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Research Article

# COMPARATIVE STUDY OF SWAB VERSUS BIOPSY OF BURNS WOUNDS IN A TERTIARY CARE HOSPITAL IN HYDERABAD

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### ABSTRACT

**Introduction:** Burn injury is a major problem in many parts of the world. It has been estimated that 75% of all deaths following burns are related to infection. Thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time, only to be replaced by newly arriving microorganisms. Introduction of new topical agents and systemic antibiotics influence the flora of the wound.

**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:** 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. Burn injuries with %TBSA 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 The concordance rates of swab and biopsy on 7<sup>th</sup> and 14<sup>th</sup> day were 83.3% and 65% respectively. Klebsiella spp. was the most common organism isolated among all samples followed by Staphylococcus aureus.

**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.

**Keywords:** Burns, Swab Sampling, Biopsy, Klebsiella.

### INTRODUCTION

Burn injury is a major problem in many parts of the world. It has been estimated that 75% of all deaths following burns are related to infection. Thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients<sup>1</sup>. The burn wound surface provides a protein rich environment consisting of avascular necrotic tissue (eschar) that provides a favourable niche for the microbial colonization and proliferation. The avascularity of the burn eschar results in impaired migration of host immune cells and restricts delivery of systemically administered antimicrobial agents to the area, while toxic substances released by eschar tissue impair local immune responses<sup>2,3,4,5,6</sup>.

The causative infective microorganisms in any burn facility change with time<sup>6,7</sup>. Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time, only to be replaced by newly arriving microorganisms. Introduction of new topical agents and systemic antibiotics influence the flora of the wound<sup>6,7</sup>. Thus, it is just not sufficient to be aware of the microorganisms that pose a problem for burn patients. To have an in-depth knowledge of the organisms that are predominant in that particular treatment facility during the particular period along with their sensitivity pattern is vital as many septic burn patients need to be treated with antibiotics before the results of microbiological cultures are available. This would be crucial to reduce the overall infection-related morbidity and mortality.

## 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.

An informed consent shall be taken from all cases of clinically suspected burn wound infection patients and the necessary clinical details shall be noted in the proforma prepared for the purpose. Specimen was collected from the depth of the wound with the help of two sterile swabs and transported immediately to the Department of Microbiology.

### Sample collection:

**Surface wound swab:** Two swabs were collected from the wound which was clinically deep on 7<sup>th</sup> and 14<sup>th</sup> 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 14<sup>th</sup> day were taken close to the area where the samples were collected on 7<sup>th</sup> 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<sup>8</sup>.

Antimicrobial susceptibility and resistance mechanisms like ESBL, MRSA etc will be detected by the standard disk diffusion method (Kirby Bauer method) on Mueller Hinton agar as recommended by the Clinical and Laboratory Standards Institute (CLSI)<sup>9</sup>.

The following commercially available antibiotic discs supplied by Himedia were used.

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

All Staphylococcal isolates were detected for Methicillin resistance using Oxacillin 10µg disc.

## RESULTS

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

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).

**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

Flames (82%) were found to be the most common cause of burns followed by electrical burns (12%) and scalds (6%) due spillage of hot water or oil or milk.

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 (Table 2),

**Table 2- Isolation of organisms in swabs**

	7 <sup>th</sup> day n =100	%	14 <sup>th</sup> day n =55	%
<b>Culture positive</b>	78	78	50	90.9
<b>Infected</b>	49	49	31	62

The concordance rates of swab and biopsy on 7<sup>th</sup> and 14<sup>th</sup> day were 83.3% and 65% respectively (Table 3)

**Table 3: Pathogen isolation: swab versus biopsy**

	7 <sup>th</sup> day (n=30)	%	14 <sup>th</sup> day (n=20)	%
Biopsy- & swab -	11	33.3	1	5
Biopsy +& swab-	1	6.6	5	25
Biopsy -& swab +	4	13.3	2	10
Biopsy & swab same	14	46.6	12	60
<b>Concordance between swab and biopsy</b>	83.3%		65%	

Note : Concordance=both samples negative or both samples positive by the same organism

**Table 4: Bacterial load in swab and biopsy**

Colony count	7 <sup>th</sup> day				14 <sup>th</sup> day			
	Swab n=18		Biopsy (n=15)		Swab (n=14)		Biopsy (n=17)	
<10 <sup>3</sup>	6	33.3%	6	54.5%	2	14.2%	2	11.7%
10 <sup>3</sup> -10 <sup>4</sup>	7	38.8%	5	83.5%	4	28.5%	4	23.5%
10 <sup>4</sup> -10 <sup>5</sup>	5	27.7%	4	26.6%	6	42.8%	5	29.4%
>10 <sup>5</sup>	0	0%	0	0%	2	14.2%	6	39.2%

Though 18.3% of the patients were septicaemic on day 7, their blood cultures were negative and of the 23.5% patients who were septicaemic on day 14, only 5.8% of their blood cultures yielded a positive culture .

On day 7, 6.6% of the patients suffered urinary tract infection and on day 14, 14.7% of the patients suffered urinary tract infection . Klebsiella spp. was the most common organism isolated among all samples followed by Staphylococcus aureus (table 5)

**Table 5: 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

**Table 6: Antibiotic susceptibility pattern of the isolated microorganisms (%)**

Organisms	AK	CF	CTR	CTX	CAZ	CFS	OX	ER	VM	PZ	I
<b>Klebsiella.spp (45)</b>	66.6	66.6	66.6	66.6	64.4	84.4	-	-	-	-	-
<b>S.aureus (25)</b>	72	72	60	68	64	80	40	52	100	-	-
<b>CONS (24)</b>	63.6	72.7	59.0	63.6	68.1	81.81	50	50	100	-	-
<b>Acinetobacter spp. (10)</b>	50	60	60	50	50	80	-	-	-	80	100
<b>P.mirabilis (10)</b>	60	50	70	60	60	70	-	-	-	-	-
<b>Pseudo. aeruginosa (9)</b>	44.4	55.5	66.6	66.6	55.5	77.7	-	-	-	88.8	88.8
<b>E.coli (9)</b>	57.6	66.6	100	100	100	100	-	-	-	-	-
<b>Providentia spp. (6)</b>	66.6	66.6		83.3	83.3	100	-	-	-	-	-
<b>Citrobacter spp. (6)</b>	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 3<sup>rd</sup> generation cephalosporins were found to produce extended spectrum beta lactamases. 37.5% of the Klebsiella spp. which were resistant to 3<sup>rd</sup> gen. cephalosporins but were 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<sup>10,11</sup>. 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<sup>11,12</sup>. PR Chalise et al study showed most commonly affected age group to be 20-30 yrs<sup>13</sup>.

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<sup>11,14</sup>.

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<sup>10,11</sup>.

Evaluation and treatment of the burn wound to prevent life-threatening complication such as sepsis are important issues in care of burn patients. The reliability of different sampling methods for the microbial assessment of wound infection is still debated. Theoretically, there should be advantages using the biopsy culture technique compared to surface swab cultures. The bacteria identified within the burned tissue are more relevant to invasive burn wound infection and possible septicaemia, than the bacterial colonization of the wound surface. Additionally, it may be more accurate to administer antibiotics based on tissue culture findings rather than surface cultures in patients who are also septic but with negative blood cultures. Although treating the surface of the burn with topical antimicrobials before swab sampling may change the micro flora on the surface even if it is washed off but the effect is not problematic on the biopsy cultures.

In our study the rates of concordance between 7<sup>th</sup> and 14<sup>th</sup> 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 7<sup>th</sup> and 14<sup>th</sup> day were 87.1% and 66.6% respectively<sup>15</sup>.

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<sup>16</sup>.

In our study 78% of the wounds were 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%)<sup>17</sup>.

In our study the most common organism isolated was *Klebsiella* spp. which correlated with that of Kehinde AO et al and Shankar Srinivasan et al<sup>12,18</sup>.

In our study prevalence of methicillin resistant *Staphylococcus aureus* was 60% whereas S. Vidhani et al reported 51.6%<sup>19</sup>. 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%.<sup>20</sup> 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<sup>21,22</sup>.

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%<sup>23</sup>.

## 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. The prevalence of *Pseudomonas aeruginosa* was less (6.7). 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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