

Research Article



Synthesis, Characterization, and Antibacterial Efficacy of Cu-ZIF-8: *In Vitro* Study of Multidrug-Resistant Gram-Positive and Gram-Negative Bacteria

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ABSTRACT

Recently, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have become multidrug-resistant (MDR) and produce many virulence factors, posing a health risk. Therefore, addressing knowledge gaps and finding solutions to the challenge caused by bacteria's increased resistance to multiple antibiotics is an urgent need. To address this risk, we aim to synthesize new nanoparticles with long-lasting, safe effects and low toxicity. Metal-organic frameworks (MOFs) are promising materials with high efficiency in inhibiting or killing MDR bacteria. In this investigation, we successfully synthesized copper-doped ZIF-8 (Cu-ZIF-8) nanocomposites using the solvothermal method. Its characteristics were studied using techniques like SEM, FTIR, EDX, and XRD. The Cu-ZIF-8-MOF exhibited high antibacterial efficacy against *S. aureus* (Gram-positive) and *P. aeruginosa* (Gram-negative), with concentrations of 12.5, 25, 50, and 100 µg/mL showing high efficiency against *P. aeruginosa*. In contrast, it was less effective against *S. aureus*. These results open up wide prospects for the application of the Cu-ZIF-8-MOF antimicrobial agent.

1. Introduction

The prevalence of multidrug-resistant Gram-positive and Gram-negative bacteria is a health risk in the management of burn hospital infections (Pachori *et al.* 2019). Bacterial infections account for 50% of deaths resulting from burn injuries. Furthermore, patients are more vulnerable to infection due to damaged skin and a weakened immune system (Mulatu *et al.* 2022). MDR *Staphylococcus aureus* and *Pseudomonas aeruginosa* are among the most significant opportunistic bacteria commonly present on skin and are major causes of burn injuries due to their high ability to form biofilms, which represent the

starting point of bacterial injuries in the deeper tissue and systemic infections, in addition to their high multidrug resistance to many antibiotics. The survival of these bacteria leads to many significant complications, the most important of which is the death of patients due to septicemia (Brandenburg *et al.* 2021; Hamza *et al.* 2026). On the other hand, antibiotic-resistant *S. aureus* and *P. aeruginosa* are a major health sector concern worldwide, as they are responsible for greater deaths and an increase in healthcare costs (Antimicrobial Resistance Collaborators 2022). These bacteria cause many hospital-acquired infections that are difficult to treat in elderly and compromised patients due to their ability to form biofilms. As a result, many researchers are exploring the use of highly active materials to eliminate MDR bacteria and destroy biofilm bacteria (Zhang *et al.* 2022). Among several nanomaterials,

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MOF-based materials are used as efficient antibacterial agents due to their distinctive chemical composition, high porosity, biocompatibility, organic and inorganic components, and effective adsorption. It was recently announced that ZIF-8 is considered an efficient photoinitiator and antibacterial agent due to its large surface area, which is attributed to its extensive nature, crystallinity, higher thermal and chemical stability, active sites, selective absorption, sodalite structure, and organic ligands (Dung *et al.* 2022). ZIF-8 is one of the compounds that can bind to other metals, such as copper, due to its electrochemical, photoelectric, and magnetic properties (Yeerken *et al.* 2023). Therefore, our investigation aimed to synthesize ZIF-8-MOF embedded with Cu nanocomposites and use as an antibacterial agent against multidrug-resistant *S. aureus* and *P. aeruginosa*.

2. Materials and Methods

2.1. Bacteriological Analysis

From February to June 2025, 150 swabs were obtained from patients who were suffering from burn infection, who were admitted to Imam Hussain Hospital and the Maternity and Pediatrics Hospital in Karbala province, Iraq. The samples were then cultured on standard culture media, such as nutrient agar, blood agar, cetrimide agar, MacConkey agar, and Mannitol salt agar. After 24 hours, *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates were identified phenotypically based on cultural characteristics, such as colony morphology, size, color, odor, elevation, the effect on the medium e.g., blood hemolysis, the appearance of pigment on cetrimide agar, the ability to ferment mannitol, biochemical tests, and Gram stain. Subsequently, they were molecularly identified using the polymerase chain reaction (PCR) technique with the 16S rRNA gene (Fadhil Abdul-Husin 2021). Finally, antibiotic susceptibility testing of the *S. aureus* and *P. aeruginosa* isolates was performed using the disc diffusion method on Mueller-Hinton agar. Five different antibiotic discs were used: Amikacin (30 µg), Ceftazidime (30 µg), Meropenem (10 µg), Ciprofloxacin (5 µg), and Aztreonam (30 µg) toward

Gram-negative bacteria. Antibiotics Benzylpenicillin (15 µg), Tetracycline (30 µg), Clindamycin (2 µg), Gentamicin (10 µg), and Levofloxacin (30 µg) were used against Gram-positive bacteria. The results were analyzed according to the Clinical and Laboratory Standards Institute (Pierce *et al.* 2023).

2.2. Polymerase Chain Reaction

We used a kit manufactured by a company (Geneaid, UK) to extract and purify DNA from *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates. Then, the purity and concentration of the DNA were measured using a NanoDrop device. Primers specific for the 16S rRNA gene, shown in Table 1, were prepared according to the manufacturer's instructions (Pioneer, Korea). A PCR reaction with a final volume of 25 µL was performed, and the DNA was then amplified under the conditions outlined in Table 1. Finally, the PCR products were analyzed by electrophoresis on an agarose gel (1.5%) in TAE buffer, and the PCR products were visualized using a UV device, which indicated positive results.

2.3. Preparation of Cu-ZIF-8 Sorbent

To prepare Cu-ZIF-8, 0.0080 mol of $\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.249 g of ZIF-8 were dissolved in 30 mL of Dimethylformamide (DMF). After that, the mixture was sterilized using an autoclave for 2 hours; the final product was collected and washed with DW water, and then it was dried in an oven at 60°C for 20 h to obtain Cu-ZIF-8 (Carson *et al.* 2009).

2.4. Characterization of the Cu-ZIF-8

The current investigation used different electrochemical characterization techniques to analyze the manufactured samples. SEM and EDX (SU8010, Japan) were used to define both the material shape and size of the Cu-ZIF-8. XRD and FT-IR (FTIR 8400S, Shimadzu, Japan) devices were used to measure the crystal structure of the samples using a Bruker D8 diffractometer. and to study the significant modifications in the surface structure and bonding of NPs, respectively (Al-Bdereh *et al.* 2024).

Table 1. Contents of the reaction mixture

Gene name	Primer sequence (5'-3')	Conditions	Size	Reference
16s rRNA <i>S. aureus</i>	F: 5-GTAGGTGGCAAGCGTTATCC-3 R: 5-CGCACATCAGCGTCAG-3	Step1: 94°C, 5 min Step 2: 58°C, 1 min	228 bp	Løvseth <i>et al.</i> 2004
16s rRNA <i>P. aeruginosa</i>	F: 5-GGGGGATCTTCGGACCTC-3 R: 5-TCCTTAGAGTGCCACCG-3	Step3:72°C, 1 min Step5: 72°C,5 min	956 bp	Spilker <i>et al.</i> 2004

2.5. Antibacterial Activity Assay

The antibacterial efficacy of Cu-ZIF-8 against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was evaluated using the standard agar well diffusion assay on Mueller–Hinton agar plates. 1 mL of the suspension of each tested bacterial culture containing 1.5×10^8 CFU/mL was spread onto the agar surface using a sterile cotton swab. Wells were prepared using a sterile disc dispenser (8 mm) in diameter. 100 μ L of 12.5, 25, 50, and 100 μ g/mL Cu-ZIF-8 was added to the wells, and saline solution was added as a negative control for Gram-negative and Gram-positive bacteria. Incubate all plates at 37°C for 24 h. Then, measure the diameter of the inhibition zone surrounding each well (Ojaimi & Shliouh 2025).

2.6. Results Analysis

We analyzed the results of this investigation using SPSS version 23 (IBM Corp., Armonk, NY, USA) and applied the Chi-square test to find the relationship between categorical variables. A P-value of 0.05 was considered statistically significant.

3. Results

A total of 150 burn swabs were cultured, 54 (36%) bacterial isolates were obtained and identified, distributed as 30/54 (55.5%) *S. aureus* and 24/54 (44.4%) *Pseudomonas aeruginosa* (Figure 1).

Phenotypic identification of *Staphylococcus aureus* appeared as large colonies with hemolytic activity on blood agar, as shown in Figure 2A. In comparison, it appeared as yellow colonies that tolerate salts on mannitol salt agar. It appeared as Gram-positive purple clusters under the microscope. *P. aeruginosa* appeared as large, smooth, elevated colonies and was pale because it is a non-lactose fermenter with an irregular edge on MacConkey agar. On blood agar, the colonies were green-black and hemolytic, as shown in Figure 2B, while on Cetrimide agar, they were mucoid, smooth, and elevated, with a creamy or green color. They appeared as Gram-negative pink bacilli under the microscope. Finally, there was genetic identification through 16s rRNA gene, as shown in Figure 3.

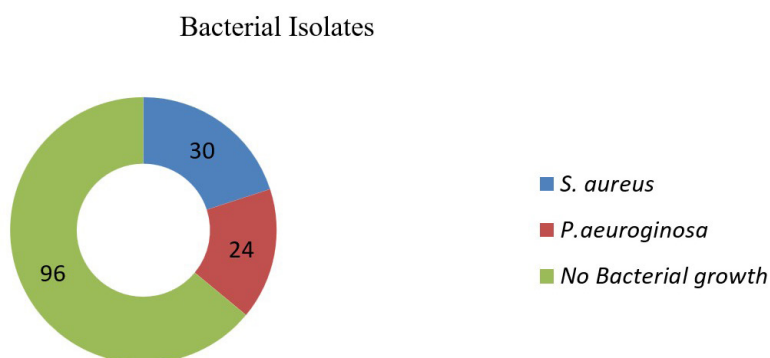


Figure 1. The percentage of clinical bacterial isolates isolated from burns



Figure 2. Cultural characterization of (A) *Staphylococcus aureus* (B) *Pseudomonas aeruginosa* on blood agar

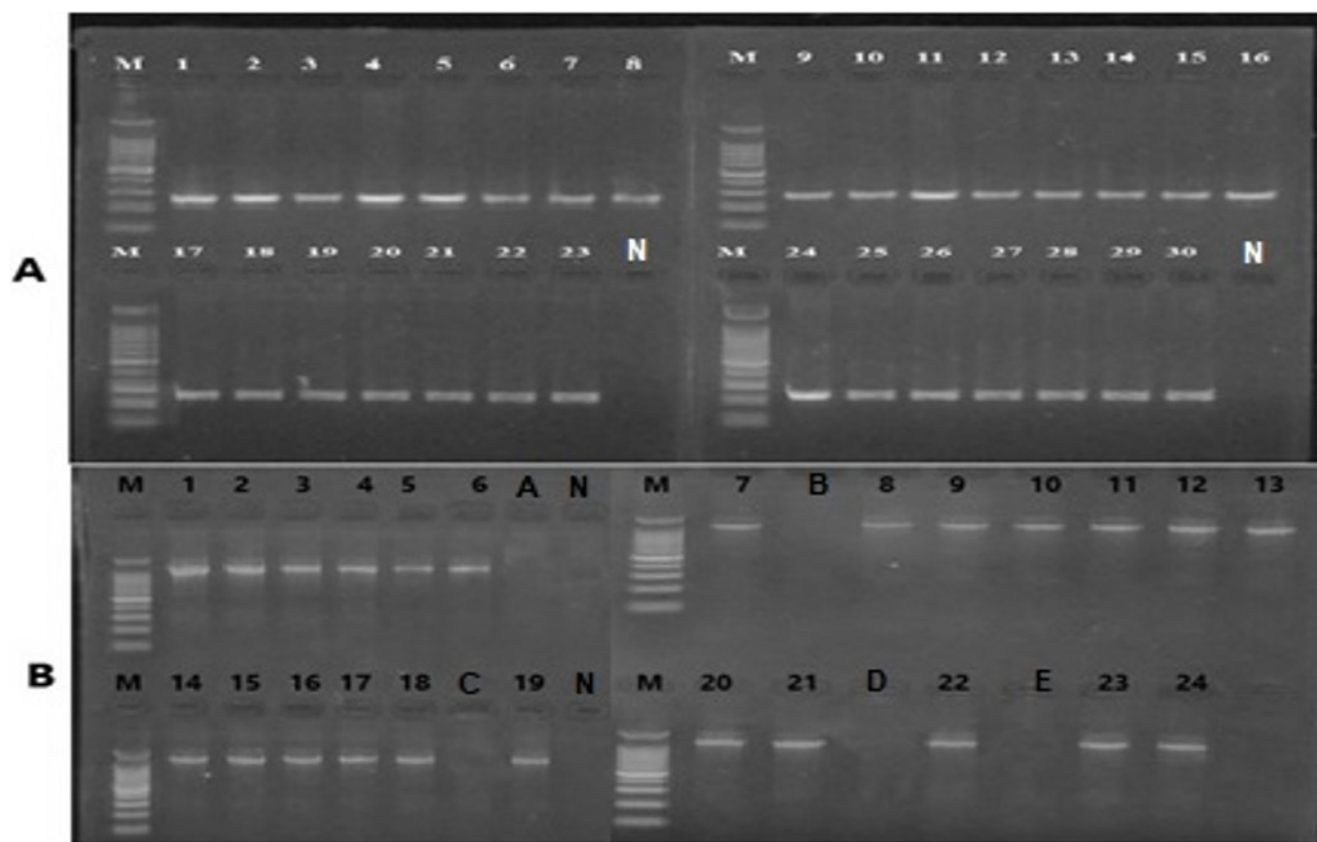


Figure 3. PCR amplicons of the 16S rRNA gene for (A) *Staphylococcus aureus* product size (288 bp), lanes 1–30= positive isolates, (B) *Pseudomonas aeruginosa* product size (956 bp), lanes 1–24= positive isolates, lanes A, B, C, D, and E = negative isolates, Lane M= reference markers (1,000 bp), lane N= negative control (double-distilled H₂O)

3.1. Antibiotic Resistance Rate

Our findings showed that *Staphylococcus aureus* has high resistance to antibiotics, 70% resistance to Benzylpenicillin, 62% to Tetracycline, 60% to Clindamycin, 55% to Gentamicin, and 50% to Levofloxacin. Resistance of *Pseudomonas aeruginosa* to the currently studied antibiotics was (67%, 100%, 25%, 71%, 75%) against Amikacin, Ceftazidime, Meropenem, Ciprofloxacin, and Aztreonam, respectively, since there are (16, 24, 6, 17, 18) resistant isolates against the tested antibiotics, respectively.

3.2. Characteristics of the Prepared Cu-ZIF-8

Figure 4 presents the SEM images of the synthesized Cu-BDC@ZIF-8 sorbent at different magnifications and synthesis stages. In the first stage, panels A–C show the successful deposition of ZIF-8 crystals onto the pencil graphite substrate. At low magnification (A), a uniform coating layer is observed. At higher magnifications (B and C),

well-defined polyhedral ZIF-8 crystals are clearly distributed on the surface, confirming the formation of the ZIF-8 structure. Following the galvanic replacement process, panels D–F demonstrate significant morphological changes. The Zn²⁺ ions were substituted with Cu²⁺ ions, which resulted in rough surfaces and defects on the crystal surfaces. This suggests that copper was incorporated and new active sites have been formed on the surface of the sorbent.

The characteristic functional groups of the electrodeposited Cu-ZIF-8 were investigated using FT-IR in the range of 500–4,000 cm⁻¹. The C-H stretching modes at 2,855, 2,925, and 2,955 cm⁻¹, as well as the aromatic C-H stretching pattern, were observed at 3,435 cm⁻¹. The N-H stretching band was observed in 2-MIM, but it disappeared in the prepared adsorbent material, leading to a specific conclusion. This finding demonstrates that there was no FT-IR-observable uncoordinated 2-MIM in the sorbent because practically all 2-MIM has

been deprotonated and unobserved in 2-MIM but disappeared in the prepared sorbent, leading to incorporation into the crystal structure as shown in Figure 5.

To determine the elemental composition of the synthesized ZIF-8 compound, EDX analysis was

performed, and the corresponding spectrum is shown in Figure 6A. The spectrum confirmed the presence of carbon (C), nitrogen (N), oxygen (O), and zinc (Zn) elements in the structure of the prepared ZIF-8. The inset table presents the atomic percentages of these constituent elements obtained from the initial

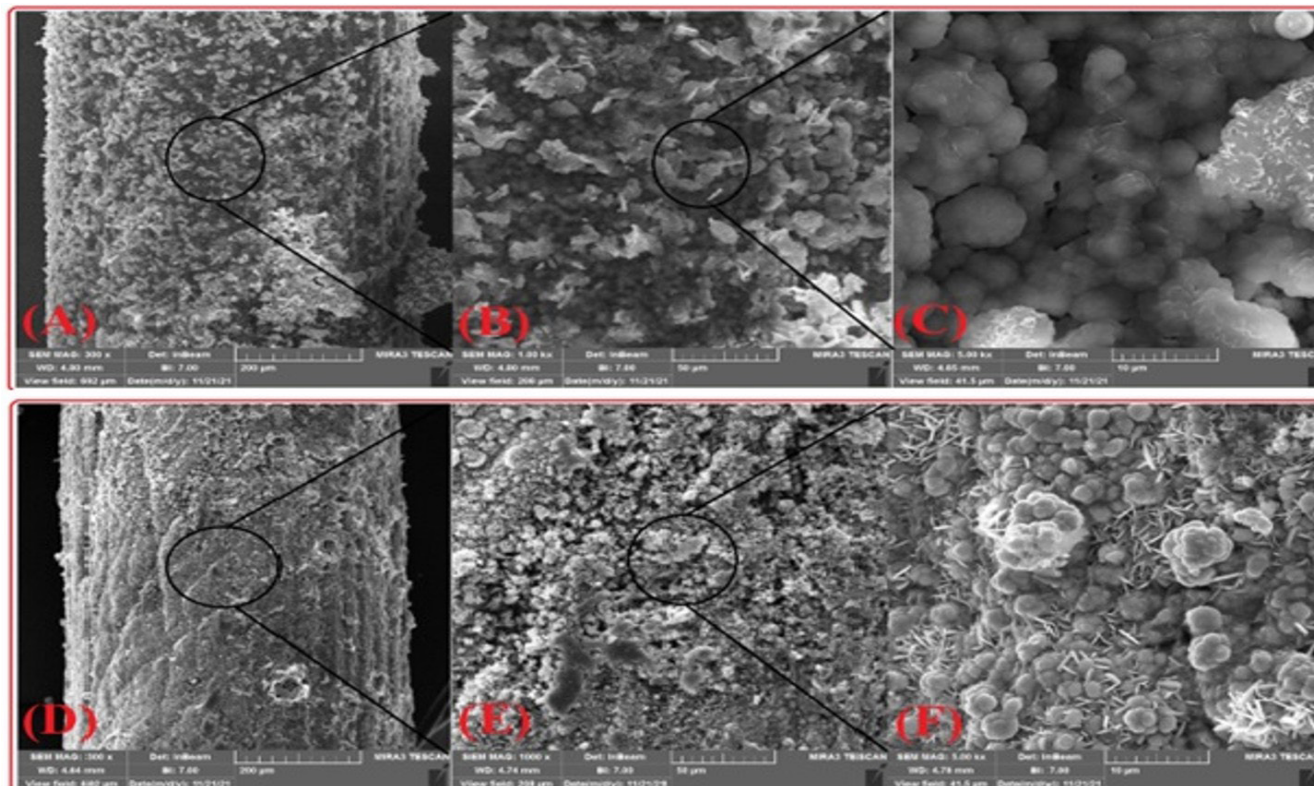


Figure 4. SEM images of the synthesized sorbent showing different morphological configurations. (A-C) ZIF-8 crystals grown on a pencil graphite substrate. (D-F) Cu/ZIF-8 after the galvanic replacement process showing morphological changes

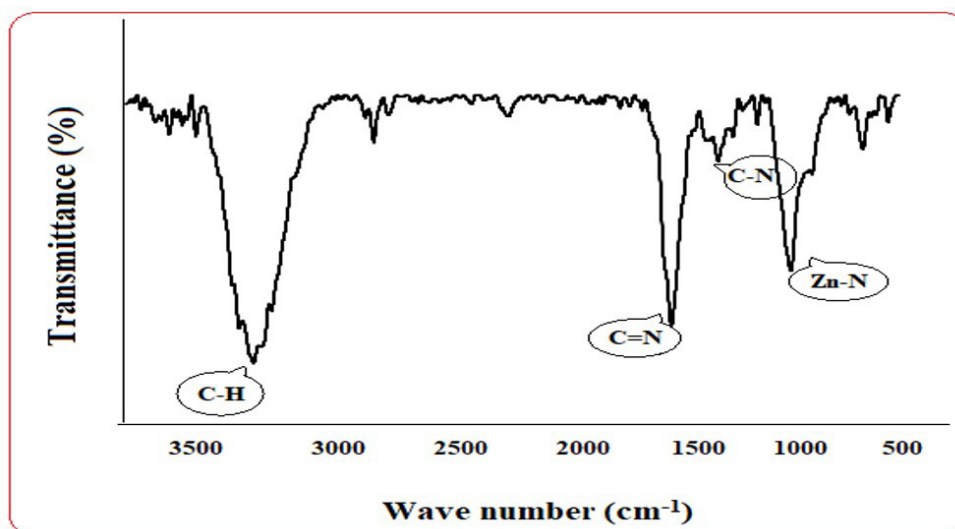


Figure 5. FT-IR spectrum of prepared Cu-ZIF-8 nanostructures electrodeposited on pencil graphite

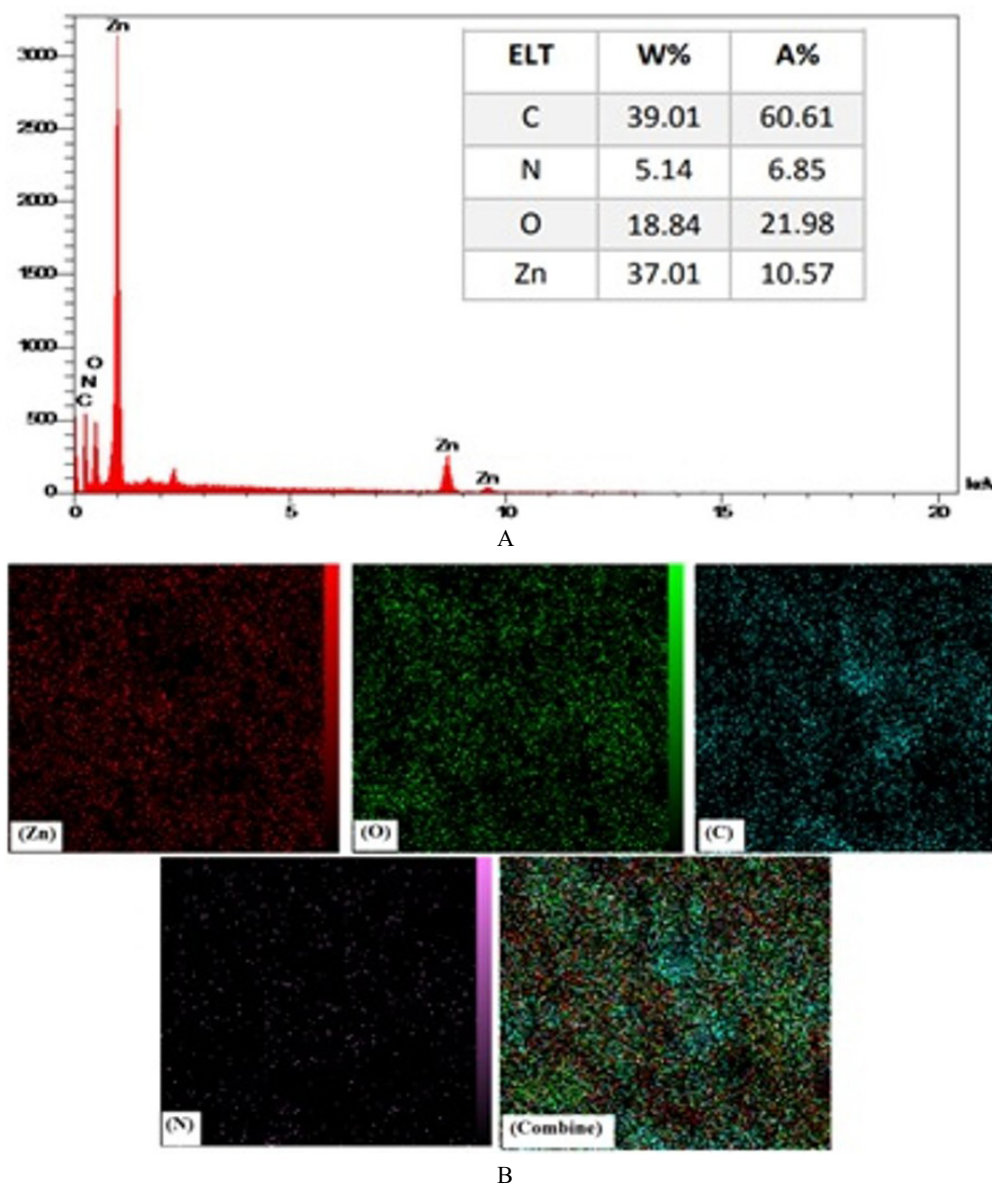


Figure 6. (A) EDX spectrum of the synthesized Cu-ZIF-8 composite with the inset table showing elemental composition, (B) elemental mapping images showing the distribution and uniform dispersion of Zn, O, C, and N elements on the surface. Color codes: C = red, N = blue, O = green, Zn = cyan, Cu = yellow

EDX analysis. To further investigate the elemental composition of the final composite sorbent, EDX analysis was also conducted, and the resulting spectrum is displayed in Figure 6B. The spectrum revealed the presence of carbon (C), nitrogen (N), zinc (Zn), and copper (Cu) incorporated into the composite structure. The atomic percentages of these elements (obtained from the EDX analysis) are given in the accompanying table. Also, the elemental mapping images show the distribution of atoms over the surface of the sorbent. In general, the EDX analysis was a useful technique for the initial elemental analysis presented in Figure 6.

In XRD, emitted X-rays are used to detect the individual elements. The various elements are related to energy peaks. The results indicated that the surface of the constructed fiber was richly oxygenated, which was indicated by the consistent delivery of oxygen over the surface. Additionally, the analytes' similar adsorption process toward the MOF involves hydrogen bonding and stacking. Thus, the extraction of the target analytes may be caused by the electron donor-acceptor interactions or hydrophobic interactions. As shown in Figure 7. The XRD pattern showed the typical peaks at 2θ with Miller indices of (001), (002), (112), (022), (012),

and (222) of ZIF-8. A slight decrease in full width at half maximum (FWHM) (011) was also observed with increasing copper doping ratio. Therefore, the substituted copper atom does not distort the lattice structure of ZIF-8 crystals, because the ionic radius of Cu^{2+} (0.71 Å) is slightly smaller than that of Zn^{2+} (0.74 Å) in the tetrahedral configuration. The prepared sorbent's excellent, sharp peaks confirmed its good crystallinity. The obtained diffraction peaks closely resemble the expected pattern.

3.3. Antibacterial Activity of Cu-ZIF-8

The antibacterial activity of Cu-ZIF-8 nanoparticles was investigated *in vitro* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, isolated from burn patients. According to our findings, Cu-ZIF-8 NPs exhibit high antibacterial activity against the examined *P. aeruginosa* strains compared with *S. aureus* at various concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL, 1,000 µg/mL). The highest bactericidal efficacy was observed at a concentration of 50 µg/mL, with *P. aeruginosa* (a Gram-negative bacterium) exhibiting an IZD of 50 mm. In contrast, *S. aureus* (Gram-positive bacterium) showed lower inhibition at the same concentration, with an inhibition zone diameter of 45 mm. This indicates that a 50 µg/mL concentration of Cu-ZIF-8 is efficient and safe, making it a viable option for potential future therapeutic applications. *P. aeruginosa* and *S. aureus* showed inhibition zones of 30 and 25 mm, respectively, at a concentration of 25 µg/mL of Cu-ZIF-8. In contrast, the inhibition zones of 44 and 40 mm at a concentration of 100 µg/mL Cu-ZIF-8 against *P. aeruginosa* and *S. aureus*, respectively, as shown in Table 2. The efficiency of the Cu-ZIF-8 was evaluated by measuring the diameter of the inhibition zone. We noted that the potency of the Cu-ZIF-8 is directly proportional to the inhibition

zone diameter, as the potency increases with increasing Cu-ZIF-8 concentration.

4. Discussion

The spread of *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibiting multidrug resistance (MDR) has led to heightened nosocomial infections in Iraq, particularly in burn hospitals. Analysing 54 *S. aureus* and *P. aeruginosa* strains isolated from burn infections provided clear insights into the prevalence in hospitals. Our ability to successfully isolate and identify these two bacteria indicates the important role of *S. aureus* and *P. aeruginosa* in causing burn infections. This calls on us to follow preventive procedures and guidelines to limit the infections. Many previous researchers have confirmed a high rate of infection with *S. aureus* and *P. aeruginosa* isolated from burns in hospitals (Tchakal-Mesbahi *et al.* 2021). This highest rise confirms proper management using antibiotics to reduce the spread of infections and control antibiotic-resistant bacteria. Therefore, the spread of MDR *P. aeruginosa* represents a substantial global health problem in managing nosocomial infections (Pachori *et al.* 2019). In the burn unit, patients are more susceptible to bacterial infections due to the destruction of skin layers and a weakened immune system, which can lead to acute symptoms and high mortality for patients,

Table 2. Bactericidal activity (mm) of Cu-ZIF-8 MOF ($p < 0.05$)

u-ZIF-8 MOF	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
100 µg/ mL	40 mm	44 mm
50 µg/ mL	45 mm	50 mm
25 µg/mL	25 mm	30 mm
12.5 µg/ mL	10 mm	15 mm
Control	0	0
χ^2		.915
P		0

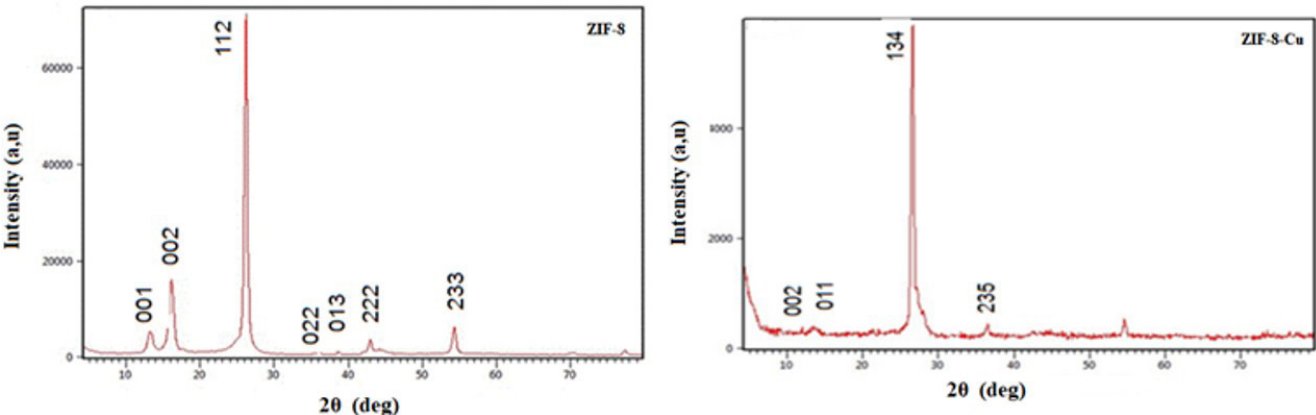


Figure 7. X-ray diffraction spectrum pattern of ZIF-8 and ZIF-8-Cu

due to the patients staying inside the wards for a long time (Norbury *et al.* 2016). In previous years, the inappropriate use of antibiotics has led to the development of MDR *P. aeruginosa* strains. This has led to increased anxiety among hospital staff due to the unavailability of antibiotics active against these bacteria (Dabbousi *et al.* 2022).

Our investigation produced *P. aeruginosa* isolates with high rates of antibiotic resistance. This constitutes a significant health challenge to Iraqi healthcare hospitals. The high rates of *P. aeruginosa* resistance to cephalosporins, monobactams, aminoglycosides, and fluoroquinolones are due to its ability to adapt and the high flexibility of its genetics. In addition, it has resistance to withstand harsh environmental conditions in hospitals, especially in the burn unit. Our findings revealed that meropenem was the most active antibiotic in this study, with an activity of 25% against *P. aeruginosa*. This result was in agreement with the study published by Sadeghi *et al.* 2025 in Iran, but higher than the results obtained by other researchers from Iraq (Polse *et al.* 2024). In contrast, our study showed the highest level of resistance rates to ceftazidime (100%), Aztreonam (75%), ciprofloxacin (71%), and Amikacin (67%). These resistance rates are consistent with the results published by (Mondol *et al.* 2025) in Bangladesh, but lower than those found by (Rout *et al.* 2025) in India, and higher than the study conducted by (Hamad & Mahmud 2025) in Iraq. The *P. aeruginosa* are characterized by possessing several mechanisms of resistance to different antibiotics. The first of these is the production of beta-lactamase enzymes (Mirghani *et al.* 2022). Furthermore, the resistance of *P. aeruginosa* to antibiotics is also enhanced by its possession of a protective layer surrounding it, known as biofilm, which acts as a physical barrier preventing antibiotics from entering the bacterial cell (Singh *et al.* 2021). *P. aeruginosa* resists antibiotics due to possessing efflux pumps that work to push the antibiotics out of the bacterial cell, thus reducing their concentration inside it (Gaurav *et al.* 2023). The emergence of MDR strains has made treating *S. aureus* infections more difficult, creating a serious concern for healthcare staff worldwide. MRSA (methicillin-resistant *S. aureus*) resists nearly all beta-lactam antibiotics, which increases its pathogenicity and allows it to cause certain clinical symptoms (Ghareeb *et al.* 2023). Our study revealed that the overall isolation rate of *S. aureus* was 30%. This rate exceeded those reported in Iraq (Hamed *et al.* 2016), who reported a rate of only 7.53%. However, this result was lower than that reported in Tunis (64%), in which samples were taken from patients with soft tissue and skin infections (Kmiha *et al.* 2023).

These discrepancies might be related to variations in the geographical region, study population, and sample type. The present study provides evidence of *S. aureus* resistance to various antimicrobial drugs. Benzylpenicillin had the highest resistance rate (70%), followed by Tetracycline (62%), clindamycin (60%), gentamicin (55%), and levofloxacin (50%). This rate exceeded those reported in Iraq by Al-Dahbi & Al Mathkhury 2013, who reported the rates were Tetracycline (34.9%), Clindamycin (29.3%), and Gentamicin (50%). A variety of factors affect the development of antimicrobial resistance, including self-medication, irrational antibiotic use, inadequate healthcare facilities, and a lack of routine susceptibility testing prior to antibiotic administration. Notably, our study revealed that approximately half of the *S. aureus* isolates were resistant to levofloxacin. These findings indicate that *S. aureus* infections within the study area can no longer be empirically treated with levofloxacin, highlighting the need for an urgent review of the current situation and control of antimicrobial use in healthcare settings. Naimi *et al.* (2017) reported that susceptibility rates of *S. aureus* isolates to the following antibiotics were, Clindamycin (50%), Cefoxitin (50%), while 75% of these isolates were resistant to Tetracycline and Gentamicin. The mechanisms of resistance to aminoglycosides such as Clindamycin in *S. aureus* include reduced uptake, target alterations in the 30S ribosomal subunit, and enzymatic modification of the aminoglycoside drugs (Vestergaard *et al.* 2019).

Recently, Cu-ZIF-8-MOF has attracted considerable attention in antibacterial studies due to its various antibacterial properties, including the continuous production of internal and external antibacterial components. Chemical catalysis to produce reactive oxygen species (ROS). Therefore, in our research, we used a Cu-ZIF-8-MOF to eliminate MDR *S. aureus* and *P. aeruginosa* using the well diffusion method. The Cu-ZIF-8-MOF demonstrated its high ability to kill MDR *S. aureus* and *P. aeruginosa*, as shown by the formation of a large inhibition zone in the medium, which was consistent with several previous investigations that resulted in the efficiency of Cu-based antibacterial agents against MDR *S. aureus* and *P. aeruginosa* (Jabbar *et al.* 2023; Yu *et al.* 2025). Our results also showed that a concentration of 50 was more effective in killing MDR *S. aureus* and *P. aeruginosa*. Our results agree with the findings of previous studies (Sheta *et al.* 2022; Wang *et al.* 2024). The antibacterial effect of Cu-ZIF-8-MOF is believed to be attributed to the production of large amounts of metallic copper ions that penetrate bacterial membranes directly, or

through ion channels or transport proteins, leading to loss of communication on the bacterial surfaces and damage to the cell membrane, in addition to the release of the outer membrane and cytosol. This enables the substance to infiltrate the cell and interact with components of the bacterial cell wall such as proteins, ribosomes, enzymes, lysosomes, and genetic material, causing oxidative stress that leads to distortion of the cell wall structure, changes in cell membrane permeability, enzyme function limitation, disruption of electrolytic activity, denaturation of proteins, and impact on gene expression (Liu *et al.* 2021). Cu-ZIF-8-MOF helps in the production of reactive oxidative species (ROS) like superoxide anions, hydroxyl radicals, and singlet oxygen, and causes mechanical spoilage to the bacterial cell. Through the binding of ROS to the bacterial cell membrane and their penetration, they then attach to the amino, hydroxyl, and sulfhydryl functional groups of the bacteria that are involved in the metabolic processes of the bacterial cell (Santo *et al.* 2012).

The growth of *S. aureus* was slightly inhibited at 12.5 µg/mL of MOF. A higher inhibition zone was observed at a concentration of 50 µg/mL; Then CFU of *S. aureus* showed Cu-ZIF-8-MOF concentration-dependent decreases. Multiple studies have demonstrated its effectiveness against various bacteria, including *S. aureus*. Binding to lipopolysaccharides, peptidoglycans, or carboxylic groups of bacteria, Cu²⁺ ions have been found to cause damage by disrupting the bacterial envelope (Mitra *et al.* 2020). This disruption is brought about by membrane depolarization due to the binding of Cu²⁺ ions to negatively charged domains, leading to a reduction in potential. Ultimately, this process may result in membrane leakiness or even complete rupture (Kumar *et al.* 2023). Bacterial cell damage can also be induced by the internalization or binding of Cu nanoparticles (NPs) by bacteria. Further, reactive oxygen species (ROS) generated by Cu NPs can lead to oxidative stress, eventually leading to deoxyribonucleic acid (DNA) damage and lipid peroxidation in the bacterial membrane (Tian *et al.* 2025). The material exhibits significant inhibition zones, making it promising for combating antibiotic-resistant strains. The results show that Cu-ZIF-8-NPs were more effective in eliminating *S. aureus* (Gram-positive) than *P. aeruginosa* (Gram-negative) because Gram-positive bacteria lack an outer cell membrane, and they are more susceptible to this invasion (Chowdhuri *et al.* 2017).

In conclusion, in our *in vitro* study, we have successfully synthesized Cu-ZIF-8-MOF. The prepared MOFs exhibited high antibacterial activity against MDR *P. aeruginosa* and *S. aureus*. Highlights the use of MOFs

as future alternatives for addressing burn infections. Cu-MOFs offer a new path to inhibit MDR Gram-positive and Gram-negative bacteria. Additionally, the unique structural characteristics of these MOFs suggest their potential for various applications, including antibacterial effectiveness and targeted drug delivery.

References

- Al-Bdere, N.M.H., Al-Janabi, N., Al-saad, N.F.N., Al-Mousawi, H.T.M. and Abbas, M.D., 2024. Enterobacter cloacae lipopolysaccharide export system protein (lptc) gene expression variation via expose to biosynthesized zinc oxide nanoparticles. *Journal of Microbiology, Biotechnology and Food Sciences*. 14, e10785-e10785. <https://doi.org/10.55251/jmbfs.10785>
- Al-Dahbi, A., Al-Mathkhury, H., 2013. Distribution of methicillin resistant *Staphylococcus aureus* in Iraqi patients and healthcare workers. *Iraqi Journal of Science*. 54, 293-300.
- Antimicrobial Resistance Collaborators 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 399, 629-655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- Brandenburg, K.S., Weaver, A.J., Jr, Karna, S.L.R., Leung, K.P., 2021. The impact of simultaneous inoculation of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* on rodent burn wounds. *Burns*. 47, 1818-1832. <https://doi.org/10.1016/j.burns.2021.02.025>
- Carson, C.G., Hardcastle, K., Schwartz, J., Liu, X., Hoffmann, C., Gerhardt, R.A., Tannenbaum, R., 2009. Synthesis and structure characterization of copper terephthalate metal-organic frameworks. *Eur. J. Inorg. Chem.* 2009, 2338-2343. <https://doi.org/10.1002/ejic.200801224>
- Chowdhuri, A.R., Das, B., Kumar, A., Tripathy, S., Roy, S., Sahu, S.K., 2017. One-pot synthesis of multifunctional nanoscale metal-organic frameworks as an effective antibacterial agent against multidrug-resistant *Staphylococcus aureus*. *Nanotechnology*. 28, 095102. <https://doi.org/10.1088/1361-6528/aa57af>
- Dabbousi, A.A., Dabboussi, F., Hamze, M., Osman, M., Kassem, I.I., 2022. The emergence and dissemination of multidrug resistant *Pseudomonas aeruginosa* in Lebanon: current status and challenges during the economic crisis. *Antibiotics*. 11, 687. <https://doi.org/10.3390/antibiotics11050687>
- Dung, N.T., Thuy, U.T.D., Thien, V.T., Thuy, N.T., Tien, N.T.C., Lin, K.Y.A., Huy, N.N., 2022. Mechanism insight into the photocatalytic degradation of fluoroquinolone antibiotics by the ZIF-8@ Bi 2 MoO 6 heterojunction. *Environmental Science: Nano*. 9, 3973-3991. <https://doi.org/10.1039/D2EN00219A>
- Fadhil Abdul-Husin, I., Sabri Abdul-Razzaq, M., 2021. Plasmid-mediated mechanism of quinolone resistance on *E. coli* isolates from different clinical samples. *Archives of Razi Institute*. 76, 561-573. <https://doi.org/10.22092/ari.2021.355392.1679>
- Gaurav, A., Bakht, P., Saini, M., Pandey, S., Pathania, R., 2023. Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. *Microbiology*. 169, 001333. <https://doi.org/10.1099/mic.0.001333>

- Ghareeb, N.H., Obaid, S.S., Jumaah, I.A.M., 2023. The prevalence of multidrug-resistant *Staphylococcus aureus* isolated from different clinical samples. *Medical Journal of Babylon*. 20, 185-187. https://doi.org/10.4103/MJBL.MJBL_239_22
- Hamad, B.K., Mahmud, M.A., 2025. Molecular detection of blaVIM and blaNDM in multidrug-resistant *Pseudomonas aeruginosa* from cancer and burn patients in Erbil, Iraq. *Frontiers in Microbiology*. 16, 1672531. <https://doi.org/10.3389/fmicb.2025.1672531>
- Hamed, S.L., Rasool, K.H., Hussein, N.H., Taha, B.M., 2016. Prevalence of bacteria isolated from patients with burn infection at a burn hospital in Baghdad and study of their antimicrobial susceptibility patterns. *Al-Kindy College Medical Journal*. 12, 64-67.
- Hamza, Z.S., Abdul-Husin, I.F., Jawad, Z.N., 2026. A review on nanoparticle biosynthesis from various organisms and their use in nanomedicine. *Microbes and Infectious Diseases*. 7, 4253-4273.
- Jabbar, A., Rehman, K., Jabri, T., Kanwal, T., Perveen, S., Rashid, M. A., Kazi, M., Ahmad Khan, S., Saifullah, S., Shah, M.R., 2023. Improving curcumin bactericidal potential against multi-drug resistant bacteria via its loading in polydopamine coated zinc-based metal-organic frameworks. *Drug Delivery*. 30, 2159587. <https://doi.org/10.1080/10717544.2022.2159587>
- Kmiha, S., Jouini, A., Zerriaa, N., Hamrouni, S., Thabet, L., Maaroufi, A., 2023. Methicillin-resistant *Staphylococcus aureus* strains isolated from burned patients in a tunisian hospital: molecular typing, virulence genes, and antimicrobial resistance. *Antibiotics*. 12, 1030. <https://doi.org/10.3390/antibiotics12061030>
- Kumar, P., Behera, A., Tiwari, P., Karthik, S., Biswas, M., Sonawane, A., Mobin, S.M., 2023. Exploring the antimicrobial potential of isoniazid loaded Cu-based metal-organic frameworks as a novel strategy for effective killing of *Mycobacterium tuberculosis*. *Journal of materials chemistry. B*. 11, 10929-10940. <https://doi.org/10.1039/D3TB02292G>
- Liu, J., Wu, D., Zhu, N., Wu, Y., Li, G., 2021. Antibacterial mechanisms and applications of metal-organic frameworks and their derived nanomaterials. *Trends in Food Science & Technology*. 109, 413-34. <https://doi.org/10.1016/j.tifs.2021.01.012>
- Løvseth, A., Loncarevic, S., Berdal, K.G., 2004. Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. *Journal of Clinical Microbiology*. 42, 3869-3872. <https://doi.org/10.1128/JCM.42.8.3869-3872.2004>
- Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S., Rijal, G., 2022. Biofilms: formation, drug resistance and alternatives to conventional approaches. *AIMS microbiology*. 8, 239-277. <https://doi.org/10.3934/microbiol.2022019>
- Mitra, D., Kang, E.T., Neoh, K.G., 2020. Antimicrobial copper-based materials and coatings: potential multifaceted biomedical applications. *ACS Applied Materials & Interfaces*. 12, 21159-21182. <https://doi.org/10.1021/acsami.9b17815>
- Mondol, S.M., Islam, M.R., Mia, M.E., Hassan, M.H., Farhad, F., Akter, K., Shakil, S.K., Islam, I., Rakhi, N.N., Mustary, J.F., Amiruzzaman, Rahaman, M.M., 2025. Molecular and genomic insights into multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Pseudomonas aeruginosa* causing burn wound infections in Bangladesh. *Scientific Reports*. 15, 25445. <https://doi.org/10.1038/s41598-025-11614-6>
- Mulatu, D., Zewdie, A., Zemedu, B., Terefe, B., Liyew, B., 2022. Outcome of burn injury and associated factor among patient visited at Addis Ababa burn, emergency and trauma hospital: a two years hospital-based cross-sectional study. *BMC Emergency Medicine*. 22, 199. <https://doi.org/10.1186/s12873-022-00758-7>
- Naimi, H.M., Rasekh, H., Noori, A.Z., Bahaduri, M.A., 2017. Determination of antimicrobial susceptibility patterns in *Staphylococcus aureus* strains recovered from patients at two main health facilities in Kabul, Afghanistan. *BMC Infectious Diseases*. 17, 737. <https://doi.org/10.1186/s12879-017-2844-4>
- Norbury, W., Herndon, D.N., Tanksley, J., Jeschke, M.G., Finnerty, C.C., 2016. Infection in Burns. *Surgical Infections*. 17, 250-255. <https://doi.org/10.1089/sur.2013.134>
- Ojaimi, R.R., Shliouh, N.H., 2025. Chemical synthesis, characterization, and antibacterial activity of copper oxide nanoparticles: *in vitro* study on uropathogenic bacteria. *BBRJ*. 9, 264-268. https://doi.org/10.4103/bbrj.bbrj_198_25
- Pachori, P., Gothwal, R., Gandhi, P., 2019. Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes & Diseases*. 6, 109-119. <https://doi.org/10.1016/j.gendis.2019.04.001>
- Pierce, V.M., Bhowmick, T., Simner, P.J., 2023. Guiding antimicrobial stewardship through thoughtful antimicrobial susceptibility testing and reporting strategies: an updated approach in 2023. *Journal of Clinical Microbiology*. 61, e0007422. <https://doi.org/10.1128/jcm.00074-22>
- Polse, R.F., Khalid, H.M., Mero, W.M.S., 2024. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from burns infections. *Journal of Contemporary Medical Sciences*. 10, 7-12. <https://doi.org/10.22317/jcms.v10i1.1415>
- Rout, S., Kumar, R., Sahu, K.K., 2025. Molecular identification of *Pseudomonas aeruginosa* isolated from burn patients by polymerase chain reaction in central Odisha, India. *Journal of Integrative Medicine and Research*. 3, 109-116. https://doi.org/10.4103/jimr.jimr_9_25
- Sadeghi, M., Mobayen, M., Yaghubi Kalurazi, T., Mehrdad, Z., Gaskarei, M.K., Moghadam, S.K., Sedigh Ebrahim-Saraie, H., Hasannejad-Bibalan, M., 2025. Epidemiological trends and evolving antibiotic resistance profiles of *Pseudomonas aeruginosa* in burn patients: a 3-year cross-sectional surveillance in Northern Iran. *Health Science Reports*. 8, e71054. <https://doi.org/10.1002/hsr.2.71054>

- Santo, C.E., Quaranta, D., Grass, G., 2012. Antimicrobial metallic copper surfaces kill *Staphylococcus haemolyticus* via membrane damage. *MicrobiologyOpen*. 1, 46-52. <https://doi.org/10.1002/mbo3.2>
- Sheta, S.M., Salem, S.R., El-Sheikh, S.M., 2022. A novel Iron (III)-based MOF: synthesis, characterization, biological, and antimicrobial activity study. *Journal of Materials Research*. 37, 2356-2367. <https://doi.org/10.1557/s43578-022-00644-9>
- Singh, S., Datta, S., Narayanan, K.B., Rajnish, K.N., 2021. Bacterial exo-polysaccharides in biofilms: role in antimicrobial resistance and treatments. *Journal, Genetic Engineering & Biotechnology*. 19, 140. <https://doi.org/10.1186/s43141-021-00242-y>
- Spilker, T., Coenye, T., Vandamme, P., LiPuma, J.J., 2004. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. *Journal of Clinical Microbiology*. 42, 2074-2079. <https://doi.org/10.1128/JCM.42.5.2074-2079.2004>
- Tchakal-Mesbahi, A., Abdouni, M.A., Metref, M., 2021. Prevalence of multidrug-resistant bacteria isolated from burn wounds in Algeria. *Annals of Burns and Fire Disasters*. 34, 150-156.
- Tian, Y., Wang, B., Zhang, Z., Kai, T., Wu, P., Ding, P., 2025. Recent advances in metal-organic frameworks for antibacterial applications: mechanisms and emerging strategies. *RSC Advances*. 15, 26710-26727. <https://doi.org/10.1039/D5RA02955D>
- Vestergaard, M., Frees, D., Ingmer, H., 2019. Antibiotic resistance and the MRSA problem. *Microbiology Spectrum*. 7, 10.1128. <https://doi.org/10.1128/microbiolspec.GPP3-0057-2018>
- Wang, X., Wang, H., Zhang, J., Han, N., Ma, W., Zhang, D., Yao, M., Wang, X., Chen, Y., 2024. Peroxidase-like active Cu-ZIF-8 with rich copper (I)-nitrogen sites for excellent antibacterial performances toward drug-resistant bacteria. *Nano Research*. 17, 7427-7435. <https://doi.org/10.1007/s12274-024-6699-x>
- Yeerken, A., Lin, J., Wang, X., Luo, Y., Ma, H., 2023. Fabrication of novel porous ZIF-67/PES composite microspheres and the efficient adsorption of triphenylmethane dyes from water. *Cryst Eng Comm*. 25, 1076-1089. <https://doi.org/10.1039/D2CE01486F>
- Yu, Z., Fu, X., Lucas, T., Zhao, H., Chen, C., Dubail, I., Chen, Y., Patriarche, G., Gateau, J., Gazeau, F., Jamet, A., Lepoitevin, M., Serre, C., 2025. MOF-enhanced phototherapeutic wound dressings against drug-resistant bacteria. *Advanced healthcare materials*. 14, e2402418. <https://doi.org/10.1002/adhm.202402418>
- Zhang, X., Peng, F., Wang, D., 2022. MOFs and MOF-derived materials for antibacterial application. *Journal of Functional Biomaterials*. 13, 215. <https://doi.org/10.3390/jfb13040215>