Original article:

Time Related Changes in Pathogenic Bacterial Patterns in Burn Wound Infections and Their Antibiotic Sensitivity Traits

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Abstract

Objectives: To examine the time related changes in the bacterial flora isolated from the burn patients and to compare the antibiograms of the predominant bacteria. Burn injuries constitute a major health concern with respect to morbidity and mortality as well as cost of management particularly in developing countries. It has been estimated that 75% of all deaths following thermal injuries are related to infection. It is essential for every hospital to determine the specific pattern of burn wound microbial colonization, time-related changes in predominant flora and anti microbial sensitivity profiles. ***Materials and Methods:*** 60 burn patients and their microbial isolates were examined during the study period. Periodic swabs were taken from burn wounds of the patients on admission and on 7th, 14th, and 21st days of hospitalization and the isolates were processed for culture, identification and antimicrobial sensitivity as per CLSI guidelines. ***Results:*** Among the 240 samples, single organism was isolated in 161 samples (67.1%) and mixed organisms in 22 samples (9.1 %) and no growth in 57 samples (23.7%). Among single isolates pseudomonas aerugenosa was predominant species (20.8%) followed by acinetobacter baumanii (15.4%), staph. aureus (14.1%) and klebsiella (7.5%). Among mixed growth pseudomonas was predominant species (30.4%) followed by acinetobacter (23.9%). klebsiella (17.4 %) and staph. aureus (10.8%). There was time related changes in bacterial isolation from burn wound during hospital stay of patients. On admission, Staph. Aureus was 18.3 %, pseudomonas was 8.3% and klebsiella was 6.6%. No growth found in 48.3% samples. These findings gradually changed with time and on day 21 Pseudomonas was 30% followed by Acinetobacter sp. (21.6%), Staph. aureus (13.3%) and Klebsella pneumoniae (6.6%). No growth was seen in 15% cases only. Antimicrobial sensitivity test showed that pseudomonas was sensitive to Imipenem, Merepenem and Ampicillin. Acinetobacter was also found most sensitive to Amikacin and Tetracycline, followed by Tobramycin. Staphylococcus aureus was sensitive to Linezolid, Gentamycin followed by Vancomycin. ***Conclusion:*** There were time-related changes in microbial colonization during hospital stay of patients. Effective protocols should be made for burn patients and effort is needed for improving overall infection related morbidity and mortality.

Keywords: Burn Injuries; Bacterial infections; Time-related changes; Antibiotic resistance.

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Introduction

The skin forms a protective barrier against invasion by bacteria, fungi and viruses; any injury to the skin therefore, causes a breach in the protective layer surrounding the body.[1] Burn injury to the tissue caused by thermal energy is one of the most common casualties suffered by people worldwide. Millions of people suffer burn injuries and thousands die of the same each year.[2] Globally, fire-related burns are responsible for about 265,000 deaths annually.[3] Over 90% of these fatalities occur in developing or low- and middle income countries (LMICs) with south-east Asia alone accounting for over half of fire-related deaths.[3] It has been estimated that 75% of all deaths following thermal injuries are related to infection.[3]

The burn wound has a much higher incidence of infections compared with other forms of trauma because of extensive skin barrier destruction as well as alteration of the cellular and humoral immune responses.[4] Thermal injury to the skin causes a massive release of humoral factors, including cytokines, prostaglandins, vasoactive prostanoids, and leukotrienes. Accumulation of these factors at the site of injury results in "spillover" into the systemic circulation, giving rise to immunosuppression. The dysfunction of the immune system, large cutaneous bacterial load, the possibility of gastrointestinal bacterial translocation, prolonged hospitalization, and invasive diagnostic and therapeutic procedures all contribute to sepsis.[5,6]

Microorganisms colonizing the burn wound originate from the patient's endogenous skin and gastrointestinal and respiratory flora.[7] Microorganisms may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers.[8] Immediately following injury, gram-positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound. Endogenous gram-negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury.[9]

Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora.[7] Patient factors such as age, extent of injury, and depth of burns coupled with microbial factors such as the type and number, enzyme/ toxin production and motility of organisms are the determinants of invasive infection.[8] Superficial bacterial contamination of the wound can easily advance to invasive infection in these patients. The degree of bacterial wound contamination has a direct correlation with the risk of sepsis.[8,9]

To establish any gains in infection control measures, it requires an 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 profiles.[10]

The increase rate of burn wound infection and sepsis is due to overcrowding (such as in developing countries), inadequate sterilization and disinfection practices, gross contamination of environment, and lack of isolation facilities, inadequate hand washing and absence of barrier nursing.[11] Patients have to stay for long period in the hospital and many intravascular and other devices are put in them.[12] Hence they are at greater risk of acquiring hospital-acquired infection.[12]

There should be continuous observation of burn infections and increase strategies for antimicrobial resistance control and treatment of infectious complications. Hence, the present study was under taken to establish the bacteriological profile of the burn wound infection and to formulate empirical treatment guidelines for these patients, so that mortality can be prevented.

Materials and Methods

A prospective cross sectional study, which was approved by ethical committee of the university, was conducted on a randomly selected 60 burn patients admitted within 24 hours of burn injury in the burn unit of the institute and who survived for at least 1 month, for a period of 1 year from April 2014 to March 2015. A detailed clinical history of the patients was collected. Two burn wound swabs were collected aseptically on admission before the start of antibiotics and there after weekly for a maximum period of three weeks, which resulted in total sample size of 240. Swab samples were taken from the wound area where the degree of burn was highest. The swabs were transported to the microbiology lab in sterile test tubes immediately. One swab was used for gram staining and the other for culture. All specimens were inoculated on 5% blood agar and Mac Conkey agar plates and incubated over night at 37^0 C. The isolates were then subjected to a battery of biochemical tests and identified by standard microbiological techniques. All the organisms isolated were subjected for antimicrobial susceptibility testing by modified

Kirby Bauer method according to CLSI guide lines.

Results

Out of 60 patients that were studied, the commonest age group was 21-40 years (50%). The second commonest and the least common age group was 0-20 years (20%) and more than 60 years (13.3%) respectively, the youngest patient being 2 Month old and the oldest being 76 years old. Maximum number of patients i.e. 25 (41.6%) suffered from fire burns, followed by 18 (30%) due to Electric burn, 11 (18.3%) were due to thermal burn and from burns due to chemicals 6 (10 %).

Majority of patients, 13 (21.6%) had burn between 31-40% of total body surface area followed by 10 patients (16.6%) who had 61-70% burns. The mean percentage of burn was 36.79% of total body surface area (TBSA) least being 10 % and maximum being more than >70% of total body surface area.

Among the total 240 swabs, single organisms were isolated in 161 samples. Mixed growth was seen in 22 samples and no growth in 57 samples. Among single isolates pseudomonas aeruginosa was leading (20.8 %) followed by Acinetobacter baumannii (15.4 %), Staphylococcus aureus (14.1 %), Klebsiella pneumoniae (7.5 %), Enterobacter (2.5%), Enterococcus (1.6 %), Escherichia Coli (1.2%), Proteus (1.2%), Coagulase Negative Staphylococcus (1.2 %) and citrobacter species (1.2%). (Table 1)

On 1st day of admission, colonization was seen in 31 patients out of which Staph. aureus was predominant. On 7th day colonization was seen in 50 patients and gram negative bacteria were predominant with Pseudomonas aeruginosa followed by Staphylococcus aureus. By the end of 3rd week, gram negative were more with Pseudomonas aeruginosa followed by Acenitobacter baumanni. No growth was seen in 29 swabs on admission but by the end of 3rd week, no growth was seen in only 9 swabs. (Table 2)

Discussion

In developing countries burn injuries are much more common than in the USA and Europe or other affluent developed countries as poverty, overcrowding, and illiteracy are the main demographic factors associated with a high risk of burn injury, creating a formidable public health problem. Additional factors include perennial fuel scarcity, erratic power supply necessitating the use of kerosene stoves and lamps, and local traditional practices such as hot water baths for mothers immediately after childbirth and the treatment of convulsions in children with fire.[12]

Lapses in child supervision, use of clothing with manmade fabrics, parental illiteracy, housing location in slums and congested areas, presence of a pre-existing impairment in a child, prior history of a sibling burn, and low socioeconomic status were reported as significant risk factors for childhood burns in Bangladesh and Pakistan.[12]

Infections remain the leading cause of death among patients who are hospitalized for burns. The risk of burn wound infection is directly correlated to the extent of the burn and is related to impaired resistance resulting from disruption of the skin's mechanical integrity and generalized immune suppression.[13]

In the present study, it was seen that the commonest age group of burns was 21-40 years (50%). The second commonest and the least common age group was 0-20 years (20%) and more than 60 years (13.3%) respectively. The findings are similar to other studies.[13,14] High incidence among young adults may be explained by the fact that they are generally active and exposed to hazards at home and at work.[14]

Pseudomonas aeruginosa isolate was most common in both single (20.8 %) and mixed (30.4 %) growth. Acinetobacter baumanni was the second most common isolate in both single (15.4 %) and mixed (23.9 %) growth. The high frequency of Pseudomonas aeruginosa might be because it is found frequently in hospital environments and burn wound are an ideal medium for their survival. Pseudomonas aeruginosa are inherently resistant to commonly used antibiotics and can even survive in common antiseptics.[15]

Regarding growth of Acinetobacter baumanni, it was similar to other studies who also reported higher frequency of Acinetobacter infections.[16] *Acinetobacter baumannii* is a rapidly emerging pathogen in the health care setting, where it causes infections that include bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. The organism's ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfaces make it a frequent cause of outbreaks of infection and an endemic, health care–associated pathogen.[17]

The spectrum of infective agents varies with time and is unique for different hospital. While evaluating microbial colonization from the 240 swabs at weekly interval it was seen, on day 0 colonization by Staphylococcus aureus was 18.3 % followed by Pseudomonas aeruginosa (8.3%) and Klebsiella pneumonia (6.6%). On day 7th the dominant isolate was Pseudomonas (20%) followed by Staph. aureus

(18.3%), Acinetobacter (16.6%) and Klebsella pneumoniae (8.3%). On day 14th Pseudomonas was 25% followed by Acinetobacter (20%), Staph. aureus (11.6%) and Klebsella pneumoniae (8.3%). On day 21st Pseudomonas was 30% followed by Acinetobacter sp. (21.6%), Staph. aureus (13.3%) and Klebsella pneumoniae (6.6%).

On day 0 there was mixed growth in 4 cases out of 60 (6.6 %), which was 10% on day 21. No growth was seen on day 0 in 29 out of 60 cases (48.3%), but by day 21 it was seen in only 9 cases (15%). It was observed in our study that on day 0 there was predominance of Staph. aureus which was gradually superceded by Pseudomonas aeruginosa on day 21. Pseudomonas colonization was dramatically increased by the day 7, where as it was 8.3% on day 0 it rose to 20% by day 7. The findings were in accordance with various studies.[16,18] The percentage of colonization by Staphylococcus aureus did not show much change in the periodic samples. The persistence of Staphylococcus throughout our study could be due to cross-infection of micro-organisms in ICU. It may also be due to the fact that proper infection control practices were not followed by relatives of patients and by health care workers.

Resistance patterns among nosocomial bacterial pathogens may vary from country to country and also within the same country, over time. In this study antibiotics sensitivity profile of the isolates were also observed. Pseudomonas aeruginosa isolates in our study were susceptible to imipenem (95%) and amikacin (90%). Acinetobacter species showed higher rate of resistance to ciprofloxacin, amikacin, ceftazidime, and piperacillin in our study. Strains showed good sensitivity to amikacin and tetracycline. (Table 3) (Table 4). Other studies have reported high degree of resistance to almost all the antibiotics.[15.16.18] We attribute these differences in the susceptibility of strains to differences in the patient population studied by us. Most of our patients were from surgical wards. Furthermore, our patients came from rural areas without much exposure to antibiotics.

Conclusion

There were time-related changes in microbial colonization during hospital stay of patients. The higher prevalence of Pseudomonas and Acinetobacter can be explained due to the prolonged stay in the hospital. The persistence of Staphylococcus till the last week explains the lack of implementation of strict infection control practices. Hence it appears absolutely essential for every hospital to conduct timely study regarding the changing pattern of the burn wound infection and their antibiotic susceptibility pattern to achieve the ultimate objective of improving infection related morbidity and mortality in burn patients.

Table 1: Organisms Isolated From Burn Wounds

Isolated Organism	**Pure Growth**		**Mixed Growth**		**Total**	
	No	**%**	**No**	**%**	**No**	**%**
Pseudomonas aeruginosa	28	46.6	18	30	46	25.9
Acinetobacter baumanni	23	38.3	12	20	35	19.7
Staphylococcus aureus	17	28.3	15	25	32	18
Klebsiella pneumoniae	17	28.3	17	28.3	34	19.2
Enterobacter species	6	10	2	3.3	8	4.5
Enterococcus species	6	10	3	5	9	5
Escherichia coli	3	5	1	1.6	4	2.2
Proteus species	3	5	0	0	3	1.6
CONS	3	5	0	0	3	1.6
Citrobacter species	0	0	3	5	3	1.6
No growth	40	66.6	….	….	40	22.5
Mixed	….	….	37	61.6	37	20.9
TOTAL	**106**	**55.2**	**71**	**36.9**	**177**	**92.1**

Table 2: Time Related Changes In Organisms Isolated From Burn Wounds

Isolated Organism	Admission Burn Unit								Total (N= 192)	
	On Admission (N= 60)		7Th Day (N= 60)		14Th Day (N= 49)		21St Day (N= 23)			
	No.	%	No.	%	No.	%	No.	%	No.	%
Pseudomonas aeruginosa	05	8.3	23	38.3	15	30.6	03	13.0	46	25.9
Acinetobacter baumanni	00	00	15	25	15	30.6	08	34.7	38	19.7
Staphylococcus aurous	10	16.6	13	21.6	6	12.2	4	17.3	33	17.1
Klebsiella Pneumoniae	6	10.0	14	23.3	11	22.4	2	8.6	33	17.1
Enterobacter species	2	3.3	3	5.0	3	6.1	0	0	8	4.1
Enterococcus species	5	8.3	3	5.0	1	2.0	1	4.3	10	5.2
Escherichia Coli	1	1.6	1	1.6	3	6.1	1	4.3	6	3.1
Proteus Species	1	1.6	2	3.3	1	2.0	0	0	4	2.0
CONS	0	0	1	1.6	2	4.0	0	0	3	1.5
Citrobacter species	2	3.3	2	3.3	1	2.0	0	0	5	2.6
No Growth	23	38.3	2	3.3	4	8.1	10	43.4	39	20.3
Mixed	9	15.0	15	25.0	11	22.4	3	13.0	38	19.7

Table 3: Percentage (%) Sensitivity Of Gram Positive Bacteria.

Organism	Vancomycin	Azithromycin	Clindamycin	Ciprofloxacin	Gentamycin	Tetracycline	Gatifloxacin	Levofloxacin	Penicillin	Rifampicin	Linezolid
Staph. Aureus	90.9 %	45.5 %	27.2 %	81.8 %	100 %	63.6 %	45.5 %	36.3 %	27.2 %	90 %	100 %
Enterococcus Species	100 %	20%	20 %	100%	100 %	60 %	40 %	60 %	20 %	0%	100 %
Cons	66.6 %	66.6%	100 %	33.3%	66.6 %	33.3 %	0%	0%	66.6 %	0%	90 %

Table 4: Percentage (%) Sensitivity of Gram Negative Bacteria

ORGANISM	Gentamicin	Ampicillin	Amikacin	Cefoperazone	Ciprofloxacin	Levofloxacin	Imipenem	Meropenem	Chloramphenicol	Kanamycin	Tetracycline	Tobramycin
Pseudomonas Aeruginosa	72.7%	100%	100%	100%	81.8%	45.4%	100%	100%	81.8%	90.9%	90.9%	100%
Acinetobacter Baumanni	27.7%	38.8%	100%	16.6%	11.1%	11.1%	33.3%	27.7%	22.2%	33.3%	100%	66.6%
Klebsiella Pneumoniae	33.3%	11.1%	100%	16.6%	22.2%	5.5%	27.7%	33.3%	11.1%	11.1%	16.6%	44.4%
Enterobacter Species	66.6%	33.3%	83.3%	50%	66.6%	16.6%	66.6%	83.3%	66.6%	33.3%	66.6%	50%
Escherichia Coli	6.6%	33.3%	100%	33.3%	66.6%	6.6%	100%	100%	66.6%	100%	6.6%	6.6%
Proteus Species	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	0%	0%
Citrobacter Species	100%	50%	100%	50%	50%	50%	100%	100%	50%	50%	50%	50%

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