



“SPECIATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF TRIBE PROTEEAEE ISOLATED FROM VARIOUS CLINICAL SPECIMEN IN GOVT MEDICAL COLLEGE, KOTA (RAJASTHAN)”

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ABSTRACT **Background:** Proteus species is commonly associated with hospital as well as community acquired infections. The increasing resistance of *Proteus* spp to commonly used antibiotics with sensitivity only to reserve drugs such as Imipenem, Ceftazidime-Clavulanic acid, Piperacillin-Tazobactam is one of the most challenging tasks which is faced in clinical practice. **Aim and objectives:** The aim of this study was to detect antimicrobial susceptibility pattern spectrum in *Proteus* species isolated from various clinical specimens at Govt. Medical College & A.G. of Hospitals, Kota. **Material and methods:** A total of 100 non-duplicate *Proteus* species obtained from various clinical samples like urine, blood, pus, sputum, endotracheal aspirate and body fluids (pleural, ascitic, peritoneal and CSF) etc. were taken for the study from November 2020 to October 2021 and identification was done as per the standard biochemical identification methods. Antimicrobial susceptibility was performed by Kirby–Bauer disc diffusion method. **Result:** Three *Proteus* species isolated were: *Proteus mirabilis* 63% (63/100), *Proteus vulgaris* 34% (34/100), and *Proteus penneri* 5% (5/100). *Proteus* species was most commonly isolated from pus (53%) followed by urine (28%), ear swab (7%), blood (5%), vaginal swab(4%) and sputum (3%) respectively. Maximum prevalence was seen in 31-45 years age group (25%) and minimum prevalence in >75 years age group (5%). Males were found to be more vulnerable than females in acquiring Proteus infections. Out of 100 *Proteus* isolates 69% were isolated from IPD cases and 31% were from OPD cases. **Conclusion:** This study highlighted the increased prevalence of *Proteus mirabilis* when compared to *Proteus vulgaris* and *Proteus penneri*. The species *Proteus penneri* was more drug resistant when compared to *Proteus mirabilis* and *Proteus vulgaris*. Piperacillin tazobactam and Imipenem were effective in treating the resistant *Proteus* species to prevent unnecessary use of antimicrobial agents, increasing drug resistance and long duration of hospital stay.

KEYWORDS : Speciation, Antimicrobial susceptibility, *P.mirabilis*, *P.vulgaris* and *P.penneri*.

INTRODUCTION:

The organisms of the *Proteus* group have been known since the earliest days of bacteriology. They are widely distributed in nature and constitute an important part of the flora of decomposing organic matter of animal origin.^[1] They are part of the colonic flora of a broad range of mammals, birds, fish and reptiles with wide distribution in soil, manure and polluted water.^[2] They are opportunistic pathogens mainly associated with urinary, skin and soft tissue infections. *Proteus* species are commonly associated with nosocomial infections.^[3]

The genus *Proteus* belong to the family *Enterobacteriaceae* and is placed under the tribe *Proteaeae* which also includes *Morganella* and *Providentia*. Members of this genus are pleomorphic, motile, aerobic and facultative anaerobic, Gram negative bacilli and include 5 named species *P. mirabilis*, *P.vulgaris*, *P.myxofaciens*, *Phauseri*, *P.penneri*, and 3 unnamed genomospecies.^[4] The distinctive feature of many of the *Proteus* species is swarming on blood agar.^[2] *Proteus* infections rank as third cause of health care associated infections and the reported prevalence worldwide is between 9.8% and 14.6%.^[5] *P.mirabilis* causes 90% of all *Proteus* infections which is both community and hospital acquired, whereas *P.vulgaris* and *P.penneri* are isolated largely from various hospitalised individuals who are having an underlying disease.^[6] In the situation of long-term catheterization the prevalence of *Proteus* UTI is 20–45% and patients may later on develop catheter obstruction, renal and bladder calculi formation, local genitourinary infections, fistula formation, incontinence and bladder cancer.^[7] *Proteus* species also accounts for around 10% of wound infections and causes significant morbidity and mortality.^[8]

Indiscriminate intake of antibiotics in the health care settings provides selective pressure leading to higher prevalence of resistant bacteria which is very common in developing countries like India. These species are potential reservoirs of resistant genes that might possibly be transferred to other bacterial pathogens.^[9]

This study was done to isolate and speciate tribe *Proteaeae* obtained from various clinical specimen in the Department of Microbiology, Government medical college and Associated group of Hospitals, Kota and To assess the antibiotic sensitivity pattern.

MATERIAL AND METHOD:

Specimen Collection

A total of 100 non-duplicate *Proteus* species obtained from various clinical samples like urine, blood, pus, sputum, endotracheal aspirate and body fluids (pleural, ascitic, peritoneal and CSF) etc. of patients attending out-patient department or inpatient department of all the associated group of hospitals (MBS Hospital, NHMC and JK Lon Hospital) were taken for the study and identification was done as per the standard biochemical identification methods.

Cultivation and Identification

All specimens were processed according to standard operating procedure of laboratory. Organisms were grown on nutrient agar, blood agar and mac conkey agar and were identified by their Motility testing by hanging drop method, Colony characteristics on solid media, Gram's staining of the isolated colonies and Battery of biochemical reactions including: Catalase test, oxidase test, indole production, methyl red, Voges-Proskauer test, citrate utilization test, urease test, triple sugar iron (TSI) test, phenylalanine deaminase test, decarboxylation of amino acids, sugar fermentation test.^[10,11,12,13]

Antimicrobial Susceptibility Test

All the identified proteus isolates were then subjected to antibiotic susceptibility testing by Kirby Bauer disk diffusion method according to CLSI guidelines using Mueller-Hinton agar medium and commercial antibiotic discs (HIMEDIA).^[14] The antimicrobial agents used were: Ampicillin(10), Amoxicillin clavulanate(20/10), Ceftriaxone (30), Ceftazidime (30), Aztreonam(30), Cefepime (30), Gentamicin (10), Amikacin (30), Imipenem (10), Ciprofloxacin (5), Piperacillin (100), Piperacillin-Tazobactam (100/10). 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:

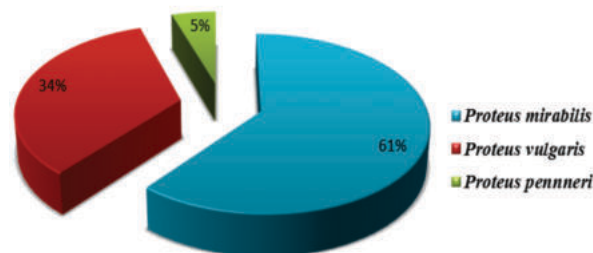
During the study period of 1 year from November 2020 to October 2021, a total of 100 proteus species were isolated from various clinical samples of patients from OPD and IPD of all the associated group of hospitals (MBS Hospital, NMCH and JK Lon Hospital). *Proteus* species were most commonly isolated from pus (44%) followed by urine (28%), ear swab (07%), blood (05%), vaginal swab(4%) and sputum (3%) respectively.

Table No: 1 Specimen wise distribution of the *Proteus* species

S. No	Specimen	P. mirabilis	P. vulgaris	P. penneri	Total	Percentage
1.	Pus	28	21	4	53	53%
2.	Urine	19	8	1	28	28%
3.	Ear Swab	5	2	-	7	7%
4.	Blood	4	1	-	5	5%
5.	Vaginal Swab	3	1	-	4	4%
6.	Sputum	2	1	-	3	3%
7.	Total	61	34	05	100	100%

The most common *Proteus* species isolated was *Proteus mirabilis*(61%) followed by *Proteus vulgaris*(24%) and *Proteus penneri* (5%).

Species wise distribution



Out of 100 *Proteus* isolates, 25 were between the age group of 31 to 45 years, 24 were between the age group of 16 to 30 years and around 22 between the age group of 46 to 75. Maximum prevalence was seen in 31-45 years age group (25%) and minimum prevalence in >75 years age group (5%). Mean age of study group was 43.96 years.

Maximum prevalence of *P.mirabilis* was in 31-45 years age group (16%) and minimum prevalence in >76 years age group (4%). Maximum prevalence of *P.vulgaris* was in 16-30 years age group (11%) and minimum prevalence in 0-15 years & >76 years age group (1 %). Maximum prevalence of *P.penneri* was in 31-45 years age groups (2%) and no *P.penneri* isolate was found in 0-15 years & >76 years age group.

Out of 100 *Proteus* isolates, 59 were males and 41 were females.

Table No: 2 Age and Sex wise distribution

Age (Years)	Male	Female	Total
0-15	5	01	6
16-30	11	13	24
31-45	16	09	25
46-60	10	12	22
61-75	13	05	18
>75	4	01	05
Total	59	41	100

Out of 100 *Proteus* isolates 69% were isolated from IPD cases and 31% were from OPD cases. 61% *P.mirabilis* were isolated from inpatient and 21% were from outpatient. 24% *P.vulgaris* were isolated from inpatient and 10% were from outpatient. 5% *P.penneri* were isolated from inpatient and none were from outpatient.

The rate of isolation of the *Proteus* species was highest from the Urology ward (19%) followed by Burn ward(15%), Surgery ward (12%), Ortho(10%), ENT and medicine(4%), O&G(3%) and Pediatrics(2%).

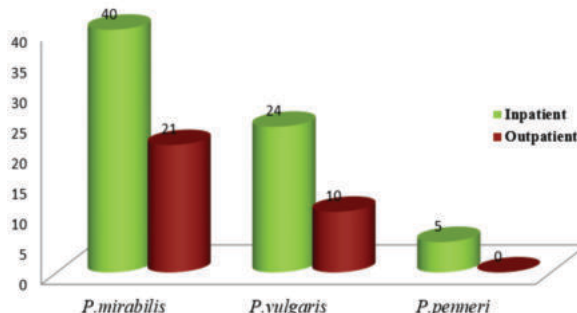
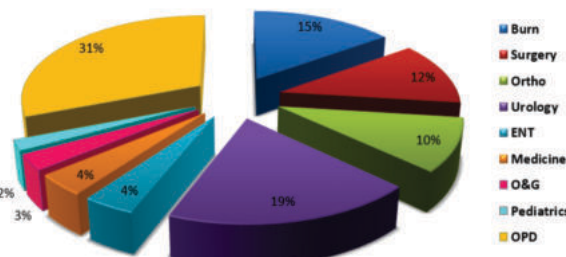


Table 3: Ward & OPD wise distribution of *Proteus* isolates

S.n	Ward	Total	Percentage
1.	Burn	15	15%
2.	Surgery	12	12%
3.	Ortho	10	10%
4.	Urology	19	19%
5.	ENT	04	04%
6.	Medicine	04	04%
7.	O&G	03	03%
8.	Pediatrics	02	02%
9.	OPD	31	31%
10.	Total	100	100%

Ward & OPD wise distribution of *Proteus* isolates



The *Proteus* isolates were most susceptible to Imipenem (94%) and Piperacillin Tazobactam(90%).

Table 4: Antibiotic susceptibility pattern of *Proteus* isolates

S. No.	Antibiotics	Number(n=100)	Percentage(%)
1.	Ampicillin(10)	39	39
2.	Amoxyclav(20/10)	52	52
3.	Ceftriaxone (30)	32	32
4.	Ceftazidime (30)	32	32
5.	Aztreonam (30)	38	38
6.	Cefepime (30)	44	44
7.	Gentamicin (10)	40	40
8.	Amikacin (30)	33	33
9.	Imipenem (10)	94	94
10.	Ciprofloxacin (5)	36	36
11.	Piperacillin (100)	27	27
12.	Piperacillin-Tazobactam (100/10)	92	92

CONCLUSION:

In conclusion, this study highlighted the increased prevalence of *Proteus mirabilis* when compared to *Proteus vulgaris* and *Proteus penneri*. The species *Proteus penneri* was more drug resistant when compared to *Proteus mirabilis* and *Proteus vulgaris*. The antibiotic resistant pattern of *Proteus* may be an indication of the resistant levels among the Enterobacteriaceae and provides selective pressure, may lead to higher level prevalence of resistant bacteria and could serve as potential reservoir of resistant genes^[15]

Species identification, surveillance and study of the epidemiology of antimicrobial resistance will assist in the therapeutic management of patients by reducing the prescription of large spectrum antibiotics control of infections.

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