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Original article

Clinical and Bacteriological Characteristics of Cutaneous Leishmaniasis at the Outpatient Clinic, Tripoli Central Hospital, Libya

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ABSTRACT

Leishmaniasis is a common health problem worldwide, endemic in nearly 90 countries, primarily in tropical and subtropical regions across four continents. An increase in the number of cutaneous leishmaniasis (CL) cases has been reported over a wide area of the South and West of Tripoli. Since most of the CL cases referred for slit and smear and management if infected, our study aimed to determine the clinical aspects of CL, to isolate and identify bacteria responsible for secondary infection in the lesions, and to study the antibiotic susceptibility of the isolated organisms to commonly used antibacterial agents. A total of 450 patients with suspected cutaneous leishmaniasis (CL) attending the dermatology outpatient clinics in Tripoli Medical Center (TMC) and Tripoli Central Hospital (TCH) over one year (June 2003 to June 2004) were examined. In each case, a detailed history and clinical examination were performed according to a protocol prepared for this study. The majority of patients were males (52%) with a male-to-female ratio of 1.1:1. Children, adolescents, and young adults—especially students and housewives—were the main age groups affected. Most patients were from the northwestern region and coastal area of Libya, and 96.7% of them were Libyans. Furunculoid and nodulo-ulcerating lesions (36.4% and 33.6%, respectively) were the main clinical types of CL reported, and most of the patients had either one or two lesions on exposed parts of the body. Samples from CL lesions for bacteriological examination were taken from 246 cases, and a positive culture was obtained in 37.4% of cases. Among the bacterial infections found, *Staphylococcus aureus* (*S. aureus*) was the most frequent isolate (62%) among children in the 0–10 years age group and young adults in the 21–30 years age group, while *Staphylococcus epidermidis* (*S. epidermidis*) was isolated less frequently (20.7%) in the 11–30 years age group. This study revealed that CL in Libya is endemic in certain areas and has a seasonal pattern, and S. aureus was the main isolate from CL lesions.

Introduction

Leishmaniasis is a common health problem worldwide and an endemic parasitic infectious disease in the Middle East, with more than 40,000 cases reported each year [1], compared to 1–1.5 million annual incidences worldwide, most of which are CL [2]. Two types commonly exist in this area: anthroponotic cutaneous Leishmania tropica and zoonotic cutaneous Leishmania major [3], the latter being more common in Libya, which is considered one of the countries with higher rates of CL, mainly in the northwestern region. The sand fly Phlebotomus papatasi is the most common species found, with the highest incidence rates in November, December, and February [4]. After the patient is bitten by the sand fly, over 2 to 6 weeks, gradually increasing papules begin to form. Then a nodule with a central crust develops, which resolves spontaneously after 6 to 9 months and, in some cases, leaves a depressed scar behind [2]. In cases where the crust peels off, an ulcer is formed, making it susceptible to bacterial infection. This superinfection has the potential to mask the initial diagnosis of amastigotes under the microscope by lowering their number. On the other hand, in countries where CL is endemic, bacterial infections could be misdiagnosed as CL and mistreated, causing iatrogenic side effects [5]. One of the most common organisms causing superinfection is *group A Streptococcus* and *S. aureus* [9–11].

Along with determining the clinical aspects of CL, and given the relatively obscure role of bacterial infection in CL, in this study we aimed to determine the clinical aspects of CL cases, to isolate and identify bacteria responsible for secondary infection in CL lesions, and to study the antibiotic susceptibility of the isolated organisms to commonly used antibacterial agents.

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Methods

study design and setting

A retrospective cross-sectional study was performed from June 2003 to June 2004 in the leishmaniasis clinic at TMC and TCH in Tripoli. A total of 450 patients clinically diagnosed with CL were enrolled in this study and were referred to us from different locations in Libya for clinical and microscopic diagnosis, as well as management. In each case, a detailed history and clinical examination were performed according to the protocol prepared for this study. Bacteriological studies were done only for 246 cases who had either never been treated before or had stopped antibiotic treatment for a few days.

Sampling procedures

Samples were taken after thoroughly cleaning the lesion and the surrounding skin using 70% alcohol. Samples were collected from the center of the ulcerated nodule or the edge of the lesion using a sterile swab, then inoculated immediately onto blood and MacConkey agar plates and transferred to the laboratory within 4 to 6 hours for processing. Slit-smear was done by making a small incision using a sterile blade No. 15 while squeezing the margin of the lesion between the thumb and index finger until the area became bloodless. The smear was taken by scraping the cut of the incision, and the tissue specimen on the blade was spread on a clean slide, left to dry, and then stained with Giemsa stain. Data were analyzed using SPSS (version [26], IBM Corp.).

Statistical analysis

A Chi-square test was used to evaluate the association between amastigote positivity and bacterial growth. A p-value of <0.05 was considered statistically significant.

Results

During the period of this study, the total number of patients attending dermatology outpatient clinics in TMC and TCH was 13,115 and 9,870, respectively. Out of the diagnosed CL cases in leishmaniasis clinics, CL accounted for about 2.7% in TMC and 0.99% in TCH. The ages of the patients ranged from three months to eighty-four years, with a mean age of 25.6 years. About 234 were males (51.8%) and 218 were females (48.2%) with male-to-female ratio was 1.1:1. The majority of cases were children in the age group (0–10 years) with an equal sex distribution, followed by the age groups (21–30) and (11–20) years, mostly seen in adolescents and young adults. Cutaneous leishmaniasis was less frequently reported in elderly people in this study. Additionally, majority of cases were from the north-western regions and coastal areas of Libya, and 96.7% of them were Libyans. Most of the patients were from EL-Nikat EL-Kamis (133, 29.6%), followed by 72 (16.1%) from Beni Walid. Out of the 450 patients with CL, 60 (13.3%) were local inhabitants of Tripoli city, and 51 (85%) of these patients gave a history of visiting endemic areas, and 132 cases (29.3%) were students. Moreover, the disease was more frequent during the winter and autumn months (Table 1).

Table 1. Demographic and baseline data

Demographics		**N= 450 (%)**
Age in years	Range	0.25 – 84
	mean	25.6
Gender	Male	234 (51.8%)
	Female	216 (48.2%)
Nationality	Libyan	435 (96.7%)
	Non-Libyan	15 (3.3%)
Residence	Tripoli	60 (13.3%)
	El-Nikat El-Kamis	133 (29.6%)
	Beni Walid	72 (16.1%)
	Girian	65(14.4%)
	El-Zawia	32 (7.1%)
	Nalot	29 (6.4%)
	Mizda	28 (6.2%)
	El-Gufra	23 (5.1%)
Occupation	Students	132 (29.3%)
	Housewives	107 (23.8%)
	Preschool age	64 (14.2%)
	Employees	51 (11.3%)

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	Teachers	28 (6.2%)
	other	68 (15%)
Most frequent months' presentation	January	125 (27.8%)
	February	114 (25.3%)
	March	76 (16.9%)
	December	55 (12.2%)

Furthermore, the duration of illness ranged from one week to three years, with a mean of 2.5 months. Twenty-two of the patients (4.9%) had lesions that existed for six months or more. Two hundred and eighteen patients (48.4%) had a family history of CL among their family members. Sixty-six of the 450 studied cases (14.7%) had an associated clinical illness: 16 of these patients (24.2%) had diabetes mellitus (DM), 10 (15.2%) had hypertension, 10 (15.2%) had both DM and hypertension, 7 (10.6%) had atopy, and 3 (4.5%) had blood diathesis. The rest of the patients had other associated diseases. This study showed that most of the patients (63.3%) had either one or two lesions, distributed mainly on exposed parts of the body, especially the face, and 167 (36.7%) had more than three lesions (Table 2).

Table 2. Patients' Clinical History

Clinical History		**N= 450**
Duration	Lesions <6 months	428 (95.1%)
	Lesions >6 months	22 (4.9%)
Family History of CL	Yes	218 (48.4%)
	No	232 (51.1%)
Associated Conditions	Total with associated illnesses	66 (14.7%)
	Diabetes Mellitus	16 (24.2%)
	Hypertension	10 (15.2%)
	Both DM & HTN	10 (15.2%)
	Atopy	7 (10.6%)
	Blood diathesis	3 (4.5%)
Lesion Count	1–2 lesions	283(63.3%)
	3 or more lesions	167(36.7%)
Slit and Smear		N= 318
Result	Positive	238 (75%)
	Negative	80 (25%)

One hundred and sixty-four cases (36.4%) had a furunculoid type of CL, 151 (33.6%) had nodulous-ulcerating (NU) lesions, 80 (17.8%) had mixed lesions, 30 (6.7%) had lesions with satellites, 8 (1.8%) had NU lesions with lymphatic infiltration, and 7 (1.3%) had sporotrichoid-type lesions, as shown in (Figures 1). Additionally, 10 (2.2%) had other unusual clinical varieties of CL, such as lupoid, carbuncle, erysipeloid, keratoacanthoma, and zosteriform-like lesions. The distribution of clinical types varied significantly between genders. Chi-square analysis showed a statistically significant association between gender and clinical types of CL (x^2 = [13.19], p = 0.04) (Figure 2).

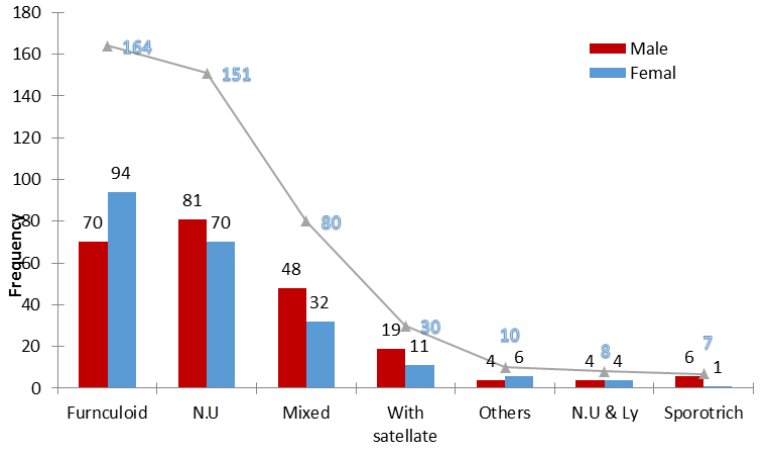

Figure 1. Clinical Types distribution of CL cases according to sex.

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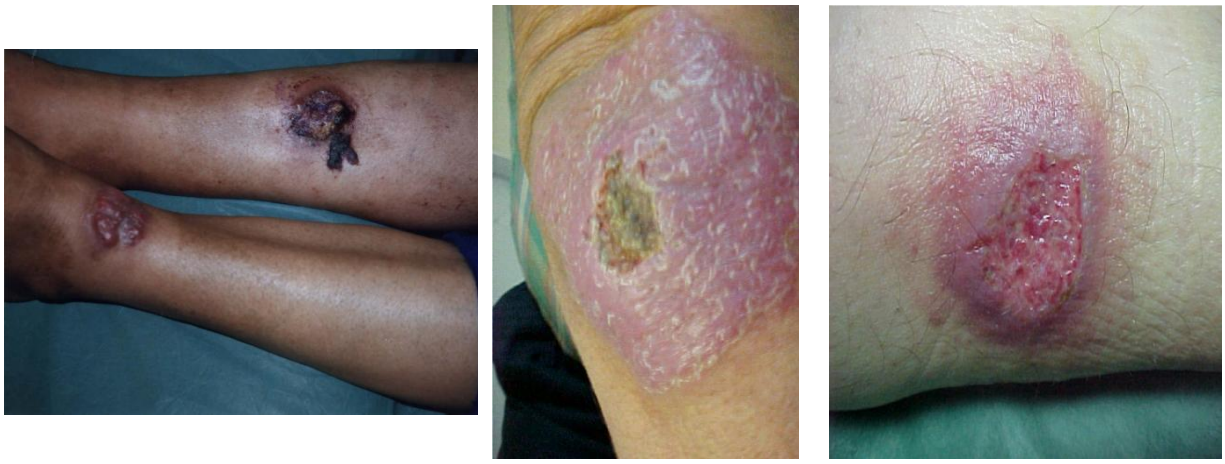

Figure 2. Clinical types of CL: (a) carbuncle-like type; (b) nodulo-ulcerative type with plaque-like infiltration; (c) infected nodulo-ulcerative type.

Samples from CL lesions for bacteriological examination were taken from 246 cases, and a positive culture was obtained in 92 (37.4%) of these cases. Among the bacterial infections found, *S. aureus* was the most frequent isolate (62%), particularly in children aged 0–10 years and young adults aged 21–30 years. *Staphylococcus. epidermidis* was isolated mainly from patients in the 11–20 and 21–30-year age groups, with a frequency of 20.7%. *Pseudomonas aeruginosa (Pseudo. aeruginosa)* and *Escherichia coli (E. coli)* were isolated at equal frequencies (5.4%), while *A B. hemolytic streptococci group A (B.H. Streptococci)*, *Klebsiella oxytoca (K. oxytoca),* and *Proteus vulgaris* (*P. vulgaris*) were isolated less frequently (3.3%, 2.2%, and 1.1%, respectively). Microscopic examination of slit smears taken from 318 CL cases revealed the presence of amastigotes in 238 (75%) of the cases, despite the presence of superinfection (Figure 3). A Chi-square test revealed a statistically significant association between amastigote positivity and bacterial growth (x^2 = [43.513], p = <0.05).

The susceptibility of each isolated antimicrobial agent was determined by the disc diffusion method (Kirby-Bauer method). Of the 57 *S. aureus* isolates tested for antibiotic susceptibility, *S. aureus* showed high resistance to sulfonamide (78.9%) and ampicillin (75.4%). Additionally, 28% of the isolated *S. aureus* strains were methicillin-resistant *S. aureus* (MRSA).

The resistance rate of *S. epidermidis*, although the number of isolates was small, was found that *S. epidermidis* showed high resistance to ampicillin (68.4%) and sulfonamide (63.2%). Both *S. aureus* and *S. epidermidis* showed low resistance to ciprofloxacin (3.5% and 10.5%, respectively) (Figure 4).

Furthermore, statistical analysis demonstrated no significant association between age group and the type of bacterial organisms isolated (p = 0.56). Similarly, no significant association was found between the month of presentation of CL and sex (p = 0.34). Gender distribution also showed no significant variation across different months (p = 0.78).

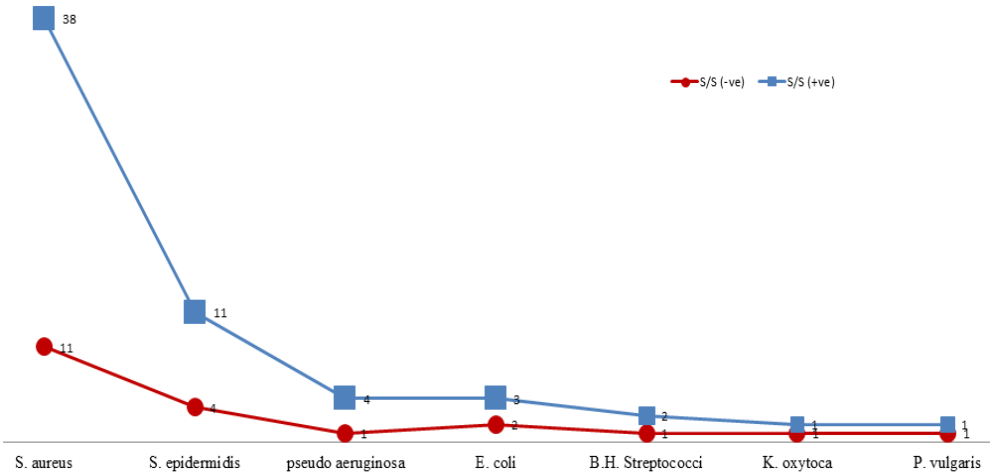

Figure 3: Percentage Distribution of isolated microorganisims from C.L. cases

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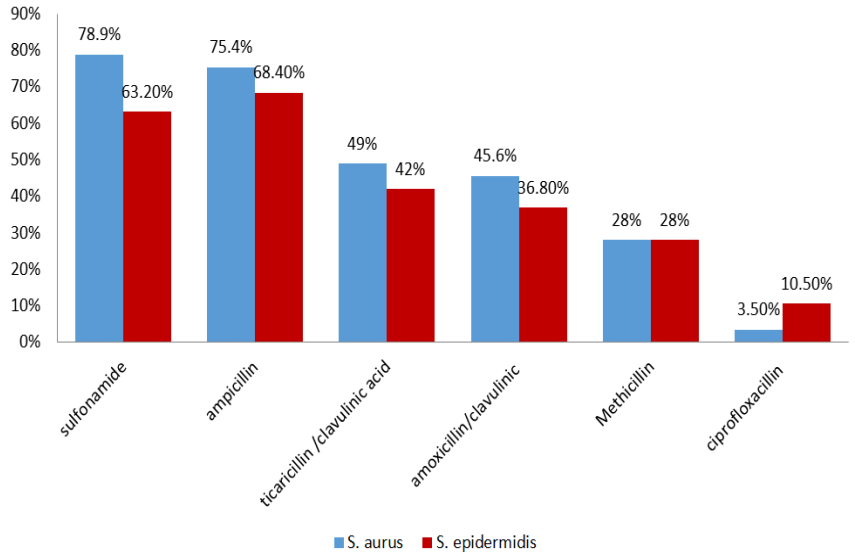

Figure 4: Percentage of resistance of S. aurus and S. epidermidis to different antibiotics

Discussion

Cutaneous leishmaniasis (CL) is a clearly significant and increasing public health problem in our country Libya. Cutaneous leishmaniasis, or oriental sore, is endemic in Libya, Kuwait, and Iraq. It is also endemic in other countries of the Middle East, and Central Asia, as well as in the countries all around the Mediterranean Coast [6]. In the present study, the clinical and bacteriological aspects of CL were carried out in 450 patients in a year study. Increase in the number of reported cases of CL in this study as compared to the previous retrospective study done by El Dwebi et al (1998) [7] in which only 443 cases were reported during 4 years. This may be explained by improved health care coverage and surveillance which have accounted for more frequent diagnoses, better communication, and reporting systems. In addition, due to new agricultural development in our country, the geographical distribution of the disease is becoming more widespread.

In the present study, most patients have one or two lesions on exposed parts of the body, especially the face. These findings concurred with studies done by Hepburn et al. (1993) [8]. This is easily explained because they are the accessible areas for the sandflies to bite. In addition, it is common to find people exposing their extremities, especially upper limbs more than any other part of the body during summer months. Also, may be related to the behavior and species of sandfly, and the immune status of the patient.

This study revealed that CL was more frequent in children, adolescents, and young adults of age group (0-10) years and (21-30) years, and less frequently seen in elderly people. This probably would indicate that this endemic disease is not a new occurrence in the involved areas. In addition, the elderly have lived in endemic areas for a long time and may have been infected in the past and develop long-lasting immunity against reinfection. Thus, younger age groups are therefore at the greatest risk of the disease because of low immunity. These findings concurred with those reported in Tripoli by El-Buni et al. (1994) [9], El-Dwebi et al. (1998) [7], and in Riyadh by Al Shammari et al. (1992) [10]. In addition, a high frequency of the disease among children has been observed in several parts of Italy and Turkey in studies done by Bettini et al. (1981) [11], and Uzun et al. (1999) [12], respectively.

This study showed that CL was more frequent in males (52%) than females (48%) with a nearly equal ratio. These findings concurred with studies reported previously by El-Buni et al. (1994) [9], and El-Dwebi et al. (1998) [7], while Al-Shammari et al. (1992) [10], in Riyadh, found almost equal male/female distribution. The reason for more CL lesions seen in males than females maybe because adult males are more exposed to infection, and/or females are not seeking medical advice, and they may find it more difficult to come to the cities to have medical examinations.

This study has reported a higher frequency of CL in rural areas (96.7%) than in urban areas and this could be because CL was endemic in certain regions of Libya a long time ago. The majority of cases were reported from the North Western region and coastal area of Libya, especially from El-Nikat El-Khamis, Beni-walid, Giarian, and El-Zawia, in addition to other geographical localities. The report of the high number of cases from these regions is unknown exactly but may be due to a lack of well-trained dermatologists to manage the cases and give them proper advice and treatment, or maybe these areas have higher populations than others. In addition, the increase in the number of cases in some regions is generally due to migration, travelling, and ecological changes. Authors found the majority of patients were students, housewives, and of preschool age. This could be explained by the fact that housewives and their children in our endemic

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areas are looking after their animals and the animal houses. In addition, children and students play outside houses even at night in those areas. This study confirmed that the CL in our country has a seasonal pattern as reported previously by El-Dwebi et al. (1998) [7]. The majority of cases were reported during winter and autumn months with a peak incidence in January and February. Al-Shammari et al. (1992) [10], and Uzun et al. (1999) [12], reported similar findings in Riyadh and Turkey respectively.

The incidence of CL caused by L. major is definitely seasonal with the peak incidence during the winter months as reported in our study and other studies and trough during summer. This is probably due to the practice of sleeping outdoors on hot summer nights provides more opportunities of infection and this correlates with the peak incidence in the winter months because the incidence period may last for several months and also correlates with the breeding of the sandfly population as reported in Saudi Arabia in Al-Hassa area [13]. This study showed that secondary bacterial infection of CL lesions accounted for about 37.4%, which was less than that reported by Vera La et al. (2002) [14], (54.2%), and Ben Ismail et al. (1999) [15], (57%), but nearly similar to that reported by Edrissian et al. (1990) [5], (35.7%) in Iran. This could be probably explained by the fact that the rate of prevalence of infection in different countries may vary according to differences in climate, exposure of the population, and health economics. Among the bacterial isolates found, *S. aureus* was the most frequent isolate (62%). These findings concurred with studies done in Iran and Brasilia, by Edrissian et al. (1990) [5], and Vera La et al. (2002) [14], respectively. Low standards of hygiene in developing countries and certain human habits may explain the higher rates of isolation of *S. aureus* from CL lesions in this study. In addition, *S. aureus* is one of the most important skin pathogens, easily transmitted and diseases caused by it are favored by a heavily contaminated environment (e.g., family member with boils), and a compromised immune system.

The clinical significance of *S. epidermidis* which was isolated from CL lesions (20.7%) in the present study is unclear, as they generally are considered to be non-pathogenic and a part of the normal skin flora; however, recent studies have suggested that *S. epidermidis* under certain conditions can cause invasive diseases and deeper parts of hidradenitis suppurativa [16,17]. Researches employed both aerobic and anaerobic cultivation methods, and could not reveal any important anaerobic bacteria, and therefore it does not appear that anaerobic bacteria were prevalent to any great extent in the CL lesions as also reported by Edrissian et al. (1990) [5]. The isolation of *B.H. Streptococci* from CL lesions in low frequency (3.3%) which does not concur with the previous studies done by Edrissian et al. (1990) [5], (10.6%), and Ben Ismail et al. (1999) [15], (22%). This could be probably due to the widely used anti-streptococcal antibiotics by dermatologists and general practitioners. The isolation of opportunist pathogenic bacteria such as *Pseudo. aeruginosa, E. coli, K. oxytoca*, and *P. vulgaris* in low frequency in our study correlates with the study done by Edrissian et al. [5]. This could be explained because most of our patients were immune competent and those bacteria are opportunistic and mainly seen in patients with altered immunity.

Direct microscopic examination was done using Giemsa stain for smears obtained from the margin of CL lesions. In this study, 75% of the cases were positive by direct microscopy in contrast to the findings in this study. Uzun et al. (1999) [12] reported that the direct microscopic examination was successful in 90% of cases, while Hepburn et al. (1993) [8], and Edrissian et al. (1990) [5] reported 61% and 51% respectively positive results on direct microscopy. Thus, direct microscopic examination using Giemsa stain remains highly successful for detecting Leishmania parasite and providing presumptive confirmation of the infection. Study revealed that the difference in the frequency of bacterial infection in CL lesions in which amastigotes were defective (70.2%), and in those in which no parasite was found (29.8%), was significant. However, Edrissian et al. (1990) [5] found the difference in the prevalence in the bacterial infection in suspected CL cases in which amastigotes were detected or not, in (26.8%) and (45.6%) respectively. This could be probably because of the large sample size in their study (2202 cases), and 788 of them were positive for pathogenic bacteria.

Analysis of antimicrobial resistance pattern reveals a remarkable resistance of *S. aureus* to ampicillin, amoxil- clavulanic acid, orbenine, and ticarcillin-clavulanic acid, which were inactive against *S. aureus* as might be expected because the majority of *S. aureus* strains are beta-lactamase producers and more than 90% of *S. aureus* contain plasmids that encode for beta-lactamases, the enzymes that degrade the beta-lactam ring of penicillin leading to inactivation of the antibiotic. In addition, the indiscriminate use of antibiotics may cause some strains of *S. aureus* to become multi-resistant as reported in our study. Some strains of *S. aureus* are resistant to the beta-lactamase-resistant penicillin such as methicillin due to changes in the penicillin-binding protein in the cell membrane which may explain the report of MRSA strains in our study which needs further studies to confirm these findings because we are not sure about bioavailability of the methicillin discs used in this study. Recently, community-acquired MRSA infections have been reported in healthy individuals even without any risk factors such as prior antibiotic use, intravenous drug use, and underlying diseases (DM, malignancy, and chronic skin disease). Ciprofloxacillin antibiotic showed potent activities against both *S. aureus* and *S. epidermidis* because this drug is still not widely used in our country. The emergence of multiple drug resistance to both *S. aureus* and *S. epidermidis*

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may be related to the wide improper use of these antibiotics by clinicians.

Conclusion

Cutaneous leishmaniasis remains a significant global health problem, influenced by travel, migration, and ecological changes. These factors explain the rise in cases in certain regions of Libya, especially the northwest of Tripoli and coastal areas. The increasing frequency of leishmaniasis highlights the need for physicians—especially dermatologists—to understand its epidemiology, transmission, and clinical management. This study showed that CL is most common among children, adolescents, and young adults, particularly in the 0–10 and 11–30 age groups. Older individuals appear to have greater immunity. Lesions were mainly found on exposed body parts, with most patients presenting one or two lesions of the furunculoid or nodulo-ulcerating type. Direct microscopic examination of lesion margins remains a useful diagnostic tool. The disease follows a seasonal pattern, peaking in the winter months. *Staphylococcus aureus* was the predominant bacterium isolated from CL lesions. Most isolates showed high resistance to commonly used antimicrobial agents, and MRSA was reported at a significant rate, requiring further study. The role of S. epidermidis remains unclear and warrants further investigation. Ciprofloxacillin was the most effective drug against both *S. aureus* and *S. epidermidis*. Bacterial cultures are recommended for cases unresponsive to initial antimicrobial therapy. In conclusion, bacterial infection should be considered in the diagnosis and management of CL, and prompt treatment may help prevent scarring.

Conflicts of Interest

The authors declare no conflicts of interest.

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