Original Article

Antimicrobial Susceptibility Pattern of Pseudomonas Aeruginosa Isolated From Clinical and Environmental Sources In Punjab, Pakistan

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ABSTRACT

The increasing incidence of antimicrobial resistance is a public health concern, and Pseudomonas aeruginosa is known to be resistant to a variety of antibiotics. **Objective:** Antimicrobial resistance and the multiple antibiotic resistance (MAR) index of *P. aeruginosa* from environmental and clinical sources were studied in the current study. **Method:** A total of 170 samples were evaluated, with 85 samples each from environmental sources and clinical settings. The isolates were subjected to microbial analysis and antimicrobial sensitivity testing. The findings revealed that 45.88 % (39) of the 85 clinical isolates tested for the presence of *P. aeruginosa* were positive. In terms of prevalence, there were significant variations (p 0.05) between the clinical samples. **Results:** Wound samples had the highest isolation rate of 28.2%, while urine samples had the lowest (12.8%). *P. aeruginosa* was found in 38.8 % (33/85) of the samples isolated from environmental sources. In terms of prevalence, there was a highly significant difference *(p 0.01)* between the isolates. All of the positive isolates were completely resistant to cefuroxime and amoxicillin (100 %). The majority were also resistant to, cotrimoxazole (82%), nalidixic acid (82%), ciprofloxacin (86%), and tobramycin (69%). There was a substantial variation in the resistance patterns of isolates. **Conclusion:** The current study demands comprehensive measures to combat antimicrobial resistance in *P. aeruginosa.*

INTRODUCTION

Pseudomonas aeruginosa are Gram-negative, non-fermentative bacteria that may be found in water, sediment, and other humid habitats. P. aeruginosa is commonly linked to human illnesses, where it acts as a nosocomial microbial pathogen that uses a functional strategic way to infect nearly any tissue or organ, especially in susceptible people or the elderly [1]. The potential of P. aeruginosa to cause illness is enhanced by the wide variety of resistance mechanisms to the antibiotics, as well as the ability to thrive in a wide range of environmental circumstances and virulence determinants [2]. P. aeruginosa thrives in both normal and low-oxygen settings

and has consequently colonized a wide range of natural and artificial reservoirs. It feeds on a variety of organic materials; in mammals, its adaptability allows it to infect injured tissues or those with weakened immune systems [3]. The bacterium is considered as a significant source of infection in a variety of vulnerable persons in hospital settings, including those undergoing critical care (ICU), with burns or neutropenia, bacteremia, wound infections, as well as other cutaneous and systemic infections. Incidence and mortality rates with P. aeruginosa infection may be on the rise among these groups of people. Because P. aeruginosa is resistant to a wide range of antimicrobials,

treating these infections might be problematic. Furthermore, the advent and dissemination of resistant organisms of the bacterium have made treatment regimens challenging[4]. Several P. aeruginosa epidemics in intensive care units (ICUs) have been documented in the literature, indicating the potential for Pseudomonas spp. to develop microbial reservoirs in the healthcare setting. In contrast to other important environmental infections, P. aeruginosa thrives in a wide range of temperatures and has an incredible ability to manipulate and thrive in nutrient-poor environments. It sticks to surfaces thanks to its capsular polysaccharide. When favorable environmental circumstances exist, cells rapidly enter the exponential phase on surfaces connected to water conveyance systems, forming biofilms [5]. The capacity of some P. aeruginosa strains to produce carbapenemases is a major threat. As a result of these difficulties, it seems fair to identify the bacterium's sources and reservoirs to avoid its acquisition by both hospitalized and non-hospitalized people [6]. Environmental factors have been responsible for many illnesses linked to P. aeruginosa outbreaks. According to emerging evidence from possible investigations, environmental factors may, however, have a role in the epidemiology of occasional P. aeruginosa infections in clinical settings [7]. Antimicrobial resistance analysis of clinical and environmental reservoirs in pseudomonas isolates will allow the possibilities of new methods and refining of current ones to stop transmission from these sources. As a result, the goal of this investigation was to determine P. aeruginosa antibiogram profiling from clinical and environmental sources.

METHODS

A total of 170 specimens were evaluated, with 85 coming from each clinical and environmental source. Two government-owned hospitals and two privately-owned hospitals in Pakistan's Punjab region provided the samples. The clinical samples included 17 urine samples, 17 sputum samples, 17 wound swab samples, 17 blood samples, and 17 eye swab samples. They came from both hospitalized and non-hospitalized people who came to the clinics. For the collection of clinical samples, ethical approval was obtained. Hospital sinks (17), garbage sites (17), and storage rooms (17) were used to collect environmental samples. All samples were taken in an aseptic manner. Isolation of P. aeruginosa: A 1.0 ml aliquot of the samples was prepared by diluting to the order of 106 following standard serial dilution techniques. Following that, 100 µl of each diluent (102-106) was inoculated on cetrimide agar and incubated for 18-24 hours at 37oC. The purified green colonies on cetrimide agar were sub-cultured and cultivated at 37oC for 18-24

hours. Following that, colonies were purified on nutrient agar and cultured at 37oC for 24-48 hours before being kept on agar slants at 4 oC until ready for analysis [8]. P. aeruginosa isolates were identified using VITEK®-MS [9]. Antibiotic Susceptibility Testing is a method of determining whether or not a person is susceptible to different classes of antibiotics. The disc diffusion (Kirby-Bauer) method was used to evaluate antibiotic susceptibility, as indicated by the Clinical and Laboratory Standards Institute's standards (CLSI, 2019). To generate a lawn of bacteria, a single inoculum of each bacterial isolate was emulsified in 5.0 mL sterile normal saline in Bijou bottles. Mueller-Hinton agar plates were inoculated with sterile cotton swabs soaked in a standardized solution of bacterial cultures. Following that, antibiotic discs (Mast Diagnostics, Merseyside, United Kingdom) containing the following antibiotics were used: amikacin (30 g), gentamicin (10 g), streptomycin (10 g), tobramycin (10 g), ceftazidime (30 g), ceftriaxone (30 g), cefuroxime (30 g), amoxicillin (10 g), imipenem (10 g), and meropenem (10g). To avoid overlapping inhibition zones, discs were spaced at least 15 mm apart and away from the plate borders. The plates were incubated for 18-24 hours at 37°C, following which the zones of inhibition (millimeters) were measured to see if the profile was sensitive (S), intermediate (I), or resistance (I). The zones were interpreted according to Clinical and Laboratory Standards (CLSI 2019) [10]. SPSS, version 26.0, was used to tabulate and analyze all of the data. The percentages of qualitative variables were used to compare them using charts and the chi-square test. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

P. aeruginosa was found in 39 of 85 clinical samples (45.8%). In terms of prevalence, there were significant variations (p 0.05) across the clinical samples. Wound samples had the highest prevalence rate of 28.2 %, while urine samples had the lowest isolation rate of 12.8 % (Table 1). P. aeruginosa was detected in 38.8% (33) of the 85 ambient samples tested. In terms of prevalence, there was a significant difference (p 0.01) between the environmental samples. (Table 2).

Sources of Clinical Samples	Total no of Samples	Positive Samples for P. aeruginosa (%)
Skin surfaces	17	6 (15.3)
Sputum	17	8 (20.5)
Urine	17	5 (12.8)
Blood	17	9 (23.0)
Wound	17	11 (28.2)
Total	85	39 (100)

Table 1: Prevalence of P. aeruginosa from Clinical isolates

Sources of Samples	Total no of Samples	Positive Samples for P. aeruginosa
Hospital sinks	17	6 (15.3)
Catheter tips of urology ward	17	8 (20.5)
Hospital walls	17	5 (12.8)
Storerooms177 (21.2)	17	9 (23.0)
Hospital bed sheets175 (15.1)	17	11 (28.2)
Total	85	39 (100)

Table 2: Prevalence of P. aeruginosa from Environmental Sources.

Table 3 shows the antibiotic susceptibility profiles of clinical and environmental isolates. All of the positive clinical isolates were completely resistant to cefuroxime and amoxicillin (100 %). The majority of the clinical isolates were also resistant to nalidixic acid (82%), cotrimoxazole (82%), and ciprofloxacin (82%). A p-value of 0.001 is indicating there was a significant variation in resistance patterns across clinical isolates. Imipenem (94 %), meropenem (77 %), ceftazidime (77 %), and amikacin (74 %) were the most sensitive antibiotics among the clinical isolates (Table 3). Cefuroxime (100%), amoxicillin (100%), tetracycline (100%), and chloramphenicol (94%) resistance were found in all environmental isolates. At p0.001, the resistance patterns of the environmental isolates showed a very high significant difference. However, ceftazidime (79%) amikacin (70%), and imipenem (64%) were all effective against some of the environmental isolates (Table 3).

Antibiotic Class	Antibiotics	Clinical (n=39)			Environmental (n=33)		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Aminoglycosides	Amikacin (AK)	10(26)	0(0)	29(74)	10(30)	0(0)	23(70)
	Gentamicin (GM)	30(77)	2(5)	7(18)	14(43)	6(18)	13(39)
	Streptomycin (S)	22(57)	4(10)	13(33)	26(79)	7(21)	0(0)
	Tobramycin (TN)	27(69)	3(8)	9(23)	21(64)	6(18)	6(18)
Cephalosporins	Ceftazidime (CAZ)	8(20)	1(3)	30(77)	6(18)	1(3)	26(79)
	Ceftriaxone (CRO)	20(51)	10(26)	9(23)	17(52)	8(24)	8(24)
	Cefuroxime (CXM)	39(100)	0(0)	0(0)	33(100)	0(0)	0(0)
Penicillins	Amoxicillin (A)	39(100)	0(0)	0(0)	33(100)	0(0)	0(0)
Monobactams	Aztreonam (ATM)	25(64)	4(10)	10(26)	27(82)	3(9)	3(9)
Folate Inhibitors	Cotrimoxazole (TS)	32(82)	1(3)	6(15)	27(82)	2(6)	4(12)
Carbapenems	Imipenem (IMI)	1(3)	1(3)	37(94)	7(21)	5(15)	21(64)
	Meropenem (MEM)	9(23)	0(0)	30(77)	7(21)	5(15)	21(64)
Tetracycline	Tetracycline (T)	15(38)	14(36)	10(26)	33(100)	0(0)	0(0)
Phenicols	Chloramphenicol ©	20(51)	5(13)	14(36)	31(94)	2(6)	0(0)
Quinolones	Ciprofloxacin (CIP)	31(80)	2(5)	6(15)	26(79)	4(12)	3(9)
	Ofloxacin (OFX)	26(67)	4(10)	9(23)	18(55)	5(15)	10(30)
	Nalidixic acid (NA)	32(82)	4(10)	3(8)	28(85)	4(12)	1(3)

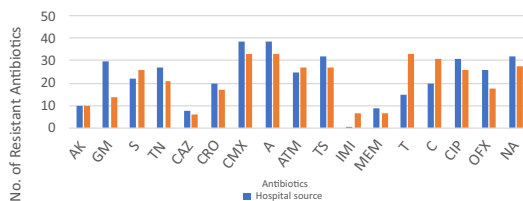**Table 3:** Antimicrobial Resistance profile of Pseudomonas aeruginosa

Figure 1: Antimicrobial Resistance profile of Pseudomonas aeruginosa

DISCUSSION

Pseudomonas aeruginosa is still a major nosocomial organism that causes substantial morbidity and mortality, especially in immunocompromised patients and those in critical care units. The study's findings highlighted the importance of clinical (sputum, wound/burns, urine, blood, and eye infection) and environmental (catheter tips, hospital walls, hospital beds, and hospital sinks) sources as a possible reservoir of the pathogen that are developing antibiotic resistance. The results showed that 38.8% of the environmental samples tested positive for P. aeruginosa. The organism's prevalence in clinical specimens (45.88 %) was not statistically different from that in environmental specimens (P > 0.05). Catheter tips (30.3 %) and storage rooms (21.2 %). had the highest occurrence. In the hospital setting, P. aeruginosa has been isolated from a wide range of reservoirs, including potable water, tap traps, showers, respiratory treatment equipment, newborn feeding basins, disinfectants, endoscopes, catheters, water baths, bathing basins, and cleaning equipment [11]. P. aeruginosa has many characteristics that help it survive in a wide variety of environments. Through the mechanism of multidrug efflux pumps, the organism is naturally resistant to a variety of disinfectants, including biguanides and quaternary ammonium compounds. In addition, the organism's capacity to develop biofilm on inanimate surfaces favors disinfectant resistance while also preventing manual removal. Its ability to survive in damp conditions is aided by the type III secretion system, which kills free-living amoeba that feeds on environmental bacteria [12]. In this study, a high %age of clinical and environmental isolates was resistant to the majority of antibiotics, including penicillin, cephalosporins, ceftriaxone, and cefuroxime (20% - 100 %), which is consistent with other studies. The formation of beta-lactamase enzymes, which break down the beta-lactam ring, may cause high resistance to beta-lactam antibiotics in those isolates. Pseudomonas species are naturally resistant to penicillins, cephems, and rifampin because they contain a moderately impermeable membrane, inducible efflux mechanisms, and a chromosomally encoded inducible beta-lactamase. [13]. In both clinical and environmental isolates, amoxicillin and cefuroxime had the highest antibiotic resistance, whereas imipenem, meropenem, amikacin, and ceftazidime had the lowest. Clinical and environmental isolates of P. aeruginosa were shown to be 100 % resistant to cefotaxime, chloramphenicol, penicillin, ampicillin, doxycycline, erythromycin, tetracycline, and cloxacillin, according to Haleem et al. (2011) [14]. Resistance to imipenem was found in 3% of clinical isolates and 21% of environmental isolates.

The limited usage of imipenem has resulted in a decrease in resistance to this antibiotic. The findings of this investigation revealed a significant rise in quinolone resistance (ciprofloxacin, ofloxacin, and nalidixic acid). For clinical and environmental isolates, nalidixic acid demonstrated the highest resistance (82 % and 85 %), followed by ciprofloxacin (80 % and 79 %) and ofloxacin (67 % and 55 %). This means that in this setting, quinolones alone cannot be relied upon as an antipseudomonal antibiotic. Because of the rising resistance of nalidixic acid in many hospitals, its empirical use is either prohibited or restricted to reduce the rising resistance rates [15]. It's worth noting that all the environmental isolates were tetracycline resistant. Tetracycline's amazing multi-resistances might be due to the antibiotic's widespread abuse in our environment, as well as an innate and acquired resistance mechanism generated primarily by an active efflux system, which efficiently expels the molecule from the cell. The resistance among P. aeruginosa to a variety of antibiotics might be the consequence of gene transfer into the hospital environment, which is a prevalent nosocomial occurrence, as well as antibiotic overuse. There was no significant difference in resistance patterns of clinical and environmental isolates to the majority of antibiotics (P>0.05) in this investigation. Multiple antibiotic resistances (MARs) were found in all of the isolates evaluated in this investigation, ranging from four to sixteen antibiotics spread over three to seven classes. A previous study reported a MAR of five to eleven antibiotics in their trials. MAR bacterial strains can also evolve as a consequence of independent processes accumulating sequentially in an organism [16-20]. The finding suggests that the isolates in this investigation came from a high-risk source(s) of contamination. Multidrug resistance in environmental isolates might be connected to the uncontrolled release of antibiotics and toxins into the environment, putting selective pressure on these medications. Antibiotic usage in hospitals and the general public exert strong selection pressure on antibiotic-resistant microorganisms.

CONCLUSIONS

The finding of the current study revealed that P. aeruginosa is very widespread, and may be isolated from a variety of clinical and environmental sources in different biological habitats. The isolates were resistant to the majority of anti-pseudomonal medications examined, and the contamination came from high-risk sources. The wide range of resistance phenotypes found in clinical and environmental samples has led to assumptions regarding inherent gene transfer in natural microbial ecosystems.

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