Original Article

DOI: 10.7860/JCDR/2014/7483.4383

Microbiology Section

Incidence of Multidrug Resistant *Pseudomonas Aeruginosa* Isolated from Burn Patients and Environment of Teaching Institution

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ABSTRACT

Context: *Pseudomonas aeruginosa* is an important pathogen which causes nosocomial infections in immunocompromised patients, especially in hospitalized burn patients. In recent times, it has emerged as a widespread Multi Drug Resistant (MDR) pathogen which requires antibiotic susceptibility testing on a regular as well as a periodic basis.

Aim of the study: The present study was undertaken to determine the antibiogram of *P. aeruginosa* which was isolated from inpatients and environmental sources, and to type the strains, based on their antibiogram patterns.

Settings and Design: A prospective study was undertaken with 525 samples (blood and wound swabs) which were taken from 60 patients who were admitted to Vardhman Mahavir Medical College and Safdarjang hospital with burn injuries and with 101 samples which were obtained from environmental sources viz. surgical instruments, dressings, suction devices, sinks, antiseptic solutions, etc.

Materials and Methods: The strains were cultured and identified by standard microbiological techniques and Kirby- Bauer disc diffusion antibiotic susceptibility testing was done for each.

Statistical analysis: Chi-squire tests were done and p- values of less than 0.05 were considered to be significant.

Results: Fifty six strains and two strains, respectively, of *P. aeruginosa* were isolated from inpatients and environmental samples (one strain from sink and one strain from door wall, among the two) respectively. In total, 58 (81%) *P. aeruginosa* strains were found to be resistant to aminoglycosides, 41-70% were resistant to beta-lactams - piperacillin, ceftazidime, and aztreonam, 34.5% were resistant to piperacillin-tazobactam, 12.06% were resistant to ciprofloxacin and 13-19% were resistant to carbapenems. All strains were sensitive to colistin. *P. aeruginosa* was resistant to three of the four 'in-use' drugs i.e. piperacillin+tazobactam, imipenem, ceftazidime, and gentamicin, which was taken as MDR, which depicted MDR percentage as 36.2 (21/58).

Conclusion: Strategies of optimal prescribing, including control of antibiotic usage, coupled with periodic studies on MDR *P. aeruginosa* infections in burn patients, appear to be leading priorities which help in improving therapeutic gains in such patients.

Keywords: *Pseudomonas aeruginosa*, Antibiotyping, Multidrug resistance

INTRODUCTION

Pseudomonas P. aeruginosa, a non-fermentative gram negative bacterium, is widely distributed in nature, including hospital environment. It is responsible for about 10% -20% of nosocomial infections which are seen as septicaemia in intensive-care units (ICUs), cystic fibrosis, burn and wound infections, etc. [1]. *P. aeruginosa* is a ubiquitous micro-organism that can rapidly acquire resistance to different broad-spectrum antibiotics. Multidrug-resistant (MDR) *P. aeruginosa* is an emerging cause of mortality and morbidity in burn patients, which causes 4-60% nosocomial infections in different parts of the world [1]. MDR *P. aeruginosa* elaborates inactivating enzymes that make beta-lactams and carbapenems ineffective, such as extended spectrum beta lactamases (ESBLs) and metallo-β-lactamases (MBLs) [2]. ESBL-producing *P. aeruginosa* was first detected in western Europe in the mid-1980s, and MBL-producing *P. aeruginosa* was first reported from Japan in 1991. They have rapidly spread over different parts of world since then [3].

MDR *P. aeruginosa* phenotype is defined as a bacterium which is resistant to anti-microbial agents which are included in three or more anti-Pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides [4]. We made an attempt to determine prevalence of MDR in our hospital, by considering MDR definition of *P. aeruginosa* as strains which were resistant to three of the 'in-use' four drugs i.e. piperacillin + tazobactam, imipenem, ceftazidime and gentamicin. The present study investigated the in-vitro activities of 12 commonly used antimicrobial agents which were used to treat *P. aeruginosa* infections in burn patients.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, and Burns and Plastic Surgery Department of Vardhman Mahavir Medical College and Safdarjang hospital, New Delhi, India, over a period of 2 years. The present study included 60 burn patients who were admitted to casualties, ICUs and wards of the hospital (group I). The patients who were included were within age range of 10-60 years, whose 20-60% body surface area (BSA) was involved in burns. Blood samples were collected from the same patients if septicaemia (fever, tachycardia, etc.) was evident. Five hundred and twenty five samples were collected from these 60 patients at different intervals- day 1, day 3 and day 7 of their admissions to the hospital. The study was approved by ethical committee of Safdarjang hospital.

A total of 101 environmental samples were collected, which included 51 hand, throat, and nasal swabs obtained from 17

medical staff (three from each) who were working in burn unit; and 50 samples from hospital environment (group II), which were taken from surgical instruments, dressings, suction devices, floors, door walls, beds, commodes, sinks, and antiseptic solutions.

Sample processing

The samples were cultured on Pseudomonas Isolation Agar (PIA), Mac Conkey's Agar, and Blood Agar and the plates were incubated overnight at 37°C. *P. aeruginosa* was identified by its colony characteristics, pigment production, grape like odour, oxidase positivity, motility, gram staining (as gram negative bacilli), ability of reducing nitrates to nitrites, non-fermentative character, along with its ability to decarboxylate arginine, liquefy gelatin and to grow at 42ºC [5]. Other bacteria which were isolated were also processed and identified by standard microbiological techniques [5].

Antibiotic susceptibility testing

Antibiotic sensitivity patterns of these isolates were studied by using Kirby Bauer Disc Diffusion method on Mueller –Hinton agar, by following CLSI 2011 Guidelines [6], by using Hi-media antibiotic discs. 11 antibiotics were tested, which included amikacin (30mcg), netilmicin (30mcg), gentamicin (10mcg), ceftazidime (30mcg), aztreonam (30mcg), ciprofloxacin (5mcg), piperacillin (100mcg), piperacillin + tazobactam (100/10mcg), imipenem (10mcg), meropenem (10mcg) and colistin (10mcg). Strains which had the same types of resistance patterns (antibiotype) were considered to be from the same clone. *Pseudomonas aeruginosa* ATCC 27853 strain was used for quality control in the study. In our work, MDR *P. aeruginosa* was detected as a bacterium which was resistant to three or more anti-Pseudomonal anti-microbial classes (piperacillin + tazobactam, imipenem, ceftazidime and gentamicin) [4].

STATISTICAL ANALYSIS

The results were statistically analyzed by Chi squire tests and p-values of less than 0.05 were considered as significant.

RESULTS

The mean age of the patients who were included in the study was 29.7 years.The incidence rate of *P. aeruginosa* was highest (33.93%) in the age group of 21-30 years, as has been shown in [Table/Fig-1]. Culture positivity rate, including all isolates from patients, was determined to be 29.5% (155 strains out of 525 samples). Of the total 155 bacterial strains obtained from patients, *P. aeruginosa* accounted for 36.12% of the bacterial cultures, followed by *Klebsiella sp.* and *Acinetobacter sp*., which accounted for 21.29% and 16.77% of the cultures respectively. Others like *Coagulase negative Staphylococci*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus sp*. accounted for the rest of the bacterial cultures, as has been described in detail in [Table/Fig-2].

Fifty six *P. aeruginosa* strains were isolated from inpatients, two were isolated from environmental samples (one from sink and other from door wall) and no *P. aeruginosa* strains were isolated from hospital staff. Of the 56 strains which were obtained from inpatients, five strains were isolated on day 1,19 strains were isolated after 48 hours of admission and 32 strains were isolated on day 7 of admission of the patients.

Antibiogram results have been described in detail in [Table/Fig-3] and they demonstrated that 58-81% strains were resistant to aminoglycosides (amikacin, netilmicin, and gentamicin), 41-70% were resistant to beta lactams-piperacillin, ceftazidime, and aztreonam, 34.5% were resistant to piperacillin-tazobactam, 12.06% were resistant to ciprofloxacin and that 13-19% were resistant to carbapenems (imipenem, meropenem). All strains were found to be sensitive to colistin (100%).

[Table/Fig-4] describes the Antibiotyping of the *P. aeruginosa* strains obtained from patients and environment. Antibiotyping was done by studying the antibiotic susceptibilities of the strains and by allotting them under different groups (Type 1-8), based on their patterns of resistance. Type 2 was the most common antibiotype which showed resistance to 7 antibiotics, and which accounted for 29.31 % of the strains. In the present study, 36.2% (21/58) *P. aeruginosa* strains were MDR and they were categorized into antibiotypes 1, 3 and 5. Among these 21 MDR isolates, 19 were clinical isolates and two were environmental strains.

DISCUSSION

There has been a rapid emergence of MDR *P. aeruginosa* in recent times, which is an important concern for clinicians who treat these infections. In the present study, 66.07% (40 /60) patients were found to be infected with *P. aeruginosa* during their stay in hospital, from among which 56 strains were isolated. Two *P. aeruginosa* isolates were obtained from environmental samples.

Among the beta-lactams, *P. aeruginosa* showed highest resistance to ceftazidime (70.68%). However, it was more sensitive to other beta-lactams i.e., piperacillin+ tazobactam, imipenem and meropenem sensitive (65.5%, 72.4% and 79.3% respectively), as has been described in [Table/Fig-3]. Colistin, although it was used only as a salvage drug, showed 100% susceptibility to all the strains. It has to be noted, that according to Srinivasan et

Age (Years)	No. of *P. aeruginosa* strains	Percentage
10-20	14	25%
21-30	19	33.93%
31-40	14	25%
41-50	06	10.71%
51-60	03	5.36%

[Table/Fig-1]: Incidence rate of 56 *Pseudomonas aeruginosa* strains in various age groups of the patients

Bacterial strains	Number (%)
Pseudomonas aeruginosa	56(36.12%)
Klebsiella spp.	33(21.29%)
Acinetobacter spp.	26(16.77%)
Coagulase negative staphylococci	17(10.96%)
Staphylococcus aureus	15(9.67%)
Escherichia coli	6(3.87%)
Proteus spp.	2(1.29%)

[Table/Fig-2]: Distribution of 155 bacterial strains isolated from sixty patients of burns injuries

Antimicrobials	Resistant	Intermediate	Sensitive
	No. (%)	No. (%)	No. (%)
Amikacin (30mcg)	47(81.03)	8(13.8)	11(18.96)
Netilmicin (30mcg)	34(58.62)	6(10.34)	18(31.03)
Gentamicin (10mcg)	47(81.03)	3(5.17)	8(13.79)
Ceftazidime (30mcg)	41(70.68)	7(12.06)	10(17.24)
Aztreonam (30mcg)	24(41.38)	20(34.5)	14(24.13)
Ciprofloxacin (5mcg)	7(12.06)	5(8.62)	46(79.31)
Piperacillin (100mcg)	37(63.79)	12(20.7)	9(15.5)
Piperacillin+Tazobactam (100/10mcg)	20(34.5)	0(0)	38(65.5)
Imipenem (10mcg)	11(18.9)	5(8.62)	42(72.4)
Meropenem (10mcg)	8(13.79)	3(5.17)	47(79.3)
Colistin (10mcg)	0(0)	0(0)	58(100)

[Table/Fig-3]: Antibiotic susceptibility results of 58 clinical isolates of *P. aeruginosa*, including fifty six strains obtained from sixty burn patients and two isolates from fifty environmental samples

Type	No. of resistant antibiotics	No. of isolates (%)	Resistance to antibiotics
1	11	11(18.97)	Ak- Nt- G-CA-Cip-CS-Pc- PT-Az -Imp –Mer
2	7	17(29.31)	Ak-Nt-G-CA-CS-Az- Pc
3	7	1(1.72)	G-CA-CS-Az-Cip-Imp-Mer
4	6	10(17.24)	Ak-Nt-G-CA-Az-Pc
5	5	9(15.5)	Ak-G-CA-Pc –PT
6	4	2(3.45)	G-Nt-Pc-Imp
7	3	6(10.34)	CS-Az-Pc
8	1	2(3.45)	Imp

[Table/Fig-4]: Antibiotyping of 58 *P. aeruginosa* strains, including fifty six strains from patients and 2 from environment

Abbreviation for the antibiotics- Ak- Amikacin, Nt- Netilmicin, G- Gentamicin, CA- Ceftazidime, Cip- Ciprofloxacin, CS- Cefoperazone+ sulbactam, Pc- Piperacillin, PT- Piperacillin+ Tazobactam, Az- Aztreonam, Imp- Imipenem, Mer- Meropenem

al., *P. aeruginosa* was resistant to beta lactams viz. cephalothin, carbeniciilin, ceftazidime (100%), and cephalexin (98%) respectively [7]. According to the study of Saha et al., it is most sensitive to beta lactams - imipenem (98.72%), followed by aztreonam (33.44%) and ceftazidime (38.32%) [8]. Studies done by Kaushik et al., [9], Singh et al., [10], Taneja et al., [11], Agnihotri et al., [12] and Ganesamoni et al., [13], which were done in Indian context, showed resistance of *Pseudomonas spp*. in the range of 13.9 - 90% to amikacin, in the range of 4 - 90% to ceftazidime,, in the range of 50 - 77.7% to gentamicin and in the range of 41 - 95.1% to ciprofloxacin, which reflected high resistance profile of this nosocomial pathogen.

In the present study, MDR rate (resistance to three or more of anti Pseudomonal antimicrobials i.e. piperacillin + tazobactam, imipenem, ceftazidime and gentamicin) was determined to be 36.2% (21/58). A study done by Unan et al., [14] in Turkey reported rates of MDR, which were as high as 60%, whereas study done by Sabir et al., in Pakistan detected lower rates of MDR (22.08%) [15]. However, the rates of our study are comparable to a study done in Egypt, where Gad et al., [16] observed 36% MDR *P. aeruginosa*.

Typing of nosocomial isolates is essential for determining the epidemiology of nosocomial infections and for designing rational pathogen control methods. Antibiotyping was very informative in our work [Table/Fig-4]. Eight different resistance patterns were identified among 58 *P. aeruginosa* isolates. MDR was categorized in antibiotypes 1, 3 and 5. The two environmental *P. aeruginosa* isolates were also MDR and they belonged to antibiotype 1(from sink) and antibiotype 5 (from wall). Antibiogram is a sensitive phenotypic marker; however, it has the disadvantage of being non-reproducible in many instances, due to the exchange of R factor among isolates (Ramprasad et al., 2010) [17].

P. aeruginosa has gradually become a major cause of nosocomial infections which occur in burn patients and which requires immediate and effective implementation of infection control strategies, to combat its spread. Environmental sources may play a significant role in spread of MDR among hospitalized patients and it has been corroborated with evidence of two MDR strains which were isolated from hospital environment. In our study, two MDR strains were isolated from hospital environment and these had similar antibiotypes as of those strains which were isolated from patients. Antibiotyping has proved to be effective in determining the sources of nosocomial infections in immunocompromised patients like burn patients, and it can guide the infection control team to take measures for regulating the spread of this pathogen. Molecular characterization of the isolates was not done in the present study, due to financial constraints, which remains a limitation of this study.

In current times, antibiotics with antiPseudomonal activity which are available, include the aminoglycosides, ticarcillin, ureidopenicillins, ceftazidime, cefepime, aztreonam, the carbapenems, and ciprofloxacin. Combination treatments are generally recommended for suspected Pseudomonas infections. It has been reported that the choice of a carbapenem, cefepime, or piperacillin+ tazobactam, in combination with amikacin or tobramycin, in current times, appears to provide the widest potential antimicrobial activity against MDR *P. aeruginosa* [16]. Interestingly, our study also revealed that carbapenems, piperacillin + tazobactam, ciprofloxacin and gentamicin combinations were very effective in providing reasonable therapeutic options.

The lack of any new compounds in the near future indicates that national and local surveillance efforts are essential, to provide clinicians with correct information for choosing right antimicrobial therapy. Rigorous monitoring for MDR among Pseudomonas isolates is very important, because outbreaks caused by strains which are resistant to potentially useful agents, including carbapenems, have been reported elsewhere [17-19].

CONCLUSION

In conclusion, restriction of 'selected antibiotic usage' and/or infection control policies must be tailored for each institution, to combat the rapid emergence of MDR *P. aeruginosa* in burn patients. The lack of newer antimicrobial agents with activities against *P. aeruginosa*, makes periodic studies on the antimicrobial resistance patterns very important.

ACKNOWLEDGEMENT

Our sincere thanks to Mrs. Lata and Mrs. Omna for their technical support which they gave during study period.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Aug 27, 2013**
Date of Peer Review: **Jan 16, 2014**
Date of Acceptance: **Mar 03, 2014**
Date of Publishing: **May 15, 2014**