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Analysis of the microbiota in the diabetic foot ulcers: Is research standardization required?

Mikrobiologiczna analiza zakażeń w zespole stopy cukrzycowej – czy konieczna jest standaryzacja badań naukowych?

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Summary

Background: Complications of infected wounds in patients with diabetic foot ulcer (DFU) are one of the greatest challenges in modern medicine. Analysis of the microbiological profile of infected ulcers may significantly improve treatment results. The aim of the study was to determine the profile of pathogens isolated in patients with DFU and to compare the results of other centers.

Materials and Methods: A retrospective study was carried out on 137 patients with DFU hospitalized at the Department of Diabetology and Internal Diseases, Medical University of Warsaw in 2011-2014. The analysis included the results of 200 microbiological cultures tested for fungi, aerobic and anaerobic bacteria. Statistical analysis was used to test differences in HbA1c values in relation to the strain of the most commonly cultured bacteria and the relationship between glycemic control and most frequently isolated pathogens.

Results: Seventy-nine bacterial species were isolated in 183 positive cultures. Gram-negative bacteria predominated with the highest percentage of representatives of *Enterobacterales*. The most often isolated bacteria were *Serratia marcescens*, *Pseudomonas aeruginos*a, *Proteus mirabilis* and methicillin-susceptible *Staphylococcus aureus*. The Kruskal-Wallis test revealed that HbA1c concentrations were different in groups infected with different strains of bacteria ($p = 0.0087$). Isolation of Escherichia coli and Morganella morganii was more often associated with poor control of diabetes.

Conclusions: The study revealed statistically significant differences in the frequency of microorganisms isolated from the wounds of patients with DFU. The discrepancies in the results of other studies published in this field indicate the need for standardization of the research design.

Keywords: **diabetic foot ulcer, microbiological culture, HbA1c values, wound infection**

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INTRODUCTION

Diabetic foot ulcers (DFUs) require intensive diagnostic and therapeutic procedures and represent one of the greatest challenges of modern medicine, both in clinical and financial aspects. This is still the most common cause of hospitalization due to complications of diabetes and one of the leading causes of amputation in the lower limbs [17]. Patients with diabetes are particularly susceptible to ulcer infections, not only because of chronic complications in the form of micro- and macroangiopathy (peripheral artery disease, PAD), but also due to hyperglycemia and related immune disorders [10]. Difficulties in the healing of chronic ulceration may be related to the size and severity of the wound, its blood supply, the presence of necrosis and also due to the persistence of lesions. While some experts consider the total microbial density to be critical in predicting delayed wound healing and infection, others regard the types of microorganisms to be of greater importance. The effect of treatment depends, among other things, on the degree of colonization of the wound and the traits of pathogens present in the wound. It is assumed that the presence of certain bacteria is clinically relevant regardless of the bacterial load, an example of which is S. pyogenes [22]. However, as numerous clinical studies have demonstrated, a measurement of the tissue microbial load in a wound can predict delayed healing or infection [3, 4, 25].

The detection of individual bacteria in the wound is the leading method and it is widely available and used in clinical practice. Rational antibiotic therapy in DFU is based on the results of antimicrobial susceptibility testing. Analysis of the microbiological profile of infected ulcers remains, therefore, one of the basic elements enabling the improvement of treatment results. IDSA guidelines 2012 describe in detail the rules for the correct collection of material for microbiological tests [16].

Over the past 25 years, the bacteriology of DFU has been reported in many studies, but the results have often been contradictory [5] and differed significantly depending on whether they concerned newly diagnosed or chronic ulcers, the effectiveness of previously used antibiotics and the frequency of hospitalization [14].

In this work, based on the analysis of the results of microbiological tests and the information on sex, age of patients, duration and type of diabetes, type of DFU and the percentage of glycated hemoglobin (HbA1c), an attempt was made to determine the profile of pathogens isolated from ulcerations in patients hospitalized in a diabetology ward of a university-affiliated hospital. A comparative analysis was performed with the results of other centers.

MATERIAL AND METHODS

A retrospective study was carried out on 137 patients aged from 27 to 82 years, with DFU, hospitalized at the Department of Diabetology and Internal Diseases of the Medical University of Warsaw in 2011–2014. We analyzed the results of 200 cultures obtained from patients, out of which 183 were positive. In some patients, depending on their clinical status, the cultures were collected more than once; however, the repeated isolates in the same patient were excluded from the statistical analysis. The analysed material consisted of ulcer swabs obtained using the Levine method (n = 197), biopsies (n = 2) and a bone fragment (n = 1). Samples for testing were taken by a trained dressing nurse. The material was routinely tested for aerobic, anaerobic and fungal microorganisms. The cultured microorganisms were identified using ATB analyzers (bio-Merieux) and MALDI-TOF MS (Bruker). Susceptibility testing was performed in accordance with the National Reference Center for Microbial Susceptibility Testing recommendations. Patients were divided into two groups: patients hospitalized once (Group 1) and patients hospitalized repeatedly (Group 2). The majority of patients were allocated to the first group (115 vs 22). Among the patients from the second group, four patients were admitted to the hospital three times, three patients were hospitalized four times, and the remaining fifteen patients were hospitalized twice.

The characteristics of patients with DFU, from whom samples were obtained and cultured, are shown in Table 1, while HbA1c values are presented in Table 2.

The statistical analysis included calculation of the mean value, standard deviation and expression of the results as percentages.

The discrepancy in the distribution of the collected HbA1c data from normality was assessed using the Shapiro–Wilk W test. The results indicated that the

Table 1. Characteristics of patients in group 1 and 2

Clinical data	Group 1 (n = 115)		Group 2 (n = 22)	
	n	%	n	%
Type 1 diabetes	13	11.3	2	9.1
Type 2 diabetes	91	79.1	20	90.9
Other specific types of diabetes	9	7.8	0	-
No data on the type of diabetes	2	1.7	0	-
Male	87	75.7	16	72.7
Female	28	24.3	6	27.3
Neuropathic foot ulcers	52	45.2	14	63.6
Neuro-ischemic foot ulcers	50	43.5	7	31.8
Ischemic foot ulcers	11	9.6	1	4.5
Indeterminate type	2	1.7	0	-
Obesity	46	40.0	14	63.6
Overweight	16	13.9	4	18.9
Normal weight	25	21.7	3	13.6
Lack of body weight data	28	24.4	1	4.5
Age in years ± SD	59.7±10.02		58.5±9.68	
Duration of diabetes in years ± SD	17.03±10.36		16.9±12.09	

Table 2. Percentage of HbA1c values of patients in the group 1 and 2

Hba1c %	<6.0	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	>12
Group 1	7.8%	34.3%	23.5%	20.6%	7.8%	2.0%	2.9%	1.0%
Group 2	2.1%	25.0%	29.2%	20.8%	20.8%	0.0%	0.0%	2.1%

distribution of the variables differed from the normal distribution. Thus, the non-parametric one-way ANOVA (Kruskal-Wallis test by ranks) was used to test whether there are significant differences in the obtained HbA1c depending on the strain of bacteria as a grouping factor. The Kruskal-Wallis test was followed by post-hoc multiple comparisons of mean ranks for each pair of groups to identify groups in which mean ranks of HbA1c were significantly different. The statistical analysis was carried out using Statistica version 10 (StatSoft, Inc., Tulsa, OK, USA). All data are presented in Table 3 as median and interquartile range, and minimum and maximum values. Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

In both groups predominated patients with type 2 diabetes (79.1% vs 90.9%). There was a majority of males (75.7% vs 72.7%). The average duration of diabetes at the time of inclusion of patients in the study in both groups was similar. The most common type of diabetic foot syndrome in both groups was the neuropathic type, with a greater predominance in Group 2 (45.22% in Group 1 vs 63.64% in Group 2). Obesity was found in Group 1 in 40.0% of patients; in Group 2 this percentage was higher and amounted to 63.64%. No major differences were found in both groups in metabolic control expressed by the mean value of HbA1c.

Table 3. Median and interquartile range and minimum and maximum values of HBA1c concentration in groups of patients infected with different strains of bacteria

Bacteria	N	Mean ± SD	Median	Min	Max	CV* [%]	SEM**
Serratia marcescens	47	7.5 ± 1.1	7.4	5.2	9.7	14.9	0.2
MSSA	43	7.9 ± 1.4	7.7	5.7	12.3	18.1	0.2
Pseudomonas aeruginosa	24	7.2 ± 0.9	7.3	6.1	9.0	12.4	0.2
Proteus mirabilis	23	7.6 ± 1	7.4	5.9	9.1	13.7	0.2
Peptostreptococcus spp.	19	7.4 ± 1.1	7.4	6.0	9.6	14.9	0.3
Escherichia coli	15	8.1 ± 1.5	8.2	5.9	11.1	18.5	0.4
Morganella morganii	12	8.8 ± 1.1	8.7	7.2	11.1	12.5	0.3

* coefficient of variation
** standard error of the mean

Table 4. Results of microbiological cultures in terms of the number of microorganisms isolated in the culture in group 1 and 2

Results of cultures (n = 200)	Group 1 (n = 137)	Group 2 (n = 63)
no growth	16 (11.7%)	1 (1.6%)
one microorganism	49 (35.8%)	21 (33.3%)
two microorganisms	33 (24.0%)	17 (27.0%)
three microorganisms	13 (9.5%)	13 (20.6%)
more than three microorganisms	26 (19.0%)	11 (17.5%)

The results of cultures, taking into account the number and percentage of microorganisms isolated in cultures of samples taken from patients in both groups, are presented in Table 4. In the majority of cultures in both groups, two or more bacterial species were isolated in culture. The percentage of cultures in which only one pathogen was found was similar in both groups. In Group 1 a higher proportion of negative cultures (11.7%) was found compared to Group 2 (1.6%).

Due to the similar characteristics of patients in both groups and a large disproportion in the number of patients between groups, a collective analysis of microbial species isolated in cultures has been performed, as shown in Table 5.

In both patient populations, isolates of 79 different bacterial species were obtained in all cultures. Gram-negative bacteria predominated among the cultivated microorganisms with the highest percentage of representatives of the order *Enterobacterales* [1]. The microorganisms most often isolated from the wounds of patients with DFU were *Serratia marescens, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcous aureus* MSSA (methicillin-susceptible *Staphylococcus aureus*). MRSA (methicillin-resistant *Staphylococcus aureus*) was isolated from 14 samples, which accounted for 3.63% of all isolates. Also noteworthy was the large percentage of anaerobic bacteria obtained in culture (21.5%). The frequency of isolation of *Candida* spp. from the diabetic foot ulcers was very low (0.78%).

Table 6 summarizes the relationship between HbA1c values and pathogens most frequently isolated in the study. Isolation of *Escherichia coli* and *Morganella* morganii in the culture was more often associated with poor control of diabetes (mean HbA1c above 8%).

The Kruskal-Wallis test revealed that HbA1c concentrations were different in groups infected with different strains of bacteria (p = 0.0087). Results of the post-hoc analysis demonstrated that significant differences in HbA1c values were between the group with *M. morganii* and groups with *S. marcescens* (p = 0.031), *P. aeruginosa* (p = 0.0045) and *Peptostreptococcus* spp. (p = 0.047).

DISCUSSION

The majority of acute infections in patients with DFU who have not recently been treated with antimicrobials are caused by aerobic Gram-positive cocci, especially staphylococci [17]. Most chronic infections, or those occurring after antibiotic treatment, are often polymicrobial, with aerobic Gram-negative bacilli joining the aerobic Gram-positive cocci [13] especially in warmer climates [24].

Table 5. Quantitative and percentage distribution of microbial species isolated from wounds in patients with DFU in both study groups

Microorganism	Number/percent	Microorganism	Number/percent
Aerobic and facultatively anaerobic Gram-negative rods: in total 187/48,44%		Facultatively anaerobic Gram-positive bacteria: in total 113/29,27%	
Enterobacterales:	145/37.56%	*Staphyloccous aureus*	46/11.92 %
1. *Enterobacteriaceae*	41/10.62%	* including 14 (3.63%) MRSA strains	
E. coli * including 1 strain ESBL(+)	15/3,89%	Coagulase negative staphylococci	
E. cloacae * including 2 strains ESBL(+)	9	*S. epidermidis*	11/2.85 %
Enterobacter spp.	2	*S. simulans*	7
E. amnigenus	1	*S. haemolyticus*	2
K. oxytoca	5	*S. cohnii*	1
K. pneumoniae * including 2 strains ESBL(+)	3	*Streptococcus* spp.	1
C. freundii	5	*S. agalactiae*	34/8.81 %
C. braakii	1	*Streptococcus* spp.	9
2. *Yersiniaceae*	49/12.69%	β-haemolytic streptococci group C,	7
S. marcescens	48/12,43%	G and F	5
Serratia spp.	1	*S. dysgalactiae*	
3. *Hafniaceae*	1/0.26%	*S. oralis*	4
H. alvei	1	*S. intermedius*	4
4. *Morganellaceae*	54/13.99%	*S. mitis, S. anginosus, S. sanguinis*	2
P. mirabilis	27	*Enterococcus* spp.	3
P. vulgaris	9	*E. faecalis*	12/3.11%
P. penneri	1	*E. faecium*	11/2,85%
M. morganii	14	Other Gram-positive bacteria:	1
P. stuartii	2	*Corynebacterium* spp., *Leuconostoc*	10/2.59%
P. rettgeri	1	spp., *D. hominis, H. kunzi, M. luteus*	
Pseudomonas aeruginosa	27/6.99%	Anaerobes: in total 83/21.5%	
* including 3 strains resistant to carbapenems, 3 strains resistant to ≥2 antibiotics			
		Peptostreptococcus spp.	21/5.44 %
Acinetobacter spp.	9/2.33 %	*F. magna*	11/2.85 %
A. baumannii * including 2 strains resistant to	7	*B. fragilis*	5
carbapenems,3 strains resistant to ≥2 antibiotics		*B. thetaiotaomicron*	4
		B. ovatus	1
		Prevotella spp.	7
A. haemolyticus	1	*Peptoniphilus* spp.	7
A. pittii	1	*Anaerococcus* spp.	7
Other Gram-negative rods:	6/1.55%	*Peptococcus* spp.	6
A. hydrophila, A. faecalis, P. multocida, B. cepacia		*Fusobacterium* spp.	5
Fungi: in total 3/0.78%		*Veillonella* spp.	4
		Actinomyces spp.	2
Candida spp.	3/0.78%	*Porphyromonas* spp.	1
C. albicans	2		
C. parapsilosis	1		

ESBL – extended-spectrum β-lactamases
MRSA – methicillin-resistant *Staphylococcus aureus*

Until the most recent decade, the majority of studies on the microbiology of DFI were conducted in North America and Europe. Investigations in warm climates (especially India, but also the Middle East and Africa) have found the most common isolates to be Gram-negative rods. Thus, clinicians in these regions should consider covering *Enterobacteriaceae* family and *Pseudomonas* spp., pending culture and susceptibility results [28]. Hospitalization and prolonged wound duration are associated with increasingly complex polymicrobial infections that often involved resistant Gram-negative microorganisms [29]. Anaerobic pathogens may occur more often in individuals with ischaemia or gangrene [17]. However, analysis of the numerous reports on the results of cultures performed in patients with DFU shows that there are significant differences in the frequency of isolated pathogens.

In our material, both in the patients who were hospitalized once, as well as in those hospitalized several times, the predominance of Gram-negative bacterial species was

Table 6. Percentage distribution of HbA1c values in patients with selected microorganisms isolated in the culture

Microorganism	Hba1c mean value	HBA1c ± SD	HbA1c <8	HbA1c 8-12,3	Lack of HbA1c measurements
Pseudomonas aeruginosa	7.2	0.9	71.4%	14.3%	14.3%
Peptostreptococcus spp.	7.4	1.1	66.7%	23.8%	9.5%
Serratia marcescens	7.5	1.1	62.0%	32.0%	6.0%
Proteus mirabilis	7.7	1.0	42.9%	39.3%	17.8%
*Staphylococcus aureus MSSA**	7.9	1.4	59.1%	38.6%	2.3%
Escherichia coli	8.1	1.5	43.75%	50.0%	6.25%
Morganella morganii	8.8	1.1	18.75%	56.25%	25.0%

*MSSA –methicillin-susceptible *Staphylococcus aureus*

observed. In the multicenter studies, performed in 2001–2004 in USA, the most often isolated were Gram-positive bacteria, including *S. aureus* and β-hemolytic *streptococci* [5], while Gram-negative bacteria predominated in tests carried out in India [21, 23].

Małecki et al. in 102 cultures found 199 different bacterial strains. There was a predominance of Gram-positive bacteria, particularly Staphylococcus aureus, coagulase-negative staphylococci, and Enterococcus faecalis, as well as Gram-negative rods, such as Pseudomonas aeruginosa, Proteus mirabilis, and Escherichia coli [19]. In our study in the cultures obtained from 137 patients, these bacterial strains constituted only 35.4% of all isolated microorganisms. Comparing the results obtained in these two centers performed in one country, the largest differences were noted in the frequency of isolation of *Enterococcus faecalis* (16.08% vs 2.85%) and *Serratia marcescens* (2.01% vs 12.43%). Compering with data from other continents, there can also be significant differences in the rate of isolation of particular pathogens in patients with DFU. A study of the occurrence of Gram-negative bacteria carried out at the University of Yenepoya (India) by Khan et al. in patients with DFU showed that *Pseudomonas aeruginosa* (39.68%), *Escherichia coli* (17.46%) and *Acinetobacter* spp. (15.41%) were the most frequently isolated bacteria. The authors also summarized the results of investigations performed in various regions of India as well as in other countries (Turkey, Iran), showing variability of *E. coli* from 9.1% to 36.5%, and *P. aeruginosa* from 8.4% to 39.7% [15]. In our material, among Gram-negative bacteria isolated from patients in the study groups, the frequency of *Escherichia coli* isolation was 8.0%, while *Pseudomonsa aeuroginosa* – 14.4%.

Citron et al. presents the results of multicenter studies carried out in the USA [5]. The authors provided the number of bacterial isolates obtained, depending on the method of material collection (aspiration, swabs, tissues). The results (expressed as percentages) obtained using these three methods were similar in the case of isolation of *Enterobacterales*, *Streptococus* spp. and *S. aureus*, but significant differences were noted in the percentage of isolated strains of *Pseudomonas* spp., *Enterococcus* spp., and particularly *S. epidermidis*. In the case of this microorganism the percentage of isolation was: from aspirates – 3.8%, from swabs – 15.9%, and from tissue samples – 18.2% [5]. According to the International Working Group on the Diabetic Foot Guidelines, for clinically infected wounds the proper tissue specimen collection for culture should be obtained aseptically by curettage or biopsy from the ulcer [18].

Table 7 presents the results of microbiological tests carried out in various countries on materials obtained from patients with DFU, comprising swabs, scrapings, purulent secretion aspirates, and tissue biopsies. It should be emphasized that in the part of the publications there was no information about the method of collecting the material for research. Based on the medical records of the patients examined in our study, it was difficult to determine whether they were previously subjected to chronic antibiotic therapy or not; however, other reports also lack information on this issue, hence the difficulties in interpreting the results.

Crouzet et al., on the basis of 14 studies conducted in 1999–2009, analyzed selected pathogens isolated from 3.119 patients with DFU [6]. Only patients with critical ischemia or persons requiring revascularization were not evaluated. In the majority of studies reviewed by these authors, the most frequently isolated pathogen in people with diabetes and foot infection was *Staphylococcus aureus* (6.5–48.8%). The most commonly cultured Gram-negative bacteria were *Enterobacterales* (7.0–33.7%). The authors, summarizing the results of the analysis of 14 studies and showing significant differences in the percentages of isolation of individual pathogens in people with DFU, emphasized the differences in research design, inclusion criteria, statistical methodology and different definitions of both clinical and microbiological endpoints used in the reviewed publications. This makes it much more difficult to compare test results.

Table 7. Microorganisms isolated from clinical samples obtained from patients with DFU in various geographical zones

Publication	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Year of publication	**2006**	**2007**	**2009**	**2011**	**2011**	**2014**	**2014**	**2015**	**2016**	**2018**	**2020**
Number of patients	**80**	**433**	**379**	**434**	**440**	**196**	**102**	**41**	**447**	**261**	**137**
Microorganism in %											
Staphylococcus aureus MSSA*	13.7	14.3	17.3	13.8	3.0	21.0	13.6	30.0	9.6	26.9	8.3
Staphylococcus aureus MRSA**		4.4	11.3	N/A	8.0	N/A	1.0	N/A	1.8		3.6
Streptococus spp.	N/A	15.5	12.6	3.0	4.0	4.7	3.0	9.0	7.2	1.7	8.8
Enterococcus spp.	11.5	13.6	7.7	9.5	6.0	3.4	16.0	N/A	4.4	12.7	3.1
coagulase-negative *Staphylococci*	6.6	15.2	14.9	5.0	0.5	N/A	11.5	N/A	1.3	N/A	2.8
Escherichia coli	12.0	1.7	N/A	16.1	6.0	28.6	7.0	4.5	15.0	12	3.9
Proteus mirabilis	12.6	2.1	N/A	8.8	6.0	4.2	7.6	11.0	9.6	3.1	7.0
Pseudomonas aeruginosa	9.8	3.5	6.9	16.9	17.0	N/A	7.5	4.5	12.4	20.9	7.0
Acinetobacter spp.	9.3	1.1	N/A	3.7	N/A	4.2	2.0	N/A	2.8	1	2.3
Klebsiella spp.	6.6	2.2	N/A	6.7	5.0	14.3	3.5	N/A	3.4	9.5	2.1
Serratia marcescens	N/A	1.2	N/A	N/A	2.0	N/A	2.0	N/A	1.6	0.3	12.4
Morganella morganii	N/A	N/A	N/A	N/A	3.0	N/A	1.5	N/A	4.9	N/A	3.6
Peptostreptococcus spp.	1.6	N/A	N/A	N/A	N/A	N/A	1.5	N/A	N/A	N/A	5.4
Bacteroides fragilis	1.6	4.1	N/A	N/A	N/A	N/A	1.0	N/A	N/A	N/A	1.3

*MSSA – methicillin-susceptible *Staphylococcus aureus*; **MRSA – methicillin-resistant *Staphylococcus aureus*; I - Gadepalli et al. India [8], II - Citron et al. USA [5], III - Yates I et al. Australia [29], IV - Ramakant et al. India [21], V - Al Benwan et al. Kuwait [2], VI - Hadadi et al. Iran [11], VII - Małecki et al. Poland [19], VIII - Perim et al. Brazil [20], IX - Hatipoglu et al. Turkey [12], X - Saseedheran et al. India [23], XI - Margas et al. Poland.
N/A – „not available"

The factor influencing the incidence of individual pathogens and the intensity of their replication may be glycemic control. Gardner et al. showed that at higher HbA1c values there was a high relative abundance of bacteria classified in the genera *Staphylococcus* and *Streptococcus* (in the study the average hemoglobin A1c was 8.5% ± 2.07) [9]. Małecki et al. showed that the deterioration of glycemic control was associated with an increase in abundance of *Entercoccus faecalis* in the ulcers [19]. In our work in patients with higher values of HbA1c, *Morganella* morganii and other Gram-negative rods were isolated with high frequency. What is more, statistical analysis revealed that HbA1c concentrations were different in groups infected with different strains of bacteria.

According to the results of molecular analyses, the complexity of the bacterial populations present in DFU is much greater than could be expected from research based solely on classical microbiological culture techniques, as they may not differentiate the diverse microorganisms that infect foot wounds [9, 26]. Unfortunately, by using this routine method, it is possible to identify anaerobes only in 25% of the samples tested. This is also confirmed by the results of our research, in which the share of anaerobic microflora was estimated at 21.5% of total number of isolates. By contrast, using the sequencing of bacterial ribosomal RNA 16S, anaerobes are detectable in over 85% of the samples tested [27]. However, traditional methods may be a useful tool for the isolation of easily cultured microorganisms, such as Staphylococcus aureus [7]. A better understanding of the composition of the DFU microbiota is particularly important for the development of new strategies for effective infection control, including the problem of biofilm formation.

The reasons for differences in the frequency of occurrence of pathogens isolated in particular healthcare centers

are complex and often difficult to explain. These discrepancies could be partly due to the shift in the causative microorganisms occurring over time, as well as geographical variations, or the types and severity of infections comprised in the studies. Diversity in research design makes comparison of results difficult.

Also, laboratory processing of the samples may have been inadequate to grow anaerobes or fastidious organisms. Lack of larger analyzes of the influence of glycemic control on the presence of individual pathogens hinders the interpretation of the results obtained, and at the same time indicates the need to continue this type of research. Another reason for the discrepancy in the results may be variable prevalence of the clinical forms of the diabetic foot syndrome (neuropathic, ischemic, neuro-ischemic). In this study neuropathic and neuro-ischemic type were predominant. But we have noted infections of mixed etiology, most often with numerous pathogens, in patients with all forms of the disease.

Our research confirms numerous reports indicating differences in bacterial culture results in DFU and points to the need for standardization of the tests performed. The question remains whether this may be due to collection and culture technique only, or are there also significant differences in the presence of pathogens in particular healthcare centers. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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