DEPARTMENT OF MEDICAL MICROBIOLOGY

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA

CARRIAGE OF MULTI-DRUG RESISTANT ENTEROBACTERALES AND *ACINETOBACTER BAUMANNII* AMONG HOSPITALISED PAEDIATRIC PATIENTS AT THE CHILD HEALTH DEPARTMENT, KORLE-BU TEACHING HOSPITAL

BY

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DECLARATION

I, Nelson Hukporti, declare that this thesis is the result of my research conducted in the Department of Medical Microbiology, College of Health Sciences, Korle-Bu, under the supervision of Prof. Japheth A. Opintan and Dr Appiah-Korang Labi, both of the University of Ghana's Department of Medical Microbiology. All sources used in this paper have been properly credited.

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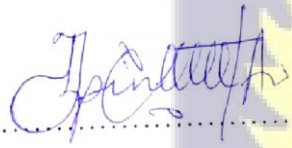
Sign:  Date: 24/08/2022

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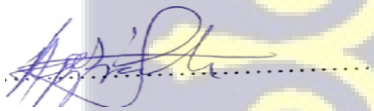
Sign  Date: 24/08/2022

Co-Supervisor: Dr Appiah-Korang Labi

DEDICATION

First and foremost, to God Almighty, the Giver life, for His never-ending love.

Secondly, to my lovely wife, Mrs Bernice Etornam Hukporti, and my adorable sons, Devine Klenam Hukporti and Joel Elikem Hukporti-Nelson.

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ABSTRACT

BACKGROUND: Infection and carriage of multidrug resistant (MDR) Enterobacterales and *Acinetobacter baumannii* are increasing globally and complicate the management of infections in children. Outbreaks of infections due to these MDR pathogens, particularly carbapenem-resistant Enterobacterales (CRE) and *A. baumannii* in hospitals are widespread and are a growing problem. Carriage of MDR pathogens is a precursor for invasive infections which are associated with high morbidity and mortality. This study determined the prevalence and epidemiology of MDR pathogens, with a focus on carbapenem-resistant Enterobacterales and *A. baumannii* among paediatric inpatients of the Korle-Bu Teaching Hospital.

AIM: This study aimed at identifying the risk factors for carriage of carbapenem-resistant Enterobacterales and *A. baumannii*, and the molecular genotypes of carbapenemase-producing isolates, among paediatric inpatients at the Korle-Bu Teaching Hospital.

METHOD: A cross-sectional study was conducted over 8 months period, from March to October 2021 at the Child Health Department, Korle-Bu Teaching Hospital. A systematic sampling method was used to recruit the participants. Relevant clinical data was extracted from participants' medical records per a structured data collection instrument. Rectal swabs were collected from participants and inoculated onto MacConkey agar and incubated at 35-37°C for 18-24hrs. Different colonial morphotypes were identified by standard bacteriological techniques and confirmed with MALDI-TOF spectometry. Antimicrobial susceptibility testing was performed on all isolates. Carbapenem resistant isolates were screened for carbapenemase production using modified Hodge test. Multiplex polymerase chain reaction (PCR) and gel imaging techniques were used to evaluate the presence, and to characterise carbapenemase genes present. Frequency tables were used to

summarize the prevalence and distribution of MDR organisms. Associations between risk factors and carriage of carbapenem resistant organisms were analysed using multinomial logistic regression.

RESULTS: A total of 344 bacteria isolates; 331 Enterobacterales and 13 *A. baumannii* were isolated from rectal swabs of 299 paediatric inpatients ≤ 13years. The most common isolates were *E. coli* (60.5%, *n* = 208), *K. pneumoniae* (29.9%, *n* = 103) and *A. baumannii* (3.8%, *n* = 13). Prevalence of MDR among the isolated organisms were 75.6% (*n* = 260); *E. coli* (74.0%, *n* = 154), *K. pneumoniae* (76.7%, *n* = 79), and *A. baumannii* (100%, *n* = 13). Carriage of ESBL producing Enterobacterales was 72.6% (*n* = 217); with *E. coli* (46.8%, *n* = 140) and *K. pneumoniae* (25.1%, *n* = 75) being the most predominant ESBL phenotypes. Faecal carriage of carbapenem resistant bacteria was 23.1% (*n* = 69). *E. coli* (11%, *n* = 33), *K. pneumoniae* (7.4%, *n* = 22), *A. baumannii* (3.3%, *n* = 10) were the most common carbapenem resistant isolates. 52.2% (*n* = 36) of these carbapenem resistant isolates expressed phenotypic carbapenemase activity by the modified Hodge test (MHT). Thirty two (46.4%) were found to harbour at least one carbapenemase gene; $bla_{Oxa\text{-}48}$ (20.3%, *n* = 14), bla_{VIM} (15.9%, *n* = 11), bla_{NDM} (4.4%, *n* = 3), and bla_{IMP} (5.8%, *n* = 4). Five (15.6%) harboured 2 carbapenemase genes, but none harboured 3 or more genes. Prior exposure to carbapenems and fluoroquinolones increased the odds of carriage of carbapenem-resistant Enterobacterales and *A. baumannii* by approximately two folds.

CONCLUSION: This study reports high faecal carriage of MDR bacteria among paediatric inpatients of the Korle-Bu Teaching Hospital. This includes carbapenem-resistant Enterobacterales and *A. baumannii* with $bla_{Oxa\text{-}48}$ and bla_{VIM} carbapenemase genes being the commonest. Prior antibiotic exposure to carbapenems and fluoroquinolones within the past year were significant risk factors for carriage of carbapenem-resistant isolates.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Test
ATCC	American type culture collection
β	Beta
CDC	Center for Disease Control
CFPD	Cefpodoxime
CFPM	Cefipime
CFTX	Cefotaxime
CFXT	Cefoxitin
CHPS	Community-Based Health Planning and Services
CIP	Ciprofloxacin
CKD	Chronic kidney disease
CLSI	Clinical and Laboratory Standard Guidelines
COT	Co-trimoxazole
CPE	Carbapenemase-producing Enterobacterales
CRE	Carbapenem-resistant Enterobacterales
DNA	Deoxynucleic acid
DNase	Deoxyribonuclease
ESBL	Extended-spectrum-β-lactamase
GEN	Gentamicin
HAI	Health associated infection
ICU	Intensive care unit

IMP Imipenemase Metallo-*β*-lactamase

IV Intravenous

KBTH Korel-Bu Teaching Hospital

KPC *Klebsiella pneumoniae* carbapenemase

MEM Meropenem

MDR Multidrug resistance

MHA Muller Hinton agar

MHT Modified Hodge Test

n Number

NDM New Delhi Metallo-*β*-lactamase

NHSN National Healthcare Safety Network

NICU Neonatal Intensive Care Unit

NMIMR Noguchi Memorial Institute for Medical Research

PICU Paediatric Intensive Care Unit

OXA-48 Oxacillinase-48

SMART Study for Monitoring Antimicrobial Resistance Trends

SPSS Statistical package for the social sciences

RS Rectal swab

µm/µl micrometer/microliter

UG University of Ghana

VIM Verona Integron Metallo-*β-lactamase*

WHO World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background

Antimicrobial resistance (AMR) is a serious public health threat, affecting global health (WHO, 2017). The emergence of multidrug resistant (MDR) Enterobacterales and *Acinetobacter baumannii* as causes of both nosocomial and community-acquired infections is a major concern globally *(Howard et al.*, 2012). Diseases caused by these bacteria are difficult to treat, particularly in low income countries, and are linked with high morbidity and mortality rates, as well as prolonged duration of hospitalisation (Agyepong *et al.*, 2018; Labi *et al.*, 2020).

MDR infections kill an estimated 700,000 people worldwide each year, with available data predicting the number could climb to 10 million by 2050 if efforts to combat resistance and development of new antibiotics are not made (O'Neill, 2014). According to the 2017 World Bank assessment, AMR could cost low-income countries over 5% of their GDP and impoverish up to 28 million people by 2050 (O'Neill, 2014).

Antimicrobial resistance to broad-spectrum antibiotics, such as the extended-spectrum cephalosporins, is a major challenge among Enterobacterales and *A. baumannii* (Labi *et al*., 2020; Olu-Taiwo *et al*., 2020). Carbapenems have predominantly been the antibiotic class of choice for treating these infections (Chiotos *et al.*, 2017; Codjoe *et al.*, 2017). However, the advent of novel *β*-lactamases with the ability to directly hydrolyse carbapenems and render it ineffective, has led to an increased prevalence of carbapenem-resistant Enterobacterales (CRE) and *A. baumannii.* This has become problematic because of the significant mortality rates associated with MDR

infections and the propensity for global spread of these resistant genes via mobile genetic elements (Nordmann *et al.*, 2013; Tzouvelekis *et al.*, 2014; Tischendorf *et al.*, 2016).

According to data from China's nationwide antimicrobial resistance surveillance, *K. pneumoniae* resistance to imipenem and meropenem grew to 25% and 26.3% in 2018, respectively, from 3.0% and 2.9 % in 2005. The rate of CRE infections has surged significantly throughout Europe. Carbapenem-resistant *K. pneumoniae* (CR-KPN) has increased to 60% in Greece and 40% in Italy (Wang, 2020). Although CRE transmission is associated with healthcare settings, the risk of spread into the general population is growing steadily. Coupled with this, are the limited therapeutic choices available to treat people, particularly children infected with these pathogens, making CRE a major global epidemiologic concern (Gupta *et al.*, 2011; Nordmann *et al.*, 2013). The World Health Organization has since 2017 categorized these pathogens as among the most serious organisms on the global priority list of pathogens (WHO, 2017).

In Africa, the epidemiology of carbapenemases is relatively unknown. New Delhi Metallo-*B*-lactamases (NDM) has been identified as the most common carbapenemase gene in Morocco, Kenya, and South Africa (Brink *et al.*, 2012; Nordmann *et al.*, 2013). In 2012, South Africa became the first African country to report a *Klebsiella pneumoniae* carbapenemases (KPC) positive organism (Brink *et al.*, 2012).

Despite the increasing carriage of MDR Enterobacterales and *A. baumannii* reports in Africa, their epidemiology remains largely unknown (Chiotos *et al.*, 2017). In Kenya for example, paediatric carriage of MDR Enterobacterales in stool has been shown to almost triple, from 21% on admission; to 57% on discharge for patients who spent 2 or more days on admission (Kagia *et al.*, 2019).

Unfortunately, no vaccines are available to prevent infections caused by these MDR pathogens. As a result, it is important everything is done to ensure common infections like urinary tract infections do not become life-threatening due to a lack of adequate treatment.

1.2 Problem Statement

There are increasing reports of MDR infections among paediatric inpatients in Ghana, particularly carbapenem-resistant Enterobacterales and *A. baumannii* infections. These are mostly associated with life-threatening nosocomial infections. (Opintan *et al.*, 2015; Codjoe *et al*., 2017; Agyepong *et al*., 2018).

In Ghana, carriage of MDR Enterobacterales and 3rd generation cephalosporin-resistant organisms are as high as 49.6% and 46.1% respectively in neonates (Labi *et al.*, 2020). Pathogen carriage is a precursor for acquiring invasive diseases with a high likelihood of poor disease outcomes (Tsai *et al.*, 2014; Doare *et al.*, 2015; Labi *et al.*, 2020). Paediatric patients are confronted with very limited antibiotic alternatives and as a result, are the worst affected group. Paediatric mortality rates due to carbapenem resistant Enterobacterales and *A. baumannii* are as high as 40–65% (Nordmann *et al*., 2011; Codjoe *et al*., 2017; Chiotos *et al*., 2017).

In addition to the high disease burden, the advent of novel *β*-lactamases with an increased rate of pathogen transmission, has contributed to the rapid global spread of MDR organisms and has increased the possibility of community transmission. Carbapenem resistant isolates are resistant to almost all available antibiotics (Doare *et al.*, 2015; Chiotos *et al*., 2017).

Despite increasing reports of infections caused by MDR Enterobacterales and *A. baumannii* in Ghanaian hospitals, surveillance studies to provide credible data to guide antibiotic stewardship strategies are lacking (Opintan *et al*., 2016; Labi *et al*., 2020). In particular, the dynamics of spread

of these MDR pathogens in Ghanaian paediatric referral centres are barely described. Meanwhile, these are the institutions already battling with an increasing number of high-risk patients and growing numbers of antibiotic-resistant pathogens (Agyepong *et al.*, 2018; Labi *et al.*, 2020). Therefore, establishing the carriage and extent of resistance determinants are required to close the knowledge gap and provide a framework for empiric therapy and antibiotic stewardship strategies (Codjoe *et al.*, 2016; Opintan *et al.*, 2015).

1.3 Justification

Despite the high clinical significance of MDR, particularly carbapenem-resistant Enterobacterales and *A. baumannii* and the public health threat they pose, very few studies have been conducted on these infections in Ghana, especially among paediatric inpatients. There is, therefore, an urgent need to investigate the carriage and molecular characteristics of these MDR pathogens in children and to identify potentially modifiable risk factors for pathogen carriage (Codjoe *et al.*, 2016; Labi *et al.*, 2020).

As the prevalence of MDR, particularly carbapenem resistance among Enterobacterales and *A. baumannii* increase, the risk for carriage among paediatric inpatients is expected to increase as well. Such an increase may result in the spread of resistance genes, including carbapenemases. A better understanding of the risk factors for CRE carriage, the dynamics of spread, as well as the molecular characteristics of the carbapenemase-producing genes present, among hospitalized paediatric patients will be useful for the management and prevention of CRE infections in Ghana (Nordmann *et al.*, 2013; Codjoe *et al.*, 2016; Chiotos *et al.*, 2017; Labi *et al.*, 2020).

There is, therefore, an urgent need to investigate the carriage and molecular characteristics of these MDR pathogens in children and to identify risk factors for pathogen carriage. Data generated from this study will be extremely useful in improving paediatric management of MDR infections as well

as improve infection prevention and control strategies in Ghana (Labi *et al.*, 2020; Codjoe *et al.*, 2016) and will provide evidence to shape policy on the allocation of scarce medical resources for antibiotic stewardship programmes.

1.4 Aim of the Study

To determine the carriage rates of MDR Enterobacterales and *A. baumannii* and associated risk factors, as well as the molecular characteristics of carbapenemase-producing genes present among paediatric inpatients at the Korle-Bu Teaching Hospital.

1.5 Specific Objectives

i. To determine the prevalence of MDR Enterobacterales and *A. baumannii* among hospitalized paediatric patients.
ii. To determine the proportion of MDR Enterobacterales and *A. baumannii* among hospitalized paediatric patients that are carbapenem-resistant, and to characterize the carbapenemase-producing genes present.
iii. To describe the risk factors for carriage of carbapenem-resistant Enterobacterales and *A. baumannii.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Multi-drug Resistance in Enterobacterales and *Acinetobacter baumannii*

MDR among Enterobacterales and *A. baumannii* has reached alarming rates. The fight to curb resistance among these groups of pathogens has assumed global importance and has taken centre stage in many clinical settings. With its "Bad Bugs No Drugs" campaign and the acronym "ESKAPE" pathogens, the Infectious Diseases Society of America, for example, has offered significant insight into this problem and the need for novel therapies (Logan, 2012; WHO 2017)

The World Health Organization (WHO) has since identified AMR among Enterobacterales and *A. baumannii* as an important barrier to public health, reaching new lows in many countries, particularly in Sub-Saharan due to existing resource constraints, resulting in poor disease outcomes, especially among vulnerable paediatric patients (WHO 2017; Adesanya *et al.,* 2020; Labi *et al.,* 2020). Broad-spectrum antimicrobial resistance remains a predominant feature within these pathogens among paediatric inpatients in low-resource countries, despite intense efforts to control their development and spread (Gupta *et al.,* 2011; Eibach *et al.,* 2016).

Carriage rates of MDR Enterobacterales and *A. baumannii* are increasing worldwide, posing a particular threat to children. Outbreaks of these MDR infections, especially carbapenem-resistant Enterobacterales and *A. baumannii* are common and are becoming a greater burden, particularly among paediatric inpatients at referral centres where these life-threatening infections are commonly preceded by pathogen carriage (Adesanya *et al.,* 2020). However, the dynamics of spread in sub-Saharan Africa is not well described and data on the prevalence, acquisition, carriage and spread of these MDR isolates among paediatric inpatients in Ghanaian referral centres, in

particular, are limited, and risk factors for transmission are not well defined (Codjoe, 2016; Kagia *et al.,* 2019; Labi *et al.,* 2020).

Carbapenems have been the antimicrobial class of the first choice for treating life-threatening infections because carbapenem resistance has been uncommon until recently. The emergence of novel *B*-lactamases with the natural ability to directly break down carbapenems has contributed to a surge in the number of carbapenem-resistant isolates (Gupta *et al.,* 2011). Given how frequently these carbapenem-resistant pathogens cause infections, in addition to the unacceptably high mortality rates, and the ease of widespread transmission of mobile genetic resistant elements, AMR among these pathogens has become a serious global health problem (Gupta *et al.,* 2011; Tzouvelekis *et al.,* 2014; Tischendorf *et al.,* 2016).

Carriage of MDR pathogens among hospitalized children increases the probability of acquiring life-threatening infections, with increased mortality and morbidity rates in this vulnerable patient demographic, and the resultant increase in transmission of these pathogens from carriers and or colonized patients to others and may remain in the environment because of unsatisfactory infection prevention and control practices (Karikari, 2017; Moshiri *et al.,* 2018). Although pathogen dissemination within the health care setting is the most important risk factor for carriage, the troubling emergence of community acquisition, probably due to contaminated public water, adds to the speed of transmission (Nordmann *et al.,* 2013; Chiotos *et al.,* 2017). It is, therefore, of paramount importance to employ specific and heightened infection control precautions and barrier nursing strategies for high-risk patients, to prevent contaminating the hospital environment and to help limit transmission to vulnerable patients (Ghaith *et al.,* 2019; Labi *et al.,* 2020).

A 2017 National Healthcare Safety Network (NHSN) data in the United States of America (USA) revealed 13% of *E. coli* and *Klebsiella*, as well as 74 % of *A. baumannii* in intensive-care units. Other studies reported an increase in the proportion of nosocomial infections caused by carbapenem-resistant Enterobacterales growing from 1.2% in 2001 to 4.2% in 2011, with *Klebsiella spp.* accounting for the largest rise (10%). Globally, meropenem non-susceptibility is found in 4% of *K. pneumoniae* and 1% of *E. coli* strains in children, while in Asia, *K. pneumoniae* resistance to cephalosporins and ampicillin among paediatric inpatients is 84% and 94% (Chea *et al.*, 2015; De Oliveira *et al.*, 2019; Yam *et al.*, 2019). MDR Enterobacterales caused 18.6% of all invasive neonatal infections in Taiwan's neonatal intensive care unit (NICU), and drug resistance was more common in neonates who had previously received broad-spectrum antibiotic treatment (Tsai *et al.*, 2014).

China's nationwide surveillance on AMR showed *K. pneumoniae* resistance to imipenem and meropenem stood at 3% and 2.9% respectively in 2005 but rose dramatically to 25% and 26.3% respectively in 2018. Similarly, the number of *K. pneumoniae* resistant to carbapenems has surged to 60% in Greece and 40% in Italy, whereas in the Republic of Korea, 69% of all bloodstream infections are caused by carbapenemase-producing Enterobacterales, with *KPC* and *NDM* being the most predominant carbapenemase genes isolated (Park *et al.*, 2019; Wang, 2020). Of the mechanisms of resistance among these pathogens, carbapenemase expression was the commonest mechanism, and the rapid global spread of carbapenem resistance is attributable to the dissemination of carbapenemase-producing strains (Eichenberger *et al.*, 2019; Wang, 2020).

In Africa, *K. pneumoniae* resistance to cephalosporins and ampicillin among pediatric inpatients reached 50% and 100%, respectively, and multidrug resistance among hospitalized paediatric inpatients, involving commonly used antibiotics; ampicillin, chloramphenicol, and cotrimoxazole

stood at 75% as opposed to 30% in Asia (Le Doare *et al.*, 2015; De Oliveira *et al.*, 2019). Rectal Carriage of MDR Enterobacterales and *A. baumannii* stood at 0% on admission in a Tunisian Intensive Care Unit (ICU) but rose sharply to 45.16% on discharge and a 24% prevalence of carbapenem-resistant Enterobacterales was recorded in an Egyptian PICU. Similarly, faecal carriage of MDR Enterobacterales was 21% on admission but rose to 57% on discharge among paediatric inpatients who spent at least 48hours on admission. The most common resistant determinants isolated were the NDM and the OXA-48 carbapenemase genes (Ghaith *et al.*, 2019; Kagia *et al.*, 2019).

In Ghana, prevalence of MDR Enterobacterales and *Acinetobacter spp.* infections in a major referral hospital were found to be as high as 89.5% and 62.1% respectively, and 59.8% of the *Acinetobacter spp.* isolates were carbapenem-resistant, whereas 8.1% harboured the *NDM* gene (Agyepong *et al.*, 2018; Olu-Taiwo *et al.*, 2020). Other studies in a NICU revealed a neonatal carriage rate of MDR Enterobacterales and organisms resistant to 3rd generation cephalosporin as high as 49.6% and 46.1% respectively. As much 75.6% and 15.6% of *Klebsiella spp.* respectively expressed phenotypic Extended-spectrum-*β*-lactamase (ESBL) and carbapenemase activity, and Codjoe *et al.* (2016) revealed a 2.9% prevalence of the carbapenemase genes; NDM, Verona Integron-encoded Metallo-*β*-lactamase (VIM) and Oxacillinase-48 (OXA-48) in Ghana (Codjoe, 2016; Labi *et al.*, 2020). Despite these alarming rates, data on AMR on paediatric referral centres is sparse, and AMR surveillance is nonexistent, not much is known about the mode of the spread of the carbapenem resistance among these MDR pathogens in paediatric inpatients (Opintan *et al.*, 2015; Codjoe, 2016; Codjoe *et al.*, 2017; Codjoe *et al.*, 2019; Labi *et al.*, 2020). These characteristics are poorly described for paediatric referral centres in Ghana, which are institutions

dealing with vulnerable patients and a surging number of MDR organisms, suggesting not much has been or is being done to contain them (Donkor *et al.,* 2013; Ampaire *et al.,* 2015).

Added to conferring resistance to almost all *B*-lactams, carbapenemase-producing organisms are linked to many other non-*B*-lactam resistance determinants, resulting in multidrug and pan drug-resistant strains (Nordmann *et al.,* 2012). The rising number of carbapenemase producers with reduced sensitivity to colistin and tigecycline; the two drugs with doubtful efficacies in previous types of CRE infections have now become first-line treatments, further restricts the already limited treatment options, giving credence to the fact that we are fast approaching, if not already at an impasse, and that totally new drugs are required urgently (Tzouvelekis *et al.,* 2014).

2.2 Infections caused by Enterobacterales and *Acinetobacter baumannii*

Enterobacterales are common colonizers of the gastrointestinal tract (GIT) of humans and are implicated in many diseases, with very alarming mortality and morbidity burdens. Similarly, the emergence *A. baumannii* has been linked with several hospital-acquired infections of clinical importance in the elderly, children, and the immunocompromised (Olu-Taiwo *et al.,* 2020). Infections acquired from these clinically relevant pathogens are increasingly becoming widespread, especially among children, and are mostly non-susceptible to several pharmacological agents, and are becoming progressively resistant to practically all existing antimicrobials, leading to poorer disease outcomes, protracted periods of hospital stay, and higher treatment costs, and may include urinary and respiratory tract infections, GIT, and skin infections, as well as septicaemia, and many other life-threatening infections (Ruppé *et al.,* 2015; Codjoe, 2016; Eibach *et al.,* 2016).

2.3 Common Antimicrobials against MDR Enterobacterales and *A. baumannii*

In the last few years, research into developing many new and modified drugs that are effective against drug-resistant Enterobacterales and *A. baumannii* has been launched, however, in most cases, no new antimicrobial class can be expected any time soon (Codjoe, 2016; Agyepong *et al.*, 2019).

Since the 1990s most low-cost broad-spectrum and first-line antibiotics have been employed as successful treatment agents for treating various kinds of infections. Some of these agents include ampicillin, trimethoprim-sulfamethoxazole, gentamicin, tetracycline, fluoroquinolones, and several cephalosporins. However, the widespread resistance of disease-causing bacteria to these drugs have necessitated the reliance on carbapenems to treat patients with these MDR pathogens. As a result, carbapenems have been such an essential antibiotic class for the effective management of MDR infections, for which reason it has been justifiably referred to as "the antibiotic of last resort" for treating life-threatening multidrug resistant infections (Eliakim-Raz *et al.*, 2015; Doi, 2019).

However, the increasing prevalence of carbapenemase-producing organisms, which are novel *B*-lactamases with the inherent ability to directly breakdown carbapenems, has resulted in the rise in bacteria strains resistant to almost all available treatment alternatives, brings the clinical efficacy of carbapenems, under immense threat, and is becoming a major setback to global public health, posing a complex clinical challenge when treating serious illnesses, particularly in children because of the already limited antibiotic options available for them (Aysegul *et al.*, 2014; Ghaith *et al.*, 2019).

Although phase III clinical trials on tigecycline in children between 8-11years suffering from life-threatening bacterial infection have yet to be completed, recent pharmacokinetic studies within

this paediatric age group suggest administering a dose at 12 hourly interval, could give satisfying results (Chiotos *et al.,* 2017). Several other innovative drugs, such as derivatives of polymyxin, *β*-lactamase inhibitors developed together with non-*β*-lactams, newer aminoglycosides are in the early phases of development (Chiotos *et al.,* 2017).

2.4 Carbapenem Structure and Mode of Activity

Carbapenems are broad-spectrum antibiotics that inhibit metallo-*ß*-lactamases (MBL) and extended-spectrum *ß*-lactamases (ESBL), both of which are produced by gram-negative bacteria, and belong to the *ß*-lactam group of antibiotics, which are similar in structure to penicillins. The name "carbapenem" describes inherent differences between penicillin and cephalosporins; "carba-" denotes the substitution of an atom of carbon for sulfur at position one, and "-penem" denotes the existence of a double bond between the second and third positions (Jeon *et al.,* 2015; Codjoe *et al.,* 2019).

Carbapenems have been until recently, highly effective in treating infections from both Gram-positive and Gram-negative bacteria isolates, in addition to anaerobes, and act by invading the bacteria cell wall and adhering to penicillin-binding proteins (PBPs) within the organism, to cause the lyse of the bacteria cell, and thereby, effectively killing the bacteria (Xu *et al.*, 2014)

2.5 Carbapenem Resistant Enterobacterales (CRE) and *A. baumannii*

CRE and carbapenem-resistant *A. baumannii* are phenotypically non-susceptible to at least one of the carbapenem antibiotics (meropenem, ertapenem, doripenem, or imipenem), and are implicated in various life-threatening nosocomial infections (Chiotos *et al.*, 2017; CDC, 2019). This arises from one of two methods: enzymatic or non-enzymatic mechanisms. Enzymatic hydrolysis involve carbapenemases; enzymes that break down the *β*-lactam ring of the carbapenem antibiotic. The

latter involves the synthesis of ESBLs and/or AmpC cephalosporinases, as well as decreased membrane permeability. The synthesis of carbapenemase enzymes is the commonest mechanism of resistance among MDR isolates and have been the key to the rapid global transmission of carbapenem resistance, due to easily transferrable mobile genetic determinants, such as plasmids and transposons that encode these carbapenemase genes (Chiotos *et al.*, 2017; Wang, 2020).

Except for the detection of *OXA-48* in Tunisia, Egypt and Morocco and *NDM* and *KPC* in Kenya and South Africa, the epidemiology of carbapenemase producing isolates in Africa is relatively unknown. In Ghana, just like in most other African countries, there is very little data on the epidemiology of carbapenemase producers; data on risk factors for pathogen carriage, dynamics of spread, treatments and disease outcomes, particularly in children is grossly limited. Meanwhile, carbapenem resistance among Enterobacterales as well as *A. baumannii* in this high risk group continue to surge, and a lack of clinical trials, assessing the possibility of new agents in the younger population worsen the already limited therapeutic choices (Kieffer *et al.*, 2016; Chiotos *et al.*, 2017).

2.6 Mechanisms of Carbapenem Resistance

Resistance to carbapenems among Enterobacterales and *A. baumannii* is facilitated by 2 main mechanisms; non-enzymatic and/or enzymatic processes (antibiotic molecule breakdown). Because of their outer membrane structure, these pathogens are frequently more resistant to antimicrobials as opposed to Gram-positive bacteria. This confers protection to the organisms' internal membrane or the peptidoglycan from dyes, drugs, detergents as well as lysozyme and penicillin leading to MDR isolates (Xu *et al.*, 2014; Codjoe, 2016).

Non-enzymatic mechanisms employ the use of efflux pumps as well as down regulation of outer membrane porins, largely from broad-spectrum antibiotic exposure. Many of these resistant mechanisms are intrinsic to the organism and can be expressed either intrinsically (chromosomal genes) or acquired. Organisms, including commensals and pathogens, with intrinsic resistance, are innately resistant to particular kinds of antimicrobial agents, complicating the selection of appropriate treatment regimens, and thereby, increasing the likelihood for acquired resistance to develop. By the selective modification of their porin channels, Enterobacterales, for example, can selectively restrict the uptake of *β*-lactam antibiotics, thereby reducing the effectiveness of the antibacterial agent. (Forsberg *et al.*, 2012; Moshiri *et al.*, 2018).

By contrast, acquired resistance involves organisms that have established numerous mechanisms of resistance, such as inactivation of enzymes, mutation of target-sites, and the activation efflux pump. Inactivating enzymes have prevailed since the clinical use of *β*-lactam antimicrobials; from penicillinases, cephalosporinases, ESBLs, and quite recently, the advent of metallo-*β*-lactamases as well as carbapenemases. Over time, these hydrolyzing enzymes have expanded their spectrum of activities. The MBLs have had a significant impact on the clinical utility of carbapenems and have the potential to render useless, the therapeutic use of these important medications (Castañeda-García *et al.*, 2013).

Horizontal transfer of mobile genetic elements harbouring resistance genes generally are made up of plasmids. Because these plasmids typically carry numerous *B*-lactamase, one plasmid conjugate may be enough in spreading resistance determinants to many antibiotic classes. There are three (3) major classes of *β*-lactamases namely AmpC, ESBL, and carbapenemase-producing organisms (Mariappan *et al.*, 2017; Labaste *et al.*, 2019).

2.6.1 AmpC *β*-lactamases

Several clinically important Enterobacterales have chromosomally encoded AmpC *β*-lactamases that easily hydrolyses broad-spectrum cephalosporins as well as penicillins and can also weakly breakdown carbapenems, acting similar to carbapenemase producers, especially when down-regulated porins are present. Antibiotic exposure can stimulate the expression of normally suppressed chromosomal AmpC enzymes, resulting in their continued expression (Malande *et al.*, 2016; Moshiri *et al.*, 2018). Many AmpC enzymes are found on mobile genetic materials and have been detected in most Enterobacterales. The spread of AmpC *β*-lactamases leads to poorer treatment outcomes because plasmid-mediated AmpC enzymes in Enterobacterales are not easily detected by phenotypic techniques. Mutations in the AmpC attenuator and promoter regions are mostly responsible for acquiring plasmid-mediated AmpC genes and their subsequent overexpression in bacterial isolates (Frye, 2013; Xu, 2014; Malande *et al.*, 2016).

2.6.2 Extended-spectrum *β*-lactamases (ESBL)

ESBL confers resistance to *β*-lactams such as aztreonam, and most cephalosporins and are most prevalent on plasmids of *Klebsiella spp*. and *E. coli* with the most prevalent ESBL genes being Temoneira-1(TEM-1) and sulfhydryl variable-1 (SHV-1) *β*-lactamases (Gupta *et al.*, 2011; Tzouvelekis *et al.*, 2014). ESBL strains whose porins have been altered or whose expression has been regulated such as in *Klebsiella spp.*, *Enterobacter spp.*, and *E. coli*, among other genera, are unlikely to spread extensively but may thrive locally within hospitals. Ertapenem is the most affected among the various carbapenem antibiotics; although bacteria pathogens may still be susceptible to other carbapenem types, lower ertapenem susceptibility is mostly linked to the presence of AmpC/ESBL and the precise modifications in bacteria porins (Bedenić *et al.*, 2014; Codjoe *et al.*, 2017).

2.6.3 Carbapenemases

Carbapenemases are *β*-lactamase enzymes that hydrolyse various types of antibiotics, including penicillins, cephalosporins, and carbapenems, giving rise to resistance to multiple *β*-lactams as well as other non-*β*-lactam resistant organisms. Carbapenemases are predominantly responsible for the development of multidrug and pan drug-resistant isolates, and the global spread of bacteria isolates nearly resistant to all known antibiotics (Nordmann *et al.,* 2012).

Carbapenemases are mostly plasmid-mediated and can spread rapidly among bacterial isolates in a variety of ways. This has become a great public health concern globally, even more so among paediatric patient populations in low resource countries because of how frequently they cause infections, the high mortality and morbidity burdens, as well as the global spread of resistant determinants via mobile genetic materials (Nordmann *et al.,* 2013; Bedenić *et al.,* 2014; Tzouvelekis *et al., 2014*; Tischendorf *et al.,* 2016).

For epidemiological purposes, it is important to distinguish carbapenemase isolates from isolates that are resistant to carbapenems as a result of other resistant mechanisms because although both types of resistant isolates require contact precautions, organisms harbouring carbapenemase genes may require more rigorous infection control methods, such as focused active surveillance (Ghaith *et al.,* 2019). The *NDM*, *KPC*, *IMP*, *VIM*, and *OXA-48* are the most widely distributed carbapenemase genes of clinical importance and have been categorized into 3 ambler classes; A, B, and D carbapenemases (Nordmann *et al.,* 2012; Kieffer *et al.,* 2016).

2.6.3.1 *K. pneumoniae Carbapenemase* (*KPC*)

The KPC is plasmid-encoded, and was first discovered in North America in *K. pneumoniae* but is now widespread in *E. coli*, *Enterobacter cloacae*, and *S. marcescens*. A wide variety of antibiotics

including aztreonam, extended-spectrum cephalosporins, and carbapenems are all hydrolysed by *KPC* carbapenemase genes (Kieffer *et al.*, 2016).

2.6.3.2 Imipenemase Metallo-*β*-lactamase (*IMP*)

A. baumannii and P. aeruginosa were the first bacteria to be found with the IMP gene. It was eventually discovered in Enterobacterales transferable plasmids. All *β*-lactams and carbapenems can be hydrolysed by *IMP* (Kieffer *et al.*, 2016).

2.6.3.3 Verona Integron-encoded Metallo- *β*-lactamase (*VIM*)

The *VIM* gene was first discovered in *P. aeruginosa*, but it was later found in plasmids from *Klebsiella spp.* and *E. coli.* It has a similar mode of action to the KPC (Kieffer *et al.*, 2016).

2.6.3.4 New Delhi Metallo-*β*-lactamase (*NDM*)

A newly discovered carbapenemase gene was discovered on the chromosomes of *A. baumannii* but later discovered on plasmids of Enterobacterales. It works in the same way as the *IMP* and the *VIM* metallo-*β*-lactamases (Kieffer *et al.*, 2016).

2.6.3.5 Oxacillinase Type Carbapenemases (*OXA*-)

OXA enzymes come in a variety of forms but do not include extended-spectrum cephalosporins and Aztreonam. Despite the discovery of OXA-48 in Enterobacterales, principally in *K. pneumoniae* and *E. coli*, OXA-type *β*-lactamases are found widely in Acinetobacter species (Kieffer *et al.*, 2016).

2.6.3.6 Molecular/Ambler classification of carbapenemase enzymes

The Ambler class A carbapenemases include chromosomal and plasmid-encoded carbapenemases, with *K. pneumoniae* carbapenemases being the most frequent. The *KPC* gene, $_{bla}KPC$, is linked

predominantly with clonal expansion and the epidemiologic success of the global spread of the KPC carbapenemase genotypes. More than 80% of carbapenem resistance in the USA is attributable to activities of *KPC*, and is also responsible for the rising prevalence of MDR globally (Chiotos *et al.*, 2017).

The *VIM*, *IMP* variants are among ambler class B carbapenemases and although have been detected globally, have been largely associated with multidrug infections within the Mediterranean sub-region as well as Asia. The *NDM*, another ambler class B variant, is prevalent in the Indian sub-region and is responsible for more than half of the CRE isolates in the region. The *NDM*, just like the other carbapenemases have recently been detected in many parts of Europe, Africa, Asia as well as the United States, usually in people who travelled to CRE prevalent areas (Chiotos *et al.*, 2017).

The *OXA-48* carbapenemases belong to the Ambler class D carbapenemases, which are oxacillinases. The most common carbapenemases found in *Acinetobacter species* are Class D carbapenemases although they are increasingly being found among Enterobacterales. Geographic disparities for the different carbapenemase genes are expected to fade away as global travel and medical tourism remain on the rise (Chiotos *et al.*, 2017).

2.7 Epidemiology of MDR Enterobacterales and *Acinetobacter baumannii* in Children

Quite possibly, the limited nature of MDR epidemiological studies in children, notably in the areas of CRE carriage and infections, may be responsible for the continuous global increase of CREs. From 2007 to 2011, 14% of neonatal invasive infections in a NICU in Kolkata, India, were caused by *NDM-1* type Enterobacterales (Johnson *et al.*, 2017). The SMART (Study of Monitoring Antimicrobial Resistance Trend) surveillance program gathered reports between 2002 and 2010,

on paediatric patients with CRE infections in five countries; India, Israel, Spain, the United States, and Greece. India had the most prevalence with 39%, followed by Israel with 29%, Spain with 19%, the United States with 11%, and Greece with 3% (Johnson *et al.*, 2017). *Enterobacter spp.*, *K. pneumoniae,* and *E. coli* were the commonest isolates detected. *NDM*, *KPC*, and *VIM* were the commonest isolated phenotypes; over 50% of the organisms were from NICUs or PICUs. All *NDM* isolates came from the Indian sub-region, all *KPC* from the USA and Israel, and all *VIM* genotypes from within Europe (Johnson *et al.*, 2017).

Nosocomial outbreaks of Carbapenem-resistant Enterobacterales (CRE) have also been recorded in paediatric health care centres in many parts of the world, notably in a California NICU, a Spanish PICU, and a Nepalese NICU. All these outbreaks were linked to carbapenemases; *IMP*, *VIM*, and *NDM-1* whereas between 2003 and 2015, records from England's Public Health unit on confirmed laboratory isolates of carbapenemase-producing Enterobacterales in the UK indicated an unprecedented spike of carbapenemase genes (Hsu *et al.*, 2014; Chiotos *et al.*, 2017). Carriage of carbapenem resistance among *K. pneumoniae* and *E. coli* accounted for 32% of all Carbapenem-resistant bacteria. With multiple reported outbreaks of carbapenemase-producing Enterobacterales and *A. baumannii* among paediatric populations in India, and many other parts of the world, children appear to be a particularly vulnerable group, who are the worst affected group in terms of mortality and morbidity burden (Chiotos *et al.*, 2017).

In Ghana and many other resource-limited countries, the mechanism of transmission is poorly defined. There exist only scanty data on the prevalence, acquisition, and carriage of these MDR pathogens among Ghanaian paediatric inpatients, and risk factors for transmission are not well established **(**Codjoe, 2016; Codjoe *et al.*, 2017; Kagia *et al.*, 2019; Labi *et al.*, 2020).

2.8 Risk Factors for Carriage of Carbapenem-resistant Isolates in Children

Carriage of these MDR pathogens is a precursor for serious systemic infections, high mortality and morbidity rates, as well as increasing hospital costs and protracted hospital stay. Several risk factors predispose children to the carriage of these pathogens including immunosuppression, prematurity, admission to the ICU, previous exposure to antibiotics, stem-cell transplant, chemotherapy, invasive medical devices, indwelling catheters, protracted hospital stay, and impaired GIT function such as necrotizing enterocolitis or Hirschprung's or surgery (Chiotos *et al.*, 2017; Codjoe *et al.*, 2017; Moshiri *et al.*, 2018; Labi *et al.*, 2020).

2.9 Laboratory Detection of MDR Enterobacterales and *A. baumannii*

Accurate detection and characterization of pathogens are crucial to aid prompt and efficient diagnoses as well as the selection of the right antimicrobials against the isolated pathogen. This is fundamental in every clinical setting the pivot of any efficient epidemiological surveillance strategies (Fournier *et al.*, 2014; Boswihi *et al.*, 2018). The CDC recommends various strategies for isolating and identifying MDR bacteria from rectal or perianal swabs in the clinical laboratory including the use of automation, disc diffusion, agar-based methods, modified Hodge test, molecular techniques and many others (Boswihi *et al.*, 2018).

2.9.1 Antimicrobial Susceptibility Testing and Diagnosis of Carbapenem-Resistant Isolates from Rectal Swabs

The Centre for Disease Control (CDC) recommends a variety of tests for in-vitro pathogen detection, including the inoculation of fresh stool specimens from rectal or perianal swabs onto MacConkey agar and the subsequent use of antibiotic discs on Mueller-Hinton agar as well as commercially available chromogenic media for the diagnoses of CRE, as well as other MDR

Enterobacterales and *A. baumannii*. They have a reasonable detection sensitivity, are reasonably affordable, and are simple to interpret (Chiotos *et al.*, 2017).

Disk diffusion is one of the most common baseline tests for pathogen detection. Impregnated discs with a standard amount of the antimicrobial agent are positioned on Mueller-Hinton agar (MHA), seeded with a 0.5McFarland Standard turbidity of the test bacteria and incubated overnight. The antimicrobial agent diffuses into the medium as the bacterium grows during incubation. The inhibition zone created by the antibiotic used is proportional to the susceptibility of the organism of interest (Chiotos *et al.*, 2017). The double-disc synergy testing is another technique for the detection of MDR organisms, particularly CRE. Automated systems are also currently commercially available to assess antibiotic susceptibility (Chiotos *et al.*, 2017).

2.9.2 Phenotypic Detection of Carbapenemase-producing Enterobacterales and *A. baumannii*

The most common phenotypic detection of carbapenemase activity is by the modified Hodge test (MHT), also called the "cloverleaf" test. It involves streaking a suspected carbapenemase-producing bacterium over MHA. The expression of carbapenemase activity is confirmed when a test isolate produces a carbapenemase enzyme that breaks down the carbapenem, permitting a carbapenem-susceptible strain to grow down the inoculum streak toward the carbapenem disc positioned at the centre of the MHA. The MHT has very high sensitivity, is affordable, as well as simple to use. It is recommended by the CLSI and the CDC for the phenotypic detection of carbapenemase producing isolates (Chiotos *et al.*, 2017).

The recently designed Carba NP test, which stands for “Carbapenemase Nordmann-Poirel”, is another very efficient technique for the detection of carbapenemase. The method is simple,

requires little expertise, can be reproduced and is highly economical (Nordmann *et al.*, 2011; Nordmann *et al.*, 2012).

2.9.3 Molecular Testing for Carbapenem Resistant Enterobacterales and *A. baumannii*

To detect and characterize common carbapenemase genes (*KPC, VIM, IMP, NDM, and OXA-48*), single and multiplex PCR techniques are highly effective and has sensitivities and specificities approaching 100%. The ability to detect specific carbapenemase genotypes, greater sensitivity and specificity, as well as quick turnaround time, are clear advantages of these molecular-based techniques. The downsides include the relatively high cost of various procedures, as well as the necessity for specialized skills and equipment (Chiotos *et al.*, 2017).

CHAPTER THREE

METHODS AND MATERIAL

3.1 Study Design

A cross-sectional study was carried out over an 8 months, from March to October 2021. A systematic sampling method was used to recruit a total of 299 participants. An interval of one month was allowed between the collection of each batch of samples to prevent duplicity of samples. The number of participants recruited per unit were proportional to the total bed capacity of the unit.

3.2 Study Site

The study was undertaken at the Department of Child Health, Korle-Bu Teaching Hospital, Accra, Ghana. The Korle-Bu Teaching Hospital is a tertiary referral hospital located in Accra, Ghana's capital city. The hospital has a Child Health Department serving children under 13years with Medical and Surgical conditions. The department has an emergency unit, a surgical unit, a 'babies' unit (for neonates), a Paediatric Intensive Care Unit (PICU), an oncology unit as well as an Out-Patient Department. The Child Health Department has an estimated daily general outpatient attendance of 120.

3.2.1 Inclusion criteria

i. Paediatric inpatients ≤ 13years for whom consent had been granted

3.2.2 Exclusion criteria

i. Paediatric inpatients with missing folders/ incomplete hospital records

ii. Paediatric inpatients ≤ 13years for whom consent had been declined

3.3 Determination of sample size

The minimum sample size was determined using an MDR carriage rate of 24% from a similar study (Ghaith *et al.,* 2019), and a 95% confidence interval with an error margin of 5%. The minimum sample size for the study was calculated using the formula;

$$N = \frac{Z^2 \times P(1-P)}{m^2}$$

Where;

N= minimum sample size

Z = 1.96, the standard score for the confidence interval at 95%.

m = margin of error at 5% (standard value of 0.05)

P = 0.24, thus, the prevalence rate (24%) of Carbapenem-resistant Enterobacterales infection in hospitalized children from a similar study (Ghaith *et al.*, 2019).

Therefore, our minimum sample size, $N = \frac{1.96^2 \times 0.24(1-0.24)}{0.05^2} = 280.28$

The minimum sample size = 280.

However, a sample size of 299 was used.

Participants' information were extracted from their clinical records per a structured questionnaire alongside the rectal swab. The number of participants from each unit/ward was proportional to the patient/bed capacity of the unit.

3.4 Stool Sample Collection, Transport, and Storage.

Using strict aseptic techniques, rectal swabs (RS) from each study participant were collected. Participants were hospitalized paediatric children 13 years and below. A total of 299 non-duplicate

RS were collected and transported in a cool box to the Department of Medical Microbiology of the University of Ghana Medical School and processed within 2hrs.

3.5 Isolation and Identification of Bacterial Isolates

Each rectal swab was directly inoculated onto MacConkey (Oxoid, Ltd Basingstoke, UK) agar and incubated at 35±2°C for 18-24hrs. This was followed by purity plating until pure bacterial isolates were obtained. Pure, non-duplicate bacteria isolates were identified presumptively using standard biochemical methods (indole test, citrate, oxidase urea, motility, Triple sugar iron (TSI) tests). Confirmation was done using MALDI spectrometry (Bruker Daltonics, Bremen, Germany).

3.6 Antimicrobial susceptibility testing

Antibiotic susceptibility test (AST) was carried out by the Kirby Bauer's disc diffusion method, per the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020). For each pure non-duplicate bacteria isolate, a 0.5 McFarland standard equivalent suspension of organisms was prepared and inoculated on Muller-Hinton agar (Oxoid, Hampshire, England) to obtain a confluent growth. Within 15 minutes of bacteria application, various antimicrobial discs were positioned on the lawn of bacterial isolates using sterile forceps and incubated aerobically within 15 minutes of antimicrobial disc application, for 16-18hrs at 37°C. Figure 2 below is a picture of Becton Dickinson Phoenix Spec™ Nephelometer, showing inoculum turbidity of 0.5 McFarland. Antimicrobial discs used for the AST were Cotrimoxazole (1.25/23.75µg), Ciprofloxacin (5µg), meropenem (10µg), gentamicin (10µg), cefepime (30µg), cefotaxime (30µg), cefpodoxime (10µg), cefpodoxime/clavulanic acid (10µg/1µg). All antimicrobials used were from BD BBL™ Sensi-Disc Antimicrobial Susceptibility Test Discs. The plates were incubated at 37˚C for 16-18 hours and the diameters of the zones of complete inhibition were measured to the nearest millimetre and compared with the CLSI guidelines to determine resistance and sensitivity states. Zone sizes within

the intermediate resistance and absolute resistant range were classified as resistant. MDR was defined as in-vitro resistance of a test organism to three or more different classes of antimicrobials (Magiorakos *et al.,* 2012). *Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* ATCC13883 were used as quality control strains for susceptibility testing.

The combined disc tests (CDT) were used for phenotypic screening and confirmation of extended-spectrum *B*-lactamases (ESBLs) expression according to CLSI (2020) recommendations. ESBL was determined by comparing the inhibition zone diameters (ZD) around cefpodoxime/clavulanic acid (10µg/1µg) disc to that of cefpodoxime (10µg) (without clavulanic acid). The test is positive if the inhibition ZD around the disc with the clavulanic acid is ≥ 5mm larger than the disc without it.

3.7 Phenotypic test for carbapenemase activity

The modified Hodge test (MHT) was used to test for phenotypic expression of carbapenemase activity, and multiplex PCR was employed to confirm the presence of specific carbapenemase-producing genes. All isolates resistant to meropenem; zone diameters (ZD) ≤ 23mm for Enterobacterales and ZD ≤ 14mm for *A. baumannii* (CLSI, 2020; Olu-Taiwo *et al.,* 2020) were phenotypically tested for carbapenemase activity by the MHT as recommended by CLSI 2020. Briefly, the indicator organism, *E. coli* ATCC 25922 was obtained by preparing an overnight broth culture, adjusted to 0.5 McFarland turbidity standard followed by a 10-fold dilution in saline. The broth was seeded onto the MHA plate (Biotec Ltd, UK) and meropenem (10µg) positioned at the centre. 3-5 colonies of the test isolate were then inoculated onto the plate in a line straight from the edge of the disc to the end of the plate and incubated overnight at 35-37^{o}C for 16-24 hours. The isolate is positive when a clover-leaf-like indentation of *the E. coli* ATCC 25922 grew along

the test organism's growth streak, within the disc diffusion zone. Figure 1 below shows positive and negative Modified Hodge Test results.

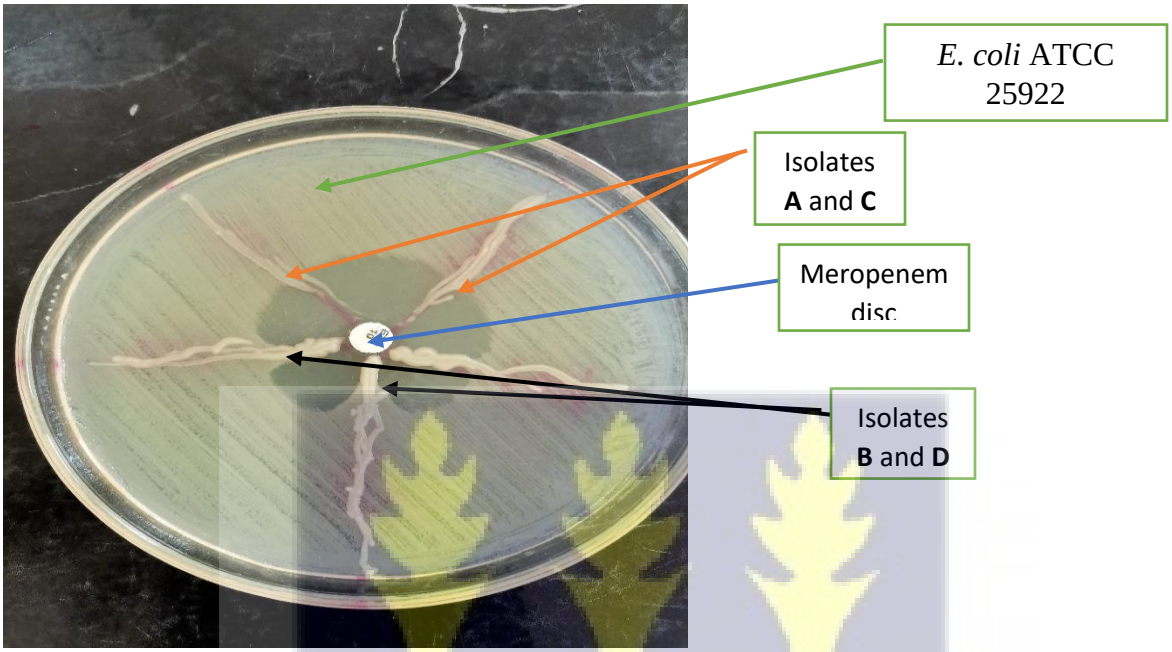

Figure 1: *Isolates A and C were Modified Hodge Test Negative, while isolates B and D were Modified Hodge Test Positive, showing clover-leaf indentation at the point of intersection between E. coli ATCC 25922 and test organism.*
Image Source: self

3.8 DNA Extraction and Analysis of Carbapenemase Genes

Extraction of DNA was done by the boiling lysis method as described by Ribeiro *et al*. (2016). All 69 carbapenem-resistant isolates were cultured overnight onto nutrient agar at 35-37ºC for 16-24 hours. Using sterile techniques, 3 colonies of the bacterial isolate was inoculated in 200µl of double distilled water and heated at 98ºC for 10 minutes, refrigerated for 10minutes at -20ºC, then centrifuged at 1,350rpm for 5minutes. 150µl of the supernatant (DNA template) was pipetted into 2ml Eppendorf tubes and stored at -20ºC until further molecular analysis.

3.8.1 Molecular characterisation of carbapenemase-producing genes

All 69 carbapenem-resistant isolates were investigated for the presence of the five most prevalent carbapenemase genes; NDM, KPC, VIM, OXA-48, and IMP by multiplex PCR as described by Obeng-Nkrumah *et al.*, (2019) and Poirel *et al* (2011). Table 1 below shows a list of primer sequences and amplicon sizes used for the detection of carbapenemase genes.

Table 1. **Table 1**: Primer sequences and amplicon sizes for carbapenemase genes

Gene	**Primer sequence (5'→3')**	**Amplicon size (bp)**	**Reference**
IMP	FP: GGAATAGAGTGGCTTAAYTCTC RP: CCAAACYACTASGTTACT	188	(Obeng-Nkrumah *et al.*, 2019)
VIM	FP: GATGGTGTTTGGTCGCATA RP: CGAATGCGCAGCACCAG	390	(Poirel *et al.*, 2011)
OXA- 48	FP: GCGTGGTTAAGGATGAACAC RP: CATCAAGTTCAACCCAACCG	438	(Poirel *et al.*, 2011)
NDM	FP: GAAGCTGAGCACCGCATTAG RP: TGCGGGCCGTATGAGTGATT	760	(Obeng-Nkrumah *et al.*, 2019)
KPC	FP: GTATCGCCGTCTAGTTCTGC RP: GGTCGTGTTTCCCTTTAGCC	683	(Obeng-Nkrumah *et al.*, 2019)

Using primers in Table 1, the PCR was run in two separate primer sets; primer sets 1 and 2. The first primer set included OXA-48 and KPC, whereas primer set 2 was made up of NDM, VIM and IMP. Each PCR reaction had 12.5µl of One Taq Quick-Load 2× Master Mix with Standard Buffer, 3.5µl of nuclease-free water, 7µl of primer, and 2µl of DNA template, to give a final PCR reaction volume of 25µl.

The applicable Biosystems Thermal cycler was used for amplification (Thermo Fisher Scientific, USA). For the first set of primers, the following cycling conditions were used; initial denaturation at 95ºC for 5 minutes, then 35 cycles of denaturation at 95ºC for 30 seconds, annealing at 60ºC for 30 seconds, and elongation at 72ºC for 1 minute, followed by a final elongation step at 72ºC for 3. The cycling conditions for the second primer set is summarised below;

Initial denaturation at 94ºC for 3 minutes, followed by 35 cycles of denaturation at 94ºC for 30 seconds, annealing at 61.6ºC for 30 seconds, and elongation at 72ºC for 1 minute, followed by a final elongation step at 72ºC for 7 minutes.

Following this, 5µl of each PCR reaction mixture was loaded onto a 2.0% agarose gel, containing SYBR red and electrophoresed for 45 minutes. The gel was visualized under UV illumination in a gel doc. The amplicon sizes were compared to a 100bp DNA ladder. Figure 2 below shows carbapenemase genes determined by multiplex PCR.

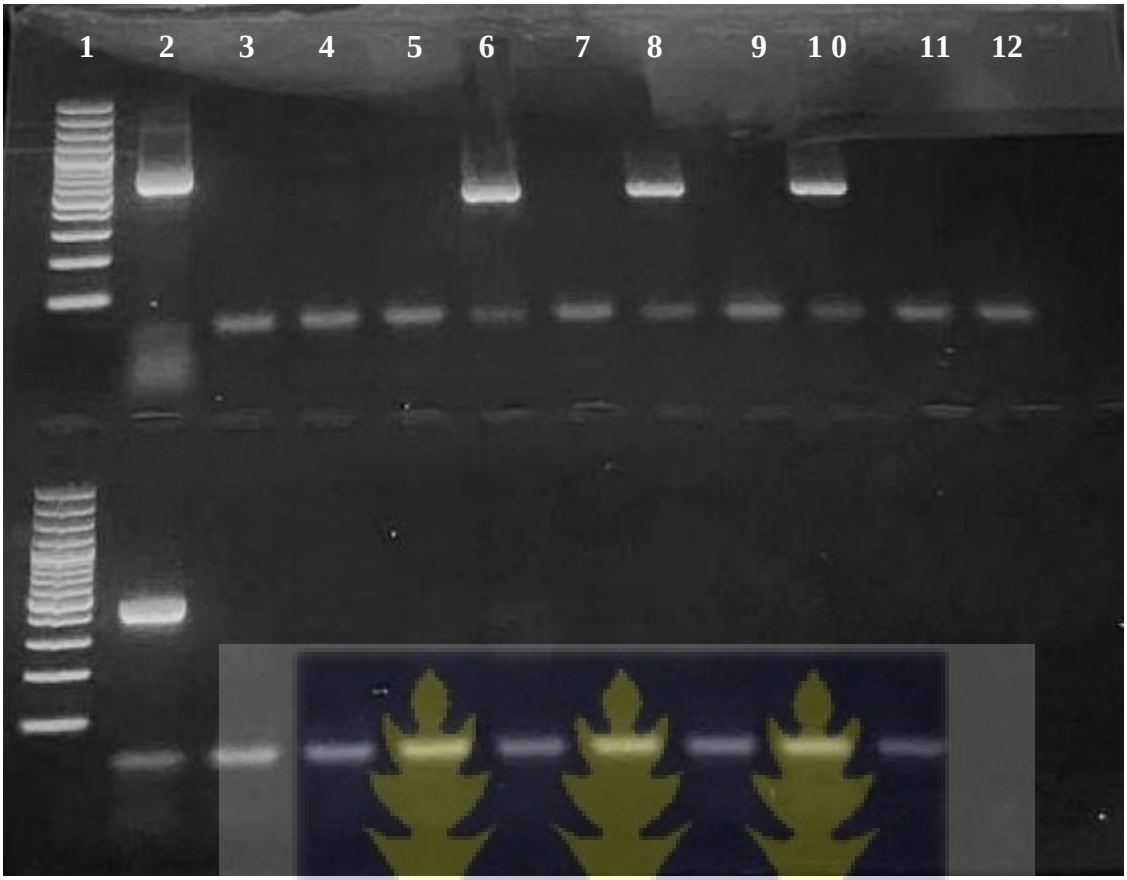

***Figure 2*:** Agarose gel electrophoresis image for amplification product of carbapenemase genes using multiplex PCR.

Lane 1: well 1= 100bp Ladder, well 2 = KPC Positive control, well 3: Negative control, wells 4 to 12: carbapenem-resistant isolates. Wells 6, 8 and 10, showing OXA-48 positive isolates (438bp)

Lane 2: well 1= 100bp ladder, well 2= OXA-48 Positive control, well 3= negative control, wells 4 to 11= carbapenem resistant isolates. No Carbapenemase gene detected.

3.9 Data Analysis

All data were entered into a Microsoft Access database, then exported and analysed using SPSS software version 25. Descriptive statistics (means, frequencies, percentages, and standard deviations) were used to summarize data on the participants' demographic and clinical characteristics, as well as the distribution of carbapenem-resistant Enterobacterales and *A. baumannii*, carbapenemase genes present, and antimicrobial resistance patterns. Also, at an alpha level of 0.05, a combination of bivariate associations and logistic regression analyses were conducted to determine risk factors for carriage with CRE among the study participants.

3.10.1 Ethical approval

The Ethical and Protocol Review Committee of the University of Ghana's College of Health Sciences granted the study ethical approval. CHS-Et/M.5- 5.10/2020-2021 is the protocol identification number. Before analysis, all data were anonymised and treated with strict confidentiality.

CHAPTER FOUR

RESULTS

4.1 Demographic and Clinical Characteristics of the Study Participants

In total, 299 paediatric inpatients aged 13 years and below (mean age = 47.8 ± 47.1 months) were recruited, with 61.9% (*n* = 155) being males, and 38.1% (*n* = 114) females. Most of the participants were referred from district healthcare facilities (42.1%, *n* = 126), and the majority of participants (24.1%, *n* = 72) recruited were from the Babies' Unit. Majority of the participants (56.5%, *n* = 169) had had a previous hospital admission within the past year (the mean previous and current hospital admission durations were 13.4 and 14.3 days, respectively), whereas (14%, *n* = 42) of the participants had previous ICU admissions, and (14%, *n* = 42) had a history of intra-nasal oxygen therapy with oxygen delivery devices in KBTH. With regards to history of invasive procedures in the past year, (91.3%, *n* = 273) had a history of intravenous device insertion over the past year, whereas (46.2%, *n* = 138) had past surgeries. 18.7% (*n* = 56) had a history of urethral catheterization, (5.4%, *n* = 16) had history of endotracheal intubation, (2%, *n* = 6) had a history of wound drain insertion, and (47.4%, *n* = 22) had had no such history. Furthermore, (7.4%, *n* = 22) and (6.4%, *n* = 19) of participants had previous exposure to chemotherapy and steroids respectively, in the past year. The clinical characteristics of the study participants are summarized in Table 2.

Table 2: Clinical characteristics of the 299 study participants

Characteristic	Number	Percentage (%)
Type of referral facility		
CHPS Compound	4	1.3
Polyclinic	74	24.7
District	126	42.1
Regional	57	19.1
Tertiary	38	12.7
Ward		
Paediatric Intensive Care Unit	20	6.7
Babies' Unit	72	24.1
Paediatric Surgical	58	19.4
Paediatric Medical	68	22.7
Oncology	33	11.0
Emergency Room	48	16.1
Previous admission within the past year		
Yes	169	56.5
No	130	43.5
Exposure to carbapenems within the past year		
Yes	37	12.4
No	262	87.6
ICU admission within the past year		
Yes	42	14
No	257	86
Comorbidities		
Malnutrition	2	0.7
Prematurity	9	3
Congenital heart disease	4	1.3
Hydrocephalus	2	0.7
CKD	6	2
Hypertension	20	6.7
History of intra-nasal oxygen use in the past year		
Yes	42	14.0
No	257	86.0
History of invasive procedure in the past year		
Nil	22	7.4
IV access	273	91.3
Surgery	138	46.2
Catheter	56	18.7
Tube	16	5.4
Wound drain	6	2.0
Exposure to immunosuppressants in the past year		
Chemotherapy	22	7.4
Steroids	19	6.4

Current admission duration = 14.3 ± 16.5 days; Previous admission duration = 13.4 ± 27.0 days

4.2 Distribution of Bacterial Isolates in Rectal Swabs of Study Participants

E. coli (60.5%, *n* = 208) was the most predominant bacteria isolated, followed by *Klebsiella pneumoniae* (29.9%, *n* = 103), then *Acinetobacter baumannii* (3.8%, *n* = 13). The distribution of the various bacteria isolates is summarized in table 3 below.

Table 3: Distribution of Bacteria Isolates

Organism	Number of Isolates	Percentage (%)
E. coli	208	60.5
Klebsiella pneumoniae	103	29.9
Acinetobacter baumannii	13	3.8
Enterobacter cloacae	8	2.3
Morganella morganii	5	1.5
Proteus mirabilis	4	1.2
Citrobacter braakii	2	0.6
Serratia marcescens	1	0.3
Total	344	100

4.3 Antimicrobial Resistance Patterns among the Isolates

The pooled rate of antimicrobial resistance for all bacteria decreased from (81.1%) cefotaxime, (77.6%) cotrimoxazole, (75%) cefpodoxime, (73.3%) cefipime, (51.7%) ciprofloxacin, (41.3%) gentamicin, and (20.1%) meropenem. The respective lowest and highest rates of antimicrobial resistance recorded for the three most predominant bacteria isolates (meropenem excluded) were as follows: *E. coli* (cefotaxime = 79.3%), *Klebsiella pneumoniae* (cefotaxime = 83.5%), and

Acinetobacter baumannii (ciprofloxacin = 90%; cefipime, cotrimoxazole, cefotaxime, gentamicin, and cefpodoxime = 100% each).

In addition, the overall prevalence of bacteria isolates that were multidrug-resistant was 75.6%, and was distributed among the most predominant bacteria isolates as; *E. coli* (74.0%), *Klebsiella pneumoniae* (76.7%), *Acinetobacter baumannii* (100%).

With regards to ESBL expression, 65.4% of Enterobacterales were ESBL producing and was distributed predominantly among *E. coli* (67.3%), and *Klebsiella pneumoniae* (72.8%). However, overall carriage of ESBL producing Enterobacterales was 72.6% (*n* = 217); with *E. coli* (46.8%, *n* = 140) and *K. pneumoniae* (25.1%, *n* = 75) being the most predominant ESBL phenotypes. The antimicrobial resistance rates and ESBL status of bacteria isolates are presented in Table 4.

Table 4: Resistance of bacterial isolates to the tested antimicrobials, as well as their MDR and ESBL proportions.

Organisms/Antimicrobials	MEM	CFPM	COT	CIP	CFTX	GEN	CFPD	MDR	ESBL
Escherichia coli	14.4%	74.5%	77.4%	49/5%	79.3%	34.1%	72.1%	74%	67.3%
Klebsiella pneumoniae	21.4%	73.8%	78.6%	56.3%	83.5%	49.5%	76..7%	76.7%	72.8%
Acinetobacter baumannii	76.9%	100%	100%	90%	100%	100%	100%	100%	NA
Morganella morganii	50%	50%	100%	25%	75%	25%	50%	75%	50%
Citrobacter braakii	0%	0%	100%	0%	0%	100%	100%	100%	0%
Proteus mirabilis	0%	0%	0%	0%	0%	50%	0%	0%	0%
Serratia marcescens	0%	100%	100%	100%	100%	100%	100%	100%	100%
Enterobacter cloacae	0%	25%	25%	25%	75%	25%	100%	50%	50%
All bacteria	20.1%	73.3%	77.6%	51.7%	81.1%	41.3%	75%	75.6%	65.4%

MEM = Meropenem; CFPM = Cefipime; COT = Co-trimoxazole; CIP = Ciprofloxacin; CFTX = Cefotaxime; GEN = Gentamicin; CFPD = Cefpodoxime, NA= Not applicable

4.4 Carriage of carbapenem-resistant Enterobacterales, *A. baumannii*, and Distribution of Carbapenemase Genes

Overall carriage of carbapenem resistant Enterobacterales and *A. baumannii* was (23.1%, *n* = 69), and was distributed among the bacteria isolates as follows: *E. coli* (11.0%, *n* = 33), *Klebsiella pneumoniae* (7.4%, *n* = 22), *Acinetobacter baumannii* (3.3%, *n* = 10) and *Morganella morganii* (1%, *n* = 3).

With regards to phenotypic carbapenemase activity, 52.2% (*n* = 36) of the carbapenem resistant isolates were MHT positive. Multiplex PCR results showed 46.4% (*n* = 32) of carbapenem resistant isolates harboured at least one carbapenemase gene. $Bla_{Oxa\text{-}48}$ (20.3%, *n* = 14) was the most widely distributed carbapenemase gene, followed by bla_{VIM} (15.9%, *n* = 11), then bla_{IMP} (5.8%, *n* = 4), and the bla_{NDM} (4.3%, *n* = 3). No carbapenem resistant isolate harboured a bla_{KPC} gene.

E. coli and *K. pneumoniae* harboured the majority of the carbapenemase genes and was distributed as follows: $bla_{Oxa\text{-}48}$ (*E. coli* = 71.4%, *n* = 10; *K. pneumoniae* = 21.4%, *n* = 3; and *M. morgannii* = 7.1%, n = 1); bla_{VIM} (*E. coli* = 54.6%, *n* = 6; *K. pneumoniae* = 36.4%, *n* = 4; and *M. morgannii* = 9.1%, n = 1); bla_{NDM} (*E. coli* = 66.7%, *n* = 2; *K. pneumoniae* = 33.3%, *n* = 1); and bla_{IMP} (*E. coli* = 50%, *n* = 2; *K. pneumoniae* = 50%, *n* = 2). No *A. baumannii* isolate harboured any carbapenemase gene.

Overall, 15.6% (*n* = 5) of bacteria isolates harboured 2 carbapenemase genes. 1 *K. pneumoniae* and 1 *E.coli* harboured both a $bla_{Oxa\text{-}48}$ and bla_{IMP}; 1 *E. coli* isolate harboured both $bla_{Oxa\text{-}48}$ and bla_{VIM}; 1 *E. coli* had a bla_{VIM} and bla_{NDM} carbapenemase genes, and 1 *K. pneumoniae* isolate harboured both bla_{NDM} and bla_{IMP} carbapenemase gene. No isolate harboured 3 or more carbapenemase genes. Table 5 below shows the distribution of the carbapenemase genes detected.

Table 5: Distribution of Carbapenemase Genes among Carbapenem Resistant Isolates

	CARBAPENEMASE GENES				
ISOLATE	***OXA-48***	***VIM***	***NDM***	***IMP***	***KPC***
E. Coli	10 (71.4%)	6 (54.6%)	2 (66.7%)	2 (50%)	Nil
K. pneumoniae	3 (21.4%)	4 (36.4%)	1 (33.3%)	2 (50%)	Nil
M. morganii	1 (7.1%)	1 (9.1%)	Nil (0%)	Nil (0%)	Nil
Total genes	**(20.3%, *n* = 14)**	**(15.9%, *n* = 11)**	**(4.3%, *n* = 3)**	**(5.8%, *n* = 4)**	**Nil**

4.5 Risk factors for Carriage of Carbapenem-resistant Enterobacterales and *A. baumannii* among the Study Participants

Of the variables investigated as potential risk factors for carriage of carbapenem-resistant Enterobacterales and *A. baumannii*, exposure to carbapenems and fluoroquinolones in the past year were associated with carriage of carbapenem-resistant Enterobacterales and *A. baumannii*. Both variables increased the odds of carriage of carbapenem-resistant isolates by approximately two folds. The results of the risk factor analysis are summarized in Table 6 below.

Table 6: Risk factors for carriage of carbapenem resistant Enterobacterales and *A. baumannii*

Risk factor	OR (95% CI)	*p* value
Exposure to carbapenems in the past year	2.178 (1.022–4.39)	0.044
Exposure to fluoroquinolones in the past year	2.420 (1.063–5.511)	0.035

CHAPTER FIVE

DISCUSSION

5.1 Prevalence and Distribution of MDR Enterobacterales and *A. baumannii* among hospitalized paediatric patients

This study investigated the risk factors for carriage of MDR pathogens, particularly carbapenem-resistant Enterobacterales and *A. baumannii*, and examined the molecular characteristics of the carbapenemase-producing genes present, among hospitalized paediatric patients in Ghana. This fills an important knowledge gap, particularly in Ghanaian paediatric referral centres where such data are grossly limited (Agyepong *et al.*, 2018; Labi *et al.*, 2020; Olu-Taiwo *et al.*, 2020).

The present study identified a high carriage of MDR Enterobacterales and *A. baumannii*, distributed among *E. coli*, *K. pneumoniae*, and *A. baumannii* as the three most predominant MDR organisms. The observed carriage is consistent with previous reports in Ghana by Agyepong *et al.* (2018) and Labi *et al.* (2020) but is significantly higher than what was reported in Tunisia (Hammami *et al.*, 2017). Antibiotic use is a significant risk factor for the development of AMR, and is known to vary between geographical locations (Chiotos *et al.*, 2017). This may be responsible for the difference in the observed prevalence.

Resistance to frequently used antibiotics in hospitalized children was high, decreasing across cefotaxime, cotrimoxazole, cefpodoxime, cefipime, ciprofloxacin, gentamicin, and meropenem. These findings are consistent with previous reports by Agyepong *et al.* (2020) in Ghana, where pathogen resistance to commonly used antibiotics was observed to be highest to most used antimicrobials, as opposed to less frequently used ones, decreasing across cotrimoxazole, cefotaxime, cefpodoxime, gentamicin, ciprofloxacin, cefipime, and meropenem. By contrast, the level of resistance observed in the present study to cefpodoxime, cefipime and meropenem were remarkably higher compared to

reports for cefpodoxime (44.4%), cefipime (8.5%), and meropenem (2.5%) from previous studies (Codjoe *et al.*, 2017; Agyepong *et al.*, 2018; Labi *et al.*, 2020). This may reflect increasing AMR among Enterobacterales and *A baumannii*, and in particular, increasing resistance of these top-priority pathogens to carbapenems in Ghana. Another reason for the disparity observed may stem from the fact that, whereas the previous studies focused on an array of clinical specimens from the general patient population, except for Labi *et al.* who focused on neonates, the present study focused exclusively on faecal carriage of these pathogens among paediatric inpatients.

5.2 Carriage and Distribution of Carbapenem-Resistant Enterobacterales and *A. baumannii* among Paediatric Inpatients

Similar to previous reports in Egypt (24%), India (39%), Israel (29%), and 19% carriage of carbapenem-resistant Enterobacterales and *A. baumannii* reported in Spain (Johnson *et al.*, 2017; Ghaith *et al.*, 2019), a high carriage (23.1%) of carbapenem-resistant Enterobacterales and *A. baumannii* was observed in the present study. However, this observation is largely at variance with the relatively lower rates (2.9%, 7.2% and 10%) previously recorded in Ghana by Codjoe *et al.* (2019), Hackman, *et al.* (2017) and Oduro (2016) respectively. The high rate observed in the current study may be suggestive of increased transmission of carbapenem-resistant isolates, particularly among paediatric inpatients at the Child Health Department, Korle-Bu Teaching Hospital. This result may also be due to the fact that this study focused exclusively on paediatric inpatients who may be at an increased risk for pathogen carriage, as opposed to the previous studies, which focussed on the general patient population.

However, carbapenem resistance was highest among *Acinetobacter baumannii*, followed by *K. pneumoniae*, and least among *E. coli*, similar to observations made in Egypt, Ghana, Tunisia, and China (Codjoe *et al.*, 2016; Hammami *et al.*, 2017; Agyepong *et al.*, 2018; Park *et al.*, 2019; Olu-Taiwo *et al.*, 2020). These carbapenem-resistant isolates were less susceptible to other commonly used

antibiotics such as cephalosporins, sulphonamides, and commonly used aminoglycosides, just as was reported by Agyepong *et al*., (2018) and Eichenberger *et al.* (2019).

5.3 Phenotypic and Molecular Characterization of Carbapenemase-producing Genes

A little over 52% of the carbapenem-resistant isolates in the present study exhibited phenotypic expression of carbapenemase activity by the MHT. However, Multiplex PCR revealed that 46.4% of carbapenem-resistant isolates harboured at least one carbapenemase gene, and less than 16% of these isolates harboured 2 carbapenemase-producing genes. None harboured 3 or more genes. This observation, although at variance with previous reports from Ghana (Codjoe *et al.*, 2016), agrees with findings in Egypt (Ghaith *et al.*, 2019), Tanzania (Mushi *et al.*, 2014) and China (Han *et al.*, 2020) where 45.3%, 35% and 49% of carbapenem resistant isolates harboured at least one carbapenemase gene respectively. This study also observed a high prevalence (15.6%) of multiple carbapenemase genes among carbapenemase producing isolates, an observation that is in variance with 7.2% prevalence observed in earlier studies in Ghana (Codjoe *et al*., 2016). This disparity may stem from the fact that the present study focused on paediatric inpatients, whereas previous studies focused on general patient populations. The increased carriage of carbapenem-resistant Enterobacterales and *A. baumannii* observed in the present study may have contributed to increased dissemination of carbapenemase genes. The present study also demonstrated that the MHT is a sensitive indicator for the detection of carbapenemase production as reported by Hara *et al.* (2013) and Nordmann *et al.* (2013).

Similar to previous reports in Ghana (Codjoe *et al.*, 2016; Codjoe *et al.*, 2019; Quansah *et al.*, 2019), the current study observed dominance of $bla_{Oxa\text{-}48}$ among the carbapenemase genes detected, occurring at a proportion of 20.3%, whereas the bla_{IMP} and bla_{NDM} genes occurred at relatively lower frequencies of 5.8% and 4.3%, respectively. Interestingly, this study detected a relatively higher proportion of bla_{VIM} (15.9%) genes compared to 7.2% reported by Codjoe *et al.*, (2016). This sharp contrast might

be indicative of increasing transmission of carbapenemases among isolates as indicated by Quansah *et al.* (2019), Labi *et al.* (2020) and El Kholy *et al.* (2020). However, no bla_{KPC} was detected in this study, which is consistent with finding in Ghana by Codjoe *et al.* (2016).

Furthermore, *E. coli* and *K. pneumoniae* were the most common carriers of carbapenemase genes. This observation is consistent with previous studies in Ghana (Codjoe *et al.*, 2019; Quansah *et al.*, 2019), Tunisia (Hammami *et al.*, 2017) and Egypt (Ghaith *et al.*, 2019). However, the carbapenemase gene distribution per species is relatively higher in the present study than previous studies in Ghana (Codjoe *et al.*, 2016; Labi *et al.* 2020), which might be giving credence to reports of increasing carbapenem-resistant determinants in the country. In particular, the increased report of *OXA-48* and *VIM* carbapenemase genes is a major cause of concern and a call for heightened surveillance of MDR pathogens, particularly surveillance of carbapenem-resistant Enterobacterales and *A. baumannii* in paediatric referral centres in Ghana, and the implementation of strict infection control measures, as well as comprehensive antimicrobial stewardship strategies.

In terms of ESBL carriage and distribution, the overall carriage of Enterobacterales that expressed phenotypic ESBL activity was almost 73%, with the most common ESBL producing pathogens being *E. coli* and *Klebsiella pneumoniae*. These observations are consistent with findings from previous studies in Ghana, Pakistan, and Egypt (Ghaith *et al.*, 2019; Labi *et al.*, 2020; Qureshi *et al.*, 2021).

5.4 Risk Factors for Carriage of Carbapenem-resistant Enterobacterales and *A. baumannii*

The findings from this study are consistent with previous studies in Ghana, China and other parts of the world, where risk factors for pathogen carriage were predominantly exposure to antibiotics, particularly carbapenems and fluoroquinolones (Chiotos *et al.*, 2017; Codjoe *et al.*, 2017; Moshiri *et al.*, 2018; Kim *et al.*, 2020; Labi *et al.*, 2020). Similarly, the current study identified previous exposure to carbapenems and fluoroquinolones in the past year as the main risk factors for carriage of carbapenem-resistant Enterobacterales and *A. baumannii*, increasing the odds of carriage of carbapenem-resistant isolates by

approximately two folds. This may be suggestive of increased use of broad-spectrum antibiotics, particularly fluoroquinolones and carbapenems in the treatment of infections. This reinforces the need for comprehensive antimicrobial stewardship strategies, especially the use of fluoroquinolones and carbapenems, to aid in the prevention and control of MDR.

CHAPTER SIX

CONCLUSION, LIMITATATION AND RECOMMENDATIONS

6.1 Conclusion

This study reports high faecal carriage of MDR Enterobacterales and *A. baumannii*, including carbapenem resistant phenotypes, with high levels of resistance to commonly used antibiotics, among paediatric inpatients at the Department of Child Health, Korle-Bu Teaching Hospital. Majority of the carbapenemase producing organisms carried $bla_{Oxa\text{-}48}$ and bla_{VIM}. Finally, the study identified previous antibiotic exposure; particularly exposure to carbapenems, and fluoroquinolones within the past year as significant risk factors for carriage of carbapenem-resistant isolates, increasing the odds of pathogen carriage by approximately two folds.

6.2 Limitations

Only a limited repertoire of carbapenemase genes were screened for, and several others may have been missed. Furthermore, the study did not determine whether or not the carbapenem resistant isolates were carried persistently.

6.3 Recommendations

Based on the findings of this study, I recommend that;

i. This study is extended to other vulnerable groups, and also continuous surveillance of MDR pathogens is enhanced, particularly among carbapenem-resistant Enterobacterales and *A. baumannii*, since this is imperative to monitor these resistance determinants.

ii. Sequencing of the carbapenemase producing isolates is carried out to help improve understanding of the molecular bases of their resistance, as well as the genetic relatedness of these genes with those identified in other geographical locations.

iii. Comprehensive antibiotic stewardship strategies are instituted to limit the clinical effects of carbapenem-resistant isolates.

iv. Additionally, the capacity and infrastructure for the detection of carbapenemase expression in clinical laboratories is be improved for the purposes of patient management and surveillance.

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APPENDIX I

CONSENT FORM

TITLE OF RESEARCH: "CARRIAGE OF MULTI-DRUG RESISTANT ENTEROBACTERALES AND *ACINETOBACTER BAUMANNII* AMONG HOSPITALISED PAEDIATRIC PATIENTS AT THE CHILD HEALTH DEPARTMENT, KORLE-BU TEACHING HOSPITAL"

Introduction:

I, Nelson Hukporti, an MPhil Medical Microbiology student of the University of Ghana, am requesting your child's participation in the research study mentioned above. I will explain the objective of this research to you, as well as the risks and benefits associated with participation so that you can decide whether or not to allow your child to partake in the study. Your decision to allow your child to participate in this research is totally voluntary. He/she is not under any compulsion to consent or partake in the study. You will not lose any privileges if you choose not to participate. You will be asked to sign this permission form in the presence of the study team if you accept to participate.

Objective of the Study: The objective of this research is to determine carbapenem-resistant Enterobacterales (CRE) and *Acinetobacter baumannii* associated risk factors as well as examine the molecular characteristics of carbapenemase-producing genes among hospitalized paediatric patients in Korle-Bu Teaching Hospital.

Carbapenem-resistant Enterobacterales (CRE) and *Acinetobacter baumannii* are highly pathogenic bacteria that are resistant to practically all antibiotics presently available. As a result, they've been linked to high rates of mortality and morbidity.

The effect of increasing rates of carbapenem resistance among Enterobacterales and *A. baumannii* and the paucity of data on these bacteria pathogens in Ghana, particularly in the paediatric population

necessitates a comprehensive study to identify CRE-associated risk factors and the molecular epidemiology of CRE infections among hospitalised paediatric patients. This study will also generate surveillance data on the infecting enterobacteriaceae, which will ultimately guide the paediatric management of CRE infections in Ghana.

The study will be carried out from February through to September, 2021. The clinical specimen which will be collected from participants is a stool specimen/rectal swab. This will be taken to the laboratory and screened for CRE. Molecular analysis will further be carried out to examine identified resistance genes.

Participant's data will be extracted from their medical records, per a structured questionnaire.

Risks and discomforts: Taking a stool sample is not an invasive procedure but could cause some inconvenience in producing the required amount of stool specimen or in taking rectal swabs.

Benefits: There is no immediate benefit to you, for your participation in this research. No gift or money will be given in exchange for the information obtained, or the stool specimen/rectal swab collected, from you. However, the data generated from this study will help the policymakers in the Ministry of Health in improving the management of diseases caused by CRE and *A. baumannii*. Furthermore, Stool specimen results will be reported to the patient's physician, to help guide his/her clinical care.
Throughout this study, information obtained on your child will be kept private, and you can be assured of the highest level of confidentiality. The samples taken from you will also be coded, and cannot be accessed by anyone who is not a part of the research team.

Complaints or Questions: For all study related issues, please contact Professor Japheth Opintan (0244789209) and Dr Appiah-Korang Labi (0244863056), all of the University of Ghana Medical School, Department of Medical Microbiology Korle-Bu or Mr Nelson Hukporti (0247736953), student, Department of Medical Microbiology, University of Ghana Medical School.

Consent for inclusion: If you agree for your child to participate in this study, please complete the form below:

I.....…… on this day …….. (Day/Month/Year) certify that I have read and understand the consent form's explanations, and that I give Mr Nelson Hukporti permission to include my child in the study titled **"Carbapenem-Resistant Enterobacteria Colonization and Infection among Hospitalised Paediatric Patients in Korle-Bu Teaching Hospital",** I understand that when the information sheet and informed consent papers have been signed, I will be given a copy to take home.

I have read the consent form and consent that my child is freely participating in this research project.

_____ _____

Signature/Thumb print of Participant Date

_____ _____

Signature/Thumb print of Obtaining Consent Date

Witness Statement: I was present when the volunteer was informed of the benefits, hazards, and procedures. All of the questions were answered, and the volunteer confirmed that his or her child would participate in the study voluntarily.

_____ _____

Signature/Thumb print of Participant Date

_____ _____

Signature/Thumb print of Obtaining Consent Date

APPENDIX II

RESEAECH QUESTIONNAIRE

UNIVERSITY OF GHANA MEDICAL SCHOOL

RESEARCH TOPIC: CARRIAGE OF MULTI-DRUG RESISTANT ENTEROBACTERALES AND *ACINETOBACTER BAUMANNII* AMONG HOSPITALISED PAEDIATRIC PATIENTS AT THE CHILD HEALTH DEPARTMENT, KORLE-BU TEACHING HOSPITAL.

STUDY ID: **DATE:**

1. What is the Age of participant?
2. What is the gender of participant? A. male **[ ]** B. female **[ ]**
3. Which level of facility has participant being referred from?
4. Which ward or unit is participant being managed?
5. How long has participant been on admission?
6. Has participant been previously admitted to another health facility?
   If yes, what level of facility?
7. Has participant ever been admitted to this facility? If yes, what was the duration of admission?
8. With regards to question 7 above, what was participant managed for?
9. What are the presenting Signs and symptoms of participant?
   ……
10. What is the primary diagnosis?
11. Any prior exposure to Carbapenems? A. Yes [ ] B. No [ ] C. Not sure [ ]
12. Any prior exposure to sulphonamides? A. Yes [ ] B. No [ ] C. Not sure [ ]
13. Any exposure to other class of antibacterial agents?

If yes, what class of antibacterial agent?

14. Has participant ever been admitted to the Intensive Care Unit (ICU)?

15. Does participant have any co-morbidities? Please specify if any……..…….

16. Any surgical history?

If yes, please specify ……

17. Any history of invasive procedure on admission?

Please state if any…….

18. Any prior exposure to immunosuppressive treatments?

If yes, please specify……

THANKS FOR YOUR PARTICIPATION

PRINCIPAL INVESTIGATOR: HUKPORTI NELSON

(MPHIL STUDENT @ UNIVERSITY OF GHANA MEDICAL SCHOOL)

PRINCIPAL SUPERVISORS: Prof. JAPHETH A. OPINTAN

Dr. APPIAH-KORANG LABI

(LECTURERS @ UNIVERSITY OF GHANA MEDICAL SCHOOL)

APPENDIX III

LABORATORY PROTOCOLS

1. Preparation of Agarose Gel (Lee et al., 2012)

i. Weigh out the appropriate mass of agarose into an Erlenmeyer flask. Agarose gels are prepared using a w/v percentage solution. The concentration of agarose in a gel will depend on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%. The volume of the buffer should not be greater than 1/3 of the capacity of the flask.

ii. Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).

iii. Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.

iv. Add ethidium bromide (EtBr) to a concentration of 0.5 µg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 µg/ml EtBr for 15-30 min, followed by destaining in running buffer for an equal length of time.

v. Allow the agarose to cool either on the benchtop or by incubation in a 65 °C water bath. Failure to do so will warp the gel tray.

vi. Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells.

vii. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use.

2. Setting up of Gel Apparatus and Separation of DNA Fragments

i. To separate the DNA samples, add loading dye. Typically, gel loading dye is generated at a concentration of 6X. (0.25 percent bromphenol blue, 0.25 percent xylene cyanol, 30 percent glycerol). The loading dye keeps track of how far your DNA sample has moved while also allowing it to sink into the gel.

ii. Set the voltage on the power supply to the required level (1-5V/cm from one electrode to the next).

iii. Add enough running buffer to completely cover the gel's surface. It's critical to utilize the same running buffer as the gel preparation buffer.

iv. iv. Connect the power supply's leads to the gel box's leads. Switch on the power supply and double-check that the gel box and the power supply are both operational.

v. Take off the cover. Load the DNA sample(s) into the gel slowly and carefully. Along with experimental materials, an appropriate DNA size marker should always be loaded.

vi. Close the gel box and replace the lid. The anode (white leads) should be closer to the wells than the cathode (black leads) (red leads). Double-check that the electrodes are inserted into the right power supply slots.

vii. Turn the power on. Continue to run the gel until the dye has migrated to the desired distance.

3. Viewing of Separated DNA Fragments

i. Turn off the power and remove the lid of the gel box when the electrophoresis is finished.

ii. Take the gel out of the gel box. Remove any excess buffer from the gel's surface. To absorb any surplus flowing buffer, place the gel tray on paper towels.

iii. Remove the gel from the gel tray and place it in front of a UV light source. The most typical method is to use a gel documentation system. Orange luminous bands should appear as DNA bands. Snap a photo of the gel.

iv. Dispose of the gel and running buffer according to the institution's rules.

APPENDIX IV

PROTOCOL FOR DNA EXTRACTION

Extraction Protocol: Modified from (Ribeiro et al., 2016) Protocol

- Pipette 200µl of double distilled water into Eppendorf tubes
- Transfer three (3) well isolated colonies in the tube and heat at 98^{o}C for 10 minutes.
- Centrifuge at 1,350rpm for 5minutes and discard supernatant
- Aliquot 150µl of the supernatant (DNA template) into 2ml Eppendorf tubes and store in -20^{o}C until further works.

APPENDIX V

PCR MASTER MIX PREPARATION

Table 7: Proportion of ingredients for the preparation of Master mix

Components	25µl Reaction	Volume (25µl)	Volume (µl) for 69 isolates
One Tag Quick-Load	12.5	12.5	825
10µM Forward primer	3.5	3.5	241.5
10 µM Reverse primer	3.5	3.5	241.5
Template DNA	2	2	X
Nuclease-free water	3.5	3.5	241.5

Table 6 Components and concentrations used in the preparation of the PCR master mix.

Table 8: Thermomocycling conditions for the carbapenemase genes (35cycles)

Components	Temperature (^{0}C)	Time
Initial Denaturation	95	5 minutes
Final Denaturation	95	30 seconds
Annealing	60	30 seconds
Extension	72	1 minute
Final Extension	72	3 minutes
Hold	4	

APPENDIX VI

GELS AND OTHER IMAGES

Figure 3: Image of gel electrophoresis

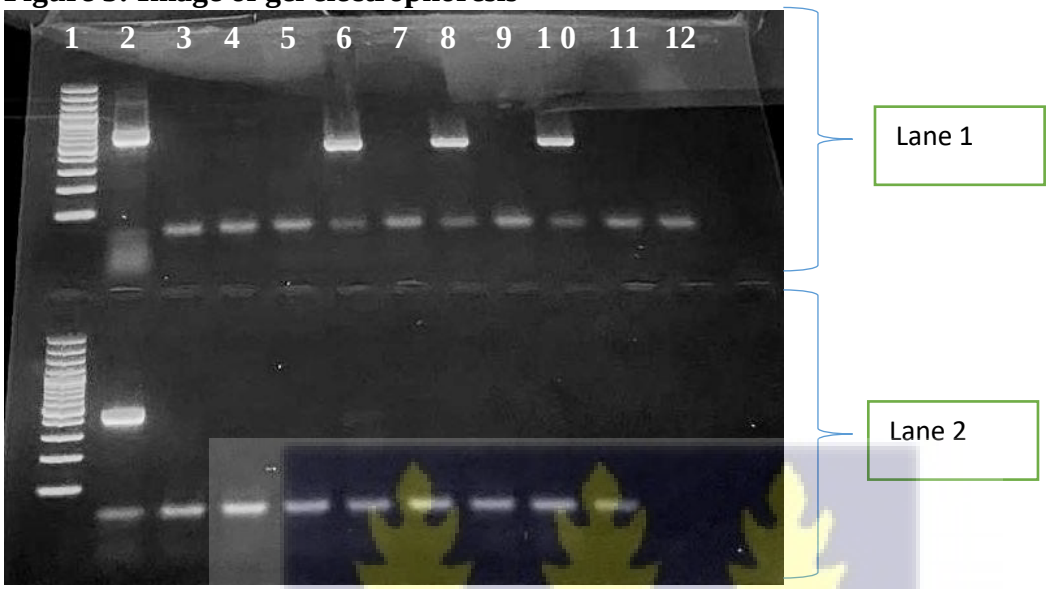


***Figure 3*: Gel 1;** *Agarose gel electrophoresis image for amplification product of carbapenemase genes using multiplex PCR*

Lane 1: well 1= 100bp Ladder, well 2 = KPC Positive control, well 3: Negative control, wells 4 to 12: carbapenem resistant isolates. Wells 6, 8 and 10, showing OXA-48 positive isolates (438bp)

Lane 2: well 1= 100bp ladder, well 2= OXA-48 Positive control, well 3= negative control, wells 4 to 11= carbapenem resistant isolates. No Carbapenemase gene detected.

Figure 4*: Gel 2;* *Agarose gel electrophoresis image for amplification product of carbapenemase genes using multiplex PCR*

GEL 2

Lanes 1and 24 – 100bp Ladder

Lane 2 & 3 – Positive controls; KPC (683bp) & OXA-48 (438bp)

Lanes 4 to 22 – samples

Lane 13 - Sample with *OXA-48* gene

Lane 23 – Negative control

Figure 5*: Gel 3;* *Agarose gel electrophoresis image for amplification product of carbapenemase genes using multiplex PCR*

GEL 3

Lanes 1 and 20 – 100bp Ladder

Lane 2 & 3 – Positive controls KPC (683bp) & OXA-48 (438bp)

Lane 4 to 19 – samples

Lanes 7, 13, 17 & 19 - Samples with OXA-48 genes

Figure 6*: Gel 4;* *Agarose gel electrophoresis image for amplification product of carbapenemase PCR* **Gel 4**

Lane 1 – Ladder

Lane 2 – sample (VIM 390bp)

Lane 3 to 11 – samples

Lane 12 – Negative control

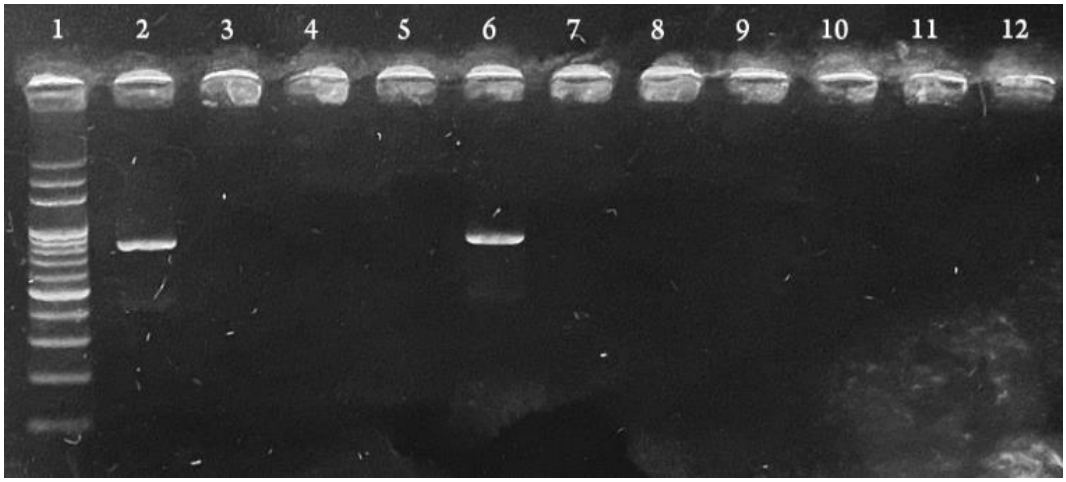


Figure 7*: Gel 5;* *Agarose gel electrophoresis image for amplification product of carbapenemase genes PCR* **Gel 5**

Lane 1 – Ladder; Lane 2 to 11 – samples

Lane 2 & 6 – sample (NDM 760bp)

Lane 12 – Negative control

Figure 8: *Image of the researcher*

Figure 9: *An image of the Becton Dickinson Phoenix Spec™ Nephelometer, showing inoculum turbidity of 0.5 McFarland.*

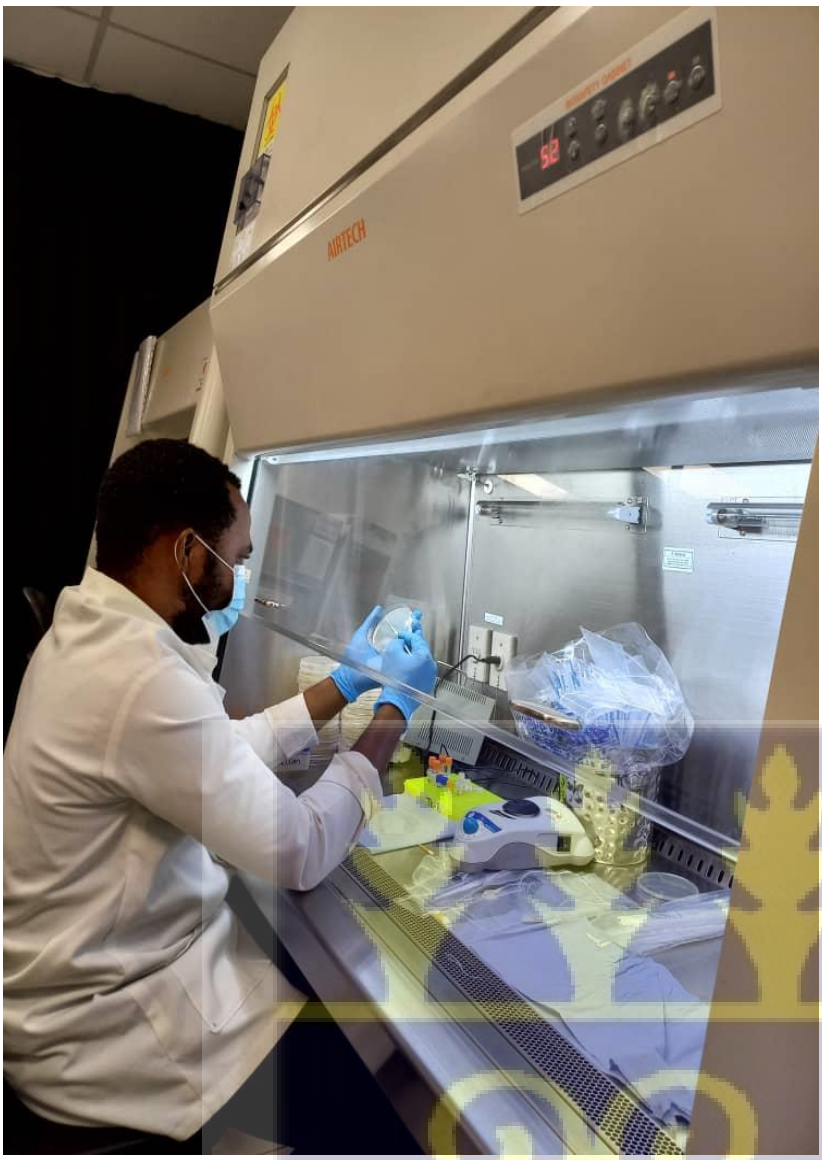
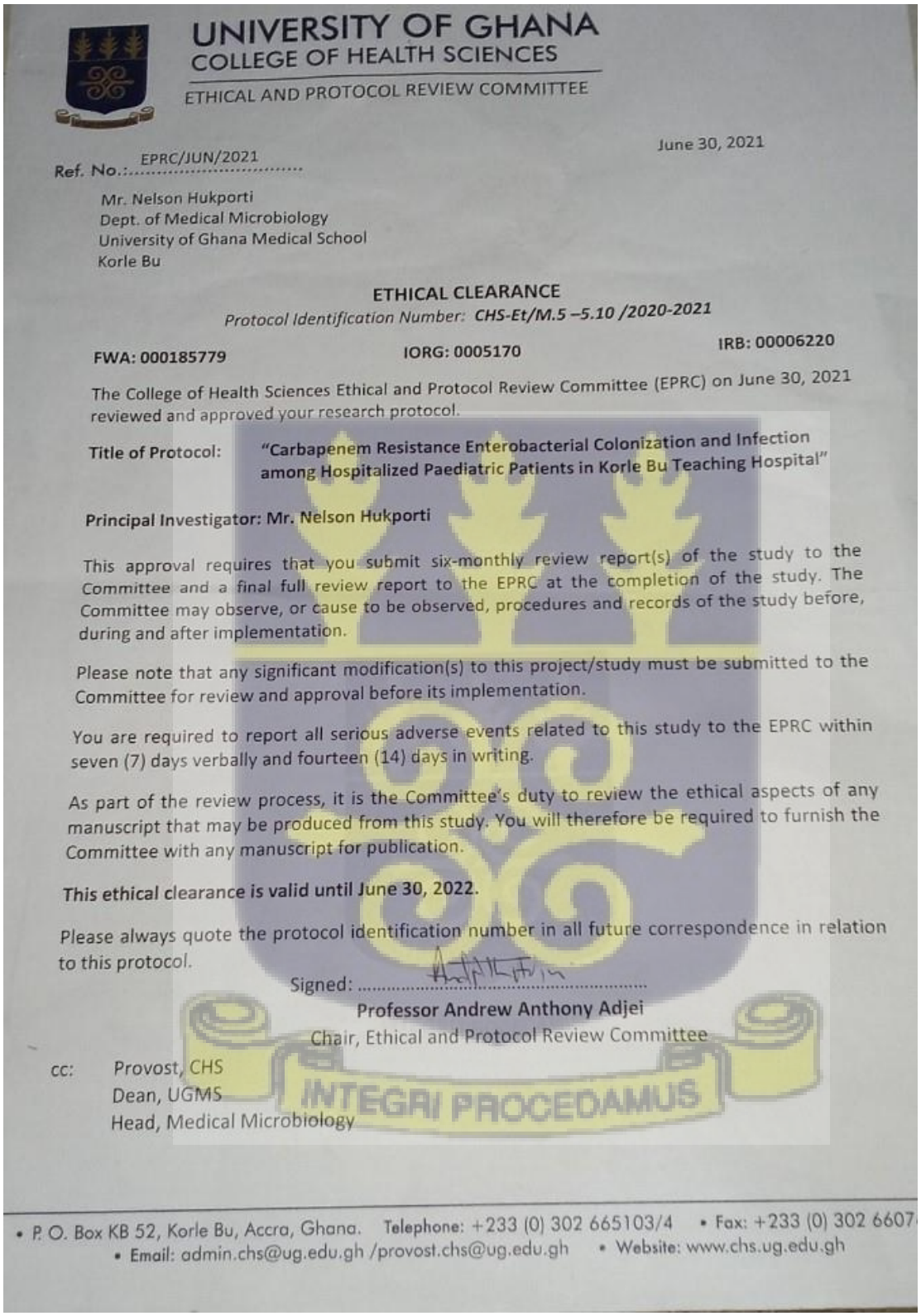


Figure 10: *A photo of the researcher working*

APPENDIX VII

ETHICAL CLEARANCE

Figure 11: Letter of approval for Ethical clearance



UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/JUN/2021

June 30, 2021

Mr. Nelson Hukporti
Dept. of Medical Microbiology
University of Ghana Medical School
Korle Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.5 –5.10 /2020-2021***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on June 30, 2021 reviewed and approved your research protocol.

Title of Protocol: **"Carbapenem Resistance Enterobacterial Colonization and Infection among Hospitalized Paediatric Patients in Korle Bu Teaching Hospital"**

Principal Investigator: Mr. Nelson Hukporti

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid until June 30, 2022.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, UGMS
Head, Medical Microbiology

INTEGRI PROCEDAMUS

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