

Full Length Research.

Aerobic Bacteria Associated With Diabetic Wounds In Patients Attending Clinic In A Rural Community In Nigeria.

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Some studies from within and outside Nigeria have documented the polymicrobial etiology of diabetic wound infections in patients. The need to ascertain the prevalent types of aerobes affecting wounds in diabetic patients in a rural community, informed this investigation. One hundred and fifty (150) wound swabs were collected from diabetic patients attending clinic in Irrua Specialist Teaching Hospital, Irrua. The swabs were inoculated into MacConkey agar, Blood agar and Chocolate agar media. Plates were incubated at 37°C for 24 hours and observed for growth. Colonies growing on culture plates were Gram stained and identified using standard bacteriological methods. Antibiotic sensitivity tests were carried out on isolates using the disc diffusion method. The occurrence of bacterial isolates in the wounds investigated was in the following decreasing order of frequency; *Staphylococcus aureus* (38%), *Escherichia coli* (24%), *Proteus* spp (20%), *Klebsiella* spp (10%) and *Pseudomonas aeruginosa* (8%). Of the 129 male patients, the percentage isolation rates of pathogens were 18.6, 2.3, 32.6, 30.2, and 16.3, for the respective age ranges of 40-49, 50-59, 60-69, 70-79 and 80-89. The respective rates for the 21 female patients were 0, 4.3, 57.1, 28.6, and 0. All the isolates were resistant to Erythromycin and Cotrimoxazole, while the relative percentage rates of sensitivity of bacterial isolates to antibiotics tested were in the decreasing order of Perfloxacin (13%), Augmentin (10%), Rocephin/Zinnacef (8%), Ciprofloxacin (6%), Gentamicin (3%), Streptomycin (2%) and Amoxicillin (1%). Knowledge of the local isolates from diabetic wounds and their susceptibility pattern would greatly assist in the proper management of patients.

Key words: Aerobes, Diabetic wound, Antibiogram, Rural community, Nigeria.

INTRODUCTION

Diabetic wound ulcers are injuries to the body tissues caused by physical trauma due to excess sugar in the blood stream (Gadepall *et al.*, 2006). The World Health Organization described diabetic foot condition as a group of syndromes that involves neuropathy and ischemic infections (WHO, 1995). Diabetes is believed to affect 2-4% of the general population (Frykberg, 2002) and as many as 15% of people with diabetes will develop foot ulceration and its related complications (Boulton *et al.*, 1999). The development of wounds is a serious complication for patients with diabetes. Numerous factors related to diabetes can impair wound healing, including wound hypoxia (inadequate oxygen delivered to the wound), infection and nutrition deficiencies (Lavery *et al.*, 2007). Boulton reported an infection rate of 2.5% in diabetic wound treated with moisture – retentive

hydrocolloid dressing compared with a 6% infection under a traditional gauze dressing (Boulton *et al.*, 1999).

Staphylococcus aureus is a prevalent isolate in diabetic foot ulcers, together with other aerobes including *Staphylococcus epidermidis*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Enterococcus* spp and coliform bacteria (Pittet *et al.*, 1999; Ozer *et al.*, 2010) Anaerobes have also been isolated from up to 95% of diabetic wounds (Karchmer and Gibbons, 1994). Also, *Propionibacterium acnes*, *P.granulosum* and *Clostridium difficile* have been isolated from diabetic wounds (Alsaimary, 2010). In view of the polymicrobial nature of diabetic foot ulcers, Karchmer and Gibbons (1994) suggested that the treatment of infection could be based on a better understanding of the general microbiology of these wounds. Consequently foot infection must be

diagnosed primarily on clinical grounds (Anandi *et al.*, 2004). Diabetic foot problems can develop extremely quickly, with tissue breakdown occurring rapidly and often complicated by infection.

Chronic wounds develop a more complex colonizing flora, including enterococci, various *Enterobacteriaceae*; obligate anaerobes, *Pseudomonas aeruginosa*, and, sometimes, other non-facultative gram-negative rods (Sapico *et al.*, 1984; Hunt, 1992; Gerding, 1995; Pathare *et al.*, 1998). Hospitalization, surgical procedures, and, especially, prolonged or broad-spectrum antibiotic therapy may predispose patients to colonization and/or infection with antibiotic-resistant organisms (e.g., MRSA or vancomycin-resistant enterococci and once ulcers are formed, they are often slow to heal (Hartemann-Heurtier *et al.*, 2004).

The aim of this study was to determine the aerobic bacteria present in diabetic wound infections and to determine the susceptibility of the isolates to popularly used antibiotics in Nigeria.

SUBJECTS, MATERIALS AND METHODS.

Informed Consent/ Sample Collection.

With the consent of the Consultant Physician and that of the patients themselves, One hundred and fifty (150) wound specimens were collected from diabetic patients attending clinic in Irrua Specialist Teaching Hospital, Irrua, Nigeria, using sterile swab sticks. Each swab was inoculated onto MacConkey, blood agar and chocolate agar media. The inoculated plates were incubated aerobically at 37°C for 24 hours. The colonial morphology, motility and gram reaction of bacterial isolates were noted and colonies were identified using biochemical tests including indole, methyl red, Voges-Proskauer, oxidase, coagulase, catalase, citrate and glucose and lactose fermentation tests as described by Cheesbrough (2004).

Susceptibility testing

Antibiotic susceptibility testing was carried out on Mueller-Hinton agar (OXOID CM 337) using the standard disk diffusion technique (Bauer *et al.*, 1966), and the following antibiotics: Pefloxacin (10µg) (May and Baker, Nigeria), Gentamicin (10µg), Augmentin (30µg), Zinacef

(20µg), Amoxicillin (30µg), Rocephin (30µg), Ciprofloxacin (10µg) (Oxoid, UK) Streptomycin (30µg), Cotrimoxazole (30µg), and Erythromycin (15µg). These antibiotics represent the major classes of antibiotics and they are commonly available and used within the state. The control strains were tested simultaneously with the test organisms.

Briefly, 3 to 5 well-isolated colonies of the test organism were selected from the agar plate. The top of each colony was touched with a sterile loop and transferred into a bijoux bottle containing 3 ml of sterile peptone water. The broth culture was then incubated at 37°C for a few hours until it achieved the turbidity of the 0.5 McFarland standard. A loopful of test organism suspension was transferred to the centre of a previously dried Mueller - Hinton agar, and with a sterile dry cotton wool swab the inoculum was evenly spread over the entire agar surface. The lid was left ajar for 3 to 5 min to allow for any excess surface moisture to be absorbed before applying the antibiotic discs. Using a sterile forceps, the antibiotic discs (previously brought to room temperature) were aseptically placed on the agar surface, and gently pressed down to make contact with the agar.

The agar plates were then incubated aerobically at 37 °C overnight. The diameters of the zones of inhibition were measured to the nearest whole millimeter using a ruler and results were interpreted according to criteria developed by Clinical Laboratory Standards (CSCL, 2010).

RESULTS

The study involved 150 patients (129 males and 21 females). Table 1 shows the frequency of bacterial isolation in relation to age and sex. The diabetic patients who reported at the clinic during the period of study were within the ages of 40 and 89 years. Among the male patients, the frequency of isolation of pathogens from diabetic wounds in relation to the age range, was in the following decreasing order: 60-69 years (32.6%), 70-79 years (30.2%), 40-49 years (18.6%). 80-89 years (16.3%), 50-59 years (2.3%). For the female patients, it was 60-69 years (57.1%), 70-79 years (28.6%), 50-59 (14.3%), while no isolates were recovered in female patients within the age ranges 40-49 and 80-89 years.

Table 1: Frequency of isolation of bacteria in relation to age and sex of patients.

Age	Male	Percentage Isolation (%)	Female	Percentage Isolation (%)
40-49	24	18.6	0	0.0
50-59	3	2.3	3	14.3
60-69	42	32.6	12	57.1
70-79	39	30.2	6	28.6
80-89	21	16.3	0	0.0

The prevalence of bacterial isolates from diabetic wounds is indicated in Table 2. *Staphylococcus aureus* was the

most prevalent isolate (38%), while the least isolated aerobe was *Pseudomonas aeruginosa* (8%).

Table 2: Prevalence of bacterial isolates from diabetic wounds.

Bacterial Isolates	Number (%) of bacteria isolated
<i>Proteus spp</i>	10(20)
<i>Escherichia coli</i>	12(24)
<i>Staphylococcus aureus</i>	19(38)
<i>Klebsiella spp</i>	5(10)
<i>Pseudomonas aeruginosa</i>	4(8)

Table 3 shows the antibiotic susceptibility profile of bacterial isolates. The number and respective percentage susceptibility rates of isolates to various antibiotics tested are shown in this Table 3. The sensitivity of bacterial

isolates to antibiotics tested was in the following decreasing order; Perfloxacin (13%), Augmentin (10%), Rocephin/Zinnacef (8%), Ciprofloxacin (6%), Gentamicin (3%), Streptomycin (2%) and Amoxicillin (1%).

Table 3: Susceptibility pattern showing number (%) of the different bacterial isolates sensitive to various antimicrobial agents.

Organisms	No of isolates	Augmentin	Amoxicillin	Erythromycin	Perfloxacin	Ciprofloxacin	Cotrimoxazole	Gentamicin	Streptomycin	Zinnacef	Rocephin
		No (%) of isolates sensitive to various antibiotics tested									
<i>Proteus spp</i>	10	3(30)	0(0)	0(0)	3(30)	0(0)	0(0)	0(0)	1(10)	2(20)	1(10)
<i>Escherichia coli</i>	12	3(25)	0(0)	0(0)	4(33.3)	2(16.7)	0(0)	1(8.3)	1(8.3)	1(8.3)	0(0)
<i>Staphylococcus aureus</i>	19	2(11)	1(5.3)	0(0)	3(16)	2(11)	0(0)	2(11)	0(0)	4(21)	5(26)
<i>Klebsiella spp</i>	5	1(20)	0(0)	0(0)	2(40)	1(20)	0(0)	0(0)	0(0)	1(20)	1(20)
<i>Pseudomonas aeruginosa</i>	4	1(25)	0(0)	0(0)	1(25)	1(25)	0(0)	0(0)	0(0)	0(0)	1(25)
Total	50	10(20)	1(2)	0(0)	13(26)	6(12)	0(0)	3(6)	2(4)	8(16)	8(16)

DISCUSSION

In this study *Staphylococcus aureus* (38%) was found to be the leading etiologic agent of diabetic wound infections. This finding agrees with the study carried out by Pittet *et al.*, (1999) in Razi, Iran. In the study, out of a total of 32 hospitalised patients with diabetic foot lesions,

16 (50%) had isolates of *Staphylococcus aureus* followed by *Escherichia coli* with a percentage frequency of 23.8%. *Staphylococcus aureus* infections have been noted to be more common among patients with diabetes (Breen and Karchmer, 1995).

Our study revealed that a preponderance of Gram – negative bacteria over the Gram-positives was isolated from diabetic wounds (Table 2); similar to the work of Shankar *et al.*, (2005) and Ozer *et al.* (2010), but in contrast with that of Frykberg (2003) who found a predominance of Gram positive aerobes infecting diabetic wounds. There is however no statistical validation of these varying observations. We are of the opinion that the type of organisms isolated from wounds may reflect the source of infection. Wounds exposed to faecal sources, for instance, may be contaminated mainly by members of the *Enterobacteriaceae*.

The results in this study is also comparable to that of Gadepalli *et al.* (2006), where male patients were predominant (85%), although *Staphylococcus aureus* exhibited a high frequency (56%) of resistance to antibiotic tests. It is also comparable to work done by Unachukwu (2005) at the University of Port Harcourt Teaching Hospital, Nigeria where *Staphylococcus aureus* was predominantly present out of seven organisms isolated from cutaneous abscesses of diabetics.

Bacterial resistance to antibiotics has become a major problem all over the globe. The present study has again exemplified this ugly trend. Interestingly all isolates in this study displayed resistance to at least three different antibiotics; thus establishing a case of multi-drug resistance among isolates. Also, *Pseudomonas aeruginosa* which is commonly isolated from wounds showed resistance to six out of the ten antibiotics tested. As also observed in a previous study by Orji *et al.*, (2009), the bacterial isolates in this study may have acquired genes for drug resistance through deliberate or inadvertent antibiotic misuse by patients.

This study shows that wound infections are polymicrobial and in most cases associated with *Staphylococcus aureus*, *Escherichia coli* and *Proteus* spp. The varieties of organisms observed in this study also support the need to obtain culture specimens from infected wounds for microbiological evaluation and antibiotic susceptibility testing so that chemotherapy can be promptly initiated. This will not only facilitate successful wound management but also assist in the control of antibiotic usage or limit bacterial resistance to antibiotics. With much diagnostic expertise by the bacteriologist and caution on the prescribing habit of the physician, a better management of diabetic wound can be achieved.

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