Pathotyping and Antimicrobial Susceptibility Profile of Diarrheagenic *Escherichia coli* Isolated from Children in an Urban Informal Settlement, Nairobi

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Research thesis presented in the department of medical microbiology and immunology in partial fulfillment of the requirement for the award of Master of Science degree in Medical Microbiology at the University of Nairobi

Declaration of originality

This research thesis is my original academic work and to the best of my knowledge, it has not been presented anywhere else.

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Dedication

I dedicate this research thesis to my entire family, especially my daughter Ciana Chemutai and mum Domtila Ngetich who have been my greatest source of motivation and inspiration to work hard. To God I dedicate this work, as this journey began with a leap of faith.

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List of Abbreviation

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility tests
CLSI	Clinical and laboratory standards institute
CDC	Center for Disease Control and Prevention
DAEC	Diffusely adherent *Escherichia coli*
DEC	Diarrheagenic *Escherichia coli*
ESBL	Extended Spectrum Beta-Lactams
E. coli	*Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
DAEC	Diffusely adherent *Escherichia coli*
PCR	Polymerase Chain Reaction
US	United States
UTI	Urinary Tract Infections
UK	United Kingdom
WHO	World Health Organization
HUS	Hemolytic Uremic syndrome

LEE	Locus for enterocyte effacement
LT	Heat labile enterotoxin
ST	Heat stable enterotoxin

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Abstract

Introduction

Diarrhea is among the primary contributors of mortality in children less than five years old globally. Diarrheagenic *Escherichia coli (DEC)* is the prime causative agent of childhood diarrhea in underdeveloped nations. The distribution of DEC pathotypes varies geographically, and in informal settlements, their occurrence is driven majorly by poor hygiene and sanitation. In addition, the lack of capacity for laboratory identification and antibiotic resistance in these settings poses a great challenge to management of childhood diarrhea. Therefore, detecting the predominant pathotypes of diarrheagenic *E. coli* informal settlements is key for proper management of childhood diarrhea.

Methodology

A laboratory-based cross-sectional study was undertaken to analyze presumptive *Escherichia coli* isolates from children below five years old presenting with acute diarrhea in three outpatient clinics at Mukuru, Nairobi, between January 2017 and September 2018. Identification, antimicrobial susceptibility testing (AST) and detection of Extended spectrum beta-lactamase (ESBL) positive DEC were determined using Vitek 2 System. Pathotyping of DEC isolates was performed using singleplex PCR and the amplicons run through gel electrophoresis. Proportions and frequency distributions of DEC pathotypes, AST profiles and ESBL positive DEC were computed and presented in tables, charts, and graphs.

Results

Of the 383 bacterial isolates, 175 were identified as *E. coli*. Among the *E. coli* isolates, 27% were DEC. These comprised of; Enteroaggregative *E. coli* (19%; 34/175), Enterohaemorrhagic *E. coli* (5%; 9/175), and Enteropathogenic *E. coli* (3%; 5/175). Overall, DEC isolates were resistant to cefazolin (90%; 43/48), ampicillin (83%; 40/48), ampicillin-sulbactam (77%; 37/48), and trimethoprim-sulfamethoxazole (83%; 40/48) but susceptible to amikacin, tigecycline, and carbapenems (100%). Resistance to ciprofloxacin (31%; 15/48), gentamicin (14%; 7/48), ceftriaxone (8%; 4/48), and cefepime (8%; 4/48) was also reported. A total of (17%; 8/48) of these isolates were ESBL positive.

Conclusion

This study reports the predominance of Enteroaggregative *E. coli*, high resistance to penicillins and cephalosporins antibiotics and ESBL positive DEC isolates in our study population.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Diarrhea is a common cause of fatalities in children under the age of five: giving rise to roughly one out of twenty seven child fatalities globally, with 80% of these happening low-middle income countries (LMICs) (WHO, 2020). In Kenya, it accounts for 17% of all childhood diseases, with children under age group zero to five experiencing at least three incidences of diarrhea yearly (KNBS, 2008; Saka et al., 2019).

The primary etiologies of diarrhea are bacteria, viruses, and parasites. Among the bacteria, diarrheagenic *Escherichia coli* (DEC) accounts for 30–40% of acute diarrhea events in children under five years in developing nations, and a consequential driver of periodical infections and diarrheal outbreaks worldwide (Saka et al., 2019). Despite being a gut microbiome, *E. coli* has the ability to integrate virulence genes, which enhances their capacity to adjust to various diverse habitats thus causing numerous diseases. To date, six DEC have been described: Enterotoxigenic *E. coli* (ETEC), Shiga-toxin producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC), and Enteroaggregative *E. coli* (EAEC), each with distinctive virulence and pathogenic mechanisms (Acosta et al., 2016). The common types of diarrhea associated with DEC include childhood and traveler's diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome, acute and tenacious diarrhea across the ages, and infant watery diarrhea linked to ETEC, EHEC, EAEC and EPEC, respectively (Acosta et al., 2016). Each of these pathotypes have been previously described in clinically symptomatic children in African countries (Sang, Oundo, et al., 2012).

The management of enteric and microbial-associated diarrhea continues to be a problem worldwide, which can be attributed in part to resistance to commonly used antibiotics (WHO, 2017b). Antibiotic resistance surveillance across 22 countries revealed widespread resistance among 500,000 patients suspected of having bacterial infections caused by *E. coli* (WHO, 2017b). Environmental factors including inadequate sanitation, hygiene practices and limited access to clean water play a crucial part in the transfer of resistant microorganisms among populations living in informal settlements. Additionally, in informal settlements, where healthcare facilities are inadequate, behaviors such as self-medication and the purchase of antibiotics over-the-counter are common, which further exacerbates the threat of antimicrobial resistance (AMR) (Kariuki et al.,

2020). The first multidrug resistant Enteroaggregative *E. coli infection* in Kenya was reported in Malindi, and these strains seem to have spread in most parts of the country 15 years later (Evalyne, 2017; Nganga, 2018; Omondi, 2018). This clearly indicates the possibility of spread of antimicrobial resistant microorganisms across the country if suitable interventions are not imposed (Sang et al., 1997; Sang, Oundo, et al., 2012). Consequently, high resistance of diarrheagenic *E. coli* to commonly dispensed antibiotics have also been reported in recent studies in Kenya (Evalyne, 2017).

Despite the existence of data on DEC pathotypes in Kenya, frequent surveillance and monitoring is critical, particularly in informal settlements where numerous diarrheal disease outbreaks are reported (Nganga, 2018). Additionally, owing to the global spread of multidrug resistant DEC pathotypes, the antibiotic susceptibility of these pathotypes must be determined to guide future management of *E. coli* related diarrhea. Although *E. coli* is routinely isolated from children presenting with diarrhea, virulence genes are not routinely detected especially in laboratories located in informal settlements in Kenya, and thus the proportion of diarrhea caused by DEC is insufficient. Hence, this study's goal was to ascertain the proportion, antimicrobial susceptibility profiles and ESBL positive DEC isolates from children attending three clinics in Mukuru, a Nairobi-based urban informal settlement.

CHAPTER TWO: LITERATURE REVIEW

2.1 *Escherichia coli*

Escherichia coli (*E. coli*) is a gram negative, rod shaped, and facultative anaerobe occurring in the intestines of animals. It was first isolated from infant stool and characterized as a short-slender rod in 1885 by Theodor Escherich; he first named it Bacterium *coli* (Escherich, 1988). It was further studied and described by other researchers and later renamed *Escherichia coli* in 1954 (Croxen et al., 2013). *E. coli* is ubiquitous in nature, which explains its diversity. It inhabits infants' gut soon after birth and afterwards establishes an association with the host resulting in a mutual benefit (Croxen et al., 2013). While being a common symbiont, pathogenic *E. coli* strains have evolved through gene gain and loss hence becoming virulent, potentially causing intestinal and extra intestinal infections which can be acquired in the community or in hospital setup. It causes disease especially when a host is debilitated, immunosuppressed or when gastrointestinal barrier is breached (Croxen et al., 2013). Extra-intestinal pathotypes of *E. coli* are key in causing non-gastrointestinal infections e.g., wound infections, urinary tract infections, and pneumonia (Robins-Browne et al., 2016).

2.2 Mode of transmission of DEC

Most pathotypes of *E. coli* are spread through contaminated food and water. Animals also act as reservoirs of some of the strains and therefore infection may occur following direct contact with infected animals. Poor sanitation and hygiene are common drivers of childhood diarrhea in slums and urban dwellings of Sub-Saharan Africa (Croxen et al., 2013). Human to human infections also occur among children in day care, schools, and nursing homes. The transmission dynamics of DEC pathotypes are illustrated in figure 1 below.

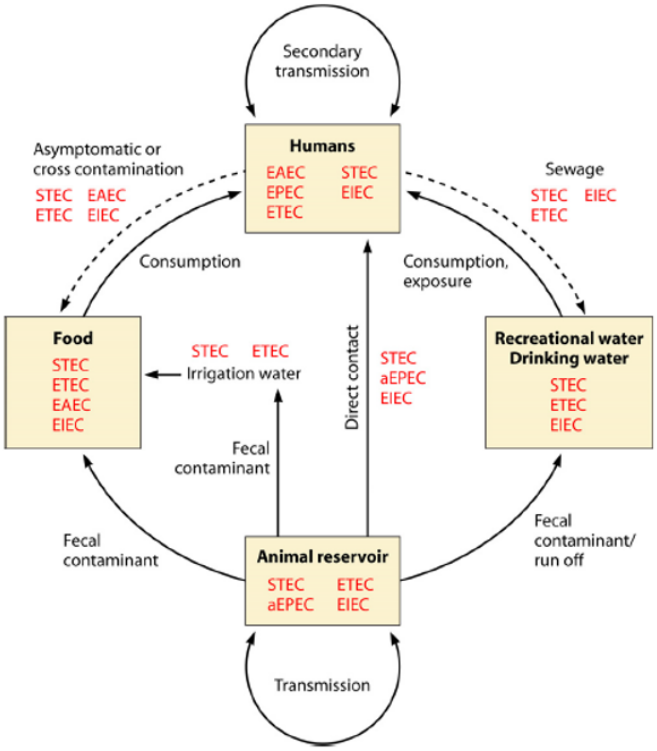


Figure 1. The potential reservoir and various modes of transmission of pathogenic *E. coli*. Animals act as reservoir of some of the pathotypes and hence can spread to humans. Humans become infected following ingestion of soiled food and water or by exposure to infected animals. Secondary spread occurs through several ways e.g., human to human transmission especially in daycares or nursing homes, food contamination due to poor cooking practices and through asymptomatic or symptomatic food handlers who contaminate food due to poor hygienic practices. Improper human waste disposal may contaminate drinking or recreational water. Adapted from (Croxen et al., 2013).

2.3 Diarrheagenic *Escherichia coli* pathotypes

2.3.1 Enteropathogenic *E. coli*

Enteropathogenic *E. coli* (EPEC) was primarily discovered and described in 1995 amid a number of infant diarrheal outbreaks in 1940s and 1950s (Neter et al., 1955). It is correlated with persistent diarrhea in adults and children but higher risk of infant mortality has been recorded worldwide (Al-Hasnawi et al., 2018; Kotloff et al., 2013). The emblem of EPEC's pathogenesis is the potential to induce diarrhea by attachment and effacing lesions (A/E) that form on intestinal epithelium (Trabulsi & Gomes, 2016). *In vitro*, they bind to cultured human epithelial cells forming micro

colonies on the surface of cells thus displaying a localized adherence pattern (LA) (Scaletsky et al., 1984). This leads to reduced absorption of food by the villi thus creating an osmotic pressure in epithelial cells resulting in loss of fluids and electrolytes (Trabulsi & Gomes, 2016). EPEC are grouped as follows; typical and atypical EPEC, contingent on the existence of virulence plasmid called EPEC adherence factor plasmid (pEAF), present in typical EPEC and absent in atypical EPEC (Trabulsi & Gomes, 2016). The plasmid consists of two loci, *bpf* and *per*; *bpf* encodes for type IV bundle forming pilus (BFP) which aid bacterial micro-colony evolution whereas *per* encrypt for transcriptional activator (plasmid regulator) of the locus for Enterocyte Effacement pathogenicity island (LEE) (Bieber et al., 1998; Gómez-Duarte & Kaper, 1995). Typical EPEC is more virulent than atypical, and this is explained by the presence of plasmid. Notably, typical EPEC form localized like adherence pattern whereas atypical EPEC forms characteristic aggregative adherence pattern on epithelial cells (Trabulsi et al., 2002). Therefore, typical EPEC causes profuse diarrhea often with mucus and its persistence can lead to severe malabsorption and severe malnutrition: the role of atypical EPEC in diarrhea is however not well understood (Fagundes-Neto & Scaletsky, 2000; Kuschner, 2017). Humans are the key reservoir of typical EPEC whereas atypical EPEC are found in healthy and sick people /animals (Gomes et al., 2016; Nataro & Kaper, 1998). EPEC consists of O and H serotypes, thus belonging to different serogroups. EPEC can be detected using PCR by detection of *eae* and *stx* genes. Typical and atypical EPEC are differentiated by detecting *EAF* or *bfp* A gene (Gomes et al., 2016).

2.3.2 Enterotoxigenic *E. coli*

Enterotoxigenic *E. coli* (ETEC) is the most genetically varying pathovar of *E. coli*, and it contains mobile elements like phages and plasmid. It is primarily distinguished by generation of plasmid-enciphered heat labile (LT) and heat stable (ST) enterotoxins (Fleckenstein & Rasko, 2016). Notably, it's not only a burden to humans but also imposes economic loses to farmers as it causes diarrhea in farm animals and majorly implicated in neonatal calves diarrhea (Dubreuil et al., 2016; Gomes et al., 2016). It contributes towards diarrheal diseases burden in developing nations where hygiene is a concern and affects travelers and tourists. Attack rate of 35% linked to ETEC infection was reported in a research conducted amongst Europeans visiting Mombasa, Kenya, in 2002 and as a result travel is a major factor for ETEC infection (Jiang et al., 2002). This pathotype preferentially affects children with astonishing number of infections exceeding 200 million cases

and 75,000 deaths in tropics with inadequate sanitary conditions (Gomes et al., 2016). It was also ranked among the dominant causes of childhood diarrhea in a global enteric multicenter study in 2012. This study emphasized on the need for prioritizing vaccine development for this pathotype (Kotloff et al., 2012). Due to the fact that heat-labile toxins of ETEC and cholera toxins share around 80% of molecular identity, both types of toxins exhibit comparable levels of pathogenicity (Fleckenstein & Rasko, 2016; Robins-Browne et al., 2016). This pathotype is non-invasive and encodes colonization factors which facilitate attachment to the epithelial cells of the small intestines, thereby producing toxins that creates an osmotic pressure in the cells evoking the release of huge amounts of fluids and electrolytes into intestinal lumen producing the characteristic watery diarrhea (Robins-Browne et al., 2016; Vidal et al., 2019). There are 3 principal colonization factor antigens (CFA) that are enciphered by ETEC namely; CFA/I, CFA/II and CFA IV; currently targets for vaccine development (Vidal et al., 2019).

Surface antigens consist of both O and H antigens, with at least 78 and 34 antigens respectively, with the common antigens being O6:H16 (LT/ST), O8:H9 (ST only), O25: NM (LT only), O78:H12 (ST only), O148:H28 (ST only), and O153:H45 (ST only). However, O169:H41 is an emerging pathotype in the US (Croxen et al., 2013). The majority of human-isolated strains contain ST toxins (Robins-Browne et al., 2016). Reportedly, children who survive repeated episodes of diarrhea caused by ETEC develop impaired growth, obesity and flattering, this underlines the need for effective interventions especially in LMICs (Zegeye et al., 2019). Detection of ETEC is achieved by evaluating the production of ST toxins.

2.3.3 Enteroaggregative *E. coli*

It was first recognized in 1987 by its Aggregative Adherence (AA) pattern which formed stacked brick structure on top of epithelial cells (Gomes et al., 2016; Nataro et al., 1987). The virulence of Enteroaggregative *E. coli* (EAEC) is primarily mediated by adhesins, toxins, and secreted proteins; the vast majority of virulence elements are encoded on the plasmid. Adhesins have been more studied because they initiate the AA pattern and the building of biofilms (Gomes et al., 2016). The formation of AA pattern is due to the existence of aggregative adherence fimbriae (AAF/I), AAF/II and AAF/ III), whose functionality is regulated by a plasmid-encoded *aggR* gene and is responsible for its pathogenicity (Dudley et al., 2006). EAEC disease is marked by mucoid watery diarrhea, and is accompanied by stomach pain, vomiting and low grade fever and can be self-limiting or

protracted depending on host factors such as immunity, nutritional status and heredity (Gomes et al., 2016; Lima & Guerrant, 1992). EAEC is often correlated with diarrhea in people of all ages worldwide, with a documented high prevalence in pediatrics in industrialized nations (Gomes et al., 2016; Rajan et al., 2018; Tobias et al., 2015). Consequently, a novel EAEC serogroup 0104:H4 has been reported to be highly virulent due to its ability to produce shiga toxins (Navarro-Garcia, 2014). It has been linked to outbreaks in Kenya (Ochi et al., 2017) and has been documented in recent Kenyan studies (Evalyne, 2017; Nganga, 2018; Omondi, 2018).

2.3.4 Enterohaemorrhagic *E. coli* / Shiga-toxin *E. coli*

Enterohaemorrhagic *E. coli* (EHEC) is a prominent foodborne pathogen originally identified in the USA amid two epidemics of hemorrhagic colitis (HC) in 1982 (Nataro & Kaper, 1998). Its virulence determinant is a phage-encoded Shiga toxin (Verotoxin) (Robins-Browne et al., 2016). It is linked to both bloody and non-bloody diarrhea and a major foodborne pathogen linked to diarrhea in most developed nations (Jiang et al., 2002; Sang et al., 2012). EHEC infections ranges from moderate diarrhea to extreme manifestations like hemorrhagic colitis and hemolytic uremic syndrome(HUS), most countries have also reported cases of acute renal impairment in babies infected with this pathotype (Gomes et al., 2016). The disease was first reported in Kenya among the Maasai children and adults presenting with bloody diarrhea (Sang et al., 2012) EHEC harbor chromosomal pathogenicity island termed “locus of enterocyte effacement” (LEE) encoding the type (III) secretion system and adhesin (intimin) and its receptor Tir; responsible for attachment and effacing phenomena on enterocytes like in EPEC (Joseph et al., 2020). Moreover, the capacity of this pathotype to bind to, colonize, and develop biofilm on any surface plays a part in its transmission (Gomes et al., 2016). Among the many serotypes causing infections in humans, serotype 0157:H7 is commonly associated with global diarrhea outbreaks, utmost cases of diarrhea, and HUS (Gomes et al., 2016).

Domestic animals like goats and cattle act as natural reservoirs of this serotype, therefore playing a role in zoonotic transmission. Previous studies have documented dairy products as vehicle of transference of EHEC to humans (Dulo et al., 2015; Sang et al., 2012). Animal products such as meat and milk are common nutritious food fed on children at an early age for their growth and development, putting them at risk for infection with this pathotype; hence, routine screening is important, particularly in children presenting with diarrhea.

2.3.5 Enteroinvasive *E. coli*

Enteroinvasive *E. coli* (EIEC) was first describe in 1947 (Ewing & Gravatti, 1946). It is a usual etiology of human dysentery in LMICs because of poor sanitation therefore children living in informal settlements are at a higher risk (Robins-Browne et al., 2016). Because of its similarity to Shigella, it is presumed to have evolved from the same pathogen source (Pasqua et al., 2017). It consists of F type plasmid termed pINV, which encodes a type 3 secretion system. This system enables EIEC to penetrate the epithelial cells and induce disease (Marteyn et al., 2012). Diarrhea occurs as a result of enterocyte invasion and penetration, where it attaches to large intestine mucosa and invades via endocytosis (Gomes et al., 2016; Pasqua et al., 2017). It is commonly linked to diarrhea outbreaks worldwide, and while the fecal oral route plays a major role in disease transmission, person to person transmission also occurs (Nataro & Kaper, 1998). In previous research, dairy products and vegetables were linked to severe diarrhea outbreaks caused by EIEC, with serogroups 0124 and 096:H19 detected in cheese and vegetables, respectively (Nataro & Kaper, 1998). The presence of the *ipa* H *iud* A (β-glucoronidase) and *Lac* Y (lactose permease) genes distinguishes it from *Shigella* species (Nataro & Kaper, 1998; Ud-Din & Wahid, 2014).

2.3.6 Diffusely adherent *E. coli*

Diffusely adherent *E. coli* (DAEC) is a unique pathotype that attaches to the epithelial cells (HeLa or HEP-2) in a pattern called “diffuse adherent pattern”, which is not a classical adherence pattern (Nataro & Kaper, 1998). This pattern uniformly covers the whole cell surface (Scaletsky et al., 1984). Afa/Dr family adhesins, which are responsible for adherence, they are found in around 75% of DAEC (Mansan-Almeida et al., 2013). The indication of DAEC in diarrhea is not well understood as it has been isolated in diarrheic and non-diarrheic children (Scaletsky et al., 2002). Equally, it’s epidemiology is also not well understood because there are no universal methods for detecting it (Croxen et al., 2013). Although the pathogenicity of DAEC that express Afa/Dr has been well documented in urinary tract infections, its function in diarrhea has not been well established (Servin, 2014).

2.4 Laboratory diagnosis of *E. coli* pathotypes

Pathogenic *E. coli* in humans is isolated from stool or rectal swab samples. The samples are initially cultured on selective media like MacConkey agar where *E. coli* grows as lactose

fermenters. Confirmatory tests for *E. coli* are then performed by use of biochemical assays such as Triple Iron Sugar test (TSI), Indole tests, and Citrate among other tests depending with the assay (Shakil et al., 2012). Pathotypes of *E. coli* are detected by use of molecular diagnostics e.g. Polymerase Chain Reaction (PCR) which can either be singleplex or multiplex and use of enzyme immunoassays such as ELISA (Croxen et al., 2013). The gold standard however for testing is PCR which involves the use of primer sequences that target virulence genes of DEC (Croxen et al., 2013). These tests are sensitive and timely; however, they are expensive, thus not routinely performed in most laboratories in LMICS. Typing of DEC can also be done by; typing of O and H antigen through agglutination assays, restriction fragment length polymorphism (RFLP), multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE). The use of serological assays in typing of DEC pathotypes is a traditional method used in most laboratories ,although it is less expensive compared to PCR, they are less sensitive, have low discriminatory power and time consuming (Croxen et al., 2013; Shakil et al., 2012). Advanced methods for detection of DEC such as sequencing, matrix-assisted laser desorption/ionization -time of flight (MALDI-TOF) mass spectrometry and single nucleotide polymorphism (SNP) and Multi Locus Sequence Typing analysis of whole genome sequences are currently being adopted in well-equipped laboratories (Elabbasy et al., 2021). Although these are the most effective methods, they are extremely expensive and most of tests are not available in underdeveloped countries. This study performed confirmatory identification of *E. coli* on Vitek 2 system using Gram negative (GN) cards and detection of virulence genes of DEC using singleplex PCR.

2.5 Management of diarrhoea in Kenya

According to WHO guidelines, childhood diarrhea is primarily managed by rehydration with Oral rehydration salts (ORS) and Zinc supplements administered for 10-14 days. These are absorbed in small intestines replacing lost water and electrolytes thus reducing duration of diarrheal episodes by 25% and stool volume by 30% (WHO, 2017a). Consequently, rehydration with intravenous fluids is also recommended in severe cases of rehydration. Additionally, WHO recommends consumption of nutrient rich foods and administration of ciprofloxacin for 3 days in patients presenting with bloody diarrhea after laboratory identification and isolation of the etiological agent (WHO, 2017a). Despite the existence of these guidelines, the inappropriate use of antibiotics is a

frequent practice in Kenya, both in the community and in hospital settings. This gears the emergence of antimicrobial resistance (AMR).

2.6 Antimicrobial resistance in *E. coli*

Antimicrobial resistance (AMR) arises when previously treatable microorganisms become resistant to the same antibiotics (WHO, 2021). AMR remains a worldwide problem requiring prompt action to curb its spread. Emergence of AMR is an intricate process resulting from the interaction of human, environmental, and pathogen-related factors, which is subsequently spread by several factors (Omulo et al., 2015). In 2014, AMR reports by WHO showed that AMR is "a global health challenge requiring intersectoral interventions within the government and society entirely" (WHO, 2017b). Bacterial resistance can either be natural (intrinsic) or acquired via mutations or gene transfer (transformation, conjugation or transduction) (Munita et al., 2016). The latter is more detrimental, as it induces AMR in other bacteria making it difficult to treat many infections. The majority of AMR genes in bacteria are located in transferable elements e.g., plasmids and transposons that can be acquired by bacteria in the environment or in humans and animals. *E. coli* is naturally sensitive to nearly all medically important antimicrobials, but it has high capacity to accommodate resistance genes through mutation or gene transfer, as seen in penicillin and third generation cephalosporin resistance (Poirel et al., 2018; WHO, 2017b). Additionally, because *E. coli* is a gut microbiome, it is easily susceptible to antibiotic selective pressure.

Drug resistant *E. coli* is problematic both in humans and veterinary practice. The most troublesome mechanisms of resistance in *E. coli* results in accession of genes encoding extended spectrum-β lactamases (conferring resistance to broad spectrum cephalosporins) , carbapenemase (conferring resistance to carbapenems), 16s rRNA methylases (conferring pan- resistance to aminoglycosides), plasmid mediated quinolone resistance (PMQR) (conferring resistance to fluoroquinolones) and *mcr* genes (conferring resistance to polymyxins) (Poirel et al., 2018). Infections caused by multidrug resistant (MDR) and ESBL producing *E. coli* are life endangering making it one of the priority bugs of concern by WHO (Pormohammad et al., 2019). In sub-Saharan Africa (SSA), the circumstance is exacerbated by insufficient sanitation, insufficient water and growing number of immunosuppressed individuals, like HIV patients, all contribute to the emergence and rapid

dissemination of resistant pathogens within the population (Kariuki & Dougan, 2014; Omulo et al., 2015).

2.7 Extended Spectrum Beta lactamase

β-lactams antibiotics are broad spectrum antibiotics which serve as alternatives for therapy when first-line antibiotics fail. Therefore, resistance to these antibiotics has a great clinical significance (Kiiru et al., 2012). β-lactamase enzymes encode for resistance to antibiotics like penicillins, cephalosporins, cephamycins and carbapenems hence are of great concern in health. They act by hydrolyzing β -lactam component on antibiotics rendering them non-functional. The commonly described ESBLs are SHV, TEM, CTX-M and OXA and have been reported worldwide (Shakil et al., 2012). Enterobacterales are common producers of these diverse enzymes, and are majorly located in genetic elements such as plasmids, which ease their dissemination across different bacterial species aggravating AMR (Kurittu et al., 2021). Some are chromosomally encoded hence less problematic compared to those located in plasmids. Bacteria acquire ESBLs genes through mutations or from other bacteria (Ojer-Usoz et al., 2017). In Kenya, ESBL producing bacteria have been isolated in humans, animals and the environment, thereby a one health problem (Muloi et al., 2022; Ojer-Usoz et al., 2017). For instance, ESBL-producing EHEC that is frequently isolated from domestic animals can spread easily to people within the homestead (Franiek et al., 2012). Human colonization with ESBL producing bacteria have also been reported by studies especially in community and hospital settings (Valverde et al., 2008; Woerther et al., 2013). Consequently, community and hospital acquired ESBL producing bacteria have been reported by various studies (Shakil et al., 2012).

Children in urban informal settlements are endangered of acquiring infections or being colonized with these bugs as poor hygiene and sanitation play a part in dissemination of these bugs. Furthermore, prior hospitalization and antibiotic use are reportedly the main risk factors for infection with these bugs in hospitals (Tornberg-Belanger et al., 2022). Therefore, insufficient infection prevention and control (IPC) practices within hospitals increases the chances for dissemination of ESBL producing bacteria. Strengthening IPC units in hospitals and improved sanitary conditions in the community are effective interventions to curb the spread of ESBL producing bacteria in these settings. In Kenya, ESBLs producing *E. coli* have been reported and understanding the epidemiology of these strains is important, particularly in low-income countries

where alternative treatment may be out of reach or unaffordable for most patients (Kiiru et al., 2012). Screening for ESBL production in bacteria is done by either disk diffusion or dilution methods. These two methods are commonly used in resource limited resources settings as they are affordable. Further confirmatory tests as per Clinical and Laboratory Standards Institute (CLSI) guidelines are performed by subjecting bacteria to combination of cephalosporin and clavulanate discs on Mueller Hinton agar, and the zones of inhibition measured and interpreted as per the principles. Broth microdilution works similar to this test. There are also commercially available ESBL detection methods such as Etests, Vitek ESBL cards, and BD Phoenix Automated Microbiology System. These tests are more effective compared to the previously explained methods, however they are expensive. PCR is also one of the efficacious methods in detection of ESBLs genes in bacteria, however it is resource intensive. This study utilized ESBL Vitek cards to phenotypically identify ESBL producers among DEC isolates.

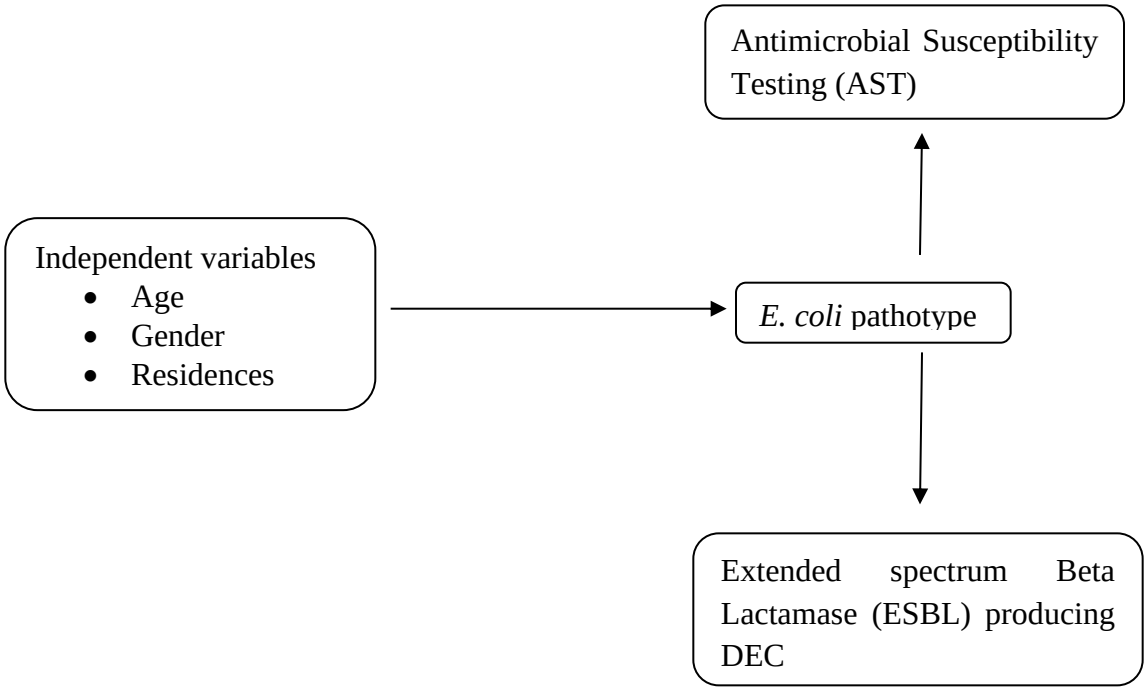
2.8 Rationale

Diarrhea is attributed to 2,195 deaths in children daily, which is more than AIDS, Malaria, and measles combined, thus second foremost determinant of mortalities in children younger than five years (CDC, 2012; WHO, 2017a). Rotavirus and *E. coli* are recurrent causes of diarrhea in children in undeveloped counties, but the successful introduction of Rotavirus vaccine has helped reduce diarrhea caused by the virus. DEC among other diarrhea causing enteric pathogens thus remains to be a threat in children. DEC alone are responsible for nearly 200,000 deaths caused by foodborne diseases globally (Mehlhorn, 2015). Even with the existing data on DEC in Kenya, the burden of DEC in children particularly in informal settlements, is not well understood since these pathotypes are spatially distributed differently depending on the seasonality, the study area and the age group (Gomes et al., 2016). In addition, poor sanitation and hygiene significantly influences the occurrence of diarrhea in urban informal settlements in Kenya and children living in these areas suffer the risk of diarrheal infections and acquiring drug resistant bugs (Ikua et al., 2021). Recent studies conducted in Kenya show that DEC are more prevalent across all ages, with multidrug resistance documented (Evalyne, 2017). Although WHO recommends prioritizing vaccines for specific DEC strains, detection of predominant pathotypes circulating in high-risk areas like informal settlements is key for planning of vaccine roll-out intervention in the near future.

Unlike other enteric pathogens, *E. coli* pathotypes cannot be identified routinely in laboratories by conventional culture, and detection relies on molecular approaches. Due to lack of resources and poor infrastructure in vast majority of African countries, laboratories located in these settings lack capacity to perform molecular diagnostics, resulting in underreporting of DEC. As a result, diarrheal infections in children are managed empirically, leading to misuse of antibiotics, thus perpetuating spread of AMR. Bacterial resistance is currently a worldwide heath menace, for instance, ESBL producing *E. coli* is among the key MDR bacteria causing extreme infections in informal settlements; it is also one of the WHO priority pathogens (Mahmud et al., 2020). Even though empirical therapy is recommended in diarrhea cases, especially in resource limited settings, antibiotic prescription should be guided by local antibiotic susceptibility profiles generated through active surveillance or testing. Therefore, this study investigated the pathotypes, antimicrobial susceptibility pattern and ESBL production in *E. coli* isolates from children under the age of 5 years in three clinics located in Mukuru slum in Nairobi County.

Conceptual framework

This illustrates the link between independent and dependent variables. As demonstrated in the figure below, independent characteristics such as age, gender, and cluster living in an urban settlement may impact the occurrence and dissemination of diarrheagenic *E. coli* pathotypes and antibiotic susceptibility.



2.9 Study questions

- What is the proportion and characteristics of diarrheagenic *E. coli* isolated from children attending the three outpatient clinics in Mukuru slum Nairobi?
- What is the antimicrobial susceptibility profile of the diarrheagenic *E. coli* isolates from the study population?
- What is the proportion of extended spectrum beta-lactamase producing diarrheagenic *E. coli* isolates in this study group?

2.10 Objectives

2.10.1 Main Objective

To determine the pathotypes, antimicrobial susceptibility profiles and ESBL positive diarrheagenic *E. coli* isolated from children attending three clinics in Mukuru, Nairobi from January 2017-August 2018.

2.10.2 Specific Objectives

- To determine the proportion of diarrheagenic *E. coli* among children attending three outpatient clinics in Mukuru slums.
- To determine antimicrobial susceptibility profiles of diarrheagenic *E. coli* recovered.
- To determine the proportion of ESBL producing strains from diarrheagenic *E. coli* isolates.

CHAPTER THREE: METHODOLOGY

3.1 Study design

This was a laboratory based cross-sectional study that was nested within a larger study titled "Integrating geographical and genetic mapping of invasive salmonella disease in children in an endemic site in Kenya: Applying genomics in the field" approved by KEMRI-SERU (SSC No.2076).

3.2 Study setting and population

Archived presumptive *E. coli* isolates from stool specimens gotten from children below five years, who had diarrhea and sought treatment at three outpatient clinics located in Mukuru informal settlement in Nairobi County: Mukuru Kwa Njenga, Mukuru Kwa Reuben and Municipal County council health clinics from January 2017 to September 2018 were analyzed. These isolates were initially identified based on their morphological characteristics on MacConkey agar plates. Mukuru is one of Kenya's largest informal settlements, located in East of Nairobi County in Embakasi constituency. It is divided into several villages and its population exceeds 100,000. The three clinics mostly serve the residents of Mukuru. Each of these facilities has at least two clinical officers and two nurses. The facilities also have a pharmacy and a laboratory and on daily basis it serves up to seven children presenting with diarrhea.

Figure 2. Map showing Mukuru informal settlement (www.google.com/maps)

3.3.1 Inclusion criteria

- Presumptive *E. coli* isolates
- Presumptive *E. coli* isolates from children ≤5 years' old

3.3.2 Exclusion criteria

- *E. coli* obtained from non-diarrheic stool
- Non-*E. coli* isolates

3.4 Sample size determination

We used Fisher's formula to calculate our desired sample size, with prevalence of 47% (Pourhoseingholi et al., 2013). The prevalence was based on a study conducted in 2018 in Mukuru by (Nganga, 2018).

$$n = \frac{t^2 p(1-p)}{m^2}$$

P=prevalence of *E. coli* 47%

q= (1-p)

m=margin of error at 5% i.e., 0.05

Therefore,

n= 383 isolates

3.5 Sampling techniques

A simple random sampling technique was utilized to select 383 *E. coli* isolates from a total of 500 presumptively identified *E. coli* archived between January 2017 and September 2018. Isolates in odd numbers were selected from freezer boxes (each with capacity of 100 isolates) till the intended sample size of 383 was achieved.

3.6 Variables

3.6.1 Independent variables

This included gender, age, and residence

3.6.2 Dependent variables

This included antimicrobial susceptibility profile, pathotypes of *E. coli* isolates and ESBL producing DEC.

3.7 Laboratory procedures

3.7.1 Revival of archived isolates

Microbiological analysis was conducted at Kenya Medical Research Institute – Centre for Microbiology Research (KEMRI-CMR) Nairobi. Prior to culture, archived isolates were retrieved from -80℃ freezer and left to thaw at 25℃ for 20 minutes. A loopful from the stock culture was sub-cultured on MacConkey agar (Oxoid, UK) and incubated at 37℃ for 18 hours. In the primary study, *E. coli* were initially identified based on their colonial morphology on MacConkey agar plate. Confirmatory testing for *E. coli* was performed on Vitek platform (GN ID, Biomerieux, France) on Vitek 2 platform.

3.7.2 Bacterial DNA extraction

Pure *E. coli* colonies were grown on Mueller Hinton agar (Oxoid, UK) and incubated at 37℃ for 18-24 hours. A loopful of the pure colonies were emulsified in 1000 µl PCR water (Invitrogen DNase RNase free water, Thermo Fisher Scientific,USA) solution and boiled for 12 minutes at 95℃ using a heating block (VWR, Avantor, USA). The solution was centrifuged (Eppendorf, UK)

for five minutes at 14000 rpm. Using pipette, 200 µl supernatant with DNA was transferred to a sterile tube for subsequent experiments.

3.7.3 DNA amplification by singleplex PCR

Singleplex PCR (PCR machine; Applied Biosystems, Thermo Fisher, USA) was used to amplify specific genes (Table 1) coding for *elt* (heat labile enterotoxins), *est* (heat stable enterotoxins), *stx* (shiga toxin), *AggR* (activator aggregative adherence regulator), *aspu* (secreted protein U gene), *IpaH* (invasion plasmid antigen), and *eae* (intimin) as described by Toma and colleagues (Toma et al., 2003). The procedure took place in a 0.2ml thin walled PCR tubes with a 27µl reaction mix that included 12µl master mix (Qiagen, Germany), 2µl primers, 12µl PCR water (Invitrogen DNase RNase free water), and 1µl DNA template (Toma et al., 2003). The cycling parameters comprised: initial denaturation at 94°C for 30 seconds, followed by 30 cycles of 94°C for 30 seconds final denaturation, 55°C (EPEC), 53°C (EAEC), 58°C (EHEC), 56°C (ETEC), 66°C (EIEC) for 1-minute annealing, 72°C for two minutes' initial extension, and final extension at 72°C for 7 minutes. Clinical isolates containing virulence genes for DEC served as positive controls whereas *E. coli* ATCC 25922 served as a negative control (Nganga, 2018).

Table 1. Diarrheagenic *Escherichia coli* pathotypes PCR primer sequences

Pathotype	Target	Sequence	References
EPEC	*eae*	F: CTGAACGGCGATTACGCGAA	(Croxen et
	881bp	R: CCAGACGATACGATCCAG	al., 2013)
EAEC	*AggR*	F: GTATACACAAAAGAAGGAAGC	(Croxen et
	254bp	R: ACAGAATCGTCAGCATCAGC	al., 2013)
	aspu	F: GCCTTTGCGGGTGGTAGCGG	(Escherich,
	282bp	R: AACCCATTCGGTTAGAGCAC	1988)
EHEC	*stx*	F: AGCTGCAAGTGCGGGTCTG	(Escherich,
	518bp	R: TACGGGTTATGCCTGCAAGTTCAC	1988)
ETEC	*est*	F: TTAATAGCACCCGGTACAAGCAGG	(Croxen et
	147bp	R: CCTGACTCTTCAAAAGAGAAAATTAC	al., 2013)
	elt	F: TCTCTATGTGCATACGGAGC	(Croxen et
	322bp	R: CCATACTGATTGCCGCAAT	al., 2013)
EIEC	*ipaH*	F: GTTCCTTGACCGCCTTTCCGATACCGC	(Kotloff et
	600bp	R: GCCGGTCAGCCACCCTCTGAGAGTAC	al., 2013)

3.7.4 Gel electrophoresis

The PCR amplicons were put through gel electrophoresis on a 2.5 % agarose gel (Agarose 0.5 × Tris-borate-EDTA) (Hi-Res Standard Agarose) for one hour. The gels were dyed with ethidium bromide (0.5 µg/ml) and gel band image viewed on ultraviolet illuminator and photographed on a gel imager (GelMax imager, Thermo Fisher,USA). The size of the amplicons was estimated using a 1kb ladder (Invitrogen, Thermo Fisher, USA, Lot number 00611464).

3.7.5 Antimicrobial susceptibility testing of *E. coli* and ESBL detection

Antimicrobial susceptibility testing (AST) and detection of ESBL positive of DEC isolates was achieved on Vitek-2 Systems (Biomérieux, France) using gram negative AST cards (AST-GN71) and examined as per 2020 Clinical and Laboratory Standards Institute guideline (CLSI M100). The list of antibacterial agents assessed against the pathotypes included penicillin (ampicillin & ampicillin/sulbactam (SAM)), cephalosporin's (cefazolin, ceftriaxone & cefepime,), aztreonam,

carbapenems (ertapenem, imipenem & meropenem), aminoglycosides (amikacin, gentamicin & tobramycin), quinolones (ciprofloxacin & moxifloxacin), tetracyclines (tigecycline), nitrofurantoin, and sulfonamide (trimethoprim/sulfamethoxazole (SXT)). *E. coli* ATCC 25922 was the control organism for this assay. Extended spectrum β-lactamase (ESBL) positive DEC isolates were identified, and results generated along with AST results (Shakil et al., 2012).

3.8 Ethical approval

The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (P512/09/2020) approved this study. Socio-demographic patient data of the archived isolates were analyzed anonymously, with no personal identifiers.

3.9 Data management and analysis

Bacterial identification and antimicrobial susceptibility outcomes were transferred from the Vitek and copied to a password protected folder in a flash disc which was then transferred to a personal computer for data cleaning. IBM SPSS Statistics version 21 was used for the analysis. Univariate analysis was done using frequency distribution and proportions for categorical variables such as gender and age category. Antimicrobial susceptibility test intervals were interpreted according to CLSI guidelines (2020).

CHAPTER FOUR: RESULTS

4.1 Patient demographics

Out of 383 revived isolates, 175 (46%) were identified as *E. coli* by Vitek 2. Majority of the *E. coli* isolates were from male children (58%) and children aged < 1 to 3 years (72%) (Table 2).

Table 2. Demographic characteristics of the study participants (N=175)

Variable	n	%
Age (years)		
< 1	34	20
1-2	46	26
2-3	48	27
3-4	25	14
4-5	22	13
Gender		
Male	101	57.7
Female	74	42.3

4.2 Pathotyping of diarrheagenic *Escherichia coli*

Out of 175 *E. coli* isolates, 48 (27%) harbored pathotype-associated virulence genes. *AggR* gene was detected in 34 isolates coding for EAEC, while *stx* and *eae* genes were detected in nine and five isolates coding for EHEC and EPEC, respectively as shown in representative gel electrophoresis images in Figure 4. EAEC was the predominant pathotype (19%; 34/175), followed by EHEC (5%; 9/175), EPEC (3%; 5/175) (Figure 3).

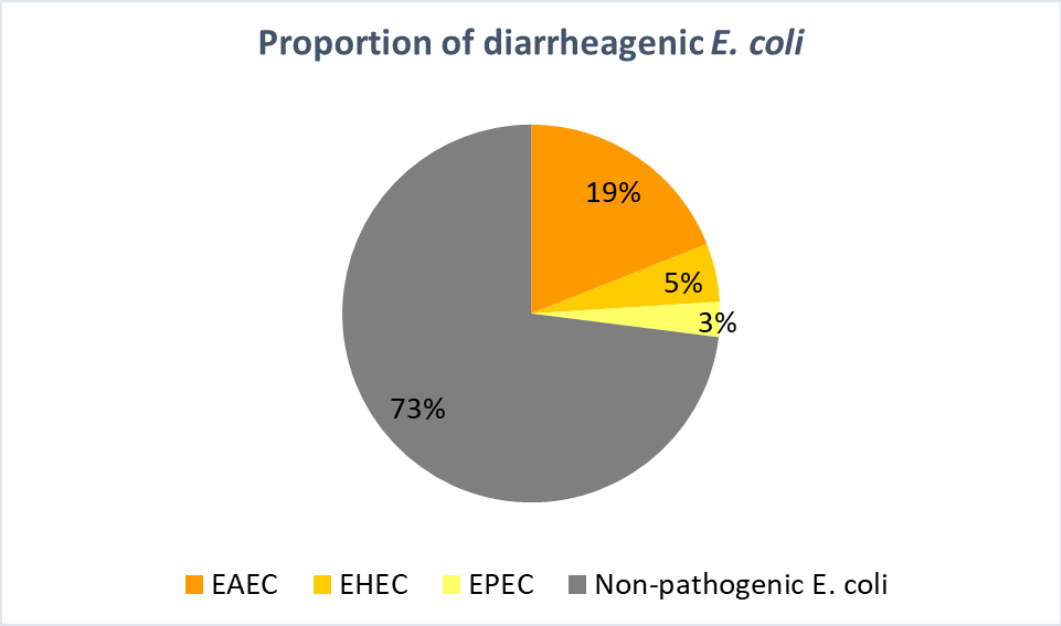
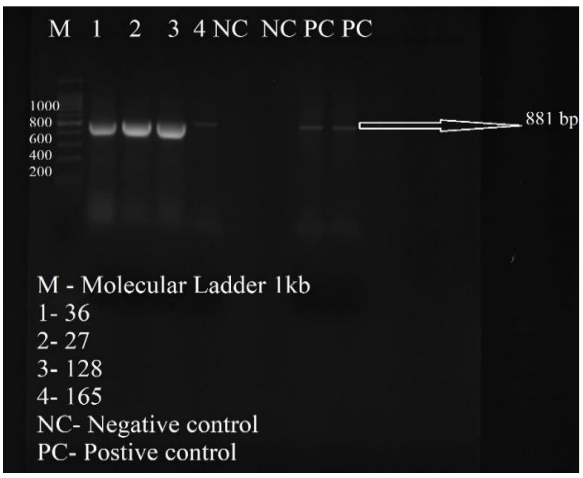
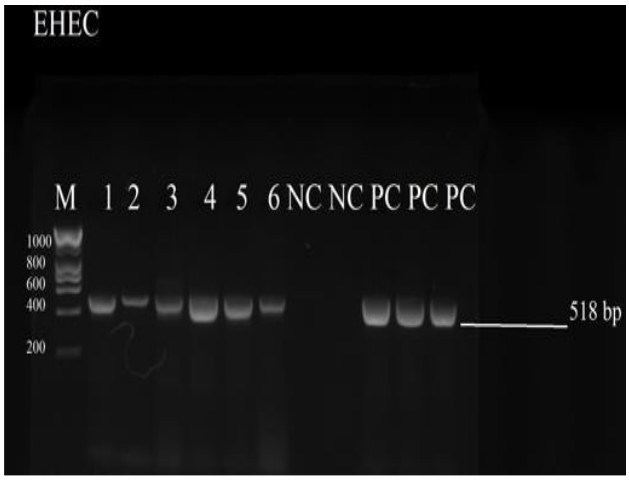


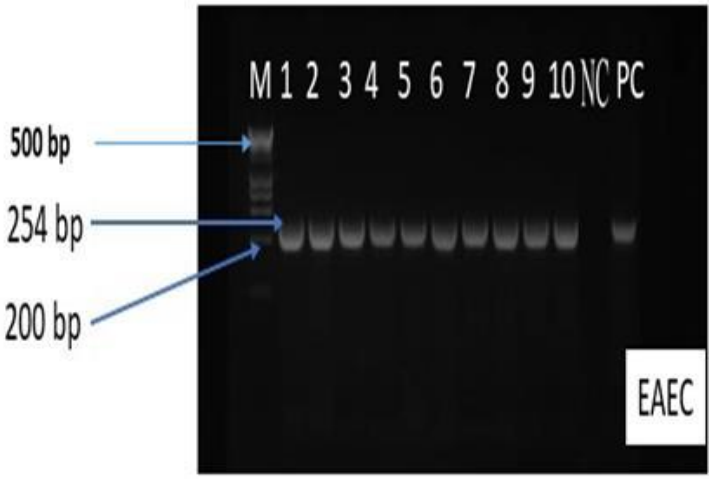
Figure 3**.** *Escherichia coli* pathotypes isolated. Abbreviations; *EAEC-Enteroaggregative E. coli, EHEC-Enterohaemorrhagic E. coli, EPEC-Enteropathogenic E. coli*



(a)



(b)



(c)

Figure 4. Gel electrophoresis representative images showing genes coding for EPEC, EHEC, and EAEC. (a) *eae* gene coding for EPEC at approximately 881 bp. (b) *stx* genes coding for EHEC at approximately 518 bp. (c) *AggR* genes coding for EAEC at approximately 254 bp. NC (negative control), PC (positive control), and M (molecular ladder).

4.3 Distribution of diarrheagenic *E. coli* pathotypes by age and residences categories

Overall, the majority of DEC (75%) occurred in children under 3 years. EAEC and EHEC were common in age group 1 to 2 years, whereas EHEC occurred in children younger than 1 year old. Most of the children who had DEC came from pipeline.

Table 3. Distribution of diarrheagenic *E. coli* pathotypes by age and residence of the children

		***E. coli* pathotypes**		
Variable		**EAEC (N=34)**	**EPEC (N=5)**	**EHEC (N=9)**
Age (years)	**n***	**n* (%)**	**n* (%)**	**n* (%)**
<1	10	6 (60)	0 (0)	4 (40)
1-2	15	11 (73)	3 (20)	1 (7)
2-3	11	8 (73)	2 (18)	1 (9)
3-4	4	2 (50)	0 (0)	2 (50)
4-5	8	7 (88)	0 (0)	1 (12)
Residences				
Bins	2	2 (100)	0(0)	0(0)
Feed the children	1	0(0)	0(0)	1(100)
Rurii	1	1 (100)	0(0)	0(0)
Falcon	5	3(60)	2(40)	0(0)
Railway	2	2(100)	0(0)	0(0)
Simbacool	2	1(50)	0(0)	1(50)
Diamond Reuben	6	2(33)	1(17)	3(50)
Pipeline	10	7(70)	2(20)	1(10)
Riara	2	2(100)	0(0)	0(0)
MCC	8	5(63)	0(0)	3(37)
Motomoto	4	4(100)	0(0)	0(0)
Sisal	1	1(100)	0(0)	0(0)
Vietnam	1	1(100)	0(0)	0(0)
48	1	1(100)	0(0)	0(0)
Wapewape	2	2(100)	0(0)	0(0)

Abbreviations: n* total DEC determined per age group and residences. EAEC (Enteroaggregative *E. coli*), EPEC (Enteropathogenic *E. coli*) and EHEC (Enterohemorrhagic *E. coli*).

4.4 Antimicrobial susceptibility profiles

Overall, pathogenic DEC isolates exhibited high resistance to cefazolin (90%), ampicillin (83%), trimethoprim-sulfamethoxazole (SXT) (83%) and ampicillin-sulbactam (SAM) (77%), but susceptible to amikacin, tigecycline, ertapenem, meropenem, and imipenem (100%). Resistance to ciprofloxacin (31%), gentamicin (14%), ceftriaxone (8%), and cefepime (8%) was also reported. 71% (34/48) of DEC were multidrug resistant (MDR), this was described by resistance more than three classes of antibiotics.

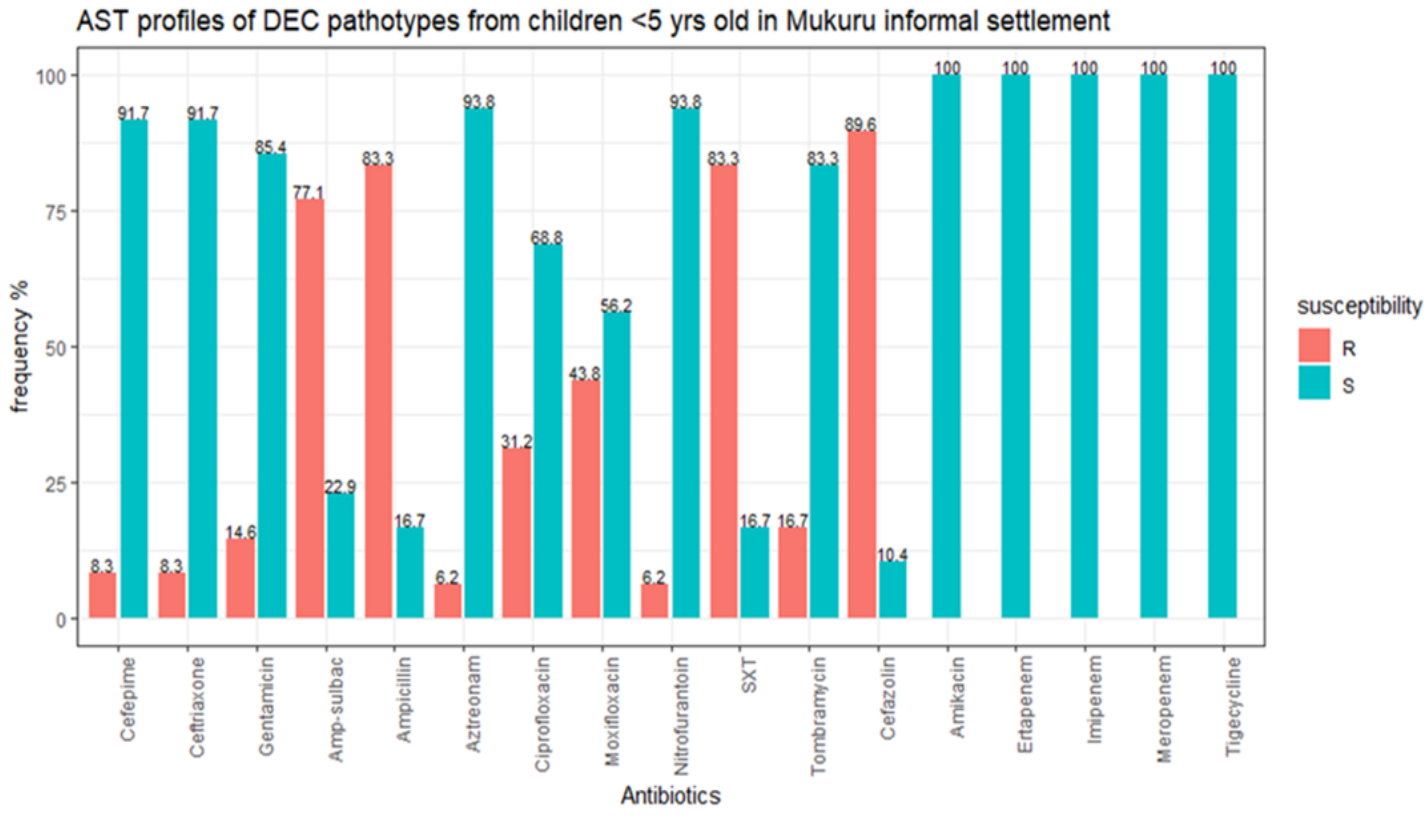


Figure 5. Overall antimicrobial susceptibility profiles of diarrheagenic *E. coli* pathotypes in children <5 years old in Mukuru informal settlement, Nairobi. Abbreviations; R-Resistant, S-Sensitive, SXT- Trimethoprim-sulfamethoxazole, AMP-sulbactam-Amppicillin-Sulbactam.

4.5 Extended spectrum beta lactamase producing DEC pathotypes

All DEC (48/48) were tested for ESBL production by use of commercial ESBL AST Vitek cards .Out of 48 DEC tested, eight (17%) were phenotypically identified as ESBL positive by Vitek as illustrated in figure 6.

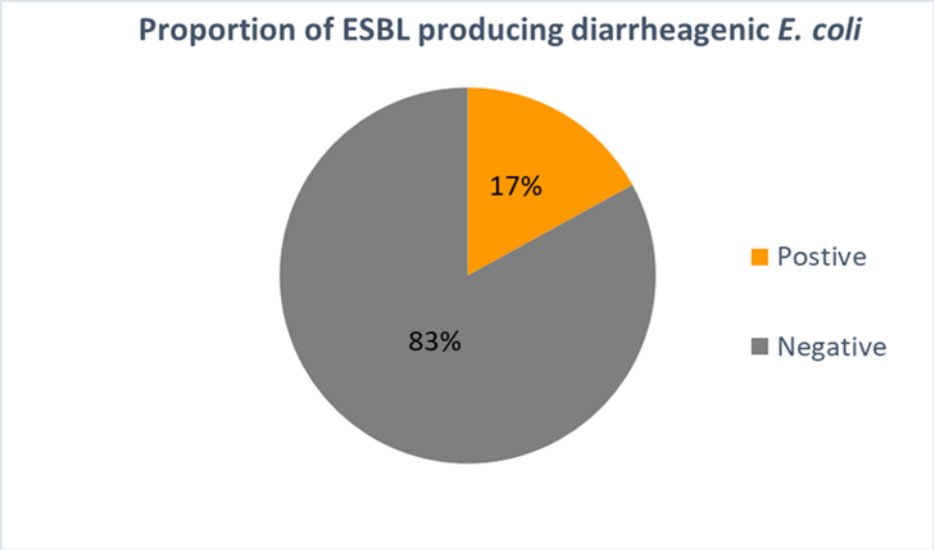


Figure 6. Proportion of ESBL producing DEC pathotypes in children <5 years old in Mukuru informal settlement, Nairobi. Abbreviation: ESBL-Extended Spectrum Beta-lactamase

CHAPTER FIVE: DISCUSSION

This study investigated the proportion of diarrheagenic *E. coli*, their antimicrobial susceptibility profiles and ESBL production among the isolates in children population below five years old in an informal settlement in Nairobi. Three pathotypes of DEC including EAEC, EHEC, and EPEC accounting for 27% of *E. coli* tested were identified. EAEC was the predominant pathotype which was similar with other studies done in Kenya (Evalyne, 2017; Shah et al., 2016b). It is a widely spread emerging pathogen (Olaniran et al., 2017; Raghavan et al., 2019; Saka et al., 2019); responsible for acute and persistent diarrhea across all ages worldwide with reported high prevalence in pediatrics in developed countries (Rajan et al., 2018; Tobias et al., 2015). This pathotype was lately outlined in diarrhea outbreak in Mandera, Kenya (Ochi et al., 2017). Among the key risk factors for EAEC infections in children include poor sanitation and hygiene, as well as being under the age of five (Evalyne, 2017; Vasudevan et al., 2003). Additionally, study by Shah and colleagues revealed that EAEC strains may persist in water for close to 60 days at standard storage temperatures, resulting in significant transmission (Shah et al., 2016a). These factors may have contributed to the high proportion of EAEC identified, hence future studies should investigate these risk factors that relate to occurrence of EAEC cases.

In contrast to previous Kenyan studies, this study documented a proportion of EHEC similar to what was reported in India, albeit with higher proportions (Raghavan et al., 2019). EHEC is a zoonotic food-borne bacteria that produces Shiga toxin and causes bloody diarrhea. In severe incidences, it might lead to life-debilitating conditions like hemolytic uremic syndrome or colitis (Ochi et al., 2017; Raghavan et al., 2019; Sang, Hamadi, et al., 2012). This pathotype has been linked to sporadic diarrhea outbreaks all over the world and as a result, seasonal variations, ecology, and host factors all play a vital role in its spread (Shah et al., 2016b). Living in close quarters with companion animals increases the risk of infection with this pathotype because these animals carry EHEC in their gut (Ferens & Hovde, 2011). However, this study did not collect data on whether households had an animal/pet, it was impossible to determine if the EHEC isolates were the result of pet ownership. This study also found that EHEC was common in children under the age of one, which might be explained by the fact that most children in this age are explorative especially within their surroundings.

In this study, EPEC was the least reported pathotype, in contrast to earlier studies done in Kenya, Nigeria, India, and Qatar (Eltai et al., 2020; Ifeanyi et al., 2015; Sang, Oundo, et al., 2012; Singh et al., 2019). EPEC is a recurrent agent of diarrhea in children globally with unravelling pathogenesis over the years, with a higher risk of deaths in infants under the age of 11 months (Chen & Frankel, 2005; Kotloff et al., 2013). A study undertaken in four provinces in Kenya in 2012 found a high prevalence of EPEC (Sang, Oundo, et al., 2012), however, its epidemiology appears to have changed, as its occurrence is rare in Kenya and even in industrialized countries where it was initially described (Nataro & Kaper, 2010). The decrease could be attributed to implementation of water, sanitation and hygiene (WASH) programs in urban informal settlements, introduction of feeding initiatives, advocacy for exclusive breasting in children since it was mostly observed in infants, the introduction of zinc supplements for management of childhood diarrhea and generally a shift in epidemiology of this pathotype (Evalyne, 2017).

In Kenya, antibiotic therapy in infant/childhood diarrhea is not recommended unless in cases of dysentery (Ministry of health republic of Kenya, 2016). However, due to lack of capacity in laboratories in low-middle income countries to perform molecular tests, particularly those in informal settlements, infections are treated empirically resulting in misuse of antibiotics (Kariuki et al., 2020). This in turn perpetuates the spread of AMR. This study reports high resistance of DEC to primary antibiotics like ampicillin, trimethoprim-sulfamethoxazole and ampicillin-sulbactam, which is in line with previous investigations in Kenya and Nigeria (Evalyne, 2017; Omondi, 2018; Saka et al., 2019). The outlined resistance to penicillin-class of antibiotics in Kenya is due to the fact that these antibiotics are routinely prescribed and readily available over the counter. On the other hand, DEC were completely susceptible to carbapenems. These are last-resort antibiotics that are not routinely prescribed in outpatient clinics in Kenya and are not readily available over the counter, resulting in the reported susceptibility. However, it is important to report on their susceptibility to measure the extent of AMR in these isolates, as gene transfer between bacteria occurs, resulting in transfer of resistant genes, escalating AMR.

In Kenya, aminoglycosides and fluoroquinolones are commonly used regimens in treatment of bacterial infections in children (Maina et al., 2020). This study found resistance to gentamicin and ciprofloxacin, which differs from the findings of a survey conducted in central region of Kenya and Burkina Faso, where all DEC isolates were susceptible to these antibiotics (Evalyne, 2017;

Konaté, Dembélé, Guessennd, et al., 2017). Aminoglycosides and fluoroquinolones resistance reported in this study has also been reflected in *Salmonella* isolates from children in this age group from same study site (Kariuki et al., 2019). This suggests resistance gene exchange between bacteria in the environment could be occurring, thereby presenting a dire health problem, that requires prompt strategies to mitigate the spread of AMR. In addition, practices such as indiscriminate antibiotic and unregulated antibiotic use, especially in developing countries, have enhanced the development of antibiotic resistance.

Cephalosporins are broad spectrum antibiotics that play a vital role in infection management. Bacterial resistance to cephalosporin's complicates illness therapy, this study found high resistance to first generation cefazolin (90%). Major factor was resistance to 4th generation cephalosporin cefepime (8%), compared to 100% susceptibility reported by Kiiru and colleagues (Kiiru et al., 2012). Hospitalization and prior antibiotic use are reportedly the main drivers for carriage and infection of cephalosporin resistant bacteria (Fulgenzio et al., 2021). The main mechanism for cephalosporin resistance in bacteria is by expression of ESBL genes which has been shown to be conjugatively transferable between bacteria (Kiiru et al., 2011). This study reports a 17% proportion of ESBL positive DEC pathotypes, this proportion is lower than the 45.2% reported in Burkina Faso (Konaté et al., 2017). *E. coli* carrying ESBL enzymes is one of the prime multidrug-resistant bacteria, linked to serious infections (Mahmud et al., 2020). ESBL positive *E. coli* among children in this study is a significant finding because Mukuru residents are at risk of acquiring drug resistant bacteria and transmitting AMR genes to other species of bacteria. As the incidence of ESBL production reported in this was low, this calls for studies to investigate the other mechanisms for resistance in these isolates as well as look into the specific ESBL gene in this region.

Study limitations

Since we used retroactive data, the findings of this study do not reflect current patient status. Moreover, due to lack of funds to collect data from the clinics we are not able to extrapolate our findings to the overall children population in Mukuru informal settlement and find the genes responsible for ESBL production. Additionally, having used archived isolates, some of the isolates lost viability and thus affected our sample size estimates. Although these restrictions had no substantial impact on our study's findings, we recommend funding for longitudinal research to provide real-time results and trends in antimicrobial resistance of DEC bacteria which is not considered routinely. This will help in management of childhood diarrhea in Mukuru informal settlements and form basis for vaccine rollout interventions in future.

Conclusion and recommendations

This study highlights the predominance of EAEC and Enterohemorrhagic *E. coli* which is a potential cause of life-threatening diseases and sporadic diarrhea outbreaks in Mukuru informal settlement. Resistance to routinely used regimens and emerging resistance to antibiotics of significant importance is also a major key finding in our study. This means that therapy is shifting towards the use of antibiotics of last resort such as carbapenems and this comes with economic implications especially in LMICs and risk of AMR to last line antibiotics. Moreover, ESBL positive *E. coli* reported in the study presents a significant public health threat in this population. As a result, there is need to increase laboratory capacity for molecular testing and continuous antibiotic resistance surveillance to aid in diagnosis and inform policy in the management of childhood diarrhea in informal settlement where the risk of diarrhea is high.

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11th November 2021

Maureen Jepleting
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APPROVED
11 NOV 2021

Dear Maureen

RESEARCH PROPOSAL: PATHOTYPING AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF DIARRHEAGENIC *ESCHERICHIA COLI* ISOLATED FROM CHILDREN IN AN URBAN INFORMAL SETTLEMENT, NAIROBI (P512/03/2020)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P512/03/2020.** The approval period is 11th November 2021 – 10th November 2022.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

PROF. M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Dean-Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
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Appendices

A. Procedure for confirmatory identification of *E. coli* on Vitek 2 system.

1. Gram negative identification cards were removed from the fridge and allowed to come to room temperature before use

2. 3mL of normal saline was aseptically transferred into a clear polystyrene 12 by 75 mm test tube using a saline dispenser.

3. 2-3 pure colonies of isolate on nutrient agar plate was picked using a sterile cotton swab then emulsified on normal saline to form a homogeneous suspension. Suspensions were placed on a cassette during preparation

4. Gram negative identification cards were removed from the box and inserted into the solution

5. Isolate identifiers were captured into a computer

6. The cassette was then placed in a filer box in Vitek 2unit for 1-2 minutes

7. Once filling was complete, the cassette was transferred into a load chamber for scanning of the cards, the cassette was then removed, and the tubes and straws disposed in a red biohazard bag.

8. Results were printed after six hours.

B. Procedure for Antimicrobial susceptibility tests on Vitek 2 system

1. Gram negative (GN71) antibiotic cards were removed from the fridge and allowed to come to room temperature before opening the package liner

2. 3ml of normal saline was aseptically transferred into a clear polystyrene 12 by 75 mm test tube using saline dispenser

3. 2-3 pure colonies of isolate on agar plate was picked using a sterile cotton swab then emulsified on normal saline to form a homogeneous organism suspension. The suspension was adjusted to attain McFarland standard using calibrated Vitek 2 DensiCHEK Plus Meter. (Range for Gram negative is 0.5-0.63). Suspensions were placed on a cassette during preparation

4. Gram negative cards were placed into the suspension

5. Isolate unique identifiable were entered into a computer

6. After data entry, the cassette was placed in a filler box on Vitek 2unit for 1-2 minutes

7. When filling was complete, the cassette was transferred into a load chamber, the cassette was then removed after loading was completed, the tubes and straw were disposed in a biohazard bag.

8. Results were printed after 6 hours.