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Antibiotic Resistant Bacteria Infecting Wounds of Rural Community Dwellers in Northern Ghana

Ezekiel K. Vicar, Samuel E. K. Acquah, Walana Williams, Eugene D. Kuugbee, Courage K. S. Saba and Gloria Ivy Mensah

ABSTRACT

To determine the prevalence, etiology and antibiotic susceptibility profile of bacterial agents of wound infection in rural community dwellers in the Northern region of Ghana. From August 2017 to July 2018, patients who reported with infected wound to four (4) primary health facilities were recruited after obtaining written informed consent. Wound swabs were taken from 93 participants: 59 (63.4%) males and 34 (36.6%) females. Wounds were aseptically swabbed and cultured. Antibiotic susceptibility tests (AST) were performed on all isolates using agar disc diffusion method according to Clinical and Laboratory Standards Institute CLSI 2013 guidelines. A total of 165 bacteria isolates were obtained from 93 wound swabs. The most predominant bacteria species were *Staphylococcus aureus* 38 (23.0%) followed by *Pseudomonas aeruginosa* 27 (19.7%), and *Klebsiella pneumoniae* 15(9.1%). Many of the isolates were from burns 61 (37.0%) and diabetic wounds 33 (20.0%), with few from motor traffic wounds 5 (3.0%). Most of the isolates were resistant to third generation cephalosporins. Notably, all (100%) of the *Acinetobacter* and *Providencia* species and 75% of Proteus species were resistant to ceftazidime and ceftriaxone. High resistance to ceftazidime and ceftriaxone was also observed in *K. pneumoniae* (53.3% and 53.3%) and *E. coli* (60.0% and 50.0%) respectively. Resistance in *Streptococcus pyogenes* to penicillin and erythromycin was 60% and 70% respectively while 10.5 % of the *Staphylococcus aureus* isolates were methicillin resistant (MSRA). This study revealed a wide range of bacterial agents are associated with wound infection and are resistant to commonly used antibiotics. Additionally, the study suggests relatively high antibiotic resistance is associated with community acquired infection of wounds.

Keywords: Antibiotic resistance, rural community dwellers, Northern Ghana, wounds

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Ezekiel Kofi Vicar
Department of Clinical Microbiology, University for Development Studies, Tamale, Northern Region, Ghana.
(e-mail: kvicar@yahoo.com)
Samuel E. K. Acquah
Department of Clinical Microbiology, University for Development Studies, Tamale, Northern Region, Ghana.
(e-mail: kvicar@yahoo.com)
Walana Williams
Department of Clinical Microbiology, University for Development Studies, Tamale, Northern Region, Ghana.
(e-mail: kvicar@yahoo.com)
Eugene D. Kuugbee
Department of Clinical Microbiology, University for Development Studies, Tamale, Northern Region, Ghana.
(e-mail: kvicar@yahoo.com)
Courage K. S. Saba
Department of Biotechnology, University for Development Studies, Tamale, Northern Region, Ghana.
(e-mail: kvicar@yahoo.com)
Gloria Ivy Mensah*
Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Greater Accra Region, Ghana

****Corresponding Author***

I. Introduction

Antibiotic resistance (AMR) and its attendant effects are of great public health concern especially in resource limited countries. A critical tool for AMR surveillance is the isolation and correct identification of bacterial agents from clinical specimens [1]. Among the varied clinical specimen received in many laboratories for bacteria isolation and antibiotic susceptibility testing (AST), wound swabs offer a unique insight of AMR in communities as wounds are usually infected by normal flora of the skin, bacteria from other parts of the body and the environment [1], [2]. Globally, wound infections are an emerging medical problem and the rising economic burden exerted by wound infection and resulting mortality rates cannot be over emphasized [1], [2].

Wounds may be colonized by several potentially pathogenic bacteria, hence easily becomes infected if proper care is not given. This polymicrobial infection, complicates bacteriologic investigations and as a result is often neglected in resource limited settings [3] where health care facilities lack very basic laboratory equipment for bacteria culture and identification. Healing takes much longer when a wound is infected and this increases treatment cost and patient suffering from the associated pain and discomfort [1], [4], [5]. This also takes a toll on the caregivers as wound management becomes more resource demanding and patience are normally stigmatized due to the stench that come with them. The occurrence and load of microorganisms is of primary importance in delaying the healing process [6]-[8]. The

situation is further worsened when these microorganisms do not respond to antibiotic treatment.

Many rural communities in Ghana are characterized by low living standards and lack of accessible healthcare. Where healthcare facilities are available at the community level, they are often not equipped with microbiology laboratory to enable them conduct bacteriological analysis of clinical samples including antibiotic susceptibility tests (AST). The low-income status, high cost of treatment and stigmatization leave many rural dwellers with no choice than to seek alternative health care from local herbalists or resort to home based traditional remedies. In most cases, wounds are later sent to the healthcare facilities in their worse state after it becomes obvious that these alternate remedies have failed.

This study identified the bacterial agents of wound infection and their antibiotic resistance profile to guide selection by health care practitioners in remote areas on choice of antibiotics for treatment of wounds.

II. Materials and Methods

A. Study Design and Site

A prospective study was conducted from August 2017 to July 2018 in four health centers namely Diare Health Center, Kpong-Tamale Health Center, Dalun Health Center and Wantungu Health Center in the Northern region of Ghana. These health centers serve the residents of the rural communities in their catchment area. All the four health centers do not conduct bacteriological analysis on samples. Even if one was provided residents may not be able to afford (GH¢55 or $10 per sample) due to their low economic status.

The study received authorization from each hospital administration (the local ethics review panel). The nature and importance of the entire study were explained to participants and those who agreed to participate, signed/thumb printed a consent form.

B. Sample Collection and Processing

Medical personnel diagnosed wounds based on their case definition. The wounds were carefully cleaned using sterile gauze moistened in sterile physiological saline. Each sample was collected by swabbing from the wound ground and edge using the Levine technique [9] and placed in Amies transport medium. These were immediately transported in an ice chest with ice packs to the University for Development Studies Microbiology laboratory for bacteriological analysis.

C. Bacteria Culture and Identification

The samples were inoculated onto Blood, Chocolate and MacConkey agar plates and incubated aerobically at 37ºC for 24 and 48 hours. Colony characteristic appearance and Gram stain reactions were used for presumptive identification of bacteria species. Specifically, Gram negative bacteria were identified using API 20E and API 20NE (bioMerieux). API-staph (bioMerieux). and API- Strep (bioMerieux) were used for identification of *Staphylococcus* and *Streptococcus* species respectively.

D. Antibiotic Susceptibility Testing

Antibiotic susceptibility tests (AST) were performed on all the isolates by agar disc diffusion method according to Clinical and Laboratory Standards Institute standards[10].

The bacteria isolates were tested for their susceptibility to Amoxicillin clavulanic acid (AMC: 30 µg), Ampicillin (AMP: 10 µg), Cefoxitin (CXT: 30 µg), Cefuroxime (CRX: 30 µg), ceftazidime (CAZ: 30 µg), Ceftriaxone (CTR: 30 µg), Chloramphenicol (CHL:30 µg), Ciprofloxacin (CIP: 5 µg), Erythromycin (ERY:15 µg), Gentamicin (GN: 10 µg), Nalidixic acid (NAL 30 µg), Nitrofurantoin (NIT: 50 µg), Penicillin G (PEN: 10 µg), Sulfamethoxazole-Trimethoprim (SXT: 23.75/11.25µg) and Vancomycin (VAN: 30µg). Cefoxitin was used as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus* as described by Fernandes et al. [11].

The results were classified as resistant (R), intermediate (I) and sensitive (S) according to the general guidelines prepared by [10]. *Escherichia coli* ATTC® 25922, *Klebsiella pneumoniae* ATTC® 13883, *Staphylococcus aureus* ATTC® 25923 and *Pseudomonas aeruginosa* ATTC® 27853, reference strains were used for quality controls.

E. Data Collation and Statistical Analysis

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

III. Results

Wound swabs were taken from 93 participants, majority of whom were male (n=59, 63.4%). Many (n=58, 62.3%) of the participants were engaged in farming or casual labour. Majority had either primary education (n=44, 47.3%) or had not received any formal education (n=30, 32.3%). Also, the majority (n=58,62.3%) whose age ranged from 20 years and 40 years had their wounds infected. Concerning the duration of wounds, 67.8% of the infected wounds were up to six-month-old and 16.1% were more than a year old (Table 1).

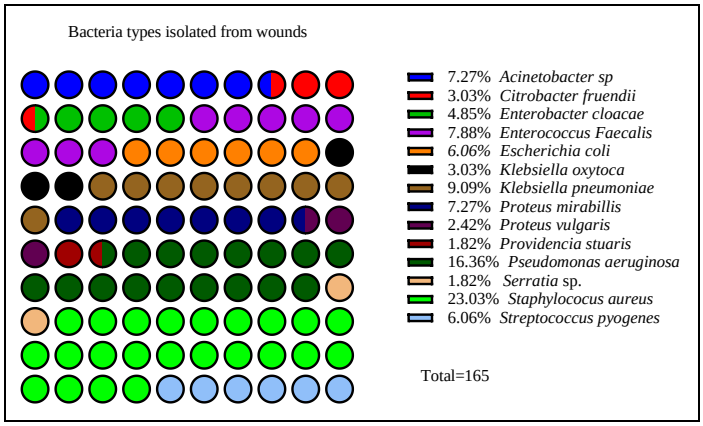

Fig. 1. Frequency distribution of bacterial etiologies isolated from would cultures.

A total of 165 bacteria pathogens comprising of 103-gram negatives and 62-gram positives were isolated from the various wound types.

In all, fourteen bacteria species were isolated from the infected wounds of the 93 participants. The most predominant species was *Staphylococcus aureus* 38 (23.0%) followed by *Pseudomonas aeruginosa* 27 (19.7%), *Klebsiella pneumoniae* 15 (9.1%), *Enterococcus faecalis* 13 (7.9%), *Proteus mirabillis* 12 (7.3%), *Acinetobacter* sp. 12 (7.3%), *Escherichia coli* 10 (6.1%), *Streptococcus pyogenes* 10 (6.1%), *Enterobacter cloacae* 8 (4.8%), *Citrobacter fruendii*

5 (3.0%), *Klebsiella oxytoca* 5 (3.0%), *Proteus vulgaris* 4 (2.4%), *Providencia stuaris* 3 (1.8%) and *Serratia* sp. 3 (1.8%). Out of the 93 specimens, monomicrobial infections was detected in 12 (12.9%) and polymicrobial infections 81 (87.1%). All Burns, cuts and diabetic wounds were characterized by polymicrobial infections. High numbers of the bacteria isolates were from burns 61 (37.0%) and diabetic wounds 33 (20.0%). The least number of bacteria isolates were from motor traffic wounds 5 (3.0%) (Table 2).

TABLE 1 : SOCIO-DEMOGRAPHIC AND WOUND CHARACTERISTICS OF PARTICIPANTS

Variable N=93	*n* (%)	
Gender		
Male	59	(63.4)
Female	34	(36.6)
Age category(years)		
<10	5	(5.4)
11-20	11	(11.8)
21-30	35	(37.6)
31-40	23	(24.7)
41-50	12	(12.9)
51-60	5	(5.4)
>60	2	(2.2)
Occupation		
Farming	35	(37.6)
Labourer	23	(24.7)
Trading	18	(19.4)
Mechanics	8	(8.6)
Employed	4	(4.3)
Others	5	(5.4)
Education		
Primary	44	(47.3)
Secondary	15	(16.1)
Tertiary	4	(4.3)
None	30	(32.3)
Duration of wound(month)		
< 1	25	(26.9)
2-6	38	(40.9)
7-12	15	(16.1)
13-18	8	(8.6)
19-24	4	(4.3)
>24	3	(3.2)
Type of wound		
Post-Surgical wounds	8	(8.6)
Bites	11	(11.8)
Burns	25	(26.9)
Cuts	10	(10.8)
Diabetic wound	18	(19.4)
Motor traffic wound	10	(10.8)
Others*	11	(11.8)

*Wound due to pressure sores and gunshot.

The isolates showed high levels of resistance to the antibiotic used for the AST. Ampicillin and Chloramphenicol resistance was high among all the gram-negative isolates as resistance ranged from 67% to 100%. Among the gram negatives, *Escherichia coli* isolates showed a relatively high resistance to all the antibiotics tested for compared to the other bacteria species; 100% to Sulfamethoxazole-trimethoprim, Chloramphenicol and nalidixic acid and 80% to Ampicillin and Cefuroxime respectively (Table 3). *Citrobacter fruendii* showed high levels of resistance to Ampicillin (100%), Cefuroxime (100%) and Chloramphenicol (80%) but were susceptible to Ceftazidime and Gentamicin with resistance of 0% and 20% respectively.

Most of the gram-negative bacteria were resistant to third generation cephalosporins. Most notable is 100% resistance showed by *Acinetobacte*r sp. and *Providencia* species to Ceftazidime and Ceftriaxone. 75% proteus vulgaris were also resistant Ceftazidime and Ceftriaxone. Resistance to Ceftazidime and Ceftriaxone was also observed in *K. pneumoniae* 53.3% and 53.3% and in *E. coli* 60.0% and 50.0% respectively (Table 3).

As shown in Table 4, *Staphylococcus aureus* showed 100% resistance to Penicillin, 78.9% to Erythromycin, 39.5% to Sulfamethoxazole-trimethoprim while 10.5% were Methicillin resistant. *Streptococcus pyogenes* showed 60% and 70% resistance to Penicillin, and Erythromycin respectively. All the *Enterococcus faecalis* were resistant to Erythromycin and Sulfamethoxazole-Trimethoprim but were susceptible to Chloramphenicol and Vancomycin.

IV. DISCUSSION

The wide variety of Gram positive and gram-negative bacteria isolated from the wounds emphasize the polymicrobial colonization of wounds. Similar reports were made in other developing countries like Tanzania [1], Nigeria [12] and Rwanda [13].

In this study, *S. aureus* and *P. aeruginosa* were the predominant bacteria isolated and these two pathogens have been frequently isolated from wounds in other studies [1], [3], [6], [13]-[15]. These two bacteria are mostly implicated in chronic wounds [6]. Similar observation was made as *S. aureus* and *P. aeruginosa* dominated in some chronic diabetic wounds and wounds from burns. While *S. aureus* produces leucocidins, catalase, coagulase and clumping-factor A, *P. aeruginosa* produce elastase. These are destructive virulent factors that interrupt healing [16], [17]. The wide range of bacteria and high levels of Gram-negative rods such as *P. aeruginosa*, *Proteus* sp. *Klebsiella* sp. and *E. coli* isolated from the wounds may suggests that the infection of the wounds may be community acquired. These predominant Gram-negative rods were also observed in other studies [18], [19]-[21]. Poor hygiene and cleaning of wounds may be the cause of the predominance of the gram negatives in the infected wounds.

Wounds resulting from burns cause severe trauma which constitute a major public health concern [22], [23]. In our study, wounds caused by burns were the most frequent and yielded more bacteria isolates similar to what has been reported in other studies [15], [24]. This could be because burns destroy the skins mechanical integrity and compromise its defense. It is known that after the skin burns, it is replaced with a protein rich layer that provides nutritional support for microbial growth [6], [22]-[24]

Studies in Tanzania [1], [21] and Turkey [24] have reported Acinetobacter sp. is one of the most prevalent pathogens found in burn wounds, however, we observed the contrary. Nonetheless, all the Acinetobacter sp. were resistant to ceftazidime and ceftriaxone, third generation cephalosporins. This observation was also made by Kumburu et al. in Tanzania [1] where 80% and 60 % resistance were recorded for ceftriaxone and ceftazidime respectively in wounds. Similar observation was made in other studies [24]-[26]. The high resistance to ceftazidime and ceftriaxone which was also observed in *K. pneumoniae* 53.3% and 53.3% and in *E. coli* 60.0% and 50.0% respectively cannot be ignored as these two microorganisms are implicated in many infectious diseases [1].

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TABLE 2: WOUND TYPE AND COMMONLY ISOLATED BACTERIAL SPECIES

Wound type	*S. aureus*		*P. aeruginosa*		*Klebsiella sp.*		*Proteus sp.*		*Acinetobacter sp.*		*E. coli*		*E. Faecalis*		*S. pyogenes*		*E. cloacae*		*Citrobacter fruendii*		*Providencia stuaris*		*Serratia sp.*	
	n (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)		*n* (%)	
Post-Surgical	4	(10.5)	1	(3.7)	4	(20.0)	1	(5.3)	1	(8.3)	0	(0.0)	2	(14.2)	1	(10.0)	0	(0.0)	0	(0.0)	2	(66.7)	1	(50.0)
Bites	3	(7.9)	2	(7.4)	2	(10.0)	0	(0.0)	0	(0.0)	1	(10.0)	1	(7.1)	2	(20.0)	1	(12.5)	0	(0.0)	0	(0.0)	0	(0.0)
Burns	15	(39.5)	8	(29.6)	6	(30.0)	10	(52.6)	8	(66.7)	3	(30.0)	4	(28.5)	4	(40.0)	2	(25.0)	1	(20.0)	0	(0.0)	0	(0.0)
Cuts	2	(5.3)	5	(18.5)	2	(10.0)	3	(15.8)	0	(0.0)	2	(20.0)	1	(7.1)	2	(20.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Diabetic wound	5	(13.2)	7	(25.9)	4	(20.0)	2	(10.5)	2	(16.7)	4	(40.0)	3	(21.4)	0	(0.0)	4	(50.0)	2	(40.0)	0	(0.0)	0	(0.0)
Motor traffic	3	(7.9)	2	(7.4)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Others*	6	(15.8)	2	(7.4)	2	10.0)	3	(15.8)	1	(8.3)	0	(0.0)	2	(14.2)	1	(10.0)	1	(12.5)	2	(40.0)	1	(33.3)	1	50.0)

TABLE 3: ANTIBIOTIC RESISTANCE PATTERN FOR GRAM NEGATIVE BACTERIA SPECIES

	AMP			CRX		SXT		NAL		CHL		CTR		CAZ		AMC		CIP		GEN	
Bacteria Isolates	N	R	(%)	R	(%)	R	(%)	R	(%)	R	(%)	R	(%)	R	(%)	R	(%)	R	(%)	R	(%)
P. aeruginosa	25	Nt		Nt		Nt		Nt		20	(80.0)	Nt		5	(20.0)	Nt		3	(12.0)	5	(20.0)
K. pneumoniae	15	15	(100.0)	10	(66.7)	11	(73.3)	8	(53.3)	6	(40.0)	8	(53.3)	8	(53.3)	10	(66.7)	7	(46.7)	6	(40.0)
P. mirabillis	12	9	(75.0)	9	(75.0)	8	(66.7)	12	(100.0)	12	(100.0)	8	(66.7)	8	(66.7)	2	(16.7)	6	(50.0)	5	(41.7)
Acinetobacter sp.	12	12	(100.0)	12	(100)	0	(0.0)	2	(100.0)	12	(100.0)	12	(100.0)	12	(100)	12	(100)	6	(50.0)	6	(50.0)
Escherichia coli	10	8	(80.0)	8	(80.0)	10	(100)	10	(100.0)	10	(100.0)	6	(60.0)	5	(50.0)	5	(50.0)	4	(40.0)	4	(40.0)
E. cloacae	8	8	(100.0)	7	(87.5)	7	(87.5)	5	(62.5)	8	(100.0)	7	(87.5)	7	(87.5)	8	(100)	3	(37.5)	3	(37.5)
C. fruendii	5	5	(100.0)	5	(100.0)	1	(20.0)	2	(40.0)	4	(80.0)	2	(40.0)	0	(0.0)	2	(40.0)	4	(80.0)	1	(20.0)
K. oxytoca	5	5	(100.0)	3	(60.0)	2	(40.0)	4	(80.0)	4	(80.0)	2	(40.0)	4	(80.0)	2	(40.0)	3	(60.0)	3	(60.0)
Proteus vulgaris	4	3	(75.0)	4	(100.0)	1	(25.0)	4	(100.0)	4	(100.0)	3	(75.0)	3	(75.0)	1	(25.0)	2	(50.0)	2	(50.0)
Providencia sp.	3	3	(100.0)	2	(66.7)	2	(66.7)	2	(66.7)	3	(100.0)	3	(100.0)	0	(0.0)	2	(66.7)	0	(0.0)	1	(33.3)
Serratia sp.	3	3	(100.0)	3	(100.0)	1	(33.3)	3	100.0)	2	(66.7)	1	(33.3)	1	(33.3)	1	(33.3)	3	(100.0)	2	(66.7)

AMC, Amoxicillin clavulanic acid; AMP, ampicillin; CRX, cefuroxime; CAZ, ceftazidime; CTR, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamycin; NAL, nalidixic acid; SXT, Sulfamethoxazole-trimethoprim., NIT, Nitrofurantoin. Nt= not tested.

TABLE 4: ANTIBIOTIC RESISTANCE PATTERN FOR GRAM POSITIVE BACTERIA SPECIES

Isolates		PEN		ERY		SXT		CTX		CHL		VA		NIT	
	N	R	%	R	%	R	%	R	%	R	%	R	%	R	(%)
Staphylococcus aureus	38	38	(100)	30	(78.9)	15	(39.5)	4	(10.5)	5	(13.2)	0	(0.0)	3	(7.8)
Enterococcus Faecalis	14	10	(71.4)	14	(100)	14	(100)	2	(14.2)	0	(0.0)	0	(0.0)	5	(35.7)
Streptococcus pyogenes	10	6	(60)	7	(70)	3	(30)	1	(10)	0	(0)	0	(0.0)	2	(5.2)

CHL, chloramphenicol; SXT, Sulfamethoxazole-trimethoprim. CXT, cefoxitin; ERY, erythromycin; PEN, penicillin G; VAN, vancomycin; NIT, Nitrofurantoin.

The 10.5 % methicillin resistant *S. aureus* (MRSA) detected in the study is surprising and a cause for concern given the rural setting. This prevalence would rather be expected from urban health centers where MRSA has been reported to be high [13], [27]. Urban areas have specialized healthcare facilities which receive many patients. This setting easily predisposes patients, staff, and care-givers to more MRSA colonization and infection compared to that experienced by those in the rural setting where there is little exposure to such health care systems [28]. MRSA infections could be ongoing in our rural areas undetected due to lack of well-equipped laboratories for detection. This dynamism could make it easier for MRSA to transfer resistance to susceptible ones. MRSA (10.5%) detected in this study may indicate a rise in the incidence of MRSA considering that in a previous study in Eikwe, a rural community in Ghana, no MRSA was detected [28]. Other studies outside Ghana have detected higher MRSA (19% -21.8%) [6], [24] in wounds, making MRSA a challenge in wound treatment.

In line with studies done in southern Ghana [28] and in other countries [1], [15], [24], there was high resistance of the isolated bacteria to third generation cephalosporins. This phenomenon must be of great public health concern as these bacteria are implicated in most community acquired infections. Self-medication, lack of proper disinfection and proper disposal of dressing material may contribute to the dissemination of these antibiotic resistant bacteria in the environment.

V. Conclusion

Our study reveals a wide range of bacterial agents associated with wound infections are resistant to the commonly used antibiotic agents. Additionally, the study suggests relatively high antibiotic resistance is associated with community acquired infectious wounds. Intensive prevention and control measures focusing on education of the community on the negative consequences of self-medication and the importance of seeking early treatment and /or management at the appropriate health facility must be implemented. This will help to control wound infection and the emergence of multi-drug resistant pathogens.

VI. Study Limitation

Due to limited funding, the study did not include detection of resistance genes. This would have enabled us identify mutations and provided information on the molecular epidemiology of wound infections in rural areas in Ghana.

VII. Acknowledgment

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VIII. Authors' Contributions

This work was carried out in collaboration with all authors. Conceptualization, GIM and EKV.; Methodology and investigation, EKV, SEKA, EDK and WW.; Formal analysis, EKV, GIM and CSKS.; Writing-Original Draft Preparation, Review & Editing, EKV, and GIM. All authors read and approved the publication of the final manuscript.

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Ezekiel Kofi Vicar is a Clinical microbiologist and lecturer at the Department of Clinical Microbiology at the University for Development Studies, Tamale, Ghana. His research interest spans infectious diseases, host and pathogen interactions and antibiotic resistance in human, animals, food, water and environmental isolates. His current research is focused on molecular basis for antibiotic resistance among enteropathogenic *E. coli* (EPEC), *Klebsiella pneumoniae, Staphylococcus aureus, Salmonella, Listeria and Shigella*.

Samuel E.K. Acquah is a Lecturer in the Department of Clinical Microbiology, School of Medical Sciences, University for Development Studies. His research interest is in pediatric bacteria and parasitic infections. His current research is in Molecular epidemiology of tuberculosis in Northern Ghana.

Dr. Williams Walana is a Lecturer in the Department of Clinical Microbiology, School of Medical Sciences, University for Development Studies. He has worked diligently as an individual and in collaboration with his peers and superiors in the areas of teaching and learning, research and community service. His research interests encompass but not limited to Infections, Immunity and Cancer (IIC). He is currently involved in teaching, research and student's supervision. He constantly seeking opportunities to partner senior scientists in the form of collaboration and mentorship.

Dr. Eugene D. Kuugbee is a Lecturer in the Department of Clinical Microbiology, School of Medical Sciences, University for Development Studies, Ghana. He holds a PhD in Biochemistry and Molecular Biology with research interest in Cancer immunobiology, infectious diseases, non-communicable diseases and Microbiome.

Dr. Courage K. S. Saba is a Senior Lecturer of Microbiology as well as the Acting Director of International Relations and Advancement of the University for Development Studies, Tamale, Ghana. He has conducted extensive research works with the one health approach by isolating, identifying and characterizing pathogenic microbes from foods, animals, humans and the environment and their resistance patterns to antibiotics in Ghana. He is currently on the roster of experts, Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (2018-2022).

Dr Gloria Ivy Mensah is a Research Fellow of the Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana and a Post-doctoral Fellow of Afrique One-ASPIRE, one of the Research Consortia under the DELTAS program of the African Academy of Sciences. She has a PhD in Medical Research-International Health from the Ludwig Maximillians University, Munich- Germany. As a one health research practitioner, she studies bacterial infections along the human, animal and environment interphase.