ORIGINAL ARTICLE

Identification and Drug Susceptibility Pattern of Pathogenic Bacterial Species among Burn Patients

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ABSTRACT

Background: Burn wound infections are medical complications that lead to sepsis, bacteremia, septicemia, prolonged hospitalization and failure of treatment.Bacterial infection of burns is an inevitable phenomenon because burn sites are ideal places for the growth and multiplication of bacteria.

Aims: To determine distribution and identification of pathogenic bacteria species among burn patients and to see the drug resistant pattern among various species of microorganisms isolated.

Study Settings: This is descriptive study was carried out in Microbiology Laboratory of Institute of Molecular biology and Biotechnology (IMBB), The University of Lahorein collaboration with Shafiq Aziz Burn Hospital from July 2014 to October 2014. Patients with only burn infections were included study.

Methods:A total of 120 burn patients of all ages were included in this study.Sterile cotton swabs were used to collect burn infection sample from each infected patient in the hospital during the study period over burn area.Samples were inoculated on nutrient agar, blood agar, and Macconkey agar and incubated for 24-48 hours at 37^0C.Purified colonies were obtained after incubation was further identified by gram staining and biochemical tests.

Results: Pediatric patients aging ≤15 years account for 35(29%) whereas adult patients >15 years were 85(71%) and the mean age of the patients was 26.2years with a range of 1 to 78 years. Demographic characteristics of burns patients belonged to poor class and illiterate people who work in industries or villages or work as chef in hotels or vendors.The overall positivity of cultures was found to be 79(66%) cases.Co-trimoxazole was found to be highly resistant among all three organisms.

Conclusion: Identification and drug susceptibility testing of burn wound infections is necessary as antibiotic resistance pattern in present study showed moderate to high antibacterial activity and it is necessary to have this pattern before prescribing any antibiotic among burn patients.

Keywords: Burn Wound Infections, BWI, Bacterial Infections, Staphylococcus, Pseudomonas.

INTRODUCTION

Burn wound infections (BWI) are medical complications that lead to sepsis, bacteremia, septicemia, prolonged hospitalization and failure of treatment[1]. It is estimated that 75% deaths in burn patients are due to infections rather than osmotic shock and hypovolemia[2]. 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 the vast majority occur in low and middle income countries. In India, over 1,000,000 people are moderately or 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[3].

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 and damaged blood vessels keep the organism from the reach of systemic administered antibiotics. Moreover, denatured protein of eschar provides nutrition for the organism[4].The sources of burn wound contaminations are hospital environment, patient's own microbial flora such as bacteria present in skin, sweat and sebaceous gland, respiratory and gastrointestinal tract, or by the contaminated hands of health care workers[5]. Cross infection may result in different burn patients due to overcrowding in burn wards. There are several factors which increases

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prevalence of burn wound infections. These factors include old age, burns exceeding 30% TBSA, increased depth of burn, invasive devices, prolonged open wound, blood transfusions, repeated exposure to hospital environment, number of ventilated days, and co morbidities like obesity, diabetes, HIV and malnutrition[6].

Burn sites remains sterile for first 24 hours but within 48 hours microorganisms from skin flora and other body systems *i.e.*GIT, respiratory tract as well as from hospital environment begins to invade burn site. Once the organism invades the wound it starts colony formation rapidly. After successful colonization bacteria forms biofilms which are enclosed in self-producing matrix or slim which renders antibiotics ineffective[6]. Severe type of infection may progress from skin surface to underlying tissues invading subeschar, subsequently prones the patients to sepsis. When the organism invades the subeschar and bacterial density exceeds 100,000/gram of tissue, it causes lethal bacteremia, delayed or non-healing of wound and eventually death. Therefore, antibiotic therapy aims to keep organism burden below 100,000 per gram of tissue[6]. The characteristic sign and symptoms of burn wound infection are presence of pus with an exudate discharge, purplish discoloration of wound, separation of eschar ,necrosis,cellutitis and other systemic symptoms like tachycardia, tachyponea, haemodynamic instability, hyper or hypothermia(>39^0C or <36^0C), anemia and mental confusion[6].

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, P. aeruginosa, Klebsiella species, Escherichia coli, proteus spp., Bacteriodesfragilis, Acinetbactorbaumannii,Peptostreptococcus, propionibacterium spp., fusobacterium spp., and various fungi Aspergilla Niger, Candida spp and zygomycetes[7]. 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 and becomes a serious threat for the life of the patient[8]. Regular monitoring of burn wound swab cultures and antimicrobial susceptibility testing in a given time is very useful for the selection of proper medicine.

This study was carried out to determine distribution and identification of pathogenic bacterial species among burn patients and to see the drug resistant pattern among various species of microorganisms isolated.

MATERIAL AND METHODS

This is descriptive study was carried out in Microbiology Laboratory of Institute of Molecular biology and Biotechnology (IMBB), The University of Lahorein collaboration with Shafiq Aziz Burn Hospital from July 2014 to October 2014. Patients with only burn infections were included in this study.

A total of 120 burn patients of all ages, admitted in burn unit of Shafiq Aziz Burn Hospital 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 etc.

Sterile cotton swabs were used to collect burn infection sample from each infected patient in the hospital during the study period over burn area. Samples were aseptically collected at the time second or third bandage when the infection had created sufficient pus. Before sampling the bandage was removed and superficial surface of wound was cleaned with 70% alcohol. Samples were inoculated on nutrient agar, blood agar, and Macconkey agar and incubated for 24-48 hours at 37^0C.Purified colonies were obtained after incubationwas further identified by gram staining and biochemical tests. The organisms were stained to see morphological characteristics under microscope by the following procedure. Several biochemical tests like catalase, coagulase, oxidase, carbohydrate fermentation, to check the availability of microorganisms for the production of various enzymes and acids.

In vitro susceptibility tests were performed on Mueller-Hinton agar by disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) standards. The inoculums prepared from the primary isolation plate or from a subculture of each strain grown on its selective agar plate. Small filter paper disks (6 mm) impregnated with a standard amount of anti-microbes were placed onto an agar plate to which bacteria have been swabbed by a bacterial suspension using distilled water comparable to 0.5 McFarland turbidity standard. The plates of Muller Hinton Agar were incubated overnight, and the zone of inhibition of bacterial growth was measured.Various antibiotics such as ciprofloxacin(5µg), gentamicin (10µg), vancomycin (30µg) ceftazidime (30µg), Amikacin (30ug) Imipenem (10ug), and Erythromycin (5ug), Doxycycllin and cotrimoxazole were the drugs used for drug sensitivity testing.

Data generated from this work were tabulated into Microsoft excel sheets and uploaded to SPSS

version 16.0 Statistical comparison of bacterial isolates and their resistance pattern was done using Chi square test. Risk factors (age, gender, TBSA, type burn sites, and burn degree) for culture results and Chi square test was used for statistical significant testing. *P*-value of <0.05 was considered as statistically significant cutoff.

RESULTS

A total of 120 samples were collected from different patients from Shafiq Aziz Burn Hospital. There were 60 females and males each with a male to female ratio of 1:1. Pediatric patients aging ≤ 15 years account for 35(29%) whereas adult patients >15 years were 85(71%) and the mean age of the patients was 26.2years with a range of 1 to 78 years. Demographic characteristics of burns patients belonged to poor class and illiterate people who work in industries or villages or work as chef in hotels or vendors. Forty percentpeople belonged to middle class who suffer from burn accident at home or any other mistakes. Highest percentage of body part affected was lower limb (48%), followed by upper limb (26%), and trunk (20%), while head and neck accounts for lowest percentage (3.2%). Scalds resulted in highest percentage 56 cases (46%), whereas open fire was responsible for 19 cases (15%), electric burn and burns due to burst of gas cylinder were found in 3 cases (2.5%). Burns caused by contact with hot object like iron and bike silencer accounted for 09 cases (7.5%).There were also burns found due to burst of gas tank of cars or in industries or pressure cooker burst at home accounted for 4 cases (3.3%) as shown in table I.

The mean burned total body surface area (TBSA) of the patient was 17.8 with a range of 1-50%, and (1-10%) category included highest percentage of patients 58% and ≥30% category showed lowest percentage of patients 10%. Second degree burn cases were highest 94(78.33%) while third degree were 26(21.67%). High no of 75(62.5%) burn injuries were noticed in age group >15 years in present study.

The overall positivity of cultures was found to be 79(66%) cases while 41(34%) samples showed no growth. Out of these 79 culture positive samples, Single isolates were present in 68 cases (86%) while 11 cases (13.9%) showed mixed growth of gram positive and negative bacteria. The most commonly isolated bacteria was *Staphylococcus aureus (S. aureus)* (62%), followed by *Pseudomonas aeruginosa (P. aeruginosa)* (35.5%), *Eschercia coli (E. coli)* (20.25%) while *Klebsiella spp., Proteus spp.* and *Enterococcus fecalis* had lowest percentages account for 5%, 2.5% *and* 5.7% respectively as shown in table II.

Table I: Demographic characteristics of the patients

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%)
Drug History of 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 site	
Head and neck	08 (6%)
Upper limb	32 (26%)
Lower limb	58 (48%)
Trunk	24 (20%)
Cause of burn	
Flame burn	19 (15%)
Scalds	56 (46%)
Chemical burn	3 (2.5%)
Contact with hot object	09 (7.5%)
Electrical burn	4 (3.3%)
Gas cylinder burst	3 (2.5%)

Various factors like age, gender, burn site, Burn TBSA and degree of burn were studied with reference to culture positivity. High culture negativity 27(77.1%) was found to be in age group <15 years as compared to culture negativity in age group >15 years. This difference showed statistical significance with P value <0.001.Higher positivity of culture in females 42(53%) as compared to 37(46%) was observed in this study. Reference to the sites of burn in the body, it was noted that that lower limb infection is significantly high (P value <0.001) in comparison to other burn sites. Highest culture positivity was

observed in 1-10% burn TBSA (68.35%) whereas TBSA above 30% showed least positive cultures (5.06%) with P value of <0.001. There was high incidence of positive 2nd degree burn cases 64(81.01%) as compared to positive 3rd degree burn cases which were about 15(18.98%) with P value<0.001.

Table II: Frequency of organisms isolate in culture positive specimens. (n=79)

Pathogens Isolated	n	%age
S.aureus	49	62
P.aeruginosa	28	35.5
E.coli	16	20.25
Proteus spp.	02	2.5
Enterococcus fecalis	06	7.6
Klebsiella spp.	04	5

Table III: Drug Resistance pattern among different organisms.

Name of drug	*S. aureus* (n= 49)	*P.aeruginosa* (n= 28)	*E. coli* (n= 16)
Imipenem	16 (32.65)	5 (17.85)	3 (18.75)
Amikacin	10 (20.40)	9 (32.14)	7 (43.75)
Gentamicin	22 (44.89)	26 (92.85)	6 (37.75)
Ciprofloxacin	28 (57.14)	12 (42.85)	6 (37.75)
Ceftazidime	30 (61.25)	20 (71.42)	10(62.50)
Cotrimoxazole	46 (93.87)	28 (100)	16 (100)
Vancomycin	0	-	-
Oxacillin	29 (40.81)	-	-

Antibiotic resistant pattern of *S.aureus, P. aeruginosa*and *E. coli* are shown in table III. Cotrimoxazolewas found to be highly resistant among all three organisms tested against. Ceftazidimewas resistant to (61.25%) isolates of *S. aureus* while this organism was least resistant to Vancomycin (0%), followed by Amikacin. (21.73%), Oxacillin (40.81%), and Ciprofloxacin (30.43%).Most effective drugs against*P.aerugionsa* were Imipenem, followed by Amikacin, and ciprofloxacin showing 17.85%, 32.14%, and 42.85% resistance respectively. Ceftazidime was found to be highly resistant both in *P.aeruginosa*(71.42) and *E. coli* (62.50).

DISCUSSION

Antibiotic resistance in burn patients delays healing and makes condition more severe. As burn patient is an immune-compromised individual because of poor vasculature and denatured protein of damaged skin provides nutrient rich environment for the growth of bacteria. All these condition favor infection to spread in blood and other organs and makes patient more ill and weak[4].Therefore, proper information regarding infecting pathogens and their resistance to various antibiotics is essential to treat patient rapidly. Sufficient research on infections in burned patients has not been done in Pakistan. Present study evaluates the bacteriology of wounds and bacterial resistance toward antibiotics.

In this study there was equal ratio (1:1) of male and female patients in burns is not in agreement witha study from Nishter Hospital Multan which reported much higher number of female patients as compared to male patientsand elaborated the fact that females work in kitchen where they are at high risk of burn[7]. Other studies from India and Iran have reported high incidence of males rather than females[9, 10].It might be expected that both male and female individuals are equally at risk as males work in industries while females work inkitchens where they are at high risk of burn accident.

High no of burn injuries (62.5%) were noticed in age group above fifteen years in present study is in agreement with an Indian study. A study in Mayo hospital Lahore is also reported similar findings regarding age group of burn patients[12]. An epidemiological study in India at Dayanand Medical College and Hospital Ludhiana, Punjab reportedmorehigher 79% patients were in the age group of 15-45 year which is not in agreement with this study[9].

Almost all 100% subjects presented accidental burn in this study is comparable with another study in same settings[12]. Another study in India also reported high cases of accidental burn (86.2%) but not comparable with present study[13]. The mean TBSA17.8% in present study is comparable with a study undertaken in southwest of Iran[14]. The most common cause of burn injuries was scalds(46%) in present study is due to careless work in kitchen and industries 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[15].This is because majority of burn cases were accidental. Careless working can result in pouring of hot liquids in limbs whereas homicidal and suicidal attempts results face and head burns. This is in agreement with the Chawla et al in which extremities were involved in majority of the cases[16].

Present study showed culture positivity in only 66% cases is in agreement with another study in Nigeria which showed positive cultures in 64.8% cases[17] whileother studies showed much higher culture positivity in 96% cases hence are not comparable with present study[2,18,].

The most common organism was *Staphylococcus aureus*(62%) followed by *Pseudomonasaeruginosa*(35.5%)are comparable with previous studiesand favor that *S. aureus* is the major organism in burns followed by *P. aeruginosa*[2,12,17,18]. Third most isolated organism was *E.coli* (20.25%) is similar withfindings of other similar sort studies under same conditions[2,7,12,].*Klebsiella*(5%), *Enterococcus fecalis*

(7.6%) and *Proteus* (2.5%)cases in present study are also in agreement with previous studies[2,7,12,17].

Multi drug resistant (MDR) *S. aureus* strains are defined as being resistant to two or more chemicals from different antimicrobial classes while MDR *P. aeruginosa* are recognized as isolates intermediate or resistant to at least three drugs in the following classes: 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[19]. Antibiotic susceptibility Pattern of *pseudomonas* 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*[20]. An older study in Tohid Burn Center Iran also stated that co-trimoxazole resistance was over 95%[21].Imipenem showed 17.85% resistance against *Pseudomonas*, proved to be the most sensitive drug in present study is comparable with a study in India at Govt. Medical College Srinagar[22].

Present study is also in agreement with a recent study undertaken in Iran[23],however much higher resistance of 65% in Pseudomonalstrians against Amikacinwas reported in an Indian study[24].*Pseudomonas* resistance against gentamicin 92.85%, ciprofloxacin 42.85%, ceftazidime 71.4%, in present study are comparable with other studies[2,7,21].

The high antibacterial activity of Imipenem may be due to the fact that this antibiotic is taking parentally and as such may not favor abuse of it in the environment. Therefore, there is need for 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 prescription of antibiotics without laboratory guidance as well as over the counter sales of antibiotics without prescription are common practices that have been implicated as possible reasons for increased antimicrobial resistance observed in present study.

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