

MICROBIOLOGY IN CHRONIC WOUNDS

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Abstract

Introduction: Chronic leg wounds are colonized by diverse microbial communities, including resident and transient flora, which significantly influence healing outcomes. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are frequently implicated, with polymicrobial infections contributing to delayed healing and antimicrobial resistance.

Aim: To investigate the microbiological agents in chronic leg wounds.

Materials and Methods: A prospective study was conducted at the University Clinic for Dermatovenerology and the Institute of Microbiology and Parasitology, Ss. Cyril and Methodius University, Skopje, following ethics approval and written informed consent. One hundred adult patients with chronic wounds localized on lower extremities (>4 weeks, >1 cm²) were included. Exclusion criteria encompassed malignancy, inflammatory dermatoses, burns, recent antibiotic therapy, and acute infections. Clinical evaluation, patient questionnaires, and wound swab samples were collected for microbial isolation and identification.

Results: Among 106 chronic wounds, microbial growth was observed in 67 samples. *Staphylococcus aureus* (MSSA 35.8%, MRSA 7.5%) was the most frequently isolated pathogen, followed by *Pseudomonas aeruginosa* (19.4%), *Enterobacter* spp. (19.4%), and *E. coli* (17.9%). Polymicrobial infections were detected in 29.8% of cases, with MSSA combined with *P. aeruginosa* or *Enterobacter* species being the most common associations.

Conclusion: Chronic leg wounds are frequently colonized by a heterogeneous mix of microorganisms, with *S. aureus* and *P. aeruginosa* predominating. Polymicrobial infections pose challenges for treatment and may influence healing outcomes. Comprehensive microbiological evaluation is essential for guiding targeted antimicrobial therapy.

Keywords: Chronic wounds; microbiology.

Introduction

The skin hosts complex microbial communities that form the cutaneous microbiota, which plays a key role in maintaining skin homeostasis and contributing to the pathogenesis of various dermatological conditions, including chronic wounds [1,2].

The healthy skin microbiome is composed of resident and transient microorganisms, predominantly commensals such as *Corynebacterium*, *Propionibacterium*, and *Staphylococcus*, with Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes representing the major bacterial phyla [3-5].

Microbial composition is influenced by anatomical site, host-related factors, and environmental conditions [1,5].

Chronic wounds are defined as wounds with impaired healing persisting beyond 3–6 weeks, in which infection and biofilm formation play a central role in delayed tissue repair [6].

Culture-independent analyses have demonstrated that chronic wounds harbor complex and dynamic microbial communities, commonly including *Staphylococcus*, *Pseudomonas*, *Corynebacterium*, and *Enterococcus* species [7,8]. Although microbial profiles appear similar across wound types and demographic groups, temporal shifts in wound microbiota have been associated with improved healing

outcomes, highlighting the importance of distinguishing pathogenic from beneficial microorganisms for prognosis and targeted therapeutic strategies [7,9,10].

Aim: To investigate the microbiological agents in chronic wounds.

Materials and methods: The prospective study was conducted at the University Clinic for Dermatovenerology and the Institute of Microbiology and Parasitology, Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, following approval by the local Ethics Committee. All participants provided written informed consent. The study included 100 adult patients of both sexes with chronic lower-extremity wounds, treated on an outpatient or inpatient basis.

Inclusion Criteria: Adults of both sexes with lower-extremity wounds persisting >4 weeks, wound size >1 cm², and who provided written informed consent.

Exclusion Criteria: Wounds caused by malignancy or inflammatory dermatoses, burns, recent (≤ 7 days) systemic or topical antibiotic therapy, inability to cooperate or lack of consent, acute phlebothrombosis or deep vein thrombosis, and acute soft tissue infections (cellulitis, erysipelas).

For each patient, a complete medical history, dermatological examination, and medical documentation related to the existing chronic wound on the lower extremities were obtained. Additionally, a questionnaire was completed for every participant, covering basic characteristics such as sex, age, nationality, occupation/hobbies, lifestyle habits, comorbidities with corresponding chronic therapy, and social factors.

A sample was taken directly from the wound for subsequent microbiological analysis aimed at isolating and identifying microbial strains. This sample was collected using a microbiological swab.

Isolation and identification of strains

From all samples collected from chronic wounds, a Gram-stained microscopic preparation was performed as an initial orientation to assess the condition of the wound, specifically the presence of bacteria (Gram-positive or Gram-negative), yeasts, and the presence or absence of leukocytes.

The samples were cultured on standard microbiological media (Columbia agar for aerobic and facultative anaerobic bacteria, Schedler agar for anaerobic bacteria, Sabouraud agar for yeasts and molds, and glucose broth). The inoculated media were incubated for 24 to 48 hours for the detection of aerobic and facultative anaerobic bacteria; 48 hours for anaerobic bacteria in anaerobic jars using the Gas Pack system; and up to 7 days for the detection of yeasts and molds [11].

Following incubation, Gram staining and standard biochemical tests were performed as needed for the final identification of microorganisms. In cases of atypical growth characteristics—particularly for inert bacteria, anaerobes, and fungi—an automated identification system (VITEK 2) was additionally used [11].

Results

Wound Microbiology

Out of the total 100 participants included in the study, 33 (33%) had a negative microbiological swab. In the remaining 67 (67%) patients, pathogenic microorganisms were isolated from the chronic wound (Figure 1)

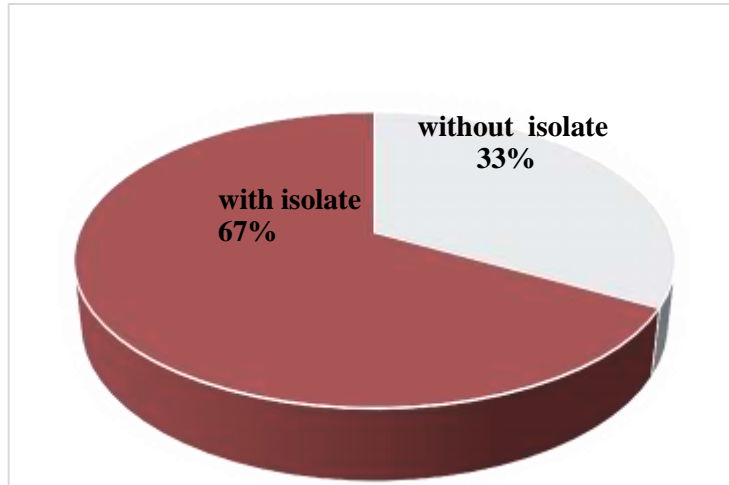


Figure 1. Presence of isolates in chronic wounds

From the chronic wounds analyzed in this study, the bacterial isolates identified are presented in Table 1.

The most frequently identified wound colonizers were MSSA (methicillin-sensitive *Staphylococcus aureus*) (35.82%), followed by *Pseudomonas aeruginosa* (19.4%), *Enterobacter* spp. (19.4%), and *Escherichia coli* (17.91%). Of all isolates, 40.21% belonged to Gram-positive bacteria, while 59.79% were Gram-negative.

The presence of polymicrobial colonization defined as more than one isolate was identified in 20 out of 67 wounds, corresponding to 29.85%. The most frequent combinations of isolates, each accounting for 2/20 cases (10%), were MSSA with *Pseudomonas aeruginosa* and MSSA with *Enterobacter* spp.

Table 1. Prevalence of specific strains in isolates from the first wound swab

Isolate (first swab)	Prevalence of Individual Strains in the Isolate from the First Swab			
	Count	Cumulative Count	Percent	Cumulative Percent
<i>MRSA</i>	2	2	2,98507	2,9851
<i>Escherichia coli</i>	4	6	5,97015	8,9552
<i>MSSA</i>	1	7	1,49254	10,4478
<i>Proteus mirabilis, Enterococcus</i>	2	9	2,98507	13,4328
<i>MSSA</i>	16	25	23,88060	37,3134
<i>Pseudomonas aeruginosa, Escherichia coli ESBL +</i>	1	26	1,49254	38,8060
<i>Pseudomonas aeruginosa, Enterobacter species, MSSA</i>	1	27	1,49254	40,2985
<i>Candida albicans, Acinetobacter, Enterococcus</i>	1	28	1,49254	41,7910
<i>Pseudomonas aeruginosa</i>	7	35	10,44776	52,2388
<i>MRSA, Escherichia coli</i>	1	36	1,49254	53,7313
<i>Enterobacter species</i>	10	46	14,92537	68,6567
<i>Proteus vulgaris</i>	1	47	1,49254	70,1493
<i>MRSA</i>	1	48	1,49254	71,6418
<i>MSSA, MRSA</i>	1	49	1,49254	73,1343
<i>Streptococcus beta haemolyticus</i>	1	50	1,49254	74,6269
<i>Escherichia coli, Enterobacter species, Enterobacter aerogenes, MSSA, Enterococcus</i>	1	51	1,49254	76,1194
<i>MSSA, Proteus vulgaris, Pseudomonas aeruginosa</i>	1	52	1,49254	77,6119
<i>Stenotrophomonas maltophilia</i>	1	53	1,49254	79,1045
<i>Candida species</i>	1	54	1,49254	80,5970
<i>Escherichia coli, Pseudomonas aeruginosa</i>	1	55	1,49254	82,0896

<i>Escherichia coli, Streptococcus agalactiae grupa B</i>	1	56	1,49254	83,5821
<i>Proteus mirabilis, Pseudomonas aeruginosa</i>	2	58	2,98507	86,5672
<i>Corynebacterium gr. JK</i>	2	60	2,98507	89,5522
<i>Corynebacterium gr. JK, Serratia species</i>	1	61	1,49254	91,0448
<i>Serratia species, Stenotrophomonas maltophilia</i>	1	62	1,49254	92,5373
<i>Enterobacter species; Stenotrophomonas maltophilia</i>	1	63	1,49254	94,0299
<i>Serratia species, undifferentiated Gram-negative bacilli</i> <i>Achromobacter</i>	1	64	1,49254	95,5224
<i>Serratia species</i>	1	65	1,49254	97,0149
<i>Escherichia coli ESBL (+), Enterococcus</i>	1	66	1,49254	98,5075
<i>Escherichia coli ESBL (+)</i>	1	67	1,49254	100,0000

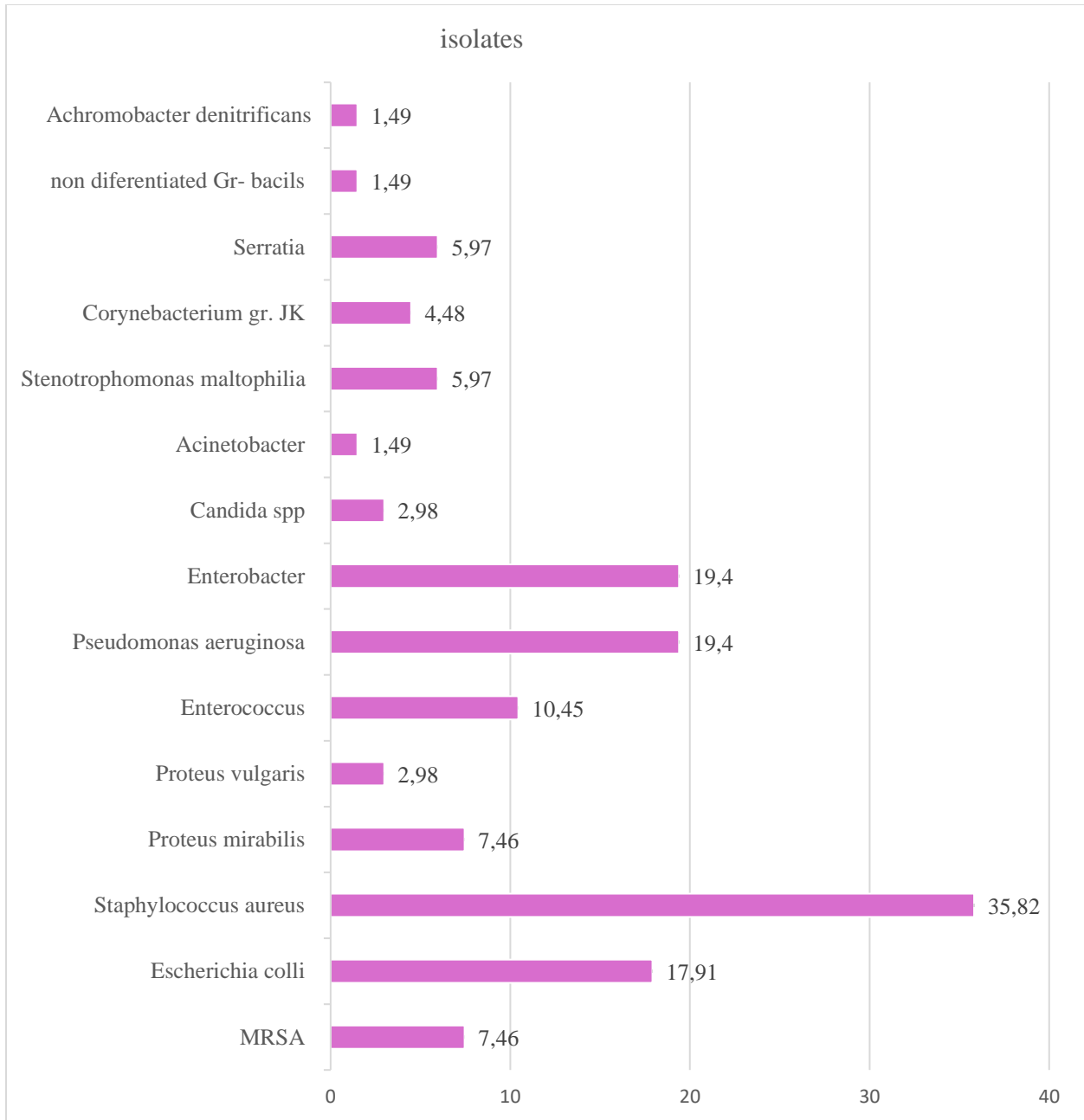


Figure 2. Percentage distribution of bacterial species isolated from chronic wounds

Discussion

Human skin and mucous membranes normally harbor diverse microbial communities composed of resident and transient flora, which play a critical role in wound healing and in the development of chronic wound infections. Infection and biofilm formation are among the main factors contributing to wound chronicity and delayed tissue repair.

The retrospective study by Bessa et al. conducted in Italy analyzed 312 wound swabs from 213 patients and identified 28 bacterial species, with *Staphylococcus aureus* being the most frequently isolated pathogen (37%), followed by *Pseudomonas aeruginosa* (17%), *Proteus mirabilis* (10%), *Escherichia coli* (6%), and *Corynebacterium* spp. (5%) [12].

In our study, MSSA was the predominant isolate (35.82%), followed by *P. aeruginosa* and *Enterobacter* spp. (both 19.4%) and *E. coli* (17.91%). Comparable bacterial heterogeneity has been reported in studies of chronic leg wounds in Germany, where *S. aureus* was detected in 50% of cases, and *P. aeruginosa* and *Enterobacter* spp. in approximately 30% [13].

Polymicrobial infections were identified in 29.8% of samples in our cohort, closely correlating with the findings of Bessa et al. (27.1%) [12]. While Ahmed et al. reported a higher prevalence of polymicrobial infections (55%) [14], other studies, including those by Hassan, Asres, and Bessa, documented a predominance of monomicrobial infections, consistent with our observations [12,15,16]. The most common polymicrobial combinations in our study involved MSSA with *P. aeruginosa* or *Enterobacter* spp., findings that parallel those reported by Puca et al. in Italy [17].

The predominance of Gram-negative bacteria in our study (59.7%) over Gram-positive organisms (40.2%) is consistent with reports from Italy and other European countries [17], although some studies have shown nearly equal distributions. Polymicrobial infections are of particular clinical importance, as they create favorable conditions for microbial synergy and horizontal gene transfer, contributing to increased antimicrobial resistance and therapeutic challenges [18].

Regarding Gram-positive pathogens, MSSA was the most frequently isolated organism, while MRSA accounted for 7.46% of isolates. This prevalence is comparable to reports from Ethiopia and England but lower than rates observed in several European countries and the United States, where MRSA prevalence ranges from 11% to nearly 20% in chronic wound infections [18-20].

Despite the relatively low prevalence observed in our study, MRSA remains a major concern due to its association with multidrug resistance, limited therapeutic options, and increased healthcare costs [21].

Among Gram-negative pathogens, *Pseudomonas aeruginosa* was one of the dominant isolates in our cohort, consistent with studies from Europe, Africa, Asia, and the United States, where it is recognized as a key pathogen in chronic wound infections due to its biofilm-forming capacity, tissue invasiveness, and resistance to multiple antibiotics [22]. The observed variability in pathogen prevalence across studies may reflect differences in patient populations, wound characteristics, healthcare environments, and methodological approaches.

Conclusion

Chronic wounds are frequently colonized by a heterogeneous mix of microorganisms, with *S. aureus* and *P. aeruginosa* predominating. Polymicrobial infections pose challenges for treatment and may influence healing outcomes. Comprehensive microbiological evaluation is essential for guiding targeted antimicrobial therapy.

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