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Original Article

Isolation and Identification of Some Bacteria Contemn in Burn Wounds in Misan, Iraq

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Abstract

The current study aimed to isolate and identify the bacteria associated with burn wounds and investigate the antimicrobial susceptibility pattern against a group of most commonly prescribed antibiotics. In total, 105 burn wound swabs were collected from burn patients admitted to the burn unit of Al-Sadr Teaching Hospital in Misan City, Iraq. The swabs had been cultured on different media; the colonies were diagnosed based on the phenotypic and culture characteristics. The bacteria were identified through cultural characters and Gram staining diagnosed by VITEK® 2 Compact Automated Systems. In total, there were nine distinct bacterial isolations, of which, *Pseudomonas aeruginosa* was the most common pathogen [20%] followed by *Staphylococcus aureus* [17.14%], *Enterobacter spp.*[16.19%], *Proteus vulgaris* [13.33%], *Proteus mirabilis* [10.47%], *Escherichia coli* [7.6%], *Klebsiella pneumoniae* [6.6%], and at last, *Staphylococcus lentus* and *Aeromonas sobria*, which had the same percentage [4.7%].Most isolates showed high resistance to Tobramycin, Trimethoprim, Cephalothin, and Imipenem while isolates mostly had high susceptibility to Amikacin, Cefotaxime, and Ciprofloxacin. Wound burn infection still represents a serious problem for burn patients with many bacteria developing different degrees of resistance to most known antibiotics.

Keywords: Antibiotics resistance, Burn wounds, *Pseudomonas aeruginosa*, Susceptibility

1. Introduction

Burns are among the most devastating forms of trauma in individuals suffering from severe thermal injury (1). Invasive infection caused by burns is responsible for 51% of death (2). Approximately, 500,000 people in the United States require medical assistance for burns annually, with 40,000 patients requiring hospitalizations to minimize bacterial infection (3, 4). The presence of microorganisms on the burn wound has a direct correlation with virulence factors. Theoretically, the high temperature sterilizes the burn wound at first. Normal skin flora and existing infections, on the other hand, grow quickly. Routine cultures demonstrate that 9 % and 54 % of patients in the pediatric burn unit are contaminated with GAS and *Staphylococcus aureus*, respectively, at the time of admission.

It should be noted that contamination is not the same as the colonization of the wound surface (5). Currently, 75% of all deaths in individuals with symptomatic burns over 40% of the body surface area are due to septic shock and infection health problems. Persistent stay in the intensive care unit, invasive preventive and surgical procedures and nature of the burn injury all lead to increased rates of nosocomial infections in burn patients (6).Wound inoculation is a valuable tool for the treatment of wounds and wound colonization. Wound invasion occurs after 5-7 days for patients with major burns. Since the majority of early diseases in burn patients are caused by endogenous bacteria, performing

initial wound culture upon admission is a good clinical practice (5).

Despite advances in topical and intravenous antimicrobial treatments, as well as the technique of prompt tangential excision, the pathogen remains a serious issue in the treatment of burn victims (7). Furthermore, due to congestion in burn hospitals, cross-infection occurs among various burn patients (8). Some germs can also be transmitted to a patient's surrounding tissue by contact with an infected person through various factors surfaces, such as water, fomite, air, and the solid hands of healthcare professionals (9). Burn wound infection is still primarily caused by *S. aureus* (6). Methicillin-resistant *S. aureus* has emerged as the most common pathogen in intensive care units in recent decades, owing to the widespread use of broad-spectrum antibiotics. In an injured person, colonization with any of these bacteria is normally asymptomatic, but they are also a source of infectious agents that can cause serious disease and death (7).

Antibiotic susceptibility patterns in burn wound infectious diseases pose a significant therapeutic issue for the caregivers of burn patients (9, 10).It is critical to understand the common organisms that cause infected burn wounds, as well as their antibiotic susceptibility patterns. These important findings could help the development of a more effective antibiotic treatment plan for burn wound infections (7).

Given that infectious organisms and their susceptibility to antibiotics change over time, it is a good idea to examine the bacterial flora of burn wounds regularly. Therefore, this study aimed to assess the infective bacteria of burn wounds and their antimicrobial susceptibility in Al-Sadr Teaching Hospital in Misan City, Iraq.

2. Materials and Methods

2.1. Samples Collection

In total, 105 burn patients from various age ranges and genders, who were admitted to the burn unit in Al-Sadr Teaching Hospital, were screened for this study during the period from October 2020 to May 2021.Wound swabs were taken from each patient. In case of direct contact with patients, a protective gown and disposable gloves were used. The specimens were transported in a sterile, leak-proof container to the laboratory. All samples were cultured on nutrient agar, MacConkey agar, 5% Blood agar, and mannitol salt agar,and incubated over night at 37 °C aerobically for 24 h.

2.2. Isolates Identification

Firstly, the colonies were diagnosed initially depending on the phenotypic and culture characteristics. Identification of the isolated bacteria was performed according to the standard microbiological methods (11), including cultural characters. Afterward, Gram stain isolates were diagnosed by VITEK® 2 Compact Automated Systems with ID-GN and ID- Gp cards based on the manufactures instructions.

2.3. Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method recommended by the Clinical and Laboratory Standards Institute (12).All aspects of the Kirby– Bauer procedure are standardized to ensure consistent and accurate results. The media used is Mueller-Hinton agar at only 4 mm deep, which was poured into either 100 mm or 150 mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4. The bacterial inoculum is prepared by diluting a broth culture to match a 0.5 McFarland turbidity standard, which is equivalent to approximately 150 million cells per mL (13). The tested antibiotics were amikacin, cefotaxime, cephalothin, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, tobramycin, imipenem, and Trimethoprim (14).

2.4. Statistical Analysis

The recorded data in the current research was analyzed using descriptive statistics generated with the help of Microsoft Excel. The quantitative data generated from the study were coded and putinto Microsoft Excel and analyzed using GraphPad Prism software (version 6). In all cases, p-values of less than

0.05 were considered statistically significant. Paired student t-test was used to test for significance between the prevalence of burns in different genders and age groups.

3. Results and Discussion

The prevalence of burn injuries and associated resistance bacteria in patients at Al-Sadr Teaching Hospital is reported for the first time in this study. In total, 105 isolates were studied, and it was found that *Pseudomomas aeruginosa* was the most common pathogen [20%] followed by *S. aureus* [17.14*%], Enterobacter spp.* [16.19%], *Proteus vulgaris* [13.33%], *P.mirabilis* [10.47%], *Escherichia coli* [7.6%], *Klebsiella pneumoniae* [6.6%], and at last, *Staphylococcus lentus* and *Aeromonas sobria*, which had the same percentage [4.76%]. Variations in both environmental factors and attitudes toward burn wound therapy could explain the variation in frequency rates (Table 1).

Table 1. Distribution of isolated bacteria and their percentages

Bacterial isolates	Number	Percentage
Staphylococcus aureus	18	17.14%
Pseudomomas aeruginosa	21	20%
Staphylococcus lentus	5	4.76%
Escherichia coli	8	7.61%
Klebsiella pneumoniae	7	6.66%
Enterobacter spp.	17	16.19%
Proteus vulgaris	14	13.33%
Proteus mirabilis	11	10.47%
Aeromonas sobria	5	4.76%

Burned surface area, along with the length of hospitalization, is one of the risk factors for infection problems, according to SOARES, MACEDO (11), which is consistent with the findings of the study performed by Zampar, Anami (15) as the majority of patients were seriously injured and had a long hospital admission. Infection complications in individuals with severe burns are caused by thermal damage of the stratum corneum and simultaneous suppression of local or systemic patient humoral immune mechanisms (11).

However, based on multiple investigations, *S. aureus* was the most common single organism colonizing burn wounds (16, 17). The results of the current study revealed that *P. aeruginosa* was the most common bacteria in burn wounds. This data supports prior research that identified these bacteria as the most common cause of burn wound infection (16).

It should be mentioned that *P. aeruginosa* is a common gamma-proteo-bacterium that can be found in a variety of environments, including soil and water. It also causes serious infections in mammals, various animals, and plants as pathogenic bacteria (8). The pathogenicity of *P. aeruginosa* is mediated by its ability to create a wide range of virulence factors, which is bolstered by its inherent resilience to environmental stressors and xenobiotics, including antibiotics, antiseptics, and heavy metals (9). By combining the data, it has been determined that these characteristics enable the pathogen to achieve efficient invasion, colonization, and persistence within the host organism (18, 19). These bacteria are transmitted through the natural gut flora of the patient and/or the environment. Regardless of the fact that these bacteria can infect burn wounds anywhere, nosocomial infection is much more common than infection in other settings for a variety of reasons, including pollution, the existence of drug-resistant bacteria, and congestion (20).

In the current study, *S. aureus* was found to be coagulase active in 17.14% of all isolates. In general, *S. Aureus* has a wide range of virulence factors that facilitate host tissue adhesion, immune response evasion, and cell/tissue death, the most prominent of which being coagulase and hemolysin (21). *S. aureus* has a variety of surface proteins that aid in the binding of the bacteria to host protein, such as laminin and fibronectin, which are found in the extracellular matrix. Fibronectin is found on the surfaces of endothelial cells, as well as in blood clots. A fibrinogen/fibrin binding protein in the bacteria helps their attachment to clots and injured tissue which makes the *S. aureus*

likely to cause wound infections, including post-surgery diseases (6).

Enterobacter spp. was one of the most frequent isolates in this investigation, represented by 16.19% of all isolates. The *Enterobacter* genus contains gram-negative homologous anaerobes that are widely spread in nature. Members of the genus *Enterobacter* had already sparked increased concern in recent decades, as they have been more linked to nosocomial infections, particularly in immunocompromised individuals (22). *Enterobacter spp*., such as *E. aerogenes* and *E. cloacae*, acquire a number of genetic mobile elements encoding resistance and virulence factors, which contribute significantly to their enhanced pathogenesis.

S. aureus (gram-positive bacterium) was the most common life-threatening burn agent before the discovery of effective antibiotics. The widespread use of broad-spectrum antibiotics has resulted in the rise of gram-negative bacteria as the most common cause of invasive burn wound infections [28]. Due to this variability in bacterial profile, an infected wound disease must be examined regularly. The majority of the bacteria found in this study (*S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Enterobacter* spp.) are known to be the most common cause of nosocomial infections worldwide.

The antimicrobial susceptibility pattern of the 9 identified bacteria against 10 selected antibiotics is presented in table 2. The investigation revealed that most of the isolates had resistance to trimethoprim and cephalothin. *P. aeruginosa* was unsusceptible to all antimicrobials, except amikacin (50.5%), ciprofloxacin (44.4%), and cephalothin (22.2%), respectively. All the isolated pathogens were 100% sensitive to Ciprofloxacin and Amikacin, and their susceptibility percentages varied from 40% to 100% as shown in table 2.

Table 2. Antimicrobial susceptibility pattern (%) of the isolates

Antibiotics	Con. /Disk(Mg/L)	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Enterobacter* species	*Proteus vulgaris*	*Proteus mirabilis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Aeromonas sobria*	*Staphylococcus lentus*
Amikacin (AN)	30 Mg/L	50.5	75.7	40	100	100	100	55.5	100	100
Cefotaxime (CTX)	30 Mg/L	0	15.2	22.2	100	100	85.7	20.2	81.8	100
Cephalothin (KF)	30 Mg/L	22.2	11.1	10.2	0	0	0	0	0	33.3
Ciprofloxacin (Cip)	5 Mg/L	41.6	85.7	54.17	77.7	66.6	55.6	77.8	88.89	100
Erythromycin €	10 Mg/L	0	50.5	0	0	22.2	44.4	33.3	77.78	88.89
Gentamicin (GEN)	10 Mg/L	0	0	66.67	50	85.7	44.4	50	44.4	28.57
Nalidixic acid (NA)	30 Mg/L	0	88.89	54.17	50	55.5	100	77.8	75.75	50
Imipenem (M)	10 Mg/L	0	0	0	0	11.1	0	33.3	22.2	44.4
Tobramycin (TM)	10 Mg/L	0	0	0	0	11.1	0	0	0	22.2
Trimethoprim (W)	1.25 Mg/L	0	0	0	0	0	0	0	16.6	27.37

S. lentus showed varied susceptibility to all antibiotics (22.2-100% sensitivity percentage). *E. coli* showed high susceptibility to Amikacin and nalidixic acid (100%) and high resistance to cephalothin, tobramycin, and trimethoprim (0%) susceptibility. *P. vulgaris* was found to be susceptible to 50% of tested antimicrobials (sensitivity percentage was 50-100%) and showed resistance to 50% against the tested antibiotics.

Receiving antibiotics before the rise of an illness, prolonged hospitalization, previous hospitalization, medical interventions, comatose state, and advanced age are all risk factors for acquiring an antibiotic-resistant bacterium (23). Antimicrobial resistance is higher in the bacteria transmitted from the healthcare setting, compared to those transferred from the intestinal microflora of the patient (24).

Multidrug-resistant microbes have been identified as a source of nosocomial infection epidemics in burn departments and as colonizers of the wounds of burn sufferers (24). Extensive and inappropriate use of a wide range of antibiotics is likely the cause of a large number of multidrug-resistant strains. These multidrug-resistant strains develop themselves in hospital environments, such as sinks, taps, railings, mattresses, and toilets, spreading from one patient to the next.

This is particularly evident in *P. aeruginosa, P. vulgaris, and S. aureus*, all of which exhibit increased resistance to antibiotics (ranging from 50% to 70%), which is due to the coordinated efforts of transcription factors with mobile genetic elements antibiotic resistance and high compressibility of the bacterial cellular envelopes. Apart from resistance development, *P. aeruginosa* can easily obtain antibiotic resistance through chromosomally-encoded gene mutations or genetic elements of drug resistance factors (25). As a result, routine microbiological monitoring and meticulous in vitro testing before using antibiotics may help reduce the risk and therapy of multidrug-resistant pathogens in burn infections.

In conclusion, burn wounds are becoming a more appropriate and variable setting for the spread of multidrug-resistant microbes. As a result, the careful antibiotic selection is necessary for optimal burn wound infection treatments to help prevent morbidity and mortality associated with multi-drug resistant microorganisms.

Authors' Contribution

Study concept and design: R. R. H.
Acquisition of data: R. R. H.
Analysis and interpretation of data: R. R. H.
Drafting of the manuscript: R. R. H.
Critical revision of the manuscript for important intellectual content: R. R. H.
Statistical analysis: R. R. H.
Administrative, technical, and material support: R. R. H.

Ethics

The current research was approved by the Ethics Committee of the Department of Biology, College of Science, University of Misan, Maysan, Iraq. Participation by the patients was voluntary in accordance with the guidelines of the Ethics Committee.

Conflict of Interest

The authors declare that they have no conflict of interest.

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