



Original Research Article

Bacteriological Profile of Burns Wound

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ABSTRACT

Introduction - Burns are one of the most common and devastating forms of trauma and a major public health concern in all around the world. Gram-positive bacteria heavily colonize the wounds within 48 hours of the injury. By day 7, the wound is colonized with other microbes Spectrum of bacterial isolates varies with time and geographical area. In addition, the problem of multi-drug resistance in gram-negative bacilli due to ESBL production and in Gram-positive organisms MRSA is becoming a serious threat as the therapeutic options to these organisms are limited.

Materials and methods - The study group consists of 100 patients admitted to burns ward in a tertiary care hospital. Surface wound swab, biopsy, blood and urine samples were collected under aseptic precautions. The samples were inoculated onto blood agar, Mac Conkey agar and the isolates were identified by standard biochemical tests. Antibiotic sensitivity was performed.

Results - Of the 100 patients, 62 were females, 38 males. The most common affected age group was 16-25 years. Flames were most common cause of burns (82%). Klebsiella was the most common isolate followed by Staphylococcus. The most effective antibiotic was cefaperazone + sulbactam.

Conclusion - Burn injuries were more common due to flames where suicidal burns constituted the major proportion.

Swab sampling can be considered a good tool for monitoring burn wounds within the first week of treatment. But for patients who remain in the burns ward for a longer period, biopsy samples are justified as it gives the microbial load in the tissue. Klebsiella spp. was the most common organism isolated

Because of the high incidence of resistant pattern of the organisms, judicious use of antibiotics based on antibiotic susceptibility pattern is recommended.

Key words – Burns, Klebsiella, biopsy

INTRODUCTION

Burns are one of the most common and devastating forms of trauma and a major public health concern in all around the world. ^[1] Burn wounds are especially prone to infection which is a major cause of morbidity and mortality in hospitalised burn

patients. Globally an estimated 195000 deaths occur annually. In India, over 1000000 people are moderately or severely burnt every year. ^[2]

Open and large wounds, make burn patients more susceptible to infection. In particular, immunosuppression caused by

impaired neutrophil function, cellular and humoral immune system can facilitate multiplication and colonization of burn wounds by different microorganisms. [3]

Gram-positive bacteria heavily colonize the wounds within 48 hours of the injury. [4] By day 7, the wound is colonized with other microbes. Invasive infection occurs when these bacteria penetrate viable tissues. [5] Infection of burn wounds may be associated with bacteremia, and interfere with the acceptance of skin grafts. [6] Despite effective topical chemotherapy, the burn wound infection still contributes to 50–75% of mortality. [7]

Spectrum of bacterial isolates varies with time and geographical area. [8] In addition, the problem of multi-drug resistance in gram-negative bacilli due to ESBL production and in Gram-positive organisms MRSA is becoming a serious threat as the therapeutic options to these organisms are limited. [8,9]

This study was conducted to detect multiple drug resistant isolates and help formulate better management of these patients.

MATERIALS AND METHODS

Study was conducted during the period January 2010 to October 2010.

The study group consists of 100 patients admitted to burns ward in Gandhi Hospital, Secunderabad.

Inclusion criteria:

All patients admitted irrespective of age and sex were included. Patients inflicted with burns of <60% of total body surface area were included.

Sample collection:

Surface wound swab: Two swabs were collected from the wound which was clinically deep on 7th and 14th day after the wound was cleansed with sterile normal saline and sterile gauze to remove the

remnants of previous days silver sulfadiazine and the colonizing bacteria.

Biopsy: Tissue biopsy samples were taken from the area where swab was collected with a 3.5mm disposable biopsy punch. The tissue was immediately placed in a sterile container in which 1ml of normal saline was added.

Swab and biopsy samples on 14th day were taken close to the area where the samples were collected on 7th day.

Urine: 5 to 10 ml urine was collected by clean catch mid stream technique.

In case of catheterized patients urine sample was collected by aspirating from an indwelling catheter using 28G needle and syringe after disinfecting the soft rubber connector between the catheter and the collecting tubing and was put into a sterile container.

Blood: Samples were collected under all aseptic precautions. The skin over the vein was cleaned with 70% alcohol and allowed to dry. Then povidone iodine was applied and allowed to dry for one minute. Then blood was collected and skin was cleansed with 70% alcohol. Blood drawn was immediately put into 50ml of brain heart infusion (BHI) broth to allow 1:10 dilution to nullify the bacteriostatic or bactericidal activity of blood and incubated at 37° c for 24hrs.

Patients were regarded as septicaemic who had signs and symptoms of fever or total leukocyte count of >10000/cmm. In these patients two blood samples were taken one hour apart and processed as above.

Processing of samples:

Swab: One swab was inoculated onto 5% sheep blood agar and Macconkey agar and a direct smear was made with another swab. Plates were incubated for 18-24hrs at 37°c.

Biopsy: The tissue along with the normal saline was placed in a sterile mortar and pestle and was homogenized to release the

bacteria trapped in the tissue and 0.01 ml of it was inoculated on 5% sheep blood agar and Macconkey agar and incubated for 18-24hrs at 37°C and the colony count was noted.

Urine: Macroscopic appearance of the urine sample was noted. Wet mount was put up to look for presence of pus cells. Samples were inoculated on 5% sheep blood agar and Macconkey agar and incubated for 18-24hrs at 37°C.

Blood: After 24hrs of incubation, sample was inoculated onto 5% sheep blood agar and Macconkey agar and incubated for 18-24hrs at 37°C and it was reinoculated after 48hrs before it was reported as no bacterial growth.

The organisms isolated were identified by standard biochemical reactions. [10]

The antibiotic sensitivity was performed according to CLSI (Clinical Laboratory Standard Institute) guidelines. [11] The following commercially available antibiotic discs supplied by Himedia were used.

Amikacin-30µg disc, Ceftazidime-30µg disc, Cefotaxime-30µg disc, Ceftriaxone-30µg disc, Cefoperazone + sulbactam-75µg+15µg, Ciprofloxacin -5µg, Piperacillin+ tazobactam 100/10µg, Imipenem-10µg.

All Staphylococcal isolates were detected for Methicillin resistance using Oxacillin 10µg disc. Quality control when performing screening and phenotypic confirmatory tests.

A non ESBL producing organism (*Escherichia coli* ATCC25922) was used as negative control and an ESBL producing organism (*Klebsiella pneumoniae* ATCC700603) was used as positive control.

Detection of extended spectrum beta lactamases

All Gram negative bacteria belonging to Enterobacteriaceae which were

resistant to cefotaxime and/or ceftazidime by Kirby Bauer disc diffusion test were screened and confirmed by disk potentiation using standard control strains.

Confirmatory methods: Disk potentiation test: In this test pair of disks containing cephalosporin with and without clavulanic acid is placed on opposite sides of the same inoculated plate. The test organism is regarded as an ESBL producer if the zone of inhibition around the combination disk is at least 5mm larger than that of the cephalosporin alone.

Detection of AmpC beta lactamases

Organisms found to be resistant to 3rd generation cephalosporin's but not inhibited by clavulanic acid were tested for production of AmpC beta lactamases by AmpC disc diffusion test.

AmpC disc test: A lawn culture of *E. coli* ATCC 25922 was prepared on Muller Hinton agar plate. Sterile blank disks (6 mm) were moistened with sterile saline (20 µl) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C. A flattening or indentation of the cefoxitin inhibition zone in the vicinity of cefoxitin disk was considered as positive and absence of distortion was considered as negative.

The above method was also performed using Tris-EDTA which permeabilises the bacterial cell and releases β-lactamases into the external environment. Filter paper discs containing Tris-EDTA were prepared in-house by applying 20 µl of a 1:1 mixture of saline and 100× Tris-EDTA. The surface of a Mueller-Hinton agar plate was inoculated with a lawn of cefoxitin susceptible *E. coli* ATCC 25922. After incubation, plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or

the absence of a distortion, indicating no significant inactivation of cefoxitin

Detection of metallo beta lactamases

Isolate of *Pseudomonas aeruginosa* resistant to imipenem was included for detection of metallo-beta-lactamase production.

Pseudomonas aeruginosa ATCC27853 was used as negative control.

Imipenem (IMP)-EDTA combined disc test: 0.5M McFarlands standardized inoculum of test organism was inoculated on 90mm Muller-Hinton agar plates. Two imipenem (10µg) discs were placed on the plate and 10µl of 0.5M EDTA solution which was prepared by dissolving 186.1g of disodium EDTA2H2O in 1000ml of distilled water and adjusting it to ph 8.0 by adding NaOH and sterilizing the solution by autoclaving, to obtain a desired concentration of 750µg. The inhibition zones of the imipenem and imipenem-EDTA discs were compared after 16-18hrs of incubation at 35°C. Increase in the inhibition zone with the Imipenem and EDTA disc was ≥7mm than Imipenem alone was considered as MBL-positive.

Imipenem-EDTA double disc synergy test (DDST): 0.5M McFarlands standardized inoculum of test organism was inoculated on 90mm Muller-Hinton agar plates. An imipenem (10µg) disc was placed 20mm from centre to centre from a blank disc containing 10µl of 0.5M EDTA (750µg). Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as positive test.

MBL E-test: The E test strip containing a double sided seven –dilution range of IPM(4to 256µg/ml) and IPM(1to 64µg/ml) in combination with a fixed concentration of EDTA was placed on a 90mm Muller-Hinton agar plates which was inoculated with a 0.5M McFarlands

standardized inoculum of the test organism. MIC ratio of IP (imipenem)/IPI (imipenem-EDTA) of >8 or > 3 log2 dilutions indicates MBL production.

RESULTS

Swabs, biopsy, blood and urine samples were collected from 100 patients admitted to Gandhi Hospital, Secunderabad, between January 2010 to October 2010.

Table 1: Age wise distribution of cases

AGE	No.	%
0-5	2	2
6-10	2	2
11-15	2	2
16-25	40	40
26-35	30	30
36-45	11	11
46-55	7	7
>55	6	6

Table 2: Aetiology of burns

	Nos	%
Flames	82	82
Electrical	12	12
Scalds	6	6
total	100	100

Table 3- Total no. of isolates

Total	Swab		Blood		Urine	
	n=133	%	n=2	%	n=9	%
Klebsiella spp.	40	30.1	2	100	3	33.3
S. aureus	25	18.7	-	-	-	-
CONS	22	16.5	-	-	2	22.2
Acinetobacter spp.	10	7.5	-	-	-	-
Proteus mirabilis	10	7.5	-	-	-	-
Pseudomonas aeruginosa	9	6.7	-	-	-	-
E.coli	7	5.2	-	-	2	22.2
Providentia spp.	6	4.5	-	-	-	-
Citobacter spp	4	3.0	-	-	2	22.2

Out of 100 patients admitted,62 were females and 38 males. Female to male ratio was 1.5:1. of all the age groups, patients in the age group of 16-25yrs were more susceptible. (Table 1)

Flames were found to be the most common cause of burns followed by

electrical burns and scalds due spillage of hot water or oil or milk.(Table 2)

Suicidal burns (56%) by self immolation by pouring kerosene on self were found to be more common than accidental burns (44%).

Burn injuries with %TBSA (Total Body Surface Area) of 31-40% were more common (29%).49% of the wounds were

infected on day 7 while 62% of the wounds were infected on day 14.

Klebsiella spp. was the most common organism isolated among all samples followed by Staphylococcus aureus (table 3)

Most of the organisms were sensitive to cefoperazone+sulbactam (Table4)

Table4: Antibiotic susceptibility pattern of the isolated microorganisms (%)

Organisms	AK	CF	CTR	CTX	CAZ	CFS	OX	ER	VM	PZ	I
Klebsiella.spp (45)	66.6	66.6	66.6	66.6	64.4	84.4	-	-	-	-	-
S.aureus (25)	72	72	60	68	64	80	40	52	100	-	-
CONS (24)	63.6	72.7	59.0	63.6	68.1	81.81	50	50	100	-	-
Acinetobacter spp. (10)	50	60	60	50	50	80	-	-	-	80	100
P.mirabilis (10)	60	50	70	60	60	70	-	-	-	-	-
Pseudo.aeruginosa (9)	44.4	55.5	66.6	66.6	55.5	77.7	-	-	-	88.8	88.8
E.coli (9)	57.6	66.6	100	100	100	100	-	-	-	-	-
Providentia spp. (6)	66.6	66.6	66.6	83.3	83.3	100	-	-	-	-	-
Citrobacter spp. (6)	75	50	100	100	100	100	-	-	-	-	-

Note : AK- Amikacin, CF – Ciprofloxacin, CTR- Ceftriaxone,CTX –Cefotaxime, CAZ- Ceftazidime, CFS-Cefperazone sulbactam, OX – Oxacillin, ER-Erythromycin, VM-Vancomycin,PZ-Piperacillin Tazobactam, I-Imipenem.

60% of the Staphylococcus aureus isolates were resistant to methicillin and 50% of the Coagulase Negative Staphylococcus isolates were resistant to methicillin.

62.5 % of the Klebsiella spp. which were resistant to 3rd generation cephalosporins was found to produce extended spectrum beta lactamases.

37.5% of the Klebsiella spp. which was resistant to 3rd gen. cephalosporins but was not inhibited by clavulanic acid was found to produce AmpC beta lactamases.

11.1% of Pseudomonas aeruginosa were found to be produce metallo beta lactamases.

DISCUSSION

In our study females were found to be more commonly affected than males. This correlated with the studies conducted by Usama B Ghaffar et al and Olive M Liwimbi et al. [12,13] Most commonly affected age group in our study was 16-25 yrs which was in contrast to the study

conducted by Olive M Liwimbi et al and Kehinde A.O et al who found most commonly affected age group to be less than 10 yrs. [13,14] PR Chalise et al study showed most commonly affected age group to be 20-30 yrs. [15]

Burns due to flames were found to be most common in our study which was similar to the study conducted by Olive M Liwimbi et al and Olaitan P B et al. [13,16]

Major % TBSA involved in our study was 31-40% which was in contrast to that found by Usama B Ghaffar et al and Olive M Liwimbi et al who found major %TBSA involved to be up to 25% and 1-5% respectively. [12,13]

In our study the rates of concordance between 7th and 14th day were 83.3 % and 65% respectively which correlated with the study conducted by Ebrahim Salehifar et al who reported that the rates of concordance between biopsy and swab on 7th and 14th day were 87.1% and 66.6% respectively. [17]

Anuradha Rajput et al concluded that wound biopsy was more representative

sample of an infected wound as it was devoid of surface contaminants and more isolates were recovered from biopsies than from wound swab. [18]

In our study 78% of the wounds was culture positive on day 7 while 90.9% of the wounds were culture positive on day 14 with prevalence of wound infection of about 62.2% and prevalence of blood stream infection and urinary tract infections in our study was 19% and 9% respectively. While Alireza Ekrami et al reported that 82.8% were culture positive on day 7 while only 17.2% were culture positive on day 14. They also reported that primary wound infection was most common (72.5%) followed by blood stream infection (18.6%) and urinary tract infections (8.9%). [19]

In our study the most common organism isolated was *Klebsiella* spp. which correlated with that of Shankar Srinivasan et al and Kehinde AO et al. [4,14]

In our study prevalence of methicillin resistant *Staphylococcus aureus* was 60% whereas S. Vidhani et al reported 51.6%. [20] A prevalence rate of 62.5% of ESBL's was found in our study while N.P Singh et al (2003) found prevalence rate of 61%. [21] Prevalence rate of AmpC beta lactamases was found to be 37.5% in our study while S. Singhal et al and Jennifer et al found the prevalence rate to be 16.18% and 31% respectively. [22,23]

In our study 11.1% of the *Pseudomonas aeruginosa* were MBL producers which co-related with that of Navaneeth BV et al who found prevalence of 12%. [24]

CONCLUSION

Females constitute a major proportion of victims of burn injuries. People in the age group of 16-25yrs were more susceptible. Burn injuries were more common due to flames where suicidal burns constituted the major proportion.

Swab sampling can be considered a good tool for monitoring burn wounds within the first week of treatment and could defer the need for invasive biopsy sampling. But for patients who remain in the burns ward for a longer period, biopsy samples are justified as it gives the microbial load in the tissue.

Klebsiella spp. was the most common organism isolated. 62.5 % and 37.5% of the *Klebsiella* spp. isolated were ESBL and AmpC positive. As this constitutes a significant proportion in our set up, it is necessary and useful to perform screening and confirmatory tests for phenotypic detection of those organisms in the routine work.

The prevalence of *Pseudomonas aeruginosa* was less (6.7) and also the prevalence of metallo beta lactamase was low (11.1%).

Because of the high incidence of resistant pattern of the organisms, judicious use of antibiotics based on antibiotic susceptibility pattern is recommended.

As resistance to various antibiotics was found among the isolated organisms new category of antibiotics with different mechanism of action is required.

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