

Implication of *Proteus* spp in the Pathology of Nosocomial Wound Infection in Northeastern Nigeria

Isyaka M. Tom^{1*}, E. B. Agbo², Umar A. Faruk², Askira M. Umore³,
Muhammad M. Ibrahim⁴, Jidda B. Umar¹, Ali B. Haruna³ and Abdullahi Aliyu²

¹Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Nigeria.

²Department of Biological Sciences, Abubakar Tafawa Balewa University, Nigeria.

³Department of Medical Laboratory Science, University of Maiduguri, Nigeria.

⁴Department of Microbiology University of Maiduguri, Nigeria.

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ABSTRACT

Aims: To determine the rate of infection and antibiogram of *Proteus* spp among hospitalised patients suffering from wound infection in Maiduguri.

Methodology: A total of 320 wound swab samples were collected from August 2016 to June 2017, and processed via microscopy, culture and further confirmed by biochemical tests. Kirby bauer disc diffusion test was used to determine the antimicrobial susceptibility pattern of *Proteus* spp isolated.

Results: Twenty eight (28) samples yielded *Proteus* spp, giving a prevalence rate of 8.75%. Male patients were more affected (60.71%) compared to females (39.29%). Difference in sex in relation to rate of infection was statistically not significant ($X^2 = 3.963$, $p < .01$). Patients within the age group of 21-30years (28.57%) recorded the highest bacterial yield while those of 61-70years and >70

*Corresponding author: Email: isyakatom77@gmail.com;

years (3.57% respectively) recorded the least. Patients suffering from Wound Sepsis and Burns recorded the most significant infection rate (28.57% respectively). *Proteus mirabilis* (53.57%) was the most isolated *Proteus* specie while *Proteus penneri* (7.14%) was the least. *Proteus* spp isolates demonstrated a marked resistance against Augmentin (89.29%) and Nalidixic Acid (85.71%) but high sensitivity was observed towards Ciprofloxacin (85.71%) and Streptomycin (78.57%).

Conclusion: We establish the role of *Proteus* spp in the infection of wounds of hospitalised patients, and the *Proteus* specie most implicated in the aetiology of wound infection is *Proteus mirabilis*. And that Ciprofloxacin and Streptomycin are the drugs of choice for the treatment of patients with *Proteus* spp wound infection in the study area, and the use of Nalidixic acid and Augmentin is highly discouraged due to high resistance.

Keywords: *Proteus* spp; wound infection; nosocomial infection; antimicrobial susceptibility

1. INTRODUCTION

Proteus is a member of the family *Enterobacteriaceae*. It is a pleomorphic, motile, non-capsulated, aerobic/facultatively anaerobic, Gram-negative, rod-shaped bacteria. The genus *Proteus* consists of five named species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. myxofaciens* and *P. hauseri* and three unnamed genomospecies: *Proteus* genomospecies 4, 5, 6 (O'Hara *et al.*, 2000).

Proteus spp are widespread in the environment and makes up part of the normal flora of the human gastrointestinal tract. *Proteus* spp are a major cause of diseases acquired outside the hospital (where many of these diseases eventually require hospitalisation) (De Champs *et al.*, 2000), and ranked third among gram negative pathogens causing hospital-acquired infections (Stamm, 1999).

Proteus spp have a diverse mode of transmission, and can cause infection in different anatomical sites of the body. Some of the incriminating sources of transmission are soil, contaminated water, food, equipment, intravenous solutions, the hands of patients and health-care personnel (Emori and Gaynes, 1993). Vatopoulos *et al.* (1996) reported that *Proteus* spp infections were associated with length of hospital stay before infection, treatment with newer antibiotics and operation during present hospitalisation (negative association).

Proteus spp possess several virulence factors which help explain their pathogenic potential. They have pili or fimbriae for adherence, elaborate cytotoxic haemolysins that lyse red blood cells and release iron as a bacterial growth factor. *Proteus* produce urease leading to the formation of struvite stone. They possess

peritrichous flagella for motility and their swarming ability enable them to colonise solid surfaces (Duguid *et al.*, 1987). *P. mirabilis*, *P. vulgaris* and *P. penneri* are opportunistic pathogens to man and are incriminated in a number of infectious diseases which includes; urinary tract infection (UTI), cystitis, pyelonephritis, prostatitis, chronic otitis media, eye infection, respiratory tract infection (RTI), wound infection, burns infection and bloodstream infection (Stock, 2003; Kim *et al.*, 2003).

Wound is a breach in the skin surface whether by trauma, accident, surgical operation, or burn provides an open door for bacterial infections (Ohalet *et al.*, 2012), the risk of wound infection increases with the degree of contamination and it has been estimated that about 50% of wounds contaminated with bacteria become clinically infected (Omole and Stephen, 2014; Sahu *et al.*, 2011). Infections of the skin and soft tissue either due to trauma, surgery, or burns may result in the generation of exudates composed of dead leucocytes, cellular debris, and necrotic tissues (Dryden, 2010).

Chronic wounds can be colonised on the surface by a wide range of organisms (Matsuura and Barg, 2013), common bacterial pathogens associated with wound infection include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus* species, *Streptococcus species*, and *Enterococcus species* (Sani *et al.*, 2012). *Proteus* species are found in long-term care facilities and hospitals (Beck-Sague and Giuliano, 1994). They cause significant clinical infections, which are difficult to eradicate especially from hosts with complicated wounds, catheterisation and underlying diseases and the immuno-compromised (Auwaerter 2008).

The widespread uses of antibiotics, together with the length of time over which they have been available, have led to the emergence of resistant bacterial pathogens leading to devastating outcome (Ohalete *et al.*, 2012). Drug resistant *Proteus* spp are difficult to eradicate and can make an outbreak difficult to control. This could lead to waste of resources, especially in resource constrained developing countries. This study therefore seeks to determine the prevalence and antimicrobial susceptibility pattern of *Proteus* spp isolated from different types of wounds among hospitalised patients.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Maiduguri, the capital of Borno state. The city is located in the northeastern part of Nigeria which lies within latitude 11.15 °N and longitude 30.05 °E in the sudano-sahelian savanna zone with a dense population that are mostly crop farmers, fishermen, herdsman and traders (Udo 1978). Based on the national census conducted in 2006, Borno state has a population of 4 151 193 (Umar *et al.*, 2016; NPC, 2006).

2.2 Sample Population

The target population for the study include in-patients and out-patients attending University of Maiduguri Teaching Hospital (UMTH), State Specialist Hospital (SSH), Mamman Shuwa Memorial Hospital (MSMH) and Umaru Shehu Ultra-Modern Hospital (USUMH). The selected hospitals serve a population of over 20 million in the North-eastern sub-region of Nigeria, comprising six states (Borno, Bauchi, Yobe, Adamawa, Taraba and Gombe) as well as a sizeable number across the borders of Cameroon, Chad and Niger Republic (Umar *et al.*, 2016).

2.3 Sample Collection and Processing

Three hundred and twenty (320) wound swab samples were collected from consented patients in both inpatient and outpatient departments. Wound bed was prepared before sample collection by using Levine's technique, where the wound surface exudates and contaminants was cleaned off with moistening sterile gauze and sterile normal saline solution. Aseptically the swab stick was rotated over 1cm² area for 5

seconds with sufficient pressure to express fluid and bacteria to surface from within the wound tissue (Gardner *et al.*, 2007). The wound swab samples were transported to Microbiology Laboratory after collection in 0.5ml sterile normal saline solution for bacterial preservation.

2.4 Microbiological Analysis

The wound swab samples were cultured by plating onto 5% Blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18-24 hours. Suspected colonies were further subcultured to obtain discrete colonies (Baker *et al.*, 2007; Cheesbrough, 2006). Gram stain was conducted as a preliminary test. Pure culture was isolated and identified based on morphological appearance on enriched (Blood agar) and differential media (MacConkey agar), motility, Gram stain reaction and reaction to biochemical tests which include, phenylalanine deaminase, urease, hydrogen sulphite production, indole, methyl red, voges proskauer, citrate, maltose fermentation and ornithine decarboxylase test (Baker *et al.*, 2007; Cheesbrough, 2006).

2.5 Antibiotic Susceptibility Test

2.5.1 Preparation of bacterial inoculum

Pure isolate of bacteria was inoculated into Nutrient broth and incubated at 37°C for up to 5 hours until turbidity equals to 0.5 Mcfarland turbidity scale. This turbidity scale was adjusted by adding 9.6 ml of 1% aqueous solution of barium chloride in 0.4ml of 1% sulphuric acid to give an approximate bacterial density of 1.2x 10⁹ cfu/ml (Cheesbrough, 2006).

2.5.2 Sensitivity testing of *Proteus* Isolates

The Kirby-Bauer disc diffusion method as described by Akubueyi *et al.* (2011) was used for this test. Pure isolates of *Proteus* spp were tested against selected antibiotics using Gram negative multidisc containing the following antibiotics; Cephalexin (10 µg/ml), Gentamicin (10µg/ml), Augumentin (30µg/ml), Nalidixic acid (30µg/ml), Streptomycin (30 µg/ml), Ofloxacin (30 µg/ml), Pefloxacin (10 µg/ml), Ciprofloxacin (10 µg/ml), Ampicillin (30 µg/ml) and Cotrimoxazole (30 µg/ml).

Prepared *Proteus* spp inoculum (1.2x 10⁹ cfu/ml) was seeded onto prepared Mueller Hinton agar (MHA) plate under aseptic condition and the

surface was allowed to absorb. Commercially obtained Gram negative antibiotics multidisc was then carefully placed onto the surface of the seeded plate with the aid of sterile forceps and incubated at 37°C for 18 -24 hours. After 24 hours, the zones of inhibition were measured. Obtained results were compared with the standard performance chart for antimicrobials susceptibility testing provided by CLSI (2014), and the frequencies of sensitivity and resistance were recorded.

2.6 Data Analysis

Data generated were analysed using Statistical Package for Social Sciences (SPSS, version 16.0). Data were presented as frequencies and percentages. Chi-square was used and evaluations were carried out at 99% confidence level and $P < 0.01$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

Twenty eight (28) out of the three hundred and twenty (320) samples collected yielded *Proteus* spp giving a prevalence rate of 8.75%. This is contrary to the findings of Moordi and Momoh (2009), Newman *et al.* (2006) and Senthamarai *et al.* (2015), but similar to the rate reported by Feglo *et al.* (2010) in a study conducted in Ghana. These variations could be attributed to difference in sample size examined as well as handling and processing techniques used.

The yield from samples collected from males was highest (60.71%) compared to females (39.29%). This is contrary to the findings 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. The predominance of males observed in this

study, is most likely due to the fact that male exposure is greater as they represent majority of workforce in Nigeria. Similar observation was made by Saleh and Hatem, (2013). Difference in sex in relation to rate of isolation was statistically not significant ($X^2 = 3.963$, $p < .01$).

Patients within the age category of 21-30yrs recorded the highest recovery rate of 28.57% and the least was observed among patients within the age category of 61-70years and >70years (3.57% respectively). The rate of isolation was highest among male patients between the ages of 21-30yrs (Table 1). This is in agreement with the findings of Omole and Stephen, (2014). They assert that age significantly affects the prevalence of wound infections, because adolescents and active age adults are usually the ones involved in strenuous activities such as sports and farming which expose them to injuries and infections. Infants and older age individuals are also considered at risk of acquiring wound infections because of the decreasing trend of their immune competence with age (Omole and Stephen, 2014; Hakim and Gress, 2007), and infants have their immune system at the developmental stage.

P. mirabilis recorded the highest yield of 4.69% followed by *P. vulgaris* 3.44% and the least was *P. penneri* with 0.62% (Table 2). This is in agreement with Feglo *et al.* (2010), who observed that *P. mirabilis* was the most common species isolated, accounting for 61.5 % of all infections and hence responsible for the majority of *Proteus* spp infections.

The prevalence of *Proteus* spp observed in this study might not be unconnected with the fact that the maintenance of sanitary system in the selected hospitals is contracted to the same company which employed unskilled and

Table 1. Age and sex distribution of patients with *proteus* infection in the study area

Age Group (Years)	Sex (n=28)		Total (%)
	Male (%)	Female (%)	
0-10	2 (7.14)	1 (3.57)	3 (10.71)
11-20	3 (10.71)	2 (7.14)	5 (17.86)
21-30	6 (21.43)	2 (7.14)	8 (28.57)
31-40	3 (10.71)	2 (7.14)	5 (17.86)
41-50	1 (3.57)	2 (7.14)	3 (10.71)
51-60	1 (3.57)	1 (3.57)	2 (7.14)
61-70	0 (0.00)	1 (3.57)	1 (3.57)
>70	1 (3.57)	0 (0.00)	1 (3.57)
Total (%)	17 (60.71)	11 (39.29)	28 (100)

($X^2 = 3.963$, $df = 7$, $p < .01$)

cheap labour. It was also observed that overstretched facilities as a result of overcrowding and inadequate infection control measures were found to be among the major problems bedeviling the selected hospitals in the study area.

The distribution of *Proteus* isolates in relation to wound types revealed that Wound Sepsis and Burns recorded the highest infection rate (28.57% respectively), and none was isolated from patients with Gunshot wounds (0.00%) (Table 2). This is contrary to the findings of Mohammed *et al.*, (2013), who claim that post-operative wounds were the most frequently infected. Burn and sepsis wounds occurring impromptu may promote multiple infections due to the damage to the skin, and this has been correlated with immune suppression (Glasser *et al.*, 2010). Post-operative wounds are generally taken care of, as those wounds are planned.

Antimicrobial susceptibility pattern of *Proteus* spp isolated revealed that the highest sensitivity/least resistance was shown towards Ciprofloxacin (85.71%/14.29%) while the least sensitivity/highest resistance was against Augmentin (10.71%/89.29%) (Table 3). It has been reported that *Proteus* spp have an intrinsic resistance to nitrofurantoin, tetracycline and polymyxins but are generally susceptible to a wide range of antibiotics such as penicillins (ampicillin, amoxicillin, and piperacillin), cephalosporins (cefazolin, ceftazidime, cefuroxime,

ceftazidime, ceftriaxone, ceftazidime, ceftriaxone, ceftazidime, and cefepime), aminoglycosides (amikacin, gentamicin, and tobramycin), imipenem, ciprofloxacin, and trimethoprim-sulfamethoxazole (O'Hara *et al.*, 2000).

Proteus mirabilis isolates were highly resistant to Augmentin (86.0%), Nalidixic Acid (80.0%), Ampicillin and Cephalexin (66.67% respectively). *Proteus vulgaris* were highly resistant to Augmentin, Nalidixic Acid, Ampicillin (90.90% respectively) and Cephalexin (81.81%). While *Proteus penneri* isolates were highly resistant to Augmentin, Nalidixic Acid and Cephalexin (100% respectively). Overall, isolates were markedly resistant against Augmentin, Nalidixic Acid, Ampicillin and Cephalexin, but moderately resistant towards Pefloxacin and Gentamicin (*P. mirabilis* and *P. vulgaris*), Streptomycin (*P. vulgaris*), Cotrimoxazole (*P. mirabilis* and *P. penneri*) and Ofloxacin (*P. penneri*) (Fig. 1). The efficacy of Quinolones and Aminoglycosides on isolates from wound infections was acknowledged by Mohammed *et al* (2013) and Taiwo *et al*, (2002) as well. Reports elsewhere indicated that most strains of *Proteus* were susceptible to Cotrimoxazole and almost all species were sensitive to gentamicin (Duguid *et al.*, 1987). The findings in this study strongly agree with this statement and does not concur with the observations of Mordi and Momoh, (2009), who reported that gentamicin is not a drug of choice for the treatment of *Proteus* infections.

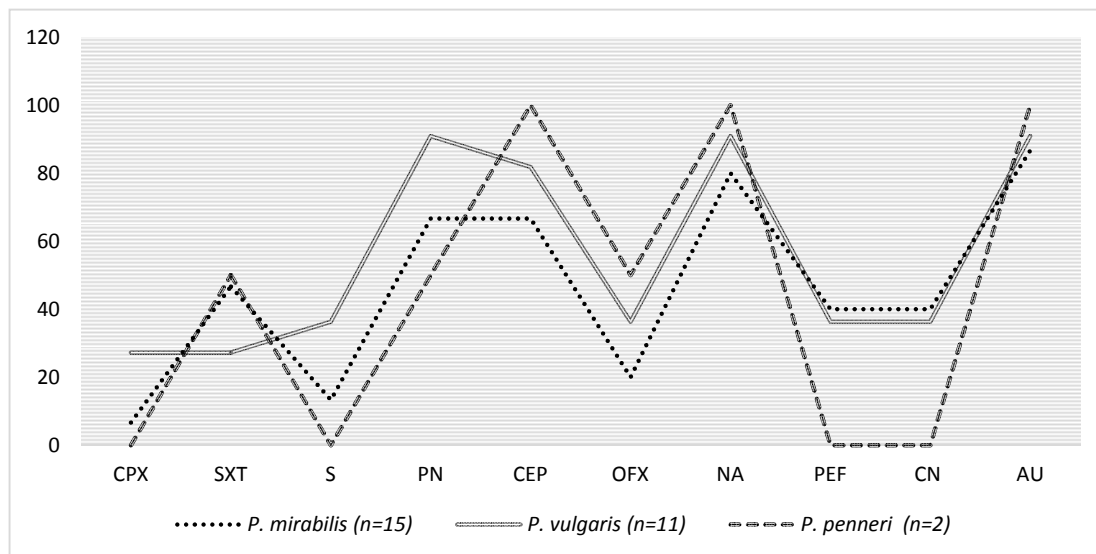


Fig. 1. Percentage resistance rate by *Proteus* spp against antimicrobial drugs tested

Table 2. Frequency of isolation of *Proteus spp* from different wound types in the study area

<i>Proteus spp</i>	Wound types						Total (%)
	Wound sepsis	Wound ulcer	Diabetic ulcer	Post-operative ulcer	Burns wound	Gunshot wound	
<i>P. vulgaris</i>	2 (7.14)	3 (10.71)	1 (3.57)	2 (7.14)	3 (10.71)	0 (0.00)	11 (39.29)
<i>P. mirabilis</i>	6 (21.43)	2 (7.14)	2 (7.14)	1 (3.57)	4 (14.29)	0 (0.00)	15 (53.57)
<i>P. penneri</i>	0 (0.00)	1 (3.57)	0 (0.00)	0 (0.00)	1 (3.57)	0 (0.00)	2 (7.14)
Total (%)	8 (28.57)	6 (21.43)	3 (10.71)	3 (10.71)	8 (28.57)	0 (0.00)	28 (100)

($\chi^2=4.769$, $df=10$, $p<.01$)

Table 3. Antimicrobial Susceptibility Pattern of *Proteus Spp* Isolated from Patients in the Selected Hospitals

Antimicrobial agent	Concentration (µg)	Antimicrobial susceptibility test	
		Sensitive (%)	Resistant (%)
Quinolones			
Ciprofloxacin (CPX)	10	85.71	14.29
Ofloxacin (OFX)	30	71.43	28.57
Pefloxacin (PEF)	10	64.29	35.71
Nalidixic Acid (NA)	30	14.29	85.71
β-Lactam Antibiotics			
Ampicillin (PN)	30	25.00	75.00
Cephalexin (CEP)	10	25.00	75.00
Augmentin (AU)	30	10.71	89.29
Aminoglycosides			
Gentamicin (CN)	10	64.29	35.71
Streptomycin (S)	30	78.57	21.43
Folate Antagonist			
Cotrimoxazole (SXT)	30	60.71	39.29

As majority of bacterial isolates observed in this study showed widespread resistance against varied classes of antimicrobial drugs, treatment of wound infections should be based on results of culture and in-vitro susceptibility test. Nevertheless, for empirical therapy, β -lactam antibiotics and Nalidixic acid are not a good choice for the treatment of *Proteus*-implicated wound infections.

Furthermore, high standards of environmental hygiene within the hospital surroundings are necessary to reduce the incidence of nosocomial infections due to *Proteus* spp. However, it is advocated that the incorporation of natural alternatives to antibiotics such as phage therapy (or other probiotics) or the use of local plant extracts with proven therapeutic and prophylactic potency will go along way in minimising the effects of drug resistance. Periodic monitoring of antimicrobial susceptibility pattern of isolates from wounds in the nosocomial setting will assist in reducing the challenges of selection of chemotherapeutic drugs for treatment especially in areas where there are no modern microbiological facilities.

4. CONCLUSION

We establish the role of *Proteus* spp in the infection of wounds of hospitalised patients. We have demonstrated that the *Proteus* specie most implicated in the aetiology of wound infection is *Proteus mirabilis*. And that Ciprofloxacin, Streptomycin and Ofloxacin are the drugs of choice for the treatment of *Proteus* spp infected wounds in the study area, and the use of Cephalexin, Nalidixic acid and Augmentin is highly discouraged due to high resistance observed.

CONSENT

All authors declare that informed consent (verbatim) was obtained from the patients for sample and data collection.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the ethics committee of the University of Maiduguri Teaching Hospital, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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