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Study of dynamics of microbial flora in burn wound cases: A hospital based descriptive study

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Abstract
Introduction: Burn wounds are the site to bacterial colonization or infection, leading to morbidity and mortality. Early identification of microbial flora provides improved management and better prognosis.
Purpose: The aim of this study was to identify the dynamics of bacteriological profile of burn cases admitted in the burn unit of SMS Medical College Jaipur. A prospective study was done in the bacteriology laboratory of the department of microbiology for 9 months.
Methods: Swabs were collected from the wounds of a total of 396 pus swabs on admission, on 3rd day of admission and on 7th day from 122 burn cases admitted in burn unit, after taking a thorough history and demographic data. The swabs were inoculated in appropriate culture media, and identification was done following the standard procedure.
Results: Among positive cultures(93.18), *Pseudomonas sp.* is the most common gram negative organism from 1st to 7th day, dominating in both GPC & GNB groups and *Staphylococcus aureus* was the common gram positive organism isolated during 1stday (1.32%), All gram positive organism were negligible by end of the 7th day. Pseudomonas with 95.19 % resistant to Ceftazidime may be potential ESBL producer. CPS are 100% resistant to Cefoxitin are MRSA.
Conclusion: The finding of the study will be helpful for identifying the dynamics of common bacteria causing burn wound infection and also to take proper precautions to prevent the emergence of antibiotic-resistant bacteria by formulating an effective antibiotic policy with the usage of combinational drugs in the management of burn wound infections.

Keywords: GNB, GPC, CPS, Burn wounds, Methicillin-resistant *Staphylococcus aureus* (MRSA), ESBL producer

1. Introduction
A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals. Skin injuries due to ultraviolet radiation, radioactivity, electricity or chemicals, as well as respiratory damage resulting from smoke inhalation, are also considered to be burns [1]. India, the second most populous country in the world with over 1.25 billion people, has an estimated annual burn incidence of 6-7 million cases per year [2].
Infection in burn patient is a leading cause of morbidity and mortality and it continues to be the challenging concern; the importance of preventing infection has been recognized in organized burn care centers starting from its inception. These included strict aseptic techniques, use of sterile gloves and dressing materials, wearing masks for dressing changes and special separation of patients, using private rooms [3].
Pseudomonas aeruginosa, Methicilin Resistant *Staphylococcus aureus* (MRSA), *Acinetobacter buamanni, Klebsiella pneumonae, Proteus mirabilis, Citrobacter sp.*, Coagulase negative Staphylococci, *Enterobacter sp.* and *Escherichia coli* were commonly associated with the wounds of burn patients [4, 5, 6, 7]. Antibacterial susceptibility patterns for microorganisms isolated from hospitalized patients are continuously evolving, and this can pose a major challenge for clinicians treating burn wound cases [8, 9].

2. Materials and Methods
Sample Population
The present study was a Laboratory based descriptive type of observational study& conducted in Bacteriology Laboratory of the Department of Microbiology SMS Medical College & Attached Hospital, Jaipur, Rajasthan from April 2017 to December 2018. A total of 392 pus samples collected from the burn patients attending burn unit were included in the study. Pus samples from burn wounds were collected on the day of admission, than on third day and on seventh day or on discharge if earlier than seven days from cases included in the study and were processed for pyogenic aerobic culture and sensitivity. Pus/ swab samples from wounds were collected in sterile universal containers. Pus/ swab samples were processed within two hours of collection and in case of delay, the samples were refrigerated at 4°C for up to six hours.

Sample Processing
All samples collected from different wound sites admitted in the burn unit in SMS Hospital, Jaipur. On day of patient's admission, on 3rd day and on 7th day, pus swabs were taken from burn cases included in the study. Aerobic pyogenic culture was done to find microbes grown on wound surface area. Swabs for aerobic culture were inoculated on Mac Conkey and blood agar as soon as possible and then immersed in Thioglycollate broth. The inoculated plates were incubated at 37°C for 24 hours. Isolates were identified by conventional methods according to the standard laboratory protocol recommended by CLSI including colony morphology, gram staining and biochemical reactions.
All gram negative bacilli were identified to species level by their characteristic appearances on the media, Gram's stain, Oxidase test, Motility and the pattern of the biochemical

reactions as per standard laboratory protocol [10-12].
All gram positive organisms were identified to species level by their characteristic appearances on the media, Gram's stain, Catalase test followed by Coagulase test. In case of Enterococci as isolate, bile esculin disc and 6.5% NaCl (salt) tolerance test was used [10-12]. The antibiotic sensitivity test was performed by modified Kirby Bauer disc diffusion technique with commercially available Hi-Media antibiotic discs according to Central Laboratory Standard Institute (CLSI) guidelines on Mueller Hinton agar plates [10-13]. The antibiotics which were used in our study were based on the standard protocol of the hospital and departmental policies (as per CLSI 2018).

3. Results

The studies revealed a higher number of cases among the age group 21-30(23.8%). Among all cases males (66.39%) outnumbered females (33.61%).(Table-1)

Table 1: Demographic characteristic features of burn patients (N=122 patients)

SN	Characteristics	Total Number (Percentage)
1	Age groups (years)	
	1-10	18 (14.8%)
	11-20	25(20.5%)
	21-30	29(23.8%)
	31-40	14(11.5%)
	42-50	19(15.6%)
	≥51	17(13.9%)
	Mean Age	29.52± 17.28
2	Gender	
	Male	81(66.39%)
	Female	41(33.61%)
	Total	122 (100%)

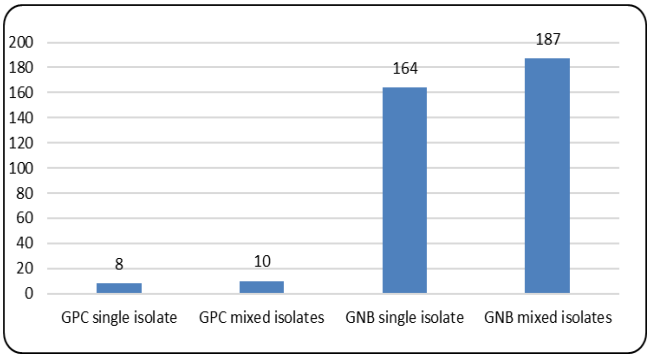

Fig 1: Distribution of GNB and GPC as single and mixed isolates

Gram negative organisms were more common 351(95.12%) than gram positive organisms 18 (4.88%). *Pseudomonas aeruginosa* 50.68% was the most predominant isolate identified throughout the study, followed by *Enterobactor 20*.33%, and E. coli 8.94%. (Table-2)
Among gram positive, *Staphylococcus aureus* was the most common organism 10(2.71%) followed by coagulase negative *Staphylococci* 4 (1.08%) and *Enterococci 4* (1.08%) *Pseudomonas aeruginosa* was most common among gram negative 187(50.68%) followed by *Enterobacter* sp 75(20.33%), *Escherichia coli* 33(8.94%), *Klebsiella pneumoniae* 21 (5.69%), *Acinetobacter sp* 10 (2.71%), *Proteus sp 18* (4.87%), and *Citrobactor* 7 (1.90%).(Table-2)

Table 2: Time-related changes in organism isolation from burn wound and dynamics of their susceptibility

SN	Microorganism	On admission (N=166)			3rd Day (N=152)			7th Day (N=51)		
		Single N (%)	Mixed N (%)	Total N (%)	Single N (%)	Mixed N (%)	Total N (%)	Single N (%)	Mixed N (%)	Total N (%)
1	*Acinetobacter spp.*	4(2.41)	1(0.60)	5(3.10)	1(0.66)	1(0.66)	2(1.32)	2(3.92)	1(1.96)	3(5.88)
2	*Citrobacter spp.*	2(1.20)	2(1.20)	4(2.41)	0	3(1.97)	3(1.97)	0	0	0
3	CoNS	1(0.60)	0	1(0.60)	1(0.66)	1(0.66)	2(1.32)	0	1(1.96)	1(1.96)
4	CPS	2(1.20)	2(1.20)	4(2.41)	2(1.32)	4(2.63)	6(3.95)	0	0	0
5	*E. coli*	7(4.22)	7(4.22)	14(8.44)	3(1.97	12(7.89)	15(9.87)	0	4(7.84)	4(7.84)
6	*Enterobacter aerogenes*	22(13.25)	19(11.45)	41(24.70)	4(2.63)	9(5.92)	13(8.55)	0	7(13.72)	7(13.72)
7	*Enterobacter cloacae*	4(2.41)	2(1.20)	6(3.61)	3(1.97)	3(1.97)	6(3.95)	0	2(3.92)	2(3.92)
8	*Enterococci*	2(1.20)	1(0.60)	3(1.81)	0	1(0.66)	1(0.66)	0	0	0
9	*Klebsiella spp.*	7(4.22)	7(4.22)	14(8.44)	21.32)	3(1.97)	5(3.29)	1(1.96)	1(1.96)	2(3.92)
10	*Proteus mirabilis*	0	5(3.01)	5(3.10)	1(0.66)	5(3.29)	6(3.95)	0	4(7.84)	4(7.84)
11	*Proteus vulgaris*	0	0	0	1(0.66)	1(0.66)	2(1.32)	0	1(1.96)	1(1.96)
12	*Pseudomonas spp.*	32(19.28)	37(22.29)	69(41.57)	57 (37.5)	34(22.37)	91(59.87)	11(21.57)	16(31.37)	27(52.94)
	Total	83(50%)	83(50%)	166(100%)	75 (49.34%)	77 (50.66%)	152(100%)	14 (27.45%)	37 (72.55%)	51(100%)

Spp. = Species; CoNS = Coagulase Negative Staphylococci; CPS = Coagulase Positive Staphylococci

In our study *Staphylococcus aureus* was the common gram-positive organism isolated during 1st day (1.32%), *Pseudomonas aeruginosa* became most common gram negative organism from 1st to 7th day and all gram positive organism were negligible by end of the 7th day. (Table 2)

Pseudomonas with 95.19% resistant to Ceftazidime may be potential ESBL producer. Cefotaxime resistant *Klebsiella* (100%), *Acinetobactor* (100%), *Enterococcus* (98.67%), *E.Coli* (90.91%), *Citrobactor* (85.71%) & *Proteus* (44.44%) are may be potential ESBL producers[26]. (Table-3)

Table 3: Potential ESBL producer among the Gram-Negative Bacteria

Antimicrobial Agent	*Pseudomonas Aeruginosa* N=187		*Esch.coli* N=33		*Klebsiella* N=21		*Proteus* N=18		*Enterobacter* N=75		*Acientobacter* N=10		*Citrobactor* N=7	
	R	%	R	%	R	%	R	%	R	%	R	%	R	%
Cefotaxime	NA	NA	30	90.91	21	100	8	44.44	74	98.67	10	100	6	85.71
Ceftazidime	178	95.19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA = Not Applicable; R = Resistant

In our study CPS are 100% resistant to Cefoxitin are MRSA (Methicilin Resistant *Staphylococcus aureus*[26]. (Table-4)

Table 4: Antibiotic resistance among the Coagulate Positive staphylococcus

Antimicrobial Agent	Coagulate Positive staphylococcus (N=10)	
	R	%
Linexolid	0	0
Vancomycin	0	0

R = Resistant

4. Discussion

In our study 23 specimens out of 392 specimens were sterile, however it is unlikely in burn patients having wounds non- colonized with microorganism. Such findings are also reported in study carried out by Saha S. K *et al*. [27]

Out of total positive culture i.e 369 (94.13%), single and mixed organisms were isolated are 172 and 197 cases respectively. Here we observed not much difference in occurrence of single or more than one organism in these cultures. It seems that wounds having single isolates are less manipulated and might be less colonized with surface bacteria, though in the study conducted by Kulkarani *et al.* [28] the wound having isolates was 27.71% & 13.34% respectively for single and mixed isolates.

Isolation of gram positive organism (*Staphylococcus aureus and Staphylococcus epidermidis*) was from 18 cases (4.8%) while gram negative organism with a number of 351 i.e 95.12% grossly out numbering the gram positives. Such observation is also supported by the study of Richcane A *et al*[32]. In the study of Datta *et al*. [29] this difference in number of gram positive and gram negative is less. Our findings are also in conjunction with many other studies like Khurram *et al*[31] and Forsan *et al*. [30].

Among the gram negative *Pseudomonas sp* is the commonest isolate both as a single and in mixed cultures, i.e 53.27% of all the gram negative isolates. This is a common observation which supported by the studies of Datta *et al*[29], Richcane *et al*, [32] Khurram *et al*, [31] and others. Other gram negative organisms were *Enterobacter spp, E. coli, klebsiella spp, Proteus spp, Acinetobacter* and *citrobacter* in descending order. *Staph. aureus* is commonest gram positive organism involved in burn wounds.

In our study we significantly observed the changing flora of burn wounds from the time of admission till the discharge or up to one week of hospital stay. It is seen from the table No.2 that most patients who had single isolate on admission with time switched to mixed type of isolates which may be due to the hospital environment facilitating the colonisation with crossover of organism among the the admitted patients. This observation is also made by Saha *et al.* [27] in their study.

The studies revealed a higher number of cases among the age group 21-30. Earlier reports also substantiate the present findings [13].

A number of reports from foreign groups indicated the predominance of *Staph. aureus* in burn wound infection [19-20]. On other hand, the reports from Indian subcontinent indicate the predominant of Gram-negative bacilli, especially *Pseudomonas spp., E. coli* and *Klebsiella spp* [20-25]. However, it is to be noted that even in the present study, the presence of *Staph. aureus* was quite significant in gram positive infection. The difference in the major etiology of burn wounds could be attributed to the socio-economic status of the individuals, society and hospital environment.

Pseudomonas with 95.19% resistant to Ceftazedime may be potential ESBL. Cefotaxime resistant *Klebsiella* (100%), *Acinetobactor* (100%), *Enterococcus* (98.67%), *E. Coli* (90.91%), *Citrobactor* (85.71%) & *Proteus* (44.44%) are may be potential ESBL producers[26]. In our study CPS are 100% resistant to Cefoxitin are MRSA (Methicilin Resistant *Staphylococcus aureus* [26].

Limitations

In our study neither fungal isolates were studied, nor was anaerobic workup done due to technical constraints.

5. Conclusion

Percentage of mixed infection increases with duration of stay in burn wards while number of pathogen decreases with duration of stay. Infections are serious problem among burn patients, therefore it is a need for every hospital to have a data on prevalent organisms and which also helps to formulate an effective antibiotic policy with care of GPC or GNB and usage of combinational drugs in the management of burn wound infections. Inadequate antimicrobial therapy for infected patients may lead to higher morbidity and mortality but inappropriate use of reserved antibiotics leads to MDR strains. The present study has revealed the emergence of MDR strains of Gram-negative bacilli and MRSA as the predominant etiological agents in burn wound infections in the hospital environment. Hence continuous microbiological surveillance and careful in vitro testing prior to antibiotic use and strict adherence to hospital antibiotic policy helps in the prevention of emergence of multidrug resistant pathogens like MRSA and potential ESBL producers, prevalence of which are at its highest.

6. References

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