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Bacteriological profile and antimicrobial resistance patterns of burn wound infections in a tertiary care hospital

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Abstract

Burns remain a significant public health problem in terms of morbidity, long-term disability and mortality. In India, over 10,00,000 people are moderately or severely burnt every year. Open and large wounds, make burn patients more susceptible to infection. In particular, immunosuppression caused by impaired neutrophil function, cellular and humoral immune system can facilitate multiplication and colonization of burn wounds by different microorganisms. The objective for the study to identify the aerobic bacterial agents responsible for burn wound infection and to study the antibiogram of bacterial isolates. The study was performed in the Department of Microbiology, Mysore Medical College and Research Institute, Mysore, for a period of one year. Wound swabs were collected from the burn wounds, were processed according to standard laboratory procedures to isolate aerobic bacterial pathogens. The isolates were tested for antimicrobial susceptibility and resistance mechanisms by Kirby -Bauer disk diffusion method as per CLSI guidelines. 90 burn patients were included in present study. A total of 114 aerobic bacterial isolates were isolated. *Staphylococcus aureus* (28.9%) was most common isolate followed by *Pseudomonas species* (26.3%), *Klebsiella species* (20.2%), *Enterococcus* species (7.1%), *Enterobacter species*, *Acinetobacter species* and *Coagulase negative Staphylococcus* (3.5%) *each, Escherichia coli and Citrobacter species* (2.6%) and *Proteus species* (1.8%). Antibiogram showed that, Imipenem (86.9%) was most effective drug against Gram negative organisms and vancomycin and linezolid (100%) were effective drugs against Gram positive organisms. It is crucial for every burn unit to determine the specific pattern of burn wound colonization and the antimicrobial resistance pattern. This will enable early treatment with proper empirical systemic antibiotics, thus improving overall infection related morbidity and mortality.

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Introduction

Infections persist as an important complication and cause of mortality in the burn patients.[1]Disrupted skin barrier, involvement of larger burnt area, immunocompromised effects of burns and prolonged stays at the hospitals were major risk factors for initiating infection[2]. The World Health Organization (WHO)has estimated that burn injury results in 265,000 deaths annually, with nearly half of these occurring in the WHOSouth-East Asia Region.[3]Burns have been documented to occur mostly outdoors for the adult male and indoors for the adult female worldwide.[4,5]

Although burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms.[6] Gram positive organisms are initially prevalent during hospital stay of patients; then gradually become superceded by Gram negative organisms that appear to have a greater propensity to invade.[7]Use of antibiotics as systemic prophylactic is a common practice with burnt patients [8]. Drug resistant bacteria with intrinsic resistance towards antibiotics, ability to survive longer in the hospital environment and hand to hand transmission of bacteria reflects their easy spread and cause outbreaks[9,10]. The present study was undertaken to study the aerobic bacterial isolates and their drug susceptibility in burn wound infections in K.R.Hospital attached to Mysore medical college and research institute, Mysore.

Materials and methods

I. Sample Collection and Transport

The area around the burn wound was cleaned with 70% ethyl alcohol and surface cleaned with sterile saline and the sample was collected from the depth of the wound using two sterile cotton swabs. The sample was transported immediately to the laboratory for further processing.

II. Processing[11]

A. Direct Microscopy

Using one swab, a smear was prepared on a clean grease free glass slide. After fixation with heat, it was stained with Gram stain. The stained smear was screened for presence or absence of pus cells and bacteria and their Gram reaction, shape, size and arrangement.

B. Bacterial culture

The specimens were inoculated onto MacConkey‟s agar, Blood agar, Chocolate agar and BHI broth. The cultures were incubated at 370 C for 18-24 hours in humid air and 5-10% CO2, and were reincubated if there was no growth and colonies were small and indistinct.108 Subculture was done from broth if required.

Growth was further identified by doing Gram stain from the colonies grown on culture media. Gram positive organisms like *Staphylococcus* was identified as per standard protocol by catalase, slide coagulase, tube coagulase and mannitol fermentation test. *Enterococcus* was identified by catalase, heat tolerance, hydrolysis of bile esculin, ability to grow in presence of 6.5% NaCl and growth at pH 9.6. Gram negative organisms were identified as per standard protocol by Gram stain, catalase, oxidase, motility, O-F, nitrate reduction, indole, MR, VP, citrate, urease, TSI, sugar fermentation and amino acid decarboxylation test.

Antibiotic susceptibility testing was done on Mueller Hinton agar using Kirby-Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI).[12]Gram positive isolates were tested against Ampcillin (10µg), Ciprofloxacin (5µg), Cotrimoxazole (1.25/23.75µg), Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg), Vancomycin (30µg), Linezolid (30µg), Gentamicin (10µg). In *Enterococcus* isolates were tested against High Level Gentamicin (120µg) and cefoxitin was excluded. Gram negative isolates were tested against Ampicillin (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Cefepime (30µg), Gentamicin (10µg), Amikacin(30µg), Piperacillin(100µg), Imipenem(10µg).

Results

Mean age of study group is 30.35 ± 13.83 In the present study, high number of cases belongs to the age group of 21-30 years 39(43.3%). Study group comprised of males 44(48.9%) and females 46(51.1%). Male-Female ratio was 1:1.04. Age and sex-wise distribution of cases are as shown in table 1. out of 90 samples studied, single isolates were found in 64 (71.1%), while 24 (26.6%) samples yielded multiple isolates and 2 (2.2%) were no growth. Culture results are as shown in table 2, a total of 114 organisms were isolated. The common isolate was *Staphylococcus aureus* 33 (28.9%) followed by *Pseudomonas* spp. 30 (26.3%), *Klebsiella* spp 23 (20.2%), *Enterococcus* spp 8 (7.1%), *Enterobacter* spp, *Acinetabacter* spp. and *CONS* 4 (3.5%) each, *Escherichia coli* and *Citrobacter* spp. 3 (2.6%) each and *Proteus* spp. 2 (1.8%) respectively. Table 3 Organisms isolated. Out of 45 Gram positive isolates, 32 (71.1%) were sensitive to clindamycin, followed by 23 (51.1%) to cotrimoxazole, 22 (48.9%) to ciprofloxacin, 18 (48.6%) to gentamicin, 16 (43.2%) to cefoxitin, 19 (42.4%) to erythromycin and 6 (13.3%) to ampicillin. All isolates were sensitive to vancomycin and linezolid. Among Enterococcus spp. 5 (62.5%) were sensitive to high level gentamicin. Table 4 shows antibiotic susceptibility pattern of Gram positive bacterial isolates. Table 5 shows Antibiotic susceptibility pattern of Gram negative bacterial isolates. Out of 69 Gram negative isolates, 60 (86.9%) were sensitive to imipenem, followed by 47 (68.1%) to amikacin, 36 (52.1%) to cefepime, 35 (50.7%) to ciprofloxacin, 32 (46.4%) to piperacillin, 31(44.9%) to gentamicin, 17(24.6%) to ceftazidime, 6(8.7%) to ampicillin.

Table 1. Age and sex-wise distribution of cases.

Age group (years)	Male n.(%)	Female n.(%)	Total n (%)
1-10	3(3.3)	5(5.6)	8(8.9)
11-20	4(4.5)	3(3.3)	7(7.8)
21-30	21(23.3)	18(20)	39(43.3)
31-40	7(7.8)	13(14.5)	20(22.2)
41-50	5(5.6)	3(3.3)	8(8.9)
51-60	4(4.4)	2(2.2)	6(6.7)
61-70	0(0)	1(1.1)	1(1.1)
71-80	0(0)	1(1.1)	1(1.1)
Total	44(48.9)	46(51.1)	90(100)

Table 2. Culture results.

Culture results	n (%)
Solitary isolates	64(71.1)
Multiple isolates	24(26.6)
No Growth	2(2.2)
Total	90(100)

Table 3. Organisms isolated.

Organisms isolated	n (%)
Staphylococcus aureus	33(28.9)
Pseudomonas species	30(26.3)
Klebsiella species	23(20.2)
Enterococcus species	8(7.1)
Enterobacter species	4(3.5)
Acinetobacter species	4(3.5)
CONS	4(3.5)
Escherichia coli	3(2.6)
Citrobacter species	3(2.6)
Proteus spp.	2(1.8)
Total	114(100)

Table 4. Antibiotic susceptibility pattern of Gram positive bacterial isolates.

Organisms	Total no. of isolates	A n.(%)	CF n.(%)	CO n.(%)	E n.(%)	CD n.(%)	CX n.(%)	VA n.(%)	LZ n.(%)	G n.(%)	HLG n.(%)
S.aureus	33	4(12.2)	14(42.4)	17(51.5)	15(45.5)	22(66.7)	14(42.4)	33(100)	33(100)	16(48.5)	NT
CONS	4	1(25)	2(50)	2(50)	1(25)	4(100)	2(50)	4(100)	4(100)	2(50)	NT
Enterococcus	8	1(12.5)	6(75)	4(50)	3(37.5)	6(75)	NT	8(100)	8(100)	NT	5(62.5)
Total	45	6(13.3)	22(48.9)	23(51.1)	19(42.4)	32(71.1)	16(43.2)	45(100)	45(100)	18(48.6)	5(62.5)

A– Ampicillin; CF – Ciprofloxacin; CO – Cotrimoxazole; E – Erythromycin; CD – Clindamycin; CX- Cefoxitin; VA– Vancomycin; LZ – Linezolid; G – Gentamicin; HLG – High Level Gentamicin.

Table 5. Antibiotic susceptibility pattern of Gram negative bacterial isolates.

Organisms	Total no. of isolates	A n.(%)	CF n.(%)	CA n.(%)	CP n.(%)	G n.(%)	AK n.(%)	PI n.(%)	I n.(%)
Pseudomonas spp.	30	4(13.3%)	11(36.7)	12(40)	18(60)	11(36.7)	20(66.7)	15(50)	26(86.7)
Klebsiella spp.	23	1(4.3)	14(60.8)	2(8.7)	7(30.4)	13(56.5)	18(78.2)	10(43.5)	20(86.9)
Enterobacter spp.	4	0(0)	2(50)	2(50)	4(100)	3(75)	4(100)	2(50)	4(100)
Acinetobacter spp.	4	0(0)	1(25)	0(0)	1(25)	2(50)	0(0)	2(50)	2(50)
E.coli	3	1(33.3)	3(100)	0(0)	3(100)	0(0)	2(66.7)	1(33.3)	3(100)
Citrobacter spp	3	0(0)	3(100)	0(0)	2(66.7)	1(33.3)	2(66.7)	2(66.7)	3(100)
Proteus spp.	2	0(0)	1(50)	1(50)	1(50)	1(50)	1(50)	0(0)	2(100)
Total	69	6(8.7)	35(50.7)	17(24.6)	36(52.1)	31(44.9)	47(68.1)	32(46.4)	60(86.9)

A– Ampicillin; CF – Ciprofloxacin; CA – Ceftazidime; CP – Cefepime; G – Gentamicin; AK- Amikacin; PI– Piperacillin; I – Imipenem.

Discussion

Bacterial infections of the burn wound still remains a major cause of morbidity and mortality in thermally injured patients. The burn site initially becomes colonized with microorganisms which if uncontrolled progresses to invasion and give rise to bacteremia and sepsis, which is a major cause of mortality in burn patients.[13]

Although the diagnosis of burn wound infections can be made clinically, additional microbiological evidence is needed for instillation of proper therapy. Among the various microbiological methods available, the swab culture technique was used because it is a simple, convenient and effective method for the identification of all potential pathogens and their antimicrobial susceptibility.[14] In the present study, the highest incidence of burns was in the age group of 21-30 years.

(Table -4) This observation is in accordance with other studies. This finding correlates with our society patterns, where adults of this age group are entrusted with the responsibilities both at home as well as outside.[12]In the present study, incidence of burn was more in females 46 (51.1%) than males 44 (48.9%) (Table-4). High incidence of burns in females is probably due to occupational hazards of working in the kitchen, as the kitchen is the most common place for accidental burns.[15] These findings were similar to the studies of Abrol *et al* and Subrahmanyam M.[16,17]

In the present study, the common isolate was *Staphylococcus aureus* 33 (28.9%), which is comparable to the studies of Altoparlak *et al*. and Chaya *et al*.[18,19]. The second common isolate was *Pseudomonas* spp. 30 (26.3%), which is comparable to the studies of Chaya *et al*. S K Saha *et al*. and Ilyas Yolbas *et al*.[19,20,21]. Prevalence of *Pseudomonas* spp. in the burn wards may be due to the fact that the organism thrives in a moist environment. *Klebsiella* spp. 23 (20.2%) was the third common isolate, which is comparable to the study of Chaya *et al*.[18]

Antibiotic susceptibility pattern

Antibiotic susceptibility was carried out for 90 isolates by Kirby-Bauer disc diffusion method. Out of 45 Gram positive isolates, 45 (100%) were sensitive to Vancomycin and Linezolid, 32 (71.1%) to clindamycin, and 23(51.1%) to cotrimoxazole (Table -3). Among 69 Gram negative isolates, 60 (86.9%) were sensitive to Imipenem, 47 (68.1%) to amikacin, 36 (52.1%) to Cefepime and 35 (50.7%) to Ciprofloxacin.

Conclusion

The present study has given us the knowledge regarding spectrum of aerobic bacterial

organisms of burn wounds in our hospital. It was seen that Gram negative organisms were more prevalent. Among the isolates, *Staphylococcus aureus* was common microorganism followed by *Pseudomonas species*. The antibiotic susceptibility testing showed that, Imipenem was the most effective drug for Gram negative isolates and Vancomycin and Linezolid for Gram positive isolates.

In conclusion, to ensure early and appropriate therapy routine microbiological surveillance and a regular update of their antimicrobial susceptibility pattern could help in prevention of development of multidrug resistance. Our results may be helpful in providing useful information regarding the pattern of burn wound microbial colonization, the dominant flora and antimicrobial resistance in burn unit and thus will help in formulation of effective guidelines for therapy, thus improving overall infection related morbidity and mortality.

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