ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical & Health Sciences Review

ISOLATION, IDENTIFICATION AND ANTIBIOGRAMS OF BACTERIA ISOLATED FROM CUTANEOUS LEISHMANIASIS WOUNDS

Sabeela Naz[1a], Niaz Muhammad[2], Shehzad Zareen*[1b], Shawana Nawaz[1c], Bibi Ibtesam Shagufta[1d], Shamim Khan[1e], Hamza Yousaf[1f], Sadia Hur[1g], Alisha khan[1h], Ayesha Siddiqua[3], Sabir Ullah[4], Sumbal Nisar[5a], Laiq Zaman*[5b], Hameed Ur Rehman[6]

[1a,b,c,d,e,f,g,h] Department of Zoology Kohat University of Science and Technology, Kohat-26000 Khyber Pakhtunkhwa, Pakistan
[1a]Email: sabeelazaman1997@gmail.com
[1b]Email: Shehzadzareen1@kust.edu.pk
[1C]Email: shawananawazkust@gmail.com
[1d]Email: ibteshagemi@gmail.com
[1e]Email: zo420211006@kust.edu.pk
[1f]Email: m.hamzayousaf671@gmail.com
[1g]Email: sadiahur512@gmail.com
[1h]Email: alishakhanbangash1@gmail.com
[2]Department of Microbiology Kohat University of Science and Technology, Kohat-26000 Khyber Pakhtunkhwa, Pakistan, Email: mniaz@kust.edu.pk
[3]Department of Chemistry, Kohat University of Science and Technology, Kohat-26000 Khyber Pakhtunkhwa, Pakistan, Email: ayeshaphd.kust@gmail.com
[4]Nowshera Medical College, Nowshera, Email: sabir3027116@gmail.com
[5a,b]Department of Zoology, University of Science and Technology Bannu (28100), KP, Pakistan, [5a]Email: sumbal018@gmail.com
[5b]Email: laiqzaman518@gmail.com
[6]Government High School Teri, B. D. Shah Karak KP Pakistan,
Email: 03449002451h@gmail.com

ARTICLE INFO

Keywords:

Cutaneous leishmaniasis, Antibiogram, Bacterial isolates, Biochemical test, District Karak.

Corresponding Authors:
Shehzad Zareen
Department of Zoology Kohat University of Science and Technology
Shehzadzareen1@kust.edu.pk

Laiq Zaman
Department of Zoology, University of Science and Technology Bannu (28100), KP, Pakistan
laiqzaman518@gmail.com

ABSTRACT

Cutaneous leishmaniasis is one of the infectious diseases which present in most of the countries included Pakistan. It transmits by the bite of infected female phlebotomine sandflies. Cutaneous Leishmaniasis is a considerable health problem in Karak District of Kohat Division. The presence of secondary bacterial infection in cutaneous Leishmaniasis wound aggravates the lesion and delays the healing process of Leishmaniasis wound. The present study was aimed at isolating and identifying the bacteria from leishmaniasis wound and checking the antibiotic sensitivity pattern. The current study was conducted on the 50 patients having clinical feature of leishmaniasis. Wound samples were collected through sterile swab from patients. After microscopically confirmed samples (Giemsa stain) were cultured on different bacteriological media (Nutrient agar, Blood agar and MacKonky agar). The colorless colonies were subjected to biochemical identification such as Gram staining, oxidase, Triple sugar iron (TSI), Methyl red (MR) and Vogues Prousker (VP) to identify potential bacterial isolates. The identified bacteria were subjected to antibiotic sensitivity test, carried out by disc diffusion method on Nutrient agar. The main isolated pathogens in this study were *Escherichia coli*, *Klebsiella* species (Gram negative) and *S. aureus* (Gram positive). Most of the isolates showed resistance against some antibiotics such as penicillin and ceftriaxone and some isolates showed intermediate susceptibility to tested antibiotics.

INTRODUCTION

Cutaneous leishmaniasis (CL) is a parasitic disease found worldwide and is considered one of the most significant vector-borne diseases. It is particularly prevalent in tropical, subtropical, and some parts of Southern Europe (Dayakar & Altiar, 2019). The disease is primarily transmitted by the bite of female phlebotomine sand flies, which belong to the Diptera order and the Psychodidae family. The occurrence of secondary bacterial infections in CL wounds can slow down the healing process. This delay is often caused by constant exposure to environmental factors, lack of cleanliness around the affected area, and the close proximity of lesions, which are commonly found on the lower limbs (Antonio et al., 2017). Wound infections contribute to higher rates of complications, prolonged hospital stays, increased mortality, and rising healthcare costs worldwide (Siddiqui, 2010). If left untreated, wounds can become colonized by various harmful microorganisms, increasing susceptibility to infections. According to the World Health Organization (WHO), failures in healthcare systems, along with the rise in mortality rates, are largely attributed to medication resistance, which continues to be a major obstacle in the fight against various diseases (WHO. 2014). The growing resistance of bacteria to antibiotics has become a serious public health challenge, increasing both morbidity and mortality rates (El Atki *et al*., 2020). Various factors contribute to bacterial resistance, including a combination of nosocomial infections, poor healthcare infrastructure, high prevalence of infectious diseases, widespread availability of inexpensive antibiotics, and rising income levels (Laxminarayan *et al*., 2016).

Cutaneous leishmaniasis (CL) affects around twelve million people worldwide, with approximately two million new cases emerging each year. This disease is endemic in nearly 100 countries, placing an estimated 350 million individuals at risk (de Vries and Schallig,

2022). In Pakistan, between 21,000 to 35,000 cases of zoonotic cutaneous leishmaniasis were recorded in 2021 (Kayani et al., 2021). Among vector-borne diseases, human CL ranks as the second most prevalent after malaria and is considered endemic in specific regions (Fiaz, Raza, and Iftikhar, 2007). The regions where the disease is most common include South Punjab, Baluchistan, Khyber Pakhtunkhwa, and parts of interior Sindh (Hussain et al., 2018). Khyber Pakhtunkhwa, situated in the northwest, has been one of the most significantly affected provinces (Khan and Wahid, 2021). In this province, the most recent outbreak led to over 28,000 cases of leishmaniasis (Khan, Afzal, and Ahmed, 2019). The disease is widely spread across districts such as Kohat, Dera Ismail Khan, Swat, Dir, Malakand, Charsadda, and Karak (Khan, Biradar, and Khan, 2021; Nawaz *et al*., 2020b; Khan *et al*., 2022). The disease's epidemiology and clinical characteristics vary significantly due to multiple factors, including the parasite, its vector, the host, and environmental conditions (Desjeux, 2004). The most prevalent form, "Cutaneous Leishmaniasis" (CL), often leads to permanent impairment, skin lesions, and scarring (Mansueto *et al*., 2014). However little efforts have been made towards the control of Cutaneous leishmaniasis (CL) and the associated pathogens. Therefore, the present research aims to focus on the isolation and identification of pathogens associated with Cutaneous leishmaniasis.

MATERIALS AND METHODS

Ethical consideration

The KUST Research Ethics Committee approved the research proposal.

Sample Collection

Wound sample were collected from CL suspected patient visiting different private and public sector hospital in District Karak and were transferred to the Laboratory of Zoology Department of Kohat University and Sciences and Technology, Kohat. A total of 50 samples were collected from May 2024 to July 2024 due to the availability of vectors.

Identification of Leishmania

For the identification of leishmania Giemsa staining was performed and thin smears were checked under microscope using different magnification lenses.

Procedure of Staining (Giemsa stain)

The swab sample were smeared on clean degreased microscopic slides, air dried and fixed with ethanol or methanol for a few seconds and air dried again. Prepared Giemsa stain solution were poured onto the slide completely covering the sample area. After 15-20 minutes of staining, the slides were slowly washed with tap water and dried in air. These giemsa stained smears were examined under the compound microscope with a 100x magnification.

Isolation and Culturing of bacteria

Nutrient broth were used to refresh the culture of bacteria. The nutrient broth media were made, media transfer in the folcan tubes and rotate or swirl the swab in the nutrient broth to release any microorganism into the medium. Incubate the tubes at 37℃ for 24 hours and after 24 hours the growth in nutrient broths were checked. After this the bacteria cultured on different bacteriological media (Nutrient agar, MacConkey agar) from the falcon tube sub-cultured and identified through Colony characteristics, microscopy (Gram staining) and series of biochemical tests were performed. In this research oxidase, TSI (Triple Sugar Iron), VP (Vogous prosker) and MR (Methyl Red) were performed.

Identification

Morphological Identification through Gram Staining Gram staining technique was performed to study the morphology of Gram positive and Gram-negative bacteria. Slides were prepared and observed under 100x of light microscope. With the help of sterile wire loop, a colony was picked and mixed in a minor drop of sterile solution of normal saline on the microscopic slide. After air drying the microscopic slide was then heat fixed to form a

smear. The primary Gram stain (Crystal Violet) was applied on the smear and held for a minute. After this the slide was washed with tap water to completely remove the excess stain. The slide was then fixed with Gram iodine for one minute which is used as a moderator to make the strong bond between the crystal violet and cell wall of bacteria. Third step was applying ethanol, a decolorizer, which washes away the stain from Gram negative cell wall, for about twenty seconds and rinsed again with water. At the end the slide was stained with secondary Gram stain safranin for 1 minute which allow the dye adherence to Gram negative cell walls and was then washed with tap water. After successful completion of these steps the slides were air dried and observed under microscope at 100x.

Biochemical testing

Based on the culture, microscopy and microbiological examination of the selected colonies, the isolated from leishmeniasis wounds were confirmed through biochemical analysis. Following the observation of Gram staining, specific biochemical test (Vogous prousker(VP), Methyl Red (MR), Triple sugar Iron (TSI) , Oxidase) were performed for further identification. The reults of the tests have been shown in Figure 04.

Antibiotic suspectibility tests

The antibiotic sensitivity test was performed on disc diffusion technique on nutrient agar. The antibiotics used were levofloxacin, Penicillin G, Ceftriaxone, Vancomycin, Amikicin, and Tetracycline. The antibiotics used in the current research work have been given in Table 01.

Table 01: Antibiotics Used for Sensittivity Test

S.No	Antibiotics name	Disc code	Disc content (µg)
1	Amikicin	AK30	30 µg
2	Ceftriaxone	CRO30	30 µg
3	Tetracycline	TE30	30 µg
4	Vancomycin	VA30	30 µg
5	Penicillin G	P10	30 µg
6	levofloxacin	LEV5	30 µg

RESULTS

In our study 50 clinical samples were collected. Gram negative isolates were more common than gram positive bacteria. In this study *E. coli, Klebsiella* species and *Staphylococcus aureus* were the major pathogens. *E. coli* was the predominant isolate with a frequency of 34%, followed by *klebsiella* species 26% and *Enterobacter* species 10% among the isolates of gram-negative isolates and *Staphylococcus aureus* 16% among gram positive isolates. *E. coli* and *Klebsiella* species were the commonest gram-negative bacteria present in leishmaniasis wound with 17 (34%) and 13 (26%) frequencies respectively and *S. aureus* with 8 (16%) frequency was the main Gram-positive bacteria present in the leishmaniasis wound.

Microscopy

Morphological Identification through Gram Staining Gram staining technique was performed to study the morphological of Gram positive and Gram negative bacteria. Slides

were prepared and observed under 100x of light microscope. Slides from each bacterial colony were prepared for microscopic investigation. The photomicrograph of the slides have been given in Figure 01.

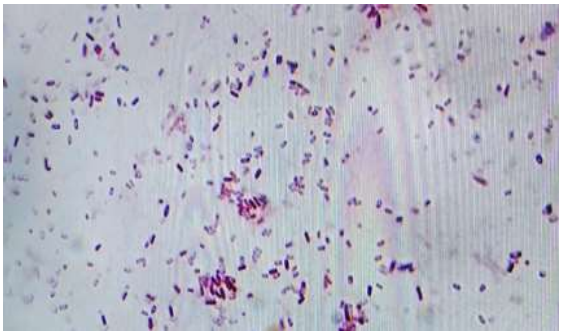

Figure 01. represents the photomicrograph of the isolates

Biochemical testing

Based on the culture, microscopy and microbiological examination of the selected colonies, the isolated from leishmeniasis wounds were confirmed through biochemical analysis. Following the observation of Gram staining, specific biochemical test (Vogous prousker(VP), Methyl Red (MR), Triple Sugar Iron (TSI) , Oxidase) were performed for further identification. The data has been given in Table 02. Photographs have also been represnted as **Figure 03**.

Frequency of bacteria isolated

E. coli and *Klebsiella* species were the commonest gram-positive bacteria present in leishmaniasis wound with 17 (36%) and 13 (24%) frequencies respectively and *S. aureus* with 8 (16%) frequency was the main Gram-negative bacteria present in the leishmaniasis wound. The data has been given in table 02.

Table 02: Frequency of Various bacteria present in Leishmaniasis wound

Gram negative bacterial isolate	Specimen type	Total n (%)
Escherichia coli	Wound	**17 (34%)**
Klebsiella species	Wound	**13 (26%)**
Enterobacter species	Wound	**5(10%)**
Pseudomonas species	Wound	**3(6%)**
Serratia species	Wound	**2(4%)**
Shigella species	Wound	**2(4%)**
Gram positive bacterial Isolate		
Staphylococcus aureus	Wound	**8(16%)**
Total		**50 (100%)**

Identification of bacterial isolates

Clinical samples were collected from hospitals which were then transferred to the Zoology Laboratory at Kohat University of Science and Technology.

The bacterial isolates were observed on incubated plates of nutrient and MacConkey agar as shown in **Figure 02**.

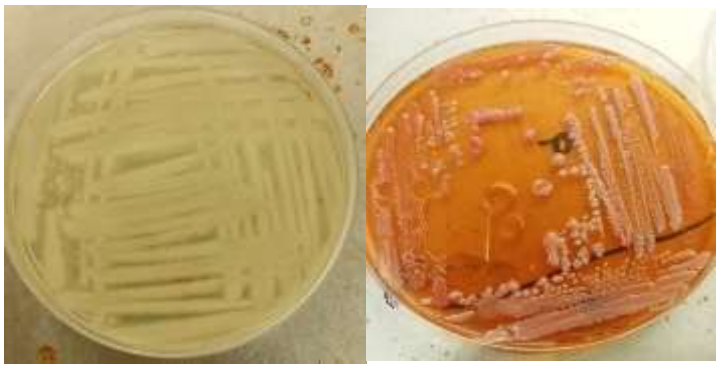

Figure 02: Bacteria Culture on Nutrient and MacConkey agar.

Biochemical Tests Results

Based on the culture, microscopy and microbiological examination of the selected colonies, the isolates from leishmaniasis wounds were confirmed through specific biochemical test including (Vogous Prousker (VP), Methyl Red (MR), Triple sugar Iron (TSI) and Oxidase) were performed for further identification. **Table 03** represents the data of isolates after performing the biochemical tests. The photos have been given in **Figure 03.**

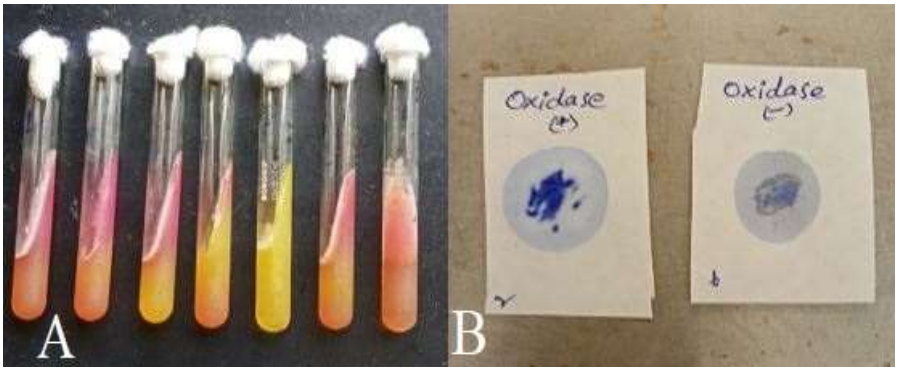

Figure 03 Represents the results of Biochemical Tests. (A) Represents the TSI test results while (B) Represents the results of Oxidase Tests.

Table 03: Biochemical Identification of Bacterial Isolates

Collected Isolates	Cell Morphology		Biochemical Tests				
	Shape	**Gram**	**TSI**		**Oxi**	**MR**	VP
			Lactose	+			

Escherichia coli	Rod	-	Sucrose	+	-	+	-
			Glucose	+			
			H2S	-			
			Gas	+			
Staphylococcus aureus	Round	+	Lactose	+	-	+	+
			Sucrose	+			
			Glucose	+			
			H2S	-			
			Gas	-			
Klebsiella species	Rod	-	Lactose	+	-	-	+
			Sucrose	+			
			Glucose	+			
			H2S	-			
			Gas	+			
Enterobacter species	Rod	-	Lactose	+	-	-	+
			Sucrose	+			
			Glucose	+			
			H2S	-			
			Gas	+			
Pseudomonas species	Rod	-	Lactose	-	+	-	-
			Sucrose	-			
			Glucose	-			
			H2S	-			
			Gas	-			
Shigella species	Rod	-	Lactose	-	-	+	-
			Sucrose	-			

			Glucose	+			
			H2S	-			
			Gas	-			
Serratia species	Rod	-	Lactose	-	-	+	-
			Sucrose	-			
			Glucose	+			
			H2S	-			
			Gas	-			

Antibiotics Sensitivity of the isolates

The modified Kirby-Buer Disc diffusion method on nutrient agar was used to determine the antibiotic sensitivity. The antibiotics used in the study were Amikacin, Ceftriaxone, Penicillin, Tetracycline, Levofloxacin and Vancomycin. The zones of inhibition were observed and measured in millimeter (mm). The antimicrobial susceptibility of the bacterial isolates is shown in Table 04. The photograph of the Antibiotic sensitivity has been shown in Figure 04. Similarly, **Table 04** and **Table 05 r**epresent the antibiograms and frequency of Inhibition Zone of the isolates.

Table 04: Antimicrobial Susceptibility Pattern of Isolates (mm)

Bacterial isolates	Zone of inhibition (mm)					
	AK30(ug)	CRO30(ug)	TE30(ug)	VA30(ug)	P10(ug)	**LEV5(ug)**
Escherichia coli	18	10	14	9	0	**18**
Staphylococcus aurous	17	18	14	30	5	**18**
Klebsiella species	20	14	26	19	10	**23**
Enterobacter species	20	15	21	18	0	**23**

Pseudomonas species	21	9	24	16	4	**22**
Shigella species	23	24	21	12	21	**30**
Serratia species	**24**	**15**	**30**	**20**	**25**	**29**

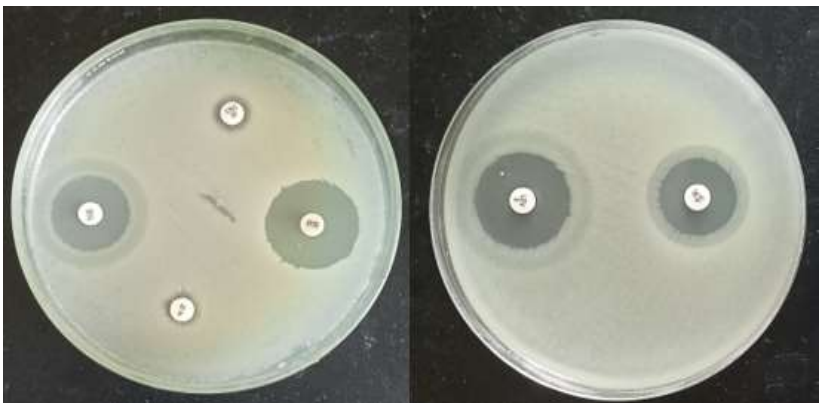

Figure 04: Represents the Antibiotic sensitivity of isolates. According to CLSI guidelines the bacteria is consider is resistance, intermediate and sensitive.

Table 04: Represents the antibiograms of isolates.

Bacterial isolate	**AK(30µg)**	**CRO(30µg)**	**TE(30µg)**	**VA(30µg)**	**P(10µi)**	**LEV(5µg)**
Escherichia coli	S	R	I	R	R	**I**
Staphylococcus aureus	I	I	R	R	R	**I**
Klebsiella specie	S	R	S	S	R	**S**
Enterobacter specie	S	R	S	S	R	**S**
Pseudomonas specie	S	R	S	I	R	**S**

Serratia specie	S	R	S	S	I	**S**
Shigella specie	**S**	**S**	**S**	**R**	**I**	**S**

* S= Sensitive R =Resistance I= Intermediate

Table 05: Shows the frequency of Inhibition Zone

S. No	Frequency of inhibition Zone					
Bacterial Isolates	AK(30µg)	CRO(30µg)	TE (30µg)	VA (30µg)	P (10µg)	**LEV(5µg)**
Escherichia coli	18.6	10.6	13.6	8.6	0	**18.3**
Staphylococcus aureus	17.3	19.6	15	10.6	4	**19.6**
Klebsiella species	19.6	13.3	27	18.6	10.3	**22**
Enterobacter species	19.3	12.6	23.3	18.3	0	**23**
Pseudomonas species	20.6	9	22.6	15.6	3	**20.3**
Serratia species	19.3	15.3	29	19	27.6	**28**
Shigella species	**22.6**	**24**	**20.3**	**11.3**	**20**	**29.6**

DISCUSSIONS

In this study the cutaneous leishmaniasis is more common among children which is similar the report made by (Mumtaz *et al*. 2016). The limbs were more likely to be exposed to the vector another parts of the body, the lesions were predominantly found there.

This investigation yielded (50) isolates that included both Gram-negative and Gram-positive bacteria from seven (7) distinct bacterial species. The results of this study are in accordance with the studies conducted in Ghana. Gram positive including *S aureous* and Gram negative including *klebsiella species, Serratia species, shigella species, Enterobacter species* and *Pseudomonas species* were identified. These findings were in accordance with the study as discussed by (Ntirenganya *et al*., 2015, Nwankwo and Edino 2014). The variety of Gram-negative bacteria discovered in the wounds could suggest that the patient

contracted illness from the local population. Numerous studies have noted this pattern of a predominance of Gram- negative infections (Bediako-bowan *et al*. 2020, Pondei *et al*. 2013). In this investigation and others, *S.aureus* was found to be the most frequently isolated species (Ereqat *et al*., 2021), Nevertheless, despite being distinct from the isolates seen in (Vicar *et al*. 2021, Salgado *et al*. 2016).

In this investigation, every sample of a wound showed signs of either mono or poly microbial infections. All isolates were tested for resistance to common antibiotics used to treat these illnesses. Among Gram negative isolates *E. coli*, *Klebsiella species*, *Enterobacter species* and *Pseudomonas species* show highly resistance to penicillin and ceftriaxone and show sensitivity for amikacin. In Gram positive isolates the *S. aureus* showed resistance to tetracycline vancomycin and penicillin and showed intermediate sensitivity to amikacin, ceftriaxone and levofloxacin. Since these antibiotics are used as primary therapies for infections in wounds, their documented resistance patterns may be related (Vicar *et al*. 2021, Kumburu *et al*. 2017). The proliferation of microorganisms resistant to antibiotics in the environment may be facilitated by self-medication and improper sanitation practices. Multiple drug resistance in bacterial isolates may because by the high prevalence of antibiotic usage in the communities and the inadequacy of microbiological diagnostic input in clinical care for such communities (Duong and Jaelin 2015). The current study concluded that the main pathogen Gram negative bacteria, particularly *E. coli* and *Klebsiella* species are the leading pathogen present in leishmaniasis wounds and *Staphylococcus aurous* involved in leishmaniasis wound among Gram positive bacteria. Most of isolates showed sensitivity to Amikacin, Levofloxacin, Tetracycline and Vancomycin and were showed highly resistant to Penicillin G and Ceftriaxone. The drug resistance of microbes is increasing at an alarming speed day by day, if this trend continues perhaps there could be a day when pathogen will be resistant to all antibiotics. To prevent such a circumstance, scientists are persuaded to believe in an alternative approach to resolve this issue. Consequently, further investigation with more samples is required, advance technologies which can provide a more detailed understanding of microbial interaction within leishmaniasis lesions which is crucial for improving diagnostic and treatment strategies for this complex infectious disease. Clinicians must first prescribe antibiotic susceptibility patterns, before providing medications for the treatment of cutaneous leishmaniasis.

REFERENCES

Antonio, L. D. F., Lyra, M. R., Saheki, M. N., Schubach, A. D. O., Miranda, L. D. F. C., Madeira, M. D. F., ... & Pimentel, M. I. F. (2017). Effect of secondary infection on epithelialisation and total healing of cutaneous leishmaniasis lesions. *Memórias do Instituto Oswaldo Cruz*, *112*, 640-646.Bediako-Bowan, Antoinette AA, Jørgen AL Kurtzhals, KåreMølbak, Appiah-Korang Labi, Enid Owusu, and Mercy J. Newman. 2020. "High rates of multi-drug resistant gram-negative organisms associated with surgical site infections in a teaching hospital in Ghana. "*BMC infectious diseases* 20, no. 1: 1-9.

Dayakar Alti, Sambamurthy Chandrasekaran, Suresh V Kuchipudi, and Suresh K Kalangi. 2019. "Cytokines: key determinants of resistance or disease progression in visceral leishmaniasis: opportunities for novel diagnostics and immunotherapy. "*Frontiers in immunology* 10:670.

de Vries, Henry JC, and Henk D Schallig. 2022. "Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments. "*American Journal of Clinical Dermatology* 23 (6):823-840.

Desjeux, Philippe.2004."Leishmaniasis: current situation and new perspectives. "*Comparative immunology, microbiology infectious diseases*27 (5):305-318.

Duong A, Jaelin M. 2015. 6 Factors that have caused antibiotic resistance. Infection Control tips:1–2. Infection control. tips/2015/11/18/6-factors-that-have-caused-antibiotic-resistance. Retrieved 15 Nov 2020

Ejaz, Amer, Naeem Raza, and Nadia Iftikhar. 2007. "Recurrent cutaneous leishmaniasis presenting as sporotrichoid abscesses: a rare presentation near Afghanistan border. "*Dermatology Online Journal* 13 (2).

El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Lyoussi, B., Oumokhtar, B., & Abdellaoui, A. (2020). Phytochemistry, antioxidant and antibacterial activities of two Moroccan Teucrium polium L. subspecies: preventive approach against nosocomial infections. *Arabian Journal of Chemistry*, *13*(2), 3866-3874.

Ereqat, S., Al-Jawabreh, A., Abdeen, Z., Al-Jwabreh, H., & Nasereddin, A. (2021). Characterization of leishmania ulcers microbiota using next generation sequencing.

Hussain, Mubashir, Shahzad Munir, Taj Ali Khan, Abdullah Khan, Sultan Ayaz, Muhammad Ameen Jamal, Irfan Ahmed, Sohail Aziz, Noha Watany, and MohamedKasbari.2018."Epidemiologyofcutaneousleishmaniasisoutbreak, Waziristan, Pakistan. "*Emerging infectious diseases* 24 (1):159.

Kumburu, Happiness Houka, Tolbert Sonda, Blandina Theophil Mmbaga, Michael Alifrangis, Ole Lund, Gibson Kibiki, and Frank M. Aarestrup. 2017. "Patterns of infections, aetiological agents and antimicrobial resistance at a tertiary care hospital in northern Tanzania."*Tropical Medicine & International Health* 22, no. 4 : 454-464.

Kayani, Behzad, Shakera Sadiq, Hamad Bin Rashid, NaseerAhmed, Altaf Mahmood, Muhammad Shakeel Khaliq, Rubab Maqsood, Haroon Rashid, Saima Hasan, and Mushtaq Muhammad Hassan. 2021. "Cutaneous Leishmaniasis in Pakistan: a neglected disease needing one health strategy."*BMC Infectious Diseases* 21:1-10.

Khan, Aakifullah, Ghufran Biradar, and Moiz Khan. 2021. "Treatment of cutaneous leishmaniasis with photodynamic therapy: The first case report from Khyber Pakhthunkhwa (KPK), Swat Pakistan."*Journal of Pakistan Association of Dermatologists* 31 (1):120-124.

Khan, Aisha, Muhammad Sohail Afzal, and Haroon Ahmed. 2019. "Leishmaniasis in Pakistan: A call for action. "*Travel Medicine Infectious Disease*:101516- 101516.

Laxminarayan, Ramanan, Precious Matsoso, Suraj Pant, Charles Brower, John-Arne Røttingen, Keith Klugman, and Sally Davies. 2016. "Access to effective antimicrobials: a worldwide challenge "*The Lancet* 387 (10014):168-175.

Mansueto, Pasquale, Aurelio Seidita, Giustina Vitale, and Antonio Cascio. 2014. "Leishmaniasis in travelers: a literature review. "*Travel medicine infectious disease* 12 (6):563-581.

Mumtaz, Shahina, Asif Hussain Munir, Muhammad Asghar and Naheed Naheed. 2016."Frequency and types of leishmaniasis in KhyberPakhtunkhwa (KPK)."*Journal of Medical Sciences*24, no. 3: 136-140.

Ntirenganya, C., Manzi, O., Muvunyi, C. M., & Ogbuagu, O. (2015). High prevalence of antimicrobial resistance among common bacterial isolates in a tertiary healthcare facility in Rwanda. *The American journal of tropical medicine and hygiene*, *92*(4), 865.

Nwankwo, Emmanuel, and Stephen Edino. "Seasonal variation and risk factors associated with surgical site infection rate in Kano, Nigeria. 2014. "*Turkish journal of medical sciences* 44, no. 4: 674-680.

Organization, World Health. 2014. *Antimicrobial resistance: global report on surveillance*: World Health Organization.

Pondei, K., Fente, B. G., & Oladapo, O. (2013). Current microbial isolates from wound swabs, their culture and sensitivity pattern at the Niger delta university teaching hospital, Okolobiri, Nigeria. *Tropical medicine and health*, *41*(2), 49-53.

Salgado, V. R., Queiroz, A. T. D., Sanabani, S. S., Oliveira, C. I. D., Carvalho, E. M., Costa, J. M., ... & Barral, A. (2016). The microbiological signature of human cutaneous leishmaniasis lesions exhibits restricted bacterial diversity compared to healthy skin. *Memorias do Instituto Oswaldo Cruz*, *111*(4), 241-251.

Siddiqui, Abdul R, and Jack MBernstein. 2010. "Chronic wound infection: facts and controversies. "*Clinics in dermatology* 28 (5):519-526.

Vicar, Ezekiel Kofi, Samuel EK Acquah, Walana Williams, Eugene D. Kuugbee, Courage KS Saba, and Gloria Ivy Mensah. "Antibiotic resistant bacteria infecting wounds of rural community dwellers in northern Ghana."*European Journal of Medical and Health Sciences* 3, no. 1 (2021): 112-117.