ORIGINAL ARTICLE

Burn wound infections: a serious threat of multidrug-resistant *Staphylococcus aureus*

KASHAF JUNAID[1*], ATA UL MUSTAFA[2], SANA ARSHAD[3], *DUNIA A. AL FARRAJ[4], SONIA YOUNAS[5], HASAN EJAZ[6]*
[1,6]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Saudi Arabia.
[2]Department of Microbiology,Government College University of Faisalabad, Pakistan.
[3]University Institute of Medical Laboratory Technology,University of Lahore, Pakistan.
[4]Department of Botany and Microbiology,College of Sciences, King Saud University, Riyadh, Saudi Arabia.
[5]Department of Pathology, Tehsil Headquarter Hospital Kamoke, District Gujranwala, Pakistan
Correspondence: Dr. Kashaf Junaid, Email: kashaf_junaid@hotmail.com Tel.+966 53 3268576

ABSTRACT

Aim: To determine the frequency and drug susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) in burn wound infections and to reportempirical treatment options.
Methods: The bacterial pathogens isolated from 140 patients of burn wounds were identified by the conventional microbiological techniques, API Staph (bioMerieux) and Clinical and Laboratory Standards Institute (CLSI) guidelines used for the in vitro antimicrobial drug resistance.
Results: Total of 190bacterial strains were isolated from 132 culture positive burn wound swabs out of which 56were *Staphylococcus aureus*. Amongst the*S. aureus*, 24 (43%) were methicillin-resistant, and 32(57%)were methicillin-sensitive. No statistical associations of MRSA infectionswere observed with age, gender, aetiology, and degree of burn ($p > 0.05$). Isolated MRSA showed antimicrobial resistance against clindamycin ($p < 0.01$) erythromycin ($p < 0.01$), levofloxacin ($p = 0.03$) and moxifloxacin ($p = 0.01$). *S. aureus* showed antimicrobial resistance predominantly against clindamycin (68%) followed by erythromycin (56%), cefoxitin(43%), levofloxacin (36%), moxifloxacin (16%), and vancomycin (4%).
Conclusion: The isolation of a high number of multidrug-resistant *Staphylococcal* strains among the burn patients is worrisome which leave us with the limited treatment options.
Keywords: Multidrug-resistance, Antibiotics, Burn wound infections, Methicillin-resistant *Staphylococcus aureus*.

INTRODUCTION

Burns, the most common and devastating forms of trauma and injury[1]. Among the mortalities that happened due to injuries, burn injury is predominantly common[2]. Wounds that result afterburn are considered as the favourable sites for bacterial multiplication[3]. In most of the patients, if the total body surface area (TBSA) is more than 40% burnt, there is a higher risk of sepsis that causes death[4]. Therefore, specialized management is necessary in order to control morbidity and mortality among burn patients[5].

A high mortality rate has been reported due to burn injuries predominantly elevated in underdeveloped countries. Reasons include insufficient facilities to control fire accidents, fewer health facilities, lack of specialized care, poor management to control the complication arise in burn patients[6]. Another reason of high mortality rate is a nosocomial infection, almost 75% deaths occur because of bacterial infection and the most common pathogens characterised from burn wounds are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella*, *Streptococcus pyogenes* and various others[7,8].

The persistence of *S. aureus* as a nosocomial pathogen is a serious concern all over the world[9]. It is the second prevalent organism causing nosocomial infection after *Pseudomonas*[10,11].Although widespread antibiotics are present to cure the bacterial infection, the emergence of drug resistance species is a common issue[12]. Globally antimicrobial resistance is a fundamental public health concern that arises due to overuse or misuse of antimicrobial compounds, poor compliance, lack of medical education, self-medication, poor choices of antibiotics at first-line treatment and many more[13].*S. aureus* is the leading cause of antibiotic resistance with continuously increasing numbers. In vitro diagnostic testing of Methicillin-resistant *S. aureus* (MRSA) is done with the cefoxitin antibiotic[14]. MRSA is an emerging problem in the field of medical microbiology, and the presence of drug resistance species at the site of burn injury made it difficult to cure the infection.Our study briefly describes the frequency of *S. aureus* and its sensitivity pattern in burn patients. The study aimed to play an important role in improving the management of patients treated with burn wound infections and to report the empirical treatment options.

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METHODS

The current cross-sectional study designed to collect the burn wound swabs from 140 patients brought to the burn unit, Allied Hospital Faisalabad, Pakistan in 2017. Only the patients with burn wounds were included in the study. The bacterial strains other than *S. aureus* were excluded from the study for further processing.This study was conducted after taking consent from the Ethical Review Committee (ERC) Punjab Medical College, Faisalabad (Ref No: 767/2017). Before taking swabs from the patients a written or informed consent was taken from patients or their relatives.

Direct swabs aseptically collected from the burn wounds were cultured on Blood, MacConkey and Mannitol salt agar medium, incubated at 35-37°C for 18-24 hours to

obtain growth of organisms and to perform qualitative and semi-quantitative analysis by colony characteristics and morphology.The individual colonies of *S.aureus* were further confirmed by Gram's staining and various biochemical tests like catalase, coagulase, and DNase. The API Staph (bioMerieux) was used for the confirmation of each bacterial strain.

The recommended guideline of the Clinical Laboratory Standard Institute (CLSI) was followed for the antimicrobial sensitivity testing[14].The Kirby-Bauer disc diffusion method was used to evaluate the antibacterial drug resistance of the *S.*aureus with CLSI recommended antibiotics. The antibiotic discs were categorised into three groups by placing two antibiotics in each group.We used erythromycin (15 µg) and clindamycin (2 µg) in Group A; cefoxitin (30 µg) and vancomycinin group B; levofloxacin (5 µg) andmoxifloxacin (5 µg) in group C. For vancomycin E-test (Oxoid) used to determine MIC (≤ 2 µg/ml sensitive; ≥ 16 µg/ml resistant). The quality control strains of *S. aureus* ATCC43300 and ATCC 25923 were used as methicillin-resistant and methicillin-sensitive *S. aureus*, respectively. The frequencies were used in terms of percentages for qualitative analysis.Descriptive analysis and Fisher's exact test was appliedto calculate the p-values using SPSS v. 24 and p-values < 0.05 was considered significant.

RESULTS

A total of 140 burn patients, admitted to Burn Centre of Allied Hospital Faisalabad, were analysed. The patients include 82(59%) males and 58(41%) females. The mean age of these patients was 20±22.5 s.d. ranging from 1 to 65 years. Aetiology of burn patients included in this study revealed 63% patients suffered from a flame burn injury, 23% with scales, 6% with an electrical burn, 4% with chemical burn and 4% had a roadside accident burn injury. Of these 140 patients, 46% had 1-25% of their Total Body Surface Area (TBSA) burnt, 40% had 26-50% TBSA, and 14% patients had more than 50% of their TBSA burnt. It was observed that 94% of total patients found positive for bacterial growth.

From the total 132 culture positive samples,190bacterial strains were isolated. It was observed that 64of the organisms were Gram-positive cocci, 28 were Gram-positive bacilli, 94 were Gram-negative bacilli, and only 4 were Gram-negative cocci. Out of the 64 Gram-positive cocci, 56 were catalase positive. Further processing revealed 56 coagulase-positive bacteria which were finally confirmed as S. aureus by API Staph.

By using in vitro antimicrobial sensitivity testing, 43% of the*S. aureus*found to be resistant and 57% sensitive to cefoxitin. Surprisingly, 4% of the *S. aureus*strains were also resistant to vancomycin, 36% against levofloxacin and 16% against moxifloxacin. Results indicate that the incidence of MRSA is not statistically significant with the age, gender, degree of burn and with the aetiology of burn. However, more cases of MRSA were observed in female patients, age less than 10 years, 25-50% TBSA and burnt by flame or liquid (Table-I).Results for the association of MRSA with different antibiotics used in this study revealed that resistance against erythromycin, clindamycin, levofloxacin, and moxifloxacinwas significantly associated with MRSA isolates p-value <0.01, <0.01, 0.03 and 0.01 respectively (Table-II).

We compared the antimicrobial sensitivity pattern between different groups of antibiotic as recommended by CLSI and observed that 36 bacterial strains were resistant to both drugs (clindamycin in combination with erythromycin) belonging to group "A" used in this research work. Only two isolates were sensitive for both antibiotics of group "A." For group "B" antibiotics (cefoxitin in combination with vancomycin) six bacterial isolates were resistant, and 24 were sensitive to both of these drugs. For group "C" antibiotics (levofloxacin in combination with moxifloxacin) 16 bacterial isolates were resistant, and 16 were sensitive to both drugs.

A multidrug-resistant pattern was observed when combinations of antibiotics of different groups were compared. We found six isolates resistant and two sensitive to four antibiotics, two of group "A" (clindamycin and erythromycin) and two of group "B" (cefoxitin and vancomycin). For the combination of group "A" and "C," it was observed that 14 isolates were resistant and only two isolates were sensitive to all four antibiotics. Maximum antimicrobial sensitivity observed for group "B" in combination with group "C". However among all isolates of *S. aureus* only two isolates were sensitive for all six antibiotics, two of each group "A, B and C" used in this study and six isolates were resistant for all of these antibiotics (Table-III).

Table-I: Association of MRSA with demographic characteristics and aetiology of burns (n=140).

Characteristics		**No. (%)n=140**	**MRSA n=24 (43%)**	**MSSA n=32 (57%)**	**p-value**
Gender	Male	82 (59%)	10 (41.7%)	22 (68.8%)	0.15
	Female	58 (41%)	14 (58.3%)	10 (31.3%)	
TBSA	1-25%	64 (46%)	8 (33.3%)	12 (37.5%)	0.94
	26-50%	56 (40%)	12 (50%)	16 (50%)	
	> 50%	20 (14%)	4 (16.7%)	4 (12.5%)	
Age	< 10 years	24 (17%)	14 (58.3%)	12 (37.5%)	0.23
	11-30 years	56 (40%)	2 (8.3%)	12 (37.5%)	
	30-60 years	58 (41.6%)	8 (33.3%)	6 (25%)	
	> 60 years	2 (1.4%)	0 (0%)	2 (6.3%)	
Etiology	Chemical	6 (4%)	2 (8.3%)	2 (6.7%)	0.44
	Electrical	8 (6%)	0 (0%)	4 (12.5%)	
	Flame	88 (63%)	10 (41.7%)	18 (56.3%)	
	Liquid	32 (23%)	10 (41.7%)	8 (25%)	
	RTA	6 (4%)	2 (8.3%)	0 (0%)	

Table-II: Association of MRSA and MSSA antimicrobial effects in vitro.

Antibiotics		Percentage (No.)	MRSA (n=24)	MSSA (n=32)	p-value
Erythromycin	Sensitive	25 (44%)	0 (0%)	8 (25%)	< 0.01
	Resistant	31 (56%)	24 (100%)	24 (75%)	
Clindamycin	Sensitive	18 (32%)	0 (0%)	18 (56.3%)	< 0.01
	Resistant	18 (68%)	24 (100%)	14 (43.7%)	
Vancomycin	Sensitive	54 (96%)	22 (91.7%)	32 (100%)	0.24
	Resistant	2 (4%)	2 (8.3%)	0 (0%)	
Levofloxacin	Sensitive	36 (64%)	10 (41.7%)	26 (81.3%)	0.03
	Resistant	20 (36%)	14 (58.3%)	6 (18.8%)	
Moxifloxacin	Sensitive	47 (84%)	16 (66.7%)	32 (100%)	0.01
	Resistant	9 (16%)	8 (33.3%)	0 (0%)	

Table-III: Multidrug resistance pattern of *S. aureus*isolated from burn wounds.

Group of Antibiotics	Sensitive	Resistant
Group A	2 (5%)	36 (95%)
Group B	24 (80%)	6 (20%)
Group C	16 (50%)	16 (50%)
Group A+B	2 (25%)	6 (75%)
Group A+C	2 (13%)	14 (87%)
Group B+C	14 (70%)	6 (30%)
Group A+B+C	2 (25%)	6 (75%)

Group A: clindamycin and erythromycin;
Group B: cefoxitin and vancomycin;
Group C:levofloxacin and moxifloxacin.

DISCUSSION

We report a high prevalence of MRSA among the burn patients. Our results indicate that burn injuries are more common in males. Aghakhani et al. in 2011 also reported gender difference in burn patients[15].In this study flame burn injury was most prevalent among all other etiological reasons. Flame on a scale is the most common type reported by others[16].

The culture positivity of 94% infections in burn patients was profoundly high in our study which indicates that the presence of any microorganism at the site of a wound which develops afterburn is highly infectious[17]. Mixed microbial flora as reported in our studies replicates previous studies. However, *S. aureus* was reported as the common isolates unlike *Pseudomonas aeruginosa* found in this study[3,18].*S. aureus* is considered a significant pathogen among burn patients across the world[19].Various studies from Pakistan have reported the different prevalence rate of MRSA in burn patients. A multicenter study has reported a prevalence of 61% MRSA in Lahore 57% in Karachi, 46% in Rawalpindi and Islamabad, 36% in Peshawar and 26% in Quetta[20,21,22]. A similar kind of study in Faisalabad reported 40% MRSA from burn patients which closely match our findings of 43% MRSA[11].

It was observed that all of the MRSA strains isolated in this study were from the mixed bacterial flora that was obtained from a single wound swab culture. Presence of MRSA and multidrug resistance at a site of infection impose problems in control of infection[23,24]. Apart from MRSA, vancomycin resistance *S. aureus* (VRSA) were also observed in these samples, which is an alarming situation for the local healthcare bodies. The vancomycin has been reported as a drug of choice in some studies without any reportable antimicrobial resistance with multidrug-resistant *Staphylococci*[25,26].

For the different groups of antibiotics, results of this study suggest multidrug resistance pattern for *S. aureus*. For the primary group (A) resistance pattern was much high, and interestingly for group C those are considered supplement antibiotics resistance pattern was also high, that indicate the misuse of antibiotics in our local population. However, among all three groups, the best combination of therapy was achieved from vancomycin. There is less literature that reports the prevalence of MRSA among burn patients, so this data is significant for healthcare bodies.

The isolation of a high number of multidrug-resistant bacterial strains of MRSA among the burn patients of a tertiary care hospital is worrisome. The reasons for this elevated pattern of multidrug resistance of *Staphylococci* might be lack of awareness in taking care of burn wounds, horizontal transmission multidrug-resistant *Staphylococci* by the healthcare staff and non-adherence to the hospital guidelines[27].

CONCLUSION

High frequency of methicillin resistant *S.aureus* was isolated from burn patients of a tertiary care hospital is worrisome. We found vancomycin is the most sensitive drug against *Staphylococci* although isolation of some vancomycin-resistant strains is alarming. Another option but with less success rate to treat the *Staphylococcal* infections was the use of levofloxacin and moxifloxacin.

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