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Original Article

Bacterial isolates from burn wound infections and their antibiograms: A eight-year study

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ABSTRACT

Background: Infection is an important cause of mortality in burns. Rapidly emerging nosocomial pathogens and the problem of multi-drug resistance necessitates periodic review of isolation patterns and antibiogram in the burn ward.
Aim: Keeping this in mind, the present retrospective study from wounds of patients admitted to burns unit was undertaken to determine the bacteriological profile and the resistance pattern from the burn ward over a period of three years (June 2002 to May 2005) and was compared with the results obtained during the previous five years (June 1997-May 2002), to ascertain any change in the bacteriological profile and antimicrobial resistance pattern.
Materials and Methods: Bacterial isolates from 268 wound swabs taken from burn patients were identified by conventional biochemical methods and antimicrobial susceptibility was performed. Statistical comparison of bacterial isolates and their resistance pattern with previous five years data was done using χ^2 test.
Results and Conclusions: During the period from 2002 to 2005 *Pseudomonas* species was the commonest pathogen isolated (51.5%) followed by *Acinetobacter* species (14.28%), *Staph. aureus* (11.15%), *Klebsiella* species (9.23%) and *Proteus* species (2.3%). When compared with the results of the previous five years i.e., 1997 to 2002, *Pseudomonas* species was still the commonest pathogen in the burns unit. However, the isolation of this organism and other gram-negative organisms had decreased in comparison to previous years. Newer drugs were found to be effective.

KEY WORDS

Antibiotics, burn, infections

Infection is an important cause of mortality in burns. It has been estimated that 75% of all deaths following thermal injuries are related to infections.[1] The rate of nosocomial infections are higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patient, invasive diagnostic and therapeutic procedures and prolonged ICU stay.[2] In addition, cross-infection results between different burn patients due to overcrowding in burn wards.[3] Complicating this high rate of infection is the fact that the spectrum of bacterial isolates varies with time and geographical area.[4] In various countries, including India, the importance of *Acinetobacter* species, as a rapidly emerging nosocomial pathogen, has been documented[5] and these bacteria are predominantly isolated from ICUs, burn units and surgical wards. In addition, the problem of multi-drug resistance in gram-negative bacilli due to extended spectrum beta lactamases (ESBL) production is becoming a serious threat to the healthcare worker, who are likely to contract the infection, as the therapeutic options to these organisms are limited.[6] This necessitates periodic review of the isolation pattern and antibiogram of the burn ward, which forms the basis for modification of drug regimen strategy. Keeping this in mind, the present study was planned to determine the bacteriological profile and the resistance pattern from outer burn ward over a period of three years (June 2002 to May 2005) and we compared this data with the results obtained during the previous five years (June 1997-May 2002), to ascertain any change in the bacteriological profile and antimicrobial resistance pattern.

MATERIALS AND METHODS

Ours is a 750-bed tertiary care hospital. This is a retrospective study of bacterial isolates from 268 wound swabs taken from patients admitted to the burn unit of the Government Medical College and Hospital, Chandigarh between June 2002 and May 2005.

The specimens were transported in sterile, leak-proof container to Department of Microbiology. All specimens were inoculated on 5% blood agar, Mac Conkey agar and Chocolate agar plates and incubated overnight at 37°C aerobically. The sample was also put into liquid media (BHI broth) and was subcultured after overnight incubation onto Blood agar and Mac Conkey agar. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques.[7]

Antimicrobial susceptibility was performed on Mueller-Hinton agar by the standard disk diffusion method recommended by the National committee for clinical laboratory standards (NCCLS).[8] The antibiotics tested for gram-positive cocci were: Amoxicillin (10 μg), cephalexin (30 μg), oxacillin (1 μg), ciprofloxacin (5 μg), erythromycin (15 μg), netilmicin (10 μg), vancomycin (30 μg), linezolid (30 μg); for gram-negative bacilli: Amoxycillin (10 μg), ceftriaxone (30 μg), cefotaxime (30 μg), cefepime (30 μg), amikacin (30 μg), gentamicin (30 μg), ciprofloxacin (5 μg), ceftazidime / clavulanic acid (30/10 μg), cefoperazone /sulbactam (75/30 μg), and imipenem (10 μg) and for non-fermenters ceftazidime, (30 μg), piperacillin (100 μg), carbenicillin (100 μg), cefepime (30 μg), amikacin (30 μg), gentamicin (30 μg), tobramycin (10 μg), ciprofloxacin (5 μg), cefoperazone / sulbactam (75/30 μg), and imipenem (10 μg) were used. The source for media and antibiotic discs was Hi-Media Ltd. India. Standard strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. Antibiogram of the bacterial isolates was done according to NCCLS/ CLSI criteria, Kirby Bauer method.[8] The zone of inhibition for gram-positive cocci, gram-negative bacilli and *Pseudomonas* species for individual antibiotics are different and standardized according to MIC value.

Statistical comparison of bacterial isolates and their resistance pattern with the data of the previous five years was done using χ^2 test and $P< 0.05$ was considered significant. We compared the difference in the profile of bacterial isolates between the year 2002-2005 and 1997-2002. Also, the resistance patterns of these isolates (1997-2002 and 2002-2005) were compared to ascertain if there is a change in the percentage of etiological agent of burn infection and their resistance pattern in our hospital.

RESULTS

Bacterial isolates were found in 260 (97.01%) samples and only eight wound swabs were sterile (2.99%). *Pseudomonas* species was the commonest pathogen isolated (51.5%) followed by *Acinetobacter* species (14.28%), *Staphylococcus aureus* (11.15%), *Klebsiella* species (9.23%) and *Proteus* species (2.3%) as shown in Table 1.

Pseudomonas species was moderately resistant to piperacillin (R-41.42%) whereas resistance was more marked with other antimicrobials like amikacin (85.18%), gentamicin (89.22%), ciprofloxacin (78.81%), carbenicillin (88.26%), tobramycin (87.52%) and ceftazidmine (79.09%), as shown in Table 2. On the other hand, *Pseudomonas* species was found to be more sensitive to newer antimicrobials as is evident by only 4.54% resistance to imipenem, 21.8% resistance to ceftazidime/clavulanic-acid, 25.67% resistance to cefoperazone/sulbactum and 50% of *Pseudomonas* species were resistant to cefepime as shown in Table 3.

Table 1: Isolation rate of organisms from wound or burn wounds swabs and comparison with previous 5 years isolation rate

Organism	*1997-02 n (%)*	*2002-05 n (%)*
Pseudomonas aeruginosa	392 (58.95)	134 (51.5)*
Staph. aureus	119 (17.89)	29 (11.15)*
Acinetobacter spp.	48 (7.22)	37 (14.23)**
Klebsiella spp.	26 (3.91)	24 (9.23)***
Enterobacter spp.	26 (3.91)	4 (1.53)*
Proteus spp.	22 (3.31)	6 (2.3)NS
Others (*E. coli*)	32 (4.81)	26 (10)**

*P<0.05; **P<0.01; ***P<0.001; NS Not significant. The two time periods were compared using χ^2 test

Table 2: Comparison of antimicrobial resistance in bacterial isolates

Organism	No. of resistant isolates (n)	
Antimicrobial agent	1997-02 n (%)	2002-05 n (%)
Amoxycillin	90(75.65)	20(69.04)NS
Erythromycin	79(66.13)	22(75.27)NS
Cephalexin	86(72.09)	17(58.72)NS
Netilmicin	39(32.47)	23(77.75)***
Ciprofloxacin	73(61.63)	21(71.60)NS
Gentamicin	247(63.16)	120(89.22)***
Amikacin	211(53.85)	114(85.18)***
Ciprofloxacin	179(45.54)	106(78.81)***
Piperacillin	154(39.25)	56(41.42)NS
Carbenicillin	171(43.52)	118(88.26)***
Tobramycin	315(80.43)	118(87.52)*
Ceftazidime	250(63.72)	107(79.09)***
Gram-negative bacilli		
Amoxicillin	145(94.1)	82(84.90)*
Gentamicin	116(75.44)	84(86.64)*
Amikacin	62(40.52)	63(64.50)***
Cefotaxime	94(60.75)	74(75.99)*
Ceftriaxone	90(58.69)	54(55.57) NS
Ciprofloxacin	75(48.44)	78(80.83)***

*P<0.05; **P<0.01; ***P<0.001; NS Not significant. The two time periods were compared using χ^2 test

Table 3: Antibiogram of gram-negative bacilli against newer drugs

	Pseudo. aeruginosa *n (%)*	*Acinetobacter* n (%)	*Enterobacteriaceae* n (%)
Cefoperazone+ sulbactam	74 (25.67)	15 (6.66)	40 (07.1)
Imipenem	66 (4.54)	19 (0)	29 (0)
Cefepime	42 (50)	11 (45.45)	45 (37.77)
Ceftazidime+ Clavulanic acid	32 (21.8)	16 (12.5)	24 (20.83)

n = no. of strains tested; % = % of strains resistant

Among gram-negative bacilli, resistance percentage varied from 64.50% to amikacin to 86.64% to gentamicin. However, not a single strain belonging to Enterobacteriaceae and *Acinetobacter* species was found to be resistant to imipenem. In addition, resistance to cefoperazone/sulbactum of *Acinetobacter* was only 6.66% and 17.5% among Enterobacteriaceae. Ceftazidime/clavulanic-acid resistance was seen in only 12.5% of *Acinetobacter* and 20.83% of Enterobacteriaceae. Resistance to fourth generation cephalosporin was 45.45% among *Acinetobacter* species and 37.77% among Enterobacteriaceae as shown in Table 3. *S. aureus*, were highly resistant to amoxycillin (69.04%), erythromycin (75.27%), and netilmicin (77.75%); and 24% of our *S. aureus* were MRSA as shown in Table 2. However, no strain of *S. aureus* was found to be resistant to linezolid or vancomycin.

DISCUSSION

In the present study, the most commonly isolated organisms from burn patients were *Pseudomonas* species followed by *Staph. aureus* and *Klebsiella* species. These results are in accordance with other studies.[6,9,10] Regarding isolation rates of organisms from our Burn ward, it was decreased for *Pseudomonas* species, *S. aureus* and *Proteus* species whereas it was increased for *Klebsiella* species as compared to the previous study. This changing trend in burn bacteriology was also seen by Singh *et al*.[6] In contrast to this, there was a significant rise in the isolation rate of *Acinetobacter* species over the last five to eight years in our burn unit. As stated by Sengupta *et al*,[11] *Acinetobacter* species are emerging as an important cause of nosocomial infection in burn units. There are a number of factors which may contribute to this increase like its presence as a normal skin commensal and its easy spread due to multi drug resistance in a hospital setting. [12]

The change in the pattern of bacterial resistance in the burn unit has importance both for clinical settings and epidemiological purpose. We saw a significantly high percentage resistance among gram-negative bacilli to aminoglycosides like gentamicin and amikacin, ciprofloxacin, carbenicillin, tobramycin, amoxicillin, cefotaxime and ceftriaxone. This alarming trend was seen for both Enterobacteriaceae group and for *Pseudomonas* species as seen in our previous study.[13] A similar report of multi drug resistant gram-negative bacilli was also reported by Singh *et al*.[6] In comparison, imipenem and combination drugs like cefoperazone/sulbactum and ceftazidime / clavulanic acid were found to be effective. This could be due to the reason that these are reserve drugs and used as last options for multi drug resistant bacteria in our hospital settings. For gram-positive cocci a significantly high resistance was seen only for netilmicin. Nevertheless, other antimicrobials tested also showed high percentage resistance. However, newer drugs like vancomycin and linezolid were shown to be highly effective.

Such high antimicrobial resistance is probably promoted due to selective pressure exerted on bacteria due to numerous reasons like non adherence to hospital antibiotic policy, and excessive and indiscriminate use of broad-spectrum antibiotics. These multi drug resistant strains establish themselves in the hospital environment in areas like sinks, taps, railing, mattress, toilets and thereby spread from one patient to another. To conclude, 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 multi-drug resistant pathogens in burn infection.

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