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

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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 Cefutaxime was 4.5%. Chloramphenicol and Septran were 100% inactive against Pseudomonas infection while > 95% strains of Pseudomonas aeruginosa were resistant to Tobramycin.

Keywords: Burns, Pseudo*monas 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*

al., 1992). 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-Conkey'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 i*solates were confirmed by certain bio-chemical 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 Pseudom-onas 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.*

Table-1
Relation of Mode of burn with Age, Sex, and Total burn surface area (TBSA) in 31 patients infected by *P. aeruginosa*

Cause of Burn	No. of Patients (%)	Age		Sex		TBSA	
		Up to 12 years	> 12 years	Male	Female	Up to 15%	>15%
		15	16	13	18	10	21
Flame	25 (80.6)	10 (40%)	15 (60%)	12 (48%)	13 (52%)	07 (28%)	18 (72%)
Scald	05 (16.2)	05 (100%)	00 (00%)	00 (00%)	05 (100)	03 (60%)	02 (40%)
Acid	01 (3.2)	00 (00%)	01 (100%)	01 (100%)	00 (00%)	00 (00%)	01 (100%)

Table-2
Isolation of *P. aeruginosa* in 52 patients

Patients (n)	Infected by *P. aeruginosa*	Cultures (n)	Positive growth n (%)	Total isolates n (%)	*P. aeuginosa* n (%)
52	31 (59.6%)	170	152 (89.4%)	190	44 (23.1%)

PTS; = Patients, INF; =Infecte

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 *P. aeruginosa.*

Table-3 shows the sensitivity and resistance pattern of *P. aeruginosa*. Imipenem was the most active drug against *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 *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 *P. aeruginosa* were resistant to Cefutaxime, Tobramycin, Gentamicin and Aztreonam.

Table 4 shows the multi-drug resistance of *P. aeruginosa* against battery of antimicrobial drugs. Out of all 44 isolates of *P. aeruginosa*, one strain was resistant to all antimicrobial drugs, 14 isolates were resistant to eleven (11) drugs, 08 isolates were resistant to nine (9) 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.

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

Table-3
Sensitivity pattern of *P. aeruginosa* isolates (n =44)

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

Table-4
Distribution of Resistance among 44 *P. aeruginosa* isolates
against 11 antimicrobial drugs

Resistant to 11	Resistant to10	Resistant to 9	Resistant to 8	Resistant to 7	Resistant to 5	Resistant to 1 or 2
01 (02.3%)	14 (31.8%)	08 (18.2%)	10 (22.7%)	08 (18.2%)	01 (02.3%)	02 (04.6%)

et al., 200). These observations are not reflected in present study, because almost all the patients were the victims of infection.

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

courses of empirical treatment (Appelgreen, 2002). 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 Ceftizoxime. 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

Ansari, M.Z. (1995). Antibiotic resistance: Epidemiology and Strategies for prevention. *J Pak Med Assoc*., **45**(1): 18-22.

Appelgren, P., Bjornhagen, V., Bragderyd, K., Jonsson, C.E. and Ransjo, U. (2002). A prospective study of infections in burn patients. *Burns*, **28**(1): 39-46.

Bang, R.L., Gang, R.K., Sanyal, S.C., Mokaddas, E. and Ebrahim, M.K. (1998). Burn septicaemia: An analysis of 79 patients. *Burns*, **24**(4): 354-361.

Barret, J.P., Gomex, P., Solano, I., Gonzalez-Derrego, M and Crisol, F.J. (1999). Epidemiology and mortality of adult burns in Catalonia. *Burns*, **25**(4): 325-329.

Calder, F. (2002). Four years of burn injuries in a Red Cross Hospital in Afghanistan. *Burns,* **28**(6): 563-568.

Douglas, M.W., Mulholland, K., Denyer, V. and Gottlieb, T. (2001). Multi-drug resistant Pseudomonas aeruginosa outbreak in a burns unit an infection control study. *Burns*, **27**(2): 131-135.

Edwards, V. and Greenwood, J. (2003). What's new in burn microbiology? James Laing Memorial Prize Essay 2000. *Burns*, **29**(1): 15-24.

Estahbanati, H.K., Kashani, P.P. and Ghanaatpisheh, F. (2002). Frequency of *P. aeruginosa* serotypes in burn wound infections and their resistance antibiotics. *Burns*, **28**(4): 340-348.

Fuchs, P.C., Kopp, J., Hafner, H., Kleiner, U. and Pallua, N. (2002). MRSA retrospective analysis of an outbreak in the burn center Aachen. *Burns*, **28**(6): 575-578.

Gang, R.K., Bang, R.L., Sanyal, S.C., Mokaddas, E. and Lari, A.R. (1999). *P. aeruginosa* septicaemia in burns. *Burns*, **25**: 611-616.

Holder, A., Volpel, K., Ronald, G. and Paranchych (1995). Studies on multiple Pseudomonas aeruginosa isolates from individual burn patients by RFLP, O antigen serotyping and antibiogram analysis. *Burns*, **21**(6): 441-444.

Kaushik, R., Kumar, S., Sharma, R. and Lal, P. (2001). Bacteriology of burn wounds the first three years in a new burn unit at the Medical College Chandigarh. *Burns*, **27**(6): 595-597.

Kirk, R.M. (2000). Clinical Surgery in General. 3rd Edition. Churchill Living-stone, pp.47-49.

Lari, A.K., Bahrami, H.H. and Alaghehbandan, R. (1998). Pseudomonas infection in Tohid Burn Centre, Iran. *Burns*, **24**(7): 637-641.

Lari, A.R. and Alaghebandan, R. (2000). Nasocomial infections in an Iranian burn care center. *Burns,* **26**(8): 737-740.

Manson, W.L., Pernot, P.C.J., Fidler, V., Sauer, E.W. and Kalasen, H.J. (1992). Colonization of burns and the duration of hospital stay of severely burned patients. *J Hosp Infect*., **22**: 55-63.

Mokadas, E.M.A., Mustafa, A.S. and Sanyal, S.C. (1996). The prevalence, antibiotic and plasmid profiles of methicillin – resistant Staphylococcus aureus (MRSA) in the Burns Unit of a Kuwaiti Hospital. *J Kuwait Med Assoc*., **28**(4): 435-439.

Nagoba, B.S., Deshmukh, S.R., Wadher, B.J. and Pathan, A.B. (1999). Bacteriological analysis of burn sepsis. *Indian J Med Sc*., **53**(5): 216-219.

Nasser, S., Mabrouk, A. and Maher, A. (2003). Colonization of burn wounds in Ain Shams University Burn Unit. *Burns*, **29**(3): 229-233.

NCCLs Performance of standard for antimicrobial susceptibility testing; eighth international supplement, (1998). **18**(1): 100-513.

Neely, A.N. and Holder, I.A. (1999). Antimicrobial resistance. *Burns*, **25**(1): 17-24.

Oraloncul, Yuksel, F., Altunay, H., Acikel, C., Celikoz, B. and Cavulu, A. (2002). The evaluation of nasocomial infection during 1st year period in the burn unit of a training hospital in Istanbul, Turkey. *Burns,* **28**(8): 738-744.

Ozumba, U.C. and Jiburum, B.C. (2000). Bacteriology of burn wound in Enugu, Nigeria. *Burns*, **26**(2): 178-180.

Prasanna, M. and Thomas, C. (1999). A profile of methicillin resistant *S. aureus* infection in the burn center of the Sultanate of Oman. *Burns,* **24**(7): 631-636.

Ronald, N.J., Pfaller, M.A., Doern, G.V., Erwin, M.E. and Hollis, R.J. (The Cefepime Study Group, 1998). Antimicrobial activity and spectrum investigation of eight broad spectrum Beta lactam Drugs: A 1997 Surveillance Trial in 102 Medical Centres in the United States. *Diagnostic Microbiol Infec Dis*., **30**(3): 215-0228.

Santucci, S.G., Gobara, S., Santos, C.R., Fontana, C. and Levin, A.S. (2003). Infections in a burn intensive care unit: Experience of seven years. *J Hosp Infect.,* **53**(1): 6-13.

Singh, N.P., Goyal, R., Manchanda, V., Das, S., Kaur, I. and Talwar, V. (2003). Changing trends in bacteriology of burns in the burns unit, Delhi, India. *Burns*, **29**(2): 129-132.

Song, W., Lee, K.M., Kang, H.J., Shin, D.H. and Kim, D.K. (2001). Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. *Burns*, **27**(2): 136-139.

Vindenes, H. and Bjerknes, R. (1995). Microbial colonization of large wounds. *Burns*, **21**(8): 575-579.

Walton, M.A., Villarreal, C., Herndon, D.N. and Heggers, J.P. (1997). The use of aztreonam as an alternative therapy for multi-resistant *P. aeruginosa*. *Burns*, **23**(3): 225-7.

Xu, W., Sun, Z. and Chen, X. (1998). An analysis of bacteria resistance to antimicrobial agents in burn center. *Chung Hua Cheng Hsing Shao Shang Wai Ko Tsa China,* **14**(3): 199-202.

Zhang, H. (1992). Sensitivity of Staph aureus and *P. aeruginosa* and clinical application of antibiotics in patients with burn septicemia. [Article in Chinese], **30**(11): 682-3, 700.