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Neonatal sepsis: Current insights and challenges

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Neonatal sepsis: Current insights and challenges

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Editorial: Neonatal sepsis: current insights and challenges

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Editorial on the Research Topic
Neonatal sepsis: current insights and challenges

The incidence of bacterial sepsis ranges from 1 to 10 cases per 1,000 live births, with a 4-fold to 10-fold increase in preterm neonates (1–4). Despite the significant progress in neonatal care, sepsis remains a leading cause of morbidity and mortality in Neonatal Intensive Care Units (NICUs), accounting for 243,000 deaths per year globally (5–8).

The World Health Organization (WHO) recently identified the need to reduce the burden of neonatal sepsis as the Sustainable Development Goal 3, aiming to decrease neonatal mortality to at least 12 per 1,000 live births by 2030 (9). The reduction of sepsis-related deaths in low-and middle-income countries is essential in order to achieve this goal (10). The incidence of neonatal sepsis widely varies among different countries and territories, reflecting differences in health resources, maternal and neonatal risk factors, and prevention strategies (5, 11). National and regional data should be considered for the implementation of successful measures for decrease of the incidence and mortality of neonatal infections. Li et al., collected data from the Global Burden of Disease 2019 to evaluate global features of incidence and mortality of neonatal sepsis and other neonatal infections (NSNIs), and to guide global and regional interventions for prevention and control of NSNIs. From 1990 to 2019, NSNI cases presented an annual global increase of 12.79%, while NSNI deaths decreased by 12.93% annually. A demographic and health survey in Ethiopia (2016) reported a significant number of neonatal deaths related to sepsis (12). Ambaye et al. conducted a trial to evaluate the time to sepsis recovery and its defining factors among neonates admitted in Woldia Comprehensive Specialized Hospital (WCSH), Northeast Ethiopia. The results indicated that time to sepsis recovery is adversely and independently associated with induction of labor and resuscitation at birth. Gezmu et al. using data from a public, tertiary-level hospital in Botswana, evaluated multiple risk factors and identified sepsis as an independent risk factor for pulmonary hemorrhage. Candida infection ranks as the third most common cause of neonatal late-onset sepsis, with Candida albicans being the most frequently isolated species. However, there has been a recent increase in the incidence of non-albicans Candida sepsis (13), including Candida

glabrata, a case of which is reported by Parramon-Teixido et al. describing a urinary tract infection in a preterm neonate.

Necrotizing enterocolitis (NEC) is another crucial complication that primarily affects premature infants and is characterized by inflammation, similar to sepsis. Additionally, both conditions present non-specific, often overlapping, clinical symptoms and signs, rendering their differential diagnosis difficult (14–16). The better comprehension of pathophysiological mechanisms of sepsis and NEC, essential for the improvement of timely and accurate diagnosis and treatment, is necessary. Jiang et al. evaluated the role of the proportion of large platelets (PLCR) and platelet crit (PCT) in prediction of NEC in low birth weight (LBW) neonates. Results of this study indicated that 2/100 LBW neonates were at risk of NEC and that sepsis and anemia were main factors associated with NEC. Stratification of confounding factors revealed the superiority of PLT activation (especially PLCR), compared to PLT count, in predicting NEC occurrence in non-septic, LBW neonates.

Early detection of neonatal sepsis is challenging (5). Delayed treatment increases mortality, while treating neonates with mild symptoms and signs or solely risk factors results in overtreatment and unnecessary use of antibiotics. The identification of an optimal biomarker for diagnosis and monitoring of neonatal sepsis is the target of ongoing research. Inflammatory biomarkers seem promising; yet, only a few have been incorporated in clinical practice. Kumar et al. analyzed hematologic and physiologic biomarkers of late-onset sepsis and NEC in very low birthweight neonates. A validated sepsis risk score (Pulse Oximetry Warning Score, POWS) (17) was used to test whether plasma biomarkers correlate with physiological biomarkers of sepsis and concluded that inflammatory biomarkers discriminated between late-onset septicemia due to Gram-negative or NEC and all other septic or non-septic conditions.

In general, the diagnostic tools currently used for sepsis are invasive and time-consuming. Newer, non-invasive analytical methods could detect an infection early and also identify the pathogen. Bous et al. developed a method to analyze the profile of volatile organic compounds of bacterial species. Multicapillary column-coupled ion mobility spectrometry was used and found appropriate for the identification and differentiation between specific bacteria, emerging as a useful *in vitro* diagnostic tool.

Purulent ophthalmic discharge poses a clinical dilemma in NICU setting, considering its potential association with late-onset sepsis. Gad et al. did not report a statistically significant correlation between purulent conjunctivitis with a positive swab culture and late-onset sepsis. Post-catheter removal sepsis (PCRS) is a notable complication of indwelling central venous catheters (CVCs) in neonates, attributed to disruption of biofilms formed along the catheter tip during removal of CVCs. The prevention of PCRS with the use of antibiotics during removal of CVCs remains debatable. Ji et al. conducted a meta-analysis, and according to their results, the use of antibiotics within 12 h of CVC removal does not significantly reduce PCRS, but is associated with reduced post-catheter removal blood stream infection.

Human milk has proved protective against neonatal sepsis and other infections, particularly in preterm neonates (18). A study by Moliner-Calderón et al. showed that any feeding with human milk was associated with a reduction in the need for vasoactive drugs in septic neonates, encouraging further research to clarify whether feeding with human milk directly affects neonatal patterns of cardiovascular maturation.

The present research topic has inspired significant focus on neonatal sepsis. Better understanding of this condition, its heterogeneity, epidemiology, and pathophysiology will help optimize short-term and long-term outcome and reduce burden on society. Neonatal sepsis has an impact that extends throughout life, and future studies should incorporate morbidity, mortality, long-term results and direct and indirect cost. This collection of sepsis-related articles in the current issue of "Frontiers of pediatrics" highlights the contemporary understanding and gaps and limitations in the diagnosis and treatment of sepsis, along with areas for further research. More studies are required on this topic, nonetheless, recent developments are quite promising and intriguing.

Author contributions

RS: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. SP: Writing – review & editing, Writing – original draft, Data curation. AK: Writing – review & editing, Data curation. AT: Writing – review & editing, Data curation. NI: Writing – review & editing, Data curation, Conceptualization.

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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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Global, regional, and national incidence and mortality of neonatal sepsis and other neonatal infections, 1990–2019

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Background: Neonatal infections, especially neonatal sepsis, are one of the major causes of incidence and mortality in pediatrics. However, the global burden of neonatal sepsis and other neonatal infections (NSNIs) remains unclear.

Methods: From the 2019 global disease burden study, we collected annual incident cases, deaths, age-standardized incidence rates (ASIRs), and age-standardized deaths rates (ASDRs) of NSNIs in the past 30 years. Analysis indicators included the percentage of relative changes in incident cases and deaths, and the estimated annual percentage changes (EAPCs) of ASIRs and ASDRs. Correlations were assessed between the EAPCs of ASIRs and ASDRs and social evaluation indicators, including sociodemographic index (SDI) and universal health coverage index (UHCI).

Results: Globally, the number of incident cases of NSNIs grew by 12.79% per year, and the number of deaths dropped by 12.93% per year. During this period, global ASIR of NSNIs increased by 46% annually on average, while ASDR decreased by 53% annually on average. The ASIR and ASDR of female NSNIs were consistently lower than that of male NSNIs. The EAPC of female ASIR was 0.61, nearly twice that of male ASIR, and female ASIR was growing rapidly. The same declining trends of ASDR were noted in males and females. The ASIR of NSNIs in high-SDI regions grew by an average of 14% annually from 1990 to 2019. Except for high-SDI regions, the ASIRs of other 4 SDI regions maintained a rising trend at a high level, and were improved in the past 10 years. The ASDRs of all 5 SDI regions generally showed a downward trend. The region with the highest ASIR of NSNIs was Andean Latin America, and Western Sub-Saharan Africa had the highest mortality. We found a negative correlation between EAPCs of ASDRs and UHCI in 2019.

Conclusion: The global health situation was still not optimal. The incidence of NSNIs remained high, and continues to rise. The mortality of NSNIs has decreased, especially in the countries/territories with high UHCI. Therefore, it is crucial to improve the overall awareness and management of NSNIs, and take interventions for NSNIs worldwide.

KEYWORDS

neonatal sepsis and other neonatal infections, global burden disease, incidence, mortality, global trend

Introduction

Neonatal infections refer to the disease caused by the infection of pathogenic microorganisms (bacterial, viral, or fungal) in newborns, including neonatal sepsis, neonatal pneumonia and other neonatal infections, the most common pathogens of which include group B Streptococcus (GBS), *Escherichia coli*, and herpes simplex virus (HSV) (1, 2). In the past decades, the mechanism of neonatal susceptibility to infection has been extensively studied. Neonatal immature immunity has been implicated for the higher neonatal infection rate (3). Preterm infants, compared with term infants, showed more immature immune responses and had a higher risk of infection (1, 4, 5). Another risk factor was neonatal environmental exposure, such as the maternal history of exposure to infectious diseases and bacterial colonization (6, 7).

Neonatal infections, especially neonatal sepsis, are one of the major causes of incidence and mortality in pediatrics (2). In 2015, 600,000 of newborns died of infection worldwide (8). The 2016 Global Burden of Disease Study illustrated that neonatal sepsis and other neonatal infections (NSNIs) ranked third in neonatal deaths (243,000 deaths per year), and also ranked at the forefront of years of lost life across all age groups (9). It was reported that South Asia and Sub-Saharan Africa had the highest burden of neonatal sepsis, and the total incidence of culture-positive sepsis reported by South Asian hospitals was 15.8 per 1,000 live births (10). This was about 2–4 times higher than the rate reported in the United Kingdom and the United States (1, 11). To reduce the incidence and mortality of NSNIs, measures should be taken based on national and regional incidence and mortality data for NSNIs and related factors.

As far as we know, there is no detailed and systematic study to illustrate the incidence and mortality of NSNIs, and the relationship with national socio-economic status and medical health level. Therefore, we collected Global Burden of Disease 2019 data to assess the global incidence and mortality characteristics of NSNIs and the relationship with national socio-economic status and medical health level, so as to provide a more comprehensive view for the development of global and regional interventions to prevent and control NSNIs health care policies.

Methods

Data source

The GBD 2019 study performed an all-round epidemiological assessment of 369 diseases and injuries (by age and sex) in 204 countries and territories worldwide in the past 30 years (12). The details of the GBD study have been reported before (12–14). The specific protocols are available in the GBD website (https://www.healthdata.org/gbd/about/protocol).

From the Global Health Data Exchange (GHDx) query tool, we collected annual incident cases, deaths, age-standardized incidence rates (ASIRs), and age-standardized deaths (ASDRs) of NSNIs in the past 30 years. A total of 204 countries and territories were included, which were divided into 5 sociodemographic index (SDI) regions and 21 GBD regions.

SDI

The SDI can quantitatively reveal the development status of the country/territory, and it is obtained by comprehensive evaluation of the overall fertility rates of women under the age of 25, the average education level of women aged 15 and above, and the per capita income (15). The SDI values range from 0 to 1. The smaller the SDI, the lower the level of sociodemographic development. The SDIs are provided in the GHDx. The SDIs of 204 countries and territories in 2019 (SDI 2019) are used for subsequent analyses.

UHCI

Universal health coverage (UHC) refers to the access of all people to the quality health services they need without experiencing financial hardship (16). The UHCI comprises 23 indicators, of which 4 are used to measure intervention coverage, and 19 are based on mortality to measure the quality of care obtained (16). The UHCI is scored on a scale of 0 (less developed) to 100 (most developed). The UHCIs are provided in the GHDx. The UHCIs of 204 countries and territories in 2019 (UHCI 2019) are used for subsequent analyses.

Statistical analysis

Analysis indicators included the percentage of relative changes in incident cases and deaths, and the estimated annual percentage changes (EAPCs) of ASIRs and ASDRs to characterize the trends in the incidence and mortality of NSNIs. The percentage of relative change in the number of incidents cases and deaths was calculated as reported (17). The formulas for ASIR and ASDR have been described previously (18, 19). EAPC is a measure that reflects trends in the age-standardized rate (ASR) in a certain interval, and its calculation method was described before (20). The EAPC and lower boundary of 95% confidence interval (CI) both >0 indicated an upward trend. Conversely, when both the EAPC and the upper boundary of the 95% CI were lower than 0, ASR showed a downward trend.

In addition, the correlations between the EAPCs of ASIRs and ASDRs and social evaluation indicators (SDI 2019; UHCI 2019) were assessed by Pearson correlation analysis in 204 countries and territories to identify potential factors affecting EAPCs. All analyses were conducted using in R (version 4.1.2). The threshold value of P is 0.05.

Results

Global trend in NSNIs from 1990 to 2019

On a global scale, the number of incident cases of NSNIs showed a rise of 12.79% (5.59 million in 1990 to 6.31 million in 2019), and the number of deaths of NSNIs declined by 12.93% (260,000 in 1990 to 227,000 in 2019) (Table 1). In males, the number of global incident cases of NSNIs grew by 10.09% annually, and the number of deaths dropped by 12.54% annually. In females, the number of global incident cases of NSNIs grew by 19.52%

TABLE 1 Incident cases and deaths of NSNIs in 1990 and 2019 and their change trends from 1990 to 2019.

Characteristic	1990, No. $\times 10^3$ (95% UI)		2019, No. $\times 10^3$ (95% UI)		Relative change, 1990–2019, %	
	Incident cases	Deaths	Incident cases	Deaths	Incident cases	Deaths
Overall	5,594.30 (4,004.81–7,551.31)	260.15 (208.54–299.46)	6,310.07 (4,506.66–8,497.41)	226.52 (190.25–275.55)	12.79	−12.93
Sex						
Female	2,522.96 (1,810.02–3,421.88)	114.37 (93.63–135.71)	2,970.03 (2,126.63–3,981.09)	100.30 (83.67–120.62)	19.52	−11.13
Male	3,071.33 (2,195.24–4,127.13)	145.78 (113.61–178.68)	3,340.03 (2,388.32–4,473.18)	126.21 (103.06–156.99)	10.09	−12.54
SDI region						
Low	854.58 (607.92–1,173.11)	85.50 (65.24–102.06)	1,510.72 (1,066.57–2,075.84)	111.77 (86.93–143.67)	60.36	30.72
Low–middle	1,364.21 (972.90–1,857.99)	102.67 (79.05–124.12)	1,591.86 (1,132.02–2,146.64)	70.06 (57.40–85.91)	11.02	−31.77
Middle	1,932.25 (1,355.95–2,606.77)	54.23 (45.03–60.96)	1,875.71 (1,290.22–2,558.78)	35.82 (29.43–43.14)	−7.66	−33.96
Middle-high	876.24 (604.27–1,204.92)	13.86 (12.29–15.48)	804.06 (555.27–1,087.96)	7.09 (5.91–8.39)	−9.10	−48.81
High	156.42 (121.64–202.45)	3.73 (3.12–4.54)	142.07 (110.56–180.43)	1.63 (1.43–1.86)	−9.17	−56.38
GBD region						
Andean Latin America	118.39 (97.19–139.17)	3.67 (2.81–4.74)	110.07 (87.09–130.06)	2.72 (1.88–3.67)	−7.03	−25.90
Australasia	2.12 (1.82–2.80)	0.06 (0.05–0.07)	2.26 (1.99–2.65)	0.02 (0.02–0.03)	3.94	−58.23
Caribbean	49.39 (39.25–59.97)	1.78 (1.40–2.22)	48.83 (39.80–57.52)	1.84 (1.25–2.59)	−1.14	3.66
Central Asia	75.46 (56.83–94.96)	1.05 (0.88–1.25)	63.10 (49.70–78.05)	1.15 (0.91–1.47)	−16.38	9.62
Central Europe	50.66 (37.67–66.71)	0.53 (0.48–0.60)	29.19 (22.17–37.76)	0.12 (0.09–0.15)	−42.38	−78.02
Central Latin America	317.61 (247.84–404.82)	8.47 (7.41–9.53)	339.61 (254.33–432.90)	6.08 (4.67–7.71)	6.93	−28.21
Central Sub-Saharan Africa	76.24 (57.99–101.70)	6.81 (3.84–10.19)	137.80 (102.03–181.84)	9.43 (5.94–14.28)	80.75	38.54
East Asia	1,054.92 (707.11–1,485.15)	4.99 (3.94–5.87)	1,111.97 (747.38–1,544.86)	2.02 (1.69–2.38)	5.41	−59.45

(Continued)

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TABLE 1 (Continued)

Characteristic	1990, No. $\times 10^3$ (95% UI)		2019, No. $\times 10^3$ (95% UI)		Relative change, 1990–2019, %	
	Incident cases	Deaths	Incident cases	Deaths	Incident cases	Deaths
Eastern Europe	213.95 (148.84–289.23)	1.76 (1.58–2.01)	142.19 (96.96–196.71)	1.04 (0.83–1.28)	−33.54	−40.69
Eastern Sub-Saharan Africa	484.38 (345.57–662.44)	36.97 (29.95–44.18)	794.52 (566.60–1,076.46)	46.26 (35.51–60.65)	64.03	25.11
High-income Asia Pacific	14.34 (11.75–17.55)	0.43 (0.34–0.54)	11.00 (9.26–13.09)	0.13 (0.11–0.15)	−23.27	−70.32
High-income North America	55.94 (40.75–77.03)	1.02 (0.94–1.11)	49.57 (36.13–66.66)	0.74 (0.66–0.82)	−11.38	−27.35
North Africa and Middle East	222.98 (159.77–298.87)	10.04 (7.29–12.75)	273.43 (199.95–358.02)	6.71 (5.11–8.69)	22.62	−33.20
Oceania	5.61 (4.14–7.32)	0.19 (0.13–0.28)	9.29 (6.82–12.22)	0.36 (0.21–0.58)	65.54	88.84
South Asia	1,161.08 (824.35–1,606.24)	91.75 (66.71–113.70)	1,428.84 (1,012.92–1,938.41)	55.81 (44.62–69.91)	23.06	−39.17
Southeast Asia	1,016.96 (732.70–1,318.02)	30.95 (23.79–37.98)	745.84 (531.27–997.51)	18.32 (14.43–23.33)	−26.66	−40.81
Southern Latin America	16.06 (13.62–18.58)	1.35 (1.12–1.60)	10.00 (8.25–11.84)	0.55 (0.41–0.73)	−37.75	−58.87
Southern Sub-Saharan Africa	58.87 (41.16–81.27)	3.13 (2.47–3.78)	71.44 (50.05–98.38)	3.43 (2.59–4.48)	21.35	9.68
Tropical Latin America	153.66 (106.36–215.71)	11.67 (10.05–13.69)	168.89 (113.54–238.49)	5.48 (4.27–6.83)	9.91	−53.03
Western Europe	36.57 (31.45–43.44)	0.90 (0.83–0.99)	33.89 (28.59–40.55)	0.44 (0.36–0.53)	−7.33	−50.61
Western Sub-Saharan Africa	409.07 (300.15–560.13)	42.63 (32.77–53.39)	728.34 (541.17–987.94)	63.84 (50.45–81.44)	78.05	49.76

GBD, Global Burden of Disease; SDI, sociodemographic index; UI, uncertainty interval.

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TABLE 2 ASIRs and ASDRs of NSNIs in 1990 and 2019 and their change trends from 1990 to 2019.

Characteristic	No. (95% UI)				No. (95% CI)	
	1990		2019		1990-2019	
	ASIR per 10^5	ASDR per 10^5	ASIR per 10^5	ASDR per 10^5	EAPC of ASIR	EAPC of ASDR
Overall	85.21 (60.99 to 114.96)	3.97 (3.18 to 4.57)	97.43 (69.58 to 131.21)	3.50 (2.94 to 4.25)	0.46 (0.43 to 0.48)	−0.53 (−0.72 to −0.35)
Sex						
Female	79.45 (56.99 to 107.74)	3.61 (2.95 to 4.28)	94.96 (68.00 to 127.27)	3.20 (2.67 to 3.85)	0.61 (0.57 to 0.65)	−0.51 (−0.67 to −0.34)
Male	90.60 (64.75 to 121.71)	4.31 (3.36 to 5.28)	99.74 (71.32 to 133.56)	3.77 (3.08 to 4.69)	0.33 (0.28 to 0.38)	−0.55 (−0.76 to −0.35)
SDI region						
Low	83.36 (60.96 to 113.77)	7.63 (5.82 to 9.10)	90.12 (65.40 to 121.37)	6.21 (4.83 to 7.98)	0.31 (0.27 to 0.36)	−0.64 (−0.72 to −0.56)
Low-middle	82.82 (60.44 to 111.40)	5.69 (4.38 to 6.88)	100.76 (73.41 to 133.61)	4.13 (3.38 to 5.06)	0.60 (0.57 to 0.64)	−1.16 (−1.31 to −1.01)
Middle	100.47 (71.52 to 133.99)	2.63 (2.18 to 2.95)	115.57 (81.09 to 156.02)	2.08 (1.71 to 2.50)	0.43 (0.36 to 0.50)	−0.83 (−1.11 to −0.56)
Middle-high	92.61 (64.75 to 126.75)	1.39 (1.23 to 1.55)	111.34 (78.46 to 148.21)	0.93 (0.78 to 1.10)	0.79 (0.73 to 0.84)	−1.59 (−1.85 to −1.33)
High	27.50 (21.39 to 35.59)	0.65 (0.55 to 0.80)	28.54 (22.20 to 36.23)	0.33 (0.29 to 0.37)	0.14 (0.07 to 0.20)	−2.77 (−2.98 to −2.55)
GBD region						
Andean Latin America	205.12 (168.34 to 241.17)	6.38 (4.89 to 8.23)	174.90 (138.40 to 206.58)	4.32 (2.97 to 5.82)	−0.52 (−0.36 to −0.68)	−1.24 (−1.38 to −1.10)
Australasia	14.22 (11.92 to 18.33)	0.37 (0.31 to 0.44)	12.72 (11.24 to 14.95)	0.13 (0.10 to 0.17)	−0.25 (−0.32 to −0.18)	−3.91 (−4.16 to −3.66)
Caribbean	49.39 (39.25 to 59.97)	1.78 (1.40 to 2.22)	48.83 (39.80 to 57.52)	1.84 (1.25 to 2.59)	0.27 (0.05 to 0.48)	0.55 (0.36 to 0.74)
Central Asia	80.66 (60.74 to 101.50)	1.12 (0.94 to 1.34)	69.61 (54.83 to 86.09)	1.27 (1.00 to 1.61)	−0.60 (−0.72 to −0.47)	0.92 (0.66 to 1.18)
Central Europe	62.90 (46.79 to 82.78)	0.65 (0.59 to 0.74)	56.35 (42.82 to 72.86)	0.22 (0.17 to 0.29)	−0.85 (−1.26 to −0.44)	−4.58 (−5.03 to −4.13)
Central Latin America	133.78 (104.38 to 170.51)	3.57 (3.12 to 4.02)	160.76 (120.44 to 204.80)	2.87 (2.20 to 3.64)	0.69 (0.45 to 0.93)	−0.82 (−1.03 to 0.62)
Central Sub-Saharan Africa	59.88 (45.54 to 79.85)	5.36 (3.03 to 8.02)	64.77 (47.96 to 85.51)	4.44 (2.79 to 6.71)	0.30 (0.26 to 0.33)	−0.44 (−0.58 to −0.29)
East Asia	87.64 (58.76 to 123.37)	0.41 (0.33 to 0.49)	149.72 (100.71 to 207.87)	0.27 (0.23 to 0.32)	2.16 (2.04 to 2.27)	−1.79 (−1.96 to −1.63)

(Continued)

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TABLE 2 (Continued)

Characteristic	No. (95% UI)				No. (95% CI)	
	1990		2019		1990-2019	
	ASIR per 10^5	ASDR per 10^5	ASIR per 10^5	ASDR per 10^5	EAPC of ASIR	EAPC of ASDR
Eastern Europe	152.01 (105.76 to 205.43)	1.24 (1.12 to 1.42)	130.81 (89.24 to 180.99)	0.95 (0.76 to 1.17)	−0.64 (−0.70 to −0.58)	−1.37 (−1.70 to −1.04)
Eastern Sub-Saharan Africa	114.12 (81.38 to 156.03)	8.77 (7.10 to 10.48)	117.92 (84.07 to 159.74)	6.88 (5.28 to 9.02)	0.18 (0.09 to 0.28)	−0.64 (−0.79 to −0.49)
High-income Asia Pacific	15.11 (12.38 to 18.49)	0.45 (0.35 to 0.57)	16.53 (13.92 to 19.66)	0.19 (0.16 to 0.22)	−0.08 (−0.02 to 0.04)	−3.69 (−4.00 to −3.39)
High-income North America	25.38 (18.49 to 34.98)	0.46 (0.43 to 0.50)	24.51 (17.87 to 32.93)	0.37 (0.33 to 0.40)	−0.11 (−0.20 to −0.01)	−0.81 (−1.05 to −0.57)
North Africa and Middle East	39.87 (28.57 to 53.46)	1.80 (1.31 to 2.29)	46.94 (34.33 to 61.47)	1.15 (0.87 to 1.49)	0.45 (0.39 to 0.51)	−1.66 (−1.73 to −1.60)
Oceania	52.76 (38.89 to 68.78)	1.79 (1.19 to 2.62)	47.19 (34.63 to 62.10)	1.83 (1.06 to 2.93)	−0.59 (−0.72 to −0.45)	0.17 (−0.02 to 0.36)
South Asia	67.82 (48.15 to 93.74)	5.37 (3.91 to 6.66)	89.21 (63.24 to 121.00)	3.49 (2.79 to 4.37)	0.77 (0.71 to 0.84)	−1.67 (−1.83 to −1.52)
Southeast Asia	168.66 (121.51 to 218.56)	5.14 (3.95 to 6.31)	142.57 (101.51 to 190.57)	3.49 (2.75 to 4.45)	−0.60 (−0.67 to −0.52)	−1.21 (−1.36 to −1.07)
Southern Latin America	31.99 (27.13 to 37.02)	2.68 (2.23 to 3.19)	21.50 (17.75 to 25.45)	1.19 (0.89 to 1.57)	−1.71 (−1.85 to −1.57)	−3.21 (−3.44 to −2.99)
Southern Sub-Saharan Africa	80.03 (55.93 to 110.49)	4.26 (3.36 to 5.14)	89.79 (62.91 to 123.63)	4.31 (3.25 to 5.63)	0.65 (0.45 to 0.84)	0.55 (0.25 to 0.85)
Tropical Latin America	89.89 (62.21 to 126.18)	6.84 (5.89 to 8.02)	109.16 (73.41 to 154.10)	3.53 (2.75 to 4.40)	0.76 (0.65 to 0.88)	−2.30 (−2.63 to −1.97)
Western Europe	16.37 (14.08 to 19.44)	0.40 (0.37 to 0.44)	16.31 (13.76 to 19.51)	0.21 (0.17 to 0.25)	−0.05 (−0.11 to 0.01)	−2.01 (−2.13 to −1.89)
Western Sub-Saharan Africa	95.84 (70.29 to 131.15)	10.05 (7.72 to 12.57)	93.05 (69.12 to 126.21)	8.18 (6.46 to 10.43)	−0.23 (−0.27 to −0.18)	−0.72 (−0.76 to −0.68)

ASIR, age-standardized incidence rate; ASDR, age-standardized deaths rate; EAPC, estimated annual percentage change; GBD, Global Burden of Disease; SDI, sociodemographic index; UI, uncertainty interval.

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annually, and the number of deaths dropped by 11.13% annually. After age standardization, the ASIR of NSNIs ascended [EAPC = 0.46 (95% CI, 0.43 to 0.48)] from 85.21 per 100,000 in 1990 to 97.43 per 100,000 in 2019 (Table 2), and the ASDR of NSNIs declined over this period by an average of 0.53 per year (95% CI, −0.72 to −0.35; from 3.97 per 100,000 in 1990 to 3.50 per 100,000 in 2019; Table 2). Overall, the ASIR showed an increasing trend year by year worldwide, with similar trend in males and females, and the ASIR of females was lower than that of males (Figure 1A). It can be noted that the ASIR of male NSNIs (ASIR: 99.74 per 100,000) was higher than that of females (ASIR: 94.96 per 100,000) in 2019 (Table 2). The EAPC of female ASIR was 0.61, nearly twice that of male ASIR, indicating that female ASIR increased rapidly. From 1990 to 2019, the global ASDR initially rose, plateaued between 1999 and 2005, and then declined (Figure 1B). The trend was the same for males and females, with males having higher ASDR than females (Figure 1B).

Regional trend in NSNIs from 1990 to 2019

In high-SDI regions, the number of incident cases of NSNIs decreased by 9.17%, and the number of deaths of NSNIs decreased by 56.38% from 1990 to 2019 (Table 1). The ASIR of NSNIs during this period increased [EAPC = 0.14 (95% CI, 0.07 to 0.20)] from 27.50 per 100,000 in 1990 to 28.54 per 100,000 in 2019, and the ASDR declined [EAPC = −2.77 (95% CI, −2.98 to −2.55)] from 0.65 per 100,000 in 1990 to 0.33 per 100,000 in 2019 (Table 2). The ASIRs of other 4 SDI regions all maintained a rising trend at a high level except for high-SDI regions, and there were heterogeneous trends between ASIRs and SDIs, for example, the ASIR of middle-SDI regions was not lower than that of low-SDI regions (Table 2, Figure 1C, Supplementary Figure 1A). The ASDR in all 5 SDI regions showed a downward trend on the whole, and the higher the SDI, the smaller the ASDR (Table 2, Figure 1D, Supplementary Figure 1B).

Among the 21 GBD regions, the number of incident cases increased in 10 regions and decreased in 11 regions; the number of deaths increased in seven regions and decreased in 14 regions (Table 1). The regions where both incident cases and deaths were increasing were Southern Sub-Saharan Africa, Eastern Sub-Saharan Africa, Western Sub-Saharan Africa, Central Sub-Saharan Africa, and Oceania (Table 1). The largest increase in incident cases was in Central Sub-Saharan Africa (relative change = 80.75%), and in deaths in Oceania (relative change = 88.84%; Table 1). Besides, the number of incident cases (relative change = −42.38%) presented the most rapid decline in Central Europe, as did the number of deaths (relative change = −78.02%; Table 1). The region with the highest incidence of NSNIs was Andean Latin America (2019 ASIR: 174.90 per 100,000), and Central Latin America ranked second (2019 ASIR: 160.76 per 100,000) (Table 2). In terms of NSNIs mortality, the threat was most severe in Western Sub-Saharan Africa (2019 ASDR: 8.18 per 100,000), and Eastern Sub-Saharan Africa ranked second (2019 ASDR: 6.88 per 100,000; Table 2). The ASIRs decreased in 10 regions, most notably in Southern Latin America [EAPC = −1.71 (95% CI −1.85 to −1.57)]; the ASIRs of 9 regions had an increasing trend, with East Asia showing highest increasing trend [EAPC = 2.16 (95% CI 2.03 to 2.27); Table 2, Figure 2A]. The ASIRs of Western Europe and High-income Asia Pacific remained stable (Table 2, Figure 2A). The ASDR generally showed a downward trend, with only three regions showing an upward trend, including Central Asia, Southern Sub-Saharan Africa, and the Caribbean (Table 2, Figure 2B). The largest decrease in ASDR was noted in Central Europe (Table 2, Figure 2B).

In order to show the recent changes in the ASIRs and ASDRs of NSNIs, we obtained data from the past 10 years (2010–2019; Supplementary Table 1). In recent 10 years, the ASIRs in regions with high-middle and low-middle SDI were still on the rise, while those in regions with high SDI, middle SDI and low SDI tended to be stable and improved compared with the overall trend (the ASIRs of all SDI regions were on the rise from 1990 to 2019; Figures 2A, C). The short-term and long-term trends of ASDRs in the 5 SDI regions were the same, showing a downward trend (Figures 2B, D). In the 21 GBD regions, East and South Asia remained the top 2 in the rapid growth of ASIRs, while Central Europe and Southern Latin America maintained a continuous decline of ASIRs (Figure 2C). Central Latin America, Eastern Sub-Saharan Africa, Tropical Latin America and Caribbean performed well. While the overall ASIRs (1990–2019) showed an upward trend, effective measures were taken in the past 10 years to successfully curb the upward trend of ASIRs, showing a downward trend (Figures 2A, C). In Southern Sub-Saharan Africa, ASIR stopped growing and remained stable over the past decade (Figure 2C). The ASDRs declined in all 21 GBD regions from 2010 to 2019, indicating that infant death caused by NSNIs has attracted attention in some regions where ASDR was still growing (Figure 2D).

National trend in NSNIs from 1990 to 2019

Among 204 countries and territories, China (1.11 million) and India (0.75 million) ranked the top 2 in the number of incident cases of NSNIs in 2019, accounting for about 29% of the number of global incident cases (6.31 million; Supplementary Table 2). The countries with the highest number of deaths were India (36,900) and Nigeria (27,200), accounting for 28% of the number of global deaths (226,500; Supplementary Table 2).

Northern Macedonia exhibited the largest increase in incident cases of NSNIs (300.45%), followed by Afghanistan (241.37%; Supplementary Table 3, Figure 3A). Mauritius had the largest ASIR (244.26 per 100,000), followed by Bangladesh (231.66 per 100,000), and Dominican Republic (230.71 per 100,000; Supplementary Table 2, Figure 3B). The ASIRs of 101 countries or territories showed an upward trend, of which North Macedonia had the largest increase [EAPC = 8.41 (95% CI, 7.72 to 9.10); Supplementary Table 4, Figure 3C]. The ASIRs of 93 countries or territories were considered to show a downward trend, with the largest decline in Serbia [EAPC = −4.76 (95% CI, −5.25 to −4.26)], followed by Poland [EAPC = −4.47 [95% CI, −5.36 to −3.58); Supplementary Table 4, Figure 3C]. The ASIRs of 25 countries or territories, including Denmark, Norway, and Greenland, remained stable (Supplementary Table 4).

North Macedonia (227.49%) had the largest increase in deaths of NSNIs, followed by Bulgaria (216.90%), and Afghanistan

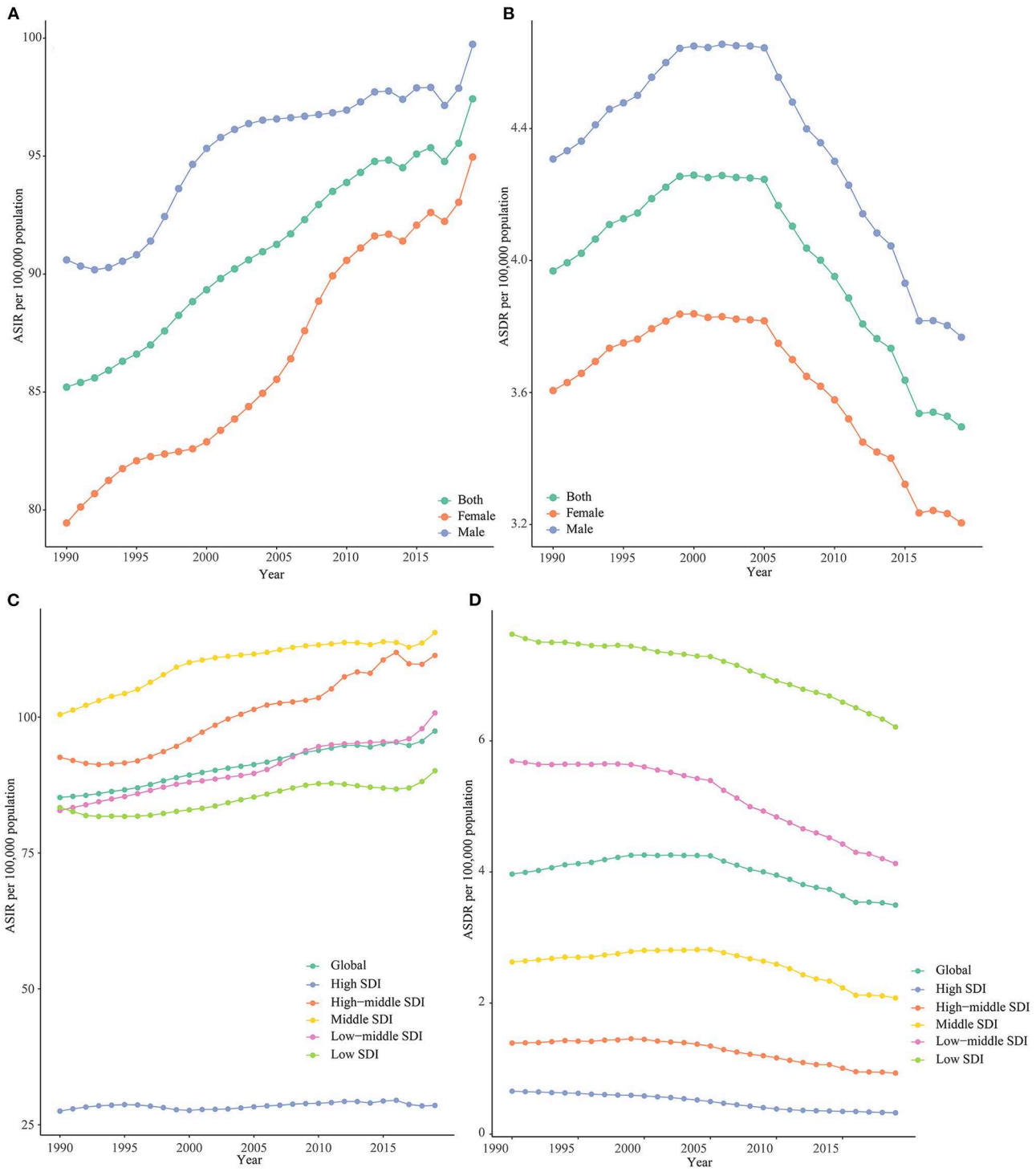

FIGURE 1
Trends of global ASIRs **(A)** and ASDRs **(B)** by gender and global ASIRs **(C)** and ASDRs **(D)** by SDIs from 1990 to 2019.

(196.38%; Supplementary Table 3, Figure 4A). In 2019, Mali (ASDR: 14.11 per 100,000) and Ghana (ASDR: 10.14 per 100,000) showed the highest ASDR (Supplementary Table 2, Figure 4B). The ASDRs were on the rise among the 38 countries or territories, with the largest increase in North Macedonia [EAPC = 9.31 (95% CI, 7.81 to −10.83); Supplementary Table 4, Figure 4C]. The ASDRs of 153 countries or territories declined, with the greatest reduction in Serbia [EAPC = −8.66 (95%CI, −9.83 to −7.47)], followed by Greece [EAPC = −7.41 (95% CI, −9.32 to −5.46); Supplementary Table 4, Figure 4C]. The ASDR remained stable in 13 countries or territories, including Yemen, Somalia, and Belize (Supplementary Table 4).

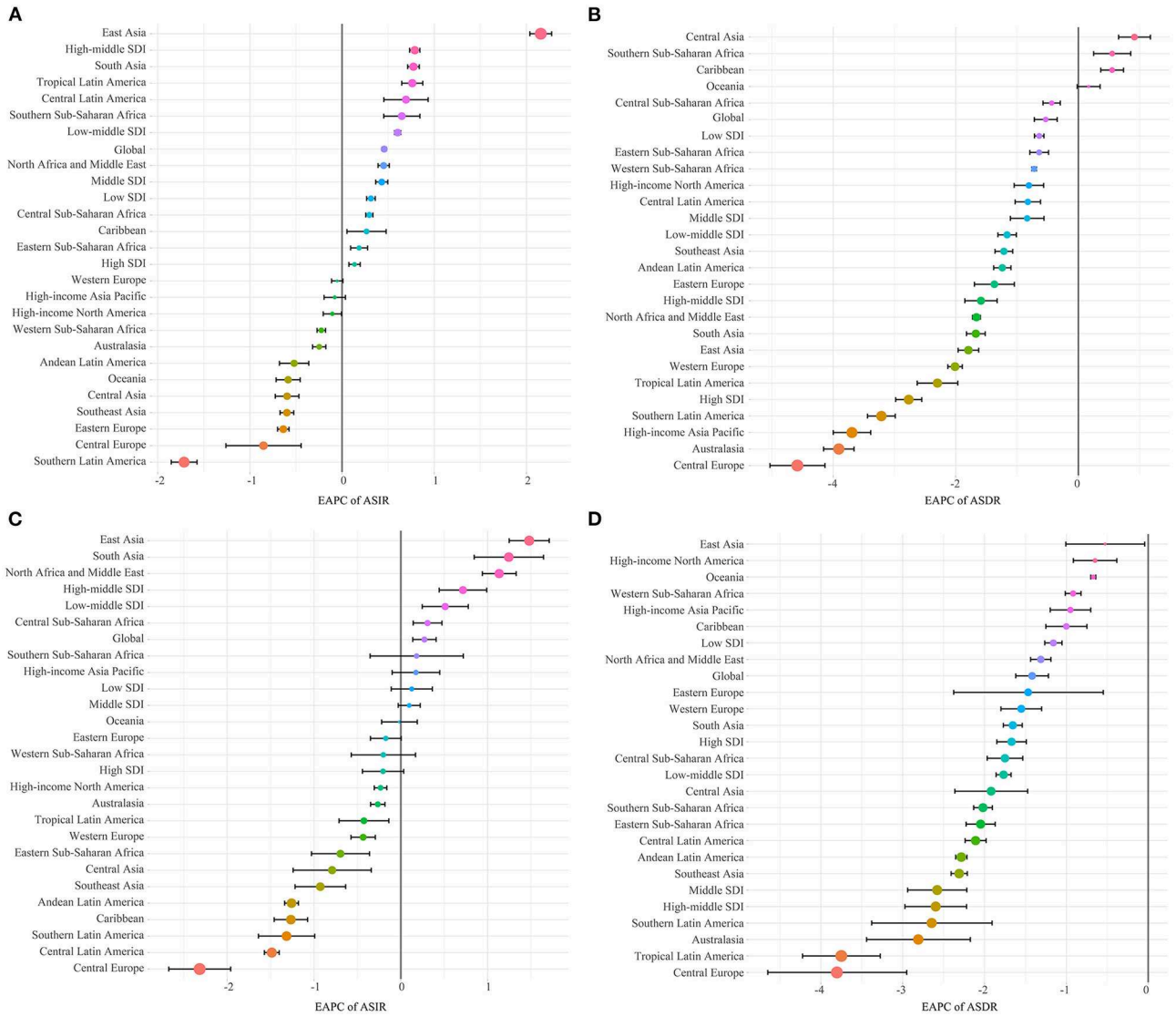

FIGURE 2
Estimated annual percentage changes (EAPCs) of ASIRs and ASDRs in NSNIs at the regional levels. **(A)** EAPCs of ASIRs from 1990 to 2019; **(B)** EAPCs of ASDRs from 1990 to 2019; **(C)** EAPCs of ASIRs from 2010 to 2019; **(D)** EAPCs of ASDRs from 2010 to 2019.

To show the effect of recent interventions, we analyzed the changes of ASIRs and ASDRs in 204 countries and territories from 2010 to 2019. The ASIRs of 74 countries or territories showed an upward trend, with the largest increase in Singapore [EAPC = 4.08 (95% CI, 3.92 to 4.24); Supplementary Table 4, Supplementary Figure 2A]. The ASIRs of 92 countries or territories were in a downward trend, with the largest decline in Estonia [EAPC = −6.21 (95% CI, −7.09 to −5.33)], followed by Serbia [EAPC = −5.22 (95% CI, −6.14 to −4.29)] and Poland [EAPC = −5.03 (95% CI, −6.32 to −3.73); Supplementary Table 4, Supplementary Figure 2A]. The ASIRs of 38 countries or territories remained stable, such as Canada and Finland (Supplementary Table 4). The ASDRs of eight countries or territories had a downward trend, and Mauritius had the largest increase [EAPC = 2.98 (95% CI, 1.23 to 4.77)], followed by Austria, Greenland and Singapore (Supplementary Table 4, Supplementary Figure 2B). The ASDRs of 177 countries or territories decreased, of which Estonia experienced the largest decline [EAPC = −7.70 (95% CI, −11.61 to −3.62)], followed by Cyrus [EAPC = −7.22 (95% CI, −8.75 to −5.67); Supplementary Table 4, Supplementary Figure 2B]. The ASDRs remained stable in 19 countries or territories, such as Sweden, France and Greece (Supplementary Table 4). Compared with the data of the past 30 years, the ASIRs and ASDRs of NSNIs in some countries and territories were under control from 2010 to 2019.

Correlations of EAPCs of ASIR and ASDR with social evaluation indicators

The EAPCs of ASIRs and ASDRs of NSNIs in 2019 were not correlated with SDI ($p > 0.05$; Figures 5A, B). The EAPCs of

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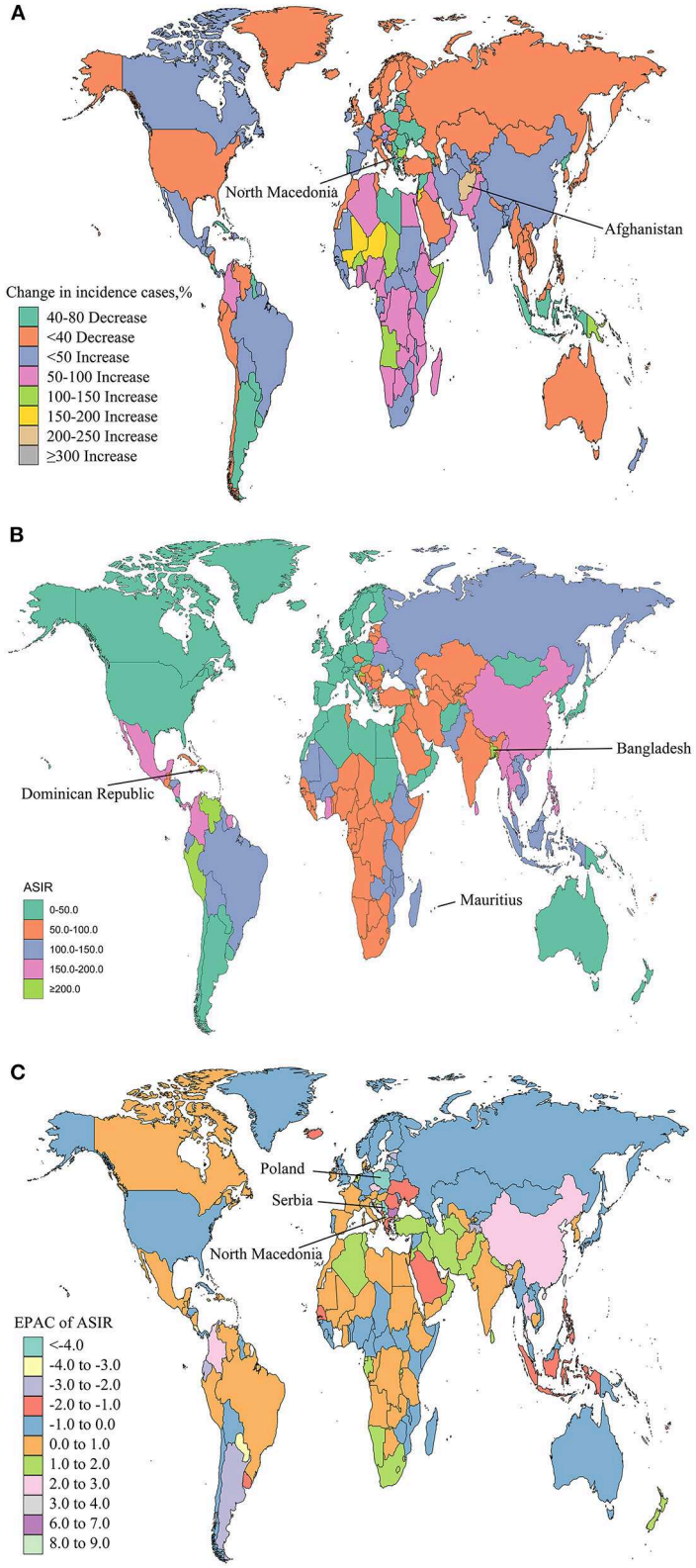

FIGURE 3
Global trends in the incidence of NSNIs in 204 countries and territories. The percentage of relative change in incident cases of NSNIs between 1990 and 2019 **(A)**, age-standardized incidence rates (ASIRs) of NSNIs in 2019 **(B)**, and estimated annual percentage changes (EAPCs) of ASIRs of NSNIs from 1990 to 2019 **(C)** were shown.

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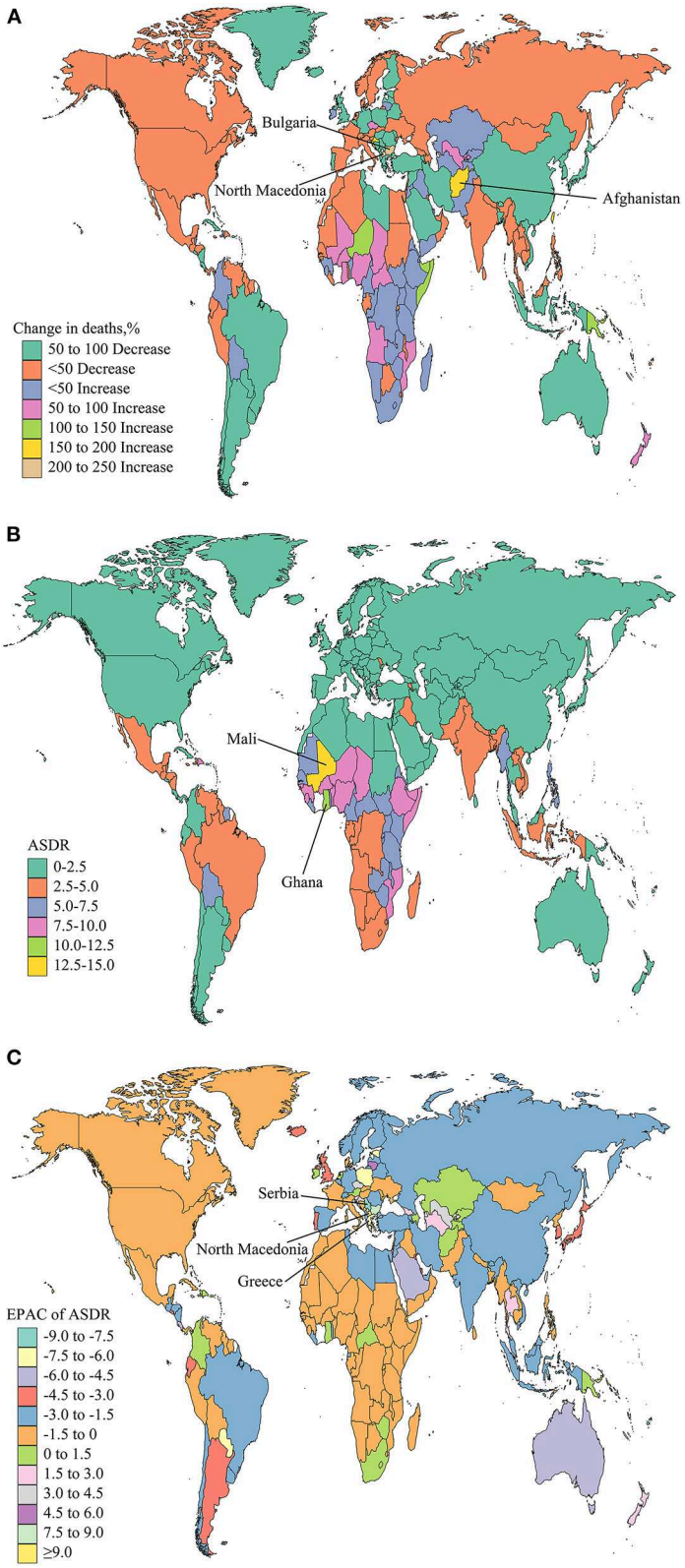

FIGURE 4
Global trends in the deaths of NSNIs in 204 countries and territories. The percentage of relative change in deaths of NSNIs between 1990 and 2019 **(A)**, age-standardized deaths rates (ASDRs) of NSNIs in 2019 **(B)**, and estimated annual percentage changes (EAPCs) of ASDRs of NSNIs from 1990 to 2019 **(C)** were shown.

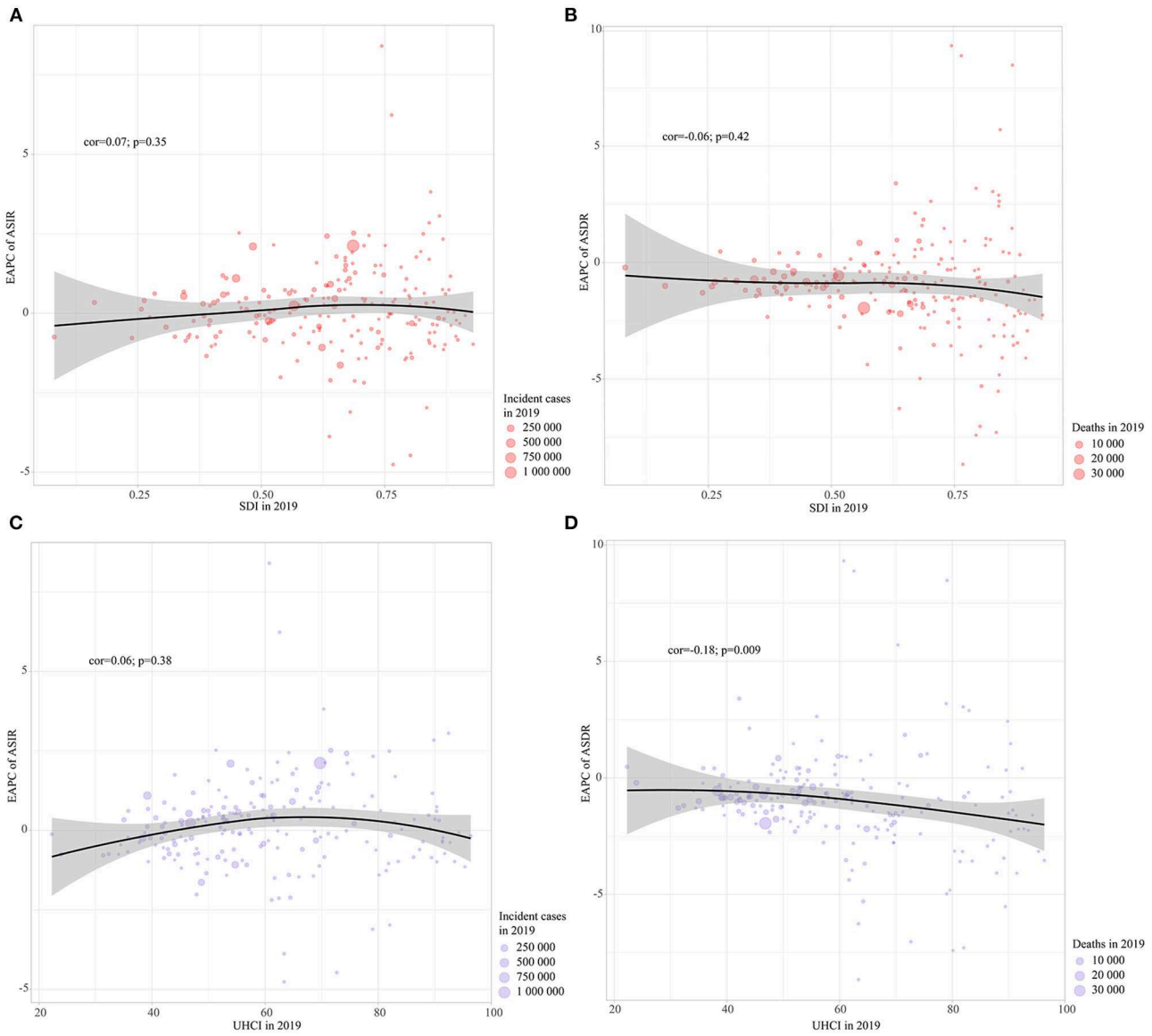

FIGURE 5
Pearson correlation was analyzed between EAPCs of ASIRs and ASDRs in NSNIs from 1990 to 2019 and SDI and UHCI in 2019 at the country and territorial levels. **(A)** SDI in 2019 and EAPC of ASIR; **(B)** SDI in 2019 and EAPC of ASDR; **(C)** UHCI in 2019 and EAPC of ASIR; **(D)** UHCI in 2019 and EAPC of ASDR. The incident cases and deaths of NSNIs from 204 countries and territories in 2019 are represented by circles. The size of the circles increased with the incident cases or deaths of NSNIs. Correlation coefficient, cor.

ASIRs of NSNIs in 2019 were not correlated with UHCI ($P > 0.05$; Figure 5C), while the EAPCs of ASDRs of NSNIs in 2019 was significantly negatively correlated with UHCI (cor = −0.18; $P = 0.009$; Figure 5D).

Discussion

To the best of our knowledge, we for the first time comprehensively assessed global and regional long-term and short-term characteristics in incidence and mortality of NSNIs. In this study, we found that the number of incident cases of NSNIs in the world grew by 12.79% per year, and the number of deaths dropped by 12.93% per year from 1990 to 2019. During this period, global ASIR of NSNIs increased by 46% annually on average, while ASDR decreased by 53% annually on average. The ASIR and ASDR of female NSNIs were consistently lower than that of male NSNIs. The EAPC of female ASIR was 0.61, nearly twice that of male ASIR, and female ASIR was growing rapidly. The same declining trends of ASDR were noted in males and females. For SDI regions, the ASIR of NSNIs in high-SDI regions grew by an average of 14% annually from 1990 to 2019. Except for high-SDI regions, the ASIRs in other 4 SDI regions maintained a rising trend at a high level. The ASDRs of all 5 SDI regions generally showed a downward trend. The largest decline was in the high-SDI regions. The highest ASIR of NSNIs was in Andean Latin America

(ASIR in 2019: 174.90 per 100,000), and the highest mortality of NSNIs was in Western Sub-Saharan Africa (ASDR in 2019: 8.18 per 100,000). From 2010 to 2019, the burden of NSNIs in several regions where the ASIRs or ASDRs were rising were under control. We found a negative correlation between EAPCs of ASDRs and UHCI in 2019.

NSNIs are one of the major causes of 2.6 million newborn deaths worldwide every year (21). It is necessary and significant to improve the current situation of NSNIs. We need to better understand the characteristics of NSNIs, and actively enhance newborn care. In this study, we found that the global incidence of NSNIs showed rapid growth, and the mortality was generally declining. The incidence and mortality of males and females were in line with global trends. The incidence and mortality of NSNIs in females were lower than in males. The continuous increase in global incidence of NSNIs may indicate that the global health environment still needs to be improved. However, the decline of global mortality of NSNIs in the short term showed that the current maternal and newborn health care has achieved positive results (22).

We found that in high SDI regions, the ASIR showed the lowest increase, while the ASDR exhibited the highest decline. In other 4 SDI regions, we found no relationship between ASIR and SDI levels. For example, the ASIR in the middle-SDI regions was higher than that in the low-SDI regions. The ASDRs of all 5 SDI regions were generally decreasing, and the higher the SDI, the smaller the ASDR. This finding indicated that NSNIs had a high ASIRs and was growing rapidly, and the mortality of NSNIs was related to the educational level and economic level of the SDI regions. Although the ASIRs of Western Sub-Saharan Africa and Eastern Sub-Saharan Africa were not the highest (the highest was Andean Latin America and Central Latin America), the ASDRs were the highest. Consistent with our findings, Sub-Saharan Africa topped the list for the mortality of NSNIs (23). One possible reason was the high antimicrobial resistance of pathogens in Sub-Saharan Africa, and the efficacy of treatment was reduced (24). Ranjeva et al. evaluated the public health burden and economic pressure caused by neonatal sepsis in Sub-Saharan Africa, and believed that it was necessary to provide reasonable and appropriate health care for neonatal infections (25).

The high incidence and its high growth rate of NSNIs may indicate that infants are generally susceptible to pathogens and their infection capacity is increasing. The death of NSNIs may be related to the lack of timely diagnosis and treatment, and antibiotic resistance (26, 27). Our findings illustrated that the mortality of NSNIs was relatively high in low- and middle-income countries. Families and health workers in low- and middle-income countries lack relevant knowledge and training to identify the early stage of infections, and the corresponding laboratory equipment is only available in hospitals, delaying the time of treatment (27, 28). In addition, many people in low- and middle-income countries may not go to hospitals due to financial hardship, or may not have access to medical care. It was estimated that there were 8 million curable deaths in 2015, of which 96% occurred in low- and middle-income countries (29). A study estimated the quality of care in 81 low- and middle-income countries, and when high-quality care was provided, it was believed that neonatal mortality would be reduced by 28% (30). UHCI is an indicator reflecting health coverage and nursing quality (16). Efforts to improve UHC can overcome these issues and promote health equity. From our results, it was observed that the UHCI was negatively correlated with the trend of ASDRs, that is, when the level of UHCI was high, the ASDR was generally in a downward trend. This was consistent with previous report (31). There is no doubt that the use of antibiotics is crucial to the treatment of infections. It is known that there is a clear correlation between antibiotic use and antibiotic resistance, and antibiotic resistance has become a persistent global health threat (32, 33). On the one hand, there is the misuse of antibiotics and population-level resistance in low- and middle-income countries (34). On the other hand, low- and middle-income countries showed high resistance to antibiotics recommended by World Health Organization (WHO) (35). These are not conducive to neonatal outcomes. Therefore, we need to improve the overall awareness, timely diagnosis and management of NSNIs.

Finally, special attention should be paid to countries or territories where ASDR is still growing, including Mauritius, Austria, and Greenland (Supplementary Table 4). In these countries and territories with ASDR on the rise, it was imperative to accelerate the promotion of universal health coverage and strengthen antibiotic management.

Limitations

This study fills a gap in this area by providing the first comprehensive assessment of global and regional long-term and short-term characteristics of incidence and mortality of NSNIs, and the relationship with socioeconomic status and medical health level. However, we should recognize several limitations. First of all, the quality of the data limited our accurate description of the results. The possible bias made the results less robust. Second, the causes of NSNIs were notoriously complex. There was no specific pathogen infection data in the GBD study, so we could not estimate the contribution of different causes.

Conclusions

To sum up, the ASIR of global NSNIs gradually increased and the ASDR gradually declined from 1990 to 2019. In addition, the ASIR and ASDR of males were higher than those of females. Except for high-SDI regions, the ASIRs of other 4 SDI regions remained at a high level in this period. The ASDRs declined in all SDI regions. The ASDRs in Western Sub-Saharan Africa and Eastern Sub-Saharan Africa were the highest. The ASDRs were still growing in some countries and territories. Therefore, it is essential to improve the overall awareness and management of NSNIs, and reduce the morbidity and mortality of NSNIs worldwide.

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

KQ: project design. JL: data curation, analysis, and manuscript writing. LS: manuscript revision. All authors read and approved the final manuscript.

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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/fpubh.2023.1139832/full#supplementary-material

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The role of human milk feeds on inotrope use in newborn infants with sepsis

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Background: Regarding neonatal hypotension, there is no certainty as to whether inotrope properties are beneficial or whether they may be harmful. However, given that the antioxidant content of human milk plays a compensatory role in neonatal sepsis and that human milk feeding has direct effects in modulating the cardiovascular function of sick neonates, this research hypothesized that human milk feeds might predict lower requirements of vasopressors in the management of neonatal septic shock.
Method: Between January 2002 and December 2017, all late preterm and full-term infants attending a neonatal intensive care unit, with clinical and laboratory findings of bacterial or viral sepsis, were identified in a retrospective study. During their first month of life, data on feeding type and early clinical characteristics were collected. A multivariable logistic regression model was constructed to determine the impact of human milk on the use of vasoactive drugs in septic newborns.
Results: 322 newborn infants were eligible to participate in this analysis. Exclusively formula-fed infants were more likely to be delivered *via* C-section, to have a lower birth weight and a lower 1-minute Apgar score than their counterparts. Human milk-fed newborns had 77% (adjusted OR = 0.231; 95% CI: 0.07–0.75) lower odds of receiving vasopressors than exclusively formula-fed newborns.
Conclusion: We report that any human milk feeding is associated with a decrease in the need for vasoactive medications in sepsis-affected newborns. This observation encourages us to undertake further research to determine whether human milk feeds mitigate the use of vasopressors in neonates with sepsis.

KEYWORDS

neonate, premature infant, breast-feeding, human milk, sepsis, hypotension, oxidative stress, dopamine

Introduction

Recent research (1) that aimed to reduce the use of inotropes resulted in a reduction of brain morbidities in premature babies; these findings add to serious efforts to clarify whether cerebral perfusion is improved or reduced with any inotrope use. Unfortunately, despite decades of use, we still have little evidence regarding the outcome of the various inotropes

Abbreviations
EF, exclusive formula feeding; GSH, reduced glutathione; GSSG, oxidized glutathione; HM, any human milk feeding; IV, intravenous; NICU, Neonatal Intensive Care Unit.

accepted for treating neonatal hypotension. A limited number of observational studies have addressed the long-term effects of vasoactive drugs. However, recruiting sick neonates for randomized controlled trials when they are unstable and require prompt intervention is extremely challenging (2). Given this, it is wise of us to try and find more ways to narrow the use of agents that may be of no benefit or potentially have adverse consequences.

Although neonates generate a relatively lower amount of inflammatory cytokines than adults, neonates with septic shock have dysregulated cytokine production, which is linked to illness severity (3). Inflammatory pathways activated in septic shock directly impact the vascular tone and cardiac function. The release of inflammatory mediators can compromise endothelial wall integrity; subsequent endothelial leakage lowers circulating blood volume and, subsequently, cardiac preload (4, 5). Bacteremic neonates have been found to have higher levels of nitric oxide and endothelin compared to their non-infected counterparts. High levels of endothelin have been correlated with myocardial dysfunction in sepsis (6).

Remarkably, systemic inflammatory response and additional compromise to perfusion occur when anti-inflammatory mechanisms do not adequately counteract pro-inflammatory pathways. Antioxidant and anti-inflammatory defenses mature during late gestation (7). Thus, preterm neonates have diminished anti-inflammatory mechanisms at birth. On the other hand, the process leading to septic shock is maintained by a cascade of inflammatory mechanisms and simultaneous increased production of free radicals. Since human milk (HM) possesses a much more potent antioxidant potential than infant formula (8), it appears to serve as the primary antioxidant source for preterm or sick neonates. Although we have not found previous research on the link between HM feeding and an overall decline in the use of vasoactive medications, the antioxidant role of HM and the critical role that oxidative stress plays in worsening the hemodynamic presentation of neonatal sepsis generate the hypothesis that HM feeding might predict lower requirements of vasopressors in the management of neonatal septic shock.

In this regard, we have analyzed the link between HM feeding and the use of vasopressors in a long-term cohort of newborn infants with sepsis admitted to our neonatal unit.

Methods

Design

This is a secondary analysis of data collected for a doctoral thesis intended to explore the clinical and laboratory characteristics of neonates with viral or bacterial sepsis (9). Previous research contains complete details of the methodological processes (10). In brief, this study was carried out using a retrospective review of electronic medical records of late preterm and full-term newborn infants.

Study population and period

Between January 2002 and December 2017, all late preterm and full-term infants up to 28 days of age with clinical and laboratory findings of bacterial or viral sepsis were identified in a cohort study. All of them attended the neonatal intensive care unit (NICU) of Sant Pau Hospital, a tertiary referral unit in Barcelona, Spain, with approximately 350 admissions per year and a bed occupancy rate of 90%; the unit consists of 10 intensive care and 7 high-care beds.

Data collection

The NICU's computerized system provided retrospective data on maternal parity and gravida, maternal diseases, infant demographic and perinatal characteristics, feeding type, and early clinical features. In addition, data on any HM or exclusive formula feeding (EF) were collected during the first month of life. All HM was mother's own milk; donor's milk is reserved for very premature infants. Fresh or frozen mother's own milk was used. These infants were not admitted to the NICU from home but from the Maternity unit. We chose to focus on the first month of life because it significantly impacts short-term outcomes. For exploratory analyses, we examined the use of antihypotensive treatments until hospital discharge.

The primary outcome of this study was to examine the use of vasopressors in newborn infants with a history of sepsis, intending to evaluate if exclusive formula feeding predicts vasopressor support practices. In this preliminary analysis, infant feeding type was an independent variable, and vasopressor support was a dependent variable.

Secondary outcomes included the following clinical characteristics based on feeding type. Neonatal status at birth, metabolic and/or respiratory acidosis, hypotension, abnormal neurological examination, abnormal brain scan, meningitis, positive blood cultures, blood cells count, type of respiratory support, discharge weight, and days spent in the hospital. The clinical information from birth until Hospital discharge has been gathered in a database, but a detail of the timing of each clinical characteristic was not available.

Statistical analysis

We used descriptive statistics to generate counts and percentages for the mode of feeding among newborn infants in the registry. Fisher's Exact or *t*-tests were used to compare infant and maternal characteristics or outcomes; *p*-values were obtained from bivariate comparisons as a function of each individual risk factor. A multivariable logistic regression model was constructed to determine the impact of milk type on the primary outcome and the independent contribution of each factor to neonatal outcomes. This model included variables associated with exposure or variables highly predictive of the primary outcomes.

Backward selection was used to remove these covariables from the multivariable logistic regression model if they no longer had significance when added to the model. Because of collinearity, heart failure was not included in the adjusted model. Outcomes were compared between the HM and EF groups with odds ratios calculated with logistic regression fit by generalized estimating equations to account for paired data. There were no methods used to account for any potential bias. The SPSS software version 21.0 (SPSS Chicago, IL, United States) was used for statistical analysis and data management.

Ethical statement

The Santa Creu & Sant Pau University Hospital's Clinical Studies Ethics Committee approved this study on December 20, 2020, with decision number: IIBSP-ENT-2020-152.

Because the study was conducted retrospectively over a two-decade period, and the data was scanned from patient files, no informed consent was obtained.

Results

During the study period (2002–2017), 4,210 neonates were admitted to the participating NICU, 7.6% ($n = 322$; 260 in HM group and 62 in EF group) of whom met inclusion criteria and were eligible to participate in the final analysis. Similar to what was published in an earlier study of these cohorts examining the relationship between type of feeding and type of respiratory support (10), baseline characteristics of neonates who received any amount of HM and neonates who received only formula are summarized in **Table 1**. The mean age of newborns on admission to the neonatal unit was 4.3 days in cases of bacterial sepsis and 12.9 days in cases of viral sepsis, and 20% of infants with bacterial sepsis required vasoactive support compared to 2% of infants with viral sepsis. EF infants were more likely to be delivered *via* C-section, had a lower birth weight, and had a lower 1 min Apgar score than HM infants. However, these relationships did not persist when introduced in the multivariate model. We found no other background differences in bivariate analysis.

Table 2 compares short-term neonatal outcomes by feeding method. On bivariate analysis, there was a higher proportion of hipotonia among EF infants than among HM infants (54% vs. 23%, $p = 0.045$); and the proportion of respiratory distress was higher among EF infants than among HM infants (56% vs. 39%, $p = 0.020$). As a result, the same applied to the proportion of infants needing some form of respiratory support (43% vs. 27%, $p = 0.019$). On the other hand, the neutrophil blood count was higher among EF infants than among HM infants (10,358/μl vs. 7,623/μl, $p = 0.005$). Interestingly, 22% of EF infants had confirmed viral or bacterial meningitis vs. 42% of HM infants ($p = 0.038$). Finally, 8.7% of HM neonates required vasopressor support, compared to 25% of EF neonates (OR = 0.28; 95% CI: 0.14–0.59; $p < 0.001$). Other short-term neonatal outcomes did not differ between the two groups.

TABLE 1 Patents' characterstics.

	Any human milk feeding, $n = 260$	Exclusive formula feeding, $n = 62$	p value
Gestational hypertension	21 (8.07%)	6 (9.7%)	0.607
Gestational diabetes	8 (3.0)	2 (3.3)	1.000
Chrioamnionitis	63 (24.0)	20 (32.2)	0.218
Group B strep positive mother	36 (13.8)	5 (8.3)	0.512
Gestational age, weeks	38.59 (2.08)	38.01 (2.27)	0.056
Delivery type: eutocic/ instrumental/C-section	147/43/72	29/3/28	0.0045**
Delivery type: vaginal/ C-section	190/72	32/28	0.0052**
Singletons/twins	249/13	53/7	0.071
Apgar 1 min	8 (2)	7 (3)	0.036*
Apgar 5 min	9 (1)	9 (2)	1.000
Birth weight, g	3,116 (639)	2,893 (637)	0.010
Height, cm	48.6 (2.84)	48.22 (2.69)	0.322
Cranial circumference, cm	33.83 (2.02)	33.37 (1.94)	0.093
Boys/Girls	136/126	34/26	0.567

Data expressed as number (%) or mean (standard deviation). Fisher's exact test or *t*-test comparison between neonates with any human milk feeding and exclusive formula feeding.
*$p < 0.05$.
**$p < 0.01$.

Logistic regression models were developed to examine the association between the use of vasopressors and feeding type. Meningitis, lower muscle tone, C-section, or postnatal steroids treatment were covariates that remained significant in the final model (**Table 3**). However, even when controlling for these covariates, HM newborns had 77% (adjusted OR = 0.231; 95% CI: 0.07–0.75) lower odds of receiving vasopressors than EF newborns.

Discussion

We found that any HM is linked to much better cardiovascular outcomes than EF. Data on exclusive HM feeding are not available in this study. However, the protecting role of any HM is supported by systematic reviews, which show that a minimum intake of 7 ml/kg/day of HM during the first 42 days is linked to a lower incidence of bronchopulmonary dyspasia and that there is conflicting evidence regarding the impact of high vs. low doses of HM on both this condition or retinopathy of prematurity (11).

This retrospective study at the level of a single NICU has explored the link between early feeding type and the administration of vasopressors. Following our initial hypothesis, after adjusting for confounding by indication, infants in the EF groups received vasopressor treatment more frequently than the HM group. As far as we know, this is the pioneer descriptive study in this field. In addition, this analysis included a large

TABLE 2 Clinical or laboratory outcomes by feeding type.

	Any human milk feeding, $n = 260$	Exclusive formula feeding, $n = 62$	p value
Apnea	47 (18.9)	17 (28.8)	0.074
Abnormal alertness state	143 (54.5)	27 (45)	0.198
Hypotonia	59 (22.5)	21 (53.8)	0.045*
Convulsions	10 (3.8)	4 (6.6)	0.304
Hypotension	43 (16.4)	16 (26.6)	0.094
Heart failure	7 (2.7)	4 (6.6)	0.128
Hepatitis	20 (7.6)	9 (15)	0.081
Fever	122 (46.5)	23 (38.3)	0.254
Rash	29 (11.0)	2 (3.3)	0.087
Vomiting	27 (10.3)	9 (15)	0.361
Neonatal acidosis	77 (29.7)	24 (40)	0.121
Positive blood culture	81 (30.9)	18 (30)	1.000
Bacterial/viral meningitis	84/113 (42.6)	7 (22.5)	0.038*
Leukocyte blood count/μl	15,317 (30,538)	23,784 (38,184)	0.103
Neutrophil blood count/μl	7,623 (5,805)	10,358 (6,980)	0.005**
Lymphocyte blood count/μl	3,896 (2,296)	4,384 (2,505)	0.197
Abnormal brain ultrasound	13 (5.9)	1 (1.9)	0.482
Pulmonary edema	13 (4.9)	7 (22.5)	0.071
Respiratory distress	103 (39.7)	34 (56.6)	0.020*
Any respiratory support	72 (27.4)	26 (43.3)	0.019*
Administered inotropes	23 (8.7%)	15/45 (25)	0.001***
Administered steroids	6 (2.2)	1 (1.9)	1.000
Administered antibiotics	212 (80.9)	53 (88.3)	0.194
Hospitalization, days	10 (9)	12 (9)	0.121
Discharge weight, g	3,425 (689)	3,256 (639)	0.083

Data expressed as number (%) or mean (standard deviation). Fisher's exact test or *t*-test comparison between neonates with any human milk feeding and exclusive formula feeding.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

TABLE 3 Multivariate logistic regression analysis of factors associated with higher vasopressor support.

Variable	Odds ratio	Standard error	z	$p > \|z\|$	95% CI
Human milk feeding	0.231	0.139	−2.43	0.015	0.071–0.752
Meningitis	0.242	0.127	−2.69	0.007	0.086–0.680
Lower muscle tone	3.239	2.276	2.43	0.015	1.308–12.203
C-section	3.426	2.080	2.03	0.043	1.042–11.264
Postnatal steroids	96.757	206.68	2.15	0.031	1.500–6239.6

CI, confidence interval; $P>|z|$, Corresponding Probability for the Reduced Logistic Model; z, Estimated Z-Score.

number of newborn infants and used multivariable statistical methods, which contributed to the findings' external validity.

We also found a positive association between any HM feeding and the rate of meningitis, a negative association between any HM feeding and hypotonia, and neutrophil counts were significantly higher in EF neonates than in HM neonates. We do not have an explanation for our results on meningitis. We also found a positive association between any HM feeding and the rate of meningitis, a negative association between any HM feeding and hypotonia, and neutrophil counts were significantly higher in EF neonates than in HM neonates. We do not have an explanation for our results on meningitis. Concerning hypotonia, no evidence that proves a direct link between lack of breastfeeding and low muscle neonatal tone but scattered data from different sources show that many neural outcomes are facilitated by HM feeding (12). On the one side, it has been reported that a high proportion of floppy infants also show shallow measurements of muscle strength (13, 14), and systematic reviews confirm the positive association between breastfeeding and body strength or balance beyond infancy (15, 16). In particular, in the long-term, adolescents who were breastfed build stronger muscles than those who were not breastfed (17). In the medium-term or short-term, there is substantial evidence of the positive impact of breastfeeding on masseter muscle function among toddlers or infants (18, 19). The combination of the negative correlation between hypotonia and muscle strength and the positive correlation between the history of breastfeeding and subsequent muscle strength may suggest some degree of correlation between lack of HM feeding and neonatal hypotonia.

Regarding our findings on neutrophil counts, we can refer to the well-known link between reactive oxygen species and high neutrophil-to-lymphocyte ratios. This association has been seen in critically ill patients, which is accompanied by a cascade of events that leads to tissue damage and increases the severity of the condition (20). In this field of study, we report no differences between the two groups of infants for the rest of the leukocytes.

While low arterial blood pressure of neonates with sepsis is linked to an increased risk of adverse outcomes (21), treating neonatal hypotension using vasopressors remains controversial (22–24). Hence, there is a consensus to promote a thorough examination of the pathophysiological mechanisms underlying low systemic perfusion to help clinicians decide which treatment strategy to use (25).

Preterm infants have myocardial dysfunction as well as immature vasculature (26). We are still learning HM feeding can have cardioprotective effects on sick neonates. The mechanism by which HM may reverse those cardiovascular changes is one of the crucial elements that is still absent. Our study may add to robust data on the benefits of HM exposure for the cardiovascular morphology of the high-risk newborn and is also consistent with findings on oxidative stress-related diseases in newborns.

The link between HM feeds and enhanced brain or lung development among high-risk neonates (27, 28) has prompted a renewed interest in optimal feeding to improve the

cardiovascular health of such neonates. Preterm infants with higher exposure to HM show enhanced cardiovascular performance by age one year (29) and a more favorable cardiac structure during adolescence and adulthood (30, 31). Our study provides further evidence of the beneficial association between early HM feeding and cardiovascular function across the neonatal developmental stage of sick neonates, which is characterized by systolic and diastolic dysfunction coupled with maladaptive vasculature (32, 33).

The massive release of pro-inflammatory molecules noted among newborn infants with bloodstream infection leads to systemic inflammatory reaction (34) in organs distant from the initial insult, involves the endothelial cells, and affects the vascular tone (3, 4, 35). Ideally, this inflammatory response is followed by a compensatory anti-oxidative response, which represents the biological attempt to prevent inflammation from becoming the dominant reaction and leading to multiorgan dysfunction (5, 36).

An increased oxidized to reduced glutathione (GSSG/GSH) ratio in blood is considered a sign of oxidative stress (37). GSSG/GSH ratios are significantly higher in each phase of septic shock in infants or children; they spike during the overspill inflammatory state and then level off (38). Accordingly, significantly higher levels of GSSG are found among critically ill infants than among matched controls. On the other hand, premature birth removes the fetus from the protected, hypoxic intrauterine milieu. It exposes the sick preterm infant to free radical injury from using high-inspired oxygen fractions or inhaled nitric oxide or from reactive oxidative species produced by septic shock (39).

Furthermore, the lack of transfer of antioxidants across the placenta during the last stages of pregnancy, coupled with their weakened capacity to synthesize antioxidant defenses, renders such infants more vulnerable to oxidative stress. As expected, it has been confirmed that preterm newborns have lower plasma GSH than full-term newborns (40). Thus, the only compensatory mechanism for the sick neonate to cope with these compromised or incomplete antioxidant systems is the free radicals scavenging ability provided by HM (41).

Studies on adults over the past two decades report that the introduction of enteral nutrition, even if the patient requires continued inotropic support, improves both cardiac index and splanchnic blood flow while restores the gastrointestinal dysfunction and reduces hospital mortality rate, which is more visible in patients treated with multiple inotropes (42–44). In respect of Pediatric Intensive Care Units, when early enteral feeding was started in critically ill children with heterogeneous diagnoses, retrospective investigations showed a decreased length of stay in the unit, decreased mortality, and reduced use of inotropes (45, 46). Consistently, the consensus is that inotropes are not a contraindication in carefully monitored early enteral feeding of unstable adults (47). Recent pediatric sepsis guidelines encourage the start of early enteral nutrition based on the child's clinical situation, given that the scant literature on the subject suggests that it is both safe and beneficial (48).

Attention should be drawn to the lack of clinical trials on early enteral feeding of neonates on inotropes. To our knowledge, only a recent randomized trial has addressed this topic. Rao et al. (49) conducted a study on 200 late preterm infants on inotropes randomly assigned to an enteral feed study group or an intravenous (IV) fluid group (one hundred each). After the infant had stable circulation for 6 h, the enteral feed group began feeding with expressed breast milk. The feeding guidelines were individualized based on the infant's clinical status, and there was no increase in the initial volume for at least 24 h. When babies were fed 80 ml/kg/day, feeding intervals of 4 h were replaced with 2 h intervals. From 100 to 160 ml/kg/day, feed volumes were advanced at less than 30 ml/kg/day. Neonates in the IV fluid group were not given any feed. All neonates in enteral feed group were expressed breast milk-fed and were closely monitored until death or discharge. Mortality in the IV fluid group was significantly higher than in the enteral group (30.4% vs. 11.7%, respectively; $p = 0.002$), and the mean duration of hospital stay was longer in the IV fluid group (15.5 days vs. 13.4 days in the enteral feed group; $p = 0.013$). As a result, the authors conclude that neonates can tolerate enteral feed up to dopamine 10 mcg/kg/min and dobutamine 7.5 mcg/kg/min. The practical advice is that enteral feed can be safely administered to neonates on inotropes.

According to currently limited evidence on unstable neonates, some neonatal guidelines favor withholding feeds for babies on inotropes (50), while others prefer to continue enteral feeds even if inotropic support occurs (51). Concerning clinical practice, a survey of 60 Spanish NICUs unveils that 100% delay enteral feeding when there is a hemodynamic failure (52). Considering that we analyzed data from a purely observational cohort, our findings do not allow at this stage to suggest different approaches to minimize inotrope treatments with uncertain cognitive outcomes (53). However, this study contributes evidence on the potential role of very early HM feeding in improving the quality of care of newborn infants with signs of shock and might boost further research on the most effective feeding practices for this group of neonates.

Our study has several limitations. First, we acknowledge that data collection on HM needs to be more specific regarding the vast variability of what this could constitute over the first weeks of life. However, the association between breastfeeding and short- or long-term health outcomes has received considerable scrutiny. A number of studies have suggested that ever vs. never HM feeding may be associated with a decreased risk of mother's carcinoma (54), cardiovascular health in schoolchildren (55), or retinopathy of prematurity among neonates (56), even after adjustment for potential confounders. Second, our study was limited because we did not include feeding duration in our definition of HM feeding. From a methods perspective, we had no information on the feeds in temporal relation to the treatment decision. Previous studies have shown that the rate of breastfeeding initiation may be the same for infants admitted to the well-newborn nursery and the NICU and also that early cessation of breastfeeding is due to the newborn's sickness in less than 5% of cases (57). Hence, other authors have pointed to

links between feeding type and neonatal conditions that are rare during the first week of life, based on data similar to ours, despite the lack of detailed information on the periods in which their patients were fed HM (56).

Third, we also had no information on the number of doses and the timeline of any vasopressor treatment. Moreover, the questionnaire did not collect the name of the vasopressor used. The reasons for prescribing vasopressors were also missing, although probably was due to hypotension. Fourth, the study reflects practices from a long time, which might have changed a decade later. Fifth, as this is a descriptive study, residual confounding may not have been identified. Sixth, we did not account for social characteristics that could impact the outcomes. Seventh, if further clinical, laboratory, or ultrasound data could be examined, our results would have been conclusive, but we must admit that hypotension was the only sign of compromised cardiovascular function that was collected.

Conclusion

Our results show that any HM feeding is associated with a decrease in the need for vasoactive medications in sepsis-affected newborns. This observation suggests that the antioxidant system that is part of HM may protect against inflammatory pathways that impact the vascular tone in sepsis and encourages further research to determine whether HM feeds directly affect neonatal cardiovascular maturation patterns.

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 human participants were reviewed and approved by IIBSP-ENT-2020-152. Written informed consent for participation was not provided by the participants' legal guardians/next of kin: Because the study was conducted retrospectively over a two-decade period, and the data was scanned from patient files, no informed consent form was obtained.

Author contributions

EM and SV had full access to all the data in the study and take responsibility for the integrity of the data. Study concept and design: JF and SV. Acquisition, analysis, or interpretation of data: JF and AL. Literature review and drafting of the manuscript: EM, GG and PM. All authors contributed to the article and approved the submitted version.

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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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Pulmonary hemorrhage and associated risk factors among newborns admitted to a tertiary level neonatal unit in Botswana

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Introduction: Pulmonary hemorrhage (PH) is a life-threatening complication seen in very sick newborns with high morbidity and mortality. There is little data on the incidence, risk factors, and ultimate survival of newborns with pulmonary hemorrhage in sub-Saharan countries, where the healthcare provision and facility differ in many ways compared to high-income countries. Hence, this study aimed to determine the incidence, identify the risk factors, and describe the outcome of pulmonary hemorrhage in newborns in a low middle income country setting.
Methods and materials: A cohort study with prospective data collection was conducted in a public, tertiary-level hospital in Botswana, the Princess Marina Hospital (PMH). All newborns admitted to the neonatal unit from 1 January 2020 to 31 December 2021 were included in the study. Data were collected using a checklist developed on the RedCap database (https//:ehealth.ub.ac.bw/redcap). The incidence rate of pulmonary hemorrhage was calculated as the number of newborns who had pulmonary hemorrhage per 1,000 newborns in the 2-year period. Group comparisons were made using X^2 and Student's *t*-tests. Multivariate logistic regression was used to identify risk factors independently associated with pulmonary hemorrhage.
Result: There were 1,350 newborns enrolled during the study period, of which 729 were male newborns (54%). The mean (SD) birth weight was 2,154(±997.5) g, and the gestational age was 34.3 (±4.7) weeks. In addition, 80% of the newborns were delivered in the same facility. The incidence of pulmonary hemorrhage was 54/1,350 {4% [95% CI (3%–5.2%)]} among the newborns admitted to the unit. The mortality rate in those diagnosed with pulmonary hemorrhage was 29/54 (53.7%). Multivariate logistic regression identified birth weight, anemia, sepsis, shock, disseminated intravascular coagulopathy (DIC), apnea of prematurity, neonatal encephalopathy, intraventricular hemorrhage, mechanical ventilation, and blood transfusion as risk factors independently associated with pulmonary hemorrhage.
Conclusion: This cohort study identified a high incidence and mortality rate of pulmonary hemorrhage in newborns in PMH. Multiple risk factors, such as low birth weight, anemia, blood transfusion, apnea of prematurity, neonatal encephalopathy, intraventricular hemorrhage, sepsis, shock, DIC, and mechanical ventilation, were identified as independently associated risk factors for PH.

KEYWORDS

pulmonary hemorrhage, premature newborns, risk factors, very low birth weight, extreme prematurity

Introduction

Pulmonary hemorrhage (PH) is diagnosed when a discharge of large amounts of bloody fluid from the endotracheal tube or respiratory tract is identified in very sick newborns who have shown acute clinical deterioration (1, 2). PH is one of the most acute and feared causes of mortality in newborns. The mortality from PH ranges from 50% to 68%, and the PH incidence rate is reported to be 1–2 per 1,000 live births occurring most commonly within the first few days of life (2, 3). PH results in an acute deterioration reflected by respiratory compromise, hemodynamic instability, and the need for ventilatory support (4).

Numerous studies have identified multiple risk factors for PH. A higher incidence of PH was observed in premature newborns with extreme prematurity and very low birth weight, as compared to newborns with higher birth weights and gestational ages (GA) (1). The literature identifies risk factors for PH as intrauterine growth restriction, respiratory distress syndrome (RDS), lung hypoplasia, sepsis, coagulopathy, thrombocytopenia, intraventricular hemorrhage (IVH), patent ductus arteriosus (PDA), ventilator treatment, and surfactant treatment (3, 5, 6).

Understanding the risk factors and identifying high-risk patients is critical for devising effective preventive and treatment measures as this would help to mitigate the high mortality and morbidity associated with PH in newborns. In a systematic review, ventilatory support, epinephrine administration, management of coagulopathy, and administration of tolazoline were all found to be effective primary treatments for PH (2).

This study aimed to determine the incidence of PH and identify risk factors associated with pulmonary hemorrhage in newborns admitted at the largest tertiary-level, public hospital in Botswana through a 2-year period.

Methods and materials

This cohort study with prospective data collection was conducted in the Princess Marina Hospital (PMH) in Gaborone, Botswana, where predominantly those with low middle income and socioeconomic status are admitted, whereas those with higher socioeconomic status or health insurance, in our experience, deliver in private hospitals. The unit has 38 beds including six neonatal intensive care beds and admits approximately 100 newborns in a month. Most of the admitted newborns are born in the facility, some are referred from local clinics and primary- or secondary-level hospitals around the capital city and southern part of Botswana. The unit provides basic and advanced clinical care for newborns with all types of medical conditions, except for advanced surgery and extracorporeal membrane oxygenation (ECMO). Due to high mortality rates, especially among the smallest, the unit provides intensive care for babies with birth weight >900 g or GA >28 weeks. All newborns admitted to the neonatal unit were enrolled if the mother gave spoken and written consent to allow the collection of clinical data. The recruitment period was from 1 January 2020 to 31 December 2021. Data were collected using a checklist developed on the RedCap database (https//:ehealth.ub.ac.bw/redcap). The data were collected by a pediatrics registrar, consultant pediatrician, and neonatologist on a daily basis for 10 days and then weekly for each admitted newborn until the occurrence of end points: discharge, death, or transfer out from the unit.

Statistical analysis

The sample size was calculated based on one sample population proportion taking the incidence of pulmonary hemorrhage at 10% from studies done in South Africa (7). Assuming that 10% of the neonates admitted in the unit have pulmonary hemorrhage, and a finite population size of 1,400 neonates admitted in the unit per year, with a 95% level of confidence and with absolute precision of 10%, the study requires a sample size of 997. After looking into the number of newborns admitted to our study, we decided to include all 1,350 study participants in the analysis.

The collected data were exported to SPSS version 27 for Mac (IBM, Chicago, United States) for analysis. Descriptive statistics were presented as frequency and percentage for categorical variables and mean and standard deviation (SD) for continuous variables. The incidence rate was calculated as a proportion with absolute precision 95% confidence interval. Group comparisons were made using X^2 and Student's t-tests for categorical and continuous variables, respectively. A multivariate logistic regression model was used to identify risk factors independently associated with pulmonary hemorrhage. Statistical significance was set at a P-value of <0.05.

Operational definitions

The following operational definitions were used.

Pulmonary hemorrhage in the newborn was defined when a discharge of large amounts of bloody fluid from the endotracheal tube or respiratory tract occurred in very sick newborns who showed acute clinical deterioration like blood aspirated from the trachea concurrent with respiratory decompensation that necessitated intubation or escalated support. When traces of blood appeared at suctioning secretions from the respiratory tract, this was not defined as PH.

Anemia was observed prior to PH in this study and according to the definitions in the unit's protocol. Early (first 1–2 days) anemia was Hb (venous or arterial) < 13.5 g/dl. Later anemia, also before PH happened, depended on the day of life (DOL) and gestational age.

Transfusion of red blood cells was performed before PH occurred when Hb level is <11–12 g/dl and the need for respiratory support, including the need for supplemental oxygen.

Apnea was defined as the absence of breathing/cessation of respiratory airflow in a neonate for a period of >15–20 s often associated with bradycardia and/or desaturation.

Presumed sepsis was defined as the presence of non-specific clinical signs and symptoms consistent with sepsis and therefore

starting treatment with antibiotics. Symptoms and signs were any five of the following: difficulty breathing, apnea, increased mean respiratory rate of more than two standard deviations (SDs) above normal for age or the need for mechanical ventilation for an acute process (not related to underlying neuromuscular disease or the need for general anesthesia), temperature lability (core temperature >38.5° or <36°C), low blood pressure and poor perfusion, abdominal distention, hyperglycemia, bloody stools, or convulsions. Abnormal whole blood cell counts and immature neutrophils were part of the evaluation for presumed sepsis.

Proven sepsis was defined when presumed sepsis and if the blood culture was positive.

Septic shock was defined if the condition required vasopressor treatment if presumed or proven sepsis.

Disseminated intravascular coagulation (DIC) was suspected and defined if simultaneous bleeding and clotting occurred during blood sampling.

PDA was confirmed by echocardiographic evaluation of each newborn when clinically suspected of having PDA, performed by a pediatric cardiologist.

Persistent pulmonary hypertension of the newborn (PPHN) in our setting is diagnosed by echocardiography. In PPHN, echocardiography demonstrates normal structural cardiac anatomy with evidence of pulmonary hypertension [i.e., elevated right ventricle pressure (RVp)]. RVp can be estimated based on Doppler measurement of the velocity of the tricuspid regurgitation (TR) jet, if present. If there is no TR, RVp can be assessed qualitatively (e.g., flattened or displaced ventricular septum).

Transient tachypnea is a mild, self-limited respiratory problem in neonates that begins after birth and lasts about 3 days, also named "wet lungs" or type II respiratory distress syndrome, often without carbon dioxide retention.

Results

There were 1,350 newborns enrolled during the study period, of which 729 (54%) were male newborns. The mean (SD) birth weight was 2,154 (±997.5) g, and the gestational age was 34.3 (±4.7) weeks. In addition, 80% of the newborns were delivered in PMH. Other deliveries were home deliveries or born at clinics outside PMH (Table 1).

Table 1 also depicts clinically suspected sepsis [886 (65.6%)], RDS [570 (42%)], and neonatal jaundice [469 (34.7%)] that were the most common clinical diagnoses observed in the admitted newborns.

The incidence of pulmonary hemorrhage among the newborns admitted to our unit was 54/1,350 {4% [95% CI (3–5.2%)]}. The incidence of PH in very low birth weight (VLBW) newborns was 2.96%. The mortality rate among all newborns diagnosed with pulmonary hemorrhage was 29/54 (53.7%). Variables, such as gestational age, birth weight, RDS, bronchopulmonary dysplasia (BPD), sepsis, Apgar score at 5 min <5, and mechanical ventilation, were included in univariate analysis. On univariate analysis, all entered variables were statistically significant at

TABLE 1 Demographic and baseline characteristics of newborns admitted to the neonatal care unit.

Variables	*n* (%)
Sex	
Male	729 (54.0)
Female	621 (46.0)
Gestational age (weeks)	
<32	412 (30.5)
≥32	938 (69.5)
Mean (SD)	34.3 (4.7)
Birth weight (grams)	
<1,500 (VLBW)	443 (32.8)
<1,000 g (ELBW)	122 (9.0)
≥1,500	907 (67.2)
Mean (SD)	2,154.3 (997.5)
Location of birth	
Inborn	1,081 (80.2)
Home delivery	30 (2.2)
Other health facilities	237 (17.6)
Admission diagnosis	
RDS	570 (42)
Congenital pneumonia	103 (7.6)
BPD	155 (11.5)
TTN	144 (10.7)
MAS	72 (5.3)
PPHN	34 (2.5)
Anemia	91 (6.7)
Laboratory-confirmed bloodstream infection (sepsis)	101 (7.5)
Clinically suspected sepsis	886 (65.6)
Shock	46 (3.4)
DIC	10 (0.7)
Pulmonary hemorrhage	54 (4.0)
Neonatal jaundice	469 (34.7)
Neonatal encephalopathy	87 (6.4)
HIE	99 (7.4)
IVH	58 (4.1)
Patent ductus arteriosus	52 (3.9)
Apgar score <5 at 5 min	
Yes	217 (16.1)
No	1,117 (82.7)

VBLW, very low birth weight; ELBW, extremely low birth weight infants; RDS, respiratory distress syndrome; BPD, bronchopulmonary dysplasia; TTN, transient tachypnea of the newborn; MAS, meconium aspiration syndrome; PPHN, persistent pulmonary hypertension of the newborn; DIC, disseminated intravascular coagulopathy; HIE, hypoxic ischemic encephalopathy; IVH, intraventricular hemorrhage.

P-value <0.05 (Table 2). Variables with *P*-value <0.1 were included for multivariate logistic regression analysis. Of these variables, birth weight [aOR (95% CI) 0.85 (0.77–0.94), $P = 0.002$], anemia [aOR (95% CI) 3.35 (1.4–7.66) $P = 0.004$], sepsis [aOR (95% CI) 2.62 (1.10–6.24), $P = 0.029$], and shock [aOR (95% CI) 5.78 (1.82–18.38), $P = 0.003$] were independently associated with risk of pulmonary hemorrhage (Table 2).

Discussion

This study found a 4% high incidence rate of PH in admitted newborns at the largest tertiary hospital in Botswana and about

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TABLE 2 Factors associated with pulmonary hemorrhage in newborns admitted to the neonatal care unit.

Variables	Pulmonary hemorrhage, *n* (%)	No pulmonary hemorrhage, *n* (%)	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Sex						
Male	27 (3.7)	702 (96.3)	1.18 (0.69–2.04)	0.547		
Female	27 (4.3)	594 (95.7)				
Gestational age (weeks)						
Mean (SD)	30.54 (3.88)	34.42 (4.6)	0.83 (0.78–0.88)	<0.001	1 (0.99–1.00)	0.505
Birth weight (g)						
Mean (SD)	1,422.4 (756.6)	2,184.8 (994.8)	1 (0.98–0.99)	<0.001	0.85 (0.77–0.94)	**0.002**
Clinical diagnosis						
Respiratory distress syndrome	43 (7.5)	527 (92.5)	5.7 (2.92–11.26)	<0.001	1.16 (0.39–3.44)	0.785
Bronchopulmonary dysplasia	12 (7.7)	143 (92.3)	2.30 (1.19–4.48)	0.012	1.62 (0.60–4.37)	0.340
Transient tachypnea of the newborn	1 (0.7)	143 (99.3)	0.15 (0.02–1.11)	0.032	0.66 (0.07–6.34)	0.720
PPHN	4 (11.8)	30 (88.2)	3.38 (1.15–9.95)	0.044	1.84 (0.26–13.22)	0.543
Anemia	20 (22.0)	71 (78.0)	10.15 (5.56–18.53)	<0.001	3.35 (1.4–7.66)	**0.004**
Sepsis	11 (10.9)	90 (89.1)	3.43 (1.71–6.88)	0.001	2.62 (1.10–6.24)	**0.029**
Clinically suspected sepsis	45 (5.1)	841 (94.9)	2.71 (1.31–5.58)	0.005	2.33 (0.73–7.46)	0.153
Shock (septic)	13 (28.3)	33 (71.7)	12.14 (5.95–24.76)	<0.001	5.78 (1.82–18.38)	**0.003**
DIC	5 (50.0)	5 (50.0)	26.35 (7.38–94.00)	<0.001	78.15 (5.90–1,035.01)	**0.001**
Neonatal jaundice	30 (6.4)	439 (93.6)	2.44 (1.41–4.23)	0.001	1.25 (0.58–5.78)	0.353
Patent ductus arteriosus	7 (13.5)	45 (86.5)	4.14 (1.77–9.67)	0.004	1.47 (0.65–3.30)	0.149
Apnea of prematurity	20 (16.5)	101 (83.5)	6.96 (3.86–12.54)	<0.001	2.30 (0.74–7.14)	**0.033**
Neonatal encephalopathy	8 (9.2)	79 (90.8)	2.68 (1.22–5.87)	0.019	2.50 (1.08–5.79)	**0.001**
IVH grade 2	4 (36.4)	7 (63.6)	14.73 (4.18–51.96)	<0.001	7.67 (2.26–25.98)	**0.050**
IVH grade 3	4 (20.0)	16 (80.0)	6.40 (2.06–19.84)	0.007	4.86 (0.99–23.94)	0.775
Apgar score <5 at 5 min	17 (7.8)	202 (92.2)	0.40 (0.22–0.73)	0.002	1.05 (0.40–2.70)	0.928
Mode of ventilation						
Spontaneous	17 (1.7)	955 (98.3)				**0.005**
Mechanical ventilation	29 (14.2)	175 (85.5)	2.54 (1.83–5.53)	<0.001	3.33 (1.43–7.79)	
CPAP	8 (7.3)	102 (92.7)				
Bradycardia needing mask/bag ventilation						
Yes	12 (14.8)	69 (85.2)	4.75 (2.39–9.42)	<0.001	1.47 (0.42–5.07)	0.547
Blood product transfusion						
Transfused	6 (28.6)	15 (71.4)	9.16 (3.37–24.92)	<0.001	4.21 (1.15–15.43)	**0.030**

SD, standard deviation; CPAP, continuous positive airway pressure; DIC, disseminated intravascular coagulation; IVH, intraventricular hemorrhage; PPHN, persistent pulmonary hypertension of the newborn.
The bold numbers indicate statistical difference.

54% mortality rate in those diagnosed with PH. This incidence and mortality rate was similar to other studies (1, 2, 8). However, there is substantial variation in reported PH incidence and associated mortality rates between high-income countries (HICs) and low middle income countries (LMICs). In a systematic review from HIC in 2012, the incidence of PH was 1–12 per 1,000 live births (9), whereas a study from China reported PH in premature newborns of BW <1,500 g at 6.1% and BW <1,000 g at 22.9%, respectively (1). Also from China, a higher neonatal mortality rate was reported in those with PH and a birth weight less than 1,500 g, 82.1% (96/117), and 67.7% (86/127) in those with a birth weight above 1,500 g (10). In a large retrospective cohort study from multiple neonatal intensive care units in the USA, mortality rates among patients with PH were 40.6% and 54.0% at 7 and 30 days of age, respectively (11).

PH can start gradually and continue for a long time, or it can be a sudden life-threatening respiratory complication associated with several risk factors (12). We identified multiple risk factors to be independently associated with pulmonary hemorrhage. In our cohort low birth weight, anemia, blood transfusion, apnea of prematurity, neonatal encephalopathy, intraventricular hemorrhage, sepsis, shock, DIC, and mechanical ventilation were all independent risk factors for PH. According to the literature (13), bleeding into the lungs occurs mainly in premature newborns with severe lung disease. Risk factors have been proposed to be respiratory problems (14) and hemodynamically significantly increased blood flow (persistent ductus arteriosus, PDA) in the blood vessels in the lungs (15), like when sepsis impacts on hemodynamics and reopens or enlarges the patent ductus arteriosus. In newborns, infection (8), in accordance with our findings of sepsis and shock being highly associated with PH, as well as the association of PH with apnea of prematurity (16), is in line with the results of our study. Our preterm babies that developed PH were critically ill with clinical conditions like sepsis or neonatal encephalopathy. A retrospective study that reported on perinatal risk factors from China identified a rate of PH in extremely low birth weight infants (ELBW, <1,000 g) to be 18.8%. These preterm babies that experienced PH were critically

ill with clinical conditions like sepsis or neonatal encephalopathy. It has been shown that small for gestational age, early onset neonatal sepsis, low birth weight, lower Apgar scores at 1 and 5 min, severe RDS, and surfactant replacement were all risk factors for PH (12). Another study from a neonatal intensive care unit in Brazil reported prior use of blood components to be an independent risk factor for PH (17). Transfusion-related PH and acute illness may be more common than previously thought (18, 19). The evidence from preclinical studies showed that blood products can directly modulate transfusion-related immunomodulation, a mechanism linking transfusion exposure with neonatal morbidities (20). Additionally, the use of blood products prior to the PH episode may cause a sudden increase in blood volume, leading to stress injury of the capillary wall, with the passage of fluid and plasma proteins, which can also lead to left ventricular failure, contributing to an increase in pulmonary capillary blood pressure (17). Preterm newborns represent one of the most frequently transfused patients (21). In accordance with our findings, a large retrospective study in preterm neonates found that those who received any RBC transfusion had a 50% increase in neonatal mortality (20).

In our cohort of 1,350 newborns, we found that recurrent apnea was highly correlated to PH. Apnea is a common condition in premature infants due to the immaturity of respiratory control mechanisms. Recurrent apnea is associated with respiratory failure, pulmonary hemorrhage, abnormal heart and lung function, intracranial hemorrhage, abnormal nervous system development, and even sudden death (22). Studies in infants indicated that negative pressure pulmonary edema because of upper airway obstruction led to pulmonary hemorrhage thought to be a stress failure caused by high negative intrathoracic pressure and mechanical disruption of the alveolar-capillary membrane leading to pulmonary bleeding (23). There could be a similar mechanism to apnea causing PH due to laryngospasm or high negative pleural pressure due to inspiratory effort during upper airway obstruction (24, 25).

In newborns, pulmonary hemorrhage is often a manifestation of pulmonary edema (26), and PDA (5) can range in severity from blood-tinged secretions in the endotracheal tube to life-threatening blood loss with hypovolemic shock. The cause of PH is thought to be due to the rapid lowering of intrapulmonary pressure, which facilitates left-to-right shunting across a PDA and an increase in pulmonary blood flow (27). In our study, we identified PDA as a risk factor for PH with statistical significance on univariate analysis, but on multivariate analysis, we could not show statistical significance.

This study has several limitations. First, this study only included study participants from a single neonatal center. Second, important variables such as the use of surfactant were not captured to give light to the impact of surfactant treatment on the incidence and outcome of PH. Finally, because of the policy of the unit, only ELBW infants of 900–1,000 g were provided intensive care and respiratory support whereas smaller ELBWs (<900 g) were provided nasal oxygen treatment, enteral and parenteral nutrition, and antibiotic treatment. We could not analyze and discuss the outcome of those ELBWs (BW < 900 g) and extremely premature babies based on the treatment provided.

Conclusion

This cohort study identified a high incidence and mortality rate in neonates with pulmonary hemorrhage. Multiple risk factors, such as low birth weight, anemia, blood transfusion, apnea of prematurity, neonatal encephalopathy, intraventricular hemorrhage, sepsis, shock, DIC, and mechanical ventilation, were identified as independently associated risk factors for PH.

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 human participants were reviewed and approved by institutional review boards at the University of Botswana, Botswana Ministry of Health, and Princess Marina Hospital, all addressed in Gaborone, Botswana. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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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Association of anemia and platelet activation with necrotizing enterocolitis with or without sepsis among low birth weight neonates: a case–control study

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Background: This study aims to evaluate the value of the proportion of large platelets (PLCR) and platelet crit (PCT) in predicting necrotizing enterocolitis (NEC) in low birth weight (LBW) neonates.
Methods: A total of 155 LBW (<2,500 g) neonates with NEC, who were admitted to the neonatal intensive care unit (NICU) of the hospital from January 1, 2017, to November 30, 2019, were included in the case group. According to the 1:3 case–control study design, a total of 465 LBW neonates without NEC (three for each LBW neonate with NEC), who were admitted to the NICU and born ≤24 h before or after the birth of the subjects, were included in the control group.
Results: During the study period, a total of 6,946 LBW neonates were born, of which 155 had NEC, including 92 who also had sepsis. Neonatal sepsis was the most important risk factor and confounding factor for NEC in LBW neonates. Further stratified analysis showed that in LBW neonates without sepsis, anemia [$P = 0.001$, odds ratio (OR) = 4.367, 95% confidence interval (CI): 1.853–10.291], high PLCR ($P < 0.001$, OR = 2.222, 95% CI: 1.633–3.023), and high PCT ($P = 0.024$, OR = 1.368, 95% CI: 1.042–1.795) increased the risk of NEC and the receiver operating characteristic curve area of PLCR, sensitivity, specificity, and cutoff value were 0.739, 0.770, 0.610, and 33.55, respectively.
Conclusions: The results showed that 2/100 LBW neonates were at risk for NEC, and the stratified analysis of the confounding factors of sepsis identified the risk factors of NEC in LBW neonates. This study first reported the significance of PLCR in the early prediction of NEC occurrence in LBW neonates without sepsis.

KEYWORDS

NEC, anemia, sepsis, the value of the proportion of large platelets, platelet crit

Introduction

Neonatal necrotizing enterocolitis (NEC) is a neonate-specific inflammatory necrotizing disease that involves the ileum and/or colon and is common among premature infants, severely threatening the life of neonates (1–3). The mortality rate has declined due to continuous advances in the treatment of premature infants in recent years, but the incidence of NEC has not decreased significantly.

Abbreviations
NEC, necrotizing enterocolitis; LBW, low birth weight; PLCR, the value of the proportion of large platelets; PCT, platelet crit; MPV, mean platelet volume; PDW, platelet distribution width; HDCP, hypertensive disorders complicating pregnancy; GDM, gestational diabetes mellitus; PP, placenta previa; PROM, premature rupture of membrane; PDA, patent ductus arteriosus.

Statistically, the incidence of NEC in premature infants weighing <1,500 g was 5%–10%, the mortality rate was 20%–30%, and >30%–50% of NEC neonates required surgical treatment (4, 5). Despite decades of research, the current understanding of the diagnosis and treatment of neonatal NEC is limited, the rate of neonatal NEC mortality remains high, and neonatal surgical advances have not significantly improved the prognosis in NEC survivors (6, 7). Therefore, NEC intervention, especially in low birth weight (LBW) neonates with NEC, should be under intensive focus with respect to identifying the causes and related factors for early diagnosis and treatment.

Hitherto, although NEC pathogenesis remains unclear, several studies have shown that it is caused by a combination of factors. Some studies have reported that preterm birth (6, 7), low birth weight (6–9), and race (8) were critical risk factors. Recent studies have shown that maternal infection (10), congenital pneumonia (11), asphyxia (12), blood transfusion (12), anemia (13, 14), and neonatal sepsis are also potential contributing factors. Furthermore, it has been suggested that NEC pathogenesis is multifactorial, involving a combination of abnormal bacterial colonization, a cascade amplification of inflammation, gut immaturity, and ischemia–reperfusion (I/R) injury. The differences in the immune response to mucosal damage and the microbiota may also be responsible for the increased inflammatory response in NEC (15). NEC induced by gut immaturity and I/R injury is not significantly associated with sepsis. Based on the epidemiological and clinical theories, sepsis can confound the diagnosis of clinical complications and the use of inflammatory proteins as the NEC marker. Both sepsis and NEC require careful differential diagnosis, as both may be lethal if not properly diagnosed and treated.

Regarding gut immaturity in NEC infants, the degree and duration of thrombocytopenia are associated with the severity of bowel injury and adverse clinical outcomes. NEC infants develop thrombocytopenia with a platelet (PLT) count of $<100 \times 10^9$/L. This low PLT count is yet an unresolved clinical dilemma (16, 17). A robust NEC biomarker different from that of sepsis could improve bedside management, reduce morbidity and mortality rates, and allow patients to select potential treatments in the clinic. Animal studies (18) have shown that PLT activation during NEC-like intestinal injury is an early, thrombin-mediated process that antedates both mucosal damage and the rise in bacterial products in plasma. Hence, it is a crucial pathophysiological event during neonatal intestinal injury. In clinical practice, the PLT count in NEC patients is monitored periodically, but PLT activation indicators, such as the mean platelet volume (MPV), platelet crit (PCT), platelet distribution width (PDW), and platelet large cell ratio (PLCR), are usually neglected (19, 20). These PLT indicators are valuable in the clinical diagnosis and prognostic prediction of cardiovascular and metabolic diseases (21, 22); however, their clinical significance, reference value, and utilization value are still being investigated.

Intriguingly, the high mortality rate of NEC patients could be ascribed to the difficulty in diagnosing and treating the condition promptly. Radiographic evidence, such as pneumatosis intestinalis, is used to diagnose severe or advanced disease but has a sensitivity of only 44% with limited specificity and a lack of interpretation concordance (2, 23). Several studies have adopted 1:1 or 1:2 case–control cohorts with low statistical efficiency (24, 25). Herein, we hypothesized that sepsis, anemia, and PLT activation index are vital NEC predictors in LBW neonates. Furthermore, a 1:3 case–control study with sufficient statistical efficiency was conducted to verify the predictive value of anemia, sepsis, and PLT activation indexes in the incidence of LBW NEC, to achieve earlier diagnosis and treatment.

Methods

Participants

A total of 155 LBW (<2,500 g) neonates with NEC, who are born in the Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China, and admitted to the neonatal intensive care unit (NICU) of the hospital from January 1, 2017, to November 30, 2019, were included in the case group. According to the 1:3 case–control study design, a total of 465 LBW neonates without NEC (three for each LBW neonate with NEC), who are admitted to the NICU and born ≤24 h before or after the birth of the subjects, were included in the control group.

The exclusion criteria for the case and control groups were as follows: neonates who were unlikely to survive or had significant gastrointestinal anomalies or those who were discharged at their own will in 3 days. Among the neonates excluded in our study, none with severe abdominal distension was suspected of having fulminant NEC.

All patient information was obtained from the hospital medical record database.

Neonates were diagnosed with NEC (stage II or above) if they met the diagnostic standard in the Practical Neonatology and Bell staging (26, 27) and had clinical symptoms such as abdominal distension, vomiting, and bloody stool triad and if the abdominal plain x-ray scan revealed abdominal intestinal aeration, intestinal obstruction, intestinal pneumatosis, or intrahepatic portal venous gas.

Sepsis in infants is defined by the presence of signs and symptoms of infection with positive blood culture. It is classified as early-onset sepsis (EOS) if symptoms start before 72 h of life and late-onset sepsis (LOS) if symptoms start after 72 h of life. NEC with sepsis is diagnosed in infants with signs and symptoms of infection and positive blood cultures before NEC.

The hospital routinely monitors the complete blood count (CBC) of high-risk infants with stable condition weekly, and the frequency of examination will be increased accordingly for infants with unstable condition. Neonatal anemia is defined as Hb levels less than the fifth percentile, with Hb levels varying with gestational age (28).

Identification of factors

The main influencing factors of the included subjects were listed, and clinical information of the subjects, including

maternal factors [age, nationality, number of fetuses, hypertensive disorders complicating pregnancy (HDCP), gestational diabetes mellitus (GDM), placenta previa (PP), placenta abruption, and premature rupture of membrane (PROM)] and neonatal factors [date of birth, birth weight, NEC diagnosis age (the timespan from the date of birth to NEC diagnosis), neonatal sepsis, patent ductus arteriosus (PDA), anemia, hypoglycemia, birth asphyxia (29), blood transfusion, mycoplasma infection (30), and hyperglycemia], were obtained from the hospital medical record database every 3 months. The complications in the subjects occurred before NEC diagnosis. PLT indicators (such as PLT count, PDW, MPV, PCT, and PLCR) of the case group were recorded as the latest data 2 days before the date of NEC diagnosis, whereas those of the control group were recorded 1 day before and after the date of NEC diagnosis of the subjects.

Assignment of main study variables

Dependent variables

NEC neonates were assigned 1, and the controls were assigned 0.

Independent variables

All independent variables (quantitative or qualitative) were converted to qualitative variables by assignment. Since there were no definitive clinical reference values for PLT indicators for neonates, especially for neonates with LBW or low gestational age, the independent variables were stratified and assigned values using the quartile (Q) method, namely, quartile 1 (Q1), quartile 2 (Q2), and quartile 3 (Q3) (**Table 1**).

Statistical analysis

The general characteristics were compared between the case and control groups. The birth weight of the NEC neonates was determined to be normally distributed by the Shapiro–Wilk test, and Student's *t*-test was used to compare the birth weight of the NEC neonates with and without sepsis. Spearman's method was used for the analysis of gestational age, birth weight, and NEC diagnostic age. In the 1:3 case–control analysis, the occurrence of NEC was evaluated by analyzing the variables using univariate non-conditional logistic regression. A factor with $P < 0.5$ was analyzed using stepwise multivariate conditional logistic regression (Cox regression). The factors, after being stratified by sepsis, were analyzed using univariate and multivariate non-conditional logistic regression. The receiver operating characteristic (ROC) curve was used to evaluate the PLCR and PCT in NEC diagnosis. SPSS 22.0 software was used for statistical analyses and image drawing. Data conforming to the normal distribution were expressed as mean ± standard deviations, while those not conforming to the normal distribution were expressed as median (minimum, maximum). The odds ratio (OR) and the 95% confidence interval (CI) were calculated. An OR with 95% CI not containing the value of 1 was

TABLE 1 Assignments of independent variables.

Variable		Assignment	Variable		Assignment
Maternal factor			Neonatal factor		
Gestational weeks	≤28	0	Gender	Male	0
	29–37	1		Female	1
	≥37	2	Birth weight (g)	<1,000	0
Number of fetuses	Single fetus	0		1,000–1,500	1
	Multiple fetuses	1		1,500–2,500	2
HDCP	No	0	Transfusion	No	0
	Yes	1		Yes	1
GDM	No	0	Sepsis	No	0
	Yes	1		EOS	1
				LOS	2
PP	No	0	PDA	No	0
	Yes	1		Yes	1
Placenta abruption	No	0	Anemia	No	0
	Yes	1		Yes	1
PROM	No	0	Hypoglycemia	No	0
	Yes	1		Yes	1
			Asphyxia	No	0
				Yes	1
			Mycoplasma infection	No	0
				Yes	1
			Hyperglycemia	No	0
				Yes	1
			PLT	<Q1: 0; Q1–Q2: 1; Q2–Q3: 2; ≥Q3: 3;	
			PDW	<Q1: 0; Q1–Q2: 1; Q2–Q3: 2; ≥Q3: 3;	
			MPV	<Q1: 0; Q1–Q2: 1; Q2–Q3: 2; ≥Q3: 3;	
			PCT	<Q1:0; Q1–Q2:1; Q2–Q3:2; ≥Q3:3;	
			PLCR	<Q1: 0; Q1–Q2: 1; Q2–Q3: 2; ≥Q3: 3	

of statistical significance. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Results of the general information

During the study period, a total of 58,507 mothers gave birth to a total of 60,182 neonates in the hospital. Among the neonates, 6,946 (11.54%) had LBW, and 155 (2.23%) had LBW and NEC [including 92 (59.35%) who also had sepsis]. Among the 465 neonate controls with LBW in this study, 58 (12.47%) also had sepsis. Among the 6,946 LBW neonates, 5,270 (75.87%) had a birth weight between 1,500 and 2,500 g [including 97 (1.84%) with NEC], 1,207 (17.38%) had a birth weight between 1,000 and 1,500 g [including 47 (3.89%) with NEC], and 469 (6.75%) had a birth weight of <1,000 g [including 11 (2.35%) with NEC], which are evaluated using the chi-square test, as shown in **Figure 1**. Moreover, one of the NEC neonates had a mother with Lahu nationality, and the others had mothers with Han nationality. There was no statistically significant difference between the case and control groups in gestational age, $P > 0.05$. The general information on LBW neonates and their mothers is shown in **Table 2**.

Univariate analysis between NEC occurrence and baseline characteristics and clinical information of mothers

The cause of NEC is complex and might be affected by multiple maternal factors (such as general characteristics and complications). The univariate analysis of the main complications revealed that the gestational week and placenta abruption were statistically correlated (**Table 3**). Placenta abruption (OR = 2.847, 95% CI: 1.517–5.343, $P = 0.001$) was identified as a risk factor for NEC occurrence, whereas gestational week (OR = 0.321, 95% CI: 0.201–0.512, $P < 0.001$) was the independent protective factor.

Univariate analysis between NEC occurrence and baseline characteristics and clinical information of LBW neonates

The literature showed that a correlation has been in the spotlight between NEC occurrence and baseline characteristics and complications in neonates. The univariate analysis revealed that in neonates, sepsis, PDA, anemia, hyperglycemia, and birth weight were statistically correlated. Sepsis ($P < 0.001$, OR = 10.247, 95% CI: 06.717–15.633), PDA ($P = 0.018$, OR = 1.680, 95% CI: 1.094–2.580), anemia ($P < 0.001$, OR = 3.482, 95% CI: 1.835–6.627), and hyperglycemia ($P = 0.005$, OR = 3.476, 95% CI: 1.447–8.351) were identified as risk factors for NEC occurrence, whereas gestational week (OR = 0.606, 95% CI: 0.453–0.812, $P = 0.001$) was the independent protective factor. Conversely, hypoglycemia (OR = 0.870, 95% CI: 0.474–1.596, $P = 0.651$), asphyxia (OR = 0.966, 95% CI: 0.575–1.623, $P = 0.895$), wet lung (31) (OR = 1.483, 95% CI: 0.986–2.231, $P = 0.058$), mycoplasma infection (OR = 2.144, 95% CI: 0.973–4.725, $P = 0.059$), and blood transfusion (OR = 0.854, 95% CI: 0.488–2.231, $P = 0.580$) were not statistically correlated with NEC occurrence in LBW neonates. In addition, the analysis of the correlation between NEC occurrence and PLT indicators in LBW neonates showed that the PLT count (OR = 1.494, 95% CI: 0.953–1.322, $P = 0.167$) was not statistically correlated, whereas PDW (OR = 1.920, 95% CI: 1.597–2.307, $P < 0.001$), MPV (OR = 2.093, 95% CI: 1.735–2.525, $P < 0.001$), PCT (OR = 1.441, 95% CI: 1.217–1.705, $P < 0.001$), and PLCR (OR = 2.156, 95% CI: 1.777–2.017, $P < 0.001$) were statistically correlated with NEC occurrence in LBW neonates (**Table 4**).

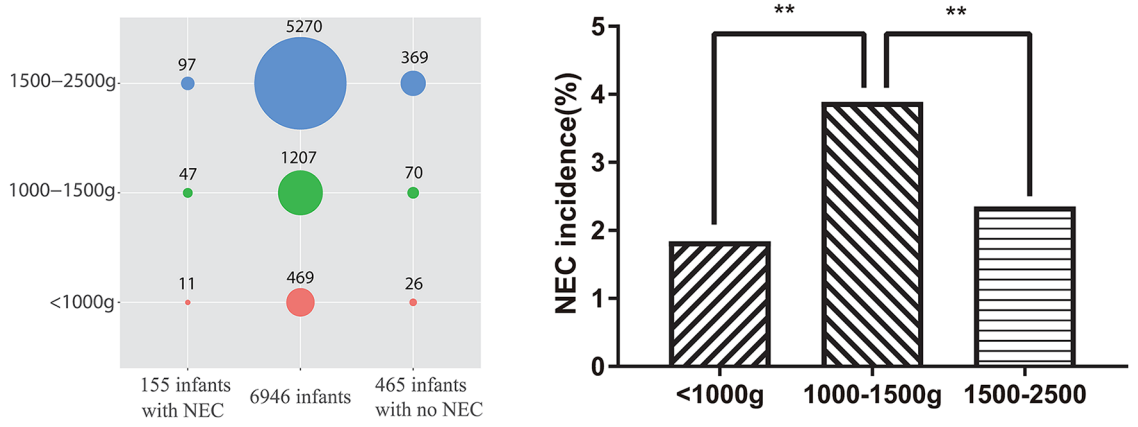

FIGURE 1
Diagnoses of enrolled infants in the 1:3 case–control study.

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TABLE 2 General information on LBW neonates and their mothers in the case–control study.

Factors		Case group ($n = 155$)	Control group ($n = 465$)
Maternal factor			
Age (years)		31.35 ± 4.59	31.10 ± 4.51
Nationality (n)	Han Chinese	154	465
	Minority	1	0
Gestational week [median (minimum, maximum)]		31 (21, 39)	34 (24, 39)
Number of fetuses (n)	Single fetus	104	288
	Multiple fetuses	51	177
HDCP (n)		18	55
GDM (n)		27	72
PP (n)		18	51
Placenta abruption (n)		20	23
PROM (n)		49	131
Neonatal factor			
Gender (n)	Male	86	226
	Female	69	239
Blood transfusion (n)		18	62
Birth weight (g)		1,588.32 ± 384.70	1,878.93 ± 449.60
Sepsis (n)		92	58
EOS		22	36
LOS		70	22
PDA (n)		34	82
Anemia (n)		21	20
Hypoglycemia (n)		15	51
Asphyxia (n)		22	68
Mycoplasma infection (n)		11	16
Hyperglycemia (n)		11	10
PLT (10^9/L)		321.73 ± 105.60	310.32 ± 110.62
PDW (fl)		14.93 ± 2.92	13.17 ± 3.02
MPV (fl)		11.77 ± 0.87	11.00 ± 1.07
PCT (%)		0.38 ± 0.11	0.34 ± 0.12
PLCR (%)		37.77 ± 6.60	31.89 ± 8.13

Analysis of the effect of multiple factors and their correlation on NEC occurrence

To avoid missing the critical clinical factors, we considered 16 variables with $P < 0.05$ in the univariate regression analysis result as independent variables for the 1:3 case–control analysis using the stepwise multivariate conditional logistic regression (the survival function was assessed by Cox regression). The results showed that sepsis, PLCR, and PCT were the final factors in the regression model. EOS (OR = 2.424, 95% CI: 1.461–4.021, $P = 0.001$) and LOS (OR = 4.291, 95% CI: 3.001–6.138, $P < 0.001$) increased the risk of NEC in LBW neonates. Increased PLCR (OR = 1.451, 95% CI: 1.220–1.724, $P < 0.001$) and increased PCT (OR = 1.225, 95% CI: 1.056–1.422, $P = 0.007$) could be the risk factors for NEC occurrence, as shown in Table 5.

TABLE 3 Univariate analysis between NEC occurrence and baseline characteristics and clinical information of mothers.

	B	SE	P	OR	95% CI	
					Lower limit	Upper limit
Gestational week	−0.272	0.036	<0.001	0.321	0.201	0.512
HDCP	−0.021	0.289	0.943	0.979	0.556	1.725
GDM	0.141	0.248	0.569	1.151	0.709	1.870
PP	0.064	0.291	0.825	1.067	0.603	1.888
Multiple fetuses	−0.226	0.196	0.249	0.798	0.544	1.171
Placenta abruption	1.046	0.321	0.001	2.847	1.517	5.343
PROM	0.16	0.201	0.414	1.18	0.800	1.750

Analysis of the effect of multiple factors and their correlation on NEC occurrence in neonates without sepsis

The effect of sepsis weighted the most among the above factors of statistical significance. Sepsis could lead to systemic inflammatory response, involving multiple organ system damages and alteration of the evaluation indicators, such as PLT count. Whereupon, the information of NEC patients without sepsis was analyzed using univariate regression, and 17 variables with $P < 0.05$ were considered as independent variables for stepwise multivariate non-conditional logistic regression analysis. The results showed that anemia, PLCR, and PCT were the final significant indicators in the model. Anemia (OR = 4.367, 95% CI: 1.853–10.291, $P = 0.001$) increased the risk of NEC in LBW neonates without sepsis, and increased PLCR (OR = 2.222, 95% CI: 1.633–3.023, $P < 0.001$) and PCT (OR = 1.368, 95% CI: 1.042–1.795, $P = 0.024$) could be the indicators in predicting the risk of NEC in LBW neonates without sepsis, as shown in Table 6.

Analysis of the effect of multiple factors and their correlation on NEC occurrence in neonates with sepsis

The information on NEC patients with sepsis was analyzed using univariate regression, and 10 variables with $P < 0.05$ were considered as independent variables for stepwise multivariate non-conditional logistic regression analysis. The results showed that only MPV was the final significant indicator entered into the model. Increased MPV (OR = 1.409, 95% CI: 1.017–1.953, $P = 0.040$) was the indicator to predict the risk of NEC in LBW neonates with sepsis (Table 7).

PLCR and PCT in predicting NEC occurrence

The ROC curve fitting analysis was used to evaluate the PLCR, PCT, and the combination of the two in NEC diagnosis. In all NEC patients in this study, the ROC curve area of PLCR diagnosis was 0.717 ($P < 0.001$), the sensitivity was 0.767, the specificity was 0.581, and the cutoff value was 33.55. The ROC curve area of PCT diagnosis was 0.606 ($P < 0.001$), the sensitivity was 0.640, the specificity was 0.560, and the cutoff value was 0.3350. The

TABLE 4 Univariate analysis between NEC occurrence and baseline characteristics and clinical information of LBW neonates.

	B	SE	*P*	OR	95% CI	
					Lower limit	Upper limit
Sepsis	2.327	0.216	<0.001	10.247	6.717	15.633
PDA	0.519	0.219	0.018	1.680	1.094	2.580
Anemia	1.249	0.328	<0.001	3.482	1.835	6.627
Hypoglycemia	−0.140	0.310	0.652	0.870	0.474	1.596
Asphyxia	−0.035	0.265	0.895	0.966	0.575	1.623
Wet lung	0.394	0.208	0.058	1.483	0.986	2.231
Mycoplasma infection	0.763	0.403	0.059	2.144	0.973	4.725
Hyperglycemia	1.246	0.447	0.005	3.476	1.447	8.351
Blood transfusion	−0.158	0.285	0.580	0.854	0.488	1.494
Birth weight	−0.500	0.149	0.001	0.606	0.453	0.812
PLT	0.115	0.084	0.167	1.122	0.953	1.322
PDW	0.652	0.094	<0.001	1.920	1.597	2.307
MPV	0.739	0.096	<0.001	2.093	1.735	2.525
PCT	0.365	0.086	<0.001	1.441	1.217	1.705
PLCR	0.768	0.099	<0.001	2.156	1.777	2.017

TABLE 5 Multivariate logistic regression (survival function Cox regression) analysis of NEC occurrence in LBW neonates.

	B	SE	*P*	OR	95% CI	
					Lower limit	Upper limit
Sepsis	1.285	0.170	<0.001	3.614	2.589	5.046
EOS	0.885	0.258	0.001	2.424	1.461	4.021
LOS	1.457	0.183	<0.001	4.291	3.001	6.138
Anemia	0.516	0.237	0.030	1.675	1.053	2.665
PLCR	0.372	0.088	<0.001	1.451	1.220	1.724
PCT	0.203	0.076	0.007	1.225	1.056	1.422

TABLE 6 Multivariate logistic regression analysis of NEC occurrence in LBW neonates without sepsis.

	B	SE	*P*	OR	95% CI	
					Lower limit	Upper limit
Anemia	1.474	0.437	0.001	4.367	1.853	10.291
PLCR	0.798	0.157	<0.001	2.222	1.633	3.023
PCT	0.313	0.139	0.024	1.368	1.042	1.795

TABLE 7 Multivariate logistic regression analysis of NEC occurrence in LBW neonates with sepsis.

	B	SE	*P*	OR	95% CI	
					Lower limit	Upper limit
MPV	0.343	0.167	0.040	1.409	1.017	1.953

ROC curve area of PLCR–PCT diagnosis was 0.719 ($P < 0.001$), the sensitivity was 0.920, the specificity was 0.423, and the cutoff value was 0.1566 (**Figure 2**).

In NEC patients without sepsis, the ROC curve area of PLCR diagnosis was 0.739 ($P < 0.001$), the sensitivity was 0.770, the specificity was 0.610, and the cutoff value was 33.55. The ROC curve area of PCT diagnosis was 0.629 ($P = 0.001$), the sensitivity was 0.672, the specificity was 0.560, and the cutoff value was 0.3350. The ROC curve area of PLCR–PCT diagnosis was 0.748 ($P < 0.001$), the sensitivity was 0.852, the specificity was 0.557, and the cutoff value was 0.1074. In conclusion, the value of the combination of PLCR and PCT and PCT alone in NEC diagnosis was not significantly higher than PLCR alone (**Figure 3**).

The values of these ROCs were acceptable.

Discussion

The present study aimed to provide a pooled estimation of NEC in LBW neonates in China. The results showed that 2/100 LBW neonates developed NEC and that sepsis and anemia were the risk factors for NEC occurrence in LBW neonates. This might be the first study to show the superior value of PLT activation (especially PLCR), rather than PLT count, in predicting NEC occurrence in LBW neonates. Most studies postulate that neonatal sepsis is a major risk factor for NEC (10, 12, 27, 32). Currently, the correlation between anemia and NEC occurrence is under intensive focus, but the findings of such studies are yet controversial. The association between PDA and the occurrence of NEC due to hemodynamic changes is consistent with the findings of most studies (26). In this study, our results are suggestive that there is a correlation between anemia and NEC occurrence. The present study identified the risk factor for NEC occurrence in LBW neonates in NICUs in China and emphasized the value of PLT activation in NEC diagnosis of LBW neonates, thereby providing a new approach for future studies on NEC pathogenesis.

Correlation between neonatal sepsis and NEC occurrence

Stratified analysis of sepsis identified the risk factors for NEC in LBW neonates. This study found that every 6/10 NEC

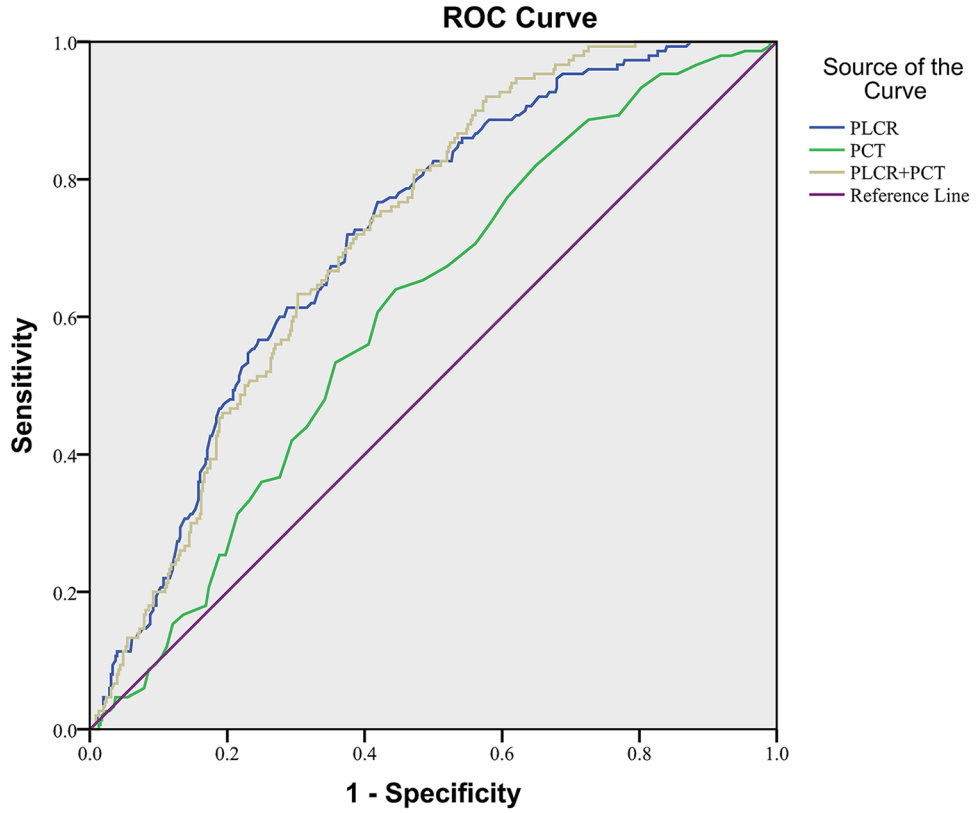

FIGURE 2
PLCR and PCT in NEC diagnosis of LBW neonates.

patients presented sepsis and both EOS and LOS were risk factors for NEC, suggesting that the control of intrauterine infection and hospital infection is crucial in preventing NEC. In the event of sepsis infection, pathogen-produced toxins may directly damage the intestinal mucosa or activate immune cells to produce cytokines, thereby altering vascular permeability and tissue damage (3). These phenomena result in the accumulation of PLTs and white blood cells in capillaries, which in turn causes intestinal damage and eventually NEC by blocking blood flow, aggravating the intestinal mucosa, and initiating excessive multiplication of intestinal bacteria. Another study showed that sepsis increases the risk of NEC by threefold (27), which is similar to the current finding. Typically, neonatal sepsis induced by different types of microbes manifests as a variety of pathophysiology and can result in several complications and outcomes (32, 33). Therefore, neonatal sepsis is deemed a vital confounding factor in the etiological analysis of NEC. Importantly, we also found that the risk factors for NEC differed between neonates with and without sepsis. These patients were further grouped into NEC subjects with and without sepsis for analysis. The results showed that NEC occurrence was correlated with anemia, PLCR, and PCT in NEC subjects without sepsis under the interplay of multiple factors. However, only MPV was weakly correlated with NEC occurrence in NEC subjects with sepsis.

Correlation between PLT activation indicators and NEC occurrence

PLT activation, rather than PLT count, was the earlier predictor for NEC occurrence. Pups with 2,4,6-trinitro-benzene sulfonic acid (TNBS)-mediated acute necrotizing ileocolitis had increased immature PLT fractions, high MPV, and increased megakaryocyte number/ploidy in the bone marrow, which are consistent with the clinical observations in human NEC (34, 35). These manifestations favored peripheral PLT consumption, but not decreased production, as the kinetic basis for thrombocytopenia (36). However, the literature showed that the PLT count decreased significantly in NEC patients compared to that in non-NEC patients and was correlated with the severity of NEC (37). Our results were different from the previous literature. The main reasons might be that the PLT in this study was counted 2 days before NEC diagnosis when NEC is in the early stage and the bone marrow is producing compensatory blood vigorously to maintain normal PLT count or increase PLT count. Additionally, PLCR and PCT were sensitive and increased at the early stage. Apparently, PLCR and PCT, not PLT count, could be used as indicators to predict the early risk in NEC patients without sepsis, indicating that PLT activation was the early predictor for NEC occurrence. Various types of sepsis affect the PLT through different pathways. PLT activation and depletion during NEC disrupt the mucosal wall established and lead to

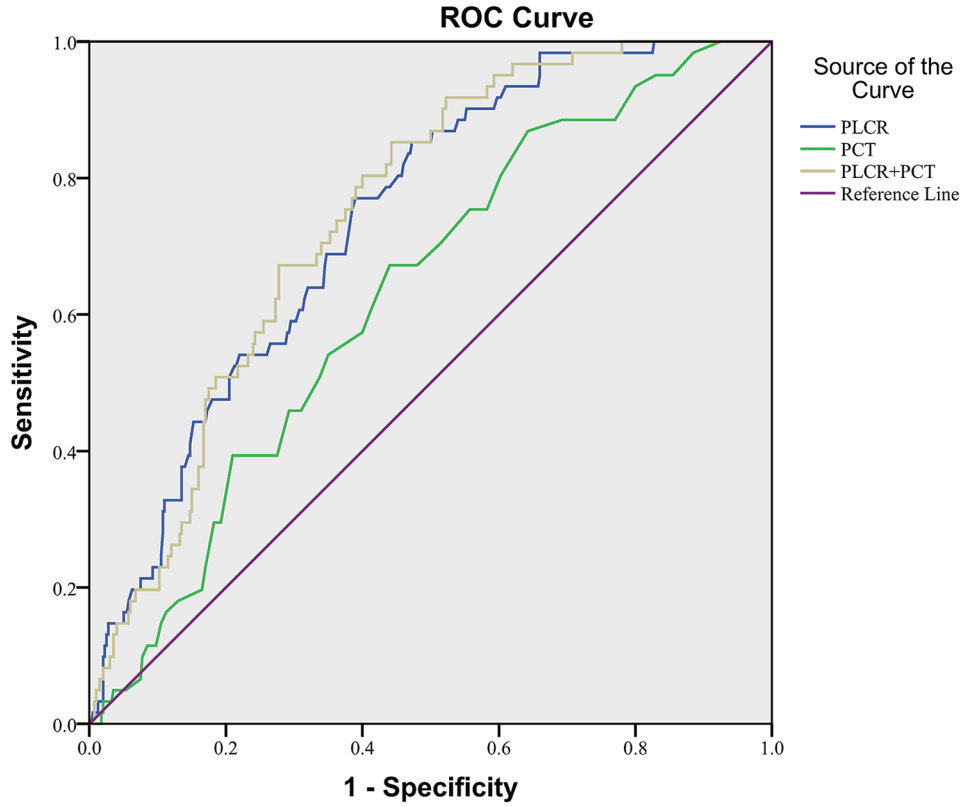

FIGURE 3
PLCR and PCT in NEC diagnosis of LBW neonates without sepsis.

bacterial translocation across the damaged mucosa (18). PLTs repair bacterial damage to the vascular endothelium, resulting in increased platelet consumption, activated immune system, and enhanced PLT apoptosis (38). Other common viral infections, such as Torch or fungal infection, directly destroy the megakaryocytes or PLTs, inhibit bone marrow hematopoiesis, induce the production of autoantibodies to accelerate PLT destruction, and affect their levels in peripheral blood. Severe bacterial infections [such as GBS (group B streptococcus) infection] disrupt bone marrow hematopoiesis, leading to thrombocytopenia. Therefore, it could be speculated that the value of PLT indicators in predicting the occurrence of NEC in neonates with sepsis was not significant.

Correlation between anemia and NEC occurrence

Anemia was a risk factor for NEC occurrence in patients without sepsis but not in those with sepsis. Previous studies on anemia promoting NEC occurrence yielded varied results. Patel et al. (13) and Singh et al. (26) speculated that anemia, rather than blood transfusion, was correlated with a high risk of NEC occurrence; however, other retrospective studies did not find such a significant effect (27, 39). Nonetheless, no stratified analysis of sepsis was performed in these studies. Herein, we proposed that anemia, rather than blood transfusion, increased the risk of NEC occurrence in patients without sepsis because anemia affects splanchnic perfusion and causes hypoxia, anaerobic metabolism, and accumulation of anaerobic metabolism products, such as lactic acids. These by-products disrupt the intestinal vascular regulation resulting in ischemic injury, thereby increasing the risk of NEC occurrence (40). Various microbes causing sepsis give rise to anemia, hypoxia, and intestinal tissue damage in the body and, hence, are deemed confounding factors that complicate the analysis of the results. In the NEC and sepsis subgroup, the lack of association between anemia and NEC development may be due to a series of severe lesions caused by sepsis that interfere with this factor; hence, more follow-up research is needed.

Nevertheless, the present study had some limitations. First, all the results were based on the largest women's hospital in Zhejiang Province (one of the largest women's hospitals in China), but the information recorded did not reflect NEC occurrence and the associated risk factors nationwide. Therefore, multicenter clinical studies are essential to further investigate the correlation between anemia, increased PLCR, increased PCT, and NEC occurrence in LBW neonates. Second, this case–control study had a retrospective design and, thus, was subject to information bias. Hence, a cohort study, as well as a randomized controlled clinical experiment, should be carried out in the future to substantiate the current findings.

Conclusion

PLCR has a significant value in the early prediction of NEC incidence in LBW neonates without sepsis.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Women's Hospital, Zhejiang University School of Medicine (IRB-20210068-R). 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 because this study was a retrospective case–control study.

Author contributions

ZJ designed the study and was the principal author of the manuscript. GY supervised data collection, did the analysis, and reviewed the manuscript. SZ supervised and contributed to the design, supervised the data analysis, and co-wrote the manuscript. LZ obtained epidemiology data and did the analysis. All authors contributed to the article and approved the submitted version.

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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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Preterm infants with positive conjunctival swab culture: risk factors and association with late-onset sepsis–a retrospective cohort study

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Introduction: Purulent conjunctival discharge in hospitalized preterm infants may indicate conjunctivitis and warrant treatment. The purpose of this study was to examine the relationship between positive conjunctival swab (CS) culture and late-onset sepsis (LOS) in preterm infants.

Methods: A retrospective cohort study was conducted to determine the relationship between positive CS culture growth results (CSP) obtained in preterm infants ≤34 weeks' gestation and the development of LOS within 120 h of obtaining CS compared with those who had negative CS culture results (CSN). Electronic medical records were reviewed from January 2015 until December 2019 for preterm infants presenting with purulent conjunctival discharge and underwent CS culture testing due to suspected conjunctivitis.

Results: Of the 234 CS cultures obtained during the study period, 145 (61.9%) were CSP compared to 89 (38.1%) CSN cultures. Gram-negative organisms accounted for 70% of all CSP cultures, with the remaining 30% being Gram-positive. Patients with CSP were smaller, younger, had lower 1-minute APGAR scores, and required respiratory support more frequently than those with CSN. Infants with CSP received antibiotics for longer periods, both topically and systemically. Infants who developed LOS were more likely to require invasive ventilation (adjusted odds ratio, 33.5; 95% CI, 2.52–446.5, $p = 0.008$). The incidence of LOS between the two groups was similar, with 6.2% observed in the CSP group compared to 3.4% in the CSN group ($p = 0.543$). Similarly, the rates of bacteremia were similar in both groups. Of the CSP patients who were presented with bacteremia, four out of seven (57%) exhibited bacteremia caused by the same organism found in their CS cultures. Similarly, within the entire cohort, respiratory cultures were performed on nine intubated patients within two weeks of obtaining CS cultures. Of these, in the CSP group, five out of six (83%) showed an organism identical to that found in the CS cultures.

Conclusion: The study found a significant proportion of positive CS cultures in preterm infants, with distinct patient characteristics and treatment compared to negative cultures. While the incidence of LOS was not significantly different between the two groups, some CSP patients demonstrated bacteremia with the same CS organism, suggesting a possible connection between conjunctival or respiratory colonization and bacteremia.

KEYWORDS

neonatal sepsis, preterm, neonatal bacteremia, conjunctivitis, conjunctival swab, conjunctival culture, eye discharge

Introduction

Conjunctivitis is a serious infection affecting up to 5% of neonates in the neonatal intensive care unit (NICU) (1), with the incidence of pathogenic bacteria in the conjunctivae increasing to 14.5% in surveillance studies (2). Hospitalized preterm infants are at a particularly high risk due to their naive immune system, immature lacrimal duct systems, prolonged hospitalization, respiratory support, invasive procedures, and colonization of conjunctivae by respiratory organisms during neonatal care (1). Conjunctival discharge is a common finding in preterm infants, particularly in the NICU. Although it can be caused by non-infectious factors like irritation or lacrimal duct blockage, it often indicates conjunctivitis when accompanied by erythema or eye edema (3). However, physical findings may not always be present in preterm infants, potentially leading to under-diagnosis of clinically significant conjunctivitis in this vulnerable population (2). Furthermore, the National Nosocomial Infection Surveillance (NNIS) definition of conjunctivitis may miss up to 38% of clinical hospital-acquired conjunctivitis in neonates (1). Treatment often involves conservative or empirical use of topical antimicrobial agents, especially if other conjunctivitis signs are absent or culture grows polymorphic or skin flora such as coagulase-negative staphylococci (CoNS). Some neonatologists may also administer topical and, in some instances, systemic antimicrobials regardless of systemic infection signs.

Whether considered colonization or active infection, the pathogens isolated could serve as a potential source of sepsis or bacteremia in vulnerable preterm and low birth weight infants, particularly those on non-invasive ventilation, and may also result in life-threatening systemic infections. Many of these hospital-acquired bacteria, such as *Escherichia coli* (*E. coli*), are invasive pathogens resistant to antimicrobials (1, 2, 4–8). As a result, some studies recommend collecting conjunctival samples for surveillance culture studies in the NICU to eradicate these pathogens and prevent sepsis or horizontal transmission of infection (9).

The association between neonatal conjunctivitis or conjunctival discharge and LOS in hospitalized preterm infants has only been reported in only a limited number of literature sources (5, 9–11). Despite the recognized significance of conjunctivitis in NICU settings, there remains a notable gap in understanding its direct relationship with LOS in hospitalized preterm infants. This deficiency in the current literature highlights the necessity of our investigation. Through this retrospective cohort study, we aimed to investigate the relationship between purulent conjunctival discharge and LOS in hospitalized preterm infants. We hypothesized that preterm infants with positive conjunctival swab (CS) cultures were more likely to develop LOS than those with negative culture outcomes.

Methods

Study population

We conducted this retrospective cohort study at the Women's Wellness and Research Center (WWRC), formerly the Women's Hospital, part of Hamad Medical Corporation (HMC) in Doha, Qatar. We searched the Microbiology Laboratory database to identify preterm neonates ≤34 weeks who had undergone CS cultures for conjunctivitis between January 2015 and December 2019. These infants are typically admitted to the NICU and often experience prolonged hospital stays. The study group was defined as preterm infants ≤34 weeks of gestation with positive conjunctival swab cultures (CSP). In contrast, the control group includes those with negative conjunctival swab cultures (CSN). Our comparison aimed to elucidate the relationship between CSP and the development of LOS in these hospitalized preterm infants within 120 h of obtaining a CS culture. The 120-hour time frame was chosen as blood cultures may require up to 5 days (or 120 h) to exhibit growth of organisms.

We excluded neonates under the following conditions: positive blood cultures >120 h of obtaining CS culture, neonates born after 34 weeks of gestation, neonates for whom a CS was not obtained, even if they were treated with local or system antimicrobials, CS cultures that grew skin flora or CoNS, CS culture obtained post antimicrobial treatment, and neonates with congenital anomalies.

The study was approved by the WWRC medical research center (MRC), the protocol number (MRC-01-20-329). Due to the retrospective nature of the study, informed consent was not required.

WWRC overview

WWRC is Qatar's largest governmental tertiary care center, with 82,860 births during the study period. Neonatal practices during the study period included using In-line closed system suctioning, shielding the eyes during suctioning in intubated infants, and sending CSs for Gram stain and bacterial culture for purulent conjunctival discharge cases. Decisions regarding

treatment were at the discretion of the physician, with topical antimicrobials usually prescribed until culture results reported.

Definition

Sepsis in neonates was defined based on two primary criteria. First, neonates who had positive blood cultures (excluding contaminants) obtained within 120 h of taking CS cultures were considered septic. Second, in the absence of a positive blood culture, sepsis was determined by a combination of symptomatic presentation and laboratory findings. Symptomatic presentation included any of the following: new onset desaturations, temperature fluctuations, tachycardia, tachypnea, apnea, feeding intolerance, lethargy, skin mottling, and other clinical conditions deemed by the neonatologist to be suspicious of sepsis, warranting further evaluation. Additionally, laboratory indicators included a high C-reactive protein (CRP) level of ≥10 mg/L and either a platelet count of $<100 \times 10^3$/ul or an absolute neutrophil count (ANC) of $<1.5 \times 10^3$/ul.

Data collection

Our primary objective was to identify late-onset neonatal bacteremia or LOS associated with CSP. Perinatal data were collected by physicians reviewing the electronic patient record, including mothers' charts for clinical and demographic data. Microbiology data was collected from the Microbiology Laboratory's information management system. Perinatal data included birth weight (BW), gestational age (GA), gender, mode of delivery, premature rupture of membranes, chorioamnionitis, Apgar scores, and Group B Streptococcus status. Our secondary objective was to evaluate outcomes associated with CSP cultures. Secondary outcomes included other neonatal infections, the development of new symptoms within 120 h of obtaining CS culture, any new need or unexplained escalation of respiratory support, feeding problems, new onset apnea, death, type of respiratory support, and duration of antimicrobial exposures. Neonatal data were collected from birth and included results of infection markers (WBC counts and CRP), conjunctival swab culture timing and results, blood cultures, and local and systemic antibiotic treatment, as well as clinical outcomes such as type of respiratory support, and feeding problems. We also collected the results of respiratory cultures if done within 2 weeks of CS cultures to determine the association or source of conjunctival bacterial organisms. Where available, the results of respiratory cultures from ventilated babies via tracheal aspirates were collected. The microbiological results were correlated clinically to determine if the organism was a pathogen, colonizer, or contaminant.

Microbiologic procedures

Conjunctival specimens were taken using an M40 Transystem™ with Amie's agar gel swab (Copan Italia, Brescia, Italy). Swabs were inoculated onto blood, chocolate and MacConkey agar plates, TM (Thayer-Martin agar). Blood and chocolate agar plates were incubated at 35–37°C in 5%–10% CO2 for 24–48 h, and MacConkey agar plates were incubated at 35–37°C for 24 h. Identification and susceptibility testing of all significant organisms was performed using the BD Phoenix™ automated identification and susceptibility testing system (Beckton Dickinson, Franklin Lakes, New Jersey, U.S.A) or for fastidious organisms, using the PhMALDI-TOF mass spectrometry (Bruker, Billerica, Massachusetts, U.S.A) with manual susceptibility testing by ETEST® (bioMérieux, Marcy-l'Étoile, France).

Statistical analysis

Descriptive analyses were performed for all patient characteristics and clinical variables. Continuous variables are presented as means and standard deviations, and categorical variables are presented as numbers and percentages. The Student *t*-test, χ^2, or Fisher's exact tests were performed, as appropriate, to determine whether variables were different between the two groups (CSP vs. CSN). Logistic regression was applied to examine the associations between various exposures and LOS while controlling for potential confounders. All *p*-values presented were two-tailed, and *p*-values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS for Windows (version 22.0, IBM Corp., Armonk, NY, USA).

Results

During the 5-year study period, we identified 285 preterm infants from whom conjunctival swab cultures were obtained (**Figure 1**). However, several infants were excluded from the study after applying our inclusion criteria. Specifically, 27 infants were above the enrollment GA cutoff, 13 had contaminated CS cultures, eight had undergone a repeat CS culture post antimicrobial treatment for the same condition, and 3 had congenital anomalies. Of the 234 preterm infants analyzed, 145 (61.9%) had positive results (CSP) and 89 (38.1%) yielded negative results (CSN), as outlined in **Table 1**. On average, eye cultures were taken at 24.5 days across the cohort, with no marked differences between the two groups. Infants in the CSP group had a lower mean BW (1,111 ± 315 g) compared to those in the CSN group (1,230 ± 470 g, $p = 0.021$). They were also younger, averaging 27.9 ± 2.6 weeks gestation against 28.7 ± 3.2 weeks for the CSN group ($p = 0.032$). Furthermore, the CSP group had a lower one-minute APGAR score with 33 infants (22.7%) compared with 9 infants (10.1%) in the CSN group, $p = 0.014$). No significant differences in other baseline demographic variables were observed between the two groups.

Table 2 compares clinical and laboratory variables in preterm infants with CSP to those in the control group. CSP patients were placed on respiratory support more frequently than CSN infants (61.4% vs. 44.9%, $p = 0.01$), primarily (noninvasive ventilation (NIV) (53.8% vs. 36.0%, respectively) and invasive mechanical ventilation (8.3% vs. 9.0%), $p = 0.001$. Neonatal

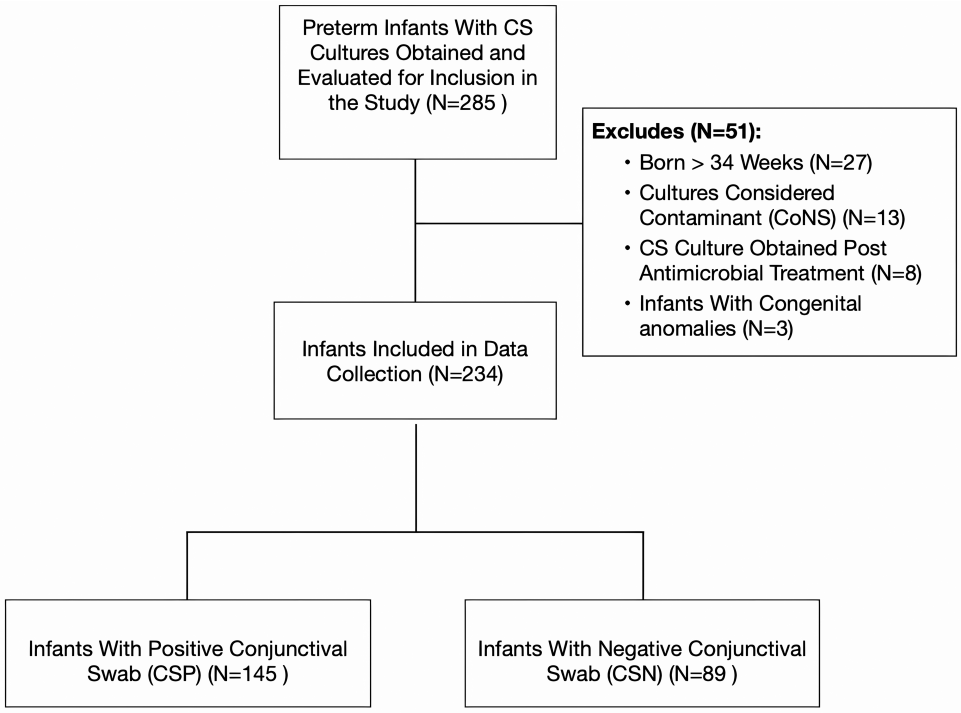

FIGURE 1
Flow diagram depicting the inclusion criteria and process of conjunctival swab culture collection. CS, conjunctival swab culture; CoNS, coagulase negative staphylococcus.

TABLE 1 Demographics of preterm infants with and without conjunctival discharge.

Variable	No growth (CSN) $N = 89$	Growth (CSP) $N = 145$	*p*-value
Males	50 (56.2%)	73 (50.3%)	0.386
Vaginal delivery	31 (34.8%)	54 (37.2%)	0.710
PROM	19 (21.3%)	34 (23.4%)	0.709
Apgar 1 min <5	9 (10.1%)	33 (22.7%)	0.014
Apgar 5 min <5	2 (2.2%)	2 (1.4%)	0.636
GA (w)	28.7 ± 3.25	27.9 ± 2.58	0.032
BW (g)	1,230 ± 470	1,111 ± 315	0.021
Eye culture day of life	22.4 ± 19.4	25.7 ± 30.4	0.345
Positive GBS status	9 (14.3%)	19 (15.1%)	0.885
Chorioamnionitis	11 (12.4%)	9 (6.2%)	0.102

GBS, group B streptococci; PROM, premature rupture of membranes; GA, gestational age; BW, birth weight.
Data are *n* (%) or mean (standard deviation) unless otherwise specified, with statistical significance. defined as $P < 0.05$.

sequential organ failure assessment (nSOFA) was higher in the CSP group (1.64 ± 3.67 vs. 0.13 ± 0.60), $p < 0.001$. Both study and control subjects received topical and systemic antibiotics frequently (97.9% vs. 87.6%, $p = 0.001$, and 38.2% vs. 26.1%, $p = 0.059$, respectively). However, the CSP group had a longer mean duration of antibiotics (topical; 8.9 vs. 5.2 days, $p < 0.001$, systemic; 4.1 vs. 1.7 days, $p < 0.001$, respectively). NIV was used by nearly half of all infants (47%), compared to 8.1% on mechanical ventilation and 44.9% on room air. Blood cultures were collected from 41% of all patients, with a higher rate observed in CSP patients (47.5%) compared to CSN patients (33.3%) ($p = 0.009$). Among the CS cultures collected, 7 (10.1%) from the CSP group tested positive, compared to three (11.1%) from the CSN group ($p = 0.714$). In the CSP group, bacteremia with identical CS organisms was identified in 4 patients, constituting 57% of that group. Similarly, LOS was diagnosed in 9 CSP patients (6.2%) compared to 3 (3.4%) CSN patients ($p = 0.543$). LOS was not shown to be a likely outcome in infants with CSP (OR, 1.89 (95% CI, 0.5–7.2, $p = 0.35$). Of the 9 patients who had respiratory cultures performed within 2 weeks of obtaining a CS culture, 5 in the CSP group (5/6 (83%) had the same organisms as in CS cultures.

After excluding samples with normal skin flora, **Figure 2** lists the isolated 174 organisms from CS cultures sent to 145 CSP patients. Gram-negative organisms accounted for 70% of CS culture results, with gram-positive organisms accounting for the remaining 30%. *Staphylococcus aureus* was the most commonly isolated organism (25%), followed by *Klebsiella pneumoniae* (17%), *Pseudomonas aeruginosa* (15%), *Serratia marcescens* (11%), *E. coli* (10%), *Acinetobacter baumannii* (5%), and *Enterobacter cloacae* (5%). Other Gram-negative bacteria (7%), *Staphylococcus aureus* (3%), and other Gram-positive bacteria (2%). There were 16 resistant organisms (9.2%) among those tested, including 7 extended spectrum beta-lactamase (ESBL) producing *E coli* (43.7%), 4 methicillin-resistant *staphylococcus aureus* (MRSA) (25%), 3 ESBL *Klebsiella pneumoniae* (18.8%), and 2 multi-drug resistant *Pseudomonas aeruginosa* (12.5%). Furthermore, 29 patients had more than one bacterial isolates.

TABLE 2 Clinical and laboratory characteristics of preterm infants with and without conjunctival discharge.

Variable	No growth (CSN) $N = 89$	Growth (CSP) $N = 145$	*p*-value
New-onset symptoms[a]	19 (21.3%)	45 (31.0%)	0.107
On respiratory support	40 (44.9%)	89 (61.4%)	0.010
Type of respiratory support			0.025
Room air	49 (55.1%)	55 (37.9%)	
Non-invasive	32 (36.0%)	78 (53.8%)	
Invasive	8 (9.0%)	12 (8.3%)	
CRP > 10 mg/L	9 (10.1%)	18 (12.4%)	0.593
Thrombocytopenia	3 (5.2%)	5 (5.0%)	0.999
Low ANC[b]	3 (5.1%)	13 (11.3%)	0.179
nSOFA, score	0.13 ± 0.60	1.64 ± 3.67	<0.001
Topical antibiotics	78 (87.6%)	142 (97.9%)	0.001
Duration of topical antibiotics, days	5.2 ± 3.17	8.9 ± 3.62	<0.001
Systemic antibiotics	23 (26.1%)	55 (38.2%)	0.059
Duration of systemic antibiotics, days	1.69 ± 3.9	4.12 ± 4.5	<0.001
Blood culture obtained	27 (30.3%)	69 (47.6%)	0.009
Bacteremia, any[d]	3 (11.1%)	7 (10.1%)	0.714
Sepsis, any	3.3 (3.4%)	9 (6.2%)	0.543
Respiratory culture sent[c]	3 (3.4%)	6 (4.1%)	0.999

Data are *n* (%) or mean (standard deviation) unless otherwise specified, with statistical significance. defined as $P < 0.05$.
[a]New-onset symptoms, CRP, C-reactive protein; ANC, absolute neutrophil count; nSOFA, neonatal sequential organ failure assessment.
[b]Low ANC, <1,500/ul.
[c]Respiratory cultures were obtained only in intubated neonates.
[d]Of blood cultures obtained within 120 h in each group.

Table 3 presents the organisms identified in both CS and blood cultures of four preterm infants, each exhibiting unique clinical features. A male infant born at 31 weeks, weighing 1,920 grams, developed sepsis due to Serratia marcescens on the 10th day, with the organism identified in CS three days prior. This infant was managed with topical gentamicin sulfate and systemic meropenem. Another male, born at 29 weeks and weighing 960 grams, showed Staphylococcus aureus in his blood culture on day 19, two days following its detection in CS, presenting symptoms like abdominal distension and cellulitis. A female infant born at 31 weeks, weighing 1,440 grams, had the same bacterium identified in her blood culture on day 10, three days prior to its appearance in CS. Treatments for these two infants included gentamicin sulfate, cloxacillin, fusidic acid, and vancomycin. Lastly, a 24-week-old female, weighing 790 grams, exhibited Escherichia coli in her bloodstream on day 1, a day subsequent to its identification in CS. Her treatment comprised gentamicin sulfate and ampicillin, reflecting her mother's cervical infection and her own severe respiratory distress.

Using univariate logistic regression analysis, we sought to identify predictors of LOS. Among various significant neonatal and clinical parameters, including CSP, only GA, (marginally significant), the requirement for any form of respiratory support, and invasive ventilation emerged as significant predictors of LOS (Table 4). Subsequently, a multivariate regression analysis, which accounted for both GA and CSP, indicated that infants developing LOS had a higher likelihood of needing invasive respiratory support [adjusted odds ratio (aOR) of 33.5; 95% CI, 2.52–446.5; $p = 0.008$]. The need for continuous positive airway pressure (CPAP) also showed an independent, albeit borderline, association with LOS (aOR, 10.5; 95% CI, 0.94–116.6; $p = 0.056$).

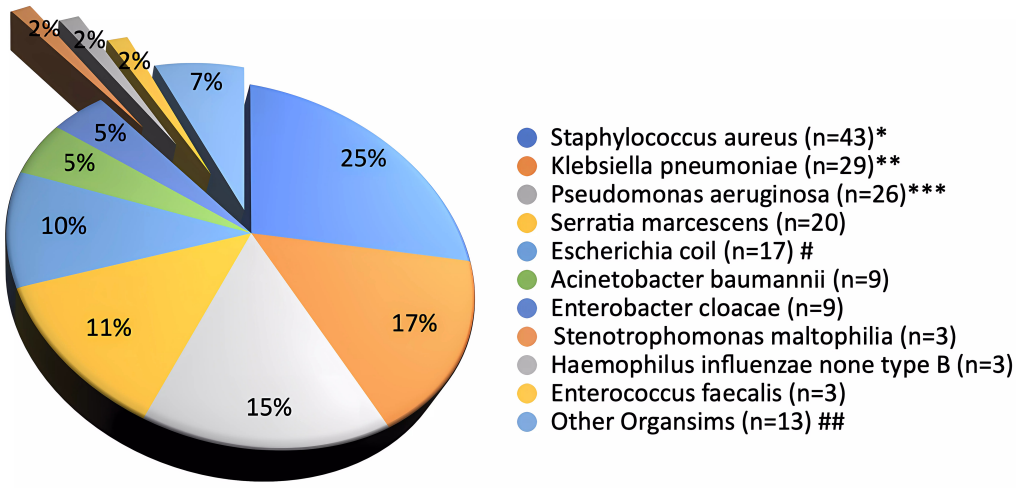

FIGURE 2
Conjunctival swab culture organisms ($N = 145$ patients and 174 cultures^).
^Cultures grow ≥ two organisms (N=29)
*Methicillin-Resistant Staphylococcus aureus (N=4/43)
**Klebsiella pneumoniae with Extended Spectrum Beta-Lactamase activity (ESBL) (N=3/29)
***Multidrug-Resistant Organisms, P. aeruginosa (N=2/26)
#ESBL Escherichia coli (N=7/17)
##Other organisms: Serratia ureilytica (n=2 (1%)), Streptococcus anginosus (n=2 (1.1%)), Group B Streptococcus (n=2 (1.1%)), Citrobacter koseri (n=2 (1.1%)), Streptococcus pneumoniae (n=1 (0.6%)), Pseudomonas stutzeri (n=2 (0.6%)), Proteus mirabilis (n=2 (1%)), Gemella haemolysans (n=2 (0.6%)), and Morganella morganii (n=2 (0.6%0).

TABLE 3 Clinical features and organisms identified in both CS and blood cultures of four preterm infants.

Organism	GA (wks)	BW (g)	Sex	CS culture day	Blood culture day	Associated symptoms	Respiratory Support	CRP	Topical Antibiotics	Systemic antibiotics
Serratia marcescens	31	1,920	Male	10	13	Tachypnea	High flow nasal cannula	8	Gentamicin sulfate for 5 days	Meropenem 10 days
Staphylococcus aureus	29	960	Male	17	19	Abdominal distention, cellulitis and feeding intolerance 3 days post eye discharge	Room Air	5	Gentamicin sulfate for 7 days	Cloxacillin 7 days
	31	1,440	Female	13	10	Fair condition	High flow nasal cannula	6	Fusidic acid for 5 days	Vancomycin 4 days then Cloxacillin 14 days
Escherichia coli	24	790	Female	2	1	Mother with cervical cerclage, cervical culture grew Escherichia coli. Baby with RDS and IVH grade II	Mechanical ventilation	68	Gentamicin sulfate for 7 days	Ampicillin 10 days

CS, conjunctival culture; GA, gestational age; BW, birth weight; RDS, respiratory distress syndrome; IVH, intraventricular hemorrhage; CRP, C-reactive protein.

TABLE 4 Univariate and multivariate logistic regression analysis of predictors associated with late-onset neonatal sepsis.

Univariate logistic regression[a]: significant covariates associated with sepsis				
	p	OR	95% C.I.	
			Lower	Upper
CSP	0.087	1.71	0.93	3.17
GA	0.052	0.79	0.64	1.00
Any respiratory support*	0.032	9.52	1.21	75.00
Invasive MV	0.002	6.48	2.03	20.71
Multivariate logistic regression[b]: significant covariates on associated with sepsis				
	p	OR	95% C.I.	
			Lower	Upper
CPAP	0.056	10.46	0.94	116.56
Invasive MV	0.008	33.55	2.52	446.49

CSP, conjunctival swab positive; GA, gestational age; aOR, adjusted odds ratio; C.I; confidence interval.
*Includes high and low flow nasal cannula; CPAP, continuous positive airway pressure; nasal intermittent ventilation; MV, mechanical ventilation.
[a]Individual variables were analyzed, and those found to be statistically insignificant ($p > 0.1$) were subsequently excluded from the analysis. These variables include gender, delivery mode, APGAR scores at 1 and 5 min, gestational age (GA), duration of membrane rupture, CS culture day, Group B Streptococcus status, chorioamnionitis, and multiple gestation.
[b]Controlled for GA and CSP.

Discussion

Purulent conjunctival discharge in preterm infants may pose a clinical quandary in terms of approach and relationship to the development of sepsis, as it may indicate an underlying infection. The high prevalence of positive results for CSP among the analyzed preterm infants, accounting for nearly 62%, indeed warrants a closer examination of the clinical implications and underscores the importance of heightened clinical vigilance in the NICU setting. This finding highlights the significance of conjunctival discharge as a potential reservoir for bacterial pathogens in these vulnerable neonates especially those on respiratory support. Despite the practice of covering the eyes with multilayer gauze during mouth and airway suctioning throughout the study years, a significant number of patients required evaluation for eye discharge, as indicated by the study. Among the patients, blood cultures were obtained in 41.5%, while CRP and complete blood count tests were conducted for 60% within 5 days of the onset of eye discharge. Furthermore, topical antibiotics were prescribed and continued in the vast majority of cases, and systemic antibiotics were initiated in one-third of all cases while awaiting the results of CS cultures and continued in a significant number of CSP patients, raising clinical concerns regarding effective antimicrobial stewardship in the NICU. It should be noted, however, that the level of compliance with the use of multilayer gauze during suctioning is not known. Therefore, there could be an increased risk of exposure to pathogens from the respiratory secretions, potentially contributing to a higher incidence of purulent eye discharge. The presence of new-onset symptoms associated with eye discharge, on the other hand, was not a statistically significant finding.

Although *Staphylococcus aureus* was the most commonly isolated organism, Gram-negative organisms overall made up more than two-thirds of the isolated organisms, particularly *Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens, and E. coli*. Furthermore, resistant strains accounted for approximately 9% of the organisms in the entire cohort. The findings of this bacterial pattern in our study, especially the predominance of Staphylococcus spp., are consistent with published surveillance and clinical reports on conjunctivitis in neonates admitted to the NICU (1, 12–14), where the most common pathogens were coagulase-negative staphylococci (25%), Staphylococcus aureus (19%), and Klebsiella spp. (10%).

According to the findings, risk factors for conjunctivitis include low BW and younger GA, a low 1-minute APGAR score, and NIV (**Tables 2**, **3**). Consistent with other studies' findings, these infants are typically the ones who require extended periods of respiratory support and recurrent ophthalmologic evaluations (1). However, as the study indicates, we were unable to demonstrate a significant independent correlation between LOS and conjunctival discharge in these infants, implying that conjunctival discharge may not be a reliable predictor of LOS in premature infants admitted to the NICU. However, as the study suggests, among other variables in

these patients, sepsis is primarily associated with invasive ventilation, whereas NIV is insignificant.

As previously stated, the study found a correlation between conjunctival discharge in hospitalized preterm infants and respiratory support, namely NIV. Respiratory secretions may be transferred from the nasopharynx to the eyes during suctioning, which is required for infants who require ventilatory assistance (1). In a study of a *P. aeruginosa* conjunctivitis outbreak in a pediatric hospital, 70% of patients with respiratory cultures obtained before the onset of conjunctivitis were colonized with this organism (15). This suggests that respiratory secretions could be the source of infection in these case. However, it is important to note that surveillance CS cultures are rarely performed in most NICUs nowadays because positive culture results in these infants have always been considered colonization from the respiratory tract if not associated with other clinical manifestations of conjunctivitis. As a result, these surveillance cultures are regarded as having little utility in the diagnosis of conjunctivitis (2). Furthermore, many cases of conjunctivitis in hospitalized preterm infants fail to meet the NNIS definition, partly due to clinicians' attitude toward eye discharge and the tendency to begin empiric treatment with or without sending CS cultures, and partly due to the absence of other clinical manifestations in preterm infants, which causes the NNIS to underestimate a significant number of cases.

Due to the retrospective nature of our study, the reliability of electronic patient records may present challenges. Potential issues include data entry errors, incomplete or inconsistent records, data loss during system upgrades, and biases in data recording. Additionally, older non-digitized records may be omitted, affecting the dataset's comprehensiveness and accuracy. It's crucial to consider these factors when interpreting the findings. Moreover, while conjunctival discharge is a recognized sign of conjunctivitis in preterm patients, there were inconsistencies in how other clinical signs were documented, complicating retrospective data retrieval. In this study, the choice to perform blood cultures in the face of conjunctival discharge varied by physician, reflecting the absence of well-established guidelines on this issue. Distinguishing between colonization and genuine conjunctivitis presents another challenge that could affect our conclusions. Additionally, sole reliance on the Microbiology Laboratory database also brings its own set of biases related to sample collection, culturing, and automated system limitations. Cross-referencing clinical data and using diverse growth mediums is crucial. Notably, while invasive ventilation is found to be a risk factor for LOS in those infants with positive CS, in most cases we are unable to confirm the presence of the same organism in respiratory samples of positive CS cultures. Furthermore, the study was conducted in a single NICU and the findings may not be generalizable to other settings.

The study sheds light on the potential relationship between the risk of LOS in ventilated preterm infants with purulent conjunctival discharge and emphasizes the importance of careful monitoring in this vulnerable population. Despite the limitations and the data presented, the potential role of respiratory secretions in neonatal conjunctival infection or colonization underscores the importance of employing proper techniques and stringent infection control measures during airway suctioning.

Conclusion

This data suggests that positive CS is common in hospitalized preterm infants with purulent eye discharge, particularly those on NIV. Although no statistically significant correlation was found between purulent conjunctivitis with a positive CS culture and LOS, the mere finding of a common pathogen isolated in both eye discharge and blood cultures in many cases signifies that there is indeed a need for further investigation in wider populations. Future studies with comprehensive documentation of clinical signs can enhance the generalizability of these findings. Such studies could further explore the relationship between purulent conjunctival discharge and LOS in ventilated preterm infants. The findings could potentially contribute to the development of evidence-based guidelines for managing conjunctival discharge in this population. Furthermore, these studies may emphasize the importance of judicious antibiotic prescribing practices in the NICU. Additionally, research focused on evaluating the effectiveness of prophylactic measures, such as eye care, in reducing the incidence of LOS in ventilated premature infants with conjunctival discharge should be considered.

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 Hamad Medical Corporation Medical Research Center (MRC). 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 due to the retrospective nature of the study.

Author contributions

AG: Conceptualization, Data curation, Methodology, Formal Analysis, Project administration, Supervision, Validation, Visualization, Writing – original draft. AK: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. MH: Data curation, Methodology. PC: Data curation, Formal Analysis, Methodology, Software, Visualization. AS: Data curation, Methodology. AR: Data curation, Methodology, MI: Data curation, Methodology. FA: Data curation, Methodology, Writing – review & editing. TA: Data curation, Investigation, Methodology. MH: Data curation, Investigation, Supervision, Methodology, Visualization, Resources, Writing – review & editing. MB: Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation,

Visualization. HP: Data curation, Investigation, Supervision, Methodology, Visualization, Resources, Writing – review & editing.

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Conflict of interest

No competing interests. All the authors are employed by Hamad Medical Corporation. 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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Detection of volatile organic compounds in headspace of *Klebsiella pneumoniae* and *Klebsiella oxytoca* colonies

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Introduction: Early diagnosis of infections and sepsis is essential as adequate therapy improves the outcome. Unfortunately, current diagnostics are invasive and time-consuming, making diagnosis difficult, especially in neonatology. Novel non-invasive analytical methods might be suitable to detect an infection at an early stage and might even allow identification of the pathogen. Our aim is to identify specific profiles of volatile organic compounds (VOCs) of bacterial species.

Methods: Using multicapillary column-coupled ion mobility spectrometry (MCC/IMS), we performed headspace measurements of bacterial cultures from skin and anal swabs of premature infants obtained during weekly screening for bacterial colonization according to KRINKO. We analyzed 25 *Klebsiella pneumoniae* (KP) cultures on MacConkey (MC) agar plates, 25 *Klebsiella oxytoca* (KO) cultures on MC agar and 25 bare MC agar plates as a control group.

Results: Using MCC/IMS, we identified a total of 159 VOC peaks. 85 peaks allowed discriminating KP and bare MC agar plates, and 51 peaks comparing KO and bare MC agar plates and 6 peaks between KP and KO *(*significance level of $p < 0.05$ after *Bonferroni post hoc analysis)*, respectively. Peaks P51 (*n-Decane*) and P158 (Phenylethyl Alcohol), showed the best sensitivity/specificity/ positive predictive value/negative predictive value of 99.9% each ($p < 0.001$) for KP. P158 showed the best sensitivity/specificity/positive predictive value/negative predictive value of 99.9% each ($p < 0.001$) for KO. Comparing KP and KO, best differentiation was enabled using peaks P72, P97 and P16 with sensitivity/specificity/positive predictive value/negative predictive value of 76.0%, 84.0%, 82.6%, 77.8%, respectively ($p < 0.05$).

Abbreviations

CI, confidential interval; DT, decision tree; IMS, ion mobility spectrometry; KO, *Klebsiella oxytoca*; KP, *Klebsiella pneumoniae*; MC, MacConkey; MCC, multi-capillary column; MCC/IMS, ion mobility spectrometer coupled to multi-capillary column; NICU, neonatal intensive care unit; P, peak; PFA, perfluoroalkoxyalkane; VOCs, volatile organic compounds.

Discussion: We developed a method for the analysis of VOC profiles of bacteria. Using MCC/IMS, we demonstrated that VOCs derived from bacteria are clearly distinguishable from a bare agar plate. Characteristic peaks obtained by MCC/IMS are particularly suitable for the species-specific identification and differentiation of KP and KO. Thus, MCC/IMS might be a useful tool for *in vitro* diagnostics. Future studies must clarify whether similar patterns of VOCs can be detected *in vivo* in patients that are colonized or infected with KP or KO to enable rapid and accurate diagnosis of bacterial colonization.

KEYWORDS

volatile organic compounds, ion mobility spectrometry, premature infant, biomarkers, *Klebsiella pneumoniae*, detection, pediatrics, non-invasive diagnostics

Introduction

Prematurity includes a state of immaturity of all organ systems including the immune system. Thus, preterm infants are at high risk for nosocomial infections. Therefore, weekly colonization screening is recommended by German Commission on Hospital Hygiene and Infection Protection (KRINKO) at the Robert Koch Institute (RKI) since 2013 as bloodstream infections and bacterial colonization pose a significant risk of morbidity and mortality for preterm infants (1). As infections of preterm infants often manifest with nonspecific symptoms, diagnosis is difficult and the time window to achieve adequate diagnosis and treatment is short. The existing diagnostic tests require painful procedures and are often linked to high costs and to limited sensitivity. Presently, acute inflammatory markers provide limited sensitivity, especially at the onset of an infection. Time consuming laboratory tests can delay adequate treatment for hours triggering empiric antibiotic treatment before knowing the laboratory results. A fast and real-time diagnostic tool could reduce unnecessary exposure to antibiotics in suspected, but unconfirmed sepsis. Rapid diagnosis and prompt initiation of therapy significantly improves outcome (2–4).

The analysis of volatile organic compounds (VOCs) represents an innovative approach for non-invasive diagnostics (5). VOCs can be produced by the host or by microbes and are emitted via body secretions or breath. Alterations in specific VOC profiles are linked to perinatal or neonatal diseases (6). Previous studies revealed the high potential of VOC analysis and its application for non-invasive diagnostics (6–9). We have developed a method using ion mobility spectrometer coupled to multi capillary columns (MCC/IMS) to measure VOC profiles and to assign individual VOCs to biochemical markers using a reference data set (BS-MCC/IMS-analyses database). Using MCC/IMS, characteristic VOC profiles can be detected in the incubator atmosphere of neonates (10). Moreover, using VOC analysis via MCC/IMS, we found that 5-methyl pentane as a potential biomarker for chorioamnionitis, a common cause of prematurity (11).

Bacteria can also produce VOCs; some VOCs originate exclusively from certain bacterial species, and analysis of VOCs profiles enables distinguishing between several bacterial species (9, 12, 13). Also, there are changes in VOCs profiles of neonates suffering from late-onset sepsis (14). Common healthcare-associated pathogens are Gram-negative bacteria such as *Klebsiella pneumoniae* (KP) and *Klebsiella oxytoca* (KO). The detection of KP is of the utmost importance as it can cause neonatal infections and sepsis, which may be difficult to treat due to the prevalence of multi-drug resistant strains (15–19). Similarly, KO can acquire antibiotic resistance and cause outbreaks in neonatal intensive care units (NICU) (20–22). The occurrence of KO is linked to antibiotic associated hemorrhagic colitis (23) and might be associated with necrotizing enterocolitis (24).

The aim of this study was to establish headspace measurements of KP and KO independent from ambient air for measuring and distinguishing bacterial cultures originating from routine anal/rectal swabs of preterm infants using MCC/IMS as a novel noninvasive, rapid and precise method.

Methods

Patients

This study was performed at the Department of Pediatrics, Saarland University Medical Center, Homburg (Germany) and at the Institute of Medical Microbiology and Hygiene, Saarland University, Homburg (Germany) after approval by the Ethical committee Saarland (reference 276/17) from April 2021 to October 2021. All acquired data were recorded and processed in an anonymized form.

Sample collection and processing

Microbial cultures of KP and KO on MacConkey (MC) agar plates (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) originated from rectal/anal swabs of preterm infants of the NICU of the Department of Pediatrics, Saarland University Medical Center, Homburg (Germany). The swabs were taken twice a week as part of the routine screening on bacterial colonization. Swabs were transferred in eSwab™ transport medium (Copan, Brescia, Italy). In the Institute of Medical Microbiology and Hygiene, Saarland University, Homburg (Germany), swabs were spread out onto MC agar

plates. Only those agar plates with growth of KP and KO cultures were included in this study. Following a Standard operating procedure (SOP), samples were processed as follows: a part of the agar with bacterial colonies on it (size: 0.5 × 0.5 cm) was cut out and placed into a laboratory bottle.

Data assignment/headspace measurement

To perform headspace measurements of VOCs derived from bacterial colonies, we used an MCC/IMS BreathDiscovery (B & S Analytik GmbH, Dortmund, Germany). It was placed on a metal cart with a laptop computer and connected to a synthetic air supply. The pre-separation was performed using an OV-5 (5% - diphenyl, 95% - dimethylpolysiloxane) multi-capillary column (MCC) (Multichrom Ltd., Novosibirsk, Russia). The device and sampling parameters are given in **Supplementary Table 1**. The methods for VOC analysis were published earlier. A laboratory bottle (100 ml) heated to 37°C served as a sample container. A closed system was established: A large laboratory bottle (1,000 ml, gas reservoir, Schott Duran®, DURAN Group GmbH, Wertheim /Main, Germany) was connected to the small bottle (100 ml, Schott Duran®, DURAN Group GmbH, Wertheim /Main, Germany) via a perfluoroalkoxyalkane (PFA) tube that was led through the caps. Both bottles were filled with synthetic air as carrier gas. Another tube connected the cap of the small laboratory bottle and the sampling input of MCC/IMS device (**Figure 1**). Data was acquired using VOCan v3.7 (B & S Analytik GmbH, Dortmund, Germany). The standard operation conditions are used as recommended by the supplier of the instrument. IMS was regularly calibrated using standardized reference mixture ("R06" calibration liquid, B & S Analytik GmbH, Dortmund, Germany, **Supplementary Table 2**).

Statistical analysis

We evaluated the data acquired by MCC/IMS using the software VisualNow 3.7 (B & S Analytik GmbH, Dortmund, Germany). All peaks were characterized by their specific combination of retention time and drift time (corresponding 1 / K_0-value, see **Supplementary Table 3**). The databank layer (BS-MCC/IMS-analyses database) was used for peak referencing and determination of retention times and 1/K_0-values. Here, pure analytes were measured 10 times each and comparison with parallel measurements using GC/MS standard procedures was performed (25, 26). Peak intensity (in volts) was considered as an indirect measure of compound concentration. A specific threshold was calculated for each peak and comparison. Box-and-Whisker plots and a rank sum test using Mann-Whitney-U test and Bonferroni *post hoc* analysis correction were performed. The *n*-value was set at $n = 25$ for KP, $n = 25$ for KO and $n = 25$ for bare MC agar plates. The α-level was defined to be 0.05, the *p*-value (one-tailed) was determined to be <0.05. Significant peaks [$p < 0.05$, 95% confidential interval (CI)] were used for further evaluation with decision trees (DT).

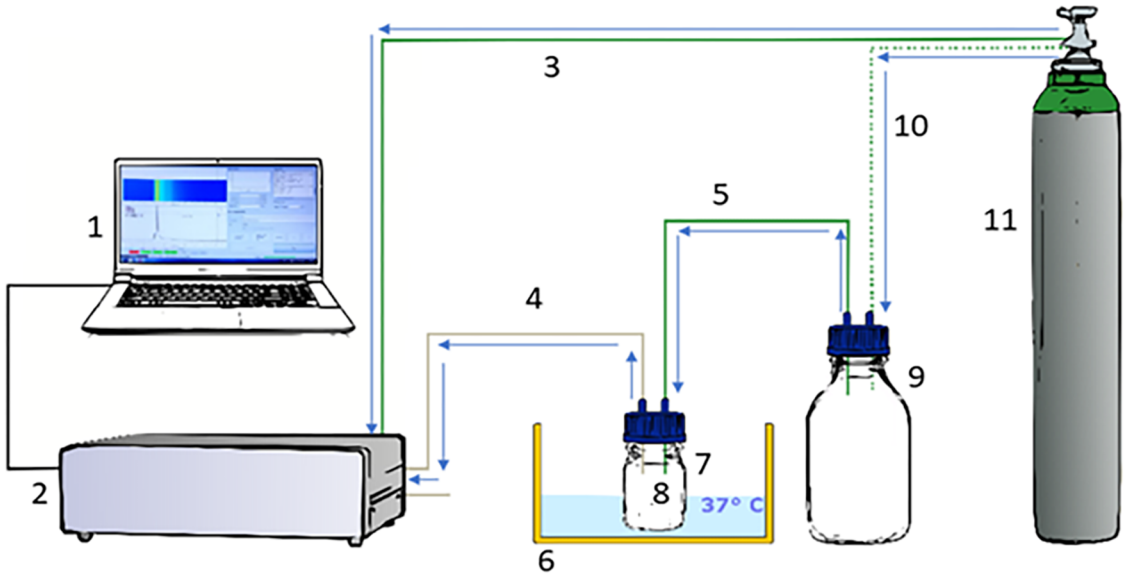

FIGURE 1
Experimental design. We used an MCC/IMS BreathDiscovery (B & S Analytik GmbH, Dortmund, Germany) to perform headspace measurements of VOCs derived from the fecal und urine samples. (1) Laptop; (2) MCC/IMS BreathDiscovery (B & S Analytik GmbH, Dortmund, Germany); (3) Connecting tube between MCC/IMS and synthetic air supply gas bottle; (4) Connecting tube between MCC/IMS and sample container; (5) Connecting tube between gas reservoir and sample container; (6) Water bath; (7) Sample container: small laboratory bottle (100 ml); (8) Diaper sample; (9) Gas reservoir: large laboratory bottle (1,000 ml); (10) Connecting tube between synthetic air supply gas bottle and gas reservoir; (11) Synthetic air supply gas bottle; PFA tubes (green) as drift gas supply; PFA tube (grey) as sample input. Data acquisition was directed and recorded using VOCan v3.7 (B & S Analytik GmbH, Dortmund, Germany).

Results

We analyzed a total of 75 agar plates with KP ($n = 25$) or KO ($n = 25$) and bare MC agar plates ($n = 25$), respectively. We identified 159 signals (peaks) from each comparison such as 85 peaks between bare MC agar plates and KP, 51 peaks between bare MC agar plates and KO and 6 between KP and KO, respectively. We found 148 peaks exclusively assigned to KO or KP and 11 peaks exclusively assigned to bare MC agar plates. 17 peaks occurred in all three groups (MC/KO/KP) and did not differ significantly between the individual groups. When comparing KP and bare MC agar plates, peak P51 and P158 showed the highest sensitivity, specificity, positive and negative predictive value after Bonferroni *post hoc* analysis correction (sensitivity and specificity 99.9%, respectively, $p < 0.001$) for KP. Comparing KO and bare MC agar plates peak P158 reaching the highest sensitivity, specificity, positive and negative predictive value of 99.9% each in a significance level with $p < 0.001$ (**Figure 2**). Regarding the comparison between KO and KP, best differentiation was enabled using peaks P72, P97 and P16 with sensitivity, specificity and positive and negative predictive value of 76.0%, 84.0%, 82.6%, 77.8%, respectively in a significance level with $p < 0.05$ after Bonferroni *post hoc* analysis correction (**Table 1**).

We established a decision tree using three peaks that enabled the differentiation of KP, KO and bare MC agar plates regarding their signal intensities. The peak P158 allows to differentiate between bare MC agar plates (with a signal intensity ≤0.011 V), KP and KO (with a signal intensity >0.011 V), respectively. Regarding signal intensities >0.487 V, KP is identified via P108. The Peak P22 enables further differentiation regarding a signal intensity ≤0.487 V: a signal intensity ≤0.010 V stands for KO and a signal intensity >0.005 V for KP (**Figure 3**).

Using our standardized database (B. Braun Melsungen-Database/BS-MCC/IMS-analyses database), two peaks were assigned to biochemical substances: P51 was assigned to *n-Decane* (CAS number 124-18-5) and P158 to Phenylethyl Alcohol (CAS number 60-12-8).

TABLE 1 Statistical analyses for the model set and validation set for peaks P158 and P51.

	P158			P51		
Best direction	**KP > MC**	**KO > MC**	**KO > KP**	**KP > MC**	**KP > KO**	**KO > MC**
Best threshold	0.012	0.015	0.082	0.008	0.017	0.007
Classified right	50	50	35	50	31	49
Classified wrong	0	0	15	0	19	1
True positive	25	25	23	25	19	25
False positive	0	0	13	0	13	1
True negative	25	25	12	25	12	24
False negative	0	0	2	0	6	0
Sensitivity [%]	99.9	99.9	92	99.9	76	99.9
Specificity [%]	99.9	99.9	48	99.9	48	96
Positive predictive value [%]	99.9	99.9	63	99.9	59	96.2
Negative predictive value [%]	99.9	99.9	86	99.9	67	99.9
Accuracy [%]	100	70	70	100	62	98
Significance level (Mann Whitney *U*)	<0.001	<0.001	/	<0.001		<0.001
Significance level (Bonferroni correction)	<0.001	<0.001	/	<0.001		<0.001

Discussion

Premature babies show an increased susceptibility to nosocomial infections. Gold standard to verify bacterial bloodstream infections are blood cultures which yield results 24–48 h or even later (27–29). Rapid diagnosis and prompt initiation of therapy significantly improves outcome (2–4). In the present study we showed that KP and KO can be distinguished based on their VOC profile using MCC/IMS. As electronic nose devices detect patterns of VOC profiles, we chose MCC/IMS to precisely identify substances. In our study, we identified two signals that could be assigned to substances using a database (B. Braun Melsungen-Database/GC/MS-MCC/IMS-analyses database):

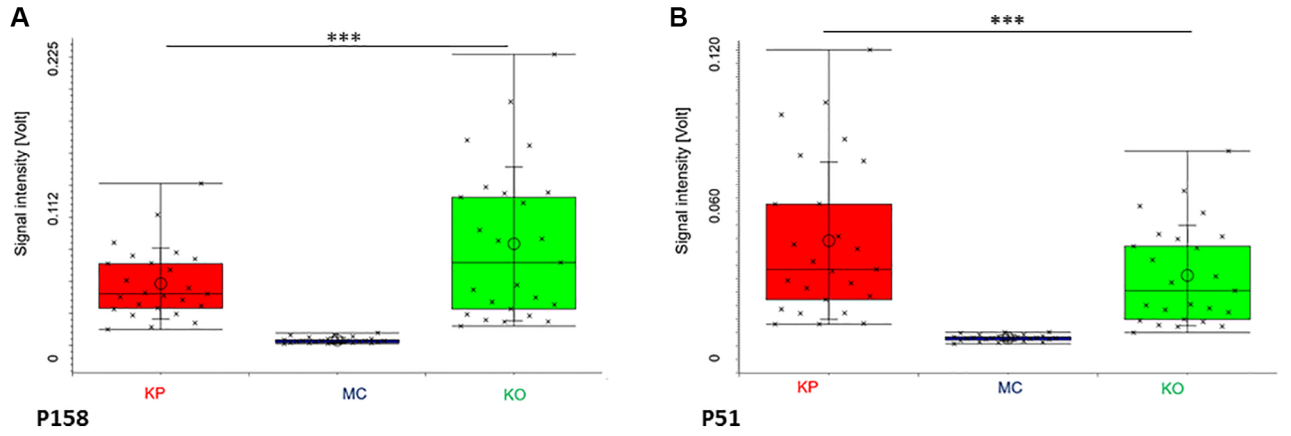

FIGURE 2
Box-and-whisker-Plot of the peaks P158 (**A**) and P51 (**B**). After Bonferroni *post hoc* analysis correction, Peaks P51 and P158 showed the highest sensitivity, specificity, positive and negative predictive value with 99.9% each in a significance level with $p < 0.001$ for *K. pneumoniae* (KP) when comparing KP and bare MacConkey agar plates (MC). Comparing *K. oxytoca* (KO) and MC P158 reaching the highest sensitivity, specificity, positive and negative predictive value of 99.9% each in a significance level with $p < 0.001$.

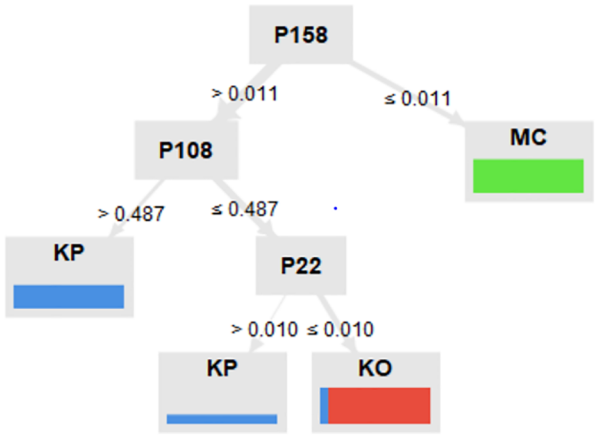

FIGURE 3
Decision tree. A total of three peaks enabled the differentiation of *K. pneumoniae* (KP), *K. oxytoca* (KO) and bare MacConkey agar plates (MC) regarding their signal intensities. With a signal intensity ≤0.011 V, P158 allows to differentiate between bare MC, KP and KO, respectively. At a signal intensitiy >0.487 V, KP is identified via P108. Peak P22 enables further differentiation regarding a signal intensity ≤0.487 V: a signal intensity ≤0.010 V represents KO and a signal intensity >0.005 V represents KP.

n-Decane and Phenylethyl Alcohol. Phenylethyl Alcohol is considered a metabolite in the Ehrlich Pathway and might derive from *Klebsiella* spp. Our future aim is to enable early diagnosis of neonatal infections by means of alterations in the VOC profile of neonates suffering from blood stream infections to be able to initiate quickly a targeted antibiotic therapy. Therefore, in the first step, we created the bacteria-specific VOC profiles using MCC-IMS in agar plates deriving from routine swabs. The next step will be the analysis of skin swabs themselves without any cultivation to differentiate KO/KP swabs from commensal bacteria – later we plan to perform measurement directly inside the incubator. As bacterial colonization is a risk factor for invasive infections, it would be favorable to quickly know about the respective colonization status without performing potential stressful swabs. If this is successful, we can focus on detecting infections in future studies.

VOC detection studies showed the great potential of VOC analysis with a wide array of techniques like breath analysis, headspace measurement of bio samples and the measurement of incubators atmosphere (7, 8, 10, 30). VOC analysis can be performed by several methods, e.g., gas chromatography coupled with mass spectrometry (GC-MS), gas chromatography time of flight- mass spectrometry (GC-ToF-MS), selected ion flow tube-mass spectrometry (SIFT-MS) and ion molecule reaction mass spectrometry (IMR-MS). Obtaining time series every 15 min is an advantage of MCC/IMS, especially in comparison to different mass spectrometric methods. The moisture content (e.g., exhaled breath samples) is a major limitation for most analytical methods, but not for MCC/IMS. On the other hand, bed side and on-site applications of MCC/IMS were reported since 2017 as medical products based on MCC/IMS technology were introduced into the market. Electronic nose devices using sensor technologies are based on pattern recognition and measurements can be conducted easily and at the bedside. As those devices do not allow qualitative VOC analysis, we decided to use an ion mobility spectrometer coupled to multi capillary columns (MCC/IMS) allowing the measurement of VOC profiles and the specification of individual VOCs using a reference data set. MCC/IMS analyses provide results within a few minutes and could therefore be used for instant bedside diagnostics. Electronic noses have been used to distinguish several bacterial strains from each other *in vitro* (9). *P. aeruginosa* was detected in human sputum and in culture via gas chromatography-mass spectrometry (GC-MS) (31). Using MCC/IMS Junger et al. showed it was also possible to distinguish individual bacterial strains based on their VOCs in pure cultures (13). We conclude that treatment and diagnostics on NICU are often linked to invasive and time-consuming procedures. Establishing a noninvasive, painless method by VOC analysis would be best for vulnerable patients like preterm infants. We were the first study to use routine swabs from preterm infants and to analyze grown pathogens on agar plates. We analyzed grown agar plates, but also empty agar plates for adjustment. Incubation of inoculated agar plates took place in microbiological incubators. In preliminary work, we also incubated empty agar plates to rule out influences of the incubator storage on the VOC profile. Further strengths of our study are that our measurements can be conducted quickly within a few minutes, even bedside diagnostics are feasible (10). MCC/IMS allows to detect substances with extremely low concentrations (pg/L) (5, 32) which enables detecting weak alterations in the VOC profile. However, due to the high sensitivity of the MCC/IMS, there is a high risk that ethanol and other environmental VOCs will interfere with the detection of VOCs that would be important for detection purposes, including bacteria. Our method uses a closed system to avoid influences by ambient air and other environmental influences. For this method, especially when developed further so it can be run without the need for an overnight culture, we would not need further consumables besides the costs for the synthetic air. Thus, this approach has the potential to be cost-effective and ecologically sustainable. Nevertheless, the present study was performed under laboratory conditions which may differ from the clinical setting. An inconvenience of our study is the measurement of bacterial colonies (KP/KO) on agar plates. The cultivation of the bacteria still takes time. For this reason, additional studies are planned to allow direct analyses of standard swabs taken from preterm infants. At this point, it is unclear if we can distinguish bacterial colonization from invasive infections via VOC analysis, these conditions may differ regarding their VOC profile based on different inflammatory responses. As bacterial colonies originate from swabs, there is an uncertainty about pure cultures of bacteria. A higher number of swab samples and measurements must be considered. *Klebsiella* spp. are a common cause of neonatal sepsis and they frequently carry resistance genes that render them multidrug-resistant (33). Currently, antibiotic susceptibility testing using electronic nose devices is still unproven. Hence, bacterial culture linked to antibiotic susceptibility testing currently remains gold standard. Under clinical conditions, influences like oral hygiene, food habits/

nutrition, age and gender were found to influence VOC profile. Nevertheless, environmental influences could be reduced following standard operating procedures.

We showed that KP and KO can be distinguished based on their VOC profile using MCC/IMS. We achieved a first step to provide a supportive tool that might quickly detect bacterial colonization on NICU.

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 Ethics Committee at the Saarland Medical Association AZ: 276 /17. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

MB: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript. MT: collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. CP: coordination of sample collection, manuscript writing, final approval of manuscript. EK, RW: manuscript writing, final approval of manuscript. JB: data analysis and interpretation, manuscript writing, final approval of manuscript. SB: coordination of sample collection, manuscript writing, final approval of manuscript. MZ: conception and design, data analysis and interpretation, financial support, manuscript writing, final approval of manuscript. SG-F: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript. All authors contributed to the article and approved the submitted version.

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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 handling editor HN declared a past co-authorship with the author MB, EK, RW, JB, MZ, and SF.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1151000/full#supplementary-material

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Antibiotic use at planned central line removal in reducing neonatal post-catheter removal sepsis: a systematic review and meta-analysis

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Background: Post-catheter removal sepsis (PCRS) is a notable complication of indwelling central venous catheters (CVCs) in neonates, which is postulated to be secondary to the disruption of biofilms formed along catheter tips up on CVCs removal. It remains controversial whether this could be prevented by antibiotic use upon CVCs removal. We aimed to evaluate the protective effect of antibiotic administration at the time of CVCs removal.

Methods: We searched through PubMed, EMBASE, Cochrane databases and reference lists of review articles for studies comparing the use of antibiotics versus no use within 12 h of CVCs removal. Risk of bias was assessed using the modified Newcastle-Ottawa Scale and Cochrane risk-of-bias tool accordingly. Results of quantitative analyses were presented as mean differences (MD) or odds ratio (OR). Subgroup and univariate meta-regression analyses were performed to identify heterogeneity.

Results: The review included 470 CVCs in the antibiotic group and 658 in the control group. Antibiotic use within 12 h of CVCs removal did not significantly reduce the incidence of PCRS (OR = 0.35, 95% CI: 0.08–1.53), but was associated with a lower incidence of post-catheter removal blood stream infection (OR = 0.31, 95% CI: 0.11–0.86). Dosage of vancomycin and world region were major sources of heterogeneity.

Conclusion: Antibiotic administration upon CVCs removal does not significantly reduce the incidence of PCRS but offers less post-catheter removal blood stream infection. Whether this will be converted to better clinical outcomes lacks evidential support. Further randomized controlled studies with longer follow-up are needed.

Summary: Results of our meta-analysis suggest that antibiotic use at planned central line removal removal does not significantly reduce the incidence of PCRS but offers less blood stream infection, which might contribute to future management of central lines in neonates.

Systematic Review Registration: https://www.crd.york.ac.uk/, PROSPERO (CRD42022359677).

KEYWORDS

neonate, sepsis, critical care, central venous catheters, meta-analysis

Introduction

Central venous catheters (CVCs) are commonly used in the neonatal intensive care unit (NICU), contributing to better survival outcomes in critically ill newborn infants. Post-catheter removal sepsis (PCRS) is an important complication of indwelling CVCs with an incidence reported varying from 1.9% to 11.6% (1, 2). PCRS is predominantly caused by late-onset central line-associated blood stream infection (CLABSI) which is defined as a primary blood stream infection developing within 48 h after CVCs removal in the absence of other known infection sites (3). It is hypothesized that a biofilm forms along the inserted catheter, which is disrupted and washed into blood stream at the removal of CVCs, leading to bacteremia (2, 4). CLABSI is correlated with increased morbidity and mortality, additional antibiotic use and prolonged hospitalization (5, 6). Fortunately, implementation of central-line bundles and prophylactic systemic antibiotics use in the 72 h preceding PICC removal may help to reduce the incidence of late-onset CLABSIs (7, 8). However, the relatively long exposure to antibiotics during infancy is challenged by the selection of antibiotic-resistant organisms and gut microbiome dysbiosis, and thus prophylactic antibiotics use is not recommended (8, 9). Hence, concurrent antibiotics given at the time of CVCs removal might be an alternative strategy. Inconsistent results have been reported in several interventional or observational studies, which underscores the need to perform a systematic review and meta-analysis to quantitatively evaluate whether antibiotics administration at the time of CVCs removal prevents late-onset sepsis in neonates.

Materials and methods

We performed the systematic review based on a protocol with the registration number CRD42022359677) and complied with the Preferred Reporting terms for Systematic Review and Meta-Analysis (PRISMA) statement (10). Reporting items were detailed in the PRISMA checklist (Supplementary Table S1).

The purpose of this review was to evaluate whether antibiotics administration within 12 h of planned CVCs removal can reduce the incidence of post-catheter removal sepsis in neonates.

Literature search

We searched through PubMed, EMBASE and Cochrane databases. The search strategy in PubMed was: [central AND (catheter OR line)] AND (removal OR remove OR removing) AND (infection OR sepsis OR bacteremia) AND (infant OR neonate OR neonatus OR neonatal OR newborn) AND (antibiotic OR prevention OR prevent OR preventing OR prophylaxis OR prophylactic). The search strategy was adapted for EMBASE and Cochrane databases. We also searched references of review articles for relevant studies. The last search update was August 2023.

Selection of studies

Studies were selected according to the PICOS (patients/participants, intervention, comparison, outcome, study type) approach. Inclusion criteria were:

Patients/participants: neonates aged ≤28 days admitted in NICU, undergoing planned removal of CVCs.

Intervention: antibiotics use within 12 h of planned CVCs removal.

Comparison: no antibiotics use within 12 h of planned CVCs removal.

Primary outcomes: PCRS which is defined as the appearance of clinical signs and symptoms of infection or the initiation of anti-infection therapy, with or without confirmatory blood markers or cultures within 72 h after catheter removal (11);

Secondary outcomes: (1) late-onset blood stream infection which is defined as clinical or laboratory signs of infection plus a positive blood culture or specific non-culture based microbiologic testing methods which is not related to the infection at another site (12); (2) CLABSI which is defined as clinical or laboratory signs of infection plus a positive blood culture developing within 48 h of CVCs removal in the absence of other known infection sites (3); (3) neonatal mortality.

Studies: retrospective or prospective human studies.

Exclusion criteria included: (1) noncomparative studies; (2) prophylactic antibiotics use for the duration of the CVCs; (3) therapeutic antibiotics use for known or suspected catheter-related bloodstream infections; (4) insufficient data for quantitative analyses; (5) grey literature lacking details or peer review. We set no restriction on language, publication type or date. Study selection was conducted by two researchers (RYJ and ZYTH) independently, with disagreements resolved through discussion with a senior investigator (LLG).

Data extraction

We extracted the following data: (1) study information: publication (article title, authors, year, journal title), study design (patient inclusion and exclusion criteria, grouping, sample size of each) and bias control; (2) baseline characteristics: gestational age, sex, birth weight, races and country or region; (3) CVCs management: type, duration of insertion and indications for removal. (4) antibiotic use: type, dosage, frequency, start and end time. (5) outcomes: incidence of PCRS, late-onset blood stream infection and CLABSIs after catheter removal, neonatal mortality. Data extraction was conducted by two researchers (RYJ and ZYTH) independently, with disagreements resolved through discussion with a senior investigator (LLG).

Risk of bias assessment

Risk of bias for randomized clinical trial (RCT) studies was assessed using the Cochrane risk-of-bias tool (13) based on seven domains: sequence generation, allocation concealment, blinding of

participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias (Supplementary Figure S1). For observational studies, risk of bias was assessed using a modified Newcastle-Ottawa Scale (NOS) (14) with the intention of best evaluating our phenomenon of interest (Supplementary Table S2). Assessment was performed based on three domains: selection, comparability and exposure, with a maximum score of 10. A total score of 5 or less, 6–7 and 8 or more was considered low, moderate and high quality, respectively. Risk of bias assessment was conducted by two researchers (RYJ and ZYTH) independently, with disagreements resolved through discussion with a senior investigator (LLG).

Statistical analysis

Basic characteristics of enrolled studies were firstly tabulated. Variables reported by three or more studies were evaluated through quantitative analyses. For continuous data, the mean differences (MD) with 95% confidence intervals (CI) were calculated as the effect measurements. Data reported as the median with interquartile range were converted into the mean with standard deviation through a recommended formula (15). For binary data, the odds ratio (OR) and 95% CI were calculated as the effect measurements. Heterogeneity across studies were evaluated by Cochrane chi-square (χ^2) and quantified with the I^2 statistics (16). I^2 values of 25, 50% and 75% represented low, moderate and high heterogeneity, respectively (17). For valuables with I^2 values ≤25%, the fixed-effect model will be used, otherwise, we used the random-effect model for data synthesis. We performed the following subgroup analyses to explore sources of heterogeneity: gestational age, birth weight, duration of catheter insertion, world region, type of study design and type, dosage and frequency of antibiotics use. Univariate meta-regression analyses were further performed to identify heterogeneity sources across studies. Multivariate meta-regression analyses were not performed due to limited number of studies. Publication bias was not evaluated as no more than ten studies were enrolled (18). All analyses were performed using Review Manager 5.3.3 (Nordic Cochrane Centre, Copenhagen, Denmark) and $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

The electronic search yielded a total of 335 potentially relevant studies (Figure 1). All records were imported into the Endnote with 37 duplicates removed. After reading the titles and abstracts, 288 irrelevant studies were further eliminated. Among the remaining 10 studies, four studies regarding routine prophylactic or therapeutic antibiotics use (2, 19–21) and one non-comparative study (22) were excluded. Therefore, a total of five studies, including one RCT (23) and four retrospective studies (1, 24–26) were ultimately enrolled in the quantitative analyses (Table 1).

All enrolled studies were conducted in the health setting of NICU. Altogether, 470 central lines in the antibiotic group and 658 central lines in the control group based on 1,054 neonates were included. The commonly used antibiotic regimen was a single dose of vancomycin (10 or 15 mg/kg) given at 2 h prior to CVCs removal. Other regimens included one dose of vancomycin plus cefazolin or two doses of cefazolin. There were no statistically significant differences between two groups in basic demographic characteristics including gestational age (Mean difference (MD) = −0.75 weeks, 95% confidence intervals (CI): −1.72 to 0.22, $P = 0.13$), male proportion (OR = 1.42, 95% CI: 0.91–2.22, $P = 0.12$) and birth weight (MD = −63.8 g, 95% CI: −232.6 to 105.0, $P = 0.46$). Also, the length of CVCs indwelling is comparable between two groups (MD = 1.61 days, 95% CI: −0.78 to 4.01, $P = 0.19$) (Table 2).

Incidence of PCRS

In total, 15 of 470 (3.2%) and 50 of 658 (7.6%) CVCs removal episodes had PCRS in the antibiotic group and control group, respectively. The random-effects meta-analysis demonstrated that antibiotics given within 12 h of CVCs removal non-significantly reduced the incidence of PCRS (OR = 0.35, 95% CI: 0.08–1.53, $P = 0.16$, $I^2 = 63\%$) (Figure 2A).

Results of subgroup analyses and univariate meta-regression were detailed in Table 3. No significant subgroup difference was tested. Regarding antibiotic regimens, the vancomycin subgroup (OR = 0.63, 95% CI: 0.02–24.37) and the cefazolin subgroup (OR = 0.07, 95% CI: 0.00–1.22) showed non-significant protective effect, while the combination subgroup suggested a near equal incidence of PCRS (OR = 1.02, 95% CI: 0.42–2.47) with and without antibiotics use. In addition, the single-dose subgroup (OR = 0.56, 95% CI: 0.15–2.15) and two-doses (OR = 0.07, 95% CI: 0.00–1.22) subgroups both demonstrated non-significant protective effect. Similar results were reached in subgroup analyses according to gestational age, birth weight, types of study design and length of CVCs indwelling. In the Asian region and low-dose (10 mg/kg) vancomycin subgroups, a single-dose of vancomycin (10 mg/kg) given at 2 h prior to CVCs removal significantly reduced the incidence of PCRS (OR = 0.12, 95% CI: 0.02–0.92), but such significant protective effect was not observed in the Western region subgroup (OR = 0.49, 95% CI: 0.10–2.47), and the high-dose (15 mg/kg) vancomycin subgroup (OR = 0.87, 95% CI: 0.18–4.12).

Incidence of post-catheter removal blood stream infection

In total, 4 of 408 (0.1%) and 15 of 481 (3.1%) CVCs removal episodes resulted in post-catheter removal blood stream infection, as was proven by blood culture, in the antibiotic group and control group, respectively. The fixed-effects meta-analysis demonstrated that antibiotics given within 12 h of CVCs removal significantly reduced the incidence of post-catheter removal blood stream infection (OR = 0.31, 95% CI: 0.11–0.86, $P = 0.03$,

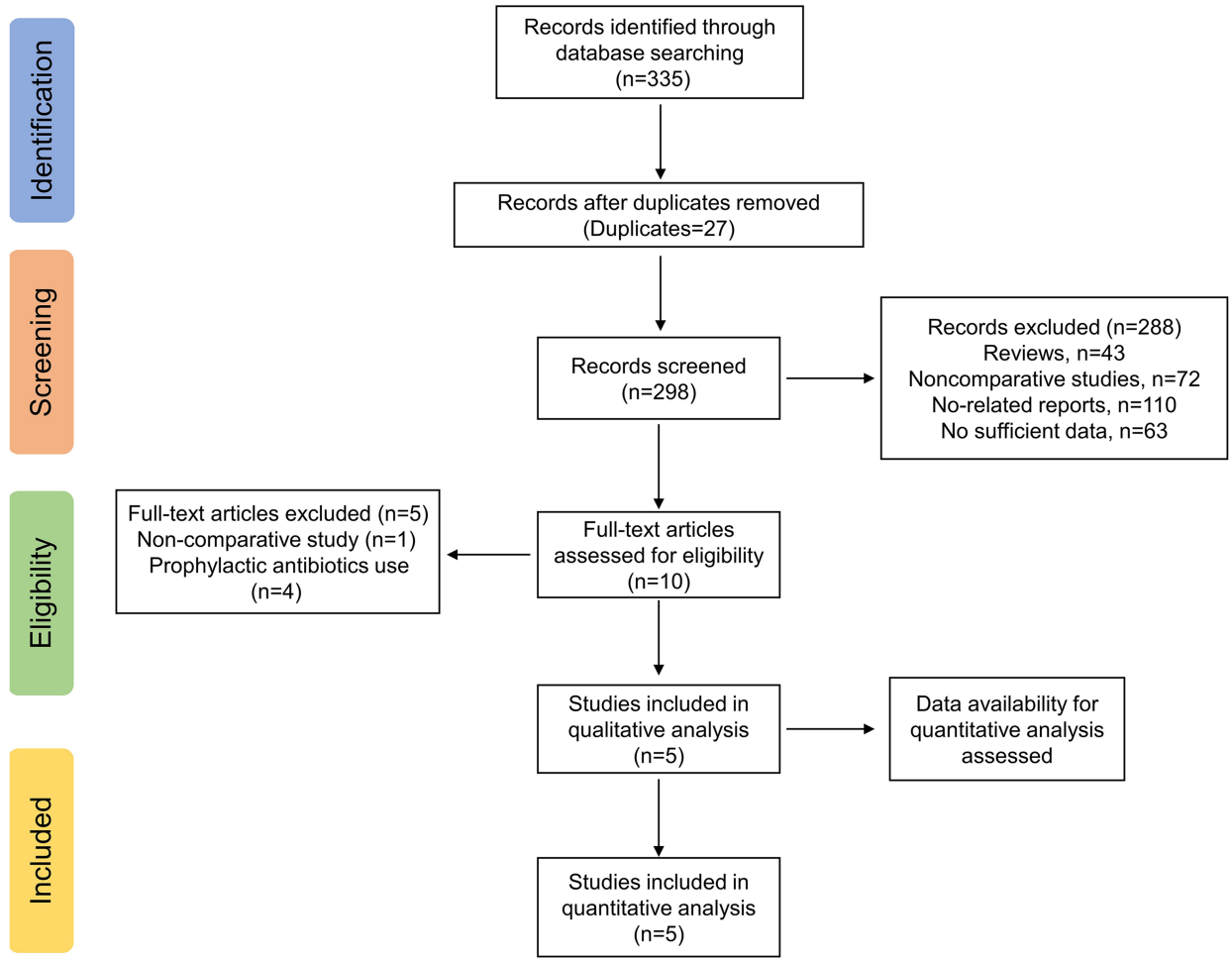

FIGURE 1
Study flow chart.

$I^2 = 0\%$) (Figure 2B). Subgroup analysis was not performed due to limited number of relevant studies.

Risk of bias assessment

Risk of bias of retrospective studies were assessed by a modified NOS (Supplementary Table S2). The total score of the four studies (1, 24–26) was 7, 8, 9, 7, respectively, indicating a moderate to low risk of bias. The risk of bias of the RCT (23) was assessed by using the Cochrane risk-of-bias tool, which was detailed in the Supplementary Table S1. This study is an open RCT with no detailed randomization and allocation procedures reported. Also, the actual enrolled number of patients was fewer than the planned value. Therefore, we considered this RCT to be at high risk of bias.

Publication bias

Publication bias was not evaluated because of a lack of test power when ten or fewer studies are enrolled (18).

Discussion

Conflicting evidence surrounds the use of antibiotic at the time of CVCs removal to prevent late-onset sepsis. In this meta-analysis, we quantitatively evaluated the preventive effect of antibiotic administration within 12 h of planned CVCs removal on late-onset sepsis based on five studies with a total of 1,128 central lines. Results demonstrated that antibiotic use upon CVCs removal did not significantly alter PCRS rates (OR = 0.35, 95% CI: 0.08–1.53), but was correlated with a lower incidence of post-catheter removal blood stream infection (OR = 0.31, 95% CI: 0.11–0.86).

Our results suggested a non-significant protective effect of antibiotic use at the time of CVCs removal in reducing rates of PCRS, with a pooled rate of 3.2%. Similar results were also found in most subgroup analyses. However, the evidence is still inadequate to examine this clinical issue as prospective, high-quality studies regarding this issue are largely insufficient. More attention has been devoted to prophylactic antibiotic use where antibiotics were given during the whole period of CVCs insertion or within 72 h prior to CVCs removal. A large retrospective study demonstrated a protective effect (OR = 0.26, $P < 0.001$) of

TABLE 1 Basic characteristics of included studies.

	Study design	Country/ region	Antibiotic group					Control group						Definition for outcomes
			Number of CVCs	Male (%)	Gestational age (wks)	Birth weight (g)	Indwelling time (days)	Antibiotics use	Number of CVCs	Male (%)	Gestational age (wks)	Birth weight (g)	Indwelling time (days)	
Reynolds 2015	Retrospective	USA	48	NA	25.7 ± 2.1	743 ± 225	27.2 ± 11.4	Vancomycin (15 mg/kg) 2 h before catheter removal OR others within 12 h before catheter removal	165	NA	27.1 ± 2.5	897 ± 197	23.4 ± 11.3	PCRS: clinical sepsis event as the performance of a sepsis workup (white blood cell count, differential, CRP, blood and/or CSF, and urine cultures) along with antibiotics given for more than 48 h initiated within 72 h of CVCs removal as indicated by the medical team.
Hemels 2011	RCT	Netherland	44	66	30.0 ± 8.4	1730 ± 1908	13.0 ± 19.2	Cefazolin (50 mg/kg) 1 h before and 12 h after catheter removal	44	46	30.0 ± 7.7	1407 ± 1326	9.3 ± 10.7	Post-catheter removal blood stream infection: clinical signs of infection accompanied by laboratory abnormalities and a positive blood culture within 48 h of CVCs removal.
Teibel 2020	Retrospective	USA	107	55	33.8 ± 4.3	2316 ± 930	12.0 ± 9.5	Vancomycin (15 mg/kg) plus cefazolin (25 mg/kg) 2 h before catheter removal	109	50	33.8 ± 4.1	2256 ± 920	12.4 ± 10.6	PCRS: by a sepsis workup including ≥2 of the following: complete blood count with differential, CRP, blood culture, urine culture, CSF culture, or antibiotics given for more than 48 h within 72 h of CVCs removal.
Tran 2021	Retrospective	USA	14	60	30.7 ± 9.0	1400 ± 1554	39.3 ± 10.6	Vancomycin (15 mg/kg) 1.5 h before catheter removal	12	60	31.0 ± 8.2	1426 ± 1471	37.7 ± 7.4	PCRS: the initiation of antibiotics within 72 h of CVC removal and the collection of at least one of the following: complete blood count and differential, CRP, or a specimen culture (blood, urine, tracheal aspirate, and/or CSF).
Yan 2021[a]	Retrospective	Taiwan	257	NA	NA	NA	NA	Vancomycin (10 mg/kg) 2 h before catheter removal OR others within 12 h before catheter removal	328	NA	NA	NA	NA	Clinical sepsis: clinical symptoms of systemic illness, with initiation of antibiotics without a positive blood culture. A workup for sepsis includes complete blood count with differential, blood, CSF, and urine cultures; CRP measurement performed within 72 h of CVCs removal; and administration of antibiotics for at least 48 h.

[a]Basic characteristics of this study were not obtained due to different groupings between this study and our meta-analysis.

RCT, randomized clinical trial; CVCs, central venous catheters; wks, weeks; hrs, hours; PCRS, post-catheter removal sepsis; CRP, C-reactive protein; CSF, cerebral spinal fluid; NA, not applicable.

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TABLE 2 Comparison of clinical characteristics.

Variables	Antibiotic vs. Control[a]	I^2(%)	*P*
Gestational age MD (95% CI), weeks	−0.75 [−1.72, 0.22]	36	0.13
Male proportion OR (95% CI)	1.42 [0.91, 2.22]	0	0.12
Birth weight MD (95% CI), g	−63.8 [−232.6, 105.0]	32	0.46
CVCs indwelling time MD (95% CI), days	1.61 [−0.78, 4.01]	22	0.19

MD, mean differences; OR, odds ratio; CI, confidence interval.
[a]A positive MD or OR favors antibiotic group.

prophylactic antibiotics in preventing culture-negative sepsis. In the intervention group, PCRS was found in 17 of the 322 (5.3%) central lines that were free from infection before removal (27). Inconsistently in an earlier RCT, infants were randomly assigned to receive amoxicillin prophylaxis or no antibiotic prior to CVCs removal (21). PCRS was found in 3 of 75 (4.0%) lines and 8 of 73 (11.0%) lines in two groups ($P = 0.107$), indicating non-significant benefit brought by routine antibiotic prophylaxis. A Cochrane meta-analysis enrolling three RCTs further affirmed the effect of prophylactic antibiotics in reducing rates of PCRS (RR = 0.40, 95% CI: 0.20–0.78), with a pooled PCRS rate of 8.8% (8). Though the absolute incidence of PCRS was comparable between antibiotic prophylaxis and antibiotics on CVCs removal, the superiority of one or another could not be determined due to a lack of comparative studies. However, there is no doubt that a single or two doses of antibiotics on CVCs removal could help to avoid antimicrobial resistance and microbiome dysbiosis brought by long-term antibiotic prophylaxis in neonates (9, 28).

Although the antibiotic use upon CVCs removal did not exhibit a significant protective effect against PCRS, it was correlated a lower risk of post-catheter removal blood stream infection (culture-positive sepsis), as suggested by our analysis. The specific mechanisms underlying this is unclear. It might be explained that the infusion of antibiotic disrupts the catheter biofilm formed along the catheter tip, decreasing the load of bacteria showered into blood stream upon catheter removal and therefore preventing culture-positive sepsis (27). However, a culture-negative sepsis could still be caused by the inflammatory response to unculturable bacteremia, especially by gram-negative bacteria which is largely non-susceptible to vancomycin. Though correlated with less blood stream infection, whether antibiotic use on CVCs removal will contribute to better clinical outcomes was not identified in our systematic review due to insufficient data reported. A recent large-scale meta-analysis indicated that despite similar mortality rate of sepsis shared by culture-positive and culture negative sepsis, patients with culture-positive sepsis had significantly longer hospitalization and mechanical ventilation duration (29). A retrospective study based on the pediatric setting reported a significantly lower mortality rate and organ-dysfunction in the culture-negative group (30). We thus speculate that antibiotic use on CVCs removal may bring clinical benefits by reducing the rate of culture-positive sepsis, which should be further examined with more relevant data reported.

To the best of our knowledge, this meta-analysis provides the most updated assessments of current evidence regarding the use of antibiotics at the time of CVCs removal in reducing late-onset sepsis. Despite this, several limitations exist. Due to the small number of published studies, we include both RCT and retrospective observational studies in our analyses, which might limit the quality of generated evidence. Even though the

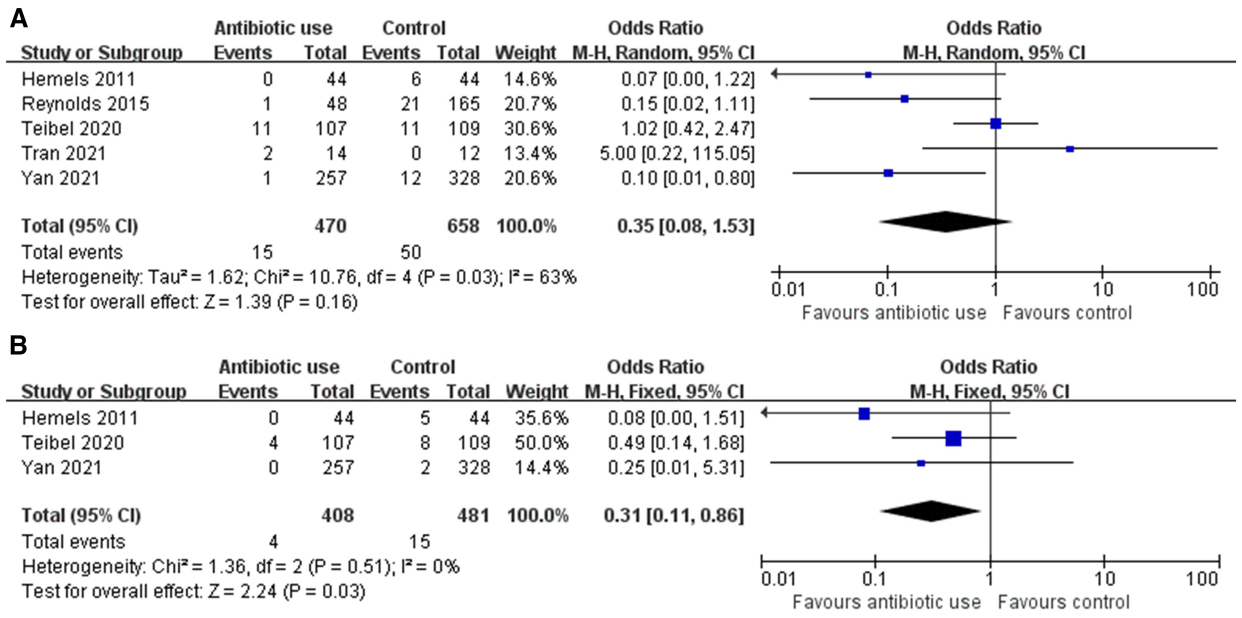

FIGURE 2
(A) Forrest plot of incidence of post-catheter removal sepsis (PCRS) for antibiotic use versus control. (B) Forrest plot of incidence of post-catheter removal blood stream infection for antibiotic use versus control.

TABLE 3 Subgroup analyses and univariate meta-regression analyses of PCRS

Groups	Subgroups	Studies (*n*)	OR [95% CI][a]	I^2 (%)	I^2_{sub} (%)[b]	P[c]
Mean gestational age	≥28 weeks	3	0.74 [0.11, 5.12]	56	22	0.26
	<28 weeks	1	0.15 [0.02, 1.11]	–		
Mean birth weight	≥1,500 g	2	0.36 [0.02, 5.50]	71	0	0.77
	<1,500 g	2	0.69 [0.02, 21.80]	71		
Types of antibiotics	Vancomycin	2	0.63 [0.02, 24.37]	74	36	0.21
	Cefazolin	1	1.02 [0.42, 2.47]	–		
	Vancomycin plus cefazolin	1	0.07 [0.00, 1.22]	–		
Frequency of antibiotic administration	One dose	4	0.56 [0.15, 2.15]	52	42	0.19
	Two doses	1	0.07 [0.00, 1.22]	–		
Dosage of vancomycin	Low dose (10 mg/kg)	1	0.12 [0.02, 0.92]	–	56	0.13
	High dose (15 mg/kg)	3	0.87 [0.18, 4.12]	40		
Length of catheter indwelling	Long length (≥20 days)	2	0.69 [0.02, 21.80]	71	0	0.77
	Short length (<20 days)	2	0.36 [0.02, 5.50]	71		
Study design	Observational	4	0.56 [0.15, 2.15]	52	42	0.19
	RCT	1	0.07 [0.00, 1.22]	–		
World region	Asian	1	0.12 [0.02, 0.92]	–	11	0.29
	Western	4	0.49 [0.10, 2.47]	49		

PCRS, post-catheter removal sepsis; OR, odds ratio; CI, confidence interval; RCT, randomized clinical trial.
[a]A positive OR favors antibiotic group.
[b]Heterogeneity across subgroups.
[c]*P* value of univariate meta-regression analyses which test for subgroup differences.

subgroup analysis based on types of study design did not detect significant interstudy heterogeneity, our results should still be interpreted with caution as the robustness and convincingness of subgroup analysis could be weakened by small number of included studies. The neonatal sepsis lacks a consensus definition, and its definition varies among enrolled studies. The updated regional practice manual and recommendations define CLABSI as a laboratory confirmed bloodstream infection where an eligible organism is identified, and an eligible central line (in place for over 48 h) is present, and further categorized the CLABSI into various types (12, 31). Once a global consensus definition is established, our outcome definition and study selection should be modified accordingly, and a re-analysis should be performed. Several critical clinical outcomes such as mortality rate, subsequent antibiotics, and other treatments for PCRS, length of stay as well as long-term outcomes are unable to evaluate due to insufficient data reported. Also, there is a moderate to high interstudy heterogeneity for the primary outcome, even though sources of heterogeneity were partly identified by subgroup analyses. Therefore, with continuous publication of articles, the update of the meta-analysis is still warranted to improve the above deficiencies.

Conclusions

In conclusion, results of our review suggests that antibiotic administration in neonates within 12 h of planned CVCs removal does not significantly reduce the incidence of PCRS but offers less post-catheter removal blood stream infection. However, whether this will be converted to clinical benefits lacks evidential support. These findings should be interpreted with caution due to limitations stated above. The update of meta-analysis is warranted with more randomized designed studies having a longer follow-up performed.

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

This study complied with the Declaration of Helsinki and the ethics approval was waived by the Ethics Committee of Peking Union Medical College Hospital and Yancheng Third People's Hospital.

Author contributions

RJ: Formal Analysis, Investigation, Methodology, Software, Writing – original draft. ZH: Formal Analysis, Investigation, Methodology, Writing – review & editing. JZ: Software, Writing – review & editing. SF: Supervision, Writing – review & editing. LG: Conceptualization, Supervision, Validation, Writing – review & editing, Resources.

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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/fped.2023.1324242/full#supplementary-material

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Inflammatory biomarkers and physiomarkers of late-onset sepsis and necrotizing enterocolitis in premature infants

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Background: Early diagnosis of late-onset sepsis (LOS) and necrotizing enterocolitis (NEC) in very low birth weight (VLBW, <1,500 g) infants is challenging due to non-specific clinical signs. Inflammatory biomarkers increase in response to infection, but non-infectious conditions also cause inflammation. Cardiorespiratory data contain physiological biomarkers, or physiomarkers, of sepsis that may be useful in combination with inflammatory hematologic biomarkers for sepsis diagnosis.
Objectives: To determine whether inflammatory biomarkers measured at the time of LOS or NEC diagnosis differ from times without infection and whether biomarkers correlate with cardiorespiratory sepsis physiomarkers in VLBW infants.
Methods: Remnant plasma sample collection from VLBW infants occurred with blood draws for routine laboratory testing and suspected sepsis. We analyzed 11 inflammatory biomarkers and a pulse oximetry sepsis warning score (POWS). We compared biomarker levels obtained at the time of gram-negative (GN) bacteremia or NEC, gram-positive (GP) bacteremia, negative blood cultures, and no suspected infection.
Results: We analyzed 188 samples in 54 VLBW infants. Several biomarkers were increased at the time of GN LOS or NEC diagnosis compared with all other samples. POWS was higher in patients with LOS and correlated with five biomarkers. IL-6 had 78% specificity at 100% sensitivity to detect GN LOS or NEC and added information to POWS.
Conclusion(s): Inflammatory plasma biomarkers discriminate sepsis due to GN bacteremia or NEC and correlate with cardiorespiratory physiomarkers.

KEYWORDS

prematurity, neonatal sepsis, biomarkers, inflammation, heart rate, oxygenation, cardiorespiratory

1 Introduction

Sepsis and necrotizing enterocolitis (NEC) lead to significant morbidity and mortality in premature infants (1). Inflammation due to sepsis and NEC can cause end-organ damage (2, 3), including brain injury, which leads to long-term impairment (4–6). Early recognition and treatment may improve outcomes, but signs and symptoms often overlap with non-infectious conditions (7). Therefore, a diagnosis is often made once the

infection is advanced, and on the other hand, empiric antibiotics are given when there is no infection (8). Clinicians have limited tests and data to guide decisions on the likelihood of infection.

Inflammatory molecules, upregulated as part of the immune response to infection, hold promise as biomarkers of neonatal sepsis. A biomarker with sufficient diagnostic accuracy could be a useful screening tool to aid in deciding whether to start antibiotics or continue monitoring for further signs of sepsis. Many studies have evaluated biomarkers for neonatal sepsis in premature infants (9–12). But, few have been translated into clinical care (13). Decisions to start or stop antibiotics in the NICU largely rely on the clinician's assessment of clinical signs and non-specific laboratory tests, such as C-reactive protein (CRP) (14) and complete blood count (CBC) components (15).

Despite extensive research, no single blood, stool, or urine biomarker has been adopted in clinical practice for its predictive accuracy to diagnose neonatal sepsis and start antibiotic therapy (16). Physiologic sepsis biomarkers, such as abnormal vital sign patterns, can be detected using analysis of continuous vital sign data from standard bedside monitors. We previously used predictive analytics and a multicenter cohort of very low birth weight (VLBW, <1,500 g) preterm infants to develop and externally validate a cardiorespiratory sepsis risk model (17). The model, called Pulse Oximetry Warning Score (POWS), detects physiologic biomarkers of abnormal heart rate and SpO2 patterns that occur near the clinical diagnosis of late-onset sepsis and calculates the relative risk of blood culture-positive sepsis in the subsequent 24 h (17). POWS has yet to be implemented in clinical practice, so we sought to understand how inflammatory biomarkers will correlate and add to cardiorespiratory markers of sepsis. Combining information from hematologic and physiologic inflammatory biomarkers could improve the accuracy of sepsis diagnosis over using either alone.

While many prior studies have analyzed inflammatory biomarkers in premature infants at the time of suspected sepsis (10, 18–20), few have analyzed baseline levels or changes over time in patients who develop sepsis (21, 22). Inflammatory biomarkers may be less useful in premature infants with non-infectious conditions that lead to inflammation, including respiratory distress syndrome and chronic lung disease. To understand the potential utility of biomarkers in VLBW infants, more data are needed. In this study, we aim to identify inflammatory biomarkers that discriminate sepsis from baseline levels and non-sepsis conditions in VLBW infants. To do this, we measured multiple biomarkers in samples collected from remnant plasma, weekly when available and at the time of evaluation for late-onset sepsis or NEC.

2 Methods

2.1 Study design

This was a prospective, observational cohort study conducted at a single center Level IV academic NICU. The Institutional Review Board approved this study with consent from a parent (IRB #HSR20902). We prospectively enrolled VLBW infants within seven days of birth and collected clinical data and remnant plasma from blood drawn for suspected sepsis and for routine laboratory testing. Clinical data and culture results were recorded from the electronic health record. We classified samples according to the indication for the laboratory tests ordered and the final diagnosis of a workup for LOS or NEC (see "clinical definitions" section below).

2.2 Sample collection

The study protocol did not require any extra blood draws and did not influence the clinical team's decisions on ordering blood tests. Nurses collected blood samples in tubes according to the clinical laboratory guidelines for the laboratory test ordered. Blood for a complete blood count was collected in EDTA tubes, and blood for a basic metabolic panel or CRP was collected in sodium heparin tubes. These were the only clinical laboratory tests used to request remnant plasma for the study. The clinical lab stores samples at 4 degrees Celsius immediately after receipt, and the leftover blood from a clinical sample was requested by the study team. The clinical lab separated plasma from whole blood for samples collected in sodium heparin tubes, while the study team separated plasma from whole blood for samples collected in EDTA tubes by centrifugation (1,800 g × 5 min). We stored all remnant plasma samples with at least 250 microliters at −80°C until analysis.

2.3 Biomarker analysis

We selected subjects with at least two collected samples of adequate plasma volume (≥ 250 µl) for biomarker analysis. The volume of blood obtained from the patient was not altered for the purpose of this study. Therefore, samples with insufficient remnant plasma were excluded from the study. The multiplex assay measured a panel of inflammatory biomarkers: interleukin (IL)-6, IL-8, IL-10, IL-18, interferon gamma inducible protein (IP)-10, tumor necrosis factor alpha (TNFα), procalcitonin (PCT), human growth factor (HGF), endothelin growth factor (EGF), soluble suppression of tumorigenicity 2 (sST-2), and IL-1 receptor antigen (IL1-ra). We selected these biomarkers based on their role in the inflammatory response to bacterial infections and the existing literature (12, 20, 23, 24). Analyte concentrations were quantified using a customized multiplex Luminex® magnetic bead-based antibody assay (R&D Systems, Minneapolis, USA). Fluorescence signals for each biomarker bead region were analyzed on a Luminex®200, a dual-laser flow-based detection instrument. Concentrations below the lowest standard were recorded as the value of the lower limit of detection for statistical analyses. We used a 10-fold dilution to analyze PCT, sST-2, and IL-1ra, and the remaining biomarkers did not require dilution. Results of CRP measurement obtained for clinical use were obtained from the electronic health record. Clinical guidelines at the time of the study recommended measuring CRP at the time of suspected infection and when determining duration of treatment, but the decision to order a CRP was left to the discretion of the clinical team.

2.4 Clinical definitions

Samples were classified as "routine" if they were obtained from blood drawn for standard laboratory monitoring, usually a basic metabolic panel, and not within 48 h of suspected infection. We classified samples obtained at the time of a blood culture using the diagnosis of the event according to the following definitions:

A. Late-onset sepsis (LOS) (25): a positive blood culture obtained after 72 h of age and treated with at least five days of intravenous antibiotics. These were further categorized as sepsis due to Gram-positive (GP) or Gram-negative (GN) bacteremia according to the organism identified by blood culture.
B. Necrotizing enterocolitis (NEC) (26): radiographic evidence of necrotizing enterocolitis and clinical illness with or without a positive blood culture.
C. Clinical sepsis (CS): negative blood and urine cultures treated with ≥5 days of antibiotics for presumed infection due to clinical illness.
D. Sepsis Ruled Out (SRO): a negative blood culture treated with <5 days of empiric antibiotics while awaiting culture results and monitoring symptoms, typically for 48 h.

For the analysis, results of samples classified as SRO and routine were grouped as "no sepsis".

2.5 Cardiorespiratory sepsis risk prediction

We previously developed a multivariable model to predict sepsis using continuous heart rate (HR) and oxygen saturation (SpO_2) data called the Pulse Oximetry Warning Score, or POWS (17). The POWS model calculates the mean, standard deviation, skewness, kurtosis, and cross-correlation of HR and SpO_2 every 10 min and uses logistic regression at each window to predict the relative risk of LOS with a positive blood culture in the next 24 h. We calculated hourly POWS values from continuous bedside monitoring data during the 12 h preceding blood cultures. POWS scores were calculated after discharge and thus did not influence decisions about clinical care. A heart rate characteristics (27, 28) monitoring system for sepsis risk prediction was in use during the study period, but was not routinely used in the decision to order a blood culture.

2.6 Statistical analyses

We compared distributions of inflammatory biomarkers and POWS data using Kruskal–Wallis analysis across the four diagnosis groups, followed by a pairwise Wilcoxon test with corrections for multiple testing. Hierarchical cluster analysis was performed on seven biomarkers in samples associated with a positive blood culture or NEC with no organism identified. Cytokine values were log transformed prior to clustering. The relationships between POWS and individual biomarker levels were assessed using univariate linear regression. The statistical significance of each model's coefficient was adjusted for repeated measures using the Huber-White method (29). A p-value <0.05 was considered statistically significant. The predictive performance of biomarkers and POWS were assessed individually or in combination using sensitivity, specificity at specific thresholds, and area under the receiver operator characteristics curve (AUC) using logistic regression to predict LOS or NEC. All statistical analysis was performed using RStudio (R Version 4.2.3, Vienna, Austria).

3 Results

3.1 Patients and samples

We enrolled 118 VLBW infants with parental consent. Of those enrolled, we had sufficient samples from 54 infants for analysis. The 54 infants included were 48% female, had a median gestational age of 26 weeks (IQR 25–27), and a median birth weight of 790 g (IQR 630–930). Compared with infants analyzed, infants excluded for insufficient samples or data were 43% female, had higher median gestational age (28 weeks, IQR 26–29) and birthweight (980 g, IQR 760–1,240). Table 1 shows clinical characteristics of infants included for sample analysis and compares those with and without LOS or NEC. In total, 188 plasma samples were analyzed, including 68 obtained near the time of blood cultures for suspected LOS or NEC and 120 at the time of routine blood sampling. The median number of samples analyzed per patient was 3 (range 2–6). Seven biomarkers (IP-10, IL-6, IL-10, IL-18, TNFa, IL-8, PCT) were analyzed at all times, while four (HGF, EGF, sST-2, IL-1ra) were successfully measured 80% of the time (152 samples in 45 patients).

Of the 67 blood cultures with study samples analyzed, 28 were diagnosed as LOS or NEC with bacteremia. Of these, there were 22 cases of Gram-positive (GP) bacteremia, 5 Gram-negative (GN) bacteremia, and 1 with NEC and GN bacteremia. Organisms in the positive blood cultures were coagulase-negative Staphylococcus (CONS) species ($n = 19$), *Escherichia coli* ($n = 2$), *Enterobacter* species ($n = 1$), Group B *Streptococcus* ($n = 1$), *Klebsiella* species ($n = 5$), and methicillin-susceptible *Staphylococcus aureus* ($n = 2$). There were 2 cases of NEC with negative blood cultures and 17 negative blood cultures diagnosed as CS. The remaining 141 samples came from blood drawn at times with NS, including routine samples and blood cultures diagnosed as SRO.

TABLE 1 Cohort characteristics overall and grouped by infants with or with late-onset sepsis (LOS) or necrotizing enterocolitis (NEC) diagnosis.

	LOS/NEC ($N = 30$)	No LOS/NEC ($N = 24$)	Overall ($N = 54$)
Gestational age (weeks), mean (SD)	25.8 (2.1)	26.2 (1.6)	25.9 (1.9)
Birth weight (grams), mean (SD)	780 (203)	831 (245)	803 (222)
Male, *n* (%)	19 (63%)	9 (38%)	28 (52%)
Race/ethnicity, *n* (%)			
Black	3 (10%)	4 (17%)	7 (13%)
Hispanic	3 (10%)	1 (4%)	4 (7%)
White	23 (77%)	18 (75%)	41 (76%)
Unknown/other	1 (3%)	1 (4%)	2 (4%)
Died, *n* (%)	5 (17%)	0 (0%)	5 (9%)

Values are presented as mean (standard deviation) or number (%).

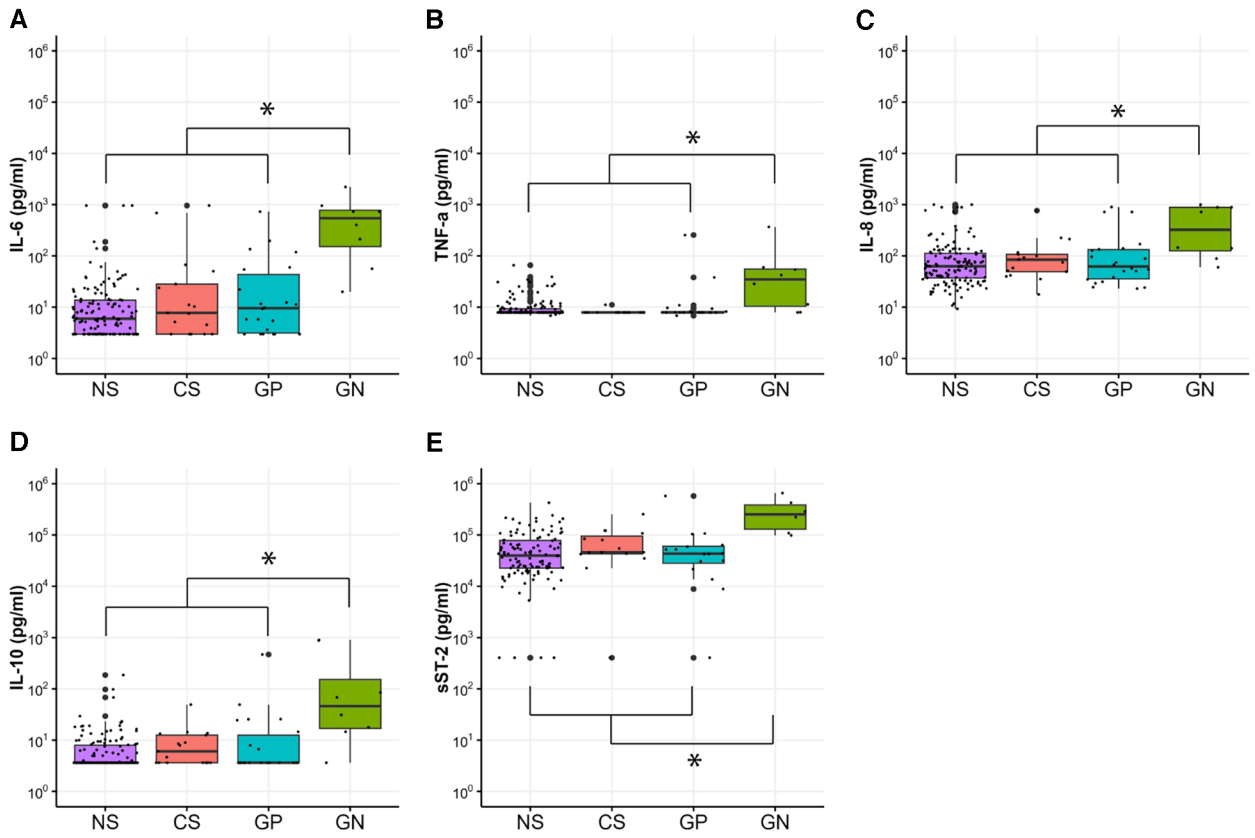

FIGURE 1
Plasma biomarker levels from remnant plasma collected at times with or without sepsis in very low birth weight (VLBW) infants. Samples were classified as clinical sepsis (CS, $n = 17$), Gram-positive bacteremia (GP, $n = 22$), Gram-negative bacteremia or Necrotizing Enterocolitis (NEC) (GN, $n = 8$), or no sepsis (NS, $n = 141$). IL-6 (**A**), TNF-a (**B**), IL-8 (**C**), IL-10 (**D**), and sST-2 (**E**) were significantly higher in patients with GN sepsis as compared with NS, CS, and GP sepsis, as indicated by asterisks. Biomarker levels were not different when comparing NS, CS, and GP sepsis samples.

3.2 Biomarkers

Five of the eleven biomarkers (IL-6, TNF-α, IL-8, IL-10, and sST-2) were significantly higher in patients with GN sepsis or NEC than those with NS, CS, and GP sepsis ($p < 0.05$). Overall, biomarkers at CS or GP diagnosis were not significantly different from NS samples (Figure 1).

CRP was ordered by the clinical team at the time of 92 (49%) of the analyzed remnant plasma samples. CRP was significantly higher in patients with GN sepsis or NEC (median CRP 4.4 g/dl, IQR 2.1–11.0) compared to patients with no sepsis [median CRP 0.1 g/dl, IQR 0.1–0.4 ($p < 0.05$)] or clinical sepsis [median CRP 0.12 g/dl, IQR 0.1–1.2 ($p < 0.05$)]. There was no statistically significant difference in CRP for GN sepsis vs. GP sepsis cases [median CRP 2.7 g/dl, IQR 0.1–3.3 ($p = 0.16$)] or clinical sepsis [median CRP 0.12 g/dl, IQR 0.1–1.2 ($p = 0.08$)].

Hierarchical cluster analysis of cytokines from 30 LOS or NEC blood samples showed two distinct clusters of biomarker profiles (Figure 2). GN bacteremia or NEC was more prevalent in the cluster of samples with the highest cytokine levels. IL-6 above 20 pg/ml had the best overall test accuracy for diagnosing gram-negative sepsis or NEC with a specificity of 78%, negative predictive value of 100%, and sensitivity of 100%.

3.3 Association with POWS, a cardiorespiratory sepsis risk prediction model

Of the 188 samples, 187 had continuous heart rate and oxygen saturation data available around the time of sample collection to calculate POWS, a pulse oximetry warning score designed as a physiomarker for impending sepsis. POWS was significantly associated with the levels of 5 biomarkers (IL-8, PCT, HGF, sST-2, and IL1-ra) irrespective of the associated diagnosis (Figure 3, $p < 0.05$). The maximum POWS within the 12 h preceding the sample had an AUC to predict LOS (GN or GP) or NEC (with or without bacteremia) of 0.610. The AUC increased to 0.680 when the IL-6 level of the sample was added to the model.

4 Discussion

We assayed inflammatory biomarkers at the time of blood culture for suspected sepsis and routine laboratory testing in VLBW infants and found differences that distinguish late-onset Gram-negative sepsis and NEC from other diagnoses, but similar biomarker distributions obtained from samples collected at

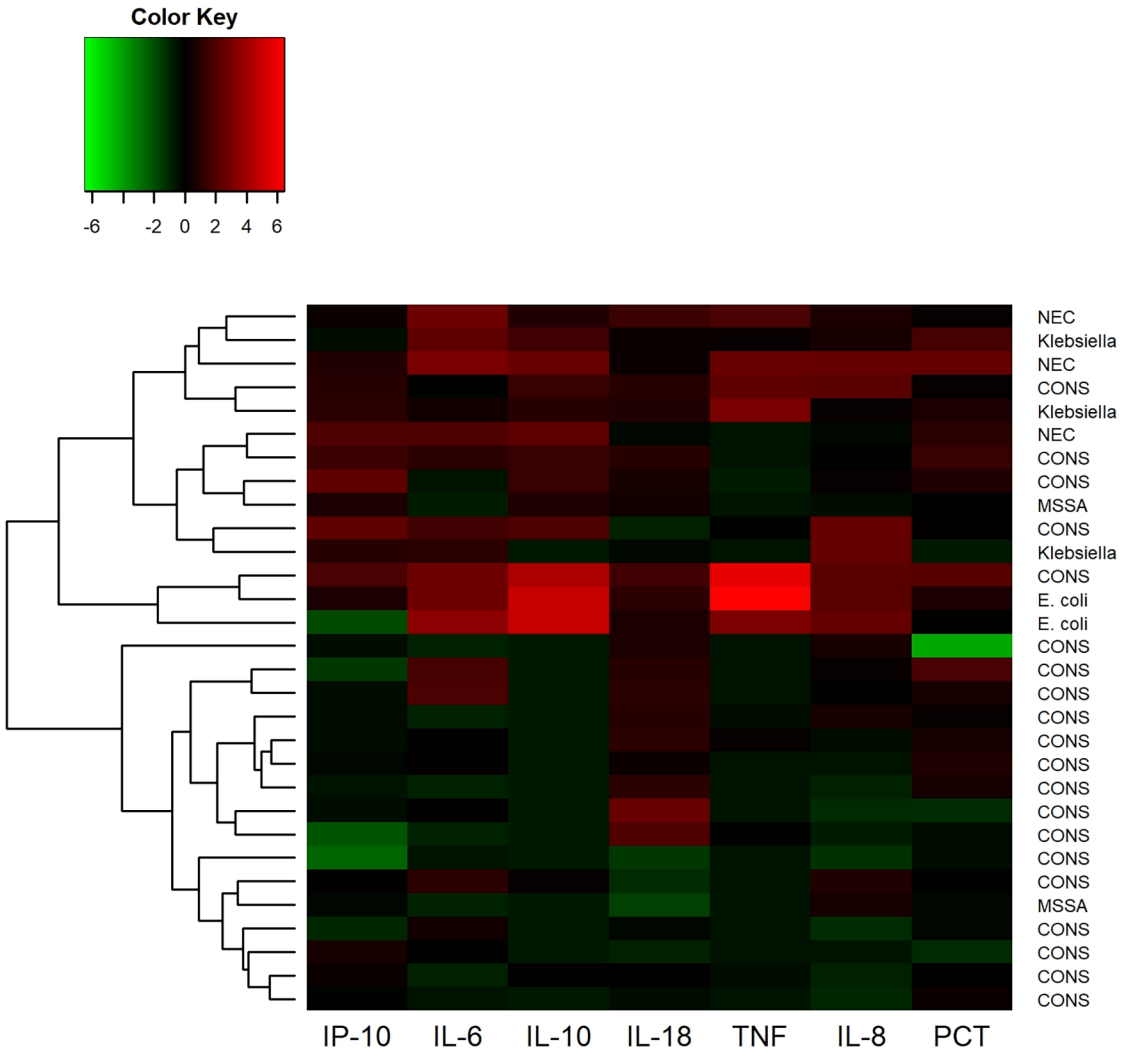

FIGURE 2
Hierarchical cluster analysis of cytokine levels in 30 cases of blood culture-positive late-onset sepsis (LOS) and necrotizing enterocolitis (NEC). The scaled natural log of each cytokine was taken prior to clustering. Higher than average cytokine levels are depicted in shades of red and lower than average levels are depicted in green. The resulting hierarchical clustering dendrogram is on the left-hand side of the heatmap.

baseline and at the time of suspected sepsis or Gram-positive sepsis. We also found correlations between inflammatory biomarkers and POWS, an algorithm that detects abnormal patterns of heart rate and oxygen saturation that we previously developed as a physiomarker of sepsis (17).

Our results confirm the findings in a previous study of VLBW infants that multiple biomarkers can discriminate between GN sepsis and GP sepsis at the time of positive blood culture (12). Since Gram-negative infections and NEC are known to carry higher morbidity and mortality, this finding might be a useful adjunct clinical decision support tool in helping pick the appropriate empiric antibiotic therapy and institute treatment early (30–32). The current study adds to our prior work (12) because we analyzed samples remote from suspected sepsis. We found no significant difference in biomarker levels in these samples compared to those obtained at the time of clinical sepsis or Gram-positive sepsis. Some VLBW preterm infants may have a chronic or subacute systemic inflammatory response associated with lung disease or intracranial hemorrhage, which could confound sepsis prediction using hematologic biomarkers or cardiorespiratory physiomarkers (33–35).

Plasma cytokines and chemokines are early markers of immune activation in response to infection, and have been shown to rise in septic premature infants (22, 36, 37). Several studies have evaluated the utility of biomarkers for diagnosing sepsis by comparing biomarker levels in cases vs. controls (11). Others have compared cytokine profiles obtained at the time of suspected infection in VLBW infants when the blood culture returns positive vs. negative (38, 39). Instead, we assessed differences in inflammatory biomarkers measured at times when there was clinical suspicion for infection or sepsis and at times remote from infection. Kuster and colleagues also took the approach of measuring cytokines at baseline and at the time of suspected sepsis in VLBW infants and found IL-1ra and IL-6 to have high sensitivity and specificity in 21 cases of late-onset sepsis, even measured from samples taken the

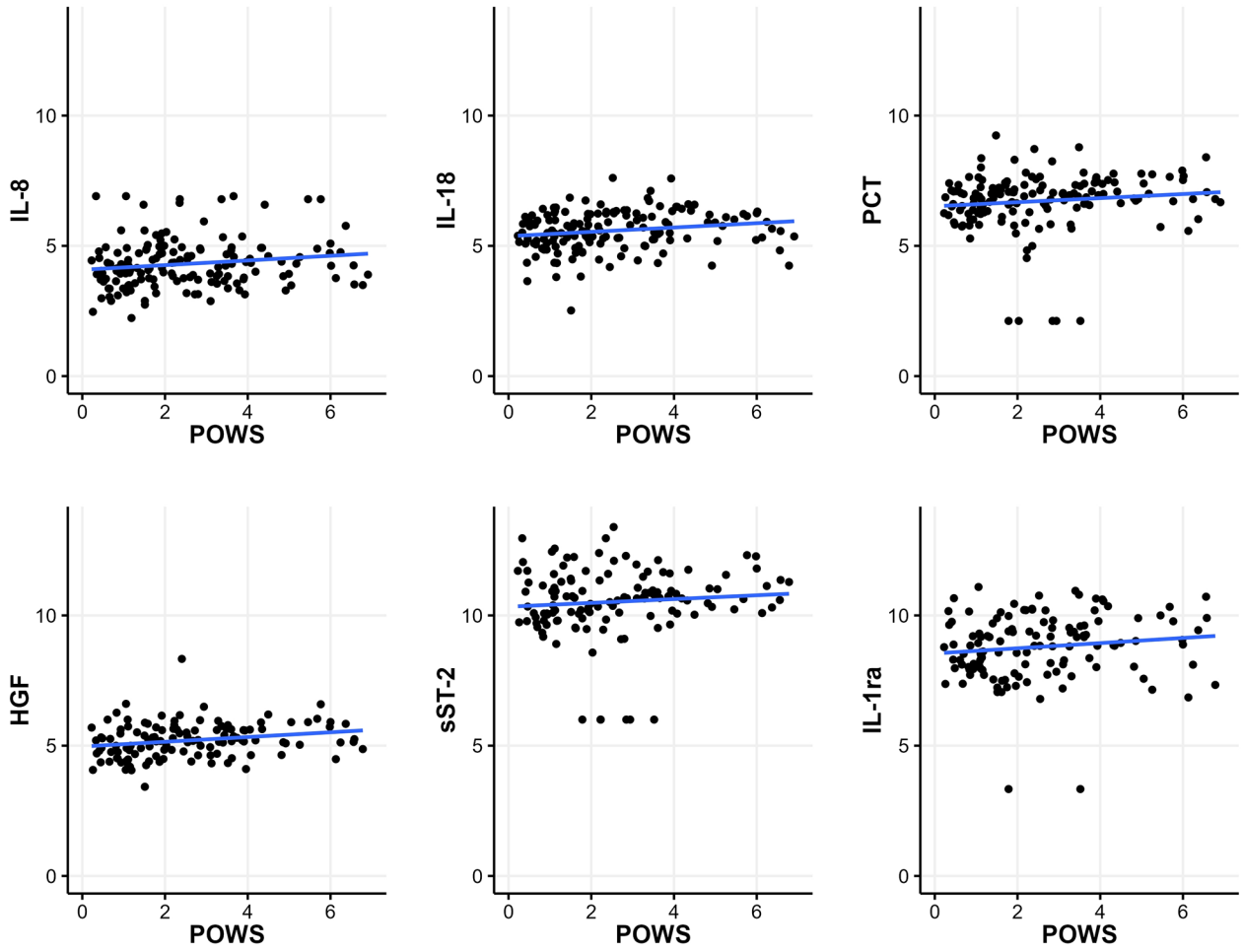

FIGURE 3
Relationship between the pulse oximetry warning score (POWS) and inflammatory biomarkers. Plots of the scaled natural log of each biomarker level on the y-axis and the maximum sepsis risk within 12 h before blood culture on the x-axis. POWS was significantly associated ($p < 0.05$) with the levels of the 5 biomarkers depicted in this figure (IL-8, PCT, HGF, sST-2, and IL1-ra), irrespective of the associated diagnosis.

day prior to blood culture (37). A longitudinal study of extremely low birth weight (<1,000 g) measured cytokines at days 1, 3, 14, and 21 after birth and found that IFN-γ, IL-10, IL-18, TGF-β, and TNF-α levels differed among infants who developed fungal or bacterial LOS compared with those who never developed sepsis (22). This study enrolled a large, multicenter cohort but did not evaluate differences relative to the timing of blood cultures. They found that infants with higher levels of immune regulatory cytokines relative to pro-inflammatory cytokines were associated with an increased risk of LOS at any time during the NICU course (21).

Of the biomarkers assayed in our study, IL-6 has been most consistently identified as a promising sepsis biomarker in previous studies, though with variable sensitivity and specificity (39). We found that IL-6 had a high specificity at a threshold set to detect 100% of Gram-negative sepsis and NEC cases but low predictive accuracy for Gram-positive cases, which were mostly due to CONS bacteremia. IL-6 has been shown to predict fatal or severe sepsis in premature infants (40), which occurs more often in Gram-negative than Gram-positive infections due to pathogen virulence factors (41). However, we also found elevated levels of IL-6 measured from samples remote from sepsis with blood draws for routine laboratory tests.

The inclusion of sST-2 as a sepsis biomarker was novel for this population as its utility has mainly been studied in adult populations. It is an IL-1 family receptor that binds IL-33 and has been implicated in diseases involving intestinal inflammation (42). Recent studies in adult patients demonstrate an association between elevated sST-2 and the severity of illness in *Clostridium difficile* colitis (43, 44). Our results indicate that this may also be a useful biomarker for diagnosing NEC and Gram-negative sepsis, where gastrointestinal dysfunction and bacterial translocation cause systemic inflammation.

Cytokines and chemokines such as IL-6, IL-1ra, and IL-8 have been demonstrated to have diagnostic utility as early sepsis markers (37, 45), while acute phase reactants such as CRP and PCT rise during the later phases of systemic inflammation (46). CRP and PCT are also examples of the few inflammatory biomarkers available in U.S. clinical laboratories, which may drive their clinical use despite evidence of low clinical utility (14). A recent meta-analysis showed overall low sensitivity and specificity of CRP for LOS diagnosis (47).

We note several limitations of the study. First, the analysis was limited by the small sample size, where most late-onset sepsis events were due to CONS bacteremia, some of which may have represented contamination and not a true infection. Second, events diagnosed as clinical sepsis are heterogeneous in severity with subjective diagnostic criteria. We collected data on clinical characteristics, but not to the level of detail to account for concurrent inflammatory processes, such as lung disease, invasive mechanical ventilation, and minor procedures. Finally, the use of remnant plasma allowed us to enroll patients and collect samples without additional blood draws, but the volume of plasma available was small and therefore did not allow assays to be run in duplicate.

Analysis of plasma biomarkers and physiomarkers of LOS using POWS, a cardiorespiratory sepsis risk score, resulted in promising correlations that warrant further confirmation in larger studies. Continuous cardiorespiratory predictive monitoring used in conjunction with biomarker testing could prove useful, both for early initiation of antibiotics when the likelihood of sepsis is high and for sparing antibiotics when biomarkers and physiomarkers indicate low sepsis risk.

5 Conclusion

In conclusion, in a prospective cohort of VLBW infants, inflammatory biomarkers discriminated between late-onset sepsis due to gram-negative bacteremia or NEC and all other samples, including those collected at times with or without suspected sepsis. Biomarkers measured at the time of sepsis due to gram-positive bacteremia did not differ from those measured at times without sepsis. Several inflammatory biomarkers, measured at baseline and at the time of suspected or confirmed sepsis, correlated with cardiorespiratory physiomarkers of sepsis.

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 University of Virginia institutional review board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

RK: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. SK: Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. AG: Data curation, Project administration, Investigation, Writing – review & editing. KF: Investigation, Methodology, Supervision, Writing – review & editing, Conceptualization. MA: Methodology, Validation, Writing – review & editing, Data curation, Formal Analysis, Investigation, Resources. WP: Supervision, Validation, Writing – review & editing, Resources. BS: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Funding acquisition, Writing – review & editing.

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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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Time to recovery from neonatal sepsis and its determinants among neonates admitted in Woldia comprehensive specialized hospital, Northeast Ethiopia: a retrospective cohort study

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Background: Neonatal sepsis is the most serious problem in neonates. It is the leading cause of neonatal death in developing countries, particularly in sub-Saharan Africa. The Ethiopian 2016 Demographic Health Survey report revealed that a high number of neonatal deaths are associated with neonatal sepsis. However, limited studies are available on exposure and time to recovery inferences in Ethiopia. Therefore, this study aimed to assess the time to recovery from neonatal sepsis and its determinants among neonates admitted to Woldia Comprehensive Specialized Hospital (WCSH), Northeast Ethiopia.
Methods: A retrospective cohort study was conducted, including 351 neonates, using systematic random sampling at WCSH from 7 to 30 March 2023. The data were entered into Epi data version 4.6 and exported to STATA 14 for analysis. Cox regression was used to identify the determinants of time to recovery from neonatal sepsis, and a variable with a *p*-value of less than 0.05, was used to declare significant association at a 95% confidence interval.
Result: Among 351 neonates with sepsis, 276 (78.63%) recovered, and the median time to recovery was 6 days. Induced labor (AHR = 0.54, 95% CI: 0.369, 0.78) and resuscitation at birth (AHR = 0.7, 95% CI: 0.51, 0.974) were significantly associated with the recovery time of neonatal sepsis.
Conclusions and recommendation: The time to recovery from neonatal sepsis is comparable to previous studies' results. The 25th and 75th percentiles were 4 and 8 days, respectively. Health professionals working in the NICU need to pay special attention to neonates born from mothers who had induced labor and those who were resuscitated at birth.

KEYWORDS

neonatal sepsis, determinants, time to recovery, Woldia, Ethiopia

Abbreviations

APGAR, activity, pulse, grimace, appearance, respiration; DOHS, duration of hospital stay; EDHS, Ethiopian demographic and health survey; EONS, early onset neonatal sepsis; LONS, late-onset neonatal sepsis; MSAF, meconium stained amniotic fluid; NICU, neonatal intensive care unit; ND, neonatal deaths; NS, neonatal sepsis; PIH, pregnancy induced hypertension; PROM, prolonged rupture of membrane; RDS, respiratory distress syndrome; SVD, spontaneous vaginal delivery; WCSH, Woldia comprehensive specialized hospital; CHR, crude hazard ratio; CI, confidence interval; AHR, adjusted hazard ratio.

Background

Neonatal sepsis (NS) refers to a systemic infection that occurs within the first 28 days of life (1). It is classified into two categories: early-onset NS, which occurs within the first 7 days, and late-onset NS, which occurs after 7 days of age (2–4). NS can be caused by bacterial, viral, or fungal pathogens (5). The clinical presentation of NS is often nonspecific and includes symptoms such as feeding difficulties, fever or hypothermia, respiratory distress, grunting, cyanosis, and apnea (6). The prevalence of specific pathogens causing NS varies across different regions, and reports from developing countries commonly indicate the presence of gram-negative organisms (7).

Sepsis is a significant contributor to global mortality, particularly in the neonatal population. Annually, there are approximately 6.3 million neonatal deaths worldwide, with the majority (73%) occurring in developing countries (8). Neonatal sepsis accounts for an estimated 26% of deaths under 5, with sub-Saharan Africa experiencing the highest mortality rates (9). Moreover, the highest incidence of sepsis is also found in neonates, affecting approximately 3 million infants globally (equivalent to 22 per 1,000 live births). The mortality rate associated with neonatal sepsis ranges from 11% to 19%, and there are also unquantified long-term neurological complications (10).

A study conducted across 12 clinical sites In middle- and low-income countries revealed that the incidence of clinically suspected sepsis was 166 per 1,000 live births, while laboratory-confirmed sepsis occurred at a rate of 46.9 per 1,000 live births. The overall all-cause mortality rate was 0.83 per 1,000 neonates. Notably, the majority of laboratory-confirmed sepsis cases occurred within the first three days of life, indicating a higher prevalence of early-onset neonatal sepsis (11). In line with these findings, the Ethiopian Demographic Health Survey 2016 reported a neonatal mortality rate of 29 per 1,000 live births. Neonatal sepsis was identified as a significant contributing factor to this high number of deaths (12, 13).

The recovery rate and duration of hospital stay for neonates with sepsis vary across different countries and regions. For instance, a study conducted in Central India reported a recovery rate of 61.8% among neonates with sepsis, with an average hospital stay of 9.7 days for those who survived (14). In Southern Ethiopia, approximately 91.4% of septic neonates were reported to recover, with a mean time to recovery of 12.74 days (15). Another study conducted in Central Gondar, Ethiopia, reported a median time to recovery from neonatal sepsis of 7 days (interquartile range, 5–10 days) (16).

Various factors can affect time to recovery from neonatal sepsis. Studies conducted in Central Gondar, Ethiopia, and Sri Lanka showed that maternal chorioamnionitis, preterm labor, intrapartum fever, and PROM are the major determinants of time to recovery from neonatal sepsis (16, 17). Additionally, a study conducted in Tikur Anbessa Specialized Hospital showed that neonates born to mothers with tract infections and prolonged hospitalization had an increased chance of hospital stay (18).

The achievement of the third Sustainable Development Goal for Child Health, which aims to eliminate preventable deaths of newborns and children under 5 by 2030, heavily relies on a substantial reduction in neonatal mortality caused by infections, particularly in developing countries (19). In Ethiopia, there is limited research available on the exposure and time-to-recovery patterns of neonatal sepsis, highlighting the need for further studies in diverse geographical areas to identify the factors influencing recovery in different populations and settings (16). Therefore, this study aimed to assess the time to recovery from neonatal sepsis and its determinants among neonates admitted to the neonatal intensive care unit of the WCSH in Northeast Ethiopia.

Methods

Study design and setting

A retrospective follow-up study was conducted from 7–30 March 2023 at Woldia Comprehensive Specialized Hospital (WCSH). It is located approximately 521 km and 360 km from Addis Ababa and Bahir Dar, respectively (20, 21). There were 10 nurses, two neonatal nurses, two general practitioners, and two pediatricians who provided services for admitted neonates. Annually, 750–1,000 neonates are admitted because of various health problems. Approximately 90% of neonates were admitted from the labor ward and approximately 10% were referred from nearby health institutions (22).

Source populations

The source populations were neonates within 28 days of diagnosis of neonatal sepsis who had been admitted to the NICU of WCSH.

Study populations

The study populations were neonates within 28 days of being diagnosed with neonatal sepsis who had been admitted to the NICU of WCSH between January 2021 and December 2022.

Eligibility criteria

Inclusion criteria

Neonates diagnosed with neonatal sepsis and admitted to the NICU of WCSH from January 2021 to December 2022 were included in the study.

Exclusion criteria

The study excluded neonates who died within 30 min of admission.

Sample size estimation and sampling techniques

The sample size was calculated using STATA software version 14, a sample size for time-to-event data, considering a 95% confidence interval (CI), alpha (0.05), probability of recovery of 0.8098, a hazard ratio for the determinant factors, percentage of recovery, and power of 80% (0.80). We used three variables to estimate the sample size. The sample sizes for the three variables, namely birth weight, intrapartum fever, and time of infection onset, were 351, 313, and 121, respectively, at the Central Gondar Public Hospital (16). Thus, the final sample size was the largest among the 351 mother–newborn pairs.

A systematic random sampling technique was used to recruit the study participants. Using medical numbers of neonates admitted to NICU from January 2021 to December 2022 with neonatal sepsis, a sampling frame was prepared separately for each year. The final sample size was proportionally allocated to each year (124 and 227 neonates in 2021 and 2022, respectively). Lastly, a systematic random sampling technique was used ($K = N/n = 774/351 = 2.205 \approx 2$) to select the study participants from the sampling frame.

Study variables and measurements

The dependent variable was the time to recovery from neonatal sepsis, defined as the time from admission to discharge when the neonate had recovered from NS (1, 15, 16, 23, 24).

Socio-demographic variables: maternal age, residence, age of neonate at admission, and neonate sex.

Maternal-related variables: parity, gravidity, onset of labor, duration of labor, mode of delivery, place of delivery, ANC visits, multiple pregnancies, Pregnancy-Induced Hypertension (PIH), antepartum hemorrhage, intrapartum fever, duration of PROM, maternal infection history, and chronic illness.

Neonate-related variables: birth weight, gestation of neonate at birth, admission weight, temperature, 1-minute and 5-minute Apgar scores, congenital anomalies, resuscitation at birth, meconium aspiration syndrome, respiratory distress, and kept in kangaroo mother care within one hour of birth.

Clinical & medical care-related and investigation variables: jaundice, cyanosis, and white blood cell count in the complete blood profile, onset of infection, enteral feeding, critical conditions, and outcome status.

Healthcare service-related variables: the prompt initiation of treatment and the timing of seeking medical care after the neonate fell ill.

Recovery: if a neonate recovered from the infection after completing treatment, according to the physician's diagnosis.

Median time to recovery: the average duration it took for neonates to be declared as recovered by the attending physicians in the unit.

Operational and term definitions

Neonatal Sepsis: if the neonate was diagnosed as having neonatal sepsis by the attending physician after taking a detailed medical history, physical examination, and laboratory tests based on the integrated management of neonatal and childhood illness criteria. The criteria include the presence of two or more persistent fevers (≥37.5°C) or persistent hypothermia (≤35.5°C) lasting for more than 1 h, rapid breathing (≥60 breaths per minute), severe chest indrawing, grunting, poor feeding, movement only when stimulated, a bulged fontanelle, convulsions, lethargy, or unconsciousness. In addition, two or more of the following hematological criteria were used: total leukocyte count (<4,000 or >12,000 cells/mm^3), absolute neutrophil count (<1,500 cells/mm^3 or >7,500 cells/mm^3), platelet count (<150 or >450 cells/mm^3), and random blood sugar (<40 mg/dl or >125 mg/dl) (4, 25, 26).

Defaulter: A neonate is considered a defaulter when they voluntarily stop or leave the treatment unit against medical advice or without completing the prescribed treatment.

Congenital anomalies: Congenital anomalies refer to structural or functional abnormalities that are present in a neonate at birth. These anomalies can involve various body systems, such as heart defects, neural tube defects, and genetic conditions like Down syndrome.

Death: In the context of this study, death refers to the unfortunate event of a neonate passing away either during the treatment period or while still admitted to the treatment unit due to neonatal sepsis.

Censored: A neonate is considered censored when they either default from the treatment, experience death, or are transferred out of the treatment unit. Censored cases are no longer actively followed up or included in the analysis of outcomes.

Length of stay: The length of stay refers to the duration in days that a neonate remains in the hospital from the time of admission until the occurrence of an event of interest (such as recovery) or until the neonate is censored (due to defaulter status, death, or transfer out).

Early initiation of treatment: Initiate broad-spectrum antimicrobials within the first hour of diagnosis.

Prolonged rupture of membrane: The time from membrane rupture to delivery >18 h (27).

Normal WBC range: the normal range of WBC is from 5,000–12,000 cells/μl.

Early initiation of treatment: initiate broad-spectrum antimicrobials within the first hour of diagnosis.

Supportive care: giving care or support with oxygen and feeding without drug management.

Critical conditions: when a neonate is hypoxic, hypoglycemic, or unable to maintain a normal body temperature.

Data collection instruments and procedures

The data for this study were collected using a checklist that was developed based on previous studies. To gather the necessary

information, the medical records of both the mothers and neonates were thoroughly reviewed. The checklist encompassed various domains, including neonatal and maternal socio-demographic characteristics, maternal health-related factors, neonatal health-related factors, healthcare service-related factors, and clinical and medical care-related factors. These domains were carefully selected to provide a comprehensive understanding of the factors that may influence the outcomes of neonates with sepsis.

Data quality assurance

Prior to commencing the data collection process, a preliminary review was conducted on a subset of the sample, specifically 15 neonates, which accounted for 5% of the total sample size. This review served as a pilot study to ensure the effectiveness and feasibility of the data collection tool. The data collection tool was prepared in English, considering the language proficiency of the nursing professionals involved in the study. These professionals were recruited specifically for the purpose of extracting variables from the medical records of the neonate and mother. To ensure the accuracy and consistency of data collection, the investigators provided a comprehensive one-day training session to the nursing professionals. This training covered the purpose and objectives of the study, the techniques for data collection, and the ethical considerations involved in handling patient information.

Throughout the data collection period, the investigators closely monitored the completeness and consistency of the collected data. Daily checks were conducted to identify any discrepancies or missing information, and necessary corrections were made promptly. By conducting a preliminary review, providing appropriate training, and implementing rigorous quality control measures, the study aimed to maintain the integrity and reliability of the collected data.

Data processing and analysis

After the data collection process, the collected data underwent a series of cleaning, coding, and entry procedures using Epi Data version 4.6. To determine the factors associated with recovery time from neonatal sepsis, Cox regression analysis was conducted. This statistical method allows for the examination of time-to-event data and was deemed appropriate for this study. Initially, variables with a *p*-value less than 0.25 were selected as candidates for the subsequent multivariate Cox regression analysis. In the multivariate analysis, variables with a *p*-value less than 0.05 were considered statistically significant, and their results were reported with 95% confidence intervals. This approach ensured that only the most relevant and impactful factors were considered in determining recovery time. Additionally, to address any potential issue of multicollinearity, the time required for recovery was taken into account during the analysis. Finally, the data were presented in figure, table, and narrative form.

Result

Socio-demographic characteristics of study participants

A total of 124 out of 273 neonates and 227 out of 501 admitted neonates in the years 2021 and 2022, respectively, were included in the study (a total of 351 neonates diagnosed with sepsis). The majority of the mothers of these neonates were aged 25–29 (31.91%), followed by those aged 20–24 (29.06%). The mean age of the neonates upon admission was 42 h (1.75 days), and the mean age of the mothers was 26.9 years. Out of the total number of neonates in the study, 270 (76.29%) were only one day old, and 221 (62.96%) were male. More than half of the respondents were living in rural areas (52.71%) (Table 1).

Maternal health-related factors of study participants

Of the neonates admitted to the NICU with NS, 299 (85.19%) were delivered at the same facility. More than half of the mothers were primiparous (51.28%), and the majority had ANC visits. Furthermore, 28 (7.98%) mothers had a history of infection during their pregnancy, and 40 (11.4%) of the mothers gave birth to twins, with either one developing neonatal sepsis. Among the mothers of admitted neonates, 78 (22.22%) had prolonged labor (Table 2).

Neonatal health-related factors of study participants

Among the neonates admitted with NS, 248 (70.66%) were at 37–42 weeks of gestational age and 134 (38.18%) weighed less than 2.5 kg at admission. In total, 127 (36.18%) of them had a 1-minute Apgar score of seven or above. Approximately 11 neonates with neonatal sepsis (3.13%) had a congenital abnormality (Table 3).

TABLE 1 Socio-demographic characteristics of neonates admitted with neonatal sepsis in WCSH, Northeast Ethiopia, 2022 ($n = 351$).

Variable	Categories	Frequency	Percentage (%)
Age of mother	15–19	34	9.69
	20–24	102	29.06
	25–29	112	31.91
	30–34	61	17.38
	35–49	42	11.97
Residence	Urban	166	47.29
	Rural	185	52.71
Neonatal age on admission	Within 1day	270	76.92
	1–6 days	47	13.39
	≥7 days	34	9.69
Sex of neonate	Male	221	62.96
	Female	130	37.04

TABLE 2 Maternal health-related factors of neonates admitted with neonatal sepsis in WCSH, Northeast Ethiopia, 2022 ($n = 351$).

Variable	Categories	Frequency	Percentage (%)
Gravidity	Prim gravida	176	50.14
	Multigravida	175	49.86
Parity	Primiparous	180	51.28
	Multiparous	171	48.72
ANC follow-up	Yes	285	81.20
	No	66	18.80
Multiple pregnancies	Yes	40	11.40
	No	311	88.60
Onset of labor	Spontaneous	297	84.62
	Induced	54	15.38
Place of delivery	Out of this hospital	52	14.81
	In this hospital	299	85.19
Mode of delivery	SVD	197	56.13
	Instrumental vaginal	64	18.23
	Cesarean section	90	25.64
Duration of labor	Normal	273	77.78
	Prolonged	78	22.22
PROM	Yes	74	21.08
	No	277	78.92
Duration of PROM	Normal	58	78.38
	Prolonged	16	21.62
Maternal fever	Yes	22	6.27
	No	329	93.73
APH	Yes	13	3.70
	No	338	96.30
PIH	Yes	20	5.70
	No	331	94.30
Maternal infection history	Yes	28	7.98
	No	323	92.02
Chronic illness history	Yes	11	3.13
	No	340	96.87

ANC, antenatal care; APH, antepartum hemorrhage; PIH, pregnancy-induced hypertension; PROM, premature rupture of membrane; SVD, spontaneous vaginal delivery.

TABLE 3 Neonatal health-related factors of neonates admitted with neonatal sepsis in WCSH, Northeast Ethiopia, 2022 ($n = 351$).

Variable	Categories	Frequency	Percentage (%)
Gestational age at birth	<37 weeks	95	27.07
	37–42 weeks	248	70.66
	>42 weeks	8	2.28
1-minute apgar score	Unknown	17	4.84
	<7	207	58.97
	≥7	127	36.18
5-minute apgar score	Unknown	17	4.84
	<7	137	39.03
	≥7	197	56.13
Birth weight	<2.5 kg	135	38.46
	2.5–4 kg	208	59.26
	>4 kg	8	2.28
Weight at admission	<2.5 kg	134	38.18
	2.5–4 kg	206	58.69
	>4 kg	11	3.13
Admission temperature (°C)	<36.5	181	51.57
	36.5–37.5	118	33.62
	>37.5	52	14.81
Admission respiratory rate	<30	30	8.55
	30–60	262	74.64
	>60	59	16.81
EBF initiate within 1st hour	Yes	257	73.22
	No	94	26.78
Kept in KMC within 1st hour	Yes	105	29.91
	No	246	70.09
Resuscitated at birth	Yes	141	40.17
	No	210	59.83
MAS	Yes	34	9.69
	No	317	90.31
RDS	Yes	77	21.94
	No	274	78.06
Congenital anomalies	Yes	11	3.13
	No	340	96.87

EBF, exclusive breastfeeding; KMC, Kangaroo mother care; MAS, meconium aspirated syndrome; RDS, respiratory distress syndrome.

Clinical and medical investigations and health service-related factors

Among the neonates admitted with sepsis, 317 (90.31%) had early-onset neonatal sepsis and 267 (76.07%) initiated treatment within 1 h. Among those with CBC profiles, 170 (48.43%) were within the normal range of WBC counts (Table 4).

Sepsis outcome and time to recovery analysis

Among the 351 study participants, 276 (78.63%) successfully recovered from NS, 45 (12.82%) died, 11 (3.13%) were referred, and 19 (5.41%) defaulted/left against medical advice (Figure 1). The mean time to recovery was nearly 7 days, with IQR = 5–10 days, and the median time was 6 days. For preterm neonates included in the study, the mean time to recovery was nearly 8 days (7.71 days) and the median recovery time was 8 days, with IQR from 6 days to 10 days.

Kaplan–Meier survival proportional hazard graph was used to check the assumption of Cox proportional hazard. The Kaplan–Meier survival curve, performed on the time to recovery in neonates with sepsis based on resuscitation at birth, showed that recovery was delayed among neonates who were resuscitated at birth compared to those who were not resuscitated (Figure 2).

The Cox proportional hazards regression survival graph shows the time to recovery of neonates with sepsis, based on the onset of sepsis. Therefore, in neonates with sepsis, EONS has a higher probability of recovery than LONS (Figure 3). In addition, the global test was performed with a χ^2 of 5.92 and a p-value of 0.2056, which is greater than 0.05. The dependent variable was std. err of 2.668803 and 95% CI: (170.2511, 180.7489).

Determinants of time to recovery from neonatal sepsis

After testing, each variable in the study with bivariable analysis ($p \leq 0.25$) was entered into the multivariable Cox regression analysis (Table 5). Multivariable Cox regression model analysis

TABLE 4 Clinical and medical investigations and health service-related factors of neonates admitted with neonatal sepsis in WCSH, Northeast Ethiopia, 2022.

Variable	Category	Frequency	Percentage (%)
Severe jaundice	Yes	24	6.84
	No	327	93.16
Cyanosis	Yes	21	5.98
	No	330	94.02
Onset of infection	EONS	317	90.03
	LONS	34	9.97
WBC in CBC profile	Normal	170	48.43
	Abnormal	149	42.45
	No information	32	9.12
Non-oral enteral feeding	Yes	149	42.45
	No	202	57.55
Being in a critical condition	Yes	143	40.74
	No	208	59.26
Antibiotics given	Yes	340	96.87
	No	11	3.13
Supportive care	Yes	177	50.43
	No	174	49.57
Blood transfusion	Yes	28	7.98
	No	323	92.02
Anticonvulsant	Yes	30	8.55
	No	321	91.45
Phototherapy	Yes	23	6.55
	No	328	93.45
Weight at discharge (in grams)	<2,500	115	32.76
	2,500–4,000	230	65.53
	>4,000	6	1.71
Early initiation of treatment	Yes	267	76.07
	No	84	23.93
Time to health facility visit after onset of illness	≤3 h	183	52.14
	>3 h	168	47.86

WBC, white blood count; CBC, complete blood count; EONS, early-onset neonatal sepsis; LONS, late-onset neonatal sepsis.

was performed to show the association between covariates and time to recovery from neonatal sepsis.

Labor Induction [AHR = 0.538, 95% CI: (0.369, 0.78)] and resuscitation at birth [AHR = 0.7, 95% CI: (0.51, 0.974)] have a significant association with the recovery time of neonates. Cox proportional hazard regression analysis showed that the chance of recovery decreased as the length of hospital stay increased.

Discussion

This study assessed determinants of time to recovery from neonatal sepsis among neonates admitted to the WCSH in Northeast Ethiopia. Among the 351 neonates with sepsis, 276 (78.63%) recovered, and the median time to recovery was 6 days. Induction of labor and resuscitation at birth have a significant association with recovery time.

This study found that 78.63% of the patients successfully recovered from neonatal sepsis. This is lower than the findings of the study conducted in Dire Dawa (23). This might be due to the higher censoring of admitted septic neonates in the study area of scarce resources because of the northern Ethiopian conflict. Moreover, a greater number of septic neonates admitted were male and rural dwellers, which is in line with studies conducted in Dire Dawa (23) and Central Gondar (16). Furthermore, it was also realized that early-onset neonatal sepsis was the predominant type (90.31%). This finding is congruent with studies conducted in different parts of Ethiopia, such as Mekelle (28), Central Gondar (16), Dire Dawa (23), and Debrezeit (29). A similar finding was also reported in a Western African study conducted in Ghana (30).

The median time to recovery from NS in this study was 6 days. This finding corresponds to the 7-day findings of Dire Dawa (23)

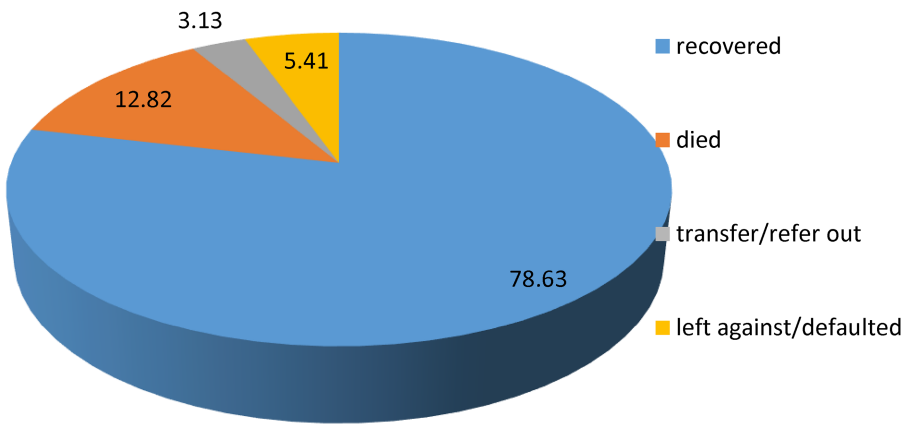

FIGURE 1
Outcomes of neonatal sepsis for neonates admitted with neonatal sepsis in WCSH, Northeast Ethiopia, 2022.

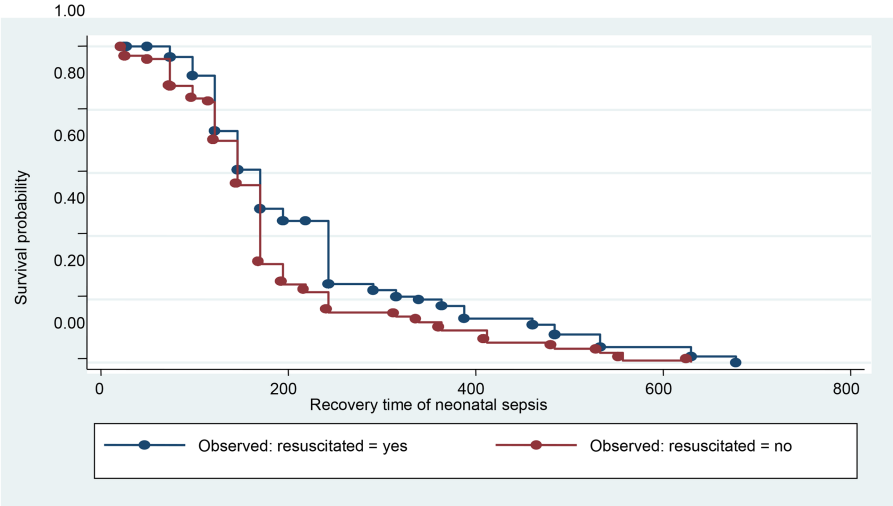

FIGURE 2
Kaplan–Meier survival curve of neonatal sepsis by resuscitation.

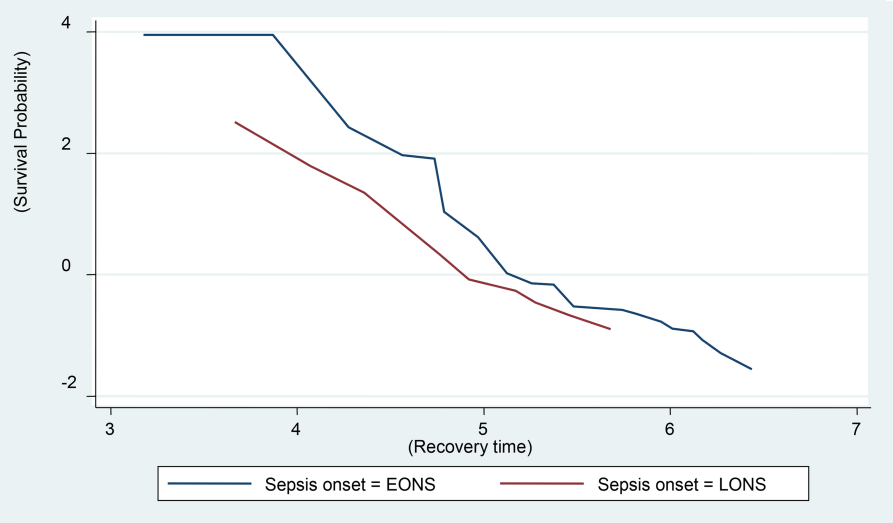

FIGURE 3
Cox proportional hazards regression survival graph of neonatal sepsis.

and Central Gondar (16). This is also comparable to the findings of earlier studies conducted in Uganda (31) and India (32), which reported median times to recovery for septic neonates of 4.5 and 5.5 days, respectively. These studies shared certain characteristics with the current investigation, such as being conducted on newborns admitted to public hospitals, having a neonate age limit of 0–28 days, having similar sample sizes, and considering clinically confirmed cases.

However, compared to a study conducted in Central India, the mean time to recovery of neonates was 9.67 days (14). This dissimilarity could be attributed to the differences in the study population. Unlike the current study, all neonates included in the Central India study were referred cases or high-risk populations who had a higher likelihood of delayed recovery due to factors such as delay in seeking health care or delay in referral. Additionally, nearly 50% of neonates had low birth weight (LBW) (14), which predisposes them to a lengthy recovery period, whereas our study had a smaller proportion of LBW neonates.

Furthermore, the current study finding was lower than that of the study conducted in the Arba Minch, Sawla, and Chencha hospitals, which indicated that the mean time to recovery of newborns was 12.74 days (15). The observed difference in this study can be attributed to differences in the diagnosis of neonatal sepsis. In the study conducted in Arba Minch, Sawla, and

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TABLE 5 Cox regression analysis showing the association between covariates and time to recovery from neonatal sepsis in WCSH ($n = 351$).

Variable	Recovery from neonatal sepsis		CHR (95% CI)	AHR (95% CI)	*p*-value
	Censored	Event			
Pregnancy					
Multigravida	6	34	0.775 (0.54, 1.11)	0.84 (.545, 1.29)	0.427
Primigravida	69	242	1	1	
Onset of labor					
Spontaneous	65	232	1	1	0.001*
Induced	10	44	.627 (0.45, 0.87)	0.54 (.369, .78)	
Duration of labor					
Normal	47	226	1	1	0.624
Prolonged	28	50	1.26 (.926, 1.72)	1.09 (.77, 1.54)	
APH					
Yes	2	11	0.79 (0.37, 1.26)	0.84 (.429, 1.65)	0.616
No	73	265	1	1	
PIH					
Yes	5	15	1.52 (0.90, 2.56)	1.12 (.61, 2.056)	0.709
No	70	261	1	1	
Gestational age at birth					
<37 weeks	22	73	0.83 (0.63, 1.08)	1.02 (.689, 1.518)	0.909
37–42 weeks	51	197	1	1	
>42 weeks	2	6	0.40 (0.18, 0.91)*	0.46 (.172, 1.259)	0.132
Birth weight					
<2.5 kg	35	98	0.67 (0.52, 0.86)*	0.31 (.089, 1.049)	0.060
2.5–4 kg	340	175	1	1	0.891
>4 kg	1	2	0.95 (0.47, 1.94)	1.07 (.41, 2.78)	
Admission weight					
<2.5 kg	33	99	0.68 (0.53, 0.88)*	2.07 (.577, 7.42)	0.264
2.5–4 kg	42	171	1	1	0.779
>4 kg	1	5	0.68 (0.37, 1.25)	1.13 (.485, 2.625)	
Temperature					
<36.5°C	45	136	1.18 (0.90, 1.54)	1.15 (1.19, 2.06)	0.090
36.5–37.5°C	24	94	1	1	0.858
> 37.5°C	6	46	0.85 (0.59, 1.22)*	1.04 (.69, 1.555)	
RR at admission					
<30	10	20	1.01 (.63, 1.59)	1.46 (.839, 2.537)	0.180
30–60	50	212	1	1	0.081
>60	15	44	0.71 (.51, .994)*	0.72 (.503, 1.04)	
KMC within 1 h					
Yes	17	88	0.80 (0.62, 1.03)	0.93 (.65, 1.329)	0.694
No	58	188	1	1	
Resuscitation at birth					
Yes	34	107	0.76 (0.59, 0.96)	0.70 (.51, .974)	0.034*
No	41	169	1	1	
RDS					
Yes	20	57	0.75 (0.55, 1.02)	0.98 (.655, 1.458)	0.910
No	55	219	1	1	
Initiation of treatment					
Early	63	204	1	1	0.165
Late	12	72	1.43 (1.09, 1.88)	1.27 (.906, 1.779)	
Time of seeking care					
3 or more hours	44	139	1	1	0.214
>3 h	31	137	0.84 (.656, 1.063)	0.83 (.61, 1.117)	
Cyanosis					
Yes	10	11	1.47 (0.80, 2.69)	1.4 (.708, 2.786)	0.331
No	65	265	1	1	
In critical condition					
Yes	50	93	0.79 (0.61, 1.02)	0.96 (.69, 1.338)	0.824
No	25	183	1	1	
IV antibiotics					
Yes	65	275	0.19 (0.023, 1.57)	0.19 (0.02, 1.604)	0.128
No	10	1	1	1	

APH, antepartum hemorrhage; PIH, pregnancy-induced hypertension; RR, respiratory rate; IV, intravenous; KMC, Kangaroo mother care; RDS, respiratory distress syndrome; 1, reference category.
*Significant at $p < 0.05$.

Chencha hospitals, neonatal sepsis was identified by blood culture (15). As a result, the study recovery time may have been longer than the median recovery time in the current study. In addition, the difference in recovery times could be attributed to the difference in the proportion of preterm neonates admitted to the NICU who had a high risk of delayed recovery, at 60% (15) compared to 22.5% in the current study. Furthermore, the proportion of LBW neonates (44%), which was larger than the current study, could also explain this disparity.

The time to recovery from NS is mainly influenced by factors such as induced labor and resuscitation at birth. Neonates delivered from mothers who had induced labor had a 46% delay in time to recovery from NS compared to those born to mothers whose labor started spontaneously. The findings of this investigation are in agreement with studies conducted in Central Gondar (16) and Arba Minch, Sawla, and Chencha hospitals (15). Since women who undergo induction of labor often have certain health problems, the neonate may be initially exposed to risks that could lead to adverse outcomes. Furthermore, it is recommended to offer induction of labor for ruptured membranes, even if it is associated with unfavorable outcomes (15, 33). This could prolong the duration of recovery from NS.

A longer time to recovery from sepsis was observed in neonates who required resuscitation at birth. Comparable findings were revealed in a study done in Central Gondar (16) and in a systematic review of the prognosis (34), indicating that resuscitation with a bag and mask was associated with a 28% delay in recovery from neonatal sepsis. Additionally, studies conducted in Ethiopia (24, 35), Ghana (30), and Tanzania (36) found that resuscitated neonates have a higher chance of developing NS than non-resuscitated neonates. Infants who require resuscitation may face problems in their respiratory or circulatory systems. Furthermore, some resuscitation methods may be invasive for newborns. In addition, neonates who required resuscitation were asphyxiated, and asphyxia lengthened the hospital stay during the NS recovery period.

Limitations of the study

The diagnosis of NS depended on clinical symptoms, which may result in an increase in false-positive cases, potentially affecting the results of the study.

Conclusions and recommendations

The time to recovery from sepsis is independently and negatively related to the induction of labor and resuscitation at birth. The final median time to recovery of neonates admitted to the WCSH neonatal intensive care unit was equivalent to that reported in studies conducted in different hospitals in Ethiopia.

Professionals working on NICU wards need to pay special attention to neonates born from mothers who had induced labor and those who were resuscitated at birth. Internal assessment of resuscitation procedures is also important. Further research on confirmed neonatal sepsis cases (EONS and LONS) using prospective follow-up studies in different geographical areas is needed to identify the different determinant factors.

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 Woldia University Institutional review board. 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

KA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HK: Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. AY: Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. EM: Data curation, Formal Analysis, Methodology, Supervision, Writing – review & editing. ZW: Data curation, Formal Analysis, Methodology, Supervision, Writing – review & editing.

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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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Case Report: Micafungin for treating *Candida glabrata* urinary infection: a clinical case in a premature neonate

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Urinary tract infections (UTIs) associated with indwelling urinary catheterization (IUC) in premature newborns (PNBs) pose a significant challenge in neonatal intensive care units (NICUs) due to the vulnerability of this population to infections and the necessity of invasive procedures. While bacterial UTIs have historically been predominant, there is a rising incidence of fungal pathogens, particularly non-*albicans Candida* strains like *Candida glabrata* and *Candida tropicalis*, attributed to broad-spectrum antibiotic use. Diagnosis of fungal UTIs in a PNB relies on culturing *Candida* spp. from properly collected urine samples, particularly critical in very low birth weight (VLBW) PNBs because of the risk of invasive candidiasis and associated complications. We present a case of an extremely premature newborn (EPNB) successfully treated for a UTI caused by *C. glabrata* with micafungin. Our case exhibits micafungin as a potentially safe and effective alternative for treating *C. glabrata* UTIs in neonates.

KEYWORDS

Candida glabrata, micafungin, urinary tract infection, premature infant, case report

Introduction

Urinary tract infections (UTIs) associated with indwelling urinary catheterization (IUC) (often required to ensure continuous drainage and measures of urinary output) in premature newborns (PNBs) represent a nosocomial infection prevalent within neonatal intensive care units (NICUs), owing to the increased susceptibility to infections in this population and the required employment of invasive procedures and supportive devices (1). Although UTIs are predominantly bacterial, the incidence of fungal pathogens in UTIs is on the rise (2, 3). Despite *Candida albicans* being the predominant pathogenic species historically, recent years have witnessed a notable surge in UTIs attributed to non-*albicans Candida* strains (30%–50%), notably *Candida glabrata* and *Candida tropicalis*, attributable to the widespread use of broad-spectrum antibiotics (3).

The acquisition of a positive *Candida* spp. culture (obtained through sterile technique) from urine in the absence of candidemia is pivotal in diagnosing UTIs caused by fungal pathogen (4). Candiduria in very low birth weight (VLBW) PNBs is associated with a significant risk of invasive candidiasis, which can lead to central nervous system involvement and mortality (4, 5). Symptoms of candidiasis in PNBs are more non-

specific compared to other populations, posing challenges in diagnosis, thus necessitating comprehensive evaluation and early initiation of antifungal therapy (4).

A definitive diagnosis of UTIs in a PNB relies on culturing any microorganism from a properly collected urine sample before treatment initiation. To obtain a urine culture, the gold standard technique is suprapubic aspiration (SPA); alternatively, sterilely collected urine via catheterization (CATH) is considered. Significant bacteriuria is defined as any bacterial count in urine obtained by SPA or >10,000 colony-forming units (CFU)/ml in samples collected by CATH (5).

In the event of a suspected UTI in a PNB, if feasible, the urinary catheter should be replaced to exclude potential colonization. Treatment of fungal UTIs is recommended solely for patients at high risk of dissemination, such as VLBW PNBs, who should be managed as though they have candidemia (4). Antifungal agents are selected based on the sensitivity of the infecting species and their ability to achieve adequate concentrations in both blood and urine (4–6). Fluconazole is the preferred treatment due to its favorable urinary excretion, enabling sufficient concentrations in urine (2, 4). Nonetheless, the rise in non-*albicans Candida* UTIs has prompted changes in resistance profiles. *C. glabrata* demonstrates dose-dependent resistance to fluconazole, while *Candida krusei* is inherently resistant (2). Other azoles (voriconazole, itraconazole, and posaconazole) are deemed less efficacious owing to their limited urinary excretion (4).

Although amphotericin B (AMB) deoxycholate has been recommended as the first-line treatment for UTI in guidelines, currently, it is no longer used in clinical practice due to its significant renal toxicity, rendering it unsuitable for PNBs (2, 4, 6). The administration of liposomal AMB is discouraged for UTI owing to its reduced renal penetration (4, 7).

These circumstances have presented a therapeutic challenge in the treatment of non-*albicans Candida* candiduria, prompting exploration of alternative options such as echinocandins. Echinocandins, through their mechanism of β-glucan synthase inhibition, exhibit fungicidal activity against *Candida* spp. (3, 4). Furthermore, they demonstrate a favorable adverse effect and drug interaction profile compared to azoles (3, 4, 8). However, their urinary excretion percentages in their active form are notably low: 1.4%, 0.7%, and 0.1% for caspofungin, micafungin, and anidulafungin, respectively, limiting their utility in UTI management (9, 10). In terms of pharmacokinetics, micafungin is rapidly and moderately distributed to renal tissues and the bladder, likely explaining its efficacy in these infections (10). In addition, it attains high plasma concentrations, which, despite its low urinary excretion, may yield sufficiently elevated levels in urine to achieve an optimal maximum concentration (Cmax) to minimum inhibitory concentration (MIC) (Cmax/MIC) ratio for UTI treatment (10).

Although recent studies have reported successful micafungin treatment of *Candida* spp. UTIs in hospitalized adults and pediatric patients (3, 8–10), there are currently no data regarding its application in PNBs. Therefore, we present a case of an extremely premature newborn (EPNB) effectively treated for fungal UTI with micafungin.

Case description

A female EPNB was born at 26 weeks of gestation with a birth weight of 709 g. Notably, severe bronchopulmonary dysplasia with secondary pulmonary hypertension was observed during the first months of life, requiring mechanical ventilation since day 74 of life (DOL). The patient presented three episodes of nosocomial sepsis within the first 3 months. The first episode was at 3 DOL with a positive blood culture for *Serratia marcescens*, the second episode at 24 DOL with a positive blood culture for *Enterobacter hormaechei* and *Enterococcus faecalis*, and the last one at 75 DOL with positive urine culture for *Klebsiella* spp. and tracheal aspiration (TA) for *S. marcescens* (Figure 1). The patient has received various antibiotics (amikacin for 5 days, vancomycin for 5 days, and meropenem for 14 and 17 days).

At 95 DOL, the patient was transferred to our center due to severe respiratory failure. Given her critical condition, a clinical and laboratory suspicion of sepsis, and a positive TA for *S. marcescens*, meropenem and vancomycin were initiated. Blood cultures, urine cultures (UC), TA, and urine sediment (US) (patient with IUC) were performed, and the previous epicutaneous catheter was removed. The patient's IUC was not removed because it was essential for monitoring urinary output. At 103 DOL, the blood culture yielded a positive result for *S. marcescens*, leading to the discontinuation of vancomycin and continuation of meropenem treatment for 14 days.

Leukocyturia, hematuria, and abundant yeasts were observed in the US. Consequently, the IUC was replaced, and a second US showed the presence of yeasts. A polymerase chain reaction (PCR) for *Candida* spp. in blood was performed, and treatment with fluconazole was initiated at a loading dose of 12 mg/kg followed by 6 mg/kg/day, as per the usual practice of our center. Three days later, the urine culture yielded a positive result for *C. glabrata* (>10,000 CFU), with a negative blood PCR. Fluconazole treatment was continued, and an invasive fungal infection workup was negative.

After 4 days of fluconazole treatment, and after the replacement of the IUC, a new urine culture showed persistently positive results for *C. glabrata* (>100,000 CFU). An antibiogram revealed intermediate sensitivity of *C. glabrata* to fluconazole (MIC: 8 μg/ml) and sensitivity to echinocandins (caspofungin: MIC 0.06 μg/ml; anidulafungin: MIC 0.03 μg/ml; micafungin: MIC 0.015 μg/ml). Given the presence of two positive urine cultures for *C. glabrata*, along with a new elevation of acute-phase reactants and no clear clinical changes, a multidisciplinary meeting was held with the NICU medical team, the Pediatric Infectious Diseases Unit, and the Pharmacy Department. A literature search for possible therapeutic alternatives suggested the use of echinocandins, leading to the discontinuation of fluconazole and initiation of micafungin treatment. Before starting the new treatment, a new *Candida*

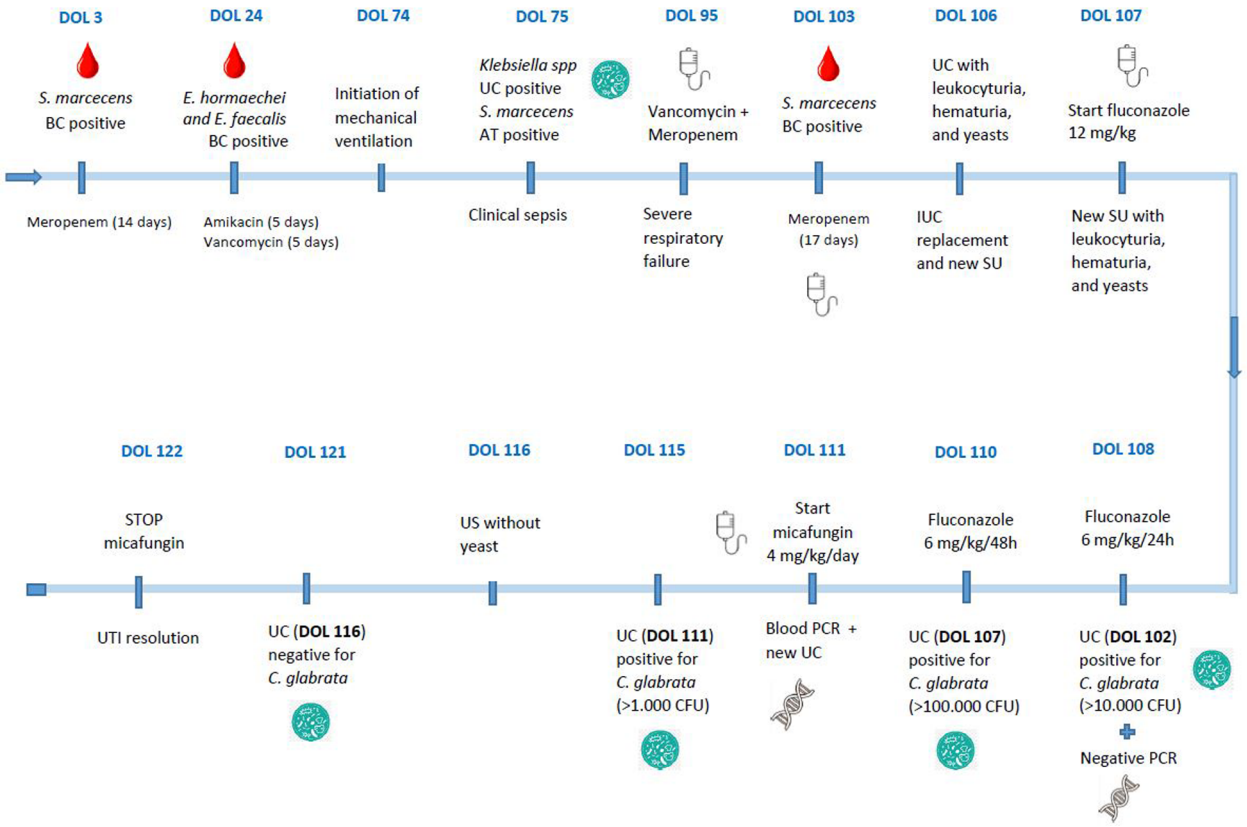

FIGURE 1
Sequence of events of the patient. BC, blood culture; CFU, colony-forming unit; DOL, day of life; IUC, indwelling urinary catheter; UC, urine culture; US, urine sediment; UTI, urinary tract infection.

spp. study in urine was conducted, which still showed positive results for *C. glabrata* (>10,000 CFU), with a negative blood PCR.

Micafungin treatment was initiated at a dose of 4 mg/kg/day. After 5 days of treatment, a urine culture with US showed the absence of yeasts. Based on these findings, it was decided to complete 14 days of micafungin at the same dose, while awaiting the urine culture result. Finally, the urine culture showed a negative result for *C. glabrata*, and clinical improvement was observed, so it was decided to discontinue antibiotic therapy, concluding the eradication of the UTI. No clinical or analytical adverse effects associated with micafungin were reported during or in the days after treatment. The patient died a week later due to pulmonary complications arising from her underlying lung pathology.

Discussion

To the best of our knowledge, we present the first reported clinical case in the literature of an EPNB with VLBW successfully treated for a UTI caused by *C. glabrata* using micafungin.

The dosing of micafungin in neonates for candidemia according to NeoFax recommendations is 4 mg/kg/day (11), as was administered in the present case. However, for the treatment of candiduria, dosing is not firmly established. Recently, Ekinci et al. (3) conducted the first retrospective cohort study in a pediatric population, where 24 patients with fungal UTIs were treated with micafungin at a dose of 2 mg/kg/day, yielding satisfactory results and only one adverse event reported (transaminase increased in one patient). It is noteworthy that in this study, the age of the youngest patient was 2 months (3), an age at which the 2 mg/kg/day dose of micafungin is appropriate (12).

On the other hand, evidence of micafungin use for treating fungal UTIs caused by various *Candida* spp. in adults is more extensive, with several publications covering hundreds of patients (3, 4, 8–10, 13). In terms of the safety profile of micafungin utilization, its propensity for inducing alterations in hepatic biomarkers is widely acknowledged (12). In this context, Auriti et al. (14) investigated the administration of micafungin at elevated doses (8–15 mg/kg/day) in neonates afflicted with systemic candidiasis, observing an elevation in hepatic enzymes, namely alkaline phosphatase and gamma-glutamyltransferase. In that case, no discernible analytical alterations were noted throughout the treatment or in the immediate aftermath of its cessation.

Given that we present a single clinical case and that there may be considerable variability in patients' evolution, especially EPNBs with VLBW, we stress the necessity for further studies and clinical trials in this population, where the available evidence is limited.

Therefore, we conclude that the administration of micafungin in neonates with UTI caused by *C. glabrata* appears to be a safe and effective alternative that could facilitate yeast eradication and patient recovery.

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

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

CP-T: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. CGE: Writing – original draft, Writing – review & editing. MF: Writing – original draft, Writing – review & editing, Supervision. CT: Writing – original draft, Writing – review & editing, Supervision. LG-G: Writing – original draft, Writing – review & editing. CR-C: Writing – original draft, Writing – review & editing, Supervision. MC-P: Writing – original draft, Writing – review & editing.

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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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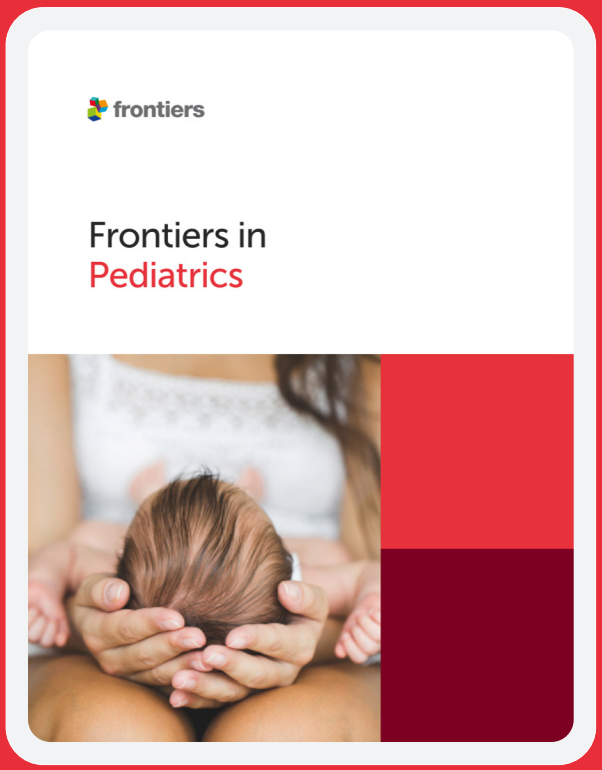

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