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Diversity and Antibigrams of Secondary Bacterial Isolates from Cutaneous Leishmaniasis Wounds

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Abstract

Background: Leishmaniasis is a vector borne disease caused by an intracellular protozoan parasite. The presence of secondary bacterial infections in cutaneous leishmaniasis wounds exacerbate lesion development and could lead to delay in the healing process. Little is also known about the different bacteria species co-infecting leishmaniasis wounds and their sensitivity patterns in Ghana. This study sought to determine the resistance patterns of bacteria co-infecting cutaneous leishmaniasis wounds from selected communities in the Nkwanta district.

Methods: Various bacteria were isolated and characterized from exudates obtained from wound swabs collected with sterile cotton tipped applicators. Confirmation of bacterial identity was done using the analytical profile index and the matrix-assisted laser desorption/ionization time of flight mass spectrometry. Antibiotic susceptibility tests were performed using agar disc diffusion method according to the Clinical and Laboratory Standards Institute breakpoint values.

Results: A total of 42 secondary bacteria were isolated from the wounds among which *S. aureus* was the most predominant (31%). Other pathogenic bacteria that colonized the wounds included *Bacillus subtilis* (23.8%), *Pantoea* spp (11.9%), *Klebsiella pneumoniae* (7.1%), *Enterobacter cloacae* (7.1%), *Aeromonas* spp (4.8%), *Serratia marcescens* (4.8%), *Serratia liquefaciens* (2.4%), *Serratia plymuthica* (2.4%), *Providencia rettgeri* (2.4%) and *Cronobacter* spp (2.4%). Majority of the isolates were obtained from Agoufie (21.4%), Baasare (19%), and Gekrong (16.7%). Most of the isolates were resistant to beta-lactam antibiotics and the third generation cephalosporin. Notably, 84.6% of the *S. aureus* isolates were methicillin and ciprofloxacin resistant whilst 92.3% were resistant to ampicillin. About

sixty-nine percent (69.2%) showed intermediate susceptibility to Erythromycin. Additionally, *S. plymutheca* was resistant to all the test antibiotics. All the *K. pneumoniae* and *E. cloacae* isolates showed resistance to ampicillin, cefotaxime, ceftriaxone, ciprofloxacin, amikacin, aztreonam and meropenem but only 66.7% of these isolates were resistant to piperacillin. All isolates of *Providencia rettgeri*, *Cronobacter* spp, *S. marcescen*, *S. liquefacien* were resistant to all the beta-lactam antibiotics.

Conclusion: This study suggests colonization of cutaneous leishmaniasis wounds with varied bacterial species that are mostly resistant to beta-lactam group of antibiotics.

Keywords: Cutaneous leishmaniasis, wounds co-infections, resistance, Neglected tropical diseases, Oti region

Background

Leishmaniasis is a vector-borne infection caused by an intracellular protozoan parasite of the genus *Leishmania* [1]. The disease affects 98 countries, with an annual incidence of 0.7–1.2 million cases of Cutaneous Leishmaniasis (CL) and 0.2–0.4 million cases of visceral leishmaniasis (VL) resulting in 20,000–40,000 deaths [2]. Cutaneous leishmaniasis is the most common form of leishmaniasis, characterized by an ulcerative skin lesion that appears as a single or multiple ulcers on the surface of the skin after being bitten by infected female sandflies. The occurrence of secondary bacterial infection in CL wounds due to the frequent environmental vulnerability, and unhygienic conditions at the lesion site coupled with ground proximity of lesions mostly found on the lower limbs, may prolong the healing process [3].

Globally, wound infections have resulted in significant morbidity, mortality, longer hospitalization, and an increase in direct and indirect healthcare expenses [4]. Wounds can be colonized by variety of potentially dangerous bacteria, making them susceptible to infection if not properly cared for. This poly-microbial infection makes bacteriologic studies more difficult, and as a result, it is frequently overlooked in resource-constrained settings [5]. When a wound becomes infected, it takes significantly longer time to heal, which increases the expense of therapy and the extent of pain and discomfort experienced by the patient [6, 7]. Drug resistance is complicating the fight against a variety of diseases, according to the World Health Organization, leading to health care failure and increased mortality [8].

Sub-Saharan Africa has a disproportionate share of the global burden of infectious disease, [9], despite this, there is a paucity of antibiotic resistance evidence from this area. In this

region, optimal infection treatment is impeded by a lack of access to diagnostic tests to detect bacteria and antibiotic susceptibility patterns, as well as a scarcity of suitably educated laboratory personnel and inadequate microbiology laboratory infrastructure [10]. These findings highlight the critical need for antimicrobial susceptibility data in African countries to guide antimicrobial therapy [11]. In Ghana, multiple drug resistance to some antibiotics have been discovered in most bacteria, especially *Staphylococcus aureus* and the Gram-negative bacteria [12, 13]. Low standards of living and lack of access to healthcare define many Ghanaian rural communities especially those with endemic neglected tropical diseases. Where community-based healthcare is accessible, it is frequently lacking a microbiological laboratory that allows for bacteriological investigation of clinical samples, including antibiotic susceptibility test (AST) [12].

Many rural residents have no choice but to seek alternative health care from local herbalists or rely on home-based traditional treatments due to their limited financial resources, high treatment costs, and stigmatization. After it is clear that these alternative therapies have failed, wounds are usually sent to healthcare institutions in their worst state. This study thus, identified and determined the susceptibility profile of bacteria co-infecting CL wounds in some endemic communities and newly identified CL endemic communities in the Oti region of Ghana. This would aid surveillance efforts and assist health practitioners optimize therapy for patients with CL wounds.

Methods

Ethical Approval

Ethical approval was obtained from the Committee on Human Research Publications and Ethics (CHRPE/AP/572/19), Kwame Nkrumah University of Science and Technology (KNUST),

Kumasi. In addition, all participants gave a written consent. The study was also conducted according to the Eastern Mediterranean Health Journal guidelines (EMHJ) on ethical conduct on the use of human subjects [14]. The purpose of the study was explained to the District Health Directorate and chiefs of the various communities and participants in English or their preferred Ghanaian language including Twi and Ewe. Participants gave both verbal and written consent and in addition, written consent was obtained from guardians of participants under the ages of 18.

Study Design and Site

This was a cross-sectional community-based study with microbiological modeling arms. Following community engagement, households with at least one case of skin lesions were selected for the study. Wound samples were collected from seven (7) communities in the Nkwanta South District, Oti region of Ghana (Figure 1). The study was conducted in the following seven communities: Baasare, Asuogya, Keri, Gekrong, Ashiabre, Agoufie and Pawa. These communities were selected because previous studies have identified them as endemic areas in Ghana [15]. According to the 2010 Population and Housing Census (PHC), the total population of the Municipality is expected to be 149,296 by 2020, with a population growth rate of 2.5 percent based on the Ghana Statistical Service's (GSS) Regional and National Growth Rates. There are 49.5% males and 50.5% females in this area with farming being their main occupation [16]. The Nkwanta South municipality, with a land area of roughly 2733 km², is situated between latitudes 7° 30' and 8° 45' North and longitudes 0°10' and 0° 45' East [15]. See Figure 1.

Inclusion and Exclusion Criteria

Participation in this study was entirely voluntary, and participants had the option to withdraw at any time. Individuals who presented with wounds were examined by a health professional and those with wounds characteristic of CL (round or oval in shape, raised borders and reddish background) [17] were included in the study while those who presented with wounds without the features of CL lesion were excluded.

Collection and confirmation of Leishmania parasite in lesions

Forty-eight (48) participants with wounds characteristic of cutaneous leishmaniasis lesions were sampled and aseptically collected with a sterile cotton tipped applicator by swabbing the surface of the wound and immersing swabs in sterile glycerol broth (Surechem Products Ltd, Needham, UK) and stored at -20°C. Filter papers were also used to collect wound exudates for PCR analysis. The samples were later transported to the Microbiology Laboratory of the Department of Microbiology, Kwame Nkrumah University of Science and Technology for bacteriological analysis.

Leishmania parasite infestation of the wounds were confirmed by the Vector-borne Infectious Diseases Laboratory, Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana[18]. Briefly, DNA was extracted from the filter paper containing the wound exudates by employing the DNeasy Qiagen protocol (cat. Nos. 69504 and 9506). The primers LINR4 (forward) (5-GGG GTT GGT GTA AAA TAG GG-3), LIN17 (reverse) (5-TTT GAA CGG GAT TTC TG-3) and LIN19 (reverse) (5-CAG AAC GCC CCT ACC CG-3) in a semi-nested PCR assay, were used to amplify the conserved area of minicircle kinetoplast DNA from *Leishmania* parasite [19]. The PCR

reaction volume was 25 μ L including, 2 μ m of primers, 2 μ m of MgCl₂, 20 μ m of dNTPs, 10x dream Taq buffer and dream Taq polymerase (2units). Given an initial incubation temperature of 95°C for 5 min, followed by denaturation at 95°C for 40 cycles at 1 minute, an annealing temperature of 54°C for 30 seconds as well as initial and final extension at 72°C for 1 min and 72°C for 5 min respectively. The PCR products were stained with ethidium bromide on a 1% agarose gel at 50 V and observed with a UV transilluminator.

Bacteria culture and identification

Ten milliliters of peptone water (Oxoid Ltd, Basingstoke, Hampshire, UK), was inoculated with 1 mL of each of the samples from the glycerol broth (Surechem Products Ltd, Needham, UK), and were incubated for 24 h at 37°C. The samples were later sub-cultured into 20 mL MacConkey agar, Mannitol Salt agar (MSA), Bismuth sulphite agar, Cetrimide agar and Trypticase soya agar (TSA) (Oxoid Ltd, Basingstoke, UK), with the aid of a sterile inoculating loop and incubated overnight at 37°C. The bacteria growth on each media were carefully fished out and purified on 20 mL blood (5% v/v) agar, incubated at 37°C for 24 h. Colony characteristic appearance and Gram-stain reactions were used for presumptive identification of bacteria species [20]. The isolates were further identified using API 20E (bioMerieux Inc., Durham, USA) biochemical assay kit and confirmed using MALDI-TOF VITEK MS (BioMérieux Corporate, Paris, France).

Antibiotic Susceptibility Testing

Antimicrobial susceptibility of all the bacterial isolates were determined using the Kirby Bauer agar disc diffusion assay guided by breakpoint values of Clinical and Laboratory Standards Institute [21]. The isolates were tested for their susceptibility to

Peperacillin/Tazobactam (36 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Ampicillin (10 µg), Tetracycline (30 µg), Trimethoprim/sulfamethoxazole (25 µg), Chloramphenicol (30 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Meropenem (10 µg), Amikacin (30 µg), Erythromycin (15 µg), Cefepime (30 µg), Ceftazidime (30 µg) and Aztreonam (30 µg) (ThermoFisher Scientific Ltd, Basingstoke, UK). Cefoxitin was used as a surrogate marker for the detection of Methicillin-resistant *Staphylococcus aureus* as described by [19]. *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 33495 and *Escherichia coli* ATCC 25922 were used as reference quality control strains.

Statistical Analysis

All data were entered into Microsoft office excel 2016 and presented in summary tables and charts. Data were also presented as percentages.

Results

A total of 48 participants with wounds characteristic of CL were recruited for the study. Thirty-three of these wounds were confirmed to be infested with Leishmania parasites [18]. Majority of the participants confirmed to have cutaneous leishmaniasis infested wounds were males (n=21, 63.6%) (Table 1). Many (n=18, 54.5 %) of the participants were students, while the others (n=9, 27.3%) were unemployed (n=6, 18.2%) and engaged in farming or casual labor. Most of them had either basic education (n=22, 66.7%) or had not received any formal education (n=12, 36.4%). Most of the participants resorted to orthodox medication obtained from community over-the-counter shops for treatment (n=25, 75.7%), whilst others relied on herbal products (n=4, 12.1%) or no form of treatment for their wounds. See Figure 2.

This study yielded (42) isolates in all, comprising eleven (11) different species of bacteria including Gram-positive and Gram-negative bacteria. Twenty-three (54.8%) Gram-positives including 13 (31%) *S. aureus* and 10 (24%) *B. subtilis*, whereas 19 (45.2%) Gram negatives comprising of *Pantoea* spp (n=5, 11.9%), *P. rettgeri* (n=1, 2.4%), *Aeromonas* spp (n=2, 4.7%), *Cronobacter* spp (n=1, 2.4%), *E. cloacae* (n=3, 7.1%), *Klebsiella pneumoniae* (n=3, 7.1%), *S. marscecens* (n=2, 4.7%), *S. liquefacien* (n=1, 2.4%) and *S. plymutheca* (n=1, 2.4%). Majority of the Isolates were from Agoufie 9 (21.4%), Baasare 8 (19%), and Gekrong 7(16.7%) (Table 2).

The susceptibility of *Enterobacterales* comprising *Enterobacter cloacae*, *Pantoea* spp, *Klebsiella pneumoniae*, *Serratia marscecens*, *Serratia liquefacien*, *Serratia plymutheca*, *Cronobacter* spp, *Aeromonas* spp and *Providencia rettgeri* were determined following the CLSI guidelines as shown in Figure 3. Ten antibiotics were screened against the isolates.

All the enteric bacteria except *Aeromonas* spp were resistant to Ampicillin and Ceftriaxone. About ninety-four percent 94.12% showed resistance to Ciprofloxacin, Cefotaxime, Meropenem and Aztreonam (Figure 3). Eighty-eight percent (88.2%) were resistant to Amikacin whilst 41.2% were resistant to chloramphenicol and Trimethoprim/sulfamethoxazole, respectively. About 11.8% and 5.9% showed intermediate susceptibility to Trimethoprim/sulfamethoxazole and Amikacin, respectively (Table 3). Additionally, *Aeromonas* spp were sensitive to all the test antibiotics.

About 92.3% of the *Staphylococcus aureus* isolates were resistant to ampicillin while 84.6% were resistant to ciprofloxacin and cefoxitin (Figure 4). Sixty-nine percent (69.2%) showed

intermediate susceptibility to Erythromycin. The *S. aureus* isolates were however, susceptible to Tetracycline (8.6%), Gentamicin (100%) and Trimethoprim/sulfamethoxazole (92.3%). All the isolates were multidrug resistant.

Discussion

Cutaneous leishmaniasis (CL), a Neglected Tropical Disease, affects a sizeable number of school children in the communities in Volta and Oti regions of Ghana [22]. Current transmission of the disease is ongoing in the Nkwanta district of the Oti region of Ghana. CL was first suspected in the Ho municipality of the Volta region in 1999 [15, 23]. Despite the fact that CL is a self-healing disease [24], many factors such as poly-microbial infections may delay the healing process and increase scarring [25]. For this reason, a better understanding of the disease and organisms co-infecting the wounds may be essential in order to provide a more effective therapeutic treatment and minimize scarring. This study sought to isolate and identify bacteria co-infecting CL wounds and determine the patterns of susceptibility to clinical antibiotics.

In this study, the disease (CL) was more common among children with ages ranging from 2-15 years (66.7%) which is similar to the report made by Kwakye-Nuako *et al.* [24] who reported that the disease was more common in children. The high prevalence in children compared to adults could be as a result of acquisition of immunity [26]. The cases were predominant in males 21 (63.6%) as compared to females (36.4%), (Table 1), which may be due to their behavioral patterns, such as the amount of time males spend outside. Since majority of participants slept regularly in treated bed net 21 (63.6%) (Table 1), exposure to the vector is more possible outside as compared to indoors. This however contradicts the

findings made by Kweku *et al.*, [15] in Ho municipality. The lesions were mostly located on the limbs, probably due to the fact that other areas of the body could be less exposed to the vector. The number of lesions ranged from 1-3 mostly in the infants (2-9yrs) but adults possessed scars indicating a previous infection.

This study yielded (42) isolates comprising eleven (11) different bacteria species including Gram-positive and Gram-negative bacteria. Gram-positives including *S. aureus* and *B. subtilis* as well as Gram-negatives comprising of *Pantoea spp*, *P. rettgeri*, *Aeromonas spp*, *Cronobacter spp*, *E. cloacae*, *K. pneumoniae*, *S. marcescens*, *S. liquefaciens* and *S. plymuthica* (Table 2) were identified. This finding is similar to microbial biodiversity data of CL lesions in related research from underdeveloped countries such as Rwanda [27] and Nigeria [28]. The large range of Gram-negative bacteria isolated from the wounds could indicate that the infection was community acquired. In this and several other research, *S. aureus* was the most commonly isolated species [12, 29, 30]. Most of the species isolated from CL wounds in this study, such as *Pantoea spp*, *P. rettgeri*, *Aeromonas spp*, *Cronobacter spp* and *E. cloacae*, have not been isolated from CL wounds in other studies, probably because of how the wounds are handled in other endemic areas [31, 25, 32].

In this study, all of the wound samples exhibited either mono-microbial and or poly-microbial infections, and there was a similar phenomenon in the report by Salgado *et al.* [29] who recorded a delayed healing in two or three patients with localized cutaneous lesion, which they attributed to the association of three different bacterial genera. High prevalence of bacteria of different genera were obtained from Agoufie community.

All isolates were screened against antibiotics that are commonly used in treating these infections. Based on the CLSI, (2018) guidelines, cefoxitin was used as a surrogate marker for

the detection of Methicillin-resistant *Staphylococcus aureus*. Methicillin resistance was high among the *S. aureus* isolates (84.1%) (Table 3). This is of concern because due to -lack of well-equipped laboratories for detection, MRSA infections may be going unnoticed in rural areas. Ninety-two (92.3%) of *S. aureus* isolates showed resistance to ampicillin whilst (84.6%) were resistant to ciprofloxacin and erythromycin. The *S. aureus* isolates were however susceptible to trimethoprim/sulfamethoxazole (92.3%) and tetracycline (84.6%), respectively. Resistance of the MRSA *strains* to beta-lactam antibiotics in the current study was higher compared to findings made by Vicar *et al.* [12]. Antimicrobial misuse resulting from self-medication could have led to the development of multidrug resistance among the *S. aureus* isolates [14]. *S. aureus* resistance to ampicillin is relatively higher in this study than that observed in a study conducted by Saana *et al.* [33] who reported about 53% resistance to the same antibiotics tested.

Among the Gram-negatives, *Aeromonas spp* showed relatively high sensitivity to all the test antibiotics compared to the other bacterial isolates. All the *Aeromonas* isolates showed susceptibility to ceftazidime, ciprofloxacin, aztreonam and trimethoprim/sulfamethoxazole (Table 3). All the *K. pneumoniae* isolates were resistant to ampicillin, cefotaxime, ceftriaxone, ciprofloxacin, amikacin, aztreonam and meropenem. *E. cloacae* was resistant to all the test antibiotics with the exception of piperacillin/tazobactam (Table 3). Similar observation was recorded by *Pantoea spp* from Ashiabre, Asuogya and Keri. All the *Providencia rettgeri*, *Cronobacter spp*, *S. marcescens*, *S. liquefacien* isolates were resistant to all the beta-lactam antibiotics with 53% showing resistance to trimethoprim /sulfamethoxazole. These findings are similar to a report made by Kumburu *et al.* [6] and Vicar *et al.* [12], who had Gram negative isolates showing significant resistance to the

penicillin and the third generation cephalosporins. Among the Gram-negative species, *S. plymutheca* showed resistance to all the test antibiotics (Table 3). The resistance trends of these antibiotics observed could be because they are employed as first-line treatments for wound infections [6, 12, 13]. Self-medication and a lack of appropriate sanitation, may all contribute to the spread of antibiotic-resistant bacteria in the environment. A greater proportion (56.3%) of participants used orthodox drugs for treating their wound infections and this may account for the higher resistance patterns of the isolates.

Multidrug resistance was observed in 89.5% (n=17/19) of the enterobacteria isolates obtained in this study. Because these bacteria are implicated in the majority of community-acquired diseases, their prevalence in clinical samples is a major public health concern. Multiple drug resistance in the bacteria isolates could be as a result of the frequency of antibiotic abuse in the communities and lack of appropriate microbiological diagnostic input in clinical care in those communities [34].

Conclusion

The study has revealed a wide range of bacteria, with an alarming rate of resistance to the commonly used antimicrobial agents. About 90% of the bacteria isolated were multidrug resistant. The study suggests the relatively high resistance to be associated with community acquired infections. MRSA and the beta-lactam antibiotics, such as the third-generation cephalosporin, were the most predominant. Good hygiene, the use of appropriate disinfectants, monitoring of antibiotic administration and prescription in hospitals and drug stores must be ensured. Additionally, regular surveillance in the endemic area will help control wound infection and the emergence of multi-drug resistant pathogens.

327 **List of abbreviations**

328 AMK Amikacin

329 AMP Ampicillin

330 API analytical profile index

331 AST Antibiotic susceptibility tests

332 ATCC American Type Culture Collection

333 ATM Aztreonam

334 CHL Chloramphenicol

335 CIP Ciprofloxacin

336 CLSI Clinical and Laboratory Standards Institute

337 CRO Ceftriaxone

338 CTX Cefotaxime

339 CL Cutaneous Leishmaniasis

340 ERY Erythromycin

341 FOX Cefoxitin

342 GEN Gentamicin

343 GSS Ghana Statistical Service

344 MSA Mannitol Salt agar

345 MALDI-ToF Matrix-assisted laser desorption/ionization time of flight

346 MEM Meropenem

347 PCR Polymerase chain reaction

348 PHC Population and Housing Census

349 SXT Trimethoprim/sulfamethoxazole

350 TET Tetracycline

351 TSA Trypticase soya agar
352 TZP Piperacillin/Tazobactam
353 WHO World Health Organization
354

355 **Declarations**

356 **Ethical approval and consent for publication**

357 Ethical approval for the study was obtained from the Committee on Human Research
358 Publications and Ethics (CHRPE/AP/572/19), Kwame Nkrumah University of Science and
359 Technology (KNUST), Kumasi. In addition, all participants gave a written consent.

360 **Availability of data and materials**

361 All data from the research are available in manuscript

362 **Competing interests**

363 The authors declare no competing interests

364 **Authors' contributions**

365 CY carried out the laboratory exercises, data analysis and drafted the manuscript. VEB was
366 involved in the conception, design, and coordination of the studies. HO, YDB and GKN
367 participated in the data analysis and editing of the manuscript. RON participated in
368 sampling, laboratory exercises and data analysis. CA participated in the conception,
369 experimental design and analysis of data. KB participated in sampling, data analysis and
370 editing of the manuscript. All the authors reviewed the manuscript.

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References

1. Sharma U. and Singh S. . Insect vectors of *Leishmania*: Distribution, physiology and their control. *Journal of Vector Borne Diseases*. 2008; 45(4):255–272. PMID: 19248652
2. Alvar J, Velez I, Bern C, Herrero M, Desjeux P. and Cano J. et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*. 2012; 7:35–671. Doi.org/10.1371/journal.pone. 0035671 PMID: 22693548
3. Antonio LDF, Lyra MR, Saheki MN, Schubach ADO, Freitas L De, Miranda C, Madeira MDF, Cristina M, Fagundes A, Aparecida É, Barreto L, Inês M. and Pimentel, F. Effect of secondary infection on epithelialisation and total healing of cutaneous leishmaniasis lesions. *National Library of Medicine*. 2017; 112(9):640–646. Doi: 10.1590/0074-02760160557. PMID: 28902290.
4. Siddiqui A. and Bernstein J. Chronic wound infection: facts and controversies. *Journal of Clinical Dermatology*. 2010; 28(5):519–26. DOI: 10.1016/j.clindermatol.2010.03.009.
5. Leopold SJ, Leth FV, Tarekegn HA. and Schultsz C. ‘Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review,’. *Journal of Antimicrobial Chemotherapy*. 2014; 69(9):2337–2353. Doi: 10.1093/jac/dku176. PMID: 24879668

- 398 6. Kumburu HH, Sonda T, Mmbaga BT, Alifrangis M, Lund O, Kibiki GA. and Aarestrup FM.
399 'Patterns of infections, aetiological agents and antimicrobial resistance at a tertiary care
400 hospital in northern Tanzania,'. *Tropical Medicine & International Health*. 2017;
401 22(4):454–464. Doi: 10.1111/tmi.12836
- 402 7. Hassan S, Reynolds G, Clarkson JA. and Brooks P. 'Challenging the dogma: relationship
403 between time to healing and formation of hypertrophic scars after burn injuries,'.
404 *Journal of Burn Care & Research*. 2014; 35(2):e118–e124.
405 DOI: 10.1097/BCR.0b013e31829b330a. PMID: 23877141
- 406 8. WHO. Antimicrobial resistance: global report on surveillance. *World Health*
407 *Organisation*. 2014b.
- 408 9. Murray C, Vos T. and Lozano R. et al. Disability-adjusted life years (DALYs) for 291
409 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global
410 Burden of Disease Study 2010. *Lancet*. 2012; 380:2197–223. Doi: 10.1016/S0140-
411 6736(12)61689-4. PMID: 23245608
- 412 10. Laxminarayan R, Matsoso P. and Pant S. et al. Access to effective antimicrobials: a
413 worldwide challenge. *Lancet*. 2016; 387:168–75. DOI: 10.1016/S0140-6736(15)00474-
414 2. PMID: 26603918
- 415 11. Lai SP, Bebell ML, Meney C, Valeri L. and White CM. Epidemiology of antibiotic-resistant
416 wound infections from six countries in Africa. *Biomedical Journal of Global Health*. 2018;
417 (3):1–7. Doi: 10.1136/bmjgh-2017-000475. PMID: 29588863
- 418 12. Vicar EK, Acquah SK, Williams W, Kuugbee ED, Saba CKS. and Mensah GI. Antibiotic
419 Resistant Bacteria Infecting Wounds of Rural Community Dwellers in Northern Ghana.
420 *European Journal of Medical and Health Sciences*. 2021; 3(1):112–117. Doi:
421 10.24018/ejmed.2021.3.1.678

13. Newman MJ, Frimpong E, Donkor ES, Opintan AJ. and Adu-Asamoah A. Resistance to antimicrobial drugs in Ghana. *Journal of Infections and Drug Resistance*. 2011; 215–220. Doi: 10.2147/IDR.S2176. PMID: 22259250
14. Emhj G. EMHJ Guidelines on Ethical Conduct and Publication of Health Research. *Eastern Mediterranean Health Journal*. 2016; 22(1):72–76.
15. Kweku MA, Odoom S, Puplampu N, Desewu K, Nuako G K, Gyan B, Raczniak G, Kronmann K C, Koram K, Botero S, Boakye D. and Akuffo H. An outbreak of suspected cutaneous leishmaniasis in Ghana: lessons learnt and preparation for future outbreaks. *Global Health Action*. 2011; 4(1):5527:10. Doi: 10.3402/gha.v4i0.5527. PMID: 21765823
16. Policy Outcome I. For 2020-2023 Nkwanta South Municipal Assembly. 2020.
17. Saúde M. da. Manual de controle da leishmaniose tegu- mentar Americana. 2nd edition. Brasília;O Ministério. 2017; 189.
18. Owusu Ntim R. Transmission of Cutaneous Leishmaniasis and Bacterial co-infection of cutaneous lesions in the Nkwanta South District of Ghana. Master of Philosophy thesis, Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. 2021; 38-48.
19. Aransay AM, Scoulica E. and Tselentis Y. Detection and identification of Leishmania DNA within naturally infected sand flies by seminested PCR on minicircle kinetoplastic DNA. *Applied and Environmental Microbiology*. 2000; 66(5):1933–1938. Doi: 10.1128/aem.66.5.1933-1938.2000. PMID: 10788363
20. Cappuccino JG. and Sherman N. Microbiology: A Laboratory Manual. Pearson Education Inc. 10th Edition. 2005; (153–175).
21. CLSI. M100 Performance Standards for Antimicrobial. 2018
22. Fryauff DJ, Health N, Klena JD, Hoel DF. and Puplampu N. Short report : ITS-1 DNA

- sequence confirmation of *Leishmania major* as a cause of cutaneous leishmaniasis from an outbreak focus in the Ho District , southeastern Ghana. *American Journal of Tropical Medicine and Hygiene*. 2006; 75(3):1–4. Doi: 10.4269/ajtmh.2006.75.502
23. Ministry of Health. The Ghana health sector annual programme of work. 2004.
24. Kwakye-Nuako G, Mosore M, Duplessi, C, Bates MD, Puplampu N, Mensah-attipoe I, Desewu K, Afegbe G, Asmah RH, Jamjoom MB, Ayeh-kumi PF, Boakye DA. and Bates PA. First isolation of a new species of *Leishmania* responsible for human cutaneous leishmaniasis in Ghana and classification in the *Leishmania enriettii* complex. *International Journal for Parasitology*. 2015; 45(11):679–684. Doi: 10.1016/j.ijpara.2015.05.001
25. Gonçalves E, Reis Filho SA, Oliveira EG, Pareira ALN, Silva AR. and Costa JML. Infecção bacteriana na leishmaniose cutânea: padrão bacteriano e sensibilidade a antibióticos. *Journal of the Brazilian Society of Tropical Medicine*. 2009; 42(2):219–21.
26. WHO. Leishmaniasis in the WHO European region. 2014a; 8. Retrieved from <http://www.euro.who.int/en/media-centre/sections/fact-sheets/2014/03/fact-sheets-world-health-day-2014-vector-borne-diseases/fact-sheet-dengue-in-the-who-european-region>
27. Ntirenganya C, Manzi O, Muvunyi CM. and Ogbuagu O. ‘High prevalence of antimicrobial resistance among common bacterial isolates in a tertiary healthcare facility in Rwanda,’. *The American journal of tropical medicine and hygiene* 2015; 92(4):865–870. Doi:10.4269/ajtmh.14-0607
28. Nwankwo EA. and Edino S. ‘Seasonal variation and risk factors associated with surgical site infection rate in Kano, Nigeria,’. *Turkish journal of medical sciences*. 2014; 44(4):674–680. Doi: 10.3906/sag-1305-47. PMID: 25551941

29. Salgado VR, de Queiroz ATL, Sanabani SS, de Oliveira CI, Carvalho EM, Costa JML, Barral-Netto M. and Barral A. The microbiological signature of human cutaneous leishmaniasis lesions exhibits restricted bacterial diversity compared to healthy skin. *Memorias do Instituto Oswaldo Cruz*. 2016; 111(4):241–251. Doi: 10.1590/0074-02760150436
30. Doudi M, Setorki M. and Narimani M. Bacterial superinfection in zoonotic cutaneous leishmaniasis. *Medical Science Monitor*. 2012; 18(9):356–61. Doi: 10.12659/MSM.883345. PMID: 22936185
31. Sadeghia G, Ziaei H, Bidabani LS. and Baghbaderani AZ. Decreased effect of glucantime in cutaneous leishmaniasis complicated with secondary bacterial infection. *Indian Journal of Dermatology*. 2011; 56 (1):37–39. Doi: 10.4103/0019-5154.77549
32. Vera L A, Santos JB, Macêdo VO, Magalhães AV, Ciuffo IA. and Santos CG. Avaliação da influência da infecção bacteriana secundária na evolução da leishmaniose cutânea em Corte de Pedra, Bahia. Vyas KS, Wong LK. Detection of biofilm in wound. *Journal of the Brazilian Society of Tropical Medicine*. 2001; 34(3):233–7.
33. Saana BSMB, Adu F, Agyare C, Gbedema SY, Boamah VE. and George DF. Antibiotic resistance patterns of strains of *Staphylococcus aureus* isolated from patients in three hospitals in Kumasi , Ghana. *Journal of Bacteriology Research*. 2013; 5(3):35–40. Doi: 10.5897/JBR2012.0081
34. Duong A. and Jaelin M. 6 Factors that have caused antibiotic resistance. *Infection Control tips*. 2015; 1–2.

493 **Figure 1**

494 **Figure 1: Distribution of towns in the Nkwanta south district where wound swabs of**
495 **Cutaneous Leishmaniasis were obtained**

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Figure 3: Resistance patterns of Enterobacterales against the selected antibiotics.

TZP: Piperacillin/Tazobactam (36 µg), AMP: Ampicillin (10 µg), CTX: Cefotaxime (5 µg), CRO: Ceftriaxone (30 µg), MEM: Meropenem (10 µg), CIP: Ciprofloxacin (5 µg), AMK: Amikacin (30 µg), CHL: Chloramphenicol (30 µg), SXT: Trimethoprim/sulfamethoxazole (25 µg) and ATM: Aztreonam (30 µg).

Figure 4: Resistance patterns of *Staphylococcus aureus* isolates against the selected antibiotic.

AMP: Ampicillin (10 µg); FOX: Cefoxitin (30 µg), CIP: Ciprofloxacin (5 µg), ERY: Erythromycin (15 µg), TET: Tetracycline (30 µg), GEN: Gentamicin (10 µg) and SXT: Cotrimoxazole (25 µg).

Table 1: Socio-demographic characteristics of CL patients and mode of community prevention and treatment of CL wounds.

Total number of participants (N=33)		
	Male	Females
Communities		
Baasare	7 (21.1)	3 (9.1)
Asuogya	3 (9.1)	2 (6.1)
Pawa	0	5 (15.1)
Keri	1(3.0)	0
Ashiabre	5 (15.1)	0
Gekrong	3 (9.1)	2 (6.1)
Agoufie	2 (6.1)	0
Total	21(63.6)	12(36.3)
Age category		
<10	11 (33.3)	4 (12.1)
11-20	9 (27.3)	5 (15.2)
21-30	0	1 (3.0)
30-45	1 (3.0)	2 (6.1)
Mode of treatment		
Orthodox	17 (51.5)	8 (24.2)
Herbal	2 (6.1)	2 (6.1)
None	2 (6.1)	2 (6.1)
Regular Use of ITN		
Yes	17 (51.5)	3 (9.1)
No	9 (27.3)	4 (12.1)

n=number of persons with cutaneous leishmaniasis lesions, N=Total number of participants,

ITN=Insecticide treated nets

Table 2: Distribution of bacterial co-infections in cutaneous leishmaniasis wounds from the various communities in the Nkwanta South District

Communities	SA	EC	P	KP	PR	AM	BS	CB	SL	SP	SM	Total
Baasare	3		1				3			1		8
Asuogya	3		1									4
Ashiabre	1	1	1	1			2					6
Keri	1		1				1					3
Pawa	2		1	1	1							5
Gekrong	1	1					2	1	1		1	7
Agoufie	2	1		1		2	2				1	9
Total	13	3	5	3	1	2	10	1	1	1	2	42

SA; *Staphylococcus aureus*, EC; *Enterobacter cloacae*, P; *Pantoea* spp, PR; *Providencia rettgeri*, KP; *Klebsiella pneumoniae*, AM; *Aeromonas* spp, BS; *Bacillus subtilis*, CB; *Cronobacter* spp, SL; *Serratia liquefacien*, SP; *Serratia plymutheca*, SM; *Serratia marscecens*,

556 **Table 3: Antibiotic susceptibility profiles of bacterial isolates co-infecting cutaneous leishmaniasis lesions from endemic communities in the**
557 **Nkwanta South District, Oti region, Ghana.**

Test antibiotics (ug/ml)		TZP		AMP		CTX		CRO		FOX		CIP		AMK		CHL		SXT		ATM		MEM		ERY		GEN		TET	
Isolate	Town	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>Aeromonas spp</i> (n=2)	Agoufie	*	*	*	*	*	*	*	*	*	*	2	0	*	*	*	*	2	0	2	0	*	*	*	*	*	*	*	*
<i>Cronobacter spp</i> (n=1)	Gekrong	0	0	0	1	0	1	0	1	*	*	0	1	1	0	1	0	0	1	0	1	0	1	*	*	*	*	*	*
<i>Providencia rettgeri</i> (n=1)	Pawa	1	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
<i>Serratia liquefacien</i> (n=1)	Gekrong	0	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
<i>Serratia plymutheca</i> (n=1)	Baasare	0	1	0	1	0	1	0	1	*	*	0	1	0	1	0	1	0	1	0	1	0	1	*	*	*	*	*	*
<i>Staphylococcus aureus-SA</i> (n=13)	Ashiabre (n=1)	*	*	0	1	*	*	*	*	0	1	0	1	*	*	*	*	1	0	*	*	*	*	0	1	1	0	0	1
	Agoufie (n=2)	*	*	1	1	*	*	*	*	1	1	1	1	*	*	*	*	2	0	*	*	*	*	1	0	2	0	2	0
	Gekrong (n=1)	*	*	0	1	*	*	*	*	0	1	0	1	*	*	*	*	1	0	*	*	*	*	0	0	1	0	1	0
	Basaare (n=3)	*	*	0	3	*	*	*	*	1	2	0	3	*	*	*	*	0	3	*	*	*	*	0	1	3	0	1	0
	Asuogya (n=3)	*	*	0	3	*	*	*	*	0	3	0	3	*	*	*	*	3	0	*	*	*	*	0	1	3	0	2	1
	Pawa (n=2)	*	*	0	2	*	*	*	*	0	2	0	2	*	*	*	*	2	0	*	*	*	*	0	0	2	0	2	0
	Keri (n=1)	*	*	0	1	*	*	*	*	0	1	0	1	*	*	*	*	1	0	*	*	*	*	0	0	1	0	0	0
Susceptibility profile of SA		*	*	1	12	*	*	*	*	2	11	1	12	*	*	*	*	10	3	*	*	*	*	1	3	13	0	8	2
<i>Pantoea spp -P</i> (n=5)	Ashiabre (n=1)	1	0	0	1	0	1	0	1	*	*	0	1	0	1	0	1	0	1	0	1	0	1	*	*	*	*	*	*
	Basaare (n=1)	0	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
	Asuogya (n=1)	1	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
	Pawa (n=1)	0	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
	Keri (n=1)	1	0	0	1	0	1	0	1	*	*	0	1	0	1	0	1	0	0	0	1	0	1	*	*	*	*	*	*
Susceptibility profile of P		3	0	0	5	0	5	0	1	*	*	0	5	0	5	3	2	3	0	0	5	0	5	*	*	*	*	*	*
<i>Klebsiella pneumoniae -KP</i> (n=3)	Ashiabre (n=1)	0	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
	Agoufie (n=1)	0	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
	Pawa (n=1)	0	1	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
Susceptibility profile of KP		0	1	0	3	0	3	0	3	*	*	*	3	0	3	3	0	3	0	0	3	0	3	*	*	*	*	*	*
<i>Enterobacter cloacae -EC</i> (n=3)	Ashiabre (n=1)	1	0	0	1	0	1	0	1	*	*	0	1	0	1	0	1	0	1	0	1	0	1	*	*	*	*	*	*
	Agoufie (n=1)	1	0	0	1	0	1	0	1	*	*	0	1	0	1	0	1	0	1	0	1	0	1	*	*	*	*	*	*
	Gekrong (n=1)	1	0	0	1	0	1	0	1	*	*	0	1	0	1	0	1	0	1	0	1	0	1	*	*	*	*	*	*
Susceptibility profile of EC		3	0		3	0	3	0	3	*	*	0	3	0	3	0	3	0	3	0	3	0	3	*	*	*	*	*	*
<i>Serratia marscecens -SM</i>	Agoufie (n=1)	0	0	0	1	1	0	0	1	*	*	1	0	0	0	1	0	0	1	1	0	0	1	*	*	*	*	*	*
	Gekrong (n=1)	0	0	0	1	0	1	0	1	*	*	0	1	0	1	1	0	1	0	0	1	0	1	*	*	*	*	*	*
Susceptibility profile of SM		0	0	0	1	1	1	0	2	*	*	1	1	0	1	2	0	1	1	1	1	0	2	*	*	*	*	*	*

558 **n: number of isolates, (*) Not Applicable, S; Susceptible, R; Resistant. SA; Staphylococcus aureus, P; Pantoea spp, KP; Klebsiella pneumoniae, EC; Enterobacter cloacae, SM;**

559 *Serratia marscecens*; TZP: Peperacillin/Tazobactam (36 µg), AMP: Ampicillin (10 µg), CTX: Cefotaxime (30 µg), CRO: Ceftriaxone (30 µg), FOX: Cefoxitin (30 µg), CIP:

560 Ciprofloxacin (5 µg), AMK: Amikacin (30 µg), CHL: Chloramphenicol (30 µg), SXT: Cotrimoxazole (25 µg), ATM: Aztreonam (30 µg), MEM: Meropenem (10 µg), ERY:

561 Erythromycin (15 µg), GEN: Gentamicin (10 µg), TET: Tetracycline (30 µg), FEP: Cefepime (30 µg) and CAZ: Ceftazidime (30 µg).