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MULTIDRUG RESISTANCE IN *PSEUDOMONAS AERUGINOSA*: *IN VITRO* EFFICACY OF CURRENT CHEMOTHERAPY AGAINST CLINICAL ISOLATES FROM NORTHERN INDIA

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ABSTRACT

Pseudomonas cause serious, life threatening infections in humans. *Pseudomonads aeruginosa*, particularly, has become an important cause of morbidity and mortality, especially in patients with compromised host defence mechanisms. Chemotherapy of infections caused due to Pseudomonas includes the use of fluoroquinolones, aminoglyosides, chloramphenicol, and tetracycline. Resistance rates in Pseudomonas towards these antibiotics are increasing day by day. The present study was aimed at evaluation of susceptibility and resistance of various clinical isolates of *P. aeruginosa* towards the commonly used antibiotics. A total of 100 clinical isolates of *P. aeruginosa* were screened for their susceptibility and resistance patterns towards several antibiotics using different concentrations i.e. 5µg/disc to 30µg/disc. The results indicated that 70% of all the clinical isolates were found resistant to ciprofloxacin with decreasing order of resistance with 58% chloramphenicol, 52% norfloxacin, 51% tetracycline, 49% ofloxacin, 42% levofloxacin, 36% getifloxacin, 20% gentamycin, 11% amikacinand 10% tazobactem. The present study suggests that majority of the *P. aeruginosa* strains shows resistance towards ciprofloxacin, whereas tazobactem and amikacin was found active against 90% and 89% of the total isolates respectively, thus, providing an important information about the preferable drugs which may be used to cure ailments caused by *P. aeruginosa*.

Keywords: Multi-Drug Resistance, *Pseudomonads aeruginosa*, Antibacterial susceptibility testing

INTRODUCTION

Pseudomonas are a diverse bacterial group of established and emergent pathogens. The members of this genus are major agents of nosocomial and community acquired infections, being widely distributed in the hospital environment where they are particularly difficult to eradicate **[1]**. Several species of the genus *Pseudomonas* are responsible for causing infection in human beings and *P. aeruginosa* is the most common in those **[2]**. *P. aeruginosa* is a Gram negative bacillus belonging to the family Enterobacteriaceae, which transmits to human beings through air, soil and water. It has increased clinical importance because of its innate resistance to multiple agents and ability to develop high level Multiple Drug Resistance (MDR). The drug resistant strains of *P. aeruginosa* are associated with significant increases in morbidity and mortality **[3]**. The drug resistance in *P. aeruginosa* may be due to altered outer membrane permeability, altered binding proteins, active efflux pumps, acquisition of resistance genes on mobile genetic elements (i.e., plasmids) or through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms **[4]**. All these factors responsible for developing drug resistance can severely limit the therapeutic options for treatment of serious infections caused by *P. aeruginosa*.

The most commonly used antibiotics in the treatment of infections caused by Pseudomonas include of fluoroquinolones, aminoglyosides, chloramphenicol, and tetracycline **[5]**. However, emergence of drug resistance in Pseudomonas towards these antibiotics has been recently reported **[6]**. There is a need to evaluate the efficacy of these commonly used antibiotics against the clinical isolates of *P. aeruginosa* to get some information about the sensitivity and resistance of those towards the antibiotics. This will help in understanding the current situation of drug resistance in *P. aeruginosa* and to vote up some new and more efficient antibiotics to replace those which are already in use and are inactive against majority of the strains. Therefore, the present study was conducted to evaluate the antibiotic susceptibility and resistance patterns among the clinical isolates of *P. aeruginosa* collected from the patients suffering from respiratory tract infections, urinary tract infections, thermal burns and cystic fibrosis.

MATERIALS AND METHODS

Materials used

Antibiotics used in the study include ciprofloxacin 5 µg/disc, ofloxacin 5 µg/disc,

levofloxacin 5 µg/disc, getifloxacin 5 µg/disc, tetracycline 30 µg/disc, chloramphenicol 30 µg/disc, amikacin 30 µg/disc, gentamicin 10 µg/disc, tazobactem 10 µg/disc and norfloxacin 10 µg/disc (Himedia, Mumbai, India). All the microbial growth media used in the study were purchased from Himedia, Mumbai, India.

Collection of Samples

A total of 100 samples of *P. aeruginosa* isolated from various clinical samples i.e. pus, pus swab, ear swab, urine, sputum and blood were included in the study. Samples were collected during January, 2012 to October, 2012 from Gian Sagar Medical College and Hospital, Patiala, Punjab; Christian Medical College, Ludhiana, Punjaband Indira Gandhi Medical College, Shimla, Himachal Pradesh, India. Written informed consent was obtained from patients before taking the samples. The study was approved by Institute ethical committee vide letter no. SUIEC/13/26. All the samples collected were cultured on appropriate culture media and biochemical analysis was performed for confirmation of *P. aeruginosa*.

Cultivation and Characterization of *P.aeruginosa*

All the clinical isolates of *P. aeruginosa* were cultivated in nutrient agar at 37°C for 24 hours. Characterization of *P. aeruginosa* was performed by Gram staining and various biochemical tests including indole test, methyl red test, Voges Proscauer test, citrate utilization test, urease test, catalase, carbohydrate fermentation, nitrate Reduction and motility test using standard procedures and also by growth on selective media.

Antibacterial susceptibility testing

The antimicrobial susceptibility testing was carried out using conventional Kirby-Bauer disk diffusion method **[7]**. All the 100 clinical isolates of *P. aeruginosa* were cultivated in nutrient broth and then the turbidity of the inoculum to be used was adjusted to 0.5 McFarland standard. Mueller Hinton Agar was used for the antimicrobial assays. The solidified media on the plates was inoculated with the help of sterilized swabs. Various antibiotic disks to be tested were placed on the respective divisions of the plate. The plates were then incubated at 37°C for 16-18 hours in inverted position. Standard strain of *P. aeruginosa* MTCC-741 was used as control. After incubation period, zone diameters around the disks were measured with a zone-scale and each zone size was compared with known standards. The isolate was then labeled as sensitive, intermediate and resistant depending upon the zone of inhibition.

RESULTS

A total of 100 clinical isolates i.e. 14 from Gian Sagar Medical College and Hospital, 36 from Christian Medical College and 50 from Indira Gandhi Medical College and Hospital, India were collected.

Cultivation and Characterization of *P. aeruginosa*

All the isolates of *P. aeruginosa* were cultivated in nutrient agar which showed optimum growth after 24 hours of incubation at 37°C. Characteristic slimy white, semi translucent, raised colonies of *P. aeruginosa* were observed on nutrient agar plate. In Gram's staining we observed reddish-pink, rod shaped cells which confirmed the presence of Gram negative bacteria.

Growth on Selective media

Simmon citrate agar is a selective media for the cultivation of *P. aeruginosa*. The green pigments of *P. aeruginosa* turned into blue after an incubation at 37°C for 24 hours, which is a characteristic feature of *P. aeruginosa*.

Biochemical Analysis

Biochemical analysis was performed for all the clinical isolates. Citrate, Catalase, Nitrate Reduction, Motility showed positive results, while Indole, Methyl Red, Voges Proskauer, Urease and Carbohydrate Fermentation (Lactose, Mannitol and Sucrose) showed negative results for all the isolates of *P. aeruginosa* (**Figure 1**).

Antibacterial Susceptibility Test

The antibacterial susceptibility assay was carried out using 10 antibiotics of different classes. We observed that majority of the isolates were resistant to ciprofloxacin, whereas susceptible towards amikacin, tazobactem and gentamycin (Figure 2). 70% of all the isolates were resistant to ciprofloxacin followed by chloramphenicol (58%), norfloxacin (52%) and tetracycline (51%), whereas, only 10-11% isolates were resistant to tazobactem and amikacin (Table 1). In case of gentamycin, 20% of all the isolates were found resistant. After antibacterial susceptibility assay the antibiogram for all the antibiotics were constructed to guide the current use of antibiotics. By the construction of antibiogram, it was concluded that NV-8, NV-11, NR-2, NR-3, NR-4 were colonially same strains as they had similar resistance pattern. Likewise NV-2, NV-3 or NV-6 or NT-16, NV-25 or NV-76 and NT-6 or NT-10 also had similar resistance pattern and both were colonially same strains (Table 2).

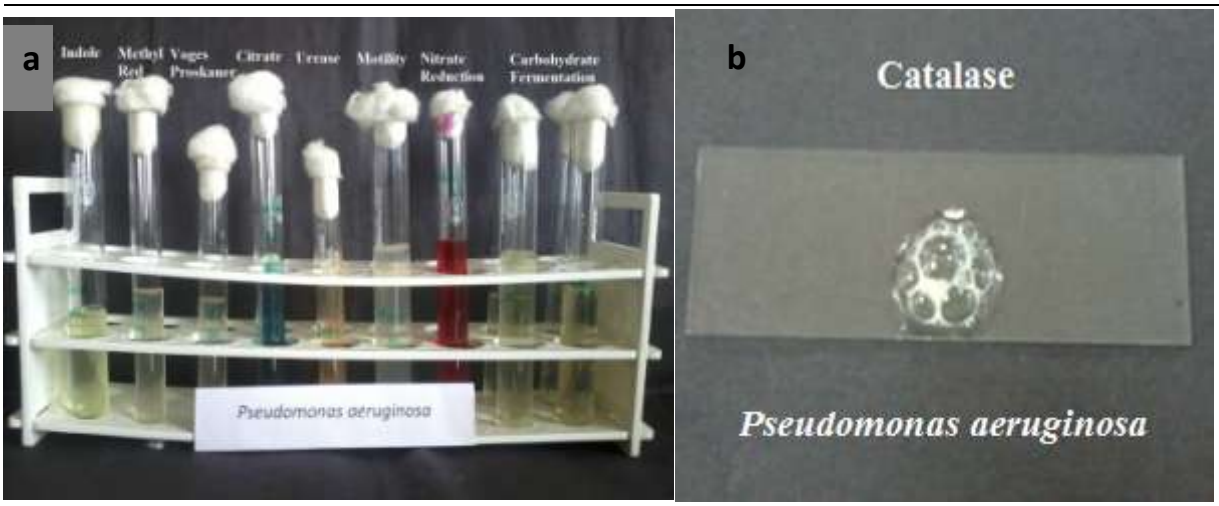

Figure 1: Results of Biochemical analysis (a and b)

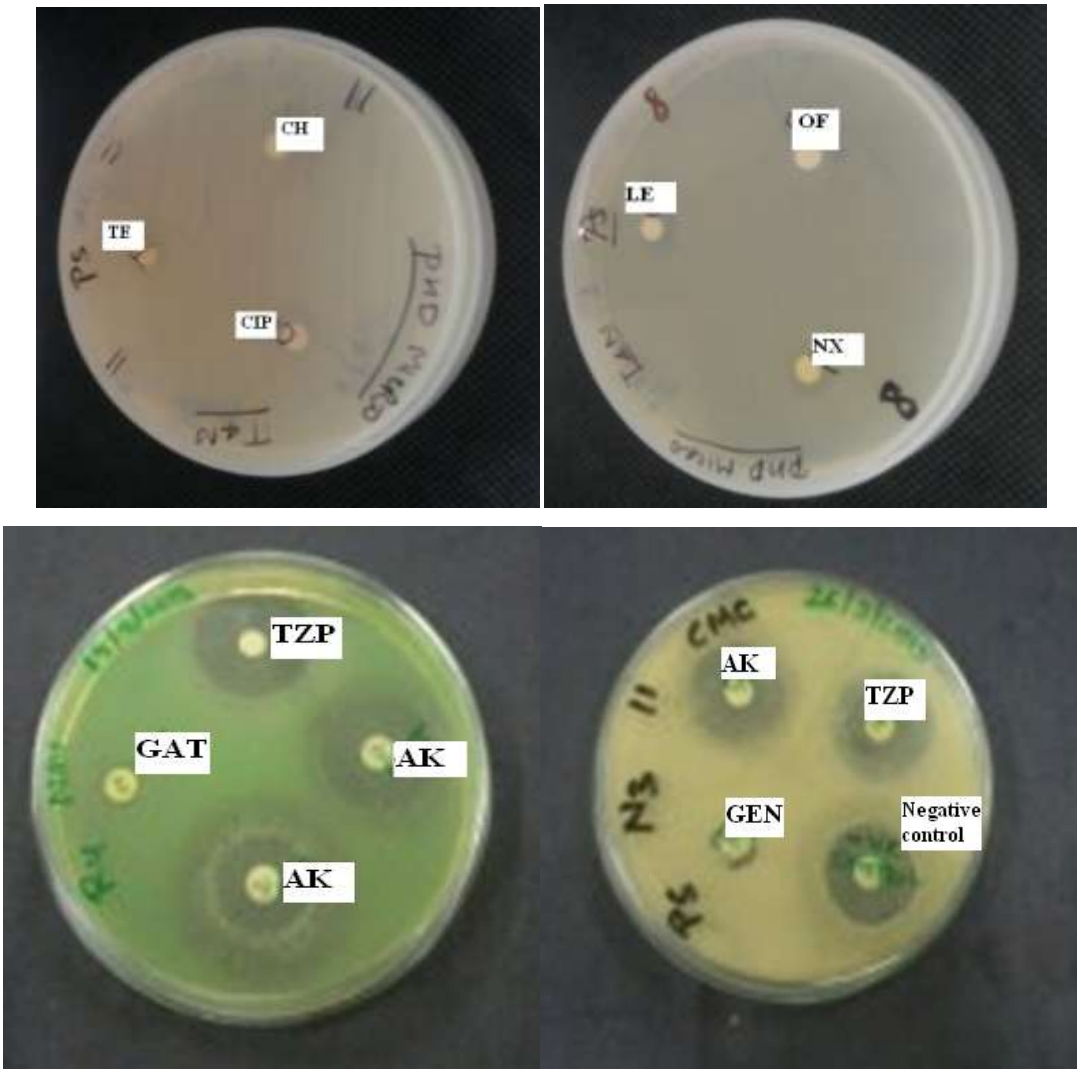

Figure 2. Drug resistant and susceptible isolates of *P. aeruginosa*. Where, CIP- ciprofloxacin, NX- norfloxacin, OF- ofloxacin, LE- levofloxacin, GAT- getifloxacin, TE- tetracycline, CH- chloramphenicol, GEN- gentamycin, AK- amikacin, TZP- tazobactem.

Table 1: Comparison of resistance pattern of *Pseudomonas aeruginosa* against commonly used antibiotics (n=100)

Antibiotics	CIP (%)	NX (%)	OF (%)	LE (%)	GAT (%)	TE (%)	C (%)	GEN (%)	TZP (%)	AK (%)
Pus/Pus swab (n=53)	64.28	40.38	16.32	28.57	41.66	60.78	44.82	70	30	36.36
Ear swab (n=24)	20	7.69	30.61	38.09	33.33	11.76	22.41	15	40	45.45
Urine (n=3)	1.42	1.92	2.04	4.76	2.77	0	1.72	0	0	0
Blood (n=12)	8.57	25	12.24	16.66	13.88	11.76	10.34	10	20	9.09
Sputum (n=8)	5.71	25	38.77	11.90	8.33	15.68	20.68	5	20	0
Total % of Resistance	70	52	49	42	36	51	58	20	10	11

Where, CIP-ciprofloxacin, NX-norfloxacin, OF-ofloxacin, LE-levofloxacin, GAT-getifloxacin, TE-tetracycline, C-chloramphenicol, GEN-gentamycin, AK-amikacin, TZP-tazobactem.

Table 2: Antibiogram of different resistance patterns of antibiotics

Serial No.	Strain name	Resistance Pattern	Source
1.	NV-2	CIP, NX, LE, TE, C	Ear swab
2.	NV-3	CIP, NX, LE, TE, C	Pus
3.	NV-6	CIP, NX, LE, TE, C, GEN	Sputum
4.	NT-16	CIP, NX, LE, TE, C, GEN	Blood
5.	NV-8	CIP, GAT, LE, TE, C	Pus
6.	NV-11	CIP, GAT, LE, TE, C	Pus
7.	NR-2	CIP, GAT, LE, TE, C	Blood
8.	NR-3	CIP, GAT,LE, TE, C	Blood
9.	NR-4	CIP, GAT, LE, TE, C	Blood
10.	NT-6	CIP, NX, GAT, LE, TE, C, AK	Blood
11.	NT-10	CIP, NX, GAT, LE, TE, C, AK	Pus
12.	NV-25	CIP, NX, OF, TE, C	Pus
13.	NV-76	CIP, NX, OF, TE, C	Urine

Where, CIP-ciprofloxacin, NX-norfloxacin, OF-ofloxacin, LE-levofloxacin, GAT-getifloxacin, TE-tetracycline, C-chloramphenicol, GEN-gentamycin, AK-amikacin.

DISCUSSION

P. aeruginosa has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. It is associated with significant morbidity and mortality in humans worldwide **[8]**. The currently used chemotherapy has several limitations including drug resistance which affects the treatment duration and efficacy. The drug resistance in *P. aeruginosa* is due to several molecular and physiological factors. Use of antibiotics without prescription and proper supervision may lead to the emergence of drug resistance making the pathogen more harmful and uncontrollable. The present study was carried out with the aim to screen commonly used antibiotics against one hundred clinical isolates of *P. aeruginosa* to find out the percentage of resistant and susceptible isolates and vote up the best antibiotic which can be used to treat infections caused by *P. aeruginosa.*

We observed that 70% of the total isolates were resistant to ciprofloxacin and most of which were pus isolates. Tazobactem and amikacin were most active against all the isolates and have shown only 10-11% resistance. Several other studies conducted in different parts of the world have reported the resistance against ciprofloxacinin *P. aeruginosa* including India **[9-10]**, Jamaica **[11]**, Latin America **[12]**, Nigeria **[13]** and Malaysia **[14]**. High frequency of prevalence of multidrug resistant strains of *P. aeruginosa* against fluoroquinolones group of antibiotics was reported from North Kerala, India **[15]** and Malaysia**[16]**. Ciprofloxacin resistant strains of *P. aeruginosa* have also been reported from Iran **[17]**. Considerably low resistance towards tazobactem has been reported in indoor-patient isolates of *P. aeruginosa* from Saudi Arabia**[18]**.

P. aeruginosa is a lethal pathogen in hospital environment especially in burn patients and ICU patients and is becoming resistant even to newer antibiotics such as ciprofloxacin and chloramphenicol. Overcrowding of patients as well as visitors in burnunit, poor isolation between patients, unhygienic conditions of patients and misuse of broad spectrum antibiotics may be some major factors behind the spreading of infections caused by *P. aeruginosa*. If timely interventions/measures are not taken to prevent the resistance, then we may have to say that we are nearing towards the end of antibiotic era. Results of the present study clearly demonstrated the occurrence of resistance to various anti-pseudomonal agents among *P. aeruginosa* isolates. Most of the commonly used anti-pseudomonal drugs including ciprofloxacin, chloramphenicol, tetracycline and norfloxacin have shown resistance towards

majority of the clinical isolates, whereas tazobactem and amikacin which are not usually used to cure pseudomonal infections have shown very strong activity and less resistance against most of the isolates. This concludes that amikacin and tazobactem may be further tested and used against pseudomonal infections which are unresponsive to commonly used antibiotics.

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