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Prevalence and Antibiotic Susceptibility Pattern of *Proteus Species* Isolated from Clinical Specimens from Selected Hospitals in Jigawa State, North-West Nigeria.

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Abstract

Proteus is a genus of Gram-negative bacteria belonging to the family of *Enterobactericeae.* Antibiotic resistance of bacteria is commonly seen in daily medical practice with multi-drug resistant Gram-negative bacteria posing the greatest threat to human health. To determine the prevalence of *Proteus species* isolated from clinical samples and antibiotics sensitivity pattern from selected hospitals in Jigawa state, Nigeria. The study was carried out on all *Proteus* organisms isolated from clinical samples from selected hospitals in Jigawa State. The clinical samples were aseptically collected and inoculated on plates of Blood agar, and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37^0C for 24 hours. Suspected *Proteus* colonies were identified through biochemical tests according to Barrow and Felthan. Modified Kirby-Bauer disk diffusion method was used to test the susceptibility of the *Proteus* isolates to nine different antimicrobial agents. The diameter of zone of growth-inhibition was observed, measured and recorded sensitive (S) or resistance (R) according to CLSI, 2012. It was found that 191 isolates were identified as *Proteus species* representing 10.3% prevalence across the state. *Proteus mirabilis* were observed to be significantly more susceptible to Ampicillin (P= 0.003), Gentamycin (P=0.024) and Cotrimoxazole (P=0.014). Compared to *Proteus mirabilis*, *Proteus vulgaris* isolates were found to be more susceptible to the cephalosporins and fluoroquinolone, with a significantly higher susceptibility profile observed specifically with cefotaxime (P = 0.041). All the *Proteus species* encountered were susceptible to Imipenem (100%). The prevalence of *Proteus species* is on the increase in Jigawa state and by extension North-west Nigeria. Prudent use of antimicrobial agents is advocated in order to tame the trend.

Keywords; *Proteus species*, prevalence, multi-drug resistant, Jigawa state

Introduction

Proteus is a genus of Gram-negative bacteria belonging to the family of *Enterobactericeae. They* are distinguishable from most other genera by their ability to swarm across an agar surface (Jacobsen *et al.*, 2008). *Proteus* is widespread in the environment and makes up part of the normal flora of the human gastrointestinal tract. *Proteus* ranks third as the cause of hospital-acquired infections (Stamm, 1999). Three species: *P. vulgaris, P. mirabilis, and P. retgerri* are opportunistic human pathogens (Guentzel, 1996). *Proteus species* are the major cause of diseases acquired outside the hospital, where many of these diseases eventually require hospitalization (De champs *et al.,* 2000).

Significantly, there has been a report in Europe on the evolution and spread of multidrug-resistant *Proteus mirabilis* clone with chromosomal AmpC-type beta-lactamase (Luzzaro *et al.*, 2009). In addition, a report exists on a wide diversity between institutions in the prevalence of pathogens and in their antimicrobial susceptibility (Fridkin, 2001) and it is said to be particularly worse in resource-poor countries where sale of antibiotics is poorly controlled (Onile, 1997).

Based on the literatures above, multidrug-resistant pathogen represents a growing public health problem in the world. In fact, with the increasing phenomenal evolution and multidrug-resistance of many bacterial pathogens (with special interest on multidrug-resistance *Proteus* spp.), there is the need for regular review of antimicrobial sensitivity pattern among clinically isolated *Proteus* spp. for accurate decision on antibiotic prescription. Resistant to commonly used antibiotics in this environment increases at an alarming rate. The broad knowledge of their occurrence and potential effects in managing infectious diseases is low among the health care providers. Antibiotic susceptibility testing is not frequently carried out in most of the health facility, proper reporting, necessary precautions to avoid their spread is lacking in many of our hospitals (Yusuf *et al*., 2013) Reports on the prevalence and antibiogram profile of *Proteus species* are few in Jigawa state, Nigeria. In view of this, this study was undertaken to determine the prevalence of *Proteus species* isolated from clinical samples and antibiotics sensitivity pattern from selected hospitals in Jigawa state, Nigeria.

Materials and methods

Study Area

This study was carried out on all *Proteus* organisms isolated from clinical samples (wound swabs, urine, ear swabs, high vaginal swab/endo-cervical swab, sputum, aspirates) from selected hospitals in Jigawa State. The hospitals include Hadeija General Hospital, Dutse General Hospital, Rasheed Shekoni Specialist Hospital Dutse (RSSH) and Federal Medical Centre (FMC) Birnin-kudu between November, 2021 to August, 2022 and sample size of 191 samples was used based on the reports of 14.6% prevalence rates of *Proteus* infections in Kano (Yusha'u *et al.*, 2010). The sample size was determined using the formula described by (Naing *et al., 2006)*.

Cultivation and Identification

The clinical samples were aseptically collected and inoculated on plates of Blood agar, and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37^{0}C for 24 hours. Suspected *Proteus* colonies were identified through biochemical tests according to Barrow and Felthan, (2003). Based on whether they were positive for Indole production; H2S gas production; Citrate utilization and urease reactions; and negative for lactose fermentation. Indole production differentiates P. vulgaris isolates from the other species; P. mirabilis and P. penneri.

Antimicrobial susceptibility test

Modified Kirby-Bauer disk diffusion method (Cheesbrough, 2012) was used to test the susceptibility of the *Proteus* isolates to different antimicrobial agents (obtained from Mast Diagnostics, UK): Amoxicillin (30 µg), ampclox (10 µg), augmentin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), co-trimoxazole (25 µg) and imipenem (10 µg). By means of Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs were applied to the surface of the inoculated agar and the plates incubated overnight at 37^{o}C. The diameter of zone of growth-inhibition was observed, measured and recorded sensitive (S) or resistance (R) according to (CLSI, 2012).

Ethical Clearance

Ethical approval was obtained from the ethical committee of Federal Medical Centre, Birnin Kudu Hospital management and Jigawa state ministry of Health, Dutse.

Data analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS® 20, USA). Descriptive statistics was used to describe the relevant variables and comparisons performed using chi-square test.

Results

Prevalence of *Proteus spp* in clinical specimens

Irrespective of location and sample type, the prevalence of *Proteus spp* in this study was 10.3%, *Klebsiella spp* was the most abundant (16.8%) isolates recovered from specimen. With respect to type of specimen, wound swab had the highest number of *Proteus spp* in all location of study, just as the least number of *Proteus spp* was recorded with High vaginal swab (Table 1).

Antibiotic susceptibility profile of bacteria isolates

All *Proteus species* were found to be susceptible to imipenem. Compared to *Proteus mirabilis*, *Proteus vulgaris* isolates were found to be more susceptible to the cephalosporins and fluoroquinolone, with a significantly higher susceptibility profile observed specifically with cefotaxime (P = 0.041). However,

they were generally observed to be more resistant to the aminopenicillins (AUG, AMX), aminoglycoside (GEN) and sulphonamides (SXT). *Proteus mirabilis* were observed to be significantly more susceptible to Ampicillin (P= 0.003), Gentamycin (P=0.024) and Cotrimoxazole (P=0.014). (Table 2).

Prevalence of MDR bacteria

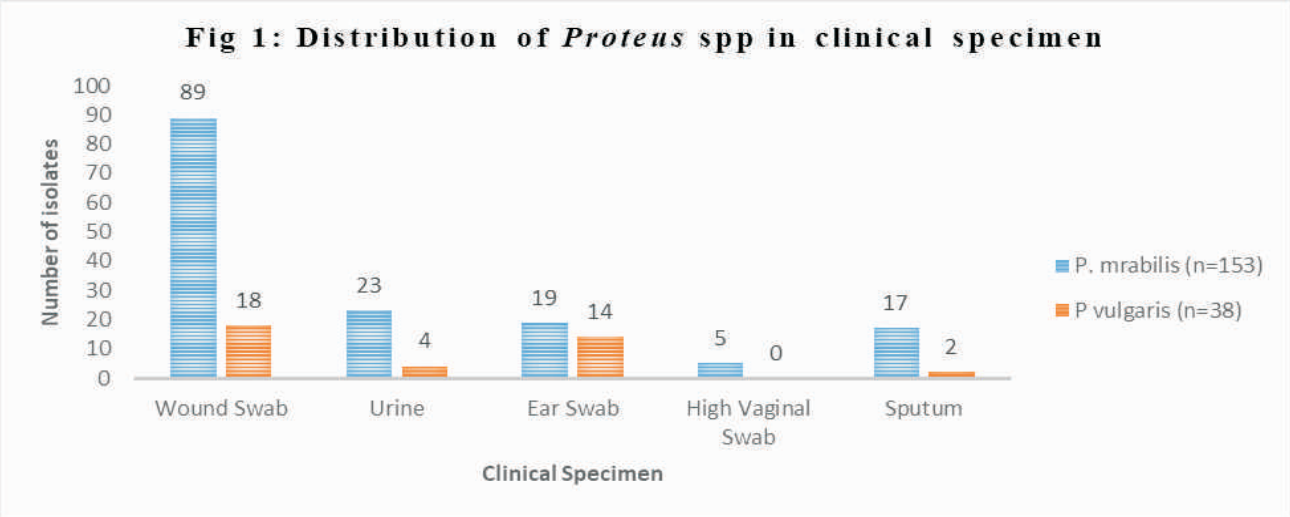
A total of 27 (17.6%) *Proteus mirabilis* isolates were found to be resistant to three or more classes of antibiotics used in this study. This was higher than 5.3% observed among *Proteus vulgaris* isolates. Compared to *Proteus vulgaris, Proteus mirabilis* were about four times (OR=3.857) more likely to be multi-drug resistant, albeit the difference was not statistically significant (P = 0.075) (Table 3).

Distribution of *Proteus species* based on clinical specimens and locations

A total of 191 *Proteus* spp (comprising of 153 and 38 isolates of *Proteus mirabilis* and *Proteus vulgaris* isolates respectively) were recovered from five (5) types of clinical samples. *Proteus mirabilis* and *Proteus vulgaris* were isolated from all clinical specimen except high vaginal swab where only *Proteus vulgaris* was found. Wound swabs had the greatest number of bacteria while high vagina swab had the least. Federal Medical Centre (FMC) Birnin-kudu had the highest proportion (36%) of *Proteus spp,* while the least (18%) was found in Rasheed Shekoni Specialist Hospital (RSSH) Dutse. (Figure 1 and 2)

Table 1: Prevalence of *Proteus species* in clinical specimens

Location	Specimen (n)	*Proteus spp (%)*	*Klebsiella spp (%)*	*E. coli (%)*	*P. aeruginosa (%)*	*C. feundii (%)*	*Enterobacter spp (%)*	*Candid albicans (%)*
FMC, BKD	Wound swab (320)	35 (10.9)	49 (15.3)	66 (20.6)	23 (7.18)	06 (1.8)	05 (1,5)	06 (1.8)
	Ear Swab (231)	17 (7.4)	42 (18.2)	25 (10.8)	11 (4.7)	03 (1.3)	05 (2.2)	08 (3.5)
	Sputum (107)	06 (5.6)	27 (25.2)	12 (11.2)	06 (5.6)	01 (0.9)	01 (0.9)	14 (13.1)
	Urine (110)	10(9.1)	12 (10.9)	15 (13.6)	03 (2.7)	04 (3.6)	03 (2.7)	12(10.9)
	HVS (54)	-	08 (14.8)	12 (22.2)	-	-	02 (3.7)	04 (7.4)
General Hospital, Hadeija	Wound swab (112)	21 (18.8)	23 (20.5)	25 (22.3)	09 (8.0)	03 (2.6)	02 (1.8)	08 (7.14)
	Ear Sw ab (96)	10 (10.4)	16 (16.6)	12 (12.5)	03 (3.1)	01 (1.0)	03 (3.1)	15 (15.6)
	Sputum (85)	04 (4.7)	13 (15.2)	04(4.7)	01(1.2)	02(2.4)	01(1.2)	17(20.0)
	Urine (68)	07 (10.3)	10 (14.7)	14 (20.5)	02 (2.9)	01 (1.4)	01 (1.4)	20 (29.4)
	HVS (20)	03(15.0)	02 (10.0)	03 (15.0)	-	-	01 (5.0)	07 (35.0)
General Hospital, Dutse	Wound swab (98)	23 (23.4)	16 (16.3)	10 (10.2)	06 (6.1)		-	-
	Ear Swab (56)	02 (3.5)	12 (21.4)	05 (8.9)	06 (10.7)	-	-	04 (7.1)
	Sputum (48)	05 (10.4)	09 (18.7)	04 (8.3)	01 (2.1)	.	01 (2.1)	12 (25.0)
	Urine (32)	05 (15.6)	04 (12.5)	06 (18.8)	-	01 (3.1)	-	06 (18.8)
	HVS (12)	02 (16.6)	08 (66.6)	01 (8.3)	-	01 (8.3)	-	03 (25.0)
RSSH, Dutse	Wound swab (115)	28 (24.3)	21 (18.3)	16 (13.9)	08 (6.9)	02 (1.7)	01 (0.8)	06 (5.21)
	Ear Swab (105)	03 (2.9)	16 (15.2)	09 (8.5)	06 (5.7)	-	-	15 (14.3)
	Sputum (94)	05 (5.3)	11 (11.7)	06 (6.4)	03 (3.2)	01 (1.1)	-	04 (4.3)
	Urine (65)	05 (7.7)	09 (13.8)	11 (16.9)	01 (1.5)	-	-	11 (16.9)
	HVS (26)	-	04 (15.4)	05 (19.2)	-	-	-	08 (30.8)
TOTAL	(1854)	191 (10.3)	312(16.8)	247 (13.3)	89 (4.8)	26 (1.4)	26 91.4)	180 (9.7)



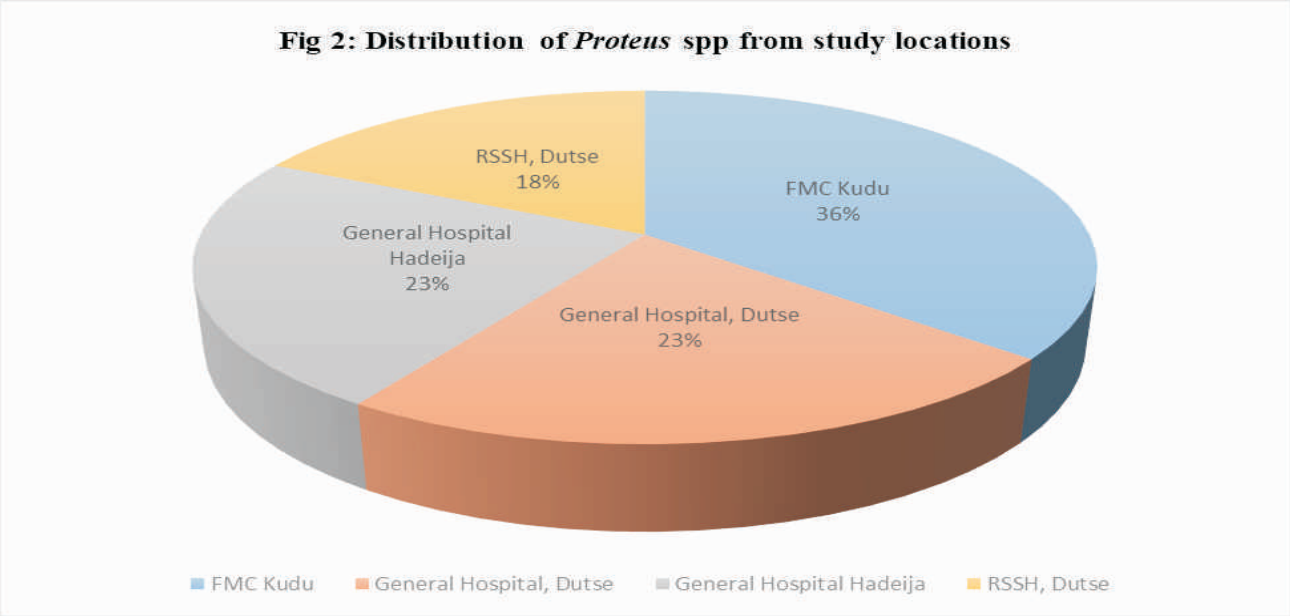


Table 2: Antibiotic susceptibility profile of bacteria isolates

Bacterial agents (N)	IMP [30mg] n (%)	CTX [30mg] n (%)	CRO [30mg] n (%)	CPX [30mg] n (%)	AUG [30mg] n (%)	AMX [30mg] n (%)	APX [10mg] n (%)	GEN [30mg] n (%)	SXT [25mg] n (%)
Proteus mirabilis (153)	153 (100%)	115 (75.2)	108 (70.6)	108 (70.6)	114 (74.5)	115 (75.2)	119 (77.8)	110 (71.9)	107 (69.9)
Proteus vulgaris (38)	38 (100%)	32 (84.2)	33 (86.8)	31 (81.6)	25 (65.8)	19 (50.0)	31 (81.6)	4 (10.5)	4 (10.5)
	ND	P=0.236	P=0.041*	P=0.173	P=0.279	P=0.003*	P=0.609	P=0.024*	P=0.014*

N- number of bacteria isolates; **n**- number of susceptible isolates; **IMP**-imipenem; **CTX**- cefotaxime; **CRO**-Ceftriaxone; **CPX**-Ciprofloxacin; **AUG**-Augmentin; **AMX**- Amoxicillin; **APX**-Ampicillin; **GEN**-Gentamycin; **SXT**- Cotrimoxazole; *- statistically significant.

Table 3: Prevalence of MDR bacteria

BACTERIA	N	MDR Pos (%)	OR	95%CI	P-value
Proteus mirabilis	153	27 (17.6)	3.857	0.875, 17.007	0.075
Proteus vulgaris	38	2 (5.3)			

N- number of isolates; **MDR**- multidrug resistant; **OR**-odd ratio; **CI**- confidence interval

Discussion

Antibiotic resistance of bacteria is commonly seen in daily medical practice with multi-drug resistant Gram-negative bacteria posing the greatest threat to human health (Gayathri *et al.*, 2012). Multi drug-resistant (MDR) Gram negative bacilli induced infections have been reported with an increasing frequency in tertiary health care providers in Nigeria and they have been found to be associated with a significant morbidity and mortality (Yusuf *et al.,* 2012). Beta lactam antibiotics are the most predominantly prescribed antibiotics to treat bacterial infections, especially in Nigeria hospitals (Yusuf and Arzai, 2011).

A total of 1854 clinical samples were collected and analyzed from November, 2021 to August, 2022 (10 months) from selected hospitals in Jigawa state. It was found that 191 isolates were identified as *Proteus species* representing 10.3% prevalence across the state. This result was in consonance with other studies, which reported a prevalence rate of 8.4% of *Proteus species* collected from clinical samples in Nigeria by (Feglo *et al.,* 2010) and prevalence rate of 9.4% in Kano by (Yusha'u *et al.*,2010). However, our finding is at variance with the reports with a prevalence rate of 1.12% in India by (Jitendra *et al.,* 2013) and 14.6% *Proteus species* in Benin, south-south Nigeria by Orhue (2014). This variation could be attributed to differences in time of collection of isolates and differences in study populations and designs. Furthermore, Jigawa state being a less developed state in this region.

Wound swabs had the highest number of bacteria while high vagina swab had the least which was in the same trend with many past studies. This result agrees with the report where wound swab recorded the highest percentage of 64.5% by (Chow, 1979) and 49.5% of wound swab (Saleh and Hatem, 2013). However, this was in contrast with similar studies conducted in Europe and Asia which showed *Proteus species* to be more commonly encountered in urine than any other clinical samples (Orret, 1999; Reslinski, 2005). Also, there is a report of higher isolation rate of 37.1% from urine samples in a research conducted in Ibadan, western Nigeria and 47% isolation rate from urine in a research conducted on Kano Northwest, Nigeria (Okesola and Adeniji,2010; Yusuf *et al.*, 2013).

It was found that 129 of positive *Proteus species* isolates were from Male patients while 62 were recovered from Female patients. Males, according to this study were therefore found to be more vulnerable than Females in acquiring *Proteus* infections. This agrees with the findings where male recorded 68% and female recorded 32% isolation rates and where male recorded 52% and female 48% rate of *Proteus species* in Kano (Jitendra *et al.,* 2013; Yusuf *et al.*, 2013). This gender distribution was different from that reported by (Feglo *et al.*, 2010) of 43% of *Proteus species* collected from clinical samples were recovered from males while 57% from females. Although, there is no adequate studies concerned with gender distribution of *Proteus* infections, the result with male patients' predominance observed in this study is most likely due to preferential treatment and attention given to females' right from childhood in this part of the country, so males are exposed more to acquiring infectious diseases and other environmental/occupational hazards.

Nine different antibiotics (IMP-imipenem; CTX- cefotaxime; CRO-Ceftriaxone; CPX-Ciprofloxacin; AUG-Augmentin; AMX-Amoxicillin; APX-Ampicillin; GEN-Gentamycin; SXT- Cotrimoxazole) representing different classes of antibiotics were tested on

Proteus species. *Proteus mirabilis* were observed to be significantly more susceptible to Ampicillin (P= 0.003), Gentamycin (P=0.024) and Cotrimoxazole (P=0.014). Compared to *Proteus mirabilis*, *Proteus vulgaris* isolates were found to be more susceptible to the cephalosporins and fluoroquinolone, with a significantly higher susceptibility profile observed specifically with cefotaxime (P = 0.041). However, they were generally observed to be more resistant to the aminopenicillins (AUG, AMX), aminoglycoside (GEN) and sulphonamides (SXT). These findings could be as a result of wide abuse and misuse of these drugs in humans and animals over a long period of time in this region. Furthermore, these drugs are generally cheap and accessible at over the counter precipitating their abuse and the pattern of result observed. This result agrees with the report from other studies. (Jitendra *et al.,* 3013; Musa *et al.,* 2019). Furthermore, it was observed from this study that all the *Proteus species* encountered were susceptible to Imipenem (100%). This result agrees with the work from Sudan where most of the *Proteus species* isolated were sensitive to meropenem (Musa *et al.*, 2019). Similar results were reported by other studies elsewhere (Chow, 1979; Newman *et al.*, 2011). The study showed that imipenem and third generation cephalosporins were still the most effective antibiotics against *Proteus species*. However, data showed that usage of these drugs in the hospitals across the state is low probably due to its high cost considering the economic status of most of the patients. Thus, these drugs could be used to treat infections caused by *Proteus species* especially in cases of life-threatening infections such as urinary tract infections and pulmonary pneumonia. However, unless antibiotic therapy is restricted and controlled legally, misuse in the form of self-medication may cause the spread of resistance, which will result in the prevalence of resistance against effective antibiotics such as third generation cephalosporin and imipenem. All the two *Proteus species* encountered were found to be multi drug resistant. Multi drug resistance was defined as resistance to at least three antibiotics (Gold and Moellering, 1996). *P. mirabilis* showed the maximum MDR with 75% and *P. vulgaris* 36%. Compared to *Proteus vulgaris, Proteus mirabilis* were about four times (OR=3.857) more likely to be multi-drug resistant, albeit the difference was not statistically significant (P = 0.075). It has been reported that MDR in bacteria generally due to multiple transposons and plasmids bearing genetic determinants for different mechanisms of resistance (Gold and Moellering, 1996).

Conclusion

This study discovered low prevalence of *Proteus species* in Jigawa state. Highest number of *Proteus species* were recovered from wound swab and male participants. Third generation cephalosporins and imipenem showed higher susceptibility against *Proteus species* in this environment. Prudent use of antimicrobial agents is advocated in order to tame the trend. This study is therefore, a step towards the generation of national data on the prevalence and antimicrobial resistance patterns of *Proteus species.*

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