Journal of Research in Applied and Basic Medical Sciences 2024; 10(1): 13-22

Original Article

Urmia University of Medical Sciences

RABMS Journal of Research in Applied and Basic Medical Sciences

Frequency and Antibiotic Resistance Patterns of Isolated Bacteria from Burn Wounds Infections in Imam Khomeini Medical Center in Urmia

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Abstract

Background & Aims: Wound infection is a major challenge in burn care and is the most common cause of death from burn. Knowledge of the major organisms that are commonly encountered in each unit is essential for effective treatment of infections. The aim of this study was to determine the type of bacterial infections in burn wounds and evaluate the frequency and antibiotic resistance of bacteria isolated from the burn section.

Materials & Methods: This descriptive study involved 120 burn patients from Imam Khomeini Hospital in Urmia, who were randomly selected from the hospital's database. We obtained samples from their burn wounds and performed conventional biochemical tests to identify the strains. We applied the disk diffusion method to determine the antibiotic resistance pattern. We used SPSS version 26 for the statistical analysis.

Results: In this study, *Acinetobacter baumannii* was the most common organism in the burn section. *Staphylococcus aureus, Pseudomonas aeruginosa, Coagulase-negative staphylococci, Acinetobacter lwoffi, Escherichia coli, Enterobacter* species, and *Klebsiella pneumoniae* were next in line. According to the antibiogram, %67.7 of the strains had Multiple Drug Resistance. Also, 42.3% of *Methicillin-Resistant Staphylococcal* isolates were identified.

Conclusion: The high frequency of Multidrug-Resistant strains in the burn ward is a serious warning in the treatment of burn wound infections in this hospital. Therefore, it seems necessary to develop new treatment strategies and follow the correct health and treatment protocols.

Keywords: Antibiotic Resistance, Bacterial Infection, Burn Unit, Urmia

Received 01 December 2022; accepted for publication 25 July 2023

Introduction

Skin is an essential component of the immune system that protects the host from potential pathogens in the environment (1). Severe burn injuries are the most vulnerable and physically debilitating injuries that affect almost any system and organ and lead to significant morbidity and mortality (2). In low-income and developing countries, burn injuries are an undeniable problem and are much more common than them in the United States and Europe or other high-income developed countries (3) Treating patients with extensive burns is still a major challenge, despite many advances in burn care in recent decades (4). The main purpose of burn treatment is rapid skin repair and wound healing to prevent secondary infections as well as reduce functional and aesthetic problems (5). Burn patients are at high risk of infection due to the nature of the burn injury, the immunizing effects of the burns, long hospital stays, and severe diagnostic and therapeutic procedures (4). The surface of the burn wound is a protein-rich environment consisting necrotic tissue that provides a good position for microbial colonization and proliferation (5). The risk of wound infection depends not only on the nature and extent of the heat injury but also on the type and number of microorganisms replaced in the burn wound. Burn wound infections have become a serious concern in the emergence of antimicrobial resistance, which greatly limits the available treatment options. Pathogenic attack under the dermis in burn patients is a precondition for impending complications including bacteremia, sepsis, and multifocal dysfunction syndrome (6). Over the years, gram-negative and gram-positive bacteria have been responsible for the most important infections transmitted by the hospital and the cause of antimicrobial treatment failure in burn infections. These bacteria have a remarkable ability to develop resistance to common antibiotics through a wide variety of mechanisms. Therefore, monitoring changes in the pattern of drug resistance is inevitable for effective treatments (7). The aim of this study was to determine the frequency and antibiotic resistance of bacteria isolated from burn wounds and to determine their antimicrobial susceptibility.

Materials & Methods

This descriptive study was performed on 120 burn patients admitted to the burn ward of Imam Khomeini Educational-Medical Center in Urmia over an 8-month period from August to March 2017.

Samples were collected from patients' wounds using sterile swabs, and transferred to the laboratory. Blood agar and McConkey agar media were used to sample collection. After purification, the bacteria obtained using standard microbiological methods such as Gram staining, colony morphology, motility test, growth on McConkey agar, blood agar, mannitol salt agar, coagulase test, catalase test, and culture of media as well as differential and biochemical tests such as oxidase test were performed. The samples were then stored in the freezer at -20$^{\circ C}$ for further steps. Antimicrobial susceptibility testing of isolated bacterial species was determined by Kirby-Bauer or diffusion disk method. Three to five colonies of bacterial culture were dissolved in 100 ml of physiological saline. Using a spectrophotometer, the light absorption intensity of this suspension was read at a wavelength of 625 nm; the resulting number was in the range of 0.08 to 0.13. Then the samples were inoculated with sterile swab on Muller Hinton agar and incubated for 24 hours at 35$^{\circ C}$. The growth halo diameter was read according to the instructions based on sensitive, semi-sensitive and resistant. Standard strains of *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 were used for quality control of antibiotic susceptibility testing. The antibiotics used in this study including Ciprofloxacin, Erythromycin, Azithromycin, Amikacin, Gentamicin, Levofloxacin, Tetracycline, Ceftazidime, Cefotaxime, Ceftriaxone, Imipenem, Meropenem, Ticarcillin, Ampicillin Sulbactam, Amoxicillin Clavulanate, Cefixime, Piperacillin, Clindamycin, Cotrimoxazole, Penicillin, Amoxicillin, and Ampicillin were bought from Padten Teb, Iran. A 30µg Cefoxitin disc was used to identify *Methicillin-Resistant Staphylococci* (MRSA) types (8). Multi-Drug Resistance strains (MDRs) were also reported based on the presence of resistance to at least one antibiotic in three or more classes of antibiotics (9). This study was done under ethical guidelines of Urmia Medical University with Ethical code of IR.UMSU.REC.1398.373.

Results

In this study, 120 patients including 77 males (64.16%) and 43 females (35.83%) participated. The highest age group was 21-40 years with 51 people (42.5%). (Table 1)

Out of 120 patients, 90 bacteria were isolated and the most common of them with 32 (35.5%) cases were *Acinetobacter baumannii*. *Staphylococcus aureus* with 15 (16.6%) cases, *Pseudomonas aeruginosa* with 12 (13.3%) cases, *Coagulase negative staphylococci (CoNS)* with 11 (12.2%) cases, *Acinetobacter lwoffii* with 7 (7.7%) cases, *Klebsiella pneumoniae* and *Enterobacter* species with 5 (5.5%) cases, and Escherichia coli with 3 (3.3%) cases were among the bacteria isolated from the patients' wounds.

Among 90 bacterial isolates, 61 (67.7%) isolates were multidrug resistant (MDR) and showed resistance to at least three different classes of antibiotics, and out of 26 isolates of *Staphylococcus,* 11 (42.3%) isolates were *Methicillin-Resistant Staphylococci* (MRSA). (Figure 1.2)

Table 1. Demographic data.

Variables	Frequency	
	N	%
Gender of total patients		
Man	77	64.16
Woman	43	35.83
Age		
≤ 20	42	35
21-40	51	42.5
40-60	22	18.33
> 60	5	4.16
Type of burns		
Flame	63	52.5
Scalds	26	21.66
oil	12	10
Electricity	8	6.66
Chemical	8	6.66
Cold burns	1	0.83
Thermal	2	1.66
Burn Percentage		
≤ 35	92	76.66
> 35	28	23.33

Gentamicin was identified as the most sensitive antibiotic for *Staphylococcus aureus* and Cotrimoxazole for *CoNS* isolates. Among gram-negative bacteria, the highest antibiotic resistance was observed in *Acinetobacter* isolates, as they showed resistance to all tested antibiotics.

The most resistant antibiotics among the isolates of *Pseudomonas aeruginosa* were Ampicillin, Amoxicillin, Tetracycline, Ampicillin Sulbactam, Amoxicillin Clavulanic acid and Cefixime. The results of the antibiogram test are shown in Tables 2, 3, and 4.

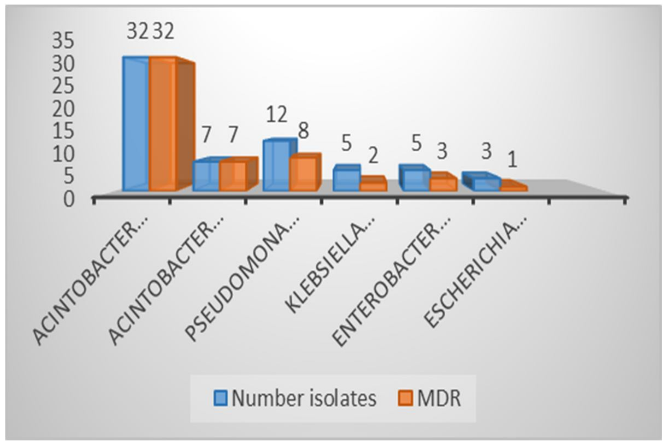

Fig. 1. Distribution of organisms gram-negative isolated with MDR cases

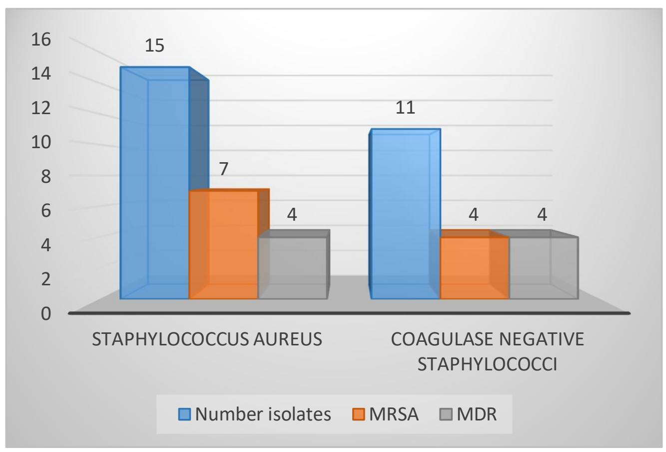

Fig. 2. Distribution of organisms gram-positive isolated with MDR and MRSA cases

Table 2. Antibiotic susceptibility pattern of gram-negative isolates

Antibiotic	Acinetobacter baumannii (n= 32)			Acinetobacter lwoffii (n= 7)			Pseudomonas aeruginosa (n=12)		
	SENSITIVE	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**
AMPICILLIN	-	-	100	-	-	100	-	-	100
Amoxicillin	-	-	100	-	-	100	-	-	100

Antibiotic	Acinetobacter baumannii (n= 32)			Acinetobacter lwoffii (n= 7)			Pseudomonas aeruginosa (n=12)		
	SENSITIVE	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**
Ceftazidime	-	-	100	-	-	100	25	16.6	58.3
Ceftriaxone	-	-	100	-	-	100	-	8.3	91.6
Cefotaxime	-	-	100	-	-	100	-	16.6	83.3
Imipenem	-	-	100	-	-	100	16.6	16.6	16.6
Meropenem	-	-	100	-	-	100	58.3	8.3	33.3
Amikacin	-	-	100	-		100	75	-	25
Gentamicin	-	-	100	-	-	100	41.6	-	58.3
Ticarcillin	-	-	100	-	-	100	-	-	100
Azithromycin	-	-	100	-	-	100	91.6	-	8.3
Ciprofloxacin	-	-	100	-	-	100	83.3	-	16.6
Levofloxacin	-	-	100	-	-	100	66.6	-	33.3
Tetracycline	-	-	100	-	-	100	-	-	100
Ampicillin-sulbactam	-	-	100	-	-	100	-	-	100
Piperacillin	-	-	100	-	-	100	8.3	-	58.3
Amoxicillin-clavlanate	-	-	100	-	-	100	-	-	100
Cefixime	-	-	100	-	-	100	-	-	100

Table 3. Antibiotic susceptibility pattern of gram-negative isolates (continue from Table 2)

Antibiotic	Klebsiella pneumoniae (n= 5)			Enterobacter spp (n= 5)			Escherichia coli (n=3)		
	SENSITIVE	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**
Ampicillin	-	-	100	-	-	100	33.3	-	66.6

Antibiotic	Klebsiella pneumoniae (n= 5)			Enterobacter spp (n= 5)			Escherichia coli (n=3)		
	SENSITIVE	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**	**SENSITIVE**	**INTERMEDIATE**	**RESISTANT**
Amoxicillin	-	-	100	-	-	100	33.3	-	66.6
Ceftazidime	20	-	80	20	-	80	66.6	33.3	-
Ceftriaxone	-	-	100	20	-	80	66.6	-	33.3
Cefotaxime	-	20	80	-	-	100	66.6	-	33.3
Imipenem	20	20	40	40	20	20	100	-	-
Meropenem	40	20	40	80	-	20	100	-	-
Amikacin	60	-	40	60	20	20	100	-	-
Gentamicin	40	-	60	20	-	80	66.6	-	33.3
Ticarcillin	-	-	100	-	-	100	-	-	100
Azithromycin	40	-	60	20	40	40	66.6	-	33.3
Ciprofloxacin	20	-	80	20	-	80	66.6	-	33.3
Levofloxacin	20	20	60	60	40	-	33.3	-	66.6
Tetracycline	-	20	80	80	-	20	33.3	-	66.6
Ampicillin-sulbactam	-	-	100	-	-	100	66.6	-	33.3
Piperacillin	20	40	40	80	-	20	33.3	-	66.6
Amoxicillin-clavlanate	-	-	100	-	-	100	33.3	33.3	33.3
Cefixime	20	-	80	20	-	80	66.6	-	33.3

Table 4. Antibiotic susceptibility pattern of gram-positive isolates

Antibiotic	Staphylococcus aureus (n=15)			Coagulase negative staphylococci (n=11)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Penicillin	-	-	100	18.1	-	81.8
Gentamicin	93.3	-	6.6	63	-	36.3
Ciprofloxacin	73.3	20	6.6	90	-	9
Levofloxacin	86.6	-	13.3	-	-	100
Tetracyclin	66.6	-	33.3	72	-	27
Azithromycin	53.3	-	46.6	72.7	9	18.1
Trimethoprim / sulfamethoxazole	73.3	6.6	20	100	-	-
Clindamycin	40	46.6	13.3	63	-	36.3
Erythromycin	60	-	40	54	9	36.3
Cefoxitin	53.3	-	46.6	63.6	-	36.3

Discussion

Burn injury is one of the most debilitating forms of trauma, and wound infection is one of its most important complications, often leading to poor healing, sepsis, disability or even death (10, 11). The biggest challenge in managing a burn infection is the proper selection and use of antimicrobial agents. Excessive use of antibiotics has been linked to the emergence of multidrug-resistant bacteria and multidrug resistance has emerged as a threat to the prognosis of burn injuries (10).

In the present study, 120 patients including 75 men and 58 women participated. This study was consistent with the study conducted by Jafari et al (2013) (12) that out of 227 samples, 57.7% of the samples were men and 42.3% of the samples were women. Also, in the study conducted in Taif (2016), out of 220 patients, 159 were male and 61 were female and number of male patients admitted to the burn ward was more than women (13). In our study, *A. baumannii* was the most common pathogen isolated from burn injury, which was similar to studies performed by Bayram et al., ALfadli et al. (9), Hegde et al. (14) and Chim et al. (15). The widespread use of broad-spectrum antimicrobials in the burn ward, as well as the susceptibility of severe burn patients to infection, provides a good basis for achieving resistance mechanisms for the formation of new strains (15). On the other hand, a study conducted by Afrasiaban et al (2009) (16), Jafari et al (2013) (12), Qazvini et al (2008) (17) showed that *P. aeruginosa* is the predominant organism isolated from burn patients, which did not agree with our study. A study conducted in Italy reported *S. aureus* as the most common organism (18).

In our study, the resistance of *Acinetobacter* was so alarmingly high that it had 100% resistance to all the antibiotics studied.

Multidrug-resistant *Acinetobacter* occurs in burn patients who are prone to infection due to the disappearance of protective barriers of the skin and mucosa (19).

In a study by Hegde et al. (2013), *Acinetobacter* showed high resistance to most drugs (14).

Out of 90 bacterial isolates, 61 (67.77%) multidrug resistant isolates (MDR) were found. The incidence of MDR was higher in *A. baumannii, P. aeruginosa* and *S. aureus*, respectively.

ALfadli et al. (9) found that 65.85% of clinical isolates were MDR and 100% of *Acinetobacter* strains were resistant to the studied antibiotics and this result was consistent with our study. In the study of Chaudhary et al., MDR was reported to be 67.74% among all isolates, so that *E. coli* isolates had the highest resistance.

In the study of Mama et al. (14), MDRs was observed in 85% of the isolates. In the study of Keen et al. (15), *A. baumannii* isolates had the highest multiple resistance with 53%.

In the case of *P. aeruginosa*, Amikacin and Azithromycin were found to be the most sensitive antibiotics. These results are consistent with studies by latika et al. (20), vimal et al. (21), forsen et al. (22), Rahimi et al. (23) and Soleimanzadeh et al. (24) who reported Amikacin as the most sensitive antibiotic. Kurds are in harmony. The most resistant antibiotics to *P. aeruginosa* were Ampicillin, Amoxicillin, Ticarcillin, Tetracycline, Ampicillin, Sulbactam and Cefixime.

In the studies of Rezaei et al. (25) the highest resistance of *P. aeruginosa* strains was related to Ceftazidime, Rajeshwar et al. (26) Imipenem, Ciprofloxacin, Piperacillin Tazobactam and Gentamicin and in the study of Mama et al. (27) Chloramphenicol, Ampicillin, Doxycycline was reported.

Among other gram-negative bacteria, the highest susceptibility of *K. pneumoniae* was to Amikacin, *Enterobacter* species to Meropenem and Tetracycline and *E. coli* to Imipenem, Meropenem and Amikacin.

In the study of Noman et al. (28) and Otta et al. (29) the highest sensitivity to Imipenem and Piperacillin Tazobactam was obtained. The most resistant antibiotics in the study of Rajabi et al. (30) and Gong et al. (18) also reported Gentamicin, Ampicillin and Amoxicillin and Clavulanic acid. In the present study, the prevalence of MRSA was 42.3%. In the study of LiLi et al. (8), 74.1% of *Methicillin-Resistant Staphylococcus aureus (MRSA)* and 74.5% of *Methicillin-Resistant Staphylococcus epidermidis (MRSE)* were reported, which was more prevalent than our results. In a study by latika et al. (20), the prevalence of MRSA was 40%, which was almost consistent with our results.

In the case of *S. aureus*, Gentamicin, Lovofloxacin, Ciprofloxacin and Cotrimoxazole were found to be the most sensitive antibiotics and Penicillin was the most resistant antibiotic. In the case of *CoNS* Cotrimoxazole, Ciprofloxacin and Tetracycline were the most sensitive antibiotics, respectively.

Akhi et al. (31) reported that the most resistant antibiotic was Chloramphenicol and the most sensitive was Vancomycin. In a study by Bayram et al. (32), the most resistant antibiotic Penicillin was reported, which was similar to our results.

Conclusion

A. baumannii is the most isolated species from the burn with high resistance to common antibiotics. Also, the high frequency of multidrug-resistant strains (MDRs) in this section is a serious warning in the treatment of burn wound infections. It is suggested that new antibiotics be used to determine the sensitivity of clinical isolates of the burn ward to develop new treatment strategies.

Acknowledgements

Authors would like to thank the financial support of Vice Chancellor for research and technology, so that it was not possible to conduct this study without their cooperation.

Conflict of Interest

The authors have no conflict of interest in this study.

Funding/Support

This article was funded by Urmia University of Medical Sciences.

Data Availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

Ethical Statement

This study was conducted after approval by the ethics committee of Urmia University of Medical Sciences with code of ethics IR.UMSU.REC.1398.373.

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