

Bacteriology and Antibiogram of Burn Infection at a Tertiary Care Center

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A prospective study was carried out in 50 burn patients admitted in Burn unit of S.S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka for over a period of two year to evaluate time-related changes in aerobic bacterial colonization and their sensitivity pattern. Periodic swabs were taken from the burn wound on Day 0, Day 7, Day 14 and Day 21 to see the changing pattern of organisms during hospital stay of patients. Among the 200 samples, single organism was isolated in 95% samples and mixed organism in 5%. Among single isolates *Pseudomonas aeruginosa* was leading (36%) followed by *Staphylococcus aureus* (20%), Coagulase negative Staphylococcus (10%) and *Klebsiella* (10%). Among mixed growth *Pseudomonas aeruginosa* was still leading organism followed by *Acinetobacter baumannii*. There was time -related changes in bacterial isolation from burn wound during hospital stay of patients. On 7th day of admission 36% of *Pseudomonas aeruginosa* was isolated followed by *Staphylococcus aureus* and only 10% each *Klebsiella pneumoniae* and *Proteus mirabilis* were isolated. These findings were gradually changing with time and on day 14th *Staphylococcus aureus* were only 4.2% whereas *Klebsiella pneumoniae* was isolated in 25% and *Pseudomonas aeruginosa* in 31.3% and *Acinetobacter baumannii* in 16.7%. On 21st day *Pseudomonas aeruginosa* in 35.7%.

Antimicrobial sensitivity test showed that *Pseudomonas aeruginosa* was highly resistant to antimicrobial agents. It was most sensitive to Imipenem (66.7%) followed by Aztreonam (55.6%) and piperacillin (55.6%). Similarly *Klebsiella pneumoniae* was highly sensitive to imipenem (41.6%). Its resistance to Ampicillin was 91.6% followed by Ciprofloxacin (83.3%), Gentamicin (75.0%) and Cotrimoxazole. *Staphylococcus aureus*, CoNS and *Enterococci faecalis* were 100% sensitive to Vancomycin. 40% of the isolates were resistant to Cephoxitin (Methicillin resistant *Staphylococcus aureus*). Among *Enterococci faecalis*, maximum resistance was seen for the drugs like Penicillin (80%), Ampicillin (80%), Cephalexin (80%) and Ceftriaxone (80%).

These bacteria, isolated from the burn patients, were almost all higher in antimicrobial resistance rate. Since these bacteria showed very high resistant rates, they must be avoided in order to control a hospital-acquired infection. Our results seem helpful in providing useful guidelines for choosing effective empiric antimicrobial therapy against bacteria isolated from the burn patients at our institute

Key words: Burn, Time-related changes in aerobic bacterial colonization, *Pseudomonas aeruginosa*.

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Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality¹. Infection is the important cause of

mortality in burns¹. It has been estimated that 75% of all deaths following thermal injuries are related to infection². One of the key areas with which surgeons treating burn patients is concerned is septic complications, as burn wound is an ideal culture medium for microorganisms³. In addition, cross infection results between different burn patients due to overcrowding in burn wards. To establish any gains in infection control measures, it requires a brief understanding of wound bacteriology. It is very crucial for every burn institution to determine the specific pattern of burn wound microbial colonization, the time related changes in the dominant flora and the antimicrobial sensitivity profiles⁴. This becomes more important because of the fact that our hospital caters to majority of burn patients of this area. This study was carried out to document burn wound infection pattern in our setup so as to enable early treatment of imminent septic episodes with proper empirical systemic antibiotics.

1. To perform aerobic culture of four samples from each burn wound case and their sensitivity pattern at a regular interval of seven days.
2. To develop a guideline for empirical treatment on the basis of time-related changes and antimicrobial sensitivity pattern of aerobic bacteria causing burn wound infection

MATERIAL AND METHODS

Ethical issues

Written or verbal consent of patient or legal guardian and permission of the respective authority of burn unit were taken.

Inclusion criteria

Patients admitted within 24 hours of burn injury.

Exclusion criteria

Patients admitted after 24 hours of burn injury.

Specimen collection and processing

The microbial colonization of wounds was studied weekly from the date of admission to the 21st day of hospitalization. This prospective study was done on 50 patients admitted in burn unit. A total of 200 surface swabs were taken using standard methods. On admission, the sampling

procedure included collection of swab from clinically deep area of burn wound site prior to any cleansing³. Later swabs were taken on occasions of surgical debridement or surgical excision and grafting. In each sampling procedure, the bandages were removed, the remnants of topical antimicrobial agents were scraped away and the wounds were swabbed before washing and applying new topical antimicrobial agents⁵. Swabs were collected by using sterile cotton tipped swabs. Specimens were immediately transferred to sterile test tube. In case of collection of sample from dry surface, swabs were moistened with sterile normal saline. The specimens were transported in sterile, leak proof container to department of microbiology. Wound swabs obtained from the burn patients were subjected to microbiological analysis. All specimens were inoculated on 5% blood agar, Mac Conkey agar and chocolate agar plates and incubated overnight at 37°C. The isolates were identified by standard microbiological techniques⁶ and their antibiotic susceptibility was determined by Kirby Bauer's disc diffusion method⁷.

RESULTS

In the present study females (68%) were affected more than that of males (32%). The pattern of organisms cultured from the wounds show that a majority (70%) were Gram negative organisms, whereas Gram positive organisms contributed 32.5% in total.

Among the total isolates, single organisms were isolated in 98% samples on 7th day of admission, 90% on 14th day of admission, and 96% on 27th day. None of the specimen collected soon after the admission showed growth for aerobic bacteria. 6% of samples showed no growth on 7th day, 20% on 14th day and 62% on 21st day of admission and no growth in 15.5% samples. 10% mortality rate was seen in the present study. Among the bacterial isolates *Pseudomonas aeruginosa* was leading (36%) followed by *Staphylococcus aureus* (20%), Coagulase negative *Staphylococcus* (10%), *Klebsiella pneumoniae* (10%), *Proteus mirabilis* (10%), *Acinetobacter baumannii* (8%), *Enterococci faecalis* (6%) and other bacteria (Table 1). Prospective study revealed time-related changes in the organism isolation.

Gram positive organisms were initially prevalent then were gradually superceded by Gram negative organisms (Table 1). Mixed organisms were absent on admission culture which were gradually increasing up to Day 21. Isolation of

Staphylococcus aureus was 20% on admission and was gradually decreased to 4.2% on Day 14th and absent on day 21st. On the other hand single isolation of *Pseudomonas aeruginosa* and *Proteus mirabilis* were 36% and 10% each on 7th day

Table 1. Time-related changes in organism isolation from burn wound

Organisms	On Admission N=50	7 th Day N=50	14 th Day N=50	21 st Day N=45
<i>Pseudomonas aeruginosa</i>	-	18 (36.0)*	15 (31.3)	05 (35.7)
<i>Staphylococcus aureus</i>	-	10 (20.0)	02 (4.2)	-
CoNS	-	05 (10.0)	01 (2.1)	-
<i>Klebsiella pneumoniae</i>	-	05 (10.0)	12 (25.0)	01 (7.1)
<i>Proteus mirabilis</i>	-	05 (10.0)	03 (6.3)	-
<i>Enterococci faecalis</i>	-	03 (6.0)	05 (10.4)	03 (21.4)
<i>Enterobacter cloacae</i>	-	02 (4.0)	02 (4.2)	-
<i>Acinetobacter baumannii</i>	-	02 (4.0)	08 (16.7)	03 (21.4)
<i>Citrobacter freundii</i>	-	-	-	02 (14.3)
Mixed growth	-	03	05	02 (04)
No growth	50	03	10	31
Mortality	-	-	-	05
Total organism	00	50	48	14

*Figures in the parenthesis indicates percentage

Table 2. Antibiotic sensitivity pattern of gram negative bacteria isolated from burn patients

Drugs		<i>Pseudomonas aeruginosa</i>	<i>Klebsiella</i> sps	<i>Proteus mirabilis</i>	<i>Acinetobacter baumannii</i>	<i>Enterobacter cloacae</i>	<i>Citrobacter freundii</i>
Ampicillin	R	18(100)*	11(91.6)	05(100)	08(100)	2(100)	1(50)
	S	0(0)	01(8.3)	0(0)	0(0)	0(0)	1(50)
Amikacin	R	16(88.8)	09(75.5)	03(60)	07(87.5)	0(0)	1(50)
	S	02(11.1)	3(25.0)	02(40)	01(12.5)	2(100)	1(50)
Cefotaxime	R	17(94.4)	09(75.5)	03(60)	06(75)	1(50)	1(50)
	S	01(5.6)	3(25.0)	02(40)	02(25)	1(50)	1(50)
Ceftriaxone	R	18(100)	08(66.6)	03(60)	07(87.5)	1(50)	1(50)
	S	0(0)	04(33.3)	02(40)	01(12.5)	1(50)	1(50)
Ciprofloxacin	R	18(100)	10(83.3)	02(40)	05(62.5)	1(50)	1(50)
	S	0(0)	02(16.7)	03(60)	03(37.5)	1(50)	1(50)
Cotrimoxazole	R	17(94.4)	08(66.6)	03(60)	06(75)	1(50)	0(0)
	S	01(5.6)	04(33.3)	02(40)	02(25)	1(50)	2(100)
Gentamycin	R	15(83.3)	09(75.5)	04(80)	07(87.5)	2(100)	1(50)
	S	03(16.7)	3(25.0)	01(20)	01(12.5)	0(0)	1(50)
Imipenem	R	06(33.3)	08(66.6)	01(20)	07(87.5)	0(0)	0(0)
	S	12(66.7)	04(33.3)	04(80)	01(12.5)	2(100)	2(100)
Aztreonam	R	08(44.4)	10(83.3)	01(20)	06(75)	0(0)	0(0)
	S	10(55.6)	02(16.7)	04(80)	02(25)	2(100)	2(100)
Piperacillin	R	08(44.4)	09(75.5)	03(60)	06(75)	0(0)	0(0)
	S	10(55.6)	3(25.0)	02(40)	02(25)	2(100)	2(100)

*Figures in the parenthesis indicates percentage

culture which were gradually decreased upto 35.7% for *Pseudomonas aeruginosa* and 6.3% for *Proteus mirabilis* respectively on day 21st and day 14th. While *Acinetobacter baumannii* was isolated in 4% on 7th day culture and increased to 16.7% on 14th day and 21.4% on 21st day.

The antibiogram of Gram negative organisms isolated from the burn wound is shown in Table II. *Pseudomonas aeruginosa* was highly sensitive to Imipenem (66.7%) followed by Aztreonams (55.6%) and Piperacillin (55.6%) but resistance to Ampicillin, Ceftriaxone and Ciprofloxacin was 100% followed by Cotrimoxazole (94.4%), Cefotaxime (94.4%), and Gentamycin (83.3%). Similarly *Klebsiella pneumoniae* was highly sensitive to imipenem (41.6%). Its resistance to Ampicillin was 91.6% followed by Ciprofloxacin (83.3%), Gentamicin

(75.0%) and Cotrimoxazole (66.7%). *Proteus mirabilis*, *Enterobacter cloacae* and *Acinetobacter baumannii* were sensitive to Imipenem.

Antimicrobial sensitivity Pattern of Gram positive organisms isolated from burn wound is shown in Table III. *Staphylococcus aureus*, CoNS and *Enterococci faecalis* were 100% sensitive to vancomycin Among *Staphylococcus aureus* 90% were resistant to Penicillin, 80% to ampicillin, cephalexin, Gentamycin. 40% of the isolates were resistant to Cephoxitin (Methicillin resistant *Staphylococcus aureus*) Among CoNS, none of the isolates were resistant to Cephoxitin. Majority of CoNS were resistant to Gentamycin, Erythromycin and ampicillin. Among *Enterococci faecalis*, maximum resistance were seen for the drugs like Penicillin (80%), Ampicillin (80%), Cephalexin (80%) and Ceftriaxone (80%).

Table 3. Antibiotic sensitivity pattern of gram negative bacteria isolated from burn patients

Antibiotics	Sensitivity	Staphylococcus aureus	CoNS	Enterococci faecalis
Penicillin	R	9(90.5)*	3(68.2)	4(80.0)
	S	1(9.5)	2(31.8)	1(10.0)
Ampicillin	R	8(23.8)	4(81.8)	4(80.0)
	S	2(76.2)	1(18.2)	1(10.0)
Cephalexin	R	8(28.6)	2(54.5)	4(80.0)
	S	2(71.4)	3(45.5)	1(10.0)
Ceftriaxone	R	7(38.1)	3(59.1)	4(80.0)
	S	3(61.9)	2(40.9)	1(10.0)
Clindamycin	R	3(33.3)	2(18.2)	3 (60.0)
	S	7(66.7)	3(81.8)	2(20.0)
Ciprofloxacin	R	6 (31.0)	2(50)	3 (60.0)
	S	4(69.0)	3(50)	2(20.0)
Amoxyclav	R	4(40.5)	1(81.8)	3 (60.0)
	S	6(59.5)	4(9.1)	2(20.0)
Erythromycin	R	8(76.2)	4(68.2)	4(80.0)
	S	2(23.8)	1(31.8)	1(10.0)
Gentamycin	R	8(78.6)	4 (72.7)	3 (60.0)
	S	2(21.4)	1(27.3)	2(20.0)
Cephoxitin	R	4 (31.0)	0(0)	NT
	S	6(69.0)	5(100)	NT
Vancomycin	R	0(0)	0(0)	0(0)
	S	10(100)	5(100)	5(100)
Tetracycline	R	4(38.1)	3(50)	2 (20.0)
	S	6(61.9)	2(50)	3(60.0)
Cotrimoxazole	R	5(40.5)	3(68.2)	3 (60.0)
	S	5(59.5)	2(31.8)	2(20.0)

*Figures in the parenthesis indicates percentage

DISCUSSION

Burn wound if not excised and grafted early becomes an ideal culture medium for the growth of microorganisms⁸. Infection is an important cause of morbidity and mortality in burns. Severe burn patients are very susceptible to infection because of wide exposed raw areas, the presence of necrotic tissue, protein rich exudates, inability of blood to reach the colonized areas of wounds and other host defense mechanisms⁹. The colonization and later invasion of tissues is from patient's normal flora of skin or from gastrointestinal tract or more usually by cross infection¹⁰. In the face of high mortality because of bacteraemia in burned patients, it is important to select antibiotics or combination of antibiotics with broad coverage for the usual pathogens. In a large number of patients this has to be empirical pending results of cultures⁵.

As the type of bacteria and their sensitivity vary from place to place analysis of burn wound microbial colonization is to be performed so that the prophylactic and therapeutic regimens could be rationalized^{6,8,11}. There are also time-related changes in burn wound microbial colonization. Different types of study on burn wound infection have been carried out in different countries of the world. Among them few were regarding time-related changes in bacterial colonization¹². In India time related changes in burn wound infection were not included in the study because patients were unable to bear the cost of treatment.

Infection with one or more organisms was present in 98% of cases. The changes in bacterial spectrum are not unexpected because of cross infections, resistance to drugs and introduction of new bacteria from other places. Hence incidence and spectrum of infection varies from place to place and country to country due to different therapeutic and preventive policy. *Pseudomonas aeruginosa* isolation was maximum in our study in both single (36%) and mixed (9%) infection (Table 1). These findings were consistent with those of other centers of different countries^{6,8}. But in a previous study in our country isolation of *Staphylococcus aureus* was leading¹⁰. In the present study *Staphylococcus aureus* was the second most common organism isolated (17.5%)

followed by *Klebsiella pneumoniae*

Analysing the results of four swabs taken from burn wound of each patient it was observed that by day 21 all the sample yielded growth. Gram negative organisms were predominant. All these changes were gradual from starting to the end of sample collection (Table 2). On the day of admission (Day 0) no bacteria were isolated. On day 7th colonization by *Pseudomonas aeruginosa* was 36% followed by *Staphylococcus aureus* 20% and *Klebsiella pneumoniae* 10%.

On Day 14, gram negative bacteria were the predominant. *Pseudomonas aeruginosa* was isolated in 31.3% followed by *Klebsiella pneumoniae* 25% and *Acinetobacter baumannii* 16.7%. On Day 21 *Pseudomonas aeruginosa* was the predominant followed by *Enterococci faecalis* (21.4%), *Citrobacter freundii* (14.3%) and *Klebsiella pneumoniae* (7.1%) On Day 0 there was no mixed growth which was 20% on Day 21. All the (100%) swabs yielded growth on Day 21. Present study revealed that Gram positive cocci (*Staphylococcus aureus*) were initially prevalent then were gradually superceded by Gram negative bacilli specially *Pseudomonas aeruginosa* throughout patients hospital stay of 21 days.

The study results of various worker revealed that the bacteriology of burn infection has been changing from time to time and also the antimicrobial sensitivity pattern^{13,14}. There are also time-related changes in burn wound microbial colonization. Gram positive cocci are initially prevalent then are gradually superceded by Gram negative bacilli throughout the patients hospital stay that have a greater propensity to invade^{3,6,8}. These time-related changes have also been found in our study (Table 2). Periodic reviews of patterns of isolation and susceptibility profiles of organisms infecting burn wounds are needed in order to modify the preventive and therapeutic strategies¹⁵. It is therefore essential for every burn institution to determine its specific pattern of burn wound microbial.

CONCLUSION

Burns are a very common injury. Minor burns will heal without much medical attention serious burn injuries are excruciatingly painful and require special care to prevent infection and reduce

the severity of scarring. They can cause lifelong disabilities and leave physical and emotional scars. Perhaps the saddest thing of all is that many burn injuries are preventable. It is quite clear that infections are a serious problem among burn patients. *P. aeruginosa* has emerged as the commonest organism causing infection and is resistant to most of the antibiotics. To keep a check on burn wound infections it is important for every hospital to have a data on prevalent organisms and their antibiotic susceptibility pattern. This study should be done frequently to check the changing pattern of the organisms and their susceptibility pattern. Based on this, the hospital should formulate an effective antibiotic policy.

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