

Original Research Article

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Study on Bacterial Flora of Burn Wound Infection: A Need for Microbiological Surveillance in Burn Units

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ABSTRACT

75% of the mortality associated with burn injuries is related to infection. The aim of the present study was to identify the bacterial profile of burn wound infection (BWI) in our setting and determine their susceptibility pattern to commonly used antibiotics. This prospective study was conducted over a period of one year in a teaching tertiary care hospital, Chennai. A total of 100 patients with burns of total body surface area (TBSA) of 20% to 40% were included. Three wound swabs on 1st, 4th and 7th day were collected aseptically and processed. Among the 274 samples collected, 191 swabs revealed growth while 83 showed no growth. Overall isolation rate was found to be 69.7% and was predominantly monomicrobial with Gram positive cocci in early swabs. Subsequent swabs showed 100% colonization with a shift to polymicrobial infection with predominant isolation of Gram negative bacilli. The most common isolate was *Pseudomonas aeruginosa* (35.84%), followed by *Klebsiella pneumoniae* (27.30%), *Acinetobacter* spp. (20.13%), *Staphylococcus aureus* (8.87%), *Escherichia coli* (2.38%). Gram negative bacteria were found to be highly susceptible to Imipenem and Piperacillin /Tazobactam. *Staphylococcus aureus* was 100% sensitive to Linezolid. Knowledge about specific pattern of burn wound infection and their resistant profile not only enable us to plan empirical antibiotics to prevent imminent septic episodes but also reduce infection related mortality in burns patients.

Keywords

Bacterial Flora, Burn Wound Infection, Microbiological Surveillance.

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Introduction

Patients with burn injuries are highly susceptible for infection as a result of disruption of the normal skin barrier and accompanying depression of immune response. The burn surface contains a large amount of necrotic tissue and the protein rich wound exudates provides a rich growth medium. So, following the initial period of shock, infection is the major complication and about 75% of the mortality associated with

burn injuries is related to infection. The organisms are mainly derived from the patient's gastro intestinal and upper respiratory tracts as well as from the hospital environment (Al-Aali *et al.*, 2016).

Infection, the risk of which is proportional to the extent of injury, continues to be the predominant determinant of outcome in thermally injured patients. Most of the

infections are thought to be of nosocomial origin wherein hand and clothing of attending staff has been implicated in many cases. The control of invasive burn wound infection through the use of effective topical chemotherapy, prompt surgical excision, and timely closure of the burn wound has resulted in unsurpassed survival rates. Even so, these measures can cause emergence of antibiotics resistant isolates and treatment failures (Saaq *et al.*, 2015).

Several studies about the microbial flora have revealed that immediately following burn injury it is predominantly Gram-positive organisms, within a week it is replaced by Gram-negative organisms. The distribution of infective agents varies with time and is unique to different hospitals (Mundhada *et al.*, 2015).

The analysis of the isolates and their sensitivity patterns helps us to track the emerging trends to formulate an institutional drug policy for the patients admitted in Burn Unit. Rational antibiotic therapy according to the prevalent strains of organisms should help in reducing the mortality and morbidity associated with burns (Shahzad *et al.*, 2012).

In view of the above literature, this study aims to identify the bacterial profile of burn wound infection (BWI) in our setting and determine their susceptibility pattern to commonly used antibiotics.

Materials and Methods

This prospective study was conducted over a period of one year in a teaching tertiary care hospital, Chennai. A total of 100 patients with burns of total body surface area (TBSA) of 20% to 40% (according to rule of nine) were included. Specimens were three wound swabs collected aseptically from burn area after thorough cleaning with sterile saline. First swab was collected immediately after

admission before start of antibiotics on Day 1` and thereafter on Day 4 and Day 10.

Sample processing

Samples were processed as per standard microbiological procedure. The specimens were subjected to direct gram staining and culture. Identification of aerobic bacteria and its antimicrobial susceptibility pattern was detected as per standard CLSI guidelines.

Antibiotic susceptibility was done by Kirby Bauer disk diffusion method. Among gram negative bacteria, Enterobacteriaceae were tested against Ampicillin 10 µg, Amikacin 30 µg, Tetracycline 30 µg, Levofloxacin 5 µg, Cefotaxime 30 µg, Ceftazidime 30 µg, Ciprofloxacin 5 µg Imipenem 10 µg, and Piperacillin-Tazobactam 100/10 µg. For Pseudomonas species and Acinetobacter species, antibiotic discs like Piperacillin-Tazobactam 100/10 µg, Cefepime 30 µg, Ceftazidime 30 µg, Imipenem 10 µg, Gentamicin 10 µg, Amikacin 30 µg and Ciprofloxacin 5 µg were used. For Staphylococcus spp., Cefoxitin 30 µg, Erythromycin 15 µg, Gentamicin 10 µg, Amikacin 30 µg, Levofloxacin 5 µg, Clindamycin 2 µg, Linezolid 30 µg, Teicoplanin 30 µg were used.

For *Enterobacteriaceae* – Isolates were considered a potential ESBL producer if the zone of inhibition for ceftazidime was observed to be <22mm. Potential ESBL producer was then subjected for ESBL Phenotypic confirmatory test –Disc Diffusion method as recommended by CLSI guidelines for antimicrobial disc susceptibility tests (NCCLS, 2003b).

Phenotypic confirmatory disc diffusion test (PCDDT) for ESBL

A Mueller Hinton agar plate was taken and a lawn culture of potential ESBL producing

isolate was made. Then ceftazidime (30µg) disc alone and with clavulanic acid (10µg) were placed at an appropriate distance from each other on the plate and incubated aerobically at 37°C overnight. A \geq 5mm increase in zone diameter for antimicrobial Ceftazidime tested in combination with clavulanic acid in comparison to the zone diameter when tested alone confirmed the organisms to be an ESBL producer by PCDDT.

Detection of MRSA

Methicillin resistant *Staphylococcus aureus* (MRSA) detection was done using cefoxitin 30 µg. Those isolates showed zone of inhibition $<$ 21 mm considered as MRSA.

Results and Discussion

A total of 100 patients (44 were males and 56 were females) with 20% to 40% burns were included in this study. Majority of the subjects included in our study had sustained second degree burns (52%) followed by first degree (34%).(Fig 1)

A total of 274 wound swabs were collected from 100 patients. The reason for less number of samples collected on day 4 and day10 were due to the fact that patients were either discharged or expired. 191 swabs revealed growth while 83 showed no growth. Isolation rate was found to be 69.7%. (Fig 2)

On admission Monomicrobial infection was common and polymicrobial type of infection was less and it was more with the patients who stayed in the hospital for more than 2 days (Table 1).

The initial swabs were predominantly monomicrobial with gram positive isolates and which is replaced by gram negative isolates in the later swabs, which were also polymicrobial. (Table2). Overall, total

number of bacterial isolates obtained was 293. Among them, the most common isolate was *Pseudomonas aeruginosa* 105 (35.84%), followed by *Klebsiella pneumoniae* 80 (27.30%) *Acinetobacter* spp. 61(20.13%), *Staphylococcus aureus* 22(8.87%), *Escherichia coli* 7(2.38%).

To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary. Therefore, a continuous surveillance of microorganisms and a regular update of their antibiotic resistance pattern is essential to maintain good infection control program in the burn unit, thus improving the overall infection-related morbidity and mortality.

In this study the pattern of burn wound microbial colonization was evaluated. The time related changes in the predominant flora was also evaluated throughout the patients hospital stay.

Our study revealed slight female preponderance (56%) compared to male. This result was in agreement with the finding reported by Mundhada *et al.*, (2015), who observed 54% in male and 46% in female. Also, Rajput *et al.*, (1998) found that burn infection in females was (60%) while burn infection in males was (40%). In contrast, DeMacedo and Santos *et al.*, (2005) found that BWI in males 59.1% was more than females 40.9%. In our country this is likely due to occupational hazards of women working in the kitchen as the kitchen is the most common place prone to burn accidents. In this study, mortality rate was low (8%) against 19.6% by Lari *et al.*, (2000). This low rate might be due the fact that we are dealing with patients having TBSA of burn between 20% and 40%. Majority of the subjects included in our study had sustained second degree burns (52%) followed by first degree (34%) (Fig. 1) This was similar to the results

reported by Al- Akayleh *et al.*, (1999) who showed highest distribution of burn wound infection in burn patients who had sustained second-degree burn (53.9%).

Isolation rate was found to be 69.7% (Fig 2) which is comparable to the isolation rate observed by Srinivasan *et al.*, (2009) (86.3%) and Modi *et al.*, (2013) (85.07). Irrespective of duration of stay, monomicrobial pattern of growth was found to be common than

polymicrobial which was in agreement with other studies by Mundhada *et al.*, (2015) and Shahzad *et al.*, (2012)(Table 1)

In a recent study on time-related changes in aerobic bacterial pattern of burn wound infection by Saha *et al.*, (2011), it was found that in burn wounds initially it was gram positive organisms which are gradually superseded by gram negative opportunists that have greater propensity to invade.

Table.1 Type of Growth on wound swab

	Day 1	%	Day 4	%	Day10	%
Monomicrobial	30	81.08%	42	55.26%	42	53.84%
Polymicrobial	7	18.91%	34	44.73%	36	46.15%

Table.2 Time related changes in bacterial profile of organisms Isolated

Organisms Isolated	Day 1	Day 4	Day 10
Monomicrobial	30	42	42
<i>Pseudomonas aeruginosa</i>	7	20	26
<i>Klebsiella</i> spp	4	13	11
<i>Acinetobacter spp</i>	2	1	3
<i>Escherichia coli</i>	--	4	--
CONS	12	---	----
<i>Staphylococcus aureus</i>	5	4	2
Polymicrobial	7	34	36
<i>Pseudomonas + Acinetobacter</i>	---	6	8
<i>Pseudomonas + Escherichia coli</i>	---	3	---
<i>Acinetobacter+ Klebsiella</i>	---	11	10
<i>Klebsiella+ CONS</i>	4	---	----
<i>Pseudomonas + Klebsiella</i>		2	6
<i>Pseudomonas + CONS</i>	2	----	----
<i>Pseudomonas + Acinetobacter+ Klebsiella</i>	-----	6	8
<i>Pseudomonas + Acinetobacter+ S. aureus</i>	-----	2	4
<i>Pseudomonas + S. aureus+ Klebsiella</i>	1	4	-----

Table.3 Resistant Profile of the Organisms

Organisms	Total No	Resistance type	Positive	Percentage
Klebsiella Species	80	ESBL	36	45
Escherichia coli	7	ESBL	4	57
Staphylococcus aureus	22	MRSA	8	36.36
CoNS	18	Methicillin resistant	5	27.77

Fig.1

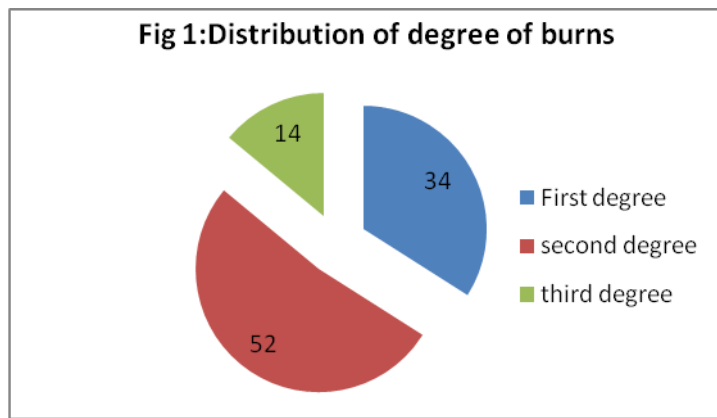


Fig.2

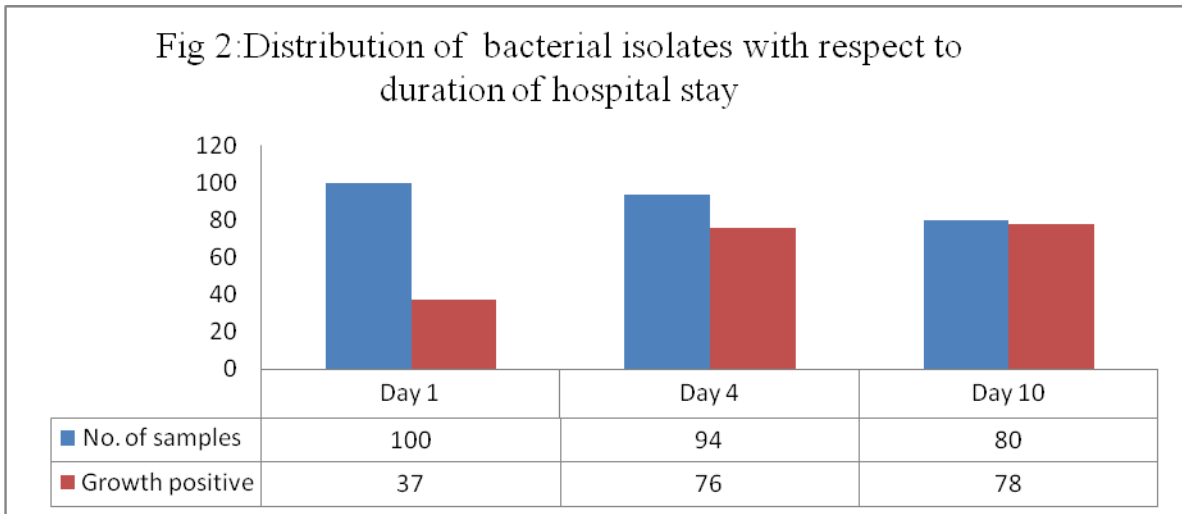


Fig.3

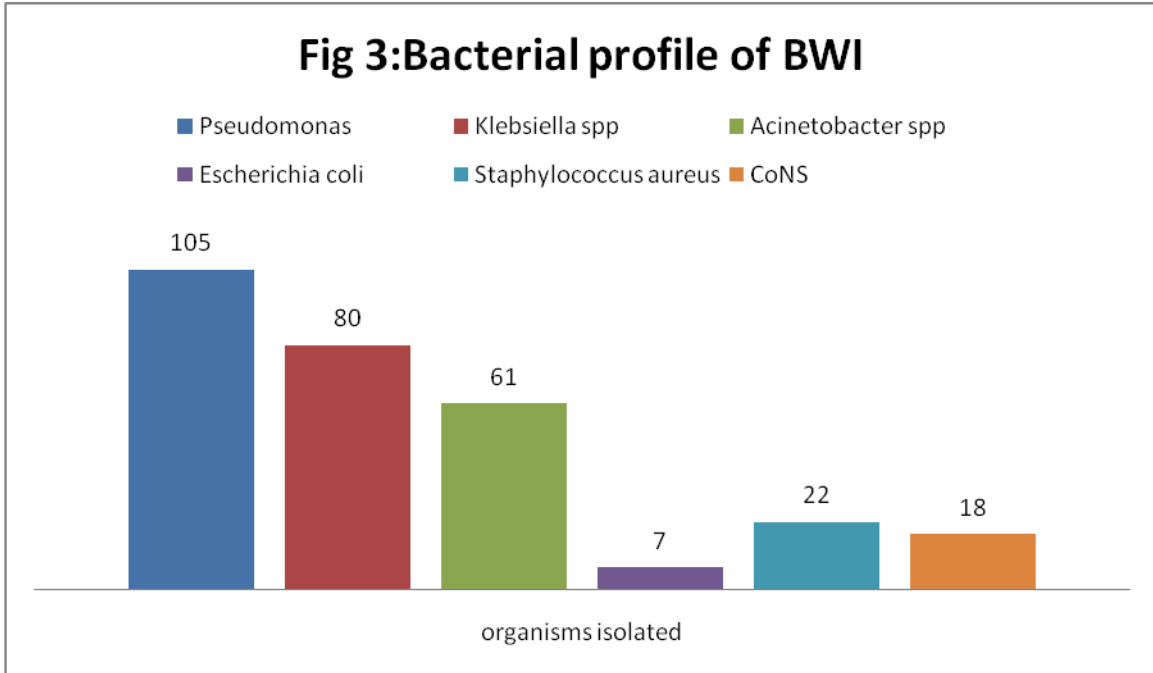


Fig.4 Antibiotic Sensitivity Pattern of Gram Negative Bacilli

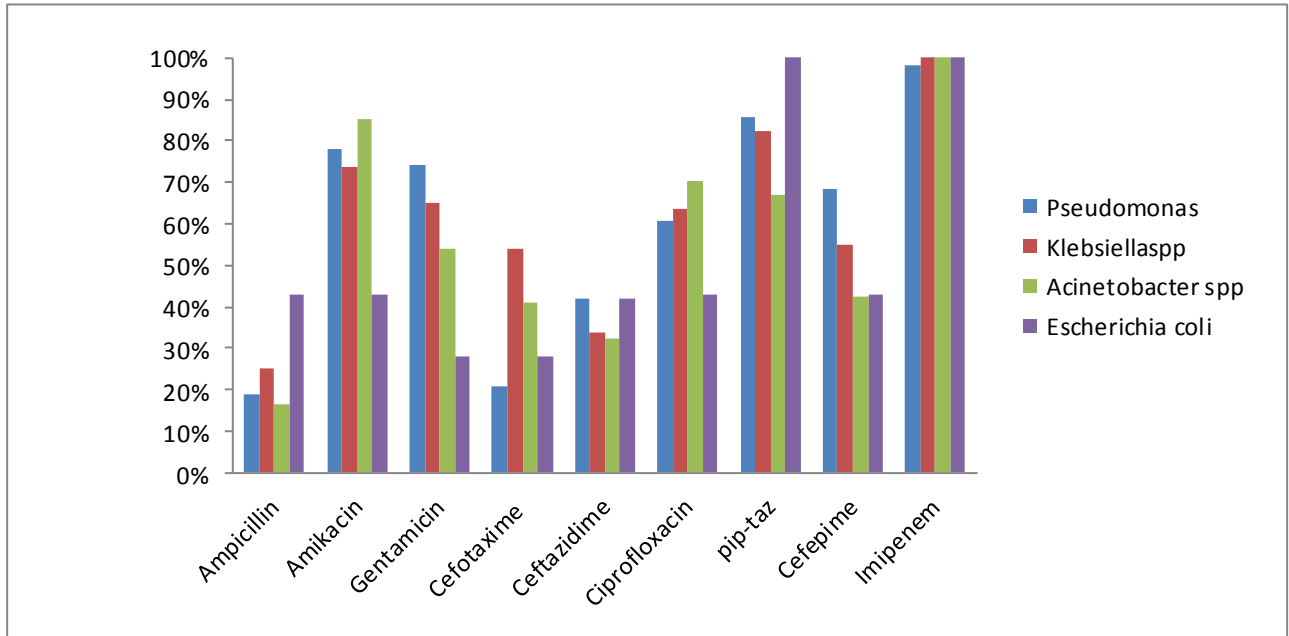
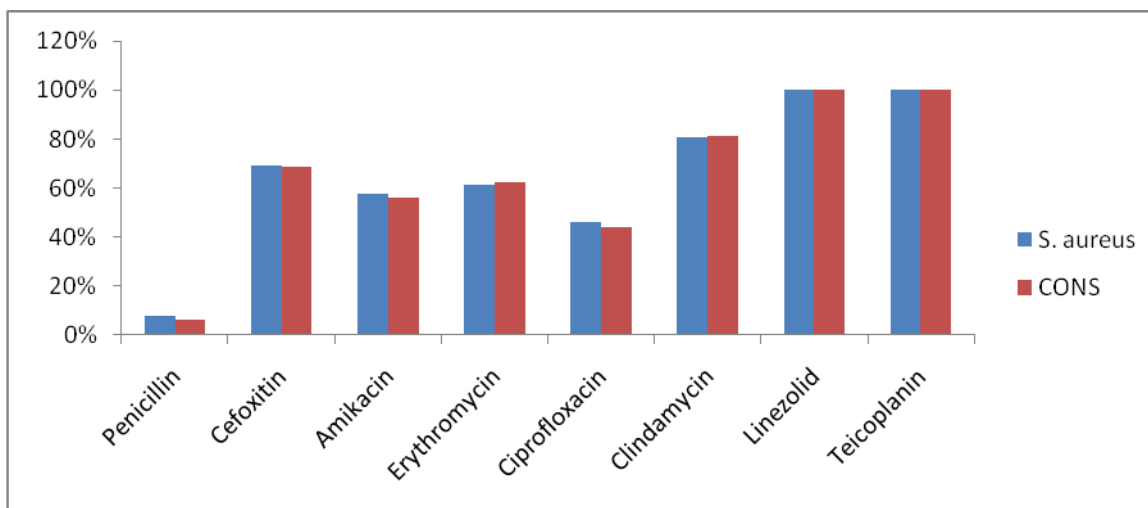


Fig.5 Antibiotic Sensitivity Pattern of Gram Positive Cocci



Even in our study similar time related changes were observed. Gram positive cocci were the most common isolate on Day 1 while gram negative bacilli were isolated more from swabs collected on Day 4 and Day 10 from the same patients (Table 2)

With the above results, it is emphasized that empirical Antibiotics on day one should focus on Gram positive agents as skin normal flora will come into act as a pathogen and from 3rd day onwards on gram negative bacilli.

In our study the predominant organisms isolated (Fig 3) were *Pseudomonas aeruginosa* [35.84%], *Klebsiella* species [27.30%], *Acinetobacter* species [20.13%], *Escherichia coli* [2.38%] *Staphylococcus aureus* [8.87%] and CONS [5.46%].

Our findings concerning the high frequency of *Pseudomonas aeruginosa* (35.84%) (Fig) coincide with many previous reports (Kaur *et al.*, (2006)., Rajput *et al.*, (1998), Mundhada *et al.*, (2015)) where this organism was held responsible for majority of burn wound infections. The most common combination was *Pseudomonas aeruginosa* with *Klebsiella* species or *Acinetobacter* species

or both. This might be probably because of its ability to resist the effect of antibiotics due to its intrinsic and acquired resistant mechanisms.

Acinetobacter species was isolated at a rate of 20.13% which is higher than the rate of isolation reported from previous studies Mundahada *et al.*, and De Macedo *et al.*, This finding is of great concern as it signifies its emerging trend as predominant pathogen in recent past.

The human skin is constantly bombarded by microbes from environment. *Staphylococcus aureus*, normal flora of healthy individual could become pathogenic when host defense is compromised as in burns patients (Chaya kumar *et al.*). Hospital environment in burn units have become reservoir for *S.aureus* which favor them to be a major nosocomial pathogen (Wildemauee *et al.*, 2004).

S.aureus was the predominant pathogen in the pre antibiotic era, still posing threat in burn patients. Isolation rate was 8.87% which is less comparable to the findings reported by Saha *et al.*, (2011) (16%). Among the *Staphylococcus aureus*-36.6% were MRSA (Table 3).

Antibiotic Susceptibility Pattern of gram negative bacteria showed high susceptibility to Imipenem (98% -100%) and Piperazillin Tazobactem (67% -100%), least susceptibility was observed for Cefotaxime (20%-53%), Ceftazidime (32% -42%) and Ampicillin (16% - 42%). According to Chayakumar *et al.*, (2010) and Saxena *et al.*, (2013), high level of resistance was observed for Ceftazidime. Our finding was also similar to these studies. Gram positive organisms were found to be susceptible to Amikacin (57%), Clindamycin(81%), Linezolid (100%), and Teicoplanin (100%) (Fig 4 & Fig 5). Least susceptibility was observed for Penicillin (8%). This is in accordance with the results of Mundahda *et al.*, (2015).

In conclusion, time related changes of bacterial flora have been observed. Based on our findings we emphasize need for every burn institute to determine its specific pattern. It is also crucial to formulate prophylactic and therapeutic strategies of burn institution.

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