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Zinc oxide nanoparticles' potential as a treatment for MDR *Klebsiella pneumoniae*, which was identified from clinical infections in Iraq

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Abstract:

Background: *Klebsiella pneumoniae* is an agent that can induce a variety of clinically significant infections, like UTI, pyogenic liver abscesses, sepsis, meningitis, pneumonia, and bacteremia. Zinc oxide nanoparticles' in vitro effectiveness against multidrug-resistant (MDR) *Klebsiella pneumoniae* isolated from different clinical illnesses is the goal of this investigation. ***Methods:*** 120 *K. pneumoniae* Isolates were obtained from individuals suffering from burns, tonsillitis, otitis media, and urinary tract infections. The samples were taken from the Burn Center in Najaf Al-Ashraf, Al-Hakim General Hospital, and Al-Sadder Medical City. They were then transferred to the University of Kufa's Faculty of Science's Advanced Microbiology Laboratory for examination. Multidrug resistance: Out of all the isolates, 60 were found to be multidrug-resistant based on biofilm formation evaluation and antibacterial susceptibility testing. ***Results:*** The findings showed that zinc oxide nanoparticles significantly decrease MDR in *K. pneumoniae*. Zinc oxide nanoparticles' bacterial inhibition zones and effect on biofilm formation also increased gradually with increasing concentrations (One, two, three, and four hundred µg/ml). ***Conclusion:*** Results demonstrate that as the concentration of zinc oxide nanoparticles increases, the inhibition zone for MDR *K. pneumoniae* progressively increases and biofilm formation decreases, further proving that the tested nanoparticles are capable of acting as a potential antimicrobial agent.

Keywords: Therapeutic Potential, Zinc Oxide, Nanoparticles, MDR, *Klebsiella pneumoniae*

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1. Introduction

The opportunistic Gram-negative bacteria *Klebsiella pneumoniae* may infect people with a variety of illnesses (1,2). K. pneumoniae has historically been linked to UTIs, pneumonia, and bacteremia, especially in immunocompromised hosts or individuals with long-term hospitalization (3,4). In addition, it may act as a commensal organism and colonize the skin, nasopharynx, and intestinal mucosa (5,6). However, in vulnerable hosts, it can infiltrate deep tissues and the circulation, leading to serious infections as bacteremia, meningitis, pneumonia, endophthalmitis, and pyogenic liver abscesses (7,8). Recently developed, but little utilized, metal oxide nanoparticles (NPs) are an antibacterial agent that incorporates an alternative method for countering multidrug-resistant infections, which is one of the major threats to global health. This simultaneous increase antibiotic antibiotic-resistant infections, while the discovery of new antimicrobials has also diminished, has led to an impending antibiotic crisis (9,10). Biofilms are structured bacterial aggregates encased in an

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extracellular polymeric substance (EPS) vital to bacterial survival and antibiotic resistance. EPS are mainly made of proteins, DNA, and polysaccharides, which account for about 90% of the biofilm architecture (11,12). Species of bacteria that reside in biofilms on hospital surfaces demonstrate extreme resistance to desiccation, disinfection with benzalkonium chloride, and ultraviolet light, which creates a strong challenge for clinicians who aim to clear pathogens from contaminated surfaces of biomaterials and tissue surfaces. By protecting bacteria from host immune responses and antimicrobial substances, biofilms contribute to their resistance and persistence, thus contributing to treatment failure and disease management (13,14). The strong antibacterial activity, biocompatibility, affordability, and durability of zinc oxide nanoparticles (ZnO NPs) have drawn a lot of attention with conventional antibiotics; their broad-spectrum activity allows them to combat multidrug-resistant pathogens such as *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli,* and *Acinetobacter baumannii*. Additionally, ZnO NPs have the advantage of being utilized in remote areas without electricity or refrigeration, representing a practical and effective approach in the general world healthcare (15-17).

2. Aim of this research: investigating how well local and imported zinc oxide nanoparticles work against MDR K. pneumoniae that has been isolated from various illnesses.

3. Materials and Methods

3.1: Specimen collection and bacterial identification

A total of 120 were collected and worked on it from patients with different clinical cases (such as tonsillitis, otitis media, UTI, burn infections, wounds, and blood) admitted to the centers for research in AL-Najaf Governorate, from October 2024 to January 2025, respectively. In order to prevent any potential contamination, all specimens were gathered carefully. They were then taken and sealed until they were moved to the University of Kufa's Faculty of Science's Advanced Microbiology Laboratory, where they were cultured on various media for 24 hours at 37°C to diagnose bacteria.

3.2: Ethical approval

Following the rules set out by human rights groups and the laws that provide sufficient information in an ethical way, the study's specimens obtained the consent of the young patients and the agreement of the adult patients.

3.3: Type of this study: Academic-Medical Research

3.4: Distribution of patient specimens for different infections

The patients' specimens of different infections were obtained from hospitals and specialized centers in Najaf province, a total of 120 specimens (100%). Out of 120, only 95 (79.16%) were considered positive growth and 25 (20.8 %) showed non-bacterial growth, On the other hand *K. pneumoniae* isolates where found in different clinical cases included 32 (53.33%) isolates in urine, 18(30 %) isolates in burn swabs,7 (11.66%) isolates in tonsils swabs, 3(5 %) isolates in otitis media swabs, Table (1)

Table 1: Numbers and percentages of positive growth specimens of *K.pneumoniae* isolates

Type of sample	No. of Samples	Male	Percentage (%)	Female	Percentage (%)	Percentage (%)
Urine Specimens	32	10	10.526%	22	23.157%	33.683%
Burn Swabs	18	10	10.526%	8	8.421%	18.947%
Tonsils Swabs	7	4	4.210%	3	3.157%	7.368%
Otitis media Swabs	3	2	2.105%	1	1.052%	3.157%
Total	60	26	27.36%	34	35.787%	63.155%

3.5: Identification of *K.pneumoniae* isolated from different infections

The first diagnosis was made using biochemical assays, selective and differential culture medium, microscopic analysis, and bacterial colony characteristics (18).

3.6: Biofilm formation detection for isolates of *K. pneumoniae*

3.6.1: Congo Red Agar (CRA) Method

By using phenotypic techniques, such as the Congo Red Agar Method (CRA), biofilm formation was identified. The *K. pneumoniae* isolates were added to CRA plates, which were then aerobically incubated for 48 hours at 37°C. The presence of black, dry, crystalline colonies on the CRA plates suggested that biofilm was being produced, whereas the colonies of biofilm that did not create biofilm remained pink or red in color (19).

3.6.2: Microtiter Plates (MTP) Method

Microtiter Plates (MTP), which are 96-well polystyrene plates, were used to quantify biofilm production semi-quantitatively in the following ways:

1. Blood agar plates were streaked with bacteria, which were then cultured at 37° C in BHI medium supplemented with 1% glucose. 2. Bacteria were diluted 1:20 in BHI 1%glucose broth with 10% glycerol during mid-logarithmic phase development (OD620=0.5), and frozen stocks were produced.

3. 270 µl of BHI in 96-well polystyrene microtiter plate wells. Thirty microliters of thawed stocks were used to inoculate 1% glucose broth. In order to check for nonspecific medium binding, a diluted culture was inoculated into each of the three wells of a microtiter plate. The plate was covered with a lid and left at 37° C for eighteen hours. 4. To get rid of planktonic cells, plates were cleaned the next day using phosphate-buffered saline (PBS). 5. After drying for an hour at 60°C, the plates were stained with a 0.5% crystal violet (CV) solution. Each well received 150 µl of CV for 30 minutes, and PBS was used for washing. 6. To get rid of any extra liquid, the microtiter plate was inverted and pressed firmly on filter paper. Air drying was done on the microtiter plates. 7. By dissolving the CV stain in 150 µl of 95% ethanol, the amount of biofilm development was measured. 8. A microtitre plate reader was used to quantify the adherent, stained biofilms' absorbance (A 630). The definition of a biofilm-positive phenotype was a value of > 0.120 at absorbance of 630 nm (20).

3.7: Susceptibility test for *K.pneumoniae* isolates

Table 2 lists the antibiotics utilized in this investigation, as reported by the firm Bio Analyses / Turkey.

Table 2: The antibiotics disk used in this study

Type of antibiotic	Antibiotic disk	Symbol	Dose
β-lactams penicillns class	Amoxicillin-clavulanic	AMC	30 µg
	Ticarcillin/Clavulanic acid	TCC	75/10µg
β-lactams carbapenems class	Meropenem	MEM	10 µg
	Imipenem	IPM	10 µg
β-lactams cephalosporines class	Cefepimeime	FEP	10 µg
Fluoroquinolones class	Ciprofloxacin	Cip	10 µg
	Norfloxacin	NX	10 µg
Quinolones class	Nalidixic Acid	NA	30 µg
Aminoglycosides class	Amikacin	AK	10 µg
	Gentamicin	CN	10 µg
	kanamycin	k	30 µg

3.8: ZnO NPs' Antibacterial Activity

ZnO NPs' antibacterial properties were evaluated against MDR *K. pneumoniae* isolated from various illnesses using Agar well diffusion techniques. A Mueller Hinton agar dish whole surface was streaked using a dipping cotton swab. Next, a sterilized cork poorer was used to make holes (5 mm) that were then filled with 80ul of ZnO NPs at four different concentrations one, two, three, and four hundred µg/ml). A meter ruler was used to measure the width of the growth inhibition zones after the Petri dishes had been incubated for 24 hours at 37°C (18)..Zinc oxide nanoparticles' antibiofilm activity against MDR *K. pneumoniae* isolates at varying concentrations. Using varying doses of zinc oxide NPs (100, 200, 300, and 400 µg/ml), the findings demonstrated that zinc oxide NPs had strong anti-biofilm action against MDR *K. pneumoniae* isolates using the plate technique (20).

3.9: The Connection Between Some Antibiotic-Resistant Imported ZnO NPs

The antibacterial activity of imported ZnO NPs with some resistance antibiotics against resistant K. pneumoniae isolates was assessed using the disc diffusion technique with MHA. Ceftazidime, CO-Trimoazole, and Ticarcillin/Clavulanic acid discs were combined with imported ZnO NPs at the optimal concentration (µg/mL). The size of the zone of inhibition for isolates of *K. pneumoniae* was measured using a meter ruler (21).

3.10: Analysis of statistics

The statistical analysis was performed using GraphPad Prism version 6, and a mean value and standard error (SE) were calculated for each piece of data. P values below 0.05 were considered statistically significant for the statistical analysis.

4. RESULTS

4.1: Identification of *K.pneumoniae* isolated from different infections

The morphological features of the colonies on MacConkey agar, CHROM agar, XLD agar, and the *K. pneumoniae* isolates were first identified using blood agar. The isolates showed up as huge, mucoid, and pink on MacConkey agar due to lactose fermentation, but as large, white, mucoid colonies without hemolysis on blood agar. However, some reports indicated that certain isolates of *K. pneumoniae* were demonstrated to be able to produce hemolysin on blood agar. The colonies appeared as metallic blue colonies on CHROM agar, and then differentiated these bacteria from *E.coli* colonies. All the isolates were cultured on Xylose lysine deoxycholate agar (XLD), and *K. pneumoniae* appeared mucoid, yellow colony on XLD agar, as shown in Figure 1.

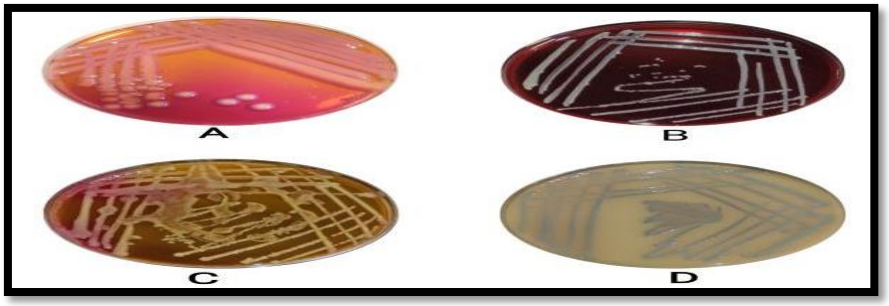

Figure 1: A variety of culture media, including MacConkey agar, Blood agar, XLD agar, and CHROM agar, were used to cultivate K. pneumoniae.

4.2: Biochemical tests of bacterial species

Table 3 shows the results of the biochemical tests of *K. pneumoniae*. The biochemical tests include the catalase test, methyl red test, motility test, production of Indole, Urease test, TSI (triple sugar iron agar), Voges-Proskauer test, Citrate, and Oxidase test.

4.3: Detection of Biofilm Formation

4.3.1: Congo Red Agar (CRA) Method

All *K. pneumoniae* isolates were assessed for biofilm formation using a phenotypic method, specifically the Congo Red Agar Method (CRA). Out of 60 (100%) *K. pneumoniae* isolates, 48 (80%) were identified as biofilm producers, indicated by the presence of black dry crystalline colonies on the CRA plates, while 12 (20%) were classified as non-biofilm producers, as their colonies remained pink or red in color, as shown in Figure 2.

4.3.2: Microtiter Plate Assay

The findings indicated the ability of some *K. pneumoniae* isolates to produce biofilms. The microtiter plate assay (MPA) identified that out of 60 (100%) isolates of *K. pneumoniae*, 32 (53.33%) were strong biofilm producers, 18(30%) were moderate, and 10(30%) were weak. It was indicated in the methods, as seen in Tables 4 and Figure 3.

Table 3: Biochemical test of bacterial species

bacterial species	Catalase	Voges- Proskauer	Indole Production	Methyl Red	Urease	Citrate	Motility	TSI	Oxidase
K.pneumoniae	+	+	-	-	+	+	-	A/A	-

Figure 2: Biofilm formation by some *K. pneumoniae* isolates (A) without Biofilm formation and (B) with Biofilm formation.

Table 4: Distribution of *K. pneumoniae* isolates based on biofilm production

Type Biofilm	NO. of *K. pneumonaei*
Strong	32 (53.33%)
Moderate	18 (30%)
Weak	10 (16.66%)
Total	60 (100%)

Figure 3: Biofilm formation of *K. pneumoniae* by the (MPA) method

4.4: Resistance to antibiotics in *K. pneumoniae*

The disk diffusion method was used to test 60 isolates of *K. pneumoniae* that resist common antibiotics.

Table 5: Antibiotic susceptibility test for 60 *K. pneumoniae* isolates

Type of antibiotic	Antibiotic disk	No.(%) of isolates exhibited	
		Resistance	sensitive
β-lactams penicillns class	Amoxicillin- clavulanic (AMC)	33 (55%)	27 (45%)
	Ticarcillin/Clavulanic acid(TCC)	55(68.75%)	25(31.25%)
β-lactams carbapenems class	Meropenem (MEM)	6 (10%)	54 (90%)
	Imipenem (IPM)	11 (18.3%)	49(81.7%)
β-lactams cephalosporines class	Cefepimeime (FEP)	15 (25%)	45(75%)
	Ceftriaone (CTR)	20 (33.33%)	40(66.66%)
	Cefotaime (CTX)	2 (3.33%)	58 (96.66%)
Fluoroquinolones class	Ciprofloxacin (Cip)	4 (6.66%)	56 (93.33%)
	Norfloxacin (NX)	10 (16.66%)	50 (83.33%)
Quinolones class	Nalidixic Acid (NA)	7 (11.7%)	53(88.3%)
Aminoglycosides class	Amikacin (AK)	2 (3.33%)	58 (96.66%)
	Gentamicin (CN)	16(20%)	64(80%)
	Kanamycin (K)	2 (3.33%)	58 (96.66%)
Sulfonamide class	CO-Trimoazole (COT)	48(60%)	32(40%)
Phenicols class	Chloramphenicol (C)	4 (6.66%)	56 (93.33%)

4.5: Antibacterial Efficacy of ZnO Nanoparticles

The antibacterial efficacy of imported ZnO nanoparticles was evaluated using the Agar well diffusion technique against multidrug-resistant *K. pneumoniae* isolated from various illnesses. The results utilizing varying concentrations (100, 200, 300, and 400) µg/ml demonstrated that the inhibition zone for *K. pneumoniae* bacteria progressively increased with higher concentrations of imported zinc oxide nanoparticles, as illustrated in Figure 4. Specifically, the inhibition zone at 400 µg/ml surpassed that at 300 µg/ml, which in turn exceeded the inhibition zone at 100 µg/ml. Consequently, the inhibition zone at 400 µg/ml was identified as the optimal concentration for the inhibitory effect of imported zinc oxide nanoparticles, as depicted in Figure 5.

4.6: The Synergistic Effect of Imported ZnO Nanoparticles with the Most Resistant Antibiotics for *K. pneumoniae*

Imported zinc oxide nanoparticles were included at four concentrations: 100, 200, 300, and 400 µg/ml, with medications resistant to *K. pneumoniae*. The combination had a good outcome, indicating an increase in the inhibitory zone relative to that of the zinc oxide nanoparticles alone, as seen in Figure 6.

4.7: Zinc Oxide Nanoparticles for Domestic Use

The results, utilizing varying concentrations (100, 200, 300, 400) µg/ml, demonstrated that the inhibition zone for *K. pneumoniae* bacteria progressively increased with higher concentrations of domestic zinc oxide nanoparticles (Figure 7). Specifically, the inhibition zone at 400 µg/ml surpassed that at 300 µg/ml, which in turn exceeded that at 200 µg/ml. Consequently, the inhibition zone at 400 µg/ml represented the optimal concentration for domestic zinc oxide nanoparticles. Furthermore, the results indicated that the inhibition zones for all four concentrations of domestic zinc oxide nanoparticles were inferior to those of imported zinc oxide nanoparticles, as illustrated in Figure 8.

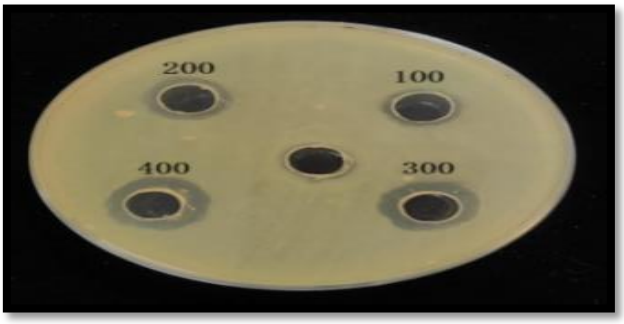

Figure 4: Effect of Different Concentrations of imported Zinc Oxide NPs against MDR *K. pneumoniae* isolates

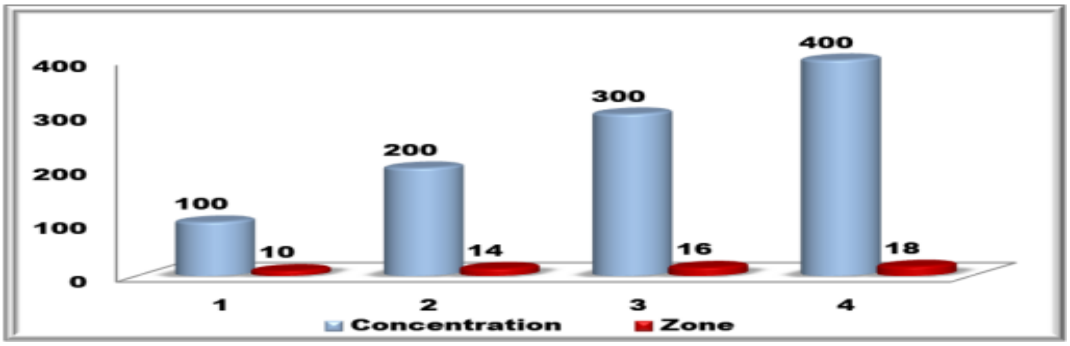

Figure 5: Effect of Different Concentrations of Zinc Oxide NPs on MDR *K. pneumoniae* isolates

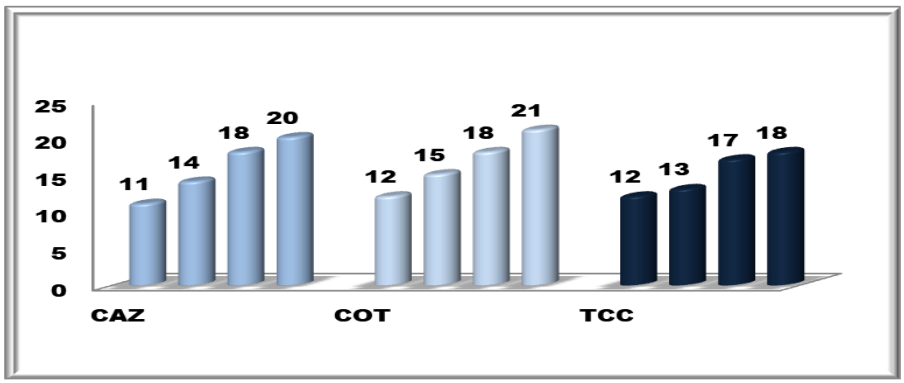

Figure (6) The Synergism Effect of Imported Zn NPs with the most Resistance Antibiotics TCC: Ticarcillin/Clavulanic acid, COT: CO-Trimoazole, CAZ: Ceftazidem against MDR *K. pneumonia*

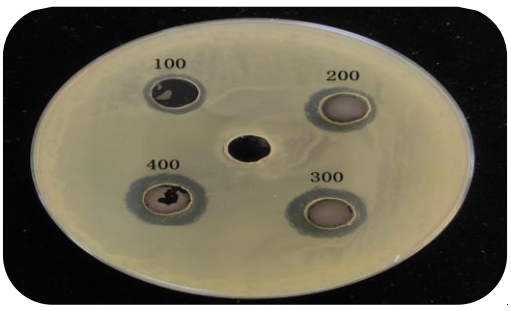

Figure 7: Effect of Different Concentrations of Domestic Zinc Oxide NPs against MDR *K. pneumoniae* isolates

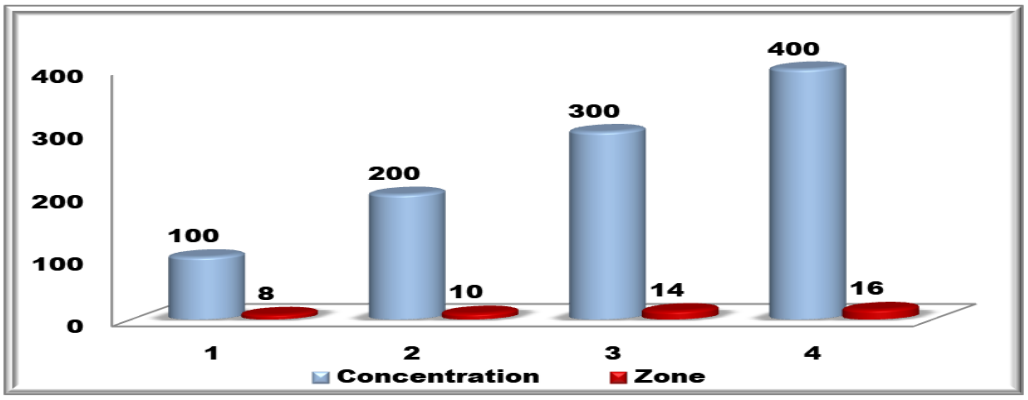

Figure 8: Effect of Different Concentrations of Domestic Zinc Oxide NPs on MDR *K. pneumoniae* isolates

4.8: The Synergistic Effect of Domestic ZnO Nanoparticles with the Most Resistant Antibiotics Against *K. pneumoniae*.

Zinc oxide nanoparticles were included at four concentrations (100, 200, 300, 400 micrograms/ml) with medications to which *Klebsiella pneumoniae* exhibits resistance. The findings indicated a beneficial impact on the inhibitory zone, shown by an increase in its area relative to the Zinc oxide particles. Isolated zinc antiparticle, as seen in Figure 9.

During the statistical analysis, the results showed significant differences in the case of mixing both imported and domestic MgO NPs together with antibiotics, while no significant differences appeared in the case of imported and domestic MgO NPs alone with antibiotics, as shown in Figures 10, 11, and 12.

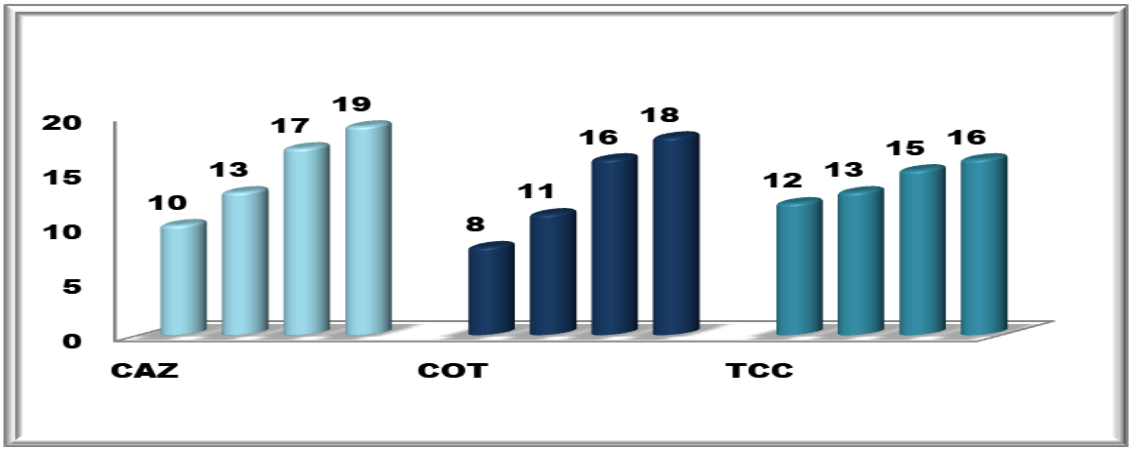

Figure 9: The Synergism Effect of Domestic ZnO NPs with the Most Resistance Antibiotics TCC: Ticarcillin/Clavulanic acid, COT: CO-Trimoazole CAZ: Ceftazidem against *K. pneumoniae.*

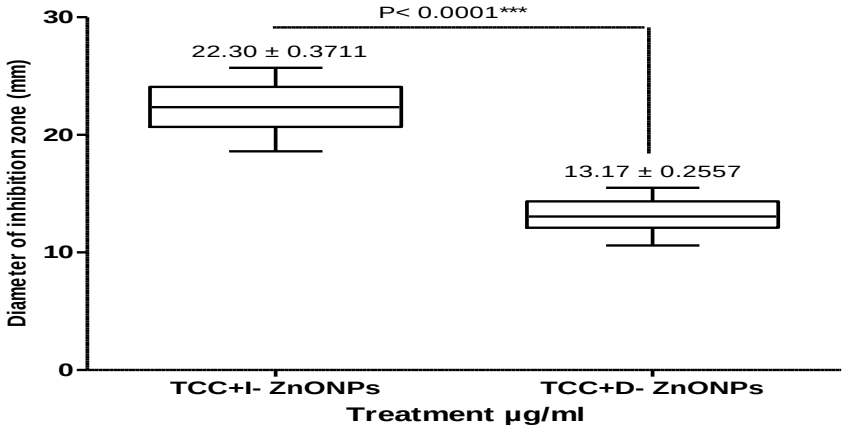

Figure 10: The combination effect of Ticarcillin/Clavulanic acid + imported ZnO nanoparticles and Ticarcillin/Clavulanic acid + domestic ZnO nanoparticles on *klebsiella pneumoniae* inhibition zone.

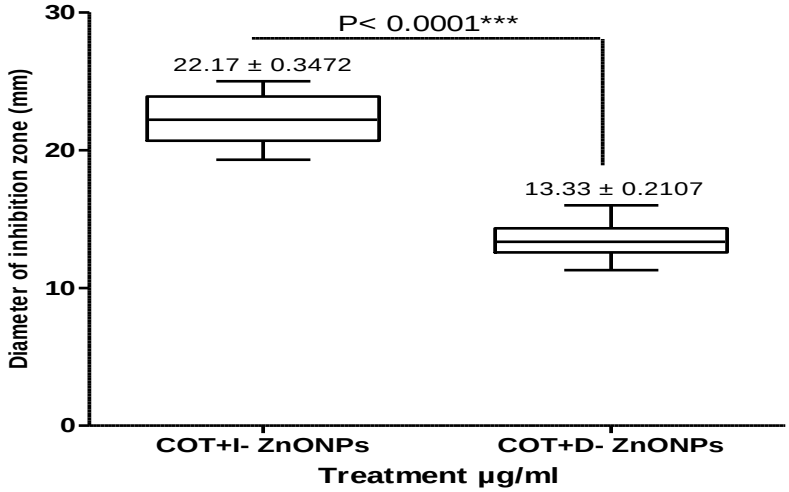

Figure 11: The combination effect of CO-Trimoazole+ imported ZnO nanoparticles and CO-Trimoazole + domestic ZnO nanoparticles on *klebsiella pneumoniae* inhibition zone.

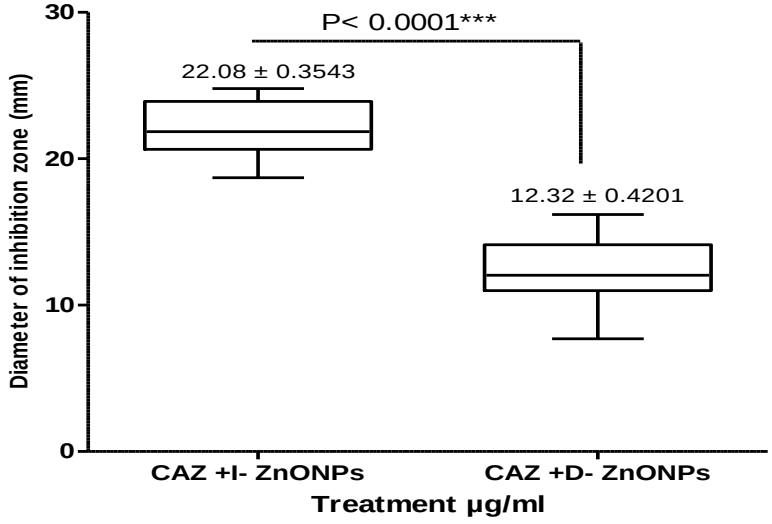

Figure 12: The combination effect of Ceftazidime+ imported ZnO nanoparticles and Ceftazidime + domestic ZnO nanoparticles on *Klebsiella pneumoniae* inhibition zone.

4.9: Antibiofilm activity of different concentrations of zinc oxide NPs against MDR *K. pneumonaei* isolates

The results showed that zinc oxide NPs expressed high anti-biofilm activity via Microtiter Plate Assay against MDR K. pneumonaei isolates using different concentrations of ZnO NPs (100, 200,300, and 400) µg/ml, as shown in Table 6.

Table 6: Antibiofilm activity of Zinc oxide NPs by absorbance at 630 nm against MDR *K. pneumoniae* isolates

No. of *K. pneumoniae* isolates	Absorbance of Zinc oxide NPs (µg/ml)			
	100	200	300	400
K. p1	0.213	0.182	0.096	0.073
K. p2	0.233	0.208	0.126	0.097
K p3	0.264	0.239	0.195	0.153
K. p4	0.333	0.273	0.227	0.145
K p5	0.401	0.294	0.210	0.148
K. p6	0.395	0.275	0.175	0.087
K. p7	0.240	0.214	0.150	0.064
K. p8	0.290	0.253	0.156	0.032
K. p9	0.380	0.324	0.159	0.047
K. p10	0.362	0.286	0.236	0.181
K. p11	0.253	0.135	0.090	0.042
K. p12	0.397	0.311	0.132	0.046
K. p13	0.287	0.233	0.128	0.051
K. p14	0.326	0.303	0.139	0.076
K. p15	0.284	0.256	0.114	0.063
K. p16	0.271	0.248	0.211	0.182
K. p17	0.368	0.321	0.244	0.132
K. p18	0.285	0.271	0.225	0.137
K. p19	0.284	0.256	0.114	0.063
K. p20	0.184	0.122	0.128	0.090

DISCUSSION

One of the most significant clinical organisms that has recently drawn a lot of attention from the public health community is *K. pneumoniae*, it is a common and global member of the Enterobacteriaceae family, it is regarded as an opportunistic pathogen that causes a wide range of illnesses and exhibits antibiotic resistance, Because they frequently progress to bacteremia, respiratory infections caused by *K. pneumoniae* that are acquired in hospitals or the community pose a major risk to public health and result in high mortality rates. Due to extensive colonization, *K. pneumoniae* is one of the primary causes of global pneumonia, also it is the primary bacterial species of community-acquired pneumonia that progresses to bacteremia in some countries (22,23).

The emergence and dissemination of antibiotic-resistant bacteria over the last decade is a major concern in clinical and public health contexts . Surveillance and surveillance of antimicrobial resistance patterns is important, especially at the national level for public health agencies (24,25). Antimicrobial resistance, a major challenge in infection treatment, is primarily driven by the overuse, underuse, and misuse of antibiotics. For example, *Klebsiella pneumoniae* utilizes a wide range of resistance mechanisms, such as antibiotic-inactivating enzymes, modification of target sites, modification of membrane permeability, activation of efflux pump systems, and changes in metabolic pathways (26,27). Moreover, while it belongs to the Enterobacteriaceae family, K. pneumoniae can synthesize Extended-Spectrum Beta-Lactamases (ESBLs), which hydrolyze various beta-lactam antibiotics. Bacteria that produce ESBLs are resistant to many antibiotics, causing these multidrug-resistant (MDR) infections to become common (28).

Mechanisms that allow *K. pneumoniae* to be recalcitrant towards β-lactam antibiotics include changes in cell permeability, induction of efflux pumps, alteration of antibiotic targets, or enzymatic inactivation of drugs. These mechanisms can function separately or together in one single *K. pneumoniae* strain. Some efflux pumps also contribute to antibiotic resistance by pumping antibiotics out of the cells. Moreover, *K. pneumoniae* produces β-lactamase enzymes, including penicillinase and cephalosporinase, that break the β-lactam ring in cephalosporins and make them ineffective (29).

Cephalosporins are a class of broad-spectrum antibiotics that are structurally related to penicillins, and they work by binding to penicillin-binding proteins (PBPs) and inhibiting bacterial cell wall synthesis, which leads to cell lysis and, in turn, bacterial death. Cephalosporin has methoxy cephalosporins and oxyimino cephalosporins in its chemical structure. They are divided into five generations according to the antibacterial spectrum. Sulfonamides are a separate class of broad-spectrum antibiotics that exert their bacteriostatic activity by inhibiting the bacterial enzyme dihydropteroate synthetase, blocking the incorporation of para-aminobenzoic acid (PABA) and thereby inhibiting folic acid biosynthesis (30). This interference causes a disturbance in nucleic acid and amino acid synthesis of the bacteria and results in bacterial cell death. *K. pneumoniae* biofilm formation is an early pathogenic step in respiratory infections. Biofilms, made of aggregated microbial cells encased in extracellular polymeric substances (EPS), augment the resistance of bacteria to host immune systems and antimicrobial agents. *K. pneumoniae* is known to form biofilms on implanted medical devices, such as intravascular catheters and endotracheal tubes, resulting in tissue colonization at distal sites and chronic infections (31,32).

We have chosen ZnO NPs as potential antibacterial agents because of their nanoscale dimensions and strong antimicrobial activity. Studies indicate that ZnO NPs have an antibacterial activity, which is mediated by their entrance into the cytoplasm or outer membrane of a bacterial cell, leading to the release of Zn^{2+} ions. It causes genomic instability, damage to membrane proteins, and damage to the bacterial cell membrane, leading to cell death. Due to their robust antibacterial characteristics, ZnO NPs already have commercial applications in diverse sectors, such as electronics, textiles, agriculture, environment, and health care (33-35). Besides ZnO NPs, other similar nanomaterials have shown powerful activity against antibiotic-resistant bacteria and viruses, including titanium dioxide (TiO_2) and silicon dioxide (SiO_2) (36). Microbial toxicity and biological responses are influenced by their unique physicochemical properties. ZnO NPs are increasingly being employed in biomedical and personal care products owing to their low toxicity, biocompatibility, and biodegradability (37,38). They may further be used in combination with anti-inflammatory agents and antibiotics to achieve antimicrobial enhancement in clinical and non-clinical settings. Furthermore, reported findings also suggest that ZnO NPs work synergistically along with Meropenem, Levofloxacin, Amikacin, and Ceftazidime against the resistant Gram-negative pathogens. Additionally, previous observations suggest that ZnO NPs nanoparticles can enhance the antibacterial property when used in combination with antibiotics like Amikacin and Meropenem against burn infection isolates, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (39,40).

CONCLUSIONS

Klebsiella pneumoniae was detected in patients who had respiratory tract, urinary tract, burn, tonsillitis, pneumonia, and otitis infections. In these, it was recognized as the most disease-causing agent of UTIs. Among the classes of antibiotics assessed, the multidisciplinary drug-resistant (MDR) K. pneumoniae isolates demonstrated the highest measure of resistance to Ticarcillin/Clavulanic acid

(TCC) and Co-Trimoxazole (COT), but showed the least resistance to Kanamycin (K). The results of the experiments exhibited that the antibacterial activity against K. pneumoniae was facilitated with increasing concentrations of zinc oxide (ZnO) nanoparticles, with an increase in the inhibition zone. Combined treatment of imported and domestic ZnO nanoparticles with antibiotics showed significant differences in statistical analysis. Nonetheless, there was no significant difference comparing the effects of both imported and domestic ZnO nanoparticles when used alone and with antibiotics.

Conflict of interest: NIL
Funding: NIL

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