Molecular characterization of multi-drug resistant (MDR) Gram-negative bacterial pathogens from environments, patients and staff in a teaching hospital in Ghana

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A thesis submitted to the Discipline of Pharmaceutical Sciences, School of Health Sciences, University of KwaZulu-Natal, for the degree of Doctor of Philosophy (Pharmacy). This is a thesis in which the chapters are written as a set of discrete research manuscripts, published or intended for submission to peer-reviewed journals, with a general introduction and final summary.

This is to certify that the content of this thesis is the original research work of Ms. Esther Eyram Asare Yeboah, carried out under our supervision at the Antimicrobial Research Unit (ARU), Discipline of Pharmaceutical Sciences, School of Health Sciences, Westville Campus, University of KwaZulu-Natal (UKZN), Durban, South Africa.

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DECLARATION

I, **Esther Eyram Asare Yeboah**, declare that

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DEDICATION

This study is dedicated to my children El-Iyanah, El-Zoe and Jireh-Manuel, who have been a motivation to complete my study in spite of a busy schedule and family life.

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LIST OF MANUSCRIPTS INCLUDED IN THIS THESIS

1. Multidrug resistant Gram-negative bacterial carriage in patients, healthcare workers and environments of a tertiary hospital in Ghana.

   Esther Eyram Asare Yeboah, Nicholas Agyepong, Daniel Gyamfi Amoako, Akebe Luther King Abia, Alexander Owusu-Ofori, Sabiha Yusuf Essack

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2. Genomic characterization of multi drug resistant ESBL-producing *Escherichia coli* isolates from patients and patient environments in a teaching hospital in Ghana.

   Esther Eyram Asare Yeboah, Nicholas Agyepong, Joshua Mbanga, Daniel Gyamfi Amoako, Akebe Luther King Abia, Arshad Ismail, Alexander Owusu-Ofori, Sabiha Yusuf Essack

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3. Genomic analysis of porin related resistance in ESBL-producing *Klebsiella pneumoniae* isolates from patients and environments in a teaching hospital in Ghana.

   Esther Eyram Asare Yeboah, Joshua Mbanga, Daniel Gyamfi Amoako, Nicholas Agyepong, Akebe Luther King Abia, Arshad Ismail, Alexander Owusu-Ofori, Sabiha Yusuf Essack

   Formatted for submission to Microbial Drug Resistance

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LIST OF ABBREVIATIONS

AAC	Aminoglycoside acetyltransferase
ABC	ATP-binding cassette
AME	Aminoglycoside-modifying enzyme
AMR	Antimicrobial resistance
AMS	Antimicrobial stewardship
ARG	Antibiotic resistant gene
BSI	Bloodstream infection
BV-BRC	Bacterial and Viral Bioinformatics Resource Center
CAT	Chloramphenicol acetyltransferase
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CRE	Carbapenem resistant Enterobacterales
ESBL	Extended-spectrum β-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GNB	Gram-negative bacteria
GRAM	Global Research on Antimicrobial Resistance
HAI	Healthcare associated infection
HCW	Healthcare worker
HeFRA	Health Facilities Regulatory Agency
HGT	Horizontal gene transfer
ICU	Intensive care unit
IPC	Infection prevention and control

iToL	Interactive tree of life
KPC	*Klebsiella pneumoniae* carbapenemase
LMIC	Low- and middle-income country
MALDI-TOF	Matrix-assisted laser desorption ionization–time-of-flight
MATE	Multi-drug and toxic compound extrusion
MBL	Metallo-β-lactamase
MDR	Multidrug resistant
MDRO	Multidrug resistant organism
MFS	Major facilitator superfamily
MGE	Mobile genetic element
MLST	Multi locus sequence typing
MS	Mass spectrometry
NDM	New Delhi metallo- β-lactamase
NICU	Neonatal intensive care unit
OR	Odds ratio
PBP	Penicillin binding-protein
PDR	Pan-drug resistance
PFGE	Pulsed-field gel electrophoresis
QAC	Quaternary ammonium compound
RND	Resistance nodulation cell division
RTI	Respiratory tract infection
SDC	Surface disinfectant cleaner
SSI	Surgical site infection
ST	Sequence type
UTI	Urinary tract infection
VIM	Verona integron encoded metallo- β-lactamase
WASH	Water, sanitation and hygiene
WGS	Whole genome sequencing
WHO	World Health Organization
XDR	Extremely drug-resistant

ABSTRACT

Multidrug resistant Gram-negative bacteria (MDR GNB) are implicated in serious infections both of community and nosocomial origin and may be disseminated in the hospital in the absence of efficient infection prevention and control (IPC) practices. The prevalence and risk factors for rectal colonization of MDR GNB among patients, the carriage of MDR GNB on healthcare workers' (HCWs') hands and the contamination patients' environments with MDR GNB were investigated in a teaching hospital in Ghana.

In this prospective study, conducted between April 2021 to July 2021, the phenotypic profiles of the MDR GNB isolates were determined using the VITEK 2 system. Risk factors for colonization with MDR GNB were assessed using univariate and multivariate analysis of associated data. The resistome, virulome, mobilome and genetic relatedness of MDR extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and ESBL-producing or carbapenem resistant *Klebsiella pneumoniae* isolates from patients and their environment were also determined using whole genome sequencing performed on the Nextseq 550 (2 x 150 bp) and bioinformatics analysis.

A total of 585 samples were collected from patients, HCWs' hands and the hospital environment within the study period. The prevalence of MDR GNB rectal colonization among patients was 50.62% on admission and 44.44% after 48 hours. MDR GNB, frequently *E. coli and K. pneumoniae* were isolated from 6 (5.26%) and 24 (11.54%) of HCW's hand swabs and environmental swabs, respectively. Previous hospitalization (*p*-value = 0.021, OR,95% CI= 7.170

(1.345-38.214) was significantly associated with colonization by MDR GNB after 48 hours of admission while age (21-30 years) (*p*-value =0.022, OR, 95% CI =0.103(0.015-0.716) was significantly identified as a protective factor associated with a reduced risk of rectal MDR GNB colonization.

Rectal carriage and acquisition of ESBL-producing *E. coli* among patients was 13.65% and 11.32% respectively. *bla*$_{\text{TEM-1B}}$ and *bla*$_{\text{CTX-M-15}}$ were commonly associated with IncFIB plasmid replicons and co-occurred with aminoglycoside, macrolide, and sulfamethoxazole/trimethoprim resistance. Multiple virulence genes, predominantly, *terC* were detected in the ESBL *E. coli* isolates. Sequence types (STs) were diverse and included one novel ST (ST13846) present in two isolates. Phylogenetic analysis grouped the ESBL *E. coli* isolates into four main clusters. High genetic relatedness was observed between two carriage isolates of ST940 and between a carriage isolate and an environmental isolate of ST648. Isolates with different STs, collected at different times and locations, also showed genetic similarities.

Of the ten selected MDR *K. pneumoniae* isolates, the β-lactamase gene, *bla*$_{\text{CTX-M-15}}$ was observed in six isolates. Mutations were found in both *ompK36* and *ompK37* in all isolates (both carriage isolates and isolates from hospital environments). Genes encoding resistance to fluoroquinolone (*qnrB*), aminoglycosides (*aadA1, aadA2, aac(3)-IIa, aac(6')-Ib-cr,aph(3'')-Ib , aph(6)-Id*) sulphamethoxazole/trimethoprim (*sul1, sul2, dfrA14, dfrA15*) were also detected. The *K. pneumoniae* isolates belonged to seventeen different STs with ST39 most commonly observed and common to both carriage isolates and isolates from hospital environments. A myriad of virulence genes, including *irp1, irp2, iutA, gndA, ompA, fes, fep, mrkD* and *fimH* were detected in both carriage and isolates from the hospital environment. IncFIB was the most abundant plasmid replicon occurring in nine (four carriage isolates and five isolates from hospital environments).

ESBL-producing *K. pneumoniae* isolates appeared to be introduced into the hospital from the community.

The high colonization of MDR GNB in patients, the carriage of MDR GNB on HCW's hands, the contamination of hospital environments and the circulation of ESBL-producing *E. coli* and *K. pneumoniae* isolates with diverse genomic characteristics, highlights the need for patient screening, and stringent infection prevention and control practices to prevent the spread of MDR GNB in hospitals. The observed clonal relatedness among isolates from patients and the hospital environment, as well as between different patients, suggests a possible transmission within and between sources, hence infection prevention and control practices need to be enhanced to prevent the dissemination and transmission of these resistant strains in the hospital. This study further highlights the usefulness of whole genome sequencing as an effective tool in AMR surveillance.

CHAPTER ONE – INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

There is increasing concern of antibiotic resistance among Gram-negative bacteria (GNB) worldwide especially as they are the leading cause of nosocomial infections with high rates of mortality and morbidity globally (Bassetti et al., 2019; Morris & Cerceo, 2020; Reynolds et al., 2022). Studies have shown that the hospital which serves as the immediate patient environment is a conduit for the spread of multidrug resistant pathogens (Huber et al., 2014; Suleyman et al., 2018). These multidrug resistant isolates can be transferred through contact with healthcare personnel, other patients as well as the hospital environment which includes but is not limited to equipment such as ventilators, endoscopes, beds, sinks, door handles and drip stands (Alrabaa et al., 2013; Bhatta et al., 2018; Sydnor & Perl, 2011). The emergence and spread of multidrug resistant microorganisms has resulted in limited antimicrobial compounds efficient enough to eliminate these microorganisms even with extensive and costly cleaning practices (Abreu et al., 2013; Church & McKillip, 2021). GNB with biocide/metal resistance genes may develop resistance to antibiotics through outer membrane changes and efflux mechanisms (Pal et al., 2015). Isolates from patients are frequently identical to other strains isolated from the immediate patient environment in nosocomial infections (Kirkgöz & Zer, 2014; Yan et al., 2019). A number of studies have indicated that hospital-acquired infections are commonly due to inefficient disinfection (Anderson et al., 2017; Doll et al., 2018; Hu et al., 2015).

Antibiotic resistant isolates have been reported in various studies in Ghana. In Ghana, resistance to antibiotics has been attributed to inappropriate use of antibiotics, inadequate monitoring and surveillance systems, unreported treatment failures as well as their use in animals (Gyansa-Lutterodt, 2013; Yevutsey et al., 2017). The highest mean resistance rate in bacterial isolates in

Ghana is reported in *Escherichia coli* (62.2%), *Klebsiella* spp. (60.4%) and *Pseudomonas* spp. (52.1%) (García-Vello et al., 2020). According to the GLASS report, a resistance of 64.3% to third generation cephalosporins was reported on *E. coli* isolates from blood stream infections in Ghana in the year 2020 (WHO, 2022). In a study to describe the prevalence and profile of infections attributable to multidrug-resistant GNB among patients at the Komfo Anokye Teaching Hospital in the Ashanti region of Ghana, multidrug resistance was evident in 89.5% in GNB from patient samples (Agyepong et al., 2018).

GNB such as *Acinetobacter* spp. and *Pseudomonas aeruginosa* have been reported to thrive even on dry surfaces in hospitals for as long as four months, and for more than 20 days on glass surfaces at room temperature as they require little to grow (Fazeli et al., 2012; Nowroozi et al., 2014; Rossolini & Mantengoli, 2005). Inanimate surfaces and equipment particularly in intensive care unit (ICUs) are commonly contaminated by bacteria, including Multidrug resistant (MDR) GNB which may contribute to ICU-acquired colonization or infection but further studies are required to determine the correlation (Russotto et al., 2015). The pre-disposing factors for infection with MDR GNB includes prolonged hospitalization, critical health conditions, invasive medical procedures such as use of mechanical ventilation and catheters, surgeries, broad spectrum antibiotic use, and co-morbidities such as diabetes, liver and renal impairment (Lat et al., 2019; Rosa et al., 2014; Vasudevan et al., 2013). Healthcare workers' (HCWs) hands are major sources of transmission of nosocomial pathogens often acquired through direct contact with patients, body fluid secretions, or touching contaminated environmental surfaces in the inpatient units (Tajeddin et al., 2016).

This study sought to phenotypically and genotypically delineate the resistance profiles among MDR GNB bacterial isolates from hospital patients, personnel and environments in a teaching

hospital in Ghana in order to evaluate and improve infection prevention and control (IPC) policies and practices in hospitals.

2.0 Literature review

This section contains a brief overview of literature on antibiotic resistance, the molecular mechanisms of resistance in GNB characterization of their resistance genes and infection prevention and control practices.

2.1 Antibiotic resistance as a global challenge

Antibiotics which became a breakthrough for treatment of infectious diseases are rapidly becoming ineffective due to the upsurge of antibiotic resistance which has been attributed to the overuse and misuse of antibiotics both in humans and in animals (Essack et al., 2016; Serwecińska, 2020).. Poor infection control practices have also been identified as a contributing factor to the spread of antibiotic resistant organisms (Sengupta et al., 2019; Tajeddin et al., 2016). AMR has threatened the efficient delivery of healthcare and has heightened the risk of infection associated with surgeries, childbirth , organ transplant and cancer treatment (Marston et al., 2016). The dearth of new antibiotics has further compounded the problem, calling for doubled efforts in managing this global health challenge (Kempf & Rolain, 2012; Tacconelli et al., 2018). Studies have shown a direct relationship between antimicrobial use (AMU) and the development of resistance (Ventola, 2015). In the ESKAPE group of organisms i.e. *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp., MDR has emerged resulting in treatment failures (De Oliveira et al., 2020; Santajit & Indrawattana, 2016).

AMR could lead to the death of an estimated 10 million people per year globally by 2050 according to the Review on Antimicrobial Resistance (O'Neill, 2014). In developing countries, increased morbidities and mortalities from drug-resistant infections have been reported with high rates of AMR to commonly prescribed antibiotics (Godman et al., 2021; Tadesse et al., 2017). According to a report from the Global Research on Antimicrobial Resistance (GRAM) project, an estimated 1.27 million deaths occurred globally in 2019 as a result of antibiotic resistance, with the greatest burden in Western sub-Saharan Africa with 27·3 deaths per 100 000 (20·9–35·3) (Murray et al., 2022), particularly in lower respiratory infections, bloodstream infections, and intra-abdominal infections (Murray et al., 2022).

The effective treatment of infections remains a burden in Ghana due to increasing AMR. Donkor et al. (2023), in a recent multicentric study of 11 hospitals in Ghana that determined AMR in GNB such as non-typhoidal salmonella and *K. pneumoniae* implicated in bloodstream infections reported high resistance to antibiotics, including penicillins, third-generation cephalosporins, and fluoroquinolones which are prescribed as first- or second-choice antibiotics for bloodstream infections in Ghana (Donkor et al., 2023).

Multidrug resistance is the resistance of microorganisms to one or more antibiotics in three or more distinct antibiotic classes. Some GNB have developed resistance to almost or all antibiotic options available and this has been described as extremely drug-resistant (XDR) phenotypes (Rossolini et al., 2014). Pan-drug resistant (PDR) bacteria exhibit resistance to all agents in all antimicrobial categories (Magiorakos et al., 2012).

In low and middle income countries, multidrug resistant bacteria are a huge problem, the spread of which is exacerbated by sub-optimal water, sanitation and hygiene (WASH), inadequate IPC

measures, lack of representative surveillance and inadequate antimicrobial stewardship programs and policies, posing a greater public health threat (Ayukekbong et al., 2017).

2.2 Mechanisms of antibiotic resistance

Bacteria may develop resistance to antibiotics either by mutations in genes associated with the mechanism of action of the compound and/or by acquiring foreign DNA coding for resistance determinants through horizontal gene transfer (HGT) (Munita & Arias, 2016). The mechanisms of resistance in bacteria include enzymatic action, modification of drug binding site, reduced uptake and prevention of entry mechanisms.

2.2.1 Enzymatic action

Bacteria may produce enzymes such as β-lactamases, aminoglycoside-modifying enzymes (AMEs), or chloramphenicol acetyltransferases (CATs) which modify or render the antibiotics inactive (Egorov et al., 2018; Santajit & Indrawattana, 2016).

CAT are acetyltransferases, classified either as Type A or Type B are usually expressed chromosomally or carried by mobile genetic elements to modify chloramphenicol. They are present in both Gram-positive bacteria and GNB (Munita & Arias, 2016).

AMEs reduce affinity of aminoglycosides and fluoroquinolones thereby reducing binding to the 30S ribosomal subunit (Kapoor et al., 2017). The AMEs which include phosphoryl-transferases, nucleotidyl-transferases or adenylyl-transferases, and aminoglycoside acetyltransferases (AACs) have been identified both in Gram-positive bacteria such as *S. aureus* and in GNB, notably *P. aeruginosa* (Ramirez & Tolmasky, 2010).

Resistance to β-lactam antibiotics is attributed largely to the presence of β-lactamases which hydrolyze the β-lactam ring including in penicillins, cephalosporins, monobactams and carbapenems (Rumbo et al., 2013; Santajit & Indrawattana, 2016). β-lactamases are classified by the Ambler (structural) and Bush–Jacoby–Medeiros (functional) systems. The Ambler classification places the β-lactamases into four groups: A, B, C and D (Meletis et al., 2012). The class A β-lactamases are commonly referred to as penicillinases and also include the extended spectrum β-lactamases (ESBLs) which hydrolyze broad spectrum cephalosporins but may be susceptible to inhibition by clavulanic acid, sulbactam, or tazobactam. The Class B β–lactamases which are referred to as the metallo-β-lactamases (MBLs) mostly require Zn^{2+} for their activity and confer resistance of bacteria to penicillins, cephalosporins and carbapenems (Santajit & Indrawattana, 2016). These enzymes such as the New Delhi metallo- β-lactamase (NDM), imipenemase metallo- β-lactamases (IMP) and Verona integron encoded metallo- β-lactamases (VIM) cannot be inactivated by clavulanate, sulbactam and carbapenems and are easily transmitted to other bacteria via plasmids (Kapoor et al., 2017). The Class C β-lactamases which are predominantly penicillinases and cephalosporinases, such as AmpC β-lactamase, confer low level resistance to the narrow-spectrum cephalosporins but are susceptible to carbapenems and not inactivated by clavulanate. Plasmid-mediated AmpC β-lactamase can be expressed in bacteria such as *K. pneumoniae* which naturally lack the encoding gene for chromosomal AmpC (El Salabi et al., 2013; Santajit & Indrawattana, 2016). Class D β-lactamases which are referred to as the oxacillinases, predominantly hydrolyze penicillin, cloxacillin, oxacillin, and methicillin. Clavulanic acid, tazobactam, and sulbactam inhibit these oxacillinases and they are commonly found in *A. baumannii* though their presence in other clinical related bacteria such as *E. coli* and

P. aeruginosa among others has been reported (Hrabák et al., 2014; Mlynarcik et al., 2020; Poirel et al., 2010).

2.2.2 Modification of drug binding site

Bacteria may develop resistance to antibiotics by altering the drug binding site. Genetic determinants involved in such target protection may be carried by MGEs though they may also be chromosomally encoded (Munita & Arias, 2016). For instance, mutations of genes encoding penicillin binding-proteins (PBPs) may cause the expression of PBP2a in *S. aureus* which results in low affinity for the β-lactam antibiotics (Ali et al., 2019). Alterations in the 30S or 50S binding subunits of bacterial ribosomes that serve as targets for antibiotics such as tetracycline, macrolides, chloramphenicol and aminoglycosides lead to resistance (Kapoor et al., 2017; Kohanski et al., 2010). Resistance may also occur in glycopeptides due to a change to D-alanyl-lactate resulting in its inability to cross link whilst mutations in topoisomerase and DNA gyrase to which quinolones bind can result in fluoroquinolone resistance due to a failure in replication (Binda et al., 2014; Majeed et al., 2017).

2.2.3 Reduced uptake and prevention of entry mechanisms

Mechanisms which decrease uptake of the antibiotic or increase efflux result in resistance to antibiotics (Kapoor et al., 2017). Porins which are found especially in GNB may result in resistance to antibiotics such as β-lactams, tetracyclines and some fluoroquinolones by reducing their uptake (Munita & Arias, 2016). The porin OprD in *P. aeruginosa* either alone or together with efflux mechanisms has been implicated in resistance to carbapenems and in *K. pneumoniae*, alteration in the porin OmpK35 to one with smaller channels OmpK36 results in resistance to several β-lactams (Li et al., 2012; Sugawara et al., 2016).

The presence of efflux pumps in both Gram-positive and Gram-negative organisms may lead to resistance to a wide range of antibiotics including β-lactams, fluoroquinolones, carbapenems and polymyxins (Giedraitienė et al., 2011). There are currently five major families of antimicrobial efflux proteins which are the ATP-binding cassette (ABC) superfamily, resistance nodulation cell division (RND) superfamily, small multi-drug resistance (SMR) family, multi-drug and toxic compound extrusion (MATE) family and the major facilitator superfamily (MFS) (Kumar et al., 2013a). The most common efflux pumps in GNB are of the resistance –nodulation division (RND) and include the AdeABC in *A. baumannii* isolates which through their presence and overexpression effect multidrug resistance (Lee et al., 2011). Some efflux pumps are chromosomally encoded accounting for the intrinsic resistance in some organisms while in others, they may be carried by mobile genetic elements (MGEs) and may be substrate-specific such as *tet* determinants for tetracycline (Santajit & Indrawattana, 2016). The MFS form the greater majority of efflux pumps in bacteria and serve as specific exporters of certain classes of drugs such as tetracycline, chloramphenicol and ciprofloxacin (Vila et al., 2007). In GNB, MFS pumps such as CmlA which mediates resistance to both chloramphenicol and florfenicol, and Flo may be chromosomally encoded or carried by plasmids (Kumar et al., 2013b). In GNB, tet efflux genes such as *tet (A)* gene which confer resistance to tetracycline can be found on transposons inserted into plasmids. The efflux determinant *tet (B)* which may be found in GNB such as *A. baumannii* affect tetracycline and minocycline but not glycylcyclines (Vila et al., 2007). Drug efflux mechanisms are particularly challenging in GNB as they may not only mediate intrinsic and acquired multidrug resistance but also are involved in other functions, such as the bacterial stress response and pathogenicity (Lin et al., 2015).

2.3 Multidrug Resistance in Gram-negative bacteria

Reports of infections caused by drug-resistant GNB have become increasingly prevalent and are considered a serious threat to public health globally (Bassetti et al., 2019; Rossolini et al., 2014; Tacconelli et al., 2018). Inadequate treatment options make such infections difficult to treat leading to high morbidity and mortality rates (Kaye & Pogue, 2015). GNB have the ability to up-regulate or acquire genes that code for antibiotic drug resistance mechanisms, and do so readily in the presence of antibiotic selection pressure (Kaur & Peterson, 2018). They often have multiple resistance mechanisms against same or different antibiotics and may also target several classes of antibiotics using a particular mechanism (Peleg & Hooper, 2010).

GNB often reported globally to be MDR include *Pseudomonas aeruginosa, Acinetobacter baumannii,* and ESBL-producing or carbapenemase-producing Enterobacterales (Agyepong et al., 2018; Peleg & Hooper, 2010; Tacconelli et al., 2018). Other antimicrobial agents to which resistance is increasingly being reported in GNB include aminoglycosides, sulphonamides and fluoroquinolones (Nseir et al., 2011; Tacconelli et al., 2018).

MDR GNB have been found to originate from long-term care facilities to hospitals and readily colonize patients with invasive medical devices (Arjun et al., 2017; Kaye & Pogue, 2015). Patients were found to be colonized on admission in a retrospective observational study in three ICUs in France, where the risk factors for the colonization and acquired infection with ESBL-producing GNB in ICUs with low ESBL-GNB prevalence rate was assessed. Four percent of the patients (131/3250) were colonized at admission, 59 acquired colonization while hospitalized (1.9%; 95% CI [1.5–2.5%]), and 15 (0.5%; 95% CI [0.3–0.8%]) acquired ESBL-GNB infections. Colonization was the main risk factor for ESBL-GNB acquired infection (OR = 9.61; 95% CI [2.86–32.29]; $p < 0.001$) (Massart et al., 2020). A study was conducted to determine the prevalence of

rectal colonization with third generation cephalosporin resistant Enterobacterales at admission to a German university hospital and to further estimate the incidence of subsequent infection as well as identify risk factors for colonization (Boldt et al. 2018). The study revealed that 10.3% (n = 415/4013) of patients were rectally colonized at admission, the incidence of nosocomial infections was 3.5 per 100 patients colonized compared to 2.3 per 100 non-colonized patients (Boldt et al., 2018). ESBL producing *E. coli* isolates colonizing hospital patients have also been reported in African countries such as South Africa, Uganda, and Tanzania (Ogunbosi et al., 2020; Eger et al., 2021; Moremi et al, 2018).

In Ghana, some studies have MDR GNB colonization in both community and hospitals. Carriage of third-generation cephalosporin-resistant *E. coli* (n=362) and *K. pneumoniae* (n=9) has been reported in 50.4% (371/736) of healthy residents in Ghanaian communities (Obeng-Nkrumah et al., 2023). High carriage rates of MDR GNB (49.6%) and third-generation cephalosporin resistant (46.1%) has also been reported in neonates in Neonatal Intensive Care Units of two tertiary hospitals in Ghana (Labi et al., 2020).

Nseir et al. (2011) in a prospective cohort study in France showed that admission to a medical and surgical intensive care unit (ICU) previously occupied by a patient with MDR GNB carried an increased risk of acquiring MDR GNB by subsequent patients. They further showed that admission to an ICU room previously occupied by a patient with MDR *P. aeruginosa* or *A. baumannii* was an independent risk factor (odds ratio of 2.3 and 4.2 respectively) for acquisition of these bacteria by subsequent room occupants. There was no such risk associated with ESBL-producing GNB (Nseir et al., 2011). In a study to assess the risk factors, antibiotic therapy and outcomes of bacteremia due to MDR GNB in a Neonatal Intensive Care Unit (NICU) patients in Taiwan, it was observed that 18.6% of all neonatal GNB bacteremias in the NICU were as a result of infection by

MDR GNB, especially in those with previous broad-spectrum antibiotic therapy and underlying renal disease (Tsai et al., 2014). ESBL production was reported to be the most frequent mechanism of resistance and neonates with MDR GNB were more likely to develop infectious complications (Tsai et al., 2014). MDR GNB in a teaching hospital in Ghana was reported at an average of 89.5%, ranging from 53.8% in *Enterobacter* spp. to 100.0% in *Acinetobacter* spp. and *P. aeruginosa* (Agyepong et al., 2018).

Efflux pumps such as AcrAB - TolC and Mex pumps of the RND superfamily are clinically important and presents an impediment to successful antibiotic therapy in GNB (Li et al., 2015). Carbapenemases have become notably common and implicated in β - lactam resistance in *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, *E. coli* and *Enterobacter* spp (El Salabi et al., 2013; Hrabák et al., 2014; Poirel et al., 2010).

Colistin which had become an important treatment option for carbapenem – resistant GNB is also threatened as the plasmid-mediated *mcr* gene which confers resistance to colistin has been identified in several countries (Hasman et al., 2015; Terveer et al., 2017). Genes that encode proteins (*lpxA, lpxC* and *lpxD*) involved in the biosynthesis of lipid A in *A. baumannii* have been shown to be involved in colistin resistance (Osei Sekyere et al., 2016). Clinical outbreaks involving colistin resistant *Klebsiella pneumoniae* carbapenemase (KPC) -producing *K. pneumoniae* and other Enterobacterales have been also been reported which is quite alarming (Ah et al., 2014; Halaby et al., 2013).

2.4 Transmission of MDR GNB

MDR GNB may be transmitted from patient-to-patient within the hospital and spread by contact with contaminated surfaces, equipment and instruments. They may also be carried via HCWs hands in their routine interaction with patients (Russotto et al., 2015; Tajeddin et al., 2016) continuing a cycle of spread as illustrated in Figure 1 below.

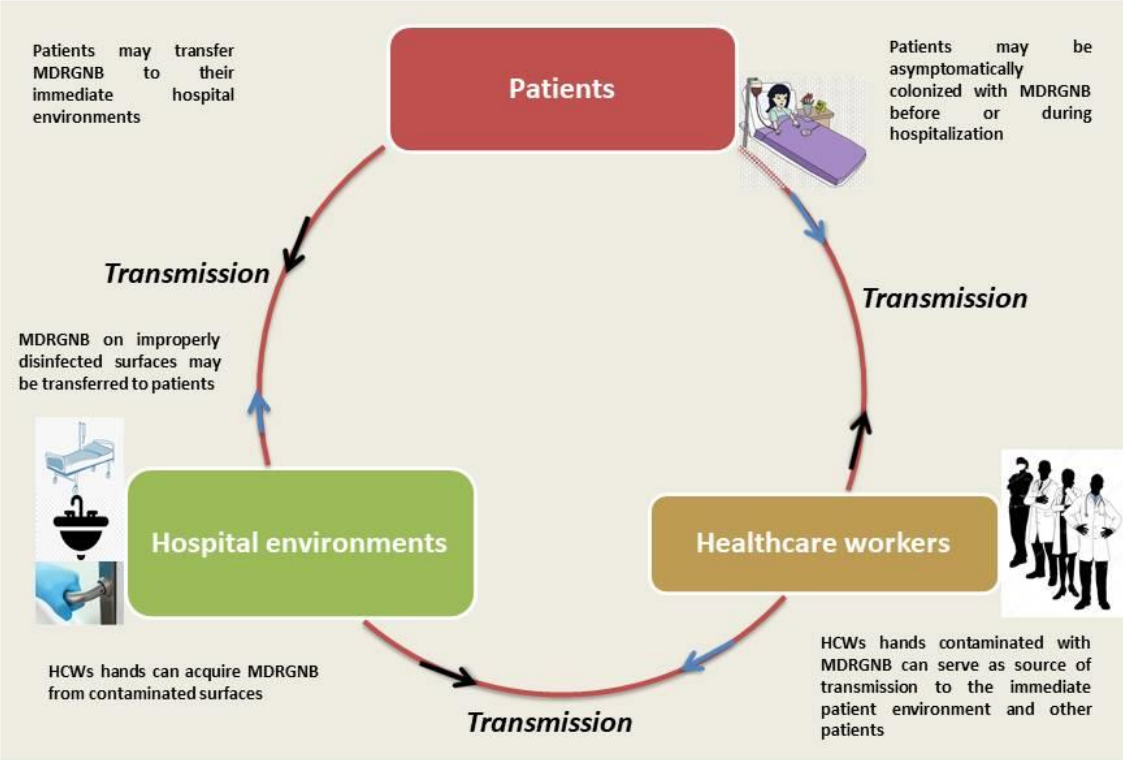

Figure 1: Spread of MDR GNB in hospital environments

The different routes of transmission of MDR GNB are discussed in the section below.

2.4.1 Hospital environments and equipment

Inanimate surfaces and equipment in in-patient units such as ICUs are heavily contaminated by bacteria, including MDR species (Hu et al., 2015). In a cross-sectional study to determine bacterial contamination, bacterial profiles, and antimicrobial susceptibility pattern of bacterial isolates from environmental surfaces and medical equipment a tertiary hospital in Ethiopia, potentially pathogenic GNB were found to contaminate ICUs and operating theaters and constituted 67.4% of all bacteria isolated from environmental surfaces in the ICU. Gram-positive bacteria were predominantly isolated from the operating theaters (Sebre et al., 2020). Bacterial strains from patients, the hands of HCWs, and the hospital environment have been demonstrated to be associated with hospital-acquired infections in several studies (El Shafie et al., 2004; Hopman et al., 2019; Shiferaw et al., 2013; Tajeddin et al., 2016; Ye et al., 2015). In a study by Tajeddin et al. (2016), to investigate the frequency and resistance patterns of bacterial responsible for hospital acquired infections (HAIs) on the hands of HCWs and the ICU environments in six ICUs in Iran, a total of 51% and 34.5% of environmental (313/605) and HCWs' (263/762) samples were reportedly contaminated with different bacterial species in the studied ICUs. Ventilators (82.91%), patient oxygen masks (81.81%) and bed linens (67.65%) accounted for sources of the most frequently contaminated samples. *K. pneumoniae*, which mainly colonized ventilators 6/11 (54.4%), was the most frequent coliform bacteria (Tajeddin et al., 2016). Eighty to ninety percent of nosocomial urinary tract infections (UTIs) are associated with the use of catheters (Al-Shenqiti et al., 2017). Frequently used hospital equipment such as stethoscopes, have also been found to be contaminated with multidrug resistant organisms (Daoudi et al., 2017; Shiferaw et al., 2013; Vajravelu et al., 2012). Out of 176 stethoscopes sampled in a cross-sectional study at a specialized hospital in Ethiopia to determine the bacterial profile and antimicrobial susceptibility pattern of isolates from stethoscopes, 85.8% were contaminated. Out of 256 isolates, 133 (52%) which

included *S. aureus*, *Proteus* spp., *Salmonella* spp., *Citrobacter* spp., *E. coli, Enterobacter* spp., *Klebsiella* spp. and *P. aeruginosa* were multidrug resistant. Stethoscopes which had never been disinfected showed high contamination rate of 90.9% (Shiferaw et al., 2013).

Frequently touched surfaces in wards have been shown to be contaminated with bacteria and may persist in the absence of effective disinfection. The bacterial contamination of frequently touched objects by patients, visitors and HCWs was investigated in a study in a Teaching Hospital in Nepal. MDR organisms including *Staphylococci* spp*, Acinetobacter* spp*., Pseudomonas* spp. and *E. coli* were isolated from door handles, elevator buttons, biometric attendance devices, telephone sets, railings and water taps. Among the GNB isolates, 52.6% (10/19) of *Acinetobacter* spp. and 46.6% (7/15) of *E coli* were ESBL producers (Bhatta et al., 2018).

In a study of ICUs and emergency rooms of four hospitals in Northern Jordan, seventy-four of 311 surface swabs from bed rails, sinks, food tables, trolley handles, sheets, ventilator inlets, blankets, sheets, door handles, light switches, bedside tables, bedside table drawers were found to be contaminated with *Acinetobacter* spp. of which 50% were from adult ICUs and 25% from emergency rooms. Ten of 24 (45.67%) *Acinetobacter* spp. isolated were XDR and more concerning, had biofilm forming capacities (Ababneh et al., 2022).

A study of environmental surfaces in five wards of the Cape Coast Teaching Hospital in Ghana showed that 12% (28/231) of surfaces swabbed were positive for at least one ESBL gene while 11% (26/231) of the surfaces were positive for the bla_{NDM-1} gene (Acolatse et al., 2022). Apenteng et al. (2022) also isolated *S. aureus* (38%) and *S. typhi* (8%) from hospital ward environments in the Tema General hospital of Ghana and found that of 57 *S. aureus* isolated, 48.7% were from

door handles and 11.7% of *S. typhi* isolates from table surfaces. The *S. aureus* (75.44%) and *S. typhi* (66.67%) isolates were highly resistant to cefuroxime (Apenteng et al., 2022).

2.4.2 Healthcare workers' hands

HCWs hands are major sources of transmission of nosocomial pathogens often acquired through direct contact with patients, body fluid secretions, or touching contaminated environmental surfaces in the inpatient units (Mehta et al., 2014; Russotto et al., 2015). From a review of studies on bacterial contamination in ICUs, it was estimated that about 20-40% of nosocomial infections are as a result of cross-infections through HCWs hands (Russotto et al., 2015).

The hands of HCWs have been found to be contaminated by bacteria in the absence of efficient hand hygiene. In a cross-sectional study to assess hand hygiene among HCWs in 18 healthcare facilities (HCF) in the Mwanza region, Tanzania, 26.4% (56/212) HCWs hands had bacterial contamination of which 17.9% (38/212) were contaminated with GNB (including coliforms, *Acinetobacter* spp. and *P. aeruginosa*). Among 74 HCWs, the overall median hand hygiene score (interquartile range) was 212.5 (Range: 190-245) and was designated at basic level in the WHO framework though about a quarter of the HCWs hands were contaminated with bacteria (Rayson et al., 2021). Similarly, in a study in a teaching hospital in Nigeria to determine the bacteria profile of HCWs hands during routine care, all of the 300 HCWs hands sampled were found to be contaminated with bacteria, predominantly coagulase negative staphylococci (67%) and 6% with Enterobacterales (Ige et al., 2021).

HCWs may acquire contaminating bacteria via patient interaction or by touching and handling contaminated inanimate surfaces in the hospital wards. Morgan et al. (2010), observed that 77

(38.7%) and 9 (4.5%) of 199 interactions between HCWs and patients colonized with MDR *A. baumannii*, resulted in HCW contamination of gloves and/or gowns, and HCW hands after glove removal and before hand hygiene respectively. Of 134 interactions with patients colonized with MDR *P. aeruginosa*, 11 (8.2%) resulted in HCW contamination of gloves and/or gowns, and one resulted in HCW contamination of hands (Morgan et al., 2010). In a similar study, 20.5% (120/585) healthcare worker/patient interactions resulted in contamination of healthcare workers' gloves or gowns. Positive environmental cultures (odds ratio [OR] 4.2; 95% CI 2.7–6.5), duration in room for >5 minutes (OR 2.0; 95% CI 1.2–3.4), performing physical examinations (OR 1.7; 95% CI 1.1–2.8), and contact with the ventilator (OR 1.8; 95% CI, 1.1–2.8) were found to be independent risk factors associated with healthcare worker contamination with MDR bacteria. Pulsed field gel electrophoresis also revealed 91% of healthcare worker isolates were related to an environmental or patient isolate indicating a likelihood of transfer of isolates from these sources (Morgan et al., 2012).

Contamination of hands of HCWs is a serious concern in critical care units such as the ICU. Ssemogerere et al., observed the fingertip stamps of HCWs in a cross-sectional study to determine the colonization of hands of HCWs in the cardiac ICU of a Heart Institute in Uganda and isolated GNB in 34% (19/56) of HCWs. Of these, 42/1% (8/19), 31.2% (6/19) and 26.3% (5/19) were non-ICU clinicians, ICU clinicians and non-clinicians respectively. Thirty-two isolates were identified, 25% from ICU staff, 47% from non-ICU staff, and 28% from others. *Acinetobacter* (34%), *Citrobacter* (21.9%), and *Pseudomonas* (21.9%) were commonly isolated from the samples and had antimicrobial resistance ranging from 4% to 90% (Ssemogerere et al., 2019).

Inanimate objects such as mobile phones, stethoscopes and other medical or non-medical devices handled by the HCWs can also be contaminated by bacteria. The rate of bacterial contamination

of HCWs mobile phones in a study in Bangladesh was 69%. GNB isolated from these included *E. coli* (14%), *P. aeruginosa* (11%) and *Salmonella* Typhi (6%) and showed varying resistance of 16% - 68.8% to different antibiotics (Debnath et al., 2018). Other similar studies have also established the isolation of MDR organisms from mobile phones as a potential risk of transmission to patients (Banawas et al., 2018; Bodena et al., 2019; Selim & Abaza, 2015). MDR GNB have also been isolated from other devices such as computer keyboards, handles of defribillators, medical charts, bronchoscopes and stethoscopes which are handled by HCWs (Mbanga et al., 2018; Morubagal et al., 2017).

Though frequently touched surfaces are often implicated in harbouring pathogens, there is evidence that portable equipment such as wheelchairs, vital signs monitors and other shared devices such as pens, tablets, touchscreens may also contribute to pathogen transmission. Hospital floors could also be contaminated with pathogens and need to be considered in disinfection routines (Donskey, 2019).

2.5 Infection Prevention and control practices

Poor infection control, unhygienic and unsanitary conditions have been implicated in the spread of drug-resistant organisms from human to human or to the environment and vice-versa (D'Agata et al., 2012; Holmes et al., 2016). Controlling resistance requires two major efforts targeted at decreasing the volumes of antibiotics used and reducing the spread of antibiotic resistant organisms as well as the genes that encode for resistance (Collignon, 2015). Knowledge on the factors contributing to the spread of infections in the hospital environment is necessary for its prevention and control and requires a concerted effort at instituting hospital IPC practices (Russotto et al., 2015; Takahashi et al., 2009). Russoto et al. in a review of bacterial contamination of inanimate

surfaces and equipment in the ICU highlighted that the contamination of hospital surfaces and equipment by GNB may contribute to ICU-acquired colonization or infection but further studies are required to determine the correlation (Russotto et al., 2015). In a study on the Efficacy of Nosocomial Infection Control (SENIC) programs in the USA, it was found that about 33% of nosocomial infections can be prevented when hospital infection control programs are instituted in hospitals (Haley et al., 1985). Harbath et al. (2003), in a review of thirty reports on nosocomial infections reported that at least 20% of all nosocomial infections, most likely, catheter related infections among others could be preventable.

In terms of the hospital environment, compliance with hand hygiene practice is essential as it is globally considered to be important for infection control and to prevent spread of resistant bacteria to patients (Khan et al., 2012). Proper hand hygiene is essentially considered to be, the simplest, most cost-effective means of reducing the prevalence of HAIs and the spread of antimicrobial resistance though there is non-adherence in many instances (Mathur, 2011). Inadequate knowledge on hand hygiene, heavy workloads, lack of disinfectants, lack of or poor design of wash stations and the use of gloves as precluding the need for hand hygiene were some factors hindering the adherence to hand hygiene practices among HCWs (Ahmadipour et al., 2022; Al Ghafari & AbuRuz, 2019).

A study in Karachi, Pakistan investigated the frequency of use of alcohol based disinfectant by HCWs and assessed their knowledge of hand hygiene, where only 12.3% of 212 hospital staff attending to patients complied with hand disinfectant use before and after every patient contact. Of the 212 participants, 62.73% of participants were aware of the WHO guidelines on hand hygiene (Ahmed et al., 2020). In Ethiopia, a study among a cross section of HCWs in public primary hospitals assessed hand hygiene compliance and associated factors and reported poor

compliance to hand hygiene with only 14.9% (50/335) adhering to hand hygiene practices (Engdaw et al., 2019).

Since the upsurge of the COVID-19 pandemic, hospitals have seen the implementation of and compliance with several IPC measures in a bid to reduce the transmission of SARS-CoV-2. Information on IPC practices at the peak of the COVID-19 pandemic in health facilities was collected in a multi-facility based cross-sectional study using a monitoring tool of the Health Facilities Regulatory Agency (HeFRA) of Ghana. IPC systems were assessed as good in most (56.3%) facilities studied noting however, that there was still the need for a revision of policies on IPC practices to strengthen IPCs in hospitals with low compliance (Bannor et al., 2021). Compliance with IPC measures such as hand hygiene for COVID-19, could have a positive impact on the containment of AMR pathogens in hospitals (Rawson, 2020). Bentivegna et al. (2021) in a case–control study to identify the incidence of MDR bacterial infections while using pandemic-related preventive measures, reported a significant decrease in the incidence of MDR bacterial infections compared to previous years in a hospital in Rome. These included MDR methicillin-resistant *S. aureus,* ESBL-producing *K. pneumoniae,* health care-associated *Clostridium difficile* (HA-CD), and *A. baumannii*. In addition to the basic prevention measures such as isolating infected patients in cohorts, wearing gloves and disposable gowns as well as surveillance, measures such as increased hand hygiene, universal masking among others were adopted in this hospital and found to be of relevance in decreasing MDR bacterial infections (Bentivegna et al., 2021).

Isolation in a single room, active screening for colonization or infection with carbapenem resistant Enterobacterales (CRE) from rectal or perirectal swabs for all patients “at-risk” for carriage of CRE as well as the observance of contact precautions are some measures recommended for the

prevention of entry of CRE in hospitals in a guidance document for IPC in patients “at-risk” for carriage of CRE (Magiorakos et al., 2017).

In addition to active surveillance for surgical site infections, device-associated infection, and, clinically defined infections, there is the need to also conduct surveillance for colonization or infections caused by multidrug-resistant organisms (MDROs) according to local epidemiology (WHO, 2018).

IPC practices have often been met with barriers which need to be addressed, particularly in developing countries and low resource settings. Inadequate hospital infrastructure, overcrowding in wards, inadequate supply of potable water, inefficient waste management systems, resource and workforce shortages, inadequate education of staff, inadequate in-service IPC training and supervision and large visitor numbers are barriers to IPC in hospitals (Lowe et al., 2021; Ogunsola & Mehtar, 2020) and requires institutional efforts such as in-service training, strengthening laboratory capacity, monitoring, audit of IPC practices and feedback for effective implementation (Sengupta et al., 2019).

Frequently used instruments and surfaces which are not properly disinfected could harbor MDR bacteria and could consequently serve as a source of contamination to patients and hospital personnel if appropriate infection control practices are not instituted. Targeted disinfection of surfaces is crucial to the prevention and control of pathogens in hospital environments (Gebel et al., 2013).

In a multicenter cross-sectional study in hospitals in China the relationship between MDRO colonization in ICU patients and ICU surface bacterial contamination status was investigated. The rate of MDRO detection on frequently touched surfaces in the ICUs was reduced remarkably from

31.77% to 13.32% after cleaning with disinfectant. Again, the cleaning reduced ICU stay and lowered mortality significantly among the cohorted group in cleaned ICU rooms (Huang et al., 2020).

Disinfection should be considered for frequently touched surfaces near patients, surfaces where contamination is assumed, surfaces with visible contamination (blood, pus, excrements) and in terminal disinfection in rooms or areas where infected or colonized patients were admitted and in outbreak situations (Gebel et al., 2013).

Surface disinfectant cleaners (SDCs) like the quaternary ammonium compounds (QACs), which are also active against MDR GNB are known to be effective in disinfection in patient areas (Reichel et al., 2014). Other disinfectants such as benzalkonium chloride (BAC), peracetic acid (PAA), and ethanol (ETH) have marked activity against GNB (Köhler et al., 2019). Though active against several bacteria, there is the need to constantly evaluate the activity of disinfectants against bacteria species as they may develop resistance to antibiotics and disinfectants used (Köhler et al., 2019).

Surface modification and/or functionalization and no-touch strategies to overcome challenges associated with surface contamination in hospitals have recently been developed. These include surfaces with anti-adhesive properties, with incorporated antimicrobial substances or modified, application of aerosols or ultraviolet light on contaminated surfaces (Querido et al., 2019).

2.6 Clinically relevant Gram-negative bacteria

GNB are commonly isolated in clinical settings. They have become resistant to antibiotics and lead to high morbidities and mortalities. The priority Gram-negative pathogens as published by the WHO based on the critical need for the research and development of new antibiotics against

them include: *Acinetobacter* (carbapenem-resistant), *Pseudomonas* (carbapenem-resistant) and various carbapenem resistant and ESBL-producing Enterobacterales (including *Klebsiella*, *E. coli*, *Serratia*, and *Proteus*) (WHO, 2017).

Majority of these bacteria listed by the WHO as critical pathogens, are also among the ESKAPE pathogens i.e. *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp., in which MDR has emerged (De Oliveira et al., 2020). These bacteria have the capacity to evade the action of antibiotics via different mechanisms thereby reducing treatment options and leading to high mortalities, hence the urgent need for new treatment options for these bacteria (Santajit & Indrawattana, 2016). The antimicrobial resistant GNB which are listed on the WHO critical priority list and are ESKAPE pathogens are elaborated in the sections below.

2.6.1 Carbapenem resistant *Acinetobacter baumannii*

Acinetobacter baumannii is a GNB of clinical concern due to its persistence in hospitals and its ability to acquire multiple resistance to antibiotics. It contaminates several equipment and surfaces in hospitals and is implicated in nosocomial infections including pneumonia, skin and soft-tissue infections, bacteraemia and urinary tract infections (Weinberg et al., 2020). *A. baumannii* infections are closely associated with surgery or the use of endotracheal tubes and intravascular, ventricular or urinary catheters, mainly in intensive care units (ICUs) (Jamal et al., 2018). The persistence of *A. baumannii* in hospital environment is facilitated by its ability to survive on dry surfaces for as long as 4 months, ability to resist disinfection, desiccation and oxidative stress, biofilm formation on abiotic surfaces and thus could colonize medical devices and equipment resulting in prolonged hospital outbreaks (Almasaudi, 2018) (Zarrilli et al., 2021).

A. baumannii is resistant to several antibiotics but particularly problematic are the carbapenem resistant *A. baumannii* (CRAB) which is included as a 'critical' pathogen in the WHO global priority list of antibiotic resistant bacteria (Zarrilli et al., 2021). In a systematic review to determine the incidence and prevalence of hospital acquired-carbapenem resistant *A. baumannii* in European, Eastern Mediterranean and African regions of the WHO, the pooled incidence of cases was 21.4 (95% CI 11.0-41.3) cases per 1,000 patients in these WHO regions (Ayobami et al., 2019). The resistance rate of *A. baumannii* isolates is estimated to be greater than 50% in many countries in Latin America according to a review by Labarca et al. (2016). In these countries, carbapenem resistance in *A. baumannii* is predominantly due to the oxacillinases OXA-23, OXA-58 and (in Brazil) OXA-143 (Labarca et al., 2016). Forty-nine percent (22/45) of *A. baumannii* isolates isolated from wound infections of patients were carbapenem-resistant in a prospective study that conducted a microbiological analysis of wound infections in a rural hospital in Ghana (Monnheimer et al., 2021).

MDR *A. baumannii* is one of the nosocomial pathogens of concern frequently acquired in high risk wards. A prospective study of the incidence, risk factors and outcome of MDR *A. baumannii* infections in a burn unit of a hospital in Paris showed that 17% (15/85) of patients had acquired MDR *A. baumannii* infection in the ICU. The *A. baumannii* infection was associated with high mortality and prolonged hospital stay with an estimated median of 10 days (Munier et al., 2019). Carbapenem resistant genes, particularly the oxacillinases have been frequently detected in carbapenem resistant *A. baumannii*. Molecular and epidemiological investigation of *A. baumannii* isolates collected from patients with hospital acquired infections in two tertiary hospitals in Tshawne, South Africa revealed a high prevalence of carbapenemases in the isolates. Prevalence of OXA-23-like β-lactamase genes was between 91%-96% in the two hospitals. OXA-58-like

genes (4%) in one of the hospitals and colistin resistance found in 1% of the isolates (*n* = 2/141) (Lowe et al., 2018). The widely reported carbapenemases NDM-1 and/or OXA-23 were detected in all carbapenem resistant *A. baumannii* isolates which were recovered from wound infections of patients in a rural hospital in Ghana. Two isolates additionally harboured OXA-420 which was first isolated in Nepal in 2014 (Monnheimer et al., 2021).

2.6.2 Carbapenem resistant *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is listed as one of the 'critical' pathogens on the WHO global priority list of antibiotic resistant bacteria. It was reported that an estimated 7.1%-7.3% of all nosocomial infections that occurred in 2011–2014 in hospitals in a study by the Centers for Disease Control CDC's National Healthcare Safety Network in the US were due to *P. aeruginosa* (Tacconelli et al., 2018; Weiner et al., 2016). A multinational study to determine the prevalence and risk factors associated with community-acquired *P. aeruginosa* in 54 countries revealed a prevalence of 4.2% for community acquired *P. aeruginosa* and 2.0% for antibiotic-resistant *P. aeruginosa*. Risk factors found to be independently associated with the community-acquired *P. aeruginosa* included prior Pseudomonas infection/colonization, tracheostomy, invasive respiratory and/or vasopressor support bronchiectasis, and severe chronic obstructive pulmonary disease (forced expiratory volume ≤30%) (Restrepo et al., 2018). *P. aeruginosa* adapts to different environments and causes severe infections mostly in immunocompromised patients or those with chronic debilitating diseases. It can have multiple intrinsic or acquired mechanisms of resistance, frequently with high resistance rates to various antimicrobial classes coupled with several virulence factors (Spagnolo et al., 2021).

Colonization with *P. aeruginosa* usually results in infections with the carbapenem resistant strains being the biggest threat to health (Lila et al., 2018; Shi et al., 2019). In a retrospective comparative

study between carbapenem susceptible and carbapenem non-susceptible *P. aeruginosa* strains isolated from bloodstream infections in China, the prevalence of carbapenem resistance was found to be 30% (101/340) in patients. Mortality was higher in patients with carbapenem non – susceptible *P. aeruginosa* (37.6% vs. 22.2%, respectively; $P = 0.003$) (Shi et al., 2019).

P. aeruginosa isolates of similar clones have been reported to be disseminated in hospitals. A prospective study was conducted to determine the clonal relationship of *P. aeruginosa* isolates from patients in a university clinical centre in Kosovo. Of the 80 *P. aeruginosa* mostly isolated from wound and respiratory tract specimens, most (47.5%) were recovered from the intensive care unit. Of the total isolates, 17.5% and 12.5% were resistant to imipenem and meropenem respectively. Of the seven Pulsed-field gel electrophoresis (PFGE) patterns observed, one was found to be common to 16 isolates which were from the ICU, neurosurgery and plastic surgery units, indicating a clonal spread within the hospital (Lila et al., 2018). A multi-centre prospective study of ten ICUs in France, evaluated the role of the environment and medical care in *P. aeruginosa* colonization or infection. The study also evaluated the risk factors associated with colonization and found the prevalence of *P. aeruginosa* colonization in patients was 15.3% and the prevalence in tap water was 17.1%. Significant risk factors for colonization or infection with *P. aeruginosa* included use of inactive antibiotics against *P. aeruginosa*, tap water contamination at the entry in the room and mechanical invasive ventilation. Of the colonized patients, 43% developed infections (Hoang et al., 2018).

Among carbapenemases, metallo-β-lactamases (MBLs) and mostly Verona integron-mediated (VIM) MBL's have played the most crucial role in the emergence of carbapenem resistant *P. aeruginosa*. $bla_{\text{VIM-2}}$ and $bla_{\text{VIM-4}}$ are usually associated with a class 1 integron (Karampatakis et al., 2018). The type of carbapenemases vary in various geographical regions. The prevalence of

carbapenemase genes in carbapenem resistant *P. aeruginosa* isolates was highest in South and Central America (69%) followed by Australia and Singapore (57%) and then in China, the Middle East and USA where the prevalence was 32%, 30% and 2% respectively according to a study investigating the prevalence of carbapenemases among carbapenem resistant *P. aeruginosa* isolates in hospitals in these geographical regions. KPC-2 (49%) and VIM-2 (36%) were the most common carbapenemases identified (Reyes et al., 2023). Carbapenemases/extended-spectrum β-lactamases including VIM, IMP, GES, PER and OXA enzymes were detected in 3.1% of *P. aeruginosa* isolates from infections in a multi-centre study exploring the molecular epidemiology and antibiotic resistance of *P. aeruginosa* isolates from patient infections in 51 hospitals in Spain (del Barrio-Tofiño et al., 2019).

Two novel *bla*$_{\text{DIM-1}}$- or *bla*$_{\text{IMP-1}}$-containing genomic islands (GIs) were detected in four extensively drug-resistant (XDR) *P. aeruginosa* isolates from inpatients at a tertiary hospital in Ghana in a study which sought to determine the molecular mechanism for carbapenem resistance in the XDR P. *aeruginosa* which were from a collection of GNB from the hospital. The strains were notably of sequence type 234 and was disseminated in the hospital (Janice et al., 2021).

P. aeruginosa can persist in hospital environments raising a concern for efficient disinfection. The prevalence of *P. aeruginosa* in isolates from wound swabs of patients with surgical site infections and from environment was 11.8% and 18.5% respectively in a study in which *P. aeruginosa* isolates were collected from the environment and burn patients to determine the molecular characteristics in a hospital in Iran. Resistance to the carbapenems; meropenem and imipenem were (51.7% and 40%), and (53.5% and 50%) in clinical and environmental samples, respectively. A similarity between the genotypes of clinical and environmental isolates was observed, suggestive of a clonal spread of the isolates within the sources (Karami et al., 2019).

Studies on the prevalence and epidemiology of *P. aeruginosa* isolates particularly in the hospital environments in Africa are minimal and hence a need for increased surveillance on these pathogens.

2.6.3 ESBL-producing and carbapenem resistant Enterobacterales

Enterobacterales are distributed widely and constitute 80% of Gram-negative isolates. They cause pneumonia, urinary tract infections, diarrhoea, meningitis, sepsis and endotoxic shock. GNB that are commonly associated with human infections include *Escherichia*, *Proteus*, *Enterobacte*r, *Klebsiella*, *Citrobacter*, *Yersinia*, *Shigella* and *Salmonella* (Oliveira & Reygaert, 2022). Though prevalence may vary in different settings, ESBL-producing Enterobacterales are a major global public health concern (Peirano & Pitout, 2019). The annual prevalence of the ESBL-producing Enterobacterales ranged from 3.8% to 10.5% (mean, 6.2%) in participating hospitals in a study assessing ESBL-producing Enterobacterales most commonly implicated in infections in 18 hospitals in France. *E. coli* (62%), *K. pneumoniae* (18.5%), and *Enterobacter cloacae* (15.5%) species constituted the highest represented species of the Enterobacterales (Robin et al., 2017).

In Ghana, the overall ESBL prevalence of 20.4% (20/98) was reported among Enterobacterales recovered from patients with diarrhoea in a general hospital and polyclinic in Accra, in a study that investigated antimicrobial resistance of ESBL-producing Enterobacterales isolated among these patients attending the two facilities. Among these were *E. coli* 13.2% (10/76), *K. pneumoniae*, 35.7%(5/14) and *Proteus mirabilis*, 57.1%(4/7). The ESBL genes bla_{TEM} , $bla_{CTX\text{-}M}$ and bla_{SHV} were the most detected in the Enterobacterales (Dela et al., 2022).

Enterobacterales, may survive on hospital surfaces, contaminating immediate patient environment. In a study in a non-outbreak setting of a UK teaching hospital that investigated the extent and

variation in endemic ESBL-producing GNB in ward environments, 3.1% of surfaces sampled were contaminated with ESBL-producing bacteria and were mainly from sink drains and floors raising a concern as long-term reservoirs for these resistant pathogens (Muzslay et al., 2017).

Carbapenem resistance in Enterobacterales has emerged in many countries in Africa with varying prevalence and carbapenemase types. *K. pneumoniae* isolates were found to cause 79.8% of bloodstream infections (n=863/1 082) and of which 89.5% (n=611/683) were healthcare associated in a study on the epidemiology of CRE bloodstream infections among hospitalized patients in South Africa conducted by the Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa (GERMS-SA). The carbapenemase genes $bla_{\text{OXA-48-like}}$, bla_{NDM} and bla_{VIM} were detected in 76.8%, 21.1% and 1.3% of the carbapenem resistant Enterobacterales respectively (Lowe et al., 2022). In Ghana, ESBL- producing Enterobacterales constituted 49.1% (113/230) of Enterobacterales from patients in a study to determine the prevalence of carbapenem-resistant Enterobacterales isolated from patients at the Cape Coast Teaching Hospital (CCTH) in the Central region of Ghana. Among the carbapenem resistant Enterobacterales showing phenotypic resistance to carbapenem, $bla_{\text{NDM-1}}$ gene was found in (11/13) of these and $bla_{\text{OXA-48}}$ gene in all thirteen isolates, raising a concern for strengthening IPC practices in Ghanaian hospitals (Sampah et al., 2022).

ESBL-producing Enterobacterales *E. coli* and *K. pneumoniae* have been reported globally to be problematic in healthcare settings as efficient treatment for infections caused by these remains a challenge (Castanheira et al., 2021; Karlowsky et al., 2022; Siriphap et al., 2022). The following sub-section provides some insight on ESBL-producing *E. coli* and *K. pneumoniae*.

2.6.3.1 ESBL *Escherichia coli*

ESBL *E. coli* have been reported in different settings and are have gradually increased (Mughini-Gras et al., 2019). Bezabih et al. (2021) in a meta-analyses of global data on faecal carriage of ESBL *E. coli* over two decades (2000-2020) found that, at least an 8-fold increase in the intestinal carriage rate of ESBL *E. coli* in the community had occurred within the period. Cumulatively, from 2003-2018, the prevalence of intestinal carriage with ESBL *E. coli* in the community was 16.5% globally. The highest carriage rates were reported in South-East Asia (27%), followed by the Western Pacific (24.5%), Africa (21.4%) and Eastern Mediterranean (20.6%) (Bezabih et al., 2021). As part of a cohort study carried out to determine the antimicrobial susceptibilities of bacteria from surgical site infections in patients at the surgical department of a Teaching Hospital in Ghana, GNB (286/352, 81%) were found predominant among the isolates. Eighty-six percent of *E. coli* isolates from surgical site infections were MDR and 61% (50/82) of the cefotaxime-resistant *E. coli* were ESBL producing (Bediako-Bowan et al., 2020).

ESBL-producing *E. coli* can be transmitted through contact with humans, animals or the environment, or via contaminated food or water. Sixty percent and 20% of community-acquired ESBL *E. coli* were attributable to human to human transmission and food respectively in a population-based modelling study of ESBL gene data of isolates from the Dutch population (Mughini-Gras et al., 2019). ESBL-producing *E. coli* were detected in 4% of abiotic surfaces (beds, treatment tables, toilets, faucets, handle doors, sinks) tested from a Tunisian hospital in a study by Dziri et al. (2016), to determine the possible role of the hospital environment in the dissemination of ESBL-producing *E. coli* isolates via hospital environment (Dziri et al., 2016).

CTX-M-producing *Escherichia coli*- s are the most common ESBL-producing globally with CTX-M-15 being the most frequently reported CTX-M worldwide (Peirano & Pitout, 2019). The

resistance phenotypes of 101 consecutive clinical isolates of *E. coli* (n = 58) and *K. pneumoniae* (n = 43) revealed that 62% of the isolates had the ESBL phenotype and the *bla*$_{\text{CTX-M}}$ gene in a study investigating the phenotypic and genomic characteristics and clonal relatedness of *E. coli* and *K. pneumoniae* isolates from a Teaching Hospital in Ghana. Most isolates also carried *bla*$_{\text{TEM}}$, *aac(3)-II, aacA4*cr, and/or *bla*$_{\text{OXA-30}}$ genes on IncF plasmids. The spread of the *bla*$_{\text{CTX-M-15}}$ was suggested to be as a result of horizontal gene transfer rather than clonal spread of the species in Ghana due to their heterogeneity (Agyekum et al., 2016).

E. coli isolates harbouring the *bla*$_{CTX-M}$ among other ESBLs *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{NDM}$ and *bla*$_{OXA}$ genes were also recovered from ward water, surfaces of faucets, sinks and drains in a study assessing biodiversity and AMR in water and surface sources in three government tertiary care hospitals in Pakistan (Aleem et al., 2021).

Though ESBL-producing *E. coli* have been reported in Africa, further studies on their transmissibility within hospital environments are needed for informing IPC policies.

2.6.3.2 ESBL *K. pneumoniae*

K. pneumoniae is widely distributed and causes common human infections such bloodstream infections (BSIs), meningitis, urinary tract infections (UTIs), respiratory tract infections (RTIs), pneumonia and surgical site infections (SSIs). It is implicated in several nosocomial infections, especially among neonates, the elderly, and immunocompromised patients (Mohd Asri et al., 2021).

ESBL producing *K. pneumoniae* are frequently the cause of severe infections and have been reported globally. Among ESBL *K. pneumoniae* isolates, the CTX-M gene is commonly reported. A study of ESBL producing Enterobacterales implicated in infections in hospitals in France, reported the presence of CTX-M genes in 81.1% of ESBL- producing *K. pneumoniae* isolates

recovered from patients (Robin et al., 2017). The CTX-M genes have also been detected in ESBL *K. pneumoniae* isolates from Africa. *bla*$_{\text{CTX-M-15}}$ genes were reported in 84% of *K. pneumoniae* isolates from patients in a study on the prevalence of ESBLs in *K. pneumoniae* isolates from patients at a Medical Centre in Ethiopia (Sewunet et al., 2021). Eighty-four percent (90/107) of *K. pneumoniae* isolates from bloodstream and urinary tract infections were ESBL producers according to a study that investigated the presence of ESBL-producing *K. pneumoniae* in clinical samples at a Teaching hospital in Cote d'Ivoire. These isolates harboured a combination of *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *bla*$_{TEM}$ in 71.1% (64/90) (Müller-Schulte et al., 2020). In Ghana, the *bla*$_{CTX-M-15}$ and *bla*$_{TEM-1B}$ genes have been reported as the most common ESBLs among MDR *K. pneumoniae* isolates from a Teaching Hospital in a study of the resistome, plasmidome and clonal relatedness in multidrug-resistant *K. pneumoniae* isolates. Multiple *K. pneumoniae* sequence types ST2171, ST2816, ST17, ST152, ST397, ST1788, ST798 and ST101 which harboured ESBL genes were found to be circulating in the hospital (Agyepong et al., 2019).

ESBL *K. pneumoniae* originating from community have also been reported. In a microbiological evaluation of sputum samples obtained from patients with suspected community-acquired pneumonia in Iraq, the prevalence of *K. pneumoniae* was reported to be 31.9% (163/511). Of these, 11.66% (19/163) harboured ESBL genes including *bla*$_{CTX-M}$ and *bla*$_{SHV}$ (Raouf et al., 2022). Similarly, in Ethiopia, the prevalence of *K. pneumoniae* among patients with community-acquired pneumonia was 18% (30/167) in a study assessing antimicrobial resistance profile of bacterial isolates from adult patients in a referral hospital in Ethiopia (Temesgen et al., 2019).

In a study in Ghana, conducted at the genitourinary clinic of the surgical department of Korle-Bu Teaching Hospital on the rectal carriage of fluoroquinolone resistant and ESBL-producing Enterobacterales in prostrate biopsy infections, 62.6% (n=226/361) of patients carried

fluoroquinolone resistant and ESBL-producing *K. pneumonia* complex. The rectal colonization by ESBL-positive *E. coli* and *K. pneumoniae* complex was identified as an independent risk factor for complications in post-biopsy infections (Labi et al., 2022).

Carbapenem resistant *K. pneumoniae* (CRKP) has also emerged and reportedly up to 54% in some WHO regions (Bassetti et al., 2019). In a 10-year (2008–2018) retrospective observational study of the prevalence, susceptibility, risk factors and molecular epidemiology of clinical CRKP isolates in China, the prevalence of ESBL-producing *K. pneumoniae* had decreased from 39.5% in 2008 to 21.5% in 2018. There was however a significant increase in prevalence for CRKP, particularly from ICUs from 2.5% to 15.8%. The decreasing trend of ESBLs-producing *K. pneumoniae* could however not be reflective of the actual situation, as some strains of *K. pneumoniae* may have harbor both carbapenemase- and ESBLs-encoding genes, one of which may predominantly be the focus of studies in some instances, thereby masking the presence of the other (Hu et al., 2020).

K. pneumoniae can persist on surfaces and can thus be disseminated within hospital environments. In a molecular surveillance study of *K. pneumoniae* on frequently touched surfaces in a non-outbreak setting involving four public hospitals, *K. pneumoniae* was isolated from 10% (75/777) of the contaminated surface samples in three wards (ICU, general, and pediatric) of the hospitals. Forty percent of the *K. pneumoniae* isolates harboured ESBLs, particularly the $bla_{CTX\text{-}M}$ genes (Malinga et al., 2022). Acolatse et al. (2022) reported that *K. pneumoniae* constituted 22.97% (17/75) of all ESBL or carbapenemase producing GNB recovered from low-touch and high-touch surfaces in a study on the environmental surveillance of ESBL and carbapenemase GNB in the Obstetrics and gynaecology, Neonatal Intensive Care Unit, Female Medical Ward, Accident and Emergency Ward and the Paediatric Ward in a Teaching hospital in Ghana. This is indicative of

K. pneumoniae isolates harbouring resistant genes which could be transmitted within the hospital and a concern for IPC (Acolatse et al., 2022).

MDR GNB have been reported in both community and nosocomial infections and are a serious public health concern (Church, 2021). Enterobacterales (ESBL-producing and carbapenem resistant) which are on the WHO list of critical pathogens requiring research and development of new antibiotics are key indicators for AMR surveillance and containment (WHO, 2017). Among the Enterobacterales, ESBL producing *K. pneumoniae* and *E. coli* have been reported globally and implicated in severe infections such as bloodstream, respiratory tract and urinary tract infections among others. Patients may also become colonized with these MDR GNB, which may be shed into their immediate environments and may be transferred to other patients in the absence of efficient IPC and specifically hand hygiene practices (Suleyman, 2018). Treatment options for these MDR GNB may be limited in the event of infection and hospital outbreaks and are thus a concern for IPC in hospitals (Bassetti, 2019). The dearth of information on molecular characterization of MDR GNB from developing countries such as Ghana is a setback for global epidemiological studies and containment of MDR GNB. There is therefore the need for increased surveillance of these pathogens, not only in infected patients but also in colonized patients and environments in non-outbreak settings to highlight areas for improving IPC. Delineating their resistance and virulence mechanisms as well as their clonal relatedness and possible transmission between various sources is important in developing strategies for the containment of AMR in Ghana.

AIM AND OBJECTIVES OF THE STUDY

3.0 Aim

To triangulate antibiotic and biocide resistance genes, virulence genes and the associated mobile genetic elements as well as the phylogenies of MDR Gram-negative bacterial isolates from hospital environments, patients and staff in a teaching hospital in Ghana to evaluate and improve IPC policies and practices.

3.1 Specific objectives

1. To recover GNB from patients rectal and hand swabs, hands of healthcare workers and ward environments (sinks, floor, drip stands, door handles and high touch surfaces in three specialties; Intensive Care Unit, Surgery and Obstetrics and Gynaecology of the Teaching Hospital.
2. To identify GNB from infected patients, healthcare workers hands and hospital environment using Vitek® 2 System.
3. To determine the prevalence and incidence of colonization due to GNB in the patients on admission and during hospital stay in the three specialties.
4. To ascertain the phenotypic characteristics of GNB isolates from, patients, staff and hospital environment by MIC determinations according to CLSI/EUCAST against a panel of 17 antibiotics (Ampicillin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefipime, Amoxicillin/Clavulanic, Doripenem, Ertapenem, Meropenem, Imipinem, Piperacillin/Tazobactam, Gentamicin, Tobramycin, Amikacin, Ciprofloxacin, Trimetoprim-sulphamethoxazole and Tigecycline).
5. To genotypically delineate antibiotic resistance mechanisms and analyze the relationship between isolates from environmental samples, patients and HCWs hands by whole genome sequencing (WGS) and bioinformatics analysis.
6. To determine the clonal and phylogenomic relationships of MDR GNB isolated from patients, HCWs hands and the hospital environments and the clonal lineages of the MDR GNB isolated from the different wards in the hospital using WGS and bioinformatics tools.

4.0 Synopsis of Methodology

4.1 Ethical considerations

The study was approved by the Institutional Review board (IRB) of the Komfo Anokye Teaching Hospital **(Reference: KATH IRB/AP/107/20)** and the Biomedical Research Ethics Committee of the University of KwaZulu-Natal **(Reference: BREC/00001917/2020)**.

4.2 General methodology

In this prospective study, we investigated the molecular epidemiology of MDR GNB isolates collected from rectal and hand swabs of all patients admitted to the ICU, Obstetrics and Gynaecology and Surgery directorates were collected on admission and 48 hours after admission over a three-month period (April 2021 to July 2021) in the Komfo Anokye Teaching Hospital in Ghana. GNB were cultured on MacConkey agar and presumptive Gram-negative bacterial isolates selected after biochemical identification and Gram staining. Identity and antibiotic susceptibilities of isolates prior to whole genome sequencing were further confirmed by VITEK. Factors that could potentially predict patient's risk of colonization with MDR GNB were assessed using a questionnaire and extracting information from patient files.

The study was narrowed down to MDR ESBL or carbapenem resistant *Escherichia coli* and *K. pneumoniae* isolates. The genomic DNA of the multidrug resistant isolates were extracted and purified and using paired-end libraries, whole genome sequencing was carried out. The resistome, virulome, mobilome, clonality and phylogeny of MDR GNB clones were ascertained using WGS and bioinformatic tools.

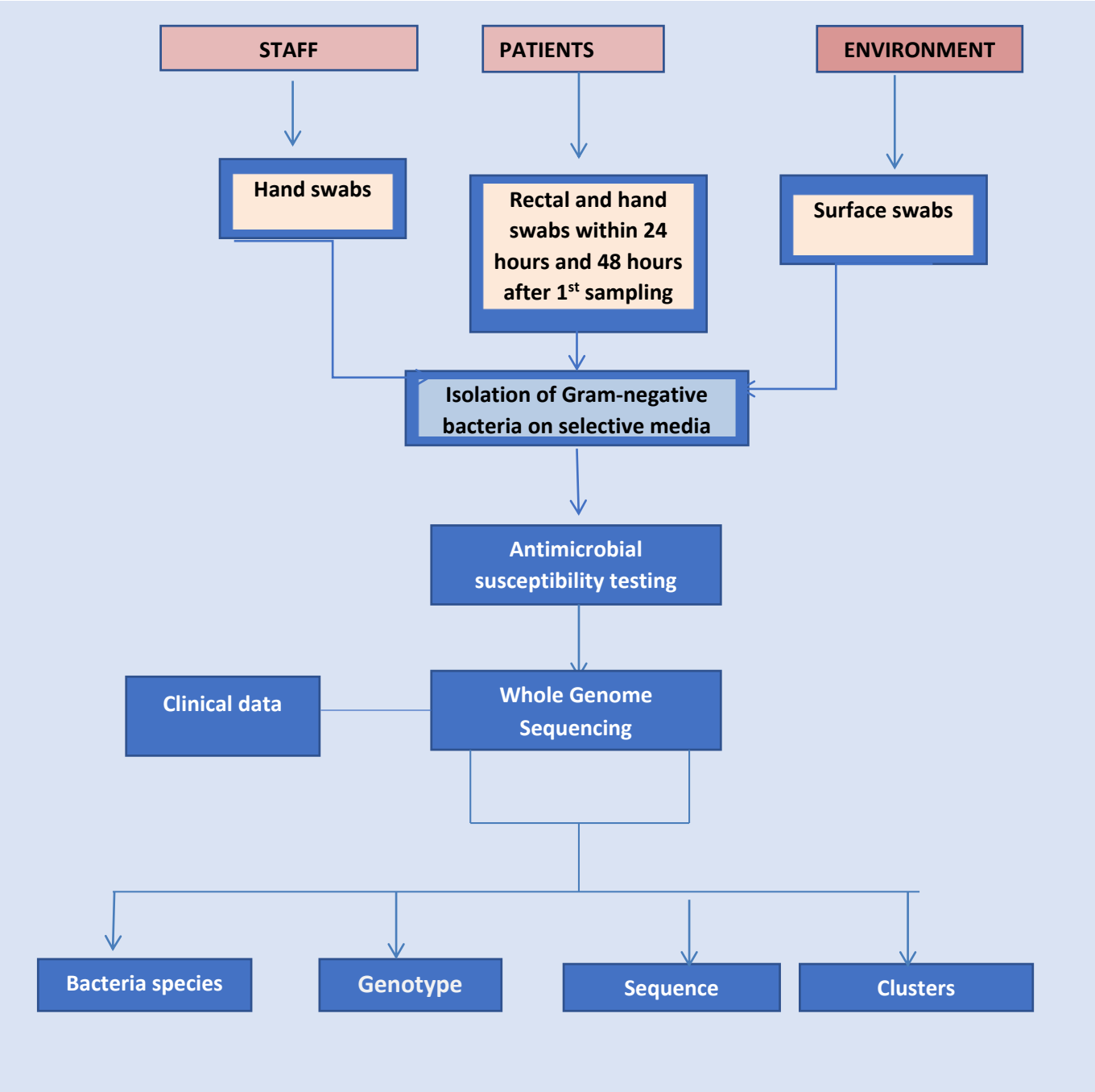

Figure 2: Summary of study methodology

5.0 Outline of the thesis

This study is presented in the form of manuscripts and comprises the following five chapters:

• **Chapter 1.** Introduction and Literature Review: This chapter gives an overview of clinically relevant MDR GNB, their transmission and their mechanisms of resistance to antibiotics. Infection prevention and control practices in the context of patients, their environment and HCWs is also discussed.

• **Chapter 2**. Manuscript 1: Multidrug resistant Gram-negative bacterial carriage in patients, healthcare workers and environments of a tertiary hospital in Ghana. This manuscript which is intended to be submitted to the Journal of Infection and Public Health discusses the prevalence of MDR GNB from patients, HCW's hands and from the hospital environments of the teaching hospital. The risk factors for rectal colonization of patients with MDR GNB on admission and after 48 hours of admission are also discussed.

• **Chapter 3**. Manuscript 2: Genomic characterization of multi drug resistant ESBL-producing *Escherichia coli* isolates from patients and patient environments in a teaching hospital in Ghana. This manuscript which has been submitted to the Journal of Infection and Public Health [Manuscript number: JI-PH-D-23-01256], describes the antibiotic resistome, mobilome, virulome and phylogenic relatedness of MDR ESBL-producing *Escherichia coli* isolates from patients and hospital environments in the Komfo Anokye Teaching Hospital in Ghana.

• **Chapter 4**. Manuscript 3: Genomic analysis of porin related resistance in ESBL-producing *Klebsiella pneumoniae* isolates from patients and environments in a teaching hospital in Ghana. This manuscript is intended for submission to Microbial Drug Resistance and describes the use of WGS and bioinformatic tools to analyze the resistance mechanisms and the phylogenomics ESBL-producing *Klebsiella pneumoniae* isolates from patients and environments in a teaching hospital in Ghana.

• **Chapter 5**. Conclusion. This chapter provides a summary of the work and the significance thereof and includes limitations of the study and recommendations for future research.

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CHAPTER TWO – MANUSCRIPT 1

MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIAL COLONIZATION IN PATIENTS, CARRIAGE BY HEALTHCARE WORKERS AND CONTAMINATION OF ENVIRONMENTS IN A TERTIARY HOSPITAL IN GHANA

AUTHOR CONTRIBUTIONS

Esther Eyram Asare Yeboah as the principal investigator, co-conceptualized the study, undertook sample collection, laboratory work and statistical analyses and drafted the manuscript.

Nicholas Agyepong – as co-supervisor co-conceptualized the study, assisted with sample collection, conducted data analysis, vetted the results and critically reviewed manuscript.

Daniel Gyamfi Amoako - conducted data analysis, vetted the results and critically reviewed manuscript.

Akebe Luther King Abia - conducted data analysis, vetted the results and critically reviewed manuscript.

Alexander Owusu-Ofori- as co-supervisor co-conceptualized the study, facilitated sample collection, vetted the results and critically reviewed manuscript.

Sabiha Yusuf Essack - as the principal supervisor, co-conceptualized the study, guided the literature review and ethical approval application, facilitated data collection and analysis, vetted the results and undertook critical revision of the manuscript.

Objectives met: This paper answers objectives 1, 2, 3 and 4.

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Original article

Multidrug-resistant Gram-negative bacterial colonization in patients, carriage by healthcare workers and contamination of hospital environments in Ghana

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ABSTRACT

Background: Patients already colonized with multidrug-resistant (MDR) Gram-negative bacteria (GNB) on admission to critical care units may be an important source of transmission of these bacteria in hospitals. We sought to determine the prevalence of MDR GNB colonization in patients, staff and the ward environment and to assess the risk factors for colonization of patients in wards.

Methods: The study was conducted from April 2021 to July 2021 in a teaching hospital in Ghana. MDR GNB were isolated from rectal, and hand swabs were taken from patients on admission and after 48 h. Swabs from HCW's hands and the ward environment were also taken. Risk factors for colonization with MDR GNB were assessed using univariate and multivariate analysis.

Results: MDR GNB rectal colonization rate among patients was 50.62% on admission and 44.44% after 48 h. MDR GNB were isolated from 6 (5.26%) and 24 (11.54%) of HCW's hand swabs and environmental swabs, respectively. Previous hospitalization (p-value = 0.021, OR, 95% CI= 7.170 (1.345–38.214) was significantly associated with colonization by MDR GNB after 48 h of admission. Age (21–30 years) (p-value = 0.022, OR, 95% CI = 0.103 (0.015–0.716) was significantly identified as a protective factor associated with a reduced risk of rectal MDR GNB colonization.

Conclusion: The high colonization of MDR GNB in patients, the carriage of MDR GNB on HCW's hands, and the contamination of hospital environments highlights the need for patient screening and stringent infection prevention and control practices to prevent the spread of MDR GNB in hospitals.

Introduction

Multidrug-resistant Gram-negative bacteria (MDR GNB) are increasingly implicated in difficult-to-treat infections, especially in critically ill patients [1,2] in low- and middle-income countries (LMICs) with inadequate infection prevention and control (IPC), surveillance, and antimicrobial stewardship (AMS) programs and policies [3,4].

Patients may be colonised with MDR organisms before admission or may acquire persistent organisms from contaminated ward environments. These MDR GNB could also be transmitted from patient to patient via the hands of staff associated with specific wards, leading to colonization and infections in new patients [5,6]. Predisposing factors for colonization proceeding to infection with GNB

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include, among others, prolonged hospitalization, critical health conditions, invasive medical procedures such as using mechanical ventilation and catheters, surgeries, broad-spectrum antibiotic use, and co-morbidities such as diabetes, liver and renal impairment [7,8]. Patients colonization by MDR GNB before or during hospitalization has prolonged effects and significantly increases the risk for subsequent MDR GNB infection, morbidity and mortality after hospital discharge [5]. Frequently used instruments (such as stethoscopes) and frequently touched surfaces which inadequately disinfected could harbor MDR bacteria, serving as a source of transmission to patients and hospital personnel [9,10].

Screening for MDR carriage in patients, staff and hospital environments in Sub-Saharan Africa, as a preventive measure, is rarely conducted due to financial constraints. However, screening patients in selected wards for MDR GNB colonization is necessary to prevent and contain the spread of these bacteria in hospitals [11].

As part of an infection prevention and control initiative at the Komfo Anokye Teaching Hospital, (KATH) in Kumasi, Ghana, patients admitted to three directorates (ICU, Obstetrics and Gynecology and Surgery) were screened for colonization with GNB on admission and after 48 h of hospitalization, respectively. This study additionally evaluated the proportions and phenotypic characteristics of MDR GNB bacteria from patients, HCW's and the ward environment. The risk factors associated with patient colonization with MDR GNB in the hospital were also analyzed to inform IPC, AMS and surveillance policies, programmes and practices.

Materials and methods

Study site

The KATH is a 1200-bed capacity government tertiary and referral hospital serving about 80% of both emergencies and regular medical cases in the Ashanti Region. The hospital also accepts referral cases from regions like the Bono, Bono East, Ahafo, Western, Eastern regions, and some parts of Ghana's Northern regions. Studied units included the hospital's Surgery, Intensive Care unit, and Obstetrics, and Gynaecology directorates.

Study design and population

This cross-sectional observational study prospectively collected rectal and hand swab samples from patients admitted to three directorates of the KATH over three months (April-July 2021). Patient samples were collected within 24 h of admission and after 48 h of admission (i.e., at 72 h). Hand swabs of staff present in the ward and swabs from environmental surfaces in the wards were taken at each patient sampling visit. A visit was defined as a patient interaction where clinical data and cultures from the patient, the ward environment and staff present during sample collected. GNB were selectively isolated, identified and subjected to antimicrobial susceptibility testing.

Demographic and clinical data

Demographic information, such as age and gender, were collected from patients' electronic folders. A questionnaire was used to collect information on potential risk factors for colonization with MDR GNB such as patient's previous hospital antimicrobial drug exposures (≤ three weeks before hospitalization), length of previous hospitalization (≤ 3 weeks before ward admission) and co-morbidities.

Sample collection and microbiological analysis

Patients

Paired samples at 2 time points (i.e., within 24 and 72 h of admission) were taken from patients' rectum and hands. Inclusion criteria included 1) patients admitted directly from the outpatient department (OPD) or consulting rooms, 2) patients ≥ 16 years old, and 3) those who voluntarily consented to participate in the study. Exclusion criteria included 1) patients who were unable to provide consent, 2) comatose patients, 3) patients who spent > 24 h at the Accident and Emergency Unit (These patients may already have been colonized or acquired GNB before admission to the wards), and 4) patients with anal atresia, ileostomy or other reasons that precluded rectal swabs during screening.

Healthcare workers

Hand swabs from healthcare workers (doctors, physician assistants and nurses) present in the ward during patient sampling were obtained by swabbing the dorsum of each finger three times (3x) and the palm of each hand two times (2x) with a twirling motion of the swab using a single swab as described previously [12]. One sample was collected per staff present at time of patient sampling.

Ward environment

Swabs were collected from the ward environmental surfaces in close contact with patients (bed rails, drip stands and frequently touched surfaces such as tap handles and door handles). The surfaces and instruments were swabbed at each time of visit to the ward for patient sample collection. At each site, an area of approximately 10 cm^2 was sampled using a sterile cotton swab. Swabs were then sent to the bacteriology laboratory in the hospital for processing within 6 h. Unoccupied beds and unused exit doors and drip stands were excluded.

All samples were transported on ice to the laboratory for processing within 6 h.

Isolation of Gram-negative bacteria

Swab samples were cultured in tryptic soy broth (Neogen, Lansing, MI, USA) overnight at 37 °C and streaked on MacConkey agar (Oxoid, Basingstoke, UK). After incubation at 37 °C for 24 h aerobically, three morphologically distinct colonies were selected for further identification. Where the colonies were not distinct, three colonies were randomly picked for Gram-staining and further identification using the Vitek® 2 System.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the isolates was determined by broth dilution using the Vitek® 2 System and Vitek® 2 Gram-negative susceptibility card (AST-N255) (BioMérieux-Vitek, Marcy-l'Étoile, France). The antibiotic panel consisted of amikacin, ampicillin, cefuroxime, doripenem, cefepime, ceftriaxone, ceftazidime, ciprofloxacin, amoxicillin/clavulanic, trimethoprim-sulphamethoxazole, ertapenem, imipenem, meropenem, gentamicin, piperacillin/tazobactam, tobramycin, tigecycline and penicillin. Isolates resistant to at least one agent in three or more distinct antibiotic classes were classified as MDR [13].

Data analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 27 (IBM., Armonk, NY, USA). Quantitative

data were expressed as frequencies and percentages while continuous variables were presented as medians (interquartile range, IQR). Chi-square and Fisher's exact tests were used to compare categorical variables. A univariate logistic regression analysis was conducted to identify factors associated with colonization by MDR GNB on admission and after 48 h of admission. A multivariate logistic regression analysis was conducted to evaluate the effect of factors previously identified in the univariate analysis model as factors with a *p*-value lower than 0.25. This was followed by an iterative process of purposeful removal of covariates in the multiple logistic regression. Results were presented as odds ratios (OR) with 95% confidence intervals (CIs). Two-tailed $p < 0.05$ was considered significant.

Results

Patient characteristics

Ninety consenting patients meeting the inclusion criteria were enrolled in the study. However, seven patients with missing sociodemographic data or missing samples were excluded from the analysis. Swabs were collected from eighty-three patients within 24 h of admission and 54 patients on follow-up after 48 h of admission, i.e., at 72 h from either rectum or hand or both (Table 1).

The average patient age was 33.74 ± 12.58; 25% of the patients had been previously hospitalized in the last three weeks or closer to admission. Antibiotic use (≤ 3 weeks) was documented in 11.90% of patients. Furthermore, 73 (87.95%) were subjected to an invasive procedure on admission or during the study.

A total of 585 samples were collected during the study. Paired hand and rectal samples were to be collected from patients, however some patients refused rectal swabbing in some instances. Rectal swabs and hand swabs were collected from 81 and 76 patients at admission, respectively, yielding 102 GNB. Fifty-seven patients were sampled after 48 h of admission, yielding 63 GNB (54 rectal and 15 hand swab isolates).

Sixteen GNB were isolated from 114 HCW hand swabs (which included 30 from nurses, 60 from midwives, 9 from medical officers and 15 from others) and 51 from 208 ward environment swabs (Fig. 1).

Table 1
Characteristics of patients on admission.

DIAGNOSIS	N	%
Tumors/ cancers	11	13,10
Pre-eclampsia/Eclampsia	62	73,81
Leg/foot ulcers	5	5,95
Others	5	5,95
INVASIVE PROCEDURES		
Urinary catheter	61	72,62
Intravenous line	63	75,00
Tracheal/nasogastric tube	2	2,38
Arterial line	2	2,38
Intracranial line	2	2,38
Surgery	5	5,95
Other invasive devices	7	8,43
Previous hospitalization (≤3 weeks)	21	25,00
Previous antibiotic use (≤3 weeks)	10	11,90
Age (years)		
18–20	4	4,81
21–30	33	39,76
31–40	30	36,14
41–50	6	7,23
51–60	2	2,41
> 60	6	7,23
Age (Mean)	33.74 ± 12.58	
DIRECTORATE		
Intensive Care Unit	5	5,95
Surgery	16	19,05
Obstetrics & Gynaecology	62	75,00

Antibiotic resistance profiles

Patients (81.8%), staff (81.3%) and environmental (68.6%) isolates were highly resistant to ampicillin. However, all isolates were susceptible to amikacin (100%) and tigecycline (99.15%) except an isolate from staff which was resistant to tigecycline. Furthermore, patients (17.7%), environmental (19.6%) and staff (12.5%) isolates exhibited resistance to the carbapenems. In the Obstetrics and Gynaecology directorate, 43.30% of GNB were MDR. Similarly, 56% and 66.67% of GNB from the surgery directorate and ICU, respectively, were MDR.

MDR GNB colonization in patients

Forty-one (41/81; 50.62%) rectal samples taken on admission were positive for one or more MDR GNB. Eight (8/81; 9.87%) patients carried MDR GNB on their hands at admission.

After 48 h of admission, twenty-three (23/53; 43.40%) patients had MDR GNB rectal colonization; 12/54; 22.22% newly acquired MDR GNB after 48 h of admission. New acquisition excluded patients colonized at baseline enrolment i.e., on admission with an MDR GNB. Twelve of 24 patients (50%) with rectal colonization of MDR GNB on admission, were colonized with different species of MDR GNB after 48 h. Of the patients in the Obstetrics and Gynaecology directorate, 23.7% had acquired MDR GNB after 48 h of admission (Fig. 2).

Risk factors for bacterial colonization upon admission

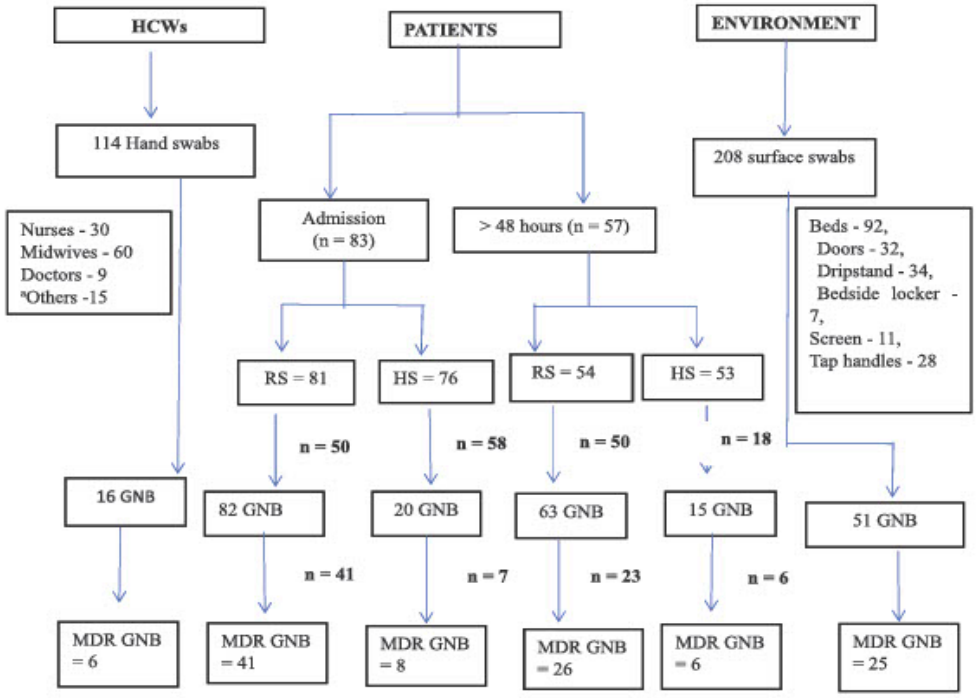
Univariate analysis revealed differences in MDRGNB-colonized and non-colonized patients, although this was not statistically significant (Table 2). Furthermore, multivariate regression analysis showed that none of the factors was statistically significantly independently associated with colonization on admission (Supplementary Table S3). Previous hospitalization (*p*-value = 0.021, OR,95% CI= 7.170 (1.345–38.214) was significantly associated with colonization by MDR GNB after 48 h of admission. Age (21–30 years) (*p*-value =0.022, OR, 95% CI = 0.103(0.015–0.716) was significantly identified as a protective factor associated with a reduced risk of rectal MDR GNB colonization (Table 3).

Hand carriage of MDR GNB by HCWs and other hospital staff

MDR GNB were isolated from six (6/114; 5.26%) healthcare workers hand swabs collected during patient sampling. Four of the six MDR GNB were isolated from midwives' hands and two from nurses' hands respectively. The MDR GNB bacteria were isolated from 5/87 (5.75%) and 1/21 (4.76%) of HCW hand samples in the Obstetrics and Gynaecology and Surgery directorates, respectively. There were no MDR GNB isolated from HCW hand samples in the ICU. Three (50%) of these isolates were *P. mirabilis*, and one each of *Providencia stuartii*, *E. cloacae* and *C. freundii*, respectively.

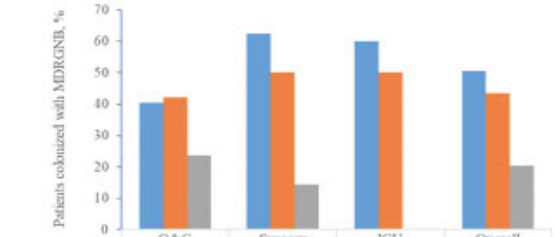
Contamination of Ward environment with MDR GNB

Twenty-five of 49 (49.01%) GNB isolates from the environmental samples collected during patient visits were MDR GNB. Fifteen (60%) MDR GNB isolates were from beds and 5 (20%) from tap handles. Eleven (44%) of the MDR GNB from environmental swabs were *E. coli*. Seventy-two percent (18/25) and 24% (6/25) of MDR GNB from environmental samples were from the Obstetrics and Gynaecology directorate and Surgery directorate, respectively (Supplementary Table S1).



HCWs: Healthcare workers, [a]- others included interns and ward assistants, RS: Rectal swab, HS: Hand swab, GNB: Gram-negative bacteria, MDR-GNB: Multidrug-resistant Gram-negative bacteria

Fig. 1. Sampling framework, HCWs: Healthcare workers, [a]- others included interns and ward assistants, RS: Rectal swab, HS: Hand swab, GNB: Gram-negative bacteria, MDR-GNB: Multidrug-resistant Gram-negative bacteria.



	O&G	Surgery	ICU	Overall
On admission	40,32	62,5	60	50,62
>48 hours	42,1	50	50	43,4
New acquisition	23,7	14,29	0	20,37

Fig. 2. Rectal colonization of patients with MDR GNB on admission and follow-up visits.

Patient colonization, staff carriage and ward environmental contamination with MDR GNB during sampling visits

At admission, MDR GNB were isolated from 15 patients' rectal and hand swabs, and the environment. In 10/28 (35.71%) patient follow-up visits where MDR GNB were isolated from patients hands or rectal swabs, MDR GNB were also isolated from ward environment and staff (Table 2). Of all the 137 patient-visits, 25 (18.25%) were associated with the isolation of one or more MDR GNB in the environment and patients (Supplementary Table, S4).

Antibiograms of MDR GNB isolates

Different antibiogram patterns were observed among MDR GNB isolates from patients, staff and the environment, with 20 antibiograms common to all sample sources. Thirteen of 25 (52%) MDR GNB isolates from the ward environment had similar antibiograms as isolates from patients (Table 4). Similar antibiogram shared by one MDR isolate from a staff. Common antibiograms were also observed in patients within and between different directorate wards.

Discussion

MDR GNB have become problematic in hospitals, causing high morbidity and mortality [1]. They may be disseminated to and from patients, HCWs and ward environments with sub-optimal IPC measures. The current study assessed the prevalence of MDR GNB in hospital ward environments, patients and on HCWs hands.

MDR GNB from patients, HCW's hands and ward environments showed similar antibiograms, indicating a likely dissemination among patients, HCW's and the environment. Patients carried MDR GNB on admission, suggesting community acquisition and eleven patients acquired MDR GNB after 48 h of admission, possibly because of inadequate IPC practices, although none developed a hospital-acquired infection.

Table 2
Univariate analysis of patients with rectal colonization of MDR GNB on admission and after 48 h of admission.

	ON ADMISSION (N = 81)			> 48 h (N = 53)		
	MDR GNB present, n (%) N = 41	MDR GNB absent, n (%) N = 40	p-value	MDR GNB present, n (%) N = 23	MDR GNB absent, n (%) N = 30	p-value
DIAGNOSIS						
Tumors/ cancers	6 (14,63)	4 (10,0)	0,737	0(0,0)	8 (26,67)	**0.007**
Pre-eclampsia/eclampsia	28 (68,29)	33 (82,5)	**0.138**	17 (73,91)	21(70,0)	0,754
leg/foot ulcers	3 (7,32)	2(5,0)	1,000	4 (17,39)	1 (3,33)	**0.154**
Others	4 (9,76)	1 (2,5)	0,359	2 (8,70)	0 (0,00)	**0.184**
INVASIVE PROCEDURE				21(91,30)	24 (80,0)	
URI catheter	29 (70,73)	31 (77,5)	0,487	14 (60,87)	20 (66,67)	0,663
IV line	32 (78,05)	30 (75,0)	0,746	19 (82,61)	22 (73,33)	0,424
Other invasive device	2 (4,88)	5 (12,5)	**0.249**	4 (17,39)	2 (6,67)	0,385
Previous hospitalization	28 (68,29)	10(25,0)	0,906	9(39,13)	6(20,0)	**0.145**
Previous antibiotic use	6 (14,63)	4 (10,0)	0,512	4 (17,39)	5 (16,67)	1,000
Obstetrics & Gynaecology	28 (68,29)	33 (82,5)	**0.138**	17(73,91)	21(70,0)	0,754
Surgery	10 (24,39)	5 (12,5)	**0.168**	5 (21,74)	8 (26,67)	0,679
Intensive Care	3 (7,31)	2 (5,0)	1,000	1 (4,35)	1 (3,33)	1,000
MDR GNB isolation on admission				12 (52,17)	13 (43,33)	0,598
Any MDR GNB isolation on admission				12 (52,17)	13 (43,33)	0,523

Statistically significant p-values of factors included in multivariate analysis are in bold

Table 3
Multivariate regression analysis of colonization of patients by MDR-GNB.

On admission	OR (95% CI)	p-value
Pre-eclampsia/eclampsia	0,703 (0,096–5,143)	0,729
Other invasive procedure	1,807 (0,274–11,915)	0,539
Surgery	1,447 (0,173–12,093)	0,733
After 48 h	**OR (95% CI)**	**p-value**
Previous hospitalization	6,803 (1,144–40,450)	**0.035**
MDR GNB on admission	3,204 (0,758–13,538)	0,113
Age (21–30) years	0,103 (0,015–0,716)	**0.022**
Age (31–40) years	3,527 (0,712–17,480)	0,123

CI; confidence interval; OR; odds ratio, Statistically significant p-values are in bold

Notwithstanding previous hospitalization, 50.62% of patients were colonized on admission, indicating community circulation of MDR GNB. This proportion is comparable to a South Africa study where MDR GNB colonization on admission was 50% among patients in a tertiary hospital compared to 29.63% in a district hospital [7]. However, a study on hospitalized patients in a Mongolian trauma centre reported a lower colonization of 29.1% on admission that escalated to 69.9% after 14 days of hospitalization [14]. Differences in IPC practices and the length of hospital stay could contribute to such differences in rectal carriage rates of MDR GNB in different settings.

Some of these patients may also have acquired MDR GNB from previous hospital stays as 68.29% of the MDR GNB-colonized patients on admission had been previously hospitalized three weeks before the current admission. This is further corroborated by the multivariate analysis, suggesting previous hospitalization was significantly associated with colonization by MDR GNB after 48 h of admission. Previous hospitalization has been linked to acquisition of MDR organisms in hospital settings [15–17]. Patients can also acquire MDR GNB shortly after admission, in the absence of IPC policies and practices.

After 48 h, the rectal carriage rate among patients in the current study was 43.40%. New acquisition of MDR GNB was observed in 11 patients, showing a likelihood of acquisition via the environment or through HCWs hands. MDR nosocomial pathogens may be acquired, especially by immunocompromised patients, in high-risk wards ICUs and surgery without efficient IPC practices. Furthermore, these patients are often subjected to invasive devices, which could be a risk factor for the development of HAIs or colonization with MDR GNB [18,19]. Although some patients on invasive devices acquired MDR GNB in the present study, the risk was not statistically significant. Obstetrics and Gynaecology directorate patients were the most colonized with MDR GNB. This poses a significant infection risk in pregnant women and neonates, as previous studies described an

Table 4
MDR GNB colonization in patients, carriage by staff and ward contamination at baseline and follow-up visits.

			Patient rectal swabs		Patient hand swab		Patient rectal or hand swab	
			MDRGNB, n (%)		MDRGNB, n (%)		MDRGNB, n (%)	
			Positive	Negative	Positive	Negative	Positive	Negative
BASELINE VISIT	**Environment contamination**	**positive**	14 (34,16)	19 (47,5)	2 (66,67)	29 (40,28)	15 (20,83)	18 (43,90)
		negative	27 (65,85)	21 (52,5)	1 (33,33)	43 (59,72)	27 (37,5)	23 (56,10)
		Total	41	40	3	72	42	41
	Staff contamination	**Positive**	15 (36,58)	15 (37,5)	1 (33,33)	27 (37,5)	15 (35,71)	15 (36,59)
		Negative	26 (63,41)	25 (62,5)	2 (66,67)	45 (62,5)	27 (64,29)	26 (63,41)
		Total	41	40	3	72	42	41
FOLLOW- UP VISIT	**Environment contamination**	**positive**	7 (30,43)	12 (40,00)	4 (66,67)	14 (29,79)	10 (35,71)	9 (31,03)
		negative	16 (69,56)	18 (60,00)	2 (33,33)	33 (70,21)	18 (64,29)	20 (68,97)
		Total	23	30	6	47	28	29
	Staff contamination	**Positive**	9 (39,13)	13 (43,33)	1 (20,00)	20 (42,55)	10 (35,71)	12 (41,38)
		Negative	14 (60,87)	17 (56,67)	5 (80,00)	27 (57,45)	18 (64,29)	17 (58,62)
		Total	23	30	6	47	28	29

P; Patient, E; Environment, ICU; Intensive Care Unit,
AMK; Amikacin, AMP; Ampicillin, CXM;Cefuroxime, DOR;Doripenem, FEP;Cefepime, CRO;Ceftriaxone, CAZ;Ceftazidime, CIP;Ciprofloxacin, AMC;Amoxicillin/clavulanic acid, SXT;Trimethoprim/Sulphamethoxazole, ERT;Ertapenem, GEN;Gentamicin, IPM;Imipenem, MEM;Meropenem, TZP;Piperacillin/Tazobactam, TOB;Tobramycin, TGC;Tigecycline, PEN;Penicillin

association between colonization in mothers and infection in neonates [20,21].

The hand carriage of MDR GNB (9.21%) among patients after 48 h presents an important source of MDR GNB transmission. Patients' hands are more likely to be a point of self-inoculation and a direct contact with HCWs, the environment and other patients, highlighting the need for increased hand hygiene practices by patients [22].

Tertiary hospitals, such as in this study, are referral points for patients from other hospitals who may have previously been exposed to antibiotics. Ten (12.35%) patients had been previously exposed to antibiotics. This may be an underestimation, where people can access unprescribed antibiotics through unauthorized outlets and may not be familiar with the names of antibiotics especially in rural areas in Ghana [23,24]. Though MDR GNB were isolated from some patients who had prior antibiotic exposure, no significant association could be established. This finding may have been affected by the fact that we explored antibiotic use only up to three weeks before hospitalization, hence, the possible exclusion of patients with antibiotic exposure of more than 3 weeks. Similarly, a study conducted in an ICU in Spain could not also confirm prior antibiotic exposure as a significant risk factor for MDR GNB colonization among admitted patients due to strict criteria used on prior antibiotic exposure in study patients [25].

Escherichia coli, *Proteus mirabilis* and *K. pneumoniae*, frequently implicated in community-acquired infections and HAIs [26–28], were the most frequently isolated GNB from patients within 24 h of admission. They may be transmitted between hospitals and communities; therefore, a potential for a cycle of transmission of these organisms between community dwellers and hospitalized patients [29].

Multidrug-resistant *E. coli*, *K. pneumoniae* and *P. aeruginosa* were the most frequently isolated organisms from patient environments. Some of these organisms, like *P. aeruginosa* could be shed from patients, and thrive in the environment, due to its biofilm formation capability [30,31]. Transmission of MDR GNB in hospitals have been linked to contamination of frequently touched surfaces in contact with patients. This underscores the need for frequent and efficient cleaning and disinfection to prevent environment-to-patient pathogen transmission, especially in high-risk wards. In Ghana, MDR GNB have been isolated as the most prevalent GNB causing infections in hospitals [32].

Healthcare workers' hand swabs were less frequently (5.26%) contaminated with MDR GNB. A positive environmental culture with multidrug-resistant organisms (MDROs) including MDR GNB has been identified as one of the risk factors for the HCW's hand contamination by MDROs [12]. The low MDR GNB isolation rate from HCWs hands could be due to compliance of the HCWs to hand hygiene practices, particularly as this was heightened due to COVID-19 pandemic. Nonetheless, the low rate of isolation of MDR GNB from HCW's hands does not preclude the likelihood of transmission, especially as a single HCW may attend to several patients in a day.

Isolates with common antibiograms were observed among patients and between wards at different time-points indicating the likelihood of MDR GNB circulating in the hospital wards. These isolates could be a source of nosocomial infections, especially in immunocompromised patients. Similar antibiograms were also shared between patients and the environment, indicating a likely transfer of the MDR isolates from patients to the environment or vice-versa. Whole genome analysis of these isolates would be useful in determining the genotypic similarity between the isolates from various sources.

Antibiograms indicated varying resistance of the isolates to some classes of antibiotics on the Watch category of the WHO AWaRe list [33]. Notably, among the MDR GNB, 45.1% were resistant to one or more of the carbapenems (meropenem, ertapenem, doripenem and imipenem) tested. Resistance to the cephalosporins was lower than that reported in a previous study by Agyepong et al. (2018) on MDR GNB from the same hospital in Ghana. This could be attributed to the differences in sample sources with isolates from this study being from colonized patients, environment and staff in contrast to the MDR GNB from clinical infections. Likewise, resistance to the carbapenems to which MDR GNB were previously reported to be susceptible to in Ghana, suggests the emergence and increase in carbapenem-resistant MDR GNB in the last five years [34,35]. The observed resistance to ciprofloxacin (27.35%) and piperacillin/tazobactam (35.90%), in the current study indicates the prevalence of MDR organisms, which could cause difficult-to-treat infections; hence, the need for increased surveillance on these antibiotics to preserve their activity.

None of the factors considered in this study were independently associated with colonization on admission, indicating that other factors such as community acquisition of resistant bacteria could be responsible for colonization of patients before hospitalization. However, age (21–30 years) was significantly identified as a protective factor associated with a reduced risk of rectal MDR GNB colonization after 48 h of admission. Though age is not a modifiable risk factor, age (> 30 years) provides a focus for targeted isolation and precautionary measures in preventing colonization and subsequent infection by MDR GNB in admitted patients [5].

Some limitations of this study were the relatively small-sample size of patients. Coupled with the exclusion of patients who had been admitted to the wards through the accident and emergency unit, the study results may underestimate the colonization of patients and cannot be generalized for the whole hospital. This, however, suggests that colonization in patients may be a bigger problem than recorded in the present study. Also, as the study period was limited and patients were not followed up beyond the second sampling i.e. after 48 h, these results may further underestimate colonization. Nonetheless, the acquisition of MDR GNB by patients in the wards has been demonstrated and this poses a significant risk of nosocomial infection. Furthermore, larger prospective studies are needed to accurately determine MDR GNB colonization at and after hospital admission and the risk factors for colonization in the hospital.

Conclusion

This study shows that MDR GNB are prevalent in patients, on HCW's hands and environment in Ghana. Isolation of MDR GNB on admission and previous hospitalization were associated with colonization by MDR GNB after 48 h of admission, stressing the need for targeted IPC interventions in hospitals to prevent and contain the transmission of MDR GNB especially in high-risk wards. Screening patients and staff for MDR GNB is an important step in preventing and containing the transmission of MDR organisms in hospitals. Routine environmental cultures are also needed to determine effectiveness of IPCs in hospitals. Transmission prevention should, thus, include hand hygiene and thorough environmental cleaning. Multi-centre studies of powered sample size would provide the necessary data required to guide policies on screening for MDR GNB on admission.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2023.10.045.

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SUPPLEMENTARY TABLES

Table S1: Proportions of MDR GNB isolated from patient, staff and environment

Organism	**Patient N (%) N=241**	**Staff N (%) N=114**	**Environment N (%) N=208**
C. freundii	0	1 (0.87)	1 (0.48)
E. cloacae	6 (2.49)	1 (0.87)	2 (0.96)
E. coli	32 (13.28)	0	11 (5.29)
K. pneumoniae	15 (6.22)	0	5 (2.40)
P. aeruginosa	1 (0.41)	0	4 (1.92)
P. mirabilis	22 (9.13)	3 (2.63)	2 (0.96)
P. stuartii	2 (0.82)	1 (0.87)	0
**Others*	6 (2.46)	0	0

**Includes Acinetobacter species, E. aerogenes, M. morgannii, P. vulgaris, P. retgerri and N. gonorrhoea*

Table S2: Distribution of MDR GNB from different environmental samples

Site	**No. of sites with MDR GNB N (%)**	**No. of MDR GNB from each site (n)**	***C. freundii* n (%)**	***E. cloacae* n (%)**	***E. coli* n (%)**	***K. pneumoniae* n (%)**	***P. aeruginosa* n (%)**	***P. mirabilis* n (%)**
Beds (N=92)	11 (11.96)	15 (29.41)	1 (33.33)	-	8 (72.73)	3 (60)	2 (66.67)	1 (25)
Door (N=32)	1 (3.1)	1(1.96)	-	-	-	1 (20)	-	-
Drip stand (N=34)	3 (8.82)	4 (7.84)	1 (33.33)	1 (33.33)	1 (9.09)	1 (20)	-	-
Bedside locker (N=7)	0	0	-	-	-	-	-	-
Screen (N=11)	0	0	-	-	-	-	-	-
Tap (N=28)	5 (17.9)	5 (9.80)	1 (33.33)	2 (66.67)	2 (18.18)	1 (20)	1 (33.33)	-
Frequency (%)	20 (9.61)	25 (49.01)	3 (12)	3 (12)	11 (44)	5 (20)	3 (12)	1 (4)

N: number of samples from the site. Frequency of the bacterial isolates among total MDR GNB from the environmental site is presented in the parenthesis. N= number of sites with MDR GNB n=number of MDR GNB isolates from site.

Table S3: Risk factors associated with carriage of MDR GNB (Multivariate Logistic Regression)

	ON ADMISSION		**> 48 HOURS**	
Risk factor	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Tumors/ cancers	1.081 (0.146-8.028)	0.939		
Pre-eclampsia/ eclampsia	0.601 (0.097-3.710)	0.584		
Surgical	2.590 (0.219-30.643)	0.450		
Previous hospitalization			7.170 (1.345-38.214)	**0.021**
ICU	2.641 (0.198-35.247)	0.463		
MDR GNB isolation on admission			6.154 (1.483-25.539)	**0.012**

Table S4: Antibiograms of all MDR GNB isolates from patients, environment and staff stratified by specialty/service

ANTIBIOGRAM	**NO. OF ISOLATES**	**SOURCE**	**SPECIALITY**
E.coli			
AMP-AMC-CXM-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-TOB-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP- CXM-CAZ-CRO-FEP-AMC-TZP-MEM-DOR-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-AMC-TZP-MEM-GEN-TOB-CIP-SXT	2	Patient	Surgery
AMP-CXM-CAZ-FEP-AMC-TZP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ- CRO- FEP-AMC-MEM	1	Patient	Obstetrics & Gynaecology

AMP-CXM-CAZ-CRO-FEP-AMC-ERT-SXT-TZP	2	Patient	Obstetrics & Gynaecology, Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-TZP-GEN-TOB-AMC-SXT	2	Patient	Obstetrics & Gynaecology (2)
AMP-CXM-CAZ-CRO-FEP-AMC-TZP-SXT	8	Patient	Obstetrics & Gynaecology(7), Intensive Care Unit (1)
AMP-CXM-CAZ-CRO-AMC-TZP	1	Environment	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-GEN-TOB-AMC-TZP-CIP-SXT	13	Environment (5)	Surgery(2),Obstetrics & Gynaecology(3)
AMP-CXM-CAZ-CRO-FEP-TZP-GEN-TOB-AMC-CIP-SXT		Patient (8)	Obstetrics & Gynaecology(7), Surgery(1)
AMP-CXM-CAZ-CRO-FEP-TZP-MEM-AMC-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-TZP-TOB-AMC-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CIP-SXT	3	Environment (2) Patient (1)	Obstetrics & Gynaecology, Surgery Obstetrics & Gynaecology
AMP-DOR-MEM-SXT	2	Environment (2)	Surgery, Obstetrics & Gynaecology
AMP-TZP-DOR-MEM-SXT	1	Environment	Obstetrics & Gynaecology
AMP-CAZ-CRO-FEP-TZP-AMC-SXT	1	Patient	Obstetrics & Gynaecology
AMP-GEN-SXT	1	Patient	Surgery
K. pneumoniae			
AMP-CXM-CAZ-CRO-TZP-AMC-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-TOB-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CRO-AMC-CIP-SXT	1	Patient	Surgery
AMP-CXM-CAZ-CRO-DOR-FEP-TZP-AMC-MEM-GEN-TOB-CIP-SXT	1	Patient	Surgery
AMP-CXM-CAZ-CRO-DOR-FEP-TZP-GEN-TOB-AMC-CIP-SXT	1	Environment	Obstetrics & Gynaecology
AMP-CXM-CAZ-FEP-TZP-AMC	1	Environment	Surgery

AMP-CXM-CAZ-CRO-FEP-AMC-TZP-SXT	3	Patient Environment	Obstetrics & Gynaecology (2) Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-TZP-AMC	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-TZP-GEN-TOB-AMC-CIP-SXT	8	Environment (2) Patient (6)	Obstetrics & Gynaecology (2) Surgery(5), Obstetrics & Gynaecology(1)
AMP-CXM-CAZ-CRO-FEP-TZP-AMC-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CAZ-CRO-FEP-TZP-GEN-TOB-AMC-SXT	1	Patient	Intensive Care Unit
E. cloacae			
AMP-AMC-SXT	2	Patient	Obstetrics & Gynaecology(2)
AMP-CXM-CAZ-CRO-AMC	1	Environment	Obstetrics & Gynaecology
AMP-CXM-DOR-MEM-AMC	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-DOR-MEM-ERT-TZP-TOB-AMC-CIP-SXT	2	Patient	Intensive Care Unit (1). Obstetrics & Gynaecology(1
AMP-CXM-CAZ-CRO-FEP-TZP-AMC-SXT	1	Patient	Surgery
AMP-CXM-CAZ-CRO-FEP-CRO-TZP-GEN-TOB-AMC-CIP-SXT	2	Patient Environment	Obstetrics & Gynaecology Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-TZP-GEN-TOB-AMC-CIP-SXT-TGC	1	Staff	Obstetrics & Gynaecology
AMP-CXM-DOR-MEM-AMC	1	Patient	Obstetrics & Gynaecology
Proteus spp			
AMP-CXM-AMC-TZP	1	Staff	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-DOR-MEM-TZP-GEN-AMC-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-ERT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-TZP-GEN-AMC-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-IPM-TZP-AMC-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-IPM-TZP-CIP-SXT	1	Patient	Obstetrics & Gynaecology

AMP-CXM-CAZ-CRO-FEP-TZP-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-TZP-SXT	2	Patient (2)	Obstetrics & Gynaecology
AMP-CXM-TZP	1	Staff	Obstetrics & Gynaecology
AMP-IPM-AMC-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-CIP-SXT	8	Patient (6) Environment (2)	Surgery (3) Obstetrics & Gynaecology (3) Obstetrics & Gynaecology (2)
AMP-IPM-CIP-SXT	2	Patient (2)	Obstetrics & Gynaecology (1),Surgery (1)
AMP-MEM-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-DOR-IPM-MER-CIP-SXT	1	Patient	Obstetrics & Gynaecology
AMP-IPM-SXT	2	Patient Staff	Obstetrics & Gynaecology Obstetrics & Gynaecology
AMP-MEM-SXT	2	Patient (2)	Surgery (1), Obstetrics & Gynaecology (1)
CAZ-MEM-SXT	1	Patient	Obstetrics & Gynaecology
Providencia spp			
AMP-CXM-CAZ-CRO	2	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-GEN-TOB-AMC	1	Patient	Obstetrics & Gynaecology
AMP-CXM-CAZ-CRO-FEP-TZP-GEN-TOB-AMC	1	Staff	Surgery
AMP-CXM-CAZ-CRO-FEP-IPM-TZP-GEN-TOB-AMC-CIP-SXT	1	Patient	Surgery
C. freundii			
AMP-CAZ-CRO-CXM-AMC	2	Environment (1) Staff (1)	Obstetrics & Gynaecology Obstetrics & Gynaecology
P. aeruginosa			
IPM-GEN-TOB-CIP	1	Environment	Obstetrics & Gynaecology
TZP-GEN-CIP	1	Environment	Surgery

DOR-TZP-FEP	1	Patient	Obstetrics & Gynaecology
DOR-IPM-MEM-GEN-TOB	1	Environment	Obstetrics & Gynaecology
CAZ-FEP-IPM-TZP	1	Environment	Intensive Care Unit
FEP-TZP-GEN-CIP-SXT	1	Patient	Obstetrics & Gynaecology
Others			
AMP-CXM-CAZ-CRO-FEP-IPM-TZP-AMC-SXT	1	Patient	Intensive Care Unit
PEN-CRO-CIP-SXT	1	Patient	Obstetrics & Gynaecology

AMK- Amikacin; AMP- Ampicillin; CXM-Cefuroxime; DOR-Doripenem; FEP-Cefepime; CRO-Ceftriaxone; CAZ-Ceftazidime; CIP-Ciprofloxacin; AMC-Amoxicillin/clavulanic acid; SXT-Trimethoprim-Sulphamethoxazole; ERT-Ertapenem; GEN-Gentamicin; IPM-Imipenem; MEM-Meropenem; TZP-Piperacillin/Tazobactam; TOB-Tobramycin; TGC-Tigecycline; PEN-Penicillin

CHAPTER THREE – MANUSCRIPT 2

Genomic characterization of multi drug resistant ESBL-producing *Escherichia coli* isolates from patients and patient environments in a teaching hospital in Ghana.

AUTHOR CONTRIBUTIONS

Esther Eyram Asare Yeboah as the principal investigator, co-conceptualized the study, undertook sample collection, laboratory work and statistical analyses and drafted the manuscript.

Nicholas Agyepong – as co-supervisor co-conceptualized the study, assisted with sample collection, conducted data analysis, vetted the results and critically reviewed manuscript.

Joshua Mbanga - conducted data analysis, vetted the results and critically reviewed manuscript.

Daniel Gyamfi Amoako - conducted data analysis, vetted the results and critically reviewed manuscript.

Akebe Luther King Abia - conducted data analysis, vetted the results and critically reviewed manuscript.

Arshad Ismail- conducted data analysis, vetted the results and critically reviewed manuscript.

Alexander Owusu-Ofori- as co-supervisor co-conceptualized the study, facilitated sample collection, vetted the results and critically reviewed manuscript.

Sabiha Yusuf Essack - as the principal supervisor, co-conceptualized the study, guided the literature review and ethical approval application, facilitated data collection and analysis, vetted the results and undertook critical revision of the manuscript

Objectives met: This paper answers objectives 3,4, 5 and 6.

Title: Genomic characterization of multi drug resistant ESBL-producing *Escherichia coli* isolates from patients and patient environments in a teaching hospital in Ghana.

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Keywords: *Escherichia coli*, multidrug-resistant, Extended spectrum β-lactamase, carbapenem resistant, genomics, mobile genetic elements; phylogeny, Ghana

ABSTRACT

Background: ESBL-producing *Escherichia coli* pose a growing health risk in community and healthcare settings. We investigated the resistome, virulome, mobilome, and genetic relatedness of multidrug-resistant (MDR) *E. coli* isolates from patients and their environment in a Ghanaian teaching hospital.

Methods: Twenty-three MDR ESBL-producing or carbapenem resistant *E. coli* isolates from a collection of MDR GNB from patients and environments were selected for genomic analyses. Whole genome sequencing and bioinformatics tools were used to analyze genomic characteristics and phylogeny.

Results: *bla*$_{\text{TEM-1B}}$ (10 isolates) and *bla*$_{\text{CTX-M-15}}$ (12 isolates) were commonly associated with IncFIB plasmid replicons and co-occurred with aminoglycoside, macrolide, and sulfamethoxazole/trimethoprim resistance. Insertion sequences, transposons, and class I integrons were found with *bla*$_{\text{CTX-M-15}}$. Carriage and environmental isolates carried multiple virulence genes, with terC being the most prevalent in 21 isolates. Seventeen sequence types (STs) were identified, including a novel ST (ST13846). Phylogenetic analysis grouped the isolates into four main clusters, with one outlier. High genetic relatedness was observed between two carriage isolates of ST940 and between a carriage isolate and an environmental isolate of ST648. Genetic similarities was also observed between isolates collected at different times and locations in the hospital.

Conclusion: We identified ESBL-producing *E. coli* with diverse genomic characteristics circulating in different directorates of the hospital. Clonal relatedness was observed among isolates from patients and the environment, as well as between different patients, suggesting transmission within and between sources.

Keywords: *Escherichia coli*, extended spectrum β-lactamase, genomics, mobile genetic elements phylogeny

1.0 INTRODUCTION

In low- middle income (LMIC) countries, antimicrobial resistance is a problem leading to high death rates and is further exacerbated by the sub-optimal surveillance and poor infection prevention and control practices [1] Multi-drug resistant Gram-negative bacteria (MDR GNB) have been implicated in critical infections in clinical settings [2].

Escherichia coli has the ability to colonize and persist in hosts and in the environment [3]. Hospitalised patients may harbour *E. coli* obtained from the community or acquire it after admission from various sources including the hospital environment via healthcare workers hands or being in close contact with other patients habouring resistant isolates or by touching contaminated surfaces [4, 5]. *E. coli* isolates may carry a repertoire of resistance and virulence genes on mobile genetic elements (MGEs). These MGEs include plasmids, integrons and insertion sequences which may be exchanged by horizontal gene transfer [6-8].

Extended spectrum β-lactamase (ESBL)–producing *Escherichia coli* which cause critical infections such as pneumonia and bloodstream infections are listed among the WHO priority organisms for which new antibiotics are required [9]. Colonization by antibiotic resistant organisms usually precedes infections with the same organisms thus the intestinal carriage of ESBL *E. coli* is a threat to health as infections caused by these result in poor treatment outcomes and high mortalities, especially among high-risk patients [5, 10]. It is estimated that globally, between 2003-2018, the cumulative global pooled prevalence of ESBL *E. coli* intestinal carriage in the community was 16.5% [5]. Colonization of hospital patients with ESBL producing *E. coli* isolates have been reported in African countries such as South Africa, Uganda, and Tanzania [11-13]. Recent studies in a Ghanaian hospital reported that about 13.2% of diarrhoegenic *E. coli*

isolates from patients were ESBL positive and cefotaxime resistant ESBL *E. coli* was found to be widely disseminated in Ghanaian hospitals [14-16].

Patients may be colonized on admission to hospitals and could be a potential source of transfer to other patients who could become infected in hospitals [10]. Resistance and virulence genes of ESBL *E. coli* isolates from patients could be shed into the hospital environment through the skin, respiratory or intestinal tract and further transferred to other patients in hospitals.

E. coli isolates showing varying resistance to β-lactams have been studied in clinical settings in Ghana but most of these have been isolates from infected patients with limited studies on the molecular epidemiology of these MDR *E. coli* isolates. There are also limited studies on colonization with ESBL *E. coli* and their potential transmission to other patients and environment, particularly in non-outbreak settings. We investigated the molecular epidemiology of MDR ESBL-producing *E. coli* colonizing patients, carried on healthcare workers hands and contaminating selected ward and intensive care unit (ICU) environments. This was done to inform infection prevention and control (IPC) measures so as to control their spread in clinical settings.

2.0 MATERIALS AND METHODS

2.1 Ethical approval

The study was approved by the Institutional Review board (IRB) of the Komfo Anokye Teaching Hospital (KATH) **(Reference: KATH IRB/AP/107/20)** and the Biomedical Research Ethics Committee of the University of KwaZulu-Natal **(Reference: BREC/00001917/2020**). Voluntary, informed written consent was obtained from participating patients and staff.

2.2 Study setting

This study was conducted at KATH which is 1200 - bed capacity government tertiary facility which serves as a referral hospital responding to the healthcare needs of about 80% of both emergencies and regular medical cases in the Ashanti region of Ghana that has a population of about 5.4 million. The hospital also attends to referral cases from other regions including the Bono, Bono East, Ahafo, Western and Eastern regions as well as some parts of the Northern regions of the country. This study was conducted in three directorates; Obstetrics and Gynaecology, Surgery and the Intensive Care Unit (ICU).

2.3 Sample collection

Samples were collected from consenting patients admitted to the ICU and Surgery, and Obstetrics and Gynaecology directorates of the hospital. Rectal swabs and hand swabs were aseptically collected from in-patients >18 years old, at admission and after 48 hours as previously described in the preceeding study. Isolation of Gram-negative bacteria and antibiotic susceptibility testing was carried out as previously described**.**

2.4 DNA extraction and Whole Genome sequencing

All MDR ESBL- producing or carbapenem resistant *E. coli* isolates were subjected to genomic DNA (gDNA) extraction using the GenElute® bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO, United States) according to the manufacturer's instructions. ESBL-*E. coli* were defined as isolates with phenotypic resistance to at least one the third generation cephalosporins on VITEK. The quantity and quality of the extracted gDNA were determined using a Nanodrop spectrophotometer Qubit (Thermo Scientific, Waltham, MA, USA). Multiplexed paired-end

libraries (2 × 300 bp) were prepared using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, United States), and sequences were determined on an Illumina MiSeq platform with 100× coverage.

2.5 Genomic analyses and annotation

Quality trimming of raw reads was done using Sickle v1.33 (https://github.com/najoshi/sickle). The raw reads were then assembled spontaneously using the SPAdes v3.6.2 assembler (https://cab.spbu.ru/software/spades/). All contiguous sequences were subsequently submitted to GenBank and assigned accession numbers under BioProject **PRJNA823741** (**Supplementary Table S1)**.

Multilocus sequence typing (MLST) of assembled genomes was determined on the MLST 1.8 database hosted by the Center for Genomic Epidemiology (CGE) (https://cge.food.dtu.dk/services/MLST/). Isolates with unknown STs were submitted to the EnteroBase *Escherichia*/*Shigella* database (https://enterobase.warwick.ac.uk/species/index/ecoli) and assigned novel STs. Resistance and virulence genes were determined using ResFinder (https://cge.food.dtu.dk/services/ResFinder/) and Virulence finder (https://cge.food.dtu.dk/services/VirulenceFinder/), and plasmids by PlasmidFinder 2.1 hosted on https:// https://cge.food.dtu.dk/services. INTEGRALL (http://integrall.bio.ua.pt/) and RAST SEEDVIEWER (https://rast.nmpdr.org/seedviewer.cgi) were used to find integrons and transposons. Insertion sequences, and prophages were determined using ISFinder (https://isfinder.biotoul.fr/) and PHASTER (https:// phaster.ca/), respectively. Plasmids of the IncF, IncH1, IncH2, IncI1, IncN, or IncA/C types were subtyped by assigning a replicon allele at

the plasmid MLST site (https://pubmlst.org/plasmid/). Phylogroups were determined via ClermonTyper (http://clermontyping.iame-research.center/).

The synteny and genetic environment of ARGs and associated MGEs was investigated using GenBank's general feature format (GFF3) files. The genetic environment of virulence genes detected in the study was also determined using a similar approach.

2.6 Phylogenomics

Phylogenomic analysis was undertaken to determine how the study isolates compare to *E. coli* genomes of human origin from South Africa and West African countries including Togo, Nigeria, Niger, Mali, Ghana, and Cameroon. All the *E. coli* genomes were reported in these countries from 2013-2021 (n =157). The genomes were downloaded from the Bacterial and Viral Bioinformatics Resource Center (BV-BRCB) website (https://www.bv-brc.org/), annotated (Table S1), and included in the analysis. The phylogenetic tree was constructed based on the maximum likelihood method using BV-BRCB. The *Escherichia coli* K12-MG1655 was used as the outgroup strain (reference genome), facilitating the configuration of the phylogenetic distance between the isolates on the branches. The Figtree software (https://tree.bio.ed.ac.uk/software/figtree/) and iTOL (https://itol.embl.de/) were used to visualize, edit, and annotate the generated phylogenetic tree.

Phandango (https://jameshadfield.github.io/phandango/#/main), was used to visualise the phylogenetic tree with corresponding metadata to get a more comprehensive insight on the relationships of the isolates.

3.0 RESULTS

3.1 Isolate characteristics

ESBL *E. coli* was isolated from 17 patients. Eleven of 83 (13.25%) patients were colonized with ESBL *E. coli* on admission. ESBL *E. coli* was also isolated from a patient's hand on admission. Six of 53 (11.32%) patients who had samples taken after 48 hours of admission, had acquired ESBL *E. coli.* Of the 208 environmental samples taken, seven (3.37%) were contaminated with ESBL *E. coli*. None of the HCW's hands were contaminated with ESBL *E. coli*. Of the seven environmental samples with ESBL *E. coli*, four were isolated from beds, two from taps and one from a dripstand.

Twenty-three MDR *E. coli* isolates which were both MDR and ESBL-producing or MDR carbapenem resistant *E. coli* isolates were subjected to WGS. Sixteen were from patients, and seven were from the hospital environment. All the carriage *E. coli* isolates were obtained from rectal swabs except one that was obtained from a hand swab. No isolates were obtained from healthcare workers' hands. Three of the environmental isolates were obtained from the surgical ward, and the remaining from the obstetrics and gynaecology ward **(Table 1)**.

3.2 Isolate antibiotic susceptibility

All of the MDR *E. coli* isolates selected were susceptible to amikacin, imipenem, and tigecycline. Highest resistance was observed against ampicillin (100%), sulphamethoxazole/trimethoprim (21/23, 91.3%), ceftazidime (21/23, 91.3%), cefuroxime (20/23, 87%), amoxicillin/clavulanic

(21/23, 91.3%). Ten (23.3%) of the isolates were resistant to the carbapenems; doripenem, ertapenem and meropenem. Six isolates from patients and four from environment were resistant to ciprofloxacin. A total of nine antibiograms were observed across the 23 selected *E. coli* isolates. The most common resistance pattern (AMP-CXM-CAZ-CRO-FEP-GEN-TOB-AMC-TZP-CIP-SXT) was observed among nine *E. coli* isolates (five from the environment and four from patients) (**Table 1)** suggesting the contamination of environmental surfaces with isolates of similar resistant patterns as those colonizing patients.

3.3 Antibiotic resistance genes

The resistance genotypes corresponded to the phenotypic resistance in most of the isolates except for a few isolates where there were no identified genotypes corresponding to phenotypic resitance to antibiotics. In one, isolate (P165) from a patient, there were no antibiotic resistance genes corresponding to its phenotypic resistance to AMP-CXM-CAZ-CRO-FEP-GEN-TOB-AMC-TZP-CIP-SXT.

ESBL genes were identified in 19 of the 23 isolates and mostly belonged to the CTX-M and TEM classes. The most common ESBL identified among the *E. coli* isolates was $bla_{\text{CTX-M-15}}$ harboured by 11 isolates from patients and one environmental isolate. $bla_{\text{TEM-104}}$ and $bla_{\text{TEM-169}}$ were each harboured by *E. coli* from a patient. The β-lactamase $bla_{\text{OXA-1}}$ was detected in 12 isolates (nine carriage and three environmental isolates). The $bla_{\text{TEM-1B}}$ gene was also detected in 10 isolates. The carbapenemase gene $bla_{\text{OXA-181}}$ was detected in two isolates from patients. In one isolate (P2R), $bla_{\text{OXA-181}}$ was detected even though there was no phenotypic expression of carbapenem resistance.

An environmental isolate, (E50-1) which showed phenotypic resistance to doripenem and meropenem had no carbapenemase or β-lactamase genes. Another isolate (P17) from a patient, harboured thirteen ESBLs (*bla*$_{\text{CTX-M-22}}$, *bla*$_{\text{CTX-M-216}}$, *bla*$_{\text{CTX-M-103}}$, *bla*$_{\text{CTX-M-176}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-156}}$, *bla*$_{\text{CTX-M-3}}$, *bla*$_{\text{CTX-M-202}}$, *bla*$_{\text{CTX-M-88}}$, *bla*$_{\text{CTX-M-203}}$, *bla*$_{\text{CTX-M-71}}$, *bla*$_{\text{CTX-M-167}}$, and *bla*$_{\text{OXA-1}}$). The ESBL *bla*$_{\text{TEM-1B}}$ and *bla*$_{\text{CTX-M-15}}$ commonly occurred with sulphamethoxazole and trimethoprim-resistant genes *sul2* and *dfrA17*, respectively **(Table 1).**

Genes conferring resistance to aminoglycosides (*aadA1, aadA2, aadA5, aph(6)-Id, aac(6')-Ib-cr, aph(3'')-Ib, aac(6')-Ib-cr, aac(3)-IId*), macrolides (*mphA* and *ermB*), tetracycline (*tetA, tetB, tet*L and *tetM*), sulphamethoxazole (*sul1, sul2, sul3*) and trimethoprim (*dfrA1, dfrA7, dfrA12, dfrA14, dfrA17 and dfrG*) were also identified in both patient and environmental isolates. The plasmid mediated quinolone resistant (PMQR) genes, *aac(6')-Ib-cr, qnrS1, qnrB19, qnrB4* and *qepA4* were also detected in the isolates. The quaternary ammonium compound resistance genes, *qacE*, were found in eight isolates (seven from patients and one from environmental isolates).

Reduced susceptibility to ciprofloxacin was observed in 10 isolates (six carriage isolates and four environmental isolates), therefore, mutations in the quinolone resistance determinant regions (QRDRs) DNA gyrase (*gyrA* and *gyrB*) and DNA topoisomerase IV (*parC* and *parE*) genes in the isolates from patients and environments were investigated. Mutations were commonly found in *gyrA* (S83L, D87N, A828S, D678E, A863V) and *parC* (E62K, S801, D475E, S80I, L440R) genes, with *gyrB* (S492N, A618T, E656D, E703D) and *parE* (T172A, S458A) having the least number of mutations. Nine isolates (four from environment and five from patients) had common mutations in *gyr A* (S83L, D87N), *parC* (S80I) and *parE* (S458A). One isolate (P51B) from a patient had mutations in all four genes: *gyrA* (S83L, D87N, A828S, D678E*), gyrB (S492N, A618T, E656D), parC (E62K, S801, D475E)* and *parE (T172A, S458A)* **(Table S4).**

Table 1: Source, antibiograms, sequence types (STs), phylogroups, resistance genes, virulence genes and plasmids found in the *Escherichia coli* isolates

ID	Source	Resistance pattern	Sequence Type	Phylogroup	ABR genes		Virulence genes	Plasmid replicon	Pmlst
					ESBLs	Others			
P51B	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	ST648	F	*bla*$_{TEM-1B}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*sul2* , *dfrA17*, *tet(B)* , *tet(M)*, *mph(A)* ,*aac(3)-IIa* , *aac(6')-Ib-cr*	*air*, *astA*, *chuA*, *eilA*, *fyuA*, *gad*, *hlye*, *hra*, *irp2*, *kpsM*, *kpsE*, *lpfA*, *papA*, *papC*, *terC*, *traT*, *yfcv*	Col(BS512) , IncFIA, IncFIB(pB171), IncFII, IncI1-I(Alpha), IncQ1 , IncX1	IncF[F2:A1:B32]
E53	Environment (Bed)	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	ST648	F	*bla*$_{OXA-1}$, *bla*$_{TEM-169}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-33,}$	*sul2*, *dfrA17*,*aac(6')-Ib-cr*, *aac(3)-IId* ,*mph(A)*, *erm(B)*, *tet(M)*, *tet(B)*	*terC*, *ipfA*, *papC*, *yfcV*, *papa_F43*, *eilA*, *hra*, *chuA*, *irp2* , *fyuA*, *astA*, *gad*, *kpsMIII_K98*, *traT*, *kpsE*	Col(BS512), IncFIA, IncFIB(pB171), IncFII, IncQ1, IncX1	IncF[F2:A1:B32]
P2R	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-SXT	ST940	B1	*bla*$_{TEM-35}$, *bla*$_{OXA-181}$	*sul2*, *dfrA12*, *dfrA1*, *aph(6)-Id*, *aadA2*, *aph(3'')-Ib*,*qnrS1*,*erm(B)*, *tet(B)*	*lpfA*, *gad*, *hlyE*, *traT*, *capU*, *terC*	IncFIA, IncFIB, IncFIC(II), ColKP3	IncF [F-:A-:B12]
P73	Patient	AMP-CXM-CAZ-CRO-FEP-MEM-AMC	ST940	B1	*bla*$_{OXA-1}$, *bla*$_{OXA-181}$, *bla*$_{TEM-35}$	*sul2*, *aph(3'')-Ib*, *aph(6)-Id*, *aadA1*,*qnrS1*, *tet(B)*	*lpfA*, *gad*, *traT*,*hlyE*, *iss*, *capU*, *terC*	ColKP3 , IncFIA, IncFIB, IncFII	IncF[F36:A1:B1]
P128	Patient	AMP-CAZ-CRO-FEP-AMC-TZP-SXT	ST13846	D	*bla*$_{TEM-1B}$, *bla*$_{CTX-M-15}$	*sul2*, *dfrA14*, *aph(6)-Id*, *aph(3'')-Ib*, *qnrS1*, *tet(A)*	*ipfA*, *eilA*, *irp2*, *chuA*, *sitA*, *fyuA*, *gad*, *kpsMIII_K96*, *ompT*, *iss*, *kpsE*, *terC*	IncY	
P166	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-SXT	ST13846	D	*bla*$_{TEM-1B}$, *bla*$_{CTX-M-15}$	*sul2*, *dfrA14*, *aph(6)-Id*, *aph(3'')-Ib*, *qnrS1*, *tet(A)*	*ipfA*, *air*, *eilA*, *sitABCD*, *irp2*, *chuA*, *fyuA*, *gad*, *kpsMIII_K96* , *ompT*, *kpsE*, *terC*	IncY	
P49	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-ERT-SXT	ST73	B2	*bla*$_{TEM-1B}$	*sul1*, *sul2*, *dfrA1*, *dfrA17*, *dfrG*, *aadA5*, *aadA1*, *aph(3'')-Ib*, *aph(6)-Id*,*erm(B)*,*tet(B)*, *tet(M)*, *tet(L)*, *qacE*	*ccI*, *cea*, *clbB*, *cnf1*, *focc*, *focG*, *hlyA*, *hra*, *ibeA*, *ireA*, *iroN*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsM*, *mchB*, *mchf*, *mchC*, *mcmA*, *neuc*, *ompT*,	Col(MG828), Col156, ColRNAI , IncFIA, IncFIB, IncFII, IncQ1	IncF[F36:A1:B1]

							papA, papC, pic, senB, sfaD, sitA, tcpC, terC, traT, usp, vat		
P142	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-DOR-MEM-SXT	ST5614	B1	*$bla_{TEM-104}$, bla_{TEM-1B}, $bla_{TEM-198}$, $bla_{CTX-M-15}$, $bla_{TEM-217}$, $bla_{TEM-234}$*	*sul1, sul2, dfrA1, dfrA17,aph(3'')-Ib, aadA5, aph(6)-Id,qnrS1, mph(A),tet(A), tet(B), qacE*	*iha, ipfA,sigA, iucC, iutA,cib, gad, traT, iss, capU, terC, hlyE*	IncB/O/K/Z, IncFII, IncQ1	IncF [F-:A-:B] *FII
P17	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-TOB-CIP-SXT	ST224	B1	*$bla_{CTX-M-22}$, $bla_{CTX-M-216}$, $bla_{CTX-M-103}$, $bla_{CTX-M-176}$, $bla_{CTX-M-15}$, $bla_{CTX-M-156}$, $bla_{CTX-M-3}$, $bla_{CTX-M-202}$, $bla_{CTX-M-88}$, $bla_{CTX-M-203}$, $bl_{aCTX-M-71}$, $bla_{CTX-M-167}$, bla_{OXA-1},*	*sul1, sul3, dfrA12, dfrA17, aadA1, aadA2, aadA5, aac(6')-Ib-cr, mph(A), erm(B), tet(A), qacE*	*ipfA, sitABCD, iucC, sitA, iutA, gad, traT, terC*	IncFIA, IncFIB, IncFII, IncI1-I(Alpha), IncY	IncF[F2:A4:B1]
P60R	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-SXT	ST1722	F	*$bla_{CTX-M-15}$, bla_{TEM-1B}*	*sul2, dfrA14, aph(6)-Id, aph(3'')-Ib, qnrS1, tet(A)*	*IpfA, afaA, afaB, afaC, afaD, air, eilA, cia, chuA, gad, traT , iss, kpsE, terC*	IncB/O/K/Z , IncI2(Delta) , IncY, pXuzhou21	
P165	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	ST3489	A			*neuC, gad, ompT, terC*	IncFIB(K), IncL, IncY	
P105	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	ST10	A	*bla_{OXA-1}, $bla_{CTX-M-15}$*	*sul1, dfrA17, aadA5,aac(3)-IIa, aac(6')-Ib-cr, mph(A), tet(B), qacE*	*hra, senB, sitABCD, iucC, irp2, sitA, iutA, fyuA, mchF, traT, terC*	IncFIA, IncFIB, IncFII	IncF[F36:A4:B1]
P159	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB -CIP-SXT	ST617	A	*bla_{OXA-1}, $bla_{CTX-M-15}$*	*sul1, sul2, dfrA17, aadA5, aph(6)-Id, aac(6')-Ib-cr, aph(3'')-Ib, mph(A), tet(B), qacE*	*sitABCD, iucC, irp2, sitA, iutA, fyuA, traT, iss, terC*	IncFIA, IncFIB	IncF[F-:A4:B1]
P72	Patient	AMP-CXM-CRO-CAZ-	ST167	A	*$bla_{CTX-M-27}$*	*sul1, sul2, dfrA12, aph(6)-Id , aph(3'')-Ib ,*	*irp2, fyuA, traT, terC*	IncFIA, IncFIB, IncFII	IncF[F48:A1:B49]

		FEP-AMC-TZP-CIP-SXT				*aadA2, qepA4, mph(A), tet(A), tet(B)*			
E56	Environment (Bed)	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TZP-CIP-SXT	ST638	A	*bla$_{TEM-1B}$, bla$_{OXA-1}$*	*aac(3)-IIa,tet(A)*	*ipfA, yfcv, terC*	Col(BS512), IncFIB(K), IncR	
E37	Environment (Tap)	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	ST410	C	*bla$_{CTX-M-15}$, bla$_{OXA-1}$*	*sul2 , dfrA14,mph(A),aac(3)-IIa, aac(6')-Ib-cr,tet(B)*	*cba, cma, cnf1, fyuA, hlya, hlyE, hra, irp2, iucC, iutA, lpfA, papA, papC, sitA, terC, traT*	IncFIA, IncFIB, IncFII, IncQ1	IncF [F104:A1:B1]
E29	Environment (Tap)	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	ST450	A	*bla$_{CTX-M-3}$, bla$_{TEM-1B}$*	*sul2, dfrA17,mph(A),aac(3)-IId ,tet(A)*	*fyuA, gad, hlyE, iha, irp2, iucC, iutA, kpsE, mchf, ompT, papA, papc, sat, senb, terC, traT*	Col(BS512) , Col156, IncFIA , IncFIB, IncFII, IncI1-I(Alpha) , IncI2(Delta)	IncF [F4:A2:B20]
E25B	Environment (Bed)	AMP-CXM-CAZ-CRO-FEP-AMC-TZP	ST127	B2	*bla$_{TEM-1D}$*	*sul1 , dfrA7,tet(B)*	*papC, sfaD, sfaE, sfaS, yfcv, papA, hra, vat, clbB, cnf1, tcpC, chuA, iroN, irp2, sitA, fyuA, usp, mcmA, gad, iss, kpsE, kpsMII_F48, ompT, traT, TerC*	IncFIA, IncFIB, IncFII	IncF[F1:A1:B20]
E50-1	Environment (Dripstand)	AMP-TZP-DOR-MEM-SXT	ST155	B1		*sul2, dfrA17, aadA5, tet(A)*	*ipfA, gad, ompT, iss, terC*	-	
E55-2	Environment (Bed)	AMP-DOR-MEM-SXT-	ST58	B1	*bla$_{TEM-1B}$*	*sul1, sul2, dfrA17, aadA5, aph(3'')-Ib, aph(6)-Id, qnrB19, mph(A), tet(A), qacE*	*iha, ipfA, papA_F43,sat, senB, iucC, sitABCD,iutA, cea, gad, traT, kpsMII_K52, iss, capU , terC*	Col(pHAD28), Col156, IncFIB, IncFI1(29)	IncF[F29:A-:B10]
P115	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-SXT	Unknown	E	*bla$_{CTX-M-15}$, bla$_{TEM-35}$, bla$_{OXA-1}$*	*sul2, dfrA1, drfA17, aadA1, aadA5, qnrS1, mph(A), tet(B)*	*IpfA, papA_F19 , papA_F20, eilA, V, terC*	Col(BS512), IncFIA, IncFIB, IncFII, IncFHI2, IncFHI2A	IncF[F36:A1:B20]

P143	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-SXT	Unknown	Unknown	*bla$_{OXA-1}$*	*sul2, dfrA14, tet(B)*	*eatA, irp2, fyuA, terC*	IncFII, IncL, IncN	IncF[[F-:A-:B-] FI
P63	Patient	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	Unknown	E	*bla$_{TEM-1B}$, bla$_{OXA-1}$, bla$_{CTX-M-15}$, bla$_{DHA-1}$*	*sul1, sul3, dfrA1, dfrA12, dfrA17, aac(6')-Ib-cr, aac(3)-IIa, aadA2, aadA5, aadA1, qnrB4, mph(A), erm(B), tet(A), tet(B)*	*ipfA, yfcv, iucC, irp2, sitABCD , iutA, fyuA, terC*	Col(BS512), IncFIA, IncFIB, IncFII, IncR	IncF[F36:A4:B1]

ampicillin (AMP), cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), amoxicillin/clavulanic (AMC), piperacillin/tazobactam (TZP), imipenem (IPM), doripenem (DOR), meropenem (MEM), ertapenem (ERT), ciprofloxacin (CIP), trimethoprim-sulphamethoxazole (SXT) and tigecycline (TGC)

3.4 Mobilome (Plasmids, Insertion Sequences, Intact Prophages, and Integrons)

Plasmid analysis revealed that all but one isolate harboured plasmid replicons. Nineteen different replicons were found in the 23 isolates. InCFIB was the most frequently found replicon and was common to 11 isolates from patients and six from environment. The IncFIA, IncFIB, IncFII plasmid replicons were found together in twelve isolates: eight in patient isolates and four in environmental isolates. Two isolates from patients and one from the environment harboured up to seven different replicons.

The CTX-M-15 gene in both patient and environmental isolates was consistently associated with an insertion sequence of the IS380 family and a transposon, commonly Tn3, while the *bla*$_{\text{TEM}}$ gene was carried mainly by transposons. The disinfectant resistant gene, *qacE*, which was found in six isolates from patients (n=5) and environmental isolates (n=1), was co-carried with *sul1* usually on a class 1 integron in all the isolates. Isolate P128, was found to have CTX-M-15 genes flanked by several transposons, insertion sequences and a recombinase and also harboured TEM-1, *qnrS1*, *aph(6)-Id*:*aph(3'')-Ib* and *sul2.* The contig harbouring the CTX-M-15 gene in P128 showed high similarity of 99.98% to the *E. coli* strain PGR46 plasmid pPGRT46 (KM023153.1) The *bla*$_{\text{OXA-1}}$ gene which was commonly flanked by the chloramphenicol resistant gene, *catB-3* and the aminoglycoside hydrolyzing gene, *aac(6')-Ib-cr5* was not associated with transposons or insertion sequences. However, the contig harbouring the *bla*$_{\text{OXA-1}}$ gene showed, showed high similarity to a plasmid **(Supplementary table S6).**

Investigation of MGEs which mobilize and transfer resistance genes between isolates revealed the presence of diverse MGEs in the isolates.

Many of the contigs of the *E. coli* isolates harbouring ARGs showed high similarity (98%-100%) to plasmids in GenBank, confirming that most of the ARGs from both patients and environment are mobilized and disseminated by plasmids **(Supplementary table S6).**

Since prophages are known to be associated with pathogenicity factors, the types of prophages in the isolates were investigated. Twenty-five different intact prophages were identified among nineteen MDR *E. coli* isolates from patient and environments. The most common prophages were Entero_mEp460 which was identified in seven isolates (Two from environment and five from patients) and Entero_BP_4795 (five patient isolates) and Klebsi_4LV2017 (three isolates: two patients and one environmental isolate). P51B from patient and E53 from environment with ST648 had the same phages (Escher_TL_2011b and Klebsi_4LV2017) (**Supplementary table S5**).

The most common integrons were of the class I type identified in sixteen isolates: nine isolates from patients and six from the environment. Similar integrons bearing the same gene cassettes were identified in isolates from both patients and the environment. The integrons commonly encoded genes for sulphamethoxazole, trimethoprim and aminoglycoside resistance. In54 and In191 were the most identified class I integron types, occurring in five and four isolates respectively. Gene cassettes of In54 frequently consisted of *AadA5*, *dfrA17, qacE* and *sul1*. The gene cassettes were common to isolates from four patients and an isolate from the environment. One other isolate from the environment (E50-1) with the In54 differed and lacked the *qacE* and *sul1* genes. The class I integron, In191 which was found with *dfrA14* occurred in four isolates from patients and one from the environment (**Table 2).**

Several different insertion sequences were detected in the *E. coli* isolates from patients and environments. Twenty isolates harboured at least one insertion sequence element. IS621 was the most commonly occurring insertion element and was found in thirteen isolates (Nine isolates from

patients and four from the environment). The insertion sequence MITEEc1 was found in eight (six from patients and two from environments) isolates **(Supplementary Table S5).**

Table 2: Integrons, gene cassettes (GCs) and sequence types found in the *Escherichia coli* isolates

ID	SOURCE	MLST	Integron	Integron	GC1	GC2	GC3	GC4	transposon
P2R	Patient	ST940	Intl2	In2-3	*dfrA1*	*sat2*	-	-	IS256, Tn7, TnsD
P73	Patient	ST940	Intl1	In757	*oxa-1*	*AadA1*	-	-	Tn3
P143	Patient	Unknown	Intl1	In757	*oxa-1*	*AadA1*	-	-	Tn3
P128	Patient	ST13846	Intl1	In191	*dfrA14*	-	-	-	
P166	Patient	ST13846	Intl1	In191	*dfrA14*	-	-	-	
P60R	Patient	ST1722	Intl1	In191	*dfrA14*	-	-	-	
E37	Environment	ST410	Intl1	In191	*dfrA14*	-	-	-	IS6, Tn3
P142	Patient	ST5614	Intl1	In54	*AadA5*	*dfrA17*	*qacE*	*sul1*	Tn3, IS6
P159	Patient	ST617	Intl1	In54	*AadA5*	*dfrA17*	*qacE*	*sul1*	
P105	Patient	ST10	Intl1	In54	*AadA5*	*dfrA17*	*qacE*	*sul1*	
E50-1	Environment	ST155	Intl1	In54	*AadA5*	*dfrA17*	-	-	Tn3
E55-2	Environment	ST58	Intl1	In54	*AadA5*	*dfrA17*	*qacE*	*sul1*	IS6
P115	Patient	Unknown	Intl2	In2-3	*dfrA1*	*sat2*	-	-	
			Intl1	In54	*AadA5*	*dfrA17*	-	-	
E29	Environment	ST450	Intl1	In987	*dfrA17*	-	-	-	
E25B	Environment	ST127	Intl1	In22	*dfrA7*	*qacE*	*sul1*	-	Tn3

3.5 Virulome, Serotypes and Phylogroups

Isolates from both patients and environments were found to harbour several virulence genes which did not differ much between sources. The most frequently identified virulence genes included *terC* which was found in 21 isolates: fourteen from patients and 7 from environment. The virulence gene *ipfA* was also harboured by 14 isolates – nine from patients and five from environment. Other frequently identified virulence genes in the isolates included, *gad* (13 isolates), *sat* (2 isolates), *papA* (7), *irp2* (11), *fyuA* (11), *iutA* (7), *traT* (12), *iucC* (7), *iss* (9), *yfcv* (6), *chuA* (6), *ompT* (7).

The highest number of virulence genes were found in an isolate P49 from a patient (33 genes) which was acquired on admission. E25B, an isolate from the environment also harboured 25 virulence genes (25) **(Table 1).**

The somatic (O) and flagellar (H) antigens were used for serotyping the *E. coli* isolates where twelve different O antigens and 17 different H types were identified across all isolates. No O type was detected for two isolates P73, from patient and E50-1, from environment which only had the H5 and H40 antigens respectively. The O antigen, O101 was common to four isolates (P2R, P105, P159 and P63) from patients. P105 (ST10) and P159 (ST617) had in common, the O101 and H10 antigens. Isolates P128 and P166 both of the novel ST 13846 had the O15 antigen but varied in the H antigen (**Supplementary Table S3**). The complexity and diversity of the virulome in isolates from patients and from environments coupled with the range of identified capsule types is a concern for IPC as they are associated with virulence.

Isolates were found to belong to six phylogroups; with six isolates (four carriage isolates and two from environments) belonging to A and to B1. Three isolates (two from patients and one from environments) belonged to the phylogroup F, two isolates each from patients belonged to D and E, while one from a patient and one from environment belonged to the frequently virulent phylogroup B2 and E **(Table 1).** All phylogroups of patient and environmental isolates harboured several virulence genes.

3.6 Sequence Types and Phylogenomic Relationships

MLST analysis showed that the isolates belonged to seventeen different sequence types (ST127, ST73, ST13846, ST1722, ST648, ST617, ST450, ST1638, ST3489, ST10, ST167, ST224, ST410,

ST155, ST58, ST5614, ST940). Three isolates (P143, P115 and P63) belonged to unknown STs **(Table 1)**. ST10, ST167, ST225, ST3489, ST617, ST940, ST 13846 were of community carriage isolates. Two isolates (P73 and P2R) from two patients had the same ST, ST 940. P73 was acquired by a patient on admission. One isolate (P51B) acquired by a patient on admission at the Obstetrics and Gynaecology directorate and one from the environment (E53) of the surgery unit had the same ST 648. Two isolates (P128 and P166) with unknown STs, both isolated from patients in different directorates after 48 hours of admission were assigned a putatively novel ST13846. These isolates with same ST showed significant clonal relatedness in the phylogenetic tree **(Figure 1)** and had similar resistance genes, virulence genes and plasmids **(Table 1)**. Analyses of the genetic environment of this new ST showed the *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{TEM-1}}$ genes were associated with Tn3::CTX-M-15:recombinase:TEM-1:IS91(transposase) on a contig which bore close resemblance with the *E. coli* strain PGR46 plasmid pPGRT46 (KM023153.1). This contig also carried the quinolone resistant gene *qnrS1* and the *aph(6)-id:aph(3'')-ib:sul2* genes close to the insertion sequence IS5075 and the Tn3 transposon. P128 and P166 which shared similar resistance genes and genetic environment as P60R (ST1722), differed in the plasmid replicons. P60R, belonging to ST1722 isolated from a patient on admission, with similar resistance genes as P128 and P166 (ST13846) had in addition to the IncY plasmid, IncB/O/K/Z, IncI2(Delta) and pXuzhou21.

Generally, isolates clustered into three main groups with one other grouping of two isolates and an outlier isolate. Group A constituted seven isolates; two isolates (E25B and E53) belonging to ST127 and ST648 from environment and five isolates (P49, P128, P166, P60R and P51B) of STs ST73, ST13846, ST1722 and ST648 from patients. Eight isolates grouped into B and included two isolates (E29 and E56) belonging to ST450 and an unknown ST from environment and six isolates

(P63, P143, P159, P165, P105 and P72) belonging to two unknown STs, ST617, ST3489, ST10 and ST167. The third group, C comprised of two isolates (P17 and P142) of STs 224 and ST5617 from patients and three isolates (E37, E50-1 and E55-2) which belonged to ST410, ST155 and ST48 from environments. P2R and P73 both from patients and belonging to ST940 clustered in one group while P115 (unknown ST) was an outlier isolate **(Figure 1).**

To visualize the genetic relatedness among *E. coli* isolates, we inferred a maximum likelihood phylogenetic tree from an alignment of 181 genomes including the complete genome of *E. coli* K12-MG1655 (reference genome) **(Figure 2)**. Isolates from this study clustered together with genomes from other countries, frequently with isolates from Nigeria and South Africa. They clustered mainly based on sequence type **(Figure 2)**. Isolates from the environment, clustered together with isolates of similar sequence types or variants of the sequence types from clinical sources. For instance, E50-1 (ST155), an isolate from the environment clustered together with other isolates of similar ST from clinical sources in other countries **(Figure 2).**

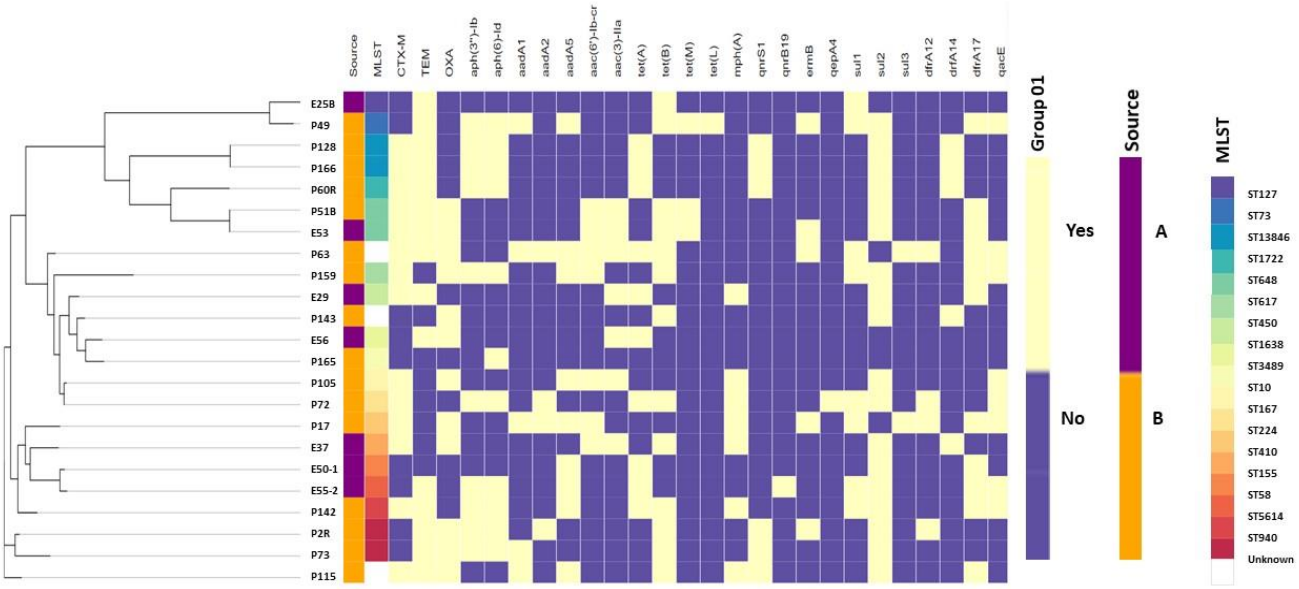

Figure 1: The phylogenetic branch and metadata [demographics, molecular typing, and antibiotic resistance genes (ARGs)] coupled by the use of Phandango (https://github.com/jameshadfield/phandango/wiki) in multidrug resistant *Escherichia coli* isolates (n = 23) from hospital patients and environments in a Teaching Hospital in Ghana.

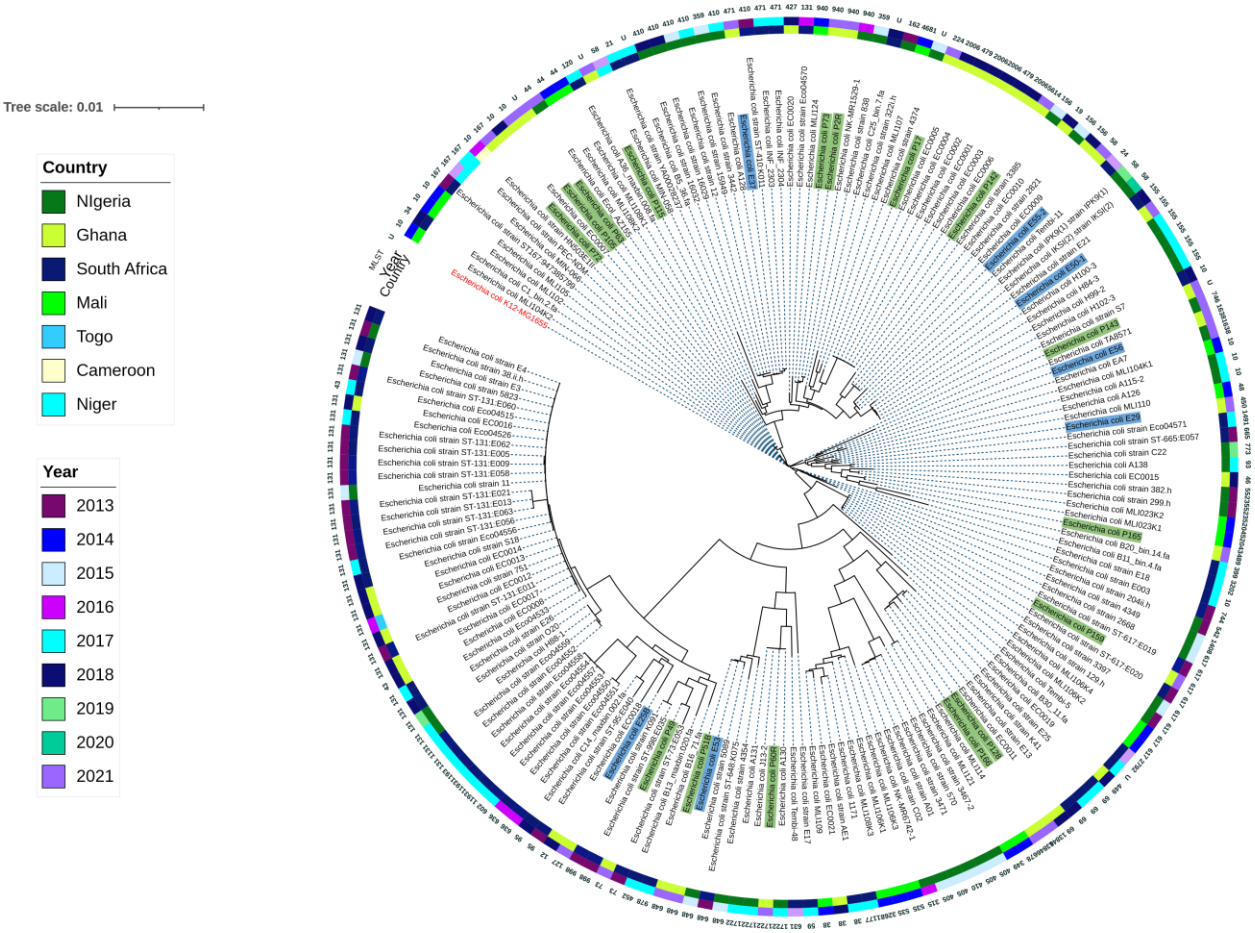

Figure 2: Maximum likelihood phylogenetic tree of *Escherichia coli* strains isolated from humans between 2013 and 2022 in selected African countries. The core-genome phylogenetic tree was drawn from 181 genomes with BV-BRC and annotated with iTOL. The tree was built with *E. coli* K12-MG1655 as the reference genome and rooted with the reference strain. The following metadata are indicated: the country of isolation on the inner colored ring, the year of isolation in the middle and the MLST in (isolates with unknown STs are indicated U) on the outer ring. Isolates from this study are labelled with green background (isolates from patients) and blue background (isolates from environment).

4.0 DISCUSSION

This study showed that patients were colonized with genetically diverse ESBL *E. coli* isolates on admission and during hospital stay. ESBL- producing *E. coli* strains were also found to be disseminated between patients and their immediate environment, suggesting the need to strengthen IPC practices to prevent infections in these patients.

The carriage of ESBL *E. coli* isolates by patients on admission in the hospital is suggestive of community carriage and subsequent introduction of these isolates into the directorates of the hospital on admission. Higher carriage rates of ESBL *E. coli* have been observed in previous studies in Tanzania (23.7%) and Iran (60.0%) [13, 17] and lower rate (10.5%) in Israel [18]. The incidence of ESBL *E. coli* acquisition among patients who previously did not harbor ESBL *E. coli* was 11.32% lower than that reported in Israel [18]. Differences in IPC practices and differences in screening time points for ESBL *E. coli* acquisition in various settings may account for the differences in carriage rates observed. Continuous screening for ESBL *E. coli* over the course of patients' stay may contribute to the detection of acquisition events.

Molecular characterization of the MDR *E. coli* isolated revealed a diversity of resistant genes in both patient carriage and environmental isolates. Among these, the ESBL gene, CTX-M was found predominantly present in the isolates, most likely borne on plasmids and commonly found together with the insertion sequence, IS1380 and the transposase Tn3 which could disseminate this gene between isolates from different sources. The presence of these CTX-M genes, colonizing patients is a concern for IPC as this suggests the prevalence and the circulation of *E. coli* containing CTX-M genes in the community. It may also have implications on the therapeutic antibiotic choices for

patients in the event of infection. The predominance of the *bla*$_{\text{CTX-M 15}}$ gene has been reported globally and in Ghana, among ESBL *E. coli* isolates [16, 19]. A study in Libya of clinical *E. coli* isolates reported a lower rate of 17.3% of isolates carrying the *bla*$_{\text{CTX-M-15}}$ gene [20] compared to the 52.2% of isolates with the *bla*$_{\text{CTX-M-15}}$ genes from this study. Though the *bla*CTX-M gene is frequently associated with the globally disseminated high-risk ST ST131 [21, 22], we found the gene present in *E. coli* strains with various ST types indicating the successful transfer of the *bla*CTX-M gene across various strains of *E. coli*. The *bla*$_{\text{CTX-M-15}}$ gene which is widespread has been found to be associated with infections of both community and hospital origins [20]. Their presence in isolates colonizing patients and among environmental isolates is thus a problem for successful IPC as they could lead to outbreaks.

There was also marked resistance to ciprofloxacin mediated by chromosomal point mutations in the *parCE* and *gyrAB* genes among isolates harbouring the *bla*CTX-M gene. Fluoroquinolone resistance has often been reported in ESBL-producing *E. coli* isolates in association with the CTX-M genes [23] raising concerns about the circulation of highly resistant clones and the limited treatment options in the event of infection with any of the ESBL-producing carriage isolates and environmental isolates.

In accordance with other studies, the class 1 integron was present in isolates, together with the CTX-M-15 genes and was found to be associated with genes encoding resistance to aminoglycosides (*aadA* gene cassettes) and/or trimethoprim (*dfrA* gene cassettes) [24, 25]. Gene cassettes with sulphonamides (*sul1*) and quaternary ammonium compounds (*qacE*) genes) were also found on class 1 integrons that were closely associated with the chromium (chrA) resistance gene. The presence of class 1 integrons in both carriage isolates and isolates from environments could facilitate the transfer of resistance genes within and between isolates of different sources as

they are noted to disseminate resistance due to their inherent mobility and are important in the development of multidrug resistance in *E. coli* [26]. Isolates bearing the CTX-M-15 genes together with *qacE* and *chrA* in both patient and environmental isolates could co-select for disinfectants, heavy metals and antibiotics [27], becoming problematic in disinfection efforts in IPCs as they are frequently associated with and mobilized by mobile genetic elements, promoting their dissemination.

An interesting finding was the presence of the carbapenemase *bla*$_{\text{OXA-181}}$ gene in two *E. coli* isolates of ST940 from patients in the same ward, one of which did not show phenotypic resistance to the carbapenems. Notably, one isolate P73 bearing the *bla*$_{\text{OXA-181}}$ was acquired on admission by a patient admitted nearly a month after the other patient. *bla*$_{\text{OXA-181}}$ has been reported among *E. coli* isolates from colonized patients, but usually among carbapenem resistant isolates, as reported recently in *E. coli* isolates of ST410 and ST940 from paediatric patients with diarrhoea in Ghana [28]. However, there were no other carbapenemases detected in contrast to studies which have found *bla*$_{\text{OXA-181}}$ together with the carbapenemase, *bla*NDM gene in *E. coli* isolates as reported in Kuwait and Korea [29, 30]. This non-expression of other carbapenemase could have resulted in one isolate not showing phenotypic resistance as co-carriage of resistant genes could impact their susceptibilities. The contigs that contained the OXA-181 gene in both isolates closely resembled the p010_B-OXA181 (CP048332.1) and pEC213_1-OXA-181(CP061102.1) plasmids suggesting they are carried by the same as reported by Prah et al. [28] showing a dissemination of an evolving phenotype of the *bla*$_{\text{OXA-181}}$ gene by same plasmids in the hospital and in Ghana.

The IncF plasmid was frequently found to be associated with resistant genes in all ST types. Acquired resistance genes in *E. coli* isolates have been found to be carried by the F plasmids and

usually with transposable elements, making the mobilization and dissemination of resistant genes between isolates from various sources possible [31].

The detection of a high number of similar virulence genes in the *E. coli* isolates from both patient carriage and environment suggests a high pathogenicity of isolates from both community and hospital environments (Table 1). There is the potential of these virulent isolates in the absence of efficient IPC practices, to cause serious infections if they are transferred from environments to patients or between patients. Virulence factors such as the adhesins *iha*, *papA*, *papC*, *sfaD*, *sfaE* and *sfaS* for adherence and invasion, as well as toxins *hylA* and *hylE* on virulence genes harboured by the isolates from both patients and environments in this study enable pathogens to survive for longer periods in colonized hosts thereby increasing their chances of eventually causing disease [32]. The phylogroups A and B1, reported among commensal *E. coli* were dominant among patient isolates in addition to other phylogroups B2 and D which are notably extra intestinal. Coupled with the presence of several virulence genes in the phylogroups A and B1 possibly acquired by horizontal gene transfer in patient carriage and environmental contamination isolates, there is the potential for increased risk of infection in patients just as isolates of the phylogroups B2 and D which are notably extra-intestinal and virulent [33, 34].

The isolates were found to belong to different STs, suggesting the circulation of various strains in the community and their subsequent introduction into different directorates of the hospital. Some STs were found common to some isolates. Two isolates, from a patient and environment of the same sequence type, ST648 carried similar antibiotic resistance genes, particularly the β-lactamases, *bla*CTX-M, *bla*TEM and *bla* OXA-1 genes **(Table 1)**. These two isolates were also 100% phylogenetically related and interestingly isolated within the same month, from different directorates, ie. from a patient in the Obstetrics and Gynaecology ward and from a bed in the ICU

(Supplementary table S1). The patient had acquired this isolate on admission, suggesting an inter-ward dissemination of the same strain spreading between patients and environment. ST648 *E. coli* strains, with their biofilm adherence virulence genes have emerged globally as a highly virulent strain likely to cause bacteremia just like the internationally circulating ST131 strain [33] which is first reported among human isolates from Ghana in this study. Such biofilm adherence genes can make isolates particularly non-susceptible to disinfection and thus remain a concern for IPC.

Similarly, two isolates from two patients in the Obstetrics and Gynaecology and the Surgical directorates belonged to ST940, one of which was acquired on admission a month after isolation of the other isolate which was carried by a patient into the ward. A novel sequence type ST13846 of the virulent phylogroup D which was detected in two isolates showed high clonal relatedness on phylogenetic analyses. Notably, these isolates were obtained from rectal swabs of two patients in two different directorates, ie. Obstetrics and Gynaecology and Surgery within the same month and both were acquired on hospitalization. The presence of ESBL-producing *E. coli* isolates with this new sequence type in two different directorates of the hospital within a week may suggest a rapid dissemination of this new ST within the hospital. The resistance genes of this new sequence type appeared to be associated with several MGEs which could facilitate the rapid spread of the antibiotic resistance genes carried by isolates of the new ST within patients in the hospital. These MGEs included the IncY plasmid which was found to be associated with the $bla_{\text{CTX-M-15}}$ gene and quinolone resistance genes (*qnrS1*) as reported in carriage isolates from community settings in Tanzania [13] and additionally the aminoglycoside resistance genes (*aph(6)-Id* and *aph(3'')-Ib*), sulphamethoxazole/trimethoprim resistance genes (*sul2* and *dfrA14*) and the tetracycline resistance gene (*tet(A)*).

The detection of isolates of common sequence types to patients and in some instances, environment in this study suggests an introduction and subsequent circulation of the ESBL *E. coli* isolates in the hospital.

Other high-risk STs detected among isolates from patients or environment in this study included ST410, ST1722 and ST10, which have also been observed among *E. coli* isolates from Ghana. The ST410 of an isolate from a tap has been reported among *E. coli* isolates in a study from two Ghanaian hospitals [15] and the ST1722 among diarrheagenic *E. coli* strains from Western Region of Ghana [35]. The isolation of the ST10 *E. coli* among colonized patients, which has previously been isolated in poultry meat and implicated in intestinal infections in other studies [19, 36] shows the circulation of this ST in various sources in Ghana and the possible acquisition of this ST by horizontal gene transfer, facilitated by plasmids from animal sources.

Phylogenetic analyses of the isolates revealed the clustering of *E. coli* isolates from patients and environments and in different directorates into similar groups **(Figure 1)**, and may suggest a possible inter-ward spread of the strains. Isolates from patient carriage clustering with isolates from patients on hospitalization is indicative of the circulation of ESBL-producing *E. coli* in community and their subsequent introduction into hospital. This is an important focus for IPC in the hospital as more stringent measures should be instituted to prevent or contain the spread of these isolates from community to hospital.

Furthermore, there was a clustering of our isolates with other genomes of similar sequence type, from African countries suggesting a possible intra-continental dissemination of the STs. The clustering of isolates from this study belonging to the high-risk sequence types ST131, ST617, ST648 among others with clinical isolates of same sequence types from other countries reveals the endemicity of these STs in the Africa. Indeed, these STs have been reported among clinical isolates

from infections in Nigeria, Ethiopia and South Africa [37-39]. An environmental isolate of ST155, which is described among food borne pathogens, common in isolates from poultry [40], clustered with clinical isolates of *E. coli* of ST155 and ST58, further raising a concern for the control of spread of this high risk clone between poultry and hospital environments. The isolate with novel ST13846 clustered together with strains of ST69, indicating a likelihood of the novel ST being a variant of the ST69 which may have been imported from other African countries. The clustering of isolates colonizing patients and contaminating environments with high-risk clones of clinical isolates from other countries suggest the potential of the isolates from this study to cause clinical infections in patients in the absence of efficient IPCs. There is the need for continuous surveillance for the timely detection of imported antibiotic resistant strains. The isolates were closely related mainly with isolates from Nigeria and South Africa noting that these countries have uploaded the largest number of genomes on the open access databases.

Since rectal screening for ESBLs is not a practice in the hospital, patients who did not consent to screening or did not meet the inclusion criteria could have been missed out as potential sources of acquisition. Environmental surfaces which were not sampled could also have harboured the isolates which were acquired.

This study has revealed the dissemination of MDR ESBL-producing *E. coli* isolates in the hospital between patients and environments, highlighting the necessity of screening patients for carriage of these pathogenic organisms.

This study has also highlighted the importance of using genomic data to detect isolates in patient carriage and environments which may have associations with strains that can cause infections.

5.0 Conclusion

MDR ESBL *E. coli* isolates were found to be circulating among hospitalized patients and their environment in Ghana. These isolates had a repertoire of resistance and virulence genes mainly associated with plasmids making them highly mobile. This is a concern for strengthening IPC practices and surveillance of not only isolates from patients but also from the environment in order to prevent and contain the spread of MDR organisms in hospitals in Ghana.

Author contributions: EEAY, NA, AOO and SYE co-conceptualized the study. EEAY undertook sample collection, laboratory and statistical analyses and wrote the original draft of the manuscript. EEAY, JM, DGA, AI, ALKA conducted data analysis. SYE, AOO, NA, ALKA, DGA, AI and JM supervised the work, vetted the results and critically reviewed the manuscript. All authors (EEAY, JM, NA, DGA, ALKA, AI, AOO and SYE) read and approved the manuscript.

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Ethical Approval statement: The study was approved by the Institutional Review board (IRB) of the KATH (Reference: KATH IRB/AP/107/20) and the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Reference: BREC/00001917/2020). Voluntary, informed written consent was obtained from participating patients and staff.

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Table S1: Characteristics of *E. coli* isolates from patients and environment

Bacterial ID	Patient no.	Date of collection	Source	Directorate	Source	Sample
P105	AP6	1/6/2021	Patient	Surgery	Rectal	admission
P128	AP63	22/7/2021	Patient	Obstetrics & gynaecology	Rectal	after 48h
P72	AP40	18/5/2021	Patient	Obstetrics & gynaecology	Rectal	admission
P60R	AP66	30/7/2021	Patient	Obstetrics & gynaecology	Rectal	Admission*
P17	BP17	30/04/2021	Patient	Obstetrics & gynaecology	Rectal	admission
P165	AP68	27/7/2021	Patient	Surgery	Rectal	admission
P142	AP67	27/7/2021	Patient	Obstetrics & gynaecology	Hand	admission
P159	AP13	30/7/2021	Patient	Obstetrics & gynaecology	Rectal	admission
P51B	AP32	15/5/2021	Patient	obstetrics & gynaecology	Rectal	after 48h
P49	AP31	15/5/2021	Patient	Obstetrics & gynaecology	Rectal	after 48h
P2R	AP2	30/4/2021	Patient	Obstetrics & gynaecology	Rectal	admission
P73	AP40	21/5/2021	Patient	Obstetrics & gynaecology	Rectal	after 48h
P166	BP13	30/7/2021	Patient	Surgery	Rectal	After 48h
P143	AP68	27/7/2021	Patient	Obstetrics & gynaecology	Rectal	admission
P115	AP56	15/6/2021	Patient	Obstetrics & gynaecology	Rectal	admission
P63	AP29	12/5/2021	Patient	Obstetrics & gynaecology	Rectal	admission
E25B	bed	21/5/2021	Environment	Obstetrics & gynaecology	Bed	
E50-1	dripstand	30/4/2021	Environment	Obstetrics & gynaecology	dripstand	
E56	bed	3/5/2021	Environment	Surgery	bed	
E37	tap	19/7/2021	Environment	Obstetrics & gynaecology	tap	
E29	tap	14/6/2021	Environment	Obstetrics & gynaecology	tap	
E55-2	bed	1/6/2021	Environment	Surgery	bed	
E53	bed	3/5/2021	Environment	Surgery/ ICU	bed	

Table S2: Genome and assembly characteristics of sequenced *E. coli* isolates from patients and environment

ISOLATE ID	SOURCE	ACCESION NO.	Antigen (Somatic O)	Flagellar (H)	SEQUENCE LENGTH	NO. OF CONTIGS	GC CONTENT %	Longest contig size (bp)	N50 value	L50 value
P51B	Patient	SAMN27356662	O102, O102	H6	5199290	136	50.5	693002	162647	10
P2R	Patient	SAMN27356667	O101	H4, H21	4734033	141	50.7	338266	107713	14
P49	Patient	SAMN27356668	O6	H1	5976865	3186	50.2	40717	2786	488
P73	Patient	SAMN27356669	No hit	H5	4793808	157	50.7	261280	93708	16
P142	Patient	SAMN27356671	O27	H14	5763277	956	50.7	76312	15233	112
P17	Patient	SAMN27356672	O8	H23	5081498	137	50.8	568303	206541	9
P60R	Patient	SAMN27356674	O1, O1	H25	5144797	78	50.4	551539	219648	7
P165	Patient	SAMN27356676	O61, O61	H34	4780747	57	50.6	646885	189779	8
P105	Patient	SAMN27356677	O101, O101	H10	5067872	181	50.5	187302	89215	19
P159	Patient	SAMN27356678	O101, O101	H10	4932348	134	50.7	221547	106189	17
P166	Patient	SAMN27356679	O15, O15	H10	5803192	1590	50.6	72453	13132	119
P128	Patient	SAMN27356680	O15, O15	H18	4967815	85	50.6	423144	186239	9
P115	Patient	SAMN27356683	O7, O53	H4	5983896	3109	50.2	252708	3104	451
P143	Patient	SAMN27356684	O99, O99, O8	H20	5657046	3098	50.5	58301	2839	465
P63	Patient	SAMN27356685	O101, O101	H4	6187348	3779	50.6	60134	2496	605
P72	Patient	SAMN31146634			4711974	124	50.8	254392	118922	14
E29	Environment	SAMN27356664	O45, O45	H19	4981043	183	50.7	180939	86806	20
E25B	Environment	SAMN27356665	O6	H31	5206655	134	50.4	423782	132761	14
E53	Environment	SAMN27356666	O102, O102	H6	5188919	152	50.5	530199	137528	12
E50-1	Environment	SAMN27356673	no hit	H40	4659426	88	50.6	387639	118999	13
E55-2	Environment	SAMN27356675	O8	H30	4947644	145	50.8	288469	105077	16
E56	Environment	SAMN27356681	O53	H18	5164605	861	50.1	208569	37344	44

Table S3: Capsular types of *E. coli* isolates

ISOLATE ID	SOURCE	MLST	Antigen (Somatic O)	Flagellar (H)
P51B	Patient	ST648	O102, O102	H6
P2R	Patient	ST940	O101	H4, H21
P49	Patient	ST73	O6	H1
P73	Patient	ST940	No hit	H5
P142	Patient	ST5614	O27	H14
P17	Patient	ST224	O8	H23
P60R	Patient	ST1722	O1, O1	H25
P165	Patient	ST3489	O61, O61	H34
P105	Patient	ST10	O101, O101	H10
P159	Patient	ST617	O101, O101	H10
P166	Patient	ST13846	O15, O15	H10
P128	Patient	ST13846	O15, O15	H18
P115	Patient	Unknown	O7, O53	H4
P143	Patient	Unknown	O99, O99, O8	H20
P63	Patient	Unknown	O101, O101	H4
P72	Patient	ST167	no hit	no hit
E37	Environment	ST410	O8, O8	H9
E29	Environment	ST450	O45, O45	H19
E25B	Environment	ST127	O6	H31
E53	Environment	ST648	O102, O102	H6
E50-1	Environment	ST155	no hit	H40
E55-2	Environment	ST58	O8	H30
E56	Environment	Unknown	O53	H18

Table S4: Chromosomal point mutations of gyrA/B, parC/E genes of the *E. coli* isolates

ISOLATE ID	**gyrA**	**gyrB**	**parC**	**parE**
P51B	S83L, D87N, *A828S, *D678E	S492N, A618T, E656D	E62K, S801, D475E	T172A, S458A
P49	S83L			
P17	S83L, D87N		S80I	S458A
P60R	*A863V	*S492N, *A618T	*E62K, *D475E	
P105	S83L, D87N		S80I	S458A
P159	S83L, D87N		S80I	S458A
P128	*D678E	*E703D	*E62K	
P166		*E703D	*E62K	
P143	D678E		E62K	
P63	S83L, D87N		E62K, S801, L440R	S458A
P72	S83L, D87N		E62K, S801	S458A
E29	S83L, D87N		S80I	S458A
E25B	S83L, D87N		S80I	S458A
E53	S83L, D87N		S80I	S458A
E50-1			E62K	
E56			E62K	
E37	S83L, D87N		S80I	S458A

**Putatively novel mutations*

Table S5: Distribution of Insertion sequences and intact prophages among *E. coli* isolates

ISOLATE ID	SOURCE	MLST	INSERTION SEQUENCES	INTACT PROPHAGES
P51B	Patient	ST648		PHAGE_Escher_TL_2011b_NC_019445(42), PHAGE_Klebsi_4LV2017_NC_047818(28)
P2R	Patient	ST940	IS609, MITEEc1, IS621, MITEYpe1	PHAGE_Entero_BP_4795_NC_004813(8), PHAGE_Entero_mEp460_NC_019716(19)
P49	Patient	ST73	IS621, ISChpi1, ISKol11, ISMsm1	PHAGE_Entero_phiFL1A_NC_013646(17), PHAGE_Escher_phiV10_NC_007804(38), PHAGE_Klebsi_4LV2017_NC_047818(21), PHAGE_Entero_P88_NC_026014(22), PHAGE_Shigel_SfII_NC_021857(23)
P73	Patient	ST940	IS621, ISNisp2, ISDin1, ISAlw6	PHAGE_Entero_BP_4795_NC_004813(7)
P142	Patient	ST5614	MITEEc1, MITEYpe1, IS621, Tn4430	PHAGE_Entero_BP_4795_NC_004813(21)
P17	Patient	ST224	MITEEc1, MITEYpe1, IS621, Tn4430	PHAGE_Salmon_SJ46_NC_031129(78)
P60R	Patient	ST1722	IS609, ISEc44, ISSen6, ISRor6,	PHAGE_Entero_SfI_NC_027339(28)
P165	Patient	ST3489	MITEEc1, IS621, IS3H, IS3F	None
P105	Patient	ST10	IS100kyp, IS100X, IS100L,IS100	PHAGE_Salmon_SEN34_NC_028699(23), PHAGE_Entero_cdtI_NC_009514(15), PHAGE_Escher_RCS47_NC_042128(53)
P159	Patient	ST617	IS3, ISEc17, IS3F, IS3H	PHAGE_Salmon_SP_004_NC_021774(19), PHAGE_Entero_BP_4795_NC_004813(9)
P166	Patient	ST13846	IS621, IS1062, ISRj1, ISAdh1	PHAGE_Entero_mEp460_NC_019716(21
P128	Patient	ST13846	MITEEc1, IS3H, IS3F, IS621	PHAGE_Entero_mEp460_NC_019716(21)
P115	Patient	Unknown	ISRaq1, ISSpr2, ISKpn34, ISKpn78, ISKpn80, IS1222, ISSen4, ISEhe4, ISYen3	PHAGE_Salmon_SSU5_NC_018843(42), PHAGE_Pseudo_phiPSA1_NC_024365(7), PHAGE_Entero_mEp460_NC_019716(16), PHAGE_Entero_BP_4795_NC_004813(7)
P143	Patient	Unknown	IS1396, ISAtsp1, IS1090, IS231K	PHAGE_Entero_lambda_NC_001416(21), PHAGE_Entero_mEp460_NC_019716(15)
P63	Patient	Unknown	ISEhe4, ISKpn80, ISKpn34, ISRaq1	PHAGE_Entero_IME10_NC_019501(13),
P72	Patient	ST167	MITEEc1, IS621, MiTEYpe1, Tn4430	None

E37	Environment	ST410	IS609	None
E29	Environment	ST450	IS2, ISEc27	PHAGE_Entero_fiAA91_ss_NC_022750(26), PHAGE_Escher_phiV10_NC_007804(40), PHAGE_Stx2_c_Stx2a_F451_NC_049924(3), PHAGE_Pseudo_phiPSA1_NC_024365(7)
E25B	Environment	ST127	ISEc42, MITEEc1, ISPa18, IS621	PHAGE_Salmon_SEN34_NC_028699(24), PHAGE_Shigel_SfII_NC_021857(32), PHAGE_Entero_mEp460_NC_019716(26), PHAGE_Entero_I2_2_NC_001332(8)
E53	Environment	ST648	IS621, ISRor3, ISHne4, ISPa126	PHAGE_Escher_TL_2011b_NC_019445(42), PHAGE_Klebsi_4LV2017_NC_047818(28)
E50-1	Environment	ST155	IS609, ISEc38, ISEc13, ISEc44	None
E55-2	Environment	ST58	MITEEc1, MITEKpn1, MITEYpe1, IS621	PHAGE_Yersin_L_413C_NC_004745(23), PHAGE_Salmon_118970_sal3_NC_031940(4), PHAGE_Entero_mEp460_NC_019716(20)
E56	Environment	Unknown	IS621, ISHch13, ISHce1, ISCosp3	PHAGE_Escher_500465_1_NC_049342(36), PHAGE_Entero_cdtI_NC_009514(5)

Table S6: Mobile genetic elements associated with antibiotic resistance

ID(ST)	Source	contig	Synteny of resistant genes	Plasmid/chromosomal sequence with closest nucleotide homology
P51B (ST648)	Patient	113	IS1:dfrA17:	*E. coli*702/18 plasmid p702_18_2 (CP074703.1)
		83	catB3:oxa-1:AAC(6')-Ib-cr5	*E. coli* JNQH498 plasmid pJNQH498-1 (CP104385.1)
		59	aac(3)-Iid:IS4	*E. coli* 702/18 plasmid p702_18_2 (CP074703.1)
		81	IS3::aac(3)-IIe	*Enterobacter hormaechei* strain 80014967 plasmid pE80014967-1 (CP104468.1)
		67	transposase::::sul2::repA	*Klebsiella quasipneumoniae* strain SWHEFF_72 plasmid unnamed1 (CP055011.1)
		71	IS1:tetC:tetB:tetR(B)::	*E. coli* D16EC0456 plasmid pD16EC0456-1 (CP088600.1)
		64	IS1380(transposase):CTX-M-15::Tn3	*Klebsiella pneumoniae* strain 2021CK-01815 plasmid unnamed1 (CP104374.1)
		75	erm(B)::transposase:	*E. coli* YJ6 plasmid pYJ6-NDM5 DNA (AP023236.1)
		69	IS6:mpR(A):mrxA:mphA	*E. coli* plasmid pM105_mF DNA (AP018137.1)
P2R(ST940)	Patient	4	IntI2(integrase):dfrA1:sat2::IS256(transposase): Tn7(transposase):TnsD(transposase):	*E. coli* Z0117EC0040 chromosome (CP098211.1)
		66	IS6(transposase):transposase::oxa-181:::ISKra4(transposase):recombinase::recombinase:QnrS1:transposase	*E. coli* 10 plasmid p010_B-OXA181 (CP048332.1)
		84	IS91(transposase):APH(6)-Id:aph(3")-Ib:sul2	*E. coli* 1EC213 plasmid pEC213_1-OXA-181 (CP061102.1)
		88	TetC:TetB:tetR(B):	*E. coli* PI24 plasmid pYLPI24a, complete sequence (CP074013.1)

P49(ST73)	Patient	1123	AadA5:dfrA17	*Shigella flexneri* 2a strain 18787_5_65 plasmid pKSR100 (CP090162.1)
		127	sul1:qacE:aadA1:dfrA1	*E. coli* EcPF5 plasmid p1 (CP054237.1)
		144	tetR(B):TetB:tetC::IS4(transposase)	*E. coli* plasmid p2 (MT077881.1)
		503	sul2::helicase	*Citrobacter youngae* strain CF10 plasmid pCF10-tmexCD1 (CP102501.1)
		1083	APH(6)-Id:aph(3")-Ib:	*E. coli* THB42-F3 plasmid pHB42-F3 (CP104331.1)
		1131	TEM-1:recombinase	*Salmonella enterica* strain s12177 plasmid ps12177-CTX (CP101349.1)
P73 (ST940)	Patient	67	transposase:Tn3(transposase)::oxa-181:EreA::ISKra4(transposase):recombinase::recombinase:QnrS1:transposase	*E. coli* 10 plasmid p010_B-OXA181 (CP048332.1)
		76	aadA1:oxa-1:intIL(integrase)::recombinase:Tn3(transposase):catA	*Salmonella enterica* subsp. *enterica serovar* Wien strain ZM3 plasmid pZM3 (MK797990.1)
		79	recombinase:TEM-190:::IS91(transposase):aph(3")-Ib:sul2	*E. coli* 1EC213 plasmid pEC213_1-OXA-181 (CP061102.1)
		83	tetR(B):tetB:tetC::IS4(transposase)	*E. coli* plasmid p2 (MT077881.1)
P142(ST5614)	Patient	86	tetA:tetR(A):relaxase:Tn3(transposase):recombinase::IntIL(integrase):dfrA17:AadA5:QacE:sul1:chrA:::IS6(transposase):MphR(A)	*E. coli* H8 plasmid B (CP010174.1)
		122	ISKra4(transposase):recombinase:QnrS1:IS3(transposase):Tn3(transposase)::CTX-M-15:::	*E. coli* O169:H41 strain 2014EL-1345-2 plasmid unnamed3(CP024226.1)
		151	sul2::IS91(transposase)::APH(6)-Id:aph(3")-Ib:	*E. coli* PMV-1 pHUSEC411like plasmid (HG428756.1)
		258	tetC:tetB:tetR(B):	*E. coli* 542093 plasmid p542093_2, (CP091411.1)

P17(ST224)	Patient	80	catB:oxa-1:AAC(6')-Ib-cr5	*E. coli* JNQH498 plasmid pJNQH498-1 (CP104385.1)
		69	sul1:QacE:AadA5:dfrA17:	*E. coli* TREC4 plasmid pTREC4 (MN158990.1)
		48	sul3::IS256(transposase):QacL:aadA1:cmlA1:aadA2:DfrA12	*E. coli* A241 plasmid pA241-TEM (MN807689.1)
		62	Erm(B)::transposase:	*E. coli* YJ6 plasmid pYJ6-NDM5 (AP023236.1)
		65	tet(A):tetR(A):relaxase	*E. coli* E1 plasmid p3 (CP104506.1)
P60R (ST1722)	Patient	32	recombinase:ISKra4(transposase):recombinase:qnrS1:IS3:Tn3::CTX-M-15:IS1380(transposase):::TEM-1:IS91(transposase):APH(6)-Id:APH(3")-Ib:sul2:IS110:Tn3:transposase	*E. coli* PGR46 plasmid pPGRT46 (KM023153.1)
		60	drfA14:intI1	*E. coli* MB50 plasmid pYLMB50a (CP073950.1)
		55	tet(A): tetR(A):relaxase	*C. sakazakii* strain Crono-589 plasmid pCrono589-1 (CP080592.1)
E55-2 (ST58)	Environment	68	IntI1(integrase):dfrA17:AadA5:QacE:sul1:chrA::IS6(transposase):mph(A):IS6(transposase)	*E. coli* SCU-103 plasmid pSCU-103-1 (CP054458.1)
		82	tetA::transposase:tetR(A):relaxase:APH(6)-Id:aph(3")-Ib:sul2:	*E. coli* SCU-103 plasmid pSCU-103-1 (CP054458.1)
P105(ST10)	Patient	105	intI1:dfrA17:AadA5:QacE:sul1:chrA:padR::	*E. coli* AH62 plasmid pAH62-3 (CP055262.1)
		127	transposase: CTX-M-15:	*Klebsiella pneumoniae* strain 2021CK-01815 plasmid unnamed1 (CP104374.1)
		145	CatB3:OXA-1:AAC(6')-Ib-cr5	*E. coli* IPCEC48 plasmid pIPCEC48_1 DNA (AP026795.1)
		140	IS3::AAC(3)-Iie	*E. coli* p11B plasmid unnamed1 (CP103756.1)
		111	tetB:tetC:AraC:Is4-like element(transposase)	*E. coli* plasmid p2 (MT077881.1)

		126	Is6(transposase):MphR(A):mrx(A):mph(A)	*E. coli* IPCEC48 plasmid pIPCEC48_1 DNA (AP026795.1)
		118	catA1::Tn3(transposase):recombinase	*E. coli* EC45 plasmid pEc45_1, (CP059126.1)
P159 (ST617)	Patient	94	catB:oxa-1:AAC(6')-Ib-cr5	*E. coli* IPCEC48 plasmid pIPCEC48_1 DNA (AP026795.1)
		88	TetR(B):TetB:	*E. coli* 1579 plasmid pMB3176_1 (CP103719.1)
		83	sul1:QacE:AadA5:dfrA17:intI1:	*E. coli* AH62 plasmid pAH62-3 (CP055262.1)
		80	sul2:::IS91(transposase):APH(6)-Id:aph(3")-Ib:	*Klebsiella pneumoniae* subsp. *pneumoniae* strain WRC18_CMC307MC plasmid pCMC307M_P4 (CP079633.1)
		89	ISEcp1(transposase):CTX-M-15::	*Klebsiella pneumoniae* strain 2021CK-01815 plasmid unnamed1 (CP104374.1)
		87	IS6(transposase):mphR(A):mrx(A):Mph(A)	*E. coli* IPCEC48 plasmid pIPCEC48_1 DNA (AP026795.1)
P166(ST13846)	Patient	488	dfrA14:intI1:	*E. coli* BL12 plasmid pBL12EC-2 (CP079777.1)
		388	IS91:aph(3")-Ib:sul2	*E. coli* 32-4 plasmid p32-4_C (CP048313.1)
		131	IS1380:CTX-M-15::Tn3:IS3:QnrS1:recombinase:ISKra4:recombinase:::	*E. coli* RIVM_C029494 chromosome (CP068823.1)
		426	TEM-1:recombinase::IS1380	*Klebsiella pneumoniae* strain 197 plasmid pMB2966_1 (CP103730.1)
		357	tet(A):tetR(A):relaxase	*E. coli* ST12468 plasmid pMB3825A_1 (CP103695.1)
P128(ST13846)	Patient	59	dfrA14:IntI1	*E. coli* BL12 plasmid pBL12EC-2 (CP079777.1)
		36	recombinase:ISKpn19(transposase):recombinase:QnrS1:IS3 Tn3::CTX-M-	*E. coli* PGR46 plasmid pPGRT46 (KM023153.1)

			15:recombinase:TEM1:IS91(transposase):APH(6)-Id:APH(3")Ib:sul2::IS5075:Tn3:	
		51	tet(A):TetR(A):relaxase	*E. coli* ST12468 plasmid pMB3825A_1, complete sequence (CP103695.1)
P115 (unknown ST)	Patient	25	recombinase:ISKra4(transposase):recombinase:qnrS1:IS3(transposase):Tn3(transposase)::CTX-M-15:IS1380(transposase):transposase	*E. coli* RM-055-WU chromosome (CP050210.1)
		103	sat2:dfrA1:IntI2(integrase)::lpfA	*E. coli* 61 chromosome (CP048326.1)
		172	catA1::Tn3(transposase):recombinase	*Klebsiella pneumoniae* strain 39427 plasmid pKPN39427.1 (CP054265.1)
		178	tetR(B):tetB:tetC::IS4(transposase)	*E. coli* plasmid p2 (MT077881.1)
		123	sul2::IS91(transposase)::recombinase:	*E. coli* plasmid p33 (MT077884.1)
		371	transposase:mphR(A):mrx:mph(A):	*E. coli* YJ3 plasmid pYJ3-a DNA (AP023228.1)
		316	TEM-190:recombinase:IS6(transposase)	*E. coli* UK_Dog_Liverpool plasmid pCARB35_02 (CP031655.1)
		417	AadA5:dfrA17:intI1(integrase):	*E. coli* IPCEC31 plasmid pIPCEC31_1 DNA (AP026784.1)
P143 (unknown ST**)**	Patient	63	aadA1:oxa-1:IntI1(integrase)::recombinase:Tn3(transposase):catA1	*E. coli* US32 chromosome (CP048606.1)
		104	sul2::IS91(transposase):APH(6)-Id:APH(3'):dfrA14:	*E. coli* RHB03-C12 plasmid pRHB03-C12_3 (CP058033.1)
		217	IS1(transposase)::tetC:tetB:tetR(B):	*E. coli* YJ3 plasmid pYJ3-a (AP023228.1)
P63 (unknown ST)	Patient	52	dfrA12::AadA3:CmlA:AadA1:QacL:IS256(transposase):sul3:::	*E. coli* CFSAN061769 plasmid pCFSAN061769_03 (CP042972.1)

		407	sul1:QacE:AadA5:drfA17:	*E. coli* IPCEC48 plasmid pIPCEC48_1 DNA (AP026795.1)
		738	catB3:oxa-1: AAC(6')-Ib-cr5	*E. coli* JNQH498 plasmid pJNQH498-1 (CP104385.1)
		877	ampR:*bla*DHA-1	*E. coli* 142 plasmid p142_A-OXA181 (CP048338.1)
		517	IS1380(transposase):CTX-M-15:	*E. coli* MS6192 chromosome (CP054940.1)
		769	TEM-1:recombinase	*E. coli* TQ2 plasmid pTQ6-tet(X4) (ON390814.1)
		272	tet(A):tetR(A):Tn3(transposase)	*E. coli* CP8-3_Sichuan plasmid pCP8-3-IncFIB (CP053738.1)
		273	tetC:tet(B):tetR(B)	*E. coli* YJ3 plasmid pYJ3-a DNA (AP023228.1)
		302	IS6(transposase):MphR(A):mrx:mphA	*E. coli* THB42-F3 plasmid pHB42-F3 (CP104331.1)
P72(ST167)	Patient	69	qacE:sul1::chrA::IS6(transposase):mphR(A):	*E. coli* Ecol_881 plasmid pEC881_1(CP019028.1)
		77	IS6(transposase):eamA:tetA:tetR(A):relaxase: APH(6)-Id: aph(3")-Ib:sul2:repC	*E. coli* HP243 plasmid pHP243 DNA(LC520289.1)
		94	AadA1: DUF1010:drfA12:	*Cronobacter sakazakii* strain Crono-589 plasmid pCrono589-1(CP080592.1)
		75	catA1:tn3(transposase)::recombinase: DUF3330:int1L(integron)	*E. coli* dm654b plasmid p_dm654b_NDM5(CP095637.1)
		85	tetR(B):tetB:tetC:AraC:IS4(transposase)	*E. coli* 2021CK-00607 plasmid unnamed1(CP104666.1)
		97	CTX-M-27: IS5/IS1182(transposase)	*E. coli* SCU-103 plasmid pSCU-103-1(CP054458.1)
		81	QepA4:IS91(transposase)	*E. coli* isolate MSB1_8B-sc-2280300 genome assembly, plasmid: 2 (LR890537.1)
E50-1(ST155)	Environment	72	sul2::IS110	*E. coli* CFS3313 plasmid pCFS3313-2 (CP026941.2)
		65	AadA5:dfrA17:intI1(integrase):recombinase:Tn3(transposase)	*E. coli* isolate J31 plasmid pJ31 (CP053788.1)

		31	relaxase:tetR(A):tet(A)::Tn3:::::::::TehB:ydcL:tnpA(transposase):insQ(transposase)::::	*E. coli* NCTC11129 genome assembly (LR134222.1)
E37 (ST410)	Environment	55	mphA:mphR(A):IS6:::dfrA14:IntI1:::Tn3:catA1	*E. coli* 165 chromosome (CP020509.1)
		82	catB-3:oxa-1:AAC(6')-Ib-cr5	*E. coli* JNQH498 plasmid pJNQH498-1 (CP104385.1)
		70	APH(6)-Id:sul2:::	*Salmonella enterica* subsp. *enterica* strain SCSM4.1 chromosome (CP047115.1)
		67	IS1380(transposase):CTX-M-15::Tn3	*E. coli* 1EC187 chromosome (CP061108.1)
		82	catB-3:oxa-1:AAC(6')-Ib-cr5	*E. coli* JNQH498 plasmid pJNQH498-1 (CP104385.1)
		73	TetC:TetB:TetR(B)::::	*E. coli* 1EC187 plasmid pEC187_1 (CP061109.1)
		79	IS3::aac(3)-Iie:	*E. coli* PM22 plasmid pYLPM22a (CP074020.1)
E29(ST450)	Environment	90	transposase:eamA:tetA:tetR(A):relaxase:APH(6)-Id: APH(3")-Ib:sul2:	*E. coli* SCU-103 plasmid pSCU-103-1 (CP054458.1)
		105	erm(B)::transposase:	*E. coli* 5M plasmid pISV_IncFII_NDM-5 (MN218686.1)
		92	TEM-1:recombinase:IS6(transposase):mphR(A):mrxA:mph(A)	*E. coli* plasmid pV021-b (AP014876.1)
		137	dfrA17:IntI1(integrase)	*E. coli* IPCEC31 plasmid pIPCEC31_1 DNA(AP026784.1)
		109	catA1::Tn3:	*Klebsiella pneumoniae* isolate 11 genome assembly, plasmid: P1 (OX030692.1)
		125	AAC(3)-Iid:IS4(transposase)	*E. coli* AH62 plasmid pAH62-3 (CP055262.1)
E25B(ST127)	Environment	83	Tet(B):TetC:IS4	*E. coli* plasmid p2 (MT077881.1)
		62	sul1:qacE:dfrA7:IntI1::recombinase:Tn3::catB3	*E. coli* SCU-397 plasmid pSCU-397-2 (CP054830.1)

		86	Tn3::TEM-1	*E. coli* 1190 plasmid p86 (CP023387.1)
E53(ST648)	Environment	69	transposase::::sul2::helicase	*Klebsiella quasipneumoniae* strain SWHEFF_72 plasmid unnamed1 (CP055011.1)
		89	catB3:oxa-1:AAC(6')-Ib-cr5:	*E. coli* strain JNQH498 plasmid pJNQH498-1 (CP104385.1)
		72	IS1(transposase:TetC:TetB:TetR(B)::	*E. coli* D16EC0456 plasmid pD16EC0456-1 (CP088600.1)
		128	IS1(transposase):dfrA17	*E. coli* 702/18 plasmid p702_18_2 (CP074703.1)
		68	IS1380(transposase):CTX-M-15::Tn3(transposase)	*Klebsiella pneumoniae* strain 2021CK-01815 plasmid unnamed1 (CP104374.1)
		61	AAC(3)-Iid:IS4(transposase)	*E. coli* plasmid p5 DNA, strain: SA1-12-GR-1 (LC318095.1)
		84	IS3(transposase)::aac(3)-Iie:	*Enterobacter hormaechei* strain 80014967 plasmid pE80014967-1 (CP104468.1)
		75	ermB::transposase	*E. coli* YJ6 plasmid pYJ6-NDM5 (AP023236.1)
		70	recombinase:IS6:mphR(A):mrxA:mphA	*E. coli* plasmid pM105_mF DNA (AP018137.1)
E56	Environment	195	IS3::aac(3)-Iie	*E. coli* 100 plasmid p100_NDM5_IncN (MT199177.1)
		167	tet(A):tetR(A):relaxase	*E. coli* CFS3273 plasmid pCFS3273-1 (CP026933.2)
		243	catB3:oxa-1	*E. coli* IPCEC48 plasmid pIPCEC48_1 (AP026795.1)

CHAPTER FOUR – MANUSCRIPT 3

Genomic analysis of porin related resistance in ESBL-producing *Klebsiella pneumoniae* isolates from patients and environments in a teaching hospital in Ghana.

AUTHOR CONTRIBUTIONS

Esther Eyram Asare Yeboah as the principal investigator, co-conceptualized the study, undertook sample collection, laboratory work and statistical analyses and drafted the manuscript.

Nicholas Agyepong – as co-supervisor co-conceptualized the study, assisted with sample collection, conducted data analysis, vetted the results and critically reviewed manuscript.

Joshua Mbanga – as co-supervisor conducted data analysis, vetted the results and critically reviewed manuscript.

Daniel Gyamfi Amoako – as co-supervisor conducted data analysis, vetted the results and critically reviewed manuscript.

Akebe Luther King Abia – as co-supervisor conducted data analysis, vetted the results and critically reviewed manuscript.

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Alexander Owusu-Ofori- as co-supervisor co-conceptualized the study, facilitated sample collection, vetted the results and critically reviewed manuscript.

Sabiha Yusuf Essack - as the principal supervisor, co-conceptualized the study, guided the literature review and ethical approval application, facilitated data collection and analysis, vetted the results and undertook critical revision of the manuscript

Objectives met: This paper answers objectives 3, 4, 5 and 6.

Title: Genomic analysis of porin related resistance in ESBL-producing *Klebsiella pneumoniae* isolates from patients and environments in a teaching hospital in Ghana.

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Abstract

Background: Multidrug resistant(MDR) ESBL-*K. pneumoniae* are widespread and implicated in both community and hospital-acquired infections. We determined the resistome, virulome, mobilome and genetic relatedness of MDR *K. pneumoniae* isolates from patients and their environment in a teaching hospital in Ghana.

Methods: Ten *K. pneumoniae* isolates which were both MDR and β-lactamase (ESBL)-producing, were selected among Gram-negative bacterial isolates from a study of multidrug resistant Gram-negative bacterial isolates colonizing patients, carried on healthcare workers hands and contaminating the patient environment. These isolates were further analyzed by whole genome sequencing (WGS) and various bioinformatics tools to identify the presence of resistance and virulence genes, mobile genetic elements and mutations in outer membrane porins.

Results: All (100%) of the selected MDR *K. pneumoniae* isolates were resistant to ceftazidime. The isolates showed varying resistance to the cephalosporins and were all susceptible to tigecycline. Three isolates showed intermediate resistance to both meropenem and doripenem. The β-lactamase gene, bla_{SHV} was the most dominant and harboured by eight isolates. $bla_{\text{CTX-M-15}}$ was observed in six isolates. Mutations in both *ompK36* and *ompK37* were found in all isolates. Genes encoding resistance to fluoroquinolone (*qnrB*), aminoglycosides (*aadA1, aadA2, aac(3)-IIa, aac(6')-Ib-cr,aph(3'')-Ib , aph(6)-Id*) sulphamethoxazole/trimethoprim (*sul1, sul2, dfrA14, dfrA15*) were also detected. The isolates were diverse and belonged to different strain types (STs), viz., ST39, ST307, ST815, ST1552, ST636, ST464 and ST1996. ST39 was the most commonly observed sequence type in four isolates (three hospital environment isolates and one carriage isolate). A myriad of virulence genes, including *irp1, irp2, iutA, gndA, ompA, fes, fep, mrkD* and

fimH were detected in both carriage and environmental isolates. IncFIB was the most abundant plasmid replicon occurring in nine (four carriage and five from the hospital environment) isolates.

Conclusion: This study showed that MDR *K. pneumoniae* isolates from patients and environment belonged to different multilocus sequence typing (MLST) lineages, predominantly ST39. Mutations were detected in the outer membrane proteins and together with ESBLs mediated resistance to cephalosporins and were hypothesized to mediate reduced susceptibility to carbapenems. A clonal relationship was established among a carriage isolate (ST39) and two isolates from hospital environment (ST39) together with an ST1996 environmental isolate. This study found that ESBL-producing *K. pneumoniae* isolates were potentially introduced into the hospital from community. Infection prevention and control practices need to be enhanced to prevent the dissemination and transmission of these resistant strains in the hospital.

Introduction

Multidrug resistant Gram-negative bacteria are a serious threat to health, and may cause life-threatening infections (Agyepong et al., 2018). Among these, ESBL- producing and carbapenem resistant *K. pneumoniae* have been listed by the World Health Organization (WHO) as pathogens of critical priority for the development of new antibiotics (WHO, 2017). It notably causes nosocomial and community-acquired infections such as pneumonia, and urinary tract infections (Mohd Asri et al., 2021). *K. pneumoniae* isolates can harbor many resistant genes and develop diverse mechanisms of resistance to antibiotics (Kashefieh et al., 2021).

In addition to its intrinsic β-lactamase gene, *bla*$_{\text{SHV-1}}$, mutations resulting in the loss, reducing the number and/or diameter of outer membrane porins, *ompK35*, *ompK36* and the quiescent *ompK37* as well as efflux pumps may contribute to the resistance of *K. pneumoniae* to β-lactams including carbapenems (Hamzaoui et al., 2018; Khalifa et al., 2021).

Antibiotic resistant genes in *K. pneumoniae* can be spread widely by the dissemination of epidemic clones and mobile genetic elements predominantly plasmids (Piccirilli et al., 2021). The Inc-groups, of plasmids IncF, IncFII(K1), IncR, IncX, IncX3, IncI2, and ColE1 have particularly been associated with rapid spread of resistance in *K. pneumoniae* isolates (Piccirilli et al., 2021).

Multilocus sequencing typing (MLST) has revealed the circulation of various STs of MDR *K. pneumoniae* isolates. Notably ST307-*K. pneumoniae* have been recognized as a globally emerging clone with genetic characteristics enabling ease of dissemination and persistence in the hospital setting (Hernández-García et al., 2022). It has been detected in Korea, Germany, South Africa and Nigeria (Afolayan et al., 2021; Baek et al., 2020; Heiden et al., 2020; Lowe et al., 2019). The ST39

has also been reported in Russia, South Africa and Ethiopia (Fursova et al., 2020; Kopotsa et al., 2020; Sewunet et al., 2021)

In a study of MDR *K. pneumoniae* isolates recovered from clinical samples in the Komfo Anokye Teaching Hospital, Ghana, 24.32% of the isolates were found to be resistant to second and third generation cephalosporins and had multiple resistant genes. MLST analyses revealed the circulation of multiple *K. pneumoniae* sequence types (ST2171, ST2186, ST17, ST152, ST397, ST101, ST1788 and STS789) in the hospital (Agyepong et al., 2019).

The presence of several virulence genes such as the fimbriae synthesis-related gene, lipopolysaccharide-related gene, capsular polysaccharide synthesis and synthesis regulation-related gene, iron uptake system, urease-related gene, tellurite resistance gene and hemolysin among others contribute to the pathogenicity of *K. pneumoniae*. Capsule and LPS antigens, K and O have been reported to contribute significantly to the pathogenicity of *K. pneumoniae* isolates (Evrard et al., 2010). The structural differences of these antigens has been useful in assigning serotypes which describe the extent of virulence among various strains of *K. pneumoniae* (Follador et al., 2016). Hypervirulent *K. pneumoniae* which are typically characterized by the presence of roB, iucA, peg-344, rmpA, and rmpA2 genes are associated with increased mortalities emerged in Asia and continue to spread globally (Russo et al,2018; Zhu et al., 2021).

Investigations on virulent *K. pneumoniae* have often been in outbreak settings. It is equally important to investigate the pathogenicity and virulence of *K. pneumoniae* isolates colonizing patients in non-outbreak settings to establish/improve infection prevention and control practice. In spite of recent studies globally on the epidemiology of ESBL-producing and carbapenem resistant *K. pneumoniae*, there is a paucity of data from Sub-Saharan African countries such as Ghana.

Using whole genome sequencing and bioinformatics analysis, we studied MDR *K. pneumoniae* isolates from patients and their hospital environments in a Teaching Hospital in Ghana to determine the antimicrobial resistance, virulence and genetic relatedness of ESBL-producing and carbapenem resistant isolates in the context of infection prevention and control (IPC).

Materials and Methods

Ethical approval

Ethical approval for the study was obtained from the Institutional Review board (IRB) of the Komfo Anokye Teaching Hospital(KATH) **(Reference: KATH IRB/AP/107/20)** and the Biomedical Research Ethics Committee of the University of KwaZulu-Natal **(Reference: BREC/00001917/2020**). Voluntary, informed written consent was obtained from participating patients and staff.

Study population and sample collection

The study focused on MDR ESBL-producing *K. pneumoniae* colonizing patients and contaminating their immediate environments at the Komfo Anokye Teaching Hospital, Kumasi. From April 2021 to July 2021. Rectal and hand swabs were collected from consenting adult patients within 24 hours of admission and after 48 hours in wards of three directorates of the hospital; Obstetrics and Gynaecology, Surgery and the Intensive Care Unit. Hand swabs were also collected concurrently from staff present at the time of patient sampling and also from the patient's immediate environment (bedrails, drip-stand, door handles and taps) in the wards.

Isolation and Identification of ESBL MDR *K. pneumoniae*

Briefly, swabs collected from 83 patients and their environments as well as from hands of healthcare workers were processed on MacConkey agar and 250 Gram-negative bacteria were identified by Gram staining. Using the VITEK 2® automated system (BioMérieux-Vitek, Marcy-l'Étoile, France), 31 isolates were identified as *K. pneumoniae* isolates among the Gram-negative bacteria.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted using the VITEK 2® automated system (BioMérieux-Vitek, Marcy-l'Étoile, France). The antibiotic panel consisted of 16 antibiotics: cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), amoxicillin/clavulanic (AMC), piperacillin/tazobactam (TZP), imipenem (IPM), doripenem (DOR), meropenem (MEM), ertapenem (ERT), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), ciprofloxacin (CIP), trimethoprim-sulphamethoxazole (SXT) and tigecycline (TGC)

DNA extraction, Genome sequencing and analysis

Genomic DNA (gDNA) from pure colonies of identified *K. pneumoniae* isolates grown overnight was extracted using the GenElute® bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO, United States) according to the manufacturer's instructions. The concentration and quality of the extracted gDNA was checked using the Nanodrop spectrophotometer Qubit (Thermo Scientific, Waltham, MA, USA). Multiplexed paired-end libraries (2 × 300 bp) were prepared using the Nextera XT DNA sample preparation kit (), and whole genome sequences were determined on an Illumina MiSeq platform (Illumina, San Diego, CA, United States) with 100× coverage.

Quality trimming of raw reads was done using Sickle v1.33 (https://github.com/najoshi/sickle). The raw reads were then assembled spontaneously using the SPAdes v3.6.2 assembler (https://cab.spbu.ru/software/spades/). All contiguous sequences were subsequently submitted to GenBank and assigned accession numbers (**Supplementary Table S1)** under BioProject **PRJNA823741.**

Molecular typing of *K. pneumoniae* isolates

Multilocus sequence typing (MLST) was performed in-silico using the WGS data online platform tool from the assembled genomes (https://bigsdb.pasteur.fr/ klebsiella/klebsiella.html). The serotypes (K types, O types, wzc and wzi allelic types) of the isolates were determined using the reference Klebsiella WGS data online platform tool, Kaptive-web (http://kaptive.holtlab.net/).

Identification of the acquired and chromosomal mutations in the isolates

Resistance and virulence genes were determined using ResFinder (https://cge.food.dtu.dk/services/ResFinder/) and VF analyzer via Virulence finder database (https://cge.food.dtu.dk/services/VirulenceFinder/) respectively. Mutations in the *ompK35, omp36* and *ompK37* genes were determined via ResFinder.

Identification of mobile genetic elements (MGEs)/genetic support

The presence of mobile genetic elements were determined using PlasmidFinder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) for plasmids, (Carattoli et al., 2014), INTEGRALL (http://integrall.bio.ua.pt/) for integrons, RAST SEEDVIEWER

(https://rast.nmpdr.org/seedviewer.cgi) for transposons and insertion sequences. Insertion sequences (IS) in genomes were predicted via the ISFinder database (https://www-is.biotoul.fr/). The PHASTER (https:// phaster.ca/) server was used for the identification and visualization of prophage sequences.

The synteny and genetic environment of antibiotic resistance genes and associated mobile genetic elements was investigated using the general feature format (GFF3) files from GenBank.

Phylogenomic analyses of the *K. pneumoniae* isolates

Phylogenomic analysis was done to determine the genetic relatedness of isolates in this study. Isolates from this study were also compared with 180 *K. pneumoniae* isolate genomes from sub-Saharan African countries that were downloaded from Bacterial and Viral Bioinformatics Resource Center (BV-BRCB) (https://www.bv-brc.org/). A maximum likelihood tree was constructed on BV-BRCB using the reference genome *K. pneumoniae* Ecl8 (accession number: HF536482 CANH01000000) for rooting. FigTree (http://tree.bio.ed.ac.uk/software/figtree/), and interactive tree of life (iTol) (https://itol.embl.de/) was used in visualization and annotation of the phylogenetic tree.

In order to get a more comprehensive insight on the relationships of isolates from this study, Phandango (https://jameshadfield.github.io/phandango/#/main) was used to visualize the phylogenetic tree with corresponding metadata.

Results

Isolates identification

Thirty-one out of 250 Gram-negative bacteria isolated from swabs were identified biochemically as *K. pneumoniae* of which 20/31(64.5%) were found to be MDR. All 20 underwent WGS, and we then excluded genomes of low quality (n=7) and those belonging to *Klebsiella variicola* (n=1) or *Klebsiella quasipneumoniae* (n=2). The remaining 10 isolates were confirmed as *K. pneumoniae*, five carriage isolates and five from environment (door handle, tap, drip-stand and bed and five from patients).

AMR profiles of MDR *K. pneumoniae* isolates from patients and environments

All (100%) of the ten selected MDR *K. pneumoniae* isolates were resistant to ceftazidime and cefuroxime. Ninety percent of the isolates were found to be resistant to piperacillin/tazobactam, cefipime, amoxicillin/clavulanic acid and sulphamethoxazole/trimetoprim. Eighty percent (8/10) of the isolates showed resistance to ceftriaxone. All isolates were however 100% susceptible to tigecycline, amikacin, ertapenem and imipenem. Two carriage isolates (P121 and P60-1) and E55-1 from hospital environment showed intermediate resistant to both meropenem and doripenem and presented with elevated minimum inhibitory concentrations (MICs) of 2-4µg/ml **(Supplementary table S2)**. Sixty percent (3/5) of carriage isolates and 60% (3/5 from hospital environments were resistant to gentamicin, tobramycin and ciprofloxacin **(Table 1).** The most common antibiogram pattern among the isolates was CXM-FEP-CAZ-CRO-AMC-TZP-GEN-CIP-GEN-SXT which was common to five isolates; two from patients (P121 and P129) and three from the hospital environment (E4, E34A and E50-2) **(Table 2)**.

WGS-based capsular serotyping and Multilocus sequence typing (MLST)

The isolates showed high variation in multilocus sequence typing (MLST) with seven sequence types identified, viz., ST39, ST307, ST815, ST1552, ST636, ST464 and ST1996). Four isolates (three from the hospital environment E4, E35, E50-2 from a bed, door handle and dripstand respectively and one carriage isolate, P60-1) belonged to the same MLST, ST 39. The isolates belonged to different capsular serotypes. Two isolates (E4 and E50-2) from the hospital environment (bed and drip-stand respectively) however shared the same MLST, ST 39 and capsular serotype wzi 2 **(Table 1)**.

The O and K capsules were diverse among the isolates. Predominantly, of the O capsules, O1/O2v1 (5/10 and O1/O2v2 (4/10) were common to the isolates. The other O-capsule types for *K. pneumoniae* was the O3b. The k capsules were more diverse with all but two isolates showing different k-capsule type. Isolates of same ST did not necessarily have the same O and K-capsules. For instance, for isolates belonging to ST 39, two isolates (E4 and E50-2 from the hospital environment and a patient respectively) had the O1/O2v1 capsule with KL2 capsules while the other two (E35 and P60-1 from the hospital environment and a patient respectively) had the same O-capsule O1/2v2 but different K-capsules (KL23 and KL149) respectively.

Resistome of the isolates

Several β-lactamase genes were identified in the isolates, with the dominant genes being the *bla*$_{SHV}$ (n=8) in four carriage and four hospital environment isolates. Six isolates with phenotypic ESBLs had ESBL genes. Predominantly, *bla*$_{CTX\text{-}M\text{-}15}$ gene was found in six isolates (three carriage and three isolates from the hospital environment). Sixteen different *bla* $_{SHV}$ genes were identified in the isolates. These included *bla*$_{SHV\text{-}40}$, *bla*$_{SHV\text{-}85}$, *bla*$_{SHV\text{-}56}$, *bla*$_{SHV\text{-}79}$ and *bla*$_{SHV\text{-}89}$ which were found together in both E50-2 and E4. One carriage isolate(P144) also harboured seven blaSHV genes (*bla*$_{SHV\text{-}26}$, *bla*$_{SHV\text{-}98}$, *bla*$_{SHV\text{-}78}$, *bla*$_{SHV\text{-}194}$, *bla*$_{SHV\text{-}199}$, *bla*$_{SHV\text{-}179}$ and *bla*$_{SHV\text{-}145}$). *bla*$_{SHV\text{-}38}$, *bla*$_{SHV\text{-}62}$, *bla*$_{SHV\text{-}172}$ and *bla*$_{SHV\text{-}106}$ were also found in one isolate each. Among these, *bla*$_{SHV\text{-}38,}$ *bla*$_{SHV\text{-}40,}$ *bla*$_{SHV\text{-}98,}$ *bla*$_{SHV\text{-}106}$ and *bla*$_{SHV\text{-}145}$ are ESBLs. The *bla*$_{SCO\text{-}1}$ gene was detected in two carriage isolates (P121 and P136) which also harboured the *bla*$_{TEM\text{-}1,}$ *bla*$_{CTX\text{-}M\text{-}15}$ genes and the *bla*$_{SHV\text{-}172}$ (in P121) and *bla*$_{SHV\text{-}62}$ (in P136) **(Table 2).** The *bla*$_{DHA}$ gene was found in a carriage isolate (P60-1) with ST39 from a patient, though other isolates of similar ST lacked the *bla*$_{DHA}$ gene. Co-production of *bla*$_{CTX\text{-}M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ was evident in three carriage isolates (P121 and P136) and two isolates (E34A and E50-2) from the hospital environment. An isolate from the environment (E35) showed no β-lactamase genes though it was resistant to cefuroxime, ceftazidime, cefipime, amoxicillin/clavulanic and piperacillin/tazobactam (**Table 2)**.

The isolates also harboured sulphamethoxazole-trimetoprim resistant genes, *sul* and *dfrA* in nine isolates. Aminoglycoside resistance was encoded by the *aac(aac(3)-IIa*, *aac(6')-Ib-cr*), *aph (aph(3")-Ib , aph(6)-Id), aad(aadA1, aadA2)* and *ant(2")-Ia* genes and were associated with increased MICs to gentamicin (≥16 µg/ml) and tobramycin (≥16 µg/ml). Other resistance genes present were the *tet* genes in four isolates (*tetA* in P60-1 and E34A and *tetD* in E35 and P136) encoding tetracycline resistance in both carriage isolates and isolates from the hospital environment. The *fosA* gene for fosfomycin resistance was harboured by all 10 isolates (five

carriage and five environmental isolates). The *QacE* genes encoding resistance to quaternary ammonium compounds were observed in three isolates (carriage isolates P121, P136 and the isolate from the hospital environment E35) **(Table 2)**.

Fluoroquinolone resistance which was observed in five (three isolates from the hospital environment and two from patients) isolates was found to be associated with the plasmid-mediated quinolone resistance (PMQR) genes, *Qnr, Oqx* and *aac(6')-lb-cr* **(Table 2**) and less frequently by chromosomal point mutations in the quinolone resistance determining regions (QRDR) of *gyrA* (S83Y, D87A) which were observed in a carriage isolate, P129 and *parC* (S80I) in P129 and E34A) **(Supplementary table S4)**. The *oqxA* and *oqxB* genes encoding the multidrug efflux pump *oqxAB* were found together in all 10 isolates (five carriage and five isolates from the environment). The *qnr* gene occurred in seven isolates (three carriage and four isolates from the hospital environment), with the most frequent being the *qnrB* genes. Five (three from the hospital environment and two carriage) isolates haboured the *aac(6')-Ib-cr* gene and was associated with high MICs to the aminoglycosides; gentamicin and tobramycin**(Table 2).**

Chromosomal point analysis indicated mutations in the *acrR* efflux pump gene in all the *K. pneumoniae* isolates. One carriage isolate, P129 had the *acrR* mutation (F204L) encoding tigecycline resistance though this was not phenotypically expressed **(Table 3)**.

Table 1: Antibiotic resistance profiles of MDR *K. pneumoniae* isolates

ID	Source	Date of isolation	Directorate	Site	Cephalosporins				β-lactamase inhibitors		Carbapenems				Aminoglycosides			FQ	Others	
					CXM	CAZ	CRO	FEP	AMC	TZP	DOR	ERT	IMI	MER	GEN	TOB	AMK	CIP	COT	TIG
P129	Patient	22/07/21	O&G	Hand *	R	R	R	R	R	R	S	S	S	S	R	R	S	R	R	S
E34A	Environment	29/06/21	O&G	Dripstand	R	R	R	R	R	R	S	S	S	S	R	R	S	R	R	S
E4	Environment	27/04/21	O&G	Bed	R	R	R	R	R	R	S	S	S	S	R	R	S	R	R	S
E50-2	Environment	30/04/2021	O&G	Dripstand	R	R	R	R	R	R	S	S	S	S	R	R	S	R	R	S
P121	Patient	15/07/21	Surgery	Rectal	R	R	R	R	R	R	I	S	S	I	R	R	S	R	R	
P136	Patient	22/07/21	ICU	Rectal	S	R	R	R	R	R	S	S	S	S	R	R	S	S	R	S
P60-1	Patient	17/05/21	O&G	Rectal*	R	R	R	R	R	R	I	S	S	I	S	S	S	I	R	
E35	Environment	29/06/21	O&G	Door handle	R	R	R	R	R	R	S	S	S	S	S	S	S	S	R	S
P144	Patient	30/07/21	O&G	Rectal*	R	R	R	S	I	S	S	S	S	S	S	R	S	R	R	S
E55-1	Environment	3/5/2021	O&G	Tap	R	R	S	R	R	R	I	S	S	I	S	S	S	S	S	S

The phenotypic resistance profile is represented as an isolate being resistant (orange) or of intermediate resistance (gold) or susceptible (white) to the antibiotic indicated on the bottom column header (and the drug class it belongs to on the top column header). FQ-fluoroquinolone, O&G-Obstetrics and Gynaecology *- isolate acquired after admission

AMK: Amikacin, CXM:Cefuroxime, DOR:Doripenem, FEP:Cefepime, CRO:Ceftriaxone, CAZ:Ceftazidime, CIP:Ciprofloxacin, AMC:Amoxicillin/clavulanic acid, SXT:Trimethoprim/Sulphamethoxazole, ERT:Ertapenem, GEN:Gentamicin, IPM:Imipenem, MEM:Meropenem, TZP:Piperacillin/Tazobactam, TOB:Tobramycin, TGC:Tigecycline

Table 2: Resistome, virulome, mobilome. OmpK mutations and clonality of MDR *K. pneumoniae* isolates

ID	Source	Resistance pattern	MLST	β-lactamases	Other ARGs	ompK mutation		Virulence genes
						ompK36	ompK37	
E50-2	Environment (Drip-stand)	AMP-CXM-CAZ-CRO-FEP-TZP-AMC-GEN-TOB- CIP-SXT	ST39	*bla*$_{SHV-40}$ *bla*$_{SHV-85}$, *bla*$_{SHV-56}$, *bla*$_{SHV-79}$, *bla*$_{SHV-89}$ *bla*$_{CTX-M-15}$ *bla*$_{OXA-1}$, *bla*$_{TEM-1B}$	*sul2, dfrA14, OqxB, OqxA , aac(3)-IIa, aac(6')-Ib-cr, aph(3'')-Ib , aph(6)-Id qnrB1 , fosA*	N49S, L59V, L191S, F207W, A217S, N218H, D224E , L228V , E232R, T254S	I70M, I128M, N230G	*fyuA, traT, irp1, irp2, iutA, icmF/tssM, clpV/tssH, tssF, vgrG/tssI, vipB/tssC, ompA, vasE/tssK, tssG, fyuA/psn, ybtQ, yagX/ecpC, yagW/ecpD, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, fur, KP1_RS17240, KP1_RS17345, KP1_RS17280, gndA, rfbK1, rfbD, rgd, KP1_RS17225, KP1_RS17340, mrkC, fimD, fimK, mrkD, fimH, rcsB,acrB, acrA, entF, fepA, entE, fes, entC, kdsA, phoP,*
E4	Environment (Bed)	AMP-CXM-CAZ-CRO-FEP-TZP-AMC-GEN-TOB- CIP-SXT	ST39	*bla*$_{SHV-40}$ *bla*$_{SHV-85}$, *bla*$_{SHV-56}$, *bla*$_{SHV-79}$, *bla*$_{SHV-89}$ *bla*$_{CTX-M-15}$ *bla*$_{OXA-1}$, *bla*$_{TEM-1B}$	*sul2, dfrA14, OqxB, OqxA , aac(3)-IIa, aac(6')-Ib-cr, aph(3'')-Ib , aph(6)-Id qnrB1 , fosA*	N49S, L59V, L191S, F207W, A217S, N218H, D224E , L228V , E232R, T254S	I70M, I128M, N230G	*ompA, irp1, irp2, fyuA, fyuA/psn, ybtQ, ybtP, yagX/ecpC, yagW/ecpD, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, iroE, iutA, phoQ, rpoS, icmF/tssM, clpV/tssH, tssF, vgrG/tssI, vipB/tssC, ompA, vasE/tssK, tssG, KP1_RS17240, KP1_RS17345, KP1_RS17280, gndA, rfbK1, rfbD, ugd, KP1_RS17225, KP1_RS17340, mrkC, fimD, fimK, mrkD, fimH, rcsB, acrB, entF, fepA, entE, fes, entC, acrA, tufA,*
E35	Environment (Door handle)	AMP-CXM-CAZ-FEP-AMC-TZP	ST39		*sul1, drfA15, OqxA, OqxB, tet(D), fosA qacE*	N49S, L59V, L191S, F207W, A217S, N218H, D224E , L228V , E232R, T254S	I70M, I128M, N230G	*iutA, traT, , kdsA, rcsA, KP1_RS17240, (KP1_RS17345), gndA, rfbD, KP1_RS17220, KP1_RS17225, ugd, KP1_RS17340, acrB, yagX/ecpC, yagW/ecpD, acrA, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, rpoS, iroE, ompA, phoP, mrkH, mrkI,*
P60-1	Patient	AMP-CXM-CAZ-CRO-FEP-TZP-AMC-SXT	ST39	*bla*$_{TEM-1B}$, *bla*$_{DHA-1}$,	*sul1, drfA7, OqxA, OqxB ant(2'')-Ia ,aph(3')-Ia, aph(3'')-Ib , aph(6)-Id, qnrB4 tet(A),fosA,*	N49S, L59V, G189T, F198Y, F207Y, A217S, T222L,	I70M, I128M, N230G	*fyuA, irp1, irp2, iutA, fur, phoQ, OmpA, phoP, kdsA, rcsA, KP1_RS17240, gndA, KP1_RS17345, rfbD, KP1_RS17220, KP1_RS17225, ugd, KP1_RS17340, ga1F, vipB/tssC, icmF/tssM, clpV/tssH, tssF, vgrG/tssI, vasE/tssK, tssG, rpoS, mrkC,*

						D223G, E232R, N304E		*fimD, fimK, mrkD, fimH, yagX/ecpC, yagW/ecpD, yagV/ecpE), yagY/ecpB,acrB, acrA, iroE, , fyuA/psn, ybtQ, ybtP, entF, fepA, entE, fes, entC, tufA*
P121	Patient	AMP-CXM-CAZ-CRO-FEP-TZP-AMC-GEN-TOB-SXT	ST815	*bla*$_{SCO-1}$, *bla*$_{TEM-1B}$, *bla*$_{CTX-M-15}$, *bla*$_{SHV-172}$	*sul1, sul2, drfA15, OqxA, OqxB aac(3)-IIa,aph(6)-Id, aadA1 , qnrS1, aph(3'')-Ib, fosA, qacE*	N49S, L59V, L191S, F207W, A217S, N218H, D224E , L228V , E232R, T254S	I70M, I128M	*iutA, icmF/tssM, tssF, impA/tssA, tssG, iroE, fur, ompA, mrkC, fimD, fimK, acrB, kdsA, entF, fepA, entE, fes, entC, clpV/tssH, vgrG/tssI, vipB/tssC, vasE/tssK, yagX/ecpC, yagW/ecpD, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, rpoS, rcsB, KP1_RS17240, KP1_RS17220, KP1_RS17225, rfbA, KP1_RS17230, frbB, tufA, KP1_RS17345, KP1_RS17340, ga1F, KP1_RS17355, phoQ, phoP, gndA, ugd,*
P136	Patient	AMP-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-SXT	ST1552	*bla*$_{SCO-1}$, *bla*$_{TEM-1B}$, *bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$, *bla*$_{SHV-62}$,	*sul1, sul2, drfA15, OqxA, OqxB aac(6')-Ib-cr , aac(3)-IIa, aph(3'')-Ib , aph(6)-Id), aadA1 OqxA, aac(6')-Ib-cr , tet(D),fosA, qacE ,*	N49S, L59V, T86V, S89T. D91K, A93S, L191Q. F207W, A217S, N218H, Q227N, L228V, E232R, H235D, T254S	I70M, I128M	*fyuA, irp1, irp2, iutA, mchF,iroE, KP1_RS17240, gndA, KP1_RS17345, rfbD, ugd, KP1_RS17225, KP1_RS17340, ga1F, , fyuA/psn, ybtQ, ybtP, acrB, yagX/ecpC, yagW/ecpD, acrA, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, rpoS, fur, mrkC, fimD, mrkD, fimH, icmF/tssM, tssF, impA/tssA, tssG, sciN/tssJ, hcp/tssD, kdsA, entF, fepA, entE, fes, entC, phoQ, phoP, clpV/tssH,ompA, vasE/tssK, vgrG/tssI,*
P129	Patient	AMP-CXM-CAZ-CRO-FEP-TZP-AMC-GEN-TOB-CIP-SXT	ST464	*bla*$_{SHV-38}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*OqxA , OqxB, dfrA15, OqxB, sul1 aac(6')-Ib-cr, aac(6')-Ib-cr , qnrB2, qnrB19 ,aac(3)-IIa, fosA*	N49S, L59V, L191S, F207W, A217S, N218H, D224E , L228V , E232R, T254S, N304E	I70M, I128M	*fyuA, irp2, iutA, irp1, fyuA, fyuA/psn, ybtQ, ybtP, entF, fepA, entE, fes, entC, KP1_RS17240, KP1_RS17280, gndA, KP1_RS17345, rfbK1, rfbD, ugd, KP1_RS17225, KP1_RS17340, yagX/ecpC, yagW/ecpD, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, ompA, iroE, mrkC, fimD, fimK, mrkD, fimH, rpoS, acrB, icmF/tssM, clpV/tssH, vgrG/tssI, tssF, ompA, vasE/tssK, tssG, acrB, tufA, phoQ, phoP, vipA/tssB, vipB/tssC,*

P144	Patient	AMP-CXM-CAZ-CRO-TOB-CIP-SXT	ST636	*bla*$_{SHV-26}$, *bla*$_{SHV-98}$, *bla*$_{SHV-78}$, *bla*$_{SHV-194}$, *bla*$_{SHV-199}$, *bla*$_{SHV-179}$, *bla*$_{SHV-145}$	*OqxA, OqxB, fosA5*	N49S, L59V, T184P	I70M, I128M	*iutA, terC, traT, iroE, KP1_RS17240, gndA, KP1_RS17345, rfbD, ugd, KP1_RS17225, KP1_RS17340, ga1F, irp1, irp2, fyuA, fyuA/psn, ybtQ, ybtP, acrB, yagX/ecpC, yagW/ecpD, acrA, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, rpoS, fur, mrkC, fimD, mrkD, fimH, icmF/tssM, tssF, impA/tssA, tssG, sciN/tssJ, hcp/tssD, kdsA, entF, fepA, entE, fes, entC, phoQ, phoP, clpV/tssH, ompA, vasE/tssK, vgrG/tssI*
E34A	Environment (Dripstand)	AMP-CXM-CAZ-CRO-FEP-AMC-TZP-GEN-TOB-CIP-SXT	ST307	*bla*$_{SHV-106}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *bla*$_{OXA-1}$	*sul2, drfA14, OqxA, OqxB aac(3)-IIa , aac(6')-Ib-cr , aph(6)-Id , aph(3'')-Ib, qnrB1, tet(A), fosA,*	N49S, L59V, T184P	I70M, I128M, N230G	*fyuA, traT, irp1, irp2, iutA, IroE, KP1_RS17240, gndA, KP1_RS17345, rfbD,KP1_RS17220, ugd, KP1_RS17225, KP1_RS17340, ga1F, acrA, yagV/ecpE, yagY/ecpB, yagZ/ecpA, ykgK/ecpR,, fyuA/psn, ybtQ, ybtP, ompA, vipB/tssC, mrkC, fimD, fimK, icmF/tssM, clpV/tssH, vgrG/tssI, tssF, vipB/tssC, ompA, vasE/tssK, tssG, fur, entF, fepA, entE, fes, entC, tufA*
E55-1	Environment (Tap)	AMP-CXM-FEP-CAZ--AMC-TZP	ST1996	*bla*$_{SHV-41}$	*OqxA, OqxB, fosA5*	N49S, L59V, L191S, F207W, A217S, N218H, D224E , L228V , E232R, T254S	I70M, I128M	*iutA, traT, KP1_RS17280, KP1_RS17345, gndA, rfbK1, ugd, KP1_RS17340, ompA, acrB, yagX/ecpC, yagW/ecpD, acrA, yagV/ecpE, yagY/ecpB, iroE, kdsA, rpoS, fur, mrkC, fimD, fimK, mrkD, fimH, icmF/tssM, tssF, impA/tssA, tssG, sciN/tssJ, clpV/tssH, vgrG/tssI, vipB/tssC, vasE/tssK, entF, fepA, entE, fes, entC, phoQ, phoP, vgrG/tssI,*

AMK:Amikacin, CXM:Cefuroxime, DOR:Doripenem, FEP:Cefepime, CRO:Ceftriaxone, CAZ:Ceftazidime, CIP:Ciprofloxacin, AMC:Amoxicillin/clavulanic acid, SXT:Trimethoprim/Sulphamethoxazole, ERT:Ertapenem, GEN:Gentamicin, IPM:Imipenem, MEM:Meropenem, TZP:Piperacillin/Tazobactam, TOB:Tobramycin, TGC:Tigecycline

Mutations in outer membrane proteins

To determine the contribution of porins to cephalosporin resistance and the reduced susceptibility to carbapenems in the *K. pneumoniae* isolates (P121, P60-1 and E55-1), all isolates were further assessed for the presence of mutations in porins that could mediate carbapenem resistance in concert with ESBLs. Investigation of major outer membrane porins showed an intact *ompK35* gene in all the MDR *K. pneumoniae* strains from patients and hospital environments. However, numerous mutations associated with resistance to cephalosporins and reduced susceptibility to carbapenems were found in *ompK36* (N49S, L59V, T86V, S89T. D91K, A93S, L191Q. F207W, A217S, N218H, Q227N, L229V, E232R, H235D, T254S, T184P, G189T, F198Y, F207Y, A217S, T222L, D223G, N304E) and *ompK37* (I70M, I128M, N230G) genes in all isolates **(Table 2)**.

Mobile Genetic elements and environment of ARGs

PlasmidFinder revealed the presence of at least two different plasmid replicon types in all ten isolates from patients and hospital environments. The plasmids included IncFIA, IncFIB, IncFII, IncHI1B, IncQ1, Col and Col440II. IncFIB was the most abundant plasmid replicon occurring in nine (four carriage and five from the hospital environment) isolates. Different insertion sequences were found in the isolates. The insertion sequences, MITEPlu5, IS*CARN29* and IS*Sph8* were found together in two ST39 isolates (E4 and P60-1) from the hospital environment and patient **(Supplementary table S4)**.

Six isolates had class I integrons. Similar gene cassettes were found in two isolates (E50-2 and E55-1) from the hospital environment bearing the *dfrA14* gene. An Intl1 integrase was associated with *dfrA15* together with the sulphamethoxazole gene *sul1* and the quaternary ammonium compound resistant gene, *QacE* on gene cassettes in the carriage isolates P121 and P136 **(Table 3)**.

The *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{TEM-1}}$ genes were associated with a transposon, recombinase or insertion sequence. The *bla*$_{\text{CTX-M-15}}$ was particularly associated with the transposon IS1380 in the environmental isolates E4, E34A and E50-2 and in the carriage isolate P121. The *bla*$_{\text{TEM-1}}$ was often bracketed by a transposase and recombinase. In E4, E34A and E50-2 *bla*$_{\text{TEM-1}}$ was associated with the transposase IS91 and a recombinase. The *bla*$_{\text{SCO-1}}$ gene was also associated with a recombinase in the carriage isolates P121 and P136. The *bla*$_{\text{SHV}}$ gene was, however, chromosomal and not carried on any mobile element. The *bla*$_{\text{OXA-1}}$ genes were commonly associated with aminoglycoside hydrolyzing gene *aac(6')-Ib-cr* and the chloramphenicol acetyltransferase *catB3*. In the carriage isolate P136, *bla*$_{\text{OXA-1}}$ was associated with the IS6 transposase with a gene cassette similar to that of *K. pneumoniae* strain KPH3 plasmid. *aph(6)-Id: aph(3'')-Ib* were associated with

the Tn3 in the carriage isolate P121. Additionally, P121 harboured resistant genes which were associated with IskrA4, IS91 and Tn3 transposons and the intI1 integrase **(Supplementary table S5)**.

Table 3: Gene cassettes of ESBL *K. pneumoniae* isolates from patients and the hospital environment

Bacterial ID	Source	Integron	Integron name	GC1	GC2	GC3	GC4
E34A	Environment	Int1l	In191	dfrA14			
E35	Environment	Int1l	ln388	dfrA15	QacE	sul1	
P121	Patient	Intl1	ln388	dfrA15	aadA1	QacE	sul1
P136	Patient	Intl1	ln388	dfrA15	aadA1	QacE	sul1
E50-2	Environment	Intl1	ln191	dfrA14			
P129	Patient	Intl1	ln388	dfrA15	aadA1		

Virulome of ESBL *K. pneumoniae* isolates

A myriad of virulence genes involved in fimbriae synthesis (*fimH*, *mrkD*), capsular polysaccharide synthesis and synthesis regulation, genes involved in iron uptake system (*iroE*, *fes*, and *fur*) aerobactin, (*iutA*, *irp1*, *irp2* and *iucA*) yersiniabactin (*ybtA*, *ybtP*, *ybtQ*), and enterobactin *entBEF*) were present in the isolates **(Table 2)**. All isolates (both carriage and from the hospital environment) haboured the *iutA*, *gndA*, *ompA* factors. *entC/E*, *fes and fep* were found in four isolates from the hospital environment and five carriage isolates but were absent in an isolate (E35) from environment which lacked any β-lactamase or ESBL genes and was carbapenem resistant. *iroE* was found in nine isolates (four from the hospital environment and five carriage isolates). *fyuA*, *irp1* and *irp2* commonly occurred together in seven isolates (three hospital environment and four carriage isolates) **(Table 2)**. Though the isolates possessed a number of virulence genes,

hypervirulent *K. pneumoniae*, which are typically characterized by the presence of *roB*, *iucA*, *peg-344*, *rmpA*, and *rmpA2* genes (Russo et al., 2018) were not found.

Phylogenomics of the isolates

Phylogenomic analyses based on differences in single nucleotide polymorphisms (SNPs) together with core genome analyses of the metadata using Phandango, revealed the isolates were diverse. Isolates of ST39 (E50-2, E4, both from the hospital environment and P121, from patient) however grouped into one cluster with E55-1(ST1996), also from the hospital environment. P144(ST636) and E34A(ST307) from a patient and the hospital environment respectively were also similar though belonging to two different sequence types **(Figure 1)**. The *bla*$_{\text{CTX-M-15}}$ gene was present in two ST39 isolates, and isolates of ST815, ST1552, ST464 and ST307. The β-lactamases were frequently associated with plasmids, particularly, the IncF1B found in all STs with the exception of an ST39 isolate (E4). The integron, Int1l was found in six isolates **(Figure 1)**.

In order to determine clonal relatedness of the *K. pneumoniae* isolates, the isolates in this study were compared to other isolates from sub-Saharan Africa. The *K. pneumoniae* isolates from the hospital environment and patient carriage were also found to cluster based on sequence types with other genomes from countries in the Sub-Saharan Africa region indicating a wide dissemination of common STs such as ST307 and ST39 in Africa. The isolates clustered with similar isolates which had been isolated from clinical sources such as blood, urine, stool in patients with infections **(Figure 2)**.

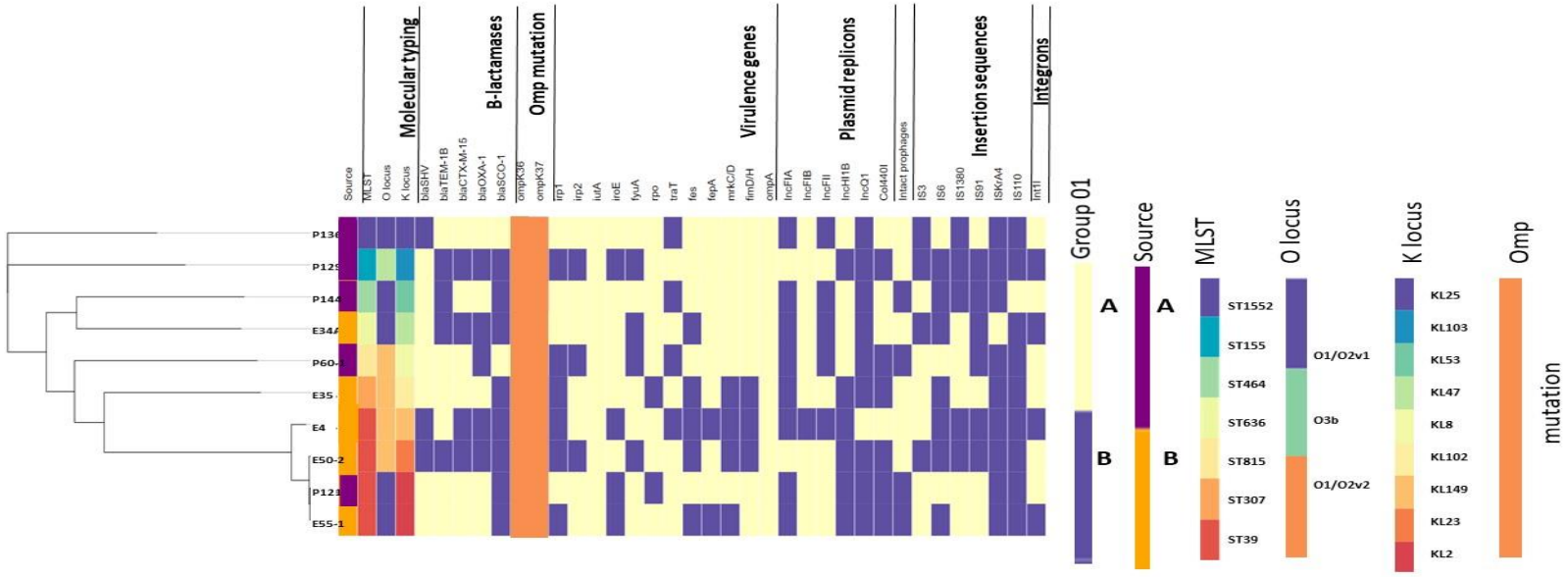

Figure 1: The core genome phylogenetic branch and metadata (source; WGS in-silico typing; β-lactamases, *ompK* mutation, plasmid replicons, integrons, insertion sequences, and intact prophages) coupled by the use of Phandango (https://github.com/jameshadfield/phandango/wiki) in MDR *K. pneumoniae* isolates (n = 10) from a teaching hospital in Ghana. The color codes for β-lactamases, plasmid replicons, integrons, insertion sequences and intact prophages (10) showed presence (yellow; A) and absence (blue; B) in the isolates. Color codes for source are patient (purple; A) and environment (orange;

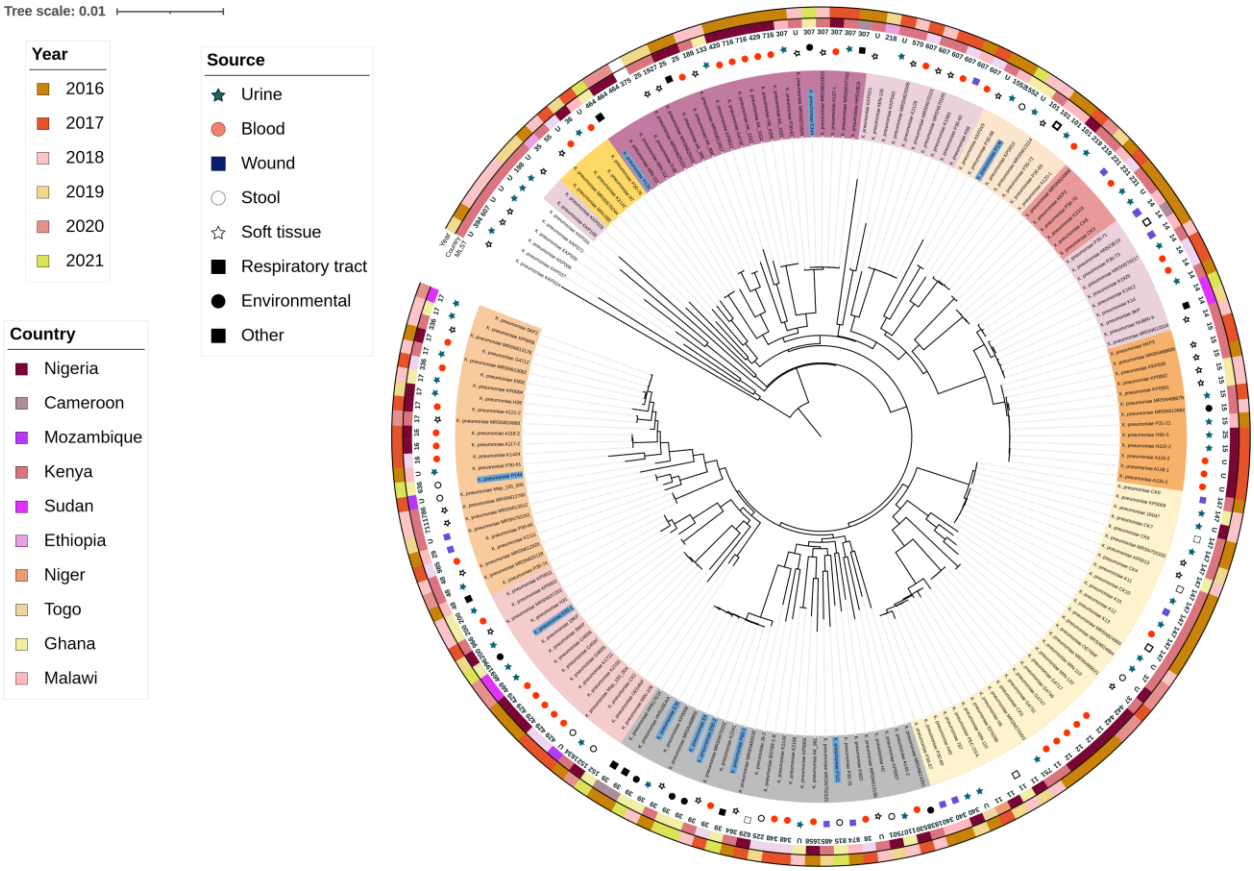

Figure 2: Maximum likelihood phylogenetic tree of *K. pneumoniae* isolates from humans between 2013 and 2022 in African countries. The core-genome phylogenetic tree was drawn from 181 genomes on BV-BRC and annotated using iTOL. The tree was built with *K. pneumoniae* Ecl8 as the reference genome and rooted with the reference strain. The following metadata are indicated: the year of isolation on the outer coloured ring, the country of isolation in the middle and the MLST in (isolates with unknown STs are indicated U) on the inner ring. The source of isolation of the isolates is indicated using shapes. Isolates from this study are labelled with a blue background.

DISCUSSION

The dissemination of antimicrobial resistant pathogenic bacteria possessing virulence factors poses a major threat to patient prognosis (Roe et al., 2019) and is thus a concern for IPC in hospitals. ESBL-producing and carbapenem resistant *K. pneumoniae* have become a serious concern in hospital and community-acquired infections (Baek et al., 2020; Legese et al., 2022).

Resistance genes were widely distributed among the *K. pneumoniae* isolates. β-lactamases commonly identified in both carriage isolates and isolates from hospital environments were *bla*$_{SHV}$ and *bla*$_{CTX-M}$ more especially, the *bla*$_{CTX-M-15}$ gene that is widespread among *K. pneumoniae* isolates (Aghamohammad et al., 2020; Founou et al., 2019; Pankok et al., 2022). These β-lactamases genes have been reported among *K. pneumoniae* isolates from South Africa and Spain, showing their widespread nature. *bla*$_{SHV}$ has been reported as commonly harboured by *K. pneumoniae* among carriage samples, confirming their constitutive chromosomal expression (Founou et al., 2019; Pérez et al., 2019). Both carriage isolates and isolates from the hospital environment additionally harboured the aminoglycoside and quinolone resistant genes *aac(6')-Ib-cr* and the multidrug efflux pumps *acrAB* and *OqxAB*. In association with the IncFII plasmid, these resistance mechanisms which are reported in other Enterobacterales (Wang et al., 2020) could also be circulated in the hospital environment and acquired by other bacteria leaving limited options for treatment of infections caused by these ESBL-producing *K. pneumoniae*.

The mutations (N49S, L59V, L191S, F207W, D224E, L228V, and E232R) in the *ompK36*, which have been previously associated with cephalosporin resistance (Piazza et al., 2022) coupled with the presence of ESBLs such as *bla*$_{CTX-M}$ may be implicated in the high resistance to cephalosporins evidenced by their increased MICs among both carriage *K. pneumoniae* and isolates from the environment (Supplementary Table S2).

The observed β-lactam, including cephalosporin resistance in the ESBL the *K. pneumoniae* isolates corroborates the report by (Humphries & Hemarajata (2017) of resistance of *K. pneumoniae* isolates to cephalosporins such as ceftazidime/avibactam associated with point mutations in *ompK36*. The mutations A217S, N218H in *ompK36* and I70M, I128M in *ompK37* reported to be associated with carbapenem resistance among *K. pneumoniae* isolates (Piazza et al., 2022), were observed in all the isolates, pointing to a developing carbapenem resistance in these ESBL- *K. pneumoniae* isolates. Yang et al also found numerous mutations in *ompK36* and *ompK37* but not in *ompK35* among all *K. pneumoniae* isolates resistant to Cefoperazone/sulbactam and piperacillin/tazobactam (Yang et al., 2022). The emerging carbapenem resistance in these *K. pneumoniae* isolates from both patients and the hospital environment, is a serious threat to IPC as they may be disseminated in the wards to cause infections which may be severe and difficult to treat. Hence there is the need for the enhancement of IPC to prevent the spread of these ESBL-producing isolates in hospitals.

The high number of virulence genes detected in both carriage and isolates from the hospital environment may contribute to the pathogenicity of the *K. pneumoniae* isolates (Parrott et al., 2021) in the colonized patients in the absence of adequate IPC practices. In this study, all the ESBL-*K. pneumoniae* isolates had the type 1 or 3 (*fim* or *mrk*) fimbrae comparable to 90% of clinical *K. pneumoniae* isolates which included ESBLs producers possessing fimbrae in a study of *K. pneumoniae* isolates from Egypt (El-Domany et al., 2021). Coupled with the different capsular serotypes, siderophores and high number of other virulence genes in the isolates, these fimbrae which are major adhesins may confer a survival advantage in these *K. pneumoniae* isolates as they are involved in bacterial colonization of host, biofilm formation and invasion (Karampatakis et al., 2023; Stahlhut et al., 2012). Though virulence genes associated with

hypervirulent *K. pneumoniae* were not found in the isolates in this study, hypervirulent *K. pneumoniae* have been identified among isolates with predominantly K1 and K2 capsules with the O1 O-antigen which have acquired the hvKP virulence plasmid (Russo & Marr, 2019) preseting the potential of isolates becoming hypervirulent if they acquire plasmids harbouring the hyper-virulence genes.

There was a high variation of STs among the isolates, showing the dissemination of different STs (ST39, ST307, ST815, ST1552, ST636, ST464 and ST1996) in the hospital. ST464 and ST636 were acquired by patients after admission and could have been circulating in the wards before patient admission, while the other STs (ST815 and ST1552) appeared to be circulating in the community. Notably, the high risk-clone ST307 was detected in one isolate from the hospital environment, suggesting the presence and possible dissemination of this global clone in Ghanaian hospitals. The ST307 which harboured the ESBL genes $bla_{\text{SHV-106}}$ and $bla_{\text{CTX-M-15}}$, and the β-lactamases $bla_{\text{TEM-1B}}$ and $bla_{\text{OXA-1}}$, compares to the ST307 among clinical *K. pneumoniae* isolates from South Africa, similarly resistant to aminoglycosides and fluoroquinolones however harbouring the carbapenemase gene, $bla_{\text{OXA-181}}$ in addition to other β-lactamases (Madni et al., 2021). The detection of this high-risk ST307 *K. pneumoniae* with β-lactamases in the hospital environment shows that it is disseminated between patients and the environment and calls for heightening of disinfection and IPC practices. To the best of the authors' knowledge, this study is the first to report ESBL *K. pneumoniae* belonging to ST307 in Ghana and calls for increased surveillance to enable the detection and control of such high-risk clones.

Phylogenetic analyses revealed a clustering of two *K. pneumoniae* isolates from the hospital environment (from a bed and dripstand) and one carriage isolate all belonging to ST39 together with an isolate from a tap (ST1996) in the Obstetrics and Gynaecology ward. Two of these ST39

isolates from hospital environments (a bed and a dripstand) in the same ward harboured the β-lactamases, *bla*$_{SHV}$ (*bla*$_{SHV-40}$, *bla*$_{SHV-85}$, *bla*$_{SHV-56}$, *bla*$_{SHV-79}$, and *bla*$_{SHV-89}$), *bla*$_{CTX-M-15}$ and same virulence genes suggesting an intra-ward circulation of the ESBL *K. pneumoniae* isolates. Notably, one ST39 isolate, E35 had no ESBLs nor any β-lactamases although phenotypically resistant to β-lactam antibiotics. This difference could possibly be attributed to other mechanisms such as the mutations in the *ompK36* porin or the presence of the efflux pumps *acrR, oqxA* and *oqxB* which were detected in the isolate, mediating resistance to the β-lactam antibiotics. In clinical settings, it is thus necessary to consider testing for other mechanisms beside the ESBLs, which are routinely tested, thus highlighting the importance of genomics in AMR surveillance. Though an ST39 isolate (P60-1) from a patient was acquired on admission, the source of acquisition could not be determined. However, the clonal relatedness of these MDR ST39 *K. pneumoniae* isolates with several ESBLs, *ompK36* mutations and virulence genes in association with plasmids and other MGEs could indicate a transfer of resistance and virulence genes among the isolates.

The ST39 clone has been previously reported in Ghana and was detected in *K. pneumoniae* isolates from a study which screened for plasmids in three *E. coli* and four *K. pneumoniae* isolates expressing ESBL mediated by the *bla*$_{CTX-M-15}$ gene from chronically infected wounds of Ghanaian patients (Pankok et al., 2022). The presence of the ESBLs in the isolates confirms the global reports of ST39 lineage of *K. pneumoniae*, which is known to be a carrier of ESBL genes notably CTX-M-15 and CTX-M-14 and has been associated with nosocomial infections (Fursova et al., 2020; Okomo et al., 2020; Villinger et al., 2022).

The dissemination of the high-risk clone ST39 *K. pneumoniae* has also been reported in infections and nosocomial outbreaks in sub-Saharan African countries; Congo, Ethiopia and The Gambia and also in China (Gala et al., 2023; Sewunet et al., 2021; Okomo et al., 2020; Du et al., 2016) and

poses a threat to patients in the hospital. Phylogenomic analyses showed a clustering of the isolates from this study with clinical isolates from other countries in sub-Saharan Africa indicating the potential of these carriage and hospital environment isolates to cause infections. There is the need for enhanced IPC practices, as patients could acquire these high-risk isolates from other patients and further disseminate them in the hospital environments.

Our study had some limitations, which includes WGS analysis on a selected few *K. pneumoniae* isolates. This study was conducted in a single tertiary hospital and is thus not representative of the epidemiology of *K. pneumoniae* isolates in Ghanaian hospitals. However, considering that the hospital serves patients from major hospitals in the Ashanti Region of Ghana and beyond, this study provides a snapshot of the state of ESBL-producing *K. pneumoniae* in hospitals in Ghana.

CONCLUSION

We have described the molecular epidemiology of MDR *K. pneumoniae* isolate, particularly of ompK mutations in combination with ESBLs and several other antibiotic resistance genes and virulence factors from hospital environments and patients. There was a dissemination of ST39 ESBL-producing *K. pneumoniae* isolates between patients and the hospital environment. Our findings re-emphasize the need for measures to contain ESBL-producing *K. pneumoniae* isolates, since their carriage in patients and dissemination in the environment of the Teaching Hospital may be an indication of an increased risk for transmission of these isolates in Ghanaian hospitals. There is the need for enhancement of infection prevention and control practices in the hospital to prevent further dissemination of these high-risk isolates and of their resistant genes.

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Table S1: Genome and assembly characteristics of sequenced *E. coli* isolates from patients and the hospital environment

Isolate ID	Source	Accession No.	Sequence Length	No. Of Contigs	GC Content %	Longest contig size (bp)	N50 value	L50 value
P60-1	Patient	SAMN27356690	5369680	85	57.4	315240	156243	12
P121	Patient	SAMN27356691	5385468	119	57.4	359829	152182	13
P136	Patient	SAMN27356692	5567722	120	57.1	431941	146906	13
P144	Patient	SAMN27356693	5666331	98	56.7	529950	205953	9
P129	Patient	SAMN27356700	5351798	96	57.4	548197	156054	10
E50-2	Dripstand	SAMN27356699	5539526	95	57.2	389286	192424	11
E4	Bed	SAMN27356686	5548621	95	57.2	453525	185184	11
E34A	Dripstand	SAMN27356687	5643793	89	57.1	496865	179681	11
E35	Door	SAMN27356688	5405239	111	57.5	305829	138484	13
E55-1	Tap	SAMN27356702	5373911	72	57.3	535663	238341	9

Table S2: Minimum inhibitory concentrations of antibiotics against ESBL *K. pneumoniae* isolates

ID	Site	Penicillin	Cephalosporins				β-lactamase inhibitors		Carbapenems				Aminoglycosides			FQ	Others	
		AMP	CXM	CAZ	CRO	FEP	AMC	TZP	DOR	ERT	IMI	MER	GEN	TOB	AMK	CIP	COT	TIG
P129	Hand	>=32	>=64	>=32	>=64	32	16	>=128	<=1	<=0.5	<=0.25	<=0.25	>=16	>=16	<=2	>=4	>=320	1
E34A	Dripstand	>=32	>=64	>=64	>=64	>=64	>=32	>=128	<=1	<=0.5	<=0.25	<=0.25	>=16	>=16	<=2	>=4	>=320	2
E4	Bed	>=32	>=64	>=32	>=64	>=16	>=32	>=128	<=1	<=0.5	1	<=0.25	>=16	>=16	<=2	>=4	>=320	<=0.5
E50-2	Dripstand	>=32	>=64	>=32	>=64	32	16	>=128	<=1	<=0.5	<=0.25	<=0.25	>=16	>=16	<=2	>=4	>=320	<=0.5
P121	Rectal	>=32	>=64	>=64	>=64	**32**	>=32	>=128	2	<=0.5	<=0.25	2	>=16	>=16	<=2	>=4	>=320	<=0.5
P136	Rectal	>=32	>=64	>=32	>=64	**32**	>=32	>=128	<=1	<=0.5	<=0.25	<=0.25	>=16	>=16	S	<=0.25	>=320	1
P60-1	Rectal	>=32	>=64	>=64	>=64	>=16	>=32	>=128	2	<=0.5	<=0.25	2	<=1	<=1	<=2	0.5	>=320	1
E35	Door handle	>=32	>=64	>=32	**>=64**	>=16	>=32	>=128	<=1	<=0.5	<=0.25	<=0.25	<=1	<=1	<=2	<=0.25	>=320	<=0.5
P144	Rectal	>=32	>=64	>=32	>=64	2	16	16	<=1	<=0.5	<=0.25	<=0.25	<=1	>=16	4	>=4	>=320	1
E55-1	Tap	>=32	16	>=32	<=1	>=16	>=32	>=128	2	<=0.5	<=0.25	4	<=1	<=1	<=2	<=0.25	<=20	<=0.5

The MIC profile is represented as an isolate being resistant (orange) or of intermediate resistance (gold) or susceptible (white) to the antibiotic indicated on the bottom column header (and the drug class it belongs to on the top column header). FQ-fluoroquinolone, O&G-Obstetrics and Gynaecology

AMK:Amikacin, CXM:Cefuroxime, DOR:Doripenem, FEP:Cefepime, CRO:Ceftriaxone, CAZ:Ceftazidime, CIP:Ciprofloxacin, AMC:Amoxicillin/clavulanic acid, SXT:Trimethoprim/Sulphamethoxazole, ERT:Ertapenem, GEN:Gentamicin, IPM:Imipenem, MEM:Meropenem, TZP:Piperacillin/Tazobactam, TOB:Tobramycin, TGC:Tigecycline

Table S3: Mobile genetic elements associated with antibiotic resistance

ID	Contig	Synteny of resistant genes	Plasmid/chromosomal sequence with closest nucleotide homology (accession number)
E4	58	CTX-M-15:IS1380(transposase)::recombinase:TEM-1:IS91transposase):APH(6)-Id:aph(3")-Ib:sul2:	*K. pneumoniae* isolate 307 genome, plasmid: P2 (OX030720.1)
	60	QnrB1::Tn3(transposase):transposase::recombinase:transposase	*K. pneumoniae* strain E17KP0053 plasmid pE17KP0053-2 (CP052219.1)
	70	IS3(transposase)::aac(3)-IIe:	*K. pneumoniae* strain EFN 299 plasmid p4 (CP092593.1)
	71	CatB3:oxa-1:AC(6')-Ib-cr5	*K. pneumoniae* strain KPH3 plasmid p1 (CP102553.1)
E34A	56	recombinase:TEM-1:IS91(transposase):APH(6)-Id:aph(3")-Ib:sul2:	*K. pneumoniae* strain 1159 plasmid pMB5730_1(CP103655.1)
	65	IS3(transposase)::aac(3)-IIe:	*K. pneumoniae* strain 197 plasmid pMB2966_1 (CP103730.1)
	67	catB3:oxa-1:AAC(6')-Ib-cr5	*K. pneumoniae* strain KPH3 plasmid p1(CP102553.1)
	64	IS1380(transposase):CTX-M-15	*K. pneumoniae* strain 2021CK-01815 plasmid unnamed1 (CP104374.1)
	54	QnrB1::Tn3(transposase):transposase:recombinase	*K. pneumoniae* strain 197 plasmid pMB2966_1 (CP103730.1)
	55	DfrA14:IntI1(integrase):	*K. pneumoniae* strain 197 plasmid pMB2966_1 (CP103730.1)
	58	tet(A):tetR(A):relaxase:Tn3(transposase):	*K. pneumoniae* isolate 392 genome, plasmid: P2 (OX030689.1)
E35	66	sul1:QacE:DfrA15:intI1(integrase):	*K. pneumoniae* strain Nord5-1_R48 plasmid pR48_2 (CP091591.1)
	72	catA1::Tn3(transposase)	*K. pneumoniae* strain 39427 plasmid pKPN39427.1 (CP054265.1)
P60-1	58	APH(6)-Id:APH(3")-Ib:sul2:	*K. pneumoniae* CC37 plasmid pCC37 (LC556212.1)
	34	tetR(A):tet(A)::EamA::Tn3(transposase):IS3(transposase):	*K. pneumoniae* strain FDAARGOS_447 plasmid unnamed3 (CP023950.1)
	68	transposase:TEM-1:recombinase	*K. pneumoniae* strain F17KP0040 plasmid pF17KP0040-1 (CP052139.1)

P121	49	IS3(transposase)::aac(3)-IIe:TEM-1:recombinase::IS1380(transposase):CTX-M-15::Tn3(transposase):QnrS1:recombinase:ISKra4(transposase):recombinase:	*K. pneumoniae* strain F16KP0096 plasmid pF16KP0096-1 (CP052151.1)
	64	IS6(transposase):::sul1:QacE:aadA1:dfrA15:intI1(integrase):recombinase:Tn3(transposase):	*K. pneumoniae* isolate BB1465 genome, plasmid:pKP-CTX-M-15_(LR822059.1)
	66	blaSCO-1:recombinase	Klebsiella sp. PO552 plasmid p1 (CP037442.1)
	73	sul2:IS91(transposase):::recombinase	*K. pneumoniae* strain Kpn223 plasmid pKPN-065 (CP015026.1)
	81	Tn3(transposase):APH(6)-Id: aph(3")-Ib	*K. pneumoniae* strain E16KP0102 plasmid pE16KP0102-2 (CP052311.1)
P136	77	Tn3(transposase):APH(6)-Id:aph(3")-Ib	*K. pneumoniae* strain CriePir26 plasmid unnamed1 (CP062987.1)
	63	blaSCO-1:recombinase	*K. pneumoniae* strain E17KP0027 plasmid pE17KP0027-1 (CP052240.1)
	88	catB3:oxa-1:AAC(6')-Ib-cr5:IS6(transposase)	Escherichia coli O25b:H4-ST131 strain U1 plasmid pU1 (MK295825.1)
	73	sul2::IS91(transposase)::recombinase	*K. pneumoniae* strain Kpn223 plasmid pKPN-065 (CP015026.1)
	62	Tn3(transposase):IS6(transposase)::sul1:QacE:aadA1:dfrA15:intI1(integrase:recombinase:Tn3(transposase)	*K. pneumoniae* strain 913 plasmid pMB2930_1 (CP103732.1)
E50-2	78	catB3:oxa-1: AAC(6')-Ib-cr5	*K. pneumoniae* strain KPH3 plasmid p1 (CP102553.1)
	57	CTX-M-15::IS1380(transposase)::recombinase:TEM-1:IS91(transposase):APH(6)-Id:APH(3")-Ib:sul2:IS110(transposase)	*K. pneumoniae* strain 197 plasmid pMB2966_1 (CP103730.1)
	62	QnrB1::Tn3)transposase):recombinase:Tn3(transposase)	*K. pneumoniae* strain E17KP0053 plasmid pE17KP0053-2 (CP052219.1)
	75	IS3(transposase)::aac(3)-Iie:	*K. pneumoniae* strain KPH3 plasmid p1 (CP102553.1)
	64	IS6(transposase):mobC(relaxome):dfrA14:IntI1(integrase)	*K. pneumoniae* strain 197 plasmid pMB2966_1(CP103730.1)
P129	55	AadA1:dfrA15:intI1(integrase):recombinase:Tn3(transposase):IS110(transposase):	*K. pneumoniae* strain CriePir26 plasmid unnamed1(CP062987.1)
	70	IS3(transposase)::aac(3)-Iie:	*K. pneumoniae* strain KPH3 plasmid p1(CP102553.1)
	71	catB3:oxa-1:AAC(6')-Ib-cr5	*K. pneumoniae* strain KPH3 plasmid p1(CP102553.1)

Table S4: Chromosomal point mutations of MDR *K. pneumoniae* isolates

ISOLATE ID	SOURCE	gyrA	gyrB	parC	parE	acrR
P129	Patient	S83Y, D87A	None	S80I	None	P161R, G164A, F172S, R173G, F204L
P60-1	Patient	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
P121	Patient	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
P136	Patient	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
P144	Patient	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
E50-2	Environment	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
E55-1	Environment	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
E4	Environment	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
E34A	Environment	S83I	None	S80I	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M
E35	Environment	None	None	None	None	P161R, G164A, F172S, R173G, L195V, F197I, K201M

Table S5: Serotype and mobile genetic elements of MDR *K. pneumoniae* isolates

ID	Serotype		Plasmid	Insertion sequences	Prophages
	O locus	K locus			
E4	O1/O2v1	KL2	IncFIB, IncFII	MITEPlu5, ISNarch1, ISCARN29, ISSph8	None
E34A	O1/O2v2	KL102	IncFIB, IncFII	ISCsr4, ISEch7, ISRor8, ISKpn21	Salmon_118970_sal3, Entero_mEp237, Klebsi_3LV2017
E35	O1/02v2	KL23	IncFIA, IncFIB, IncFII	MITEEc1, TnXax1, ISButh2, ISAusP1	Salmon_vB_SosS_Oslo, Pseudo_phiPSA1
P60-1	O1/O2v2	KL149	Col440, IncQ1	MITEPlu5, ISCARN29, ISSph8, ISAs6	Entero_HK140
P121	O1/O2v2	KL8	IncFIB, IncHI1B	MITEYpE1, MITEEc1, TsAn2, ISStma11	None
P136	O1/O2v1	KL25	Col440I, Col440II, IncFIB, IncHI1B	ISSty2, IS1230B, IS3F, ISeAl1	Klebsi_ST512_KPC3phi13.2, Entero_mEp390
P144	O1/O2v1	KL47	Col, IncFIB, IncHI1B	ISRor8, ISKqu3, ISSen13, ISPa40	Klebsi_ST15_OXA48phi14.1, Salmon_118970_sal3, Salmon_SSU5
E50-2	O1/O2v1	KL2	IncFIB, IncFII	ISkqu3, IS609, TnShfr1, ISArp14	None
P129	O1/O2v1	KL53	Col, IncFIB, IncHI1B	ISEhe4, ISKpn80, ISKpn34, ISRaq1	None
E55-1	O3b	KL103	IncFIA, IncFIB, IncFII	ISEc14, ISEam1, ISEc24, ISEc15	Cronob_ENT47670

CHAPTER FIVE- CONCLUSION

This study undertook the molecular characterization of multi-drug resistant (MDR) Gram-negative bacterial pathogens from hospital environments, patients and HCWs in a teaching hospital in Ghana.

5.1 Conclusion

The following were the main findings from the study according to the study objectives.

Objectives 1 and 2: To recover GNB from patients rectal and hand swabs, hands of healthcare workers and ward environments (sinks, floor, drip stands, door handles and high touch surfaces in three specialties; Intensive Care Unit, Surgery and Obstetrics and Gynaecology of the Teaching Hospital. To identify GNB from patients, healthcare workers hands and hospital environment using Vitek® 2 System

- Five hundred and eighty-five samples were collected from patients, healthcare workers and the hospital environment within the study period. From these, MDR GNB were isolated from 5.26% (6/114) of healthcare workers hand swabs, 38.29% (81/211) of swabs collected from patients and 12.01% (25/208) of environmental swabs. Fifteen (60%) MDR GNB isolates from the hospital environment were from beds, five (20%) from tap handles, four (16%) from dripstands and one (4%) from a door handle.
- *Escherichia coli* and *Klebsiella pneumoniae* were the most frequently isolated MDR GNB from all samples collected and were predominantly from the Obstetrics and Gynaecology directorate.

Objective 3: To determine the prevalence and incidence of colonization due to GNB in the patients on admission and during hospital stay respectively in the three directorates.

- The overall MDR GNB rectal colonization rate among patients was 50.62% at admission and 44.44% after 48 hours of admission.

- Previous hospitalization (p-value = 0.021, OR,95% CI= 7.170 (1.345-38.214) was significantly associated with colonization by MDR GNB after 48 hours of admission. Age (21-30 years) (p-value =0.022, OR, 95% CI =0.103(0.015-0.716) was significantly identified as a protective factor associated with a reduced risk of rectal MDR GNB colonization.

Objective 4: To ascertain the phenotypic characteristics of GNB isolates from, patients, HCW's and hospital environment by MIC determinations according to CLSI/EUCAST against a panel of 17 antibiotics (ampicillin, cefuroxime, ceftazidime, ceftriaxone, cefipime, amoxicillin/clavulanic, doripenem, ertapenem, meropenem, imipinem, piperacillin/tazobactam, gentamicin, tobramycin, amikacin, ciprofloxacin, trimetoprim-sulphamethoxazole and tigecycline).

- Isolates from patients (81.8%), HCW's hands (81.3%) and the hospital environment (68.6%) were highly resistant to ampicillin. However, all isolates were susceptible to amikacin (100%) and tigecycline (99.15%) with the exception of an *E. cloacae* isolate from a HCW's hand which was resistant to tigecycline. A few isolates from patients (17.7%), the hospital environment (19.6%) and HCW's (12.5%) exhibited resistance to the carbapenems.
- Different antibiogram patterns were observed among MDR GNB isolates from patients, HCW's and the environment. Twenty antibiograms were common to isolates from patients

and environments or HCW's. The most common antibiogram was AMP-CXM-CAZ-CRO-FEP-GEN-TOB-AMC-TZP-CIP-SXT which was observed among thirteen *E. coli* isolates from patients and environments.

- In the Obstetrics and Gynaecology directorate, 43.30% of GNB were MDR. Similarly, 56% and 66.67% of GNB from the surgery directorate and ICU, respectively, were MDR.

Objective 5: To genotypically delineate antibiotic resistance mechanisms and analyze the relationship between isolates from environmental samples, patients and HCWs hands by whole genome sequencing (WGS) and bioinformatics analysis.

- ESBL-producing *E. coli* rectal carriage and acquisition among patients were 13.65% and 11.32% respectively. Of the 23 whole genome sequences, *bla*$_{\text{TEM-1B}}$ and *bla*$_{\text{CTX-M-15}}$ were commonly associated with IncFIB plasmid replicons and co-occurred with aminoglycoside, macrolide, and sulfamethoxazole/trimethoprim resistance.
- Insertion sequences, transposons, and class I integrons were found with *bla*$_{\text{CTX-M-15}}$. The *E. coli* isolates from carriage and hospital environments carried multiple virulence genes, with *terC* being the most prevalent.
- Sequence types (STs) of the *E. coli* isolates were diverse and included a novel ST (ST13846) which was observed among two isolates.
- The *bla*$_{\text{SHV,}}$ was the most dominant β-lactamase gene among ten (10) ESBL-producing *K. pneumoniae* isolates sequenced. These included the ESBLs *bla*$_{\text{SHV-38,}}$ *bla*$_{\text{SHV-40,}}$ *bla*$_{\text{SHV-98,}}$ *bla*$_{\text{SHV-106}}$ and *bla*$_{\text{SHV-145.}}$ One carriage isolate also harboured seven blaSHV genes (*bla*$_{\text{SHV-26}}$, *bla*$_{\text{SHV-98}}$, *bla*$_{\text{SHV-78}}$, *bla*$_{\text{SHV-194}}$, *bla*$_{\text{SHV-199}}$, *bla*$_{\text{SHV-179}}$ and *bla*$_{\text{SHV-145}}$).

- *bla*$_{\text{CTX-M-15}}$ was also commonly observed in the isolates in association with genes encoding resistance to fluoroquinolone, aminoglycosides, sulphamethoxazole/trimethoprim.
- Mutations were found in both *ompK36* and *ompK37* in all *K. pneumoniae* isolates (both carriage isolates and isolates from the hospital environment) and together with β-lactamases were found to possibly mediate resistance to cephalosporins and developing resistance to carbapenems.
- The *K. pneumoniae* isolates were diverse and belonged to different STs with the ST39 being the most commonly observed sequence type in four isolates (three hospital environment isolates and one patient isolate).
- A myriad of virulence genes, including *irp1*, *irp2*, *iutA*, *gndA*, *ompA*, *fes*, *fep*, *mrkD* and *fimH* were detected in both carriage and environmental isolates. IncFIB was the most abundant plasmid replicon occurring in nine isoaltes (four carriage and five from hospital environments).
- ESBL *K. pneumoniae* isolates appeared to be introduced into the hospital from the community.

Objective 6: To determine the clonal and phylogenomic relationships of MDR GNB isolated from patients, HCWs' hands and the hospital environments and the clonal lineages of the MDR GNB isolated from the different wards in the hospital using WGS and bioinformatics tools.

- Phylogenetic analysis grouped the *E. coli* isolates into four main clusters. High genetic relatedness was observed between two carriage isolates of ST940 and between a carriage isolate and an environmental isolate belonging to ST648. Isolates with different STs, collected at different times and locations, also showed genetic similarities.

- Clonal relatedness was observed among ESBL-producing *K. pneumoniae* isolates from patients and the hospital environment, as well as between different patients, suggesting a possible transmission within and between sources. A clonal relationship was established among a carriage isolate and two environmental isolates of ST39 together with an ST1996 environmental isolate.
- Infection prevention and control practices need to be enhanced to prevent the dissemination and transmission of these resistant strains in the hospital.

5.2 Limitations

The main limitation of this study is the relatively small number of isolates investigated and the short duration of study of three months which made it difficult to assess long-term effects of colonization of patients with MDR GNB.

The results cannot be generalized to all hospital settings in Ghana since the study was carried out at a single centre. Nonetheless, these interesting findings from the tertiary hospital which is a referral centre for most secondary and primary facilities in about four regions of Ghana, provides a snapshot of the situation in Ghana.

Recommendations

Screening and surveillance for MDR GNB in patients, health care workers and the hospital environment should be routinely implemented, especially in high-risk wards in hospitals to monitor the emergence and spread of drug-resistant pathogens.

Infection prevention and control practices should be heightened to contain the spread of drug-resistant bacteria in Ghanaian hospitals.

Multi-center studies are needed to determine the colonization of patients, carriage by HCW's hands and contamination of hospital environments with MDR GNB on a larger scale for situational analysis.

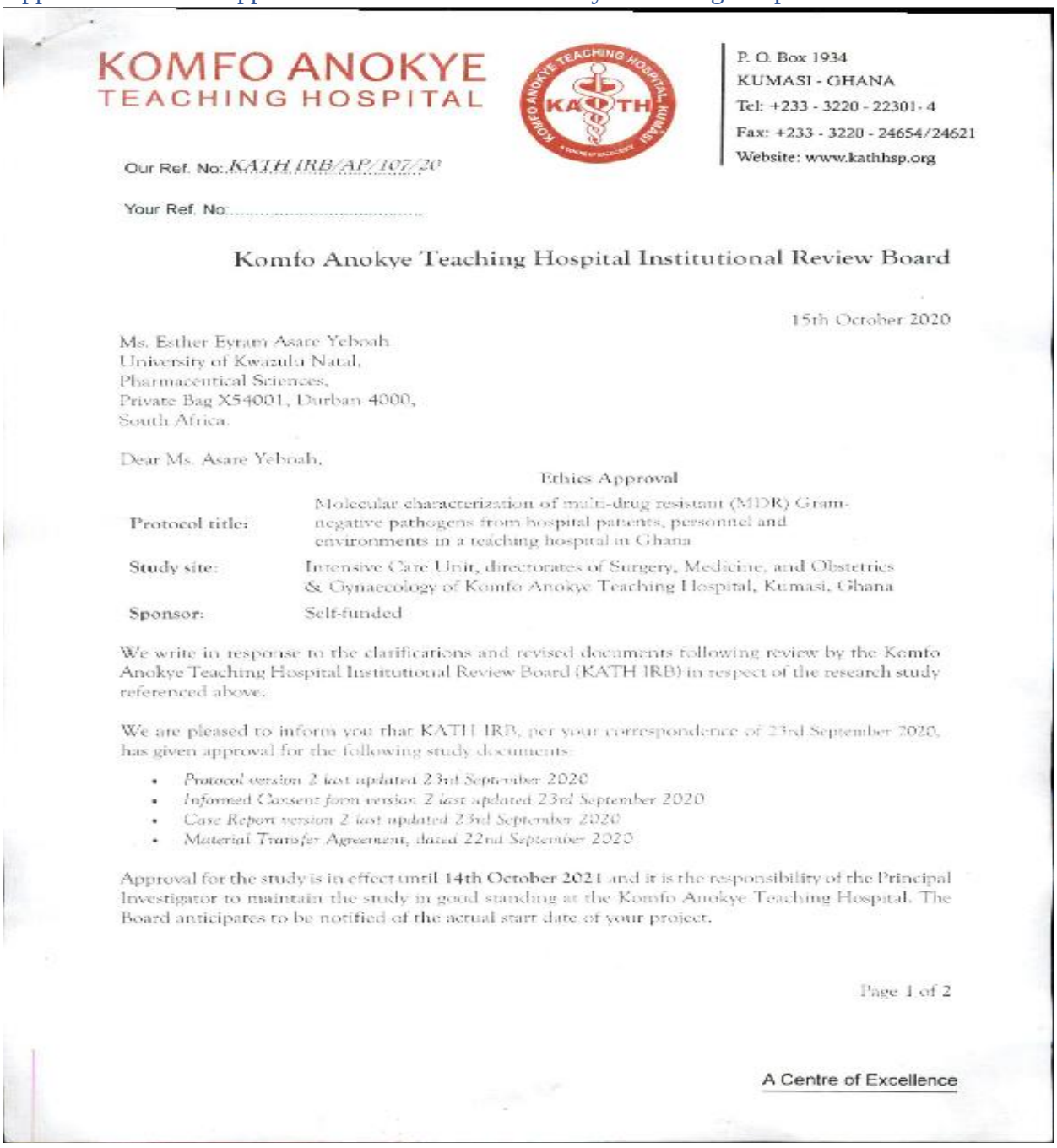
There is the urgent need to design policies to promote the rational use of antibiotics in Ghana and to control the spread of AMR in Ghana.

Significance of study

This study revealed a high prevalence of MDR GNB colonization in patients. Carriage on healthcare workers hands and contamination of hospital environments with MDR GNB though minimal could be disseminated in hospitals. *E. coli* and *K. pneumoniae* were most frequently isolated among the MDR GNBs. A clonal relationship was found within and between isolates from patients and hospital environments presenting the potential for the dissemination of MDR GNB between patients, HCWs and hospital environments in the absence of efficient infection prevention and control practices. Screening for MDR GNB should be implemented in Ghanaian hospitals, particularly in high-risk wards to enable the early detection of drug-resistant starins in order to prevent and contain the spread of these infectious pathogens in hospitals.

APPENDICES

Appendix 1: Ethics Approval letter from Komfo Anokye Teaching Hospital



KOMFO ANOKYE
TEACHING HOSPITAL

P. O. Box 1934
KUMASI - GHANA
Tel: +233 - 3220 - 22301- 4
Fax: +233 - 3220 - 24654/24621
Website: www.kathhsp.org

Our Ref. No: KATH IRB/AP/107/20

Your Ref. No:.....

Komfo Anokye Teaching Hospital Institutional Review Board

15th October 2020

Ms. Esther Eyram Asare Yeboah
University of Kwazulu Natal,
Pharmaceutical Sciences,
Private Bag X54001, Durban 4000,
South Africa.

Dear Ms. Asare Yeboah,

Ethics Approval

Protocol title:	Molecular characterization of multi-drug resistant (MDR) Gram-negative pathogens from hospital patients, personnel and environments in a teaching hospital in Ghana
Study site:	Intensive Care Unit, directorates of Surgery, Medicine, and Obstetrics & Gynaecology of Komfo Anokye Teaching Hospital, Kumasi, Ghana
Sponsor:	Self-funded

We write in response to the clarifications and revised documents following review by the Komfo Anokye Teaching Hospital Institutional Review Board (KATH IRB) in respect of the research study referenced above.

We are pleased to inform you that KATH IRB, per your correspondence of 23rd September 2020, has given approval for the following study documents:

- *Protocol version 2 last updated 23rd September 2020*
- *Informed Consent form version 2 last updated 23rd September 2020*
- *Case Report version 2 last updated 23rd September 2020*
- *Material Transfer Agreement, dated 22nd September 2020*

Approval for the study is in effect until **14th October 2021** and it is the responsibility of the Principal Investigator to maintain the study in good standing at the Komfo Anokye Teaching Hospital. The Board anticipates to be notified of the actual start date of your project.

Page 1 of 2

A Centre of Excellence

Appendix 2: Ethics approval letter from BREC- UKZN

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

17 November 2020

Mrs Esther Eyram Asare Yeboah (214584425)
School of Health Sciences
Westville

Dear Mrs Yeboah,

Protocol reference number: BREC/00001917/2020
Project title: Molecular epidemiology of multi-drug resistant (MDR) Gram-negative pathogens from hospital patients, personnel and environments in a teaching hospital in Ghana.
Degree: PhD

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 17 November 2020. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations dated 10th November 2020, see (http://research.ukzn.ac.za/Libraries/BREC/BREC Lockdown Level 1 Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 17 November 2020. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 08 December 2020.

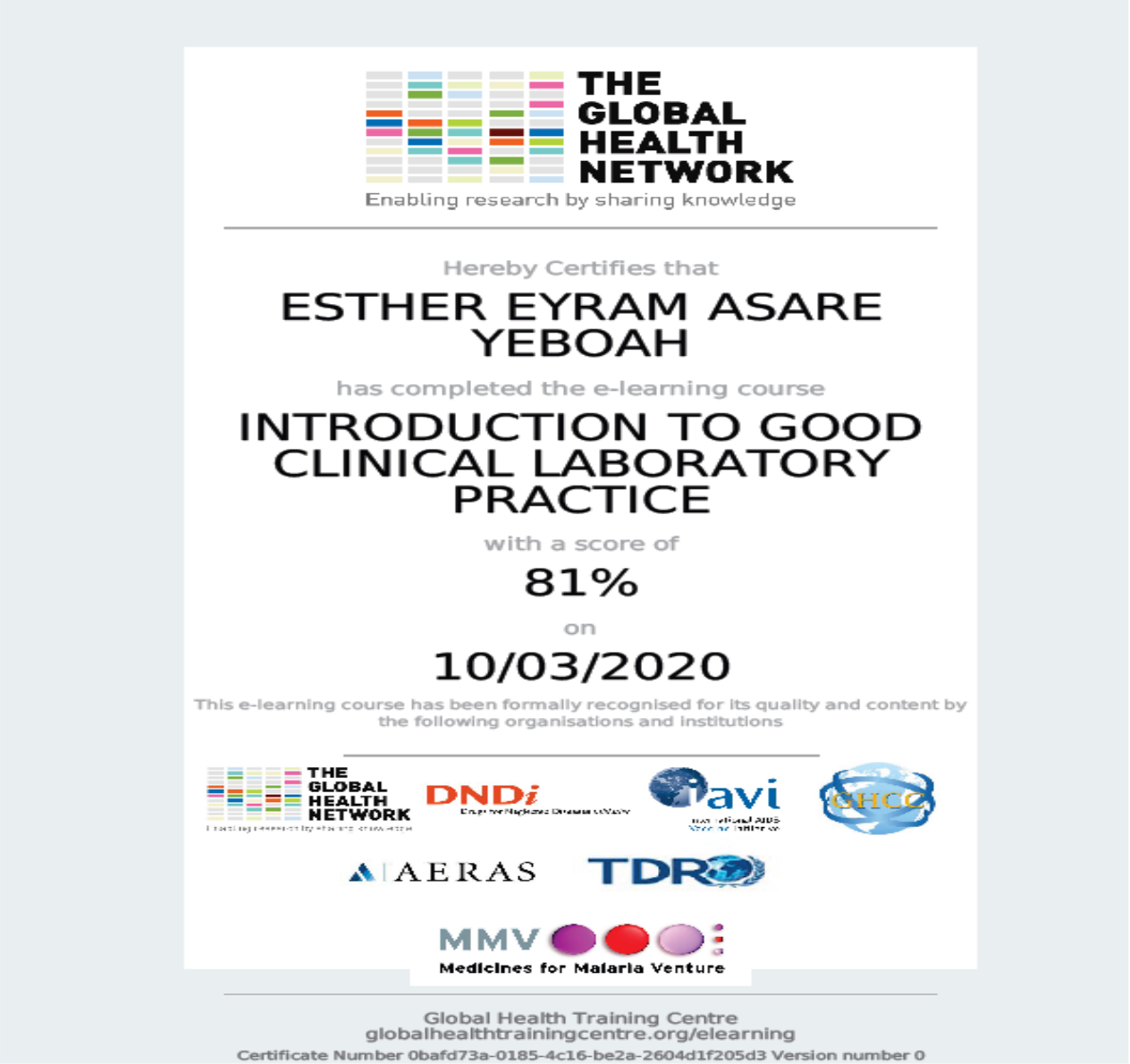
Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Appendix 3: Introduction to Good Clinical Laboratory Practice certificate



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Hereby Certifies that

ESTHER EYRAM ASARE YEBOAH

has completed the e-learning course

INTRODUCTION TO GOOD CLINICAL LABORATORY PRACTICE

with a score of

81%

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This e-learning course has been formally recognised for its quality and content by the following organisations and institutions

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Certificate Number 0bafd73a-0185-4c16-be2a-2604d1f205d3 Version number 0

Appendix 4: TRREE training certificates

TRREE

Zertifikat
Certificat
Certificado
Certificate

Promouvoir les plus hauts standards éthiques dans la protection des participants à la recherche biomédicale
Promoting the highest ethical standards in the protection of biomedical research participants

Clinical Trials Centre
The University of Hong Kong

Certificat de formation - Training Certificate

Ce document atteste que - this document certifies that

Esther Eyram Asare Yeboah

a complété avec succès - has successfully completed

Introduction to Research Ethics

du programme de formation TRREE en évaluation éthique de la recherche
of the TRREE training programme in research ethics evaluation

[redacted]

Release Date: 2020/03/10

CID : P6NV9dEv7

Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

FMH Continuing Education Program (5 Credits)
Programme de Formation continue (5 Crédits)

Foederatio Pharmaceutica Helvetiae FPH Programmes de formation continue
Continuing Education Programme
Programmes de formation continue

Ce programme est soutenu par - This program is supported by :

European and Developing Countries Clinical Trials Partnership (EDCTP) (www.edctp.org) - Swiss National Science Foundation (www.snf.ch) - Canadian Institutes of Health Research (http://www.cihr-irsc.gc.ca/e/2691.html) - Swiss Academy of Medical Science (SAMS/ASSM/SAMW) (www.samw.ch) - Commission for Research Partnerships with Developing Countries (www.kfpe.ch)

[RDV : 20170310]

TRREE

Zertifikat
Certificat
Certificado
Certificate

Promouvoir les plus hauts standards éthiques dans la protection des participants à la recherche biomédicale
Promoting the highest ethical standards in the protection of biomedical research participants

Clinical Trials Centre
The University of Hong Kong

Certificat de formation - Training Certificate

Ce document atteste que - this document certifies that

Esther Eyram Asare Yeboah

a complété avec succès - has successfully completed

Research Ethics Evaluation

du programme de formation TRREE en évaluation éthique de la recherche
of the TRREE training programme in research ethics evaluation

[redacted]

Release Date: 2020/04/30
CID : 2nMKPY6d

Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

FMH Continuing Education Program (5 Credits)
Programme de Formation continue (5 Crédits)

Fédération Pharmaceutique Helvétique FPH Programmes de formation continue
Continuing Education Programme
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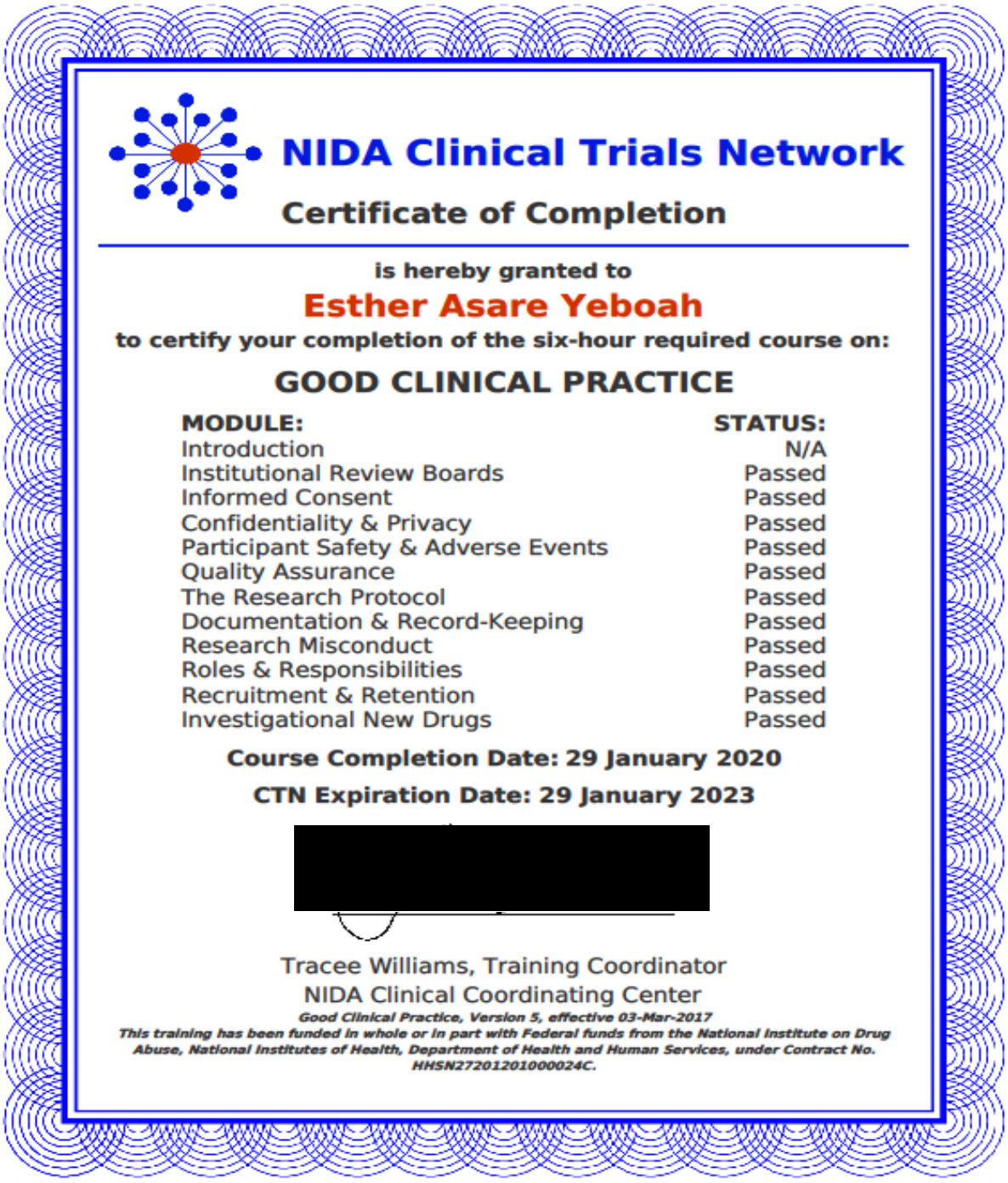
[REV : 20170310]

Appendix 5: Good Clinical Practice certificate

PhD/Pharm/CHS/Version 1 05/08/2020



NIDA Clinical Trials Network

Certificate of Completion

is hereby granted to

Esther Asare Yeboah

to certify your completion of the six-hour required course on:

GOOD CLINICAL PRACTICE

MODULE:	STATUS:
Introduction	N/A
Institutional Review Boards	Passed
Informed Consent	Passed
Confidentiality & Privacy	Passed
Participant Safety & Adverse Events	Passed
Quality Assurance	Passed
The Research Protocol	Passed
Documentation & Record-Keeping	Passed
Research Misconduct	Passed
Roles & Responsibilities	Passed
Recruitment & Retention	Passed
Investigational New Drugs	Passed

Course Completion Date: 29 January 2020

CTN Expiration Date: 29 January 2023

[redacted]

Tracee Williams, Training Coordinator
NIDA Clinical Coordinating Center

Good Clinical Practice, Version 5, effective 03-Mar-2017

This training has been funded in whole or in part with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272012010000024C.

Appendix 6: Informed consent form and study participant information document

Appendix 6.1: Informed consent form and patient information document

CONSENT FORM

Infections are caused by small living things/microorganisms called bacteria and many different bacteria may cause the same infection at different times.

We treat the infection with antibiotics, medicine that will kill the bacteria and make you better.

The best way to do this is to find out what kind of bacteria may cause or maybe causing your infection, to test many different antibiotics against the bacteria and then make sure that the medicine the doctor gave you is the right one to kill the bacteria that is causing your infection.

For us to be able to do this, we need your permission to take samples from your hands and rectal area. The doctor/nurse who is highly qualified to this will take the sample.

If you are diagnosed of an infection, we will need to isolate bacteria from the sample collected and processed at the diagnostic laboratory.

We also need some information from your medical records to fill out a questionnaire. This will be done by the research assistant who is trained to do this and legally and ethically bound to ensure that all your personal details will be kept confidential.

Your information will help us to learn which antibiotic is the best for different kinds of infections in different parts of the body and other patients with the same type of infection will benefit from your participation.

Your participation is completely voluntary, i.e., you don't have to participate in this study if you don't want to. Your hospital care and medical/drug treatment will not be jeopardized if you choose not to participate.

You can withdraw from the study at any time, again with no impact on your hospital care and medical/drug treatment at the hospital.

If you kindly agree to participate, we need you to sign this consent form giving us permission to take samples from your infection site and taking information from your medical records.

Please do not hesitate to contact the researcher on 233249533781 or the Institutional Review board (KATH) and the UKZN Biomedical Research Ethics Committee (contacts as stated below) should you have any queries or concerns related to your voluntary participation:

Institutional Review Board
Research and Development Unit
Komfo Anokye Teaching Hospital
P.O. Box 1934

Kumasi, Ghana.
Tel: 233 266083585 or 233 556490029
Email: kathirb25@gmail.com, kathirb@kathhsp.org

Biomedical Research Ethics Administration
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, South Africa
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:
I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I ——————————— (Name of Witness) certify that information given to

_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

SAMPLE COLLECTION SHEET- PATIENTS

PLEASE RECORD ALL INFORMATION AS AT TIME AND DATE OF SPECIMEN COLLECTION	
Identifier	
Patient Number	
Patient Name	
Ward	
Date of Birth/Age	
Gender	Male Female
Date of Admission	
Hospital Type	
Ward Type	
Date of Specimen Collection	
	Rectal swab
Diagnosis	respiratory tract infection gastro-intestinal infection urinary tract infection skin infection nosocomial infection Other - list
Co-morbidity	HIV/AIDS Diabetes Hypertension Cardiac Asthma
Invasive Procedures	Urinary catheter IV line Central venous/arterial line Intrathecal line Endotracheal - ventilator

	Endotracheal - tracheotomy tube Nasogastric tube Blood transfusion Surgical	
Previous Hospitalization	No Yes - in the last week Yes - in the last month Yes - in the last 3 months	**1 2 3 4**
Previous Antibiotic Treatment	No Yes - in the last week Yes - in the last 2 weeks Yes - in the last 3 weeks	
Duplicate Isolate Screen	Same patient Identical isolates Within 5 days	

SAMPLE COLLECTION SHEET – ENVIRONMENT

PLEASE RECORD ALL INFORMATION AS AT TIME AND DATE OF SPECIMEN COLLECTION	
Identifier	
Ward	
Other wards Hospital Type Ward Type	**1 2 3 4**
Date of Specimen Collection	

Specimen Source	Bed Drip stand Table Sink Tap handle Door handle Other
Duplicate Isolate Screen	

Appendix 6.2: Informed consent form and staff information document

CONSENT FORM

Infections may be caused by bacteria and many different bacteria may cause the same infection at different times.

We treat the infection with antibiotics, medicine that will kill the bacteria and make patients better.

The best way to do this is to find out what kind of bacteria may cause infections, to test many different antibiotics against the bacteria and then make sure that the antibiotics are active against the bacteria. We will also be able to determine the kind of bacteria circulating in the hospital environment.

For us to be able to do this, we need your permission to take samples from your hands. A study nurse/laboratory technician who is highly qualified to this will take the sample.

We also need some information from you to fill out a questionnaire. This will be done by the research assistant who is trained to do this and legally and ethically bound to ensure that all your personal details will be kept confidential.

Your information will help us to learn which kind of bacteria may be circulating in the wards and also to determine which antibiotics are active against them. This is important for Infection Control and patients with similar bacteria species will benefit from your participation.

Your participation is completely voluntary, i.e., you don't have to participate in this study if you don't want to.

You can withdraw from the study at any time, again with no impact on your hospital care and medical/drug treatment at the hospital.

If you kindly agree to participate, we need you to sign this consent form giving us permission to take samples from your infection site and taking information from your medical records.

Please do not hesitate to contact the researcher on 233249533781 or the Institutional Review board (KATH) and the UKZN Biomedical Research Ethics Committee (contacts as stated below) should you have any queries or concerns related to your voluntary participation:

Institutional Review Board
Research and Development Unit
Komfo Anokye Teaching Hospital
P.O. Box 1934
Kumasi, Ghana.
Tel: 233 266083585 or 233 556490029
Email: kathirb25@gmail.com, kathirb@kathhsp.org

Biomedical Research Ethics Administration
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, South Africa
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:
I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

WITNESS' SIGNATURE _____

SAMPLE COLLECTION SHEET- HEALTHCARE WORKER

PLEASE RECORD ALL INFORMATION AS AT TIME AND DATE OF SPECIMEN COLLECTION

Identifier

Staff type

Ward

Gender — Male / Female

Years of practice

Status — Permanent / Part-time / Intern

Other wards

Ward Type

1 2 3 4

Date of Specimen Collection

Specimen Source — Hand swab

Duplicate Isolate Screen

Appendix 6.3: Sample collection sheet – environment

SAMPLE COLLECTION SHEET – ENVIRONMENT

PLEASE RECORD ALL INFORMATION AS AT TIME AND DATE OF SPECIMEN COLLECTION

Identifier

Ward

Other wards

Hospital Type

Ward Type

1 2 3 4

Date of Specimen Collection

Specimen Source

- Bed
- Drip stand
- Table
- Sink
- Tap handle
- Door handle
- Other

Duplicate Isolate Screen

Appendix 7: Manuscript submission confirmation

Submission confirmation from Journal of Infection and Public Heath

De : Journal of Infection and Public Health <em@editorialmanager.com>
À : Akebe Luther King Abia <lutherkinga@yahoo.fr>
Envoyé : lundi 14 août 2023 à 17:32:25 UTC+2
Objet : Submission Confirmation

Dear King,

We have received your article "Genomic characterisation of multidrug-resistant ESBL-producing Escherichia coli isolates from patients and patient environments in a teaching hospital in Ghana" belonging to "ARTICLE_TYPE" for consideration for publication in Journal of Infection and Public Health.

Your manuscript will be given a reference number once an editor has been assigned.

To track the status of your paper, please do the following:

1. Go to this URL: https://www.editorialmanager.com/ji-ph/

2. Enter these login details:

3. Click [Author Login]
This takes you to the Author Main Menu.

4. Click [Submissions Being Processed]

By submitting your paper to Journal of Infection and Public Health you agree and acknowledge the journal is an Open Access journal. If your paper is successful and accepted by the Editor your article will be subject to an article processing fee.

An invoice will be issued post acceptance.

More information about the journal and the APC can be found here: https://www.elsevier.com/journals/journal-of-infection-and-public-health/1876-0341/guide-for-authors

Thank you for submitting your work to this journal.

Kind regards,

Editorial Manager
Journal of Infection and Public Health

****
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