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Bacteriological Profile and Antimicrobial Resistance Patterns in Diabetic Foot Infections among Patients with Diabetes at CNHU-HKM, Benin

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Abstract

Objective: To investigate the bacteriological characteristics of infected diabetic foot lesions at the CNHU-HKM in Cotonou. **Patients and Methods**: We conducted a descriptive cross-sectional study over a 5-year period, from January 1, 2015 to December 31, 2019, including 102 patients admitted to the Endocrinology and Metabolism Department of CNHU-HKM, Cotonou, for infected diabetic foot lesions and who had undergone bacteriological sampling. **Results**: A total of 143 bacteria were identified, with 34.3% of samples containing at least two bacteria. Gram-negative bacilli predominated, and the most frequently isolated organisms were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. A high frequency of bacterial resistance to antibiotics was observed, including 38.5% and 42.3% of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae*, respectively. **Conclusion**: The bacterial flora, predominantly Gram-negative, and the high frequency of bacterial resistance to commonly used antibiotics, highlights the need to reconsider empirical antibiotic therapy choices.

Keywords

Infection, Diabetic Foot, Bacteria, Antimicrobial Resistance

1. Introduction

Chronic diabetic foot ulcers are one of the most common complications among

people with diabetes. Nearly a third of people with diabetes suffer from foot ulcers at the course of their lifespan, and over 50% of these ulcers are infected [1]. Diabetic foot infections are associated with high morbidity and mortality and can lead to lower limb amputation if not treated effectively [2]. These lesions are often polymicrobial, with Gram-positive or Gram-negative aerobic bacteria [3] [4] as well as anaerobes involved. To prevent antibiotic resistance, reduce costs and minimise adverse effects associated with antibiotic use, the recommendations of the Infectious Diseases Society of America (IDSA) for the diagnosis and treatment of diabetic foot infections advocate the prescription of antibiotics that have proven effective in treating diabetic foot infections, with coverage of Gram-positive cocci and limited coverage of Gram-negative bacteria. Generally, the empirical antibiotics used orally are clindamycin, cotrimoxazole, levofloxacin and amoxicillin + clavulanic acid [5]. However, the rapid emergence of antibiotic resistance to these drug families among the most common bacteria represents the greatest challenge in the management of these infections, especially carbapenem resistance and, in some cases, multidrug resistance [6].

The aim of this study is therefore to determine the profile of the identified bacteria and their sensitivity to the antibiotics used in the treatment of infected diabetic feet.

2. Material and Method

The study was conducted in the Endocrinology and Metabolism Department of the Hubert Koutoukou Maga National University Hospital Hubert Koutoukou Maga (CNHU HKM) in Cotonou. This was a descriptive cross-sectional study based on retrospective data involving diabetic patients admitted to the diabetic foot infection unit between 1 January 2015 and 31 December 2019. All patients admitted during the study period who had bacteriological testing of pus with an antibiogram were included. Patients admitted for a diabetic foot infection with incomplete medical records were excluded from the study.

The socio-demographic, clinical, laboratory and therapeutic data were collected using a dedicated data collection form. Diabetic foot was defined as an infection, ulceration or destruction of deep foot tissues associated with neuropathy and/or peripheral arterial disease of the lower limbs in a diabetic patient (International Consensus on the Diabetic Foot). Infection was diagnosed on the basis of the presence of fever, suppuration of the foot, leukocytosis on complete blood count, elevated C-reactive protein (CRP), with or without the isolation and identification of bacteria in bacterial culture of the pus. Bacteriological samples have been done by swab. Existing dressings were removed using aseptic precautions. The wounds surface was irrigated with sterile normal saline. Necrotic tissue, slough and superficial debris were removed by sharp debridement. And after cleansing and debridement, a sterile swab was applied using Levine technique. When feasible, deep tissue specimens were obtained using sterile curettes. Specimen were immediately placed into transport media and delivered promptly to the microbiology laboratory. Aerobic and anaerobic cultures were performed according to local laboratory procedures. The results of the susceptibility testing were interpreted in accordance with the recommendations of the French Society of Microbiology's Susceptibility

Testing Committee and the European Committee on Antimicrobial Susceptibility Testing (CASFM-EUCAST) of February 2018 [7].

The different lesions were graded using the Wagner classification [8]. Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* (MRSA) were classified as multidrug-resistant bacteria (MDRB). In terms of treatment, an amputation was classified as minor if it was partial and major if it was total (leg or thigh).

Statistical analyses: the data collected were analysed using Epi Info software version 5.4. The results were presented as proportions or means with their standard deviation. The frequency of resistance was calculated by dividing the number of bacteria resistant to the tested antibiotic by the total number of that type of bacteria isolated.

3. Results

3.1. Characteristics of the Study Population

A total of 138 patients were received during the period study and 102 were included. The mean age of the patients was 56.13 ± 10.57 years, ranging from 29 to 83 years. The sex ratio (M/F) was 1.68, with a predominance of males (62.7%). The mean duration of diabetes was 26.77 years. More than half of the patients had had diabetes for over 15 years (58.8%). Patients with type 2 diabetes were the most common group, accounting for 97.1%. Regarding medical history, 9.8% had a history of foot ulcers, and 8.8% had a history of foot amputation. Self-medication was reported by 47.1% of patients. The antibiotics most commonly used by patients were amoxicillin + clavulanic acid, metronidazole and cloxacillin (Table 1).

Table 1. General characteristics of patients hospitalised for diabetic foot conditions, N = 102.

	Mean ± SD or n (%)
Age	56.13 ± 10.7
Male gender	64 (62.7)
Type of diabetes	
Type 1	3 (2.9)
Type 2	99 (97.1)
Duration of diabetes	26.77 ± 25.51
History of foot ulcer	10 (9.8)
History of foot amputation	9 (8.8)
Self-medication with antibiotics	48 (47.1)
Antibiotics used for self-medication	
Amoxicillin	9 (8.8)
Amoxicillin and clavulanic acid	16 (15.7)
Metronidazole	15 (14.7)
Cloxacillin	15 (14.7)
Others*	13 (12.7)

*Others: Ceftriaxone, Ciprofloxacin, azithromycin.

3.2. Clinical and Laboratory Findings

The most common type of lesion was a spontaneous phlycten (35.3%). The mean time to onset of the lesion was 5.67 ± 5.37 weeks, ranging from 1 to 32 weeks. Diabetic peripheral neuropathy (DPN) was present in 86.3% of patients. Foot lesions were classified as Wagner grade III in 35.3% of cases, and grades IV and V in 39.9% of cases. The mean glycated haemoglobin (A1c) was 9.73 ± 2.59% and 32.4% had an HbA1c level above 7%. Hyperleukocytosis was present in 91.2% of patients; elevated CRP in 65.7% and osteitis on X-ray in 70.6% of patients.

A total of 143 bacteria were identified. The culture was single-microbial in 65.7% of cases, bi-microbial in 28.4% of cases and contained three organisms in 5.9%. The most commonly found bacteria were *Pseudomonas aeruginosa* (18.90%), *Escherichia coli* and *Klebsiella pneumoniae* in equal proportions (18.18%); *Staphylococcus aureus* (15.38%), *Acinetobacter* sp. (7%), and *Enterococcus* sp. (4.19%) (Table 2).

Table 2. Clinical and laboratory characteristics of patients hospitalised for diabetic foot complications, N = 102.

	Mean ± SD or n (%)
Mechanism of injury	
Unperceived trauma	9 (8.8)
Perceived trauma	28 (27.5)
Spontaneous blister	36 (35.3)
Undetermined	15 (14.7)
Other*	14 (13.7)
Duration of injury (weeks)	5.67 ± 5.37
Presence of NDP	88 (86.3)
Wagner classification	
II	25 (24.5)
III	36 (35.3)
IV + V	41 (39.9)
A1c	9.73 ± 2.59
Leukocytosis	93 (91.2)
Elevated CRP	67 (65.7)
Presence of osteitis	72 (70.6)
Isolated bacteria N = 143	
Gram-negative bacilli	
Enterobacteriaceae	
Escherichia coli	26 (18.18)
Klebsiella pneumoniae	26 (18.18)
Enterobacter sp.	6 (4.19)

Continued

Morganella morgani	5 (3.50)
Proteus sp.	6 (4.19)
Citrobacter koseri	2 (1.40)
Providencia stuartii	1 (0.7)
Non-fermenting Gram-negative bacilli	
Pseudomonas aeruginosa	27 (18.90)
Acinetobacter sp.	10 (7)
Gram-positive cocci	
Staphylococcus aureus	22 (15.38)
Enterococcus sp.	6 (4.19)
Staphylococcus saprophytus	4 (2.79)
Coagulase-negative Staphylococcus	1 (0.7)
Streptococcus agalactiae	1 (0.7)

3.3. Profile of Antibiotic Resistance

The most frequently isolated bacteria showed a high frequency of resistance to beta-lactams, ranging from 50% to 65.4% for *Klebsiella pneumoniae*, from 53.8% to 69.2% for *Escherichia coli*, and 68.2% for MRSA. More Specifically, 7.7% of *Escherichia coli* strains and 7.4% of *Pseudomonas aeruginosa* strains isolated were resistant to imipenem. The findings were similar for fluoroquinolones, with average resistance rates of 62.82% and 59.56% for ciprofloxacin and ofloxacin respectively. The average resistance to aminosides was 42% for gentamicin and 36.32% for netilmicin (**Table 3**). The mechanism of resistance in Enterobacterales strains was primarily the production of an ESBL. We found that 42.3% of *Klebsiella pneumoniae* strains and 38.5% of *Escherichia coli* strains were ESBL-producing (**Table 4**).

4. Discussion

This study identified the main bacteria involved in infected feet, as well as their behaviour in relation to the antibiotics used. It emerged that the most common bacteria were *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. All these bacteria showed a high level of resistance to the antibiotics tested.

In this study, as in series on diabetic foot, some patients hospitalised for diabetic foot had already experienced a foot ulcer and an amputation in the context of diabetic foot [9] [10]. Patients had self-medicated with antibiotics in 47.1% of cases, and amoxicillin + clavulanic acid was the most commonly used molecule, followed by metronidazole and cloxacillin. This almost systematic use may be due to the relative ease of access to these drugs, although this has since been regulated and strictly controlled by the country's health authorities.

Table 3. Antibiotic resistance profile of bacteria tested in 102 patients hospitalised for diabetic foot, N = 102.

	Bacteria			
	Pseudomonas aeruginosa (%)	*Klebsiella pneumoniae* (%)	*Escherichia coli* (%)	*Staphylococcus aureus* (%)
Beta-lactams				
Amoxicillin + clavulanic acid	-	65.4	53.8	68.2
Cefuroxime	-	57.7	69.2	-
Cefixime	-	50	57.7	-
Cefotaxime	-	65.4	65.4	-
Ceftriaxone	-	57.7	65.4	-
Imipenem	7.4	0	7.7	-
Aminoglycosides				
Gentamicin	40.7	42.3	57.7	27.3
Netilmicin	33.3	46.2	38.5	27.3
Fluoroquinolones				
Ciprofloxacin	48.1	65.4	92.3	45.5
Ofloxacin	-	69.2	73.1	36.4
Other antibiotics				
Cotrimoxazole	-	65.4	76.9	31.8

Table 4. Multidrug-resistant bacteria (MDRB) in 102 patients hospitalised for diabetic foot, N = 102.

Bacteria	MDRB n (%)
Gram-negative bacilli	
Enterobacteriaceae	
Escherichia coli N = 26	10 (38.5)
Klebsiella pneumoniae N = 26	11 (42.3)
Enterobacter sp. N = 6	2 (33.33)
Morganella morgani N = 5	1 (20.0)
Proteus sp. N = 6	0 (0.0)
Citrobacter koseri N = 2	0 (0.0)
Providencia stuartii N = 1	1 (100)
Non-fermenting Gram-negative bacilli	
Acinetobacter sp. N = 10	1 (10.0)
Gram-positive cocci	
Enterococcus sp. N = 6	0 (0.0)

We found that 28.4% of samples contained two organisms and 5.9% contained

three organisms. The polymicrobial nature observed in diabetic foot infections is well-known and described in the published literature [11]-[14] with varying frequencies.

Regarding the bacterial ecology identified in this study, a study conducted by Alassani *et al.* in 2014 [15] in the same department found a similar bacterial ecology among 26 identified a similar bacterial flora, dominated by Gram-negative bacteria, among the 26 identified pathogens. Other authors have confirmed this bacteriological profile dominated by Gram-negative bacteria in infected diabetic feet [6] [10] [16] [17]. In contrary to the findings of our study and those of Asian and Turkish studies [14] [18], European and American studies have found a bacteriological profile dominated by Gram-positive bacteria [9] [19] [20]. The presence of Pseudomonas among the common bacteria, as seen in our study, is attributed to hospital flora resulting from prolonged hospital stays and the chronic nature of the lesions [17] [21].

The antibiotic resistance profile of the most commonly isolated bacteria revealed a high frequency of ESBL-producing Enterobacteriaceae (E. coli 38.5%, *Klebsiella pneumoniae* 42.3%) and MRSA (68.2%). Multiple studies have reported a high frequency of antibiotic resistance in bacteria from infected diabetic feet [2] [17] [22]. More specifically, higher resistance to beta-lactam antibiotics is observed, with the exception of imipenem, to which the bacteria remain highly susceptible. This might be partly explained by the high prevalence of ESBL-producing bacteria found: 38.5% of *Escherichia coli* strains and 42.3% of *Klebsiella pneumoniae* strains. ESBL-producing strains of *Klebsiella pneumoniae* and *Escherichia coli*, as well as multidrug-resistant Pseudomonas, have also been reported by other studies [10] [23] in infected diabetic feet. Resistance to the second-generation fluoroquinolones tested remains high for the three Gram-negative bacteria, with the highest frequency observed in *Escherichia coli*. Mahamane Sani *et al.* [16] reported 52.7% resistance among Enterobacteriaceae to first- and second-generation fluoroquinolones in their 2017 study conducted in Ivory Coast. This high frequency of bacterial resistance to antibiotics might make it more difficult to choose the initial empirical antibiotic therapy, which is based primarily on beta-lactams ± beta-lactamase inhibitors [5], as resistance is higher and there few matches with antibiotic susceptibility testing results, which accounted for 3.9% in our study. In African studies, the most commonly used combinations are penicillin-imidazoles and fluoroquinolones-imidazoles [16] [24] [25]. The combination of amoxicillin + clavulanic acid and metronidazole was the most commonly used in our study (32.4%). Moreover, the prolonged length of stay observed in this study could be explained by the high frequencies of deeper lesions (Wagner grades III, IV, V) and osteitis (70.6%). However, this finding remains consistent with those reported by Sidibé *et al.* in 2006 [24] and Mamane Sani *et al.* in 2017 [16], which were 54 ± 29 and 59.4 ± 31.6 days respectively. The amputation rate (41.2%) remains similar to that of 41.38% reported by Sidibé *et al.* [24] but higher than the 22% reported by Nese Saltoglua *et al.* in 2018 [10].

Our study, however, has certain limitations that should be noted. Its single-center design may not accurately represent the country's ecology. Furthermore, the study location (the country's first reference center) and the sometimes lengthy patient journey involving the use of inappropriate and often incompletely documented empirical antibiotics could also contribute to an increased incidence of bacterial resistance.

5. Conclusion

The bacteriological profile in this study is dominated by Gram-negative bacteria. The bacteria were multi-drug resistant, with a significant frequency of ESBL-producing strains. There is a low degree of consistency between empirical antibiotic therapy and the results of the antibiotic sensitivity test. It may therefore be necessary to review practices regarding the choice of antibiotics in empirical therapy in order to improve patient outcomes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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