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Multidrug Resistance and ESBLs Production in *Proteus* species from Wound Infections in a Tertiary Hospital in South-East of Nigeria

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A B S T R A C T

Wound infections caused by drug-resistant *Proteus* species pose a global health threat, including in Nigeria. This study aimed to determine the prevalence, antibiotic susceptibility, and ESBL production in *Proteus* species from wound infections in a Nigerian tertiary hospital. The descriptive cross-sectional study spanned eight months (September 2022 to April 2023) at Alex-Ekwueme Federal University Teaching Hospital in Ebonyi State, Nigeria. 322 wound samples from in and out-patients were analyzed using disc diffusion for antimicrobial susceptibility, the double disc-synergy test for phenotypic ESBL production, and PCR for ESBL gene screening. Out of the 322 samples, 61 (18.9%) were *Proteus* species, with 43 (70.5%) identified as *Proteus* mirabilis and 18 (29.5%) as *Proteus vulgaris*. Surgical wounds had the highest number of *Proteus* isolates (41%), followed by wound ulcers (34.4%), and trauma wounds had the least (24.68%). The relationships between wound type and *Proteus* prevalence, patient category and *Proteus* prevalence, and age of patients and *Proteus* prevalence were not statistically significant ($p > 0.05$). However, the relationship between patients' sex and *Proteus* prevalence was significant, with males (73.8%) having higher isolates than females (26.2%). Ceftazidime and levofloxacin showed the highest activity against *Proteus* isolates, while nalidixic acid, cefotaxime, imipenem, and nitrofurantoin exhibited the highest resistance. There were significant differences in antibiotic sensitivity and resistance between *P. mirabilis* and *P. vulgaris* ($p < 0.05$). 80% of *Proteus* isolates were multidrug-resistant (MDR), and this relationship was statistically significant ($p < 0.05$). 55% of *P. mirabilis* and 55.6% of *P. vulgaris* were confirmed to produce ESBLs. PCR detected ESBL genes: TEM (37.5%), CTX-M (37.5%), and SHV (25.0%). *Proteus* isolates from the studied wound infections demonstrated a high level of multidrug resistance, and ESBL genes were detected.

Introduction

A wound results in tissue damage which stimulates a coordinated physiological response to provide haemostasis and initiate the processes of inflammation, proliferation and remodeling (Young and McNaught, 2011). Wounds can be broadly categorized as having either an acute or a chronic etiology. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and

those caused by crush or gunshot injuries (Davis *et al.*, 1992). Chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue (Davis *et al.*, 1992). Pathophysiological abnormalities that may predispose to the formation of chronic wounds such as leg ulcers, foot ulcers, and pressure sores include compromised tissue perfusion as a consequence of impaired arterial supply or impaired venous drainage and metabolic diseases such as diabetes mellitus. Advancing age, obesity, smoking, poor nutrition, and immunosuppression associated with disease or drugs may also exacerbate chronic ulceration (Siddiqui and Bernstein, 2010). Wound infection is the invasion of a wound by proliferating microorganisms to a level of a systemic response in the host. The presence of microorganisms within the wound causes local damage and impedes wound healing (Siddiqui and Bernstein, 2010). The common bacterial pathogen associated with wound infections includes *Staphylococcus aureus, Proteus species, Escherichia coli, Pseudomonas eruginosa, Klessiella pneumoniae* and *Streptococcus species* (Mohammed *et al.,* 2013).

Proteus is a member of the Enterobacteriaceae family. The genus *Proteus* consists of motile, facultative anaerobic Gram-negative rods. The genus *Proteus* currently consist of five named species; *P. mirabilis*, *P. vulgaris*, *P.penneri*, *P.myxofaciens* and *P.hauseri* and three unnamed *Proteus* genomospecies 4, 5 and 6 and consist of 80 O-antigenic serogroups (O'Hara *et al.*, 2000). The First isolates were reported and characterized by Hauser in the late 19th Century. The genus name *Proteus* originates from mythological Greek sea god. A striking characteristic of *Proteus* species is their swarming activity. *Proteus* swarms across agar surfaces, overtaking any other species present in the process (Hyun *et al.*, 2016).

Proteus species have several virulent factors some of which are fimbriae and adhesion, flagella for swimming and swarming growth, urease production, biofilm production and proteases production (Armbruster *et al.*, 2018). *Proteus* species are naturally found in the intestinal tract of humans, animals, water and soil (Drezweicka, 2016). They are also found in long term care facilities and hospital environments (Kramer *et al.,* 2006). They cause significant clinical infections, which are difficult to eradicate especially from host with complicated wounds, catheterization, underlying diseases and immunocompromised (Gadepalli *et al.*, 2006). They usually contaminate wounds through the environment. With the help of their virulent factors, once they gain access into the body, they develop mechanism to exploit the host for continuous survival and dissemination. The dissemination of this organism tend to be associated with bacteremia, septicaemia, shock and prolonged hospital stay with an increasing chance of developing drug resistant and prevent wound healing especially drug resistant strains. *Proteus* species can be naturally resistant to antibiotics, such as benzylepenicillin, oxacillin, tetracycline, macrolides and nitrofurans (Stock, 2003).

Proteus species can acquire resistance to beta-lactams antibiotics through plasmid mediated beta-lactamases. In the last decade there have also been numerous reports of production of extended spectrum beta-lactamases (ESBLs) by *Proteus* species. The ESBLs can confer resistance to third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime as well as monobactam (Stock, 2003). Wound infections are becoming increasingly difficult to treat due to this emergence and widespread of antibiotic resistance. Those caused by drug resistant *Proteus* species are becoming an expanding public health threat globally including Nigeria.

They are particularly resistant to beta-lactams antibiotics through the production of plasmid mediated beta-lactamases (ESBLs). A regular research to review the antimicrobial resistant pattern and ESBL production in *Proteus* species can be helpful in formulating antibiotic policy and also in controlling resistance in *Proteus* species. Therefore

the aim of this work was to determine the prevalence, antibiotic susceptibility pattern, phenotypic and genotypic ESBL production in *Proteus* species from wound samples in a tertiary hospital in South-East of Nigeria.

Materials and Methods

Study Design

This was a descriptive cross-sectional study carried out within a period of eight months, from September, 2022 to April, 2023, in Alex-Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State, Nigeria. A random sampling of in-patients and out-patients, males and females of all aged groups that attend the hospital within the period of this study were carried out.

Sample Size Determination

Three hundred and twenty two (322) wound samples were used for this study. Calculated using this formula ($N = Za2P (1-P)/D^{2)}$ by fisher for minimum sample size, given score for 95% confidence interval =1.96, P = prevalence, and D = acceptable error (5%). 8.75% prevalence was used for this study, which was obtained from a research work by Torm *et al.,* (2008).

Calculations

The sample sizes for this study was determined using the fisher's formula, $N = Za2P (1-P)/D^2$

Where Za =significant level set at 95% confidence level. Za is 1.96 for two tailed test.

P = prevalence of the attribute under study. P is 8.75 % (0.0875)

D = margin of error tolerated. D is 5% (0.05)

N = minimum sample size $=Za2P (1-P)/D^2$

Substituting in the formula,

$N = 1.96 \times 2 \times 0.0875 (1- 0.0875)/ (0.05)^2 = 125$ approximately.

Allowing 10% non –responses, N= 10 X 125/100 = 12.5

N = 125+ 12.5 = 138 approximately

Sampling Procedure / Sample collection

Consent was obtained from the patients prior to sample collection. Well structured questionnaire was used to obtain information on the patient demographic characteristics, use of antibiotics, type of patients and type of wound samples. Sterile swab sticks were used for sample collection.

The wound was first cleaned with moisten sterile gauze and sterile normal saline solution before using the sterile swab stick to rotate over the wound. Criteria for inclusion in this study include: Willingness to participate in the study, patients that are not on antibiotics and no history of antibacterial therapy within two weeks prior to attendance to hospital.

Isolation and identification of the bacterial pathogens

Isolation and identification of bacteria were done at the Microbiology unit of the hospital. The wound samples were cultured on freshly prepared and dried blood agar and macConkey agar, and then incubated at 37^0C for 24hours. The isolates were identified on the basis of their morphology, swarming on blood agar, Gram stain reaction, biochemical tests and those ones showing the identity of *Proteus* further underwent the following stages: Antimicrobial susceptibility testing, check for extended spectrum beta-lactamases, Polymerase chain reaction and Gel electrophoresis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was tested by the disc diffusion method according to the Clinical and

Laboratory Standard Institute guideline (CLSI, 2020). Overnight cultures of each *Proteus* isolates were adjusted to 0.5 McFarland turbidity standards and using sterile swabs, the test organisms were smeared onto Mueller Hinton agar. Sterile forceps were used to distribute the antibiotic disc on the inoculated plates.

After proper diffusion of the antibiotic into the agar, the plates were incubated at 37^0C for 18h. The zone of inhibition was measured and the values recorded. Zone of inhibition were categorized as either sensitive intermediate or resistant. The resistance, intermediate and sensitivity were interpreted according to Clinical and Laboratory Standards Institute guideline (CLSI, 2020).

The antimicrobial discs used include ceftriaxone (30ug), ceftazidime (30µg), cefotaxime (30µg) cefixime (5ug), cefuroxime (30ug), augumentin (30 µg), levofloxacin (5ug), ofloxacin (30µg), ciprofloxacin (5 µg), imipenen (10ug), nitrofurantoin (300µg), gentamicin (10 µg) and nalidixic acid (30 µg).

Primary test for production of ESBLs

Antibiotic susceptibility testing was done to three types of third generation cephalosporins antibiotics: ceftazidime, cefotaxime, and ceftriaxone. If inhibition zone for bacterial isolates were: ≤ 22 mm for cefotaxime, ≤ 17 mm for ceftazidime and ≤ 19 mm for ceftriaxone, the results were considered as positive result for production of extended spectrum beta-lactamase (CLSI, 2020).

Confirmatory test for ESBLs

Augumentin disc (30 µg) was placed in the center of Mueller Hinton agar plate (Oxoid, UK). Around the three sides of augumentin disc (30 µg), a disc of ceftazidime (30 µg), ceftriaxone (30 µg) and cefotaxime (30 µg) were placed with distance of 30mm from center to center of augumentin disc. Then the plate was incubated at 37°C for 24 h. If inhibition zone was increased towards the augumentin disc that considered as positive results for production of ESBL. This was performed using double disc-synergy test (Drieux and Jarler, 2008).

Polymerase Chain Reaction

Multidrug resistant (MDR) isolates were selected for amplification of resistant genes using polymerase chain reaction technique. Multidrug resistant isolates are those isolates that were resistant to more than three classes of antibiotics. Bacteria DNA extraction was done using the Thermo Scientific GeneJET Genomic DNA Purification Kit. The polymerase chain reaction was carried out using the one Taq Quick load 2X Master Mix with Standard Buffer (NewEngland Biolabs, MA, U.S.A.), which is composed of; 20 mMTris-HCl, 1.8 mM MgCl2, 22 mMNH4Cl, 22 mMKCl, 0.2 mM DNTPS, 5% glycerol, 0.06% IGEPAL CA-630, 0.05% Tween 20,Xylene Cyanol FF, Tartrazine and 25 units/ml Taq DNA polymerase.

Detection of Extended spectrum beta-lactamase genes

Multidrug resistant isolates that were positive for ESBLs test were screened by PCR for blaTEM, blaSHV and blaCTX-M genes using specific primers. BlaCTX-M: Forward; TTTGCGATGTG CAGTACCAGTAA, Reverse; CGATATCGT TGGTGGTGCCATA, blaTEM: Forward; TACG ATACGGGAGGGCTTAC, Reverse; TTCCTGTTT TTGCCACCCA, blaSHV: Forward; TCAGCGAAA AACACCTTG, Reverse; TCCCGCAGATAAATC ACCA.

Data Analysis

All statistical analyses were conducted using SPSS Windows version 22. Descriptive statistics (frequencies and percentages) were used to describe categorical variables. The Pearson Chi-square (x^2) test was employed to examine significant differences in proportions with a confidence interval of 95%. A p-value < 0.05 indicated a statistically significant relationship.

Limitation of the study

Materials used in the microbiological culture are basically for the isolation of aerobic pathogens incriminated in wound infections, thus may not take into account, the anaerobic pathogens.

Ethics of study

The study received ethical approval from the Research and Ethics Committee of Alex-Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State, Nigeria, with registration number AE-FUTHA/REC/VOL 3/2022/070.

Results and Discussion

Demographic characteristics

Regular research to review antimicrobial pattern of *Proteus* species and other bacteria causes of wound infections can be helpful in prevention of resistance and effective management of wounds especially in health care settings thereby reducing morbidity and mortality caused by wound infections. From this study, Out of the 322 wound samples analyzed 257 (79.8%) yielded bacteria growth. A total of 61 (18.9%) isolates of *Proteus* species of which 43 (70.5%) and 18 (29.5%) isolates were *Proteus mirabilis* and *Proteus vulgaris* respectively were isolated. In concordance with previous research *P. mirabilis* is the most isolated *Proteus* species. *P. mirabilis* is the most extensively dispersed in the environment. Surgical wound recorded the highest *Proteus* isolates with 25 (41%) followed by wound ulcer: 21(34.4%) the least was trauma wounds: 15 (24.68%) (Table1). The relationship between type of wound and prevalence of *Proteus* species was not statistically significant ($p > 0.05$, $p=0.987$, $X^2=0.338$, $df=4$). Surgical wounds recording the highest isolates of *Proteus* species agrees with Mohammed *et al.,* (2013) studies in Nigeria and Zafer *et al.,* (2019) studies in Ouetta, Pakistan but disagrees with Torm *et al.,* (2008) studies in Northern part of Nigeria in which wound ulcer recorded the highest isolates. Surgical wound supposed to be clean wound compared to other wound types as those wounds are planned, but high contamination of surgical wounds sites constitute a serious problem in the hospital indicating the need to improve the standard of surgical procedures and the standard of environmental hygiene in the hospital. In-patient recorded higher isolates with 42 (68.9%) than out-patients 19 (31.1%). 31 (73.8%) isolates of *P. mirabilis* were isolated from in-patients while 12 (63.2%) were isolated from out-patients. *P. vulgaris* recorded 11 (26.2%) isolates from in-patients and 7 (36.8%) isolates from out-patients (Table 2). The relationship between patients category and prevalence of *Proteus* species was not statistically significant ($p > 0.05$ $p=0.611$, $X^2=0.984$, $df=1$). More isolates of *Proteus* species were recorded from in-patients compared to out-patients. This shows that most of wound infections were contaminated from the hospital environment. *Proteus* infections acquired in the hospital can be through environmental contamination (person to person spread or through medical advices). According Kramer, *et al.*, (2006) studies, *P. vulgaris* can persists on dry hard surfaces for up to 2 days. Gender distribution of *Proteus* isolates (Table 3) showed that 45 (73.8%) were from male patients, 32 (71.1%) *P. mirabilis* and 13 (28.8%) *P. vulgaris, w*hile 16 (26.2%) *Proteus* isolates were from female patients, 11 (68.2%) being *P. mirabilis* and 5 (31.2%) *P. vulgaris*. The relationship between sex of patients and prevalence of *Proteus* species was statistically significant ($p<0.05$, $p=0.005$, $X^2=10.433$, $df=2$). Male patients recording higher isolates agrees with previous research of Akambi *et al.,* (2017); Feglo *et al.,* (2010); Bashwan and El-shafi (2013) and Zafer *et al.,* (2019). This may be likely due to the fact that male exposure is greater as they represents majority of the workforce, so they are exposed more to acquiring wound infections, to road accidents, more bone fractures and other operations. Isolated *Proteus* species were highest among patients aged 31-40 years followed by age group 21-30, 41-50 and 51-60 and lowest among patients aged <1 year and 71- 80 years (Table 4). The relationship between age of patients and prevalence of *Proteus* species was not statistically

significant ($p > 0.05$, $p=0.837$, $X^2=12.195$, $df=18$). The age groups 21-60years also represents the most active and work force age groups, who are prone to injuries, accidents, diseases and surgical operations.

Other bacteria isolates

Bacteria isolated in decreasing other of occurrence include: *Staphylococcus* spp; 105 (35.4%), *Proteus* species; 61(20.5%), *Klebsiella* spp; 46 (15.5%), *Escherichia coli;* 36 (12.1%), *Pseudomonas* spp; 17 (5.7%), *Streptococcus* spp; 13(4.4%), *Morganella* spp; 9 (3.0%), *Enterobacter* spp; 4 (1.4%), *Providencia* spp; 3 (1.0%), *Citrobacter* spp; 3 (1.0%) (Figure1). From this study *Proteus* specie ranks the highest frequency among the Gram negative bacteria isolated. This is in accordance with Mordi and Mono (2008) studies in Benin City Nigeria, in which *Proteus* species recorded highest frequency with (26.8%), and also with Raja studies (2007) in Malaysia in which *Proteus* species was the highest (28%) among the Gram negative bacteria. However Mohammed *et al.*, (2013) and Torm *et al.*, (2008) reported less. Infections due to *Proteus* are increasing and this has significantly increases health care cost. Control of *Proteus* infections have become more challenging due to the antibiotic resistance of infections.

Antibiotic susceptibility profile of the *Proteus* isolates

Ceftazidime (30µg) showed the highest activity against *P. mirabilis* isolates with percentage sensitivities of 74.4% followed by levofloxacin (5ug) with sensitivity of 58.1%. Levofloxacin was most sensitive against *P. vulgaris* isolates with percentage sensitivity of 61.1% followed by ceftazidime and gentamicin with sensitivity of 38.9% each. Nitrofurantoin had the highest resistance on *P. mirabilis* with percentage resistance of 93% followed by cefotaxime and nalidixic acid with percentage resistance of 90.7% each. Similarly, nalidixic acid and nitrofurantoin had the highest resistance on *P. vulgaris* with percentage resistance of 94.4% and 88.9% respectively (Table 5b). There was a statistically significant difference in the sensitivity and resistance of the tested antibiotics to *P. mirabilis* ($p < 0.05$, $p=0.000$, $X^2=157.379$, $df=12$). There was also a statistically significant difference in the sensitivity and resistance of the tested antibiotics to *P. vulgaris* ($p < 0.05$, $p=0.000$, $X^2=42.923$, $df=12$). *P. mirabilis* isolated were more susceptible to antibiotics used than *P. vulgaris* isolates. *P vulgaris* produces a chromosomally encoded class A cefuroximase conferring resistance to aminopenicillin, first and second generation cephalosporins, with the exception of cefoxitin. *P. mirabilis* does not produce any chromosomally encoded beta-lactamase, resulting in full susceptibility to all beta-lactams for wild type (Girlich, 2020). The *Proteus* isolates were more susceptible to ceftazidime and levofloxacin but showed decreased susceptibility to the following antibiotics, ceftriaxone, ofloxacin, gentamicin and ciprofloxacin and high resistance to cefotaxime, cefixime, nacidixic acid, augumentin, cefuroxime, imipenem and nitrofurantoin. *Proteus* species are naturally resistant to nitrofurantoin and imipenem. Resistance to imipenem may be due to loss of porins, reduce expression of penicillin binding protein or acquisition of several antibiotic resistance gene including carbapenemase genes (Girlich, 2020). The high resistance of *Proteus* isolates to most of the antibiotics tested resulted in most of the isolates being found to be multidrug resistant.

Eighty percent (49/61) of the *Proteus* isolates were found to be multidrug resistant (MDR). Out of 43 isolates of *P. mirabilis,* 31 (72.1%) were found to be MDR, while all the 18 (100%) isolates of *P. vulgaris* were found to be MDR (Table 6) The relationship between *Proteus* isolates and multi-drug resistance ability was statistically significant. ($p < 0.05$, $p=0.012$, $X^2=6.253$, $df=1$). All the isolates of *P. vulgaris* were found to be multidrug resistance. *P. vulgaris* showed higher level of MDR than *P. mirabilis*. This is also related to the fact that *P. vulgaris* were more resistant to the antibiotics tested than *P. mirabilis*. *Proteus* isolates from in-patients showed higher level of multi drug resistance than isolates from out-patients.

Table.1 Distribution of isolates of *Proteus* species according to wound types

Samples	*Proteus* species		Total	X^2	P value
Wound types	*P. mirabilis*	*P. vulgaris*			
Surgical wound (n=125)	17 (68%)	8 (32%)	25 (41%)	0.338	0.987
Wound ulcer (n=118)	15 (71.4%)	6 (28.6%)	21 (34.4%)		
Trauma wound (n=79)	11 (73.3%)	4 (26.7%)	15 (24.6%)		
Total	43 (70.5%)	18 (29.5%)	61(100%)		

n=number tested, Pearson Chi-square test was used to compare the association between variables. P < 0.05 was considered statistically significant.

From Table 1, a total of 61 *Proteus* species were isolated of which *Proteus mirabilis* recorded 43 (70.5%) while *Proteus vulgaris* recorded 18 (29.5%). Surgical wound recorded the highest *Proteus* isolates with 25 ((41%) followed by wound ulcer: 21 (34.4%) the least was trauma wound: 15 (24.6%). The relationship between type of wound and prevalence of *Proteus* species was not statistically significant (p > 0.05, p=0.987, X^2=0.338, df=4).

From Table 2, a total of 42 (68.9%) isolates of *Proteus* species were from In-patient while 19 (31.1%) were from out-patients. 31 (73.8%) isolates of *P. mirabilis* were isolated from in-patients while 12 were isolated from out-patients. *P. vulgaris* recorded 11 isolates from in-patients and 7 isolates from out-patients. The relationship between patients category and prevalence of *Proteus* species was not statistically significant (p > 0.05 p=0.611, X^2=0.984, df=1).

From Table 3, a total of 45 (73.8%) isolates of *Proteus* species were isolated from male patients while 16 (26.2%) were isolated from female patients. Out of 45 isolates of *Proteus* species from male patients, *P. mirabilis* recorded 32 (71.1%) while *P. vulgaris* recorded 13 (28.8%). Out of 16 isolates of *Proteus* species isolated from female patients, *P. mirabilis* recorded 11 (68.8%) while *P. vulgaris* recorded 5(31.2%). The relationship between sex of patients and prevalence of *Proteus* species was statistically significant (p<0.05, p=0.005, X^2=10.433, df=2).

Table.2 Distribution of isolates of *Proteus* species according to patient category

	Proteus species		Total	X^2	P value
Patient category	*Proteus mirabilis*	*Proteus vulgaris*			
In-patient (n=212)	31 (73.8%)	11 (26.2%)	42 (68.9%)	0.984	0.611
Out-patient (n=110)	12 (63.2%)	7 (36.8%)	19 (31.1%)		
Total	43 (70.5%)	18 (29.5%)	61 (100%)		

n=number tested, Pearson Chi-square test was used to compare the association between variables. P < 0.05 was considered statistically significant.

Table.3 Distribution of isolates of *Proteus* species according to patients' sex

	Proteus species		Total	X^2	P value
Sex	*Proteus mirabilis*	*Proteus vulgaris*			
Male (n=178)	32 (71.1%)	13 (28.8%)	45 (73.8%)	10.433	0.005
Female (n=144)	11 (68.8%)	5 (31.2%)	16 (26.2%)		
Total	43 (70.5%)	18 (29.5%)	61(100%)		

n=number tested, Pearson Chi-square test was used to compare the association between variables. P < 0.05 was considered statistically significant.

Table.4 Distribution of isolates of *Proteus* species according to patients' age groups

Age groups (yrs)	*Proteus* species		Total	X^2	P value
	Proteus mirabilis	*Proteus vulgaris*			
<1 (n=10)	1(100%)	0 (0%)	1 (1.6%)	12.195	0.837
1 – 10 (n=37)	5 (83.3%)	1 (16.7%)	6 (9.8%)		
11 – 20 (n=31)	3 (60%)	2 (40%)	5 (8.2%)		
21 – 30 (n=64)	4 (50%)	4 (50%)	8 (13.1%)		
31 – 40 (n=92)	15 (71.4%)	6 (28.6%)	21 (34.4%)		
41 – 50 (n=33)	5 (62.5%)	3 (37.5%)	8 (13.1%)		
51 – 60 (n=30)	7 (87.5%)	1 (12.5%)	8 (13.1%)		
61 – 70 (n=14)	1 (50%)	1 (50%)	2 (3.3%)		
71 – 80 (n=4)	0 (0%)	0 (0%)	0 (0%)		
81 – 90 (n=7)	2 (100%)	0 (0%)	2 (3.3%)		
Total	43 (70.5%)	18 (29.5%)	61 (100%)		

n=number tested, Pearson Chi-square test was used to compare the association between variables. $P < 0.05$ was considered statistically significant.

From Table 4, the number of isolated *Proteus* species was highest among patients aged 31-40 years and lowest among patients aged <1 years and those between 71-80 years. The relationship between age of patients and prevalence of *Proteus* species was not statistically significant ($p > 0.05$, $p=0.837$, $X^2=12.195$, $df=18$).

Table 5a shows the sensitivity, intermediates and resistance profile of the *Proteus* isolates while table 5b compares sensitivity and resistance profile *Proteus* isolates.

From Table5b, Ceftazidime (30µg) showed the highest activity against *P. mirabilis* isolated with percentage sensitivities of 74.4% followed by levofloxacin (5ug) with sensitivity of 58.1%. Levofloxacin was most sensitive against *P. vulgaris* isolated, with percentage sensitivity of 61.1% followed by ceftazidime and gentamicin with sensitivity of 38.9% each. Nitrofurantoin had the highest resistance on *P. mirabilis* with percentage resistance of 93% followed by cefotaxime and nalidixic acid with percentage resistance of 90.7% each. Similarly, nalidixic acid and nitrofurantoin had the highest resistance on *P. vulgaris* isolated with percentage resistance of 94.4% and 88.9% respectively. There was a statistically significant difference in the sensitivity and resistance of the tested antibiotics to *P. mirabilis* ($p < 0.05$, $p=0.000$, $X^2=157.379$, $df=12$). There was also a statistically significant difference in the sensitivity and resistance of the tested antibiotics to *P. vulgaris* ($p < 0.05$, $p=0.000$, $X^2=42.923$, $df=12$).

Table.5a Antibiotic Susceptibility profile of *Proteus species* isolates

		Proteus mirabilis			***Proteus vulgaris***		
Antibiotics	**Disc conc**	Sensitive (%)	Intermediate (%)	Resistant (%)	Sensitive (%)	Intermediate (%)	Resistant (%)
Levofloxacin	LEV (5ug)	25 (58.1%)	8 (18.6%)	10 (23.3%)	11 (61.1%)	3 (16.7%)	4 (22.2%)
Ceftazidime	CAZ(30ug)	32 (74.4%)	2 (4.7%)	9 (20.9%)	7 (38.9%)	2 (11.1%)	9 (50%)
Ceftriaxone	CTR(30ug)	19 (44.2%)	11 (25.6%)	13 (30.2%)	3 (16.7%)	2 (11.1%)	13 (72.2%)
Ofloxacin	OFL (5ug)	14 (32.6%)	10 (23.3%)	13 (30.2%)	4 (22.2%)	3 (16.7%)	11 (61.1%)
Ciprofloxacin	CPR (5ug)	17 (39.5%)	3 (7.0%)	24 (55.8%)	1 (5.6%)	3 (16.7%)	14 (77.8%)
Gentamicin	GEN (10ug)	10 (23.3%)	4 (9.3%)	30 (69.8%)	7 (38.9%)	2 (11.1%)	9 (50%)
Cefixime	CXM (5ug)	13 (30.2%)	1 (2.3%)	29 (67.4%)	2 (11.1%)	3 (16.7%)	13 (72.2%)
Cefuroxime	CRX (30ug)	5 (11.6%)	9 (20.9%)	31 (72.1%)	2 (11.1%)	2 (11.1%)	14 (77.8%)
Cefotaxime	CTX (30ug)	3 (7.0%)	1 (2.3%)	39 (90.7%)	3 (16.7%)	2 (11.1%)	13 (72.2%)
Augmentin	AUG (20ug)	4 (9.3%)	1 (2.3%)	38 (88.4%)	2 (11.1%)	2 (11.1%)	14 (77.8%)
Imipenem	IMP (10ug)	3 (7.0%)	2 (4.7%)	38 (88.4%)	2 (11.1%)	1 (5.6%)	15 (83.3%)
Nacidixic acid	NA (10ug)	3 (7.0%)	1 (2.3%)	39 (90.7%)	1 (5.6%)	0 (0%)	17 (94.4%)
Nitrofurantoin	NIT (300g)	1 (2.3%)	2 (4.7%)	40 (93.0%)	1 (5.6%)	0 (0%)	16 (88.9%)

From Table 6, a total of 49 (80.3%) of the *Proteus* isolates were found to be multidrug resistant (MDR). 31 (72.1%) MDR strains of *P. mirabilis* were isolated while 18 (100%) MDR strains of *P. vulgaris* were isolated. The relationship between *Proteus* isolates and multi-drug resistance ability was statistically significant. ($p < 0.05$, $p=0.012$, $X^2=6.253$, $df=1$). From Table 7, out of the 31 MDR strains of *P. mirabilis* isolated, 17 (54.8%) were confirmed to produce ESBL while 14 (45.2%) were confirmed to be ESBL non-producers. Out of the 18 MDR strains of *P. vulgaris* isolated, 10 (55.6%) were confirmed to produce ESBL while 8 (44.4%) were confirmed to be ESBL non-producers. The relationship between MDR stains of *Proteus* species and ESBL production was not statistically significant ($p > 0.05$, $p=0.961$, $X^2=0.002$, $df=1$).

Table 8 show gene frequency and percentage frequency. TEM and CTX-M genes recorded the highest frequency with 37.5% each while SHV genes recorded 25.0%.

Table.5b Comparing the Sensitivity and Resistance profile of *Proteus* isolates

		Proteus mirabilis			***Proteus vulgaris***		
Antibiotics	**Disc conc**	Sensitive (%)	Resistant (%)	P-value	Sensitive (%)	Resistant (%)	P-value
Levofloxacin	LEV (5ug)	25 (58.1%)	10 (23.3%)	0.000	11 (61.1%)	4 (22.2%)	0.000
Ceftazidime	CAZ(30ug)	32 (74.4%)	9 (20.9%)		7 (38.9%)	9 (50%)	
Ceftriaxone	CTR(30ug)	19 (44.2%)	13 (30.2%)		3 (16.7%)	13 (72.2%)	
Ofloxacin	OFL (5ug)	14 (32.6%)	13 (30.2%)		4 (22.2%)	11 (61.1%)	
Ciprofloxacin	CPR (5ug)	17 (39.5%)	24 (55.8%)		1 (5.6%)	14 (77.8%)	
Gentamicin	GEN (10ug)	10 (23.3%)	30 (69.8%)		7 (38.9%)	9 (50%)	
Cefixime	CXM (5ug)	13 (30.2%)	29 (67.4%)		2 (11.1%)	13 (72.2%)	
Cefuroxime	CRX (30ug)	5 (11.6%)	31 (72.1%)		2 (11.1%)	14 (77.8%)	
Cefotaxime	CTX (30ug)	3 (7.0%)	39 (90.7%)		3 (16.7%)	13 (72.2%)	
Augumentin	AUG (20ug)	4 (9.3%)	38 (88.4%)		2 (11.1%)	14 (77.8%)	
Imipenem	IMP (10ug)	3 (7.0%)	38 (88.4%)		2 (11.1%)	15 (83.3%)	
Nalidixic acid	NA (10ug)	3 (7.0%)	39 (90.7%)		1 (5.6%)	17 (94.4%)	
Nitrofurantoin	NIT (300g)	1 (2.3%)	40 (93.0%)		1 (5.6%)	16 (88.9%)	

Table.6 Distribution of MDR isolates of *Proteus* species

	Multi-Drug Resistance				
***Proteus* species**	MDR	Non-MDR	Total	X^2	P value
P. mirabilis	31(72.1%)	12 (27.9%)	43 (100%)	6.253	0.012
P. vulgaris	18 (100%)	0 (0%)	18 (100%)		
Total	49 (80.3%)	12 (19.7%)	61(100%)		

Pearson Chi-square test was used to compare the association between variables. $P < 0.05$ was considered statistically significant.

Table.7 Distribution of ESBL producers and ESBL non-producers among MDR strains of *Proteus* species

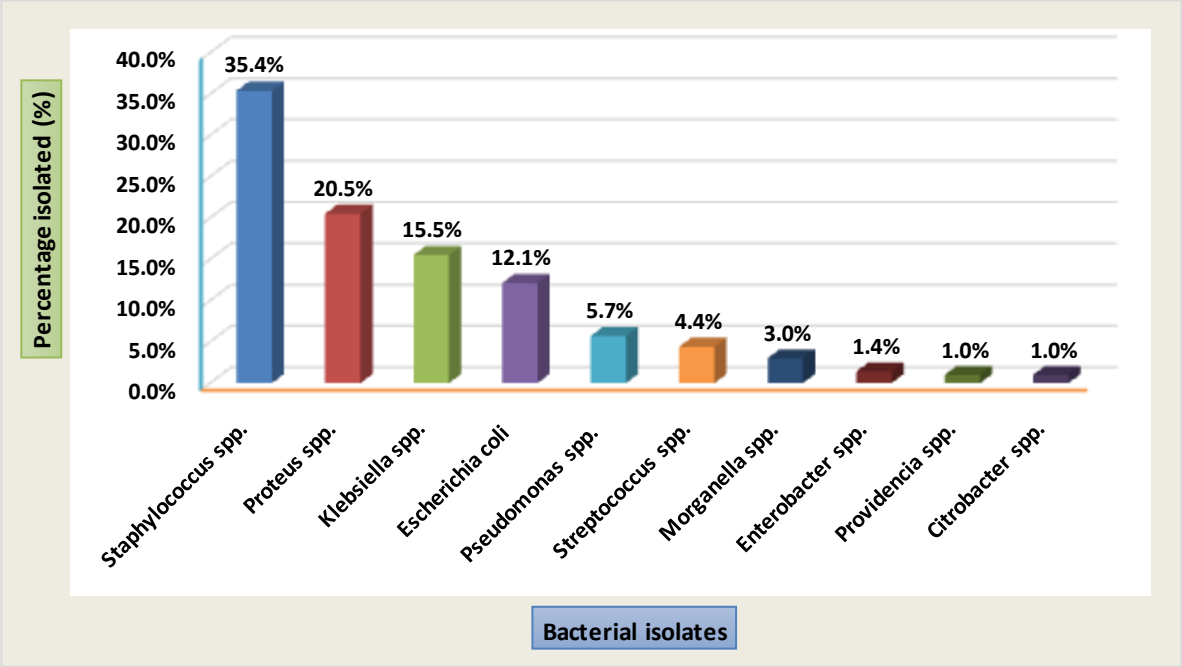
		ESBL production			
***Proteus* species**	MDR Strains	ESBL producer	ESBL non-producer	X^2	P value
P. mirabilis	31 (63.3%)	17 (54.8%)	14 (45.2%)	0.002	0.961
P. vulgaris	18 (36.7%)	10 (55.6%)	8 (44.4%)		
Total	49 (100%)	27 (55.1%)	22 (44.9%)		

Pearson Chi-square test was used to compare the association between variables. $P < 0.05$ was considered statistically significant.

Table.8 Gene frequency and percentage frequency of the *Proteus* species

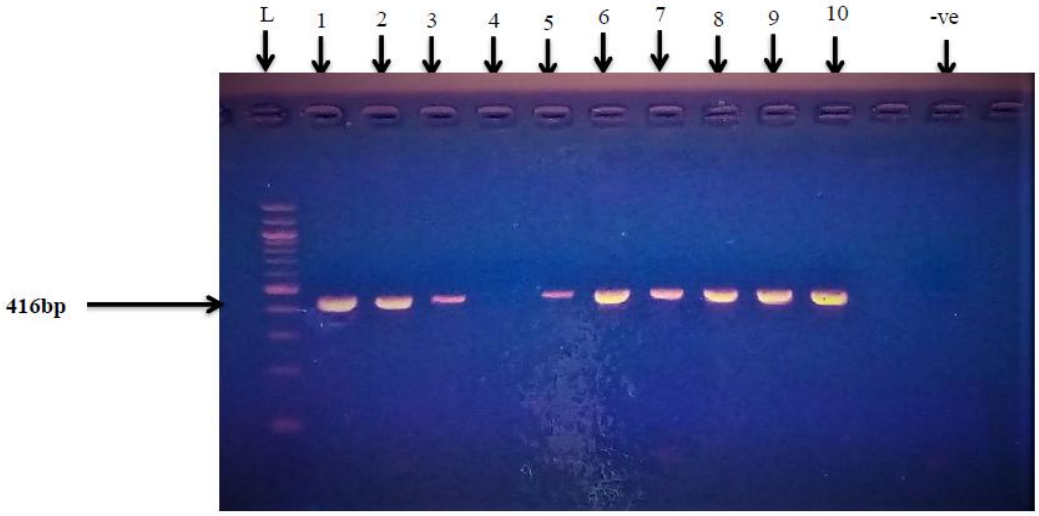
Gene type	Specific Gene	Proteus species		Total frequency		% frequency
		P. mirabilis	*P. vulgaris*			
ESBL gene	TEM	7	2		9	37.5
	SHV	4	2		6	25.0
	CTX-M	7	2		9	37.5
Total		**18**	**6**		**24**	**100**

Fig.1 Distribution of bacteria isolates



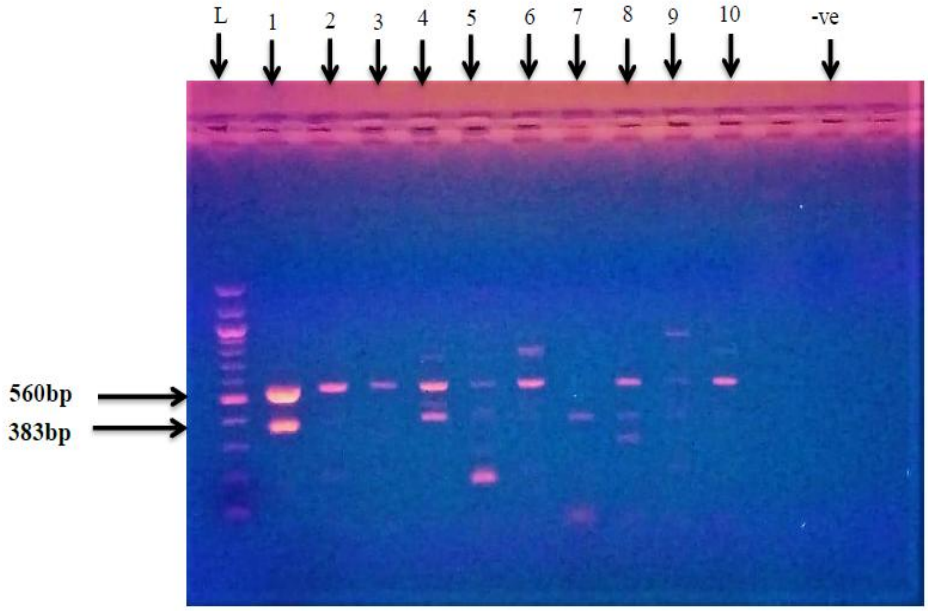
Staphylococcus spp was the most isolated bacteria with a frequency of 105 (35.4%), followed by *Proteus* spp: 61 (20.5%), *Klebsiella* spp: 46 (15.5%), *Escherichia coli*: 36 (12.1%). *Providencia* spp and *Citrobacter* spp were the least isolated bacteria with the frequency of 3 (1.0%) each.

Fig.2 BlaTEM gene (416bp) gel image



All the screened DNA, except the DNA loaded in Lane 4, were **positive** for the Bla TEM ESBL gene

Fig.3 CTX-M gene (560bp) and SHV gene (383bp) gel image



All the screened DNA, except the DNA loaded in Lane 7, were *positive* for the Bla CTX-M gene
*The DNA loaded in lanes 1, 4,5,7, 8, and 9 were *positive* for Bla SHV gene, while the DNA loaded in lanes 2,3,6 and 10 were *negative* for the Bla SHV gene
*The other unaccounted bands in the gel could as a result of primer dimmers.

There was a case of 2 days old baby in newborn intensive care unit, whose *Proteus* isolates were also found to be multidrug resistance, thus a case of nosocomial infection. This should be a worrisome development to health care professionals, highlighting that the control of nosocomial infection must be drastically improved in Nigerian hospitals.

The high level of multidrug resistant observed in *Proteus* isolates from this study confirms earlier

reports that multidrug resistant is increasing in Nigeria among *Proteus* species and other members of the Enterbactereceae family (Alabi *et al.,* 2017; Ogbulu *et al.,* 2011; Okesola *et al.,* 2009). Also according to Antimirobial Resisitant Collabolators (2019) 4.95 million deaths were associated with bacterial antimicrobial resistant globally in 2019. Death rates attributes to resistant is highest in Western-Sub Sahara Africa at 27.3 deaths per 100,000 and lowest in Australia at 6.5 deaths per 100,000.

Antimicrobial resistance poses a major threat to human health around the world. 17 (54.8%) of *P. mirabilis* were confirmed to produce ESBL while 14 (45.2%) were confirmed to be ESBL non-producers. Out of the 18 MDR isolates of *P. vulgaris*, 10 (55.6%) were confirmed to produce ESBL while 8 (44.4%) were confirmed to be ESBL non-producers (Table 7). The relationship between MDR stains of *Proteus* species and ESBL production was not statistically significant ($p > 0.05$, $p=0.961$, $X^2=0.002$, $df=1$). Phenotypic detection of ESBL production from this study showed that more than fifty percent of the *Proteus* isolates were positive for ESBL production. This is in line with Feglo *et al.,* (2010) studies in Ghana in which *P. mirabilis* and *P. vulgaris* were 77% and 65.4% positive for ESBL production.

From this study not all the multidrug resistant isolates were positive for ESBL production which shows that there other means of acquiring resistant in bacteria apart from ESBL production. The emergence of antibiotic resistance is highly correlated with selective pressure resulting from inappropriate use of these drugs. In addition to selective pressure, change in cell permeability, efflux or altering of antigenic target and horizontal gene transfer also contribute to antibiotic resistant (Livermore, 2003).

Molecular detection of ESBLs genes

Polymrase chain reaction revealed the presence of ESBLs genes: TEM (37.5%), CTX-M (37.5%) and SHV (25.0%) (Table7). ESBL genes like TEM, SHV and CTX-M are the main beta-lactameses especially CTX-M with an emergence prevalence reaching rates over 85% in some region of the world (Conton *et al.,* 2012). In the USA, the US centers for Disease Control and Prevention estimated that 197, 400 infections and 9100 deaths occurred in hospitalized patients in 2017 owing to Enterobacterales producing ESBL (CDC, 2019). ESBL production provides a high level of resistance to third generation cephalosprorins and aztreonan in *Proteus* species. The first ESBL producing *P. mirabilis* isolates was described in France in 1999 (Dechamps *et al.,* 2000). In Belley *et al.,* studies in USA and Europe, ESBL was the most prevalence beta-lactan resistant determinant with frequency of 11.9% and the ESBL genes were present in 33.3% of third generation cephalosporin resistant *P. mirabilis* isolates (Belly *et al.,* 2021). In Taiwan, ESBL (mainly CTX-M) producing *Proteus* specie isolates has increased approximately three fold from 6.2% in 2005 to 20% in 2009 (Huang *et al.,* 2015). In Nigeria, some authors (Alabi *et al.,* 2017; Ogbulu *et al.,* 2011; Okesola *et al.,* 2009) also reported high level of ESBL production in *Proteus* specie and other Enterobacterales.

Proteus species ranks the highest Gram negative bacteria that cause wound infections and most strains harbour ESBL genes and have develop multidrug resistance. The rate of development of new antimicrobial agents has failed to keep pace with the rate at which bacteria become resistant to antibiotics. There is need to reduce unnecessary antibiotic prescribing, both qualitatively and quantitatively. There is also need to optimize control measures to minimize the risk of spread of resistant bacteria and need for early detection of pathogens. These approaches will help to prevent spread of multidrug resistance.

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