Diabetic foot infection and osteomyelitis. Are deep-tissue cultures necessary?

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Abstract

Introduction **Diabetic foot infections (DFIs) are common and difficult to treat. The objective of this study was to compare swab and tissue cultures as indicators of appropriate treatment of DFIs.**

Methods **This is a prospective study conducted during a 4-year period. All patients with DFIs and/or diabetic foot osteomyelitis (DFO) admitted to the University Hospital of Heraklion, Greece, were included. Clinical data were collected, while cultures taken with swabs and/or tissue biopsies were used as indicators of the microbiological cause and the appropriate treatment.**

Results **In total, 83 individuals (62.7% males) with mean age of 72 years, were enrolled. Coexisting osteomyelitis was present in 18.1%. From tissue and pus cultures, 131 and 176 pathogens, respectively, were isolated. Gram-positive aerobes were the most common microorganisms, followed by Gram-negatives. Infection was polymicrobial in 40 (70.2%) out of 57 patients with tissue culture and in 54 (75.0%) out of 72 with pus culture. Microbiological results from tissue cultures were compatible with those from pus at a rate of 80%, while in cases of osteomyelitis concordance reached 100%. Multidrug-resistant organisms (MDROs) were isolated from 32 (24.4%) tissue and 44 (25%) pus cultures (p=0.910). Initial empirical antimicrobial treatment was considered inappropriate in 44.6% of cases.**

Conclusions **A high concordance between easily taken swab cultures and those taken by biopsy was noted, especially in DFO. This was helpful for early change to appropriate treatment in cases where MDROs were isolated and empirical treatment was inappropriate. Further research is needed to confirm this observation in clinical practice.**

Keywords **Diabetic foot osteomyelitis, multidrug resistant, ulcer infections, superficial and deep tissue cultures.**

Introduction

Diabetic foot infection (DFI) represents a major cause of morbidity in patients with diabetes mellitus (DM).[1] DFIs, an important subset of complicated skin infections, are common and often difficult to treat, being the leading cause of non-traumatic lower limb amputations. They are associated with prolonged in-hospital stay, economic healthcare burden, as well as psychological morbidity.[2]

DFI is the invasion of a foot wound by pathogenic microbes, leading to local tissue

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damage, favored by peripheral neuropathy, peripheral arterial disease and hyperglycemia-associated deranged host defenses. These infections begin as a minor problem and later on progress involving deep tissues, joints, or bones, especially if they remain un-or-mis treated.[3] DFIs typically begin in a wound, most often a neuropathic ulceration. Like all wounds, they are colonized with microorganisms, however, when the infection occurs, it is defined by signs of inflammation and purulence.[3]

DFIs are usually polymicrobial. The most common pathogens are *Staphylococcus aureus*, *Proteus* spp., and *Escherichia coli*, while wounds involve deep tissues, or in cases of ischemic necrosis, obligate anaerobes also play their role.[3] The selection of the initial empirical antimicrobial regimen is challenging, based mainly on the infection's severity, likely pathogens and the local antimicrobial resistance patterns. Inappropriate initial treatment may allow the infection to progress, requiring hospitalization, surgical resections and/or amputation, or may lead to patient's death.[4]

For identification of the causative pathogens, a deep tissue specimen culture, obtained by biopsy, after the wound has been cleansed and debrided, is recommended.[4] However, easily obtained swab cultures are also still used, although data on their reliability in identifying pathogens and not colonizers, are inconclusive.[4]

The aim of this study was to present the clinical and microbiological characteristics of DFIs of hospitalized patients in a region with high rate of antimicrobial resistance, and to evaluate and compare the effectiveness of swab and tissue cultures for the successful identification of organisms causing DFI and diabetic foot osteomyelitis (DFO).

Methods

Study population

This is a prospective study that was conducted during a 4-year period (2011–2014). All patients with DFIs or diabetic foot osteomyelitis (DFO) that were admitted to the Department of Vascular Diseases of the University Hospital of Heraklion, Crete, Greece, during the study period, were enrolled in the study. The patients' clinical characteristics, causative microorganisms, their empirical and/or targeted treatment, and the infections' outcomes were recorded and evaluated. Clinical data collection included patients' demographics, predisposing factors and Charlson Comorbidity index.[5] Swabs and/or biopsy material cultures were used for microbiological evaluation. Diabetic foot ulcers were graded as uninfected or mildly, moderately and severely infected using the Infectious Diseases Society of America (IDSA) classification system.[6] DFOs were diagnosed based on clinical and radiological criteria [positive probe-bone test or MRI (magnetic resonance imaging)], while the positive bone culture confirmed the diagnosis of DFO. A patient was considered to have a prior hospitalization if he/she had been hospitalized during the three previous months. Empirical antimicrobial treatment was considered appropriate if the isolated causative organisms were sensitive to the antimicrobials given. Empirical treatment given for DFIs and DFOs followed a local protocol that included clindamycin with either beta-lactams (aminopenicillins or cephalosporins) or quinolones, while the results of cultures were pending. Eradication of the infection was defined as complete disappearance of signs and symptoms of the infection. Persistence was defined as the continuation of signs and symptoms, confirmed by positive cultures of the lesion.

The present study has been approved by the University Hospital of Heraklion ethical committee.

Microbiological methods

Before initiation of the empirical antimicrobial treatment, two samples for culture were obtained from the majority of patients. The first was a superficial and the other a deep tissue sample; microbiological cultures and antimicrobial susceptibilities were performed on both of them. Correlation between the microbiome phenotype of the two samples was also performed. Pus and exudates were collected by using a sterile cotton swab, which was immediately placed in Amies transport medium (bioMérieux SA, Marcy L'Étoile, France). Tissue

biopsy specimens were placed in sterile normal saline solution. The samples were promptly transported to the Microbiology Laboratory for further processing.

For specimen processing and culture, routine laboratory methods were used. Standard biochemical methods were used for the identification of bacterial species: API system (bioMérieux), and Vitek2 automated system (bioMérieux). The Vitek2 automated system was also used for testing the antimicrobial sensitivity. The Clinical and Laboratory Standards Institute (CLSI) breakpoints (M100-S26) were applied to interpret susceptibility results of all antimicrobial agents, except for tigecycline and colistin.[7] The MICs of tigecycline and colistin were interpreted following the U.S. Food and Drug Administration (FDA) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, respectively.[8,9] As quality control strains, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. aureus* ATCC 43300 were used.

For identification of extended-spectrum-beta-lactamases (ESBLs), AmpCs and carbapenemases in Enterobacteriaceae, additional phenotypic tests were applied. ESBL production was tested with the modified CLSI ESBL confirmatory test.[10] Phenotypic detection of AmpC production was carried out by using the Etest AmpC and the cefoxitin-cloxacillin double-disk synergy method.[11] For detecting and differentiating the production of MBL, KPC or both MBL and KPC carbapenemases, a phenotypic method was applied using disks of MER (10 μg) alone and with phenylboronic acid (PBA), ethylenediaminetetraacetic acid (EDTA), or both PBA and EDTA.[12] *S. aureus* isolates were phenotypically classified as methicillin-susceptible *S. aureus* (MSSA) or methicillin-resistant (MRSA), based on the cefoxitin disk diffusion test and the latex agglutination test for PBP2a (bioMérieux). *Enterococcus* isolates were defined as vancomycin-resistant enterococci (VRE), based on vancomycin resistance determined by the disk diffusion method, as recommended by CLSI[7] and the E-test method (BioMérieux). Consequently, established criteria described by Margiorakos et al. were used to classify antimicrobial agents.[13]

Statistics

Categorical data were analyzed with Fisher's exact test or Pearson's chi-square test, as appropriate. Continuous variables were compared using Student's t-test for normally distributed variables and the Mann–Whitney U-test for non-normally distributed variables. All tests were two-tailed and p-values <0.05 were considered to be significant. Data are presented as number (%) for categorical variables and median (interquartile range, IQR) or mean (±standard deviation, SD) for continuous variables. All the above-mentioned statistics were calculated with GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA). A multivariate logistic regression analysis model was developed to evaluate the effect of gender, age, duration of DM, prior hospitalization, prior antimicrobial treatment and fever with infection by MDROs with a $p<0.05$. Furthermore, a multivariate logistic regression analysis model was developed to evaluate the effect of gender, age, duration of DM, prior hospitalization, prior antimicrobial treatment, fever, infection by MDR pathogens, effective empirical antimicrobial treatment and duration of antimicrobial treatment with eradication of infection with a $p<0.05$. Finally, a multivariate logistic regression analysis model was developed to evaluate the above-mentioned parameters with the need for amputation of lower extremity with a $p<0.05$. Multivariate analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

Results

In total, 83 individuals (52 males, 62.7% and 31 females, 37.3%) with a mean age of 72±10.6 years and a mean duration of DM 18.4±11.2 years were evaluated. The most common comorbidities were: peripheral arterial disease in 82 patients (98.8%), diabetic neuropathy in 80 (96.4%), cardiovascular disease in 53 (63.9%) and chronic kidney disease in 21 (26.5%), while the study population's median Charlson comorbidity index was 6 (IQR): 5-7 (Table 1). The DFIs were classified as mild in 7 (8.4%),

moderate in 75 (90.4%) and severe in 1 patient (4.8%). Among all patients, 15 (18.1%) had coexisting osteomyelitis.

Table 1. Medical history of patients with DFI and DFO

	DFI & DFO patients (n=83)
Peripheral artery disease, n (%)	82 (98.8)
Diabetic neuropathy, n (%)	80 (96.4)
Cardiovascular disease, n (%)	53 (63.9)
Chronic kidney disease, n (%)	21 (26.5)

DFI – diabetic foot infection; **DFO** – diabetic foot osteomyelitis.

Table 2 highlights the differences between patients with DFI and DFO. Antimicrobials had been given during the last 3 months to 57.8% of patients (DFI=54.4%, compared to 73.3% of DFO; p=0.251), while a total of 37.3% of patients had been hospitalized during the same period (DFI=39.7%, compared to 26.7% of DFO; p=0.394). The median duration of hospitalization was 17 days (IQR: 9.5-21 days).

Gram-positive aerobes were the most frequently isolated organisms identified in 71 out of 139 (51.1%) tissue cultures, and 89 out of 182 (48.9%) pus cultures. Gram-negatives were isolated from 60 (43.2%) tissue cultures and 87 (47.8%) pus cultures and anaerobes were isolated in 8 (5.7%) tissue cultures and 6 (3.3%) pus cultures. Infection was polymicrobial in 40 (70.2%) out of 57 patients with tissue culture and in 54 (75.0%) out of 72 with pus culture. A total of 307 organisms [superficial swabs=176 (57.3%) and deep tissue=131 (42.7%)] have been isolated. The most frequently isolated pathogens were *Enterococcus faecalis* (50, 16.3%), *Staphylococcus aureus* (34, 11%), *Proteus mirabilis* (27, 8.8%), *Staphylococcus epidermidis* (20, 6.5%) and *Pseudomonas aeruginosa* (18, 5.86%) (Table 3). The concordance rate of pathogens isolated from deep tissue with those isolated from superficial swab cultures was 80% in all cases, while in patients with DFO it was 100%.

Multidrug-resistant organisms (MDROs) were isolated in 32 patients (24.4%) from tissue and in 44 (25%) from pus (p=0.910). Among Gram-negative bacteria, 37 (12.1%) were MDR, 4 extensively drug-resistant (XDR) (1.3%) and 1 pan-drug-resistant (PDR) (1.3%). Among Gram-positive organisms, methicillin-resistant *Staphylococcus* spp. represented 43.4% of isolates while only one vancomycin resistant *Enterococcus* (VRE) spp. has been isolated. The resistance to antimicrobials observed is presented in detail in Table 3. The mean number of isolates per patient was 2.4 by superficial swab and 2.3 by deep tissue sampling, while in 36 cases (43.4%), cultures yielded at least one MDRO. The duration of hospitalization of patients suffering from at least one MDRO was 17.3±9.4 days, as compared to 16.9±9.8 days for patients suffering from infection with sensitive pathogens (p=0.900). A multivariate logistic regression analysis model identified young age and previous antimicrobial treatment as independent factors associated with infection by MDROs. The results of the regression analysis are shown in Table 4.

Empirical treatment was appropriate in 55.4% of all cases. Among all patients, 54 (65.1%) underwent lower extremity amputation. Among those with amputation, only 9 (16.7%) had a major amputation. A multivariate logistic regression analysis model identified only age to be negatively associated with the need for amputation [p=0.044, OR=0.93 per year, (95% CI 0.87-1)]. In the majority of patients (77; 92.8%) an ankle-brachial index <0.9 was found. Regarding outcome, eradication of infection was observed in 72 patients (86.7%), persistence in 10 (12%), superinfection in 1 (1.2%) and death due to septic shock in 3 (3.6%). A multivariate logistic regression analysis model identified the duration of diabetes and effective empirical antimicrobial treatment as independent factors associated with eradication of infection. The results of the logistic regression analysis are shown in Table 5.

Discussion

The results of the present study showed a concordance rate of 80% in isolated pathogens between superficial swabbing and deep tissue

Table 2. Characteristics of the 83 patients and results of statistical differences between patients with DFIs and DFOs

	DFI (n=68)	DFO (n=15)	Total (DFI+DFO) (n=83)	p
Age, mean (SD)	73.1 (10.6)	67.7 (9.9)	72.1 (10.6)	0.074
Charlson comorbidity index, median (IQR)	6 (5-7)	5 (4-6)	6 (5-7)	0.162
Male, n (%)	42 (61.8)	10 (66.7)	52 (62.7)	0.777
Prior hospitalization, n (%)	27 (39.7)	4 (26.7)	31 (37.3)	0.394
Prior antimicrobial use, n (%)	37 (54.4)	11 (73.3)	48 (57.8)	0.251
Oral hypoglycemic drugs, n (%)	18 (26.5)	6 (40)	24 (28.9)	0.350
Insulin, n (%)	47 (69.1)	9 (60)	56 (67.5)	0.549
Appropriate empiric antimicrobial treatment, n (%)	40 (58.8)	6 (40)	46 (55.4)	0.253
Duration of hospitalization, median (IQR)	15.5 (8.8-21)	19 (10-24)	17 (9.5-21)	0.284

DFI – diabetic foot infection; **DFO** – diabetic foot osteomyelitis; **IQR** – interquartile range; **SD** – standard deviation.

Statistical tests performed for comparison of characteristics among patients with DFI and DFO included Student's t-test for comparison of age, Mann-Whitney for comparison of Charlson's Comorbidity Index and duration of hospitalization, and Fischer's exact test for comparison of proportions of male, prior hospitalization, prior antimicrobial use, oral hypoglycemic drugs, insulin use, and appropriateness of antimicrobial treatment.

cultures in DFIs, while in DFOs, swabs identified all microorganisms that were isolated from bone biopsy. Furthermore, DFIs were often caused by MDROs. *Enterococcus faecalis* was the most commonly isolated pathogen, while infections were frequently polymicrobial. Finally, duration of diabetes and appropriate empirical antimicrobial treatment were found to be independently associated with eradication of infection.

The reliability of the easily obtained swab culture method in DFIs remains uncertain. Only few studies have compared superficial swab and deep tissue culture.[14-16] The present results are in agreement with those of Slater et al.[14] and Bozukurt et al.[15] that reported a 90% and 89% concordance rate between swab and tissue culture respectively. Similar results have been reported also by Pellizzer et al.[16] who have found that the mean number of isolates per patient was 2.34 by swabbing and 2.07 by tissue culture, with no differences between the two procedures in terms of isolated microorganisms and their frequencies. However, our results differ from those of other older studies[17] as well as from the IDSA guidelines.[6] IDSA in particular suggests only deep tissue biopsies for microbiological diagnosis in such cases.

DFOs are almost always caused by the contiguous spread of an infection from a chronic ulcer. They occur in up to 15% of cases with a diabetic foot ulcer, while about 20% of all DFIs have osseous involvement at presentation.[18,19] In the present study 18% of patients with DFI suffered also from osteomyelitis, as proved clinically by positive probe-test or radiologically by a positive MRI confirming bone infection. In these patients, the concordance rate between swabbing and bone biopsy culture was 100%. The results of this study are not in agreement with that of Slater et al.[14] who showed that bone and swab cultures had a concordance of only

Table 3. Microorganisms isolated from deep tissue and superficial swab of 83 patients with DFI

Pathogen	Total number of isolates n=307	Culture deep tissue n=131 (%) / swab n=176 (%)	Resistance deep tissue n=131 (%)	Resistance swab n=176 (%)
Gram-positive				
Enterococcus spp.	59	30 (51) / 29 (49)		
E. faecalis	50	26 (52) / 24 (48)	0	1 (4.2) MDR
E. avium	6	2 (33.3) / 4 (66.7)	0	0
E. faecium	3	2 (66.7) / 1 (33.3)	1 (50) VRE	0
Staphylococcus spp.	76	32 (42.1) / 44 (57.9)		
S. aureus	34	12 (35.3) /22 (64.7)	2 (16.7) MRSA	7 (31.8) MRSA
S. epidermidis	20	11 (55) / 9 (45)	8 (72.7) MRSE	8 (88.9) MRSE
[a]Other CNS	22	9 (40.1) /13 (59.8)	3 (33.3) MRS	5 (38.5) MRS
Streptococcus spp.	12	4 (33.3) / 8 (66.7)		
S. agalactiae	5	1 (20) / 4 (80)	0	0
S. constellatus	4	2 (50) / 2 (50)	0	0
[b]Other streptococci	3	1 (33.3) / 2 (66.7)	0	0
Gram-negative				
Proteus spp.	34	15 (44.1) / 19 (55.9)		
P. mirabilis	27	12 (44.4) / 15 (55.6)	3 (25) MDR 1 (8.3) XDR	2 (13.3) MDR 1 (6.7) XDR
P. vulgaris	5	2 (40) / 3 (60)	0	0
P. penneri	2	1 (50) / 1 (50)	0	0
Pseudomonas spp.	20	10 (50) /10 (50)		
P. aeruginosa	18	9 (50) / 9 (50)	1 (1) MDR 1 (%) XDR	1 (1) MDR
P. putida	2	1 (50) / 1 (50)	0	0
Klebsiella spp.	21	8 (38) / 13 (62)		
K. pneumoniae	14	5 (36%) / 9 (64)	3 (60) MDR	6 (66.7) MDR
K. oxytoca	7	3 (42.8) / 4 (57.2)	0	0
Escherichia coli	17	7 (41) / 10 (59)	1 (14.3) MDR	1 (10) MDR
Morganella spp.	13	6 (46) / 7 (54)	1 (16.7) MDR 1 (16.7) XDR	1 (14.3) MDR
Enterobacter spp.	11	4 (36.4) / 7 (63.6)	2 (50) MDR	2 (28.6)
Citrobacter spp.	5	2 (40) / 3 (60)	0	0
Serratia spp.	6	2 (33.3) / 4 (66.6)	0	2 (50) MDR
Acinetobacter spp.	5	3 (60) / 2 (40)	2 (66.7) MDR 1 (33.3) XDR	2 (100) MDR
Providencia spp.	5	1 (20) / 4 (80)	1	1
[c]Other Gram-negative	7	1 (14.3) / 6 (85.7)	1 (100) MDR	1 (16.7) MDR
[d]Anaerobes	16	5 (31.3) / 11 (68.7)	1 (20) MDR	1 (9.1) MDR

CNS – coagulase negative staphylococci; **MRSA** – methicillin resistant *Staphylococcus aureus*; **MRSE** – methicillin resistant *Staphylococcus epidermidis*; **VRE** – vancomycin resistant enterococci.

[a]Other CNS: *Staphylococcus saprophyticus, Staphylococcus lugdunensis, Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus hominis, Staphylococcus simulans, Staphylococcus cohnii, Staphylococcus sciuri, Staphylococcus* coagulase-negative. [b]Other streptococci: *Streptococcus bovis, Aerococcus viridans, Streptococcus dysgalactiae*. [c]Other Gram negative: *Alcaligenes faecalis, Finegoldia magna, Prevotella oralis, Stenotrophomonas* spp. [d]Anaerobes: *Corynebacterium minutissimum, Corynebacterium* group I, *Clostridium sordellii, Clostridium perfringens, Bacteroides uniformis, Bacteroides ureolyticus, Bacteroides fragilis, Peptostreptococcus* spp.

67%. However, our results suggest that in patients with DFOs, targeted antimicrobial treatment can be guided by using superficial cultures alone, without the need for the more invasive and laborious bone biopsy. This could be cost and time saving in cases of patients with

Table 4. Logistic regression analysis of infection by MDROs

	Univariate analysis p	Multivariate analysis p	OR (95% CI)
Age (years)	0.050	0.046	0.92 (0.85-1)
Gender (female)	0.404	0.864	1.14 (0.26-4.89)
Duration of diabetes (years)	0.643	0.451	0.97 (0.91-1.04)
Prior hospitalization	0.001	0.697	1.34 (0.3-5.92)
Prior antimicrobial treatment	<0.001	0.002	28.62 (3.43-238.65)
Fever	0.647	0.266	2.3 (0.53-10.01)

MDROs – multidrug resistant microorganisms.

Table 5. Logistic regression analysis of eradication of infection

	Univariate analysis p	Multivariate analysis p	OR (95% CI)
Age (years)	0.278	0.183	0.9 (0.77-1.05)
Gender (female)	0.294	0.517	0.46 (0.04-4.86)
Duration of diabetes (years)	0.044	0.050	1.25 (1-1.58)
Prior hospitalization	0.679	0.863	1.5 (0.02-152)
Prior antimicrobial treatment	0.458	0.936	0.82 (0.007-99.1)
Fever	0.176	0.307	0.18 (0.007-4.74)
MDR	0.180	0.924	1.17 (0.04-31)
Effective empirical antimicrobial treatment	0.003	0.040	34.77 (1.18-1027.6)
Duration of antimicrobial treatment	0.840	0.986	1 (0.86-1.16)

MDR – multidrug resistant.

DFOs, since a bone biopsy requires a surgical procedure that may delay appropriate antimicrobial treatment.

Several studies have investigated the microbiology of DFIs.[6,20,21] In developed countries, aerobic Gram-positive cocci are the predominant organisms for acute DFIs, with *S. aureus* being the most frequently isolated pathogen,[6,20] while chronic wound infections are usually polymicrobial with several causative organisms, including Gram negative bacilli and anaerobes.[6,21] Moreover, in recent studies, a lower proportion of *S. aureus* and an increasing proportion of Gram-negative microorganisms, including *Pseudomonas aeruginosa*, has been reported,[21,22] as in the present study in which more than 40% of the isolates from DFIs were Gram negative.

Many studies have demonstrated that severe and moderate DFIs are usually polymicrobial, which is important for the consideration of the proper antimicrobial treatment, since it means that a single antimicrobial regimen may be inadequate.[6,22,23] Gadepalli et al. showed that 82.5% of DFIs demonstrated polymicrobial flora, with an average of 2.3 species per patient, while the aerobic to anaerobic ratio was 5.5.[22] The pathogens most commonly isolated were *Staphylococcus aureus*, *Proteus* spp., and *Escherichia coli*. In a study by Zubair et al., polymicrobial infection was reported in 65% of cases of DFIs, with a predominance of *Escherichia coli* and *Staphylococcus aureus* among aerobes and

Peptostreptococcus spp. among anaerobes.[23] In this series almost 90% of patients had moderate DFIs; the infection was polymicrobial in 70% and 75% of the patients with tissue and pus culture respectively, with *Enterococcus faecalis* being the most frequently isolated pathogen in almost 16% of the cases. Our results are in line with those studies, since, in the present one, 70% of infections were polymicrobial.

DFIs usually begin as local infections; however, they may progress and involve deep tissues or/and bones. Sepsis syndrome may occur, while surgical resections and/or amputation may be required, if the patient is not treated appropriately.[24] Therefore, as many different organisms either alone or in combination can cause DFIs, empirical antimicrobial treatment remains a challenge. Initial empirical treatment should be based mainly on the severity of the infection and on the local prevalence of resistant pathogens. Mild to moderate infections can usually be treated with oral antimicrobials, while the severe ones require hospitalization and parenteral treatment.[6]

Multidrug-resistant organisms, particularly MRSA, ESBL–producing Gram-negative bacilli and highly resistant *Pseudomonas aeruginosa* represent an increasing problem in most settings.[6,21] The present study has revealed a high prevalence (40%) of MDROs in DFIs that could explain the high rate of inappropriate empirical antimicrobial treatment that has been observed (42.5%). This is in contrast with another study from a different region in Greece that did not show significant resistance rates, even though in that study diabetic foot osteomyelitis was an exclusion criterion.[25] Interestingly, in our study, a multivariate logistic regression analysis model identified young age and previous antimicrobial treatment to be independently associated with infection by MDROs. This is in accordance with other studies, where previous antimicrobial use has been associated with infection by MDROs.[26] This implies that, maybe, current empirical antimicrobial treatment selection is inadequate for the treatment of these infections at least in our region. Thus, a change of practice regarding the empirical antimicrobial treatment, according to the local antimicrobial resistance, could be an important step for more appropriate and timely management of these infections. To that end, according to our results, DFIs that are severe enough requiring hospitalization could be treated with regimens other than the current one in our hospital that consists of clindamycin and either beta-lactams (aminopenicillins or cephalosporins) or quinolones, as this was found in this study to be inadequate, based on the local antimicrobial resistance rates. The administered antimicrobial regimen should provide appropriate coverage for both Gram-positive (that may include vancomycin, linezolid or daptomycin) and Gram-negative microorganisms (that may include a beta-lactam with anti-pseudomonal activity, like cefepime), since these infections are commonly polymicrobial.

Since appropriate antimicrobial treatment plays an extremely important role in the management of DFIs, especially in the era of antimicrobial resistance, it may not be a surprise that appropriate empirical antimicrobial treatment was found to be an independent factor associated with eradication of DFIs. On the other hand, the identification of the duration of DM as an independent factor associated with eradication of DFIs seems like an unexpected finding, since duration of DM is positively associated with the development of DM complications, and one would anticipate that a patient with more vascular complications may have worse prognosis in case of DFI.

Some of this study's limitations include the relatively small sample size, mainly regarding those with osteomyelitis, and that the study was performed in a single center that may limit the generalization of the results and conclusions. A strength of the study is the lack of antimicrobial administration at the time of swabbing, deep tissue or bone culture.

Conclusions

In conclusion, this study has shown a high concordance rate between easily obtained swab and deep tissue cultures. Thus, swab cultures may be of great help for early change to appropriate treatment since empirical antimicrobial treatment of DFIs can often be inadequate due to frequent

isolation of MDRO pathogens and the polymicrobial nature of these infections. Finally, the increased antimicrobial resistance in the present setting, that was higher than in other Greek centers, should be a reminder for consideration of the local microbiology when treating patients with such infections in order to ensure successful treatment. Further research involving large numbers of patients is needed to confirm the present findings.

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