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RESEARCH PAPER

The Influence of 530nm Semiconductor Lasers and CdS Nanoparticles on Antibiotic Susceptibility of P. Aeruginosa and E. Coli Bacteria

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ABSTRACT

Two isolates of *Pseudomonas aeruginosa* and *E.coli* were obtained. Ten types of antibiotics were examined for their effect on these two strains. The Cadmium sulfide nanoparticles (CdSNPs) prepared by the hydrothermal method with a crystal size of 22.7 nm and absorption peak at 556 nm were used in this study. It was found that the solution of CdS nanoparticles has a strong inhibitory effect for *P. aeruginosa* reached inhibition zones diameters of (11, 12, and 15) mm at concentrations (100, 200, and 300 µg/ml), and (12, 14 and 17) mm and for the same concentrations for *E. coli* bacteria respectively. The study showed that the absorbance of bacteria decreases when irradiating the 530nm laser, at an exposure time of 5 minutes, the highest inhibition rate was found to be 15 % for E. coli bacteria, while the highest inhibition rate was 15% for Pseudomonas aeruginosa bacteria, and that increasing the exposure time is sufficient to damage the living cell and kill the bacteria.

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INTRODUCTION

Gram-negative *Pseudomonas aeruginosa* and *E. coli* bacteria can be found in various environments including freshwater and foodstuff [1]. *P. aeruginosa* is an opportunistic pathogen that is a primary source of disease and death in people with cystic fibrosis and immunocompromised patients. *P. aeruginosa* eradication has grown more challenging owing to its extraordinary drug resistance. Most antibiotics don't work on *P. aeruginosa* strains because they have a lot of built-in and acquired resistance [2]. *Escherichia coli* is a leading cause of urinary tract infections, newborn meningitis, and septicaemia. Human and animals are known to be the source of pathogenic *E. coli* in the human intestine. Genetic plasticity enables evolution and a wide range of variation *in Escherichia coli* strains ranging from avirulent to highly pathogenic strains, including the creation of virulent hybrid microorganisms. This capacity also aids in the development of antibiotic resistance. [3, 4]. One of the concerns for global health worldwide is the emergence of antibiotic-resistant bacteria. Antibiotic resistance genes are found in many bacterial communities in the environment and are not limited to clinical settings. The determinants of resistance in bacteria that encode the self-resistance mechanism present in all or most non-producing environmental bacteria should be studied. While the presence of resistance determinants in soil and environmental bacteria does not pose a risk to human health, their transfer to new hosts may constitute a risk or threat to human health [5]. As a result,

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materials and methods that affect these bacteria rather than antibiotics are required. Nanoparticles emerged as the savior that may solve this problem [6]. Ciprofloxacin is likely to form a compound with Zn2+ ions released from the surface of ZnO NPs, increasing its antibacterial action. ZnO NPs may also block efflux transporters, increasing antibiotic effectiveness against *P. aeruginosa* [7]. Nanoparticles can target bacteria as an alternative to antibiotics and may be useful in treating bacterial infections. Examples include antibacterial coatings for implantable devices, drugs used to prevent infection and promote wound healing, antibiotic delivery systems, bacterial detection systems for antibacterial vaccines and microbial diagnosis to stop bacterial infections [8]. The antibacterial efficacy of magnesium oxide nanoparticles was tested using two methods: agar diffusion and plate casting. It was observed that these nanoparticles are capable of producing reactive oxygen species that damage the cell membrane, leading to leakage of cell contents and cell death. Based on these findings, a highly effective and cost-effective antibacterial agent was developed [9]. CdS nanoparticles (pure and 1 percent Cu doped) were tested for antibacterial susceptibility against the pathogens Staphylococcus aureus, Salmonella typhimurium, *Pseudomonas aeruginosa*, *E. coli*, and Klebsiella pneumoniae. The antibacterial activity of nanoparticles was determined using the well diffusion technique. CdSNPs (pure and 1% Cu doped) show a wide range of antibacterial activity.

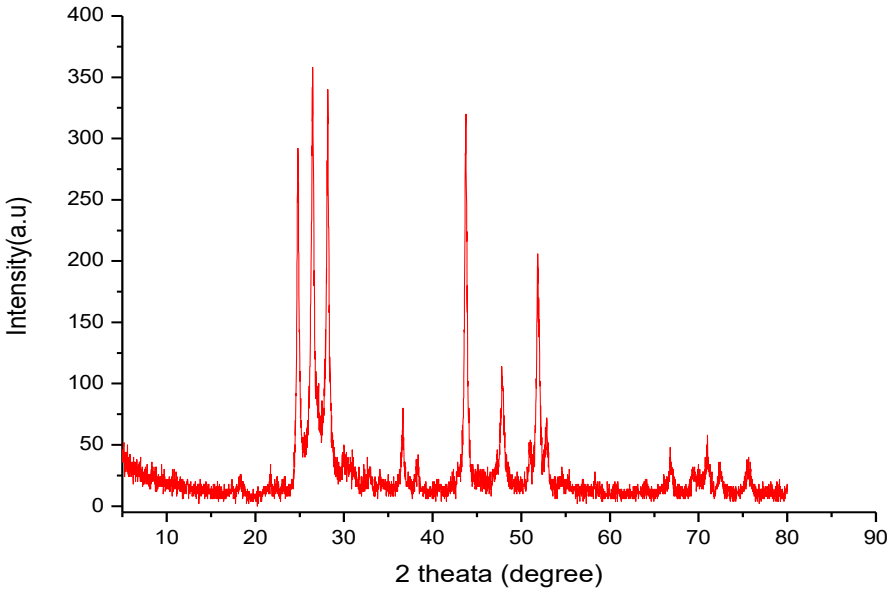

Fig. 1. The XRD peaks of CdSNPs [12].

Table 1. Antibiotics susceptibility test of *P. aeruginosa* and *E. coli* bacteria.

Antibiotics	Inhibition zone diameter (mm)	
	P. aeruginosa	*E. coli*
MEM	32	
TOB	16	15
CAZ	0	10
CIP	40	15
LEV	20	15
IPM	40	35
AK	16	16
CN	11	15
ATM	20	22
PRL	13	15

The antibacterial impact of doping has been improved [10]. Physical and chemical conditions affect the growth and activity of bacteria, and these conditions include the transmission and emission of energy through the material medium containing bacteria, which is known as irradiation, where the laser is used to sterilize water and some foodstuffs, which is One of the well-known sterilization methods, which has a severe effect on microorganisms, requires the influence of radiation directly, and its effect increases by increasing the radiation dose, i.e., the time of irradiation. The bacteria's ability to survive decreases as the laser exposure period increases [11].

MATERIALS AND METHODS

Isolation of the bacteria

Samples were taken from people with ear infections and different kinds of burns. The bacteria were then isolated and identified in the microbiology laboratory at the College of Medicine, University of Babylon. The most common bacterial spp. Isolated from the burns belong to *P. aeruginosa* and *E. coli*.

Synthesis of CdS nanoparticles

CdSNPs with a hexagonal wurtzite phase were produced using the hydrothermal method [12]. The crystallite size and the average particle size were equal to 22.7 nm and 50 nm, respectively. CdSNPs have a peak absorption at the wavelength of 556 nm. It has an indirect energy gap of 2.25 eV.

Antibiotics susceptibility assay

Muller Hinton agar (Himedia, India) were prepared, sterilized, poured into Petri dishes, and

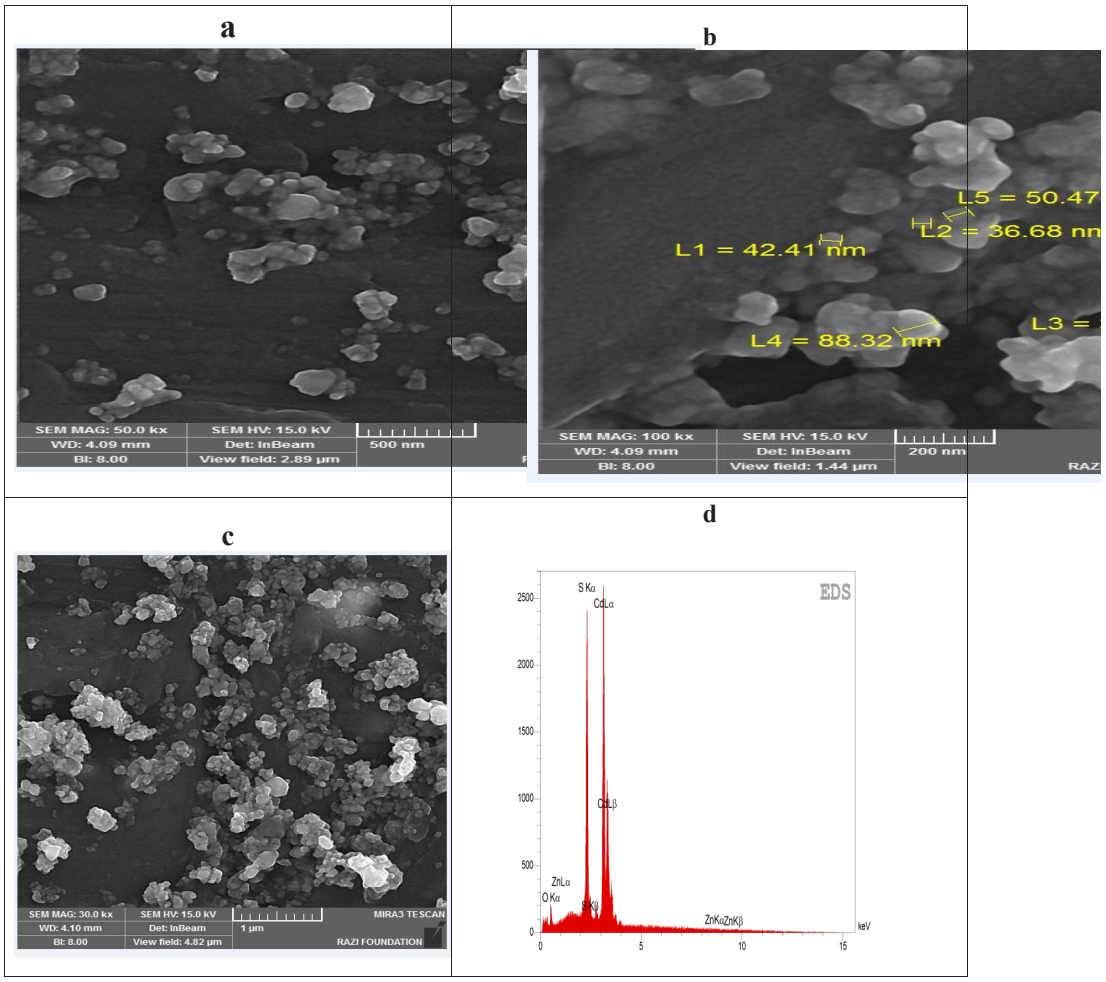

Fig. 2. FESEM and EDS of CdSNPS by hydrothermal method at (a-500 nm, b- 200 nm, c- 1µm and d- EDS.

incubated overnight before use. Isolated bacteria were spread on the surfaces of the agars. Antibiotic disks were located on the surface of each culture medium and pressed slightly, gestated for 24 hours at 37 C^0. After incubation, the inhibition diameter of the zone was calculated using a ruler and the results were recorded.

Testing the sensitivity of bacteria to nanomaterials

The suspension of CdS nanoparticles was syntheses by dissolving (2 mg) of CdSNPs in (4 ml) of nutrient broth (NB) to obtain a concentration of (500 µg/ml), and from it, the concentrations of 100 mg/ml, 200 mg/ml, and 300 mg/ml were obtained. Four holes were made in each plate containing Muller Hinton agar by a cork piercing. In each well, 100 µl of each concentration (100, 200, and 300) was added. The fourth well received 100 µl of NB without any NPs and was used as a control for comparison. The dishes were incubated for 24 hours at 37°C. After incubation, a plastic ruler was used to measure the size of the inhibition zone for each concentration against each bacterium.

Preparation of bacteria for laser irradiation

Sterile liquid NB medium was prepared in test tubes, one tube was inoculated with *E. coli* and the other with *P. aeruginosa*. After that, the inoculated media were distributed in a sterile 96-well plates (200 µl in each well). The wells were divided into four groups (N=5); Control group, Group of 1 minute laser irradiation, Group of 3 minutes laser irradiation, and Group of 5-minute laser irradiation.

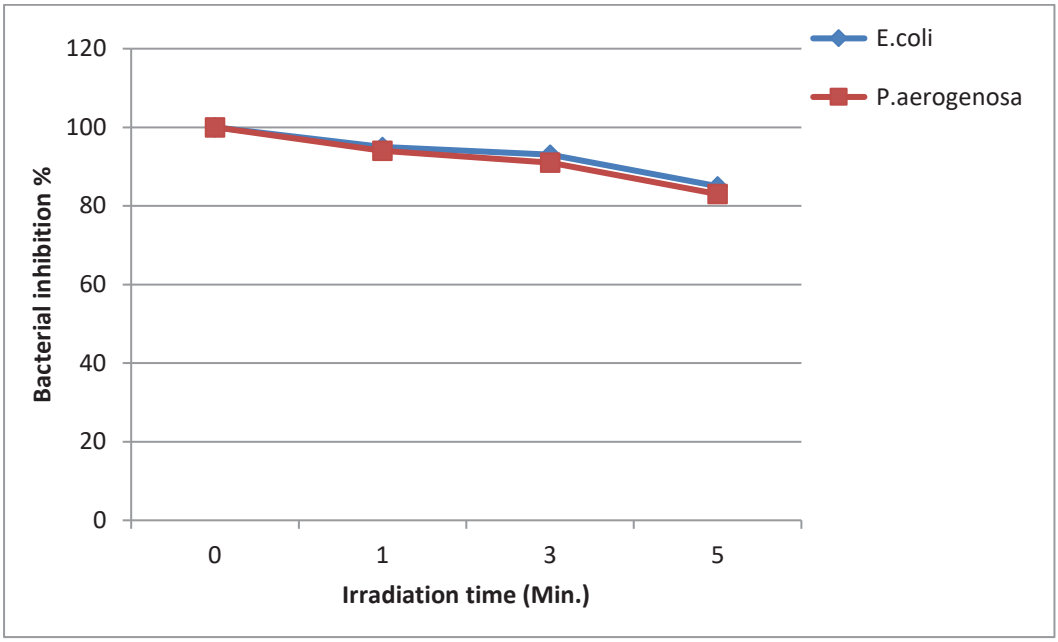

Fig. 3. The survival rate of *E. coli* and *P. aeruginosa* as a function of irradiation time.

Table 2. Effect of CdS NPs on *P. aeruginosa* and *E. coli* bacteria evaluated using disk diffusion method.

NPs Concentration (µg/ml)	The diameter of Inhibition zone (mm)	
	P. aeruginosa.	*E. coli*.
Control	0	0
100	11	12
200	12	14
300	15	17

A 530 nm semiconductor laser and an output power of 300 mW was used. Laser light was projected vertically on the wells of 96 well plate containing the bacterial suspension, and a convex lens was used to focus the laser beams on the sample. To calculate the percentage of inhibition, the optical density of the bacterial suspension was measured immediately after inoculation. Then the wells are exposed to laser light for (0, 1, 3, and 5 minutes) and incubated for 24 hours. Then, the optical densities of the cultured wells were measured again. Bacterial growth rate was calculated using the following equation:

Bacterial growth % = (OD of treated wells before incubation - OD of treated wells after incubation) / (OD of control wells before incubation/ OD of control wells after incubation) x 100

Effect of nanomaterials on antibiotic sensitivity

To study the effect of nanomaterial on the sensitivity of bacteria to antibiotics, the nanomaterials were added to the culture medium (Muller-Hinton agar) at a final concentration of (100 ng/ml). The medium was sterilized by autoclaving and poured into dishes. Then the media was streaked with the bacteria and the antibiotic disks were attached to the surface of the medium. Inhibition zones were measured using a ruler after the plates were incubated for 24 hours at a temperature of 37°C.

Effect of laser on bacterial antibiotic sensitivity

The two types of bacteria were exposed to a laser beam for 3 minutes before culturing on Muller-Hinton agar medium. Then the same antibody discs used before were attached to the medium surface and gestated for 24 hours, then the inhibition zones diameters were then measured.

RESULTS AND DISCUSSION

Antibiotics Susceptibility Test

The susceptibility assay for *P. aeruginosa* and *E. coli* isolates to the antibiotics (MEM, TOB, CAZ, CIP, LEV, IPM, AK, CN, ATM, PRL) was passed out using the "disk diffusion method by measuring area of inhibition is shown in Table 1. The result shows that *P. aeruginosa* was highly sensitive for IPM, CIP, and MEM while the lowest sensitivity was to PRL, CN, and CAZ.

While it is clear from Table 1 that *E. coli* shows high sensitivity towards MEM, IPM, and ATM and low sensitivity for CAZ.

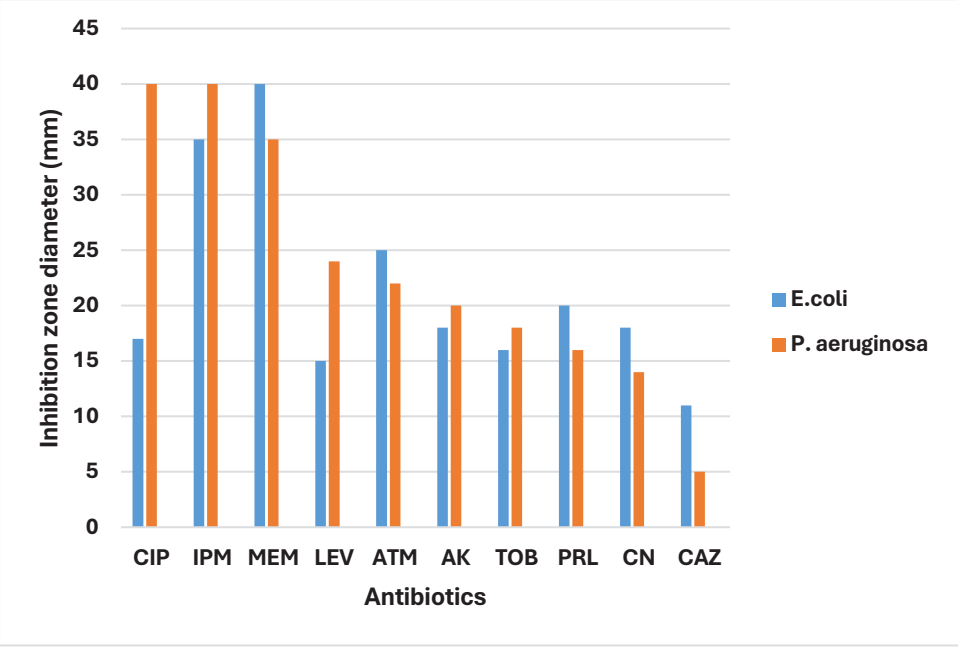

Fig. 4. Antibiotics sensitivity of *P. aeruginosa* and *E. coli* treated with CdSNPs.

The reason of bacterial resistance to antibiotics might be the result of a change in the genes located on the chromosomes, or because of a mutation that leads to a damage or loss of penicillin binding proteins, a loss of permeability to antibiotics, or the production of beta-lactam enzymes, especially for beta-lactam group [13, 14, 15].

Characterization of CdSNPs prepared by the hydrothermal method as indicated in reference [12]

XRD of the CdSNPs

The peaks located at 2θ corresponding to the following lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (203), (210), (211), (114) and (105) respectively indicates the formation of a hexagonal wurtzite phase of cadmium sulfide nanoparticles of (CdSNPs) as shown in the Fig. 1 and its agreement with reported in ICDC(No.00-041-1049) file card and Scherer's formula has been used to calculate the crystallite size of CdS nanoparticles and its equal to 31.8 nm.

Field Emission Scanning Electron Microscope (FESEM) of CdSNPs

Scanning electron microscopy images of CdSNPs are shown in Fig. 2a-c. The nanoparticles appear to be aggregated and irregularly distributed across the entire surface, with an average particle size of approximately 50.8 nm as measured using the image J software. The EDS spectrum of the hydrothermally prepared cadmium nanoparticles reveals that the predominant quantity is due to cadmium and sulfur particles, with very small trace amounts of zinc and oxygen present due to minor contamination at the sampling site, as shown in Fig. 2d. XRD and EDS results indicate that the cadmium sulfide nanoparticles exhibit high purity.

Effect of CdS nanoparticles on bacteria

It was found that CdS NPs have high activity on *Pseudomonas aeruginosa* and *E. coli* bacteria, as shown in Table 2. At concentration of (300 µg/ml), the diameter of inhibition zone reaches (15 mm) for *P. aeruginosa*, while for *E. coli* bacteria, it reaches 17 mm. Cytotoxic effect appear even at lower concentrations (100 and 200 µg/ml) used.

The large surface area to volume ratio of nanomaterials affects the permeability of the bacterial plasma membrane, leading to cell death [16] [17]. Cadmium nanoparticles carry a positive charge, while the bacterial cell wall (teichoic acid) carries a negative charge. This creates an electromagnetic attraction between them, leading to a decrease in membrane permeability and potentially causing cell disruption and death [18]. The variation in cell wall structure leads

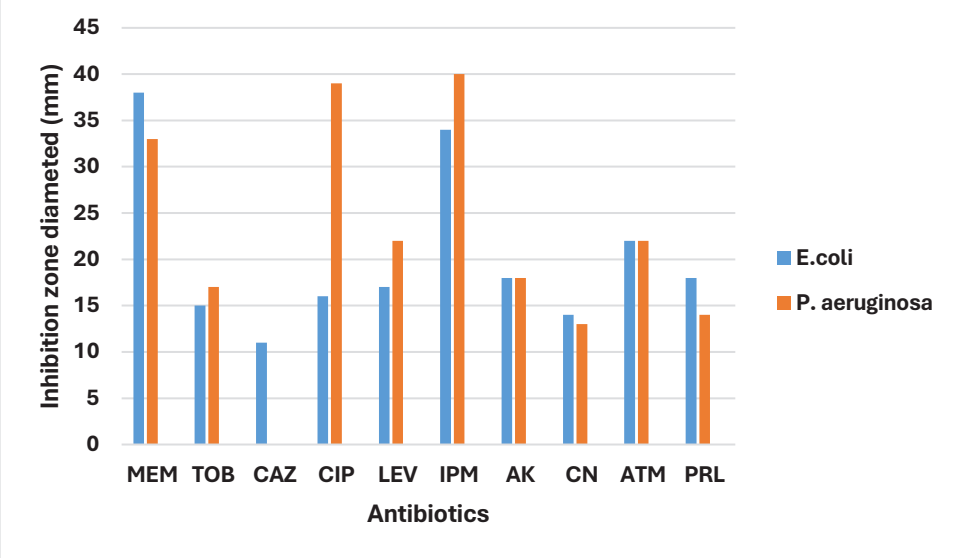

Fig. 5. Antibiotics sensitivity of *P. aeruginosa* and *E. coli* treated with 532 nm laser light.

to differences in bacterial sensitivity and thus determines cell permeability. The outer membrane of bacteria contains Lipopolysaccharides, which impede the entry of large molecules. Nanoparticles may also affect bacterial growth signaling pathways, impacting cell viability, as indicated by the researcher in the reference [19].

Effect of laser irradiation of bacterial samples

Bacterial isolates were irradiated for different periods using a semiconductor laser (λ=530 nm and a power of 300 mW). After incubation, the inhibition rate was evaluated according to the culture's optical density. Results are illustrated in Fig. 3. It is clear that bacterial survival was reduced with increasing irradiation time. According to previous studies, the respiratory chain components are the primary photoreceptors for visible light [20]. Laser light affects bacterial cells via the generation of reactive oxygen species, which are directly proportional to the light intensity and irradiation dose [21].

Effect of nanomaterials on antibiotic sensitivity

Bacterial sensitivity to the antibiotics in the presence of CDSNPs varied depending on the type of antibiotic as well as the type of bacteria as shown in Fig. 4. The highest sensitivity shown by *P. aeruginosa* was to the antibiotics (CIP and IPM) and it did not affect by CdSNPs, while the lowest sensitivity was to the antibiotics (CAZ and CN). Sensitivity to these antibiotics was increased by CdSNPs co-treatment. While it is clear from Fig. 4 that the highest sensitivity shown by *E. coli* to the antibiotics (MEM and IPM) and the lowest by the CAZs and LEV. Sensitivity to these antibiotics did not improve by nanoparticle co-treatment.

Nanomaterials can augment the effectiveness of antibiotics directly via deforming and disrupting cell walls of bacterial by extending the bacteria over the attraction from diverse interaction points, or when the NPs surfaces jerk the bacteria close, these structures puncture into the bacteria and create cell destructive pores. NPs can infiltrate through bacterial cell wall and membrane, disrupting important cellular mechanisms [22], enabling the nonselective penetration of the antibiotics inside the bacterial cell [23]. In addition, NPs can promote antibiotic effect by other mechanisms such as causing toxicity by releasing of free metal ions elevating the oxidative stress [24]. These mechanisms may help antibiotics to work better by facilitating their penetration and action.

Effect of laser on antibiotic sensitivity

Bacteria were exposed to the laser for three minutes, before antibiotics sensitivity testing. Results show that the highest resistance shown by *P. aeruginosa* is to the antibiotics (IPM and CIP), while the lowest resistance shown to the antibiotics (CAZ and CN).

Fig. 5 shows that the highest resistance shown by *E. coli* to antibiotics (MEM and IPM) and the least resistance shown to antibiotics (CAZ and CN), and through the results, it was shown that the resistance of bacteria to antibiotics decreases when exposed to the laser beam.

Recent studies showed that laser light has the potency to increase antibiotic susceptibility. Laser light affects Gram-positive bacteria more than Gram-negative bacteria. Green laser (532 nm) for example, can inhibit bacterial growth or even destruct the entire bacterial cell according to laser energy [25]. The effect of laser on antibiotic susceptibility may be related to the alteration in membrane efflux pump mechanism responsible for some antibiotics resistance [26]. Laser light caused alteration in many enzymatic activities, this in turn can modulate bacterial resistance to certain antibiotics [27]. There are also recent studies on the use of nanoparticles alone in removing pollutants, as mentioned in the references [28-31].

CONCLUSION

The efficiency of CdSNPs in affecting *E. coli* and *Pseudomonas aeruginosa* bacteria that have resistance to antibiotics is high, and the effect of *E. coli* bacteria on nanomaterials is greater than that of *Pseudomonas aeruginosa* bacteria. The laser irradiation of bacteria leads to a decrease in the live numbers of bacteria with increasing dose or exposure period. The possibility of obtaining a killing rate of (16%) for *P. aeruginosa* bacteria and (15%) for *E. coli* bacteria by using the laser beam compared to the CdSNPs. The effect of bacteria exposed to laser radiation for antibiotics is greater than the nanomaterial for antibiotics. The possibility of using the laser beam in sterilization and treatment of pathological conditions improves antibiotics.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this

manuscript.

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