Effect nanoparticles zirconium on bacteria growth multidrug resistance *pseudomonas aerginosa* isolated from burns patients

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Abstract. A total of 153 samples (swabs) were taken from burned patients between the ages of 2 and 75, representing a variety of age groups and body regions. All samples were obtained from Baghdad's government hospitals. Beginning in early September 2022 and ending in early January 2023, (48/153) isolates (31.37%) were identified as P. aeruginosa Testing for antibiotic susceptibility It was conducted using various antibiotic classes (8 antibiotics), which were assessed using the VITEK 2 compact system. The results revealed that P. aeruginosa isolates were resistant to ceftazidime and cefepime (100%) in the same rate, the resistance to Tobramycin and Ciprofloxacin was (77.08), and the maximum sensitivity to Colistin was (79.1) and resistance to imipenem, meropenem, and Levofloxacin was (52.08%), (79.18%), and (72.92%), respectively. In this study used zirconium nanoparticles for inhibition growth bacteria. The efficacy of the synthetic nanoparticles against P. aeruginosa was tested as five different concentrations (1,2.5,5,10 and 15) mg/ml were adopted, and concentrations of (5,10 and 15) mg/ml showed efficacy in inhibiting bacterial growth while (1 and 2.5) mg/ml was not given any effectiveness, the results of minimum inhibitory concentration was (5) mg\ml.

1 Introduction

Burns were a widespread health issue around the world because they result in the rupturing of the skin, the body's first line of defense against infection. As a result, the patient is exposed to numerous microbes, including the bacterium *Pseudomonas earuginosa*, which is known to be antibiotic-resistan [1]. Antimicrobial resistance to several different drug families, including carbapenems, fluoroquinolones, cephalosporins, and aminoglycosides, has been created and spread by MDR *P. aeruginosa*. This resistance makes treatment more difficult, due to its ability to develop resistance through a number of mechanisms, including efflux pumps, the secretion of alginate during biofilm formation, the production of -lactamases and aminoglycoside modifying enzymes, and the production of penicillin-binding proteins, *P. aeruginosa* infections are difficult to treat [2]. Nanotechnology is a novel technique that provided multiple methods in treating chronic human diseases through directing the drug at a specific location to facilitate delivery of accurate medicines. The important applications of

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nanotechnology in this field are biosensing, anticancer therapy and drug delivery, nanoparticles are clusters of atoms, with sizes ranging between 1 and 100 nm, whereas a "nano" is used to indicate one billionth of a meter [3]. In the current study has concentrated on biogenic ZrO2NP synthesis and promoting their biological applications, with a focus on their antibacterial properties [4].

2 Materials and methods

2.1 Collecation samples and identification

Samples (153) were collected. Under the supervision of the responsible doctor, samples were collected from burn patients using a transporter media cotton swab. The burned area was cleaned thoroughly with sterile normal saline, and a sterile cotton swab was gently passed over the burned area. The samples were then transferred to the lab and incubated at 37 °C for 24 hours before being cultured. Identified *P. aeruginosa* isolates by diagnostic tests including biochemical tests, culture media like (cetrimide agar) and VITEK-2 Compact System

2.2 Antibiotic Susceptibility Assays by Using VITEK2 Compact System

Based on a determination of the MIC approach utilizing AST cards, antibiogram testing was carried out using the automated VITEK-2 compact system. This card listed the antibiotics in tables (1) Most recent recommendations by consensus [5].

Table 1. Antibiotic used in this study.

NO.	Antibiotic Group	Antibiotic Disk	Concentration
1	Carbapenem	Imipenem	10 µg
		Meropenem	10 µg
2	Lipopeptides	Colistin	10 µg
3	Aminoglycosides	Tobramycin	10 µg
4	Fluoroquinolones	Levofloxacin	5 µg
		Ciprofloxacin	5 µg
5	Cephems (Cephalosporin IV)	Cefepime	30µg
		Ceftazidime	30µg

2.3 Nanoparticles (ZrO_2)

2.3.1 Tests and Characterization of ZrO2 nanoparticles

Three tests to verify the properties and efficiency of nanoparticles sach as (X- Ray Diffraction Technique, Scanning electron microscope and Energy-dispersive X-ray spectroscopy) based on the sources [6] [7] [8] respectively.

2.3.2 Determination of Minimum Inhibitory Concentration (MIC) of ZrO_2NPs against P. aeruginosa

The minimal inhibitory concentration (MIC) value is the lowest concentration of compounds that can completely prevent visible growth of bacteria. An equal number of bacteria was added into 1ml of broth media that contained different concentrations of ZrO2NPs in each

test tube (1,2.5,5,10 and 15 mg/ml) and then incubation at 37ºC for 24, MIC values for each strain of bacteria were determined by choosing the lowest concentrations reported there was no visible growth. The minimal bactericidal concentration (MBC) values were determined as well by sub-culturing each experiment sample on a specific strain of *P. aeruginosa* and monitoring for bacterial growth [9].

3 Results and discussion

3.1 Specimens collection from burn patients

A total of 153 samples (swabs) were collected by the sterile cotton swab from September 2022 to January 2023, the specimens Among 153 (48/153) isolates (31.37%) were diagnosed as *P. aeruginosa* based on phenotypic methods by streaking on different culture media then screening on selective medium such as (cetrimide agar) and confirming with Vitek 2 and the rest were distributed between no growth (41\153) (26.83%) and other gram negative bacteria (64\153) (41.8%) *Proteus spp*. (5\64) (7.81%), *Klebsiella spp*. (27\64) (42.18%), *E. coli* (24\68) (37.5%) and *Acintobacter spp*. (8\68) (12.5%). Shown table (2). The findings revealed that burns accounted for (48/153) (31.37%) of the *P. aeruginosa* isolates. This may be because this pathogen has a number of potentially virulent components that aid in its ability to colonize and infect mammalian tissues, including hemolysin, pyoverdine, and protease, which encourage adhesion to host cells, damage host tissue, and interfere with the immune system [10]. Clinical samples included (23\48) (47.9%) samples taken from male and (25\48) (52.1%) samples taken from females. This study's findings are consistent with those of Kirkuk University [11], who found that females were more than men were (52.9% vs. 47.1%) figure (1). The possible reasons for high percentage in curruent study to female may be due to the types of populations studied, females may have routine indoor work and are often at risk of infection from flame, oil and hot water, the age ranged between from (2 to 75 years). All samples were collected from Governmental hospitals in Baghdad. this study agree with [12], collected and diagnosed as *P. aeruginosa (*26\68) (38.23%) from burn patients. This study disagree with [13] showed higher rates of infection with *P. aeruginosa* (91.6%).

Table 2. Number and percentage of bacteria that isolated in this study.

Bacteria	NO. of isolates	Percent %
P. aeruginosa	48	31.37
Klebsiella spp.	27	17.64
E. coli	24	15.68
Acintobacter spp.	8	5.22
Proteus spp.	5	3.26
No growth	41	26.83
Total	153	100

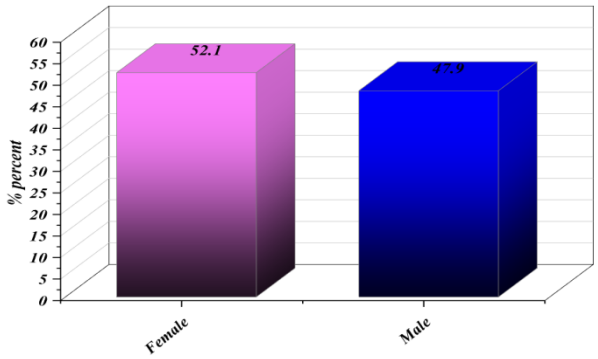

Fig. 1. Percentage of gender that isolated in this study.

3.2 Antibiotic Susceptibility of *P. aeruginosa*

The results of the present study shown indicated that from all *P. aeruginosa* (48) were tested for their antibiotic resistance to (8) antibiotics, using VITEK 2 compact system in recommendation with the clinical and laboratory standards institute [14], guidelines depending on the diameter of inhibition zone (mm). The antimicrobial potency of selected antibiotics against the *P. aeruginosa* summarized in Table (1). This study's percentage of imipenem users concur with [15], finding which resulted in resistance (53.3%) for Imipenem in study of *P. aeruginosa* isolated from contaminated burns and wounds in Baghdad, this study Disagree with the results obtained by [16] in Babylon who showed extremely low resistance rate to Imipenem (8%). In this research the percentage of resistance for meropenem agree with [17], who found resistance to meropenem was (73%) in Iran. While disagree with This study [18] in Baghdad, he found resistant rate Meropenem 35% respectively. High-level Carbapenem resistance include (Imipenem and Meropenem) reported in this study as shown in a table (1) may be multiple mechanisms including Carbapenemase production and efflux-pump over-expression according [19]. The percentage of resistance for colistin agree with [20] findings about the degree of resistance to the antibiotic Lipopeptides (colistin).

Rate resistance of colistin was (18.5%) in Saudi Arabia. Resistance to colistin is caused by the phosphoethanolamine transferase enzyme, its encoded gene is located on mobile genetic elements [21]. According to [22], who showed that the rate of tobramycin resistance was (79.6%), the proportion of resistance for aminoglycoside antibiotics (Tobramycin) is similar to that found in this study. This study contrast the findings of [23] in Saudi Arabia, he discovered that the rate of tobramycin resistance was (12%). Certain genetically encoded enzymes, such as N-acetyl-transferases, adenyl-transferases, and phosphosphor-transferases, may be the cause of the bacterial resistance to this family of antibiotics. These enzymes are recognized as particular AMEs (aminoglycoside-modifying enzymes) [24]. And alteration of the ribosomal binding site by mutation expression of 16S ribosomal RNA methylases [25].

The percentage of resistance for fluoroquinolone drugs (ciprofloxacin and levofloxacin). Levofloxacin antibiotic was agreement with [26] found rate resistance of levofloxacin was (72.83%) from isolated *P. aeruginosa* from clinical samples in diyala and while the result of [27] in duhok city, iraq disagree with current study score low resistance (13.3%). For the antibiotic ciprofloxacin, the percent resistance was identical to that shown by [28] found that almost all *P. aeruginosa* isolates were (75.5%). This study disagree with [29], the researcher found the resistance of *P. aeruginosa* isolates against Ciprofloxacin (26%). *P. aeruginosa* have two types essential mechanisms of resistance to fluoroquinolone; structural

modification of target enzymes and efflux pump [30]. Targets of the fluoroquinolone drugs (levofloxacin and ciprofloxacin) include DNA gyrase (type II topoisomerase, topoisomerase IV), Fluoroquinolone resistance is mostly caused by mutations in the fluoroquinolone resistance-determining region (FRDR) of the DNA gyrase subunit-coding genes *parC* and *gyrA* or *gyrB*. The topoisomerase II (*gyrA*), topoisomerase IV (*parC*), and efflux regulatory (mexR) gene alterations are therefore the primary causes of fluoroquinolone resistance in gram-negative bacteria. In gram-negative bacteria, such as *P. aeruginosa*, amino acid changes discovered in the genes *gyrA*, *gyrB*, *parC*, and *mexR* are linked to high levels of fluoroquinolone [31].

In this study the percentage of resistance for Cephems (Cephalosporin IV) including (Cefepime and Ceftazidime) Cefepime agree with [32] rate resistance of cefepime was (100%) and In this study disagree with [33] the rate was (23.5%) resistance. In this study the percentage of resistance for Ceftazidime agree with the results of [34] in Basrah, that *P. aeruginosa* were resistant to ceftazidime, (100%).

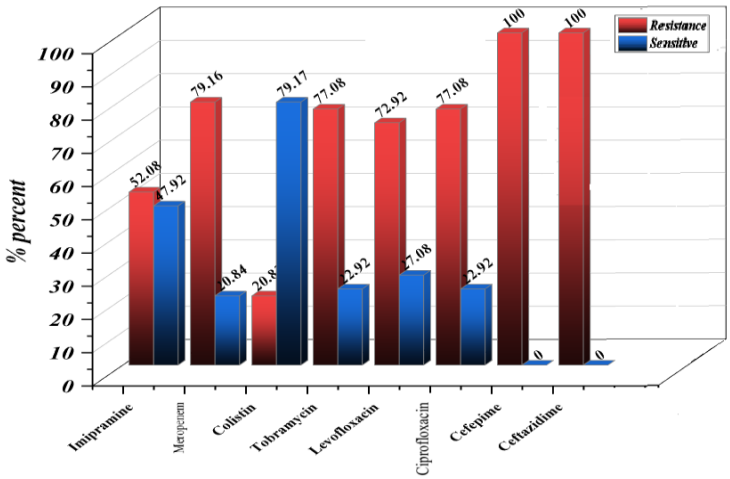

Fig. 2. Percentage the antibiotic susceptible test

3.3 ZrO2 nanoparticles characters

3.3.1 X- Ray Diffraction Technique (XRD)

The prepared sample's XRD pattern was recorded in order to verify the phase development of ZrO2. When compared to Tetragonal JCPDS No. 70-1769, XRD reveals a single broad peak associated with tetragonal ZrO2 nanoparticles, 300 of which belong to the plan. Using Scherrer's equation, this broad peak figure (3), indicates a crystallite size of 2 nm. Additionally, there are no additional unidentified peaks, indicating that the prepared ZrO2 is single crystalline.

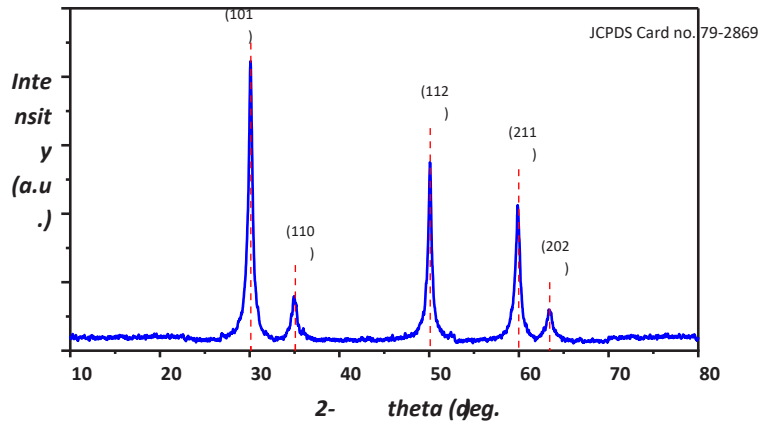

Fig. 3. X-ray diffraction patterns of ZrO2.

3.4 Energy-dispersive X-ray spectroscopy (EDX)

While the EDX spectra of pure ZrO2 show peaks for the elements Zr and O, the spectra of composites show peaks for the elements Zr, O, F, Ca, C, and Si. Due to the small peaks of C, Ca, and Si in the spectrum, negligible levels of contaminants have been discovered. As seen in (figure 4), the Zr/O weight ratio between the O and Zr peaks is approximately (40.5:30.5), and the intensities of the O and Zr peaks vary linearly with the concentrations of their respective predecessors of ZrO2.

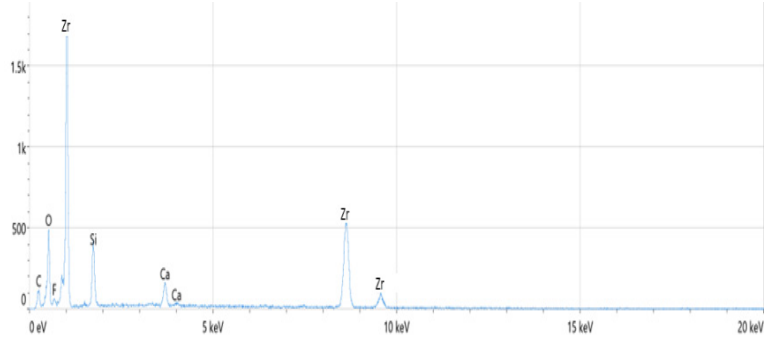

Fig. 4. EDX spectrum of the proposed ZrO2.

Table 3. ESX spectrum of the proposed ZrO_2.

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	25.2	1.2	12.7	0.6
O	45.3	0.8	30.5	0.5
F	5.0	0.8	4.0	0.6
Si	7.9	0.2	9.3	0.2
Ca	1.7	0.1	2.9	0.1
Zr	14.8	0.3	40.5	0.8

3.5 FESEM characterization of zirconia nanoparticles

The form and microstructure of the material were examined with SEM. Particles in this zirconia sample range in size from 30 to 65 nm. The XRD results were discovered to be entirely consistent with the pictures of the zirconia samples. The size distribution of zirconia particles as seen through a SEM is shown in Figures (5). Zirconia particles, whose size distribution is roughly (45 nm), as previously mentioned, will fill the holes in the resin specimen

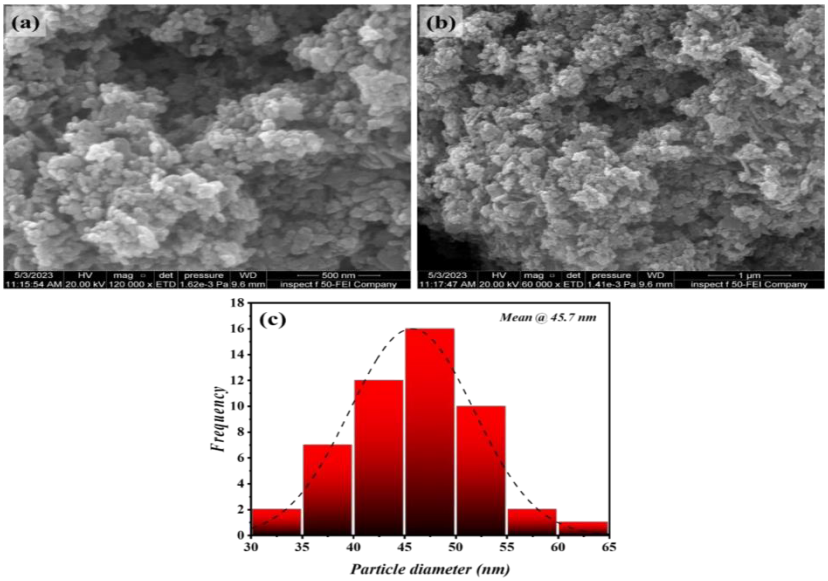

Fig. 5. FESEM topographies of ZrO2 with scale bar of (a) 500 nm and (b) 1mm, while a histogram distribution illustration is demonstrated in (c).

3.6 Determination the Minimum Inhibitory Concentration (MIC)and Minimum Bactericidal Concentration (MBC) of ZrO2NPs against *P. aeruginosa*

Multi-drug resistant microorganisms have emerged as a result of excessive or inappropriate usage of antimicrobials. Nanotechnology offers an alternate approach for creating alternative antimicrobial agents that can effectively destroy bacterial cells and show enormous potential for usage in both medical and veterinary applications. This will help to overcome the drawbacks of existing synthetic antimicrobial chemicals. The result in Figure (6) showed that there was no visible growth of bacteria with concentrations of ZrO2NPs 15, 10 and 5 mg/ml suggested that the MIC of all *P. aeruginosa* strains was (5) mg/ml.

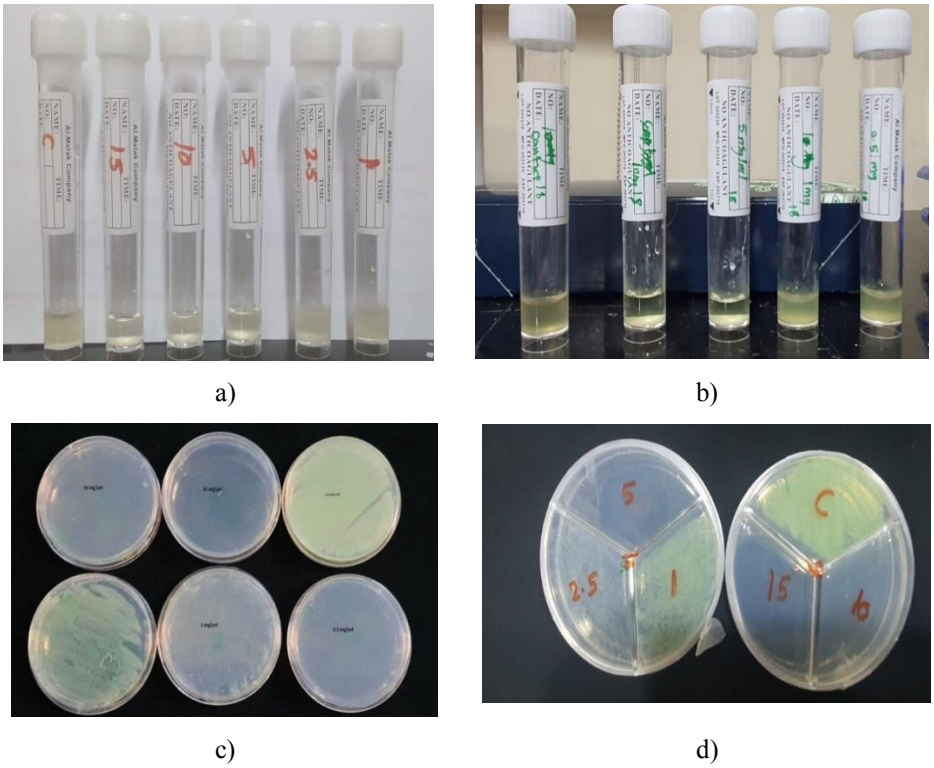

Fig. 6. A and B MIC of ZrO2 effect on bacteria growth, C and D MBC of ZrO2 effect on bacteriagrowth cultured on selective media.

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