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REVIEW ARTICLE

Effect of ZnO Nanoparticles as Antimicrobial on Multidrug Resistance *Klebsiella Pneumonia*: A Review

Review: Pengaruh Nanopartikel ZnO sebagai Antimikroba pada *Multidrug Resistance Klebsiella Pneumonia*

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ABSTRACT

Klebsiella pneumoniae (K. pneumoniae) is a common microorganism for different infections, including respiratory, urinary tract, and biliary diseases, where immunocompromised individuals are directly affected by this bacterium. However, with the emergence of hypervirulent strains, individuals, whether healthy or immunocompetent, are equally susceptible to *K. pneumoniae* infections. This problem is diagnosed by the wide spreading of multidrug-resistant bacteria in the environment with multiple resistance mechanisms, which are the main challenges for an effective treatment. This put physicians in an inflexible confusion because of the limitation of medical treatment options. Among many remedy substances, zinc oxide (ZnO) nanoparticles (NPs) showed remarkable antibacterial properties versus many Gram-positive and Gram-negative bacteria. This work focuses on presenting the influence of these NPs on the expression of genes in charged in generating the *K. pneumoniae* capsule. Furthermore, perspectives for applying ZnO NPs in clinical practices are also discussed. As the in vivo studies show a powerful impact of ZnO on bacteria, it is anticipated that this method of treatment will be utilized by hospitals.

Keywords: zinc oxide; nanoparticles; Klebsiella pneumoniae; capsule genes; antibiotic resistance

INTRODUCTION

K. pneumoniae is classified as a Gram-negative pathogen that belongs to the Enterobacteriaceae family. Commonly, it is associated with the nosocomial infections; this type of infections is mainly affecting individuals who are immunocompromised. Hence, urinary tract, respiratory tract, bloodstream, and surgical site infections are mostly caused by *K. pneumoniae*.[1]

This bacterium contains a large genome system of both plasmids and chromosomal gene loci.[2] Depending on this system, the type of *K. pneumoniae* is identified: opportunistic, hypervirulent, or multidrug-resistant. Moreover, it makes the bacteria efficient to induce community-acquired infections.[3]

The global concern about the evolution of multidrug-resistant pathogens in humans is critical, where *K. pneumoniae* is one of these pathogens. This bacterium causes a consequential public health issue by limiting the medical treatment options. The important *K. pneumoniae* resistance mechanisms are those that employ the β-lactamase enzymes, where they can hinder the activity of β-lactam antibiotics.[4] β-lactams work as transpeptidases that resemble the sequence of d-Ala-d-Ala amino-acids and change the substrate's D-alanyl-D-alanine transpeptidases. This action will interrupt the bacterial cell wall synthesis.[5] Moreover, *K. pneumoniae* strains that have a carbapenem resistance possess an ex-genome-(plasmid) that encodes carbapenemase; the carbapenemase makes all of the carbapenem antibiotics functionally ineffective.[4,6] In addition, traditional medical treatment methods fail in enucleating the multidrug resistant infections, not only for *K. pneumoniae* but for all other bacteria.[7] Finding new curing strategies became urgent due to the limitation in current options, increase in mortality rates among patients, and evolution of genes against the antimicrobial drugs. Here, we provide an overview on the use of nanotechnology as one of the recent strategies used to treat multidrug resistant *K. pneumoniae*. Based on the reported studies, this technique is showing interesting results and more progress could be witnessed in the future.

Resistance Genes of *K. pneumoniae*

In severe infections of skin and soft tissues, *K. pneumoniae* strains with hypervirulent phenotypes (hvKp) and aggressive capsular serotypes, such as K1 and K2, were detected to arise frequently. The expression of the armA gene, which encodes the 16SrRNA methylase enzyme and prevents binding to the bacterial ribosome, was found to be sufficient for initiating phenotypes resistance, even with the reduced use of antibiotics such as the aminoglycosides.[8] However, there is a polysaccharide capsule that encases the whole cell's surface of *K. pneumoniae* and contains large amounts of glucuronic and pyruvic acids.[1] This cover gives the colonies their glistening and mucoid appearance on agar plates, and also responsible for the biofilm and virulence factors of this bacteria.[2] *K. pneumoniae* was historically classified based on the capsule (K antigen) serotyping; to date, 79 capsule types were recognized.[3] These strains, especially the clinical isolates, produce a viscous polysaccharide capsule. The biochemical complication of these capsules promotes the production of strain-specific antigenics of capsular components. The distinct antigenic types of capsules produced by *Klebsiella* strains have been used for strain recognition during clinical infections.[4] Capsule polysaccharide (CPS) is known as one of the most significant virulence factors of *K. pneumoniae* that has a density of 160 nm; the capsular is an acidic polysaccharide complex.[5] Capsular polysaccharide genes comprise a set of genes that are responsible for the synthesis of different serotypes of CPS.[6] Some of these genes, including the magA, k2A, rmpA, and kfu, have been classified as markers of virulence.[7] However, the function of this virulence factor is not the only concern in *Klebsiella's* pathogenicity, the multidrug resistance genes are another concern that cannot be ignored. Likewise other organisms, bacteria have a genomic flexibility for adaption and

protect themselves from toxic chemicals. So, the bacteria are well developed for antimicrobial-resistant genetic system. The first system that bacteria developed to be antimicrobial resistant is the genes transmission that partially involve the mobile genetic elements (MGE, transposons, plasmids, and integrons). Plasmid-borne genes are another system responsible for the initiation of resistance. *Klebsiella* as a gram-negative bacteria shows class 1 integrons, which are associated with a variety of resistance gene cassettes like spectinomycin and trimethoprim resistances.[8] *K. pneumoniae* strain FFUL 22K has class 3 integrons included with blaGES-1 gene. A report investigated 587 Gram-negative bacteria showed a remarkable resistance to ceftazidime and sulbactam-cefoperazone, where 0.7% (4/587) isolates possessed class 3 integron. The incidence and detection rates of class 3 integron varied from 0 to 10%.[9] Moreover, *K. pneumoniae* has a chromosomal system for antibiotics resistance like strain HS11286 chromosome that has a putative type II TA.[10] Furthermore, the chromosomal encoded kacAT bicistronic operon of strain HS11286 has a functional locus with kacA encoding the antitoxin to the toxic product of kacT has a remarkable impact in multidrug bacterial toleration.[11] Examples of virulence and drug resistance-related genes for *K. pneumoniae* are shown in Table 1.

Table 1. Examples of virulence and drug resistance-related genes reported for *K. pneumoniae*

Genes Product	Product Function	Reference
GmrkABCDF	Encodes fimbriae type 1 and 3	Lin TH, et al.[12]
Cps	Encodes polysaccharide capsule	Martin RM, et al.[13]
rmpA	Synthesis of capsular compounds	Wan G, el al.[14]
magA,k2A;wcaG;wabG;uge; ycfM	Formation of capsule	Candan ED, et al.[15]
wbbY; wbbZ	Modify LPS composition	Follador R, et al.[16]
entS	Production of enterobactin	Farahpour MR, et al.[17]
armA; aacA4;aacC2;aadA1; aac(6')-Ib	Aminoglycosides resistance	Horn J, et al.[18]
blaKPC-2;blaKPC-3	Carbapenem, clavulanic acid	Yoong P, et al.[19]
acrAB,qnrB; qnrS	Quinolones resistance	Horn J, et al.[18]
blaSHV; blaTEM ; blaCTX-M	Carbapenems resistance	Vena A, et al.[20]
lpxM	Polymyxin resistance	Horn J, et al.[18]
amR;rpsJ;tetA	Tigecycline resistance	Ahn C, et al.[21]

Nanotechnology

Nanotechnology could be assumed as the most innovative progress in the twenty-first century in all fields of technology, science, and industry.[8] Nanoparticles (NPs) are defined as particles of 1-100 nm diameter, where this small size altered their known physicochemical properties and pursued new approaches.[9] Yet, nanotechnology is considered as a recent class of defense against bacterial infections due to the unique antibacterial characteristics that some nanomaterials own. It was found that the highly effective nanomaterials in this regard are metals, metal oxides, and carbon nanotubes; the antibacterial properties are contributed to the size and morphology influence. This process is represented by three steps: 1) the production of reactive oxygen species (ROS) and/or metallic ions; 2) the produced moieties affect bacteria's protein and DNA destruction, and 3) the NPs accumulate on the bacterial cell membrane that finally deforms it and annihilates the bacteria.[10]

Zinc oxide is an inorganic chemical compound that used widely in life, and the development in nanotechnology led to advancing its nano-size preparation methods.[22] ZnO-NPs diameter is less than 100 nm with superior catalytic properties, with a large ratio of surface to volume.[23] Depending

on the synthesis method of ZnO-NPs, the physicochemical properties can be manipulated.[24] ZnO NPs are among the metal oxides that showed an effective antibacterial potential versus several Gram-positive and -negative bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.[11]

Antibacterial Activity of ZnO Nanoparticles

Several strategies have been employed to control the microbial infections. One of these strategies is the utilization of metal oxide nanoparticles, which is recognized as a strong approach used in health-related applications.[25,26] The good antibacterial feature of ZnO-NPs have been demonstrated by many studies as shown in Table 2.

Table 2. Some studies shown that ZnO-NPs have antimicrobial properties.

Tested bacteria	Reference
S. aureus and *E. coli*	Redy KM, et al.[27]
S. enterica serovar Enteritidis, Salmonella Suspensions	Xie Y, et al.[28]
Pseudomonas aeruginosa	Chitra K, et al.[29]
Klebsiella pneumoniae, Bacillus subtilis, Salmonella typhi	Jeeva LV, et al.[30]

Many research groups have examined the bactericidal potency of ZnO NPs. According to a recent research, NPs is an effective bactericidal agent for antibiotics treatment and bacterial growth detection when the culture turbidity and viable cells ratio tests are used in colony counts.[22] However, the modification inside the bacterium increased the antibacterial activity of ZnO NPs.[23] As a result, the antibacterial activity level was calculated; results showed that it can be increased as the number of starting bacterial cells drops from 106 to 102 colony-forming units (CFU).[24]

The effect of ZnO NPs was studied on the cell wall, which is a complex of surface proteins used for adhesion and colonization, and contains components like polysaccharides and teichoic acid. These components protect the bacteria against host defenses and environmental conditions.[31] Chemically, these components are charged macromolecules; so, to disrupt these molecules and their functions and location, scientists triggered this disruption on specific groups on the surface of the microorganism cell wall.[32] A study by Pati et al showed that ZnO NPs can alter the membrane's integrity of a bacterium cell, reduce the surface hydrophobicity, and disorganize the oxidative stress-resistance genes transcription.[33] In addition, other studies show that ZnO NPs own a crucial role in the disruption of the genomic material of the bacteria. For example, *Pseudomonas aeruginosa* bacteria mRNA pslA gene is in charged in the biofilm formation. These gene levels have been affected by treating these bacteria for 30 minutes with ZnO NPs.[34] Another study found that the antibacterial activity of ciprofloxacin and ceftazidime antibiotics increased when exposed to a small amount of ZnO NPs.[35]

ZnO NPs and other metal oxides are promising biomedical treatment agents because of their ability to attack more than one target of the bacteria. Here, they became suitable antibacterial and antifungal agents, or supporters for the antibiotics. More studies are needed to uncover more mechanisms and modes of ZnO NPs action on *Klebsiella pneumonia* and other bacteria. Figure 1. shows the distinctive bacteria cell structures

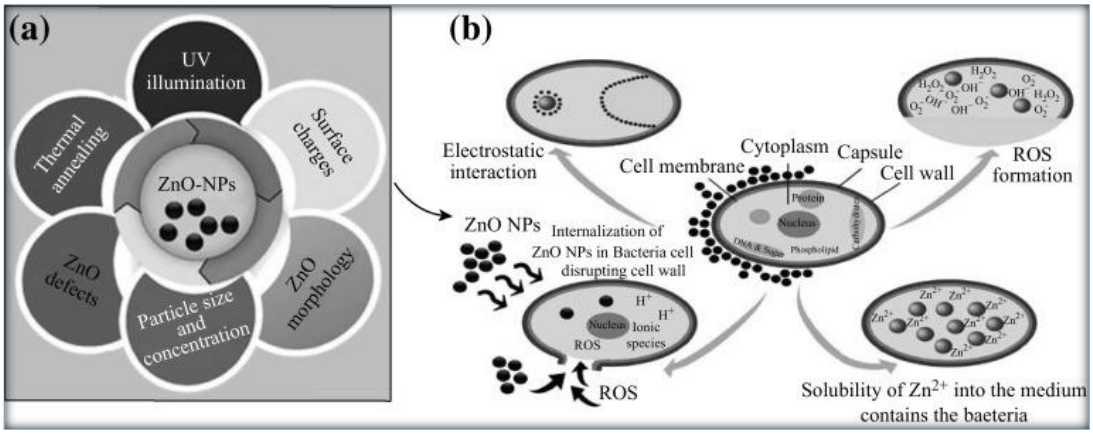

Figure 1. (a) relationship between the a major ZnO-NPs factors that affect the antibacterial response, and (b) different potential mechanisms of ZnO-NPs antibacterial activity, such as ROS generation, Zn^{2+} release, incorporation of ZnO-NPs into bacteria, and electrostatic contacts.

CONCLUSION

Previous studies have shown the impact of nano-size ZnO NPs as an antibacterial due to their unique properties that enable them to eliminate virulence factors in pathogenic bacteria. This type of NPs affects the components that build the bacteria's cells walls, and the impact extends to the whole cell. It is recommended to conduct the research on various types of bacteria to understand the mechanisms of impact and comprehend them. Other chemical and physical properties of ZnO NPs, such as the size, shape, pore size, surface area, hydrophilicity, etc. could also influence the activity and need to be furtherly investigated. It is anticipated to apply this treatment method in hospitals soon.

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AUTHORS CONTRIBUTION

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CONFLICT OF INTEREST

The authors declare that there is no known conflict for this work

REFERENCES

1. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical infectious diseases, 2009;48(1):1-12. doi: 10.1086/595011
2. Martin RM, Bachman MA. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Frontiers in cellular and infection microbiology, 2018;8:4. DOI: 10.3389/fcimb.2018.00004
3. Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS microbiOL rev. 2017;41(3):252-75. doi: 10.1093/femsre/fux013.
4. Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrobial agents and chemotherapy. 2001;45(4):1151-61. doi: 10.1128/AAC.45.4.1151-1161.2001.
5. Drawz SM, Bonomo RA. Three decades of β-lactamase inhibitors. Clin Microbiol Rev. 2010;23(1):160-201. doi: 10.1128/CMR.00037-09
6. Thakur PK, Kumar J, Ray D, et al. Search of potential inhibitor against New Delhi metallo-beta-lactamase 1 from a series of antibacterial natural compounds. J Nat Sci Biol Med. 2013;4(1):51-6. doi: 10.4103/0976-9668.10726
7. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int. 2016;2016:2475067. DOI: 10.1155/2016/2475067
8. Wang G, Zhao G, Chao X, et al. The Characteristic of Virulence, Biofilm and Antibiotic Resistance of Klebsiella pneumoniae. Int. J Environ. Res Public Health. 2020;17(17):6278. doi: 10.3390/ijerph17176278.
9. Collis CM, Kim MJ, Partridge SR, et al. Characterization of the class 3 integron and the site-specific recombination system it determines. J Bacteriol. 2002;184(11):3017-26. doi: 10.1128/JB,184.11.3017-3026.2002
10. Harms A, Maisonneuve E, Gerdes K. Mechanisms of bacterial persistence during stress and antibiotic exposure. Science. 2016;354(6318):aaf4268. doi: 10.1126/science.aaf4268.
11. Hall AM, Gollan B, Helaine S. Toxin–antitoxin systems: reversible toxicity. Curr Opin Microbiol. 2017;36:102-10. doi:10.1016/j.mib.2017.02.003.
12. Lin TH, Chen Y, Kuo JT, et al. Phosphorylated OmpR Is Required for Type 3 FimbriaeExpression in Klebsiella pneumoniae Under Hypertonic Conditions. Front. Microbiol. 2018;9:2405. https://doi.org/10.3389/fmicb.2018.02405
13. Martin RM, Bachman MA. Colonization, Infection, and the Accessory Genome of Klebsiella pneumoniae. Front Cell Infect Microbiol. 2018;8:4. doi: 10.3389/fcimb.2018.00004.
14. Wan G, Zhao G, Chao X, et al. The Characteristic of Virulence, Biofilm and Antibiotic Resistance of Klebsiellapneumoniae. Int J Environ Res Public Health. 2020;17(17):6278. doi: 10.3390/ijeph17176278.
15. Candan ED, Aksoz N. Klebsiella pneumoniae: Characteristics of carbapenem resistance and virulence factors. Acta Biochim Pol. 2015;62(4):867–74. doi: 10.18388/abp.2015_1148.
16. Follador R, Heinz E, Wyres KL, et al. The diversity of Klebsiella pneumoniaesurface polysaccharides. Microb Genom. 2016;2(8):e00007. doi: 10.1099/mgen.0.000073.
17. Farahpour MR, Vahid M, Oryan A. Effectiveness of topical application of ostrich oil on the healing of Staphylococcus aureus-and Pseudomonas aeruginosa-infected wounds. Connect Tissue Res. 2018;59(3):212–22. doi: 10.1080/03008207.2017.1350174.
18. Horn J, Stelzner K, Rudel T, et al. Inside job: Staphylococcus aureus host-pathogen interactions. In J Med Microbiol. 2018;308(6):607–24. doi: 10.1016/j.ijmm.2017.11.009.
19. Yoong P, Torres VJ. The effects of Staphylococcus aureus leukotoxins on the host: Cell lysis and beyond. Curr Opin Microbiol. 2013;16(1):63–9. doi: 10.1016/j.mib.2013.01.012.
20. Vena A, Castaldo N, Bassetti M. The role of new beta-lactamase inhibitors in gram-negative infections. Curr Opin Infect Dis. 2019;32(6):638–46. DOI: 10.1097/QCO.0000000000000600

21. Ahn C, Yoon SS, Yong TS, et al. The Resistance Mechanism and Clonal Distribution of Tigecycline-Nonsusceptible Klebsiella pneumoniae Isolates in Korea. Yonsei Med J. 2016;57(3):641–6. doi: 10.3349/ymj.2016.57.3.641.
22. Espitia PJP, Soares NFF, Coimbra JSR, et al. Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. Food and bioprocess technology. 2012;5(5): 1447-64. DOI: 10.1007/s11947-012-0797-6.
23. Khan ZUH, Sadiq HM, Shah NS, et al. Greener synthesis of zinc oxide nanoparticles using Trianthema portulacastrum extract and evaluation of its photocatalytic and biological applications. J Photochem Photobiol B. 2019;192:147-57. doi: 10.1016/j.jphotobiol.2019.01.013.
24. Bharat TC, Shubam, Mondal S, et al. Synthesis of doped zinc oxide nanoparticles: a review. Materialstoday: Proceedings. 2019;11(2):767-75. https://doi.org/10.1016/j.matpr.2019.03.041
25. Azam A, Ahmed A, Oves M, et al. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. Int J Nanomedicine, 2012;7:6003-9. doi: 10.2147/IJN.S35347.
26. Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Sci Techno Adv Mater. 2008;9(3):035004. doi: 10.1088/1468-6996/9/3/035004
27. Reddy KM, Feris K, Bell J, et al. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl Phys Lett. 2007;90(213902):213902-1-3. doi: 10.1063/1.2742324.
28. Xie Y, He Y, Irwin PL, et al. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. Appl Environ Microbiol. 2011;77(7):2325–31. doi:10.1128/AEM.02149-10.
29. Chitra K, Annadurai G. Antimicrobial activity of wet chemically engineered spherical shaped ZnO nanoparticles on food borne pathogen. Int Food Res J. 2013;20(1):59–64.
30. Jeeva LV, Sharath R, Chandraprabha MN, et al. Synthesis, characterization and evaluation of antimicrobial activity of zinc oxide nanoparticles. J Biochem Tech. 2012;3(5):S151-S154
31. Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater. 2004;8:37-57. doi: 10.22203/ecm.v008a05.
32. Pontes JTC, Borges ABT, Roque-Borda CA, et al. Antimicrobial Peptides as an Alternative for the Eradication of Bacterial Biofilms of Multi-Drug Resistant Bacteria. Pharmaceutics. 2022;14(3):642. doi: 10.3390/pharmaceutics14030642.
33. Pati R, Mehta RK, Mohanty S, et al. Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. Nanomedicine. Nanotechnology. 2014;10(6):1195-208. doi: 10.1016/j.nano.2014.02.012.
34. Sangani MH, Moghaddam MN, Forghanifard MM. Inhibitory effect of zinc oxide nanoparticles on pseudomonas aeruginosa biofilm formation. Nanomed J. 2014;2(2):121-8.
35. Mishra PK, Mishra H, Ekielski A, et al., Zinc oxide nanoparticles: a promising nanomaterial for biomedical applications. Drug Discov Today. 2017;22(12):1825-34. doi: 10.1016/j.drudis.2017.08.006.