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The Emergence of MDR *Escherichia coli* Isolates in Baghdad Province and Attempts to Control These Isolates Using Nanoparticals

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Abstract: Nanotechnology offers new perspectives on the efficient treatment and control of disease caused by bacteria that are resistant to antibiotics. Various nanoparticle conjugates have shown wide-ranging antibacterial activity. The synthesis of copper-cobalt oxide (CuO–CoO) nanoparticles was achieved using a photo irradiation approach. The structure and crystallographic phase of the nanoparticles were determined using X-ray diffraction analysis (XRD). The size and shape of the product were examined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The antibacterial efficacy of CuO–CoO NPs was assessed against multidrug resistance (MDR), extensively drug-resistant (XDR), and potentially pans drug-resistant (PAN) microorganisms using the agar well diffusion technique. It was found that the average crystallite size of binary CuO-CoO NPs was 16 nm. The range of particle sizes shown in the (TEM) image is 25- 35 nm. Several agglomerated nanoparticles with various sizes caused by various metal oxide nanoparticles are apparent in the SEM image of the binary CuO-CoO NPs. The concentrations of nanoparticles were 10000 µg/ml, which exhibited the highest inhibitory impact against the MDR isolate, measuring 18 mm However, the least inhibitory effect against the XDR isolate was seen at a concentration of 10000 µg/ml, measuring 13 mm. The minimum inhibitory concentration MIC of CuO–CoO NPs was determined using the micro-dilution technique. The findings revealed that the (MIC) for Cuo-CoO NPs against the (MDR) isolate was 1250 µg/ml, but the MIC for the (XDR) isolate was 2500 µg/ml. The bacteria that were tested exhibited notable variations in their susceptibility to Cuo-CoO NPs the bacteria that displayed the highest resistance to Cuo-CoO NPs in this study were also resistant to all antibiotics (PAN). Conversely, the MDR strain demonstrated the highest sensitivity to Cuo-CoO NPs.

Keywords: Nanoparticles, Antimicrobial resistance, Antibacterial activity.

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Introduction

Uropathogenic *Escherichia coli* (UPEC) strains have been identified as the main etiological cause responsible for urinary tract infections (UTIs) in the human population (1). The overall prevalence of (UTIs) attributed to uropathogenic *E. coli* (UPEC) strains ranges from approximately 30% to 70% according to multiple sources (2, 3). (UTIs) caused by uropathogenic *E. coli* (UPEC) strains typically necessitates antibiotic treatment. The effective control and treatment of (UTIs) can be achieved through the accurate prescription of various groups of antibiotics, including beta-lactams, aminoglycosides, quinolones, sulfonamides, tetracyclines, penicillins, and cephalosporins. The emergence of antibiotic resistance among pathogenic organisms has become a global issue that significantly impacts the efficacy of treating infectious diseases (4).

The increased utilization and potential misapplication of antibiotics in the fields of human medicine, agriculture, and veterinary practices are the main factors contributing to this phenomenon. There has been a concerning rise in antibiotic resistance observed among bacteria responsible for both community-acquired infections and nosocomial infections. *E. coli*, a group of multidrug-resistant pathogens, is of particular interest (5, 6).

A particular type of UPEC can be classified as a Multi-Drug Resistant (MDR) bacterium if it exhibits resistance to a minimum of 3 distinct antibiotic classes. On the other hand, it would be categorized as an Extensively Drug Resistant (XDR) bacterium if it demonstrates sensitivity to solely 1 class of antibiotics (5) and PDR defined as non-susceptibility to all agents in all antibiotic classes (7). Uropathogenic *E. coli* (UPEC) isolates can acquire antimicrobial resistance through two mechanisms: DNA mutation or horizontal gene transfer (HGT). Mutations manifest in a spontaneously, exhibiting a variable frequency contingent upon the specific antibiotic and microorganism involved (8).

In certain cases, bacteria must undergo a gradual accumulation of mutations in order to acquire complete clinical resistance. This is observed, for instance, in the resistance to fluoroquinolones, where the inactivation of hydrolytic enzymes by β-lactamases and the alteration of permeability through active efflux pumps both contribute to the development of resistance (9).

Materials and methods Study subjects

An overall of 210 samples of urine were collected from UTI patients who were hospitalized at AL-Elwia_ Pediatrics.

Nanotechnology represents a promising strategy for the creation of innovative nontraditional antimicrobial agents known as nano-antibiotics. These nano-antibiotics offer an effective means of treating infectious diseases and possess numerous advantages over conventional antimicrobial agents. These advantages include the absence of adverse effects, enhanced efficacy against drug-resistant species, and the ability to impede the development of resistance by interfering with multiple biological pathways According to the research carried out by Kumar *et al.* (10).

The bactericidal mechanisms employed by these nanoparticles (NPs) encompass several processes. Firstly, they generate reactive oxygen species (ROS), including H2O2, $O2^{-}$, and OH^{-}. Additionally, they disrupt the membrane of bacterial cell walls. Furthermore, they inhibit DNA synthesis and intracellular enzyme activity. Lastly, they interfere with energy transduction (11).

The antibacterial efficacy of CuO nanoparticles, which were manufactured using co-doping, was effectively evaluated against several strains of multidrug-resistant bacteria. These strains included Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, as well as Gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa* (12).

The objective of this study is to identify the presence of (MDR) and (XDR) (PDR) UPEC strains that have been isolated from urinary tract infections in the Baghdad province of Iraq and the role of Nanotechnology against these resident isolates.

Teaching Hospital and Teaching Laboratories in Medical City between January and May 2021. The study

protocol was approved by the Ethics Committee of the Iraqi Ministry of Health and Environment, and written informed consent was obtained from all participants before entering the study.

The detection of UPEC was conducted using established microbiological and biochemical procedures (13). The Gram staining approach and other biochemical assays were primarily used to eliminate microorganisms other than

E. coli. The suspected cases were then validated using the VITEK® 2 system (bioM´erieux, Inc., Hazelwood, Mo., U.S.).

The isolates underwent a sensitivity test towards 15 antibiotics by using the Kirby-Bauer disc diffusion method on Muller-Hinton agar. The width of the zone of inhibition (ZOI) generated by every antibiotic disc was assessed and documented, and the isolates were categorized as "R, resistant", "I, intermediate", or "S, sensitive" based on ZOI in accordance with the recommendations set by the Clinical and Laboratory Standards Institute CLSI, (14).

The isolates exhibiting the resistance pattern of (MDR), (XDR), and (PDR) (isolates no.24, 25, and 1 respectively) and were subjected to nanoparticle exposure using the agar well diffusion method. Holder and Boyce (15) conducted assays to examine the antibacterial properties of Cuo-CoO NPs towards multi-drug resistant (MDR), extensively drug resistant (XDR), and pan-drug resistant (PDR) *E.coli* isolates. The first well was filled with 100 µL of Cuo-CoO-NPs (concentration 10000 µg/ml) while the second well was filled with 100 µL of Cuo-CoO-NPs (concentration 1000 µg/ml) the third well was filled with 100 µL of distilled water (D.W) as a negative control.

Antibiotic Susceptibility

The antimicrobial susceptibility of 15 antibiotics was assessed using agar disc diffusion. Antibiotic discs originated from Bioanalyse, a company based in Turkey. The antibiotic discs used in this study include Amikacin (10µg), Gentamicin (10µg), and Tobramycine (10µg), all of which are classified as aminoglycoside antibiotics.In addition, βeta-Lactam antibiotics such as Piperacillin (10µg), Augmentin (30µg), and Cephalosporins from the 3rd generation class comprising Cefotaxime (30µg), Ceftriaxone (30µg), and Ceftazidime (30µg).The fourth generation of cephalosporins includes Cefepime (10µg). The carbapenem class includes Meropenem (10µg) and Imipenem (10µg). Tetracyclines are represented by Tetracycline (30µg) and Doxycycline (30µg). The Fluroquinolone class consists of Ciprofloxacin (5µg) and Levofloxacine (5µg).

The standardized overnight cultures of every specimen were used to inoculate the melted Mueller-Hinton agar (MHA). The inoculated MHA was then placed onto sterilized plates in triplicate, following strict aseptic conditions. The ingredients were allowed to solidify, after which the antibiotic disks were meticulously and aseptically placed on the surface of the culture media. Afterward, the MHA plates were incubated at a temperature of 37 degrees Celsius for 24 hours. The assessment and analysis of the areas of inhibition were performed after a 24-hour of cultivation (14).

Synthesis and characterization of binary Copper-Cobalt Oxides (Cuo-CoO) Nanoparticles

The binary copper-cobalt oxides(CuO– CoO) nanoparticles were manufactured using the photo

irradiation technique. Individually the source of metals (copper $Cu(No_3)_2$ and cobalt $Co(No_3)_2$) was used with 1:1 mole ratio in order to prepare binary oxides of Cuo-CoO Nanoparticles. The X-ray powder diffraction (XRD) analysis was used to evaluate the structure and crystallographic phase of the product. Additionally, the product's size and morphology were examined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Antibacterial activity of binary Copper-Cobalt Oxides (CuO-CoO) Nanoparticles

The MIC refers to the lowest concentration of a substance that is required to inhibit the growth of a microorganism. Under sterile conditions, 100 µl of MHB (Muller Hinton Broth) was introduced into every well of a 96-well microtiter plate. Afterward, 100 µl of Cuo-CoO -NPs (10000 µg/ml) was added to the first vertical row (A1-A10) and well mixed. Afterward, a sterile micropipette tip was used to transfer a 100µl volume from one well to the next well in a horizontal direction within the same row. Once again, a 100 µl amount of the mixture was transferred from the second well to the third well and well mixed. Similarly, the dilution procedure was performed consecutively until the 8th well, at which point 100µl was extracted and, after that, discarded. This research focuses on the concentration of Cuo-CoO-NPs. The concentration of nano-particles (Cuo- CoO NPs) was halved in each well. In the end, a 5 µl amount of bacterial solution was used.

A suspension including about 1×10^6 cells per milliliter was added to all wells, except for row A11-H11, which acted as the negative control and contained only Mueller-Hinton broth (MHB). The row A12-H12 served as the growth control in the experiment. After 24 hours, a quantity of 10 µl of resazurin (337.5 mg dissolved in 50 ml of distilled water, produced and stored in the absence of light, and well mixed) was added to each well. The samples were subjected to incubation at a temperature of 37 ◦C for a length of 4 hours to facilitate their preparation for further analysis.

The antibacterial effectiveness of nanomaterials was assessed using the agar well diffusion technique, as outlined by Yaseen *et al*. (15), at a concentration of 10000 µg/ml.Three isolates of *E.coli*, exhibiting (MDR), (XDR), and (PDR) (isolates no.24, 25, and1 respectively), were suspended in 5 ml of normal saline. Next, a fraction of the bacterial suspension was meticulously transferred and equally distributed over the Mueller-Hinton agar medium. Subsequently, the dishes were allowed to air dry. 8 mm wells were created on Mueller-Hinton agar plates and filled with 100 µL of Nanoparticle solutions. Well No. 1 was filled with a concentration of (10000 µg/ml), while the second well was filled with 100 µL of Cuo-CoO-NPs (concentration 1000 µg/ml), the third well was filled with 100 µL of distilled water (D.W) as a negative control. The plates were placed in an incubator at a temperature of 37 ◦C for 18- 24 hours in order to quantify the size of the inhibitory zone, measured in millimeters.

Ethical Approval: The experimental work was approved by the Ethical Committees of the hospital and in compliance with recommendations of the Ethical Committees Committee; privacy was maintained regarding patient data.

Results and Discussion

The present investigation included gathering 50 clinical isolates of *E.coli* from patients with urinary tract infections (UTIs) who were hospitalized at AL-Elwia_ pediatrics teaching hospital and Teaching Laboratories in Medical City. *E.coli* is a common bacterium species often linked to several types of diseases, such as urinary tract infections (UTIs). *E.coli* is recognized as the primary cause of about 80 to 90 percent of urinary tract infections (UTIs) in the community, and 30 to 50 percent of UTIs in a hospital environment. The coordinates are (16, 17). Other studies conducted locally and globally have also indicated a comparably high incidence of urinary tract infections (UTIs) (18,19).Various variables contribute to the spread and dispersion of urinary tract infections (UTIs), including socioeconomic and demographic data, hospitalization, age, gender, marital status, clinical characteristics, habits, practices, genitourinary abnormalities, and seasonal fluctuation (20). Moreover, the isolates examined in this research were gathered between January and May 2021. Concerning the seasonal risk factor in Iraq, the average temperature fluctuates between 48oC in July and August and below freezing in January (21). Multiple studies conducted in Iraq and elsewhere have shown that UPEC and other viruses exhibit seasonal patterns, with the highest number of cases seen during the summer months. Notably, there was a notable increase in frequency throughout February and May (22, 23). One possible explanation for this phenomenon is the *E. coli's* capacity to endure greater temperatures in the environment. The number is 24.

Diagnostic procedures performed in a laboratory setting. The bacterial isolates were first cultivated on MacConkey agar, Eosine Methylene Blue agar (EMB), and Chromo agar during the laboratory examination. The *E.coli* isolates had a round, convex morphology and a pink color on MacConkey agar plates due to their ability to ferment lactose. *E.coli* was also cultivated on EMB, a selective medium used to distinguish *E.coli* from *Klebsiella pneumoniae* and other lactose-fermenting bacteria. The colonies exhibited a metallic sheen with a dark center. On CHROM agar plates, the colonies appeared as greenish-blue colonies (Figure 1).

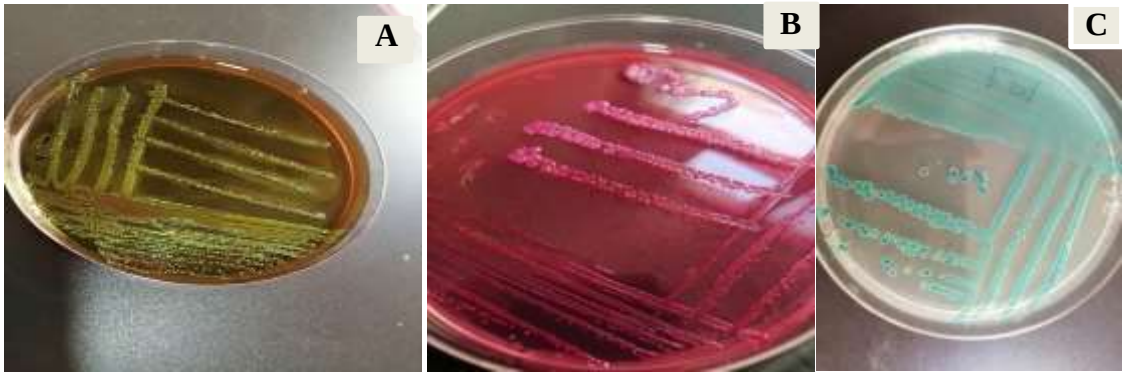
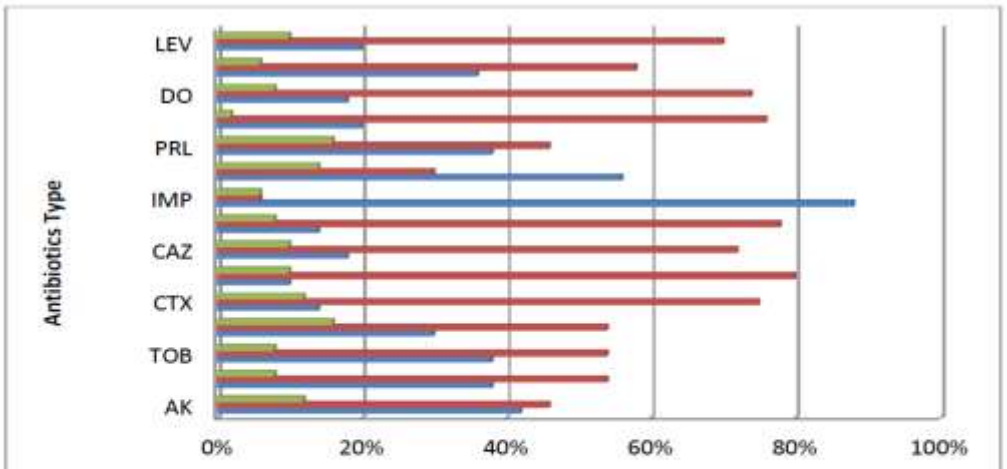


Figure (1): *E.coli* on different culture media.
(A) Eosin Methylene Blue agar, (B) MacConkey agar, and (C) CHROM agar , after a period of 18h of incubation at 37ºC.

Vitek-2 Compact automated system tests were also used to confirm the identification of *E.coli* isolates.

Antimicrobial Susceptibility of *E.coli* isolates

The isolates exhibited varying degrees of antibiotic resistance in this investigation. Specifically, the isolates have shown resistance to Amikacin (46%), Gentamicin (54%), and Tobramycine (54%), all of which are aminoglycoside antibiotics.In addition, βeta-Lactam antibiotics consist of Piperacillin (46%), Augmentin (54%), and the 3rd generation family of Cephalosporins, which includes Cefotaxime (75%), Ceftriaxone (80%), and Ceftazidime (72%).The fourth generation of cephalosporins consists of Cefepime (78%). The Carpenems class includes Meropenem (30%) and Imipenem (6%). Tetracyclines are represented by Tetracycline (76%) and Doxycycline (74%). The fluoroquinolone class consists of Ciprofloxacin (58%) and Levofloxacine (70%). Susceptibility patterns of the isolates are shown in Figure (2).



	AK	GEN	TOB	AUG	CTX	CRO	CAZ	CFM	IMP	MEM	PRL	TE	DO	CIP	LEV
Intermediated	12%	8%	8%	16%	12%	10%	10%	8%	6%	14%	16%	2%	8%	6%	10%
Resistance	46%	54%	54%	54%	75%	80%	72%	78%	6%	30%	46%	76%	74%	58%	70%
sensitive	42%	38%	38%	30%	14%	10%	18%	14%	88%	56%	38%	20%	18%	36%	20%

Figure (2): Percentage of antibiotic resistance of *E.coli*,

AK =Amikacin , GEN=Gentamicin, TOB=Tobramycine, PRL= Piperacillin , AUG=Augmentin , CTX=Cefotaxime , CRO=Ceftriaxone, CAZ=Ceftazidime, CFM=Cefepime , IMP=Meropenem , MEM=Imipenem , TE=Tetracyclins ,DO=Doxycyclin, Fluroquinolone ,CIP=Ciprofloxacin ,LEV= Levofloxacine.

Furthermore, 44% of the (50) *E. coli* isolates showed (MDR), with 20 (40%) exhibiting (XDR) characteristics, 3(6%) potentially displaying pan drug-resistant (PDR) traits, and 5 (10%) not demonstrating any drug resistance (Non-MDR). This phenomenon may be attributed to the emergence of antibiotic resistance in *E. coli* pathogens against all categories of antibiotics used for the treatment of human and animal infections (25). Consequently, these infections pose significant difficulties in terms of treatment (26).

The findings were consistent with a research done by Aabed *et al*. (27), which reported that almost all *E.coli*

isolates (95.8%) obtained from urine samples exhibited (MDR). In a separate investigation conducted by Saeed *et al*. (28), it was discovered that every single *E. coli* isolate tested exhibited resistance to a minimum of three antimicrobial drugs from different classes. AL-khazraji1 (29) demonstrated that 58.2% of the *E. coli* isolates exhibited (MDR). Furthermore, research carried out by Sabir *et al*. (30) revealed that 81% of the *E.coli* samples tested exhibited (MDR), whereas 8.7% of the samples showed (XDR). The discrepancies in population demographics, geographical distribution, time intervals between examinations, and the kinds and magnitudes of clinical specimens might account for the divergent findings seen across many research.

Cuo-CoO NPs synthesis and characterization

The structure and crystallographic phase of the binary CuO–CoO nanoparticles were investigated using XRD analysis. The diffraction pattern can be seen in Figure (3). The diffraction peaks seen in the CuO sample only correspond to the monoclinic CuO phase (JCPDS: 48–1548), with no presence of impurity peaks. The diffraction peaks observed at 35.74, 38.96, 59.32, and 64.95 correspond to the crystal planes (002), (111), (113), and (311) of the monoclinic CuO phase in the binary CuO-CoO nanoparticles. Similarly, the diffraction peaks at 36.77, 44.7, 69.2, and 76.3 represent the crystal planes (111), (200), (311), and (222) of the cubic CoO phase (JCPDS: 75-0393).

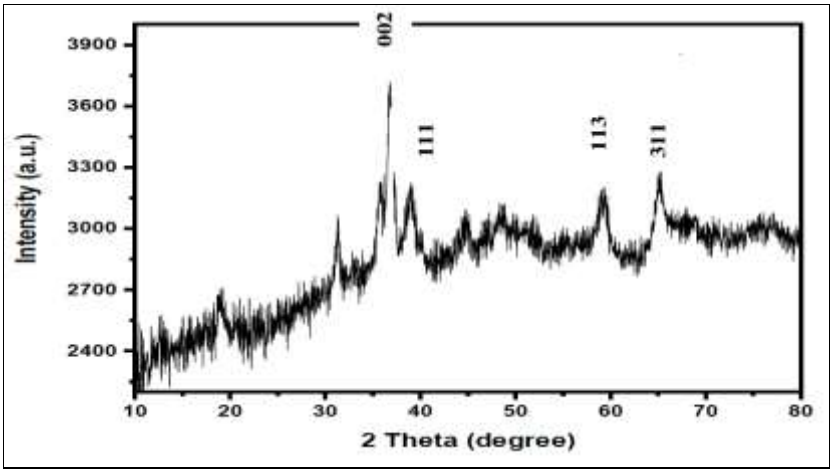


Figure (3): X-ray diffraction analysis pattern of Cuo-CoO nanoparticles.

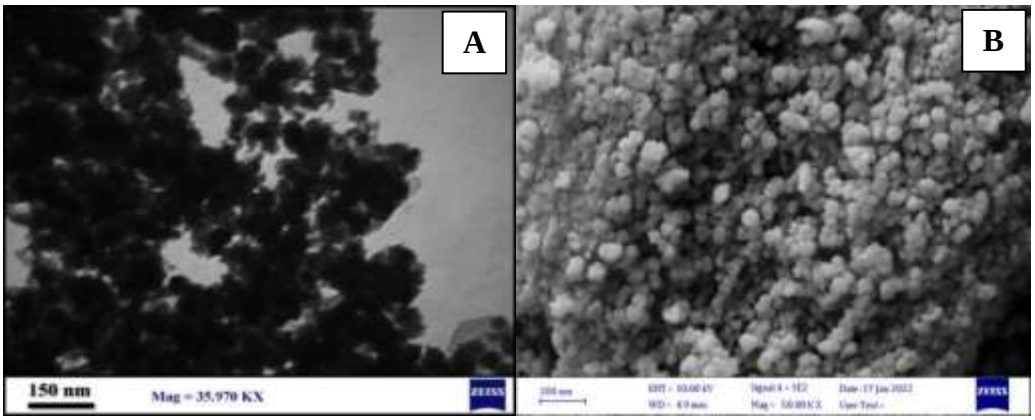


Figure (4): The electron microscope Images of binary CuO-CoO nanoparticles a) TEM and b) SEM.

The level of purity of the nano-synthesised material is demonstrated by the lack of peaks for any contaminants. The Scherrer equation (31, 32) was used to determine the crystallite size of the sample. It was discovered that the average crystallite size of binary CuO-CoO nanoparticles was 16 nm.

Transmission and scanning electron microscopy are used to examine the product's size and morphology (TEM and SEM). The TEM image of the synthesized binary CuO-CoO nanoparticles is presented in Figure (4A). The result is made up of spherical particles with a consistent shape and narrow size distribution, as seen by the TEM image. The range of particle sizes shown in TEM image with a size 25- 35 nm. The inset Figure (4B) reveals that several agglomerated nanoparticles with various sizes caused by various metal oxide nano-particles are apparent in the SEM image of the binary CuO-CoO nanoparticles.

CuO-CoO nanoparticles have shown antimicrobial activity against the selected isolates using an agar well diffusion assay**.** It was observed that the growth of multiple drugs resist (MDR, XDR) bacteria was inhibited at 10000 µg/ml of CuO-CoO NPs, the nanoparticles concentrations had a maximum inhibitory effect against MDR isolate (18 mm) followed by XDR isolate (13 mm) at (10000 µg/ml) while there was no effect on the possible PDR isolate Figure (5) (Table 1).

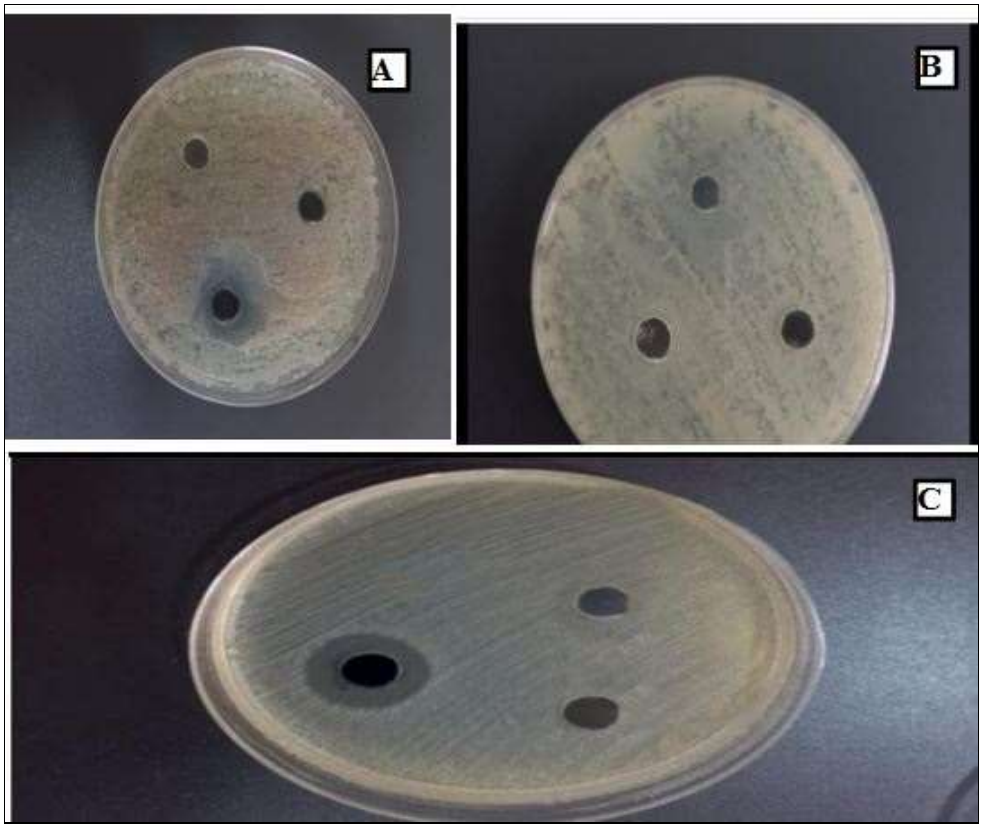


Figure (5): Antibacterial effect of CuO-CoO-NPs (A) shows the inhibition zone of CuO-CoO-NPs against *E.coli* (MDR isolate) full with 1000 µg/ ml in well 1, 10.000 µg/ ml in well 2 while well 3 full with D.W as a control in agar well diffusion method (B) inhibition zone of CuO-CoO-NPs against *E.coli* (PDR isolate) (C) inhibition zone of CuO-CoO-NPs against *E.coli* (XDR isolate).

Antibacterial activity of CuO- CoO Nanoparticles

The determination of (MIC) of Cuo-CoO-NPs was done using the micro dilution method. A series of different concentrations from (10000-19.5) µg/ml were performed. The result showed the MIC for Cuo-CoO-NPs for the MDR was 1250µg/ml while the MIC for XDR was 2500 µg/ml, Figure (6) (Table 1).

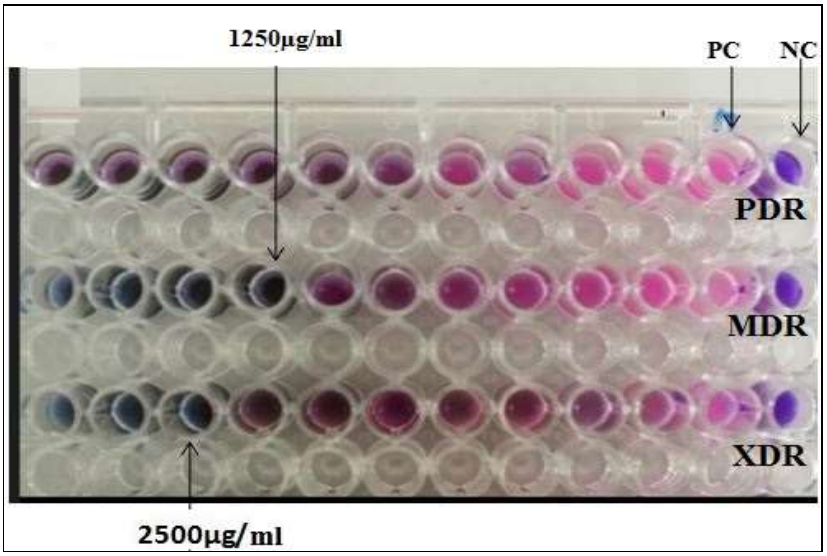


Figure (6): Minimum inhibitory concentrations of *E. coli* isolate (PDR, XDR, and PDR).

Table (1): Source of infection, Antibiotic sensitivity test.

No.	*E.coli* Isolates No.	Résistance pattern	MIC of Cuo-CoO NPs	Inhibition zone at 1000 µg/ml	Inhibition zone at 10000 µg/ml
1	1	Possible PDR	----------	----------	----------
2	24	MDR	1250µg/ml	----------	18mm-
3	25	XDR	2500µg/ml	----------	13mm

Furthermore, the proliferative capacity of *E.coli* was consistently diminished after being exposed to Cuo-CoO-NPs for 14 hours.

The total eradication of *E. coli* cells was seen at a concentration of 10,000 µg/ml. Based on these data, our work has shown that the use of Cuo-CoO-NPs nanocomposite has the potential to eliminate MDR *E. coli* cells. Furthermore, nanoparticles possess distinctive physiochemical characteristics that might be further investigated for their potential to develop novel antibacterial mechanisms (10).

The copper/copper oxide nanoparticles (CuNps/CuONps) have antibacterial properties (33, 34). According to the available study, the antibacterial activity of the nanoparticle is assumed to be caused by the electrostatic interface between the particle and the cell (35). The Cu2+ ion may in addition traverse the lipid bilayer and enter the cell. Ahead of entering the cell, it stimulates the synthesis of reactive oxygen species (ROS). The presence of lipid peroxidation and protein oxidation is also seen (36). The antibacterial action of Cu is attributed to its capacity to transition between

oxidation states of +1 and +2. A study revealed that CuONps are capable of cooperateing with amino acids, which significantly affects their bacterial activity (37).

The findings of the current research were consistent with those of Elwakil *et al*. (38), who investigated the antibacterial effects of CuO-NPs on multi-drug resistant (MDR) *E. coli*. Elwakil *et al.* (38) observed that CuO-NPs inhibited bacterial growth after 8 hours of exposure. Similarly, Ali *et al*. (39) carried out a study in Baqubah and Documented that CuO-NPs exhibited antimicrobial activity against MDR bacteria.

Conclusion

The findings indicated that the studied bacteria exhibited variability in their susceptibility to CuO-NPs. The results demonstrated a direct correlation between the type of bacterial resistance to antibiotics and its resistance to CuO-NPs. In this study, isolate number 1 exhibited the highest resistance to CuO-NPs, as well as resistance to all antibiotics (possible PAN). On the other hand, MDR *E. coli* was found to be the most susceptible bacteria to CuO-NPs at a concentration of 10000 μg/ml.

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