The Antimicrobial Resistance Pattern in the Clinical Isolates of Pseudomonas Aeruginosa in a Tertiary Care Hospital; 2008–2010 (A 3 Year Study)

K.M. MOHANASOUNDARAM

ABSTRACT

Introduction: Pseudomonas aeruginosa is an opportunistic human pathogen and is the leading cause of nosocomial infections especially among patients admitted to intensive care units. (ICU).It has been implicated in diverse nosocomial infections. In recent years, a considerable increase in the prevalence and multidrug resistance (MDR) P.aeruginosa has been noticed with high morbidity and mortality. So we aimed in the present study to determine the status of antimicrobial resistance to individual antipseudomonal agents and the magnitude of multidrug resistance in these organisms. The aim of the study was to retrospectively analyze and determine the distribution rate and antimicrobial resistance pattern in P.aeruginosa among clinical specimens for a period of 3 years.

Methods: P.aeruginosa were isolated and identified by conventional methods. The antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method. The clinical and specimen distribution properties of P.aeruginosa were evaluated based on their resistance.

Results: The isolation rate of P.aeruginosa in this study was 5%, 6.8% and 5% in 2008, 2009 and 2010 respectively. Pus, tracheal aspirates and urine were important sources of P.aeruginosa isolation in ICU and non ICU inpatients. Resistance rates of pseudomonas varied with the antibiotics and the high resistance observed was related to the increased use of broad spectrum antibiotics. Multidrug resistance P.aeruginosa is on the rise especially in nosocomial infections. Hence rigorous monitoring of MDR strains, restriction of inappropriate use of antimicrobial agents and adherence of infection control practices should be emphasized to delay the emergence of clinically significant MDR-P.aeruginosa

Conclusion: To conclude, although multidrug resistance has commonly been reported in nosocomial P.aeruginosa, community acquired data are less frequently reported. For this reason epidemiological studies on the prevalence and antimicrobial susceptibility pattern of resistant isolates in different geographical settings would provide useful information to guide clinicians in their choice of therapy and to contribute to the global picture of antimicrobial resistance. Rigorous monitoring of MDR in P.aeruginosa, restriction of the inappropriate use of antimicrobial agents and adherence of infection control practices should be emphasized to delay the emergence of clinically significant P.aeruginosa.

Key Words: Pseudomonas aeruginosa, ICU, multidrug resistance

INTRODUCTION

Pseudomonas aeruginosa is an aerobic, motile, gram negative rod that belongs to the family, pseudomonadaceae[1]. Being an opportunistic human pathogen, it is the leading cause of nosocomial infections, especially among patients who are admitted to intensive care units. (ICU). It has been implicated in diverse nosocomial infections like nosocomial pneumonias, urinary tract infections (UTIs), skin and soft tissue infections, in severe burns and in infections in immunocompromised individuals. Of particular concern is the limited number of effective antipseudomonal agents which are used in the therapeutic practice, due to the constitutive low level resistance to several agents and the multiplicity of the mechanisms of resistance in *Pseudomonas aeruginosa* [2].

Its general resistance is due to a combination of factors [3]. It is intrinsically resistant to antimicrobial agents, due to the low permeability of its cell wall. It has the genetic capacity to express a wide repertoire of resistance mechanisms. It can become resistant through mutations in the chromosomal genes which regulate the resistance genes. It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages. In recent years, a considerable increase in the prevalence of multidrug resistance (MDR) in *P.aeruginosa* has been noticed, which is related to high morbidity and mortality [2], [4]. Regional variations in the antibiotic resistance exist for different organisms, including *P.aeruginosa* and this may be related to the difference in the antibiotic prescribing habits.

The periodic testing and analysis of antibiotic resistance would enable the physicians to detect the trends in the resistance pattern to the commonly prescribed antibiotics in a given organism. So, we aimed in the present study, to determine the status of antimicrobial resistance to individual antipseudomonal agents and the magnitude of the multidrug resistance in these organisms.

MATERIAL AND METHODS

The patient's clinical data (including age, sex, location in the ICU or the non-ICU, clinical diagnosis and specimen type) and the results of the *P.aeruginosa* susceptibility testing were obtained during the period from 2008-2010.When there were identical isolates from the

same patient, only one isolate was included. The bacterial isolates were considered as inpatient isolates if they were cultured more than 48 hours after admission.

A total of 370 clinical isolates from various samples such as pus, urine, sputum, blood, ET tubes and other specimens from the in and out patients which were received over a period of 3 years from 2008-2010, were analyzed.

IDENTIFICATION

The isolates were identified by conventional methods. The strains were identified as *P.aeruginosa*, based on the colony morphology, gram staining, oxidase reaction, the production of the pyocyanin pigment, nitrate reduction, the use of citrate and malonate as carbon sources, and its ability to grow at 5°C and 42°C. The *P.aeruginosa* ATCC 27853 strain was used as the quality control. The susceptibility testing of *P.aeruginosa* was performed by the Kirby-Bauer disc diffusion method and the results were interpreted according to the CLSI guidelines5. The antibiotics which were tested were ciprofloxacin(Ce), levofloxacin(Le), gentamicin(G), amikacin(Ak), ceftazidime(Ca), cefepime(Cpm), piperacillin/ tazobactum(Pt), cefeperazone /sulbactum(Cfs) and imepenem(I).

RESULTS

The results which were obtained are depicted in the table as follows: [Table/Fig-1], [Table/Fig-2], [Table/Fig-3], [Table/Fig-4], [Table/ Fig-5], [Table/Fig-6]

DISCUSSION

Bacterial resistance has been emerging and this has both clinical and financial implications for the therapy of infected patients3,4. *P.aeruginosa* is a major cause of nosocomial infections. Despite the advances in sanitation facilities and the introduction of a wide variety of antimicrobial agents with antipseudomonal activities, life threatening infections which are caused by *P.aeruginosa* continue to be hospital based infections.

The distribution of the isolates is significantly affected by the type of hospital from which they have been isolated. The isolation rate due to nosocomial infections was 3-16% in multi centre studies6. In this study, the isolation rate was 4%, 6.8% and 4% in 2008, 2009 and 2010 respectively, which was relatively low when compared to similar studies with a high prevalence.

The isolation rate was significant in the ICUs in our study (p0.05). Intensive care patients create an environment for infection because of the debilitating effect of a prolonged hospitalization and the application of medical equipment (airway, catheters etc). A high prevalence of pseudomonas infections was found in the 35-50 years age group. The prevalence rate of *P.aeruginosa* ranged from 10.5 to 30% and one study from India reported a prevalence rate of 20.3%7.

The prevalence of the *P.aeruginosa* isolates varied with the clinical conditions and the specimens. The highest number of isolates was from pus and endotracheal tubes.

Year	Total No of Isolates	Specimen Type					
		Pus	Et	Urine	Blood	Sputum	Others
2008	102	29	25	17	10	10	11
2009	156	46	55	26	5	14	10
2010	112	36	28	17	16	10	12

[Table/Fig-1]: Distribution Of P.Aeruginosa Among Clinicl Isolates

Characteristics	2008 N=102(4%)	2009 N=156(6%)	2010 N=112(4%)
Mean age(years)	43.56 %	41.49%	38.85%
Male: female	80:19	77:22	68:31
ICU patients	59.78%	37%	47.7%
Non-ICU patients			
Out patients	4.3%	6%	11.5%
Inpatients	35.86%	57%	40.7%
Underlying conditions			
Surgery	31.42%	26.6%	24.5%
Diabetes	13.33%	15.15%	13.5%
Respiratory diseases	10.47%	9.69%	11%
Renal disease	7.6%	10.3%	13.5%
Road traffic accident	15.23%	18.78%	19.49%
Burns	11.42%	10.3%	6.7%
others	10.47%	9.09%	11%
Specimens			
pus	25%	29.5%	31.8%
ET	21.74%	35.3%	24.8%
urine	16.3%	16.7%	15%
blood	9.78%	3.2%	8.8%
sputum	9.78%	8.9%	8.8%
others	16	6.4%	10.6%

[Table/Fig-2]: Characteristics of Patients Infected with *P.Aeruginosa* 2008-2010

The unique feature of *P.aeruginosa* is its resistance to a variety of antibiotics, which is attributed to a low permeability of the cell wall, the production of inducible cephalosporinsaes, an active efflux and a poor affinity for the target (DNAgyrase)8.

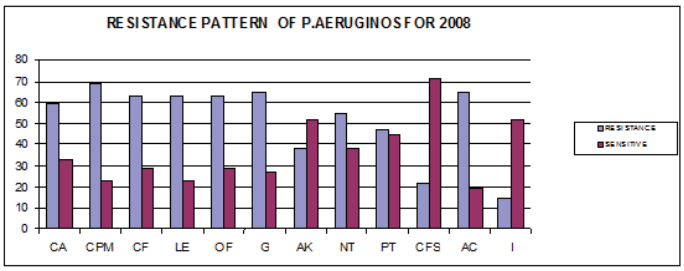
More resistant strains in the ICU and increasing morbidity and mortality with a high multiresistance among bacteria seem to be an important problem.

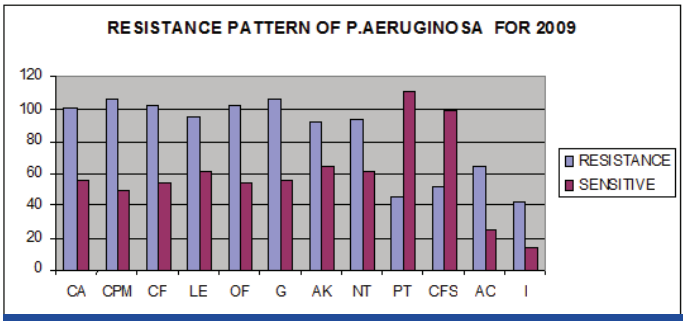
In various studies which investigated the resistance of *P.aeruginosa* to ciprofloxacin, the proportion was reported to be 0-89%9. In the present study, the overall resistance to ciprofloxacin was around 63.1%.

Levofloxacin has some added advantages such as its effectiveness against gram positive bacteria, its ability to concentrate more in the urine and its use in the form of single daily doses. The activity of levofloxacin is equal to or less than ciprofloxacin. We found that the resistance to levofloxacin was 60% in 2008 and 2009 and that it drastically reduced to 50% in 2010.

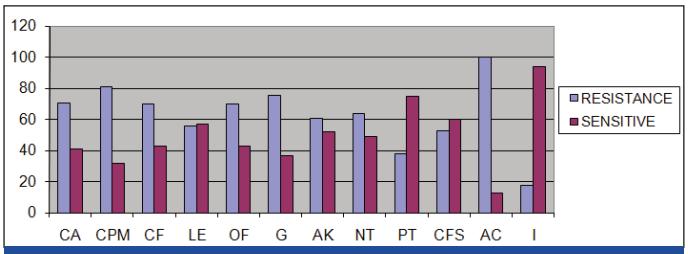
Year	Total no. of isolates	Ca	Cpm	Cf	Le	Of	G	Ak	Pt	Cfs	Ac	I
2008	102	57.8	67.6	61.7	61.7	61.7	63.7	37	46	20.5	63	14.7
2009	156	64.7	67.9	65.3	60.8	65.3	67.9	58.9	29	32.6	42	27
2010	112	63.3	72.3	62.5	50	62.5	67.8	54.4	34	47	89	16

[Table/Fig-3]: Percentage Resistance To Antimicrobial Agents For Clinical Isolates Of P.Aeruginosa 2008-2010

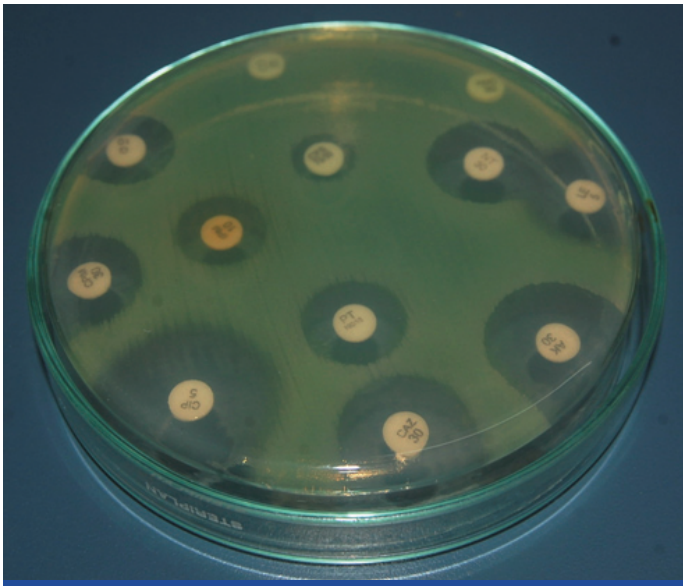




[Table/Fig-4]: The resistance pattern of P.aeruginosa for 3 years are depicted as bar diagram



[Table/Fig-5]: Antimicrobial resistance pattern of p.Aeruginosa for 2010



[Table/Fig-6]: Antibiogram Showing Multidrug Resistant P.Aeruginosa

Because of the increasing resistance to fluroquinolone in many hospitals, its empirical usage is either banned or restricted, to bring the developing resistance rates under control9.

Ceftazidime and cefepime are the most frequently prescribed third and fourth generation cephalosporins respectively. The resistance to ceftazidime was reported as 4-18%, but in our study, it was 57% to 63.3 % and the resistance to cefepime was found to increase from 67% to 72%. These high values of resistance which were observed were comparable to those of the reports from Gujarat, with a resistance value of 75% [10]. The increased prevalence of ceftazidime resistant *P.aeruginosa* is related to the increased use of beta lactam antibiotics such as amoxicillin and ceftazidime. Selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains.

The amino glycosides inhibit protein synthesis by binding to the 30 S subunit of the ribosome and the inactivation of the amino glycosides occurs through the production of enzymes which transfer acetyl, phosphate or adenylyl groups to the amino acid hydroxyl substituents on the antibiotics11. Over the 3 year period, the resistance to gentamicin was steady, while that of amikacin was highest during the year 2009(58.9), but was low as compared to the resistance to gentamicin.

In our study, the resistance rate to imipenem was 27% in 2009 and 16% in 2010 respectively. Various studies have shown the resistance to imipenem to be up to 31.6%12. Our report was in concordance with that of the above study.

A decline in the resistance to imipenem could be attributed to the restricted use of this antimicrobial agent.

The rate of the MDR-in *P.aeruginosa* (resistant to more than 2 classes of antibiotics) is increasing in many parts of the world and it poses a serious therapeutic dilemma3. In some institutes, the treatment of the MDR-in *P.aeruginosa* is being limited to polymixin B. We observed that the percentage of the MDR in the *P.aeruginosa* strains had increased from 64% in 2008 to 71% in 2010. Most of the isolates were from pus and endotracheal aspirates. The fact that most of the multiresistant strains were isolated from the ICUs is worrisome for the future. The development and the application of antimicrobial usage policies along with the aid of the Hospital Infection Control Committee will decrease the appearance and the spread of the nosocomial infection epidemics.

In this study, the inpatient isolates, particularly in the ICU patients, were more resistant to most of the antibiotics as compared to the outpatient isolates. We also observed that the highest rate of resistance was among the isolates from the ICU patients, followed by the isolates from the non –ICU patients and the least resistance was observed in the outpatient isolates. We also found no increase in the antibiotic resistance in the outpatient isolates over the 3 year period, except for the urinary isolates which showed an increase in the resistance rates. This observation correlates with the high exposure of the inpatient isolates to broad spectrum antibiotics and their overuse due to a prolonged stay in the hospital.

To conclude, although multidrug resistance has commonly been reported in nosocomial *P.aeruginosa* infections, community acquired data have less frequently been reported. For this reason, epidemiological studies on the prevalence and antimicrobial susceptibility pattern of the resistant isolates in different geographical settings would provide useful information in order to guide clinicians in their choice of therapy and to contribute to the global picture of antimicrobial resistance.

Rigorous monitoring of the MDR in *P.aeruginosa*, the restriction of the inappropriate use of antimicrobial agents and adherence to infection control practices should be emphasized in order to delay the emergence of clinically significant *P.aeruginosa*.

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AUTHOR:
1. Dr. K.M. Mohanasoundaram

NAME OF DEPARTMENT(S)/INSTITUTION(S) TO WHICH THE WORK IS ATTRIBUTED:
Department of Microbiology, Vinayaka Missions Medical College Salem, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr.K.M. Mohanasoundaram M.D., (Micro)
Associate professor, Department of Microbiology
Vinayaka Missions Medical College, Salem
Email-mohanapalani@gmail.com
Mobile-94431-32290

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