

Original Research Article

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Prevalence and Antimicrobial Susceptibility Pattern of *Proteus* Species in Clinical Samples

Rajiv Ranjan Prasad^{1*}, Vijay Shree², Satyendu Sagar¹, Sunil Kumar⁴ and Prabhat Kumar¹

¹Department of Microbiology, NMCH, Patna, India

²Department of Community Medicine, IGIMS, Patna, India

*Corresponding author

ABSTRACT

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Different *Proteus* species may vary with the type of infections they cause in both the community and hospital environments. However, in many laboratories in developing countries, differentiation of the genus *Proteus* into species is not generally done during bacteriological diagnosis due to high cost and special skills involved. This study aimed at determining the prevalence of different *Proteus* species in Nalanda Medical College and Hospital, PATNA, their antibiotic resistance pattern and how they relate to patients' demographic data. This is a prospective study involving the analyses of clinical samples for *Proteus* species and determining their antimicrobial susceptibility pattern. Thirty two *Proteus* species were isolated from 1910 clinical specimens obtained from patients suspected of bacterial infection, giving 1.67 % prevalence of *Proteus* infections. Wound isolates were the highest followed by urine. Three *Proteus* species; *P. mirabilis*, *P. vulgaris* and *P. penneri* were recovered from the samples. *P. mirabilis* was the commonest species (59.38 %), and hence the causative species of a majority of *Proteus* infections followed by *P. vulgaris* (34.37 %), and *P. penneri* (6.25 %). The three *Proteus* species recovered were highly resistant to ampicillin, cefuroxime, netillin and pefloxacin. This study has also provided information for use in generating national data.

Introduction

Proteus is a genus of Gram-negative bacteria belonging to the family of *Enterobacteriaceae*. *Proteus* species are distinguishable from most other genera by their ability to swarm across an agar surface (Jacobsen *et al.*, 2008). *Proteus* is widespread in the environment and makes up part of the normal flora of the human gastrointestinal tract. *Proteus* ranks third as the cause of hospital-acquired infections (Stamm, 1999). Three species: *P. vulgaris*, *P. mirabilis*, and *P. penneri* are opportunistic human pathogens (Guentzel, 1996).

Proteus species are the major cause of diseases acquired outside the hospital, where many of these diseases eventually require hospitalization (De Champs *et al.*, 2000). *Proteus* species, particularly *P. Mirabilis*, is believed to be the most common cause of infection-related kidney stone, one of the most serious complications of unresolved or recurrent bacteruria (Coker *et al.*, 2000). *P. mirabilis* has been implicated in meningitis, empyema, osteomyelitis and gastroenteritis. Also, it frequently causes nosocomial infections of the urinary tract (46%),

surgical wounds (24%) and lower respiratory tract (30%). Less frequently, *proteus species* cause bacteraemia (17%), most often in elderly patients (Mansy, 2001). The phenomenal evolution and increase of multidrug-resistance of many bacterial pathogens is increasing and representing a growing public health problem in the world. Evolution and spread of a multidrug-resistant *Proteus mirabilis* clone with chromosomal AmpC-type beta-lactamase was reported in Europe (Luzzaro *et al.*, 2009; D'Andrea *et al.*, 2011).

Multidrug-resistance of *Proteus* spp. calls for regular review of antimicrobial sensitivity pattern among clinically isolated *Proteus* spp. in order to be able to decide which antibiotic to be prescribed.

Materials and Methods

Specimens' Collection

Different clinical samples such as sputum, wound swab, cerebrospinal fluid (CSF), tracheal aspirate (Tr. asp.), throat aspirate, pus, abdominal abscess ear swab, bed sores, peritoneal wound swab, pleural fluid were collected from 1910 patients (Inpatient and Outpatient) of Nalanda Medical College and Hospital, Patna, Bihar, India. Demographic data such as sex of the patients was recorded prior to sample collection.

Cultivation and Identification

The clinical samples collected were aseptically inoculated on plates of Blood agar, Chocolate agar Cystine-Lactose-Electrolyte-Deficient (CLED) agar and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37°C for 24 h. The morphological characteristics of the colonies including size, shape, colour, pigmentation and haemolytic nature were recorded. Suspected *Proteus* colonies were isolated

and identified through biochemical tests according to Barrow and Feltham:[9] based on whether they were positive for nitrate reduction; H₂S gas production; methyl-red and urease reactions; and negative for lactose fermentation.

Antimicrobial Susceptibility Test

Modified Kirby-Bauer disk diffusion method (Cheesebrough, 2000) was used to test the susceptibility of the *Proteus* isolates to different antimicrobial agents (obtained from BDH London, UK): ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), gentamicin (10 µg), amikacin (10 µg) and co-trimoxazole (25 µg). The inocula were prepared by growing the various *Proteus* species on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 ml of normal saline in a test tube. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs were applied to the surface of the inoculated agar and the plates were incubated overnight at 37°C. The diameter of zone of growth-inhibition observed was measured and compared to the chart provided by National Committee for Clinical Laboratory Standards (NCCLS).

Results and Discussion

Three *Proteus* species were recovered from 32 of the 1910 clinical samples collected (Table 1) and this gave a prevalence rate of

1.67%. 23 of these samples (71.87 %) were taken from male patients and 9 (28.13 %) from females. All the age groups except 90-99 years age group had at least one species present. *P. mirabilis* being the highest with 59.38% (Figure 1) that could be detected among all the age groups (Table 2) except <1 years old and 90-99years old age groups. *P. vulgaris* accounted for 34.37 % of the *Proteus* isolates and was present in all the age groups except 1-9 years, 80-89 years and 90 - 99 years age group. *P. penneri* (6.25 %) was absent in samples obtained from < 1years, 1-9 years, 10-19 years, 30-39 years, 50 - 59 and 90 - 99 years age groups. Wound samples contributed the highest percentage of *Proteus* followed by urine.

Antimicrobial Susceptibility of the *Proteus* Isolates

The *Proteus* isolates recovered were highly

susceptible to Cefotaxime, Ofloxacin, Gentamycin, Amikacin, Lomefloxacin, Ciprofloxacin and Cefaperazone . However, 53.12% of *Proteus* isolates exhibited resistance to ampicillin, 28.12% to Netilline and 18.76% each Cefuroxime and Pefloxacin(Figure -2) Species identification and surveillance of antimicrobial resistance is essential in management and control of infections. These practices are usually absent in most of our hospitals mainly due to the high costs involved. Three *Proteus* species (*P. mirabilis*, *P. vulgaris* and *P. penneri*) were identified to be responsible for causing infections in various anatomical sites. *P. mirabilis* was the most common species isolated, accounting for 59.38 % of all the infections and hence responsible for the majority of *Proteus* infections. This result agrees with similar studies conducted in England, Wales and Northern Ireland [Chow, 1979; Jones, 2003].

Table.1 Distribution of *Proteus* Species among In-patient and Out-patient in Relation to Specimen Type

Samples	<i>Proteus</i> species	In- patients	Out-patients	Total no. of Species	Total no. of Isolates
Pus	Pm	9	3	12	19
	Pv	4	2	6	
	Pp	0	1	1	
Urine	Pm	2	1	3	8
	Pv	3	1	4	
	Pp	0	1	1	
Sputum	Pm	0	1	1	1
	Pv	0	0	0	
	Pp	0	0	0	
Ear swab	Pm	1	1	2	2
	Pv	0	0	0	
	Pp	0	0	0	
Blood	Pm	0	1	1	2
	Pv	1	0	1	
	Pp	0	0	0	
		20/32(62.50%)	12/32(37.50%)		32

Fig.1 Species Distribution

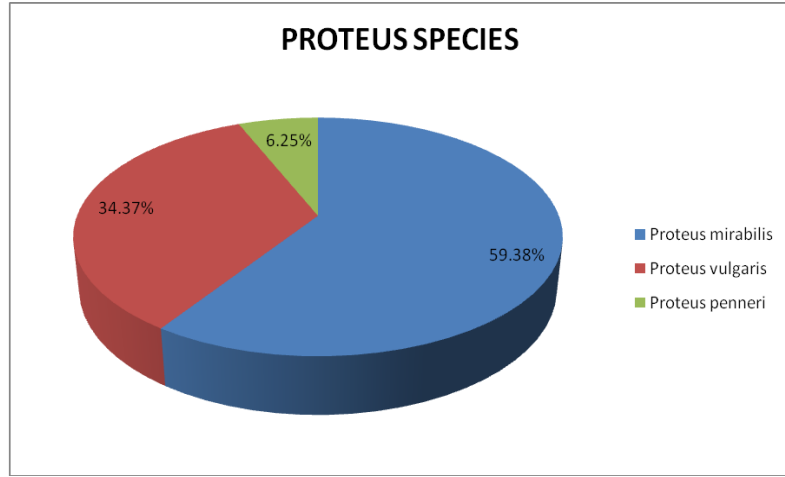
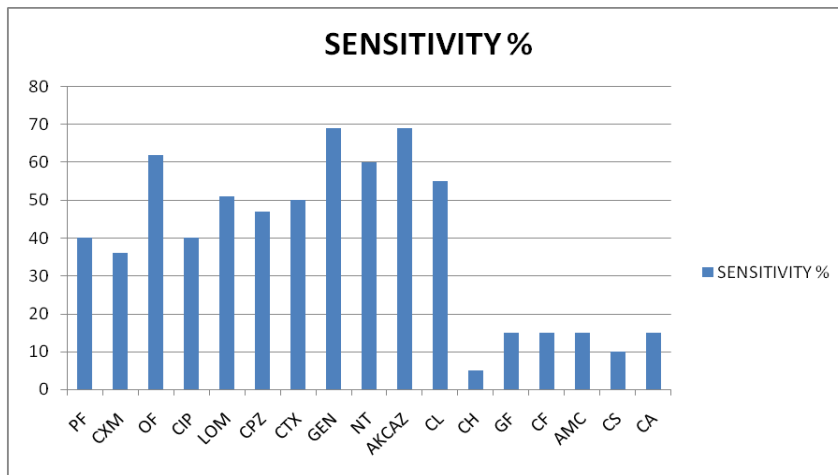


Fig.2 Showing Antibiotic Sensitivity Pattern of Proteus Mirabilis



Wounds recorded the highest percentage of *Proteus* isolates (67.85 %) followed by urine (19.64%). Our findings thus partially supports the findings of those from Europe and Asia; [Reslinski, 2005; Chung, 1999] which showed *Proteus* species to be more commonly encountered in urine than in other clinical specimens. *P. mirabilis* has a higher propensity for colonizing the urinary tract due to difference in its pathogenicity (Mobley, 1994). *Proteus* infections were also common among the in-patients (62.50 %) as compared to out-patients (37.50 %). Out of the 32 clinical specimens from which *Proteus* was recovered, 23 (71.87 %) were

collected from males and 9 (28.13 %) from females. The study showed a significant difference between the males and females infected with *Proteus*. The *Proteus* infections were detected in all age groups from <1 to 99 years where 60-69 years age group registering as the highest group infected (23.21 %). The *Proteus* species isolated were found to have high antimicrobial resistance against third generation of Cephalosporin antibiotics. All the *Proteus* species were highly sensitive to Cefotaxime, Ciprofloxacin, Lomefloxacin, Cefoperazone, Cefuroxime, Ofloxacin, Ceftazidime, Gentamycin,

Netilline and Amikacin.

In conclusion, the high antibiotic resistance of *Proteus* may be an indication of the resistance levels among the enterobacteriaceae and perhaps salmonellae since indiscriminate ingestion of antibiotics provides selective pressure, leading to a higher prevalence of resistant bacteria (Levy, 1999) which is very common in developing countries like India. Not only are these species potential causes of infections but also potential reservoirs of resistance genes that could be transferred to other bacterial pathogens.

P. mirabilis, *P. vulgaris* and *P. penneri* are the species implicated in *Proteus* infections; wounds recorded the highest incidence of *Proteus* infection at NMCH Patna, Bihar. The species were susceptible to Cefotaxime, Ofloxacin, Gentamycin, Amikacin, Lomefloxacin, Ciprofloxacin and Cefaperazone. They were, however resistant to ampicillin, Netilline and Cefuroxime and Pefloxacin and hence these must not form part of the empirical antibiotics for the treatment of *Proteus* infections at NMCH Hospital.

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