ORIGINAL PAPERS

BACTERIAL PROFILE AND ANTIBIOTIC RESISTANCE IN PATIENTS WITH CHRONIC VENOUS LEG ULCERS IN A DERMATOLOGY SERVICE IN ROMANIA: A MATTER OF NATIONAL IMPORTANCE

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Summary

Antibiotic susceptibility is essential in the prescription of empirical antibiotics before the results of cultures of wound samples are available [1]. For patients with chronic leg ulcers (CLU), increasing resistance to commonly used antibiotics has been observed. The aim of our study was to isolate the bacteria that infect CLU, determine their antimicrobial resistance profile and show how quickly patients develop resistance to the antibiotics used. Gram-negative bacteria (Proteus mirabilis and Pseudomonas aeruginosa) were more frequently associated with CLU than Gram-positive bacteria (Staphylococcus aureus). We observed that the antibiotics most likely to induce resistance in a short period of time for Gram-negative bacteria are cefepime and ceftazidime and for Gram-positive bacteria, fluoroquinolones, followed by aminoglycosides and macrolides, mostly in the urban area.

Keywords: *chronic leg ulcer, antibiotic resistance, bacterial culture.*

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Introduction

Chronic leg ulcers (CLU) constitute a global health problem with a large socioeconomic impact. It is estimated that active ulcers occur in approximately 2% of the population and are mostly observed in individuals between 60 and 80 years of age [2].

CLU represents a skin defect below knee level that persists for more than six weeks and shows no tendency of healing after three or more months. Common causes are neuropathy, venous and arterial diseases. Infections in leg ulcers can be caused by Gram-positive and Gram-negative bacteria, which can represent a major challenge for therapy when they develop resistance to one or more antibiotics [3]. Of the many presentations across the clinical spectrum of chronic venous disease, venous leg ulcers can be considered amongst the most important [4].

Long-term empirical antibiotic therapy of wounds and their chronicity may favor the selection of drug-resistant microbial strains within the wound, especially if the leg ulcer was infected with alert pathogens [5]. Colonization of pathogenic bacteria at the wound site is associated with wound chronicity. A bacterial culture study recognized *Staphylococcus aureus* at the surface of wounds and *Pseudomonas aeruginosa* in deeper layers of tissue [6]. Colonization of the ulcer does not necessarily result in clinically overt infection [5]. Recently, it has been observed that bacteria in chronic wounds develop biofilms that contribute to a delay in healing. Resistance to

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antibiotics in biofilm-producing bacteria is a crucial problem in the management and treatment of chronic wounds [6].

In order to minimize colonization and possible infection, a careful cleaning of the wound using physiological solutions or anti-microbial agents is important [3]. In the event of an infection, certain signs and symptoms should be carefully evaluated, such as edema, erythema, pain, granulation tissue with a friable characteristic, high white blood cell count, delayed healing under adequate topical therapy and changes in exudate characteristics [3]. The presence of those symptoms supports the clinical diagnosis of wound infection and the primary objective in such cases is targeted antibacterial treatment [5].

To date, there is a lack of consensus regarding the proper use of antibiotics (indication, dosage, type of antibiotic, duration of therapy) in CLU. Therefore, they are frequently prescribed without an accurate selection or clinical indication [7].

Materials and methods

A total of 50 patients, 27 males (54%) and 23 females (46%) were randomly selected every 5th patient based on the following inclusion criteria: age between 35 and 90 years old with chronic venous leg ulcer for more than 6 months, without tendency to epithelialization, admitted in our hospital between January 2019 and December 2022. The exclusion criteria were: other types of ulcers, ulcers lasting less than 6 months, pregnant women. Data collected included: age, sex, living environment, number of hospital admissions, number of cultures made.

Samples were collected by rotating the swab firmly in a circular motion, over a 1 cmḥ area of the ulcer after it was cleaned of exudate, necrotic tissue and remnants of the dressing.

The microorganisms from the swabs were incubated on selective media under standard conditions and then processed on Vitek2. Statistical data were firstly analyzed by using Multiple Linear Regression (Excel app – "Data Analysis/Regression" feature) taking into consideration no. of strains with resistance to antibiotics as depended variable, and two independent variables as input: no. of hospitalization and current living environmental area (Urban versus Rural). The method has been chosen in order to confirm if both independent variables can predict the depended variable within one regression model.

Results

A total of 195 bacterial cultures were performed from which 265 bacterial strains were isolated as follows: in 109 cultures (55,89%) a single bacterial strain was present, in 59 cultures (30,25%) two bacterial strains were present, in 13 cultures (6,66%) three or more bacterial strains were present and in 14 cultures (7,17%) bacterial strains were absent. Among these, there were 55 (20,75%) Gram-positive bacteria (26 bacterial strains were MRSA) and 210 (79,24%) Gram-negative bacteria (31 bacterial strains were MDR and 7 bacterial strains were ESBL) (Table 1).

The most common bacterial strain was *Proteus mirabilis*, closely followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 1). The most common combinations of two bacterial strains were *Pseudomonas aeruginosa* and *Proteus mirabilis* in 21 cultures, *Pseudomonas aeruginosa* and *Providencia stuartii* in 6 cultures, *Proteus mirabilis* and *Klebisella* spp. in 5 cultures. The most common combination of three bacterial strains was *Proteus mirabilis, Pseudomonas aeruginosa* and *Providencia stuartii* in 2 cultures.

Gram-negative bacteria were more resistant to: cephalosporins, carbapenems, piperacillin, colistin and aminoglycosides. Gram-positive bacteria were more resistant to: fluoroquinolones, macrolides, clindamycin and gentamycin (Figure 1). All MRSA bacterial strains were sensitive to vancomycin, so it continues to be the drug of choice for most MRSA infections.

Note: Rate of bacterial resistance takes into consideration only data for 3 bacteria (*Pseudoonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus*) that count for almost 70% of total isolated pathogens within January 2019 – December 2022 period of time, by using Vitek2 as method of resistance determination. The percentages assigned to each antibiotic were calculated by dividing the number of strains resistant to the

Table 1. Profile of bacteria isolated from 50 patients with chronic venous leg ulcer

Bacteria category	**Number of pathogens**	**% from Total***
Sub-category 1 - Gram-negative bacteria	**210**	**79,25**
Proteus mirabilis	*68*	*25,66*
Pseudomonas aeruginosa	*67*	*25,28*
Klebsiella spp.	*12*	*4,52*
Escherichia Coli	*15*	*5,66*
Acinetobacter spp.	*16*	*6,03*
Providencia spp.	*10*	*3,77*
Enterobacter spp.	*6*	*2,26*
Others	*16*	*6,03*
Sub-category 2 - Gram-positive bacteria	**55**	**20.75**
Staphylococcus aureus	*48*	*18,11*
Streptococcus spp.	*7*	*2,64*
Total*	**265**	**N/A**

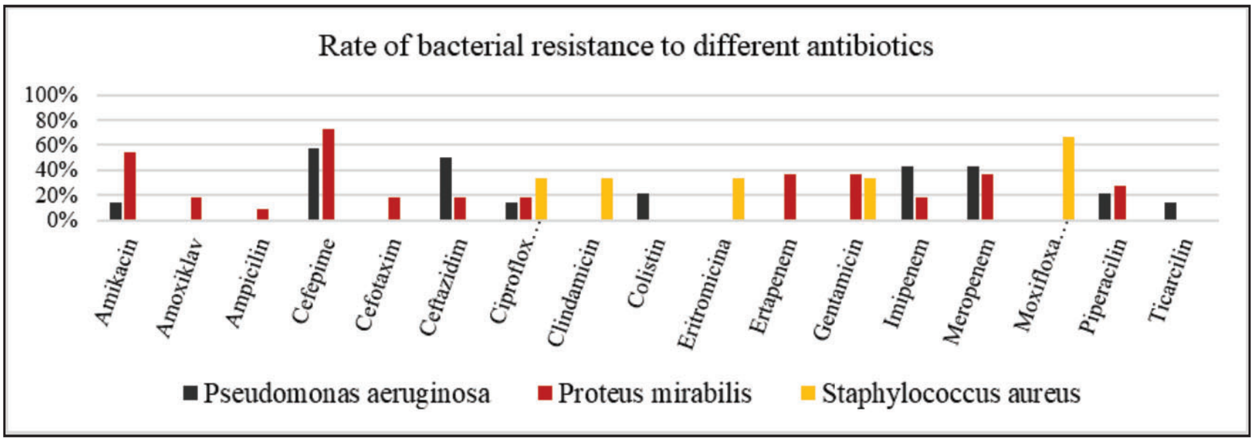

Figure 1. Rate of bacterial resistance to antibiotics.

antibiotic by the number of strains resistant to one or more of the antibiotics considered in this study (see example in Table 2).

Of the total number of patients, 4 patients had only one hospital admission, 12 patients had between 2 and 5 hospital admissions and 34 patients had more than 5 hospital admissions during the period mentioned above. The mean age of hospitalized patients was 68 years.

Table 2. Rate of bacterial resistance to Cefepime

	Pseudomonas aeruginosa	*Proteus mirabilis*	*Staphylococcus aureus*
No. of identified strains	67	68	48
No. of strains with at least one resistance to antibiotics	14	11	6
No. of resistance occurrences for Cefepime	8	8	0
Bacteria resistance for Cefepime	**(8/14) * 100**	**(8/11) * 100**	**(0/6) * 100**

Most isolated bacteria have developed resistance to common antibiotics within about 6 months.

Multiple linear regression analysis was performed for the number of strains with resistance to antibiotics as the dependent variable, and the number of hospitalizations and social environment (urban and rural) as independent variables, results being presented in Table 3. This analysis has been applied for the same 3 bacteria: *Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus*

The coefficient of determination (the Multiple R) value, simply tells us that almost 50,5% of no. of antibiotics, for which patients developed resistance, can be explained using the regression model. However, the overall F-test p-value of 0,001 indicates that the null hypothesis can be reasonable rejected, that means model fits the data better than the intercept-only model. However, as far as independent variables, the no. of hospitalizations coefficient has a range of confidence interval between 0.088 and 0.266 which means the „0" value of it are excluded from probability point of view. The intercept and Social Environment („1" for Urban and „2" for Rural) have both in range the „0" value for their own coefficient. That means that we cannot accurately predict the no. of strains with resistance to antibiotic based on those two independent variables in one regression model.

Going further with raw data analysis, we've noticed that between urban and rural environments, there are some differences in term of average no. of strains with resistance to antibiotics. Data from Figure 2 indicate a significant difference between urban and rural environments as far as resistance occurrence rate is concerned. Looking for potential regression models, we've applied the Simple Linear Model with no. of strains with resistance to antibiotics as depended variable, and no. of hospitalizations as independent variable, separately for each social environment. Results are presented in Table 4.

Following the same criteria to analyze the data (Multiple R, p-value and confidence intervals), the only model that eliminates the null hypothesis is the one related to rural social environment. In this case, a linear model based on no. of hospitalizations (linearity coefficient = 0,2329) seems to highly predict the no. of strains

Table 3. Multiple Linear Regression Model for no. of strains with resistance to antibiotic as dependent variable

Regression Statistics						
Multiple R	**0,505**					
R Square	0,255					
Adjusted R Square	0,223					
Standard Error	2,796					
Observations	50					
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	2	125,85	62,9250	8,0491	**0,0010**	
Residual	47	367,43	7,8177			
Total	49	493,28				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0,9622	1,3432	0,7164	0,4773	-1,7399	3,6643
No. of hospitalization	0,1773	0,0442	4,0120	**0,0002**	**0,0884**	**0,2662**
Social Environment	-0,4895	0,8119	-0,6029	0,5495	-2,1229	1,1439

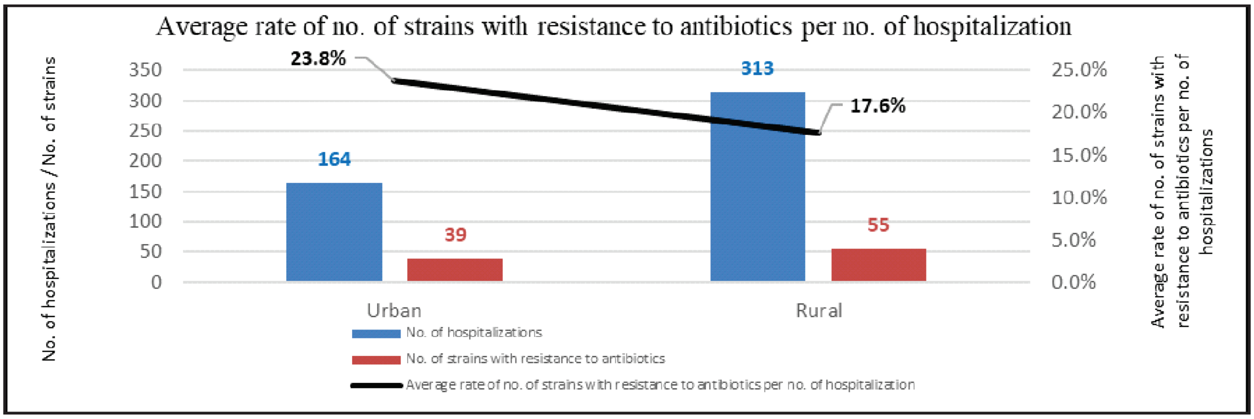

Figure 2. Impact of social environment on no. of strains with resistance to antibiotics

Table 4. Simple Linear Regression model for no. of strains with resistance to antibiotics

	Urban	Rural
Multiple R	0,095	**0,839**
R Square	0,009	**0,704**
Adjusted R Square	-0,043	0,693
Standard Error	3,571	1,652
Observations	21,000	29,000
ANOVA	**Urban**	**Rural**
Significant F	0,681	**0,000**

Rural				
Rural	*Coefficients*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0,6167	0,1715	1,5175	0,2841
No. of hospitalization	**0,2329**	**0,0000**	**0,1732**	**0,2925**

Urban				
	Coefficients	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2,275	0,089	-0,379	4,929
No. of hospitalization	-0,053	0,681	-0,322	0,215

with resistance to antibiotics. For instance, in this social environment, after four hospitalizations, we might expect to observe at least one strain, among *Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus*, with resistance to one of antibiotics taken into consideration in this survey. However, further investigations should be conducted to develop a more reliable model based on other independent variables, such as the category of antibiotics prescribed during and after hospitalization, individual historical data on antibiotics consumption and so on.

Discussion

The majority (92,8%) of CLU showed positive cultures. As with other studies, we observed that infection was more frequently monomicrobial (55,89%), with the predominance of Gram-negative bacteria (*Proteus mirabilis* was the dominant bacterial strain) resistant to one or more antibiotics. Our study demonstrated the presence of: *Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumanii, Klebsiella* spp., *Enterobacter* spp., *Streptococcus* spp.

Staphylococcus aureus infections usually originate from asymptomatic colonization [8]. Colonization is harmless, but colonizing strains lead to biofilm formation which confers protection against antimicrobial therapeutics and host immune clearance [9]. Alert pathogens account for 24% of all bacterial strains identified in our leg ulcers cultures, comparative to 28.6% in other studies [5].

Ceftriaxone and ceftazidime are the most commonly used injectable cephalosporins in Romanian hospitals [8]. Our study suggests that third generation cephalosporins are potent inducers of extended-spectrum beta-lactamases and should therefore be avoided because of the major risk of selecting resistant germs. According to the national antibiotic therapy guide, for *Pseudomonas aeruginosa* infections, colistin is the most effective solution, and in the case of *Staphylococcus aureus* infections, cotrimoxazole has proven very effective. When CLU are superinfected with mixed bacterial flora it is recommended to use combinations of antibiotics depending on the results of the antibiogram [10].

In our study, for Gram-negative bacteria the antibiotics most likely to induce resistance in a short period of time are cefepime and ceftazidime, followed by carbapenems (imipenem, meropenem). The remaining antibiotics (piperacillin, colistin, amikacin, gentamicin, ampicillin) are the least likely to induce resistance. With regard to *Staphylococcus aureus,* we find that the first resistances occur with fluoroquinolones, followed by aminoglycosides and macrolides. On the other hand, *Staphylococcus aureus* does not induce resistance to third and fourth generation of cephalosporins or to carbapenems. Furthermore, can show resistance to almost all available antibiotics. Vancomycin remains the antibiotic of last resort for MRSA infections [11].

Historically, antibiotic resistance has always been present, given that many antibiotic molecules are found in natural environments and have been interacting with germs. Thus, a certain level of intrinsic resistance is expected in most microorganisms [12]. Intrinsic resistance is a naturally occurring phenomenon that is independent of antibiotic exposure and is universally found within of a group of bacteria or within a bacterial species [13]. Acquired resistance is more clinically critical because we encounter bacteria that were initially susceptible but then become resistant to antibiotics and may be one of the reasons for the lack of effective treatment [14].

We observed that patients from rural areas (58%) might be more affected by the number of hospitalizations when analyzing the impact of multiple hospitalizations on their level of antibiotic resistance. Although for this social area the simple linear model analyzed can reasonably predict the level of antibiotic resistance, further studies that will include other variables could provide solutions to mitigate this trend.

The primary treatment of infected leg ulcer is often empirical and based on existing clinical guidelines. Hence, knowing the prevalence of pathogenic bacteria and their antibiotic resistance patterns in a local area, it is crucial to have the best empiric antibiotics for its residents [15]. Proper wound dressing (foam dressings, alginate dressings, hydrocolloids) and regular cleaning are essential for preventing infection, facilitating healing and promoting a moist wound environment [4]. It is necessary to detect the specific pathogens and their susceptibility pattern to initiate early treatment with the appropriate antibiotics [16].

Conclusions

In our country, antimicrobial resistance represents a great challenge to therapy. We observe that many bacteria developed resistance to one or more antibiotics, especially in urban area. Any administration of antibiotics, whether clinically indicated or not, contributes to increasing the level of bacterial resistance to antibiotics.

We do not suggest broad-spectrum antibiotics in the first instance, because bacteria quickly acquire resistance and antibiotics become ineffective. Cephalosporins should only be used in absolutely necessary cases and not as a first option. In ulcers with a duration of more than 6 months, the administration of ampicillin, cefotaxime, aminoglycosides for Gram-negative bacteria and the administration of cephalosporins, ampicillin and carbapenems for Gram-positive bacteria should be considered until the antibiogram arrives.

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Conflict of interest
NONE DECLARED

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