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Etiological Factors of Infections in Diabetic Foot Syndrome – Attempt to Define Optimal Empirical Therapy

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Diabetic foot syndrome (DFS) represents one of the most frequent reasons for lower limb amputation in developed countries. In most cases, it is associated with bacterial infection, requiring optimal antibiotic therapy.
Objectives. The aim of this study was to identify the most frequent pathogens responsible for infections associated with DFS, establish the optimal protocol of empirical therapy, and ascertain the clinical variables that may determine the choice of the appropriate antibacterial agent.
Material and Methods. The analysis included hospital records of patients treated at the Department between 2008 and 2010. A total of 102 individuals were identified; their material was cultured and tested for antibiotic susceptibility.
Results. A total of 199 bacterial strains were isolated. There was a predominance of Gram-positive bacteria, particularly *Staphylococcus aureus*, *Staphylococcus* coagulase-negative strains, and *Enterococcus faecalis*. Of note was the high percentage of *E. faecalis* infection (16.08%). One can speculate on the potential etiological factors in the case of some bacteria, e.g. patients infected with *S. aureus* were characterized by higher monocytosis and lymphocytosis as compared to other patients. Analysis of drug susceptibility revealed that ciprofloxacin has the highest (but still only 44%) efficacy of all agents tested as monotherapy, and a combination of piperacillin and tazobactam or amoxicillin and clavulanate with aminoglycosides is particularly beneficial.
Conclusions. *Staphylococcus* spp. predominates amongst the etiological factors of DFS infection; however, the rate of *E. faecalis* infection is alarmingly high. Monotherapy enables effective treatment in a minority of cases; therefore, at least two-drug protocols should be implemented from the very beginning of the therapy (**Adv Clin Exp Med 2014, 23, 1, 39–48**).

Key words: antibiogram-based therapy, antibiotics, diabetic foot syndrome, *Enterococcus faecalis.*

Diabetes affects more than 3 m patients in Poland, and it is estimated that this number will increase to 3.4 m by 2030. The prevalence of diabetes slightly exceeds 10%. Impaired glucose tolerance (IGT) is detected in more than 5 m patients. The number of diabetes-related deaths is reaching 30,000 and the costs of treatment calculated per diabetic patient are estimated at USD 1143 per year in Poland [1]. Diabetic foot syndrome (DFS) is one of the chronic complications of diabetes. According to the definition, this condition pertains to infection, ulceration, or destruction of deep tissues of the foot, resulting from the injury of peripheral nerves or blood vessels. Depending on its etiology, DFS is divided into neuropathic, ischemic, or mixed type [2]. DFS constitutes the principal reason of non-injury amputations of legs in developed countries, and the related infections represent the most frequent cause of diabetes-related hospitalizations [3]. The results of a Canadian study suggest that diabetic patients have a higher risk of infection, which is 1.21-fold higher than in

the controls [4]. Antibiotic therapy, initially empirical and subsequently antibiogram-based, is the most important treatment modality in the case of DFS infection. The aim of this retrospective study was to determine the types of pathogens that are most frequently isolated from DFS patients treated at the Department of Angiology, Systemic Hypertension and Diabetology of Wroclaw Medical University, analyze their drug resistance, propose the most potentially effective protocol of empirical antibiotic therapy, and verify any potential associations between the type of pathogen and clinical manifestation and laboratory findings.

Material and Methods

The analysis included 4500 hospital records of patients treated at the Department of Angiology between 2008 and 2010. The inclusion criteria for further analysis included the diagnosis of type 2 diabetes, co-existing DFS, and a bacterial culture with antibiotic susceptibility testing. The material for microbiological culture and antibiotic susceptibility testing (fragments of tissues, surface biopsies, aspirates) was obtained from deeper layers of the ulceration, following disinfection of the skin surface. The susceptibility of microorganisms to antibiotics and chemotherapeutics was determined qualitatively by means of a diffusion method with paper discs impregnated with antibiotic at a given concentration. Under such conditions, the susceptibility of the analyzed strain is determined based on the size of the inhibition zone around the disc impregnated with a given antibiotic. A detailed protocol of the testing can be found in relevant literature [5].

Aside from detected pathogens and their resistance, the analysis included the patient's age, location of the ulceration, presence of obliterative atherosclerosis (abnormal values of the ankle-brachial index or abnormalities on a Doppler ultrasonography), type of DFS, concentration of glycated hemoglobin, glomerular filtration rate (eGFR) estimated on the basis of the MDRD (Modification of Diet in Renal Disease) formula, presence of microalbuminuria or proteinuria, ketonuria, glycosuria, anemia, concentration of C-reactive protein (CRP), peripheral monocyte, neutrophil, eosinophil, basophil, and lymphocyte count, and fasting glucose concentration.

The results were subjected to statistical analysis. In the case of normally distributed variables (identified by the Shapiro-Wilk test) and homogeneity of variance (confirmed by the Levene test), intergroup differences were analyzed by means of simple analysis of variance (ANOVA). Whenever at least one of the abovementioned conditions was not satisfied, the non-parametric Kruskal-Wallis rank test (in the case of more than two groups, with subsequent post-hoc testing) or the Mann-Whitney *U*-test (in the case of two groups) was applied. Intergroup differences in the percentage distributions of dichotomous variables were analyzed with the Pearson's $\chi 2$ test. P value < 0.05 was considered statistically significant. Due to widely accepted recommendations, 95% confidence intervals (CI) were calculated for each distribution aside from means and standard deviations. Additionally, logistic regression and cluster analysis were employed. Cut-off values characterized by maximal sensitivity and specificity were identified on the basis of a Receiver Operating Characteristic (ROC) curve analysis. All calculations were conducted with the Statistica 10 package (Stat Soft Inc.).

Results

The analysis included 4500 hospital records of patients treated at the Department of Angiology between 2008 and 2010. Overall, 102 individuals (67 men and 35 women) with an average age of 65 years were included. One-hundred and 99 bacterial strains were isolated, including 8 (4%) alert pathogens [6]: *Enterobacter aerogenes* extended-spectrum beta-lactamase (ESBL) (n = 2), *Escherichia coli* ESBL (n = 2), methicillin-resistant *Staphylococcus aureus* (MRSA) (n = 2), *Acinetobacter baumannii* (n = 1), and *Enterobacter cloacae* (n = 1). Only one strain was isolated from 38 individuals (37.5%), two strains – in 33 patients (32%), three strains – in 17 (16.3%), four strains – in 4 (4%), and five strains – in 9 (9%); in only one case (1.2%) the culture was sterile. Gram-positive bacteria comprised 52.76% of all recovered strains, whereas the corresponding value was equal to 47.24% in the case of Gram-negative bacteria. Facultative anaerobes were most prevalent (64.32%), followed by aerobes (29.14%), and anaerobes (6.54%). Nearly two-thirds of infections were caused by one of the following 6 bacterial strains: *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus* coagulase-negative species, *Pseudomonas aeruginosa*, *Proteus mirabilis*, or *Escherichia coli* (Fig. 1). *Staphylococcus spp*. corresponded to 26.63% of all etiological factors; specifically, coagulase-positive *Staphylococcus aureus* was recovered in the majority of cases. The percentages of various microorganisms isolated from the analyzed patients are presented in Table 1.

Analysis of laboratory and clinical data revealed that individuals with *E. faecalis* had a higher percentage of glycated hemoglobin as compared to

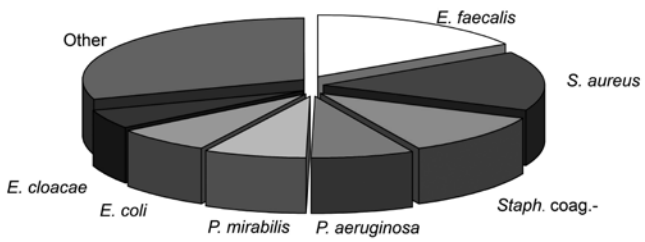

Fig. 1. Percentage of various bacterial strains isolated from the infection of diabetic foot syndrome

Table 1. Percentage distribution of cultured microorganisms

Bacterium	N	%	Comments
Enterococcus faecalis	32	16.08	10 HLAR
Staphylococcus aureus	31	15.58	27 MSSA, 2 MRSA (including 2 alert)
Staphylococci coagulase-negative	22	11.05	MRCNS, MSCNS, *Staphylococcus epidermidis, carnosus, simulans, lentus, haemoliticus, capitis*
Pseudomonas aeruginosa	15	7.54	
Proteus mirabilis	15	7.54	
Escherichia coli	14	7.04	2 alert ESBL
Enterobacter cloacae	10	5.03	1 alert ESBL
Klebsiella oxytoca	7	3.52	
Streptococcus pyogenes	6	3.02	
Serratia marcescens	4	2.01	
Klebsiella pneumoniae	4	2.01	
Enterococcus faecium	4	2.01	2 HLAR
Enterobacter aerogenes	4	2.01	2 alert ESBL
Acinetobacter baumani	4	2.01	1 alert
Streptococcus agalactiae	3	1.51	
Stenotrophomonas maltophilia	3	1.51	
Peptostreptococcus spp.	3	1.51	
Morganella morganii	3	1.51	
Citrobacter freundii	3	1.51	
Prevotella melaninogenica	2	1.01	
Bifidobacterium spp.	2	1.01	
Bacteroides fragilis	2	1.01	
Providencia rettgeri	1	0.50	
Kluyvera ascorbata	1	0.50	
Eubacterium aerofaciens	1	0.50	
Clostridium bifermentans	1	0.50	
Citrobacter braakii	1	0.50	
Alcaligenes faecalis	1	0.50	
Total	199	100.00	

ESBL – extended-spectrum beta-lactamase; HLAR – high-level aminoglycoside resistance; MRCNS – methicillin resistant coagulase-negative *Staphylococci*; MSCNS – methicillin-sensitive coagulase-negative *Staphylococci*.

other infections [mean 9.0% (95% CI: 7.96–10.05) vs. 7.47% (95% CI: 7.00–7.93), Mann-Whitney *U* test p = 0.002] (Fig. 2). Logistic regression analysis showed that an increase in the glycated hemoglobin fraction by one point was associated with a 1.38 odds ratio of infection with this bacteria (95% CI: 1.10–1.74), p < 0.01). Moreover, the fraction of glycated hemoglobin HbA1c ≥ 12.6% was associated with 98% specificity for *E. faecalis* infection; however, the sensitivity was low and amounted to only 15% (likelihood ratio, LR = 9.23).

Compared to individuals infected with other bacteria, patients with *S. aureus* infection were characterized by a higher estimated glomerular filtration rate (eGFR) determined by means of the MDRD method, and higher fasting glycemia [eGFR 68.97 mL/min, 95% CI: 61.90–76.03 vs. 57.08 mL/min, 95% CI: 51.43–62.72, Mann-Whitney *U* test p < 0.01; fasting glycemia 160.38 mg/dL, 95% CI: 140.44–180.31 vs. 133.25 mg/dL, 95% CI: 120.59–145.91, Mann-Whitney *U* test p < 0.01]. Additionally, infection with *S. aureus* was most frequent in patients with generalized atherosclerotic process (chi-square test; p < 0.01, $\phi = -0.27$). In turn, patients infected with coagulase-negative *Staphylococci* were characterized by an enhanced immune response of the monocyte and lymphocyte system as compared to other bacterial infections (monocyte count: 0.96 G/l, 95% CI: 0.84–1.09 vs. 0.79 G/L, 95% CI: 0.73–0.85, Mann-Whitney *U* test p < 0.05; lymphocyte count: 2.88 G/L, 95% CI: 2.06–3.70 vs. 1.71 G/L, 95% CI: 1.53–1.88, Mann-Whitney *U* test p < 0.001; Fig. 3). Lymphocyte count ≥ 3.11 G/l was characterized by 96% specificity (but only 40% sensitivity) with regards to the infections with coagulase-negative *Staphylococci* (LR = 10).

Although the activation of the immune response was also observed in individuals with *P. mirabilis* infection, it involved monocytes and neutrophils rather than lymphocytes (monocyte count: 0.99 G/L, 95% CI: 0.88–1.10 vs. 0.79 G/l, 95% CI: 0.73–0.85, Mann-Whitney *U* test p < 0.01; neutrophil count: 8.48 G/l, 95% CI: 7.32–9.63 *vs.* 5.36 G/L, 95% CI: 4.81–5.91, Fig. 4, Mann-Whitney *U* test p < 0.0001). Importantly, the neutrophil count of one patient (7%) with this infection fit within the normal range. Neutrophil count above 8.84 G/l was characterized by 57% sensitivity and 91% specificity with regards to *P. mirabilis* infection (LR = 6.12), and monocyte count ≥ 2 G/L was 98% specific (but only 7% sensitive) for infection with this bacteria (LR = 5.43). All infections with *Proteus mirablis* were associated with anemia; however, this relationship did not prove statistically significant due to the high prevalence of anemia in the examined population (chi-square test with Yates' correction p = 0.06; $\phi = 0.22$).

No other significant relationships were identified between infection with a given pathogen and laboratory or clinical characteristics.

Data on the chemotherapeutic susceptibility of various strains is summarized in Table 2. An analysis that was inclusive of all the pathogens revealed that the fraction of sensitive strains was the highest in the case of piperacillin/tazobactam, gentamycin,

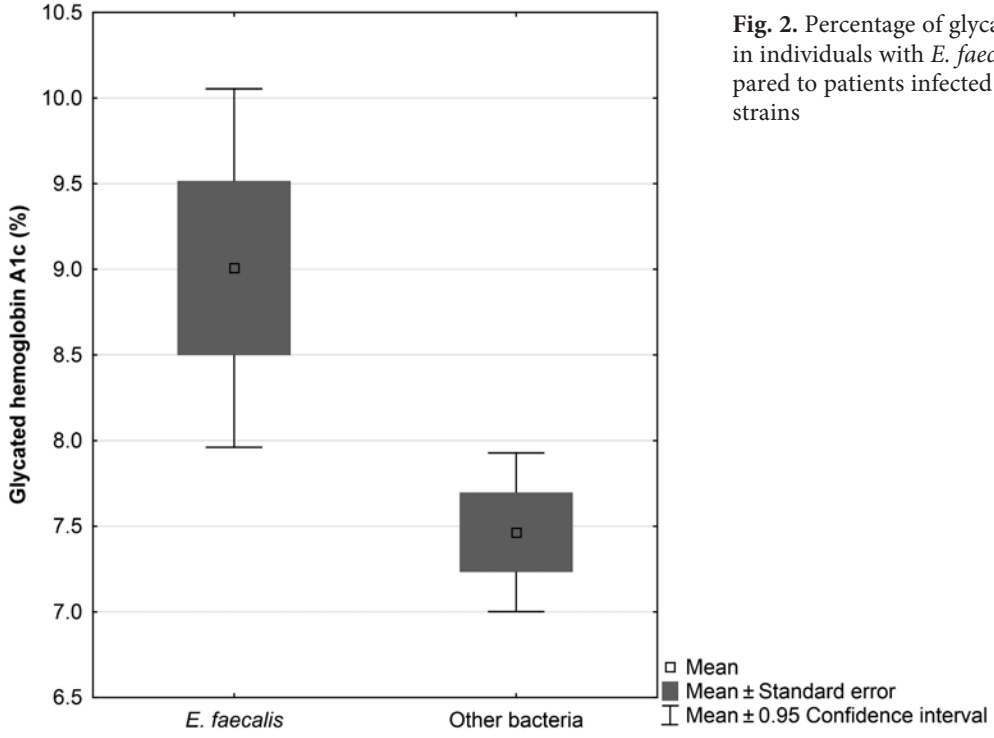

Fig. 2. Percentage of glycated hemoglobin A1c in individuals with *E. faecalis* infection compared to patients infected with other bacterial strains

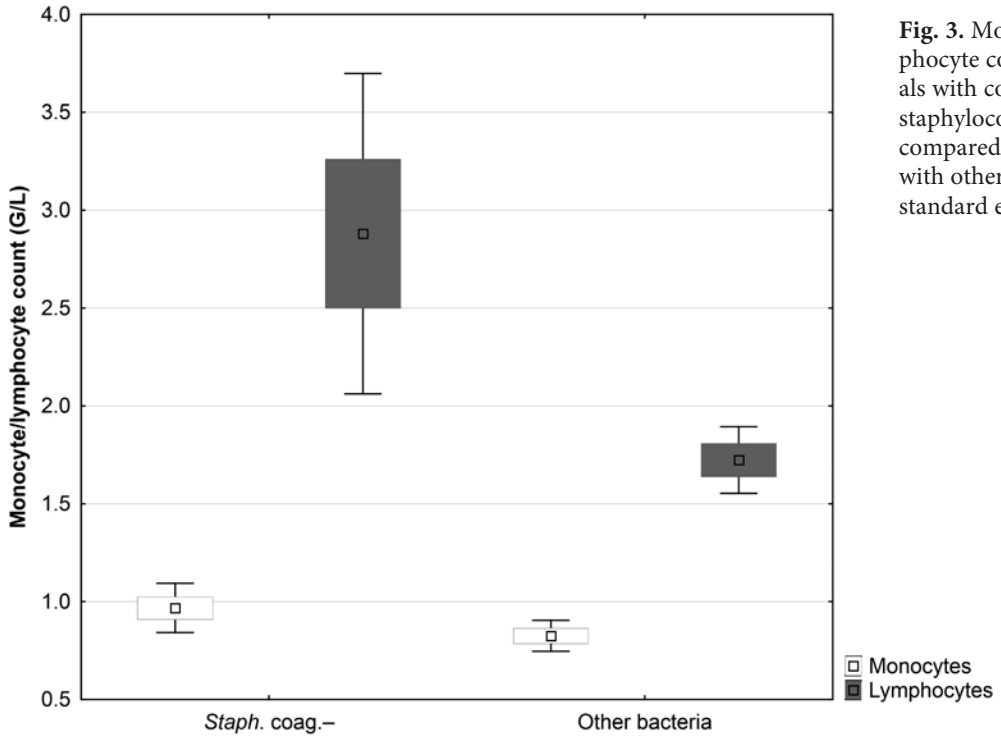

Fig. 3. Monocyte and lymphocyte count in individuals with coagulase-negative staphylococcal infection compared to patients infected with other bacteria (mean, standard error, 95% CI)

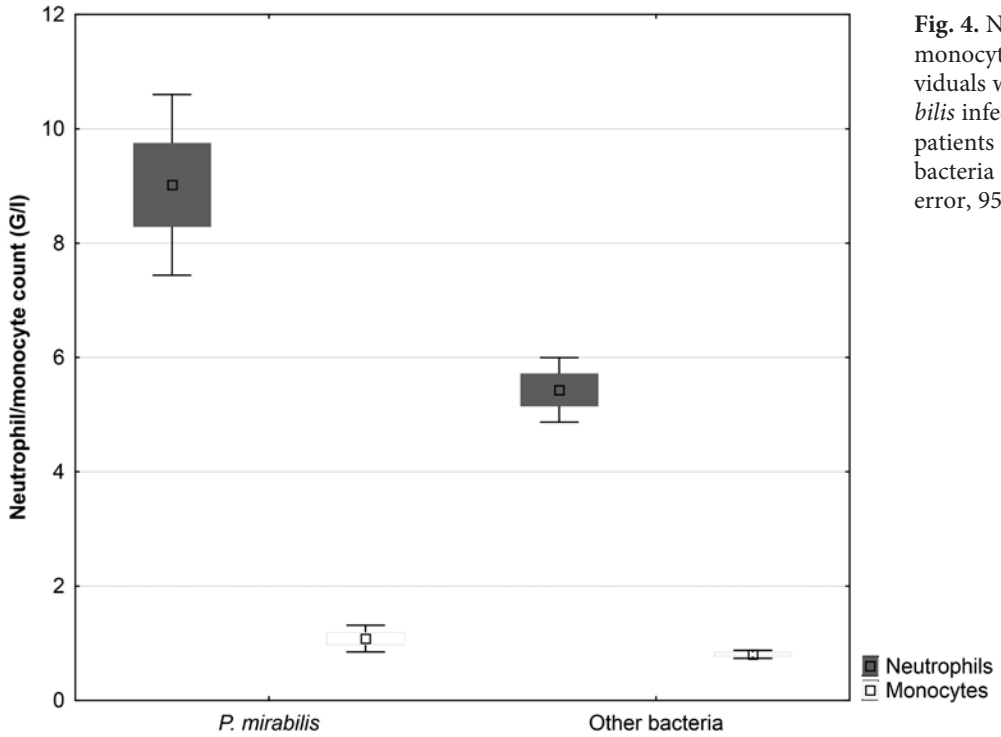

Fig. 4. Neutrophil and monocyte count in individuals with *Proteus mirabilis* infection compared to patients infected with other bacteria (mean, standard error, 95% CI)

and ciprofloxacin, while the lowest number of strains showed resistance to carbapenems, cefotaxime, and piperacillin/tazobactam.

Taking into account the fraction of sensitive and resistant strains, a cluster analysis was conducted in order to significantly optimize empirical therapy. As a result, four principal groups of chemotherapeutics were identified (Fig. 5):

1) Nethylmycin, cefuroxime, trimethoprim/sulfamethoxazole, clindamycin, erythromycin, ampicillin – characterized by a relatively low percentage of sensitivity and relatively high resistance rate; not recommended.

2) Doxycycline, amoxicillin/clavulanic acid – characterized by a relatively high percentage of sensitivity, but also high resistance rate; rather not recommended

3) Ciprofloxacin, gentamycin – antibiotics characterized by a high fraction of sensitivity and relatively low resistance rate; recommended.

Table 2. Sensitivity of all analyzed strains to various chemotherapeutics

No.	Chemotherapeutic	Sensitive strains (%)	Resistant strains (%)
Penicillins			
1.	Piperacillin/tazobactam	39.44	5.60
2.	Amoxicillin/clavulanate	30.52	25.60
3.	Ampicillin	20.19	16.80
4.	Penicillin	16.43	–
Cephalosporins			
5.	Ceftazidime	35.68	4.00
6.	Cefetamet	30.52	–
7.	Cefotaxime	30.05	3.20
8.	Cefuroxime	13.62	12.00
9.	Cefoperazone/sulbactam	5.16	–
Carbapenems			
10.	Imipenem	32.86	3.20
11.	Meropenem	28.17	2.40
Macrolides			
12.	Erythromycin	17.84	18.40
Lincosamides			
13.	Clindamycin	21.13	21.60
Aminoglycosides			
14.	Gentamycin	51.64	16.00
15.	Amikacin	40.85	7.20
16.	Nethylmycin	22.54	5.60
Tetracykliny			
17.	Doxycycline	21.13	31.20
Glycylcyclines			
18.	Tigecycline	3.76	–
Glycopeptides			
19.	Vancomycin	33.80	–
20.	Teicoplanin	29.11	–
Fluoroquinolones			
21.	Ciprofloxacin	42.72	20.80
Sulphonamides			
22.	Trimethoprim/sulfamethoxazole	31.46	16.00

4) Meropenem, cefotaxime, imipenem, ceftazidime, amikacin, piperacillin/tazobactam – antibiotics characterized by a high fraction of sensitive strains and extremely low percentage of resistant strains; definitely recommended.

The list of less and more recommended antibiotic combinations (Table 3) was developed after considering solely the *in vitro* efficacy, i.e. the percentage of strains sensitive to a given antibiotic combination, estimated on the basis of the

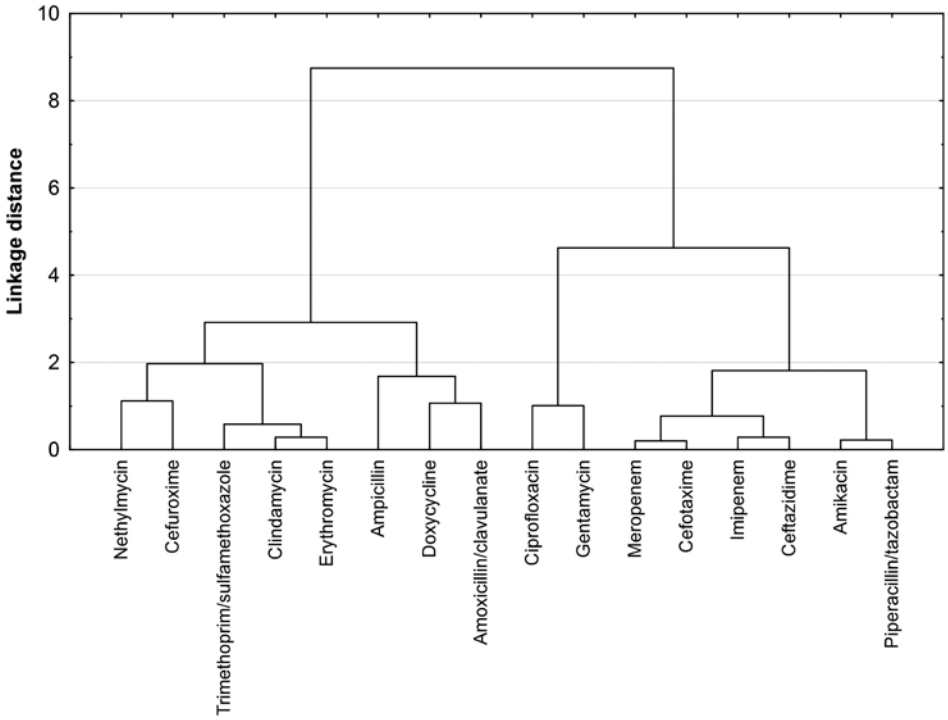

Fig. 5. Results of a cluster analysis with regards to the sensitivity and resistance of isolated strains to various chemotherapeutics (tree diagram, Ward's method, Euclidean distance)

sensitivity to a single antibiotic determined by the disc method, and the loss, i.e. the percentage of strains sensitive to both antibiotics (in situations where application of one chemotherapeutic would be theoretically sufficient).

Discussion

A EURODIALE study revealed that bacteria can be recovered from 58% of newly-diagnosed cases of diabetes in which material was obtained from developing ulcerations [7]. The participation of various etiological factors depends on geographical latitude, as well as on the cultural and hygienic customs of a given region. Western countries are predominated by Gram-positive bacteria, mostly *Staphylococcus aureus* and beta-hemolytic *Streptococcus* spp. [8, 9]. In contrast, the Gram-negative bacteria, mostly *Pseudomonas aeruginosa* and *Escherichia coli*, predominate in Eastern countries, particularly in India. The most prevalent Gram-positive bacteria observed in this region include *Staphylococcus aureus* and *Enterococcus* spp. [10]. Taking the percentage distribution of microorganisms into account, the situation in Poland approaches European trends. However, the high fraction of enterococci requires further research, mostly with regards to *Enterococcus faecalis* (16.8%), whose prevalence, together with *Enterococcus faecium*, exceeds 18%, and thus is higher than in the case of *Staphylococcus aureus* (slightly above 15%). During our search through the available literature, we did not find any report in which the prevalence of *Enterococci* would be higher than that of *Staphylococcus aureus*, which predominates amongst the Gram-positive microflora [10, 11]. There are several potential reasons behind such a high fraction of *Enterococci*, including severe dental caries [12], insufficient personal hygiene, co-existing urinary tract infection, or contact with poultry/poultry meat [13]. However, the selection of strains resulting from empirical antibiotic therapy, started prior to hospitalization and based mostly on β-lactams, to which *Enterococci* show natural resistance, seems the most rational explanation. About 30% of the enterococcal strains isolated from our patients were revealed as high level aminoglycoside resistant (HLAR); in contrast, there were no alarm strains resistant to glycopeptides (VRE – vancomycin-resistant *Enterococcus*). These values are lower than previously presented in the available literature [14–17]. In most cases the material was obtained immediately on admission to the clinic, and a relatively low fraction of HLAR strains were recovered, both of which contradict the nosocomial source of enterococcal infection.

The presented associations between the etiological factors and clinical data analyzed in this

Table 3. Recommended combinations of antibiotics and their effect on treatment efficacy

No.	Combination of antibiotics	Efficacy (%)	"Loss" (%)
β-lactam antibiotic and aminoglycoside			
1.	Amoxicillin/clavulanate + amikacin	61.5	9.9
2.	Piperacillin/tazobactam + amikacin	52.6	27.7
3.	Ceftazidime + amikacin	51.6	24.9
4.	Cefetamet + amikacin	50.2	21.1
5.	Cefotaxime + amikacin	50.1	20.2
6.	Amoxicillin/clavulanate + gentamycin	73.7	8.5
7.	Piperacillin/tazobactam + gentamycin	70.4	20.7
8.	Ceftazidime + gentamycin	68.1	19.2
9.	Cefetamet + gentamycin	64.3	17.8
10.	Cefotaxime + gentamycin	66.2	15.5
β-lactam antibiotic and fluoroquinolone			
11.	Amoxicillin/clavulanate + ciprofloxacin	63.4	9.8
12.	Piperacillin/tazobactam + ciprofloxacin	57.7	24.4
13.	Ceftazidime + ciprofloxacin	53.4	24.4
14.	Cefetamet + ciprofloxacin	51.2	22.1
15.	Cefotaxime + ciprofloxacin	53.5	19.2
β-lactam antibiotic and macrolide			
16.	Amoxicillin/clavulanate + erythromycin	45.1	3.3
17.	Piperacillin/tazobactam + erythromycin	57.2	0
18.	Ceftazidime + erythromycin	53.5	0
19.	Cefetamet + erythromycin	48.4	0
20.	Cefotaxime + erythromycin	47.9	0
β-lactam antibiotic and lincosamide			
21.	Amoxicillin/clavulanate + clindamycin	44.6	7.0
22.	Piperacillin/tazobactam + clindamycin	59.6	0.9
23.	Ceftazidime + clindamycin	56.8	0
24.	Cefetamet + clindamycin	51.2	0
25.	Cefotaxime + clindamycin	51.6	0
Aminoglycoside and fluoroquinolone			
26.	Gentamycyna + ciprofloxacin	62.4%	31.9%
27.	Amikacyna + ciprofloxacin	60.1%	23.5%
Aminoglycoside and macrolide			
28.	Gentamycyna + erythromycin	56.9	12.7
29.	Amikacyna + erythromycin	54.5	4.2
Aminoglycoside and lincosamide			
30.	Gentamycyna + clindamycin	59.6	13.1
31.	Amikacyna + clindamycin	57.3	4.7
Macrolide and tetracycline			
32.	Erythromycin + doxycycline	30.5	8

paper have purely practical applications, enabling the selection of a "better" protocol of antibiotic therapy when the results of a culture are unavailable. Although we did not obtain sufficiently high sensitivity of the analyzed parameters, their high specificity and acceptable likelihood ratio (LR) are worth mentioning [18], particularly with regards to a ≥ 12.6% fraction of glycated hemoglobin in individuals with *E. faecalis* infection (LR = 9.23), lymphocytosis ≥ 3.11 G/L in the course of *S. aureus* infection (LR = 10), and monocytosis ≥ 2 G/L in *P. mirabilis* infection (LR = 5.43). The complex and variable interaction between a given etiological factor and the host's immune system, particularly in diabetic patients, can be responsible for these clinically important findings [19, 20].

In view of the fact that even up to 34% of patients with ulceration associated with DFS require amputation [5], early, appropriate antibiotic therapy plays an important role, aside from rational insulin therapy, potential revascularization, decompression of the foot, and local treatment. Therefore, we analyzed the antibiotic susceptibility for all microorganisms isolated from our material rather than for individual strains. Such an approach is clinically justifiable since the empirical antibiotic therapy should be started before the etiological factors can be identified based on the culture results. Antibiotics that proved to be particularly valuable in the monotherapy of our patients included ciprofloxacin, aminoglycosides (typically not implemented as monotherapy), amoxicillin/clavulanate, and piperacillin/tazobactam; however, it should be emphasized that the efficacy of any single chemotherapeutic did not exceed 43% of the cases. Therefore, it is quite obvious that combined therapy should be implemented at the very beginning of severe infection. Our analysis revealed that in such cases a combination of amoxicillin/clavulanate or piperacillin/tazobactam with aminoglycosides is most efficient; this is generally consistent with the recommendations of PDA and monographs dealing with antibiotic therapy [21]. Of note are the small benefits associated with combining ciprofloxacin with chemotherapeutics from other groups.

The fact that the data presented originates from a single center is unambiguously the principal limitation of this study. Nevertheless, it is likely that the significance of at least some of the hereby identified problems, such as the high prevalence of *E. faecalis* infection, laboratory abnormalities specific to certain types of infection, and analysis of drug susceptibility spreads beyond the Wrocław center.

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