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Prevalence of Bacterial Infections among Cutaneous Leishmaniasis Patients

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Abstract

Cutaneous leishmaniasis (CL) is an infectious disease transmitted by female sandflies that are infected with protozoan parasites belonging to the genus Leishmania. This vector-borne illness manifests in humans through the formation of one or multiple skin lesions, which may present as ulcers, nodules, or papules. A significant complication of CL is the occurrence of secondary bacterial infections, which can exacerbate tissue damage and lead to the formation of scars. These bacterial infections are particularly prevalent in regions with inadequate sanitation and hygiene practices. The presence of secondary bacterial infections can impede the healing process of CL lesions and heighten the likelihood of scarring. The main objective of the present investigation was to examine the frequency of bacterial infections in patients with cutaneous lesions who were admitted to the Dermatology Clinic of the National Center for Disease Control for the treatment of Cutaneous Leishmaniasis. This cross-sectional study was conducted at the NCDC Dermatology Clinic. and included 81 patients with confirmed CL. Samples were collected from all patients and analyzed using standard bacteriological and parasitological methods. Out of the 81 patients, leishmania was detected in all cases. Bacteriological experiments revealed that 58 cases (71.06%) were also infected with bacterial infections. The most common bacterial isolates were *Staphylococcus aureus* (39.8%), *Pseudomonas auroginosa* (22.6%), and *Staphylococcus epidermidis (*8.6%). These findings highlight the ongoing issue of bacterial infections in patients with cutaneous leishmaniasis and emphasize the importance of considering them during treatment.

Keywords: Bacteria, *Cutaneous leishmaniasis*, infection, National Center for Disease Control (NCDC).

Introduction

Leishmaniasis is a parasitic disease caused by parasites of the genus Leishmania. Leishmania causes a variety of clinical symptoms based on the parasite's features and the host's immunological response. The most prevalent disease manifestation is cutaneous leishmaniasis (CL), which manifests as lesions that frequently ulcerate and typically heal spontaneously within weeks to months [1,2]. *Leishmania major* and *tropica* are the species responsible for the majority of cutaneous leishmaniasis (CL) infections in Libya, where the disease is a public health problem [3]. A typical lesion is a painless ulcer at the site of the parasite delivery, characterized by a raised, hardened edge and a necrotic base covered in dried, yellowish exudate [3,4]. It is usually devoid of pain. Many patients have one or two skin lesions, usually found on exposed areas, ranging in size from half a centimeter to four centimeters in diameter. When the crust covering the

nodule of the lesion breaks, it becomes vulnerable to colonization or infection by various microorganisms, including pathogenic or opportunistic fungi and bacteria, which may cause additional infections [4]. Behrman and Vaughan (1983), noted that secondary infections may contribute to the size and shape of the lesion, as well as scar formation. However, data on the specific microorganisms involved are limited, and it is thought that they are primarily derived from the transient or indigenous skin microbiota [5]. Numerous bacteria live on the skin, and their type and density are influenced by anatomical factors, humidity, sebum production, and hormonal changes within the host. The bacteria in the skin microbiota are often commensal, symbiotic, or parasitic; their relationship to the host may be intrinsic or influenced by changes in the host's immune-mediated states. It has been shown that secondary infections can decrease the number of amastigotes in microscopic specimens obtained from cutaneous lesions [6]. Besides causing the intensity and paroxysms of the disease, bacterial infections have a negative influence on disease diagnosis [7]. A limited number of studies have been identified in the literature regarding the prevalence of bacteria in lesions of CL [8,9]. reported the predominant presence of coagulase-positive *staphylococci* in ulcers of CL in Iran, as well as *Streptococcus* species, *Pseudomonas* species, *Klebsiella* species, and *E. coli* [9,10]. also observed the prevalence of *Staph. aureus* in cutaneous leishmaniasis lesions in Maranha˜o and Minas states, Brazil, respectively. However, these reports did not determine the presence of obligate anaerobic bacteria infection [10,11]. The main aim of the current study was to investigate the prevalence of bacterial infections among individuals with cutaneous lesions who were admitted to the National Center for Disease Control Dermatology Clinic to treat Cutaneous Leishmaniasis.

Materials and Methods

Study Design: A cross-sectional study was conducted at the National Center for Disease Control (NCDC) Leishmania Dermatology Clinical Tripoli. A total of 81 patients diagnosed with cutaneous leishmaniasis were referred to the Dermatology and Leishmaniasis Clinic between November 2021 and February 2022. Prior to the study, ethical approval was obtained from the research committee. The study objectives were explained to all participants, and informed consent was obtained from each individual. All relevant data were collected using a questionnaire, which included demographic information such as age, gender, medical history, location, and number of lesions, as well as details of any previous antibiotic treatment. Additionally, laboratory tests were conducted at the parasitology and vector-borne diseases laboratory. The diagnosis was initially made clinically and then confirmed through parasitological studies by direct skin–slit smear stained with Giemsa.

Inclusion criteria consisted of patients with a history of recent antibiotic therapy or concurrent use of topical medications with antibiotic or antiseptic effects were excluded from the study.

Sampling procedures: Sample collections were assisted by trained health workers in line with ethical standards. The lesions were cleaned using sterile gauze and physiologic saline and disinfected five times in an outward circular motion with 70% alcohol-immersed cotton. Using a sterile lancet or sterile surgical blade, 2-3 mm long superficial incisions were made on the margins of the lesion, and pressure was maintained with a finger to achieve hemostasis. Then smears were studied by gram stain and direct microscopy techniques.

Bacteriological culture is then accomplished using the streak method according to standard methods [12,13]. Culture plates are incubated, and daily monitoring of culture media is essential, colony morphology and description are observed for 24-48 hours. Each sample (Swab) was plated onto a culture media, including Mannitol Salt Agar, MacConkey Agar, Blood Agar, Nutrient Agar, Muller-Hinton Agar,

Triple Sugar Iron Agar, and Nutrient Broth within 24 hours of sampling, and morphologically identified by smearing on the slide for bacterial Gram's stain, and the culture media. Bacterial genus and species were identified by standard identification testing according to certain guidelines.[14]

Statistical method: The data was entered in Excel sheets and SPSS (IBM Statisticstics for Windows, version 21.0, IBM Corp., Armonk, New York, USA), and both programs were used to conduct data analysis. The ANOVA test (Analysis of Variance) was used to test if there are statistically significant differences between demographic characteristics and categorical dependent variables of interest.

Results: In this study, a total of 81 patients who confirmed Cutaneous Leishmaniasis (CL) were enrolled. The male population exhibited a higher prevalence, with 64 individuals (79%) affected, compared to 17 females (21%). The age range of the patients varied from 1 month to 70 years or older. The age group of 20-29 years exhibited the highest prevalence, with 15 individuals (18.5%), followed by the 40-49 age group with 14.9 individuals (18.2%) and the 30-39 age group with 12 individuals (14.8%). The prevalence of CL cases within the age groups showed significant associations ($P < 0.05$). Additionally, the results indicated that 42% of the patients who had multiple exposed parts of the body were infected with CL, with 58% exhibiting a single lesion Table 1.

Table 1. Demographic characteristics of the studied population

Characteristic	Percent (%)
Sex	
Male	79%
Female	21%
Age groups	
0-9	8.6%
10-19	16%
20-29	18.5%
30-39	14.8%
40-49	18.2%
50-59	14.8%
60-69	4.9%
≥70	3.7%
Face	1%
Neck	1%
Ear	2%
Arm	9%
Hand	10%
Leg	15%
Foot	20%
Multiple exposed parts	42%
1 lesion	58.0%
2 lesions	35.8%
≥ 3 lesions	6.2%

The results of the culture were positive in 71.60% of the patients (58 patients). Bacteria isolated from the lesions were as follows: *Citrobacter breakii* 2 (2.2%), *Enterobacter clocea* 6 (6.5%), *Escherichia coli* 7 (7.5%), *Klebsiella pneumonia* 2(2.2%), MRSA 1 (1.1%), *Pantoa sp* 2 (2.2%), *Pasteurella multocida* 2 (2.2%), *Protus mirabilis* 2 (2.2%), *Protus vulgaris* 1 (1.1%), *Pseudomonas aeruginosa* 21 (22.6%), *Serratia plymutica* 1 (1.1%), *Staphylcoccus areus* 37 (39.8%), *Staphylococcus epidermidis* 8 (8.6%), *Streptococci pyogenes* 1 (1.1%). Figure 1.

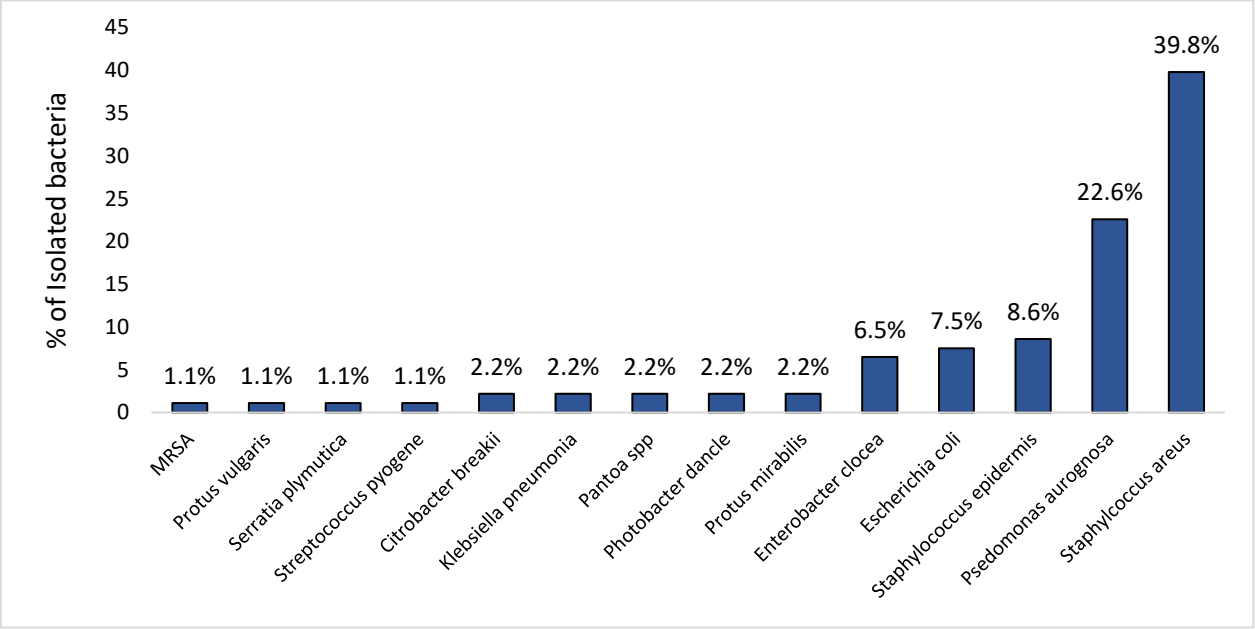

Figure 1. Isolates bacteria from patients' cutaneous leishmaniasis lesions.

Discussion

Cutaneous leishmaniasis (CL) is a prevalent public health concern in numerous countries worldwide, including Iraq, Kuwait, Iran, Afghanistan, countries in the Middle East, and North Africa [14]. Additionally, the occurrence of secondary bacterial infection is a complication associated with CL, leading to the deterioration of infected leishmaniasis ulcers. This secondary bacterial infection exacerbates the condition and results in further tissue destruction and necrosis. Despite the varying manifestations of cutaneous leishmaniasis, ranging from self-healing cutaneous lesions to chronic forms of the disease, the outcome depends on the infecting Leishmania spp. and the host's immunological protection [15]. To enhance therapeutic treatment efficacy and minimize scarring, a deeper understanding of the infection and the organisms co-infecting the wounds is necessary. This study aims to isolate and identify bacteria co-infecting CL wounds. The study was conducted prospectively at the NCDC Leishmania Dermatology Clinic in Tripoli, involving eighty-one patients with cutaneous leishmaniasis who were referred to the clinic between November and February. These patients exhibited symptoms of the disease, had a portion of their infected skin lesion sampled, and underwent smear analysis to ascertain the prevalence and nature of the disease. Additionally, demographic, clinical, and microbiological information of patients with ulcerative cutaneous leishmaniasis was evaluated.

The results indicate a significant disparity in leishmaniasis infection rates between males and females. The prevalence of cases was higher in males, accounting for 79.01% (64 cases), compared to females at 20.99% (17 cases). This observation aligns with previous studies conducted in Libya, where males were found to

be more significantly affected than females. In a study of 140 cutaneous leishmaniasis cases, 57.1% were males and 42.9% were females [15]. This difference may be attributed to variations in behavioral patterns, such as males spending more time outdoors, engaging in more physical activity, and being exposed to diverse environments due to their occupation, such as farmers in rural areas. Conversely, females may be more protected due to their clothing choices, which provide coverage [16]. However, it is important to note that this finding contradicts studies conducted in Yemen [17] and Morocco [17], where cutaneous leishmaniasis was observed to be more prevalent in females than in males. The observed discrepancy may be attributed to variations in the sociodemographic characteristics of the study participants and the employed study designs. However, studies conducted in Nigeria have reported no gender disparity in the incidence of the disease[19]. The present study has revealed that the location of lesions in different regions is influenced by sand fly activity, clothing culture, and exposure to the carrier. Patients predominantly exhibited lesions on their limbs, such as the foot (20%), leg (15%), hand (10%), and arm (9%), with 42% of patients exhibiting multiple lesion sites on their trunk and extremities. Approximately 12% of the lesions were observed on the hands, face, and neck, which may be attributed to sleeping outside without using linen and lace and improper body covering during the summer. These findings are consistent with those of a previous study conducted in Iran, which demonstrated that CL lesions primarily occur on exposed areas of the body, such as the face, hands, and feet [20]. The present study has also revealed that different age groups exhibit varying rates of CL disease. Although no significant association was observed between the prevalence distribution of the isolated bacteria and age ($P > 0.05$), a significant association was observed between the clinical form of the disease and various age groups ($P = 0.0038$). The youngest patient was under the age of one, while the oldest was 90 years old. Less than a quarter of patients were above 42 years of age, and more than half of the patients were under the age of 28. In a study conducted by Hamzavi and Khademi, the highest number of cases (24.7%) was observed in the age group of 20-29 years [21]. In contrast, Delgado et al. reported a mean age of 35 years for CL cases, which differs from the present study [22]. The prevalence of CL was found to be statistically significant in different age groups ($P < 0.001$) [23]. No statistically significant difference was observed between the number of lesions (one, two, three, four, and more lesions) and gender ($P = 0.170$). All of the ulcerated lesions exhibited subsequent bacterial infections. Non-ulcerated lesions did not yield any bacterial isolates. Based on these findings, it can be concluded that the degradation of the epidermis in the ulcerated lesions predisposed the patients to subsequent bacterial infection. In this study, 58 (71.60%) out of the eighty-one patients with confirmed cutaneous leishmaniasis were found to have secondary bacterial infections. A total of ninety-three (93) isolates from fourteen different bacterial species, including both Gram-positive and Gram-negative bacteria, were obtained in this study. The study revealed that S. aureus was the most frequently isolated species among the Gram-positive bacteria [24, 25]. In an additional study on impetiginized forms of leishmaniasis, Staphylococcus aureus was identified as the causative pathogen, followed by Staphylococcus epidermis, Streptococcus pyogenes, and MRSA [26]. Furthermore, Pseudomonas aeruginosa was the most common Gram-negative bacteria, which contradicts a study conducted in Sudan where E. coli was found to be the most common Gram-negative bacteria, followed by Enterobacter cloacae, Citrobacter braakii, Klebsiella pneumonia, Pantoa sp., Pasteurella multocida, Proteus mirabilis, Proteus Vulgaris, and Serratia plymutica. Another study conducted by El-Safi et al., 1991 investigated bacterial species but did not identify the harmful organism [27]. Similarly, El-On et al., 1992 isolated bacterial infections from clinically infected lesions, including Proteus Vulgaris, Pasteurella multocida, Staphylococcus aureus, Staphylococcus albus, E. coli, and Pseudomonas aeruginosa [28]. However, the

wide variety of Gram-negative bacteria isolated from the lesions may suggest that the infection was acquired in the community. Our findings demonstrate that all of the lesion samples were infected with either mono-microbial or poly-microbial organisms and there was no significant association between the prevalence distribution of the isolated bacteria with the age, sex, location, number, and duration of the disease ($P > 0.05$). With regard to the findings of our study, it is recommended that topical antiseptic solutions be employed for ulcerated lesions of cutaneous leishmaniasis in order to mitigate the risk of secondary bacterial infection, which has the potential to expedite tissue deterioration.

Conclusion:

The prevalence of cutaneous leishmaniasis remains a significant concern in Libya. Moreover, the presence of concurrent infections caused by pathogenic or opportunistic bacteria further complicates this issue, as these bacteria have a detrimental impact on the diagnosis and treatment of leishmaniasis. Consequently, in cases where cutaneous leishmaniasis lesions are accompanied by alternative infections, it is imperative to simultaneously address the diagnosis and treatment of the parasite through the administration of appropriate antibiotics. Overall, healthcare professionals should prioritize efforts to prevent and minimize this problem, thereby mitigating the occurrence of concurrent infections in these lesions.

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