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Characterization of *Proteus* Spp. And Plasmid Mediated Resistance Among Hospitalized Patients in Eku, Delta State, Nigeria

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ABSTRACT

The study was conducted to analyze the occurrence and antimicrobial susceptibility profile of Proteus species in blood and wound samples from patients who visited Eku General Hospital in Delta State, Nigeria. A total of 60 samples, 30 from blood and 30 from wound, were collected for the study. The bacteria were identified through bacteriological and biochemical analyses, while antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Plasmid curing was carried out using a sub-inhibitory concentration of 10% sodium dodecyl sulfate on resistant organisms. The study found three isolates including Proteus mirabilis, P. vulgaris, and P. penneri. The results showed that P. mirabilis was the most prevalent in blood (32.3%) and wound (22.6) samples, followed by P. penneri (11.3%), and P. vulgaris (9.7%) in blood sample. The study also found that the highest rate of isolation of the bacterium occurred among individuals aged between 21-35 years, followed by 12-20 years. Result of antibiotic susceptibility test showed high level of resistance in the two most commonly prescribed antibiotics cephalosporins and ampicillin. No resistance was observed in gentamycin making it the best drug for treating empirical infections. However, resistance was mostly carried on plasmids as shown by the loss of resistance after curing by the isolates. Harbouring of resistance on plasmids as huge public health implications including but not limited to spread of resistance genes, as such measures should be put in place to prevent spread.

KEYWORDS: Proteus mirabilis, P. vulgaris, P. penneri. blood, wound, infection.

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INTRODUCTION

Proteus species are peritrichously flagellated bacteria belonging to the Morganellaceae family. They are identified from other Enterobacterales representatives by their intense and remarkable swarming growth on solid medium, which is a consequence of the multicellular differentiation phenomenon [1, 2]. *Proteus mirabilis* is a common bacterium found in the gastrointestinal tracts of humans and animals, but it may also be found in a wide range of habitats, such as soil, water sources, and sewage (3). It has been shown to induce significant infections when combined with Escherichia coli and Klebsiella species (4).

They are opportunistic pathogens that may affect mainly immunocompromised individuals and cause infections of the urinary tract and wounds, bacteremia, abscesses in many organs, or other infections (5). Among the pathogenic species, P. mirabilis is the most common, followed by less frequently isolated *P. vulgaris, P. penneri, P. hauseri*.

P. mirabilis and P.vulgaris are associated with various infections [14]. *P.mirabilis* infection can lead to severe and persistent respiratory, skin, eye, wound, and gastrointestinal infections. Notably, it is responsible for 90% of Proteus infections and is classified as community-acquired infection (6). P. mirabilis is the third most prevalent cause of urinary tract infections (UTIs) and the second most common cause of catheter-associated urinary tract infections (CAUTIs) in long-term catheterized patients, it is responsible for 12% of complicated UTIs (7).

Similar to other gram-negative bacteria, Proteus species can cause sepsis, or systemic inflammatory response syndrome (SIRS), a serious illness with a 20% to 50% chance of death, by releasing endotoxin, a component of the gram-negative bacterial cell wall, into the bloodstream and inciting further inflammatory reactions in the host [8]. The majority of P. mirabilis bacteremia and sepsis cases have a high fatality rate and are related to UTIs [8, 9]. In the past, most P. mirabilis isolates were susceptible to standard antibiotic classes. However, recent studies indicate that antibiotic resistance is increasing among P. mirabilis isolates in different countries. (10,11) Like many other members of the Enterobacteriaceae family, P. mirabilis harbors plasmids and integrons that code for antimicrobial resistance. Resistant P. mirabilis isolates have been associated with nosocomial outbreaks, and ESBL-producing P. mirabilis has been linked to nosocomial outbreaks in neonatal ICUs in India (12).

Do to rising resistance in proteus sp. it becomes important to characterize and determine the genetic location of resistance. So as to carry out effective therapy. This study therefore analyzed the prevalence of Proteus species in blood and wound samples and their antimicrobial susceptibility profile.

MATERIALS AND METHODS

Isolation of *Proteus* spp.

The study includes clinical isolates of *Proteus* species obtained by screening a total of sixty blood and wound samples from patients.

Clinical samples were obtained from Eku General Hospital in Delta State. The hospital is known to provide healthcare facilities to people from different areas and also serves as a referral hospital. The clinical isolates were identified based on their morphological behavior on various differential media at the Microbiology Laboratory of Delta State University, Abraka. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 minutes at 15 lb pressure. Further identification was then carried out by standard biochemical testing using the methods described by Cheesebrough (2004).

Susceptibility testing before Plasmid curing

The susceptibility test was conducted using the Kirby-Bauer method of sensitivity determination. Petri dishes of Mueller Hinton agar were prepared according to the manufacturer's instructions. About 0.1 ml of Proteus spp. equivalent to 0.5 McFarland standard was seeded into each of the Petri dishes containing Mueller-Hinton agar using sterile swabs. These were allowed to stand for 45 minutes to enable the inoculated organisms to pre-diffusion. The antibiotics used for sensitivity were ampicillin (10 µg), ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), and nitrofurantoin (300 µg). Escherichia coli ATCC 25922, the control strain, was aseptically placed on the surfaces of the sensitivity agar plates. These were incubated for 18–24 hours at 37 °C, and the radial zone of inhibitions was taken. The results were expressed in accordance with the 2018 Clinical and Laboratory Standards Institute (CLSI).

Plasmids Curing with Sodium Dodecyl Sulfate

Plasmid curing was carried out using a sub-inhibitory concentration of 10% sodium dodecyl sulfate on resistant organisms. An overnight broth culture was inoculated into 4.5 milliliters of nutrient broth. After adding 0.5 ml of 10% SDS, the mixture was incubated for 48 hours at 37°C. 0.5 ml of the newly prepared 4.5 ml nutrient broth was added, and the mixture was then incubated for a further 24 hours at 37°C.

Post-curing susceptibility test

This was done following the standard method mentioned previously. We adhered to the 2018 recommendations published by the Clinical and Laboratory Standards Institute (CLSI). An antibiotic susceptibility test was carried out on each bacterium. The suspension was uniformly spread onto Mueller-Hinton agar. The antimicrobial impregnated discs were placed using sterile forceps on the agar surface, and the plates were incubated at 37°C for 24 h.

RESULTS

The distribution of *Proteus* spp among in-patients in relation to their age is as shown in Table 1. The study found that the bacterium was most prevalent among individuals aged between 21-35 years, followed closely by 12- 20 years. Represented in Table 2 is the percentage prevalence of isolates from the different clinical samples investigated, According to the results, *P. mirabilis* 34 (54.8%) was the most common followed by *P. vulgaris* 17 (27.4%) and *P. penneri* (17.7%). The distribution of these isolates as per the clinical samples indicated that *P. mirabilis* was most prevalent in both blood (32.3%) and wound (22.6%). *P. penneri* was the least common in both blood and wound. The percentage antibiogram susceptibility test before curing is presented in Table 3. It was observed that the isolates showed very high level of resistance of ≥ 76% to the cephalosporins tested. Resistance to the cephalosporins was as high as 90% and 100% in *P. vulgaris* and *P. penneri.* Another class of antibiotics that high resistance was observed was the penicillin, ampicillin. Resistance to ampicillin ranged from 58.8% to 82.3%. No resistance was observed in gentamycin. This is a positive observation. After antibiotic sensitivity, plasmid curing was done to determine the genetic location of resistance. Result of plasmid curing presented in table 4 showed that the resistance displayed by the isolates were carried on plasmid as most of the isolates were no longer resistance after curing.

Table 1: DISTRIBUTION OF ISOLATED *PROTEUS SPECIES* AMONG IN-PATIENTS IN RELATIONS TO AGE.

Sex/Age	*P. mirablis*	*P. vulgaris*	*P. penneri*
1-11	3	5	2
12-20	10	4	4
21-35	11	7	3
36-44	8	1	2
45-53	2	0	0
Total	34	17	11

Table 2: PERCENTAGE PREVALENCE OF ISOLATES FROM CLINICAL SAMPLES COLLECTED FROM EKU HOSPITAL

n(%)

Isolates	*P. mirabilis*	*P. vulgaris*	*P. penneri*	
Blood	20(32.3)	6(9.7)	7(11.3)	33(53.2)
Wound	14(22.6)	11(17.7)	4(6.5)	29(46.8)
Total	34(54.8)	17(27.4)	11(17.7)	62(100.00)

Table 3: PRE- CURING ANTIBIOGRAM SUSCEPTIBILITY TEST OF ISOLATES

n(%)

Isolates	**CAZ**	**CXM**	**AM**	**OFX**	**CPX**	**GN**	**NIT**	**AMX**
P. mirabilis(34)	26 (76.5)	24(70.5)	28(82.3)	14(41.2)	14(41.1)	0(0.00)	12(35.29)	6(17.6)
P. Vulgaris (17)	17(100.0)	12(70.5)	10(58.8)	8(47.1)	7(41.1)	0(0.00)	8(47.06)	4(23.5)
P. penneris(11)	10(90.9)	8(72.7)	8(72.7)	5(45.5)	6(54.55)	0(0.00)	4(36.36)	11(100.00)

Key: Ceftazidime (30ug) = CAZ; Cefuroxime (30ug) = CXM; Ampicillin (10ug) = AM; Ofloxacin (5ug) = OFX; Ciprofloxacin (5ug) = CPX; Gentamycin (10ug) = GN; Nitrofuratoin (300ug) = NIT; Amoxicillin (10ug) = AMX

Table 4: POST-CURING ANTIBIOGRAM ANTIBIOTIC SUSCEPTIBILITY TEST OF BACTERIAL ISOLATES

n(%)

Isolates	**CAZ**	**CXM**	**AM**	**OFX**	**CPX**	**GN**	**NIT**	**AMX**
P. mirabilis (34)	3(8.82)	2(2.94)	3(8.82)	3(8.82)	3(8.82)	0(0.00)	4(11.76)	3(8.82)
P. Vulgaris (17)	4(23.53)	2(11.76)	1(5.88)	1(5.88)	0(0.00)	0(0.00)	1(5.88)	0(0.00)
P. penneris (11)	3(27.27)	2(18.18)	1(9.09)	1(9.09)	1(9.09)	0(0.00)	1(9.09)	2(18.18)

Key: Ceftazidime (30ug) = CAZ; Cefuroxime (30ug) = CXM; Ampicillin (10ug) = AM; Ofloxacin (5ug) = OFX; Ciprofloxacin (5ug) = CPX; Gentamycin (10ug) = GN; Nitrofuratoin (300ug) = NIT; Amoxicillin (10ug) = AMX

DISCUSSION

Proteus species are commonly responsible for infections that occur in healthcare settings. The bacterium can spread to different parts of the body using various methods of dissemination (Kuruvilla et al., 2023). The study found that the highest rate of isolation of the bacterium occurred among individuals aged between 21-35 years, followed closely by 12- 20 years. This finding is similar from previous studies conducted by Omole and Stephen (2014), who also studied the isolation of *Proteus* species from clinical samples and observed a high rate of isolation among individuals aged between 21-30 years, particularly for wound infections. They noted that age plays a significant role in the frequency of wound infections, as teenagers and active individuals are more likely to engage in physically demanding activities like farming and sports, which increases their risk of infections and accidents. Infants and elderly individuals are also at risk of getting wound infections as their immune system weakens with age (Omole and Stephen, 2014; Nawaz *et al*. 2019). Additionally, Zafar *et al.* (2019), in their study on the characterization of *Proteus mirabilis* isolated from patient wounds, mentioned that *Proteus* species, especially *P. mirabilis*, affected all age groups, with the highest prevalence among individuals aged between 16-30 years (6.70%), followed by 5-10 years old (5%) and 30-50 years old (1.60%).This study revealed that *P. mirabilis* (32.3%) was the most frequently isolated bacterium from the blood, followed by *P. penneri* (11.3%) and *P. vulgaris* (9.7%) This is in line with Feglo *et al*. who found that *P. mirabilis, P. vulgaris and P. penneri* were the species most commonly associated with *Proteus* infections.

The antibiotics Ceftazidime (30ug), Cefuroxime (30ug), Ampicillin (10ug), Ofloxacin (5ug), Ciprofloxacin (5ug), Gentamycin (10ug), Nitrofurantoin (300ug) and Amoxicillin (10ug) were used for this study. With regards to the three most used antibiotics of proteus infection which are the c penicillins, cephalosporins and quinolones, the cephalosporins had the highest levels of resistance in this study followed by ampicillin. The efficacy of the cephalosporins has been reported to be reduced in proteus species over the past decade. This is occasioned by an extraordinary rise in microbial resistance (Akortha and Egbule 2008; Egbule, 2016; Egbule et al., 2016; Hatfull et al., 2022; Iweriebor,2022;). We reported as high as 100% resistance in ceftazidime and 70.5% in cefuroxime. The results are comparable to those of Facciolà etal., 2022, Girlich et al., 2020. This high level of resistance observed in this study to commonly prescribed antibiotics for *Proteus sp.* are becoming unable to cope with the situation, complicates the treatment of proteus infection infections and prolongs hospital stay.

Owing to the role plasmids play in acquiring genes encoding antimicrobial resistance and the transfer of resistance in *Proteus sp.* Plasmid curing experiment was done to determine the invovement of plasmids in resistance in this study. Result indicated that resistance in most antibiotics investigated were plasmid borne, as resistance were lost after curing experiment that eliminated the plasmid. Plasmid mediated resistance is an issue in both clinical practice and animal husbandary and has been reported by a number of researchers in Nigeria (Egbule, 2016; Egbule and Yusuf 2019; Iweriebor et al., 2021; Iweriebor et al., 2022; Egbule, 2022; Morka (2020).) A number of antibiotic resistance determinants are present in *proteus* strains and are mediated by mobile genetic elements such as plasmids and integrons (Szabo et al., 2018). This study, therefore, necessitates the quest for safe and efficient antibiotics.

CONCLUSION

The high prevalence of *Proteus* spp. and resistance profile in clinical samples of blood and wound indicates that there is a need to find safe and efficient antibiotics. Moreso, .harbouring of resistance on plasmids as huge public health implications including but not limited to spread of resistance genes, as such measures should be put in place to prevent spread.

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