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STUDY OF ANTIBIOTIC SENSITIVITY TO PSEUDOMONAS AERUGENOSA IN HOSPITALISED PATIENTS

Microbiology

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ABSTRACT

P. aerugenosa may cause chronic debilitating pulmonary infections. Judicial use of antibiotics should be encouraged. Hence this study was conducted to observe hundred isolates of P. aeruginosa in various clinical samples. Among β-lactams group, the isolates of *P. aeruginosa* showed maximum resistance to ticarcillin /clavulanic acid (79.0%) . Among aminoglycosides group, the isolates of *P. aeruginosa* showed maximum resistance to gentamicin (74.0%) . Among fluoroquinolones group, isolates of *P. aeruginosa* showed maximum resistance to ciprofloxacin (64.0%) . Out of the total hundred *P. aeruginosa* isolates 74(74.0%) were MDR isolates , while 60 (60.0%) were XDR.

KEYWORDS

P. aeruginosa- Pseudomonas aeruginosa, MDR- Multi drug resistance, XDR- Extensive drug resistance,

INTRODUCTION:

P.aerugenosa causes infections particularly in burns patients where the skin host defenses are destroyed, orthopedic related infections, respiratory diseases, immunosppressed and catheterized patients. It may be the cause of the chronic debilitating pulmonary infections, which is one major cause of death in patients with cystic fibrosis.Generally, it contributes substantially to wound related morbidity and mortality worldwide.[1]

There are a limited number of antimicrobial agents with reliable activity against *P. aeruginosa*, including antipseudomonal penicillins, cephalosporins, carbapenems, and fluoroquinolones, particularly ciprofloxacin. Aminoglycosides are frequently used as part of combination regimens for treatment of serious pseudomonal infections but are generally not recommended as single drugs. For each of these agents, emergence of resistance during therapy has been described and has been recognized as a cause of treatment failure.[2]there are various mechanisms are known to cause resistance to antibiotics by P. aerugenosa eg. Extended spectrum beta-lactamase which is responsible for resistance to beta lactam antibiotics, similarly AmpC beta lactamase, Metallo beta lactamase, D-oxacillinase. Hence this study has been provided information about sensitivity and resistance pattern of antibiotics to P. aerugenosa in northern part of India.

MATERIAL AND METHODS:

This study was conducted in the Department of Microbiology, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak over a period of one year.

A total of 100 isolates of *P. aeruginosa* isolated from various clinical specimens like urine, pus, blood, body fluids, sputum, etc collected from patients, irrespective of age and sex, were identified by standard microbiological procedures.[2,3]

Collection of specimen

1. Urine: clean catch midstream urine samples were collected.
2. Pus: aspirated samples of pus or swabs were collected.
3. Blood: blood samples were collected by aseptic venipuncture.
4. Body fluids: body fluids were aspirated under aseptic conditions.
5. Sputum: expectorated sputum samples were collected.

Processing of samples

Microscopy and culture of all the above mentioned samples were done. Cultures were performed on blood agar and MacConkey agar. Inoculated media was examined for growth after overnight incubation at 37^{0}C. Blood samples were cultured in glucose broth and subcultured on blood agar and MacConkey agar after incubation at 37^{0}C for 24 hours, 48 hours, 72 hours and on 7th day.The evaluation of colony morphology on the plating media was done and the subsequent identification procedures was carried on the isolated bacteria, using standard procedures.[2,3,4]

CULTURE OF SPECIMEN

Blood agar and MacConkey agar were inoculated within half an hour of collection with the specimen. Inoculation of samples on culture medium was done using an ordinary reusable inoculating loop.

Antimicrobial Susceptibility Testing

A total of 100 isolates of *P. aeruginosa* were subjected to antimicrobial susceptibility testing performed by Kirby-Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) criteria. Mueller-Hinton Agar (MHA) was used for antibiotic sensitivity testing. Antibiotic discs used in the study were procured from Hi-media® Laboratories, Mumbai, India and from BD diagnostics® USA. American Type Culture Collection (ATCC) strain viz *P. aeruginosa* ATCC 27853 strain was employed as control strain.[5,6] Preparation and inoculation of test plates were done as standardised method.

Application of discs to inoculated agar plate.

The antibiotic discs were applied using a sterile forcep and were gently pressed down to ensure complete and uniform contact of the disc with agar surface.The discs of following antimicrobials with their disc concentrations in brackets, were put up :

ceftazidime (30µg), cefepime (30µg), ceftriaxone (30µg), ceftizoxime (30µg), imipenem (10µg), meropenem (10µg), piperacillin/ tazobactam (100/10µg), ticarcillin/clavulanic acid (75/10µg), gentamicin (10µg), amikacin (30µg), netilmicin (30µg), ciprofloxacin (5µg), aztreonam (30µg), polymyxin B (300 units), colistin (10µg), cefoxitin (30µg). In case of urinary isolates, ofloxacin (5µg) and norfloxacin (10µg) were also added.

Incubation

The plates were incubated within 15 minutes of disc application, in the incubator at 37^{o}C in ambient air for 24 hours.

Interpretation Of The Results

The plates were examined carefully in transmitted light. The diameter of the zones of complete inhibition (as judged by unaided eye) was measured to nearest whole millimeter using a scale, which was held on the back of inverted petri dish. Any presence of small colonies (>1 colony) or a light film of growth within the zone of inhibition indicated resistance of the isolate to that antimicrobial agent.

The zones of inhibition were measured and were interpreted as sensitive (S), intermediately sensitive (IS) or resistant (R) according to disc manufacturer information tables. An isolate was considered as multidrug resistant (MDR) if it was resistant to at least three classes of antimicrobial agents viz. all penicillins and cephalosporins (including inhibitor combinations), fluoroquinolones, and aminoglycosides. Extensively drug resistant (XDR) *Pseudomonas spp.* was the *Pseudomonas spp.* isolate that was resistant to three classes of antimicrobials described above (MDR) and also resistant to carbapenems.

RESULTS:

Antimicrobial susceptibility testing of 100 clinical isolates of *P. aeruginosa* was determined by performed by Kirby-Bauer disc diffusion method.

Table 1: Antimicrobial Resistance Pattern Of 100 Clinical Isolates of *P. aeruginosa*

Antimicrobial drugs	*P. aeruginosa* isolates(n= 100)	
	Number of resistant isolates(n)	Percentage of resistant isolates (%)
β-lactams		
Ceftizoxime(30µg)	50	50.0
Ceftazidime(30µg)	73	73.0
Ceftriaxone(30µg)	48	48.0
Cefepime(30µg)	45	45.0
Cefoxitin(30µg)	68	68.0
Aztreonam(30µg)	78	78.0
Imipenem(10µg)	65	65.0
Meropenem(10µg)	66	66.0
Piperacillin/ tazobactam(100/10µg)	12	12.0
Ticarcillin/ clavulanic acid(75/10µg)	79	79.0
Aminoglycosides		
Gentamicin(10µg)	74	74.0
Amikacin(30µg)	69	69.0
Netilmicin(30µg)	64	64.0
Fluoroquinolones		
Ciprofloxacin(5µg)	64	64.0
Norfloxacin(10µg)	67	67.3
Others		
Polymyxin B(300 units)	2	2.0
Colistin(10µg)	3	3.0

Table 1 shows the antimicrobial resistance pattern of hundred clinical isolates of P. aeruginosa. Among β-lactams group, the isolates of P. aeruginosa showed maximum resistance to ticarcillin /clavulanic acid (79.0%) followed by aztreonam(78.0%), ceftazidime(73.0%), cefoxitin(68.0%), meropenem(66.0%), imipenem (65.0%), ceftizoxime (50.0%), ceftriaxone (48.0%), cefepime (45.0%), while resistance to piperacillin/ tazobactam was seen in 12% isolates.

Among aminoglycosides group, the isolates of P. aeruginosa showed maximum resistance to gentamicin (74.0%) followed by amikacin (69.0%) and netilmicin (64.0%). Among fluoroquinolones group, isolates of P. aeruginosa showed maximum resistance to ciprofloxacin (64.0%) followed by norfloxacin (33.0%). Among others group, resistance to colistin and polymyxin B was seen in 3.0% and 2.0% strains respectively.

Table 2: Distribution Of MDR And XDR Isolates Among The *P. aeruginosa* Isolates

Total number of P. aeruginosa isolates	MDR P. aeruginosa isolates		XDR P. aeruginosa isolates	
	n	%	n	%
100	74	74.0	60	60.0

Table 2 shows distribution of MDR and XDR isolates among the *P. aeruginosa* isolates. Out of the total hundred *P. aeruginosa* isolates 74(74.0%) were MDR isolates , while 60 (60.0%) were XDR.

Table 3: Distribution Of MDR *P. aeruginosa* Isolates Among Different Samples

Total MDR isolates	Urine		Pus		Blood		Sputum		Body fluids	
	n	%	n	%	n	%	n	%	n	%
74	35	47.3	14	18.9	16	21.6	9	12.2	0	0.0

Table 3 shows the distribution of MDR *P. aeruginosa* isolates among different samples. Out of total seventy four MDR isolates, maximum number of samples were from urine samples 35(47.3%), followed by blood samples (21.6 %) , pus samples (18.9%) ,sputum(12.2%) and fluid samples (0.0%).

DISCUSSION:

the purpose of this study to observe antibiotics resistance pattern to P. aerugenosa in hospitalised patient in northern India.

On performing antimicrobial susceptibility testing of 100 isolates of *P. aeruginosa* , high resistance to β-lactams was seen viz 73.0% resistance to ceftazidime, 68.0% resistance to cefoxitin, 50.0% resistance to ceftizoxime, 48.0% to ceftriaxone, 45.0% to cefepime and 78.0% resistance to aztreonam. Other studies on *P. aeruginosa* isolates have depicted similar results with respect to these antibiotics. Basak et al have reported 64.0% resistance to ceftazidime and 66.0% resistance to cefepime.[7] Similarly Behera et al have also reported high level resistance to β- lactams viz.70.0% resistance to ceftazidime and 81.0% resistance to cefepime.[8] Upadyay et al also reported 97.0% resistance to cefoxitin, 82.7% resistance to ceftazidime and 89.1% resistance to ceftizoxime.[9] Our results were also in accordance with Kumar et al who reported 66.8% resistance to ceftazidime, 68.3% resistance to cefepime and 48.5% resistance to ceftriaxone.[10] However , Agarwal et al have reported lower level of resistance to ceftazidime (10.35%) as compared to our study.[11] Similarly, Khan et al also have reported low level resistance to cefepime (31.7%), ceftazidime (30.2%) and aztreonam (37.0%) as compared to the present study[1]. This discordance may be due to greater use of cephalosporins for empirical therapy in almost all the patients admitted in this institute.

In the present study , a moderately high resistance was observed for aminoglycosides in isolates of *P. aeruginosa* viz 74.0%, 69.0%,64.0% resistance was observed for gentamicin, amikacin, and netilmicin respectively. Similar results have been reported by other authors.[10, 12, 13] However other authors have reported higher resistance to aminoglycosides as compared to our study.[8, 14, 15]This can be due to the reason that aminoglycosides are injectable antibiotics, so they are not much used in the OPD setting and ward settings in our institute, which could be the reason of lower resistance to these drugs in our study as compared to other studies. As the use of various antimicrobials in empirical therapy and treatment varies from place to place, so the levels of resistance may also vary.[14]

The prevalence of multidrug resistant *P. aeruginosa* isolates in present study was 74.0% which was in accordance with studies by other authors who have reported prevalence of 84.5% and 68.75% multidrug resistant *P. aeruginosa* isolates.[16, 17] However Tavajjohi et al reported 32.5% multidrug resistant *P. aeruginosa* isolates and Amutha et al reported 45.2% multidrug resistant *P. aeruginosa* isolates which was lower as compared to our study. This discordance may be due to delays in starting appropriate therapy may contribute to increased length of hospital stay and persistence of multidrug resistant *P. aeruginosa* isolates infection.[18,19]

In the present study, MDR *P. aeruginosa* isolates were mainly from urine sample (47.3%) followed by blood samples (21.6%), pus(18.9%) and sputum samples(12.2%). This was in accordance with a study by Mahmoud et al who also reported maximum MDR *P. aeruginosa* isolates from urine (44.4%) followed by pus (37.0%) and sputum samples (11.2%).[20]

CONCLUSION:

This study concluded that, P. aeruginosa was maximally resistant to beta lactam antibiotic (Ticarcilline/ clavulinic acid) and presence of MDR and XDR species of P. aeruginosa were high in this area.

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