Multidrug Resistance in Gram-Negative Bacteria isolated in a Tertiary-Care Hospital in Northern Ghana from 2017-2019

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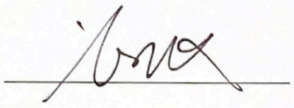
DECLARATION

This certifies that the Master's Thesis of

Osei Kennedy Mensah is approved.



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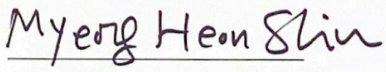

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DEDICATION

I dedicate this dissertation to the Almighty God for the gift of life and the opportunity to broaden my knowledge.

I also dedicate it to my Mother who has been a rock in my life and education and has never relented in her support of my aspirations.

Finally, i would like to dedicate my dissertation to the government and people of Korea for the warm reception during my study.

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LIST OF ABBREVIATIONS

AST	Antimicrobial Susceptibility Test
AMR	Antimicrobial resistance
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
ESBL	Extended-spectrum ßeta-lactamases
GIT	Gastrointestinal tract
MDR	Multidrug Resistance
MDR-GNB	Multidrug resistant gram-negative bacteria
UTI	Urinary tract infections
WHO	World Health Organization

ABSTRACT

Background: Multidrug resistance exist within a wide variety of clinically significant pathogens and poses a serious and growing global public health threat. This study aimed to determine the incidence, patterns, and trends of multidrug resistance of gram-negative bacterial isolates in clinical specimens cultured at the Tamale Teaching Hospital Laboratory.

Methods: This retrospective study analyzed gram-negative bacteria isolates and sensitivity test results of patients who visited the Tamale Teaching Hospital laboratory in Ghana, West Africa from 2017 to 2019

Results: A total of 2769 gram-negative bacteria isolates, and their phenotypic AST results were analyzed in this study. A total of 1297 gram-negative bacteria were isolated from urine samples representing 46.8% of all the isolates followed by isolates from sputum samples and wound swabs. *Escherichia coli* (23.8%) is the highest isolated gram-negative bacteria in all samples with, predominance from urine samples making up 33.2%. All gram-negative bacteria isolated from 2017-2019 showed significant multidrug resistance between 60% and 95.5%. *Klebsiella pneumonia* similarly showed increased multidrug resistance levels year on year; 2017(84%), 2018(89.5%) and 95.1% in 2019. *Pseudomonas aeruginosa* which showed relatively low multidrug resistance rates (65.8%) was, still determined to demonstrate increased resistance from 2017(59.5%) to 2019(78.7%). Gram-negative bacteria showed the highest susceptibility to antibiotics in the aminoglycoside group with amikacin the most effective. *Enterobacter* spp resistance to amikacin was as low as 16.2%, *Escherichia coli* (11.8%) and *Klebsiella pneumoniae* was determined to be 17.7%.

Conclusion: The study has shown high levels of multidrug-resistant gram-negative bacteria commonly isolated as the causative organisms in a range of infections. There is high resistance to penicillins, cephalosporins, and fluoroquinolones among major gram-negative pathogens. Aminoglycosides exhibited the least levels of resistance to isolated gram-negative bacteria in the Tamale Teaching Hospital.

Keywords: Gram-negative bacteria, multidrug resistance, Tamale Teaching Hospital

1 INTRODUCTION

1.1 GENERAL INTRODUCTION

Multidrug resistance (MDR) exist within a wide variety of clinically significant pathogens and poses a serious and growing global public health threat.[1]In the 2014 global surveillance report on Antimicrobial resistance, the WHO warns of an apocalyptic post-antimicrobial era, a possible reality in the 21st century. Antimicrobial resistance is a sgnificant threat to the prevention and treatment of the wide range of diseases caused infectious pathogens. Viruses, bacteria, parasites, and fungi are increasingly becoming resistant to the currently available antimicrobial previously effective at treating medical conditions due to diseases caused by these microorganisms.Currently , MDR is estimated to cause an estimated seven hundred thousand deaths globally.If the situation of the trend of resistance is allowed to persist MDR could result in the deaths of over 10 million people per year [2].

Infections caused by multidrug-resistant bacteria has been particularly challenging in developing countries and are mostly accompanied by increased hospital stay and high rates of morbidity and mortality [3]. The threat of antimicrobial resistance in low and middle is significantly exacerbated due to several practices pervasive in these countries. A study published in 2017 outlined the causes of antimicrobial resistance in developing countries to include; widespread unnecessary and overuse of antimicrobials due to lack of appropriate regulations in the sales and administration of antimicrobials, counterfeit and low-quality antimicrobials resulting in sub-inhibitory concentration of in vivo and high rate of self-

medication among others.[4]. Ironically countries in the WHO African region has a problem with unavailability of data on AMR[5].All the reasons ascribed to be causes of Antimicrobial Resistance in developing countries are prevalent in Ghana and studies into AMR have reveal the existence of increasing trends of resistance among several common pathogens.[6, 7].

Over the years the WHO has develop a list of priority of organisms which it deems as iminent global health threats to guide research and new antibiotic development. The categorization of these priority pathogens in three levels of severity, includes *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Enterobacteriaceae,* which all fall within the critical priority class of organisms. Other description highlights the threat of these pathogens to positive clinical outcomes. Several studies have espoused the clinical significance of gram-negative ESKAPE organisms like *Klebsiella pneumoniae* in hospital settings and Latin America and Asia[8, 9]. ESKAPE gram-negative bacteria are among some of the most common organisms implicated in health-care-associated infections. They are documented to be rapidly becoming resistant to commonly used broad spectrum antibiotics. Not only are these infections prone to negative health outcomes, they are contributors to adverse socio-economic impacts by putting significant burden on health care systems in low-and-middle-income countries[10]. Due to the high prevalence of multidrug resistance among ESKAPE bacteria, as defined by the Infectious Diseases Society of America such as *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa,* and *Enterobacter* spp., these pathogens

feature prominently in the global Priority Pathogen List (PPL) of antibiotic-resistant bacteria.

1.2 RESEARCH PURPOSE

Multidrug resistance is a major public health threat globally and there is an urgent need to reverse the current trend of increasing resistance to multiple drugs by organisms. MDR issues in low- and middle-income countries will require a coordinated effort from all stakeholders. In 2015, the World Health Organization published a global action plan. It implores sovereign countries to develop their individual action plans on combating antimicrobial resistance using the global action plan as a template. However, the absence of surveillance data on the extent of AMR in low-and-middle-income-countries has hampered public health experts to be able to formulate the required measures intended to counter the threat of AMR. There is a need to be abreast with the current prevalence and patterns of resistance among priority pathogens. This study seeks to provide insight into the prevalence and resistance patterns of gram-negative in the northern part of Ghana.

1.3 SPECIFIC OBJECTIVES

- To determine the incidence of multidrug resistance of gram-negative bacterial isolates in clinical specimen cultured at the Tamale Teaching Hospital Laboratory
- To determine the profile of antimicrobial resistance among gram-negative bacterial isolates in clinical specimen cultured at the Tamale Teaching Hospital Laboratory

- To determine the trend in Multidrug resistance among gram-negative bacteria from 2017 to 2019 in the Tamale Teaching Hospital.

2 LITERATURE REVIEW

2.1 BRIEF HISTORY OF THE DEVELOPMENT OF ANTIMICROBIAL AGENTS

Infectious diseases have accounted for a large proportion of all diseases over the years. By the latter part of the 19th century, it was discovered that the causative agent of infectious diseases were microorganisms. As a result, a curative agent aimed at microorganism was developed. The first antimicrobial agent in the world was salvarsan, a remedy for syphilis synthesized by Ehrlich in 1910. In 1935, sulfonamides were developed by Domagk and other researchers. These drugs were synthetic compounds and had limitations in terms of safety and efficacy. In1928, Fleming discovered penicillin. He found that the growth of Staphylococcus aureus was inhibited in a zone surrounding a contaminated blue mold (a fungus from the Penicillium genus) in culture dishes, leading to the finding that a microorganism would produce substances that could inhibit the growth of other microorganisms. The antibiotic was named penicillin, and it came into clinical use in the 1940s. Penicillin, which is an outstanding agent in terms of safety and efficacy, led in the era of antimicrobial chemotherapy.

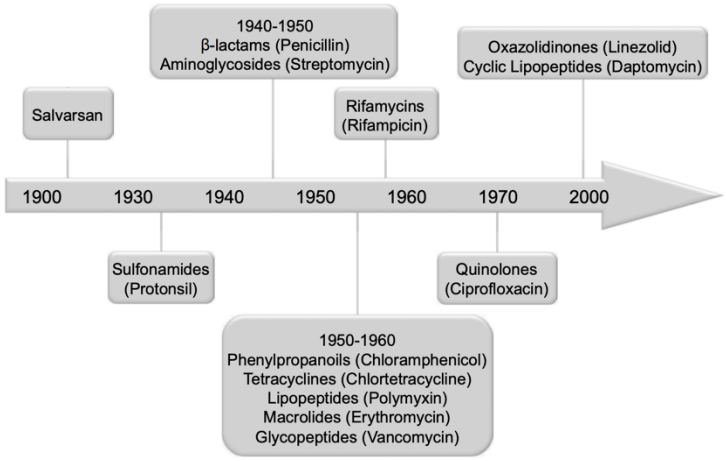

Figure 1. Timeline of antibiotic discovery[11]

2.2 CLASSIFICATION OF ANTIBACTERIAL DRUGS

Therapeutic antimicrobial agents could be of either natural or synthetic origin. They generally work by inhibiting or disrupting vital metabolic processes within the mostly bacterial cell, targeting structures or pathways sufficiently different or absent in mammalian cells.

Antibiotics can be grouped according to several criteria: inhibitory effect, the spectrum of activity, and molecular target. Some antimicrobial compounds are bactericidal at clinically used concentrations and capable of killing the infecting bacteria, whereas others are bacteriostatic, inhibiting the growth or reproduction of the bacterial cells[12]. Some drugs are regarded as having a broad spectrum of clinical activity and are used against a wide range of gram-negatives and gram-positives, in contrast others have a relatively narrow spectrum of clinical activity. Antibacterial drugs also differ in their bacterial targets and

mechanisms of action. Important targets for clinical antibacterial drugs include cell wall biosynthesis and membrane integrity, folic acid metabolism, protein synthesis, and DNA replication and transcription.

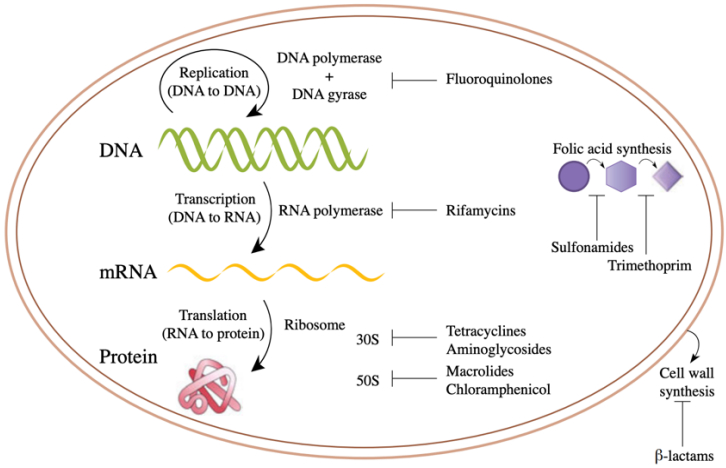

Figure 2. Antibiotic classification and mechansim[11]

2.3 GRAM-NEGATIVE BACTERIA

Gram-negative bacteria are distinct by virtue of their comparably thin peptidoglycan cell wall which does not retain the crystal violet stain used in Gram staining[13]. Gram-negative bacteria are ubiquitous. The gram-negative bacteria include the model organism *Escherichia coli*, and many pathogenic bacteria, such as *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Acinetobacter baumanii*. They are an important public health challenge. Their outer membrane protects them from many antibiotics (including penicillin), detergents that would normally damage the peptidoglycans of the (inner) cell

membrane, and lysozyme, an antimicrobial enzyme produced by animals that form part of the innate immune system. Gram-negative bacteria (GNB) differ from gram-positive bacteria with respect to the structure of the cell wall. This results in differences in the penetration and retention of chemical agents. Gram-negative bacteria have what is referred to as an envelope, consisting of three principal layers: the outer membrane, containing the lipopolysaccharide/endotoxin, the peptidoglycan cell wall with peptide chains, partially cross-linked, and the cytoplasmic or inner membrane.shock[14, 15]. Several antibiotics classes have been designed to target gram-negative bacteria, including aminopenicillins, ureidopenicillins, cephalosporins, ßeta-lactam-betalactamase inhibitor combinations (e.g., piperacillin-tazobactam).

2.4 MULTIDRUG RESISTANCE IN GRAM-NEGATIVE BACTERIA

The internationally accepted definition for multidrug resistance is the non-susceptibility of a bacteria isolate to at least one agent in three or more antimicrobial classes. Other categorizations are extensively drug-resistant (XDR) is defined as non-susceptibility to at least one agent in all, but two or fewer antimicrobial categories, and pan drug-resistant as non-susceptibility to all agents in all available antimicrobial categories [16]. Antibiotic resistance can be naturally occurring to specific bacteria, generally due to their structural and metabolic composition. Gram-negative Organisms are naturally resistant to Vancomycin., for example, is due primarily to the inability of the compound to permeate the cell wall of the organism. ßeta-lactamase production by *Klebsiella* species renders it

resistant to ampicillin. *Pseudomonas aeruginosa* is naturally insensitive, e.g., to sulphonamides, tetracycline, chloramphenicol, and trimethoprim[15].

Some bacteria develop resistance over time due to mutations in bacterial DNA, thereby rendering antibiotics ineffective, conveying a survival advantage to the mutated bacterial strain. This means that bacteria without these advantages die or cannot reproduce in the presence of antibiotic agents, while resistant bacteria can proliferate with less competition. Mutations in chromosomal genes can induce an increase in the expression of intrinsic resistance mechanisms (antibiotic-inactivating enzymes or efflux pumps)[17]. Some resistance is conferred on bacteria from other bacteria either of the same species or form other species or genus. The Mechanism used for the transfer of resistance genes from one bacterium to the other is conjugation, transduction, and transformation. Vectors carrying one or more resistance genes may be plasmids (resistance plasmid 1 is a typical example in GNB), transposons (e.g. Tn5053), or integrons e.g. (Verona integron-encoded metallo-ßeta-lactamase producing GNB)[15]. As for GNB, particulary for *Enterobacteriaceae*, there is evidence suggesting that resistance genes and associated insertion elements carried on plasmids are often found concentrated in large multi-resistance regions (MRR) [18]. For instance, ESBL- and carbapenemase-encoding plasmids may carry resistance determinants for other antimicrobial groups, including aminoglycosides and fluoroquinolones[17].

Recent studies have established the possibility of plasmid-mediated horizontal transmission of resistant genes e.g., ESBL-genes, from animals to humans through the food chain[19, 20].

2.4.1 Acinetobacter baumannii

Acinetobacter baumannii is an aerobic gram-negative bacterium and the major caused of healthcare-associated infections worldwide. It is one of the ESKAPE pathogen that threatens the fight against infectious disease control due to the high non-susceptibilty to may class of anitibiotics [21].*A. baumannii* rapidly develops resistance to antimicrobials by different mechanisms such as the inactivation of β-lactams by β-lactamases which is considered as a major MDR mechanism in *A. baumannii*.

Another resistance way is multidrug efflux pumps against many different classes of antibiotics classes, including tigecycline or imipenem resistance in *A. baumannii*. The resistance of *A. baumannii* to aminoglycoside is mediated by three classes of enzymes, including acetyltransferases, adenyltransferases, and phosphotransferases[22]. These enzymes chemically modify aminoglycosides. The coding genes can be transferred through plasmids, transposons, and integrons. Another is the alterating of target sites, such as penicillin-binding proteins (PBPs), mutations of DNA gyrase, and others, alter the target sites for antibiotics. Overexpression of certain PBPs results in imipenem resistance and mutation in DNA gyrase as in the cases of quinolone and tetracycline resistance in *A. baumannii*[23].

2.4.2 Enterobacteriaceae

Enterobacteriaceae is a large family of organisms such as *Escherichia coli*, *Klebsiella sp*., and *Enterobacter* spp. is the primay cause of urinary tract infections (UTIs), blood-stream infections, hospital, and healthcare-associated pneumonia. Resistance is mainly related to the production of ESBLs, but other mechanisms of resistance are also emerging, leading to multidrug-resistance (MDR) [24]. There is resistance of Enterobacteriaceae to third generation Cephalosporin-Resistant with the employment of mechanism like mutation of genes encoding TEM-1, TEM-2, or SHV-1 gives rise to new β-lactamases that can hydrolyze them. Enterobacteriaceae may also expresses other types of ESBLs like CTX-M that hydrolyzes cefotaxime more efficiently than ceftazidime and carbapenem hydrolyzing oxacillinases (OXA), which are mainly found in *P. aeruginosa* and rarely in *Enterobacteriaceae*. In addition to ESBLs, AmpC β-lactamases are also able to hydrolyze third-generation cephalosporins and are resistant to inhibition by clavulanate and other β-lactamase inhibitors [24]

2.4.3 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative aerobic bacterium commensally found in the intestinal flora. However, it is is also classified as an ESKAPE organism responsible for ICU-acquired infections in critically ill patients globally. Several mechanisms account for its antibiotic resistance: innate resistance of *P. aeruginosa* such as over-expression of efflux pumps and decreasing outer membrane permeability and acquired resistance mechanisms

like the acquisition of resistance genes or mutation in genes encode for porins and other proteins these all can make this microorganism challenging to treat.

2.5 EPIDEMIOLOGY OF MULTI-DRUG RESISTANT GRAM-NEGATIVE BACTERIA

The morbidity and mortality associated with gram-negative multidrug-resistant bacteria (GNB-MDR) are particularly concerning as new antimicrobial agents, with susceptibility ability to inhibit proliferation of these organisms have not been discovered and developed as fast as might have been required[25].Health-care-acquired infections continuously remain a global public health concern, particularly for critically ill inpatients and for patients requiring placement of invasive devices or surgical procedures. According to the WHO, carbapenem resistance in *Klebsiella pneumoniae* a last-resort treatment, has spread to all regions of the world. *Klebsiella pneumoniae* is a major cause of hospital-acquired infections such as pneumonia, bloodstream infections, and infections in newborns and intensive-care unit patients. In some countries, carbapenem antibiotics do not work in more than half of people treated for *Klebsiella pneumoniae* infections.

Resistance in *E. coli* to one of the most widely used medicines for treating of urinary tract infections (fluoroquinolone antibiotics) is pervasive. There are countries in many parts of the world where this treatment is now ineffective in more than half of patients. serious therapeutic dilemmas because of their complex resistance profiles[25, 26]

Studies conducted in other countries of the WHO African Region have shown a significant increase in the prevalence of MDR on the continent. A 12-month AMR survey conducted from October 2011 to September 2012 at a tertiary facility in Cape Town, South Africa, found that for healthcare-associated *Enterobacteriaceae* bloodstream isolates, susceptibility rates were 58.5%, ceftriaxone, 64.6%, gentamicin, and 70%, ciprofloxacin. The study also found that for healthcare-associated *Pseudomonas* and *Acinetobacter* strains; they showed less than 80% susceptibility to all antibiotics tested except colistin[21]. This finding was consistent with other studies done in Rwanda and Kwazulu-Natal. It was concluded that there is an urgent need for infection control intervention in these countries due to high prevalence rate of MDR among gram-negative bacteria. The study in Rwanda, which retrospectively reviewed AST results over a five year period found *Klebsiella spp*. be most susceptible to colistin (99.8%), imipenem (89.4%), and norfloxacin (69.8%); and least susceptible to piperacillin (18.2%), amoxicillin–clavulanate (24.6%), ceftriaxone (24.8%), and cotrimoxazole (28.4%)[27]. The same study also reported significant resistance among commonly used antibiotics in most gram-negative pathogens. Studies on multidrug resistance conducted in Ghana in a tertiary healthcare facility by Agyepong et al. also reported findings similar to what has been observed across the continent. The study found that isolates showed high resistance to ampicillin (94.4%), trimethoprim/sulfamethoxazole (84.5%), cefuroxime (79.0%) and cefotaxime (71.3%) but low resistance to ertapenem (1.5%), meropenem (3%) and amikacin (11%). It also determined the average multi-drug

resistance was 89.5% and ranged from 53.8% in *Enterobacter spp*. to 100.0% in *Acinetobacter spp.* and *P. aeruginosa*.[28].

3 MATERIALS AND METHODS

3.1 STUDY DESIGN

This retrospective study analyzed gram-negative bacteria isolates and sensitivity test results of patients who visited the Tamale Teaching Hospital laboratory in Ghana, West Africa, from 2017 to 2019.

3.2 STUDY SITE

This study was conducted with data from the Tamale Teaching Hospital Laboratory. The Tamale Teaching Hospital is the only tertiary health care and referral facility responsible for serving patients from Northern, Upper West, Upper East, Northern Volta, and parts of the Bono Ahafo Regions of Ghana.

Figure 3. Map of Depicting Northern Ghana and patient referral coverage area of Tamale Teaching Hospital. Adapted from Antwi et al.[29]

3.3 SAMPLE SIZE

The sample size included Antimicrobial Susceptibility Testing results of gram-negative bacteria isolates conducted in the Tamale Teaching Hospital Laboratory from January 2017 to December 2019. The list of WHO priority gram-negative bacteria include *Acinetobacter baumanii, Pseudomonas aeruginosa, Enterobacteriaceae [Klebsiella pneumoniae, Escherichia coli, Enterobacter spp., Serratia spp., Proteus spp., and Providencia spp., Morganella spp*.] which classified as critical priority according to WHO priority list[30]. High Priority gram-negative organisms include *Helicobacter pylori, Campylobacter, Salmonella* spp. and *Neisseria gonorrhoeae.* The final category of priority pathogens classified as medium priority has two gram-negative bacteria; *Haemophilus influenzae* and *Shigella spp*[30]. Information on diagnosis, sex, age, and ward type was obtained from patients' records from the laboratory sample registration system.

3.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST) AND DETERMINATION OF MULTIDRUG-RESISTANCE

The AST was done on each isolated gram-negative bacteria using the disk diffusion method.[31] Minimum inhibitory concentrations (MICs) of a panel of antibiotics were determined and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [32].

Multidrug resistance in this study was defined as isolates that are resistant to at least one agent in three or more antibiotic classes according to Magiorakos et al. [16]

3.5 STATISTICAL ANALYSIS

The antimicrobial susceptibility will be expressed as a percentage of the total same species isolates that were resistant to a particular antimicrobial. This will be calculated on an annual basis (calendar year- 2017,2018,2019), and trends will be assessed from year to year over the entire study period for each bacterial organism. Average annual susceptibility over the entire study period will be calculated to provide insight on overall antimicrobial susceptibility patterns.

3.6 ETHICAL CONSIDERATION

Ethical review was obtained from the Institutional Review Board (IRB) of Yonsei University (IRB No: Y-2020-0129)

4 RESULTS

4.1 AGE DISTRIBUTION AMONG GENDER AND SAMPLES CULTURED

The gender distribution of clinical samples cultured with the isolation gram-negative bacteria is almost equal proportions between males and females. Sample cultured from females amounted to 1406, constituting about 51% of the total cultures that isolated gram-negative bacteria, as shown in Table 4.1. A total of 1297 gram-negative bacteria were isolated from urine samples representing 46.8% of all the isolates followed by isolates, from sputum samples and wound swabs, as shown in Table 4.1. Most bloodstream gram-negative infections were realized in children below the age of 10, with a high of 52 gram-negative isolates making up 67% of all gram-negative bloodstream infections.

The Elderly above the age of 60 were found to be susceptible to respiratory infections accounting for the highest gram-negative isolates from sputum. Gram-negative isolates from urine samples were more common in individuals between the ages of 20 and 49.

Table 4.1 Age distribution among gender and samples cultured

	Age								
Characteristics	<10	10-19	20-29	30-39	40-49	50-59	60-69	≥ 70	TOTAL
Gender									
Female	162	88	413	301	161	82	75	106	1406
Male	159	79	123	159	132	132	168	411	1363
Total	321	167	536	460	293	214	243	517	2769
Clinical Samples									
Aspirates & Fluids*	6	7	12	17	9	5	6	4	66
Blood	52	4	4	5	5	4	3	2	79
Semen		1	1	11	3	1	1		18
Sputum	20	32	56	61	101	76	86	148	580
Stool	1		3	6	2				12
Swabs*	12	21	152	99	28	5	5	2	324
Tissue	2	2		1					5
Urine	195	56	213	183	84	99	113	354	1297
Wound	30	41	84	76	63	30	37	37	398
TOTAL	321	167	536	460	293	214	243	517	2769

*Abscess, Ascetic Fluid, Aspirates, Pleural effusions, CSF ** Vaginal swab, Endocervical swab, Anal swab, Urethral swab, Ear swab, Eye swab, Buccal swab, Throat swab

4.2 DISTRIBUTION OF GRAM-NEGATIVE BACTERIA FROM CLINICAL SAMPLES CULTURED FROM 2017 -2019

Escherichia coli (23.8%) is the highest isolated gram-negative bacteria in all samples with predominance from urine samples making up 33.2%. most gram-negative bacteria were isolated from urine samples. As shown in Table 4.2 bloodstream infections were dominated by *Enterobacter sp.* (22.0%) followed by *Klebsiella sp*. with (20.7%). Respiratory isolates were dominated by *Klebsiella sp.* (21%).

Table 4.2 Distribution of GNB from clinical samples cultured from 2017 -2019

	No. of isolates in clinical sample (%)					
Spieces	Blood	GIT	Respiratory	Urine	Wound	Total
A. baumannii	3(3.7)	1(1.7)	3(0.5)	6(0.4)		13(0.5)
Citrobacter spp	9(11.0)	2(3.3)	44(7.2)	214(13.3)	41(10.1)	310(11.1)
Enterobacter spp	18(22.0)	8(13.3)	94(15.3)	159(9.9)	39(9.6)	318(11.5)
E. coli	11(13.4)	15(25.0)	43(7.0)	534(33.2)	57(14.1)	660(23.8)
K. pnuemoniae	17(20.7)	15(25.0)	129(21.0)	388(24.1)	77(19.0)	626(22.6)
M. morganii	1(1.2)	1(1.7)	9(1.5)	45(2.8)	5(1.2)	61(2.2)
Proteus spp	1(1.2)	3(5.0)	20(3.3)	84(5.2)	66(16.3)	174(6.3)
P. rettgeri				12(0.8)	5(1.2)	17(0.6)
P. aeruginosa	16(19.5)	9(15.0)	87(14.2)	152(9.5)	111(27.4)	375(13.5)
Salmonella spp	2(2.4)	2(3.3)		1(0.1)		5(0.2)
Serratia spp	1(1.2)		1(0.2)	7(0.4)	3(0.7)	12(0.4)
Shigella spp	3(3.7)	4(6.7)		4(0.3)		11(0.4)
Total	82(100)	60(100)	615(100)	1607(100)	405(100)	2769(100)

Abbreviations: Gram-negative bacteria (GNB), Gastrointestinal Tract (GIT), Percentage of species isolated in particular clinical sample (%)

4.3 MDR AMONG ISOLATED GRAM-NEGATIVE BACTERIA

All gram-negative bacteria isolated from 2017-2019 showed significant multi-drug resistance between 60% and 95.5%. *Moraxella catarrhalis* showed the highest multi-drug resistance (95.5%) of all bacteria with 100 and above isolates. Priority gram-negative bacteria were all determined to have resistance to above 60% of drugs tested. *Acinetobacter baumanii* (83.3%), *Citrobacter sp*. (91.4%), *Enterobacter spp*. (84.2%), *Escherichia coli* (84.2%), and *Klebsiella pneumoniae* (88.8%) were all determined to have multidrug resistance above 80%. *Pseudomanas aeruginosa* isolated from 2017 to 2019 was determined to have an average multidrug resistance of 65.8% as shown in Table 4.3.

The data showed a progressive increment in the levels of resistance among gram-negative bacteria from 2017 to 2019. *Citrobacter* spp showed an increase in multidrug resistance from 89.1% in 2017 to 98% in 2019. *Klebsiella pneumoniae* similarly showed increased levels of multidrug resistance year on year; 2017(84%), 2018(89.5%) and 95.1% in 2019. *Pseudomanas aeruginosa* which showed relatively low levels of multidrug resistance (65.8%), was still determined to demonstrate increased resistance from 2017 (59.5%) to 2019 (78.7%).

Table 4.3 MDR among isolated Gram-negative Bacteria (2017-2019)

	2017		2018		2019		Total (2017-2019)	
Speices	n	MDR (%)	n	MDR (%)	n	MDR (%)	n	MDR (%)
A. baumanii	5	4(80.0)	3	3(100)	4	3(75.0)	12	10(83.3)
Citrobacter spp	124	108(89.1)	140	130(92.9)	51	50(98.0)	315	288(91.4)
Enterobacter spp	111	87(78.4)	128	112(87.5)	84	73(86.9)	323	272(84.2)
Escherichia coli	230	193(83.9)	285	261(91.6)	147	133(90.5)	662	587(88.7)
K. pneumoniae	181	152(84.0)	351	314(89.5)	101	96(95.1)	633	562(88.8)
M. morganii	11	9(81.8)	41	38(92.7)	8	8(100)	60	55(91.7)
Proteus spp	58	47(81.0)	89	80((89.9)	33	31(93.9)	180	158(87.8)
P. rettgeri	4	4(100)	9	9(100)	4	3(75.0)	17	16(94.1)
P. aeruginosa	153	91(59.5)	161	106(65.8)	75	59(78.7)	389	256(65.8)
Salmonella spp	2	1((50.0)	3	2(66.7)			5	3(60.0)
Serratia spp	2	1((50.0)	10	10(100)			12	11(91.7)
Shigella spp	4	4(100)	6	5(83.3)	1	1(100)	11	10(90.9)

number of species isolated in year (n), number of Multidrug resistant organisms isolated (MDR), percentage of multidrug resistant organisms (%)

4.4 RESISTANCE PROFILE OF GRAM-NEGATIVE BACTERIA (2017-2019)

Resistance to Ampicillin was highest among all *Enterobacteriales* tested. *Enterobacter* spp, *Escherichia coli* and *Klebsiella pneumoniae* were demonstrated to have a resistance of above 97% to ampicillin as shown in Table 4.4. Gram-negative bacteria showed the highest susceptibility to antibiotics in the aminoglycoside class, with amikacin the most effective. *Enterobacter* spp resistance to Amikacin was as low as 16.2%, *Escherichia coli* (11.8%) and *Klebsiella pneumoniae* were determined to be 17.7%, as shown in Table 4.4. *Acinetobacter baumannii* demonstrated the highest resistance to amikacin with a resistance of 42.8%. All major gram-negative bacteria were shown to have significant resistance against Tetracyclin, trimethoprim-sulfamethoxazole, and Nalidixic Acid, as shown in Table 4.4.

There is varied suceptibilty to fluoroquinolones among different gram-negative bacteria. *Acinetobacter baumanii* was determined to have a resistance of 28.6 % and 30.0% levofloxacin and ciprofloxacin, respectively. *Pseudomonas aeruginosa* was shown to be more resistant to levofloxacin (53.7%) as compare to ciprofloxacin with a resistance rate of 39.6% as shown in Table 4.4

Table 4.4 Resistance Profile of major gram-negative pathogens (2017-2019)

	No. of isolates (% of isolates resistant)				
Antibiotics	*A. baumannii*	*Enterobacter* spp.	*Escherichia coli*	*Klebsiella pneumoniae*	*P. aeruginosa*
AMK	7(42.8)	198(16.2)	383(11.8)	425(17.7)	242(19.4)
AMP	NT	156(98.7)	241(97.9)	272(97.4)	NT
CFP	NT	69(84.1)	186(77.4)	188(88.3)	NT
CTX	8(87.5)	196(76.0)	448(69.2)	369(77)	NT
CRO	9(55.5)	242(73.6)	477(68.6)	416(76.7)	NT
CXM	NT	120(89.2)	190(77.4)	206(84.4)	NT
CHL	NT	76(56.6)	136(50.)	181(67.4)	NT
SXT	5(40.0)	166(68.7)	275(89.5)	273(79.5)	NT
CIP	10(30.0)	287(42.5)	591(65.3)	555(54.1)	354(39.6)
GEN	12(33.3)	302(45)	644(31.7)	579(46.1)	355(42.5)
LVX	7(28.6)	185(45.4)	410(70.7)	415(55.5)	203(53.7)
NAL	NT	91(82.4)	262(90.1)	222(78.8)	NT
NIT	NT	117(88.0)	325(53.2)	269(90.7)	NT
TZP	1(100)	95(83.2)	263(72.2)	204(77.5)	88(77.3)
TET	8(87.5)	144(95.1)	309(96.8)	354(96.1)	NT

Abbreviations: Not tested (NT), amikacin (AMK), ampicillin (AMP), cefoperazone (CFP), cefotaxime (CTX), ceftriazone (CRO), chloramphenicol (CHL), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), gentamicin (GEN), levofloxacin (LVX), nalidixic acid (NAL), nitrofurantoin (NIT), Piperacillin-tazobactam (TZP), tetracycline (TET)

5 DISCUSSION

Epidemiological and laboratory surveillance of bacterial infection and multidrug resistance to antibiotics are essential for informing antimicrobial stewardship and implementation of infection prevention and control measures. This becomes more imperative in low-and-middle-income countries where the studies have shown the lack of laboratory diagnostic capacity, poor implementation of infection control measures, and a deficit in the awareness of infections caused by multidrug-resistant bacteria, which have resulted in high mortality and morbidity.[33, 34].

This study's main purpose was to determine the rate of multidrug resistance in gram-negative bacteria in a tertiary healthcare facility. The study observed a high prevalence of infections among children below the age of 10 and the elderly above 60. This distribution of bacterial is similar to the finding by Agypepong et al.[28]. The elderly and infants are usually more disposed to infections due to their incompetent immune status. The advancing age is commonly associated with risk factors including reduced immunity, co-morbidity with other diseases such as non-communicable diseases like diabetes, cardiovascular diseases, and renal disorders while in infants, lack of fully developed immunity, malnutrition and inadequate hygiene put them at greater risk of infections.[35-37]. This study observed a urinary tract infection (58.0%) to be the most common form of gram-negative bacteria infection with a high disease burden on Northern Ghana's population. Similar studies in Ghana and Sub saharan African region depicts similar findings. The finding of this study is comparable to other studies that determined similar trends in gram-

negative infection patterns.[38-40]. Studies in Korea also came to the finding implicating *Enterobacterales; Escherichia coli,* and *Klebsiella pneumoniae* as the major causative organism in urinary tract infections[41]. Other studies have associated the high levels of urinary tract infections, especially among females in middle age groups to be associated with sexual activity and poor personal hygiene[38]. *Escherichia coli* (23.8%) and *Klebsiella pneumoniae* (22.6%) were observed to be the most common gram-negative bacteria pathogen responsible for infections among patients at the health facility. Several similar studies conducted in other healthcare facilities in Ghana observed the same trend, with *Escherichia coli* and *Klebsiella pneumoniae* been the most predominant of the pathogens responsible for infections [28, 42]. *Klebsiella pneumoniae* and *Pseudomonas aureginosa* were determined to be the highest causes of blood stream infections accounting for 20.7% and 19.5% respectively of all bloodstream infections observed. The results agrees with a similar study done in Rwanda and Gabon which observed the major cause of bloodstream infections to be *Klebsiella pnuemoniae* [43, 44]. However, a multicenter hospital survellaince study conducted in Korea observed *Escherichia coli* (47.1%) was the major cause of bacteremia in community-acquired bloodstream infection followed by *Klebsiella pneumoniae* (12.6%) [45]. The major causative organisms for wound infection in this study was observed to be *Pseudomonas aureginosa* (27.4%), *Klebsiella pneumoniae* (19.0%) and *Proteus spp* (16.3%).This results is comparable to results found in a study of wound infection in Ghana where it was observed that the major gram-negative bacteria responsible for infection in burns patients was *Escherichia coli*, *Klebsiella pneumoniae* and

Pseudomonas aeruginosa [46]. The factors associated with wound infections in hospital setting has been suggested to be poorly implemented infection prevention and control, personal hygiene, and a lack of proper wound care. This study observed increase in the trend of the rate of resistance to all antibiotic groups among major gram-negative bacteria isolated in northern Ghana from 2017 to 2019.

This study determined that the resistance of gram-negative bacteria to commonly prescribed antimicrobial is extensive. This study indicated multidrug resistance rates of between 60.0% in *Salmonella typhi* to and 95.5% in *Moraxella catarrhalis.* Major clinically significant gram-negative bacteria like *Pseudomonas aureginosa* (65.8%), *Escherichia coli* (88.7%), *Acinetobacter baumannii* (83.3%), and *Klebsiella pneumoniae* (88.8%) as reported in Table 4.3. These results should be interpreted with the backdrop that the study site is a tertiary health care referral facility. That will suggest that majority of the samples received are from severly ill patients who may have received empirical antimicrobial therapy at different levels of the healthcare system. The other factors associated with the observed high levels of multidrug resistance is accounted for by widespread unnecessary and overuse of antimicrobials due to lack of appropriate regulations in the sales and administration of antimicrobials, counterfeit and low-quality antimicrobials resulting in a sub-inhibitory concentration of in vivo and the high rate of self-medication as stipulated in a 2017 study of the use of antimicrobials in low-and-middle-income countries[47]. This result is comparable to studies done in Ghana in a similar setting with the same definition for mutltidrug resistance [38, 42]. Agyepong et al. showed MDR rates in a tertiary healthcare

facility in Ghana to be 89.9% in *Escherichia coli* , 94.7% in *Klebsiella pnuemoniae* ,100% in *Acinetobacter baumanii* and 100 % in *Pseudomonas aeruginosa* [28] . However, the MDR rates in this study (65.8%) was found to be lower than what was found in the study by Agyepong et al. (100%).

The resistance profile of major gram-negative bacteria isolated displayed high-level multidrug resistance with high resistance to ampicillin; *Enterobacter spp* (98.7%), *Escherichia coli* (97.9%), and *Klebsiella pnuemoniae* (97.4%). The degree of resistance showed in the study was consistent with studies done in Ghana[28, 38, 42] and also agreed with other studies conducted in the sub-Sahara African countries such as Ethiopia, Zimbabwe and Rwanda [27, 48, 49]. Resistance to fluoroquinolones among major gram-negative was reported in this study to be range between 28.6% *Acinetobacter baumannii* resistance to levofloxacin to 65.3% *Escherichia coli* resistance to Ciprofloxacin. As shown in Table 4.4. Comparatively, major gram-negative pathogens exhibited the least resistance to aminoglycosides. Resistance to amikacin among major pathogens isolated were observed to be 42.8% in *Acinetobacter baumannii* and 19.4% in *Pseudomanas aeruginosa.* Enterobacteriacaea resistance to amikacin ranged from 11.8% in *Escherichia coli* to 17.7% in *Klebsiella pnuemoniae* as shown in Table 4. These findings were consistent with studies conducted in Ghana [28] and also comparable to studies in other parts of Africa[50, 51].

The observation of a increased resistance trend in the African sub-region is indicative of high antibiotic selection pressure mainly due to availability and easy accessibility of

counterfeit antimicrobials with suboptimal inhibitory concentrations, high levels of self-medication and the lack of antimicrobial stewardship programs in both communities and health care setting [47]. This study limitations includes the use of retrospective data which may have varying consistency. The study site used disk diffusion method which is qualitative in its interpretation of minimum inhibitory concentration using CLSI guidelines. The samples were cultured, and an antimicrobial susceptibility test performed in a referral tertiary healthcare facility, and as such the results should be interpreted in that context. Based on the observed high level of, and increasing trend of resistance among gram-negative pathogens, there is an urgent need for the institution of Antimicrobial Stewardship Programs in hospitals and the community. The is also a need for regulation and education on the appropriate use of antibiotics in the animal husbandry industry in Ghana.

6 CONCLUSIONS

The study has shown high levels of multidrug-resistant gram-negative bacteria commonly isolated as the causative organisms in a range of infections. The predominant gram-negative bacteria implicated in infections in the Tamale Teaching Hospital were *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. There is high resistance to penicillins, cepholosporins and fluoroquinolones among major gram-negative pathogens. Aminoglycosides exhibited the least levels of resistance to isolated gram-negative bacteria in the Tamale Teaching Hospital.

The conduction of nationwide surveilance of multidrug resistance is a resource intensitive endeavour and have proven to be difficult to undertake in low- and middle-income countries like Ghana. This study will be helpful in informing policy direction on infection prevention and control, and antimicrobial stewardship programmes.

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