International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 8, Issue 11, 2016, pp.-804-806.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

DETECTION OF RESISTANT PHENOTYPES OF PSEUDOMONAS AERUGINOSA IN TERTIARY CARE HOSPITAL

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Received: October 23, 2016; Revised: October 26, 2016; Accepted: October 27, 2016; Published: October 28, 2016

Abstract-: Background: *Pseudomonas aeruginosa* cause infections of all sites and is common cause of nosocomial infections. **Objectives:** Detection of resistant phenotypes of *P.aeruginosa* clinical isolates in rural tertiary care hospital. **Materials and methods:** A total of 88 *P.aeruginosa* were included in the study and antibiotic susceptibility of the isolates was determined by Clinical laboratory and standard institute [CLSI] guidelines. Betalactames, aminoglycosides and quinolones resistant phenotypes of *P.aeruginosa* are detected on the basis of antibiotic susceptibility profile. Extended spectrum betalactamases (ESBL), AmpC betalactamases and Carbapenemases are detected using standard microbiology guidelines. **Result:** Out of 88 *P.aeruginosa* isolates, majority were isolated from sputum followed by urine and endotracheal tube. Betalactams resistant phenotypes: Out of 88, 71 (81%) were natural wild strain, 4(5%) were ESBL, 3 (4%) were carbapenamase, 1 (1%) was penicilinase, oprJ, oprN, and D2 resistant phenotype. Aminoglycosides resistant phenotype: Out of 88, 64 (73%) were natural wild strain, 6 (7%) were G phenotype, 5 (6%) were impermeability phenotype, and 1 (1%) was GNtT phenotype. Quinolones resistant phenotype: Out of 88, 74 (84%) were natural wild strain, 4(5%) were IV phenotype, and 2 (2%) were efflux phenotype. **Conclusion:** Early identification of resistant phenotypes of *P.aeruginosa* based on antimicrobial susceptibility result would guide clinician to start early appropriate therapy that lead to good clinical outcome, and to reduce spread of nosocomial infections

Keywords- AmpC betalactamases, Carbapenemases, Extended spectrum betalactamases, *Pseudomonas aeruginosa*, Resistant phenotypes

Citation: Oza Sweta S., et al., (2016) Detection of Resistant phenotypes of Pseudomonas Aeruginosa in Tertiary Care Hospital. Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 8, Issue 11, pp.-804-806.

Academic Editor / Reviewer: Kunjan Kikani, Amitabha Dan, Dr Vinod Kumar Singh, Senouci-rezkallah Khadidja

Introduction

Pseudomonas aeruginosa cause infections of all sites; most common are lungs, skin and soft tissues. Most of the infections occurred in hospitalized patients. It causes ventilator associated pneumonia (VAP), catheter associated urinary tract infections (CAUTI), infective endocarditis, and burn wound infections and chronic respiratory tract infections in patients with underlying bronchiectasis, cystic fibrosis. *P. aeruginosa* is multi-disinfectant resistant, thus increasing infection in hospital [1, 2].

Natural inherent resistance is known as wild - type phenotype that is chromosomal mediated. Acquired resistances are either by enzymatic mechanisms like ESBL, AmpC betalactamases, class A and class B (Metallo-betalactamases [MBL] carbapenemases or and non-enzymatic mechanisms alike to impermeability efflux and targets change [3]. These mechanisms of resistance can present simultaneously, and leads to multidrug resistant phenotypes which are most worrisome part [4].

Antimicrobial susceptibility testing and resistant phenotype detection of *P. aeruginosa* is therefore crucial in clinical practice. Empirical treatment of infections by *P. aeruginosa* usually selected on the basis of known local epidemiology. Colistin have proven useful against multidrug resistant strains, but newer treatments options for the future are scarce, therefore, selection of appropriate antibiotics and optimize their use currently remains the best way of coping with pseudomonas infections[5].

Awareness of resistant phenotypes of *P. aeruginosa* is very important as it help in choosing appropriate treatment.

Materials and Methods

A total 900 various specimens from patients were received in microbiology laboratory between July 2014 to January 2015. All the specimens were process and isolates that were identified as *P. aeruginosa* using standard microbiological guidelines (n=88) were incorporated in study [6].

Antimicrobial agents

Antibiotic disks used in present study were piperacillin (100µg), piperacillin/ tazobactam (100/10µg), cefoperazone (75ug), ceftazidime (30µg), cefepime (30µg), cefpirome (30µg), aztreonam (30µg), imipenem (10µg), meropenem (10 µg), cefoperazone/sulbactam(75/10µg), gentamicin (10µg), tobramicin (10µg), amikacin (30µg), netilmycin (30µg) and ciprofloxacin(5µg), ofolxacin (5µg), norfloxacin (10µg), levofloxacin (5 µg). All antibiotic disks were obtained from Himedia laboratories.

Antimicrobial susceptibility test

Antibiotic susceptibilities of bacterial isolates were determined according to standard guidelines suggested by the Clinical and Laboratory Standards Institute [7]. Isolated and identified colonies of *P. aeruginosa* were inoculate and antibiotic disks were placed on MHA plate. The MHA plate was incubated at 37°C for 18-20 hrs. Result was recorded as sensitive, resistant and intermediate according to diameter of zone of inhibition. The standard strain of *P. aeruginosa* (ATCC27853) was used as a control.

Resistant phenotype detection:

Betalactams resistant phenotypes [Table-1], aminoglycosides resistant phenotypes [Table-2] and quinolones resistant phenotypes [Table-3] of *P. aeruginosa* were identified based on antibiotic susceptibility result of following antibiotics [4].

Table-1 *Betalactams resistant phenotypes of P.aeruginosa*

Drug	Wild type	Penicil linase	AmpC	ESBL		Carbapenemase			Efflux			D2
				CTX-M	PER	A	B	D	OprM	OprJ	OprN	
Ticarcillin	S	R	R	R	R	R	R	R	R	S	S	S
Ticarcillin/ Clavulanic acid	S	S	R	S	S	R	R	R	R	S	S	S
Piperacillin	S	R	R	R	R	S	R	R	S	S	S	S
Piperacillin/ Tazobactam	S	S	R	S	S	S	R	R	S	S	S	S
Cefoperasone	S	R	R	R	R	R	R	S	S	S	S	S
Ceftazidime	S	S	R	R	R	R	R	S	S	S	S	S
Cefepime	S	S	S	S	R	R	R	S	S	R	S	S
Cefpirome	S	S	S	S	R	R	R	S	S	R	S	S
Aztreonam	S	S	S	S	R	S	R	R	S	S	S	S
Imipenem	S	S	S	S	S	R	S	S	R	S	R	R

Table-2 *Aminoglycosides resistant phenotypes of P.aeruginosa*

Drug	Gentamycin	Amikacin	Tobramycin	Netilmycin
Wild type	S	S	S	S
G	R	S	S	S
GNt	R	S	S	R
GT	R	S	R	S
GNtT	R	S	R	R
TNtA	S	R	R	R
GTNtA	R	R	R	R
Impremeability	R	R	R	R

Table-3 *Quinolones resistant phenotypes for P.aeruginosa*

Drug	Ciprofloxacin	Lovofloxacin	Ofloxacin	Norfloxacin
I (Wild type)	S	S	S	S
II	S	I	I	I
III	S	R	R	R
IV	R	R	R	R
Efflux	R	S	S	R

Resistant phenotype confirmation [7].

Extended-spectrum betalactamases (ESBLs) detection

Screening test: Isolates of *P. aeruginosa* that exhibit resistance to cefotaxime (<27 mm) and ceftazidime (<22mm) were considered as screening tests positive.

Phenotypic confirmation test:

Combined disc method:Two disks, ceftazidime (CAZ) and ceftazidime+clavulanic acid (CAZ-CAC) disks were placed on 30 mm apart from centre to centre on the surface of aMHA plate.

Zone of inhibition of ceftazidime+clavulanic (CAZ-CAC)disk was ≥ 5 mm than that of ceftazidime (CAZ) disk alone, it was considered as ESBLs positive.[8]

AmpC detection

Screening test: Isolates of *P.aeruginosa* that exhibit resistance to cefoxitin (30µg) were considered as screening test positive.

Phenotypic confirmation test: Two disks, cefoxitin and cefoxitin+boronicacid disks were placed 30 mm apart from centre to centre on the surface of aMHA plate.

Zone of inhibition of cefoxitin+ boronic acid disk was ≥ 5 mm than that of cefoxitin disk alone, it was considered as AmpC positive, and confirming that the strain produces AmpC [9].

Carbapenemase detection

Screening tests: A *P. aeruginosa* strains that were produces < 21 mm diameter to meropenem, and imipenem were considered as screening test positive [4].

Confirmation tests:

a. Class A carbapenemases confirmation test: Meropenem 10µg disks and meropenem10µg + boronic acid 600µg disks were placed on Mueller Hinton agar. A 5 mm or larger diameter between the meropenem disc and the meropenem+boronic acid disc confirms that the strain produces class A carbapenemase [10].

b. Class B (MBL) carbapenemases confirmation test: Meropenem 10µg disks and meropenem 10µg + EDTA 750µg discs were placed on Mueller Hinton agar. The MBL detection is based on the 7 mm or larger difference between the meropenem disc and the meropenem+EDTA disc [11, 12].

Result

Total 88 *P. aeruginosa* isolated from various clinical samples of patients coming to rural tertiary care center.

Out of 88, majority were isolated from sputum samples 62 (72%), followed by urine 8 (9%), pus 7 (8%), ET 6 (7%), and least were isolated from swab 3 (4%), stool 1 (1 %), and blood 1 (1 %) as shown in [Table-1]

Out of 88, 75 (85%) were isolated from indoor patients and 13 (15%) were from outdoor patients

Table-4 *Antibiogram of isolated P.aeruginosa*

Antibiotics	Sensitive (%)
Ceftazidime	89
Cefoperazone	89
Piperacillin/Tazobactam	98
Cefoperazone/Sulbactam	88
Imipenem	90
Colisin	100
Amikacin	89
Gentamycin	84
Netilmycin	85
Tobramycin	88
Ciprofloxacin	92
Levofloxacin	93
Norfolaxacin	89
Ofloxacin	92

P. aeruginosa isolates exhibits 100% sensitivity to colistin, 98% to piperacillin/ tazobactam, 92-93% to quinolones, 90% to imipenem, 84% to gentamicin, and 88-90% to antipseudomonal cephalosporins.

Out of 88, 71 (81%) were natural wild strain, 4(5%) were ESBL, 3 (4%) were carbapenamase, 1 (1%) was penicilinase, oprJ, oprN, and D2 resistant phenotype. None of them was AmpC and oprMproducer.

Out of 88, 64(73%) were natural wild strain, 6 (7%) were G phenotype, 5(6%) were impermeability phenotype, and 1 (1%) was GNtT phenotype. None of them was GNt, GT, and TNt phenotype producer.

Table-5 *Betalactams resistant phenotypes of P.aeruginosa*

Betalactams resistant phenotypes	No.	%
Natural wild strain	71	81
Penicillinase	1	1
ESBL	4	5
Carbapanemase	3	4
Class B (MBL)	2	2
Class D	1	1
EFFLUX		
OprJ	1	1
OprN	1	1
D2	1	1
Unidentified	7	8

Table-6 *Aminoglycosides resistant phenotypes of P. aeruginosa*

Aminoglycosides resistant phenotype	No.	%
Wild type	64	73
G	6	7
GNtT	1	1
Impermeabilty phenotype	5	6
Unidentified	9	10

Table-7 *Quinolones resistant phenotypes of P. aeruginosa*

Quinolones resistant phenotype	No.	%
I	74	84
IV	4	5
Efflux	2	2
Unidentified	8	9

Out of 88, 74(84%) were natural wild strain, 4(5%) were IV phenotype, and 2(2%) were efflux phenotype. None of them was II and III resistant phenotype producer.

Discussion

In present study, Out of 88 *P. aeruginosa* isolates, 62 (72%) from sputum samples, 8 (9%) from urine, 6 (7%) from endotracheal tube ET, 1 (1 %) from stool, and blood samples isolated. Majority were from sputum followed by urine and ET. In Oana Alexandra et al reported, maximum isolates of *P. aeruginosa* from urine samples followed by sputum, pus and blood [3]. Hamze et al. study showed majority of *P. aeruginosa* were isolated from broncho-alveolar secretions (34.1%) and urine samples (26.1%).[13] *P. aeruginosa* infection commonly occurs in patients with underlying bronchiectasis, cystic fibrosis, catheterized and intubated patients [2,14].

In Hamze at al study, antibiotic susceptibility of the *P. aeruginosa* isolates was 87.5% towards meropenem, 80.7% towards cefepime, 78.4% towards imipenem, 77.4% towards ceftazidime, and 66% towards ticarcillin.[13] In present study, *P. aeruginosa* isolates exhibits 100% sensitivity to colistin, 98% to piperacillin/ tazobactam, imipenem (90%) 92-93% to quinolones, 90% to imipenem, 89% to ceftazidime , 89% to cefoperazone, 89% to amikacin.

In present study, 71 (81%) were beta lactams natural wild strain, 4(5%) were ESBL, 3 (4%) were carbapenamase, 1 (1%) was penicilinase, oprJ, oprN, and D2 resistant phenotype.

In Zhilong Chen study, ESBLs production was variable from 35.3% in the burn wards to 64.7%[15] and whiles in present study the frequency of ESBLs production by *P.aeruginosa* in the hospital were 4% that is very much less than Chen study. Nirav Pandya et al study report 9.9% MBL production in pseudomonas sp. [12] while in present study it was 2% in *P.aeruginosa*.

In present study, 64 (73%) were aminoglycosides natural wild strain, 6 (7%) were G phenotype, 5 (6%) were impermeability phenotype, and 1(1%) was GNtT phenotype. In present study, 74 (84%) were quinolones natural wild strain, 4(5%) were IV phenotype, and 2(2%) were efflux phenotype. There was maximum wild type phenotype isolated.

P. aeruginosa is one of the most challenging pathogenic bacteria. There is the constant evolution of resistance in *P. aeruginosa* that leads to appearance of new resistance mechanisms, so there is need to early diagnose the *P.aeruginosa* resistant phenotype for good clinical outcome. This study helps in early identification of P. *aeruginosa* antibiotypes and their resistance pattern based on antibiotic susceptibility result and guide clinician to start early appropriate therapy that lead to good clinical outcome, and reduce spread of nosocomial infections.

Source of support: Nil

Conflict of interest: None

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