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Bacteriology of Diabetic Foot Ulcers with Reference to Multidrug Resistance Strains at the Yaounde Central Hospital (Cameroon)

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Abstract: Introduction. Foot infections are a major complication of diabetes mellitus and eventually lead to development of gangrene and lower extremity amputation. Many studies reported the bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory. Aims and Objectives. This study was carried out to determine the bacterial profiles of infected ulcers and the antibiotic resistance pattern of the isolates at the endocrinology and metabolic diseases unit of the Yaoundé Central Hospital. Materials and Methods. Samples were collected from 59 patients with diabetic foot ulcers by using sterile swabs and they were processed. Results. A total of 148 bacterial isolates were obtained from 56 positive cultures, with an average of 2.5 organisms per case. The age group of these patients ranged from 14 to 75 years and the maximum number of patients was in the age group of 51 to 60 years. Gram negative bacilli were more prevalent (65.5%) than gram positive cocci (36.4%). Polymicrobial growth was observed in 84.48% of the specimen, Monomicrobial growth in 18.59% and sterile growth in 6.25% of the isolates. The commonest isolates among the gram negative bacteria's were Proteus spp. (21.6%), Escherichia coli (18.9%), Klebsiella. spp (16.9%) and Pseudomonas aeruginosa (8.1%) while among the gram positive bacteria's Staphylococcus aureus was predominant (17.6%), followed by Staphylococcus epidermidis (10.1%) then by Streptococcus pyogenes (6.8%). Antimicrobial susceptibility results showed that Gram negative bacterial isolates were 100% sensitive to Imipenem and 86.5% resistance to Ampicillin, while for the Gram positive bacterial, they were 44.5% sensitive to Ciprofloxacin and 46.8% resistance to Oxacillin. Conclusion. This study showed a preponderance of gram negative bacilli among the isolates from the diabetic foot ulcers. Knowledge on the antibiotic sensitivity pattern of the isolates will be helpful in determining adequate drugs for the empirical treatment of diabetic ulcers.

Keywords: Diabetic Foot Ulcer, Bacterial Profile, Antibiotic Sensitivity

1. Introduction

A diabetic foot is one of the most feared complications of diabetes and it is the leading cause of the hospitalization among diabetic patients [1]. It is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, which leads to the development of gangrene and which even necessitates limb amputation. The individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes [2]. The impaired micro-vascular circulation in patients with a diabetic foot limits the access of phagocytes, thus favoring the development of an infection [3]. The local injuries and the improper foot wear further compromise the blood supply in the lower extremities [1]. While the foot infections in persons with diabetes are initially treated empirically, a therapy which is directed at the known causative organisms may improve the outcome [4].

Many studies have reported the bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory [4]. These discrepancies could partly have been due to the differences in the causative organisms, which had occurred over time,

geographical variations, or the type and the severity of the infection, as were reported in the studies [4]. Mostly, the diabetic foot infections are mixed bacterial infections and the proper management of these infections requires an appropriate antibiotic selection, based on the culture and the antimicrobial susceptibility testing results [5]. Now, medical and research communities are beginning to realize that the diversity of the bacterial populations in chronic wounds may be an important contributor to the chronicity of the wounds, such as diabetic foot ulcers. The current study was undertaken as an attempt to examine the major populations of bacteria which were associated with the bio burden of infected diabetic foot ulcers. By performing a survey on the wounds from different subjects, an attempt was made to identify the genera or the noted pathogens that were consistently present in diabetic ulcers [6] and also to note the changes in the bacteriological profiles of the infected foot ulcers, as compared to those which were seen in previous studies. This study was undertaken with the aim of determining the bacterial profile of infected diabetic foot ulcers and the antibiotic resistance pattern of the bacterial isolates.

2. Materials and Methods

59 diabetic patients with foot ulcers were included in this study, which was conducted for a period of 6 months at the Yaounde Central Hospital Cameroon. A clinical history was elicited with regards to the duration of diabetes, the type of treatment that the patient received and the presence of other systemic illnesses. The patients were also assessed clinically and the ulcers were graded according to Wagner's grade. The samples were collected after obtaining informed consents from the patients.

Samples were collected from the deeper portion of the ulcers by using 2 sterile swabs which were dipped in sterile glucose broth. The samples were collected by making a firm, rotatory movement with the swabs. One swab was used for Gram staining and the other was used for culture. A direct Gram stained smear of the specimen was examined. The specimens were inoculated onto blood agar, chocolate agar, Chapman agar and eosine methylene blue agar. The inoculated plates were incubated at 37 oC overnight and the plates were examined for growth, the next day. The further processing was done according to the nature of the isolate, as was determined by Gram staining and the colony morphology. The organisms were identified on the basis of their Gram staining properties and their biochemical reactions.

Antibiotic Susceptibility testing

The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method, as per the CLSI guidelines, 2011 [7]. The antimicrobial discs which were used were those of Ampicillin (10µg), Aztreonam (30µg), Gentamicin (10µg), Ceftazidime (30µg), Cefoxitin (30µg), Imipenem (10µg), and Colistin (10µg), for the Gram negative bacilli. Ampicillin, Cefoxitin (30µg), Chloramphenicol (30µg), Clindamycin (2µg), Erythromycin (15µg), Oxacillin (1µg), Vancomycin (30µg), Ciprofloxacin (30ug), Ofloxacin (5µg), and Tetracycline (30µg) were used to study the susceptibility patterns of the Gram positive cocci. Data and entered into a computer database through the Epi-info 3.4.5 Software. Rates and proportions were calculated and the results presented using frequency tables, from which histograms were plotted using the Microsoft Office Excel Software.

3. Results

3.1. Samples

During the study period, 59 patients with ulcers were registered, of which 32 were males (54.23%) and 27 were females (45.76%), the male to female ratio was 1:1.85. Their age ranged from 14 to 75 years and the maximum number of patients (32.2%) was in the age group of 51 to 60 years (Fig 2). The next most prevalent age group was between 41 to 50 years (16.9%) and 61 to 70 years (16.9%) (Fig.1 and Fig. 2)

Among the total patient population 47 (79.66%) had type 2 diabetes mellitus, whereas only 12 (20.34%) had type 1 diabetes mellitus.

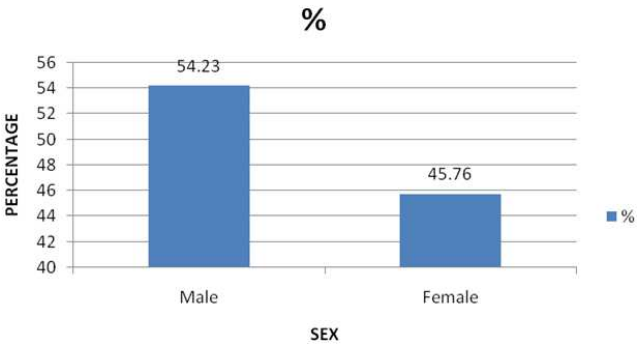

Fig. 1. *Sex proportion plot.*

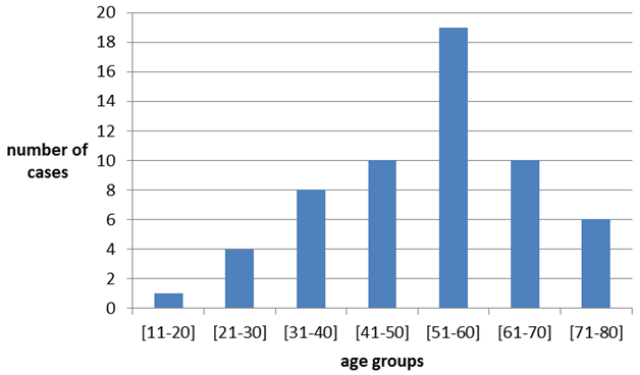

Fig. 2. *Histogram showing the various age groups of the study population.3.2. Diabetes Type*

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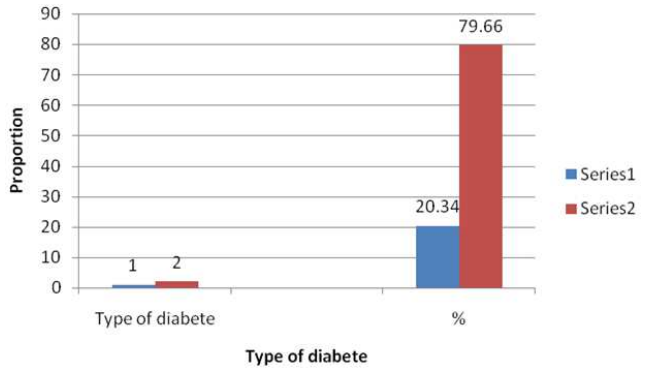

Fig. 3. Proportions of the types of diabetes.

3.3. Complications

Out of the 59 patients, 26 (30.9%) had retinopathy, 30 (35, 7%) had neuropathy, 6 (7.1%) had cardiopathy while 4 (4.8%) had nephropathy and 18 (21.45%) had both retinopathy and neuropathy (Fig 4). Moreover, maximum number of patients was isolated from grade III ulcers and minimum in grade V ulcers (Fig 5). The grading was done according to the Wagner classification system.

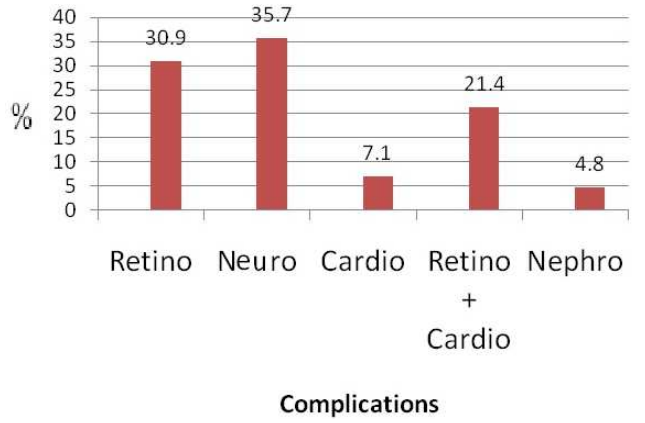

Fig. 4. Percentage of diabetic patients with respect to complications.

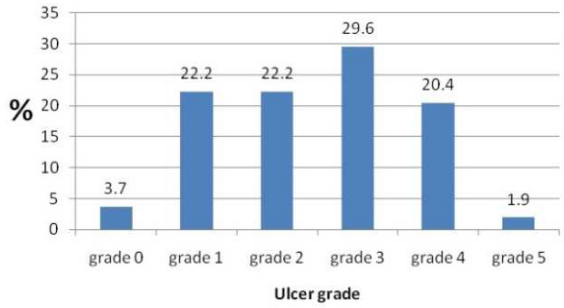

Fig. 5. Number of diabetic patients with respect to grades.

3.4. Microbiological Observations

3.4.1. Frequency of Isolated Bacteria

Of the 59 patients studied, 148 organisms (97 gram negative bacilli (65.5%) and 51 gram positive cocci (36.4%) were isolated in 56 positive cultures, which represent an average of 2.5 organisms per case. Bacteriological culture revealed Monomicrobial growth in 7 (18.59%) specimen, polymicrobial growth was isolated in 49 (84.48%) specimens and 3 (6.25%) specimens had sterile growth (fig 11). These three (3) specimens with sterile growth were from patients of grade 0 and 1. The most frequently isolated pathogens among the Gram negative bacteria (65.54%) included Proteus spp. (21.6%), Echerichia coli (18.9%), Klebsiella.spp (16.9%) and Pseudomonas aeruginosa (8.1%) respectively. Gram positive bacteria accounted for 34.46% of all bacterial isolates. Staphylococcus aureus was predominant (17.6%) among Gram positive bacteria, followed by Staphylococcus epidermidis (10.1%) and Streptococcus pyogenes (6.8%) as shown in Fig 6A and 6B.

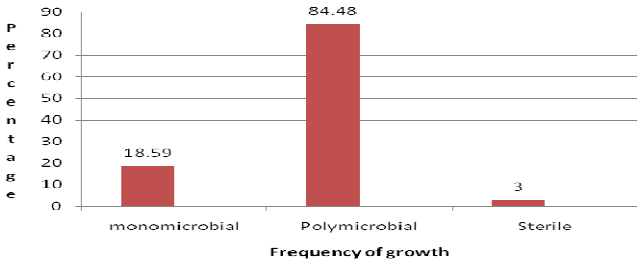

Fig. 6A. Frequency of growth.

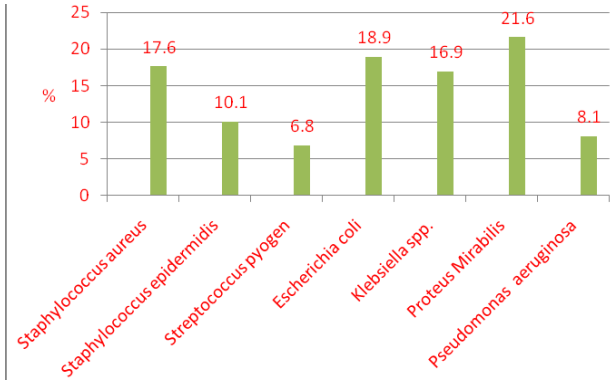

Fig. 6B. Bacteria distribution.

3.4.2. Susceptibility Pattern of Isolated Bacteria

For gram positive cocci

The percentage of sensitivity, resistance and intermediate for gram positive cocci are 44.5%, 46.8% and 29.8% respectively (Fig 7). Most of the gram positive cocci identified from ulcer swab cultures were sensitive to Ciprofloxacin antibiotics, resistance to Oxacillin and intermediate to Ciprofloxacin antibiotics as shown in Figure 8.

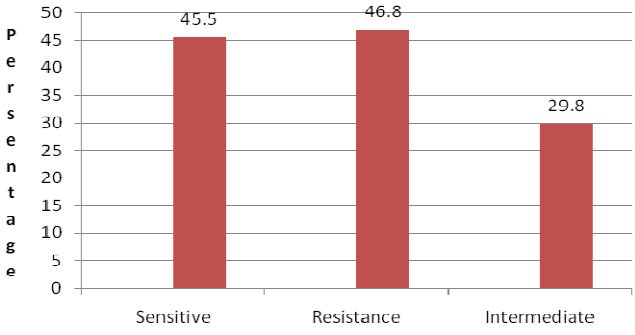

Fig. 7. Susceptibility testing for gram positive cocci.

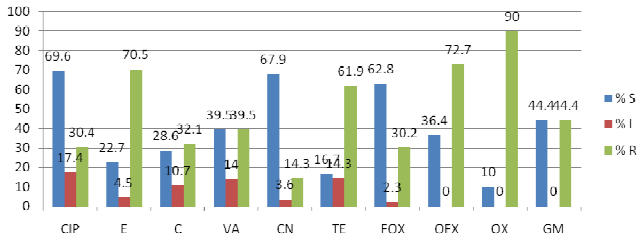

Fig. 8. *Susceptibility pattern for gram positive cocci.*

For gram negative bacilli

The degree of antibiotics sensitivity, resistance and intermediate was high in gram negative bacilli with a percentage of 55.5%, 53.2% and 70.2% respectively (Fig 9). Most of the gram negative bacilli were 100% sensitive to Imipenem (IPM) and 86, 5% resistance to Ampicillin (AMC) and 40% intermediate to Colitine (CT) as shown in Figure 10.

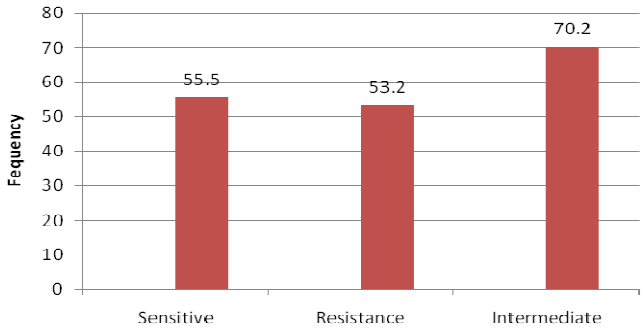

Fig. 9. *Susceptibility pattern for gram negative bacilli.*

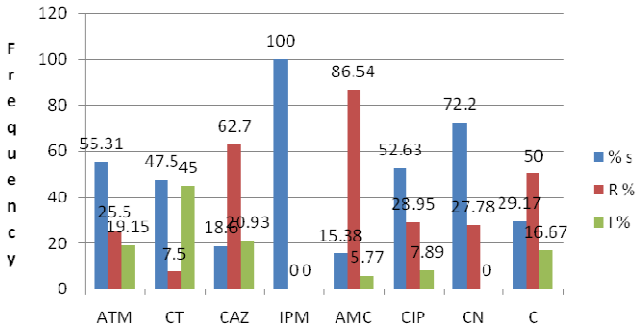

Fig. 10. *Susceptibility pattern for gram negative bacilli.*

4. Discussion

The prevalence of diabetic foot ulcers among male subjects was found to be 54.23% in males against 45.76% in females and their age ranged from 14 to 75 years, this may be due to higher level of outdoor activity among males compared to females. This is in support with study findings of Ahmed and co-workers [7], Sharma and co-workers [8]; Hena and co-workers [9]; Hayat and co-workers [10] as they indicate males dominate in having diabetes with foot infections when compared to females. Majority of patients are in age group falling in between 51 to 60 (32,2%), followed by 61 to 70 (16,9%) and 41 to 50 (16,9%) and it clearly explains the fact that age factor plays an important role in occurrence to infections, while this may be explained by other factors including non-compliance due to poly pharmacy and economic issues. More over the elderly are likely to have lived with diabetes mellitus for a longer duration than the younger patients thereby making them more prone to the complications that predispose to foot ulceration. Our study results are in accordance with the study findings of Vishwanathan and co-workers [11].

Our results show that 79, 66% of our study population had type 2 diabetes while 20, 34% had type 1 diabetes. This is in accordance with the work of Ravisekhar and co-workers [12] who had a prevalence of 88,8% of type 2 diabetes. This may be due to increases in life expectancy, obesity and sedentary lifestyle.

The increasing life expectancy of diabetic patients, there is an increased risk of diabetes related complications especially chronic complications like sensory neuropathy and retinopathy. This study also reports a high prevalence of neuropathy (35.7%) and of retinopathy (30.9 %). The prevalence of sensory neuropathy in earlier studies shows is marked with a lot of variation. Ako and co-workers found 77.8% in Nigeria [13], Shankar and co-workers found 56.8% in southern India [14] while Mohammad and co-workers found 66.6% for neuropathy and 52.7% for retinopathy in a northern India [5]. This variation in the prevalence may be due to difference in the methods used for the diagnosis of bacterial infections.

The maximum number of patients with infected diabetic foot ulcers belongs to Wagner grade 3 and 4 and minimum in Wagner grade 5. This is in accordance with the work of Mohammad and co-workers [15] in which, most patients had ulcers of grade 3 and 4. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery units in the country, attempted self-medication and trust in traditional healers. Moreover inadequate antibiotic treatment and the use of non sterile instruments for dressing wounds results in the growth of multi resistant organisms necessitating hospital admission and surgical intervention.

Further more in our study, a total of 148 organisms were isolated from 56 samples with an average of 2.5 organisms per case. This is in accordance with the work of Ravisekhar and co-workers [12],who had a total sample of 183 isolates from 80 specimens with an average of 2,3 and with the work of Kavita and co-workers [16], yielding an average of 2.56 organisms per case. The 2.56 value is higher, as compared to the findings of vishwanathan and co-workers, Bansal and co-workers, which yielded 1.21 and 1.52 organisms per case, respectively[11][17]. These differences may be due to the differences in sample sizes of each study.

Wound cultures revealed 84.48% patients had polymicrobial isolates (both Gram positive and negative organism) and 18.59% had monomicrobial infection and no organism was found in 3 swabs. Mohd and co-workers [5], Anandi and co-workers [18], Rama and co-workers [19], Pappu and co-workers [20], Citron and co-workers [21] have respectively reported 33%, 67%, 66%, 7.7% and 83% of

polymicrobial infections and 56.6%, 19 %, 23%, 92% and 16.2% monomicrobial infections respectively. In some studies, monomicrobial isolates were most common like that of Mohd and co-workers [5], Pappu and co-workers [20]. This kind of discrepancy could be due to geographical variations, or the types and severity of infection. Overall, Gram-negative microbes were the predominant pathogens isolated with 65.5%, followed by Gram-positive microbes with 34.5%. A similar result was observed in a studies carried out in India [12] [14] [17]. Raja and co-workers also documented more Gram-negative bacteria than Gram-positive bacteria (52 vs. 45% and 67 vs. 33%, respectively) [22]. Thus, in the Yaoundé city it is essential for clinicians following diabetic patients to select and prescribe antibiotics that are more effective against Gram-negative bacteria.

Proteus spp. (21.6%), Escherichia coli (18.9%) and Klebsiella spp (16.9%) were the majority of the causative Gram-negative microorganisms. Among the Gram-positive microorganisms, Staphyloccocus aureus (17.6 %), Staphyloccocus epidermidis (10.1%) Streptococcus pyogenes (6.8%) were more predominantly isolated. These pathogens were believed to have colonized the superficial foot ulcers. These results are comparable with those of Raja and co-workers [22] and of Bansal and co-workers [17], who also reported 28%, 25%, 15% and 9% for Proteus spp, Pseudomonas spp, Klebsiella pneumonia and Escherichia coli respectively and 44%, 25% and 9% for Staphylococcus aureus, group B streptococcus and Enterococcus spp respectively. Renin and co-workers [22] reported 24%, 21%, 9% and 12% for Proteus spp, Enterobacter spp, Citrobacter spp and Escherichia coli respectively and 29% and 3% for Staphylococcus aureus and Staphylococcus epidermidis respectively. Banal and co-workers [17] reported 22%, 17%, 18% and 11% respectively for Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus spp and 19% to Staphylococcus aureus.

With regards to sensitivity patterns, the study revealed an outstanding sensitivity of gram positive cocci to the Ciprofloxacin (69.6%) antibiotics. There was no marked difference in sensitivity of the isolates to macrolid (clindamycin), cephalosporin (cefoxitin) and macrolides (gentamycin). This finding is consistent with that of Orji and co-workers [23] who reported a significant sensitivity to ciprofloxacin (78%) among diabetic mellitus foot ulcers isolates.

Resistance in this present study was highest for the penicillins (oxacillin) (90%) and least for the macrolid (clindamycin representing 14.3%). This high resistance to the penicillin may be linked to the in excessive and bad use of this group of drugs in the Cameroonian society, thus increasing the selective pressure in the bacterial populations. Moreover the penems (imipenem) were the best choice for gram negative bacilli with a sensitivity of 100 %, thus it can be used to treat severe diabetic foot infection. This finding is in accordance with a recent study done by Banashankari and co-workers, who reported 100% susceptibility to imipenem when tested for Enterobacteriacea. Ampicillin was the most resistant to all isolated gram negative bacteria. In Cameroon, antibiotics are permanently available with or without a physician's prescription. They are found all over the cities, at places such as markets, malls etc. This gives rise to inadequate dosing, incomplete courses, and indiscriminate drug use that ultimately leads to the emergence and spread of antimicrobial resistance.

5. Conclusion

Our study has showed that the bacteria profile for diabetic patients with foot ulcers are mostly polymicrobial. Proteus spp and Staphylococcus aureus were the most commonly identified in Diabetic foot ulcers. Ciprofloxacin and Imipenem were the most effective antimicrobial therapy against Gram-negative and Gram-positive microorganisms, respectively. Penicillin (oxacillin and ampicillin) were the most resistant antibiotics. Because of the limited suitability of these antibiotics, choosing empiric antibiotic therapy should depend upon the clinical features of the infections and the local pattern of bacterial etiology and its antibiogram.

Acknowledgements

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