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# Emergence of Multi-Drug Resistant Strains among Bacterial Isolates in a Burn Care Facility

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***Abstract:***
*Infection is one of the most common causes of mortality and morbidity following burn injury. The study compared the results obtained in 5 years data (Jan 2008– Dec 2012) with the previous 5 years data (Jan 2003– Dec 2007) from the burn ward to determine the changing patterns and emerging trends of bacterial isolates and also emergence of multi-drug resistant strains among bacterial isolates. From 2008-12, Pseudomonas was found to be most common isolate (37.2%) followed by Staphylococcus aureus (17.5%), Klebsiella (14.6%) and Acinetobacter (12.7%). A constant and significant increase was found in the incidence of Acinetobacter (from 6.1% in 2003–07 to 12.7% in 2008–12) and Klebsiella(from 10.7% in 2003–07 to 14.6% in 2008–12). Most of the gram-negative isolates obtained were found to be MDR. Acinetobacter spp. was the most common MDR organism and has shown little susceptibility to most antibiotics. Imipenem have been very reliable reserve antibiotics throughout the study period for gram negative bacilli with susceptibilities of or near 100%. Isolation of MRSA was 68.8%, but all were sensitive to Vancomycin. Hence in-depth knowledge of the bacteria causing infectious complications and their antibiotic susceptibilities is a prerequisite for treating burn patients. Our study shows frequent shifts in microbial spectrum and their antibiogram, which mandate frequent reassessments of empirical antimicrobial therapy and patient management decisions during treatment of burned patients.*

## 1. Introduction

India has an estimated annual burn incidence of 6-7 million, based on data from major hospitals, which is the second largest group of injuries after road accidents [1]. Nearly 10% of these are life threatening and require hospitalization[1]. Infections leading to septicemia are the common cause of morbidity and mortality following burn injury. Burn injuries involve destruction of the skin barrier and concomitant depression of local and systemic host cellular and humoral immune responses [2]. The burn wound surface provides a protein-rich environment consisting of avascular necrotic tissue that provides a favourable environment for microbial colonization and proliferation. Although burn wound surfaces are sterile immediately following thermal injury, these wounds usually become infected with microorganisms [3]. Hospital acquired infections in burn patients can be endogenous or exogenous. Endogenous infections are caused by the patient's own flora, exogenous infections result from transmission of organisms from a source other than the patient [4]. Indiscriminate use of antibiotics over time has remarkably influenced the pattern of pathogens causing burn infections. The organisms isolated and the antimicrobial sensitivity profile varies from hospital to hospital. The worldwide emergence of antimicrobial resistance among a wide variety of burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections. Methicillin-resistant staphylococcus aureus (MRSA), vancomycin-resistant enterococci, and multi-drug resistant Gram-negative bacteria that possess several types of beta-lactamases, including extended spectrum beta-

lactamases (ESBL) and metallo beta-lactamases (MBL) have been emerging as serious challenges in hospitalized patients. These organisms can be transmitted easily from one patient to another. Thus, burn units are common places where explosive and prolonged outbreaks of infections caused by resistant organisms occur [5]. Emergence of multi-drug resistant organisms necessitates a planned surveillance and infection control practices to reduce the incidence of hospital acquired infections amongst burn patients [6]. As the spectrum of micro-organisms causing infections in burn patients varies not only with geographical location, but also with time, it is necessary to carry out reviews of the bacterial flora present in burn unit. The aim of this retrospective study was to elucidate shifts in spectrum of major aerobic bacteria and their corresponding antimicrobial susceptibilities isolated from pus/wound swabs taken from patients admitted in Burn ward of PGIMER & Dr. RML Hospital, New Delhi, India, over a period of 10 years from Jan 2003 to Dec 2012.

**2. Materials and Methods**

The microbiological results of pus/wound swabs from burn patients collected from the Burn ward and processed in the Microbiology department, Dr. RML hospital, between 2003 and 2012 were analysed retrospectively. The standard practice of inoculation of swabs in the department was followed. The samples received were cultured on 5% sheep Blood Agar and MacConkey Agar plates & also immersed in BHI broth. Both plates and broth were incubated at 37°C for 24hrs, and then plates were examined for growth. Cultures were considered negative if no growth was observed in plates & broth. Bacterial isolates were identified according to standard methods and tested against various antibiotics by Kirby Bauer's disc diffusion method & zone diameters were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. The same laboratory protocols were followed during the whole period. The results obtained in 5 years data (Jan 2008– Dec 2012) were compared with the previous 5 years data (Jan 2003– Dec 2007) from the same ward to determine the changing patterns and emerging trends of bacterial isolates and also emerging drug resistance among isolates. Any repeat isolate from the same patient obtained on more than one occasion was not included in the study. Statistical comparison of bacterial isolates and their resistance pattern with the data of the previous five years was done using $\chi^2$ test, and $P< 0.05$ was considered significant.

**3. Results**

Out of 4398 samples processed there were 3590 (81.6%) identified positive wound cultures. (2155 out of 2588 from 2008-12 and 1435 out of 1810 from 2003-07). Pseudomonas spp. was the commonest isolated pathogen followed by Staphylococcus aureus, Klebsiella species and Acinetobacter spp. Comparison of organisms isolated from wound swabs is shown in table 1. The incidence of gram positive and gram negative organisms changed over the 10 year period. A constant and significant increase was found in the incidence of Acinetobacter spp. (from 6.1% in Jan 2003– Dec 2007 to 12.7% in Jan 2008– Dec 2012) and Klebsiella spp. (from 10.7% in Jan 2003– Dec 2007 to 14.6% in Jan 2008– Dec 2012) . Most of the gram-negative isolates obtained were found to be multi-drug resistant. Acinetobacter spp. has shown little susceptibility to most antibiotics. Carbapenems have been very reliable reserve antibiotics throughout the study period for gram negative bacilli, with susceptibilities of or near 100% (Table 2). Because Staphylococcus aureus showed high resistance to our standard antibiotics so second line drugs, Vancomycin (Glycopeptide) & Linezolid (Oxazolidinone) were also included for MRSA suspected isolates. All strains were susceptible to Glycopeptides and oxazolidinone (Table 3).

| Organism | 2008-2012 (2155/2588) | 2003-2007 (1435/1810) | Stastical analysis P value |
|---|---|---|---|
| Pseudomonas spp | 802(37.2%) | 672(46.8%) | 0.000*** |
| Staphylococcus aureus | 378 (17.5%) | 266 (18.5%) | $0.446^{NS}$ |
| Klebsiella species | 314 (14.6%) | 154 (10.7%) | 0.001*** |
| Acinetobacter spp | 273 (12.7%) | 88 (6.1%) | 0.000*** |
| Enterobacter spp | 149 (6.9%) | 91 (6.3%) | $0.501^{NS}$ |
| Proteus spp | 121(5.6%) | 89 (6.2%) | $0.463^{NS}$ |
| Escherichia coli | 106 (4.9%) | 49 (3.4%) | $0.440^{NS}$ |
| Enterococcus fecalis | 12(0.55%) | 11 (0.8%) | - |
| Others | - | 15 (1%) | - |

*Table 1: Comparison of Organisms Isolated From Wound Swabs*

** Others Include Citrobacter Spp., Providencia Spp Etc.*

**P<0.05; **P<0.01; ***P<0.001; NS Not Significant. The Two Time Periods Were Compared Using $X^2$ Test*

| Antimicrobials | Pseudomonas spp | | Klebsiella species | | Acinetobacter spp. | |
|---|---|---|---|---|---|---|
| | 2008-12 n=802(%) | 2003-07 n=672(%) | 2008-12 n=314(%) | 2003-07 n=154(%) | 2008-12 n=273(%) | 2003-07 n=88(%) |
| G | 697(86.9) | 537(79.9)*** | 270(86) | 123(79.9)*** | 256(93.8) | 76(86.3)* |
| Ak | 625(77.9) | 416(61.9)*** | 122(38.8) | 57(37) NS | 240(87.9) | 70(79.5)* |
| Cf | 441(55) | 262(38.9)*** | 119(37.9) | 45(29.2)* | 258(94.5) | 62(70.4)*** |
| Ce | - | - | 276(87.9) | 117(76)*** | 259(94.9) | 81(92) NS |
| Ca | 657(81.9) | 530(78.8) NS | - | - | - | - |
| Pc | 658(82) | 530(78.8) NS | - | - | - | - |
| PT | 296(36.9) | 151(22.5)*** | 53(16.9) | 21(13.6) NS | 54(19.8) | 15(17) NS |
| Imp | 3(0.3) | 0 | 2(0.6) | 0 | 5(1.8) | 0 |

*Table 2: Comparison of Antimicrobial Resistance in Gram-Negative Bacteria:*
**G-Gentamicin(10µg), Ak-Amikacin(30µg), Cf-Ciprofloxacin(5µg), Ce-Cefotaxime(30µg), Ca-Ceftazidime(30µg), Pc-Piperacillin(100µg), PT- Piperacillin- Tazobactam(100/10µg) , Imp- Imipenem(10µg)*
**P<0.05; **P<0.01; ***P<0.001; NS Not Signi›Cant. The Two Time Periods Were Compared Using $X^2$ Test*

| Antimicrobial agent | Number of resistant isolates | |
|---|---|---|
| | 2008-2012 | 2003-2007 |
| **S. aureus** | **378/2155** | **266/1435** |
| Cefoxitin (30µg) | 260(68.8%) | 167(62.8%) NS |
| Tetracycline(30µg) | 178(47%) | 122(45.8%) NS |
| Erythromycin(15µg) | 283(74.8%) | 191(71.8%) NS |
| Cotrimoxazole (1.25/23.75µg) | 298(78.8%) | 204(76.7%) NS |
| Ciprofloxacin(5µg) | 163(43.1%) | 117(44%) NS |
| Vancomycin(30µg) | 0 | 0 |

*Table 3: Comparison of Antimicrobial Resistance In S.Aureus*
**P<0.05; **P<0.01; ***P<0.001; NS Not Signi›Cant. The Two Time Periods Were Compared Using $X^2$ Test*

**4. Discussion**

The main objective was to analyse the changes in bacterial isolates from burn wounds and their antibiograms. In this study, considerable shifts were seen both in the spectrum of bacteria isolated and in their antibiotic susceptibility. The infectivity rate in our study was 81.6%. This high rate is because we receive the wound swabs from Burn unit mostly after three days of admission. Infection rate was 83.3% from 2008-2012 than 79.3% from 2003-2007. This could be explained by the fact that sample collection method was re-emphasised intermittently from 2008-2012. Bacteria rapidly colonise burn wounds following injury. They originate from the patient's endogenous skin, gastrointestinal and respiratory flora, and may also be transferred via contact with contaminated external surfaces and soiled hands of healthcare workers [8]. Prevalence of Gram-positive and gram-negative bacteria is known to be highly variable, differing greatly not only between burn centres, but also within individual hospitals. In the present study, Pseudomonas spp., Staphylococcus aureus and Klebsiella spp. remained the most common isolates in burn patients. These results were similar to results from other studies [9, 10, 11, 12]. As reported in other studies, multi-drug resistant Acinetobacter spp. have emerged as a significant cause of wound infection in our burn unit. According to Singh et al and others, presence of Acinetobacter spp. as normal skin flora, its easy transmissibility and ability to remain viable in a hospital environment due to its multi-drug resistant status and several other factors have been implicated in the increased incidence of nosocomial infections due to this organism [12, 13, and 14]. Isolation rates of Klebsiella spp. & Acinetobacter spp. have increased in 2008-2012 when compared with data from 2003-2007 from our burn unit. Conversely, isolation rates of Pseudomonas spp., Proteus spp and Enterococcus fecalis have decreased over the same period. The pattern of bacterial resistance is important for epidemiological and clinical purposes. The resulting antibiogram give serious cause for concern because the predominant bacterial isolates were highly resistant to the commonly available antimicrobials. The incidence of Pseudomonas, resistant to ceftazidime (81.9%), gentamicin (86.9%), amikacin (77.9%), ciprofloxacin (55%) and piperacillin-tazobactam (37%) is higher in our study and resistance has significantly increased in comparison to previous 5 years which is comparable to other studies [15,16,17]. Treatment options for multi-drug resistant strains are very limited. Ninety four percent of Acinetobacter spp. was resistant to most of the antibiotics tested which is similar to other studies, Sengupta et al and others particularly from India [14, 15, 17]. Recently, MRSA has emerged as a significant nosocomial pathogen. In the present study, the incidence of MRSA among Staphylococcus aureus isolates was 68.8% which is similar to reports particularly from India [14]. Presently, Carbapenems, Glycopeptides and Oxazolidinone are effective drugs for these drug resistant pathogens. Such high antimicrobial resistance reported is probably due to empirical use of broad spectrum antibiotics which exerts selective pressure on bacteria thereby promoting multi-drug resistant (MDR) strains. The early detection of isolates is very important to prevent treatment failure as the time involved in isolation, identification and performing antibiotic sensitivity can take as long as 48 h from the time sample is received. This time period may be enough to allow a sub clinical infection to become life threatening illness. Another factor

adding to the complication is multi-drug resistance of the organism. These MDR strains once established in hospital environment can persist for months in a unit and can infect patients being treated there [15, 16].

**5. Conclusion**

In our study we found there was a considerable shift in the spectrum of major aerobic bacteria infecting the burn wounds from Jan 2003-Dec 2007 to Jan 2008- Dec 2012. Isolation of Klebsiella spp. & Acinetobacter spp. increased while Pseudomonas spp., Proteus spp and Enterococcus faecalis decreased from 2003-07 to 2008-12. Acinetobacter spp. emerged as a significant cause of burn wound infection. Also significant difference in the resistance pattern of various gram negative isolates was found. The commonly isolated gram negative bacilli showed increase resistance to amikacin and ciprofloxacin, also the resistance to piperacillin-tazobactam increased substantially which is comparatively a recent antibiotic. Hence detailed knowledge of the organisms causing infectious complications and their antimicrobial susceptibility profile is a prerequisite for treating burn patients. Our study also shows that a continuous monitoring of microbial spectrum and their antibiogram is absolutely essential to determine changing trends in resistance pattern and to plan empirical drug use policy.

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