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Research Paper

A Study of Bacterial Profile and Antibiotics Resistance Pattern of Various Clinical Isolates in Wound Swabs of Hospitalised Burn Patients at Tertiary Care Hospital

Medical Science

KEYWORDS : Bacterial profiling, Wound Swab, Antimicrobial sensitivity

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ABSTRACT

Introduction: Burn injuries are common throughout the world, especially in developing and underdeveloped countries. The survival rate for burn patients has substantially improved in the past several decades due to advancement in medical care and centers specialized in burn care; however due to the increased survival rates, infections are becoming a leading cause of mortality rather than hypovolaemia and shock. Currently 75% of all deaths are due to infectious complications such as sepsis and bacteremia 1

Aim & objectives: This study was conducted for isolation, identification, determination of frequency, spectrum and antibiotic sensitivity pattern of aerobic bacterial isolates from the wound swab samples of burn patients.

Material Method: Study was carried out in Department of Microbiology, over a period of one year, from May 2014 to May 2015. A total of 100 patients of all age groups and both sexes admitted in our Burn Care Unit, from J.L.N Hospital, were selected for this study.

Result and Observation: From 100 patients, a total of 133 samples were taken and 156 organisms were isolated. The overall isolation rate was 87.96%. Solitary isolates (58%) were seen more commonly than multiple isolates (30%). Overall, Gram negative organisms (66.66%) were more common than Gram positive organisms (33.33%). Pseudomonas (P. aeruginosa and Pseudomonas species) (42.94%) was the most common isolate followed by Staphylococcus aureus (19.23%), Klebsiella pneumoniae (14.74%), Coagulase Negative Staphylococcus (14.10%), Escherichia coli (5.12%), Proteus mirabilis (2.04%), Citrobacter freundii (1.28%). Imipenem (100%) was the most effective drug against the Pseudomonas aeruginosa and other Pseudomonas species. Imipenem (100%) was also the most effective drug against the other Gram Negative Organisms. It was seen that vancomycin and linezolid (100%) were the most effective drugs for the Gram positive organisms. Incidences of Methicillin Resistant Staphylococcus aureus were 37% incidences of Methicillin Resistant CONS were 18.18%.

Conclusion: The routine microbiological surveillance and adherence to restrictive antibiotic policy may be useful in reducing infection related mortality and morbidity in burn patients. Our results may be of help in providing useful information regarding formulation of effective guidelines for therapy, thus improving overall infection related morbidity and mortality.

INTRODUCTION

Extensive burns contribute to local and systemic immune-suppression. Also the burn wound surface provides a favourable niche for microbial colonization and proliferation due to destruction of skin surface, while the avascularity of the eschar causes decreased immunity due to impaired migration of host cell, which in turn restricts the delivery of antimicrobial agents and releases toxic substances that weaken host immune response[2].

The risk of infection is directly proportional to the extent of injury and continues to be the predominant determinant for outcome in burn patients[3]. Depth of the injury and age of the patients are the main risk factors in burn wound infections. The colonizing microorganism and its invasive potential also influence the risk of infection[4].

In burn wound patients organisms responsible for infections may be endogenous or exogenous which can change over the time in the individual patient[5].

Typically, the burn wound is sterile immediately after thermal injury, but after 48 hours the wound is colonized with skin commensals. After one week or so, the wounds become colonized with organisms from endogenous or exogenous sources such as the host's gastrointestinal or respiratory tracts or from the hospital environment[1]. This uncontrolled colonization may lead to invasion with systemic complications and death[6].

The most commonly recovered pathogens depend on the site of burn wounds and reflect the hospital's nosocomial pathogens. In the pre antibiotic era, *Streptococcus pyogenes* was the predominant pathogen but now it has been replaced with *Staphylococcus aureus*, *Pseudomonas aeruginosa, Proteus mirabilis* & *Klebsiella pneumoniae*[1].

To suggest the preventive measures, an insight into the pattern of burn injury is necessary[7] and therefore the present study was done to acquire a clear picture of the bacteriological spectrum of infection of burns in our burn care unit and their anti-microbial sensitivity pattern for proper appraisal of the problem and institution of effective therapy and control.

Aim & objectives

- To isolate and identify aerobic bacterial isolates from the wound swab samples of burn patients.
- To determine the frequency and spectrum of aerobic bacterial isolates in the wound swab samples of burn patients.
- To study their antibiotic sensitivity pattern.

Material and Method:

A total of randomly 100 burn patients' wound samples were collected from various wards of J.L.N hospital, Ajmer, to identify the common aerobic bacterial isolates

Sample Collection and Transport:

Wound swab samples were collected from the hospitalized burn patients. The area around the burn wound was cleaned with 70% ethyl alcohol and the sample was collected from the depth of the wound using sterile cotton swabs. The sample was transported immediately to the laboratory for further processing. Samples were collected immediately after the patients were admitted to the burns unit and every week thereafter until discharge or death of the patient[8].

Inclusion criteria: All the aerobic bacterial isolates from wound samples of burn patients were included for the study.

Exclusion criteria: All the clinical isolates other than aerobic bacteria from wound samples of burn patients were excluded from the study.

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The samples were processed immediately in the following manner:

a. Direct microscopic examination
b. Inoculation on different other media
c. Preliminary identification of the growth
d. Biochemical tests
e. Antimicrobial susceptibility

Isolation for aerobic bacteria

Two sterile swab sticks were used to collect the discharge from the affected wound.

1. One swab stick used for Grams staining.
2. Other swab stick was used for inoculation on MacConkey agar (MA), Blood agar (BA) & Brain Heart Infusion (BHI) broth.

MacConkey agar, Blood agar & BHI were incubated at 37^0C. After 24-48 hours of incubation, the plates were examined for growth. If there was no growth on MA, BA & BHI was turbid, then subculture was done on MA & BA .The colony morphology was studied and processed.

Gram-negative bacilli- motility was done by hanging drop method, oxidase and catalase tests were done. Biochemical tests were done and interpreted according to Lactose fermenting and Non-lactose fermenting colonies appeared on MA. These tests are:

- Indole test
- Methy red test
- Simmon's Citrate test
- Christensen's Urease test
- Phenylalanine deaminase test
- Triple sugar iron test
- Glucose fermentation
- Lactose fermentation
- Sucrose fermentation.

Gram-positive cocci

For gram positive cocci – catalase test, oxidase tests were done. For cocci in clusters which are oxidase negative & catalase positive--slide and tube coagulase, Mannitol fermentation tests were done.

Antibiogram Testing

The antibiotiogram testing was done as per CLSI guidelines using modified Kirby-Bauer method. Bacterial suspension was prepared by inoculating 4-5 isolated colonies in the peptone water and incubated at 37^0C for 2 hours. Turbidity was set to that of 0.5 Mc Farland standards and a lawn culture was made on Muller Hinton Agar plate using sterile cotton swabs. The required antibiotic discs were then placed aseptically on this using sterile forceps. The plates were then incubated 24 hours at 37^0C. Zone size was measured the next day and reported as sensitive or resistant by comparing the zone size to the Kirby-Bauer chart.

Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion methods according to CLSI protocol. Depending on the isolate, antibiotic discs were selected from among the following: Co-trimaxazol (25µg), Erythromycin (15µg) , Gentamicin (10µg),Ciprofloxacin (5µg), Oxacillin(1µg),Amoxyclav (30 μg), Linazolid (30 μg),Vancomycin (30µg), Tetracycline (30µg),Cefotaxime(30µg),Amikacin(30µg),Amoxyclav(30 μg),Ceftazidime (30 μg),Imipenem (10 μg), Pipercilline (100 μg).

Result & Observation:

The age of the patient ranged from 6 months to 80 years. Maximum incidences were noted during the second and third decades. Incidences were more in females than in males. Most common cause for burn wounds was burns due to flames (85%). Mortality rate was found to be 34%. It was seen that the mortality rate increased with increase in TBSA. TBSA >60% showed 72.41% mortality rate. From 100 patients, a total of 133 samples were taken and 156 organisms were isolated. The overall isolation rate was 87.96%. It was observed that solitary isolates (58%) were seen more commonly than multiple isolates (30%).Overall, Gram negative organisms (66.66%) were more common than Gram positive organisms (33.33%). *Pseudomonas* (*P. aeruginosa* and *Pseudomonas* species) (42.94%) was the most common isolate followed by *Staphylococcus aureus* (19.23%), *Klebsiella pneumoniae* (14.74%), Coagulase Negative Staphylococcus (14.10%), *Escherichia coli* (5.12%), *Proteus mirabilis* (2.04%), *Citrobacter freundii* (1.28%).Even though Gram positive organisms were common in first weeks and subsequently decreased in later weeks, Gram negative organisms especially *Pseudomonas* were more predominant in first as well as in later weeks.

Imipenem (100%) was the most effective drug against the *Pseudomonas aeruginosa* and other *Pseudomonas* species. Least effective drug against *Pseudomonas* was Ceftazidime (17.91%). Ciprofloxacin (71.74%) and gentamicin (50.74%) were moderatlely effective. Imipenem (100%) was also the most effective drug against the other Gram Negative Organisms. Least effective drug against other Gram Negative Organisms was Ceftriaxone (35.13%). Gentamicin, tetracyline and aztreonam were moderately effective.It was seen that vancomycin and linezolid (100%) were the most effective drugs for the Gram positive organisms. Least sensitive drug against Gram positive organisms was ampicillin (3%). Gentamicin, amoxyclav and clindamycin were moderately effective. Incidences of Methicillin Resistant Staphylococcus aureus were 37% incidences of Methicillin Resistant CONS were 18.18%.

Discussion:

In the present study an attempt was made to know the aerobic bacteriology of the burn wounds with antimicrobial susceptibility testing of the bacterial isolates. The results were compared with other studies and discussed as follows:

Age distribution pattern revealed 48.23% of the patients were in the age group of 21 to 40 years, which is in accordance to Sadeghi-Bazargani H et al[9], and Jaiswal AK et al[10] the average age of the patient varies from 19 to 35 & 21– 30 years of age, respectively, in the different studies they reviewed. Similar results were seen by Chakraborty S et al[11] who reported that 56.6% of the cases were of 20-39 years age.(TABLE 1)

The reason of high incidences in this age group can be given as patients in this age group are more prone to occupational hazards as well as household accidents than children or elderly.

Incidence was more in females (54%) than males. This is similar to findings by Kaur H et al[12], Rajput A et al[64] and Ganesamoni S et al[7]. In contrast, Agnihotri N et al[14], Lari AR et al[15], Ramakrishnan MK et al[16] and Ekrami A et al[17], reported that the incidence was higher in males in their studies.High incidence of burns in females is probably due to domestic accidents in the household and constantly working with the fire during cooking.

The overall mortality rate was 34%. The mortality rate in burn wounds with TBSA > 60% was maximum (72.41%). This is in accordance with Branski et al[18] who reported a mortality rate of 50% and in burns with > 50% TBSA it reached 60 – 80%. Other studies also reported high mortality rates ranging from 40 - 65%[7,10,19,20]. However, Sadeghi-Bazargani H et al[9] (18%), Lari AR et al[15] (19.6%), Alaghehbandan R et al[21] (10.3%) have reported lower mortality rates.

The overall isolation rate was found to be 87.96%. This was comparable with findings of Srinivasan S et al (86.3%)[23]. Others have reported higher isolation rates such as 93% by Ramakrishnan MK et al 57, 95% by Kaur H et al[24] and 97.01% by Mehta M et al[25].

We noted that solitary isolates were more common (58%) than multiple (30%). This is comparable to other studies by Jefferson Lessa Soares de Macedo et al[27], Ramakrishnan MK et al[16], Kaushik R et al[27] and Dhar S et al[28] who reported solitary isolation rates of 89.3%, 84%, 78% and 58.42% respectively.

Pseudomonas (42.94%) *(P. aeruginosa* and other *Pseudomonas* species) (TABLE 2) was the most common isolate in burn patients. These results were similar to results from other studies[12,27,29]. In contrast, some other reports indicated a decrease in burn wound colonization with *P. aeruginosa*. Source of *Pseudomonas* infection may be either endogenous like patient's gastrointestinal flora or exogenous[2]. There are opinions that with the initiation of antibiotics against Gram positive organisms a significant rise in Pseudomonas infection of burned patients had occurred[30]. Predominance of *Pseudomonas* species in the burn wards maybe due to the fact that the organism thrive in the moist environment[13,29].

The second most common isolate was *Staphylococcus aureus* (19.23%), again similar to reports from other studies[13,16,25,27,28,29]. This is in contrast, however to some other studies especially from developed countries which report *S. aureus* as the most predominant organism in burn patients[31]. Staphylococcus was the predominant cause of burn wound infection in the pre-antibiotic era and remains an important pathogen at present. However, Srinivasan S et al stated that the percentage incidence of staphylococci is on the decline from 2002 – 2005[22].

As for *Klebsiella pneumoniae*, they accounted for 14.74% of all the organisms isolated in our study. Our results were comparable with those of Singh N P et al[32], Nasser S et al[33], M K et al[16], Rajput A et al[13] who reported moderate incidences of *Klebsiella*. In our study, Coagulase Negative Staphylococci (CONS) were recovered at a frequency of 14.10%. 95% of these were isolated in the first week, followed by 5% in the second week and none in the third week. This is in agreement with Altoparlak U et al[34], 11% by Kaur et al[24], 11.6% by Nasser S et al[33] and 15.2% by Jefferson Lessa Soares de Macedo et al[26]. Regardless of the incidence, it is advisable in view of the immune-compromised status of the burned patients that Coagulase Negative Staphylococci should be considered a significant pathogen[33].

Escherichia coli accounted for 5.12% of the total isolates. This low incidence of *E. coli* is in agreement with other studies in which the frequency of the organism does not exceed 5%[26,17,25,35]. Nasser S et al however, reported a higher incidence of *E. coli* (13.6%)[33]. Srinivasan S et al stated that the prevalence of *E. coli* was on the rise from 2001 to 2004 and it has started to wean off from 2005 and 2006 in the successive years[22].

Isolation rate of *Proteus mirabilis* was 2.04%. This is comparable to other studies that report a similar isolation rate[13,25,26,31]. The incidence of *Proteus* species is reported at frequencies as high as 11% to no incidence at all[14].

The incidences of *Citrobacter freundii* were 1.28% this is comparable to study by Ekrami A et al.[17], S. Rajeshwar Rao et al[35] showed 3.6% incidences of Citrobacter species.

Contrary to the findings in the pre-antibiotic era, the isolation of beta haemolytic streptococci from burn wounds has now become rare[13,36]. In this study we also did not find any isolates of beta haemolytic streptococci. Other studies have also reported no isolation of the microorganism[14,47].

We found in our study that even though Gram positive organisms were common in first weeks and subsequently decreased in later weeks, Gram negative organisms especially *Pseudomonas* were more predominant in first as well as in later weeks. This finding is in accordance with other studies such as Rastegar Lari et al[37] and Kaur H et al[24]. Early infection due to *P. aeruginosa* and other Gram negative bacilli might be due to immune-compromised status of burn patients and/or overcrowding and person to person spread in the burn unit.

The antimicrobial sensitivity testing was done by Kirby Bauer's disc diffusion method according to CLSI guidelines. Among the gram negative isolates the most effective drug was imipenem showing 100% sensitivity. This is in accordance with a study by Guggenheim M et al[38].

The most common isolates *P. aeruginosa* and *Pseudomonas spp.* showed 100% sensitivity to Imipenem. Sensitivity to commonly used drugs were like 71.74% to ciprofloxacin, 50.74% to gentamicin, 46.26% to piperacillin, 44.74% to amikacin, 37.31% to aztreonam, 34.32% to cefotaxime, cefepime 31.34% and low sensitivity to amoxyclav, ceftazidime, ceftriaxone and tetracycline. This high level of resistance to commonly used antibiotics is comparable with Agnihotri N et al[14], Kaur H et al[24], Mohammad Imran et al[31].

Klebsiella pneumoniae accounted for 14.74% of all the isolates. All the isolates showed high resistance to ciprofloxacin, cefotaxime, ceftazidime, ceftriaxone and amoxyclav, aztreonam and gentamicin showed 47.82% sensitivity and cefepime and amoxyclav showed 43.47% sensitivity. While 65.21% were sensitive to amikacin and 60.89% were sensitive to tetracycline. Sensitivity to imipenem was 100%.

The other Gram negative isolates, namely *E. coli*, *Prot. mirabilis* and *C. freundii* showed good sensitivity to imipenem, amikacin, amoxiclav, gentamicin and aztreonam and moderate sensitivity to tetracycline, ciprofloxacin, cefotaxime and ceftazidime, ceftriaxone and cefepime.

Mehta M et al[25] saw a significantly high percentage of resistance among gram negative bacilli to aminoglycosides, ciprofloxacin, carbenicillin and cephalosporins. But in comparison, imipenem was found to be effective.

Jefferson Lessa Soares de Macedo et al[26], Singh NP et al[32] and Rastegar Lari AR et al[37] also reported a high degree of resistance to antimicrobial agents.

The Gram positive isolates showed 100% sensitivity to vancomycin and linezolid, followed by 76.92% sensitivity to gentamicin and 75% to amoxyclav and 67.30% to clindamycin. Only 3% of the isolates were sensitive to ampicillin.

Similar findings were seen by Rastegar Lari AR et al[37] and Kaushik R et al[27]. However, several other studies have observed a higher level of resistant of these organisms to these antimicrobials[28,29,36].

We found that 37% of the isolates of *S. aureus* were methicillin resistant. This is comparable with other studies on MRSA in burn patients by Rajput A et al[13] and Oncul O et al[39]. They both reported a 40% incidence of MRSA. 18.18% of the CONS isolates were methicillin resistant. This finding is similar to that by Altoparlak U et al[30] who reported 20.9% isolates of CONS to be methicillin resistant.

The high percentage of multi drug resistance is probably due to irrational use of broad spectrum antibiotics in hospitals[14]. The high prevalence of MRSA in our setting calls for routine testing of all *S. aureus* isolates from wound specimens for methicillin resistance. This will help to curtail the spread of this organism and also to start early and appropriate therapy which will result in reduction in overall morbidity and mortality.

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CONCLUSION

Infection in the burn patients has been the major cause of morbidity since long. The present study has given us the knowledge regarding incidence of bacterial colonization of burn wounds in our hospital. It was seen that Gram negative organisms were more prevalent. *Pseudomonas aeruginosa* was the most common microorganism followed by *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Burn patients can easily acquire infections from the surroundings as well as disperse them too. *Pseudomonas* remains the most common pathogen in early and in later weeks. Early infection due to *P.aeruginosa* is a matter of concern. Hence we strongly recommend that *P.aeruginosa* should be considered the main nosocomial pathogen in our burn care unit and preventive actions such as hand hygiene and proper infection control measures should be taken to reduce its spread.

The antibiotic susceptibility testing showed that imipenem was the most effective drug for Gram negative isolates and vancomycin and linezolid for Gram positive isolates. Emerging multidrug resistant strains are of foremost concern in burn patients. Once multi drug resistant strains become established in the hospital settings it can persist for a long time. Therefore it is needed to do careful microbiological surveillance and proper testing before the start of antibiotic therapy.

In conclusion, routine microbiological surveillance and adherence to restrictive antibiotic policy may be useful in reducing infection related mortality and morbidity in burn patients. Our results may be of help in providing useful information regarding formulation of effective guidelines for therapy, thus improving overall infection related morbidity and mortality.

TABLE 1 Distribution of cases according to age

Age Group (years)	No. of patients
0-10	14
11-20	15
21-30	34
31-40	15
41-50	13
51-60	6
61-70	2
>71	1

TABLE 2 DISTRIBUTION OF THE ISOLATES

Distribution of the isolates	No. of patients
Pseudomonas aeruginosa	55
Pseudomonas spp.	12
Staphylococcus aureus	30
CONS	22
Klebsiella pneumoniae	23
Echerichia coli	8
Proteus mirabilis	4
Citrobacter freundii	2

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