International Journal of Pathogen Research

1(2): 1-9, 2018; Article no.IJPR.44305

Plasmid Profile Analysis of Multi-drug Resistant *Proteus* spp isolated from Patients with Wound Infection in Northeastern Nigeria

Isyaka M. Tom[1*], E. B. Agbo[2], Umar A. Faruk[2], Muhammad M. Ibrahim[3], Askira M. Umoru[4], Jidda B. Umar[1], Abdullahi Aliyu[2] and Ali B. Haruna[4]

[1]Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Nigeria.
[2]Department of Biological Sciences, Abubakar Tafawa Balewa University, Nigeria.
[3]Department of Microbiology, University of Maiduguri, Nigeria.
[4]Department of Medical Laboratory Sciences, University of Maiduguri, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author IMT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EBA and UAF participated in the design of the study and supervised the study. Author MMI managed the analyses of the study, wrote the final draft of the manuscript. Authors AMU, JBU, AA and ABH managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2018/v1i21245
Editor(s):
(1) Dr. Wan Mazlina Md Saad, Lecturer, Department of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, Malaysia.
Reviewers:
(1) Nwibo Daniel Don, Teikyo University, Japan.
(2) Oluyele, Olumide, Federal University of Technology Akure, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/26344

Original Research Article

Received 2nd July 2018
Accepted 18th September 2018
Published 22nd September 2018

ABSTRACT

Aims: We analysed the prevalence of plasmid mediated multidrug resistance among *Proteus* spp isolated from wound infection patients attending healthcare centers in Maiduguri.
Methodology: 320 wound swab samples were collected from August 2016 to June 2017, and investigated via microscopy, cultured on Blood agar and MacConkey agar. Suspected *Proteus* spp isolates were further confirmed using biochemical tests. Kirby bauer disc diffusion test was used to determine the antimicrobial susceptibility pattern. Isolates confirmed to be multidrug resistant (MDR) were subjected to gel electrophoresis for the determination of plasmid profile.
Results: Twenty eight (28) samples yielded *Proteus* spp, giving a prevalence rate of 8.75%.

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

Proteus mirabilis was the most significant specie isolated (32.14%). Isolates were most sensitive/least resistant to Ciprofloxacin (85.71%/14.29%) and most resistant/least sensitive to Augmentin (10.71%/89.29%). 64.28% of isolates observed were MDR strains and were quite significant among patients between the ages of 21-30years (21.43%). MDR *Proteus mirabilis* was most the significant and highly prevalent among patients suffering from wound sepsis and burns (10.71% respectively). The association between MDR *Proteus* spp and wound types was statistically not significant (X^2 =7.342, p>0.01). Plasmid profile analysis revealed that 72.22% of the MDR isolates harbour plasmids with a DNA fragment size of 100 bp and a molecular weight of 31ng/10µl. An average of 7.22% of MDR isolates were cured of their plasmids while an average of 56.67% of MDR isolates resisted curing.

Conclusion: Here, we report a high prevalence of multidrug resistance and a high rate of plasmid carrying strains of *Proteus* spp in wounds of hospitalised patients. We suggest that there is a role played by plasmid in the mediation of multidrug resistance among the MDR *Proteus* spp isolated, where the majority of the MDR isolates observed carry plasmids.

Keywords: Proteus spp; plasmid profile; multi-drug resistance; wound infections; plasmid curing.

1. INTRODUCTION

Wound infection can be regarded as the invasion and proliferation by one or multiple species of microorganisms on wound, which could lead to pus formation [1]. Infection can be regarded as the attachment of microorganisms to host cells where they proliferate and become better placed to cause damage to host tissues [2]. Wounds can be infected by a variety of bacteria, the most common of which are *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp and *Proteus* spp [3].

Proteus spp is a rod-shaped pleomorphic, motile, non-capsulated, aerobic/facultatively anaerobic, gram-negative bacterium that has been implicated in hospital and community acquired infections. They are widespread in the environment and make up part of the normal flora of the human gastrointestinal tract. Although *Escherichia coli* accounts for the largest percentage of cases of uncomplicated infections, *Proteus* spp ranks third particularly in the nosocomial setting [4].

Proteus spp have been implicated in the infection of wounds, especially diabetic wounds, along with *Escherichia coli*, *Enterobacter* spp, *Klebsiella* spp and *Serretia marcescens* [5,6,7]. They cause significant clinical infections which are difficult to eradicate, especially from hosts with complicated wounds, catheterisation and underlying diseases as well as the immunocompromised [8]. *Proteus* spp can be naturally resistant to antibiotics; some strains produce penicillinase and almost all are resistant to the polymyxins such as colistin and polymyxin B [9], but the most disturbing aspect was the emergence of multi-drug resistant strains that are spreading incessantly [10].

The prevalence of multi-drug resistant (MDR) bacterial isolates has increased in the last few years, affecting the prognosis and survival of hospitalised patients especially in developing countries like Nigeria. Like many other members of the family *Enterobacteriaceae*, *Proteus* spp can harbour numerous plasmids and integrons that houses the genetic determinants of antimicrobial resistance [11]. Multidrug-resistant (MDR) strains of *P. mirabilis*, for instance, produce extended-spectrum ß-lactamases (ESBLs) or the AmpC-type cephalosporinase and rarely carbapenemases, and their prevalence in some settings is relatively high [12,13].

We investigated a cohort of hospitalized patients suffering from wound infection to ascertain the prevalence of multidrug resistant *Proteus* spp from the various wound types, and to look at the plasmid profile of the MDR isolates.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Maiduguri, located in the northeastern part of Nigeria which lies within latitude 11.15 ºN and longitude 30.05 ºE in the sudano-sahelian savanna zone, with a dense population that are mostly crop farmers, fishermen, herdsmen and traders [14].

2.2 Study Population

The target population for the study includes in-patients and out-patients attending four healthcare centers within Maiduguri metropolis. These centers 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 [15].

2.3 Sample Collection and Processing

Swab sample of wounds were collected at random from consented patients in both inpatient and outpatient departments. Wound beds were prepared before sample collection using Levine`s technique as described by [16]. The wound surface exudates was cleaned off with moistened sterile gauze and sterile normal saline solution. The wound area was disinfected. Aseptically, the swab stick was rotated over 1 cm^2 area for 5 seconds with sufficient pressure to express fluid and bacteria to the surface from within the wound tissue. The wound swab samples were transported in 0.5ml sterile normal saline solution for microbiological analysis.

2.4 Microbiological Analysis

The wound swab samples were cultured onto 5% Blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18-24 hour. Suspect colonies were further subcultured to obtain pure culture. Gram stain was conducted as a preliminary test. Pure culture of *Proteus* spp was isolated and identified based on morphological appearance, motility, Gram staining reaction and biochemical tests which include; phenylalanine deaminase, urease, hydrogen sulphite production, indole, methyl red, voges proskauer, citrate, maltose fermentation and ornithine decarboxylase test [17,18].

2.5 Antibiotics Susceptibility Test

2.5.1 Preparation of bacterial inoculum

Isolates of *Proteus* spp were inoculated into Nutrient broth and incubated at 37°C for 5 hours until turbidity was adjusted to 0.5 Mcfarland turbidity scale. This turbidity scale was prepared by adding 9.6 ml of 1% aqueous solution of barium chloride ($BaCl_2$) in 0.4 ml of 1% sulphuric acid (H_2SO_4) giving an approximate bacterial density of $1.2x10^9$ cfu/ml [18].

2.5.2 Antibiotics sensitivity test

The Kirby-Bauer disc diffusion method as described by [19] was used for this test. Pure isolate of *Proteus* were tested against selected antibiotics using Gram negative multidisc cartridge containing the following antibiotics; Ceporex (10 µg/ml), Gentamicin (10 µg/ml), Augumentin (30 µg/ml), Nalidixic acid (30 µg/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).

The inoculum prepared ($1.2x10^9$ cfu/ml) was seeded onto prepared Mueller Hinton agar (MHA) under aseptic conditions and the surface is allowed to absorb. The antibiotic-impregnated multidisc was carefully placed onto the surface of the seeded plate with the aid of sterile forceps and incubated at 37°C for 18-24 hour. The zones of inhibition were then measured and interpreted according to the Standard Performance Chart by Clinical Laboratory Standards Institute [20].

2.6 Plasmid Curing Analysis

Multidrug resistant isolates were subjected to plasmid curing using the method of [21]. Overnight cultures in nutrient broth were diluted 10-fold and 1 ml inoculum was added to 30 ml of nutrient broth (pH=7.6). 1ml of 10%w/v Sodium Dodecyl Sulfate (SDS) solution was added to the broth and incubated for 24 hours. The overnight broth cultures were diluted with sterile distilled water and inoculated onto Mueller Hinton agar plates. The colonies were then sub-cultured onto Mueller Hinton agar plates and were again screened for antibiotic resistance by the disk diffusion method. Resistance markers expressed after curing were regarded as being chromosome-mediated while those not expressed were regarded as plasmid mediated.

2.7 Plasmid DNA Extraction and Gel Electrophoresis

Plasmid DNA extraction was performed using the alkaline lysis method described by [22]. *Proteus* spp isolates were sub-cultured onto a nutrient agar plate and incubated for 24 hour at 37°C, 3-5 colonies of the pure isolates from the overnight culture was inoculated into Luria bertani broth (LB) and incubated overnight at 37°C. The overnight broth culture was transferred into 1.5ml eppendorf tube and was spun at high speed for 1

minute with table top centrifuge, the supernatant was discarded to remove the liquid completely by turning the tube upside down on a piece of paper towel for a few seconds.100 µl of resuspension (P1 buffer) solution was added into the tube, and vortexed to completely resuspend cell pellet. 100 µl of lysis solution (P2 buffer) was added and mixed gently by inverting the tube 5-6 times. The solution quickly turn transparent and became more viscous which indicates bacterial lysis has taken place.150 µl neutralising solution (P3 buffer) was added and mixed by inverting the tube several times.

At this point bacterial chromosomal DNA was seen as a white precipitate, the tube is then centrifuged at high speed for 10 minutes. The supernatant was transferred carefully to a new 1.5 ml eppendorf tube with a 1 ml pipette. 2.5-3 mls of 200-proof cold ethanol (stored at -20°C) was added to each tube and mixed by inverting the tube few times. The plasmid DNA precipitate was spun down at high speed for 10 minutes, the supernatant was discarded and the remaining liquid was removed as much as possible by leaving the tube upside down on a piece of paper towel, then the tube was kept in a tube holder and was allowed to air dry for 10-20 minute or on a heat blocker at 37°C to dry faster, DNA precipitate turns white when dry. The DNA pellet was resuspended in 50 µl of tris borate EDTA, the solution was completely dissolved by pipetting the solution several times. A large amount of RNA was present in the DNA sample. Therefore, for subsequent use 1-5 ul of (1 mg/ml) RNAase was added to completely remove RNA.

Afterwards, agarose gel electrophoreses was conducted using the method described by [23]. 1% agarose was prepared by dissolving 1g of agarose powder in 100 ml Tris Borate Ethylene Diamine Tetra acetic acid (TBE = 1X), it was swirled to thoroughly mix. The solution was then dissolved in microwave for 3 minute and allowed to cool on the bench to 60°C or just when hot enough to hold with bare hands. 10 µl of ethidium bromide was added and mixed avoiding bubbles. The gel cassette (tray) was assembled and the gel was poured into the gel trough, the gel comb was then inserted and the gel was allowed to set for about 30 minute.

The electrophoreses tank was filled up to the mark with 1X TBE buffer and the comb was removed and side rubber seal was disassembled from the solidified agarose. The set agarose was transferred to the gel tank ensuring the buffer floods at least 2mm above the gel and the comb grooves (wells) was labelled in order of the samples to be loaded. On a separate micro titre plate 2 µl of the loading dye (it contain dye such as Xylene cyanol and bromophenol blue, and glycerol) and the extracted plasmid DNA was mixed using a 10µl micropipette and the mixture was transferred to the gel wells and the standard DNA molecular marker (DNA ladder) was run along with the test. The gel tank was closed and the power means was connected and switched on at constant voltage of 90v for 60 minutes. The gel tank was switched off and unplug and the gel was carried in its holder to the UV trans-illuminator, the gel documentation system was assembled and the image (bands) was photographed.

The DNA bands were interpreted according to the Solis BioDyne DNA Ladder molecular weight marker which range in size from 100 bp to 3000 bp, and results recorded.

2.8 Data Analysis

Data generated were analysed using Statistical Package for Social Sciences (SPSS). 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

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

Twenty eight (28) out of the three hundred and twenty (320) samples analysed yielded *Proteus* spp, giving a prevalence rate of 8.75%, which was more significant among males (60.71%) than females (32.29%). Rate of infection was highest among patients of 21-30 years (28.57%). Male patients between the age categories of 21-30 years recorded the most significant infection rate (21.43%) on sex versus age group basis (Table 1). Similar findings by [24] was reported and they assert that age significantly affects the prevalence of wound infections, since adolescents and active-age adults are usually the ones involved in much physical activities such as sports and farming which may expose them more to injuries and infections. Infants and older age individuals are also considered at risk of acquiring wound infections because of the

decrease in immune competence with age [25,26], and infants have their immune system at the developmental stage.

3.2 Antimicrobial Susceptibility Pattern of *Proteus* spp Isolated

Antimicrobial susceptibility pattern of *Proteus* spp isolated showed 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 2). Cumulatively, Isolates were most sensitive/least resistant to Quinolones (with the exception of Nalidixic acid against whom a marked resistance was observed) and Aminoglycosides class of drugs, with an average sensitivity/resistance rate of 73.81%/26.19% and 71.43%/28.57% respectively. Resistance by isolates was highly pronounced against β-lactam antibiotics with an average resistance/sensitivity rate of 79.76%/ 20.24%.

Our results demonstrated a marked resistance by *Proteus* spp towards Nalidixic acid. It has been reported that the emergence and prevalence of Nalidixic acid–resistant clones have been more problematic in hospitals than in any other environments [27]. Poor adherence to recommended dosage by patients and widespread, indiscriminate use of antibiotics, which are freely available and accessible with high potential for abuse, are part of the reasons why drug resistance is on the rise.

Although variation in resistance exist between *Proteus* isolates examined, the overall most effective antibiotics were the Flouroquinolones and Aminoglycosides. The low level of resistance to these antibiotics may be due to their better efficacy and to certain extent, high price. This makes them not readily available and affordable and by extension, lower the chances of abuse. As such, the restriction of over-the-counter prescription of antibiotics and general awareness about the higher possibility of resistance by bacterial species to these drugs can help reduce the level of abuse in the society.

64.27% of the isolates were multidrug resistant. Patients within the age category of 21-30 years recorded the highest yield of 21.43% and the least was observed among patients within the age category of 0-10 yrs (0.00%) (Table 3). This is in concurrence with the findings of [28] in a study carried out in Ghana, and that of [29] in India.

The latter concluded that resistance to antibiotics is an increasingly common problem and its management is a subject of concern. And that species identification and routine study of the epidemiology of antimicrobial resistance will assist in the management and control of infections, and in therapeutic management of patients by shifting the mode of prescription of antibiotics from broad spectrum to narrow spectrum.

The evolution and spread of multi-drug resistant *Proteus mirabilis* clone with chromosomal AmpC-type beta-lactamase has been reported in Europe. Resistance to high levels of antibiotics has been ascribed in most instances to the presence of plasmids [30,13].

Table 1. Distribution of *Proteus* spp according to patients' demographic properties

Age group (Years)	Sex of patients		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)

$*X^2$ =3.041; df=7; p<0.01

Table 2. Antimicrobial susceptibility pattern of *Proteus* spp isolated

Antimicrobial agents	Concentration (µg)	Susceptibility pattern	
		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			
Ampicillin (PN)	30	25.00	75.00
Ceporex (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

Table 3. Percentage occurrence of MDR *Proteus* spp among patients based on their age

Age group (Years)	Multidrug resistant *Proteus* spp Isolated (%)
0-10	0 (0.00)
11-20	2 (7.14)
21-30	6 (21.43)
31-40	3 (10.71)
41-50	3 (10.71)
51-60	2 (7.14)
61-70	1 (3.57)
>70	1 (3.57)
Total (%)	18 (64.29)

Percentage rate of occurrence of multi drug resistance among *Proteus* spp showed that *Proteus mirabilis* (32.14%) recorded the most multidrug resistant (MDR) strains while *Proteus penneri* recorded the least (3.57%) (Table 4).

Table 4. Percentage occurrence of multidrug resistance according to *Proteus* specie type isolated

Proteus spp	Wild strains (%)	Multidrug resistant strains (%)
P. mirabilis	15 (53.57)	9 (32.14)
P. vulgaris	11 (39.29)	8 (28.57)
P. penneri	2 (7.14)	1 (3.57)
Total (%)	28 (100)	18 (64.29)

Distribution of MDR *Proteus* spp isolated from wound types examined showed that Wound sepsis and Burns wound recorded the highest prevalence (17.86% respectively), while Gunshot wound recorded the least (0.00%). MDR *Proteus mirabilis* was isolated the most from Wound sepsis and Burns wound (10.71% respectively) whereas MDR *Proteus vulgaris* was isolated the most from Wound sepsis, Wound ulcer and Burns wound (7.14% respectively). MDR *Proteus penneri* was only isolated from wound ulcer (3.57%). The association between MDR *Proteus* spp and the various wound types examined was statistically not significant (X^2 =7.342, p<0.01) (Table 5).

Plasmid curing on the 18 MDR isolates revealed an average of 7.22% of isolates were cured of their plasmids while an average of 56.67% of MDR isolates resisted curing (Fig. 1). Plasmid profile analysis revealed that 72.22% of the multidrug resistant isolates harbour similar plasmids with a molecular weight of 31 ng/10 µl and DNA fragment size of 100 bp (Fig. 2). Resistance to high levels of antibiotics has been ascribed in most instances to the presence of plasmids [30].

A study conducted by [31] reported that plasmids were able to move genetic antibiotic resistant determinants among various bacterial strains and contribute to overall pathogenic potential of disease causing bacteria. A principal mechanism for the spread of antibiotic resistance is by horizontal transfer of genetic material through conjugation, transformation, or transduction. Resistance genes can be further incorporated into the recipient chromosome (in the form of integrons) by recombination, these genes may contain single mutations or more severe sequence changes [32]. [33] were able to

Table 5. Frequency of isolation of MDR *Proteus* spp among various wound types examined

Wound types examined	Specie type (%) (n=28)			Total (%)
	P. mirabilis	***P. vulgaris***	***P. penneri***	
Wound Sepsis	3 (10.71)	2 (7.14)	0 (0.00)	5 (17.86)
Wound Ulcer	0 (0.00)	2 (7.14)	1 (3.57)	3 (10.71)
Diabetic Ulcer	2 (7.14)	1 (3.57)	0 (0.00)	3 (10.71)
Post-Operative Wound	1 (3.57)	1 (3.57)	0 (0.00)	2 (7.14)
Burns Wound	3 (10.71)	2 (7.14)	0 (0.00)	5 (17.86)
Gunshot Wound	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total (%)	9 (32.14)	8 (28.57)	1 (3.57)	18 (64.29)

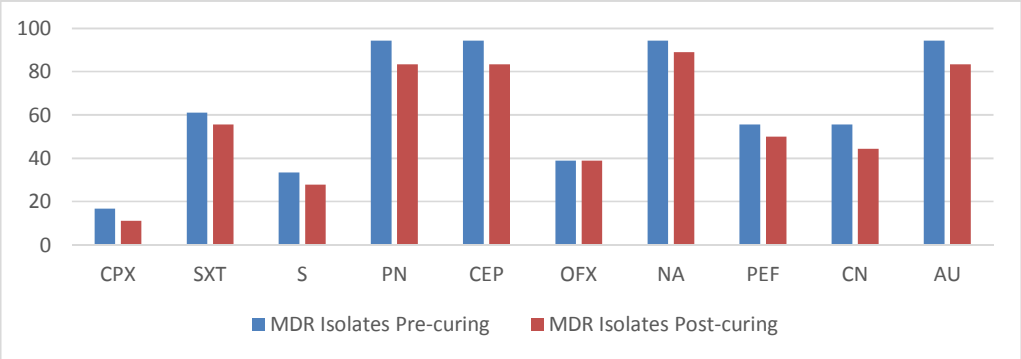

Fig. 1. Plasmid curing analysis of multidrug resistant *Proteus* strains isolated from the study area

**CPX=Ciprofloxacin, SXT=Cotrimoxazole, S=Streptomycin, PN=Ampicillin, CEP=Ceporex, OFX=Ofloxacin, NA=Nalidixic Acid, PEF=Pefloxacin, CN=Gentamicin and AU=Augmentin*

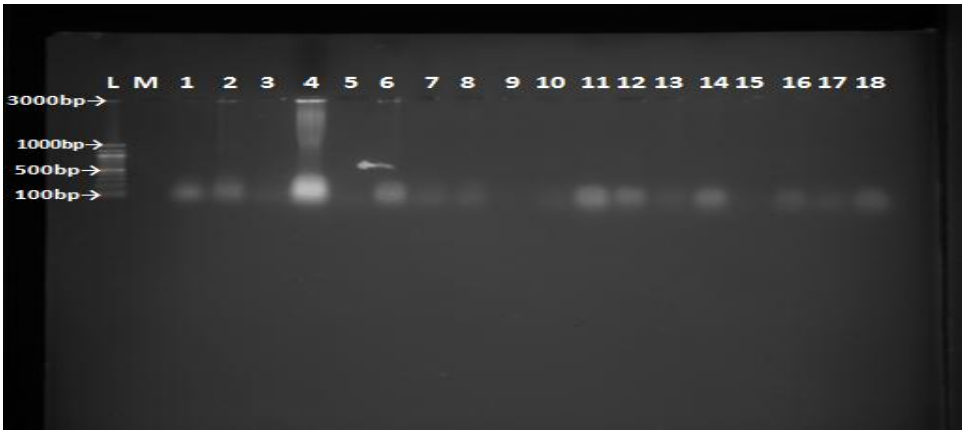

Fig. 2. The agarose gel electrophoresis profile of plasmid DNA from *Proteus* spp isolated

demonstrate that plasmids containing class 1 or 2 integrons and bla_{TEM-1} were able to be transferred from *Proteus mirabilis* isolates into *Escherichia coli* by conjugation, indicating that conjugal transfer could contribute to the dissemination of antibiotic resistance genes between the *Enterobacteriaceae* species.

4. CONCLUSION

Here, we report a high prevalence of multidrug resistance and a high rate of plasmid carrying strains of MDR *Proteus* spp in wounds of hospitalised patients. We suggest that there is a role played by plasmid in the mediation of

multidrug resistance among MDR *Proteus* spp isolated, where the majority of the MDR isolates observed in this study carry similar plasmids. But since most isolates resisted curing, the phenotype observed in those isolates could be chromosome mediated.

CONSENT

All authors declare that informed consent was obtained from the patients (or other approved parties) 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.

REFERENCES

1. Calvin M. Cutaneous wound repair. Wounds. 1998;10(1):12-32.
2. Collier M. Understanding wound inflammation. Nurs. Times. 2003;99(25): 63-64.
3. Gus G, Bronze MS, Drevets DA, Glatt A, Mylonakis E, Burke AC. *Proteus* infections. Drugs and Diseases; 2017.
   Available:https://emedicine.medscape.com/article/226434-overview
   (Accessed on July 2016)
4. Stamm WE. Urinary tract infections. In clinical infectious disease: A practical approach, Root, K. (ed). Oxford University Press, Inc, New York; 1999.
5. Goldstein EC, Citron DM, Nesbitt CA. Diabetic foot infection. Diabetes Care. 1996;19:638- 641.
6. Mutta RN, Oliveira MN, Magathaes PSF, Dias AM, Aragho LP, Forti AC, Carlvalho CBM. Plasmid mediated extended spectrum beta lactamase producing strain of enterobacteriaceae isolated from diabetic foot infections in Brazilian Diabetic center. Br. J. Infect. Dis. 2003;7(2):129-134.
7. Ravisekhar G, Bernu D, Vishnub hartla S, Arti K, Ammini AC, Rama C. Clinical microbiological study of diabetic foot ulcers in an Indian tertiary-care hospital. Diabetic Care. 2006;29:1727- 1732.
8. Auwaerter P. Antibiotic guide. Johns Hopkins ABX (antibiotic) Guide, Baltimore, MD; 2008.
9. Duguid JP, Marmion BP, Swain R.A. Medical microbiology. 13th Ed. Published by ELBS Low Price Edition; 1987.
10. Armbruster CE, Mobley HL. Merging mythology and morphology: The multifaceted lifestyle of *Proteus mirabilis*. Nat. Rev. Micro. 2012;10:743-754.
11. Hall RM, Collis CM. Antibiotic resistance in gram-negative bacteria: The role of gene cassettes and integrons. Drug Resist. Updat. 1998;1:109-119.
12. Cohen-Nahum K, Saidel-Odes L, Riesenberg K, Schlaeffer F, Borer A. Urinary tract infections caused by multi-drug resistant *Proteus mirabilis*: risk factors and clinical outcomes. Infection. 2010;38: 41-46.
13. D'Andrea MM, Literacka E, Zioga A, Giani T, Baraniak A. Evolution and spread of a multidrug-resistant *Proteus mirabilis* clone with chromosomal AmpC-type cephalosporinases in Europe. Antimicrob. Agents Chemother. 2011;55:2735-2742.
14. Udo RK. A comprehensive geography of West Africa. 1st ed. Heinemann Educational Books Nig. Ltd.; 1978.
15. Umar JB, Ibrahim MM, Tom IM, Umoru AM, Isa T. *Pseudomonas aeruginosa* in otitis media. International Journal of Medicine. 2016;4(2) 55-57.
16. Gardner SE, Frantz R, Hillis SL, Park H, Scherubel M. Diagnostic validity of semiquantitative swab cultures. Wounds. 2007;19:31-38.
17. Baker FJ, Silverton RE, Pallister CJ. Baker and Silverton's Introduction to Medical Laboratory Technology. Publisher: Bounty Press Limited, Nigeria. 2007;285-311.
18. Cheesbrough B. District Laboratory practice in Tropical Countries Part 2, Second Edition Cambridge University Press, Cambridge; 2006.
19. Akubuenyi FC, Arikpo GE, Ogugbue CJ, Mfongeh JF, Akpanumun EV. Antibiotic resistance profile of waste water isolates obtained from University of Calabar Teaching Hospital and General Hospital Calabar, Nigeria. Nig. J. of Microbiol. 2011;25: 2243-2250.
20. Clinical Standard Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing:

Seventeenth international supplement. National Committee for clinical laboratory standards document M100-S13, Wayne Pennslyvania, USA; 2016.
21. Isibor JO, Uwabor C. Antibiotic susceptibility pattern and plasmid profiles of *Pseudomonas aeruginosa* isolated from some hospital patients in Benin City, Nigeria. New York Science Journal. 2015;8(7)14.
22. He F. Plasmid DNA extraction from *E. coli* using alkaline lysis method. Biology Protocol. 2011;30:1-3.
23. Meyer JM, Neely A, Stintzi A, Georges C, Holder IA. Pyoverdin is essential for virulence of *Pseudomonas aeruginosa*. Infection and Immunity. 1996;64(2):518-23.
24. Torpy JM, Alison B, Richard MG. Surgical wound infections. JAMA. 2005;294:21-22.
25. Omole A, Stephen E. Antibiogram profile of bacteria isolated from wound infection of patients in three hospitals in Anyigba, Kogi Sate, Nigeria. FUTA Journal of Research in Sciences. 2014;2:258-266.
26. Hakim FT, Gress, RE. Immunosenescence: Deficits in adaptive immunity in the elderly. Tissue Antigens. 2007;70(3):179-189.
27. Soto SM, Gonza´lez-Hevia MA, Mendoza MC. Antimicrobial resistance in clinical isolates of *Salmonella enterica* serotype *Enteritidis*: relationships between mutations conferring quinolone resistance, integrons, plasmids and genetic types. J. Antimicrob. Chemother. 2003;51:1287-1291.
28. Feglo PK, Stephen YG, Solomon AQ, Clement O. Occurrence of spp distribution and antibiotic resistance of *Proteus* isolates: A case study at the Komfo Anakye Teaching Hospital (KATH) Ghana. Int. J. of Pharma Sci. and Res. 2010; 1(9):347-352.
29. Nita P, Nikita S, Rajni S, Saroj H, Rakesh KM. Prevalence of Multidrug (MDR) and Extensively Drug Resistant (XDR) *Proteus* species in a tertiary care hospital, India. Int. J. Curr. Microbiol. App. Sci. 2014; 3(10):243-252.
30. Daini OA, Ogbolu DO, Ogunledun A. Plasmid determined resistance to quinolones in clinical isolates of Gram-negative enteric bacilli. Afr Med Sci. 2006; 35:437-441.
31. Yah SC, Eghafona NO, Oranusi S, Abouo AM. Widespread plasmid resistance genes among *Proteus* species in diabetic wounds of patients in the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. African J. of Biotech. 2007;6(15):1757-1762.
32. Dzidic S, Suskovic J, Kos B. Antibiotic Resistance in Bacteria, Food Technol. Biotechnol. 2008;46(1):11–21.
33. Shin-Hee K, Cheng-I W, Haejung A. Molecular Characterization of Multidrug-Resistant *Proteus mirabilis* Isolates from Retail Meat Products. Journal of Food Protection. 2005;68(7):1408-1413.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26344