BACTERIOLOGICAL AND MYCOLOGICAL PROFILE OF BURN WOUND INFECTION IN A TERTIARY CARE HOSPITAL IN KATIHAR, BIHAR

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ABSTRACT

BACKGROUND

Injuries secondary to severe burns rank among the most serious forms of trauma resulting in anatomic, physiologic, endocrinologic and immunologic stress, especially when burns involve more than 20% of Total Body Surface Area (TBSA). In patients surviving the initial burn and resuscitative phase, infections are a leading cause of mortality. The burnt surface is sterile immediately following thermal injury but after 48 hours the wound is colonised with skin pathogens that typically reside in sweat glands and hair follicles before the burn. After 5 to 7 days, wounds become colonised with yeast and/or Gram-positive and Gram-negative organisms from the host's intestinal and upper respiratory tracts, or from the hospital environment and health care workers' hands.

MATERIALS AND METHODS

Surface swabs were collected from 200 patients with burn wound infection of both sexes and all age groups. The microorganisms were identified as per standard protocol based on colony morphology; Gram's staining findings and biochemical tests. ESBL (Extended Spectrum Beta-lactamases) detection was done using PCDDT (Phenotypic Confirmatory Disc Diffusion Test).

RESULTS

A total of 268 organisms were isolated from 200 samples. Out of the 268 isolates, 213 (79.48%) were Gram-negative bacilli, 42 (15.67%) were Gram-positive cocci and 13 (4.85%) were fungi. Among the Gram-negative bacilli, *Pseudomonas aeruginosa* was the most common bacteria isolated at 45.15% (121/268). The isolates exhibited maximum resistance to 3rd generation cephalosporins with good response to piperacillin/tazobactam and carbapenems. Among all the Gram-negative bacilli, 30.99% (66/147) were ESBL producers.

CONCLUSION

The study showed that burn wound infection was more common in females than males. Majority of cases had a burn sustained from an open flame, mostly in winters. Gram-negative bacilli were the predominant isolates (80%) from patients with burn wound infection. Fungi were isolated in about 5% of cases. Pseudomonas aeruginosa was the commonest isolate (45%). Amongst the Gram-positive cocci, staphylococci and enterococci were isolated consistently from patients.

KEYWORDS

Burns, Infections, Gram-Negative Bacilli, Gram-Positive Cocci.

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BACKGROUND

Burn wound infection develops in patients after admission to the hospital. Injuries secondary to severe burns rank among the most serious forms of trauma resulting in anatomic, physiologic, endocrinologic and immunologic stress, especially when burns involve more than 20% of Total Body Surface Area (TBSA).[1]

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After the initial burn and resuscitative phase, infections are a leading cause of mortality. It has been estimated that 75% of all deaths following injuries are due to infections.[2]

The infecting pathogens differ from one hospital to another. The burnt surface is sterile immediately following thermal injury but after 48 hours the wound is colonised with skin pathogens that typically reside in sweat glands and hair follicles before the burn. After 5 to 7 days, wounds become colonised with yeast and/or Gram-positive and Gram-negative organisms from the host's normal intestinal and upper respiratory tracts, or from the hospital environment and health care workers' hands. Fungal infections typically involve the skin or lungs and occur in elderly patients with greater percentage of TBSA and hospital stays longer than 2 to 4 weeks.[1]

Burns are a global public health problem, accounting for an estimated 2,65,000 deaths annually. Non-fatal burns are a leading cause of morbidity, including prolonged

hospitalisation, disfigurement and disability, often with resulting stigma and rejection.

Diagnosis of burn wound infection based on clinical signs and symptoms alone is difficult. Regular sampling of the burn wound either by surface swab or tissue biopsy for culture is done to monitor the presence of infection. Superficial swabs provide an adequate sampling of the microbial flora present on the wound surface and are the most convenient and least invasive approach currently available for excised burn areas.[3]

The present study was taken up with a view to determining the spectrum of microorganisms responsible for causing burn wound infection in our hospital setting and gain knowledge about their antimicrobial susceptibility pattern to help the clinician in deciding on empirical treatment options in cases of severe burn wound infection and imminent septic episodes without having to wait for culture results.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Microbiology, Katihar Medical College, Katihar, Bihar. All patients admitted to the Department of Surgery with burn wound infection were inducted into the study. Institutional ethical committee clearance was obtained before conducting the study. Surface swabs were collected from 200 patients with burn wound infection of both sexes and all age groups. As far as possible, three swabs were collected for microscopy, bacterial and fungal culture. Culture was done on routine bacteriological and fungal culture media.

The microorganisms were identified as per standard protocol based on colony morphology, Gram's staining findings and biochemical tests.[4,5]

All bacterial and yeast isolates were tested against different antimicrobial agents on Mueller-Hinton agar plates by modified Kirby-Bauer disc diffusion technique.[6]

Extended Spectrum Beta-lactamase (ESBL) detection was done using PCDDT (Phenotypic Confirmatory Disc Diffusion Test) with Cefotaxime, Cefotaxime/Clavulanic Acid and Ceftazidime, Ceftazidime/Clavulanic Acid.[6]

Statistical analysis of results was done using the chi square test. P-value ≤ 0.05 was considered to be significant and p- value ≤ 0.001 was considered to be highly significant. All statistical analysis was carried out using online statistical software (http://www.physics.csbju.edu/stats/contingency_NROW_NCOLUMN_form.html; accessed on 18.02.2017).

All media, reagents and antibiotic discs were procured from HiMedia Laboratories, Mumbai.

RESULTS

Out of the total of 200 samples processed in the laboratory, 132 samples showed pure growth of a single organism and 68 showed growths of two organisms.

Table 1 shows the age and sex wise distribution of burn cases. Maximum number of burn cases (69/200) were seen in the age group 21-30 years; out of which 58 (84.06%) were female and 11 (15.94%) were male. Least number of cases (8/200) were seen in the age groups 41 – 50 years; out of which 6 (75%) were female and 2 (25%) were male. The difference in the number of females (58) as compared to the males (11) in the age group 21-30 yrs. was found to be statistically highly significant (p value=0.000).

Table 2 depicts the mechanism of burns. In most of the cases i.e. 88% (176/200), an open flame was responsible for the burns, chemical burns accounted for 1.5% (3/200) of cases. The difference in the number of cases caused by an open flame as compared to other three mechanisms viz. hot liquid, electrical and chemical was found to be statistically highly significant (p value=0.000).

Figure 1 shows the season wise distribution of microorganisms isolated from burn wound cases. Maximum number of cases 40.5% (81/200) was seen in the winter followed by 25% (50/200) of cases seen in the spring season.

Table 3 shows the distribution of microorganisms isolated from burn wound infections. Out of the 268 isolates, 213 (79.48%) were Gram-negative bacilli, 42 (15.67%) were Gram-positive cocci and 13 (4.85%) were fungi. Among the Gram-negative bacilli, *Pseudomonas aeruginosa* was the most common bacteria isolated 45.15% (121/268) and *Citrobacter freundii* was least common 1.49% (4/268). Among the Gram-positive cocci, *Staphylococcus aureus* was the most common isolate 8.21% (22/268) and *Enterococcus faecium* was least common 1.12% (3/268). Among the fungi, *Candida albicans* 2.98% (8/268) was the most common isolate.

Table 4 denotes antimicrobial susceptibility pattern of isolates. Isolates were found to be universally resistant to amoxicillin with significant resistance to 3rd generation cephalosporins [ceftriaxone-100% (92/92), cefuroxime-97.83% (90/92) and cefotaxime-92.86% (198/213) were resistant]. Response to imipenem [84.51% (180/213) were sensitive] and piperacillin/tazobactam [74.65% (159/213) were sensitive] was good among the Gram-negative bacilli. The Gram-positive cocci were found to be universally resistant to cephalexin, significantly resistant to erythromycin 88.10% (37/42). A good number of Gram-positive isolates were found to be sensitive to linezolid 80.95% (34/42) and vancomycin 73.81% (31/42). The enterococci were universally resistant to penicillin, sensitivity to high content gentamicin was 57.14% (4/7) and streptomycin was 42.86% (3/7) respectively whereas sensitivity to teicoplanin was 100% (7/7).

Figure 2 shows the ESBL production among Gram-negative isolates including Pseudomonas species. Maximum ESBL production was noted in *Citrobacter freundii* 50% (2/4) and least in *Pseudomonas aeruginosa* 25.62% (31/121).

Age	Female (%)	Male (%)
0-10	20 (74.07)	07 (25.92)
11-20	21 (55.26)	17 (44.74)
21-30	58 (84.06)*	11 (15.94)*
31-40	15 (45.45)	18 (54.55)
41-50	06 (75.00)	02 (25.00)
51-60	05 (33.33)	10 (66.67)
>60	03 (30.00)	07 (70.00)
Total	**128**	**72**

Table 1. Age and Sex wise Distribution of Burn Cases

*p= 0.000.

Mechanism	No. of Cases	Percentage (%)
Flame*	176	88
Hot liquid	08	04
Electrical	13	6.5
Chemical	03	1.5
Total	**200**	**100**

Table 2. Mechanism of Burns

*p= 0.000.

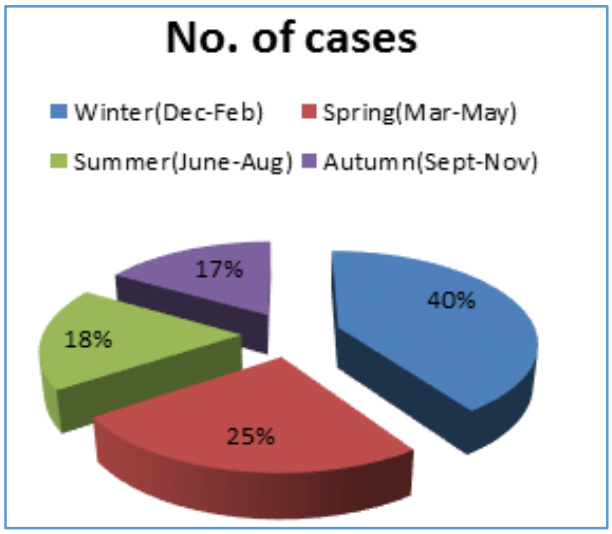

Figure 1. Seasonal Distribution of Burn Cases

Isolate	No.	Percentage (%)
Pseudomonas aeruginosa	121	45.15
Escherichia coli	28	10.45
Proteus mirabilis	25	9.33
Klebsiella pneumoniae	22	8.21
Staphylococcus aureus	22	8.21
Staphylococcus epidermidis	13	4.85
Klebsiella oxytoca	07	2.61
Acinetobacter baumannii	06	2.24
Citrobacter freundii	04	1.49
Enterococcus faecalis	04	1.49
Enterococcus faecium	03	1.12
Candida albicans	08	2.98
Candida tropicalis	05	1.86

Table 3. Microorganisms Isolated from Burn Wound Infections

Antibiotic	*Pseudo. aeruginosa* n=121 (%)	*Esche. coli* n=28 (%)	*Proteus mirabilis* N=25 (%)	*Kleb. pneumoniae* n=22 (%)	*Staph. aureus* n=22 (%)	*Staph. epidermidis* n=13 (%)	*Kleb. oxytoca* n=07 (%)	*Acineto. baumannii* n=06 (%)	*Citrobacter freundii* n=04 (%)	*Entero. faecalis* n=04 (%)	*Entero. faecium* n=03 (%)
CAZ	28 (23.1)										
PI	39 (32.2)										
CIP	24 (19.8)	7 (25.0)	10 (40)	10 (45.5)	10 (45.4%)	3 (23.1)	2 (28.6)	0 (0)	0 (0)	2 (50)%	0 (0)
CPZ	15 (12.4)	1 (3.6)	1 (4)	0 (0)			0 (0)	0 (0)	0 (0)		
CTX	15 (12.4)	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)		
CPM	14 (11.6)	0 (0)	4 (16)	1 (4.5)			2 (28.6)	0 (0)	0 (0)		
AK	49 (40.5)	12 (42.9%)	7 (28)	2 (9.1)	14 (63.6%)	5 (23.07)	5 (71.4)	0 (0)	0 (0)		
GEN	21 (17.3)	6 (21.4)	2 (8)	5 (22.7)	8 (36.4)	0 (0)	0 (0)	0 (0)	0 (0)		
TOB	29 (24.0)										
PIT	100 (82.6%)	21 (75.0)	22 (88)	9 (40.9)			5 (71.4)	0 (0)	2 (50)		
CFS	18 (14.9)	2 (7.1)	2 (8)	1 (4.5)			5 (71.4)	0 (0)	0 (0)		
IPM	118 (97.5%)	17 (60.7%)	22 (88)	9 (40.9)			7 (100)	5 (83.3)	2 (50)		
CXM		0 (0)	2 (8)	0 (0)			0 (0)	0 (0)	0 (0)		
CTR		0 (0)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)		
CN					0 (0)	0 (0)					
E					3 (13.6)	0 (0)				1 (25%)	1 (33.3%)
NET					21 (95.4%)	11 (84.6)					
VA					19 (86.4%)	7 (31.8)				2 (50%)	3 (100%)
LZ					19 (86.4%)	11 (84.6)				2 (50)%	2 (66.6%)
CD					7 (31.8)	5 (38.4)					
TEI										4 (100%)	3 (100%)
TE										2 (50%)	1 (33.3%)
HLG										3 (75%)	1 (33.3%)

Table 4. Antibiotic Susceptibility Pattern of Isolates

CAZ- Ceftazidime; PI- Piperacillin; CIP- Ciprofloxacin; CPZ- Cefoperazone; CTX- Cefotaxime; CPM- Cefepime; AK- Amikacin; GEN- Gentamicin; TOB- Tobramycin; PIT- Piperacillin/Tazobactam; CFS- Cefoperazone/ Sulbactam; IPM- Imipenem; CXM- Cefuroxime; CTR- Ceftriaxone; CN- Cephalexin; E- Erythromycin; NET- Netilmicin; VA- Vancomycin; LZ- Linezolid; CD- Clindamycin; TEI- Teicoplanin; TE- Tetracycline; HLG- High level Gentamicin.

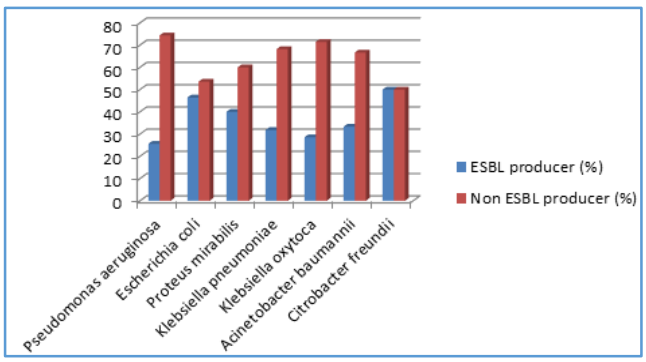

Figure 2. ESBL Production among Gram-negative Isolates

DISCUSSION

Majority of patients (69/200) in this study were in the age group 21-30 yrs, out of which 84.06% were female. This was followed by the age group 11-20 yrs. (38/200) among which 55.26% were female and 44.74% were male. Similar findings have been reported by other authors.[7,8,9] A few authors on the other hand have reported more number of males.[10,11,12]

The preponderance of female patients may be due to the fact that they tend to spend more time in the kitchen cooking over open flames.

In most cases, the cause of burn production was an open flame - 88%. The electric burns accounted for 6.5% followed by scalding (in hot liquid) 4% and chemical burns 1.5%. Other authors have also reported maximum number of burns due to flame injury (66.0%) followed by scalding (16%).[10] Likewise another group of authors have reported 87.81% of flame burns, followed by scalds 10.08% and electrical burn 2.11%.[7] The findings of these studies are more or less similar to the findings of the present study except that in our study, the number of electrical burns was more than the number of burns caused by scalding.

Majority of burn cases were seen in winter (40.50%). The cases correspondingly reduced to 25% in spring, 18% in summer and 16.50% in autumn. The greater number of cases in winter can be explained by the fact that people, especially in rural areas have a habit of sitting around open flames for warmth.

Pseudomonas aeruginosa was the most common isolate in cases of burn wound infection 45.15%, followed by *Escherichia coli* 10.45%, *Proteus mirabilis* 9.33% and *Staphylococcus aureus* and *Klebsiella pneumonia* 8.21% each. *Candida albicans* accounted for 2.98% and *Candida tropicalis* for 1.86% of isolation. (Table 3)

Similar reports were made by other authors with *Pseudomonas aeruginosa* (30%) being the most common isolate followed by *Staphylococcus aureus* (28%) and Klebsiella species (16%).[13] A few others have, however, reported *Staphylococcus epidermidis* (56.63%) as the most common isolate followed by *Pseudomonas aeruginosa* (18.18%) and *Staphylococcus aureus* (13.63%).[14] A retrospective study of isolations from burn wound infection from 1997 to 2002 and from 2002 to 2005, however, reported that the number of Pseudomonas infections reduced from 58.95% in the 1st study to 51.5% in the 2nd study. There was increase in the number of Acinetobacter infections from 7.22% to 14.23%.[2] The incidence of *Acinetobacter baumannii* infection in our study was found to be 2.24%.

In another study, Staphylococcus was the most common isolate 47.8%, followed by *Pseudomonas aeruginosa* 23.0% and *Candida albicans* and *Escherichia coli* 5.3% each.[11] The variations seen in the isolation pattern of different organisms in all these studies could be due to the geographical locations in which these studies were conducted and the differences in hospital microenvironment in different hospitals. However, *Pseudomonas aeruginosa* appears to be a major pathogen in all these studies as seen in the present study.

Gram-negative bacilli showed maximum resistance to amoxicillin and 3rd generation cephalosporins. Least resistance was seen with imipenem and piperacillin/tazobactam. (Table 4).

The high degree of resistance exhibited by these isolates was probably due the fact that they were hospital-acquired strains. Another reason could be the gross misuse of antibiotics in hospital settings especially cephalosporin group of antibiotics.

Other authors have also reported 100% resistance to ampicillin in *Escherichia coli* and Proteus species which is quite similar to the findings of present study where all Gram-negative isolates including *Escherichia coli* and Proteus species showed 100% resistance to amoxicillin.[13] Few authors have reported 100% sensitivity to imipenem which is bit different from the findings of present study where 15.49% of the strains were found to be resistant to imipenem.[2] Another study reported that 93.34% strains of Klebsiella species were resistant to ceftriaxone which is again similar to the findings of the present study where 100% resistance was seen with ceftriaxone, 97.83% with cefuroxime and 92.86% with cefotaxime.[8]

42.86% of Staphylococci were methicillin resistant and 23.81% were vancomycin resistant as detected by disc diffusion test. Maximum resistance was seen with Amoxicillin and Cephalexin (100%). Least resistance was seen with Netilmicin (8.57%) and Linezolid (19.05%).

Some authors have reported 35.6% of MRSA strains and others 40.0% methicillin-resistant strains in Staphylococci which is similar to the findings of the present study. Vancomycin resistance was, however, not encountered in these studies.[9,8]

Among all the Gram-negative bacilli, 30.99% were ESBL producers. Maximum ESBL production was seen in *Citrobacter freundii* in which 50% of the strains were ESBL producers. However, as the number of Citrobacter isolated were very few (only 4 isolates), this finding is probably not a true representation of ESBL production in this species. ESBL production was seen in 46.43% of *Escherichia coli*, 40.00% of *Proteus mirabilis* and 31.82% of *Klebsiella pneumoniae*, 28.57% of *Klebsiella oxytoca*, 25.62% of *Pseudomonas aeruginosa* (Figure 2).

Others have found 42.9% of Klebsiella spp. to be ESBL producers, followed by *Escherichia coli* 25.0% and Proteus spp. 21.4%. Overall 21.4% of their isolates were ESBL producers.[15] The findings of this study are different from those of the present study.

Another group of authors found ESBL production in 39.8% of Gram-negative bacilli as compared to 30.99% in our study. In their study, however, *Pseudomonas aeruginosa* was the predominant ESBL producer 20.5% followed by *Klebsiella pneumoniae* 7.2%.[16]

CONCLUSION

In the present study, it was seen that burn wound infection was more common in females than males. Majority of cases had burn sustained from an open flame with most cases occurring in winter. As seen in all types of health care associated infections, Gram-negative bacilli were the predominant isolates from patients with burn wound infection accounting for 80% of cases. This is because Gram-negative bacilli are hardier and adapt better to hospital environment than Gram-positive cocci, with a few exceptions like staphylococci and enterococci. Fungi were isolated in about 5% of cases indicating that they may play a greater role in burn wound infection especially in this era of widespread use of antibiotics. As expected Pseudomonas aeruginosa was the commonest isolate accounting for 45% of total isolations. Amongst the Gram-positive cocci, it was seen that the hardier ones like Staphylococci and Enterococci were isolated consistently from patients with burn wound infection.

Infection control measures like strictly enforced hand washing and universal use of personal protective equipment like gowns, gloves and mask needs to be strictly adhered to. Practices like early excision therapy can be performed as a bedside procedure to prevent cross-infection. Role of infection control practices, regular surveillance cultures especially for detection of MRSA, VRE, ESBL producing Gram-negative bacilli also need to be highlighted. Routine cultures from burn wounds and other sources like blood, respiratory and urine samples should be monitored to identify epidemic pathogens and antibiotic resistant strains so that infection control measures can be immediately implemented.

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