

## Pattern of Microorganisms Isolated from Flame Burn Wounds, and Their Trends of Susceptibility to Antibiotic During Past Three Years

Ehmer Al-Ibran,<sup>1</sup> Sidra Meraj,<sup>2</sup> Mahwish Nasim,<sup>2</sup> Maria Khan<sup>2</sup> and Masood Hussain Rao<sup>3</sup>

### ABSTRACT

**Objective:** To determine the prevalent bacteria causing wound infection and their trend of susceptibility to antibiotics over a period of last three years in flame burn cases. This is an observational descriptive study conducted at the Burns Center, Civil Hospital Karachi.

**Methods:** All available wound swabs culture and sensitivity reports of flame burn victims admitted during the period of January 2009 - December 2011 were included. Wound swab was usually taken from the burnt area that appears clinically deep, with pus or with thick eschar. In laboratory, microbial growth on any of the inoculated plates gram staining, biochemical identification and antibiotic sensitivity by disc diffusion method was done. Statistical analysis was done through SPSS version 17.0 to determine the frequencies, mean  $\pm$  SD and association of these culture and sensitivity of isolated organisms with antibiotics.

**Result:** Wound swab culture and sensitivity reports of 382 flame burnt patients were assessed. No significant relation was found between age, gender and duration of stay of a patient and bacterial colonization. Culture and sensitivity reports showing isolates of Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Proteus as major microorganisms. Pseudomonas Aeruginosa was found to be the most commonly isolated microorganism 36.6% (f=140). It was found to be highly sensitive with Imipenam throughout our period of study whereas Staphylococcus Aureus was found highly sensitive with vancomycin, doxycillin and Amoxicillin+ clavulanic acid (in sequence with their percentage sensitivity in 2011).

**Conclusion:** During the last three years, a change in susceptibility of antibiotic was found. Some of the antibiotics which were 100 sensitive with common bacteria's were posted to resistant during this period. Therefore, repeated analysis of microbes prevalent in Burn Care facility should be carried out in order to determine change in their sensitivity spectrum to commonly prescribed antimicrobials to rule out wrong selection of empirical antibiotics.

**Key words:** Sensitivity, Antibiotics, pattern of antibiotic susceptibility, pattern of microorganisms.

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### INTRODUCTION

Microbial infection is the commonest and the most serious complication of burn injury.<sup>1</sup> it accounts for approximately 75% of all deaths in burn victims.

Although, the burned area is sterile but later on gram positive bacteria, normally residing in hair follicles and sebaceous glands of skin invade exposed underlying structures.<sup>2</sup> Other potential pathogens are acquired by the hospital environment, patient's digestive and respiratory tracts. The severity of patient immune suppression, local wound factors and invasive capacity of organism determine the degree of penetration in to viable tissue. Disseminated infection is likely to occur if sub eschar tissue is invaded. Therefore, burns wound is the most frequent cause of origin of sepsis in these patient.<sup>3</sup> Bacterial isolates from wounds of burn victim are known to vary with time and geographical location in every burn unit.<sup>4</sup>

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1 Burns Center, Civil Hospital & Dow University of Health Sciences, Karachi, Pakistan.

2 Student, Fourth Year MBBS, Dow University of Health Sciences, Karachi, Pakistan.

3 Principal Research Officer, PMRC Research Center, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan.

**Correspondence:** Dr. Ehmer Al-Ibran, Burns Center, Civil Hospital & Dow University of Health Sciences, Karachi, Pakistan.

Email: dr.ehmer@yahoo.com

In clinical and non clinical setting all over the world, bacteria are increasingly becoming resistant to the

conventional antibiotics. Previously, gram positive bacteria, particularly methicillin resistant staphylococcus aureus and vancomycin resistant enterobacter species were the main concern in burns ward. However, multidrug resistant gram negative bacteria due to extended spectrum beta lactamase production pose the major threat for infection and sepsis now days. There are fewer new antibiotics active against gram negative bacteria, although increase in resistance is faster than in gram positive.<sup>5</sup>

In a study,<sup>8</sup> it was reported that prevalence of multidrug resistant *Acinetobacter baumani* is increased. This has also been documented as a rapidly emerging nosocomial pathogen in various countries, including India.<sup>4</sup> However, multiple studies conducted all over the world have reported *Pseudomonas aeruginosa* and staphylococcus aureus to be the most prevalent, followed by *Klebsiella*.

Rational of this study is that we want to determine the prevalent bacteria causing wound infection and their trend of susceptibility to antibiotics in our setup over a period of last three years, which will help in planning drug regimen for burn victims.

## PATIENTS AND METHOD

An observational descriptive study on retrospective data was conducted on wound swab Culture and sensitivity reports of admitted patients of Burns' centre, Civil hospital, Karachi which receives burden of burn patients not only from Karachi but from all over Sindh and Baluchistan. The study was conducted on data of patients admitted from January 2009 to December 2011. All the patients suffering with flam burnt patients of all age groups and both sexes having complete Culture and sensitivity reports were included. However Cases of scald burn, electric burn and chemical burn were excluded because bacteriology and bacterial sensitivity against antibiotics varies with the type of burn. Cases of fire burn victims reported in outpatient department were also excluded.

During dressing, every wound is carefully monitored for signs of infection includes presence of swelling, pus and redness. If signs are positive, wound swab is taken and sent immediately for culture and sensitivity. If any signs of systemic infections such as fever is found, broad spectrum antibiotics are employed as empirical treatment before culture and sensitivity report.

Wound swab is usually taken from the burnt area that appears clinically deep, with pus or with thick eschar. Sampling is done before wound cleansing through sterile cotton swab, wetted with sterile normal saline

which is rubbed on the wound surface. It is inoculated by conventional T- method on blood agar, MacConkey agar and on cooked meat medium. Inoculated plates are incubated at 37°C aerobically overnight. At the end of 24 hours, plates are examined for microbial growth and gram staining is done for bacteria. After 48 hours, cooked meat medium is observed for autolysis and gas production. Sub-culturing is done from the cooked meat medium on blood agar and MacConkey agar. Sub-cultured plates are incubated at 37°C aerobically. Plates are examined for any microbial growth and if found on any of the inoculated plates gram staining, biochemical identification and antibiotic sensitivity by disc diffusion method is done. The data was analyzed through SPSS version 17. in order to determine frequencies, mean ± SD and cross tabs are done for culture and sensitivity of isolated organisms with antibiotics.

## RESULT

Over all Wound swab culture and sensitivity reports of 382 fire burnt patients were assessed in our study. Majority of them were for the year 2009 in which 196 reports were found. The lowest reports were found in 2010 where only 70 reports were found. Majority reports found were belongs to male (53.4%). Mean age of the patients was 28.7 ± 13.3 Years.(Range 69 i.e. 3 - 72 years) Majority of the patients were from the age group of 16-30 years. Most of the patients fall in the group having 11-20% of burned body surface area 110 cases i.e. 28.9%. Most of the patients 142 i.e. 37.2% stayed in the hospital for more than 30 days and maximum stay duration was 278 days.(Table I)

Figure 1. Frequency with percentage of Microorganisms isolated from wound swabs

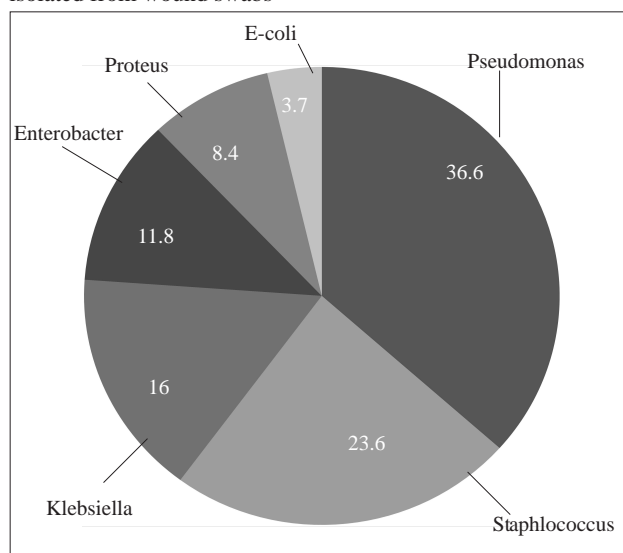


Table I: Demographic Distribution (n=382)

	Frequency	Percentage
<b>Age distribution of the patients</b>		
1. < 15 years	12	03.1
2. 16-30 Years	224	58.7
3. 31-45 Years	77	20.2
4. 46-60 years	48	12.6
5. 60 + years	21	05.4
<b>Gender</b>		
1. Male	204	53.4
2. Female	178	46.6
<b>Year wise Admission status</b>		
1. 2009	196	51.0
2. 2010	70	18.6
3. 2011	116	30.4
<b>Hospital stay</b>		
1. Up to 10 days	126	32.9
2. 11-30 Days	114	29.9
3. More than 30 days	142	37.2
<b>Body surface area</b>		
1. Up to 10%	042	10.9
2. 11-20 %	110	28.8
3. 21-30%	084	22.1
4. 31-40%	064	16.7
5. More than 40 %	082	21.5

*Pseudomonas Aeruginosa* was found to be the most commonly isolated microorganism which was found in 140 cases (36.6%). Other isolated organisms were *Staphylococcus*, and *Enterobacter*. (Table. 2a and 2b)

Stains of *E.coli* were isolated only during the year 2011. No sample was found having this bacterium during 2009 and 2010.

Figure 2: Antibiotics susceptibility trends for *Pseudomonas Aeruginosa*

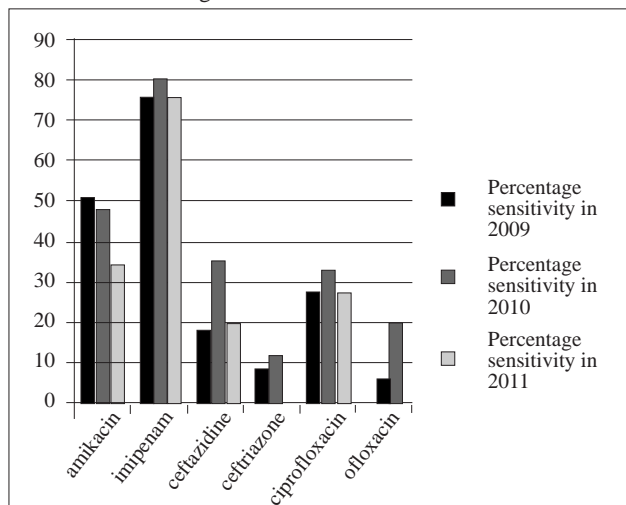
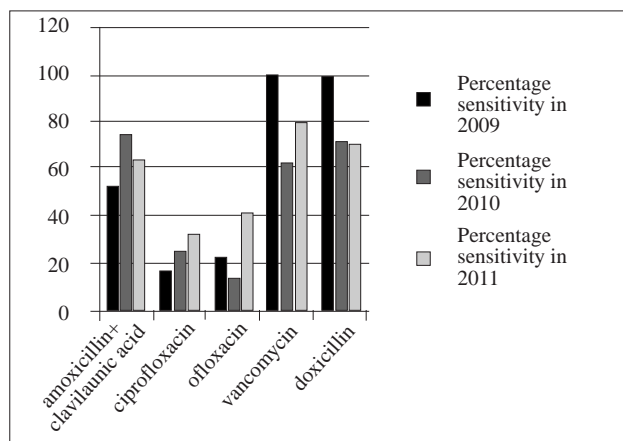


Table 2: Culture and sensitivity pattern of isolated organisms with antibiotics

Antibiotics	% sensitivity in 2009	% sensitivity in 2010	% sensitivity in 2011
<b>1. STAPHYLOCOCCUS</b>			
Amoxicillin+ clavilaunic acid.	52.7	75	64.1
Ciprofloxacin	17.07	25	32.4
Ofloxacin	22.5	14.2	41.6
Vancomycin	100	62.5	79.48
Doxicillin	100	71.4	71.05
<b>2. PSEUDOMONAS</b>			
Amikacin	50	47.82	34.48
Imipenam	75	80	75
Ceftazidine	18.18	35	20.6
Ceftriaxone	8.86	12.5	-
Cefotaxime	5.08	14.28	-
Ciprofloxacin	27.16	33	27.5
Ofloxacin	6.55	20	0
<b>3. KLEBSIELLA</b>			
Ceftazidine	100	11	33
ceftriaxone	100	20	12
cefotaxime	0	20	16.66
ciprofloxacin	100	60	28.57
Ofloxacin	100	70	54.16
<b>4. ENTROBACTER</b>			
Ceftazidine	18.18	100	No sample found in 2011
ceftriaxone	28.57	100	
cefotaxime	12.5	50	
ciprofloxacin	92.3	50	
Ofloxacin	90	100	
<b>5. PROTEUS</b>			
ceftriaxone	16.6	0	50
cefotaxime	12.5	0	-
ciprofloxacin	71.4	100	40
Ofloxacin	87.5	100	40
<b>6. E.COLI</b>			
Ceftriaxone	No sample of E.coli was found in 2009 and 2010		14.28
ceftazidine			14.28
cefotaxime			7
ciprofloxacin			30.7
Ofloxacin			21.42

Figure 3: shows antibiotics susceptibility trends for *Staphylococcus Aureus*



*Pseudomonas Aeruginosa* isolated from wound swab was found to be highly sensitive with Imipenam throughout our period of study. *Staphylococcus Aureus* was found highly sensitive with vancomycin, doxicillin and Amoxicillin+ clauvilaunic acid (in sequence with their percentage sensitivity in 2011). (Figure 3, 4, and Table 5).

The results were also analyzed for assessment of any association. However No significant relation was found between age, gender and duration of hospital stay of a patient and bacterial colonization.

## DISCUSSION

Delay in wound healing and deep scar formation has been found to be associated with higher mortality rates in studies because of sepsis.<sup>6</sup> Mortality rate in fire burn victims in our setting has been reported to be 18.9% due to infection.<sup>7</sup> In addition, Oncul from Turkey calculated the nosocomial infection rate of 18.2 per1000 patients in their hospital.<sup>8</sup> It is thus essential to know the prevalent microorganisms and their antibiotic susceptibility in an effort to prevent sepsis in infected fire burn victims. Various researchers have reported limited use of prophylactic systemic antibiotics in non septicemia fire burn victims<sup>9</sup> where general hygiene, high humidity and deep burn over 30% is present.<sup>22</sup>

We investigated three hundred eighty two (382) wound swab culture and sensitivity reports of fire burn victims admitted during three (3) year`s duration (2009-2011) at Burns Center and included six (6) most common bacteria isolated thereof; namely *Pseudomonas Aereginosa* (36.6%), *Staphylococcus Aureus* (23.6%), *Klebseilla* (16%), *Enterobacter* (11.8%), *Proteus* (8.4%) and *E. Coli* (3.7%). Most of the swabs revealed single microorganisms. Mean age was  $28.7 \pm 13.2$  years and type of bacteria isolated showed statistically insignificant correlation with age variable. Age group of 16- 30 years including both genders, was most common (58.7%). The incidence of burn injury among males and females patients was comparable (male to female ratio: 1.4:1), this finding has also been reported previously in other studies from our region.<sup>2,7</sup> Mean %TBSA was 28% in both males and females.

Studies have described epidemiology of burns, microorganisms from wound isolates and their antibiogram on national level.<sup>2,5,7</sup> in other Asian countries,<sup>3,9,10,18,20,23</sup> and fewer studies belong to the developed/developing countries.<sup>6,8,11,19</sup> Recent studies from our setting reported the most common infective agent from all type of infected samples like blood, wound swab and urine to be *staphylococcus aureus* during the first week of injury.<sup>7,13-15</sup> This is contrary to our finding which reports *Pseudomonas aeruginosa*

to be the most common bacteria isolated from the wound swabs; that might be due to the fact that we took the swabs after 3 to 7 days of hospitalization. Ahmed et al.<sup>2</sup> from our country and few others<sup>2,3,11-16</sup> also reported most common isolate to be *Pseudomonas aeruginosa* after the 6<sup>th</sup> day. Studies reporting gram positive bacteria like *staphylococcus aureus* as the most prevalent bacteria in burn wounds are also present<sup>6</sup> whereas Shankar S. et al, in their 13 year`s study found *Klebseilla* as the most consistent microorganism.<sup>10</sup>

Burn tissue is initially sterile, due to impact thermal injury, however afterwards gram positive bacteria residing on skin and its appendages colonize the exposed subcutaneous structures.<sup>18</sup> Thus during early 2-7 days; most of the researchers have found the gram positive microbes that are normal residents of human skin; such as *staphylococcus aureus* and *streptococci*. Later on normal flora of gastrointestinal and urinary tract is found to infect the wound such as *E. Coli*, *Klebseilla* and *Proteus* species.<sup>6</sup>

Methicillin resistance *staphylococcus aureus* (MRSA) a gram positive bacteria that is known to show high resistance to most of the currently available antibiotics and ESBL producing *Pseudomonas aeruginosa* also have shown resistance against anti gram negative biotic.

We currently have limited options available for multidrug resistant strains in view of ever increasing antimicrobial resistance.<sup>6</sup> In this study *Pseudomonas A.* was most sensitive (76.6%) to Imipenem throughout the three years` duration (also consistent with other reports<sup>6,11,12</sup> whereas sensitivity to Amikacin decreased gradually during this time; on average it showed 44% sensitivity; Cefazidime, Ceftriaxone, Cefotaxime and Ciprofloxacin showed on average < 30%. Bayat et al from Iran have declared 100% resistance of *pseudomonas* in their setting to Imipenem.<sup>14</sup> Other researchers have also reported total resistance (100%) to Gentamycin FOR *Pseudomonas*<sup>9,12,14</sup> thought it was not checked continually in our study.

Identification of *staphylococcus* species as having ability to develop changes in sensitivity to antimicrobial<sup>14</sup> calls for extra insight into use of broad spectrum antibiotics against this microbe. It has been investigated for long time and results have shown various mutated transcription factors and other genetic alteration. In our study 25 isolates consisted of MRSA (Multi Resistance Staph Aureus), others have reported much higher prevalence.<sup>6</sup> *Staphylococcus aureus* was most sensitive to (80%)Vancomycin and (80%)Doxycycline consistently over 3 years similar to other studies, followed by Amoxiclav (64%); some researchers reported 90% resistance to Penicillins.<sup>6</sup>

Most of the organisms responsible for severe burn wound infection are gram negative bacteria,<sup>2</sup> and these also possess greater ability to invade deeper and cause sepsis.<sup>12</sup> Multi drug resistant gram negative bacilli have been reported by many researchers which is alarming<sup>3,9,12</sup> for burn victim care givers. However Ciprofloxacin and Ofloxacin comparatively showed least resistance to gram negative bacilli in ours as well as other reports.<sup>6</sup>

To conclude, repeated analysis of microbes prevalent in Burn Care facility should be carried out in order to determine change in their sensitivity spectrum to commonly prescribed antimicrobials; this will rule out wrong selection of empirical antibiotics.<sup>2</sup> Further, in vitro studies and strict adherence to hospital antibiotic policy and justified use of broad spectrum systemic antibiotics should be practiced.<sup>3</sup> Overall burns wound management should be based on surgical debridement of wounds, early grafting and topical antibiotics and invasive infections should be monitored keenly through clinical parameters.<sup>9</sup>

## CONCLUSION

During the last three years, a change in susceptibility of antibiotic was found. Some of the antibiotics which were 100 sensitive with common bacteria's were posted to resistant during this period. Therefore, repeated analysis of microbes prevalent in Burn Care facility should be carried out in order to determine change in their sensitivity spectrum to commonly prescribed antimicrobials to rule out wrong selection of empirical antibiotics.

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