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Original Research Paper

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PHENOTYPIC DETECTION AND ANTIBIOTIC RESISTANCE PATTERN OF ESBL PRODUCING PROTEUS MIRABILIS IN VARIOUS CLINICAL ISOLATES

Dr. Shireen Rana — Assistant Professor, Dept. of Pharmacology, KBN University- Faculty of Medical Sciences, Kalaburagi, Karnataka. India

Dr. Bilal Ahmad Mir* — Professor, Dept. of Microbiology, KBN University- Faculty of Medical Sciences, Kalaburagi, Karnataka. India *Corresponding Author

ABSTRACT Despite the advent of various antimicrobials, nowadays, the community acquired and health care associated infections are relatively frequent with Proteus species. Its resistance to various groups of antibiotic has been increasing, which needs a continuous survey for better therapeutic response. The Emergence of infections caused by extended-spectrum beta-lactamase (ESBL) Proteus species is alarming which creates serious health problem resulting in an enormous burden in health care setup and cost. From total 5055 samples, 591 (11.7%) *Proteus mirabilis* isolates were obtained. 186 (5.4%) from urine samples, 120 (18.5%) from wound 105 (30.7%) from blood, 172 (28.5%) from pus were isolated. Out 591 samples of Proteus mirabilis 185 (31.3%) were ESBL positive. Maximum samples were obtained from wound (37.5%), followed by pus (36.0%), urine (31.7%), and blood (16%). Statistical analysis was done using chi – square test and p value was found to be significant The frequency of ESBL producing strains in our study was 31.3 %. In the present study, presence of ESBLs was determined with CLSI phenotypic confirmatory test (PCT), double disc diffusion test (DDST) and E-test. PCT detected 185 (31.3%) isolates, DDST detected 169 (28.5%) isolates and E - test detected 185 (31.3%) isolates. Thus a higher rate of ESBL detection was found with CLSI phenotypic confirmatory test. Thus, in this study it was observed that ESBL production among *Proteus mirabilis* isolates were more frequently detected by the combination disc (PCT) method than the double disc approximation test. All the positive isolates detected in PCT were confirmed by MIC using E-test. The clinical laboratory standards institute (CLSI) also recommends the use of the combination disc method for the phenotypic confirmation of ESBL production among Enterobacteriaceae.

KEYWORDS : Extended-spectrum Beta-lactamase (ESBL), *Proteus mirabilis*, Antibiogram, Clinical Laboratory Standard Institute (CLSI) guidelines, Enterobacteriaceae.

INTRODUCTION:

Among members of Enterobacteriaceae family, *Proteus mirabilis* is one of the common causes of urinary tract infections (UTIs) and nosocomial infections. [1] Wild-type strains of *P. mirabilis* are generally susceptible to beta lactams. 90% of Proteus infections are caused by *P. mirabilis* and it is thought to be common cause of infection-related kidney stone and recurrent bacteruria. [2]

Prevalence of ESBL production in members of Enterobacteriaceae family is high and for detecting these enzymes different laboratory methods are used so that they can be properly managed. Concentrating on this ever growing issue of ESBL and taking into consideration that there is scarcity of data regarding ESBL producing *Proteus mirabilis* in our region, the current study was undertaken to add a few drops of knowledge into the vast ocean of ESBL[3]

MATERIALS AND METHODS:

A total of 5055 samples of urine, wound, pus, sputum & blood were collected from past three years. Laboratory Samples were processed and isolates were identified as per standard methods[4]

All the samples were collected under all aseptic conditions and inoculated onto nutrient agar, blood agar, MacConkey agar and incubated at 37°C, overnight. The colonies were tested for the standard biochemical reactions for *Proteus mirabilis*. The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercially available disc (Hi media) on Muller Hinton agar and results were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines. Those strains showed resistance to 3rd generation cephalosporins were subjected to ESBL detection methods using double disc synergy test and confirmed by phenotypic confirmatory test and MIC (using E test).

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined by Kirby-Bauer disk diffusion method as per CLSI guidelines [5]. Antimicrobial disks used were Ampicillin (10µg), Amoxycillin-clavulanic acid (20/10µg), Piperacillin (100µg), Piperacillin-tazobactam (100/10µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg),Ofloxacin (5µg), Cefuroxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Gentamicin (10µg), Amikacin (30µg), Tobramycin (30µg, Co-trimoxazole (1.25/23.75 µg), Aztreonam (30µg) and Imipenem (10µg). [Hi Media, Mumbai]

Screening test for ESBLs

Screening of ESBLs was done as per CLSI guidelines, isolates showing inhibition zone size of <22 mm with Ceftazidime (30 µg), <25 mm with Ceftriaxone (30 µg), and <27 mm with Cefotaxime (30µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production

Confirmatory tests for ESBLs

1. Double disc synergy test:

Organisms to be tested were inoculated onto Muller-Hinton agar plate by lawn culture. A disc containing amoxyclav (Amoxycillin + clavulanic acid) was placed at center of the plate. Ceftazidime, Ceftriaxone andCefotaxime were placed with the interdisc distance (edge to edge) of 15 mm from the amoxyclav disc. The plates were incubated at 37°C for overnight. Enhancement of zone of inhibition towards amoxyclav by any one of these drugs such as ceftazidime, cefotaxime, ceftriaxone was considered as positive result.

2. Phenotypic confirmatory test with combination disc: Disk of Ceftazidime (30µg) and a disk of Ceftazidime +Clavulanic acid (30 µg/10 µg), cefotaxime (30mcg) and a disk of cefotaxime + clavulanic acid (30mcg/10mcg) were used. Both the disks were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Muller Hinton Agar plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured.

When there was an increase of > 5 mm in inhibition zone diameter around combination disk with clavulanic acid versus

the inhibition zone diameter in disk alone was confirmed positive for ESBL production Figure 1.

3. MIC reduction test (E test): The isolates positive with combination disk test were further confirmed for ESBL production by this test. Minimum inhibitory concentration of the isolates was determined by E test method. Concerntration range of antibiotic used are as follows Ceftazidime: 0.25-16 □g/ml Ceftazidime + Clavulanic acid: 0.016-1 □g/ml When the ratio of the value obtained for Ceftazidime (CAZ): The value of Ceftazidime in combination with Clavulanic acid (CAZ+) is more than 8 or no zone is obtained for CAZ and Zone obtained in CAZ+ indicates that the strain is an ESBL producer [6].

RESULTS:

1. Isolation of *Proteus mirabilis* from various clinical samples:

From total 5055 samples, 591 (11.7%) Proteus mirabilis isolates were obtained. 186 (5.4%) from urine samples, 120 (18.5%) from wound 105 (30.7%) from blood, 172 (28.5%) from pus were isolated (Figure 40). Statistical analysis is shown in table 1.

2. Distribution of *Proteus mirabilis* isolates producing ESBL:

Out 591 samples of *Proteus mirabilis* 185 (31.3%) were ESBL positive. Maximum samples were obtained from wound (37.5%), followed by pus (36.0%), urine (31.7%), and blood (16%). Statistical analysis was done using chi – square test and p value was found to be significant (Table 2).

3 Antimicrobial susceptibility:

Table 3/figure 1 shows the highly resistant drugs for Proteus mirabilis ESBL isolates were Ampicillin (100%), Piperacillin (91.6%), Amoxycillin + clavulanic acid (83.0%). Non ESBL producing isolates were (100%), (75.6%), (82.2%) resistant to the same antibiotics respectively. Significant difference in resistant pattern between ESBL and Non ESBL isolates was found in case of beta lactam drugs Amoxycillin + clavulanic acid, Ciprofloxacin, Ofloxacin, Cefuroxime, Gentamicin, Ceftazidime, Ceftriaxone, Piperacillin + Tazobactam, Amikacin, Nitrofurantoin, Tobramycin and Co – trimoxazole. The ESBL isolates of Proteus mirabilis were highly resistant to these set of antibiotics; however, non-ESBL isolates showed relatively higher sensitivity to them. The most effective drugs found in antibiotic resistance testing against *Proteus mirabilis* ESBL isolates were Imipenem and Aztreonam showing 0% resistance each. The percentage of all resistant antibiotics among ESBL and Non-ESBL *Proteus mirabilis* is shown in Figure 2.

Table 1:	Proteus mirabilis from various clinical samples		
Sample	Proteus mirabilis		Total samples
	Positive (%)	Negative	number
Urine	186 (5.44)	2959	3415
Wound	120 (18.5)	529	649
Blood	105 (30.7)	237	342
Sputum	8 (17.8)	37	45
Pus	172 (28.5)	432	604
$\chi 2 = 444.202$; $p < 0.001$			

Table 2:	Distribution according to clinical sources of Proteus mirabilis isolates producing ESBL		
Clinical source	Number of ESBL producing isolates P. mirabilis		
	Tested	Positive (%)	Negative
Urine	186	59 (31.7)	127
Wound	120	45 (37.5)	75
Blood	105	17 (16.1)	88
Sputum	8	02 (25.0)	6
Pus	172	62 (36.0)	110
Total	591	185 (31.3)	406
$\chi 2 = 15.257$; $p = 0.004$			

Table 3: Antibiotic resistance pattern of Proteus mirabilis				
Antibiotic	Code	ESBL producer n=185 n (%)	Non-ESBL producer n= 406 n (%)	P - value based on 2 test
Ampicillin	A	185 (100)	406 (100)	-
Piperacillin	B	169 (91.3)	307 (75.6)	<0.001
Amoxicillin + clavulanic acid	C	153 (83)	334 (82.2)	<0.001
Ciprofloxacin	D	76 (41.0)	110 (27.0)	0.897
Ofloxacin	E	60 (32.4)	75 (18.4)	0.001
Cefuroxime	F	43 (23.2)	36 (8.8)	<0.001
Gentamicin	G	38 (20.5)	43 (10.5)	<0.001
Ceftazidime	H	36 (19.4)	41 (10.0)	0.001
Ceftriaxone	I	35 (18.9)	29 (7.1)	0.002
Piperacillin + tazobactam	J	35 (18.9)	8 (1.9)	<0.001
Amikacin	K	23 (12.4)	20 (4.9)	<0.001
Nitrofurantoin	L	23 (12.4)	36 (8.8)	0.001
Tobramycin	M	22 (11.8)	29 (7.1)	0.180
Co-trimoxazole	N	19 (10.3)	39 (9.6)	0.057
Aztreonam	O	0	0	-
Imepenam	P	0	0	-

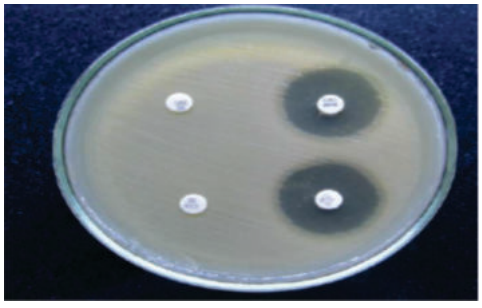

Figure 1: ESBL detection by phenotypic confirmation test showing zone of inhibition.

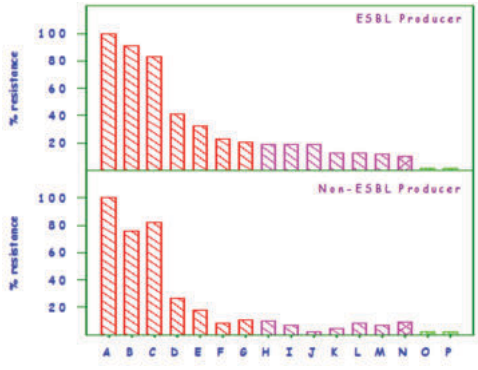

Figure 2. Percent resistance of ESBL and Non-ESBL producers of *Proteus mirabilis*.

DISCUSSION:

In the present study, the prevalence rate of Proteus among 5055 various clinical specimens was 591 (11.7%). in other studies proteus rate was 3.04% - 28.75% [7-9]. The same has been reported with In the present study, out of 591 P. mirabilis, 185 (31.3%) were ESBL producers. wound (37.5%), pus (36.0%) and urine (31.7%) formed the bulk of the samples that yielded culture of ESBl producing Proteus mirabilis.

In the present study, urine (31.7 %) was the one of the predominant samples of isolation after wound, sputum and pus (Table 2). It is comparable with other studies [10-11]. Proteus is one of the important organisms in causing UTI. Its urease enzyme cause polyvalent cations such as Mg+, Ca+ which will precipitate the urine and form struvite stones causes

obstruction of the urinary tract, leads to persistence of the bacterium and makes the treatment difficult.

In this study, Proteus spp were equally isolated (36.0%) from pus (Table 2), whereas the other studies observed maximum isolates in pus samples [12 -13]. There is lack of data regarding prevalence of ESBL producing P. mirabilis, studies conducted outside India have reported prevalence of 3% to 20% [14-15]. In the present study highest resistance was seen with Ampicillin (100%), Piperacillin (91.3%) and Amoxycillin-clavulanic acid (83%), least with Aztreonam and Imipenem (Table 3/ Figure 2) After Impinem and Aztreonam, the most sensitive drugs for P. mirabilis were Aminoglycosides and Nitrofurantoin. Similar findings were reported by other authors [16-17]

In our study, results of antimicrobial susceptibility test revealed that Imipenem was the most effective antibiotic against Proteus spp which is followed by Piperacillin-Tazobactam (Table 3). Similar pattern were observed with other studies[18]. Though Imipenem was found to be unaffected by the enzymes in our study, the variation in the resistance reports could be due to the study environment. All the isolates showed high resistance rate of 100 % & 83% to Ampicillin & Amoxyclav respectively. It is in accordance with the other studies [19]. Attention should be focused on the decreasing trend of susceptibility to this group of drugs because prescription of these antibiotics to Proteus infections will end up with multi drug resistance (MDR), extended drug resistance (XDR) and pan drug resistance (PDR) which is worrisome. MDR is pervasive and emerging clinical problem, which causes significant morbidity, mortality and increased economical burden which stems from the inappropriate, excessive use of antibiotics. The frequency of ESBL producing strains in our study is 31.3 %. In the present study, presence of ESBLs was determined with CLSI phenotypic confirmatory test (PCT), double disc diffusion test (DDST) and Etest. PCT detected 185 (31.3%) isolates, DDST detected 169 (28.5%) isolates and E-test detected 185 (31.3%) isolates. Thus a higher rate of ESBL detection was found with CLSI phenotypic confirmatory test. Additional 2.8 % cases were detected by CLSI phenotypic confirmatory test than DDST method. Thus, in this study it was observed that ESBL production among *Proteus mirabilis* isolates were more frequently detected by the combination disc (PCT) method than the double disc approximation test. All the positive isolates detected in PCT were confirmed by MIC using E-test. The clinical laboratory standards institute (CLSI) also recommends the use of the combination disc method for the phenotypic confirmation of ESBL production among Enterobacteriaceae.

CONCLUSION:

It gives details on the identification of ESBL producing *Proteus mirabilis* by phenotypic methods. Maximum numbers of ESBL producing Proteus mirabilis isolates were obtained from wound followed by pus, urine, sputum and blood. When the ESBL producing *Proteus mirabilis* were subjected to antibiotic susceptibility testing, highest resistance was seen with Ampicillin, Piperacillin and Amoxycillin plus clavulanic acid respectively.

All of ESBL producers were susceptible to Imipenem and Aztreonam and many of them were also susceptible to Tobramycin and Nitrofurantoin suggesting that these drugs continue to be effective against ESBL producers. By Phenotypic confirmatory test more number of ESBL producing P. mirabilis was detected when compared to double disc synergy test.

Conflict of Interest: None

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