



PREVALENCE OF BACTERIAL PATHOGENS ASSOCIATED WITH WOUND INFECTIONS FROM DIABETIC OUT-PATIENTS AT PLATEAU SPECIALIST HOSPITAL, JOS, NIGERIA

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ABSTRACT

This study was carried out to determine the frequency and antibiotic susceptibility profile of bacterial agents associated with diabetic patients attending out-patients Department in Plateau Specialist Hospital Jos. Wound swabs and pus were collected from the wounds of 251 out-patients' department (OPD) over a 14 months' period. Subjects were recruited upon approval by the Ethical Research Committee of the UCTH and after obtaining written or oral informed consent from the subject. Samples were cultured and microbial isolates identified using standard microbiology methods. Antibiotic susceptibility testing was carried out on the bacterial isolates. Six bacterial isolates were identified from wounds of diabetic patients viz: *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The most frequently encountered bacterial pathogen of wound infection among subjects was *Pseudomonas aeruginosa* (26.67%) followed by *Staphylococcus aureus* (23.33%) while the lowest was *Klebsiella pneumonia* (6.67%). The antibiotic susceptibility profile of the bacterial isolates to commonly used antibiotics ranged between 0.0% to 100%. The flouroquinolones were the most active antimicrobial agents observed among bacterial isolates from diabetic out-patients studied. *S. aureus* [7(100%) and 6(85.7%)] was the highest susceptible bacteria followed by *P. aeruginosa* [7(87.5%) and 5(62.5%)], *E. coli* 6(100%) against Ciprofloxacin (10%) and Augmentin (30%) respectively. Meanwhile, *K. pneumonia* and *S. pneumonia* were not susceptible each (0.0%) against Doxycycline and Chloramphenicol at 10% and 30% concentrations respectively. The study showed a high index of wound contamination with bacteria pathogens and resistance pattern to standard and commonly used antibacterial agents among diabetic out-patient from the study area.

Keywords: Antimicrobial resistances, prevalence, wounds infections, Jos.

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INTRODUCTION

A wound is a type of injury which happens relatively quickly in which skin is torn, cut or punctured (an open wound) or where blunt force trauma causes a contusion (a closed wound) (Adegoke *et al.*, 2010). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin and therefore compromises its protective function, as a result wound can be contaminated by microorganisms like bacteria and infection occurs when such organisms multiply and cause damage (Bowler *et al.*, 2001; Arabishahi and Koohpayezade, 2006). Infection also occurs when virulence factors produced by the microorganisms overwhelm the host natural resistance (Bowler *et al.*, 2001). Wound infection is characterized by the presence of pus in lesions with pyrexia, pain and induration. The unbroken skin is the first line of defence and a barrier against microbial invasion which serves as host to a variable number of transient or contaminating bacteria (Stephan and Landis, 2008). Although its low surface pH, sebaceous fluid, and fatty acids produced inhibits the colonization and growth of pathogenic organisms, exposure of subcutaneous tissue following loss of skin integrity provides a moist, warm, and nutritious environment that is conducive for microbial colonization and proliferation (Bowler *et al.*, 2001). The contamination or mere presence of pathogenic organisms in wound without local or systemic tissue involvement may not result in infection (Sule *et al.*, 2002). The organisms can cause destruction if left untreated (AWMA, 2011). Bacterial wound infections are important because they can slow down the healing process, lead to wound breakdown, prolonged hospital stay and increase in the cost of treatment (Alexander, 1994; Sule *et al.*, 2002). High index of wound contamination with bacteria are the third most frequent nosocomial infections (exposure to dirty environment, contaminated water or materials used for treatment and even from the hospital) (Dionigi, *et al.*, 2001; Ducel *et al.*, 2010).

However, the clinical significance of the type of microorganism present is reduced if there are limited signs of infection, which is common in people with infected diabetic ulcers (Bowler 2001). Delayed healing is more likely to occur in people with diabetic foot infections, even when less pathogenic microorganisms are present (Williams *et al.*, 2018).

Epidemiological surveillance of infection is indispensable for effective management of diseases, and the creation and implementation of control measures. High prevalence of open wound infections has been reported as a matter of national health emergency (Mofikoya, 2009). Studies have shown that most hospitals in developing countries especially Africa, have rudimentary and highly compromised infection control programmes due to lack of awareness of the problem, lack of personnel, poor water supply, erratic electricity supply, poor laboratory back-up and funding, traumatic and surgical site infections (Fadeyi *et al.*, 2008; Mehta *et al.*, 2007; Anguzu and Olila, 2007; Samuel *et al.*, 2010). These factors are rife in most rural health care centres in Nigeria, and underscore the need for this study. The etiology of wound infection, regional and local variations occur among causative micro-organisms from country to country and from hospital to hospital even within the same region (Wariso and Nwachukwu, 2003; Biadlegna *et al.*, 2009; Egbe *et al.*, 2011). With the surge of multi-drug resistant bacteria, health care providers have become increasingly challenged in effective wound management (AWMA, 2011). Accurate information of the incidence and etiology of infections acquired within a hospital is essential for articulation of effective preventive or management measures (Akinjogunla *et al.*, 2009; Sanjay *et al.*, 2010). Against this background, this study was aimed at determining

the prevalence, causative organisms and profile of associated bacterial isolates among out-going patients in Plateau State Specialist Hospital, Plateau State, Nigeria.

MATERIALS AND METHODS

STUDY AREA

Plateau is a state in the Middle-belt region of Nigeria with a population of about 3,206,531 people (NPC, 2006; NBS, 2009). The state lies between longitude 8°22'N, 8°35'E and latitude 10°26'N, 10°40'E and it has an area of 26,899 square kilometres (NBS, 2009). The altitude ranges from around 1,200 meters (about 4000 feet) to a peak of 1,829 metres above sea level in the Shere Hills range near Jos. Plateau State is situated in the tropical zone and it shares boundaries with Bauchi state to the North-East, Kaduna State to the North-West, Nasarawa State to the South-West and Taraba State to the South-East (C-GIDD 2008). Jos South, a metropolitan city is one of the local government areas headquarter of Plateau State, Nigeria (National Bureau of Statistic (NBS) 2009). Majority of the residents of Jos South are farmers with few civil and public servants, teachers and students making less than 10% of the community. Plateau Specialist Hospital is the only State-owned tertiary health care provider in Jos South, Plateau State. Some people from all neighbouring LGAs also attend the Hospital.

STUDY POPULATION AND DESIGN

Clinical samples of cases and symptoms of wound infections from both male and female volunteers with age range between 18-80 years in the out-patient departments (OPD) of Plateau State Specialist Hospital (PSH) were recruited for this study. The prospective, hospital-based parallel studies from clinical cases that suffered diabetic wound infections ran for 6 months from July 2019 to December 2019.

Subjects were recruited upon approval by the Ethical Research Committee of Jos University Teaching Hospital (JUTH) and Plateau State Specialist Hospital, Jos, Nigeria. Verbal informed consent was obtained from all participating subjects prior to specimen collection. A structured questionnaire was administered to study subjects for information on demographic data (age, gender, marital status, site of wound, wound type and the type of prophylactic antibiotics). Subjects who could read and write completed the forms while the semi literates and the illiterates were assisted following verbal response to questions.

CULTURE MEDIA AND PREPARATION

Media used include MacConkey agar, blood agar, Manitol salt agar, Cetrimade agar, Simmon citrate, Sabouraud dextrose agar, of Urease agar, Nutrient agar, Mueller Hilton Agar and Peptone water were prepared according to the method adopted by Microbiology Society (MS 2016).

COLLECTION OF SPECIMENS

Wound swabs and pus were obtained by doctors and nurses in the clinics and wards, and by Medical Laboratory Scientists in the laboratory using commercially available sterile cotton swabs from 220 subjects suspected to be infected or suffered diabetic clinical cases of wound infections were included in the study. Suspicion was dependent

on the history of patients with delayed or inability of wounds to heal properly, swelling, foul odour, discharge, enlargement of wound lesions alongside exudation and painful wounds in Plateau Specialist Hospital (PSH), under standard universal aseptic precautions. Only two swabs per patient were collected. The samples collected were transported immediately to the Department of Clinical Microbiology Laboratory at the Faculty of Pharmaceutical Sciences, University of Jos to prevent drying (Pondei *et al.*, 2013).

ISOLATION AND IDENTIFICATION OF ISOLATES

Bacterial isolates were identified using standard laboratory techniques (Collee *et al.*, 1996; Sule *et al.*, 2002; Barrow and Feltman, 2003; Cheesbrough, 2006; Cappuccino and Sherman, 1996; MS, 2016). One of the wound swabs was used to make film and stained by gram's stain. The second isolates from the swabs were immediately inoculated on blood, MacConkey, Centrimide and Mannitol Salt agar and incubated aerobically and in carbondioxide jar for 24 to 48 hours at 37°C (Pondei *et al.*, 2013). Anaerobic cultures were not done due to lack of anaerobic jar in the laboratory at the time of this study. Bacterial isolates were isolated and identified based on the streak plate method and colonial characteristics, Gram staining and biochemical tests. Candidiasis was diagnosed by the presence of yeast-like cells as well as identification of isolates from culture. All yeast isolates were inoculated on CHROM Agar Candida™ and incubated for 48 hours at 37° C. The colour produced by each colony was used to identify the yeast.

ANTIBIOTIC SUSCEPTIBILITY TESTING AND STANDARDIZATION OF ISOLATES

Antibiotic susceptibility testing was done using the Kirby-Bauer Disc Diffusion Method on Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB) in distilled water at 15 lbs psi for 25-30 min. They were further maintained on the different prepared media at 37°C for 48 hours and stored at 4°C, and interpreted according to the recommendations of the Microbiology Society's Standards (MS 2016) and (Howe and Andrews, 2012). Exactly 0.2ml of overnight cultures of each organism was dispensed into 20ml of sterile nutrient broth and diluted to standard inoculum of 10⁶ cfu/ml. A loopful of the standard inoculum was used for the antimicrobial assay (Collins *et al.*, 1995).

STATISTICAL ANALYSIS

These parameters were tested in triplicates. Data obtained in the study were analysed with Epi Info 2012 software. Differences in infection rates between age groups and sex were analysed using χ^2 test. A p-value ≤ 0.05 was considered significant. Percentage susceptibility was calculated.

RESULTS

Total number of patients and demographic data

A total of 220 (108 females and 112 males) were recruited from clinical cases and symptoms of wound infections from both male and female volunteer in the out-patient departments (OPD) of Plateau State Specialist Hospital (PSH).

The age range of the study population was from 18 - 80 years.

PERCENTAGE FREQUENCY OF OCCURRENCE OF WOUND BACTERIAL ISOLATES

The result of the percentage frequency of occurrence of the organisms showed the presence of six (6) bacteria isolates from wounds of diabetic patients. They are; *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. *Pseudomonas aeruginosa* (26.67%) was the most predominant isolate followed by *Staphylococcus aureus* (23.33%), while *Klebsiella pneumonia* was the least (6.67%). (Table 1).

The frequency and percentage distribution of wound bacterial infection among males and females' sample in out-patients from 2019 did not differ significantly ($p>0.05$). However, male patients had a slightly higher prevalence than female patients in the year 2019 as indicated in Table 1. Age and gender affected the prevalence of wound bacterial infection among male and female outpatients as shown in Table 1 &2.

SENSITIVITY TEST RESULT

The fluoroquinolones (antibiotics used in treatment of systemic infection by interfering with DNA replication) were the most active antibacterial agents against bacterial isolates from in and out patients studied. A generally higher resistance pattern was observed among nosocomial bacterial pathogens as indicated in Table 2. Gentamycin most effective against aerobic Gram-negative rods.

Table 1: Frequency and percentage distribution of Isolates from male and female wound Samples.

Gender	<i>E. coli</i>	<i>P. mirabilis</i>	<i>S. aureus</i>	<i>S. pyogene</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>
Males	4(12.00)	3(9.00)	4(18.33)	2(8.0)	3(9.67)	1(3.33)
Females	2(6.00)	1(4.0)	3(5.0)	1(2.0)	5(17.0)	1(3.34)
Total	6(20.00)	4(13.0)	7(23.33)	3(10.0)	8(26.0)	2(6.67)

Table 2: Number of wounds Examined from male and female Subjects as per their ages.

Age	No. of wounds	
	Examined in Males	Examined in Females
18-50	17	21
51-60	24	22
61-70	30	27
71-80	41	38
Total	112	108

Table 3: Susceptibility profile of bacterial isolates Against some selected Antibiotics

Bacterial agents	Antibiotic Agents				
	CIP 10%	AUG 30 %	GEN 10%	CHL 30%	DOX 10%
<i>Pseudomonas aeruginosa</i> (8)	7(87.5)	5(62.5)	4(50.0)	0.0	0.0
<i>Streptococcus pneumonia</i> (3)	3(100)	2(66.7)	1(33.3)	0.0	0.0
<i>Proteus mirabilis</i> (4)	3(75.0)	2(50.0)	1(25.0)	1(25.0)	0.0
<i>Klebsiella pneumonia</i> (2)	2(100)	2(100)	1(50.0)	0.0	0.0
<i>Staphylococcus aureus</i> (7)	7(100)	6(85.7)	4(57.1)	2(28.6)	1(14.2)
<i>Escherichia coli</i> (6)	6(100)	4(66.7)	3(50.0)	1(16.7)	0.0

CIP - Ciprofloxacin; GEN- Gentamicin; AUG-Augmentin; DOX- Doxycycline and CHL- Chloramphenicol, 0.0 – denotes not susceptible.

DISCUSSION

In this study, the findings demonstrated the predominance of *Pseudomonas aeruginosa* *Staphylococcus aureus* and *E. coli*. *P. aeruginosa* was the commonest isolate, accounting for 8 (26.7%), followed by coagulase negative *Staphylococcus aureus* and *Escherichia coli* with 7 (23.3%) and 6 (20.0%) respectively. These results were in agreement with results from previous studies conducted globally and in various parts of the country (Isibor *et al*, 2008).

However, the result of this study is at par with Oladeinde *et al.* (2013) and Imarenezor (2017) who reported that *S. aureus* was the most prevalent pathogen with the frequency of occurrence (62.7% and 52.8%) followed by *P. aeruginosa* (23.1% and 9.9%) in male and female subjects when compared with the result of our investigation respectively.

Findings from Adegoke *et al.* (2010) carried out at a University hospital in Nigeria showed that the commonly isolated bacteria were *S. aureus* (25%) and *P. aeruginosa* (20%) which when compared with the result of our investigation are of lower prevalence rate. The high prevalence of *P. aeruginosa* among subjects may be attributed to contamination of wounds with soils, medical devices or the existence of locality variability.

Ogba *et al.* (2014) also reported in their study of bacterial pathogens associated with wound infections in Calabar, Nigeria that *Staphylococcus aureus* was the most prevalent pathogen 91 (30.8%) among both category of patients, followed by *Pseudomonas aeruginosa* 51 (17.3%). *Streptococcus* species were the lowest isolated pathogens 4(1.3%) agreed with this result. Another study at a University teaching hospital in Iran, also reported *Staphylococcus aureus* to be the commonest bacteria isolated (43%). These slight variations may be due to the difference in study areas and populations. Although a number of studies have been conducted on wound infections in some hospital and

clinics in Nigeria, to the best of our knowledge, none has been done in a tertiary health care facility (Plateau Specialist Hospital) on the prevalence of wound infections among diabetic out patients in Jos South LGA, Plateau State.

Jos South and other neighbouring communities are strictly urban settings with inhabitants being largely civil servants and farmers. In other words, the difference may be due to the fact that subjects enrolled for the study had wounds and are diabetic patients suspected to be infected as opposed to all wounds examined by many reported studies by scientists (Ogba *et al.*, 2014).

The bacteria isolates obtained in this study includes pathogens such as *E. coli* known to cause food poisoning, diarrhea, abdominal pain, fever, nausea, vomiting and about 75% to 95% of urinary tract infections. *E. coli* infection is contagious and can be spread from person to person by faecal contamination. *K. pneumonia* that causes health care-associated infections in form of pneumonia, sepsis, wound and urinary tract infections (Long *et al.*, 2017). It has caused a global explosion of drug-resistant and are increasingly difficult to treat because they are resistant to many of the available antibiotics (Dromigny *et al.*, 2005; Long *et al.*, 2017). Until now, *K. pneumonia* was thought to be the culprit in most *Klebsiella* infections. It is also capable of causing invasive and severe infections in patients with the same rate of mortality as other species of the *Klebsiella*. Our finding is in agreement with the work of Long *et al.* (2017), who stated that three *Klebsiella* species caused life threatening infections and shared drug resistance genes in a study in Houston, Texas.

P. aeruginosa is an opportunistic human pathogen. It is “opportunistic” because it seldom infects healthy individuals (Botzenhardt and Doring, 1993; Costerton and Anwar, 1994).

Furthermore, any *P. aeruginosa* already present on a burn victim’s skin before the injury can transform from an innocuous organism on the surface of the skin to a source of infection in the bloodstream and body tissues of the same individual (Fick, 1993; Lyczak *et al.*, 2000). High rate of occupational, nutritional, unhealthy life styles related diseases, diabetics, poor hygiene and accessibility to health care facility may be responsible for the observed trend. In this study, we observed that there was a link between age and gender of the diabetic wound infections in out-patients. Age did not significantly affect the prevalence of wound infection in our study. However, gender affects the prevalence of the wound infection from our study. These have been previously confirmed in two separate Nigerian studies by Ezebialu *et al.* (2010) and Egbe *et al.* (2011). This finding does not agree with the study by Ogba *et al.* (2014) who reported that age have no bearing on wound infections with people in their second to fourth decades of life being more prone since this is the most productive age range. This is inconsistent with the report of Pondei *et al.* (2013), but different from the reports of Egbe *et al.* (2011) in Bayelsa, Nigeria.

Generally, antibiotic susceptibility of isolates to commonly used antibiotics was low; the quinolones (Ciprofloxacin), Augmentin and Gentamycin were the most potent antimicrobial agents observed in our study. Meanwhile, a high level of resistance of isolates tested with Doxycycline and Chloramphenicol was also observed. This may be attributed to the fact that Doxycycline and Chloramphenicol derivatives have been widely abused and frequently implicated in self-medication in Nigeria. The multiple resistance of isolates, especially *S. pneumonia*, *K. pneumonia* and *P. aeruginosa* to commonly used antibiotics in the locality of the study calls for an immediate action on the controlled use of antimicrobials in the hospitals and the need to monitor resistance. Good antimicrobial use is necessary for effective wound management. The susceptibility rate of bacterial isolates observed in this study, agrees

with the reports of Ogba *et al.* (2014), Sule *et al.* (2002), Oladeinde *et al.* (2013) and Pondei *et al.* (2013) in Nigeria and Anguzu and Olila (2007) in Uganda.

Odugbemi (1981) and Pondei *et al.* (2013) also reported that high level of antibiotic abuse in Nigeria arise from self-medication which is associated with inadequate dosage and failure to comply with treatment regimen. These antibiotics are being sold over the counter with or without prescription (Anguzu and Olila, 2007; Oladeinde *et al.*, 2013; Ogba *et al.*, 2014).

CONCLUSION AND RECOMMENDATION

This study has shown a high index or prevalence of wound contamination of diabetes patients with *P. aeruginosa* and *S. aureus* being the most predominant etiologic agents of wound infection observed among out-patients with significantly higher prevalence of 8(26.7%) and 7 (23.3%) respectively. The flouroquinolones were the most active antimicrobial agents observed among bacterial isolates from out-patients studied. *S. pneumonia*, *K. pneumonia* and *P. aeruginosa* showed low susceptibility to Doxycycline and Chloramphenicol which are commonly used in our locality. However, a generally higher antimicrobial resistance pattern was observed among diabetes patients in our study.

In conclusion, *P. aeruginosa* was confirmed to be the most prevalent bacteria in diabetic wound infection of out-patient from this study. Contracting wound infection remains an ongoing problem when an injury is sustained and the rate of getting infection when a wound is contaminated is greatly increased, when risk factors are present. For example, diabetic patients are at high risk of getting infection because they have a weakened immune defence. To avoid wound infection in diabetic, one of the most importance things to do, is to practice careful foot care, in addition to wearing shoes and socks to avoid minor bumps and scrapes. the feet should be examined daily for any blisters, cuts, scrap, sore or other skin problems that could allow an infection to develop. Wound specimens should also be collected for culture and susceptibility testing to help the clinicians before appropriate antibiotic selection and chemotherapeutic management of wound infections is recommended. It is essential that people who have diabetes keep their blood sugar levels under control to reduce the risk of slow-healing wounds and complications, including foot ulcers. Hence good hygiene and proper care of wound infection plus in cooperation of antimicrobial drugs during treatment is recommended. The multiple antibiotic resistance of isolates calls for an immediate action on the control of antimicrobial usage and the need to monitor resistance.

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