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Research Article

Multi-drug Resistant *Pseudomonas aeruginosa* Isolated from Intensive Care Burn Unit

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Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen that develops life-threatening infections in patients with immunological system defects like burn patients. *P. aeruginosa* is naturally resistant as well as able to get acquired resistance to effective antibiotics which lead to problematic conditions. This study was designed to isolate *P. aeruginosa* from burn patients and to evaluate drug susceptibility for determination of multidrug-resistant isolates of *P. aeruginosa*.

Materials and Methods: A prospective study was carried out on the patients visiting Victoria Hospital, Kalasipalayan, Bangalore, India from Nov 15, 2010 to June 15, 2011. The pus sample was collected using sterile cotton swab from 100 patients with burn wound infections. *P. aeruginosa* was identified by standard bacteriological methods. The drug susceptibility pattern using 12 different antimicrobial agents (Amikacin, Ciprofloxacin, Gentamicin, Cefotaxime, Imipenem, Meropenem, Cefoperozone, Tobramycin, Piperacillin/Tazobactum, Cefepime, Ceftazidime, and Norfloxacin) was performed for all the isolates using Kirby Bauer's Disc Diffusion Method.

Results: *P. aeruginosa* were isolated from 17 clinical samples and all of these isolates were Multidrug Resistance *P. aeruginosa* (MDRPa). Resistance rates to various antibiotics were as follows: Amikacin (47.1%) Ciprofloxacin (35.5%) Gentamicin (47.1%) Cefotaxime (76.5%) Imipenem (88.2%) Meropenem (94.1%) Cefoperozone (94.1%) Tobramycin (100%) Piperacillin/Tazobactum (82.4%) Cefepime (64.7%) Ceftazidime (70.6%) Norfloxacin (70.6%).

Conclusion: Optimization of using antimicrobial agents and control of infection is recommended to prevent the increasing population of MDRPa in the new burn centre setting in this study.

Keywords: Multidrug resistance, *Pseudomonas aeruginosa*, burn patients

1. Introduction

Burn injury, one of the significant public health problems worldwide, is at high risk for nosocomial infections.[1] According to Swedish study, the most common infection was burn wound infection (60%) followed by blood stream infection (20%), urinary tract infection (20%), and pneumonia (10%).[2] The burn wound represents a susceptible site of opportunistic colonization by organisms of endogenous and exogenous origin such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* spp., and various coliform bacilli. Fungi like *Candida albicans*, *Aspergillus fumigatus* are also responsible for causing infection.[3,4] Burn patients are at risk for developing sepsis secondary to pneumonia, catheter-related infections, and suppurative thrombophlebitis.[5] Although present techniques of burn wound care have significantly reduced the incidence of infections, severely burned patients may still develop life-threatening infections.[6] *P. aeruginosa* is a leading cause of nosocomial infection, ranking second among the gram negative pathogens reported to National Nosocomial Infectious Surveillance (NNIS) system.[7] *P.aeruginosa* is an opportunistic pathogen causing severe, acute and chronic nosocomial infections in immunocompromised as well as catheterized or burn patients especially in developing countries.[8,9]

P.aeruginosa infections are problematic due to its intrinsic as well as acquired resistance to many effective groups of antibiotics.[6] Multi drug resistance *P. aeruginosa* (MDRPa) is defined as an isolate intermediate or resistant to at least three groups of antibiotics among β-lactams, carbapenems, aminoglycosides, and Fluoroquinolones.[10] Intrinsic MDRPa is attributed by limited permeability of outer membrane, production of inducible β- lactamase and Multidrug Efflux system.[7,11,12] Among four MDR efflux system in *P. aeruginosa*, MexAB-OprM and MexXY-OprM contribute to intrinsic resistance whereas hyperexpression of MexCD-OprJ and MexEF-OprN leads to acquired MDRPa.[13] Plasmid and integron have a crucial role in acquisition of mobile elements.[8]

Many nosocomial infection surveillances of Intensive Care Units (ICUs) and nosocomial outbreaks have demonstrated that *P.aeruginosa* is capable of long term persistence in hospital environment.[14,15] High prevalence of nosocomial infections and the presence of MDRPa suggest continuous surveillance of burn infections and develop strategies for antimicrobial resistance control and treatment of infectious complications.[16]

2. Materials and Methods

This prospective study was conducted on burn patients visiting Victoria Hospital, Kalasipalayan, Bangalore, India from Nov 15, 2010 to June 15, 2011. A total of 100 samples were collected from the burn patients. Pus sample were collected from wound using a sterile cotton swab and transported to the laboratory using Stuart's medium without delay. Speciemen were inoculated on MacConkey agar, Blood agar and Nutrient agar plates and the 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, haemolysis on Blood agar, pigment production on nutrient agar and smell in cultures. *P. aeruginosa* isolates were confirmed by biochemical tests including Oxidase test, Citrate utilization, Aesculin hydrolysis, Gelatin hydrolysis, Nitrate reduction and growth at 42°C. Sugar fermentation tests including Glucose, Sucrose, and Maltose were also performed. The antibiotic susceptibility test of *P. aeruginosa* isolates were performed by modified Kirby Bauer's Disc Diffusion method using 12 different antibiotics (Amikacin(30mcg), Ciprofloxacin(5mcg), Gentamicin(10mcg), Cefotaxime(30mcg), Imipenem(10

mcg), Meropenem (10mcg), Cefoperozone (75mcg), Tobramycin (10mcg) Piperacillin/Tazobactum (100mcg/10mcg), Cefepime (30mcg), Ceftazidime (30mcg) and Norfloxacin (10mcg) in accordance with NCCLs guidelines. The Statistical software namely SPSS 15.0, was used for the analysis of the data.

3. Results

Out of 100 clinical samples processed from the burn patient, *P. aeruginosa* was isolated from 17 samples followed by *Staphylococcus aureus Klebsiella* spp,*Proteus* spp *Enterococcus faecalis*,*Escherichia coli*, *Acinetobacter* spp.

The antibiotic susceptibility pattern showed 100% of the isolates were resistant to Tobramycin, and 94.1% were resistance to Cefoperazone and Meropenem followed by Amikacin and Gentamicin (47.1%) whereas the organism was least resistant to Ciprofloxacin (35.3%).

Antibiotic resistance pattern shows that all of the 17 isolates were Multidrug resistance *Pseudomonas aeruginosa* (MDRPa).

Fig1: Prevalence of *Pseudomonas aeruginosa* in samples studied

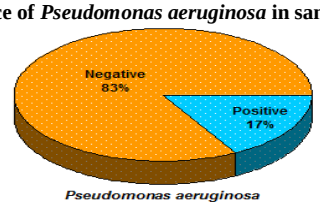
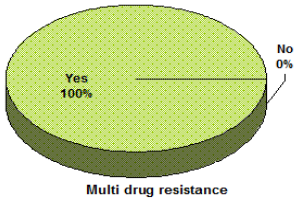


Table 1: Resistance pattern of isolates

S.N.	Antibiotics	Number of samples (n=17)	%	95%CI
1	Amikacin	8	47.1	26.17-69.04
2	Ciprofloxacin	6	35.3	17.31-68.70
3	Gentamicin	8	47.1	26.17-69.04
4	Cefotaxime	13	76.5	52.74-90.44
5	Imipenem	15	88.2	65.66-96.71
6	Meropenem	16	94.1	73.02-98.96
7	Cefoperazone	16	94.1	73.02-98.96
8	Tobramycin	17	100.0	81.57-100.00
9	Piperacillin-tazobactam	14	82.4	58.97-93.81
10	Cefepime	11	64.7	41.30-82.69
11	Cefatazidime	12	70.6	46.87-86.72
12	Norfloxacin	12	70.6	46.87-86.72

Figure 2: Multidrug resistance pattern of isolates



4. Discussion

Infection due to MDRPa has become a challenge in clinical practice and is not uncommon.[18] In MDRPa infections, susceptibility testing for antimicrobials that are not tested routinely and antibiotic synergy studies should be considered for synergistic effects. Furthermore, combination therapy may exert a selection pressure that allows only sub populations with the reduced virulence to be expressed.[3]

Out of 100 samples, the prevalence of *P.aeruginosa* was 17%. Similar study by Ahmad *et al.*, (2003) revealed higher prevalence of *Pseudomonas* Spp. (36%) followed by *Staphylococcus aureus* (19%), *Klebsiella* spp. (15.54%), *Proteus* spp. (11.19%), *Enterococcus faecalis* (8.5%), *Escherichia coli*(5.1%), *Acinetobacter* spp. (1.1%), *Salmonella senftenberg* (0.8%) and others (3%)[23]. Naser *et al.*, (2003) from Egypt have also reported *P. aeruginosa* as the most frequent isolate 21.6% .[18]

MDRPa have commonly been reported as colonizers of the wounds or the cause of nosocomial outbreaks infection in burn units. Among all MDRPa isolates, the highest resistance was shown by Tobramycin (100%), Meropenem (94.1%), Cefoperozone (94.1%), Imipenem (88.2%) Piperacillin/Tazobactum (82.4%), Cefotaxime (76.5%) Ceftazidime (70.6%) Norfloxacin (70.6%). The organisms were intermediately resistance to Cefepime (64.7%) and least resistance against Amikacin (47.1%), Gentamicin (47.1%), Ciprofloxacin (35.3%). Iraj *et al.*, (2013) showed that *P. aeruginosa* acquired high level resistance against Piperacillin (69.7%) Ceftazidime (68.6%) and Ciprofloxacin (63.3%). A low level resistance was recognized for Imipenem (23.3%) and Gentamicin (37.2%), while an intermediate level resistance was found against the Amikacin (48.8%) and Tobramycin (58.2%).[19] Higher rates of resistance to Aminoglycosides antibiotics, including Tobramycin (82 %), Amikacin (73%), and Gentamicin (80%), was reported by Bojary Nasrabadi *et al.*, (2012).[20]

In the present study, Imipenem was the most effective antibiotic against *P. aeruginosa* that is in consistent with other studies conducted at Iranian burn centers.[21,22] However, increasing Imipenem resistant strains have also been reported in other Iranian burn care centers in recent studies.[23,24] Similar studies by Ahmed *et al* (2013) showed that Amikacin was the most effective drug against all *P. aeruginosa* isolates with maximum sensitivity (80.5%) followed by Imipenem (66.7%) and Gentamicin (56.1%). On the other hand, *P. aeruginosa* had high resistance rates to Cefepime (98%) followed by Piperacillin\Tazobactum (94.7%), Ceftazidime (91%).[25] Study by Siva Gowri(2009) shows Piperacillin-Tazobactam as the most active antimicrobial agent with 91.8% susceptibility, followed by the Aminoglycosides (Amikacin, 86.6% and Gentamicin, 84.5%), the Quinolone (Ciprofloxacin, 83.5%) and the beta-lactams (Cefepime, 80.4%, Ceftazidime, 80.4%, Imipenem, 79.4% and Meropenem, 77.3%).[26]

5. Conclusion

High prevalence of nosocomial infections and the presence of MDRPa suggest continuous surveillance of burn infections and develop strategies for antimicrobial resistance control and treatment of infectious complications. The choice of therapy for MDRPa often becomes very limited and an additional matter of concern is represented by the fact that no new antimicrobial agents, active against MDRPa are in advanced stages of development as therapeutic options.

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