ORIGINAL PAPER
Polish Journal of Microbiology
2023, Ahead of Print
https://doi.org/10.33073/pjm-2023-014

The Pathogen Isolates in Chronic Wound Infections in Poland

MARCIN MALKA[1], ARKADIUSZ KRAKOWIECKI[1], MAGDALENA CHOJAK[1], MAREK PŁAWSKI[1], MARIUSZ WĄDOŁEK[1], AGNIESZKA WOŁOWICZ[1], ALEKSANDRA DYCZEWSKA[1], ALEKSANDRA PAŹ[2], KATARZYNA PAWLIK[3] and TOMASZ GRZELA[4]

[1] PODOS Wound Care Clinic, Warsaw, Poland
[2] Medical University of Warsaw, Chair and Department of Experimental and Clinical Pharmacology, Warsaw, Poland
[3] Microbiological Laboratory, Independent Public Specialist Healthcare Centre in Lebork, Lebork, Poland
[4] Medical University of Warsaw, Chair and Department of Histology and Embryology, Warsaw, Poland

Submitted 7 February 2023, accepted 13 March 2023, published online 30 March 2023

Abstract

Chronic wound infection is one of the factors that hinder or prevent its healing. The incidence of infection may vary depending on the type of wound. It is estimated that clinically significant infection in diabetic foot syndrome occurs in up to 30% of patients. Accurate diagnosis of infection features and proper microbiological tests are crucial for introducing of appropriate local and often systemic treatment. The aim of the study was a comparative analysis of the microbiota found in infected chronic wounds in patients from Poland, consulted on an outpatient basis at a wound care center in 2013–2021. The indication for microbiology culture tests was the detection of local signs of infection, and sampling was preceded by appropriate wound debridement. The standard culture technique was a deep-tissue biopsy. Material for the study was collected from 1,199 patients. Overall, 3,917 results of microbiological tests were subjected to retrospective analysis. The paper presents the results in the form of the number of cultured microorganisms and their relative incidence as percentages, considering the division into the types of wounds from which the material was obtained. The most frequently isolated microorganisms in the analyzed group were *Staphylococcus aureus* (14.3% of this group were MRSA – methicillin-resistant *Staphylococcus aureus*) and *Enterococcus faecalis* (2.4% of this group were VRE – vancomycin-resistant *Enterococcus*).

Further analysis of such an extensive database, especially regarding drug susceptibility of isolated microorganisms, seems crucial to elaborate new recommendations for empirical antibacterial treatment of infected chronic wounds.

K e y w o r d s: deep-tissue biopsy, infection, chronic wound

Introduction

Regardless of the constant development of techniques for chronic wound treatment and the availability of more and more modern dressings and antibacterial products, wound care is a medical, organizational, and economic challenge (EWMA 2008). In Germany, in 2012, 1.04% of insured patients were diagnosed with a wound, of which the vast majority were leg ulcers (0.7%) (Heyer et al. 2016). In turn, 739,000 patients were found to have leg ulcers in the UK in 2019 (Graves et al. 2022). There are no precise data on the number of patients with chronic wounds in Poland. Some authors say it may amount to 500,000 (Sopata et al. 2020).

Most frequently observed chronic wounds include diabetic foot syndrome, venous leg ulcers, wounds during critical limb ischemia, pressure ulcers, neoplastic wounds, but also postoperative wounds. Infection is one factor that hinders or prevents wound healing and deteriorates the patient's prognosis. It is estimated that clinically significant infection in the course of diabetic foot syndrome occurs in up to 30% of patients (Mrozikiewicz-Rakowska et al. 2021).

Accurate diagnosis of infection features and proper microbiological tests are crucial for introducing appropriate local and often systemic treatment. Antibacterial management and appropriate causal treatment lead to infection control and initiation of the healing process.

* Corresponding author: M. Malka, PODOS Wound Care Clinic, Warsaw, Poland; **e-mail: marcinmalka@gmail.com**

Proper debridement before diagnostic material sampling and the recommended best form of culture by deep-tissue biopsy is rarely performed, mainly in specialist centers that treat chronic wounds (Tedeschi et al. 2017; García et al. 2020).

The selection of antibiotics in the empirical therapy of chronic wounds is based on standards developed on data collected abroad. Knowledge of the epidemiology of chronic wounds in Polish patients is necessary to create national standards of empirical therapy that should be applied before the results of bacterial cultures are obtained. Improving the quality of initial treatment in this area can significantly affect the infection control rate and the time when regeneration occurs in damaged tissues and reduce the risk of severe complications, including sepsis or limb amputation.

The aim of the study was a descriptive analysis of the microorganisms found in infected chronic wounds in patients from Poland, consulted on an outpatient basis at a wound care center from February 26, 2013, to June 29, 2021.

Experimental

Materials and Methods

Collecting material. The retrospective analysis included 3,917 results of microbiological tests performed on cultures from chronic wounds. The material for the study was collected from 1,199 patients consulted and treated on an outpatient basis at a specialist wound care center from 2013 to 2021. The division of wounds and the definitions for which the wound was classified into a given group are presented in Table I.

Sampling. The indication for the material sampling for microbiological analyses following the recommendations of the Infectious Diseases Society of America (IDSA) was the presence of at least local symptoms of infection, i.e.: redness, excessively warm in touch, oedema, tenderness or pain, and purulent exudate (Lipsky et al. 2012). Colonized wounds without signs of infection were excluded from the analysis.

Table I
Type of wound definition.

Type of wound	Definition
Neuropathic diabetic food syndrome	Wounds that are difficult to heal within the foot in diabetic patients with neuropathy as the dominant cause of the wound
Ishemic diabetic food syndrome	Wounds in the foot area in diabetic patients with ischemic features in vascular examination
Mixed diabetic food syndrome	A combination of the two above mentioned
Foot deformity with neuropathy	Foot deformities with symptoms of neuropathy, causing tissue overload and ulceration, but without diabetes
Wounds in the course of critical limb ischemia	Lower limb ulcers not subjected to conservative treatment and requiring revascularization procedures to initiate healing, regardless of the presence of diabetes
Venous leg ulcers	Leg ulcers causally related to chronic venous insufficiency (confirmed by Doppler examination)
Mixed leg ulcers	Leg ulcers causally related to chronic venous insufficiency (confirmed in a Doppler examination), but at the same time features of chronic ischemia requiring or not requiring revascularization (some patients were treated with first-degree compression and walking training without revascularization)
Lymphatic leg and/or foot ulcers	Leg and/or foot ulcers not causally related to chronic venous insufficiency (confirmed absence of chronic venous insufficiency in Doppler examination), clinical features of lymphedema (positive Stemmer test, and/or lymphoscintigraphy confirming lymph stasis, and/or ultrasound description or clinical picture typical of lymphatic insufficiency)
Pressure ulcers	Wounds in places typical for pressure ulcers (most often: trochanters, sacrum, ischial tuberosities, heels) associated with pressure, friction, and shearing forces in people who are completely or partially immobilized
Postoperative wounds	Hard-to-heal wounds, the beginning of which was related to a surgical procedure in any area (usually abdominal integuments, groin, lower limbs)
Post-traumatic wounds	Chronic wounds whose onset was related to an injury, and there is no cause typical for chronic wounds (e.g., ischemia, venous insufficiency)
Neoplastic wounds	Wounds in which the presence of neoplastic cells was confirmed by histopathological examination or in which the examination was not performed, but the description of the surgical procedure that preceded the occurrence of the wound indicated incomplete resection of the neoplastic lesion
Nail fold wounds	Wounds of the nail fold associated with the pressure of the nail plate on the nail fold (so-called ingrown nail)
Other soft tissue infections	Wounds within soft tissues, in which no cause typical for chronic wounds qualifying to the above groups was found, and clinical symptoms indicated infection

All cultures were taken sterilely by deep-tissue biopsy (Hryniewicz et al. 2012; Tedeschi et al. 2017; García et al. 2020). The collection of the material was preceded by appropriate wound debridement. The activities in question were carried out under the standards applicable in the centre. All employees of the center have undergone mandatory training in the use of these standards.

Before starting each procedure, the peri-wound skin was cleansed using an Octenisan® glove (Schülke & Mayr GmbH, Germany) or cleaning foam and non-sterile gauze. Next, the wound was prepared for debridement using the sterile technique. From that moment, all activities were performed by staff wearing sterile gloves after hand disinfection. The intact skin around the wound was washed three times with an alcohol preparation (Kodan® (Schülke & Mayr GmbH, Germany) or Skinsept® Color (Ecolab Sp. z o.o., Poland)) using sterile gauze. The treatment area and the wound area were covered with sterile drapes, and adhesive drapes were used if the skin condition allowed it. The wound bed and margins were subjected to lavaseptics prior to culture sampling. For lavaseptics, only sterile agents were used before the culture was taken, without adding a bactericidal substance, usually saline. Then, depending on the clinical condition of the wound, it was debrided using one or more tools. For this purpose, the following were used: Schülke® or Debrisoft® wound pad, Volkmann type curette, Luer Bone Rongeur, dissection scissors, and scalpels (Mrozikiewicz-Rakowska et al. 2021). The procedure used assumed the collection of material for examination without necrotic tissues or other impurities, i.e., from a possibly well-debrided wound bed. The standard technique used to collect the culture was deep-tissue biopsy, recommended as a diagnostic procedure in the center represented by the authors (Tedeschi et al. 2017; García et al. 2020). Cultures were collected using a sterile Volkmann curette. In the case of bone cultures, bone tissue was collected using a Luer Bone Rongeur. The collected material was transferred from the tool to the transport medium. In order to minimize the risk of contamination of the collected material, in the case of e.g., an applicator with a viscose swab and the collected material touching the edge of the tube, the collection process was repeated. At least one sample was taken from each patient at one appointment. In 68% of patients, one sample was collected during one appointment, and two samples were collected in 30% of patients. In the remaining 2% of cases, at least three samples were taken from the same wound for bacteriological examination, with a maximum of 12 samples taken from one patient at a time.

The material collected for bacteriological examination was transferred to an Amies transport medium and then sent to one (always the same) laboratory for microbiological diagnostics. Until reception, the samples were stored at room temperature. The medium used allows the sample to be stored at room temperature for up to 72 hours; however, according to the manufacturer's recommendation, for optimal growth, the culture should be started within 24 hours after material collection (Amies – Agar Swabs; deltalab, Spain).

In the evaluated material, the average storage time of samples from collection to registration in the laboratory was 12 hours and 46 minutes. The average culture time from registration to the result was 4 days and 13 hours.

Microbiological analysis. The collected biopsies were cultured on the following media: chocolate agar with PolyViteX (incubation in an atmosphere of increased carbon dioxide concentration – 5%), chromogenic agar for urine culture, and initial identification of *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Citrobacter* spp., *Proteus* spp., *Morganella* spp., *Providencia* spp., Columbia Sheep Blood, Columbia CNA (nalidixic acid and colistin), and Schaedler's broth with 0.02% agar and vitamin K_3. All media were incubated for 48 h at 36 ± 1°C. Fungal cultures were grown on Sabouraud medium with gentamicin and chloramphenicol and incubated at 30°C for five days. When testing for anaerobic bacteria, the material was cultured on Schaedler's medium with 5% sheep blood, and Schaedler's agar with neomycin and vancomycin with 5% sheep blood and incubated under anaerobic conditions. Schaedler's broth after 48 hrs. was subcultured onto Columbia agar with sheep blood as an aerobic control and Schaedler's agar with 5% sheep blood and Schaedler's agar with neomycin and vancomycin with 5% sheep blood. The final negative result for aerobic bacteria was issued after 48 hours, and for anaerobic bacteria and fungi after 5 days of incubation.

The cultured microorganisms were identified using biochemical tests on the VITEK® 2 Compact apparatus (bioMérieux, France) until 2016; the identification was also performed using the mass spectrometry method (VITEK® MS by bioMérieux (France) until 2018, and from February 2018 MALDI-TOF MS by Bruker (USA)). Latex tests were also used to determine serogroup affiliation according to the Lancefield classification for beta-hemolytic streptococci.

The cultured isolates were tested for susceptibility by broth microdilution using automated identification, and susceptibility testing systems on VITEK® 2 Compact (bioMérieux, France) and MicroScan WalkAway *plus* (Beckman Coulter, USA) instruments. In addition, drug susceptibility was tested by the disc diffusion method, using strips impregnated with a gradient of antibiotic concentrations and microdilutions in broth in the case of colistin. Resistance phenotypes were verified by methods specified by the National Reference Center for Antimicrobial Susceptibility Testing. Susceptibility results were interpreted based on EUCAST guidelines (EUCAST 2017).

Table II
The quantitative distribution of the type of material used for microbiological examination depending on the type of wound.

Type of wound	Site of the material sampling			Total 100%
	Soft tissue scrapings	Bone scrapings	Other	
	n (%)	n (%)	n (%)	n
Neuropathic diabetic food syndrome	925 (80.5)	217 (18.9)	7 (0.6)	1,149
Ishemic diabetic food syndrome	139 (72.8)	51 (26.7)	1 (0.5)	191
Mixed diabetic food syndrome	434 (83.6)	85 (16.4)	0 (0.0)	519
Foot deformity with neuropathy	102 (77.9)	29 (22.1)	0 (0.0)	131
Wounds in the course of critical limb ischemia	502 (81.8)	112 (18.2)	0 (0.0)	614
Venous leg ulcers	554 (97.9)	12 (2.1)	0 (0.0)	566
Mixed leg ulcers	108 (95.6)	4 (3.5)	1 (0.9)	113
Lymphatic leg and/or foot ulcers	89 (100.0)	0 (0.0)	0 (0.0)	89
Pressure ulcers	126 (83.4)	25 (16.6)	0 (0.0)	151
Postoperative wounds	111 (90.2)	11 (8.9)	1 (0.8)	123
Post-traumatic wounds	74 (85.1)	13 (14.9)	0 (0.0)	87
Neoplastic wounds	38 (100.0)	0 (0.0)	0 (0.0)	38
Nail fold wounds	47 (95.9)	2 (4.1)	0 (0.0)	49
Other soft tissue infections	96 (99.0)	1 (1)	0 (0.0)	97
Total	3,345 (85.4)	562 (14.3)	10 (0.3)	3,917

Statistical analysis. The paper presents the results in the form of the number of cultured microorganisms and their relative incidence as percentages. Calculations were performed using IBM® SPSS® Statistics version 26.0.

Results

In the analyzed population of patients with infected chronic wounds, the largest group (n = 446; 36.6%) were patients with all types of DFS and patients with VLU (n = 225; 18.4%). The quantitative distribution of the material used for microbiological examination depending on the type of wound is presented in Table II.

Soft tissues were used in 85.4% (n = 3,345) of cultures. Bone scrapings accounted for 14.3% (n = 562) of cultures. This material was collected when bones were visualized at the bed of an infected wound, most often in the case of ischemic DFS (26.7%) and the group of wounds defined as foot deformity with neuropathy (22.1%).

Table I also includes microbiological tests for which the material is not specified. They accounted for 0.3% (n = 10) of all tests and concerned cultures for which the type of material was not specified in the available documentation or the material was not collected by deep-tissue biopsy due to the small amount of available material or anatomical conditions.

Overall, 2,837 aerobic (72.4%), 1,062 anaerobic (27.1%), and 18 mycological (0.5%) cultures were performed.

Among all performed tests, every third culture (n = 1,235; 31.6%) was negative, i.e., one in which no microorganism was grown. A quantitative summary of negative cultures depending on the type of wound is presented in Fig. 1.

Noteworthy is a relatively large number of negative cultures obtained from chronic post-traumatic wounds (42.5%), other infections of soft tissues (40.2%), and post-operative wounds (38.2%). Among the aerobic cultures, only 12.7% of the results were negative, and in the group of anaerobic tests, the lack of bacterial growth was reported in as many as 81.2% of the tests. In the remaining tests, from 1 to 7 pathogens were isolated. The quantitative distribution of tests depending on the number of isolated microorganisms in relation to the type of culture (aerobic, anaerobic, or mycological) is presented in Table III. On average, 1.2 pathogens were grown from one culture. After the exclusion of negative cultures from the analysis, an average of 1.75 pathogens was obtained.

Following the standards in force in the microbiological laboratory in which all the analyzed tests were performed, samples from which four or more isolates were cultured were marked as suspected of contamination. In the analyzed material, 4 and more isolates were cultured in 153 samples (all in aerobic tests) which constituted 5.4% of the entire group of aerobic cultures. It was confirmed that all standards of wound debridement for sampling and the sampling itself were met. After consultation with the micro-

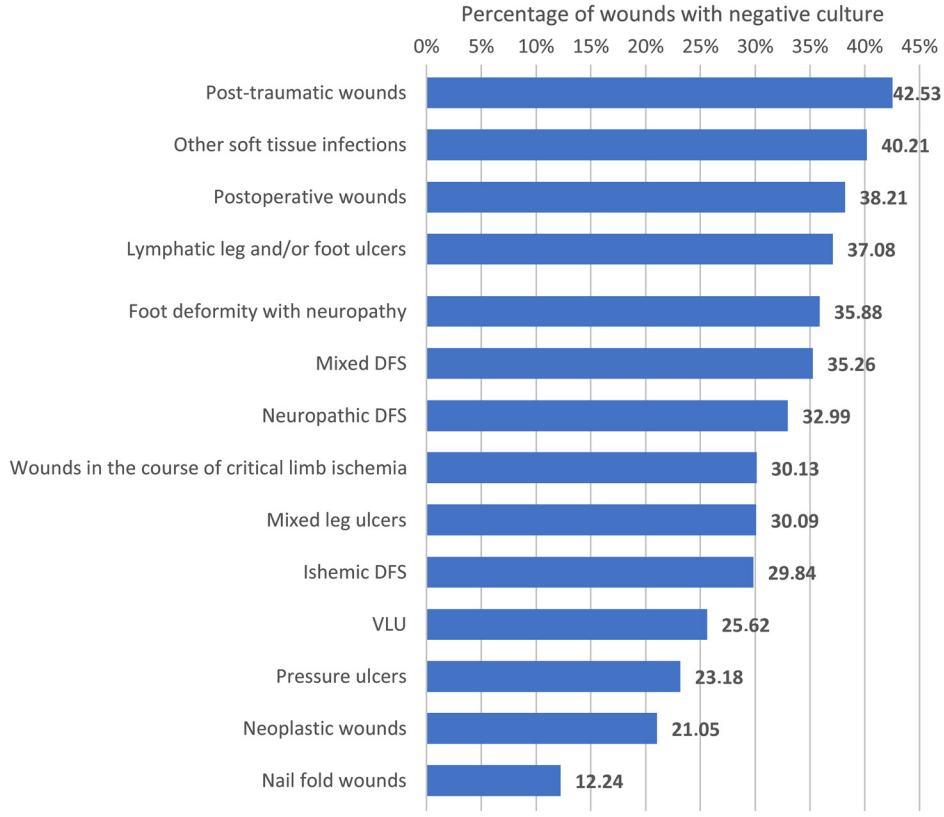

Fig. 1. Quantitative summary of negative cultures depending on the type of wound.
DFS – diabetic food syndrome, VLU – venous leg ulcers

biology laboratory, it was concluded that the cultured bacteria could be considered an actual component of the infected wound microbiome. There was no suspicion of contamination in the description of the anaerobic culture results. Most often, four or more organisms were cultured from wound material in VLU (7.6% of all studies in this group), critical ischemia of the lower extremities (5.5% of all studies in this group) and neuropathic DFS (2.9% of all studies within this group).

The distribution of the number of cultured microorganisms in one culture depending on the type of wound is presented in Fig. 2.

A total of 120 different species of microorganisms were identified in the materials tested. The 15 most frequently isolated microorganisms from the analyzed material are presented in Fig. 3. *Staphylococcus aureus* was found in 31.3% of aerobic cultures, *Enterococcus faecalis* in 26.3%, *Escherichia coli* in 13.3%, and *Pseudomonas aeruginosa* in 12.5%.

Table III
The quantitative distribution of tests depending on the number of isolated microorganisms with the type of culture (the shaded area corresponds to the isolation of four or more microorganisms).

Number of isolated microorganisms	Type of culture						Mycological	
	Aerobic		Anaerobic		Total			
	n	%	n	%	n	%	n	%
0	361	12.7	862	81.2	12	66.7	1,235	31.6
1	1,217	42.9	167	15.7	6	33.3	1,390	35.5
2	734	25.9	30	2.8	0	0.0	764	19.5
3	372	13.1	3	0.3	0	0.0	375	9.5
4	124	4.4	0	0.0	0	0.0	124	3.2
5	25	0.9	0	0.0	0	0.0	25	0.6
6	3	0.1	0	0.0	0	0.0	3	0.1
7	1	0.0	0	0.0	0	0.0	1	0.0
Total	2,837	100.0	1,062	100.0	18	100.0	3,917	100.0

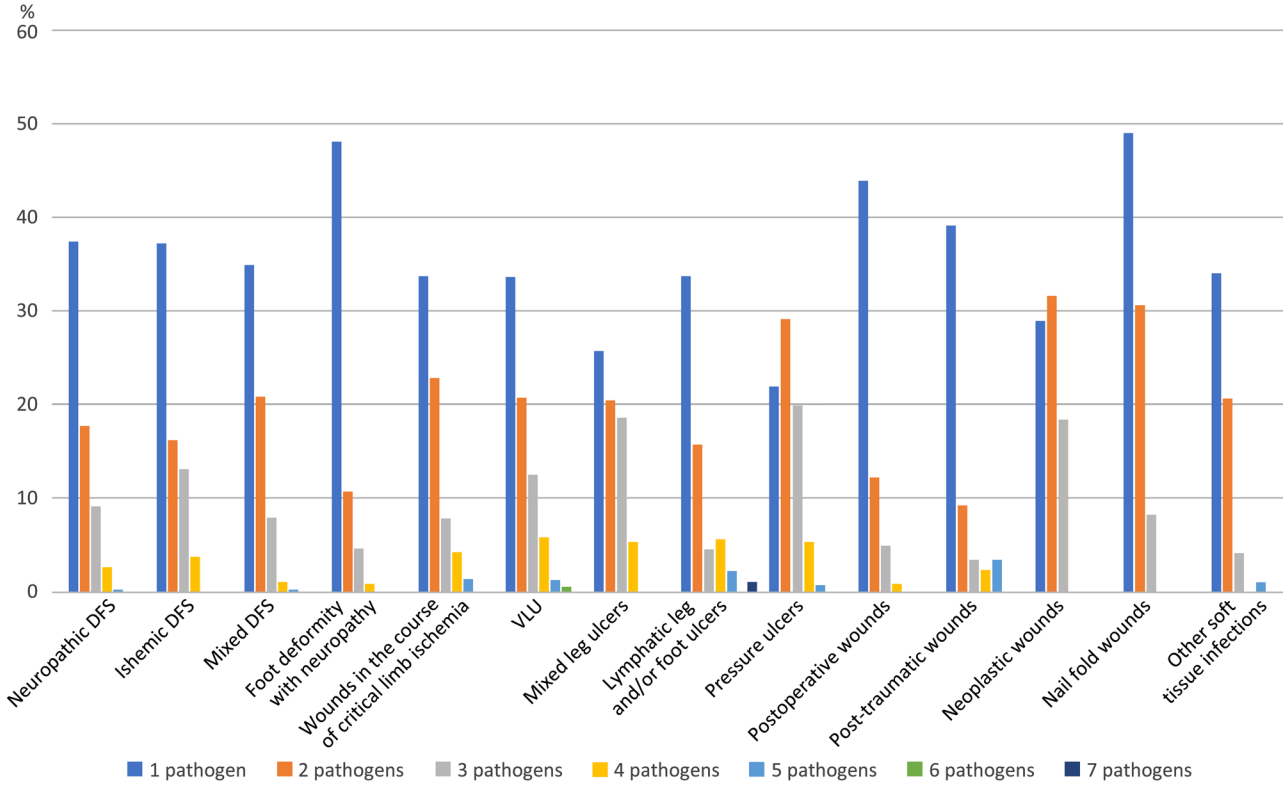

Fig. 2. The distribution of the number of cultured microorganisms in one culture depending on the type of wound.

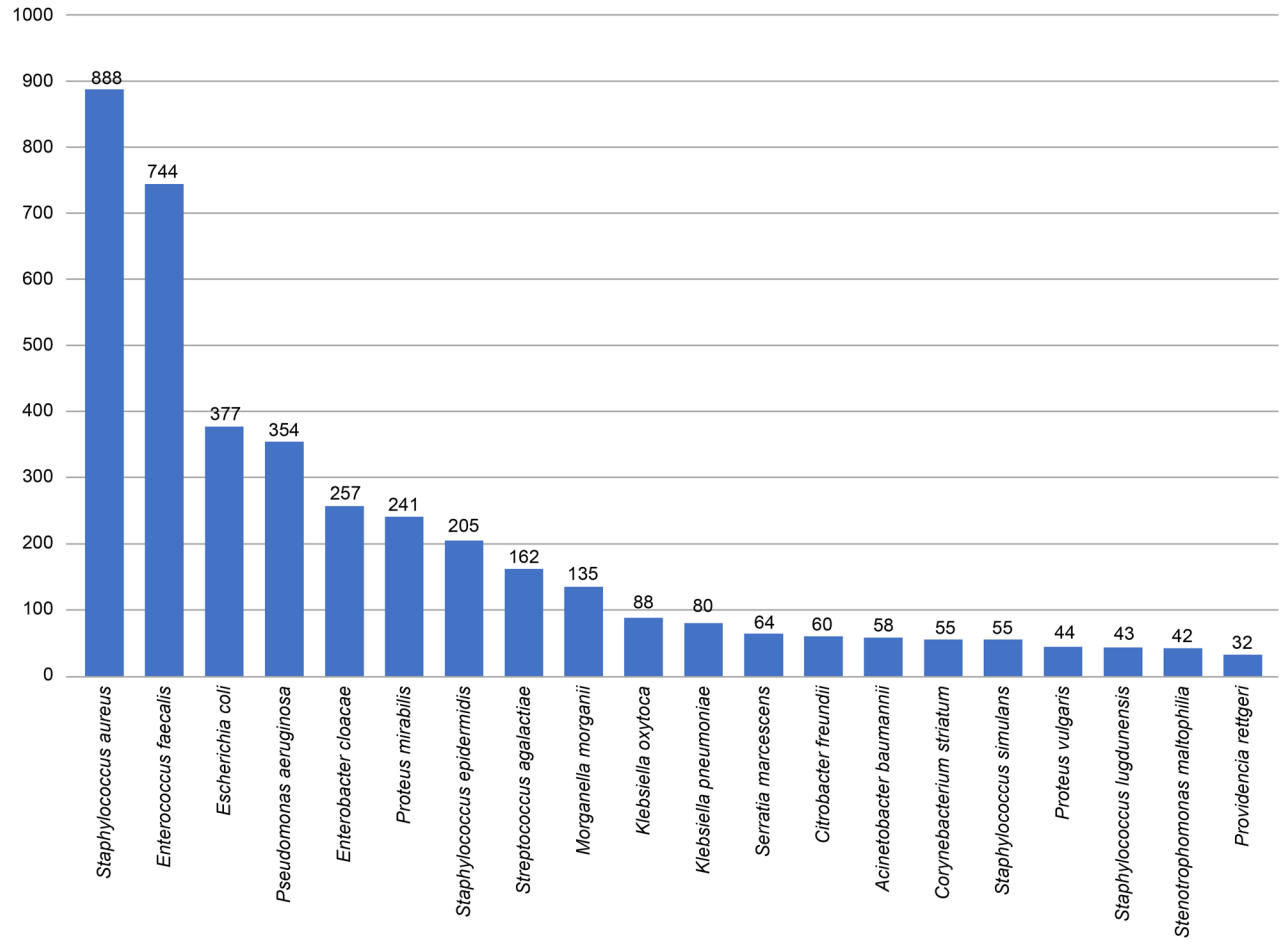

Fig. 3. Most frequently isolated pathogens in the materials.

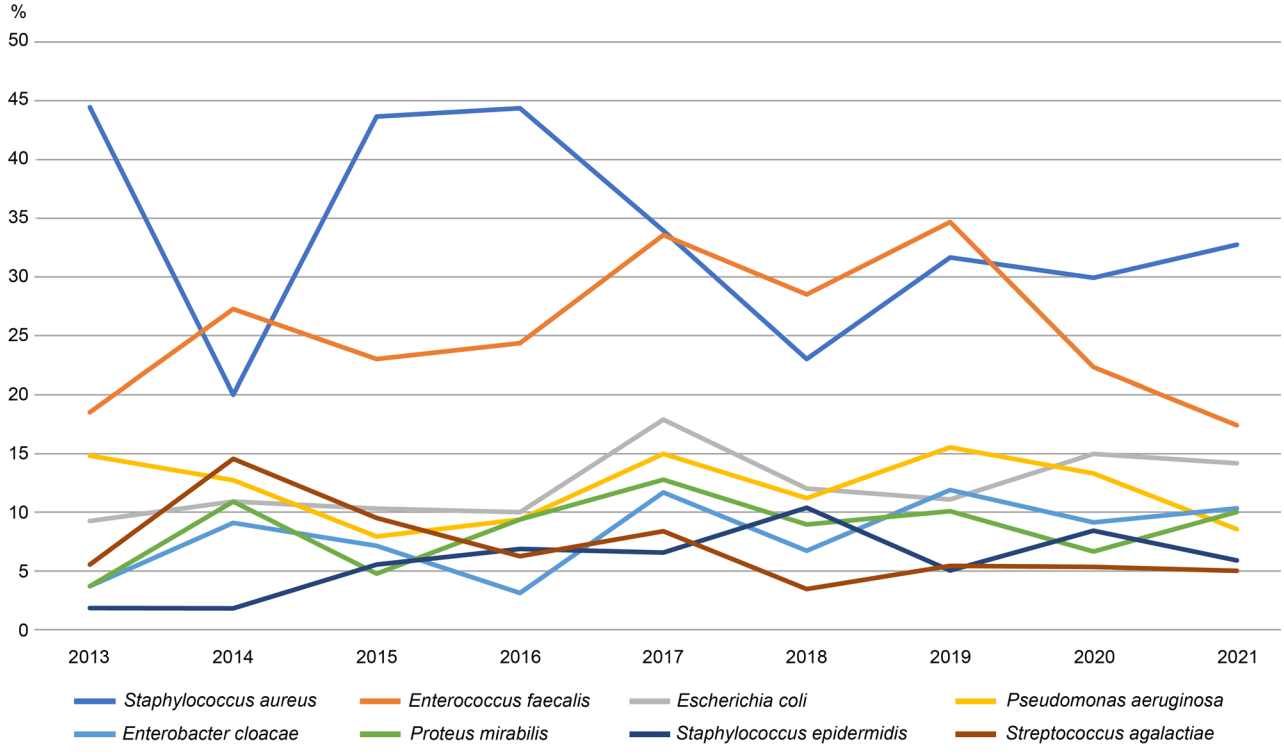

Fig. 4. The dynamics of changes in the etiology of chronic wound infections.

The most frequently isolated organism was *S. aureus*. Eighty-six percent of these isolates were sensitive to methicillin (MSSA – methicillin-sensitive *Staphylococcus aureus*). In the remaining 14% of cases, *S. aureus* resistant to methicillin (MRSA – methicillin-resistant *Staphylococcus aureus*) were isolated. *S. aureus* was most common as an etiological agent of chronic wound infection in nail fold wounds (71.4%), mixed leg ulcers (35.6%), lymphatic leg and/or foot ulcers (35.5%), and VLU (33.9%). Further in the frequency of occurrence, according to the type of wound, this microorganism was a component of the microbiome in infections 34.9% of wounds during critical ischemia of the lower limbs, 34.4% of post-traumatic wounds, and 32.9% of wounds in neuropathic DFS.

In addition to *S. aureus*, coagulase-negative *Staphylococci* (CoNS) were observed in the materials studied. The presence of *Staphylococcus epidermidis* (7.3%), *Staphylococcus simulans* (1.9%), *Staphylococcus lugdunensis* (1.6%), and *Staphylococcus hemolyticus* (0.7%) was most frequently found. Considering the whole group of coagulase-negative staphylococci, we isolated them from 13.7% of aerobic cultures.

The second most common microorganism was *E. faecalis*. Vancomycin-resistant *Enterococcus* (VRE) was found in 2.4% of these isolates. *E. faecalis* was the most common component of the wound microbiome in ischemic DFS (39.1%), neoplastic wounds (37.9%), pressure ulcers (34.8%), leg and/or foot lymph ulcers (29.0%), and VLU (28.0%).

It is worth noting that another microorganism frequently found in infected pressure ulcers (33.9%), as well as in infected neoplastic wounds (27.6%) was *E. coli*.

In turn, *P. aeruginosa* was most often isolated from the material obtained from infected leg ulcers of mixed (42.5%) or venous (24.8%) etiology.

The analysis presented above indicated that the most common etiological factor of infections in the course of neuropathic DFS was one of the four following microorganisms *S. aureus* (32.85%), *E. faecalis* (26.96%), *E. coli* (9,75%), *Streptococcus agalactiae* (8.66%). In the mixed DFS, the microorganisms most often cultured were: *E. faecalis* (28.00%), *S. aureus* (22.86%), *E. coli* (15.14%), *S. epidermidis* (11.43%). The most common cultures in the wounds in the course of critical ischemia of the lower limbs were *S. aureus* (34.92%), *E. faecalis* (25.62%), *P. aeruginosa* (13.38%), *E. coli* (12.24%), and *E. cloacae* (6.6%). The most common cultures in the wounds in patients with venous leg ulcers were *S. aureus* (33.64%), *E. faecalis* (28.04%), *P. aeruginosa* (24.77%), and *E. coli* (15.89%).

The data presented above concerned the cumulative results of microbiological tests carried out in outpatients from 2013 to 2021. However, it was observed that in the following years, the microbiological profile of infections and the percentage share of individual pathogens underwent visible changes. Fig. 4 presents the dynamics of these changes concerning eight microorganisms most frequently found in the results of microbiological tests in the analyzed period. Throughout the

period covered by the analysis, the most frequently detected microorganisms were *S. aureus* (31.3%) and *E. faecalis* (26.3%).

Discussion and Conclusions

Treatment of chronic wounds requires knowledge of their microbiome to implement appropriate antibacterial treatment. Recommendations for empirical antibiotic therapy are often based on data from centres located in remote parts of the world with different climates and concern other types of microorganisms detected in locally infected wounds (Abdu et al. 2019; Schaumburg et al. 2022).

In addition to the significantly smaller number of patient groups analyzed in such studies, collecting material for microbiological tests often needs to meet the current standards. To the best of our knowledge, the material presented in this study is the largest database of cultures taken by deep-tissue biopsy from a single center in Poland. Special care was given to the material quality sent to the microbiology laboratory analyzed in this study. Standard technique used to collect the culture in patients with chronic wounds is deep-tissue biopsy (Hryniewicz et al. 2012; Tedeschi et al. 2017; García et al. 2020). It is worth emphasizing that the material for microbiological tests was collected only from wounds with clinical signs of infection. Colonized wounds were excluded from the evaluation.

In the analyzed material, a relatively large number of negative cultures was observed, obtained from chronic post-traumatic wounds (42.5%) and postoperative wounds (38.2%). Gitajn et al. (2016) described 9% of negative cultures among patients with infected postoperative wounds after bone fracture stabilization procedures. Rondas et al. (2015) found 18.9% of negative cultures taken from chronic wounds with clinical signs of infection.

In the presented material, only 12.7% of the tests were negative among aerobic cultures. In contrast, in the group of cultures performed for anaerobic bacteria, no growth was found in as many as 81.6% of the tests. It may result from a low prevalence of anaerobic microbiota in the etiology of diagnosed infections. However, the impact of using non-optimal transport media in the microbiology laboratory serving the center cannot be ruled out. Routine use of specialized tubes (e.g., Venturi Transystem®) or liquid transport media (e.g., eSwab®) may increase the detection of anaerobic microorganisms responsible for infections of chronic wounds (Hudspeth et al. 1997; van Horn et al. 2008; Tyrrell et al. 2016; Demuyser et al. 2018).

In the analyzed material, four or more types of microorganisms (maximum seven) were isolated in 153 aerobic culture samples (5.4% of the tested sample). Such a small percentage may indicate a significantly good debridement of wounds for culture collection and a sufficiently high data reliability. On average, 1.2 pathogens were isolated from one culture. In studies by Han et al. (2011), standard bacteriological cultures from chronic wounds showed the presence of an average of three pathogens. Interestingly, high-throughput next-generation sequencing of the same material detected an average of 17 microbes in each wound. Although the cited work was based on analyzing samples from only 15 patients, it made us realize how complex and diverse the wound microbiome can be, potentially responsible for its infection (Han et al. 2011).

A total of 120 different microorganisms were identified in the analyzed material. *S. aureus* (31.3%) had the largest share of infections of chronic wounds. This observation is consistent with the data presented in the literature. In the meta-analysis by Howell-Jones et al. (2005) *S. aureus* and coagulase-negative staphylococci were the dominant pathogens isolated from infected wounds.

S. aureus was isolated from infected leg ulcers with an incidence of 43%, while *S. epidermidis* was detected in 14% of VLUs and 20.6% of DFS cultures (Howell-Jones et al. 2005). Similarly, coagulase-negative *Staphylococci* (CoNS) were relatively common in our analysis. The entire CoNS group accounted for 13.7% of all isolated microorganisms in our material. The most common CoNS were: *S. epidermidis*, *S. simulans*, *S. lugdunensis*, and *S. hemolyticus*.

In the study by Puca et al. (2021), *S. aureus* was present in 38.5% of samples collected using the "Z" technique, obtained from wounds in outpatients. Also, in our material, *S. aureus* was the most common microorganism, and its incidence in the examined cultures was similar (31.3%).

In the already cited study, Rondas et al. (2015) isolated most frequently: *S. aureus* (51.1%), *S. agalactiae* (12.2%), *P. aeruginosa* (8.9%), *E. coli* (7.8%), and *P. mirabilis* (7.8%) regardless of the type of wound. *S. aureus*, *E. coli*, and *P. aeruginosa* were also dominant in our material. Significant discrepancies concerned *S. agalactiae*. This microorganism was detected most often among all streptococcal in our material but significantly less often than in the cited publication (5.7% vs. 12.2%). *E. faecalis* was the second pathogen after *S. aureus* and was present in 26.2% of the tested samples from our center, compared to 6.7% of pathogens isolated from infected chronic wounds in the study by Rondas et al. (2015). Also, MacDonald et al. (2002) observed a higher incidence of *S. aureus* (42%) and *E. faecalis* (29%) in infected wounds during DFS.

In our material, methicillin-resistant *S. aureus* (MRSA) accounted for 14.0% of all *S. aureus* isolates. Data from the literature indicate a much higher percent-

age of these isolates in infections of chronic wounds. In the study by Yates et al. (2009), MRSA accounted for as much as 39.6% of all isolated *Staphylococci aureus*, and in Kasithevar et al. (2017) this percentage was 48.1%.

Anaerobic organisms were also observed among the microorganisms detected in infected chronic wounds. Positive cultures for anaerobes were obtained in 18.8% of cultures. These affected 13.2% of VLU samples and 18.9% of DFS wound samples (all types combined), respectively. Trengove et al. (1996) detected anaerobes in a quarter of samples from infected and uninfected VLU. In the observations of MacDonald et al. (2002), they accounted for only 6% of isolates from infected wounds in the course of DFS, while in the work of Rondas et al. (2015), anaerobic bacteria were isolated from only 2% of chronic wounds. In this context, an interesting observation was made by Bowler and Davies (1999), who found anaerobes in as many as 82% of infected leg ulcers. Noteworthy, this study dates back to 1999, when different standards of wound debridement for material collection and microbiological examination were in force. The wounds included in this study were only cleansed with saline before the culture was taken. The cultures were collected using techniques similar to Levin's, including necrotic tissue. Since the results of the cited study differ significantly from those presented by other authors and us, it should be assumed that they reported wound-colonizing microorganisms and not those responsible for the infection.

Although proper surgical debridement of the wound is of crucial importance for treating chronic wounds, in some clinical cases, it is also necessary to include antimicrobial treatment. The idea is then to use targeted therapy. However, this is possible only after obtaining the microbiological test result. In many cases, the rate of infection-induced inflammation causes rapid tissue loss. Hence the need for immediate antibacterial treatment. In selecting antibiotics in the empirical therapy of chronic wounds, we rely on standards developed based on data from outside Poland. The constantly growing population of patients with infected wounds, the growing resistance of microorganisms, and the shortage of optimal systemic solutions require several actions to organize the management of this group of patients (WHO 2019; World Union of Wound Healing Societies 2020).

Further analysis of the extensive database of reliable cultures obtained by deep-tissue biopsy is crucial to create new recommendations for empirical antibacterial treatment of infected chronic wounds, especially in terms of drug susceptibility of isolated microorganisms.

Abbreviations

CoNS – coagulase-negative *Staphylococci*
DFS – diabetic foot syndrome
IDSA – Infectious Diseases Society of America
MRSA – methicillin-resistant *Staphylococcus aureus*
MSSA – methicillin-sensitive *Staphylococcus aureus*
VLU – venous leg ulcers
VRE – vancomycin-resistant *Enterococcus*

ORCID

Marcin Malka https://orcid.org/0000-0003-3605-6789
Magdalena Chojak https://orcid.org/0009-0005-8329-0270
Aleksandra Dyczewska https://orcid.org/0009-0008-4772-3675
Tomasz Grzela https://orcid.org/0000-0001-8519-4868
Arkadiusz Krakowiecki https://orcid.org/0009-0003-2197-1535
Katarzyna Pawlik https://orcid.org/0009-0007-7521-8345
Aleksandra Paź https://orcid.org/0009-0002-2511-6811
Marek Pławski https://orcid.org/0009-0000-2163-2777
Mariusz Wądołek https://orcid.org/0009-0003-4857-1451
Agnieszka Wołowicz https://orcid.org/0009-0002-8761-5018

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Abdu AB, Egbagba J, Fente BG. Identification and antimicrobial susceptibility profile of bacterial pathogens isolated from wound infections in a tertiary hospital, Bayelsa, South southern, Nigeria. Trop J Pathol Microbiol. 2019 Dec;5(12):966–975. https://doi.org/10.17511/jopm.2019.i12.01

Bowler PG, Davies BJ. The microbiology of infected and noninfected leg ulcers. Int J Dermatol. 1999 Aug;38(8):573–578. https://doi.org/10.1046/j.1365-4362.1999.00738.x

Demuyser T, De Geyter D, Van Dorpe D, Vandoorslaer K, Wybo I. Extensive evaluation of fastidious anaerobic bacteria recovery from the Copan eSwab® transport system. J Microbiol Methods. 2018 Jan; 144:73–78. https://doi.org/10.1016/j.mimet.2017.11.009

EUCAST. EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0. Basel (Switzerland): The European Committee on Antimicrobial Susceptibility Testing; 2017 [cited 2021 Jan 29]. Available from https://www.eucast.org/resistance_mechanisms/

EWMA. Hard-to-heal wounds: a holistic approach. European Wound Management Association (EWMA). London (UK): MEP Ltd.; 2008 [cited 2023 Jan 02]. Available from https://ewma.conference2web.com/#resources/215693

García HAL, Febles SRJ, García OY, Moliner MC. [Cultivo mediante hisopado superficial versus cultivo de la biopsia de tejidos profundos en la infección del pie diabético] (in Spanish). Rev Méd Electrón. 2020;42(5).

Gitajn IL, Heng M, Weaver MJ, Ehrlichman LK, Harris MB. Culture-negative infection after operative fixation of fractures. J Orthop Trauma. 2016 Oct;30(10):538–544. https://doi.org/10.1097/BOT.0000000000000618

Graves N, Phillips CJ, Harding K. A narrative review of the epidemiology and economics of chronic wounds. Br J Dermatol. 2022 Aug; 187(2):141–148. https://doi.org/10.1111/bjd.20692

Han A, Zenilman JM, Melendez JH, Shirtliff ME, Agostinho A, James G, Stewart PS, Mongodin EF, Rao D, Rickard AH, et al. The importance of a multifaceted approach to characterizing the microbial flora of chronic wounds. Wound Repair Regen. 2011 Sep–Oct;19(5):532–541. https://doi.org/10.1111/j.1524-475X.2011.00720.x

Heyer K, Herberger K, Protz K, Glaeske G, Augustin M. Epidemiology of chronic wounds in Germany: analysis of statutory health insurance data. Wound Repair Regen. 2016 Mar;24(2):434–442. https://doi.org/10.1111/wrr.12387

Howell-Jones RS, Wilson MJ, Hill KE, Howard AJ, Price PE, Thomas DW. A review of the microbiology, antibiotic usage and resistance in chronic skin wounds. J Antimicrob Chemother. 2005 Feb; 55(2):143–149. https://doi.org/10.1093/jac/dkh513

Hryniewicz W, Kulig J, Ozorowski T, Mól A, Kulig P, Wąchol D. [Stosowanie antybiotyków w wybranych zakażeniach skóry i tkanek miękkich] (in Polish). Warsaw (Poland): Narodowy Instytut Leków; 2012. [cited 2023 Jan 12]. Available from http://antybiotyki.edu.pl/wp-content/uploads/Rekomendacje/rekomendacje-stosowanie-ant-w-wybranych-zak-skory.pdf

Hudspeth MK, Citron DM, Goldstein EJ. Evaluation of a novel specimen transport system (Venturi Transystem) for anaerobic bacteria. Clin Infect Dis. 1997 Sep;25(Suppl 2):S132–S133. https://doi.org/10.1086/516198

Kasithevar M, Periakaruppan P, Muthupandian S, Mohan M. Antibacterial efficacy of silver nanoparticles against multi-drug resistant clinical isolates from post-surgical wound infections. Microb Pathog. 2017 Jun;107:327–334. https://doi.org/10.1016/j.micpath.2017.04.013

Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2012 Jun;54(12):e132-73. https://doi.org/10.1093/CID/CIS346

MacDonald YGD, Hait H, Lipsky B, Zasloff M, Holroyd K. Microbiological profile of infected diabetic foot ulcers. Diabetic Med. 2002 Dec;19(12):1032–1034. https://doi.org/10.1046/j.1464-5491.2002.00696_1.x

Mrozikiewicz-Rakowska B, Jawień A, Szewczyk M, Sopata M, Korzon-Burakowska A, Dziemidok P, Gorczyca-Siudak D, Tochman-Gawda A, Krasiński Z, Rowiński O, et al. [Postępowanie z chorym z zespołem stopy cukrzycowej – wytyczne Polskiego Towarzystwa Leczenia Ran 2021: część 1] (in Polish). Leczenie Ran. 2021; 18(3):71–114. https://doi.org/10.5114/lr.2021.110995

Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, Prezioso S, Muraro R, Di Giovanni P. Microbial species isolated from infected wounds and antimicrobial resistance analysis: data emerging from a three-years retrospective study. Antibiotics. 2021 Sep 24;10(10):1162. https://doi.org/10.3390/antibiotics10101162

Rondas AA, Halfens RJ, Schols JM, Thiesen KP, Trienekens TA, Stobberingh EE. Is a wound swab for microbiological analysis supportive in the clinical assessment of infection of a chronic wound? Future Microbiol. 2015;10(11):1815–1824. https://doi.org/10.2217/fmb.15.97

Schaumburg F, Vas Nunes J, Mönnink G, Falama AM, Bangura J, Mathéron H, Conteh A, Sesay M, Sesay A, Grobusch MP. Chronic wounds in Sierra Leone: pathogen spectrum and antimicrobial susceptibility. Infection. 2022 Aug;50(4):907–914. https://doi.org/10.1007/s15010-022-01762-6

Sopata M, Jawień A, Mrozikiewicz-Rakowska B, Augusewicz Z, Bakowska M., Samson I, Gabriel M, Grzela T, Karpiński T, Kuberka I, et al. [Wytyczne postępowania miejscowego w ranach niezakażonych, zagrożonych infekcją oraz zakażonych – przegląd dostępnych substancji przeciwdrobnoustrojowych stosowanych w leczeniu ran. Zalecenia Polskiego Towarzystwa Leczenia Ran] (in Polish). Leczenie Ran. 2020;17(1):1–21. https://doi.org/10.5114/lr.2020.96820

Tedeschi S, Negosanti L, Sgarzani R, Trapani F, Pignanelli S, Battilana M, Capirossi R, Brillanti Ventura D, Giannella M, Bartoletti M, et al. Superficial swab versus deep-tissue biopsy for the microbiological diagnosis of local infection in advanced-stage pressure ulcers of spinal-cord-injured patients: a prospective study. Clin Microbiol Infect. 2017 Dec;23(12):943–947. https://doi.org/10.1016/j.cmi.2017.04.015

Trengove NJ, Stacey MC, McGechie DF, Stingemore NF, Mata S. Qualitative bacteriology and leg ulcer healing. J Wound Care. 1996 Jun;5(6):277–280. https://doi.org/10.12968/jowc.1996.5.6.277

Tyrrell KL, Citron DM, Leoncio ES, Goldstein EJ. Comparison of the Copan eSwab System with an agar swab transport system for maintenance of fastidious anaerobic bacterium viability. J Clin Microbiol. 2016 May;54(5):1364–1367. https://doi.org/10.1128/JCM.03246-15

Van Horn KG, Audette CD, Sebeck D, Tucker KA. Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. J Clin Microbiol. 2008 May;46(5):1655–1658. https://doi.org/10.1128/JCM.02047-07

WHO. Ten threats to global health in 2019 [Internet]. Geneva (Switzerland): World Health Organization; 2019 [cited 2023 Jan 12]. Available from https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019

World Union of Wound Healing Societies. Optimising wound care through patient engagement. London (UK): Wounds International; 2020 [cited 2023 Jan 12]. Available from: www.woundsinternational.com

Yates C, May K, Hale T, Allard B, Rowlings N, Freeman A, Harrison J, McCann J, Wraight P. Wound chronicity, inpatient care, and chronic kidney disease predispose to MRSA infection in diabetic foot ulcers. Diabetes Care. 2009 Oct;32(10):1907–1909. https://doi.org/10.2337/dc09-0295