

## ANTIBIOTIC SUSCEPTIBILITY AND RESISTANCE OF BACTERIAL PATHOGENS IN CHRONIC LEG ULCERS: A RETROSPECTIVE COHORT STUDY

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### Abstract

Antimicrobial resistance has recently become a significant challenge in the medical field, affecting among others the management of chronic wounds. It is also frequently observed in dermatology, studies showing that up to 100% of chronic leg ulcers (CLU) are at some point colonized with bacterial pathogens. The aim of our study was to describe the spectrum of bacterial colonization and their antibiotic susceptibility in patients with CLU from a tertiary referral center from Romania. A total of 150 patients with at least one CLU and with a microbiological culture performed from the lesions, were included in the study. 9 bacterial species and 217 strains were identified. The most frequent bacterial pathogen was *Staphylococcus aureus* (42.4%), followed by *Pseudomonas aeruginosa* (23.04%) and *Proteus* spp. (13.82%). High resistance to oxacillin, erythromycin and clindamycin were found among methicillin-resistant *Staphylococcus aureus* strains, contrary to methicillin-susceptible *Staphylococcus aureus* strains that depicted high resistance to ampicillin, clindamycin and erythromycin. *Pseudomonas* spp., *Klebsiella* spp. and *E. coli* strains showed resistance to ciprofloxacin. In summary, these findings underline the importance of bacterial profiling and their antibiotic susceptibility in CLU. Due to the emergence of resistant bacterial strains, swab tests should be performed whenever suspecting infections in CLU before the initiation of antibiotics.

### Rezumat

Rezistența antimicrobiană a devenit în ultimii ani o provocare pentru domeniul medical, afectând printre altele și managementul rănilor cronice. Frecvent observată și în dermatologie, studiile au arătat că până la 100% din ulcerurile cronice de gambă (UCG) sunt la un moment dat colonizate cu bacterii. Scopul studiului nostru a fost descrierea spectrului colonizării bacteriene și a susceptibilității la antibiotice la pacienții cu UCG dintr-un spital terțiar din România. În studiu au fost incluși 150 de pacienți cu cel puțin un UCG și o cultură microbiologică efectuată. 9 specii bacteriene și 217 tulpini au fost izolate. Cel mai frecvent microorganism identificat a fost *Staphylococcus aureus* (42,4%), urmat de *Pseudomonas aeruginosa* (23,04%) și *Proteus* spp. (13,82%). Rezistența crescută la oxacilină, eritromicină și clindamicină a fost identificată la tulpinile de *Staphylococcus aureus* metilino-rezistent, în timp ce tulpinile de *Staphylococcus aureus* metilino-sensibil au prezentat rezistență la ampicilină, clindamicină și eritromicină. *Pseudomonas* spp., *Klebsiella* spp. și *E. Coli* au demonstrat rezistență la ciprofloxacina. În concluzie, rezultatele obținute subliniază importanța actualizării periodice a profilului bacterian și a susceptibilității la antibiotice în UCG. Datorită apariției tulpinilor bacteriene rezistente, este imperios necesară efectuarea de culturi bacteriene de fiecare dată când se suspectează prezența infecțiilor în UCG, înainte de inițierea antibioticoterapiei.

**Keywords:** chronic leg ulcer, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, antibiotic resistance

### Introduction

Antimicrobial resistance (AMR) has become a salient challenge in the medical field of the past decades. According to the European Center for Disease Prevention and Control, AMR is responsible for almost 33,000 deaths *per* year in the European Union, causing productivity losses and healthcare costs of over 1.5 billion euros/year [2, 6, 12]. Despite significant taken measures, this issue did not cease or lessen in severity, causing serious concerns [2, 11, 33]. Many factors are thought to be involved, the most

important being the irresponsible use of antibiotics, either self-administrated by patients or irrationally prescribed by doctors. In addition, misuse of antimicrobial agents in agriculture and farming, easy travel routes, spontaneous mutations with subsequent evolution of bacteria, and reduced research activity in this field due to high costs, have also been incriminated [7, 11]. Since chronic wounds represent an important aspect in dermatology [1, 20], AMR constitutes a pressing problem including in this field.

About 1% of the European population is suffering from chronic leg ulcers (CLU), and up to 80% are the result of venous disease [14, 24]. Chronic wound surface can become the perfect microenvironment for bacterial growth. Studies show that 80% to 100% of CLU are at some point colonized with bacteria [7, 14, 24, 29], yet the bacterial involvement in the pathogenesis of chronic ulcerations remains unclear [24]. The spectrum of bacterial involvement ranges from a simple uncomplicated colonization to a manifest infection, and in rare cases, sepsis. Furthermore, not only CLU impacts the quality of life but is also associated with significant economic burden due to its recurrent nature [28, 29]. To date, there is a lack of consensus regarding the proper use of antibiotics (indication, type of antibiotic, dosage, duration of therapy) in CLU [24, 23]. Therefore, these agents are frequently prescribed without an accurate selection, regimen or clinical indication.

The aim of our study was to describe the spectrum of bacterial colonization in patients with CLU and their susceptibility pattern to antibiotics.

## Materials and Methods

### Study design

We performed an observational, retrospective cohort study based on medical records of hospitalized patients at the Department of Dermatology, "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania, from January to December 2019.

CLU was defined as a skin defect in the area below the knee with a persistence of more than 6 weeks, and showing no tendency to heal.

Only patients with at least one CLU regardless of the etiology, with microbiological culture performed from the lesions and corresponding antibiogram, were included in the study. Swab tests were obtained before any antiseptic local therapy or topic and/or systemic antibiotic treatment. One single admission was randomly chosen for patients with several admissions during that year.

Patients with any documented antimicrobial therapy prior to admission and those with malignant ulcerations were excluded from the study.

Obtained swab tests were examined at the Laboratory of Bacteriology of the Emergency County Hospital Cluj-Napoca, Romania. We recorded the following information: age, gender, ulcer type, wound duration, comorbidities (the presence or absence of diabetes mellitus), subcutaneous tissue infections (cellulitis, erysipelas) and laboratory results regarding microbiological cultures and corresponding antibiograms.

### Statistical analysis

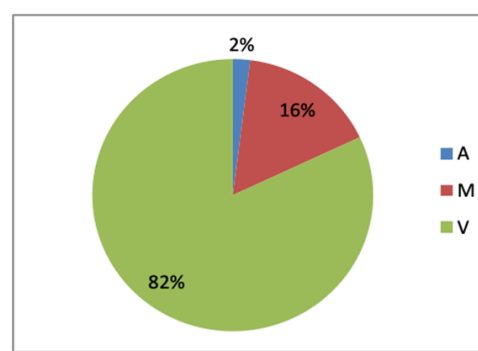
Statistical analysis was performed using the R software. Qualitative variables were described as frequencies and percentages, while quantitative ones as means, medians and standard deviations.

## Results and Discussion

One hundred and fifty patients were included in our study, of them 77 were male (51.33%) and 73 female (48.67%). The mean age at diagnosis was 69.93 years (range 39 - 90).

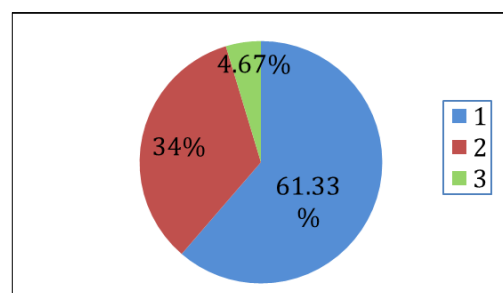
Regarding the etiology of CLU, the most frequently incriminated cause was chronic venous insufficiency (82%), whereas a mixed type of ulcer was observed in 16% of patients. Advanced peripheral arterial occlusive disease was seen only in 2% of cases (Figure 1). As for comorbidities, 19,33% had a positive diagnosis of diabetes mellitus.

The spectrum of identified bacterial pathogens included 9 different bacterial species and 217 isolated strains. 92 patients presented solely one bacterium on the wound surface, whereas 51 had two and 7 patients presented three bacteria (Figure 2).



**Figure 1.**

Distribution of patients (%) according to the type of ulcer (A = arterial, M = mixed, V = venous)



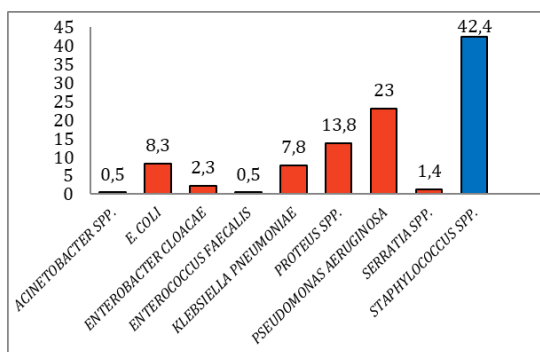
**Figure 2.**

Distribution of patients (%) according to the number of bacterial isolates (1, 2 and 3 different types of bacterial isolates)

The most frequent identified microorganism was *Staphylococcus aureus* (42.4%), followed by *Pseudomonas aeruginosa* (23.04%), *Proteus* spp. (13.82%), *E. coli* spp. (8.29%), *Klebsiella pneumonia* (7.83%), *Enterobacter cloacae* (2.3%), *Serratia* spp. (1.38%), *Acinetobacter* spp. (0.48%) and *Enterococcus faecalis* (0.46%) (Figure 3).

Based on the Gram stain characteristics, Gram-negative bacteria were the most frequently identified pathogens, whereas *Staphylococcus aureus* and *Enterococcus*

*faecalis* were the only Gram-positive isolated species. Of the 92 *Staphylococcus aureus* isolates, 37 were methicillin-resistant *Staphylococcus aureus* (MRSA).



**Figure 3.**

Percentage of bacterial species identified in CLU (blue = Gram-positive; red = Gram-negative)

Among the least common identified bacterial species, *Acinetobacter* spp. was resistant only to ciprofloxacin, while *Enterococcus faecalis* only to erythromycin. One third of the *Serratia* strains were resistant to doxycycline, while 40% of *Enterobacter* spp. showed resistance to amoxicillin/clavulanic acid (Table I). *Pseudomonas* spp. demonstrated a high sensitivity to carbapenems (imipenem-92%, meropenem-88%), piperacillin/tazobactam (88%), colistin (86%), ceftazidime (86%) and amikacin (78%). 24% of the strains were resistant to cefepime and ciprofloxacin. Regarding *Proteus* spp., a high resistance to doxycycline was observed (63.33%), followed by trimethoprim/sulfamethoxazole (33%) and levofloxacin (26.67%). Cephalosporin resistance has also been observed, while a high percentage of the isolated strains were sensitive to aminoglycosides (amikacin – 93.33%, gentamicin – 63.33%), carbapenems (imipenem – 83.33%, meropenem – 96.67%) and piperacillin/tazobactam (96.67%).

*Klebsiella* spp. presented a high resistance to amoxicillin/clavulanic acid (41.18%), with some strains also resistant to ceftazidime, ciprofloxacin and trimethoprim/sulfamethoxazole. However, *Klebsiella* spp. showed the highest sensitivity to aminoglycosides and fluoroquinolones.

All *E. coli* isolates were sensitive to piperacillin/tazobactam and a great majority also to ciprofloxacin (94.44%). Reduced sensitivity was observed for amoxicillin/clavulanic acid, ampicillin, doxycycline, colistin and tetracycline.

*Staphylococcus aureus* other than MRSA depicted the following pattern: high sensitivity for amoxicillin/clavulanic acid (94.55%), oxacillin (90.91%), quinolones (ciprofloxacin – 85.45%, levofloxacin – 81.82%), and high resistance to ampicillin (54.55%), clindamycin (49.09%), erythromycin (58.18%) and penicillin (63.64%). A lower resistance was observed for doxycycline (10.91%) and gentamicin (12.73%).

MRSA strains presented a good sensitivity for linezolid, trimethoprim/sulfamethoxazole, gentamicin, chloramphenicol, ciprofloxacin and vancomycin. Its bacterial resistance was high for oxacillin (97.3%), erythromycin (94.59%), ampicillin (91.89%), amoxicillin/clavulanic acid (89.19%), clindamycin (83.78%), doxycycline (37.84%) and ciprofloxacin (40.54%).

**Table I**

Antibiotic susceptibility pattern of CLU isolated bacteria

Isolates	Most Sensitive	Most Resistant
<i>Staphylococcus aureus</i>	AMC OXA LZD	PEN ERY AMP
MRSA	SXT LZD LVX	OXA ERY AMP
<i>Acinetobacter</i> spp.	AMC GEN MEM	CIP
<i>E. Coli</i> spp.	CIP IPM MEM	AMC SXT DOX
<i>Enterobacter cloacae</i>	LVX AMK CRO	AMC CIP TET
<i>Enterococcus faecalis</i>	AMC LVX VAN	ERY
<i>Klebsiella pneumoniae</i>	MEM TZP CIP	AMC CAZ FEP
<i>Proteus</i> spp.	TZP MEM AMK	DOX SXT LVX
<i>Pseudomonas aeruginosa</i>	IMP TZP CST	FEP CIP LVX
<i>Serratia</i> spp.	FEP LVX TZP	DOX

Abbreviations: AMC, amoxicillin/clavulanic acid; AMP ampicillin; AMK amikacin; CHL, chloramphenicol; CST, colistin; CAZ, ceftazidime; FEP, cefepime; CRO, ceftriaxone; CFP/S, cefoperazone/sulbactam; CIP, ciprofloxacin; CLI, clindamycin; DOX, doxycycline; ERY, erythromycin; OXA, oxacillin; GEN, gentamicin; LZD, linezolid; LVX, levofloxacin; IPM, imipenem; MEM, meropenem; MRSA, methicillin-resistant *Staphylococcus aureus*; PEN, penicillin; TZP, piperacillin/tazobactam; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; VAN, vancomycin.

CLU represent a debilitating condition, quite common in the dermatological practice. Each wound is thought to be colonized at some point by at least one bacterium [3, 9, 24]. Even though various clinical guidelines on CLU management are available, they are not entirely evidence-based, but rather expert's opinions [30]. Thus, there is a lack of consensus regarding the proper antibiotic use in CLU [1, 23]. Questions such as

when to administer antibiotic treatment, what kind of antibiotic to use and what treatment regimen to follow, remain to be addressed.

Our study illustrates the bacterial colonization pattern in CLU, as well as their antibiotic susceptibility in a Romanian cohort of patients. *Staphylococcus aureus* was the most prevalent bacteria on microbiological cultures (42.2%). Similar findings have been described in many other studies [31, 17, 34]. Beside a well-known commensal species, *Staphylococcus aureus* is also a true pathogen responsible for a wide variety of community-associated and nosocomial infections, the latter being associated with high morbidity and mortality rates [19]. Persistent colonization with *Staphylococcus aureus* is observed in approximately 20% of the general population, while 30% carry *Staphylococcus aureus* only transiently [25]. The high prevalence of MRSA observed in this study (17.05%) may suggest possible nosocomial infections. MRSA is associated with a greater risk of mortality, longer duration of hospitalization, and higher hospital costs [10]. In the Mediterranean region, MRSA rates showed a prevalence of up to 39% [4]. Our results highlight that linezolid, trimethoprim/sulfamethoxazole and vancomycin are good first-choice treatments for MRSA skin infections. However, MRSA was also fairly susceptible to both gentamicin (64.86%) and levofloxacin (67.57%), but choosing these drugs as first therapeutic options should be done with great caution given the increasing antibiotic resistance among MRSA strains [27].

Similar to previously published studies, *Pseudomonas aeruginosa*, a Gram-negative bacterium, was the second most frequent species in our study (23.04%) [17, 34]. It has been shown that *Pseudomonas* infections might lead to a slower healing of CLU, and thus, reduce treatment responses [8]. This is probably due to its high capacity of developing complex biofilm structures on wound surfaces, thus being considered one of the ESKAPE pathogens [15]. The latter is an acronym for multidrug-resistant bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) that are highly capable of “escaping” the destructive action of antimicrobial agents [26]. In our study, *Pseudomonas aeruginosa* presented a good susceptibility to cephalosporin (ceftazidime – 86%), carbapenems (meropenem – 88%, imipenem – 92%), piperacillin/tazobactam (88%) and colistin (86%). The greatest resistance for this species was to quinolones (levofloxacin – 18%, ciprofloxacin – 24%) and cefepime (24%). Studies have reported a strong correlation between increased use of ciprofloxacin and a high prevalence of ciprofloxacin resistant strains [16]. Between 2006 and 2009, studies have reported an increasing tendency of antimicrobial resistance of *Pseudomonas aeruginosa*

to all antibiotics, the highest being to ciprofloxacin (33%) [22].

We also observed that a vast majority of the identified strains showed significant resistance to some of the most commonly prescribed antibiotics, such as amoxicillin/clavulanic acid, ampicillin, ceftazidime and ciprofloxacin. A possible explanation for this finding might reside in the indiscriminate use of these antimicrobials. Gram-negative species were susceptible to amikacin and piperacillin/tazobactam, contrary to Gram-positive that were sensitive to linezolid, chloramphenicol and gentamicin. These results may be explained by the fact that these types of antibiotics are more expensive, therefore being often prescribed in a closed circuit, and also used with more caution.

Since microbial flora is present in every wound at some point, many authors recommend using antimicrobial treatment only in CLU with clinical and laboratory signs of infections, such as the presence of local purulence and systemic symptoms, but also based on patient's underlying risk factors and community rates of drug-resistant pathogens [3]. Contrarily, other studies suggest that the presence of the bacterium itself is a sign of secondary infection interfering with the healing dynamics [5, 32]. Regardless of the recommendations, empiric antibiotic therapy should aim the most prevalent flora and be based mainly on local antimicrobial susceptibility [23]. In a century marked by a global antibiotic resistance, it is absolutely necessary to correctly assess this problem in order to ensure proper management of infected CLU, especially since the current context of the COVID-19 pandemic might contribute to a rise in AMR [13, 21]. According to Langford *et al.* [18], a minority of COVID-19 diagnosed patients associated a bacterial co-infection. However, up to 72% have received antibiotic treatment even in the absence of clinical indication, thus promoting among other factors AMR [7].

Regarding the limitations of the study, we should firstly mention its retrospective nature. Secondly, the small number of participants. Thirdly, the antibiotics used for bacterial susceptibility assessment were not always the same, depending on the laboratory's availability. Lastly, the history of antibiotic treatment prior to swab sampling, unless documented, was self-reported, thus potentially leading to bias. Despite all these limitations, presenting the current spectrum of bacterial colonization in patients with CLU and their antibiotic susceptibility, provides a valuable information with significant practical impact on the therapeutic approach of infections in CLU.

## Conclusions

The results of this study demonstrate the wide spectrum of bacterial colonization in patients with CLU and their susceptibility pattern to antibiotics. The high prevalence of drug-resistant *Pseudomonas aeruginosa*

and MRSA strains should discourage antibiotic initiation in CLU before obtaining proper microbiological cultures. Antibiotics should be accurately chosen based on specific clinical data, swab test result and corresponding antibiogram. This study could aid the physicians in choosing the correct empirical antibiotic treatment in CLU infections, thus contributing to lower AMR rates.

### Conflict of interest

The authors declare no conflict of interest.

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