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Retrospective Observational Study on Microbial Contamination of Ulcerative Foot Lesions in Diabetic Patients

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Abstract: According to recent studies, there are almost 435 million people worldwide with diabetes mellitus. It is estimated that of these 148 million will develop Diabetic foot ulcers (DFUs) during their lifetime, of which 35 to 50% will be infected. In this scenario, the presence and frequency of pathogenic microorganisms and their level of susceptibility to the most frequent classes of antibiotics used to treat this pathological condition from patients with DFUs admitted to the outpatient clinic of vascular surgery of the Federico II University Hospital of Naples from January 2019 to March 2021 were investigated. Furthermore, the diabetic population characteristics under study (i.e., general, clinical, and comorbidities) and the pathogenic bacteria isolated from lesions were also considered. Bacterial strains poorly susceptible to antibiotics were more frequent in polymicrobial infections than in monomicrobial infections. β-Lactams showed the highest levels of resistance, followed by fluoroquinolones, aminoglycosides, and finally macrolides. The main findings of the study demonstrated that the occurrence of resistant microorganisms is the dominant factor in ulcer healing; thus it is essential to investigate the antibiotics' susceptibility before setting antibiotic therapy to avoid inappropriate prescriptions that would affect the treatment and increase the development and spread of antibiotic resistance.

Keywords: DFU; antimicrobial-resistant bacteria; epidemiology

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1. Introduction

Diabetic foot infections have been defined by the Infectious Diseases Society of America (IDSA) as "any infra-malleolar infection that occurs in a person with diabetes mellitus" [1].

According to recent studies, there are almost 435 million people worldwide with diabetes mellitus [2]; it is estimated that of these 148 million will develop Diabetic foot ulcers (DFUs) during their lifetime [3,4], of which 35 to 50% will be infected [5].

The chronic condition of uncontrolled hyperglycaemia is often complemented by peripheral polyneuropathy, which is the primary cause of ulcer formation (neuropathic ulcer). Once the ulcerative condition has set in, bacteria can colonize the lesion (more than 50% of cases), with an inflammatory response and systemic infections [6].

The most common clinical signs and symptoms of diabetic sensory neuropathy infection are lack of pain, vascular disease, and impaired cellular immunity. Microbiological and other diagnostic laboratory tests should confirm the diagnosis. According to IWGDF (International Working Group on the Diabetic Foot) guidelines, infection is present if there are two or more signs of inflammation, including erythema, pain, tenderness, warmth, induration, and purulent discharge [7].

Foot infections are among the most common causes of hospitalization in the diabetic population, accounting for 20% of all diabetes-related hospitalizations; in the United States alone, diabetic foot infections account for one fifth of the causes of hospitalization [8]. The care of DFUs is an issue strongly felt also among Mediterranean countries due to the high prevalence of the pathology in this area [9,10].

In most cases, this condition can represent a significant threat to the patient's life and the risk of amputation of the lower limbs, which is 155 times greater than for non-diabetics [9]. The risk of death also drastically increases; it is estimated that 1 in 4 patients and up to 1 patient in 8 can die within 1 year according to the infection severity [11]. Moreover, 5 years mortality rate in patients with ulcer and diabetic foot infection is close to 50%, much higher than other oncological diseases (i.e., breast cancer, prostate cancer, Hodgkin's lymphoma) [12,13].

In 80% of cases, bacterial infections may affect soft tissues and, in the remaining 20%, bone structure, with a significantly increased risk of skin infection compared to non-diabetic patients [14–16].

Localization and severity of the infection can also influence the composition of pathogenic ulcer microbiota, so the epidemiology of diabetic DFUs is highly variable.

As the skin has its own microbiota, microorganisms are always present on wounds [17]. In this scenario, the diagnosis of DFUs must be based not only on individual microbiological findings but also substantiated by clinical signs and symptoms. However, due to the local and systemic inflammatory response mediated by the neuropathic and ischemic course of the disease, it is often difficult to diagnose DFUs at an early stage (critical colonisation) [18]. Therefore, in order to differentiate real DFUs from non-infected ones (colonised), both clinical and microbiological criteria are crucial.

While most acute superficial infections are monomicrobial with a greater frequency of Gram-positive cocci, chronic and deep infections are commonly polymicrobial with a greater prevalence of Gram-negative bacilli [19,20].

Polymicrobial infections consist of a complex and varied bacterial ecosystem that can vary from two to several microbial species. In this scenario, several bacterial virulence factors can complicate the infection progression and its healing, leading to its chronicity [19,21,22].

For these reasons, knowing the microbiological aetiology of the lesion plays a critical role in clinical practice since identifying possible correlations between the epidemiology of the diabetic population and the microbiology of the DFUs can implement prevention and treatment strategies with a reduction of complications and healthcare costs [23].

To this end, a retrospective observational study aiming to identify and analyse the presence and frequency of pathogenic microorganisms and their level of susceptibility to the most frequent classes of antibiotics used to treat this pathological condition was conducted on clinical samples from patients with DFUs admitted to the outpatient clinic of vascular surgery of the Federico II University Hospital of Naples from January 2019 to March 2021.

2. Materials and Methods

2.1. Patients

Forty-three DFUs patients, both male (n.31) and female (n.12) aged from 42 to 94 years were admitted to the outpatient clinic of vascular surgery of the Federico II University Hospital of Naples from January 2019 to March 2021. All subjects under study had a diagnosis of diabetic pathology and classified with Rutherford Category IV–V peripheral artery disease with the need for revascularization and/or other interventions.

2.2. Population Characterization

Pathologies that can affect the course and severity of complications in diabetic pathology have been taken into account (i.e., arterial hypertension, kidney failure, Chronic Ob-

structive Pulmonary Disease (COPD), Coronary Atherothrombotic Disease (CAD), arterial thrombotic coronary disease), together with Body Mass Index (BMI) and smoking habit.

According to the BMI patients were classified as [24]:

- Normal weight
- Overweight
- I obesity

According to the smoking habit, patients were classified as:

- smokers
- non-smokers
- former smokers

2.3. Sample Collection

DFUs microbiological sample collection was performed during the first admission visit to ensure appropriate microbial isolation without any alteration due to antibiotic therapy. All samples were swabbed without the use of saline or other solutions that could affect microbiological analysis. Only in a few cases when an eschar was present was the lesion debrided in order to allow suitable sampling.

2.4. Microbial Identification and Antibiotic Susceptibility

Microbial identification and antibiograms were carried out by the microbiology laboratory of Federico II University Hospital of Naples using VITEK® (bioMérieux SA Marcy l'Etoile, France) automatic platform. Microorganisms' antibiotic susceptibility was expressed as MIC (μg/mL) according to Clinical Laboratory Standards Institute (CLSI) breakpoints [25].

The antibiotics tested were:

- ✓ β-lactams:
  - Penicillin
    - MIC Oxacillin
    - Ampicillin
    - Piperacillin
  - Cephalosporins
    - Ceftaroline
    - Cefepime
    - Ceftazidime
    - Cefotaxime
  - Carbapenems
    - Ertapenem
    - Imipenem
    - Meropenem
- ✓ Aminoglycosides
    - Gentamicin
    - Amikacin
    - Tobramycin
- ✓ Fluoroquinolones
    - Levofloxacin
    - Ciprofloxacin
- ✓ Macrolides
    - Erythromycin
- ✓ Sulphonamide
    - Cotrimoxazole

✓ Glycopeptide
- Vancomycin

2.5. Data Collection and Analysis

All records were collected anonymously and under current Italian privacy laws (Legislative Decree no.196 of 2003), with prior approval of the facility management. Only the information strictly relevant for the investigation were examined, namely:

- Date of birth
- Sex
- Body Mass Index (BMI)
- Concomitant pathologies, such as:
  - Arterial hypertension;
  - Renal failure;
  - Chronic obstructive pulmonary disease (COPD), or chronic obstructive pulmonary disease);
  - Coronary Atherothrombotic Disease (CAD), arterial thrombotic coronary artery disease;
  - Any other comorbidities.

The information collected for each subject was reported and reorganized with the help of a spreadsheet. A unique alphanumeric code was assigned for each patient, and a multilevel database was created to reorganize all the information. The patient's general information, physical characteristics, lifestyles, and co-morbid conditions were reported in the first level. A further level was devoted to clinical samples and microbiology lesions. The organization of the data consists of several parts:

- the first level presented general information such as execution date, reporting date, and reporting code;
- the second data set on the sample's microbiology; for each microorganism, a unique numerical code has been assigned to facilitate its identification and information management;
- the last, concerning information about antibiotic susceptibility.

3. Results

3.1. Gender and Age

A total of 43 subjects were included in the study, 11 female (26%) and 32 males (74%). Patient's age ranged from 42 to 94 years. The mean age was 68.81 ± 10.73, 73.27 ± 13.90 years for females and 65.59 ± 9.41 for males (Table 1).

Table 1. DFUs population gender and age.

	Total	**Male**	**Female**
DFUs Patients *n* (%) [a]	43 (100)	32 (74)	11 (26)
Mean age *n* ± D.S.	68.81 ± 10.73	73.27 ± 13.90	65.59 ± 9.41
Minimum age *n*	42	42	57
Maximum age *n*	94	81	94

[a] Percentage referred to the total population (*n* = 43). D.S. Standard Deviation.

3.2. Smoking Habit

The total number of smokers was 63% (*n* = 27), former smokers were 16% (*n* = 7) and non-smokers 21% (*n* = 9). Among smokers, 51% (*n* = 22) were male and 12% (*n* = 5) were female. Among non-smokers, 7% (*n* = 3) were men, 14% (*n* = 6) women. There were no female patients among former smokers, while of these 16% (*n* = 7) were male (Table 2).

Table 2. DFUs and population lifestyle: smoking habit and BMI.

	Total	Male	Female
Smokers *n* (%) [a]	27 (63)	22 (51)	5 (12)
Non-smokers *n* (%)	21 (9)	3 (7)	6 (14)
Former smokers *n* (%)	7 (16)	7 (16)	0
Normal weight *n* (%) (BMI 18.50–24.99)	3 (7)	1 (2)	2 (5)
Overweight *n* (%) (BMI 25–29.99)	32 (74)	24 (56)	8 (18)
I obesity *n* (%) (BMI 30–34.99)	8 (19)	7 (17)	1 (2)

[a] All percentages referred to the total population (*n* = 43).

3.3. BMI

Most patients, 74% (*n* = 32), were overweight (BMI between 25 and 29.99), 19% (*n* = 8) were classified as I obesity (BMI between 30 and 34.99) and only 7% (*n* = 3) were normal weight (BMI between 18.50 and 24.99). The overweight condition was predominant in males, 56% (*n* = 24), compared to females 18% (*n* = 8). As regards grade I obesity, 17% (*n* = 7) were male and 2% (*n* = 1) female. Finally, only 7% (*n* = 3) were normal weight, of which 2% (*n* = 1) were male and 5% (*n* = 2) were females (Table 2).

3.4. Comorbidities

Cardio-vascular and kidney diseases were the most frequent comorbidities retrieved. 88% (*n* = 38) of the sample population had arterial hypertension, followed by CAD, COPD, and kidney failure (respectively 56% (*n* = 24), 39% (*n* = 17) and 28% (*n* = 12)). Only 5% (*n* = 2) presented other comorbidities, such as vasculitis and anaemia (Table 3).

Table 3. DFUs and population comorbidities.

	Total	Male	Female
Arterial hypertension *n* (%) [a]	38 (88)	27 (63)	11 (25)
CAD *n* (%)	24 (56)	18 (42)	6 (14)
COPD *n* (%)	17 (39)	11 (25)	6 (14)
Kidney failure *n* (%)	12 (28)	10 (23)	2 (5)
Other *n* (%)s	2 (5)	1 (2.5)	1 (2.5)
NuD 1 *n* (%)	10 23)	8 (19)	2 (5)
NuD 2 *n* (%)	10 (23)	6 (14)	4 (9)
NuD 3 *n* (%)	9 (21)	5 (12)	4 (9)
NuD 4 *n* (%)	8 (19)	7 (16)	1 (2.5)
NuD 5 *n* (%)	1 (2.5)	1 (2.5)	0
No other pathologies *n* (%)	5 (12)	4 (9)	1 (2.5)

[a] All percentages referred to the total population (*n* = 43).

19% (*n* = 8) of male patients has a Number Disease (NuD) of 1, 14% (*n* = 6) a NuD of 2, 12% (*n* = 5) NuD of 3, 16% (*n* = 7) NuD of 4 and 2.5% (*n* = 1) NuD of 5, while only 9% (*n* = 4) of the male population had no concomitant pathology. As regards the female population, 5% (*n* = 2) had 1 NuD 9% (*n* = 4) 2, 9% (*n* = 4) 3 and 2.5% (*n* = 1) 4; there was no NuD of 5 for female patients. Finally, only 2.5% (*n* = 1) of female subjects did not have comorbidities. The highest NuD for male was 1 (19% (*n* = 8)), while for female this was 2 and 3 (9% (*n* = 4)) (Table 3).

3.5. DFUs Microbiology

A total of 99 strains (95 bacteria and 4 fungi) belonging to 30 different species (28 bacteria and 2 fungi) were isolated from the 43 patients examined. Among bacterial species, 39% (*n* = 11) were Gram + and 61% (*n* = 17) of Gram—(Table 4).

Table 4. DFUs and microorganism distribution.

	Strain Isolated	Species	Gram +	Gram −
Total *n*	99	30		
Bacteria *n* (%)	95 (96) [a]	28 (93) [b]	11 (39) [c]	17 (61) [c]
Fungi *n* (%)	4 (4) [a]	2 (7) [b]		

[a] Percentages refer to total microorganisms isolated (*n* = 99). [b] Percentages refer to microorganism species isolated (*n* = 30). [c] Percentages refer to bacterial species isolated (*n* = 28).

The epidemiologic distribution of the 99 strains isolated was as follows: *Staphylococcus aureus* 22% (*n* = 22); *Pseudomonas aeruginosa* 15% (*n* = 15); *Escherichia coli* 9% (*n* = 9); *Corynebacterium* spp. 8% (*n* = 8); *Proteus mirabilis* 6% (*n* = 6); *Staphylococcus epidermidis* 4% [*n* = 4]; *Serratia marcescens*, *Enterococcus faecalis*, 3% (*n* = 3); *Streptococcus agalactiae*, *Enterobacter cloacae* sbp. *cloacae*, *Cryseobacterium indologenes*, *Morganella morganii* sbp. *morganii*, *Klebsiella pneumoniae*, 2% (*n* = 2); *Staphylococcus warneri*, *Staphylococcus lugdunensis*, *Staphylococcus cohnii* sbp. *urealyticum*, *Alcaligenes faecalis*, *Staphylococcus simulans*, *Enterococcus avium*, *Citrobacter koseri*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Pseudomonas putida*, *Kocuria* spp., *Pseudomonas oleovorans*, *Stenotrophomonas maltophilia*, *Pseudomonas fluorescens* and *Prevotella disiens* respectively 1% (*n* = 1). The fungal species isolated were *Candida parapsilosis* 3% (*n* = 3) and *Candida krusei* 1% (*n* = 1) (Table 5).

Table 5. DFUs' bacterial epidemiologic distribution.

	Number	Percentage (%) [a]
Staphylococcus aureus	22	22
Pseudomonas aeruginosa	15	15
Escherichia coli	9	9
Corynebacterium spp.	8	8
Proreus mirabilis (%)	6	6
Staphylococcus epidermidis	4	4
Serratia marcescens	3	3
Enterococcus faecalis	3	3
Cryseobacterium indologenes	2	2
Streptococcus agalactiae	2	2
Enterobacer cloacae sbp. *cloacae*	2	2
Morganella morganii sbp. *morganii*	2	2
Klebsiella pneumoniae	2	2
Staphylococcus warneri	1	1
Staphylococcus lugdunensis	1	1
Staphylococcus cohnii sbp. *urealyticum*	1	1
Staphylococcus simulans	1	1
Alcaligenes faecalis	1	1
Enterococcus avium	1	1
Citrobacter koseri	1	1
Acinetobacter baumanii	1	1
Citrobacter freundii	1	1
Pseudomonas putida	1	1
Pseudomonas oleovorans	1	1
Pseudomonas fluorescens	1	1
Kocuria spp.	1	1
Stenotrophomonas maltophilia	1	1
Provetella disiens	1	1
Candida krusei	1	1
Candida parapsilosis	3	3

[a] Percentages refer to total microorganisms isolated [*n* = 99]. Microorganisms have been listed in descending order of isolation.

3.5.1. Polymicrobial and Monomicrobial Infections

Among the DFUs analysed, 33% (n = 14) were diagnosticated as Mono-Microbial infections (MMI), while 67% (n = 29) as Poly-Microbial infections (PMI).

As regard PMI, 34% (n = 18) of DFUS were infected by two different microorganisms, with 15% (n = 8) by three and 8% (n = 4) by four microorganisms (Table 6).

Table 6. Polymicrobial and Mono-microbial infections.

		Number	Percentage %
MMI		14	33 [a]
PMI		29	67 [a]
	2 microorganisms	15	51 [b]
	3 microorganisms	10	34 [b]
	4 microorganisms	2	7 [b]
	5 microorganisms or more	2	7 [b]

[a] percentages refer to the total population (n = 43) [b] percentages refer to PMI (n = 26).

3.5.2. Antibiotic Susceptibility

All antibiotic susceptibility is reported in Table 7. A summary of resistant strains is listed below.

- Oxacillin

Six microbial species were tested. Three resistant *S. aureus* 66% (n =15) MIC50 0.5 MIC 90 < 2, *S. epidermidis* 100% (n = 4) MIC50 and MIC 90 > 2; and *S. lugdunensis* 100% (n = 1) MIC 50 and MIC90 > 23.

- Ampicillin

Six microbial species were tested. One resistant *E. coli* 100% (n = 9) MIC50 and MIC90 > 8;

- Piperacillin

Only one microbial species was tested. None resistant

- Ceftaroline

Only one microbial species was tested *S. aureus* susceptible 100% (n = 22) MIC50 0.25 and MIC90 0.5.

- Cefepime

Eight microbial species were tested. One resistant *P. aeruginosa* 40% (n = 6) MIC50 0.5 MIC90 8. One intermediate *E. coli* 66% (n = 6) MIC50 2 MIC90.

- Ceftazidime

15 microbial species were tested. Six resistant *P. aeruginosa* 33% (n = 5) MIC50 2 MIC90 > 8 *E. coli* 33% (n = 3) MIC50 0.5 MIC90 8, *P. mirabilis* 17% (n = 1) MIC50 $\leq$ 0.12 MIC90 $\leq$ 0.5 *C. indologenes* 100% (n = 2) MIC50 and MIC 90 > 32 *E. cloacae* sbp. *cloacae* 50% (n = 1) MIC50 0.5 MIC90 > 32 *C. freundii* 100% (n = 1) MIC50 and MIC90 > 32.

- Cefotaxime

10 microbial species were tested. Five resistant *E. coli* 66% (n = 6) MIC50 > 4 MIC90 > 32, *P. mirabilis* 17% (n = 1) MIC50 $\leq$ 0.25 MIC90 $\leq$ 1 *C. indologenes* 100% (n = 2) MIC50 and MIC 90 > 4 *E. cloacae* sbp. *cloacae* 50% (n = 1) MIC50 $\leq$ 0.25 MIC90 > 32 *C. freundii* 100% (n = 1) MIC50 and MIC90 > 32.

- Ertapenem

Nine microbial species were tested. One resistant *C. indologenes* 100% (n =2) MIC50 and MIC90 $\leq$ 0.12

- Imipenem

Eight microbial species were tested. One resistant *P. aeruginosa* 20% (*n* = 3) MIC50 0.5 MIC90 2

- Meropenem

16 microbial species were tested. Two resistant *P. aeruginosa* 20% (*n* = 3) MIC50 $\leq$ 0.25 MIC90 2 and *A. baumannii* 100% (*n* = 1) MIC50 and MIC90 > 8

- Gentamicin

21 microbial species were tested. Eight resistant *S. aureus* 9% (*n* = 2) MIC50 and MIC $90 \leq 0.5$ *P. aeruginosa* 27% (*n* = 4) MIC50 2 MIC90 8 *Corynebacterium* spp. 38% (*n* = 3) MIC50 and MIC90 1.5 *P. mirabilis* 33% (*n* = 2) MIC50 $\leq$ 1 MIC90 8 *S. epidermidis* 100% (*n* = 4) MIC50 4 MIC90 8 *C. indologenes* 100% (*n* = 2) MIC50 and MIC90 > 8 *M. morganii* sbp. *morganii* 100% (*n* = 2) MIC50 and MIC90 > 8 and *A. baumannii* 100% (*n* = 1) MIC50 and MIC90 > 8

- Amikacin

14 microbial species were tested. Two resistant *P. aeruginosa* 13% (*n* = 2) MIC50 4 MIC90 8 and *A. baumannii* 100% (*n* = 1) MIC50 and MIC90 > 32

- Tobramycin

Six microbial species were tested. None resistant, all susceptible

- Levofloxacin

Nine microbial species were tested. Two resistant *S. aureus* 32% (*n* = 8) MIC50 0.25 MIC 90 > 4 and *S. epidermidis* 100% (*n* = 4) MIC50 and MIC90 4

- Ciprofloxacin

16 microbial species were tested. Nine resistant *P. aeruginosa* 67% (*n* = 10) MIC50 1 MIC90 > 2 *E. coli* 78% (*n* = 7) MIC50 1 MIC90 > 2 *Corynebacterium* spp. 100% (*n* = 8) MIC50 and MIC90 > 1 *P. mirabilis* 50% (*n* = 3) MIC50 $\leq$ 0.25 MIC90 >2 *C. indologenes* 100% (*n* = 2) MIC50 and MIC90 > 2 *M. morganii* sbp. *morganii* 100% (*n* = 2) MIC50 and MIC90 > 2 *A. faecalis* 100% (*n* = 1) MIC50 and MIC90 > 2 *A. baumannii* 100% (*n* = 1) MIC50 and MIC90 > 2 *P. fluorescens* 100% (*n* = 1) MIC50 and MIC90 > 2

- Erythromycin

Six microbial species were tested. Four resistant *S. aureus* 55% (*n* = 12) MIC50 1 MIC90 > 4 *S. epidermidis* 75% (*n* = 3) MIC50 and MIC90 > 4 *S. aga-latiae* 100% (*n* = 2) MIC50 and MIC90 2 *S. lugdunensis* 100% (*n* = 1) MIC50 and MIC90 > 4

- Cotrimoxazole

Only one microbial species was tested. None resistant

- Vancomycin

11 microbial species were tested. One resistant *Kocuria* spp. 100% (*n* = 1) MIC50 and MIC90 2.

3.5.3. DFUs' Microbiology and Diabetic Population

Regarding monomicrobial infection 20% (*n* = 9) of the DFUs' population were male while 12% (*n* = 5) were woman. Polymicrobial infections were reported according to the number of microorganisms isolated.

- Two microorganisms isolated (P2), 28% (*n* = 12) male and 7% (*n* = 3) female;
- Three isolated microorganisms (P3), 14% (*n* = 6) male and 9% (*n* = 4) female;
- Four isolated microorganisms (P4), 5% (*n* = 2) in the male gender and none female;
- Five+ microorganisms (P5+) 5% (*n* = 2) male and none female (Figure 1).

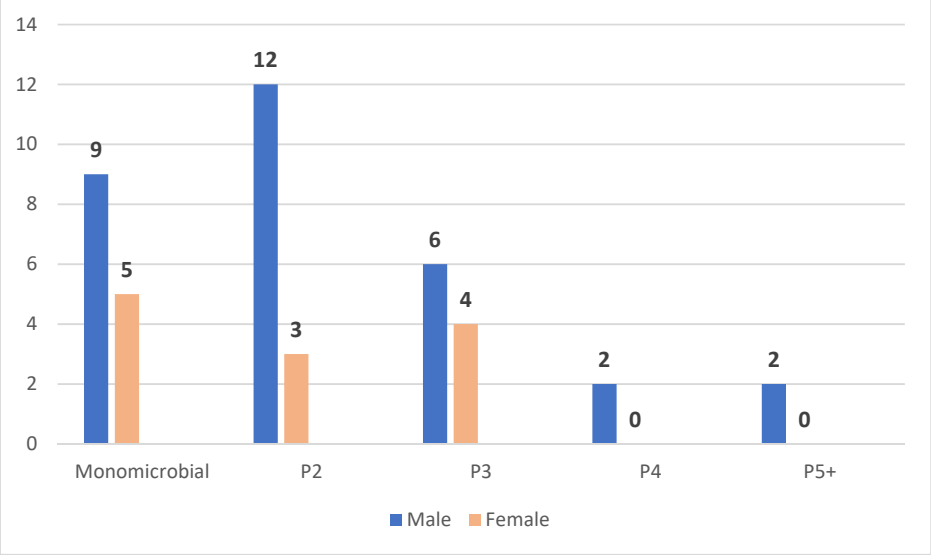

Figure 1. Mono and Polymicrobial infections and DFU patients' gender. P2: polymicrobial infection colonized by 2 microorganism; P3: polymicrobial infection colonized by 3 microorganism; P4: polymicrobial infection colonized by 4 microorganism; P5+: polymicrobial infection colonized by 5 or more microorganism.

Results for cigarette smoking and mono-/polymicrobial infections are shown in Figure 2. For monomicrobial infections, 19% (n = 8) of patiets were smokers (S), 7% (n = 3) were ex-smokers (EX) and non-smokers (NS), respectively. Regarding polymicrobial infections:

- Two P 18% (n = 8) S, 5% (n = 2) EX and 12% (n = 5) NS;
- Three P 16% (n = 7) S, 5% (n = 2) EX and 2% (n = 1) NS;
- Four P 5% (n = 2) S;
- Five+ P 5% (n = 2) S.

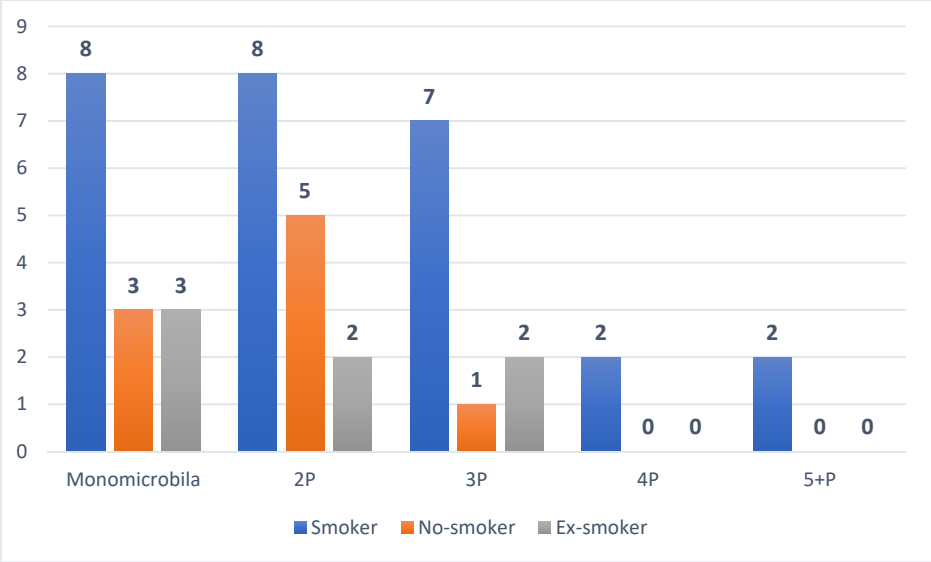

Figure 2. Monomicrobial and polymicrobial infections and DFU patient lifestyle: cigarette smoking. P2: polymicrobial infection colonized by 2 microorganisms; P3: polymicrobial infection colonized by 3 microorganisms; P4: polymicrobial infection colonized by 4 microorganisms; P5+: polymicrobial infection colonized by 5 or more microorganisms.

Table 7. Bacterial antibiotic susceptibility. [a] Clinical Laboratory Standards Institute (CLSI) breakpoints [25]. All percentages refer to the single bacterial specie tested.

	MIC (μg/mL) Breakpoints [a]	*S. aureus*	*P. aeruginosa*	*Escherichia coli*	*Corynebacterium* spp.	*P. mirabilis*	*S. epidermidis*	*Serratia marcescens*	*E. facalis*	*C. indologenes*	*S. agalatiae*	*E. cloacae* sbp. *cloacae*	*M. morganii* sbp. *morganii*	*K. pneumoniae*	*S. werneri*	*S. lugdunensis*	*S. cohnii* sbp. *urealyticum*	*S. simulans*	*A. faecalis*	*E. avium*	*Citrobacter koseri*	*A. baumannii*	*C. freundii*	*P. putida*	*P. oleovorans*	*P. fluorescens*	*Kocuria* spp.	*S. maltophilia*	*P. disiens*
Oxacillin	**S**[susceptible] ***n*** **(%)**	7 (32)													1 (100)		1 (100)	1 (100)										
	I[intermediate] ***n*** **(%)**																											
	R[resistant] ***n*** **(%)**	15 (66)					4 (100)									1 (100)												
	MIC 50	0.5					>2								0.5	>2	0.5	≤0.25										
	MIC 90	<2					>2								0.5	>2	0.5	≤0.25										
Ampicilin	**S**[susceptible] ***n*** **(%)**								3 (100)		2 (100)								1 (100)							1 (100)		1 (100)
	I[intermediate] ***n*** **(%)**																											
	R[resistant] ***n*** **(%)**			9 (100)																								
	MIC 50			>8					≤2		≤0.06								≤2							0.125		0.064
	MIC 90			>8					≤2		0.12								≤2							0.125		0.064
Piperacillin	**S**[susceptible] ***n*** **(%)**																											
	I[intermediate] ***n*** **(%)**																											
	R[resistant] ***n*** **(%)**																											1 (100)
	MIC 50																											0.125
	MIC 90																											0.125
Ceftaroline	**S**[susceptible] ***n*** **(%)**	22 (100)																										
	I[intermediate] ***n*** **(%)**																											
	R[resistant] ***n*** **(%)**																											
	MIC 50	0.25																										
	MIC 90	0.5																										
Cefepime	**S**[susceptible] ***n*** **(%)**		9 (60)	3 (33)		6 (100)															1 (100)			1 (100)	1 (100)	1 (100)	1 (100)		
	I[intermediate] ***n*** **(%)**			6 (66)																								
	R[resistant] ***n*** **(%)**		6 (40)																									
	MIC 50		4	2		≤0.12															≤0.12			2	8	4	0.75		
	MIC 90		8	4		≤0.12															≤0.12			2	8	4	0.75		

Table 7. *Cont.*

	MIC (μg/mL) Breakpoints [a]	*S. aureus*	*P. aeruginosa*	*Escherichia coli*	*Corynebacterium* spp.	*P. mirabilis*	*S. epidermidis*	*Serratia marcescens*	*E. facalis*	*C. indologenes*	*S. agalatiae*	*E. cloacae* sbp. *cloacae*	*M. morganii* sbp. *morganii*	*K. pneumoniae*	*S. iverneri*	*S. lugdunensis*	*S. cohnii* sbp. *urealyticum*	*S. simulans*	*A. faecalis*	*E. avium*	*Citrobacter koseri*	*A. baumannii*	*C. freundii*	*P. putida*	*P. oleovorans*	*P. fluorescens*	*Kocuria* spp.	*S. maltophilia*	*P. disiens*
Ceftazidime	**S**^{susceptible} ***n*** **(%)**		10 (67)	6 (66)		5 (83)		3 (100)				1 (50)	2 (100)	2 (100)					1 (100)		1 (100)			1 (100)	1 (100)	1 (100)	1 (100)		
	I^{intermediate} ***n*** **(%)**																												
	R^{resistant} ***n*** **(%)**		5 (33)	3 (33)		1 (17)				2 (100)		1 (50)											1 (100)						
	MIC 50		2	0.5		≤0.12		≤0.12		>32		0.5	≤0.12	≤0.12					4		0.25		>32	2	2	8	8		
	MIC 90		>8	8		≤0.5		≤0.12		>32		>32	≤0.12	≤0.12					4		0.25		>32	2	2	8	8		
Cefotaxime	**S**^{susceptible} ***n*** **(%)**			3 (33)		5 (83)		2 (67)				1 (50)	2 (100)	2 (100)					1 (100)		1 (100)								
	I^{intermediate} ***n*** **(%)**							1 (33)																					
	R^{resistant} ***n*** **(%)**			6 (66)		1 (17)				2 (100)		1 (50)											1 (100)						
	MIC 50			>4		≤0.25		≤0.25		>32		≤0.25	≤0.25	>32					1		≤0.25		>32						
	MIC 90			>32		≤1		≤0.25		>32		>32	≤0.25	>32					1		≤0.25		>32						
Ertapenem	**S**^{susceptible} ***n*** **(%)**			9 (100)		6 (100)		3 (100)				2 (100)	2 (100)	2 (100)					1 (100)				1 (100)						
	I^{intermediate} ***n*** **(%)**																												
	R^{resistant} ***n*** **(%)**									2 (100)																			
	MIC 50			≤0.25		≤0.12		≤0.12		>4		≤0.12	≤0.12	≤0.12					≤0.12				0.25						
	MIC 90			≤0.25		≤0.12		≤0.12		>4		≤0.12	≤0.12	≤0.12					≤0.12				0.25						
Imipenem	**S**^{susceptible} ***n*** **(%)**		12 (80)	9 (100)					3 (100)											1 (100)	1 (100)			1 (100)	1 (100)	1 (100)			
	I^{intermediate} ***n*** **(%)**																												
	R^{resistant} ***n*** **(%)**		3 (20)																										
	MIC 50		0.5	≤0.25					≤1											≤1	≤0.25			≤0.25	≤0.25	0.5			
	MIC 90		2	0.5					≤1											≤1	≤0.25			≤0.25	≤0.25	0.5			
Meropenem	**S**^{susceptible} ***n*** **(%)**		12 (80)	9 (100)		6 (100)		3 (100)		2 (100)		2 (100)	2 (100)	2 (100)					1 (100)		1 (100)		1 (100)	1 (100)	1 (100)	1 (100)			1 (100)
	I^{intermediate} ***n*** **(%)**																												
	R^{resistant} ***n*** **(%)**		3 (20)																			1 (100)							
	MIC 50		≤0.25	≤0.25		≤0.25		≤0.25		4		≤0.25	≤0.125	≤0.25					≤0.25		≤0.25	>8	≤0.25	2	≤0.25	≤0.25			1
	MIC 90		2	≤0.25		≤0.25		≤0.25		4		≤0.25		≤0.25					≤0.25		≤0.25	>8	≤0.25	2	≤0.25	≤0.25			1

Table 7. *Cont.*

	MIC (μg/mL) Breakpoints [a]	*S. aureus*	*P. aeruginosa*	*Escherichia coli*	*Corynebacterium* spp.	*P. mirabilis*	*S. epidermidis*	*Serratia marcescens*	*E. facalis*	*C. indologenes*	*S. agalatiae*	*E. cloacae* sbp. *cloacae*	*M. morganii* sbp. *morganii*	*K. pneumoniae*	*S. iverneri*	*S. lugdunensis*	*S. cohnii* sbp. *urealyticum*	*S. simulans*	*A. faecalis*	*E. avium*	*Citrobacter koseri*	*A. baumannii*	*C. freundii*	*P. putida*	*P. oleovorans*	*P. fluorescens*	*Kocuria* spp.	*S. maltophilia*	*P. disiens*
Gentamicin	**S**^{susceptible} ***n* (%)**	20 (91)	11 (73)	9 (100)	5 (62)	4 (67)		3 (100)				2 (100)		2 (100)			1 (100)	1 (100)	1 (100)		1 (100)		1 (100)	1 (100)	1 (100)	1 (100)			
	I^{intermediate} ***n* (%)**																										1 (100)		
	R^{resistant} ***n* (%)**	2 (9)	4 (27)		3 (38)	2 (33)	4 (100)			2 (100)			2 (100)									1 (100)							
	MIC 50	≤0.5	2	≤1	1.5	≤1	4	≤1		>8		≤1	>8	≤1			≤0.5	≤0.5	2		≤1	>8	≤1	≤1	2	≤1	0.38		
	MIC 90	≤0.5	8	≤1	1.5	8	8	≤1		>8		≤1	>8	≤1			≤0.5	≤0.5	2		≤1	>8	≤1	≤1	2	≤1	0.38		
Amikacin	**S**^{susceptible} ***n* (%)**		13 (87)	6 (66)		6 (100)		3 (100)				2 (100)	2 (100)	2 (100)					1 (100)		1 (100)		1 (100)	1(1)	1(1)	1(1)			
	I^{intermediate} ***n* (%)**			3 (33)																									
	R^{resistant} ***n* (%)**		2 (13)																			1 (100)							
	MIC 50		4	4		4		≤1				≤1	4	≤1					4		≤1	>32	≤1	≤1	4	2			
	MIC 90		8	8		4		4				≤1	4	≤1					4		≤1	>32	≤1	≤1	4	2			
Tobramycin	**S**^{susceptible} ***n* (%)**		15 (100)	9 (100)																	1 (100)			1 (100)	1 (100)	1 (100)			
	I^{intermediate} ***n* (%)**																												
	R^{resistant} ***n* (%)**																												
	MIC 50		≤1	1 (1)																	≤1			≤1	≤1	≤1			
	MIC 90		2	1 (1)																	≤1			≤1	≤1	≤1			
Levofloxacin	**S**^{susceptible} ***n* (%)**	15 (68)									2 (100)				1 (100)	1 (100)	1 (100)	1 (100)											
	I^{intermediate} ***n* (%)**																										1 (100)		
	R^{resistant} ***n* (%)**	8 (32)					4 (100)																						
	MIC 50	0.25				≤0.5	4				0.5				0.25	0.25	0.5	≤0.12									0.75		
	MIC 90	>4				≤0.5	4				0.5				0.25	0.25	0.5	≤0.12									0.75		

Table 7. *Cont.*

	MIC (μg/mL) Breakpoints [a]	*S. aureus*	*P. aeruginosa*	*Escherichia coli*	*Corynebacterium* spp.	*P. mirabilis*	*S. epidermidis*	*Serratia marcescens*	*E. facalis*	*C. indologenes*	*S. agalatiae*	*E. cloacae* sbp. *cloacae*	*M. morganii* sbp. *morganii*	*K. pneumoniae*	*S. werneri*	*S. lugdunensis*	*S. cohnii* sbp. *urealyticum*	*S. simulans*	*A. faecalis*	*E. avium*	*Citrobacter koseri*	*A. baumannii*	*C. freundii*	*P. putida*	*P. oleovorans*	*P. fluorescens*	*Kocuria* spp.	*S. maltophilia*	*P. disiens*
Ciprofloxacin	**S**^{susceptible} ***n*** **(%)**		5 (33)	2 (22)		3 (50)		3 (100)				2 (100)		2 (100)								1 (100)		1 (100)	1 (100)		1 (100)			
	I^{intermediate} ***n*** **(%)**																													
	R^{resistant} ***n*** **(%)**		10 (67)	7 (78)	8 (100)	3 (50)				2 (100)			2 (100)						1 (100)		1 (100)				1 (100)				
	MIC 50		1	1	>1	≤0.25		≤0.06		≥2		≤0.06	≥2	≤0.06					≥2		≤0.06	>2	0.25	0.12	≥2	0.5			
	MIC 90		>2	>2	>1	>2		≤0.06		≥2		≤0.06	≥2	≤0.06					≥2		≤0.06	>2	0.25	0.12	≥2	0.5			
Erythromycin	**S**^{susceptible} ***n*** **(%)**	10 (45)					1 (25)								1 (100)			1 (100)											
	I^{intermediate} ***n*** **(%)**																													
	R^{resistant} ***n*** **(%)**	12 (55)					3 (75)				2 (100)					1 (100)													
	MIC 50	1					>4				2				1	>4		≤0.25											
	MIC 90	>4					>4				2				1	>4		≤0.25											
Cotrimoxazole	**S**^{susceptible} ***n*** **(%)**																													1 (100)
	I^{intermediate} ***n*** **(%)**																													
	R^{resistant} ***n*** **(%)**																													
	MIC 50																													≤20
	MIC 90																													≤20
Vancomycin	**S**^{susceptible} ***n*** **(%)**	22 (100)			7 (100)		4 (100)			3 (100)	2 (100)				1 (100)	1 (100)	1 (100)	1 (100)		1 (100)										
	I^{intermediate} ***n*** **(%)**																				-						1 (100)			
	R^{resistant} ***n*** **(%)**																				-						-			
	MIC 50	1			0.25		2			1	0.5				≤0.5	≤0.5	1	≤0.5			≤0.5						2			
	MIC 90	1			0.5		2			4	0.5				≤0.5	≤0.5	1	≤0.5			≤0.5						2			

Body Mass Index (BMI) in monomicrobial infections showed that 2% (n = 1) of population was normal weight, 24% (n = 10) was overweight, and 7% (n = 3) was in a grade I obesity condition. On the other hand, for polymicrobial infections:

- Two P 5% (n = 2) were normal-weight subjects, 26% (n = 11) overweight, and 5% (n = 2) in a grade I obesity condition;
- Three P 16% (n = 7) was represented by overweight and 7% (n = 3) in a grade I obesity condition subjects;
- Four and five+ P, were 5% (n = 2) in an overweight condition (Figure 3).

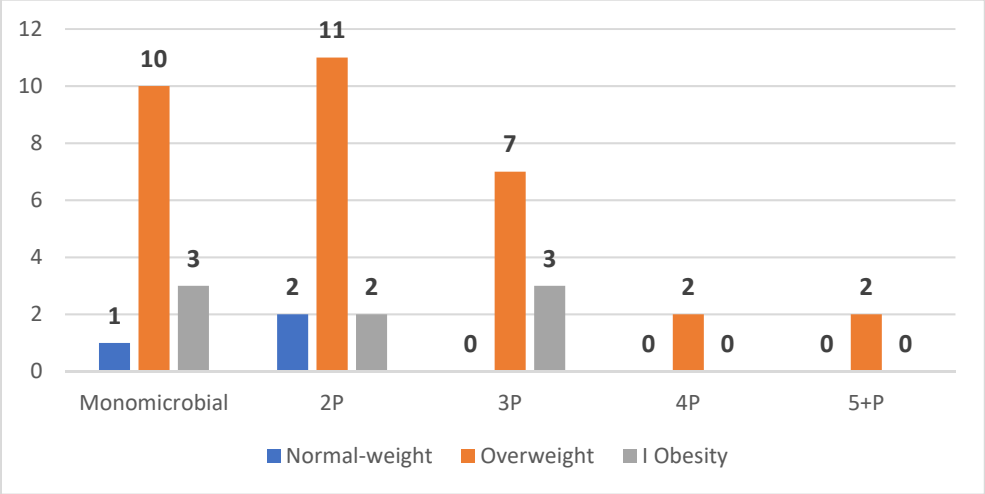

Figure 3. Monomicrobial and polymicrobial infections and DFU patient lifestyle: BMI. P2: polymicrobial infection colonized by 2 microorganisms; P3: polymicrobial infection colonized by 3 microorganisms; P4: polymicrobial infection colonized by 4 microorganisms; P5+: polymicrobial infection colonized by 5 or more microorganisms.

Lastly, monomicrobial infections on comorbidities revealed that 5% (n = 2) had no NuD, 7% (n = 3) had one NuD, 5% (n = 2) had two NuD, 7% (n = 3) had three NuD, and 9% (n = 4) had four NuD. In polymicrobial infections NuD distribution was:

- TwoP, 2% (n = 1) had no NuD, 7% (n = 3) had one NuD, 15% (n = 6) had two NuD, 5% (n = 2) had three NuD, and 7% (n = 3) had four NuD; for a total of 36% (n = 15);
- ThreeP 5% (n = 2) have no NuD, 7% (n = 3) have one NuD, 2% (n = 1) have two NuD, 5% (n = 2) have three NuD, 2% (n = 1) have four NuD, and 2% (n = 1) have five NuD. For a total of 23% [n = 10];
- FourP 2% (n = 1) have one NuD and 2% (n = 1) have three NuD. For a total of 4% (n = 2);
- Five+ P 2% (n = 1) have 2 NuD and 2% (n = 1) have three NuD. For a total of 4% (n = 2) (Figure 4).

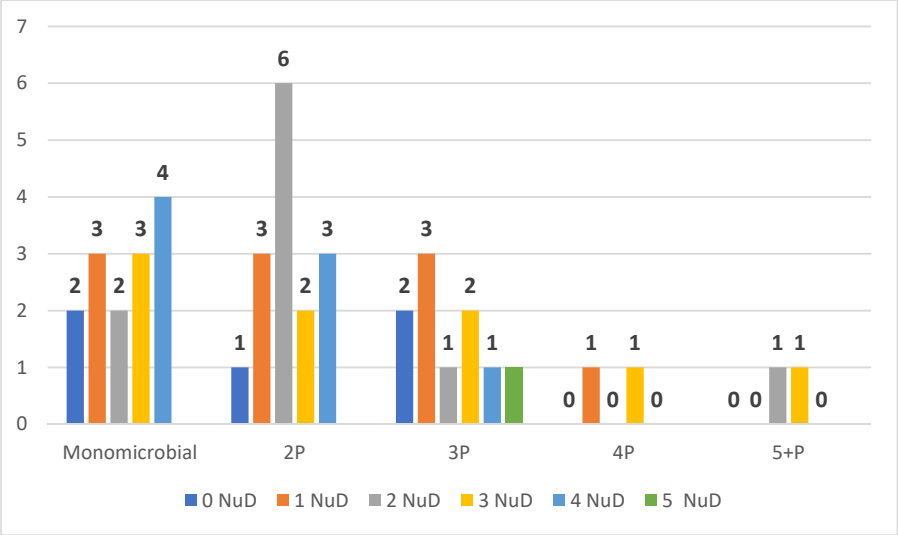

Figure 4. Monomicrobial and polymicrobial infections and DFU patient comorbidities. P2: polymicrobial infection colonized by 2 microorganisms; P3: polymicrobial infection colonized by 3 microorganisms; P4: polymicrobial infection colonized by 4 microorganisms; P5+: polymicrobial infection colonized by 5 or more microorganism.

4. Discussion

Previous studies have established that several factors such as age sex and lifestyle influence the epidemiology of DFUs. In relation to gender, a 2017 systematic review published by Zhang et al. revealed that the prevalence of diabetic foot ulceration is higher in males than females [26]. These findings were confirmed in this study where a higher prevalence of male subjects (76%) was observed. Nevertheless, the reason for this prevalence is still not entirely clear, although the higher engagement of males in physical labour could be a possible cause [27] (Table 1).

The mean sample age of patients (68.81 ± 10.73) mirrored that reported in DFUs cases from economically developed countries. Furthermore, also the gender age agreed with literature data, and males (73.27 ± 13.90) are generally older than females (65.59 ± 9.41) (Table 1) [28–30].

As regards the population lifestyle, cigarette smoking was considered. Our data showed that 63% of the patients were smokers, and of these 51% were male and 12% female (Table 2). Several studies have identified cigarette smoking as a significant risk factor for diabetic foot ulceration, as daily tissue hypoxia can cause or amplify vascular and neuropathic disorders in the lower limbs of diabetic patients [26,31,32].

These findings were also confirmed by DFU prevalence in diabetic patients from North America, Belgium, and Norway, where a higher percentage of smokers than in Europe is recorded [26].

An additional aspect of DFU patient's lifestyle investigated was nutrition. 74% of the population was overweight (BMI between 25 and 29.99), whereas 19% was grade I obese (BMI between 30 and 34.99). Both these conditions were more frequent in males (56% and 17%, respectively). On the other hand, females had a frequency percentage of 18% and 2% (Table 2).

The correlation between BMI and the risk of DFUs developing is still not completely clear, although some studies have shown that overweight and obesity (BMI between 25 and 45 kg/m^2) are probably related to a higher frequency of foot ulcers in diabetics [33–35]. All these factors are well-proven conditions that expose diabetic subjects to an increased risk of ischemic pathology and foot injuries [27].

In this scenario, comorbidities and specifically the presence of cardiovascular, occlusive, thrombotic, and renal diseases represent a critical risk factor in the onset and prognosis of diabetic disease and its complications, particularly for the onset of DFUs [36]. Retrospec-

tive observation revealed that arterial hypertension is the most frequent comorbidity (88% of the sample population), followed by CAD (56%), COPD (39%) and renal failure (28%). Only 5% of the population had any other conditions. In males, the most frequent condition was one NuD (19%), while for females, two and three NuD (9%) (Table 3).

In addition to the sample population characteristics, DFU microbiota was also analyzed. The microbiological analysis revealed that, in most cases, infections were due to bacteria (96% of cases) (Table 4). A higher prevalence of these was polymicrobial than monomicrobial (67% and 33%, respectively) (Table 5). Scientific evidence also confirms that severe chronic ulcers are often colonized by several microorganisms at the same time [21,22,37,38]

The most recurrent microbial species and the frequency between Gram-positive and Gram-negative bacteria were also taken into account. In this scenario, epidemiological studies on DFUs have reported a higher frequency of Gram-positive bacteria in Western countries, and a higher frequency of Gram-negative bacteria in Eastern ones (India, Middle East, China, Africa) [39–41]. Possible explanations could be found in the excessive sweating of the feet caused by the hot climate of eastern countries, in association with the high level of self-treatment of the ulcerative lesion with antibiotics without any medical criteria, as well as the poor hygienic conditions, especially at the perineal and hand level [42]. These literature data were confirmed in this study, where 39% of the 28 bacterial species isolated were Gram-positive and 61% Gram-negative (Table 4).

Regarding the literature data on microbial species colonizing DFUs, the most frequent isolates were *S. aureus* [1,7,43], *P. aeruginosa* and *E. coli* [20,40]. Our data agreed with the literature; thus, the most frequent microbial species were *S. aureus* (22%), followed by *P. aeruginosa* (15%), *E. coli* (9%), *Corynebacterium* spp. (8%) and *P. mirabilis* (6%) (Table 6).

As shown in Table 7, microbial susceptibility to antibiotics was assessed for the 99 bacterial strains isolated. β-lactam presented the highest percentage of resistant bacteria (69%), followed by fluoroquinolones (47%), aminoglycosides (22%) and macrolides (18%) (Table 7). Antimicrobial-resistant bacteria were more frequent in polymicrobial infections than monomicrobial, as confirmed by a systematic review published in 2020 about the prevalence of antibiotic resistance in DFUs [44]. In this scenario, it is clear that in DFUs clinical management, microorganisms' epidemiology is crucial to determine the lesion severity and possible therapeutic strategies.

The type of infection (poly- or monomicrobial) and the frequency of bacteria poorly sensitive to antibiotics were also correlated with the characteristics of the sample population (gender, cigarette smoking, BMI and number of comorbidities) to identify patterns that could be a starting point for further epidemiological and clinical investigations.

These findings allow us to distinguish two typologies of DFU patient, according to infection type. The polymicrobial patient infected by two concomitant bacteria (Figures 1–4) was male (Figure 1), smoker (Figure 2), overweight (Figure 3), with a NuD of 2 (Figure 4). On the other hand, the monomicrobial patient was also male (Figure 1), smoker (Figure 2), overweight (Figure 3) with NuD 4.

These findings emphasize once again that antibiotic therapy for DFU is an arduous clinical pathway, despite that to date there are still no clinical guidelines, but only recommendations [45–47].

5. Conclusions

Knowledge of DFU microbiological epidemiology is a crucial aspect for appropriate clinical management. Indeed one of the main factors affecting the healing of the ulcer is undoubtedly the presence of resistant microorganisms. Among these, antibiotic susceptibility of DFU colonizing microorganisms allows to provide the most appropriate antibiotic therapy with a reduction of health care costs and in the development of resistant strains. In this scenario, the DFU microbiota analysis together with the antibiotic susceptibility profiles allows personalizing, as much as possible, the diagnostic, therapeutic and care process.

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References

1. Lipsky, B.A.; Berendt, A.R.; Deery, H.G.; Embil, J.M.; Joseph, W.S.; Karchmer, A.W.; LeFrock, J.L.; Lew, D.P.; Mader, J.T.; Norden, C. Diagnosis and treatment of diabetic foot infections. *J. Am. Podiatr. Med. Assoc.* **2005**, *95*, 183–210. [CrossRef]
2. Vos, T.; Allen, C.; Arora, M.; Barber, R.M.; Bhutta, Z.A.; Brown, A.; Carter, A.; Casey, D.C.; Charlson, F.J.; Chen, A.Z. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: A systematic analysis for the Global Burden of Disease Study 2015. *Lancet* **2016**, *388*, 1545–1602. [CrossRef]
3. Armstrong, D.G.; Boulton, A.J.; Bus, S.A. Diabetic foot ulcers and their recurrence. *N. Engl. J. Med.* **2017**, *376*, 2367–2375. [CrossRef]
4. Lazzarini, P.A.; Pacella, R.E.; Armstrong, D.G.; van Netten, J.J. Diabetes-related lower-extremity complications are a leading cause of the global burden of disability. *Diabet. Med.* **2018**, *35*, 1297–1299. [CrossRef]
5. Prompers, L.; Huijberts, M.; Apelqvist, J.; Jude, E.; Piaggesi, A.; Bakker, K.; Edmonds, M.; Holstein, P.; Jirkovska, A.; Mauricio, D. High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. *Diabetologia* **2007**, *50*, 18–25. [CrossRef]
6. Bader, M.S. Diabetic foot infection. *Am. Fam. Physician* **2008**, *78*, 71–79.
7. Lipsky, B.A.; Senneville, É.; Abbas, Z.G.; Aragón-Sánchez, J.; Diggle, M.; Embil, J.M.; Kono, S.; Lavery, L.A.; Malone, M.; van Asten, S.A. Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes Metab. Res. Rev.* **2020**, *36*, e3280. [CrossRef] [PubMed]
8. Hobizal, K.B.; Wukich, D.K. Diabetic foot infections: Current concept review. *Diabet. Foot Ankle* **2012**, *3*, 18409. [CrossRef]
9. Lavery, L.A.; Armstrong, D.G.; Wunderlich, R.P.; Mohler, M.J.; Wendel, C.S.; Lipsky, B.A. Risk factors for foot infections in individuals with diabetes. *Diabetes Care* **2006**, *29*, 1288–1293. [CrossRef]
10. Bracale, U.M.; Ammollo, R.P.; Hussein, E.A.; Hoballah, J.J.; Goeau-Brissonniere, O.; Taurino, M.; Setacci, C.; Pecoraro, F.; Bracale, G.; Giribono, A.M.; et al. Managing peripheral artery disease in diabetic patients: A questionnaire survey from vascular centers of the Mediterranean Federation for the Advancing of Vascular Surgery (MeFAVS). *Ann. Vasc. Surg.* **2020**, *64*, 239–245. [CrossRef] [PubMed]
11. Fincke, B.G.; Miller, D.R.; Turpin, R. A classification of diabetic foot infections using ICD-9-CM codes: Application to a large computerized medical database. *BMC Health Serv. Res.* **2010**, *10*, 192. [CrossRef]
12. Armstrong, D.G.; Wrobel, J.; Robbins, J.M. Guest editorial: Are diabetes-related wounds and amputations worse than cancer. *Int. Wound J.* **2007**, *4*, 286–287. [CrossRef] [PubMed]
13. Robbins, J.M.; Strauss, G.; Aron, D.; Long, J.; Kuba, J.; Kaplan, Y. Mortality Rates and Diabetic Foot UlcersIs it Time to Communicate Mortality Risk to Patients with Diabetic Foot Ulceration? *J. Am. Podiatr. Med. Assoc.* **2008**, *98*, 489–493. [CrossRef] [PubMed]
14. Lavery, L.A.; Armstrong, D.G.; Wunderlich, R.P.; Tredwell, J.; Boulton, A.J. Diabetic foot syndrome: Evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. *Diabetes Care* **2003**, *26*, 1435–1438. [CrossRef] [PubMed]
15. Wukich, D.K.; McMillen, R.L.; Lowery, N.J.; Frykberg, R.G. Surgical site infections after foot and ankle surgery: A comparison of patients with and without diabetes. *Diabetes Care* **2011**, *34*, 2211–2213. [CrossRef] [PubMed]
16. Wukich, D.K.; Lowery, N.J.; McMillen, R.L.; Frykberg, R.G. Postoperative infection rates in foot and ankle surgery: A comparison of patients with and without diabetes mellitus. *JBJS* **2010**, *92*, 287–295. [CrossRef]
17. Magnifico, I.; Petronio Petronio, G.; Venditti, N.; Cutuli, M.A.; Pietrangelo, L.; Vergalito, F.; Mangano, K.; Zella, D.; Di Marco, R. Atopic dermatitis as a multifactorial skin disorder. Can the analysis of pathophysiological targets represent the winning therapeutic strategy? *Pharmaceuticals* **2020**, *13*, 411. [CrossRef]
18. Sotto, A.; Richard, J.-L.; Messad, N.; Molinari, N.; Jourdan, N.; Schuldiner, S.; Sultan, A.; Carrière, C.; Canivet, B.; Landraud, L.; et al. Distinguishing colonization from infection with Staphylococcus aureus in diabetic foot ulcers with miniaturized oligonucleotide arrays: A French multicenter study. *Diabetes Care* **2012**, *35*, 617–623. [CrossRef]

19. Charles, P.G.; Uçkay, I.; Kressmann, B.; Emonet, S.; Lipsky, B.A. The role of anaerobes in diabetic foot infections. *Anaerobe* **2015**, *34*, 8–13. [CrossRef]
20. Sadeghpour Heravi, F.; Zakrzewski, M.; Vickery, K.; Armstrong, D.G.; Hu, H. Bacterial diversity of diabetic foot ulcers: Current status and future prospectives. *J. Clin. Med.* **2019**, *8*, 1935. [CrossRef]
21. Rhoads, D.D.; Wolcott, R.D.; Sun, Y.; Dowd, S.E. Comparison of culture and molecular identification of bacteria in chronic wounds. *Int. J. Mol. Sci.* **2012**, *13*, 2535–2550. [CrossRef]
22. Citron, D.M.; Goldstein, E.J.; Merriam, C.V.; Lipsky, B.A.; Abramson, M.A. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. *J. Clin. Microbiol.* **2007**, *45*, 2819–2828. [CrossRef] [PubMed]
23. Joseph, W.S.; Lipsky, B.A. Medical therapy of diabetic foot infections. *J. Vasc. Surg.* **2010**, *52*, 67S–71S. [CrossRef]
24. Weir, C.B.; Jan, A. *BMI Classification Percentile and Cut off Points*; StatPearls Publishing: Treasure Island, FL, USA, 2019.
25. Weinstein MP, L.J.; Bobenchik, A.M.; Campeau, S.; Cullen, S.K.; Galas, M.F.; Gold, H.; Humphries, R.M.; Kirn, T.J.; Limbago, B.; Mathers, A.J. CLSI M100-ED30:2020 Performance Standards for Antimicrobial Susceptibility Testing. Available online: http://em100.edaptivedocs.net/GetDoc.aspx?doc=CLSI%20M100%20ED30:2020&scope=user (accessed on 20 May 2020).
26. Zhang, P.; Lu, J.; Jing, Y.; Tang, S.; Zhu, D.; Bi, Y. Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. *Ann. Med.* **2017**, *49*, 106–116. [CrossRef] [PubMed]
27. Moura Neto, A.; Zantut-Wittmann, D.E.; Fernandes, T.D.; Nery, M.; Parisi, M.C. Risk factors for ulceration and amputation in diabetic foot: Study in a cohort of 496 patients. *Endocrine* **2013**, *44*, 119–124. [CrossRef] [PubMed]
28. Lauterbach, S.; Kostev, K.; Becker, R. Characteristics of diabetic patients visiting a podiatry practice in Germany. *J. Wound Care* **2010**, *19*, 140–148. [CrossRef]
29. Sämann, A.; Tajiyeva, O.; Müller, N.; Tschauner, T.; Hoyer, H.; Wolf, G.; Müller, U.A. Prevalence of the diabetic foot syndrome at the primary care level in Germany: A cross-sectional study. *Diabet Med.* **2008**, *25*, 557–563. [CrossRef]
30. Morbach, S.; Lutale, J.K.; Viswanathan, V.; Möllenberg, J.; Ochs, H.R.; Rajashekar, S.; Ramachandran, A.; Abbas, Z.G. Regional differences in risk factors and clinical presentation of diabetic foot lesions. *Diabet Med.* **2004**, *21*, 91–95. [CrossRef]
31. Jensen, J.A.; Goodson, W.H.; Hopf, H.W.; Hunt, T.K. Cigarette smoking decreases tissue oxygen. *Arch. Surg.* **1991**, *126*, 1131–1134. [CrossRef] [PubMed]
32. Obaid, H.; Eljedi, A. Risk factors for the development of diabetic foot ulcers in Gaza Strip: A case-control study. *Int. J. Diabetes Res.* **2015**, *4*, 1–6.
33. Boyko, E.J.; Ahroni, J.H.; Cohen, V.; Nelson, K.M.; Heagerty, P.J. Prediction of diabetic foot ulcer occurrence using commonly available clinical information: The Seattle Diabetic Foot Study. *Diabetes Care* **2006**, *29*, 1202–1207. [CrossRef]
34. Pham, H.; Armstrong, D.G.; Harvey, C.; Harkless, L.B.; Giurini, J.M.; Veves, A. Screening techniques to identify people at high risk for diabetic foot ulceration: A prospective multicenter trial. *Diabetes Care* **2000**, *23*, 606–611. [CrossRef]
35. Sohn, M.W.; Budiman-Mak, E.; Lee, T.A.; Oh, E.; Stuck, R.M. Significant J-shaped association between body mass index (BMI) and diabetic foot ulcers. *Diabetes Metab. Res. Rev.* **2011**, *27*, 402–409. [CrossRef]
36. Saeedi, P.; Petersohn, I.; Salpea, P.; Malanda, B.; Karuranga, S.; Unwin, N.; Colagiuri, S.; Guariguata, L.; Motala, A.A.; Ogurtsova, K.; et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes Res. Clin. Pract.* **2019**, *157*, 107843. [CrossRef]
37. Al Benwan, K.; Al Mulla, A.; Rotimi, V.O. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. *J. Infect. Public Health* **2012**, *5*, 1–8. [CrossRef]
38. Miyan, Z.; Fawwad, A.; Sabir, R.; Basit, A. Microbiological pattern of diabetic foot infections at a tertiary care center in a developing country. *Age Years* **2017**, *53*, 10–20.
39. Tascini, C.; Piaggesi, A.; Tagliaferri, E.; Iacopi, E.; Fondelli, S.; Tedeschi, A.; Rizzo, L.; Leonildi, A.; Menichetti, F. Microbiology at first visit of moderate-to-severe diabetic foot infection with antimicrobial activity and a survey of quinolone monotherapy. *Diabetes Res. Clin. Pract.* **2011**, *94*, 133–139. [CrossRef] [PubMed]
40. Viswanathan, V.; Pendsey, S.; Radhakrishnan, C.; Rege, T.D.; Ahdal, J.; Jain, R. Methicillin-resistant Staphylococcus aureus in diabetic foot infection in India: A growing menace. *Int. J. Low. Extrem. Wounds* **2019**, *18*, 236–246. [CrossRef] [PubMed]
41. Jaju, K.; Pichare, A.; Davane, M.; Nagoba, B. Profile and Antibiotic Susceptibility of Bacterial Pathogens Associated With Diabetic Foot Ulcers From a Rural Area. *Wounds* **2019**, *31*, 158–162.
42. Uçkay, I.; Aragón-Sánchez, J.; Lew, D.; Lipsky, B.A. Diabetic foot infections: What have we learned in the last 30 years? *Int. J. Infect. Dis.* **2015**, *40*, 81–91. [CrossRef]
43. Lipsky, B.A.; Berendt, A.R.; Cornia, P.B.; Pile, J.C.; Peters, E.J.; Armstrong, D.G.; Deery, H.G.; Embil, J.M.; Joseph, W.S.; Karchmer, A.W. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin. Infect. Dis.* **2012**, *54*, e132–e173. [CrossRef]
44. Zubair, M. Prevalence and interrelationships of foot ulcer, risk-factors and antibiotic resistance in foot ulcers in diabetic populations: A systematic review and meta-analysis. *World J. Diabetes* **2020**, *11*, 78. [CrossRef] [PubMed]
45. Pérez-Panero, A.J.; Ruiz-Muñoz, M.; Cuesta-Vargas, A.I.; Gónzalez-Sánchez, M. Prevention, assessment, diagnosis and management of diabetic foot based on clinical practice guidelines: A systematic review. *Medicine* **2019**, *98*, e16877. [CrossRef]

46. Kasiya, M.M.; Mang'anda, G.D.; Heyes, S.; Kachapila, R.; Kaduya, L.; Chilamba, J.; Goodson, P.; Chalulu, K.; Allain, T.J. The challenge of diabetic foot care: Review of the literature and experience at Queen Elizabeth Central Hospital in Blantyre, Malawi. *Malawi Med. J.* **2017**, *29*, 218–223. [CrossRef] [PubMed]
47. Crouzet, J.; Lavigne, J.; Richard, J.; Sotto, A.; Nîmes University Hospital Working Group on the Diabetic Foot. Diabetic foot infection: A critical review of recent randomized clinical trials on antibiotic therapy. *Int. J. Infect. Dis.* **2011**, *15*, e601–e610. [CrossRef] [PubMed]