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Antibiotic susceptibility of vancomyin and nitrofurantoin in *Staphylococcus aureus* isolated from burnt patients in Sulaimaniyah, Iraqi Kurdistan

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SUMMARY

Burns remain a significant public health problem in terms of morbidity and mortality throughout the world, especially in low and middle-income countries. Burning raptures the skin barriers that normally prevent invasion by microorganisms and infection is a major complication in burn patients. Methicillin resistant *Staphylococcus aureus* (MRSA) is the most important nosocomial pathogen. This retrospective analysis was conducted in the burn unit of the Department of Microbiology in the Sulamaini *Plastic Surgery and Burns Hospital.* The analysis is based on data collected from the medical records of 2938 burn patients, hospitalized between May 2008 and December 2011. The clinical samples were taken from various body sources for microbiological tests. Patients with a high percentage of total body surface area burnt ($P<0.001$) and a longer hospital stay ($P<0.001$) were more likely to have infection compared to other patients. In addition, among all tested antibiotics, vancomycin and nitrofurantion seem to be the most effective antibiotics for MRSA. Furthermore there was a significant association between age and antibiotic resistance for all antibiotics except for vancomycin and nitrofurantoin. Resistance to antibiotics increased with advancing age. The wide use of antibiotics in the treatment of bacterial infections has probably led to the emergence and spread of resistant strains. Routine microbiological surveillance and careful in vitro testing prior to antibiotic use and strict adherence to hospital antibiotic policy may help in the prevention and treatment of antibiotic resistant pathogens in burn infections.

KEY WORDS: Infection, Antibiotic resistance, *Staphylococcus aureus*, Burns, Sulaimaniyah, Iraqi Kurdistan.

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INTRODUCTION

Infection remains the leading cause of morbidity and death among burn patients. Treatment and prevention of invasive nosocomial wound and bloodstream infection among burn patients presents a difficult challenge in low-income and middle-income countries. A particular challenge is the emergence of highly drug-resistant bacterial pathogens (Kumarasamy *et al.,* 2010). Burns remain a significant public health problem in terms of morbidity, long-term disability and mortality throughout the world, especially in developing countries **(**Othman and Kendrick, 2010; Othman and Kendrick, 2011). Thermal injury destroys the skin barriers that normally prevent invasion by microorganisms (Singh *et al*., 2003; Barret *et al*., 1999; Murray and Finegold 1984; Lari AK *et al*., 1998; Nasser *et al*., 2003). Burn patients become susceptible to infection due to the loss of this protective barrier and decreased cellular and hu-

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moral immunity (Wong *et al*., 2002). Infection remains a major complication in burn patients after the initial period of shock and the chance of infection persists until complete wound healing (Kaushik *et al*., 2001; Pallua *et al*., 1999 and Gang *et al*., 1999). On the other hand, extensive full thickness burn wounds especially have a high incidence of sepsis and other physical, pathological and psychological complications. It is estimated that infection in burn patients is responsible for as high as 70% of the mortality whereas the remaining 30% is attributable to other causes (Singh *et al*., 2003; Nasser *et al*., 2003; Kaushik *et al*., 2001; Gang *et al*., 1999; Tang *et al*., 1999; Bang *et al*., 1999; Oraloncul, *et al*., 2002; Nakae and 2000). Gram-positive bacteria in the depth of sweat glands and hair follicles may survive the heat of initial injury. Following colonization, these surface organisms start to penetrate the burn eschar and the viable surrounding tissues (Nasser *et al*., 2003 and Pallua *et al*., 1999). Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most important pathogen among Gram-positive cocci in burn infection (Oraloncul *et al*., 2002) and patients with extensive burn injuries are especially susceptible to infection with this organism, therefore infection with MRSA requires special management. The percentage of these patients with proven microbiological MRSA infection in industrial countries has increased dramatically (Fuchs *et al*., 2002). Burn units within hospitals have become a major reservoir for MRSA with a special potential for spreading quickly in the hospital environment (Mokaddas *et al.,* 1999). This nosocomial pathogen causes outbreaks of infection that result in serious problems in the management of burn patients because of the presence of many strains becoming multiresistant to several classes of antibiotics (Gang *et al*., 1999; Mokaddas *et al.,* 1999). The aim of the present study was to provide the epidemiology of the Methicillin-resistant *Staphylococcus aureus* infection in burn patients and identify effective antibiotics against MRSA to treat infections in our burn center.

MATERIAL AND METHODS

Data management and statistical analysis

This retrospective analysis was conducted in the Sulaimani Burns and Plastic Hospital (the burn centre). The epidemiological analysis is based on data collected from the medical records of 2938 burn patients, hospitalized between May 2008 and December 2011. Data collected included age, sex, percent total body surface area burnt (TBSA), residence, season of injury, mechanism of injury, and outcome. The clinical samples were taken for microbiological tests from various body sources (blood, urine, burn wound) at different time intervals during the patients' stay in hospital. Culture and sensitivity tests were undertaken at the centre's microbiology laboratory. Analysis was undertaken using Stata version 9 (Statacorp 2006). Descriptive analysis was performed; numeric data were summarized as means or medians depending on normality. Associations between categorical variables were tested by chi-squared test and p values equal to or smaller than 0.05 were reported a significant.

Microbiological assessment

A) Bacterial isolation and identification

For isolation, all the samples were inoculated on a selective and differential medium (mannitol salt agar), enriched medium (blood agar) and incubated at 37°C for 24 hours, colonies of mannitol fermenter and beta hemolysis of staphylococci were submitted to identification tests according to Bergey's manual of determinative bacteriology (Holt *et al.,* 1994; Atlas *et al.,* 1995; Mahon *et al.,* 2000), and finally analytical profile index (API staph system) and latex agglutination test (Staphurex) were used as a confirmation step of identification (BioMerieux SA, Lyon, France).

B-Antibiotic sensitivity test (Kirby-Bauer method)

All isolated *S. aureus* were tested against eight commonly used antibiotic discs (vancomycin, oxacillin, nitrofurantion, clindamycin, rifampicin, gentamycin, cotrimoxazol, and erythromycin) (Table 2). Inoculums from the tested bacterium were prepared depending on Kirby-Bauer antibiotic testing. The bacterial suspensions were prepared from fresh single colonies and adjusted by comparison with 2 McFarland turbidity standard ($6x10^8$ cells/ ml) tubes. Sterile cotton swab was dipped into the inoculum and then swabbed evenly across the surface of a Mueller-Hinton agar plate, after that within 15 minutes of inoculation, the antimicrobial-containing discs are applied to

the agar with a forceps pressed firmly to ensure contact with the agar and then plate inverted and incubated at 37°C for 18 hours (Verhaegen *et al.,* 2003)**.** Inhibition zones were expressed in (mm) as the diameters of clear zones around the discs (CLSI, 2007). The most common antibiotic disc according of the (Fluka company) are vancomycin (VAN), oxacillin (OXA), nitrofurantion (F), clindamycin (CLI), rifampicin (RA), gentamycin (CN), co-trimoxazol, and erythromycin (ERY) and their international standard concentrations in (µg /disc) were 30 , 1, 300, 2, 5, 10, 25, and 15, respectively.

RESULTS

1. Main characteristics of participants

Of 2938 patients who were admitted in the burns centre during the study period, a total of 654 patients (males 42%, females 58%) yielded at least one positive culture for *Staphylococcus aureus* accounting for 22% of all patients. Among S. *aureus* positive cases 88% were MRSA. The age of these patients was not normally distributed ranging from under one to 86 years (median 18, IQR 4, 26) with 39% of patients being children below 15 and 38% being aged 15-29 years. The majority of burns was caused by flame (68%) followed by scalds (26%). The total body surface area ranged from 1 to 83% with a mean of 26.8% (SD 15.3). The mean hospital stay was not normally distributed and ranged from 0-86 days with a median of 17 days (IQR 9, 28). See Table 4 for other characteristics of the samples.

2. Factors associated with *S. aureus* infection

Detailed data for patient characteristics of those with and without *S. aureus* infection were available for 609 patients admitted during 2008 which enabled a comparison of the two groups. There was no significant difference in gender in terms of *S. aureus* infection (28% of males and 29% of females had infection). Age, mechanism of burn and season were also not associated with infection (Table 1). Patients coming from Sulaymaniyah city were significantly less likely to be infected compared to patients coming from outside the city and from other provinces (20%, 29% and 37% respectively, P=0.002). Patients with total body surface area (%TBSA) burnt of 25-50% were more likely to have infection compared to patients with %TBSA <25% (68% vs. 25%, P<0.001). Infection was also associated with a longer hospital stay. While 84% of patients staying over 3 weeks had infection, only 6% of those staying up to 7 days had infection (P<0.001).

TABLE 1 - *Main characteristics of participants.*

Characteristics	*Number*	*Percent*
Total	654	100
Year		
2008	173	26.5
2009	209	32.0
2010	136	20.8
2011	136	20.8
Sex		
Male	273	41.7
Female	381	58.3
Age		
0-5 years	166	25.5
6-14 years	86	13.2
15-29 years	249	38.3
30-59 years	126	19.4
60 and over	24	3.7
Residence		
Sulaymaniyah city	183	28.0
Outside Sulaymaniyah city	260	39.8
Other provinces	210	32.2
Season of injury		
Winter	140	21.4
Spring	175	26.8
Summer	167	25.5
Autumn	172	26.3
Mechanism of injury		
Flame	441	67.7
Scald	167	25.7
Other	51	6.6
Mortality rate	98	15.0
Median age in years (IQR)	18 (4, 26)	
Median TBSA% burnt (IQR)	25 (16,37)	
Median hospital stay in days (IQR)	17 (9, 28)	

The main characteristics of all burn patients. The table gives both the number and percentage of the patients (years, sex, age, residence, season of injury, mechanism of injury and mortality rate) and the median of age, total body surface area, and hospital stay.

TABLE 2 - *Antibiotic resistance and sensitivity in 1063 positive S. aureus cultures.*

Antibiotic	*Resistant Number (%)*	*Sensitive Number (%)*
Vancomyin	5 (0.5)	1057 (99.5)
Oxacillin	944 (88.9)	118 (11.1)
Nitrofurantoin	27 (2.5)	1035 (97.5)
Clindamycin	605 (57.0)	457 (42.5)
Rifampicin	608 (57.0)	454 (43.0)
Gentamicin	866 (81.5)	197 (18.5)
Cotrimoxazole	881 (82.9)	182 (17.1)
Erythromycin	929 (87.4)	134(12.6)

Antibiotic resistance and sensitivity in 1063 positive *S. aureus* cultures**.** All isolated *S. aureus* were tested against 8 commonly used antibiotic discs (vancomycin, oxacillin, nitrofurantion, clindamycin, rifampicin, gentamycin, cotrimoxazol and erythromycin). Percentage for both resistance and sensitive MRSA is also reported in the table.

3. Sites of the positive specimens

The study included 1063 instances of positive cultures for *S. aureus* from 654 patients. Number of positive samples per patient ranged from 1 to 9 samples. There were 426 patients (65%) with one sample, 122 patients (19%) with two sample, 65 patients (10%) with 3 samples and fewer patients with more positive samples. The majority of the samples were taken from the lower limbs and feet (36%) followed by the upper limbs and hands (24%) and the trunk (24%). See Table 5 for more details.

4. Antibiotic resistance

Antibiotic sensitivity was undertaken for vancomycin, oxacillin, nitrofurantoin, clindamycin, rifampicin, gentamicin, cotrimoxazole and erythromycin. Table 2 shows more details on the antibiotic resistance and sensitivity to these antibiotics in 1063 positive *S. aureus* cultures. Overall, vancomycin seems to be the most effective antibiotic with only 0.5% of cultures showing resistance while oxacillin is the least effective with 89% resistance. Nitrofurantoin is the second most effective with 2.5% resistance while resistance to gentamicin is also high at 82%.

5. Antibiotic resistance according to site of infection

Table 6 shows antibiotic resistance in relation to various infection sites.

Cultures from the earlobe have the highest resistance to vancomycin with 4% of cultures being resistant to this antibiotic. Earlobe infection seems to be more resistant to most other antibiotics compared to other sites too. Infections of the hand are less resistant to most antibiotics compared to other sites.

6. Antibiotic resistance according to sex and age of patients

Resistance to antibiotics was also compared ac-

TABLE 3 - *Percentage of S aureus samples resistant to antibiotics according to sex and age of patients.*

	Percentage S. aureus samples resistant to antibiotics							
	Vancomycin	*Oxacillin*	*Nitrofurantoin*	*Clindamycin*	*Rifampicin*	*Gentamicin*	*Cotrimoxazole*	*Erythromycin*
Sex								
Male	0.0*	87.1*	1.9*	54.8*	56.0*	79.3*	80.1*	83.3†
Female	0.6	90.1	3.0	58.4	58.1	82.9	84.8	90.1
Age								
0-5 years	0.0*	80.7†	0.8*	52.2*	47.1†	70.2†	74.0†	78.6†
6-14 years	0.0	86.0	2.9	51.9	52.2	79.4	79.4	84.6
15-29 years	0.7	92.5	2.3	59.3	60.8	84.8	86.7	91.6
30-59 years	0.5	91.4	4.8	60.3	64.1	87.1	85.2	89.0
60 and over	0.0	97.1	2.9	60.0	60.0	91.4	97.1	97.1

*P value bigger than 0.05, difference between groups is not significant. †P value smaller than 0.05, difference between groups is significant. Percentage of *S. aureus* samples resistant to antibiotics according to sex and age of patients.

cording to gender and age of patients. There was no significant difference between males and females in terms of antibiotic resistance for all antibiotics except erythromycin (Table 3). While resistance to erythromycin was 83% in males, it was 90% in females (χ^2=10.6, 1 df, P=0.001). In terms of age, there was a significant association between age and antibiotic resistance for all antibiotics except for vancomycin and nitrofurantoin. Resistance to antibiotics increased with advancing age. For example resistance to oxacillin was 81% in children aged 0-5 while this was 97% in patients aged 60 and over. Likewise, resistance to gentamicin was 70% in children and 91% in the older persons. See Table 3 for more information.

DISCUSSION AND CONCLUSION

Open and large areas of burn injuries create favorable conditions for the penetration of noso-

TABLE 4 - *Association between S. aureus infection and different patient characteristics.*

Characteristic	*Infection Number (%)*	*No infection Number (%)*	*P value*
Sex			
Male	71(27.7)	185 (72.3)	χ^2=0.05, 1 df, P=0.81
Female	101 (28.6)	252 (71.4)	
Age			
0-5 years	42 (28)	108 (72)	
6-14 years	11 (16.4)	56 (83.6)	
15-29 years	73 (29.8)	172 (70.2)	χ^2=0.27, 4 df, P=0.18
30-59 years	41 (32.5)	85 (67.5)	
60 and over	5 (23.8)	16 (76.2)	
Residence			
Sulaymaniyah city	41 (20.2)	162 (79.8)	
Outside Sulaymaniyah city	75 (29.3)	181 (70.7)	χ^2=12.7, 2 df, P=0.002
Other provinces	56 (37.3)	94 (62.7)	
Mechanism of injury			
Flame	119 (30.0)	278 (70.0)	
Scald	12 (30.8)	27 (69.2)	χ^2=2.5, 2 df, P=0.3
Other	41 (23.7)	132 (76.3)	
Season of injury*			
Spring	38 (24.8)	115 (75.2)	χ^2=1.4, 2 df, P=0.5
Summer	64 (30.6)	145 (69.4)	
Autumn	70 (28.3)	177 (71.7)	
Hospital stay			
0-7 days	19 (6.1)	294 (93.9)	
8-14 days	52 (32.9)	106 (67.1)	χ^2=225.0, 3 df, P<0.001
15-21 days	35 (59.3)	24 (40.7)	
22 days and over	66 (83.5)	13 (16.5)	
%TBSA burnt			
0-25%	92 (25.2)	273 (74.8)	
25.1-50%	70 (68.0)	33 (32.0)	χ^2=116.0, 3 df, P<0.001
50.1-75%	9 (14.5)	53 (85.5)	
75.1-100%	1 (1.3)	78 (98.7)	

*This group of patients did not include any from winter. The association between *S. aureus* infection and different patient characteristics like (sex, age, residence, mechanism of injury, season of injury, hospital stay, and total body surface area). This association was ascertained for both infected and non-infected persons by methicillin-resistant *Staphylococcus aureus*. Associations between categorical variables were tested by chi-squared test and p values equal to or smaller than 0.05 were reported a significant.

TABLE 5 - *Site of the positive specimens.*

Sample site	*Number*	*(%)*
Total	1063	100
Lower limb excluding foot	355	33.4
Trunk	252	23.7
Upper limb excluding hand	152	14.3
Hand	105	9.9
Blood	78	7.3
Head and neck excluding earlobe	60	5.7
Foot	30	2.8
Earlobe	26	2.5
Urine	4	0.4

Sites of positive specimens. The table shows the total number and percentage of the main infected sites by *S. aureas* (lower limb excluding foot, trunk, upper limb excluding hand, hand, blood, head and neck excluding earlobe, foot, earlobe, and urine) for 1063 burn patients.

comial pathogens. For this reason, special attention to hospital infection should be paid in burns centers. According to the data provided in literature, the most common infection in burn patients is that with *S. aureus* especially MRSA (Buzaid *et al.*, 2011). In the Plastic Surgery and Burns Hospital of our hospital MRSA was found in 88% of samples tested and the mortality rate was 15% (Table 4). Total burn surface area has been found to be most important risk factor for nosocomial infection (Oraloncul *et al*., 2002) which supports the result of the present study that showed the patients with %TBSA burnt of 25-50% were more likely to have infection compared to patients with %TBSA <25% (68% vs. 25%, P<0.001). Infection was also associated with a longer hospital stay (Hershow *et al.*, 2010). The outcome of the present study showed that the 84% of patients staying over 3 weeks had infection, only 6% of those staying up to 7 days had infection (P<0.001) (Table 1). In case of a severe burn, the probability of MRSA infection increases, since the patients spend more time in the hospital, and undergo

TABLE 6 - *Percentage S. aureus samples resistant to antibiotics according to site of infection.*

Site of infection	*Percentage S. aureus samples resistant to antibiotics*							
	Vancomycin	*Oxacillin*	*Nitrofurantoin*	*Clindamycin*	*Rifampicin*	*Gentamicin*	*Cotrimoxazole*	*Erythromycin*
Lower limb excluding foot	0.3	88.2	3.1	56.1	57.2	81.4	81.7	85.9
Trunk	0.0	88.1	1.6	56.4	56.0	78.2	83.3	88.1
Upper limb excluding hand	0.7	92.8	2.6	68.4	66.5	84.2	87.5	89.5
Hand	1.0	78.1	2.9	41.4	43.8	75.2	72.4	79.1
Blood	0.0	96.2	3.9	65.4	61.5	88.5	89.7	92.3
Head and neck excluding earlobe	0.0	95.0	1.7	56.7	65.0	88.3	85.0	96.7
Foot	0.0	82.8	0.0	50.0	51.7	70.0	76.7	80.0
Earlobe	3.9	96.2	3.9	53.9	46.2	100.0	92.3	96.2
Urine	0.0	100.0	0.0	50.0	50.0	75.0	75.0	75.0

Antibiotic resistances in relation to various infection sites (lower limb excluding foot, trunk, upper limb excluding hand, hand, blood, head and neck excluding earlobe, foot, earlobe, and urine) and the percentage of *S. aureus* samples resistant to all antibiotics.

more frequent dressings. Therefore, surgery should be performed as early as possible and patients with diagnosed MRSA infection should be isolated and dressed using disposable materials. In this study antibiotic sensitivity was undertaken for vancomycin, oxacillin, nitrofurantoin, clindamycin, rifampicin, gentamicin, cotrimoxazole and erythromycin (Table 2). Overall, vancomycin seems to be the most effective antibiotic with only 0.5% of cultures showing resistance and nitrofurantoin is the second most effective with 2.5% resistance as compared with the other used antibiotics (Table 2). This is probably because of only occasional use of these two antibiotics versus prolonged and widespread use of other antibiotics without culture and sensitivity tests which has led to emergence of more resistant strains. In terms of age, there was a significant association between age and antibiotic resistance for all antibiotics except for vancomycin and nitrofurantoin. Resistance to antibiotics increased with advancing age (Table 3). For example resistance to oxacillin was 81% in children aged 0-5 while this was 97% in patients aged 60 and over. Likewise, resistance to gentamicin was 70% in children and 91% in older persons (Table 3). Furthermore, Edwards *et al*. concluded that infection is more common at extreme ages (Edwards *et al.,* 2003). So the reason for this finding could also be the excessive and irrational use of antibiotics in our study population because the frequent usage of antibiotics stimulates the development of antibiotic resistant microbes, increases treatment costs, and causes side-effects. All these could probably be avoided if antibacterial preparations are administered rationally meaning that antibiotics must be used only when necessary, with a careful choice of the suitable medicine, dose and optimal duration of treatment. Late arrival in hospital, home use of traditional remedies for burn wounds and the contaminated hospital environment could all favor colonization and infection of the burn wounds. Prophylactic use of antibiotics in burn patients is hardly justified. Antibiotics are better used when the causative agent is known, and antibiotic sensitivity test is undertaken, starting with lower generation antibiotics, and in the presence of the symptoms of wound infection. The high incidence of MRSA in our burns centre (88%) supports the opinion that transmission from patient to patient in the hospital is the most likely source of the MRSA infection (Boyce *et al.,* 1981). Infection by MRSA leads to significant burn wound morbidity, with ongoing sepsis, graft loss, and the ever-present threat of invasive burn wound infection with an associated mortality of 20 to 40% (Collopy *et al.,* 1984; Crossley *et al.,* 1979). Commonly used anti-staphylococcal antiseptics and topical agents have bactericidal activity against MRSA, a significant number of these organisms are not eliminated (Hayley *et al.,* 1985). Based on our findings, only vancomycin and nitrofurantoin seem to be of value in the treatment of MRSA amongst the 8 antibiotics tested. However, because of its high concentrations in the urine, nitrofurantoin could be a good choice in urinary tract infections caused by this organism. In this study vancomycin was highly effective against MRSA in vitro and therefore could be a good choice for MRSA infections in burn patients. Use of less effective antibiotics including rifampicin, clindamicin and gentamicin (Table 2) in patients suspected of or having MRSA infections has to be justified. The risk of infection with this multidrug resistant microorganism could be reduced by forceful implementation of strict infection control measures. This may not only reduce the diagnostic and therapeutic cost but it can also help in delaying the process of development of drug resistance in clinical isolates.

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