Study of the antibiotic sensitivity pattern of Pseudomonas aeruginosa and its mechanism of resistance

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Abstract

Introduction: The multidrug resistant nature of P. aeruginosa is a major cause of morbidity in immunocompromised patients. The mechanisms which play a role in causing multidrug resistance of P.aeruginosa includes lower outer membrane permeability, increased expression of efflux pumps, presence of Amp C Beta lactamase enzyme. The present retrospective study aims to analyse the antibiotic sensitivity pattern of P.aeruginosa in our clinical setting in various clinical specimens and to study the various mechanisms playing a role in resistance to antibiotics.

Materials and methods: The retrospective study included all the Psedomonas aeruginosa isolates from clinical specimens received in our microbiology department between June 2015 to June 2016. The specimens included pus, aural swab, conjunctival swab, blood, urine and endotracheal tube. A total of 40 cases were included in the study.

Results: highest sensitivity was observed for amikacin(65%), Piperacillin/ tazobactum(52.5%),Gentamycin(47.5%) Highest resistance (77.5%) was to Trimethoprim/ sulphamethaxazole, 62.5% to nalidixic acid, 57.5% cases had resistance to quinolones. Impermeability to carbapenems accounted for 42.5% cases, followed by carbapenamase(metallo-oxa) resistance mechanism in 37.5%.

Conclusion: Growing resistance to commonly used antibiotics is a major concern to clinicians as several complex mechanisms are involved in the multi-drug resistance nature of P.aeruginosa. Careful use of antibiotics, culture and sensitivity testing and prudent selection of drugs and effective infection control measures would help the clinicians in planning and executing effective treatment thus limiting the emergence of multidrug resistant strains of Pseudomonas aeruginosa.

Keywords: P. aeruginosa, Resistance mechanism, AAC, ANT, Carbapenemase, AmpC

Introduction

Peudomonas aeruginosa is a gram negative bacillus known to be resistant to many antibiotics. It is a major cause of nosocomial infections specially in immunocompromised patients.(1) The multidrug resistant nature of P. aeruginosa is a major cause of morbidity in immunocompromised patients. P.aeruginosa causes pneumonia, bacteremia, urinary tract infections, skin and soft tissue infections, otitis media and a host of other infections which are difficult to eradicate owing to its multidrug resistance.

The mechanisms which play a role in causing multidrug resistance of P.aeruginosa includes lower outer membrane permeability, increased expression of efflux pumps,presence of Amp C Beta lactamase enzyme which leads to resistance to commonly used antibiotics like Penicillin G, cefalosporins and quinolones.(2)

The broad spectrum activity of carbapenems makes it a choice of treatment for Pseudomonas infections when all other drugs become resistant. Therapeutic options for treating P.aeruginosa are limited because of its innate resistance to several antibiotics, caused mainly because of the indiscriminate use of antibiotics, incomplete therapy, inadequate dosage and over-the-counter use of drugs.(3)

The present retrospective study aims to analyse the antibiotic sensitivity pattern of P.aeruginosa in our clinical setting in various clinical specimens and to study the various mechanisms playing a role in resistance to antibiotics.

Materials and Methods

The retrospective study included all the Psedomonas aeruginosa isolates from clinical specimens received in our microbiology department between June 2015 to June 2016.The specimens included pus, aural swab, conjunctival swab, blood, urine and endotracheal tube. A total of 40 cases were included in the study.

Specimens were collected by standard protocol and for catheterized patients urine samples were collected in sterile screw capped wide mouth container after clamping the catheter for 30 minutes. All the specimens were inoculated on Blood and MacConkey agar plates and incubated at 37^0C for 24 hours.

The bacteria were identified on the basis of Gram staining and colony morphology and accordingly the panel N280 for identification & susceptibility was chosen to be processed on Vitek II (Biomerieux).

Results

A total of 40 patients with growth of Pseudomonas aeruginosa in different clinical specimens between June 2015 to June 2016 were included in the study. The patients were divided into < 20, 21-40, 41-60, 61 to80

and more than 80 years age group in both the sexes.(Table 1).

5% patients were in > 80 years age group, followed by 12.5% in 41-60, 20% in61-80 years age group, 30% below 20 years and maximum 32.5% in 21-40 years age group.

67.5%(27/40) patients were males and 32.5%(13/40) were females. Male to female ratio 9 was 2.07:1(Table 1).

Table 1: Demographic data of pseudomonas aeruginosa

Age (Years)	Male	Female	Total
<20	5	7	12
21-40	11	3	14
41-60	4	1	5
61-80	5	1	6
>80	2	1	3
Total	27	13	40

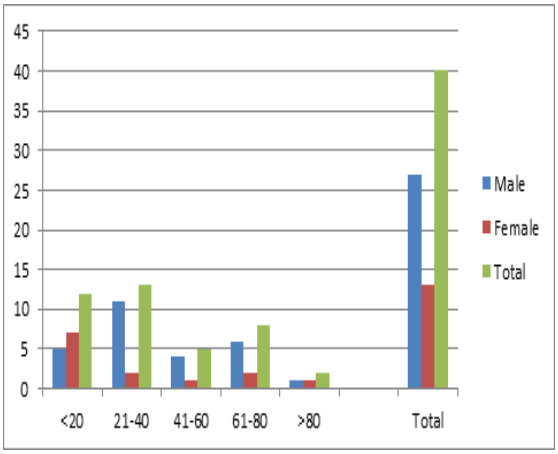

Fig. 1

Pseudomonas aeruginosa was grown in 2.5% patients with aural and conjunctival swab as specimen, 7.5% in blood, 12.5% in endotracheal tube, 20% in pus and maximum 55% in urine(Table 2).

Table 2: Demographic data of pseudomonas aeruginosa in different specimens

Specimen	Male	Female	Total
Aural Swab	1	0	1
PUS	6	2	8
Blood	1	2	3
Conjuctival Swab	1	0	1
Endotracheal Fluid	4	1	5
Urine	12	10	22
Total	25	15	40

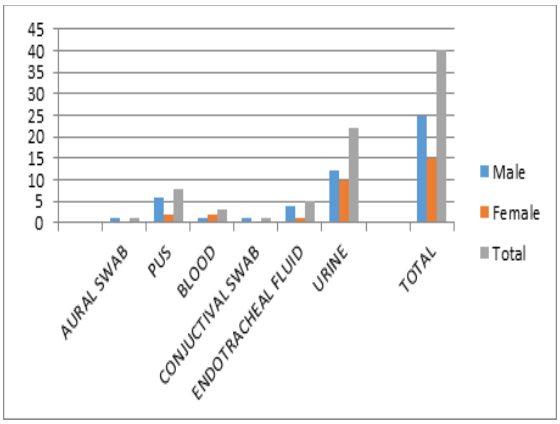

Fig. 2

Table 3 shows the MIC value of sensitive and resistant antibiotics.

Table 3: Showing MIC value of antibiotics

Antibiotic	Sensitive MIC Value	Resistant MIC Value
AM-Ampicillin	4	>=32
AMC-Amoxicillin/ Clavulanic Acid	4,8	>=32
AN-Amikacin	<=2	>=64
CAZ-Ceftazidime	<=1	>=64
CFM-Cefixime	<=0.25	>=4
CIP-Ciprofloxacin	<=0.25	>=4
CRO-Ceftriaxone	<=1	>=64
CS-Colistin	<=0.5	>=64
CXM-Cefuroxime	<=1	>=64
CXMA-Cefuroxime Axetil	<=1	>=64
DOR-Doripenem	0.5	>=8
FEP-Cefepime	<=1	>=64
FOS-Fosfomycin	<=16	>=256
FOX-Cefoxitin	<=4	>=64
FT-Nitrofurantoin	<=16	>=512
GM-Gentamicin	<=1	>=16
IPM-Imipenem	<=0.25	>=16
LEV-Levofloxacin	0.25	>=8
MEM-Meropenem	<=0.25	>=16
MNO-Minocycline	<=1	>=16
NA-Nalidixic Acid	<=2	>=32
NOR-Norfloxacin	<=0.5	>=16
OFL-Ofloxacin	<=0.25	>=8
SFP-Cefoperazone/ Sulbactam	<=8	>=64
SXT-Trimethoprim/ Sulfamethoxazole	<=10	>=320
TCC-Ticarcillin/ Clavulanic Acid	64	>=128
TGC-Tigecycline	<=0.5	>=8
TIC-Ticarcillin	<=8	>=128
TZP-Piperacillin/ Tazobactam	<=4	>=128

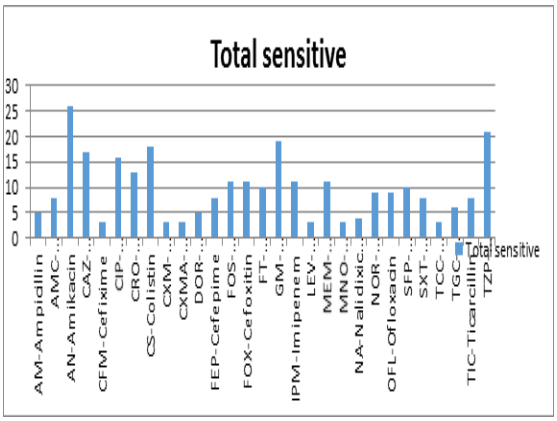

Fig. 3

The study of sensitivity pattern of P.aeruginosa showed highest sensitivity for amikacin (65%), Piperacillin/ tazobactum (52.5%), Gentamycin (47.5%), colistin (45%), Ceftazidime (42.5%), ciprofloxacillin (40%), ceftriazone (32.5%), fosfomycin, cefoxitin, Imipenem, Merpoenem (27.5%), ofloxacin, norfloxacin (22.5%), trimethoprim/sulfamethaxazole, Ticarcillin, cefipime and amoxyclavulinic acid (20%), tigecycline (15%), Doripenem and ampicillin (12.5%) Nalidixic acid (10%), nitrofurantoin, cefoperazone/ sulbactum (9%), and lowest sensitivity (5%) to ceftazidime, cefuroxime, cefuroxime axetil, Ticarcillin/ clavulinikc acid and minocycline.(Table 4)

Table 4: Showing data of sensitive antibiotics

Antibiotic	Total sensitive
AM-Ampicillin	5
AMC-Amoxicillin/ Clavulanic Acid	8
AN-Amikacin	26
CAZ-Ceftazidime	17
CFM-Cefixime	3
CIP-Ciprofloxacin	16
CRO-Ceftriaxone	13
CS-Colistin	18
CXM-Cefuroxime	3
CXMA-Cefuroxime Axetil	3
DOR-Doripenem	5
FEP-Cefepime	8
FOS-Fosfomycin	11
FOX-Cefoxitin	11
FT-Nitrofurantoin	10
GM-Gentamicin	19
IPM-Imipenem	11
LEV-Levofloxacin	3
MEM-Meropenem	11
MNO-Minocycline	3
NA-Nalidixic Acid	4
NOR-Norfloxacin	9
OFL-Ofloxacin	9
SFP-Cefoperazone/ Sulbactam	10
SXT-Trimethoprim/ Sulfamethoxazole	8
TCC-Ticarcillin/ Clavulanic Acid	3
TGC-Tigecycline	6
TIC-Ticarcillin	8
TZP-Piperacillin/ Tazobactam	21

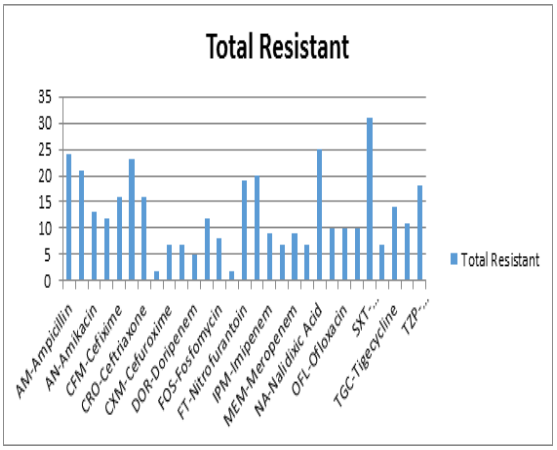

Fig. 4

Highest resistance (77.5%) was observed to Trimethoprim/sulphamethaxazole, followed by 62.5% to nalidixic acid, 60% to ampicillin, 57.5% to ciprofloxacin, 52.5% to amoxyclavulinic acid, 50% to gentamycin, 47.5% to 9 nitrofurantoin, 45% to piperacillin/ tazobactum, 40% to cefixime and ceftriaxzone, 35% to tigecycline, 32.5% amikacin, 30% to ceftazidime and cefipime, 27.5% to ticarcillin, 25% to norfloxacillin, ofloxacillin and cefoperazone/ sulbactum, 22.5% to imipenem, meropenem, 20% to fosfomycin, 17.5% to levofloxacin, minocycline, cefuroxime and cefuroxime axetil, 12.5% to Doripenem, 11.5% to ticarcillin/ clavulinic acid and lowest resistance to colistin and cefoxitin(5%).(Table 5)

Table 5: Showing data of resistant antibiotics

Antibiotic	Total Resistant
AM-Ampicillin	24
AMC-Amoxicillin/ Clavulanic Acid	21
AN-Amikacin	13
CAZ-Ceftazidime	12
CFM-Cefixime	16
CIP-Ciprofloxacin	23
CRO-Ceftriaxone	16
CS-Colistin	2
CXM-Cefuroxime	7
CXMA-Cefuroxime Axetil	7
DOR-Doripenem	5
FEP-Cefepime	12
FOS-Fosfomycin	8
FOX-Cefoxitin	2
FT-Nitrofurantoin	19
GM-Gentamicin	20
IPM-Imipenem	9
LEV-Levofloxacin	7

MEM-Meropenem	9
MNO-Minocycline	7
NA-Nalidixic Acid	25
NOR-Norfloxacin	10
OFL-Ofloxacin	10
SFP-Cefoperazone/ Sulbactam	10
SXT-Trimethoprim/ Sulfamethoxazole	31
TCC-Ticarcillin/ Clavulanic Acid	7
TGC-Tigecycline	14
TIC-Ticarcillin	11
TZP-Piperacillin/ Tazobactam	18

In the study of mechanism causing resistance to antibiotics against Pseudomonas aeruginosa, it was observed that 57.5% cases had resistance to quinolones. Impermeability to carbapenems in the beta lactam family accounted for 42.5% cases, followed by carbapenamase(metallo-oxa) resistance mechanism in 37.5% along with resistance to Gentamycin, netilmycin, amikacin and tobramycin(aminoglycoside6) and Resistance to Gentamycin, amikacin, netilmycin(aminoglycoside 7). Resistance to fosfomycin was observed in 32.5% cases. Low level non enzymatic ticarcillin resistance was observed in 25% cases. Acquired penicillinase (beta lactam -6) resistance was seen in 22.5%, followed by 20% as Acquired pase+impermeability to carbapenems and high level resistance to beta lactams(family-15). Resistance to gentamycin/netilmycinand tobramycin, gentamycin, netilmycin (aminoglycoside 8, 9, 10, 11) and impermeability to carbapenems (beta lactam 24) accounted for 10% cases. Only 7.5% cases observed had resistance to tobramycin/ netilmycin/ amikacin(aminoglycoside-12 family).

Table 6 shows the resistance mechanism in different clinical specimens.

Table 6: Resistant Mechanism of Pseudomonas aeruginosa in various clinical specimens

SPECIMENS	Family - AMINOGLYCOSIDES - 6	Family - AMINOGLYCOSIDES - 7	Family - AMINOGLYCOSIDES - 8	Family - AMINOGLYCOSIDES - 9	Family - AMINOGLYCOSIDES - 10	Family - AMINOGLYCOSIDES - 11	Family - AMINOGLYCOSIDES - 12	Family - BETA-LACTAMS - 3	Family - BETA-LACTAMS - 6	Family - BETA-LACTAMS - 8	Family - BETA-LACTAMS - 14	Family - BETA-LACTAMS - 15	Family -BETA-LACTAMS - 20	Family - BETA-LACTAMS - 24	Family - FOSFOMYCIN - 1	Family - QUINOLONES - 2
	Resistant (GENNETAMITOB)	Resistant (GENNETAMI)	Resistant (GENNET)	Resistant (GEN)	Resistant (TOBgennet)	Resistant (TOBGEN)	Resistant (TOBNETAMI)	ACQPASE + RCARBAPENEMS (IMPERMEABILITY)	ACQUIRED PENICILLINASE	CARBAPENEMASE (METALLO-OROXA)	High Level R + RCARBAPENEMS (IMPER)	High Level RESISTANCE	Low Level Non Enzymatic TICAR Resistance	Resistant CARBAPENEMS (IMPERMEABILITY)	Resistant	Resistant
AURAL SWAB	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
PUS	5	5	0	0	0	0	0	2	0	4	5	0	0	0	0	5
BLOOD	0	0	1	1	1	1	0	1	2	0	0	0	2	0	0	1
CONJUCTIVAL SWAB	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1
ENDOTRACHEAL FLUID	5	5	0	0	0	0	0	2	0	5	5	0	0	0	0	5
URINE	5	5	3	3	3	3	2	3	6	6	7	8	6	4	13	11

Highest resistance mechanisms were observed in urine followed by endotracheal tube, pus, blood, conjunctival swab and least in aural swab.

Discussion

In our study, majority of the Pseudomonas isolates were in males as compared to females, with maximum patients in 21 to 40 years age group. Urine was the most common specimen in which Pseudomonas was isolated because this age group is more prone to urinary tract infections as this age group is more active sexually.

Amikacin, Piperacillin/ Tazobactum, gentamycin, colistin and ceftazidime showed highest sensitivity while trimethoprim/ sulfamethaxazole, nalidixic acid, ampicillin, ciprofloxacin, nitrofurantoin showed maximum resistance to P.aeruginosa. According to the study conducted by Aizaz Shah et. al., they observed that Imipenem had minimum resistance (10.4%), followed by Piperacillin/ Tazobactum(19.6%) and Amikacin (25.3%).[4] Our study correlates with this study. A study by Naeem et al showed Amikacin to be 99-100% sensitive followed by Piperacillin/ Tazobactum against P.aeruginosa.[5] A study conducted by Hasan AS et al showed high level resistance to Amikacin.[6] In the study by Bouza E et al in Europe, P. aeruginosa showed 72% resistance to Gentamycin, 69.2% to Tobramycin, 40% to amikacin.[7] This could be due to the fact that Amikacin is used only in severe infections because of its high treatment cost and intravenous route of administration. Thus, resistance to amikacin emerges slowly.

There are several mechanisms which cause resistance to develop against P.aeruginosa. The resistance to aminoglycosides, Gentamycin, is mainly due to enzymatic N-acetylation of deoxystreptamine moiety.[8] Acetylation of aminoglycosides (AACs) can be seen at 1, 3, 6' and 2' amino groups and include gentamycin, tobramycin, netilmycin and amikacin.

APHs (aminoglycoside phosphoryl transferases) cause inactivation of aminoglycosides such as kanamycin, neomycin and streptomycin.[9,10,11] The inactivation is due to phosphoryl transferases(APH (3')) modifying the 3' OH of these antibiotics. APH (3')-I and II are predominant in Pseudomonas isolates.

ANTs (aminoglycoside nucleotidyl transferase) along with AAC represents commonest resistance mechanism in P.aeruginosa.[12,13] The ant (2")-I enzyme causes inactivation of gentamycin and Tobramycin but not netilmycin and amikacin. Reduced uptake of aminoglycosides due to impermeability causes resistance to amikacin, gentamycin and tobramycin.

Recent studies focus on the involvement of efflux mechanism which results in aminoglycoside resistance.[14] Few studies emphasise on the role of ribosomal changes and defects in the electron transport affecting the aminoglycoside uptake.[15,16]

P.aeruginosa has an inherent resistance to several beta lactam antibiotics due to mutations in the transfer of genetic elements like chromosomally encoded inducible AmpC beta lactamase and efflux pump mechanism.[17,18] The extended spectrum beta lactamases (ESBL)are inhibited by clavulinic acid. The metallo beta lactamases (MBL), the cefalosporinase(AmpC) and oxacillinases (oxa) are other mechanisms causing resistance to P.aeruginosa.

All these resistance mechanisms were observed to play a role in causing multi drug resistance to P.aeruginosa in our clinical setting specially in urinary isolates. Probably due to the fact that UTI is the commonest infection.

Conclusion

Growing resistance to commonly used antibiotics is a major concern to clinicians as several complex mechanisms are involved in the multi-drug resistance nature of P.aeruginosa. Careful use of antibiotics, culture and sensitivity testing and prudent selection of drugs and effective infection control measures would help the clinicians in planning and executing effective treatment thus limiting the emergence of multidrug resistant strains of Pseudomonas aeruginosa.

Conflict of interest: None

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