

Original Research Article

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## Characterization of *Proteus* Species and Detection of Multi Drug Resistant (MDR) with Special Reference to ESBL Strains

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### ABSTRACT

#### Keywords

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Extended-spectrum  $\beta$ -lactamase (ESBL) continues to be a major problem in healthcare settings. The current study was carried out to assist the clinicians to prescribe appropriate antibiotics against gram-negative clinical isolates. Different *Proteus species* are commonly implicated pathogens in hospital as well as community acquired infections. A total of 172 *Proteus species* isolated from exudates and urine were speciated and tested for antibiogram. ESBL screening was done using 3<sup>rd</sup> generation Cephalosporin's and confirmed by double disc diffusion test. Among the 172 *Proteus* isolates, exudates (68.02%) showed the predominant cases, with *Proteus mirabilis* 117(62.20%) as predominant isolated organism followed by *Proteus vulgaris* 56 (32.55%), *Providencia stuartii* 6 (3.48%) and *Morganella morganii* 3 (1.74%). They were highly sensitive to Imipenem. *Proteus species* were highly resistant to ampicillin, Ceftazidime, Aminoglycosides and Quinolones showing 70% of multiple drug resistant (MDR) and 3.4% Pan drug resistant (PDR) of which 24% of *Proteus* isolates were confirmed ESBL producers.

### Introduction

The tribe *Proteus* consists of a unique group of bacteria within the family *Enterobacteriaceae*. *Proteus* can be found in animals, soil and in the intestinal tract of humans (Kamga). The tribe *Proteus* includes of the genus *Proteus*, *Providencia* and *Morganella* in which *Proteus* was found to be mostly involved in many of the *Proteus* infections and Hospital acquired infection/nosocomial infection. The genus *Proteus* forms the part of the normal microbial flora of the gastrointestinal system and plays a crucial pathogenic role in human beings, both in the community as well as hospital

premises. *Proteus* has been ranked third position in causing nosocomial infection (Nachammai; De Champs *et al.*, 2000). *Proteus mirabilis* and *Proteus vulgaris* are widely recognised as human pathogens in which *Proteus mirabilis* is the most common causative agent of infection-related kidney stone and recurrent bacteriuria (Coker *et al.*, 2000). It was also found to cause pyogenic lesions, wound infections, septicaemia and rarely meningitis (Orett, 1999). There has been a high prevalence of *Proteus mirabilis*, in skin abscesses, which had potential implications for selection of antimicrobial therapy and a case suppurative pericarditis

caused by *Proteus mirabilis* was also identified (Mistry *et al.*, 2010; Sawaya *et al.*, 2009). Occurrence of various virulence factors like fimbrial antigen adhering to uroepithelium, outer membrane proteins (OMP), hemolysin production, cellular invasive property, urease and DNase enzyme production, swarming motility through quorum sensing, adds up for its colonization and progressive disease.

Wild type strains of *Proteus mirabilis* are usually sensitive to  $\beta$ -lactum group of antibiotics. However, due to indiscriminate use of  $\beta$ -lactum antibiotics have led to the development of resistance to this group of antibiotics due to various type of  $\beta$ -lactamase production. Since ESBL producing *Proteus species* are also found to be Multidrug resistant, it is important to know the prevalence of ESBL producing *Proteus* in a hospital setting, to prevent spread of nosocomial infection caused by MDR organisms.

With this background, this study was carried out to determine prevalence of various *Proteus species* causing infections and its antibiogram susceptibility pattern in a tertiary care hospital as well as ESBL production in them.

## Materials and Methods

This laboratory based study was being done for over a period of six months from July 2015 to December 2015 in the Department of Microbiology, Saveetha Medical College and Hospital, Thandalam, Chennai, India to determine the prevalence of Multidrug resistant and ESBL producing *Proteus species* causing Urinary Tract Infection (UTI), Wound Infection and nosocomial infection.

A total of 172 isolates of Gram- negative bacilli obtained from clinical specimens like

urine and wound/ pus swab of both hospitalized patients and out-patients were identified biochemically by the standard microbiological methods (Collee *et al.*, 1996). Isolates from mixed culture were excluded from the study but those that show non-lactose fermenting colonies on MacConkey agar and swarming on Blood agar were isolated and identified by biochemical tests based on whether they were positive for Phenylalanine deaminase production; H<sub>2</sub>S gas production; and urease reactions. Isolates were also tested for virulence factors including production of enzymes like Urease and DNase. The antibiogram of the isolates was determined by the Standard Kirby Bauer's disc diffusion method and Clinical Laboratory Standards Institute (CLSI) recommendations using following antibiotics discs (Hi-Media, India) Ampicillin (10 $\mu$ g), Amikacin (30 $\mu$ g), Ceftazidime (30 $\mu$ g), Ciprofloxacin (5 $\mu$ g), Cephalexin (30 $\mu$ g), Cotrimoxazole (1.25/23.73 $\mu$ g), Cefepime (30 $\mu$ g), Cefotaxime (30 $\mu$ g), Gentamicin (10 $\mu$ g), Ofloxacin (5 $\mu$ g), Imipenem (10 $\mu$ g). The zone diameters were interpreted as per Clinical Laboratory Standards Institute (CLSI) recommendations (2012).

ATCC *Escherichia coli* 25922 strain was used for quality control.

## ESBL screening test

According to CLSI guidelines (2012), in disc diffusion test, those *Proteus* isolates with a zone inhibition of

$\leq 27$  mm with Cefotaxime,  
 $\leq 25$  mm with Ceftriaxone,  
 $\leq 22$  mm with Ceftazidime,  
 $\leq 17$  mm with Cefpodoxime, were considered potential ESBL producers and were further confirmed for ESBL production by doing double disc diffusion method (CLSI).

### **ESBL confirmatory test (Double disc diffusion test)**

Here overnight culture suspension of the test isolates adjusted to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on a Muller Hinton Agar plate. The cefotaxime (30µg) and cefotaxime-clavulanic acid (30/10 µg) discs were placed 20 mm apart. Similarly ceftazidime ((30µg) and ceftazidime-clavulanic acid (30/10 µg) discs were also placed 20mm apart.

After overnight incubation at 37°C, the test organism was considered as a confirmed ESBL producer if the zone size around the clavulanic acid combination disc is increased >5 mm vs. the 3<sup>rd</sup> generation Cephalosporin's disc alone.

### **Results and Discussion**

A total of 172 *Proteus species* were isolated from various clinical samples in which majority cases were isolated in Exudates (68.02%) which is in accordance with other studies (Mordi *et al.*, 2009; Newman *et al.*, 2006) done worldwide. *Proteus mirabilis* was the most frequently isolated species 107 (62.20%) followed by *Protues vulgaris* 56 (32.55%), *Providencia stuartii* 6 (3.48%) and *Morganella morganii* 3 (1.74%) as shown in Figure 1.

Highest prevalence of *Proteus* infection was observed in the age group ≥50 year age group (50% ) followed by 41-50 years age group in which prevalence was 14% as (Table-1). All the isolates of *Protues mirabilis* (62.20%) and *Protues vulgaris* (32.55%) were found to be positive for production of virulence factors e.g. Urease and DNase. *Proteus* isolates from inpatient was highest from exudates accounting for 103 (60%) of the total followed by urine 39 (22%)-Table 2, as compared to out-patient (Felgo *et al.*, 2010; Luelmi *et al.*, 2014).

This finding indicates that *Proteus* may become a major cause hospital acquired infection in our set up if not detected and treated on time.

Isolates showing resistance to either three groups or more standard antibiotics is considered as “Multidrug resistant organism” (MDR). In this study, Multidrug resistance was observed in 122 (70.9%) *Proteus* isolates of which maximum cases were found in *Proteus mirabilis* 79 (46%) (Figure-2), with commonest phenotype being co-resistance to ampicillin, Ceftazidime and Aminoglycosides i.e. (Amikacin, Gentamicin). High Resistance to Quinolones (43.6%) was also observed among the isolates as seen in Table-3. Pan-drug resistance was observed in 6 (3.4%) isolates which needs further testing by molecular methods to detect different resistance genes. Resistance to Imipenem was found in 16 isolates (9.3%) of which maximum cases were *Proteus mirabilis* i.e.12 (75%).High antibiotic resistance was found among *Proteus* in other studies also (Reslinki *et al.*, 2005; Rozalski *et al.*, 2012; Sekowska *et al.*, 2004; Shenoy *et al.*, 2013).

In our study Out of 93 (54%) screen positive *Proteus* isolates, ESBL production was confirmed by the phenotypic method in 22 (24%) isolates with highest incidence in *P.mirabilis* 18 (82%) followed by *Proteus vulgaris* 4(18%) –Table 4. Maximum ESBL positive isolates were recovered from exudates 19 (86.3%), in admitted patients. Although finding of 24% ESBL producers in our study was low in comparison to different part of the world and country (50-60%). However it is similar to a study done by Senthamarai *et al.*, (24.8%) in 2015 from South India. None of the four screen positive *Providencia stuartii* isolates of our study showed ESBL production by confirmatory test. All the ESBL producing *Proteus* isolates were found to be multidrug resistant

with maximum resistance against Ampicillin (100%), Cefotaxime (100%), Ceftazidime (91%), and Quinolones (68%). Only one ESBL producing *Proteus isolate* (4.5%) was found to be resistant to Imipenem which indicates that Carbapenems are still the most effective antibiotic against these infections in our set up (El-Tahawy, 2000).

According to our study maximum infection in urine sample was caused by *P.mirabilis* (61%) which is in accordance with Mobley at al (22) where it was found that *P. mirabilis* has a higher propensity for colonizing the urinary tract due to difference in its pathogenicity .

**Table.1** Distribution of *Proteus* species in different age groups

AGE GROUP ( in years)	Positive isolates (n=172)	%
0-10	17	9.88%
11-20	6	3.48%
21-30	18	10.46%
31-40	21	12.20%
41-50	24	13.95%
≥50	86	50%
<b>TOTAL</b>	<b>172</b>	<b>100%</b>

**Table.2** Distribution of *Proteus* isolates among inpatients and outpatients

Isolates	Exudate				Urine			
	IP	%	OP	%	IP	%	OP	%
<i>P.mirabilis</i>	64	62.13%	11	78.57%	22	56.41%	14	87.5%
<i>P.vulgaris</i>	34	33%	3	21.42%	13	33.33%	2	12.5%
<i>Providencia stuartii</i>	4	3.88%	0	0%	2	5.12%	0	0%
<i>Morganella morganii</i>	1	0.97%	0	0%	2	5.12%	0	0%
<b>TOTAL</b>	<b>103</b>	<b>100%</b>	<b>14</b>	<b>100%</b>	<b>39</b>	<b>100%</b>	<b>16</b>	<b>100%</b>

NOTE: *Proteus* isolates were found to be most common among the in-patients (both from exudates and urine specimens) with 103(59.88%) and 39(22.67%) isolates respectively.

**Table.3** Pattern of antimicrobial resistance among *Proteus* isolates

ANTIBIOTICS	<i>P.mirabilis</i> (n=107)	<i>P.vulgaris</i> (n=56)	<i>Prov.</i> <i>Stuartii</i> (n=6)	<i>M.morganii</i> (n=3)
AMP	87 (81.30%)	44 (79%)	5 (83.33%)	2(67%)
AK	25 (23.36%)	7 (13%)	2 (33.33%)	0
CAZ	16 (15%)	9 (16%)	1(17%)	1 (33.33%)
CIP	28 (26.16%)	13 (23.21%)	3 (50%)	0
CN	58 (54.20%)	28 (50%)	4 (67%)	1 (33.33%)
COT	83 (78%)	37 (66%)	4 (67%)	2 (67%)
CPM	36 (34%)	17 (30.35%)	0	0
CTX	43 (40%)	21 (38%)	2 (33.33%)	0
GEN	37 (35%)	10 (18%)	2 (33.33%)	1 (33.33%)
IPM	7 (7%)	5 (9%)	1 (17%)	0
OF	52 (49%)	19 (34%)	4 (67%)	0

**Table.4** Confirmed ESBL *Proteus* isolates

Organisms (n=22)	ESBL Producing %
<i>P.mirabilis</i>	82%
<i>P.vulgaris</i>	18%

**Fig.1** Species distribution of *Proteus*

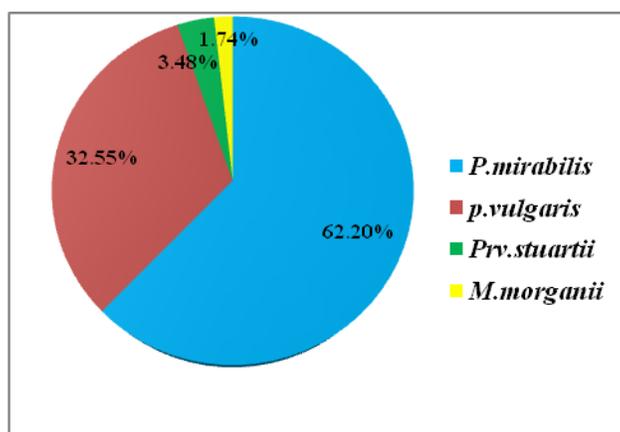


Fig.2 MDR *Proteus* species

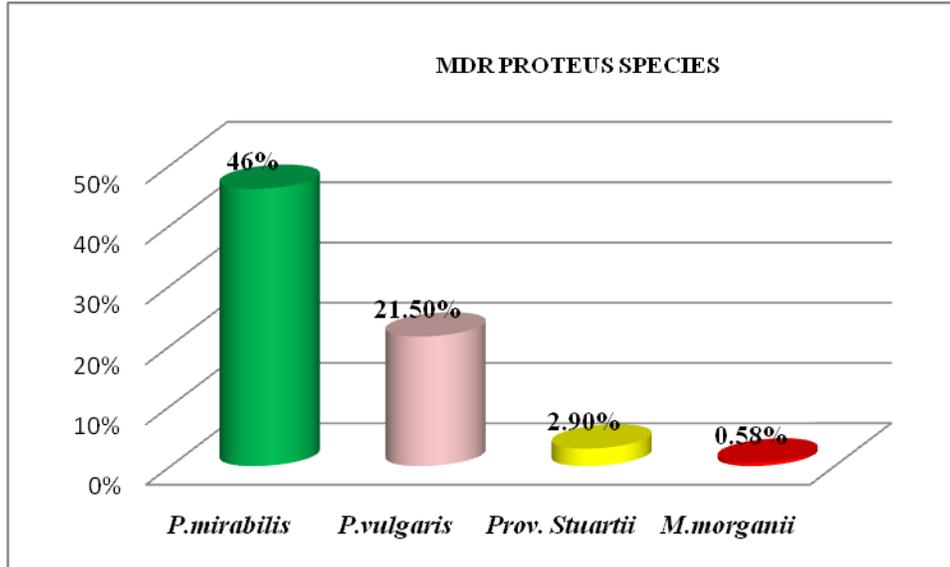


Image.1 Double disc diffusion test showing ESBL positive strain



Although *Escherichia coli* accounts for the major cases of uncomplicated urinary tract infections *Proteus* ranks third as the cause of these infections particularly in hospital acquired set up (Bahaswan *et al.*, 2013) Indiscriminate use of antibiotics particularly third generation Cephalosporins provide selective pressure among Enterobacteriaceae which is the possible cause of multidrug resistance observed among these organisms including *Proteus* (Levy, 1999). Since plasmids are responsible for ESBL

production tend to be large (80Kb or more) and carry resistance to several other antimicrobial agents. These organisms carrying these plasmids are also potential reservoirs of resistance genes that could be transferred to other bacterial pathogens. ESBL producing organisms cause a major problem for clinical therapeutics. The incidences of ESBL producing *Proteus* among clinical isolates have been steadily increasing over the past few years resulting in limited therapeutic options. Though in our

study, ESBL producing organisms were found to be restricted to inpatients only, there is still risk of spread of these organisms to community. With the spread of ESBL producing strains in hospitals all over the world, it is necessary to know the prevalence of ESBL positive strains in a hospital so as to formulate antibiotic policy for empirical therapy in high risk units where infections due to these resistant organisms is much higher (Mathur *et al.*, 2002).

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