

Correlation between superficial and intra-operative specimens in diabetic foot infections: results of a cross-sectional Tunisian study

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Abstract:

Objective: To determine the correlation between superficial, and intra-operative specimens in diabetic foot infections (DFIs).

Methods: We conducted a cross-sectional study in patients with DFIs hospitalized in a Tunisian teaching hospital. Superficial specimens were collected for all patients, and intra-operative specimens were collected in operated patients. The specimens were processed using standard microbiology techniques. Antimicrobial susceptibility testing was carried out according to the protocol established by the European Committee on Anti-microbial Susceptibility Testing. Intra-operative and superficial specimens were considered correlated if they isolated the same microorganism(s), or if they were both negative.

Results: One hundred twelve patients, 81 males and 31 females, mean age 56 years, were included. Superficial samples were positive in 77% of cases, and isolated 126 microorganisms. Among the positive samples, 71% were monomicrobial. The most frequently isolated microorganisms were *Enterobacteriaceae* (53%), followed by *streptococci* (21%) and *Staphylococcus aureus* (17%). Nine microorganisms (7%) were multi-drug resistant. Intra-operative samples were positive in 93% of cases. Superficial specimens were correlated to intra-operative specimens in 67% of cases. Initial antibiotic therapy was appropriate in 70% of cases. The lower-extremity amputation and the mortality rates were 41% and 1%, respectively.

Conclusion: In our study, DFIs were most frequently caused by *Enterobacteriaceae* and superficial specimens were correlated to intra-operative specimens in only two thirds of cases. Clinicians should emphasize on the systematic practice of intraoperative specimens in all patients with DFIs treated surgically, while well-performed superficial specimens could be useful for prescribing appropriate antibiotic therapy in other patients.

Keywords: Diabetic foot infections, superficial specimens, intra-operative specimens.

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Introduction

Diabetic foot infections (DFIs) are common and serious. They occur in 15-25% of diabetic patients in the course of their disease, and are associated with high rates of lower extremity amputation (LEA) and mortality. The annual incidence of LEA ranges from 3.6% in Germany to 6.7% in France and 12% in India, and the annual mortality ranges from 7.8% in France to 9.6% in Scotland¹⁻⁵.

The average direct cost for LEA ranges from € 19472 per patient in Germany, to \$ 33500 per patient in the USA^{6,7}. In Tunisia, the prevalence of type 2 diabetes mellitus in adults is as high as 10.9%⁸.

In addition to surgical procedures, podiatric care and arterial re-vascularization, antibiotic therapy is the cornerstone in the management of DFIs. For most patients, initial antibiotic therapy is empirical, hence the need for local bacteriological data. To the best of our knowledge, only two studies reported bacteriological profile in DFIs in Tunisia^{9,10}. Furthermore, most bacteriological studies in DFIs were based on superficial specimens, using cotton swab over the wound, often contaminated with normal skin flora or colonizers. Swab specimens should be avoided as they provide less accurate results¹¹. The aim of this study was to determine the bacteriological profile, and the correlation between superficial and intra-operative specimens in DFIs in a Tunisian tertiary hospital.

Methods

Study design and population

We conducted an observational cross-sectional study in adult patients hospitalized for DFIs in the Departments of Infectious Diseases, General Surgery and Endocrinology, at Farhat Hached hospital in Sousse - Central Tunisia, between October 2011 and December 2012.

DFIs were defined by the presence of purulent secretions or at least two of the following signs: pain, redness, warmth, swelling, and fever. They were graded according to the Infectious Diseases Society of America/International Working Group on the Diabetic Foot (IDSA/IWGDF) system^{12,13}. Socio-demographic, clinical, and bacteriological data was collected for each patient. Antibiotic therapy was considered appropriate if the microorganisms isolated from superficial and intra-operative specimens were susceptible to the prescribed antibiotics. The patients' outcome was assessed within one month after discharge.

Specimens' collection and bacteriological study

On admission, superficial specimens were collected for all patients. Swabs after debridement and cleansing with saline imbibed sterile compress were taken for open wounds, and needle aspirates after cleansing with polyvidone-iodine solution were taken for closed lesions (abscesses and other fluctuant infected tissues). In operated patients, intra-operative specimens were obtained by pus needle aspirates, infected soft tissues biopsies, or bone

biopsies, in case of osteomyelitis. All specimens were sent within one to two hours to the laboratory of Microbiology in our hospital^{14,15}.

The specimens were transported in sterile tubes without transport medium, and were processed immediately upon arrival at the laboratory. Superficial specimens were immediately plated onto blood agar and supplemented chocolate agar. Intra-operative specimens were cut, crushed, and plated systematically on the same agars as above, added to thioglycolate medium, and Brain Heart Infusion (BHI). A direct Gram stained smear of the specimen was examined.

The inoculated plates were incubated at 37°C 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. Based on Gram-staining and colony morphology, bacterial isolates were identified, and biochemical reactions were performed for confirmation Api systems (bioMérieux, Marcy l'Etoile, France). Species which were considered systematically as pathogens spp were: *Staphylococcus aureus*, beta hemolytic *Streptococcus* and Gram negative bacilli (GNB). Commensal bacteria were taken into account, if they were isolated in pure culture on repeated and good quality samples by taking into account the direct Gram stained.

All isolated strains which were considered as pathogens were subjected to susceptibility testing against antimicrobial agents by the disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocols. The following multidrug resistant (MDR) organisms were screened as recommended: extended-spectrum beta lactamases (ESBL)-producing *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae*, MDR *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus* (VRE). The following risk factors for infection with MDR bacteria were screened : hospitalization, antibiotic therapy, proton pump inhibitors use, or urinary catheter in the preceding six months, and current hemodialysis.

Correlation between superficial and intra-operative specimens

Intra-operative and superficial specimens were considered correlated if they isolated the same microorganism(s) or if they were both negative, and not correlated if they iso-

lated different microorganisms or if one specimen was positive and the other negative.

Statistical study

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) version 10.0. Qualitative variables were expressed as percentages, and quantitative variables were expressed as means \pm standard deviation. Significance of the study variables was tested by using the Chi-square test, Fisher's exact test, or Yates test for qualitative variables, and Student's t-test for quantitative variables. A p value < 0.05 was considered to be statistically significant.

Ethical considerations

As our study didn't involve changes to the patients' usual medical management, no study protocol had been submitted to our hospital Ethics Committee approval. However, patients were provided with oral information on the interest of the bacteriological documentation of the infection, and gave their verbal consent before being included in the study.

Results

Baseline characteristics

During the study period, 112 patients were hospitalized for DFI. Of these patients, 81 (72%) were male and 31

(28%) were female (sex-ratio 2.3). The mean age of the patients was 56 years, the mean duration of diabetes was 14 years, and the mean A1c hemoglobin (HbA1c) level was 11.5%. The mean duration of the infection was 10 days. The infection involved the toes in 47 cases (42%) and the forefoot in 46 cases (41%). Forty eight patients (43%) had grade 4 infection, 45 (40%) had grade 3 infection, and 19 (17%) had grade 2 infection. Twenty two patients (20%) had a history of anti-biotic treatment within the previous 7 days. A total of 17 intravenous antibiotic regimens were prescribed, on the day of admission to hospital in 106 patients (95%) and after the results of the bacteriological study in 6 patients (5%). Monotherapy with amoxicillin-clavulanic acid (55/106; 52%) and the combination amoxicillin-clavulanic acid with ciprofloxacin (25/106; 24%) were the most frequently prescribed antibiotics. The initial antibiotic therapy, assessed in the 87 patients, whose bacteriological specimens were positive, was appropriate in 61 cases (70%). Seventy four patients (66%) underwent surgery. LEA was performed in 46 patients (41%), while conservative surgery was performed in 28 patients (25%). Amputation was limited to the toes in 32 patients (28%), and transmetatarsal in one patient (1%), while leg amputation was performed in 13 patients (12%). One patient (1%) died of severe acute lung edema. The baseline characteristics are shown in Table 1.

Table 1. Baseline characteristics of the study group (N=112)

Parameters	Patients (n, %)	Mean (\pm SD) [range]
Gender		
male	81 (72)	
female	31 (28)	
Age (years)		56 \pm 13 [23-86]
Department		
Infectious Diseases	73 (65)	
General Surgery	25 (22)	
Endocrinology	14 (12)	
Diabetes mellitus		
type 2	92 (82)	
type 1	20 (18)	
duration (years)		14 \pm 9 [0-42]
Hb A1c (%)		11.5 \pm 2.2 [7.2-16]
Cardiovascular risk factors		
tobacco use	47 (42)	
hypertension	36 (32)	
dyslipidemia	24 (20)	
Foot infection site		
toes	47 (42)	
forefoot	46 (41)	
heel	19 (17)	
Foot infection grade		
grade 4	48 (43)	
grade 3	45 (40)	
grade 2	19 (17)	
Foot infection duration		10 \pm 9 [1-60]
Antibiotic regimens		
AMC*	55 (52)	
AMC + ciprofloxacin	25 (24)	
others	26 (24)	
Surgery		
lower extremity amputation	74 (66)	
toes	46 (41)	
transmetatarsal	32 (28)	
leg	1 (1)	
leg	13 (12)	
Conservative	28 (25)	
Death	1 (1)	

*: amoxicillin - clavulanic acid

Bacteriological study

Superficial specimens

Initial superficial specimens, taken in 112 patients, mostly by swabs (110 cases, 98%), yielded a positive culture in 86 cases (77%). Antibiotic therapy prior to hospitalization was noted in 17% (15/86) of patients with positive culture, and in 27% (7/26) of patients with negative culture ($p=0.18$). In 11 patients with negative initial specimen, a second specimen was performed. Culture was positive in 7 cases. Overall, 93 superficial specimens (/112 patients, 83%) were positive and isolated 126 micro-organisms (Figure 1). The mean number of isolates per specimen

was 1.35 (range, 1-3). One micro-organism was isolated in 66 cases (71%), 2 micro-organisms were isolated in 21 cases (23%), and 3 micro-organisms were isolated in 6 cases (6%). The most frequently isolated micro-organisms were GNB (60%), mainly *Enterobacteriaceae* (53%), followed by Gram positive bacteria (40%), mainly *Streptococci* (21%) and *Staphylococcus aureus* (17%). The predominance of GNB was observed, regardless the duration of infection: ≤ 7 days (57%) compared to > 7 days (64%) ($p=0.15$), and ≤ 15 days (68%) compared to > 15 days (50%) ($p=0.96$). A predominance of GNB (69%) compared to Gram positive bacteria (31%) ($p=0.004$) was

noted in grade 2 and grade 3 infections, while no significant difference (46% and 54% respectively, $p=0.056$) was noted in grade 4 infections. The antibiotic susceptibility rates of isolated micro-organisms were as follows: piperacillin-tazobactam (99%), imipenem (98%), cefotaxime (93%), ertapenem (93%), amoxicilline-clavulanic acid (87%), cotrimoxazole (86%), ciprofloxacin (58%). Nine micro-organisms (7%), including 8 GNB (/75, 11%) and

one methicillin-resistant *Staphylococcus aureus* (MRSA) (/22, 4%), were multidrug resistant (MDR). The risk factors of infection with a MDR bacteria were hospitalization in the preceding six months (7 patients), antibiotic therapy in the preceding six months (5 patients), proton pump inhibitors use in the preceding six months (3 patients), and urinary catheter in the preceding six months (2 patients). The bacteriological data of the superficial specimens are summarized in Table 2 and Table 3.

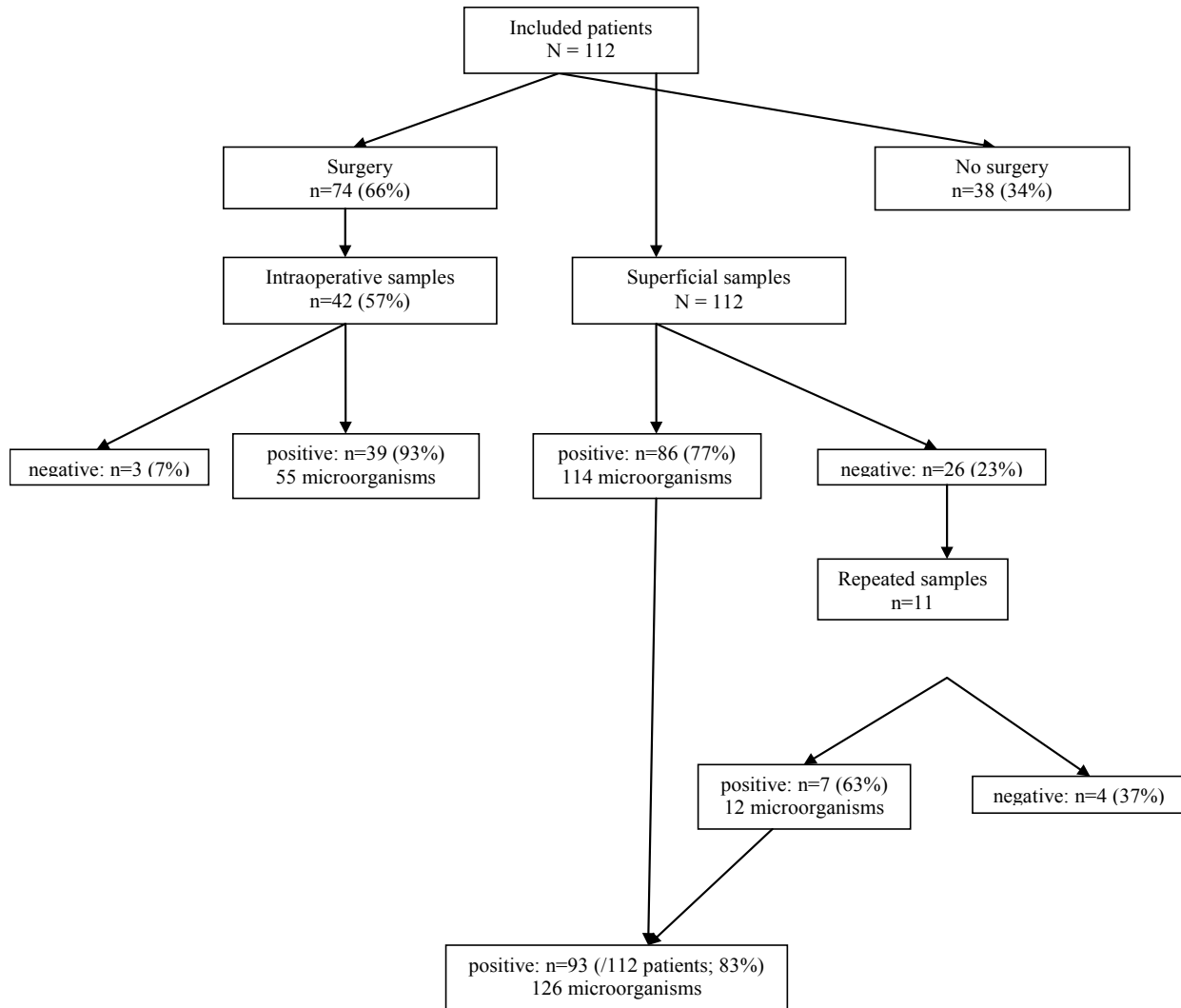


Figure 1. Bacteriological study of superficial and intraoperative specimens

Table 2. Distribution of microorganisms obtained from superficial and intra-operative specimens

Variable	superficial specimens (n,%)	Intra-operative specimens (n,%)	p
No. of samples	93	42	
No. of isolates	126	55	
Mean no. of isolates per sample	1.35	1.41	0.32
Monomicrobial	66 (71)	25 (64)	0.55
Multidrug resistant	9 (7)	5 (9)	0.37
Gram negative aerobic bacilli	75 (60)	37 (67)	0.97
<i>Enterobacteriaceae</i>	67 (53)	32 (58)	0.38
<i>Proteus spp</i>	22 (17)	9 (16)	
<i>Klebsiella spp</i>	11 (10)	7 (13)	
<i>Morganella morganii</i>	9 (7)	5 (9)	
<i>Citrobacter spp</i>	8 (6)	5 (9)	
<i>Enterobacter cloacae</i>	8 (6)	4 (7)	
others	9 (7)	2 (4)	0.5
<i>Pseudomonas aeruginosa</i>	8 (6)	5 (9)	
Gram positive cocci	48 (38)	16 (29)	0.31
<i>Streptococci</i>	26 (21)	9 (16)	0.5
group B	15 (12)	6 (11)	
group A	8 (7)	1 (2)	
others	3 (2)	2 (3)	
<i>Staphylococcus aureus</i>	22 (17)	7 (13)	0.34
Gram positive aerobic bacilli	3 (2)	2 (3)	
<i>Corynebacterium spp</i>	3 (2)	2 (3)	

Table 3. Susceptibility of isolated microorganisms to antibiotics

Antibiotic	superficial samples (N=126) susceptible (n,%)	intraoperative samples (N=55) susceptible (n,%)	p
amoxicillin-clavulanic acid	110 (87)	47 (85)	0.72
cefotaxime	117 (93)	50 (91)	0.61
piperacillin-tazobactam	125 (99)	55 (100)	NA
ertapenem	118 (93)	51 (93)	0.55
imipenem	123 (98)	53 (96)	0.50
amikacin	116 (92)	51 (93)	0.82
gentamicin	112 (89)	49 (89)	0.67
ciprofloxacin	67 (58)	39 (70)	0.07
cotrimoxazole	109 (86)	50 (91)	0.34
fosfomycin	116 (92)	54 (98)	0.21
vancomycin	48 (38)	16 (29)	0.09
pristinamycin	47 (37)	19 (34)	0.56
fusidic acid	19 (15)	6 (10)	0.52
rifampicin	44 (39)	15 (27)	0.21

NA : not applicable

Intra-operative specimens

Intraoperative specimens, taken in 42 patients (among 74 operated, ie 57%), were positive in 39 cases (93%) and isolated 55 micro-organisms. The mean number of isolates per sample was 1.41 (range,1-3). One micro-organism was isolated in 25 cases (64%), 2 micro-organisms were isolated in 12 cases (31%), and 3 micro-organisms were isolated in 2 cases (5%). The most frequently isolated microorganisms were GNB (67%), mainly *Enterobacteriaceae* (58%), followed by Gram positive bacteria (29%), mainly *Streptococci* (16%) and *Staphylococcus aureus* (13%). A predominance of GNB (78%), compared to Gram positive bacteria (22%) ($p=0.002$) was noted in grade 2 and grade 3 infections, while no significant difference (48% and 52% respectively, $p=0.09$) was noted in grade 4 infections. The antibiotic susceptibility rates of isolated micro-

organisms were as follows: piperacillin-tazobactam (100%), imipenem (96%), cefotaxime (91%), ertapenem (93%), amoxicilline-clavulanic acid (85%), cotrimoxazole (91%), ciprofloxacin (70%). Five microorganisms (9%), including 3 GNB (/37; 8%) and 2 MRSA (/7; 29%), were MDR. The bacteriological data of the intraoperative specimens are summarized in Table 2 and Table 3.

Correlation between superficial and intraoperative specimens

A correlation between intra-operative and superficial specimens was noted in 28 cases (67%). This rate was higher for GNB (78%), than for Gram positive cocci (50%) ($p=0.27$) (Table 4). There was no significant difference in the bacteriological profile, and in the antibiotic susceptibility rates between superficial, and intra-operative specimens (Table 2 and Table 3).

Table 4. Correlation rates between superficial and intraoperative specimens

Microorganisms isolated from intraoperative specimens	number of specimens (%)	correlation rate (%)
Gram negative aerobic bacilli	23	78
<i>Proteus spp</i>	5	
<i>Klebsiella spp</i>	4	
<i>Morganella morganii</i>	4	
<i>Citrobacter koseri</i>	3	
others	7	
Gram positive cocci	12	50
Streptococci	4	
<i>Staphylococcus aureus</i>	3	
others	5	
Others	7	57
mixed flora	3	
<i>Corynebacterium spp</i>	1	
negative	3	
Total	42 (100)	67

Discussion

In our study, both superficial and intra-operative specimens were frequently positive (77% and 93%, respectively), even in patients who received antibiotics within the previous days. Most bacteriological samples were monomicrobial (71%), and the most frequently isolated micro-organisms were GNB (60%), mainly *Enterobacteriaceae* (53%), followed by Gram positive cocci (38%) mainly streptococci (21%), while *Staphylococcus aureus* was less frequently isolated (17%). The predominance of GNB was noted, regardless of the duration of infection. In our study, no anaerobe was isolated. Indeed, the majority of cultures were performed from superficial specimens, and both transportation media, and culture conditions for anaerobes are not available in the laboratory of Microbiology in our hospital.

In published series, the pre-dominance of mono-microbial or poly-microbial specimens and the distribution of isolated micro-organisms varied considerably. Gram positive cocci were most frequently (52.6 to 68%) isolated in some studies, while GNB were most frequently (56 to 80%) in others [10, 16-22]. In a Turkish study, Gram positive cocci (48.7%) and GNB (48.4%) were isolated at an equal frequency²³. Among the Gram positive cocci, the most commonly isolated micro-organism was *Staphylococcus aureus*, followed by *Streptococci* and *enterococci*, while among the GNB, the most commonly isolated micro-organisms were the *Enterobacteriaceae* (mainly *Proteus spp*, *Klebsiella spp*, and *Escherichia coli*) and *Pseudomonas aeruginosa*¹⁶⁻²³. Despite anaerobes were frequently (13.6 to 25%) isolated from DFIs in some studies, these microorganisms were not or rarely (0.3 to 2%) isolated in most other studies^{10,16,17,19-22, 24-26}. This may be attributed to the difficulty in ensuring adequate sampling, transportation, and culture conditions for these fastidious pathogens. The bacteriological data of different studies are shown in Table 5.

Table 5. Distribution of the microorganisms isolated from diabetic foot infections in different studies

Variable	Our study	Ben Moussa et al. Tunisia [10]	Richard et al. France [16]	Tascini et al. Italy [17]	Hatipoglu et al. Turkey [23]	Sekhar et al. India [22]	Perim et al. Brazil [19]
Study period	2011-12	2011-13	2007-09	2006-08	1989-2011	2011	2013
Number of patients	112	100	291	1295	2097	108	41
Number of samples	93	124	251	1295	-	108	41
Number of isolates	126	136	351	1159	1974	150	89
Mean No. of isolates per sample	1.35	1.1	1.4	0.9	-	1.39	2.17
Monomicrobial (%)	71	55	68	60.5	-	44	30
Gram negative aerobic bacilli(%)	60	80	35.9	40.6	48.4	56	31
<i>Enterobacteriaceae</i>	53	71	27.1	23.5	28.5	28	26.5
<i>Proteus spp</i>	17	27	8.5	-	5.3	8	11
<i>Klebsiella spp</i>	10	17	1.1	-	6.5	12	0
<i>Morganella spp</i>	7	0	4.3	-	-	0	0
<i>Citrobacter spp</i>	6	3	1.1	-	-	0	1.0
<i>Enterobacter spp</i>	6	6	4.8	-	4.0	0	10
<i>Serratia spp</i>	-	3	1.4	-	-	0	0
<i>Escherichia coli</i>	-	7	4.8	-	12.5	8	4.5
Others	7	6	0.9	-	-	0	0
<i>Pseudomonas aeruginosa</i>	6	8	6.6	10.3	13.7	24	4.5
<i>Acinetobacter spp</i>	0	1	0.9	1	1.9	4	0
Other Gram negative aerobic bacilli	0	0	1.4	2	4.6	0	0
Gram positive cocci (%)	38	19	59.8	52.6	48.7	44	68
<i>Staphylococcus aureus</i>	17	9	32.5	29.9	23.8	32	30
Coagulase-negative <i>Staphylococci</i>	0	0	4.6	4.6	8.9	0	29
<i>Streptococci</i>	21	10	13.7	4.6	6.5	4	9
group B	12	5	-	2.8	-	-	7
groupe A	7	0	-	-	-	-	0
others	2	5	-	1.8	-	-	2
<i>Enterococci</i>	0	0	8.0	9.7	8.6	8	0
Others	0	0	-	-	1.0	-	0
Gram positive aerobic bacilli (%)	2	0	2.3	3.5	-	0	0
<i>Corynebacterium spp</i>	2	0	2.3	3.5	-	0	0
Anaerobes (%)	0	0	2	0.3	2.3	0	0
Fungus (%)	0	0	0	6.4*	0.5	0	0

- : not available ; * : all of *Candida* genus

The differences in DFIs bacteriological profile noted between the different studies may be explained by many factors such as the duration and the severity of the infection, prior antibiotic therapy, hospital or out-patient care, bacteriological sampling (superficial swabbing, needle puncture, bone percutaneous biopsy, intra-operative pus swabbing or tissue biopsy), and bacteriological study (transportation medium and culture conditions).

In our study, a correlation between superficial and intra-operative specimens was noted in only 67% of cases, with no significant difference between GNB, Gram positive cocci or mixed isolates. In two other studies, the overall correlation rate was as low as 50%, between superficial swabs and deep tissue percutaneous biopsy, and 62% between superficial swabs and deep tissue surgical biopsy^{27,28}. Intra-operative specimens are more reliable and must be performed whenever possible, even in patients undergoing anti-biotic therapy. However, in our study, intra-operative specimens were performed in only 57% of

cases. Thus, in daily practice, superficial specimens could provide useful information to guide antibiotic therapy in patients with DFIs.

In the present study, 17 antibiotic regimens were prescribed as first-line therapy, 66% of them were appropriate. The most frequently prescribed regimens were amoxicillin-clavulanic acid (52%) and the association of amoxicillin-clavulanic acid with ciprofloxacin (24%). In a Tunisian study, initial antibiotic therapy was appropriate in only 56% of cases, and in a French study, 62 combinations of antibiotics were prescribed as first-line therapy, of whom 56% were changed, mainly due to a mismatch in susceptibility results^{10,16}. These high rates of inappropriate initial antibiotic therapy may be explained by the lack of local bacteriological data, to guide initial antibiotic therapy in DFIs. In our study, the initial antibiotic therapy was empirical in almost all cases (95%), because the DFIs were frequently serious (grade 3 or grade 4 in 83% of cases) on admission. This may be explained by the long dura-

tion of diabetes, the poor glycemic control, the frequent association of cardiovascular risk factors, and the delay in consultation.

In this study, the overall antibiotic susceptibility rates of the isolated microorganisms were high (87% for amoxicillin-clavulanic acid, 86% for cotrimoxazole, 99% for piperacillin-tazobactam, and 92% for amikacin) except for fluoroquinolones (58%), and the rates of infection with MDR microorganisms were low both for GNB (11%) and *Staphylococcus aureus* (4.5%). In other studies, the rates of infection with MDR microorganisms ranged from 9.5% in Tunisia to 12% in France and 18.9% in Portugal.^{10,16,20}

According to these data, we could recommend as first-line antibiotic therapy for DFIs in our setting either amoxicillin-clavulanic acid or cotrimoxazole in patients without severe sepsis, and the association of piperacillin-tazobactam with amikacin in patients with severe sepsis or septic shock. Fluoroquinolones could be prescribed only after the isolation of a susceptible microorganism from bacteriological sample, especially in patients with osteomyelitis. However, since this study was conducted 5 years ago, the bacteriological resistance may have evolved. Thus, further studies are needed to establish more recent bacteriological data in DFIs in our setting.

This study has potential limitations since some data such as clinical outcome, interval between patient admission and surgical treatment, antibiotic treatment duration and revascularization procedures were not available.

Conclusion

In the present study, DFIs were most commonly caused by GNB, mainly *Enterobacteriaceae*, regardless the duration of the infection. The isolated microorganisms were frequently susceptible to first-line prescribed antibiotics, except for fluoroquinolones. Superficial and especially intra-operative samples yielded positive cultures in the majority of cases, even in patients who received antibiotics within the previous few days, and the correlation rate between the two sampling techniques was low. In conclusion, clinicians should emphasize on the systematic practice of intra-operative specimens in all patients with DFIs treated surgically, while well-performed superficial specimens could be useful for prescribing appropriate antibiotic therapy in other patients.

Conflicts of interest

None.

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