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By

Alsadig Mohammed Abdalla
Sheren Ahmed Saeed
Mawia Mukhtar

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Detection of Antibiotic Resistance of Pathogenic Bacteria Recovered from Cutaneous Lesions of Human Leishmaniasis Patients in Khartoum State (Sudan)

Alsadig Mohammed Abdalla*[1], Sheren Ahmed Saeed[2], Mawia Mukhtar[2]

[1]Department of Microbiology-Faculty of Medicine-Sebha University-Libya.
[2]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

*Corresponding Author's Email: alsadig58@yahoo.com

ABSTRACT

The purpose of this work was to investigate the presence of pathogenic bacteria in lesions of patients with cutaneous leishmaniasis wounds and the pattern of susceptibility to antibiotics, in order to study the prevalence of secondary bacterial infection in ulcerated lesions and its relationship to the healing process. Twenty samples from leishmaniotic patients attended at the Institute of Endemic Diseases-University of Khartoum were evaluated. The criteria for inclusion of patients in this work were based on a positive clinical diagnosis of CL ulcer together with laboratory analysis. The bacteria isolated were Staphylococcus aureus in 9 cases (45%), coagulase negative Staphylococcus in 11 cases (55%).

Forty four percent of the S. aureus were resistant to methicillin and vancomycin, 56% to erythromycin, and 78% to amoxicillin. 45% of the coagulase negative Staphylococci were resistant to methicillin, 55% to vancomycin and amoxicillin. All the Staphylococcus isolates were highly sensitive to meropenem and amikacin (99-100%).

Keywords: *Cutaneous leishmaniasis*, bacterial, secondary infection.

INTRODUCTION

Cutaneous leishmaniasis (CL) is a worldwide public health and a social problem in many developing countries. It can affect the skin and mucous membranes, and is caused by different Leishmania species widespread in 88 countries in the New and Old World.

Old World Cutaneous Leishmaniasis (OWCL) is present in many endemic areas in North Africa, the Mediterranean, the Middle East, the Indian subcontinent and Central Asia, (González et al., 2008). Leishmaniases are a group of diseases caused by several species of the genus Leishmania. It has been estimated that 1.5 million new cases of cutaneous leishmaniasis occur annually and more than 80.0% of the total cases affect individuals in developing countries (Neupane et al 2008). Brazil, Iran, Afghanistan and Sudan suffer the highest prevalence and the disease is a priority for public health in all hyper endemic regions of the world (Vega-Lopez and Hay, 2004).

Human leishmaniasis is usually classified as cutaneous and visceral or old world type and new world type. The species involved in old world type are *L. major, L. tropica, L. aethiopica, L. donovani* and *L. infantum*. The species responsible for new world type of cutaneous leismaniasis are *L. mexicana* and *L brasiliensis* (Vega-Lopez and Hay, 2004). Cutaneous leishmaniasis (CL) in Sudan is caused by Leishmania major, zymodeme LON-1. The disease is endemic in many parts of the country. The vector is *Phlebotomus papatasi* and the animal reservoir is probably the Nile rat *Arvicanthis niloticus*. Clinically, patients usually present with papules, nodules, or nodulo-ulcerative lesions, mainly on the exposed parts of the skin. In 20% of cases the parasite disseminates through the lymphatics, producing sporotrichoid-like lesions (El-Hassan and Zijlstra, 2001). Elamin et al. (2008) in their study found that *L. donovani* is also a cause of CL in Sudan. The disease mechanisms responsible for healing or chronicity of experimental and human leishmaniasis are essentially confined to the immune system (Lezama-Dávila et al., 1998). Bacterial, fungal, viral and parasitic infections sometimes result as illnesses affecting the integrity of skin and immune system (Grasa et al., 2000); Berhe et al., 2001). When nodules due to infection with *Leishmania* parasites ulcerate, they become susceptible to colonization with a number of microorganisms, such as pathogenic fungus and

bacteria that could provoke secondary infections. Studies carried out in Iran and Ecuador have documented the presence of bacterial infections associated with cutaneous lesions in patients with CL that include a number of pathogenic bacteria (Issae-Marquez and Lezoma-Davila, 2003). Most of these contaminating micro-organisms are known to be derived from the transient or indigenous skin microbiota. In virtually all studies of skin and soft-tissue infections, *Staphylococcus aureus* is the most common pathogen. In most studies, *Streptococcus pyogenes* ranks second in frequency (Summanen et al., 1995). Because the skin is a fairly dry habitat, Gram-negative bacteria are, with one exception, rarely found on the skin in comparison with the Gram-positive bacteria. The exception is the genus *Acinetobacter*, which is found colonizing the moister areas of skin such as axillae, groin and antecubital fossa. A number of *Enterobacter* species can be found on the hands, which become temporarily colonized and constitute a source for cross-infection. However, *Pseudomonas aeruginosa* and *Proteus mirabilis* can be found in the toe webs of normal individuals and can be found as skin invaders in persons with very moist feet (Tannock, 1999). A recent study on the major pathogens isolated from skin and soft-tissue infections showed that, in Latin America, the most common pathogens, in decreasing order of prevalence, were *Staph. aureus* (32.8 %), *Escherichia coli* (13.1 %), *Pseud. aeruginosa* (11.9 %), *Enterococcus* species (7.7 %), *Klebsiella pneumoniae* (5.8 %), *Enterobacter* species (5.6 %) and *Acinetobacter* species (4.1 %) (Sader et al., 2002). Anaerobes play an important role in complicating skin infections, and peptostreptococci typically are the most common of the anaerobic isolates (Bowler et al., 2001). Secondary infection or mistreatment can alter the clinical picture of CL and cause difficulty in diagnosis and delay in treatment. In such cases the diagnosis should be confirmed by examination of smears from lesions, culture, and histopathological examination (Singh and Sivakumar, 2003).

We designed the present study to investigate the presence of secondary bacterial infection in lesions of patients with cutaneous leishmaniasis ulcer, in Khartoum state (Sudan), and the pattern of susceptibility to antimicrobials using conventional culture methods.

MATERIALS AND METHODS

Samples were collected from 20 patients with clinically suspected cutaneous leishmaniasis lesions attended at the Institute of Endemic Diseases-University of Khartoum. The ages of the patients ranged from 4 to 63 years, 15 (75%) of them were men and 5 (25%) were women. Small 1-5 chronic ulcerative lesions at skin site were observed in the different patients. Diagnosis of the secondary infection was made by bacterial aerobic culture of peripheral tissue specimen of the ulcer.

METHODOLOGY

The criteria for inclusion of patients in this work were based on a positive clinical diagnosis of CL ulcer together with laboratory analysis. These laboratory tests included parasite identification by lesion smears stained with Giemsa, Bacterial culture and identification of bacteria present in CL ulcers was performed as follows:

After appropriate cleaning, ulcer's specimens were obtained by rubbing sterile cotton swabs moistened with sterile saline solution over the edge of ulcerated lesions. They were Gram stained, cultured in blood agar and Mannitol salt agar then incubated at 37ºC for 24 h. Isolates were preserved by sub culturing from blood agar plates into nutrient agar slant Bijou screw capped bottles, then subjected to comprehensive bacteriological studies (catalase, Dnase and coagulase tests) to determine their biochemical characteristics according to procedures described by Cowan and Steels (Barrow and Feltham, 2003 and Cheesbrough, 2000)..

Antibiotic sensitivity testing of the isolated bacteria was carried out against 6 antibiotics (Methicillin, vancomycin, meropenem, Amikacin. Erythromycin, and Amoxycillin) using standard disc diffusion method.

RESULTS

All 20 patients tested for parasite identification by lesion smears stained with Giemsa were positive for CL infection with *Leishmania major*. Results of bacterial culture were positive in all the twenty samples collected and the isolated bacteria were Gram positive cocci identified as staphylococci. Nine were *Staphylococcus aureus* bacteria and 11 were coagulase negative *Staphylococci*.

Forty four percent of the *Staphylococcus aureus* were resistant to methicillin and vancomycin, 56% were resistant to erythromycin, and 78% were resistant to amoxicillin. All the isolates 100% were sensitive to meropenem and 99% were sensitive to amikacin (Table 1).

Results of sensitivity testing of coagulase negative *Staphylococci* isolates showed that 45% were resistant to methicillin, 55% were resistant to vancomycin, 64% were resistant to erythromycin and 55% were resistant to amoxicillin. All the isolates 100% were sensitive to meropenem and amikacin (Table 2).

(Table 1): Results of antibiotic sensitivity testing

	***Staphylococcus aureus* isolates**				**coagulase–ve *Staphylococcus* isolates**			
Antibiotic	**Resistant**		**Sensitive**		**Resistant**		**Sensitive**	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
Methicillin	4	44	5	56	5	45	6	55
Vancomycin	4	44	5	56	6	55	5	45
Meropenem	0	0	9	100	0	0	11	100
Amikacin	1	1	8	99	0	0	11	100
Erythromycin	5	56	4	44	7	64	4	36
Amoxycillin	7	78	2	22	6	55	5	45

DISCUSSION

Cutaneous Leishmaniasis (CL) is a parasitic disease characterized by single or multiple ulcerations. Secondary bacterial infection is one of the complications of the disease that can increase the tissue destruction and the resulting scar (Hengameh and Sadeghian, 2008).

In this study all of the 20 patients with confirmed CL were positive for secondary bacterial infections. The bacterial isolate were staphylococci. Nine were *Staphylococcus aureus* and 11 were coagulase negative *Staphylococci*. This is in agreement with Alsamarai and AlObaidi (2009) who in their study in Iraq found that secondary bacterial infection occurred in 42% of CL lesions and *Staphylococcus epidermidis* was the most common bacteria (55%) isolated from lesions, followed by *Staphylococcus aureus* (33%). Hengameh and Sadeghian (2008) in their study in Iran, found that the most common bacterial isolate from CL ulcers was *S. aureus*, other pathogens included coagulase negative *Staphylococcus, E. coli, P. vulgaris*, and *Klebsiella.* These microorganisms are regarded as normal flora in a healthy person; therefore, the presence of *Leishmania* allows these opportunist micro organisms to invade either unprotected injured areas or at times when the immunity of a person was decreased (Alsamarai and AlObaidi, 2009).

Unfortunately, failure to treatment in CL is becoming a problem in many endemic areas, occurring in 5–70% of the patients. In some instances, this could be attributed to re-infection or immunologic, physiologic, and pharmacokinetic deficiencies in the host (Grogi et al., 1991).

In a study from Mexico, the pathogenic role of bacteria in skin lesions of patients with chiclero's ulcer (one of the forms of CL due to Leishmania Mexicana), reluctant to antimonial treatment was determined which suggested the need of elimination of bacterial infection before starting treatment (Isaae-Marquez and Lezoma-Davila, 2003).

In this study, the pattern of resistance of isolated bacteria to common antibiotics was also performed. We observed that 44% of the *S. aureus* were resistant to methicillin and vancomycin, 56% to erythromycin, and 78% to amoxicillin. This is in contrast to results obtained by Claúdia et al., (2005) in their study in Brazil who found that *Staph. aureus* strains isolated from cutaneous ulcers were susceptible to almost all tested drugs. Isaac-Márquez and Lezama-Dávila (2003) in their study found that 44% of Species of *Staphylococcus* from ulcerated CL lesions including *S. aureus* also presented resistance to erythromycin. The increase in resistance to methicillin and other tested antibiotics seen in our results may be due to overuse and misuse of these drugs in our country.

The genus Staphylococcus includes pathogenic organisms in which *S. aureus* is the most important one that has become resistant to most of the therapeutic agents that have been developed in the recent years (Jun et al, 2004). The most notable example of this phenomenon was the emergence of methicillin resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the launch of methicillin (Qureshi et al, 2004). Overuse of antibiotics and the selection of broad, rather than narrow spectrum agents, have contributed to the high prevalence of methicillin-resistant *S. aureus* (MRSA) colonization in wounds. Many of these MRSA isolates are becoming multi-drug resistant, and are susceptible only to glycopeptide antibiotics such as vancomycin (Mehta et al,1998). Low-level resistance to vancomycin is emerging at present (Assadullah et al, 2003). The multi-drug resistant strains isolated from CL lesions in this study could favor the indication of antibiotic susceptibility test to improve drug therapy.

In conclusion, the results of the present study show that microbial secondary contaminants, particularly *Staphylococci*, should be considered in the diagnosis and treatment of CL lesions.

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