

Spectrum of Bacterial Pathogens Isolated from Burn Wound Patients

Sadia Ikram*, Niveen Asher, Bilal Farooq*, Aksa Rehman***, Shagufta Hussain**, Sohaila Mushtaq*

Author's Affiliation

*Azra Naheed Medical College, Lahore

**Usra Medical College, Islamabad

***CMH Medical College, Lahore

Author's Contribution

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Address of Correspondence

Dr. Sadia Ikram

Email: dr_sadia.sajid@hotmail.com

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ABSTRACT

Objectives: To find out the spectrum of bacterial pathogens isolated from pus samples of infected burn sites and to come across the sample collection according to post burn day and distribution of Gram positive and negative isolates in samples in relation to time of collection.

Methodology: It is a prospective, non-randomized, descriptive study conducted at Microbiology laboratory, Pathology department and burn care center (BCC), Pakistan Institute of Medical Sciences Islamabad for 4 months, from 2nd April to 3rd August 2013. One hundred and ten clinical isolates from 68 patients were collected. Sample collection from referred patients was done at the time of admission and from admitted patients was done during changing their dressings. The sample collected was immediately transferred to the Pathology laboratory and submitted for Culturing. The samples were inoculated on Blood agar and MacConkey agar (Oxoid USA) and incubated aerobically at 35+ 2 0C for 18 -48 hours. After incubation the pathogens were identified with the help of colonial Morphology, gram stain reaction, biochemical tests, and API 20E.

Results: Out of 68 samples 110 burn wound pathogens were isolated. 47% of the samples yielded single etiological agent whereas the rest of 53 % had polymicrobial etiology. Gram negatives were in majority 81.82% and Gram positives were 18.18 %. The samples in which only one isolate was obtained were 32. Amongst them *P. aeruginosa* was isolated in (65.6%) of samples. In 31 samples two isolates were obtained. In such samples coexistence of *P. aeruginosa* and *K. pneumoniae* was most prevalent i.e. in 10 (32.25%). There were only five samples in which more than two isolates were obtained. Amongst Gram negatives were the predominant bacterial pathogens out of them *P. aeruginosa* were 53 %. MRSA made the major bulk of Gram positives that is 65 %, the rest 35% were *Staph aureus* MSSA. Gram positives were isolated more in the first week samples i.e. 30% as compared to the subsequent weeks.

Conclusion: It was concluded that more than 80 % of bacterial isolates of burn wound infections were Gram negative and less than 20% were Gram positive i.e. *Staphylococcus aureus*.

Key words: MRSA, *P. aeruginosa*, *Staphylococcus aureus*, Gram positive.

Introduction

Burn is damage to skin or other human tissue caused by heat.¹ The burn wounds are protein rich, full of moisture allowing bacteria to reside and multiply and cause infection freely.² Burn patients are at an increase risk of developing local and systemic infections which is the most common cause of death after burn injury.² Bacterial infection of burn wound not only increase the degree of wound invasiveness but also delay wound healing, it worsens the patient's condition and prolongs hospital stay.³

The foremost causative factor of burn globally is fire, constituting 44% (American Burn Association Repository Report 2011). According to centre of fire statistics, the average number of fires worldwide is 7-8 million per year resulting 195000 deaths in a year.⁴ Majority of burn incidence i.e. about 90 % occurs in moderate and low income countries (WHO 2004). 1.3/ 100,000 population as compared to high income countries where it is 0.14 per 100,000 populations.

Southeast Asia is a marked region for burning women as a routine part of domestic violence, being male dominated and lacking legislation it represents 25 % of burns globally in which 70% are only females of South East Asia.⁴ In Pakistan, an estimated 300 women are burned to death each year by their husband's families and these grave incidents are sometimes disguised as accidents, such as an 'exploding stove' (Progressive Women's Association quoting BBC NEWS 1999).

Globally the burn wound pathogens are more or less similar. Microorganisms routinely isolated from burn wounds include aerobic organisms like *Staphylococcus aureus*, *Streptococcus pyogenes*, *E.coli*, *Klebsiella Spp*, *Proteus* etc. Anaerobic organisms like *Bacteroides fragilis*, *Peptostreptococcus*, *Propionibacterium Spp*, *Fusobacterium Spp* and fungi like *Aspergillus niger*, *Candida Spp* and Zygomycetes.⁵

Pseudomonas aeruginosa has emerged as a predominant member of the burn wound flora and in the absence of topical therapy is cultured from the burn injuries of 70% patients by the third week.⁶ Many studies support the fact that due to patho-physiological reasons the bacterial pathogens isolated in the first week after second, third degree burn injury are Gram positive like *Staphylococci*. In case infection develops during second to third week the predominant isolates are either *Pseudomonas* or members of *Enterobacteriaceae*. Beyond this time period, fourth week onwards, polymicrobial infections develop in burn wounds being a blend of *Pseudomonas aeruginosa*, members of *Enterobacteriaceae*, *Acinetobacter Staphylococci*, *Streptococci* and sometimes *fungi* like *candida spp*, in any combination.⁷

Due to gravity of the outcomes in burn patients' studies have been carried out around the world in an attempt to isolate offending bacterial pathogens. As it is a well known fact that there are regional variations within the same country not only in terms of the pathogens responsible for infection. Hence taking the opportunity of having a state of the art burn care center with a well equipped set up, a large catchment area and management strategies quite in accordance with International standards a study was conducted focusing on finding out the spectrum of infecting organisms in burn wounds.

Methodology

It is a prospective, non-randomized, descriptive study conducted at Microbiology laboratory, Pathology department and burn care center (BCC), Pakistan Institute of Medical Sciences Islamabad for 4 months, from 2nd April to 3rd August 2012. Inclusion criteria: Pus samples from infected burn wounds, received from burn care center PIMS, with no age or gender

restriction. The samples were collected by doctor in burn care center.

Exclusion criteria: Only one sample from each patient was included and any duplicate sample from the same patient was excluded from the study. Information regarding name, age, gender, sampling date, date of burn, cause of burn, degree of burn, date of admission, referral status, was obtained and filled in the prepared research Performa.

110 clinical isolates from 68 patients were collected. Sample collection from referred patients was done at the time of admission and from admitted patients was done during changing their dressings. The sample collected was immediately transferred to the Pathology laboratory and submitted for Culturing. The samples were inoculated on Blood agar and MacConkey agar (Oxoid USA) and incubated aerobically at 35+ 2 OC for 18-48 hours. After incubation the pathogens were identified with the help of colonial Morphology, gram stain reaction, biochemical tests i.e. Catalase, coagulase and DNase test (for Staphylococci), Oxidase, Urease, and API 20E. The results of API was compiled by interpreting the positive results seven digits numerical profile was determined which was looked up for interpretation in an index called Analytical profile Index. Quality control: *S aureus* ATCC 25923 was run as positive control and *S faecalis* (known negative) was taken as negative control. *E.coli* ATCC 25922 was used as a control strain in every Gram negative organism other than *Pseudomonas*. *P aeruginosa* ATCC 27853 was used as a control strain for every *Pseudomonas*. Data analysis was performed SPSS version 16

Results

Out of 68 samples 110 burn wound pathogens were isolated. 47% of the samples yielded single etiological agent whereas the rest of 53 % had polymicrobial etiology. Within the polymicrobial samples majority had two isolates and only five samples had more than two isolates. Gram negatives were in majority 81.82% and Gram positives were 18.18%. The samples in which only one isolate was obtained were 32. Amongst them *P. aeruginosa* was isolated in 21(65.6%) of samples, followed by *MRSA* 5 (15.6%), and then *K. pneumoniae*, and *E coli* 3(9.3%) each. (Table I)

Table I: Percentage Break down of single clinical isolates from burn wound pus samples (n=32)

Isolate	n (%)
<i>P. aeruginosa</i>	21(65.6%)
Methicillin Resistant Staph aureus (MRSA)	5 (15.6%)
<i>K. pneumoniae</i>	3(9.3%)
<i>E coli</i>	3(9.3%)

In 31 samples two isolates were obtained. In such samples, coexistence of *P. aeruginosa* and *K. pneumoniae* was most prevalent i.e. in 10 (32.25%). In another 48.3% of the samples, *P. aeruginosa* was isolated with *E. coli*, *P. mirabilis*, *MRSA*, or *Staph aureus MSSA*, in percentages varying from (10%-13%). In another 25% *K. pneumoniae* was isolated along with *P. mirabilis*, *MRSA*, *E. coli* and *Staph aureus*. In one sample both *Staph aureus MSSA* and *MRSA* were isolated

There were only five samples in which more than two isolates were obtained. Four samples had three organisms and only one had four isolates. All these organisms as mentioned above were found in different combinations.

Apart from the distribution of isolates in samples, when total isolates obtained were analyzed it was found that out of the total 110 isolates, *Pseudomonas aeruginosa* was 43 % and was the main pathogen responsible for burn wound infections. Next organism contributing as pathogen was *K. pneumoniae* ie 21%, followed by *Staph aureus* 18 % in which two others were *MRSA*, Remaining 17% were from *E. coli* and *Proteaceae* family. Overall as already mentioned Gram negative organisms were 81.82%.

As already mentioned Gram negatives were the predominant bacterial pathogens out of them *P. aeruginosa* were 53 % followed by *K. pneumoniae* which were 25.5%. The rest of gram negatives *E. coli*, *P. mirabilis*, *P. stuartii* were quite less in number and made the remaining 22% of Gram negative bacterial clinical isolates. (Figure 1)

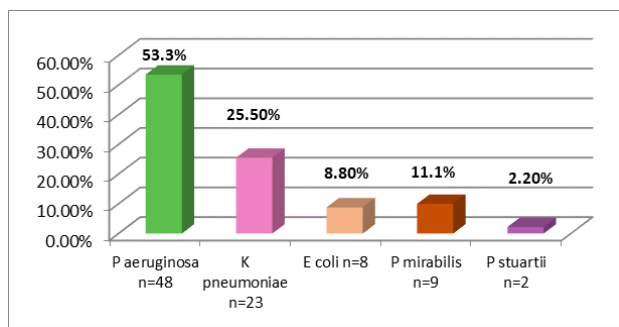


Figure 1: Percentage distribution of Gram negative isolates. (n=90)

MRSA made the major bulk of Gram positives that is 65 %, the rest 35% were *Staph aureus MSSA*. No other Gram positives were isolated.

Data was analyzed to find out the distribution of samples collected in relationship to the age of burn wound. 20 20.5% samples were collected in the first week of burning, 49 42.6% were second to third week old burn, and the rest of 41 (36.7 %) samples were from third to six weeks old burns.

Predominantly Gram negatives were obtained in each week of samples collected, Gram positives were isolated more in the first week samples i.e. 6 30% as compared to the subsequent weeks (Figure 2).

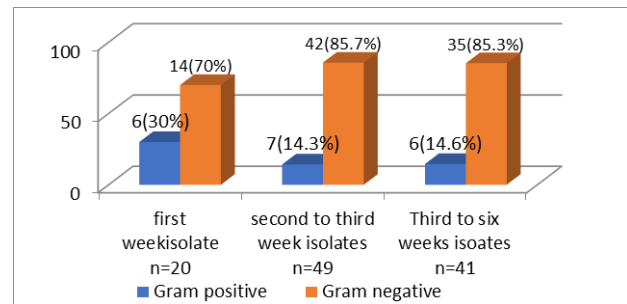


Figure 4. Distribution of Gram positive & negative isolates in samples in relation to time of collection

Discussion

In our study polymicrobial growth occurred in 52.7 % of total samples while 47.3% had single isolates. In a similar study from Nishtar hospital Multan, polymicrobial growth occurred in 34.64% of cases in the second to third week mainly⁸, similarly 37.5% polymicrobial was reported in a study⁹. Poly microbial growth was slightly more in our study probably because most of the samples received were from patients in second to third weeks and more; as advancing burn age is associated with more polymicrobial growth⁷.

In the present study predominant isolates were Gram negative (81.82%) versus Gram positive organisms (18.18%). It was consistent with findings of another study in India where Gram negatives were 80.71% and Gram positives were 19.29 % .⁹

The predominant isolate in the present study was *Pseudomonas aeruginosa* constituting 43 % of the total isolates and 53% of Gram negative isolates it was followed by *Klebsiella pneumoniae* which was (21%) and *Staphylococcus aureus* which was (18.18%), within the *Staphylococci* *MRSA* was predominant (65% of *Staphylococci* and 11.8% of the total). Numerous studies have reported that *P. aeruginosa* is a predominant isolate from infected burn wounds mainly because protein rich granulation tissue in burn wound helps the organism to flourish .Shahzad, N et al⁸ reported 54% *P. aeruginosa* out of the total burn isolates and Rajput A et al⁹ in 2006 reported *Pseudomonas* as 55% of the total burn isolates. Sabzghabee A et al 2012¹⁰ reported 47.3% *Pseudomonas*, 23% *Klebsiella* and *Staph aureus* 19% respectively.

Rani et al¹¹ who studied burn wound bacterial isolate in India reported *Staphylococcus aureus* as the most frequently isolated burn wound pathogen (24%), followed by *Klebsiella*

pneumoniae(20.8%) and *Pseudomonas aeruginosa* only (19.8%). Ahmad M et al in 2006 reported¹²23% *Klebsiella* and 11.8% *Methicillin resistant Staph aureus*. Sirinivas et al¹³ from India reported *Klebsiella* as most frequently isolated pathogen.

In contrast to various studies from India, Turkey and Singapore,¹⁴ various studies^{15,16} which reports *Acinetobacter* isolated from burn wounds, no *Acinetobacter* spp was isolated from the present study.

In addition to major pathogens in the present study 7.2% *E.coli*, 8% *Proteus mirabilis* were also isolated. Consistently Rajput et al reported 2.85% *E coli* and 4.29 % *Proteus mirabilis*

Detail of post burn day sampling revealed that the predominant bacterial population in the first week and thereafter was of Gram negative isolates. In the first week samples Gram positive isolates mainly *Staphylococcus aureus* were (30%) and their number decreased to (14.2%) in the second and third week. This fact coordinated with a study carried out few years back in PIMS hospital. In which *Staphylococcus aureus* was cultured from (24.4%) of first week samples while decreasing to (18.2%) in second to third week sample.¹²

Conclusion

It is concluded at the end of the study that Most common bacterial isolates of burn wound infections were Gram negative and less than 20% were Gram positive. i.e. *Staphylococcus aureus*.

Ratio of the isolation of Gram positives was more in the first week and decreased gradually from second to third week. Samples with single isolates or poly microbial isolation were almost equal in number. *Pseudomonas aeruginosa* is the predominant bacterial isolate from the burn wound. *Klebsiella pneumonia* was the next most frequently isolated pathogen, followed by *Gram positive* isolates, most common of which were *Staph aureus*.

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