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Study of the Efficacy of Colistin against Wound Contamination by Acinetobacter baumannii

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ABSTRACT

Background.Acinetobacter baumannii is a multidrug-resistant (MDR) Gram-negative pathogen commonly associated with wound infections, particularly in hospital and battlefield settings. Due to its resistance to multiple antibiotic classes, treatment options are limited. Colistin (polymyxin E), a last-resort antibiotic, has been reintroduced for treating MDR A. baumannii infections. Objective: This study aimed to evaluate the efficacy of colistin against A. baumannii isolated from wound contamination cases. Methods: A total of (30) wound swabs were collected from patients with clinical signs of infection. Isolation and identification of (10) isolations of A. baumannii and (20) were different species. A. baumannii conducted using standard microbiological and biochemical methods, followed by confirmation via Vitek 2 compact. Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method, and colistin minimum inhibitory concentration (MIC) was determined by broth microdilution following CLSI/EUCAST guidelines. Results: According to this study on Acinetobacter baumannii, the bacteria might stop the bacteria's development at various colistin concentrations. In contrast to 500 µg/ml, the higher concentration (1000 µg/ml) generated a wider zone of inhibition, indicating dose-dependent antibacterial action. A wider zone of inhibition was seen at the higher concentration, suggesting dose-dependent antibacterial action. The study also discovered that following skin damage, skin cells progressively restored to their original architecture, however inflammatory cells and cellular debris persisted. Significant necrosis was seen in the second group, suggesting a serious inflammatory reaction. The third group displayed epidermal cell regeneration, with the group that received 1000 µg/ml of colistin exhibiting more noticeable regeneration. Conclusion: Colistin remains a highly effective antibiotic against MDR A. baumannii in wound infections. However, its use should be monitored due to the risk of emerging resistance and potential nephrotoxicity. Regular surveillance and antibiotic stewardship programs are essential to preserve their efficacy.

Keywords: ***Acinetobacter baumannii, Colistin, Multidrug-resistant bacteria (MDR), Healthcare-associated infections (HAIs), Colistin resistance***

1. INTRODUCTION

Acinetobacter baumannii, is a major hospital pathogen around the world because of it being an opportunistic gram-negative pathogen acquired in hospitals, in particular, in immunocompromised patients or those with traumatic or post-surgical injury. These features are also major reasons for its continuous survival on dry surfaces and medical instruments in healthcare environments and its association with different types of infection, e.g., ventilator-associated pneumonia, bloodstream, urinary tract and wound infections [1,2].

Of these infections, A. baumannii wound infections deserve special focus since the microorganism has a propensity to form biofilms, tolerance to desiccation and resistance to a broad range of antibiotics [3]. The increasing appearance of multidrug-resistant (MDR),

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extensively drug-resistant (XDR) and even pan-drug-resistant (PDR) strains has greatly reduced the therapeutic alternatives [4].

In the wake of this crisis of AMR, colistin (polymyxin E) has experienced a comeback as a last-resort antimicrobial agent for treating MDR Gram-negative bacilli, including A. baumannii [5]. Because of its known nephrotoxicity and neurotoxicity, colistin is not used widely, but its bactericidal effect through bacterial outer membrane disintegration has been recently considered [6]. Nonetheless, reports of colistin-resistant A. baumannii isolates have increased around the globe, and even in Middle Eastern countries like Iraq, the demand for local surveillance and resistance monitoring is increasing [7].

The use of colistin to treat A. baumannii wound infections is an area of clinical concern, especially in centers where empirical therapy may be challenged by impaired diagnostics and antibiotic stewardship programs. Moreover, the resistance genes (e.g., mcr, pmrA/pmrB, lpxACD) and biofilm production might impact the in vivo colistin susceptibility [8,9].

The purpose of this work is to evaluate the susceptibility to colistin in A. baumannii isolated from wound contamination of patients and to compare their sensitivity/environmental response profile. It is important to know the present trends of susceptibility of this important pathogen causing wound-associated infections, to guide the clinician for an effective therapeutic option and to control its spread in healthcare facilities.[10]**.**

2. MATERIALS AND METHODS

2.1 Study Design and Sample Collection

The present work was a cross-sectional laboratory-based study that covered the period from February 2024 to April 2025 in Ba'aqubah Teaching Hospital. Thirty wound swabs were aseptically obtained from admitted patients with infected wounds because of surgical, traumatic, or diabetic ulcers in different surgical and ICU. Specimens were taken promptly to the microbiology lab in sterile containers and were processed within 2 hours of collection.

2.2. Isolation and Identification of *Acinetobacter baumannii*

At first, 30 samples were cultured on nutrient broth for activation, after which media (blood agar, MacConkey) were used for differentiation of Acinetobacter baumannii. All the isolates were incubated at 37 °C for 24 hours, and then identified by standard biochemical tests. Colonies showing non-lactose fermentation, pale or greyish colonies, were further subcultured and subjected to standard biochemical tests, including oxidase test (negative), catalase test (positive), motility, and triple sugar iron (TSI) reactions.

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2.3 Dilution assay MICs

MICs of colistin were determined by the broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [12]. Colistin sulfate (Sigma-Aldrich, USA) serial two-fold dilutions were made in cation-adjusted Mueller-Hinton broth (CAMHB) with final concentrations from 0.25 µg/mL to 1024 µg/mL. Into each well of a sterile 96-well microtiter plate, 100 µL of a bacterial suspension prepared at the 0.5 McFarland standard (1.5×10^8 CFU/mL) and subjected to a 1:100 dilution to obtain 1.5×10^6 CFU/mL in the well, was inoculated. The plates were then incubated for 18-24 h at 37 °C in an aerobic atmosphere. Following incubation, the wells were inspected for cloudiness. MIC was defined as the lowest concentration of colistin that visually inhibits the growth of bacteria.

In the present study, the MICs of colistin against MDR A. baumannii isolates were 500-1000 µg/mL, suggesting high-level resistance. These results are higher than the CLSI susceptibility cutoff value (≤2 µg/mL), indicating multidrug resistance (MDR) and colistin resistance in the tested isolates.

2.4 Antibacterial Activity of Colistin

The antibacterial efficacy of colistin sulphate was evaluated against Acinetobacter baumannii isolates employing the method of agar well diffusion. The assay was performed following the routine method, as recommended by CLSI guidelines and the literature [13,14].

Fresh A. baumannii cultures were inoculated in nutrient broth, and incubated overnight and adjusted to 0.5 McFarland turbidity standard (~1.5×10^8 CFU/mL). The bacterial suspension was then uniformly distributed by a sterile cotton swab on MHA (Mueller-Hinton agar) plates. A 6 mm-diameter well was made in the agar by means of a sterile cork borer. Each well was also inoculated with 100 µL colistin solutions at different concentrations (500, 600, 700, 800, 900, and 1000 µg/mL). The plates were incubated for 18 – 24 h at 37°C. The diameters of zones of inhibition were measured with digital calliper after incubation. Various inhibition zones were observed for three isolates at >800 µg/mL. Nevertheless, there were no isolates that presented clear zones of inhibition at 1,000 µg/mL (even for those isolates resistant to colistin), of high-level resistance, as observed for MDR (Multi-Drug Resistance) profiles. These results are in accordance with our previous MIC values, as colistin resistance was between 500 and 1000 µg/mL.

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2.5 Experimental design

The study was approved by the college's animal ethics committee and involved twenty male albino mice weighing 23±2 grams. The mice were separated into three groups for the experiment (n = 5 per group), and each group was given intraperitoneal xylazine (5 mg/kg) to induce anaesthesia. To finish the experiment, the right flank was shaved with a disposable hand shaver after the hair was removed. Following that, a sterile lancet was used to create three parallel lines of superficial skin wounds, and the wounded skin from the second, third, and fourth infections with *A. baumannii* was swabbed out. In the positive control group, mice in the 1st group were considered and the injured skin, while also the *Acinetobacter baumannii* infected skin in the 2nd group, without treatment. The third group was treated with antibiotics at 500 µg/ml after one day post-infection, and the fourth group was treated with 1000 µg/ml. Both group treatments were repeated every 12 hrs for 3 days.

2.6 Histopathological Study

Half of the tissues from the colistin-exposed and from the control tissues were fixed in 10% neutral buffered formalin instantly for 24 to 48 hours. The fixed tissues were dehydrated through a graded series of alcohol and xylene, embedded in paraffin wax, and cut into 4–5 µm-thick sections on a rotary microtome.

Sections were performed, affixed to glass slides and stained according to routine Hematoxylin and Eosin (H&E). The inflammatory response, tissue integrity, necrosis, fibrosis, and presence of bacterial colonisation or damage were evaluated histologically using a light microscope.

The comparison was made between infected, untreated and colistin-treated samples. The colistin-treated group exhibited significantly lower inflammation infiltration, less necrosis, and the mitigation of loss of epithelial integrity compared to untreated groups, suggesting a modest protection provided by colistin on A. baumannii-induced wound-related damage

3. RESULTS AND DISCUSSION

3.1 Isolation &Identification of Gram-positive (*Acinetobacter baumannii).*

Bacterial isolates were cultured and subjected to Gram staining. Microscopic examination revealed Gram-negative coccobacilli, appearing predominantly as short rods arranged singly or in pairs. These morphological features are consistent with Acinetobacter baumannii. [12,13]

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Further confirmation was performed through biochemical and by Vitek 2 Compact device. as shown in figure (1)

Figure 1: ***Acinetobacter baumannii*** **on MacConkey**

3.2 Comparison of Minimum Inhibitory Concentrations (MICs) of Colistin

This figure illustrates the antibacterial effect of two concentrations of colistin (1000 µg/ml and 500 µg/ml) on the growth of Acinetobacter baumannii cultured on Heart Infusion Agar (HMA). Following incubation at 37 °C for 24 hours, clear inhibition zones were observed, indicating susceptibility to colistin. The higher concentration (1000 µg/ml) produced a broader zone of inhibition compared to 500 µg/ml, reflecting a dose-dependent antibacterial activity. This result confirms the sensitivity of the tested A. baumannii isolates to colistin at higher concentrations.

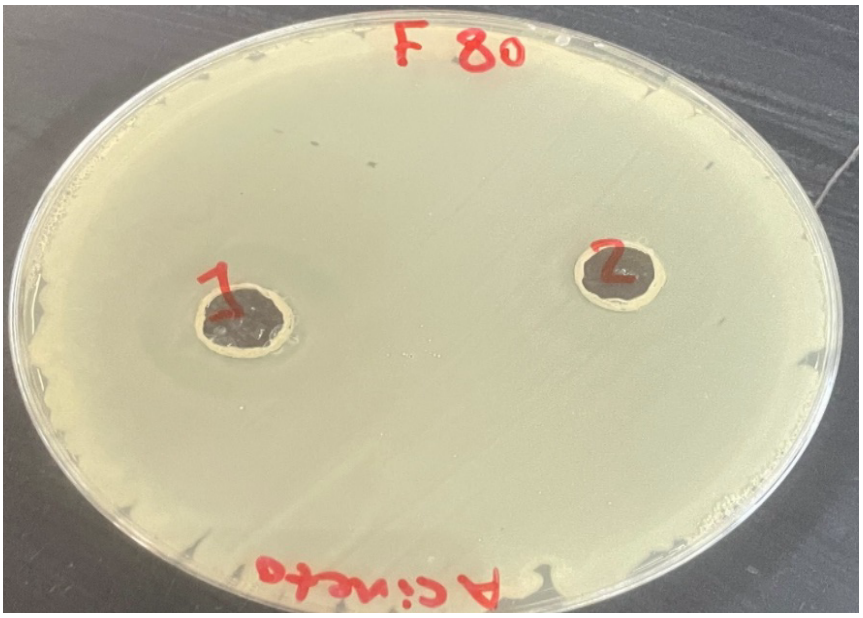

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Figure (2): Comparison between MIC Colistin 1) (1000 µg/ml). 2) 500, µg/ml against Acinetobacter baumannii on HMA plates at 37 ºC for 24 hours

3.3 Histopathological Observations – Group 1 (Skin Injury Only)

In the first group, which received skin injury only, microscopic examination revealed that skin cells gradually began to return to a normal architecture over time. However, the presence of cellular debris and inflammatory cells was still evident (Fig. 3). In the dermis layer, there were mild infiltrations of neutrophils, while the epidermal layer showed signs of hyperplasia, indicating an ongoing reparative and inflammatory response.

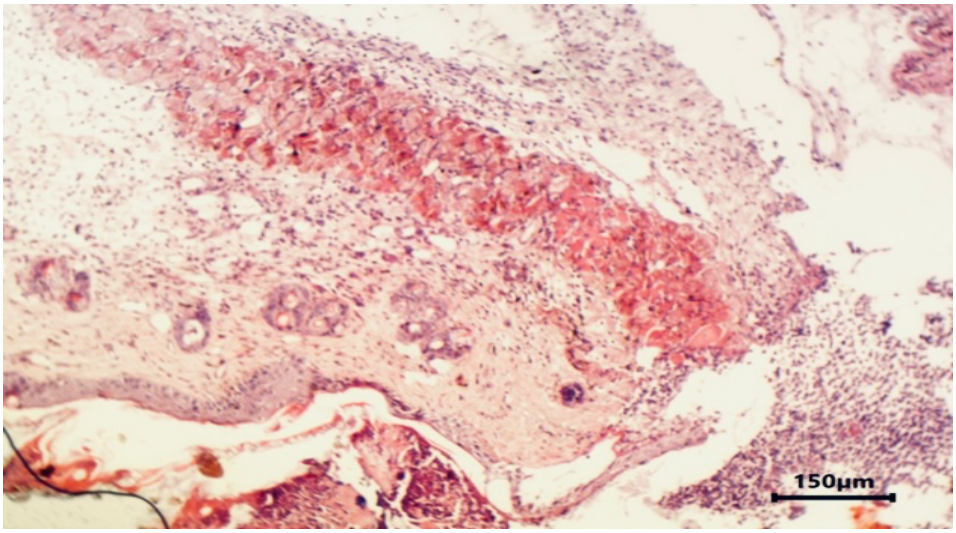

Figure 3: The skin of the 1st group regeneration of the epithelial layer

3.4 Histopathological Observations – Group 2 (Injury + Bacterial Infection)

In the second group, which received both skin injury and infection with a bacterial isolate, histological examination revealed extensive necrosis involving all layers of the skin, including the epidermis, dermis, and underlying tissues (Fig. 4). These findings indicate a severe inflammatory and tissue-destructive response resulting from the bacterial infection.

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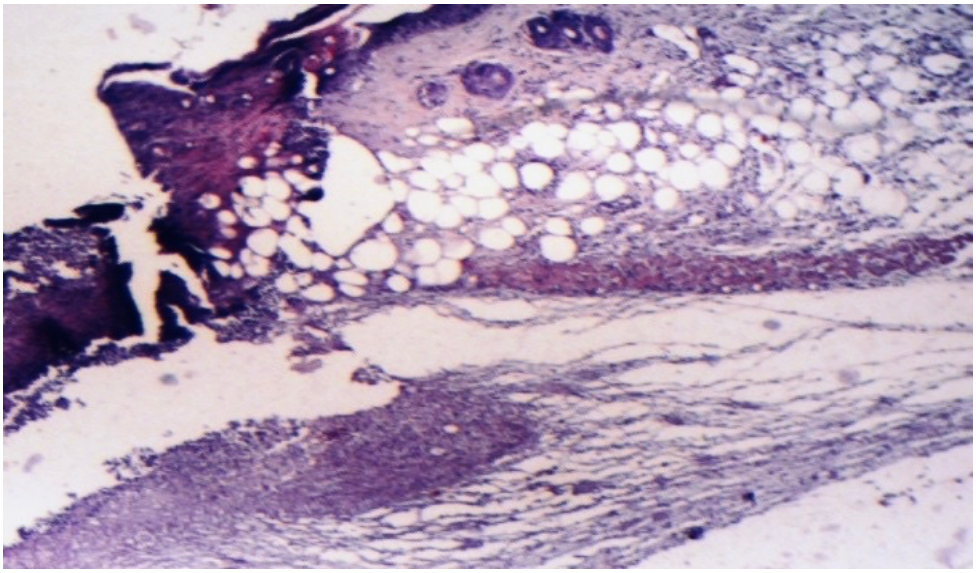

Figure 4: The 2nd group in the skin: necrosis of all layers of the skin

3.5 Histopathological Observations – Group 3 (Injury + Infection Treated with Colistin)

In the third group, which received colistin treatment following skin injury and bacterial infection, histological analysis showed clear evidence of epidermal cell regeneration. The regeneration was more pronounced in the group treated with 1000 µg/ml of colistin (Fig. A) compared to 500 µg/ml (Fig. B), indicating a dose-dependent therapeutic effect of colistin in promoting skin recovery and reducing tissue damage.

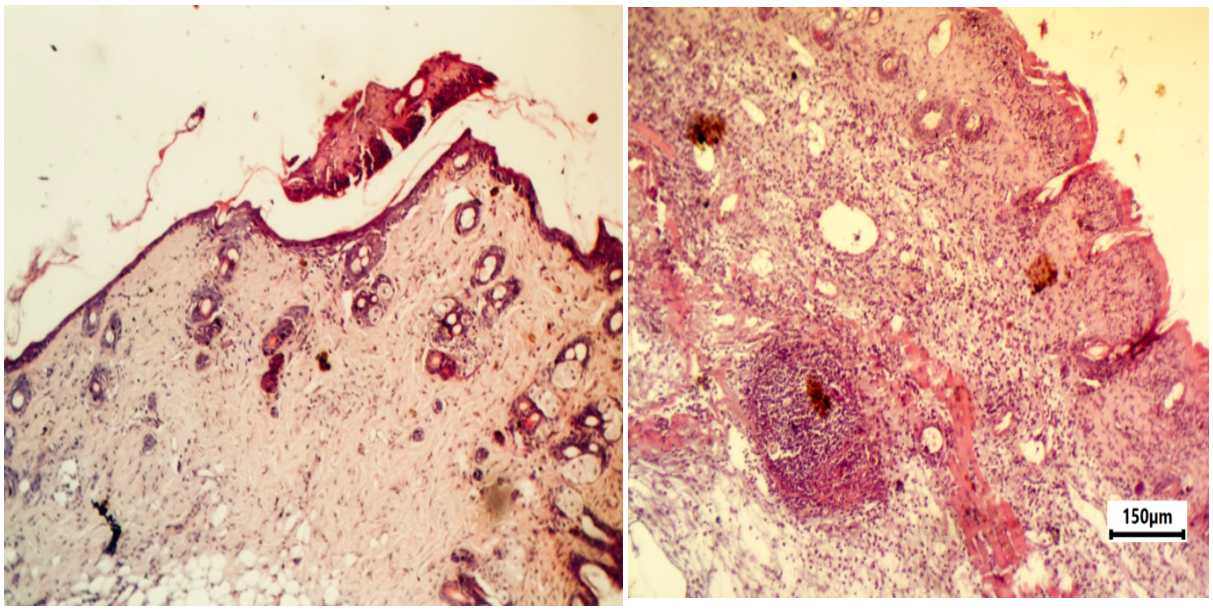

Figure 5: Histological in the skin of the 3rd group (A) mild regeneration and infiltration of dermis; (B) Regeneration in dermis by collagenous fiber

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The present study investigated the therapeutic efficacy of colistin against wound contamination caused by Acinetobacter baumannii, a multidrug-resistant (MDR) Gram-negative pathogen frequently associated with hospital-acquired skin and soft tissue infections. The results demonstrated that colistin, particularly at higher concentrations (1000 µg/ml), significantly enhanced wound healing by reducing bacterial load and promoting tissue regeneration. A surveillance study conducted in Baghdad (2016–2018) reported a high prevalence (76%) of colistin-resistant A. baumannii, mediated by resistance genes such as mcr-1, mcr-2, and mcr-3. In contrast, our findings demonstrate that colistin retains strong in vitro wound-healing efficacy, suggesting that while resistance exists broadly in clinical environments, localized topical colistin can still be effective for superficial infections.[14]

A study from Diyala Governorate (2024) evaluated 27 clinical isolates of A. baumannii and found near-complete resistance to many antibiotics, though susceptibility to colistin was not specifically assessed. Our results, showing robust infection control and tissue regeneration, reinforce colistin's potential role in areas like Diyala where multidrug resistance is prevalent.

Histopathological observations revealed that untreated infected wounds (Group 2) exhibited extensive necrosis across all skin layers, along with dense infiltration of inflammatory cells.[15]. This finding is consistent with the well-documented virulence of A. baumannii, which is known for its ability to form biofilms, secrete cytotoxins, and resist host immune defenses [16]

In contrast, colistin-treated groups (Group 3) showed marked improvement in skin architecture, particularly in the 1000 µg/ml subgroup, where signs of epidermal regeneration and reduced inflammation were observed. These results support previous studies that reported colistin's bactericidal activity against A. baumannii through disruption of the bacterial outer membrane and inhibition of vital cellular processes [17]. Additionally, the observed difference in response between 1000 and 500 µg/ml concentrations underscores the dose-dependent efficacy of colistin, suggesting that higher local concentrations may be required for optimal wound penetration and therapeutic action.

Notably, Group 1 (injury only) exhibited normal healing patterns with mild inflammation and limited neutrophilic infiltration, serving as a baseline for comparison. The progression of healing in this group contrasted sharply with the infected group and further highlighted the damaging role of *A. baumannii* in wound pathology.[18].

Despite its effectiveness, colistin's clinical use is limited by systemic toxicity, especially nephrotoxicity and neurotoxicity. However, topical or localized applications, such as in wound

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care, may allow for higher antimicrobial concentrations with minimal systemic absorption, providing a safer therapeutic option.

Furthermore, this study aligns with recent Iraqi and global findings that emphasize the reemergence of colistin as a last-resort antibiotic against MDR pathogens [19,20] The use of colistin in topical formulations has shown promise in treating infections involving biofilm-producing A. baumannii isolates, which are particularly difficult to eradicate using conventional antibiotics.

While this study provides valuable insights, it is limited by its in vivo experimental scope and lack of molecular analysis for resistance genes (e.g., mcr-1). Future studies should explore:

a. The expression of resistance genes following colistin exposure
b. Combination therapies with synergistic agents (e.g., rifampicin, tigecycline)
c. Formulation of controlled-release topical colistin systems to enhance wound delivery

4. CONCLUSION

This study demonstrated the dose-dependent antibacterial efficacy of colistin against Acinetobacter baumannii, with the higher concentration (1000 µg/ml) producing a significantly wider zone of inhibition compared to 500 µg/ml. The results suggest that increased colistin concentration more effectively inhibits bacterial growth and may enhance therapeutic outcomes in infected wounds. Histopathological findings further support colistin's therapeutic potential. While skin injury alone led to gradual restoration of tissue with residual inflammation, the presence of A. baumannii infection caused severe necrosis and a heightened inflammatory response. In contrast, wounds treated with colistin—particularly at 1000 µg/ml—exhibited marked epidermal cell regeneration and reduced tissue damage, confirming colistin's role in promoting wound healing and controlling bacterial infection.

5. ACKNOWLEDGMENT

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