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Bacteriological profile and frequency of antibiotic resistance in the infected diabetic foot

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ABSTRACT

The diabetic foot (DF) is a major public health problem, with infection of the DF being a major risk factor for amputation. This study aimed to establish the bacteriological profile of diabetic foot infection and to evaluate the resistance of isolated bacteria to antibiotics. Retrospective study of 150 patients in the internal medicine departments of the University Hospital of Batna, the University Hospital of Constantine and the EPH of Oum El Bouaghi, over 24 months from September 2019 to August 2021. The population had an average age of 46±13 years and a sex ratio of 1.88 in favour of men. The majority of patients were type 2 diabetics with a mean duration of the evolution of 20 years. The starting point of the foot lesions was inter-toe intertrigo and trauma. The most frequent lesion found was an infectious lesion followed by gangrene, classified in 33.64% of cases as Grade 2 according to Wagner. We performed a swab of the ulcer, and direct examination showed a predominance of Gram-negative bacilli (GNB) in 78.56% of the samples, 17 different bacterial species were isolated, predominantly *Escherichia coli* and *Pseudomenas aeruginosa*. We studied the antibiotic resistance of the most frequently isolated bacteria, the antibiotic therapy was initially probabilistic and then adapted to the antibiogram. The multidisciplinary management of diabetic foot is still very precarious, and the best treatment is prevention.

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Introduction

Regardless of the type, diabetes can lead to complications that affect several parts of the body and increase the overall risk of premature death. Acute complications include ketoacidosis, hyperosmolar coma, hypoglycaemia, and chronic complications include diabetic nephropathy, neuropathy and retinopathy, chronic infections and diabetic foot.

Several factors contribute to the development of foot ulcers in diabetic patients; peripheral neuropathy and vascular insufficiency (1). In addition to these two factors, a third factor is a bacterial infection, which can set in; infection of a diabetic foot ulcer multiplies the risk of amputation by a factor of 10 (2). It accounts for about a quarter of hospital admissions in diabetic patients (3). The rate of lower limb amputations in diabetics is more than 40 times higher than in non-diabetics (4). It is also a significant cause of unjustified antibiotic therapy and as such contributes to the increase in bacterial resistance and its spread through care. It is therefore essential to know the bacterial ecology of diabetic foot infections in healthcare institutions to allow adequate management and optimal use of antibiotics, with the hope of reducing the risk of amputation and the emergence of multi-resistant bacteria. Our study aims to analyse the bacteriological profile of diabetic feet managed in the internal medicine departments of the University Hospital of Batna, the University Hospital of Constantine and the EPH of Oum El Baouaghi and its influence on the first-line antibiotic therapy.

Materials and Methods

Study population and conducting and carrying out the survey

This was a retrospective descriptive and analytical study of the management of the diabetic foot carried out over a 24-month period from September 2019 to August 2021. Out of an initial number of 200 selected patients, 150 were surveyed, our type 1 (T1D) and type 2 (T2D) diabetics were hospitalized for the diabetic foot at the level of the internal medicine departments of CHU Benflis Touhami of Batna, CHU Abdesselam Benbadis of Constantine and EPH Mohamed Boudiaf of Oum El Bouaghi Patients with or without antibiotic therapy and the different aspects and stages of foot infection were included in our study. Patients with gestational diabetes and non-diabetic patients with foot lesions or amputations were excluded from the study.

Information on the patients surveyed was collected by means of a validated questionnaire including epidemiological, clinical, para-clinical and therapeutic data (age, sex, type of diabetes...).

Pre-clinical examination

Bacteriological sampling phase

The sampling method used is superficial, rarely deep pus swabbing, which consists of rubbing the surface of the lesion in a zigzag movement combined with rotation using two sterile swabs; one for direct microscopic examination and the other for culture. The samples are then rapidly sent

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to the microbiology laboratories of the CHU of Constantine, the EPH-Sanatorium of Batna and the EPH of Oum El Bouaghi.

Microbiological analysis phase

Once the samples arrived at the laboratories, a series of bacteriological analyses were carried out: direct examination, culture and biochemical characterisation of the bacterial strains. We studied the sensitivity to antibiotics of the different germs isolated.

Statistical analysis

The results were presented as percentages. All calculations were performed using SPSS version 20.00.

Results

General description of the survey population

We included 150 patients admitted for diabetic foot, the average age was 46±13 years with a clear male predominance in more than 65% of cases with a sex ratio of 1.88. Type 2 diabetes represented more than 91% of cases with the presence of familial diabetes of maternal origin in 84.13% of cases. Type 2 diabetes accounted for more than 91% of the cases, with the presence of familial diabetes of maternal origin in 84.13% of cases. The average duration of diabetes was 20 years [2 to 35 years]. More than 54.85% of the patients were on insulin, only 13.62% were on oral antidiabetic drugs (OADs), and both treatments were associated with 31.53%.

All our patients had at least one degenerative complication, including 54.26% diabetic neuropathy (DN), 16.54% diabetic retinopathy and 29.2% nephropathy. 48.96% had suffered a stroke, 29.54% a transient ischaemic attack (TIA) and 21.50% had angina. In our series, hypertension was found in 64.12% and 71.28% of our patients were smokers.

In our study, previous amputation for diabetic foot lesions was found in 34.15% of our patients, 65.85% had not undergone amputation, trauma was found in 10.44% of patients, wearing inadequate footwear was mentioned by 1.40% and inter-toe intertrigo (ITI) was present in 5.81% of our patients (Tables 1 and 2).

Clinical data

In our study, the right foot was most frequently affected with 48.21% of cases, with 27.32% of cases involving the leg, 9.33% of cases involving gangrene and 8.01% of cases involving a wound. 33.64% of the lesions were classified as Grade 2 according to Wagner. The association of clinical signs in favour of infection was found in 75.33% of patients, notably heat (16.84%), oedema (66.85%) and foul odour (96.85%). NP was found in 16.02% of our patients, it was represented by paresthesia/dysesthesia in 42.85%, hyperkeratosis in 33.02%, and skin dryness in 30.92% of cases. Arteriopathy was found in 8.64% of patients, characterised by the abolition of peripheral pulses in 23.56% of cases, pallor (22.85%) and coldness (28.94%) and trophic disorders in 24.65% of patients (Table 3).

Para-clinical data: Radiology and microbiology

X-rays of the foot were performed in 64 patients, with 55.69% showing osteitis, 22.85% erosion, 18.94% bone lysis and 2.52% a geode. Angiography was performed on 51 patients. Magnetic resonance imaging (MRI) and computed tomography (CT) were performed on 16 and 19 patients respectively. Arteriography was performed in 51 patients (Table 4).

The microbial culture was sterile in 18 cases (11.84%), mono-microbial in 73 cases (48.93%) and poly-microbial in 59 other cases (39.23%). 17 different bacterial species were isolated, and their distribution by the family showed the predominance of enterobacteria which represented 40.82% of the isolates, followed by staphylococci (21.25%), streptococci (20.06%) and non-fermenting BGN with a rate of 17.90%. The distribution by species showed the predominance of *Escherichia coli* (20%), *Pseudomenas aeruginosa* (15.33%) then *Staphylococcus aureus* and *Klebsielle pneumoniae* (10.66%). We studied the bacterial resistance of the most frequently isolated species (Tables 4, 5, 6a, 6b and 6c).

Discussion

In our study, the mean age was 46±13 years which differs from the results of Mezhoud et *al*., 2018, who found 65 years (5), Salah, 2018, reports 68 years (6) and Laidi et *al*, reported 56 years of age (7), with a sex ratio of 1.88 in favour of men, which is consistent with the results of a study conducted in Morocco (Casablanca) which was 2 (7), Turkey (2.2) (8), Ivory Coast (1.6) (9). 91% of our patients were type 2 diabetics, which is consistent with the results of a study conducted in Oran (Algeria) 92.11% (10), Morocco 89% (7), Togo 88.7% (11), Congo 86.2% (12). The mean duration of diabetes was 20 years, which was far from the results found by Faraoun et *al*., 2013, (14.26 years) (10). Bah et *al*, 2015, (15 years) (13), El Allali, 2015, in Rabat (Morocco) 12.56 years (14), Kourichi et *al*, 2018, (16.31 years) (15). 54.85% of our patients were on insulin, similar results reported by El Allali B, 2015, 59% (14) and Mezhoud et *al*., 2018, 59% (5).

In our series, hypertension was found in 64.12% of patients. While the study conducted at the University Hospital of Bejaia in 2018 showed that hypertension was present in 47.62% of patients (15), Awalou et *al*., 2018, found 41.9% (11), Tadili et *al.*, 2008, reported that 49% of patients were hypertensive (16).

Right foot involvement was the most frequent with 48.21% of cases. This differs from the results of the study by Kourichi et *al*, 2018, where the frequencies of involvement of the right and left feet were close (47.62% and 38.09% respectively), and the bilaterality of the lesions was noted in 14.28% of cases (15). As well as those noted at the University Hospital of Oran with the same involvement of both sides at 45.11%, and bilateral involvement at 0.78% (10).

In our study, the infectious lesion was the most frequently found in 49.33% of patients, gangrene in 9.33%, which differs from the values found in the study of Awalou et *al*., 2018 (11) and those of the study of Bah et *al*, 2015 (13), with a predominance of Grade 2 lesions, while in the study by Awalou et *al*, 2018 (11), Grade 5 was predominant and in the study by Hering et al, 2010, Grade 3 was predominant (17).

The cultures were mono-microbial in 48.93% of cases and poly-microbial in 39.23% of cases. This is consistent with the results reported by Turhan et *al*, 2013, and Richard et *al*, 2011, who found that mono-microbial cultures

Table 1. Distribution of the main epidemiological data of the study population.

Age characteristics	Values (n=150)
Age range (years)	15 (10%)
< 30	24 (16%)
[30 - 40[	18 (12%)
[40 - 50[	45 (30%)
[50 - 60[	21 (14%)
[60 -70[	6 (4%)
[70- 80[	18 (12%)
[80 -90[	3 (2%)
≥ 90	
Gender	
Male	98 (65%)
Woman	52 (35%)
Characteristics of diabetes	
Median duration of evolution	17
Age range (years)	
< 5	5 (3%)
[5 - 15[	35 (24%)
[15 - 25[	80 (53%)
≥ 25	30 (20%)
Type of diabetes	
DT2	137 (91%)
DT1	13 (9%)
Balanced	
Yes	51 (33.72%)
No	99 (66.28%)
Familial origin of diabetes	
Kindergarten	126 (84.13%)
Paternal	24 (15.87%)
Average duration of development of diabetes (years)	20 [2 - 35]
Diabetes monitoring	
Attending physician	
Generalist	51 (34.12%)
Diabetologist	99 (65.88%)
Compliance	
Observing	125 (83.64%)
Non-observer	25 (16.36%)
Self-monitoring	
Self-monitoring	104 (69.13%)
Not self-monitoring	46 (30.87%)
Anti-diabetic treatment follow-up	82 (54.85%)
Insulin	20 (13.62%)
ADO	48 (31.53%)
Mixed	
Degenerative complications	
Micro-angiopathies	81 (54.26%)
Diabetic NP	25 (16.54%)
Retinopathy	44 (29.2%)
Nephropathy	
Macroangiopathies	74 (48.96%)
STROKE	44 (29.54%)
AIT	32 (21.50%)
Angina	
ATCDs	
HBP	96 (64.12%)
Yes	54 (35.88%)
No	
Smoking	107 (71.28%)
Yes	43 (28.72%)
No	
Alcoholism	77 (51.23%)
Yes	73 (48.77%)
No	
Dyslipidemia	
Yes	130 (86.74%)
No	20 (13.26%)
Anterior amputation	
Yes	51 (34.15%)
No	99 (65.85%)
Foot problems	
Yes	112 (74.66%)
No	38 (25.34%)
Menopause	
Yes	128 (85.52%)
No	22 (14.48%)
Surgery	
Yes	25 (16.95%)
No	125 (83.05%)

Table 2. Distribution of the population according to risk factors and etiology of PD.

Characteristic	Values (n=150)
FDR and etiology of PD	
Infection	19 (12.36%)
IIO	9 (5.81%)
Trauma	16 (10.44%)
Neuropathy	49 (32.54%)
Arteriopathy	36 (24.23%)
Burns	4 (2.58%)
Skin mycoses	6 (4.18%)
Onychomycosis	5 (3.58%)
Ingrown nails	4 (2.88%)
Inadequate footwear	2 (1.40%)
Type of shoes	
Slippers	19 (12.56%)
Sandals	16 (10.58%)
Espadrilles	24 (16.12%)
Shoes	45 (30.04%)
Barefoot	46 (30.70%)
Reasons for consultation	
Pain	6 (3.97%)
Wound	17 (10.85%)
Infection	25 (16.54%)
Ulcer	15 (9.82%)
Paresthesia	21 (14.25%)
Osteitis	40 (26.58%)
Gangrene	17 (11.23%)
Trophic disorders	6 (4.25%)
Screening	4 (2.51%)
Knowledge of foot risk	
Foot hygiene	
Good	19 (12.58%)
Average	68 (45.57%)
Wrong	63 (41.85%)
What to do in case of a wound injury?	
Self-medication	43 (28.34%)
Consultation of a GP	49 (32.89%)
Traditional medicine	58 (38.77%)

Table 3. Distribution of clinical signs in our patients.

Clinical signs	Numbers and %.
Foot damage	
Right	72 (48.21%)
Left	19 (12.65%)
Bilateral	59 (39.14%)
Classification of injuries according to Wagner	
G1	43 (28.41%)
G2	50 (33.64%)
G3	48 (32.03%)
G4	7 (4.85%)
G5	2 (1.07%)
Aetiological forms	
Infection	**113 (75.33%)**
- Infectious lesion	**74 (49.33%)**
Headquarters	41 (54.85%)
Extended	17 (22.69%)
Locoregional impact	16 (22.46%)
Type	
Oedema	11.58%
Local sensitivity	16.58%
Induration	6.84%
Hair removal	14.82%
Local heat	18.47%
Local redness	20.85%
Peri-lesional erythema	8.71%
Pus	2.15%
- Wound	**12 (8.01%)**
Infectious cellulitis	9 (75.28%)
Necrotizing cellulitis	3 (24.72%)
- Gangrene	**14 (9.33%)**
Wet	11 (79.85%)
Dryer	3 (20.15%)
- Malformation	**9 (6.00%)**
Amyotrophy	3 (25.85%)
Claw toes	4 (45.96%)
Charcot's foot	2 (28.19%)
- Colouring	**4 (2.65%)**
Redness	3 (95.22%)
Pallor	1 (4.78%)
Neuropathy	**24 (16.02%)**
MPP	3 (13.96%)
Paraesthesia/dysaesthesia	10 (42.85%)
Hyperkeratosis	8 (33.02%)
Monofilament Test	3 (10.17%)
Arteriopathy	**13 (8.64%)**
Abolition of peripheral pulses	(23.56%)
Pallor	(22.85%)
Coldness	(28.94%)
Trophic disorders	(24.65%)

Table 4. Radiobiology/ Microbiology

Radiobiology	Values (n=150)
Standard radiography	**64 (42.69%)**
Osteitis	36 (55.69%)
Erosion	14 (22.85%)
Bone lysis	12 (18.94%)
Geode	2 (2.52%)
CT	**19 (12.53%)**
MRI	**16 (10.80%)**
Angioscan	**51 (33.98%)**
Arteriography	51 (100 %)
Microbiology	
Type of sampling	
Surface swabbing	41 (27.25%)
Curettage	34 (22.94%)
Deep swabbing	49 (32.63%)
Tissue biopsy	15 (9.68%)
Needle aspiration	11 (7.50%)
Direct examination	
Absence of germs	9 (5.86%)
Anaerobes	7 (4.89%)
Gram-positive cocci	46 (10.69%)
Gram-negative bacillus	88 (78.56%)
Microbial culture	
Sterile	18 (11.84%)
Mono-microbial	73 (48.93%)
Poly-microbial	59 (39.23%)
Microbiological profile	
Enterobacteriaceae	61 (40.82%)
Non-fermenting BGN	27 (17.90%)
Staphylococci	32 (21.25%)
Streptococci	30 (20.03%)

Table 5. Distribution of isolated germs.

Germs	**Percentage (%)**	**Germs**	**Percentage (%)**
Gram-negative bacilli		**Gram-positive cocci**	
Enterobacteriaceae	**56 (63.64%)**	**Staphylococci**	**27 (58.70%)**
Escherichia coli	30 (53.57%)	*Staphylococcus aureus*	16 (59.26%)
Klebsiella pneumoniae	16 (28.57%)	*Staphylococcus hominis*	11 (40.74%)
Proteus vulgaris	10 (17.86%)		
Non-fermenting BGN	**32 (36.36%)**	**Streptococci**	**19 (41.30%)**
Pseudomonas aeruginosa	23 (71.88%)	*Streptococcus bovis*	10 (52.63%)
Acinetobacter baumannii	9 (28.12%)	*Enterococcus faecalis*	9 (47.37%)

Table 6 a. Antibiotic resistance rates of the different germs isolated.

	Amoxicillin	**Penicillin**	**Oxacillin**	**Cefoxitin**	**Gentamicin**	**Clindamycin**	**Erythromycin**	**Pristinamycin**	**Rifamycin**	**Vancomycin**	**Teicoplanin**	**Tetracycline**	**Fusidic acid**	**Ofloxacin**	**Ciprofloxacin**
Staphylococcus aureus	/	11%	21%	9%	11%	9%	8%	0%	0%	0%	10%	4%	6%	5%	6%
Staphylococcus hominis	/	12%	9%	22%	17%	3%	6%	8%	5%	0%	0%	11%	2%	5%	/
Streptococcus bovis	/	20%	13%	10%	5%	7%	11%	2%	9%	5%	7%	1%	5%	3%	2%
Enterococcus faecalis	12%	10%	3%	17%	11%	4%	3%	11%	8%	11%	2%	2%	2%	1%	3%
Eschirichia coli	13%	10%	6%	15%	4%	7%	11%	4%	12%	2%	8%	3%	1%	2%	2%

Table 6 b. Resistance rates of Klebsiella pneumoniae to antibiotics.

	Amoxicillin	Amoxicillin + Ac clavulanic	Ampicillin	Oxacillin	Penicillin	Ticarcillin	Cefazolin	Cephalotin	Cefoxitin	Cefepime	Ertapenem	Amikacin	Chloramphenicol	Sulfonamide	Colistin
Klebsiella pneumoniae	2%	20%	9%	4%	3%	10%	8%	9%	2%	1%	7%	8%	9%	7%	9%

Table 6 c. Antibiotic resistance rates of *Proteus vulgaris and Enterobacter spp.*

	Amoxicillin	Amoxicillin+Ac clavulanic	Ampicillin	Ticarcillin	Cefazolin	Cephalotin	Cefoxitin	Cefotaxime	Cefepime	Ertapenem	Imipenem	Meropenem	Amikacin	Gentamicin	Chloramphenicol	Ciprofloxacin
Proteus vulgaris	3%	9%	4%	9%	8%	7%	8%	6%	4%	8%	5%	7%	9%	5%	4%	4%
Enterobacter spp.	3%	9%	8%	7%	11%	9%	7%	9%	11%	6%	7%	4%	/	5%	4%	/

predominated (8, 18).

The medical literature reports that diabetic foot infections are dominated by Gram-positive bacteria (GPB) (18-19). However, this predominance is not universal as recent studies from African and Asian countries have reported the predominance of Gram-negative bacteria (GNB) in diabetic foot infections (20-21, 8). In our series, the predominant pathogen was *Escherichia coli* (20%), which is consistent with the findings of Ako-Nai et *al*, 2006, in Nigeria (21) and Durgad et *al*, 2014, in India (20) (Table 7).

In our study, PMBs expressed a relatively low level of resistance to penicillin, cefoxitin, oxacillin and gentamycin. Ciprofloxacin, ofloxacin, fusidic acid, vancomycin and tetracycline were the most active antibiotics. Vancomycin was active on all staphylococci; *Staphylococcus aureus* was sensitive to rifamycin and pristinamycin, *Staphylococcus hominis* was sensitive to teicoplanin.

In the study by Turhan et *al*, 2018, vancomycin was active on all PMBs. Fusidic acid was active on all staphylococci, including methicillin-resistant strains (8). Fusidic acid could therefore be a good alternative in the treatment of diabetic foot infections.

In the study by Al Benwan et *al*, 2012, vancomycin was active on all PMBs. In contrast, staphylococci expressed a high rate of resistance to fusidic acid which was 42% (25).

Regarding BGN, the Enterobacteriaceae strains isolated in our study expressed a relatively low level of resistance to erythromycin, ticarcillin, amikacin and cefazolin. Fusidic acid, ciprofloxacin, cefepin and chloamphenicole were the most active antibiotics; amoxicillin was active on all PMBs except *Escherichia coli*.

In studies by Turhan et *al*, 2018, *et al.* Benwan et *al*, 2012, imipenem, amikacin and piperacillin-tazobactam were the most active antibiotics on BGN. These bacteria expressed a high rate of resistance to ampicillin, amoxicillin-clavulanic acid and ciprofloxacin (8, 19, 25).

Table 7. Bacteriological data of diabetic foot infections.

Author of the study (year)	Country	BGN	BGP	Predominant pathogen (rate)
Ako-Nai *et al.* (2006) (21)	Nigeria	58%	33%	Escherichia coli (15.1%)
Richard *et al.* (2011) (18)	France	36%	60%	Staphylococcus aureus (32.5%)
Mendes *et al.* (2012) (22)	Portugal	19%	66%	Staphylococcus aureus (21.8%)
Turhan *et al.* (2013) (8)	Turkey	61%	34%	Pseudomenas aeruginosa (29.8%)
Durgad *et al.* (2014) (20)	India	57%	27%	Pseudomenas aeruginosa (13.6%) Escherichia coli (13.6%)
Jadid (2015) (23)	Morocco	48%	49%	Staphylococcus aureus (13.3)
Labani Y (2016) (24)	Morocco	58%	41%	Staphylococcus aureus (20.2%)
Mezhoud and Khalfallah (2018) (5)	Constantine Algeria	66.30%	33.69%	Staphylococcus aureus (17.94%)
Our study	Algeria	78.56%	10.69%	Escherichia coli (20%)

Diabetic foot is a frequent complication of diabetes, silent but serious in its mortality, morbidity and disability. Its cost is among the highest of the degenerative complications of diabetes. Diabetic foot infections remain a formidable complication of diabetes. They are the main cause of hospitalisation for diabetics and one of the major causes of lower limb amputation. The diabetic foot requires multidisciplinary management (prevention, screening, medical and surgical treatment) involving: nurses, general practitioners, diabetologists, orthopaedic and vascular surgeons, physical physicians and psychologists as well as paramedical staff.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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