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### Bacterial Isolates and Antimicrobial Resistance in Chronic Venous Leg Ulcers: Rethinking Empirical Antibiotic Therapy

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#### Introduction

Chronic venous leg ulcers (VLU) represent a significant clinical challenge due to prolonged healing, frequent bacterial colonization, and the increasing burden of antimicrobial resistance. Because colonization alone does not justify systemic antibiotic therapy, accurate differentiation between colonization and clinically significant infection is essential. This study aimed to characterize the microbiological profile and resistance burden in chronic VLUs using repeated deep tissue biopsy sampling, an approach rarely reported in longitudinal wound microbiology studies.

#### Materials and Methods

We conducted a one-year longitudinal observational pilot study involving 10 patients with chronic VLUs at the Department of Dermatovenerology, Clinical Hospital Center Rijeka. The study population comprised an equal distribution of male and female patients, with a mean age of 74 years (range 51–84 years). A total of 17 serial deep tissue wound biopsy specimens were collected across 10 patients during follow-up, yielding 30 bacterial isolates for microbiological culture and antimicrobial susceptibility testing. Resistance profiles were interpreted according to EUCAST criteria.

#### Results

Polymicrobial colonization was observed in most patients (80%), most commonly involving two bacterial species, frequently resistant to two or more antibiotics. The microbiological profile was dominated by Gram-negative organisms, with *Pseudomonas aeruginosa* (70%) being the most prevalent pathogen, followed by *Escherichia coli* (40%), *Enterococcus faecalis* (30%), and *Proteus mirabilis* (30%), whereas *Staphylococcus aureus* was isolated in only one patient (10%). Across consecutive biopsies, isolates demonstrated a trend toward increasing antimicrobial resistance during follow-up. Ciprofloxacin resistance was the most common phenotype (43.3% of isolates) and was detected in 80% of patients, followed by resistance to piperacillin-tazobactam and imipenem/cilastatin (each 13.3%). Notably, multidrug-resistant Gram-negative isolates were identified in a substantial proportion of patients (60%), including carbapenem-resistant *Acinetobacter baumannii* complex in 20%.

#### Conclusions

Our findings support current international recommendations advocating restrictive and rational systemic antibiotic use in chronic VLUs. Serial biopsy-based monitoring demonstrated persistence of resistant organisms and detection of multidrug-resistant Gram-negative pathogens over follow-up, highlighting the importance of targeted diagnostics in guiding antibiotic stewardship. Optimal VLU management therefore requires accurate clinical differentiation of infection from colonization and a multidisciplinary approach including compression therapy, debridement, and strict adherence to antimicrobial stewardship principles.

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