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Prevalence and Antibiotic Susceptibility Pattern of Pathogens Isolated from Post-Operative Wounds of Diabetes Mellitus Patients in Uyo, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author EDN supervised and performed the laboratory work while author NAJ managed the literature searches and statistical analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at investigating the prevalence and antibiotic susceptibility of isolates from wounds of diabetic and non diabetic patients in University of Uyo metropolis. Eighty five wound swab samples were collected from the culture bench of University of Uyo Health Center and three neighboring hospitals. The isolates were characterized and identified using standard microbiological methods. The antibiotic susceptibility pattern was determined using the disc diffusion method. Oral interview was conducted on patients to ascertain their medical history and samples taken to conduct HbA1c test at the time of wound swab collection. Out of the samples collected, 62.5% were infected with bacteria (80% Gram negative and 20% Gram positive) featuring frequency of occurrence percent of *Staphylococcus aureus* (28.8%), *Pseudomonas* spp. (33.1%), *Escherichia coli* (25.0%),

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Proteus spp. (8.3%) and *Klebsiella* spp. (2.6%). There was significant difference in the antibiotic sensitivity of *S. aureus* to ciprofloxacin and ofloxacin comparing the two groups. Similarly, there was significantly lower sensitivity to ofloxacin shown by *E. coli* and *Klebsiella* spp. in the DM group compared with the control. There were no significant differences in the antibiotic sensitivities of the isolated organisms for the cephalosporin and aminoglycoside antibiotics. The study showed that isolates from SWI demonstrated significant differences in susceptibility to fluoroquinolones but no differences to the aminoglycoside and cephalosporin antibiotics based on the glycaemic levels.

Keywords: Antibacterial sensitivity; surgical wound infection; diabetes mellitus.

1. INTRODUCTION

There is a complex interplay between the host, microbial and surgical factors that ultimately determines the establishment of a wound infection. A wound is any injury that damages the skin compromising its protective function [1]. Microbiological and patient's systemic factors may pose some risks affecting the healing of wounds. The successful invasion and proliferation by one or more species of microorganisms of the breached protective function of the skin, as it is in most surgeries, lead to wound sepsis [2-4].

The potentials for infections depend on some variables such as the state of hydration, nutrition and existing medical conditions. The micro-organisms that typically infect wounds depend on some environmental influence, the state of the host immune system and the depth of the wound. Strict aerobes require oxygen while some organisms survive in environment with or without oxygen and are known as facultative anaerobes. Fungi, protozoa and viruses have been isolated in wounds existing in a polymicrobial community, however bacteria remains the mainstream pathogenic stay in SWI [5,6].

Diabetes mellitus (DM) is a chronic disease that is due primarily to a disorder of carbohydrate metabolism and caused by a deficiency or diminished effectiveness of insulin resulting in hyperglycaemia and glycosuria [7,8]. Secondary changes as a result of DM may occur in the metabolism of proteins, fats, water and electrolytes with grave consequences. Hyperglycaemia in DM occurs as a result of decreased and impaired transport or uptake of glucose into muscles and adipose tissues along with depression of key gluconeogenic enzymes (*i.e.*, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose biphosphatase and glucose-6-phosphatase). These significant biochemical steps promote gluconeogenesis or repression of glycolytic enzymes (*i.e.*, glucokinase, phosphofructokinase and pyruvate kinase). The reduced amino acid levels in the peripheral tissues due to depressed amino acid transport and uptake lead to increased amino acid levels in blood (*i.e.*, alanine) providing fuel for gluconeogenesis and decreased protein synthesis in all tissues. All of these metabolic changes and hydrolysis of stored lipids cause interference at several steps of carbohydrate phosphorylation in muscles further contributory to hyperglycaemia [9].

The metabolic changes in DM have been reported to cause a delay in wound healing and the consequent reduction of immunity. It is believed that this health condition may allow the colonization of microorganisms leading to SWI.

This work seeks to assess the prevalence of bacterial pathogens in diabetic and non diabetic SWI and the antibacterial susceptibility to the commonly employed agents in the study centre.

2. MATERIALS AND METHODS

2.1 Methods

2.1.1 Study location

The study was carried out at the University of Uyo Health Services Centre, Uyo Nigeria being the central collating centre with 3 sub-centres (Hospitals in Uyo metropolis as sample collecting centre).

2.1.2 Sample collection

Wound swabs were collected from 85 surgical wound sites from in-patients and out-patients visiting the collection centres within the period of May 2012 to January 2013. Blood samples were also collected for the determination of HbA1c [7,8]. The samples were transported aseptically to the collating centre for analysis.

2.1.3 Sampling and isolation

The employed media (*i.e.*, MacConkey agar, nutrient agar, blood agar) were prepared according to the method of Collee *and co-workers* [10]. The specimens were inoculated on blood and MacConkey agar plates and incubated aerobically at 37℃ for 48 h. The colony forming units (cfu) of the organisms were determined. Colonies having cfu above 10^3 organisms per colony were set aside for further antibiogram analysis [11]. The bacterial colonies obtained were sub-cultured on nutrient agar by streak plate technique and incubated at 37℃ for 24 h. The pure colonies of the organisms obtained were kept in nutrient agar slants and stored in the refrigerator at 4℃ prior to characterization and identification.

2.1.4 Characterization and identification

The bacterial growth was identified by colony characteristics, blood haemolysis, microscopic examination of Gram stained preparations and motility techniques. The biochemical characteristics employed to confirm the identity of the isolates included hydrogen sulphide production, indole production, glucose, lactose and mannitol fermentation, gelatin liquefaction, nitrate reduction, urease and oxidase test and catalase activity according to the methods of Manual of Methods for General Bacteriology [12]. The isolates were identified by comparing their characteristics with those of known taxonomy according to the scheme of Cowan and Steel, 1993 [13]. Biomerieux test kit was also employed to characterize the organisms according to the method of Odumeru et al. [14].

2.1.5 Antibacterial susceptibility test

The antibacterial sensitivity test employed was paper disc diffusion performed according to Kirby-Bauer techniques. The identified isolates were tested against a total of 7 commonly prescribed and employed antibacterial agents in the locality ampicillin (30 µg), ciprofloxacin (10 µg), ofloxacin (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), streptomycin (30 µg) and gentamycin (30 µg). The results were interpreted using chart of CLSI, 2012 [15]. The susceptibility profiles of the isolates from the surgical wounds were determined by standard methods.

2.2 Statistical Analysis

Data was processed using SPSS Statistics version 20, IBM Corporation, USA. Paired samples t-test was used to determine any difference between the antibiotic susceptibility of the organisms in the DM and the NDM (control) groups while significant differences was taken at $p<0.05$.

3. RESULTS

Out of the 85 patients from whom swab samples were collected (49 males and 36 females), 60 and 25 swabs were obtained from in-patients and out-patients, respectively. The HbA1c level (≥ 7%) significant of uncontrolled DM was observed in 24.1% of the infected patients (43.8% among males and 18.8% among females). A total of 54 (63.5%) presented post-operative wound infections with bacterial count in excess of 10^3 organisms count in some colonies. A total of 5 organisms were identifiable by Gram staining in this study with (20% and 80% being Gram positive and Gram negative, respectively). The Gram negative organisms isolated from the DM group were *Ps. aeruginosa*, *E. coli*, *Proteus* spp. and *Klebsiella* spp. The only Gram positive microbe was *S. aureus*. All microbes featured in the DM and control group except *Ps. aeruginosa* that was conspicuously absent in the NDM group.

The female patients had higher infection rate (77.6%) compared to male (50.0%). Table 1 gives the demographics and distribution of the patients based on the study centres, sex and the glycaemic disposition. Fig. 1 presents the distribution of the surgical wound sites in the study population while Table 2 gives the distribution of the isolated pathogens in the DM and NDM SWI groups. Fig. 2 presents the prevalence of the organisms implicated in the SWI. There was generally lower percentage susceptibility to the antibiotics by the organisms in the DM group compared with the NDM group, for most of the organisms. Table 3 presents the antibiotic sensitivity for the isolated organisms for the groups while Table 4 gives the statistically computed values evaluating the differences in the susceptibility values to the antibiotics for the isolated organisms in the DM and the NDM groups.

Comparing among the organisms for the DM group, the Gram negative organisms were least sensitive to ampicillin and highest sensitivity to ceftazidime. *Ps. aeruginosa* was conspicuously absent from the NDM group in this study.

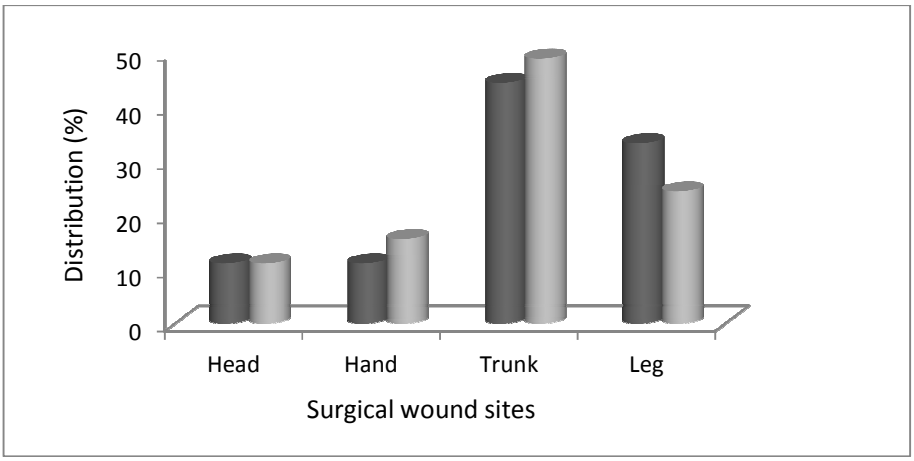

Fig. 1. Distribution of the SWI sites (DM■, NDM □)

Table 1. Demographics and distribution of the SWI patients in the study

Parameters	DM group		Non DM (Control) group	
Sex distribution	**Male n(3)**	**Female n(6)**	**Male n(13)**	**Female n(32)**
Mean age of patient (years)	37.5±4.5	43.0±6.5	41.5±2.0	44.5±4.5
Number of patients in each study center				
1	1	2	6	12
2	2	1	4	9
3	-	2	1	7
4	-	1	2	4
Mean ± SEM HbA1c (%)	10.2±0.6	11.5±1.5	6.0±0.4	6.5±0.7
Number of different organisms isolated	5	5	4	4

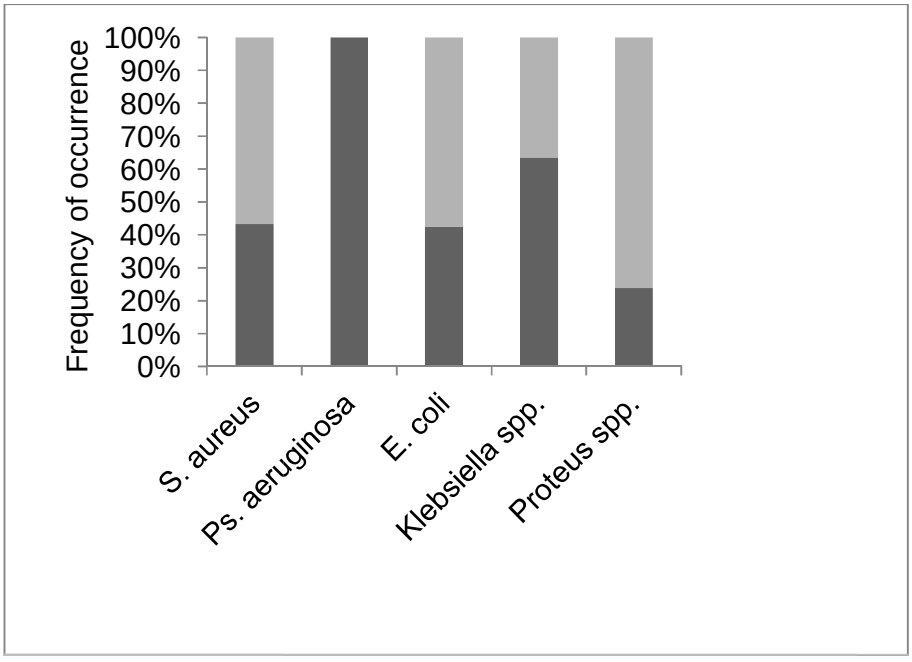

Fig. 2. Distribution of the isolated organisms in the DM and the (NDM) control group (DM■, NDM □)

Table 2. The frequency of occurrence (%) of the isolated organisms from the SWI

Organisms	DM group		Non DM (Control) group	
	Male	Female	Male	Female
S. aureus	13 (48.1)	16 (36.4)	19 (46.3)	26 (37.1)
Ps. aeruginosa	6 (22.2)	15 (34.1)	12 (29.3)	18 (26.1)
E. coli	4 (14.8)	11 (25.0)	7 (17.1)	17 (24.6)
Klebsiella spp.	2 (7.4)	2 (4.5)	-	-
Proteus spp	2 (7.4)	-	3 (7.3)	8 (11.6)

4. DISCUSSION

The investigation of antimicrobial susceptibility of bacterial pathogens for possible susceptibility profile changes based on prevailing or co-existing clinical condition(s) is necessary for adequate infection treatment. This study was conducted against the backdrop of the clinical conditions wherein the ambulatory and hospitalized patients employed in this study presented with signs of SWI in spite of the different pre and post-operative antibacterial agents administered to prevent infection. In the present study, there was higher growth and prevalence of the infecting organisms in the DM group compared to the control. This is in agreement with the study by Presutti and Millo [16] which concluded that there was the need to control blood sugar levels to reduce infections. Glycosylated haemoglobin levels for the DM patients were determined to ascertain the glycaemic controls more importantly for the DM patients. The delay in wound healing observed may be attributed to the high HbA1c levels of the DM group. Based on the conclusions of Presutti and Millo, uncontrolled and consistently elevated glycaemic levels may increase infection and possibly the pattern of resistance to antoibacterial agents

S. aureus was observed as the predominant organism followed by *Ps. aeruginosa* in this study. This is also in agreement with the study conducted by Mashita et al*.* [17] but contrary to the report by Sani et al. [18] and Mohammed et al*.* [16]. No isolates of *Citrobacter* spp*.* was found in the samples in our study but *Klebsiella* spp*.* was isolated. Similar work by Sani and co-workers featured these Gram negative organisms without *Klebsiella* spp*.* from wound infection [18].

Previous studies on SWI have reported the importance of Gram negative organisms causing delay in wound healing. The ratio of Gram negative to Gram positive organisms implicated in this study was 4:1. The presence of *S. aureus* has been demonstrated severally as a predominant organism in wound sepsis [19-21] while *Ps. aeruginosa* and *E. coli* have also been commonly isolated from diabetic ulcers in similar studies conducted by Fadeyi et al*.* [22]. The fundamental infecting organisms in post-operative DM are therefore premised on the trio of *S. aureus*, *E. coli* and *Ps. aeruginosa*, adjudging from the outcome from across a wide geographical study [23].

The proportion of location of surgical wounds shows that the site was more located in the trunk region in both groups in this study. It has been reported that the infecting organisms in surface wounds are usually the patient's endogenous flora [24]. Gram negative organisms tend to be endemic in hospital environment as they are easily transferred from object to object. It suffices to propose that most of the invading organisms from surgical wounds were contacted from the hospital or point of surgery.

The co-existence of *S. aureus* and *Ps. aeruginosa* has been reported in wound sepsis by many researchers [24,25]. Our study has also presented the neigbourliness of these pathogens. *Ps. aeruginosa* was not observed in the DM group. The organism is known to colonize diverse environmental habitat ad able to persist and grow eve under extremely poor ad hostile conditions [26]. The preferably chosen sources of carbon or nitrogen include short-chain fatty acids, amino acids and polyamines [27]. Although *Ps. aeruginosa* metabolizes sugars, sugars represent less preferred substrates which are degraded via the Entner-Duodoroff pathway [28]. The uncontrolled sugar levels in these SWI patients may have an association with the unobserved growth of this opportunistic human pathogen.

There was widespread resistance to ampicillin by the organisms from both groups. This feature is characteristic of certain antibacterial which are prone to abuse in communities due to the manner of prescribing, sales and use of the antibiotics involved [28].

Table 3. Antibiotic susceptibility pattern of the SWI isolates

Isolates	Sensitivity to antibacterial agents (S %)													
	AMP		OFL		CPF		CTX		CFM		STR		GTN	
	DM	NDM	DM	NDM	DM	NDM	DM	NDM	DM	NDM	DM	NDM	DM	NDM
S. aureus (N=40)	11 (27.5)	10 (25.0)	17 (42.5)	22 (55.0)	16 (40.0)	24 (60.0)	18 (45.0)	22 (55.0)	16 (40.0)	20 (50.0)	10 (25.0)	12 (30.0)	12 (30.0)	12 (30.0)
P. aeruginosa (N=40)	6 (15.0)	-	23 (57.5)	-	21 (52.5)	-	20 (50.0)	-	28 (70.0)	-	14 (35.0)	-	14 (35.0)	-
E. coli (N=30)	9 (30.0)	21 (70.0)	16 (53.3)	14 (46.7)	20 (66.7)	21 (70.0)	26 (86.7)	26 (86.7)	26 (86.7)	24 (80.0)	21 (70.0)	20 (66.7)	24 (80.0)	20 (66.7)
Klebsiella spp. (N=30)	6 (20.0)	15 (50.0)	15 (50.0)	21 (70.0)	18 (60.0)	18 (60.0)	20 (66.7)	28 (93.3)	24 (80.0)	26 (86.7)	12 (40.0)	18 (60.0)	15 (50.0)	18 (60.0)
Proteus spp. (N=30)	5 (16.7)	11 (36.7)	12 (40.0)	16 (53.3)	24 (80.0)	26 (86.7)	16 (53.3)	21 (70.0)	24 (80.0)	22 (73.3)	18 (60.0)	24 (80.0)	22 (73.3)	22 (73.3)

**DM and CT represent Diabetes Mellitus and the non diabetes mellitus (control) group, respectively. AMP, OFL, CPF, CTX, CFM, STR and GTN represent ampicillin, ofloxacin, ciprofloxacin, ceftriaxone, ceftazidime, streptomycin and gentamycin, respectively.*

Table 4. The probability values for the comparison of the antibiotic susceptibility of the isolated organisms for the DM and the NDM (Control) group

Organisms	Ampicillin	Ofloxacin	Ciprofloxacin	Ceftriaxone	Ceftazidime	Streptomycin	Gentamycin
S. aureus	0.12	0.01	0.04	0.20	0.20	0.07	0.68
Ps. aeruginosa	-	-	-	-	-	-	-
E. coli	0.23	0.04	0.12	0.30	0.33	0.18	0.06
Klebsiella spp.	0.02	0.02	0.84	0.03	0.15	0.06	0.42
Proteus spp.	0.01	0.2	0.27	0.09	0.09	0.51	0.057

- *Significant difference in antibiotic sensitivity is taken for values P<0.05*

S. aureus showed significant antibiotic susceptibility to the fluoroquinolones (ciprofloxacin and ofloxacin) in the control group than the DM while *E. coli* and *Klebsiella* spp. had the same trend for ofloxacin. The organisms isolated had no significant difference in their antibiotic sensitivity to the cephalosporin (ceftriaxone and ceftazidime) for the DM and the NDM group. The same trend was observed for the organisms with respect to the aminoglycoside (streptomycin and gentamycin) for the DM and NDM) group. The clinical condition with DM presenting with uncontrolled blood sugar have been reported to feature episodes of urinary tract infections and often prescribed fluoroquinolones or aminiglycosides. The resistance pattern therefore of these SWI isolates may be an indication of the use of these drugs in the study area.

5. CONCLUSION

The findings of this study revealed that Gram negative organisms (*E. coli, Klebsiella* spp*., Ps. aeruginosa and Proteus* spp.) were prevalent in SWI with *S. aureus* co-existing with these organisms in both study group. *Ps. aeruginosa* was absent in the non-diabetic group.

The Gram negative isolates from the non diabetic SWI sites revealed significantly lower antibiotic susceptibility to the fluoroquinolone drugs but no difference to the cephalosporin and aminoglycoside drugs when compared with the presentation in the diabetic group.

CONSENT

All authors declare that written informed consent was obtained from the volunteers who participated in the study.

ETHICAL APPROVAL

In accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, approval for this work was obtained from the University Health Centre Research Advisory Committee and the Clinical Pharmacy and Biopharmacy Departmental Research Committee, Faculty of Pharmacy University of Uyo. This work therefore has been performed in accordance with the ethical standards laid down in the 2003 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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