

## Study of Secondary Bacterial Infections Among the Patients with Cutaneous and Mucocutaneous Leishmaniasis in Tamar City- Yemen.

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### Abstract

The main objective of this study was to determine the how common secondary bacterial infections that are isolated from patients with cutaneous and mucocutaneous leishmaniasis in Tamar City- Yemen. This study included patients admitted to the Skin Disease Unit and the Tamar General Hospital lab between August 2020 and January 2021. Clinical samples were examined using standard parasitological and bacteriological methods. The study included 81 patients, and *leishmania* was found in 60 (74 percent) of them. Bacterial infections had also infected 60 cases. The most common secondary infection bacterial causative agents found in this study were 30 isolates (38%) of *kokuria* spp out of the total number 79 bacterial isolates, 28 (35.4%) isolates of *Bacillus* spp., 12 (15.2%) isolates of *P. aeruginosa* and 9 (11.4%) isolates of *S. aureus*. Sensitivity testing revealed that 16% of *S. aureus* was resistant to Azithromycin, 35% of *Kocuria* spp. were resistant to Cefotaxime and 30% were resistant to Ceftriaxone, 36% of *Bacillus* spp. were resistant to Azithromycin, and 20% were resistant to Ceftriaxone, 8.3% of *P. aeruginosa* were resistant to Azithromycin and 10% were resistant to Ceftriaxone. The majority of isolates (35–50%) were sensitive to Ofloxacin and Ciprofloxacin. The recent article's results indicate that bacterial infections are still a significant problem in patients with cutaneous leishmaniasis in Yemen, and should be considered when treating these patients.

**Keywords:** Cutaneous and Mucocutaneous leishmaniasis, secondary bacterial infections, Yemen

**الملخص:** كان الهدف الرئيسي من هذه الدراسة هو تحديد مدى انتشار العدوى البكتيرية الثانوية التي عزلت من مرضى داء الليشمانيات الجلدي والجلد المخاطي في مدينة ذمار - اليمن. تضمنت هذه الدراسة المرضى الذين تم إدخالهم إلى وحدة الأمراض الجلدية ومختبر مستشفى ذمار العام بين أغسطس 2020 ويناير 2021. تم فحص العينات السريرية باستخدام الطرق البكتريولوجية القياسية. شملت هذه الدراسة 81 مريضاً، وتم العثور على الليشمانيا في 60 (74 بالمائة) منهم. كما أصابت العدوى البكتيرية 60 حالة. أكثر العوامل المسببة للعدوى الثانوية شيوعاً التي تم العثور عليها في هذه الدراسة هي 30 عزلة (38%) من نوع بكتيريا كوكوريا من العدد الكلي 79 عزلة بكتيرية، 28 (35.4%) عزلة من الباسلاس، 12 (15.2%) عزلة من سيدوموناس ابروجنوزا و 9 (11.4%) عزلة من بكتيريا المكورات العنقودية. أظهر اختبار الحساسية أن 16% من بكتيريا العنقودية الذهبية كانت مقاومة للأزيتروميسين، و35% من كوكيورا كانت مقاومة ل سيفتوكسيم و30% مقاومة ل سيفترياكسون و36% من الباسلاس. كانت مقاومة لأزيتروميسين، و20% مقاومة لسيفترياكسون، و8.3% من سيدوموناس ابروجنوزا كانت مقاومة لأزيتروميسين و10% مقاومة لسيفترياكسون. كانت غالبية العزلات (35-50%) حساسة لأوفلوكساسين وسيفيروفلوكساسين. تشير النتائج إلى أن الالتهابات البكتيرية لا تزال مشكلة كبيرة لدى مرضى داء الليشمانيات الجلدي في اليمن، ويجب أخذها في الاعتبار عند علاج هؤلاء المرضى.

### 1.Introduction

Leishmania is an obligate intracellular parasite that is transmitted from animal to human or human to human by bites from an infected female sandfly. Whereas the older literature focused primarily on three species, *L. donovani* (visceral leishmaniasis), *L. tropica* (cutaneous leishmaniasis), and *L. braziliensis* (cutaneous leishmaniasis), the current taxonomy of leishmaniasis is in a state of flux [1].

One of the complications of leishmaniasis that can increase tissue injury and scarring is a secondary

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bacterial infection. Secondary bacterial infection is an illness that occurs during or after leishmaniasis treatment. It could be caused by the first treatment (alternative medicine) or by immune system changes [2].

Bacterial secondary infection may cause local pain and start producing serious and purulent exudate that either entirely or partially heals the ulcer and then dries into crusts, as well as interfere with the healing process. The incidence of secondary infection in cutaneous leishmaniasis (CL) lesions range from 23.6-81% [3]. The most frequently isolated bacteria from cutaneous lesions of leishmaniasis are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Corynebacterium diphtheriae* [4]. Antimicrobial Surveillance Program study on the pathogens responsible separated from skin and soft-tissue infections, the most common pathogens in Latin America, in decreasing order of prevalence, were *S. aureus* (32.8 percent), *Escherichia coli* (13.1 percent), *P. aeruginosa* (11.9 percent), *Enterococcus* species (7.7 percent), *Klebsiella pneumoniae* (5.8 percent) and *Enterobacter* species (5.6 percent) [5].

*Kocuria* spp. is Gram-positive cocci that live on the human and animal skin and mucous membranes. *Kocuria* was also separated from various environmental and natural niches [6]. Infections that can be caused by *Kocuria* spp. have recently increased in frequency, having caused both superficial and depth infections. *Kocuria* species have now arisen as pathogenic strains, primarily in compromised hosts suffering from severe underlying diseases [7].

## 2. Materials and Methods

### Study population

The study was conducted in the period from August 2020 to January 2021. The site of the study was the outpatient Dermatology Clinic at Thamar General Hospital, Yemen. The patients registered in the clinical and parasitological diagnosis of CL and referred to Skin Disease Unit and Thamar General Hospital lab. The patients belonged to both sex and different age groups and had different clinical forms of CL.

### Specimen Collection

Both the lesion and skin area around the lesion are disinfected by 70% alcohol-soaked cotton in circular motion. Two sterile cotton swabs moistened with sterile saline solution are to be rotated over the edge of ulcerated lesions. One of which is for media inoculation and, with the remaining swab is for performing two smears over glass slides to be stained with Gram and Giemsa stain to detect bacterial [8].

### Direct Detection of Leishmania

The sample smeared or impressed directly on the slide. Examination of Giemsa stained slides is the technique most commonly used to visualize the amastigote form, the parasite tissue form [9].

### Isolation and Identification of Bacteria

Specimens are to be inoculated onto a rich and selective media; 5% sheep Blood agar and MacConkey agar respectively and incubated aerobically at  $36\pm 1^{\circ}\text{C}$  for 24-48 hours [10].

### Staining and Biochemical Tests

Gram stain and biochemical tests for identification purposes are to be done according to standard diagnostic references [11].

## 3. Results and Discussion

### Prevalence of Bacterial Secondary Infections

Table 1 shows a total of 81 leishmanial cases were sampled for detecting a possible bacterial secondary infection of the lesion. Only 60 cases of bacterial secondary infections were detected from 79 isolates. Table 1 shows the adults age group (18 to 40 years old) was the most affected group while the age group elderly (> 65 years) was the least affected group.

Table 1. Distribution of secondary infection bacterial of Cutaneous and mucocutaneous leishmaniasis among cases.

Age group (years)	No. of leishmanial cases	No. of bacterial secondary infection
Preschool Children	9	9
Children	21	12
Adolescents	6	6
Adults	24	18
Middle-Aged	12	12
Elderly	9	3
<b>Total</b>	<b>81</b>	<b>60</b>

\* Age grouping is based on Yemeni demographic criteria: neonates < 28 days; infants < 1 year; preschool 1-5 years; Child 5-12 years; adolescent 12-18 years; adult 18-40 years; middle-aged 40-65 years; elderly > 65 years.

### Distribution of Secondary Infections among Leishmanial Type

The proportion male of patients with mucocutaneous leishmaniasis was the highest group in terms of the secondary infections reaching approximately 40%. Details of the prevalence of the secondary infections are summarized in cutaneous and mucocutaneous leishmaniasis in males and females cases in Table 2.

Table 2. Details of the prevalence of secondary infection in males and females cases

Gender	Type of Leishmania	Positive cases of Leishmania	Negative cases of Leishmania	Total cases
Male	CL	12	0	12
	MCL	24	9	33
Female	CL	15	12	27
	MCL	9	0	9
<b>Total</b>		<b>60 (74%)</b>	<b>21 (26%)</b>	<b>81</b>

\* CL: Cutaneous leishmaniasis & MCL: Mucocutaneous leishmaniasis

### Detected Bacteria and their Susceptibility Patterns

Among 81 positive *leishmania* cases, 60 cases (74%) had positive microbial culture, these bacterial infections including pathogens and opportunistic bacteria as follows:

The most common secondary infection bacterial causative agents found in this study were 30 isolates (38%) of *kocuria* spp from 79 bacterial isolates, 28 (35.4%) isolates of *Bacillus* spp., 12 (15.2%) isolates of *P. aeruginosa* and 9 (11.4%) isolates of *S. aureus* in (Table 3). There were 14 patients infected with two bacteria species, 2 patients with three bacteria species, and the rest of the patients with one bacterium species.

Table 3. The bacterial isolated from cases in Cutaneous and Mucocutaneous leishmaniasis.

Bacteria	Type of Leishmania	No. of isolates	Total of isolates	Total of cases
<i>Kocuria</i> spp.	CL	14	30 (38%)	24 (40%)
	MCL	16		
<i>Bacillus</i> spp.	CL	10	28 (35.4%)	24 (40%)
	MCL	18		
<i>P. aeruginosa</i>	CL	9	12 (15.2%)	6 (10%)
	MCL	3		
<i>S. aureus</i>	CL	3	9 (11.4%)	6(10%)
	MCL	6		
<b>Total</b>			<b>79 (100%)</b>	<b>60 (100%)</b>

The sensitivity testing revealed that 16% of *S. aureus* was resistant to Azithromycin, 35% of *Kocuria* spp. were resistant to Cefotaxime and 30% were resistant to Ceftriaxone, 36% of *Bacillus* spp. were resistant to Azithromycin, and 20% were resistant to Ceftriaxone, 8.3% of *P. aeruginosa* were resistant to Azithromycin and 10% were resistant to Ceftriaxone. The majority of isolates (35–50%) were sensitive to Ofloxacin and Ciprofloxacin.

Secondary bacterial infections are one of the complications of CL. Although some authors emphasize the scarcity of this finding [12], 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 tissue destruction and necrosis.

This study showed the difference in the affluence of a variety of aerobic bacteria in the lesions from which it was separated amastigote (74%) as well as in the cases from which it wasn't separated the parasite (26%).

**Edrissian et al** [13] showed the affluence of a variety of pathogenic bacteria such as beta-hemolytic *Streptococcus* and coagulase-positive *Staphylococcus* in cutaneous leishmaniasis lesions in Iran. These bacteria are demonstrated to be present in our research too.

**Angelica and Claudio Manuel** [14] detected in Mexico, the bacteria like *Staphylococcus aureus* and *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Enterococcus*, and *Enterobacter* species in cutaneous leishmaniasis lesions.

In our study, it was observed the rate of bacterial *Kocuria* spp. infections in the cases of positive leishmania more than other bacteria. After that, *Bacillus* spp, *P. aeruginosa* occupied the second place. *S. aureus* was also seen among the found pathogenic bacteria in these lesions.

In this study, the infection rate of *Staphylococcus aureus* was 11.4%. In one research the same infection rates (27.8) reported by **Edrissian et al** [13] in Iran. The comparison of these findings to each other illustrates that the alternative infection of cutaneous leishmaniasis lesions with these pathogen bacteria has been reduced in the country.

Mainly, the results showed that the aerobic bacteria, particularly coagulase-positive were found among important pathogenic bacteria in the lesions suspected of leishmania in Yemen and this must be considered and taken into account when treating the disease.

Cutaneous leishmania is still considered a serious problem in Yemen and furthermore, alternative infections of pathogenic or opportunist bacteria make this problem more complex and the bacteria make a negative effect on leishmania diagnosis and treatment.

Thus, in cutaneous and mucocutaneous leishmania lesions having alternative infections, it is necessary that the diagnosis and the treatment of the parasite are simultaneously accomplished by using suitable antibiotics. Overall, health care should help this problem to be prevented and reduced and avoids alternative infections happening in the lesions. 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.

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