



Isolation, Identification and Antibiotic Susceptibility Profile of *Proteus* Species Isolated from Patients Attending Saint John's Hospital, Gboko, Benue State

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ABSTRACT

Urinary tract infection (UTI) is one of the most common bacterial infections. It is a broad term that encompasses asymptomatic bacteriuria and symptomatic infection with bacterial invasion and inflammation of the urinary tract. This study was carried out to determine the phenotypic characteristics and antibiotic susceptibility pattern of *Proteus* species isolated from the urine of patients attending Saint John's Hospital, Gboko, Benue State. Eighty-two (82) midstream urine samples were collected in sterile universal bottles from males and females of all ages. Blood agar base was prepared according to the manufacturer's instructions. The urine samples were inoculated onto Blood agar and the inoculated plates were incubated for 24 h at 37°C. The colonies obtained were sub-cultured repeatedly on nutrient agar to obtain pure isolates. The isolates were identified using morphological and biochemical tests. The antibiotic susceptibility pattern of the isolates was determined using the Kirby-Bauer disc diffusion method. Thirty-three (33) *Proteus* isolates were obtained, giving an overall infection rate of 40.24%. The age group ≥ 51 years (13.41%, n=11) had the highest rate of infection while the age group 10-20 years (1.22%, n=1) had the lowest. There was a statistically significant difference in the infection rate between the age groups examined [$\chi^2 = 16.015$, df = 4, p = 0.001]. Female subjects (29.27%, n=24) had a higher occurrence rate than male subjects (10.97%, n=9), and the difference in infection rate between male and female subjects was statistically significant [$\chi^2 = 9.437$, df = 1, p = 0.002]. Among the six antibiotics tested, the isolates showed the highest susceptibility to Ciprofloxacin (27.3%), followed by Cefixime (21.2%), Ofloxacin (15.2%) and Pefloxacin (15.2%), while susceptibility was lowest to Sparfloxacin (12.1%) and Cefotaxime (9.1%). Resistance was highest against Ciprofloxacin (40.0%), followed by Cefixime (26.7%), Ofloxacin (20.0%) and Pefloxacin (13.3%); no isolate was classified as resistant to Sparfloxacin or Cefotaxime. The high resistance rates recorded against the fluoroquinolones and Cefixime suggest that these drugs have limited efficacy against urinary tract infections, particularly *Proteus* species infection, and should be less considered in treatment regimens. The infection rate recorded in this study indicates a significant level of *Proteus*-associated urinary tract infection among patients attending Saint John's Hospital, Gboko, and this signifies a public health hazard. The level of resistance recorded could be attributed to the abuse of drugs among the study population. Therefore, there is a need for strong collaboration between physicians and the laboratory in the choice of antibiotics for the treatment of urinary tract infections.

Keywords: UTI, antibacterial, phenotypic, resistance, susceptibility

INTRODUCTION

Proteus species is an important cause of urinary tract infection (UTI), particularly complicated and catheter-associated UTIs. Its clinical importance is closely related to its production of urease. *Proteus* species, especially *P. mirabilis*, produce the enzyme urease, which hydrolyzes urinary urea. Ammonia reacts with water to form ammonium and hydroxide ions, causing the urine pH to rise, often producing markedly alkaline urine. The

elevated urinary pH promotes precipitation of magnesium ammonium phosphate (struvite) and calcium carbonate/phosphate crystals. Consequently, *Proteus* infections are strongly associated with infection/struvite stones. *Proteus* is particularly important in indwelling urinary catheters, where urease-mediated alkalization can cause mineral crystals to precipitate within the catheter and on its surface. *P. mirabilis* has marked motility, including characteristic swarming growth, which may facilitate spread along urinary devices and the urinary tract. It can also form biofilms, allowing persistence on catheter surfaces and contributing to antimicrobial tolerance.

The interaction between bacterial pathogens and their human hosts is a dynamic, co-evolutionary process (Brites and Gagneux, 2015), and the emergence of resistance is an inevitable evolutionary consequence of antimicrobial exposure (Andersson, 2020). Antimicrobial resistance remains a significant threat to the treatment of bacterial infections globally, most importantly in low- and middle-income settings including Africa (Ayukekbong et al., 2017). The AMR threat adds to the existing higher burden of bacterial infections in such settings and low access to adequate diagnostics, specifically at intermediate and peripheral levels of the health system (Ayukekbong et al., 2017).

The hospital environment is a major driver of resistance. Inappropriate use of antibiotics in hospitals creates a complex relationship between antibiotic consumption and the emergence of antimicrobial resistance (Cantón et al., 2013), while prescribing behaviour among clinicians is itself shaped by social and institutional norms (Charani et al., 2013). Antibiotic stewardship programmes have been shown to reduce the incidence of infection and colonization with antibiotic-resistant bacteria (Baur et al., 2017). Resistance also spreads beyond the clinical setting: environmental development and transfer of resistance determinants is now recognized as a human health risk (Ashbolt et al., 2013), and proposed environmental limits for antibiotics predicted to select for resistant bacteria have been developed (Bengtsson-Palme and Larsson, 2016). Aquaculture has been identified as an additional environmental gateway to the development and globalisation of antimicrobial resistance (Cabello et al., 2016). Among Gram-negative organisms, extended-spectrum β -lactamases and related mechanisms are of particular concern in urological practice (Boyle and Zembower, 2015).

Antibiotic susceptibility testing (AST) remains the standard diagnostic method for detecting bacterial resistance and guides clinicians in the appropriate and timely treatment of bacterial infections. Antibiotic susceptibility testing results are crucial for surveillance studies to generate epidemiological data on bacterial pathogens and associated antimicrobial resistance. These data constitute the baseline information for national, regional and global strategies to contain the spread of antimicrobial resistance (Ayukekbong et al., 2017). Understanding the recent status of antimicrobial resistance surveillance and trends of resistance in Africa could improve clinical practice by guiding the clinician's choice of the right antibiotic and informing decision-making. The rationale for this study is to determine the antibiotic susceptibility pattern of *Proteus* species isolated from the urine of patients attending Saint John's Hospital, Gboko, Benue State.

MATERIALS AND METHODS

Study Area

Gboko is a Local Government Area in Benue State, North-central Nigeria. Gboko LGA was created on 11th May, 1970. The majority of Gboko LGA residents work in agriculture, and the region is well-known for cultivating crops such as rice, yams, maize, and cassava in significant quantities. Several marketplaces, including the Ortese, Ikpa, and Tarukpe markets, where locals can buy and sell a wide range of goods, are located in the Gboko LGA, where trade is also thriving.

Inclusion Criteria, Exclusion Criteria, and Ethical Approval

Patients presenting with symptoms suspected of urinary tract infection were recruited for the study, while those without such symptoms were excluded. Ethical approval was obtained from the Ethical Clearance Committee of Saint John's Hospital, Gboko, Benue State, prior to the commencement of the study, to ensure that the rights, dignity, and autonomy of individuals were respected, including obtaining informed consent, maintaining confidentiality, and avoiding harm.

Sample Collection

Eighty-two (82) midstream urine samples were collected in sterile universal sample bottles. The samples were collected from males and females of all ages attending Saint John's Hospital, Gboko, Benue State. The samples collected were appropriately labeled and transported to the laboratory for immediate microbiological analysis and processing.

Preparation of Media

Blood agar base was prepared according to the manufacturer's instructions; the dehydrated medium was weighed and dissolved in the appropriate volume of sterile distilled water. The suspension was heated to boiling and sterilized using an autoclave at 121°C for 15 minutes as described by Cheesbrough (2006).

Sample Inoculation

The urine samples were inoculated onto Blood agar. The inoculated plates were incubated for 24 h. The colonies obtained were sub-cultured repeatedly on nutrient agar to obtain pure isolates. The growth characteristics of the isolates were observed and recorded.

Morphological Identification

Gram Staining

The colony of each isolate was emulsified using a wire loop in a drop of normal saline on a clean microscope slide (smear). It was allowed to air dry and was heat-fixed by passing the slide across a flame three times. The slide was placed on the staining rack and flooded with crystal violet for 1 minute and rinsed with clean water. It was covered with Lugol's iodine (mordant) for 1 minute and rinsed with clean water. It was rapidly decolorized with acetone and rinsed immediately with clean water. It was counterstained with safranin (secondary dye) for 30 s. The back of the slide was wiped with cotton wool and allowed to air dry; the slide was mounted on the microscope stage and a drop of immersion oil was added. The specimen was brought into focus using a $\times 100$ objective with the iris diaphragm completely open to increase the amount of light entering the objective. Gram reaction was determined on the basis of the colour of dye retained: Gram-positive organisms retain the colour of the primary dye (crystal violet) and appear purple, while Gram-negative organisms lose the colour of the primary dye after decolourization and pick up the colour of the secondary dye (safranin) and appear red, as described by Cheesbrough (2006).

Biochemical Tests

Sugar Fermentation Test

The medium (Triple Sugar Iron Agar) was prepared in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the isolate and incubated at 37°C for 24 h. The tube was observed for lactose, sucrose and glucose fermentation on the basis of colour change resulting from acid production in the medium due to fermentation of the sugars by the test isolate, as described by Cheesbrough (2006).

Indole Test

The test organisms were inoculated into a test tube containing 5 ml of sterile peptone water (Cheesbrough, 2006) and incubated at 37°C for 48 h; 0.5 ml of Kovac's reagent was then added and shaken gently, and the tube was examined for red ring colour formation on the surface layer within 10 minutes. The formation of a red ring colour on the surface layer was taken as an indole-positive result.

Catalase Test

A drop of hydrogen peroxide was placed on a microscope slide and the isolate was picked using a wire loop and emulsified in the drop of hydrogen peroxide, and was observed for immediate bubble formation, which indicated a catalase-positive result, as described by Cheesbrough (2006).

Citrate Utilization Test

The medium was prepared in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the organism and incubated at 37°C for 48 h. A bright blue colouration in the medium was taken as a citrate-positive result, as described by Cheesbrough (2006).

Urease Test

The medium (urea agar base) was prepared in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the isolate and incubated at 37°C for 18 h. The tube was observed for colour change. Red/pink colour was taken as a urease-positive result, as described by Cheesbrough (2006).

Identification of Proteus Isolates

Proteus species was identified using different biochemical assays: citrate utilization, D-mannose fermentation, gelatin liquefaction (22°C), H₂S production (TSI), swarming, and urea hydrolysis. The bacterial isolates were inoculated individually into Sulfide-Indole-Motility (SIM) medium, incubated for 24-48 h at 37°C, and then 0.5 ml of Kovac's reagent was added. The tubes were gently shaken and allowed to stand for a few minutes. The formation of a cherry red ring layer on the medium's surface indicated that the isolate belonged to *P. vulgaris*, whereas *P. mirabilis* isolates yielded negative results (Cheesbrough, 2006).

Antibiotic Susceptibility Testing

The antibiotic susceptibility pattern of the isolates was determined using the Kirby-Bauer disc diffusion method as described by Cheesbrough (2006). The isolates were suspended in phosphate-buffered saline (PBS) and adjusted to McFarland 0.5 turbidity standard in order to obtain an approximate concentration of 1.5×10^8 CFU/ml. A sterile cotton swab was immersed in the PBS suspension, and the swab was pressed against the inside walls of the test tube above the fluid level to remove excess fluid. The swab was streaked on the surface of a Mueller-Hinton agar (MHA) plate such that a uniform distribution was achieved. Standard antibiotic discs containing Ciprofloxacin (20 µg), Cefixime (10 µg), Ofloxacin (5 µg), Pefloxacin (30 µg), Sparfloxacin (10 µg) and Cefotaxime (30 µg) (Bioanalyse and Oxoid discs) were placed on the inoculated Mueller-Hinton agar medium. The plates were incubated at 37°C for 24 h. The zones of growth inhibition were measured to the nearest millimetre using a transparent ruler, and the mean of triplicate readings was taken as the zone diameter. Isolates were classified as Susceptible (S), Intermediate (I) or Resistant (R) according to CLSI (2024), using the following zone diameter breakpoints: ≥ 18 mm = susceptible, 13-17 mm = intermediate, and < 13 mm = resistant. Isolates showing resistance to at least three of the antibiotics tested were regarded as multi-drug resistant. Laboratory strains of known sensitivity (*S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*) were used as quality control strains for the antimicrobial discs.

Statistical Analysis

Data were expressed in percentages and analyzed using Statistical Product and Service Solutions (SPSS) version 29. Pearson's Chi-square test was used for comparison, with significance set at $p < 0.05$. The results of this study are summarized in Tables 1–5. Table 1 presents the Gram stain reaction and biochemical test profiles of seven representative *Proteus* isolates, confirming all as Gram-negative bacilli with typical reactions for *P. mirabilis* and *P. vulgaris*. Table 2 shows the infection rate by age group, with the highest rate in those ≥ 51 years (13.41%) and the lowest in the 10–20 years group (1.22%). Table 3 presents the infection rate by gender, with females (29.27%) showing a higher rate than males (10.97%). Table 4 details the in-vitro antibiotic susceptibility profile, showing that susceptibility was highest to Ciprofloxacin (27.3%) and lowest to Cefotaxime (9.1%). Table 5 summarizes the resistance rates, with resistance highest against Ciprofloxacin (40.0%) and no resistance recorded for Sparfloxacin or Cefotaxime.

RESULTS

Table 1: Gram Stain Reaction and Biochemical Tests in Proteus Species Isolates

Test	<i>P. mirabilis</i>	<i>P. mirabilis</i>	<i>P. mirabilis</i>	<i>P. mirabilis</i>	<i>P. vulgaris</i>	<i>P. mirabilis</i>	<i>P. vulgaris</i>
Gram reaction	GNB	GNB	GNB	GNB	GNB	GNB	GNB
Citrate	+	+	+	+	+	+	+

Test	<i>P. mirabilis</i>	<i>P. mirabilis</i>	<i>P. mirabilis</i>	<i>P. mirabilis</i>	<i>P. vulgaris</i>	<i>P. mirabilis</i>	<i>P. vulgaris</i>
Indole	–	–	–	–	+	–	+
TSI	red (alkaline)	red (alkaline)	red (alkaline)	acid butt	red (alkaline)	yellow	yellow (alkaline)
Methyl red	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+
Motility test	+	+	+	+	+	+	+
Oxidase	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–
H ₂ S	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+
Voges Proskauer	–	–	–	–	–	–	–
Gas from Glucose	+	+	+	+	+	+	+
Gelatin	–	–	–	–	–	–	–

Key: TSI = Triple Sugar Iron Agar; + = Positive; – = Negative; H₂S = Hydrogen Sulphide; GNB = Gram Negative Bacteria.

Note: Seven representative isolates are shown. TSI reactions are reported as slant/butt colour changes; where a single entry is given, the isolate produced the stated reaction in both the slant and the butt.

Table 2: Infection Rate with Respect to Age

Age group	Number of samples examined	Number of positive samples (%)
10-20	17	1 (1.22)

Age group	Number of samples examined	Number of positive samples (%)
21-30	16	5 (6.10)
31-40	17	9 (10.97)
41-50	15	7 (8.54)
≥51	17	11 (13.41)
Total	82	33 (40.24)

$\chi^2 = 16.015$, $df = 4$, $p = 0.001$ (< 0.05 ; significant difference). Percentages were calculated using the total number of specimens examined ($N = 82$).

Table 3: Infection Rate with Respect to Gender

Gender	Number of samples examined	Number of positive samples (%)
Male	41	9 (10.97)
Female	41	24 (29.27)
Total	82	33 (40.24)

$\chi^2 = 9.437$, $df = 1$, $p = 0.002$ (< 0.05 ; significant difference). Percentages were calculated using the total number of specimens examined ($N = 82$).

Table 4: In-vitro Antibiotic Susceptibility Profile of *P. mirabilis* and *P. vulgaris* Isolates (Number Susceptible)

Isolates	N	CIP (20 µg) n (%)	CEF (10 µg) n (%)	OFL (5 µg) n (%)	PEF (30 µg) n (%)	SPA (10 µg) n (%)	CTX (30 µg) n (%)
<i>P. mirabilis</i>	21	6 (28.6)	4 (19.0)	3 (14.3)	3 (14.3)	3 (14.3)	2 (9.5)
<i>P. vulgaris</i>	12	3 (25.0)	3 (25.0)	2 (16.7)	2 (16.7)	1 (8.3)	1 (8.3)
All isolates	33	9 (27.3)	7 (21.2)	5 (15.2)	5 (15.2)	4 (12.1)	3 (9.1)

Key: CIP = Ciprofloxacin; CEF = Cefixime; OFL = Ofloxacin; PEF = Pefloxacin; SPA = Sparfloxacin; CTX = Cefotaxime; N = number of isolates tested; n = number susceptible; µg = microgram. Percentages for *P. mirabilis* were calculated using $N = 21$; for *P. vulgaris*, $N = 12$; and for all isolates, $N = 33$. Isolates not classified as susceptible were either intermediate or resistant (see Table 5).

Table 5: Resistance of the Isolates to the Antibiotics Tested

Antibiotic	Concentration (µg)	Zone of inhibition cut-off for resistance (mm)	Number resistant	(%)
CIP	20	≤ 8	6	40.0
CEF	10	≤ 10	4	26.7
OFL	5	≤ 6	3	20.0
PEF	30	≤ 9	2	13.3
SPA	10	< 13	0	0.0
CTX	30	< 13	0	0.0

Key: CIP = Ciprofloxacin; CEF = Cefixime; OFL = Ofloxacin; PEF = Pefloxacin; SPA = Sparfloxacin; CTX = Cefotaxime; mm = millimetre; µg = microgram. Zone diameter breakpoints: ≥18 mm = susceptible; 13-17 mm = intermediate; <13 mm = resistant (CLSI, 2024). Percentages were calculated using N = 15 resistant isolates recovered across the four antibiotics for which resistance was recorded (CIP = 6, CEF = 4, OFL = 3, PEF = 2). No isolate was classified as resistant to Sparfloxacin or Cefotaxime.

DISCUSSION

Urine samples from patients attending Saint John's Hospital, Gboko, Benue State were bacteriologically examined for *Proteus* species infection. The isolates obtained were subjected to antibiotic susceptibility testing. The present study showed that the rate of urinary tract infection caused by *Proteus* species among patients attending Saint John's Hospital, Gboko was 40.24%. The rate of infection recorded in this study was high compared with what was recorded in previous studies from other parts of the country: 37.5% in Ebonyi, Eastern Nigeria (Onoh et al., 2013), 32.7% in South West Nigeria (Oluwafemi et al., 2018) and 31.6% in Kano, Northern Nigeria (Muhammad et al., 2019). The high rate of urinary tract infection recorded in this study is of public health concern.

The rate of infection was higher among the age categories 31-40 years and ≥51 years. This finding agrees with the findings of Nwachukwu et al. (2018) and Azami et al. (2019), which show that urinary tract infections were more prevalent among the age group between 30 and 39 years. The high incidence of urinary tract infection among the 31-40 years age group may be due to a high rate of previous untreated urinary tract infection and lack of personal hygiene, as reported by Ranjan (2018). However, the higher rate of infection observed among the age group greater than 51 years could be attributed to weaker immunity, which predisposes this group more to infections than those with active immunity.

The study shows a higher rate of infection among female subjects than among male subjects. This agrees with the findings of Kabujo et al. (2016) and Khanal et al. (2016). The higher infection rate among the female gender could be due to the close proximity of the anus to the vagina and the ability of microorganisms to move from the perineal areas to the moist, warm environment of the female genitalia, as reported by Tibyange et al. (2015) and Odoki et al. (2015). Among the six antibiotics tested, the isolates showed the highest susceptibility to Ciprofloxacin (27.3% of all isolates), followed by Cefixime (21.2%), Ofloxacin (15.2%) and Pefloxacin (15.2%), while susceptibility was lowest to Sparfloxacin (12.1%) and Cefotaxime (9.1%). Resistance was highest against Ciprofloxacin (40.0%), followed by Cefixime (26.7%), Ofloxacin (20.0%) and Pefloxacin (13.3%). No isolate was classified as resistant to Sparfloxacin or Cefotaxime; isolates that were not susceptible to these two agents fell within the intermediate category. This pattern of reduced susceptibility to fluoroquinolones and third-generation cephalosporins among Gram-negative uropathogens is consistent with the report of Folliero et al. (2020). The high resistance rates recorded against Ciprofloxacin and Cefixime suggest that these drugs have limited efficacy against urinary tract infections, particularly *Proteus* species infection, and should be less considered in treatment regimens. Conversely, the comparatively lower resistance rates observed against Pefloxacin, Ofloxacin, Sparfloxacin and Cefotaxime may reflect the fact that these are reserved agents and are not commonly used in health facilities, and therefore have fewer opportunities for resistance development, as noted by Folliero et al. (2020).

The high resistance rates observed in this study could be attributed to irrational use of antibiotics (overuse and abuse), inexact diagnosis and improper antibiotic prescription, loss of patient sensitivity, self-medication, poor healthcare environments, poor personal hygiene, and widespread agricultural use which could lead to genetic modification, all of which have been identified as drivers of antimicrobial resistance (Cantón et al., 2013; Charani et al., 2013; Ashbolt et al., 2013; Cabello et al., 2016; Bengtsson-Palme and Larsson, 2016; Ayukekbong et al., 2017; Baur et al., 2017; Andersson, 2020). The burden of resistance is compounded in low-resource settings by limited access to adequate diagnostics (Boyle and Zembower, 2015; Ayukekbong et al., 2017). The findings of this study therefore support the need for continuous local surveillance to guide empirical therapy.

CONCLUSION

This study recorded an overall *Proteus*-associated urinary tract infection rate of 40.24% among patients attending Saint John's Hospital, Gboko, Benue State, with the highest rates in the ≥ 51 years age group and among female subjects. The isolates showed the highest susceptibility to Ciprofloxacin and Cefixime and the lowest susceptibility to Sparfloxacin and Cefotaxime, while resistance was highest against Ciprofloxacin and Cefixime. There is a need for strong collaboration between physicians and the laboratory in the choice of antibiotics for the treatment of urinary tract infections, and for the rational use of antibiotics to limit the further emergence of resistance.

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