MULTI DRUG RESISTANT PSEUDOMONAS AERUGINOSA: A NOSOCOMIAL INFECTION THREAT IN BURN PATIENTS

ZULFIQAR A. NAQVI, KHURSHEED HASHMI*, QAZI M.RIZWAN** AND SALEEM A. KHARAL**

Department of Pathology, Fatima Jinnah Dental College, Karachi, Pakistan
*Department of Pathology, Sindh Medical College, Dow University of Health Sciences, Karachi, Pakistan
**Department of Microbiology, Basic Medical Sciences Institute, Jinnah Post-Graduate Medical Centre, Karachi, Pakistan

ABSTRACT

Pseudomonas aeruginosa remains the leading pathogen causing burn wound infection. It is found as major colonizer of the burn wound because it thrives on moist burn wound surface and survives well in the hospital environment, once it is established, it can persist for months within a unit, and poses as multi drug resistant nosocomial infection threat for patients being treated there. The emergence of multi drug resistant Pseudomonas aeruginosa in burn wound is becoming a challenging problem in infection control programmes. A total of 44 isolates of Pseudomonas aeruginosa were recovered from burn patients. Most of them were resistant to multiple antibiotics. Their sensitivity against Imipenem was over all better than the other drugs i.e. 77.3%. Ciprofloxacin was the second most effective drug against this organism with a sensitivity of 54.5% while a 4th generation cephalosporin, Cefepime was effective against 22 (50%) isolates. About 30% Pseudomonas aeruginosa were sensitive to Amikacin. Aztreonam showed inhibitory activity against (6.8%) strains. Piperacillin activity was 18.2%. The efficacy of Ceftriaxone was 4.5%. Chloramphenicol and Septran were 100% inactive against Pseudomonas infection while > 95% strains of Pseudomonas aeruginosa were resistant to Tobramycin.

Keywords: Burns, Pseudomonas aeruginosa, multiple drug resistance, Karachi.

INTRODUCTION

Burn injury is a major public health problem in many countries of the world (Song and Lee, 2001). It is one of the most common injuries accounting for 3% of total admission (Calder, 2002). These injuries still show significant morbidity and mortality in developing countries (Barret et.al.1999). Infection in burn patients is difficult to control due to the presence of dead and denatured burn eschar, and moist environment, that act as a good growth medium for microbes. Prolonged hospital stay and invasive diagnostic and therapeutic procedures (Gang et al., 1999; Bang and associates, 1998). Impaired cellular and humoral immunity in these patients with lymphocytopenia, decreased IL2, inflammatory reaction, neutrophil chemotactic, phagocytic intracellular enzyme activity bactericidal activity, immunoglobulin and complement favor the origin and continuity of infection (Kirk, 2000, Oralankul et al., 2002 and Fuchs et al., 2002).

Due to prolonged hospital stay these patients are at high risk of nosocomial infection. In this situation topical antimicrobial agents play a limited role that reduces the incidence of septic complication but the incidence of bacterial colonization had not decreased (Gang et al., 1999 and Manson et
Multi drug resistant Pseudomonas aeruginosa

It is estimated that as many as 75% of all deaths following burn injury are related to infection (Vindenes et al., 1995).

Pseudomonas aeruginosa is found as major colonizer of the burn wound because it thrives on moist burn wound surface and usually gains access to burn patients through cross contamination. It persists as a major nosocomial infection threat to burn patients. Arising of resistance against multiple antimicrobial drugs frequently complicates the treatment of Pseudomonas aeruginosa infection. This may lead to serious infection and thus mortality rate in these patients becomes high (Holder et al., 1995; Lari and associates, 2000; Esthabanati et al., 2002).

The emergence of multi-resistance Pseudomonas aeruginosa in burn wound is becoming a challenging problem in infection control programmes (Douglas, 2001). It is almost always predominant in monobacterial as well as polybacterial infection (Nagoba et al., 1999).

MATERIAL AND METHODS

This study was conducted between November 2002 to February 2003 in the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi.

A total of 52 infected patients irrespective of age, sex, degree and percentage of burn, admitted in three different hospitals of Karachi, were included in this study. On the basis of clinical judgment of infection, 170 swabs of pus from infected burn wound were collected at the time of change of dressing. Swabs were collected every week for up to four weeks (Song and Lee, 2001; Fuchs et al., 2002).

Following collection, Specimen was immersed in Stuart’s transport medium and transported to laboratory without delay. In the laboratory swabs were inoculated on Mac-Key’s agar, Blood agar and Nutrient agar and plates were incubated at 37°C over night. Initial diagnosis of isolates was made on the basis of Gram’s staining of pus and culture, colonial morphology on different media, hemolysis on Blood agar, pigment production on nutrient agar and smell in cultures, and Oxidase test. Pseudomonas aeruginosa isolates were confirmed by certain biochemical tests including Citrate utilization, Ausculin hydrolysis, Gelatin hydrolysis, Nitrate reduction and growth at 42°C. In addition to these tests, Sugar fermentation tests including Glucose, Sucrose, Maltose were also performed. The susceptibility test of Pseudomonas aeruginosa isolates were performed by Kirby Bauer method in accordance with NCCLS guidelines (NCCLS, 1998).

RESULTS

Table 1 shows the relation of mode of burn with age, sex and total burnt surface area (TBSA) in 31 patients infected by P. aeruginosa. Flame burn was the over all predominant cause of burn injuries in 25 (80.6%) patients. Scalded burn was the second common cause of burn injury in 05(16.2%) of patients. Remaining one patient (3.2%) got chemical (acid) burn. Ten out of 25 patients (40%) who acquired burn injury by flame, were aged up to 12 years. While, remaining 15 (60%) patients were older than 12 years.

Patients affected by flame burn belonged to both sexes in which Male were 12 (48%) and Female were 13 (52%). In all patients affected by flame burn, TBSA up to 15% was found in 07(28%) and TBSA > 15% was found in 18(72%) patients. Scalded burn was found in 05 patients. All female patients were older than 12 years. In these patients TBSA up to 15% was found in 03 (60%) patients and TBSA > 15% found in 02(40%) patients. One patient that was affected by acid burn was male having age >12 years and TBSA > 15%.

Table-2 shows the summary of the study in which 52 patients were included. Out of these 31(59.6%) patients were infected by P.
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Table-1
Relation of Mode of burn with Age, Sex, and Total burn surface area (TBSA) in 31 patients infected by \textit{P. aeruginosa}

<table>
<thead>
<tr>
<th>Cause of Burn</th>
<th>No. of Patients (%)</th>
<th>Age</th>
<th>Sex</th>
<th>TBSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Up to 12 years</td>
<td>&gt; 12 years</td>
<td>Male</td>
</tr>
<tr>
<td>Flame</td>
<td>25 (80.6)</td>
<td>15 (60%)</td>
<td>10 (40%)</td>
<td>13</td>
</tr>
<tr>
<td>Scald</td>
<td>05 (16.2)</td>
<td>05 (100%)</td>
<td>00 (00%)</td>
<td>00</td>
</tr>
<tr>
<td>Acid</td>
<td>01 (3.2)</td>
<td>00 (00%)</td>
<td>01 (100%)</td>
<td>01</td>
</tr>
</tbody>
</table>

Table-2
Isolation of \textit{P. aeruginosa} in 52 patients

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Infected by \textit{P. aeruginosa}</th>
<th>Cultures (n)</th>
<th>Positive growth n (%)</th>
<th>Total isolates n (%)</th>
<th>\textit{P. aeruginosa} n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>31 (59.6%)</td>
<td>170</td>
<td>152 (89.4%)</td>
<td>190</td>
<td>44 (23.1%)</td>
</tr>
</tbody>
</table>

\textit{PTS;} = Patients, \textit{INF;} = Infection

\textit{P. aeruginosa}. A total of 170 burn wound swabs were collected in which 152 (89.4%) yielded positive growth and total 190 organisms were isolated and identified in which 44 (23.1%) organisms were \textit{P. aeruginosa}.

Table-3 shows the sensitivity and resistance pattern of \textit{P. aeruginosa}. Imipenem was the most active drug against \textit{P. aeruginosa} that was active against 34 (77.3%) isolates. Ciprofloxacin was the 2nd active drug being effective against 24 (54.5%) isolates and Cefepime was the 3rd active drug against 22 (50.0%) isolates of \textit{P. aeruginosa}. Amikacin and Piperacillin were active only against 13 (29.5%) and 08 (18.2%) isolates respectively. Inactivity of Septran and Chloramphenicol was absolute, while > 91% strains of \textit{P. aeruginosa} were resistant to Cefutaxime, Tobramycin, Gentamicin and Aztreonam.

Table 4 shows the multi-drug resistance of \textit{P. aeruginosa} against battery of antimicrobial drugs. Out of all 44 isolates of \textit{P. aeruginosa}, one strain was resistant to all antimicrobial drugs, 14 isolates were resistant to eleven (11) antibiotics, 10 isolates were resistant to eight (8) drugs, 08 isolates were resistant to seven (7) drugs, one isolates was resistant to 05 drugs while two (02) organisms were resistant to one to two drugs respectively.

\textbf{DISCUSSION}

It is generally recognized that heavy bacterial wound colonization is more likely to lead to wound sepsis; this may reflect the current status of the wound. Colonization may occur more rapidly when the condition of wound is poor (Ozumba and Jiburum, 2000).

Infection is common in extreme of ages (Edwards and Greenwood, 2003). Total burn surface area is found to be the most important risk factor for nosocomial infection (Oralankul...
P. aeruginosa remains the leading pathogen causing burn wound infection (Lari and Bahrami, 1998). It survives well in the hospital environment. Once it is established, it can persist for months within a unit, posing as multi-drug resistant nosocomial infection risk for patients being treated there. Hands of staff members can become transiently contaminated and transfer infection among patients (Douglas, 2001; Mokadas and Mustafa, 1996; Edwards 2003).

In the present study 44 (23.1%) isolates of P. aeruginosa were recovered from burn patients. This finding is similar to several other studies Edwards and Greenwood, 2003, Nasser, 2003; Santucci et al., 2003). However, some other studies have reported the lower (15% or less) recovery rate of P. aeruginosa (Bang, 1998; Vindenes, 1995; Appelgreen, 2002). A significant number of P. aeruginosa (74%) was found in a study conducted in Tohid Burn Centre Tehran Iran (Lari and Bahrami, 1998). Authors suggested that this high frequency of P. aeruginosa might be due to prolonged hospital stay and intensive use of antibiotics.

There is no antimicrobial drug to which resistance has not eventually appeared (Neely and Holder, 1999). High frequency and nature of antibiotic resistance may be due to over usage of antibiotics such as Ciprofloxacin, Gentamicin and Amikacin as well as non-availability and high cost of preferred antibiotics of choice (Lari, 2000). β-Lactam antibiotics have been shown to cause Gram-negative problems with high number of

### Table-3

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disk content</th>
<th>Sensitive (%)</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>10 µg</td>
<td>03 (6.8)</td>
<td>41 (93.2)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>100 µg</td>
<td>08 (18.2)</td>
<td>36 (81.8)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 µg</td>
<td>13 (29.5)</td>
<td>21 (70.5)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>30 µg</td>
<td>03 (6.8)</td>
<td>41 (91.2)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>30 µg</td>
<td>22 (50.0)</td>
<td>22 (50.0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>05 µg</td>
<td>24 (54.5)</td>
<td>20 (45.5)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>10 µg</td>
<td>34 (77.3)</td>
<td>10 (32.7)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>10 µg</td>
<td>02 (4.5)</td>
<td>42 (95.5)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30 µg</td>
<td>02 (4.5)</td>
<td>42 (95.5)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>00</td>
<td>44 (100)</td>
</tr>
<tr>
<td>Septran</td>
<td>05 µg</td>
<td>00</td>
<td>44 (100)</td>
</tr>
</tbody>
</table>

### Table-4

Distribution of Resistance among 44 P. aeruginosa isolates against 11 antimicrobial drugs

<table>
<thead>
<tr>
<th>Resistant to 11</th>
<th>Resistant to 10</th>
<th>Resistant to 9</th>
<th>Resistant to 8</th>
<th>Resistant to 7</th>
<th>Resistant to 5</th>
<th>Resistant to 1 or 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 (02.3%)</td>
<td>14 (31.8%)</td>
<td>08 (18.2%)</td>
<td>10 (22.7%)</td>
<td>08 (18.2%)</td>
<td>01 (02.3%)</td>
<td>02 (04.6%)</td>
</tr>
</tbody>
</table>
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Increasingly bacteria are becoming multiple antibiotic resistant, leaving little or no effective systemic treatment option (Edwards & Greenwood, 2003). This bacterial resistance to antimicrobial agents is an important public health problem in both the developing and the developed countries, in which many of these organisms are multiple drug resistant i.e., resistant to two or more antibiotics to which the bacteria were usually susceptible (Neely and Holder, 1999; Ansari, 1995; Parsanna and Thomas, 1999). Immuno-compromised burn patients, who receive multiple antibiotics, are essentially incubator for antibiotic resistant strains. The development of resistance is progressive, evolving from low level through intermediate to high levels with the exception of direct transfer of genetic information, which can result in immediate high resistance (Neely and Holder, 1999).

Pseudomonas is very resistant to most antibiotics and the resistance in this organism develops very rapidly. The rate of development of resistance to new antibiotics is much faster than the rate of invention and development of new antibiotics (Estahbanati, 2002 and Zhang, 1992).

Carbepenems are useful in treatment of some cases of multi-drug resistant strains of Pseudomonas aeruginosa (Douglas, 2001). In this study also most of Pseudomonas aeruginosa strains were MDR and their sensitivity against Imipenem, though not ideal, was comparatively better than the other drugs i.e., 77.3%.

In some studies the sensitivity of Imipenem against Pseudomonas aeruginosa was relatively more i.e. 86% 78%, 88% and 91.6% respectively (Neely and Holder, 1999; Xu and Sun, 1998; Mokadas and Mustafa, 1996, Ronald et al., 1998). But the resistance of P. aeruginosa was much higher (48%) against this drug in a study conducted by Singh et al. in Korea in 2001 (Song et al. 2001). In an Indian study conducted in 1999, Imipenem was active against 43% isolates of P. aeruginosa (Nagoba, 1999).

Ciprofloxacin has been reported as the second most effective drug against Pseudomonas aeruginosa (Kaushik and Kumar, 2001). In the present study also it was the second most effective drug against this organism i.e. 54.5% isolates were sensitive to Ciprofloxacin.

In a study conducted between 1995 and 1997 at Tohid burn centre Tehran Iran by Lari and colleagues, 82% strains of Pseudomonas aeruginosa were resistant to Amikacin.33 While in another study conducted in a burn center of Tehran 67.4% strains were resistant to Amikacin (Estahbanati, 2002). The findings in the present study are in accordance with these studies, with about 70.5% resistance in P. aeruginosa against this drug. Interestingly 100% isolates of Pseudomonas aeruginosa were resistant to Amikacin in a retrospective report of ministry of health, Muscat Oman (Prasanna and Thomas, 1999).

In the present study 93.2% P. aeruginosa isolates showed resistance against Gentamicin. This finding is similar to the study conducted in Tohid burn centre Tehran Iran, where more than 95% strains of Pseudomonas aeruginosa were resistant to Gentamicin (Lari, 1998). Gentamicin is a cheap and easily available drug that is used extensively in general and hospital practice in clinically suspected Gram-negative infections. This may be the main reason for the development of resistance in bacteria against this drug.

Aztreonam is a monobactam β-lactam drug. It has excellent activity against Pseudomonas species but has a limited treatment option against MDR strains of Pseudomonas aeruginosa (Douglas, 2001). Same was the case in the present study where Aztreonam was active only against 6.8% P. aeruginosa isolates and most isolates were MDR strains.
Piperacillin was active only against 18.2% Pseudomonas aeruginosa isolates. This finding is unique from other studies in which Pseudomonas aeruginosa remained 80-90% susceptible to Piperacillin (Mokadas and Mustafa, 1996; Walton et al., 1997).

The present data regarding the efficacy of Cefutaxime i.e., 4.5% against P. aeruginosa correlates with the study conducted in Tohid Burn Centre Tehran, Iran where P. aeruginosa were recovered as an agent of out break and over 95% Pseudomonas aeruginosa were resistant to Cefixoxime. Over usage of the antibiotics was the main reason of resistance suggested by the authors (Lari, 1998). This seems to be the case in the present study as well.

In a study from United States of America in 1997 Cefutaxime was effective against only 18% strains of non-enteric Gram-negative bacilli (Ronald, 1998). Another study conducted in New Delhi India by Ronald et al., 66% strains of P. aeruginosa were resistant to Cefutaxime (Singh et al., 2003).

This study indicates that the 4th generation cephalosporin, Cefepime was effective against 22 (50%) isolates. Chloramphenicol and Septran were totally inactive against Pseudomonas isolates while > 95% strains of P. aeruginosa were resistant to Tobramycin.

The factors which might have resulted in infection by multi-resistant micro-organisms lack of knowledge about infection control measures in hospital workers and absence of infection control program in hospitals. Over crowding of patients as well as visitors in burn unit, poor isolation between patients, unhygienic conditions of patients as well as burns unit and misuse of broad spectrum antibiotics may be some other factors.

A strict antibiotic policy and establishment of infection control programs, will help to lower the incidence of resistance in hospitalized, especially burn patients.

REFERENCES


