Diversity And Antibiotics of Secondary Bacterial Isolates From Cutaneous Leishmaniasis Wounds

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Diversity and Antiograms 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 liquefacien (2.4%), Serratia plymutheca (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. marcescens*, *S. liquefaciens* 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
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),
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 km$^2$, 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 whiles 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 Quiagen protocol (cat. Nos. 69504 and 9506). The primers LINR4 (forward) (5-GGG GTT 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 µL including, 2µm of primers, 2µm of MgCl₂, 20µm 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 \textit{S. aureus} and \textit{B. subtilis} as well as Gram-negatives comprising of \textit{Pantoea spp}, \textit{P. rettgeri}, \textit{Aeromonas spp}, \textit{Cronobacter spp}, \textit{E. cloacae}, \textit{K. pneumoniea}, \textit{S. marscecens}, \textit{S. liquefacien} and \textit{S. plymutheca} (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, \textit{S. aureus} was the most commonly isolated species [12, 29, 30]. Most of the species isolated from CL wounds in this study, such as \textit{Pantoea spp}, \textit{P. rettgeri}, \textit{Aeromonas spp}, \textit{Cronobacter spp} and \textit{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. plymuthica* 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.
List of abbreviations

AMK  Amikacin
AMP  Ampicillin
API  analytical profile index
AST  Antibiotic susceptibility tests
ATCC  American Type Culture Collection
ATM  Aztreonam
CHL  Chloramphenicol
CIP  Ciprofloxacin
CLSI  Clinical and Laboratory Standards Institute
CRO  Ceftriaxone
CTX  Cefotaxime
CL  Cutaneous Leishmaniasis
ERY  Erythromycin
FOX  Cefoxitin
GEN  Gentamicin
GSS  Ghana Statistical Service
MSA  Mannitol Salt agar
MALDI-ToF  Matrix-assisted laser desorption/ionization time of flight
MEM  Meropenem
PCR  Polymerase chin reaction
PHC  Population and Housing Census
SXT  Trimethoprim/sulfamethoxazole
TET  Tetracycline
TSA  Trypticase soya agar

TZP  Piperacillin/Tazobactam

WHO  World Health Organization

Declarations

Ethical approval and consent for publication
Ethical approval for the study 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.

Availability of data and materials
All data from the research are available in manuscript

Competing interests
The authors declare no competing interests

Authors’ contributions
CY carried out the laboratory exercises, data analysis and drafted the manuscript. VEB was involved in the conception, design, and coordination of the studies. HO, YDB and GKN participated in the data analysis and editing of the manuscript. RON participated in sampling, laboratory exercises and data analysis. CA participated in the conception, experimental design and analysis of data. KB participated in sampling, data analysis and editing of the manuscript. All the authors reviewed the manuscript.

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Figure 1: Distribution of towns in the Nkwanta south district where wound swabs of Cutaneous Leishmaniasis were obtained
Figure 2. Examples of skin ulcers which tested positive for Cutaneous Leishmaniasis and Kirby Bauer disk diffusion susceptibility assay on bacterial isolates. A. Location: Left lower and upper leg (dimension: 17.0 mm by 13.1 mm) and B. Location: Left lower leg (dimension: 19.0 mm by 16.3 mm.)
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.

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

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

<table>
<thead>
<tr>
<th>Communities</th>
<th>SA</th>
<th>EC</th>
<th>P</th>
<th>KP</th>
<th>PR</th>
<th>AM</th>
<th>BS</th>
<th>CB</th>
<th>SL</th>
<th>SP</th>
<th>SM</th>
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</table>

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 plymetheca*, SM; *Serratia marscecens*,
### Table 3: Antibiotic susceptibility profiles of bacterial isolates co-infecting cutaneous leishmaniasis lesions from endemic communities in the Nkwanta South District, Oti region, Ghana.

<table>
<thead>
<tr>
<th>Test antibiotics (µg/ml)</th>
<th>TGP</th>
<th>AMP</th>
<th>CTX</th>
<th>CRO</th>
<th>FOX</th>
<th>CIP</th>
<th>AMK</th>
<th>CHL</th>
<th>SXT</th>
<th>ATM</th>
<th>MEM</th>
<th>ERY</th>
<th>GEN</th>
<th>TET</th>
</tr>
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<tbody>
<tr>
<td><strong>Isolate</strong></td>
<td><strong>Town</strong></td>
<td><strong>S</strong></td>
<td><strong>R</strong></td>
<td><strong>S</strong></td>
<td><strong>R</strong></td>
<td><strong>S</strong></td>
<td><strong>R</strong></td>
<td><strong>S</strong></td>
<td><strong>R</strong></td>
<td><strong>S</strong></td>
<td><strong>R</strong></td>
<td><strong>S</strong></td>
<td><strong>R</strong></td>
<td><strong>S</strong></td>
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<td>Aeromonas spp (n=2)</td>
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<td>*</td>
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<td>Keri (n=1)</td>
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<td>*</td>
<td>*</td>
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<td><strong>Susceptibility profile of SA</strong></td>
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<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

| Pantoena spp-P (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 | 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=3) | 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 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 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 | 1 | 0 | 0 | 0 | 1 | 0 | * | * | 0 | 0 | 5 | 0 | 5 | 3 | 2 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Klebsiella pneumoniae-KP (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 | * | * | * | * | * |
| Agouifie (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 | 0 | 3 | 0 | 3 | 0 | 3 | 0 | 3 | 0 | 3 | 0 | 3 | 0 |

| Enterobacter cloacae-EC (n=3) | Ashiabre (n=1) | 1 | 0 | 0 | 1 | 0 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| Agouifie (n=1) | 1 | 0 | 0 | 1 | 0 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| Gekron (n=1) | 1 | 0 | 0 | 1 | 0 | 1 | 0 | * | * | 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 | 0 | 3 | 0 | 3 |

| Serratia marcescens-EC | Agouifie (n=1) | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | * | * | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| Gekron (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 SMC** | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 2 | * | * | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

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