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Antimicrobial Susceptibility Pattern of *Proteus* Species Isolated From Various Clinical Samples in a Tertiary Care Hospital

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Abstract: *Proteus* species are an important cause of community- and health care-associated infections, including those involving the urinary tract, the abdominal cavity, and the blood stream. This study was undertaken to isolate and identify different *Proteus* species from various clinical samples and to analyse their antimicrobial suceptibility pattern. One hundred ten isolates from various clinical samples were identified as per conventional phenotypic methods and antimicrobial susceptibility testing was done by Kirby-Bauer Disc diffusion method as per CLSI guidelines**.** Most common species isolated from various samples was *P. mirabilis* 80.90% (89/110); followed by *P. vulgaris* 14.54% (16/110); and *P. penneri* 4.54% (5/110). Piperacillin-tazobactam was the most sensitive drug for all isolates 95.45% (105/110) followed by imepenem 87.27% (96/110). We concluded that species identification and surveillance of antimicrobial resistance is essential in management and control of infections.

Keywords: Antimicrobial susceptibility, *Proteus*, muiti-drug-resistance, extensively-drug-resistance.

INTRODUCTION

Proteus species *are* an important cause of community- and health care-associated infections, including those involving the urinary tract, the abdominal cavity, and the blood stream. The genus *Proteus* belongs to the tribe Proteeae of the family Enterobacteriaceae [1]. The most characteristic feature which distinguishes *Proteus* spp from other members of this family is the swarming phenomenon, a multicellular differentiation process of short rods to elongated swarmer cells [2].

The genus *Proteus* currently consists of five named species (*P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. myxofaciens* and *P. hauseri*) and three unnamed genomospecies (4, 5, and 6) [3]. These microorganisms cause a number of infections including urinary tract infections (UTIs), wound infections and meningitis especially in neonates or infants. It causes complicated UTIs with a higher frequency, compared to other uropathogens. *Proteus* infections are accompanied by a formation of urinary stones, containing struvite and carbonate apatite [4]. Three species namely *P. vulgaris*, *P. mirabilis*, and *P. penneri* are opportunistic human pathogens. Among the all *Proteus* species, *P. mirabilis* causes 90% of *Proteus* infections and is believed to be the most common cause of infection-related kidney stones, one of the most serious complications of unresolved or recurrent bacteruria [5]. *Proteus vulgaris* is most frequently isolated from immuno-compromised patients or those on long-term antibiotic regimen [6]. *P. penneri* has ability to cause major infectious diseases and nosocomial outbreaks and carries similar pathogenic determinants to *P. mirabilis* and *P. vulgaris* [7]. The susceptibility of members of the Proteeae to concentrations of antibiotic attainable in the body is highly variable. All species of the tribe are resistant to nitrofurantoin, tetracyclines, tigecyclines and polymyxins [8]. In general, *P. mirabilis* is the most sensitive species of the genus. *P. mirabilis* is susceptible to β-lactams, aminoglycosides, fluoroquinolones, and trimethoprim/sulfamethoxazole [9].

MATERIALS AND METHODS

The present study was carried out over a period of one year (April 2016 to March 2017) in the Department of Microbiology, SMS Medical College, Jaipur (Rajasthan). One hundred ten clinical isolates of *Proteus* species isolated from various clinical samples were included in the study. The clinical samples included were mainly pus/wound swab, urine, blood, tracheal swab, ear pus/discharge, and stool. *Proteus* species were also isolated from few other samples like tissue, pleural fluid, catheter tip, umbilical tip, ET tube aspirate etc. Samples were inoculated on blood agar

and MacConkey agar. The isolates were identified by colony morphology, Gram's staining, and biochemical reactions. The isolates were further speciated on the basis of carbohydrate fermentation test and ornithine decarboxylase test [3].

Antimicrobial susceptibility testing [ampicillin (10µg), piperacillin (100µg), cefpodoxime (10µg), cefotaxime (30µg), ceftazidime (30µg), imipenem (10µg), meropenem (10µg), gentamicin (10µg), amikacin (30µg), amoxicillin-clavulanate (20/10µg), piperacillin-tazobactam (100/10µg), aztreonam(30µg), ciprofloxacin(5µg), levofloxacin (5µg), trimethoprim-sulfamethoxazole(1.25/23.75µg)] of the isolated *Proteus* species was done by Kirby-Bauer Disc diffusion method and results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines[10]. The isolate was considered as multi-drug-resistant (MDR) when non-susceptible to at least one agent in three or more antimicrobial categories and extensively-drug-resistant (XDR) when non-susceptible to at least one agent in all but two or fewer antimicrobial categories. Isolate non-susceptible to all agents in all antimicrobial categories was considered as pandrug-resistant (PDR) [8].

RESULTS

Out of 110 of *Proteus* isolates obtained, majority of the *Proteus* species were isolated from pus/ wound swab 40.90% (45/110) followed by urine 34.54% (38/110), tracheal swab 10% (11/110), ear swab 3.63%(4/110), blood and stool 2.73% (3/110) each, tissue 1.82% (2/110), pleural fluid 0.91% (1/110). Other samples including umbilical catheter tip, foley's catheter tip and ET tube aspirate accounted for 2.73% (3/110). Most common species isolated from various samples was *P. mirabilis* 80.90% (89/110), followed by *P. vulgaris* 14.54% (16/110); and *P. penneri* 4.54% (5/110).

All three *Proteus* species were maximally isolated from pus/wound swab obtained from inpatients accounting for 39.09% (43/110) followed by urine 16.36% (18/110) (Table No. 1). *Proteus* isolates were predominantly isolated from male patients (70.90%) as compared to female patients (29.10%).

Piperacillin-tazobactam was the most sensitive drug for all the three species, *P. mirabilis* being 94.38% sensitive while *P. vulgaris* and *P. penneri* were 100% sensitive followed by imepenem (80-93.75%). *P. penneri* was completely resistant to amoxyclave, piperacillin, ceftazidime and cefpodoxime (100% each). (Table No. 2). Among all 110 isolates 83.63% were found to be multi-drug-resistant (MDR) and 30% were possible XDR. No PDR was observed in this study. (Table No. 3)

Table-1: Sample-wise distribution of *Proteus* species among In-patients and Out-patients

Samples	*Proteus* Species	In-patients(N=83)	Out-patients(N=27)	Total No. of Species	Total No. of Isolates
Pus/Wound swab	Pm	35	2	37	45
	Pv	5	0	5	
	Pp	3	0	3	
Urine	Pm	15	13	28	38
	Pv	3	5	8	
	Pp	0	2	2	
Tracheal Swab	Pm	9	0	9	11
	Pv	2	0	2	
	Pp	0	0	0	
Ear swab	Pm	1	3	4	4
	Pv	0	0	0	
	Pp	0	0	0	
Blood	Pm	3	0	3	3
	Pv	0	0	0	
	Pp	0	0	0	
Stool	Pm	1	1	2	3
	Pv	0	1	1	
	Pp	0	0	0	
Tissue	Pm	2	0	2	2
	Pv	0	0	0	
	Pp	0	0	0	
Pleural fluid	Pm	1	0	1	1
	Pv	0	0	0	
	Pp	0	0	0	
Others	Pm	3	0	3	3
	Pv	0	0	0	

	Pp	0	0	0	

Table-2: Antimicrobial susceptibility pattern of Different *Proteus* species

Antibiotics	*P. mirabilis* (89)		*P. vulgaris* (16)		*P. penneri* (5)		P Value LS
	S	R	S	R	S	R	
Ampicillin	22(24.72%)	67(75.28%)	*	*	*	*	
Piperacillin	35(39.33%)	54(60.67%)	9(56.25%)	7(43.75%)	0(0%)	5(100%)	0.078
Cefotaxime	56(62.92%)	33(37.08%)	12(75%)	4(25%)	1(20%)	4(80%)	0.085
Ceftazidime	30(33.71%)	59(66.29%)	7(43.75%)	9(56.25%)	0(0%)	5(100%)	0.195
Cefpodoxime	26(29.21%)	63(70.79%)	5(31.25%)	11(68.75%)	0(0%)	5(100%)	0.353
Imepenem	77(86.52%)	12(13.48%)	15(93.75%)	1(6.25%)	4(80%)	1(20%)	0.641
Meropenem	51(57.30%)	38(42.70%)	12(75%)	4(25%)	1(20%)	4(80%)	0.087
Aztreonam	64(71.91%)	25(28.09%)	11(68.75%)	5(31.25%)	3(60%)	2(40%)	0.832
Amoxycillin-clavulanate	34(38.20%)	55(61.80%)	5(31.25%)	11(68.75%)	0(0%)	5(100%)	0.206
Piperacillin-Tazobactam	84(94.38%)	5(5.62%)	16(100%)	0(0%)	5(100%)	0(0%)	0.539
Ciprofloxacin	46(51.69%)	43(48.31%)	10(62.5%)	6(37.5%)	3(60%)	2(40%)	0.697
Levofloxacin	38(42.70%)	51(57.30%)	8(50%)	8(50%)	1(20%)	4(80%)	0.496
Amikacin	59(66.29%)	30(33.71%)	12(75%)	4(25%)	1(20%)	4(80%)	0.073
Gentamicin	49(55.06)	40(44.94%)	12(75%)	4(25%)	1(20%)	4(80%)	0.082
Cotrimoxazole	28(31.46%)	61(68.54%)	4(25%)	12(75%)	1(20%)	4(80%)	0.771

**P. Vulgaris* and *P. penneri* are intrinsically resistant to ampicillin.

Table-3: MDR, XDR and PDR in *Proteus* isolates

Total Isolates	MDR	XDR	PDR
110	92(83.63%)	33(30%)	0

DISCUSSION

Presence of *Proteus* in clinical samples is of great importance since like other members of Enterobacteriaceae, they are opportunistic pathogens and may cause morbidity and mortality particularly in hospitalized patients. Drug resistance among these pathogens is on an increase particularly by production of extended spectrum β-lactamases.

The present study comprised of 110 *Proteus* isolates from various clinical samples. In the present study it was observed that *Proteus* species were maximally isolated from pus/wound swab samples (40.90%) followed by urine samples (34.54%). Our study correlates with the study done by Vinoth J *et al*. Pandey JK *et al*. Feglo *et al.* which also observed *Proteus* as the common cause of wound infections in India[11-13] while in few other studies *Proteus* was commonly encountered in urine than in other clinical specimens[14,15].

In present study around 75% (83/110) of *Proteus* isolates were from samples obtained from indoor patients with pus/ wound swab being the predominant sample (43/83) as compared to samples from outdoor patients and this difference between isolates from indoor and outdoor patients with respect to pus/wound swab was found to be statistically significant (P<0.001). Most of the indoor samples were obtained from burn unit. Burns provide a suitable site for bacterial colonization, multiplication and infection, mainly because of the larger area involved and longer duration of patient stay in the hospital [1].

Age wise distribution of *Proteus* isolates in the present study revealed that the maximum no. of *Proteus* spp. were isolated from samples observed in the age group 1-10 years (18.18%) and age group 51-60 years (15.45%). Gender based distribution showed that *Proteus* species were more commonly isolated from male patients (70.90%) than female (29.10%). Similar findings were observed in Pandey J K *et al*. and Pal N *et al*. study [12, 16].

In the current study *Proteus* species recovered from 110 specimens were *Proteus mirabilis* (80.90%), *Proteus vulgaris* (14.55%), and *Proteus penneri* (4.55%). Similar results have been reported in various other studies [12-16], On the contrary, Nachammai SM *et al*. reported *P. vulgaris* (74.3%) as most commonly isolated species [18].

Proteus mirabilis were predominantly isolated in all clinical samples obtained mainly from indoor patients including pus /wound swab, urine, tracheal swab, blood and pleural fluid supporting the fact that this species is implicated in causing wound infection, UTI, respiratory infections and blood stream infections particularly in hospitalized patients. These organisms are capable of colonizing and causing disease among hospitalized patients due to virulence factors including adhesion, motility, and immunoavoidance as well as biofilm formation [7].

Antimicrobial sensitivity pattern of *Proteus* isolates in our study for commonly used antibiotics

showed that piperacillin-tazobactam was the most sensitive drug with 94.38%, 100% and 100% sensitivity for *P. mirabilis*, *P.vulgaris* and *P. penneri* respectively followed by imepenem revealing 86.52%, 93.75% and 80% sensitivity. Most of the other commonly used antimicrobials revealed moderate to low sensitivity (Table No.2).

The isolates were further categorized into MDR, XDR and PDR on the basis of standardized international criteria [8]. We found 83.63% of all isolates to be multi-drug resistant (MDR), 30 % possible XDR and no PDR in our study. (Table No. 3) A high rate of MDR *Proteus* species (82-90%) have been reported worldwide particularly among various Asian and African studies [13, 16, 18]. Perween N *et al*. in their study from India reported 55% MDR and 39.4% XDR in *Proteus* spp. isolated from burn wound infections [1]. MDR bacteria are one of the most important current threats to public health and they are associated with nosocomial infections.

CONCLUSION

Proteus species is emerging as an important pathogen in capable of causing community acquired as well as hospital acquired infections including urinary tract, wound, and blood stream infections. *Proteus mirabilis* (89/110) was the commonest species isolated accounting for 80.90% and was also found to be predominant among IPD samples. All the three *Proteus* species isolated showed maximum susceptibility to piperacillin-tazobactam followed by imipenem and meropenem. Most of the isolates (83.63 %) were found to be multiple-drug-resistant (MDR). Decreasing susceptibility of *Proteus* group and the potential for emerging resistance needs for regular review of antimicrobial susceptibility pattern to institute appropriate antibiotic therapy. This study is therefore a step towards the generation of data on the prevalence of antimicrobial resistance patterns of *Proteus* species.

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