Multidrug Resistant *Pseudomonas aeruginosa* and *Klebsiella* spp Dual Isolate, causing Hospital Acquired Burn Wound Infection in Burn Care Unit of a Tertiary Care Hospital in North India

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**Abstract**

Hospital acquired infections (HAI) affect 1 in 10 patients admitted to hospital. They are associated with prolonged hospital stay, mortality and health care costs. If patients survive the initial burn and resuscitative phase, infections are a leading cause of mortality. Also Burn patients are at a higher risk of acquiring HAI with Multidrug resistant (MDR) organisms. Furthermore, infections with MDR pathogens increase morbidity, decrease treatment success, reduce hospital turn-over rate and increase cost of patient care. Samples falling under the inclusion criteria of the study and manifesting any symptoms and signs of hospital acquired burn wound infection during the management of burns were included in the study. HAI were defined based on CDC criteria. Swabs were collected from the wound showing signs of infection and were transported to microbiology laboratory for processing. Positive Isolates were confirmed by conventional biochemical tests. And isolates exhibiting ambiguous taxonomic classification were confirmed by Vitek-2 Compact Automated Identification System. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. A total of 71 percent (71/100) of patients developed HAI out of these 71 patients majority (74%) developed burn wound infection. Among the total of 92 pus samples processed 80.43% were positive while as 19.56 % were negative. Out of the total culture positive samples 42.3% showed multiple isolate which mostly comprised of two organisms. While as 19.56 % showed no growth. Dual isolates of *P.aeruginosa* + *Klebsiella* spp constituted a majority (50%) of the positives. *P.aeruginosa* alone was isolated from 16.21% samples followed by *Klebsiella* (12.16%) and *E .coli* (5.4%).All the isolates of most frequently isolated organism *Pseudomonas aeruginosa* were sensitive to polymyxin –b .6.9% were sensitive to imipenem and only 4 % were sensitive to levofloxicin. This isolated Pseudomonas was seen to be resistant to most of the other tested drugs. Out of the 46 *Klebsiella* isolates all were sensitive to polymyxin –b and to tigecycline, 15(32.6%) were sensitive to minocycine and 2(4.3%) were sensitive to ceftriaxone. HAI with MDR organisms and lack of newer antimicrobial agents with activity against them continues to be a major problem as well as a challenge for microbiologists and clinicians alike .Antibiotic stewardship, tailored infection control policies, regular screening of ICU’S are essential to combat this challenge.

**Keywords**

Hospital Acquired Infection, Burn Wound Infection, *Pseudomonas Aeruginosa*, *Klebsiella*, MDR, Burn Care Unit

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Introduction

Hospital acquired infections (HAI) are defined as infections that are not present or incubating at the time of hospital admission and develop after 48 hours or more of admission, 3 days of discharge or 30 days of an operation (Chin-Hong and Guglielmo, 2017; Mayhall, 2003). They affect 1 in 10 patients admitted to hospital. They are associated with prolonged hospital stay, mortality and health care costs. If patients survive the initial burn and resuscitative phase, infections are a leading cause of mortality (75% of cases) in these patients (Lari and Alaghehbandan, 2000). The longer the patient stays in the hospital, the higher the chances for the patient to acquire hospital acquired infections in the wound (Anon Bhat and Vasaikar 2010; Church et al., 2006; Ugburo et al., 2004).

The most common types hospital acquired infection in the hospital setting are blood stream infection, urinary tract infections, pneumonias, and skin and soft tissue infections (Mayhall, 2003). Burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury. Burn wound infection is a serious problem because it causes a delay in epidermal maturation and leads to additional scar tissue formation. Invasion of microorganisms into the tissue layers below the dermis may also result in bacteremia, sepsis, and multiple-organ dysfunction syndrome (Church et al., 2006). Although the initial burn wound is sterile, immediately following thermal injury, these wounds become colonized with microorganisms (Cen et al., 2015). Gram positive bacteria that survive the thermal insult such as staphylococci located deep within sweat glands and hair follicles heavily colonize the wound surface within the first 48 hours (Soares de Macedo and Santos, 2006; Wang et al., 2010). Microorganisms transferred to a patient’s skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers can further contaminate the wound (Cen et al., 2015). Wound colonization by yeasts and fungi usually occurs later due to the use of broad-spectrum antibiotic therapy (Soares de Macedo and Santos 2006; Wang et al., 2010)

Immune suppression, intestinal bacterial translocation, extended hospitalization and invasive diagnostic and therapeutic procedures including intubation and catheterization can all contribute to contamination of burn wounds and development of systemic infection (Cen et al., 2015; Soares de Macedo and Santos, 2006). Changes in resistance pattern may be attributed to factors such as, cross infection, inappropriate use of antibiotics, selective pressure and even mutations in bacterial genome (Anon Lunawat et al., 2015) Prolonged courses of antibiotics, often in combination result in selection of multidrug resistant nosocomial strains (Leseva et al., 2013). Compared to other patients, burn patients are at a higher risk of acquiring hospital acquired infection from multidrug resistant gram negative bacilli, thereby limiting the choice of empirical therapy (Anon Gang et al., 1999; Glik et al., 2012; Yali et al., 2014). In addition, the profile of organisms causing burn wound infections changes with time and geographical location, and from primarily gram positive to gram negative (Agnihotri, Gupta, and Joshi 2004; Altoparlak et al., 2004). Various factors have been shown to increase the prevalence of BWIs leading to Blood Stream Infections (BSIs) in patients and these can be grouped into two; patient and microbial factors. Patient factors include: size of the injury Total Burn Surface Area (%TBSA), degree of burn, anatomical location, duration of hospital stay, systemic prophylaxis, co-morbidities (i.e. obesity, diabetes, immunosuppression,
malnutrition, HIV) and the extremes of age (Al Laham et al., 2013; Ngugi, 2013). The microbial factors are: virulence, numbers of organisms, motility, extra-cellular products such as proteinases, collagenases, hyaluronidase, exotoxins and antimicrobial resistance (Edwards-Jones, Greenwood, and Manchester Burns Research Group, 2003).

Antibiotic-resistant organisms have been implicated in infections of the burn wound, blood and other anatomic sites in patients with major thermal injury (Church et al., 2006; Embil et al., 2001; Hsueh et al., 1998).

Furthermore, infections with multi-drug resistant pathogens whether in hospitals or in the community increase morbidity, decrease treatment success, reduce hospital turn-over rate and increase cost of patient care (Alebachew et al., 2012).

Materials and Methods

This prospective study was conducted in the Department of Microbiology, Government Medical College Srinagar Kashmir India. The patients admitted to burn care unit with the following criteria were included in the study: No infection at the time of admission and up to 48 hrs (cultures negative); Length of stay in the hospital more than 48 hrs; Signs and symptoms suggestive of infection. Exclusion criteria: Patients referred from other hospitals; Infection acquired before 48 hours of admission.

Samples from patients falling under the inclusion criteria of the study and manifesting any symptoms and signs of hospital acquired infection during the management of burns were included in the study. Hospital acquired infections were defined based on CDC criteria as described below (Latham, 1996).

Surface swabs were collected from burn wounds after removing any dressings and topical antimicrobial agents and cleansing of the wound surface with sterile normal saline (Church et al., 2006). Swabs were collected from the depth of the wound showing signs of infection by Gently rolling swab over the surface of the wound approximately five times, focusing on area where there is evidence of pus or inflamed tissue. Specimen were places in the Stuarts transport media whenever indicated and transported to the microbiology laboratory for processing as soon as possible (Belba et al., 2013).

Positive Isolates were confirmed by conventional biochemical tests (Cheesbrough, 2006) and isolates exhibiting ambiguous taxonomic classification were confirmed by Vitek-2 Compact Automated Identification System following the manufacturer’s instructions.

Antimicrobial susceptibility testing were performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (clinical laboratory standards institute, 2015) Briefly, from a pure culture, 3- 5 selected colonies of bacteria were picked and transferred to a tube containing 5 ml sterile normal saline and mixed gently until a homogenous suspension was formed. Turbidity of the culture suspension was equilibrated to match 0.5 McFarland standards (Clinical laboratory standards institute, 2015). A sterile cotton swab was used and the excess suspension removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar (MHA). The plates were incubated at 37 °C for 18-24 hours and the diameters of the zone of inhibition around the disk were measured using verniercalipers in millimeters and interpreted according to CLSI 2015 criteria; sensitive, intermediate, and resistant. P. aeruginosa American Type
Culture Collection (ATCC) 35218, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as control organism.

Multidrug resistant (MDR) was defined as resistance to at least one antibiotic agent in three or more antimicrobial classes (Magiorakos *et al.*, 2012).

Antimicrobial classes tested were Penicillin class (ampicillin, penicillin); Cephalosporin class (ceftazidime, ceftriaxone); Aminoglycosides class (gentamicin); Tetracycline class (Tetracycline); fluoroquinolones class (ciprofloxacin); folate pathway inhibitors class (sulfamethoxazole-trimethoprim); phenicols class (chloramphenicol); macrolides class (erythromycin) (Magiorakos *et al.*, 2012).

**Results and Discussion**

A total of 100 patients were taken and 71 percent developed HAI and 29 percent didn’t develop any of the HAI. 71 percent (71/100) of patients developed HAI out of these 71 patients majority (74%) developed burn wound infection. Among the total of 92 pus samples processed, Majority of the samples 80.43% were positive while as 19.56 % were negative. Out of the total culture positive samples 42.3% showed multiple isolate which mostly comprised of two organisms (chart: 1). While as 19.56 % showed no growth.

### Table 1: Sensitivity profile of gram negative organisms isolated from pus

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>Pseudomonas</em> (n=49)</th>
<th><em>E.coli</em> (n=4)</th>
<th><em>Acinetobacter</em> (n=2)</th>
<th><em>Klebsiella</em> (n=46)</th>
<th><em>Proteus</em> (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>-</td>
<td>4</td>
<td>2</td>
<td>46</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin/tazobactum</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Polymyxin-b</td>
<td>49</td>
<td>4</td>
<td>2</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>0</td>
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<td>-</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Amoxicillin + clavulenic acid</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ampicillin/sulbactum</td>
<td>-</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Gentamicin</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Ertaopenem</td>
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<td>-</td>
<td>-</td>
<td>2</td>
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<tr>
<td>Cotrimoxazole</td>
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<td>-</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Tobramycin</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Cefepime</td>
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<tr>
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<td>Minocycline</td>
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<td>-</td>
<td>15</td>
<td>-</td>
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<td>Cefotaxime</td>
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<td>0</td>
<td>1</td>
<td>-</td>
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</tbody>
</table>
Dual isolates of *P. aeruginosa* + *Klebsiella* spp constituted a majority (50%) of the positives. *P. aeruginosa* alone was isolated from 16.21% samples followed by *Klebsiella* (12.16%) and *E. coli* (5.4%). (Chart: 2)

Sensitivity profile of organisms isolated from pus: All the isolates of most frequently isolated organism *Pseudomonas aeruginosa* were sensitive to polymyxin –b. 6.9% were sensitive to imepenem and only 4% were sensitive to levofloxacin. This isolated *Pseudomonas* was seen to be resistant to most of the other tested drugs. Out of the 46 *Klebsiella* isolates all were sensitive to polymyxin –b and to tigecycline, 15(32.6%) were sensitive to minocycine and 2(4.3%) were sensitive to ceftriaxone (Table 1).

HAI are a significant problem for health services in all countries, with important effects on the survival of high-risk patients, such as burn patients. Infections of burn sites are very dangerous problems that can compromise the patient’s survival and the outcome of reconstructive treatment (Anon n.d.).

Among the 100 HAI in our study, burn wound infection constituted 74%. Our study indicates here that the rate of development of HAI are substantially higher than similar studied from India (Taneja *et al.*, 2004) and also from other parts of the world (58,69).

Infection remains a foremost concern in the management of burn wounds because the
large raw area with its serous exudate may act as a huge culture plate on which organisms can establish and multiply (Gang et al., 1999). Fresh burns are usually sterile but progressively become colonized with one or more bacterial species as the patient’s stay in burn unit increases.

In our study, 38.04% of swab cultures yielded single isolates whereas multiple isolates were seen in 42.3% of the swab cultures. Overall, *P. aeruginosa*+ *Klebsiella* were the commonly isolated organism (50%) followed by pseudomonas alone (16.2%), *Klebsiella* alone (12.16%), *E. coli* (5.4%) MRSA (4%) and *Acinetobacter* spp. (2.70%) *Pseudomonas* spp has been found to be the major pathogen (66.2%) in burn patients and was also the most important cause of sepsis in them. As far as, the overall colonizing organism is concerned our findings are consistent with those reported by Kaushik et al., (Revathi, Puri, and Jain 1998). These authors reported that *Pseudomonas* was the most commonly cultured organism (54.2%) followed by *S. aureus* (20.8%). Similar observations were made by Revathi et al., who reported *P. aeruginosa* as the most common isolate (36%) from the burn patients followed by *S. aureus* (19%). Our observations are in contrast to a number of studies from various countries which reported *S. aureus* as the most common colonizing organisms in their patients et al., 1992) Ozumba and Jiburum (Ozumba and Jiburum, 2000) from Africa however, reported most common colonizing organism as *Klebsiella* species (26.7%) followed by *S. aureus*(25.6%).

In pus samples, commonly isolated organism *P. aeruginosa* was sensitive to polymyxin B in all the isolates (100%), 6.9% were sensitive to imipenem and only 4 % were sensitive to levofloxacin. Out of the 46 *Klebsiella* isolates from pus all were sensitive to polymyxin –b. 15(32.6%) were sensitive to minocycline and 2(4.3%) were sensitive to ceftriaxone. Hospital acquired isolates of *Klebsiella* are resistant to most antibiotic as a result of acquisition of multidrug resistance plasmids.

*Klebsiella* is known to carry plasmids encoding extended spectrum beta lactamases and carbapenemases and treatment with such strains is associated with treatment failure and death.

This study provides assessment of important aspects of hospital acquired burn wound infections. Gram negative organisms predominated in hospital acquired burn wound infections, with *P. aeruginosa*+ *Klebsiella* spp dual isolate being the most common. We found these isolates showed multiple drug resistance to commonly prescribed antimicrobial agents.

This study concludes that HAI’s with MDR organisms in particular continues to be a major problem for the healthcare facilities and a challenge for the microbiologists and clinicians alike. Lack of newer antimicrobial agents with activity against MDR organisms makes periodic studies on antimicrobial susceptibility patters very important. Were commend regular screening of ICU’s to give early warnings for the presence of MDR pathogens. Antibiotic stewardship and tailored infection control policies for each institution are essential to combat this challenge.

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