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Characterization of plasmids in bacterial isolates in a tertiary health care facility in Kilimanjaro region, Tanzania

Sengeruan, Lameck

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CHARACTERIZATION OF PLASMIDS IN BACTERIAL ISOLATES IN A TERTIARY HEALTH CARE FACILITY IN KILIMANJARO REGION, TANZANIA

Lameck Pashet Sengeruan

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of

Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

August, 2022

ABSTRACT

Plasmids are infectious extra-chromosomal DNA molecules found within bacteria. Their detection is important for guiding clinical treatment and controlling resistant disease outbreaks in hospital and community settings. Plasmids were characterized in bacterial isolates recovered from inpatient retrospective specimens admitted at the Kilimanjaro Christian Medical Centre (KCMC) from August 2013 to August 2015 in Kilimanjaro, Tanzania; to determine their prevalence, effects on the risks of antibiotic resistance development and spread. Bacterial isolates were fully genome sequenced. The risk factors for infections with pathogens resistant to at least one antibiotic were examined using logistic regression models. Results showed that, 56.2% (159/283) of bacterial isolates were found to carry plasmids. Twenty-six (86.7%) multiple-replicon plasmids and 4 (13.3%) single-replicon plasmids were found to carry both resistance and virulence genes. There was no statistically significant correlation found between the number of antibiotic resistance and virulence genes in plasmids ($r = -0.14$, $p > 0.05$). Moreover, adjusted for other factors, lower odds of infection with pathogens resistant to at least one antibiotic for males (0.16 [95% CI, 0.05 - 0.49]; $p = 0.001$) was found. However, non-significant difference odds of carrying resistant pathogens was found between those transferred and non-transferred patients, and those sought and did not sought medical services. The findings show a relatively high proportion of plasmid-carrying isolates in the study area, suggesting selection pressure due to antibiotic use in the hospital. Co-occurrence of antibiotic resistance and virulence genes found in the studied clinical isolates, affirming that this is a public health concern that needs an immediate attention.

DECLARATION

I, Lameck Pashet Sengeruan, declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this Master's dissertation is my original work, and that it has not been submitted or presented for a similar degree award in any other university or institution.

02.08.2022

Lameck Pashet Sengeruan

(Candidate)

Date

The above declaration is confirmed by:

02.08.2022

Dr. Tolbert Sonda

(Supervisor 1)

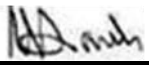
Date

02.08.2022

Dr. Katharina Kreppel

(Supervisor 2)

Date



02.08.2022

Dr. Elingarami S. Nkya

(Supervisor 3)

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CERTIFICATION

The undersigned supervisors certify that they have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology the dissertation entitled "*Characterization of plasmids in bacterial isolates in a tertiary health care facility in Kilimanjaro Region, Tanzania*" in partial fulfillment of the requirements for the degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology.

02.08.2022

Dr. Tolbert Sonda

Date

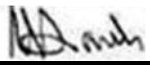
(Supervisor 1)

02.08.2022

Dr. Katharina Kreppel

Date

(Supervisor 2)



02.08.2022

Dr. Elingarami S. Nkya

Date

(Supervisor 3)

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DEDICATION

To my Mama Mary and the family.

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LIST OF ABBREVIATIONS AND SYMBOLS

AOR	Adjusted Odds Ratio
ARGs	Antibiotic Resistance Genes
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Test
BAP	Bacterial Analysis Pipeline
cvaC	Colicin V
DDD	Defined Daily Doses
DNA	Deoxyribonucleic Acid
F	Fertility Factor
GRSM	Gentamicin-Resistant *Serratia Marcescens*
GSF	Good Samaritan Foundation
GTDB-Tk	Genome Taxonomy Database Toolkit
HGT	Horizontal/Lateral Gene Transfer
HIV	Human Immuno-deficiency Virus
ICU	Intensive Care Unit
IHI	Ifakara Health Institute
IQR	Interquartile Range
IRB	Institutional Review Board
iutA	Aerobactin
KCMC	Kilimanjaro Christian Medical Centre
KCRI	Kilimanjaro Clinical Research Institute
LMICs	Low- and Middle-Income Countries
MD	Medical Doctor
MDR	Multi-drug Resistant
MGEs	Mobile Genetic Elements
MRSA	Methicillin-Resistant *Staphylococcus Aureus*
MSA	Multi-Locus Sequence Alignment
NM-AIST	Nelson Mandela African Institution of Science and Technology
OR	Odds Ratio

PhD	Doctor of Philosophy
RNA	Ribonucleic Acid
SOS	Save Our Souls (Bacterial response to DNA damage)
SD	Standard Deviation
SKESA	Strategic K-mer Extension For Scrupulous Assemblies
SSA	Sub-Saharan Africa
TB	Tuberculosis
USA	United States of America
VFs	Virulence Factors
WGS	Whole Genome Sequencing
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Antibiotic resistance of human pathogens is becoming a serious threat globally and is causing morbidity and mortality (Wang *et al*., 2018), particularly in resource-limited countries like Tanzania, with fewer treatment options. Predictions suggest that, 10 million people will die globally of infections resistant to antimicrobials by 2050, and economic losses of US $60–100 trillion will occur annually due to antimicrobial resistance (AMR) (Promite *et al*., 2017). The African continent is reported to have a higher proportion (49.9%) of antimicrobial use to treat human infections compared to other continents around the world (< 43%) (Versporten *et al*., 2018).

In Tanzania, AMR is a serious problem as reported by the 2016 Global Health Security Agenda assessment due to the antimicrobials overuse in the human, plant and veterinary sectors (Azabo *et al*., 2022; Mdegela *et al*., 2021; WHO, 2017). Mbwasi *et al*. (2020) reported that on average the defined daily doses (DDD) per 1 000 inhabitants per day (DDD/1 000/D) for antimicrobials imported in Tanzania is 80. Poor utilization of antimicrobial susceptibility test (AST) services was reported with high empirical use of antibiotics across hospitals in Tanzania (Seni *et al*., 2020). Furthermore, AMR surveillance implementation in Tanzania is at low level and variable across public and private health care facilities (Sangeda *et al*., 2020).

Antimicrobials are frequently used in hospitals for prophylaxis and treatment of both community-acquired and nosocomial infections. Multiple studies have shown that there is incorrect antimicrobial selection, dose use, administration route, and the duration of the treatment that contribute to an antimicrobial-resistance evolvement and spread (Rahal *et al*., 2002; Sousa *et al*., 2021). Multi-drug resistance is the main issue in tertiary hospitals in Tanzania (Silago *et al*., 2020).

Transmission of resistance to antimicrobials within or between bacterial species can either be vertical or horizontal. Vertical transmission enables the transmission of resistance traits from bacteria to their offspring, while horizontal gene transfer enables the transmission of resistance traits among the bacterial community (Davies, 2010; Paramasivam, 2013).

Horizontal gene transfer (HGT) involves three mechanisms: transformation, conjugation, and transduction (Fig. 1; Bello-l *et al*., 2019). The transformation involves bacterial uptake of free DNA from the surroundings. Conjugation involves gene transfer through donor and recipient cells with direct contact. Donor cells promote gene transfer due to the presence of a fertility (F) factor. Transduction involves viruses carrying bacterial genes infecting bacteria.

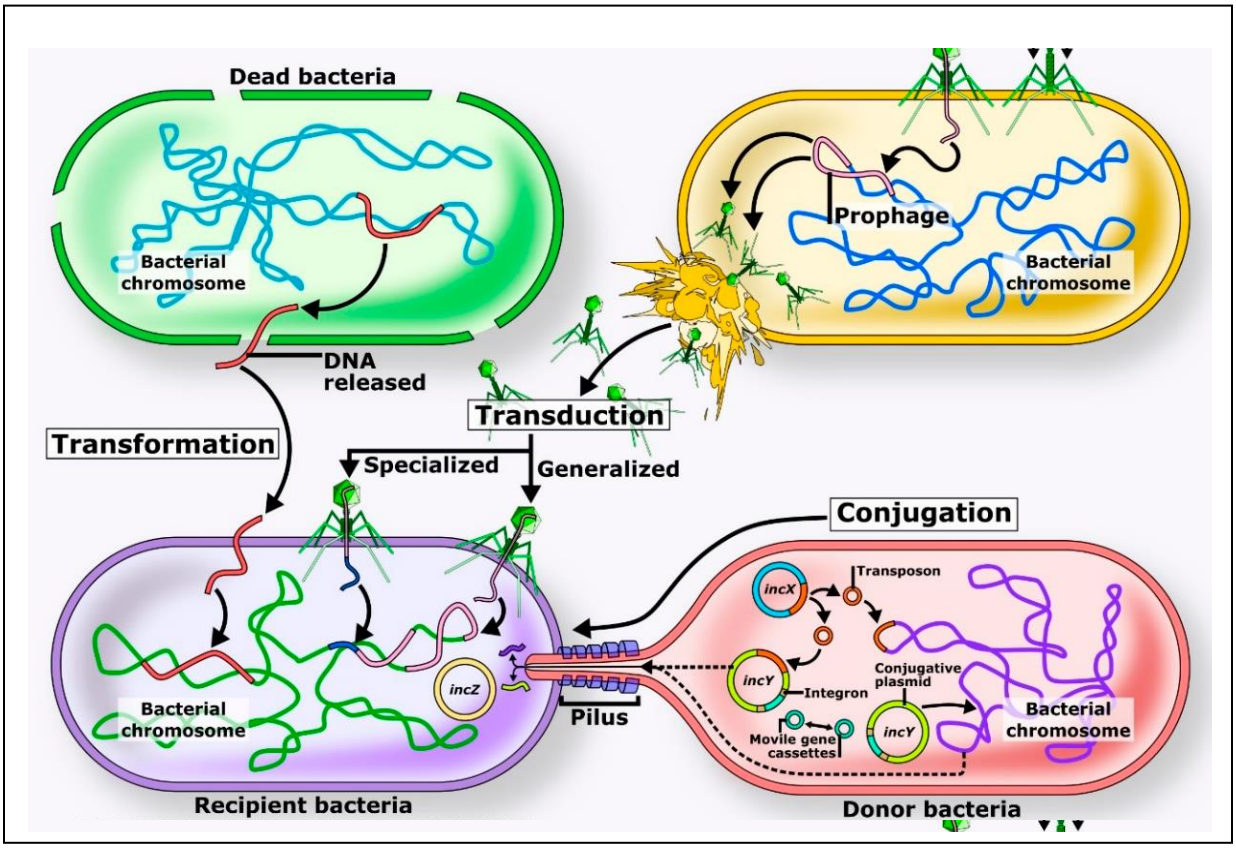

Figure 1: Transduction, conjugation, and transformation mechanisms (Bello-l *et al*., 2019)

Mobile genetic elements such as plasmids, transposons, gene cassette, integrons, insertion sequences and bacteriophages are transferred between bacteria via HGT (Paramasivam, 2013). It is important to monitor antibiotic resistance development in bacteria and analysis of drug-resistant bacteria looking on mobile genetic elements (MGEs). Therefore, for the purpose of this study, MGEs of relevance are plasmids.

Plasmids can contain the following genes: antibiotic resistance genes and virulence factors, which allow bacteria to adapt different environments and niches (Carattoli, 2009; Ramirez *et al*., 2014). Antibiotic resistance genes in plasmids contribute to inactivation of antibiotics in four ways: drug modification, drug degradation, drug efflux and drug target alteration (Carattoli, 2009).

Drug modification and degradation processes involve enzymes that discern, cover and change antibiotic molecules, preventing them from impacting the targets. Drug efflux is the situation of removing antibiotics from inside to outside of the bacterial cell. The drug target alteration is the process of altering a cell target by mutation to prevent drug binding, which results in ineffectiveness. Thus, antibiotic resistance genes in plasmids regulate these mechanisms (Paramasivam, 2013).

According to their mobility between bacteria, plasmids are grouped into conjugative and non-conjugative plasmids. Conjugative plasmids possess *tra* genes responsible for conjugation while non-conjugative plasmids lack *tra* gene functions, which need the assistance of conjugative plasmids to be transferred (Bennett, 2008; Salyers & Amábile-Cuevas, 1997). Conjugative plasmids are further categorized into broad host range and narrow host range, depending on the number of bacterial species that they can be transferred to (Bennett, 2008).

Furthermore, plasmids are also classified as virulent when they turn a bacterium into a pathogen that causes a disease. For instance, *Escherichia coli (E. coli)* without virulence plasmids found in humans' and animals' intestine is not pathogenic, but it can cause severe diarrhea and vomiting after acquiring virulence plasmids. *Salmonella enterica* also contains virulence plasmids (Fluit, 2005).

Therefore, limited knowledge regarding the spread of antibiotic resistance genes (ARGs) and virulence genes among clinical isolates in Tanzania, plasmids were characterized in bacterial isolates from inpatients at Kilimanjaro Christian Medical Centre to establish their resistance and virulence in humans.

1.2 Statement of problem

Plasmids are important vehicles in disseminating and acquiring antibiotic resistance and virulence genes, and can thus constitute a major burden on human health (Foley *et al*., 2021). Recent studies have suggested that the prevalence of AMR is higher in low- and middle-income countries (LMICs) compared to European countries and the United States (Iskandar *et al*., 2020; Neill *et al*., 2016). There is however, limited knowledge regarding the dissemination of antibiotic resistance genes (ARGs) and virulence genes among clinical isolates in sub-Saharan Africa (SSA). This study was therefore, conducted to determine the proportion of bacterial isolates carrying plasmids, to identify plasmids that mediated resistance and virulence genes, to investigate the relationship between antibiotic resistance genes and virulence genes within plasmids, and to identify the association between socio-demographic characteristics and antibiotic resistance using whole genome sequenced data of clinical isolates from inpatients admitted to Kilimanjaro Christian Medical Centre (KCMC) in Tanzania.

1.3 Rationale of the study

Better knowledge of the dynamics and biology of plasmid-mediating resistance and virulence genes in bacterial species isolated from patients is essential for guiding appropriate drug development, clinical treatment, detecting and controlling resistant disease outbreaks in hospital and community settings, thus reducing morbidity, mortality and hospital costs from hospital stays.

1.4 Research Objectives

1.4.1 General Objective

Characterizing plasmids of public health relevance in bacterial isolates to determine their prevalence, effects on pathogenicity and the risks of antibiotic resistance development and spread among inpatients admitted to KCMC in Moshi, Kilimanjaro region, Tanzania.

1.4.2 Specific Objectives

(i) To determine the proportion of bacterial species carrying plasmids

(ii) To identify plasmid mediated resistance to antibiotics and virulence among bacterial species isolated from clinical samples in a tertiary hospital in Moshi, Kilimanjaro, Tanzania

(iii) To determine the relationship between resistance and virulence genes within plasmids from bacteria isolated from clinical samples

(iv) To determine the association between socio-demographic characteristics and antibiotic resistance

1.5 Research Questions

The study aimed to answer the following questions:

(i) What is the proportion of bacterial species among all isolated species from clinical samples carried plasmids?

(ii) Which plasmids mediated resistance and virulence genes among bacteria in clinical samples from a tertiary hospital in Moshi, Kilimanjaro, Tanzania?

(iii) Is there any relationship between plasmid mediated resistance and virulence genes in bacteria isolated from clinical samples?

(iv) What are the socio-demographic characteristics associated with antibiotic resistance?

1.6 Significance of the study

This research study adds to a body of knowledge important information on plasmids profiles from clinical isolates at KCMC in Moshi, Kilimanjaro, Tanzania. Furthermore, this study offers an insight on the risks of carrying a pathogen with resistance to at least one antibiotic among inpatients.

1.7 Delineation of the study

The study used bacterial whole genomes data with associated patients' socio-demographic and clinical characteristics to characterize plasmids to provide up-to-date information that will contribute to the understanding of the spread of antimicrobial resistance in health care settings in Tanzania. However, there are a number of limitations in the present study that warrant careful interpretation. There was no antimicrobial susceptibility testing done that could provide insights on validating the presence of resistance and virulence genes and actual resistance phenotypes. Bioinformatics analysis was performed on Illumina short reads, which limited the ability to assemble completed plasmid genomes, and consequently the ability to 'tease out' individual plasmids from assembled contigs. Assembly graphs were classified by Gplas+PlasFlow for plasmid prediction. As for any machine learning-based approach or indeed any method based on inference from similarity with known sequences, including tools such as PlasmidFinder, the predictive ability of the model is strongly dependent on the data in its reference database or training set. A bias toward plasmids in well-studied organisms is therefore likely to occur.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of terms

Alarmones – are the intracellular signal molecules that are produced in bacteria that help them to react against harsh environments.

Antibiotics – are the medicinal drugs that fight bacterial infections in both people and animals.

Antibiotic Resistance Genes – these are the mobile genetic elements that can be shared between microorganisms through lateral gene transfer and can allow resistance to antibiotics.

Antimicrobial Resistance – is the ability of microorganisms such as bacteria, viruses, fungi, and parasites to stop the effects of antimicrobials against them.

Antimicrobials – are the medicinal drug agents that kill or stop the growth of microorganisms.

Bacterial Isolates – separated bacterial strains from clinical samples.

Defined Daily Doses – this is the statistical measure of drug consumption.

Health Seeking Behavior/Seeking Medical Services – is the admission of individuals to the dispensaries/clinics and pharmacies/maduka ya dawa while having health problems for the purpose of finding appropriate treatment before attending KCMC.

Horizontal Gene Transfer – is the transfer of genetic material between bacteria.

Multidrug Resistance – is the acquired resistance to at least one medicinal drug agent in three or more antimicrobial classes.

Patient Hospital Transfer – in this context is the transfer of a patient from different health care facilities to KCMC for diagnostic procedures and more advanced care.

Pathogenicity – is the microorganism's capability to cause a disease that is determined by its virulence factors.

Sequenced Data - data in which the order of nucleotides in DNA are determined.

SOS system – this is the pivotal system for bacterial adaptation, pathogenesis and diversification.

Virulence – is the degree of damage that is caused by a microorganism to its host.

Virulence Factors – these are bacterial molecules that are required for a bacterium to cause a disease to the host.

Whole Genome Sequencing – is the determination of the complete DNA sequence of an organism's genome at a single time.

2.2 Plasmids' ecology

Plasmids in bacteria are small, often circular DNA molecules which are separate from the bacterial chromosome and can replicate independently. Although they carry only a small number of genes, some have been associated with antibiotic resistance (Millan, 2018). However, plasmids without antibiotic resistance genes may harbor other genes advantageous to the host bacteria, including virulence genes (Mcmillan *et al*., 2019).

Plasmids can acquire resistance genes from transposons or insertion sequences and replicate independently in various hosts (Rozwandowicz *et al*., 2018). Many plasmids with broad host ranges, such as IncR, IncW, IncQ, IncU and IncY, which carry multiple antimicrobial resistance genes are less reported in the literature (Rozwandowicz *et al*., 2018). Probably, less reporting may limit understanding and thus contributes to the continuing spread of resistance genes unknowingly.

2.3 Role of plasmids for public health

In Asia, several studies found plasmids facilitating the spread of resistant pathogens that cause infections such as typhoid, bacillary dysentery and gonorrhea among people. In Africa, evidence suggests that a huge burden of infectious diseases and their spread is also associated with presence of plasmids (Kivata *et al*., 2020; Sekyere & Reta, 2020).

Plasmids have a significant impact on public health. For example, patients in several districts of Southern Vietnam experienced the relapsed typhoid fever because of resistant *Salmonella typhi* (*S. typhi)* isolates to antibiotics, such as chloramphenicol, ampicillin, trimethoprim, sulfonamides, and tetracycline, that were associated with acquisition of an incompatibility group HI plasmid (Butler *et al*., 1973). Interestingly, Vietnamese resistant *S. typhi* isolates belonged to 11 different phage types, which indicated the spread of the HI plasmid among *S. typhi* organisms (Goldstein *et al*., 1986).

Huang *et al*. (2005) reported resistant *Shigella sonnei* isolates to ceftriaxone, which were mediated by a plasmid that encoded CMY-2-type AmpC beta-lactamase. The isolates were identified in a bacillary dysentery outbreak in two elementary schools in Yu-Li, Taiwan, where 182 children were affected after a typhoon hit the area in 2001.

In Liberia, 100 patients in the pediatric population were reported from October 1980 to August 1982 at Curran Lutheran Hospital to have multiple drug-resistant and virulent *Salmonella enteritidis* serotype, which was mediated by a 120-megadalton conjugative plasmid that conferred resistance to streptomycin, tetracycline, chloramphenicol, carbenicillin, penicillin, ampicillin, and sulfadiazine (Monson *et al*., 1985).

Sarafian and Knapp (1989) described the spread of spectinomycin-resistant *Neisseria gonorrhoeae* strains in the United States and Republic of Korea in 1985 through military activities. The strains isolated in both countries carried beta-lactamase plasmids. In Australia, high-level penicillin resistance in *Neisseria gonorrhoeae* was observed in clinical isolates and that was conferred by plasmid producing penicillinase. The genes within these plasmids were *bla*TEM-1gene and gonococcal *bla*TEM-135 gene (Müller *et al*., 2011).

In addition, John and McNeill (1981) reported gentamicin-resistant *Serratia marcescens* (GRSM) strains in a crowded neuro-surgical unit and spread at several hospitals in Charleston, South Carolina. Nearly all patients with these strains had colonization or urinary tract infections associated with indwelling bladder catheters. Gentamicin-resistant *Serratia marcescens* strains carried a 41-megadalton conjugative plasmid that conferred resistance to ampicillin, carbenicillin, tetracycline, kanamycin, gentamicin and tobramycin antibiotics.

Virulence genes in bacterial plasmids contribute to increased pathogenic bacteria (Smalla *et al*., 2015b; Smillie *et al*., 2010) that places everyone at risk. For example, colicin V (*cva*C) and aerobactin (*iut*A) virulence factors potentiates *Escherichia coli* pathogenicity (Koga *et al*., 2014). They cause morbidity, mortality, and increased healthcare costs (Partridge *et al*., 2018), which makes their detection and monitoring an important intervention (Puustusmaa, 2018).

2.4 The link between plasmid prevalence and antibiotic resistance

Han *et al*. (2018) suggested that multiple plasmids carriage may positively affect the transfer of ARGs. Several different incompatibility plasmid groups have been associated with multiple ARGs in bacteria (Carattoli & Elena, 2009). Plasmids reported to carry ARGs in nosocomial infections and community-acquired outbreaks (Li *et al*., 2017; Weingarten *et al.*, 2016; Whichard *et al.*, 2018). A study conducted in Cairo, Egypt reported that 43.3% of 150 bacterial isolates recovered from urine samples were multi-drug resistant and 60% harbored plasmids (Elshamy *et al*., 2020). Talukdar *et al*. (2013) reported that 36% (84/233) of multi-drug resistant (MDR) *E. coli* isolates from household water supply in Bangladesh carried multiple plasmids (2 to 8). Moreover, more than 80% of ARGs were reported in a plasmid sequence that identified in *S. enterica* isolates (Mcmillan *et al*., 2019).

2.5 Relationship between resistance and virulence genes within plasmids

Resistance and virulence in bacteria are two dependent features. A negative or positive relationship can be found between these features. Furthermore, the relationship mainly depends on bacterial species type, mechanism of resistance and virulence, ecological niche and environmental conditions as well as the host immune system (Cepas & Soto, 2020).

Plasmids disseminate antibiotic resistance and virulence determinants concurrently among bacterial populations (Martínez & Baquero, 2002; Mccarthy & Lindsay, 2012). Plasmids that encode both resistance and virulence genes in bacteria leads to selection of resistance determinants in bacteria in non-infective environments subjected to antibiotic pressure. Conversely, in infectious conditions, concurrent selection of virulence and resistance determinants can occur in bacteria, even in the absence of antibiotic selective pressure.

In some cases, increase in resistance comes together with an increase in virulence in plasmids when carrying both determinants (Merino *et al*., 2010; Szczepanowski *et al*., 2005; Woodford *et al*., 2009); such as during activation of the SOS system, which facilitates dissemination of resistance and virulence encoding genes simultaneously (Hacker *et al*., 1996; Úbeda *et al*., 2007); and alarmones accumulation in *E. coli*, which linked to biofilm formation and tolerance to beta-lactams (Joseleau-petit *et al*., 1994).

Tartor *et al*. (2021) reported a significant relationship between virulence and resistance genes within plasmids in Gram-negative bacteria recovered from milk samples. Moreover, Dlamini *et al*. (2018) reported a significant correlation between antibiotic resistance and virulence genes among *Salmonella* strains in South Africa. Even though the relationship between ARGs and virulence factors (VFs) has been sporadically reported in bacterial isolates (Pan *et al*., 2020a), this study intended to determine the relationship between the number of ARGs and virulence genes within plasmids.

2.6 Importance of antimicrobial resistance surveillance in low and middle income countries

Antimicrobial resistance surveillance implementation is variable among LMICs (Seale *et al*., 2017). It is important to support and strengthen AMR surveillance among LMICs due to different challenges that face individual, community and national levels. For example, poor hygiene and sanitation, antibiotic overuse in animals, counterfeit medications and human overconsumption of antibiotics driven by infectious diseases. Additionally, over the counter use of antibiotics due to non-stringent regulations and poor diagnostic services that favors large-scale empirical antibiotic use are among the challenges in LMICs that necessitate AMR surveillance.

Furthermore, AMR surveillance provides information that influences infectious disease management (Hay *et al*., 2018), and better understanding of regional and global AMR spread, that potentially saving millions of lives (Johnson & Johnson, 2015). Therefore, surveillance of AMR is essential particularly in LMICs where the burden of infections is high and health systems governance is weak.

CHAPTER THREE

MATERIALS AND METHODS

This chapter describes the study area, study design and study dataset

3.1 Study area

The study was conducted at the Kilimanjaro Christian Medical Centre (KCMC) (Fig. 2). Kilimanjaro Christian Medical Centre is among the six Tanzania's zonal referral hospitals, located in Moshi, northern Tanzania -3°19'15.09" S latitude and 37°19'46.03" E longitude at 966 m (3 169 ft) altitude. The hospital is a semi-public/private teaching hospital with 1 300 staff (*KCMC*, 2022), with a capacity of 650 inpatient beds and receives approximately 500 outpatients daily from different parts of Tanzania (Sonda *et al*., 2018).

It serves a catchment area of about 15 million people with the following specialties; emergency medicine, internal medicine, obstetrics/gynecology, pediatrics, dermatology, urology, ear-nose and throat, physiotherapy, ophthalmology, orthopedics, general surgery, anesthesia, pathology and oncology. Kilimanjaro Christian Medical Centre is linked to the Kilimanjaro Clinical Research Institute (KCRI), which is a medical research institute. Both KCRI and KCMC are among the pillars of the Good Samaritan Foundation (GSF) in which KCRI is concentrating on research and KCMC is based on patient care (*KCMC*, 2022). Thus, KCRI is integrated within a hospital care system to facilitate a suitable environment for conducting medical research.

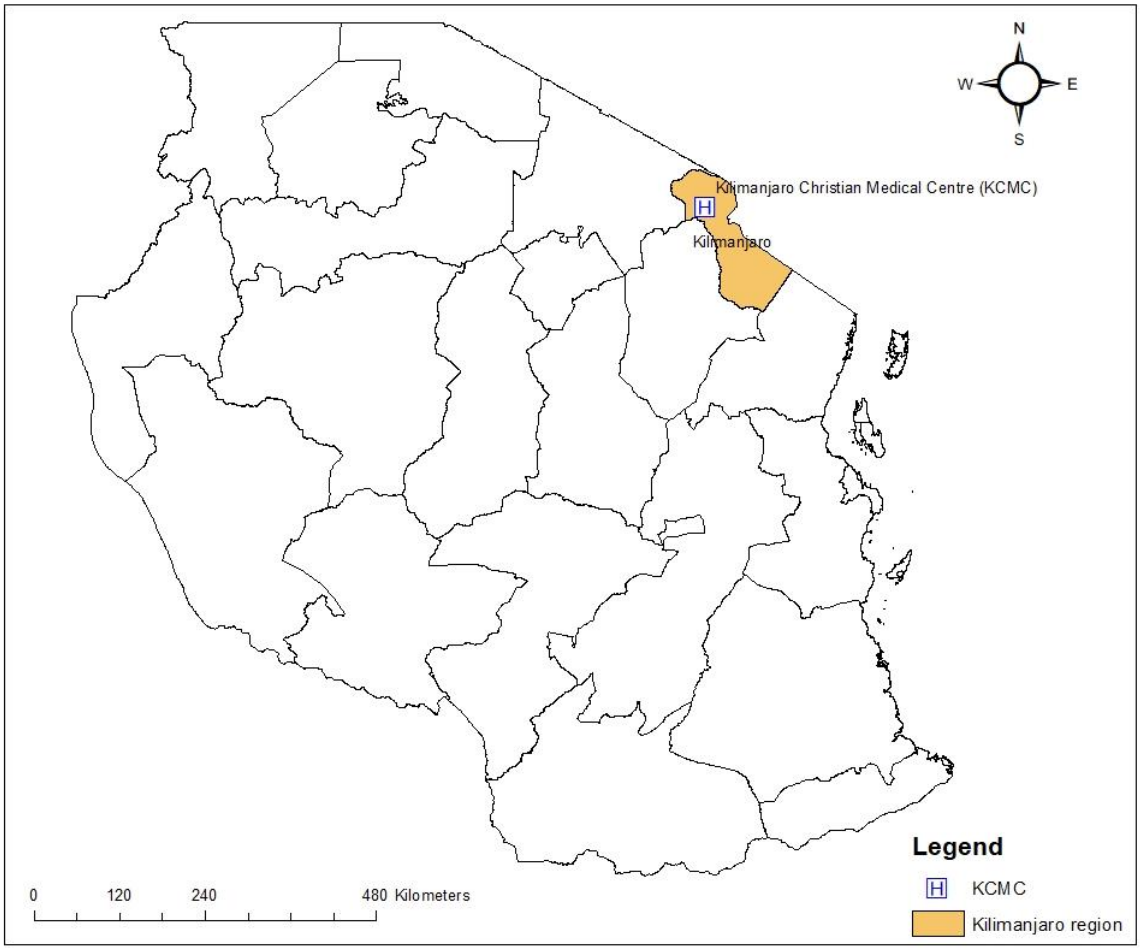

Figure 2: Map of Tanzania showing the location of the Kilimanjaro Christian Medical Centre (KCMC)

3.1.1 Study design and dataset

A retrospective study was conducted. The secondary data that was analyzed originated from a prospective cross-sectional study (Kumburu *et al*., 2017), that was conducted at KCMC from August 2013 to August 2015. A total of 377 bacterial whole genomes with associated metadata were retrieved for analysis from KCRI Compute cluster. Additional ethical approval was obtained from the Ifakara Health Institute Research Ethics Committee (IHI/IRB/No: 14-2021) for plasmid characterization.

Below is the schematic representation of the in-house bacterial analysis bioinformatics pipeline (BAP) and statistical analysis used for assembly, plasmid extraction, validation, and identification of ARGs and virulence genes, descriptive statistics, the relationship between antibiotic resistance genes and virulence genes, and association between patients' socio-demographic characteristics and antibiotic resistance (Fig. 3).

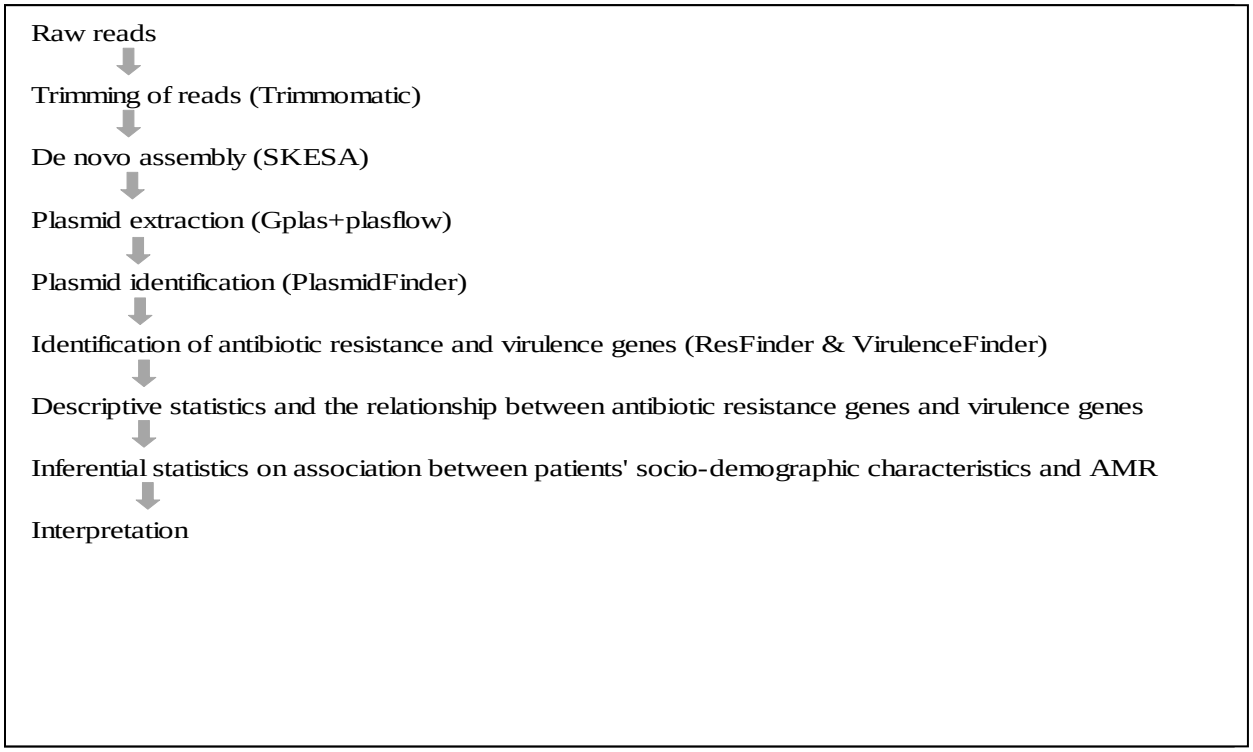

Figure 3: Schematic diagram showing analysis steps

3.2 Bioinformatics Analysis

3.2.1 Quality Control and Trimming of Illumina Sequences

The following steps were followed:

(i) All bacterial raw reads from 377 bacterial whole genomes were submitted to in-house bacterial analysis pipeline (BAP), available at https://github.com/zwets/kcri-cge-bap. Assembly was performed using SKESA 2.4.0 (Souvorov *et al*., 2018).

(ii) All resulting assemblies were then processed in batch by the Genome Taxonomy Database Toolkit (GTDB-Tk) 0.3.2 (Chaumeil *et al*., 2020) for detailed taxonomic assignment.

(iii) Metrics produced by the BAP and GTDB-Tk were then used to assess the quality of each assembly. Assessment was based on read counts, coverage depth, assembly structure (contig count, N1, N50, L50), deviation of assembly length from reference, GTDB alignment fraction, and GTDB Multi-Locus Sequence Alignment (MSA) coverage. A six-point scale was used for assembly quality rating: 0 (Unusable), 1 (Mix), 2 (Bad), 3 (Usable), 4 (Good), and 5 (Excellent).

(iv) Finally, categories 0 to 2 were excluded, while categories 3 through 5 were used for subsequent analysis. Every assembly in these categories was for a single isolate that had (nearly) complete genome coverage, at sufficient sequencing depth. A total of 283 bacterial isolates were recovered.

3.2.2 Plasmid extraction and validation

Raw reads assembly was repeated with Unicycler 0.4.7 (Wick *et al*., 2017) for its ability as a "SPAdesoptimiser" to produce long and, in the ideal case, circular contigs. Assembly graphs (GFA) were submitted to Gplas 0.6.1 + Plasflow 1.1 (Arredondo-alonso *et al*., 2020; Krawczyk *et al*., 2018) for plasmid prediction. GPlas and Plasflow taken into account the connected components in the assembly graph when predicting plasmids. The components predicted to be plasmids were extracted from the assemblies and submitted to PlasmidFinder1.3 (Carattoli *et al*., 2014) for validation.

3.2.3 Identification of antibiotic resistance genes (ARGs) and virulence genes in plasmid replicon types

To identify antibiotic resistance and virulence genes carried in plasmid replicons, the assembled putative plasmid sequences for each isolate were submitted to Resfinder 4.0 (Bortolaia *et al*., 2020) and VirulenceFinder 1.4 (Joensen *et al*., 2014), respectively. In both Resfinder and VirulenceFinder, 90% identity and 60% coverage settings to call a gene were selected.

3.2.4 Statistical Analysis

Stata14 (College Station, TX, 77845, USA) was used for descriptive statistics and determination of the relationship between antibiotic resistance and virulence genes in plasmid replicons. Additionally, resistance genes transfer was assessed in plasmids. Each plasmid that carried by an isolate was classified as resistant to none of the determined antibiotic versus resistant to at least one of the determined antibiotic (response variable).

The association between the response variable (resistance to at least one antibiotic) and individual independent variables linked to the samples such as the patient's age group, sex, ward of admission, occurrence of ward transfer and/or hospital transfer, as well as current infection status, health seeking behavior before attending KCMC and hospitalization history, was done using Fisher's exact test or Chi-square test. Multivariable Logistic regression was performed to determine the risk factors for infections with pathogens resistant to at least one antibiotic among inpatients at KCMC hospital. In the multivariable analysis, any variable with p-value ≤ 0.2 (Ziaul *et al*., 2020) and those regarded as key risk factors despite their p-values being > 0.2 in univariate analysis were included. A significant association was decided based on two-tailed p-values and the respective 95% confidence intervals.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Study population

In total, 128 patients whose whole genome bacterial isolates were analyzed were included in this study (Table 1). One hundred twenty-eight selected patients had positive plasmid isolates. The mean age in years (SD) was 46.2 (18.0). There were 77 (60.2%) male patients and 47 (36.7%) females, while 4 (3.1%) missed sex identification. Among the study population whose samples were used in this study, most patients were Chagga and Pare, however, other ethnic groups were Maasai, Meru, Iraq (Wamburu), Sambaa, Sukuma, Nyaturu and Rangi. The main economic activities conducted by these ethnic groups include agriculture, livestock keeping, tourism and fishing. Household income is mainly generated by the sale of cash or food crops, livestock or livestock products and fishing activities.

A total of 28 (21.9%) patients were diabetic, 6 (4.7%) were cancer patients, 6 (4.7%) were suffering from TB, 2 (1.5%) were HIV positive and 86 (67.2%) were others. Among those, 61 (47.7%) had no underlying conditions and 25 (19.5%) had other underlying conditions and infections (multiple conditions) such as asthma, hypertension, stroke, necrotic pyomyositis, pneumonia, peptic ulcer disease, cellulitis and esophageal candidiasis. Moreover, twenty-four (18.8%) were wound swabs from patients with diabetic wounds, 11 (8.6%) from patients with burn wounds, 10 (7.8%) from patients with post-surgical wounds, 6 (4.6%) from patients with motor traffic accident wounds and 77 (60.2%) were others. Of those, 35 (27.3%) were from patients with other wounds from bed sores, skin disease, kaposi sarcoma lesion, abscess, gangrene, osteomyelitis, tetanus, septic lymphadenitis, ulcer-rated umbilical mass and 42 (32.8%) had no wounds (Table 1).

Table 1: Demographic and clinical characteristics of study patients

Patient characteristics	Missing [a]	Total (%)
Number of patients		128 (100)
Mean age in years (SD)		46.2 (18.0)
Sex	4 (3.1)	
Female		47 (36.7)
Male		77 (60.2)
Ward of admission	1 (0.8)	
Surgical		62 (48.4)
Surgical ICU		9 (7.1)
Medical		52 (40.6)
Medical ICU		4 (3.1)
Specimen collected	1 (0.8)	
Blood		8 (6.3)
Sputum		13 (10.2)
Stool		19 (14.8)
Swab		87 (67.9)
Underlying conditions		
Cancer		6 (4.7)
Diabetes		28 (21.9)
HIV		2 (1.5)
TB		6 (4.7)
Others		86 (67.2)
Type of wound		
Burn wound		11 (8.6)
Diabetic wound		24 (18.8)
Motor traffic wound		6 (4.6)
Post-surgical wound		10 (7.8)
Others		77 (60.2)
History of hospitalization	8 (6.3)	
No		78 (60.9)
Yes		42 (32.8)
Patient hospital transfer	3 (2.3)	
No		44 (34.4)
Yes		81 (63.3)
Patient ward transfer	5 (3.9)	
No		110 (85.9)
Yes		13 (10.2)
Current infection status	1 (0.8)	
No		26 (20.3)
Yes		101(78.9)
Seeking medical services/health seeking behavior	4 (3.1)	
No		31(24.2)
Yes		93 (72.7)
Median time in days stayed in the hospital before survey (IQR)	8 (6.3)	8 (4-11.5)

SD = standard deviation; ICU = intensive care unit; IQR = interquartile range; Missing [a]=were the missing values in each variable.

4.1.2 Proportion of bacterial species carrying plasmids

A total of 283 whole genome bacterial sequences were analyzed. One hundred fifty-nine (56.2%) bacterial isolates were detected to carry plasmid replicons. Out of 159 plasmid replicons, 93 non-repetitive plasmids were predicted. Of 93 plasmid replicons, 48 (51.6%) occurred in isolates carrying single replicons and 45 (48.4%) were in multiple-replicon isolates. *Klebsiella pneumoniae* isolates were the most carriers of multiple-replicons plasmids (17, 28.3%), followed by *S. aureus* (15, 25.0%) and *E. coli* (15, 25.0%). *Escherchia coli* isolates were the most single plasmid carriers (23, 23.2%), followed by *S. aureus* (15, 15.2%) and *P. mirabilis* (14, 14.4%) (Table 2).

Table 2: Proportion of bacterial isolates carrying plasmid replicons

			Plasmid replicons	
	Isolates		Single	Multiple [b]
Species	**N**	**%**	**N**	**N**
Enterobacter asburiae	1	0.6	1	0
Enterobacter cloacae	3	1.9	1	2
Enterobacter hormaechei	10	6.3	6	4
Enterobacter kobei	1	0.6	0	1
Enterobacter roggenkampii	1	0.6	1	0
Enterobacter soli	1	0.6	1	0
Enterobacter sp. n18-03635	1	0.6	1	0
Enterococcus faecalis	7	4.4	5	2
Enterococcus faecium	3	1.9	2	1
Enterococcus gallinarum	1	0.6	1	0
Escherichia coli	38	23.9	23	15
Klebsiella michiganensis	2	1.3	1	1
Klebsiella oxytoca	2	1.3	1	1
Klebsiella pneumoniae	25	15.7	8	17
Klebsiella variicola	2	1.3	2	0
Micrococcus sp. Kbs0714	1	0.6	1	0
Morganella morganii	4	2.5	4	0
Proteus columbae	1	0.6	1	0
Proteus mirabilis	14	8.8	14	0
Proteus penneri	1	0.6	1	0
Proteus vulgaris	1	0.6	1	0
Pseudomonas aeruginosa	2	1.3	2	0
Shewanella algae	1	0.6	1	0
Staphylococcus aureus	30	18.9	15	15
Staphylococcus capitis	1	0.6	1	0
Staphylococcus epidermidis	1	0.6	1	0
Staphylococcus haemolyticus	3	1.9	3	0
Staphylococcus hominis	1	0.6	0	1

N, total number of isolates or replicons; Multiple [b], multiple-replicon plasmid types in an isolate.

4.1.3 Plasmid replicon types mediated resistance and virulence genes concurrently

A total of 30 isolates with plasmid replicons were identified to carry both resistance and virulence genes, of which 26 (86.7%) were multiple-replicon plasmids and 4 (13.3%) were single-replicon plasmids (Table 3 and Table 4). All four single-replicon plasmids were carried by *E. coli*. Resistance gene *Sul1* was found the most common across three single-replicon plasmid types IncFII, IncQ1171 and IncFII(pRSB107). Virulence genes iucC *and iutA* were also seen the most common across three single-replicon plasmid types IncQ1, IncFII(pRSB107) and IncFIA (Table 3).

Among the 26 multiple-replicon plasmids, 11 (42.3%) were carried by *E. coli* isolates, 10 (38.4%) by *K. pneumoniae* isolates, 2 (7.6%) by *E. hormaechei* isolates, 1 (3.9%) by *E. cloacae* isolate, 1(3.9%) by *K. oxytoca* isolate and 1 (3.9%) by *K. michiganensis* isolate. Virulence gene *traT* was seen in 18 (69.2%) of the 26 multiple-replicon plasmids, followed by *terC* which was identified in 7 (26.9%) multiple-replicon plasmids. Resistance genes in multiple-replicon plasmids, *sul2* was observed in 17 (65.4%) replicon types, followed by *blaTEM-1B* in 15 (57.7%) replicon types, followed by *blaCTX-M-15* in 14 (57.7%) replicon types and *blaOXA-1* in 13 (50.0%) replicon types (Table 4).

Table 3: Single-replicon plasmid types mediating both resistance and virulence genes

Single plasmid	Resistance genes	Virulence genes
IncFII	*aac(3)-IIa,aadA5,blaCTX-M15,dfrA17,qacE,sul1,tet(B)*	*traT*
IncQ1	*aph(3'')-Ib,aph(6)-Id,blaTEM-1B,dfrA7,qacE,sul1,sul2*	*cea,focCsfaE,focG,focI,iha,ireA,iucC,iutA,mchB,mchC,mchF,mcmA,papA_F48,papC,sat*
IncFIA	*sitABCD,tet(A)*	*iucC,iutA,sitA*
IncFII(pRSB107)	*dfrA5,qacE,sul1,sul2*	*capU,iroN,iss,iucC,iutA,mchB,mchC,mchF,mcmA,vat*

Table 4: Multiple-replicon plasmids mediated both resistance and virulence genes

Multiple-replicon plasmids	Resistance genes	Virulence genes
IncFIB(K),IncFII(pKP91),IncR	*ARR-2,aac(3)-IIa,aac(6')-Ib-cr,aadA1,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,catB3,cmlA1,dfrA14,ere(A),qacE,sul1,sul2,tet(A)*	*traT*
IncFIB(K)(pCAV1099-114),IncHI2,IncHI2A,IncX3	*aac(6')-Ib-cr,aadA5,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaTEM-1B,catB3,dfrA17,qacE,qnrB1,sul1,sul2,tet(A),tet(B)*	*terC*
Col156,IncFIA,IncFIB(AP001918),IncFII(pRSB107)	*aac(3)-IIa,aac(6')-Ib-cr,aadA5,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,catB3,dfrA17,mph(A),qacE,sul1,sul2,tet(A)*	*hra,iha,iucC,iutA,sat,senB,traT*
IncFIB(K),IncFII(K),IncQ1,IncR	*ARR-2,aac(3)-IIa,aadA1,aph(3'')-Ib,aph(3')-Ia,aph(6)-Id,blaCTX-M-15,blaTEM-1B,cmlA1,ere(A),qacE,qnrB1,sul1,sul2*	*traT*
IncFIA(HI1),IncFIB(K),IncFII(Yp),IncHI2,IncHI2A,IncN3,pKP1433	*aac(3)-IIa,aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaTEM-1B,catB3,qacE,qnrB1,sul1,sul2,tet(A)*	*terC*
IncFIB(K),IncFII(K)	*aac(3)-IIa,aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaTEM-1B,catB3,dfrA14,qnrB1,sul2,tet(A)*	*traT*
IncHI2,IncHI2A	*aac(3)-IIa,aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaTEM-1B,catB3,dfrA14,qnrB1,sul2,tet(A)*	*terC*
Col156,IncFIA,IncFIB(AP001918),IncFII	*aac(3)-IIa,aac(6')-Ib-cr,aadA5,blaCTX-M-15,blaOXA-1,catB3,dfrA17,mph(A),qacE,sitABCD,sul1,tet(A)*	*capU,fyuA,irp2,iucC,iutA,senB,sitA,traT*
IncFIB(pECLA),IncFII(pECLA),IncHI2,IncHI2A	*aac(3)-IIa,aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,catB3,dfrA14,qnrB1,sul2,tet(A)*	*terC*
Col156,IncFIA,IncFIB(AP001918)	*aac(6')-Ib-cr,aadA5,blaCTX-M-15,blaOXA-1,catB3,dfrA17,mph(A),qacE,sitABCD,sul1,tet(A)*	*iucC,iutA,senB,sitA,traT*
IncFIA,IncFIB(AP001918)	*aac(3)-IIa,aac(6')-Ib-cr,aadA5,blaCTX-M-15,blaOXA-1,catB3,dfrA17,qacE,sitABCD,sul1*	*fyuA,irp2,iucC,iutA,sitA,traT*

Table 4: Continues

Multiple-replicon plasmids	Resistance genes	Virulence genes
IncFIA,IncFII	*aac(6')-Ib-cr,aadA5,blaCTX-M-15,blaOXA-1,catB3,dfrA17,mph(A),qacE,sul1,tet(A)*	*afaA,afaC,afaD,iha,iucC,iutA,nfaE,papA_F43,sat,traT*
IncFIA,IncFIB(AP001918),IncFII(pAMA1167-NDM-5)	*aac(3)-IId,aadA2,blaTEM-1B,catA1,dfrA12,mph(A),qacE,qepA4,sul1*	*traT*
IncFIA(HI1),IncFIB(K),IncFII(pKP91),IncR	*aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaTEM-1B,catB3,dfrA14,sul2*	*traT*
IncFIA,IncFIB(AP001918),IncFII	*aac(6')-Ib-cr,aadA5,blaOXA-1,catB3,dfrA17,qacE,sul1,tet(B)*	*traT*
IncFIB(K),IncFII(K),IncQ1	*aph(3'')-Ib,aph(3')-Ia,aph(6)-Id,blaTEM-1B,dfrA14,mph(A),sul2*	*traT*
Col156,IncFIB(AP001918),IncFII	*aph(3'')-Ib,aph(6)-Id,blaTEM-1B,catA1,dfrA7,sul2,tet(D)*	*afaA,afaB,afaC,afaD,afaE,hra,iha,iss,iucC,iutA,papA_F43,sat,senB,traT*
Col156,IncFIA,IncFIB(AP001918),IncQ1	*aph(3'')-Ib,aph(6)-Id,blaTEM-1B,dfrA17,sul2,tet(B)*	*iha,iucC,iutA,papA_F43,sat,senB*
IncFIB(K)(pCAV1099-114),IncHI1B(pNDM-MAR)	*aph(3'')-Ib,aph(6)-Id,dfrA15,qacE,sul1,sul2*	*terC*
IncFIB(K),IncFII(K),IncR	*aac(3)-IId,blaCTX-M-15,blaTEM-1B,dfrA30,sul2*	*traT*
IncFIB(K)(pCAV1099-114),IncHI1B(pNDM-MAR),IncR	*blaTEM-1B,dfrA5,qacE,sul1,tet(D)*	*fyuA,irp2,traT*
IncFII(K),IncR	*aac(3)-IId,blaCTX-M-15,blaTEM-1B,dfrA30,sul2*	*traT*
IncFIB(AP001918),IncFII,IncQ1	*aph(3'')-Ib,aph(6)-Id,blaTEM-1B,dfrA5,sul2*	*cia,cvaC,etsC,hlyF,ireA,iroN,iss,iucC,iutA,mchF,ompT,papA_F11,papC,traT*
IncFIB(K)(pCAV1099-114),IncY	*sul2,tet(D)*	*terC*
IncFIB(pHCM2),IncHI2,IncHI2A	*blaTEM-1B*	*terC*
IncFIB(pB171),IncFII(pCoo)	*mdf(A)*	*eae,espA,espF,nleB,nleC,perA,tir,traT*

4.1.4 Correlation between antibiotic resistance and virulence genes

The relationship between the number of antibiotic resistance genes and virulence genes in 26 multiple-replicon plasmids was explored using Pearson correlation approach. There was a non-significant weak negative relationship between antibiotic resistance and virulence genes' existence in plasmids ($r = -0.14$, $p > 0.05$).

4.1.5 Association between socio-demographic characteristics and antibiotic resistance

Significant statistical association was observed between antibiotic resistance and sex ($\chi^2 = 8.2889$, $p = 0.004$) (Table 5). However, other variables were not associated with antibiotic resistance ($p > 0.05$).

Table 5: Association between risk factors and antibiotic resistance

Variable	Chi-square (χ2)	*p*-value
Age category		0.778
Current infection	2.450	0.117
Hospital transfer	0.028	0.868
Hospitalization history	1.124	0.289
Ward transfer		0.157
Seek medical services	0.794	0.373
Gender	8.289	0.004
Bed transfer		0.677
Ward of admission	2.075	0.354

4.1.6 Risk factors for carrying a pathogen with resistance to at least one antibiotic

Table 6 shows crude odds ratio and adjusted odds ratio (AOR) for various patient and hospital characteristics. After adjusting for other characteristics (risk factors), male patients showed a relatively reduced risk of carrying a pathogen with resistance to at least one antibiotic compared to female patients AOR 0.16, 95% CI (0.05 – 0.49), $p = 0.001$. Those who were transferred from another hospital to KCMC had no sufficient evidence of carrying a pathogen with resistance to at least one antibiotic compared to those who were not transferred; AOR 8.82, 95% CI (0.98 – 79.72), $p = 0.05$. Also, there is insufficient evidence to conclude that the risk of carrying a pathogen with resistance to at least one antibiotic is different between those who had the history of seeking medical services at different or same clinics, dispensaries or pharmacies before attending the hospital (KCMC) and those who had no the history of seeking medical services in these places; AOR 0.11, 95% CI (0.01 – 1.03), $p = 0.05$.

Table 6: Multivariable logistic regression showing risk factors for carrying AMR plasmids among inpatients

Univariate analysis				Multivariable regression analysis			
Variable	COR[a]	*P*-value	95% CI	Variable	AOR[b]	*P*-value	95% CI
Age category	0.82	0.46	0.48 - 1.39				
Current infection status	0.46	0.12	0.17 - 1.23	Current infection status	0.31	0.1	0.07 - 1.27
Hospitalization history	0.57	0.29	0.19 - 1.63	Hospitalization history	0.57	0.41	0.15- 2.17
Gender	0.27	0.005	0.11- 0.67	Gender			
				Female	1	ref	
				Male	0.16	0.001	0.05 - 0.49
Patient hospital transfer	0.93	0.87	0.38 - 2.28	Patient hospital transfer			
				No	1	ref	
				Yes	8.82	0.05	0.98 - 79.72
Patient bed transfer	2.09	0.41	0 .36 - 12.08				
Patient ward transfer	3.39	0.15	0 .65 - 17.83	Patient ward transfer	1.52	0.68	0.21 - 11.04
Seek medical services	0.65	0.37	0.25 - 1.68	Seek medical services			
				No	1	ref	
				Yes	0.11	0.05	0.01 - 1.03
Ward of admission	0.96	0.89	0.53 - 1.73				

[a] Crude odds ratio, [b] Adjusted odds Ratio

4.2 Discussion

This study was conducted to characterize plasmids of public health relevance from bacterial isolates to determine their prevalence, effects on pathogenicity and the risks of antibiotic resistance development and spread among inpatients in a tertiary health care facility in northern Tanzania.

In the present study a high proportion of clinical bacterial isolates from inpatients at KCMC hospital was found to carry plasmids. The present findings are in concordance with previous studies elsewhere (Devi *et al*., 2019). The observed high carriage of plasmids by the analyzed isolates might plausibly be a reflection of resistance selection pressure due to high antibiotic exposure in hospital settings (Sonda *et al*., 2016).

Escherichia coli isolates were the most prevalent carriers of single-replicon plasmids followed by *S. aureus* and *P. mirabilis.* On other hand, *K. pneumoniae* were the most prevalent carriers of multiple-replicon plasmids, followed by *S. aureus* and *E. coli*. The present study findings are in line with study results in a tertiary care hospital in south India (Devi *et al*., 2019). Possible explanation could be that the mentioned bacterial species have great medical relevance and thus are relatively more often isolated in hospital settings compared to other species (Malachowa & Deleo, 2010; Ramirez *et al*., 2019). Additionally, single and multiple-replicon plasmids carrying resistance and virulence genes in *E. coli* and *K. pneumoniae* may reflect their ability to infect humans and resist against antibiotic classes, but also converting other susceptible bacterial strains to be resistant and virulent through sharing of the genes.

Furthermore, the present study findings show a larger proportion of *P. mirabilis* carrying plasmids than other relevant studies in South India (Devi *et al*., 2019). This difference might be due to the fact that majority of the present study isolates were from wound specimens in which *P. mirabilis* were identified (Endimiani *et al*., 2005; Mordi & Momoh, 2009), the sample size and the population of interest. Additionally, larger proportion of *P. mirabilis* carrying plasmids probably indicates high rate of sharing *P. mirabilis* intrinsic resistance genes to tetracyclines, nitrofurantoin, colistin, clindamycin, polymyxins, fusidic acid, glycopeptides, macrolides, rifampin with other bacterial species through HGT (Decôme *et al*., 2020; Gogry *et al*., 2021; Nte *et al*., 2021).

This study identified bacterial species with low plasmid prevalence including *Enterobacter sp. n18-03635, Enterobacter kobei, Klebsiella variicola* and *Klebsiella oxytoca.* The study findings are consistent with other studies conducted in Canada, Greece and Mexico (Boyd *et al*., 2020; Rodríguez-Medina *et al*., 2019; Tsakris *et al*., 2011) where these microbes had low plasmid prevalence. Also, the study revealed low plasmid prevalence species that were reported elsewhere in soil, fish and pigeon samples (Bhassu *et al*., 2019; Dai *et al*., 2018; Manter *et al*., 2011) which are *Micrococcus sp. Kbs0714, Enterobacter soli* and *Proteus columbae*. The observed species with low plasmid prevalence might be due to their rarity and in most cases misidentification in clinical isolates (Jousset, 2019; Potter *et al*., 2018). However, reports of bacterial species with low plasmid prevalence might indicates the possible emerging and transmission of bacterial pathogens in humans' community and hospital settings (Rodríguez-medina *et al*., 2019).

Previous studies reported that the IncF plasmid group in *E. coli* carried resistance and virulence genes concurrently more often (Beceiro *et al*., 2013). Reciprocally, IncQ1 plasmid in *E. coli* in this study reported to carry the highest number of both resistance and virulence genes. Studies conducted in Brazil agreed with findings of this study (Cassiano *et al*., 2018; Cerdeira *et al*., 2019) where IncQ1 plasmid was predominant carrier of both resistance and virulence genes. This is possibly due to the fact that IncQ1 plasmids have high-level mobility, stability, replication at high copy number and transferred in wide range of bacterial species through conjugative plasmids (Erchia & Pazzani, 2016; Lima *et al*., 2020; Mcmillan *et al*., 2020; Rawlings & Tietze, 2001). Furthermore, IncQ1 plasmids in *E. coli* isolates may facilitate sharing of resistance and virulence genes with other more different species in hospital settings in Tanzania that can challenge the treatment of bacterial infections compared to IncF narrow range and low copy number plasmids.

In this study it was also identified that there are different multiple-replicon plasmids ranging from two to seven plasmids. This is probably an indicative of bacterial evolution to adapt and thrive in hospitals where they are excessively exposed to antimicrobials, antiseptics and disinfectants (Donnell, 1999; Smalla *et al*., 2015a; Xie *et al*., 2018). A similar distribution of some multiple-replicon plasmids in other regions carrying similar or different antibiotic resistance and virulence genes was noted in the present study.

This suggests that resistant bacteria arising in one geographical area can spread countrywide/worldwide either by direct exposure or through the food chain or climate change and the environment (Iskandar *et al*., 2020).

There was no significant relationship found in the present study between numbers of antibiotic resistance and virulence genes in plasmids ($r = -0.14$, $p > 0.05$). However, this might indicate that acquisition of antibiotic resistance can induce the loss of virulence factors. Previous studies support this study finding (Pan *et al*., 2020b), but does not agree with a study by Dionisio (2019). This discordance might be due to the fact that in other studies the relationship between resistance and virulence genes was determined at species level and were from gut and environmental samples (Beceiro *et al*., 2013).

The present study investigated the possible risk factors for carrying a pathogen with resistance to at least one antibiotic. The findings showed that sex is significantly associated with carrying pathogens with resistance to at least one antibiotic among inpatients. The findings are consistent with other reports on risk factors for antibiotic resistance development in healthcare settings reported by Chen *et al*. (2021). The study reported that males had lower likelihood risk of carrying pathogens with resistance to at least one antibiotic compared to females. The reason for the female susceptibility to microbial resistance may be attributed to more frequent use of antibiotics by females, who tend to more frequently attend hospitals/or seek health care services when they fall sick compared to male counterparts. This behavior may lead to more frequent and sometimes unnecessary prescription of antibiotics to females. The study findings contrast with a report from Switzerland which shows males had 2 fold higher resistance rates of their pathogens than females (Erb *et al*., 2018). The difference might also be linked to biological or social factors. For example anatomical and hormonal differences that can influence the course of infections, pregnancy, abortion and childbirth that can contribute exposure to antibiotics that more likely lead the development of antibiotic resistance (ReAct, 2020).

Furthermore, the difference in risk factors for resistance across Tanzania and Switzerland might be due to difference in antimicrobial use guidelines/policies (Seni *et al*., 2020), expensive and unavailability of reserve antibiotics in Tanzania's health care facility settings (Id *et al*., 2020).

There was no significant risk difference of carrying pathogens with resistance to at least one antibiotic between patients transferred from another health care facility to KCMC and those who were not transferred. This might indicates that AMR is so widespread and most likely exists in all hospitals in Tanzania (Haber *et al*., 2010; Mulvey & Simor, 2009; Sangeda *et al*., 2021; Seni *et al*., 2020; Sindato *et al*., 2020), and that an effect cannot be seen anymore.

Interestingly, non-significant risk difference of carrying pathogens with resistance to at least one antibiotic between patients that sought health care services before attending KCMC and those who came to KCMC directly was found. This may be explained by the fact that in Tanzania taking antibiotics is not always linked to a visit to healthcare services (Mburu *et al*., 2021).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Klebsiella pneumoniae isolates were found to be the most carriers of multiple-replicon plasmids (17, 28.3%), while *E. coli* isolates carried most single-replicon plasmids (23, 23.2%). This underscores clinical relevance of monitoring these common pathogens in poor resource settings, looking on plasmids that might contribute to their dominance. Furthermore, females were found to carry pathogens with more resistance compared to males in the current study probably because of biological and social factors.

5.2 Recommendations

Following the results, the recommendations are to:

(i) Implement hospital antimicrobial surveillance and stewardship to limit inappropriate use of antimicrobials.

(ii) Use new antibiotic drugs and anti-virulence molecules to fight against the emergence and dissemination of antibiotic-resistant strains with virulence factors. However, previous drugs that have not been used for sometime may be used for medication.

(iii) Conduct further research to collect more evidence that will help to curb the spread of these public health relevant plasmids.

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RESEARCH OUTPUTS

(i) Publication

Sengeruan, L. P., van Zwetselaar, M., Kumburu, H., Aarestrup, F. M., Kreppel, K., Sauli, E., & Sonda, T (2022). Plasmid characterization in bacterial isolates of public health relevance in a tertiary healthcare facility in Kilimanjaro, region Tanzania. *Journal of Global Antimicrobial Resistance*, 30 (384 - 389). https://doi.org/10.1016/j.jgar.2022.06.030

(ii) Poster Presentation