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Application of nano zinc in controlling of multi drug resistant pathogenic Escherichia coli

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Abstract: Multi drug resistance (MDR) is becoming a global problem for public health which threatens the lives of hospitalized individuals as well as increases health care costs and long-time treatment. Further spread of the antimicrobial resistance occurs owing to misapplication of antimicrobial medications and hurries the development of drug-resistant strains. Zinc oxide nano particles (ZnO NPs) are used as alternative antibacterial agent for controlling MDR pathogenic E. coli. About 75 clinical E. coli isolates were collected from patients admitted to Mansoura University Hospitals and Mansoura Insurance Hospital. Antibiotic susceptibility pattern was tested by the disc diffusion method. We selected only 40 MDR isolates. All 40 MDR isolates were tested for the effect of different concentrations of ZnO NPs alone as an antibacterial agent and the combined effect of antibiotics and ZnO NPs as an antibacterial agent by the agar well diffusion method. E. coli isolates showed the highest resistance to azteronam, cefipime, cefotaxime, amoxicillin clavulanic acid, ciprofloxacin and cefaclor with (100%) followed by piperacillin tazobactam with (95%), followed by norfloxacin, tetracycline and sulphamethoxazole trimethoprim with (92.5%), Of all concentrations of ZnO NPs, the stock (4.176g/l) was the most active one with inhibition zones diameter ranged between (14-26mm) and the second concentration (1.392 g/l) caused inhibition zones diameter ranged between (14-24mm), followed by (0.464, 0.154, 0.051, 0.017, 0.0057 g/l) respectively. The antibacterial activity of antibiotics such as meropenem, piperacillin tazobactam, cefipime, amoxicillin clavulanic acid, ciprofloxacin, gentamycin, amikacin, azteronam, nitrofurantoin, sulphamethoxazole trimethoprim, cefaclor, tetracyclin and norfloxacin respectively against E. coli was increased considerably in the presence of ZnO NPs while the antibacterial activity of cefotaxime was not increased considerably in the presence of ZnO NPs**.**

keywords: Multi drug resistance (MDR), *Escherichia coli (E. coli)* and Zinc oxide nanoparticles (ZnO NPs)

1.Introduction

Escherichia coli (*E. coli*) is a common constituent of the gastrointestinal flora of most vertebrates, including humans, and may be isolated from a variety of environmental sources. While most strains are nonpathogenic, certain ones can cause a variety of intestinal and extra-intestinal infections **(1)**.

E coli are Gram-negative bacteria, which belong to the family *Enterobacteriaceae* and are considered the most prevalent infecting organism **(2)**. Certain strains of *E. coli* were responsible for infant diarrhea and gastroenteritis **(3)**. In addition, it is a facultative anaerobe, motile, non-sporulating, lactose fermenter and oxidase negative rode **(4)**. Normally originate in the inferior intestine of warm-blooded organisms. The greatest *E. coli* strains are inoffensive, it is a part of the normal flora of the gut, which can advantage their hosts by producing vitamin K2 and stopping the formation of pathogenic bacteria in the gut but some serotypes can reason undecorated food exterminating in humans **(5)**. Some *E. coli* are useful, although certain reason infections other than intestinal infections, such as urinary tract infections **(6)**, Pneumonia, septicemia **(7)**,

anemia, kidney failure and neonatal meningitis. Symptoms of these diseases include abdominal cramps, fever, hemorrhagic colitis, vomiting and diarrhea, which may be bloody **(8)**. Multi-drug resistant (MDR) is a bacterium growing in presence of several drugs or carrying several resistances genes **(9)**. MDR in Gram-negative bacteria, chiefly *Enterobacteriaceae* such as *E. coli*, has converted one of the major global concerns. Infections with these bacteria lead to prolonged hospital charges and higher mortality rates **(10)**. Added banquet of the antimicrobial resistance occurs due to misuse of antimicrobial drugs and accelerates the emergence of drug-resistant strains. Also, poor infection control practices, inadequate sanitary conditions and inappropriate food handling participate in spreading resistance **(11)**. Newly, nanotechnology has developed progressively central in the biomedical and salutary areas as alternate antimicrobial policy due to recurrence infectious diseases and the entrance of antibiotic-resistant strains particularly within Gram-negative microorganisms **(12)**. Nanoparticles (NPs) are classically no superior than 100 nm in scope and their biocidal efficiency is recommended to be owed to a grouping of their minor size and tall surface-to-volume ratio, which enable intimate connections with microbial tissues **(13. 14. 15 and 16)**. Finally, due to the problem of MDR strains of *E. coli* we need to find an alternative safe agent for controlling these strains so, we examine the susceptibility of these strains to ZnO NPs and examine combination between antibiotics and ZnO NPs for controlling these strains.

2.Materials and methods

Collection of samples and identification of *E. coli*:

Medical samples (urine, blood, sputum, wounds) were collected from patients who were admitted to different Diagnostic Departments of Mansoura University Hospitals and Mansoura Insurance Hospital (Specialized medical, Convalesce and critical care, Pediatric and Emergency). These tasters were cultured using the standard media (CLED agar for urine samples, Blood and MacConkey`s agar for blood, sputum and wound swabs) and incubated aerobically at 37^{o}C overnight. The identification of *Escherichia coli* isolates was done by colony morphology, microscopic examination after Gram staining, and biochemical tests including Kliger Iron Agar(KIA), Lysine Iron Agar (LIA), Motility Indole Ornithine medium (MIO), Urease and Citrate utilization tests **(17)**.

Antibiotic susceptibility test:

Antibiotic liabilities of *E. coli* isolates were done by Kirby–Bauer disc diffusion method **(18)** using Muller Hinton agar. The antibiotics tested were gentamicin (10 µg), amikacin (30 µg) from aminoglycosides, norfluxacin (10 µg), ciprofloxacin (5 µg) which belong to 1st and 2nd generation of quinolones respectively, azteronam (30 µg) which belongs to monobactam, cefipime (30 µg), cefotaxime (30 µg) and cefaclor (30 µg), which belong to 4th, 3rd and 2nd generations of cephalosporines respectively, meropenem (10 µg) which belongs to carbapenems, which belong to penicillins, these are B-lactams, tetracycline (30 µg) which belongs to tetracyclines, sulphamethoxazole trimethoprime (25 µg) which belongs to sulphonamides and nitrofurantoin (300 µg) **(19)**

Preparation of ZnO NPs:

ZnO NPs dispersion was prepared from (SIGMA –ALDARICH)

Characterization of ZnO NPs:

Product name: Zinc oxide, dispersion-nanoparticles

Color: Off- White to Tan

Form: Dispersion

Size: <100 nm particle size (TEM)

Average Particle Size(APS): ≤ 40 n m

pH: 7.5 ± 1.5

Concentration ZnO %: 20 wt.% in H2O

Density: 1.7g/ml ±0.1 g/ml at 25°C

Antibacterial activity of ZnO NPs

After the preparation of different concentrations of ZnO NPs, the antibacterial activity of ZnO NPs against MDR *E. coli* isolates was determined by using the agar well diffusion method according to **(20)**. Muller Hinton Agar medium was prepared and inoculated with MDR *E. coli* suspension by streaking the sterile non-toxic cotton swab pre-

moistened with *E. coli* suspension in three directions over the entire surface of the agar plates to obtain a uniform inoculum. The density of the *E. coli* suspension was equivalent to that of 0.5 MacFarland standard (1.5 x 10^8 CFU/mL). A sterile cork borer was used to make wells of 6mm in diameter in the agar plate. 100 µl of each concentration of ZnO NPs were introduced into each well using a sterile Pasteur pipette and allowed to stand for 1 hour at room temperature to diffuse the dispersion into the medium. The sterilized water was used in the same manner as negative control which did not affect the growth of microorganisms. The dishes were then hatched at 37ºC for 18-24 h. After cultivation, the entire distance of the inhibition zone was measured in three different directions on all 3 replicates and the average value was tabulated.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ZnO NPs:

MIC and MBC were dogged by a method suggested in **(21)** with some modifications. Temporarily, the sterile tubes were gestated aerobically at 37°C for 24 h, which contained 5 ml Muller-Hinton (MH) broth (Difco, USA) with ballpark 5×10^9 CFU bacterial cells and 0 (the control group), 4.176, 1.392, 0.464, 0.154, 0.051and 0.017g/l ZnO NPs. The attentiveness of the tube without observable growth of the bacterial cells was the MIC. To appraise the MBC, 100 µl of sample from each tube without visible growth was transported into MH agar plate (Oxoid, UK), and then raised aerobically for another 24 h. The attention of the tube without growth was the MBC (in this test, the populace in agar plate less than 10 was regarded as no growth). All the measures were triplicate.

Determination of colony forming unit (CFU):

Cubicles (1×10^8 CFU/ml) were gathered from exponentially grown cultures (MDR *E. coli* isolates). The cells were frozen with ZnO NPs in increasing attentions from 0.0057 to 4.176 g/l and both the treated and untreated cells were then gilded on nutrient agar saucers to scrutinize the colony founding skill. Plates were gestated overnight at 37°C and the colonies tallied. All experiments were appreciated in triplicate and modes were obtained **(23)**.

Characterization of antibacterial activity of ZnO NPs by Transmission Electron Microscope (TEM):

Steps were done in Electron Microscope Unit in Faculty of Agriculture, Mansoura University.

According to **(24)** *Escherichia coli* O157:H7 cultures, untreated or frozen with ZnO NP were reared overnight at 37C and then placed into the primary fixative and microwaved (MW) under vacuum conditions in a Pelco Biowave (Ted Pella, Inc.,

Determination of the combined effect of antibiotics and ZnO NPs against MDR *E. coli* :

Briefly, according to **(25)** Muller Hinton Agar medium was equipped and immunized with MDR *E. coli* strains by flashing the sterile non-toxic cotton swab pre moisturized with *E. coli* postponement in three guidelines over the entire surface of the agar plates to obtain an unchanging inoculum. The thickness of the *E. coli* suspension was equal to that of 0.5 MacFarland standard (1.5 x 10^8 CFU/mL). A sterile cork borer was used to make wells of 6mm in width in the agar plate (the number of wells depends on the number of resilient antibiotic discs for each strain). After the knowledge of each strain with a plate, put resistant antibiotic discs on wells (one on each well) using sterilized forceps, then pour 100 µl of concentration no.4 (0.154g/l) (as median concentration which causes inhibition for *E. coli* isolates) on each antibiotic disc, was presented into each well using sterile Pasteur tube and permissible to stand for 1 hour at room temperature to diffuse the dispersion into the medium. The plates were then gestated at 37ºC for 18-24 h. After incubation, the entire diameter of the inhibition zone was unhurried in three different guidelines on all 3 replicates and the normal value was presented.

3.Results

***E. coli* isolates:**

In this study, forty clinical *E. coli* isolates were obtained from patients admitted to different Mansoura University Hospitals and

Mansoura Insurance Hospital. Each sample was cultured on a specific medium. Results recorded in **Table (1)** shows that urine samples were the commonest samples giving positive *E. coli* growth while sputum gave the lowest number of *E. coli.*

Table (1): Distribution of *E. coli* strains among different clinical samples.

Sample	Number of *E. coli* Total = 40	Percentage (%)
Urine	17	42.5%
Blood	12	30%
Wound	6	15%
Sputum	5	12.5%
Total	40	100%

Table (2) shows that the clinical *E. coli* isolates include 22 (55%) isolates from females and 18 (45%) isolates from males.

Table (2): Percentage of clinical samples collected from different genders

Samples	Gender				Total	
	Female		Male			
	No.	%	No.	%	No.	%
Urine	12	70.5%	5	29.4%	17	**42.5%**
Blood	5	41.6%	7	58.3%	12	**30%**
Wound sab	3	50%	3	50%	6	**15%**
Sputum	2	40%	3	60%	5	**12.5%**
Total	**22**	**55%**	**18**	**45%**	**40**	**100%**

Response of *E. coli* against different antibiotics:

Forty *E. coli* isolates, recorded resistance to most of antibiotics that were tested for susceptibility as shown in **Photo (1)** and **Table (3)** which showed that the maximum zones of inhibition observed for meropenem was 36,33,32,30and 28 mm in diameter with *E. coli* no. 19,18,12,1,10 and14 respectively. followed by gentamycin which yields an inhibition zone of 26 mm in diameter with *E. coli* no. 13.

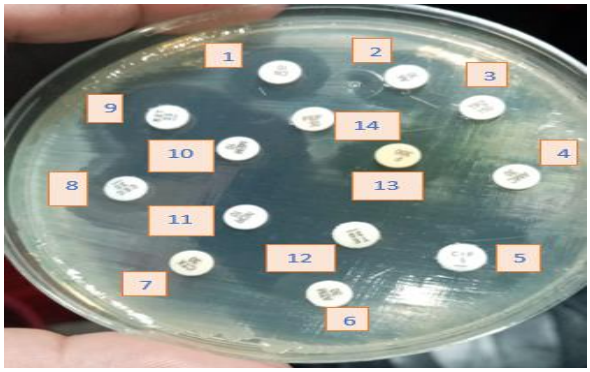

Photo (1) Antibiotic susceptibility test of E. coli isolate on Muller Hinton agar as (1)Gentamycin,(2)Amikacin,(3)Piperacyllin tazobactam,(4) Amoxycillin clavulanic acid ,(5) Ciprofloxacin,(6)Azteronam,(7) Cefotaxime, (8) Cefaclor,(9) Sulphamethoxazole trimethoprim,(10) Meropenem, (11) Norfloxacin,(12) Tetracyclin,(13) Nitrofurantoin and (14)Cefipime

Table (3): Response of the tested clinical *E. coli* isolates against different antibiotics

S. No.	S. T.	CN 10µg	AK 30 µg	ATM 30µg	FEP 30µg	CTX 30µg	TZP 110µg	AMC 30µg	MEM 10µg	NOR 10µg	CIP 5µg	SXT 25µg	TE 30µg	F 300µg	CEC 30µg
1	U	20(S)	20 (S)	0 ®	0 ®	0 ®	0 ®	0 ®	30 (S)	0 ®	0 ®	0 ®	0 ®	15 (I)	0®
2	U	0 ®	15 (I)	0 ®	0 ®	0 ®	0 ®	0 ®	24 (S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
3	B	0 ®	10®	0 ®	0 ®	0 ®	12®	0 ®	20 (I)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
4	B	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	12®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
5	S	0 ®	18(S)	0 ®	14 ®	0 ®	12®	0 ®	20 (I)	0 ®	0 ®	0 ®	0 ®	12®	0 ®
6	B	10®	12®	0 ®	0 ®	0 ®	0 ®	0 ®	20 (I)	10 ®	0 ®	0 ®	0 ®	10 ®	0 ®
7	U	0 ®	0 ®	0 ®	0 ®	10®	0 ®	0 ®	12®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
8	W	0 ®	20(S)	0 ®	0 ®	0 ®	0 ®	0 ®	16 ®	0 ®	0 ®	0 ®	0 ®	15(I)	0 ®
9	B	16(S)	15(I)	0 ®	10 ®	0 ®	19 ®	0 ®	23 (S)	20 (S)	20 ®	0 ®	0 ®	15(I)	0 ®
10	U	0 ®	20(S)	10 ®	15 ®	0 ®	14 ®	0 ®	30 (S)	0 ®	0 ®	0 ®	0 ®	10 ®	0 ®
11	S	20(S)	14®	0 ®	18 ®	0 ®	20 ®	0 ®	18®	0 ®	0 ®	0 ®	0 ®	18 (S)	0 ®
12	U	20(S)	18(S)	0 ®	0 ®	0 ®	20 ®	0 ®	32 (S)	0 ®	0 ®	0 ®	0 ®	20 (S)	0 ®
13	U	26(S)	24(S)	0 ®	18 ®	0 ®	20 ®	0 ®	24(S)	16 (I)	0 ®	0 ®	0 ®	22 (S)	0 ®
14	W	14(I)	24(S)	12 ®	18 ®	0 ®	25(S)	0 ®	28(S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
15	U	10®	24(S)	0 ®	0 ®	0 ®	14 ®	0 ®	24(S)	0 ®	0 ®	0 ®	0 ®	15 (I)	0 ®
16	U	14(I)	20(S)	0 ®	0 ®	0 ®	0 ®	0 ®	22 (I)	0 ®	0 ®	20(S)	0 ®	14 ®	0 ®
17	U	0 ®	15(I)	0 ®	0 ®	0 ®	0 ®	0 ®	23 (S)	0 ®	0 ®	20(S)	20 s	15 (I)	0 ®
18	B	10®	25(S)	0 ®	0 ®	0 ®	20 ®	0 ®	33(S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
19	U	0 ®	15(I)	0 ®	12 ®	0 ®	20 ®	0 ®	36(S)	0 ®	0 ®	0 ®	24 s	20(S)	0 ®
20	U	0 ®	20(S)	0 ®	14 ®	0 ®	20 ®	0 ®	22 (I)	0 ®	0 ®	0 ®	18 s	0 ®	0 ®
21	U	20(S)	20(S)	0 ®	0 ®	0 ®	20 ®	0 ®	25 (S)	0 ®	0 ®	0 ®	0 ®	15 (I)	0 ®
22	S	12®	15(I)	0 ®	0 ®	0 ®	0 ®	0 ®	18 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
23	W	0 ®	18(S)	0 ®	0 ®	0 ®	0 ®	0 ®	22 (I)	0 ®	0 ®	0 ®	0 ®	12 ®	0 ®
24	U	15(S)	18(S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	14 ®	0 ®
25	S	12®	18(S)	0 ®	0 ®	0 ®	18 ®	0 ®	22 (I)	0 ®	0 ®	0 ®	0 ®	14 ®	0 ®
26	B	12®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	12 ®	0 ®
27	U	12®	17(S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	14 ®	0 ®
28	B	14(I)	17(S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
29	B	0 ®	20(S)	0 ®	0 ®	0 ®	18 ®	0 ®	24 (S)	0 ®	0 ®	0 ®	0 ®	11 ®	0 ®
30	W	14(I)	18(S)	12 ®	12 ®	0 ®	18 ®	0 ®	22 (I)	0 ®	0 ®	0 ®	0 ®	18(S)	0 ®
31	B	14(I)	18(S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
32	W	14(I)	14 ®	0 ®	0 ®	0 ®	0 ®	0 ®	16 ®	0 ®	0 ®	0 ®	0 ®	15(I)	0 ®
33	B	0 ®	14 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®
34	U	14(I)	16(I)	10®	0 ®	0 ®	14®	0 ®	20 (I)	0 ®	0 ®	0 ®	0 ®	15(I)	0 ®
35	U	14(I)	15(I)	0 ®	0 ®	0 ®	18 ®	0 ®	22 (I)	0 ®	0 ®	0 ®	0 ®	20(S)	0 ®
36	B	0 ®	16(I)	0 ®	0 ®	0 ®	18 ®	0 ®	22 (I)	16(I)	18 ®	20(S)	0 ®	14 ®	0 ®
37	W	13®	16(I)	0 ®	0 ®	0 ®	0 ®	0 ®	24(S)	0 ®	0 ®	0 ®	0 ®	12 ®	0 ®
38	S	0 ®	0 ®	12 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	12 ®
39	U	12®	12®	12 ®	0 ®	0 ®	12 ®	0 ®	18 ®	0 ®	0 ®	0 ®	0 ®	14 ®	0 ®
40	B	15(S)	18(S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	18(S)	0 ®

.No. ⇒Sample number, S.T.⇒Sample type, U ⇒ Urine , B ⇒ Blood , S ⇒ Sputum , W ⇒ Wound , (S) ⇒Sensitive , (I) ⇒ Intermediate , ® ⇒ Resistant ,CN ⇒ Gentamycin , AK ⇒ Amikacin , FEP ⇒ Cefipime ,CIP ⇒ Ciprofloxacin , ATM ⇒ Azteronam , NOR ⇒ Norfloxacin ,CTX ⇒ Cefotaxime , TPZ ⇒ Piperacillin Tazobactam ,AMC ⇒ Amoxicillin Clavulanic acid , CEC ⇒ Cefaclor , F ⇒ Nitrofurantoin , TE ⇒ Tetracycline , SXT ⇒ Sulphamethoxazole Trimethoprime , MEM ⇒ Meropenem

Antibacterial activity of ZnO NPs against clinical *E. coli* isolates:

In the current study, 10 concentrations of ZnO NPs were screened for their antibacterial activity against clinical *E. coli* by the agar well diffusion method. The diameter of the inhibition zones of different concentrations of ZnO NPs was tabulated in **Table (4)** and shown in **Photo (2).** Of all concentrations of ZnO NPs, the stock with a concentration 4.176 g/l was the most active one with inhibition zones diameter ranging between 14mm-26mm and the second with a concentration 1.392 g/l caused inhibition zones diameter ranging between 12mm-24mm and so on. *E. coli* was more resistant to 0.0019 g/l, 0.00063 g/l and 0.00021 g/l. The results indicated that there was a decrease in the growth of *E. coli* with an increase in ZnO NPs concentration and vice versa.

Table (4): Antimicrobial activity of different concentrations of ZnO NPs against clinical *E. coli* isolates

	Diameter of inhibition zone (mm) of different concentrations of ZnO NPs											
NO.	S.T.	4.176	1.392	0.464	0.154	0.051	0.017	0.0057	0.0019	0.00063	0.00021	C
1	U	20	19	17	14	12	10	R	R	R	R	R
2	U	22	20	16	12	R	R	R	R	R	R	R
3	B	16	15	14	12	10	R	R	R	R	R	R
4	B	18	17	16	15	12	12	10	R	R	R	R
5	S	18	17	15	12	10	R	R	R	R	R	R
6	B	17	16	15	13	12	10	10	R	R	R	R
7	U	15	14	12	11	10	10	R	R	R	R	R
8	W	20	19	18	16	15	12	10	R	R	R	R
9	B	20	18	16	15	14	12	10	R	R	R	R
10	U	25	24	20	19	16	12	R	R	R	R	R
11	S	26	24	22	20	14	12	R	R	R	R	R
12	U	23	22	19	16	10	R	R	R	R	R	R
13	U	22	20	18	16	13	10	R	R	R	R	R
14	W	24	22	20	18	12	10	R	R	R	R	R
15	U	24	22	20	18	12	10	R	R	R	R	R
16	U	18	16	13	12	R	R	R	R	R	R	R
17	U	24	22	19	18	12	10	R	R	R	R	R
18	B	24	20	18	16	12	10	R	R	R	R	R
19	U	24	23	21	18	10	R	R	R	R	R	R
20	U	22	21	20	19	18	16	15	R	R	R	R
21	U	22	20	18	16	10	R	R	R	R	R	R
22	S	14	13	12	11	11	R	R	R	R	R	R
23	W	15	14	13	12	R	R	R	R	R	R	R
24	U	17	16	15	14	13	11	R	R	R	R	R
25	S	18	16	15	14	13	12	11	R	R	R	R
26	B	16	15	14	13	11	R	R	R	R	R	R
27	U	18	16	15	14	13	11	R	R	R	R	R
28	B	16	15	14	13	13	12	11	R	R	R	R
29	B	14	12	R	R	R	R	R	R	R	R	R
30	W	16	15	13	11	R	R	R	R	R	R	R
31	B	17	16	15	14	13	12	11	R	R	R	R
32	W	16	15	14	13	11	R	R	R	R	R	R
33	B	14	13	12	11	R	R	R	R	R	R	R
34	U	18	16	15	14	R	R	R	R	R	R	R
35	U	16	15	14	12	R	R	R	R	R	R	R
36	B	16	15	13	12	R	R	R	R	R	R	R
37	W	15	14	13	12	R	R	R	R	R	R	R
38	S	18	16	15	14	R	R	R	R	R	R	R
39	U	17	16	14	13	R	R	R	R	R	R	R
40	B	16	15	14	12	R	R	R	R	R	R	R

NO. ⇒ Number of resistant *E. coli* isolates , S.T. ⇒ Sample type , U ⇒ Urine , B ⇒ Blood , W ⇒ Wound , S ⇒ Sputum

Table (5): Comparison between the activity of antibiotics and different concentrations of ZnO NPs against MDR *E. coli* isolates

E. coli isolates No.	Diameter of inhibition zone(mm)																	
	S.T	CN 10µg	AK 30 µg	ATM 30µg	FEP 30µg	CTX 30µg	TZP 110µg	AMC 30µg	MEM 10µg	NOR 10µg	CIP 5µg	SXT 25µg	TE 30µg	F 300µg	CEC 30µg	4.176g/l ZnO NP	1.392 g/l ZnO NP	0.0464 g/l ZnO NP
2	U	0 ®	15 (I)	0 ®	0 ®	0 ®	0 ®	0 ®	24 (S)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	22	20	16
3	B	0 ®	10®	0 ®	0 ®	0 ®	12®	0 ®	20 (I)	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	16	15	14
4	B	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	12®	0 ®	0 ®	0 ®	0 ®	0 ®	0 ®	18	17	16
5	S	0 ®	18(S)	0 ®	14 ®	0 ®	12®	0 ®	20 (I)	0 ®	0 ®	0 ®	0 ®	12®	0 ®	18	17	15
6	B	10®	12®	0 ®	0 ®	0 ®	0 ®	0 ®	20 (I)	10 ®	0 ®	0 ®	0 ®	10 ®	0 ®	17	16	15

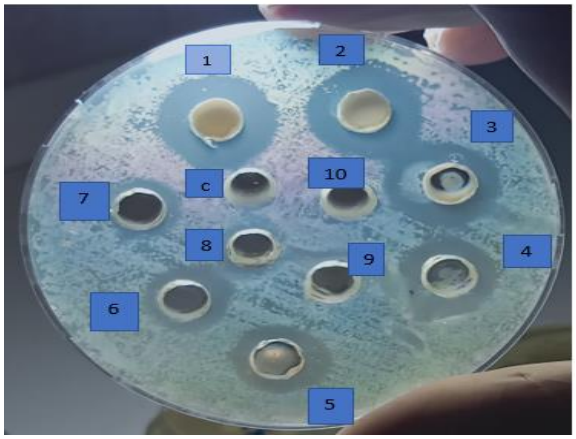

Photo (2) Inhibition zones of different concentrations ((1)4.176, (2)1.392, (3)0.464, (4)0.154, (5)0.051, (6)0.017, (7)0.0057, (8)0.0019, (9)0.00063, (10)0.00021g/l and ©control(dist.H2O)) of ZnO NPs against clinical *E. coli* isolates

As shown in **Table (5)** even some concentrations of ZnO NPs showed good activity against resistant *E. coli* strains whilst modern antibiotic therapy has limited effect

Minimum inhibitory concentration (MIC) of the effective ZnO NPs against clinical *E. coli* isolates:

The most effective ZnO NP concentration was the first and second concentrations against MDR *E. coli* isolates. The value of MIC was determined by the lowest concentration required to arrest the growth of the bacteria at the end of 24 h of incubation. The results demonstrated that MIC was 0.051g/l as shown in Photo (3) and Photo (4). As (MBC) endpoint is defined as the lowest concentration of antimicrobial agent that kills 100% of the initial bacterial population.

The results demonstrated that MBC was 1.392 g/l as shown in Photo (5).

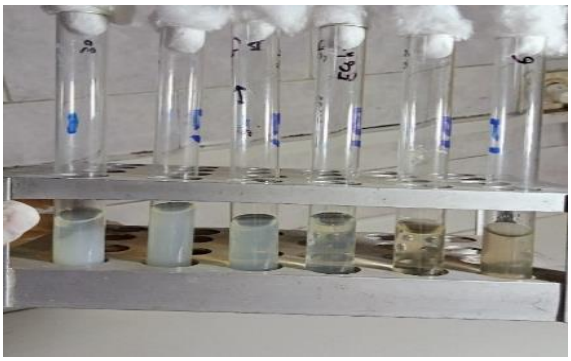

Photo (3): Determination of minimum inhibitory concentration (MIC) of ZnO NPs by nutrient broth media as (1)4.176, (2)1.392, (3)0.464, (4)0.154, (5)0.051 and (6)0.017g/these concentrations were mixed with nutrient broth media

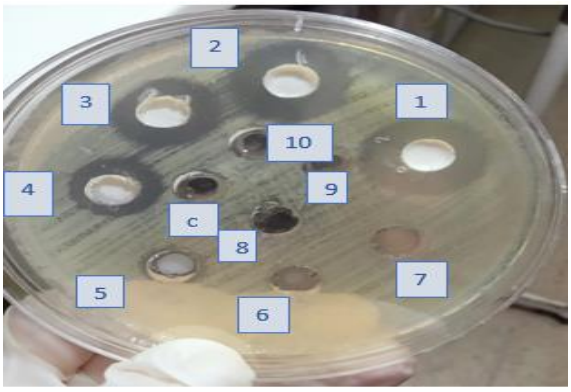

Photo (4): Determination of minimum inhibitory concentration (MIC) of ZnO NPs on Muller Hinton Agar (MHA) as ((1)4.176, (2)1.392, (3)0.464, (4)0.154, (5)0.051, (6)0.017, (7)0.0057, (8)0.0019, (9)0.00063, (10)0.00021g/l and ©control(dist.H2O)) of ZnO NPs

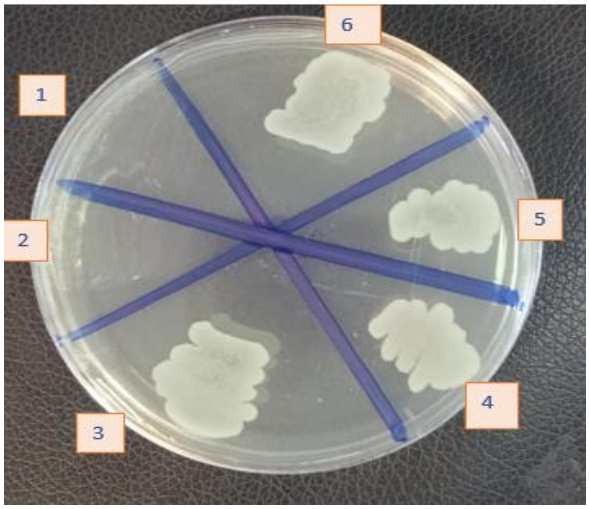

Photo (5): Determination of minimum bactericidal concentration (MBC) of ZnO NPs on Muller Hinton Agar (MHA)as ((1)4.176, (2)1.392, (3)0.464, (4)0.154, (5)0.051and (6)0.017g/l of ZnO NPs mixed with bacterial suspension of *E. coli* isolate

Determination of colony forming unit (CFU):

The number of CFU of *E. coli* after overnight incubation in the presence of different concentrations of ZnO NPs was shown in Figure (1). The numbers of CFU has been observed to reduce significantly with increasing concentrations of ZnO NPs in *E*

coli .

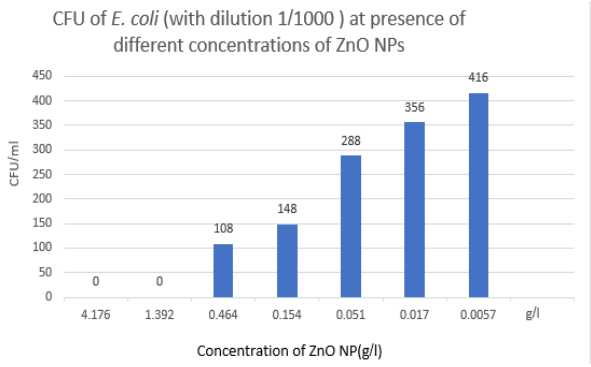

Fig (1): Colony forming unit (CFU) of *E. coli* (with 1/1000 dilution) in the presence of different concentrations of ZnO NP

Effect of ZnO NPs on MDR *E. coli* isolates represented by Transmission Electron Microscope (TEM):

The contrast between Photo (6) which signify *E. coli* cells grown up in the regulator (ZnO NP -free media) and Photo (7) which signify *E. coli* cells grown in the media covering ZnO NPs showed that ZnO NP slanted & spoiled the microbial cell sheath, subsequent in seepage of cytoplasmic insides, while the external entrance of the bacterial cell was hardly exaggerated. This established that ZnO NP transformed the cell membrane machinery with lipids and proteins, while no significant morphological deviations were experimental**.**

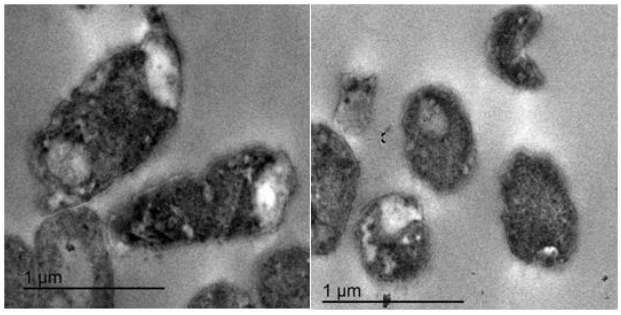

Photo (6) TEM images of *Escherichia coli* cells grown in the control (ZnO NP-free media).

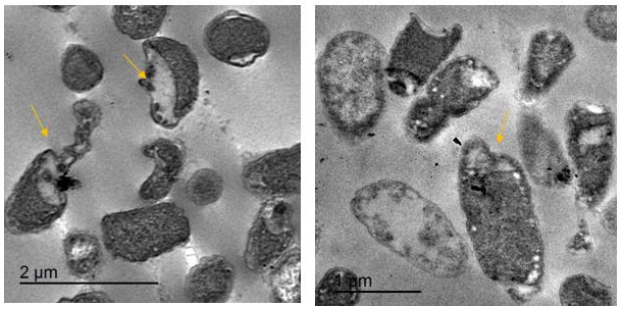

Photo (7) TEM images of *Escherichia coli* cells grown in the media containing ZnO NPs. Arrows indicate the leakage of intracellular contents and disorganization of cell membranes.

Combination effect of antibiotics and ZnO NPs on *E. coli*:

The results in Table (6) and Photo (8) determined that, the antibacterial activity of antibiotics such as meropenem, piperacillin tazobactam, cefipime ,amoxicillin clavulanic acid, ciprofloxacin, gentamycin, amikacin, azteronam, nitrofurantoin, sulphamethoxazole trimethoprim, cefaclor, tetracyclin and norfloxacin respectively against *E. coli* was increased considerably in the presence of ZnO NPs; therefore, the combination of ZnO NPs with these antibiotics can be recommended for possible combination therapy. On the other hand, the antibacterial activity of cefotaxime was not increased considerably in the presence of ZnO NPs; therefore, the combination of ZnO NPs with this antibiotic cannot be recommended for possible combination therapy.

Table (6): Response of clinical MDR isolates of *E. coli* against resistant antibiotics uploaded with concentration NO. (4) = 0.154 g/l of ZnO NPs

NO.	S.T.	CN	AK	ATM	FEP	CTX	TZP	AMC	MEM	NOR	CIP	SXT	F	CEC	TE
1	U	-	-	18	14	R	22	16	-	15	14	15	20	14	14
2	U	12	-	14	12	R	20	12	-	R	12	14	14	12	14
3	B	15	-	15	22	13	-	12	-	14	16	15	22	16	12
4	B	20	16	16	18	14	24	16	-	23	15	16	17	18	16
5	S	16	-	16	-	15	-	16	-	18	14	17	-	16	20
6	B	-	-	16	20	15	22	15	-	-	22	16	-	16	16
7	U	22	22	18	22	-	20	18	-	20	20	18	20	14	15
8	W	16	-	16	15	R	15	14	-	17	14	16	-	16	13
9	B	-	-	20	-	13	-	20	-	-	-	18	-	18	16
10	U	16	-	-	-	12	-	16	-	13	15	13	-	16	16
11	S	-	-	14	-	R	-	15	-	13	12	18	-	16	12
12	U	-	-	20	15	R	-	16	-	14	15	14	-	16	16
13	U	-	-	15	-	13	-	16	-	-	16	16	-	15	14
14	W	-	-	-	-	11	-	14	-	14	13	14	17	14	13
15	U	-	-	16	16	13	-	16	-	16	15	14	-	15	15
16	U	-	-	16	24	16	22	15	-	14	R	-	-	R	18
17	U	15	-	15	14	R	14	16	-	13	13	-	-	16	-
18	B	-	-	15	15	R	-	15	-	16	12	14	16	16	16
19	U	12	-	13	-	R	-	13	-	12	12	13	-	13	-
20	U	18	-	18	-	14	-	21	-	18	18	18	22	17	-
21	U	-	-	18	20	16	-	12	-	12	12	13	-	13	13
22	S	-	-	16	12	R	24	13	-	14	16	16	18	14	14
23	W	14	-	16	13	R	16	R	-	14	12	14	-	13	14
24	U	-	-	R	13	R	20	18	15	15	12	12	-	17	12
25	S	-	-	13	14	R	-	14	-	12	14	14	-	14	12
26	B	-	16	16	R	R	R	R	15	R	12	R	-	R	R
27	U	-	-	R	R	R	R	R	R	R	R	R	-	R	R
28	B	-	-	R	R	R	R	R	R	R	R	R	18	R	R
29	B	R	-	R	R	R	-	R	-	R	R	18	-	R	R
30	W	-	-	-	-	R	-	14	-	14	14	18	-	12	14
31	B	-	-	17	16	R	15	18	16	16	16	17	18	16	16
32	W	-	-	16	R	R	20	R	-	18	16	20	-	R	R

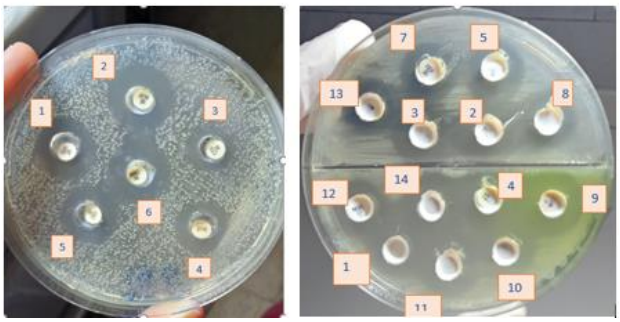

Photo (8) Combination effect of antibiotics and ZnO NPs on *E. coli* as (1) Sulphamethoxazole trimethoprime,

(2) Azteronam, (3) Cefotaxime, (4) Tetracycline, (5) Cefaclor, (6) Amoxycillin clavulanic acid, (7) Cefipime,

(8) Amikacin, (9) Nitrofurantoin, (10) Piperacillin tazobactam, (11) Ciprofloxacin, (12) Norfloxacin, (13) Gentamycin and (14) Meropenem (antibiotics uploaded with conc. No. (4)= 0.154g/l of ZnO NPs

Disscusion of the results:

Struggle to antibacterial mediators is exceedingly established in bacterial isolates worldwide, mainly in emerging realms. Normal colonic flora is a reservoir for fighting genes; the pervasiveness of resistance in *E. coli* is a useful indicator of antibiotic fight in bacteria in the community. In this study, 42.5 % of *E. coli* were isolated from urine samples in agreement with (26), 17 *E. coli* isolates from urine samples include 12 (70.5%) isolates from females, this was nearly in agreement with (27) and 5 (29.4%) isolates from males (28). The *E. coli* isolates were collected from different clinical specimens showed deferent degree of susceptibility to different antibiotics. *E. coli* were highly resistant to azteronam, cefipime, cefotaxime, amoxicillin clavulanic acid, ciprofloxacin and cefaclor with (100%)(these data were in disagreement with (29) who found that the highest resistance of *E. coli* was detected with ciprofloxacin (89%)) followed by piperacillin tazobactam with (95%), followed by norfloxacin, tetracycline and sulphamethoxazole trimethoprim with (92.5%), followed by Nitrofurantoin with (62.5%) ,followed by gentamycin with (60%) , followed by meropenem with (37.5%) and finally amikacin with (25%) . The prevalence and rate of resistance among pathogenic bacteria differ vastly based on geographical location and hospital type, but it is raising enough to be considered a health threat (26). In the present study, of all concentrations of ZnO NPs, the stock (4.176g/l) was the most active one with inhibition zones diameter ranged between (14-26mm) and the second concentration (1.392 g/l) caused inhibition zones diameter ranged between (14-24mm), followed by (0.464, 0.154, 0.051, 0.017, 0.0057 g/l) respectively. On the other hand, no antimicrobial activity was detected in concentrations (0.0019, 0.00063, 0.00021 g/l) against all forty clinical isolates of *E. coli* at the same dose (100µ). In this study, MDR *E. coli* strains were found sensitive to some of tested concentrations of ZnO NPs. This has clearly indicated that these concentrations might have different modes of action than that of antibiotics of tested isolates. It was found that the antibacterial activity of ZnO NPs increased with increasing powder concentration. As it was also shown in the study of (31) it has been seen in this study that by increasing the concentration of ZnO NPs in wells, the growth inhibition has also been increased. The size of inhibition zone was different according to the concentrations of ZnO NPs. Even some concentrations of ZnO NPs showed good activity against resistant *E. coli* strains whilst modern antibiotic therapy has limited effect. In this search, the numbers of CFU have been observed to reduce significantly with increasing concentrations of ZnO NPs in *E. coli*. The internal characteristics were demonstrated for *E. coli* isolate before and after treatment with ZnO NPs by Transmission Electron Microscope (TEM). The sheaths of the microbial cells were distorted and intracellular meetings were jumbled. Many null cells were creating in the bacterial samples conserved with ZnO NPs, demonstrating that the intracellular contents had trickled out of the cells owing to the injury and disorganization of the cell crust. The antimicrobial mechanism of ZnO NP was consequently thought to be supplementary to the eradication of lipids and proteins on cell casing by ZnO NP, producing a escape of intracellular contents as shown in TEM results, these results were in agreement with (32).Combination of antibiotics and synthetic organic or inorganic nanomaterials are of great importance. The antibacterial activity of antibiotics such as meropenem, piperacillin

tazobactam, cefipime, amoxicillin clavulanic acid, ciprofloxacin, gentamycin, amikacin, azteronam, nitrofurantoin, sulphamethoxazole trimethoprim, cefaclor, tetracyclin and norfloxacin respictevily against *E. coli* was increased considerably in the presence of ZnO NPs; therefore, the combination of ZnO NPs with these antibiotics can be recommended for possible combination therapy. On the other side, the antibacterial activity of cefotaxime was not increased considerably in the presence of ZnO NPs; therefore, the combination of ZnO NPs with this antibiotic cannot be recommended for possible combination therapy.

Conclusion

Multi drug resistant (MDR) is becoming a global problem for public health, which threatens the lives of hospitalized individuals as well as health care cost and long-time treatment. The uncontrolled use of antibiotics shares significantly to their resistance among *E. coli* isolates. Contrary to the synthetic drugs, some inorganic nanoparticles (NPs) are not associated with many side effects and have a great therapeutic potential to treatment of infectious caused by resistant microbes. Scientists have realized an immense potential in synthetic products from zinc oxide nanoparticles (ZnO NPs) to serve as substitute cause of opposing infections in human being which may also be of lower cost and less deadliness specially in controlling of MDR *E. coli* strains.

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