

Original Research Article

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Study of Prevalence of *Pseudomonas aeruginosa* in Various Clinical Samples and Investigation of its Antibiotic Sensitivity Pattern

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ABSTRACT

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Management of *Pseudomonas aeruginosa* becomes a challenge because of its ability of development of resistance by intrinsic and acquired mechanisms. This resistance mechanism makes the treatment of such pathogen difficult, thus multidrug resistance is acquiring in many strains. The present study aimed to generate the sufficient data regarding the prevalence and antibiotic sensitivity data. Various clinical samples of Blood, Pus, Sputum and Urine were collected and then subjected to their identification by Cultural, Biochemical, Morphological identification and then identified by Vitek 2 system. Then, the identified isolates were screened for the antibiotic sensitivity pattern by disc diffusion method. The results were interpreted according to CLSI 2007 guidelines. Out of 300 isolates tested significant resistant to antibiotics, CAZ (73 isolates) then CPZ (56 isolates), DOR (54 isolates), LE & GEN (53 isolates), PB (52 isolates), CTX (46 isolates), MZ (39 isolates), CTR (16 isolates) and CB (15 isolates) were detected. The results obtained in the study shows many antibiotics are losing efficacy. Thus, the result says that it proper legislation, practice and awareness among the community is needed use antibiotic as therapeutic agent in future generation.

Introduction

Pseudomonas aeruginosa is one of the most common opportunistic pathogen which is often infects people with low immune power. High fatality rate of *P. aeruginosa* infection is reported in burn patients or the people assisted

with ventilation. Prevalence of *P. aeruginosa* is also encountered in respiratory infection (Bodnar *et al.*, 2016). Though resistance to antibiotic can be acquired in *P. aeruginosa* through continuous exposure to antibiotics but some of the intrinsic factors are also responsible for resistance against antibiotics.

The intrinsic factors such, the production of an AmpC, low outer membrane permeability, the presence of genes for efflux pumps makes the *P. aeruginosa* resistant (Livermore, 2002).

Reduction in emergence of resistance can be achieved by raising awareness about antibiotic use, improvement of hygiene to reduce spreading of infection, reduction in overuse of antibiotics, continuous surveillance of drug resistance pattern and choice and invention of new diagnostics (Bush, 2004). Because of drug abuse of antibiotics by unawareness of population and unethical medical practice in clinics some strains of *P. aeruginosa* acquire resistance which is creating severe in treatment of such pathogen. Various mechanisms are adopted by pathogen which is providing resistance power against wide range of antibiotics. One of the key mechanisms of resistance can be considered as selective mutations in the chromosome. Resistance to cephalosporin acquired due to lytic enzyme AmpC. Which is found on the chromosome of *Enterobacteria*, *P. aeruginosa*, and other non fermentative Gram-negative bacilli which is induced by Beta-lactam (Xia *et al.*, 2016). Bacterial resistance to antimicrobial agents cause serious public health problem in current day scenario globally. Selective pressure is generated on discovery and use of antibiotics (Normark and Normark, 2002).

Horizontal gene transfer mechanism and mutation in chromosomal genes are responsible for resistance in *P. aeruginosa*. Alterations in the genes of DNA gyrase and topoisomerase IV play critical role in *P. aeruginosa*. The evolutionary processes of antimicrobial resistance in *P. aeruginosa* persist majorly less studied. The resistance is acquired for cephalosporins, fluoroquinolones, and carbapenems, in *P. aeruginosa*. Lipocalins proteins present in both gram-positive and – negative pathogen provides resistance against

hydrophobic antibiotics. Investigation on *Pseudomonas aeruginosa* PAO1 reported that transcription of *BcnA*, gives protection against hydrophobic antibiotics considerably decrease in sensitivity polymyxin B treatment (Jalaland Wretling, 1998).

Increased resistance to antibiotics has been widely reported in clinical isolates of *P. aeruginosa* (Mohammadinia *et al.*, 2012) rather than environmental isolates and the resistance is due to genes encoded by chromosome (Livermore, 2002)also by the capacity of acquiring mobile genetic elements(Kung *et al.*, 2010). The rate of drug resistance globally demands study of prevalence of *P. aeruginosa* and study of antibiotic resistance (Willcox, 2011; Abidi, 2013).

The current study aims to investigate the prevalence of *P. aeruginosa* in clinical samples and to study is resistance pattern. The present study would help researchers to consider the problem of drug resistance and formulating the alter strategy or new idea to deal with such resistant *P. aeruginosa*.

Materials and Methods

Collection of clinical samples

From different pathology laboratories of Nagpur (MS), India clinical samples of urine (U), pus (P), blood (B) and sputum (S) were collected aseptically.

Enrichment of clinical sample containing pathogens

The sterile cotton swab was prepared by using surgical cotton and sterilized it in autoclave. The sterile swab dipped in samples and then transferred in sterile Tryptone Soy Broth. The tubes were inoculated at 37°C for 48 hrs in biological incubator.

Isolation of *P. aeruginosa*

The aliquot of culture from enriched Tryptone Soy Broth was inoculated on Pseudomonas isolation agar by streak plate method and incubated at 37⁰C for 48 hrs. The typical colonies on selective media showing luxurious growth with green pigmentation were maintained and used for further study.

Microscopic examination of *P. aeruginosa*

The standard Gram staining procedure was used for isolated colonies for finding Gram character. The slides were focused on microscope for observation.

Biochemical identification of *P. aeruginosa*

Isolated colonies were identified by biochemical tests; Indole test, Methyl red test, Catalase test, Citrate utilization test, Urease test, Oxidase test, Triple sugar iron test, and pigment production was studied. The outcome of the results was compared with Bergey's Manual of Determinative Bacteriology 9th edition. The further biochemical identification was done by using Vitek 2 System.

Preparation of inoculums

The inoculum of 0.5 McFarland standards was prepared which correspond to size of 1.5×10⁸ CFU/ml.

Antimicrobial susceptibility testing

Commercially available discs (HiMedia, Mumbai, India) of antimicrobial susceptibility testing was used for susceptibility testing; Amikacin (AK) 30 mcg, Gatifloxacin (GAT) 5 mcg, Doripenem (DOR) 10 mcg, Tobramycin (TOB) 10 mcg, Aztreonam (AT) 30 mcg, Polymyxin-B (PB) 300 units, Cefoperazone (CPZ) 75 mcg, Levofloxacin (LE) 5 mcg, Cefotaxime (CTX) 30 mcg,

Gentamycin (GEN) 10 mcg, Piperacillin/Tozobactam (PIT) 10 mcg, Ticarcillin (TI) 75 mcg, Carbencillin (CB), Norfloxacin (NX) 10 mcg, Cetriaxone (CTR) 30 mcg, Imepenem (IPM) 10 mcg, Netillin (NET) 30 mcg, Aziocillin (AZ) 75 mcg, Meropenem (MRP) 10 mcg, and Ceftizoxime (CZX) 30 mcg, Ceftazidime (CAZ) 30 mcg, Mezlocillin (MZ) 75 mcg

100 µl of broth culture was inoculated by spread plate method on solidified Hi sensitivity test agar plates (sterilised in autoclave) and discs were placed equidistly under laminar air flow. The plates were incubated at 37⁰C for 18-24hrs. After incubation, zones were compared with standard CLSI recommendation (CLSI 2007).

Results and Discussion

Total 300 isolates from different clinical samples; Pus, Urine, Blood and Sputum were screened for isolation of *P. aeruginosa*. Initially the enriched culture were streaked on Pseudomonas Isolation Agar were the isolates of *P. aeruginosa* shows luxurious growth with green pigmentation. These isolates were subjected to identification by cultural, biochemical and morphological analysis. On specific media, Pseudomonas Isolation Agar, *P. aeruginosa* shows green colour pigmentation. Investigation of biochemical results showed Indole test: Negative, Methyl red test: Negative, Citrate utilization test: Positive, Catalase test: Positive, Oxidase test: Positive, Urease test: Negative, Triple sugar iron test K/K or K/no change without H₂S gas production, pigment production. The colonies identified by cultural and biochemical method was subjected for morphological identification by Gram staining, the results obtained to be a prominent Gram negative Rod shaped bacteria. After analysis of complete 300 isolates the different statistics are analysed. The maximum number of isolates were found

in Urine (133) followed by Pus (116), Blood (36) and Sputum (15). The percentage of prevalence is shown in Table 1.

The each isolates of *P. aeruginosa* obtained from different clinical samples were studied for antimicrobial susceptibility against different antibiotics. The results of all tested *P. aeruginosa* were categorised according to their susceptibility pattern and results are noted. The pattern of susceptibility was noted for each antibiotic under study. The results of the study are given in Table 2 and represented graphically in Graph 1 for better understanding.

The data from Table 1 can be summarised to see the significant result obtained in the study where maximum number of resistance obtained in CAZ (73 isolates) then CPZ (56 isolates), DOR (54 isolates), LE & GEN (53 isolates), PB (52 isolates), CTX (46 isolates), MZ (39 isolates), CTR (16 isolates) and CB (15 isolates). These are the significant numbers which alarm the population that emergence of drug resistance is increasing remarkably against different antibiotics.

The continuous monitoring of result is necessary for the real-time study of the drug resistant pathogen which will give strategies for choice of other drug or choice of new therapy.

In study of Javiya *et al.*, (2008) and Chaudhari *et al.*, (2013) found that the most number of *P.*

aeruginosa isolated was found in pulmonary samples, followed by pus and urine (Javiya *et al.*, 2008; Chaudhari *et al.*, 2013). The variation in the result is due to the research were aimed to study the *P. aeruginosa* in Hospital of Pulmonary care. Significant population of *P. aeruginosa* is detected in urine (Ojo and Anibijuwon, 2010) which corroborate with present study. *P. aeruginosa* has been found more prevalently in the age group of 21-40 years (Mohanasoundaram, 2011). The more prevalence of in urine is because it causes Urinary Tract Infection in most of the people (KO, 2009).

Multidrug resistance *P. aeruginosa* are most commonly reported in pus. The major percentage of resistance is found in Amikacin, Aztreonam, Ceftazidime and Piperacillin found in the study of Kamaria *et al.*, (Kamaria *et al.*, 2016). *P. aeruginosa* showed the highest sensitivity to piperacillin/tazobactam followed by meropenem, ciprofloxacin, cefepime, amikacin, and gentamicin and colistin was in susceptible range in all organisms (Abdul Wahab *et al.*, 2017). Intermediate pattern of resistance was found in *P. aeruginosa* against Cefepime (66.7%) and Ciprofloxacin (58.3%). The higher resistance pattern is found against Ciprofloxacin and cefepime (Upadhaya, 2014; Iraj *et al.*, 2013). Less resistance against antibiotics; Imipenem, Colistin, Piperacillin/Tazobactum detected in study by Iraj *et al.*, (2013).

Table.1 Prevalence of *P. aeruginosa* in various clinical samples

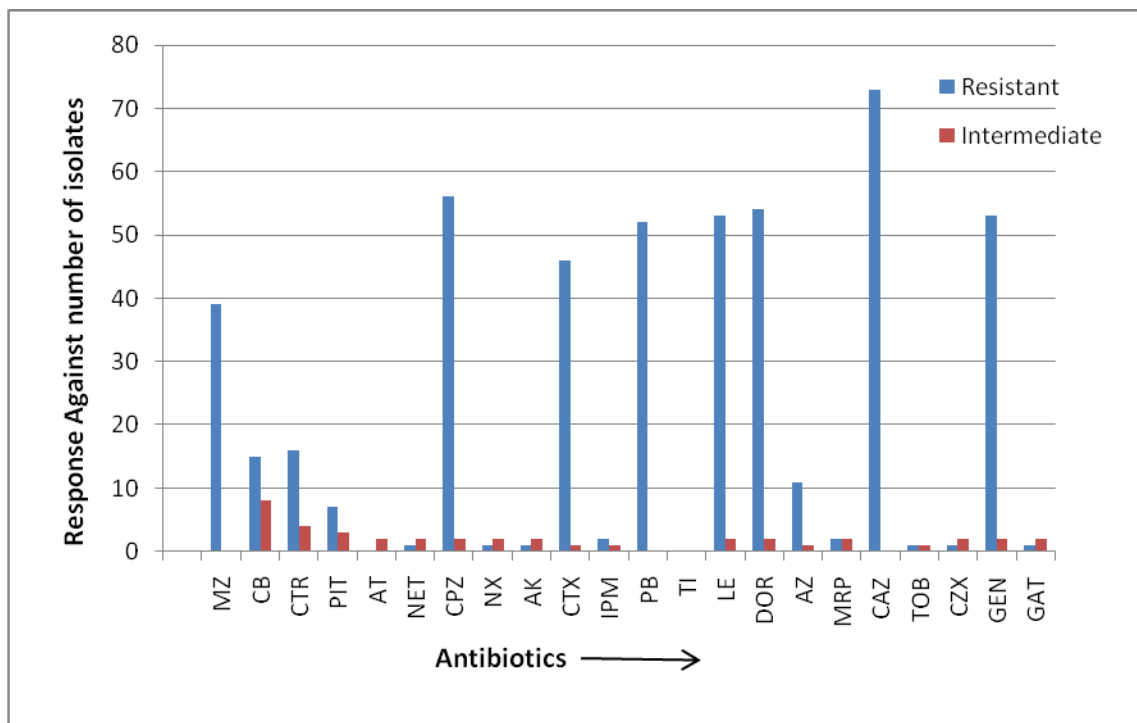
Clinical Samples	No. of Isolates	% of prevalence
Urine	133	44.33%
Pus	116	38.66%
Blood	36	12%
Sputum	15	5%

Table.2 Number of isolates showing response as resistant or intermediate out of total 300 isolates

Antibiotic	Response to individual antibiotics (r and i) of total 300 isolates		Antibiotic	Response to individual antibiotics (r and i) of total 300 isolates	
	R	I		R	I
MZ	39	00	PB	52	00
CB	15	08	TI	00	00
CTR	16	04	LE	53	02
PIT	07	03	DOR	54	02
AT	00	02	AZ	11	01
NET	01	02	MRP	02	02
CPZ	56	02	CAZ	73	00
NX	01	02	TOB	01	01
AK	01	02	CZX	01	02
CTX	46	01	GEN	53	02
IPM	02	01	GAT	01	02

Where; r- Resistant and i-intermediate

Graph.1 Comparative analysis of sensitivity pattern among different isolates against *P. aeruginosa*



Against imipenem, flouoroquinolones and aminoglycosides *P. aeruginosa* strains exhibits an intrinsic sensitivity. Widespread emergence of resistance in *P. aeruginosa* is detected (Pagani *et al.*, 2005). Seeing the connection between antibiotic usage and development of resistance against antibiotics, the results achieved in present work recommend not using antibiotics by self medication.

Resistance to the aminoglycosides was not found in some study (Anjun and Mir, 2010). Whereas, high resistance rate is detected against gentamicin (Strateva *et al.*, 2007). According to Boffi *et al.*, (2000) the resistance pattern is different in different country (Boffi *et al.*, 2000) this may be because of different regulation, ethics followed by medical practitioners and awareness among the population.

The continuous monitoring of antibiotic resistance will help the physicians to perceive the resistance trends in scenario that will help the medical practitioner to prescribe the antibiotics and following of treatment strategy to cure the person with pathogen infection. This study aimed to know the status of antimicrobial resistance in community against the antipseudomonal agents. The data obtained during the study gives the overview of resistance in *P. aeruginosa* along with the prevalence pattern in various clinical samples.

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