EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 11
551-554
Year: 2017

PREVALENCE OF PSEUDOMONAS AERUGINOSA AND ITS RESISTANCE PATTERN IN COMMUNITY BASED CLINICAL SAMPLES

Nilay Chatterjee and Dr. Somasri Dam*

Department of Microbiology, University of Burdwan, Burdwan – 713104, West Bengal, India.

***Corresponding Author: Dr. Somasri Dam**
Department of Microbiology, University of Burdwan, Burdwan – 713104, West Bengal, India.

Article Received on 12/09/2017 **Article Revised on 03/10/2017** **Article Accepted on 24/10/2017**

ABSTRACT

Background: The aim of our present study is to determine the prevalence of *Pseudomonas aeruginosa* from various clinical samples and its resistant pattern to commonly used antibiotics. **Material and Methods:** A total of 3003 clinical samples (urine, blood, pus/wound swab, sputum) were collected from a modern Diagnostic Centre in Kolkata during the period of July to December 2016. *P. aeruginosa* were preliminary identified according to the morphological characteristics and traditional biochemical test. Then confirm by VITEK- 2 (Biomerieux) compact systems a fully automated growth based technology. Antibiogram was also studied by VITEK- 2 (Biomerieux). **Result:** Out of 3003 clinical samples, a total of 34 strains of P. aeruginosa were isolated and the prevalence rate was 1.13%. Maximum number was from samples of urine (64.7%) followed by pus (32.45%). The maximum number of samples from males and the infection was seen maximum among females of age group of >61. Out of 34 strains of Pseudomonas aeruginosa 22 were found Multi Drug Resistant (MDR) strains and 11 were found Extensive Drug Resistance (XDR). Predominant MDR strains isolated from urine. Pseudomonas aeruginosa has shows 100% resistant to Minocycline, Tigecycline and Cotrimoxazole. The only promising antibiotic was Colistin. **Conclusion:** *P aeruginosa* is a common pathogen isolated from clinical samples of patients. Colistin, imipenem, pipercillin and tazobactum were most sensitive for multidrug resistant strains. Periodic susceptibility testing should be carried out over a period of two to three years, to detect the resistance trends. Also, a rational strategy on the limited and prudent use of anti-Pseudomonal agents is urgently required.

KEYWORDS: *Pseudomonas aeruginosa*, Multi Drug Resistance, Colistin.

INTRODUCTION

Pseudomonas aeruginosa is an aerobic, motile, gram negative rod that belongs to the family, pseudomonadaceae.[1] The Pseudomonads are a diverse bacterial group of established and emergent pathogens.[2] The genus *Pseudomonas* accounts for the largest percentage of all non-fermenters isolated from clinical specimens. *P. aeruginosa*, although not an obligate parasite, is the species amongst the Pseudomonads most commonly associated with human diseases.[3] It needs minimal nutritional requirements for growth. *P aeruginosa* emerged as a major human pathogen since 1940 because of its ability to cause infection in immunocompromised and burned hosts as well as cystic fibrosis patients.[4] *P. aeruginosa* is known to cause a wide spectrum of diseases. It can be isolated from various body fluids such as sputum, urine, wounds, and eye or ear swabs and from blood.[3] It is also being increasingly implicated in community acquired infections.

In recent years, drug resistant bacteria have given rise to several serious outbreaks of infections with many deaths.[5] Unfortunately, *P. aeruginosa* demonstrates resistance to multiple antibiotics, thereby jeopardizing the selection of appropriate treatment.[6] Resistance to most antipseudomonal agents has increased by >20%, over the last five years.[7] *P. aeruginosa* is usually inherently resistant to many antimicrobial agents, treatment of pseudomonal infections is usually difficult, and mortality is usually high.[8] *P. aeruginosa* develops resistance by various mechanisms like multi-drug resistance efflux pumps, biofilm formation, production of β-lactamases and aminoglycoside modifying enzymes.[9] Its general resistance is due to a combination of factors.[10] 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.

So, the aim of our present study is to determine the prevalence of *P. aeruginosa* in clinical samples and their

antimicrobial resistance pattern to individual antipseudomonal agents and the magnitude of the multidrug resistance in these organisms.

MATERIAL AND METHODS

The present study was conducted from June 2016 to November 2016 in the department of microbiology in a multi-speciality diagnostic centre in central Kolkata. Common specimens like urine, blood, wound swab/pus and sputum from both community as well as hospitalized patients were received in the department of microbiology were collected. A total of 3003 clinical specimens (176 blood samples, 2645 urine samples, 117 samples of pus and wound swab and 65 lower respiratory secretion samples) were processed for isolation and identification of *Pseudomonas aeruginosa* as per standard procedures. Urine, sputum and pus/wound swabs were inoculated on nutrient, MacConkey and blood agar respectively. After overnight incubation only pure growth of NLF (Non Lactose Fermenters) colonies were processed for identification of Pseudomonas. The single isolated colonies obtained from the primary culture were subjected to gram staining. Only pure growth of gram negative bacilli was subjected to oxidase test.

All blood samples were collected aseptically and introduced into BacT / ALERT PF & FA PLUS bottles and incubated in BacT / ALERT 3D system (Biomerieux). The BacT / ALERT Microbial Detection System utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) dissolved in the culture medium. If microorganisms are present in the test sample, CO_2 is produced as the organisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO_2, the colour of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow. The lighter colour results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes. The positive blood culture samples were subculture in blood agar and MacConkey agar respectively. The pure colonies obtained from the subculture were subjected to gram staining followed by oxidase test.

The pure growths of NLF oxidase positive gram negative bacilli from all samples were utilized for identification using Biomerieux GN – ID cards in VITEK 2 machine. The VITEK- 2 (Biomerieux) compact systems is a fully automated growth based technology that performs bacterial / yeast identification by biochemical analysis using colorimetric method. The gram negative bacteria identification card uses 47 biochemical tests for identification of the organism. The susceptibility tests were done by using Biomerieux AST – N281 cards in VITEK- 2 machine. The antibiotics against which susceptibility are checked by MIC were Amikacin, Aztreonam, Cefepime, Cefoperazone/Sulbactam, Ciprofloxacin, Colistin, Doripenem, Gentamicin, Imipenem, Levofloxacin, Meropenem, Piperacillin/ Tazobactam, Ticarcillin/ Clavulanic acid, Tigecycline and Trimethoprim/ Sulfamethoxazole. Results were interpreted according to CLSI guidelines (2015).

RESULT

A total 34 *Pseudomonas aeruginosa* strains were isolated from 3003 various clinical specimens. Prevalence of *Pseudomonas aeruginosa* is 1.13%. The highest isolation rate of *Pseudomonas aeruginosa* was observed from Urine (64.7%) followed by Pus/wound swab (32.35%) and Blood (2.94%).

Table 1: Pseudomonas aeruginosa isolates among all specimens (n = 34).

Name of the Specimen	Number of Specimen (%)	*Pseudomonas aeruginosa* isolates (%)
Urine	2645 (88.07%)	22 (64.7%)
Blood	176 (5.86%)	1 (2.94%)
Pus/Wound Swab	117 (3.89%)	11 (32.35%)
Lower Respiratory Secretion	65 (2.16%)	X
Total	3003	34

The highest percentage of isolates was from patients > 61 years old 21 (61.76%) followed by 40 – 60 years old 9 (26.47%). Specimens isolated from male patients 20 (58.82%) and female patients 14 (41.7%) where the male and female ratio was 1: 0.7.

Table 2: Age wise distribution of *Pseudomonas aeruginosa* (N = 34).

Age in Years	Males (%)	Females (%)	Total (%)
0 to 15	0	0	0
16 to 30	1 (2.94%)	1 (2.94%)	2 (5.88%)
31 to 45	2 (5.88%)	0	2 (5.88%)
46 to 60	8 (23.52%)	1 (2.94%)	9 (26.47%)
> 61	9 (26.47%)	12 (35.29%)	21 (61.76%)
Total	20 (58.82%)	14 (41.7%)	34 (100%)

The resistance pattern of *P. aeruginosa* shows 100% resistance to Minocycline, Tigecycline and co-trimoxazole followed by Levofloxacin, Ticarcillin/ Clavulanic acid, Gentamicin (41.1%), Ciprofloxacin (38.2%), Aztreonam (35.2%), Cefoperazone/Sulbactum (32.3%), Meropenem (29.4%), Piperacillin/Tazobactam, Imipenem, Ceftazidime (26.4%), Amikacin and Cefepime (23.5%). The most effective antibiotic was Colistin shows only 11.7% resistance.

Table 3: Anti-microbial resistant pattern of *Pseudomonas aeruginosa* (n = 34).

Antibiotics Group	Antibiotic Agent	MIC Value (µg/ml)		Resistance (%)
		S	R	
Penicillin	Piperacillin/Tazobactam (PIT)	≤64/4	≥128/4	9 (26.4%)
	Trimethoprim/Sulfamethoxazole (COT)	≤2/38	≥8/152	34 (100%)
	Ticarcillin/Clavulanic acid (TCC)	≤64/2	≥128/2	15 (44.1%)
Cephalosporin	Cefoperazone/Sulbactam (CS)	≤16	≥32	11 (32.3%)
	Ceftazidime (CAZ)	≤8	≥32	9 (26.4%)
	Cefepime (CPM)	≤8	≥32	8 (23.5%)
Monobactam	Aztreonam (AT)	≤4	≥16	12 (35.2%)
Carbapenem	Imipenem (IPM)	≤4	≥16	9 (26.4%)
	Meropenem (MRP)	≤4	≥16	10 (29.4%)
	Doripenem (DOR)	≤4	≥16	10 (29.4%)
Aminoglycosides	Amikacin (AK)	≤16	≥32	8 (23.5%)
	Gentamicin (GEN)	≤4	≥8	14 (41.1%)
Glycylcyclines	Tigecycline (TGC)	≤4	≥16	34 (100%)
	Minocycline (MI)	≤4	≥16	34 (100%)
Polymyxin	Colistin (CL)	≤2	≥8	4 (11.7%)
Fluoroquinolones	Ciprofloxacin (CIP)	≤1	≥4	13 (38.2%)
	Levofloxacin (LE)	≤2	≥8	15 (44.1%)

Bacterial strains that show resistance to three or more categories of antibiotics are referred as Multi Drug Resistant (MDR). More number of MDR strains are isolated from urine15 (68.18%) followed by pus 7 (31.81%).

Table 4: Multi Drug and Extensive Drug Resistant strains among *Pseudomonas aeruginosa* (N = 34).

Name of the Specimen	MDR Strains (%)	XDR Strains (%)
Urine	15 (68.18%)	6 (54.54%)
Blood	X	1 (9.09%)
Pus/Wound Swab	7 (31.81%)	4 (36.36%)
Lower Respiratory Secretion	X	X
Total	22	11

DISCUSSION

Pseudomonas aeruginosa has emerged as an important pathogen and is responsible for the nosocomial infections that is one of the important causes of morbidity and mortality among hospital patients.[11] Despite advance in sanitation facilities and introduction of a wide variety of antimicrobial agents with anti-pseudomonal activities, life threatening infections caused by *P. aeruginosa* continue to be increasing.[12]

In our present study we isolated 22 MDR strains out of 34 strains of *P. aeruginosa* among 3003 community based clinical samples from various patients. The prevalence of *P. aeruginosa* was 1.13%. Most isolates in this study were obtained from urine samples, accounting for 64.7% of the total. This is not surprising considering the fact that most samples from community patients. When factors such as age and sex of patients were considered, we found the occurrence of *P. aeruginosa* to be higher in females and inpatients in the age group of >61 years.

Drug resistance pattern of *P. aeruginosa* isolated from clinical specimens we found to be highly variable. 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).[13] 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 antibiotics.[14] In our study aminoglycoside like amikacin and gentamicin shows 23.5% and 41.1% resistance respectively similar finding shown by L.tif. Savap, Nizami et al,[12] amikacin 42.2%, gentamicin70.7%. Ceftazidime and cefepime are the most frequently prescribed third and fourth generation cephalosporins respectively. In our study Ceftazidime resistance was 26.4%, L.tif.Savap, Nizami et al.,[12] shown 48.9%, resistance of ceftazidime was 15 – 22% in the world.[15] In our study Piperacillin resistance was 26.4%, L.tif.Savap, Nizami et al.,[12] obtained 28%, Bouza E et al.[16] in Spain 10%, Bongiflio G et al[15] in Italy 12%.

In various studies which investigated the resistance of *P.aeruginosa* to ciprofloxacin, the proportion was reported to be 0-89%. In the present study, the overall resistance to ciprofloxacin was around 38.2%. 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 44.1. 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 control.[17]

CONCLUSION

The antimicrobial agents are losing their efficacy because of the spread of resistant organism due to indiscriminate use of antibiotics, lack of awareness and non-availability of antimicrobial testing facilities. Empirical antibiotic treatment should be avoided and treatment should be carried out using antibiotic susceptibility tests and continuous monitoring and comparative analysis of emergence of resistance trends of *Pseudomonas aeruginosa* is essential in health care centres.

ACKNOWLEDGEMENT

Dr. Kumkum Bhattacharyya, Department of Microbiology, IPGMER, Kolkata for collection of samples and analysis.

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