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Antibiotic Resistance Pattern of Bacterial Isolates from Burn Wounds at a Private Hospital in Lahore, Pakistan

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ABSTRACT

Burn wound infections are medical complications that lead to sepsis, bacteremia, septicemia, prolonged hospitalization and failure of treatment. Present study was aimed to isolate and identify bacterial isolates from burn patients and to determine the antibiotic resistance pattern of isolated bacteria. A total of 120 burn patients of all ages were included in this study. Bacterial isolation and identification was performed using nutrient agar, blood agar, MacConkey agar and different biochemical tests. Antimicrobial susceptibility testing was performed on Mueller-Hinton agar by disk diffusion method. Younger patients aging ≤15 years accounted for 29% whereas adult patients >15 years were 71%. Most of the burn patients were illiterate and belonged to poor class who work in industries, villages, chef in hotels or vendors. A total of 79 bacterial strains were isolated from all clinical samples and *Staphylococcus aureus* was found most abundant (53%) strain whereas the prevalence of *Pseudomonas aeruginosa strain were* 30% and *Escherichia coli* was 17%. Most of the isolates were resistant to co-trimoxazole although 76%, 68%, 58%, 33% and 30% of isolates expressed resistance to ceftazidime, gentamicin, ciprofloxacin, amikacin and imipenem, respectively. Conclusively, highly resistant isolates were involved in burn wound infections which could be resulted in prolonged and ineffective therapeutics.

Keywords: Burn wound infections, Antibiotic sensitivity, *Staphylococcus aureus*, multidrug resistant, Lahore.

INTRODUCTION

Burn wound infections (BWI) are medical complications that lead to sepsis, bacteremia, septicemia, prolonged hospitalization and failure of treatment (Obiazi *et al.,* 2007). It is estimated that 75% deaths in burn patients are due to infections rather than osmotic shock and hypovolemia (Rajput *et al.,* 2008). Globally burns are one of the serious health care problems. Burn injuries rank among the most severe types of grievances agonized by human body with an associated high mortality and morbidity rate. According to World Health Organization an estimated 265,000 deaths every year are caused by burns and majority of the cases occur in low and middle income countries. In India, over one million people are moderately to severely burnt every year. Nearly 173,000 Bangladeshi children are moderately or severely burnt every year. In Bangladesh, Colombia, Egypt and Pakistan, 17% of children with burns have a temporary disability and 18% have a permanent disability. Burns are the second most common injury in rural Nepal, accounting for 5% of disabilities. In 2008, over 410,000 burn injuries occurred in the United States of America, with approximately 40,000 requiring hospitalization (WHO, 2014). Bacterial infection of burns is an inevitable phenomenon because burn sites are ideal places for the growth and multiplication of bacteria. Burned patient becomes immune-compromised due to loss of physical barrier (skin) that protects the body. Damaged blood vessels of the burn patients keep the organism safe from administered antibiotics. Moreover, denatured protein of eschar provides

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nutrition for the organism (Kehinde *et al.,* 2004). The sources of burn wound contaminations are hospital environment, patient's own microbial flora such as bacteria present on skin, sweat and sebaceous glands, respiratory and gastrointestinal tract, or by the contaminated hands of health care workers (Church *et al.,* 2006). Cross infection may result in different burn patients due to overcrowding in burn wards. There are several factors involved in enhanced prevalence of burn wound infections which include old age, burns exceeding 30% total body surface area (TBSA), depth of burn, invasive devices, prolonged open wound, blood transfusions, repeated exposure to hospital environment, number of ventilated days, and comorbidities like obesity, diabetes, HIV and malnutrition (Rode *et al.,* 2009).

Studies in Pakistan, India, Britain, Turkey, Palestine, Bangladesh, Nigeria, Iran, America, Europe and many other countries demonstrated that common pathogens causing burn infections are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli, Bacteriodes fragilis*, *Acinetobacter baumannii*, *Peptostreptococcus* spp., *Propioni bacterium* spp., *Fusobacterium* spp*., Klebsiella* spp., *Proteus* spp., various fungi like *Aspergillus niger, Candida* spp. and *Zygomycetes* spp., (Shahzad *et al.,* 2012).

However, bacterial flora of different burns unit is different because it varies with time and geographical location and depends upon pre-existing disease, types of antibiotic therapy, and the residential flora of the burn unit. Antibiotic resistance in burn patients prolongs illness and increases health care cost hence complicates the treatment which ultimately happens a serious life threat of the patient (Savas *et al*., 2004).

The present study was aimed to determine distribution and identification of bacterial species isolated from burn patients admitted in a private Burn Hospital, Lahore and antimicrobial susceptibility pattern of isolated bacteria.

MATERIALS AND METHODS

Sample collection

A total of 120 burn patients of all ages, admitted in burn unit of Shafiq Aziz Burn Hospital, Lahore, Pakistan admitted from December 2014 to March 2015 were included in this study. A semi-structured questionnaire was used to gather the information regarding demographic characteristics, clinical assessment of the wound, cause of burn, site affected, total body surface area (TBSA), degree and complications. Chronological data, dates of admission and discharge were also recorded.

Sterile cotton swabs were used to collect the sample from each infected patient in the hospital during the study period from burn area. Samples were aseptically collected at the time of second or third bandage when the wound was purulent. Before sampling the bandage was removed and superficial surface of wound was cleaned with 70% alcohol.

Isolation and identification of bacteria

Isolation was performed on nutrient agar, blood agar, and MacConkey's agar. Purified culture obtained after incubation was further identified by microscopy (Gram's staining) and characterized by biochemical tests. Several biochemical tests like catalase, coagulase, oxidase, carbohydrate fermentation were conducted to evaluate the production of various enzymes and acids by bacterial isolates.

Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed on Mueller-Hinton agar by disk diffusion method following guidelines of Clinical and Laboratory Standards Institute (CLSI, 2014). Antibiotics included in this study for susceptibility testing were ciprofloxacin (5µg), gentamicin (10µg), vancomycin (30µg), ceftazidime (30µg), amikacin (30µg), imipenem (10µg), and co-trimoxazole (Trimethoprim/ Sulfamethoxazole) (1.25/23.75µg). Freshly prepared inoculum from the primary isolation plate or from a subculture of each strain grown on its selective agar plate was used for this test. Bacterial suspension using normal saline was prepared comparable to 0.5 McFarland of turbidity standard, inoculated on plates of Muller Hinton agar, antibiotic discs are placed on inoculated plate and incubated overnight. Zones of inhibition were measured, recorded and results were interpreted according to guidelines of CLSI.

Statistical comparison of bacterial isolates, their resistance pattern, risk factors (age, gender,

TBSA, type burn sites, and burn degree) was compared using Chi square test. *P*-value of <0.05 was considered as statistically significant cutoff.

RESULTS

Younger patients aging ≤ 15 years accounted for 29% whereas adult patients above 15 years were 71%. Demographically, 40% of people belonged to middle class who suffered from burn accident at home. Highest proportion of body parts affected were lower limbs (48%) followed by upper limbs (26%) and trunks (20%), while head and neck accounted for lowest percentage (3%). Scalds resulted in highest proportion *i.e.,* 46%, whereas open fire was reasoned in 15% cases, electric burn and burns due to blow of gas cylinder were found in 3% cases. Burns caused by contact with hot object like iron and bike silencer accounted for 8% cases. Burns due to blow of gas tank of cars, industries or pressure cooker exploded at home were observed in 3% cases as shown in Table 1.

The mean burned TBSA of the patients was 17.8 with a range of 1-50% whereas TBSA between 1-10% were recorded among highest proportion of patients (58%). TBSA ≥30% was observed among lowest number of patients that was only 10%. High number of second degree burn cases were 78% while third degree cases were 22%). High number of (63%) burn injuries was noticed in age group below 15 years in present study.

Overall 83% isolates were identified, out of these 73% were infected with single type of bacteria while 12% cases showed mixed growth of gram positive and negative bacteria. The most prevalent isolate was *Staphylococcus aureus* (53%), followed by *Pseudomonas aeruginosa* (30%) and *Escherichia coli* (17%) as shown in Table 2.

Various factors like age, gender, burn site, burn TBSA and degree of burn were studied with reference to culture positivity. High culture negativity (77%) was found in age group below15 years as compared to culture negativity in age group above15 years. This difference showed statistical significance (*P*<0.001). Higher positivity of culture (53%) was observed in case of females as compared to 46% cases of males included in this study.

Table 1: Demographic characteristics of the patients.

Demographic factors	Characteristics	Value (%)
Gender	Male	60 (50)
	Female	60(50)
Marital status	Married	75(62.5)
	Unmarried	45 (37.5)
Mean age in years	Male	30.4
	Female	34
	Total	26.2
	Range	1-78
Education	Illiterate	47(39)
	Primary	38 (31)
	Middle	24 (20)
	Matric	12 (10)
Occupation	Chef/cooking	16 (13.33)
	Industrial work	58 (48.33)
	others	46(38.33)
H/O antibiotic treatment	Present	29 (24)
	Absent	56 (46)
	Not established	35 (29)
Socio economic status	Poor class	80 (66.67)
	Middle Class	40 (33.33)
Mode of burn injury	Accidental	115 (95.86)
	Suicidal	02 (1.6)
	Homicidal	03 (2.5)
Burn sites	Head and neck	08 (6.0)
	Upper limb	32 (26.0)
	Lower limb	58 (48.0)
	Trunk	24 (20.0)
Cause of burn	Flame burn	19 (15.0)
	Scalds	56 (46.0)
	Chemical burn	3 (2.5)
	Contact with hot object	09 (7.5)
	Electrical burn	4 (3.3)
	Gas cylinder burst	3 (2.5)

Table 2: Distribution of isolates recovered from burn wounds (n=93).

S. No.	Bacteria	Occurrence %
1	*Staphylococcus aureus*	52.6
2	*Pseudomonas aeruginosa*	30.4
3	*Escherichia coli*	16.9

It was noted that higher isolation of bacteria from lower limb infection was statistically significant (*P*<0.001) in comparison to other burn sites. Highest culture positivity was observed in 1-10% burn TBSA which was accounted for 68% whereas cases with burn TBSA above 30% showed least positive cultures which was only 5% (*P*<0.001). There was higher incidence of positive cultures among 2nd degree burn cases, 64 (81%) as compared to positive cultures in 3rd degree burn cases which were about 15 (19%) (*P*<0.001).

Table 3: Resistance Pattern of bacterial isolates against some antibiotics.

Antibiotics	Resistance Percentage		
	Staphy. aureus	***Pseud. aeruginosa***	***E. coli***
Imipenem	33	18	19
Amikacin	20	33	44
Gentamicin	45	93	38
Ciprofloxacin	57	43	38
Ceftazidime	61	71	63
Cotrimoxazole	94	100	100
Vancomycin	0	-	-

Antibiotic susceptibility pattern depicted that all isolates were highly resistant to cotrimoxazole. Nearly, 61%, 22% and 30% of *S. aureus* isolates were resistant to ceftazidime, amikacin and ciprofloxacin respectively, whereas all of *S. aureus* isolates were susceptible to vancomycin. However, most of *P. aeruginosa* were resistant to gentamicin but 71, 43, 32 and 18% of *P. aeruginosa* isolates were resistant to ceftazidime, ciprofloxacin, amikacin and imipenem respectively. Around 63% of *E. coli* isolates were showing resistance against ceftazidime whereas 44% of *E. coli* isolates expressed resistance against gentamicin and ciprofloxacin. Moderate resistance against amikacin was observed by *E. coli*, 44% whereas most effective antibiotic noted against *E. coli* was imipenem; 71% of isolates were susceptible.

DISCUSSION

In this study there was equal number of male and female patients which is not in agreement with a study from Nishter Hospital Multan, Pakistan which reported a slightly high number of female burn patients as compared to male patients and elaborated the fact as females work in kitchen where they are at high risk of burn (Shahzad *et al.,* 2012). Other studies from India and Iran have reported high incidence of males rather than females (Bayat *et al.,* 2011; Gupta *et al*., 2012). It might be expected that both male and female individuals are equally at risk as males work in industries while females work in kitchens where both are at equal risk of burn accident.

High number of burn injuries (62.5%) was noticed in age group above fifteen in present study which is in agreement with another study carried out in India (Rajput *et al.,* 2008). A study in Mayo hospital Lahore also reported similar findings regarding age group of burn patients (Saad *et al.,* 2009). An epidemiological study in India at Dayan and Medical College and Hospital Ludhiana, Punjab reported higher 79% patients were in the age group of 15-45 year which is not in accordance our findings.

Almost all 100% subjects presented accidental burn in this study is comparable with another study in same settings (Saad *et al.,* 2009). Higher number of accidental burn cases (86%) were reported (Shirkhoda *et al.,* 2011). The mean TBSA (18%) in present study is comparable with a study undertaken in southwest of Iran (Shirkhoda *et al.,* 2011). The most common cause of burn injuries was scalds (46%) in present study which is due to careless work in kitchen and industries. This finding is in agreement with the report of center of disease control (CDC) that reported scalds account for 33%-58% of all patients hospitalized for burns in USA (CDC, 2009). Limbs burn may occur due to falling hot liquid while, whereas homicidal and suicidal attempts results face and head burns. It has been reported that extremities were involved in majority of the burn cases (Chawla *et al.,* 2005).

Present study showed culture positivity in 66% which is supported by with another study in Nigeria that showed positive cultures in 65%.Much higher culture positivity (96%)has been reported in different studies (Agnihotri *et al.,* 2004, Rajput *et al.,*2008 ; Egbe *et al.,* 2011,).

The most common organism isolated was *S. aureus* (62%) followed by *P. aeruginosa* (35%). These findings are comparable with previous studies and favor that *S. aureus* is the major organism in burns followed by *P. aeruginosa* (Agnihotri *et al.,* 2004, Rajput *et al.,* 2008, Saad *et al.,* 2009, Egbe *et al.,* 2011).

Multi drug resistant (MDR) *S. aureus* strains are defined as being resistant to two or more chemicals from different antimicrobial classes. MDR *P. aeruginosa* has been recognized as isolate intermediate or resistant to at least three classes of drugs *i.e*., β-lactams, carbapenems,

aminoglycosides, and fluoroquinolones. Multi-drug resistant organisms pose a great problem in burn units because it reduces effectiveness of treatment and increase morbidity and mortality (Komolafe *et al.,* 2003). Antibiotic sensitivity pattern of *Pseudomonas* spp. showed 100% resistant to co-trimoxazole in present study is in agreement with a recent study published in Iran on 81 burn patients at Isfahan University Hospital which also revealed 100% resistant to co-trimoxazole against *Pseudomonas spp.* (Sabzghabaee *et al.,* 2012). *Pseudomonas* spp. also showed 32% resistant against amikacin in the present study which is slightly higher than previous studies carried out in India that reported 30% resistance *Pseudomonas* strains isolated during this study were resistant to amikacin. Present findings were similar to another study undertaken in Iran (Taherzadeh *et al*., 2011). However, much higher resistance of 65% in *Pseudomonas* strains against amikacin was reported in India (Dhar *et al.,* 2007).

The findings of the study emphasize the need of laboratory guidance before prescription of antibiotics to treat wound/burn infections in order to preserve these effective antibacterial agents to become multi-drug resistant agents. This is particularly important in our environment where antibiotics are prescribed without laboratory guidance as well as over the counter sales of antibiotics is common practices. The above factors have been implicated as possible reasons for increased microbial resistance observed in the present study.

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