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Concurrent Extended Spectrum Beta-lactamase Production and Multidrug Resistance among *Proteus* Species isolated from Clinical samples of patients attending selected Hospitals in Northeastern Nigeria

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Abstract

Proteus species are rod-shaped, Gram-negative bacteria that cause opportunistic infections in the urinary tract and occasionally in the gastrointestinal tract. They are implicated in infections like cystitis and pyelonephritis, particularly in immunocompromised individuals, and are frequently present in cases of asymptomatic bacteriuria. Herein, we aimed to investigate the co-occurrence of extended-spectrum beta-lactamase (ESBL) enzyme production and multidrug resistance (MDR) among Proteus spp. Isolated from patients attending selected hospitals in Northeastern Nigeria. A total of 1,500 clinical samples from consenting patients across six states in the Northeastern region of Nigeria were collected. The samples were cultured on Blood agar, and growth resembling that of Proteus species were again subcultured onto MacConkey agar to obtain discrete colonies, further confirmed using biochemical tests. Antibiotics susceptibility test was carried out for all isolates using the Kirby-Bauer disc diffusion method, coupled with a screening of the production of extended-spectrum beta-lactamase using the Combined Disc Diffusion Method. Of the 1500 samples collected, 144 yielded positive growth for Proteus spp., resulting in a prevalence rate of 9.60%. Among these Proteus isolates, three species were identified, with Proteus mirabilis (90.97%) being the most abundant, followed by Proteus vulgaris (8.33%) and Proteus penneri (0.70%). The Proteus isolates displayed significant resistance to ß-lactam antibiotics, with a Mean ± SD of 96.64 ± 22.73. A substantial portion of the Proteus spp. Isolated exhibited multidrug resistance (87.89%), with Proteus mirabilis (82.27%) being the most prevalent MDR species. Moreover, about 71.0% of the Proteus spp were ESBL producers, with Proteus mirabilis (64.54%) being the most predominant. Furthermore, 67.38% of all isolates exhibited MDR and ESBL production, and Proteus mirabilis (62.41%) was the most significant among the three Proteus species. These findings highlight the occurrence of multidrug resistance and ESBL production among Proteus spp. in Northeastern Nigeria, with Proteus mirabilis particularly noteworthy. This information is crucial for guiding clinical decision-making, especially in managing infections caused by multidrug-resistant and ESBL-producing Proteus strains.

Keywords: Proteus species, Extended-spectrum beta-lactamase, Multidrug resistance, hospitals

INTRODUCTION

Human populations from time immemorial have been significantly faced with infectious diseases, which cause immeasurable morbidity and death. The discovery of antibiotics at the beginning brings lots of expectations and hopes to see the end of effectively tackling this menace (Ribeiro da Cunha *et al.*, 2019). However, antibiotic resistance, defined as the ability of microbes to withstand the effects of drugs formulated to prevent and restrain their growth or kill them (CDC, 2021), soon became a challenge. Bacteria have shown the capacity to evolve and develop antibiotic resistance (Davies, 1994; Tenover, 2001; Livermore, 2003).

The situation even became worse due to poorly regulated antibiotic use, inadequate surveillance and misuse of antibiotics in clinical medicine and the livestock industry, resulting in the emergence and spread of multidrug resistant (MDR) bacteria worldwide (Medina and Pieper, 2016; Cerceo *et al.*, 2016; WHO, 2019; Pacios *et al.*, 2020).

Beta-lactam antibiotics possess a beta-lactam ring and are one of the most commonly prescribed worldwide due to their efficacy, broad spectrum, and low toxicity (Oberoi *et al.*, 2013). Resistance to this very important group of antibiotics is pervasive, further constricting the therapeutic options available (Versalovic *et al.*, 2011). The Resistance mechanisms encompass various factors, one of the most crucial being the production of enzymes encoded by specific genes carried on bacterial plasmids, including ß-lactamase and extended-spectrum ß-lactamase. The prevalence of MDR bacterial strains producing extended-spectrum beta-lactamase (ESBL) has been on the rise in recent years, particularly affecting the prognosis and survival of hospitalized patients in developing countries such as Nigeria (Senthamarai *et al.*, 2015; Tom *et al.*, 2018).

Extended-spectrum ß-lactamases (ESBLs) have emerged as a significant resistance mechanism against cephalosporins and other ß-lactam antibiotics, primarily in Gram-negative bacteria, especially those of the *Enterobacteriaceae* family such as *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus* spp (Mendelson *et al.*, 2005; Paterson and Bonomo, 2005; Falagas and Karageorgopoulos, 2009), and other bacterial species such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Jacoby and Munoz-Price, 2005). ESBLs are often plasmid-mediated and can hydrolyze and inactivate a wide range of ß-lactam antibiotics, including various Penicillins, broad-spectrum cephalosporins, and monobactams. They are generally derived from Temorina (TEM) and sulfhydryl variable (SHV) type enzymes (Lautenbach *et al.*, 2001).

Proteus species, like many other members of the *Enterobacteriaceae* family, can harbor numerous plasmids and integrons housing genetic determinants of antimicrobial resistance (Tom *et al.*, 2018). Different kinds of infectious diseases such as cystitis, pyelonephritis, prostatitis, chronic otitis media, eye infections, respiratory tract infections (RTI), wound infections, burn infections, and bloodstream infections are in some cases linked to pathogenic *Proteus* (Stock, 2003). Treating such infections can be challenging if the etiologic agent happens to be multidrug resistant ESBL-producing phenotype. In this study, we aim to describe the prevalence of MDR and ESBL production by *Proteus* spp. circulating in hospitals in the Northeastern region of Nigeria. The data generated from this study can be valuable to clinical microbiologists, clinicians, and infection control professionals. It can also be used for empirical treatment in areas with limited laboratory services.

MATERIALS AND METHODS

Study Population

The study focused on patients receiving care at six healthcare facilities in the North-eastern part of Nigeria, catering to a population exceeding 40 million across six states (Borno, Bauchi, Yobe, Adamawa, Taraba, and Gombe) and including a significant number from neighboring Cameroon, Chad, and Niger Republic (Umar *et al.*, 2016).

Ethical Consideration and Consent

Ethical approval for the study was obtained from the hospitals while patients' informed consent was sought before demographic data and sample collection. All experiments have been examined and approved by the ethics committee of the various Hospitals management and co-signed by the State Ministries of Health.

Sample Collection and Processing

A total of 1,500 clinical samples were randomly collected from consented patients from six (6) states of the North-eastern region, including wounds, sputum, urine, ear, catheter tip, genital swabs, and stools. Two hundred and fifty (250) samples each were collected from six (6) hospitals, which comprised of the following: Abubakar Tafawa Balewa Teaching Hospital (ATBU-TH), Bauchi, State Specialist Hospital, Maiduguri (SSH MAID), Federal Medical Centre, Yola (FMC Yola), State Specialist Hospital, Damaturu (SSH DMT), Federal Medical Centre, Jalingo (FMC Jalingo), and Federal Medical Centre, Gombe (FMC Gombe). Demographic data was collected and recorded before sample collection from patients.

Microbiological Analysis

The 1,500 clinical samples were processed and inoculated on to 5% Blood agar, MacConkey agar, and Cystine Lactose-Electrolyte-Deficient (CLED) and incubated aerobically at 37ºC for 18-24 hours (Chessbrough, 2006; Baker *et al.*, 2007). Colonies with characteristic colonial morphology were subcultured onto MacConkey agar to obtain discrete colonies.

Following the gram staining technique, the pure cultures of *Proteus* spp were identified using morphological appearance, swarming motility on Blood agar, Secondary Gram stain reaction for confirmation of characteristic colonies, and biochemical tests, which included phenylalanine deamination, urease production, sugar fermentation on Triple Sugar Ion (TSI) Agar, indole, Methyl Red, Voges Proskauer, Citrate utilization test and Ornithine Decarboxylase est (Chessbrough, 2006; Baker *et al.*, 2007).

Antibiotic Susceptibility Test

Standardization of Bacterial Inoculum

A few colonies of pure isolates of *Proteus* spp cultured on an agar plate were picked with a sterile wire loop and placed into a tube containing sterile normal saline and homogenized to give a turbid solution which was compared to the Mcfarland turbidity scale by continuously adjusting the turbidity until turbidity equals to 0.5 Mcfarland turbidity scale (Ejikeugwu, 2023). This turbidity scale was prepared by adding 0.6 ml of 1% aqueous solution of barium chloride in 99.4 ml of 1% sulphuric acid, giving an approximate bacterial density of 1.5 x 10^8 cfu/ml (Cheesbrough, 2006).

Determination of the Sensitivity Pattern of *Proteus* spp

The Kirby-Bauer disc diffusion method, outlined by Akubuenyi *et al.* (2011), was employed in this assessment. Pure *Proteus* spp isolates were examined against a range of commonly Zprescribed antibiotics in the region. The antibiotic discs contained varied concentrations: Cephalexin (10µg), Gentamicin (10µg), Augmentin (30/10µg), Amoxicillin (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), cefotaxime (30µg), Meropenem (30µg), Ertapenem (30µg), Aztreonam (30µg), Nalidixic acid (30µg), Streptomycin (30µg), Norfloxacin (30µg), Ofloxacin (30µg), Pefloxacin (10µg), Ciprofloxacin (10µg), Levofloxacin (10µg), Chloramphenicol (10µg), Rifampicin (10µg), Erythromycin (10µg), Ampiclox (30µg), Ampicillin (30µg), and Cotrimoxazole (30µg) (Fondoz laboratories, Nigeria; Oxoid Limited, UK).

The 0.5 McFarland standardized *Proteus* spp inoculum containing 1.5 X 10^8 cfu/mL was inoculated onto Mueller Hinton agar (MHA) plates, maintaining aseptic conditions, and allowed to absorb at room temperature for two minutes. Subsequently, commercially obtained antibiotic discs were meticulously positioned on the plate surface using sterile forceps. The plates were then incubated at 37°C for 24 hours. Post-incubation, the zones of inhibition were measured precisely in millimeters. The inhibition zones were interpreted using the CLSI manual (CLSI, 2023).

Determination of Multidrug-resistant *Proteus* spp

The determination of MDR among *Proteus* spp was done according to the definition proffered by Magiorakos *et al.* (2012). MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories or classes.

Preliminary Screening for Extended Spectrum ß-lactamase Production

Proteus spp were screened for ESBL production following CLSI's (2023) recommendations. A prepared inoculum was flooded onto Mueller Hinton agar (MHA) plates and left to absorb at 37°C for two minutes. Antibiotic discs containing Ceftriaxone (30µg), Ceftazidime (30µg), and Cefotaxime (30µg) were then carefully placed on the seeded plate using sterile forceps. After 24 hours of incubation at 37°C, inhibition zones were measured. Isolates showing ≤ 25 mm (Ceftriaxone), ≤ 22 mm (Ceftazidime), and ≤ 27 mm (Cefotaxime) were considered suspected ESBL producers.

Phenotypic Confirmatory Test for ESBL

The Combined Disc Diffusion Method recommended by CLSI (2023) was also employed. A 0.5 McFarland turbidity standard suspension was prepared from MDR *Proteus* spp colonies, creating a lawn culture on MHA plates. Discs of Ceftazidime and Ceftazidime + Clavulanic acid (30µg/10µg) were aseptically placed on the MHA surface with a 15 mm distance between them. After overnight incubation at 37°C, an increase of ≥ 5 mm in the zone diameter of Ceftazidime + Clavulanic acid compared to Ceftazidime alone confirmed ESBL production by the isolates.

Data Analysis

The data were analyzed utilizing the Statistical Package for Social Sciences (SPSS, version 21.0) and presented in frequencies and percentages. Chi-square analysis was employed, conducting 99% confidence level evaluations, with statistical significance set at $P<0.01$.

RESULTS

One thousand and five hundred samples were collected from patients attending selected Hospitals in the North-Eastern states of Nigeria, comprising of State Specialist Hospital (SSH), Maiduguri, SSH, Damaturu, Federal Medical Centre (FMC), Yola, Abubakar Tafawa Balewa Teaching Hospital (ATBU-TH), Bauchi, FMC, Gombe and FMC, Jalingo, and examined for pathogenic *Proteus* species.

The clinical samples collected from patients for the study include, Wound swabs, urine, Ear swabs, Catheter tips, Sputum, Faeces, Genital swabs and Body fluids. Out of the 1500 samples collected, 144 were positive for *Proteus* infections, with a prevalence rate of 9.6% (Figure 1).

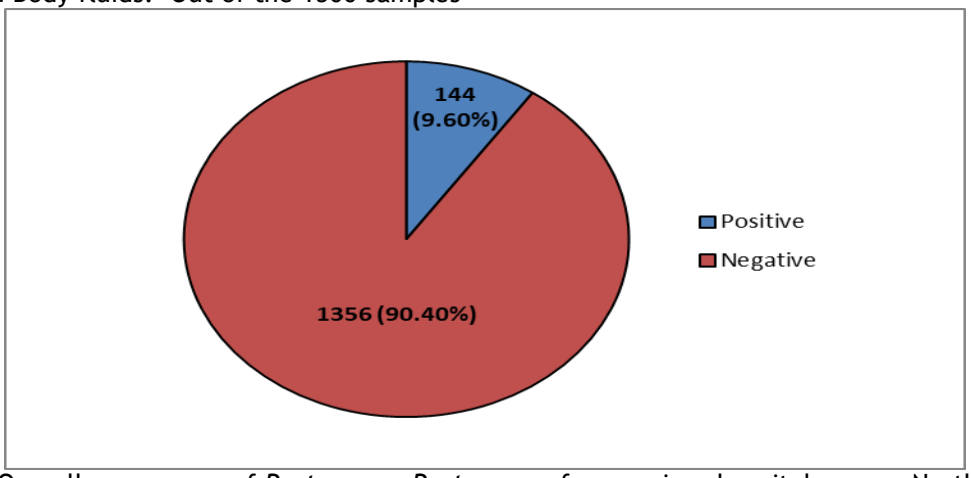


Figure 1: Overall occurrence of *Proteus* spp *Proteus* spp from various hospitals across North-eastern Nigeria

The highest infections rate was recorded among patients attending State Specialist Hospital (SSH), Maiduguri, with 31 (2.07%), followed by FMC, Gombe 27 (1.80%), SSH, Damaturu 26 (1.73%), ATBU-TH, Bauchi 21 (1.40%), FMC, Yola 20 (1.33%) and the least FMC, Jalingo with 19 (1.27%) (Table 1). Out of the three pathogenic species of *Proteus* isolated, *P. mirabilis* gave the highest yield of 131 (8.73%), followed by *P. vulgaris* 12 (0.80%), and the least was *P. penneri* with 1 (0.07%) (Table 2).

Table 1: Occurrence of *Proteus* spp from various hospitals across the North-eastern Nigeria

Hospital	Number of Samples collected	No. positive	Occurrence (%)
SSH Maiduguri	250	31	2.07
FMC Yola	250	20	1.33
SSH DMT	250	26	1.73
ATBU-TH Bauchi	250	21	1.40
FMC Gombe	250	27	1.80
FMC Jalingo	250	19	1.27
Total (%)	1500	144	9.60

(X^2= 5.740, df = 10, p-value = 0.837)

Table 2: Distribution of *Proteus* species isolates from sampled hospitals in North-eastern Nigeria

Proteus spp	Frequency	Percentage (%)
Proteus mirabilis	131	90.97
Proteus vulgaris	12	8.33
Proteus penneri	1	0.70
Total	144	100

The demographic distribution of *Proteus* infections among patients in the study area revealed that *Proteus* infection was highest among males, 81 (56.25%), compared to females, their counterpart with 63 (43.75%) (p=0.728). Patients within the age category of 31-40 years recorded the highest infection rate of 30 (20.83%), followed by those aged 21 and 30 years with 27 (18.75%), and the least were patients within the age category of >60 years 13 (9.03%) (Table 3).

Table 3: Relationship between Age and Gender of patients in the Distribution of *Proteus* spp in North-eastern Nigeria

Age Group (years)	Gender		Total (%)
	Male (%)	Female(%)	
0 10	11 (7.64)	7 (4.86)	18 (12.50)
11 20	9 (6.25)	7 (4.86)	16 (11.11)
21 - 30	15 (10.42)	12 (8.33)	27 (18.75)
31 40	16 (11.11)	14 (9.72)	30 (20.83)
41 50	12 (8.33)	14 (9.72)	26 (1.8.06)
51 60	8 (5.56)	6 (4.17)	14 (9.72)
>60	10 (6.94)	3 (2.08)	13 (9.03)
Total (%)	81 (56.25)	63 (43.75)	144 (100.0)

(X^2 =3.621, df =6, P- value 0.728)

The antimicrobial susceptibility pattern of *Proteus* spp isolated showed the highest resistance against all the ß-lactam antibiotics used in this study, except for Meropenem, which proved moderately sensitive with 97 (67.36%). The highest resistance of *Proteus* species was recorded against Ampicillin 122 (84.72%), followed by Amoxicillin, Ceporexin, and Cefotaxime with 116 (80.56%) each. Resistance against other groups of antibiotics stood at 188 (81.94%), 115 (79.86%), 74 (51.39%), and 71 (49.31%) to Nalidixic acid, Norfloxacin, Chloramphenicol, and Erythromycin respectively. *Proteus* species isolated were highly susceptible to Levofloxacin 126 (87.50%) and Ciprofloxacin 105 (72.92%), while exhibited intermediate sensitivity pattern to Rifampicin 86 (59.72%) and Streptomycin 75 (54.86%) (Table 4). The frequency of isolation of multi-drug resistant *Proteus* spp from samples collected in the selected Hospitals showed that patients attending SSH, Maiduguri, recorded the highest rate of isolation, 26 (20.60%), and the least was found among patients who attended FMC, Yola, and ATBU-TH, Bauchi with17 (13.60) each (Table 5).

Table 4: Antibiotic Susceptibility Pattern of *Proteus* spp isolated from patients attending sampled hospitals in North-eastern Nigeria

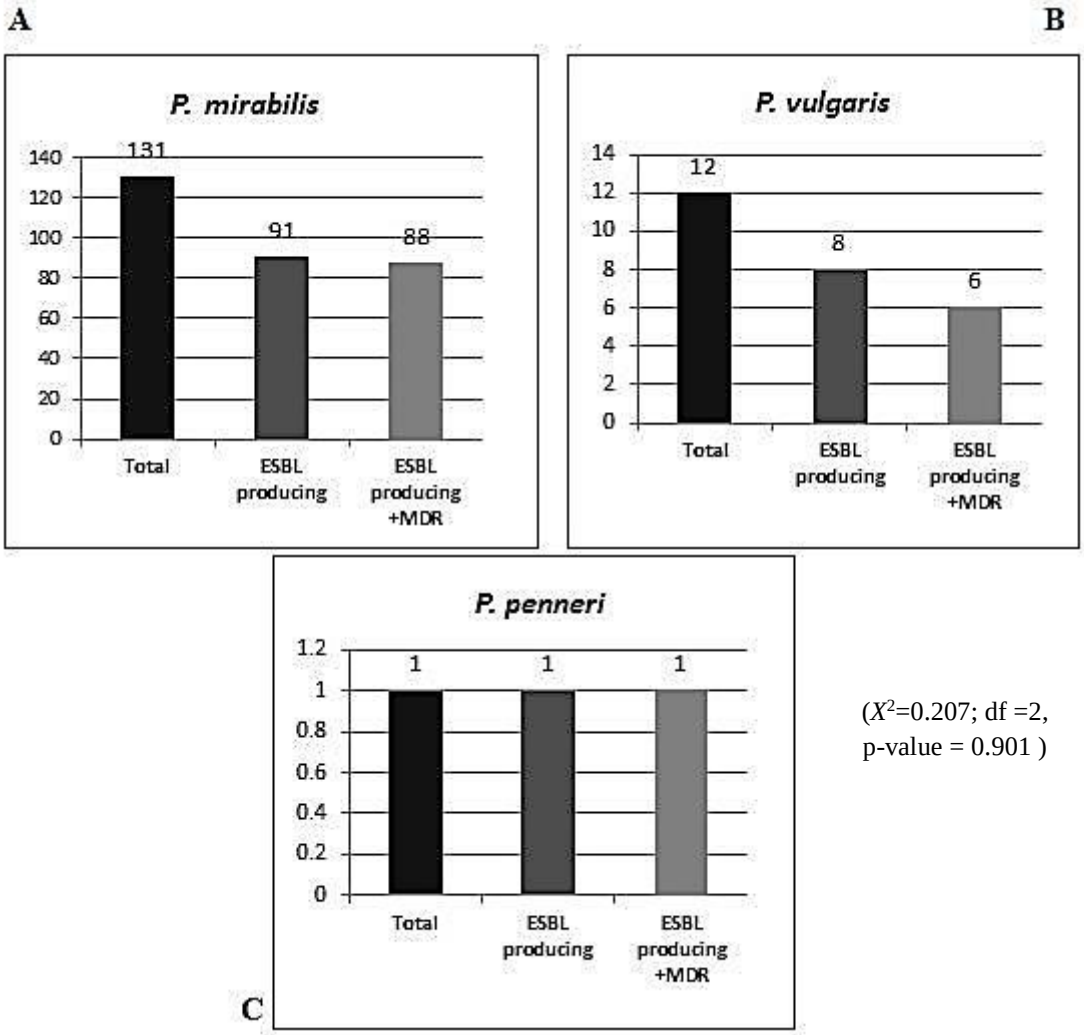
Antimicrobial Agents tested	Susceptibility pattern	
	Sensitivity (%)	Resistance (%)
Quinolones		
Ciprofloxacin (CPX)	105 (72.92)	39 (27.08)
Ofloxacin (OFX)	94 (65.28)	50 (34.72)
Pefloxacin (PEF)	77 (53.47)	67 (46.52)
Nalidixic acid (NA)	26 (18.06)	118 (81.94)
Norfloxacin (NB)	29 (20.14)	115 (79.86)
Levofloxacin (LEV)	126 (87.50)	18 (12.50)
ß-lactam Antibiotics		
Ampicillin (PN)	22 (15.28)	122 (84.72)
Cephalexinin (CEP)	28 (19.44)	116 (80.56)
Amoxicillin (AMX)	28 (19.44)	116 (80.56)
Augmentin (AU)	51 (35.42)	93 (64.58)
Ampiclox (APX)	41 (28.47)	103 (71.53)
Ceftazidime (CAZ)	38 (26.39)	106 (73.61)
Cefotaxime (CTX)	28 (19.44)	116 (80.56)
Ceftriazone (CRO)	55 (38.19)	89 (61.81)
Meropenem (MEM)	97 (67.36)	47 (32.64)
Ertapenem (ETP)	65 (45.14)	79 (54.86)
Aztreonam (ATM)	68 (47.22)	76 (52.78)
Aminoglycosides		
Gentamicin (CN)	68 (47.22)	76 (52.78)
Streptomycin (S)	79 (54.86)	65 (45.14)
Sulphonamide		
Cotrmoxazole (SXT)	74 (51.39)	70 (48.61)
Rifampins		
Rifampicin (RD)	86 (59.72)	58 (40.28)
Chloramphenicols		
Chloramphenicol (CH)	70 (48.61)	74 (51.39)
Macrolides		
Erythromycin (E)	73 (50.69)	71 (49.31)

Table 5: Distribution of Multidrug-resistant *Proteus* spp among patients attending selected hospitals in North-eastern Nigeria

Proteus species	Hospital						Total
	SSH Maiduguri	FMC Yola	SSH Damaturu	ATBUTH Bauchi	FMC Gombe	FMC Jalingo	
P.mirabilis	23 (18.40)	15(12.00)	22 (17.6)	15(12.00)	23(18.40)	18 (14.4)	116(92.80)
P. vulgaris	2 (1.60)	2 (1.60)	0 (0.00)	2 (1.60)	1 (0.80)	1 (0.80)	8 (6.40)
P. penneri	1 (0.80)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.80)
Total	26 (20.60)	17(13.60)	22 (17.6)	17(13.60)	24 (19.2)	19(15.20)	125 (100)

(X^2=7.321, p= 0.695

This study showed that out of the 144 *Proteus* ssp isolated, 100 (69.44%) were ß-lactamase enzymes producers, with only one isolate of *P. penneri* being the highest 1(100.0%), followed by *P. mirabilis* 91 (69.47%) and *P. vulgaris* having the least 8 (66.67%). A total of 95 (65.97%) of the isolates that are ß-lactamase enzymes producers are simultaneously showing phenotypic MDR, with only one isolate of *P. penneri* being the highest 1(100.0%), followed by *P. mirabilis* 88 (67.18%) and *P. vulgaris* having the least 6(50.0%) (Figure 2).



(X^2=0.207; df =2, p-value = 0.901)

Figure 2: Analysis of Extended spectrum beta lactamase production and Multidrug resistance among (A) *Proteus mirabilis*, (B) *Proteus vulgaris*, and (C) *Proteus penneri* identified from patients attending hospitals in Northeastern Nigeria

The distribution rate of ß-Lactamase enzyme production among MDR *Proteus* spp. isolated from patients attending the selected Hospitals in the study area revealed that SSH, Maiduguri has the highest number of isolates, 24 (25.26%), followed by SSH, Damaturu, and FMC Jalingo with 17(17.89%) each and ATBU-TH, Bauchi the least with 8 (8.42%) (Table 6).

Table 6: Rate of Distribution of ß-Lactamase Enzyme Production among MDR *Proteus* Spp. Isolated from Patients Attending the Selected Hospitals in the Study Area

Proteus species	Hospital						Total
	SSH Maiduguri	FMC Yola	SSH Damaturu	ATBU-TH	FMC Gombe	FMC Jalingo	
P. mirabilis	21(22.11)	12(12.65)	17(17.89)	8(8.42)	14(14.74)	16(16.84)	88(92.63)
P. vulgaris	2(2.11)	2(2.11)	0(0.00)	0(0.00)	1(1.05)	1(1.05)	6(6.32)
P. penneri	1(1.05)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.05)
Total	24(25.26)	14(14.74)	17 (17.89)	8(8.42)	15(15.79)	17(17.89)	95(100)

(x^2=6.40, df =10, p-value=0.781)

DISCUSSION

Prevalence of *Proteus* spp in Relation to Patients' Demographic Properties

In this study, out of the one thousand five hundred (1500) clinical samples examined for *Proteus* spp, one hundred and forty-four (144) yielded positive bacterial growth, which translates into a prevalence rate of 9.60% (Table 1). This is similar to the findings of Feglo *et al*. (2010), with 8.4% from 2361 clinical specimens. Contrasting results were obtained by Abera and Biadglegne (2009), Yusha'u *et al*. (2010), and Senthamarai *et al*. (2015) with 37.3%, 17.05%, and 3.04% in studies conducted from Ethiopia, Nigeria, and India respectively. These variations in the prevalence might not be unconnected with a difference in the type of clinical samples, the sample size examined, and the handling and processing techniques. The sample size could affect the prevalence rate because the larger the sample size, the smaller the estimation error and as such, the true reflection of the characteristic in a population can be obtained when the sample size is larger. State Specialist Hospital (SSH) Maiduguri recorded the highest frequency of *Proteus* species 31 (2.07%), while the least was obtained from FMC Jalingo with 19 (1.27%). The distribution of *Proteus* spp. in various hospitals was statistically insignificant (p-value = 0.837). It was observed that overstretched facilities as a result of overcrowding and inadequate infection control practice were found to be a common problem affecting the selected hospitals in the study area (Table 1). Three (3) species of *Proteus* were identified in this study, with *Proteus mirabilis* (90.97%) being the most abundant. This is followed by *Proteus vulgaris*; the least was *Proteus penneri* (0.70%) (Table 2).

Gender-based distribution of the occurrence of *Proteus* showed that males were most affected 81 (56.25%) compared to females 63 (43.75%). This study revealed that the difference in gender in relation to the occurrence of *Proteus* spp. Infection was statistically not significant (p-value=0.728) (Table 3). This is in contrast to the reports of Feglo *et al*. (2010), who reported that 43.0% of *Proteus* species isolated from clinical samples were from male patients while 57.0% were from females. This ascendancy is most likely due to the fact that the degree of exposure of maless is higher as they represent the majority of the the labour force in Nigeria. This can expose an individual to various kinds of risks, such as injuries and interaction with contaminated objects, and since *Proteus* species are quite ubiquitous in nature, the rate of *Proteus* infection becomes very high. A similar observation was made by Bashwan and Shafey (2013) in Saudi Arabia, who reported an occurrence of 75% for males and 25% for females. The age group with the highest occurrence of *Proteus* spp was 31-40 years (20.83%), and the least was patients within the age category of 51-60years and >60years with 14 (9.72) and 13 (9.03%), respectively (Table 3). This concurred with the reports of Torpy *et al*. (2005) and Omole and Stephen (2014), that age significantly affects the prevalence of infections since adolescents and active ages adults are usually the ones involved in stressful activities such as searching for daily bread and farming (Hakim and Gress, 2007; Omole and Stephen, 2014), and with high tendency of socialization with peer group which may expose them more to infectious diseases.

Antimicrobial Susceptibility Pattern of *Proteus* spp Isolated

A high level of antibiotic resistance of *Proteus* spp to all the Beta-lactam antibiotics used in this study was observed (96.64% ± 22.73). This implies that the mean resistance rate of *Proteus* spp towards ß-lactam antibiotics was 96.64%. This is quite high and very significant because eleven (11) ß-lactam antibiotics were tested. Meropenem was an exception, to which 67.36% of the isolates were susceptible. Individually, the highest resistance rate by the isolates was demonstrated against Ampicillin (84.72%), Amoxicillin, Cephalexin, and Cefotaxime, recorded at 80.56% each. Others with high resistance rates included Ceftazidime, Ceftriazone, and Ertapenem (73.61%, 61.81%, and 54.86%, respectively). Resistance of *Proteus* spp. to drug combinations was observed against Augmentin (64.58%) and Ampiclox (71.53%).

The penicillin and cephalosporin resistance observed in this study can be explained by the excessive use of Cefotaxime, Ceftazidime, and Ceftriazone for empirical therapy in hospitals. The production of ESBL as a resistance mechanism against these three antibiotics has been documented. Infection with ESBL-producing *Enterobacteriaceae*, where plasmid-carrying genes that encode ESBLs can be easily transmitted horizontally between different bacteria in the hospital environment (Schaufler *et al.*, 2016; Vubil *et al.*, 2017; Ghenea *et al.*, 2022; Van-Almsick *et al.*, 2022) Furthermore, antimicrobial susceptibility tests revealed that Levofloxacin (LEV) and Ciprofloxacin (CPX) antibiotic were the most effective antibiotics against *Proteus* spp. with a susceptibility rate of 126 (87.50%) and 105 (72.92%) respectively, followed by Ofloxacin (OFX) 94 (65.28%). The *Proteus* species isolated were found to have highly resistant to Nalidixic acid 118 (81.94%) and Norfloxacin 115 (79.86%), while moderately resistant against Gentamicin (CN) 76 (52.78%), Chloramphenical (CH) 74 (51.39%), Erythromycin (E) 71 (49.31%) and Cotrimoxazole (SXT) 70 (48.61%). In this study, members of the family Quinolones such as Levofloxacin, Ciprofloxacin, and Ofloxacin proved to have high sensitivity rates as most efficacious against *Proteus* infections while Rifampicin and Streptomycin showed intermediate sensitivity pattern (Table 4).

Analysis of Co-occurrence of Multidrug resistance and Extended-spectrum ß-lactamase production among *Proteus* spp identified

The prevalence rate for the Proteus spp MDR strains in this study was 86.81%. The distribution of MDR strains of *Proteus* spp among the selected Hospitals shows that SSH, Maiduguri had the highest percentage of MDR strains (20.60%), followed by FMC, Gombe (19.2%), SSH, Damaturu (17.6%), FMC, Jalingo (15.20%) and ATBU-TH, Bauchi and FMC, Yola each recorded 13.60% (Table 5). The prevalence of confirmed ESBL-producing *Proteus* spp. based on Combined Disc Diffusion Test recorded in this study was 69.44% with *P. mirabilis* having 91 (69.47%) and *P. vulgaris* 8 (66.67%). The occurrence could be attributed to the acquisition of plasmids carrying ESBL genes leading to the emergence of β-lactamase producing *Proteus* strains (Bradford, 2001; Fam *et al.*, 2011; Sah and Hemalatha, 2015) that can deactivate extended spectrum Cephalosporins, Penicillins, and Aztreonam. These enzymes might be coded on bacterial chromosomes or be plasmid-mediated, allowing their transfer between different bacterial populations. Up to 95 (65.97%) of the confirmed ESBL-producing *Proteus* spp. were phenotypically MDR strains. Multidrug-resistant strains with confirmed ß-lactamases were higher among only one isolate of *P. penneri* (100%), followed by *P. mirabilis* (67.18%) and *P. vulgaris* (50.0%) (Table 6; Figure 2). Extended Spectrum ß-lactamases producing organisms also have the potential capacity to acquire resistance to other antimicrobials such as Quinolones, Tetracyclines, Cotrimoxazole, Trimethoprim, and Aminoglycosides (Dhillon and Clark, 2011). The highest prevalence of confirmed MDR *Proteus* spp. producing ß-lactamase enzymes was observed among isolates from SSH, Maiduguri at 25.26%, with *P. mirabilis* having 22.11%, *P. vulgaris* at 2.11%, and. *P. penneri* the least (1.05%). This is followed by SSH, Damaturu, and FMC, Jalingo 17.89%. The prevalence of a particularly resistant strain in a particular hospital is related to the frequency of antibiotic usage, and the predominance of a multi-resistant strain may be maintained by the widespread use of any one of the antibiotics to which it is resistant. This study shows higher levels of resistance due to ESBL production with resistance to Nalidixic acid, Norfloxacin, Gentamicin, Chloramphenicol, Cotrimoxazole, and Erythromycin. Hence, this cross-resistance astonishing features is worrisome because it restricts greatly the treatment options in patients infected with ESBL-producing *Proteus* spp.

Conclusion

In conclusion, this study revealed a notable prevalence of *Proteus* spp, at 9.60%. *Proteus mirabilis* emerged as the predominant species, constituting a significant majority of the isolates. Alarmingly, a substantial proportion displayed resistance to ß-lactam antibiotics. Multidrug resistance was prevalent in most of Proteus isolated, and *Proteus mirabilis* exhibited the highest frequency. Furthermore, a concerning majority of *Proteus* spp. demonstrated extended-spectrum beta-lactamase production, underscoring the urgent need to address this region's antimicrobial resistance. Regional epidemiological surveillance of ESBL-encoding genes is crucial, along with an investigation into the transmission mode within hospitals. Implementing effective antimicrobial stewardship is imperative to mitigate antibiotic resistance due to ESBL-producing *Proteus* in the region.

Competing Interests

The authors have declared that no competing interests exist.

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