Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.917
*Biol. Clin. Sci. Res. J., Volume, **2024**: 917*
Original Research Article

DETECTION OF blaCTX-M AND blaSHV GENES OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING *Klebsiella pneumoniae*

ZAHOOR S[1], SAGHEER S[*1], ANWAR R[2], MAHMOOD S[3], PARA M[4], TABASSUM I[1], MUGHAL VA[5]

[1]*Lahore College for Women University, Lahore, Pakistan*
[2]*Department of Physics, University: Government College Women University Sialkot, Pakistan*
[3]*Institute of Microbiology, Government College University Faisalabad 38000, Pakistan*
[4]*Department of Zoology, Lahore College for Women University, Lahore, Pakistan*
[5]*Department of Microbiology, University: Quaid-I-Azam, Islamabad, Pakistan*
**Corresponding author`s email address:* ssagheer73@gmail.com

(Received, 15th April 2024, Revised 09th June 2024, Published 25th June 2024)

Abstract:*Antimicrobial resistance poses a significant challenge in many hospitals, increasing infection morbidity and mortality. It is a global issue with far-reaching implications for human and animal health, the environment, agriculture, and the economy. The ineffective use of antibiotics in treating infectious diseases is a significant driver of antimicrobial resistance, which can be either innate or acquired.* ***Objective:*** *This study aimed to detect the presence of blaCTX-M and blaSHV genes in extended-spectrum beta-lactamase (ESBL) producing Klebsiella pneumoniae and to evaluate the antibacterial activity of zinc oxide (ZnO) nanoparticles synthesised using Calotropis procera extract.* ***Methods:*** *A cross-sectional study was conducted at Lahore College for Women University, Lahore, from January to December 2023. A total of 20 clinical isolates of Klebsiella pneumoniae were collected and identified using standard microbiological techniques. The presence of ESBL-producing strains was detected using the double disc synergy test. Polymerase chain reaction (PCR) was used to detect blaCTX-M and blaSHV genes. ZnO nanoparticles were synthesised using Calotropis procera extract and tested for antibacterial activity against ESBL-producing strains at different concentrations (20 mg/ml, 25 mg/ml, 30 mg/ml, and 35 mg/ml). The antibacterial activity was evaluated using the disc diffusion method, and data were analysed using SPSS version 23.0.* ***Results:*** *Of 20 clinical isolates, 15 (75%) were ESBL positive, and 5 (25%) were ESBL negative. PCR results showed that 80% of the ESBL-producing strains contained the blaCTX-M gene, and 83% included the blaSHV gene. The antibacterial activity of ZnO nanoparticles was concentration-dependent, with the highest inhibitory zones observed at a concentration of 35 mg/ml. The synergistic effect of ZnO nanoparticles with cefotaxime and ceftriaxone significantly increased the antibacterial activity against ESBL-producing strains.* ***Conclusion:*** *The study highlights the significant presence of blaCTX-M and blaSHV genes in ESBL-producing Klebsiella pneumoniae. The green synthesis of ZnO nanoparticles using Calotropis procera extract demonstrated enhanced antibacterial activity, especially when combined with beta-lactam antibiotics. These findings suggest that ZnO nanoparticles could be an alternative strategy to combat antimicrobial resistance.*

Keywords: Antimicrobial Resistance, Beta-Lactamase, ESBL, Klebsiella Pneumoniae, Blactx-M, Blashv, Zinc Oxide Nanoparticles, Calotropis Procera, PCR, Synergistic Effect

Introduction

Antimicrobial resistance poses a significant challenge in many hospitals, increasing infection morbidity and mortality (1). It is a global issue with far-reaching implications for human and animal health, the environment, agriculture, and the economy (2, 3). The ineffective use of antibiotics in treating infectious diseases is a significant driver of antimicrobial resistance, which can be either innate or acquired (4, 5).

The spread of antibiotic resistance among clinical strains of various bacteria, including Acinetobacter, Klebsiella, Pseudomonas, and Escherichia coli, has become a significant concern (6). This resistance is often transmitted by Motile Genetic Elements (MGE) (7). The overuse of antibiotics has led to the emergence of multidrug-resistant bacterial strains, posing a severe threat to public health (8). Furthermore, the discovery and development of antibiotics have been essential in combating microbial infections (9). However, the widespread use of broad-spectrum antibiotics has resulted in the emergence of multidrug-resistant isolates, complicating the clinical landscape. Antibiotics are classified based on their target, molecular structure, and mode of action, with different classes of antibiotics targeting cell membrane assembly, supermolecule synthesis, and nucleic acid synthesis (10, 11).

In addition to antibiotic resistance, beta-lactamases, enzymes produced by bacteria, present another challenge. These enzymes compete with beta-lactam antibiotics, such as penicillins and cephalosporins, rendering them ineffective by disrupting their molecular structure.

Addressing antimicrobial resistance and finding alternative strategies for combating bacterial infections are critical priorities for the global healthcare community.

Methodology

The study was conducted at the Department of Zoology, Lahore College for Women University, Lahore, to identify and characterise bacterial strains that produce beta-lactamase enzymes and assess their susceptibility to zinc oxide (ZnO) nanoparticles. Materials utilised in the study included various microbiological media such as Nutrient

[Citation: Zahoor, S., Sagheer, S., Anwar, R., Mahmood, S., Para, M., Tabassum, I., Mughal, V.A. (2024). Detection of blactx-m and blashv genes of extended spectrum beta lactamase producing *klebsiella pneumoniae*. *Biol. Clin. Sci. Res. J.*, **2024***: 917*. doi: https://doi.org/10.54112/bcsrj.v2024i1.917]

Agar, MacConkey Agar, Muller Hinton Agar (Oxoid), and Luria Bertani (LB) Broth (Sigma-Aldrich), along with antibiotic sensitivity discs for Imipenem (10 µg), Ciprofloxacin, Ceftriaxone, and Ampicillin (30 µg). Equipment used ranged from standard laboratory apparatus to specific molecular biology tools supplied by vendors, including Sartorius, Germany (balances); Capp, UK (micropipettes); and Memmert, USA (incubators).

For the synthesis of ZnO nanoparticles, zinc acetate (Sigma-Aldrich) was used, employing leaves of Calotropis procera as the biological precursor. Bacterial strains were collected from General Hospital, Lahore, from patient samples showing beta-lactamase activity. Fifteen strains, predominantly Klebsiella pneumoniae, were selected for further analysis.

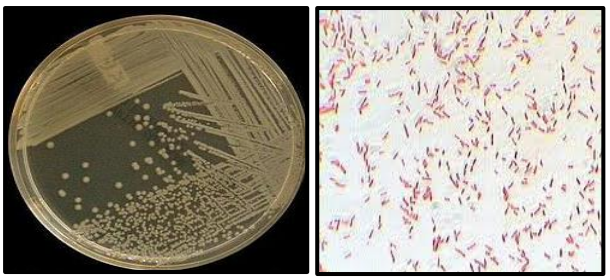
All glassware and instruments were sterilised by autoclaving at 121°C and 15 psi for 20 to 30 minutes. Media were prepared according to the manufacturers' specifications and autoclaved under the same conditions. The bacterial strains underwent morphological and biochemical identification based on protocols from Bergey's Manual of Determinative Bacteriology. Molecular identifications and resistance mechanism investigations were conducted using PCR assays targeting blaCTX-M and blaSHV genes, optimised specifically for this study.

The strains' antibacterial susceptibility to standard antibiotics and synthesised ZnO nanoparticles was assessed using the Kirby-Bauer disc diffusion method and healthy diffusion techniques, adhering to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The green synthesis of ZnO nanoparticles was carried out using a method described by Vidya et al. (2013) with Calotropis procera leaf extract, and the nanoparticles were characterised using spectroscopic techniques.

Data from the study were analysed using Microsoft Excel 2010 and SPSS version 16.0, with the statistical significance of differences in inhibition zones determined by one-way ANOVA and post-hoc tests. The Institutional Review Board of Lahore College for Women University granted ethical approval for the study, ensuring compliance with ethical standards for research involving bacterial isolates from human sources.

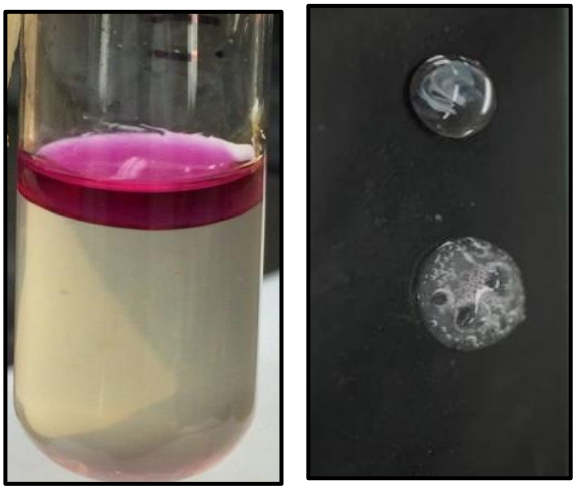
Results

Klebsiella pneumonia colonies appeared to be large and mucoid. Following gram staining, the colonies were coloured pink, showing that they were gram-negative rods. In the catalase test, K. pneumoniae formed bubbles, indicating reduced hydrogen peroxide. There was no response from K. pneumoniae in an oxidase test. The indole test came up negative.



I **II**

Fig.1: I Growth of *K. pneumoniae* of Nutrient Agar II Microscopic examination



III **IV**

Fig. 2: III. Indole test negative, IV. Catalase test positive

The double disc synergy test (Jarlier et al., 1988) was used to detect ESBL-generating gram-negative bacterial strains. Figure 4.3 shows that after phenotypic identification of ESBLs, 15 (75%) out of 20 clinical isolates were ESBL positive, while 5 (25%) were ESBL negative.

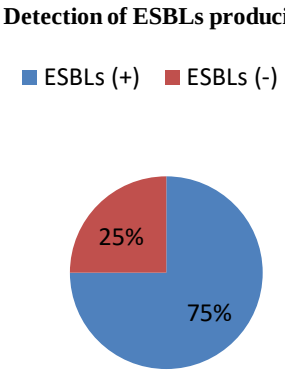
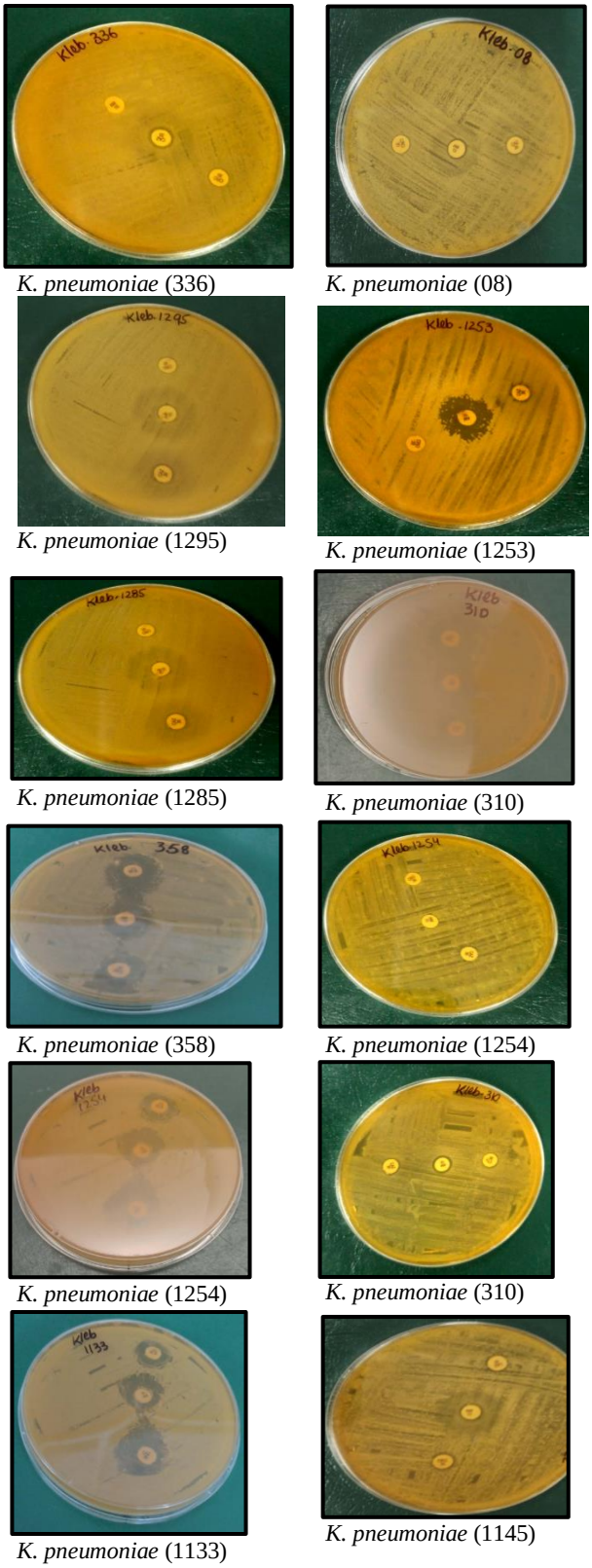


Fig 3: Percentage of ESBL (+ve) and (-ve) clinical strains (n=20)

Gram-negative bacterial strains were tested to see how ZnO nanoparticles in DMSO at various doses (20 mg/ ml, 25 mg/ ml, 30 mg/ ml, and 40 mg/ ml) affected them. The maximum inhibitory zones were attained at a 40 mg/ml nanoparticle concentration. Figures and tables show how nanoparticles have antibacterial properties. This was also seen on the graph, where the maximum bar was produced at a concentration of 40 mg/ml nanoparticles and the lowest at 20 mg/ml.

Table 4.1 shows the zones of inhibition formed when ZnO nanoparticle concentrations of 20mg/ml, 25mg/ml, 30mg/ml, and 35 mg/ml were used. The results showed that at 35 mg/ml concentration of ZnO NPs, K. pneumoniae (1245) had the highest zone of inhibition of 13mm, and *K. pneumoniae* (336) had the lowest zone of inhibition of 7mm.

[Citation: Zahoor, S., Sagheer, S., Anwar, R., Mahmood, S., Para, M., Tabassum, I., Mughal, V.A. (2024). Detection of blactx-m and blashv genes of extended spectrum beta lactamase producing *klebsiella pneumoniae*. *Biol. Clin. Sci. Res. J.*, **2024***: 917*. doi: https://doi.org/10.54112/bcsrj.v2024i1.917]



K. pneumoniae (336) *K. pneumoniae* (08)

K. pneumoniae (1295) *K. pneumoniae* (1253)

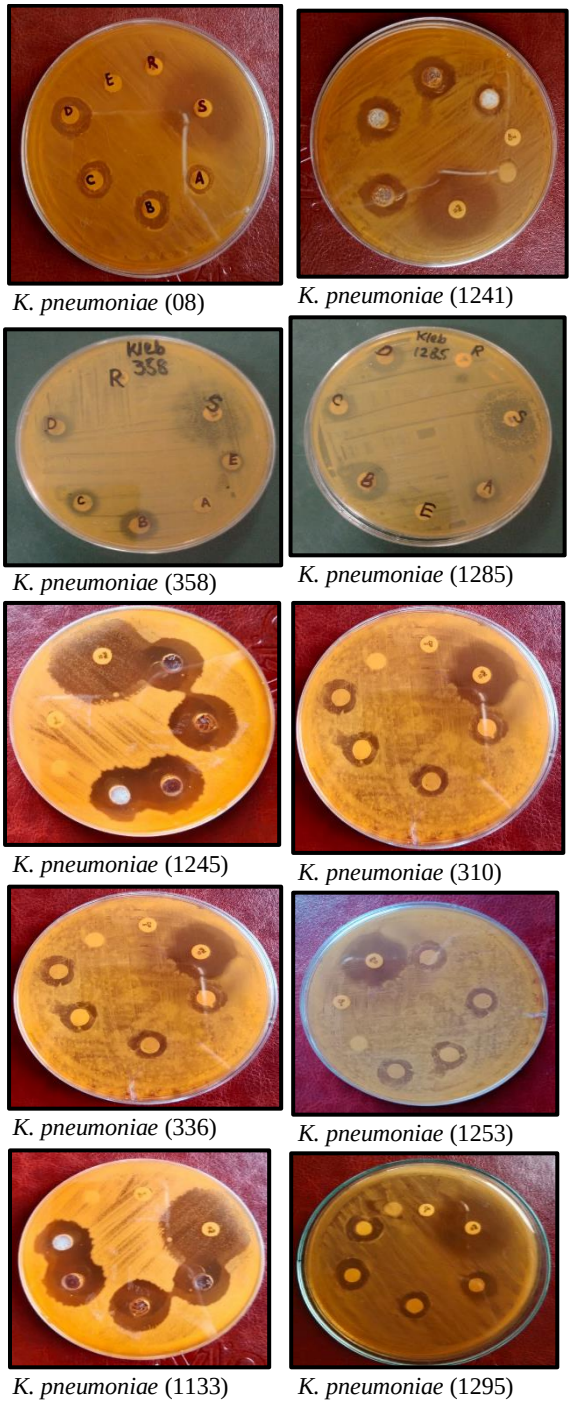
K. pneumoniae (1285) *K. pneumoniae* (310)

K. pneumoniae (358) *K. pneumoniae* (1254)

K. pneumoniae (1254) *K. pneumoniae* (310)

K. pneumoniae (1133) *K. pneumoniae* (1145)

Fig. 4: Detection of ESBLs Producing Strains



K. pneumoniae (08) *K. pneumoniae* (1241)

K. pneumoniae (358) *K. pneumoniae* (1285)

K. pneumoniae (1245) *K. pneumoniae* (310)

K. pneumoniae (336) *K. pneumoniae* (1253)

K. pneumoniae (1133) *K. pneumoniae* (1295)

Fig. 5: Antimicrobial activity of ZnO Nanoparticles against bacterial strains

Where: A: 20 mg/ml of ZnO, B: 25 mg /ml of ZnO, C: 30 mg/ml of ZnO, D: 35 mg/ml of ZnO, E: Negative Control (water), S: Sensitive antibiotic (Imipenem) and R: Resistant antibiotic (Ciprofloxacin)

Table 1: Antimicrobial activity of ZnO nanoparticles against bacterial strains by disc diffusion method

Strain no.	Zone of inhibition in mm					
	Concentration of ZnO NPs (mg/ml)				Antibiotics	
	20	25	30	35	IMP (10ug/disc)	CIP

K. pneumoniae (310)	8 plus8±0.4	10 plus10±0.4	12 plus12±0.4	13±0.4	16±0.2	R
K. pneumoniae (1253)	9±0.3	11±0.5	12±0.3	12±0.2	25±0.1	R
K. pneumoniae (336)	7±0.3	10±0.4	11±0.5	14±0.3	16±0.5	R
K. pneumoniae (1245)	7±0.3	8±0.5	10±0.3	11±0.5	30±0.3	R
K. pneumoniae (358)	8±0.4	10±0.4	12±0.4	13±0.4	20±0.2	R
K. pneumoniae (1285)	13±0.5	14±0.3	15±0.5	16±0.2	25±0.5	R
K. pneumoniae (1241)	8±0.4	9±0.4	10±0.3	11±0.3	26±0.4	R
K. pneumoniae (08)	9±0.3	11±0.5	13±0.4	15±0.5	20±0.3	R

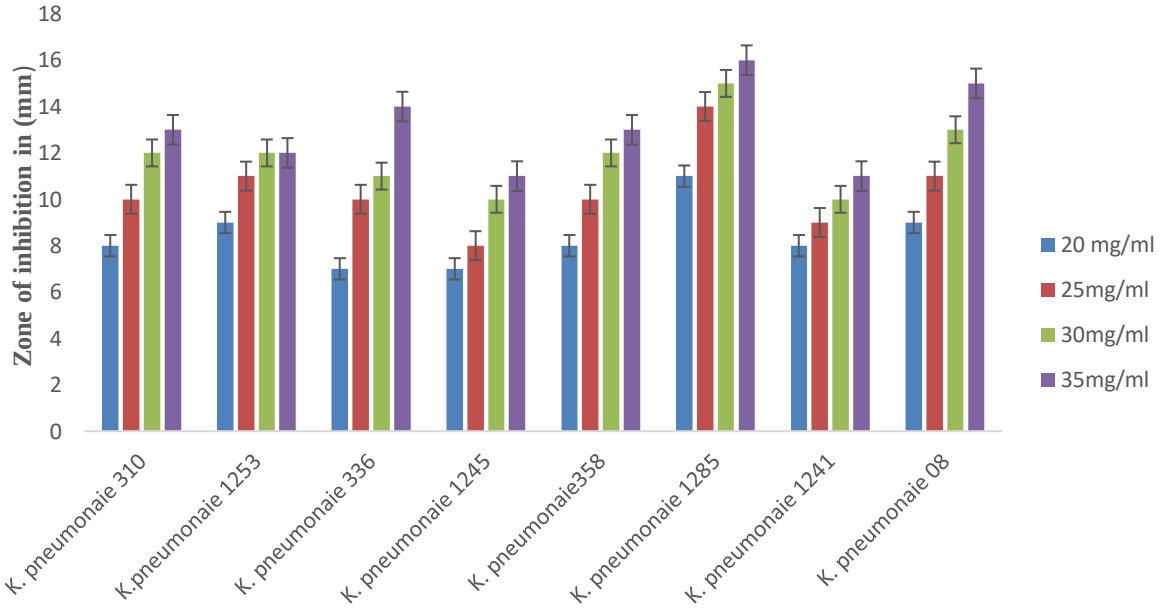


Fig. 6: Antimicrobial activity of ZnO nanoparticles against bacterial strains by disc diffusion method:

Table 2: Synergistic effect of nanoparticles with antibiotics (CTX and CRO)

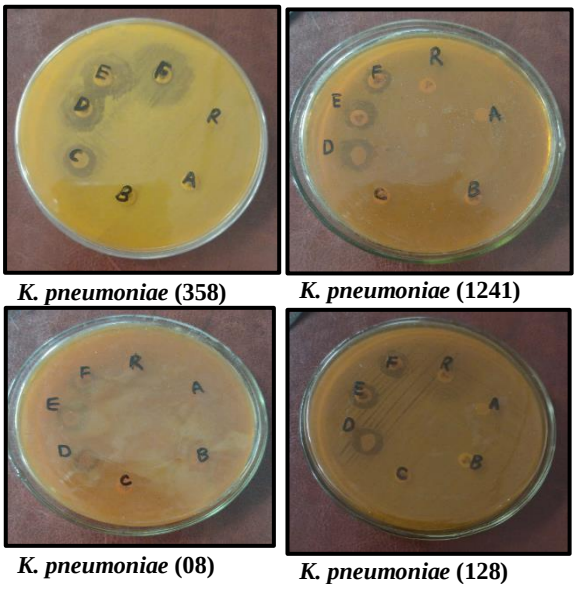
Strain no	Zone of inhibition (mm)					
	ZnO	**CTX**	**ZnO+CTX**	**CIP**	**CRO**	**ZnO+CRO**
K. pneumoniae 310	8±0.2	R	15±0.2	R	R	11±0.1
K. pneumoniae 1253	11±0.1	R	12±0.1	R	R	10±0.4
K. pneumoniae 336	10±0.4	R	16±0.5	R	R	12±0.5
K. pneumoniae 1245	8±0.2	R	18±0.5	R	R	8±0.2
K. pneumoniae 358	7±0.2	R	18±0.1	R	R	15±0.3
K. pneumoniae 1285	9±0.5	R	12±0.1	R	R	13±0.2
K. pneumoniae 1241	9±0.3	R	13±0.2	R	R	12±0.5
K. pneumoniae 08	8±0.2	R	14±0.4	R	R	13±0.2

Table 3. Concentration and respective ratios of bacterial DNA samples

Sample ID	**260/280 ratio**	**Conc. Of DNA (ng/ul)**
K. pneumoniae 310	1.28	23.8
K. pneumoniae 1253	0.81	183.9
K. pneumoniae 336	0.83	34.1
K. pneumoniae 1245	1.05	236.7
K. pneumoniae 358	0.82	72.9
K. pneumoniae 1285	1.24	38.4
K. pneumoniae 1241	0.96	106.6
K. pneumoniae 08	1.13	35.5
K. pneumoniae 1133	0.79	60.6
K. pneumoniae 1295	0.81	183.9

[Citation: Zahoor, S., Sagheer, S., Anwar, R., Mahmood, S., Para, M., Tabassum, I., Mughal, V.A. (2024). Detection of blactx-m and blashv genes of extended spectrum beta lactamase producing *klebsiella pneumoniae*. *Biol. Clin. Sci. Res. J.*, **2024***: 917*. doi: https://doi.org/10.54112/bcsrj.v2024i1.917]

The increased antibacterial properties of the nanoparticle with drugs including cefotaxime, ceftriaxone, and ciprofloxacin against particular bacterial strains were also assessed. The outcomes demonstrated that the presence of ZnO NPs improved the antibacterial activity of cefotaxime and ceftriaxone. The outcome demonstrated that antibiotics and NPs work together to produce greater antibacterial effects. The table data were presented according to zone of inhibition.



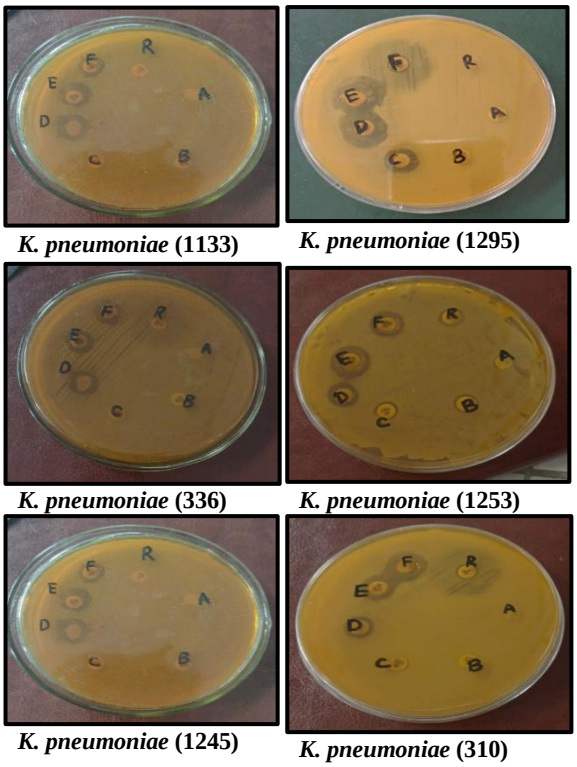


Fig. 7: Synergistic effect of Nanoparticles against bacterial strains
Where: A: Control, B: CTX, C: CRO, D: Nanoparticles, E: CTX+ Nanoparticles, F: CRO+Nanparticles and R: Resistant antibiotic (Ciproflaxcin)

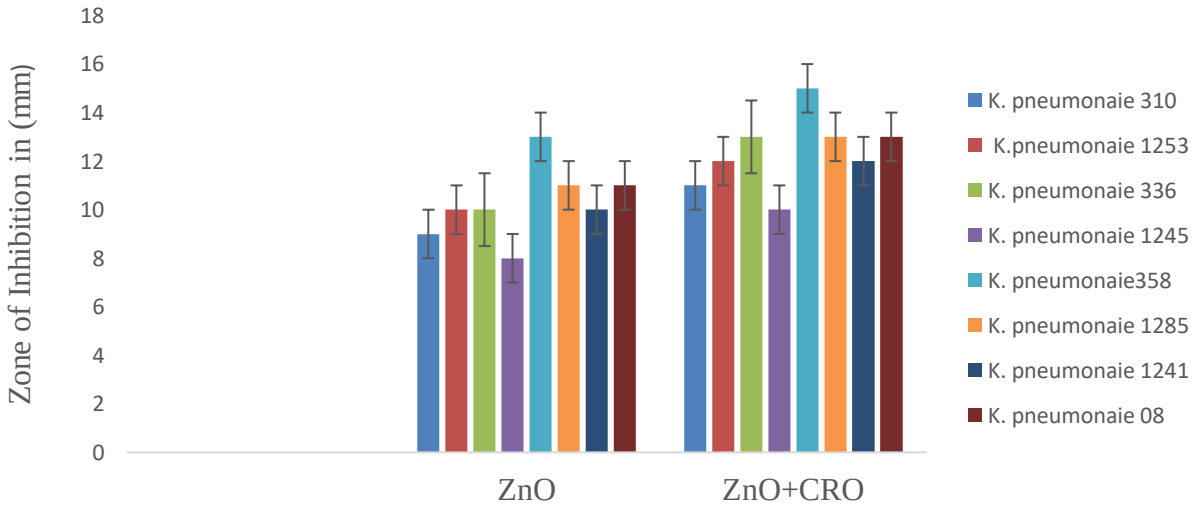


Fig 8: Graphical presentation of Synergistic effect of nanoparticles with antibiotics (CRO)

The alkaline lysis method was used to isolate plasmid DNA. An ultraviolet (UV) light source, such as a transilluminator 1, is used to excite the fluorescent molecules in order to visualize the DNA segments. Ethidium Bromide (EtBr) is the most commonly used to fluorescent DNA. Intercalation occurs when EtBr molecules squeeze between neighboring base pairs in a DNA double helix. Any EtBr intercalated into the DNA fluoresces and emits a bright orange light when exposed to UV light. Figure 4.14 shows the bacterial bands on an agarose gel illuminated by a UV Tran illuminator. DNA was isolated from 20 clinical isolates'. DNA was visible in 10 clinical isolates only. To determine the prevalence of blaCTX-M gene, PCR was performed on bacterial strains that were ESBLs positive. A total of 8 (80%) clinical strains contained the gene, while 2 (20%) clinical isolates did not contain the blaCTX-M. The size of

the PCR product was estimated using DNA markers and a known DNA ladder. For blaCTX-M, the product size observed on gel was approximately 600bp.

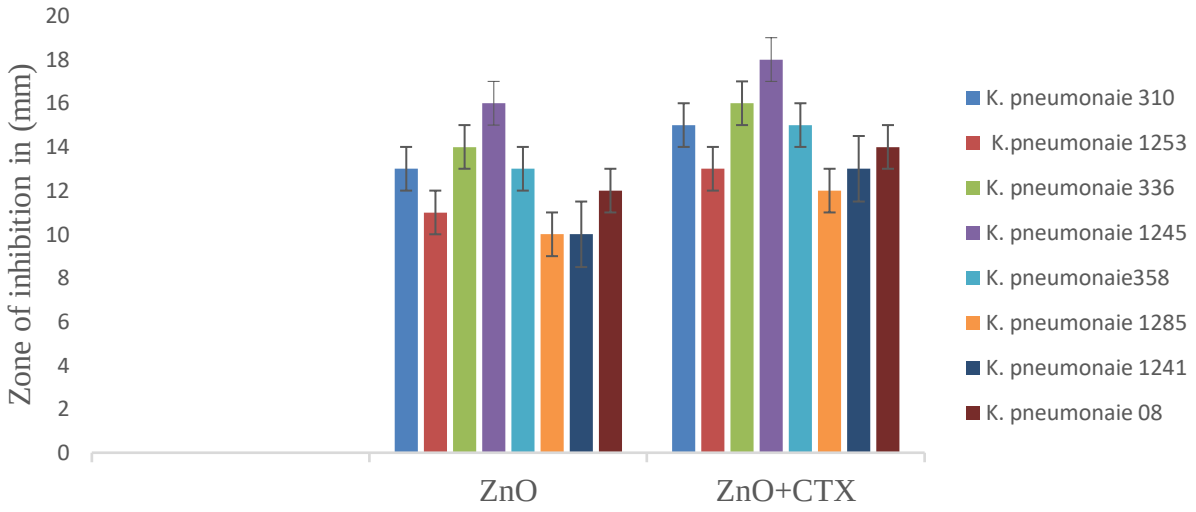


Fig 9: Graphical presentation of Synergistic effect of nanoparticles with antibiotics (CTX)

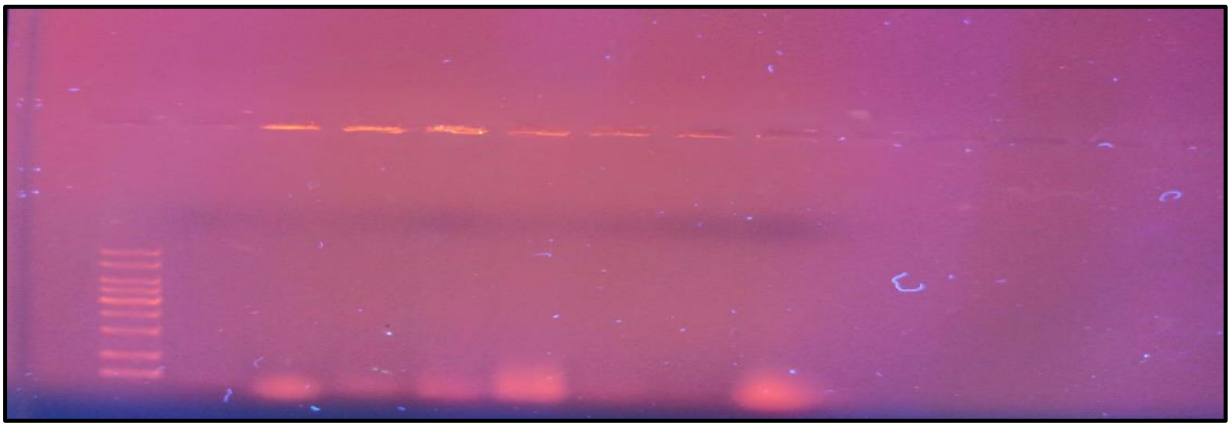


Fig 10: Plasmid DNA bands visualization by UV trans illuminator
Visualization of plasmid DNA of clinical isolates: (From left to right) Lanes: 1=*K. pneumonaie*1253, 2=*K. pneumoniae* 310, 3=*K. pneumoniae* 08, 4= *K. pneumoniae* 336, 5= *K. pneumoniae* 1241, 6= *K. pneumoniae* 1285, 7= *K. pneumoniae* 1245, 8= *K. pneumoniae* 1133, 9= *K. pneumoniae* 1295, 10= *K. pneumoniae* 358,
M-100 bp DNA Ladder

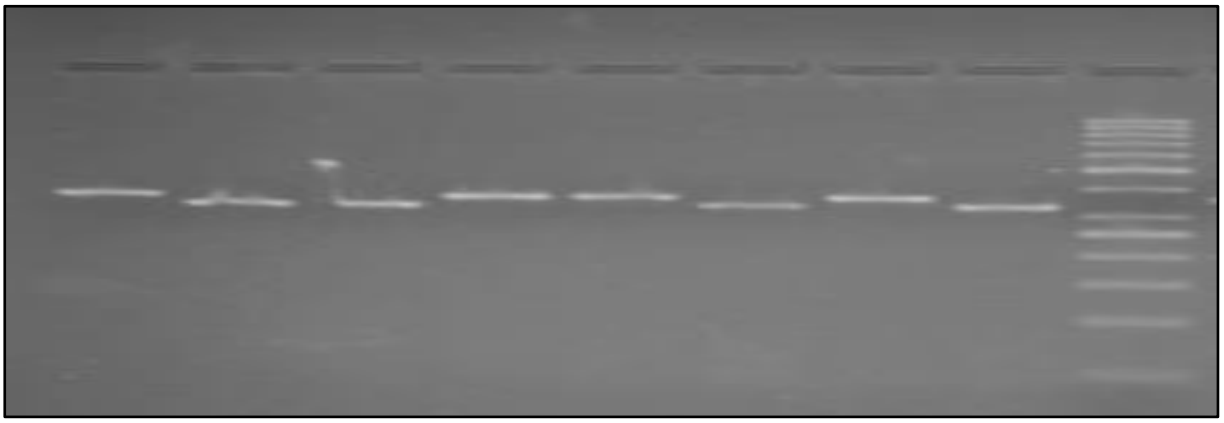


Fig 11: PCR Amplification for BlaCTXM gene (from right to left)
1= K. pneumoniae 310, 2= K. pneumoniae 1253, 3= K. pneumoniae 336, 4= K. pneumoniae 1245, 5= K. pneumoniae 358, 6= K. pneumoniae 1285, 7= K. pneumoniae 1241, 8= K. pneumoniae 08
Product size 600bp, M 100 bp ladder

[Citation: Zahoor, S., Sagheer, S., Anwar, R., Mahmood, S., Para, M., Tabassum, I., Mughal, V.A. (2024). Detection of blactx-m and blashv genes of extended spectrum beta lactamase producing *klebsiella pneumoniae*. *Biol. Clin. Sci. Res. J.*, **2024***: 917*. doi: https://doi.org/10.54112/bcsrj.v2024i1.917]

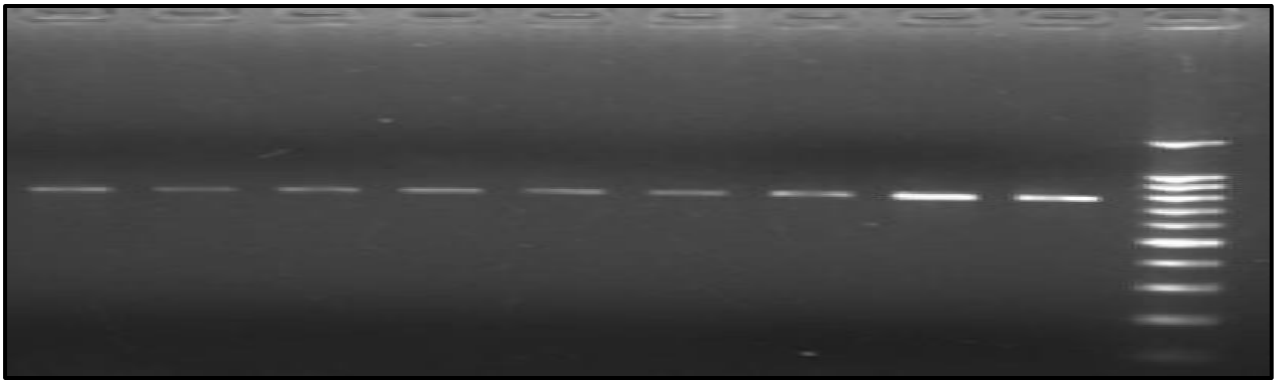


Fig 12: PCR Amplification for blaSHV gene (from right to left)
1= *K. pneumoniae* 310, 2= *K. pneumoniae* 1253, 3= *K. pneumoniae* 336, 4= *K. pneumoniae* 1245, 5= *K. pneumoniae* 358, 6= *K. pneumoniae* 1285, 7= *K. pneumoniae* 1241, 8= *K. pneumoniae* 08, 9= *K. pneumoniae* 1295
Product size 716bp, M 100 bp ladder

To determine the prevalence of the SHV gene, PCR was performed on bacterial strains that were ESBLs positive. A total of 6 (83%) clinical strains contained the CTX-M gene, while 2 (20%) clinical isolates did not contain the SHV gene. The size of the PCR product was estimated using DNA markers and a known DNA ladder. For, SHV gene the product size observed on gel was approximately 716.

Discussion

The current study was designed to isolate and characterize the blaSHV and blaCTX-M gene and green synthesis s of ZnO using *Calotropis procera* extract. These nanoparticles were tested for antibacterial activity against gram negative bacterial strains such as *K. pneumoniae* which were sensitive to the beta-lactam antibiotic Imipenem but resistant to Ciprofloxacin. The double disc test was used to identify ESBLS-producing strains. Bacterial susceptibility to nanoparticles was tested using a disc diffusion assay with four different nanoparticle concentrations (20mg/ml, 25 mg/ml, 30 mg/ml, and 35 mg/ml). When observed individually, NPs showed antibacterial effects as indicated by the zone of inhibition, but the beta-lactam antibiotics cefotaxime and ceftriaxone comparatively enhanced this effect. The highest zones of inhibition were obtained at the highest concentration of nanoparticles, 35 mg/ml. The difference between the means seemed statistically significant at $p \leq 0.05$.

Antibiotic resistance was now recognised as a widespread issue that affects both hospital-acquired diseases and non-hospital strains that could be treated with antibiotics. Numerous MDR-based infections not only reduce the effectiveness of current treatments but also result in thousands of fatalities. To address this issue, rapid modifications were required (12).

Carbapenem resistance Enterobacteriaceae (CRE) was increasingly isolated from community-acquired and nosocomial infections (13, 14). CRE could spread from person to person, and the genes encode carbapenems could spread horizontally between strains (15). As a result of its ease of spread, it has become a significant public health concern. Previous research has shown that plasmids carrying the carbapenemase gene frequently carry resistance genes to other antibiotics (16).

Beta-lactam antibiotics, commonly used to treat bacterial infections, were becoming less effective against Enterobacteriaceae. The production of ESBLs by these bacteria is the primary cause of this decrease in efficacy (17). Various beta-lactamases have emerged due to the widespread use of beta-lactam antibiotics in clinical practice over the last several decades. Production was the most common resistance mechanism to beta-lactam antibiotics in gram-harmful bacteria.

The production of ESBLs facilitated beta-lactamase enzyme resistance to third-generation cephalosporins (18), BlaTEM type ESBLs had recently increased rapidly in Enterobacteriaceae, *K. pneumoniae*, and *E. coli.* In the current study, 83% of (*K. pneumonia-producing* ESBLs tested positive for the blaSHV and 80% for blaCTX-M genes present. (Figure 16, 17). These values were similar to those found in another study. Another study from Pakistan found that only 53% of bacterial isolates had the blaSHV and blaCTX-M genes, whereas our study found a higher incidence. Our findings demonstrated that the rate of ESBL-producing *K. pneumoniae* harbouring the blaSHV and blaCTX-M genes increased rapidly. In 2005, the values for the blaSHV and blaCTX-M genes were 4% and 43%, respectively. This rising trend was also mentioned in several other comparative studies (19).

According to the findings of this study, the most common ESBL gene found in *K. pneumoniae* isolates was blaCTX-M. Antimicrobial susceptibility testing of *K. pneumoniae* isolates producing 80% ESBLs. Most continents were concerned about the global spread of blaCTX-M-producing *K. pneumoniae*. According to Eskandari-Nasab *et al*. (2018), the prevalence of blaCTX-M was 10.0, 30.0, 35.3, 56.7, 64.4, 96.9, and 100.0% in Bahrain, Turkey, Saudi Arabia, Iran, United Arab Emirates, Pakistan and Kuwait, respectively (20). While international studies found varying percentages of this gene present in ESBL isolates from North Africa, America, Russia, Latin America, Brazil, and Europe, the percentages were 7.4, 26.4, 34.9, 61.1, 62.1, and 84.5%, respectively, according to Raouf et al., (2022). Even though blaTEM and blaSHV variants were the most common ESBLs, they appear to have become less common over the last decade than blaCTX-M. The findings of this study were consistent with previous studies that found the blaCTX-M gene to be the most common ESBL type in *K. pneumoniae* isolates. In contrast to our findings, Ferreira *et al*. (2019) from Brazil and Kerluku et al. (2023) from Portugal found a higher prevalence of blaSHV than blaCTX-M in *K. pneumoniae (21, 22)*. Many factors could

[Citation: Zahoor, S., Sagheer, S., Anwar, R., Mahmood, S., Para, M., Tabassum, I., Mughal, V.A. (2024). Detection of blactx-m and blashv genes of extended spectrum beta lactamase producing *klebsiella pneumoniae*. *Biol. Clin. Sci. Res. J.*, **2024***: 917*. doi: https://doi.org/10.54112/bcsrj.v2024i1.917]

contribute to these differences, including sample origin, sample size, studied population, and detection methods.

Finally, our findings revealed the presence of ESBL genes in 80% of *K. pneumoniae* isolates. Previous research from Brazi l10, China 16, 17, and Portugal18 found multiple ESBL genes in clinical isolates of *K. pneumoniae*.

According to our findings, green-synthesized ZnO outperformed chemically synthesised ZnO regarding antimicrobial activity. Chemical composition, concentration, size, shape, and photo-activation were some of the factors that influence metallic nanoparticle antimicrobial properties. Because the composition and size of chemical and green synthesised NP differ, they exhibit different antimicrobial activity against the same pathogens. Antibiotics were tested for antimicrobial activity against the bacterial strains used in the study. Imipenem was the only antibiotic that was effective against all strains. *K. pneumoniae* was more sensitive to Imipenem with a maximum zone of inhibition of 30mm. A study by Bradley et al., 2016 found that meropenem was slightly more active against gram-positive than gram-negative pathogens. Unlike Imipenem, all bacterial strains were resistant to the antibiotic ciprofloxacin, with no zone of inhibition observed. Aliakbar Nasiri *et al*. (2016) conducted a study in which 20 strains of P. aeruginosa were isolated, and their susceptibility to different antibiotics was tested, one of which is Ciprofloxacin, and all strains were resistant to ciprofloxacin. Zhang *et al*. (2009) recommended the potential mechanisms involving the interaction of nanomaterials with biological molecules. The author documented that the negative and positive charges of microorganisms and metal oxides create an electromagnetic attraction between the microbe and the treated surface. When the contact is made, the microbe is oxidised and dead instantly.

According to Glover *et al*. (2006), among many other NPs, ZnO NPs were being studied for their photocatalytic antimicrobial activity. In the current study, green synthesised ZnO had the highest zone of inhibition, 12mm, with disc diffusion assay among all strains tested at the highest concentration, 35 mg/ml. Haghi *et al*. (2011) used the double disc diffusion test to examine the antibacterial effect of 0.01, 0.5, 1, and 1.5% ZnO NPs on *K. pneumoniae*. The maximum 5mm zone of inhibition was observed in 1.5% ZnO NPs. The current study observed a maximum inhibition zone of 12mm against *K. pneumoniae* 35mg/ml of ZnO NPs. Russell *et al*. (1994) discovered that a substantial hindrance of nanoparticles to the outer membrane inhibits active transport, dehydrogenase and periplasmic enzyme activity and eventually inhibits RNA. The synthesis of DNA and proteins caused cell lysis.

The current study showed that ZnO nanoparticles had a synergistic effect with the antibiotics imipenem, ciprofloxacin, ceftriaxone, ceftriaxone, ceftriaxone, ceftriaxone, ceftriaxone, ceftriaxone, i.e., they formed a zone of inhibition against all the bacterial strains. Ciprofloxacin, ceftriaxone, and cefotaxime had no inhibitory activity when used alone, but when combined with ZnO, the zone of inhibition was increased against *K. pneumoniae*.

Alekish *et al*. (2018) investigated the effect of ZnO nanoparticles with different antibiotics against MDR S. aureus (23). Among the antibiotics, cefotaxime formed a zone of inhibition of 20mm when used alone but 24mm when combined with ZnO nanoparticles. Their findings may be related to the current findings, which show a synergistic effect of cefotaxime and ZnO nanoparticles.

When ZnO nanoparticles were combined with the beta-lactam antibiotics cefotaxime and ceftriaxone, the antimicrobial activity of these antibiotics against all bacterial isolates was increased. The zone of inhibition was 18mm (ZnO-CTX) and 15mm (ZnO+CRO) against *K. pneumoniae* (358). Other nanoparticles have been studied similarly. Nazari *et al*. (2012) conducted a similar study in which gold NPs combined with cefotaxime antibiotics increased the antibacterial effect of antibiotics at test concentration (40g/disc). In this study, 20 clinical isolates were initially screened for carbapenemase production. In our current study, ESBLs in clinical isolates yielded 75% favourable and 25% negative results. Various studies conducted by multiple researchers showed different results such as Jacobson showed lower ESBL production in P aeruginosa, 7.7%, 4.2%, 3.7%, and 9.2%, respectively (24, 25). The rapid spread of blaCTX-M and blaSHV-producing Enterobacteriaceae, particularly K. pneumonia, was a worldwide public health concern. An earlier study from another Nepalese tertiary care hospital found that respiratory tract specimens had a lower incidence of producing gram-negative bacteria (1.3%). (26). Another recent news report from Brazil was reported in 2008. According to Alkhudhairy *et al., 38.9% of gram-negative* bacterial isolates tested positive for blaCTX-M and 29.8% for blashv positive (27). This study produced similar results because all blaCTX-M and blush-positive strains were gram-negative bacteria.

Antibacterial activity increases with the increase in concentration of nanoparticles. Therefore, we use ZnO NPs to hinder the growth of bacteria. This study will be helpful for the formulation of novel antibacterial agents that were required to develop a generation of therapies to combat anti-microbial resistance. The antibacterial properties of these nanoparticles could be explored for use in various medical and industrial applications, such as the renewal of the antibiotic development strategy to overcome antimicrobial resistance. Antimicrobial awareness campaigns at the country level should be established, suggesting that DNA sequence analysis should be done to check the nucleotide sequence changes in blaCTX-M and blaSHV due to ZnO nanoparticles.

Conclusion

Bacteria are becoming increasingly resistant to antibiotics. This study emphasises the significance of the growing threat posed by ESBL-producing *K. pneumoniae*. This results in resistance to third-generation cephalosporins such as cefotaxime and ceftriaxone. *Calotropis procera*-derived ZnO nanoparticles are more durable, eco-friendly, cheap, and affordable. The antimicrobial activity of biosynthesised ZnO nanoparticles increased with increasing surface-to-volume ratio due to a decrease in particle size of nanoparticles. ZnO nanoparticles improved the efficacy of previously resistant antibiotics CTX and CRO against ESBL-positive bacterial strains. That is the conclusion. At various concentrations, ZnO nanoparticles can inhibit the growth of gram-negative bacteria such as *K. pneumoniae*.

[Citation: Zahoor, S., Sagheer, S., Anwar, R., Mahmood, S., Para, M., Tabassum, I., Mughal, V.A. (2024). Detection of blactx-m and blashv genes of extended spectrum beta lactamase producing *klebsiella pneumoniae*. *Biol. Clin. Sci. Res. J.*, **2024***: 917*. doi: https://doi.org/10.54112/bcsrj.v2024i1.917]

Recommendations

Antibacterial activity increases with the increase in concentration of nanoparticles. Therefore, we use bio-synthesized zinc oxide NPs to hinder the growth of bacteria. This study will be helpful for the formulation of novel antibacterial agents that are required to develop a new generation of therapies to combat anti-microbial resistance. The antibacterial properties of these nanoparticles can be explored in the future for use in various medical and industrial applications—renewal of the antibiotic development strategy to overcome antimicrobial resistance. Antimicrobial awareness campaigns at the country level should be established. It is suggested that DNA sequence analysis should be done to check the nucleotide sequence changes in blaCTX-M and blaSHV due to ZnO nanoparticles

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department concerned. (IRB/no-23339/LCWL dated 5-2-22)
Consent for publication
Approved
Funding
Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

Author Contribution

SUMBAL ZAHOOR
Coordination of collaborative efforts.
SADIA SAGHEER
Conception of Study, Development of Research Methodology Design, Study Design,, Review of manuscript, final approval of manuscript.
RAJIA ANWAR
Study Design, Review of Literature.
SARA MAHMOOD
Conception of Study, Final approval of manuscript.
MAH PARA
Data entry and Data analysis, drafting article.
IQRA TABASSUM
Manuscript drafting.
VANEEZA ARSHAD MUGHAL
Manuscript revisions, critical input.

References

1. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, et al., editors. Antimicrobial resistance: a growing serious threat for global public health. Healthcare; 2023: MDPI.
2. Silbergeld EK. One health and the agricultural transition in food animal production. Global transitions. 2019;1:83-92.
3. Rohr JR, Barrett CB, Civitello DJ, Craft ME, Delius B, DeLeo GA, et al. Emerging human infectious diseases and the links to global food production. Nature sustainability. 2019;2(6):445-56.
4. Haney EF, Hancock RE. Addressing antibiotic failure—beyond genetically encoded antimicrobial resistance. Frontiers in Drug Discovery. 2022;2:892975.
5. Subramaniam G, Girish M. Antibiotic resistance—A cause for reemergence of infections. The Indian Journal of Pediatrics. 2020;87(11):937-44.
6. Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: The most critical pathogens. Pathogens. 2021;10(10):1310.
7. Hall JP, Harrison E, Baltrus DA. Introduction: the secret lives of microbial mobile genetic elements. The Royal Society; 2022. p. 20200460.
8. Terreni M, Taccani M, Pregnolato M. New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. Molecules. 2021;26(9):2671.
9. Lewis K. The science of antibiotic discovery. Cell. 2020;181(1):29-45.
10. Skwarczynski M, Bashiri S, Yuan Y, Ziora ZM, Nabil O, Masuda K, et al. Antimicrobial activity enhancers: Towards smart delivery of antimicrobial agents. Antibiotics. 2022;11(3):412.
11. Fereshteh S, Noori Goodarzi N, Kalhor H, Rahimi H, Barzi SM, Badmasti F. Identification of Putative Drug Targets in Highly Resistant Gram-Negative Bacteria; and Drug Discovery Against Glycyl-tRNA Synthetase as a New Target. Bioinformatics and Biology Insights. 2023;17:11779322231152980.
12. Martin I, Kenna DT, Morales S, Alton EW, Davies JC. Variability in bacteriophage and antibiotic sensitivity in serial Pseudomonas aeruginosa isolates from cystic fibrosis airway cultures over 12 months. Microorganisms. 2021;9(3):660.
13. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, et al. Epidemiology of carbapenem-resistant Enterobacteriaceae infections: report from the China CRE Network. Antimicrobial agents and chemotherapy. 2018;62(2):10.1128/aac. 01882-17.
14. Kelly AM, Mathema B, Larson EL. Carbapenem-resistant Enterobacteriaceae in the community: a scoping review. International journal of antimicrobial agents. 2017;50(2):127-34.
15. Zhang R, Liu L, Zhou H, Chan EW, Li J, Fang Y, et al. Nationwide surveillance of clinical carbapenem-resistant Enterobacteriaceae (CRE) strains in China. EBioMedicine. 2017;19:98-106.
16. Rozwandowicz M, Brouwer M, Fischer J, Wagenaar J, Gonzalez-Zorn B, Guerra B, et al. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy. 2018;73(5):1121-37.
17. Russo A, Berruti M, Giacobbe DR, Vena A, Bassetti M. Recent molecules in the treatment of severe infections caused by ESBL-producing bacteria. Expert Review of Anti-infective Therapy. 2021;19(8):983-91.
18. Lin X, Kück U. Cephalosporins as key lead generation beta-lactam antibiotics. Applied Microbiology and Biotechnology. 2022;106(24):8007-20.
19. Abrar S, Ain NU, Liaqat H, Hussain S, Rasheed F, Riaz S. Distribution of bla CTX− M, bla TEM, bla SHV

and bla OXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. Antimicrobial Resistance & Infection Control. 2019;8:1-10.
20. Eskandari-Nasab E, Moghadampour M, Tahmasebi A. Prevalence of blaCTX-M gene among extended-spectrum β-lactamases producing Klebsiella pneumoniae clinical isolates in Iran: A meta-analysis. Iranian Journal of Medical Sciences. 2018;43(4):347.
21. Ferreira APdS, Szwarcwald CL, Damacena GN, Souza PRBd. Increasing trends in obesity prevalence from 2013 to 2019 and associated factors in Brazil. Revista Brasileira de Epidemiologia. 2021;24(suppl 2):e210009.
22. Kerluku M, Jankuloski D, Manovska Ratkova M, Prodanov M, Dimzoska Stojanovska B, Dodovski A, et al. β-Lactamase genes (blaCTX-M, blaSHV, blaTEM, blaOXA1 AND blaOXA2) and phylogenetic groups in ESBL producing commensal Escherichia coli isolated from faecal samples from dairy farm in the Municipality of Debar. Mac Vet Rev. 2023;46:89-97.
23. Alekish M, Ismail ZB, Albiss B, Nawasrah S. In vitro antibacterial effects of zinc oxide nanoparticles on multiple drug-resistant strains of Staphylococcus aureus and Escherichia coli: An alternative approach for antibacterial therapy of mastitis in sheep. Veterinary world. 2018;11(10):1428.
24. Kumar A, Das S, Anjum N, Oraon V, Das S. Antimicrobial susceptibility pattern of extended spectrum beta-lactamase (ESBL) and non ESBL producing Pseudomonas aeruginosa, isolated from pus samples from a tertiary care hospital in Bihar. Int J Curr Microbiol App Sci. 2020;9(6):3646-55.
25. Zha L. Treatments of nosocomial pneumonia in the era of antimicrobial resistance: The University of Liverpool (United Kingdom); 2022.
26. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. Antimicrobial Resistance & Infection Control. 2017;6:1-9.
27. Alkhudhairy MK, Alshammari MMM. Extended spectrum β-lactamase-producing Escherichia coli isolated from pregnant women with asymptomatic UTI in Iraq. EurAsian Journal of BioSciences. 2019;13(2):1881-9.