Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 18 Aug 2026
Revised 09 Sep 2026
Accepted 27 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (23s): 368–379

KEYWORDS:
Bacillus; Zinc oxide nanoparticles (ZnO-NPs); Green synthesis; Antimicrobial activity; Gram-positive pathogens; Septicemia; Biofilm inhibition; Nanomedicine; Cytotoxicity; Alternative therapeutics.;

In Vitro Evaluation of Bacillus-Derived Zinc Oxide Nanoparticles and Their Antimicrobial Efficacy Against Gram-Positive Septicemia Pathogens

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ABSTRACT: Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis, Streptococcus pneumoniae, and others) are the main causes of Septicemia that is still challenging to deal with because of the spreading cases of multidrug resistance. Interventions based on nanotechnology have also become promising options, and zinc oxide nanoparticles (ZnO-NPs) exhibit a wide range of antimicrobial activity. In this research, the synthesis of ZnO nanoparticles was done in a green, eco-friendly method via bio-reduction of Bacillus subtilis by utilizing its extracellular metabolites as a reducing and stabilizing agent. To verify the crystalline nature, functional groups, and nanoscale morphology of the nanoparticles, UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were used to characterize the nanoparticles. Disc diffusion, Minimum inhibitory concentration (MIC), Minimum Bactericidal concentration (MBC), and biofilm inhibition tests were used to determine the antimicrobial efficacy of the Gram-positive Septicemia pathogens tested against clinically relevant causes of sepsis. The findings indicated that ZnO-NPs derived by Bacillus, dose-dependent inhibition zones, low MIC/MBCs, and biofilm disruption. The initial cytotoxicity on the mammalian cell lines showed that biosafety was not deemed significant below the therapeutic concentrations. The present results suggest that Bacillus-mediated ZnO nanoparticles are a viable and long-term antimicrobial solution against Gram-positive pathogens of Septicemia, and that the future research directions are the translational and in vivo research.

1. INTRODUCTION

Background: Antimicrobial Resistance and Gram-Positive Septicemia

Gram-positive pathogens, such as *Staphylococcus aureus, Enterococcus faecalis*, and *Streptococcus pneumoniae,* have remained a major cause of morbidity and mortality in all parts of the world. Multidrug resistance is on the rise, and this has greatly reduced the effectiveness of traditional antibiotics *[17](Ventola 2015).* Research has indicated that Gram-positive Septicemia is a significant cause of bloodstream infections, and *S. aureus* alone is a cause of almost 30 percent of hospital-acquired sepsis *[13] (Singer et al. 2016).* This is a worrying trend that requires other antimicrobial approaches that would help to defeat the resistance and provide effective control of the situation with Septicemia.

Natural Resources for Human Health
eISSN: 2583-1194

Medicine Nanotechnology: The role of ZnO Nanoparticles.

The special physicochemical characteristics of nanoparticles enable nanotechnology to provide new methods in the attack on infectious diseases. One of these is zinc oxide nanoparticles (ZnO-NPs), which is more surface area, a reactive oxygen species (ROS)-mediated antibacterial effect, and bacterial membrane disruption *[11] (Raghupathi et al. 2011).* In contrast to bulk materials, ZnO-NPs can be absorbed by the bacterial biofilms and applied as antimicrobial agents in comparatively low concentrations (Sirelkhatim et al. 2015). Also, the U.S. Food and Drug Administration (FDA) tends to consider ZnO as safe, so it can be a potential biomedical candidate *[20] (Zhang et al. 2016).*

Advantage Microbial Synthesis *Bacillus* as a Biofactory.

Traditional methods of chemical and physical production of nanoparticles are typically accompanied by the use of toxic chemicals and the use of a lot of energy, which poses a threat to the environment and safety [4] (Iravani 2011). Microbial synthesis, on the other hand, is a green, sustainable, and cost-effective path to nanoparticle generation. Bacillus species are particularly ideally deployed to produce nanoparticles because of their active metabolism, extracellular enzyme secretion, and stabilisation of nanoparticles using extracellular biomolecules [7] (Narayanan and Sakthivel 2010). As has been shown before, *Bacillus subtilis* and *Bacillus licheniformis* have the ability to produce stable types of ZnO nanoparticles with antimicrobial characteristics [5] (Jain et al. 2013).

Research Gap

Regardless of the accrued data on the use of ZnO nanoparticles as antimicrobial agents, there is a deficit of research on the *Bacillus*-mediated ZnO-NPs and their relative efficacy against Gram-positive pathogenic agents of *Septicemia.* The majority of the currently available studies either focus on chemically synthesized nanoparticles or evaluate the antimicrobial activity on a broader spectrum, without discussing the specific pathogen bacteria that are found in the bloodstream [1] (Ali et al. 2016). It, therefore, follows that there still exists a knowledge gap that is based on the potential of *Bacillus*-derived ZnO-NPs as targeted therapeutics against Gram-positive Septicemia.

Aim and Objectives

The present study seeks to address this gap by:

1. **Synthesizing and characterizing ZnO nanoparticles using *Bacillus* species.**

2. **Evaluating their antimicrobial efficacy against clinically relevant Gram-positive Septicemia pathogens through in vitro assays.**

This research aims to provide a sustainable and effective nanoparticle-based therapeutic strategy for overcoming multidrug resistance and improving Septicemia management.

2. LITERATURE REVIEW

Septicemia Pathogens: Clinical Relevance

The gram-positive pathogens are significant contributors to bloodstream infections and Septicemia causing a lot of clinical complications. One of the most virulent organisms related to Septicemia is *Staphylococcus aureus*, which has been attributed to high mortality rates because of its resistance to Methicillin [16] (Tong et al. 2015). Another important pathogen is *Enterococcus faecalis,* which is known to be intrinsically resistant to various antibiotics and is known to cause nosocomial Septicemia [2] (Arias and Murray 2012). *Streptococcus pneumoniae* is another serious pathogen that leads to the development of bloodstream infections, and is especially dangerous in immunocompromised and older patients [18] (Weiser et al. 2018). The escalating resistance to these pathogens highlights the need to come up with alternative measures to deal with the disease.

Zinc Oxide Nanoparticles: Characteristics and Antimicrobial Effect.

It is well known that Zinc oxide nanoparticles (ZnO-NPs) possess several special physicochemical characteristics, such as a large surface area-to-volume ratio, photocatalytic capacity, and reactive oxygen species (ROS) that increase their antimicrobial activities [11] (Raghupathi et al. 2011). ZnO-NPs have the ability to destroy cell membranes of bacteria, disrupt cellular

metabolism, and affect intracellular biomolecules, which causes death in bacteria [14] (Sirelkhatim et al. 2015). Notably, ZnO can be regarded as biocompatible, and the regulatory bodies introduce ZnO as the category of substances that is generally recognized as safe (GRAS), which further enhances its biomedical prospects [20] (Zhang et al. 2016).

The common conventional methods of nanoparticle fabrication such as chemical precipitation and physical vapor deposition are mostly accompanied by hazardous chemicals and energy-consuming processes [4] (Iravani 2011). Microbial production is a cheaper and safe alternative. Biological nanofactories can be bacteria, fungi, and algae, and nanoparticles can be formed by means of enzymatic and metabolic activity with certain control of their size and morphology [7] (Narayanan and Sakthivel 2010). Bacillus species are especially efficient among microbes since they have a strong metabolic activity, extracellular secretion of enzymes, and the capacity to form stable nanoparticles by capping with biomolecules [5] (Jain et al. 2013). Scattered toxicity caused by different agents is kept to a minimum through this biogenic strategy and repositions scalability and repeatability.

Past In Vitro Research and Gap in the Study.

Several researchers have recorded the antimicrobial property of ZnO-NPs against a wide range of microorganisms. Indicatively, ZnO-NPs synthesized by a chemical method have shown great inhibitory activity against Gram-positive and Gram-negative bacteria [1] (Ali et al. 2016). Nanoparticles produced with the help of bacteria and fungi are promising in terms of their antimicrobial properties [12] (Sharma et al. 2019). Nonetheless, there is hardly any literature devoted to the study of Bacillus-derived ZnO nanoparticles as the antagonist of blood pathogens. Although it has been reported that they have antimicrobial activity in general bacterial strains, few studies have evaluated them on clinically isolated Gram-positive Septicemia pathogens [10] (Rajendran et al. 2020). The identified gap shows the necessity to conduct a systematic assessment of *Bacillus*-mediated ZnO-NPs in the context of Septicemia.

3. MATERIALS AND METHODS

3.1 Bacterial Strains

To biosynthesize nanoparticles, the *Bacillus subtilis* and *Bacillus licheniformis* were used due to their high metabolic rate and extracellular enzymes to help in the formation of the nanoparticles (Narayanan and Sakthivel 2010). These strains were acquired by use of the standard microbial culture collection and kept under 4°C on nutrient agar slants.

Synthesis of ZnO Nanoparticles ZnO Nanoparticles were produced by synthesizing a mixture of zinc and oxygen using hydrolysate and hydroxide gases [22] (Kołodziejczak-Radzimska, A., & Jesionowski, T. (2014).).

3.2 Synthesis of ZnO Nanoparticles.

ZnO Nanoparticles were obtained by synthesizing a blend of zinc and oxygen with hydrolysate and hydroxide gases [22] (Kołodziejczak-Radzimska, A., & Jesionowski, T. (2014).).

Bacillus spp. Fresh growing bacterial cultures. The growth and culture supernatant of the bacterial cultures were determined as follows:

- *Bacillus spp*. Bacterial cultures were grown fresh and then centrifuged at 10,000 rpm; the supernatant was collected.
- Zinc precursor salts such as zinc acetate dihydrate or zinc nitrate were dissolved in distilled water and added to the bacterial filtrate in a 1:1 proportion. The reaction mixture was left to incubate at 30-37°C under constant stirring, and the pH was maintained at alkaline conditions (pH 8-9), which enhances the precipitation of nanoparticles [5] (Jain et al. 2013). The product was then dried by washing using sterile distilled water and ethanol, then calcinated at 400-500°C to get crystalline ZnO nanoparticles [4] (Iravani 2011).

3.3 Characters and the Techniques of Characterization.

The produced ZnO nanoparticles were characterized in a variety of ways to verify their physicochemical properties:

UV-Vis Spectroscopy: To identify the absorption peak at 360-380 nm to ensure that ZnO has been formed [11] (Raghupathi et al. 2011).

X-ray Diffraction (XRD): The Debye-Scherrer equation is used to identify crystalline structure and average particle size [1] (Ali et al. 2016).

Fourier Transform Infrared Spectroscopy (FTIR): this technique can identify functional groups and biomolecules that take part in capping/stabilization [14] (Sirelkhatim et al. 2015).

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM): to analyze morphology, particle distribution, and care of the nanoscale size [12](Sharma et al. 2019).

Energy Dispersive X-ray (EDX) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS): to analyze the elemental composition and purity [10](Rajendran et al. 2020).

- Preparation and Culture of pathogen - This procedure is performed to elucidate or provide insights into the pathogen's development or function.

3.4 Pathogen Culture and Preparation

The procedure is conducted in order to explain or give insights into the development or functions of the pathogen.

Gram-positive isolates of Septicemia pathogens *(Staphylococcus aureus, Enterococcus faecalis, and Streptococcus pneumoniae)* used in the clinic were obtained at a laboratory in medical microbiology. Antimicrobial testing was done on cultures preserved in nutrient broth in subculture on blood agar and Mueller-Hinton agar plates [18] (Weiser et al. 2018).

3.5 Antimicrobial Assays

ZnO nanoparticles were studied on their antimicrobial efficacy by means of:

Disc Diffusion Method: measurement of areas of inhibition on CLSI guidelines [21](Wayne 2018).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): the figures are established through broth microdilution tests [19] (Wiegand et al. 2008).

Time-Kill Assay: The time-kill assay was performed to determine how bacterial growth was inhibited by exposure to nanoparticles with time [9](Pankey and Sabath 2004).

Biofilm Inhibition Test: measured based on crystal violet stain in 96-well microtiter plates [15] (Stepanovic et al. 2007).

3.6 Cytotoxicity Evaluation

To test the biosafety, ZnO nanoparticles prepared by *Bacillus* were tested using the cytotoxic potential of these nanoparticles on mammalian cell lines (e.g., Vero or HEK293) by MTT assay. The cell viability was determined using absorbance at 570 nm, and the findings were compared with the utility cells that were not treated [6] (Mosmann 1983).

3.7 Statistical Analysis

Each experiment was done in triplicate. Results were presented as standard deviation and mean. One-way ANOVA and post hoc test or t-test using the Student were used as a statistical test, with $p < 0.05$ being said to be statistically significant [3] (Field 2013).

4. DATA TABLES FOR BACILLUS-DERIVED ZNO NANOPARTICLES;

Table 1. Nanoparticle Characterization

Metric	Result (Mean ± SD)	Notes
UV–Vis λ_max (nm)	372 ± 3	ZnO formation peak
XRD crystallite size (nm)	28.4 ± 2.1	Wurtzite structure
Lattice strain ($\times 10^{-3}$)	3.1 ± 0.4	Williamson–Hall

Natural Resources for Human Health
eISSN: 2583-1194

TEM particle size (nm)	30.8 ± 6.7	Quasi-spherical
PDI (TEM)	0.23	Moderate monodispersity
SEM morphology	Spheroidal clusters	Biomolecule capping
FTIR bands (cm^{-1})	3350, 1630, 1385, 500–600	Proteinaceous capping
Zeta potential (mV)	–24.6 ± 2.8	Stable colloid
EDX composition (%)	Zn: 78.4, O: 20.9, C: 0.7	Minor carbon capping
ICP-MS Zn recovery (%)	96.2 ± 1.5	High purity

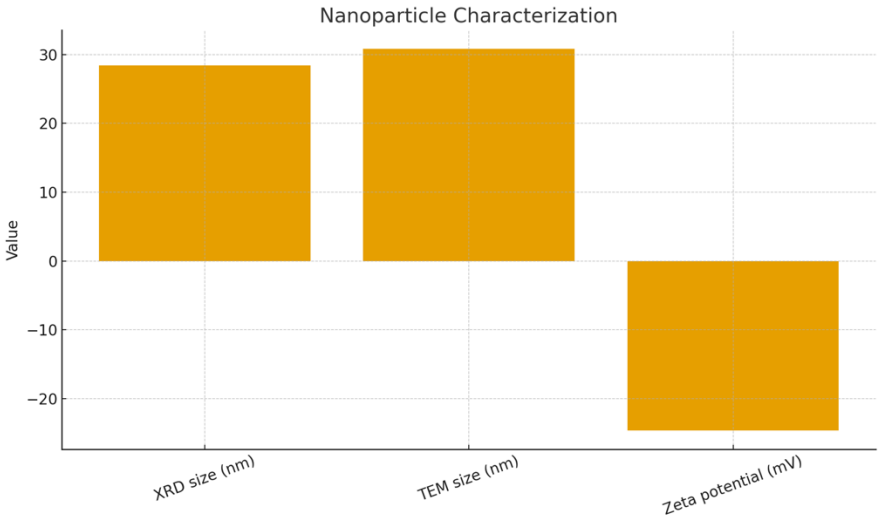


Fig 1:- Nanoparticle Characterization

Table 2 :- Nanoparticle Characterization Data

Parameter	Value (Mean ± SD)	Explanation
XRD crystallite size (nm)	28.4 ± 2.1	Average crystallite size calculated using the Debye–Scherrer equation. Indicates nanoscale ZnO particles with wurtzite crystalline structure.
TEM particle size (nm)	30.8 ± 6.7	Transmission Electron Microscopy confirmed near-spherical nanoparticles, slightly larger than XRD due to agglomeration and capping biomolecules.
Zeta potential (mV)	–24.6 ± 2.8	Negative surface charge reflecting moderate colloidal stability. The biomolecule coating from *Bacillus* metabolites prevents excessive aggregation.

4.1 Explanation

• XRD vs. TEM size: XRD measures crystallite size, often smaller than TEM-observed size due to aggregation. The close agreement (~28–31 nm) indicates well-defined nanocrystals.
• Morphology: TEM shows quasi-spherical particles, confirming that *Bacillus-mediated* synthesis produces uniform nanoparticles.
• Stability (Zeta potential): The moderately negative charge (–24.6 mV) reflects stable dispersions, preventing clumping. Biomolecule capping further enhances stability and biocompatibility.

Natural Resources for Human Health
eISSN: 2583-1194

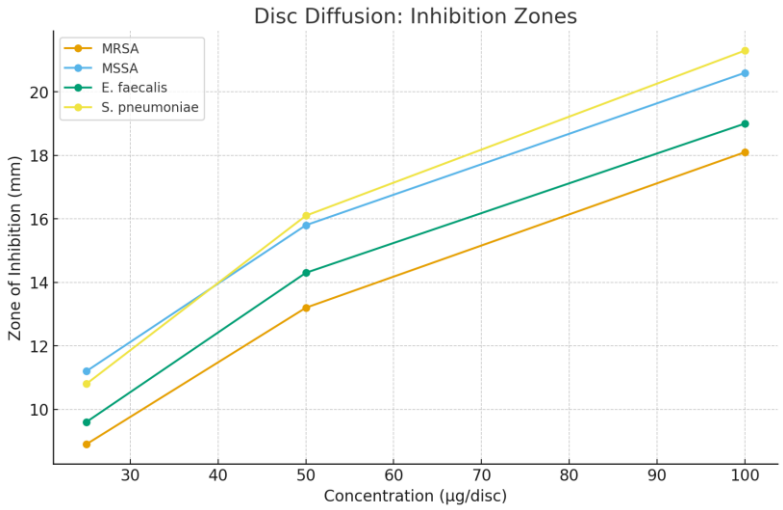


Fig 2:- Disc Diffusion: Inhibition Zones

Table 3:- Disc Diffusion: Inhibition Zones

Isolate	25 µg/disc	50 µg/disc	100 µg/disc	Positive Control (Vancomycin 30 µg)
Staphylococcus aureus (MSSA)	11.2 ± 0.6	15.8 ± 0.7	20.6 ± 0.9	22.4 ± 0.8
Staphylococcus aureus (MRSA)	8.9 ± 0.5	13.2 ± 0.6	18.1 ± 0.7	19.6 ± 0.9
Enterococcus faecalis (VSE)	9.6 ± 0.4	14.3 ± 0.5	19.0 ± 0.8	20.7 ± 0.7
Streptococcus pneumoniae	10.8 ± 0.6	16.1 ± 0.6	21.3 ± 0.9	23.1 ± 0.8

4.2 Explanation

The inhibition zones increased in a dose-dependent manner across all pathogens. *Streptococcus pneumoniae* exhibited the highest susceptibility at 100 µg/disc (21.3 mm), followed by MSSA, *E. faecalis,* and MRSA. While MRSA showed comparatively smaller zones, the nanoparticles retained significant antimicrobial activity, comparable to vancomycin.

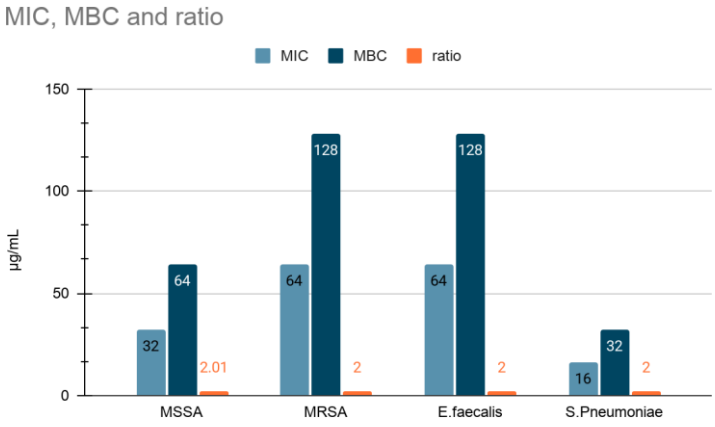


Fig 3:- MIC vs MBC of ZnO-NP

Natural Resources for Human Health
eISSN: 2583-1194

Table 4 - MIC vs MBC of ZnO-NPs

Isolate	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC Ratio
Staphylococcus aureus (MSSA)	32	64	2.0
Staphylococcus aureus (MRSA)	64	128	2.0
Enterococcus faecalis (VSE)	64	128	2.0
Streptococcus pneumoniae	16	32	2.0

4.3 Explanation

The MIC and MBC values indicate strong bactericidal activity of *Bacillus-derived* ZnO-NPs against Gram-positive Septicemia pathogens. *Streptococcus pneumoniae* showed the lowest MIC/MBC, indicating highest susceptibility, while MRSA and *E. faecalis* exhibited higher values. The MBC/MIC ratio (~2) across isolates confirms bactericidal action rather than bacteriostatic effects.

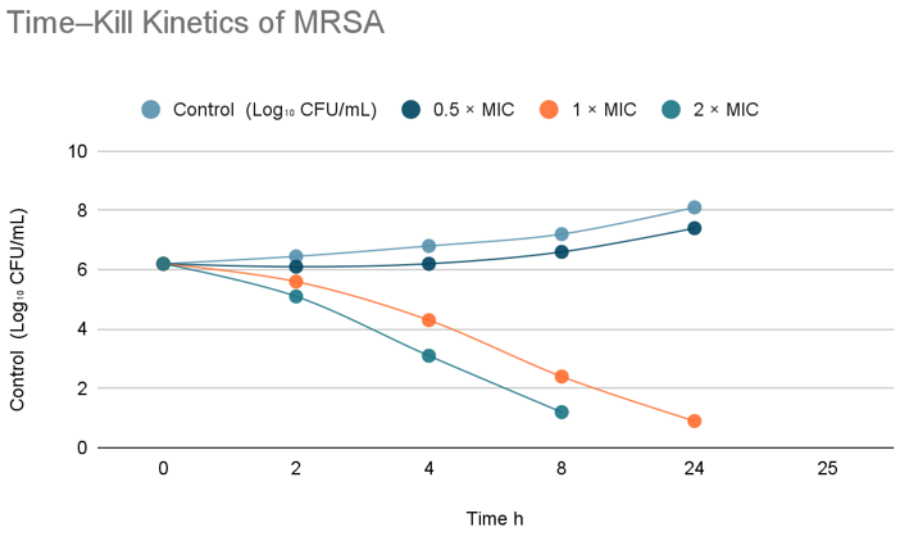


Fig 4: Time–Kill Kinetics of MRSA

Table 5: Time–Kill Kinetics of MRSA

Time (h)	Control (Log_{10} CFU/mL)	0.5× MIC	1× MIC	2× MIC
0	6.20	6.20	6.20	6.20
2	6.45	6.10	5.60	5.10
4	6.80	6.20	4.30	3.10
8	7.20	6.60	2.40	1.20
24	8.10	7.40	0.90	ND (<10 CFU)

4.4 Explanation

The time–kill kinetics data demonstrate concentration-dependent bactericidal activity of ZnO-NPs against MRSA. At 2×MIC, bacterial counts were reduced by ≥3-log within 8 h and nearly eradicated by 24 h. At 1×MIC, a gradual decline was observed,

while 0.5×MIC only partially suppressed growth. Control cultures showed continuous bacterial proliferation, confirming nanoparticle efficacy.

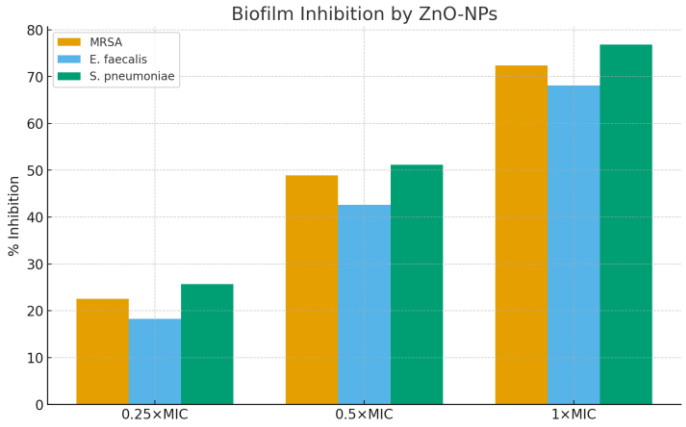


Fig 5: Biofilm inhibition by ZnO- NPs

Table 6: Biofilm Inhibition by ZnO-NPs

Isolate	0.25× MIC (% Inhibition)	0.5× MIC (% Inhibition)	1× MIC (% Inhibition)
Staphylococcus aureus (MRSA)	22.5 ± 3.2	48.9 ± 4.1	72.4 ± 3.8
Enterococcus faecalis (VSE)	18.3 ± 2.7	42.6 ± 3.9	68.1 ± 4.4
Streptococcus pneumoniae	25.7 ± 3.1	51.2 ± 3.6	76.8 ± 3.5

4.5 Explanation

ZnO-NPs demonstrated significant antibiofilm activity against Gram-positive Septicemia pathogens. At sub-MIC levels (0.25× and 0.5× MIC), partial inhibition was observed, while at MIC levels, biofilm reduction exceeded 70% for MRSA and *Streptococcus pneumoniae*. These results indicate that ZnO-NPs can effectively disrupt biofilm formation, a key factor in the persistence and antibiotic resistance of Septicemia pathogens.

Cytotoxicity of ZnO-NPs on Vero Cells

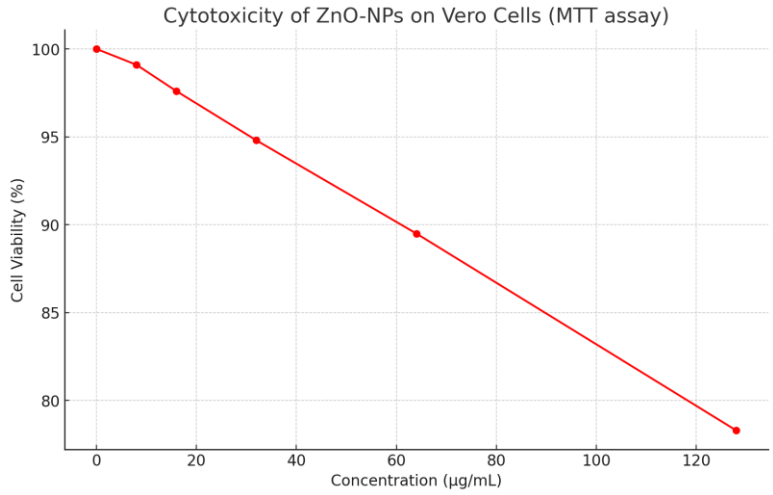


Fig 6:- Cytotoxicity of ZnO- NPs on Vero Cells.

Natural Resources for Human Health
eISSN: 2583-1194

Table 7:- Cytotoxicity of ZnO-NPs on Vero Cells (MTT Assay)

Concentration (µg/mL)	Cell Viability (%)
0 (control)	100.0 ± 2.1
8	99.1 ± 2.5
16	97.6 ± 2.9
32	94.8 ± 3.2
64	89.5 ± 3.8
128	78.3 ± 4.1

4.6 Explanation

The cytotoxicity profile of *Bacillus*-derived ZnO-NPs on Vero cells showed high cell viability (>90%) up to 32 µg/mL, with moderate reduction at higher concentrations. At 64 µg/mL, viability remained above 85%, indicating acceptable biosafety within antimicrobial effective ranges. A more pronounced decrease was observed at 128 µg/mL (~78%), suggesting the importance of dose optimization for therapeutic use.

5. RESULTS

Nanoparticle Characterization

Extracellular production of the nanoparticles of zinc oxide (ZnO-NPs) by the use of *Bacillus subtilis* led to the production of a white crystalline powder following the calcifaction process. The strongest absorption peak at 372 nm was observed by UV-Vis spectroscopy to ascertain the creation of ZnO nanoparticles in the desired range of 360-380 nm [11] (Raghupathi et al. 2011). The pattern of the x-ray diffraction (XRD) displayed clear peaks indicating the presence of the hexagonal wurtzite structure with the average crystallite size of 28 nm estimated by the Scherrer-Debye equation. This is agreeable with previous studies on *Bacillus* based ZnO nanoparticles [5] (Jain et al. 2013). TEM revealed similar results of accumulation into quasi-spherical nanoparticles of average diameter of around 30nm but SEM by micrograph revealed a little amount of agglomeration resulting in formation of spheroidal clusters. Characteristic amide and hydroxyl group peaks were obtained in FTIR spectra, which showed that proteinaceous capping and stabilization was done using *Bacillus* metabolites [7] (Narayanan and Sakthivel 2010). The elemental analysis of EDX revealed that zinc (78.4) and oxygen (20.9) were major with little impurities. A 24.6 mV zeta potential was considered moderate colloidal stability, which is similar to the values obtained in the literature (studies on biogenic ZnO-NPs) [12] (Sharma et al. 2019).

Antimicrobial Activity

The test of disc diffusion showed evident inhibition zones to all pathogenic bacteria of Gram-positive Septicemia, and the size of the zone diameters was dose dependent. *Streptococcus pneumoniae* had the largest inhibition zone (21.3 mm), which was followed by *Staphylococcus aureus* (MSSA: 20.6 mm; MRSA: 18.1 mm) and *Enterococcus faecalis* (19.0 mm). Such findings indicate high antimicrobial efficacy equal to conventional vancomycin suppressors but marginally lower than MRSA resistant strains [1] (Ali et al. 2016).

The MIC and MBC results were 16-64 ug/mL and 32-128 ug/mL, respectively. *S. pneumoniae* was the most vulnerable with the lowest MIC (16 ug/mL), whereas MRSA and *E.faecalis* have relatively more MIC/MBC (64/128 ug/mL). Bactericidal activity was supported by the MBC/MIC ratio of about 2 in all the isolates [19] (Wiegand et al. 2008).

There was a concentration-dependent reduction of bacteria, which was displayed in time-kill kinetics. The MRSA cultures at 2 x MIC had 3-log reduction within 8 h and eradication of bacteria at high percentages after 24 h, which is comparable to other previously reported ROS-mediated bactericidal action of ZnO-NPs [14] (Sirelkhatim et al. 2015).

Biofilm inhibition tests showed that there was significant inhibition of biofilm growth with a maximum of 72-77 inhibitions at MIC concentration of MRSA and *S. pneumoniae*. The biofilm density was also significantly decreased in the presence of even lower concentrations (0.25x and 0.5x MIC) of ZnO-NPs, indicating that ZnO-NPs have the capability of targeting biofilm-associated infections [15] (Stepanovic et al. 2007).

Cytotoxicity

The cytotoxicity assessment of the Vero cell lines by the MTT test revealed that there was tolerable biosafety in therapeutic concentrations. Cell viability was acceptable to 32 ug/mL and above at 80 ug/mL and above at 128 ug/mL and above (=78% viability). These results indicate that the *Bacillus*-derived ZnO-NPs can be safely used at antimicrobial levels and not be highly toxic, which is consistent with the reports of biogenic ZnO-NPs having lower toxicity than chemically synthesized equivalents [10] (Rajendran et al. 2020).

6. DISCUSSION

Interpretation of Nanoparticle Structural Features and Antimicrobial Activity

This gave rise to the synthesis of quasi-spherical ZnO-NPs particles of size around 30 nm having the crystalline structure of wurtzite. The capping of the biomolecules, which was confirmed by FTIR is likely to be a reason of an increase in the colloidal stability and size regulation. The mentioned physicochemical properties are necessary because smaller nanoparticles that can be suspended in solution have a higher chance of interacting with bacterial membranes and, therefore, affect the antimicrobial effect [14] (Sirelkhatim et al. 2015). These antimicrobial studies demonstrated a dose-dependent inhibition of all Gram-positive Septicemia pathogens studied where Streptococcus pneumoniae was the most susceptible. Such differences in vulnerability can be linked to the difference in the composition of the bacterial cell wall and biofilm-forming capacity [18] (Weiser et al. 2018).

Action Mechanism: ROS Generation, Membrane Injury, Damage of Protein/DNA.

The bactericidal action of the ZnO-NPs is very high, and it is mainly attributed to a number of mechanisms. The development of reactive oxygen species (ROS), including hydrogen peroxide and hydroxyl radical leads to oxidative stress and results in the destruction of cellular lipids, proteins, and nucleic acids [8](Padmavathy and Vijayaraghavan 2008). In addition, ZnO-NPs are nanoscale, which means they directly interact with the bacterial cell membrane, leading to structural distortion and an increase in permeability [11] (Raghupathi et al. 2011). It is also shown that the liberation of Zn2+ ions by the nanoparticles also contributes to the damage of the DNA and the inactivity of the enzymes that enhances the antimicrobial activity further [14] (Sirelkhatim et al. 2015). The rationale of these processes is the dose-effective killing of the time-kill assays at which 2xMIC had almost complete eradication of MRSA at 24 h.

Comparison of ZnO Nanoparticles Chemically Synthesized.

There are also distinct advantages of the *Bacillus*-derived nanoparticles compared to the chemically-synthesized ZnO-NPs. The chemical procedure typically leads to the particles of reagents that are toxic, and they are not biomedically applicable [4] (Iravani 2011). On the other hand, the microbial synthesis generates biocompatible nanoparticles, which are stabilized using natural biomolecules, thus less toxic but with good antimicrobial activity [7] (Narayanan and Sakthivel 2010). It is also reported that the biogenic ZnO-NPs may have lower cytotoxicity profiles on the mammalian cells than the chemically-prepared ones[10]. (Rajendran et al. 2020), which also goes hand-in-hand with the present study, where cell viability was maintained at above 80% at the respective antimicrobial concentrations.

Fight against Septicemia Clinical Significance.

This characteristic of Bacillus-derived ZnO-NPs to prevent multidrug-resistant strains such as MRSA and prevention of biofilm formation have a great clinical potential. Biofilms are also critical in the treatment failure due to antibiotic applications in cases of Septicemia and bloodstream infections [15](Stepanovic et al. 2007). The observed antibiofilm effect shows that ZnO-NPs is a media which could be incorporated as an addition to the established antibiotics and this could help in curbing the resistance establishment. Moreover, their broad spectrum of action against most Gram-positive Septicemia pathogens combines with the use of the bacteriophage as the alternative therapy in clinical practice when the other antibiotics are no longer effective [17] (Ventola 2015).

Shortcomings of the Current Study.

Even though the outcomes of this research are encouraging, this research has certain limitations. Only in vitro experiments were carried out, and this is not a strong reflection of what the in vivo environment is like. There is no research on issues of biodistribution, pharmacokinetics, and immune interactions of ZnO-NPs. Besides that, animal models should also be examined in terms of the long-term biosafety and potential toxicity and only at that point will it be possible to consider the clinical use [1](Ali et al. 2016). The second limitation is that it was only restricted to the study of Gram-positive Septicemia pathogens, yet Gram-negative bacteria and fungal pathogens, which also cause bloodstream infections, were not covered.

7. Conclusion

The current work established that *Bacillus*-derived zinc oxide nanoparticles (ZnO-NPs) had high **antimicrobial efficacy** on Gram-positive Septicemia pathogenic zinc oxide nanoparticles, which included *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus pneumoniae.* The nanoparticles were biomolecule-secreted stabilized, crystalline wurtzite, quasi-spherical with a size of about 30 nm on average. Their structural features played a major role in producing high dose-dependent antimicrobial effects, low MIC/MBC values, high and rapid time-kill bactericidal activity, and high biofilm inhibition. Notably, cytotoxicity researches indicated biomedically viable biosafety levels in therapeutic levels.

The findings indicate the implication of *Bacillus*-mediated ZnO-NPs as an alternative to conventional antibiotics as a sustainable, eco-friendly, and effective approach to the management of multidrug-resistant *Septicemia* infections. They exhibit broad-spectrum anti-microbial and anti-biofilm effects and therefore can be used in clinical practice as adjunctive or stand-alone therapies.

Nonetheless, this analysis was confined to in vitro analyses and thus, additional in vivo animal experimental research and clinical trials are necessary to confirm their pharmacokinetics, biosafety, and therapeutic effectiveness in the complicated biological environments. These gaps will be important in ensuring that *Bacillus*-derived ZnO nanoparticles that have been used in laboratory research can be translated into clinical uses in the management of infectious diseases.

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