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Prevalence of antibiotic resistance in Pseudomonas aeruginosa isolated from clinical samples

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Abstract

Objective: To determine the frequency and susceptibility pattern of *Pseudomon aeruginosa* isolated from clinical specimens .

Methods: This cross sectional study was conducted in the Department of Microbiology at Agartala government medical college, under Gobind ballobh pant hospitals, from 1st January 2016 to 31st december 2016. Clinical specimens were collected from diffirent deperments of Gobind ballobh pant hospitals. Clinical isolates were identified by standard and specific microbiological methods. The antibiotic susceptibility pattern was determined by Kirby Bauer Disc diffusion method. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to determine the results.

RESULTS: We isolated 151 p aeruginosa from 5762 specimens comprising prevalence of 2.620 %. Amikacin was found to be most sensitive drugs followed by meropenem 70.19% ,imipenem 69.53 % and ofloxacin 64.23% .

Conclusion: Antibiotic resistant *P. aeruginosa* are emerging as a critical human health issue. There is an urgent need to resolve the issue by taking some preventive measures. Combined efforts of health care professionals and researchers are required to educate people about the proper use of antibiotics and other infection control measures.

Key words : Multi Drug resistant Pseudomonas aeruginosa, Amikacin, 3rd generation cephalosporins, β lactam.

Introduction :

Pseudomonads are diverse group of established and emerging pathogen and are major causes of nosocomial and community acquired infections.This organism is widely distributed in the hospital environment where they are particularly difficult to eradicate[1] .Majority of the infections caused by P. aeruginosa are often severe, life threatening and are untreatable because of the higher resistance to antimicrobial agents and lack of new drugs development[2,3].

There are various factors responsible to the emergence of resistance such as, misuse and overuse of antibiotics, patient related factors, inappropriate prescriptions by the physicians, self medications especially young adults, use of broad spectrum antibiotics and synergistic combinations, un necessary promotions by pharmaceutical industry, untrained staff in microbiological testing laboratories, lack of awareness with the new guidelines recommended for antimicrobial testing etc.[4]

Over all, resistance rates keep on increasing and differ according to epidemiology of different geographical locations. Multi drug resistance is getting common phenomenon and resistance of almost all anti-pseudomonal agents are being reported worldwide. There is debatable issue of using combination of antimicrobial agents against complicated infections, but usually single antimicrobial agents are recommended for uncomplicated infection.[5]Despite advances in medical and surgical care and

wide variety of anti pseudomonal agents, life threatening infections caused by *P.aeruginosa* is still considered as most challenging pathogen.

The present study was conducted to detect the prevalence and antibiotic susceptibility profile of P. aeruginosa isolated from different clinical samples, collected from GBP hospitals of Agartala.

METHODS

This cross sectional one year study was conducted in Microbiology Department of Agartala Government Medical College, from 1st January 2016 to 31st December 2016. Routine clinical samples (urine, pus, wound and conjunctival swabs, sputum and blood) were collected from different departments. All samples were inoculated on primary culture plates blood agar, Mac Conkey agar and cystine lactose electrolyte deficiency agar and incubated for overnight at 37ºC. Isolates were identified on the basis of colony morphology, Gram staining and biochemical tests including Catalase, Oxidase, Indole, Motility, Citrate, Urea, TSI reaction, and Pyocin production.

And this overnight culture was further incubated on water bath for 2 h, the turbidity of inocula was matched with 0.5 MacFarland standard suspension. The turbidity of standard was comparable to bacterial suspension containing 1.5×10^8 CFU/ml. Mueller Hinton plates were seeded with 0.5 MacFarland suspension matched turbidity inocula and antibiotic disc were placed on them used. The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercially available discs (Hi-Media) on Muller Hinton Agar using Amikacin (30mcg), Ciprofloxacin (5mcg), Ofloxacin (5mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Piperacillin (10mcg), Piperacillin Tazobactam (100/10mcg), Ceftriaxone-Sulbactam (75/15mcg), Imipenum (10mcg), Meropenem, Nitrofurantoin (300mcg- for urinary isolates). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Screening of *P.aeruginosa* for ESBLs production was performed according to the procedures as recommended by the CLSI, using indicator cephalosporins, ceftriaxone (30µg), ceftazidime (30µg), and cefotaxime (30µg). Isolates exhibiting zone size ≤ 25 mm with ceftriaxone , ≤ 22 mm for ceftazidime and ≤ 27mm with cefotaxime were considered as ESBLs producer.

Results

Among the 5562 total clinical samples, 151 isolates of *P.aeruginosa* were isolated (2.62%).Urine (32.45%) was the predominant sample of isolation, which was followed by pus (24.50%), sputum (19.86%) and ear swab(13.24%).

Table 1: Distribution of Pseudomonas aeruginosa in different samples

Name of the ward	Number of isolate of Pseudomona aeruginosa	Percentage %
Pus	37	24.52
Sputum	30	19.86
Aural swab	20	13.24
Blood	5	3.31
Urine	49	32.45
Wound swab	9	5.96
Eye	1	0.66
total	151	100%

Table 2:Age distribution

Age	Male	Female	Total
0-20	15(48.38%)	16(51.61%)	31(20.52%)
21-40	25(49.01%)	26(50.98%)	51(33.77%)
41-60	28(70%)	12(30%)	40(26.49%)
>61	20(68.96%)	9(31.03%)	29(19.20%)
0-->61	88(58.27%)	63 (41.72%)	151

Males (58.27%) were commonly affected and maximum number of cases were seen between age group 21-40 years.

It was observed that isolates of P.aeruginosa were resistant to most of the routinely used anti-pseudomonal drugs.

Highest resistance were observed for Ceftriaxone (95.36%), Cefazolin 80.14% Piperacillin (72.85%), Ciprofloxacin(70.20%) and Cetriaxone / sulbactum (68.875%). Those strains showed resistance to Ceftazidime & Ceftriaxone were subjected to ESBL detection tests. Among 151 strains of *P.aeruginosa*, which were screened phenotypically for various mechanisms of resistance, 31.13 % showed ESBL production.

Table 3: Antibiogram of P aeruginosa

SN	Name of drugs	Sensitive %	Resistant %
1	Amikacin	74.84	25.15
2	Piperacillin	27.15	72.85
3	Piperacillin /tezobactum	34.00	66.00
4	Meropenem	70.20	29.80
5	Ofloxacin	64.24	35.76
6	Imipenem	69.54	30.46
7	Nitrofurantoin	41.06	58.94
8	Cetriaxone / sulbactum	31.13	68.87
9	Ciprofloxacin	29.80	70.20
10	Cefazolin	19.86	80.14
11	Ceftriaxone	4.64	95.36
12	Cetazidime	19.86	80.14

Out of 151 isolates, 43 were found to be MDR *P.aeroginusa* . MDR P. aeruginosa was difined as a bacterium which was resistant to three or more antiPseudomonal antimicrobial classes.(carbapenems, fluoroquinolones,penicillins /cephalosporins and aminoglycosides).

Discussion:

In India, prevalence rate of *P.aeruginosa* infection varies from 10.5% to 30%. It ranged from 3 to 16%, in a multicentric study conducted by Ling J M et al. The prevalence in our study was found to be 2.62 % which is comparable to above study[6].

In the present study, highest percentage of P.aeruginosa was recorded from urine samples(32.45%) followed by pus samples(24.52%).These results are in line with various other studies where prevalence was also found higher in samples of urine and pus. JS Gill[7], et al shown in their study that Urine (47 out of 88) and wound (38 out of 88) samples accounted for the majority of the positive isolates.

Male preponderance (58.27%) was noted in this study. Similar observations were made by, Anupurba et al., (60%)[8]& Siti Nur et al (57%)[9].Outdoor activity, personal habits, nature of work and exposure to soil, water and other areas which are inhabited by organism could be the reason for male preponderance. More no of cases cases 51(33.77%), were seen between 21-40 years. This is in accordance with other studies reported by Okon K.O et al., (24.6%)[10] and Anupurba S et al.,(45.88%)[8], the common age group was between 21–40 in these studies too.

Increasing resistance of beta-lactam in nosocomial *P. aeruginosa* has become a serious threat particularly against third and fourth generation Cephalosporins, is of major concern. There are a lot of molecular mechanisms to develop resistance against these antibiotics; generation of extended-spectrum beta-lactamases (ESBL), by incorporation of bla genes in integrons and sites.[11]

Present study showed that *P. aeruginosa* was found to be highly resistant against cephalosporin group of antibiotics where Cefazolin Ceftriaxone and Cetazidime were highly resistant to P aeruginosa infection showing resistant rates of 80.14%,95.36% and 80.14%. Among these three Ceftriaxone was highly resistant. Similar results were also seen in study conducted by Senthamarai HYPERLINK

"https://www.ncbi.nlm.nih.gov/pubmed/?term=S.%20S%5BAuthor%5D&cauthor=true&cauthor_uid=24995180" S. et al[12] , Ceftazidime (65.38%), & Ceftriaxone (55.76%). Indiscriminate use of 3rd generation cephalosporin as broad spectrum empirical therapy and the secretion of ESBL enzymes mediate the resistance by hydrolysis of β-lactam ring of β-lactam antibiotics. Other mechanisms of drug resistance to β-lactam group of antibiotics are loss of outer membrane protein, production of class C AmpC β-lactamase and altered target sites.

In our study 31.13 % isolates were ESBL producers . whichwas slightly lower than the study conducted by VarunGoel et al[13]., where ESBL producers were 42.30%.

In present study 29.80% of the isolates were resistant to Imipenem & 29.80% were Meropenem where Rodríguez-Martínez JM et al[14], showed that 87% of strains of *P. aeruginosa* were resistant against Imepenem . Another study reported 100% resistance against Carbapenems,[15] it is very obvious that efficacy of this particular antibiotic is declining. Clonal spread contributes lesser importance in the statistics and epidemiology of infections caused by P. aeruginosa, and the main mechanism associated with increased resistance to Imipenem was reduced expression of OprD (outer membrane protein) found in the isolate.[14]

Aminoglycosides are broad-spectrum antibiotics with a peculiar structure of an aminocyclitol ring. They are very effective against aerobic and facultative aerobic Gram-negative bacteria. They mainly act by inhibiting protein synthesis and break cell membrane. Our data showed amikacin became the most effective drug to treat p aeruginosa infection which is quiet surprising compared to other studies But Fouzia Khan et al[16] showed that the hightest efficacy of Amikacin in their study where only 10% of the p aeruginosa was resistant to Amikacin in our present study. One study declared 21% resistance against Aminoglycoside.[17] Moreover, one more study explained 83% resistance to Amikacin. The resistance of clinical isolates to Aminoglycoside antibiotics varies with the specific drug, the microorganism, its mechanism of resistance, the geographic area and many other factors.[18]

Fluroquinolone compounds are one of the important antimicrobial agents that have been used for variety of infections. New groups of Fluroquinolone are beneficial against Gram-negative and Gram-positive bacteria as far as older Fluroquinolones are concerned, they were effective against aerobic Gram-negative bacteria[17.] Present study showed 7o% resistance against Ciprofloxacin while 100% resistance against Ciprofloxacin was exhibited in one study[15]. Similarly, 87.8% resistance was also claimed by another study.[19]

CONCLUSION

As the problem of antibiotic resistance in Pseudomonas aeruginosa has increased to an alarming stage it is necessary to use antibiotics wisely in all fields and efforts should also been made towards early detection and prevention of emergence of antibiotic resistance in Pseudomonas aeruginosa.

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