frontiers in Nanotechnology

ORIGINAL RESEARCH
published: 13 November 2020
doi: 10.3389/fnano.2020.576342

ZnO Quantum Dots: Broad Spectrum Microbicidal Agent Against Multidrug Resistant Pathogens *E. coli* and *C. albicans*

Preeti[1], *V. S. Radhakrishnan*[1], *Sushanto Mukherjee*[2], *Sujata Mukherjee*[2], *Surinder P. Singh*[3]* *and Tulika Prasad*[1]*

[1] *Special Centre for Nano Sciences and AIRF, Jawaharlal Nehru University, New Delhi, India,* [2] *Gitanjali Patho Diagnostics, Vivekananda Marg, Patna, India,* [3] *CSIR-National Physical Laboratory, Dr. K. S. Krishnan Marg, New Delhi, India*

Infectious microbial diseases are leading causes of morbidity and mortality worldwide. Further, emergence of Multidrug resistance (MDR) in microbes has posed a critical concern for public healthcare and microbial therapeutics. Nano-based drug compositions might be ideal candidates to address the challenges of microbial drug resistance. Herein, we synthesized monodispersed, spherical ZnO quantum dots (QDs) of average diameter 5–6 nm as biosafe, nanomicrobicidal agent against MDR pathogens. The broad spectrum microbicidal potential of ZnO QDs was evaluated against Extended Spectrum Beta Lactamase (ESBL) producing MDR isolates of *E. coli* (from UTI patients with resistance to antibiotics of different classes viz. third & fourth generation of cephalosporin, penicillin, monobactams, and quinolones) and MDR isolates of *C. albicans* (from three different AIDS patients who during therapy acquired drug resistance and sensitive isolates evolved into MDR strains). ZnO QDs exhibited dose dependent, broad spectrum microbicidal activity against all MDR isolates of *E. coli* and *C. albicans*. Highly reduced growth indicating highly reduced cell viability was observed in all MDR isolates of *E. coli* and *C. albicans* at minimum inhibitory concentration (MIC) of 200 μg/ml ZnO QDs and the growth/ cell viability was further reduced in presence of 250 and 400 μg/ ml of ZnO QDs for *E. coli* and *C. albicans*, respectively. To the best of our knowledge, microbicidal potential of ZnO QDs against microbial strains exhibiting MDR for currently used drugs has not been studied. Results of present study indicate that ZnO QDs might be promising as next generation broad spectrum alternative for combating MDR in microbial pathogens.

Keywords: ZnO QDs, multi drug resistance (MDR), antimicrobial therapy, broad spectrum, microbicidal

OPEN ACCESS

Edited by:
Yogendra Kumar Mishra, University of Southern Denmark, Denmark

Reviewed by:
Won Min Park, Kansas State University, United States
Mubarak A. Mujawar, Florida International University, United States

***Correspondence:**
Tulika Prasad
prasadtulika@hotmail.com;
prasadtulika@mail.jnu.ac.in
Surinder P. Singh
singh.uprm@gmail.com

Specialty section:
This article was submitted to Biomedical Nanotechnology, a section of the journal Frontiers in Nanotechnology

Received: *25 June 2020*
Accepted: *14 October 2020*
Published: *13 November 2020*

Citation:
Preeti, Radhakrishnan VS, Mukherjee S, Mukherjee S, Singh SP and Prasad T (2020) ZnO Quantum Dots: Broad Spectrum Microbicidal Agent Against Multidrug Resistant Pathogens E. coli and C. albicans. Front. Nanotechnol. 2:576342. doi: 10.3389/fnano.2020.576342

INTRODUCTION

Infectious microbial diseases remain the leading cause of human mortality worldwide (Bloom and Cadarette, 2019). Among the diseases caused by various microbial species viz. bacteria, viruses, fungi, or parasites, most frequently encountered are bacterial infections such as diarrhea, cholecystitis, bacteraemia, cholangitis, urinary tract infections (UTI), meningitis, and pneumonia (Zhou et al., 2018). World Health Organization (WHO) reported millions of global deaths in

2016 due to diarrhoeal diseases, lower respiratory infections and tuberculosis (Global Health Estimates, 2016). One of the most common bacterial pathogens particularly responsible for UTI, sepsis/ meningitis and enteric/ diarrhoeal diseases is *Escherichia coli* (*E. coli*) (Allocati et al., 2013; Saeed et al., 2015). Of these, UTI is the second most common community-acquired infections and commonly treated by drugs such as penicillins, cepahlosporins (includes third and fourth generation agents), trimethoprim/ sulfamethoxazole, and quinolones (Picozzi et al., 2014). It has been observed that UTI is frequently caused by Extended Spectrum Beta Lactamase (ESBL) producing *E. coli*, which often acquires resistance to all penicillins, cephalosporins, and cross-resistance to trimethoprim/ sulfamethoxazole and quinolones (Picozzi et al., 2014). Other than bacterial infections, fungal infections also are life threatening and responsible for 1.5–2 million deaths annually (Denning and Bromley, 2015). As per Global Action Fund for Fungal Infections (GAFFI), more than 300 million people of all ages suffer from serious fungal diseases, but still fungal infections remain the most neglected among microbial infections (Rodrigues and Nosanchuk, 2020). Common fungal infections such as oral or vaginal candidiasis and life-threatening systemic infections (systemic candidiasis) are caused by *Candida albicans* (*C. albicans*) and around 400,000 cases of *Candida* bloodstream infections are annually recorded worldwide (Pal, 2017). *C. albicans* normally resides as commensal microbial flora of the human gastrointestinal (GI) tract but turns pathogenic in immunocompromised individuals (Mayer et al., 2013). The most prevalent and recurrent fungal infection in HIV-infected patients caused by *C. albicans* is oropharyngeal candidiasis (OPC) (Vazquez, 2010). Prolonged azole drug therapy for OPC often leads to the emergence of drug resistance in *C. albicans* and has emerged as a recognized problem in AIDS patients (Patil et al., 2018).

Various antimicrobial drugs are available for the treatment of microbial infections and each class of drug has a particular mode of action and definite cellular target such as cell wall, membrane lipids, translational machinery, and DNA replication (Liwa and Jaka, 2015; Silver, 2016). The extensive deployment and indiscriminate use of these drugs lead to the emergence of multi drug resistance (MDR) in microbial pathogens wherein microbes evolve with adaptive strategies either to subvert or evade host immune responses or to counter the antibiotics. The primary adaptive strategies of the microbes include drug target modifications, drug degradation, and overexpression of drug efflux pumps (Munita and Arias, 2016). Increasing incidence of microbial MDR is a critical issue in public healthcare and microbial therapeutics. The continuous evolution of drug resistant microbial strains therefore, constantly demands for new and safe antimicrobial agents with broad spectrum activity and minimal host toxicity.

Recent advances in nanotechnology have led to the inception of Nanomedicine with a promise for improved drug delivery, imaging, medical implants, antimicrobials, biosensing platforms for detection of microbial diseases, cancer therapeutics etc. (Nune et al., 2009; Holzinger et al., 2014; Singh et al., 2014; Zhu and Liao, 2015). In order to improve the treatment outcome of microbial infections, nano-based drug compositions might be ideal candidates to address the challenges of microbial drug resistance by favoring multi targeted action, broad spectrum activity and lower host toxicity (Singh et al., 2014). Several metal and metal oxide nanoparticles such as silver, gold, titanium oxide, and zinc oxide are known to possess antimicrobial properties (Singh et al., 2014; Gold et al., 2018). Among various nanoparticles, Zinc oxide (ZnO) nanoparticles maybe a novel antimicrobial agent of choice because of its non-toxic nature as compared to other competitive nanomaterials; further it can also act as micronutrient for regulation of innate and adaptive immune responses (Roy et al., 2014). Although, studies are available on antimicrobial activity of nano ZnO (Joshi et al., 2009; Xie et al., 2011; Ansari et al., 2012; Wahab et al., 2014; Hameed et al., 2016; Farzana et al., 2017; Duffy et al., 2018; Gupta et al., 2018; Hosseini et al., 2018; Jalal et al., 2018), no study so far has reported the antimicrobial activity of ZnO quantum dots (QDs) against MDR pathogens.

In order to develop a biosafe, nano-based therapeutic microbicidal agent, we investigated the broad spectrum microbicidal activity of ZnO QDs on MDR isolates of microbial pathogens (*E. coli* and *C. albicans*). The ZnO QDs were synthesized using wet chemical route and their microbicidal potential were demonstrated against ESBL producing MDR isolates of *E. coli* (with resistance to most of the currently used antibacterial drugs of different classes such as third & fourth generation of cephalosporin, penicillin, monobactams and quinolones) and MDR isolates of *C. albicans* (MDR strains evolved from sensitive strains by acquiring resistance). ZnO QDs appear as promising new-generation broad spectrum microbicidal agent to combat MDR pathogens.

MATERIALS AND METHODS

Materials

For synthesis of ZnO QDs, zinc acetate dihydrate and lithium hydroxide monohydrate were procured from Sigma Aldrich (MO, USA), absolute ethanol from (Alfa Aesar, MA, USA) and hexane from Merck (NJ, USA). Media chemicals for culture of the microbial cells namely, Luria Broth from BD Difco (TX, USA), yeast extract, peptone and glucose were obtained from Fisher Scientific (Hampton, NH, USA) and agar from Hi-Media (Mumbai, India).

Microbial Strains

Bacterial Strains

Wild type *Escherichia coli* (DH5α) (Monk et al., 2016) and ESBL producing isolates of *E. coli* (16, 36, 105, and 153) were used in this study as representatives of bacterial pathogens. The *E. coli* isolates (16, 36, 105, and 153) were from Pathological and Diagnostics Laboratory (Gitanjali Patho Diagnostics Laboratory, Patna, India). Antibiotic susceptibility testing of the *E. coli* isolates was performed in accordance with the recommendations of Clinical Laboratory Standard Institute (CLSI) using Kirby-Bauer Method (Pouladfar et al., 2017). Kirby-Bauer Method is based on agar disc diffusion assay and is a widely used method for antimicrobial susceptibility testing. Herein, the filter paper discs impregnated with antimicrobial agents are applied onto the agar

surface already inoculated with test microbe. On incubation, the antimicrobials diffuse out from the disc into the agar creating a concentration gradient and the area of no microbial growth around the disc is called zone of inhibition. The Antibiogram for the isolates of *E. coli* used in this study was performed using proper quality control protocols on Muller-Hinton agar disc diffusion plate and is shown in **Table 1**.

Fungal Strains

Wild type *Candida albicans* (SC5314) and three isogenic matched pairs of isolates (G1 & G5, F1 & F5, and GU4 & GU5) of *C. albicans*, used in this study are listed in **Table 2** and the strain descriptions are already reported in many studies previously (Fonzi and Irwin, 1993; Franz et al., 1998, 1999). These three isogenic matched pairs of strains were provided by Joachim Morschhauser, University of Wurzburg, Germany and originally isolated from three different AIDS patient suffering from recurrent episodes of OPC. The azole therapy given over a period of time to these AIDS patients for OPC resulted in the acquisition of MDR by the sensitive strains and subsequent evolution of the azole sensitive G1, F1, and GU4 to highly resistant G5, F5, and GU5, respectively (Franz et al., 1998, 1999).

TABLE 1 | Antibiogram of MDR isolates of *E. coli*.

Antibacterial drug (pharmacological name)	Drug content on disc (μg)	Interpretation reference for zone of inhibition (mm)		MDR isolates of *E. coli*			
		Sensitive (*S*)	Resistant (*R*)	16	36	105	153
Cefazolin	30	23	19	R	R	R	R
Cefotaxime	30	26	22	R	R	R	Least S
Cefpirome	30	15–21	<14	R	R	R	R
Cephalexin	30	14	<14	R	R	R	R
Cotrimoxazole	25	16	10	R	R	Moderately S	R
Cefdinir	5	20	16	R	R	Less S	R
Ticarcillin + Clavulanic acid	75/10	20	14	R	R	R	R
Aztreonam	30	28	17	R	R	R	R
Nalidixic acid	30	19	13	R	R	R	R
Norfloxacin	10	17	12	R	R	R	R

TABLE 2 | Azole sensitive and resistant *C. albicans* strains.

Antifungal drug (pharmacological name)	Sensitive strain	MIC (μg/ ml)	Resistant strain	MIC (μg/ ml)	References
Fluconazole	G1	0.39	G5	≥50	Franz et al., 1998
	F1	3.12	F5	≥50	Franz et al., 1998
	GU4	3.12	GU5	>100	Franz et al., 1999

Growth Media and Culture Conditions

Wild type *Escherichia coli* (DH5α) and ESBL producing isolates of *E. coli* (16, 36, 105, and 153) were grown at 37°C in Luria Broth (LB) media for 12 hrs under continuous shaking at 140–150 rpm and these exponentially growing log phase cells were used for all experiments. Wild type SC5314 and drug sensitive-resistant isogenic strains (G1 & G5, F1 & F5, and GU4 & GU5) were grown on 1% (w/v) Yeast extract, 2% (w/v) Peptone and 2% (w/v) Dextrose (YEPD) media. The cells were grown at 30°C with continuous shaking at 140–150 rpm for 14–16 h and these exponentially growing cells were used for all experiments.

Synthesis of ZnO QDs

ZnO QDs were synthesized by wet chemical route (Spanhel and Anderson, 1991). A 0.1 M ethanolic solution of zinc acetate dihydrate was prepared in absolute ethanol at 90°C under continuous refluxing for complete dissolution, then cooled at room temperature and this solution was designated as Sol A. In a separate beaker 0.14 M ethanolic solution of lithium hydroxide monohydrate was prepared in absolute ethanol at room temperature using sonication. This solution was designated as Sol B. Sol A and Sol B were mixed at room temperature (25°C) under vigorous stirring for 1 h to form ZnO QDs. The QDs were precipitated using hexane and centrifuged to collect the particles. These QDs were dried at 60°C under vacuum. The obtained powder was characterized for their structural functional and optical properties using following techniques. A stock solution of ZnO QDs in deionized water (milli Q) was prepared for all the experiments.

Characterization of ZnO QDs

The structural aspects of QDs were studied using X-ray powder diffractometer (PANalyticalX'Pert PRO diffractometer, Almelo Netherlands) with Cu Kα radiation ($\lambda = 1.5418$ Å) in the scan range of 20°–80° diffraction angle and step size of 0.016°. The optical property, specifically excitonic absorbance of the ZnO QDs was analyzed using UV-Vis spectrophotometer (Perkin Elmer, UV-Vis Spectrophotometer Lambda 35, Singapore). The morphology and chemical composition of ZnO QDs were examined by Scanning Electron Microscopy (SEM) (Carl Zeiss E®AG-EVO®40 SEM, Jena, Germany) equipped with EDX Spectrometer (EDS; E-sprit 1.8 X-ray microanalysis, Quantax 200; Bruker Nano GmbH, Berlin, Germany). Further, a 200 kV Transmission Electron Microscope (TEM) (JEM 2100F; JEOL, Tokyo, Japan) having Image Tool Software (multipoint image database software for grain and particle analysis; Dietermann & Heuser Solution GmbH, Greifenstein, Germany) was used to determine mean particle size. High resolution TEM (HRTEM) analysis was used to visualize planar lattice spacing (d spacing) to ascertain the crystalline nature.

Assessment of Microbicidal Potential of ZnO QDs

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent necessary to inhibit visible microbial growth, while minimum microbicidal concentration is the minimum concentration of an antimicrobial

agent that results in microbial death. The closer the MIC is to the MBC, the more microbicidal the compound is. MIC is an endpoint measurement and represents microbial growth in the presence of antimicrobial agent and thus, the static/cidal effects of the antimicrobial agent over time and is employed both as the criteria for determination of susceptibility/ resistance/ static or cidal effect of compounds and also as the single, quantitative pharmacodynamic (PD) parameter formally used for the rational design of antibiotic treatment protocols.

In this study, the MIC and microbicidal activity of the ZnO QDs against the microbial pathogens *E. coli* and *C. albicans* were determined using Internationally accepted methods viz. Broth Microdilution Assays (Clinical and Laboratory Standards Institute, 2006, 2008; Holla et al., 2012; Preeti et al., 2020) and Spot Assays (Clinical and Laboratory Standards Institute, 2006; Tong, 2012; Thomas et al., 2015; Wang et al., 2017; Radhakrishnan et al., 2018b).

In broth microdilution assay, the antimicrobial agent is serially diluted in multiple tubes or wells of microtitre plate from very high to low concentrations to obtain a gradient. Here, 5×10^5 CFU/ ml and $0.5–1 \times 10^4$ CFU/ ml are standard inoculum for antimicrobial susceptibility testing of bacterial and fungal pathogens, respectively. This assay is used to determine the percentage of cell killing and growth inhibition at the respective concentration of the antimicrobial agent. On the other hand, the spot assay is a sensitive method which involves serial dilutions of fixed number of microbial cells as per standard protocol, followed by spotting of these dilutions of microbial cells on solid agar containing plates at concentrations of antimicrobial agents determined by broth microdilution assay. Spot assay is used to compare the viability of cultivable microbial cells under different conditions.

Broth Microdilution Assay

MIC of ZnO QDs for *E. coli* (DH5α) and *C. albicans* (SC5314) was determined by broth microdilution assay, which was performed in 96 well microplate, following the protocol described by CLSI (Clinical and Laboratory Standards Institute, 2006, 2008; Oh et al., 2012; Preeti et al., 2020). The exponentially growing log phase cells of *E. coli* and *C. albicans* were resuspended in 0.9% saline to give an optical density of 0.1 at 600 nm (OD_{600}), which correspond to cell number of 5×10^7 CFU/ ml for *E. coli* and $0.5–1 \times 10^6$ CFU/ ml for *C. albicans*. The cells were subsequently diluted 100-fold in LB and YEPD media, respectively for *E. coli* and *C. albicans* to achieve the final concentration of cells 5×10^5 and $0.5–1 \times 10^4$ CFU/ ml. Thereafter, different concentrations of ZnO QDs (0–400 μg/ ml) were added to the respective media containing cells and incubated at 37°C for *E. coli* and at 30°C for *C. albicans*. Respective controls for growth of the microbial cells were maintained without ZnO QDs. Readings were then recorded at 600 nm and the differences in optical densities were compared with the control grown without ZnO QDs.

Spot Assay

Spot assay was used to confirm the susceptibility of wild type, drug sensitive and resistant isolates of *E. coli* and *C. albicans* toward ZnO QDs (Mukhopadhyay et al., 2004; Clinical and Laboratory Standards Institute, 2006; Thomas et al., 2015; Wang et al., 2017; Radhakrishnan et al., 2018b).

Overnight grown microbial strains of *E. coli* and *C. albicans* were resuspended in 0.9% saline and the OD_{600} of the cell suspension was first adjusted to 0.1 and CFU/ ml was calculated. Then, 5-fold serial dilutions of cell suspension containing 5×10^5 cells/ ml of *E. coli* and 1×10^6 cells/ ml of *C. albicans* were prepared. Subsequently, 5 μl of each serially diluted cell suspension were spotted onto LB plates (containing 0, 200, and 250 μg/ ml of ZnO QDs) for bacterial and YEPD plates (containing 0, 200 and 400 μg/ ml of ZnO QDs) for fungal strains, respectively. The LB plates were incubated for *E. coli* at 37°C for 24 h and for *C. albicans* at 30°C for 48 h. Growth controls without ZnO QDs were maintained for both *E. coli* and *C. albicans* separately. The plates were then imaged and reduction in cell viability in presence of ZnO QDs was evaluated by comparing with the control grown without ZnO QDs.

Statistical Analysis

All experiments were performed in triplicates and the results were represented as mean ± standard deviation. GraphPad Prism version 6.0 (GraphPad software, CA) was used to calculate *p*-value in Student *t*-test and for validating reproducibility and significance of experiments. Intergroup differences were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical Properties of ZnO QDs

The crystalline structure of synthesized ZnO QDs was evaluated using X-Ray diffraction (XRD) technique by recording the diffraction pattern between 20 and 80° (**Figure 1A**). The XRD of ZnO QDs displayed Braggs reflections at 32.01, 34.48, 36.50, 47.71, 56.87, 62.99, and 68.34° corresponding to the planes (100), (002), (101), (102), (110), (103), and (112), respectively and confirmed the formation of single phase hexagonal wurtzite structure of QDs (**Figure 1A**). The diffraction planes were in accordance with JCPDS (Joint committee on powder diffraction standards) file no. 36–1451. No other characteristic XRD peaks were observed, indicating the phase purity of the synthesized ZnO QDs. The lattice spacing $d_{(101)}$ was found to be 0.245 nm, which was in agreement with the wurtzite ZnO nanoparticles (NPs) (Siddiqi et al., 2018). UV-Visible absorption spectrum (**Figure 1B**) exhibited the absorption maxima at 360 nm, which corresponded to enhanced band gap of 3.4 eV as a result of smaller size of the ZnO QDs. SEM micrographs revealed formation of spherical ZnO QDs with smooth surface (**Figure 2A**). Presence of strong peaks in the EDS spectrum were observed at around 1 keV from the Zn L lines, at 8.601 keV from Zn Ka, at 9.5 keV from Zn Kb line and at around 0.5 keV from O Ka (**Figure 2B**). EDS spectrum indicated that the elements present were only Zinc and Oxygen. TEM image confirmed synthesis of mono-dispersed and spherical ZnO QDs of average size ∼5–6 nm which was in agreement with XRD results (**Figure 2C**). HRTEM clearly revealed the lattice structure and the average distance (*d*) between atomic planes

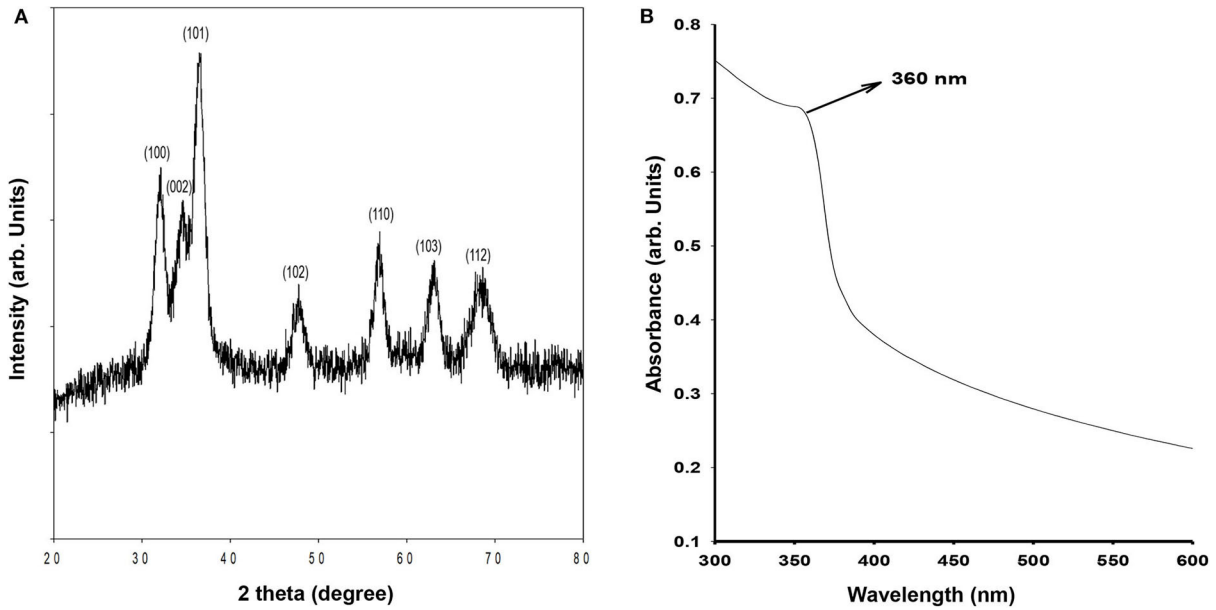


FIGURE 1 | (A) X-ray diffraction of ZnO QDs; **(B)** Absorbance spectrum for ZnO QDs showing absorbance maxima at 360 nm.

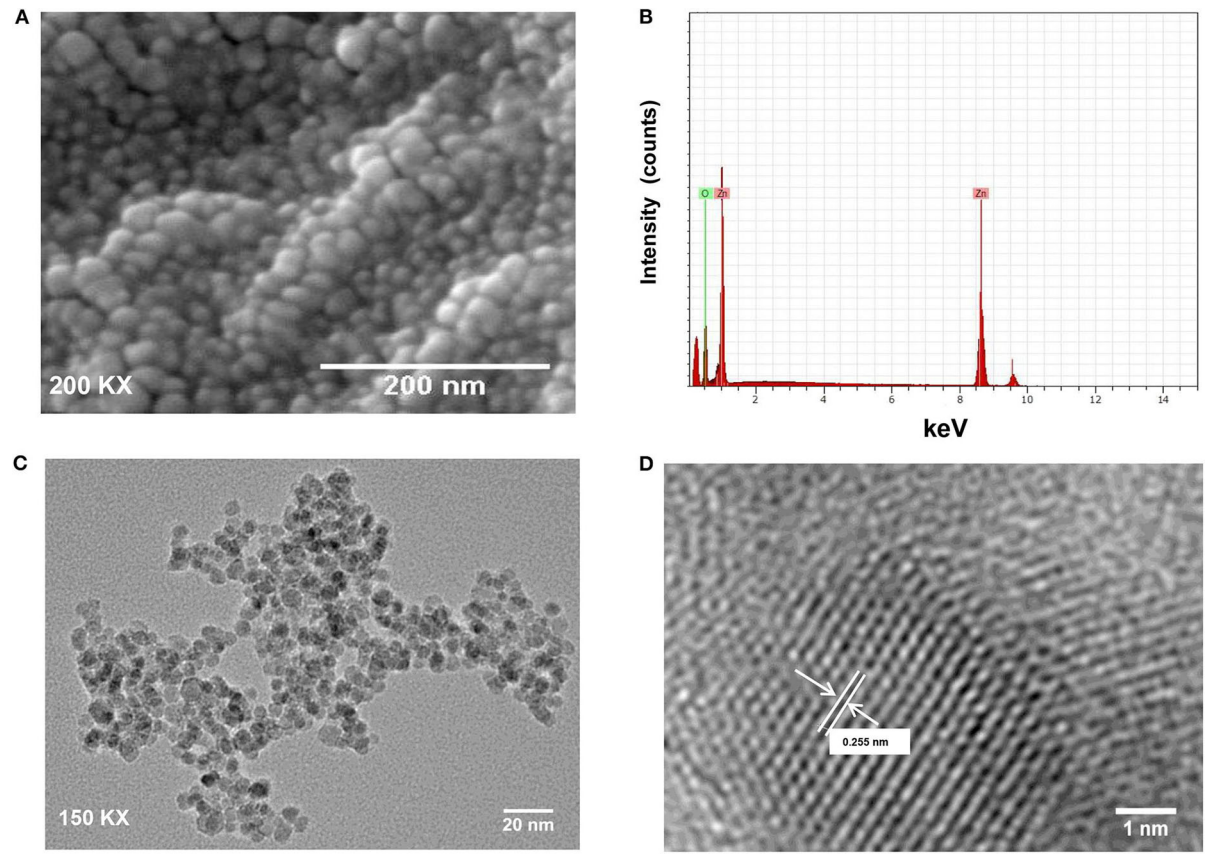


FIGURE 2 | (A) SEM image of ZnO QDs; **(B)** SEM-EDS spectrum for ZnO QDs with strong peaks at 0.1, 8.6, and 0.5 keV; **(C)** TEM image showing spherical, mono-dispersed ZnO QDs of average size 5–6 nm; **(D)** High-resolution TEM image for ZnO QDs showing lattice spacing ($d = 0.255$ nm, where d represents the distance between two lattice fringes).

of ZnO QDs as 0.25 nm (**Figure 2D**), which corroborated with XRD data.

Monodispersed, spherical ZnO QDs with average diameter 5–6 nm were thus synthesized, which exhibited hexagonal wurtzite structure and showed typical absorption peak at 360 nm corresponding to a band gap of 3.4 eV.

Microbicidal Potential of ZnO QDs

ZnO QDs showed dose dependent toxicity against wild type strains of *E. coli* and *C. albicans*, used as model microbial pathogens in this study. Here in this study, broth microdilution assay performed in presence of different concentrations of ZnO QDs (0–400 μg/ ml) revealed that the percentage of growth inhibition at 100 μg/ ml ZnO QDs was 44.76% for *E. coli* and 53.89% for *C. albicans* cells whereas 200 μg/ ml ZnO QDs was able to inhibit 70.87% (MIC_{70}) growth of *E. coli* and 90.57% (MIC_{90}) growth of *C. albicans* cells (**Figures 3A,B**). Thus, MIC_{70} and MIC_{90} of ZnO QDs were found to be 200 μg/ ml for *E. coli* and *C. albicans*, respectively, as determined by the reduction in respective optical densities measured at 600 nm (Optical density of 0.1 at 600 nm corresponds to 5×10^7 CFU/ ml for *E. coli* and $0.5–1 \times 10^6$ CFU/ ml for *C. albicans*). **Figure 3** represents the optical density of microbial cells grown in presence of ZnO QDs at MIC concentration and at half the MIC concentration. The data represented was reproducible in least three independent set of experiments.

Interestingly, a recent study reported that smaller size ZnO QDs exhibited better bactericidal activity than larger ZnO NPs (da Silva et al., 2019). This was in agreement with our results wherein we observed 70 and 90% reduction in *E. coli* and *C. albicans* cells, respectively at MIC_{70} and MIC_{90} values of 200 μg/ ml ZnO QDs, demonstrating promising broad spectrum microbicidal potential of smaller sized 5–6 nm ZnO QDs against both bacterial and fungal pathogens.

Microbicidal Potential of ZnO QDs Against MDR Isolates of *E. coli* and *C. albicans*

Microbicidal activity of ZnO QDs was evaluated against MDR isolates of *E. coli* and *C. albicans*. Antibiotic susceptibility of ESBLs producing *E. coli* (16, 36, 105, and 153) revealed their resistance toward penicillins, cepahlosporins and cross-resistance to quinolones (**Table 1**). ESBL producing MDR isolates of *E. coli* acquire resistance to β-lactam antibiotics such as penicillin derivatives (penams), cephalosporins (cephems), monobactams, carbapenems, and carbacephems etc. through degradation of β-lactam rings of the antibiotics by production of extended spectrum beta lactamase (Shaikh et al., 2015). The three isogenic matched pairs (G1 & G5, F1 & F5, and GU4 &

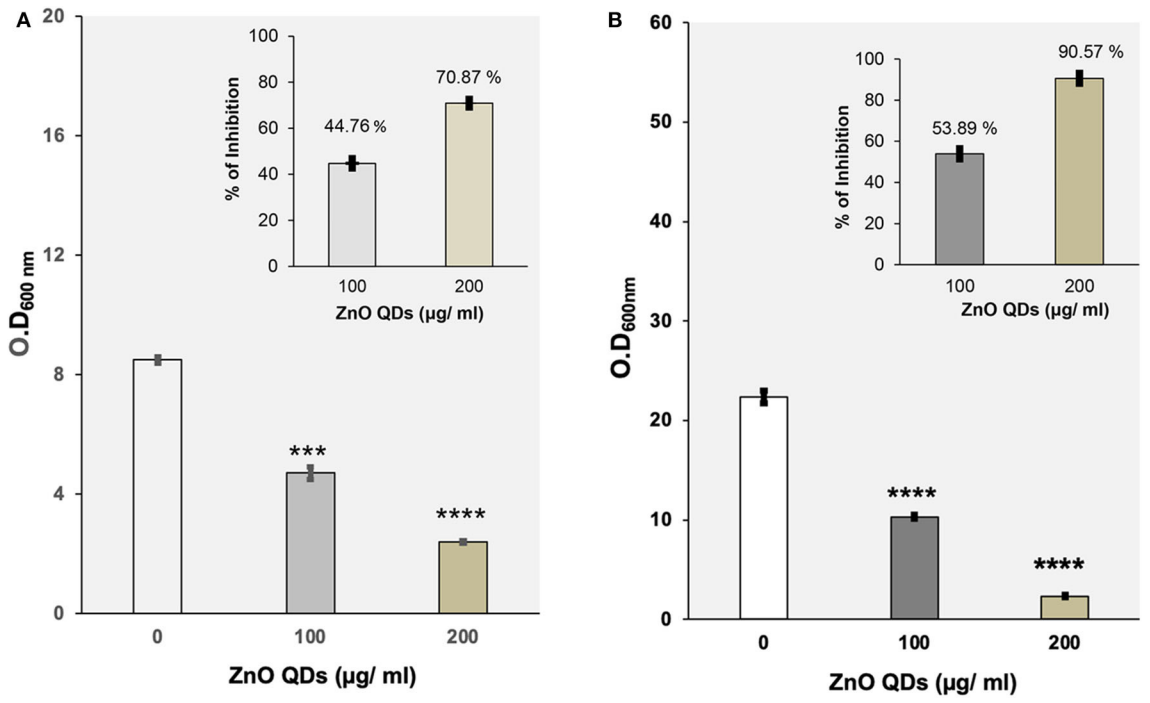


FIGURE 3 | (A) Evaluation of antibacterial potential of ZnO QDs. Determination of growth by measurement of OD_{600nm} using broth microdilution method for *E. coli* cells grown at 37°C in the presence of 0, 100, and 200 μg/ ml of ZnO QDs, respectively. The values are mean ± *SD* of three independent sets of experiments. ***represents $p = 0.001$ and ****represents $p < 0.0001$, calculated with respect to control. Inset shows the percentage of dose dependent growth inhibition of *E. coli* cells in presence of respective concentrations of ZnO QDs; **(B)** Evaluation of antifungal activity of ZnO QDs. Determination of growth by measurement of OD_{600nm} using broth microdilution method for *C. albicans* cells grown at 30°C in the presence of 0, 100, and 200 μg/ ml of ZnO QDs, respectively. The values are mean ± *SD* of three independent sets of experiments. ****represents $p < 0.0001$, calculated with respect to control. Inset shows the percentage of dose dependent growth inhibition of *C. albicans* cells in presence of respective concentrations of ZnO QDs.

GU5) were originally collected from three different AIDS patient suffering from OPC (Franz et al., 1998, 1999). Due to continuous administration of high doses of azoles for recurrent episodes of OPC, the azole sensitive G1, F1, and GU4 acquired resistance during therapy and evolved to become highly azole resistant G5, F5, and GU5, respectively (**Table 2**). The isolates of *Candida* used in this study were reported to acquire resistance due to increase in amount of cellular ergosterol and overexpression of drug efflux pumps (Franz et al., 1998, 1999).

The microbicidal activity of ZnO QDs was further confirmed by spot assay. ZnO QDs exhibited dose dependent, broad spectrum microbicidal activity against all MDR isolates of *E. coli* and *C. albicans* tested (**Figures 4**, **5**). Here it clearly showed significantly reduced cell viabilities for MDR strains of both microbial pathogens in comparison to the control plate (without ZnO QDs), which was indicated by the disappearance of cells on agar plates containing ZnO QDs. No such microbicidal effect or reduction in cell viability was observed on respective Luria Bertani (LB) and Yeast Extract, Peptone and Dextrose (YEPD) control plates, maintained without ZnO QDs. Spot assay involves spotting of dilutions of microbial cells at concentrations of antimicrobial agents determined by broth microdilution assay. In this study, the spot assay was performed at different concentrations between MIC values and four times MIC values. Of these, the best results for spot assay obtained at two concentrations (i.e., MIC concentration and the next higher concentration are represented in **Figures 4**, **5**, to demonstrate significant loss of cell viability which is indicated by the significant disappearance of cells in presence of ZnO QDs). Studies evidence that microbicidal concentration is usually either equal to/ 2-fold/ 4-fold > MIC values (Microbicidal concentration ≥ 1x or 2x or 4x MIC) (Morrissey et al., 2014). This validated our results that highly reduced cell viability was observed for MDR isolates of both *E. coli* and *C. albicans* cells at MIC concentration of 200 μg/ ml ZnO QDs, which was further reduced in presence of 250 and 400 μg/ ml of ZnO QDs for *E. coli* and *C. albicans*, respectively (**Figures 4**, **5**). Complete growth inhibition was observed at 4x MIC_{70} of ZnO QDs for *E. coli* and at 4x MIC_{90} of ZnO QDs for *C. albicans* cells, thus confirming the microbicidal potential of ZnO QDs (data not shown). The data represented was reproducible in at least three independent set of experiments. Therefore, our spot assay results demonstrated that ZnO QDs were able to counter MDR in microbial isolates tested and could render susceptibility to the drug resistant microbials cells.

Several studies have documented size dependent microbial toxicity of ZnO NPs (Sirelkhatim et al., 2015). In our study, the synthesized 5–6 nm ZnO QDs exhibited significant microbicidal

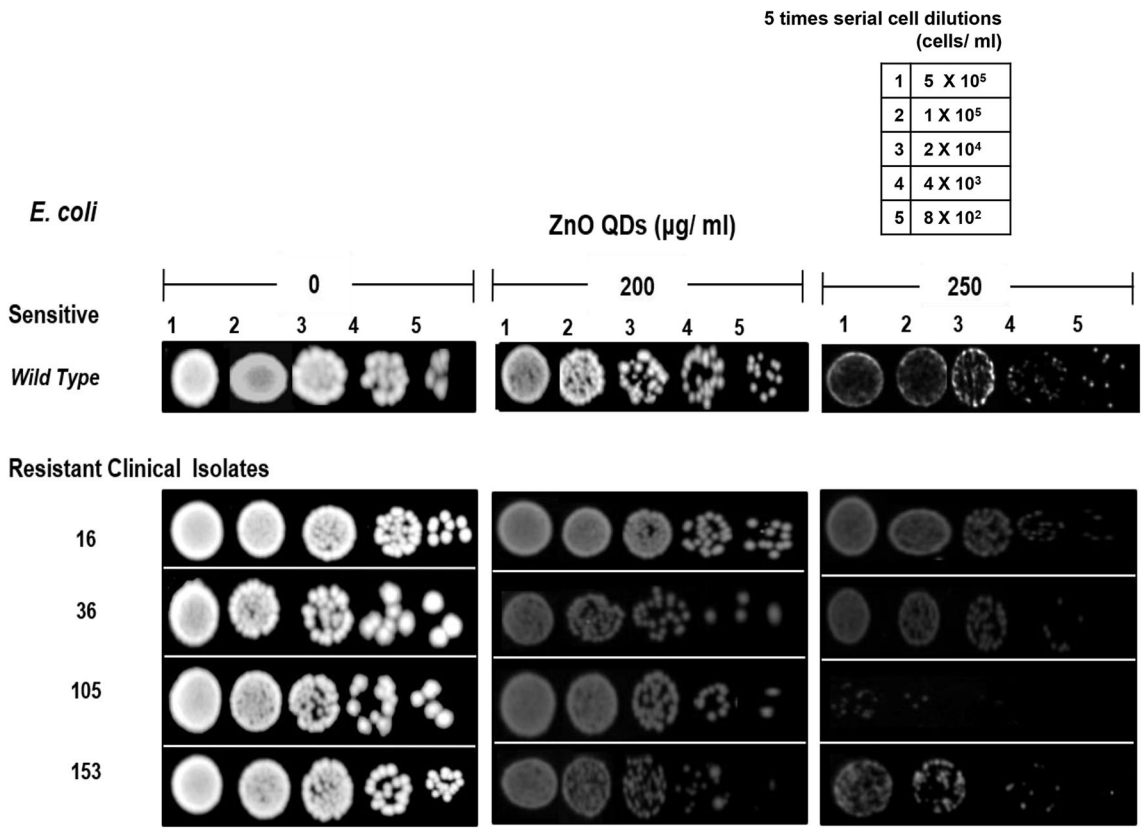


FIGURE 4 | Antimicrobial activity of ZnO QDs against MDR strains of *E. coli*. Spot assay showing of ZnO QDs against MDR isolates (16, 36, 105, and 153) of *E. coli* in the presence of 0, 200, and 250 μg/ ml of ZnO QDs.

activity against both drug sensitive and resistant isolates of *E. coli* and *C. albicans*. The small size of ZnO QDs might be responsible for this promising microbicidal activity. It is possible that antimicrobial or microbicidal activity of metal oxide NPs results from the interaction of metal ions (resulting from dissolution of metals ions from the surface of NPs) with cells,

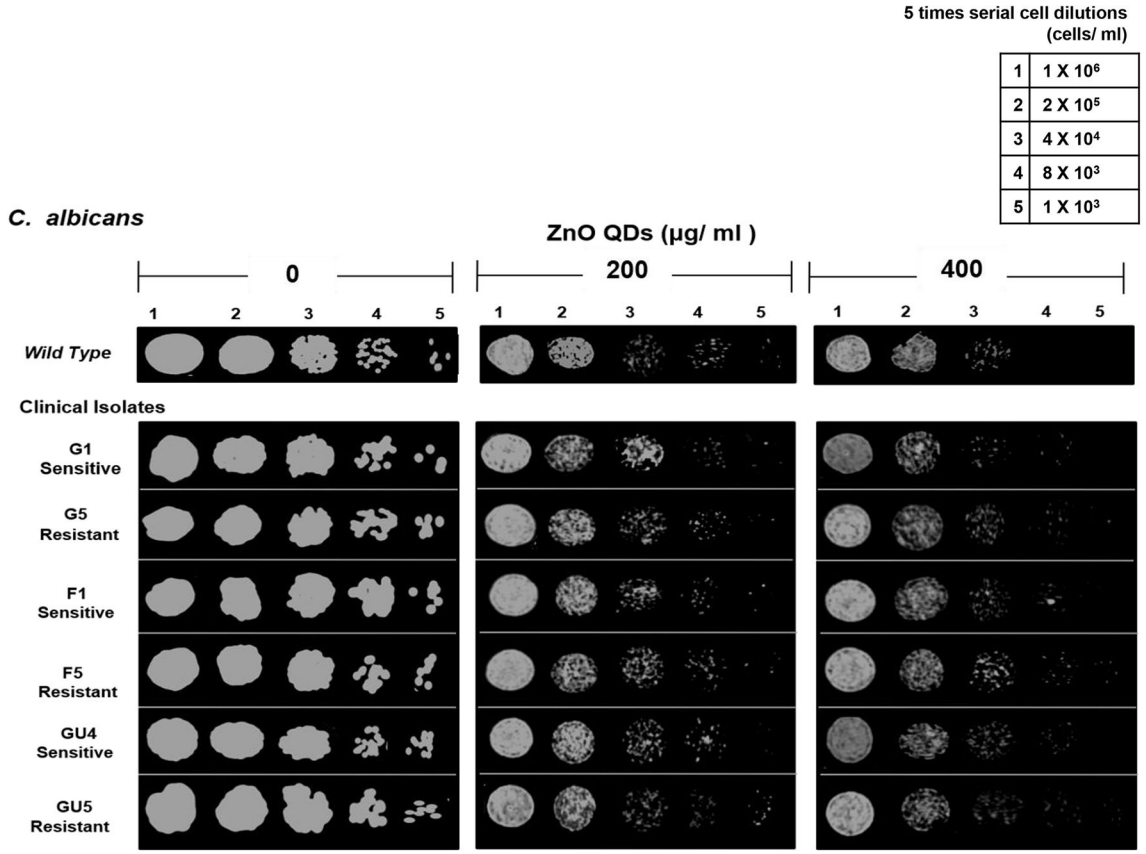
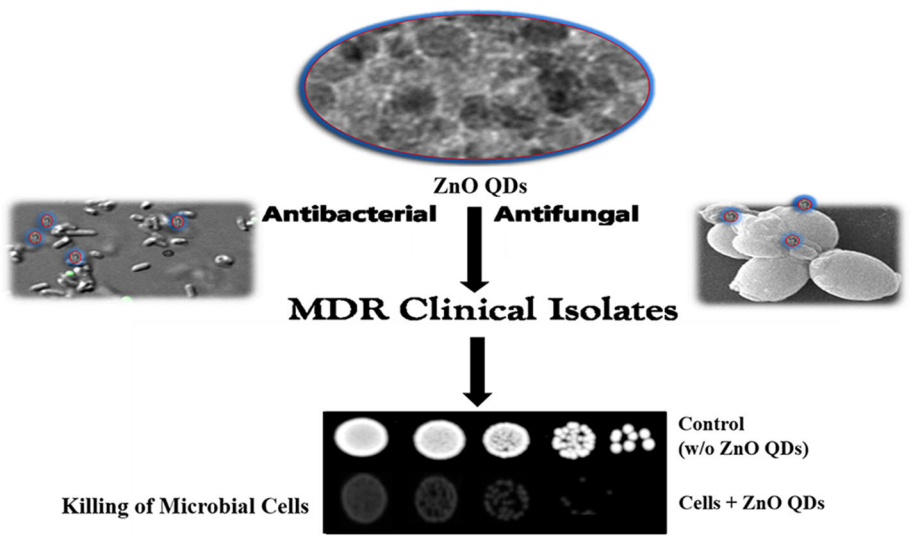


FIGURE 5 | Antimicrobial activity of ZnO QDs against azoles resistant *C. albicans*. Spot assay showing anti-*Candidal* activity of ZnO QDs against azoles sensitive (G1, F1, and GU4) and azoles resistant (G5, F5, and GU5) isolates in the presence of 0, 200, and 400 µg/ ml of ZnO QDs.



SCHEME 1 | Graphical illustration of this study.

TABLE 3 | Comparison of antimicrobial activity of ZnO NPs and ZnO QDs reported in other studies.

Pathogen	Size of ZnO NPs/QDs	Remarks	References
ANTIMICROBIAL POTENTIAL OF ZnO NPs AND ZnO QDs AGAINST DRUG SUSCEPTIBLE MICROBIAL ISOLATES			
Bacterial			
Staphylococcus aureus *Streptococcus pyogenes* *Bacillus cereus* *Pseudomonas aeruginosa* *Proteus mirabilis* *Escherichia coli*	50–90 nm (NPs)	Good antibacterial activity at 1,500 μg/ ml	Gupta et al., 2018
Campylobactor jejuni	30 nm (NPs)	MIC between 25 and 50 μg/ ml	Xie et al., 2011
Campylobactor *Salmonella* (Strains isolated from poultry)	50 nm (NPs)	MIC between 312.5 and 625 μg/ ml	Duffy et al., 2018
E. coli	5–6 nm (QDs) 10–15 nm (NPs)	Growth curve assessment indicated MIC value at 25 μg/ ml for both ZnO QDs and NPs But QDs and NPs at 25 μg/ ml showed only around 10 and 5% growth inhibition, respectively	Wahab et al., 2014
E. coli	3–5 nm (QDs) (from acetate adsorbed) 4–7 nm (QDs) (from nitrate adsorbed)	MIC at 2.5 mM in light and 3 mM in dark MIC at 6 mM in light and no significant bacterial growth inhibition was observed upto 30 mM under dark	Joshi et al., 2009
E. coli	**5–6 nm (QDs)**	**MIC_{70} at 200 μg/ ml**	**This study**
Fungal			
C. albicans	10–30 nm (NPs)	MIC at 250 μg/ ml	Jalal et al., 2018
C. albicans isolated from urinary catheters	20–50 nm (NPs)	Reduction in biofilm formation after treatment with 28 ± 1.2 μg/ ml	Hosseini et al., 2018
C. albicans	**5–6 nm (QDs)**	**MIC_{90} at 200 μg/ ml**	**This study**
ANTIMICROBIAL POTENTIAL OF ZnO NPs AND ZnO QDs AGAINST MDR ISOLATES			
ESBL producing *E. coli* and *Klebsiella pneumonia*	Around 19 nm (NPs) as determined by SEM in the study	500–8,000 μg/ ml	Ansari et al., 2012
ESBL producing *E. coli* and *Klebsiella pneumoniae* (Imipenem sensitive and Ciprofloxacin resistant)	Size of NPs not reported	MIC determined by agar dilution method was 80 μg/ ml for *E. coli* and 50 μg/ ml for *Klebsiella pneumoniae*	Farzana et al., 2017
ESBL producing *E. coli* and *Klebsiella pneumonia*	47 nm (NPs)	MIC_{90} at 800 μg/ ml for E. coli and at 1,000 μg/ ml for Klebsiella pneumoniae	Hameed et al., 2016
MDR pathogens *P. mirabilis* *E. coli*	Irregular ZnO nanosheets of size range between 500 nm to few micron	Showed antibacterial activity (assessed by Kirby-Bauer disc diffusion method) with max. zone of inhibition of 27 ± 0.5 mm for *P. mirabilis* and 24 ± 0.5 mm for *E. coli* at 25 μg/mL ZnO nanosheets Nanosheets displayed antibiofilm activity	Rajivgandhi et al., 2018
ESBL producing MDR isolates of *E. coli* (16, 36, 105 and 153) resistant to most of the different classes of currently used antibacterial drugs which include third & fourth generation of cephalosporin, penicillin, monobactams, and quinolones)	**5–6 nm (QDs)**	**Significant growth inhibition and loss of cell viability of all MDR isolates at 200 μg/ ml**	**This study**
C. albicans (Fluconazole-resistant isolates isolated from urinary catheters)	20–50 nm (NPs)	Reduction in biofilm formation after treatment with 47± 2.8 μg/ ml	Hosseini et al., 2018
MDR isolates of *C. albicans* (G5, F5, and GU5 collected from three different AIDS patients who over the period of therapy had acquired drug resistance wherein sensitive isolates G1, F1, and GU4 evolved into MDR strains)	**5–6 nm (QDs)**	**Significant growth inhibition and loss of cell viability of all susceptible and MDR isolates at 200 μg/ ml**	**This study**

Bold values are meant to highlight the findings and significance of this study in the table for comparison with other studies.

which lead to reactive oxygen species (ROS) generated oxidative stress. The dissolution of metal and metal oxide nanoparticles remains highly dependent on NPs type, concentration, size, coatings and pH of aqueous media. Higher dissolution of Zn^{2+} ions was reported from 4 nm ZnO QDs than larger NPs, resulting in presence of more Zn^{2+} ions in ZnO QDs solution

(Mudunkotuwa et al., 2012), which in turn might be responsible for the observed higher antimicrobial activity of QDs against both drug susceptible and resistant pathogens (Siddiqi et al., 2018). Moreover, the quantum confinement of ZnO QDs creates surface defects on ZnO QDs and produces large number of electron donor or acceptor active sites, which react with oxygen and hydroxyl ions (in aqueous suspension) to produce highly reactive superoxide and hydroxyl radicals. These highly reactive free radicals contribute to enhanced ROS generation and result in peroxidation of lipids, nucleic acid and proteins, disruption of cell membrane and leakage of cytoplasmic content, thus leading to cell death (Asok et al., 2015; Gold et al., 2018; Radhakrishnan et al., 2018a; Tiwari et al., 2018).

In addition, the small size of ZnO QDs also provide larger surface area for their higher interaction with microbial cells. Furthermore, the surface defects of ZnO QDs might also increase the density of positive surface charge as particle size decreases, which might increase interaction with microbial cells (possessing negative charge). This can lead to higher agglomeration of ZnO QDs on the surface of microbes leading to altered metabolic activity and increased antimicrobial activity (Asok et al., 2015). Thus, small size of ZnO QDs might exert excellent antimicrobial activity through multiple mechanisms.

However, the major limitation of microbial therapeutics is that each class of antimicrobial drug has a particular mode of action and definite cellular target such as cell wall, membrane lipids, translational machinery, or DNA replication (Liwa and Jaka, 2015; Silver, 2016). For example, the mode of action of β-lactam antibiotics is through inhibition of bacterial cell wall biosynthesis and that of antifungal azoles is via inhibition of fungal membrane ergosterol biosynthetic pathway (Prasad et al., 2011; Shaikh et al., 2015). But on emergence of MDR in microbial pathogens, drug therapy becomes ineffective and poses a bottleneck in microbial therapeutics. MDR being multifactorial, microbial pathogens acquire resistance by various adaptive strategies such as drug target modifications, drug degradation, target amplification, alterations in cell wall, modifications in cell membrane, and overexpression of drug efflux pumps (Shaikh et al., 2015; Munita and Arias, 2016). The challenges faced due to acquisition of microbial drug resistance can likely be overcome by favoring multi-targeted action of antimicrobial agents. In view of the previous reports that interactions of nanoparticles with microbes lead to membrane lipid peroxidation, cell membrane disruption, leakage of cytoplasmic contents, and cell death (Jalal et al., 2018; Radhakrishnan et al., 2018b), it appears that small sized ZnO QDs can address the multifactorial challenges of microbial drug resistance (Graphical illustration in **Scheme 1**) by favoring multi targeted action, broad spectrum activity and lower host toxicity. In the light of such multi targeted action, it is unlikely for the microbes to simultaneously develop a host of cellular changes or mutations to exhibit resistance.

CONCLUSION

In this study, monodispersed ZnO QDs of average particle size ∼5–6 nm were synthesized using wet chemical route and the broad spectrum, microbicidal activity of ZnO QDs was demonstrated against both drug susceptible and drug resistant isolates of *E. coli* and *Candida albicans*. No study so far has reported the antimicrobial activity of ZnO QDs against MDR pathogens. **Table 3** gives a comparison of our study with previous studies reported on antimicrobial potential of ZnO NPs/ ZnO QDs against drug susceptible and MDR microbial isolates. In our study, ZnO QDs were found to counter MDR in both bacterial and fungal pathogens tested (ESBL producing MDR isolates of *E. coli* which showed resistance to most of the currently used antibacterial drugs of different classes such as third and fourth generation of cephalosporins, penicillins, monobactams, and quinolones and MDR isolates of *C. albicans* collected from three different AIDS patients who during therapy for OPC had acquired drug resistance). The study clearly indicates that ZnO QDs possess broad spectrum microbicidal potential and could serve as a next generation alternative of antimicrobial therapy by combating MDR in microbes and help in limiting the extensive use of antibiotics.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

Preeti, SS, and TP contributed to the concept and design of the study. Preeti conducted the experiments. Preeti and VR participated in the acquisition of data and statistical analysis. Preeti, SS, and TP participated in interpretation of data and drafting of the manuscript. SS and TP contributed to editing the manuscript and revised it critically for significant intellectual content. SusM and SujM isolated and performed antibiotic susceptibility profiling of the *E. coli* strains and completed the antibiogram. All authors read and approved the final manuscript.

FUNDING

This work has been supported by grants to TP from Department of Biotechnology (BT/PR5110/MED/29/497/2012 and BT/BI/12/045/2008), JNU-UPOE II scheme (ID-161), and JNU-DST-PURSE Phase-II, India.

ACKNOWLEDGMENTS

JNU for providing infrastructural support and Council of Scientific and Industrial Research (CSIR), India for the award of Junior Research Fellowship to Preeti are gratefully acknowledged. We acknowledge Joachim Morschhauser, University of Wurzburg, Germany for generously providing clinical isogenic isolates of *Candida albicans*. SS acknowledges CSIR-NPL for providing the nanoparticle synthesis and characterization infrastructure.

REFERENCES

Allocati, N., Masulli, M., Alexeyev, M. F., and Di-Ilio, C. (2013). *Escherichia coli* in Europe: an overview. *Int. J. Environ. Res. Public Health* 10, 6235–6254. doi: 10.3390/ijerph10126235

Ansari, M. A., Khan, H. M., Khan, A. A., Sultan, A., and Azam, A. (2012). Synthesis and characterization of the antibacterial potential of ZnO nanoparticles against extended-spectrum β-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from a tertiary care hospital of North India. *Appl. Microbiol. Biotechnol.* 94, 467–477. doi: 10.1007/s00253-011-3733-1

Asok, A., Ghosh, S., More, P. A., Chopade, B. A., Gandhi, M. N., and Kulkarni, A. R. (2015). Surface defect rich ZnO quantum dots as antioxidants inhibiting α-amylase and α-glucosidase: a potential anti-diabetic nanomedicine. *J. Mater. Chem. B* 3, 4597–4606. doi: 10.1039/C5TB00407A

Bloom, D. E., and Cadarette, D. (2019). Infectious disease threats in the 21st century: strengthening the global response. *Front. Immunol.* 10:549. doi: 10.3389/fimmu.2019.00549

Clinical and Laboratory Standards Institute (2006). *Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI Document M100-S16CLSI*. Wayne, PA. Available online at: https://clsi.org/media/1928/m07ed11_sample.pdf

Clinical and Laboratory Standards Institute (2008). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard-Second Edition. CLSI document M38-A2*. Wayne: Clinical and Laboratory Standards Institute. Available online at: https://clsi.org/media/1455/m38a2_sample.pdf

da Silva, B. L., Caetano, B. L., Chiari-Andréo, B. G., Pietro, R. C. L. R., and Chiavacci, L. A. (2019). Increased antibacterial activity of ZnO nanoparticles: influence of size and surface modification. *Colloids Surf. B Biointerf.* 177, 440–447. doi: 10.1016/j.colsurfb.2019.02.013

Denning, D. W., and Bromley, M. J. (2015). How to bolster the antifungal pipeline. *Science* 347, 1414–1416. doi: 10.1126/science.aaa6097

Duffy, L. L., Osmond-McLeod, M. J., Judy, J., and King, T. (2018). Investigation into the antibacterial activity of silver, zinc oxide and copper oxide nanoparticles against poultry-relevant isolates of *Salmonella* and *Campylobacter*. *Food Control* 92, 293–300. doi: 10.1016/j.foodcont.2018.05.008

Farzana, R., Iqra, P., Shafaq, F., Sumaira, S., Zakia, K., Hunaiza, T., et al. (2017). Antimicrobial behaviour of Zinc oxide nanoparticles and β-lactam antibiotics against pathogenic bacteria. *Arch. Clin. Microbiol.* 8:57. doi: 10.4172/1989-8436.100057

Fonzi, W. A., and Irwin, M. Y. (1993). Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 134, 717–728.

Franz, R., Kelly, S. L., Lamb, D. C., Kelly, D. E., Ruhnke, M., and Morschhauser, J. (1998). Multiple molecular mechanisms contribute to a stepwise development of fluconazole resistance in clinical *Candida albicans* strains. *Antimicrob. Agents. Chemother.* 42, 3065–3072. doi: 10.1128/AAC.42.12.3065

Franz, R., Ruhnke, M., and Morschhauser, J. (1999). Molecular aspects of fluconazole resistance development in *Candida albicans*. *Mycoses* 42, 453–458. doi: 10.1046/j.1439-0507.1999.00498.x

Global Health Estimates (2016). *Deaths by Cause Age, Sex, Country and by Region, 2000-2016*. Geneva: World Health Organization 2018. Available online at: http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html

Gold, K., Slay, B., Knackstedt, M., and Gaharwar, A. K. (2018). Antimicrobial activity of metal and metal oxide based nanoparticles. *Adv. Ther.* 1:1700033. doi: 10.1002/adtp.201700033

Gupta, M., Tomar, R. S., Kaushik, S., Mishra, R. K., and Sharma, D. (2018). Effective antimicrobial activity of green ZnO nanoparticles of *Catharanthus roseus*. *Front. Microbiol.* 9:2030. doi: 10.3389/fmicb.2018.02030

Hameed, A. S. H., Karthikeyan, C., Ahamed, A. P., Thajuddin, N., Alharbi, N. S., Alharbi, S. A., et al. (2016). *In vitro* antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*. *Sci. Rep.* 6:24312. doi: 10.1038/srep24312

Holla, G., Yeluri, R., and Munshi, A. K. (2012). Evaluation of minimum inhibitory and minimum bactericidal concentration of nano-silver base inorganic anti-microbial agent (Novaron®) against *Streptococcus mutans*. *Contemp. Clin. Dent.* 3, 288–93. doi: 10.4103/0976-237X.103620

Holzinger, M., Le Goff, A., and Cosnier, S. (2014). Nanomaterials for biosensing applications: a review. *Front. Chem.* 2:63. doi: 10.3389/fchem.2014.00063

Hosseini, S. S., Ghaemi, E., and Koohsar, F. (2018). Influence of ZnO nanoparticles on *Candida albicans* isolates biofilm formed on the urinary catheter. *Iran. J. Microbiol.* 10, 424–432.

Jalal, M., Ansari, M. A., Ali, S. G., Khan, H. M., and Rehman, S. (2018). Anticandidal activity of bioinspired ZnO QDs: effect on growth, cell morphology and key virulence attributes of *Candida* species. *Artif. Cells. Nanomed. Biotechnol.* 46, 912–925. doi: 10.1080/21691401.2018.1439837

Joshi, P., Chakraborti, S., Chakrabarti, P., Haranath, D., Shanker, V., Ansari, Z. A., et al. (2009). Role of surface adsorbed anionic species in antibacterial activity of ZnO quantum dots against *Escherichia coli*. *J. Nanosci. Nanotechno.* 9, 6427–6433. doi: 10.1166/jnn.2009.1584

Liwa, A. C., and Jaka, H. (2015). "Antimicrobial resistance: mechanisms of action of antimicrobial agents," in *The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs*, ed A. Méndez-Vilas (Badajoz: Formatex Research Center), 876–885.

Mayer, F. L., Wilson, D., and Hube, B. (2013). *Candida albicans* pathogenicity mechanisms. *Virulence* 4, 119–128. doi: 10.4161/viru.22913

Monk, J. M., Koza, A., Campodonico, M. A., Machado, D., Seoane, J. M., Palsson, B. O., et al. (2016). Multi-omics quantification of species variation of *Escherichia coli* links molecular features with strain phenotypes. *Cell Syst.* 3, 238–251. doi: 10.1016/j.cels.2016.08.013

Morrissey, I., Oggioni, M. R., Knight, D., Curiao, T., Coque, T., Kalkanci, A., et al. (2014). Evaluation of epidemiological cut-off values indicates that biocide resistant subpopulations are uncommon in natural isolates of clinically-relevant microorganisms. *PLoS ONE* 9:e86669. doi: 10.1371/journal.pone.0086669

Mudunkotuwa, I. A., Rupasinghe, T., Wu, C. M., and Grassian, V. H. (2012). Dissolution of ZnO nanoparticles at circumneutral pH: a study of size effects in the presence and absence of citric acid. *Langmuir* 28, 396–403. doi: 10.1021/la203542x

Mukhopadhyay, K., Prasad, T., Saini, P., Pucadyil, T. J., Chattopadhyay, A., and Prasad, R. (2004). Membrane sphingolipid–ergosterol interactions are important determinants of multidrug resistance in *Candida albicans*. *Antimicrob. Agents. Chemother.* 48, 1778–1787. doi: 10.1128/AAC.48.5.1778-1787.2004

Munita, J. M., and Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiol Spectr.* 4, 1–37. doi: 10.1128/microbiolspec.VMBF-0016-2015

Nune, S. K., Gunda, P., Thallapally, P. K., Lin, Y. Y., Laird Forrest, M., and Berkland, C. J. (2009). Nanoparticles for biomedical imaging. *Expert Opin. Drug Deliv.* 6, 1175–1194. doi: 10.1517/17425240903229031

Oh, H. Y., Lee, J. O., and Kim, O. B. (2012). Increase of organic solvent tolerance of *Escherichia coli* by the deletion of two regulator genes, fadR and marR. *Appl. Microbiol. Biotechnol.* 96, 1619–1627. doi: 10.1007/s00253-012-4463-8

Pal, M. (2017). Morbidity and mortality due to fungal infections. *J. Appl. Microbiol. Biochem.* 1:2. doi: 10.21767/2576-1412.100002

Patil, S., Majumdar, B., Sarode, S. C., Sarode, G. S., and Awan, K. H. (2018). Oropharyngeal candidosis in HIV-infected patients-an update. *Front. Microbiol.* 9:980. doi: 10.3389/fmicb.2018.00980

Picozzi, S. C., Casellato, S., Rossini, M., Paola, G., Tejada, M., Costa, E., et al. (2014). Extended-spectrum beta-lactamase-positive *Escherichia coli* causing complicated upper urinary tract infection: urologist should act in time. *Urol. Ann.* 6, 107–12. doi: 10.4103/0974-7796.130536

Pouladfar, G., Basiratnia, M., Anvarinejad, M., Abbasi, P., Amirmoezi, F., and Zare, S. (2017). The antibiotic susceptibility patterns of uropathogens among children with urinary tract infection in Shiraz. *Medicine* 96:e7834. doi: 10.1097/MD.0000000000007834

Prasad, T., Sethumadhavan, S., and Fatima, Z. (2011). "Altered ergosterol biosynthetic pathway-an alternate multidrug resistance mechanism independent of drug efflux pump in human pathogenic fungi C. albicans," in *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances, Microbiology Series*, ed A. Méndez-Vilas (Badajoz: Formatex Research Center), 757–768.

Preeti, Prasad, T., Magoo, D., Meena, K., Ghorai, S. M., and Kaur, H. (2020). Elucidation of the antimicrobial capabilities of spirooxindole fused heterocycles synthesized via catalyst free method. *Indian J. Nat. Sci.* 10, 27323–27330.

Radhakrishnan, V. S., Dwivedi, S. P., Siddiqui, M. H., and Prasad, T. (2018a). *In vitro* studies on oxidative stress independent, Ag-nanoparticles induced cell toxicity of *Candida albicans*, an opportunistic pathogen. *Int. J. Nanomed.* 13, 91–96. doi: 10.2147/IJN.S125010

Radhakrishnan, V. S., Mudiam, M. K. R., Kumar, M., Dwivedi, S. P., Singh, S. P., and Prasad, T. (2018b). Silver nanoparticles induced alterations in multiple cellular targets, which are critical for drug susceptibilities and pathogenicity in fungal pathogen (*Candida albicans*). *Int. J. Nanomed.* 13, 2647–2663. doi: 10.2147/IJN.S150648

Rajivgandhi, G., Maruthupandy, M., Muneeswaran, T., Anand, M., and Manoharan, N. (2018). Antibiofilm activity of zinc oxide nanosheets (ZnO NSs) using *Nocardiopsis* sp. GRG1 (KT235640) against MDR strains of gram negative *Proteus mirabilis* and *Escherichia coli*. *Process Biochem*. 67, 8–18. doi: 10.1016/j.procbio.2018.01.015

Rodrigues, M. L., and Nosanchuk, J. D. (2020). Fungal diseases as neglected pathogens: a wake-up call to public health officials. *PLoS Negl. Trop. Dis.* 14:e0007964. doi: 10.1371/journal.pntd.0007964

Roy, R., Kumar, S., Verma, A. K., Sharma, A., Chaudhari, B. P., Tripathi, A., et al. (2014). Zinc oxide nanoparticles provide an adjuvant effect to ovalbumin via a Th2 response in Balb/c mice. *Int. Immunol.* 26, 159–172. doi: 10.1093/intimm/dxt053

Saeed, A., Abd, H., and Sandstrom, G. (2015). Microbial aetiology of acute diarrhoea in children under five years of age in Khartoum, Sudan. *J. Med. Microbiol.* 62, 432–437. doi: 10.1099/jmm.0.000043

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., and Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. *Saudi J. Biol. Sci.* 22, 90–101. doi: 10.1016/j.sjbs.2014.08.002

Siddiqi, K. S., Rahman, A. U., and Tajuddin, Husan, A. (2018). Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Res. Lett*. 13:141. doi: 10.1186/s11671-018-2532-3

Silver, L. L. (2016). Appropriate targets for antibacterial drugs. *Cold Spring Harb. Perspect. Med.* 6:a030239. doi: 10.1101/cshperspect.a030239

Singh, R., Smitha, M. S., and Singh, S. P. (2014). The role of nanotechnology in combating multi-drug resistant bacteria. *J. Nanosci. Nanotechnol.* 14, 4745–4756. doi: 10.1166/jnn.2014.9527

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., et al. (2015). Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano Micro Lett*. 7, 219–242. doi: 10.1007/s40820-015-0040-x

Spanhel, L., and Anderson, M. A. (1991). Semiconductor clusters in the sol-gel process: quantized aggregation, gelation, and crystal growth in concentrated ZnO colloids. *J. Am. Chem. Soc.* 113, 2826–2833. doi: 10.1021/ja0008a004

Thomas, P., Sekhar, A. C., Upreti, R., Mujawar, M. M., and Pasha, S. S. (2015). Optimization of single plate-serial dilution spotting (SP-SDS) with sample anchoring as an assured method for bacterial and yeast cfu enumeration and single colony isolation from diverse samples. *Biotechnol. Rep*. 8, 45–55. doi: 10.1016/j.btre.2015.08.003

Tiwari, V., Mishra, N., Gadani, K., Solanki, P. S., Shah, N. A., and Tiwari, M. (2018). Mechanism of anti-bacterial activity of zinc oxide nanoparticle against carbapenem-resistant *Acinetobacter baumannii*. *Front. Microbiol*. 9:1218. doi: 10.3389/fmicb.2018.01218

Tong, Z. (2012). Spot assay for yeast. *Bio Protocol.* 2:e16. doi: 10.21769/BioProtoc.16

Vazquez, J. A. (2010). Optimal management of oropharyngeal and esophageal candidiasis in patients living with HIV infection. *HIV/AIDS* 2, 89–101. doi: 10.2147/HIV.S6660

Wahab, R., Khan, F., Singh, R. B., and Khan, A. (2014). Enhance antimicrobial activity of ZnO nanomaterials (QDs and NPs) and their analytical applications. *Physica E Low Dimens. Syst. Nanostruct*. 62, 111–117. doi: 10.1016/j.physe.2014.04.017

Wang, J., Woo, M., and Yan, C. (2017). Spot plating assay for the determination of survival and plating efficiency of *Escherichia coli* in sub-MIC levels of antibiotics. *JEMI Methods*. 1, 26–29.

Xie, Y., He, Y., Irwin, P. L., Jin, T., and Shi, X. (2011). Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 77, 2325–2331. doi: 10.1128/AEM.02149-10

Zhou, Y., Zhu, X., Hou, H., Lu, Y., Yu, J., Mao, L., et al. (2018). Characteristics of diarrheagenic *Escherichia coli* among children under 5 years of age with acute diarrhoea: a hospital-based study. *BMC Infect. Dis.* 18:63. doi: 10.1186/s12879-017-2936-1

Zhu, Y., and Liao, L. (2015). Applications of nanoparticles for anticancer drug delivery: a review. *J. Nanosci. Nanotechnol.* 7, 4753–4773. doi: 10.1166/jnn.2015.10298

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.