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Antibiotic sensitivity pattern of clinical isolates of Proteus species with special reference to ESBL and Amp C production

KEYWORDS: Proteus, ESBL, AmpC

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ABSTRACT *Proteus spp are gram negative bacilli which can cause both community acquired and nosocomial infections. This study was conducted to note the antibiotic sensitivity pattern, production of ESBL and AmpC by clinical isolates of Proteus spp in a tertiary care center. Antibiotic sensitivity testing was done according to CLSI guidelines. The proteus isolates were sensitive to third generation cephalosporins, carbepenems and piperacillin/tazobactam. 20% of the isolates were ESBL and 28.5% were AmpC positive. 50% of the isolates were resistant to fluoroquinolones. 21 proteus isolates exhibited multidrug resistance. This may be due to the transfer of multiple genes from one bacterial population to another. Recognition of resistance pattern of bacteria is necessary from time to time to give appropriate treatment and avoid adverse clinical outcomes.*

Introduction:
Proteus spp. are part of Enterobacteriaceae family of gram negative bacilli found in the human intestinal flora and also in multiple environmental habitats, including long term care facilities and hospitals. They are the common cause of nosocomial infections including urinary tract, wound, and bloodstream infections (BSI)(1). This organism is intrinsically resistant to nitrofurantoin and tetracycline, but it is naturally susceptible to β-lactams, aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole(2). However, drug resistance has been increasingly reported for this genus, and the diffusion of resistance to extended-spectrum cephalosporins due to the production of extended-spectrum β-lactamases (ESBLs) has become of great concern (3). Emergence of ESBLs in gram negative bacteria has become a major Public health concern. It is clinically important to detect and specifically target ESBL because ESBL producers may be clinically resistant to many beta-lactams, including the penicillins, aztreonam and cephalosporins. This study was undertaken to determine the various infections caused by Proteus species, their sensitivity pattern to commonly used antibiotics with special reference to ESBL and AmpC production.

Materials and Methods:
This prospective hospital based study was carried out in Microbiology, KMC, Mangalore over 9 months. All non-duplicate consecutive proteus isolates from urine, blood and exudate samples were included in the study. The isolates were identified by standard biochemical reactions and antibiotic sensitivity testing was done for Ampicillin, Amoxycillin/ clavulanate, Ceftazidime, Cefotaxime, Ceftriaxone, Cefuroxime, Cefazolin, Cefoxitin, Piperacillin, Piperacillin/Tazobactum, Cotrimoxazole, Norfloxacin, Ciprofloxacin, Ofloxacin, Amikacin, Chloramphenicol, Meropenem, Imipenem by Kirby Bauer Disk Diffusion Method. The ESBL detection was done by CLSI phenotypic confirmatory method(4). AmpC detection was done by Amp C disk test(5). The data collected was analysed using SPSS version 11.5 and results were expressed by using proportion of Sensitive(S), Resistant(R) and Intermediates(I).

Results:
A total of 84 Proteus isolates were collected from urine (29), Blood (1), Pus/swab (47), tissue (5) and respiratory secretions (2). Maximum isolates were obtained from pus or swab collected from wounds both ulcers and postoperative wound infections. 13 of the specimens of the pus/swab had multiorganisms isolated. The associated organisms were Streptococcus spp, Klebsiella spp, E.coli, Pseudomonas spp and Citrobacter spp. The incidence of Proteus infection was more common in males (76.1%) when compared to female patients. The infection with Proteus was observed in the elderly age group who had some predisposing factors like surgery, sepsis, catheterization, diabetes mellitus or CVA. The Proteus spp showed high resistance to ampicillin, amoxyclav, cefuroxime, cefazolin, cefoxitin, cotrimoxazole and chloramphenicol. Resistance to fluoroquinolones seemed to be rising with nearly 50% isolates showing resistance. (Table 1) 17 isolates of Proteus were found to be ESBL and 24 AmpC positive.

Discussion:
Proteus bacilli, under favorable conditions, are able to cause community acquired and nosocomial infections, most often in immunocompromised. The intestine forms the major reservoir of these bacteria in humans, and can result in autoinfections or transmission of the bacteria from patient to patient in hospitals (6).

Proteus bacilli play a particularly important role in urinary tract infection specially when there are any predisposing factors like surgery or catheterization. UTI due to proteus is more common in males whereas infections caused by E.coli are more common in female patients (7) In our study we found similar findings with more isolates from male patients (76.1%) with UTI. The virulence factors like adhesion factors, flagella, IgA protease, urease enzyme help the organism to establish an infection. The formation of bladder or kidney stones obstructs the urinary tract or the catheters which makes the treatment difficult and also persistence of the bacterium (8).

Proteus species are part of the gram-negative bacilli found implicated in diabetic wounds along with Escherichia, Klebsiella, Enterobacter, and Serratia species(9). Proteus species was highest isolated from pus/swab in our study. There were multi-organisms isolated from these specimens like in other studies(10). Post-surgical wound infections were mostly associated with other gram negative organisms like E.coli, Klebsiella spp. or Pseudomonas spp. whereas with diabetic ulcers or injection abscess it was associated with gram positive cocci like Streptococcus spp. or S.aureus.

The Proteus spp. showed high resistance to ampicillin, amoxyclav, cefuroxime, cefazolin, cefoxitin, cotrimoxazole and chloramphenicol. The isolates were resistant to the 2nd generation cephalosporins may be due to the extensive use of these antibiotics for all the gram positive infections also. The

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isolates were sensitive to ceftazidime, cefotaxim, ceftriaxone, piperacillin, piperacillin/tazobactum, amikacin, imipenem and meropenem. Resistance to meropenem was seen only in one isolate of Proteus isolated from a patient with abdominal wound infection. The isolate was sensitive to piperacillin/tazobactum. The isolates were sensitive to amikacin which is the drug of choice mostly in our geographic area for post-operative wound infections. Resistance to this antibiotic was observed in the isolates from respiratory tract and from urine of catheterized patients.

Resistance to fluoroquinolones seemed to be rising with nearly 50% isolates showing resistance. Fluoroquinolones are used widely in the hospital for resistant UTI, respiratory tract infections and empirically in case of suspected gram negative infections. This may have contributed to the development of resistance to this group among the organisms of Enterobacteriaecae group. With the rise of ESBL positive organisms, fluoroquinolones may be the drug of choice before use of carbapenems. The resistance is not only chromosome mediated, but also plasmid mediated (qnr) which may help in rapid spread of resistant strains due to widespread use of quinolones in animal feeds also. The risk factor associated with infection with fluoroquinolone resistant Proteus species was prior antibiotic treatment. Judicious use of this group of antibiotics goes a long way in preventing widespread resistance.

ESBLs are mutant plasmid mediated beta lactamases derived from older, broad spectrum beta lactamases and confer resistance to all extended spectrum cephalosporins and aztreonam, except cephamycins and carbepenems(11). 17 isolates (20%) demonstrated the presence of ESBL. Most of the ESBL isolates were from pus/swab (9) specimens. 24 (28.5%) isolates were Amp C positive. 10 isolates were positive for both ESBL and Amp C production. All the patients from whom these isolates were obtained were exposed to previous antibiotics for more than five to seven days and had undergone some invasive procedure or were admitted in the ICU. Recognition of all ESBL and Amp C producers is of major clinical concern, as inappropriate treatment of invasive infections with cephalosporins can lead to therapeutic failures and adverse clinical outcome.(12)In case of both ESBL and Amp C producing isolates carbepenem like meropenem is the drug of choice.

The rapid emergence of antibiotic resistant transfer genes among bacterial population are becoming important causes of clinical infections. In this study we found 21 Proteus isolates resistant to multidrugs. 8 isolates from urine, 12 from pus/swab and one of the isolate from endotracheal secretions of a ventilated patient demonstrated multidrug resistance to the cephalosporins, fluoroquinolones, cotrimoxazole, and chloramphenicol. The isolates were sensitive only to combination antimicrobials and the carbepenems which are the only treatment option. This not only increases the morbidity of the patient but also increases the cost of treatment which is not very favourable in developing countries like ours.

Table 1: Antibiotic sensitivity pattern of the Proteus isolates

Antibiotics		Urine (29)	Blood (1)	Pus/ swab (47)	Tissue (5)	Respiratory secretions (2)
Ampicillin	S(23)	9	1	11	2	-
	R(61)	20	-	36	3	2
Amoxyclav	S(29)	9	1	17	2	-
	R(55)	20	-	30	3	2
Ceftazidime	S(65)	25	1	34	4	1
	R(19)	4	-	13	1	1
Cefotaxime	S(68)	26	1	36	4	1
	R(16)	3	-	11	1	1
Ceftriaxone	S(60)	23	1	31	4	1
	R(24)	6	-	16	1	1
Cefuroxime	S(31)	11	1	17	2	-
	R(53)	18	-	30	3	2
Cefazolin	S(24)	8	1	13	2	-
	R(60)	21	-	34	3	2
Cefoxitin	S(29)	11	1	13	4	-
	R(55)	18	-	34	1	2
Piperacillin	S(70)	24	1	39	5	1
	R(14)	5	-	8	-	1
Piperacillin Tazobactum	S(77)	25	1	44	5	2
	R(7)	4	-	3	-	-
Cotrimoxazole	S(51)	20	1	27	3	-
	R(33)	9	-	20	2	2
Norfloxacin	S(57)	19	1	33	3	1
	R(27)	10	-	14	2	1
Ciprofloxacin	S(57)	19	1	32	3	2
	R(27)	10	-	15	2	-
Ofloxacin	S(57)	19	1	33	3	1
	R(27)	10	-	14	2	1
Amikacin	S(68)	25	1	37	4	1
	R(16)	4	-	10	1	1
Chloramphenicol	S(60)	22	1	32	4	1
	R(24)	7	-	15	1	1
Meropenem	S(82)	28	1	46	5	2
	R(2)	1	-	1	-	-
Imipenem	S(84)	29	1	47	5	2

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