Burn Wound Microbiology and the Antibiotic Susceptibility Patterns of Bacterial Isolates in Three Burn Units of Abbottabad, Pakistan

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Infection is the leading cause of morbidity and mortality among burn patients and is accentuated multifold by the emergence of antimicrobial resistance among the nosocomial isolates. It is vital to know the common organisms involved in infected burn wound etiology and their respective antibiotic susceptibility patterns. These crucial findings can help in formulating a better and more efficient antimicrobial therapy plan for controlling burn wound infections. The current study was conducted to identify the common bacteria involved in causing infections in wounds of burn patients and their respective antibiotic susceptibility patterns in three hospitals of Abbottabad, Pakistan. A total of 100 patients were included from the burn units of three hospitals in Abbottabad. Wound swabs were taken from the deepest portions of infected burns, and the organisms involved were isolated via standard microbiological techniques. The Kirby-Bauer disc diffusion technique was used to monitor antibiotic susceptibility. Gram-positive organisms were found readily in infected burn wounds. *Staphylococcus aureus* (46%) was the most common isolate followed by *Staphylococcus epidermidis* (17%), *Escherichia coli* (16%), *Proteus spp.* (12%), *Klebsiella pneumoniae* (10%), and *Pseudomonas aeruginosa*, which was only 7%. Gram-positive bacteria were sensitive to amikacin, gentamicin, cefotaxime, and norfloxacin. In contrast, the gram-negative isolates were sensitive to amikacin, chloramphenicol, and nalidixic acid. *Pseudomonas* was resistant to most of the antibiotics tested in the present study.

Burn injury is one of the most debilitating forms of trauma, with wound infections being one of its most daunting complications.[1] The burn injury renders the skin open to the exposure of several different microbes. The increased susceptibility of infections among burn patients is primarily due to the suppressed immunity because of the ruptured protective barrier of the skin as well as the augmenting systemic inflammatory response.[2] In addition, the burn wound site is highly moist due to the exudation and is rich in nutrients needed for bacterial growth.[3] As observed, nosocomial infections are more common in burn patients when compared with patients of other wards.[4] The extreme degree of bacterial wound infection, if left untreated, can transform into life-threatening "sepsis".[5] Sepsis is mainly responsible for more than 73% mortalities within the first 5 days post-burn.[6]

Burn wounds are the perfect adobes for bacterial proliferation. The bacterial profile in burn wound changes significantly over time. The burn site remains sterile for the first 24 hours of the burn injury; however, when this period subsides, the gram-negative bacteria readily colonize the wound area.[7] Within a few days of the burn, the gut flora of the patient also colonizes the wounds. These organisms are mostly gram-negative bacteria. Gram-positive bacteria, on the other hand, typically comprise the normal skin flora.[8]

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The biggest challenge in managing burn infections is the appropriate selection and use of antimicrobial agents. The burn wound microbial dynamics evolve rapidly, with multiple bacterial species invading the tissue at one time. The colonizing organisms include both the patient's body flora as well as the nosocomial species. Unlike in other diseases, the use of antibiotics is thus rendered quite complicated in infected burn wounds. The basic pharmacokinetic parameters of antibiotics (absorption, distribution, metabolism, and excretion) are significantly hampered in burn patients, and significant variations exist between individuals.[9] Therefore, constant monitoring of infections and antibiotic susceptibility patterns in burn patients becomes critically important.

The ineffective, non-regulated use of antimicrobials has been associated with the emergence of multidrug-resistant bacteria. Multidrug resistance has emerged as a notorious threat to the prognosis of the burn injuries. This vexing phenomenon calls for the establishment of a strict antibiotic usage policy in hospitals. The rational administration of antibiotics should be entertained. The dosage should be specific to eradicate the culprit organisms and must never be subtle enough to escalate the colonization of the antibiotic-resistant strains. Only a few studies in Pakistan have been done which assess the fluctuating trends in the burn wound microbiology and their respective antibiotic susceptibility patterns. In our study, we evaluated the microflora in burn wounds of 100 patients admitted in the burn wards of three different hospitals of Abbottabad. The bacterial isolates then underwent thorough antimicrobial susceptibility testing. This study aims to evaluate the antibiotic susceptibility patterns of the organisms involved in deteriorating burn injury in order to formulate a better and well-controlled antibiotic administration policy for future management of these patients.

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METHODS

The present study involves data collection from 100 patients admitted in the burns and surgical units of three different hospitals in Abbottabad over a 7-month period between July 2016 to January 2017. Samples were collected from the patients directly admitted in the burns unit of Ayub teaching hospital, District Headquarter hospital, and Combined Military Hospital Abbottabad; 57 patients from Ayub teaching hospital, 30 patients from District Headquarter hospital, and 13 patients from Combined Military Hospital Abbottabad were included. All individuals less than 60 years of age were included in the study, irrespective of their gender. Patients who were previously treated in other medical centers were excluded from participating. Moreover, patients with chronic ailments, such as diabetes and tuberculosis, were also excluded from the study. The patients were stratified via age, sex, occupation, detailed history of the accident, cause, and degree of burn, thorough clinical assessment of wounds, site of the burn injury, and total body surface area (TBSA) affected by the injury. Lund and Browder's charts were used to estimate the TBSA involved in the burn injury. SPSS 10.05 was used for statistical analysis. The level of significance was standardized as 0.05.

Samples were taken with sterilized swabs from the deepest part of the infected wounds of burn patients and were taken to the microbiological laboratory for further process. For each patient, sampling was done on third day post-admission, and sterile swabs were used. One swab was used per patient, and 100 swabs were collected in total. For the identification of bacterial species, samples were then inoculated on nutrient agar, MacConkey agar, and Blood agar at 37°C for 24 hours. Culture and staining were carried out to identify the pathogens, and their antibiotic sensitivity was determined via the Kirby-Bauer disc diffusion technique. Microorganisms were grown in Brain Heart Infusion broth and inoculated on Mueller–Hinton agar plates by sterile swabs, and then antibiotic discs were placed on media and pressed gently and left for incubation overnight. Commercially available antibiotic discs such as Gentamicin (GEN) 10 μgm, Ciprofloxacin (CIP) 5 μg, Tetracycline (TE) 30 μg, Vancomycin (VA) 5 μg, Amikacin (AK) 30 μg, Ceftazidime (CAZ) 30 μg, Cefotaxime (CTX) 5 μg, Norfloxacin (NOR) 30 μg, Cefaclor (CEC) 30 μg, Ampicillin (AM) 10 μg, Nalidixic acid (NAL) 100 μg, Tobramycin (TOB) 30 μg, Chloramphenicol (CHL) 5 μg, Erythromycin (ERY) 15 μg, and Carbapenem (CPO) 10 μg were used. The bacterial inoculums that were isolated from collected samples were prepared in 5 ml nutrient broth with three to five bacterial colonies of each strain. A sterile cotton swab was dipped into the cell suspension of the respective isolate and inoculated on the entire agar surface of each plate, in both horizontal and vertical directions. Right after 5 min, the antibiotic discs were placed. The inoculated plates were left for incubation at 37°C for 24–48 hours to get samples approximately close to 0.5% McFarland standard for susceptibility testing in an inverted position, and the zone of inhibition was recorded. These zones were compared to standardized charts to ascertain antibiotic sensitivity and resistance patterns.

RESULTS

Out of the total 100 cases, the majority of the burn cases were observed in the male population (n = 63) (Table 1). The age spectrum included patients between 1 to 60 years. Twenty-three percent of the total study population belonged to the age group of less than 20 years old, whereas 68% of the patients were between 20 to 50 years old, making it the modal age group. Patients who were greater than 50 years comprised only 9 percent of the total study population.

The most common cause of burns among patients was the scald burns, which accounted for 72% of the total cases. The scald burns when compared to open fire burns, were statistically significant (P-value < .001). Open fire burns contributed to 28% of the total burns. For both scald burns and open fire burns, the majority of the patients belonged to the age group of greater than 20 years, as shown in Table 1.

Second-degree burns were three times more prevalent in the population when compared with third-degree burns (Table 1).

Twenty to Twenty-nine percent of TBSA was the modal group of the surface area involved. Greater than 30% TBSA involvement was a rare entity with an incidence of less than one-tenth of the assessed population (Table 1). Overall, the majority of burns in patients involved the trunk (36%), followed by lower limb (27%), upper limb (23%), and the head and neck region (14%).

The percentages of different bacterial isolates found in the wounds of burn patients are depicted in Table 2. Overall, the gram-positive organisms comprised more of the infection, causing flora than the gram-negative ones. It was found that *Staphylococcus aureus* was the most common strain that was identified in the wounds. *Pseudomonas aeruginosa* was the least prevalent organism in burn wounds.

Table 1. Patient demographics

Burn Patients (N = 100)	n (%)
Gender	
Males	63 (63.0)
Females	37 (37.0)
Age	
<20 yr	40 (40.0)
Open fire burns	7 (17.5)
Scald burns	33 (82.5)
>20 yr	60 (60.0)
Open fire burns	21 (35.0)
Scald burns	39 (65.0)
Cause of burn injury	
Open fire burns	28 (28.0)
Scald burns	72 (72.0)
Severity of burns	
Second-degree burns	78 (78.0)
Third-degree burns	22 (22.0)
TBSA (%)	
1–9	30 (30.0)
10–19	15 (15.0)
20–29	56 (56.0)
>30	9 (9.0)

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Table 2. Common bacterial isolates causing burn wound infections and their prevalence

Bacterial Isolates	Percentage (%)
Gram-positive	
Staphylococcus aureus	46
Staphylococcus epidermidis	17
Gram-negative	
Escherichia coli	16
Proteus spp.	12
Klebsiella pneumoniae	10
Pseudomonas aeruginosa	7

Table 3 illustrates the antibiotic sensitivity patterns for all the six bacteria that were isolated from the wounds of the burn patients. *Staphylococcus aureus* was most sensitive to vancomycin (89%), followed by gentamicin (88.5%). On the other hand, *Staphylococcus epidermidis* was most sensitive to gentamicin (82.5%), followed by vancomycin (80.2%). Gram-positive organisms showed the highest sensitivity to gentamicin, vancomycin, and norfloxacin.

Among the aminoglycosides, the gram-positive bacteria showed the highest sensitivity to gentamicin, while gram-negative bacteria were most sensitive to amikacin. Among the fluoroquinolones, the gram-positive bacteria were highly sensitive to norfloxacin, whereas gram-negative bacteria showed better sensitivity to ciprofloxacin.

The gram-negative bacteria were most sensitive to amikacin, chloramphenicol, and nalidixic acid.

Out of all the organisms, *P. aeruginosa* showed the greatest resistance to the majority of antibiotics. The highest sensitivity it showed was with aminoglycosides: amikacin and tobramycin (11% with each).

Table 3. Antibiotic sensitivity patterns (in percentage) of common burns wound isolates

	Drug Class	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Escherichia Coli*	*Proteus spp.*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
AK	Aminoglycosides	60	60.5	82	86.5	91	11
AM	Penicillin	17.5	12	12.2	17	13	9
CEC	Cephalosporin	26	25.3	50.5	63	56	2
CHL	Lincosamide	–	–	72	81	80.7	4
CIP	Fluoroquinolone	70	68	46.7	43	59.2	7
CTX	Cephalosporin	76.4	78	15	20	21	7
CAZ	Cephalosporin	68.8	72	–	–	–	–
ERY	Macrolide	–	–	57	68	62	2
GEN	Aminoglycosides	88.5	82.5	62	59	51	–
NAL	Quinolone	–	–	87	89	90	5
TOB	Aminoglycosides	–	–	65	51	55	11
NOR	Fluoroquinolone	85	80	–	–	–	1
TE	Tetracycline	16.6	18	–	–	–	2
VAN	Glycopeptide	89	80.2	–	–	–	3
CPO	Carbapenem	–	–	–	–	–	8

AK, Amikacin; AM, Ampicillin; CEC, Cefaclor; CHL, Chloramphenicol; CIP, Ciprofloxacin; CTX, Cefotaxime; CAZ, Ceftazidime; ERY, Erythromycin; GEN, Gentamicin; NAL, Nalidixic acid; TOB, Tobramycin; NOR, Norfloxacin; TE, Tetracycline; VAN, Vancomycin; CPO, Carbapenem.

DISCUSSION

The present study aims to highlight the common bacterial pathogens involved in causing burn wound infections in burns patients. Three burn centers in the Abbottabad strip were included in the study. Overall a similar microbial trend was seen in the wound samples, which were tested from all three hospitals. We further investigated the antibiotic resistance profile against some common antibiotics for the isolated bacterial genera at our burn units in order to inculcate a better future therapeutic approach towards preventing and catering burn wounds at hospitals in Pakistan since studies are scarce on the subject.

In this study, the majority of burns patients were males (63%) when compared with females (37%). This finding is in accordance with studies done by Anwer et al and Li et al.[10,11] The contributing factor to the predominance of burns in males might be that most of the men tend to indulge in self-repair of kitchen and heating appliances without taking sufficient precautionary measures.[10] Ultimately they come in contact with hot fluids or steam, which can cause severe scald burns.

The majority of the burn patients were adults, and only 23% of the total population were teenagers and children. This trend was coherent to another study in which the adults were

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affected more than the pediatric age group.[12] Our data are consistent with the fact that the modal age group affected by burns was 20 to 50 years old, which was precisely compatible with the results of a study conducted by Khan et al.[13] Only 9% of the patients in our study were greater than 50 years old, as in other studies.[14] A probable reason for this finding might be that people in Pakistan live in huge joint family setups in which treatment at home for children and the elderly is encouraged, especially for emergencies like burns.

Our study declared that the scald burns were the more common etiology of burn injuries with fire burns being the second, contributing to 72 and 28% of the total burn injuries, respectively. The study by Qtait et al also had similar patterns of results where 79% of the burns were scald burns, and 20% were flame burns.[15] Frequent exposure to the hot water, primarily in kitchen and washrooms, is a key cause of scald burns. Moreover, infants and preschool children are left alone wandering in the kitchen, and in the proximity of hot fluids like tea and coffee, the contact of which results in scald burns. Cooking and heating instruments are a significant cause of fire burns. The females working in the kitchen are prone to fire burns as they are actively involved in domestic chores. Moreover, in Pakistan, gas cylinders are used as a core household equipment and are installed in kitchens. There are high chances of fire accidents in these conditions, and hence gas cylinders are a significant risk factor for fire burns.

When the degree of the burns was assessed, about 78% of the total cases reported of second-degree burns. This pattern coincided with the study by Frans et al[16] in which the second-degree burns were the most frequent. More than half of the admitted patients in this study (56%) presented with burns that occupied the TBSA of 20–29%. A study conducted in India also had similar results, with 21–40% as the highest TBSA.[17]

We concluded in our study that the trunk was the most frequently burned region (36%), followed by the lower limb (27%). This trend was in contrast to the study done by Tian Hao et al[18] in which the extremities of the lower limb were affected more than the trunk. The face and neck, torso, and limbs are prone to burn injuries in patients of all age groups, and this finding is comparable with the results of a study in Shanghai, China.[19] Collectively, the limbs were affected the most. This finding is of crucial importance. Attention needs to be paid to protect and treat the burned extremities effectively, or else complications like cicatrix and contractures arise, jeopardizing the quality of life of the patients. It is observed that individuals consciously protect their heads in a burn injury, and this might be the reason that the head and neck region was seen to be least involved (14%) as a burn injury zone in our study.

In our study, *S. aureus* was found to be the most common organism isolated in burn patients (46%). This finding is in contrast to multiple studies in which *P. aeruginosa* was the most common pathogen isolated in burn wounds. In a study conducted in Cairo Egypt, Nasser et al[20] concluded that the gram-negative strains were more common in burn patients than gram-positive ones; *P. aeruginosa* was the most frequent isolate (21.6%), followed by *Klebsiella pneumoniae* (15.2%). In another study, Sharma, Latika Sahni et al[21] reported that *Pseudomonas* was the most common organism involved in burn wounds (38%), whereas *S. aureus* was the second most common strain identified (35%). This entirely contradicts our findings in which *Pseudomonas* was the least prevalent organism reported (only 7%). Moreover, in our study, no isolate of *β-hemolytic Streptococci* was found, and this finding is in agreement with the previous studies done on burn wounds.

Antibiotic sensitivity patterns found in our study revealed that the isolates were frequently resistant to antibiotics belonging to penicillin and tetracyclines. This is quite daunting as these drugs are among the most commonly prescribed antibiotics and are indiscriminately used as first-choice options for antibacterial treatment. Both *S. aureus* and *S. epidermidis* showed high sensitivity to gentamicin and vancomycin. The resistance of *S. aureus* to gentamicin was only 11.5%, which is similar to a study in which the same strains showed 18% resistance to gentamicin.[22] Gentamicin is a vital antibiotic that is used as a component of combination therapies globally for the treatment of *S. aureus* infections.[23] In our study, *S. aureus* was most sensitive to vancomycin (89%), which is in contrast with other studies in which vancomycin is found to be effective in 100% of gram-positive organisms.[24]

Gram-negative bacteria showed the highest sensitivity to amikacin. Amikacin is a second-generation aminoglycoside. These findings were consistent with the study done by Agnihotri et al[25] in which amikacin was the most effective therapy for gram-negative species; however, in contrast to our study, *Pseudomonas* was the most common isolate in burn wounds.

Antibiotic resistance is slowly and gradually swooping down on all the antibiotic classes one by one. The phenomenon of multidrug-resistant is not something hidden. Once multidrug-resistant strains become strong enough, they are inevitable and may persist for months in a patient's body. Therefore, strict microbiological surveillance and correct detection of strains involved should be done before commencing antibiotic therapy. Moreover, the use of inappropriate drugs should be avoided as much as possible. Pathogenic flora of the burn wound is a dynamic entity, changing and diversifying along the course of time. The antibiotic susceptibility patterns of the burn wound isolates are the biggest obstacle in the swift treatment of burn injuries and the most significant loophole in a nosocomial environment. Physicians must ascertain proper, regular evaluation of the wound culture to spot the most common organisms causing infections. Each burn center must run regular checks on the changing antibiotic sensitivity profiles for these organisms. Identification of the infectious strains and their antibiotic susceptibility patterns should, therefore, be recognized as a core component of the burn treatment protocol.

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