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Prevalence and Antimicrobial Resistance Patterns of Proteus Species Isolated from Clinical Specimens in a Tertiary Care Hospital Kanpur

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Abstract

Introduction:

Proteus species are opportunistic pathogens known to cause both community-acquired and healthcare-associated infections (HCAIs). The increasing incidence of antimicrobial resistance in these organisms poses a significant public health threat.

Aim:

To assess the prevalence and antibiotic resistance patterns of *Proteus* species isolated from various clinical specimens using conventional culture methods.

Materials and Methods:

This cross-sectional study was conducted between March 2024 and February 2024 at Rama Medical College Hospital and Research Centre, Mandana, Kanpur. *Proteus* isolates were obtained from clinical samples such as wound swabs, pus, urine, cerebrospinal fluid (CSF), tracheal swabs, endotracheal aspirates, vaginal swabs, blood, body fluids, ear swabs, and tissue. Phenotypic identification and antibiotic susceptibility testing were performed using standard culture techniques and disc diffusion methods in accordance with CLSI guidelines.

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Results:

Out of 100 *Proteus* isolates, the majority were recovered from pus (35%), wound swabs (22%), and urine samples (25%). A higher prevalence was observed in male patients (65%). *Proteus mirabilis* was the predominant species (89%). Among the isolates, 39% were confirmed as Extended Spectrum Beta-Lactamase (ESBL) producers, 20% were AmpC producers, and 1% was a carbapenemase producer. The highest antibiotic sensitivity was observed with piperacillin-tazobactam and tigecycline (100%), followed by meropenem and imipenem (98%).

Conclusion:

The study highlights a rising trend in antimicrobial resistance among *Proteus* species, primarily driven by ESBL, AmpC, and carbapenemase enzyme production. While initial screening tests are useful, confirmatory phenotypic testing is essential for accurate detection and effective management of resistance in clinical settings.

Keywords:

Antimicrobial resistance, non-lactose fermenter, *Proteus mirabilis*, *Proteus vulgaris*, swarming motility.

Introduction

Proteus species, members of the tribe Proteeae within the family Enterobacteriaceae, are pleomorphic, gram-negative bacilli commonly found in environmental reservoirs such as sewage and decaying organic matter (1). In humans, they are considered normal flora of the gastrointestinal tract but act as opportunistic pathogens when host defences are compromised. Clinically, *Proteus mirabilis* is the most frequently isolated species, associated predominantly

with urinary tract infections (UTIs), wound infections, empyema, bacteraemia, and complications such as renal calculi. *P. vulgaris* is typically encountered in immunocompromised individuals, particularly those with prolonged antibiotic exposure, while *P. penneri* is implicated in sporadic outbreaks of nosocomial infections. The prevalence of *Proteus*-related UTIs is notably high in chronically catheterized patients, with *P. mirabilis* accounting for up to 45% of such infections (2-3). Additionally, *Proteus* species have been isolated from a variety of clinical conditions, including otitis media, soft tissue infections, and sepsis. Their virulence is attributed to several factors, including swarming motility, urease production, biofilm formation, and proteolytic enzymes (4).

A significant concern in clinical microbiology is the increasing incidence of multidrug-resistant (MDR) *Proteus* strains. Resistance mechanisms include the production of Extended Spectrum Beta-Lactamases (ESBLs), AmpC β-lactamases, and carbapenemases (5). These enzymes contribute to treatment failure, longer hospital stays, and increased healthcare costs. While ESBLs confer resistance to third-generation cephalosporins, AmpC enzymes can hydrolyze a broader spectrum, and carbapenemases nullify the effectiveness of even last-resort antibiotics. Despite the clinical importance of *Proteus* species, data on their resistance mechanisms remain limited. This study seeks to fill that gap by examining the prevalence and resistance profiles of *Proteus* isolates from diverse clinical specimens, with an emphasis on phenotypic detection of key β-lactamase enzymes (6-7).

MATERIALS AND METHODS

Study Design and Setting

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A cross-sectional study was conducted from March 2024 to February 2024 at Rama Medical College Hospital and Research Centre, Mandana, Kanpur.

Inclusion Criteria

All non-duplicate clinical isolates of *Proteus* species obtained from various specimens including pus, sputum, urine, CSF, tracheal swabs, endotracheal aspirates, vaginal swabs, blood, body fluids, ear swabs, and tissue were included. Only samples collected prior to the initiation of antibiotic therapy were considered.

Exclusion Criteria

All isolates not identified as *Proteus* species were excluded from the study.

Isolation and Identification

Clinical samples were collected aseptically and processed using standard microbiological techniques. *Proteus* species were identified based on colony morphology—swarming on blood agar and non-lactose fermenting colonies on MacConkey agar—and further confirmed by biochemical tests. These included a positive phenylalanine deaminase (PPA) test, abundant hydrogen sulfide (H_2S) and gas production in Triple Sugar Iron (TSI) agar, and rapid urease activity. Indole production distinguished *P. vulgaris* (positive) from *P. mirabilis* (negative), while maltose fermentation and absence of ornithine decarboxylase were used to identify *P. penneri*.

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Antibiotic Susceptibility Testing (AST)

AST was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Zone sizes were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines. Antibiotics tested included:

- **Penicillins and β-lactam combinations**: ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam
- **Cephalosporins**: ceftazidime, cefepime, ceftriaxone, cefuroxime, cefotaxime, cefoxitin, cefazolin
- **Carbapenems**: meropenem, imipenem, ertapenem
- **Others**: gentamicin, amikacin, chloramphenicol, ciprofloxacin, tetracycline, tigecycline, nitrofurantoin

Phenotypic Confirmatory Testing

To confirm the presence of β-lactamase enzymes:

- **ESBL Detection**: The combined disc test using ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/20 µg) was employed. A ≥5 mm increase in the inhibition zone around the clavulanate combination disc indicated ESBL production.
- **AmpC Detection**: The modified three-dimensional test involved placing a cefoxitin disc on a lawn culture and introducing enzyme extract in a slit parallel to the disc. A characteristic “flattening” or indentation in the zone of inhibition suggested AmpC production.
- **Carbapenemase Detection**: The Modified Hodge Test (MHT) was performed using a meropenem disc in the centre of the agar plate inoculated with *E. coli* ATCC 25922. A

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cloverleaf-like indentation in the zone of inhibition along the test organism's streak indicated carbapenemase activity. *Klebsiella pneumoniae* ATCC 1705 served as the control strain.

Results

Age Group	Males	Females
0–15	4	0
16–30	2	2
31–45	9	6
46–60	16	18
61–75	30	9
>75	4	0

Table 1: Demographic Distribution by Age and Gender

The age-wise breakdown reveals a clear trend: the prevalence of *Proteus* infections increases significantly with age. The highest frequency is observed in the 61–75-year age group, contributing to 39% of all cases. This trend is not surprising. Age is a known risk factor for many infections, particularly in organisms like *Proteus*, which are opportunistic pathogens. Elderly patients are more likely to have comorbid conditions such as diabetes, chronic kidney disease, and prostatic hyperplasia, which predispose them to urinary tract infections (UTIs) and wound infections—two major sites from which Proteus was isolated in this study (7-10).

Moreover, older individuals often undergo surgical procedures or are subjected to prolonged catheterization, both of which are well-known risk factors for *Proteus* infections due to their nosocomial association (11). Also, immune senescence in the elderly—characterized by a

natural decline in immune system efficiency—can contribute to increased susceptibility to bacterial infections (12).

Interestingly, 65% of isolates were from males, and only 35% from females. The trend persisted across most age groups, especially in the 61–75 age range (30 males vs. 9 females). The only age group where females slightly outnumbered males was 46–60 years. Statistically, this difference in distribution between genders and age groups was found to be significant ($Chi^2 = 12.12$, $p = 0.033$). This means the association is not due to random variation but reflects a real difference.

There are multiple plausible explanations:

- Males, particularly elderly males, are more prone to complications like prostatic hypertrophy, which leads to urinary retention—a key factor in UTI pathogenesis (13).
- Anatomically, the female urinary tract is shorter and hence more prone to ascending infections in the general population. However, when considering hospital-acquired infections or infections among the elderly, this anatomical advantage is often neutralized by the presence of catheters and systemic immunosuppression.
- Males may have higher exposure to surgical interventions or trauma in certain settings (e.g., chronic non-healing wounds, diabetic ulcers), where Proteus is often isolated.

The age-gender association suggests that empirical treatment and diagnostic vigilance should be heightened in elderly males presenting with symptoms of UTI or wound infections. Since these patients are more likely to harbor *Proteus* spp., clinicians must consider this in their empirical antimicrobial selection. It also supports the need for antimicrobial stewardship targeting these demographics, including preventive strategies such as:

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- Reducing unnecessary catheter use
- Timely wound debridement
- Enhanced hygiene protocols in elder care facilities

Further, this demographic information is crucial for hospital infection control teams to target screening and preventive programs effectively. It may also indicate a need for specific protocols in geriatric wards and surgical units, where elderly male patients are admitted (14).

From a broader health systems standpoint, understanding demographic patterns allows for better resource allocation. Hospitals can prioritize diagnostics (e.g., urine cultures, AST) for high-risk groups, potentially reducing the burden of empiric broad-spectrum antibiotic use and mitigating antimicrobial resistance (AMR). In future studies, it would be beneficial to examine other risk factors within each age and gender group, such as diabetes, renal insufficiency, catheterization status, hospitalization duration, or immunosuppressive therapy. This can refine our understanding of how these demographic variables interact with other clinical factors in determining *Proteus* infection risk (15-18).

Sample Type	*P. mirabilis*	*P. vulgaris*
Pus	29	6
Urine	24	1
Wound swab	21	1
Blood	1	0
Ear swab	3	2
Tissue	9	1
Tracheal aspirate	2	0

Table 2: Sample Type vs. Proteus Species

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A significant highlight of this data is the dominance of *Proteus mirabilis*, which constituted 89% of total isolates in the study. Across all sample types—whether pus, urine, or wound swabs—*P. mirabilis* outnumbered *P. vulgaris* considerably. This prevalence aligns well with global literature. *P. mirabilis* is known to be the most common *Proteus* species involved in clinical infections, particularly UTIs and wound infections (19). It is a frequent colonizer of indwelling catheters and surgical wounds, due in part to its ability to form biofilms and its characteristic swarming motility which helps it colonize tissue and medical devices effectively.

Pus (35% of total isolates): The most common specimen yielding *Proteus* isolates. A total of 35 isolates were from pus, with *P. mirabilis* accounting for 29. This aligns with *P. mirabilis*' known pathogenic role in post-surgical wound infections, diabetic foot infections, and abscesses.

Urine (25%): Again, *P. mirabilis* (24/25) dominates. Its strong urease activity enables it to alkalinize the urine, facilitating the formation of struvite stones and biofilms on catheters—key reasons for its UTI prevalence (17).

Wound Swab (22%): The third highest source of isolates, showing similar trends. Swab cultures from infected wounds yielded predominantly *P. mirabilis* (21 out of 22).

Blood, Tissue, Tracheal Aspirates: These sterile-site specimens produced relatively fewer isolates, but still, *P. mirabilis* was more prevalent, supporting its capacity to cause invasive infections under favorable (or compromised host) conditions.

Ear Swabs: This was the only sample type where *P. vulgaris* had a notable presence (2 of 5 isolates). This could suggest a niche role for *P. vulgaris* in localized ENT infections, particularly in immunocompromised or pediatric populations.

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Despite the clear trend of *P. mirabilis* being dominant, the Chi-square test for independence did not reveal statistical significance ($Chi^2 = 8.21$, $p = 0.223$). This means the distribution of species across sample types could have occurred by chance based on the sample sizes used.

The low number of *P. vulgaris* isolates (only 11%) makes it statistically difficult to detect significant distributional differences. Some categories like blood and tracheal aspirate have very small counts, limiting the power of the test. More granular classification (e.g., segregating community vs. hospital-acquired cases or surgical vs. non-surgical wounds) might have helped establish a stronger correlation. Despite the lack of statistical significance, the clinical significance remains strong, especially due to the known pathogenic mechanisms of *P. mirabilis* and its known role in UTI and wound infections (20). Clinically, this distribution pattern suggests that when a *Proteus* species is isolated from urine, pus, or wound swabs, it is highly likely to be *P. mirabilis*. From a diagnostic standpoint, this knowledge can aid in: Guiding early, presumptive therapy before species identification is finalized. Risk stratifying patients, particularly in urology and surgery wards, where the presence of *P. mirabilis* may predict more severe or persistent infections due to its biofilm-forming capacity. Additionally, while *P. vulgaris* was found less frequently, its significance should not be undermined. *P. vulgaris* is intrinsically more resistant to antibiotics and is often isolated in immunocompromised individuals or those with prolonged hospital stays. Thus, when *P. vulgaris* is isolated, clinicians should exercise more caution and possibly consider broader antimicrobial coverage. The proteolytic, haemolytic, and urease-producing capacities of *P. mirabilis* contribute to its virulence. In wound infections, it facilitates tissue destruction and delays healing. In the urinary tract, it promotes calculus formation and persistent bacteriuria. These features make infections difficult to eradicate and frequently recurrent, especially in the elderly or catheterized patients.

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Resistance Mechanism	Screening (%)	Confirmatory (%)
ESBL	41%	39%
AmpC	25%	20%
Carbapenemase	2%	1%

Table 3: Resistance Mechanism Detection

The analysis in this study focuses on three major β-lactamase-mediated mechanisms of resistance in *Proteus* species: Extended Spectrum Beta-Lactamases (ESBL), AmpC β-lactamases, and Carbapenemases. These enzymes are responsible for hydrolyzing and inactivating a wide range of β-lactam antibiotics, making infections more difficult to treat and contributing to the growing global burden of antimicrobial resistance (AMR).

- ESBLs confer resistance to penicillin's and cephalosporins (especially third generation), but their activity can be inhibited by β-lactamase inhibitors like clavulanic acid.
- AmpC β-lactamases are cephalosporinases that confer resistance to cephalothin, cefazolin, cefoxitin, most penicillin's, and β-lactamase inhibitor combinations.
- Carbapenemases hydrolyze a broad spectrum of β-lactams, including carbapenems, which are typically used as last-line treatments.

ESBLs were confirmed in 39% of isolates, making this the most prevalent resistance mechanism among *Proteus* species in this cohort. This is a worrying statistic. ESBL-producing organisms are resistant to multiple classes of antibiotics and are often co-resistant to aminoglycosides and fluoroquinolones, further complicating treatment. In a clinical setting, identifying ESBL-producing *Proteus* is crucial because such infections do not respond to third

generation cephalosporins, even if in-vitro sensitivity is seen. Piperacillin-tazobactam and carbapenems remain the mainstay treatments, although the former is often ineffective in high-inoculum infections like abscesses or pneumonia. The presence of ESBL producers emphasizes the need for strict antibiotic stewardship, including the prudent use of cephalosporins and early implementation of phenotypic confirmatory testing (20).

AmpC β-lactamases were confirmed in 20% of isolates, which is a significant finding. AmpC-producing strains are often resistant to β-lactam/β-lactamase inhibitor combinations like amoxicillin-clavulanate and cefoxitin, which limits treatment options. These enzymes are not inhibited by clavulanic acid, and their presence usually predicts resistance to a wide range of β-lactam agents. Furthermore, detection is clinically tricky (11). Routine laboratory screening for AmpC is not universally practiced, leading to underreporting. From a treatment standpoint, AmpC-producing isolates are best treated with carbapenems or fourth generation cephalosporins like cefepime. However, with resistance mechanisms overlapping, therapy selection must be guided by susceptibility profiles.

Carbapenemase production was rare in this study—detected in only 1% of isolates. While the number is low, its significance cannot be overstated. Carbapenemase-producing organisms (CPOs) are usually resistant to almost all β-lactams and often carry multiple resistance genes, leaving very few therapeutic options such as colistin, tigecycline, or newer β-lactam/β-lactamase inhibitor combinations like ceftazidime-avibactam. Early and accurate detection of CPOs is critical to avoid treatment failures. The presence of even a single carbapenemase-producer implies that these enzymes are in circulation and may spread horizontally through plasmids, especially in environments with high antibiotic pressure (e.g., ICUs).

The study's use of double-disc synergy tests, the modified three-dimensional test (for AmpC), and the Modified Hodge Test (MHT) represents a gold-standard approach for resource-limited settings. However, the incorporation of molecular diagnostics (e.g., PCR for bla genes) could further improve diagnostic accuracy. With nearly 40% of *Proteus* isolates harboring ESBLs and an additional 20% showing AmpC activity, this study points to a significant burden of β-lactam resistance in a single tertiary center. Extrapolated across the region or country, the impact could be immense. Routine surveillance of resistance patterns is essential to inform empirical antibiotic guidelines.

Discussion

Proteus species are widely distributed in the environment and are recognized as normal inhabitants of the human gastrointestinal tract. While *Escherichia coli* remains the leading cause of uncomplicated urinary tract infections (UTIs) such as cystitis and pyelonephritis, *Proteus* species—particularly *Proteus mirabilis*—are considered the third most common uropathogen, especially in hospital-acquired infections (20).

In the present study, the majority of *Proteus* isolates were obtained from individuals aged 61–75 years (39%), followed by those aged 46–60 years (34%) and 31–45 years (15%). This age distribution aligns with the findings reported by studies (21-22), indicating that older age groups are at increased risk of *Proteus*-associated infections, possibly due to underlying comorbidities, frequent hospital visits, or use of indwelling devices.

A striking finding in this study was the predominance of *P. mirabilis*, which accounted for 89% of the total isolates, while *P. vulgaris* represented only 11%. This distribution is consistent with earlier studies (62.37%) (23-25). The variation in prevalence across studies may be attributed

to demographic differences, institutional infection control practices, or evolving pathogenic potential of *Proteus* species.

Species-level identification is clinically relevant, as resistance patterns can vary significantly. *P. vulgaris* is known to exhibit a broader resistance profile compared to *P. mirabilis*, which was found to be relatively more susceptible in this study. Among the clinical samples, pus was the most common source of *Proteus* isolates (35%), followed by urine (25%) and wound swabs (22%). Lower frequencies were observed in blood, ear swabs, tissue specimens, and tracheal aspirates. These findings mirror those of Bahashwan SA who also reported pus and urine as the predominant specimen types for *Proteus* isolation (26).

Proteus species play a critical role in UTIs, particularly in patients with predisposing conditions such as catheterization, anatomical abnormalities, or recent surgeries. Their virulence is largely attributed to several factors: swarming motility, the production of urease and proteolytic enzymes, IgA protease, and fimbriae. Urease, in particular, facilitates the formation of struvite stones by increasing urinary pH and promoting the precipitation of magnesium and calcium salts, which can lead to obstruction and persistent infection. These mechanisms are especially problematic in catheterized patients.

In the current study, a male predominance (65%) was observed among patients with *Proteus* infections, especially among elderly individuals. This gender trend may be linked to higher rates of urinary retention and catheter use in older men.

Antibiotic susceptibility testing revealed the highest sensitivity rates for piperacillin-tazobactam and tigecycline (100% each), followed by meropenem and imipenem (98%). These findings are consistent with those reported by Kengne M et al. and Preethishree P et al., who

also observed complete susceptibility to piperacillin-tazobactam and carbapenems (27-28). Amikacin demonstrated good activity (88% sensitivity), while gentamicin showed moderate effectiveness (72% sensitivity). Notably, ampicillin resistance was found in 64% of isolates, highlighting its limited clinical utility.

Regarding resistance mechanisms, the screening tests identified ESBL production in 41% of isolates, while confirmatory tests verified this in 39%. AmpC β-lactamase production was observed in 25% by screening and confirmed in 20% of cases. Carbapenemase producers were rare—detected in 2% by screening and confirmed in just 1% of isolates. These results closely align with previous studies such as Maheswary D and Chitralekha S (29).

The consistency between screening and confirmatory results, particularly for ESBLs, reinforces the reliability of initial detection methods. However, the discrepancies observed for AmpC and carbapenemase highlight the limitations of routine screening and underscore the importance of phenotypic confirmatory tests to avoid false positives or negatives. The ability to accurately detect and differentiate resistance mechanisms is essential for guiding targeted antimicrobial therapy and for implementing effective infection control measures.

Conclusion

This study emphasizes the importance of monitoring antimicrobial resistance at the species level. Understanding the prevalence and resistance patterns of *Proteus* species supports more

rational antibiotic use, minimizes the risk of therapeutic failure, and contributes to overall antimicrobial stewardship efforts.

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