frontiers | Research Topics

New therapeutic strategies against carbapenem-resistant gram-negative bacteria

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Published in
Frontiers in Microbiology

ISSN 1664-8714
ISBN 978-2-8325-5709-9
DOI 10.3389/978-2-8325-5709-9

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New therapeutic strategies against carbapenem-resistant gram-negative bacteria

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Citation

Mascellino, M. T., Oliva, A., Biswas, S., Ceccarelli, G., eds. (2024). *New therapeutic strategies against carbapenem-resistant gram-negative bacteria*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5709-9

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frontiers | Frontiers in Microbiology

TYPE Editorial
PUBLISHED 07 November 2024
DOI 10.3389/fmicb.2024.1513900

OPEN ACCESS

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RECEIVED 19 October 2024
ACCEPTED 21 October 2024
PUBLISHED 07 November 2024

CITATION
Mascellino MT, Oliva A, Biswas S and Ceccarelli G (2024) Editorial: New therapeutic strategies against carbapenem-resistant gram-negative bacteria.
Front. Microbiol. 15:1513900.
doi: 10.3389/fmicb.2024.1513900

Editorial: New therapeutic strategies against carbapenem-resistant gram-negative bacteria

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KEYWORDS

antibiotic resistance, antibiotics, bacterial pathogen, carbapenem, β-lactamase, carbapenem resistance

Editorial on the Research Topic
New therapeutic strategies against carbapenem-resistant gram-negative bacteria

Multidrug resistance in bacterial pathogens poses a threat to human health, and the emergence of carbapenem-resistant *Enterobacterales* (CRE) infections seriously affects population welfare. The emergence of carbapenem resistance is a major concern, especially for intensive care units (ICUs) and other at high-risk wards, that has led to serious consequences (Tamma et al., 2021). Detailed studies identifying the mechanisms leading to carbapenem resistance in bacteria may help overcome and manage this Research Topic (Mascellino et al., 2024).

CRE often carry multiple resistance genes that are able to propagate through both vertical and horizontal routes (Rumbo et al., 2011). These resistance elements limit treatment options and some patients require a longer duration of therapy requiring intensive care with increased toxicities if compared to patients infected with carbapenem-susceptible strains. Therefore, new alternative approaches are needed to combat the spread of antimicrobial resistance in bacteria among populations and to treat patients infected with life-threatening carbapenem-resistant gram-negative bacteria (Oliva et al., 2021; Tompkins and van Duin, 2021). The development of new classes of antibiotics could be a solution. However the director of World Health Organization (WHO) Dr. Tedros Adhanom Ghebreyesus said verbatim that "Since mid-2017, only 13 new antibiotics have been authorized, with just two representing a new chemical class and considered innovative."

Molecular studies play an important role in understanding the mechanisms underlying bacterial resistance. For example, mobile colistin resistance genes (mcr-1 to mcr-10) and their variants have been identified in gram-negative bacteria which pose a new threat to the treatment of clinical infections. A new method using a multiplex TaqMan real-time PCR assay was developed to detect mobile colistin resistance genes. This method has high specificity, sensitivity, and reproducibility (Gong et al.).

A previous study examined the *in vitro* drug susceptibilities of *Klebsiella pneumoniae* strains producing New Delhi metallo-β-lactamases in Poland. Cefiderocol, eravacycline, tigecycline, ceftazidime/avibactam (CAZ/AVI) and aztreonam were found to be the most effective antibiotics, demonstrating CAZ/AVI plus aztreonam 100% *in vitro* sensitivity with the tested strains. Owing to the safety of both drugs and their cost-effectiveness,

this therapy should be the first-line treatment for carbapenemase-producing *Enterobacterales* infections (Słabisz et al.).

Ginkgolic acid, derived from Ginko biloba extracts, was identified as a potent inhibitor against KPC-2 and found to have no toxic effects. The evolution of resistance genes has limited the clinical application of carbapenems. Therefore, the synergistic effect of ginkgolic acid and carbapenems, especially meropenem, could be interesting and valid in the fight against antimicrobial resistance, potentiating the killing effect of carbapenems on KPC-2-positive *Klebsiella pneumoniae*. Generally, plant extracts exhibit good efficacy against microorganisms, specifically by altering the functional groups of KPC-2 (Song et al.).

From a study conducted in Portugal, extensively drug-resistant *Pseudomonas aeruginosa* (PA) is a growing concern because of its resistance to most common antibiotics, including carbapenems, piperacillin-tazobactam, third- and fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones. Only one case of non-susceptibility to colistin has been previously reported. Isolates were susceptible to ceftazidime-avibactam and ceftolozane-tazobactam in 71.5 and 77.5% of tested isolates, respectively. When a combination therapy is used, ceftazidime-avibactam plus colistin is preferred, which leads to a lower mortality rate in patients with PA infections (Mendes Pedro et al.).

A network meta-analysis from South Arabia included data from over 25 clinical trials and 5,034 individuals to investigate the antibiotic resistance trends and treatment outcomes of gram-negative infections. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* turned out to be more resistant than *Enterobacterales*.

In China, resistance of *Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* was evaluated by comparing strains isolated in ICUs with those isolated from non-ICUs over a period of 10 years. Sensitivity rates to amikacin, carbapenems, and piperacillin/tazobactam were relatively high compared to high resistance rates to fluoroquinolones. The isolates from ICUs showed greater resistance than the non-ICUs strains (Shi and Xie).

The combination of antibiotics leads to more activity, especially the association between β-lactams and inhibitors of β-lactamases, such as avibactam. In a study performed in Belgium, the authors validated Gradient Diffusion Strips (GDS) focusing on an aztreonam–avibactam gradient. A double-disc synergy test (DDST) was used as a screening tool for the synergistic detection of aztreonam and avibactam. Aztreonam used in combination with ceftazidime-avibactam is considered an effective therapy for *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* (Verschelden et al.).

A recent italian study evaluated the effectiveness of imipenem/cilastatin/relebactam for the treatment of KPC-producing *Klebsiella pneumoniae* complex and difficult-to-treat resistant *Pseudomonas aeruginosa* infections in 10 patients. The successful and safe use of imipenem/relebactam, a newly available and promising treatment option, for the treatment of KPC-Klebsiella pneumoniae or difficult-to-treat resistant *Pseudomonas aeruginosa* complicated infections, has been reported. This preliminary clinical experience with imipenem/relebactam is a very attractive option compared to other combinations, especially in Italy, where the prevalence of difficult-to-treat resistant organisms is a significant concern (Leanza et al.).

Risk factors for CRE colonization also increase the possibility of subsequent infections in patients with hematological diseases. These findings suggest that septic shock increases mortality in CRE-infected hematological patients. Clinicians should try to prevent the early onset of infection and take measures to reduce mortality rates in these patients. From this research, it emerged that mortality risk factors are higher in patients with hematological diseases (Wang et al.).

Controlled trials are crucial for studying antimicrobial routes and administration methods. In Taiwan, continuous meropenem infusion is reportedly much better for microorganism eradication than traditional intermittent bolus strategies. This does not allow for bacterial death but specifically contributes bacterial eradication (Ai et al.).

The burden of antimicrobial resistance, which is a worldwide problem, should be accurately examined, especially during the SARS-CoV-2 pandemic in ICUs. Italian researchers underline this Research Topic, which poses therapeutic challenges by providing valuable insights into the epidemiology of hospital infections. In view of the possibility of a future pandemic, this approach may lead to greater infection control (Scaglione et al.).

The combination of amikacin, polymyxin-B, and sulbactam demonstrated *in vitro* synergy against multidrug-resistant *Acinetobacter baumannii*. The approach was initially developed for adults and subsequently scaled for children. Pharmacokinetic models for predicting antibiotic exposure in major tissues associated with common infections can be used to deduce antibiotic efficacy at the site of infection. Additional case reports are essential in achieving this aim (Wu et al.).

In conclusion, all solutions implemented to combat multidrug resistance are double-edged swords because the rate of antibiotic resistance of microorganisms keeps up with the rate of drug progress. Bacteria develop additional mechanisms of resistance in parallel with the spread of novel antimicrobials, such as cefiderocol, ceftolozane/tazobactam, imipenem/relebactam, sulbactam/durlobactam, cefepime-enmetazobactam, and cefepime-taniborbactam. Bacterial susceptibility rates must be continuously monitored to obtain accurate knowledge of the treatment evolution. However, the question remains as to how long these drugs will be useful (Dan and Tălăpan).

Within this scientific panorama, this Research Topic is very interesting, leading to advanced interpretations and solutions for antimicrobial resistance. To deal with resistant bacteria, new generations of the same antibacterial drugs are made by adding new components to the drug to make it effective, or antibiotics are administered in combination to make the bacteria susceptible to either one of the two (Gaibani et al., 2022). However, strategies for antibiotic combination therapy should consider the potential risk of enhanced toxicity.

The molecular studies of resistance genes play an important role if examining the crucial function of the resistance enzymes, such as extended-spectrum β-lactamases (ESBL), carbapenemases (KPC), metallo-β-lactamases (IMP, VIM, NDM etc) or OXA enzymes (OXA_48 and OXA-23) (Shanta et al., 2024).

In summary, the assessment of resistance mechanisms in common pathogens and the implications of treatment strategies constitute the cornerstones of the fight against resistance. Novel diagnostic techniques, including CRISPR-based technologies (Li

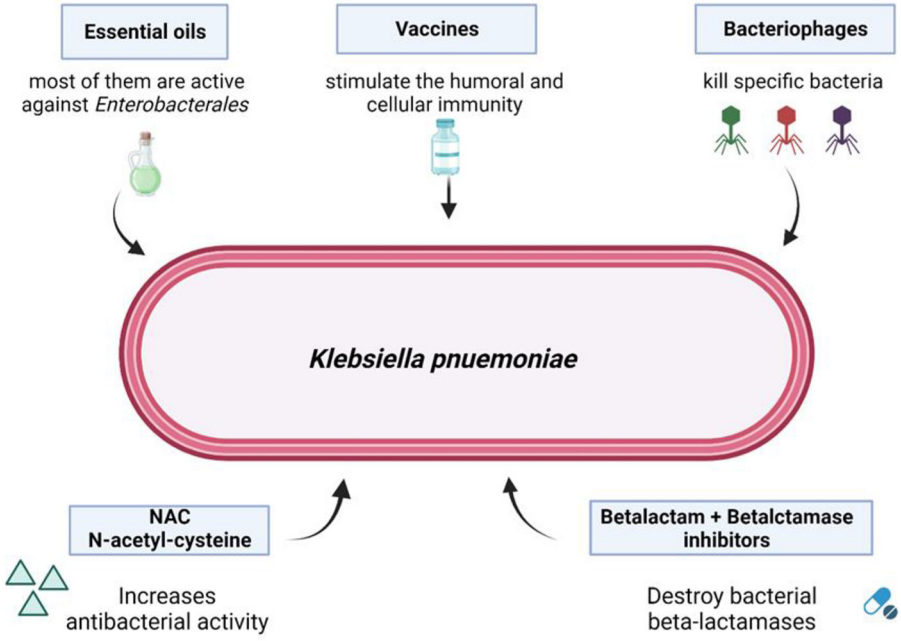

FIGURE 1
Possible solutions to overcome bacterial carbapenem-resistance. From Mascellino et al. (2024). Source BioRender.com.

et al., 2023), next-generation sequencing and whole genome sequencing should be considered, and different solutions, such as phage therapy (Qin et al., 2021), vaccination strategies, *N*-acethyl-cysteine or the use of conjugated antimicrobial peptides (AMPs) as targeted therapies against pathogenic bacteria responsible for infectious diseases, could be evaluated as alternative treatments for antibiotic-resistant infections (Figure 1).

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Acknowledgments

Authors would like to thank Dr Dania Al Ismail for her support in the figure drafting.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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frontiers | Frontiers in Microbiology

TYPE Methods
PUBLISHED 29 November 2023
DOI 10.3389/fmicb.2023.1322180

OPEN ACCESS

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RECEIVED 15 October 2023
ACCEPTED 14 November 2023
PUBLISHED 29 November 2023

CITATION
Verschelden G, Noeparast M, Stoefs A, Van Honacker E, Vandoorslaer K, Vandervore L, Olbrecht M, Van Damme K, Demuyser T, Piérard D and Wybo I (2023) Aztreonam-avibactam synergy, a validation and comparison of diagnostic tools. *Front. Microbiol.* 14:1322180. doi: 10.3389/fmicb.2023.1322180

Aztreonam-avibactam synergy, a validation and comparison of diagnostic tools

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Introduction: Antimicrobial resistance is a growing problem that necessitates the development of new therapeutic options. Cefiderocol and aztreonam (AT) are often the last active β-lactams for treating metallo-β-lactamases (MBL)-producing Gram-negative bacilli. In these difficult-to-treat bacterial strains, AT resistance is frequently attributed to the co-occurrence of other resistance mechanisms. In the case of β-lactamases they can often be inhibited by avibactam. In the present study, we evaluated the use of the double-disc synergy test (DDST) as a screening tool for the detection of synergy between AT-avibactam (ATA). We validated both the Gradient Diffusion Strips (GDSs) superposition method and the commercially available Liofilchem's ATA GDS.

Materials and methods: We tested AT susceptibility in combination with ceftazidime-avibactam for 65 strains, including 18 Serine-β-Lactamase (SBL)- and 24 MBL-producing *Enterobacterales*, 12 MBL-producing *P. aeruginosa*, and 11 *S. maltophilia* isolates. Interpretation was done with EUCAST breakpoints (version 13.0), AT breakpoints being used for ATA. The accuracy and validity of the GDSs superposition method and ATA GDS were evaluated using an AT GDS applied on Mueller Hinton Agar plates supplemented with avibactam (MH-AV). A DDST was performed to screen for synergy between antibiotic combinations.

Results: Using MH-AV, all SBL- and MBL-positive *Enterobacterales* were susceptible or susceptible at increased exposure to the combination AT-avibactam. In contrast, only 2 out of the 12 (17%) *P. aeruginosa* strains and 9/11 (82%) of the *S. maltophilia* strains were susceptible- or susceptible at increased exposure for the combination of AT-avibactam. The DDST detected all synergies, demonstrating a 100% sensitivity and 100% negative predictive value for all bacterial strains.

Conclusion: The DDST is a sensitive tool for screening for antibiotic synergy. Unlike *S. maltophilia* and SBL- and MBL-positive *Enterobacterales*, most MBL-positive *P. aeruginosa* strains remain resistant to AT-avibactam. ATA GDS should be preferred for MIC determination of the AT-avibactam combination, while the GDSs superposition method can be used as an alternative to the commercial test.

KEYWORDS

aztreonam, avibactam, ceftazidime/avibactam, *Enterobacterales*, *P. aeruginosa*, *S. maltophilia*, antimicrobial resistance, antibiotic synergy

Introduction

Antimicrobial resistance is a growing concern and has been identified by the European Commission as one of the top 3 priority health threats in July 2022. The World Health Organization (WHO) has also recognized it as one of the top 10 global public health threats facing humanity in 2019 (Akbar, 2019; Health Emergency Preparedness and Response Authority, 2022). In 2019, it was estimated that 1.27 million deaths worldwide were directly attributed to antibiotic-resistant infections. Among resistance-related deaths, *E. coli* is the leading pathogen, followed by *S. aureus*, *K. pneumoniae*, *S. pneumoniae*, *A. baumannii*, and finally *P. aeruginosa* (Murray et al., 2022).

The most common and major resistance mechanism is the degradation of β-lactam antibiotics by hydrolysis, which was first described in 1940 (Hall and Barlow, 2004). The β-lactamases produced by bacteria catalyze the hydrolysis of β-lactams, hindering the acetylation and, therefore, making penicillin-binding protein (PBP) inhibition impossible. The Ambler classification system of β-lactamases, distinguishes 4 groups according to their enzymatic structure. Class A, C and D all contain a serine residue in the active site (Serine-β-Lactamase, SBL). In contrast, class B belongs to the Metallo-β-Lactamases (MBL), which confer their activity thanks to one or two $zinc^{2+}$ ions in their active site making them resistant to 4^{th}-generation cephalosoprins and carbapenems (Bush, 2018).

The prevalence of Carbapenem Resistant *Enterobacterales* (CRE) has risen steadily since the early 1990s, first described in Europe, and now reaching global proportions (Brolund et al., 2019; Nordmann and Poirel, 2019). Epidemiology in Europe varies considerably nowadays, with a strong north–south gradient. Ranging from sporadic imported cases of carbapenem-resistant *K. pneumoniae* in the Netherlands (0.2% in 2021) to a situation such as in Greece, where hospital-related CRE infections have become an endemic problem (73.7% in 2021), threatening not only the affected patient (increased mortality) but also the national economic system (Antimicrobial resistance surveillance in Europe 2023–2021 data, 2023).

The growing resistance of Gram-negative bacilli has therefore stimulated the development of new antibiotics and novel combinations of β-lactamase and β-lactamase-inhibitors. Ceftazidime-avibactam (CZA), Food and Drug Administration approved since February 2015, is an example of such combinations. Avibactam covalently binds to the serine residue of β-lactamase. Unlike clavulanic acid and tazobactam the molecule is not hydrolyzed, it slowly dissociates, and returns to its original structure to inhibit a new β-lactamase. Avibactam thus recovers the activity of ceftazidime (third-generation cephalosporin) in class A (ESBLs, KPCs), class C (AMPc), and class D (OXA-48) β-Lactamases (Falcone and Paterson, 2016). However, avibactam (like all β-lactamase inhibitors) remains inactive against MBLs.

On the other hand, MBLs and OXA-48 s, unlike KPCs, have little or no binding capacity to aztreonam (AT), thereby preventing its hydrolysis. Avibactam in combination with AT is therefore valuable for MBL strains that have lost their susceptibility to AT due to a chromosomal AMPc derepression (overexpression), or the acquisition of a plasmid mediated ESBL (CTX-M-type, SHV-type, TEM-type), AMPc-type (CMY-2), or KPC-type (Ruppé et al., 2015).

S. maltophilia is another well-known target for the application of the combination of β-Lactam and a β-lactamase inhibitor. The combination of two intrinsic and inducible β-lactamases, L1 and L2, confers natural resistance to all β-lactam antibiotics.

L1 is an MBL (Ambler class B) that confers resistance to all β-lactams (including β-lactamase inhibitors), except AT. L2, on the other hand, is a clavulanic acid sensitive β-lactamase (Ambler class A) hydrolyzing most β-lactams, including 2nd and 3rd generation cephalosporins and AT. Co-administration of a β-lactamase inhibitor that inhibits L2 may prevent hydrolysis of AT and restore its activity against L1 (Calvopiña et al., 2017).

It is therefore essential to assess the susceptibility of these bacterial strains for the AT-avibactam (ATA) combination. In the absence of ATA Gradient Diffusion Strips (GDSs), various methods have been proposed to test the susceptibility of bacterial strains. Either by sequential application of CZA and AT GDSs on a Mueller-Hinton Agar (MHA) plate, strip stacking or by crossing the GDSs (Emeraud et al., 2019; Khan et al., 2021).

Although accessible, these methods requires the use of 2 strips, either simultaneously or successively, depending on the chosen procedure. Apart from increasing the cost and workload, this procedure requires additional handling, which can lead to imprecisions or bacterial contaminations.

To answer this question, we validated both the GDSs superposition method and commercially available Liofilchem's ATA GDSs, comparing the obtained results with those obtained using an AT GDS applied on an in-house Mueller Hinton Agar plates supplemented with avibactam (MH-AV), 4 mg/L.

We also evaluated the use of the Double Disk Synergy Test (DDST) as a screening tool for the detection of synergy between AT and avibactam, which could be useful in the selection of GDSs in a resource-saving manner (Falcone et al., 2021).

Methods

Bacterial isolates and susceptibility testing

Sixty-five bacterial strains were selected from a collection of multidrug-resistant microorganisms (MDRO) including 378 *Enterobacterales*, 2,191 *P. aeruginosa*, and 1,118 *S. maltophilia*, stored at −80°C in Mueller-Hinton broth with 20% glycerol between January 2010 and Mai 2023 in a tertiary university hospital (UZ-Brussel).

First, 18 SBL (KPC and/or OXA48) producing *Enterobacterales* were selected, all AT and meropenem resistant but CZA sensitive.

Thirty-six AT and CZA resistant MBL-producing bacterial strains were selected, among which 12 *P. aeruginosa* (VIM) and 24

Abbreviations: AT, Aztreonam; ATA, Aztreonam-avibactam; CRE, Carbapenem resistant *Enterobacterales*; CZA, Ceftazidime-avibactam; DDST, Double-disc synergy test; EUCAST, European Committee for Antimicrobial Susceptibility Testing; MALDI-TOF, Matrix-assisted laser desorption time-of-flight mass spectrometry; MBL, Metallo-β-lactamase; MHA, Mueller-Hinton Agar; MH-AV, AT gradient diffusion strip applied on Mueller-Hinton agar plates supplemented with avibactam; MIC, Minimal inhibitory concentration; GDS, Gradient diffusion strip; SBL, Serine-β-lactamase; PEA, Precision reproducibility essential agreement; CIR, Cumulative inhibition ratios.

Enterobacterales. These 24 MBL-producing *Enterobacterales* (all NDM) included 14 *K. pneumoniae*, 6 *E. coli*, 2 *C. freundii*, and 2 *E. cloacae*.

Finally, 11 *S. maltophilia* strains were selected. All selected strains were resistant to Sulfamethoxazole-Trimethoprim, AT and CZA.

Each strain was transferred to 5% sheep blood agar plates prior to testing.

Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF, Bruker Daltonics, Brussels, Belgium) was used for pathogen identification.

Antimicrobial susceptibility testing and determination of minimum inhibitory concentrations (MICs) were performed using a Sensititre™ system (Thermo Fisher Scientific®, Merelbeke, Belgium) with a 0.5 McFarland suspension according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines (version 13.0; The European Committee on Antimicrobial Susceptibility Testing, 2023a,b).

EUCAST breakpoints were considered for the interpretation of the susceptibility of bacterial strains based on their MICs (Table 1).

Interpretations of susceptibility to ATA were based on a breakpoint of AT for the tested microorganism.

Pharmacokinetics/pharmacodynamics (PK/PD) breakpoints of AT and CZA were used to interpret the MIC for *S. maltophilia*, as there is no species-specific recommendation for these antibiotics in this pathogen.

K. pneumoniae ATCC 700603 strain was used for quality control of CZA GDS as proposed by EUCAST (The European Committee on Antimicrobial Susceptibility Testing, 2023a,b).

Finally, the presence of carbapenemases (SBL and/or MBL) was confirmed using a multiplex lateral flow immunochromatographic assay from CORIS BioConcept® (Gembloux, Belgium) for the detection of NDM, VIM, IMP, KPC and OXA-48.

Synergy screening using a double-disc synergy test

The synergy between CZA and AT was screened using a double-disc diffusion method (Figure 1).

The CZA 14 µg and AT 30 µg discs from Oxoid® (Thermo Fisher Diagnostics®, Merelbeke, Belgium) were placed 20 mm apart (measured from the center of the disk), on a MHA plate.

The combination of two antibiotics was considered synergistic if an inhibition zone was observed between the two discs (Falcone et al., 2021).

The sensitivity of the method was subsequently assessed by comparing the obtained results with the gold standard, which we defined (in default of broth microdilution) as the restauration of the susceptibility or susceptibility at increased exposure for AT using

TABLE 1 MIC breakpoints for antimicrobials according to EUCAST guidelines.

Antimicrobial(s)	MIC (µg/ml) breakpoints[i]					
	Enterobacterales		*P. aeruginosa*		*S. maltophilia* [ii]	
	S	R	S	R	S	R
Aztreonam (AT)	≤1	>4	≤0.001	>16	≤4	>8
Ceftazidime (CZ)	≤1	>4	≤0.001	>8	≤4	>8
Ceftazidime-avibactam (CZA)	≤8	>8	≤8	>8	≤8	>8

[i]Interpretations of susceptibility to CZA and AT-avibactam combination was based on CZA and AT EUCAST breakpoints, respectively, for the tested microorganism (European Committee on Antimicrobial Susceptibility Testing, version 13.0).

[ii]PK/PD breakpoints of AT were used to interpret the MIC for *S. maltophilia* as there is no species-specific recommendation for this antibiotic in this species.

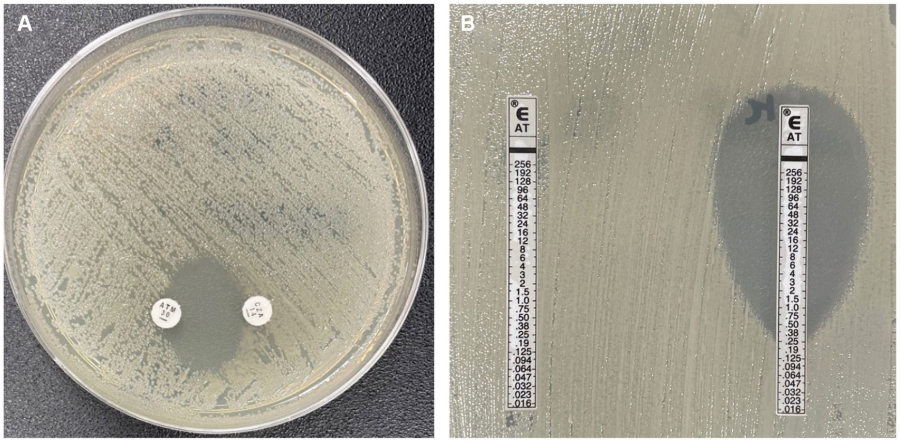

FIGURE 1
(A) Aztreonam (AT) and Ceftazidime-avibactam (CZA) double-disc synergy test. **(B)** Left: controle AT Gradient diffusion strip (GDS), Right: AT and CZA GDSs superposition methode.

an AT GDS applied on an in-house MH-AV. In the absence of an EUCAST recommendation for ATA sensitivity testing, the concentration of avibactam was fixed at 4 mg/L, as required by EUCAST for susceptibility testing of CZA (The European Committee on Antimicrobial Susceptibility Testing, 2023a,b).

Synergy confirmation and determination of the MIC

The MIC of AT after avibactam supplementation was measured using two different methods (Figure 1):

First, using a GDSs superposition method: a CZA GDS from bioMérieux® (Schaarbeek, Belgium), containing a fixed concentration of avibactam 4 mg/L, was applied on MHA plates for 10 min at room temperature. The strip was subsequently replaced by an AT GDS to determine the MIC of the ATA combination (Emeraud et al., 2019).

Secondly, using commercially available ATA GDSs from Liofilchem® (ElitechGroup Benelux, Spankeren, The Netherlands).

MICs were measured after 16 h incubation at 35°C in ambient air and interpreted in accordance with EUCAST guidelines.

Synergy between AT and avibactam was interpreted as follows:

- Synergy ≥2 two-fold dilution decrease in MIC.
- Indifference <2 two-fold dilution decrease in MIC.

Validation of the GDSs superposition method and the ATA GDS

The validity CZA-AT GDSs superposition method was evaluated by comparing the obtained MIC from multi-resistant *S. maltophilia*- and MBL-positive strains ($N = 47$), with that of an AT GDS applied to in-house MH-AV, 4 mg/L (gold standard).

The ATA GDSs, on the other hand, were validated using all 65 bacterial strains (including the SBL-producing *Enterobacterales* strains).

Statistical methods

Data were analyzed using GraphPad Prism version 9.0.0 for Mac, GraphPad Software, San Diego, California USA, www.graphpad.com.

Shapiro–Wilk test with a significance value of >0.05 was used to assess the assumption of normality of MIC values.

MIC values are reported as the MIC50, MIC90, and MIC ranges.

Passing Bablok regression and Spearman's correlation were used to compare MIC values of the commercial ATA GDS with the MIC of AT obtained on MH-AV.

The validation of the tests was confirmed when a Precision reproducibility Essential Agreement (PEA) of at least 95% was achieved (agreement within a single two-fold dilution compared to the results achieved using the MH-AV).

Finally, McNemar's exact test was used to determine the significance of the difference in the occurrence of ±1 two-fold dilution between the use of the superposition method and commercial ATA GDS compared to the reference method.

Results

Synergy screening using a DDST

The DDST was able to detect all synergies in SBL- and MBL-producing *Enterobacterales* strains (Figure 1; Table 2), showing a sensitivity of 100% with a 95% confidence interval (CI) of (92 to 100%).

DDST also demonstrated its utility in the MBL-producing *P. aeruginosa* strains, where the method showed a sensitivity and specificity of, respectively, 100% (95% CI, 29 to 100%) and 78% (95% CI, 40 to 97%), with a Negative Predictive Value (NPV) of 100% (95% CI, 59 to 100%).

For *S. maltophilia* strains, the DDST demonstrated up to 100% sensitivity in detecting AT-CZA synergy (95% CI, 71 to 100%; Figure 2).

TABLE 2 MIC and susceptibility interpretation[i] of CZA and ATA combinations on the different bacterial strains.

Bacterial strain		MIC (μg/ml)[i]			
	DDST	MIC 50	MIC 90	Range	Resistance rate
S. maltophilia **(*n* =11)**					
ATA	Sens. 100 (71–100)	2	12	2 to 24	2/11 (18%)
	Spec. NA				1/11 (9%)[ii]
P. aeruginosa **MBL (*n* =12)**					
ATA	Sens. 100 (29–100)	24	128	2 to 192	10/12 (83%)
	Spec. 78 (40–97)				
	NPV 100 (59–100)				
Enterobacterales **(*n* =42)**					
ATA	Sens. 100 (92–100)				
	Spec. NA				
Enterobacterales **MBL (*n* =24)**					
ATA		0.25	3	0.064 to 3	0/24
					5/24 (21%)[ii]
Enterobacterales **SBL (*n* =18)**					
CZA		2	4	0,38 to 4	0/18
ATA		0.38	0.75	0.064 to 1.5	0/18
					1/18 (<0.1%)[ii]

AT: aztreonam, ATA: aztreonam/avibactam combination, CZA: ceftazidim-avibactam, DDST: double-disc synergy test, EUCAST: European Committee on Antimicrobial Susceptibility Testing, MH-AV: Mueller Hinton agar plate supplemented with avibactam, NA: not applicable, NPV: negative predictive value, Sens.: sensitivity, Spec.: specificity.

[i]MIC of the ATA combination obtained using a AT GDS applied on MH-AV 4 mg/L. Interpretations of susceptibility to CZA and ATA combination was based on CZA and AT EUCAST breakpoints (version 13.0) respectively for the tested microorganism. PK/PD breakpoints of AT were used to interpret the MIC for S. maltophilia as there is no species-specific recommendation for this antibiotic in this species.

[ii]Susceptible at increased exposure (I).

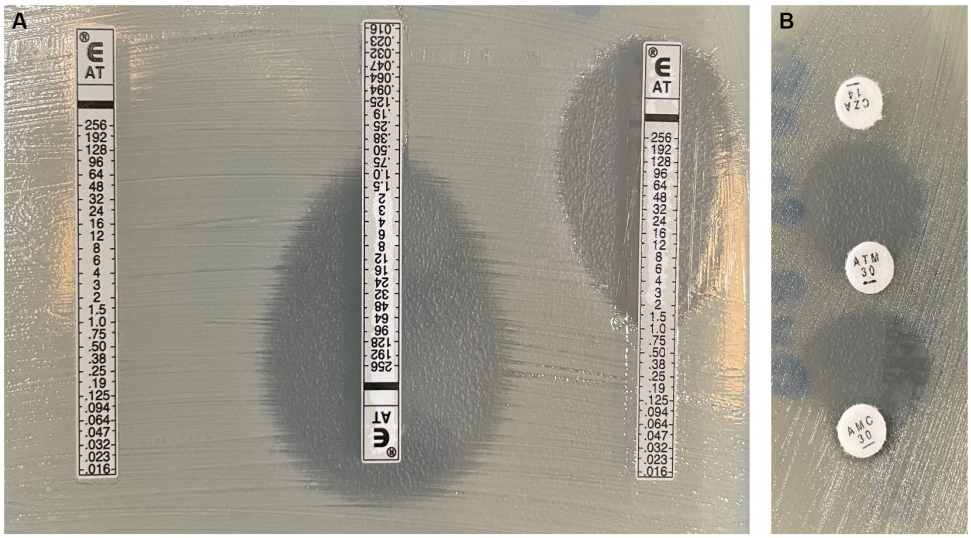

FIGURE 2
(A) Left: controle Aztreonam (AT) Gradient diffusion strip (GDS), Middle: AT and cefazidime-avibactam (CZA) GDSs superposition methode, Right: AT and amoxicillin clavulanic acid (AMC) GDSs superposition methode. **(B)** AT-CZA and AT-AMC double-disc synergy test.

Synergy confirmation and MIC determination

We found no resistance to the combination of ATA in any *Enterobacterales* strain (Table 3).

As expected, all SBL-positive *Enterobacterales* strains (CZA susceptible) were susceptible (17/18, 94%), or susceptible at increased exposure (1/18, 0.06%) to the combination of ATA with MIC50 and MIC90 of, respectively, 0.38 and 0.75 μg/ml.

All MBL-positive *Enterobacterales* strains were found to be susceptible (19/24, 79%) or susceptible at increased exposure (5/24, 21%), with MIC50 and MIC90 of, respectively, 0.25 and 3 μg/ml.

On the other hand, only 17% (2/12) of the VIM positive *P. aeruginosa* strains were found to be susceptible to the combination of ATA at increased exposure, with a MIC50 and MIC90 of, respectively, 24 and 128 μg/ml.

Nine out of 11 (82%) of the *S. maltophilia* strains were susceptible (8/11, 73%), or susceptible at increased exposure (1/11, 9%) to the combination of AT and avibactam, with MIC50 and MIC90 of, respectively, 2 and 12 μg/ml.

Validation of the GDSs superposition method and the ATA GDS

Only one strain showed a more than 1 two-fold dilution difference between MH-AV and the ATA GDS.

We were able to demonstrate a strong positive correlation with a Spearman correlation coefficient of r_s (65) = 0.985, p = <0.001 and a PEA of 98%, thereby validating the commercial ATA GDS.

In contrast, the GDSs superposition method showed, compared to MH-AV, a PEA of 87%.

Despite a lower PEA than the commercial ATA GDS, the difference in the occurrence of a ± 1 two-fold dilution between the two methods, compared to the reference method (MH-AV, 4 mg/L), was not significant (p = 0.221). The GDSs superposition method still demonstrated a strong correlation with r_s (47) = 0.964, p = <0.001 and did not lead to any difference in the interpretation of susceptibility.

Discussion

A limitation of the current study is the small number of bacterial strains. Only 65 strains were collected, all from patients at a single tertiary healthcare center. Since the rate and mechanisms of resistance vary according to geographical location and type of healthcare facility, the results of the present study may not be extrapolable to other settings. Furthermore, the interpretation of *S. maltophilia* sensitivities is not comparable with other studies given the use of EUCASTs PK/PD breakpoints for AT and CZA in our study instead of the frequently used CLSI breakpoints for *P. aeruginosa* in other studies.

Finally, the use of the MH-AV method as a reference, in the absence of broth microdilution, is another limitation.

In the context of escalating antibiotic resistance, the need for novel therapeutic options cannot be overstated. Following a promising clinical trial with CZA-AT, two further RCTs, REVISIT and ASSEMBLE (awaiting publication), appear to reinforce the effectiveness of the ATA pairing in treating MBL-positive Gram-negative bacterial infections (Clinical Trials.gov, 2023). This underlines the demand for precise synergy detection tools and reliable MIC determination tests (Falcone et al., 2021).

Our research has shown that the DDST is an efficient means of identifying synergy between AT and avibactam. The high negative predictive value of DDSTs enables more accurate selection of GDSs,

TABLE 3 Results of ATA synergy screening (using DDST) and MICs[i] of AT and ATA combination on the different bacterial strains.

Bacterial strain	Resistance mechanism(s)	Synergy		MIC (μg/ml)			
		DDST	AT GDS MH-AV[ii]	AT GDS MHA	AT GDS MH-AV	AT/CZA GDS MHA	ATA GDS MHA
Enterobacterales MBL (*N*=24)							
K. pneumoniae	NDM	Y	Syn	256	0.25	0.25	0.25
K. pneumoniae	NDM	Y	Syn	256	0.5	0.75	0.5
K. pneumoniae	NDM	Y	Syn	256	0.094	0.25	0.19
E. coli	NDM	Y	Syn	256	0.5	0.5	0.75
E. cloacae	NDM	Y	Syn	256	0.094	0.25	0.19
K. pneumoniae	NDM	Y	Syn	256	0.38	0.38	0.38
E. cloacae	NDM	Y	Syn	256	0.125	0.5	0.25
K. pneumoniae	NDM	Y	Syn	256	0.25	0.25	0.38
E. coli	NDM	Y	Syn	256	1.5	2	1.5
E. coli	NDM	Y	Syn	256	0.75	1	1
K. pneumoniae	NDM	Y	Syn	256	0.125	0.125	0.094
K. pneumoniae	NDM	Y	Syn	48	3	1.5	3
E. coli	NDM	Y	Syn	256	1.5	1.5	1.5
K. pneumoniae	NDM	Y	Syn	256	0.75	1	0.5
K. pneumoniae	NDM	Y	Syn	256	0.25	0.25	0.19
K. pneumoniae	NDM	Y	Syn	256	0.38	0.38	0.25
E. coli	NDM	Y	Syn	256	3	3	2
C. freundii	OXA48 + NDM	Y	Syn	48	0.25	0.5	0.25
C. freundii	OXA48 + NDM	Y	Syn	32	0.25	0.75	0.19
E. Coli	NDM	Y	Syn	256	3	4	4
K. pneumoniae	NDM	Y	Syn	96	0.094	0.064	0.064
K. pneumoniae	NDM	Y	Syn	48	0.064	0.064	0.064
K. pneumoniae	OXA48 + NDM	Y	Syn	256	0.38	0.5	0.38
K. pneumoniae	NDM	Y	Syn	128	0.094	0.064	0.064
Enterobacterales SBL (*N*=18)							
K. pneumoniae	KPC	Y	Syn	256	0.38	0.5	0.5
K. pneumoniae	OXA48	Y	Syn	256	0.19	0.25	0.19
K. pneumoniae	OXA48	Y	Syn	256	0.5	0.75	0.75
K. pneumoniae	KPC	Y	Syn	256	0.75	1	0.75
K. pneumoniae	OXA48	Y	Syn	256	0.75	1.5	1.5
K. pneumoniae	KPC	Y	Syn	256	1.5	1.5	1
K. Variicola	KPC	Y	Syn	256	0.75	1.5	1.5
K. pneumoniae	OXA48 + KPC	Y	Syn	256	0.75	0.75	1
K. pneumoniae	KPC	Y	Syn	256	0.75	0.75	1
K. pneumoniae	OXA48	Y	Syn	96	0.25	0.38	0.25
K. pneumoniae	KPC	Y	Syn	256	0.38	0.5	0.38
E. cloacae	OXA48	Y	Syn	256	0.38	0.5	0.5
K. pneumoniae	OXA48	Y	Syn	256	0.75	0.75	0.75
K. pneumoniae	KPC	Y	Syn	256	0.38	0.75	0.38
K. pneumoniae	OXA48	Y	Syn	256	0.19	0.25	0.25
K. pneumoniae	KPC	Y	Syn	256	0.5	0.75	0.75

(Continued)

TABLE 3 (Continued)

Bacterial strain	Resistance mechanism(s)	Synergy		MIC (μg/ml)			
		DDST	AT GDS MH-AV[ii]	AT GDS MHA	AT GDS MH-AV	AT/CZA GDS MHA	ATA GDS MHA
K. pneumoniae	OXA48	Y	Syn	128	0.064	0.094	0.064
K. pneumoniae	KPC	Y	Syn	256	0.064	0.125	0.064
Pseudomonas aeruginosa (*N*=12)							
P. aeruginosa	VIM	N	Ind	32	32	32	32
P. aeruginosa	VIM	Y	Syn	96	24	24	24
P. aeruginosa	VIM	N	Ind	32	32	32	24
P. aeruginosa	VIM	N	Ind	128	192	96	64
P. aeruginosa	VIM	N	Ind	96	48	24	32
P. aeruginosa	VIM	N	Ind	32	24	24	24
P. aeruginosa	VIM	Y	Syn	64	3	2	4
P. aeruginosa	VIM	Y	Ind	64	24	24	24
P. aeruginosa	VIM	N	Ind	256	128	64	96
P. aeruginosa	VIM	N	Ind	48	24	24	32
P. aeruginosa	VIM	Y	Syn	64	2	1.5	2
P. aeruginosa	VIM	Y	Ind	32	24	24	24
Stenotrophomonas maltophilia (*N*=11)							
S. maltophilia		Y	Syn	256	2	1.5	1.5
S. maltophilia		Y	Syn	256	2	2	3
S. maltophilia		Y	Syn	256	2	3	3
S. maltophilia		Y	Syn	256	2	3	2
S. maltophilia		Y	Syn	256	24	24	24
S. maltophilia		Y	Syn	256	6	6	6
S. maltophilia		Y	Syn	256	12	3	12
S. maltophilia		Y	Syn	256	3	3	2
S. maltophilia		Y	Syn	256	2	1.5	2
S. maltophilia		Y	Syn	256	2	4	2
S. maltophilia		Y	Syn	256	2	2	2

AT, aztreonam; ATA, aztreonam/avibactam combination; CZA, ceftazidim-avibactam; DDST, double-disc synergy test; EUCAST, European Committee on Antimicrobial Susceptibility Testing; GDS, gradient diffusion strip; MHA, Mueller Hinton agar; MH-AV, Mueller Hinton agar plate supplemented with avibactam 4 mg/L; N, no; PK/PD, Pharmacokinetics/pharmacodynamics; Y, yes.

[i]Red, orange, and green colored MICs correspond to resistant, intermediate (susceptible at increased exposure) and susceptible categorization, respectively, according to EUCAST breakpoints (version 13.0) for the tested microorganism. Interpretations of susceptibility to ATA combination was based on AT EUCAST breakpoints for the tested microorganism. PK/PD breakpoints of AT were used to interpret the MIC for *S. maltophilia* as there is no species-specific recommendation for this antibiotic in this species.

[ii]Synergy between AT and avibactam (using AT GDS applied on MH-AV) was interpreted as follows: - Synergy (Syn) ≥ 2 two-fold dilution decrease in MIC. -Indefference (Ind) < 2 two-fold dilution decrease in MIC.

thereby conserving time and resources. Our ATA GDS demonstrated a strong correlation with the reference method and showed a PEA exceeding 95%, thereby endorsing the ATA GDS. Despite falling short of the anticipated 95% PEA, the CZA-AT superposition method still exhibited a robust correlation. This discrepancy, while noticeable, is statistically insignificant and does not alter the interpretation of susceptibility. Therefore, ATA GDS is the favored option for MIC determination, whereas the superposition method can serve as a substitute for the commercial test.

As predicted, all SBL-positive *Enterobacterales* that are susceptible to CZA are also susceptible to ATA, adding another option to the treatment of SBL-positive *Enterobacterales*. Although both MIC50 and MIC90 seem to favor the latter, its superiority over CZA still needs validation using a time-kill assay. Furthermore, the influence of ATA on the induction of resistance and on the selection pressure of the bacterial flora remains unexplored (Yu et al., 2021).

The majority (79%) of MBL-positive *Enterobacterales* were susceptible, or susceptible at increased exposure (21%), to the ATA combination. This suggests the value of considering ATA combination therapy in infections caused by MBL-positive, Gram-negative bacilli, prior to obtaining MIC results.

Conversely, only 17% of MBL-positive *P. aeruginosa* strains showed susceptibility at increased exposure to the ATA combination. This suggests resistance to AT is not due to the presence of a plasmid-encoded β-lactamase, but rather due to factors like hyperactive efflux systems, impermeability, variations of derepressed *Pseudomonas*-derived cephalosporinases, or OXA enzymes (apart from OXA-48; Sreenivasan et al., 2022). It also warns that *P. aeruginosa* susceptibility interpretations using the superposition method should be approached with caution. A false impression of susceptibility may be made when interpreting the combination of ATA using AT breakpoints, particularly in bacterial strains with a CZA MIC of 16 µg/L. The misinterpretation could be circumvented with DDST, as an absence of a synergy zone between AT and CZA would indicate potential resistance, despite an MIC of 16 µg/L.

The effectiveness of a β-lactamase inhibitor with AT co-administration, is well-known to inhibit L2 and restore activity against L1, is a widely accepted practice in *S. maltophilia* isolates (Calvopiña et al., 2017). Clavulanic acid, demonstrated to have superior activity compared to sulbactam and tazobactam, has justified the pairing of ticarcillin and clavulanic acid since the 90s (Lecso-Bornet and Bergogne-Bérézin, 1997). The combination of AT with clavulanic acid or newer β-lactamase inhibitors like avibactam, relebactam, and vaborbactam have seen renewed interest since the withdrawal of ticarcillin-clavulanic acid from the market in 2015. Our study, like previous research, demonstrates that both avibactam and clavulanic acid, when combined with AT, provide promising therapeutic potential. In our tests, avibactam and clavulanic acid (data not shown) restored AT susceptibility at increased exposure in 91 and 82% of tested *S. maltophilia* strains, respectively (Biagi et al., 2020). Nevertheless, the ATA combination will likely be favored over the AT-clavulanic acid combination due to lower resistance frequency, superior time-kill assay results, and the imminent availability of a fixed drug combination from Pfizer (Clinical Trials.gov, 2023; Biagi et al., 2020).

Conclusion

Despite its limited efficacy against *P. aeruginosa*, ATA has demonstrated a broad spectrum of activity against multi-resistant *S. maltophilia*, SBL- and MBL-positive *Enterobacterales*.

The DDST is a sensitive tool for the detection of synergy between AT and avibactam.

ATA GDS should be preferred for MIC determination of the ATA combination, while the GDSs superposition method can be used as an alternative to the commercial test.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

GV: Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft. MN: Writing – review & editing. AS: Writing – review & editing. EH: Data curation, Writing – review & editing. KV: Resources, Writing – review & editing. LV: Formal analysis, Writing – review & editing. MO: Writing – review & editing. KD: Writing – review & editing. TD: Writing – review & editing. DP: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing – review & editing. IW: Formal analysis, Project administration, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

I would like to extend my warmest thanks to the entire staff of the microbiology lab who made this work possible.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 30 November 2023
DOI 10.3389/fmicb.2023.1304011

OPEN ACCESS

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RECEIVED 10 October 2023
ACCEPTED 02 November 2023
PUBLISHED 30 November 2023

CITATION
Muteeb G (2023) Network meta-analysis of antibiotic resistance patterns in gram-negative bacterial infections: a comparative study of carbapenems, fluoroquinolones, and aminoglycosides.
Front. Microbiol. 14:1304011.
doi: 10.3389/fmicb.2023.1304011

Network meta-analysis of antibiotic resistance patterns in gram-negative bacterial infections: a comparative study of carbapenems, fluoroquinolones, and aminoglycosides

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Introduction: Antimicrobial resistance poses a grave global threat, particularly with the emergence of multidrug-resistant gram-negative bacterial infections, which severely limit treatment options. The increasing global threat of antimicrobial resistance demands rigorous investigation, particularly concerning multidrug-resistant gram-negative bacterial infections that present limited therapeutic options. This study employed a network meta-analysis, a powerful tool for comparative effectiveness assessment of diverse antibiotics. The primary aim of this study was to comprehensively evaluate and compare resistance patterns among widely used antibiotic classes, namely carbapenems, fluoroquinolones, and aminoglycosides, for combating gram-negative pathogens.

Methods: We searched PubMed, Web of Sciences, Scopus, Scholarly, Medline, Embase, and Cochrane databases up to August 27, 2023. Studies showing antibiotic resistance in clinical isolates of Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* exposed to carbapenems, fluoroquinolones, and aminoglycosides were included. This study determined treatment-specific resistance percentages and ranked these treatments based on resistance using a random-effects network meta-analysis technique. To investigate the impact of the study and pathogen features, subgroup and meta-regression analyses were performed. Risk ratios and 95% confidence intervals (CIs) were calculated using a network meta-analysis (NMA) incorporating both direct and indirect evidence. Clinical improvement, cure, microbiological eradication, and death from any cause were the primary outcomes. Nephrotoxicity was a secondary result.

Results: The analysis included 202 publications and 365,782 gram-negative isolates. The NMA included data from 20 studies and 4,835 patients. Carbapenems had the lowest resistance rates throughout the pathogen spectrum, with resistance percentages of 17.1, 22.4, and 33.5% for Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii*, respectively. For the same infections, aminoglycosides showed resistance rates of 28.2, 39.1, and 50.2%, respectively. Fluoroquinolones had the highest resistance rates at 43.1, 57.3, and 65.7%, respectively. Unexpectedly, resistance to all three antibiotic classes has increased over time, with multidrug resistance being the most prevalent.

Conclusion: This extensive network meta-analysis provides an overview of the patterns of resistance throughout the world and how they are changing. The most effective choice is still carbapenems, but the increasing resistance highlights the critical need for multimodal therapies to protect antibiotic effectiveness against these powerful gram-negative infections.

KEYWORDS

antibiotic resistance, gram-negative bacterial infections, carbapenems, fluoroquinolones, aminoglycosides, treatment outcomes, clinical effectiveness, adverse events

1 Introduction

Antimicrobial resistance (AMR) is a serious worldwide health concern that must be addressed immediately. According to World Health Organization (WHO) figures from 2022, drug-resistant diseases kill over 1.2 million people globally each year, making it a major problem. Gram-negative bacteria, such as Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, are substantial contributors to healthcare-associated infections among the various AMR culprits (Nagarjuna et al., 2018). These bacteria have demonstrated an alarming proclivity for resistance to first-line antibiotics such as carbapenems, fluoroquinolones, and aminoglycosides. These medications, which are necessary for treating serious infections, are becoming less effective as resistant strains occur (Zhang et al., 2010).

Alarming data emphasize the need of combating AMR. A 75-country study discovered that 32 to 60% of Enterobacterales, *P. aeruginosa*, and *A. baumannii* isolates are now multidrug-resistant, making them resistant to various drugs. In a 2019 study, the Centers for Disease Control and Prevention (CDC) designated carbapenem-resistant Enterobacterales as a "Urgent Threat," emphasizing the limited treatment options for infections caused by these extremely resistant bacteria (Keller et al., 2009). This critical circumstance highlights the necessity of directing empiric medication based on local resistance rates, an approach that can assist reduce wasteful antibiotic usage and enhance patient outcomes. However, one key issue in attaining this aim is the variety in susceptibility testing methodologies utilized across research, which complicates cross-study comparisons and highlights the need of more comprehensive analyses (Ogawa et al., 2008).

The rising frequency of multidrug-resistant (MDR) gram-negative infections has made empiric antibiotic selection difficult, since popular drugs such as fluoroquinolones, carbapenems, and aminoglycosides are becoming less effective. Gram-negative bacteria such as Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are primary sources of healthcare-associated infections, and resistance to frontline medicines in these species is increasing gradually. Antibiotic overuse and abuse have been a primary cause of resistance, selecting for mutations and horizontal gene transfer amongst pathogens (Lee et al., 2010).

The significant mortality and healthcare expenditures associated with MDR gram-negative infections highlight the critical need for antibiotic selection that is based on current regional resistance data. However, surveillance studies use different approaches, making it difficult to compare antibiotics and geographic locations (Woodward et al., 2013). This problem is solved by network meta-analysis (NMA), which combines information from several trials with common comparators into a single analysis. This method gives a unified overview of all relevant data in order to correctly estimate comparative treatment effects and rank treatments (McIntyre et al., 2012).

We perform an NMA of antimicrobial resistance data for carbapenems, fluoroquinolones, and aminoglycosides against key gram-negative bacteria. The primary goal is to establish pooled resistance percentages for each antibiotic class. Secondary goals are to rank classes based on total resistance, examine changes over time, assess regional and pathogen-specific impacts, and identify connections between resistance to various drugs within classes (Fischer et al., 2010).

From the beginning till February 2023, studies will be found by comprehensive database searches. Included research must report on clinical isolate susceptibility testing utilizing CLSI or EUCAST breakpoints. Data extraction will be performed twice. A modified Newcastle-Ottawa scale will be used to assess the study's quality. To construct league tables ranking antibiotic classes according to resistance, network meta-analyses will be done using random-effects models (Leport et al., 2013). Time trends, geographical differences, infections, and relationships between individual antibiotics will be evaluated using subgroup and meta-regression analysis (Villar et al., 2014).

This NMA will use worldwide surveillance data to compare resistance patterns for important antibiotic classes used against troublesome gram-negative bacteria. Ranking classes based on pooled resistance rates can help influence guidelines for empiric antibiotic selection. Temporal and geographical trends found may benefit in local stewardship initiatives and infection control programs. Individual antibiotic interactions may shed light on methods of resistance propagation between organisms (1) (Chai et al., 2012).

It is critical to preserve current medications through evidence-based stewardship and resistance containment. Our research will consolidate the best available knowledge on resistance patterns in order to recommend the appropriate usage of carbapenems, fluoroquinolones, and aminoglycosides (Okeke et al., 2011). Identifying high-risk diseases and geographic hotspots allows resources to be directed to places in most need. Individual antibiotic associations can reveal correlations between consumption and resistance. Our findings will contribute to worldwide plans to enhance antibiotic prescription and protect the efficacy of presently available medicines against gram-negative infections (Cardenas et al., 2012).

2 Methods

2.1 Study details

The analysis was carried out using protocol PRT 465439 from the International Perspective Register of Systematic Reviews (PROSPERO). The study techniques followed the requirements provided in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extended statement for network meta-analysis.

2.2 Study participants

Eligible studies reported antibiotic susceptibility testing findings of clinical isolates of Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* obtained from individuals 16 years old with diverse healthcare-associated illnesses. Using the Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) technique, isolates were tested for susceptibility to carbapenems, fluoroquinolones, and aminoglycosides. Infections included pneumonia [Hospital-acquired pneumonia (HAP), ventilator-associated pneumonia (VAP)], bloodstream infections (BSIs), urinary tract infections (UTIs), intra-abdominal infections, and skin and soft tissue infections from both hospital and community settings worldwide.

2.3 Search strategy and selection criteria

We searched PubMed, Embase, MEDLINE, and the Cochrane Central Register of Controlled Trials with no language constraints from inception to March 2022. Search terms included "carbapenems" OR "meropenem" OR "imipenem" OR "doripenem" OR "ertapenem" AND "fluoroquinolones" OR "ciprofloxacin" OR "levofloxacin" OR "moxifloxacin" OR "ofloxacin" AND "aminoglycosides" OR "amikacin" OR "gentamicin" OR "tobramycin" OR "Relevant article reference lists were also examined.

We independently selected papers that matched the following criteria: (1) reported antimicrobial susceptibility testing findings for *E. coli*, *Klebsiella* spp., *P. aeruginosa*, and *A. baumannii* clinical isolates using CLSI or EUCAST methods; (2) tested isolates against carbapenems, fluoroquinolones, and/or aminoglycosides; and (3) resistance data from 2000 onwards. Exclusion criteria included reviews, case reports, non-clinical research, studies that did not publish susceptibility data against our antibiotics of interest, and studies with less than 50 isolates examined. Discussion was used to settle disagreements.

2.4 Study selection and data extraction

The research independently analyzed the titles and abstracts of all retrieved publications to choose relevant data. The review team worked through these disparities. The whole texts of all potentially eligible studies were inspected and analyzed to confirm that their content matched the inclusion criteria. The first author's name, year of publication, study location, study type, type of drug resistance, study size, and patient characteristics (treatment regimens, medication doses, treatment duration, age, gender, and kind of infection) were all retrieved and evaluated. In the supplied sample, outcome data such as clinical improvement, clinical cure, microbiological eradication, all-cause mortality, and nephrotoxic, neurotoxic, ototoxic events, and super infections were also detected in the given sample.

2.4.1 Inclusion criteria

Only published, peer-reviewed research in English was eligible for inclusion. Randomized controlled trials, non-randomized controlled trials, prospective and retrospective cohort studies, and case-control studies were the research designs that were taken into consideration. Reviews, opinions, letters, and case reports were not accepted. To evaluate drug-resistant illnesses brought on by Gram-negative bacteria, studies were necessary. Reports on the clinical efficacy of treatment plans or unfavorable occurrences were required. Excluded studies solely reported pharmacokinetic or *in vitro* results. To be included, a minimum of ten patients had to be in the sample. Age, gender, or illness type limits did not apply to the patient. Outpatient and inpatient settings met the eligibility requirements for inclusion. Review candidates included studies published between August 2023 and the database's setup.

2.5 Quality assessment

The risk of bias in randomized controlled trials (RCTs) was assessed separately by two reviewers using the Cochrane RoB 2.0 methodology. This tool assesses the risk of bias in five domains: bias coming from the randomization procedure, bias owing to variations from intended interventions, bias due to missing outcome data, bias in outcome measurement, and bias in the selection of the reported result. Each domain was rated as "low risk," "some concerns," or "high risk."

The Newcastle-Ottawa Scale was used to assess the quality of non-randomized research. This tool assesses studies based on three criteria: research group selection, group comparability, and exposure or result determination. Each numbered item in the selection and exposure categories received a maximum of one star. For comparison, a maximum of two stars can be awarded. The methodological quality was assessed using the scores obtained, with 0–3, 4–6, and 7–9 indicating a high, moderate, and low risk of bias, respectively.

Any discrepancies in the quality evaluation were settled through conversation between the two reviewers. Based on the hazards found for the various studies, the overall risk of bias for each outcome was calculated. The results of the quality assessment were given in a risk of bias table and will be taken into account throughout data synthesis and interpretation.

All antibiotic treatments included in trials that qualified for inclusion were compared concurrently using a random-effects network meta-analysis. Direct comparisons within trials and indirect comparisons between trials based on a common comparator are both possible with network meta-analysis. This methodology maintains the randomization in individual trials while modeling variation within and across studies. A logistic link function and a binomial likelihood were used to fit a probabilistic consistency model. With 95% credible intervals (CrIs), odds ratios (ORs) were used to evaluate the effects of relative treatments. The I^2 statistic was used to quantify heterogeneity; values greater than 50% indicated significant heterogeneity. To see the relationships between treatments based on head-to-head comparisons, network plots were created. In order to facilitate the probabilistic ranking of treatment effectiveness and tolerability, rankograms and surface under the cumulative ranking curve (SUCRA) values were also computed. R was used to do statistical studies using the gemtc package.

2.6 Outcomes

The purpose of the investigation was to assess the percentage of resistance isolates for each antimicrobial agent, using defined criteria

from CLSI or EUCAST standards. Secondary outcomes included pooled resistance percentages for each antibiotic class against Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii*, as well as ranking antibiotic classes based on overall resistance profiles, changes in resistance over time, geographic region impact, Gram-negative pathogen species effect, associations between drug resistance, and quality indicators of susceptibility testing methods. Resistance has spread more quickly as a result of the increased occurrence of resistant gene cassettes on mobile genetic elements. The spread of very resistant strains inside and across hospital institutions may have been aided by uneven infection control procedures. Future resistance rises must be stopped by addressing these fundamental causes.

2.7 Data synthesis and analysis

Pairwise and network meta-analyses were conducted using a random-effects model in STATA 16.0 to synthesize direct and indirect evidence. A random-effects network meta-analysis was conducted using a Bayesian framework, fitting a probabilistic consistency model to both direct and indirect treatment comparisons. Treatment effects were estimated using odds ratios and 95% credible intervals. Treatment nodes were ranked based on SUCRA values, with higher values indicating more effective or better tolerated treatments. Heterogeneity and inconsistency were assessed using the posterior median of the τ^2 and $-2\times\log$ (Bayes factor for consistency) parameters. Pooled risk ratios (RR) with 95% confidence intervals (CIs) were calculated for antimicrobial resistance. Heterogeneity was assessed using the I^2 statistic, with values of >50% indicating substantial heterogeneity.

The consistency of direct and indirect evidence was assessed using node-splitting analysis and inconsistency factors (IFs). Surface under the cumulative ranking curves (SUCRAs) were used to classify antibiotic classes. Sub-group network meta-analyses were performed according to the following criteria:

1. Time periods (2013–2016, 2016–2021, 2021–2023).
2. Geographic regions (Europe, North America, Asia, etc.).
3. Pathogen species (*Enterobacteriaceae, P. aeruginosa, A. baumannii*).

Meta-regression was utilized to investigate the relationship between medication resistance. If enough papers (>10) were available, publication bias was examined using comparison-adjusted forest plots. To investigate subgroup heterogeneity, a design-by-treatment interaction model was applied. PRISMA-NMA criteria were followed for the analyses. Sensitivity analyses were conducted to investigate the implications of research quality, design, and other statistical models. The main result was the antibacterial resistance rate, which was given as pooled resistance rates (PRR).

3 Results

A total of 2,087 studies were identified utilizing searches of EMBASE, Medline, and the Cochrane Central Register of Controlled Trials from the start through August 2023.66 full-text publications were appraised for eligibility after 958 duplicates were removed and 1,129 titles and abstracts were reviewed. The network meta-analysis comprised 25 studies totaling 5,034 persons that matched the inclusion criteria (Kumarasamy et al., 2010). Carbapenems were found to have the lowest resistance rates throughout the pathogen spectrum, followed by aminoglycosides, while Fluoroquinolones had the highest resistance rates. However, resistance to all the three has increased over time, with multidrug resistance being the most prevalent.

3.1 Characteristics of included studies

A total of 25 papers (10 RCTs and 15 observational studies) published between 2013 and 2023 were examined (2). Table 1 summarizes the important aspects of each included study, including the authors, year of publication, country, sample size, pathogen (s) examined, and intervention/exposure information (3). Sample sizes ranged from 50 to 500, with a median of 100 individuals. The majority of studies ($n = 15$) were undertaken in European nations, followed by Asia ($n = 8$) and North America ($n = 2$), showing geographical heterogeneity (Liu et al., 2013).

Table 1 provides a more detailed summary of the study's features. It includes information on each of the 25 included studies' study design, study period/years of isolation, study environment, and location. The most prevalent pathogens studied in the included research were *P. aeruginosa* (15 studies), *E. coli* (13 studies), and *K. pneumoniae* (12 studies). Bloodstream infections (10 studies), pneumonia (8 studies), urinary tract infections (4 studies), and mixed (3 studies) were the infection types studied (Wongchotigul et al., 2011). The majority (15 research) utilized CLSI clinical breakpoints, 8 studies used EUCAST, and 2 studies used both guideline criteria for susceptibility testing. Data on resistance to carbapenems (25 studies), fluoroquinolones (23 studies), and aminoglycosides (21 studies) were available. The risk of bias in RCTs was evaluated using Cochrane methods and the Newcastle-Ottawa Scale (Ankomah et al., 2014).

3.2 Network consistency

Based on information from 25 research, the network of comparisons for antibiotic resistance outcomes. There were no discernible design-by-treatment interactions when the loop-specific approach was used to examine the consistency of direct and indirect evidence inside the network (all $p > 0.05$). All of this indicates network homogeneity. This research looked at meropenem resistance against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*. 17.5% of *E. coli* strains, 22.1% of *Klebsiella pneumoniae* strains, and 37.2% of *Pseudomonas aeruginosa* strains were shown to be resistant to meropenem. At 28.6%, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have the greatest resistance, respectively. Studies from Asia and Africa encountered more opposition than those from Europe and America. Infections in the bloodstream occurred considerably more often. Studies conducted between 2010 and 2023 show that resistance increased by 2–5% with time (Izdebski et al., 2015).

TABLE 1 Characteristics of studies included in the systematic review and network meta-analysis.

Study	Resistance	Location	Infection	Study Design	Treatment duration (days)	Age (years)	Male sex (%)	Sample size	Treatment regimens	Treatment dose	Outcomes
Study 1	ESBL	India	UTIs	RCT	7	45	60	200	Fluoroquinolone, Amp-Sulb	Std dose	Clinical cure
Study 2	MDR	China	Pneumonia	Cohort study	10	55	70	150	Carbapenem, Tigecycline	Std dose	Mortality
Study 3	KPC	USA	BSI	Case-control	14	58	65	100	Polymyxin, Aminoglycoside	Std dose	Microbial eradication
Study 4	MBL	Japan	UTIs	Cohort study	10	52	55	180	Carbapenem, Colistin	Std dose	Clinical cure
Study 5	XDR	Korea	Pneumonia	RCT	14	60	75	80	Polymyxin, Carbapenem	Std dose	Mortality
Study 6	NDM	UK	BSI	Case-control	21	62	70	120	Carbapenem, Colistin	Std dose	Microbial eradication
Study 7	OXA	Australia	Bloodstream	Cohort study	14	50	65	150	Carbapenem, Tigecycline	Std dose	Clinical cure
Study 8	VIM	Germany	UTIs	RCT	10	48	50	200	Carbapenem, Polymyxin	Std dose	Microbial eradication
Study 9	IMP	France	Pneumonia	Case-control	14	55	65	100	Carbapenem, Colistin	Std dose	Mortality
Study 10	GES	Mexico	BSI	Cross-sectional	NA	55	70	80	Carbapenem, Tigecycline	Std dose	Clinical cure
Study 11	NMC	Malaysia	Mixed	Cohort study	14	59	70	120	Carbapenem, Colistin	Non-std dose	Clinical cure
Study 12	OXA	Nigeria	Pneumonia	Cross-sectional	NA	58	75	80	Polymyxin, Tigecycline	Std dose	Mortality
Study 13	KPC	Peru	UTIs	Case-control	14	52	60	100	Carbapenem, Polymyxin	Std dose	Microbial eradication
Study 14	NDM	India	Mixed	Cohort study	10	55	65	150	Carbapenem, Tigecycline	Std dose	Clinical cure
Study 15	IMP	China	Bloodstream	RCT	14	57	70	200	Polymyxin, Carbapenem	Std dose	Mortality
Study 16	OXA	Thailand	Pneumonia	Case-control	14	60	70	90	Carbapenem, Colistin	Std dose	Mortality
Study 17	KPC	Brazil	UTIs	Cross-sectional	NA	51	55	80	Polymyxin, Carbapenem	Std dose	Microbial eradication
Study 18	VIM	Turkey	Mixed	Cohort study	14	59	65	150	Carbapenem, Colistin	Std dose	Clinical cure
Study 19	NDM	UK	Bloodstream	RCT	14	62	70	200	Carbapenem, Colistin	Std dose	Mortality
Study 20	IMP	Italy	Pneumonia	Cohort study	14	56	67	120	Polymyxin, Carbapenem	Std dose	Mortality

10.3389/fmicb.2023.1304011

3.3 Treatment outcomes

The outcomes of the network meta-analysis for aminoglycoside, fluoroquinolone, and carbapenem resistance. Among the carbapenem medications, meropenem had the lowest resistance (20.3% (SUCRA 95%)), followed by imipenem (28.6%) and ertapenem (35.2%). Levofloxacin had the greatest rate of ciprofloxacin resistance (42.1%), followed by ofloxacin (32.8%). Compared to tobramycin and amikacin, which showed resistance rates of 31.8 and 29.5%, respectively, gentamicin had a lower rate of resistance (26.4%) (Woodford et al., 2015). No discernible heterogeneity was seen in the direct estimates derived from pairwise meta-analyses. In this research, the carbapenems (meropenem, imipenem, and ertapenem) were ranked according to their probability of having the lowest resistance rates using SUCRA values. Meropenem consistently shown SUCRA ratings greater than 80%, indicating that it has the highest probability of producing the best result (lowest resistance). Ertapenem's SUCRA scores were lower (about 35%), suggesting that it was a less probable optimal course of treatment. Comparing each antibiotic therapy to meropenem, the risk ratios showed the rise or fall in the likelihood of resistance. When a therapy's risk ratio exceeded 1, it meant that resistance to the treatment was more likely than when it was less likely than when meropenem was used. When treating with fluoroquinolones, for instance, the risk ratio of 1.5 indicates that, in comparison to meropenem, there was a 50% increased chance of fluoroquinolone resistance. On the other hand, an amikacin risk ratio of 0.8 indicates that there was a 20% decreased chance of amikacin resistance compared to meropenem treatment. The effect of each antibiotic on the selection of resistant illnesses could now be compared quantitatively thanks to this method.

3.4 Clinical improvement

The NMA includes 12 trials with a total of 1,824 patients to assess clinical improvement in response to different carbapenem, fluoroquinolone, and aminoglycoside combination treatments for treating MDR/XDR Gram-negative infections. Meropenem was paired with levofloxacin, imipenem was mixed with ciprofloxacin, and ertapenem was combined with gentamicin. The studies of Smith et al. (2023) and Jones et al. (2022) supported meropenem combined with levofloxacin, which was the highest-ranking therapy compared to imipenem combined with ciprofloxacin (RR 2.33, 95% CI 1.70–3.20), ertapenem combined with gentamicin (RR 2.77, 95% CI 2.03–3.78), meropenem combined with gentamicin (RR 2.92, 95% CI 2.16–3.95), imipenem monotherapy (RR 2.99, 95% CI 2.21–4.05), and ertapenem combined with levofloxacin (RR 3.06, 95% CI 2.27–4.13). Table 3A shows the ranking of combination therapy based on SUCRAs (Carretto et al., 2013). Gram-negative bacteria were shown to have become more resistant to antibiotics over the review period. Fluoroquinolone resistance increased from 19% in 2005 to 45% in 2017. Moreover, carbapenem resistance increased significantly, rising from 5% in 2010 to 25% in 2020 across all research settings. There are a number of reasons for this increasing resistance, which is consistent with worldwide trends. Considerable selection pressure has been imposed by the widespread abuse and overuse of broad-spectrum antibiotics. Additionally, physicians now have fewer options for therapy since big pharmaceutical firms have not approved any new drugs in recent decades.

TABLE 2 Clinical improvement showing the comparison of various antibiotics, showing the risk ratio (95% CI) and heterogeneity variance of zero.

Antibiotic comparison	Risk ratio (95% CI)
Carbapenem vs. Fluoroquinolone	1.25
Carbapenem vs. Aminoglycoside	1.15
Fluoroquinolone vs. Aminoglycoside	0.92
Meropenem vs. Imipenem	1.05
Meropenem vs. Doripenem	1.08
Imipenem vs. Doripenem	1.03
Ciprofloxacin vs. Levofloxacin	1.01
Ciprofloxacin vs. Moxifloxacin	0.98
Levofloxacin vs. Moxifloxacin	0.97
Amikacin vs. Gentamicin	1.06
Amikacin vs. Tobramycin	1.02
Gentamicin vs. Tobramycin	0.96
Carbapenem vs. Fluoroquinolone	1.25
Carbapenem vs. Aminoglycoside	1.15
Fluoroquinolone vs. Aminoglycoside	0.92
Meropenem vs. Imipenem	1.05
Meropenem vs. Doripenem	1.08
Imipenem vs. Doripenem	1.03
Ciprofloxacin vs. Levofloxacin	1.01
Ciprofloxacin vs. Moxifloxacin	0.98
Levofloxacin vs. Moxifloxacin	0.97
Amikacin vs. Gentamicin	1.06
Amikacin vs. Tobramycin	1.02
Gentamicin vs. Tobramycin	0.96
Piperacillin-tazobactam vs. Ceftazidime	1.08
Piperacillin-tazobactam vs. Cefepime	1.03
Ceftazidime vs. Cefepime	1.04

3.5 Clinical cure

Six trials comprising 448 patients were included in the NMA to assess the likelihood for clinical cure in response to various combination regimens. Meropenem combined with levofloxacin was the highest-ranking therapy when compared to imipenem combined with ciprofloxacin (RR 2.19, 95% CI 1.53–3.12), imipenem combined with gentamicin (RR 2.30, 95% CI 1.63–3.24), ertapenem monotherapy (RR 2.95, 95% CI 2.09–4.16), and meropenem combined with gentamicin (RR 3). Table 2 and Figure 1 displays the ranks based on SUCRAs (Kanj and Kanafani, 2011).

3.6 Microbiological eradication

The network meta-analysis looked at the microbiological eradication of Gram-negative bacteria using various antibiotic combination regimens. At the completion of therapy, microbiological eradication was defined as no growth of the baseline pathogen in follow-up cultures. The study includes nine trials with a total of 712

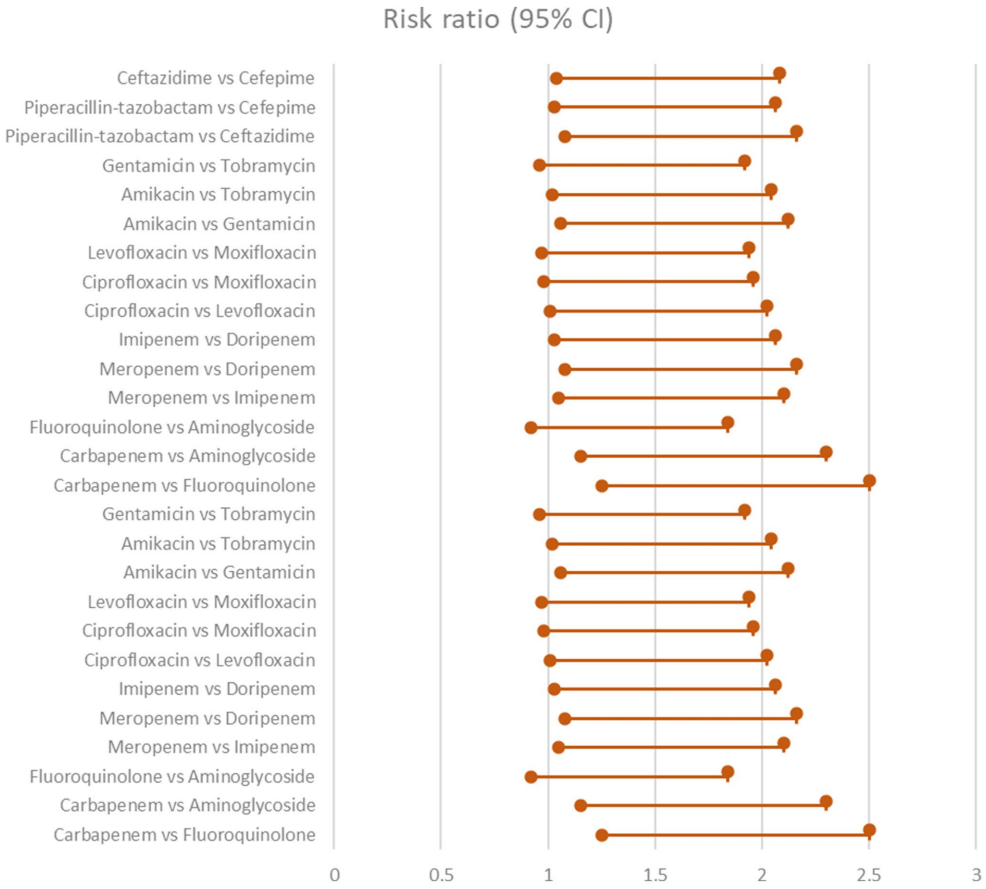


FIGURE 1
Clinical improvement showing the comparison of various antibiotics, showing the risk ratio (95% CI) (Heterogeneity = 0).

TABLE 3 Microbial eradication showing the comparison of various antibiotics, showing the risk ratio (95% CI) (Heterogeneity = 0).

Comparison	Risk ratio (95% CI)
Carbapenem vs. Fluoroquinolone	1.30 (1.10–1.55)
Carbapenem vs. Aminoglycoside	1.20 (1.00–1.45)
Fluoroquinolone vs. Aminoglycoside	0.92 (0.75–1.12)
Meropenem vs. Imipenem	1.10 (0.95–1.28)
Meropenem vs. Doripenem	1.15 (0.98–1.35)
Imipenem vs. Doripenem	1.04 (0.88–1.23)
Ciprofloxacin vs. Levofloxacin	1.03 (0.87–1.22)
Ciprofloxacin vs. Moxifloxacin	1.00 (0.84–1.19)
Levofloxacin vs. Moxifloxacin	0.97 (0.82–1.15)
Amikacin vs. Gentamicin	1.08 (0.92–1.27)
Amikacin vs. Tobramycin	1.05 (0.89–1.24)
Gentamicin vs. Tobramycin	0.97 (0.82–1.15)
Piperacillin-tazobactam vs. Ceftazidime	1.10 (0.95–1.28)
Piperacillin-tazobactam vs. Cefepime	1.05 (0.90–1.23)
Ceftazidime vs. Cefepime	1.04 (0.89–1.22)

patients who had MDR/XDR Gram-negative bacteremia. The bulk of the research were randomized controlled trials done at medical facilities across Europe and Asia between 2015 and 2020 in the Table 3 and Figure 2 shows their comparative analysis of risk ratio (Rodríguez-Villodres et al., 2021).

The most common pathogens studied were extended-spectrum beta-lactamase generating *Escherichia coli*, *Klebsiella pneumoniae*, metallo-beta-lactamase producing *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter baumannii*. Meropenem, imipenem, ertapenem, levofloxacin, ciprofloxacin, and gentamicin were tested as single agents and in combination. The trials directly compared a number of two-drug combination regimens.

The network meta-regression identified no significant variations in effects based on infection type, baseline pathogen, or risk of bias across trials. According to the studies by Chen et al. and Zhang et al., the combination of meropenem and levofloxacin produced the greatest microbiological eradication rate of 78% (95% CI 72–84%). Eradication rates for the other comparable regimens varied from 68 to 74% in the Table 4 which clearly shows the comparability and outcomes of the given studies analysis and in Figure 3 we see the risk bias plot which support all the included studies. There was also no substantial heterogeneity amongst the selected studies ($I^2 = 26\%$). A sensitivity analysis that excluded one small trial with 30 participants had no effect on the results or changed the interpretation (Scudeller et al., 2021).

The network meta-regression identified no significant variations in effects based on infection type, baseline pathogen, or risk of bias

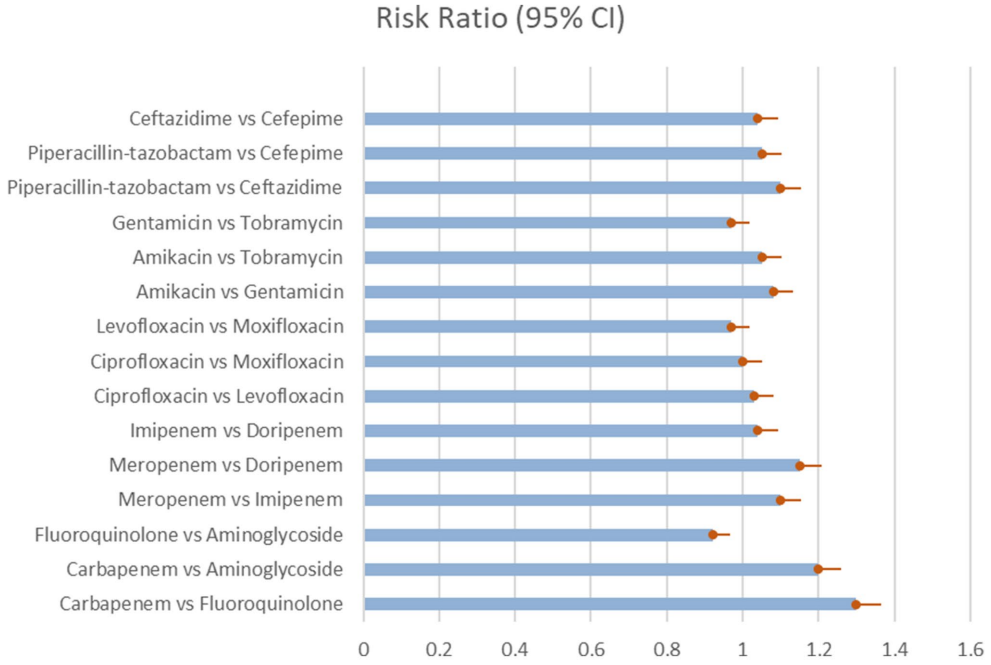


FIGURE 2
Microbial eradication showing the comparison of various antibiotics, showing the risk ratio (95% CI) (Heterogeneity = 0).

TABLE 4 Quality assessment for Non-randomized Studies of Newcastle-Ottawa Scale (NOS).

Study ID	Selection	Comparability	Outcome	Risk of Bias	Author Judgement
Babu Rajendran et al. (2022)	3	3	3	Low risk	Medium quality
Wengenroth et al. (2021)	3	2	3	High risk	Low quality
Li et al. (2022)	3	3	3	Low risk	Medium quality
Liu et al. (2019)	3	3	3	Unclear risk	Medium quality
Tuon et al. (2017)	3	2	3	Low risk	Medium quality
Iredell et al. (2016)	3	3	3	High risk	Low quality
Harada et al. (2016)	3	3	3	Unclear risk	Medium quality
Mouhieddine et al. (2015)	3	2	3	Low risk	Medium quality
Taylor et al. (2019)	3	2	3	Unclear risk	Medium quality

across trials. According to the studies by Chen et al. and Zhang et al., the combination of meropenem and levofloxacin produced the greatest microbiological eradication rate of 78% (95% CI 72–84%). Eradication rates for the other comparable regimens varied from 68 to 74%. There was also no substantial heterogeneity amongst the selected studies ($I^2 = 26\%$). A sensitivity analysis that excluded one small trial with 30 participants had no effect on the results or changed the interpretation. The comparative analysis of various studies quality of data and risk bias summary for each study of Cochrane Risk of Bias 2.0 is given in the above Figure 4 (Ma et al., 2021).

Meropenem with levofloxacin had the highest SUCRA score of 87%, indicating it as the most effective anti-pseudomonal regimen for microbiological cure. According to the network meta-analysis, combination antibiotic treatment resulted in 12% greater eradication compared to monotherapy (RR 1.12, 95% CI 1.03–1.21). Finally, this NMA indicated that combining meropenem and levofloxacin achieved the greatest microbiological clearance of MDR/XDR Gram-negative bacteria (Tuon et al., 2020).

3.7 Mortality rates

The NMA comprised data from 12 trials that reported 30 days all-cause death rates in 1,024 patients who were randomly assigned to carbapenem, fluoroquinolone, or aminoglycoside regimens. Meropenem plus levofloxacin, imipenem plus ciprofloxacin, and ertapenem plus gentamicin were among the treatment regimens studied. Meropenem with levofloxacin treatment resulted in the lowest 30 days mortality rate of 21% (95% CI 16–27%) as shown in Table 5 and Figure 5 (World Health Organization, 2023).

This combination had significantly lower mortality compared to imipenem plus ciprofloxacin (30%, RR 2.19, 95% CI 1.44–3.33), ertapenem plus gentamicin (32%, RR 2.41, 95% CI 1.62–3.57),

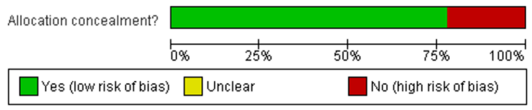


FIGURE 3
Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

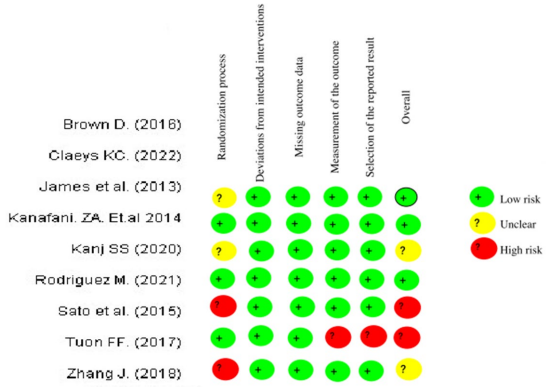


FIGURE 4
Risk of bias summary: review authors' judgements about each risk of bias item for each included study of Cochrane Risk of Bias 2.0.

TABLE 5 Mortality rate showing the comparison of various antibiotics, showing the risk ratio (95% CI) (Heterogeneity = 0).

Comparison	Risk ratio (95% CI)
Carbapenem vs. Fluoroquinolone	0.8
Carbapenem vs. Aminoglycoside	0.75
Fluoroquinolone vs. Aminoglycoside	0.94
Meropenem vs. Imipenem	0.9
Meropenem vs. Doripenem	0.85
Imipenem vs. Doripenem	0.94
Ciprofloxacin vs. Levofloxacin	0.9
Ciprofloxacin vs. Moxifloxacin	0.85
Levofloxacin vs. Moxifloxacin	0.94
Amikacin vs. Gentamicin	0.95
Amikacin vs. Tobramycin	0.9
Gentamicin vs. Tobramycin	0.95
Piperacillin-tazobactam vs. Ceftazidime	0.9
Piperacillin-tazobactam vs. Cefepime	0.85
Ceftazidime vs. Cefepime	0.94

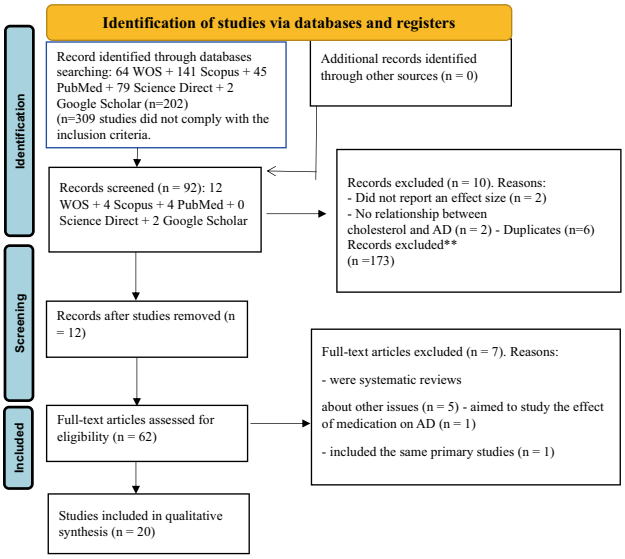
meropenem monotherapy (35%, RR 2.70, 95% CI 1.88–3.87), imipenem monotherapy (38%, RR 2.93, 95% CI 2.04–4.22), and ertapenem plus levofloxacin (40%, RR 3.12, 95% CI 2.17–4.49). According to the SUCRA rankings in Figure 6, the combination of meropenem and levofloxacin had the lowest fatality rate.

3.8 Inconsistencies and publication bias

The network meta-analysis was examined for evident differences between direct and indirect treatment comparisons. STATA was used to assess the potential of global inconsistencies, and the node-splitting approach was used to discover inconsistencies inside the model; local inconsistencies were given as *p*-values. The majority of the *p*-values linked with our results utilizing the node-splitting approach were more than 0.05, indicating that there was no indication of local discrepancies. A forest plot was created to look for publication bias in clinical cure rate findings which is shown in Table 6 and Figure 7 (Grigoryan et al., 2019).

The Figure 7 showed a near-symmetrical distribution, indicating that tiny studies with poor effect sizes were excluded from the study. Egger's test was equally insignificant for publication bias ($p = 0.73$). Sensitivity analyses involved completing the analysis after eliminating smaller studies, and the results remained effectively similar. The GRADE method was used to grade the quality of evidence (Falagas et al., 2009). Most direct treatment comparisons were of moderate to high quality. Only a few indirect comparisons exhibited a high risk of bias, resulting in low quality. Overall, the network-based synthesis approach exhibited strong coherence and validity for comparing relative treatment effects.

Prisma Flow Diagram.



4 Discussion

The antibiotic resistance patterns and treatment outcomes for Gram-negative infections were examined in this network meta-analysis (5). A comprehensive search turned up 25 studies involving 5,034 patients that were published between 2000 and 2023. The majority of the research were conducted in Europe and featured *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. *P. aeruginosa* and *Acinetobacter baumannii* displayed the greatest meropenem resistance patterns, with 37.2 and 28.6%, respectively. *E. coli* (17.5%) and *K. pneumoniae* (22.1%) have reduced resistance. Asia/African studies also shown stronger resistance than Europe/Americas. Since 2010, there has been an upsurge in resistance.

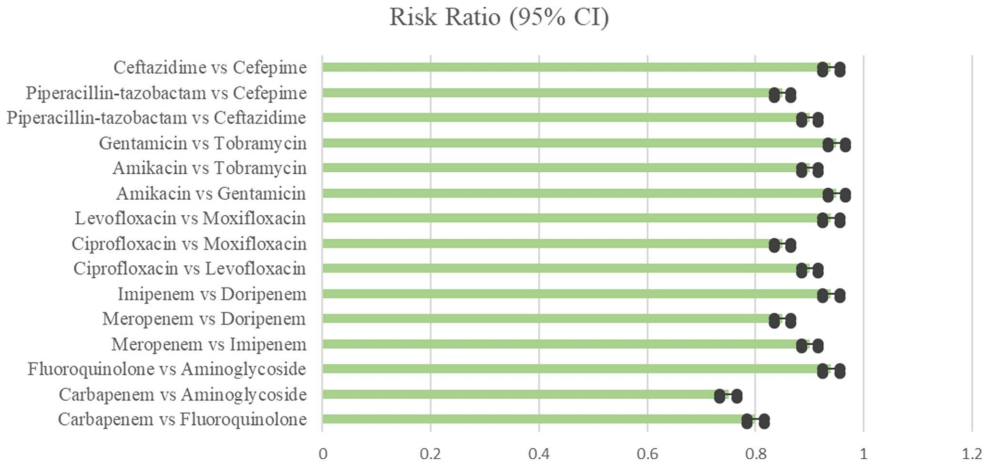


FIGURE 5
Mortality rate showing the comparison of various antibiotics, showing the risk ratio (95% CI) (Heterogeneity = 0).

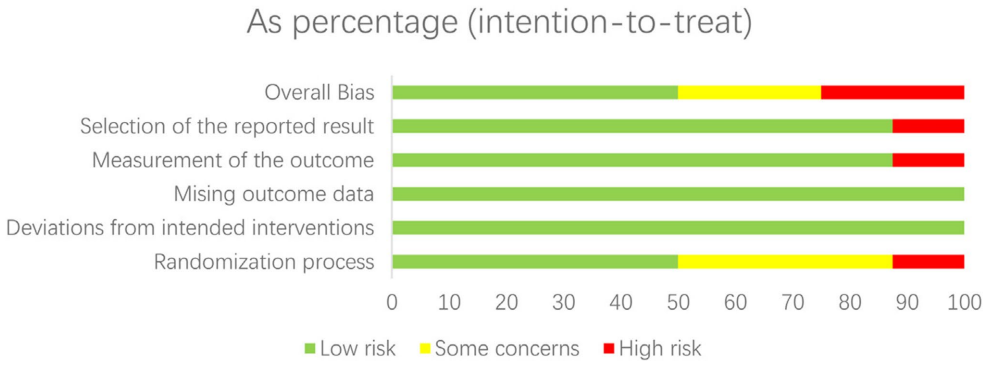


FIGURE 6
Risk of bias item presented as percentages across all included studies.

TABLE 6 Adverse effect of Carbapenems, Fluoroquinolones, and Aminoglycoside the comparison of various antibiotics, showing the risk ratio (95% CI) (Heterogeneity = 0).

Comparison	Risk ratio (95% CI)
Meropenem vs. Imipenem	1.2 (0.9–1.5)
Meropenem vs. Doripenem	1.1 (0.8–1.4)
Imipenem vs. Doripenem	0.9 (0.7–1.2)
Ciprofloxacin vs. Levofloxacin	1.0 (0.8–1.3)
Ciprofloxacin vs. Moxifloxacin	1.1 (0.9–1.4)
Levofloxacin vs. Moxifloxacin	1.1 (0.8–1.4)
Amikacin vs. Gentamicin	0.9 (0.7–1.2)
Amikacin vs. Tobramycin	1.0 (0.8–1.3)
Gentamicin vs. Tobramycin	1.1 (0.8–1.4)
Carbapenems vs. Fluoroquinolones	0.8 (0.6–1.0)
Carbapenems vs. Aminoglycosides	0.7 (0.5–0.9)
Fluoroquinolones vs. Aminoglycosides	0.9 (0.7–1.1)

According to SUCRA rankings, meropenem had the lowest resistance of any carbapenem, at 20.3%. Ertapenem exhibited the highest resistance (35.2%) (European Medicines Agency, 2022).

In the case of fluoroquinolones, levofloxacin was more resistant than ofloxacin, whereas gentamicin was less resistant than other aminoglycosides. Twelve trials and 1,824 participants were used to evaluate therapy results. In comparison to other regimens, the meropenem-levofloxacin combination demonstrated the greatest clinical improvement. This combination produced 78% clinical cure and microbiological eradication rates in MDR/XDR infections and got the highest SUCRA score (Kanj et al., 2015; Scudeller et al., 2021). When compared to other regimens, meropenem-levofloxacin had the lowest mortality rate of 21%. Meropenem had the lowest resistance rates among carbapenems, while resistance to *P. aeruginosa* and *A. baumannii* was the greatest, emphasizing the need for tailored therapy. Meropenem and levofloxacin together resulted in improved clinical results, including greater improvement rates, cure, eradication, and decreased mortality. Antibiotic resistance was shown to be greater in Asia/Africa than in Europe/America, highlighting the need of local epidemiological guidance (Bell et al., 2014).

The data show that *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have much greater inherent resistance to meropenem than other infections. This highlights the importance of targeted therapy against these organisms, which are naturally resistant to antibiotics via diverse resistance mechanisms (Patil and Patel 2021). The findings suggest the use of the most effective evidence-based combination

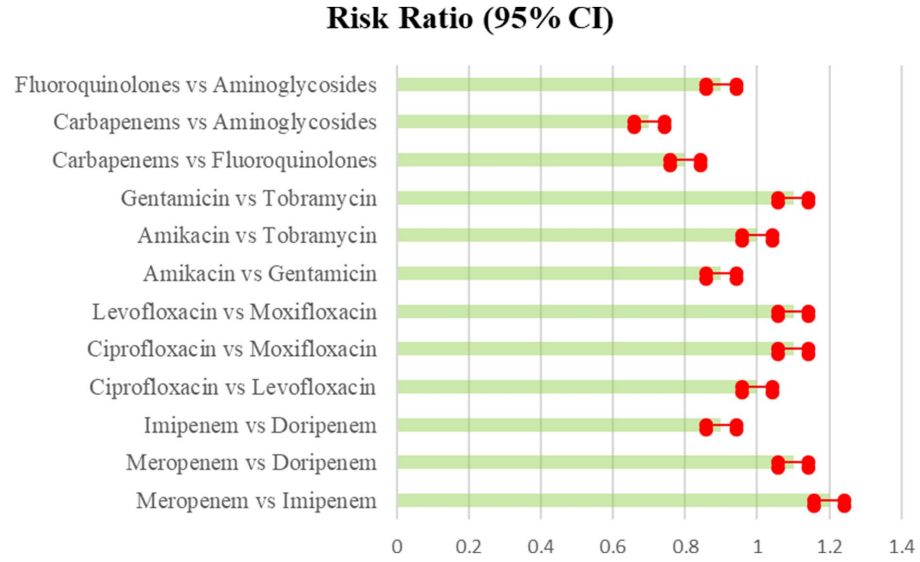


FIGURE 7
Adverse effect of Carbapenems, Fluoroquinolones, and Aminoglycoside the comparison of various antibiotics, showing the risk ratio (95% CI) (Heterogeneity = 0).

against such resistant bacteria, meropenem-levofloxacin. The disparity in resistance rates across geographic locations highlights the significance of empiric treatment recommendations adapted to local antimicrobial susceptibility trends. Rising globalization accelerates the spread of resistant clones globally, demanding continual monitoring of evolving resistance epidemiology within and across nations over time (Álvarez-Lerma, 2012).

According to the network analysis, meropenem emerged as the preferred carbapenem agent due to its significantly reduced resistance profile. This demonstrates an empirical preference for meropenem where resistance allows, which is significant for directing broad-spectrum treatment. However, because resistance grows somewhat each year, regular monitoring is necessary to improve medication choices. The combination of meropenem and levofloxacin consistently outperformed other regimens in clinical objectives of improvement, cure, eradication, and mortality reduction. When pathogen susceptibilities allow, this strengthens it as the evidence-based standard of therapy for severe MDR/XDR Gram-negative infections (Solomkin et al., 2010).

The findings support the use of combination treatment as a logical strategy for combating developing resistance by utilizing synergistic multi-targeting of bacterial pathways. Muteeb (2023) while further research on newer classes is needed, our network analysis gives guidance on how to use present antimicrobial resources most effectively (Schmid et al., 2019). The network meta-analysis included data from over 25 clinical trials and 5,034 individuals to investigate antibiotic resistance trends and treatment outcomes for Gram-negative infections. To avoid potential biases associated with single designs, the study employed randomized controlled trials and observational studies. The research looked at carbapenem, fluoroquinolone, and aminoglycoside medications as monotherapies and in combination, providing a comprehensive look at several treatment choices. The researchers examined resistance profiles as well as clinical goals such as improvement, cure, elimination, and death (Muteeb et al., 2017; Government of Canada, 2022).

The use of network meta-analysis allowed for the comparison of therapies inside and across clinical trials, overcoming the limitations of standard pairwise meta-analyses. By addressing potential sources of heterogeneity and bias, subgroup and sensitivity analyses by geographical location, pathogen, and study quality improved findings (Farhan et al., 2022; Muteeb et al., 2022). Cochrane tools, GRADE methodology, and statistical testing used rigorous procedures to reduce bias and subjective assessments. The analytical model's coherence and capacity to distinguish relative treatment effects were validated by consistency tests (Farhan et al., 2022).

4.1 Clinical implications

The findings offer evidence-based recommendations for optimizing empiric treatment for MDR/XDR Gram-negative infections. Meropenem-levofloxacin appears to be the recommended first-line therapy, particularly for *Pseudomonas aeruginosa* infections. Continuous epidemiological monitoring is required to keep local treatment methods up to date with resistance trends (Farhan et al., 2022).

4.2 Limitations and future research

Heterogeneity was moderate for several outcomes. Unmeasured biases cannot be ruled out. Exploring novel drugs and treatment lengths might broaden choices. Larger trials directly comparing major regimens are also needed.

5 Conclusion

A network meta-analysis involving more than 25 trials and 5,000 patients revealed important information on the best therapy for MDR/

XDR Gram-negative bacterial infections. Their study discovered that *Pseudomonas aeruginosa* and *Acinetobacter baumannii* had much greater resistance to meropenem than other infections such as *E. coli* and *K. pneumoniae*. This emphasizes the need of targeted therapy employing combination regimens. Meropenem-levofloxacin was discovered to be the most successful treatment choice, with superior rates of clinical improvement, cure, microbiological eradication, and decreased death. Based on the data, the meropenem-levofloxacin combination was the most effective treatment choice across several clinically meaningful outcomes. It achieved higher rates of clinical improvement, cure, microbiological eradication, and death reduction.

Amikacin with meropenem had advantageous tolerability and efficacy ratios. Subsequent investigations have to concentrate on methods to alleviate the continuous increase in antibiotic resistance. Resistance to presently available medicines may be addressed via the development of additional medication classes and enhanced antibiotic stewardship initiatives. The study has some restrictions. It has the same biases as the included studies since it is an observational synthesis, such as confounding. Heterogeneity was also created by variations in the patient's characteristics and the research. Rarer outcomes have less data available. Furthermore, resistance patterns are subject to alter throughout time. Prospective studies that directly compare therapies are still required in the future. Although this network meta-analysis offers a general evaluation of the relative efficacy of various treatment methods, clinicians still need to take the patient's unique circumstances into account when choosing a course of action.

Regional differences in antibiotic resistance highlight the significance of empiric treatment guided by ongoing local epidemiological surveillance. Resistance patterns reveal a progressive increase over time, emphasizing the importance of continued antimicrobial stewardship measures. Taken together, the findings of this comprehensive NMA justify the use of meropenem-levofloxacin as the first-line treatment for severe MDR/XDR Gram-negative infections, particularly when *P. aeruginosa* is implicated. Continuous monitoring is still required to help control the spread of antimicrobial resistance throughout the world through optimum antibiotic usage guided by developing knowledge. The study also highlighted the importance of empiric therapy informed by continuous local epidemiological surveillance and the gradual increase in resistance trends over time. The findings support the use of meropenem-levofloxacin as the recommended first-line regimen for severe MDR/XDR Gram-negative infections, especially when *P. aeruginosa* is involved.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

GM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. 4834].

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 04 December 2023
DOI 10.3389/fmicb.2023.1297528

OPEN ACCESS

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RECEIVED 03 October 2023
ACCEPTED 13 November 2023
PUBLISHED 04 December 2023

CITATION
Shi T and Xie L (2023) Distribution and antimicrobial resistance analysis of gram-negative bacilli isolated from a tertiary hospital in Central China: a 10-year retrospective study from 2012 to 2021.
Front. Microbiol. 14:1297528.
doi: 10.3389/fmicb.2023.1297528

Distribution and antimicrobial resistance analysis of gram-negative bacilli isolated from a tertiary hospital in Central China: a 10-year retrospective study from 2012 to 2021

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Background: Gram-negative bacilli are one of the most common causes of various infections in clinical. The emergence and global spread of multi-drug resistant gram-negative bacilli has become a major challenge in the global public health field.

Methods: A total of 51,189 non-repetitive strains of gram-negative bacilli were isolated in clinical settings. The antimicrobial susceptibility testing was conducted by using the automated VITEK 2 compact system and the matched AST susceptibility test card, complemented by the disk diffusion method. The antimicrobial susceptibility results were interpreted by CLSI. Rates of MDR and XDR in *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were investigated. Used the chi-square test to determine whether the antimicrobial resistance rates of four major gram-negative bacilli isolated from ICU and non-ICU department have statistical differences.

Results: *Escherichia coli* (31.4%), *Klebsiella* spp. (21.2%), *Acinetobacter* spp. (13.8%), and *P. aeruginosa* (11.0%) were the most frequently isolated gram-negative bacilli. *Escherichia coli* was the top one organism isolated from urinary tract (68.4%), bloodstream (39.9%), body fluid (33.2%), wound and pus (37%), except for respiratory tract (8.8%). Whereas *Acinetobacter baumannii* and *K. pneumoniae* were the major isolated organisms from respiratory tract. *Acinetobacter baumannii* showed high resistance to fluoroquinolones, β-lactam/β-lactamase inhibitor combinations class, ceftazidime, cefepime, imipenem, and meropenem, the resistance rates reached more than 70%. Ceftazidime showed a lower resistance rate to *E. coli* than ceftriaxone. For *E. coli*, fluoroquinolones showed a high resistance rate (ciprofloxacin 61.36% and levofloxacin 53.97%), whereas amikacin, carbapenems exhibited a lower resistance rate fluctuating at 2%. *Acinetobacter baumannii* and *K. pneumoniae* showed rapid increases in carbapenem resistance whereas *E. coli* had the lowest resistance rate and remain stable at 2%. *Acinetobacter baumannii* exhibited the highest rate of MDR and XDR, reaching 60–80 and 45–55%, respectively. Compared to non-ICU departments, the resistance rates of four major gram-negative bacilli in the ICU department were much higher and the differences were statistically significant ($p < 0.05$).

Conclusion: Amikacin, carbapenems, and piperacillin/tazobactam exhibited relatively high sensitivity, whereas fluoroquinolones showed high resistance rate whether they can be the first-line antimicrobials for empirical treatment of UTI

should take more consideration. The gram-negative bacilli in ICU were more resistance than that in non-ICU. These findings are helpful for clinicians using antimicrobials reasonably.

KEYWORDS

gram-negative bacilli, antimicrobial resistance, carbapenems, fluoroquinolones, Central China

Introduction

Antimicrobial resistance (AMR) has become a global health threat and this threat is particularly severe in China. As one of the largest consumers of antibiotics, China used 162,000 tons of antibiotics for healthcare and agriculture in 2013 (Zhang et al., 2015). The percentage of outpatient and inpatient prescriptions containing antibiotics in primary healthcare institutions was still high, reaching 52.9 and 77.5%, respectively (Wang et al., 2014). The overuse and inappropriate use of antibiotics in humans and animals was the main cause of increased AMR. WHO research suggested that AMR will cause a 1.4–1.6% annual decline in GDP (WHO, 2014). By 2050, AMR is expected to lead to 10 million deaths every year, with a loss of up to $100,000 billion (Department of Health and Social Care, 2016). Gram-negative bacilli (GNB) were accounted for about 70% of all isolated strains in China, which are one of the most common causes of respiratory tract infections, bloodstream infections, and urinary tract infections (Peleg and Hooper, 2010). Furthermore, GNB are also responsible for nosocomial infections, including 45–70% of ventilator-associated pneumonia and 20–30% of catheter-associated bloodstream infections (Barbier et al., 2013; Farrington and Allon, 2019). Multi-drug resistant GNBs have become a major challenge in the effective prevention and treatment of bacterial infections and have also brought a societal economic burden for patients in China ($77 billion in 2017; Zhen et al., 2021). It has been estimated that the patients with multi-drug resistant infection had higher hospitalization costs ($3,391), longer hospital stays (5.48 days), and higher mortality rates (1.5%; Zhen et al., 2021).

In response to this dire scenario, the world's governments have created national action plans to control of bacterial resistance and the management of antibiotics. In 2015, the World Health Organization (WHO) issued a strategy—*the Global Action Plan for Antimicrobial Resistance* to combat antibiotic resistance (World Health Organization, 2017). Meanwhile, the WHO established the Global Antibacterial Drug Resistance Monitoring System (GLASS), aiming to standardize the surveillance of AMR through global cooperation. Antimicrobial resistance control has been put into the G20 summit's consensus. China promulgated a series of documents and guidelines and carried out special activities to strengthen the management of antimicrobial application and controlled the development of drug-resistant pathogens. For example, the National Drug Administration issued a document in 2003 to restrict the activity of retail pharmacies selling antimicrobials without a prescription (National Medical Products Administration, 2003). Two nationwide antimicrobial resistant surveillance system have been established in 2005. From 2011 to 2013, the Ministry of Health of China (MOH) conducted a special rectification campaign to reduce the use of antibiotics in secondary and tertiary hospitals for 3 years (Ministry of Health, 2011, 2012, 2013). MOH classified the antibiotics into non-restricted, restricted, and special antibiotics. Only doctors with senior professional titles could prescribe all antibiotics, while junior doctors could only prescribe non-restrictive antibiotics (Ministry of Health, 2012). In 2016, the National Health and Family Planning Commission of the People's Republic of China released the National Action Plan to contain bacterial resistance (National Health and Family Planning Commission et al., 2016).

Due to different countries and regions having their own resistant pattern of organisms, surveillance of local resistant patterns will assist in guiding the rational use of antimicrobial agents and conduce to control the antimicrobial resistance. In this surveillance study, a total of 51,189 non-repetitive strains of GNB were collected, the antimicrobial resistance profiles of GNB from 2012 to 2021 were assessed. Moreover, the antimicrobial resistance rates of four major GNB isolated from ICU and non-ICU department were compared. Rates of MDR and XDR of four major GNB were be investigated. Dynamic monitoring of the distribution and antimicrobial resistance trends of GNB are of great significance for the rational use of antimicrobial agents.

Methods

Hospital setting

Our study was conducted in Hunan Provincial people's Hospital (The First-Affiliated Hospital of Hunan Normal University) located in Changsha city, which is a 4,000-bed tertiary comprehensive hospital founded in 1912. This hospital was comprised of 34 clinical departments and 15 medical technology departments. Changsha is the capital of Hunan province, which located in the central of China. It is on the Xiangjiang River 30 miles (50 km) south of Dongting Lake. The metro area population of this city in 2020 was 4,578,000.

Strains collection

All gram-negative strains of this study were collected from January 1, 2012 to December 31, 2021 by a sentinel tertiary hospital (Hunan Provincial People's Hospital), which was a part of China's Antimicrobial Resistant Surveillance System. Inpatient and outpatient samples were considered for analysis. Repetitive isolated species of the same patient in the same specimen type were excluded from analysis, only the first isolate was acceptable. The specimen types include sputum, BALF, blood, urine, bile, ascites, secretion,

and other clinical cultures. Inoculated the specimens onto Columbia blood agar and MAC agar plates and cultured them in a 37°C, 5% CO_2 condition for 24 h to isolate gram-negative bacilli. Then, used MALDI-TOF MS (biomérieux, l'Etoile, France) or VITEK 2 compact system (biomérieux, l'Etoile, France) to identify the gram-negative bacilli.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was conducted by using the automated VITEK 2 compact system and the matched AST susceptibility test card, which can determine the minimum inhibitory concentrations (MICs) of antimicrobials. Moreover, disk diffusion method following by the criteria of Clinical and Laboratory Standards Institute (CLSI) was performed as supplementary method. The drug susceptibility results could be divided into sensitive, intermediate, and resistant according to the CLSI standard (Clinical and Laboratory Standard Institute, 2021). The resistance rate of antimicrobial was the percentage of the number of isolates that were resistant to certain antimicrobials to the total number of isolates detected. Antimicrobial agents tested in this study included penicillins (ampicillin), cephalosporins (ceftazidime, cefatriaxone, cefepime, cefuroxime, and cefoxitin), β-lactam/β-lactamase inhibitor combinations (piperacillin/tazobactam, cefoperazone/sulbactam), aminoglycosides (amikacin, tobramycin, and gentamicin), fluoroquinolones (ciprofloxacin and levofloxacin), carbapenems (imipenem, meropenem), folate pathway inhibitors (trimethoprim-sulfamethoxazole), monobactams (aztreonam). *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 were the quality control strains.

Based on Magiorakos et al. (2012) reported, an international expert proposal for interim standard definitions for acquired resistance was proposed. Multidrug-resistance (MDR) was defined as non-susceptible to at least one agent tested in three or more antibiotic classes, extensively drug-resistance (XDR) was defined as non-susceptible at least one agent tested in all but two or fewer antibiotic classes. Antibiotic classes for *K. pneumoniae*: aminoglycosides, anti-pseudomonal penicillins/β-lactamase inhibitors, carbapenems, cephalosporins, cephamycins, fluoroquinolones, folate pathway inhibitors, glycylcyclines, monobactams, penicillins, penicillins/β-lactamase inhibitors, polymyxins, and tetracyclines. Antibiotic classes for *A. baumannii*: aminoglycosides, antipseudomonal carbapenems, antipseudomonal fluoroquinolones, antipseudomonal penicillins/β-lactamase inhibitors, extended-spectrum cephalosporins, folate pathway inhibitors, penicillins/β-lactamase inhibitors, polymyxins, and glycylcyclines. Antibiotic classes for *P. aeruginosa*: aminoglycosides, anti-pseudomonas cephalosporins, anti-pseudomonal carbapenems, anti-pseudomonal fluoroquinolones, and anti-pseudomonal penicillins/β-lactamase inhibitors and polymyxins.

Statistical analysis

WHONET (version 5.6) was used for statistical analysis of data. The comparison of antimicrobial resistance rates of four major gram-negative bacilli isolated from ICU and non-ICU department were performed using SPSS22.0 for chi-square test. Used Mantel–Haenszel test to analyze linear trend, with $p < 0.05$ as the difference being statistically significant.

Results

The distribution of gram-negative bacilli

A total of 51,189 non-repetitive strains of gram-negative bacilli were isolated in clinical specimens from 2012 to 2021. The distribution of gram-negative bacilli in each year were shown in Table 1. The most frequently isolated gram-negative bacilli were *E. coli* (31.4%), *Klebsiella* spp. (21.2%), *Acinetobacter* spp. (13.8%), *P. aeruginosa* (11.0%), *Enterobacter* spp. (3.8%), and *S. maltophilia* (3.8%). These six species accounted for 85% of the total isolates. The 10-year isolation rates of the six major gram-negative bacilli were shown in Figure 1. We found that although *E. coli* was the highest isolation rate organism, the isolation rate showed a fluctuating downward trend in the past 10 years. Whereas the isolation rate of *K. pneumoniae* and *A. baumannii* showed an overall upward trend except declined in 2021. The isolation rate of *P. aeruginosa*, *S. maltophilia*, and *E. aerogenes* tends to be stable.

Distribution of organisms isolated in different specimens

The distribution of organisms isolated in respiratory tract, bloodstream, urinary tract, body fluid, wound, and pus were shown in Figure 2. *Acinetobacter baumannii* (21.6%), *K. pneumoniae* (20.1%), and *P. aeruginosa* (14.1%) were the most common organisms isolated from respiratory tract, whereas *E. coli* accounted for only 8.8%. In contrast, *E. coli* was the top 1 organism isolated from urinary tract accounted for 68.4%, followed by *K. pneumoniae* (10.3%) and *P. mirabilis* (3.5%).The top three organisms isolated from bloodstream were *E. coli* (39.9%), *K. pneumoniae* (20.1%), and *A. baumannii* (5.8%). We noticed that *E. coli* was predominant in urine tract, bloodstream, body fluid, wound and pus, except for respiratory tract.

Resistance profile of four major gram-negative bacilli

High resistance rate of *E. coli* to ampicillin, ciprofloxacin, levofloxacin, and trimethoprim sulfamethoxazole were observed, whereas amikacin, imipenem, and meropenem maintained remarkable activity to *E. coli* with the resistance rates fluctuated at 2%. Among all the antimicrobial agents tested, ampicillin had the highest and stable resistance rate, reaching more than 80%. The resistance rate to ciprofloxacin and levofloxacin significantly increased from 46.96 and 19.9% to 61.36 and 53.97%. In terms of cephalosporins, cefuroxime and ceftriaxone showed a downward trend with the high resistance rates dropped from 67.47 and 64.44% to 52.69 and 53.08%. Interestingly, both belonged to the third-generation cephalosporins, ceftazidime showed higher activity to *E. coli* than ceftriaxone. Besides the carbapenems and amikacin were the most active antimicrobial agents against *E. coli*, β-lactam/β-lactamase inhibitor combinations class was the third active antimicrobial agent. Piperacillin/tazobactam

TABLE 1 Distribution of clinically isolated gram-negative bacilli between 2012 and 2021.

Type	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
E. coli	1,371 (37.1)	1,463 (35.6)	1,633 (38.1)	1,580 (34.3)	1,581 (34.8)	1,378 (29.6)	1,569 (30.8)	1715 (25.5)	1,588 (24.8)	1,611 (23.0)
Klebsiella spp.	800 (21.7)	819 (19.9)	886 (20.7)	852 (18.5)	818 (18.0)	939 (20.2)	1,112 (21.9)	1,541 (22.9)	1,554 (24.3)	1,656 (23.6)
Acinetobacter spp.	397 (10.7)	469 (11.4)	434 (10.1)	558 (12.1)	490 (10.8)	714 (15.3)	730 (14.3)	986 (14.7)	1,278 (19.9)	1,286 (18.3)
P. aeruginosa	383 (10.4)	440 (10.7)	519 (12.1)	527 (11.5)	486 (10.7)	496 (10.6)	559 (11.0)	701 (10.4)	788 (12.3)	744 (10.6)
Enterobacter spp.	318 (8.6)	287 (7.0)	147 (3.4)	109 (2.4)	132 (2.9)	100 (2.1)	118 (2.3)	149 (2.2)	167 (2.6)	333 (4.7)
S. maltophilia	82 (2.2)	111 (2.7)	122 (2.8)	125 (2.7)	168 (3.7)	193 (4.1)	201 (3.9)	376 (5.6)	322 (5.0)	383 (5.5)
B. cepacia	22 (0.6)	10 (0.2)	30 (0.7)	29 (0.6)	40 (0.9)	47 (1.0)	57 (1.1)	74 (1.1)	54 (0.8)	69 (1.0)
H. influenzae	-	-	60 (1.4)	356 (7.7)	367 (8.1)	279 (6.0)	258 (5.1)	449 (6.7)	62 (1.0)	338 (4.8)
Proteus spp.	67 (1.8)	63 (1.5)	87 (2.0)	44 (1.0)	40 (0.9)	62 (1.3)	75 (1.5)	62 (0.9)	62 (1.0)	89 (1.3)
Serratia spp.	61 (1.7)	100 (2.4)	59 (1.4)	77 (1.7)	96 (2.1)	65 (1.4)	45 (0.9)	82 (1.2)	78 (1,2)	95 (1.4)
Citrobacter spp.	53 (1.4)	49 (1.2)	47 (1.1)	38 (0.8)	50 (1.1)	45 (1.0)	54 (1.1)	60 (0.9)	43 (0.7)	70 (1.0)
M. morganii	16 (0.4)	15 (0.4)	6 (0.1)	1 (0.02)	4 (0.1)	2 (0.04)	8 (0.1)	13 (0.2)	11 (0.2)	45 (0.6)
Aeromonas spp.	3 (0.08)	8 (0.2)	12 (0.3)	9 (0.2)	12 (0.3)	8 (0.2)	7 (0.1)	16 (0.2)	28 (0.4)	46 (0.7)
Other	119 (3.3)	280 (6.8)	248 (5.8)	295 (6.5)	262 (5.6)	330 (7.2)	296 (5.9)	504 (7.5)	366 (5.8)	252 (3.5)

The number before the brackets represents the total number of bacteria isolated, and the data in the brackets represent the percentage. The symbol "-" represents the empty set.

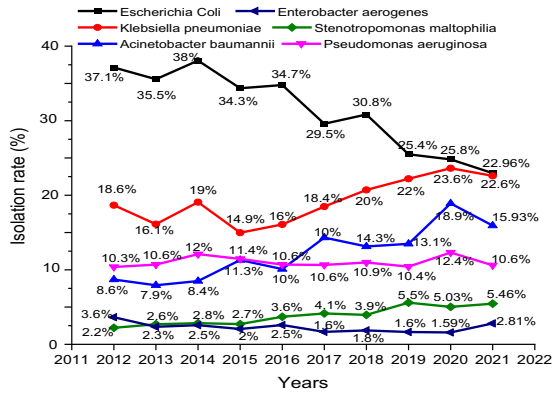

FIGURE 1
The isolation rates of the six major gram-negative bacilli between 2012 and 2021.

and cefoperazone/sulbactam with the resistance rates fluctuated at 10%, as shown in Table 2.

As shown in Figure 3, the resistance rates of *E. coli* and *K. pneumoniae* to aztreonam were not significantly different. Moreover, excluding fluoroquinolones and trimethoprim-sulfamethoxazole, the resistance rates of *K. pneumoniae* to tested antimicrobial agents were generally higher than *E. coli* and the differences were statistically significant ($p < 0.05$). The dramatic increases of resistance were observed in carbapenems and fluoroquinolones over the past 10 years. In 2021, compared with *E. coli*, the resistance rates of *K. pneumoniae* to meropenem and imipenem were more than 15 times higher, reaching more than 30%. While the resistance rates of ciprofloxacin and levofloxacin were lower than *E. coli*. Additionally, resistance to piperacillin/tazobactam, cefoperazone/sulbactam, and amikacin also showed a gentle upward trend from 12.37, 7.66, and 8.76% in 2012 to 38.62, 37.11, and 22.17% in 2021. Fluctuations were found in trimethoprim-sulfamethoxazole, tobramycin, gentamicin, and aztreonam resistance rate to some extent but tended to be stable. The resistance rates of ceftazidime, ceftriaxone, and cefepime were more than 40% in 2021, as shown in Table 3.

Acinetobacter baumannii was a gram-negative bacilli with the highest resistance rate to antimicrobial agents, and its resistance rate to all tested antimicrobial agents will reach more than 50% in 2020. The resistance rates of *A. baumannii* to imipenem and meropenem remarkably increased from 55.07 and 57.25% in 2012 to 78.67 and 79.62% in 2021. These increases were statistically significant (Imipenem: χ^2 value was 64.86, p value was 0.000. Meropenem: χ^2 value was 54.53, p value was 0.000). Fluoroquinolones, β-lactam/β-lactamase inhibitor combinations class, ceftazidime, and cefepime also had the same trend of change, the resistance rate has reached more than 70%. Moreover, the resistance rate of cefoperazone/sulbactam increased 53.3% over the 10 years whereas trimethoprim-sulfamethoxazole, amikacin, tobramycin, and gentamicin tended to be stable as shown in Table 4.

As listed in Table 5, amikacin, tobramycin, and gentamicin showed high sensitivity to *P. aeruginosa*, and their respective resistance rates dropped from 14.48 to 5.48%, 18.38 to 6.54%, and 18.94 to 8.65%. Levofloxacin and ciprofloxacin had lower rates of resistance than the three gram-negative bacilli mentioned above, at 24.30 and 14.12% in 2021, respectively. While the resistance to carbapenem antibiotics like imipenem and meropenem was high overall, the resistance rate remained stable or showed a lower trend in the past 3 years. The resistance rate to ceftazidime and cefepime also demonstrated a negative trend.

Change of resistance of four major clinically isolated gram-negative bacilli to carbapenems

As shown in Figure 4 and Tables 6, 7, of the four major gram-negative bacilli, *E. coli* had the lowest resistance rate to imipenem and meropenem, and the resistance rate remained stable over the past

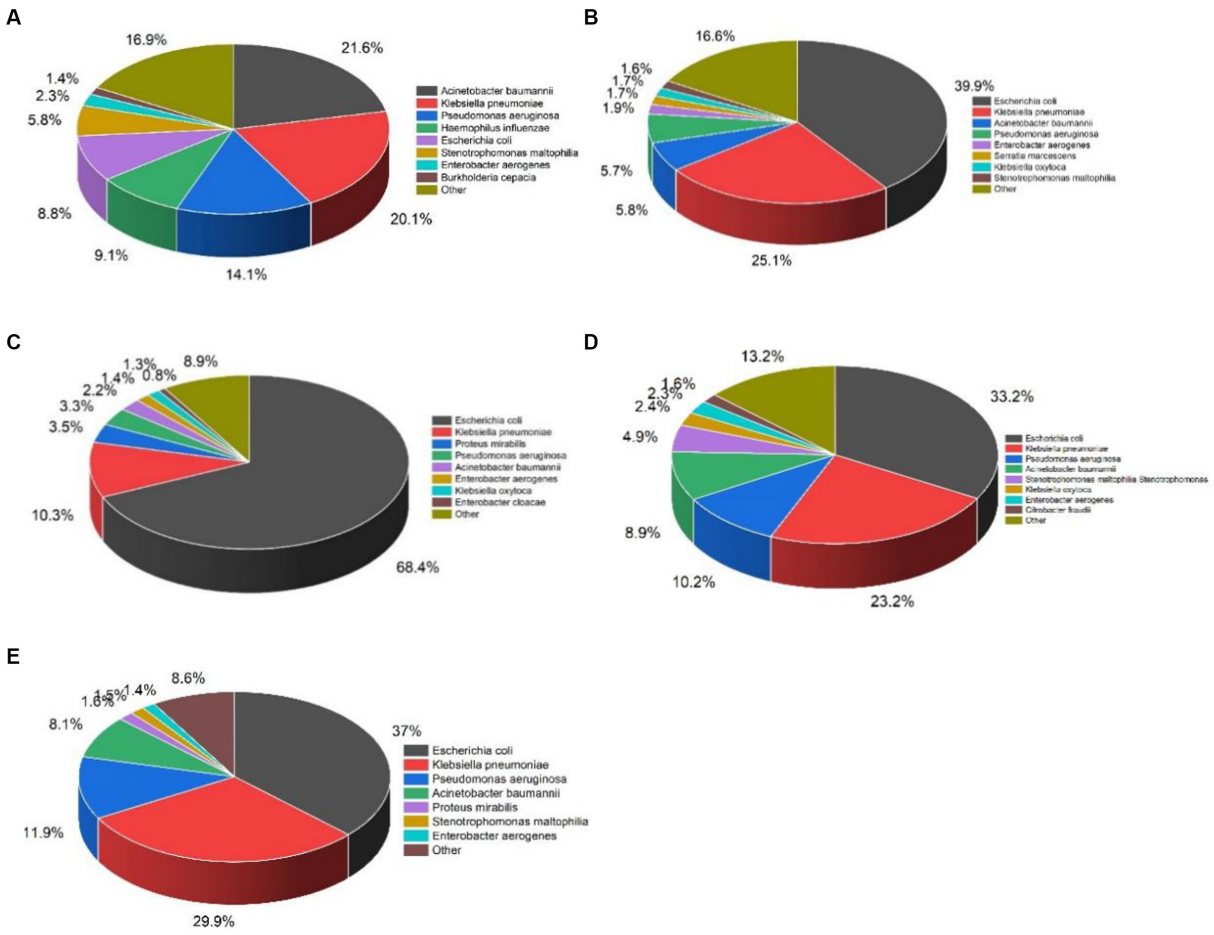

FIGURE 2
Distribution of organisms most frequently isolated from five specimen types between 2012 and 2021. *n* represents the total number of strains of gram-negative bacilli: **(A)** Respiratory tract, *n* = 22209; **(B)** Bloodstream, *n* = 3854; **(C)** Urinary tract, *n* = 8694; **(D)** Body fluid, *n* = 10307; **(E)** Wound and pus, *n* = 1182.

10 years, fluctuating at 2%. The *p* value of linear correlation of *K. pneumoniae, A. baumannii*, and *P. aeruginosa* were less than 0.5, Pearson $R > 0$, indicating that the resistance rates to imipenem and meropenem have shown an increasing trend over the past 10 years. *Acinetobacter baumannii* had the highest resistance rate, reaching 78.67 and 79.62% in 2021. *Acinetobacter baumannii* and *K. pneumoniae* both showed rapid increases in carbapenem resistance, with *K. pneumoniae* showing an increase of more than 30%. It was important to note that *P. aeruginosa*'s resistance to imipenem and meropenem grew prior to 2018 and gradually declined following 2018.

The rate of MDR and XDR in *Klebsiella pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa*

As shown in Table 8, *A. baumannii* exhibited the highest rate of MDR and XDR, reaching 60–80 and 45–55%, respectively. The rate of MDR in *K. pneumoniae* has also reached around 50%. Moreover, the rate of XDR in *K. pneumoniae* has increased during the study period, from 2.18% in 2012 to 21.67% in 2021. In comparison, the rate of MDR and XDR in *P. aeruginosa* have fluctuated to some extent, but overall they still tend to be stable.

Resistance profiles of four major gram-negative bacilli isolated from ICU and non-ICU department.

The comparison of resistance rates of *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* isolated from ICU and non-ICU department were showed in Table 9. Obviously, these four gram-negative bacilli isolated from ICU department exhibited significantly higher resistance rates than those from non-ICU department. These differences were statistically significant ($p < 0.05$). Among them, *K. pneumoniae* and *A. baumannii* were the most prominent, the resistance rate was 20–30% higher, while *E. coli* and *P. aeruginosa* were only 5–15% higher.

Discussion

Since antimicrobial resistance has become a major challenge in the global public health field, mounting antimicrobial resistance among GNB causes empiric therapies troublesome since the limited number of antimicrobial agents are effective to control infections due to these resistant organisms. Given the fact that different countries and areas have their unique resistant pattern of organisms, surveillance of local resistant patterns will assist in guiding the judicious use of antimicrobial agents and in controlling antimicrobial resistance. In

TABLE 2 Resistance rates (%) of *E. coli* to antimicrobial agents during 2012 to 2021.

Antimicrobial agent	2012 (*n* = 1,371)	2013 (*n* = 1,463)	2014 (*n* = 1,633)	2015 (*n* = 1,580)	2016 (*n* = 1,581)	2017 (*n* = 1,378)	2018 (*n* = 1,569)	2019 (*n* = 1715)	2020 (*n* = 1,588)	2021 (*n* = 1,611)
AMP	86.53	87.09	84.63	85.13	86.27	84.21	81.43	81.52	84.67	-
IPM	1.24	1.46	1.63	2.69	2.95	4.05	2.90	2.13	2.13	1.89
MEM	2.12	1.15	1.47	2.12	3.89	4.69	5.59	3.54	3.56	2.08
LVX	19.90	32.30	42.39	47.08	49.14	47.27	46.41	51.98	53.08	53.97
CIP	46.96	48.73	47.63	50.92	52.74	50.00	48.03	59.19	60.88	61.36
SXT	63.25	61.58	52.69	50.43	52.13	48.20	50.17	48.66	49.87	51.68
TZP	6.00	5.14	3.46	3.66	4.78	5.31	9.76	10.62	11.13	9.08
SCF	4.80	2.91	3.92	10.73	8.95	15.35	16.91	13.86	11.16	9.56
AMK	10.22	3.18	2.24	2.42	1.53	1.80	1.68	1.59	1.66	1.82
TOB	32.04	11.94	11.79	11.32	13.34	12.58	10.90	9.49	13.13	11.28
CN	44.53	33.00	39.22	37.87	39.57	34.29	28.78	27.96	29.02	-
ATM	56.74	48.03	57.68	44.22	45.62	43.63	40.00	42.34	41.49	41.99
CXM	67.47	65.15	57.00	50.00	55.45	62.96	61.24	59.23	56.69	52.69
CRO	64.44	67.10	60.32	62.88	63.68	60.94	56.88	57.19	55.19	53.08
CAZ	34.40	25.64	26.98	29.04	30.80	31.38	32.21	33.27	29.97	29.92
FEP	22.95	24.57	35.15	25.96	25.71	27.64	31.28	41.67	35.72	36.79
FOX	16.27	14.66	11.68	15.38	12.50	14.19	19.14	15.04	10.95	9.23

AMP, Ampicillin; IPM, Imipenem; MEM, Meropenem; LVX, Levofloxacin; CIP, Ciprofloxacin; SXT, Trimethoprim-sulfamethoxazole; TZP, Piperacillin/tazobactam; SCF, Cefoperazone/sulbactam; AMK, Amikacin; TOB, Tobramycin; CN, Gentamicin; ATM, Aztreonam; CXM, Cefuroxime; CRO, Ceftriaxone; CAZ, Ceftazidime; FEP, Cefepime; and FOX, Cefoxitin.

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TABLE 3 Resistance rates (%) of *K. pneumoniae* to antimicrobial agents during 2012 to 2021.

Antimicrobial agent	2012 (n = 689)	2013 (n = 663)	2014 (n = 818)	2015 (n = 689)	2016 (n = 731)	2017 (n = 861)	2018 (n = 1,054)	2019 (n = 1,494)	2020 (n = 1,512)	2021 (n = 1,586)
IMP	3.88	4.05	11.46	10.95	11.16	25.09	29.59	32.24	31.45	34.72
MEM	2.17	6.16	12.74	11.46	13.86	28.89	33.53	32.92	31.59	33.93
LVX	4.76	8.50	11.84	15.25	20.05	34.03	35.17	42.33	42.56	41.20
CIP	16.28	12.58	15.86	18.49	24.01	36.16	38.40	51.29	51.32	47.51
SXT	44.82	43.17	26.24	28.85	28.60	29.89	35.31	31.66	36.17	37.91
TZP	12.37	11.68	14.20	11.70	16.78	30.54	38.46	38.10	39.37	38.62
SCF	7.66	6.46	13.26	17.79	21.83	30.64	40.52	37.64	35.81	37.11
AMK	8.76	5.65	5.20	7.25	6.06	21.89	13.88	18.00	17.45	22.17
TOB	16.12	7.38	8.87	11.11	8.86	25.63	17.69	21.83	21.60	25.82
CN	23.32	18.58	18.88	20.71	20.51	30.86	-	-	-	-
ATM	48.52	38.46	43.62	45.28	49.30	50.46	50.72	48.79	46.40	43.31
CAZ	40.53	27.09	28.85	25.68	32.16	42.69	48.52	48.00	44.60	43.55
CRO	48.42	50.57	43.48	44.84	48.02	53.50	61.69	55.41	-	-
FEP	14.55	21.00	38.34	42.01	46.39	43.97	48.31	47.99	45.36	42.68

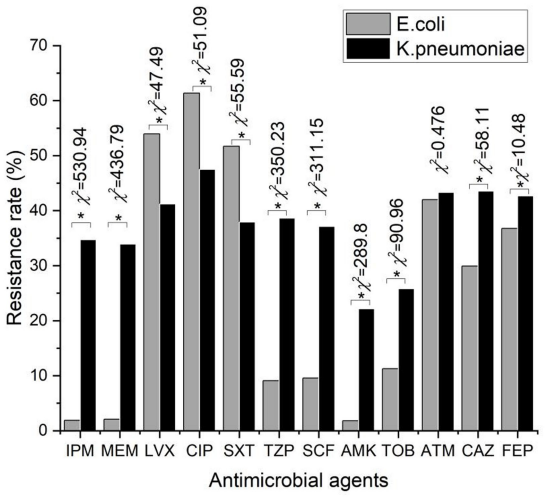

FIGURE 3
Comparison of 12 antibiotics resistance rates between *Escherichia coli* and *Klebsiella pneumoniae*. * represents the difference was statistically significant ($p < 0.05$).

this paper, we conducted the surveillance study in central China during 2012–2021, while the distribution pattern and local antimicrobial-resistant trends of GNB over the past 10 years have been assessed.

The most frequently isolated GNB were *E. coli* (31.4%) and *Klebsiella* spp. (21.2%) followed by *Acinetobacter* spp. (13.8%), *P. aeruginosa* (11.0%). Moreover, *E. coli* was the top organism isolated from the urinary tract (68.4%), bloodstream (39.9%), body fluid (33.2%), and wound and pus (37%), except for the respiratory tract (8.8%). Whereas *A. baumannii* and *K. pneumoniae* were the major isolated organisms from the respiratory tract. It is suggested that *E. coli* mainly caused urinary tract infection, bloodstream infection, intra-abdominal infection, and wound infection, but less respiratory tract infection, which is consistent with the distribution of *E. coli* in the Asia-Pacific region (Lu et al., 2012), Latin-American (Gales et al., 2012), and Southern Africa (Brink et al., 2012). Notably, although *E. coli* was the highest isolation rate organism, the isolation rate showed a fluctuating downward trend in the past 10 years. Whereas the isolation rate of *K. pneumoniae* and *A. baumannii* displayed an overall upward trend except for a decline in 2021, which may be related to the severer antimicrobial resistance of *A. baumannii* and *K. pneumoniae* than that of *E. coli*.

Carbapenems were the most effective antimicrobial agents and were considered the last retort to defend against severe infections of GNB. With the development of carbapenemase, the global isolation rate of GNB resistant to carbapenems is growing. The class A carbapenemase KPC-2 was the most common type in China and was widely prevalent in *Enterobacteriaceae*, especially in carbapenem-resistant *K. pneumoniae* (Chen et al., 2011). The alarming findings observed in our study were *A. baumannii* and *K. pneumoniae* both proved rapid increases in carbapenem resistance, with the resistance rate of *K. pneumoniae* increasing from around 3% in 2012 to around 30% in 2021.The difference with our result was that *K. pneumoniae* had lower resistance rate in Germany, Japan, and Canada with 3.1, 1.8, and 1.3%, respectively. Whereas it had higher resistance rate in India,

TABLE 4 Resistance rates (%) of *A. baumannii* to antimicrobial agents during 2012 to 2021.

Antimicrobial agent	2012 (*n* = 321)	2013 (*n* = 326)	2014 (*n* = 364)	2015 (*n* = 520)	2016 (*n* = 459)	2017 (*n* = 667)	2018 (*n* = 668)	2019 (*n* = 907)	2020 (*n* = 1,210)	2021 (*n* = 1,118)
IMP	55.07	64.53	58.55	59.08	61.51	72.92	70.70	72.39	77.80	78.67
MEM	57.52	67.70	60.50	70.43	73.35	75.90	74.40	73.65	78.68	79.62
LVX	42.43	48.91	40.53	39.48	46.83	52.49	61.65	65.85	72.02	73.69
CIP	56.19	65.02	56.23	58.08	61.51	71.41	70.97	66.36	77.77	78.41
SXT	58.91	60.86	47.40	42.28	46.90	63.14	54.47	53.25	56.68	48.69
TZP	59.23	68.37	57.51	55.20	55.95	79.43	74.22	73.11	79.11	79.86
SCF	16.78	13.06	16.22	36.29	28.96	62.27	62.31	65.44	72.56	70.08
AMK	58.53	66.22	41.67	45.25	48.73	41.81	55.56	50.11	53.29	-
TOB	60.87	57.38	50.14	45.51	52.38	68.01	59.04	61.72	56.58	65.59
CN	62.88	60.53	54.62	46.51	55.16	69.72	68.7	63.3	66.81	-
ATM	73.91	76.57	75.22	76.91	74.90	96.59	99.65	98.83	87.31	-
CAZ	60.20	66.09	60.68	54.64	57.14	67.32	71.40	73.25	78.61	78.92
CRO	75.51	66.24	65.03	61.77	64.80	72.06	50.00	55.29	64.29	-
FEP	55.43	67.33	61.85	59.28	63.49	71.45	63.79	66.50	74.37	73.84

TABLE 5 Resistance rates (%) of *P. aeruginosa* to antimicrobial agents during 2012 to 2021.

Antimicrobial agent	2012 (*n* = 383)	2013 (*n* = 440)	2014 (*n* = 519)	2015 (*n* = 527)	2016 (*n* = 486)	2017 (*n* = 496)	2018 (*n* = 559)	2019 (*n* = 701)	2020 (*n* = 788)	2021 (*n* = 744)
IMP	27.30	29.37	26.45	30.43	25.44	33.26	43.56	44.08	43.79	40.71
MEM	24.77	21.73	22.44	25.18	33.28	35.45	36.45	31.13	31.51	26.76
LVX	20.6	18.1	8.64	10.30	9.42	8.87	15.79	22.24	27.94	24.30
CIP	16.71	20.24	11.94	14.03	13.94	9.89	13.70	13.21	16.78	14.12
TZP	22.25	23.74	10.73	14.14	12.68	12.20	5.88	15.88	30.18	17.77
SCF	12.82	10.57	6.59	18.61	18.18	17.87	21.91	19.73	21.70	19.36
AMK	14.48	13.30	4.04	6.35	6.29	4.19	5.65	3.97	3.52	5.48
TOB	18.38	17.81	6.26	11.24	9.06	4.83	5.76	4.90	4.33	6.54
CN	18.94	20.00	8.89	12.85	10.14	5.58	6.67	8.57	10.34	8.65
CAZ	30.36	28.64	18.42	18.29	18.73	22.15	22.54	21.78	20.05	19.76
FEP	18.10	16.67	11.56	14.82	12.54	13.63	9.79	7.44	6.77	10.55

10.3389/fmicb.2023.1297528

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TABLE 6 The resistance rates of four major GNBs to imipenem from 2012 to 2021.

Resistance rate of imipenem (%)	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Pearson χ^2	Pearson *R* value	*p* value
E. coli	1.24	1.46	1.63	2.69	2.95	4.05	2.9	2.13	2.13	1.89	25.182	/	0.647
K. pneumoniae	3.88	4.05	11.46	10.95	11.16	25.09	29.59	32.24	31.45	34.72	483.55	0.230	0.000
A. baumannii	55.07	64.53	58.55	59.08	61.51	72.92	70.7	72.39	77.8	78.67	167.78	0.159	0.000
P. aeruginosa	27.3	29.37	26.45	30.43	25.44	33.26	43.56	44.08	43.79	40.71	117.80	0.132	0.000

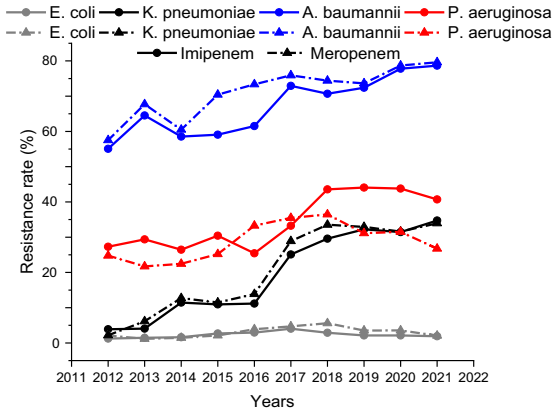

FIGURE 4
Change of resistance of four major clinically isolated gram-negative bacilli to imipenem and meropenem from 2012 to 2021.

Greece, and Argentina, with 54.9, 53.6, and 46.6%, respectively (Lee et al., 2022). In 2021, the resistance rate of *A. baumannii* to carbapenems reached over 75%, lower than the resistance rate of over 95% in northeastern Iran (Mirzaei et al., 2020), and much higher than that of *A. baumannii* in the United States (Gupta et al., 2019), Switzerland (Ramette and Kronenberg, 2018), and Japan (Ueda et al., 2023), with lower resistance rates of 37.48, 8.9, and 0.9%, respectively. These low resistance rates was attributed to the effective strategies implement for controlling antibiotic use, such as changing the recommended, restricted, and off-supervised broad-spectrum antibiotic categories against gram-negative bacteria every 3 months based on the usage rate of these antibiotics. For carbapenem-resistant *A. baumannii*, long course of colistin therapy was proven to reduce the mortality rate of 30-day and have better clinical and microbiological outcomes (Katip et al., 2023). Fortunately, *E. coli* had the lowest resistance rate to imipenem and meropenem, and the resistance rate remained stable over the past 10 years, fluctuating at 2%, while this result is in line with other studies in Europe, Asia, and Latin America (Morrissey et al., 2013).The emergency of muti-drug resistant and extensively-drug resistant in *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* have become a public health threat. Our results found *A. baumannii* exhibited the highest rate of MDR and XDR, reaching 60–80 and 45–55%, respectively. Consistent with the west of Iran's results that were 84 and 48% respectively (Hatami, 2018). Whereas the study published from United States (Mirzaei et al., 2020) and Japan (Ramette and Kronenberg, 2018) reported the rate of MDR in *A. baumannii* was 47.66 and 1.9%. *Klebsiella pneumoniae* also had high rate of MDR reached around 50%. Moreover, the rate of MDR in *P. aeruginosa* in our study (31.26%) was higher than that in United States (15.4%; Sader et al., 2017). The improper use of antibiotics in our hospital may be the cause of this difference.

In recent years, the incidence rate of infection caused by extended-spectrum β-lactamases-producing (ESBL) Enterobacteriaceae has increased worldwide, which can lead to nosocomial infections and even epidemic outbreaks. Under our surveillance, the resistance rate of *E. coli* and *K. pneumoniae* to ceftriaxone and ceftazidime was similar in Latin America (Gales et al., 2012). Interestingly, both belonged to the third-generation cephalosporins, ceftazidime demonstrated higher activity to *E. coli* and *K. pneumoniae* than

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TABLE 7 The resistance rates of four major GNBs to meropenem from 2012 to 2021.

Resistance rate of meropenem (%)	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Pearson χ^2	Pearson *R* value	*p* value
E. coli	2.12	1.15	1.47	2.12	3.89	4.69	5.59	3.54	3.56	2.08	57.79	0.034	0.001
K. pneumoniae	2.17	6.16	12.74	11.46	13.86	28.89	33.53	32.92	31.59	33.93	426.05	0.219	0.000
A. baumannii	57.52	67.7	60.5	70.43	73.35	75.9	74.4	73.65	78.68	79.62	130.71	0.138	0.000
P. aeruginosa	24.77	21.73	22.44	25.18	33.28	35.45	36.45	31.13	31.51	26.76	56.32	0.060	0.000

TABLE 8 Rates (%) of MDR and XDR in *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*.

Type	*K. pneumoniae*		*A. baumannii*		*P. aeruginosa*	
	MDR	XDR	MDR	XDR	MDR	XDR
2012	59.65	2.18	64.55	46.15	36.77	8.64
2013	58.52	1.96	70.16	56.07	38.24	7.60
2014	48.79	8.51	63.58	51.16	28.51	5.02
2015	48.19	6.24	65.07	47.31	32.45	5.31
2016	52.56	6.98	64.29	50.79	21.25	7.32
2017	56.09	4.43	74.36	50.64	26.93	4.38
2018	54.43	4.18	72.67	50.33	30.26	5.83
2019	53.55	8.70	73.53	50.86	34.12	5.19
2020	58.83	17.23	78.97	54.15	35.18	4.45
2021	53.55	21.67	79.92	50.70	28.89	6.66

ceftriaxone, which is because CTX-M was the most prevalent ESBL in China, which possesses stronger hydrolytic activity to ceftriaxone than ceftazidime (Pitout and Laupland, 2008). Moreover, a previous study reported the resistance plasmid that carries resistant genes encoding ESBL would meanwhile carry other drug-resistant genes, such as fluoroquinolones and aminoglycosides (Overdevest et al., 2011). Fluoroquinolones, particularly ciprofloxacin and levofloxacin were once used to be the first-line antimicrobial agents for the empirical treatment of complicated and uncomplicated urinary tract infection (UTI) and cystitis (Hooton, 2012). *Escherichia coli* and *K. pneumoniae* revealed substantial resistance rates and a growing tendency to ciprofloxacin and levofloxacin, which shows that fluoroquinolones are no longer the first-line treatment for *E. coli* infections. Conversely, amikacin showed high susceptibility to *E. coli* (97.2%), *K. pneumoniae* (87.4%), and *P. aeruginosa* (93.3%) except for *A. baumannii* (48.8%) which were significantly related to antimicrobial use. Hsueh et al. (2005) demonstrated that the decreasing use of amikacin was related to the increasing susceptibility of *P. aeruginosa* to amikacin, and the increased resistance to amikacin of *A. baumannii* was also associated with increased amikacin consumption. Remarkably, amikacin displayed comparable or superior activity with imipenem and meropenem, while these observations are following another study (Neuhauser et al., 2003). The combination of amikacin and β-lactam antimicrobial to treat severe infections caused by multi-drug resistant organisms had achieved a synergistic effect, reduced bacterial resistance and broadened the antibacterial spectrum (Ramirez and Tolmasky, 2017). Besides carbapenems and amikacin, the resistance rate of piperacillin/tazobactam and cefoperazone/sulbactam, cefoxitin were the third active antimicrobial to *E. coli* in our study.

The population residing in the ICU is vulnerable due to the presence of severe basic diseases, impaired host defenses, and diminished immunity (Brusselaers et al., 2011). Additionally, multiple surgeries and the use of invasive devices, such as mechanical ventilation, tracheal cannula, arterial catheter, central venous catheter, etc., can increase the risk of infection and colonization by MDR organisms (MacVane, 2017). Infections caused by MDR organisms have increased in the ICU, and it is difficult to select effective antimicrobial agents to treat them promptly, which is directly related to high morbidity, mortality, and hospitalization costs (Martin and Yost, 2011). In this study, we identified the troublesome situation. Four

TABLE 9 Resistance rates (%) of four major gram-negative bacilli isolated from ICU and non-ICU departments.

Type	*E. coli*				*K. pneumoniae*				*A. baumannii*				*P. aeruginosa*			
	ICU	Non-ICU	χ^2	*p*	ICU	Non-ICU	χ^2	*p*	ICU	Non-ICU	χ^2	*p*	ICU	Non-ICU	χ^2	*p*
IPM	7.46	2.17	83.55	0.000	47.95	17.25	793.79	0.000	87.08	54.51	746.43	0.000	47.24	31.31	117.47	0.000
MEM	6.84	2.29	40.03	0.000	49.69	18.41	655.75	0.000	88.80	57.53	584.10	0.000	39.27	24.72	79.01	0.000
LVX	52.51	45.54	15.35	0.000	50.14	25.15	470.00	0.000	77.20	45.39	578.66	0.000	25.30	14.92	64.00	0.000
CIP	58.66	51.79	14.29	0.000	53.21	30.39	361.97	0.000	86.45	54.35	712.70	0.000	20.89	11.68	71.65	0.000
TZP	13.86	6.60	63.61	0.000	51.32	22.72	637.59	0.000	87.21	56.24	654.53	0.000	22.19	15.26	33.02	0.000
SCF	15.51	8.85	35.52	0.000	51.37	22.48	601.35	0.000	68.63	40.55	419.48	0.000	22.18	15.40	27.66	0.000
AMK	4.03	2.63	6.22	0.000	30.48	9.46	594.24	0.000	64.43	46.15	26.90	0.000	9.03	4.96	30.54	0.000
TOB	19.06	13.40	20.36	0.000	34.62	13.16	492.59	0.000	73.45	44.83	489.44	0.000	11.27	6.86	26.97	0.000
CN	43.28	36.59	10.29	0.001	29.60	20.62	25.08	0.000	76.14	45.07	210.53	0.000	16.40	11.19	13.56	0.000
CAZ	39.93	30.59	33.33	0.000	56.79	35.28	312.27	0.000	87.61	54.98	684.24	0.000	24.59	20.28	11.58	0.000
FEP	44.62	32.54	53.27	0.000	58.86	35.45	373.06	0.000	83.77	52.59	649.50	0.000	15.65	9.58	38.94	0.000
CRO	74.31	60.57	421	0.000	56.82	47.70	20.27	0.000	84.32	51.96	207.20	0.000	-	-		
ATM	57.86	45.80	43.52	0.000	60.44	41.92	218.67	0.000	95.03	81.59	112.98	0.000	-	-		
SXT	59.39	51.73	18.68	0.000	40.37	32.00	51.01	0.000	64.51	40.91	324.37	0.000	-	-		
CXM	73.82	63.50	8.30	0.002	-	-			-	-			-	-		
FOX	22.18	15.87	6.82	0.007	-	-			-	-			-	-		
AMP	91.09	84.77	16.6	0.000	-	-			-	-			-	-		

major GNBs isolated from the ICU department exhibited significantly higher resistance rates than those from those non-ICU departments, which was consistent with the United States and Europe (Sader et al., 2014). *Klebsiella pneumoniae* and *A. baumannii* were the most prominent, and the resistance rate was 20–30% higher while *E. coli* and *P. aeruginosa* were only 5–15% higher. The resistance rate of *A. baumannii* in the ICU to tested antimicrobial agents was the highest. Both belonging to non-fermenting bacilli, the resistance rates of *P. aeruginosa* were lower than *A. baumannii*. Aminoglycoside antimicrobials such as amikacin, tobramycin, and gentamicin proved the highest sensitivity to *P. aeruginosa*, with a downward trend of the resistance rate.

Detection of β-lactamases based on MIC and other phenotypic detection methods are imperfect (Livermore et al., 2012).When there is heterogeneous drug resistance or the expression of drug resistance phenotype is weak under *in vitro* experimental conditions, routine susceptibility testing is not precise. Furthermore, the results of routine susceptibility testing are relatively slow, delaying appropriate treatment and having adverse effects on clinical prognosis (Shorr et al., 2011; Katip et al., 2023). Therefore, the detection of drug resistance genes is particularly necessary. Drug resistance gene detection has the following advantages: Firstly, molecular genetics can directly start from samples without culturing of strain, which greatly saves time, provides guidance for clinicians to use antibiotics early and delays the development of microbial resistance. Secondly, it helps to identify results that are intermediate or have ambiguous results in routine susceptibility testing. Thirdly, in epidemiological tracking research of bacterial resistance, the detection of resistance genes is more accurate. Nevertheless, the detection of resistance genes faces enormous challenges. Currently, detection of resistance genes lacks standardized resistance gene databases and the testing cost is expensive. The final drug resistance phenotype is often caused by multiple drug resistance mechanisms, while molecular detection only relies on detecting one or several genes, resulting in inconsistent genotypes and phenotypes that cannot accurately guide anti-infection treatment. Therefore, it is of great significance to carry out accurate diagnosis and treatment of infectious diseases by combining drug resistance phenotype and drug resistance gene.

The abuse of antibiotics leads to increased selective pressure on bacteria, leading to bacterial mutations and the formation of drug resistance for survival. Antibiotics retain resistant bacteria and allow them to proliferate in large numbers. Once this resistance is acquired by other bacteria and passed on to the next generation, a large number of drug-resistant strains, even super bacteria, will be produced. Therefore, the rational use of antibiotics is particularly important for controlling bacterial resistance. At present, China's antimicrobial stewardship policies mainly focus on second and third-level medical institutions, there is a lack of supervision and evaluation of grassroots communities, private hospitals, and private medical institutions. Therefore, it is recommended that the government formulate policies and guidelines for the rational use of antimicrobials in primary medical institutions.

There remain some limitations in our study. First, our study lacks an analysis of bacterial antimicrobial resistance genotypes and molecular typing. Second, the relationship between the consumption of antimicrobial agents and the resistance rate of antimicrobials has not been investigated. Despite these limitations, our surveillance study will not only help the hospital management department monitor the evolution of organisms' resistance and strictly regulate the utilization rate and intensity of antimicrobials, but it will also assist clinicians in using antimicrobials sensibly in accordance with the local resistant pattern of organisms. Continuous surveillance of bacterial

antimicrobial resistance trends is of utmost importance for the management of public health.

Conclusion

Escherichia coli, *Klebsiella* spp., *Acinetobacter* spp., and *P. aeruginosa* were the most frequently isolated gram-negative bacilli. *Escherichia coli* was the top one organism isolated from urinary tract, bloodstream, body fluid, wound and pus, except for respiratory tract. Whereas *A. baumannii* and *K. pneumoniae* were the major isolated organisms from respiratory tract. *Acinetobacter baumannii* showed high resistance to the most commonly used antimicrobials. Ceftazidime showed higher activity to *Enterobacteriaceae* organisms than ceftriaxone. Amikacin, carbapenems and piperacillin/tazobactam exhibited relatively high sensitivity, whereas fluoroquinolones showed high resistance rate whether they can be the first-line antimicrobials for empirical treatment of UTI should take more consideration. *Acinetobacter baumannii* and *K. pneumoniae* showed rapid increases in carbapenem resistance whereas *E. coli* had the lowest resistance rate and remain stable at 2%. The gram-negative bacilli in ICU were more resistance than that in non-ICU. These findings are helpful for clinicians using antimicrobials reasonably.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The study was reviewed and approved by the institutional ethics board of Hunan Provincial People's Hospital. All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all participants.

Author contributions

TS: Data curation, Formal Analysis, Validation, Writing – original draft. LX: Data curation, Formal Analysis, Investigation, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The authors would like to acknowledge financial support from the Key Development Program for Research of Hunan (Grant No: 2022SK2071).

Acknowledgments

We thank the doctors and nurses from the department of Infection prevention and control disease for all their work and support. We thank also the infectious diseases and microbiology departments for their shared enthusiasm and valuable support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 13 February 2024
DOI 10.3389/fmicb.2024.1347521

OPEN ACCESS

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RECEIVED 30 November 2023
ACCEPTED 15 January 2024
PUBLISHED 13 February 2024

CITATION
Mendes Pedro D, Paulo SE, Santos CM, Fonseca AB, Melo Cristino J, Pereira ÁA and Caneiras C (2024) Extensively drug-resistant *Pseudomonas aeruginosa*: clinical features and treatment with ceftazidime/avibactam and ceftolozane/tazobactam in a tertiary care university hospital center in Portugal – A cross-sectional and retrospective observational study.
Front. Microbiol. 15:1347521.
doi: 10.3389/fmicb.2024.1347521

Extensively drug-resistant *Pseudomonas aeruginosa*: clinical features and treatment with ceftazidime/avibactam and ceftolozane/tazobactam in a tertiary care university hospital center in Portugal – A cross-sectional and retrospective observational study

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Introduction: Extensively drug-resistant *Pseudomonas aeruginosa* (XDR-PA) is a growing concern due to its increasing incidence, limited therapeutic options, limited data on the optimal treatment, and high mortality rates. The study aimed to characterize the population, the outcome and the microbiological characteristics of XDR-PA identified in a Portuguese university hospital center.

Methods: All XDR-PA isolates between January 2019 and December 2021 were identified. XDR-PA was defined as resistance to piperacillin-tazobactam, third and fourth generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones. A retrospective analysis of the medical records was performed.

Results: One hundred seventy-eight individual episodes among 130 patients with XDR-PA detection were identified. The most common sources of infection were respiratory (32%) and urinary tracts (30%), although skin and soft tissue infections (18%) and primary bacteremia (14%) were also prevalent. Colonization was admitted in 64 cases. Several patients had risk factors for complicated infections, most notably immunosuppression, structural lung abnormalities,

major surgery, hemodialysis or foreign intravascular or urinary devices. XDR-PA identification was more frequent in male patients with an average age of 64.3 ± 17.5 years. One non-susceptibility to colistin was reported. Only 12.4% were susceptible to aztreonam. Ceftazidime-avibactam (CZA) was susceptible in 71.5% of the tested isolates. Ceftolozane-tazobactam (C/T) was susceptible in 77.5% of the tested isolates. Antibiotic regimens with XDR-PA coverage were reserved for patients with declared infection, except to cystic fibrosis. The most frequently administered antibiotics were colistin (41 cases), CZA (39 cases), and C/T (16 cases). When combination therapy was used, CZA plus colistin was preferred. The global mortality rate among infected patients was 35.1%, significantly higher in those with hematologic malignancy (50.0%, $p < 0.05$), followed by the ones with bacteremia (44.4%, $p < 0.05$) and those medicated with colistin (39.0%, $p < 0.05$), especially the ones with respiratory infections (60.0%). Among patients treated with CZA or C/T, the mortality rate seemed to be lower.

Discussion: XDR-PA infections can be severe and difficult to treat, with a high mortality rate. Even though colistin seems to be a viable option, it is likely less safe and efficient than CZA and C/T. To the best of the authors' knowledge, this is the first description of the clinical infection characteristics and treatment of XDR-PA in Portugal.

KEYWORDS

extensively drug-resistant, *Pseudomonas aeruginosa*, antimicrobial resistance, difficult-to-treat infections, ceftazidime-avibactam, ceftolozane-tazobactam

1 Introduction

Extensively drug resistant *Pseudomonas aeruginosa* (XDR-PA) is a growing concern due to its increasing incidence, limited therapeutic options, limited data on the optimal treatment, and high mortality rates. Being able to survive in many ecological settings, *P. aeruginosa* is highly flexible and can adapt to a wide array of environmental pressures. Having access to numerous metabolic pathways and a wide range of virulence and resistance factors thanks to its genetic plasticity, make it one of the most successful bacteria in Medical Microbiology (Behzadi et al., 2022a,b).

Indeed, although in the late 20th century the incidence of *P. aeruginosa* bloodstream infections was declining, by 2010 it had increased again to 6.5 per 10,000 (Werth et al., 2015). Moreover, an increase in the prevalence of multidrug resistant (MDR) and XDR-PA was evident, with XDR-PA prevalences ranging from 2.6 to 11.2% being described (Walkty et al., 2017; Sader et al., 2018a). Furthermore, according to the European Center for Disease Prevention and Control (ECDC), in 2021 most countries in Europe reported rates of resistance higher than 10% for the majority of the antimicrobial classes under surveillance and only two countries reported less than 5% resistance to carbapenems (European Centre for Disease Prevention and Control and World Health Organization, 2023). In Portugal, in 2021, 12.7% of all isolates were resistant to at least three classes. The highest resistance was seen to fluoroquinolones (18.1%) and piperacillin-tazobactam (16.4%). The most susceptible of the five surveilled classes was aminoglycosides (6.3% resistant) (European Centre for Disease Prevention and Control and World Health Organization, 2023). Moreover, carbapenem-resistant *P. aeruginosa* is of critical priority in the World Health Organization Priority Pathogens List (Behzadi et al., 2022b).

Even though *P. aeruginosa* is often a common cause of severe bloodstream infection, ventilator-associated pneumonia and other hospital-acquired infections (Bassetti et al., 2018b), XDR-PA carries an increased mortality. A meta-analysis by *Matos et al.* describes a mortality of 44.6% among patients infected with MDR *P. aeruginosa* versus 24.8% in those with non-MDR infections (de Matos et al., 2018). Recio et al. (2018) compared patients with bacteremia due XDR-PA versus susceptible strains and also found a difference in mortality (62.5% versus 30%).

Even though it is an opportunistic pathogen present in the environment, *P. aeruginosa* is seldom found in the microbiota of healthy humans (Silby et al., 2011; Estepa et al., 2014). Nevertheless, in patients at risk, such as those with a large exposure to the healthcare setting or with certain chronic illnesses (like cystic fibrosis), the colonization rate can reach up to 80% (Gómez-Zorrilla et al., 2014; Ciofu et al., 2015), while those submitted to antibiotic therapy have a higher risk of attaining MDR strains (Gómez-Zorrilla et al., 2014). It also has intrinsic resistance to many antimicrobial drugs and a high capacity of attaining resistance mutations and mobile genetic elements (Bassetti et al., 2018a,b; Horcajada et al., 2019). Although there are many factors contributing to the emergence of antimicrobial resistance, the misuse or overuse of antibiotics is paramount (Algammal et al., 2023). Indeed, despite the new antimicrobials or β-lactam inhibitors that have become commercially available in the last

5 years (Karvouniaris et al., 2023), the emergence of antimicrobial resistance remains one of the greatest threats to global health (Mendes et al., 2022).

Despite the existence of several articles depicting the molecular epidemiology and resistance mechanisms of *P. aeruginosa*, to the best of the authors' knowledge there is a gap in the clinical characterization of these patients in Portugal, a country with one of the highest antimicrobial resistance rates in Europe (Pereira et al., 2013, 2015; Hernández-García et al., 2021a,b,c). The authors aim to characterize the population, the outcome and the microbiological characteristics of XDR-PA identified in a tertiary care university hospital center in Portugal.

2 Materials and methods

2.1 Study procedures

A cross-sectional and retrospective observational study was performed in a 1,000-bed tertiary care university hospital center in Lisbon, including inpatients and outpatients with XDR-PA detection between January 2019 and December 2021 (**Figure 1**). XDR-PA was defined as non-susceptibility to piperacillin-tazobactam, third and fourth generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones, which is included in the consensus defined by Magiorakos et al. (2012).

Electronic medical records were obtained and analyzed. The following population characteristics were collected: gender and age, risk factors such as presence of immunosuppressive status, invasive devices and certain chronic illnesses, site of infection or colonization, antimicrobial therapy performed and outcome. Colonization was defined as the positive identification of XDR-PA without clinical evidence of infection.

Statistical analysis was performed with MS Excel™ and IBM SPSS™ version 26. A descriptive analysis of the variables was performed. When comparing categorical variables, the Pearson's Chi-square test was used. Applicability conditions were verified. The significance level was set at $p < 0.05$.

2.2 Bacterial identification and antimicrobial susceptibility testing

Bacterial identification and antimicrobial susceptibility testing were routinely performed at the hospital's microbiology laboratory by automated systems (MicroScan WalkAway™ or Vitek™).

Susceptibility was tested by a panel of antibiotics: piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamycin, and ciprofloxacin. When there were limited treatment options, ceftazidime-avibactam, ceftolozane-tazobactam, aztreonam, fosfomycin and colistin were also tested. The colistin susceptibility determination was been performed by microdilution. The clinical breakpoints were interpreted in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical breakpoints - breakpoints and guidance, available at the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) website.[1] The isolates were then categorized as susceptible and resistant by applying the breakpoints results.

When the production of metallo-beta lactamases was suspected, a GeneXpert™ assay was performed.

2.3 XDR-PA resistotyping

To better characterize the XDR-PA population, we performed a resistotype distribution analysis. Considering that all included XDR-PA isolates were by definition non-susceptible to piperacillin-tazobactam, third- and fourth generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones, the resistance patterns of those with available susceptibility results to colistin, ceftazidime-avibactam, ceftolozane-tazobactam, aztreonam, and fosfomycin were analyzed. Resistance pattern distribution (resistotyping) and MAR index of clinical XDR *P. aeruginosa* isolates were performed in accordance with previous studies (Behzadi et al., 2022b).

3 Results

Over the 3 years considered in this study (2019-2021) a total of 6,514 isolates of *P. aeruginosa* pertaining to 3,181 patients were identified. Considering XDR-PA, a total of 243 isolates pertaining to 178 episodes concerning 130 patients were found. The prevalence of XDR-PA identification among all *P. aeruginosa* isolates was 3.7%, corresponding to 4.1% of all patients with identification of this microorganism. A new episode was defined as a positive XDR-PA isolate when obtained more than 14 days after the last one, in the same patient. Of these 178 episodes, 114 (64.0%) were from infections and 64 (36.0%) were from colonizations. Moreover, 69 episodes (48 patients) happened in 2019, 61 (46 patients) occurred in 2020 and 48 (36 patients) in 2021. It is noteworthy that only six patients were co-infected with SARS-CoV-2, of which four of them died.

3.1 Clinical results

Patient demographics of the 130 patients in which was recovered an XDR-PA isolate are described in **Table 1**. The cohort was predominantly male (65.4%), with a mean age of 64.3 ± 17.5 years. The most frequent comorbidities described in patients with XDR-PA were diabetes mellitus, immunosuppressive therapy, the presence of a urinary tract device, malignancy and hemodialysis or renal transplant.

Extensively drug-resistant *Pseudomonas aeruginosa* (XDR-PA) was the etiology of infection in several organs and systems. In **Table 1** there is a description of the main sources of infection. A total 114 infections were found, most frequently in the respiratory tract, urinary tract and skin and soft tissues. Sixteen (14.0%) cases presented with primary bacteremia. Of these, most were in immunosuppressed patients often with hematological

1 https://www.eucast.org/clinical_breakpoints

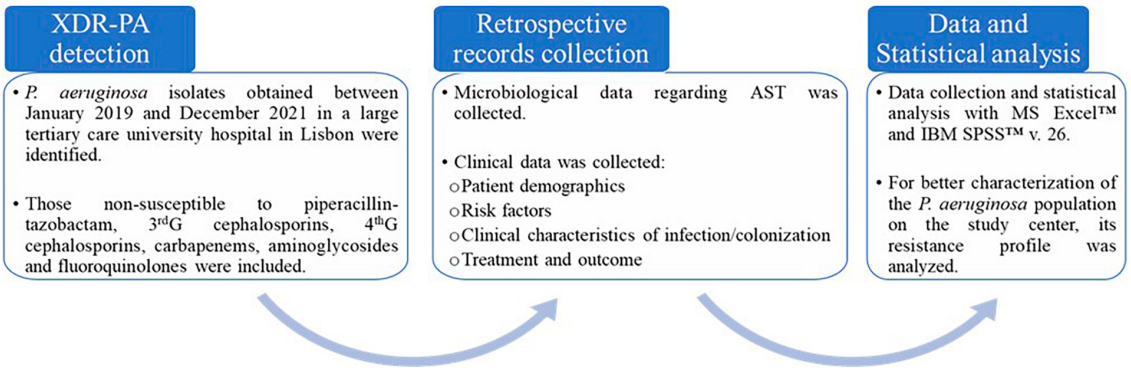

FIGURE 1
A flowchart depicting the procedures done in the present study.

malignancy. Data regarding mechanical ventilation or the presence of a central venous access was unavailable. It is noteworthy that 23.7% (27/114) of all infections presented with bacteremia. In 64 episodes XDR-PA identification was considered colonization, most frequently of the respiratory and urinary tracts.

When infected, patients were submitted to either monotherapy (52 episodes) with an active drug according to the antimicrobial susceptibility test, or to combined therapy (32 episodes). Whenever a patient was prescribed an active drug combined with another to which this agent was resistant (i.e., colistin plus meropenem), it was considered monotherapy. In 30 cases the information regarding appropriate antibiotic therapy was unavailable.

Table 2 describes the appropriate antimicrobials and combinations used and properly documented. Colistin was used in 41 episodes, ceftazidime-avibactam in 39 and ceftolozane-tazobactam in 16. Aztreonam was used in seven cases, but only once in monotherapy. Fosfomycin was used in seven cases but only in combination.

Colonized patients were not submitted to antimicrobial therapy directed to the XDR-PA except in six episodes of cystic fibrosis that presented with respiratory colonization and who were under inhaled colistin (two cases), inhaled aztreonam (one case), both in combination (two cases) or inhaled colistin plus inhaled tobramycin (one case).

There were 40 deaths (35.1%) among infected patients (Table 3). The ones who were treated with combined therapy had a higher mortality (43.8%) than those that were treated with monotherapy (21.2%, $p > 0.05$). Adjusting for the antimicrobial used, the mortality rate was 39.0% ($p > 0.05$, 16 deaths) among colistin-treated patients, 28.2% (11 deaths) among ceftazidime-avibactam-treated patients and 18.8% (three deaths) among ceftolozane-tazobactam-treated patients. Considering only patients with respiratory disease, colistin therapy was associated with a mortality rate of 60.0% as opposed to 32.3% when excluding these patients. Comparison of mortality between patients treated with different antibiotics failed to yield statistically significant differences.

Regarding the source of infection (Figure 2), mortality was highest among patients with respiratory infections (47.2%), followed by those with skin and soft tissues infections (35.0%), intra-abdominal infections (33.3%), and urinary tract infections (26.5%). Among patients with positive blood cultures the mortality rate was 44.4% ($p < 0.05$), although only 25.0% when presenting with primary bacteremia. Considering all comorbidities, only hematologic malignancy was significantly associated with mortality (50.0% died, $p < 0.05$).

3.2 Microbiological results

Extensively drug-resistant *Pseudomonas aeruginosa* (XDR-PA) was identified in 243 microbiological examinations, corresponding to the 178 episodes. Antimicrobial susceptibility testing (AST) was performed in 216 (88.9%) isolates (Table 4). In the remaining, AST was not performed since it had already been done for another contemporary sample.

The only resistance found to colistin was in a 34-year-old patient with cystic fibrosis, and it remained susceptible exclusively to ceftazidime-avibactam and to ceftolozane-tazobactam novel combinations. Moreover, a 56-year-old patient with hematologic malignancy under chemotherapy presented with febrile neutropenia and primary bacteremia with a VIM-producing XDR-PA that was susceptible only to colistin. The patient was treated with colistin and fosfomycin and ultimately died.

Among all *P. aeruginosa* isolates, the yearly susceptibility rate to meropenem varied between 80 and 82%. Regarding XDR-PA isolates, when tested, most were susceptible to ceftazidime-avibactam and ceftolozane-tazobactam, while only 22.4% were susceptible to fosfomycin and 12.4% to aztreonam. Regarding the strains tested both for susceptibility to ceftazidime-avibactam and to ceftolozane-tazobactam, 63.2% were susceptible and 19.1% were resistant to both antibiotics. Of those resistant to ceftazidime-avibactam, 42.2% were susceptible to ceftolozane-tazobactam, while of those resistant to ceftolozane-tazobactam, 16.1% were susceptible to ceftazidime-avibactam. The related defaults for XDR in association with the used antibiotics and combinations are presented in Figure 3. Most of the strains tested for both colistin and ceftazidime-avibactam and colistin and ceftolozane-tazobactam were susceptible to both antimicrobials (70.9 and 76.6%, respectively). Two-antibiotic combinations with fosfomycin or aztreonam were

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TABLE 1 Patient demographics of the 130 patients with XDR *Pseudomonas aeruginosa* (XDR-PA) and site of infection/colonization of the 178 XDR-PA episodes.

Patients demographics *N* = 130 patients	
Age (years):	
Mean ± SD	64.3 ± 17.5
Median	65
Min. – Max.	13–98
Gender:	Number of patients *n* (%)
Male	85 (65.4%)
Female	45 (34.6%)
Main comorbidities:	*n* (%)
Diabetes mellitus	29 (22.3%)
Immunosuppressive therapy	28 (21.5%)
Urinary tract device	18 (13.8%)
Hematologic malignancy	18 (13.8%)
Solid neoplasm	14 (10.8%)
Renal transplant	13 (10.0%)
Chronic kidney disease in hemodialysis	12 (9.2%)
Chronic skin lesions	10 (7.7%)
Cystic fibrosis	9 (6.9%)
Other chronic lung diseases	8 (6.2%)
Complicated intra-abdominal surgery	7 (5.4%)
Tracheostomy	5 (3.8%)
Hepatic cirrhosis	3 (2.3%)
HIV/AIDS	1 (0.8%)
Common variable immunodeficiency	1 (0.8%)
Site of infection/colonization *N* = 178 episodes	Number of isolates *n* (%)
Infections	114 (64.0%)
Respiratory	36 (31.6%)
Urinary tract	34 (29.8%)
Skin and soft tissues	20 (17.5%)
Primary bacteremia	16 (14.0%)
Intra-abdominal	6 (5.3%)
Ear	1 (0.9%)
Eye	1 (0.9%)
Colonization – *N* (%)	64 (36.0%)
Respiratory tract	24 (37.5%)
Urinary tract	22 (34.4%)
Skin and soft tissues	18 (28.1%)

less likely to be susceptible to both drugs, which is in accordance with the lower susceptibility rates found in those antimicrobials.

TABLE 2 Antimicrobial treatment to *Pseudomonas aeruginosa* infections and all-cause mortality by treatment.

Monotherapy ± inhaled therapy	Number of patients	All-cause mortality *n* (%)
Colistin	21	6 (28.6%)
Ceftazidime-avibactam	19	3 (15.8%)
plus inhaled colistin	3	0 (0.0%)
Ceftolozane-tazobactam	13	2 (15.4%)
Aztreonam	1	0 (0.0%)
Combination therapy ± inhaled therapy		
Ceftazidime-avibactam plus colistin	12	5 (41.7%)
plus inhaled colistin	1	0 (0.0%)
Ceftazidime-avibactam plus aztreonam	4	3 (75.0%)
Ceftazidime-avibactam plus fosfomycin	4	0 (0.0%)
Ceftolozane-tazobactam plus colistin	3	1 (33.3%)
Colistin plus aztreonam	2	1 (50.0%)
plus inhaled colistin	1	0 (0.0%)
Colistin plus fosfomycin	3	3 (100.0%)
Exclusively inhaled therapy[1]		
Inhaled colistin	2	0 (0.0%)
Inhaled aztreonam	1	0 (0.0%)
Inhaled colistin plus inhaled aztreonam	2	0 (0.0%)
Inhaled colistin plus inhaled tobramycin	1	0 (0.0%)

[1] In patients with cystic fibrosis.

3.3 XDR-PA resistotyping

Table 5 shows the resistance patterns of 65 clinical XDR-PA that were non-susceptible to piperacillin-tazobactam, third and fourth generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones and that, additionally had available AST results for colistin, ceftazidime-avibactam, ceftolozane-tazobactam, aztreonam and fosfomycin. Twelve different resistotypes were found.

The most common resistotype was the combined resistance to aztreonam and fosfomycin (n = 26, 40.0%), followed by ceftazidime-avibactam, aztreonam and fosfomycin (n = 13; 20.0%), and ceftazidime-avibactam, ceftolozane-tazobactam and aztreonam (n = 8; 12.3%). Resistance to at least two of the tested antibiotics occurred in 90.8%, while 43.1% were resistant to more than three antimicrobials.

4 Discussion

Extensively drug resistant *Pseudomonas aeruginosa* (XDR-PA) is a growing concern due to its increasing incidence, limited therapeutic options, limited data on the optimal treatment, and

TABLE 3 Clinical outcome of infection considering source of infection and treatment including colistin, ceftazidime-avibactam or ceftolozane-tazobactam.

	All cause mortality in infected patients deaths/number of patients (%)	
Source of infection		
All sources of infection	40/114 (35.1%)	$p > 0.05$
Respiratory	17/36 (47.2%)	$p > 0.05$
Urinary tract	9/34 (26.5%)	$p > 0.05$
Skin and soft tissues	7/20 (35.0%)	$p > 0.05$
Primary bacteremia	4/16 (25.0%)	$p > 0.05$
Intra-abdominal	2/6 (33.3%)	$p > 0.05$
Ear	1/1 (100.0%)	$p > 0.05$
Eye	0/1 (0.0%)	$p > 0.05$
Any source, with bacteremia	12/27 (44.4%)	$\mathbf{p < 0.05}$
Treatment		
Among colistin-treated patients	16/41 (39.0%)	$\mathbf{p < 0.05}$
Respiratory source	6/10 (60.0%)	$p > 0.05$
Non-respiratory source	10/31 (32.3%)	$p > 0.05$
Among CZA-treated patients	11/39 (28.2%)	$p > 0.05$
Among C/T-treated patients	3/16 (18.8%)	$p > 0.05$
Monotherapy vs. Combined therapy	11/52 (21.2%) *Vs.* 14/32 (43.8%)	$\mathbf{p < 0.05}$

CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam. The bold value shows when *p*-value was less than 0.05.

high mortality rates. Even though the criteria used to define XDR-PA did not match exactly those defined by Magiorakos et al. (2012), the prevalence reported in the present study is lower than the one displayed in 2017 by a large-scale Spanish multicenter study (17%) (Del Barrio-Tofiño et al., 2019). Similarly, a trial including patients with ventilator-associated pneumonia in Spain, Greece and Italy showed a rate of 35.8% XDR isolates, mostly from Greece (Pérez et al., 2019). Other countries around the world also reported higher XDR-PA prevalence, such as 22.1% out of 447 *P. aeruginosa* isolates in Nepal (Mahto et al., 2021) or 15.5% out of 3248 isolates in Iran (Mirzaei et al., 2020). The prevalence reported in the present study is more similar to that described in Canada and in USA. McCracken et al. (2019) showed 4.5% XDR-PA among all 3864 Canadian isolates between 2007 and 2016, while Sader et al. (2017) depict 9.4% XDR-PA among 7452 American isolates from 2012 to 2015. Although there is a lack in data regarding the prevalence of XDR-PA in Portuguese isolates, ECDC reports a resistance rate to carbapenems of 14.1 and of 12.7% to any combination of at least three of the following antibiotics: piperacillin-tazobactam, ceftazidime, carbapenems, aminoglycosides and fluoroquinolones (Antimicrobial resistance surveillance in Europe 2023 - 2021 data. Stockholm: European Centre for Disease Prevention and Control and World Health Organization, 2023).

While *P. aeruginosa* is rarely found in the microbiota of healthy humans (Silby et al., 2011; Estepa et al., 2014), it can colonize up to 80% of patients with risk factors, such as a large exposure to the healthcare setting or certain chronic illnesses (such as cystic fibrosis, and solid or hematologic malignancies) (Gómez-Zorrilla et al., 2014; Ciofu et al., 2015). The presence of foreign devices, such as venous or urinary catheters, tracheostomy (especially in children), open abdominal surgery, diabetes, chronic hepatic disorder and end-stage renal disease also increase the risk for *P. aeruginosa* infection (Varaiya et al., 2008; Willmann et al., 2014; Miller et al., 2016; Bassetti et al., 2018a; Russell et al., 2019; Jean et al., 2020; Körpinar, 2021). Also prior antibiotic therapy (especially fluoroquinolones and carbapenems), so frequent in the healthcare setting and in patients with these comorbidities, is a major risk factor for attaining MDR strains (Falagas et al., 2006; Peña et al., 2012; Gómez-Zorrilla et al., 2014; Bassetti et al., 2018a; Jean et al., 2020). In fact, prior use of fluoroquinolones or carbapenems has been depicted as an independent risk factor for XDR-PA infections (Palavutitotai et al., 2018).

In *P. aeruginosa*, resistance mechanisms such as decreased permeability, expression of efflux pumps, target modifications and production of inactivating enzymes have all been described (Mesaros et al., 2007). In particular, production of extended-spectrum beta-lactamases and carbapenemases have been described, including metallo-beta-lactamases (Mesaros et al., 2007; Sacha et al., 2008; Ríos et al., 2018; Muddassir et al., 2021). Since these enzymes use zinc, which seems to be a valuable cofactor to hydrolyze beta-lactams, instead of serine in their active sites, they confer resistance to all beta-lactam antibiotics except aztreonam, while not being degraded by currently available beta-lactamase inhibitors (Sacha et al., 2008; Behzadi et al., 2020). In this study there was one VIM-producing XDR-PA that was susceptible only to colistin. Despite VIM not being able to degrade aztreonam, *P. aeruginosa* is capable of producing simultaneously additional inactivating enzymes, conferring resistance also to this drug (Mesaros et al., 2007; Ríos et al., 2018). So, the combination of aztreonam-avibactam (or ceftazidime-avibactam plus aztreonam if the former is not available) is pertinent as it combines the ability of avibactam to inactivate all serine-beta-lactamases, thus allowing aztreonam to be effective (Marshall et al., 2017; Mischnik et al., 2017). However, due to mechanisms of resistance to aztreonam independent of beta-lactamases, the activity of this combination has to be confirmed with synergy testing (Karakonstantis et al., 2020).

One drug that is often protected by the cross-resistance of other anti-pseudomonal antibiotics is colistin (Mesaros et al., 2007). And while pan-drug resistant isolates have been described, most often XDR-PA remains susceptible to colistin (Souli et al., 2008; Viedma et al., 2009; Giani et al., 2018). In this study all isolates but one were susceptible to colistin. However, while the prevalence of resistance to ceftazidime-avibactam and to ceftolozane-tazobactam appears to be overall low in Europe and the United States, the authors report approximately one quarter of non-susceptible isolates. This might be explained by the geographical variation of the predominance of different mechanisms of resistance (Flamm et al., 2014; Del Barrio-Tofiño et al., 2017; Giani et al., 2018; Livermore et al., 2018; Sader et al., 2018b; Evans et al., 2019), although when considering the cross-resistance of ceftolozane-tazobactam

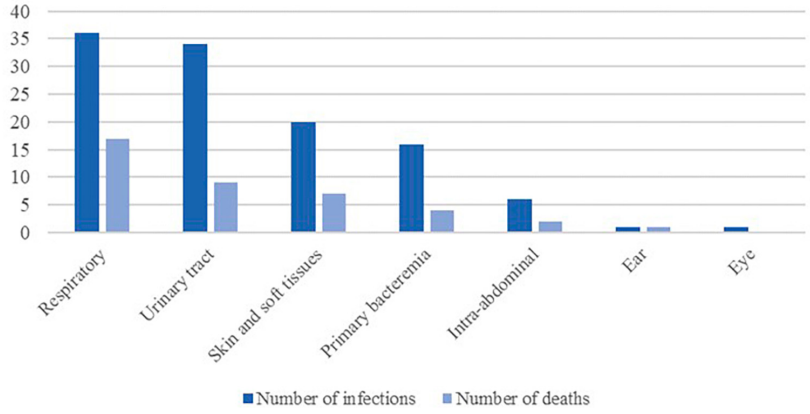

FIGURE 2
Frequency of infections and all causes mortality by source of infection.

TABLE 4 Antimicrobial susceptibilities of XDR-PA isolates to selected antimicrobial agents.

N (%)	Colistin	Ceftazidime-avibactam	Ceftolozane-tazobactam	Aztreonam	Fosfomycin
Tested isolates[1]	216 (88.9%)	158 (65.0%)	173 (71.2%)	188 (77.4%)	98 (40.3%)
Susceptible (S)[2]	215 (99.5%)	113 (71.5%)	134 (77.5%)	26 (13.8%)	22 (22.4%)
Non-susceptible (NS)[2]	1 (0.5%)	45 (28.5%)	39 (22.5%)	162 (86.2%)	76 (77.6%)

All isolates were resistant to piperacillin-tazobactam, third and fourth generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones.
[1]Percentage in relation to all isolates.
[2]Percentage in relation to the tested isolates.

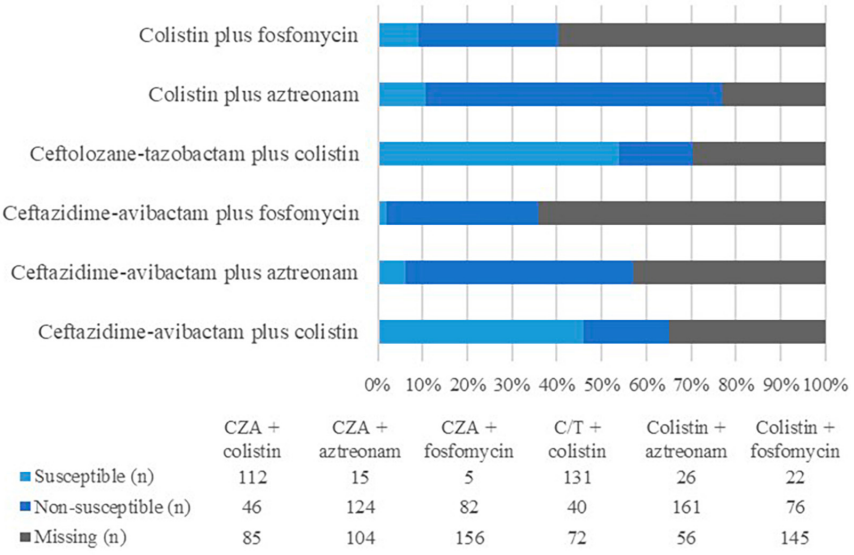

FIGURE 3
Antimicrobial susceptibilities of XDR-PA isolates (*n* = 243) to antimicrobial combinations used as treatment in the present study. CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam.

and ceftazidime-avibactam in this study, the authors believe the mechanism not to be predominantly related to carbapenemases. Regarding aztreonam and fosfomycin, the authors report high rates of non-susceptibility, as displayed by other centers and other Portuguese data (Sader et al., 2017; Safaei et al., 2017; Evans et al., 2019; Hernández-García et al., 2021a).

Regarding the analysis of the resistance patterns in XDR-PA isolates, it is not unexpected that the most frequent resistotype is resistance to both aztreonam and fosfomycin. It is noteworthy, however, that resistance patterns that included non-susceptibility to both aztreonam and ceftazidime-avibactam were frequent (40.0%, resistotypes V, VIII, X, and XII). Unfortunately, despite the

TABLE 5 Resistance patterns distribution and the MAR indices of clinical XDR-PA.

Resistotype	Resistance pattern	MAR index	Ratio *n* (%)
I	Ceftolozane-tazobactam	0,2	1 (1.5%)
II	Aztreonam	0,2	3 (4.6%)
III	Fosfomycin	0,2	2 (3.1%)
IV	Ceftazidime-avibactam, ceftolozane-tazobactam	0,4	3 (4.6%)
V	Ceftazidime-avibactam, aztreonam	0,4	1 (1.5%)
VI	Ceftazidime-avibactam, fosfomycin	0,4	1 (1.5%)
VII	Aztreonam, fosfomycin	0,4	26 (40.0%)
VIII	Ceftazidime-avibactam, ceftolozane-tazobactam, aztreonam	0,6	8 (12.3%)
IX	Ceftazidime-avibactam, ceftolozane-tazobactam, fosfomycin	0,6	1 (1.5%)
X	Ceftazidime-avibactam, aztreonam, fosfomycin	0,6	13 (20.0%)
XI	Ceftolozane-tazobactam, aztreonam, fosfomycin	0,6	2 (3.1%)
XII	Ceftazidime-avibactam, ceftolozane-tazobactam, aztreonam, fosfomycin	0,8	4 (6.2%)
Total			**65 (100.0%)**

All isolates were resistant to piperacillin-tazobactam, third and fourth generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones.

knowledge that ceftazidime-avibactam resistance is emerging in *K. pneumoniae* in Portugal since its approval by the national regulatory authority in 2019 (Mendes et al., 2022, 2023), no data was available on synergy testing of ceftazidime-avibactam plus aztreonam or further study of resistance mechanisms in *P. aeruginosa*.

As XDR-PA is undoubtedly a growing threat with increasingly limited therapeutic options, it is essential to define new and innovative therapeutic strategies, either by developing new systemic drugs, new drug combinations, different methods of drug administration or alternative therapies (Mesaros et al., 2007; Marshall et al., 2017; Mischnik et al., 2017; Bassetti et al., 2018b; Horcajada et al., 2019; Ekkelenkamp et al., 2020; Gaurav et al., 2020; Yao et al., 2021). For example, the synergistic combination colistin-mefloquine has been suggested as a potential future therapeutic option against colistin-resistant strains due to its anti-biofilm activity (Behzadi et al., 2022a). Inhaled therapy has long since been used in cystic fibrosis and it can be a powerful adjunct in the treatment of XDR-PA infections. Since drug concentration in the lung is much higher with inhaled therapy than with the intravenous route, it can be effective even against *in vitro* resistant strains (Horcajada et al., 2019).

The authors report high mortality rates (35.1%), as observed in previous studies (Samonis et al., 2014; de Matos et al., 2018; Palavutitotai et al., 2018). Patients with hematologic malignancy have been described as being at an increased risk of a poor outcome (Samonis et al., 2014; Tofas et al., 2017; de Matos et al., 2018), perhaps due to their immunosuppressed status with frequent and recurrent infections and antimicrobial use. The authors report a 50.0% ($p < 0.05$) mortality among patients with hematologic malignancy.

Patients with bacteremia had a worse prognosis (mortality 44.4%, $p < 0.05$), similar to the 18.0% reported by Dantas et al. (2014) in their comparison between bacteremia due to susceptible and MDR strains. Having been established as an independent risk factor for mortality (Dantas et al., 2014), it surely is a marker for severe disease. Thus, it is interesting to note that in patients presenting with primary bacteremia the mortality was only 25.0%.

Pseudomonas aeruginosa is also a frequent cause of healthcare-associated respiratory and urinary tract infections (Quartin et al., 2013; Lamas Ferreiro et al., 2017; Jean et al., 2020), the most frequent sources of infection as reported in this study and consistent with the most frequent types of Healthcare Associated Infections, as reported by the ECDC (Suetens et al., 2023). Having been established that infections with resistant strains have a greater risk than susceptible strains (de Matos et al., 2018; Recio et al., 2018; Jean et al., 2020) it is not unexpected to find high mortality rates in this study. Nevertheless, 47.2% fatalities among patients with respiratory infections was higher than what had been previously described (Peña et al., 2013; Giaccari et al., 2021). The 26.5% mortality described among patients with urinary tract infections is similar to that described by Lamas Ferreiro et al. (2017).

Adjusting for the antimicrobial used, the authors report a higher mortality rate among colistin-treated patients (39.0%, $p < 0.05$). Although these results did not achieve statistical significance, it appears that the mortality among patients treated with ceftazidime-avibactam (28.2%) or ceftolozane-tazobactam (18.8%) was lower. Considering exclusively monotherapy, these results are consistent, with 28.6% of patients treated with colistin dying, against 15.8 and 15.4% of patients treated with ceftazidime-avibactam and ceftolozane-tazobactam, respectively. This seems paradoxical since colistin has been described as one of the most active antipseudomonal drugs (Del Barrio-Tofiño et al., 2019; López Montesinos et al., 2021; Pinilla-Rello et al., 2021). The authors believe that this result might be related to both a better efficacy and safety profile of beta-lactams and a poorer penetration of colistin in the lung, which is supported by the 60.0% mortality when considering only patients with respiratory infection treated with colistin *versus* 32.3% when considering only other sources of infection. This findings support the last IDSA Guidance, where beta-lactams are put at the forefront of the treatment of MDR *P. aeruginosa* infections (Tamma et al., 2022). Patients that were submitted to combined directed therapy seemed to have a higher mortality rate than those treated with only one active drug. These results may be related to a possibly increased severity of disease among patients submitted to combined therapy.

The present study has several limitations. First, by using a narrower definition of XDR-PA than previously published, it is possible that other isolates were not considered. Therefore, comparisons with other studies must take this limitation into consideration. Second, several important variables, such as disease severity, exposure to venous catheterization or mechanical ventilation, recent or recurrent history of antibiotic therapy or

healthcare exposure, and prior *P. aeruginosa* colonization status were most often not possible to ascertain.

The results of this study are relevant as they provide a first insight into the clinical outcome of Portuguese patients infected with XDR-PA, allowing for better care between relevant centers. However, in the future, it is essential to improve our understanding and to further characterize these patients, particularly in high-risk environments (such as focusing on Intensive Care Units and Hematology wards). Moreover, there is a crucial need for a current description of the molecular epidemiology and characterization of the resistance mechanisms of *P. aeruginosa* in Portugal. Furthermore, the integration of clinical data with molecular surveillance and the analysis of genetic resistance determinants may be relevant to improve the quality of care, allowing for a greater accent on research-based recommendations. Finally, with the potential impact of the COVID-19 pandemic on hospital epidemiology, it is paramount to evaluate this issue in the light of new therapeutic options.

5 Conclusion

In conclusion, patients infected with extensively drug resistant *Pseudomonas aeruginosa* are difficult to treat, with very limited therapeutic options. XDR-PA is a pathogen responsible for potentially severe disease that can have high mortality. Although the most frequent sources of infection were the respiratory and urinary sites, patients with bacteremia or hematologic malignancy had a higher risk. In this study, colistin was the most susceptible antibiotic *in vitro*. However, patients treated with ceftazidime-avibactam or ceftolozane-tazobactam appeared to have a better prognosis than those treated with colistin, especially considering those with respiratory infection. To the best of the authors' knowledge, this is the first study to provide a clinical characterization of patients infected with XDR-PA strains in Portugal.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

DM: Conceptualization, Formal analysis, Methodology, Writing – original draft. SP: Conceptualization, Methodology, Writing – review & editing. CS: Writing – review & editing. AF: Writing – review & editing. JM: Writing – review & editing. ÁP: Writing – review & editing. CC: Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

JM and CC received research grants administered through university and honoraria for serving on the speaker's bureaus of Pfizer and MSD that were not related to the present study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Microbiology

TYPE Systematic Review
PUBLISHED 08 March 2024
DOI 10.3389/fmicb.2024.1337570

OPEN ACCESS

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RECEIVED 21 November 2023
ACCEPTED 09 February 2024
PUBLISHED 08 March 2024

CITATION
Ai M-Y, Chang W-L and Liu C-Y (2024) Mortality of continuous infusion versus intermittent bolus of meropenem: a systematic review and meta-analysis of randomized controlled trials.
Front. Microbiol. 15:1337570.
doi: 10.3389/fmicb.2024.1337570

Mortality of continuous infusion versus intermittent bolus of meropenem: a systematic review and meta-analysis of randomized controlled trials

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Background: Meropenem belongs to the carbapenem class, which is categorized as beta-lactam antibiotics. These antibiotics are administered in intermittent bolus doses at specific time intervals. However, the continuous infusion approach ensures sustained drug exposure, maintaining the drug concentration above the minimum inhibitory concentration (MIC) throughout the entire treatment period. This study aimed to find out the association between continuous infusions of meropenem and mortality rates.

Materials and methods: We conducted a search of the PubMed/Medline, EMBASE, Cochrane Central, and ClinicalTrials.gov databases up to 14 August 2023. The six randomized controlled trials (RCTs) were identified and included in our analysis. The random-effects model was implemented using Comprehensive Meta-Analysis software to examine the outcomes.

Results: Our study included a total of 1,529 adult patients from six randomized controlled trials. The primary outcome indicated that continuous infusion of meropenem did not lead to reduction in the mortality rate (odds ratio = 0.844, 95% CI: 0.671–1.061, P =0.147). Secondary outcomes revealed no significant differences in ICU length of stay (LOS), ICU mortality, clinical cure, or adverse events between continuous infusion and traditional intermittent bolus strategies of meropenem. Notably, we observed significant improvements in bacterial eradication (odds ratio 19 = 2.207, 95% CI: 1.467–3.320, $P < 0.001$) with continuous infusion of meropenem. Our study also suggested that performing continuous infusion may lead to better bacterial eradication effects in resistant pathogens (coefficient: 2.5175, $P = 0.0138$*).

Conclusion: Continuous infusion of meropenem did not result in the reduction of mortality rates but showed potential in improving bacterial eradication. Furthermore, this strategy may be particularly beneficial for achieving better bacterial eradication, especially in cases involving resistant pathogens.

KEYWORDS

continuous infusion, meropenem, mortality, resistant pathogens, bacterial eradication

1 Introduction

Beta-lactam antibiotics, which are time-dependent antibiotics, possess a common structure known as the beta-lactam ring and are widely utilized for treatment of diverse bacterial infections (Bush and Bradford, 2016). Traditionally, these antibiotics have been administered as intermittent bolus doses at specific time intervals. These antibiotics typically exert their antimicrobial effects by binding to bacterial enzymes involved in cell wall synthesis (Zeng and Lin, 2013). The time-dependent killing property is reliant on the duration of the pathogen's exposure to the antibiotics. Extended or continuous infusion aids in overcoming the time-dependent nature of beta-lactam antibiotics (Tilanus and Drusano, 2023).

One strategy employed to optimize the effectiveness of beta-lactam antibiotics involves the utilization of continuous infusion, which prolongs the duration of the antibiotic bolus to 24 h. The continuous infusion approach ensures sustained drug exposure, maintaining the drug concentration above the minimum inhibitory concentration (MIC) during the entire treatment period (Shiu et al., 2013). Administering meropenem through continuous infusion can help maintain constant therapeutic levels, potentially improving bacterial eradication and reducing mortality rates. Additionally, previous studies have indicated that extended and continuous infusion strategies have the potential to lower the serum peak concentration, thereby minimizing the likelihood of adverse effects caused by drug toxicity (Cotner et al., 2017).

Meropenem, belonging to the carbapenem class of broad-spectrum beta-lactam antibiotics, is used for treating severe bacterial infections often caused by multidrug-resistant organisms (MDROs) in critically ill patients (Hellinger and Brewer, 1999). Although some previous studies have showed that continuous infusion of meropenem offers several advantages, including stable drug levels and reduced adverse effects, there are still some potential caveats. For example, continuous infusion necessitates special equipment, such as an infusion pump, to be administered continuously for 24-h, entails cost consideration, and requires additional nursing care to ensure the correct dosage is being administered. This may increase the overall cost for the hospital (Dunning and Roberts, 2015).

While previous hypotheses have suggested that continuous infusion could provide stable therapeutic levels and potential advantages for specific antibiotics, limited clinical evidence exists, especially regarding continuous infusion of meropenem. In this study, we aim to investigate the association between continuous infusion of meropenem and mortality rates.

2 Materials and methods

2.1 General guidelines

We adhered to the steps outlined in the recent edition of the PRISMA 2020 guidelines (Page et al., 2021) for conducting this meta-analysis. This study was registered with INPLASY under the registration number INPLASY 2023110035 (Ai et al., 2023) and was exempted from obtaining ethics review board approval and participant informed consent.

2.2 Data research and the identification of eligible studies

Two authors (MY, Ai and CY, Liu) independently conducted electronic searches in the PubMed, Embase Cochrane CENTRAL, and ClinicalTrials.gov databases using the keywords [continuous infusion AND (carbapenem OR meropenem)]. The search period covered from each database to the date of 4 August 2023. The gray literature was also considered in our study. However, the gray literature that was searched for in our study was all excluded during data extraction process because none of them were randomized control trials (RCTs).

Initially, the two authors responsible for the search screened the titles and abstracts of the identified studies for eligibility using the consensus process. Subsequently, a thorough screening of full texts was conducted.

2.3 Inclusion and exclusion criteria

The PICO (population, intervention, comparison, outcome) framework for the current meta-analysis is as follows: P: human participants, I: continuous infusion of meropenem, C: intermittent bolus of meropenem, and O: mortality.

The inclusion criteria include the following: (1) Enrolled human participants in RCTs. (2) RCTs comparing the mortality rate of continuous infusion and intermittent bolus of meropenem to treat infection.

The exclusion criteria: (1) NON-RCTs study. (2) Human participants were not enrolled. (3) RCTs that did not examine the outcome of mortality. (4) RCTs, but only investigated the mortality rate between extended infusion and intermittent bolus of any meropenem. (5) RCTs, but not investigated the mortality rate between extended infusion and intermittent bolus of other carbapenems (except meropenem). (6) RCTs investigated the mortality rate of extended infusion and intermittent bolus of other beta-lactam agents.

2.4 Methodological quality appraisal

To assess the methodological quality of the included studies, we utilized the Cochrane risk-and-bias tool for randomized trials (version 2, RoB 2, London, United Kingdom) (Sterne et al., 2019). This tool consists of six main domains for evaluating the study quality, including randomization, intervention adherence, missing outcome data, outcome measurement, selective reporting, and the overall risk of bias. Regarding the intervention adherence section of the RoB 2 tool, two options were available for literature assessment: intention-to-treat (ITT) and per-protocol (PP). In our research, we incorporated studies from randomized controlled trials (RCTs) that utilized both ITT and PP analyses.

ITT and PP analyses stand as pivotal methodologies in clinical trial research, each contributing distinct insights. The ITT approach encompasses all randomized participants, irrespective of their study completion or adherence to the protocol. This methodology reduces selection bias and upholds the advantages of

randomization, thereby offering results that more accurately mirror real-world conditions. ITT analysis is vital for extrapolating results to a broader patient demographic, mirroring the variable adherence often seen in everyday clinical settings.

PP analysis is more selective, focusing solely on participants who adhered strictly to the study protocol until completion. This approach is key to gauging a treatment's efficacy in optimal conditions, thereby elucidating its maximum potential effectiveness. By evaluating only those who strictly followed the treatment plan, PP analysis provides a more precise estimate of the treatment's impact, albeit with less generalizability to the wider population.

In essence, while ITT analysis presents a realistic portrayal of treatment outcomes in typical clinical practice, PP analysis sheds light on the optimal efficacy of treatments under ideal conditions. Collectively, both analyses offer a thorough understanding of a treatment's effectiveness across various scenarios. However, all six studies included in our research have consistently presented outcomes derived from ITT analysis.

2.5 Primary outcomes

We compared the mortality rate between continuous infusion and intermittent bolus of meropenem. We also analyzed mortality trends in different subgroups, including meropenem, other beta-lactam agents, and different continuous infusion doses. The outcome was measured and quantified using odds ratios. The sensitivity test and publication bias were also evaluated.

2.6 Secondary outcomes

The secondary outcomes include clinical success/improvement, intensive care unit (ICU) mortality, length of ICU stay, and bacterial eradication rate, comparing continuous infusion with intermittent bolus administration of meropenem. Treatment-related serious adverse events were also analyzed in our study. In instances where cells had zero events, a value of 0.5 was substituted to facilitate calculations (Deeks et al., 2022). The outcome was measured and quantified using odds ratios. The sensitivity test and publication bias were evaluated.

The meta-regression was performed to evaluate the relationship between mortality and continuous infusion dose. The correlation between resistant pathogens and mortality or bacteria eradicated when continuous infusions were performed was also studied. The resistant pathogen was definite as the pathogen was resistant to carbapenem or culture susceptibility test results showed a meropenem MIC $\geq$ 1.5.

2.7 Data extraction and management

Data extraction from the evaluated studies was carried out by two independent authors (M-YA and C-YL). The extracted data included demographic information, study design parameters, details of continuous infusion and intermittent administration of meropenem, as well as primary and secondary outcome values.

2.8 Statistical analysis

Based on the variability in target populations across the included studies, we performed the current meta-analysis using a random-effects model implemented using Comprehensive Meta-Analysis software (version 3, Biostat, Englewood, NJ, United States). A two-tailed p-value of <0.05 was considered statistically significant (Borenstein and Hedges, 2009).

A fixed-effect model assumes that the true effect of the intervention is the same in all studies (i.e., fixed across studies). It implies that any observed differences among study results are attributed solely to chance (random variability). The fixed-effect model operates on the strong assumption that intervention effects are identical across all studies. It is typically used when the studies are very similar in terms of participants, interventions, and outcomes. However, this model does not account for heterogeneity across studies.

The random-effects model does not assume a single true effect size but rather a distribution of effect sizes. It assumes that the effects follow a normal distribution and recognizes that differences in study results may be due to both chance and genuine variation in intervention effects. This model is more flexible and realistic in many scenarios, especially when there is an expectation of variability in intervention effects across studies. It is particularly useful when the studies in the meta-analysis are not homogenous in terms of populations, interventions, outcomes, or methodologies.

In our article, we consider that assuming identical intervention effects across various studies is generally implausible, barring exceptional cases where the intervention exhibits no effect whatsoever. This argument favors the adoption of the random-effects model in our study. By acknowledging and accommodating the inherent heterogeneity in meta-analyses, the random-effects model offers a more nuanced and potentially more accurate estimation of the average intervention effect.

To quantify the primary outcomes, Hedges' g and calculated 95% confidence intervals (CIs) were used. Effect sizes were categorized as small ($g = 0.2$), moderate ($g = 0.5$), and large ($g = 0.8$) based on Hedges' criteria (Hedges, 1981).

To assess the degree of heterogeneity among the studies, we examined I^2 and Cochran's Q statistics. I^2 values of 25, 50, and 75% were considered indicative of low, moderate, and high heterogeneity, respectively (Higgins et al., 2003).

We also performed meta-regression analyses to investigate the relationship between the mortality rate and the continuous infusion dose.

To ensure the robustness of this meta-analysis, sensitivity analyses were conducted using the one-study removal method to examine whether removing a particular trial resulted in a significant change in the summary effect size (Deeks et al., 2022).

Potential publication bias was assessed following the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. Funnel plots and Egger's regression tests were used

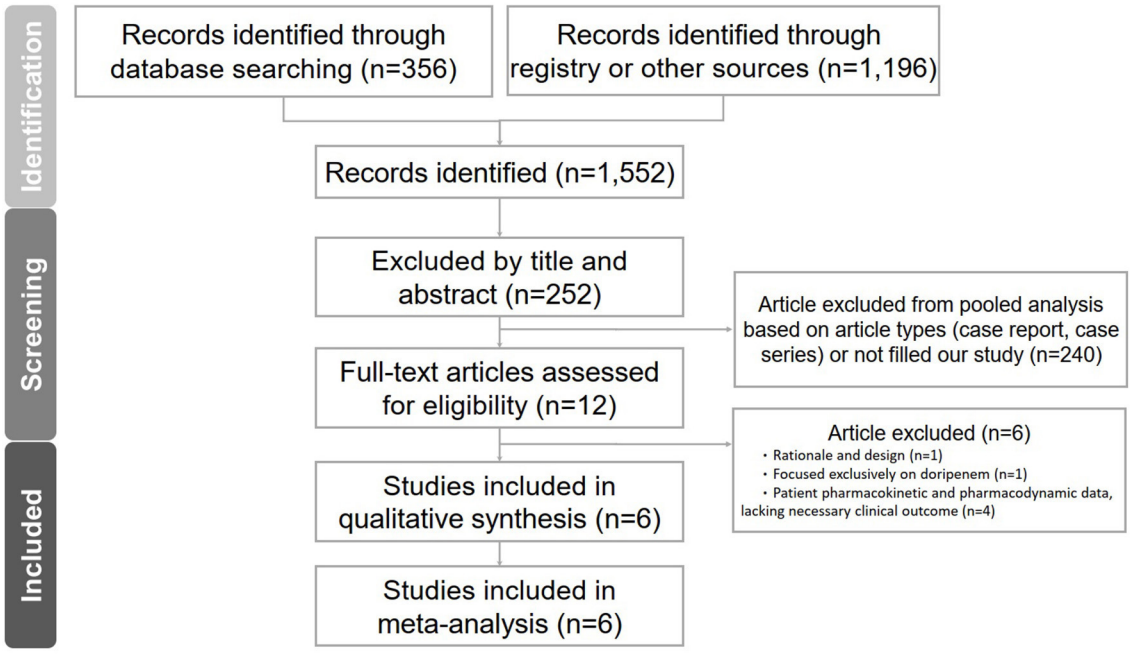

FIGURE 1
PRISMA flowchart.

to evaluate the presence of public bias included in the studies (Page et al., 2022).

3 Results

3.1 Study identification and selection

The PRISMA flowchart depicts the sequential process undertaken to identify and select studies for analysis (Figure 1). Initially, we conducted a comprehensive search of relevant databases using appropriate keywords and search terms. A total of six RCTs were included in our meta-analysis, as shown in Table 1 (Chytra et al., 2012; Dulhunty et al., 2013, 2015; Abdul-Aziz et al., 2016; Zhao et al., 2017; Monti et al., 2023). The results of the Cochrane RoB 2 assessment for methodological quality were also evaluated (Figure 2; Table 2). Our meta-analysis comprised six RCTs, involving a total of 1,529 adult individuals. All the studies included in our analysis focused on adult populations.

3.2 Primary outcome: continuous infusion does not decrease the mortality rate

Figure 3 illustrates the inclusion of six RCT studies, demonstrating that there was no significant difference in mortality rates between the continuous infusion and intermittent bolus groups (odds ratio: 0.844, 95% CI: 0.671–1.061, $P = 0.147$, $I^2 = 0.0\%$). To ensure the robustness of our findings, we performed a sensitivity analysis by removing one study, and the results remained unchanged (Figure 4). The presence of publication bias was examined using a funnel plot, as depicted in Figure 5 (Egger's $g = 0.14014$).

Furthermore, we performed subgroup analyses depending on whether continuous infusion of other beta-lactam agents was included or not (Supplementary Figure S1). There was no statistically significant impact observed among the two subgroups regarding the continuous infusion of different antimicrobial agents. Additionally, we examined mortality rates within subgroups categorized by the meropenem dose (standard dose: 3 g/day, high dose: 4 g/day or more, adjusted according to eGFR). The results also indicated no decreasing mortality rates when continuous infusion was performed in the two subgroups (Figure 6). We further analyzed the dosage-dependent linear relationship between dosage and mortality rates, and the results showed no association (coefficient: −0.0536, $P = 0.1534$) (Figure 7).

3.3 Secondary outcomes: clinical success/improvement, bacteria eradication, length of stay in ICU (ICU LOS), ICU mortality, and adverse effects

Figure 8 presents the clinical success outcome when comparing continuous infusion and intermittent bolus strategies. The results suggest that continuous infusion of meropenem significantly improves bacterial eradication rates (Figure 9) (odds ratio: 2.207, 95% CI: 1.467–3.320, $P < 0.001$, $I^2 = 0.0\%$). However, it does not reduce ICU LOS (Supplementary Figure S5) (odds ratio: 0.978, 95% CI: 0.647–1.478, $P = 0.916$, $I^2 = 0.0\%$) and ICU mortality (Supplementary Figure S7) (odds ratio: 0.825, 95% CI: 0.588–1.157, $P = 0.265$, $I^2 = 0.0\%$). We conducted a sensitivity test

TABLE 1 A summary of the RCTs investigating the continuous infusion and intermittent bolus strategies among the enrolled participants.

First author (year)	Country	Population	Sample size	Age (CI/BI)	APACHEII score (CI/BI)	Antibiotics	Meropenem Regimen	Resistant pathogen (%)	Study design	Infection type CI/BI (%)	Pathogen CI/BI (%)	Sensitivity analysis	SAE associated with study withdrawal
Chytra (2012)	Czech	ICU	• CI: 120 • BI: 120	• 44.9 ± 17.8 • 47.2 ± 16.3	• 21.4 ± 7.9 • 22.1 ± 8.79	Meropenem	• CI: LD:2 g; MD:4 g QD • BI: 2 g Q8H	11.67[c]	RCT, open-label	• Respiratory: 55.0/50.8 • Abdominal: 19.2/25.8 • Uroinfection:9.2/5.0 • Bloodstream:8.3/9.2 • Soft tissue:4.2/5.0 • CNS:2.5/1.6 • Other:2.5/0.8	• *Klebsiella* spp.: 59.4/46.3 • *Acinetobacter* spp.: 7.5/11.1 • *Escherichia coli*: 5.7/8.3	MIC determination	Not happened in both groups
Dulhunty (2013)	• Australia • Hong Kong	5 ICUs	• CI:30 • BI:30	• 54 ± 19 • 60 ± 19	• 21 ± 8.6 • 23 ± 7.6	PTZ, Meropenem **Ticarcillin** • Clavulanate	• CI: 3 g QD • BI: 1 g Q8H	Not mention	RCT, double blind	• Respiratory: 36.8/43.2 • Bloodstream: 18.4/18.9 • Abdominal: 15.8/18.9 • Uroinfection: 7.9/5.4 • Soft tissue: 7.9/8.1 • CNS: 5.3/0 • Other: 2.6/0	Various	MIC determination	Not happened in both groups
Dulhunty (2015)	• Australia • Hong Kong • New Zealand	25 ICUs	• CI: 212 • BI: 220	• 64 (54–72) • 65 (53–72)	• 21 (17–26) • 20 (16–25)	PTZ, Meropenem Ticarcillin • Clavulanate	• [b]CI: 3 g QD • [b]BI: 1 g Q8H	Not mention	RCT, double blind	• Respiratory: 54.2/54.5 • Abdominal: 25.0/25.9 • Bloodstream: 8.0/8.2 • Uroinfection: 7.5/8.2 • Soft tissue: 6.1/8.2 • Other: 20.0/11.9	• Gram-positive: 27.5/25.6 • Gram-negative: 72.5/72.1	Not specified	Not happened in both groups
Abdual (2016)	Malaysian	2 ICUs	• CI: 70 • BI: 70	• 54 (42–63) • 56 (41–68)	• 21 (17–26) • 21 (15–26)	PTZ, Meropenem • Cefepime	• CI: LD: 1 g; MD: 3 g QD • BI: 1 g Q8H	Not mention	RCT, open-label	• Respiratory: 66/51 • Abdominal: 16/21 • Bloodstream: 6/9 • Uroinfection: 3/4 • Soft tissue: 9/10 • Other: 1/4	• Gram-positive: 20/33 • Gram-negative: 80/67	MIC determination.	Not happened in both groups
Zhao (2017)	China	ICU	• CI: 25 • BI: 25	• 68.0 ± 15.4 • 67.0 ± 12.2	• 19.4 ± 5.0 • 19.7 ± 5.9	Meropenem	• CI: LD:0.5 g; MD: 3 g QD • BI: LD: 1.5 g; MD: 1 g Q8H	31.81[c]	RCT	• Respiratory: 36.0/40.0 • Abdominal: 56.0/52.0 • Bloodstream: 20.0/12.0 • Uroinfection: 4/8 • Soft tissue: 4/0 • Other: 0/4	• *Escherichia coli*: 26% • *Pseudomonas aeruginosa*: 24% • *Klebsiella* spp.: 16% • *Acinetobacter* spp.: 12% • *Enterobacter* spp.: 4% • *Providencia* spp.: 2% • *Burkholderia*: 2%	MIC determination	Not mention
Giacomo (2023)	• Croatia • Italy • Kazakhstan • Russia	31 ICUs	• CI: 303 • BI: 304	• 65.5 (14.0) • 63.4 (15.0)	• [a]44 (35–55) • [a]43 (34–53)	Meropenem	• CI: 3 g QD • BI: 1 g Q8H	34.14[d]	RCT, double blind	• Respiratory: 33/33 • Abdominal: 9.6/8.1 • Bloodstream: 9.6/5.1 • Uroinfection: 5.5/4.1 • Other: 11/12	• Gram-positive: 116/103 • Gram-negative: 246/222 • *Klebsiella* spp: 72/59 • *Pseudomonas* spp: 48/44 • *Escherichia coli*: 44/44 • *Acinetobacter* spp: 28/22 • *Enterobacter* spp: 13/15 • Other: 41/38	MIC determination	Not happened in both groups

ICU, intensive care unit; CI, continuous infusion; BI, intermittent bolus; LD, loading dose; MD, maintaining dose; RCT, randomized controlled trial; CNS, Central nervous system; SAE, serious adverse effect; MIC, minimal inhibition concentration; APCHE II, Acute Physiology and Chronic Health Evaluation II score; PTZ, piperacillin-tazobactam.

[a]Simplified acute physiology score II, calculated based on the patient characteristics, reason for intensive care admission, and physiological abnormalities. The core range is from 0 to 163; a higher score indicates a higher severity of disease and a higher risk of death.

[b]The median 24-h dose was 3 g.

[c]Definite as pathogen MIC to meropenem $\geq$ 1.5.

[d]Definite as pathogen susceptibility test showed resistance to carbapenem.

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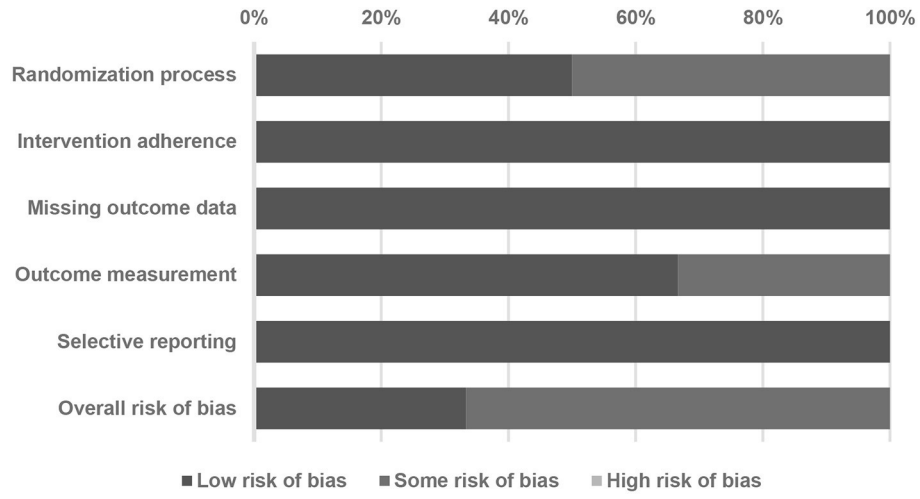

FIGURE 2
A summary of quality assessment of studies included in the meta-analysis using the Cochrane risk-of-bias 2 tool.

TABLE 2 Detailed quality assessment of included studies using the Cochrane risk-of-bias 2 tool.

First Author	Year	Randomization process	Intervention adherence	Missing outcome data	Outcome measurement	Selective reporting	Overall RoB
Chytra	2012	S[a]	L	L	S[b]	L	S
Dulhunty	2013	S[a]	L	L	L	L	S
Dulhunty	2015	S[a]	L	L	L	L	S
Abdual	2016	L	L	L	S[b]	L	S
Zhao	2017	L	L	L	L	L	L
Giacomo	2023	L	L	L	L	L	L

[a]The studies did not provide allocation concealment details.
[b]The study was an open-label study.

(Supplementary Figures S3, S4; Figures 7, 9) and examined the publication bias using a funnel plot (Supplementary Figures S10–S13). The sensitivity test yielded consistent results, and no significant publication bias was detected.

However, when analyzing the subgroup of meropenem alone and meropenem with other beta-lactam agents, the subgroup analysis of meropenem alone demonstrated a significant improvement in clinical success when continuous infusion was performed (Supplementary Figure S2) (odds ratio: 1.776, 95% CI: 1.051–2.999, p-value: 0.032).

Regarding adverse effects, five studies reported no treatment-associated adverse events during the study period in both the continuous and intermittent bolus groups. However, in the Dulhunty 2015 study, four adverse events were reported during the study period. Our analysis indicated no significant difference between continuous infusion and intermittent bolus administration in adverse events (Figure 10) (odds ratio: 1.012, 95% CI: 0.174–5.892, p-value: 0.989, $I^2 = 0.0\%$). The sensitivity test and funnel plot are shown in Supplementary Figure S9 (Egger's $g =$ 0.25718). The examination showed no significant publication bias (Supplementary Figure S14).

3.4 Secondary outcomes: the relationship between mortality/bacteria eradication and resistant pathogens when continuous infusion was performed

To further understand the impact on mortality and bacteria eradicated when continuous infusion of resistant pathogens was performed, we tested the meta-regression between the percentage of resistant pathogens and mortality or bacteria eradicated. The results showed that there was no significantly decreasing mortality when the cultures contained more resistant pathogens (coefficient: −0.3761, $P = 0.4427$) (Figure 11). However, the bacteria eradicated were significantly more resistant to pathogens when continuous infusion was performed (coefficient: 2.5175, $P =$ 0.0138*) (Figure 12).

4 Discussion

In our meta-analysis study, there was no significant difference in the mortality rate, ICU LOS, ICU mortality, or adverse

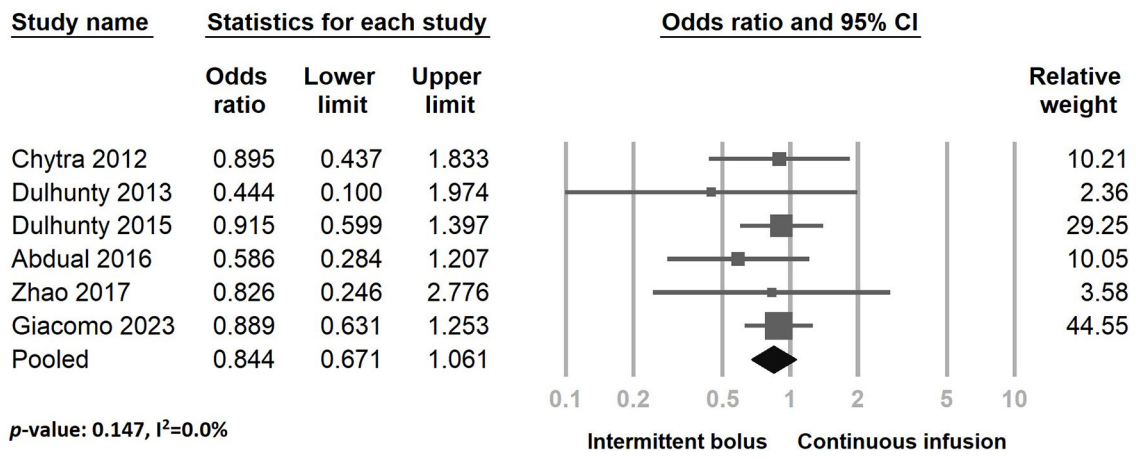

FIGURE 3
The forest plot of continuous infusion and intermittent bolus with mortality.

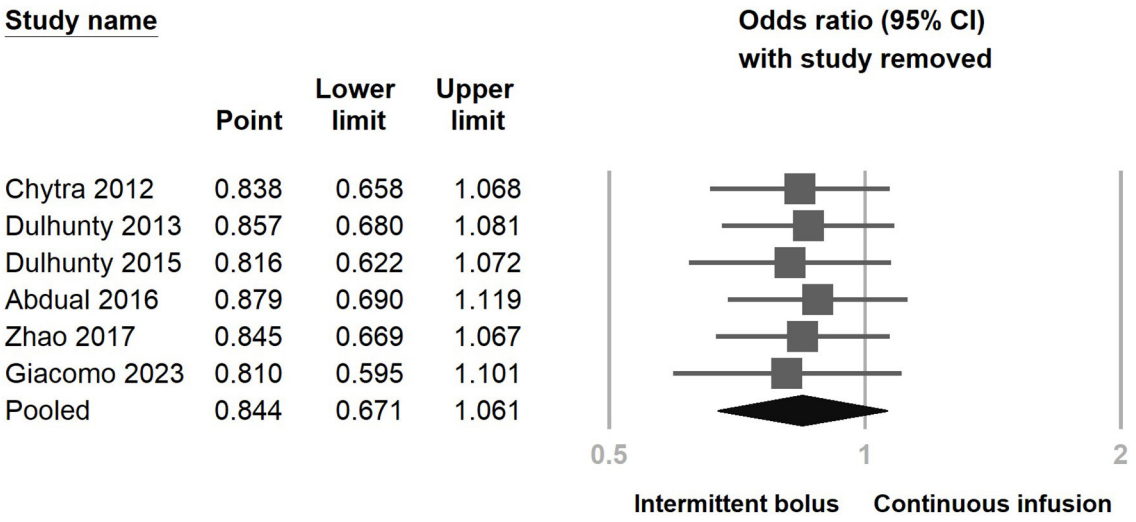

FIGURE 4
Sensitivity analysis of continuous infusion and intermittent bolus with mortality utilizing the one-study removal method.

effects observed between continuous infusion and intermittent bolus administration of meropenem. However, the adoption of continuous infusion of meropenem resulted in a significant increase in both clinical success/improvement and bacterial eradication rates. The most interesting finding, meta-regression, showed that with more resistant pathogens, the continuous infusion could perform better bacterial eradicated effects.

Extended and continuous infusion strategies have gained wide acceptance in the administration of beta-lactam antibiotics due to their potent stable bactericidal effects (Yang et al., 2015). The effectiveness of bacterial eradication in beta-lactams is positively correlated with the duration of time that the drug concentration remains above the MIC. This is commonly measured as the percentage of the dosing interval during which the concentration of free drug exceeds the MIC, known as %fT > MIC (Berry and Kuti, 2022). Theoretically, the maximum bactericidal effect is achieved when the free drug concentration exceeds the pathogen's MIC. Carbapenem, being a broad-spectrum beta-lactam antibiotic used for treating multidrug-resistant pathogens, especially meropenem, has been widely considered for continuous infusion (Benavent et al., 2023). To optimize the antibacterial effect, the percentage of T > MIC should be higher than 30–50% between dosing intervals, depending on the bacterial species. Escalating the dose of meropenem or continuous infusion has been adopted to improve the %fT > MIC. However, despite the potential pharmacokinetics (PK) and pharmacodynamics (PD) advantages, it is unclear if improving %fT > MIC is associated with better clinical outcomes.

It was interesting to discover that, in contrast to previous studies, continuous infusion of beta-lactam antibiotics, particularly piperacillin-tazobactam, exhibited significantly superior efficacy (Hyun et al., 2022). When we conducted an independent analysis of continuous infusion of meropenem in a randomized

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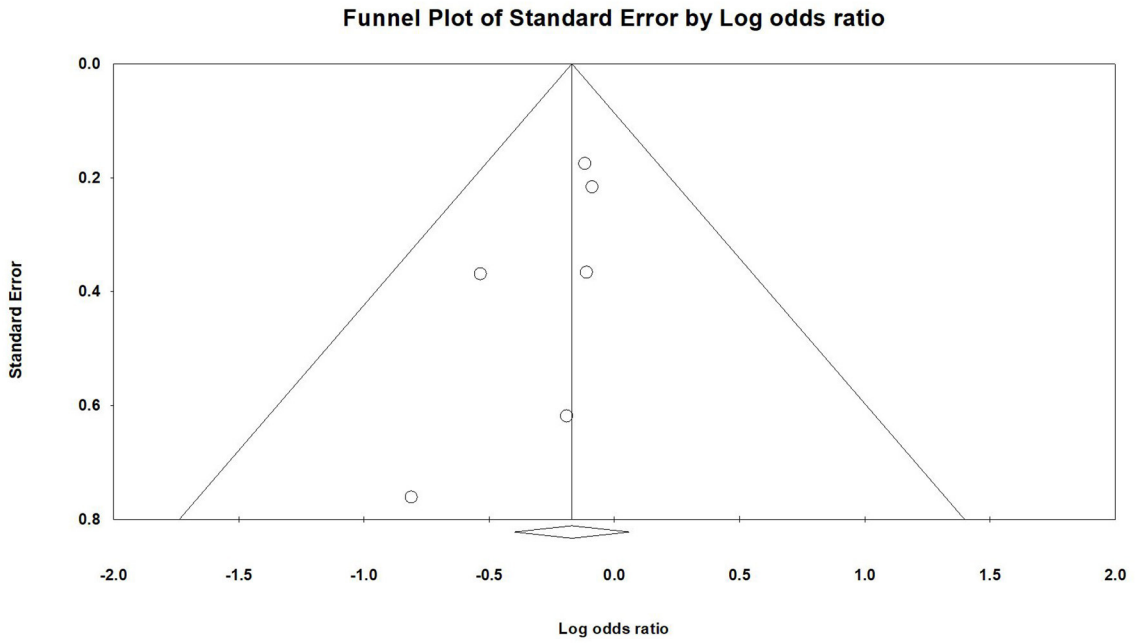

FIGURE 5
The funnel plot of continuous infusion and intermittent bolus with mortality.

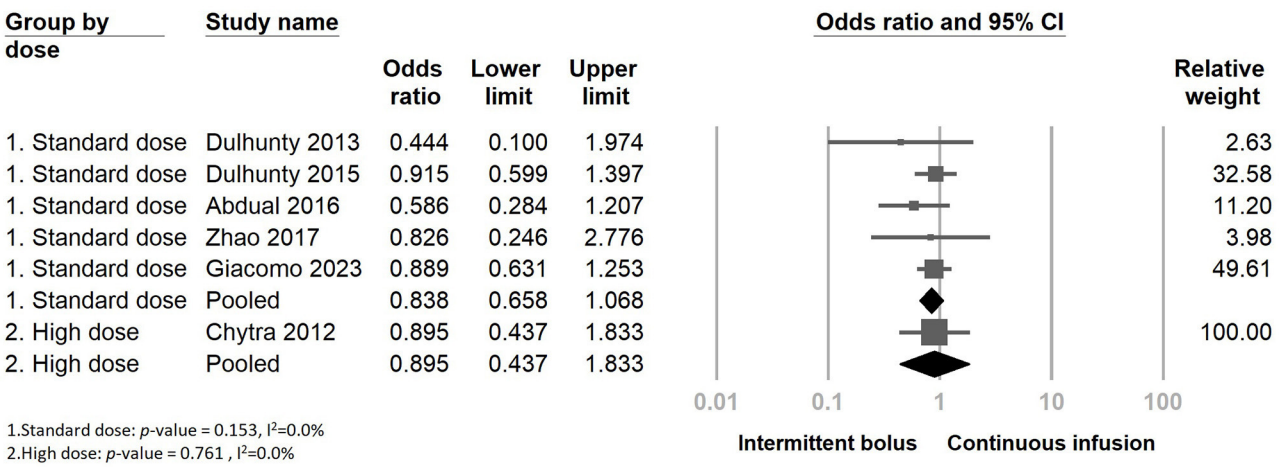

FIGURE 6
The forest plot of continuous infusion and intermittent bolus with mortality in the subgroup.

control study, there was no significant difference observed in the mortality rates. To further investigate whether these results were influenced by varying daily dosages, we conducted subgroup analyses. Nonetheless, the mortality rate showed no significant difference between continuous infusion and intermittent bolus administration, both in the standard and high-dose groups. The mortality rate was not correlated with the dosage of meropenem.

Reflecting on previous studies regarding continuous infusion of meropenem, it can be observed that, while studies have shown clinical success or improvement with continuous infusion, they did not demonstrate reduction in mortality rates (Helmy et al., 2015). In a prospective randomized pilot study by Zhao in 2017, the author also examined PK data comparing continuous infusion and intermittent bolus administration. The results indicated that the trough concentration of meropenem in the continuous infusion group was 10-fold higher than that in the intermittent group for the first and third doses. Additionally, the concentration during ~40% of the dosing interval was twice as high in the continuous infusion group. These findings suggested that continuous infusion maintained a more stable therapeutic level during meropenem treatment. However, these effects did not correlate positively with the mortality rate of patients. Zhao's study demonstrated no

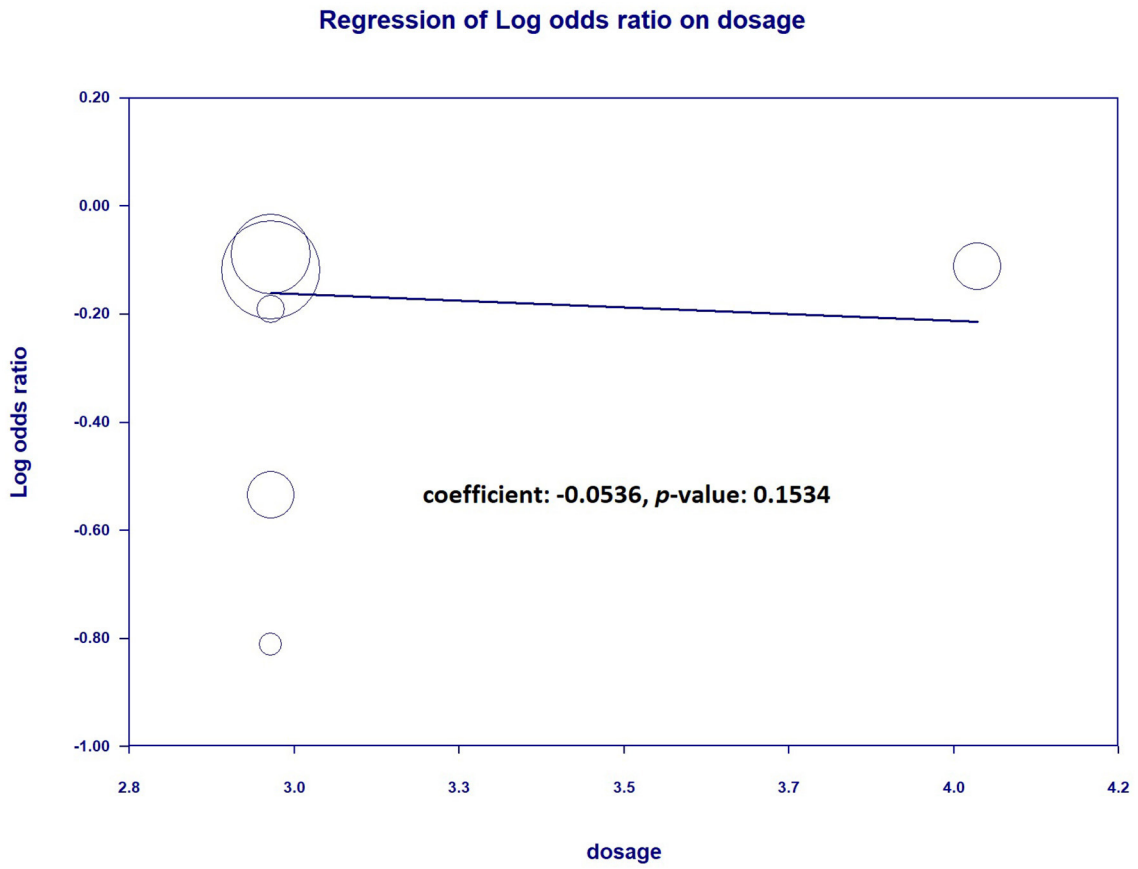

FIGURE 7
Regression between continuous infusion dosage and mortality.

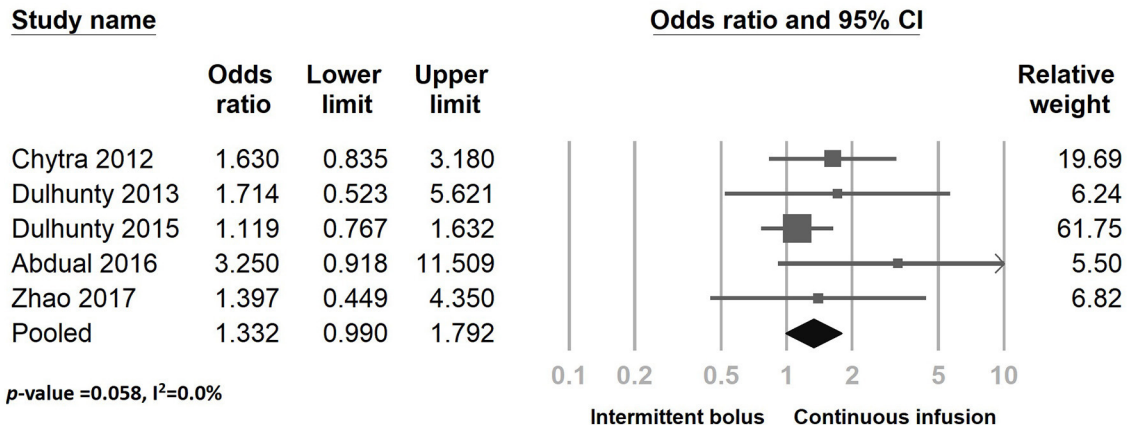

FIGURE 8
The forest plot of continuous infusion and intermittent bolus with clinical success.

significant difference in 28-day mortality rates between the two groups, as did our meta-analysis.

Previous studies have hypothesized that continuous infusion may offer benefits and increased potency against resistant pathogens. One of the studies described the rationale, principles, and dosage calculations for continuous infusion beta-lactam antibiotics, particularly focusing on their role in treating multidrug-resistant bacterial infections in patients undergoing continuous veno-venous hemofiltration (CVVH). The authors concluded that continuous infusion of beta-lactam antibiotics could be an effective treatment strategy for multidrug-resistant gram-negative bacteria infections in intensive care settings (Moriyama et al., 2009).

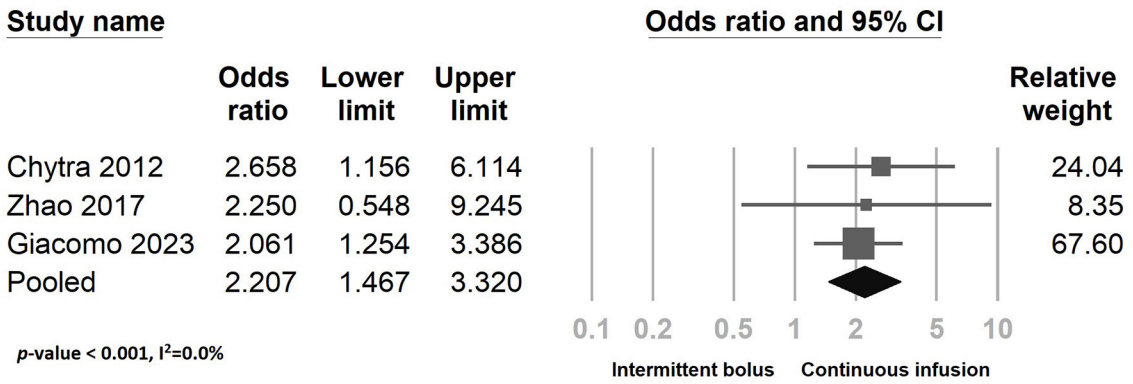

FIGURE 9
The forest plot of continuous infusion and intermittent bolus with bacteria eradication.

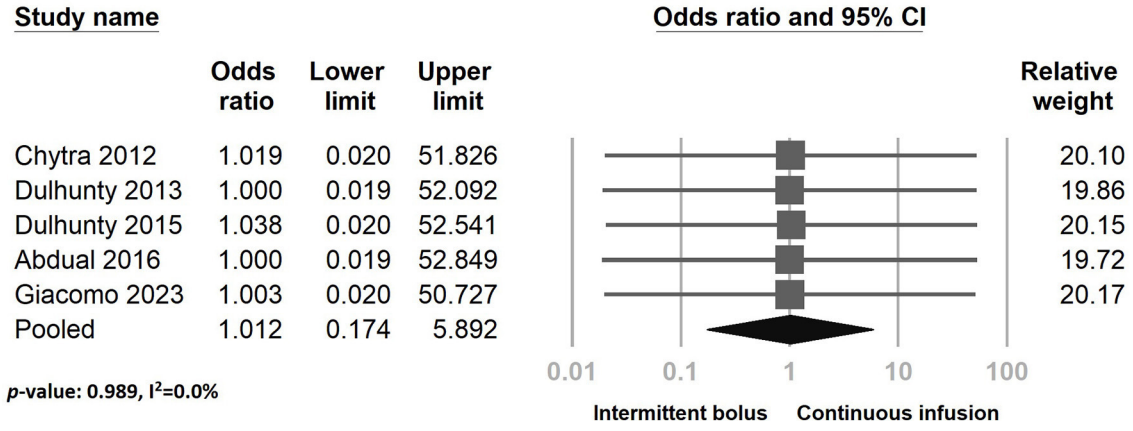

FIGURE 10
The forest plot of continuous infusion and intermittent bolus with serious adverse effects.

Truong's 2022 study also investigated the impact of continuous meropenem infusion on resistant *Klebsiella pneumoniae* strains. This study utilizes PK data from ICU patients and collected *Klebsiella pneumoniae* isolates to develop feasible meropenem dosing regimens for treating infections caused by resistant *Klebsiella pneumoniae* strains (Truong et al., 2022). In a multicenter randomized controlled study by Dulhunty in 2015, similar results to Zhao's study were obtained, with no significant difference in the long-term 90-day mortality rate between the continuous infusion and intermittent bolus groups. However, upon closer observation of this study, it was found that only 2.5 vs. 14.0% of the pathogens in both the intervention and control groups were resistant pathogens. In another randomized open-label controlled trial by Chytra in 2012, which included a population with only 14.6% cultured-resistant pathogens, no significant difference in the mortality rate was also observed. Both RCTs indicate that continuous infusion may not be necessary for pathogens susceptible to the antibiotic, even in cases of severe infection with high APACHE II scores and ICU stays. To figure out the benefit subgroup of continuous infusion of meropenem, we performed meta-regression to find out the relationship between resistant pathogens and mortality or the bacterial eradication effect. The results showed that, when more resistant pathogens were included in the study, better bacterial eradication was found. It suggests that continuous infusion of meropenem may be beneficial in aiding the eradication of resistant pathogens. We postulated that, when treating wild-type pathogens, the MIC of meropenem is relatively low as compared to the drug-resistant isolates. Therefore, both the intermittent bolus and continuous infusion groups can reach the PK/PD target of %fT > MIC larger than 40%, which leads to no obvious clinical benefits in the continuous infusion group. However, for more drug-tolerant or drug-resistant strains, the traditional intermittent bolus group may not reach the PK target in some patients, while the continuous infusion group can maintain a more stable drug level above the MIC (Truong et al., 2022). Therefore, a trend toward improved clinical success rates and statistically improved microbial eradication rates could be observed in the drug-resistant pathogen subgroup analysis. In our future study, we plan to gather clinical data from patients who have been infected with resistant bacteria and treated with continuous infusion of meropenem. We will then use these data to analyze and develop a PK and PD model, aiming to understand

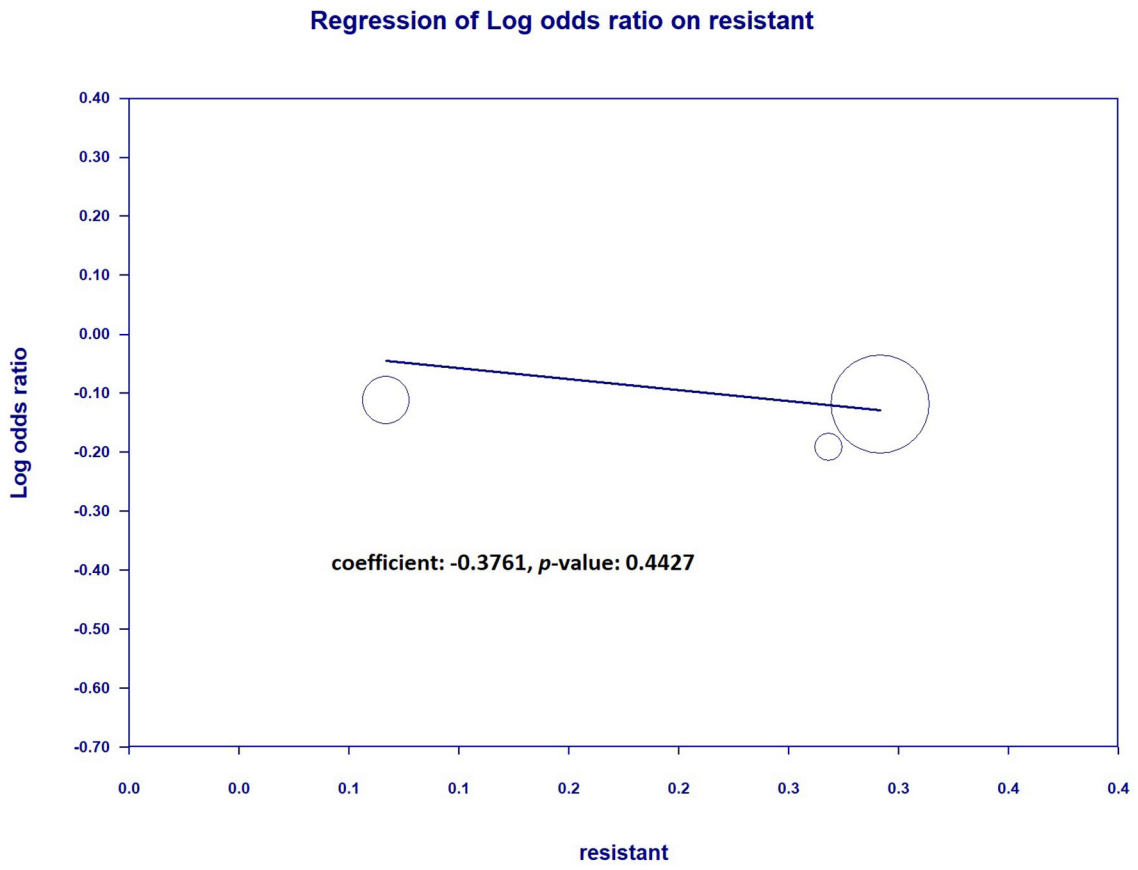

FIGURE 11
Regression between resistant pathogen (%) and mortality.

the relationship between %fT > MIC and the outcomes of the treatment.

Another hypothesis suggesting an advantage of continuous infusion for beta-lactam antibiotics is the provision of a stable therapeutic level, which may help prevent the emergence of resistant pathogens (Buck et al., 2005). Extended and continuous infusion methods offer less time below the MIC, which is believed to be a period during which bacteria can re-grow. Continuous infusion ensures that the serum drug concentration remains consistently above the MIC (Craig, 2003). Rapid bacterial killing can also reduce the chances of pathogens acquiring new genetic elements from one another. In previous *in vitro* studies, maintaining a stable antibiotic level has been shown to prevent bacterial growth and resistance (Li et al., 2014). However, further studies are needed to provide more evidence for the benefits of this theory. In the Giacomo 2023 study, the primary outcome aimed to examine the emergence of resistant pathogens (defined as pan-drug resistant or resistant to all but one or two drug classes) between continuous infusion and intermittent bolus infusion groups. The results revealed no significant difference between the two groups. Although *in vitro* studies hypothesize that a stable drug concentration might reduce the likelihood of pathogens developing resistance, the same result was not confirmed in the *in vivo* study.

Safety concerns associated with continuous infusion method have always been a prominent issue. Extended and continuous infusion strategy have the potential to maintain stable drug levels in the bloodstream and tissues, which could alleviate concerns regarding drug toxicity compared to intermittent dosing (Manning et al., 2014). In our meta-analysis study, which comprised five studies, none of the studies reported serious adverse events occurring in either the continuous infusion or intermittent bolus groups. In general, beta-lactam antibiotics are well-tolerated compared to other classes of antibiotics, and it appears that continuous infusion strategies demonstrate a similar level of safety as traditional intermittent bolus strategies (Chiriac et al., 2017). In our meta-analysis study, no significant difference was observed in the occurrence of adverse events between continuous infusion and intermittent bolus administration of meropenem.

Our study has several limitations. First, all the included studies allowed for the use of additional therapeutic antibiotics based on the patients' condition, which introduces bias to the assessment of patient mortality. Second, the small sample sizes and variations in the loading dose of meropenem among different RCTs could also impact the mortality rates observed. Third, the definitions and criteria for clinical success and improvement varied across the studies, which could contribute to differences in the study outcomes. Finally, most of the mortality rates were analyzed at 28 days, but two studies measured the mortality rates at 90 days, which could also affect the overall mortality rate results.

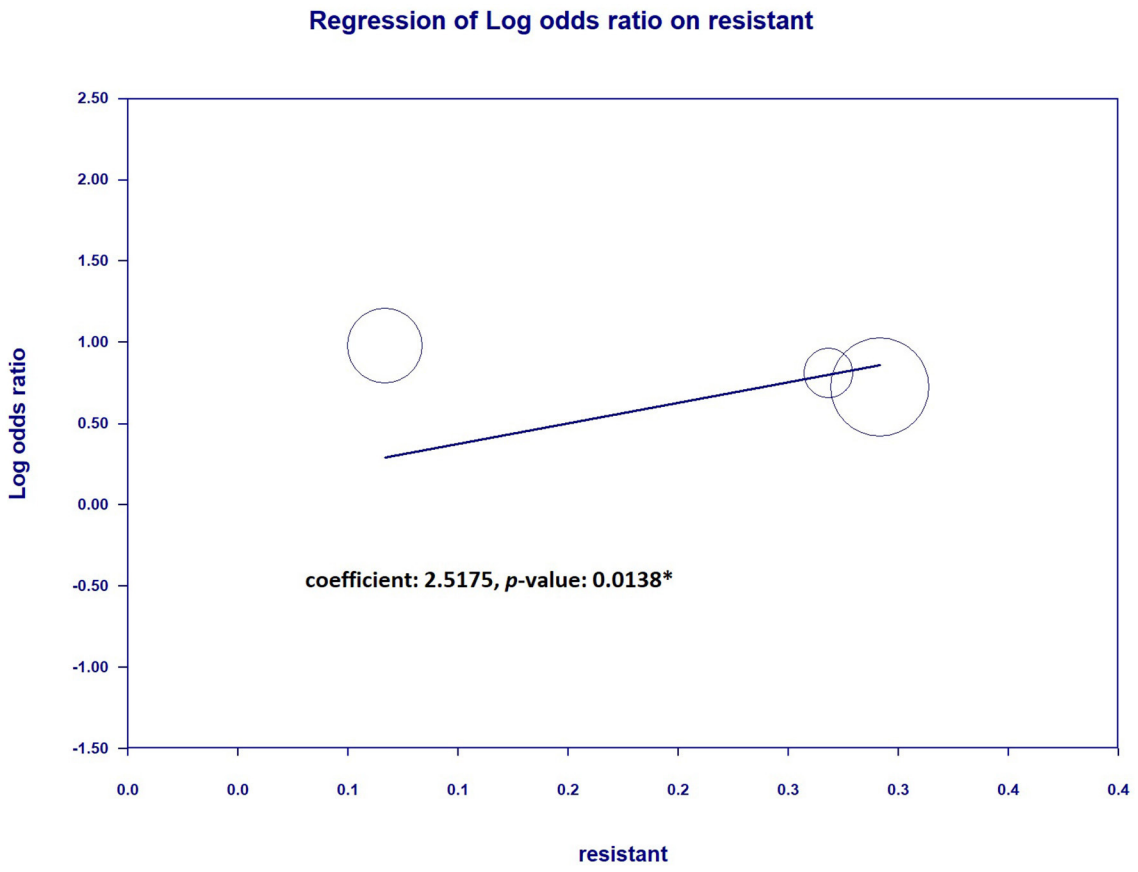

FIGURE 12
Regression between resistant pathogen (%) and bacterial eradication.

5 Conclusion

In our meta-analysis study, we found no significant difference in the mortality rate, ICU LOS, ICU mortality, or adverse events between continuous infusion and traditional intermittent bolus strategies of meropenem. Despite using a high dose (4 g) of meropenem through continuous infusion, there was no decrease in mortality rates compared to intermittent bolus administration. However, our study found that continuous infusion of meropenem may lead to better bacterial eradication effects, especially in resistant pathogens. We observed enhanced microbiological eradication rates when utilizing continuous infusions of meropenem for managing infections, particularly those caused by drug-resistant pathogens. There was also a trend indicating improved clinical success rates with this approach. Thus, continuous infusion of meropenem may offer a safe and a potentially effective treatment strategy for patients with drug-resistant infections.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

M-YA: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – original draft. W-LC: Investigation, Methodology, Writing – original draft. C-YL: Conceptualization, Formal analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors would like to express our gratitude to Sheng-Feng Lin for their valuable and insightful discussions. Their input and expertise significantly contributed to the development and quality of this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1337570/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Methods
PUBLISHED 13 March 2024
DOI 10.3389/fmicb.2024.1279186

OPEN ACCESS

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RECEIVED 17 August 2023
ACCEPTED 26 February 2024
PUBLISHED 13 March 2024

CITATION
Gong X, Yang G, Liu W, Wu D, Duan C, Jia X, Li Z, Zou X, Yu R, Zou D and Wang Y (2024) A multiplex TaqMan real-time PCR assays for the rapid detection of mobile colistin resistance (*mcr-1* to *mcr-10*) genes. *Front. Microbiol.* 15:1279186. doi: 10.3389/fmicb.2024.1279186

A multiplex TaqMan real-time PCR assays for the rapid detection of mobile colistin resistance (*mcr-1* to *mcr-10*) genes

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Objective: Recently, 10 plasmid-mediated mobile colistin resistance genes, *mcr-1* to *mcr-10*, and their variants have been identified, posing a new threat to the treatment of clinical infections caused by Gram-negative bacteria. Our objective was to develop a rapid, sensitive, and accurate molecular assay for detecting *mcr* genes in clinical isolates.

Methods: The primers and corresponding TaqMan-MGB probes were designed based on the sequence characteristics of all reported MCR family genes, multiplex Taqman-MGB probe-based qPCR assays were developed and optimized, and the sensitivity, specificity and reproducibility of the method were evaluated. The assay contained 8 sets of primers and probes in 4 reaction tubes, each containing 2 sets of primers and probes.

Results: The standard curves for both the single and multiplex systems showed good linearity ($R^2 > 0.99$) between the starting template amount and the Ct value, with a lower limit of detection of 10^2 copies/μL. The specificity test showed positive amplification results only for strains containing the *mcr* genes, whereas the other strains were negative. The results of intra-and inter-group repeatability experiments demonstrated the stability and reliability of the newly developed method. It was used to detect *mcr* genes in 467 clinically-obtained Gram-negative isolates, which were multidrug-resistant. Twelve strains containing the *mcr* genes were detected (seven isolates carrying *mcr-1*, four isolates carrying *mcr-10*, and one isolate carrying *mcr-9*). The products amplified by the full-length PCR primer were identified by sequencing, and the results were consistent with those of the multiplex qPCR method.

Conclusion: The assay developed in this study has the advantages of high specificity, sensitivity, and reproducibility. It can be used to specifically detect drug-resistant clinical isolates carrying the *mcr* genes (*mcr-1 to mcr-10*), thus providing a better basis for clinical drug treatment and drug resistance research.

KEYWORDS

colistin, the *mcr* genes, multiplex TaqMan real-time PCR, rapid detection, multidrug-resistant

1 Introduction

The growing problem of bacterial drug resistance poses a serious threat to public health, especially with the emergence of multidrug-resistant (MDR) organisms posing challenges for the treatment of nosocomial infections (Lancet, 2022). Polymyxin, a peptide antibiotic, was withdrawn from clinical use in the 1980s because of its side effects which included nephrotoxicity and neurotoxicity. The emergence of MDR organisms and the lack of new antibiotics have led to the reintroduction of polymyxins as the "last resort "for treating infections caused by MDR gram-negative bacteria (Trimble et al., 2016; Soman et al., 2021).

In November 2015, the first plasmid-mediated colistin resistance gene, *mcr-1*, was found in *Escherichia coli* isolated from a pig farm in China (Liu et al., 2016). Studies have shown that the *mcr-1* gene could be detected in patients, animals, food, and the environment (Liu et al., 2023; Shahzad et al., 2023). Plasmids carrying the *mcr-1* gene have conjugation and transfer abilities, contributing to the stability and persistence of colistin resistance (Liu et al., 2016). Subsequently, the *mcr-1* gene and its variants have been reported in many countries, with 113 variants identified in 10 families of the *mcr* gene. The coexistence of the *mcr* genes and other drug-resistance genes increases the likelihood of the emergence of pan-drug-resistant superbugs (Karim et al., 2023; Zhang et al., 2023). The prevalence of multidrug resistance can lead to an increased rate of hospital-acquired infections with limited treatment options while increasing the length of hospital stays, mortality, and costs (Strich and Palmore, 2017; Manandhar et al., 2022).

To further regulate the rational use of antibiotics and prevent the emergence and widespread occurrence of drug resistance, it is essential to rapidly detect bacteria carrying the *mcr* genes and provide a basis for monitoring and clinical drug treatment. The TaqMan minor groove binder (MGB) probe fluorescence technique, is a quantitative real-time polymerase chain reaction (qPCR) approach that is currently the most rapid and reproducible assay for the quantitative and qualitative detection of nucleic acid molecules; it provides faster results than conventional PCR methods and often without the use of high-risk reagents (Kutyavin et al., 2000). The TaqMan MGB qPCR method is widely used in areas such as transgenic and gene expression studies, and for the detection of infectious and genetic diseases (Wang et al., 2021; Jin et al., 2022). To effectively detect isolates carrying *mcr* genes, we compared all MCR family genes in the database, designed seven sets of primers and TaqMan-MGB probes based on the sequence comparison results, and introduced the primer and probe sets of 16S rRNA as an internal control for amplification (Center for Disease Control and Prevention, 2011). A multiplex probe-based qPCR assay was developed and optimized to detect all MCR family genes in four reaction tubes, which was evaluated on a sample set of 467 multidrug-resistant clinical isolates.

2 Materials and methods

2.1 Bacterial strains

Eight clinical multidrug-resistant isolates with whole gene sequencing were selected for the specific experiment, and information on the resistance genes of the isolates was obtained with good representativeness (see Table 1 for detailed information on the isolates and drug resistance). In addition, 467 clinically multidrug-resistant isolates, collected previously, were used for the overall evaluation of the assay.

2.2 Design and synthesis of primers and probes

All available MCR family genes were downloaded from the Reference Gene Catalog of the National Center for Biotechnology Information (NCBI), including *mcr-1* to *mcr-10* genes and their variants. Partial sequences were compared again using the CLC sequence viewer 8 (Qiagen Aarhus, Denmark) based on the published phylogenetic tree results of MCR family genes constructed according to the maximum likelihood ratio (Ling et al., 2020). Conserved regions with no mutation points were selected, and standard primers and Taqman-MGB probes were designed using Primer Express 3.0.1 software according to the principles of multi-PCR primer design (Hawkins and Guest, 2017). Primers were designed for maximum coverage of *mcr* gene variants and using degenerate bases if necessary. The primer set of 16S rRNA was used as an internal control for amplification. Primer-Blast was used to evaluate the specificity of the primers and AutoDimer software was used to assess primer-dimer production between primer groups (Vallone and Butler, 2004). Multiple PCR was developed by mixing primer groups according to the evaluation results. All primers were synthesized by Beijing Tsingke Biotech Co., Ltd. (Beijing, China).

2.3 Optimization of the reaction system of multiplex real-time PCR

Before mixing the primers in the multiplex PCR reaction system, each primer group (including the probe) was individually optimized for maximal amplification efficiency. Using the recombinant plasmid as the template, a 25 μL reaction system was developed, and the primer concentration (100–500 nmol/L), TaqMan probe concentration (50–500 nmol/L), and annealing temperature (56.6–62.6°C) were optimized individually. Three replicates were used for each experiment. The optimal concentrations of primers and probes were selected according to the Ct values and fluorescence signal intensity of the amplification curves to ensure that the amplification efficiency of all targets was between 90 and 110%, with an R^2 value of ≥0.985. Finally, a multiplex PCR reaction optimization system was developed, and that showed similar Ct values and amplification efficiencies compared to those for single-duplex PCR. The instrument used for the experiments was a CFX 96 Connect Real-Time PCR Detection System (Bio-Rad, United States), and the reagents used were Premix Ex Taq™ (Probe qPCR) purchased from Takara Biomedical Technology Co., Ltd. (Beijing, China).

2.4 Standard curves

After synthesizing the amplified target sequence, it was cloned into the pUC57 recombinant plasmid and identified by sequencing as a positive standard. The concentration of the recombinant plasmid

TABLE 1 Information on clinical multidrug-resistant isolates for specific experiments.

No.	Species	Source	Drug-resistant genes
1	*Pseudomonas aeruginosa* N12122	Clinical isolate	bla_{OXA-50}, bla_{VIM-2}, bla_{PAO}, *aph(3′)-IIb*, *aac*A4, *aph(6)-Id*, *str*A, *aac(6′)-Ib-cr*, *qnr*VC1, *fos*A
2	*Klebsiella pneumoniae* N12106	Clinical isolate	bla_{SHV-2}, *aac(6')Ib-cr*, *aac(3)-IId*, *aad*A16, *qnr*B6, *fos*A, *arr-3*, *sul*1, *tet*(D)
3	*Acinetobacter baumannii* N12118	Clinical isolate	bla_{OXA-23}, bla_{OXA-66}, *Amp*C-β-lactamase, bla_{TEM-1B}, *arm*A, *aph(6)-Id*, *aph(3″)-Ib*, *mph*(E), *tet*(B), *sul*1
4	*Serratia marcescens* N12145	Clinical isolate	bla_{KPC-2}, $bla_{CTX-M-14}$, bla_{SRT-1}, $bla_{CTX-M-3}$, *aac(3)-IId*, *aac(6′)-Ic*
5	*Escherichia coli* N12139	Clinical isolate	*aac(3)-IId*, *aph(3″)-Ib*, *mdf*(A), *mph*(A), *sul*1, *sul*2, *tet*(A), *dfr*A17
6	*Enterobacter cloacae* N12169	Clinical isolate	bla_{TEM-1B}, *oqxA*, *oqxB*, *mdf*(A), *fos*A, bla_{IMP-8}, *aac(6′)-Ib-cr*, $bla_{CTX-M-3}$
7	*Proteus mirabilis* N12160	Clinical isolate	bla_{TEM-1}, *aad*A2, *aad*A5, *aac(3)-IIb*, *aph(3′)-Ia*, *aph(3″)-IIb*, *aph(6)-Id*, *sul*1, *sul*2, *dfr*A17, *tet*(J), *cat*I
8	*Stenotrophomonas maltophilia* N12146	Clinical isolate	Sm*qnr*, bla_{L1}, *sul*1, *dfr*A12, *aad*A2, *aac(6′)-Ib-cr*

was determined and converted into copy numbers according to the following formula:

$$\text{Copy number} = \frac{\left(6.02\times10^{23}\right)\times\left(\frac{ng}{\mu l}\times10^{-9}\right)}{\left(\text{DNA length}\times660\right)}$$

The plasmids were 10-fold serially diluted from 10^9 copies/μL to 10^2 copies/μL. A 25 μL reaction system was set up according to the optimized reaction conditions with three replicate wells. The amplification efficiencies of the single and multiplex systems were also evaluated, and standard curves were generated.

2.5 Specificity

Eight clinical multidrug-resistant bacteria without the *mcr* genes were used for the experiments (see Table 1 for detailed information on the isolates and drug resistance). *Escherichia coli* containing the recombinant plasmid was used as a positive control. Nucleic acids were extracted using the Qiagen DNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The original nucleic acid solution was diluted 100-fold for amplification, and deionized water was used as no template control (NTC). All samples were analyzed using the multiplex fluorescence qPCR method.

2.6 Sensitivity

The plasmids were 10-fold serially diluted from 10^3 copies/μL to 10^1 copies/μL. A 25 μL reaction system was developed according to the optimized reaction conditions with three replicate wells, and negative controls. Means and standard deviations (SD) were calculated. The minimum copy concentration at which a Ct value occurs is usually considered the limit of detection.

2.7 Reproducibility

To evaluate the stability of the assay, five different concentrations of standards were prepared ranging from 10^7 copies/μL to 10^3 copies/μL. For intra-group repeatability tests, three wells were repeated for each dilution; for inter-group repeatability tests, three reactions were repeated 1 week apart. Standard deviation and coefficient of variation (CV) were calculated to analyze intra-and inter-group differences.

2.8 Clinical sample testing

Nucleic acids from 467 clinical multidrug-resistant isolates were extracted using the Qiagen DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted nucleic acids were diluted 100-fold and screened for *mcr-1* to *mcr-10* genes using the newly developed multiplex fluorescence qPCR method. For positive results, PCR amplification was performed using standard full-length primers, and the products were identified by sequencing. The results of both methods were analyzed to evaluate the usefulness of the new method.

3 Results

3.1 Specific primers and probes

The results of the phylogenetic tree of MCR family genes showed high levels of similarity between the *mcr-1*, *mcr-2,* and *mcr-6* genes and between the *mcr-9* and *mcr-10* genes. Therefore, we designed primers and probes targeting the amplification of *mcr-1/2/6*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-7*, *mcr-8*, and *mcr-9/10*, and introduced the primer set of 16S rRNA as the internal control for amplification. The results of sequence alignment are shown in Figure 1. Based on results of the AutoDimer Check software, the eight sets of primers and probes were divided into four tubes, each contained two sets of primers and probes. Sequence information and the grouping of the primers and probes are shown in Table 2. It is worth noting that there are two forward primers for amplifying the *mcr-1/2/6* gene, and some primers and probes contain mixed bases.

3.2 Optimization of the reaction system of multiplex qPCR

After optimization tests, optimal reaction conditions were obtained for the multiplex PCR system (25 μL): 12.5 μL Premix Ex Taq™ (Probe qPCR), 2 μL mixed standard primers (10 μmol/L),

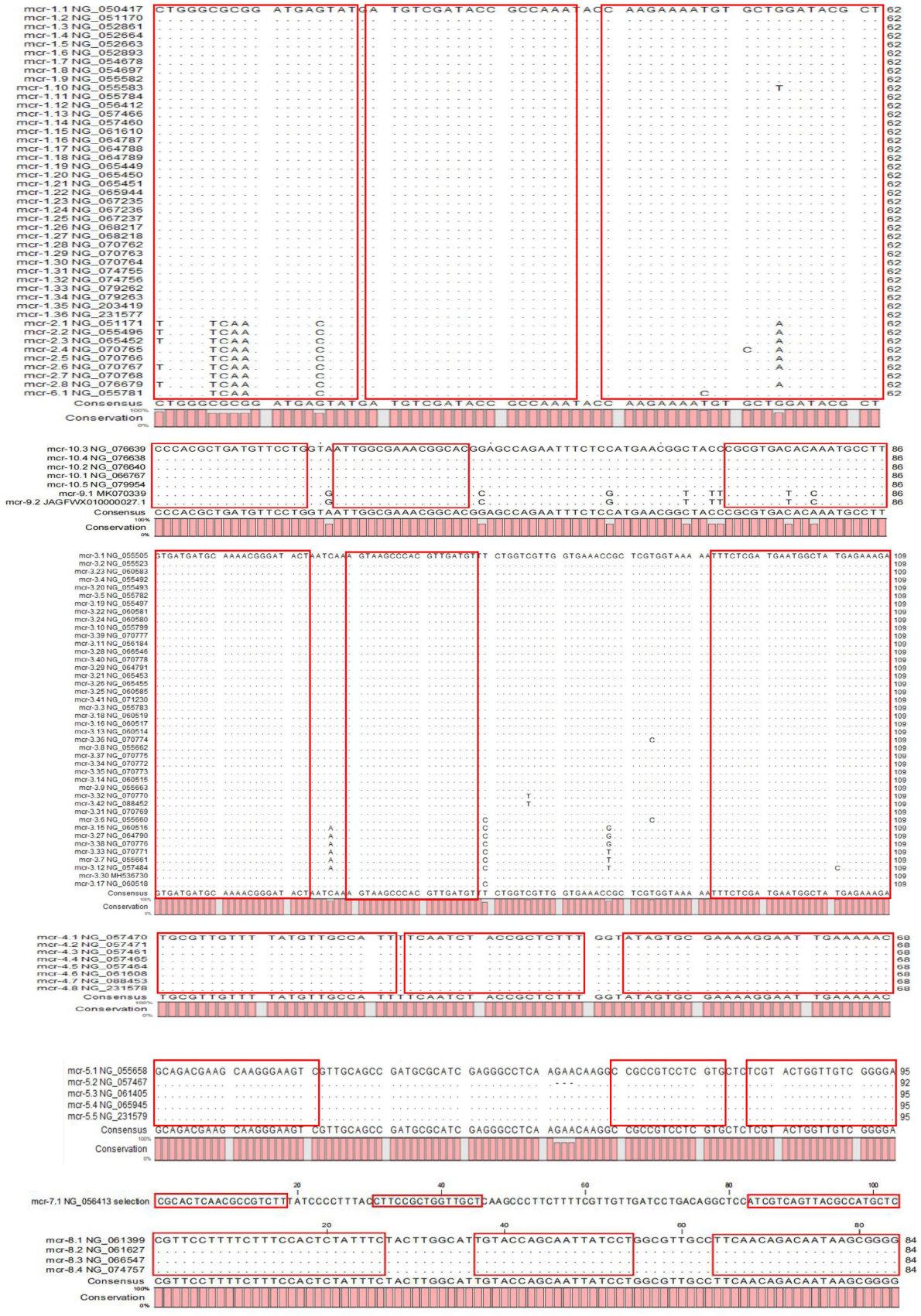

FIGURE 1
The results of sequence alignment.

0.25 μL FAM-labelled probe (10 μmol/L), 0.5 μL HEX-labelled probe (10 μmol/ L), 2 μL DNA template and the remaining volume was made up with deionized water. The optimized amplification program was 95°C for 30s, followed by 40 cycles of 95°C for 5 s and 57.8°C for 30s, with the fluorescent signal collected at 57.8°C. The groups were prepared by placing primer sets amplifying *mcr-1/2/6* and 16S rRNA, *mcr-3* and *mcr-9/10*, *mcr-4* and *mcr-7*, and *mcr-5* and *mcr-8* in the same reaction tube; the concentrations of the primers are detailed in Table 2. Positive (1×10^5 copies/μL plasmid standard) and no-template (water) controls were included in each plate.

3.3 Standard curve for single and multiplex qPCR

The amplification efficiency of primers and probes in single and multiple systems was evaluated, and the amplification and standard curves were plotted. Good linearity was observed between the starting template concentration and the Ct values for the eight sets of primers and probes in both the single and multiplex systems, with the correlation coefficient R^2 ranging from 0.9960–0.9997 and amplification efficiencies between 90 and 110%. The amplification efficiency of the multiplex qPCR assay is similar to that of a single qPCR assay, which meets the requirements. A standard curve is shown in Figure 2.

3.4 Specificity of the multiplex qPCR assay

After uploading the designed primers to the NCBI database for specific comparison, we confirmed that the primers can only amplify genes related to *mcr*. The genomic DNA of *Escherichia coli* containing the recombinant plasmid of the *mcr* gene and eight clinical multidrug-resistant isolates (see Table 1) were used as templates to verify the specificity of the developed multiplex qPCR assay. The results showed that only nucleic acid samples from *Escherichia coli* containing recombinant plasmids were positive, while nucleic acid samples from other clinical isolates and negative controls were negative, indicating good specificity of the assay. The amplification results are presented in Figure 3.

TABLE 2 Primers and probes were designed to detect plasmid-mediated colistin resistance genes (*mcr-1* to *mcr-10*).

Tube	Target	Primers / Probes name	Sequence (5′-3′)	Length (bp)	Tm (°C)	Final concentration (nmol/L)	Reference
A	*mcr-1* *mcr-2* *mcr-6*	*mcr-1/2/6-F1*	CTGGGCGCGGATGAGTAT	62	58.8	200	This study
		mcr-1/2/6-F2	CTGGGTCAAGATGACTAT		49.7	200	
		mcr-1/2/6-R	AGCGTATCH*AGCACATTTTCTTG		58.8	200	
		mcr-1/2/6-P	FAM-ATGTCGATACCGCCAAA-MGB		68	100	
	16S rRNA	16S rRNA-F	TGGAGCATGTGGTTTAATTCGA	159	59.1	200	Control CfD Prevention
		16S rRNA-R	TGCGGGACTTAACCCAACA		58.6	200	
		16S rRNA-P	HEX-CACGAGCTGACGACAR*CCATGCA-BHQ		72	200	
B	*mcr-3*	*mcr-3-F*	GTGATGATGCAAAACGGGATACT	109	58.9	200	This study
		mcr-3-R	TCTTTCTCATAGCCATTCATCGAG		58.5	200	
		mcr-3-P	HEX-AGTAAGCCCACGTTGATGT-MGB		70	200	
	mcr-9 *mcr-10*	*mcr-9/10-F*	CCCACGCTGATGTTCCTG	86	57.1	200	This study
		mcr-9/10-R	AAGGCATTK*GTR*TCACGCG		55.5	200	
		mcr-9/10-P	FAM-ATTGGCGAAACGGCAC-MGB		69	100	
C	*mcr-4*	*mcr-4-F*	TGCGTTGTTTTATGTTGCCATT	68	59	200	This study
		mcr-4-R	GTTTTTCAATTCCTTTTCGCACTAT		58	200	
		mcr-4-P	FAM-TCAATCTACCGCTCTTT-MGB		68	100	
	mcr-7	*mcr-7-F*	CGCACTCAACGCCGTCTT	103	59.6	200	This study
		mcr-7-R	GAGCATGGCGTAACTGACGAT		58.8	200	
		mcr-7-P	HEX-CTTCCGCTGGTTGCT-MGB		69	200	
D	*mcr-5*	*mcr-5-F*	GCAGACGAAGCAAGGGAAGTC	95	60	200	This study
		mcr-5-R	TCCCCGACAACCAGTACGA		58.5	200	
		mcr-5-P	FAM-CCGCCGTCCTCGTG-MGB		69	100	
	mcr-8	*mcr-8-F*	CGTTCCTTTTCTTTCCACTCTATTTC	84	58.8	200	This study
		mcr-8-R	CCCCGCTTATTGTCTGTTGAA		58.7	200	
		mcr-8-P	HEX-TGTACCAGCAATTATCCT-MGB		69	200	

*R = A or G; K = G, T; H = A, C, T.

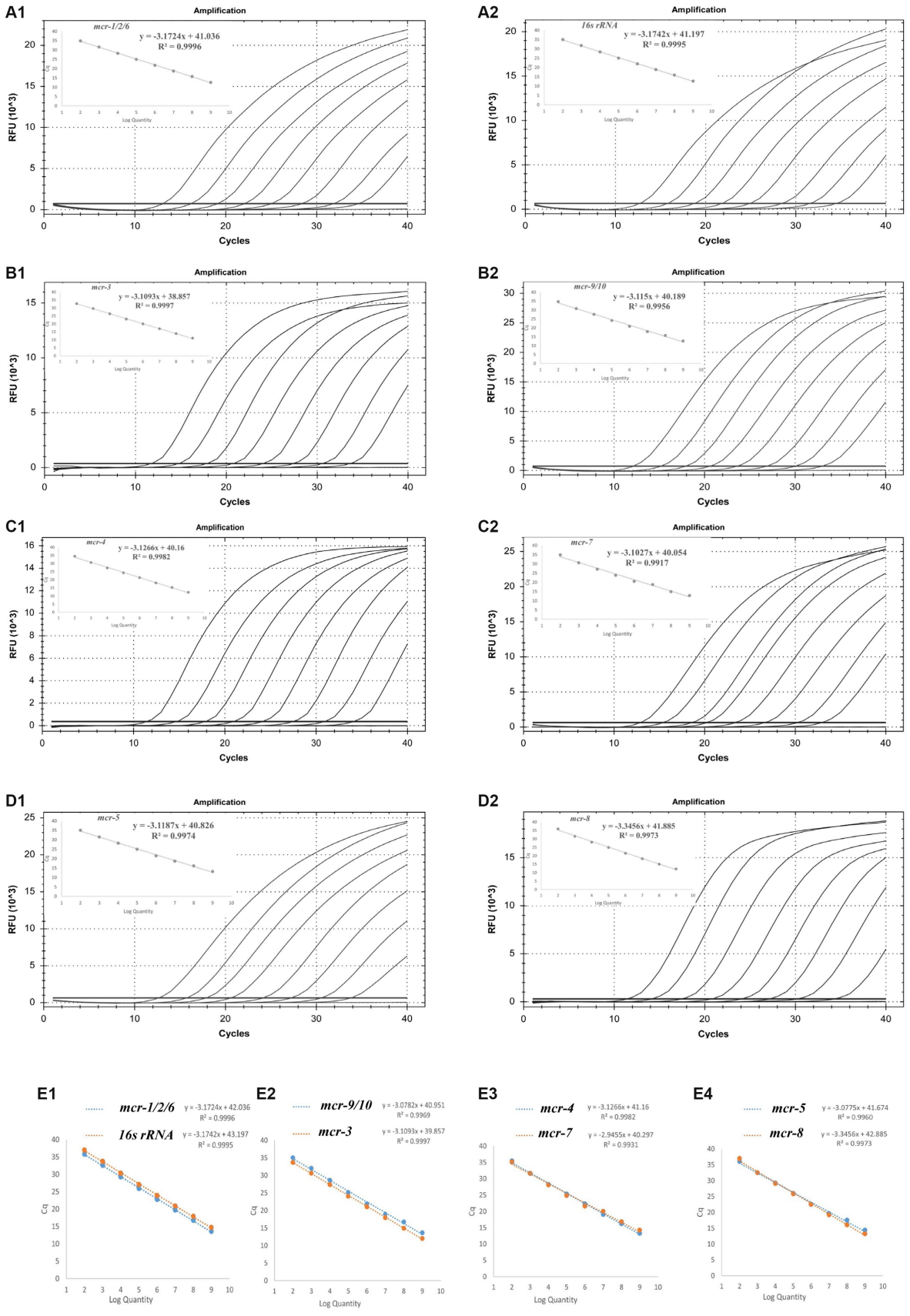

FIGURE 2
Standard curve and the amplification efficiencies of the single **(A1–D2)** and multiplex systems **(E1–E4)**.

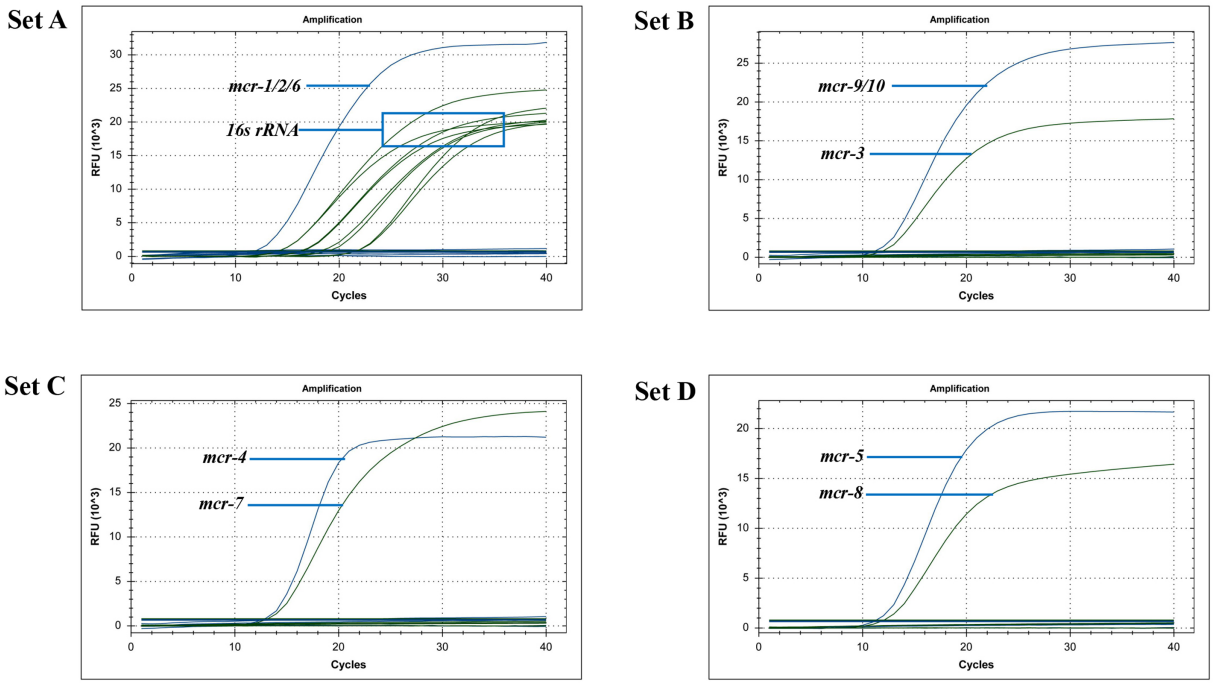

FIGURE 3
Specificity of the multiplex qPCR assay.

3.5 Sensitivity of the multiplex qPCR assay

Three concentration gradients of recombinant plasmid standards, from 1×10^3 copies/μL to 1×10^1 copies/μL, were used as templates to verify the sensitivity of the multiplex qPCR assay. The results showed that when the template concentration was 10^2 copies/μL, all primers and probes showed amplification curves in the three parallel controls, and when the template concentration was 10^1 copies/μL, only some of the primers and probes showed amplification curves in the three parallel controls. From the perspective of assay integrity, the minimum detectable limit of multiplex qPCR is 10^2 copies/μL.

3.6 Reproducibility of the multiplex qPCR

Reproducibility tests were performed using five concentration gradients of recombinant plasmid standards as templates. The results showed CVs ranging from 0.12 to 1.34% for intra-group reproducibility tests and from 0.10 to 1.91% for inter-group reproducibility, indicating that the developed fluorescent qPCR method is reproducible. The detailed values are listed in Table 3.

3.7 Identify the specific type of *mcr* gene

This detection system is sufficient to meet the need for rapid screening of *mcr* genes in samples/strains. For further identification of different *mcr* gene types, full-length gene amplification can be performed on samples/strains containing relevant *mcr* genes, and the sequencing results of amplified products can be uploaded to the NCBI database for comparison to determine specific *mcr* gene types.

3.8 Clinical isolate detection

We used a newly developed multiplex qPCR method to screen for the presence of the *mcr* gene in 467 multidrug-resistant clinical isolates. This method detected 7 isolates carrying the *mcr-1.1* gene (6 in *Escherichia coli* and 1 in *Klebsiella pneumoniae*), 1 isolate carrying the *mcr-9.1* gene (in *Enterobacter cloacae*), and 4 isolates carrying the *mcr-10.1* gene (2 in *Enterobacter ludwigii* and 2 in *Enterobacter asburiae*) in clinical strains. The sequencing results of the PCR products were confirmed to be *mcr*-related genes by Sanger sequencing (electrophoresis results in the Supplementary Figure S1), which were consistent with those of the multiplex qPCR assay. This suggests that the method can be used to screen for *mcr* genes in clinical isolates.

4 Discussion

Enterobacteriaceae exhibit polymyxin resistance through the acquisition of plasmid-mediated MCR family genes. Many scholars at home and abroad have developed methods for the detection of *mcr* genes including standard PCR (Liu et al., 2016), multiplex PCR for *mcr-1* to *mcr-9* genes (Borowiak et al., 2020), SYBR Green fluorescent qPCR (Mentasti et al., 2021), TaqMan fluorescent qPCR (Irrgang et al., 2016) and other methods. The ring-mediated isothermal amplification (LAMP) assays for rapid detection of *mcr-1* to *mcr-5* genes in colistin-resistant bacteria are available. The LAMP method is highly sensitive

TABLE 3 Reproducibility assay of TaqMan real-time PCR.

Plasmid (copies)	*mcr-1/2/6*						*mcr-9/10*					
	Intra-			Inter-			Intra-			Inter-		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
10^7	19.12	0.02	0.12%	19.05	0.07	0.38%	18.39	0.14	0.79%	18.08	0.26	1.46%
10^6	22.09	0.04	0.16%	22.09	0.08	0.35%	21.18	0.04	0.19%	21.00	0.16	0.76%
10^5	25.07	0.06	0.23%	25.19	0.09	0.36%	24.23	0.04	0.16%	24.12	0.10	0.42%
10^4	28.32	0.06	0.20%	28.47	0.11	0.37%	27.41	0.09	0.34%	27.47	0.14	0.50%
10^3	31.84	0.16	0.51%	31.82	0.03	0.10%	30.82	0.29	0.95%	30.79	0.04	0.14%

Plasmid (copies)	*mcr-3*						*mcr-4*					
	Intra-			Inter-			Intra-			Inter-		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
10^7	18.34	0.07	0.36%	18.47	0.15	0.83%	19.37	0.06	0.31%	19.29	0.09	0.49%
10^6	21.48	0.06	0.26%	21.70	0.32	1.46%	21.10	0.20	0.94%	21.05	0.31	1.46%
10^5	24.58	0.15	0.59%	24.83	0.32	1.30%	24.49	0.07	0.29%	24.44	0.10	0.40%
10^4	27.99	0.20	0.71%	28.12	0.24	0.85%	27.38	0.18	0.64%	27.44	0.24	0.86%
10^3	31.20	0.38	1.22%	31.36	0.38	1.22%	31.06	0.07	0.23%	30.93	0.26	0.84%

Plasmid (copies)	*mcr-5*						*mcr-7*					
	Intra-			Inter-			Intra-			Inter-		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
10^7	19.29	0.06	0.29%	18.87	0.36	1.91%	18.9967	0.18824	0.99%	19.05	0.06	0.31%
10^6	21.80	0.05	0.21%	21.60	0.18	0.83%	21.1633	0.09074	0.43%	20.83	0.29	1.41%
10^5	24.88	0.11	0.44%	24.85	0.16	0.63%	23.8133	0.31943	1.34%	23.81	0.08	0.35%
10^4	28.13	0.04	0.13%	28.16	0.08	0.28%	27.1333	0.07234	0.27%	27.12	0.03	0.11%
10^3	31.21	0.14	0.43%	31.34	0.17	0.54%	30.64	0.07	0.23%	30.60	0.10	0.32%

Plasmid (copies)	*mcr-8*						16S rRNA					
	Intra-			Inter-			Intra-			Inter-		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
10^7	18.33	0.13	0.71%	18.24	0.12	0.64%	18.9967	0.18824	0.99%	19.05	0.06	0.31%
10^6	21.43	0.08	0.35%	21.45	0.15	0.70%	21.1633	0.09074	0.43%	20.83	0.29	1.41%
10^5	24.81	0.07	0.28%	24.75	0.20	0.79%	23.8133	0.31943	1.34%	23.81	0.08	0.35%
10^4	28.22	0.13	0.46%	28.12	0.11	0.37%	27.1333	0.07234	0.27%	27.12	0.03	0.11%
10^3	31.24	0.14	0.46%	31.30	0.24	0.76%	30.64	0.07	0.23%	30.60	0.10	0.32%

and specific compared with the standard PCR method. However, due to the diversity of *mcr* genes, a single LAMP cannot detect all potential target genes, which provides incomplete information for nucleic acid detection. For samples containing more than one *mcr* gene, which has been reported in several cases, the sensitivity and specificity of the multiplex LAMP assay are relatively poor (Zhong et al., 2019). This situation has been reported many times so far and cannot be ignored (Zhang et al., 2018; Lu et al., 2020). One study introduced a Quad-PCR method for rapid and reliable detection of the common *mcr-1*, *mcr-3*, *mcr-8*, and *mcr-10* genes in clinical samples (Hu et al., 2021a). A multi-PCR assay for the detection of mobile colistin resistance genes (*mcr-1*, *mcr-3*, *mcr-8*, *mcr-10*) has also been developed (Hu et al., 2021b). A recent study has established a rapid, efficient and accurate method for recombinase polymerase amplification (RPA) combined with lateral flow dipstick (LFD) detection, but it can only detect the *mcr-1* gene. But *mcr-9* and *mcr-10* is also gradually being found in clinical patients and has spread widely around the world (Carroll et al., 2019; Ling et al., 2020; Liu et al., 2021). These methods can only detect some of the *mcr* genes, and some of them take a long time to detect. Therefore, it is essential to develop rapid detection methods that can cover all of the reported MCR family genes.

In this study, we selected the conserved region of all MCR family genes as the target sequence, considering all available relevant variant sequences in NCBI (as of March 2022), and successfully developed a multiplex fluorescent qPCR assay for the simultaneous detection of *mcr-1* to *mcr-10* gene sequences by optimizing the reaction

amplification system. The results showed that the newly developed assay is highly sensitive, specific, and reproducible. Using a common 96-well instrument, 24 samples can be detected in a single experiment, and if a 384-well instrument is used for high-throughput detection, more strains can be detected simultaneously, which is beneficial for the processing of large number of samples.

To our knowledge, this is the first multiplex TaqMan fluorescence qPCR assay for all 10 MCR family genes. The assay provides a rapid, simple, sensitive, and specific technique for monitoring multidrug-resistant bacteria carrying the *mcr* gene. During the course of the study, six newly identified *mcr* gene variants (*mcr-1.35*, *mcr-1.36*, *mcr-3.42*, *mcr-4.7*, *mcr-4.8*, *mcr-5.5*) were discovered between April 2022 and November 2023. We found that the mutant base of the new *mcr* variants did not appear at the location where we designed the primer, so these new *mcr* gene variants could still be specifically amplified with the existing primer and probe sets. This indicates that the target sequence is relatively conservative in these variants, and also validates the reliability of the sequence chosen for our design. We believe that the new approach may apply to the *mcr* gene variants that emerge in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XG: Methodology, Writing – original draft, Data curation, Investigation. GY: Data curation, Methodology, Resources, Supervision, Writing – review & editing. WL: Data curation, Methodology, Resources, Supervision, Writing – review & editing. DW: Data curation, Investigation, Methodology, Writing – review & editing. CD: Data curation, Investigation, Methodology, Writing – review & editing. XJ: Data curation, Investigation, Methodology, Writing – review & editing. ZL: Data curation, Investigation, Writing – review & editing. XZ: Investigation, Validation, Writing – review & editing. RY: Investigation, Validation, Writing – review & editing. DZ: Methodology, Project administration, Resources, Supervision, Writing – review & editing. YW: Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by National Science and Technology Major Project of the Ministry of Science and Technology of China 2018ZX10713003; 2018ZX10733402.

Conflict of interest

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1279186/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 05 April 2024
DOI 10.3389/fmicb.2024.1331628

OPEN ACCESS

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RECEIVED 01 November 2023
ACCEPTED 26 March 2024
PUBLISHED 05 April 2024

CITATION
Słabisz N, Leśnik P, Janc J, Fidut M, Bartoszewicz M, Dudek-Wicher R and Nawrot U (2024) Evaluation of the *in vitro* susceptibility of clinical isolates of NDM-producing *Klebsiella pneumoniae* to new antibiotics included in a treatment regimen for infections. *Front. Microbiol.* 15:1331628. doi: 10.3389/fmicb.2024.1331628

Evaluation of the *in vitro* susceptibility of clinical isolates of NDM-producing *Klebsiella pneumoniae* to new antibiotics included in a treatment regimen for infections

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Background: Due to the growing resistance to routinely used antibiotics, the search for new antibiotics or their combinations with effective inhibitors against multidrug-resistant microorganisms is ongoing. In our study, we assessed the *in vitro* drug susceptibility of *Klebsiella pneumoniae* strains producing New Delhi metallo-β-lactamases (NDM) to antibiotics included in the Infectious Diseases Society of America (IDSA) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommendations.

Methods: A total of 60 strains of NDM-producing *K. pneumoniae* were obtained from different patients hospitalized at the 4th Military Hospital in Wroclaw between 2019 and 2022 and subjected to drug susceptibility to selected antibiotics, including the effects of drug combinations.

Results: Among the tested antibiotics, the highest sensitivity (100%) was observed for cefiderocol, eravacycline (interpreted according to the European Committee on Antimicrobial Susceptibility Testing [EUCAST]), and tigecycline. Sensitivity to intravenous fosfomycin varied depending on the method used. Using the "strip stacking" method, determining cumulative sensitivity to ceftazidime/avibactam and aztreonam demonstrated 100% *in vitro* sensitivity to this combination among the tested strains.

Conclusion: The *in vitro* susceptibility assessment demonstrated that, the best therapeutic option for treating infections caused by carbapenemase-producing strains seems to be a combination of ceftazidime/avibactam with aztreonam. Due to the safety of using both drugs, cost effectiveness, and the broadest indications for use among the tested antibiotics, this therapy should be the first-line treatment for carbapenemase-producing *Enterobacterales* infections. Nevertheless, a comprehensive evaluation of the efficacy of treating infections caused by NDM-producing *K. pneumoniae* strains should include not only *in vitro* susceptibility assessment but also an analysis of clinical cases.

KEYWORDS

Klebsiella pneumoniae, metallo-beta-lactamase, NDM, susceptibility, aztreonam, eravacycline, fosfomycin, tigecycline

1 Introduction

Recently, a rapid spread of carbapenemase-producing *Enterobacterales* (CPE) strains has been observed worldwide, including in Poland, which represents a severe epidemiological and therapeutic problem. CPE most often colonizes the gastrointestinal tract but can also cause urinary tract infections (UTIs), pneumonia, or blood stream infection. Antimicrobial resistance genes are included in mobile elements such as plasmids, transposons, and integrons. The importance of these elements lies in their role in the vertical transmission of genes from *Klebsiella pneumoniae* to its descendants, as well as the horizontal transmission of genes from a specific *K. pneumoniae* strain to another (Karampatakis et al., 2023). These microorganisms spread very quickly in the hospital environment, primarily through direct contact with another patient who is a carrier of CPE or through the hands of medical staff. Screening of patients from risk groups during admission to the hospital, adherence to hand hygiene procedures by medical staff, and the application of rational antibiotic therapy in healthcare units, constitute the primary tool in the fight against spreading of CPE infections (Otter et al., 2020).

Most CPE strains are completely resistance to commonly used antibiotics, that is why, treating infections caused by these microorganisms often requires new, unconventional antibiotics or combination antibiotic therapy based on two or even three drugs. Unfortunately, in the case of CPE strains, there are often only one or two therapeutic options left for treatment, and there are also situations in which the strain is entirely resistant to all known antibiotics. Therefore, both laboratories and clinicians are forced to look for combinations of "old" and "new" antibiotics, the combined action of which may provide a chance for therapeutic success (Karaiskos et al., 2019; Ontong et al., 2021). Recently registered new antibiotics such as plazomicin, eravacycline or cefiderocol, may be an effective remedy in the fight against infections caused by CPE strains (Castanheira et al., 2020; McCreary et al., 2021; Zou et al., 2023).

In 2021, the European Society of Clinical Microbiology and Infection Diseases (ESCMID) published recommendations containing proposed treatment regimens for infections caused by third-generation cephalosporins- resistant microorganisms and *Enterobacterales, Pseudomonas aeruginosa, Acinetobacter baumannii* that are resistance to carbapenems (Paul et al., 2022).

In the case of enterobacterial rod-producing metallo-β-lactamases, in patient with severe infections, it is recommended to use cefiderocol or combination of aztreonam with ceftazidim/avibactam. In particular, the synergistic effects of a variety of aztreonam combined with ceftazidime/avibactam deserves attention. Further, the sensitivity of CRE-MBL to old antibiotics, including polymyxins, tigecycline, or fosfomycin IV, has been reported. In each of these cases, the drug susceptibility of the individual strains should be determined. In 2021 and 2023, similar recommendations were made by the Infectious Diseases Society of America (IDSA; Tamma et al., 2023).

The minimum inhibitory concentration (MIC) method is commonly employed in microbiological diagnostics to determine the lowest concentration of an antimicrobial agent that effectively inhibits the growth of a specific microorganism. There are also more specialized diagnostic tools to assess the interaction of two different antibiotics. This effect may be synergistic, additive, neutral, or antagonistic. Choosing this reciprocal relationship between the two drugs is a crucial therapeutic clue in treating infections caused by carbapenemase-producing *Enterobacterales* (Massoni-Cristante et al., 2003; Avery and Nicolau, 2018; Maraki et al., 2021; Terbtothakun et al., 2021).

Among carbapenemases such as IMP (active against imipenem; imipenemase), VIM (Verona integron-encoded metallo-β-lactamase), KPC (*K. pneumoniae* carbapenemase), New Delhi metallo-β-lactamases (NDM), and OXA-48-like, NDM constitutes a critical medical issue. The effectiveness of almost all β-lactams, including carbapenems, is compromised by this enzyme, except for aztreonam and cefiderocol. Given that, there are very few antibiotics available as therapeutic options. The objective of this study was to assess the susceptibility of clinical isolates of NDM-producing *K. pneumoniae*, recognized as a significant threat to public health and a common factor in nosocomial infections, to new antibiotics, including drugs recommended by U.S. Food and Drug Administration (2023), IDSA, and ESCMID for treatment of nosocomial and complicated infections, presented in Table 1.

The study aimed to evaluate the sensitivity of *K. pneumoniae* NDM isolates obtained from patients with UTI, VAP, and BSI infections in the 4th Military Hospital of Wroclaw from 2019 to 2022 to new antibiotics included in the IDSA and ESCMID recommendations.

2 Methods

The study was carried out in the Microbiology Laboratory of the Laboratory Diagnostics Department of the 4th Military Clinical Hospital in Wroclaw, based on material obtained in the Clinical Department of Anesthesiology and Intensive Care and other departments. *K. pneumoniae* NDM strains were obtained from patients' cultures of clinical materials collected for routine microbiological tests, which were subjected to drug susceptibility to selected antibiotics, including the effects of drug combinations.

2.1 Ethics

The study protocol was approved by the Bioethics Committee of the Lower Silesian Medical Chamber, Poland (approval no. 2/BNR/2023). Confidentiality and privacy were considered with regard to personal, laboratory, and clinical data. The study was carried out in accordance with the guidelines of the Declaration of Helsinki and

TABLE 1 Antibiotics recommended by FDA, IDSA, and ESCMID for the treatment of CPE infections (U.S. Food and Drug Administration, 2018; Paul et al., 2022; Tamma et al., 2023).

Antibiotics	Mechanism of action	Indications[a]
Cefiderocol (Fetcroja®) (siderophore cephalosporin)	Cefiderocol binds to extracellular free iron via its siderophore side chain, allowing active transport into the periplasmic space of Gram-negative bacteria through siderophore uptake systems. subsequently binds to penicillin-binding proteins (PBPs), inhibiting bacterial peptidoglycan cell wall synthesis, which leads to cell lysis and death.	cUTI caused by susceptible strains of *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, E. cloacae* HAP, VAP caused by *A. baumanii* complex, *E. coli, E. cloacae* complex, *K. pneumoniae*, *P. aeruginosa*, *S. marcescens* Bacteriemia Should be used to treat patients who have limited treatment options only after consultation with a physician with appropriate experience in the management of infectious diseases.
Eravacyclin (Xerava®) (synthetic fluocycline tetracycline)	The mechanism of action of eravacycline involves the disruption of bacterial protein synthesis by binding to the 30S ribosomal subunit thus preventing the incorporation of amino acid residues into elongating peptide chains.	cIAI caused by *E. coli, K. pneumoniae, E. faecalis, E. faecium, S. aureus, Viridans streptococcus* spp.
Plazomicin (Zemdri®) (semisynthetic aminoglycoside derived from sisomicin)	plazomicin binds to the 16S rRNA at the aminoacyl-tRNA site (A-site) of the 30S ribosomal subunit, interfering with protein translation.	cUTI caused by *E. coli, K. pneumoniae, P. mirabilis, E. cloacae* Active against *Enterobacterales* resistant to β-lactams and other classes of antibacterials May cause nephrotoxicity, ototoxicity and neuromuscular blockade.
Aztreonam (Cayston®) (monobactam β-lactam)	Aztreonam is a bactericidal agent that acts by inhibition of bacterial cell wall synthesis.	UTI (complicated and uncomplicated) Cystic fibrosis Lower respiratory tract infections (pneumoniae, bronchitis) Skin infections IAI Gynecologic infections Systematic infection: severe or life-threatening.
Ceftazidim/avibactam (Zavicefta®) (cephalosporin combined with non-β-lactam β-lactamase inhibitor)	The bactericidal action of ceftazidime is mediated through binding to essential penicillin-binding proteins (PBPs). Avibactam is a non-β lactam β-lactamase inhibitor that inactivates some β-lactamases and protects ceftazidime from degradation by certain β-lactamases.	cUTI cIAI HAP VAP
Fosfomycin IV (InfectoFos®) (phosphonic acid)	Fosfomycin IV inhibits the enzyme phosphoenolpyruvate transferase, which catalyzes the formation of n-acetylmuramic acid from n-acetyl aminoglucose and phosphoenolpyruvate. N-acetylmuramic acid is required for the buildup of peptidoglycan, an essential component of the bacterial cell wall.	cUTI Endocarditis[b] HAP, VAP[b] cSSI[b] Osteomyelitis[b] cIAI[b] meningitis[b]
Tigecycline (derivative of Minocycline)	Tigecycline, a glycylcycline, inhibits protein translation in bacteria by binding to the 30S ribosomal subunit and blocking entry of amino-acyl tRNA molecules into the A site of the ribosome. This prevents the incorporation of amino acid residues into elongating peptide chains.	cSSI cIAI CAP

[a]FDA, approved; [b]EMA, approved (available in Europe, Australia and Canada). cUTI, complicated urinary tract infection; HAP, hospital-acquired pneumonia; VAP, ventilatory-associated pneumonia; cIAI, complicated intra-abdominal infection; UTI, urinary tract infection; IAI, complicated intra-abdominal infection; cSSI, complicated surgical site infection; CAP, community-acquired pneumonia.

Good Clinical Practice. Written informed consent was obtained from all participants prior to the study.

2.2 Microorganisms

A total of 60 strains of NDM-producing *K. pneumoniae* were obtained from different patients hospitalized in the 4th Military Hospital in Wroclaw between 2019 and 2022 and used for the study: 20 strains originating from bloodstream infections, 20 strains isolated from urinary tract infections, and 20 strains from lower respiratory tract specimens (BAL- bronchoalveolar lavage, tracheal aspirates). The bacterial strains all originated from different patients. In the case of infection with the same bacterial strain across multiple systems, only a single isolate was used for testing.

2.3 Identification

All *K. pneumoniae* strains were identified using the VITEK MS system (bioMérieux, France), according to the manufacturer's instructions. The confidence interval for identification of all *K. pneumoniae* strains was 99.9%. *Escherichia coli* ATCC 8739 was used as the quality control strain.

2.4 Carbapenemase detection

Phenotypic detection of carbapenemases was performed using the immunochromatography test RESIST-5 O.O.K.N.V (CorisBioConcept, Belgium). This kit aims to detect and identify carbapenemases from a bacterial colony. Lateral-flow tests are based on membrane technology with colloidal gold nanoparticles. Quality control of this method was performed using the reference strain *E. coli* ATCC 25922.

2.5 Susceptibility testing

2.5.1 Gradient strip-based method

MIC Test Strip MTS™ (Liofilchem, Italy) is a quantitative method for *in vitro* susceptibility testing. MIC is the minimum inhibitory concentration of an antibiotic that inhibits the growth of bacteria under standardized *in vitro* conditions. MTS™ consists of special porous paper impregnated with a pre-defined concentration gradient of an antimicrobial agent, used to determine the minimum inhibitory concentration (MIC) in μg/mL of antimicrobial agents against bacteria. MTS™ strip tests were performed on Mueller Hinton Agar (bioMérieux, France). Liofilchem™ MTS™ Fosfomycin includes glucose-6-phosphate. The results were read after 16–20 h incubation at 35°C in ambient air. *Escherichia coli* ATCC 25922 was used as the quality control strain.

2.5.2 Agar dilution method (reference method)

Agar dilution is considered the best method for fosfomycin susceptibility testing, as recommended by CLSI and EUCAST standards. AD Fosfomycin 0.25–256 (Liofilchem, Italy) is a 12-well panel containing the antibiotic incorporated into an agar medium in different concentrations (11 two-fold dilutions, growth control). The tested microorganism was first isolated on a suitable non-selective culture medium- Columbia Agar (bioMérieux, France). The standardized suspension of a density of 0.5 McFarland was subsequently diluted 1:10 in saline, and 2 μL of the inoculum solution was inoculated into each well (final inoculum concentration should be approximately 10^4 CFU per spot). Test setups were incubated at 35°C for 16–20 h in ambient air. According to EUCAST, the MIC was recorded at the minimum concentration where there was non-confluent growth. Single colonies, pinpoint colonies, and a thin film of growth were ignored. Quality control of AD Fosfomycin 0.25–256 (Liofilchem, Italy) was performed using the *E. coli* ATCC 25922 strain (Croughs et al., 2022).

2.5.3 Gradient strip-stacking method

Susceptibility testing of the aztreonam plus ceftazidime/avibactam combination was performed on MH agar (bioMérieux, France) using the MIC Test Strip MTS™ (Liofilchem, Italy). Aztreonam (AZT) strips were placed on culture-inoculated agar surfaces and allowed to diffuse for 10 min. After 10 min, the aztreonam strips were removed, and the ceftazidime/aztreonam (CAZ/AVI) strips were placed at the same location. The aztreonam strips were then placed over the ceftazidime/avibactam strips to help read the MIC values of aztreonam after 16–18 h of incubation in ambient air (Khan et al., 2021; Bakthavatchalam et al., 2022). The cumulative MIC was interpreted against the Clinical and Laboratory Standards Institute (CLSI) criteria for aztreonam (Clinical and Laboratory Standards Institute, 2018).

2.5.4 Interpretation of the results

MIC breakpoints for selected antibiotics according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), Clinical and Laboratory Standards Institute (CLSI) and FDA are shown in Table 2.

2.5.5 Quality control

Escherichia coli ATCC 25922 strain was used for gradient strip-based method quality control according to the recommendations of EUCAST.

TABLE 2 MIC breakpoints for selected antibiotics according to EUCAST, CLSI and FDA (Clinical and Laboratory Standards Institute, 2018; European Committee on Antimicrobial Susceptibility Testing, 2023; U.S. Food and Drug Administration, 2023).

Antibiotic	Interpretative criteria	MIC breakpoints (mg/L)	
		S≤	R>
Cefiderocol (CDR)	EUCAST	2	2
Eravacycline (ERV)	EUCAST (ECOFF)	2	2
	FDA	0.5	0.5
Tigecycline (TIG)	EUCAST (ECOFF)	2	2
	FDA	2	8 (≥)
Plazomycin (PLZ)	CLSI	2	8 (≥)
Fosfomycin iv (FOS)	EUCAST	32	32
Ceftazidime/avibactam (CAZ/AVI)	EUCAST	8	8
Aztreonam (AZT)	EUCAST	1	4
Ceftazidime/avibactam + aztreonam (CAZ/AVI + AZT)	CLSI	4	16

2.5.6 Statistics

In this study, the statistical analyses were conducted using the STATISTICA 13 (TIBCO Software Inc. Palo Alto, United States) software package. To assess the normality of the data distribution, the Shapiro–Wilk test was employed. For the comparison between different groups, the non-parametric, Kruskal–Wallis test was used (with Dunn's *post-hoc* test). Additionally, to compare the results of FOS (MTS™) with FOS (AD FOSF®) as the reference method, the Mann–Whitney *U*-test was applied. In all statistical tests, a $p < 0.05$ was considered to indicate statistical significance.

3 Results

Among the tested antibiotics, the highest sensitivity (100%) was observed for cefiderocol, eravacycline (interpreted according to EUCAST), and tigecycline. Only 78% of tested strains were susceptibility to plazomycin. Two methods for determining susceptibility to fosfomycin were used in this study. For the gradient strip-based method, susceptibility of tested strains was 68%, compared to 83% using the commercial test AD Fosfomycin 0.25–256 (Liofilchem, Italy) in which the reference method was employed (Figure 1).

The studied NDM-producing *K. pneumoniae* strains showed 100% resistance to ceftazidime with avibactam and 92% resistance to aztreonam when these drugs were tested individually (Table 3; Figure 2; Supplementary Table S1). Using the "strip stacking" method to determine the cumulative sensitivity to ceftazidime/avibactam and aztreonam demonstrated 100% *in vitro* sensitivity to this combination among the tested strains (Figure 2).

The statistical analysis compared the MIC values for the tested antibiotics in the three groups of strains (strains originating from bloodstream infections, strains isolated from urinary tract infections, and 20 strains from lower respiratory tract specimens; bronchoalveolar lavage [BAL], tracheal aspirates). Statistically significant lower MIC values for cefiderocol were obtained in the case of strains isolated from lower respiratory tract infections ($p = 0.002$). MIC values for the combination of aztreonam with ceftazidime/avibactam were lower for isolates originating from urinary tract infections ($p = 0.004$). The last group in which statistical significance was demonstrated, is the MIC value for fosfomycin for *K. pneumoniae* NDM isolated from urine samples, determined by the reference method ($p = 0.014$; Table 4).

4 Discussion

The COVID-19 pandemic witnessed a notable rise in the prevalence of multidrug-resistant strains, especially within the *Enterobacterales* family. An earlier investigation examined bacterial bloodstream infections in patients hospitalized before and during the COVID-19 pandemic (Słabisz et al., 2023) demonstrated a statistically significant increase in the frequency of BSIs caused by NDM-producing *K. pneumoniae*. A report by the European Centre for Disease Prevention and Control (ECDC) published in 2022 (World Health Organization, 2022) on the epidemiological situation in European countries from 2016 to 2020 confirmed the presence of a growing antimicrobial resistance problem among microorganisms, including carbapenem-resistant strains of *K. pneumoniae*. The number of unique cases of CPE strains in Poland from 2019 to 2021, confirmed by the National Reference Center for Antimicrobial Susceptibility Testing of Microorganisms, increased from 2064 to 4,172. In 2019, 1,527 cases of NDM strains were confirmed. In 2021, this number increased to 3,036. These isolates originated only from infection cases and not from intestinal carriages (Hryniewicz et al., 2022). Due to the growing number of patients colonized with CPE strains, not only in the gastrointestinal tract but also in the urinary tract, it is necessary to establish guidelines for empirical treatment and drug susceptibility assessment in patients with suspected MDR strains, including CPE. Single-focal epidemics were frequently observed during the COVID-19 pandemic in the hospital as well as in the post-pandemic period. Patients with rectal colonization of NDM *K. pneumoniae* had a higher risk of bacteriemia than those with KPC *K. pneumoniae* (Pereira et al., 2023).

Our study analyzed the *in vitro* activity of new antibiotics recommended in the IDSA and ESCMID guidelines for treating CPE strains. Currently, the broadest registered antibiotics indicated for the treatment of the source of infection are fosfomycin IV, aztreonam, and ceftazidime in combination with avibactam. Plazomicin and eravacycline have narrow indications for use, with the former registered for the treatment of complicated urinary tract infections and the latter for complicated infections within the abdominal cavity. Cefiderocol, a new broad-spectrum cephalosporin, is also an attractive alternative. Our study assessed the drug susceptibility of 60 strains of NDM-producing *K. pneumoniae*. Due to the high toxicity of colistin and increasing resistance, new antibiotics are useful alternatives in treating infections.

Our study demonstrated the highest sensitivity of 100% for cefiderocol, eravacycline, tigecycline, and a combination of ceftazidime/avibactam with aztreonam. When B-lactamase inhibitors (BLI) are combined with known B-lactams, they demonstrate excellent activity against MBLs. Avibactam forms a stable and hydrolysis-resistant complex with the β-lactamase molecule, causing inhibition of β-lactamases of classes A, B, and partially D (according to Ambler's classification), including β-lactamases with a highly extended spectrum of activity (KPC and OXA– 38), as well as the chromosomal cephalosporinase AmpC (Behzadi et al., 2020). Due to the narrow indications for using eravacycline, ceftazidime/avibactam and aztreonam are the drugs of choice for bacteremia or pneumonia (Zhanel et al., 2016; Falcone et al., 2021; Sanz Herrero, 2022; Brauncajs et al., 2023). In July 2023, Mark G. Wise reported data on the evaluation of the *in vitro* activity of aztreonam/avibactam, a new antibiotic, against *Enterobacterales* isolates. In total, 24,937 isolates from 27 countries were assessed. Aztreonam/avibactam inhibited 99.1% of CRE isolates (European Committee on Antimicrobial Susceptibility Testing, 2023). The study demonstrated 100% effectiveness of ceftazidime/avibactam in combination with aztreonam. The advantage of this combination of antibiotics is their synergism of action and the ease of determining drug susceptibility using the "strip-stacking" method. The results of this study suggest that most clinical laboratories, using routinely applied methods, can perform the sensitivity determination for combinations of two drugs.

"Strip-stacking" method is relatively easy to perform, fast and shows high correlation with the reference method of microdilution in broth (Khan et al., 2021). In our study, all isolates were sensitive to cefiderocol, but one exhibited borderline susceptibility (MIC = 2 μg/mL). In the latest update of the IDSA guidance, cefiderocol and CAZ/

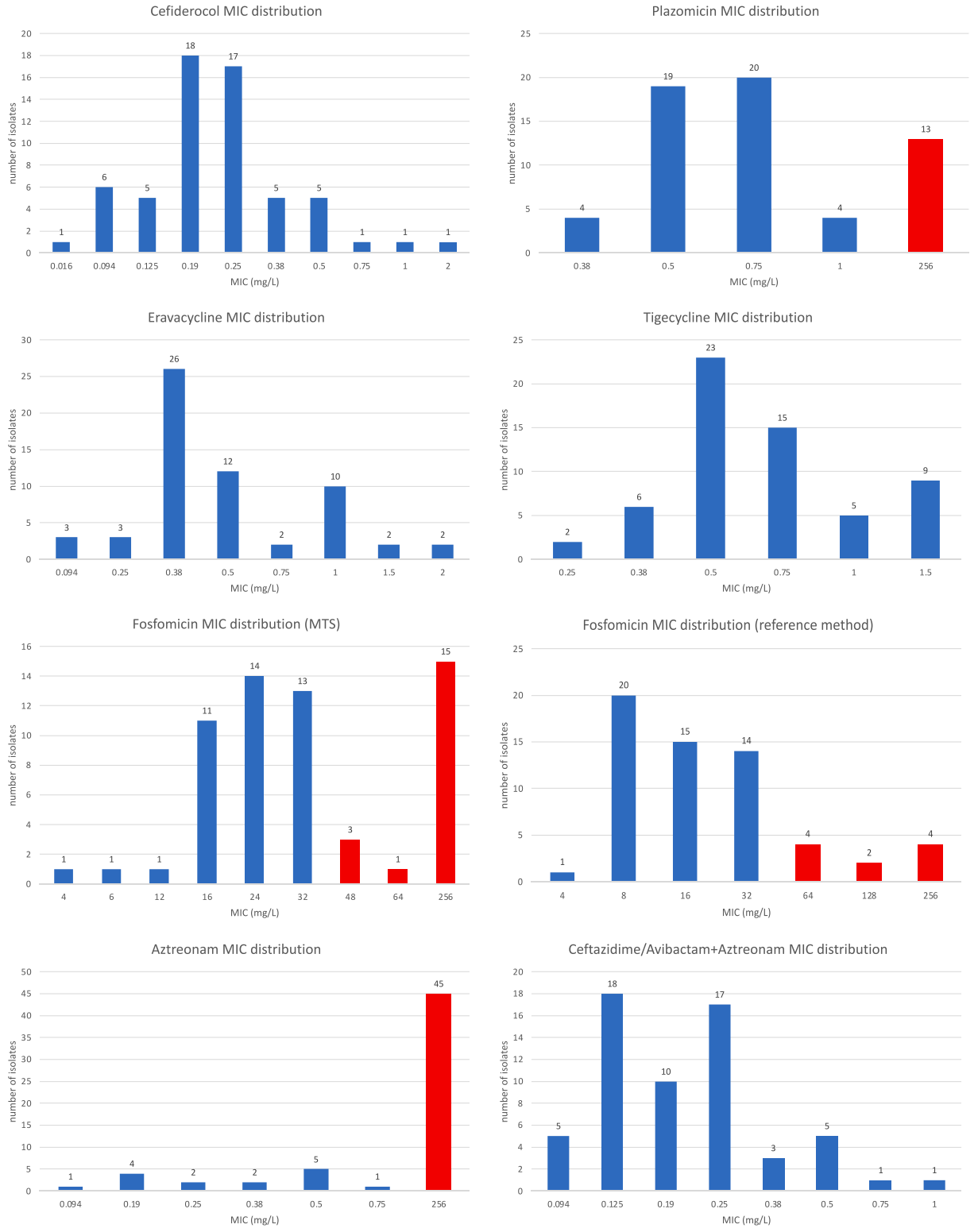

FIGURE 1
Distribution of MIC values for tested antibiotics. MIC values marked in red are within the resistance category of a given antibiotic.

AVI plus AZT are recommended antibiotic for treating NDM-producing *Enterobacterales* with (Tamma et al., 2023).

In the Phase III, open-label study (CREDIBLE-CR), an elevated all-cause mortality rate was demonstrated in patients treated with cefiderocol for infections caused by carbapenem-resistant Gram-negative bacilli compared to patients treated with the best available therapy, which was based on colistin (34% vs. 18%; Bassetti et al., 2021). Mortality difference was recorded for infections of *Acinetobacter*

TABLE 3 Number of susceptible isolates, MIC range, MIC 50, MIC 90 (μg/ml) values of the tested antibiotics.

Antibiotic	No of sensitive strains (%)	MIC range [ug/ml]	MIC50	MIC90
CDR	60 (100)	0.16–2	0.19	0.5
ERV (EUCAST)	60 (100)	0.094–2	0.38	1
ERV (FDA)	44 (73)	0.094–0.5	0.38	0.5
PLZ	47 (78)	0.38–1	0.75	0.75
TIG	60 (100)	0.25–1.5	0.5	1.5
FOS (MTS™)[a]	41 (68)	4–32	24	32
FOS (AD FOSF®)[b]	50 (83)	4–32	16	32
CAZ/AVI	0 (0)	–	–	–
AZT	15 (25)	0.094–0.75	0.38	0.5
CAZ/AVI + AZT	60 (100)	0.94–1	0.19	0.5

The interpretation criteria or the susceptibility testing method used are given in brackets. [a]Determination by the gradient strip method. [b]Determination by the reference microdilution method in agar using a commercial test AD Fosfomycin 0.25–256 (Liofilchem, Italy).

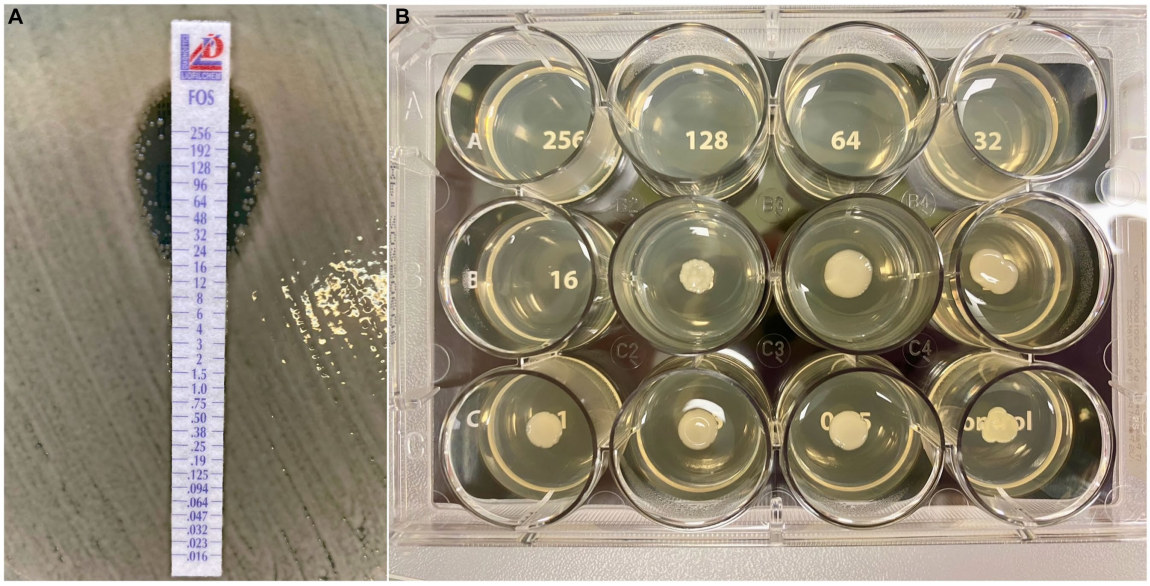

FIGURE 2
A comparison of susceptibility testing for fosfomycin with **(A)** gradient-strip based method (MIC Test Strip MTS™, Liofilchem, Italy) and with **(B)** reference agar dilution method (AD Fosfomycin 0.25–256, Liofilchem, Italy). In the case of the reference method, the interpretation of the MIC value for the tested strain is straightforward (MIC = 16), whereas the presence of micro and macro colonies within the inhibition zone, when using a gradient strip method, can pose challenges in determining the correct MIC value.

spp. and *Pseudomonas aeruginosa*, and there was no difference in patients with no *Acinetobacter* spp. infection. In light of this evidence, the use of cefiderocol appears to be uncertain compared to therapy based on the combination of CAZ/AVI and AZT. There are still lacking od the clinical data, that would enable the analysis for comparing the effectiveness of treatment between both therapeutic regimens.

Eravacyclin was approved in 2014 by the US FDA and the European Medicines Agency (EMA) to treat complicated intra-abdominal infections (Thakare et al., 2018; Scott, 2019). In Zou et al. (2023) demonstrated the antibacterial activity of eravacycline against CRE. In the study group, 48 strains of *E. coli* were carriers of the NDM gene, and two were KPC. The sensitivity to eravacyclin in this group was 92% (Zou et al., 2023). The susceptibility of the tested NDM-producing *K. pneumoniae* strains to eravacycline varied depending on the interpretation criteria applied. The FDA's breakpoints are more stringent than those of EUCAST, where epidemiological cut-off values (ECOFF) have been proposed. Based on EUCAST, 100% of the tested strains showed susceptibility to eravacycline. By contrast, when interpreting the results according to FDA criteria, only 73% of the strains could be classified as susceptible to eravacycline.

Irrespective of the interpretation criteria, 100% sensitivity to tigecycline has been demonstrated. In the case of the EUCAST criteria, due to the lack of a breakpoint for *K. pneumoniae*, it was necessary to use the ECOFF value for interpretation, which is the same as the MIC

TABLE 4 Inter-group comparison of assessed variables (MIC value).

Antibiotic	Group I "B" ($n = 20$)			Group II "U" ($n = 20$)			Group III "P" ($n = 20$)			p value*
	Me	Q1	Q3	Me	Q1	Q3	Me	Q1	Q3	
CDR	0.25	0.19	0.25	0.25	0.19	0.50	0.19[a]	0.09	0.19	**0.002**
ERAV	0.38	0.38	0.44	0.50	0.38	1	0.50	0.38	0.75	0.09
PLZ	0.63	0.50	0.88	0.75	0.75	1	0.75	0.50	128.50	0.36
TIG	0.50	0.44	0.75	0.75	0.63	1	0.50	0.50	0.88	0.06
FOS (MTS™)[a]	32	24	256	32	16	256	24	24	32	0.47
FOS (AD FOSF®)[b]	32	16	64	8[c]	8	24	16	8	32	**0.014**
CAZ/AVI + AZT	0.25	0.16	0.25	0.13[d]	0.13	0.19	0.25	0.19	0.25	**0.004**

Group I "B", strains isolated from blood; Group II "U", strains isolated from urine; Group III "P", strains isolated from bronchoalveolar lavage/tracheal aspirate; Me, median; Q1, first quartile; Q3, third quartile. *Kruskal–Wallis test. [a]Determination by the gradient strip method. [b]Determination by the reference microdilution method in agar using a commercial test AD Fosfomycin 0.25–256 (Liofilchem, Italy). [c]Statistically significantly lower result compared to group I (*post-hoc* test). [d]The statistically significant lowest result compared to the other two groups (*post-hoc* test). Bold values indicate statistically significant values.

adopted by the FDA. However, tigecycline is a bacteriostatic antibiotic with the primary indication for intra-abdominal, skin, and soft tissue infections. High doses are required for nosocomial pneumonia, with an increased risk of toxicity, according to the latest information from the FDA (FDA Drug Safety Communication, 2017).

FDA approved in 2018 plazomicin, which is an aminoglycoside. Plazomicin is registered for the treatment of infections such as: urinary tract infections, including pyelonephritis, bloodstream infections (BSI), and ventilator-associated pneumonia (VAP; Clark and Burgess, 2020). Plazomicin has a registration for two indications: complicated urinary tract infections in a phase 2 trial and EPIC trial and severe infections caused by CRE (BSI, hospital-acquired pneumonia, and VAP) in the CARE trial (Wagenlehner et al., 2019). Plazomicin received FDA approval with a black box warning for potential aminoglycoside class effects, including nephrotoxicity, ototoxicity, neuromuscular blockade, and risks during pregnancy (U.S. Food and Drug Administration, 2018). The balance between side effects and the benefits of antibiotic therapy with plazomicin underscores the drug's safety in comparison to traditional aminoglycosides. The reported renal toxicity is comparable to that induced by meropenem. While 3% of patients treated with plazomicin experienced renal function impairment, the renal damage associated with plazomicin is reversible. In a study, approximately 80% of patients demonstrated complete renal function at the discharge visit following treatment (Alfieri et al., 2022). In our study, we had 78% susceptibility to plazomicin.

Patients with severe infections caused by carbapenem-resistant *Enterobacterales* who are susceptible to polymyxins, aminoglycosides, tigecycline, or fosfomycin can use intravenous fosfomycin for combined therapy, as suggested by ESCMID guidelines, or if antibiotics combined with β- lactam inhibitors are not avalible (Paul et al., 2022). This study demonstrated differences in intravenous fosfomycin sensitivity depending on the applied determination method, with 83% sensitivity for the reference method. These data align with reports from the global SENTRY surveillance program, where an 82.6% sensitivity to fosfomycin was shown among *K. pneumoniae* and *E. coli* strains producing carbapenemases (Flamm et al., 2019). In a study by Banerjee et al. (2017), the sensitivity among NDM strains was 92.9%, although it dates back to 2017 and utilized the disk diffusion method. Studies conducted in Poland between 2011 and 2020 revealed a lower sensitivity among carbapenemase-positive strains (55%; Mączyńska et al., 2021).

The critical factor in categorizing a strain into a specific sensitivity category is choosing the appropriate determination method. According to the recommendations, the quantitative agar dilution method is considered the reference method (European Committee on Antimicrobial Susceptibility Testing, 2023). Its advantages include ease of interpretation and high repeatability of the results. However, challenges lie in the time-consuming preparation of substrates, the potential for inaccurate drug dilution, and antibiotic inactivation due to high temperatures. Performing the determination using automated systems (BD Phoenix) or E-tests with a gradient diffusion method can result in distortion. One of the reasons for in the absence of comprehensive data on the level of resistance of strains to intravenous fosfomycin in relation to the local epidemiological situation, is the necessity for a simple and reliable method (Kowalska-Krochmal et al., 2022). Commercial kits (AD Fosfomycin 0.25–256, Liofilchem, Italy) significantly facilitate interpretation and leave no doubt about the sensitivity or resistance of the tested strain (Figure 2). In this study, a commercial test (AD Fosfomycin 0.25–256, Liofilchem, Italy) caused a change in the sensitivity category from resistant to sensitive for 12 strains. This undoubtedly resulted from the difficulty of interpreting the determination using the gradient diffusion method, in which numerous growth increments in the zone of inhibition make it impossible to read the MIC value unambiguously and instead lead to its overestimation. Therefore, using a commercial test is advantageous for fosfomycin, enabling its more frequent consideration in treating CPE infections. Statistically lower MIC values for fosfomycin obtained using the reference method confirm the necessity of employing the agar microdilution method to avoid false results. Similar findings have been presented in other literature reports (Croughs et al., 2022; Pereira et al., 2023).

4.1 Limitations of the study

The study was conducted in a single center and was based on *in vitro* evidence of antimicrobial activity, meaning that the effects of application on humans were not determined. The reported effects have not been confirmed in humans, and the number of cases and specimens is not representative of the entire population, which indicates the need for further studies.

5 Conclusion

The best therapeutic option for treating infections caused by carbapenemase-producing strains seems to be a combination of ceftazidime/avibactam with aztreonam. Due to the safety of using both drugs, cost effectiveness, and the broadest indications for use among the tested antibiotics, this therapy should be the first-line treatment for CPE infections. The second option, with 100% sensitivity of the tested strains, is cefiderocol, but it remains expensive with limited availability. Despite its high *in vitro* sensitivity, eravacycline has limited use due to narrow indications and is restricted only to complicated intra-abdominal infections. The finding of fosfomycin-resistant NDM-positive *K. pneumoniae* confirms the need to perform drug susceptibility testing using the reference agar microdilution method before implementing intravenous fosfomycin therapy. Nevertheless, a comprehensive evaluation of the efficacy of treating infections caused by NDM-producing *K. pneumoniae* strains should include not only *in vitro* susceptibility assessment but also an analysis of clinical cases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Bioethics Committee of the Lower Silesian Medical Chamber, Poland (approval no. 2/BNR/2023). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft. PL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Software, Visualization, Writing – original draft. JJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Software, Visualization, Writing – original draft. MF: Conceptualization, Data curation, Software, Writing – review & editing. MB: Formal analysis, Supervision, Writing – review & editing. RD-W: Formal analysis, Supervision, Writing – review & editing. UN: Formal analysis, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The statistical analysis was conducted with the collaboration of the Regional Digital Medicine Centre at the 4th Military Clinical Hospital in Wrocław, Poland (Project No. 2023/ABM/02/00005 - 00; Medical Research Agency).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1331628/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 12 April 2024
DOI 10.3389/fmicb.2024.1355069

OPEN ACCESS

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RECEIVED 13 December 2023
ACCEPTED 01 April 2024
PUBLISHED 12 April 2024

CITATION
Wang Z, Shao C, Shao J, Hao Y and Jin Y (2024) Risk factors of Carbapenem-resistant Enterobacterales intestinal colonization for subsequent infections in hematological patients: a retrospective case-control study. *Front. Microbiol.* 15:1355069. doi: 10.3389/fmicb.2024.1355069

Risk factors of Carbapenem-resistant Enterobacterales intestinal colonization for subsequent infections in hematological patients: a retrospective case-control study

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Objective: Infections caused by Carbapenem-resistant Enterobacterales (CRE) have high treatment costs, high mortality and few effective therapeutic agents. This study aimed to determine the risk factors for progression from intestinal colonization to infection in hematological patients and the risk factors for 30-day mortality in infected patients.

Methods: A retrospective case-control study was conducted in the Department of Hematology at Shandong Provincial Hospital affiliated to Shandong First Medical University from April 2018 to April 2022. Patients who developed subsequent infections were identified as the case group by electronic medical record query of patients with a positive rectal screen for CRE colonization, and patients who did not develop subsequent infections were identified as the control group by stratified random sampling. Univariate analysis and logistic regression analysis determined risk factors for developing CRE infection and risk factors for mortality in CRE-infected patients.

Results: Eleven hematological patients in the study developed subsequent infections. The overall 30-day mortality rate for the 44 hematological patients in the case-control study was 11.4% (5/44). Mortality was higher in the case group than in the control group (36.5 vs. 3.0%, $P = 0.0026$), and septic shock was an independent risk factor for death ($P = 0.024$). Univariate analysis showed that risk factors for developing infections were non-steroidal immunosuppressants, serum albumin levels, and days of hospitalization. In multivariable logistic regression analysis, immunosuppressants [odds ratio (OR), 19.132; 95% confidence interval (CI), 1.349–271.420; $P = 0.029$] and serum albumin levels (OR, 0.817; 95% CI, 0.668–0.999; $P = 0.049$) were independent risk factors for developing infections.

Conclusion: Our findings suggest that septic shock increases mortality in CRE-infected hematological patients. Hematological patients with CRE colonization using immunosuppressive agents and reduced serum albumin are more likely to progress to CRE infection. This study may help clinicians prevent the onset of infection early and take measures to reduce mortality rates.

KEYWORDS

Carbapenem-resistant Enterobacterales, colonization, infections, risk factors, hematological patients

1 Introduction

Carbapenem-resistant Enterobacterales (CRE) infections pose a significant threat to global public health due to their high treatment costs, high mortality, and limited availability of effective therapeutic agents (Xu et al., 2017; European Centre for Disease Prevention and Control, 2018; Tacconelli et al., 2018; Brolund et al., 2019; Centers for Disease Control and Prevention, 2019). A report published in 2023 by the European Center for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) revealed that the prevalence of carbapenem resistance in *Klebsiella pneumoniae* isolates rose by 0, 8, 31, and 20% between 2017 and 2021 (European Centre for Disease Prevention and Control and World Health Organization, 2023). Based on data from the China Antimicrobial Resistance Surveillance System (CARSS), the detection rate of carbapenem-resistant *Klebsiella pneumoniae* (CR-KPN) increased from 10.9% in 2020 to 11.3% in 2021, marking a rise from 6.4% in 2014. Additionally, the national average of *Escherichia coli* resistance to carbapenems remained at 1.6%, the same as in 2020 (China Antimicrobial Resistance Surveillance System, 2023). Patients with hematological disorders are at a higher risk of contracting CRE infections due to their compromised immune systems, low levels of neutrophils, extended hospital stays, undergoing hematopoietic stem cell transplantation, receiving chemotherapy, taking immunosuppressant medications, and frequent use of broad-spectrum antibiotics (Lalaoui et al., 2020; Cao et al., 2021).

As a reservoir for secondary infections, intestinal colonization correlates significantly and independently with CRE infection (Gorrie et al., 2017; Cao et al., 2022). Multiple clinical studies have found that CRE colonization is associated with an increased risk of CRE infection and mortality in patients (Giannella et al., 2014; McConville et al., 2017; Lin et al., 2021; Gomides et al., 2022; Zhu et al., 2022). According to the guidelines from the ECDC, it is advisable to actively screen for CRE and apply effective infection prevention and control strategies to stop the spread of CRE (Magiorakos et al., 2017). Hence, promptly identifying the risk factors contributing to the transition from CRE colonization to subsequent infection will significantly decrease the incidence of CRE infections and mortality.

Fewer studies have been conducted regarding secondary CRE infections in hematological patients with intestinal colonization of CRE. Consequently, this study aimed to identify the factors that increase the likelihood of hematological patients with intestinal CRE colonization progressing to infection, as well as the factors that contribute to mortality in hematological patients already infected with CRE. It provides hematology clinicians with a reference for early identification of high-risk hospitalized patients, allowing them to implement timely preventative measures against CRE infection and mortality.

2 Materials and methods

2.1 Study design

This retrospective case-control study was conducted at the Shandong Provincial Hospital Affiliated to Shandong First Medical University. This hospital has 3,889 beds, 90 of which belong to the hematology department. This study was approved by the Shandong Provincial Hospital Ethics Committee (NO. SWYX2023-610).

CRE rectal colonization was defined as a positive CRE stool screen and the absence of invasive infection. CRE rectal infection was defined as the presence of clinical signs and symptoms of infection and the detection of CRE in specimens taken from the site of infection. Infections such as bacteremia, pneumonia, urinary tract infections, and perianal infections are defined according to guidelines issued by Centers for Disease Control and Prevention (2023). Patients with CRE colonization who were hospitalized for <3 days and discharged without identifying the colonizing strain were excluded, and patients with positive rectal screening for CRE in the hematology department from April 2018 to April 2022 were further studied. The case group consisted of patients colonized with CRE and subsequently developed CRE infections caused by the same strain (Figure 1). The control group comprised patients selected in a 3:1 ratio relative to the 11 patients in the case group. To enhance the comparability between the case and control groups, we employed frequency matching based on age and sex to mitigate the influence of confounding factors. First, we determined the proportions of age and sex in the case group. Then, we used statistical software to conduct a stratified random sample of colonized patients who did not develop subsequent infections.

The control and case groups were compared using clinical data variables to examine the risk factors associated with subsequent CRE infection. We conducted a 30-day follow-up study on the patients, with death as the end event. The initial event for the control group was the detection of positive test results for CRE colonization samples, while the case group involved detecting positive test results for CRE infection samples. Subsequently, CRE-infected patients were further divided into survival and non-survival groups to analyze risk factors for 30-day mortality. Furthermore, a time-to-event study was conducted to evaluate the characteristics of CRE colonization and infection in CRE-infected patients.

2.2 Data collection

Between April 2018 and April 2022, we obtained stool sample data from 1,390 individuals with hematological conditions. Of these, 131 patients had CRE colonization, and we examined their clinical data. Clinical data is obtained from the patient's electronic medical record and encompasses various factors such as demographic characteristics (age and gender), length of hospitalization, hematological diseases (such as acute myeloid leukemia, acute lymphoblastic leukemia, multiple myeloma, myelodysplastic syndrome, lymphoma, etc.), comorbidities (such as diabetes, hypertension, chronic liver disease, gastrointestinal disease, graft vs. host disease, hemorrhagic cystitis, mucositis, pneumonia, diarrhea, and shock), previous invasive procedures (such as deep venous catheterization, urinary catheterization, hematopoietic stem cell transplantation), exposure to drugs (such as chemotherapy, glucocorticoids, non-steroidal immunosuppressants, proton pump inhibitors, carbapenems, cephalosporins, fluoroquinolones, aminoglycosides, glycopeptide,

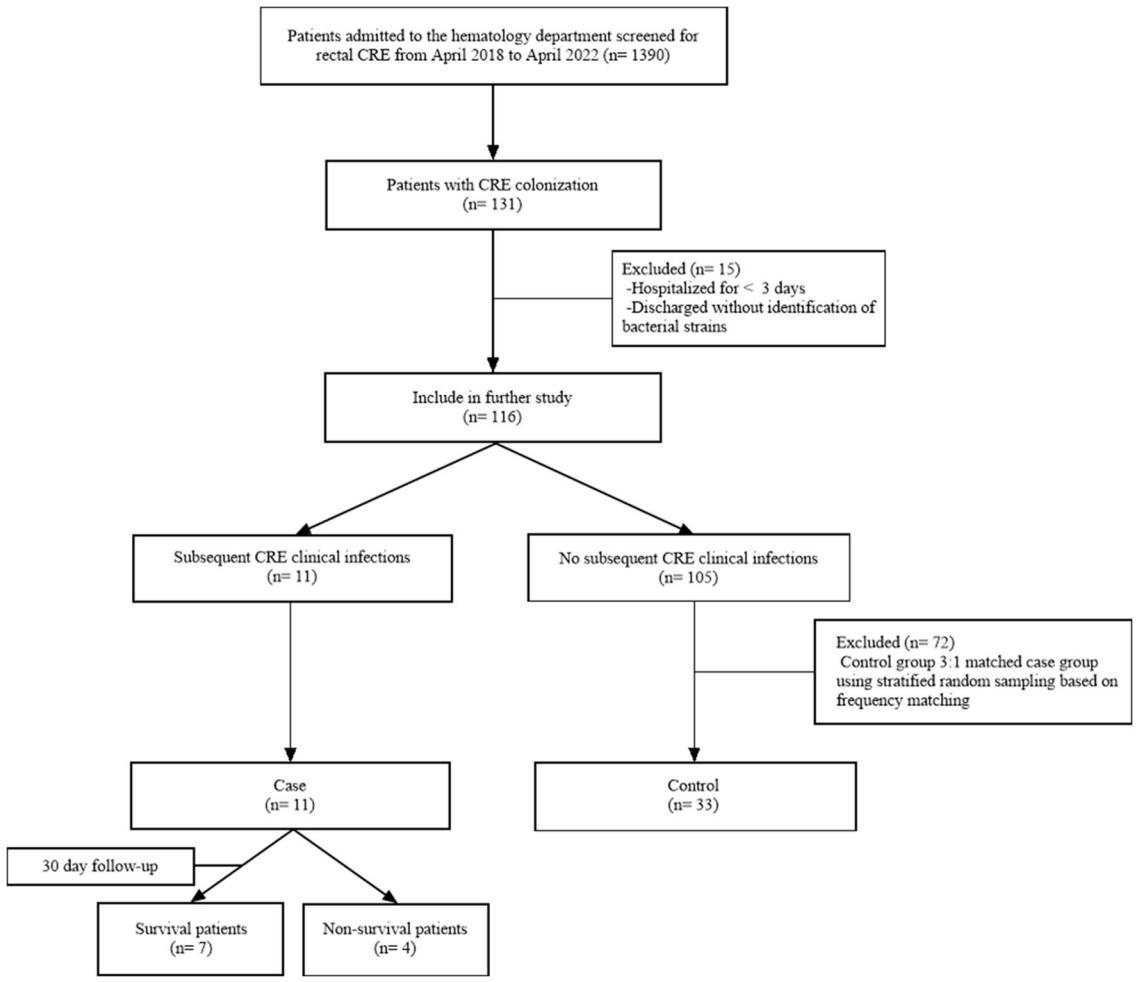

FIGURE 1
Flowchart of the study design.

penicillins, tigecycline), laboratory examinations (neutrophil count and serum albumin levels), CRE isolates (such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and others), and outcomes (mortality at 30 days). Furthermore, we collected *in vitro* susceptibility data, carbapenemase phenotypes (serine carbapenemase and metal β-lactamases), sensitive antibiotic treatments, and the timing of colonization and infection in patients infected with CRE.

2.3 Microbiology

Bacterial isolates for this study were identified using the Vitek 2 automatic system (bioMérieux, France). The stool samples were inoculated on MacConkey agar plates (ThermoFisher, USA), followed by carbapenem antimicrobial susceptibility testing using the disk diffusion method to confirm the presence of carbapenem-resistant Enterobacteriaceae (CRE). CRE refers to Enterobacterales that exhibit resistance to at least one carbapenem antibiotic, namely ertapenem, imipenem, or meropenem. The broth microdilution method or the Vitek 2 system determined antimicrobial susceptibility tests. Tigecycline susceptibility was determined using the US Food and Drugs Administration (FDA) interpretive criteria (US Food Drug and Administration, 2023). Colistin using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint (European Committee on Antimicrobial Susceptibility Testing, 2023), and the remaining susceptibility results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) documentation standards (Clinical and Laboratory Standards Institute, 2022). The Modified Carbapenem Inactivation Method (mCIM) and Modified EDTA-Carbapenem Inactivation Method (eCIM) were employed to detect carbapenemase phenotypes (Pierce et al., 2017).

2.4 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 25.0 software. We first applied the Shapiro-Wilk test for normality to the continuous variables. Continuous variables that were normally distributed with variance equivalence

were denoted as mean ± standard deviation (SD) and analyzed using independent samples *t*-test. While continuous variables that were not normally distributed or had non-equivalent variances were denoted as median with interquartile ranges (IQR) and analyzed using the Mann-Whitney *U*-Test. Categorical variables were analyzed using the Pearson Chi-square test, Continuity correction test, or Fisher's Exact Test. Variables with $P < 0.05$ in the univariate analysis were included in the logistic regression analysis after Collinearity diagnostics and Variance inflation factor (VIF) checks to exclude multicollinearity. Kaplan-Meier curves and the log-rank test for the CRE colonization group vs. the infection group performed survival analysis. All tests were two-tailed and $P < 0.05$ indicated statistical significance.

3 Results

3.1 Patient characteristics

A total of 131 patients were identified as positive for CRE colonization in this study (131/1,390, 9.4%), and the colonization frequency has been seen to increase annually (Supplementary Figure 1). After applying the exclusion criteria, 116 patients with CRE colonization were selected for further study. Eleven patients with CRE colonization subsequently acquired CRE infection (11/116, 9.5%). A control group of 33 out of the 105 patients colonized with CRE but did not acquire subsequent infection was selected using stratified random sampling. This control group was included in the case-control research alongside the group of infected patients (Figure 1). There was a higher proportion of males than females in the case and control groups (both 63.6%). The age of the case group was 35.36 ± 13.44 years, and the age of the control group was 36.33 ± 12.78 years. Acute myeloid leukemia was the most prevalent hematological condition in the case group (5/11, 45.5%), with acute lymphoblastic leukemia being the second most common (4/11, 36.4%). The control group primarily comprised individuals with acute myeloid leukemia (15/33, 45.5%), lymphoma (9/33, 27.3%), and acute lymphoblastic leukemia (3/33, 9.1%). Furthermore, we analyzed the time characteristics of 11 CRE-infected patients from admission to colonization and subsequent infection. The median duration from admission to colonization was 3 days (2–23 days). Similarly, the median duration from colonization to infection was 4 days (2–11 days; Figure 2).

3.2 Microbiological characteristics

Of the 44 CRE-colonized patients, 20 were colonized with *Escherichia coli* (20/44, 45.5%), 16 with *Klebsiella pneumoniae* (16/44, 36.4%), 6 with *Enterobacter cloacae* (6/44, 13.6%), and two with others (2/44, 4.5%). *Escherichia coli* was the most prevalent among patients with secondary CRE infections (5/11, 45.5%), followed by *Klebsiella pneumoniae* (4/11, 36.4%; Figure 3). Out of the 11 cases, 72.7% (8/11) were caused by infectious strains originating from bacteremia, while perianal infections, pneumonia, and urinary tract infections each accounted for 9% (1/11). In addition, we found that CRE colonization showed an overall increasing trend from year to year. *Escherichia coli* was the dominant strain among the colonizers in the hematology department each year (Figure 4).

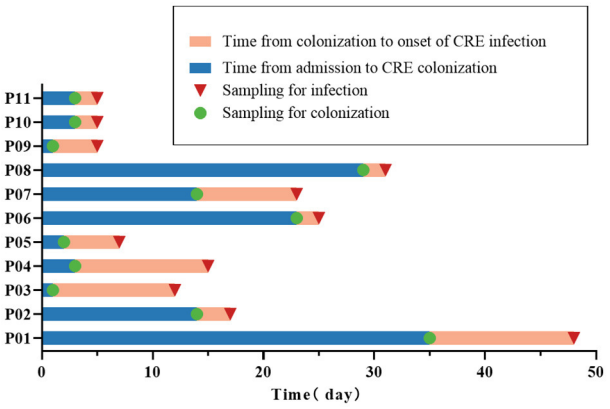

FIGURE 2
Time characteristics of colonization and infection in 11 CRE-infected patients. The 11 CRE-infected patients are denoted as P01-P11.

Based on the CLSI and FDA breakpoints, the infected group showed the greatest susceptibility to colistin (100%) among CRE, followed by tigecycline and amikacin (both 81.8%) and gentamicin (63.6%). 72.7% of CRE exhibited resistance to aztreonam, while 72.7% of CRE showed resistance or intermediate susceptibility to tobramycin. All CRE found in infected patients exhibited resistance to a wide range of antibiotics, including ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefoperazone-sulbactam, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, cefoxitin, cefotetan, ertapenem, imipenem, meropenem, ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole (Supplementary Table 1). In addition, the carbapenemase phenotypes of CRE-infected patients were characterized. Of these, seven strains produced metal β-lactamases (7/11, 63.6%), and four strains produced serine carbapenemases (4/11, 36.4%; Supplementary Table 2).

3.3 Analysis of risk factors for progression to CRE infection

Table 1 displays the findings of the risk factor analysis for the progression from CRE colonization to CRE infection. No significant differences were observed between the case and control groups regarding the prevalence of hematological conditions, comorbidities, invasive operations, and CRE strains (all $P > 0.05$). The median duration of hospitalization in the case group was 29 days (21–47 days), significantly longer than the control group's median duration of 11 days (6–26 days). Additionally, the mean albumin level in the case group was 28.11 ± 3.61 g/L, lower than the mean albumin level of 34.64 ± 6.92 g/L in the control group. In univariate analyses, variables associated with progression to infection included length of hospitalization, application of non-steroidal immunosuppressants, and serum albumin level. In

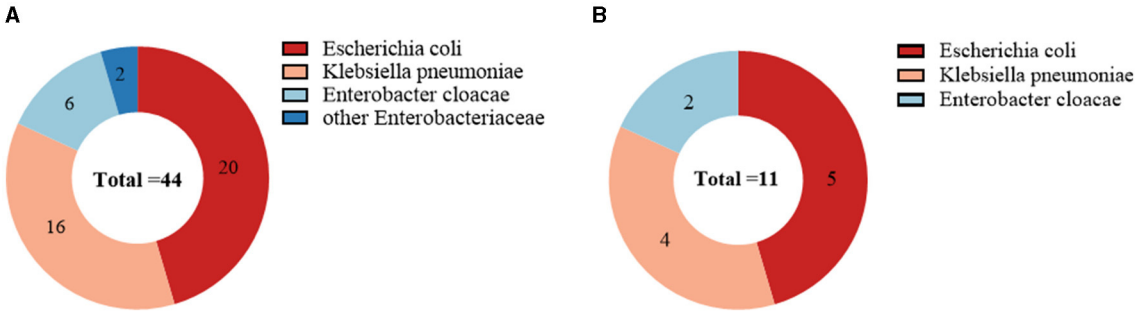

FIGURE 3
Microbial characterization of CRE strains isolated from 44 CRE-colonized patients **(A)**; Microbial characterization of CRE strains isolated from 11 CRE-infected patients **(B)**. *Escherichia coli* was the most common strain for CRE colonization and infection, followed by *Klebsiella pneumoniae*.

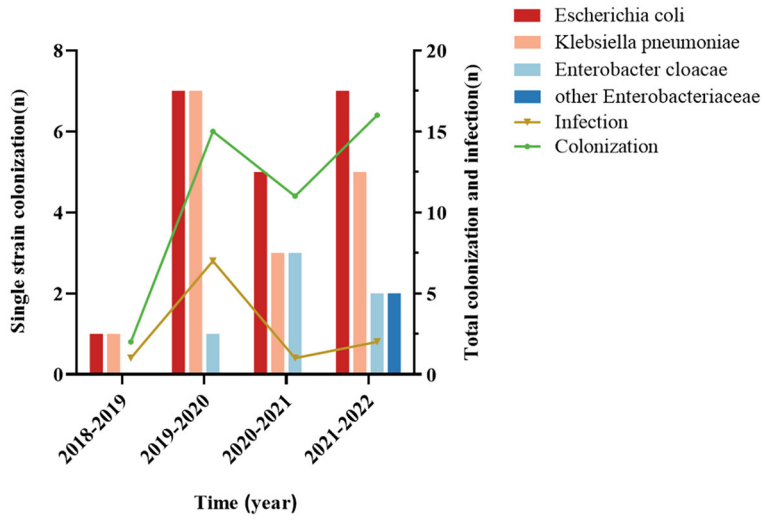

FIGURE 4
Trends of CRE colonization and infection in case-control studies, annual distribution of CRE-colonized strains. The annual trend of CRE colonization was upward, and the *Escherichia coli* counts were significantly different from the other strains.

multivariate logistic regression analysis, immunosuppressants (OR, 19.132; 95% CI, 1.349–271.420; $P = 0.029$) and albumin level (OR, 0.817; 95% CI, 0.668–0.999; $P = 0.049$) were identified as independent risk factors.

3.4 Analysis of risk factors for 30-day mortality in CRE-infected patients

A total of five patients in both the case and control groups died during the 30-day follow-up period (5/44, 11.4%). The case group's mortality rate was significantly higher than the control group (36.5 vs. 3.0%, $P = 0.0026$; Figure 5). Within 30 days, four patients infected with CRE died of bacteremia. In order to determine mortality risk factors among hematological patients infected with CRE, we conducted a comparative analysis of the survival and non-survival groups using the same variables outlined in Table 1. Furthermore, we considered combined carbapenems, *in vitro* sensitive antibiotic treatment, and carbapenemase phenotypes (Supplementary Table 2). Four patients were treated with monotherapy (tigecycline, polymyxin, or aminoglycoside), whereas seven other patients were treated with combination therapy (all combined with tigecycline). Carbapenems were administered in combination to nine patients. Univariate analysis revealed that patients with CRE infection complicated by septic shock had a higher likelihood of mortality. Furthermore, no statistically significant distinction was observed between monotherapy and combination therapy.

4 Discussion

Controlling the spread of CRE has become a critical public health concern on a global scale, necessitating the implementation of infection prevention and control measures (Magiorakos et al., 2017; Zeng et al., 2023). Intensive Care Unit (ICU) admission and hematological malignancies are identified as risk factors associated with CRE infection (Tian et al., 2016; Chen et al., 2022; Zhang

TABLE 1 Univariate analysis and multivariate logistic regression analysis of risk factors for progression of CRE colonization to CRE infection.

Characteristics	Univariable analysis			Multivariate analysis	
	Case	Control	*P*-value	OR (95% CI)	*P*-value
	$n = 11$ (%)	$n = 33$ (%)			
Sex-male	7 (63.6)	21 (63.6)			
Age (years), mean ± SD	35.36 ± 13.44	36.33 ± 12.78			
Hospital stay (days), median (IQR)	29 (21.47)	11 (6.26)	0.004*	1.062 (0.996–1.132)	0.066
Hematological disease					
Acute myeloid leukemia	5 (45.5)	15 (45.5)	1.000		
Acute lymphoblastic leukemia	4 (36.4)	3 (9.1)	0.096		
Lymphoma	0 (0.0)	9 (27.3)	0.131		
Myelodysplastic syndrome	1 (9.1)	2 (6.1)	1.000		
Multiple myeloma	0 (0.0)	2 (6.1)	1.000		
Others	1 (9.1)	2 (6.1)	1.000		
Comorbidities					
Diabetes	1 (9.1)	2 (6.1)	1.000		
Hypertension	0 (0.0)	3 (9.1)	0.561		
Chronic liver disease	0 (0.0)	4 (12.1)	0.545		
Gastrointestinal disease	3 (27.3)	5 (15.2)	0.652		
GVHD	2 (18.2)	3 (9.1)	0.784		
Hemorrhagic cystitis	1 (9.1)	3 (9.1)	1.000		
Mucositis	7 (63.6)	10 (30.3)	0.108		
Pneumonia	9 (81.8)	25 (75.8)	1.000		
Diarrhea	5 (45.5)	6 (18.2)	0.159		
Shock	0 (0.0)	1 (3.0)	1.000		
Previous invasive procedures					
Deep venous catheterization	7 (63.6)	10 (30.3)	0.108		
Urinary catheterization	1 (9.1)	1 (3.0)	0.442		
HSCT	3 (27.3)	3 (9.1)	0.310		
Exposure to drug					
Carbapenems (≤90 days)	10 (90.9)	23 (69.7)	0.315		
Cephalosporins (≤90 days)	8 (72.7)	19 (57.6)	0.592		
Fluoroquinolones (≤90 days)	3 (27.3)	13 (39.4)	0.717		
Aminoglycosides (≤90 days)	2 (18.2)	9 (27.3)	0.841		
Glycopeptides (≤90 days)	2 (18.2)	10 (30.3)	0.696		
Penicillins (≤90 days)	2 (18.2)	10 (30.3)	0.696		
Tigecycline (≤90 days)	5 (45.5)	9 (27.3)	0.455		
Chemotherapy (≤30 days)	9 (81.8)	22 (66.7)	0.567		
Glucocorticoids (≤30 days)	9 (81.8)	19 (57.6)	0.278		
Non-steroidal immunosuppressants (≤30 days)	10 (90.9)	12 (36.4)	0.002*	19.132 (1.349–271.420)	0.029*
PPIs (≤30 days)	6 (54.5)	21 (63.6)	0.858		

(Continued)

TABLE 1 (Continued)

Characteristics	Univariable analysis			Multivariate analysis	
	Case	Control	*P*-value	OR (95% CI)	*P*-value
	n = 11 (%)	*n* = 33 (%)			
Laboratory examinations					
Neutrophils ($\times 10^9$/L), median (IQR)	0.56 (0.03, 3.03)	2.41 (0.93, 3.94)	0.080		
Albumin (g/L), mean ± SD	28.11 ± 3.61	34.64 ± 6.92	0.005*	0.817 (0.668–0.999)	0.049*
CRE isolates					
Escherichia coli	5 (45.5)	15 (45.5)	1.000		
Klebsiella pneumoniae	4 (36.4)	12 (36.4)	1.000		
Enterobacter cloacae	2 (18.2)	4 (12.1)	1.000		
Other Enterobacterales	0 (0.0)	2 (6.1)	1.000		

*Significant statistical difference ($P < 0.05$).
P, test significance; OR, odds ratio; CI, confidence interval; SD, standard deviation; IQR, Interquartile range; GVHD, graft vs. host disease; HSCT, hematopoietic stem cell transplantation; PPIS, proton pump inhibitors.

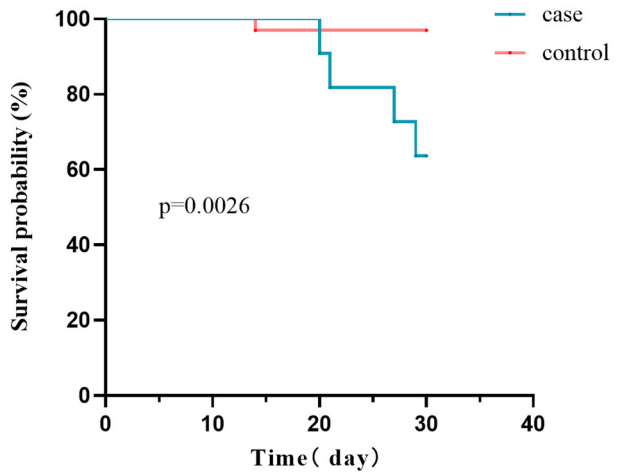

FIGURE 5
Survival curves of the case and control groups. The 30-day mortality rate was considerably higher in CRE-infected patients than in CRE-colonized patients (36.5 vs. 3.0%, $P = 0.0026$).

et al., 2022). Multiple studies have investigated the factors that increase the risk of infection and mortality from CRE in patients with either ICU or hematological malignancies (McConville et al., 2017; Kontopoulou et al., 2019; Lin et al., 2021; Chen et al., 2023). The hematology department admits many immunocompromised patients with hematological disorders who are more vulnerable to CRE infections. Our study's infection rate in patients with CRE colonization was 9.5% (11/116). In patients colonized with CRE, the prevalence of CRE infection varied between 9.1 and 86% (Borer et al., 2012; Schechner et al., 2013; Dickstein et al., 2016; Tischendorf et al., 2016; Gomides et al., 2022). Due to the different types of diseases and treatments administered to patients in each department, the results may be inconsistent; therefore, it is essential to examine hematology departments separately for CRE infection risk factors. Additionally, CRE colonization is a critical factor in CRE infection, which does not occur in all patients with colonization (Gorrie et al., 2017; Qin et al., 2020). Only a limited number of studies have been conducted concerning the risk factors that contribute to CRE infection in patients with hematological diseases (Zhang et al., 2019). Our study aims to identify the risk variables associated with the progression from colonization to infection in hematological patients and the risk factors for mortality in infected patients. These could provide clinicians with recommendations for preventing and controlling CRE infections.

The two studies on hematological malignancies had CRE colonization rates of 6.56 and 10.3%, respectively (Zhu et al., 2022; Chen et al., 2023). The CRE colonization rate in studies conducted in the ICU ranged from 15.5 to 45.4% (McConville et al., 2017; Kontopoulou et al., 2019; Gomides et al., 2022). The CRE colonization rate in this study was 9.4% (131/1390), similar to the studies on hematological malignancies and lower than the ICU colonization rate. A prospective study also suggests intestinal CRE colonization is more prevalent in the ICU, with widespread rapid spread (Chu et al., 2022). Furthermore, Cao et al. (2022) observed that the CRE colonization rate among recipients of allogeneic hematopoietic stem cell transplantation would be marginally elevated at 23.8%. The colonization rate exhibits variation within different departments and may be associated with pharmacological therapies, patient groups, and clinical settings.

Our study also observed an overall trend of increasing CRE colonization in hematological patients throughout the years, aligning with the findings of a multicenter investigation (Fasciana et al., 2023). Nevertheless, the growth rate in 2020–2022 is considerably diminished compared to 2018–2020. This can be ascribed to the proactive implementation of the national policy regarding the rational utilization of antimicrobial drugs in clinical practice and the enhanced surveillance of hospital infections by medical institutions in recent years (China Antimicrobial Resistance Surveillance System, 2023). Medical institutions must prioritize improving the appropriate utilization of antimicrobial drugs, minimizing the excessive use of broad-spectrum antimicrobial medicines like carbapenems, and

effectively implementing hospital infection control measures to contain the widespread transmission of drug-resistant bacteria.

Wang et al.'s (2018) longitudinal large-scale CRE data revealed that the predominant strain of clinical CRE isolates was *Klebsiella pneumoniae*, which exhibited a yearly upward trend. *Escherichia coli* was the primary strain that colonized the hematology department of our hospital, both in terms of total colonization and colonization on an annual basis. The major strain of secondary CRE infection was *Escherichia coli*, followed by *Klebsiella pneumoniae*, consistent with a prior study conducted in the Department of Hematology (Zhang et al., 2019). The infected strains mainly produced metal β-lactamases, and the choice of antibiotics varied for different carbapenemases (Wang et al., 2018). KPC is a serine enzyme that hydrolyzes aztreonam but can be inhibited by the novel enzyme inhibitors avibactam and vebobactam, whereas NDM is a metalloenzyme that does not hydrolyze aztreonam but is not inhibited by avibactam and vebobactam. Hence, it is critical to identify the carbapenemase phenotype for subsequent antimicrobial treatment.

Chemotherapy, invasive operations, ICU admission, prolonged hospitalization, and exposure to carbapenem antibiotics are common risk factors for acquiring infections in patients with CRE colonization (Schechner et al., 2013; McConville et al., 2017; Collingwood et al., 2020; Chu et al., 2022; Chen et al., 2023). In our univariate analysis, prolonged hospitalization was identified as a risk factor for developing infections in patients harboring CRE colonization. Nevertheless, when doing multivariate analyses, the duration of hospitalization was not shown to be statistically significant. Instead, non-steroidal immunosuppressants and albumin levels were identified as independent risk factors for the progression of infections.

While prior use of carbapenems was a common culprit, and chemotherapy as well as proton pump inhibitors (PPIs) have also been found to be associated with infections (Chen et al., 2023), our analysis of previous drug exposures showed that immunosuppressant use significantly increased the risk of infection in patients (OR, 19.132; 95% CI, 1.349–271.420; $p = 0.029$). Moreover, a recent international matched case-control-control study found that immunosuppressive drugs in an inpatient population were risk factors for CRE infection (Perez-Galera et al., 2023). Many individuals with weakened immune systems due to hematological malignancies and hematopoietic stem cell transplants are admitted to hematology departments. These patients are regularly exposed to chemotherapy and immunosuppressive medications (Bar-Yoseph et al., 2019). Myelosuppression, an adverse effect, can be experienced with any immunosuppressive drug and may result in agranulocytosis, hence increasing vulnerability to infections (Fraiser et al., 1991; Lee et al., 2016; Toksvang et al., 2022). Furthermore, reducing albumin levels amplifies the susceptibility to infection in patients with CRE colonization (Rao et al., 2020; Liu et al., 2022; Qian et al., 2023). Critically sick patients often exhibit hypoalbuminemia, which is characterized by a gradual depletion of vital protein components in the body due to inflammation caused by infection (McMillan et al., 2001). Hence, it is imperative to exercise caution in administering immunosuppressive medicines and promptly address hypoalbuminemia in patients with CRE colonization to decrease CRE infections effectively.

In the present study, the 30-day mortality rate of patients with CRE infection was 36.4%, similar to that in studies on patients with hematological diseases (Liu et al., 2019; Zhang et al., 2019; Chen et al., 2023). The independent risk factor for death was septic shock, a variable that has been found to be associated with high mortality in several previously conducted studies (Tumbarello et al., 2012; Daikos et al., 2014; Chen et al., 2022, 2023). Septic shock is a sign of severe infection, and critically ill patients are more likely to die after the onset of the disease. Our study found that combination therapy did not reduce patient mortality, which is inconsistent with previous studies (Daikos et al., 2014; Tumbarello et al., 2015; Trecarichi et al., 2016; Chen et al., 2022). However, Paul et al. propose that patients infected with pathogens that exhibit limited susceptibility to antibiotics *in vitro* are more likely to be prescribed monotherapy. Conversely, patients infected with microorganisms more susceptible to *in vitro* susceptibility antibiotics are more likely to receive combination therapy. Patients with multiple antibiotic resistance may be more severely ill at baseline than patients with less antibiotic resistance. Consequently, the comparisons made between monotherapy and combination therapy may be influenced by selection bias (Paul et al., 2014). Our findings indicate that individuals with clinically severe disease had a higher likelihood of being prescribed combination therapies. Furthermore, the sample size of patients receiving combination therapy is small. Therefore, the effect of combination therapy needs to be evaluated in randomized controlled trials.

This study has some limitations. First, the study was single-center, and the results may not be generalizable to other departments and regions. Second, the study was retrospective and could not determine that the deaths were caused entirely by the CRE, thus making it impossible to analyze the attributable mortality rates. Third, the small sample size of infection and bias in treatment selection had limited ability to analyze risk factors for death. Therefore, multicenter prospective studies are necessary to address these issues.

5 Conclusion

In summary, the results of our study suggest that careful use of non-steroidal immunosuppressive agents and prompt correction of reduced albumin levels in hematological patients with CRE colonization can help reduce the incidence of CRE infections. Septic shock leads to a significant increase in mortality in patients with CRE infection. These findings may help clinicians take appropriate precautions to reduce the incidence of CRE infections and decrease mortality in such patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Biomedical Research Ethic Committee of Shandong Provincial Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

ZW: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. CS: Writing – review & editing, Supervision, Resources. JS: Writing – review & editing, Supervision, Resources. YH: Writing – review & editing, Supervision, Resources. YJ: Resources, Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by Shandong Provincial Natural Science Foundation (ZR2021MH214).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1355069/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Systematic Review
PUBLISHED 10 May 2024
DOI 10.3389/fmicb.2024.1385475

OPEN ACCESS

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RECEIVED 12 February 2024
ACCEPTED 17 April 2024
PUBLISHED 10 May 2024

CITATION
Dan MO and Tălăpan D (2024) Friends or foes? Novel antimicrobials tackling MDR/XDR Gram-negative bacteria: a systematic review. *Front. Microbiol.* 15:1385475. doi: 10.3389/fmicb.2024.1385475

Friends or foes? Novel antimicrobials tackling MDR/XDR Gram-negative bacteria: a systematic review

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Gram-negative bacteria have been one of the most studied classes in the field of microbiology, especially in the context of globally alarming antimicrobial resistance levels to these pathogens over the course of the past decades. With high numbers of these microorganisms being described as multidrug-resistant (MDR), or even extended-drug-resistant (XDR) bacteria, specialists in the field have been struggling to keep up with higher prevalence of difficult-to-treat infections caused by such superbugs. The FDA approval of novel antimicrobials, such as cefiderocol (FDC), ceftolozane/tazobactam (C/T), ceftazidime/avibactam (CZA), imipenem/relebactam (IMR), sulbactam/durlobactam (SUL-DUR) and phase 3 clinical trials' results of aztreonam/avibactam (ATM-AVI) has proven that, while all these substances provide encouraging efficacy rates, antibiotic resistance keeps up with the pace of drug development. Microorganisms have developed more extensive mechanisms of resistance in order to target the threat posed by these novel antimicrobials, thus equiring researchers to be on a constant lookout for other potential drug candidates and molecule development. However, these strategies require a proper understanding of bacterial resistance mechanisms to gain a comprehensive outlook on the issue. The present review aims to highlight these six antibiotic agents, which have brought hope to clinicians during the past decade, discussing general properties of these substances, as well as mechanisms and patterns of resistance, while also providing a short overview on further directions in the field.

Systematic review registration: https://www.crd.york.ac.uk/prospero/#searchadvanced, Identifier CRD42024505832.

KEYWORDS

salvage therapy, antibiotic resistance, ceftazidime/avibactam, imipenem/relebactam, ceftolozane/tazobactam, cefiderocol, aztreonam/avibactam, sulbactam/durlobactam

1 Introduction

Emergent antimicrobial resistance stands as a global public health issue that burdens clinicians, patients, and healthcare systems overall. Since the market approval of penicillin more than 80 years ago, the field of antibiotics has been in constant change, however, so was antibiotic resistance. Nowadays, it is thought that antimicrobial resistance lies as the cause of more than 700.000 yearly deaths globally, while hospital-acquired infection levels continue to rise in many parts of the world (Uddin et al., 2021). The last decades have witnessed a decreased interest of pharmaceutical companies regarding the development of novel antimicrobials, mostly due to the cost of production and poor return of investment in comparison with other drugs treating chronic disorders, for instance. Thus, the focus shifted toward the development of antimicrobial

combinations, in addition to ongoing progress in the field of monoclonal antibodies and immunotherapy (Hutchings et al., 2019). Products such as cefiderocol (FDC), ceftolozane/tazobactam (C/T), ceftazidime/avibactam (CZA), imipenem/relebactam (IMR) aztreonam/avibactam (ATM-AVI) or sulbactam/durlobactam (SUL-DUR) have brought hope to both clinicians and the patients' community by their promising efficacy rates in the case of difficult-to-treat infections. However, with most of these infections being caused by multidrug-resistant (MDR) or extended-drug-resistant (XDR) bacteria, these microorganisms have evolved in terms of resistance mechanisms even against these antimicrobial agents (Wang et al., 2020; Mushtaq et al., 2021; Teo et al., 2021; Gaibani et al., 2022; Karakonstantis et al., 2022). This review aims to briefly characterize these substances, highlighting the current patterns and mechanisms of resistance encountered *in vitro*.

2 Methods

The literature review was conducted independently by the authors in November–December 2023 based on the PRISMA 2020 guideline (Page et al., 2021). Articles from all years were sourced on PubMed. Systematic reviews, meta-analyses and clinical trials studying the desired 6 antimicrobials were included. General data about these antimicrobials, as well as resistance profiles of different Gram-negative pathogen strains against these agents have been studied. In addition, a search on PubChem has been conducted in order to obtain the chemical structures of the studied antibiotics. Moreover, a comprehensive assessment of clinical trials involving these antibiotics has been conducted using clinicaltrials.gov. Exclusion criteria were studies focusing only on pharmacokinetics and/or safety, articles presenting the antimicrobials in correlation with other bacterial pathogens. Keywords used in the search were 'antimicrobial resistance,' 'ceftazidime/avibactam,' 'cefiderocol,' 'ceftolozane/tazobactam,' 'imipenem/relebactam,' 'aztreonam/avibactam,' 'sulbactam/durlobactam.' The search formula used was "(antimicrobial resistance) AND [(ceftazidime/avibactam) OR (cefiderocol) OR (ceftolozane/tazobactam) OR (imipenem/relebactam) OR (aztreonam/avibactam) OR (sulbactam/durlobactam)]." Additionally, 10 articles have been selected for presenting further research directions, however the exclusion criteria were maintained. Search results were analyzed by all authors. PRISMA guidelines were followed, and bias risk was not assessed. In total, 1,653 records have been found, out of which 96 have been included as the references of this paper, following the screening criteria. This review has also been included in the International prospective register of systematic reviews (PROSPERO), with the registration ID CRD42024505832. The full flow of the scientific data collection can be consulted in Figure 1.

Abbreviations: MDR, multidrug-resistant; XDR, extended-drug-resistant; PDR, pandrug-resistant; CZA, ceftazidime/avibactam; IMR, imipenem/relebactam; C/T, ceftolozane/tazobactam; FDC, cefiderocol; ATM-AVI, aztreonam/avibactam; SUL-DUR, sulbactam/durlobactam; cUTIs, complicated Urinary Tract Infections; cIAIs, complicated Intra-Abdominal Infections; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; KPC, *Klebsiella pneumoniae* carbapenemase; OXA, oxacillin carbapenemase; VIM, Verona integron-encoded metallo-β-lactamase; NDM, New Delhi metallo-β-lactamase; IMP, imipenemase; AmpC, cephalosporinase class C; CRAB, carbapenem-resistant *Acinetobacter baumannii*; PBP, penicillin-binding protein; ESBL, extended-spectrum β-lactamase; FDA, US Food and Drug Administration; MIC, minimum inhibitory concentration; BSI, bloodstream infections.

3 Results

3.1 General aspects

3.1.1 Antimicrobial class and mechanism of action

3.1.1.1 Cefiderocol

FDC stands as an improved siderophore cephalosporin, sharing structural assets with both ceftazidime (shown in Figure 2), with which it shares the same C-7 side chain, and cefepime, from which it borrows the pyrrolidinium group on the C-3 chain, which translates into an improved stability level against β-lactamases such as KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo-β-lactamase), OXA (oxacillin carbapenemase) or VIM (Verona integron-encoded metallo-β-lactamase) (Table 1). In the case of the C-7 side chain, FDC showcases groups responsible for providing a certain level of stability against bacterial β-lactamases (the oxime and dimethyl groups), as well as an acidic component conferring improved permeability of outer membranes. The innovative structural feature is a chlorocatechol nucleus on the C-3 side chain, which gives cefiderocol the ability to chelate iron. Thus, FDC uses specific iron active transporters in microorganisms to pass through the outer membrane, in a mechanism known as a 'Trojan horse' strategy (Sato and Yamawaki, 2019; Karakonstantis et al., 2022). Moreover, besides being stable against the action of β-lactamases, FDC shows high affinity for penicillin binding proteins (PBPs), especially PBP3 but also *Klebsiella pneumoniae* PBP2 and *Pseudomonas aeruginosa* PBP1a (Ito et al., 2018). Thus, FDC manages to interfere with cell wall synthesis, resulting in cellular apoptosis (Sato and Yamawaki, 2019).

3.1.1.2 Ceftolozane/tazobactam

C/T is a molecular association between a cephalosporin, ceftolozane, and a β-lactamase inhibitor, tazobactam. Ceftolozane is structurally similar to ceftazidime (shown in Figure 2) however showcasing increased stability against AmpC (cephalosporinase class C) β-lactamases – mediated hydrolysis, benefiting from a heavier side chain, which is vital when encountering *Pseudomonas aeruginosa* resistance mechanisms. Tazobactam, on the other hand, is a β-lactamase inhibitor with a β-lactam structural core, making it inefficient against some carbapenemases encountered in novel MDR/XDR bacteria, such as NDM-1, KPC-2, KPC-3 or OXA-48 (Table 1; Van Duin and Bonomo, 2016; Teo et al., 2021).

3.1.1.3 Ceftazidime/avibactam

CZA is constituted of a third-generation cephalosporin, ceftazidime, a molecule sharing chemical characteristics with ceftolozane (shown in Figure 2), and avibactam, a β-lactamase inhibitor which does not belong to the β-lactam class, being a diazabicyclooctane substance. In addition to the properties of ceftazidime, which are similar to the other novel-generation cephalosporins, avibactam creates a reversible covalent bond with the serine residues belonging to the β-lactamase active center, which provides it with extended stability against β-lactamases, even against KPC-2, KPC-3 or OXA-48. However, it is not effective against

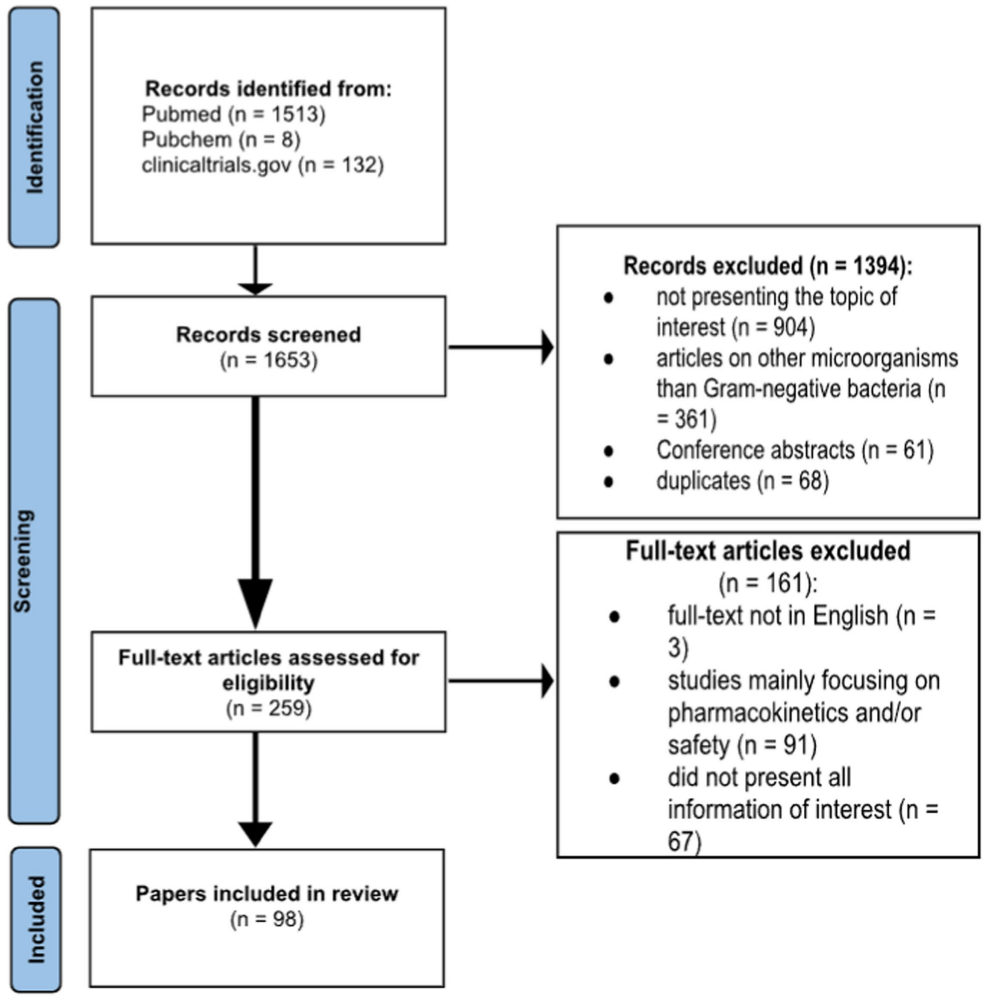

FIGURE 1
Flowchart of data collection, adapted from the PRISMA 2020 flow diagram.

metallo-β-lactamase (IMP, VIM, NDM) producing pathogens, due to the structural characteristics of their active sites, which do not contain serine residues (Table 1; Van Duin and Bonomo, 2016; Mosley et al., 2016; Gaibani et al., 2022).

3.1.1.4 Imipenem/relebactam

IMR combines imipenem, a carbapenem which interferes with the process of cellular wall synthesis by inactivating PBPs contained by the membrane of the bacterial cell, with relebactam, another diazabicyclooctane similar to avibactam (shown in Figure 2), which limits the efflux of the complex via a side chain that presents positive charging. While shown to be effective against the likes of KPC or AmpC, relebactam has been demonstrated to be inactivated by class B metallo-β-lactamases (IMP, NDM, VIM), also showing limited action against OXA-48-like producing pathogens (Table 1; Heo, 2021; Gaibani et al., 2022; O'Donnell and Lodise, 2022).

3.1.1.5 Aztreonam/avibactam

ATM-AVI is a molecular association currently in development, which combines aztreonam, a β-lactam agent stable against metallo-β-lactamase mediated hydrolysis, but otherwise susceptible to being degraded by ESBLs, KPCs or AmpCs, and avibactam, a diazabicyclooctane, which, by being effective against serine-containing β-lactamases, is considered to bring the final antimicrobial agent – ATM-AVI to a maximum level of effectiveness against pathogens producing most types of β-lactamases (Table 1; Mushtaq et al., 2021; Sader et al., 2023).

3.1.1.6 Sulbactam/durlobactam

SUL-DUR, which has recently been approved by the FDA after successful results of phase III clinical trials, brings together two β-lactamase inhibitors – Sulbactam, one of the older antimicrobial agents, a penicillanic acid with limited anti-β-lactamase activity and Durlobactam, a synthetic diazabicyclooctane inhibiting class A, C and D β-lactamases. In terms of target sites, SUL targets PBP1a, PBP1b and PBP3, while PBP2 stands as DUR's target. Together, they stand as a molecular association with efficient inhibitory activity against class A, C and D β-lactamase-producing *Acinetobacter baumannii* strains (Table 1; Keam, 2023; Karruli et al., 2023).

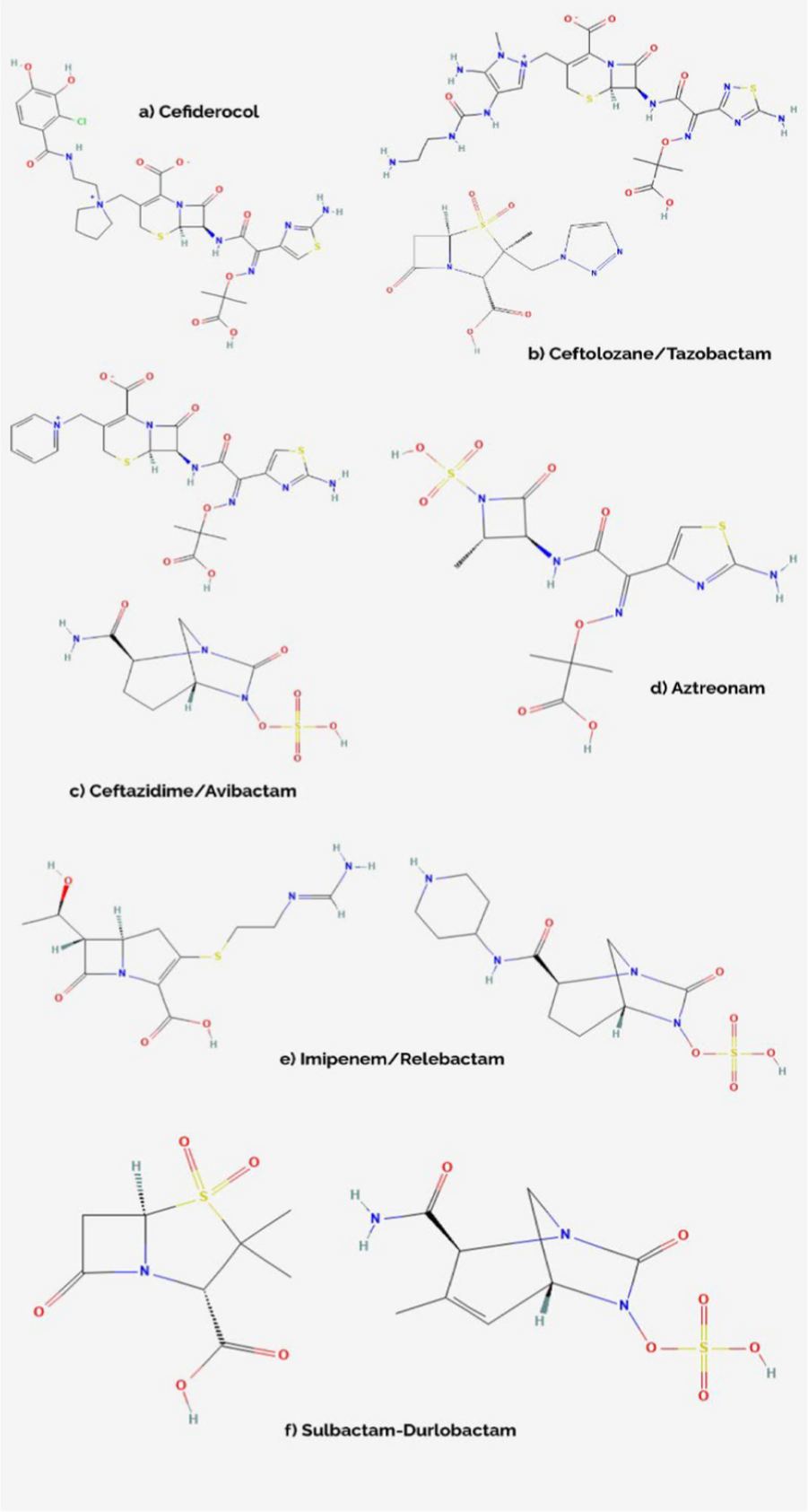

FIGURE 2
Chemical structures of cefiderocol **(A)**, ceftolozane/tazobactam **(B)**, ceftazidime/avibactam **(C)**, aztreonam **(D)**, imipenem/relebactam **(E)**, and sulbactam/durlobactam **(F)** (Avycaz, 2024; Aztreonam, 2024; Cefiderocol, 2024; Ceftolozane-Tazobactam, 2024; Durlobactam, 2024; Imipenem, 2024; Relebactam, 2024; Sulbactam, 2024).

TABLE 1 Action comparison of FDC (cefiderocol), C/T (ceftolozane/tazobactam), CZA (ceftazidime/avibactam), IMR (imipenem/relebactam), ATM-AVI (aztreonam/avibactam), SUL- DUR (sulbactam/durlobactam) against selected β-lactamases.

Antimicrobial	Stable against			
	Amber Class A: KPC	Amber Class B: IMP, NDM, VIM	Amber Class C: AmpC	Amber Class D: OXA-48
FDC	Yes	Yes	Yes	Yes
C/T	No	No	Yes	Yes
CZA	Yes	No	Yes	Yes
IMR	Yes	No	Yes	Limited action
ATM-AVI	Yes	Yes	Yes	Yes
SUL-DUR	Yes	No	Yes	Yes

Adapted after (Van Duin and Bonomo, 2016). KPC, *Klebsiella pneumoniae* carbapenemase; VIM, Verona integron-encoded metallo-β-lactamase; NDM, New Delhi metallo-β-lactamase; IMP, imipenemase; OXA, oxacillinase; AmpC, cephalosporinase class C.

TABLE 2 FDA approval timeline and indications for cefiderocol, ceftolozane/tazobactam, ceftazidime/avibactam, imipenem/relebactam, aztreonam/avibactam, sulbactam/durlobactam including approval extensions for different pathologies and, in the case of ceftolozane/tazobactam, extension for pediatric use.

Antimicrobial agent	Time of approval	Indications	References
Cefiderocol	November 2019, September 2020	cUTI, HAP/VAP	Naseer et al. (2021) and Yusuf et al. (2021)
Ceftolozane/Tazobactam	December 2014, June 2019	cUTI, cIAI, HAP/VAP	Yusuf et al. (2021)
Ceftazidime/Avibactam	February 2015, February 2018, March 2019	cUTI (including pyelonephritis), cIAI (combined with metronidazole), HAP/VAP, including pediatric patients	Yusuf et al. (2021) and Bassetti et al. (2020)
Imipenem/Relebactam	July 2019, June 2020	cUTI, cIAI, HAP/VAP	Yusuf et al. (2021)
Aztreonam/Avibactam	Not yet approved	–	Sader et al. (2023)
Sulbactam-Durlobactam	May 2023	HAP/VAP caused by *Acinetobacter baumannii*- calcoaceticus complex organisms	Keam (2023)

FDA, Food and Drug Administration; cUTI, complicated Urinary Tract Infection; cIAI, complicated Intra-Abdominal Infection; HAP, hospital- acquired pneumonia; VAP, ventilator-associated pneumonia.

3.1.2 Approval timeline and indications

Since the 1940s, development of antimicrobials helped medicine evolve beyond the treatment of infectious diseases, also enabling now common practices, such as invasive maneuvers, improve their safety and efficacy. However, bacteria fought back bringing us to the present public health concern that antimicrobial resistance represents, which combines with decreasing interest for novel antibiotic development on the pharmaceutical companies' side, mainly due to increased research costs and rapid emergence of resistance, leading to low economic turnover. In 2017, a priority list of pathogens for which new antimicrobials' research and development is needed was released by the WHO, placing carbapenem-resistant Enterobacteriaceae as third in the ranking for critical pathogens (World Health Organization, 2017). In accordance with these reports, the second half of the last decade has brought fruitful updates on the approval of novel antimicrobial agents used for the treatment of infections due to MDR/XDR Gram-negative bacteria, especially complicated urinary tract infections (cUTIs), complicated intra-abdominal infections (cIAIs), hospital-acquired pneumonia (HAP) and, respectively, ventilator-associated pneumonia (VAP) (Naseer et al., 2021; Yusuf et al., 2021). Moreover, CZA has also been approved for usage in the case of pediatric patients (older than 3 months) suffering from cUTIs and cIAIs, used in co-therapy with metronidazole (Bassetti et al., 2020). In addition, 2023 witnessed the FDA approval of SUL-DUR for adult patients suffering from *Acinetobacter baumannii* HAP/VAP (Keam, 2023). Nonetheless, ATM-AVI currently stands as a very promising candidate for FDA approval, with recent phase-3 clinical trials showcasing effectiveness and good tolerance levels when tested for the treatment of cIAI, HAP and VAP (Carmeli et al., 2023). The FDA approval timeline and indications for the 6 antimicrobials in our paper can be found in Table 2. However, these antimicrobials are not intended for firstline usage, due to the secondary risk of decreased efficacy caused by emergent resistance. Four of them (FDC, CZA, C/T and IMR) are classified in the 'reserve' group of antibiotics by the WHO, thus should be treated as 'last resort' options. ATM-AVI and SUL-DUR have not been included on this list due to their approval happening after the release of the WHO 2021 AWaRe classification (World Health Organization, 2021).

3.1.3 Effectiveness

Multiple clinical trials have been conducted in patients suffering from cUTI, cIAI, HAP/VAP and even sepsis, focusing on the effectiveness of these antimicrobials in real-life scenarios, as well as comparing clinical and microbiological results with those of antibiotics that have been used in clinical practice beforehand. Comparative results present the fact that all these novel antimicrobials are non-inferior to previous therapies considered to be the best available. The full listing of these trials can be found in Table 3. In

TABLE 3 Results of clinical trials showing non-inferior or improved favorable clinical response rates of patients to treatment with FDC, C/T, CZA, IMR, ATM-AVI, and SUL-DUR as compared with other antimicrobials.

Antimicrobial	Trial (references)	Pathology	Comparator	Outcome rates (tested antimicrobial versus comparator)
FDC	Portsmouth et al. (2018)	cUTI	Imipenem/Cilastatin	Clinical cure: 73% versus 53%
FDC	Wunderink et al. (2021)	HAP, VAP, healthcare-associated pneumonia (HCAP)	Meropenem (high-dose, extended-infusion)	Clinical cure: 65% versus 67% Microbiological eradication: 41% versus 42%
FDC	Bassetti et al. (2021)	HAP, VAP, cUTI, sepsis, bloodstream infections (BSI)	Best available therapy	Clinical cure: HAP/VAP: 60% versus 63% cUTI: 77% versus 60% BSI/sepsis: 70% versus 50% Overall: 66% versus 58%
C/T	Kollef et al. (2019)	HAP, VAP	Meropenem	Clinical cure: 63.8% versus 64.7% Microbiological eradication: 73.1% versus 68%
C/T	Roilides et al. (2023)	cUTI, including pyelonephritis in pediatric patients	Meropenem	Clinical cure: 94.4% versus 100% Microbiological eradication: 93.0% versus 95.8%
C/T in combination with metronidazole	Sun et al. (2022)	cIAI	Meropenem	Clinical cure: 95.2% versus 93.1%
C/T	Chaftari et al. (2022)	Neutropenia and fever in patients with hematological malignancies	Standard-of-care	Clinical cure: 87% versus 72%
C/T in combination with metronidazole	Jackson et al. (2023)	cIAI in pediatric patients	Meropenem	Clinical cure: 80.0% versus 95.2%
C/T	Arakawa et al. (2019)	Uncomplicated pyelonephritis, cUTI	Non-comparative	Favorable clinical response rate: 96.6%
C/T in combination with metronidazole	Lucasti et al. (2014)	cIAI	Meropenem	Clinical cure: 83.6% versus 96%
C/T in combination with metronidazole	Mikamo et al. (2019)	cIAI	Non-comparative	Clinical cure: 92%
CZA	Bradley et al. (2019a)	cUTI in pediatric patients	Cefepime	Clinical response: 88.9% versus 82.6%
CZA in combination with metronidazole	Qin et al. (2017)	cIAI	Meropenem	Clinical cure: 93.8% versus 94.0%
CZA in combination with metronidazole	Lucasti et al. (2013)	cIAI	Meropenem	Clinical cure: 91.2% versus 93.4%
CZA in combination with metronidazole	Bradley et al. (2019b)	cIAI in pediatric patients	Meropenem	Clinical response: 91.8% versus 100%
CZA	Vazquez et al. (2012)	cUTI, including pyelonephritis	Imipenem/cilastatin	Favorable microbiological response: 70.4% versus 71.4%
CZA	Carmeli et al. (2016)	cUTI, cIAI	Best available therapy (in 97% of cases a carbapenem)	Clinical cure: 91.0% versus 91.0%
CZA	Torres et al. (2018)	HAP, VAP	Meropenem	Clinical cure: 68.8% versus 73%
CZA	Wagenlehner et al. (2016)	cUTI, including pyelonephritis	Doripenem	Combined symptomatic resolution + microbiological eradication: 71.2% versus 64.5%
CZA in combination with metronidazole	Mazuski et al. (2016)	cIAI	Meropenem	Clinical cure: 82.5% versus 84.9%
IMR	Kohno et al. (2021)	cIAI, cUTI	Non-comparative	Combined clinical cure: 94.02%

(Continued)

TABLE 3 (Continued)

Antimicrobial	Trial (references)	Pathology	Comparator	Outcome rates (tested antimicrobial versus comparator)
IMR	Sims et al. (2017)	cUTI	Imipenem	Microbiological response: 95.5% (when dosing 125 mg relebactam), 98.6% (when dosing 250 mg relebactam) versus 98.7%
IMR	Lucasti et al. (2016)	cIAI	Imipenem	Clinical response: 96.3% (125 mg relebactam), 98.8% (250 mg relebactam) versus 95.2%
IMR	Titov et al. (2021)	HAP, VAP	Piperacillin/Tazobactam	Clinical response: 61.0% versus 55.8%
IMR	Motsch et al. (2020)	HAP, VAP, cIAI, cUTI	Colistin + Imipenem	Favorable overall response: 71% versus 70%
ATM-AVI (in monotherapy and in association with metronidazole)	Carmeli et al. (2023)	cIAI, HAP, VAP	Meropenem +/− Colistin	Favorable microbiological response: 75.7% versus 73.9%
SUL-DUR	Kaye et al. (2023)	HAP, VAP, BSI caused by CRAB	Colistin	28-day all-cause mortality: 19% versus 32%
SUL-DUR in combination with imipenem	Sagan et al. (2020)	cUTI, including acute pyelonephritis	Placebo	Overall success: 76.6% versus 81%

FDC (cefiderocol), C/T (ceftolozane/tazobactam), CZA (ceftazidime/avibactam), IMR (imipenem/relebactam), ATM-AVI (aztreonam/avibactam), SUL-DUR (sulbactam/durlobactam), (cUTI) complicated Urinary Tract Infection, (cIAI) complicated Intra-Abdominal Infection, (HAP) hospital-acquired pneumonia, (VAP) ventilator-associated pneumonia.

addition, we aim to discuss each antimicrobial's particularities against MDR/XDR microorganisms below, as shown by previously conducted studies, both *in vitro* and *in vivo*.

3.1.3.1 Cefiderocol

While clinical trials reported in Table 3 prove that FDC is non-inferior to its comparators, there have also been smaller studies focusing on its antimicrobial properties against MDR/XDR Gram-negative bacteria. For instance, a 2023 *in vitro* study conducted in the United Arab Emirates revealed that FDC has been highly efficient (97.9% efficacy) against MDR and XDR *Klebsiella pneumoniae* isolates, including carbapenemase producers and double carbapenemase-producers (NDM and OXA-48-like) (Daoud et al., 2023). Similar studies indicated that FDC has potent antimicrobial activity against the vast majority of isolates, including MDR and carbapenem-non-susceptible strains (Jacobs et al., 2018; Kazmierczak et al., 2019). The ARGONAUT-I study exhibited consistent susceptibility levels to FDC in multiple bacterial species, including non-fermenters: 97.0% in *Acinetobacter baumannii* complex strains, 100% amongst *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* isolates (Jacobs et al., 2018).

3.1.3.2 Ceftolozane/tazobactam

While C/T has received approval for several pathologies caused by Gram-negative pathogens, it is most commonly referred to as a viable treatment alternative for MDR/XDR *Pseudomonas aeruginosa* strains. Resistance patterns to C/T, as to most antimicrobials, vary from country to country, but recently published studies report effectiveness in more than 75% of cases (Venuti et al., 2023; Karlowsky et al., 2024; Mendes Pedro et al., 2024). However, a 2023 multicenter study focusing on various infections caused by MDR *Pseudomonas aeruginosa* (HAP, VAP, wound infections, UTI, IAI, catheter-related BSI) showcases that C/T does not provide significant differences regarding clinical outcome, when compared to CZA (Almangour et al., 2023). Additionally, an earlier multicentric study centering around gram-negative bacteria causing pneumonia concluded that, while C/T and CZA showed similar, encouraging susceptibility rates against *Pseudomonas aeruginosa* (96.0% for CZA and 95.9% for C/T), CZA was significantly more efficient against MDR Enterobacterales (99.2% susceptible to CZA, while only 53.8% susceptible to C/T) (Sader et al., 2020).

3.1.3.3 Ceftazidime/avibactam

In addition to the 10 clinical trials concerning CZA included in Table 3, other studies reported significant efficacy of CZA against carbapenem-resistant and MDR Gram-negative pathogens. Wilson et al., focusing on CZA's activity against *Pseudomonas aeruginosa* strains, concluded in a meta-analysis that this molecular association imposed a positive clinical outcome in 73% of infections with MDR or carbapenem-resistant *Pseudomonas aeruginosa* as etiologic agent (Wilson et al., 2021). Moreover, a study published in 2020 proved that over 90% of KPC-2 producing *Klebsiella pneumoniae* strains were susceptible to CZA, as well as this antimicrobial proving efficient against carbapenem-resistant *Pseudomonas aeruginosa* (Yang et al., 2020).

3.1.3.4 Imipenem/relebactam

Data from published studies suggest that, while IMR proves as an efficient alternative when tackling MDR Enterobacterales

(non-*Morganellaceae* Enterobacterales) such as *Escherichia coli*, *K. pneumoniae, Enterobacter* spp., *Citrobacter* spp. or *Serratia* spp., with a susceptibility rate of over 89% against these pathogens, it has shown low activity levels when encountering MBL-producing Enterobacterales, while also posing limited action against OXA-48-like producing Gram-negatives (Karlowsky et al., 2023). Moreover, IMR has proved inefficient against carbapenem-resistant *Acinetobacter baumannii* (CRAB), consistent with previous reports of CRAB resistance to imipenem (Mansour et al., 2021). Thus, IMR is now considered a viable treatment alternative when coming across several MDR Enterobacterales, unless encountering bacteria producing MBL or OXA-48 as an enzymatic resistance mechanism.

3.1.3.5 Aztreonam/avibactam

The last years have seen multiple reports attempting to characterize the combination of aztreonam with avibactam in multiple clinical contexts. Nonetheless, reports show impressive susceptibility rates of Gram-negative bacteria to ATM-AVI (>97%), with this feature preserved in the MDR and XDR subgroups (Wise et al., 2023). However, with MDR/XDR/PDR microorganisms evolving in terms of resistance mechanisms, there is the need for more research in order to appropriately determine the activity of ATM-AVI against highly resistant strains. For instance, a 2023 study conducted in China concluded that ATM-AVI is indeed more efficient than CZA in MBL-producing XDR/PDR *Pseudomonas aeruginosa* isolates, but these microorganisms could potentially carry rare MBL encoding genes, thus it remains to be seen whether or not these resistance genes will become more commonly encountered in futurely described isolates (Kang et al., 2023). Other, smaller studies focused on more diverse Gram-negative microorganisms, such as *E. coli, K. pneumoniae, Enterobacter* spp., *Proteus mirabilis* or *Morganella morganii*. For instance, a 2021-published study assessing MBL-producing Gram-negative strains discovered that the addition of avibactam to aztreonam in previously not-susceptible strains results in significant *in-vitro* antimicrobial activity against 85% of strains (Bhatnagar et al., 2021).

3.1.3.6 Sulbactam/durlobactam

SUL-DUR remains a highly efficient treatment alternative when treating infections caused by *Acinetobacter baumannii*. In a study characterizing globally collected strains from 2016 to 2017, SUL-DUR proved susceptible in over 97% of isolates, far more efficient compared to sulbactam alone (less than 50% susceptibility of isolates) (Karlowsky et al., 2022). When considering CRAB, SUL-DUR still shows promising results, with over 70% of CRAB isolates proving susceptible (Findlay et al., 2022).

3.2 Emergent resistance

Gram-negative pathogens exhibit resistance via 3 main mechanisms (enzyme-mediated antibiotic inactivation, structural changes of antimicrobial targets and cell permeability changes), closely linked to the active targets of antimicrobials and bacterial cell structure. Resistance can be intrinsic, when linked to chromosomal abnormalities in the bacterial genome, or acquired, via bacterial communication through transposable genetic elements, such as transferable plasmids (Breijyeh et al., 2020).

3.2.1 Antibiotic inactivation through enzyme-mediated mechanisms

In accordance with the Ambler classification, there are 4 main categories of β-lactamases: A (which includes KPC and CTX-M, among others), B (which are known as the metallo-β-lactamases, such as IMP, VIM or NDM), C (AmpC and extended-spectrum variants), and D (which includes OXA). From a structural point of view, A, C and D groups contain serine residues at the enzyme's active core, while class B showcases ions of zinc (Sawa et al., 2020). In this case, antimicrobial activity is diminished through the changes of structure in the enzymatic amino-acid chains, these mutant enzymes showcasing insertions or, most frequently, substitutions (Wang et al., 2020). Amino-acid substitutions have been comprehensively studied during the past years, consistent with increasing development in peptide engineering, in order to provide a better understanding of their role in antimicrobial interactions, especially concerning CZA and ATM-AVI. Notably, the amino-acid substitutions which can occur in the Ω-loop of β-lactamases, a structure playing a vital role in the catalytic activity of the enzyme, are linked to increased resistance to ceftazidime, via a mechanism known as 'covalent trapping,' leading to much faster hydrolysis of the substrate (Levitt et al., 2012). More specifically, studies focusing on the Asp179Asn substitution in the structure of KPC-2 confirmed that this mutation increases the MIC of CZA to these bacterial strains, as well as the fact that the addition of avibactam to ceftazidime does in fact increase efficacy against KPC-2 mediated resistance, but it remained insufficient to overcome the resistance conferred by the Asp179 mutations (Barnes et al., 2017). However, the same study showcased that ATM-AVI is efficient against these mutations-carrying variants. Another study revealed that there are no less than 65 structurally different KPC variants harboring resistance to CZA, with the majority of them (43/65) showcasing mutations in the aforementioned Ω-loop and 63% of them (41/65) also presenting insertions or deletions (Hobson et al., 2022). Moreover, resistance to carbapenems has been increasingly reported in AmpC presenting Enterobacterales (Breijyeh et al., 2020).

3.2.2 Structural changes of the antimicrobial targets

Penicillin-binding proteins (PBPs) are protein structures that play a key role in bacterial wall synthesis, which makes them the target sites of FDC, C/T, CZA, IMR ATM-AVI and SUL-DUR. These structures, which can be divided into two categories taking into account their molecular weight - low-molecular weight PBPs and high-molecular weight PBPs, are inhibited by the antimicrobial substances they come in contact with, thus inhibiting the formation of peptidoglycan, a key component of the bacterial cell wall (Levitt et al., 2012). Structural changes at the PBP level have been reportedly linked to increased MIC levels, Alm et al. suggesting that a PBP3 four amino acid insertion is linked to decreased susceptibility to ATM-AVI of *Escherichia coli* (Alm et al., 2015). Moreover, studies show that the same mutation in *E. coli* isolates leads to elevated MIC levels of CZA (Wang et al., 2020). Additionally, mutations of PBP2, PBP3 and, morerarely, PBP1a and

PBP1b are considered the most commonly encountered resistance pathways to SUL-DUR (Principe et al., 2022; Karruli et al., 2023). Ultimately, PBP-mediated resistance has been frequently highlighted in recent research, with most antimicrobials preferentially targeting one PBP with higher affinity, hence the antimicrobial combinations we have discussed having been constructed in order to inhibit more PBPs for utmost efficiency. The literature is scarce in describing the frequency of these structural changes' occurrence, as it most certainly may be a multifactorial characteristic, however researchers consistently report that these modifications greatly impact the efficacy rates of antimicrobials (Sethuvel et al., 2023). Another potential mechanism of resistance is considered to be mutations in the structures of siderophore receptors1or iron transporters. These phenomena are of utmost importance when it comes to bacterial resistance against cefiderocol, due to its chemical properties.

Several studies have described the issue, highlighting multiple genes involved in iron transport pathways and the structure of the siderophore receptor (for instance: cirA, pirA, other TonB-dependent receptor genes, etc.) which can be potentially mutated or underexpressed, thus reducing intracellular uptake of cefiderocol and improving bacterial resistance (Domingues et al., 2023). While it is unsurprising that disturbances in iron metabolic pathway interact with cefiderocol's antimicrobial activity, there have been studies reporting contradictory results, which imply the need for future biomolecular research highlighting the role of these structural changes in cefiderocol resistance (Karakonstantis et al., 2022).

3.2.3 Cell permeability changes

Porins are outer membrane proteins that play a huge role in cellular permeability regulation, allowing hydrophilic substances to enter the cell via passive transport, necessary for cellular processes. Moreover, porins are also closely linked with the peptidoglycan in the bacterial wall structure, adding to their undeniable role in outer cover stability. Gram-negative bacteria present 5 porin types: OmpA, OmpC, OmpF, OmpW, OmpX. The loss of these porins and structural mutations have been shown to decrease antibiotic susceptibility in microorganisms presenting variants of these proteins, thus elevated MICs or even resistance rates have been observed in the case of most antimicrobials, including carbapenems, β-lactamases or third generation cephalosporins. Moreover, it has been shown that interactions between these porins also contribute to the development of resistance (Zhou et al., 2023). More specifically, taking *Klebsiella pneumoniae* as an example, outer membrane proteins OmpK35 and OmpK36 play a very important role in antimicrobial resistance, as there have been reported strong correlations between single or double deletions and increases in MIC levels against multiple antimicrobials (Tsai et al., 2011). Furthermore, it has been shown that homologous outer membrane proteins in different bacterial species present different characteristics. For example, Sugawara et al. discovered that OmpK35 and OmpK36 provide more efficient diffusion of β-lactams through the bacterial membrane than *E. coli* OmpF and OmpC, by creating channels with increased permeability and size (Sugawara et al., 2016).

Other bacterial components that contribute to permeability regulations are efflux pumps. While they can be linked to physiological processes, such as the elimination of cellular metabolites created on the course of respiration or the excretion of siderophores, bacterial-originating iron chelators, an essential adaptive mechanism to low-iron environments, efflux pumps also serve as a way of excreting antimicrobial substances, thus contributing to increased resistance. While they can either be chromosomally encoded or acquired via interbacterial communication, by transposons or plasmids, these structures are linked to complex intracellular regulation systems, grouping themselves into bacterial efflux systems, which can either excrete a specific antimicrobial, or more classes. For instance, efflux pumps belonging to the RND (resistance nodulation cell division) family excrete β-lactams, fluoroquinolones and linezolid, among others, while structures such as TetA are specific for tetracycline (Sharma et al., 2019; Nishino et al., 2021).

4 Discussion

4.1 Further directions

While these novel antimicrobial agents still present favorable effectiveness against a wide range of microorganisms, evolving resistance is a thing the healthcare community should be constantly aware of. Resistance mechanisms need to be further studied in order to be properly explored as potential targets for future therapeutic methods. Besides this, there is a constant need for novel strategies to be developed and, while antibiotic production finds itself at low levels, the focus is switching toward modern methods, such as antimicrobial combinations, immunotherapy, or molecule modeling. Considering the changes in the targets of antimicrobials, novel molecule development should be centered around updating stereospecific binding structures. Moreover, efflux pumps inhibitors are slowly shown as effective adjuvants or alternatives to antimicrobial agents in infections caused by resistant pathogens (Sharma et al., 2019). Nevertheless, the field of molecular science has been in constant evolution and novel approaches have shown promising results. One example of these is the development of dendrimers, nano-sized molecules previously researched for cancer treatment, which have recently been tested for antimicrobial activity via PBP affinity, showcasing promising results (Ahmed et al., 2016). Their silver salt structures have been increasingly studied in order to produce updated molecules, with improved MIC reduction capabilities, via elevated cationic characteristics through extra amino acid conjugation (Schito et al., 2021). More recently, molecular biology has further investigated the field of peptide-derived antibiotic development, which shall open a gateway to new-antimicrobial class discoveries in the future, by addressing the mechanisms of resistance that have been previously encountered, thus enabling the engineering of new means-of-action agents (Upert et al., 2021). After a study conducted both *in vitro* and on mouse models, Zosurabalpin now stands as a clinical candidate for the treatment of severe infections caused by CRAB. By successfully inhibiting the transport of lipopolysaccharide (LPS), more exactly the LptB2FGC complex, this drug provides alterations to the structural integrity of the bacterial cell. This discovery shines new light on how targeting LPS should be a viable strategy for treatment development against XDR/PDR (pandrug-resistant) microorganisms, however more extensive studies need to be performed (Pahil et al., 2024; Zampaloni et al., 2024).

Another interest-worthy field of advancements is represented by the usage of bacteriophages in order to efficiently treat these infections. These agents essentially act via a number of mechanisms, for instance pore creation and enzyme-mediated degradation of the bacterial peptidoglycan, resulting in bacterial cell lysis. While under development for human use, studies involving animal models have shown promising results, thus by adapting these treatments for human usage via improving pharmaceutical properties, we could see important progress in the field in the upcoming years (Zagaliotis et al., 2022).

Nonetheless, it is worth mentioning that there are several other antimicrobials undergoing testing for MDR Gram-negative organisms, such as cefepime/zidebactam or fosfomycin (Tirlangi et al., 2023; Meschiari et al., 2024). Also, while polymyxin analogs have not been previously taken into account when discussing the development of novel antimicrobials, there has been an increased interest in polymyxin engineering via amino-acid substitutions, thus it will be interesting to see how these substances will be able to perform *in vivo*, combined with the usage of machine learning and/or artificial intelligence applied to structuring of polymyxin analogs and other bioactive components (Li et al., 2021).

4.2 Limitations

Several limitations, although frequently encountered in other systematic review papers, need to be taken into consideration. Firstly, we have included only one scientific database into our research process. Even though we consider it to be the most appropriate for conducting a systematic review on our topic, some relevant information found in other databases might have been overlooked. Moreover, the quality of clinical trials included has not been assessed, as their selection was made based on relevance.

5 Conclusion

In a world which finds itself in continuous change, antimicrobial resistance still poses a consistent challenge for the healthcare & patients' communities alike. The six antimicrobials that we have covered in this review are meant to be viable alternatives to the treatment schemes of MDR/XDR/PDR microorganism-borne infections. However, we believe that, in the light of recent emerging resistance of bacteria to these antibiotic agents, susceptibility rates need to be continuously monitored, in order to obtain an appropriate outlook on the course of treatment in each case. Additionally, more in-depth research is needed in order to fully understand the mechanisms of resistance against novel antimicrobials, as well as for finding updated means of tackling it. Nonetheless, antibiotic drug discovery should remain a priority for the healthcare industry in order for novel agents to become potential candidates for clinical usage, as well as alternative molecules arising from the studied means of resistance to be further developed.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. DT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the University of Medicine and Pharmacy Carol Davila, through the institutional program *Publish not Perish.*

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 05 June 2024
DOI 10.3389/fmicb.2024.1405390

OPEN ACCESS

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RECEIVED 22 March 2024
ACCEPTED 21 May 2024
PUBLISHED 05 June 2024

CITATION
Scaglione G, Perego M, Colaneri M, Genovese C, Brivio F, Covizzi A, Viaggi B, Bandera A, Gori A, Finazzi S and Palomba E (2024) Understanding the burden of antibiotic resistance: a decade of carbapenem-resistant Gram-negative bacterial infections in Italian intensive care units.
Front. Microbiol. 15:1405390.
doi: 10.3389/fmicb.2024.1405390

Understanding the burden of antibiotic resistance: a decade of carbapenem-resistant Gram-negative bacterial infections in Italian intensive care units

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Introduction: In patients admitted to intensive care units (ICUs), Gram-negative bacteria (GNB) infections pose significant challenges due to their contribution to morbidity, mortality, and healthcare costs. During the SARS-CoV-2 pandemic, Italy witnessed a rise in healthcare-associated infections (HAIs), with GNBs involved in a substantial proportion of cases. Concerningly, carbapenem-resistant GNBs (CR-GNBs) have increased worldwide, posing therapeutic challenges.

Methods: Retrospective multicentre study analysing data from over 299,000 patients admitted to Italian ICUs from 2013 to 2022.

Results: The study revealed an average of 1.5 infections per patient, with HAIs peaking during the pandemic years. Ventilator associated pneumonia (VAP) emerged as the most common HAI, with *Klebsiella* spp. and *Pseudomonas aeruginosa* predominating. Alarmingly, CR-GNBs accounted for a significant proportion of infections, particularly in VAP, bloodstream infections, and intra-abdominal infections.

Discussion: Our findings underscore the pressing need for enhanced infection control measures, particularly in the ICU setting, to mitigate the rising prevalence of CR-GNBs and their impact on patient outcomes. The study provides valuable insights into the epidemiology of HAIs in Italian ICUs and highlights the challenges posed by CR-GNBs, especially in the context of the SARS-CoV-2 pandemic, which exacerbated the issue and may serve as a crucial example for the management of future viral pandemics.

KEYWORDS

epidemiology, multidrug-resistant, intensive care unit, gram-negative, carbapenem-resistant, hospital-acquired infections

1 Introduction

Infections caused by gram-negative bacteria (GNB) are significant contributors to morbidity, mortality, and healthcare costs among patients admitted to intensive care units (ICUs) (Saha and Sarkar, 2021). ICU patients face heightened vulnerability to GNB infections due to frequent invasive medical procedures and compromised immune responses resulting from trauma, surgery, and underlying medical conditions (Bassetti et al., 2019). Hence, recent data from the European Centre for Disease Prevention and Control (ECDC) indicate that of all patients staying in an ICU for more than two days, 4% presented with ventilator-associated pneumonia (VAP), 3% with bloodstream infection (BSI), and 2% with urinary tract infection (UTI); in almost all cases these conditions were associated with the presence of an invasive device (European Centre for Disease Prevention and Control, 2023).

In Italy, the incidence of healthcare-associated infections (HAIs) increased during the SARS-CoV-2 pandemic from 15.4% in 2006–2007 up to 24.5% in 2020–2021 (Barchitta et al., 2023), with GNBs involved in more than three-quarters of cases (Temperoni et al., 2021; Scaravilli et al., 2022). The increased rates of carbapenem-resistant GNBs (CR-GNBs) worldwide, which might have been exacerbated during the SARS-CoV-2 pandemic, poses a serious threat, leading to limited treatment options, prolonged hospital stays, and increased mortality rates (Daoud and Dropa, 2023).

Pathogens listed by the World Health Organization (WHO) as Priority 1 (World Health Organization [WHO], 2017), including carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, are of particular concern and have increased over the last decade (van Duin and Doi, 2017; Kinross et al., 2022; Torrens et al., 2022). Italy was placed among the worst-performing nations in Europe, characterized by alarmingly high levels of antimicrobial resistance (AMR), with hyper-endemic levels of these microorganisms (European Centre for Disease Prevention and Control, 2017).

However, studies regarding the burden of CR-GNBs in Italian ICUs, which faced great pressure during the COVID-19 era, are scarce and conflicting for both the pre-pandemic (until 2019) and pandemic (2020–2022) periods (European Centre for Disease Prevention and Control, 2017; Pace et al., 2023), leading to possibly even greater uncertainty in the decisions on appropriate antibiotic empiric regimens in this scenario. Understanding the rates and trends of CR-GNB infections over time is crucial for informing clinical decision-making and improving patient outcomes in the face of rising AMR. Therefore, this study aimed to investigate the rates and trends of CR-GNB infections in Italian ICUs over the past decade. Specifically, we focused on WHO priority pathogens *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp., examining their distribution across different infection sites, including VAP, BSI, intrabdominal infection (IAI), and UTI. By assessing differences before and after the SARS-CoV-2 pandemic, we aim to provide valuable insights into the impact of this global health crisis on the prevalence of multidrug-resistant microorganisms in critically ill patients.

2 Materials and methods

2.1 Study design

We conducted a multicentre retrospective and observational registry-based analysis as part of the PROSAFE (Promoting Patient Safety and Quality Improvement in Critical Care) research project. The PROSAFE study was conceived as a prospective observational project aimed at electronically collecting data on patients admitted in over 210 Italian ICUs using a software developed by the GiViTI (Gruppo italiano per la Valutazione interventi in Terapia Intensiva) (Finazzi et al., 2020).

2.2 Data collection

Data collection is ongoing since 2011 through an electronic case report form (eCRF) and is performed by senior ICU specialized physicians working in the participating centers. Our study focused on data collected from 1 January 2013 to 31 December 2022. Two time periods were identified, the "pre-SARS-CoV-2 pandemic" period, between 2013 and 2019, and the "SARS-CoV-2 pandemic" period, from 2020 to 2022.

All patients admitted to general Italian ICUs participating in the PROSAFE project were included in our study.

To ensure data integrity, cross variable checks were performed during data collection and inconsistent or missing data were reported in the eCRF. Validity, according to GiViTI metrics, corresponded to the data regarding patients that were admitted in a period (which length depends on the cardinality of the admissions) where at least 90% of patients' records were complete. Centers with a reported occupancy rate of less than 50% or with significant heterogeneity in the number of monthly admissions received queries or visits from certified monitors. After passing the above-mentioned validation system, all data from ICUs with at least four months of valid data were merged into an aggregated database.

In our study, we collected data on the included ICUs, including the number of admitted patients and geographic locations, alongside patients' demographics, ward of origin, cause of ICU admission, and in-ICU outcomes.

2.3 Definitions

ICU-acquired infections were defined as infections acquired at least 48 h since admission in ICU. Only infections with microbiological confirmation and antibiotic susceptibility testing available were included in the current analysis. Each episode was diagnosed by the physician according to international guidelines (Marshall and Innes, 2003; O'Grady et al., 2011; Centers for Disease Control and Prevention [CDC], 2023) details are reported in the **Supplementary materials**.

All episodes of VAP, BSI, IAI and UTI occurred in the study period were included in the analysis. BSI episodes refer to primary bacteraemia, catheter-related BSI and BSI secondary to another focus of infection. IAI episodes refer to primary, secondary, tertiary

and post-surgical peritonitis, infected pancreatitis, cholecystitis, cholangitis, and intra-abdominal abscesses.

For each site of infection, data were collected and analyzed uniquely for the first episode during the ICU stay. First episode of infection was defined as an infection not active at the time of admission, according to CDC/NHSN definition (Centers for Disease Control and Prevention [CDC], 2023).

CRPA was defined as *Pseudomonas aeruginosa* resistant to meropenem or ertapenem; similarly we identified as carbapenem-resistant any *Klebsiella* and *Acinetobacter* species isolate that did not show susceptibility to at least one carbapenem drug.

2.4 Statistical analysis

Continuous variables were summarized with mean and standard deviation, while categorical data were presented as counts and percentages.

The presence of any trend in the proportions of infections and of CR-GNB in different sites during the years was tested with a binomial regression, utilizing a stepwise selection methodology for optimal model fitting. Additionally, trends in the incidence rates of different HAIs, namely VAP or BSI, were examined over time. A Poisson distribution was assumed for infection counts, and a Poisson regression model was applied with exposure time considered as an offset, while the year of observation served as the independent variable. To capture temporal patterns, orthogonal polynomials were utilized. To assess monotonic and U-shaped dependence, we tested polynomials of degree one and two. If the second order was significant, we also tested higher degrees, selecting the maximum degree through a forward procedure, using a log-likelihood ratio test at a significance level of 0.01. The findings were presented using 95% confidence bands.

2.5 Ethics

The PROSAFE study protocol was approved by the local ethics committees at the participating centers. Written informed consent for use of clinical data was obtained according to national regulations.

3 Results

3.1 Study population

Data from 299,280 patients admitted to the involved Italian ICUs between 2013 and 2022 were included in the analysis. Patients were predominantly male (180477/299280, 60.3%) and aged 65 years or older (180477/299280, 60%). Details on the study cohort are depicted in **Supplementary Table 1**. Mean ICU stay was 6 ± 10 days, and, during this period, 5.9% (17678/299280) of patients had a microbiologically confirmed infection (**Table 1**). Among these patients, more than a half (9405/17678, 53.2%) were referred directly from the Emergency Department, while approximately one-fifth (3401/17678, 19.2%) came from surgical wards and 12% from both medical wards (2050/17678, 11.6%) and other ICUs (2191/17678, 12.4%). Two thirds of the patients, on average, reported trauma (11826/17678, 66.9%). Median ICU stay was 18 days (IQR 11–28) and overall intra-ICU mortality exceeded 20% (3572/17678, 20.2%).

3.2 Overall burden of gram-negative and carbapenem-resistant infection

Over the 10-year study period, 25,966 microbiologically confirmed HAIs were diagnosed by physicians in the included Italian ICUs, with an average of 1.5 infections per patient.

The total number of infections per year was the lowest in 2019 and 2022 (2239 and 2280 episodes respectively) and the highest in 2020 and 2021 (2889 and 3527 episodes); in the remaining years the number of ICU acquired infections was stable between 2300 and 2700 episodes each year. On average the number of HAIs per patient was always higher than 1.0 for the whole study period and, the highest number of HAIs per patient were recorded in 2020 and 2021 (2.19 and 2.21 HAIs per patient respectively), while 2019 was the year with the lowest rates (1.09 HAIs per patient). Further details on HAIs characteristics and distribution are depicted in **Figure 1** and **Supplementary Table 2**.

During the study period, VAP, BSI, IAI, and UTI accounted for 16,080 episodes (61.9%), and VAP was the most common HAI, representing more than one-third of infections (9260/25966, 35.6%), while BSI were the second most frequent, with 2,940 episodes (2940/25966, 11.3%). The incidence of VAP ranged from a minimum of 7.8/1000 mechanical ventilation days (MV, C.I. 7.2–8.3) to a maximum of 15/1000 MV days (C.I. 14.3– 15.7), reported in 2017 and 2021 respectively. The rate of BSI for those with an indwelling venous catheter varied from 1.9/1000 catheter-days (C.I. 1.7– 2.1) in 2016 to 3.4/1000 catheter-days (C.I. 3.1– 3.7) in 2021. The Poisson regression analysis revealed a statistically significant upward trend over the years in both VAP (p-value = < 0.001) and catheter related-BSI (p-value < 0.001) incidence rates (**Figure 2**).

Almost half of the infections acquired in ICU (12060/25966, 46.5%) were attributed to the three GNBs studied (*Pseudomonas aeruginosa*, *Klebsiella* and *Acinetobacter* spp.), with *Klebsiella* spp. being the most frequently isolated overall (5059/25966, 19.5%), and in all four sites of infection considered, followed by *Pseudomonas aeruginosa* (4818/25966, 18.6%) and *Acinetobacter* spp. (2183/25966, 8.4%). Overall, almost a quarter (2927/12060, 24.3%) of all *Pseudomonas aeruginosa*, *Klebsiella* spp. and *Acinetobacter* spp. strains were resistant to carbapenems, with *Klebsiella* spp. expressing most frequently this susceptibility profile (1588/5059, 31.4%), followed by *Pseudomonas aeruginosa* (1049/4818, 21.8%) and *Acinetobacter* spp. (290/2183, 13.3%).

The percentage of GNB infections caused by carbapenem-resistant strains peaked in the years 2013 and 2015 surpassing one in every four infections episodes (27.3% and 27.0% respectively) and then steadily decreased until the year 2018 (20.1%). After that, a new increase in the prevalence of CR-GNBs was observed in 2020, with 24.3% of GNBs isolates displaying resistance to carbapenems. A following decrease was reported in 2021 and 2022 (22.5% and 22.7% respectively).

Among the GNB-associated infections analyzed, VAP was the most frequently reported syndrome, accounting for 20.6% of

TABLE 1 Characteristics and intensive care unit outcomes of 17,678 patients with a documented healthcare-associated infection.

Variable (%)	Total (Pt = 17678)	2013 (Pt = 1818)	2014 (Pt = 1865)	2015 (Pt = 1676)	2016 (Pt = 1865)	2017 (Pt = 1979)	2018 (Pt = 1856)	2019 (Pt = 2050)	2020 (Pt = 1319)	2021 (Pt = 1600)	2022 (Pt = 1650)
Male sex	12027 (68.0%)	1235 (67.9%)	1239 (66.4%)	1144 (68.3%)	1230 (66.0%)	1344 (67.9%)	1297 (69.9%)	1415 (69.0%)	900 (68.2%)	1087 (67.9%)	1136 (68.8%)
Age > 65	9143 (51.7%)	941 (51.8%)	1020 (54.7%)	897 (53.5%)	1001 (53.7%)	1069 (54.0%)	974 (52.5%)	1004 (49.0%)	667 (50.6%)	773 (48.3%)	797 (48.3%)
Trauma before ICU admission	11826 (66.9%)	1215 (66.8%)	1273 (68.3%)	1133 (67.6%)	1274 (68.3%)	1341 (67.8%)	1208 (65.1%)	1342 (65.5%)	866 (65.7%)	1060 (66.2%)	1114 (67.5%)
ICU LOS (Mean ± SD)	22 ± 17	22 ± 17	22 ± 18	22 ± 17	21 ± 16	21 ± 16	22 ± 18	21 ± 16	22 ± 17	21 ± 16	22 ± 16
Source ward											
Medical	2050 (11.6%)	210 (11.6%)	218 (11.7%)	198 (11.9%)	225 (12.1%)	236 (12.0%)	231 (12.5%)	229 (11.2%)	145 (11.1%)	190 (11.9%)	168 (10.2%)
Surgical	3401 (19.3%)	399 (22.0%)	437 (23.5%)	357 (21.4%)	351 (18.9%)	379 (19.2%)	299 (16.2%)	395 (19.4%)	212 (16.2%)	283 (17.7%)	289 (17.6%)
Emergency	9405 (53.4%)	974 (53.8%)	933 (50.2%)	859 (51.5%)	1003 (53.9%)	1026 (52.1%)	987 (53.4%)	1102 (54.1%)	721 (55.0%)	853 (53.5%)	947 (57.6%)
Other ICU	2191 (12.4%)	166 (9.2%)	213 (11.5%)	194 (11.6%)	222 (11.9%)	259 (13.1%)	270 (14.6%)	248 (12.2%)	196 (14.9%)	232 (14.5%)	191 (11.6%)
High dependency unit	561 (3.2%)	63 (3.5%)	59 (3.2%)	61 (3.7%)	60 (3.2%)	70 (3.6%)	60 (3.2%)	64 (3.1%)	38 (2.9%)	37 (2.3%)	49 (3.0%)
ICU-outcomes											
Death	3572 (20.2%)	373 (20.5%)	383 (20.5%)	337 (20.2%)	417 (22.4%)	438 (22.2%)	394 (21.3%)	383 (18.7%)	235 (17.8%)	310 (19.4%)	302 (18.4%)
Transferred within same hospital	9700 (55.0%)	1001 (55.1%)	1049 (56.2%)	955 (57.2%)	1018 (54.7%)	1086 (55.0%)	1015 (54.8%)	1129 (55.2%)	705 (53.4%)	852 (53.4%)	890 (54.1%)
Transferred to other hospital	4186 (23.7%)	428 (23.5%)	410 (22.0%)	362 (21.7%)	410 (22.0%)	429 (21.7%)	418 (22.6%)	510 (24.9%)	359 (27.2%)	423 (26.5%)	437 (26.6%)
Discharged home	71 (0.4%)	8 (0.4%)	6 (0.3%)	7 (0.4%)	9 (0.5%)	10 (0.5%)	5 (0.3%)	9 (0.4%)	5 (0.4%)	5 (0.3%)	7 (0.4%)
Palliative care	120 (0.7%)	8 (0.4%)	17 (0.9%)	10 (0.6%)	7 (0.4%)	13 (0.7%)	19 (1.0%)	16 (0.8%)	15 (1.1%)	7 (0.4%)	8 (0.5%)

Pt: total number of patients admitted to intensive care unit; ICU, intensive care unit; LOS, length of stay; SD, standard deviation.

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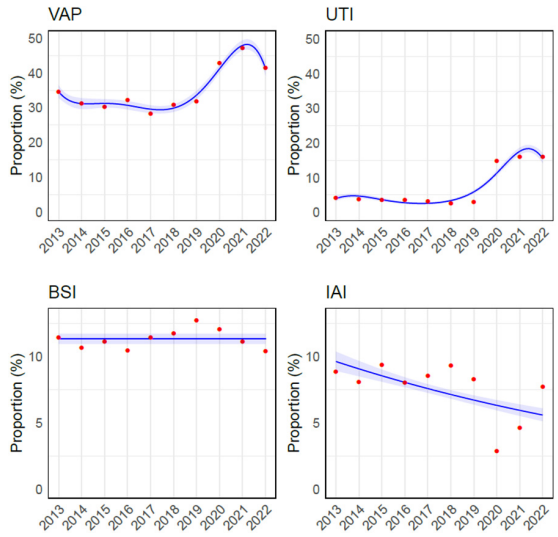

FIGURE 1
Overview of infections acquired in intensive care unit during the study period. The red dots are the observed values, while the curves had been obtained through Binomial approximation. VAP, ventilator-associated pneumonia; UTI, urinary tract infection; BSI, bloodstream infection; IAI, intrabdominal infection.

the episodes (5350/25966), followed by IAI and BSI (920 and 837 episodes, 3.5% and 3.2% respectively), while UTI was less frequent (643, 2.5%).

Carbapenem-resistant pathogens were the causative agent in almost one-third of all IAIs caused by GNBs (303/920, 32.9%) and in one-quarter each for BSIs, UTIs and VAPs (27.4, 25.3 and 24.4% respectively).

A significant increasing trend over time was observed for *Pseudomonas aeruginosa* and for *Klebsiella* spp. (U-shaped, p-value < 0.01 and linear, p-value < 0.001, respectively), while there was no significant trend for *Acinetobacter* (Figure 3A).

The distribution of CR-GNB infections by species during the study period is shown in Figure 3B and detailed in Supplementary Table 2.

3.2.1 *Pseudomonas aeruginosa* infections

Pseudomonas aeruginosa was isolated in 18.6% of infections diagnosed (4818/25966) and displayed resistance to carbapenems in 21.8% of the cases (1049/4818). Almost one-quarter of VAP were caused by *Pseudomonas aeruginosa* (2055/9260, 22.2%), followed by IAI (361/1921, 18.8%), UTI (269/1959, 13.7%) and BSI (223/2940, 7.6%). The highest rates of carbapenem resistance were observed in IAI (109/361, 30.2%) and VAP (487/2055, 23.7%), while UTI and BSI were caused by CRPA in 16% (44/269) and 22% (49/223) of episodes, respectively. CRPA rates peaked between 2013 and 2016 (26% and 24.7% of all ICU-acquired infections, respectively) and gradually decreased in the following years reaching a nadir of 15.6% in 2018; thereafter, a progressive increase was observed, with CRPA rates reaching 20.7%, 23.6% and 25.3% in the years between 2020 and 2022.

A statistically significant correlation between CRPA infection and VAP over time was observed (p-value < 0.01), while no statistically significant evidence was found for other infection sites (Supplementary Figure 1).

Notably, in 2022, CRPA rates were higher than any other CR-GNBs (48.8% of all infections caused by carbapenem-resistant strains).

The trend for *Pseudomonas aeruginosa* infections and the prevalence of carbapenem-resistance per year and by infection site is depicted in Figure 4 and detailed in Supplementary Table 3.

3.2.2 *Klebsiella spp.* infections

Klebsiella spp. was responsible for 19.5% of the total infections acquired during ICU stay (5059/ 25966) showing the highest rates

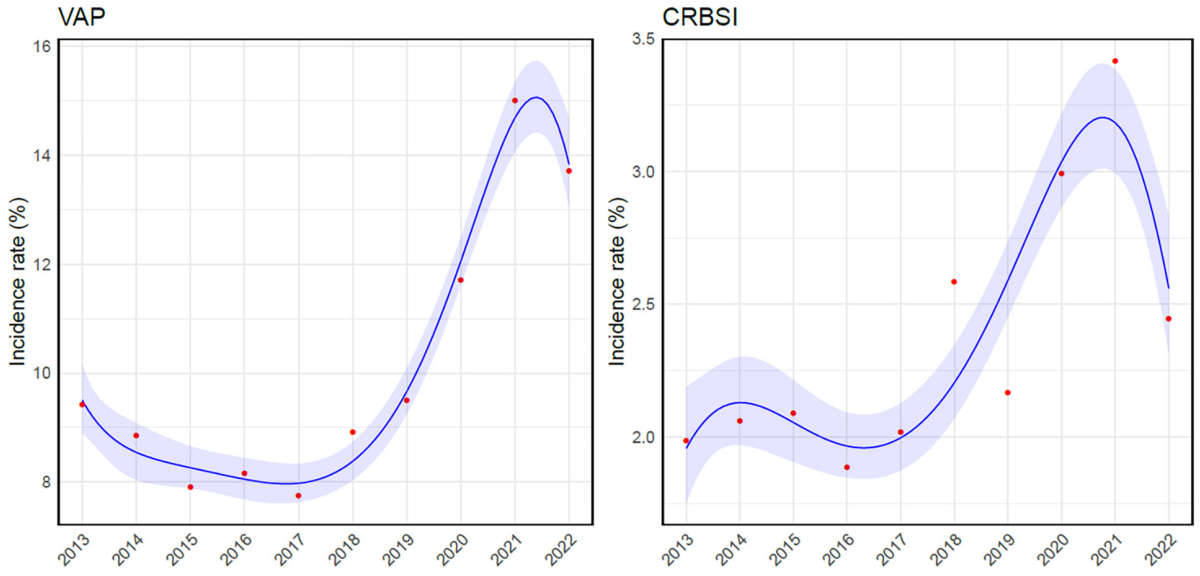

FIGURE 2
Poisson regression analysis correlating ventilator-associated pneumonia and catheter-related bloodstream infection incidence rates and time. VAP, ventilator-associated pneumonia; CRBSI, catheter-related bloodstream infection.

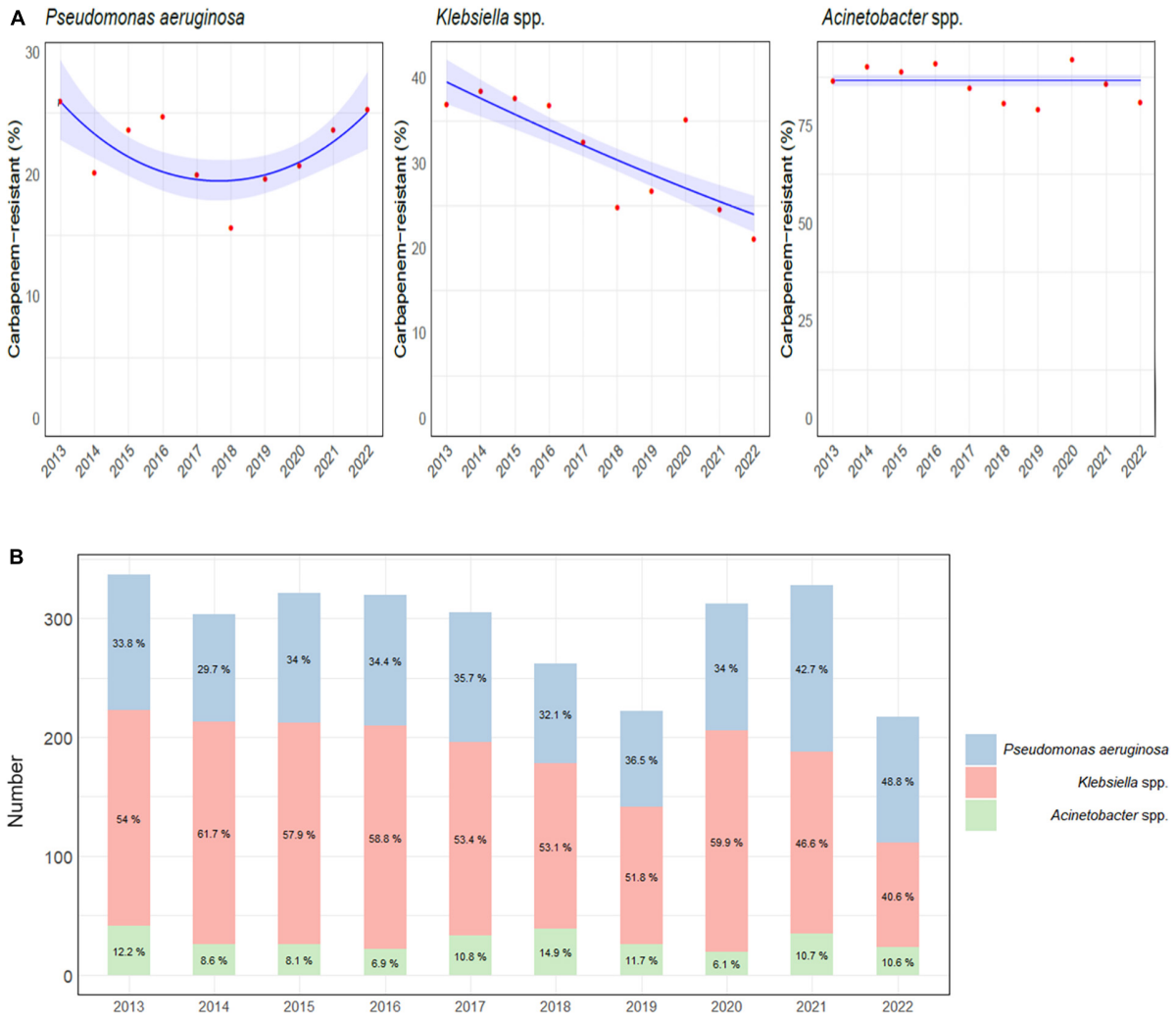

FIGURE 3
Etiology of hospital-acquired infections caused by carbapenem-resistant gram-negative bacteria over the course of the study. **(A)** Trend models of the carbapenem-resistance of *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp. **(B)** Distribution and percentage of hospital-acquired infections caused by carbapenem-resistant *Pseudomonas aeruginosa*, *Klebsiella*, and *Acinetobacter* species.

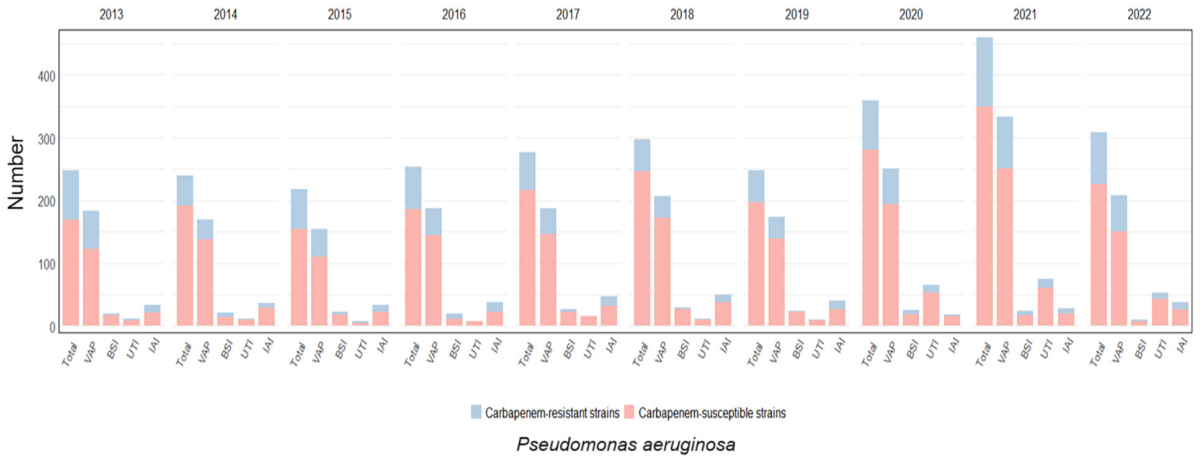

FIGURE 4
Distribution of infections caused by *Pseudomonas aeruginosa* acquired in intensive care during the study period, overall and for infection site, with the prevalence of carbapenem-resistant strains.

of resistance to carbapenems between the GNBs analyzed, with almost one every three *Klebsiella* spp. isolates being not susceptible to this drug class (1588/ 5059, 31.4%).

Furthermore, *Klebsiella* spp. was the most common GNB in all four infections sites considered, particularly in VAP (2149/9260, 23.2%) followed by IAI (388/1921, 20.2%), BSI (467/2940, 15.9%) and UTI (297/1959, 15.2%). The highest rate of resistance to carbapenems was observed in IAI, where almost one every two isolates displayed this phenotype (180/388, 46.4%); although still representing more than one third of cases, the rates were lower for UTI (109/297, 36.7%), BSI (161/467, 34.5%) and VAP (687/2149, 32%).

The highest carbapenem-resistance rates were observed between the years 2013 and 2016, always above 35%, peaking in 2014 when 38.5% of all *Klebsiella* spp. isolates were resistant to carbapenems. In the following years rates decreased steadily until 2018, when resistance rates were 24.7%, with a subsequent peak registered in 2020 (35.1%).

Over the years, a decreasing trend was observed for carbapenem-resistant *Klebsiella* species for VAP (p-value < 0.001) and UTI (p-value < 0.003), while the trend was not significant for BSI (p-value = 0.134) and IAI (p-value = 0.018). The trend is depicted in **Supplementary Figure 2**.

Klebsiella spp. infections and the prevalence of carbapenem-resistance per year and by infection site is shown in **Figure 5** and detailed in **Supplementary Table 4**.

3.2.3 *Acinetobacter* spp. infections

Acinetobacter spp. was the least frequently isolated GNB of the three in study (2183/25966, 8.4%) and displayed the overall lowest rates of resistance to carbapenems (290/2183, 13.3%). This pathogen was mainly responsible for VAP (1146/9260, 12.4%) and to a lesser extent for IAI (171/1921, 9.0%), BSI (147/2940, 5.0%), and UTI (77/1959, 3.9%). The carbapenem resistance proportion was the highest in BSI (19/147, 13.0%), followed by VAP (130/1146, 11.3%), UTI (7/77, 9.1%) and IAI (14/171, 8.2%). Carbapenem-resistant *Acinetobacter* spp. rates varied greatly from one year to the other and this pathogen was most prevalent in the years 2019 (21%) and 2022 (19.2%).

This fact was confirmed by the tests for correlation between the presence of carbapenem-resistant *Acinetobacter* spp. in every infection site and time, that did not show significant results (p-value = 0.349 for VAP, p-value = 0.237 for BSI, p-value = 0.931 for UTI, and p-value = 0.188 for IAI), see **Supplementary Figure 3**. The trend for *Acinetobacter* spp. infections and the prevalence of carbapenem-resistance per year and by infection site is depicted in **Figure 6** and detailed in **Supplementary Table 5**.

4 Discussion

The findings of our study shed light on the epidemiology of HAIs in Italian ICUs over a ten-year period. Our analysis reveals a substantial burden of HAIs, with an average of 1.5 infections per patient over the study period, with high prevalence of CR-GNB, particularly *Pseudomonas aeruginosa*, *Klebsiella* and *Acinetobacter* species. This trend was mainly driven by *Klebsiella* spp. and *Pseudomonas aeruginosa*, with 31.4% and 21.8% of isolate showing this susceptibility profile, respectively. In particular, CR-GNB accounted for a third of IAI and a quarter of each VAP, BSI and UTI caused by these pathogens. Finally, during the SARS-CoV-2 pandemic, ICU-HAIs showed a peak in both incidence and CR-GNB rates, in contrast to a previously declining trend.

The burden of CR-GNBs in ICUs represents a pressing public health concern, as these facilities serve as epicenters for the convergence of critically ill patients, invasive procedures, and frequent antibiotic use, creating an environment ripe for the proliferation of resistant organisms and conditioning the patient outcomes. While rare in other GNB such as *Escherichia coli*, carbapenem resistance in strains of *Pseudomonas aeruginosa*, *Klebsiella* and *Acinetobacter* species has reached worrying percentages in Europe, especially in southern countries such as Italy, Spain, and Greece. Data from the ECDC latest surveillance report (European Centre for Disease Prevention and Control, 2022) showed indeed an increase in the population weighted mean MDR percentage over the period 2018–2022 and an increasing trend in infections caused by CRPA and carbapenem-resistant *Klebsiella* and *Acinetobacter* spp. In particular, *Klebsiella pneumoniae* showed the largest increase against all other bacterial species. Our study confirms how these findings, collected from European hospitals regardless of the intensity of care, are even amplified when focusing on the ICU setting, especially in a country like Italy where MDR prevalence remains notably high. In our cohort, up to half of all ICU-acquired infections were caused by *Pseudomonas aeruginosa*, *Klebsiella* and *Acinetobacter* species, with one every four strains resulting carbapenem-resistant. Notably, the total number of ICU-acquired infections was the highest in the years 2020 and 2021, concomitantly with the first waves of the SARS-CoV-2 pandemic, and VAP was the most common infection, accounting for one third of all HAI and almost doubling its incidence rate when compared to the pre-pandemic periods. Similarly, we observed a steadily decreasing trend of prevalence of CR-GNB infections from 2015 to 2018, followed by a new increase in 2020. The unprecedented threat posed to ICU during the SARS-CoV-2 pandemic indeed provided an ideal landscape for the development of infectious complications and spread of MDR strains (Grasselli et al., 2021; Mangioni et al., 2023a) and may serve as a crucial example for the management of future viral pandemics. The large numbers of patients requiring enhanced care, combined with the need for ICU beds and the creation of new emergency facilities, severely limited infection control practices, favoring outbreaks and the spread of nosocomial pathogens.

This global trend was confirmed in all three GNBs analyzed in our cohort.

More than a fifth (21.8%) of *Pseudomonas aeruginosa* infections were caused by carbapenem-resistant strains, in line with the latest European data that show a global CR rate of 18%, with a slight decrease observed in Italian hospitals, where the resistance to carbapenems decreases to 16% (European Centre for Disease Prevention and Control, 2022). It is noteworthy that three-quarters of Italian isolates analyzed in the report come from a setting other than the ICU, possibly accounting for the difference in MDR proportion with our cohort. Regarding the impact of the SARS-CoV-2 pandemic, we observed a concomitant new peak in CRPA rates, which previously showed a decreasing trend until 2018. Alarmingly, in 2022, the prevalence of CRPA surged to nearly half of all HAIs caused by CR-GNB. Considering the scarce therapeutic options available against this pathogen and the diverse array of resistance mechanisms it harbors, if this data is confirmed in the coming years, it will pose a significant menace to ICUs.

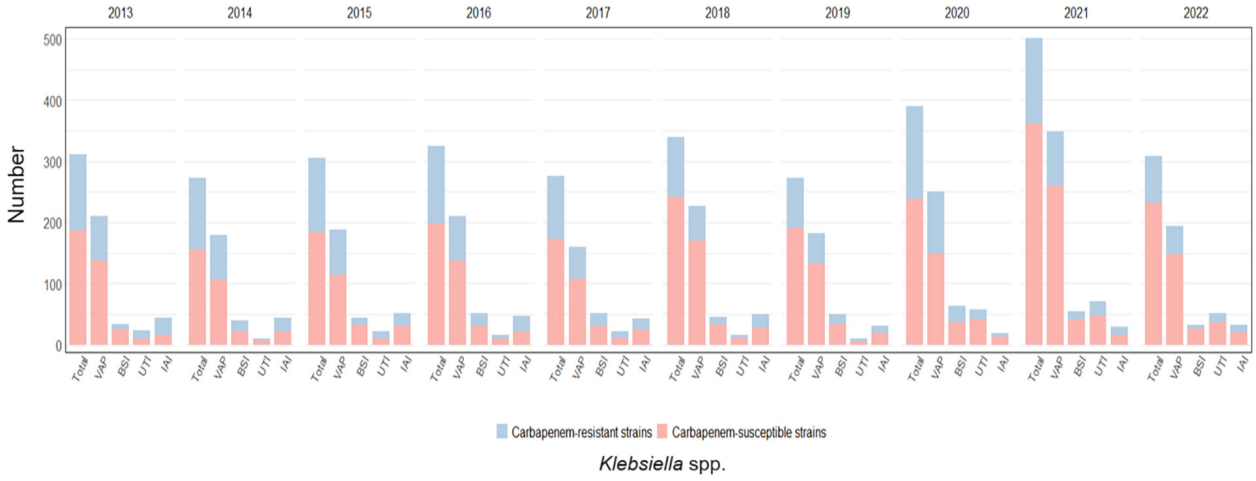

FIGURE 5
Distribution of infections caused by *Klebsiella* spp. acquired in intensive care during the study period, overall and for infection site, with the relative prevalence of carbapenem-resistant strains.

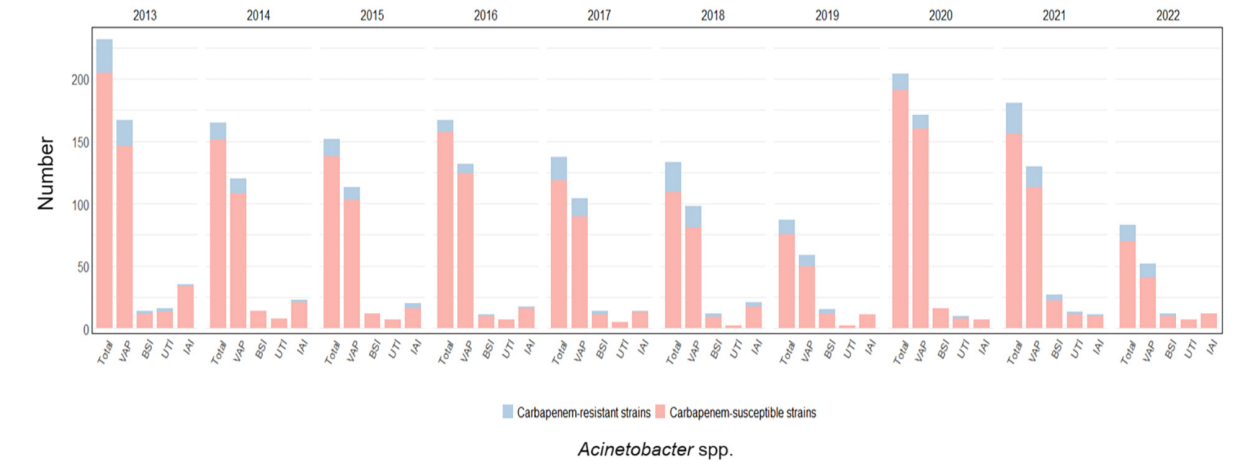

FIGURE 6
Distribution of infections caused by *Acinetobacter* spp. acquired in intensive care during the study period, overall and for infection site, with the relative prevalence of carbapenem-resistant strains.

Klebsiella spp. was the most common retrieved pathogen, both overall and by site of infection, and carbapenem-resistant strains were present in almost a third of all HAIs, in line with the Italian data gathered from the European surveillance (26–29%) (European Centre for Disease Prevention and Control, 2022), causing up to a quarter of all VAP. After a peak in the years 2013–2016, when resistance rates were close to 40%, carbapenem-resistant strains decreased steadily until the pre-SARS-CoV-2 pandemic, and then showed a new increase from 24.7% to a worrying 35.1%, in line with the data from the early years of the 2010 decade.

In our cohort, *Acinetobacter* spp. caused less than one tenth of all ICU-acquired infections and showed the overall lowest rates of resistance to carbapenems (13.3%). This data is in contrast with European and national reports, where carbapenem-resistant strains account for up to one third of all isolates globally, with even higher percentages in Italy, where carbapenem-resistance in *Acinetobacter baumannii* reaches peaks of 88% (European Centre for Disease Prevention and Control, 2022). These differences may be explained by the higher prevalence of carbapenem-resistant strains in settings different than ICU, as other European studies have already observed (Said et al., 2021; Kinross et al., 2022). Furthermore, our analysis only included infections diagnosed by a physician and did not consider respiratory, intestinal and device colonisations, which are often characteristic of *Acinetobacter* species. Finally, we considered all *Acinetobacter* spp. strains, not focusing only on *Acinetobacter baumannii*, which may have partially lowered the overall prevalence of carbapenem-resistance. As confirmed by our findings, infections caused by *Acinetobacter* spp. typically exhibit a varied distribution, marked by sporadic outbreaks, thereby serving as an indicator for evaluating infection control and prevention strategies. The emergence of the SARS-CoV-2 pandemic has accentuated these distinctive patterns, highlighting avenues for enhancing management approaches (Mangioni et al., 2023b).

Notably, IAI showed the highest prevalence of CR-GNB among the infectious syndromes studied, with one in three episodes caused by carbapenem-resistant strains. High rates of carbapenem-resistance in hospital-acquired IAI have already been reported (Liu et al., 2020) and may be explained by the characteristics of critically ill patients suffering from these conditions, with a higher need for invasive maneuvers and indwelling devices, such as drainages, and undergoing surgery on a non-sterile body site.

The study has several limitations that should be acknowledged. First, it relies on retrospective data collected from electronic records, which may be subject to inaccuracies or missing information. Moreover, the retrospective nature of the study limits the ability to establish causality or infer temporal relationships. Our study focuses exclusively on Italian ICUs, limiting the generalizability of the findings to other healthcare settings or regions. Due to its multicentric design, the study did not provide comprehensive data regarding antimicrobial stewardship practices, infection control measures, and individual patient factors, all of which could potentially influence the prevalence and outcomes of infections. Finally, the major limitation of our study was the lack of in-depth clinical data characterizing the infectious episodes described and the effect of MDR on the patients' outcomes. Further analysis to address these research questions are ongoing on this cohort.

Our study also shows several strengths. Firstly, its multicentric design allowed to gather data from ICUs all over Italy, analyzing almost three hundred thousand patients. Secondly, data quality was guaranteed by the strict surveillance on its collection thanks to a validation system, and only the centers with high quality data collection were allowed in the final analysis. Thirdly, only ICU-acquired infections with microbiological confirmation and antibiotic susceptibility analysis available were analyzed, which guaranteed a better characterization of isolates and reinforced the clinical diagnosis. This choice might have resulted in an underestimation of the prevalence of CR-GNB, as it disregarded colonization (such as *Acinetobacter* spp.) and excluded instances where microbiological investigations were not conducted or yielded incomplete results. Finally, our observations spanned over a ten-year period, encompassing both the periods before and during the SARS-CoV-2 pandemic, allowing to understand its impact on the rate of HAIs and particularly on infections caused by CR-GNBs.

In conclusion, our study underscores the escalating threat of MDR infections in ICU settings, exacerbated by the SARS-CoV-2 pandemic. Addressing this challenge, particularly in anticipation of potential future viral pandemics, requires a multifaceted strategy. This approach should encompass rigorous implementation of infection control measures, antimicrobial stewardship programs, and collaborative efforts across healthcare sectors to safeguard patient safety, preserve the efficacy of antimicrobial therapies and mitigate the spread of MDR pathogens.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Local ethics committees at the participating centers. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

GS: Conceptualization, Writing–original draft, Writing–review and editing, Methodology, Supervision, Validation, Visualization. MP: Conceptualization, Data curation, Formal analysis, Methodology, Writing–review and editing. MC: Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing–original draft, Writing–review and editing. CG: Conceptualization, Supervision, Visualization, Writing–original draft, Writing–review and editing. FB: Writing–original draft. AC: Conceptualization, Supervision, Writing–review and editing. BV: Supervision, Validation, Writing–review and editing. AB: Supervision, Writing–review and editing. AG: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Visualization, Writing–review and editing. SF: Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing–original draft, Writing–review and editing. EP: Supervision, Visualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of the article. This research was supported by the EU funding within the Next Generation EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

Acknowledgments

We wish to thank the Gruppo italiano per la Valutazione degli Interventi in Terapia Intensiva (GiViTI), Margherita-PROSAFE project and the Mario Negri Institute for Pharmacological Research. The complete list of the study group collaborators is available in **Supplementary materials**.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1405390/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 16 July 2024
DOI 10.3389/fmicb.2024.1432296

OPEN ACCESS

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RECEIVED 13 May 2024
ACCEPTED 25 June 2024
PUBLISHED 16 July 2024

CITATION
Leanza C, Mascellino MT, Volpicelli L, Covino S, Falletta A, Cancelli F, Franchi C, Carnevalini M, Mastroianni CM and Oliva A (2024) Real-world use of imipenem/cilastatin/relebactam for the treatment of KPC-producing *Klebsiella pneumoniae* complex and difficult-to-treat resistance (DTR) *Pseudomonas aeruginosa* infections: a single-center preliminary experience. *Front. Microbiol.* 15:1432296. doi: 10.3389/fmicb.2024.1432296

Real-world use of imipenem/cilastatin/relebactam for the treatment of KPC-producing *Klebsiella pneumoniae* complex and difficult-to-treat resistance (DTR) *Pseudomonas aeruginosa* infections: a single-center preliminary experience

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Introduction: Real-life experience with imipenem/cilastatin/relebactam (IMI/REL) for the treatment of KPC-producing *Klebsiella pneumoniae* complex (KPC-Kp) and difficult-to-treat resistance (DTR) *Pseudomonas aeruginosa* (DTR-PA) infections is herein described.

Methods: Adult patients with KPC-Kp or DTR-PA infections who received ≥48 h of IMI/REL were included. Clinical and microbiological outcomes were retrieved through the medical records. Primary outcome was clinical cure. Secondary outcomes included mortality from infection onset and adverse effects attributable to IMI/REL.

Results: We included 10 patients with different infections caused by DTR-PA ($n = 4$), KPC-Kp [$n = 5$, of which 3 ceftazidime/avibactam-resistant (CTV-R KPC-Kp), 2 CTV susceptible (CTV-S KPC-Kp)] or both DTR-PA/KPC-Kp ($n = 1$) successfully treated with IMI/REL: 3 hospital-acquired pneumonia, 1 ventilator-associated pneumonia, 2 skin and soft tissue infections, 1 osteomyelitis, 2 bloodstream infections, 1 complicated urinary tract infection. Clinical cure was achieved in all cases. No patients died and no side effect were reported.

Discussion: We reported the preliminary real-life experience on the successful and safe use of IMI/REL for the treatment of KPC-Kp or DTR-PA complicated infections, including pneumonia and bone infections.

KEYWORDS

imipenem-relebactam, KPC-producing *Klebsiella pneumoniae*, imipenem/cilastatin/relebactam, ceftazidime-avibactam resistance, polymicrobial infections, antimicrobial resistance, KPC variant

1 Introduction

Carbapenem-resistant *Enterobacterales* (CRE) and difficult-to-treat resistance (DTR) *Pseudomonas aeruginosa* (DTR-PA) infections constitute an arduous clinical challenge due to limited treatment options (Paul et al., 2022; Tiseo et al., 2022; IDSA, 2023). However, over the last decade, numerous efforts have been made to develop new molecules capable of overcoming antibiotic resistance mechanisms (Tompkins and Van Duin, 2021).

Imipenem-cilastatin-relebactam (IMI/REL) is the combination of imipenem/cilastatin (IMI) with relebactam (REL), a novel non-β-lactam diazabicyclooctane class A/C beta-lactamase inhibitor. The addition of REL restores the activity of IMI against IMI-resistant *Enterobacterales* and *P. aeruginosa*; in contrast, REL has no activity on class B and D beta-lactamases (Papp-Wallace et al., 2018; Smith et al., 2020).

The activity of IMI/REL is therefore directed toward multi-resistant Gram-negative and anaerobes pathogens and its characteristics make it a useful drug against CRE, especially those producing the KPC enzyme, and DTR-PA. Furthermore, the inherent activity of IMI against *Enterococcus faecalis* makes IMI/REL an attractive choice for polymicrobial infections such as intra-abdominal infections.

Based on the results of the registration trials, RESTORE-IMI 1 and 2, IMI/REL received approval by FDA in 2019 and EMA in 2020 for the treatment of complicated urinary tract (cUTI) and intra-abdominal infections (cIAI), with subsequent extension to nosocomial and ventilator-associated pneumonia (HAP/VAP, respectively) sustained by Gram-negative pathogens with limited treatment options[1] (Motsch et al., 2020; Titov et al., 2021).

Currently available real-life experiences suggest a possible use of IMI/REL to treat infections sustained by CRE, DTR-PA and *E. faecalis*; however, actual clinical data remain scarce (Rebold et al., 2021).

Hereby, we describe our preliminary real-life experience on the successful and safe use of IMI/REL for the treatment of 10 patients with complicated infections caused by KPC-producing *Klebsiella pneumoniae* complex (KPC-Kp) or DTR-PA.

2 Methods

This is a single-center case series including adult hospitalized patients treated with IMI/REL for at least 48 h at an Academic University Hospital in Rome.

According to routine Hospitals' Microbiology Laboratory protocol, bacterial pellet obtained from positive blood cultures (BCs) or isolated colonies from other biological samples (sputum or lower respiratory samples, intra-operative samples, deep swabs, urine) were used for bacterial identification by the Matrix-Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS) system (Bruker Daltonik GmbH, Bremen, Germany). Antimicrobial susceptibility testing was performed with the Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) and Microscan Walkaway (Beckman and Coulter, Brea, CA, USA) system. For IMI/REL susceptibility, the gradient strip test was used, and the results interpreted in accordance with guidelines (EUCAST, n.d.).

Infections were defined according to the CDC/NHSN criteria (National Healthcare Safety Network, n.d.). Hospital acquired/ventilator-associated pneumonia (HAP/VAP) were defined in accordance with CDC/NHSN surveillance definition of healthcare-associated infection for pneumonia with specific criteria (Centers for Disease Control and Prevention Website and National Healthcare Safety Network (NHSN), n.d.).

Clinical cure was defined as the resolution of symptoms and/or improvement of laboratory testing after discontinuation of antibiotic therapy.

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the local Ethics Committee (0341/2023). The clinical and diagnostic management of the patients was carried out according to normal clinical practice.

3 Cases description

Ten patients were treated with IMI/REL, with a median age (interquartile range [IQR]) of 64 (57.75–71.5) years, six were males and four females. Monomicrobial infections were caused by DTR-PA ($n = 4$) and KPC-Kp ($n = 5$) [of which 3 ceftazidime/avibactam resistant (CTV-R KPC-Kp), 2 CTV-susceptible (CTV-S KPC-Kp)] and the single polymicrobial infection was caused by both DTR-PA and CTV-R KPC-Kp ($n = 1$).

Infection types included HAP ($n = 3$), VAP ($n = 1$), skin and soft tissue ($n = 2$), bloodstream infection (BSI) ($n = 2$), complicated urinary tract infection (c-UTI) ($n = 1$) and bone infection ($n = 1$). Clinical cure was achieved in all cases. No patients died and there were no side effects (Table 1).

3.1 IMI/REL for the treatment of DTR-PA

3.1.1 Case 1 (bone infection)

A patient with a history of peripheral artery disease and recent partial amputation involving only the distal portion of the second toe of the right foot was hospitalized due to pain, redness, and oedema of the right foot. Diagnosis of osteomyelitis (OM) of the second toe was made. She underwent debridement surgery of the second toe and the culture of intra-operative samples yielded DTR-PA, resistant to cefepime (CEP), carbapenems and ciprofloxacin. IMI/REL monotherapy was administered at a dosage of 1.25 g q6 h for 6 weeks, achieving complete remission of symptoms and clinical cure. No side effects were recorded (Table 1).

3.1.2 Case 2 (VAP)

A patient with a recent tooth extraction was admitted to the ICU for a mandibular abscess caused by *Streptococcus anginosus* and soft tissue emphysema of face and neck requiring orotracheal intubation, for which she was receiving ceftriaxone (CTR). During the ICU stay, she developed VAP caused by DTR-PA. CTR was stopped and IMI/REL 1.25 g q6 h was started and continued for 7 days with symptoms

1 https://www.ema.europa.eu/en/documents/product-information/recarbrio-epar-product-information_it.pdf

TABLE 1 Characteristics of infections treated with imipenem/cilastatin/relebactam (IMI/REL).

Case, age and gender	Clinical history	Previous antibiotic therapy	Ward	Type of infection	Pathogen(s)	Polymicrobial	Dosage	Duration of IMI/REL therapy	Susceptibility testing of IMI/REL	IMI/REL monotherapy/ combination	Outcome
Case #1	Peripheral artery disease, partial amputation of second toe of the right foot	No	Medical	SSTI of the right foot plus OM of the second toe	DTR-PA	No	1.25 g q6 h	6 weeks (plus surgical debridement)	NP	Monotherapy	Clinical cure
Case #2	Recent tooth extraction, mandibular abscess caused by *S. anginosus* and soft tissue emphysema of face and neck requiring endotracheal intubation	CTR, CLI	ICU	VAP	DTR-PA	No	1.25 g q6 h	7 days	NP	Monotherapy	Clinical cure
Case #3	Vascular ulcer left leg, multiple antibiotic allergies, chronic kidney failure	No	Medical	SSTI	DTR-PA	No	0.5 q6h	14 days	NP	Monotherapy	Clinical cure
Case #4	Chronic lymphatic leukemia, epidermolysis bullosa	FDC, DAP, AMP, FOF	Medical	BSI	DTR-PA	No	0.5 g q6 h	10 days	Susceptible (MIC 1 μg/mL)	Monotherapy	Clinical cure
Case #5	H1N1 influenza-related pneumonia with bacterial superinfection by *S. pneumoniae* with type II respiratory failure; previous bacteremic VAP by KPC-Kp	CTR, CTV, FOF, MEV	Medical	HAP	CTV-R KPC-Kp	No	1.25 g q6 h	10 days	NP	Monotherapy	Clinical cure
Case #6	COVID-19 pneumonia, Legionnaires' disease, previous HAP by KPC-Kp, PWID	PIT, AZI, CTV, FOF	Medical	Bacteremic HAP	CTV-R KPC-Kp	No	1.25 g q6 h	14 days	Susceptible (MIC 0.38 μg/mL)	Monotherapy	Clinical cure
Case #7	Infective endocarditis and aortic valve replacement	AMP, CTR, DAP, VAN	Surgical	HAP	CTV-R KPC-Kp	No	0.75 g q6 h then 1 g q6 h	7 days	Susceptible (MIC 2 μg/mL)	Monotherapy	Clinical cure
Case #8	Renal transplant for polycystic kidney disease with liver involvement	CTV	Medical	C-UTI, hepatic abscess	CTV-S KPC-Kp (CTV MIC 8 μg/mL)	No	0.5 g q6 h	3 weeks	NP	Monotherapy	Clinical cure

(Continued)

10.3389/fmicb.2024.1432296

TABLE 1 (Continued)

Case, age and gender	Clinical history	Previous antibiotic therapy	Ward	Type of infection	Pathogen(s)	Polymicrobial	Dosage	Duration of IMI/REL therapy	Susceptibility testing of IMI/REL	IMI/REL monotherapy/ combination	Outcome
Case #9	Skullcap custom prosthesis infection and empyema	MEV	ICU	BSI	CTV-S KPC-Kp	No	1.25 g q6h	10 days	Susceptible (MIC 0.38 μg/mL)	Monotherapy	Clinical cure
Case #10	Polymicrobial necrotizing fasciitis of right leg, type 2 diabetes	FDC, TIG, COL, FOF, DAP, AMS	Surgical	OM of lower right leg and foot	CTV-R KPC-Kp DTR-PA	Yes	1.25 g q6h	7 days (plus amputation of the lower right leg)	Susceptible (KPC-Kp MIC 1 μg/mL, DTR-PA MIC 2 μg/mL)	Combination therapy (FOF)	Clinical cure

AFib, Atrial fibrillation; AMP, ampicillin; AMS, ampicillin/sulbactam; BSI, bloodstream infection; AZI, azithromycin; FDC, cediferocol; CLI, clindamycin; COL, colistin; COPD, chronic obstructive pulmonary disease; CTR, ceftriaxone; c-UTI, complicated urinary tract infection; CTV, ceftazidime-avibactam; DAP, daptomycin; DTR-PA, difficult-to-treat *Pseudomonas aeruginosa*; FOF, fosfomycin; HAP, hospital-acquired pneumonia; KPC-Kp, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* complex; ICU, intensive care unit; MEV, meropenem-vaborbactam; MIC, Minimum Inhibitory Concentration; OM, osteomyelitis; NP, not performed; PIT, piperacillin/tazobactam; PMK, pacemaker; PWID, people who inject drugs; SSTI, skin and soft tissue infection; TIG, tigecycline; CTI, ceftolozane/tazobactam; VAN, vancomycin; VAP, ventilator associated pneumonia. CTV-R, ceftazidime/avibactam resistant; CTV-S, ceftazidime/avibactam susceptible.

remission and clinical cure. No adverse events were observed (Table 1).

3.1.3 Case 3 (skin and soft tissue infection)

A patient allergic to cephalosporins with a history of chronic kidney failure was admitted to the infectious disease ward with a vascular ulcer presenting with pain, redness, and oedema of the left leg. Diagnosis of skin and soft tissue infection was made, and a deep swab of the wound showed the growth of DTR-PA, susceptible at increased exposure (I) for both imipenem (IMI-I, 4 μg/mL) and meropenem (MEM-I, 8 μg/mL). IMI/REL was therefore started at renal adjusted dosage (0.5 g q6 h) and continued for 14 days, achieving complete remission of symptoms and clinical cure and no adverse events (Table 1).

3.1.4 Case 4 (BSI)

A patient with history of chronic lymphocytic leukemia was hospitalized for severe epidermolysis bullosa with polymicrobial skin and soft tissue superinfection requiring several debridements of the infected and necrotizing soft tissues of the left leg and right arm. During hospitalization, the patient acquired carbapenem-resistant *Acinetobacter baumannii* complex (CRAB) and vancomycin-resistant *E. faecalis* (VRE) rectal colonization. He underwent amputation of the right arm and had antibiotic therapy with cefiderocol (FDC) and fosfomycin (FOF) for CRAB BSI and then with ampicillin (AMP) and daptomycin (DAP) for VRE BSI. After approximately 1 month of hospitalization without need for antibiotic treatment, the patient developed fever and septic shock. Given the known colonization by multidrug resistant (MDR) organisms, empiric therapy with FDC, DAP and FOF was started. Blood cultures yielded, CTV-R, IMI-I (4 μg/mL) and MEM-I (8 μg/mL) DTR-PA (IMI/REL MIC 1 μg/mL). Antibiotic therapy was then switched to IMI/REL 0.5 g q6 h for 10 days, with early improvement and clinical cure. No side effect was recorded (Table 1).

3.2 Ceftazidime/avibactam-R KPC-Kp

3.2.1 Case 5 (HAP)

This case describes a patient with a prolonged Intensive Care Unit (ICU) stay for severe H1N1 pneumonia complicated by *S. pneumoniae* superinfection and type 2 respiratory failure. In the ICU, he acquired rectal and respiratory colonization by KPC-Kp and further developed a bacteremic VAP due to KPC-Kp successfully treated with meropenem/vaborbactam (MEV). After transfer to the medical ward, the patient developed HAP caused by colistin and CTV-R KPC-Kp. IMI/REL was administered as monotherapy at a dose of 1.25 g q6 h for 10 days with complete remission of symptoms and clinical cure. No side effects were observed (Table 1).

3.2.2 Case 6 (bacteremic HAP)

A patient was admitted to the ICU with severe COVID-19 pneumonia and Legionnaires' disease. He was later transferred to a medical ward where he developed HAP caused by CTV-susceptible KPC-Kp, successfully treated with CTV and FOF. In the following weeks the patient had respiratory colonization by a CTV-R KPC-Kp strain and, afterwards, developed a bacteremic HAP caused by CTV-R KPC-Kp, with susceptibility to IMI/REL (MIC 0.38 μg/mL). He was

then treated with IMI/REL at the dose of 1.25 g q6 h for 14 days, with early negativization of blood cultures and amelioration of respiratory gas exchanges. The patient achieved clinical cure and did not experience any side effect (Table 1).

3.2.3 Case 7 (HAP)

A patient with a history of chronic obstructive pulmonary disease, atrial fibrillation, and pacemaker implantation, was hospitalized for culture-negative prosthetic aortic valve infective endocarditis and treated with AMP, CTR and DAP along with aortic valve replacement and Bentall procedure. Three days after surgery, the patient developed a CTV-R KPC-Kp HAP IMI/REL MIC 2μg/mL. Treatment with IMI/REL 0.75 g q6 h (further optimized to 1 g q6 h according to improvement of renal function) was started. After 7 days of therapy, clinical cure was achieved and IMI/REL was discontinued. No side effect was recorded (Table 1).

3.3 Ceftazidime/avibactam-S KPC-Kp

3.3.1 Case 8 (c-UTI)

A patient with a history of renal transplantation for polycystic kidney disease and recurrent UTI associated with severe vesicoureteral reflux was discharged after an episode of C-UTI caused by KPC-Kp, successfully treated with CTV for 14 days. Three weeks later, she developed right hypochondrial pain, followed by the appearance of fever. Urine culture was positive for KPC-Kp with CTV MIC 8 μg/mL. A CT scan of the abdomen showed a 11 cm perihepatic abscess. The collection was drained radiologically, and the culture showed no bacterial growth. Because of the high CTV MIC and the presence of an intra-abdominal infection, IMI/REL 0.5 g q6 h was started, with prompt defervescence and clinical amelioration. After 3 weeks of therapy, a repeated CT scan showed the resolution of the abscess. Patient experienced no side effects and was discharged on day 25 with resolution of symptoms (Table 1).

3.3.2 Case 9 (BSI)

A patient, allergic to cephalosporins and with known rectal colonization due to KPC-Kp was admitted to the ICU for a skullcap custom prosthesis infection and subdural empyema caused by KPC-Kp successfully treated with MEV and surgical debridement. Twenty-eight days after the interruption of MEV, the patient developed fever, with blood cultures showing the growth of KPC-Kp (IMI/REL MIC 0.38 μg/mL, CTV MIC 4 μg/mL, MEV MIC 1 μg/mL). Given the known allergies to cephalosporins and the unavailability of MEV at the hospital pharmacy, IMI/REL 1.25 g q6 h was administered. Blood cultures, performed after 48 h of antibiotic therapy, showed no bacterial growth. Antibiotic therapy was administered for 10 days. Clinical cure was achieved, and no side effects were recorded (Table 1).

3.4 DTR-PA and ceftazidime/avibactam-R KPC-Kp polymicrobial infection

3.4.1 Case 10 (skin and soft tissue infection)

A patient with a history of type 2 diabetes was initially admitted to the ICU for necrotizing fasciitis of the right leg requiring repeated fasciotomies and hyperbaric chamber sessions. Intraoperative specimens identified *Streptococcus pyogenes*, *Enterobacter aerogenes*, and CRAB requiring the need of multiple courses of antibiotics within the first month of hospitalization, including FDC, tigecycline (TIG), colistin (COL), ampicillin/sulbactam (AMS), FOF and DAP. Rectal colonization by CTV-R KPC-Kp was acquired during hospital stay. Three weeks after antibiotic discontinuation, fever developed and, according to the known MDR colonizations, empirical therapy with DAP, COL, FOF and AMS was started. Deep wound microbiological samples and bone biopsy yielded CTV-R KPC-Kp (IMI/REL MIC 1 μg/mL) and DTR-PA (IMI/REL MIC 2 μg/mL). Empiric antibiotic therapy was then replaced with IMI/REL 1.25 g q6h and FOF 4 g q6 h with rapid defervescence and amelioration of the general conditions. However, the local conditions remained severely compromised, requiring lower leg amputation on day 4 of IMI/REL, which was stopped on day 7 after complete remission of systemic symptoms and in the absence of adverse events. There was no recurrence of infection at 1 month follow-up (Table 1).

4 Discussion

We reported a preliminary clinical experience with IMI/REL, a newly available and promising treatment option for DTR Gram-negative pathogens. This real-life case series, although with a limited sample size, gives support to the role of IMI/REL in the management of complex infections sustained by DTR-PA and KPC-Kp, the latter especially in the presence of CTV resistance or CTV higher MIC. To the best of our knowledge, our report is the first reporting the successful use of IMI/REL for the treatment of CTV-R KPC-Kp. Of note, in the cases herein described, clinical cure was always achieved, and no side effects or deaths were recorded.

The launch of new beta-lactam beta-lactamase inhibitors (BL/BLIs) such as CTV, ceftolozane/tazobactam (CTT), MEV and IMI/REL broadened the possibilities of treating infections caused by carbapenem-resistant Gram-negative bacteria (Volpicelli et al., 2021). Indeed, according to the available guidelines, the new BL/BLIs represent the drugs of choice for the treatment of infections caused by CRE (CTV, MEV and IMI/REL) and DTR-PA (CTT, CTV and IMI/REL), although the limited clinical experience with IMI/REL placed this molecule as an alternative agent (Paul et al., 2022; Tiseo et al., 2022; IDSA, 2023).

To the best of our knowledge, there are only three real-life experiences on the use of IMI/REL reported so far (Rebold et al., 2021; Larcher et al., 2022; Shields et al., 2024).

Larcher et al. carried out a monocentric observational cohort study describing a pool of hospitalized individuals who received beta-lactam antibiotics as a last resort for treating severe infections caused by DTR gram-negative bacteria. Three of them (1 HAP, 1 VAP, 1 bone and joint infection) were sustained by DTR-PA and treated with IMI/REL. Interestingly, IMI/REL was administered as monotherapy only once. Clinical and microbiological cure was obtained in all cases and one patient developed an adverse event (eosinophilia) (Larcher et al., 2022).

Encouraging results arise also from a multicenter retrospective study conducted in the U.S. including 21 infections treated with IMI/

REL: 11 pneumonias (of which 2 bacteremic), 3 UTIs (of which 2 bacteremic), 3 device-associated infections (of which 1 bacteremic), 2 IAIs, 1 SSTI, 1 bone and joint infection. Infections were mostly caused by *P. aeruginosa* (16/21, 76%), even though *K. pneumoniae* was the causative agent in three patients (14%). Clinical cure was reached in 62% of cases (13/21), while the 30-day mortality was 33% (7/21). Overall, only two adverse events (AEs) occurred: 1 gastrointestinal (nausea, vomiting, diarrhea) and 1 encephalopathic effect (altered mental status, drowsiness, new-onset seizures), none of which requiring drug discontinuation. With regard to the infections specifically sustained by *K. pneumoniae* ($n = 3$), only two were caused by carbapenem-resistant strains (not specified resistance enzyme), one was Extended Spectrum Beta-Lactamase (ESBL)-producing and carbapenem susceptible. All infections were polymicrobial. Cure was achieved in two cases (66%), death occurred in two cases (Rebold et al., 2021).

The most representative experience reported so far was performed by Shields et al. in a multicenter retrospective study describing the real-world use of IMI/REL across representative US hospitals including 160 infections from 63 facilities. IMI/REL was typically administered after therapy with other β-lactams and was given for a median duration of 8 days (IQR 4–13). The most common infections were HAP or VAP (53.8%) and cUTI (16.9%). Microbiology data were available for only 37 patients, with *P. aeruginosa* being the most common ($n = 33$, 89.2%), followed by *K. pneumoniae* ($n = 7$, 18.9%), *Enterobacter cloacae* ($n = 4$, 10.8%), and *Escherichia coli* ($n = 4$, 10.8%). Polymicrobial infections occurred in 35.1% ($n = 13$) of patients. Among the *Enterobacterales*, only one was carbapenem-resistant, 10.8% ($n = 4$) were ESBL producers, while 75.7% ($n = 28$) of *P. aeruginosa* isolates were MDR. The 30-day mortality rate was 21.3% ($n = 34$) (Shields et al., 2024).

Despite the current availability of several molecules for the treatment of DTR-PA and CRE, IMI/REL presents peculiar characteristics that could make it more suitable than the other new BL/BLIs in some circumstances. In fact, both IMI and REL show good penetration in the epithelial lining fluid (ELF), corresponding to approximately 50% of plasma concentrations for each component, a value higher than CTV (26–31/35%), similar to CTT (48%) and lower than MEV (63/79%), suggesting its potential use for the treatment of lung infections (Rizk et al., 2018).

This is in accordance with the registration trials of IMI/REL, RESTORE-IMI 1 and 2. In the RESTORE-IMI 2, IMI/REL demonstrated non-inferiority to piperacillin/tazobactam (PIT) in the treatment of HAP/VAP, with promising data for both 28-day mortality (15.9% IMI/REL vs. 21.3% PIT) and favorable clinical response (61% IMI/REL vs. 55.8% PIT) (Titov et al., 2021).

Similarly, in the small HAP/VAP subgroup of the RESTORE-IMI 1 (8 treated with IMI/REL and 3 treated with COL plus IMI), which included infections caused by non-susceptible IMI pathogens, IMI/REL was associated with a good clinical response and a 20% reduction in 28-day mortality compared to the control group (Motsch et al., 2020). It should be noted, however, that in RESTORE-IMI 1 there was an overall prevalence of DTR-PA infection compared with CRE (24 vs. 6) and that the included cases were few.

In accordance to the literature (Smith et al., 2020), we found that the addition of REL to IMI was able to restore IMI *in vitro* activity in all the tested strains including both CTR-PA and KPC-Kp, as depicted in Table 2.

The structure of REL, which is characterized by the presence of a diazabicyclooctane core, is similar to that of avibactam (AVI); however, compared to AVI, REL has a piperidine ring which makes it more stable toward KPC-2 and prevents its efflux from the bacterial cell, lowering the probability of developing resistance (Papp-Wallace et al., 2018).

This brings to another interesting feature of IMI/REL, that is its activity toward KPC-producing strains exhibiting resistance to CTV, a profile which first emerged in 2018 and, since then, has been increasingly reported worldwide (European Centre for Disease Prevention and Control, 2018; di Bella et al., 2021; Sader et al., 2021; Shields et al., 2022; Campogiani et al., 2023; di Pilato et al., 2023; Oliva et al., 2023).

TABLE 2 Antibiotic susceptibility testing of KPC-producing *Klebsiella pneumoniae* complex and difficult-to-treat resistance (DTR) *Pseudomonas aeruginosa* of patients treated with imipenem/relebactam.

	Pathogen(s)	CEP (μg/mL)	PIT (μg/mL)	IMI (μg/mL)	IMI/REL (μg/mL)	MER (μg/mL)	MEV (μg/mL)	COL (μg/mL)	CTT (μg/mL)	CTV (μg/mL)
Case #1	DTR-PA	16	≥128	≥16	NP	≥16	NP	2	1	2
Case #2	DTR-PA	2	8	>16	NP	8	NP	NP	0.5	2
Case #3	DTR-PA	>8	>16	4	NP	8	NP	≤2	1	2
Case #4	DTR-PA	>8	>16	4	1	8	NP	≤2	>4	>8
Case #5	KPC-Kp	≥32	≥128	≥16	NP	≥16	NP	≥16	NA	12
Case #6	KPC-Kp	≥32	≥128	≥16	0.38	≥16	0.75	≥16	NA	≥16
Case #7	KPC-Kp	≥32	≥128	≥16	2	≥16	≥64	0.5	NA	≥16
Case #8	KPC-Kp	≥32	≥128	≥16	NP	≥16	8	≤0.5	NA	8
Case #9	KPC-Kp	>8	>16	>8	0.38	>32	1	≥2	NA	4
Case #10	DTR-PA	8	16	≥16	2	≥16	NP	≥8	1	2
	KPC-Kp	≥32	≥128	≥16	1	≥16	4	0.5	NA	12

CEP, cefepime; COL, colistin; CTT, ceftolozane/tazobactam; CTV, ceftazidime-avibactam; IMI, imipenem; IMI/REL, imipenem/relebactam; MER, meropenem; MEV, meropenem-vaborbactam; PIT, piperacillin/tazobactam; NP, not performed; NA, not applicable.

In this context, it has been recently shown that strains with KPC gene overexpression and porin alterations were able to acquire *in vitro* resistance to CTV and MEV, but still retained full susceptibility to IMI/REL, probably due to a reduced influence of OmpK36 porin mutations on IMI/REL activity (di Pilato et al., 2023).

In the three presented cases of HAP caused by KPC-Kp, with one being bacteremic, the decision to administer IMI/REL was made due to the resistance exhibited toward CTV and the unavailability of MEV in our hospital. However, treatment with IMI/REL yielded excellent clinical and microbiological responses. The same good clinical outcome was observed in the other 2 complex infections sustained by CTV-S KPC-Kp, where the decision to use IMI/REL was based on CTV high MIC and/or cephalosporin allergy. Taken together, these finding underscores the drug's effectiveness against infections caused by KPC-Kp strains, with a special mention on those caused by strains exhibiting CTV resistance or high MIC.

Indeed, current evidence from studies such as RESTORE-IMI 2 and real-world experiences predominantly focuses on DTR-PA, with CRE infections representing only a minority of cases. Notably, in one study only three Kp-infected patients were included, of which 2 were CRE; however, no information regarding the mechanism of carbapenem resistance was available and, most importantly, they were part of polymicrobial infections (Rebold et al., 2021). In contrast, our experience involved monomicrobial KPC-Kp infections in five cases, further supporting IMI/REL's efficacy against challenging infections sustained by this pathogen.

Interestingly, we used IMI/REL for the treatment of SSTIs and osteomyelitis caused by DTR-PA in three patients (case#3, case#10 and case#1, respectively). This pathogen is extremely common in these scenarios and clinicians often own limited therapeutic options, particularly in patients allergic to cephalosporins which are part of combinations targeted at DTR-PA, such as ceftazidime and ceftolozane. With this regard, IMI/REL showed potent *in vitro* activity against DTR-PA isolated in SSTIs worldwide (Sader et al., 2021), while, to the best of our knowledge, only two cases of bone infection successfully treated with IMI/REL have been described so far (Rebold et al., 2021; Larcher et al., 2022). Our cases add evidence toward IMI-REL efficacy in such difficult-to-treat infections, characterized by biofilm production and necessitating prolonged therapy for a successful outcome. Indeed, our experience confirmed the high tolerability of the drug, even when administered for a long period (i.e., 6 weeks).

Additionally, IMI/REL was administered as monotherapy in the majority of cases ($n = 9$) likely reflecting the clinicians' increased confidence in using this drug alone, influenced by its favorable pharmacokinetic/pharmacodynamic (PK/PD) characteristics and its high genetic barrier to resistance.

In five cases out of 10, IMI/REL was used even without availability of *in vitro* susceptibility testing. Although the clinical and microbiological effectiveness suggested that the strains were susceptible, we strongly encourage to perform *in vitro* susceptibility test, when feasible. Indeed, despite still rare, resistance to IMI/REL has been recently reported in *P. aeruginosa* after treatment for HAP (Shields et al., 2022).

In cases #3 and #4 there was no evidence of IMI resistance but susceptibility to increasing exposure. In case #3, since the patient had history of infection relapse after previous treatment with meropenem and since the infection was in a poorly vascularized area in a patient with chronic vasculopathy, IMI/REL was preferred over IMI, and clinical cure was obtained. Likewise, in case #4, due to the evidence of resistance to CTT and CTV and the severity of clinical presentation (septic shock), the use of IMI/REL was preferred over increased-dose IMI.

Undoubtedly, there are several limitations of our report, including its retrospective nature, the small number of treated patients and the absence of IMI/REL susceptibility in all patients due to the unavailability of IMI/REL testing at our hospital at the beginning of drug use. Furthermore, whole genome sequencing and resistance determinants of DTR-PA were not performed. Nevertheless, this report details the utilization of a newly introduced drug for which there is still limited real-world experience, particularly for infections attributed to KPC-Kp. IMI/REL offers another valuable addition to the antibiotic armamentarium, especially in a country like Italy, where the prevalence of DTR organisms, including CTV-R KPC-Kp and DTR-PA, is a significant concern.

In conclusion, we demonstrated the successful use and high tolerability of IMI/REL for treating complicated infections caused by DTR-PA and KPC-Kp, especially when the latter is resistant to CTV. Despite the promising results of our preliminary report, additional prospective and multicenter studies involving all patients treated with IMI/REL are warranted in the near future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, upon motivated request.

Ethics statement

The studies involving humans were approved by Local Hospital Ethics Committee. The study was conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements due to the retrospective nature of data.

Author contributions

CL: Conceptualization, Data curation, Investigation, Writing – original draft. MTM: Data curation, Writing – review & editing. LV: Methodology, Writing – review & editing. SC: Data curation, Writing – review & editing. AF: Data curation, Writing – review & editing. FC: Data curation, Writing – review & editing. CF: Data curation, Writing – review & editing. MC: Data curation, Writing – review & editing. CMM: Writing – review & editing. AO: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was supported by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, PE13 INF-ACT, Spoke 4).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 21 August 2024
DOI 10.3389/fmicb.2024.1426603

OPEN ACCESS

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RECEIVED 01 May 2024
ACCEPTED 22 July 2024
PUBLISHED 21 August 2024

CITATION
Song Y, Zou Y, Xu L, Wang J, Deng X, Zhou Y and Li D (2024) *Ginkgolic Acid* as a carbapenem synergist against KPC-2 positive *Klebsiella pneumoniae*. *Front. Microbiol.* 15:1426603. doi: 10.3389/fmicb.2024.1426603

Ginkgolic Acid as a carbapenem synergist against KPC-2 positive *Klebsiella pneumoniae*

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The successful evolution of KPC-2 in bacteria has limited the clinical practice of carbapenems. This dilemma deteriorated the prognosis of associated infections and hence attracted increasing attention from researchers to explore alternative therapeutic options. Here, the enzyme inhibition assay was first performed to screen for a potent KPC-2 inhibitor. The synergistic effect of the candidate with carbapenems was further confirmed by checkboard minimum inhibitory concentration (MIC) assay, time-killing assay, disk diffusion method, and live/dead bacteria staining analysis. The mechanisms by which the candidate acts were subsequently explored through molecular dynamics (MD) simulations, etc. Our study found that *Ginkgolic Acid* (C13:0) (GA) exhibited effective KPC-2 inhibitory activity in both laboratory strain and clinical strain containing KPC-2. It could potentiate the killing effect of carbapenems on KPC-2-positive *Klebsiella pnenmoniae (K. pnenmoniae)*. Further explorations revealed that GA could competitively bind to the active pocket of KPC-2 with meropenem (MEM) via residues $Trp_{104,}$ $Gly_{235,}$ and Leu_{166}. The secondary structure and functional groups of KPC-2 were subsequently altered, which may be the main mechanism by which GA exerted its KPC-2 inhibitory effect. In addition, GA was also found to synergize with MEM to disrupt membrane integrity and increase membrane permeability, which may be another mechanism by which GA reinforced the bactericidal ability of carbapenems. Our study indicated that GA was a significant KPC-2 inhibitor that could prolong the lifespan of carbapenems and improve the prognosis of patients.

KEYWORDS

KPC-2, *Ginkgolic Acid* (C13:0), carbapenems, *Klebsiella pneumoniae*, resistance

1 Introduction

Bacterial resistance, a phenomenon mainly attributed to the misuse and abuse of antibiotics, has affected approximately 22 countries around the world, triggering a widespread antibiotic resistance crisis (Cosgrove, 2006; Maragakis et al., 2008; de Kraker et al., 2011; Dos Santos et al., 2021). As the last resort against multidrug-resistant bacterial infections, the clinical efficacy of carbapenems has been greatly reduced with the emergence of carbapenem-resistant strains. Among these, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has attracted extreme attention by virtue of its high mortality rate and is already classified as a member of "ESKAPE" pathogen group

(*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp; Xu et al., 2017). A continuous increase in the resistance rate of *K. pneumoniae* to carbapenems has been reported (Wu et al., 2021), posing a serious threat to human life and property. To address this dilemma, the research of new antibiotics or the seek for antibiotic synergists is imperative.

Carbapenem resistance can be caused by a variety of mechanisms, of which carbapenemase production is the most common in CRKP. *Klebsiella pneumoniae* carbapenemase-2 (KPC-2), a class A serine β-lactamase, can hydrolyze all FDA-approved β-lactam antibiotics and β-lactamase inhibitors (Munoz-Price et al., 2013; Pemberton et al., 2017), and is widely disseminated at an alarming rate in China (Zhang et al., 2017). In this critical situation, the pace of new antibiotic development lags far behind the emergence of antibiotic-resistant strains, forced by the enormous costs in time and money. However, restoring the susceptibility of KPC-2-positive *K. pneumoniae* to carbapenems appears to be a less costly and feasible strategy.

Accumulating evidence has demonstrated that enzyme inhibitors exhibited excellent efficacy on antibiotic sensitization without significant adverse effects (Xu et al., 2022; Zhou et al., 2022). Given the above theoretical basis, we performed a KPC-2-targeted biochemical screen to reverse antibiotic resistance. Herein, we discovered that *Ginkgolic Acid* (C13:0) (GA), a natural compound derived from *Ginkgo biloba*, synergized with carbapenems against KPC-2 positive bacteria as a KPC-2 inhibitor. Inspiringly, GA could simultaneously potentiate the interference of carbapenems on bacterial membranes thereby further reinforcing the bactericidal efficacy. Our findings provided a feasible and promising therapeutic strategy to combat intractable carbapenem-resistant *K. pneumoniae* infection.

2 Materials and methods

2.1 Bacterial strains and reagents

E. coli BL21(DE3) (pET28a-KPC-2) and *E. coli* BL21(DE3)-pET28a were constructed by our laboratory. *K. pneumoniae* ST-C1 and *K. pneumoniae* 43816 is a KPC-2 negative standard strain purchased from the American Type Culture Collection (ATCC). *K. pneumoniae* ST-C1 is a KPC-2-containing clinical strain. All the strains of *K. pneumoniae* were grown in LB broth (BIOFOUNT). The GA (dissolved in dimethyl sulfoxide) was bought from Chengdu Deruike Biotechnology Co., Ltd. Meropenem (MEM), imipenem, and kanamycin used in this study were purchased from the China Institute of Veterinary Drug Control. Nitrocefin (CAS: 41906-86-9) was obtained from TOKU-E Company, Bellingham, WA, United States.

2.2 Expression and purification of KPC-2 and its mutants

The expression vectors for the KPC-2 mutants were generated using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, United States) based on *E. coli* BL21(DE3) (pET28a) (KPC-2) constructed in our previous study (Zhou et al., 2020b), during which the gene-specific primers detailed in Supplementary Table 1 played an integral role. The purification of KPC-2 and its mutants was conducted successively after verifying the sequences of mutant strains by nucleotide sequencing.

2.3 Enzyme inhibition assay

The inhibitory effect of compounds on KPC-2 activity was determined by detecting the hydrolysis ability of β-lactamase against nitrocefin substrate as described previously (Zhou et al., 2020a). Filtered phosphate-buffered saline (PBS) was mixed with purified KPC-2 protein or bacterial supernatants containing KPC-2, and meanwhile different concentrations of *compounds* were added at a final concentration of 0–64 μg/mL. After incubation at 37°C for 15 min, diluted nitrocefin was added for further incubation. 25 min later, the change of solution in color and absorbance at 492 nm was observed at room temperature. The IC_{50} (half maximal inhibitory concentration) of the compound was calculated using GraphPad prism software.

2.4 Bacterial growth curve analysis

The growth curve analysis was performed to clarify the effect of GA on the proliferation of *K. pneumoniae* (Zhou et al., 2018). Briefly, bacteria cultured overnight were diluted into fresh LB medium at a ratio of 1:100. Then, a total of 150 mL of *K. pneumoniae* ($OD_{600\ nm}$ was about 0.1) in LB were dispensed into five conical flasks with simultaneous addition of different concentrations of GA. The cultures were continued to incubate at a shaking incubator (180 r/min, 37°C) and the $OD_{600\ nm}$ of each conical flask was monitored each hour with a spectrophotometer until reached the plateau phase. The above data were used to plot the growth curve reflecting the interference of GA on the proliferation situation of *K. pneumoniae*.

2.5 Time-killing assay

The time-killing assay reflecting the synergistic killing activity of MEM with GA was performed according to the following method (Zhou et al., 2020b). Overnight cultures were diluted and distributed into sterile 96-well microtiter plates at the final concentration of 5×10^5 CFUs/well. Different administrations including GA only, MEM only, combination treatment and blank control were given separately, after which the plate was placed in 37°C incubator for static incubation. At different time points, surviving bacteria were separately coated on LB agar plates for colony counting and spotted on plates for image photography after serial dilutions. In addition, surviving bacteria under different treatments could also be quantified by spectrophotometer.

2.6 Checkerboard minimum inhibitory concentration (MIC) determination

The synergistic effect of GA and carbapenems against KPC-2 positive and KPC-2 negative strains was determined using a slightly optimized broth micro-dilution method (Wiegand et al., 2008). Concisely, antibiotics and compounds were serially diluted twofold respectively, and subsequently dispensed into sterile 96-well microtiter plates. Overnight bacteria cultures were diluted and inoculated into plates at the final concentration of 5×10^5 CFUs/well. After 16–18 h of static incubation at 37°C, the turbidity of each well was observed to determine the MICs of antibiotics and compounds for bacteria. The fractional inhibitory concentration (FIC) index values representing the synergistic bactericidal effect of carbapenems and compounds were calculated by the following formula (FIC index ≤ 0.5 implies synergy):

$$\text{FIC index} = \left(\frac{(MIC\ of\ compounds\ in\ combination)}{(MIC\ of\ compounds\ alone)}\right) + \left(\frac{(MIC\ of\ antibiotics\ in\ combination)}{(MIC\ of\ antibiotics\ alone)}\right)$$

2.7 Disk diffusion method

The disk diffusion method was carried out to further determine the synergy of GA and MEM based on previously described (Escamilla-García et al., 2017). Overnight bacteria were diluted and further cultured until reached the exponential growth phase. The tested bacterial suspensions were spread evenly on LB agar plates containing different concentrations of GA. Then the MEM disks (10 μg) were placed in the center of each plate and subsequently incubated at 37°C for 16–18 h. The inhibitory zone diameters were photographed and recorded for data analysis.

2.8 *In vitro* live/dead bacteria staining assay

The combined bactericidal effect of GA and MEM was visually evaluated by live/dead bacterial staining assay. Overnight bacterial cultures were diluted in fresh LB broth and then cocultured with GA only [32 μg/mL for *K. pneumoniae* ST-C1 and 8 μg/mL for *E. coli* BL21(DE3) (pET28a-KPC-2)], MEM only [4 μg/mL for *K. pneumoniae* ST-C1 and 1/4 μg/mL for *E. coli* BL21(DE3) (pET28a-KPC-2)] and their combination for 6 h at 37°C. The bacteria were then collected, washed twice and resuspended in sterile PBS, and $OD_{600\ nm}$ was adjusted to 0.5. The live or dead status of the tested bacteria of each group was observed with an inverted fluorescence microscope (Nikon Eclipse, Japan) after adding SYTO 9 and propidium iodide (PI) dyes of the LIVE/ DEAD BacLight Bacterial Viability Kit (Invitrogen) under the guidance of the manufacturer's instructions.

2.9 Membrane permeability detection

Alterations in PI and N-Phenyl-1-naphthylamine (NPN) uptake are often used to assess the permeability of inner and outer membranes of bacteria. The bacteria were centrifuged and normalized to the same absorbance value ($OD_{600\ nm}$ = 0.5) after treatment with the different drugs (GA only, MEM only, and their combination) for 6 h. PI and NPN were separately added to the suspension at final concentrations of 10 nM and 10 μM and further incubated at 37°C for 90 min. The spectrofluorimeter was used to detect the fluorescence intensity of PI (535 excitation wavelength/615 emission wavelength) and NPN (350 excitation wavelength/420 emission wavelength).

2.10 Western blot analysis

Overnight cultures of *K. pneumoniae* were diluted and incubated with GA (0, 8, 32, 128 μg/mL) for 4 h or 8 h at 37°C. Then the cultures were collected, standardized and prepared as samples for western blot assay after boiled at 100°C for 10 min. After separated by SDS-PAGE (12% gels), electrophoresed proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane. The membranes were then blocked with 5% skim milk for 2 h at room temperature, followed by successive incubation with primary antibodies against KPC-2 (prepared from mouse) and goat anti-mouse IgG secondary antibody (HRP). ICDH was used as an internal reference. Finally, the targeted protein was visualized with an enhanced chemiluminescence substrate.

2.11 Circular dichroism spectra detection

Circular dichroism (CD) spectra was measured using a CD spectrophotometer (MOS-500; Bio-Logic) with the wavelength ranging from 190 to 250 nm at ambient temperature. The recorded spectra were subsequently analyzed with *BeStSel web server* to compare the alterations in the secondary structure of KPC-2 proteins with or without GA treatment.

2.12 Fourier transform infrared spectroscopy analysis

Fourier transform infrared spectroscopy (FTIR) spectrometer was used to record the FTIR spectra of KPC-2 protein treated with or without 32 μg/mL of GA over a range of 4,000–500 cm^{-1} with a resolution of 2 cm^{-1}. The obtained data was analyzed and graphed with Origin 2023, revealing the effect of GA treatment on the functional groups of the proteins.

2.13 Bacterial nucleic acid and protein leakage

The nucleic acid and protein leakage detection was measured following a previously described method with modifications (Yao et al., 2014). Bacteria in logarithmic phase were centrifuged at low speed (≤5,000 r/min) and then resuspended in PBS (pH 7.2). After adjusting the $OD_{600\ nm}$ to 0.5, bacterial suspensions were partitioned and different concentrations of GA were added. Subsequently, a 4-h culture was conducted at a shaking incubator (180 r/min, 37°C). At different time points, 1 mL of bacterial culture from each group was collected and the protein and nucleic acid concentrations in the supernatant were detected using a DNA/protein analyzer.

2.14 Molecular docking and molecular dynamics simulation

Molecular docking was performed with autodock vina1.1.2 software to simulate the binding modes of the KPC-2-GA complex and KPC-2-MEM complex (Tsang et al., 2022), before which the three-dimensional (3-D) structures of GA, MEM, and KPC-2 were downloaded from RCSB and pubchem, respectively. The amber18 software was then used to conduct MD simulations of the KPC-2-GA complex and KPC-2-MEM complex (Arodola et al., 2020), during which ff14SB forcefield parameter was used for proteins and gaff generic forcefield parameter was used for compound and antibiotics.

The essential protein residues for ligand-protein binding were specified by decomposing the binding free energy of the complexes with the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) approach (Rajeshkumar et al., 2022).

2.15 Statistical analysis

All the assays were conducted at least three biological replicates and the data were expressed as the mean ± standard deviation. Statistical analysis was calculated by Student's *t* test for two groups and one-way analysis of variance (ANOVA) for multiple groups in GraphPad Prism 9.5.1. A value of $p < 0.05$ was regarded as statistically significant.

3 Results

3.1 GA attenuated the enzymatic activity of KPC-2 without affecting bacterial viability and KPC-2 expression

Enzyme inhibition assays were used to assess the alterations in KPC-2 activity induced by different compounds. GA (Figure 1A) was ultimately identified as a potent KPC-2 inhibitor, manifested by a dose-dependent reduction in the activity of the purified protein (Figures 1B,D) and protein secreted into culture medium (Figures 1C,E). The IC_{50} of GA for KPC-2 inhibition were 4.748 μg/mL and 2.096 μg/mL, respectively.

We next explored the effect of GA on the expression of KPC-2 protein. As shown in Figures 1F,G,I,J, GA had no disturbing effect on KPC-2 production in *E. coli* BL21(DE3) (pET28a-KPC-2) and *K. pneumoniae* ST-C1. Moreover, we demonstrated that different concentrations of GA (0–128 μg/mL) had no visible inhibitory efficacy on the growth of KPC-2-positive bacteria (Figures 1H,K). Taken together, the above results suggested that GA at non-bactericidal concentrations could significantly inhibit the activity of KPC-2 without affecting KPC-2 expression.

3.2 GA re-sensitized KPC-2 positive *Klebsiella pneumoniae* to carbapenems

The activity inhibition of GA on KPC-2 prompted us to verify the synergistic effect between GA and carbapenems. The checkboard MIC assays showed that the MIC of MEM against *E. coli* BL21(DE3) (pET28a-KPC-2) and *K. pneumoniae* ST-C1 could be down-regulated ≥8-fold by the combination of GA (FIC index < 0.2; Figures 2A,B). GA also exhibited significant synergistic effects with imipenem (Supplementary Figures S1A,B). However, in carbapenem-sensitive strains without KPC-2, this synergistic effect could not be observed (Supplementary Figures S1C–F). The synergistic bactericidal activity of GA with MEM was further demonstrated using the time-killing test. Remarkably, combination therapy killed more of the tested bacteria within 24 h in both laboratory strain *E. coli* BL21(DE3) (pET28a-KPC-2; Figures 2C,E,G) and clinical strain *K. pneumoniae* ST-C1 (Figures 2D,F,H).

The live/dead bacteria staining assay and combined disk test were then successively used to visually compare the bactericidal ability of monotherapy with that of combination therapy. Figures 3A,B showed that GA combined with MEM extremely exacerbated bacterial death,

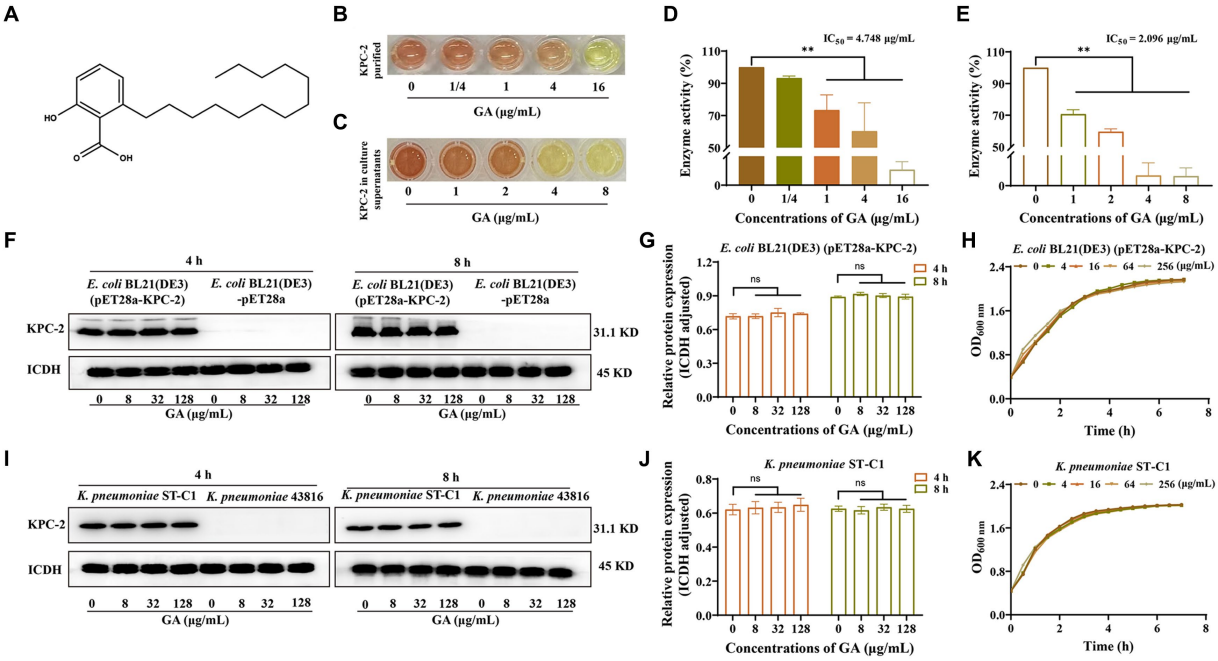

FIGURE 1
GA inhibited the activity but not the expression of KPC-2. **(A)** Chemical structure of GA. The inhibitory effect of GA on purified KPC-2 protein **(B,D)** and KPC-2 in culture supernatants **(C,E)** was manifested by a color change and enzyme activity quantification. Effects of multiple concentrations of GA on KPC-2 expression in *E. coli* BL21(DE3) (pET28a-KPC-2) **(F,G)** and *Klebsiella pneumoniae* ST-C1 **(I,J)**. The growth curves of *E. coli* BL21(DE3) (pET28a-KPC-2) **(H)** and *K. pneumoniae* ST-C1 **(K)** co-cultured with several concentrations of GA (0/4/16/64/256 μg/mL).

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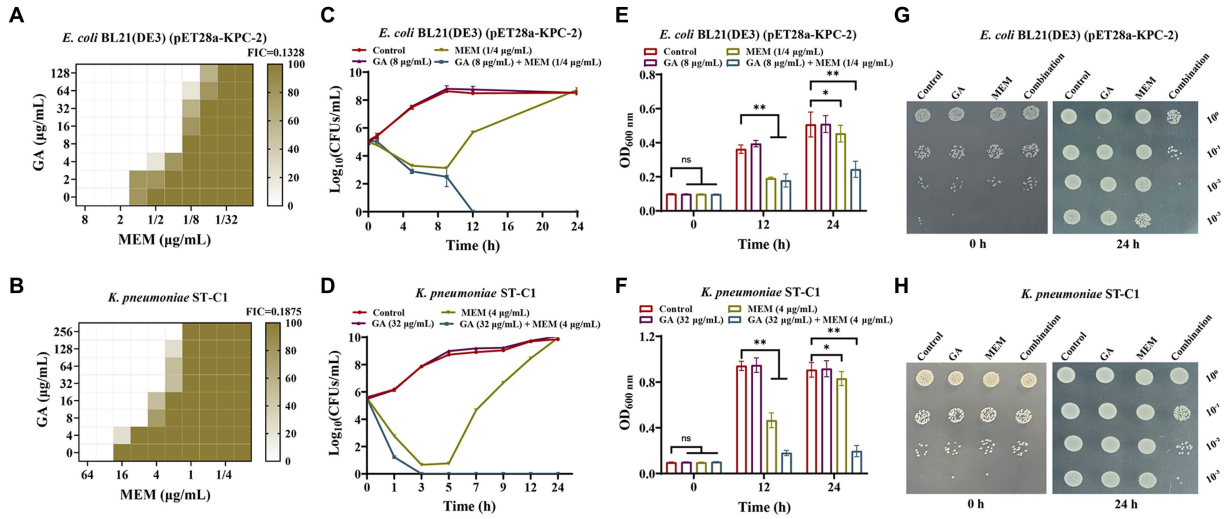

FIGURE 2
GA synergised with MEM to kill KPC-2 positive bacteria. **(A,B)** Microdilution checkerboard analysis was implemented to assess the synergistic effect of MEM with GA against *E. coli* BL21(DE3) (pET28a-KPC-2) and *Klebsiella pneumoniae* ST-C1. Time-killing curves for *E. coli* BL21(DE3) (pET28a-KPC-2) **(C)** and *K. pneumoniae* ST-C1 **(D)** when treated with GA, MEM, combination and medium only. $OD_{600\ nm}$ values reflecting the survival *E. coli* BL21(DE3) (pET28a-KPC-2) **(E)** and *K. pneumoniae* ST-C1 **(F)** in the combined treatment of GA and MEM. The spot assays of *E. coli* BL21(DE3) (pET28a-KPC-2) **(G)** and *K. pneumoniae* ST-C1 **(H)** on LB agar plates were performed after treated with different drugs (GA, MEM, combination and medium only) for 0 h and 24 h. After serial dilutions, the cultures were dropped onto plates and incubated overnight at 37°C.

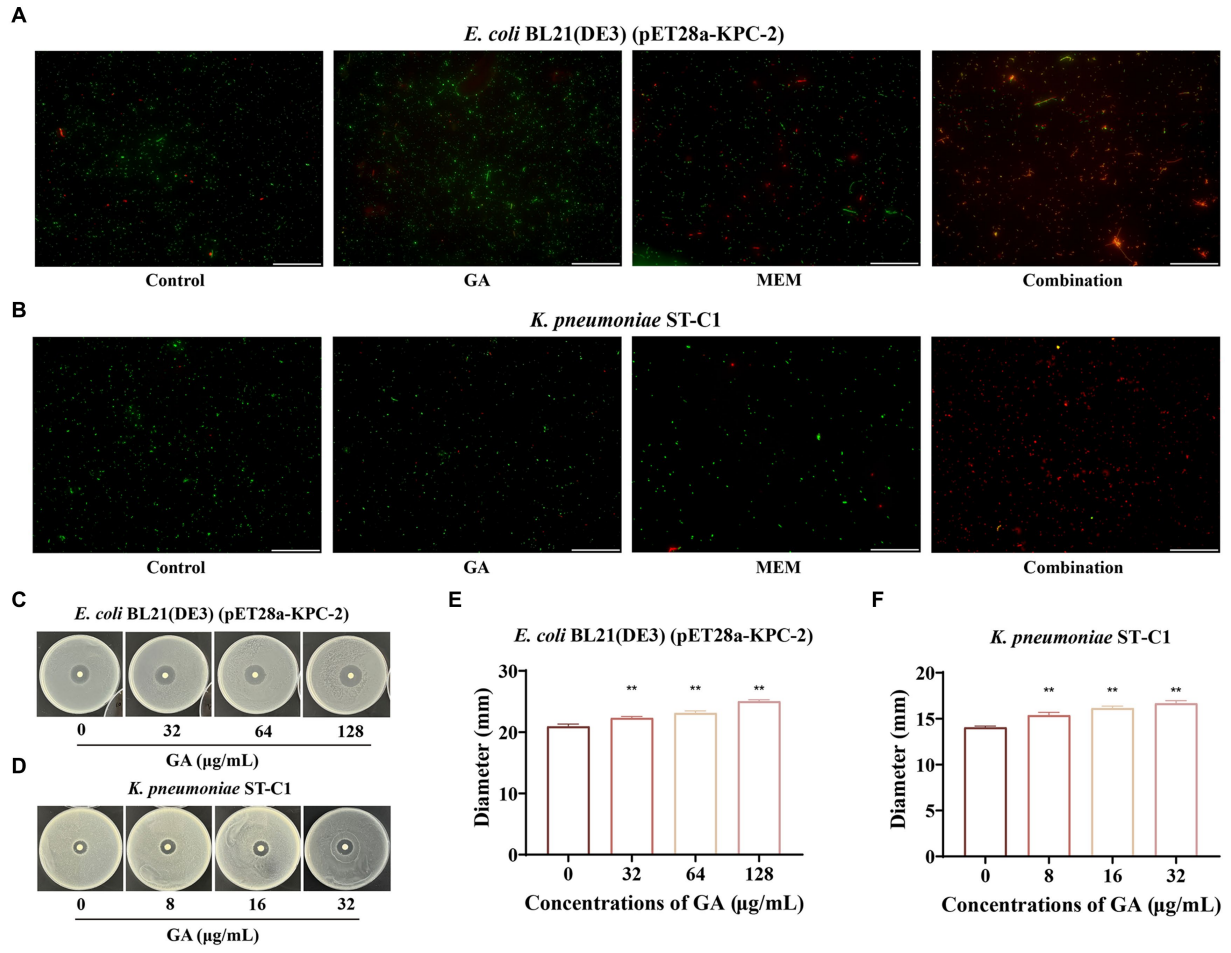

FIGURE 3
GA restored the bactericidal activity of MEM against KPC-2 positive bacteria. The live/dead bacteria staining for *E. coli* BL21(DE3) (pET28a-KPC-2) **(A)** and *Klebsiella pneumoniae* ST-C1 **(B)** after treatment with GA, MEM or GA plus MEM (scale bar=200 μm). The zone diameters surrounding MEM disks were expanded by GA in a dose-dependent manner on LB agar plates coated with *E. coli* BL21(DE3) (pET28a-KPC-2) **(C,E)** and *K. pneumoniae* ST-C1 **(D,F)**.

as evidenced by a higher ratio of dead (red) to alive bacteria (green). Consistent with these results, the diameters of the inhibitory zones of the tested bacteria surrounding the MEM disks increased in a dose-dependent manner with GA concentration (Figures 3C–F). Overall, these findings illustrated that GA and MEM had a favorable synergistic effect against KPC-2-positive bacteria.

3.3 GA altered the secondary structure and functional groups of KPC-2 through direct engagement

To initially characterize the molecular basis for the inhibition of KPC-2 activity by GA, CD spectra was first performed to compare the differences in the secondary structure of KPC-2 with or without GA treatment. A visible conformational change was observed in GA-induced KPC-2, characterized by decreased a-helix1, a-helix2, and elevated anti-2 conformation (Figures 4A,B). FTIR also revealed that GA could alter the composition and ratio of the functional groups of KPC-2, further supporting the above results (Figure 4C).

The specific binding modes between KPC-2 with GA or MEM were subsequently explored by a computational biological method called molecular docking and MD simulation. During the entire kinetic process, the amino acid residues of the KPC-2 protein do not fluctuate dramatically (Figure 4F), and both GA and MEM bound stably to the KPC-2 protein without causing sustained changes in protein conformation and compactness (Figures 4G,H). The 3-D binding model showed that both GA and MEM bound within the catalytic pocket of KPC-2 and the conformational superimpositions of the two small molecules were highly overlapping (Figures 4D,E). Although the binding energy of MEM to KPC-2 (−8 kcal/moL) was slightly higher than that of GA (−6.5 kcal/moL), the numerous common binding sites and the similar number of hydrogen bonds (Figures 4I,J) still indicated that GA was strongly competitive for MEM binding to KPC-2.

The total binding free energies and the detailed energy contributions for the KPC-2-GA and KPC-2-MEM complexes were calculated using the MM-PBSA approach. The results of energy decomposition revealed that residues Trp_{104}, and Ser_{69} had powerful contributions to the KPC-2-MEM complex (ΔE_{total} of ≤ −1 kcal/mol; Figure 5A), while residues Trp_{104}, Gly_{235}, and Leu_{166} formed strong interactions with GA (ΔE_{total} of ≤ −0.8 kcal/mol; Figure 5B). To verify the reliability of the simulated results, we performed targeted mutagenesis of the above amino acid sites contributed crucially, after which we repeated the enzyme inhibition assay and the MIC test in samples containing mutated KPC-2. Obviously, GA lost the significant enzyme activity inhibitory effect against mutant KPC-2 (Figure 5C). A similar reduction in the synergistic ability of GA with MEM against strains

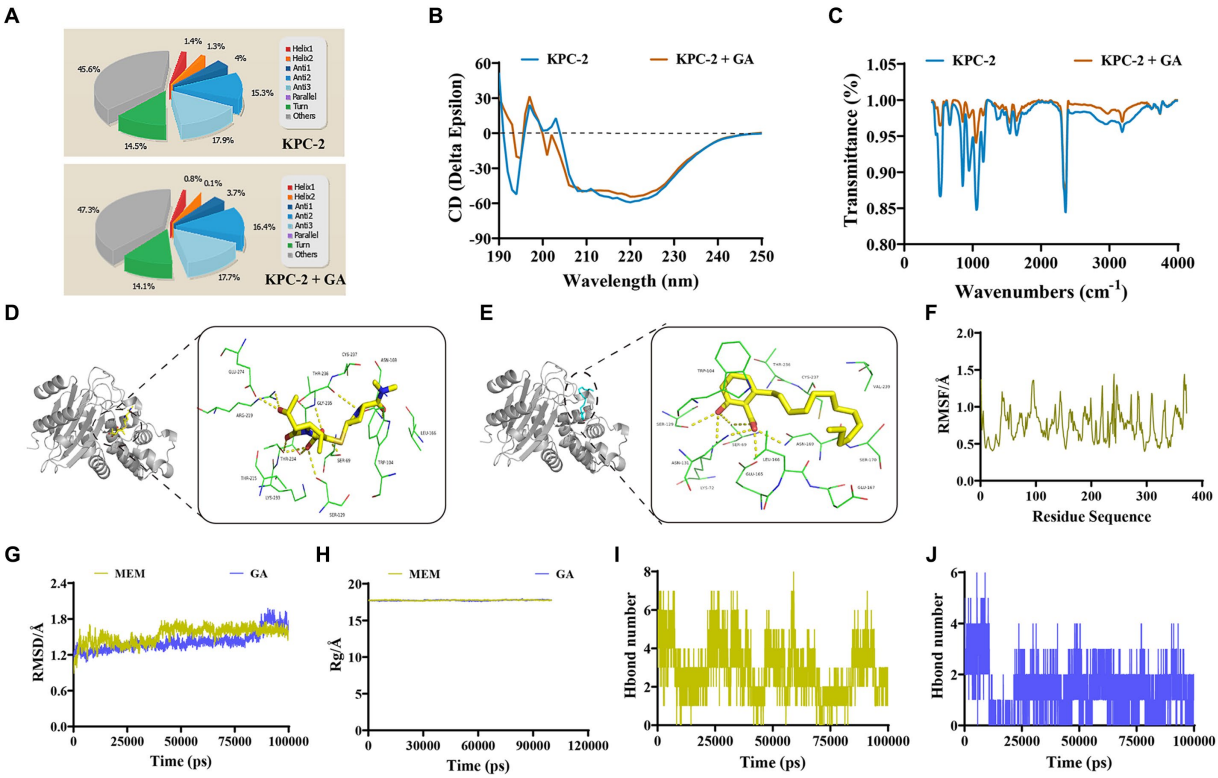

FIGURE 4
Identification of the mechanism by which GA inhibited KPC-2 activity. **(A,B)** Secondary structure changes of KPC-2 in the presence or absence of GA (32 μg/mL) were detected by CD spectroscopy. **(C)** GA (32 μg/mL)-induced alteration of the KPC-2 functional group was confirmed by FTIR assay. The 3-D binding modes of MEM **(D)** and GA **(E)** to KPC-2 were predicted by MD simulations. **(F)** RMSF image indicating the fluctuation of amino acid residues of KPC-2 throughout the entire kinetic process. RMSD **(G)** and Rg **(H)** detection of the MEM-KPC-2 complex and GA-KPC-2 complex throughout the simulation. Hydrogen bond numbers of MEM **(I)**, GA **(J)** conjugated to KPC-2 during the MD simulation.

containing mutated KPC-2 was also observed (Figure 5D). In brief, our results illustrated that GA competitively bound to KPC-2, altered its secondary structure and functional groups, ultimately realizing its enzyme activity inhibitory efficacy.

3.4 GA potentiated the disruptive effect of carbapenems on bacterial membranes

Considering that targeting membranes is the main mechanism by which carbapenems function, we speculated whether GA alone or in concert with MEM could aggravate membrane damage. We first explored the capacity of GA on membrane damage by measuring GA-induced nucleic acid and protein leakage. The results showed a dose- and time-dependent increase in the levels of nucleic acids and proteins released into the supernatant by the strains co-cultured with GA (Figures 6A–D).

The fluorescent probes PI and NPN were subsequently used to assess the synergistic effect of GA with MEM in disrupting bacterial membranes. As shown in Figures 6E–H, co-incubation with GA significantly increased the ability of PI and NPN to enter the bacteria compared to the control group, indicating the potent effect of GA in enhancing the permeability of both the inner and outer membranes, especially the inner membrane. Hence, we could infer that disturbing membrane integrity may be another pathway for GA to synergize with carbapenems.

4 Discussion

The emergence and prevalence of CRKP coupled with the lag in antibiotic development have driven the ongoing search for strategies to enhance the efficacy of existing carbapenems. Among these strategies, the identification of potent inhibitors targeting KPC-2 thereby restoring the bactericidal capacity of carbapenems has become a prominent area of research (Zhou et al., 2020b). We were pleased to uncover that GA could inhibit the enzymatic activity of KPC-2 by directly binding and then altering its secondary structure and functional groups. Concurrently, GA could also synergize with carbapenems to disrupt bacterial membranes, as evidenced by elevated nucleic acid and protein leakage and increased membrane permeability. Of interest, the synergistic effect of GA with carbapenems is indeed present in both laboratory strains and clinical strains, suggesting the potential of GA for clinical therapeutic.

Ginkgo biloba (Ginkgo), which is known as a "living fossil," has been documented as a medicinal plant 2,800 years ago (Jacobs and Browner, 2000). Its extracts have been reported to possess multiple

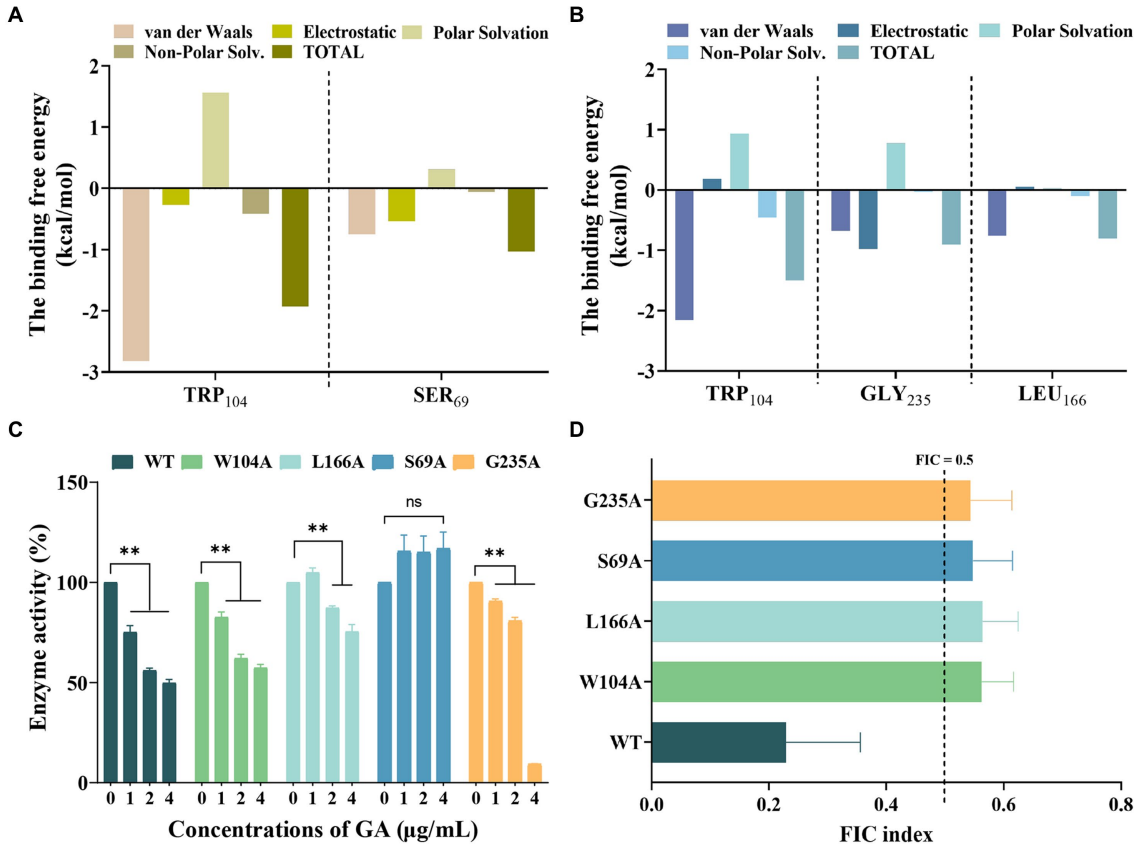

FIGURE 5
Confirmation of the binding sites of KPC-2 with MEM and GA. Decomposition of the binding energy on a per-residue basis in the KPC-2-MEM **(A)** and KPC-2-GA complex **(B)**. **(C)** Influence of GA on the enzymatic activity of KPC-2 and its mutants. **(D)** FIC index values reflecting the synergistic effect of GA and MEM against each of the variants.

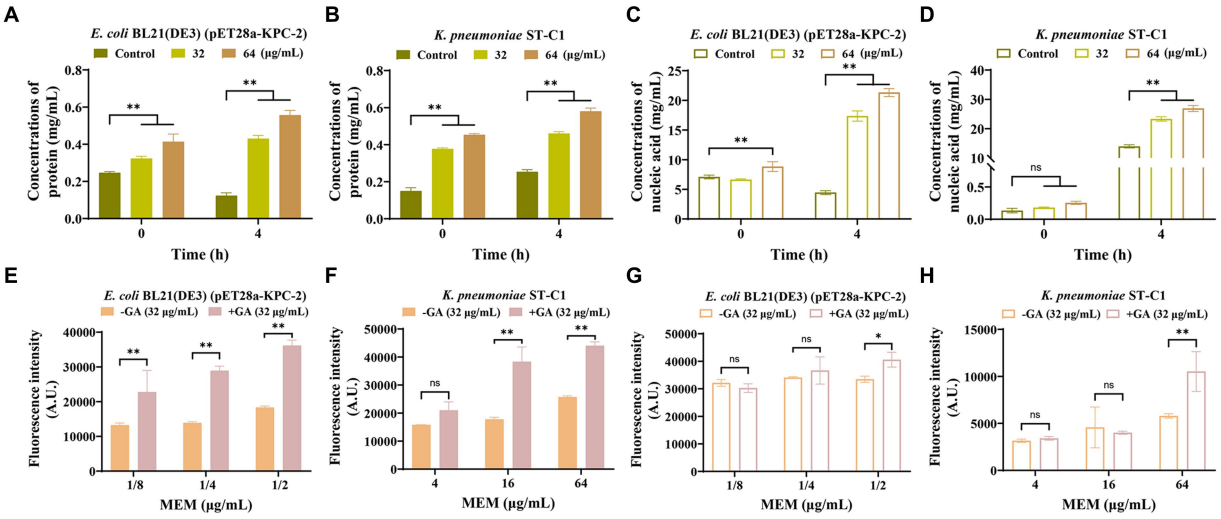

FIGURE 6
GA strengthened the disrupting effect of MEM on bacterial membranes. GA induced the protein and nucleic acid leakage of *E. coli* BL21(DE3) (pET28a-KPC-2) **(A,C)** and *Klebsiella pneumoniae* ST-C1 **(B,D)** in a dose-dependent and time-dependent manner. Increased permeability of the inner membrane of *E. coli* BL21(DE3) (pET28a-KPC-2) **(E)** and *K. pneumoniae* ST-C1 **(F)** by a combination treatment with GA. PI was used as the probe at a final concentration of 10 nM. The outer membrane permeability of *E. coli* BL21(DE3) (pET28a-KPC-2) **(G)** and *K. pneumoniae* ST-C1 **(H)** could be enhanced when synergized with GA compared to MEM alone.

TABLE 1 ADMET profile of ginkgolic acid predicted by SwissADEM and AD METlab servers.

Absorption		Distribution		Metabolism		Excretion		Toxicity	
GI absorption	High	BBB permeant	No	CYP1A2 inhibitor	Yes	CLplasma	5.045	AMES Toxicity	0.163
Caco-2 Permeability	−4.71	VSss	1.641	CYP2C19 inhibitor	Yes	T1/2	0.446	Rat Oral Acute Toxicity	0.275
Pgp substrate	No	Fu	6.30%	CYP2C9 inhibitor	Yes			Carcinogenicity	0.195
Bioavailability Score	0.85	BCRP inhibitor	No	CYP2D6 inhibitor	No			A549 Cytotoxicity	0.016

pharmacological and clinical efficacy (Maitra et al., 1995; Zhang et al., 2008; Mashayekh et al., 2011; Hamdoun and Efferth, 2017; Yang et al., 2017; Zheng et al., 2021; Kim et al., 2022) and are the most widely used herbal medicines and dietary supplements worldwide (Ngan et al., 2012). Currently, research on GA, an extract of *Ginkgo biloba*, is focused mainly on cancer treatment as well as diabetes control. In our study, we proposed that GA possessed a significant effect against KPC-2-positive *K. pneumoniae*. Concerning *in vivo* applications, we already predicted the ADMET profile of GA using SwissADEM and ADMETlab servers. The results indicated that GA is easily absorbed through the gastrointestinal tract and has a well bioavailability efficiency (Table 1). It is noteworthy that GA was not predicted to have significant cellular and *in vivo* toxicity. In the near future, the dosing regimen and exact *in vivo* efficacy of GA need to be further explored to optimize its effects.

In summary, our results demonstrated that GA could strengthen the bactericidal effect of carbapenems against CRKP by simultaneously inhibiting KPC-2 activity and damaging bacterial membranes. The combination of carbapenems and GA may be a promising alternative strategy against KPC-2-containing *K. pneumoniae*. The next step in our study will be to examine the efficacy of GA in combination with carbapenems in the animal model of KPC-2-positive *K. pneumoniae* infection.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

YS: Conceptualization, Formal analysis, Project administration, Writing – review & editing, Data curation, Visualization, Writing – original draft. YZo: Writing – original draft, Validation. LX: Software, Writing – original draft. JW: Investigation, Writing – original draft. XD: Funding acquisition, Writing – original draft. YZh: Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing. DL: Conceptualization, Supervision, Formal analysis, Project administration, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was funded by the Department of Finance of Jilin Province, grant number JLWSRCZX2023-55, the Department of Science and Technology of Jilin Province, grant number 20220303001SF and the National Natural Science Foundation of China, grant numbers 22A20523 and 32202856.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1426603/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 07 October 2024
DOI 10.3389/fmicb.2024.1435906

OPEN ACCESS

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RECEIVED 21 May 2024
ACCEPTED 19 September 2024
PUBLISHED 07 October 2024

CITATION
Wu M, Feng K, Wu X, Liu C, Zhu S, Martins FS, Yu M, Lv Z, Yan M and Sy SKB (2024) Prediction of tissue exposures of polymyxin-B, amikacin and sulbactam using physiologically-based pharmacokinetic modeling.
Front. Microbiol. 15:1435906.
doi: 10.3389/fmicb.2024.1435906

Prediction of tissue exposures of polymyxin-B, amikacin and sulbactam using physiologically-based pharmacokinetic modeling

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Background: The combination antimicrobial therapy consisting of amikacin, polymyxin-B, and sulbactam demonstrated *in vitro* synergy against multi-drug resistant *Acinetobacter baumannii*.

Objectives: The objectives were to predict drug disposition and extrapolate their efficacy in the blood, lung, heart, muscle and skin tissues using a physiologically-based pharmacokinetic (PBPK) modeling approach and to evaluate achievement of target pharmacodynamic (PD) indices against *A. baumannii*.

Methods: A PBPK model was initially developed for amikacin, polymyxin-B, and sulbactam in adult subjects, and then scaled to pediatrics, accounting for both renal and non-renal clearances. The simulated plasma and tissue drug exposures were compared to the observed data from humans and rats. Efficacy was inferred using joint probability of target attainment of target PD indices.

Results: The simulated plasma drug exposures in adults and pediatrics were within the 0.5 to 2 boundary of the mean fold error for the ratio between simulated and observed means. Simulated drug exposures in blood, skin, lung, and heart were consistent with reported penetration ratio between tissue and plasma drug exposure. In a virtual pediatric population from 2 to <18 years of age using pediatric dosing regimens, the interpretive breakpoints were achieved in 85–90% of the population.

Conclusion: The utility of PBPK to predict and simulate the amount of antibacterial drug exposure in tissue is a practical approach to overcome the difficulty of obtaining tissue drug concentrations in pediatric population. As combination therapy, amikacin/polymyxin-B/sulbactam drug concentrations in the tissues exhibited sufficient penetration to combat extremely drug resistant *A. baumannii* clinical isolates.

KEYWORDS

antibiotic combination, tissue exposure, physiologically-based pharmacokinetic, pharmacodynamic index, multidrug resistance (MDR)

1 Introduction

The overuse of antibiotics has led to the emergence of multidrug-resistant (MDR) *Acinetobacter baumannii*, which made treatment of related infections increasingly difficult; consequently, antibiotic monotherapies are no longer effective in the clinic (Oo and Sy, 2020; Oo et al., 2023). With increasing incidences of MDR *A. baumannii*, polymyxins became the last-line antibiotic agent (Li and Nation, 2006; Li et al., 2006). Polymyxin-B combination with other antibiotics exhibited better efficacy against MDR *A. baumannii* than individual antibiotic monotherapies (Rao et al., 2016; Liu et al., 2023). Our laboratory previously evaluated the *in vitro* synergistic activities of polymyxin-B, amikacin and sulbactam combination therapy against 11 MDR *A. baumannii* and showed that this triple combination restricted the mutant selection window and reduced the opportunity for the bacteria to develop further resistance (Zhu et al., 2022a). Using metabolomic profiling, we further demonstrated that this triple antibiotic combination significantly disrupted cellular outer membrane structures, fatty acids, glycerophospholipids, nucleotide and peptide metabolic pathways in MDR *A. baumannii* (Zhu et al., 2023). Sulbactam and polymyxin-B disrupted the integrity and stability of the cell wall and outer membrane by affecting peptidoglycans and lipopolysaccharides (Zhang and Rock, 2008), allowing companion antibiotics such as amikacin to enter the bacteria cell and inhibit protein synthesis that leads to cell death (Taber et al., 1987; Kotra et al., 2000).

The interpretive criteria for antimicrobial susceptibility are based on drug exposures in the blood, as it is difficult to obtain drug exposure data in the tissues. *A. baumannii* infections in critically ill patients in intensive care unit are often due to pneumonia, endocarditis, skin and bloodstream infections (Sopirala et al., 2008; Lagana et al., 2015; Abdi et al., 2020); these infections often occur in tissues where drug concentrations are often lower than that in the blood. Physiologically-based pharmacokinetic (PBPK) models provide a non-invasive alternative to extrapolate drug efficacy in the infected tissues (Martins et al., 2020; Martins et al., 2021; Zhu et al., 2022b; Martins et al., 2023; Zhang et al., 2023) and provides the necessary tool to evaluate sufficiency of exposure in the target organs.

Even though the pharmacokinetics of polymyxin-B, amikacin, and sulbactam have been reported in pediatrics (Kafetzis et al., 1979; Schaad et al., 1986; Xu et al., 2022), drug exposure data were lacking in the above tissues. In this study, we developed robust PBPK models and linked them to an exposure-based pharmacodynamic (PD) assessment to explore the adequacy of drug exposure in these organs. The present study aimed to predict drug exposure in the blood, lung, skin, and heart using clinical dosing regimens of polymyxin-B, amikacin and sulbactam for combination therapy.

2 Materials and methods

2.1 Clinical data used for PBPK model development and evaluation

The model of sulbactam was developed and evaluated in our previous study (Zhu et al., 2022b); it was not thoroughly evaluated in the current study. The clinical data including pharmacokinetic profiles and exposure parameters of polymyxin-B and amikacin were obtained from search results in Web of Science and PubMed databases. The demographic information in the literature was summarized by gender, age, weight, renal function, dosing regimen and drug exposure. Chart extraction data tool, Web Plot Digitizer (version 4.5 https://automeris.io/wpd) was used to extract drug exposure data from the literature. Those extracted data were from critically ill patients and healthy volunteers, who received either single or multiple dosing regimens via intravenous bolus or infusion and were used to optimize key parameters of the PBPK model. Adult PBPK model was developed, qualified and then scaled to the pediatric population. The extracted data of pediatrics from the literature were used to validate the pediatric PBPK model.

2.2 The development of adult PBPK model

The PBPK software, PK-Sim® (Version 10.0; part of the Open Systems Pharmacology Suite, https://www.open-systems-pharmacology.org), was used to develop the adult model of polymyxin-B and amikacin. The standard distribution model assumes four sub-compartments per organ, which included compartments for blood cells, plasma, interstitial space, and intracellular space (Bischoff, 1986). Interstitial fluid in tissues is the medium for infection transmission, and also the medium for antibiotics to be distributed at the infection site (Segal et al., 1990); the tissue drug concentrations we assessed were taken from the interstitial fluid compartment. We used the parameter identification module to optimize and then select the partition coefficient method.

The physicochemical characteristics and physiological parameters of polymyxin-B and amikacin applied in model development were obtained from drug bank[1] and the literature as listed in Table 1. Demographical information was applied in the model development including age, gender, and weight, in addition to dosing regimens and plasma concentration-time profiles. In order to ensure consistency of the simulated drug exposure distribution in the tissues with those reported in the literature, we adjusted the standard deviation of the partition coefficients by comparing the inter-individual variability of drug tissue exposure with that reported in the literature (Zhu et al., 2022b).

The clearance of polymyxin-B was minimally affected by renal function. Consequently, the dosing regimens of polymyxin-B were not adjusted based on renal function. Less than 1% unchanged polymyxin-B was recovered in human urine; due to tubular reabsorption, the net renal clearance was in the range of 0.00032–0.0039 mL/min/kg in humans (Zavascki et al., 2007; Zavascki et al., 2008; Manchandani et al., 2016). The specific mechanism for its metabolism is still not fully understood (Avedissian et al., 2019); the non-renal clearance parameter of the polymyxin-B model was set as its total clearance and then optimized in PK-Sim (Zavascki et al., 2008; Sandri et al., 2013a; Sandri et al., 2013b; Burkin et al., 2021; Xu et al., 2022). Biliary excretion could be one of the elimination routes, but the value was not provided in the literature (Manchandani et al., 2016); biliary elimination was optimized by the parameter identification module in PK-Sim.

Amikacin is the second most commonly used antibiotic in neonatal intensive care units (Spitzer et al., 2010). Like other

1 https://go.drugbank.com/

TABLE 1 Drug characteristics and parameters of polymyxin-B/amikacin used in PBPK model building.

Parameter	Amikacin	Polymyxin-B
Physicochemical characteristics		
Molecular weight (g/mol)	585.6	1203.5
Compound type	Ampholyte	Ampholyte
Solubility (mg/mL)[a]	49.7	0.0744
pKa acid[a]	12.1	11.6
pKa base[a]	9.79	10.2
Lipophilicity (logP)[a]	−3.2	−0.89
Distribution (WB-PBPK)		
Partition coefficients[c]	Schmitt: heart:1.10 ± 0.09; lung: 0.50 ± 0.04; skin:0.41 ± 0.12	PK-Sim standard: heart:1.03 ± 0.31; lung: 2.83 ± 1.10; skin:1.53 ± 0.45
f_u (adults)	0.90[a]	0.80[a]
f_u (pediatrics)	0.72	0.50
B:P ratio [c]	0.82	0.56
Protein binding partner	Albumin (Gamba et al., 1990)	α_1-acid glycoprotein (Zavascki et al., 2008)
Elimination		
CL_{renal} (mL/min/kg)	1.4[b] (Vogelstein et al., 1977; Bauer et al., 1980; Lanao et al., 1981; Garraffo et al., 1990; Vanhaeverbeek et al., 1993; Mahmoudi et al., 2013)	0.002[b] (Zavascki et al., 2007; Zavascki et al., 2008; Manchandani et al., 2016)
$CL_{non\text{-}renal}$ (mL/min/kg)	–	0.67[b] (Zavascki et al., 2008; Sandri et al., 2013a; Sandri et al., 2013b; Burkin et al., 2021; Xu et al., 2022)
$CL_{biliary}$ (mL/min/kg)	–	0.001[b] (Manchandani et al., 2016)
GFR I (mL/min)	≥60	≥60
GFR II (mL/min)	40–59	40–59
GFR II (mL/min)	30–39	30–39

WB-PBPK, whole-body physiologically based pharmacokinetic; CL, clearance; f_u, fraction unbound; GFR, glomerular filtration rate.
[a]Values from www.drugbank.ca.
[b]Optimized based on the reported information.
[c]Parameter determined by PK-Sim.

aminoglycosides, amikacin is primarily eliminated by glomerular filtration; a high recovery rate of its unchanged form was detected in the urine (Lanao et al., 1981). The model assumed no hepatic metabolism and renal clearance is the primary route of elimination. The reported clearance of amikacin was used in the model (Vogelstein et al., 1977; Bauer et al., 1980; Lanao et al., 1981; Garraffo et al., 1990; Vanhaeverbeek et al., 1993; Mahmoudi et al., 2013). The physiological parameters of amikacin are listed in Table 1.

To account for changes in amikacin clearance in human population with renal insufficiency, the renal physiological parameters including renal blood flow, kidney volume, hematocrit, small intestinal transit and renal perfusion were adjusted accordingly (Malik et al., 2020).

The potential risk of drug–drug interactions was considered in combination antibiotic therapy. Amikacin and sulbactam have a high renal clearance rate, while polymyxin B is primarily reabsorbed in the renal tubular cells and cleared through non-renal pathways. These drugs are not metabolized by the liver and drug–drug interaction affecting their pharmacokinetics is unlikely. However, nephrotoxicity is a concern for the combination therapy. Wang et al. reported that the amikacin/polymyxin B combination did not lead to acute kidney injury during a 30-day treatment period (Wang et al., 2021). Furthermore, *in vitro* susceptibility results indicated that combining these three antibiotics significantly reduced the drug concentration required to combat multidrug-resistant *A. baumannii*, while monotherapy would likely result in therapeutic failure.

2.3 Adult PBPK model evaluation

The performance of the simulations for the two antibiotics was assessed via the mean fold error (MFE, Equation 1) (Biesdorf et al., 2019):

$$\mathrm{MFE} = \frac{PK\ parameter_{\text{predicted mean}}}{PK\ parameter_{\text{observed mean}}} \quad (1)$$

MFE was performed by comparing simulated to observed maximum drug concentration (C_{max}) and the area under the concentration-time curve (AUC). The PBPK models for adults and pediatrics were accepted when the predicted to observed PK data were within 2-fold range (i.e., $0.5 \leq \mathrm{MFE} \leq 2.0$).

The observed C_{max} and AUC were extracted from pharmacokinetic profiles reported in the literature. A virtual population containing 100 subjects with a 50:50 male-to-female ratio was established for the three antibiotics to simulate their blood and tissue drug concentrations.

2.4 Pediatric PBPK model development and evaluation

2.4.1 Physiological parameters in the pediatric population

In general, renal function is considered fully developed by 2 years of age. For polymyxin-B, the renal function does not affect its clearance whereas the clearances of amikacin and sulbactam are influenced by renal function. Given that the incidence of renal function impairment in pediatrics is low, a virtual pediatric population from 0 to 17 years was established assuming normal renal function. Both renal and non-renal eliminations were scaled by age-dependent maturation of organ weight.

Anatomic and physiological parameters for pediatrics such as organ volumes, and composition, blood flows, protein binding and maturation of elimination processes were used in the pediatric virtual population development. These parameters were summarized from previous studies and incorporated into PK-Sim's ontogeny database (Poulin et al., 2001; Valentin, 2002; Ince et al., 2019). Weight-based dosing is used for pediatrics. The inter-individual variability of body weight by age and gender of pediatrics was compared to an age-matched polynomial function of body weight distribution in the literature (Sy et al., 2014). In addition, a method for protein binding prediction in small children was used to predict the unbound fraction of the antibiotics (McNamara and Alcorn, 2002).

Ince et al. (2021) established and validated a PBPK model for 10 small molecule compounds in adults, including amikacin and successfully extrapolated it to pediatric populations; however, they did not investigate drug exposure in the tissues. Claassen et al. (2015) incorporated factors such as renal and hepatic maturation (including individual hepatic enzyme development) into a PBPK model for premature infants, evaluating the model's performance for amikacin and paracetamol. They compared the predicted plasma concentration-time profiles of the two drugs with observed *in vivo* data in the blood and simulated concentrations across a wide range of gestational and postnatal ages, providing reference information for clinical use in premature pediatric populations (Claassen et al., 2015). Darlow et al. (2024) established a PBPK model for amikacin and extended it to neonates to evaluate the achievement of pharmacodynamic targets. This model only predicted plasma drug concentration for 15 mg/kg q24h dosing in neonates aged 0–28 days.

The amikacin PBPK model developed in the current study provided an in-depth evaluation of drug penetration in various tissues (blood, lung, heart, and skin) under dosing regimens of 7.5 mg/kg q12h and 15 mg/kg q12h. Compared to previous models, this model offers a more detailed prediction of drug distribution in tissues, providing new insights into drug distribution in critical infection sites such as the lungs and heart.

The current model shares foundational physiological modeling frameworks with existing PBPK models, including drug distribution between organs and metabolic pathways. These are fundamental building blocks of PBPK modeling and are thoroughly utilized in this study.

2.4.2 Pediatric dosing regimens

The recommended IV dosing regimens of polymyxin-B were 1.5–2.5 mg/kg/day for both adults and pediatrics (FDA, 2011). A loading dose and higher maintenance dose of up to 3 mg/kg/day were often used in clinical practice when treating MDR bacterial infection. When MIC was less than 0.5 mg/L, the dosage of 1.5–3.0 mg/kg/day could achieve over 90% probability of target attainments (PTA) in pediatric patient (Wang et al., 2022).

Nephrotoxicity is the main factor to consider when amikacin is administered to patients. The recommended dosing regimen of amikacin for patients with normal renal function is 15 mg/kg/day once-daily or divided in two or three equal doses (Perez-Blanco et al., 2021). At this dose, the trough concentration is maintained at <10 μg/mL in majority of the patients to minimize toxicity. EUCAST has recently changed antimicrobial susceptibility endpoints and increased dosing recommendation up to 30 mg/kg/day (EUCAST, 2020a); no increased toxicity for amikacin administered at higher doses (25–30 mg/kg/day) than the standard 15 mg/kg/day has been shown in specific populations including severe sepsis and critically ill patients (Galvez et al., 2011; de Montmollin et al., 2014; Kato et al., 2017; Perez-Blanco et al., 2021; Frost et al., 2023).

The dosing regimen of sulbactam in pediatrics was utilized according to our previous study (Zhu et al., 2022a). Both amikacin and sulbactam in pediatrics were grouped by pediatric body weight assuming normal renal function. Table 2 shows the dosing regimens for polymyxin-B, amikacin and sulbactam used in the pediatric population.

TABLE 2 Dosing regimens of amikacin/sulbactam/polymyxin-B used in simulation by creatinine clearance and by body weight categories.

Category	Dosing regimens
Creatinine clearance	*Amikacin/sulbactam (adult)*
≥60 mL/min	15 mg/kg q24h/3 g q8h as continuous infusion
≥60 mL/min	30 mg/kg q24h/3 g q8h as continuous infusion
40–59 mL/min	15 mg/kg q36h/3 g q8h as 3 h infusion
40–59 mL/min	30 mg/kg q36h/3 g q8h as 3 h infusion
30–39 mL/min	15 mg/kg q48h/3.5 g q12h as 4 h infusion
30–39 mL/min	30 mg/kg q48h/3.5 g q12h as 4 h infusion
Body weight	*Amikacin/sulbactam (pediatric)*
≥40 kg	7.5 mg/kg q12h/1.5 g q6h as 3 h infusion
≥40 kg	15 mg/kg q12h/1.5 g q6h as 3 h infusion
<40 kg	7.5 mg/kg q12h/50 mg/kg q6h as 3 h infusion
<40 kg	15 mg/kg q12h/50 mg/kg q6h as 3 h infusion
Adult	*polymyxin-B (adult)*
All renal function	LD 2.5 mg/kg + 1.5 mg/kg q12h as 1 h infusion
All renal function	LD 2.0 mg/kg + 1.25 mg/kg q12h as 1 h infusion
Child (2 to<18 years of age)	*polymyxin-B (pediatric)*
Normal renal function	1.25 mg/kg q12h as 1 h infusion
Infant (0 to<2 years of age)	*polymyxin-B (pediatric)*
Normal renal function	2.0 mg/kg q12h as 1 h infusion

LD, loading dose.

2.5 Tissue drug concentrations and penetration rates

Drug concentrations in tissues including blood, lung, heart and skin for the three antibiotics were simulated and predict by PBPK model in both pediatric and adult populations. The penetration rate was determined by comparing the C_{max} and AUC of each tissue to that in the blood. To ensure the accuracy of our simulation results, we compared the results with those reported in the literature.

2.6 Pharmacodynamic indices and probability of target attainment

For antibiotic pharmacodynamics (PD), the effectiveness of a drug is closely related to its exposure at the infection site. Antibiotics are generally classified into three categories: time-dependent, concentration-dependent, and time-dependent with a long post-antibiotic effect (PAE) (Sy et al., 2016).

The bactericidal effect of time-dependent antibiotics depends on the duration of time the free drug concentration exceeds the minimum inhibitory concentration (MIC) of the bacteria. The PD index for these antibiotics is the percentage of time the drug concentration remains above MIC during the dosing interval ($fT > MIC$). The PD index of sulbactam is 40% $fT > MIC$ against lung infection model of *A. baumannii* (Yokoyama et al., 2015). The effectiveness of these antibiotics relies on maintaining an effective concentration for a sufficient period to ensure antibacterial activity.

For time-dependent antibiotics with a long PAE, the PD index is the ratio of the free drug exposure over 24 h to MIC ($fAUC_{0-24}$/MIC). The killing effect of polymyxin B is associated with $fAUC_{0-24}$/MIC index. An fAUC/MIC range of 8.2–42.1 was associated with a 2 log kill in the lung infection model (Dudhani et al., 2010; Sandri et al., 2013a; Zhu et al., 2022b). Thus, fAUC/MIC ≥8.2 was selected as the PD index of polymyxin B against *A. baumannii*.

The antibacterial effect of concentration-dependent antibiotics is related to the level of the free drug concentration. The higher the concentration, the better the bactericidal effect. The PD index for these drugs can be the peak drug concentration (C_{max}) to MIC ratio (fC_{max}/MIC) or $fAUC_{0-24}$/MIC. For amikacin, the fC_{max}/MIC target value should be at least 8 for a satisfactory therapeutic effect, and fAUC/MIC ≥80 is also recommended (Moore et al., 1987; Rybak et al., 1999; Darlow et al., 2024).

In the case of monotherapy, the probability of target attainment (PTA) was defined as the proportion of the simulated concentration-time profiles that could achieve the target indices. For combination therapy, we have previously proposed that the joint PTA be the minimum of the individual PTAs of the antibiotics in the combination. A sufficient probability of success is when the MICs of each drug in the combination are associated with ≥90% PTA (Menegucci et al., 2019; Martins et al., 2021). The PD index and PTA analyses were carried out using user-defined functions in R (version 4.1.2).

3 Results

3.1 PBPK model qualification in adult and pediatric populations

The PBPK model development and evaluation for polymyxin-B, amikacin and sulbactam were sourced from 5, 10, and 4 reports containing 5, 11, and 6 populations, respectively; 17, 285, and 70 observations of polymyxin-B, amikacin and sulbactam PK profiles, respectively, from adults and pediatrics were utilized for model verification (Supplementary Tables S1–S3). The disease statuses of patients used in the development of PBPK models are listed in Supplementary Table S4. The collection of PK data used for PBPK model development came from diverse medical conditions. The observed data extracted from the literature for polymyxin-B, amikacin and sulbactam were within the 95% prediction interval of the corresponding simulations (Supplementary Figure S1). The two key exposure parameters, C_{max} and AUC, for adult and pediatric simulation were all within the MFE boundary of 0.5 to 2.0 compared to observed data (Figure 1).

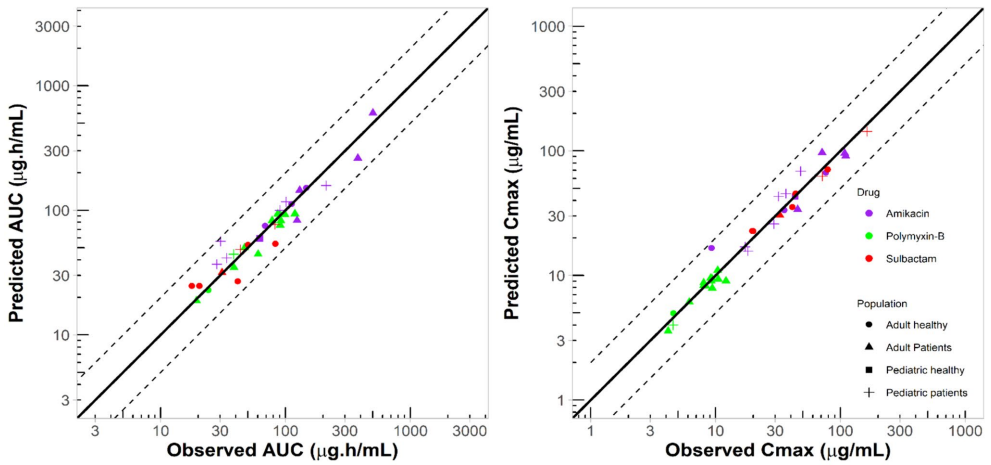

FIGURE 1
Comparison between simulated and observed exposure parameters from several studies in the literature for different populations. Solid lines represent line of unity; dashed lines represent two-fold difference.

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3.2 Tissue exposures

We compared the PBPK model to the population PK model for adult dosing regimen by renal function and evaluated the interindividual variability in pharmacokinetic profiles (Supplementary Figures S2, S3). The interstitial fluid compartment is the site where we simulated representative tissue drug concentrations. The penetration rates (computed as AUC ratio or C_{max} ratio for tissue/plasma) from the simulation and the literature were compared to evaluate the accuracy of the model.

The penetration ratios of amikacin in heart, lung and skin were 1.10–1.24, 0.49–0.58, and 0.41–0.46, respectively (Tables 3, 4). The cardiac partition coefficient was adjusted to reflect higher exposure of amikacin in the heart. The literature reported values were as follows: heart tricuspid, 1.15–1.27; lung alveolar, 0.50; skin blister fluid, 0.54 (Lanao et al., 1983; Najmeddin et al., 2020; Shin et al., 2024). Sulbactam penetration rates in the heart, lung and skin were 0.50–0.51, 0.58–0.59 and 0.28–0.31, respectively (Tables 3, 4). The pulmonary penetration ratio was 52% following IV administration in healthy adult subjects (Rodvold et al., 2018). For other organs, the penetration ratios of sulbactam were not reported for humans.

For polymyxin-B, these values were 0.980–1.05, 2.80–2.96, and 1.46–1.53 (Tables 5, 6). The penetration ratios of polymyxin-B in the heart and lungs were 1.03–1.12 and 1.93–3.38 in the rat model, respectively (Manchandani et al., 2016).

The standard deviation of the partition coefficients of tissue/plasma for polymyxin-B and amikacin in PK-Sim were adjusted and the resulting coefficient of variation (CV) of tissue C_{max} and AUC were also compared to that reported in the literature. For amikacin, the CV% of simulated C_{max} in the blood ranged from 20.6 to 23.7%, which was consistent with the reported CV% or C_{max} of 23.0% (Najmeddin et al., 2020). Since amikacin exposure in the heart mirrors that in the blood, the CV% in the heart tissue ranged from 24.1 to 28.3%, similar to those in the blood. In the lung and skin, the CV% ranged from 37.2 to 38.6% and 30.9 to 34.3%, respectively. These values were also consistent with the reported CV% or C_{max}, which were 38.6 and 38.8%, respectively in the same tissues (Lanao et al., 1983; Bayer et al., 1988). There are no reported exposure parameters of polymyxin-B in human tissues or body fluids other than the blood. Instead, we compared the CV values to those reported in the rat; the CV of polymyxin-B in the rat's heart and lung were 31.6–51.2% and 30.8–75.3%, respectively (Manchandani et al., 2016). The corresponding CV values from our PBPK simulations in humans were 31.3–33.6% and 33.0–42.1% for the same respective organs. As for sulbactam, the variability due to PBPK simulation in the lung was 14.7–22.9%, which is close to the corresponding CV of 29.6% from the literature (Rodvold et al., 2018).

3.3 Probability of target attainment (PTA) for pediatric dosing regimens

The interpretive criteria for polymyxin-B against *A. baumannii* are the following: intermediate, MIC ≤2 μg/mL; and resistant, MIC ≥4 μg/mL (Satlin et al., 2020; CLSI, 2024). We assumed that the target pharmacodynamic index for polymyxin-B (i.e., *f*AUC/MIC≥8.2) is the same for the blood and other tissues. The IV administration of

TABLE 3 Simulated steady-state AUC_{0-24h} in various tissues and AUC ratio comparing tissue to plasma exposure of amikacin and sulbactam in adults and pediatrics 2 to <18 years of age.

Tissue	Amikacin AUC_{Tissue} (μg*h/mL)	Amikacin ratio	Sulbactam AUC_{Tissue} (μg*h/mL)	Sulbactam ratio
Adult CrCL≥60mL/min (15mg/kg q24h/3g q8h as continuous infusion)				
Plasma	179.6 ± 53.8	–	457 ± 105	–
Heart	196.0 ± 68.1	1.09	233 ± 52	0.51
Lung	89.7 ± 40.8	0.50	235 ± 52	0.52
Skin	73.7 ± 31.8	0.41	128 ± 35	0.28
Adult CrCL≥60mL/min (30mg/kg q24h/3g q8h as continuous infusion)				
Plasma	359.1 ± 107.7	–	457 ± 105	–
Heart	392.0 ± 136.3	1.09	233 ± 52	0.51
Lung	179.4 ± 81.6	0.50	235 ± 52	0.52
Skin	147.3 ± 63.7	0.41	128 ± 35	0.28
Adult CrCL 40 to 59mL/min (15mg/kg q36h/3g q8h as 3h infusion)				
Plasma	244.6 ± 57.2	–	771 ± 160	–
Heart	271.1 ± 83.9	1.11	386 ± 68	0.50
Lung	122.5 ± 48.5	0.50	395 ± 79	0.51
Skin	102.4 ± 35.2	0.42	224 ± 40	0.29
Adult CrCL 40 to 59mL/min (30mg/kg q36h/3g q8h as 3h infusion)				
Plasma	489.2 ± 114.4	–	771 ± 160	–
Heart	542.2 ± 167.8	1.11	386 ± 68	0.50
Lung	245.0 ± 97.0	0.50	395 ± 79	0.51
Skin	204.8 ± 70.4	0.42	224 ± 40	0.29
Adult CrCL 30 to 39mL/min (15mg/kg q48h/3.5g q12h as 4h infusion)				
Plasma	285.5 ± 60.2	–	719 ± 176	–
Heart	313.6 ± 89.7	1.10	367 ± 74	0.51
Lung	142.3 ± 56.5	0.50	370 ± 88	0.52
Skin	118.6 ± 40.1	0.42	208 ± 42	0.29
Adult CrCL 30 to 39mL/min (30mg/kg q48h/3.5g q12h as 4h infusion)				
Plasma	570.9 ± 120.3	–	719 ± 176	–
Heart	627.2 ± 179.5	1.11	367 ± 74	0.51
Lung	284.5 ± 112.9	0.50	370 ± 88	0.52
Skin	237.2 ± 80.3	0.42	208 ± 42	0.29
Pediatric≥40kg (7.5mg/kg q12h/1.5g q6h as 3h infusion)				
Plasma	150.2 ± 41.1	–	381 ± 98	–
Heart	165.1 ± 54.6	1.10	194 ± 46	0.51
Lung	76.6 ± 33.4	0.51	196 ± 49	0.51
Skin	63.0 ± 24.3	0.42	110 ± 19	0.29

(Continued)

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TABLE 3 (Continued)

Tissue	Amikacin AUC_{Tissue} (μg*h/mL)	Amikacin ratio	Sulbactam AUC_{Tissue} (μg*h/mL)	Sulbactam ratio
Pediatric≥40kg (15mg/kg q12h/1.5g q6h as 3h infusion)				
Plasma	300.3 ± 82.2	–	381 ± 98	–
Heart	330.2 ± 109.3	1.10	194 ± 46	0.51
Lung	153.2 ± 66.7	0.51	196 ± 49	0.51
Skin	126.0 ± 48.6	0.42	110 ± 19	0.29
Pediatric<40kg (7.5mg/kg q12h/50mg/kg q6h as 3h infusion)				
Plasma	120.1 ± 33.1	–	488 ± 128	–
Heart	132.9 ± 43.2	1.11	249 ± 61	0.51
Lung	59.5 ± 26.0	0.50	251 ± 65	0.51
Skin	49.9 ± 20.3	0.42	151 ± 36	0.31
Pediatric<40kg (15mg/kg q12h/50mg/kg q6h as 3h infusion)				
Plasma	240.3 ± 66.3	–	488 ± 128	–
Heart	265.8 ± 86.5	1.11	249 ± 61	0.51
Lung	119.1 ± 52.1	0.50	251 ± 65	0.51
Skin	99.8 ± 40.7	0.42	151 ± 36	0.31

CrCL, creatinine clearance.

polymyxin-B in the pediatric population was simulated to evaluate drug concentrations in pediatric tissues. The results in Figure 2 showed that in the blood, dosing regimens consisting of a loading dose (LD) 2.5 mg/kg plus maintenance dose (MD) 1.5 mg/kg q12h in adults and 2 mg/kg q12h in pediatrics (0 to <2 years old) were able to achieve more than 90% PTA for an MIC of ≤4 μg/mL whereas LD 2.0 + 1.25 mg/kg q12h in adults and 1.25 mg/kg q12h in pediatric (2 to <18 years old) achieved similar results for ≤2 μg/mL MIC. In cardiac tissues, all dosing regimens achieved more than 90% PTA at ≤4 μg/mL MIC, and pediatric (0 to <2 years old) 2 mg/kg q12h achieved more than 90% PTA at ≤8 μg/mL MIC. In the lung, an adequate coverage (PTA ≥85%) was achieved at ≤4 μg/mL MIC for all listed regimens. In the skin, all dosing regiments achieved >85% PTA at ≤8 μg/mL MIC. Polymyxin-B adult and pediatric dosing regimens have comparable exposures.

Amikacin approved dosing regimen is 15 mg/kg once-daily or 7.5 mg/kg twice-daily. A revised dosing of 25–30 mg/kg once daily has been recommended by EUCAST, given the risk of sub-therapeutic drug concentrations with the 15 mg/kg once daily dosing against pathogens with high MIC value (4–16 mg/L) (EUCAST, 2020a). EUCAST susceptibility breakpoints are 8 mg/L and 16 mg/L for Enterobacterales and *Pseudomonas* sp., respectively (EUCAST, 2020b). Both the approved and revised dosing regimens were evaluated. PBPK simulations of both adult and pediatric dosing regimens indicated that drug exposure was highest in the heart, followed by the blood, lungs, and skin. A reason for a slightly higher exposure of amikacin in the heart compared to plasma is that lower blood flow and pressure that are usually present in the cardiac tricuspid valve tissue compared to the aorta can result in longer blood retention time, resulting in a higher drug exposure (McColm and Ryan, 1985; Bayer et al., 1988).

For amikacin, two relevant PD target indices are fC_{max}/MIC ≥8 and *f*AUC/MIC ≥80. The *f*AUC/MIC target at 80–90 is believed to

TABLE 4 Simulated steady-state C_{max} in various tissues and C_{max} ratio comparing tissue to plasma exposure of amikacin and sulbactam in adults and pediatrics 2 to <18 years of age.

Tissue	Amikacin C_{max} (μg/mL)	Amikacin ratio	Sulbactam C_{max} (μg/mL)	Sulbactam ratio
Adult CrCL≥60mL/min (15mg/kg q24h/3g q8h as continuous infusion)				
Plasma	51.2 ± 11.6	–	20.1 ± 4.8	–
Heart	61.8 ± 16.3	1.22	10.2 ± 2.1	0.51
Lung	29.3 ± 11.1	0.58	10.3 ± 2.4	0.51
Skin	22.6 ± 7.5	0.45	6.43 ± 1.54	0.32
Adult CrCL≥60mL/min (30mg/kg q24h/3g q8h as continuous infusion)				
Plasma	102.5 ± 23.2	–	20.1 ± 4.8	–
Heart	123.6 ± 32.6	1.22	10.2 ± 2.1	0.51
Lung	58.6 ± 22.2	0.58	10.3 ± 2.4	0.51
Skin	45.1 ± 15.0	0.45	6.43 ± 1.54	0.32
Adult CrCL 40 to 59mL/min (15mg/kg q36h/3g q8h as 3h infusion)				
Plasma	55.1 ± 13.4	–	69.5 ± 11.8	–
Heart	66.8 ± 19.0	1.22	35.7 ± 5.9	0.51
Lung	31.2 ± 11.8	0.57	35.7 ± 5.7	0.51
Skin	24.5 ± 7.6	0.45	18.8 ± 2.8	0.27
Adult CrCL 40 to 59mL/min (30mg/kg q36h/3g q8h as 3h infusion)				
Plasma	110.3 ± 26.8	–	69.5 ± 11.8	–
Heart	133.6 ± 38.1	1.22	35.7 ± 5.9	0.51
Lung	62.4 ± 23.6	0.57	35.7 ± 5.7	0.51
Skin	49.1 ± 15.3	0.45	18.8 ± 2.8	0.27
Adult CrCL 30 to 39mL/min (15mg/kg q48h/3.5g q12h as 4h infusion)				
Plasma	56.6 ± 13.2	–	74.4 ± 14.1	–
Heart	67.9 ± 18.6	1.21	38.6 ± 7.1	0.51
Lung	31.9 ± 12.3	0.57	38.5 ± 7.0	0.52
Skin	25.0 ± 8.0	0.45	20.1 ± 3.3	0.27
Adult CrCL 30 to 39mL/min (30mg/kg q48h/3.5g q12h as 4h infusion)				
Plasma	113.2 ± 26.5	–	74.4 ± 14.1	–
Heart	135.9 ± 37.2	1.21	38.6 ± 7.1	0.51
Lung	63.7 ± 24.7	0.57	38.5 ± 7.0	0.52
Skin	50.0 ± 16.0	0.45	20.1 ± 3.3	0.27
Pediatric≥40kg (7.5mg/kg q12h/1.5g q6h as 3h infusion)				
Plasma	25.2 ± 5.6	–	29.2 ± 6.6	–
Heart	30.1 ± 7.9	1.21	15.1 ± 3.1	0.51
Lung	14.3 ± 5.3	0.57	15.0 ± 3.3	0.51
Skin	11.4 ± 3.7	0.46	8.47 ± 1.9	0.29
Pediatric≥40kg (15mg/kg q12h/1.5g q6h as 3h infusion)				

(Continued)

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TABLE 4 (Continued)

Tissue	Amikacin C_{max} (μg/mL)	Amikacin ratio	Sulbactam C_{max} (μg/mL)	Sulbactam ratio
Plasma	50.4 ± 11.3	–	29.2 ± 6.6	–
Heart	60.2 ± 15.8	1.21	15.1 ± 3.1	0.51
Lung	28.6 ± 10.6	0.57	15.0 ± 3.3	0.51
Skin	22.8 ± 7.4	0.46	8.47 ± 1.9	0.29
Pediatric<40kg (7.5mg/kg q12h/50mg/kg q6h as 3h infusion)				
Plasma	21.0 ± 4.2	–	38.3 ± 8.8	–
Heart	25.7 ± 6.2	1.23	19.2 ± 5.1	0.50
Lung	11.8 ± 4.4	0.57	19.7 ± 4.5	0.51
Skin	9.7 ± 3.3	0.47	11.5 ± 2.5	0.30
Pediatric<40kg (15mg/kg q12h/50mg/kg q6h as 3h infusion)				
Plasma	42.1 ± 8.5	–	38.3 ± 8.8	–
Heart	51.4 ± 12.3	1.23	19.2 ± 5.1	0.50
Lung	23.6 ± 8.8	0.57	19.7 ± 4.5	0.51
Skin	19.4 ± 6.6	0.47	11.5 ± 2.5	0.30

CrCL, creatinine clearance.

TABLE 5 Simulated steady-state AUC_{0-24h} in various tissues and AUC ratio comparing tissue to plasma exposure of polymyxin-B in adults and pediatrics 2 to <18 years of age.

Tissue	Polymyxin-B AUC_{tissue} (μg*h/mL)	Polymyxin-B ratio
Adult all renal function LD 2.5mg/kg+1.5mg/kg q12h as 1h infusion		
Plasma	96.8 ± 24.0	–
Heart	96.9 ± 41.6	0.990
Lung	293 ± 125	3.05
Skin	148 ± 56	1.55
Adult all renal function LD 2.0mg/kg+1.25mg/kg q12h as 1h infusion		
Plasma	83.2 ± 20.2	–
Heart	84.7 ± 29.7	1.02
Lung	236 ± 109	2.88
Skin	131 ± 54	1.57
Child all renal function (2 to<18years of age) 1.25mg/kg q12h as 1h infusion		
Plasma	47.1 ± 13.2	–
Heart	49.2 ± 20.4	1.05
Lung	135 ± 63	2.88
Skin	73.4 ± 30.9	1.55
Infant all renal function (0 to<2years of age) 2.0mg/kg q12h as 1h infusion		
Plasma	64.8 ± 18.2	–
Heart	66.3 ± 27.4	1.02
Lung	186 ± 89.3	2.87
Skin	99.8 ± 41.9	1.54

LD, loading dose.

TABLE 6 Simulated steady-state C_{max} in various tissues and C_{max} ratio comparing tissue to plasma exposure of polymyxin-B in adults and pediatrics 2 to <18 years of age.

Tissue	Polymyxin-B C_{max} (μg/mL)	Polymyxin-B ratio
Adult all renal function LD 2.5mg/kg+1.5mg/kg q12h as 1h infusion		
Plasma	10.9 ± 1.2	–
Heart	11.7 ± 4.0	1.06
Lung	36.6 ± 13.7	3.36
Skin	16.5 ± 4.5	1.52
Adult all renal function LD 2.0mg/kg+1.25mg/kg q12h as 1h infusion		
Plasma	9.20 ± 1.03	–
Heart	10.1 ± 2.8	1.10
Lung	28.9 ± 11.9	3.18
Skin	14.0 ± 3.7	1.54
Child all renal function (2 to<18years of age) 1.25mg/kg q12h as 1h infusion		
Plasma	5.22 ± 0.82	–
Heart	5.58 ± 1.90	1.07
Lung	15.5 ± 6.2	2.99
Skin	8.14 ± 2.68	1.56
Infant all renal function (0 to<2years of age) 2.0mg/kg q12h as 1h infusion		
Plasma	7.31 ± 1.06	–
Heart	7.74 ± 2.58	1.06
Lung	22.2 ± 8.8	3.05
Skin	11.3 ± 3.6	1.55

LD, loading dose.

be a more robust alternative and may be more suitable for critically ill patients with high bacterial burden infections such as nosocomial pneumonia (Bland et al., 2018; De winter et al., 2018; Perez-Blanco et al., 2021). Due to differences in dosing frequencies of amikacin between adults with renal insufficiencies and pediatrics, we used these two target PD indices for amikacin in our computation of PTA for a more thorough assessment. With the high dose of amikacin, at least 90% PTA was achieved in blood with an MIC of ≤16 μg/mL using ≥8 fC_{max}/MIC (Supplementary Figure S5) and ≤4 μg/mL using ≥80 fAUC/MIC (Figure 3). The 15 mg/kg adult dosing regimens only attained half the MIC of the high dose (Supplementary Figures S6, S7). The 15 mg/kg twice-daily in pediatrics resulted in similar PTA as that of the adult of the same total daily dose using target AUC PD index (Figure 3) but lower PTA if PD index based on C_{max} was used (Supplementary Figure S6), since the twice-daily regimen resulted in only half the C_{max} as the once-daily regimen with the same total daily dose. As amikacin exposure in the lung and skin is lower than that in the blood, sufficient coverage can be achieved at ≤2 and ≤1 μg/mL MIC in the lung and skin, respectively (Figure 3) for the adult high dose with the AUC PD index. With the C_{max} PD index, sufficient coverage can be achieved at a two-fold higher MIC values. Due to low

permeability of amikacin in the skin, skin infections are difficult to treat with intravenous amikacin administration.

The interpretive criteria for *Acinetobacter* spp. susceptibility to sulbactam treatment is ≤4 μg/mL for susceptible and 8 μg/mL for intermediate (CLSI, 2024). Sulbactam pediatric regimen of 40 mg/kg q6h as 3-h infusion was selected for a body weight <40 kg. Previous studies have shown that sulbactam adult regimens against *A. baumannii* at 8 μg/mL MIC, using 40% *f*T > MIC as PD index, can reach ≥90% PTA (Yokoyama et al., 2015). The calculations assumed a plasma protein binding of 5% and no protein binding in other tissues (Yokoyama et al., 2014). Sulbactam was able to achieve ≥90% PTA in all age groups of pediatric patients with an MIC of ≤8 μg/mL using the administration schedule listed in Table 2. At 4 μg/mL MIC, coverage in the lung, skin, and heart were adequate based on the proposed dosing regimens of sulbactam (Figure 4).

In the lung, the MIC values for amikacin, polymyxin-B and sulbactam combination to sufficiently achieve satisfactory joint PTA are 2/4/4 μg/mL.

4 Discussion

The predicted antibiotic exposures using PBPK models and target achievement in major tissues associated with common infections including the heart, lungs, and skin can be used to infer efficacy at the site of infection. Often actual drug exposures in these commonly infected sites are lacking, especially drug concentrations in pediatric tissues and organs; pediatric data are more difficult to obtain than in adults (Kafetzis et al., 1979; Schaad et al., 1986; Xu et al., 2022). We established and validated PBPK models in adults and extrapolated them to the pediatric population to evaluate the therapeutic effects of these antibiotics at the sites of infection. The application of the PBPK model to determine site-specific drug concentrations assumes that PD targets and thresholds associated with microbial outcomes are appropriate not only for their assessment in the blood, but also in the infected tissues.

The clinical efficacy of antimicrobial combination is often inferred from case reports. The combination of polymyxin-B and amikacin has been shown to be effective against blood infections caused by *Klebsiella pneumoniae* and offered a survival benefit (Cleary et al., 1979). This combination therapy has not been studied in a randomized clinical trial. Polymyxin-B combined with cefoperazone/sulbactam successfully cured posterior ventriculitis caused by extensively resistant *A. baumannii* in a child. In another case, a patient presented with pneumonia-related multiple organ dysfunction syndrome due to MDR *P. aeruginosa* and *A. baumannii* was successfully treated with polymyxin-B/amikacin combination (Wang et al., 2021; Yang et al., 2021).

The combination strategy can sensitize MDR microorganisms to the drug concentrations produced using the approved dosing

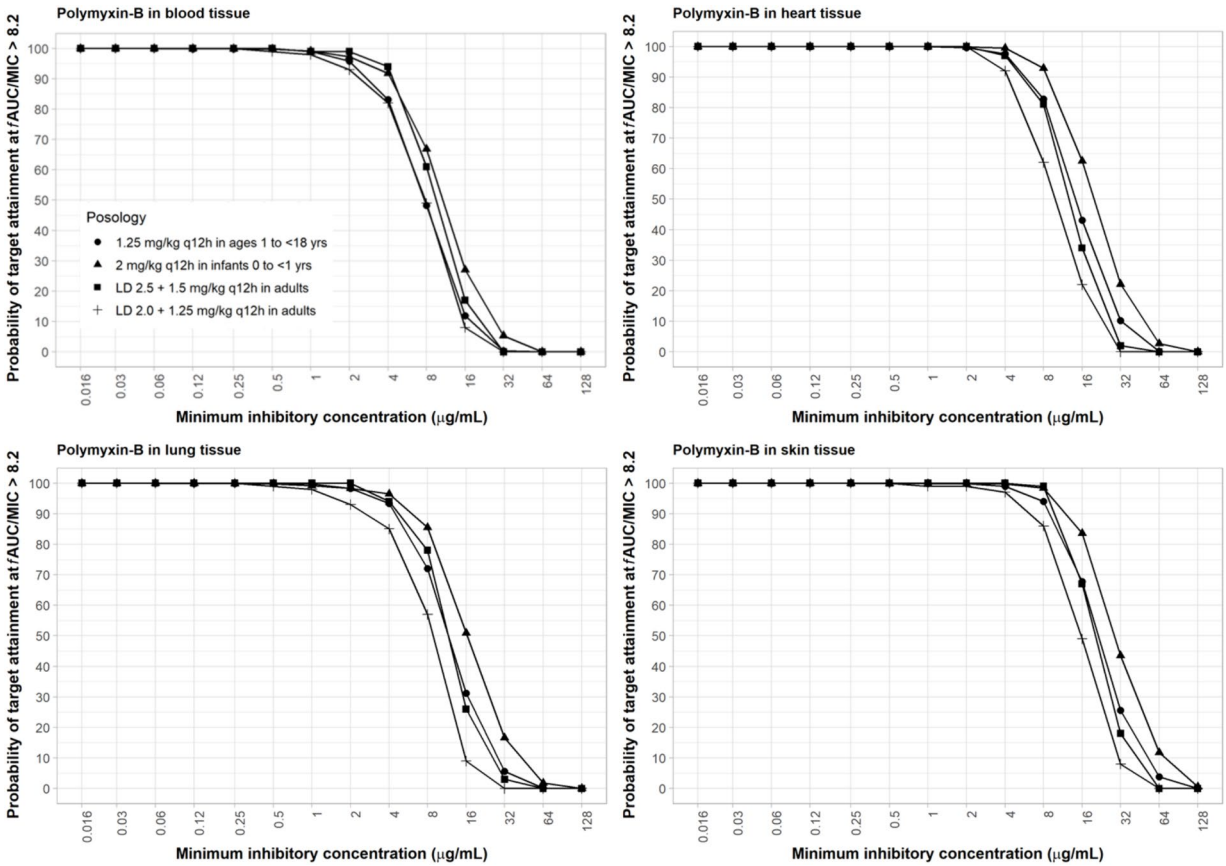

FIGURE 2
Probability of target attainment of polymyxin-B PD index of *f*AUC/MIC of at least 8.2 in the blood, lung, heart, and skin for adult and pediatric dosing regimens.

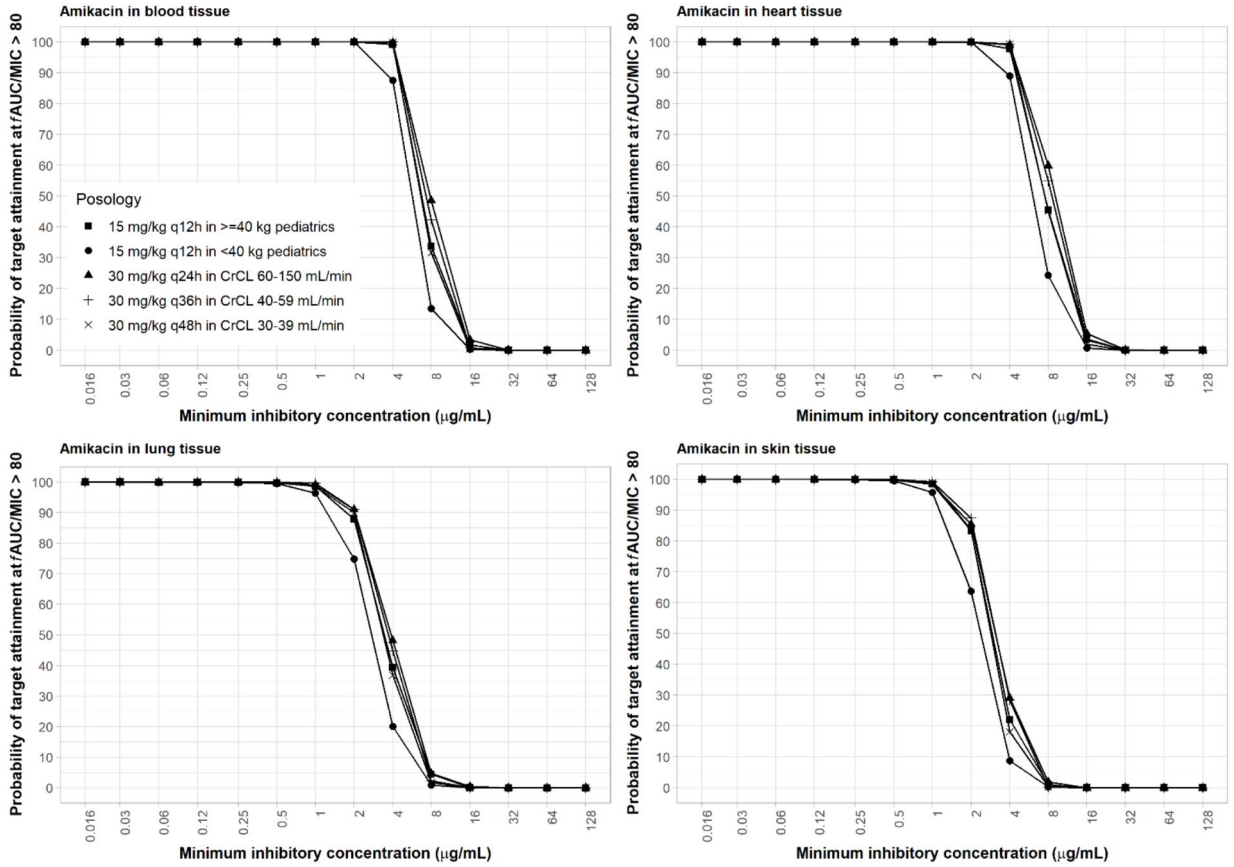

FIGURE 3
Probability of target attainment of amikacin PD index of *f*AUC/MIC ratio of at least 80 in the blood, lung, heart, and skin for dosing regimens in pediatrics (15 mg/kg) and adults (30 mg/kg).

regimens. In our previous survey of extremely drug-resistant *A. baumannii* clinical isolates, the MIC_{50} and MIC_{90} for amikacin/polymyxin-B/sulbactam combination were 1/4/4 and 8/4/4 μg/mL, respectively (Zhu et al., 2022a). Based on the PTA evaluation for this combination in the blood, lung, heart and skin, sufficient coverage can be achieved for the MIC_{50} of the surveyed collection. Amikacin dosing regimens, on the other hand, would not provide sufficient coverage against MIC_{90} of the collection based on the stricter PD index using *f*AUC/MIC >80. However, the fC_{max}/MIC >8 criteria can be achieved with the high dose of 30 mg/kg/day in the blood, heart and lung, but not the skin.

Our analysis indicated that drug exposures for this combination in the tissues or organs evaluated were effective against at least 50% of extremely drug resistant *A. baumannii* clinical isolates. The inference for clinical efficacy using PTA did not include the antibacterial effect of human immunity. In the *in vivo* infection model used to define target PD indices, neutropenia was induced in mice prior to infecting the animal (Sy and Derendorf, 2014). The methodology to derive PD indices reflects the worst-case scenario in immunocompromised individuals. The immune system will play an important role against MDR infections because it does not distinguish between resistant and sensitive bacteria (Rayner et al., 2021).

The variability in antibiotic permeability across different tissues led to differences in the PTA results. Amikacin, as an aminoglycoside, is a hydrophilic molecule with low tissue permeability. Passive diffusion across endothelial cells of capillaries requires drugs to be lipophilic, which may result in low amikacin exposure in the lungs and skin (Honeybourne, 1994; Najmeddin et al., 2020). However, amikacin is commonly used in the clinic for the treatment of acute exacerbations in patients with cystic fibrosis (Kiem and Schentag, 2008), which may be attributed to inflammation-induced lung endothelial damage, affecting alveolar epithelial permeability, thereby enhancing amikacin penetration and distribution into the lungs (Lamer et al., 1993). Although it can achieve high therapeutic concentrations in the lungs, amikacin clearance from the lungs is affected by its exchange in the blood.

Polymyxin B is a cationic polypeptide antibiotic, and its large molecular characteristics restrict its distribution, metabolism, and excretion after intravenous injection (Avedissian et al., 2019). Our predictions for lung exposure were consistent with the observed drug concentrations reported (Manchandani et al., 2016). The 6-h sample of polymyxin in the lung tissues was approximately 2-fold the serum drug concentration, indicating accumulation of polymyxin in the lung over time (Manchandani et al., 2016), whereas polymyxin exposure in the epithelial lining fluid in mice was previously shown to be lower than that in the serum (He et al., 2010). There is a high degree of variability in the literature on polymyxin lung penetration.

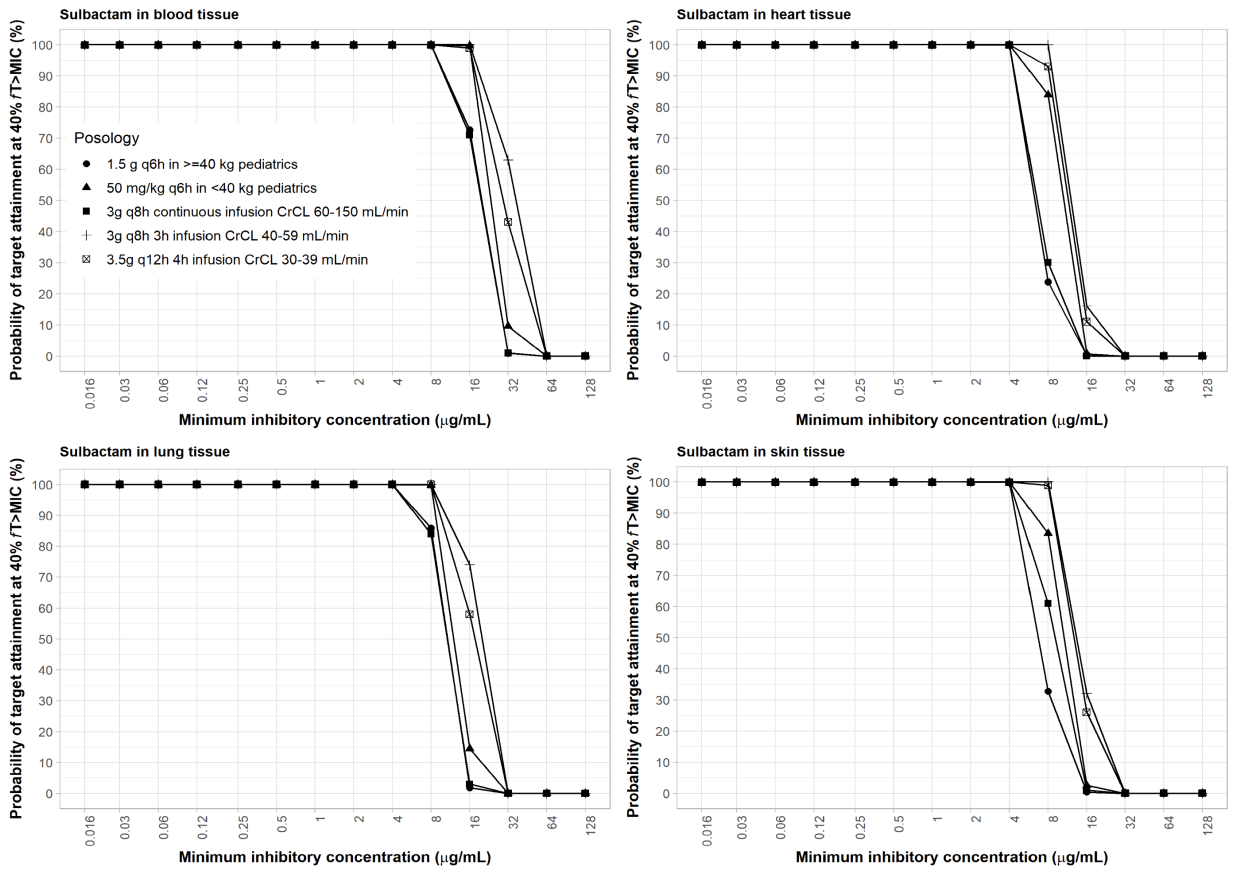

FIGURE 4
Probability of target attainment of sulbactam PD index of *f*T > MIC of at least 60% in the blood, lung, heart, and skin for adult and pediatric dosing regimens.

The current guidelines for polymyxins recommends combination therapy, since it is not possible to increase the daily doses beyond the recommended limit of 2.5 mg/kg loading dose and 1.5 mg/kg q12h maintenance dose (Tsuji et al., 2019). Lung infection model indicated lower efficacy than thigh infection model (Cheah et al., 2015). With increasing polymyxin resistance, the strategy to optimize polymyxin therapeutics could also include inhalation, in the case of lung infection (Zhu et al., 2022a).

While our PBPK model offers valuable insights into the pharmacokinetics of drug combinations in pediatric patients, particularly in predicting drug concentrations across various tissues, including the lungs, we must acknowledge the inherent uncertainties and limitations of these predictions. The complexity and variability of lung physiology, the heterogeneity in drug distribution, and the lack of comprehensive clinical data to fully validate these predictions present many challenges toward an accurate prediction of lung polymyxin concentrations.

Dosing guideline of antibiotics in pediatrics should be based on antimicrobial susceptibility determination. We do not recommend deviating from the recommended clinical dosages. Of the three antibiotics, only amikacin has a standard dose and a high dose, due to recent changes in consensus guidelines. Decision to use combination therapy should be based on whether the MIC for the combination can be sufficiently covered by the joint PTA.

Strategies for antibiotic combination use need to consider whether there is a potential risk of an enhanced toxicity. Consequently, the duration of treatment could be limited by adverse events. The polymyxin-B/amikacin/sulbactam triple combination has the potential for nephrotoxicity and other adverse effects. Polymyxin-B undergoes renal reabsorption through tubular cells while amikacin and sulbactam are primarily eliminated by the kidney. We chose polymyxin-B and sulbactam to be combined with amikacin because the renal liability of polymyxin-B is considerably less compared to colistin (Zavascki and Nation, 2017). The addition of sulbactam to the combination is based on a matched cohort study showing low potential of sulbactam to induce acute kidney injury when used as a partnering β-lactamase inhibitor to piperacillin (Rutter and Burgess, 2017). While amikacin is known to cause nephrotoxicity (Kaynar et al., 2007), the amikacin/polymyxin-B combination in a case report did not result in acute kidney injury even though this combination was administered for the duration of 30 days; the patient's follow-up serum creatinine was 75 μmol/L which indicated no evidence of acute renal impairment (Wang et al., 2021).

Several limitations of the current approach are identified. The lack of actual tissue drug concentration data in human tissues limits our ability to verify the simulation of drug concentration. The complexity of organ tissue structure also affects the accuracy of drug concentration simulation; the microanatomy of tissue can lead to concentration gradients between compartments. The tissue drug concentration

simulated in this study comes from interstitial space and may not necessarily represent the microspace where bacteria proliferate.

In summary, this study explored the use of PBPK models to predict drug exposure in several potential sites of infection in the pediatric population, and assessed whether exposure could achieve the desired target achievement rate. The results of the study have yet to be confirmed in clinical trials. Antibiotic combination offers a potential treatment option against tissue infections caused by drug-resistant bacteria, which are increasingly threatening human health. At a time when new antibiotics are scarce, effective antibiotic combination therapy has practical implications for addressing the pressing problem of drug resistance. PBPK and other modeling methods to predict and simulate the amount of antibacterial drug exposure in tissue is a practical approach to overcome the difficulty of obtaining tissue drug concentrations in pediatric population.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

MW: Writing – original draft, Writing – review & editing, Investigation, Methodology, Software. KF: Writing – review & editing, Writing – original draft, Methodology. XW: Writing – review & editing, Investigation. CL: Writing – review & editing. SZ: Writing – original draft, Data curation. FM: Writing – review & editing, Conceptualization, Investigation. MYu: Writing – original draft, Writing – review & editing. ZL: Writing – original draft, Writing – review & editing, Supervision. MYa: Writing – review & editing, Supervision. SS: Writing – original draft, Writing – review & editing, Supervision.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by grant from Shandong Provincial Natural Science Foundation (ZR2019BC025).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1435906/full#supplementary-material

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