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Antibiogram Profiles of Bacteria Isolated from Cutaneous Leishmaniasis Patients' Lesions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cutaneous Leishmaniasis is a vector-borne disease that arises from the presence of an intracellular protozoan parasite. The occurrence of secondary bacterial infections in wounds caused by cutaneous leishmaniasis not only worsens the development of lesions but also hinders the healing process. Additionally, there is limited knowledge regarding the various bacterial species that co-infect leishmaniasis wounds and their susceptibility patterns in Tripoli. This study aimed to ascertain the resistance patterns of bacteria co-infecting cutaneous leishmaniasis wounds in patients seeking treatment at the NCDC Dermatology clinic.

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Methods: A study at the NCDC Dermatology Clinic involved 81 patients with confirmed CL. Bacteria were isolated and characterized from wound swabs collected using sterile cotton-tipped applicators. The isolates were cultivated on various agar plates and tested for bacterial identification using tests like oxidase, catalase, and coagulase slide tests and also biochemical tests. Antibiotic susceptibility tests were performed using the agar disc diffusion method according to Clinical and Laboratory Standards Institute breakpoint values.
Results: The study identified 93 secondary bacteria in Cutaneous leishmaniasis lesions, with Staphylococcus aureus (39.8%) and Pseudomonas aeruginosa (22.6%) being the most prevalent. Other pathogenic bacteria included Staphylococcus epidermidis (8.6%), Streptococci pyogenes (1.1%), and Methicillin-resistant Staphylococcus aureus (MRSA). Other pathogenic bacteria included Escherichia coli (7.5%), Enterobacter cloacae (6.5%), Citrobacter braakii (2.2%), Klebsiella pneumonia (2.2%), Pantoa sp. (2.2%), Pasteurella multocida (2.2%), Proteus mirabilis (2.2%), Protus Vulgaris (1.1%), and Serratia plymutica (1.1%). The study found that Ciprofloxacin had the most effective impact on bacterial isolates, followed by Rifampicin and Bactrim.
Conclusion: The findings of this study indicate that cutaneous leishmaniasis wounds are colonized by a diverse range of bacterial species, which exhibit a high degree of resistance to Augmentin, Amoxicillin, and Fusidic acid antibiotics.

Keywords: Cutaneous leishmaniasis; wounds co-infections; lesion; resistance; neglected tropical diseases; Libya.

1. INTRODUCTION

Cutaneous leishmaniasis (CL) represents the most prevalent clinical presentation of leishmaniasis, a neglected tropical disease [1]. Despite not posing a threat to life, CL results in unsightly scarring, which can lead to social ostracism [2]. This affliction is endemic in over 70 countries, primarily affecting socioeconomically disadvantaged populations [3]. Cutaneous leishmaniasis typically manifests as painless ulcerated skin lesions, usually appearing as one or multiple ulcers in areas of the skin exposed to insect bites [3]. These ulcers have a rounded or oval shape, an infiltrated base, well-defined and raised erythematous borders, a reddish background with noticeable granulations, and minimal secretion[4]. The most commonly employed treatment for CL is meglumine antimoniate. Following treatment, the average time for the lesions to epithelialize is between 30 and 60 days [4,5]. The presence of a bacterial secondary infection can result in local pain and the production of serous and purulent exudate, which can completely or partially cover the ulcer and subsequently dry into crusts [4]. This infection may also impede the healing process [6,7].

The incidence of secondary infection in CL lesions ranges from 23.6% to 81% [8,9]. The most commonly isolated bacteria from cutaneous leishmaniasis lesions are Staphylococcus aureus, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* [8,9]. These bacteria, along with other Gram-positive and Gram-negative types, are frequently isolated and are some of the most concerning antibiotic-resistant organisms we face today [8,9,10].

The determination of bacterial type is influenced by various factors, such as the age of the patient, the location of the lesion, the gender of the patient, and personal hygiene practices [11,12]. Research has indicated that microbial patterns differ across different sites of infection within the body, including lesion infections [13]. Furthermore, the response of bacterial isolates to antimicrobial agents varies depending on the specific isolate, the type of antimicrobial agent used, and the site of the lesion and infection [13]. Due to the significant burden of infections, inappropriate use of antibiotics, unrestricted availability of drugs, and limited access to antimicrobial susceptibility testing, the issue of drug resistance poses a significant challenge in Libya [14,15-17].

The National Center for Disease Control has recommended early detection and response as a key strategy to combat the spread of antimicrobial resistance (AMR) [18]. However, there is a lack of comprehensive data on the antibiogram of bacterial pathogens isolated from various infection sites in Africa, including Libya. Additionally, there is a dearth of documented information regarding bacterial isolates and their antibiotic resistance profiles specifically within the Dermatology clinic at the NCDC. Given the

increasing number of patients seeking care at the Dermatology clinic each year, it is imperative to consider implementing culture and drug susceptibility tests. Therefore, this study aims to determine the antibiotic sensitivity pattern (antibiogram) of the isolated bacteria to facilitate the selection of the most effective therapy.

2. MATERIALS AND METHODS

The study was done on Cutaneous leishmaniasis lesion swabs taken from 81 confirmed CL patients attending the NCDC Dermatology clinic from November 2021 to February 2022. 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. The study protocol was reviewed and approved by the Ethics Committee of the Research Council of the National Center for Disease Control. After enrolment, all data were recorded into a well-designed questionnaire, Primary characterization of isolates was based on the microscopic Gram stain examination performed in the Parasitology and vector-borne disease laboratory.

2.1 Sample Collection Culture and Identifications

Samples of cutaneous leishmaniasis lesions were collected in a sterile manner using a cotton-tipped applicator. The surface of the wound was swabbed, and the swabs were then immersed in sterile glycerol broth provided by Surechem Products Ltd, located in Needham, UK. Each swab was cultivated on various agar plates including g Mannitol Salt Agar, MacConkey Agar, Blood Agar, Nutrient Agar, Muller-Hinton Agar, Triple Sugar Iron Agar, and Nutrient Broth. These plates were incubated at a temperature of 37◦C for 24 hours. Bacterial identification was carried out through the performance of several tests including the oxidase test, catalase test, and coagulase slide test. Furthermore, the isolates were subjected to further identification using the API 20E biochemical assay kit provided by bioMerieux Inc., located in Durham, USA.

2.2 Antibiotic Susceptibility Testing

Antimicrobial susceptibility of all the bacterial isolates was determined using the Kirby Bauer agar disc diffusion assay guided by breakpoint values of the Clinical and Laboratory Standards Institute (CLSI M100-S26). The isolates were tested for their susceptibility to Bactrim (25ug), Ciprofloxacin (10ug), Augmentin (30ug), Amoxicillin (25ug), Erythromycin (15ug), Fusidic acid (10ug), and Rifampicin (5ug).

3. STATISTICAL METHODS

The data was entered into Microsoft Office Excel 365 and presented in summary tables and charts. Data were also presented as frequencies and percentages.

4. RESULTS AND DISCUSSION

4.1 Bacterial Profile

In this study, a total of 58 specimens (71.60%) were found to be culture positive. The proportion of bacterial isolates was significantly higher in males 36 (62.1%) compared to Females 22 (37.9%) ($P<0.05$). There was no significant association observed between different isolated bacteria-positive samples within age groups ($P>0.05$). This suggests that different types of bacteria infect individuals of different ages, indicating that they are not specifically linked to a particular age group (Table 1).

Table 1. The prevalence of different isolated bacteria-positive samples within age groups

Age Group (Years)	Positive Culture	
	Gram-Positive	**Gram-Negative**
0-9	4 (8.5%)	4(8.6%)
10-19	7(14.8%)	7(15.2%)
20-29	10(21.2%)	5(10.8%)
30-39	7(14.8%)	7(15.2%)
40-49	4(8.5%)	7(15.2%)
50-59	11(23.4%)	10(21.7%)
60-69	2(4.2%)	2(4.3%)
≥ 70	2(4.2%)	4(8.6%)
Total	**47**	**46**

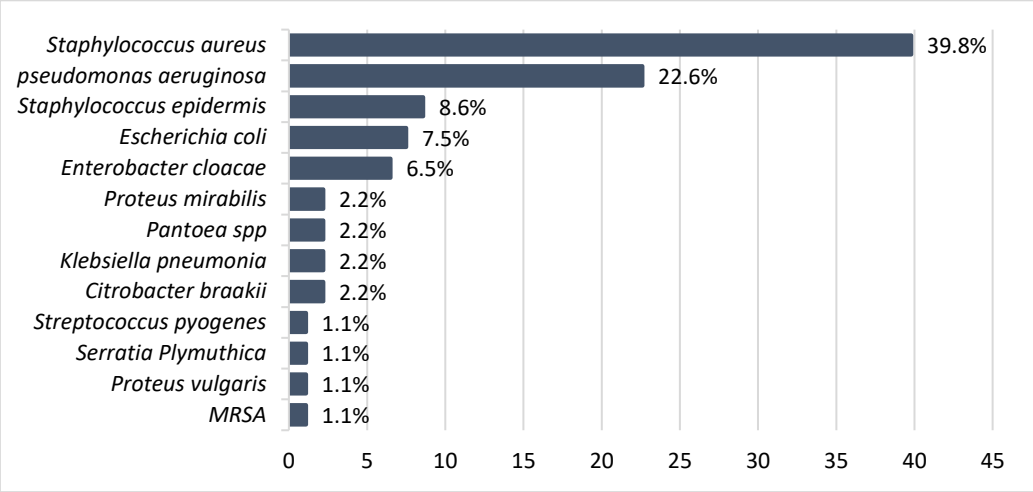

Fig. 1. Bacteria isolated from lesions of cutaneous leishmaniasis patients

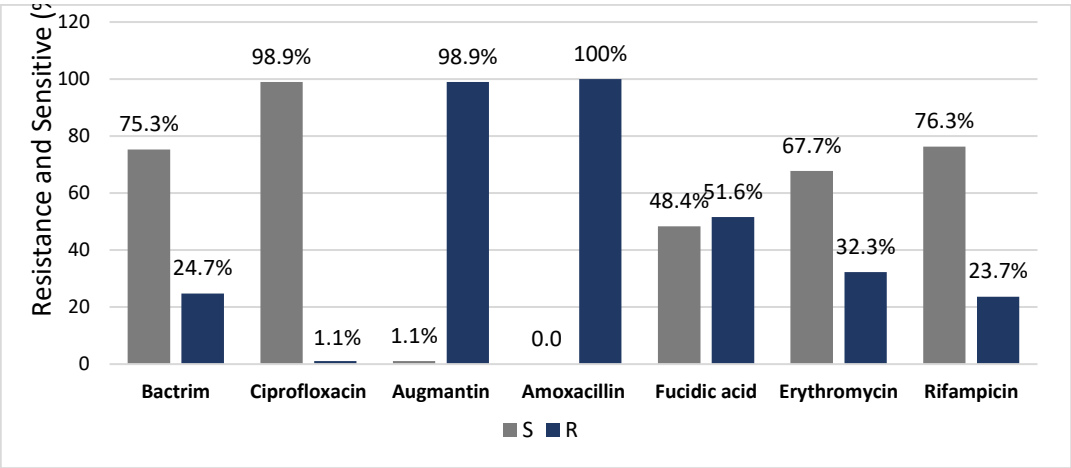

Fig. 2. Percentage of antibiotic resistance and susceptibility of bacterial species among cutaneous leishmaniasis patients

Table 2. The Antibiotic susceptibility of isolated bacteria from cutaneous leishmaniasis patients

Bacterial isolates	Antibiotics						
	AML	AUG	SXT	CIP	E	FA	RD
Staphylococcus aureus	R	R	S	S	S	R	S
Pseudomonas aeruginosa	R	R	R	S	R	R	R
Staphylococcus epidermis	R	R	S	S	S	R	S
Escherichia coli	R	R	S	S	R	R	S
Enterobacter cloacae	R	R	S	S	S	R	S
Proteus mirabilis	R	R	S	S	S	R	S
Pantoea spp	R	R	S	S	R	R	S
Klebsiella pneumonia	R	R	S	S	R	R	S
Citrobacter braakii	R	R	S	S	S	R	S
Streptococcus pyogenes	R	R	S	S	S	R	S
Serratia plymuthica	R	R	R	R	S	S	S
Proteus vulgaris	R	R	S	S	S	R	S
MRSA	R	R	R	S	R	R	R

Amoxicillin (AML), Augmentin (AUG), Bactrim (SXT), Ciprofloxacin (CIP), Erythromycin(E), Fusidic acid (FA), and Rifampicin (RD)

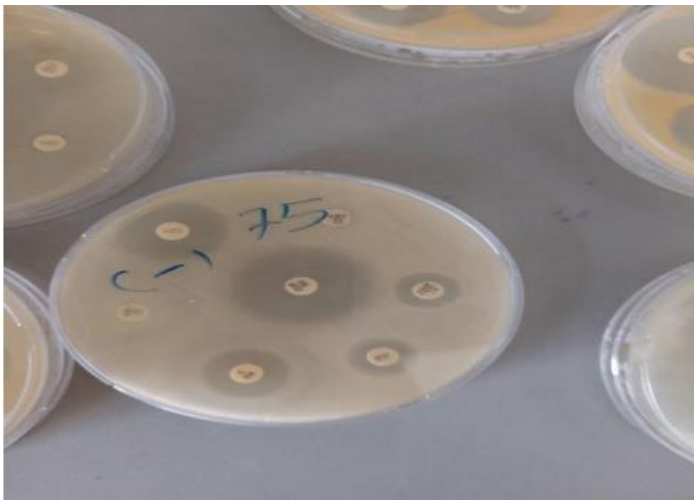

Fig. 3. Antibiotic sensitivity test (Zone of inhibition)

The study involved the isolation of 93 strains from patients who tested positive for cl, encompassing four distinct bacterial species. Out of these isolates, 47 were identified as Gram-positive bacteria, while the remaining 46 were Gram-negative bacteria consisting of ten different species. The Gram-positive bacteria isolated from the lesions included *Staphylococcus aureus* 37 (39.8%), *Staphylococcus epidermidis* 8 (8.6%), *Streptococci pyogenes* 1 (1.1%), and *Methicillin-resistant Staphylococcus aureus (MRSA)* 1 (1.1%). The Gram-negative bacteria isolated from the lesions included *Pseudomonas aeruginosa* 21 (22.6%), *Escherichia coli* 7 (7.5%), *Enterobacter cloacae* 6 (6.5%), *Citrobacter braakii* 2 (2.2%), *Klebsiella pneumonia* 2 (2.2%), *Pantoea spp* 2 (2.2%), *Pasteurella multocida* 2 (2.2%), *Proteus mirabilis* 2 (2.2%), *Proteus vulgaris* 1 (1.1%), and *Serratia Plymuthica* 1 (1.1%) (Fig. 1).

4.2 Antibiograms of Bacterial Isolates from lesions of cutaneous leishmaniasis patients

With regards to the susceptibility of bacterial isolates to various antibiotics, the results of the antimicrobial susceptibility testing (AST) presented in Table 2 indicate that all Gram-positive Cocci species were susceptible to Ciprofloxacin, Bactrim, Fusidic acid, Erythromycin, and Rifampicin. The highest resistance was observed towards Amoxicillin, Augmentin, and Streptococcus pyogenes was found to be susceptible to Ciprofloxacin, Bactrim, Erythromycin, and Rifampicin, while resistant to Amoxicillin, Augmentin, and Fusidic acid. Methicillin-resistant *Staphylococcus aureus* (MRSA) was susceptible to Ciprofloxacin, but exhibited the highest resistance towards Amoxicillin, Augmentin, Bactrim, Erythromycin, Fusidic acid, and Rifampicin. Gram-negative bacteria, on the other hand, displayed varying responses and resistance to antibiotics. In this study, *Pseudomonas aeruginosa* was found to be susceptible to Ciprofloxacin, but exhibited the highest resistance towards Amoxicillin, Augmentin, Bactrim, Erythromycin, Fusidic acid, and Rifampicin. *Escherichia coli, Klebsiella*. were susceptible to Ciprofloxacin, Bactrim, and Rifampicin, but resistant to Amoxicillin, Augmentin, Fusidic acid, and Erythromycin. Furthermore, *Enterobacter cloacae, Citrobacter braakii, Proteus mirabilis*, and *Proteus vulgaris* were found to be susceptible to Ciprofloxacin, Bactrim, Erythromycin, and Rifampicin, but resistant to Amoxicillin, Augmentin, and Fusidic acid. Pasteurella multocida was sensitive to Bactrim, Ciprofloxacin, Fusidic acid, and Rifampicin. Lastly, Serratia plymutica exhibited resistance to Ciprofloxacin, Bactrim, Amoxicillin, and Augmentin, but was susceptible to Erythromycin, Fusidic acid, and Rifampicin, as determined by this study Fig. 2.

The first recorded instance of Cutaneous Leishmaniasis (CL) in Libya was documented in 1930, which was then followed by its subsequent dissemination across the municipalities of the northwestern region [19,20]. Although CL is a self-healing ailment, various factors, such as poly-microbial infections, can potentially impede the healing process and exacerbate scarring. Therefore, gaining a comprehensive understanding of the disease and the organisms that co-infect the wounds is

imperative to develop more effective treatments and minimize scarring. The objective of this study was to isolate and identify the bacteria that co-infect CL wounds and determine their susceptibility patterns to clinical antibiotics. The prevalence of culture-confirmed bacterial infection was significantly higher in males than females in the present study. The finding is in agreement with a report from Iran and Ethiopia [21,22,23]. This may be due to Men are more likely to work in occupations that involve exposure to Leishmania parasites and other infectious agents, such as agriculture and construction, Men are more likely to engage in outdoor activities that put them at risk of insect bites and hormonal differences such as Testosterone play a role in the increased prevalence of secondary bacterial infection in cutaneous leishmaniasis lesions in male. The results of our study indicate that there was no significant difference ($p \geq 0.05$) in age between the positive-culture and negative-culture groups. This finding is consistent with a previous study conducted by Layegh *et al*., which suggested that the lack of significant differences in gender and age in the isolation of gram-negative bacteria can be attributed to the equal likelihood of wound contamination in both sexes and age groups. Furthermore, our study revealed that the most frequently isolated bacterial pathogens from cutaneous leishmaniasis lesions were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This finding aligns with previous reports from Iran [22], Iraq [24], and Sudan [25], where gram-negative bacteria were more commonly isolated compared to gram-positive bacteria. The predominance of gram-negative bacteria in these regions may be attributed to their simple nutritional requirements, frequent presence in clinical settings, and ability to resist multiple antibiotics. These findings are consistent with microbial biodiversity data from other studies conducted in underdeveloped countries such as Rwanda [26] and Nigeria [27]. The wide range of gram-negative bacteria isolated from the wounds suggests that the infection may have been acquired from the community. Additionally, *S. aureus* was consistently identified as the most frequently isolated species in our study, as well as in several other studies [22,23].

The present study has revealed the antibiotic susceptibility pattern of patients suffering from ulcerative cutaneous leishmaniasis. This information has been utilized to determine the most appropriate empirical antibiotic treatment for each case. It is well-established that microbial isolates and their antibiotic resistance exhibit high variability, and therefore, antibiotics commonly used to treat such infections were tested on all isolates. The Clinical and Laboratory Standards Institute guidelines, CLSI M100-S26 (2018), were followed to detect the sensitivity and resistance of S. aureus, S. epidermidis, Streptococcus pyogenes, *MRSA, P. aeruginosa, E. coli, Pantoa sp., K. pneumoniae, E. cloacae, Citrobacter braakii, Protus mirabilis*, and *Protus vulgaris* to various antibiotics. The results indicated that Ciprofloxacin, Bactrim (Trimethoprim/Sulfamethoxazole), Erythromycin, Fusidic acid, and Rifampicin were effective against *S. aureus* and *S. epidermidis* isolates, while Amoxicillin and Augmentin were ineffective. *Streptococcus pyogenes* exhibited similar results, except for resistance to Fusidic Acid. MRSA was only sensitive to Ciprofloxacin and resistant to other antibiotics*. P. aeruginosa* showed high sensitivity to Ciprofloxacin and resistance to other antibiotics. However, all isolates of *E. coli, Pantoa sp*., and *K. pneumoniae* were resistant to Amoxicillin, Augmentin, Erythromycin, and Fusidic acid*. E. cloacae* and *Citrobacter braakii, Proteus mirabilis*, and *Proteus vulgaris* were susceptible to Ciprofloxacin, Bactrim, Erythromycin, and Rifampicin, but had already acquired resistance to other antibiotics. The presence of multiple drug resistance in bacterial isolates may be attributed to the frequent abuse of antibiotics in communities and the lack of appropriate microbiological diagnostic input in clinical care [27,28]. Bactrim, which demonstrates efficacy against both Gram-positive bacteria and Gram-negative isolates, is the prescribed treatment in Dermatology and Leishmaniasis Clinics, despite the presence of significant resistance to Augmentin and Amoxicillin in our study. Furthermore, our study findings indicate that the antibiotic Ciprofloxacin exhibits the most favorable effect on bacterial isolates, followed by Rifampicin and Bactrim. Although Fusidic acid is commonly utilized for bacterial infections, our study reveals higher rates of resistance compared to antibiotic response. The issue of antibiotic resistance must be carefully considered, particularly when dealing with Gram-negative bacteria. Monitoring antibiotic resistance in such cases is an emerging problem. In our investigation, the Gram-negative bacterium *P. auroginosa* demonstrated the highest resistance to all antibiotics except for Ciprofloxacin, as it typically exhibits a more aggressive nature [29,30].

5. CONCLUSION

The prevalence of secondary bacterial infection in lesions of cutaneous leishmaniasis (CL) was found to be 71.60%. The most commonly isolated pathogens were *Staphylococcus aureus* and *Pseudomonas aeruginosa.* These findings emphasize the significance of *S. aureus* and *P. aeruginosa* as major risk factors for the rapid growth of large CL ulcers, highlighting the need for public health education regarding these pathogens. In addition, our study suggests that ulcerated lesions of CL should be treated with topical antiseptics to prevent subsequent bacterial infections that may accelerate tissue degradation. Furthermore, the administration of antibiotics, particularly those targeting *Staphylococcus*, would be advisable in cases of secondary bacterial infection symptoms and indications. It is reassuring to note that the current practice of prescribing Bactrim in our country is appropriate and effective and can be largely continued. However, Ciprofloxacin may be a more effective option to prevent any future shifts in antimicrobial sensitivity and should be monitored for antibiotic resistance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

The study goals were explained to all patients and informed consent was obtained from each participant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

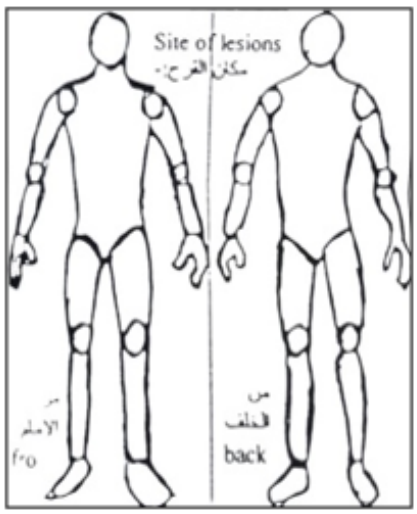
Zoonotic Disease Control Administration
National Program for Control of Leishmaniasis Patients
Leishmaniasis Reporting Questionnaire

Information Demographic:						
File Number	Age of Birth ...../...../.....	Age ( )	Name			
	Phone No.	Nationality	Sex	Male ○	Female ○	
Registration Date ...../...../.....	Occupation	Municipality	Residence			
Patient's signature						
Epidemiological						
Discovery Date	...../...../...20					
Lesion(s)first noticed by patient	Days ( ) Weeks ( ) Months ( ) Years ( )					
Location (Write down the number of Lesions)	Trunk (.....)	Face/Ears (.....)	Head/Neck (.....)			
	Fingers (.....)		Upper limbs (.....)			
	Toes (.....)		Lower limbs (.....)			
largest lesion size	○ ≤ 4 cm		○ > 4 cm			
Past cutaneous leishmaniasis History:						
Any history of the disease?	No ○	Yes ○				
Any previous treatment for cutaneous leishmaniasis?	No ○	Yes ○				
Onset of the treatment:	...../...../.....					
Duration of the treatment	○ Days	○ Weeks	○ Months	○ Years		
Type of the treatment	Cryotherapy ○	Intralesional	○ Antibiotics ○			
Intramuscular or Intravenous ○	Notes:					
Any history of travel in last 6 months?	○ Yes	○ No				
Where (Municipality)?						

Zoonotic Disease Control Administration
National Program for Control of Leishmaniasis Patients
Leishmaniasis Reporting Questionnaire

Past Medical History				
Do you have any chronic disease?		Respiratory diseases ○	Arthritis ○	
Yes ○	No ○	Heart disease ○	Diabetes ○	Renal disease ○
Hypertension ○	Cancer ○	Immunocompromised ○	Specify	
Diagnosis and Management:				
Was sample been taken?	Yes ○	No ○	Note:	
Was treatment been prescribed?	Yes ○	No ○	Note:	
Type of treatment?				
Local treatment	Cryotherapy ○	Disinfectant ○		
Topical antibiotics (ointment/cream)/type:				
Systemic treatments	Antibiotics: ○	Intramuscular or Intravenous ○	Intralesional ○	
Topical antibiotics (ointment/cream)/type				

Investigations:	Liver Function Test ○	Renal Function Test ○	Chest X Ray ○	CBC ○	Test ECG ○
Physician name:			**Prepared by:**		



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