

Full length Research Paper

Bacteriological profile and antibiogram of aerobic burn wound isolates in a tertiary care hospital, Odisha, India

Muktikesh Dash^{1*}, Pooja Misra², Siddhartha Routaray³

¹Department of Microbiology, Maharaja Krishna Chandra Gajapati Medical Collage and Hospital, Berhampur University, Berhampur, Odisha, India.

²Department of Radiodiagnosis, Maharaja Krishna Chandra Gajapati Medical Collage and Hospital, Berhampur University, Berhampur, Odisha, India.

³Department of Anesthesia, SCB Medical College and Hospital, Utkal University, Cuttack, Odisha, India.

Accepted 09 July, 2013

Approximately 73 percent of all post-burn deaths are directly or indirectly related to septic processes. The objective of this retrospective study at a tertiary care hospital, Odisha, India was to isolate aerobic bacterial pathogens and study its antimicrobial resistant pattern in order to establish empiric antimicrobial strategies for the early treatment of imminent septic events. During three year period (January 2010 to December 2012), 193 burn wound swabs were collected from 187 hospitalized patients. Isolation and identification of microorganisms was done using standard procedure and each isolate's antimicrobial resistant pattern was determined by Kirby-Bauer disc diffusion technique. From 193 swabs, 171 (88.6%) culture positive swabs and 176 isolates were obtained. The most common isolate was *Pseudomonas aeruginosa* (49.4%), followed by *Staphylococcus aureus* (22.2%), *Klebsiella pneumoniae* (13.1%) and *Acinetobacter baumannii* (4.5%). *P. aeruginosa* was least resistant to piperacillin/tazobactam (12.6%) and imipenem (9.2%) and 59 percent methicillin resistant *S. aureus* (MRSA) was 100% sensitive to vancomycin and linezolid. High prevalence of multidrug resistant bacteria in our hospital setting suggest continuous surveillance of burn wound infections and need for development of strict infection control practices.

Key words: Antimicrobial resistance; burn wound; swabs; aerobic bacteria; *Pseudomonas aeruginosa*; *Staphylococcus aureus*.

INTRODUCTION

Burns are one of the most common and devastating form of trauma. Burn patients are at risk for infection because of their destroyed skin barrier, suppressed immune system compounded by prolonged hospital stay and invasive therapeutic and diagnostic procedures (Mayhall, 2002). Infection is a major cause of morbidity and mortality in hospitalized burn patients (Mc Manus et al., 1994). Approximately 73 percent of all post-burn deaths have been shown to be directly or indirectly caused by

septic processes (Tancheva and Hadjiiski, 2005). The pattern of microbial pathogen differs from hospital to hospital; the varied bacterial flora of infected wound may change considerably during the healing period (Kumar et al., 2001).

Multidrug resistant bacteria have frequently been reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of the wounds of the burn patients (Karlowsky et al., 2004; Agnihotri et al., 2004). Similarly, antimicrobial resistant pattern among most frequently isolated bacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and other Gram-negative bacteria has reached to a alarmingly high level in different studies conducted within India (Nagoba et al., 1999, Shahid and Malik 2005 and

*Corresponding author E-mail: - mukti_mic@yahoo.co.in
Tel. +91 9861046640, 0680-2292526

Table 1. Gender wise distribution of swabs collected from burn patients in a tertiary care hospital, Odisha, India (n=187).

| Gender | Total number of burn wound swabs | | |
|--------|----------------------------------|----------------------------|----------------------------|
| | No. tested (%) | No. of growth positive (%) | No. of growth negative (%) |
| Male | 86 | 77 (89.5) | 09 (10.5) |
| Female | 101 | 88 (87.1) | 13 (12.9) |
| Total | 187 (100) | 165 (88.2) | 22 (11.8) |

Rajput et al., 2008). Keeping all these facts in view, the present study was carried out to determine the aerobic bacterial burn wound isolates in our hospital setting and describe their resistance patterns, which would enable the determination of empiric antimicrobial strategies for the early treatment of imminent septic events.

MATERIALS AND METHODS

Study area, population and methodology

A total of 187 patients were admitted to the burn unit of a tertiary care hospital, Odisha, India during three year period from January 2010 to December 2012. The retrospective evaluation of patient's age, sex, severity of burn injury and hospital stay was carried out on the basis of case histories.

Data about pathogens were obtained by microbiological analysis of swabs taken from burn patients. A total of 193 surface swabs were taken from all 187 burn patients. Swabs were taken when the wound infection was clinically diagnosed after admission in the burn unit. Clinical diagnosis of burn wound infection relied on regular monitoring of vital signs and inspection of entire burn wound surface, during each dressing change. Local signs of burn wound infection with invasion included conversion of a partial-thickness injury to a full-thickness wound, rapidly extending cellulitis of healthy tissue surrounding the injury, rapid eschar separation and tissue necrosis. Repeated pathogens for the same patient were not included in the study. Verbal informed consent was obtained from all patients prior to swab collection. The study was conducted after due approval from institutional ethical committee.

Sample collection and processing

Whenever applicable, wound surface swabs were collected from partial-thickness to full-thickness wound (from 2° to 4° burn). The infected area was swabbed by using alginate swabs and then cultured aerobically on 5% sheep blood agar and MacConkey agar. Isolation and

identification was done according to standard procedure (Baron and Finglod, 1996). All isolates were tested for antimicrobial susceptibility testing by the standard Kirby-Bauer disc diffusion method according to Bauer et al (Bauer et al., 1966). A suspension of each isolate was made so that the turbidity was equal to 0.5 McFarland and then plated onto Muller-Hinton agar plate. The following standard antibiotic discs were used; ampicillin (10mcg), carbenicillin (100mcg), sulphamethoxazole/trimethoprim i.e., co-trimoxazole (23.75/1.25mcg), ciprofloxacin (5mcg), gentamicin (10mcg), amikacin (30mcg), tobramycin (10mcg), cephalothin (30mcg), ceftazidime (30mcg), cefoperazone (75mcg), cefoperazone/sulbactam (75/30mcg), piperacillin (100mcg), piperacillin/tazobactam (100/10mcg), imipenem (10mcg), oxacillin (1mcg), clindamycin (2mcg), vancomycin (30mcg) and linezolid (15mcg). All dehydrated media and antibiotic discs were procured from Himedia Labs., Mumbai, India. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009). The control strains used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923.

Statistical analysis

The data were analyzed for mean, median and standard deviation by using GraphPad QuickCalcs statistical software Inc., 2236 Avenida de la Playa La Jolla, CA 92037 USA.

RESULTS

The mean age of patients was 26.7±15.8 years (median 24, 95% confidence intervals 24.4 to 28.9 and range was from minimum 2 to maximum 75 years). There were 101 females and 86 males with female to male ratio 1.17:1 [Table 1]. The mean total body surface area (TBSA) percentage among burn patients was 43.5±23.3 % (median 36, 95% confidence intervals 36.9 to 50.1 and range was from minimum 10 to maximum 100 percentage).

Table 2. Isolation of aerobic bacterial pathogens from burn wound swabs in a tertiary care hospital, Odisha, India (n=176).

| Gram reaction | Type of isolates | No. of isolates | Percentage (%) |
|-----------------------|--------------------------------|-----------------|----------------|
| Gram-negative bacilli | <i>Pseudomonas aeruginosa</i> | 87 | 49.4 |
| | <i>Enterobacteriaceae</i> * | 42 | 23.9 |
| | <i>Acinetobacter baumannii</i> | 08 | 4.5 |
| Gram-positive cocci | <i>Staphylococcus aureus</i> | 39 | 22.2 |

**Enterobacteriaceae* included - *Klebsiella pneumoniae* 23 (13.1%), *Escherichia coli* 7 (3.9%), *Proteus mirabilis* 5 (2.8%), *Enterobacter aerogenes* 3 (1.7%), *Citrobacter freundii* 3 (1.7%) and *Serratia marcescens* 1 (0.6%)

Table 3. Antimicrobial resistant pattern (%) among bacterial isolates in burn patients.

| Antibiotic | Concentration of disc (mcg) | Resistant organism (s) | | | |
|--------------------------------|-----------------------------|---|---|------------------------------------|--|
| | | No. (%) of <i>Pseudomonas aeruginosa</i> (n=87) | No. (%) of <i>Enterobacteriaceae</i> (n=42) | No. (%) of <i>S. aureus</i> (n=39) | No. (%) of <i>Acinetobacter baumannii</i> (n=08) |
| Ampicillin | 10 | NT | 39 (92.9) | 27 (69.2) | NT |
| Carbenicillin | 100 | 51 (58.6) | 21 (50) | NT | 05 (62.5) |
| Piperacillin | 100 | 46 (52.9) | 17 (40.5) | NT | 06 (75) |
| Piperacillin/tazobactam | 100/10 | 11 (12.6) | 04 (9.5) | NT | 01 (12.5) |
| Gentamicin | 10 | 59 (67.8) | 23 (54.8) | 17 (43.6) | 07 (87.5) |
| Amikacin | 30 | 43 (49.4) | 09 (21.4) | NT | 05 (62.5) |
| Tobramycin | 10 | 48 (56.3) | 13 (30.9) | NT | 06 (75) |
| Ciprofloxacin | 5 | 53 (60.9) | 27 (64.3) | 23 (59) | 07 (87.5) |
| Sulphamethoxazole/trimethoprim | 23.75/1.25 | NT | 29 (69) | 26 (66.7) | NT |
| Cephalothin | 30 | NT | 33 (78.6) | 29 (74.4) | NT |
| Ceftazidime | 30 | 63 (72.4) | 26 (61.9) | NT | 07 (87.5) |
| Cefoperazone | 75 | 61 (70.1) | 18 (42.9) | NT | 06 (75) |
| Cefoperazone/sulbactam | 75/30 | 24 (27.6) | 07 (16.7) | NT | 02 (25) |
| Imipenem | 10 | 08 (9.2) | 0 | NT | 0 |
| Oxacillin | 1 | NT | NT | 23 (59) | NT |
| Clindamycin | 2 | NT | NT | 13 (33.4) | NT |
| Vancomycin | 30 | NT | NT | 0 | NT |
| Linezolid | 15 | NT | NT | 0 | NT |

NT- Not tested, mcg- micrograms, *Enterobacteriaceae* included - *Klebsiella pneumoniae* -23, *Escherichia coli* -7, *Proteus mirabilis*- 5, *Enterobacter aerogenes*- 3, *Citrobacter freundii* -3 and *Serratia marcescens*- 1 isolates.

A total of 193 surface swabs were taken from burn wounds, out of which 171 (88.6%) swabs were culture positive and rest 22 (11.4%) were sterile. From 171 culture positive swabs only 5 swabs yielded more than one isolate, thus a total of 176 bacterial isolates were obtained. The most predominant bacterial isolate was *Pseudomonas aeruginosa* 87 (49.4%), followed by other gram negative *Enterobacteriaceae* 42 (23.9%) including *Klebsiella pneumoniae* 23 (13.1%), *Escherichia coli* 7 (3.9%), *Proteus mirabilis* 5 (2.8%), *Enterobacter aerogenes* 3 (1.7%), *Citrobacter freundii* 3 (1.7%) and *Serratia marcescens* 1 (0.6%). Other bacteria isolated

were *Staphylococcus aureus* 39 (22.2%) and *Acinetobacter baumannii* 8 (4.5%) [Table 2].

Table 3 illustrates the antimicrobial resistance profiles of the bacterial isolates. The most predominant bacterial isolate *Pseudomonas aeruginosa* was resistant to commonly used antipseudomonals i.e., ceftazidime (72.4%), cefoperazone (70.1%), gentamicin (67.8%), ciprofloxacin (60.9%), carbenicillin (58.6%), tobramycin (56.3%), piperacillin (52.9%), amikacin (49.4%) and cefoperazone/sulbactam (27.6%). *P. aeruginosa* was least resistant to piperacillin/tazobactam 12.6% followed by imipenem 9.2%. It is worth to note that 23 (59%) of

Staphylococcus aureus were methicillin resistant. However, these isolates were 100% susceptible to vancomycin and linezolid.

DISCUSSION

Burns become infected because of thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humoral immune responses (Alexander, 1990). The burn wound surface is a protein-rich environment consisting of avascular necrotic tissue that provides a favorable niche for microbial colonization and proliferation. Despite significant improvement in the survival of burn patients, infectious complication continues to be the major cause of morbidity and mortality. The goal of burn wound management is to reduce the onset and density of bacterial contamination through early microbiological diagnosis, strict isolation technique and proper implementation of infection control policies (Amin and Kalantar, 2004).

In our study, female : male ratio of total burn patients was 1.17:1, similar to that of other studies conducted by Akhter et al. and Batra et al. in India (Akhter et al., 2010 and Batra 2003). In comparison, studies done by Ekrami et al. in Iran and Bagdonas et al. in Lithuania showed higher incidence of burn injuries among males (Ekrami and Kalantar, 2007 and Bagdonas et al., 2004). In India higher incidence of burn injuries among females may be related to inadequate precautions during cooking, wearing of loose Indian saris, inability to cope with the physical and psychological stress of marriage and harassment from parents-in-law (Bilwani and Gupta, 2003). The mean age of burn patients admitted to hospital was 26.68 years, similar to that of other studies (Akhter et al., 2010, Ekrami and Kalantar, 2007 and Bagdonas et al., 2004). High incidence among young adults may be explained by the fact that they are generally active and are exposed to hazardous situations both in home and at work.

In this present study, high culture positivity of 88.6% was found in the samples collected from burn patients. This is in agreement with other studies (Ekrami and Kalantar, 2007 and Bagdonas et al., 2004). Our finding showed that *P. aeruginosa* (49.4%) was the most common isolate which coincides with many previous studies within and outside India (Nagoba et al., 1999, Rajput et al., 2008 and Ekrami and Kalantar, 2007). However, study conducted by Bagdonas et al. had revealed that *S. aureus* (52.1%) was the most predominant isolate (Bagdonas et al., 2004). Although *S. aureus* remains a common cause of early burn wound infection, *P. aeruginosa* from patient's endogenous gastrointestinal flora or moist environmental source is the most common

cause of burn wound infections. The second most common isolate in our study was *S. aureus* (22.2%) followed by *K. pneumoniae* (13.1%) and *A. baumannii*, which is similar to other studies (Nagoba et al., 1999, Rajput et al., 2008 and Ekrami and Kalantar, 2007).

Our study showed *P. aeruginosa* was highly resistant to commonly used antipseudomonals and was least resistant to piperacillin/tazobactam (12.6%) and imipenem (9.2%). Similar finding of least resistant antimicrobial imipenem against *P. aeruginosa* was reported by Rajput et al. in Lucknow, India (Rajput et al., 2008). Other Gram-negative bacilli were highly resistant to commonly used ampicillin (92.9%), co-trimoxazole (69%), ciprofloxacin (64.3%) and gentamicin (54.8%). Similarly, piperacillin/tazobactam (90.5%) and imipenem (100%) were found to be most effective antimicrobial agents against Gram-negative bacilli. Multidrug resistant Gram-negative bacilli that possesses several types of beta-lactamases including extended spectrum beta-lactamases, ampC beta-lactamases, and metallo beta-lactamases, have been emerging as serious pathogens in hospitalized patients (Clark et al., 2003). Low resistant pattern shown by imipenem and piperacillin/tazobactam may be due to its restricted use because of higher cost and limited availability.

In our study, 59 percent of *S. aureus* were methicillin resistant (MRSA). This result is in agreement with studies done by Ekrami et al. and Rajput et al. where they had found out 58% and 40% of MRSA isolates respectively. (Rajput et al., 2008 and Ekrami and Kalantar, 2007). These MRSA isolates showed 100% susceptibility to both vancomycin and linezolid.

The high percentage of multidrug resistant isolates is probably due to empirical use of broad-spectrum antimicrobials prior to development of infection, extended duration or previous hospitalization and non-adherence to hospital antimicrobial policy. Strict infection control practices i.e., physical isolation in a private room, use of gowns and gloves during patient contact, hand washing before and after each patient visit and appropriate antimicrobial therapy are essential tools to reduce the incidence of infections due to these multidrug resistant organisms (Elliot and Lambert, 1999). Therefore, routine institutional laboratory surveillance system involving periodic sampling of burn wounds would facilitate the selection and administration of appropriate empirical systemic antimicrobial agents prior to the availability of microbiological culture and susceptibility test results.

CONCLUSION

To conclude, burn patients were most commonly infected with *P. aeruginosa* and *S. aureus*. Majority of these

isolates were multidrug resistant. In Gram-negative isolates, imipenem or piperacillin/tazobactam and in Gram-positive, vancomycin or linezolid can be used as empirical antimicrobial therapy prior to the availability of microbiological culture and susceptibility test results. A burn unit-specific nosocomial infection surveillance system may be introduced to reduce the incidence of multidrug resistant infections among burn patients, and for selecting appropriate antimicrobial agents.

REFERENCES

- Agnihotri N, Gupta V, Joshi RM (2004). Aerobic bacterial isolates from burn wound infections and their antimicrograms-a five-year study. *Burns* ;30:241-3.
- Akther JM, Nerker NE, Reddy PS, Khan MI, Chauhan MK, Shahapurkar VV (2010). Epidemiology of Burned Patients Admitted In Burn Unit of A Rural Tertiary Teaching Hospital. *Pravara Med Rev*;2:11-7.
- Alexander JW (1990). Mechanism of immunologic suppression in burn injury. *J Trauma*;30:70-5.
- Amin M, Kalantar E (2004). Bacteriological monitoring of hospital borne septicemia in burn patients in Ahvaz, Iran. *Burn Surgical Wound Care*;3:4-8.
- Bagdonas R, Tamelis A, Rimdeika R, Kiudelis M (2004). Analysis of burn patients and the isolated pathogens. *Lithuanian Surg*;2:190-3
- Baron J, Finglod S (1996). Methods for identification of etiologic agents of infectious diseases. In: Baily & Scott's diagnostic microbiology. 10th ed. St Louis, USA: Mosby, Inc.p.327-529.
- Batra AK (2003). Burn mortality, Recent trends and socio-cultural determinants in rural India. *Burns*;29:270-5.
- Bauer AW, Kirby WM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*;45:493-6.
- Bilwani PK, Gupta R (2003). Epidemiological profile of burn patients in LG Hospital, Ahmadabad. *Indian J Burns*;11:63-4.
- Clark NM, Patterson J, Lynch JP (2003). Antimicrobial resistance among gram-negative organisms in the intensive care unit. *Curr Opin Crit*;9:413-23.
- Clinical and Laboratory Standards Institute (2009). Performance standards for antimicrobial susceptibility testing: Nineteenth Informational Supplement. CLSI document M100-S19. Wayne; PA: USA. Clinical and Laboratory Standards Institute,.
- Ekrami A, Kalantar E (2007). Bacterial infections in burn patients at a burn hospital in India. *Indian J Med Res*;126:541-4.
- Elliot TS, Lambert PA (1999). Antibacterial resistance in the intensive care unit: mechanisms and management. *Br Med Bull*;55:259-76.
- Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF, Volturo GA (2004). Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Ann Clin Microbiol Antimicrob*;3:3-7.
- Kumar V, Bhatnagar SK, Singh AK, Kumar S, Mishra RK (2001). Burn Wound Infection: A study of 50 cases with special reference to antibiotic resistance. *Indian J Bio Res*;46:66-9.
- Mayhall CG (2002). The epidemiology of burn wound infections: then and now. *Burns*;29:738-44.
- Mc Manus AT, Mason AD jr, Mc Manus WF, Pruitt BA (1994). A decade of reduced Gram-negative infections and mortality improved isolation of burned patients. *Arch Surg*;129:1306-9.
- Nagoba BS, Deshmukh SR, Wadher BJ, Pathan AB (1999). Bacteriological analysis of burn sepsis. *Indian J Med Sci*;53:216-9.
- Rajput A, Singh KP, Kumar V, Sexena R, Singh RK (2008). Antibacterial resistance pattern of aerobic bacteria isolates from burn patients in tertiary care hospital. *Biomed Res*;19:1-4.
- Shahid M, Malik A (2005). Resistance due to aminoglycoside modifying enzymes in *Pseudomonas aeruginosa* isolates from burn patients. *Indian J Med Res*;122:324-9.
- Tancheva D, Hadjiiski O (2005). Effect to early nutritional support on clinical course and septic complications in patients with severe burns. *Ann Burns Fire Disasters*;18:74-9.