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RESEARCH ARTICLE

Antimicrobial Activity of Zinc Oxide Nanoparticles against ESBL Producing *Klebsiella pneumoniae* Isolated from Equines in Egypt

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A B S T R A C T

Antibiotic resistance of *Klebsiella pneumoniae* (*K. pneumoniae*) has become alarming to public health, moreover biofilm production is one of the virulence mechanisms that contributes to antibiotic resistance. Metal oxide nanoparticles are showing promise as antibacterial agents. The purpose of this study was to assess the antibacterial activities and dosage effect of ZnO-NPs against ESBL *K. pneumoniae* isolates recovered from horses exhibiting respiratory symptoms. The antibiotic sensitivity of ESBL producing *K. pneumoniae* strains was tested using the Kirby Bauer disc diffusion method, which showed 100% resistance to cefotaxime and erythromycin while resistance to tetracycline, ampicillin, amoxicillin / clavulanic acid, ceftriaxone, ceftazidime, amikacin and ciprofloxacin were 85.7, 71.42, 42.85, 28.57, 28.57, 14.28 and14.28%, respectively. Regarding the biofilm production genes scanning, *K. pneumoniae* isolates were positive for *luxS* gene (100%), while only 85.7% of *K. pneumoniae* were positive for *Uge* gene and none of them having *mrk*D gene. ZnO-NPs were tested against a local isolate of ESBL-producing *K. pneumoniae* for their antibacterial properties. *K. pneumoniae* had MIC and MBC values of 5ug/ml and 3ug/ml, respectively. In the current study, antibiotic abuse leads to an increase in the population of ESBL-producing *K. pneumoniae*, posing a concern to public health. ZnO-NPs, a new material, exhibit significant antibacterial activity against hypermucoviscosity *K. pneumoniae* isolate.

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INTRODUCTION

K. pneumoniae is one of the top six pathogenic microorganisms deemed to be multidrug resistant (MDR) and the most virulent bacteria, known as ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*) (Mulani *et al.,* 2019). *K. pneumoniae* is one of the most significant bacteria that cause nosocomial infections (Wareth *et al.,* 2021), produces respiratory symptoms as hemorrhagic nasal discharge and pneumonia in equines (Estell *et al.,* 2016) as well as the ability to cause abortion in pregnant mares (Silva *et al.,* 2020). However, antimicrobial resistance is widely distributed in equine populations, such as those in housing (stables) or hospitals, through the horizontal transfer of resistance genes from one bacterial strain to another, or even from one bacterial population to another, from an infected animal to another, or even to a human and vice versa. (Van Spijk *et al.,* 2019). Antimicrobial resistance has emerged as a major medical and veterinary issue in this species, among MDR *K. pneumoniae*, Carbapenemase and extended spectrum blactamase (ESBL) producing bacteria have been identified as an urgent threat by health agencies worldwide (CDC, 2019). MDR *K. pneumoniae* infection in animals can cause life-threatening illnesses such as severe mastitis in cows and hemorrhagic pneumonia, raising treatment expenses, especially when the infection is resistant to frequently prescribed antibiotics (*e.g.,* trimethoprim or gentamicin) (Estell *et al.,* 2016; Trigo da Roza *et al.,* 2019).

Biofilm production, which acts as a barrier to antibiotic action and can also develop resistance due to cell interaction and DNA acquisition from nearby bacterial communities, is one of *K. pneumoniae*'s virulence mechanisms that contributes to resistance (Das *et al.,* 2020). There are genes in *K. pneumoniae* that are connected to biofilm formation, including capsular genes like *rmp*A, which is linked to the K1/K2 virulent type and fimbriae genes like *mrk*D and *fim*H, which detect fimbrial types I

and 3, respectively and other genes that influence *K. peumoniae's* uptake of iron include aerobactin and *kfu* genes(Ma *et al*., 2005), as well as genes that encode the necessary enzymes for the lipopolysaccharide manufacture, like the *uge* gene, which is responsible for the uridine diphosphate galacturonate 4 epimerase (Alcántar-Curiel *et al*., 2013).

In Egypt, there have been very few studies on drug-resistant *K. pneumoniae* in equines (Fawzy *et al.*, 2021). Therefore, strategies for the formulation of novel, safe, and reasonably priced antibiotics must be quickly developed (Guo *et al.*, 2017). Metal oxide nanoparticles have become effective antibacterial agents in this situation (Luo *et al.*, 2014). Zinc (Zn) is an essential mineral that may assist injury repair, immune response modulation, fertility and metabolism, growth, and free radical scavenging (Kujur *et al.*, 2016). The ZnO-NP is a more effective compound than ZnO. Furthermore, it has an excellent permeability, increasing its potential as a therapeutic component (Baltić *et al*., 2013). Metal oxides, specifically zinc oxide nanoparticles, make up these nanomaterials (ZnO-NPs). The total annual production of ZnO-NP is predicted to be between 550 and 33400 tones worldwide (Czyzowska and Barbasz, 2020; Faizan *et al.*, 2020). Nanomaterials are utilized in production and animal medicine to enhance infection detection and treatment, enhance feeding and feed digestion, and health promotion (Bogdan *et al*., 2017). This study aimed to evaluate the antibacterial abilities and dosage impact of ZnO-NPs against a *K. pneumoniae* isolate recovered from horses suffering from respiratory manifestation.

MATERIALS AND METHODS

Ethical approval: This study was approved by the NRC's Medical Research Ethics Committee (permission no. 19153).

Bacterial isolates: The current seven strains of ESBL produce *K. Pneumonia* was isolated and identified in a previous study from horses with respiratory disorders in Cairo Governorate, Egypt (Arafa *et al*., 2022).

Testing the antimicrobial susceptibility: ESBL producing *K. pneumoniae* strains were examined against 12 antimicrobial drugs using Kirby-Bauer's standard disc diffusion technique in accordance with CLSI; 2015.

DNA extraction: Following the manufacturer's instructions, DNA was extracted from bacterial cultures using the QIAamp DNA Mini kit from Qiagen, Germany, GmbH.

Molecular detection of biofilm genes using conventional Polymerase Chain Reaction (PCR): The PCR was carried out in a final volume of 25 µl reaction containing 1µl of target DNA, 0.5 µl (10 M) of each primer, and 12.5 µl of 2x COSMO PCR RED Master Mix (Cat. W1020300X, Willofort Co., UK), to identify the biofilm production genes (*luxS*, *uge*, *mrkD*). The InGenius3 gel documentation system was used to photograph and analyse the gels. Table 1 and 2 include a list of the primers and cycle conditions employed.

Synthesis of zinc oxide nanoparticles (ZnO-NPs): $ZnCl_2$, LiOH, KOH, NaOH, ammonia water and analytical grade of glycerol (Nanjing chemical reagent factory, China) were utilized without further purification. ZnO nanoparticles preparation in different conditions were shown in Table 3 (Wang *et al*., 2018).

Characterization of ZnO-NPs: The dispersion and morphology of ZnO-NPs were studied using high-resolution transmission electron microscopy (HR-TEM) at an acceleration voltage of 200 kV. A PANalytical (Empyrean) X-ray diffractometer was used to detect Patterns of X-ray diffraction (XRD) via Cu K1 radiation (wavelength 1.5406) at a voltage of 40 kV, a scan angle range of 5-80°, a current of 30 mA, and a scan step of 0.02°. The particle size distribution was determined using the Zetasizer nano-Zs90 (Malvern, UK).

Antibacterial activity of zinc oxide nanoparticles against *Kebesilla* species: The antibacterial test was carried out quantitatively according to the AATCC test method100-1999 for Bacterial Counting (Chun *et al*., 2009). All tests were examined by standard plate count technique. Antibacterial properties are evaluated by a percentage of decrease in the number of organism cells that survived after coming into touch with the ZnO-NPs as opposed to those that survived after coming into contact with the control. The following equation was used to express all of the results:

$$\text{Reduction (\%)} = (B\text{-}A/B) \text{ X } 100$$

Where B: the number of microorganisms survive without contact with ZnO-NPs, and A: the number of microorganisms survive after contact with ZnO-NPs.

To determine the MIC, with minor modifications, the microdilution method with 96-well microplates was applied (Paredes *et al*., 2014). In brief, 95 µl of MH broth medium and 5 µl of bacterial inoculum into each well were dispensed to produce 96-well plates. Furthermore, the wells were filled with 100 µl of each manufactured ZnO-NPs (1-50 mg/ml) solution to ascertain the MIC of ZnO-NPs. After that, the microplates were incubated for 24 hours at 37°C. A microplate reader was used to measure the absorbance at a wavelength of 600 nm in order to calculate the growth rate.

When compared to a positive control (no ZnO-NP treatment), the MBC value was determined as the ZnO-NP concentration that completely inhibited bacterial growth.

RESULTS

Testing of antimicrobial susceptibility: Antibiotic sensitivity tests were carried out on ESBL producing *K. pneumoniae* strains against 12 antimicrobial drugs. According to Table 4, seven *K. pneumoniae* showed complete resistance (100%) to cefotaxime and erythromycin. On the other side, tetracycline, ampicillin, amoxicillin / clavulanic acid, ceftriaxone, ceftazidime, amikacin and ciprofloxacin showed different percentage of resistance 85.7, 71.42, 42.85, 28.57, 28.57, 14.28 and14.28%, respectively. The sensitivity of tested strains to Chloramphenicol, sulphamethoxazole/trimethoprim and gentamicin were 85.7, 85.7 and 57.14%, respectively.

Table 1: Primers used for molecular detection of biofilm genes

Gene	Sequence (5´-3´)	Amplicon size (bp)	Reference
luxS	GCC GTT GTT AGA TAG TTT CACAG CAG TTC GTC GTT GCT GTT GATG	447bp	Shadkam *et al.* (2021)
Uge	TCT TCA CGC CTT CCT TCA CT GAT CAT CCG GTC TCC CTG TA	535bp	Shah *et al.* (2017)
mrkD	CCACCAACTATTCCCTCGAA ATGGAACCCACATCGACATT	226bp	Shah *et al.* (2017)

Table 2: Cycling conditions for molecular detection of biofilm genes

Gene	Init. Denat.	Denat.	Anneal.	Extention	Final Ext.	Cycles
luxS	95°C 2min	95°C 20sec	53°C 30sec	72°C 45sec	72°C 7min	35
Uge	95°C 2min	95°C 20sec	55°C 30sec	72°C 45sec	72°C 7min	35
mrkD	95°C 2min	95°C 20sec	54°C 30sec	72°C 45sec	72°C 7min	35

Table 3: Preparation of the ZnO nanoparticles in different conditions

Mole ratio		$ZnCl_2$ solution	Hydroxides	Concentration of
glycerol	Zn^{2+}	concentration (wt%)		hydroxide (wt%)
0	1	65	NaOH	50
3.33	1	65	NaOH	50
1	1	65	NaOH	50
1.67	1	65	NaOH	50
3.33	1	65	NaOH	50
1	1	50	NaOH	50
1	1	65	NaOH	50
1	1	80	NaOH	50
3.33	1	65	LiOH	8
3.33	1	65	NH_4OH	25
3.33	1	65	NaOH	50
3.33	1	65	KOH	60

Table 4: Antibiotic sensitivity test of *K. pneumoniae* isolated from equines in Egypt

Sample No	AMP	AMC	CTX	CAZ	CRO	AK	C	CIP	Tet	E	CN	SXT
1	R	I	R	I	S	S	S	S	R	R	I	S
2	I	I	R	I	S	S	S	S	R	R	S	S
3	I	R	R	I	R	R	I	S	I	R	S	I
4	R	R	R	R	R	S	S	S	R	R	S	S
5	R	I	R	I	S	S	S	S	R	R	I	S
6	R	R	R	R	S	S	S	S	R	R	S	S
7	R	I	R	I	S	S	S	R	R	R	I	S

cefotaxime(CTX -30 µg),ceftriaxone(CRO -30 µg), ceftazidime (CAZ -30 µg), ciprofloxacin (CIP-5 µg), tetracycline (Tet-30 µg), gentamicin (CN-10 µg), erythromycin (E-15 µg), ampicillin (AMP -10 µg), amikacin (AK-30 µg), amoxicillin / clavulanic acid(AMC-10 µg), Chloramphenicol(C-30 µg) , sulphamethoxazole/ trimethoprim (SXT-25 µg).

Molecular detection of biofilm genes: Regarding the biofilm production genes scanning, all the seven *K. pneumoniae* isolates were positive for *luxS* gene (100%), while only 6 out of 7 (85.7%) were positive for *Uge* gene. None were positive for *mrkD* gene (0%).

Powder X-ray diffraction (XRD) studies: XRD pattern was shown in Fig. 1 XRD analysis reveals monophasic poly crystalline structure of for wurtzite structure in accordance with JCPSD code no. 36-1451 which confirm crystalline structure and hexagonal phase. The reflections observed at 31.58°, 34.25°, 36.07°, 47.32°, 56.33°, 62.57°, 66.07°, 67.64°, 68.78° and 76.26° correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201) and (202). The mean particle size of the NPs was calculated using Debye-Scherrer formula.

$$d = \frac{0.89\lambda}{\beta \cos\theta},$$

Where D is the size of the crystallites in the particle, the Scherrer constant is denoted by K and is approximately 0.9, λ is the wavelength of light used for diffraction (λ = 1.54 Å), β is the FWHM (full width at half maximum) of the diffraction peak, and θ is the reflection angle. The average size of the ZnO NPs calculated from the XRD pattern was 48 nm.

High Resolution Transmission electron microscopy (HRTEM): The morphology of the synthesized ZnO nanoparticles was estimated via HRTEM as depicted in Fig. 2 the HRTEM images showed that ZnO nanoparticles are nano sheet like shaped structure with a particle size range from 2 nm to 40 nm where some of the observed nano sheers are agglomerated showing bigger size. Selected area electron diffraction (SAED) showed a ring pattern characteristic with some brighter and more distinct spot in the rings that indicates the presence of some larger crystallites, while the ring was relatively continuous indicates crystallites with small size.

Field Emission Scanning electron microscope (FESEM): FESEM studies were carried out to investigate the superficial topography of synthesized zinc oxide. FESEM micrographs of the zinc oxide NPs have been represented in Fig. 3. FESEM image–showed nano sheet like structure due to the presence of capping agent, the nanoparticles appeared spherical with inclined surfaces in SEM images.

Antibacterial activity of ZnO-NPs against *K. pneumonia:* As each concentration of ZnO-NPs were performed against *K. pneumoniae* in triplicate so the average of reduction percent was recorded in each concentration as follow 100% reduction at 20 ug/ml, 15 ug/ml, 7 ug/ml and 5 ug/ml while the reduction percent was 90% at 3 ug/ml. The lowest reduction was 40% at 1 ug/ml. For *K. pneumoniae*, the MIC and MBC values with ZnONPs were 5 ug/ml and 3 ug/ml, respectively.

DISCUSSION

Klebsiella pneumoniae is the most prominent pathogen in the genus *Klebsiella*; it can withstand high quantities of disinfectants (Wareth *et al*., 2021). The World Health Organization (WHO) classified *K. pneumoniae* as a life-threatening bacteria due to the fact of the widespread distribution of highly resistant strains not just against beta-lactams but also against the last-resort treatment carbapenems, which were considered as the preferred recommended medicine (WHO, 2020). In this study, all strains of *k. pneumoniae* showed complete resistance (100%) to cefotaxime and erythromycin. On the other side, tetracycline, ampicillin, amoxicillin / clavulanic acid, ceftriaxone, ceftazidime, amikacin and ciprofloxacin showed different percentage of resistance 85.7, 71.42, 42.85, 28.57, 28.57, 14.28 and14.28%, respectively. The sensitivity of tested strains to Chloramphenicol, sulphamethoxazole/ trimethoprim and gentamicin were 85.7, 85.7 and 57.14%, respectively.

Garcia-Fierro *et al*. (2022) revealed that all noticed isolates were derived from diseased companion animals infected with the *K. pneumoniae* species complex, with

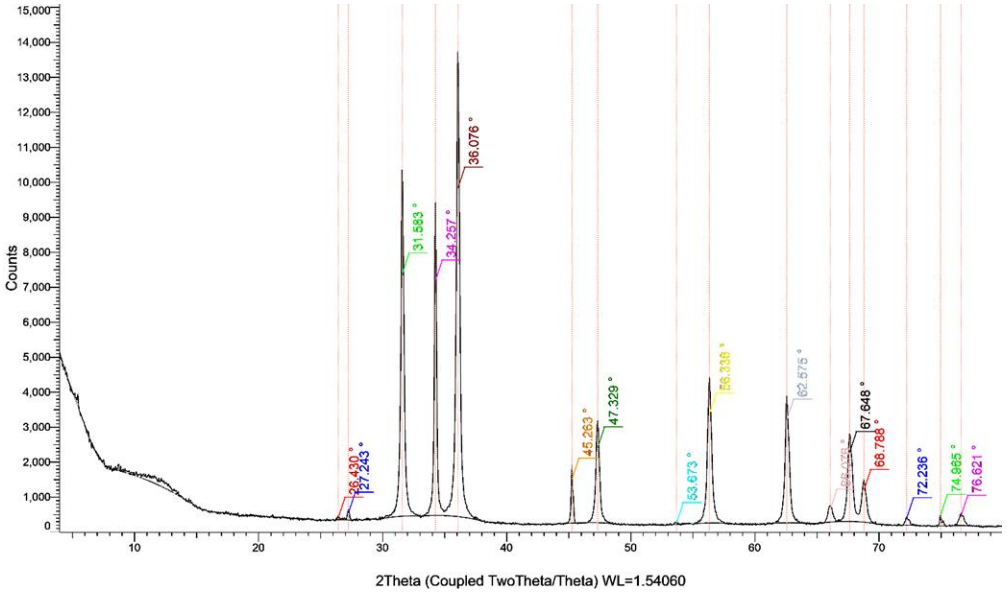


Fig. 1: XRD pattern of ZnO NPs

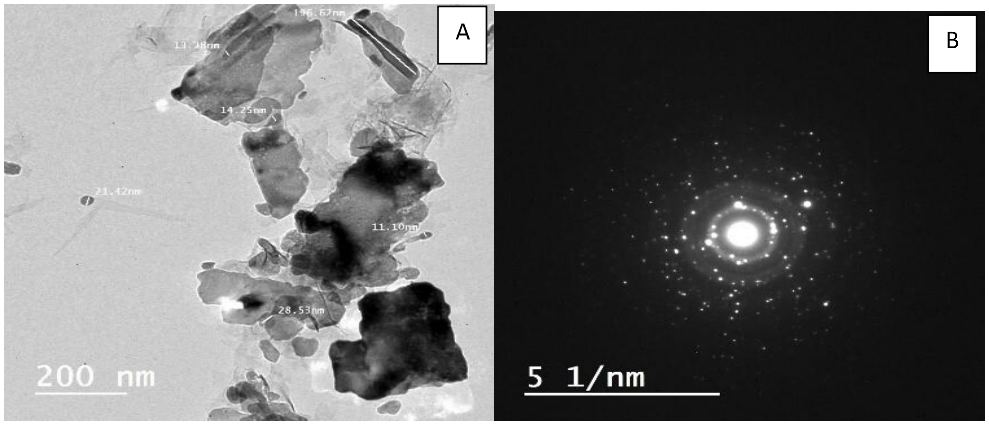
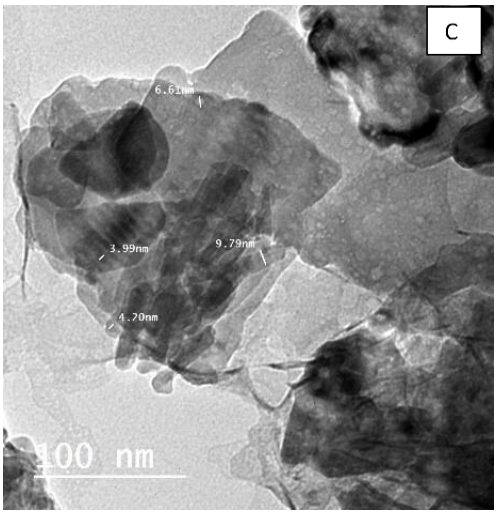
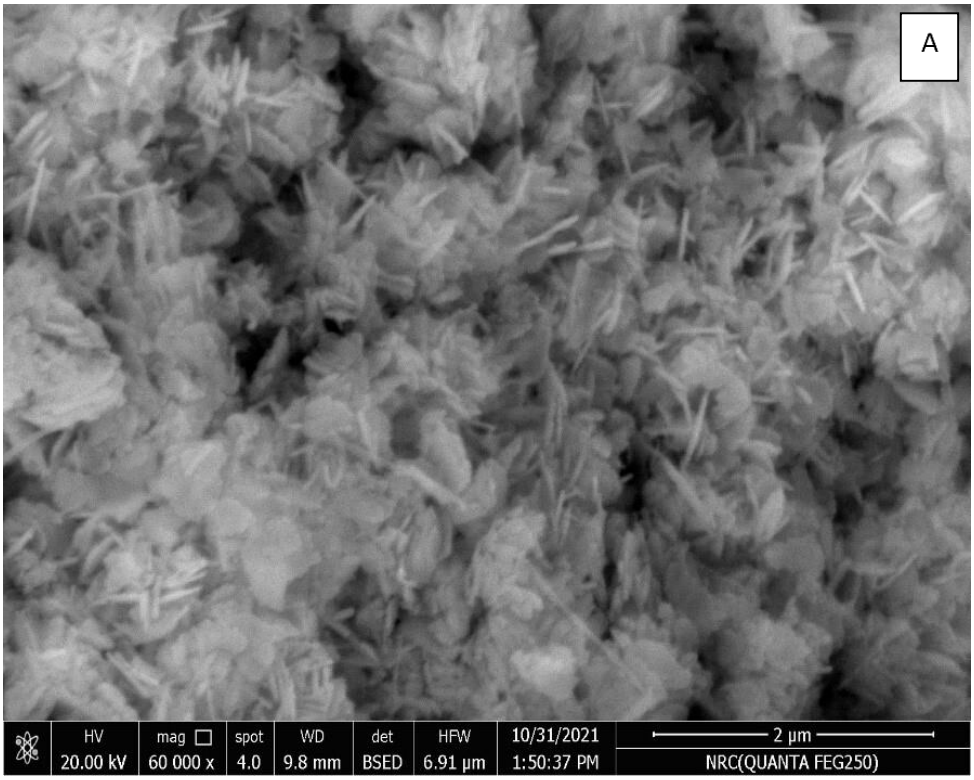


Fig. 2: TEM images of zinc oxide nanoparticles at different magnifications (a, c) and SAED pattern (b)



K. pneumoniae dominating (98%). Furthermore, all isolates of *K. pneumoniae* were resistant to cefoxitin, ceftiofur, or both. The majority of the antimicrobial substances examined were ineffective against isolates of *K. pneumoniae* isolated from the horse with surgical site infection (Trigo da Roza *et al*., 2019). In another study showed that all isolates were sensitive to colistin and tigecycline, two medicines used as a last resort for Carbapenem-resistant Enterobacterales infection (Rodriguez-Bano *et al*., 2018) while Wang *et al*. (2021); reported that *K. Pneumoniae* isolate obtained from fecal horse sample showing 100% resistance to beta-lactam antibiotics and aminoglycoside antibiotics such as amikacin.

The persistence of *K. pneumoniae* species is increased by the formation of biofilm, which shields it from host immunological responses and antibiotic action (Karigoudar *et al.*, 2019). Regarding the biofilm production genes survey, all the seven *k.pneumoniae* isolates were positive for *luxS* gene (100%), while only 6 out of 7 (85.7%) were positive for *Uge* gene. None were positive for *mrkD* gene (0%) as shown in Fig. 1 and Fig. 3. Hamam *et al.* (2019) revealed that 98% of the isolates of *K. pneumoniae*



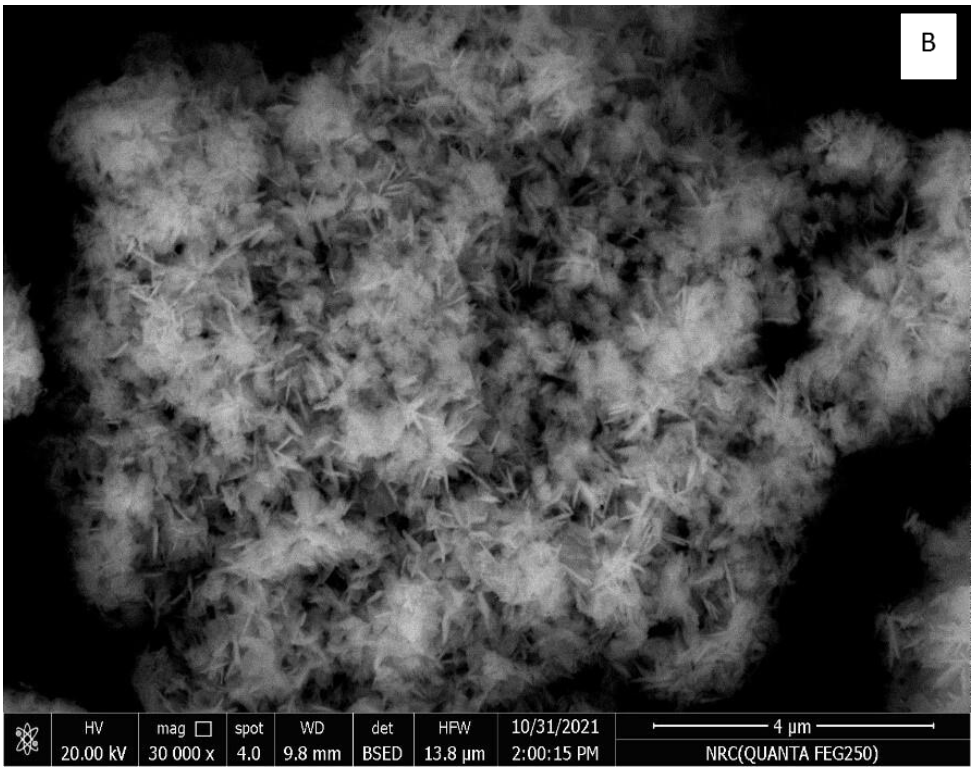


Fig. 3: SEM images of Zinc oxide at magnifications (a) 60K and 30K

of *K. pneumoniae* carried the *lux*S gene. The most common virulent genes were *fimH* (76%), *uge* (70%), *rmpA* (62%), mag (59%) and wzy (59%) while aerobactin gene (50%) and *kfa* gene (33%) had the lowest prevalence (Elbrolosy *et al.*, 2020). Similar prevalence rates regarding *fimH*, *uge*, and *rmpA* genes were reported (Ferreira *et al.*, 2019). Priyanka *et al.* (2022) revealed that *mrkA* and *mrkD* gene expression declined while *fimH* gene expression increased. Shakib *et al.* (2018) also reported reduced *mrkD* gene prevalence percentages. These results coincide with ours that none of the seven strains were positive for the *mrkD* gene (0%).

The consequences of biofilm-associated *K. pneumoniae* infections in overcoming the effects of antibiotic therapy have not been thoroughly investigated (Devanga *et al.*, 2020). The number of scientific articles devoted to the discovery of new antimicrobial compounds is estimated to be around 99000 in 2018-2020, with 5900 of them devoted to the discovery of metal-based antimicrobial compounds (NCBI, 2020).

The morphology of the synthesized ZnO nanoparticles was assessed via HRTEM as depicted in fig 3. The HRTEM images showed that ZnO are nano sheet like shaped structure and it has particles that range in size from 2 nm to 40 nm where some of the observed nano sheers are agglomerated showing bigger size. SAED (Selected area electron diffraction) showed ring pattern characteristic with some brighter and more distinct spot in the rings that indicates the presence of some larger crystallites, while the ring was relatively continuous indicates crystallites with small size.

Zinc oxide nanoparticles crystallize in a variety of morphologies, depending on the synthesis procedure and circumstances. Many scientists attribute ZnO-NPs antibacterial capabilities to their shape. ZnO-NPs exhibit their most frequent nanoconfiguration in terms of morphologies when compared to other nano-metal oxides, including nanocombs, nanocages, nanobelts, and nanosprings (Wang, 2004).

In accordance with Yang *et al.* 2009, ZnO-NPs with rod-like and wires forms enter bacterial walls more readily than spherical-shaped ZnO-NPs. Moreover, Talebian *et al.*, 2013 discovered that ZnO-NPs with a flower-like shape had stronger biocidal activity towards the test bacteria than ZnO-NPs with a rod or spherical shape. In comparison to nanorods, ZnO nanotubes shown better antibacterial activity against the chosen test microorganisms, and Elkady *et al.* (2015) found that this improvement was due to the ZnO nanotubes' increased surface area. The conditions under which nanoparticles are created determine their sizes and shapes (Soren *et al*., 2018). Nanoparticles' antibacterial capabilities vary depending on their size (Yousefi *et al*., 2017). The smallest size of some nanoparticles results in the highest antibacterial activity (Bai *et al*., 2020). The best antibacterial activity of several nanoparticles appears at their lowest sizes. In this work, *K. pneumoniae* were sensitive to ZnO-NPs. These findings are comparable with those observed by Danial and Yousef, 2014 and those reported by Hozyen *et al*. (2019). The toxicity of nanoparticles is directly influenced by their concentration, particularly at high concentrations large quantities of ions can be released by the Gomes ions of nanoparticles. (Tamayo *et al*., 2014). ZnO nanoparticle concentrations that are bactericidal typically fall within a range that is much smaller than 4 (Lallo da Silva *et al*., 2019). In the present work, ZnO-NPs exhibited antibacterial effects at concentrations of 3-5 ug/ml against *K. pneumoniae* recovered from horses with respiratory manifestation as reported by Hozyen *et al.* (2019), that Gram-negative bacteria as *K. pneumoniae*, *P. aeruginosa* and *E. coli* were more resistant to MBC and MIC values than Gram-positive bacteria as *S. aureus*. This phenomenon is thought to be caused by variations in Gram-negative and Gram-positive cell wall structures (Slavin *et al.*, 2017).

Nanoparticles interacting with the bacterial cell wall, on the other hand, provide a concentrated source of ions that are constantly released, increasing cell toxicity (McQuillan *et al.*, 2012). Although explanation is challenging due of the numerous pathways that appear to be simultaneously engaged by nanoparticles, these pathways are additionally constitute the reason why exposure to nanoparticles works so well. It appears that the mixture itself is harmful; a single ingredient probably won't be enough to kill the germs. Nanoparticles are effective for treating infections and destroying pathogens due to their multi-target activity (Slavin *et al*., 2017).

Conclusions: In conclusion, a population of ESBL-producing *K. pneumoniae* could emerge as a result of incorrect antibiotic use and a lack of veterinary services, putting public health at risk. Biofilm formation in *K. pneumoniae* may operate as a barrier to antibiotic activity and can potentially lead to resistance, therefore further research is needed to fully understand the virulence and spread of *K. pneumoniae*. Zinc oxide nanoparticles stand out among metal oxides. It is cost-effective, non-toxic, chemically stable, and biosafe. Additionally, it could potentially work as an antimicrobial agent. In the present study, ZnO-NPs, a novel material, were found to be effective antibacterial agents against *K. pneumoniae* isolates from horses suffering from respiratory manifestations; which has zoonotic implications due to the frequent contact between horses and people.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions: AAA and MMK participated in the design of the study, AAA conducting the PCR genetic markers of virulence, Project administration and Funding. MMK was responsible for detection of the antibacterial activity of ZnO-NPs.

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REFERENCES

Alcántar-Curiel MD, Blackburn D, Saldaña Z, *et al.*, 2013. Multi-functional analysis of *Klebsiella pneumoniae* fimbrial types in adherence and biofilm formation. Virulence 4:129-38.

Arafa AA, Hedia RH, Dorgham SM, *et al*., 2022. Determination of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolated from horses with respiratory manifestation. Vet World 15:827-833.

Bai X, Li L, Liu H, *et al*., 2020. Solvothermal synthesis of ZnO nanoparticles and anti-infection application in Vivo. ACS Appl Mater Interfaces 7:1308–17.

Baltić MŽ, Bošković M, Ivanović J, *et al.*, 2013. Nanotechnology and its potential applications in meat industry. Tehnologija Mesa 54:168–175.

Bogdan J, Pławińska-Czarnak J and Zarzyńska J, 2017. Nanoparticles of titanium and zinc oxides as novel agents in tumor treatment: A review. Nanoscale Res Lette 12:225.

CDC, 2019. Antibiotic resistance threats in the United States. U.S. Department of Health and Human Services, CDC, Atlanta, GA. https://doi.org/10.15620/cdc:82532.

Chun DTW, Foulk JA and McAlister DD, 2009. Testing for antibacterial properties of cotton/flax denim. Indus Crops Prod 29:371-376.

CLSI, 2015. clinical and laboratory standards institute (2015) performance standards for antimicrobial susceptibility testing; 25th Informational Supplement. CLSI Document M100-S25, Clinical and Laboratory Standards Institute, Wayne, PA.

Czyzowska A and Barbasz A, 2020. A review: Zinc oxide nanoparticles-Friends or enemies? Int J Environ Health Res 32:885–901.

Danial EN and Yousef JM, 2014. Comparative studies between zinc oxide and manganese oxide nano-particle for their antimicrobial activities. J Pure Appl Microbiol 8 :293-300.

Das CGA, Kumar VG, Dhas TS, *et al*. 2020. Antibacterial activity of silver nanoparticles (biosynthesis): A short review on recent advances. Biocatal Agric Biotechnol 27:101593.

Devanga RNK, Muthuirulandi SDP, Triplicane DH, *et al*., 2020. The influence of biofilms on carbapenem susceptibility and patient outcome in device associated *K pneumoniae* infections: insights into phenotype vs genome-wide analysis and correlation. Front Microbiol 11:3220. https:// doi. org/ 10. 3389/ fmicb. 2020. 591679

Elbrolosy AM, Eissa NA, Al-Rajhy NA, *et al*. 2020. *MrkD* Gene as a Regulator of Biofilm Formation with Correlation to Antibiotic Resistance among Clinical *Klebsiella pneumoniae* Isolates from Menoufia University Hospitals. Egypt J Med Microbiol 29:137-144.

Elkady MF, Hassan HS, Hafez EE, *et al*., 2015. Construction of zinc oxide into different morphological structures to be utilized as antimicrobial agent against multidrug resistant bacteria. Bioinorg Chem Appl 2015:536854.

Estell KE, Young A, Kozikowski T, *et al*., 2016. Pneumonia caused by *Klebsiella spp.* in 46 horses. J Vet Intern Med 30:314–321.

Faizan M, Hayat S and Pichtel J, 2020. Effects of zinc oxide nanoparticles on crop plants:A perspective analysis. Sustainable agriculture reviews 41, 1st ed.; Hayat S, Pichtel J, Faizan M, Fariduddin Q, Eds.; Springer Nature AG: Cham, Switzerland. pp.83-99

Fawzy NM, Osman KM, Farag AN, *et al*., 2021. Phenotypic study on the bacterial isolates from equine with respiratory disorders regarding antimicrobial drug resistance. World 11(1):98–109.

Ferreira RL, da Silva BCM, Rezende GS, *et al*., 2019. High prevalence of multidrug-resistant *Klebsiella pneumoniae* harboring several virulence and β-Lactamase encoding genes in a Brazilian intensive care unit. Front. Microbiol 9:3198.

Garcia-Fierro R, Drapeau A, Dazas M, *et al*. 2022. Comparative phylogenomics of ESBL-, AmpC- and carbapenemase-producing *Klebsiella pneumoniae* originating from companion animals and humans. J Antimicrob Chemother 77:1263-1271.

Guo Y, Wang S, Zhan LY, *et al*., 2017. Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. Front. Cell Infect Microbiol 7:24.

Hamam SS, El Kholy RM and Zaki MES, 2019. Study of various virulence genes, biofilm formation and extended-spectrum β-lactamase resistance in *Klebsiella pneumoniae* isolated from urinary tract infections. Open Microbiol J DOI:10.2174/1874285801913010249.

Hozyen HF, Ibrahim ES, Khairy EA, *et al*., 2019. Enhanced antibacterial activity of capped zinc oxide nanoparticles: A step towards the control of clinical bovine mastitis. Vet World 12:1225-1232.

Karigoudar RM, Karigoudar MH, Wavare SM, *et al*. 2019. Detection of biofilm among uropathogenic *Escherichia coli* and its correlation with antibiotic resistance pattern. J Lab Physicians 11:17-22.

Kujur K, Ghosh S, Batabyal S, *et al*., 2016. Effect of micronutrient supplementation on the hormonal profile of local goat and sheep breeds of West Bengal. Indian J Anim Sci 86 :224–225.

Lallo da Silva B, Caetano B, Chiari-Andréo BG, *et al.* 2019. Increased antibacterial activity of ZnO nanoparticles: influence of size and surface modification. Colloids Surf B Biointerfaces 177:440–7.

Luo Y, Wang Y, Ye L, *et al*., 2014. Molecular epidemiology and virulence factors of pyogenic liver abscess causing *Klebsiella pneumoniae* in China. Clin. Microbiol Infect. 20:O818-O824.

Ma LC, Fang CT, Lee CZ, *et al*., 2005. Genomic heterogeneity in *Klebsiella pneumoniae* strains is associated with primary pyogenic liver abscess and metastatic infection. J Infect Dis 192:117-28.

McQuillan JS, Infante HG, Stokes E, *et al*., 2012. Silver nanoparticle enhanced silver ion stress response in *Escherichia coli* K12. Nanotoxicology 6 :857-866.

Mulani MS, Kamble EE, Kumkar SN, *et al* ,2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance:a review. Front Microbiol 10:539.

NCBI, 2020. Available at:https://pubmed.ncbi.nlm.nih.gov/?termanti bacterial&filteryears.2017-2021&sortdate

Paredes D, Ortiz C and Torres R, 2014. Synthesis, characterization, and evaluation of antibacterial effect of Ag nanoparticles against *Escherichia Coli* O157:H7 and methicillin-resistant *Staphylococcus aureus* (MRSA). Int J Nanomed 9:1717-1729.

Priyanka A, Vijaya KD, Anusha R, *et al*., 2022. Biofilm formation and associated gene expression in multidrug-resistant *Klebsiella pneumoniae* isolated from clinical specimens. Curr Microbiol 79:73

Rodriguez-Bano J, Gutierrez-Gutierrez B, Machuca I, *et al*., 2018. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and Carbapenemase-producing *Enterobacteriaceae*. Clin Microbiol 14; 31e00079-17. doi:10.1128/CMR.00079-17.

Shadkam S, Goli H, Mirzaei B, *et al*., 2021**.** Correlation between antimicrobial resistance and biofilm formation capability among *Klebsiella pneumoniae* strains isolated from hospitalized patients in Iran. Ann Clin Microbiol Antimicrob 20:13.

Shah RK, Ni ZH, Sun XY, *et al*., 2017. The determination and correlation of various *virulence genes*, ESBL, serum bactericidal effect and biofilm formation of clinical isolated classical *klebsiella pneumoniae* and hypervirulent *klebsiella pneumoniae* from respiratory tract infected patients. Pol J Microbiol 66:501-508.

Shakib P, Kalani M, Ramazanzadeh R, *et al*., 2018. Molecular detection of virulence genes in *Klebsiella Pneumoniae* clinical isolates from Kurdistan Province, Iran. Biomed Res 5:2581-2589.

Silva AAD, Villalobos EMC, Cunha EMS, *et al*., 2020. Causes of equine abortion, stillbirth, and perinatal mortality in Brazil. Arq Inst Biol **https://** doi. org/ 10. 1590/ 1808- 16570 00092 020

Slavin YN, Asnis J, Häfeli UO, *et al*., 2017. Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. J Nanobiotechnol 15:65.

Soren S, Kumar S, Mishra S, *et al*., 2018. Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method. Microb Pathog 119:145-151.

Talebian N, Amininezhad SM, Doudi M, *et al*., 2013. Controllable synthesis of ZnO nanoparticles and their morphology-dependent antibacterial and optical properties. J Photochem Photobiol 66-73.

Tamayo LA, Zapata PA, Vejar ND, *et al*., 2014. Release of silver and copper nanoparticles from polyethylene nanocomposites and their penetration into *Listeria monocytogenes*. Mater Sci Eng. C Mater Biol Appl, 40:24-31.

Trigo da Roza F, Couto N, Carneiro C, *et al*., 2019. Commonality of multidrug-resistant *Klebsiella pneumoniae* ST348 isolates in horses and humans in Portugal. Front Microbiol 18:10:1657.

Van Spijk JN, Schmitt S and Schoster A, 2019. Infections caused by multidrug resistant bacteria in an equine hospital (2012–2015). Equine Vet Educ 31:653–658.

Wang H, Li X and Liu BT, 2021. Occurrence and characterization of KPC-2-producing ST11 *Klebsiella pneumoniae* isolate and NDM-5-producing *Escherichia coli* isolate from the same horse of equestrian clubs in China. Trans bound Emerg Dis 68:224–232.

Wang ZL, 2004. Zinc oxide nanostructures: growth, properties and applications, J Phys Condens Matter 16:R829–R858, https://doi.org/ 10.1088/0953

Wang Z, Li H, Tang F, *et al*., 2018. A facile approach for the preparation of Nano-size Zinc Oxide in Water/Glycerol with extremely concentrated zinc sources. Nanoscale Res Lett 13 :202.

Wareth G, Sprague LD, Neubauer H, *et al*., 2021. *Klebsiella pneumoniae* in Germany:an overview on spatiotemporal distribution and resistance development in humans. Ger J Microbiol **https://** doi. org/ 10. 51585/gjm. 2021. 0004

World Health Organization (WHO) 2020. Antimicrobial resistance. https:// www.who. int/ news- room/ fact- sheets/ detail/ antimicrobial- resistance

Yang H, Liu C, Yang D, *et al*., 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. J Appl Toxicol 29:69–78.

Yousefi M, Dadashpour M, Hejazi M, *et al*., 2017. Anti-bacterial activity of graphene oxide as a new weapon nanomaterial to combat multidrug-resistance bacteria. Mater Sci Eng C Mater Biol Appl 74:568–81.