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Original Research Article

Antibiotic profiling of *Pseudomonas aeruginosa* isolates from pus sample of rural tertiary care hospital of Western Maharashtra, Loni, India

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ABSTRACT

Background: *Pseudomonas aeruginosa (P. aeruginosa)* considered as an opportunistic pathogen which can be isolated from various kinds of infection. The risk of emergence of antibiotic resistance is based on different antibiotic treatments. Antibiotic resistance and flexibility to adapt changing environment renders the pathogens a matter of concern in hospital acquired infections. Changing pattern of antimicrobial resistance pose challenge in treating pyogenic infections, hence periodical monitoring of bacterial profile and their antibiotic susceptibility pattern is important. This study deals with the infectious and drug resistance nature of *P. aeruginosa* with effectiveness of antimicrobial agents against it.
Methods: Present study was conducted in Centre for Biotechnology, Pravara Institute of Medical Sciences, Loni, Maharashtra, India. A total of 763 pus samples were received in the bacteriology section of department of microbiology, rural medical college, Loni from the various wards of Pravara Rural Hospital. The colonial morphology and identification was done as per standard microbiology procedures. Antibiogram testing was done as per Kirby Bauer disc diffusion method.
Results: Out of 763 pus samples 154 were *Pseudomonas aeruginosa* thus showing 20.19% prevalence. In this study, it was observed that isolates were sensitive to Ciprofloxacin (76.63%) followed by Amikacin. However, showed 90.90 % resistant to Cefazolin followed by Co-trimoxazole 75.97% was observed. Multi drug resistance (MDR) strain 68.83% (N=106) was detected from 154 isolates strains of *Pseudomonas aeruginosa*. Prevalent resistance pattern was found to be GENr, AKr, CAZr, CZr, COTr for 10 (9.43%) isolates followed by GENr, CAZr, CZr, MRPr, COTr, CIPr for 9 (8.49%) isolates.
Conclusions: Present study focused on antibiotic resistance pattern of *P. aeruginosa* from pus sample. This study contributes in understanding the emergence of MDR strains which can be considered for judicial usage of antibiotics in hospital settings.

Keywords: Antibiotics, Clinical isolate, MDR, *Pseudomonas aeruginosa*, Pus sample

INTRODUCTION

Examination of various clinical specimens is required for accurate diagnosis and then to decide accurate treatment strategy. Various clinical condition leads to pus accumulation, acting as a major source of infection as it provide moist environment for pathogens growth, spread an infection. Pus samples represent a pyogenic infection which is characterized by local inflammation usually caused by any pyogenic bacteria; it leads to accumulation

of dead leucocytes and infectious agent.[1] A break or abrasion in the skin can provide an entryway for these surface bacteria into the body, this stick and moist environment of abrasion allow bacteria to grow exponentially into the cut. The body's defence starts acting by recruiting immune cells into the site attacking bacteria. Eventually, accumulation of these cells produces the thick whitish liquid that we call pus.[2]

Enterococci sp. and *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella sp*. and *Proteus sp*. are commonly observed pus forming microorganism. Among this *Pseudomonas* are extremely notorious. This organism is highly pathogenic due to its ability to produce a variety of toxins and proteases and on its ability to resist phagocytosis, a survival strategy.[2] Genus *Pseudomonas* consist of more than 140 species including *P. aeruginosa*is primary human pathogen in genus *Pseudomonas*.[3]

It is an invasive, gram negative pathogen dually complicating treatment by its drug resistance profile, leading to treatment failure and/or expose patients to adverse effects from advanced antibiotic drug regimens. *P. aeruginosa* tends to grow in moist environment hence can potentially colonize hospital environment. *Pseudomonas aeruginosa* is the leading cause of death as it is associated with high mortality rate. Resistance to many currently available antibiotics is one reason for high death rate.[4]

It is referred to as intrinsic resistance. Its rare occurrence has been observed as normal flora of humans, usually isolated from patients with burns, cystic fibrosis, and neutropenia.[5] *Pseudomonas aeruginosa* is commonly resistant to antibiotics, and because of this it is a dangerous and dreaded pathogen. The strains are regularly sensitive to cephalosporins, carbenicillin, colistin, gentamicin, polymyxin, quinolones, and streptomycin; but a degree of cross-resistance among these agents has been reported.[6]

There are numerous studies across the globe reporting on bacterial profile in the pyogenic wound infections. Case-fatality rates are due to drug resistance profile of *Pseudomonas*. Beta-lactamases, along with penicillinases, cephalosporinases and carbapenems are part of its resistance mechanism.[7] The increasing resistance was observed for drug like carbapenems, Quinolones and 3rd generation Cephalosporins for *Pseudomonas aeruginosa*.[8] This kind of study is crucial for a clinician who intends to start first-hand treatment to the patients. This study was designed to evaluate the profile of *Pseudomonas aeruginosa* along with their susceptibility to antimicrobial agents. Thus, the present study reported on isolation and antibiogram analysis of most notorious *Pseudomonas aeruginosa* from pus samples of Pravara Rural Hospital, Loni, Maharashtra, India.

METHODS

A total of 763 pus sample were received from department of microbiology, rural medical college, from various wards of Pravara Rural Hospitals, Loni, Maharashtra, India.

Sample Processing and identification of organism: two sterile swab sticks were used to collect the pus samples. 1st swab stick was used for gram staining and 2nd swab stick was used for culture. Direct smear with gram stain were screened for the presence of inflammatory cells and type of microbial flora. 2nd swab was inoculated on MacConkey agar (MA) and Blood agar (BA). It was incubated at 37ºC for 24-48 hrs. After observing the growth on MA and BA, it was then subculture on MA and BA. The colonial morphology and identification was done as per standard microbiology procedures.[9, 10]

Antibiogram testing

Selective colonies from the culture plate were inoculated into 2 ml of peptone water. Incubated at 37^0C for 2 hours. Turbidity was compared to that of 0.5 McFarland standards. A cotton swab was immersed and rotated in this inoculum, the swab was then pressed to the inner surface of the tube to remove excess inoculums. It was then used for carpet streaking on Muller Hinton agar plate. The required antibiotic discs were then placed aseptically on this medium with sterile forcep. The plate was incubated 24 hrs at 37^0C. Next day the zone size was recorded and reported as sensitive or resistant by comparing the zone size to the Kirby-Bauer chart. If the organism were not sensitive to any of the drug, then a second line of drug is put up using the same procedures as above. Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion method according to CLSI protocol.[12] Depending on the isolate that is *Pseudomonas aeruginosa*, antibiotic discs were selected from among the following to determine antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolates: Gentamicin (10 mcg/disc), Amikacin (10 mcg/disc), Ceftazidime (30 mcg/disc), Meropenem (10 mcg/disc), Ciprofloxacin (5 mcg/disc), Cefazolin (30 mcg/disc), Co-trimoxazole (25 mcg/disc) were tested (Himedia, Mumbai, India).

RESULTS

A total of 763 pus samples were received from Department of Microbiology laboratory for a period of February-October 2016. *Pseudomonas aeruginosa* was isolated in 154 pus samples (20.19%) out of total 763 samples of the cases studied (Figure 1).

Table 1: Antibiogram of *Pseudomonas aeruginosa*.

Name of antibiotics	Standard antibiotic concentration (10mcg/disc)	*Pseudomonas aeruginosa*, N= 154	
		Sensitive	Resistance
Gentamicin (GEN)	10	65 (42.20%)	89 (57.80%)
Amikacin (AK)	10	100 (64.94%)	54 (35.06%)
Ciprofloxacin (CIP)	05	118 (76.63%)	36 (23.37%)
Ceftazidime (CAZ)	30	66 (42.85%)	88 (57.15%)
Meropenem (MRP)	10	85 (55.20%)	69 (44.80%)
Co-Trimoxazole (COT)	25	37 (24.03%)	117 (75.97%)
Cefazolin (CZ)	30	14 (9.10%)	140 (90.90%)

Table 2: Multidrug resistance (MDR) patterns of *P. aeruginosa*.

Pattern no.	Antibiotic resistant pattern	Number of isolates (N= 106)	Isolates
5	GENr, AKr, CAZr, CZr, MRPr, COTr, CIPr	2	P33, P253
5	GENr, CAZr, CZr, MRPr, COTr, CIPr	9	P259, P263, P305, P657, P834, P876, P890, P899, P965
5	GENr, AKr, CAZr, MRPr, COTr, CIPr	2	P382, P716
5	GENr, AKr, CZr, MRPr, COTr, CIPr	3	P611, P286, P31
4	GENr, AKr, CAZr, CZr, MRPr, CIPr	4	P309, P348, P921, P983
4	GENr, AKr, CAZr, CZr, COTr, CIPr	4	P73, P308, P381, P468
4	GENr, AKr, CAZr, CZr, MRPr, COTr	2	P466, P659
4	GENr, AKr, CZr, MRPr, COTr	5	P88, P335, P409, P758, P779
4	CAZr, CZr, MRPr, COTr, CIPr	2	P896, P464
4	GENr, CAZr, CZr, MRPr, CIPr	1	P923
4	AKr, CAZr, CZr, COTr, CIPr	1	P105
4	GENr, CAZr, CZr, MRPr, COTr	7	P311, P312, P430, P245, P260, P414, P939
4	CAZr, MRPr, COTr, CIPr	1	P999
4	CZr, MRPr, COTr, CIPr	1	P90
4	GENr, AKr, CZr, COTr, CIPr	1	P281
4	GENr, CZr, MRPr, COTr	3	P802, P855, P859
3	GENr, CAZr, CZr, COTr	6	P119, P357, P402, P505, P818, P874
3	GENr, AKr, CAZr, COTr	1	P897
3	GENr, AKr, CAZr, CZr, MRPr	2	P679, P680
3	CAZr, CZr, MRPr, COTr	4	P95, P107, P287, P459
3	CAZr, CZr, COTr, CIPr	2	P69, P101
3	GENr, AKr, CZr, COTr	3	P104, P403, P637
3	GENr, CAZr, CZr, MRPr	8	P109, P340, P350, P355, P443, P489, P512, P596
3	GENr, AKr, CZr, MRPr	1	P356
3	AKr, CAZr, CZr, COTr	5	P46, P293, P294, P295, P477
3	AKr, CAZr, CZr, MRPr	1	P562
3	GENr, AKr, CAZr, CZr, COTr	10	P23, P244, P261, P264, P374, P408, P469, P775, P914, P963
3	GENr, CZr, MRPr	1	P940
3	GENr, CZr, COTr	3	P85, P656, P770
3	GENr, COTr, CIPr	1	P111
3	AKr, CZr, COTr	1	P351
3	CZr, MRPr, COTr	6	P209, P406, P449, P461, P782, P966
3	CZr, COTr, CIPr	1	P426
3	CAZr, COTr, CIPr	2	P603, P706

Thus, antibiogram analysis of *Pseudomonas aeruginosa* was carried out of 154 isolates. The antibiogram of *Pseudomonas aeruginosa* (Table 1) revealed that Ciprofloxacin (CIP) (76.63%) was the most susceptible drug followed by Amikacin (AK) (64.94%), 100% sensitivity to any antibiotics was not observed for *P.*

aeruginosa strains. Highest resistance was observed for Cifazolin (CZ) (90.90%) followed by Co-Trimoxazole (COT) (75.97%). A basic discrimination for sensitive and resistance outlined in Figure 2.

A total of 106 (68.83%) were found to be resistance for ≥ 3 antibiotics group and are called as MDR. This MDR strains were detected from 154 isolates of *Pseudomonas aeruginosa* among 763 pus samples. Among the resistance pattern (Table 2) highly prevalent pattern was found to be GEN^r, AK^r, CAZ^r, CZ^r, COT^r followed by GEN^r, CAZ^r, CZ^r, MRP^r, COT^r, CIP^r.

The following data represents multi drug resistant (MDR) strains based on their resistant to three or more classes of antibiotics (Table 2).

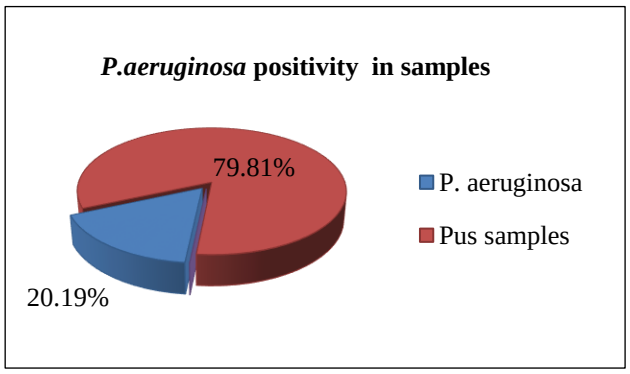

Figure 1: Culture positivity among pus sample.

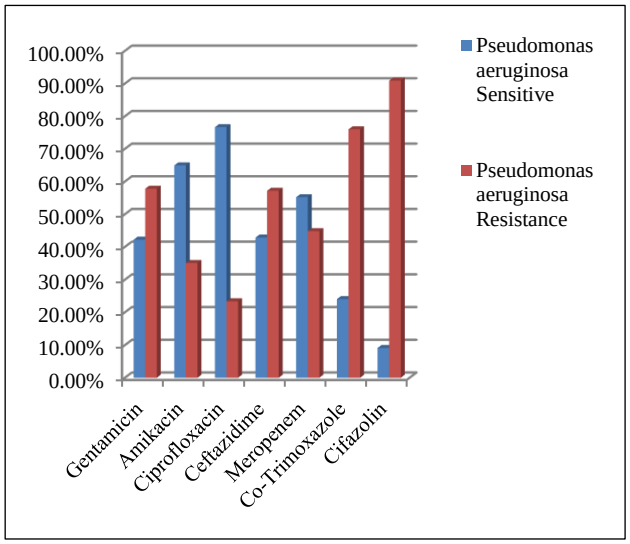

Figure 2: Antimicrobial susceptibility patterns of *P. aeruginosa*.

Among 154 isolates strains of *P. aeruginosa* from 763 pus samples 68.83% (N=106) MDR strain was detected. Most prevalent pattern was found to be GEN^r, AK^r, CAZ^r, CZ^r, COT^r for 10 (9.43%) isolates in106 followed by GEN^r, CAZ^r, CZ^r, MRP^r, COT^r, CIP^r for 9 (8.49%) isolates.

DISCUSSION

A species of substantial medical importance, *P. aeruginosa* is a prototypical "multidrug resistant (MDR) pathogen" recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms. *P. aeruginosa* is a reason for high fatality rate, as it has arisen as a vital pathogen for nosocomial infection in hospital settings.[13] Life threatening infection rate caused by *P. aeruginosa* continue to rise though there is improvement in sanitation facilities and the introduction of a wide variety of antimicrobial agents with antipseudomonal activities.[14]

Recently, it has been proposed that the term “extensive drug resistance” should be used to indicate resistance to all, but one or two classes of antimicrobial agents.[15] Due to the use of various invasive devices the first line of defense like normal skin and mucosal barrier are more prone to infection hence contributes to susceptibility of hospital patients towards nosocomial infection.[16] Observation was comparable with the earlier studies by various authors. Gram negative bacilli dominance in the aerobic growth in pus has been highly recorded by studies reported by Ghosh et al and Zubair et al.[17,18]

As per our present study we isolated 106 MDR strains from 154 isolates of *P. aeruginosa* out of 763 pus samples obtained from various patients of the hospital. In accordance with the data the prevalence rate of *P. aeruginosa* was 20.19% thus data can be correlated to the earlier study conducted like, Sharma et al reported-on *Pseudomonas aeruginosa* to be 20%, *P. aeruginosa* was 21.49% in a study of Rao et al, a private hospital in Chennai reported 29.8% strains among diabetic foot ulcer patients were *P. aeruginosa*. Sivanmaliappan et al reported 14.30% *P. aeruginosa* out of 270 pus samples in an Indian scenario.[19-22] Whereas, Al-Marzoqi et al reported 22.46% in an Iraq report.[23] Al-Ibran E et al a Karachi report focused on *P. aeruginosa* to be the most commonly isolated microorganism 36.6%, therefore in this study of wound infection in fire burnt patients *Pseudomonas* was categorized as a prevalent pathogen.[24] Similarly 25% *Pseudomonas* were isolated in pus samples as reported by Ahmend et al.[25]

The present study discloses the prevalence of infections due to *P. aeruginosa* and their propensity for drug resistance. Multidrug resistant bacteria are usually complicating course of therapy and thus are causing major public health problems-an emerging challenge to health care. As per the Antibiogram data of present study *P. aeruginosa* (N=154) 90.90% resistance was observed for Cefazolin (CZ), followed by 75.97% Co-Trimoxazole (COT), Gentamicin (GEN) 57.80%, Ceftazidime (CAZ) 57.15%, Meropenem (MRP) 44.80%, Amikacin (AK) 35.06%, with lowest of Ciprofloxacin (CIP) 23.37% and at the same time highest sensitive was observed for Ciprofloxacin (CIP)76.63%, followed by Amikacin (AK) 64.94%. As per earlier study sharma et al reported 100%

sensitivity to Amikacin and Ciprofloxacin (60%) in their study.[22] On the contrary Al-Ibran E et al stated reduction in sensitivity to Amikacin (44%), Ceftazidime, Ciprofloxacin showed on average < 30%.[24] Moradian F et al reported for highest resistance rate to ceftizoxime 92.6% followed by resistances against Cefazolin 92.3% and the lowest were against ciprofloxacin 38.9%, amikacin 46.3% ,thus resistance to Cefazolin is comparable to present study.[34]

Further, Meropenem (MRP) 55.20%, and sensitivity was further reduced for other drugs as resistance increased. Whereas in earlier studies by Sivanmaliappan et al, showed 66.6% resistance to gentamicin, amikacin, ceftazidime, resistance to Ceftazidime 60.87% was reported by Rao et al.[20-21] Ciprofloxacin (CIP) showed highest antipseudomonal activity as per above data. The resistance pattern is responsible for the emergence of multidrug-resistant strains (MDR). Thus, a treatment to be based on antibiotic resistance profile rather than empirical treatment said by Sava P et al.[26]

Various reports have figured out for the Multi drug resistance pattern association with gram negative bacilli increasing at alarming rate.[27-29] The percentage for MDR *P. aeruginosa* fall in range of 11.36% stated by SitiNurAtiquahIdris et al to 91.6% reported by Panranjothi S et al.[30,31] As per the present study conducted in Pravara rural hospital, Loni, Maharashtra, India settings 68.83% (N =106) MDR strain was detected from 154 isolates strains of *P. aeruginosa* among 763 pus samples. Thus, this study is in accordance to above range. Multidrug resistance 55.5% of the strains of *P. aeruginosa* reported by Sivanmaliappan et al.[20] In the study reported by Gill and colleagues 22.7% multi drug resistant (MDR) *P. aeruginosa* was estimated.[32, 33]

As per 68.83% (N =106) MDR strain in the study. Thus, highly prevalent pattern was found to be GEN^r, AK^r, CAZ^r, CZ^r, COT^r for 10 (9.43%) isolates in 106 followed by GEN^r, CAZ^r, CZ^r, MRP^r, COT^r, CIP^r for 9 (8.49 %) isolates in 106 of drug resistance. Whereas Moradian F et al reported FEP-CAZ-CZ-CT-CTX-CRO-AN-GM-TIC-IPM-OFX-CIP to be 12 (22.2%) out of 54 isolated MDR bacterial strains.[34]

CONCLUSION

This study focused on isolation of opportunistically pathogenic bacteria namely *P. aeruginosa* from pus sample, those are responsible for causing various human illness. This is commonest isolates of pus forming infection. Hence, Knowledge of the most common causative agents of infection and their antimicrobial susceptibility pattern is a crucial step for the empirical therapy before the culture results are available. Antimicrobial susceptibility pattern of microorganisms varies with time, place and depend on emergence of new resistance strain. As antimicrobials is the mainstay of therapy. It is important to consider antibiotic resistance when selecting the regimen. Thus, consistent data on antibiogram analysis is mandatory for clinicians to decide appropriate treatment strategy. This will eventually help in time management, accurate administration of drug; reduce possibility of drug resistance and therapy failure. The widespread use of broad-spectrum antibiotics in the hospital is probably responsible for the emergence of resistant strain. Frequent monitoring of the MDR in *P. aeruginosa*, the restriction of the inappropriate use of antibiotics and involvement of infection control practices, strict impetus for patient personal hygiene should be implemented to avoid emergence of clinically significant *P. aeruginosa*.

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