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Antibiotic Resistance And Prevalence Of The Tribe Proteeae (Proteus Species, Morganella Morganii, Providencia Species) In Various Age Groups: A Three-Year Review.

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Abstract:
The aim of this retrospective study was to determine the prevalence of the tribe Proteeae, which includes the genera Proteus, Morganella, and Providencia species, in various clinical specimens and to assess their antimicrobial susceptibility patterns in Delhi during January 2020 to December 2022. The tribe Proteeae comprises a group of bacteria within the family Enterobacterales and is responsible for most cases of nosocomial infections in hospital settings. Specimens were collected and screened for Proteeae using the current MALDI-TOF methods for the identification of species within the three genera.
Out of 141,526 positive bacterial growth samples, 3,973 (2.8%) were identified as members of the tribe Proteeae, of which 2,643 (66.5%) were from urine, 658 (16.5%) from pus, 222 (5.6%) from fluid, 183 (4.6%) from respiratory samples, 32 (0.8%) from genital samples, and 20 (0.5%) from blood samples. Speciation of the Proteeae isolates revealed that 3,058 (76.9%) were Proteus mirabilis, 37 (0.9%) were Proteus vulgaris, 709 (17.8%) were Morganella morganii, 134 (3.4%) were Providencia rettgeri, and 35 (0.8%) were Providencia stuartii.
In total, 3,973 patients were identified from whom Proteeae isolates were obtained, with the elderly population being at the highest risk. Proteus mirabilis generally exhibited susceptibility. Drugs such as Piperacillin/tazobactam, Cefoperazone/sulbactam, Ertapenem, Meropenem, and Amikacin were used to treat the Proteeae group of organisms in this region. However, Ampicillin, Amoxiclav, Cephalosporins, Quinolones, Imipenem, and TM/SXT exhibited a high level of resistance, which was observed in various samples.

Keywords: *Proteeae(Proteus spp., Providencia spp., and Morganella morganii)*

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I. Introduction:

Discussing the taxonomy and nomenclature of the genera *Proteus, Providencia,* and *Morganellamorganii* independently can be quite challenging. Historically, these three genera belonged to the tribe*Proteeae* within the *Enterobacterales* family and are significant opportunistic pathogens capable of causing various nosocomial infections. The tribe designation is not often used; however, as a sake of convenience, this term used in this study. *Proteeae* are widespread in the environment and constitute a part of the normal flora in the human gastrointestinal tract. They possess the ability to oxidatively deaminate a broad range of amino acids, especially phenylalanine, and are responsible for most nosocomial infections in hospital settings, as indicated by several references [3, 9, 11].

These infections, resulting from *Proteus mirabilis'* unique capacity to form crystalline biofilms, can ultimately lead to catheter surface crust formation and obstruction. This obstruction may result in urinary retention, reflux, and potentially life-threatening complications such as sepsis and septic shock if the infection ascends, causing cystitis and pyelonephritis [2, 4, 13]. Furthermore, the removal of the crystalline catheter may cause damage to the urethra and bladder mucosa.

Although Escherichia coli is the most common cause of uncomplicated cystitis, pyelonephritis, and prostatitis, Proteus mirabilis ranks fourth in terms of causing these infections. This bacterium was initially

described in 1885 by German microbiologist Gustav Hauser, who observed its ability to swarm on solid surfaces [3, 9].

Morganella morganii and *Providencia spp.*, despite their wide distribution, are infrequent causes of community-acquired infections. They are considered non-negligible opportunistic nosocomial pathogens due to increased resistance levels and virulence, primarily leading to post-operative wound and urinary tract infections [4]. In some cases, these bacteria result in a high mortality rate among patients. It is widely acknowledged that *P. mirabilis* displays the most favourable resistance patterns within the *Proteeae* group, while *P. vulgaris*, *Providencia species*, and *Morganella species* exhibit somewhat higher resistance levels. However, their inherent resistance too many antibiotics, such as Beta-lactam antibiotics, Tetracyclines, Colistin, and Nitrofurantoin, severely limits therapeutic alternatives [4].

The retrospective study's objective was to evaluate the prevalence of different genera within the *Proteeae* tribe, namely Proteus, Morganella, and Providencia species, and to focus on antibiotic resistance levels among various clinical isolates over a three-year period at *Dr. Lal Path Labs.*

II. Materials and Methods:

A retrospective study conducted in 3973 (2.8%) patients attending the *Dr Lal path lab*, NRL, Delhi,a tertiary health care Lab which providing a full range of test facilities. The study was carried out from January 2020 to December 2022. Specimens were plated on cysteine lactose-electrolyte-deficient (CLED) agar, CHROMagar and blood agar. All cultured plates were observed for colonial morphology of the bacteria such as sizeon agar, blood agar plates were used to detect swarming and hemolysis and the characteristic smell of *Proteeae* isolates was also noted. The inoculated plates incubated at 37°C for 24 hrs and those cultures that becomes negative at the end of 24 hours incubations were further incubate for 48 hours.

Bacterial identification was made using MALDI-TOF and antimicrobial susceptibility was evaluated by VITEK® 2 with respective susceptibility cards (AST 405, BioMerieux, India) as per as CLSI M100-S-32[15]. The following antibiotics were used for the isolates, Ampicillin (AMP), Amoxycillin/clavulanic acid (Amoxyclav), Piperacillin/Tazobactam (TZP), Cefepime, Ceftriaxone (CFX), Cefoperazone sulbactam, Ciprofloxacin (CIP), Amikacin (AMK), Gentamicin (GEN), Ertapenem, Imipenem(IMP),Meropenem (MRP), Trimethoprim/Sulphomethoxazole(TM/SXT) and Fosfomycin (FOS). Standard strains of *E. coli* (ATCC 25922) were used routinely in this study as control. No data collected on the clinical background of the patients.

Statistical analysis: The analysis done using the statistical software package Myla (Biomerieux). Age, organisms causing various infections, their antibiotic susceptibility and resistance with MIC were included as variables in this study.

III. Results:

A laboratory based retrospective study design was used to conduct this study from 1st of January 2020–31st of December 2022 at *Dr Lal Path labs*, Delhi. The present study describes the distribution and antimicrobial susceptibility of *Proteeae* group of organism isolated from a large number of clinical samples collected over a 36 month period, as part of routine analyses, from unselected community patients (male and female of any age and clinical condition) living in the north India. During 3 year the Microbiology departmentidentified 1, 41,526 positive bacterial growth, of which 3973 (2.8%) were identified as members of *Proteeae* of which 2643 (66.5%) were from urine, 658 (16.5%) from Pus, 222 (5.6%) from fluid, 183(4.6%) from respiratory, 32(0.8%) from Genital, 20 (0.5%) from blood respectively [Table 1].

Table 1: Frequency of isolation of *Proteeae* species from clinical specimens.

Source	Total Number of *Proteeae* isolates (%)	*Proteeae* isolates identified		
		Proteus spp. *N=3095 (77.9%)*	*Morganella morganii* *N=709 (17.8%)*	*Providencia spp.* *N=169 (4.2%)*
Blood	**20 (0.5)**	**8**	**6**	**6**
Pus	**658 (16.5)**	**491**	**141**	**26**
Fluid	**222 (5.6)**	**168**	**36**	**18**
Urine	**2643 (66.5)**	**2130**	**430**	**83**
Respiratory	**183 (4.6)**	**134**	**28**	**21**
Genital	**32 (0.8)**	**28**	**4**	**0**
Other	**215 (5.4)**	**136**	**64**	**15**

In this study, Urinary tract infections are mainly caused by *Proteeae* group of organisms. Out of 709 isolates of *Morganella morganii* accounted for most of these isolates were isolated from Urine, followed by Pus, other sample [Table 1]. The maximum number of at the same time, there have only been 169 cases of

Providencia spp. (i.e., *P. rettgeri* and *P. stuartii*) can cause clinical infections in the Urine followed by pus, respiratory and fluid, such cases are rare and considered opportunistic.

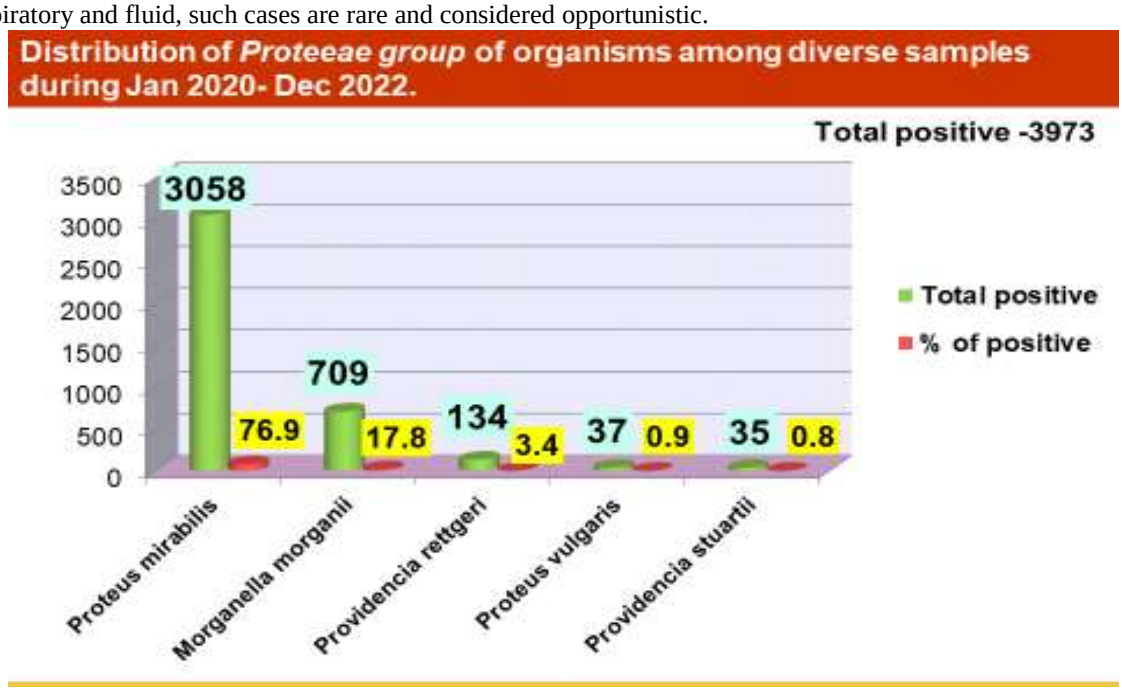

Figure 1: The most predominant *Proteeae* group of organisms.

3973 *Proteeae* group of organismisolated from various clinical samples, and the infection rate was estimated to be 2.8%. 3 genera were identified, Among these, *Proteus mirabilis* was the commonest 3058 (76.9%) species isolated, followed by *Morganella morganii* 709 (17.8%) and *Providencia spp*. (169; 4.2%) are isolated much less frequently and account for less than 5% of clinical isolates [Fig 1].

Table 2: Distribution of *Proteeae*group of organisms by age of patient.

Age	Total No. of isolatesN= 3973 (%)	*Proteus species N=3095 (77.9%)*	*Morganella morganii N=709 (17.8%)*	*Providencia species N=169 (4.2%)*
0-12	429(10.8)	353	62	14
13-35	579 (14.6)	478	101	0
36-50	449 (11.3)	369	80	0
51-65	802 (20.2)	618	160	24
>=65	1714 (43.1)	1277	306	131

The Distribution of *Proteeae* isolates by age in relation is shown in Table2. Out of the 3973 positive isolates, The highest susceptible age group of *Proteeae* group of organismsinfected irrespective of gender was found to be >50 years (63.3%) followed by ≤13-35 (14.6%), ≤ 36-50 (11.3%), and then 0-12 years (10.8%) respectively (Table 2).

2159 (54.3%), of the *Proteeae* group of organism were isolated from the males, and 1814 (45.6%) were isolated from female patients. Compared with other species, *Proteus mirabilis* occurred at a much higher frequency, especially among males.

Table3:Cumulative interpretation of antimicrobial susceptibility (%) with MIC 50/90 among *Proteeae* group of organisms in diverse samples.

Cumulative interpretation of antimicrobial susceptibility (%) among *Proteeae group* of organisms Isolates with cumulative MIC (50/90) in diverse samples during Jan 2020- Dec 2022									
Antibiotics	*Morganella morganii* N=709			*Proteus species* N=3095			*Providencia species* N=169		
	S%	R%	MIC 50/90	S%	R%	MIC 50/90	S%	R%	MIC 50/90
Ampicillin	7.8	92.2	32/32	30	70	32/32	3.4	96.6	32/32
Amoxyclav	8.5	91.5	32/32	58	42	8/32	4.5	95.5	32/32
Piperacillin/ tazobactum	91.7	8.3	<=4/8	94	6	<=4/8	75.3	24.7	<=4/128
Cefepime	80	20	<=1/16	69.4	30.6	1/16	75	25	1/32
Ceftriazone	65	35	1/64	63.3	36.7	1/64	55.2	44.8	1/64
Cefoperazone/ sulbactam	89.3	10.7	<=8/32	90.9	9.1	<=8/16	78.3	21.7	<=8/64
Ertapenem	96.5	3.5	0.5/0.5	97.5	2.5	0.5/1	67.5	32.5	0.5/8
Imepenem	34.5	65.5	2/8	48	52	2/8	61.9	38.1	1/16
Meropenem	90.3	9.7	<=0.25/1	91.1	8.9	<=0.25/1	78.9	21.1	<=0.25/16
Amikacin	91.8	8.2	2/16	87.2	12.8	2/32	72.6	27.4	2/64
Gentamicin	71.2	28.8	<=1/16	62.9	37.1	<=1/16	56.1	43.9	<=1/16
Ciprofloxacin	18.8	81.2	4/4	26.8	73.2	4/4	38.2	61.8	2/4
TM/SXT	48.2	51.8	160/320	45.1	54.9	320/320	59.9	40.1	<=20/320
Fosfomycin	26	74	256/256	74.6	25.4	<=16/256	74.2	25.8	32/256

Tested antibiotics against *Proteus species*: Out of 3095 (77.9%) tested isolates of *Proteus species*were least sensitive to the Ampicillin, Ciprofloxacin, Imepenem and Trimethoprim Sulphomethoxazole (TM/SXT) (Table.3). 50% isolates having MIC 32µg/ml, 4µg/ml, 2µg/ml, 320 µg/ml, and 90% of isolates was having MIC 32µg/ml, 4µg/ml8µg/ml and 320 µg/ml against Ampicillin, Ciprofloxacin, Imepenem and Trimethoprim Sulphomethoxazole (TM/SXT) respectively. Amoxyclav were sensitive to 58% of the infected individuals and resistant to the remaining. Cefepime, Ceftriazone and Gentamicin found to be sensitive for <70% of the *Proteus species* infected individuals.

Among the systemically active antimicrobial agents, Piperacillin/tazobactum,Cefoperazone/sulbactam, Meropenem, Ertapenem andAmikacin appear to be the most active against this important pathogen.

Tested antibiotics against *Morganella morganii*: In this study *Morganella morganii* were recorded high resistance rate (92.2 and 91.5%) against Ampicillin and Amoxyclav (MIC50/90 32/32) demonstrated that 50 % and 90% of isolates were within 32 µg/ml (Table 3) because of their intrinsic non-susceptibility to these antibiotics.The least susceptible and highly resistant drugs were, Ciprofloxacin, Imepenem and Fosfomycin. Ninety percent of *Morganella morganii* isolates was tested against Ciprofloxacin, Imepenem and Fosfomycin having MIC 4µg/ml, 8µg/ml and 256µg/mlrespectively. Gentamicin and Ceftriaxonewas sensitive against <=70% of the infected individuals.

Ertapenemfound to be sensitive against 96.5% of the *Morganella morganii* infected individuals followed by Amikacin, Piperacillin/tazobactumand Meropenem having ≥ 90%sensitivity, Cefoperazone/sulbactam, susceptible against ≤ 90% whereas 80% of the *Morganella morganii*infected individuals was sensitive to Cefepime with MIC <=1µg/ml.

Tested antibiotics against *Providencia species*: In this study *Providencia species* were recorded high resistance rate (96.6 and 95.5%) against Ampicillin and Amoxyclav (MIC50/90 32/32) demonstrated that 50 % and 90% of isolates were within 32µg/ml because of their intrinsic non-susceptibility to these antibiotics. Ceftriaxone, Gentamicin and TM/SXT having <60% susceptibility with (MIC50/90) demonstrated that 50% of isolates were within 1µg/ml, <=1µg/ml, <=20µg/ml MIC and 90% isolates were within 64µg/ml, 16µg/ml and 320µg/ml respectively(Table 3).

Most of the drugs showed reduce susceptibility in *Providencia species* includingPiperacillin/tazobactam, Cefoperazone sulbactam, Cefepime and Meropenem found to be sensitive <=80% of the *Providencia species* infected individuals followed by Fosfomycin,Amikacin,Ertapenem and

Imepenem groups having <75% sensitivity, whereas least susceptible drugs were Ciprofloxacin (38.2%) demonstrated that 50% were within 2µg/ml and 90% isolates were within 4µg/ml, respectively.

In this study, Amikacin, Piperacillin/tazobactum, Cefoperazone/sulbactam, Meropenem, and Ertapenem, appear to be the most active antimicrobial agents were recordedagainst *Proteeae* group of organism.WhileCefepime, Ceftriaxone, and Gentamicinshowed reduce susceptibility <=75% against these organisms except *Providencia* species. Meanwhile the high resistance rate were recorded to Ampicillin,Amoxyclav, Ciprofloxacin, Imepenem and Trimethoprim Sulphomethoxazole (TM/SXT) in tribe *Proteae.*

IV. Discussion:

In light of the findings from our retrospective study, it becomes evident that the *Proteeae* group of organisms constitutes a significant pathogenic threat affecting individuals of all age groups, with an estimated prevalence of 2.8%. Our study aligns with the findings of other studies [3, 4]. To the best of our knowledge, this is the first investigation to identify the emergence of Multidrug-resistant *Proteeae* within Delhi, North India. Nevertheless, research conducted both domestically and internationally has demonstrated substantial variation in species distribution and susceptibility patterns of pathogens across different regions. Therefore, it is imperative to advocate for continuous and rigorous surveillance efforts [4, 6, 8, 9, 11, 13].

Global reports indicate that *Proteus mirabilis* ranks as the fourth most common pathogen in urinary tract infections (UTIs), with *Proteus spp.* being the predominant bacteria isolated in cases involving bladder and kidney stones (accounting for 70% of cases). However, complex UTIs, especially those caused by *Proteus mirabilis*, present increasingly challenging medical scenarios [4]. In our study, the isolation rate of *Proteus mirabilis* exceeded that of other *Proteeae* members, a trend consistent with results reported in other studies [2, 4, 6, 7, 8, 13]. Most studies concur with our findings, identifying *Proteeae* as the most prevalent uropathogens, particularly among males in the community [2, 3, 14]. Notably, our study reveals a higher incidence of *Proteeae* infections in elderly adults, a conclusion shared with studies conducted in South India and abroad [2, 4].

The isolation rate of *Proteeae* group organisms was highest in urine samples (66.5%), followed by pus samples (16.5%), mirroring similar findings in other studies [4, 7, 11, 14]. However, some studies have reported a higher prevalence of isolation from pus samples compared to other clinical specimens in both Indian and international contexts [2, 8, 10]. Thus, our results, in conjunction with previous studies, underscore the influence of geographic location and the choice of clinical samples on the isolation rate [2, 4, 6-8, 10, 11].

Our study reports the highest resistance to Ampicillin and Amoxyclav among the *Proteeae* group, including *Proteus species*, *Morganella morganii*, and *Providencia species* isolates, consistent with findings from India and abroad, where Ampicillin and Amoxyclav resistance rates ranged from 70% to 100% [6, 10, 13, 14]. Moreover, our results indicate an alarming rise in third-generation and fourth-generation Cephalosporin resistance (20-45%) among Proteeae species, a trend supported by studies conducted in India and abroad [4, 6, 10, 11, 14], although some reports indicate even higher Cephalosporin resistance rates (80-100%) [1, 10]. Given the escalating Cephalosporin resistance, prudent antibiotic stewardship coupled with targeted antibiotic therapy is essential for managing these frequently encountered infections.

Furthermore, our study reveals decreased susceptibility to Quinolones, Gentamicin, and TM/SXT compared to previous years [6, 8, 14]. Overall, our findings align with previous studies conducted in various regions, which have reported Ciprofloxacin and TM/SXT resistance rates as high as 70% and 80-90%, respectively [1, 10]. However, some studies have observed higher susceptibility to Quinolones [8, 11, 13]. Notably, our study shows a *Proteeae* resistance rate of 8-28% to Amikacin, consistent with other studies [1, 6, 10, 12], although some reports suggest higher resistance to Amikacin [11], indicating a potential concern for increased Amikacin usage in Delhi.

In our study, Meropenem-resistant strains were detected at a rate of 8-9.7%, suggesting that Meropenem may still be a viable option for treating Proteus species and *Morganella morganii* infections, in line with another study [7]. However, it is important to note that Imipenem exhibits only marginal effectiveness against *Proteeae* [5]. Our study highlights that over 50% of *Morganella morganii* and *Proteus species* isolates from diverse samples were resistant to Imipenem, consistent with findings from studies conducted abroad [5]. In contrast, some studies have reported Proteus isolates as highly sensitive to Imipenem [1, 10, 11, 13].

The emergence of Multidrug-resistant (MDR) Proteeae species is a significant concern, as they may potentially share their resistance genes with other bacterial strains [5, 14]. Meanwhile, *Providencia* species exhibited resistance rates of 25-40% to major antibiotics, including Piperacillin/tazobactam, Cefoperazone sulbactam, Ertapenem, Meropenem, and Amikacin, contrasting with *Proteus* and *Morganella morganii*, which displayed susceptibility. These findings align with another study [6] and correspond to reports indicating greater resistance within the Penem group among *Providencia* compared to *Morganella* and *Proteus isolates* [4, 6, 9,

12]. This worrisome resistance pattern leaves us with limited therapeutic options for treating *Providencia species*infections and raises concerns about the potential development of extensively drug-resistant strains that could be more virulent than other *Proteeae*.

Higher resistance rates were observed for Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, and TM/SXT among the *Proteeae* group. The increasing trend in resistance among these isolates is a cause for concern in the future [4, 7, 11, 15]. Consequently, we conclude that these antibiotics are not suitable for treating infections caused by the *Proteeae* group in Delhi.

Based on our data, Piperacillin/tazobactam, Cefoperazone/sulbactam, Ertapenem, Meropenem, and Amikacin appear to be effective treatment options for clinical infections caused by *Morganella morganii* and *Proteus mirabilis* isolates in Delhi. These findings align with previous studies conducted in India and abroad [1, 4, 6, 7, 8, 10, 14].

Our local resistance results mirror the global trend of increasing drug resistance in *Proteeae* against various antimicrobial agents. This underscores the importance of continuous local surveillance to guide treatment recommendations and monitor trends over time. Such efforts are essential for assisting clinicians in making empirical medication selections. Nevertheless, we must acknowledge certain limitations of this retrospective study, including its design and the limited access to individual patient medical records and information on underlying illnesses, aside from age, inpatient/outpatient status, and catheterization.

V. Conclusion:

In summary, our study underscores the substantial concern posed by *Proteeae* group members, which are likely to become a significant challenge in the forthcoming years in North India due to the rising annual resistance rates to Ampicillin, Amoxyclav, Cephalosporins, Aminoglycosides, and TM/SXT. The emergence of multidrug-resistant (MDR) *Providencia species*, exhibiting increasing virulence compared to other *Proteeae*, has already begun to give rise to extensively drug-resistant (XDR) strains, leaving us with limited treatment options at our disposal. Consequently, further studies are imperative to better understand this evolving situation,todefine the epidemiology, risk factors and antimicrobial resistance patternwhich is essential for management and prevention of these *Proteeae* group in Delhi.

Ethical Approval: It is not applicable.

Conflicts of Interest: There are no conflicts of interest.

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