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Antibacterial Activity of Different Nanoparticles on Local Pathogenic Multi-Drug Resistant *Escherichia Coli*

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Abstract: Nano technology has emerged as a promising tool against *Escherichia coli* as a result of increased resistance of *E. coli* to antibiotics. The present study was designed to evaluate the antibacterial activity of ZnO, TiO2, Ag-doped ZnO and Ag-doped TiO2 nanoparticles (synthesized by using sol-gel method) against *E. coli* isolated from animals suffering from diarrhea. Structural and morphological properties of these nanoparticles were investigated using, Transmission Electron Microscopy (TEM) and X-ray diffraction (XRD) which revealed that ZnO NPs size was 26 nm and Ag-doped ZnO NPs size was 19 nm. In both ZnO and Ag-doped ZnO NPs, the hexagonal wurtzite structure was observed. TiO2 NPs only have the anatase phase with size 32 nm but Ag-doped TiO2 has both rutile and anatase phases with size 15 nm. The morphologies of TiO2 and ZnO were influenced by doping with Ag. Three local pathogenic multi-drug resistant (MDR) strains of *E. coli* ($O_{157}H_7$, O_{125} and O_{44}) and reference strain of *E. coli* (ATCC25922) were used to assess the antibacterial activity of the synthesized materials. Antibacterial activity was determined by using disc diffusion assay and MIC was measured using micro well dilution method. The results showed that MIC of ZnO was 31.25µg/ml for *E. coli*$O_{157}H_7$, While MIC of Ag-doped ZnO was 7.8µg/ml for *E.coli* O_{125}. In addition, the MIC for TiO2 was15.6 µg/ml for *E. coli*O_{44}, While Ag-doped TiO2 showed MIC 3.9µg/ml for *E. coli* (ATCC25922). In conclusion, ZnO and TiO2 nanoparticles showed antibacterial activity against *E. coli* which can be increased by doping with silver.

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Keywords: *E. coli*, ZnO NPs, TiO2 NPs, AgNPs, TEM

1. Introduction

Escherichia coli are significant commensal inhabitants of the intestinal tract of most mammals, including animals and humans (**Dean-Nystrom, *et al.*, 1997**). It is associated with several disease conditions including, hemorrhagic colitis, thrombotic thrombocytopenic purpura and hemorrhagic uremic syndrome in humans, diarrheal diseases in cattle especially young calves and small ruminants (**Duhamel, *et al.* 1992, Naylor, *et al.*, 2005, Nasr, *et al.*, 2014).** It is a Gram-negative rod-shaped bacterium belonging to *Enterobacteriaceae* family. It represents a public health concern as animals might serve as a reservoir of the pathogenic *E. coli*, which might be transmitted to humans by ingestion of contaminated food or water or by contact with infected animals or their environment (**Fairbrother and Nadeau, 2006**).

The misuse of antibiotics in veterinary and human medicine resulted in increased *E. coli* resistance to antibiotics (**Alonso *et al.*, 2017**). With the evolution of *E. coli*'s multi-drug resistant strains, Nano technology has emerged as a promising tool against *E. coli*. Several nanoparticles (NPs) are available including silver, zinc, titanium, and copper NPs, which are known by their specific properties and selectivity on pathogenic microorganisms (**Kumar *et al.*, 2015**).

It gives its action on bacteria through different mechanisms including destruction of peptidoglycan layer and the cell wall, destruction of protons efflux pumps resulting in changes in the membrane charges, releasing of toxic ions, reactive oxygen species (ROS) formation, which degrade cell wall, reactive oxygen species (ROS) degrading DNA, RNA and proteins and lowering the adenosine triphosphate (ATP) production (**Nguyen *et al.*,2019**). ROS is the predominant antibacterial mechanism in case of metallic oxide nanoparticles especially for nano-ZnO and nano-TiO2. TiO2 and ZnO NPs characterized by their antibacterial activity, high photosensitivity and oxidizing power, being nontoxic, relatively inexpensive, having unique optical, electrical properties and chemical stability (**Luis *et al.*, 2019**). Noble metal nanoparticles, such as

silver nanoparticles (AgNPs), can attack effectively against both Gram-negative and Gram-positive bacteria (**Le Ouay and Stellacci *et al.*, 2015**). Furthermore, TiO2 and ZnO were doped with various materials having antimicrobial activity such as Ag **(Fu, *et al.*, 2015)**, MgO **(Ashok *et al.*, 2015)** and copper **(Janczarek, *et al.*, 2018)** to improve their efficiency. Several studies shown that Ag/TiO2 NPs exhibit a wide spectrum of antibacterial activity against numerous Gram-positive and Gram-negative bacteria including highly resistant strains such as methicillin-resistant *S. aureus* and enteropathogenic *E. coli*, this significant effect is related to the role of silver as electron traps in the TiO2 band gap (**Aditya *et al.*, 2018) and (Trilok *et al.*, 2019).**

The sol-gel method is an interesting synthesis method for the predation of hybrid materials (mixed oxide systems and/or inorganic-organic systems), involving hydrolysis and condensation reactions on the precursors (**Catauro, *et al.*, 2018).** This method characterized by simplicity, mild reaction conditions, high homogeneity of the product, low energy cost **(Kubiak, *et al.*, 2018).** This study was designed to investigate the antibacterial activity of different nanoparticles including ZnO, TiO2, Ag-doped ZnO and Ag-doped TiO2 on local pathogenic MDR strains of *E. coli* ($O_{157}H_7$, O_{125} and O_{44}) and reference strain (ATCC25922) isolated from animals suffering from diarrhea.

2. Materials and Methods

2.1. Tested strains of *E. coli*:

Three local pathogenic MDR strains of *E. coli* ($O_{157:H7}$, O_{125} and O_{44}) and reference strain (ATCC25922) isolated from animals suffering from diarrhea and obtained from (**Abd El Twab *et al.*, 2020**).

2.2. Synthesis of Nanoparticles:

The sol-gel method was used for synthesis of NPs as described **by (Gupta *et al.*, (2013**) and (**Gebretinsae *et al.*, 2018**).

2.3. Characterizations of TiO2, ZnO, Ag doped TiO2 and Ag doped ZnO Nanoparticles:

The structural and morphological properties of these nanoparticles were investigated using:

2.3.1. X-ray diffraction*:* XRD patterns collected between the angles 20 to 80 degrees at a scan rate of 1 degree per minute using XRD Shimadzu 6000 diffractometer.

2.3.2. Transmission electron microscope Negative staining method (Yashroy, 1990) using (JEOL JEM 1400 Transmission electron microscope at Cairo University Research park (CURP). Nanoparticles were suspended in de-ionized water and ultrasonicated for about 10 min and a clear dispersion of which is drop casted on to a carbon film coated Cu grids for collecting high resolution transmission electron micrographs images (HRTEM).

2.4. Antibacterial Activity Test:

Disc diffusion assay to assess the antibacterial activity of the synthesized NPs on three Local pathogenic MDR strains of *E. coli* ($O_{157}H_7$, O_{125} and O44) and Reference strain (ATCC25922) **(Abd El Twab *et al.*, 2020**). It was performed according to (**Jing, *et al.*, 2018**) with modification; sterile standard filter paper discs (4 mm in diameter) were impregnated with sterile sonicated aqueous suspensions of Nano materials at concentration (10, 5, 2.5, 1.25, 0.625) mg/ml and placed onto the inoculated plates using sterile forceps, and incubated at 37°C for 24 h followed by measuring of the zone of inhibition around the discs by mm. The procedures described above were repeated3 times for each NPs treatment and each bacterium.

2.5. Determination of Minimum Inhibitory Concentration (MIC):

The modified micro dilution method as described by (**Tayel *et al.*, 2011**) was used. The MIC was defined as the least concentration of TiO2, ZnO, Ag doped ZnO and Ag doped TiO2 NPs that visually inhibited the bacterial growth after 24 h of incubation.

2.6. Transmission electron microscope

Negative staining method **(Yashroy, 1990)** using JEOL JEM 1400 Transmission electron microscope was used to examine the morphological characters and changes of bacterial cells before and after treatment with synthesized NPs.

3. Results

3.1. Nano Material Characterization:

3.1.1. X-ray diffraction (XRD):

The synthesized nanoparticles diameter were calculated using Debye-Scherrer formula: $\mathbf{D} = \frac{0.9\,\lambda}{\beta \cos\theta}$ **(Cullity, 1967).** Where **D** is the average diameter of the crystals, 0.9 is Scherrer's constant, λ is the wavelength of X-rays, β is the full width at half-maximum (FWHM) of the diffraction peak and θ is the Bragg diffraction angle.

XRD pattern of the prepared ZnO and Ag-doped ZnO powder revealed the hexagonal wurtzite phase and verified that the synthesized Nano powder was free of impurities, as it doesn't contain any characteristic XRD peaks other than ZnO peaks. With size equal to 26 nm and 19 nm respectively, Figures (1a & 1b). But TiO2 has anatase phase with size equal 32nm and Ag-doped TiO2 has both rutile and anatase phases with size equal 15nm shown in Figure (1c & 1d**).** The morphologies of TiO2 and ZnO were influenced by doping with Ag.

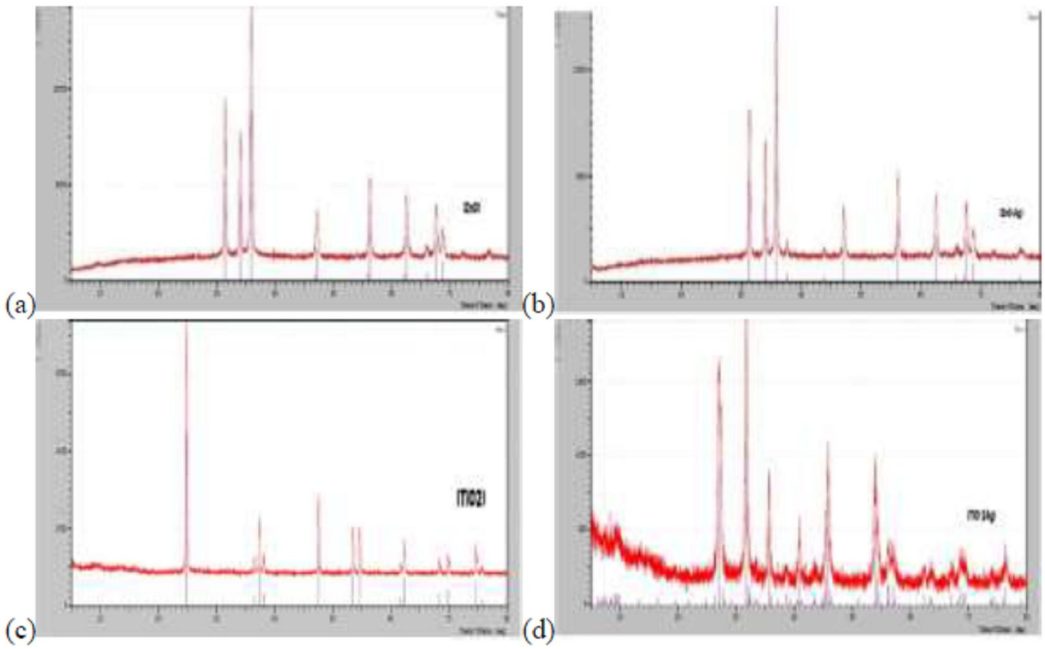

Figure 1: XRD pattern synthesized nanoparticles:
(a) ZnO. (b) Ag-doped ZnO (c) TiO2. (d) Ag-doped TiO2

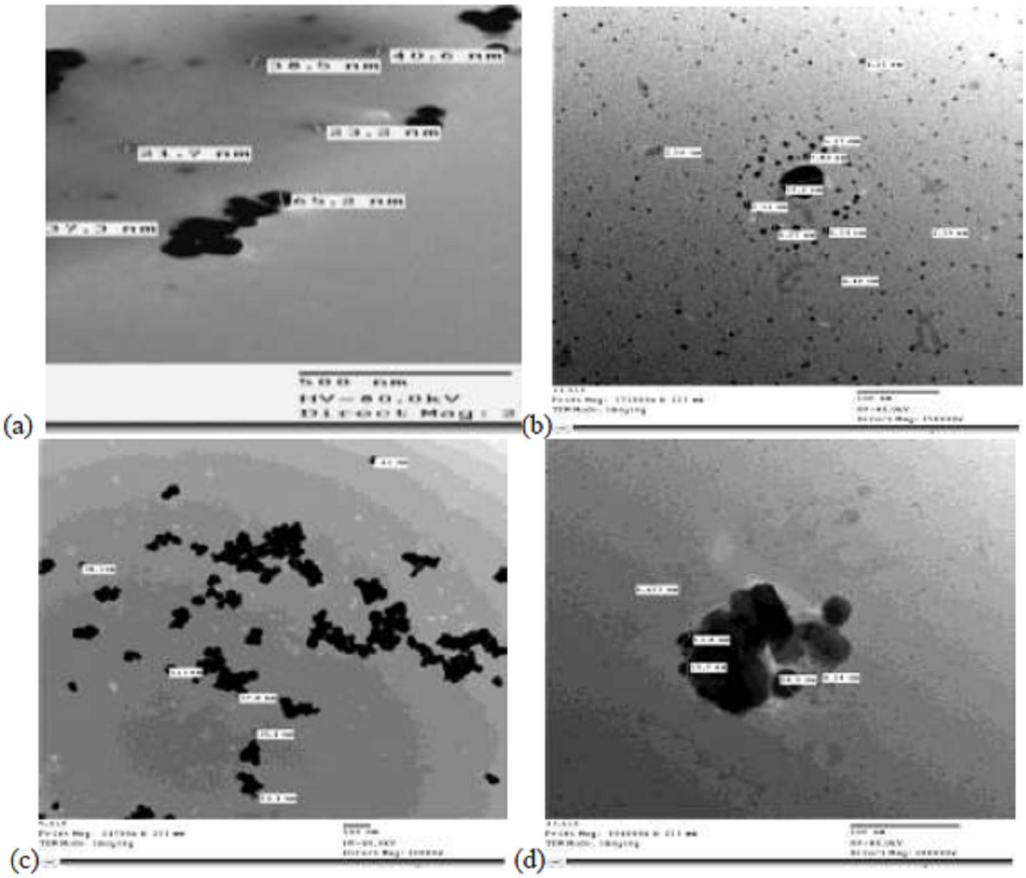

Figure 2: Tem analysis of synthesized nanoparticles.
(a) ZnO (b) TiO2 (c) Ag doped Zno. (d) Ag-doped TiO2

3.1.2 TEM analysis

TEM images of ZnO and Ag doped ZnO nanoparticles showing the particles are scattered in the case of ZnO and Ag-doped ZnO nanoparticles figure (2a & 2b), Ag NPs (black spots <10 nm), ZnO nanoparticles (15–50 nm). The shape and size of TiO2 and Ag-doped TiO2 nanoparticles were analyzed by TEM images in Figure (2c & 2d) showed the particles are irregular in shape agglomerated in the case of TiO2 whereas they are scattered in the case of Ag-doped TiO2nps, particles size about (25–60 nm). The bigger nanoparticles are assigned to nano-TiO2 and the smaller ones are Ag NPs.

3.2. Antibacterial activity of TiO2, ZnO, Ag doped TiO2 and Ag doped ZnO Nanoparticles:

3.2.1. Agar disc diffusion assay:

The antibacterial activities of different concentrations (10- 5- 2.5- 1.25- 0.625) mg/ml from each synthesized NPS (ZnO, TiO2, Ag-doped ZnO and Ag-doped TiO2) against 3 *E. coli* strains and (1) reference strain were evaluated by using disc diffusion assay. The results showed that pure ZnO and TiO2 formed poor inhibition zones in comparison with Ag doped ZnO and Ag doped TiO2 respectively. Ag doped TiO2 treatments showed the most significant antibacterial activities against *E. coli* and the highest inhibition zone was observed for reference strain (28mm) followed by Ag doped ZnO and the highest inhibition zone was observed for O_{125} strain (23mm), then TiO2 and the highest inhibition zone was observed for O_{44} strain (18mm), while ZnO have the lowest antibacterial activities and the highest inhibition zone was observed for $O_{157:H7}$ strain (14 mm) Table (1).

Table (1): Antibacterial Activity of Zno, Tio2, Zno Doped Ag and Ag-Doped Tio2 NPS against Isolated *E. Coli* Strains.

NPs conc. \ *E.coli*		O_{125} (ZOI)	O_{44} (ZOI)	$O_{157:H7}$ (ZOI)	*Referance strain* (ZOI)
TiO2/Ag	10	21	21	24	28mm*
	5	18	17	20	23
	2,5	15	12	16	18
	1,25	11	9	13	16
	0,625	0	6	10	13
ZnO/Ag	10	23mm*	17	19	20
	5	19	14	16	18
	2,5	15	12	13	15
	1,25	12	10	11	14
	0,625	6	0	0	5
TiO2	10	16	18mm*	17	15
	5	15	17	14	12
	2,5	13	15	12	11
	1,25	11	10	9	9
	0,625	0	8	0	7
ZnO	10	12	13	14mm*	13
	5	11	10	12	11
	2,5	9	8	10	10
	1,25	8	7	6	6
	0,625	0	0	0	0

(ZOI): Zone Of Inhibition By mm, *: the highest inhibition zone was observed.

3.3. MIC of Tio2, Zno, Ag Doped Tio2 And Ag Doped Zno Nanoparticles On *E.Coli* Strains:

MIC of ZnO occurred at (31,25µg/ml) for O_{157H7} and 62,5 µg/ml for O_{125}, O_{157H7} and (ATCC25922)., while MIC of TiO2 occurred at (15,6 µg/ml) for O_{44} and (31,25µg/ml) for O_{157H7}, O_{125} and (ATCC25922), MIC of Ag-doped ZnO occurred at (7,8 µg/ml) for *O125*and at (15,6 µg/ml) for O_{44}, O_{157H7} and (ATCC25922). and MIC of Ag-doped TiO2 showed at 3,9 µg/ml for (ATCC25922) and at (7,8 µg/ml) for *O125*, O_{44} and O_{157H7}. (Table2).

Table (2): MIC of TiO2, ZnO, Ag-doped TiO2 and Ag-doped ZnO NPS on *E.coli* strains

e.coli strains	O_{125}(A)	O_{44} (B)	O_{157H7} (C)	Referance strain (D)
ZnO	62,5 µg/ml	62,5 µg/ml	31,25*µg/ml	62,5 µg/ml
ZnO/Ag	7,8*µg/ml	15,6 µg/ml	15,6 µg/ml	15,6 µg/ml
TiO2	31,25µg/ml	15,6*µg/ml	31,25 µg/ml	15,6 µg/ml
TiO2/Ag	7,8 µg/ml	7,8 µg/ml	7,8 µg/ml	3,9*µg/ml

3.4. Transmission Electron Microscope

TEM analyses was used to assess the morphological change of bacterial cells induced by the treatment of ZnO, TiO2, Ag doped ZnO, Ag doped TiO2 NPS. The leakage of intracellular contents and membrane disorganization were observed in bacterial cells treated with mentioned NPs**,** Figure 3 (a, b, c, d, e).

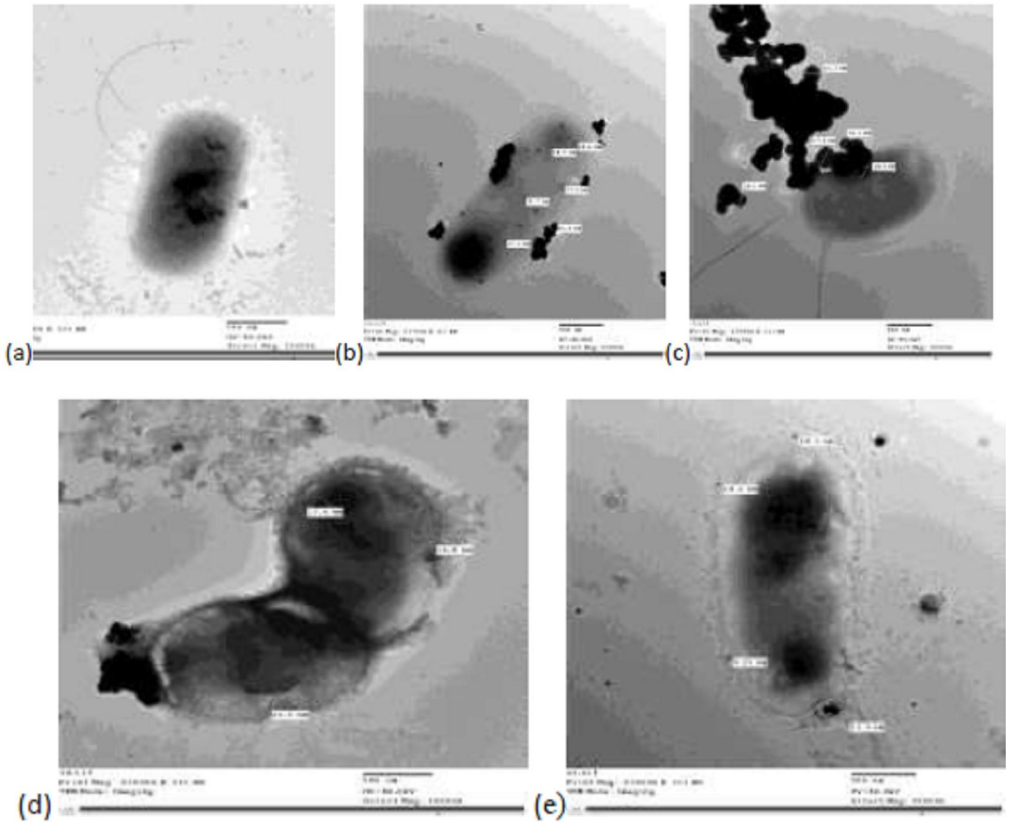

Figure (3): TEM analyses of the morphological change of bacterial cells induced by the treatment of *E. coli* strains by synthesized NPS*:*

(a) Control untreated *E. coli (*ATCC25922): the bacterial cells were in normal sizes with smooth cell line, defined intracellular structures and absence of cellular materials outside the cell

(b) ZnO NPS *on (*$O_{157:H7}$) membranes of the bacterial cells were deformed and intracellular structures were disorganized.

(c) TiO2 on *(*O_{44}): lysis of cell membrane and destruct flagellum.

(d) Ag doped ZnO on O125 cell rupture, presence of cellular material including chromatin bodies outside the damaged cell membrane.

(e) Ag doped TiO2 on (ATCC25922): cellular components and ribosome present in the surrounding fluids.

4. Discussion

TiO2 has anatase phase with size equal 32nm while Ag-doped TiO2 has both rutile and anatase phases with size equal 15nm. The morphologies of TiO2 and ZnO were influenced by doping with Ag. This result was agreed with that of **Zhang *et al.*, (2005)** and **Gebretinsae *et al.*, (2018**).

In contrast, **Gupta *et al.*, (2013**) observed the typical anatase phase in Ag-doped TiO2, while in case of pure TiO2 both anatase and rutile phases were present with crystal size 18 nm and 22 nm

respectively. **Xu *et al.* (2008)** reported that a mixture of anatase and rutile phases of Ag-doped TiO2 possesses greater antibacterial and photocatalytic activity than the pure anatase phase.

XRD pattern of the prepared ZnO and Ag-doped ZnO powder revealed the hexagonal wurtzite phase with size equal to 26 nm and 19 nm respectively and this result was agreed with that of **(Prabhu and Poulose, 2012), Jamil *et al.*, (2014) and Gebretinsae *et al.*, (2018).**

TEM images of ZnO and Ag doped ZnO NPs showing The particles are scattered in the case of ZnO and Ag-doped ZnO, these results are in accordance with the values founded by **(Cheng *et al.*, 2010)** and **(Luis *et al.*, 2019).**

The shape and size of TiO2 and Ag-doped TiO2 NPs were analyzed by TEM showing The particles are irregular in shape agglomerated in the case of TiO2 whereas they are scattered in the case of Ag-doped TiO2 NPs, this result was agreed with that of **(Gupta *et al.*, (2013**).

Using disc diffusion assay to assess the antibacterial activity of synthesized NPS, the results showed that pure ZnO and TiO2 formed poor inhibition zones in comparison with Ag-doped ZnO and Ag-doped TiO2 respectively. These results were in accordance with the values founded by (**Zhang *et al.*, 2005), (Gupta *et al.*, 2013**). **(Hidayati *et al.*, 2013)**, (**Nguyen *et al.*,2019)**, **(Luis *et al.*, 2019)** and also **Banerjee *et al.* (2006)** who demonstrated that doping of silver on the surface of metal or metal oxide (TiO2 and ZnO NPs) increase in the antibacterial activity.

MIC of ZnO occurred at (31,25µg/ml) for O_{157H7} this result of ZnO-NPs was agreed with findings reported previously by **(Reddy *et al.*, (2007), (Liu *et al.*, 2009), (Ibrahem *et al.*,2017) and (Myassar *et al.*, 2018)**. In contrast, this result wasn't agreed with the MIC of ZnO Nps was founded by **Lanka *et al.*, (2014)** at 40 µg/ml. **Mahmood *et al.*, ((2020)** studied the antimicrobial activity of ZnO and TiO2 NPs suspensions against *E. coli* (ATCC-25922), *S.* enteritidis (ATCC-49221), *L.monocytogenes* (ATCC-13932) and *S. aureus* (ATCC-33591), MIC values for gram-negative and gram-positive bacteria to ZnO NPs were found to be ~2.5-3 mg/mL and ~1.5-2 mg/mL, respectively.

MIC of Ag-doped ZnO occurred at (7,8 µg/ml) for O125, this result wasn't agreed with (**Gebretinsae *et al.*, (2018)** who reported that the reduction in the viability of *S. aureus, P. aeruginosa* and *E. coli* bacteria, to zero using Ag-doped ZnO occurred at 60 µg/mL.

MIC of TiO2 occurred at (15,6 µg/ml) for O44, this result wasn't agreed with **(Mojtaba *et al.*, 2018)** who reported that TiO2 NPs have no antibacterial activity, while ZnO NPs had antibacterial effects at 0.1 µg/ml and 0.01 M. **Mahmood *et al.*, ((2020)** reported that MIC values for *E. coli*, *S.* enteritidis to TiO2 NPs were found to be ~2-2.5 mg/mL and for *L. monocytogenes* and *S. aureus* was ~1-1.5 mg/ml.

The MIC of TiO2/Ag Nps was3,9 µg/ml for (ATCC25922) this result was nearly similar to **(Kedziora *et al.*, 2012)** who recorded that MIC value for (*S. aureus, E. coli and K. pneumoniae*) was 0.4 µg/ml. On the other hand MIC value of TiO2/Ag wasn't agreed with **(Keleher *et al.*, 2002)** who obtained MIC values for the crystalline TiO2/Ag particles 6.4 µg/ml for *E. coli* and 3.9 µg/ml for *S. aureus* strains, and also (**Gebretinsae *et al.*, 2018)** who reported that the reduction in the viability of *S. aureus, P. aeruginosa* and *E.coli* bacteria, to zero using Ag-doped TiO2 occurred at 80 µg/mL, TEM analyses was used to assess the morphological change of bacterial cells induced by the treatment of ZnO, TiO2, Ag-doped ZnO, Ag-doped TiO2 NPS. TEM analysis of control untreated *E. coli (*ATCC25922) showing that the bacterial cells were in normal sizes with smooth cell line, defined intracellular structures and absence of cellular materials outside the cell, this result agreed with **(Liu *et al.*,2009)**. On the contrary, *E. coli* cells growing in the TSB media containing NPs were clearly damaged by ZnO NP, the membranes of the bacterial cells were deformed and intracellular structures were disorganized, this result agreed with **(Liu *et al.*, 2009),** (**Tayel *et al.*, 2011**) TiO2 NPS cause lysis of *E. coli* (O44) cell membrane and destruct flagellum. and that agreed with **(Azam, *et al.*, 2012)** and **(Simonsen *et al.*, 2010).**

Many null cells were found in the bacterial samples treated with Ag doped ZnO owing to the damage of the cell membrane, promoting the release of the intracellular contents and death of the bacterial cells that agreed with (**Nguyen *et al.*,2019**).

Ag doped TiO2 NPS incorporated into cell membrane and RNA cause pores in membrane disrupt ione exchange, and this was agreed with **(Luis *et al.*, 2019**) and **Standard and White LED (2019).**

5. Conclusion:

In this study, the antibacterial effects of ZnO-TiO2, Ag doped ZnO and Ag doped TiO2 nanoparticles were investigated against multidrug-resistant *E. coli* ($O_{157:H7}$-O_{125}-O_{44}) also Reference strain (ATCC25922) was used as control. Ag/TiO2 nano exhibited higher antibacterial activity against *E. coli* than the Ag/ZnO nanohybrids followed by TiO2 NPS and ZnO NPS have the least antibacterial effects.

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