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Research Article

COMPARATIVE STUDY OF *PSEUDOMONAS AERUGINOSA* ISOLATE RECOVERED FROM CLINICAL AND ENVIRONMENTAL SAMPLES AGAINST ANTIBIOTICS

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ABSTRACT

Pseudomonas aeruginosa has emerged as one of the most potential problematic gram negative pathogen, the present study to investigate the prevalence of *P. aeruginosa* from the various environmental and clinical samples. The highest isolation rates of *P. aeruginosa* was found in clinical 71%, followed by industrial effluent 58.8%, soil 50%, water 45.45% and air 43.45%. The antibiotic susceptibility test was performed by the disc diffusion method according to NCCLS (National Committee for Clinical and Laboratory Standard) guidelines. The traditionally clinical pertinent antibiotics like Amikacin (Ak), Ceftazidime (Ca), Netilmicin (Nt), Gentamicin (G), Piperacillin (Pc), Ciprofloxacin (Cf), and Imipenem (I) were tested against *P. aeruginosa.* Among the antibiotics, the most effective antibiotic were carbapanems and aminoglycosides and the resistance rates were detected as 18% and 28%, respectively among 50 *P. aeruginosa* strains. Over 20% of the isolates were exhibited multi-drug resistance to five (or) more antibiotics, especially clinical isolates. In conclusion, the results indicates, the excessive use and disposing of antibiotic and chemicals leads to the emergence of antibiotic resistance in the environment and hospital. So that proper monitoring and optimization should be adopted.

Keywords: *P. aeruginosa,* Antibiotic resistance, Susceptibility, Multi drug resistance

INTRODUCTION

Pseudomonas aeruginosa belongs to a vast genus of obligate aerobic, non-fermenting, saprophytic, Gram-negative bacilli widespread in natural environment such as soil, plant surfaces, fresh vegetables, sewage, waste water, sink, moist environment, and river water[1]. Obviously, this organism is endowed with weak pathogenic potential. However, its profound ability to survive on inert materials, minimal nutritional requirement "growing in distilled water", which is evidence of its minimal nutritional needs[2], tolerance to a wide variety of physical conditions and its relative resistance to several unrelated antimicrobial agents and antiseptics, contributes enormously to its ecological success and its role as an effective opportunistic pathogen.

Generally *P. aeruginosa* is environmentally acquired and spread person-to-person rarely[3]. These bacteria can be transmitted through respiratory care equipment, irrigating solutions, catheters, infusions, cosmetics, dilute antiseptics, cleaning liquids, and even through toilet soaps[4, 6]. It exhibits considerable rate of nosocomial infection in prolonged admission of patients in hospital and tendency of nosocomial pathogenic to acquire new antibiotic resistance traits poses a great problem in their treatment and control[7]. According to CDC (Centre of Diseases Control and Prevention) reported that *P. aeruginosa* is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospitals – acquired infections.

The resistance capability of *P. aeruginosa* towards a range of antibiotics is possible by several mechanisms. The major mechanism of resistance to ß lactam antibiotics is beta-lactamase production, cell wall permeability and aminoglycoside-modifying emzymes[8]. More than 340 ß-lactamase enzymes have been detected to date. Although not completely understood, several factors have been identified as virulence determinants of *P. aeruginosa*. These include *rhl/las* otherwise known as quorum-sensing system, [9] types III secretion system[10]; multidrug efflux system[11] and biofilm forming system.

As the strain resistance develops to "first-line" antibiotics, followed by therapy with new, broader spectrum antibiotic, more expensive antibiotics, at last leading to the development of resistance to new class of drugs. In the present study, the susceptibility patterns of *P. aeruginosa* isolates to some commonly used antibiotics in Cameroon in order to update our knowledge on the use of antibiotic policies and guidelines to prevent the unnecessary and indiscriminate use of antibiotics to reduce morbidity and mortality rates in *Pseudomonas* infection in patients, thus facilitating health care services and improving cost effectiveness of the treatment[12].

In the present study, we aimed at finding out the isolation rates of *P. aeruginosa* from the clinical and environmental sources, and also to detect the sensitive and resistance pattern of isolated *P. aeruginosa* against different antimicrobial agents.

MATERIALS AND METHODS

Study design

The study was carried out over a period of five months, that is, between January-May 2010 in Cuddalore SIPCOT (The State Industries Promotion Corporation of TamilNadu), area a chemical industrial estate located 8 km from south of Cuddalore on the seaward side south east coast of India. Various industries like pesticides, pharmaceuticals, dyes, paints and other chemicals factories are located in this estate. They discharge untreated effluents into the environment it contains various chemical and antibiotics. Environmental samples that include industrial effluent, soil and air were collected from the above location and water sample were collected from the lake, pond, and also from paddy field situated near SIPCOT. Clinical samples were collected from a tertiary care Govt. hospital Salem (patient caused from urinary tract infection). A total of 50 isolates of *P. aeruginosa* recovered from environmental and clinical sources. The sources include 10 isolates each from industrial effluents, soil, water, air and clinical.

Bacteriological Analysis

The collected microbial source was transported to the laboratory following Cheesbrough[13] method. Samples were plated primarily onto nutrient agar and Mac conkey agar which was incubated at 37^{0}C for 24–48 h. The bacterial isolates were observed for morphological characters and identified by using the tests guided by Bergey's Manual of Systemic Bacteriology. Suspicious isolates were presumptively identified by using colony morphology, pigment formation, mucoidy, haemolysis on blood agar, positive oxidase test, growth at 42^{0}C on nutrient agar, motility test, and Gram reaction[13]. Further, the *P. aeruginosa* isolate was confirmed by using a rapid NEFERM-24 (LA CHE MA) biochemical kit according to the manufacturer's instruction.

Antibiotic Susceptibility Testing

The agar disc diffusion method of Bauer[14] modified based on National Committee for Clinical Laboratory Standards[15] CLSI was followed to perform the susceptibility test for the *P. aeruginosa*

isolates (Fig.1). A uniform spread plate of *P.aeruginosa* was done using sterile cotton swab on Mueller-Hinton plate and the plates were allowed to dry. Thereafter, the clinically pertinent 7 antibiotic discs with the following drug contents Amikacin (Ak), Ceftazidime (Ca), Netilmicin (Nt), Gentamicin (G), Piperacillin (Pc), Ciprofloxacin (Cf), and Imipenem (I) were placed on the plate. After 24 hrs, clinical interpretation [resistant (R), and sensitive (S)] of the size of the zone was evaluated based on the MIC susceptibility value as determined by the diameter from the zone of inhibition (Tabel-1) and compared with ATCC 27589 strain of *P. aeruginosa*. All the reagents and antibiotic susceptibility test discs used in the test were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai.

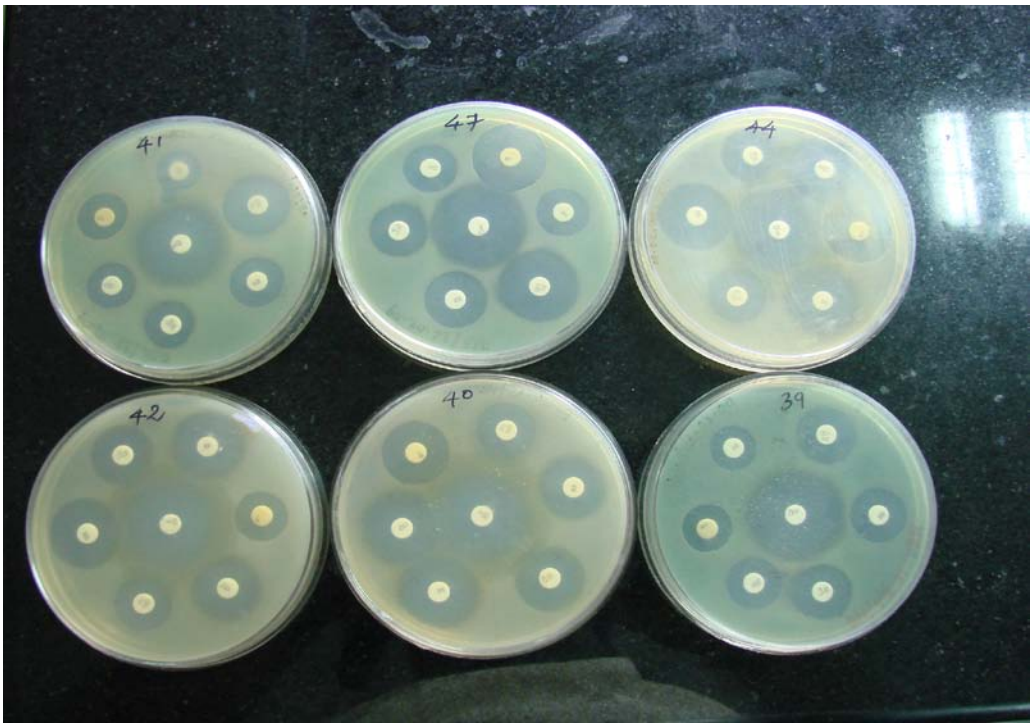

Fig. 1: Antibiotic sensitive and resistance test of *P. aeruginosa*

Table 1: Standard zone chart of different antimicrobials for *P. aeruginosa*

S. No.	Drug	Code	Resistance (mm) Less than	Sensitive (mm) More than
1	Amikacin 30 mcg	Ak	14	17
2	Nitilmicin 30 mcg	Nt	12	15
3	Pipercillin 100 mcg	Pc	17	18
4	Imipenem 10mcg	I	13	16
5	Gentamycin 10 mcg	G	12	15
6	Ciprofloxacin 5 mcg	Cf	15	18
7	Ceftazidimine 30 mcg	Ca	14	18

RESULTS

A total of 50 *P. aeruginosa* strains were isolated from environmental and clinical sources. The highest isolation rates of *P. aeruginosa* strains was found in clinical (71%) followed by industrial effluents (58.8%), soil (50%), water (45.45%), and air (43.45%). The Isolation rates of *P. aeruginosa* recovered from environmental and clinical sources is given in Table-2.

The antibiotic sensitivity and resistance patterns of various source isolates are shown in Figure 2&3. The most commonly applying drugs for *Pseudomonas* infection was used for antibiotic susceptibility assay.

This antibiotic was tested against 50 isolates recovered from clinical and environmental isolates. Among three aminoglycosides, amikacin showed 72% susceptibility, nitilmycin showed 60% sensitivity and gentamycin showed 52% susceptibility. Among the quinolones groups, ciprofloxacin showed 68% sensitivity. The ceftazidime of cephalosporins showed 64% sensitivity. Imipenem (carbapenems) was found to be the most effective antibiotic, which showed 82% susceptibility.

Table 2: Isolation rates of *P. aeruginosa* recovered from environmental and clinical sources

Site of collection	No. of sample examined	No. of positive isolates (%)
Water	25	10 (45.45)
Air	23	10 (43.47)
Soil	20	10 (50)
Industrial effluents	17	10 (58.8)
Clinical	15	10 (66.6)

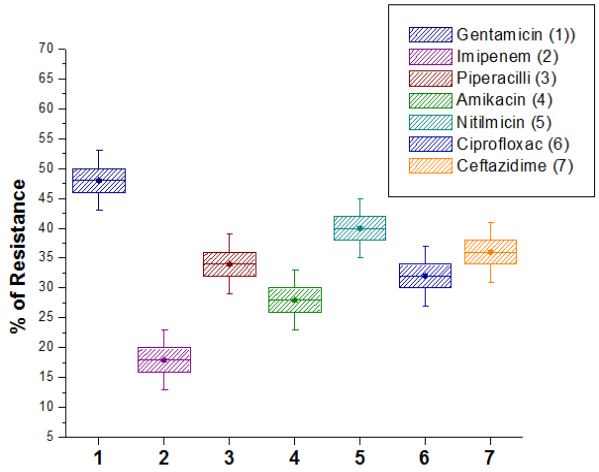

Fig. 2: Antibiotic sensitive pattern of *P. aeruginosa*

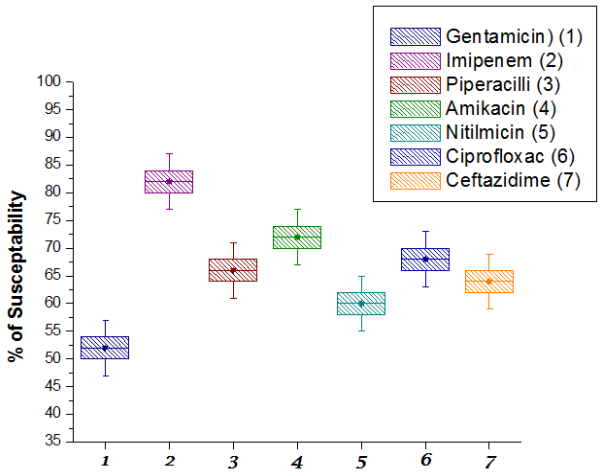

Fig. 3: Antibiotic resistance pattern of *P. aeruginosa*

With respect to resistance pattern, the most effective antibiotics were carbapenems and aminoglycosides (imipenem and amikacin) and the resistance rates were detected as 18% and 28%, respectively over 50 *P. aeruginosa* strains. While the other antibiotics, the resistance rates of *P.aeruginosa* were in the following order: quinolones (ciprofloxacin) 32%, ß-lactamase inhibitor (pipercillin) 34%, third generation (ceftazidime) 36%, nitilmicin & gentamicin were recorded 40 % & 48 % respectively.

In Multi drug resistance, around 42% of the isolates were resistant to three or more antibiotics; of this, 20% of isolates were resistance to five or more antibiotics. The majority coming from industrial effluents, there was no pronounced variation between industrial effluent and clinical sources and the resistance rates were detected as 12% and 10%, while other sources were decrease in the order of multiple drug resistance such as soil 8%, water 8% and air 2%.

DISCUSSION

In the present study, seven antimicroloial agents were tested against *P. aeruginosa* from different samples. They were (ß-lactamases) piperacillin, imipenem, third generation (ceftazidime) cephalosporins, (aminoglycosides) gentamicin, amikacin, netilmicin and (quinolones) ciprofloxacin. The reason choosing this antimicrobial was their wide use in the hospital as antipsedudomonal agents. Therefore, this kind of study could provide appropriate guidelines to the hospital regarding the prescription of these antimicrobials according to their sensitivity to *P. aeruginosa*.

Among the seven antibiotics, maximum sensitivity was found with imipenem (82%) followed by amikacin (72%) while other drugs showed decrease in susceptibility pattern. Maximum sensitivity was demonstrated by these drugs in comparison to other antibiotics used in our study. One of the reasons for these drugs still remaining sensitive might be due to their restricted use in ICU and also limited use in critical care unit.

In earlier studies[19, 20], it was reported that increased resistance rates of *Pseudomanas aeruginosa* have been detected against carbapenems, quinolons and third-generation cephalosporins across the globe. In the present study, resistance rates against carbapenems such as imipenem, aminoglycosides such as amikacin was 18% and 28% respectively. In yet another study [21-23], it was reported that resistance to imipenem was 14% in Spain, 19.3% in Italy and 68% in Saudi Arabia. The National Nosocomial Infections Surveillance (NNIS) system reported that the incidence of imipenem resistance as 18.5% among isolates of *Pseudomanas aeruginosa* from ICU patients[24].

The resistance of *P. aeruginosa* to the antibiotic in the quinolone group is variable in different centers. In a prospective study, resistance to ciprofloxacin was reported as 8-31% in ICU patients (26, 27). The present study revels that the resistance rate against ciprofloxacin was found as 32%. while it was 23% in Spain21, 31.9% in Italy22, and 28.8% in Latin America. Similarly, the pipercillin resistance rate was 10% in Spain21, 12% in Italy22, 14% in Latin America28, but it was 34% in our study. Based on the result, the resistance rate of *P.aeruginosa* varies with time and geographical location 29.

The resistant rate of ceftazidime (36%) was slightly increased compared to Ciprofloxacin. According to earlier reports, resistance to ceftazidime was 15%-22% in the world[28]. Resistance to

piperacillin was higher, similar to ceftazidime. Resistance rates of anti-pseudomonal antibiotics were quite low in the United Kingdom: 5% for ceftazidime, 7% for piperacillin, 10% for ciprofloxacin, and 11% for imipenem[30].

Clinical isolates were highly resistance to the antibiotic when compared to the environmental isolates; this may be due to the constant exposure to the antibiotic in the hospitals environment. Although, there was no pronounced difference between the resistance pattern of clinical and industrial isolates, while others in ascending order such as soil, water and air isolates. Among the sources, least resistance was found in air isolates; this may be due to the less exposed to chemical/antibiotic stress showed least resistant. Among the 10 air isolates two were having resistant capacity; this may be due to mutation in the gene sequences.

Approximately 42% of isolates were resistant to three or more antibiotics; of this 20% of isolates were resistance to five or more antibiotic. The majority coming from industrial effluent might be linked to the uncontrolled disposing of chemicals and antibiotic into the environment creating a selective pressure on these microbes leads to multiple drug resistance. In case of hospitals use of antibiotics and the community at large serves as a major selective pressure for antibiotic resistant bacteria[31]. Often they carry drug resistance gene and transfer them rapidly among themselves leads to the multiple drug resistance to the hospitals.

Multi-drug-resistant to nosocomial infectious pathogen has been increasing around the world[32]. The existence of metallo-blactamases and extended-spectrum ß-lactamase-producing strains exhibiting resistance to most b-lactams antimicrobial agents greatly complicate the clinical management of patients infected with such multi-drug-resistant strains [31, 33].

In the earlier studies, the range of Multi-drug-resistance ranging was 50% in Turkey and 3% in Spain, UK, and Malta [34, 35]. In our study, 7.2% MDR *P. aeruginosa* was recorded and maximum number of clinical sample (12%) followed by industrial effluent (10%), soil (8%), water (8%) and air (2%).

CONCLUSION

Our results indicate that the resistance of *P. aeruginosa* leads by the uncontrolled disposing of chemicals and antibiotic in the environment. In addition, immunization may fail to recover by constant exposure of resistance microbes. Even though of medical improvement, the antimicrobial resistance still becomes an age - old problem. So, proper implementation of antibiotic policies and guideline must be there in every hospital to local susceptibility pattern. Currently, the treatment of *P. aeruginosa* infections is based on combination antibiotic therapy that traditionally includes ß-lactam agents and aminoglycosides, in addition to this; treatment with fluoroquinolones has offered new perspectives. The development of effective vaccine against *P. aeruginosa* is necessary in the modern world.

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