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▣ BRIEF COMMUNICATION

Distribution Frequency of Pathogenic Bacteria Isolated from Cutaneus Leishmaniasis Lesions

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Abstract: Cutaneous leishmaniasis (CL) is a parasitic disease characterized by single or multiple ulcerations. Secondary bacterial infections are one of the complications that can increase the tissue destruction and the resulting scar. To better determine the incidence of real secondary bacterial infections in CL, we designed the current study. This was a cross-sectional study performed in Skin Diseases and Leishmaniasis Research Centre, Isfahan, Iran. A total of 1,255 patients with confirmed CL enrolled in the study. Sterile swaps were achieved for ulcer exudates and scraping was used for non-ulcerated lesions. All samples were transferred to tryptic soy broth medium. After 24 hr of incubation at 37℃ they were transferred to eosin methylene blue agar (EMB) and blood agar. Laboratory tests were used to determine the species of bacteria. Among 1,255 confirmed CL patients, 274 (21.8%) had positive cultures for secondary bacterial infections. The bacteria isolated from the lesions were *Staphylococcus aureus* in 190 cases (69.3%), coagulase negative *Staphylococcus* in 63 cases (23.0%), *E. coli* in 10 cases (3.6%), *Proteus* sp. in 6 cases (2.2%), and *Klebsiella* sp. in 5 cases (1.9%). The results show that the overall incidence of secondary bacterial infections in the lesions of CL was 21.8%, considerably high. The incidence of secondary bacterial infections was significantly higher in ulcerated lesions compared with non-ulcerated lesions.

Key words: *Leishmania*, cutaneous leishmaniasis, bacteria, *Staphylococcus aureus*

Leishmaniasis is a parasitic disease transmitted by sandflies. It is clinically characterised by a spectrum of cutaneous, mucocutaneous and visceral manifestations that depend largely on the species of parasite involved and the host immune response. According to recent estimates, 1.5 million new cases of cutaneous leishmaniasis (CL) cases occur each year. More than 90% of cases occur in 5 countries in the Old World (Afghanistan, Algeria, Iran, Iraq, and Saudi Arabia) and 2 countries in the New World (Brazil and Peru) [1]. CL in the Old World is caused by *Leishmania major*, *Leishmania tropica*, *Leishmania infantum*, and *Leishmania aethiopica*, which are found in southern Europe, the Mediterranean basin, the Middle-East and Africa [2]. CL in the New World is mainly caused by members of the *Leishmania braziliensis* complex (*L. braziliensis* and *Leishmania peruviana*), *Leishmania mexicana*, *Leishmania amazonensis*, and the *Leishmania guyanensis* complex (*L. guyanensis* and *Leishmania panamensis*).

CL of the Old World eventually heals. The rate of spontaneous healing depends on several factors, including parasite load and virulence, host immune responses, location of the lesion, and the presence or absence of secondary bacterial infections. Lesions caused by *L. major* heal spontaneously after about 18 wk [3]. An important part of therapy for CL is local care along with anti-leishmania therapy. Treatment of secondary bacterial infections is essential for healing. On the other hand, secondary bacterial infections of CL will increase the tissue destruction and the resulting scar [4]. To better determine the incidence of real secondary bacterial infections in CL, we designed the current study.

This was a cross-sectional study performed at Skin Disease and Leishmaniasis Research Centre (SDLRC), Isfahan, Iran. The patients enrolled in this study with clinical and parasitological diagnosis of CL and referred to SDLRC from August 2006 to January 2008. The patients belonged to both sex and different age groups and had different clinical forms of CL. The patients or their guardians were informed of this study and they signed the consent forms. Pregnant women, patients with underlying diseases, more than one lesion, and using topical or systemic antibiotics in recent week were not included in the study. The skin areas surrounding the lesions were thoroughly cleaned using cotton wool moistened with alcoholic iodine. After appropriate cleaning, ulcer specimens were obtained by rubbing sterile saline solution over the edge of ulcerated lesions and samples were collected aseptically with scraping the non-ulcerated lesions. All samples were transferred to tryptic soy broth medium. After 24 hr incubation at

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Table 1. Distribution frequency of bacteria in positive cultures

Species of bacteria	Clinical form of lesions			Total (%)
	Ulcerated plaque	Non-ulcerated plaque	Ulcerated papule or nodule	
Staphylococcus aureus	180 (65.7)	4 (1.4)	6 (2.2)	190 (69.3)
Coagulase negative *Staphylococcus*	63 (23.0)	-	-	63 (23.0)
Escherichia coli	10 (3.6)	-	-	10 (3.6)
Proteus sp.	6 (2.2)	-	-	6 (2.2)
Klebsiella sp.	5 (1.9)	-	-	5 (1.9)
Total no. of positive cultures	264 (96.4)	4 (1.4)	6 (2.2)	274 (100.0)

37℃, the samples were transferred to eosin methylene blue agar (EMB) and blood agar. Gram staining, oxidase test, indole test, urease test, catalase test, and coagulase test were used to determine the species of bacteria. All collected data were statistically analyzed by SPSS and the χ^2 test.

A total of 1,255 patients confirmed as CL enrolled in the study. The age range of patients was 2 months to 85-yr-old, and the mean age of the patients was 27.21 ± 11.28 yr. Of them 510 patients (40.6%) were women and 745 (59.4%) were men. The mean duration of the disease was 1.5 ± 0.8 month. The clinical features of lesions included non-ulcerated plaque in 351 patients (28.0%), ulcerated plaque in 401 (32.0%), non-ulcerated papule in 112 (8.9%), ulcerated papules 87 (6.9%), non-ulcerated nodules 175 (13.9%), and ulcerated nodules 129 (10.3%). The sites of the lesions were face in 439 patients (35.0%), upper limbs in 313 (24.9%), lower limbs in 263 (21.0%), and trunk in 240 (19.1%). The results of the culture were positive in 21.8% (274) of the patients. The bacteria isolated from the lesions were *Staphylococcus aureus* in 190 cases (69.3%), coagulase negative *Staphylococcus* in 63 cases (23.0%), *E. coli* in 10 cases (3.6%), *Proteus* sp. in 6 cases (2.2%), and *Klebsiella* sp. in 5 cases (1.9%). The distribution frequency of the isolated bacteria by the clinical from of leishmaniasis lesions are shown in Table 1.

Secondary bacterial infections are one of the complications of CL. Although some authors emphasize on the rarity of this finding [3], our clinical findings are contrary to this. In our practice, we encountered many cases of infected leishmaniasis ulcers. In fact, secondary bacterial infections can exacerbate the disease and the final scar, because it will increase the tissue destruction and necrosis. In these cases, painful ulcers with purulent discharges and with surrounding inflammation would be resulted. In addition, the duration of the disease would be prolonged [4]. An appropriate use of antibiotics will decrease the resultant infections in these cases. One study in Sudan has shown the prevalence of the secondary infections to be 18% of 736 evaluated patients [5]. The pathogenic organism was not identified in this study [5]. In another study, bacteria including *Proteus vulgaris, Pasturella multisided, S. aureus, Staphylococcus albus, E. coli,* and *Pseudomonas aeruginosa* were isolated from clinically infected lesions [6]. In another study that was performed in the impetigized forms of leishmaniasis, *S. aureus* was recognized to be the responsible pathogen [4]. In a study performed in the Yucantan peninsula of Mexico, some pathogenic bacteria were detected from the skin lesions of patients with chiclero's ulcers (a form of CL due to *L. mexicana*) reluctant to antimonial treatment, and need to eliminate bacterial infections by antibiotic therapy before starting antimonial administration was suggested [7]. In our study, out of the 1,255 patients with confirmed CL, 274 (21.8%) had confirmed secondary bacterial infections. The most common bacterial isolate was *S. aureus*. Other pathogens included coagulase negative *Staphylococcus, E. coli, P. vulgaris,* and *Klebsiella*. There was no significant association between the prevalence distribution of the isolated bacteria with the age, sex, location, number, and duration of the disease ($P > 0.05$). However, there was a significant association between the clinical form of the disease and the isolated bacteria ($P = 0.00001$). All lesions that had secondary bacterial infections were ulcerated. There was no bacterial isolates from the lesions that were not ulcerated.

Regarding these facts we can conclude that destruction of the epidermis in the ulcerated lesions had predisposed the patients to secondary bacterial infections. Regarding the results of our study, we suggest that topical antiseptic solutions are needed for ulcerated lesions of CL to prevent secondary bacterial infections that may accelerate tissue destruction. In addition, in the case of symptoms and signs of secondary bacterial infections, use of antibiotics especially against *S. aureus* would be logical.

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