Bacterial Flora of Leg Ulcers in Patients Admitted to Department of Dermatology, Poznań University of Medical Sciences, during the 1998-2002 Period

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SUMMARY Venous leg ulcers are an important cause of morbidity in a significant percentage of the world population. The percentage of leg ulceration, either active or healed, in the European population is about 1%-2%. The aim of this study was to analyze the rate of colonization and qualitative composition of the bacterial flora isolated from leg ulcers in patients admitted to Dermatology Department, Poznań University of Medical Sciences, during the 1998-2002 period, with special reference to the infection risk factors. Bacteriological diagnosis of 175 wound swabs was performed in compliance with compulsory laboratory methods. In 173 positive results, the predominant culture composition included Staphylococcus aureus (56.57%), Pseudomonas aeruginosa (37.14%), Enterococcus faecalis (22.29%), Proteus mirabilis (13.71%) and Escherichia coli (12.57%). There was a significant increase in the incidence of Pseudomonas aeruginosa, Enterococcus faecalis and Escherichia coli, along with a decrease in the incidence of Staphylococcus aureus isolation during the study period. The rate of yeast-like fungi strains, mainly Candida albicans, recorded in culture composition showed a systematic increase. Changes in the qualitative and quantitative composition of bacterial flora, presence of multiple isolates, and concomitant diseases that may influence the characteristics of leg ulcer disease were closely monitored.

KEY WORDS leg ulcers, bacterial flora, wound healing

INTRODUCTION

Chronic venous leg ulcers are an important cause of morbidity in a significant percentage of the world’s population. The prevalence of lower extremity ulceration is estimated to be approximately 1% in the European population. Up to 80% of all leg ulcers are caused by venous disease (1-4).

Leg ulcers appearing in the course of chronic venous insufficiency frequently recur after healing. The pathophysiology of venous ulceration is multifactorial and complex. The bacterial influence on the pathogenesis of chronic venous ulcers is unclear (5,6). Chronic wound surface can become...
a moist medium for bacterial growth. Very often the microorganisms cultured from chronic ulcers are of endogenous origin, mainly fecal, oral and cutaneous. In most cases, the nature of chronic venous ulceration is polymicrobial (5-10). Various reports indicate that 80% to 100% of chronic ulcers are at some point colonized with bacteria. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are predominantly isolated from leg ulcers (5-8).

Delayed wound healing has been associated with aerobic or facultative pathogens (5-7,9). The presence of *Proteus mirabilis* and *Pseudomonas aeruginosa* in wound swabs has been linked to poorly healing ulcers (11). Other authors suggest a positive correlation between prolonged wound healing and higher quantitative bacterial count or the presence of four and more bacterial groups (7,9,11).

In this study, the rate of colonization and qualitative composition of leg ulcer bacterial flora were analyzed according to characteristic features of the leg ulcer syndrome and concomitant diseases.

**MATERIAL AND METHODS**

Patients suffering from leg ulcers admitted to Dermatology Department of Poznań University of Medical Sciences between 1998 and 2002 were included in the study.

Microbiological examination of wound swab samples was performed at the Laboratory of Bacteriology, Clinical Hospital No. 2 in Poznań. A total of 175 wound swabs were obtained from patients aged 34-93 prior to any antimicrobial therapy.

Columbia agar with 5% sheep blood, Chapman medium, Sabouraud medium with antibacterial agent, medium for gram-negative bacteria culture, coccosal medium and enriched broth were inoculated for the isolation of aerobic bacteria. The incubation took place at 37° C for 48 h. For anaerobes, isolation specimens were plated onto Columbia agar with 5% sheep blood, selective medium for anaerobic gram-negative bacteria and multiplying broth. The plate media were incubated at 37° C in anaerobic conditions. The identification of both aerobic and anaerobic organisms was performed by use of the ATB Plus bioMerieux computer method.

**RESULTS**

Microorganisms were cultured in 173 (99%) of 175 samples. Women accounted for 80% of study patients. The predominant pathogens were *Staphylococcus aureus* (56.57%), *Pseudomonas aeruginosa* (37.14%), *Enterococcus faecalis* (22.29%), *Proteus mirabilis* (13.71%), *Escherichia coli* (12.57%) and *Candida* spp. (6.86%) (Fig. 1).

A significant increase was recorded in the rate of *Pseudomonas aeruginosa* from 27.78% in 1998 to 42.42% in 2002, *Enterococcus faecalis* from 8.33% in 2000 to 39.39% in 2002, *Escherichia coli* from 11.11% in 2000 to 18.18% in 2002, *Staphylococcus haemolyticus* from 2.78% in 2000 to 15.15% in 2002, *Candida* spp. from 2.78 in 2000 to 12.12% in 2002, and *Enterobacter cloacae* from 2.78% in 2000 to 6.06% in 2002. An interesting pattern was observed in the concurrent decrease in the incidence of *Staphylococcus aureus* isolation from 69.44% in 2000 to 48.48% in 2002 (Fig. 2).

**Figure 1.** Percentage composition of predominant bacterial strains cultured between 1998 and 2002.

**Figure 2.** Variation in the incidence of bacterial isolation during the 1998-2002 period.
The number of isolates per specimen varied from one to six. Two and three microorganisms were most frequently cultured from one ulcer (Fig. 3). The number of polymicrobial specimens increased with the duration of ulcer disease. The presence of three or four organisms was observed in ulcers non-healing for 10 or more years. Single or double isolates were predominant in the samples cultured from ulcers lasting for less than 5 years (Fig. 4).

Figure 3. Percentage of polymicrobial isolates.

Figure 4. Correlation between polymicrobial samples and duration of venous leg ulcer

The presence of *Proteus mirabilis* and *Pseudomonas aeruginosa* in wound swabs could be associated with delayed wound healing. *Proteus mirabilis* and *Pseudomonas aeruginosa* were mainly cultured from patients suffering from leg ulcers for 10 years and more. This correlation was more evident for *Proteus mirabilis* than for *Pseudomonas aeruginosa*, where positive cultures were also frequently obtained from patients with a shorter history of leg ulcer disease (Fig. 5).

Figure 5. Correlation between the presence of *Proteus mirabilis* and *Pseudomonas aeruginosa*, and duration of leg ulcer disease.

The coexisting diseases such as diabetes mellitus or erysipelas did not alter the percentage of isolation and had no effect on either quantitative or qualitative composition of the bacterial flora. Surprisingly, diabetes mellitus was not a factor in increasing the incidence of *Candida* spp. colonization.

DISCUSSION

Venous leg ulcers are associated with medical, social and emotional burden, thus reducing the patient's quality of life. Chronic wounds very often present a non-healing and recurrent medical problem.

Our study confirmed results reported elsewhere on *Staphylococcus aureus* as the predominant microorganism cultured from leg ulcers swabs. On the other hand, we observed a decrease in the incidence of *Staphylococcus aureus* isolation, along with an increase in the rate of *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* during the study period. The increasing number of *Candida* spp. isolates was seen in patients without diabetes mellitus as the concomitant disease. Polymicrobial results of swab cultures were obtained from patients suffering from leg ulcers for many years.

Patients diagnosed with chronic venous insufficiency suffer from impaired wound healing in the affected extremity. The mechanism of delayed healing is unknown. The risk factors for the development of chronic venous insufficiency include age, female sex, obesity, lack of physical activity, and family history (1-5). A chronic, long-lasting...
venous insufficiency may lead to premature fibroblast aging, which is strongly connected with impairment of the wound healing processes. In addition, insufficient blood supply is present due to the pathophysiologic mechanisms of chronic venous insufficiency in lower extremities (12).

The following mechanisms should be taken in consideration when discussing the bacterial role in the wound healing process: contamination, where the wound has non-replicating organisms on its; colonization takes place when bacteria capable of replicating on the ulcer surface inhibit non-viable tissue in the wound in the absence of immune response. Clinically significant infection is defined as a sudden onset of pain or increased pain, spreading erythema, swelling, cellulitis, purulent exudation and odor (13-16). These situations can take place in leg ulcers.

Differentiation between the infected and non-infected leg ulcers may pose a dilemma and is a matter of much discussion, because treatment is determined by the diagnosis. In our retrospective study, we were not able to correlate our data with detailed clinical characteristics of the examined leg ulcers. Some authors suggest that the presence of bacteria in chronic wounds can be identified as a secondary infection, which can also result in impaired wound healing. The presence of more than $10^5$ bacteria per gram of tissue may impede wound healing and epithelial regeneration (17). Most leg ulcers are characterized by complex bacterial population. It is suggested that microbial interactions may contribute to the pathogenesis of chronic wound infections, leading to deterioration of such wounds (6).

Moreover, it should be emphasized that infected ulcers may not only become a serious and dangerous source of nosocomial infections but chronic wounds may also be colonized with nosocomial bacterial strains causing systemic complications in elderly patients with impaired immunity system.

**CONCLUSIONS**

*Staphylococcus aureus* was the main bacterial factor cultured from leg ulcer swabs obtained from patients admitted to Dermatology Department of Poznań University of Medical Sciences between 1998 and 2002. The study period was characterized by a significant increase in the incidence of *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli* and *Candida* spp. isolation. The finding of *Pseudomonas aeruginosa, Proteus mirabilis* and polymicrobial samples in patients with chronic leg ulcer disease may confirm the potential role of bacterial synergism in delayed healing of chronic wounds.

**References**


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