

Escherichia coli Pathotypes in Children with Acute Diarrhea in an Informal Settlement in Nairobi, Kenya

Moureen Jepleting^{1*}, Winnie Mutai^{2,3}, Victor Moses Musyoki³, Beatrice Oduor¹, Charchil Ayodo¹, Robert Mugoh⁴, Samuel Kariuki⁴, Sylvia Omulo^{1,2,5}

¹Washington State University Global Health-Kenya, Nairobi, Kenya

²University of Nairobi Institute of Tropical and Infectious Diseases, Nairobi, Kenya

³Department of Medical Microbiology, University of Nairobi, Kenya

⁴Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya

⁵Paul G. Allen School for Global Health, Washington State University, Pullman, USA

Email: *moureen.jepleting@wsu.edu

How to cite this paper: Jepleting, M., Mutai, W., Musyoki, V.M., Oduor, B., Ayodo, C., Mugoh, R., Kariuki, S. and Omulo, S. (2023) *Escherichia coli* Pathotypes in Children with Acute Diarrhea in an Informal Settlement in Nairobi, Kenya. *Advances in Microbiology*, **13**, 181-192.

<https://doi.org/10.4236/aim.2023.134011>

Received: March 28, 2023

Accepted: April 24, 2023

Published: April 27, 2023

Copyright © 2023 by author(s) and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Diarrhea is among the leading causes of morbidity and mortality in children aged < 5 years globally. In underdeveloped countries, diarrheagenic *Escherichia coli* (DEC) accounts for 30% - 40% of childhood diarrhea cases. To identify the pathotypes involved in diarrheal outbreaks in Kenya, we analyzed archived *E. coli* isolates from children < 5 years old presenting with diarrhoea at three outpatient facilities in an informal settlement between January 2017 and September 2018. *E. coli* confirmation and antimicrobial susceptibility testing were done using the VITEK[®]2 instrument. Pathotype identification was performed via conventional polymerase chain reaction. Of 175 *E. coli* isolates, 48 (27%) were DEC pathotypes, with enteroaggregative *E. coli* (EAEC) predominating (71%, 34/48). Enterohemorrhagic (EHEC) and enteropathogenic *E. coli* (EPEC) represented 19% and 10% of isolates, respectively. Enteroinvasive and enterotoxigenic pathotypes were not identified. All DEC isolates were susceptible to amikacin, ertapenem, imipenem, meropenem and tigecycline. Conversely, most (>80%) isolates were resistant to ampicillin, ampicillin-sulbactam and sulfamethoxazole-trimethoprim. Half of all EAEC and EPEC strains were resistant to cefazolin while half of EHEC isolates were resistant to ciprofloxacin and moxifloxacin. In total, 18 resistance phenotypes were identified with “ampicillin-cefazolin-ampicillin/sulbactam-sulfamethoxazole/trimethoprim” predominating (33%, 16/48). The majority (81%) of DEC isolates were multidrug-resistant, with extended-spectrum beta-lactamase production identified in 8% of these isolates. This study highlights the predominance of Enteroaggregative *E. coli* and mul-

tidrug resistance of DEC pathotypes. Studying the epidemiology of diarrheal disease and antimicrobial resistance surveillance, will aid in identifying dominant etiological agents of diarrhea and newly emerging resistant strains in informal settlements.

Keywords

E. coli Pathotypes, Children, Diarrhoea, Informal Settlement, Multidrug Resistance

1. Introduction

Diarrhoea is the second leading cause of death among children < 5 years of age. It accounts for one out of twenty seven child fatalities globally, with 80% of these occurring in low-middle-income countries [1]. In Kenya, diarrhoea is responsible for 17% of all childhood diseases, with children < 5 years experiencing, on average, three incidences of diarrhoea annually [2] [3]. The common etiologies of diarrhoea are bacteria, viruses, and parasites. Among bacteria, diarrheagenic *Escherichia coli* (DEC) strains are responsible for 30% - 40% of acute diarrhoea episodes, and drive periodic infections and diarrheal outbreaks worldwide [2]. The introduction of the rotavirus and cholera vaccines reduced the burden of diarrhea in children by 52% - 59% globally [4] [5]. However, bacteria-associated diarrhea remains a problem in informal settlements, which have poor sanitation and hygiene, and variable access to vaccines.

Five common DEC pathotypes with distinct virulence and pathogenic characteristics have been described. These include enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (ETEC) [6]. These are associated with, among others, travellers' diarrhoea, haemorrhagic colitis, haemolytic-uremic syndrome and acute and persistent diarrhoea [6].

Antibiotic-resistant DEC isolates have been reported globally complicating the management of bacterial diarrhoea [7]. In Kenya, the first multidrug-resistant EAEC infection was reported in Malindi in 1997 [8] and is currently widely disseminated. Recent studies have reported high rates of resistance to ampicillin (AMP) and sulfamethoxazole/trimethoprim (SXT) among DEC strains [9], indicating increased transmission and the possible emergence of novel resistant strains. Nevertheless, frequent monitoring of DEC pathotypes is not conducted, and diarrhoea infections are treated empirically, potentially enhancing the selection of resistant strains. Identifying these pathogens is critical, particularly in informal settlements where diarrhoea outbreaks are commonly reported [10], and where inadequate sanitation, poor hygiene and limited access to clean water can enhance their transmission and maintenance [11]. This study investigated the DEC pathotypes associated with acute diarrhoea infections and their antimicrobial susceptibility profiles among children attending three outpatient healthcare facili-

ties within the Mukuru informal settlement in Nairobi, Kenya, to guide the management of *E. coli*-related diarrhoea.

2. Materials and Methods

Study population. We analysed archived *E. coli* isolates from stool samples of children aged < 5 years, who presented with diarrhoea at three outpatient clinics—Mukuru Kwa Njenga, Mukuru Kwa Reuben, and Municipal County Council—located within the Mukuru informal settlement in Nairobi County. These children were enrolled in an enteric fever surveillance study [12]. *E. coli* isolates collected between January 2017 and September 2018 were included.

Isolate revival. Isolates were retrieved from -80°C freezer and thawed at room temperature for 20 min. These were then plated on MacConkey agar (Oxoid, UK) and incubated at 37°C for 18 h. Single colonies were obtained from each plate and sub-cultured on Mueller Hinton agar (Oxoid, UK) at 37°C for 18 h. Confirmatory identification of pure isolates was done using the VITEK[®]2 Gram-negative identification cards (Biomérieux, France).

DNA extraction. Once confirmed as *E. coli*, isolates were sub-cultured overnight on Mueller Hinton agar. Single colonies were collected from each plate and emulsified in 1 mL of Invitrogen DNase/RNase free water (Thermo Fisher Scientific, USA) in a sterile microcentrifuge tube and boiled at 95°C for 12 min. Upon cooling, the suspension was centrifuged for 5 min at $15,366 \times g$ and 200 μL of the supernatant containing DNA transferred into a sterile Eppendorf tube for downstream analyses.

Molecular analysis. The Applied Biosystems PCR machine (Thermo Fisher, USA) was used to amplify genes 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) using select primers (Table 1). Polymerase chain reaction (PCR) was done following the protocol by Toma *et al.* [13], *i.e.*, initial denaturation at 94°C , 30 s, 30 cycles of amplification at 94°C for 30 s, annealing at 55°C (EPEC), 53°C (EAEC), 58°C (EHEC), 56°C (ETEC), 66°C (EIEC) for 1 min, initial extension at 72°C for 2 min, and final extension at 72°C for 7 min. Clinical isolates containing DEC virulence genes were used as positive controls and *E. coli* ATCC 25922 was used as a negative control [10].

Amplified PCR products were run on a 2.5% Hi-Res Standard Agarose gel (Agarose $0.5 \times$ Tris-borate-EDTA) for 1 h. The gel was stained using 0.5 $\mu\text{g}/\text{mL}$ ethidium bromide and visualized under an ultraviolet illuminator. PCR amplicon sizes were confirmed using a 1 kb Invitrogen ladder (Thermo Fisher, USA).

Antibiotic susceptibility testing. All DEC isolates were tested for susceptibility to 17 antibiotics—belonging to 11 antibiotic classes [16]—using the VITEK[®]2 AST-GN71 cards. Interpretation of antibiotic susceptibility results was based on the 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. Quality control testing for the VITEK[®]2 cards was done using manufacturer

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

Pathotype	Target	Sequence	Size
EAEC	<i>aggR</i> [‡]	F: GTATACACAAAAGAAGGAAGC R: ACAGAATCGTCAGCATCAGC	254 bp
	<i>aspu</i> [†]	F: GCCTTTGCGGGTGGTAGCGG R: AACCCATTGCGTTAGAGCAC	282 bp
EHEC	<i>stx</i> [†]	F: AGCTGCAAGTGCGGGTCTG R: TACGGGTTATGCCTGCAAGTTCAC	518 bp
EIEC	<i>ipaH</i> [†]	F: GTTCCTTGACCGCCTTCCGATACCGC R: GCCGGTCAGCCACCCTCTGAGAGTAC	600 bp
EPEC	<i>eaE</i> [‡]	F: CTGAACGCGGATTACGCGAA R: CCAGACGATACGATCCAG	881 bp
ETEC	<i>est</i> [‡]	F: TTAATAGCACCCGGTACAAGCAGG R: CCTGACTCTTCAAAAGAGAAAATTAC	147 bp
	<i>elt</i> [‡]	F: TCTCTATGTGCATACGGAGC R: CCATACTGATTGCCGCAAT	322 bp

Enteroaggregative-(EAEC), enterohemorrhagic-(EHEC), enteroinvasive-(EIEC), enteropathogenic-(EPEC) and enterotoxigenic *E. coli* (ETEC). [‡][13], [†][14], ^{*}[15].

recommended ATCC strains. Multidrug resistance was defined as resistance to at least one antibiotic in three or more antibiotic classes.

Data analysis. Data was analysed using frequency distributions and proportions for pathotypes and AST results on SPSS version 21 and Microsoft Excel Spreadsheet Software (2016).

Ethics statement. This study was approved by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (P512/09/2020).

3. Results

Diarrheagenic *E. coli* pathotypes

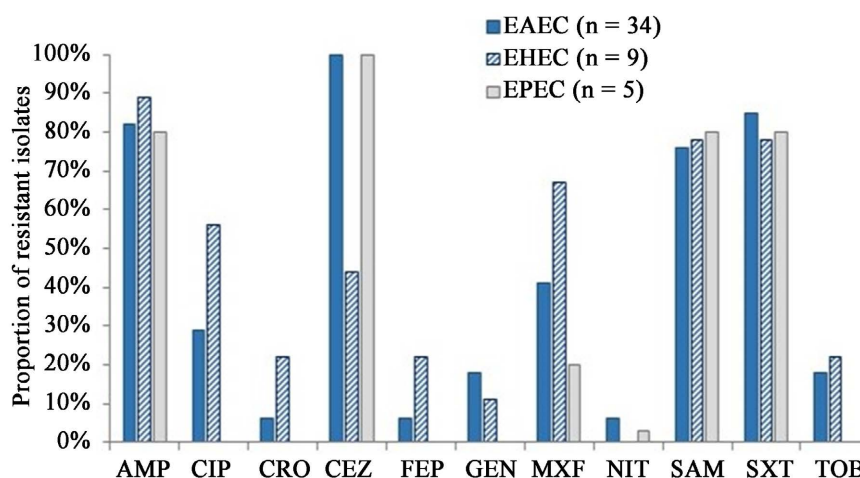
Of the 175 *E. coli* isolates analysed, 48 (27%) were pathogenic including 34 EAEC (71%), nine EHEC (19%), and five EPEC (10%). EIEC and ETEC pathotypes were not identified. Among EAEC isolates, only the *aggR* gene was detected.

Antibiotic susceptibility profiles of *E. coli* pathotypes

All DEC isolates were fully susceptible to amikacin, ertapenem, imipenem, meropenem and tigecycline. Most (>80%) were resistant to ampicillin, ampicillin-sulbactam and sulfamethoxazole-trimethoprim. All EAEC and EPEC isolates were resistant to cefazolin while EHEC where < 50% of isolates were. In general, <30% of EAEC and EHEC isolates were resistant to ceftriaxone, cefepime, gentamycin and tobramycin; EPEC strains were susceptible to these antibiotics, and ciprofloxacin. All EHEC isolates were susceptible to nitrofurantoin (**Figure 1**).

Resistance phenotypes

In total, 18 resistance phenotypes were identified among the 48 isolates (**Table 2**). The most common resistance phenotype was “AMP-CEZ-SAM-SXT”, which



AMP, ampicillin; ATM, aztreonam; CIP, ciprofloxacin; CRO, ceftriaxone; CEZ, cefazolin; FEP, cefepime; NIT, nitrofurantoin; GEN, gentamicin; MXF, moxifloxacin; SAM, ampicillin-sulbactam; SXT, sulfamethoxazole-trimethoprim; TOB, tobramycin.

Figure 1. Proportion of resistant diarrheagenic *E. coli* pathotypes identified among children < 5 years old in the Mukuru informal settlement, Nairobi.

Table 2. Resistance phenotypes of diarrheagenic *E. coli* isolates.

Resistance phenotype	EAEC N = 34 n (%)	EHEC N = 9 n (%)	EPEC N = 5 n (%)	Total N = 48 n (%)
AMP-ATM-CIP-CRO-CEZ-FEP-MXF		1 (11)		1 (2)
AMP-ATM-CIP-CRO-CEZ-FEP-MXF-SAM-SXT	1 (3)			1 (2)
AMP-ATM-CIP-CRO-CEZ-FEP-MXF-SXT	1 (3)			1 (2)
AMP-CEZ-GEN-MXF-SAM-SXT-TOB	1 (3)			1 (2)
AMP-CEZ-MXF-SAM	1 (3)			1 (2)
AMP-CEZ-MXF-SAM-SXT	1 (3)		1 (20)	2 (4)
AMP-CEZ-NIT-GEN-SXT-TOB	1 (3)			1 (2)
AMP-CEZ-NIT-MXF-SAM-SXT	1 (3)			1 (2)
AMP-CEZ-NIT-SAM-SXT			1 (20)	1 (2)
AMP-CEZ-SAM-SXT	13 (38)	1 (11)	2 (40)	16 (33)
AMP-CIP-CEZ-GEN-MXF-SAM-SXT-TOB	4 (12)	1 (11)		5 (10)
AMP-CIP-CEZ-MXF-SAM-SXT	4 (12)			4 (8)
AMP-CIP-CRO-CEZ-FEP-MXF-SAM-TOB		1 (11)		1 (2)
AMP-CIP-MXF-SAM-SXT		2 (22)		2 (4)
AMP-MXF-SAM-SXT		1 (11)		1 (2)
AMP-SAM-SXT		1 (11)		1 (2)
CEZ	4 (12)		1 (20)	5 (10)
SXT	2 (6)	1 (11)		3 (6)

Enteroaggregative-(EAEC), enterohemorrhagic-(EHEC), enteroinvasive-(EIEC), enteropathogenic-(EPEC) and enterotoxigenic *E. coli* (ETEC). AMP, ampicillin; ATM, aztreonam; CIP, ciprofloxacin; CRO, ceftriaxone; CEZ, cefazolin; FEP, cefepime; NIT, nitrofurantoin; GEN, gentamicin; MXF, moxifloxacin; SAM, ampicillin-sulbactam; SXT, sulfamethoxazole-trimethoprim; TOB, tobramycin.

was observed in 33% of isolates, and was the only phenotype identified in all three pathotypes. “AMP-CIP-CEZ-GEN-MXF-SAM-SXT-TOB” and “CEZ” were the next most common phenotypes each accounting for a 10% representation. Overall, 81% of all DEC were multidrug-resistant. Four (8%) of the 48 isolates tested positive for ESBL-production two EAEC (6%, n = 34) and two EHEC (22%, n = 9) isolates.

4. Discussion

Diarrheagenic *E. coli* are among the primary causes of diarrhea in low-middle-income countries [15] [18]. The overall diarrhea burden associated with DEC is not well defined in Sub-Saharan Africa due to poor testing infrastructure [19]. This study reports a proportion of 27% of DEC, higher than what was previously reported in four provinces in Kenya, Zambia and South Africa [11] [20] [21]. However, this is lower than the proportions reported in Uganda and central regions of Kenya [22] [23]. The distribution of DEC pathotypes varies geographically due to seasonal variations, animal interactions and poor sanitation and hygiene [9] [11] [24]. Moreover, host factors such as malnutrition and HIV status are associated with some DEC pathotypes [22] contributing to the regional and global variation of these pathotypes. The most effective therapy for DEC-related diarrhea is vaccination, but the immunological heterogeneity of the strains has, for instance, challenged the development of ETEC vaccine [25].

Three *E. coli* pathotypes were associated with diarrhoea among children aged < 5 years in our study population. EAEC was the common pathotype, consistent with previous studies in Kenya and Uganda [9] [22] [24]. EAEC is an emerging pathogen responsible for acute and persistent diarrhea across all ages worldwide [2] [26] [27], although a higher prevalence is reported among young children in developing nations [28]. Poor sanitation, inadequate water supply and compromised hygiene—conditions that are prevalent in informal settlements and which promote recurrent diarrheal episodes among children—are some of the risk factors associated with EAEC infections in children [9] [29]. Further, EAEC strains may persist in water for almost 60 days at standard storage temperatures, enhancing their transmission [29].

EHEC, which was the second most common pathotype, is typically a zoonotic food-borne pathogen which causes bloody diarrhea and kidney failure through the expression of a Type III secretion system and Shiga toxins, respectively [30]. In India, living in close quarters with domestic animals has been associated with increased risk of EHEC infections in humans [31]. Environmental transmission could also be a source for EHEC infection in children especially during their explorative stages [32]. In the Mukuru informal settlement, only 23% and 12% of households keep cats and dogs, respectively, with <10% owning livestock, suggesting that contact with domestic animals may not be a significant risk factor for EHEC infections in this setting [33]. Nevertheless, its comparatively lower detection relative to EAEC may indicate low-level exposure to animal faeces

within the environment or an alternative exposure.

EPEC was the least reported pathotype in this study contrary to earlier studies done in Kenya, Nigeria, India, and Qatar [20] [34] [35]. EPEC is a recurrent agent of diarrhoea and is associated with high mortality among children age < 11 months [36] [37]. A previous study in four counties in Kenya reported an overall prevalence of 51% among children < 5 years [20] suggesting that its epidemiology might be changing. This may explain the decreasing number of reports of its occurrence in industrialized countries than initially described [38]. In Kenya, the changing epidemiology may be attributed to overall improvements in water, sanitation, and hygiene, advocacy for exclusive breastfeeding among infants and the introduction of zinc supplements for the management of childhood diarrhea [9].

More than half of all DEC pathotypes were resistant to ampicillin, ampicillin-sulbactam, cefazolin and sulfamethoxazole-trimethoprim, consistent with previous studies in Kenya and Nigeria [2] [9] [39]. Most of these antibiotics are inexpensive oral formulations that are easily accessible and may thus be frequently used within this population. Further, most EHEC isolates were resistant to ciprofloxacin, an antibiotic to which increasing resistance has been reported among *Salmonella* sp. isolated in the same study area. This suggests possible gene transfer between bacteria in this setting and, possibly, antibiotic selective pressure [40]. In Kenya, antibiotic therapy for infant or childhood diarrhoea is recommended for the treatment of dysentery and salmonellosis, while oral rehydration solutions and zinc sulphate are recommended for the management of DEC-related diarrhoea [41]. However, low diagnostic capacities in low-middle income countries promote empiric treatment of childhood diarrhoea with antibiotics, which likely contributes to the emergence of resistant strains [42]. For informal settlements, where dense populations and unsanitary environmental conditions can facilitate the transmission of multidrug-resistant bacteria [43] [44] [45], antibiotic stewardship efforts are best complemented with water, sanitation and hygiene interventions to reduce childhood diarrhea-related deaths.

We observed resistance—albeit low—to aminoglycosides (gentamicin and tobramycin), fluoroquinolones (ciprofloxacin and moxifloxacin) and a 4th-generation cephalosporin (cefepime) among EAEC and EHEC isolates, in contrast to studies in Kenya and Burkina Faso that reported complete susceptibility to these antibiotics [9] [46]. Further, we found a low occurrence of ESBL-positive DEC pathotypes (in EHEC and EAEC only) unlike in the Burkina Faso and Uganda studies [22] [46]. Even at low proportions, ESBL-producing *E. coli* are an important cause of life-threatening infections in informal settlements, whose spread is facilitated by poor sanitation and hygiene, hospitalization and indiscriminate antibiotic use which are prevalent in these settings [47]. Their occurrence, therefore, present a public health threat that necessitates interventions to curb further emergence and spread within communities.

The observed susceptibility to carbapenems and amikacin may be due to limited access to these antibiotics as they are not prescribed at outpatient clinics in Kenya. However, misuse of locally available β -lactam antibiotics such as ceftri-

axone, ampicillin and amoxicillin may select for carbapenemase-expressing strains. For instance, hospital and community colonization with carbapenem resistant Enterobacterales have been recently reported in Kenya [16], suggesting changing antimicrobial resistance trends that requires active surveillance, monitoring and antimicrobial stewardship in the community and hospital set ups.

Multidrug resistance was common among the DEC isolates, as has been shown in studies in Kenya, Qatar, Iran and Burkina Faso [34] [48] [49] [50]. The observed resistance to penicillin, sulfonamide, cephalosporin, and quinolone drug combinations has been reported in other countries at variable levels [21] [50]. Interestingly, recent studies in Kenya that involve DEC isolates have not reported multidrug resistance phenotypes that include cephalosporin and quinolone drug combinations [39] [48], suggesting the possible emergence of novel strains. In contrast, other studies in Africa have reported multidrug-resistant phenotypes that involved penicillin, sulfonamide, colistin and tetracycline drug combinations [9] [39] [46]. Since our antibiotic panel did not include colistin and tetracycline we did not identify these phenotypes and cannot, therefore, confirm their presence in our study population.

Our study had a few limitations. By using archived isolates, we were unable to collect other data (e.g., participant demographics, persistent vs. acute diarrhea, location, antibiotic use, or coinfections) that may have helped to explain the observed findings. Further, the isolates analyzed in this study were collected from sick children who presented themselves at the study facilities, who represent a non-random subset of the population living in the Mukuru informal settlement. Consequently, our results may not be generalizable to the entire child population in this setting. Lastly, given that the DEC strains isolated may have been a secondary cause of diarrhea among the enrolled children, we were unable to conclusively assess the severity of these pathogens in this population. Longitudinal cohort studies that include a randomized population of children may be best suited to address these limitations.

5. Conclusion

This study found that EAEC contributes most to DEC-related diarrheal infections among children living in the Mukuru informal settlement in Kenya. The presence of multidrug resistant strains, particularly phenotypes that are resistant to cephalosporin and quinolone drug combinations, suggests that enhanced water, sanitation, and hygiene programs remain the best strategy for preventing the emergence and spread of diarrhoeal diseases in these settings. Understanding the epidemiology of diarrheal disease and improving antimicrobial resistance surveillance can help identify the etiological agents of diarrhea and identify newly emerging resistant strains before they disseminate widely. This will inform targeted treatment guidelines for better management of childhood diarrhea and form a basis for future vaccine interventions.

Acknowledgements

We acknowledge the Centre for Microbiology Research in KEMRI for providing the samples analyzed in this study, Washington State University Global Health-Kenya and the University of Nairobi for providing the resources to analyse these samples.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] World Health Organization (2020) Children: Improving Survival and Well-Being. <https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality>
- [2] Saka, H.K., Dabo, N.T., Muhammad, B., García-Soto, S., Ugarte-Ruiz, M. and Alvarez, J. (2019) Diarrheagenic *Escherichia coli* Pathotypes from Children Younger than 5 Years in Kano State, Nigeria. *Frontiers in Public Health*, **7**, Article 348. <https://doi.org/10.3389/fpubh.2019.00348>
- [3] Kenya National Bureau of Statistics (2008) Kenya Demographic and Health Survey 2008-2009.
- [4] Das, J.K., Tripathi, A., Ali, A., Hassan, A., Dojoseandy, C. and Bhutta, Z.A. (2013) Vaccines for the Prevention of Diarrhea Due to Cholera, Shigella, ETEC and Rotavirus. *BMC Public Health*, **13**, Article No. S11. <https://doi.org/10.1186/1471-2458-13-S3-S11>
- [5] Burnett, E., Parashar, U.D. and Tate, J.E. (2020) Global Impact of Rotavirus Vaccination on Diarrhea Hospitalizations and Deaths among Children < 5 Years Old: 2006-2019. *The Journal of Infectious Diseases*, **222**, 1731-1739. <https://doi.org/10.1093/infdis/jiaa081>
- [6] Acosta, G.J., *et al.* (2016) Diarrheagenic *Escherichia coli*: Prevalence and Pathotype Distribution in Children from Peruvian Rural Communities. *The American Journal of Tropical Medicine and Hygiene*, **95**, 574-579. <https://doi.org/10.4269/ajtmh.16-0220>
- [7] World Health Organization (2017) Global Antimicrobial Resistance Surveillance System (GLASS) Report.
- [8] Sang, W.K., *et al.* (1997) Multidrug-Resistant Enteroaggregative *Escherichia coli* Associated with Persistent Diarrhea in Kenyan Children. *Emerging Infectious Diseases*, **3**, 373-374.
- [9] Evalyne, K. (2017) Characterization and Antimicrobial Susceptibility Pattern of Diarrheagenic *E. coli* in Thika Level 5 Hospital. Master's Thesis, Jomo Kenyatta University of Agriculture and Technology, Thika.
- [10] Nganga, G.W. (2018) Bacterial and Parasitic Co-Infections, Antimicrobial Susceptibility and ESBL Genes Carriage in Bacteria Isolated from Children below 5 Years with Diarrhea Attending Mukuru Slum Health Clinics. Master's Thesis, Jomo Kenyatta University of Agriculture and Technology, Thika.
- [11] Nguyen, T.Y.C., *et al.* (2021) Diarrhoea among Children Aged under Five Years and Risk Factors in Informal Settlements: A Cross-Sectional Study in Cape Town, South Africa. *International Journal of Environmental Research and Public Health*, **18**, Article 6043. <https://doi.org/10.3390/ijerph18116043>

- [12] Kariuki, S., *et al.* (2020) High Relatedness of Invasive Multi-Drug Resistant Non-Typhoidal Salmonella Genotypes among Patients and Asymptomatic Carriers in Endemic Informal Settlements in Kenya. *PLOS Neglected Tropical Diseases*, **14**, e0008440. <https://doi.org/10.1371/journal.pntd.0008440>
- [13] Toma, C., *et al.*, (2003) Multiplex PCR Assay for Identification of Human Diarrheagenic *Escherichia coli*. *Journal of Clinical Microbiology*, **41**, 2669-2671. <https://doi.org/10.1128/JCM.41.6.2669-2671.2003>
- [14] Escherich, T. (1988) The Intestinal Bacteria of the Neonate and Breast-Fed Infant. *Reviews of Infectious Diseases*, **10**, 1220-1225. <https://doi.org/10.1093/clinids/10.6.1220>
- [15] Kotloff, K.L., *et al.* (2012) The Global Enteric Multicenter Study (GEMS) of Diarrheal Disease in Infants and Young Children in Developing Countries: Epidemiologic and Clinical Methods of the Case/Control Study. *Clinical Infectious Diseases*, **55**, S232-S245. <https://doi.org/10.1093/cid/cis753>
- [16] Ita, T., *et al.*, (2022) Prevalence of Colonization with Multidrug-Resistant Bacteria in Communities and Hospitals in Kenya. *Scientific Reports*, **12**, Article No. 22290. <https://doi.org/10.1038/s41598-022-26842-3>
- [17] Clinical & Laboratory Standards Institute (2020) CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing. 30th Edition.
- [18] World Health Organization (2017) Diarrhoeal Disease.
- [19] Kariuki, S., Kering, K., Wairimu, C., Onsare, R. and Mbae, C. (2022) Antimicrobial Resistance Rates and Surveillance in Sub-Saharan Africa: Where Are We Now? *Infection and Drug Resistance*, **15**, 3589-3609. <https://doi.org/10.2147/IDR.S342753>
- [20] Sang, W.K., Oundo, V. and Schnabel, D. (2012) Prevalence and Antibiotic Resistance of Bacterial Pathogens Isolated from Childhood Diarrhoea in Four Provinces of Kenya. *The Journal of Infection in Developing Countries*, **6**, 572-578. <https://doi.org/10.3855/jidc.2196>
- [21] Chiyangi, H., *et al.* (2017) Identification and Antimicrobial Resistance Patterns of Bacterial Enteropathogens from Children Aged 0-59 Months at the University Teaching Hospital, Lusaka, Zambia: A prospective Cross Sectional Study. *BMC Infectious Diseases*, **17**, Article No. 117. <https://doi.org/10.1186/s12879-017-2232-0>
- [22] Masiga, F., Kigozi, E., Najjuka, C.F., Kajumbula, H. and Kateete, D.P. (2022) Diarrhoeagenic *Escherichia coli* Isolated from Children with Acute Diarrhoea at Rakai Hospital, Southern Uganda. *African Health Sciences*, **22**, 581-588. <https://doi.org/10.4314/ahs.v22i1.67>
- [23] Mbuthia, O.W., Mathenge, S.G., Oyaro, M.O. and Ng'ayo, M.O. (2018) Etiology and Pathogenicity of Bacterial Isolates: A Cross Sectional Study among Diarrheal Children below Five Years in Central Regions of Kenya. *The Pan African Medical Journal*, **31**, Article 88. <https://doi.org/10.11604/pamj.2018.31.88.15644>
- [24] Shah, M., *et al.* (2016) Prevalence, Seasonal Variation, and Antibiotic Resistance Pattern of Enteric Bacterial Pathogens among Hospitalized Diarrheic Children in Suburban Regions of Central Kenya. *Tropical Medicine and Health*, **44**, Article No. 39. <https://doi.org/10.1186/s41182-016-0038-1>
- [25] Qadri, F., *et al.* (2020) Safety and Immunogenicity of the Oral, Inactivated, Enterotoxigenic *Escherichia coli* Vaccine ETVAX in Bangladeshi Children and Infants: A Double-Blind, Randomised, Placebo-Controlled Phase 1/2 Trial. *The Lancet Infectious Diseases*, **20**, 208-219. [https://doi.org/10.1016/S1473-3099\(19\)30571-7](https://doi.org/10.1016/S1473-3099(19)30571-7)
- [26] Olaniran, A.O., Kistnasamy, E.J. and Ramlal, P.S. (2017) An Ecological Study of Diarrhoeagenic *E.coli* Associated with Indiscriminate Waste Dumps and under Five

- Diarrhoea in Six Informal Settlements in Durban, eThekweni Municipality, South Africa. *International Journal of Environment and Waste Management*, **20**, 300-323. <https://doi.org/10.1504/IJEWEM.2017.10011365>
- [27] Raghavan, P.R., Roy, S., Thamizhmani, R. and Purushothaman, A.S. (2017) Diarrheogenic *Escherichia coli* Infections among the Children of Andaman Islands with Special Reference to Pathotype Distribution and Clinical Profile. *Journal of Epidemiology and Global Health*, **7**, 305-308.
- [28] Rajan, A., *et al.* (2018) Novel Segment- and Host-Specific Patterns of Enteraggregative *Escherichia coli* Adherence to Human Intestinal Enteroids. *mBio*, **9**, e02419-17. <https://doi.org/10.1128/mBio.02419-17>
- [29] Vasudevan, P., Annamalai, T., Sartori, L., Hoagland, T. and Venkitanarayanan, K. (2003) Behavior of Enteraggregative *Escherichia coli* in Bottled Spring and Mineral Water. *Journal of Food Protection*, **66**, 497-500. <https://doi.org/10.4315/0362-028X-66.3.497>
- [30] Cameron, E.A., Curtis, M.M., Kumar, A., Dunny, G.M. and Sperandio, V. (2018) Microbiota and Pathogen Proteases Modulate Type III Secretion Activity in Enterohemorrhagic *Escherichia coli*. *mBio*, **9**, e02204-18. <https://doi.org/10.1128/mBio.02204-18>
- [31] Shrivastava, A.K., Mohakud, N.K., Panda, S., Patra, S.D., Kumar, S. and Sahu, P.S. (2020) Major Enteropathogens in Humans, Domestic Animals, and Environmental Soil Samples from the Same Locality: Prevalence and Transmission Considerations in Coastal Odisha, India. *Epidemiology and Health*, **42**, Article ID: e2020034. <https://doi.org/10.4178/epih.e2020034>
- [32] Ferens, W.A. and Hovde, C.J. (2011) *Escherichia coli* O157:H7: Animal Reservoir and Sources of Human Infection. *Foodborne Pathogens and Disease*, **8**, 465-487. <https://doi.org/10.1089/fpd.2010.0673>
- [33] Mbae, C., *et al.* (2020) Factors Associated with Occurrence of Salmonellosis among Children Living in Mukuru Slum, an Urban Informal Settlement in Kenya. *BMC Infectious Diseases*, **20**, Article No. 422. <https://doi.org/10.1186/s12879-020-05134-z>
- [34] Eltai, N.O., Al Thani, A.A., Al Hadidi, S.H., Al Ansari, K. and Yassine, H.M. (2020) Antibiotic Resistance and Virulence Patterns of Pathogenic *Escherichia coli* Strains Associated with Acute Gastroenteritis among Children in Qatar. *BMC Microbiology*, **20**, Article No. 54. <https://doi.org/10.1186/s12866-020-01732-8>
- [35] Ifeanyi, C.I.C., Ikeneche, N.F., Bassey, B.E., Al-Gallas, N., Aissa, R.B. and Boudabous, A. (2015) Diarrheogenic *Escherichia coli* Pathotypes Isolated from Children with Diarrhea in the Federal Capital Territory Abuja, Nigeria. *The Journal of Infection in Developing Countries*, **9**, 165-174. <https://doi.org/10.3855/jidc.5528>
- [36] Chen, H.D. and Frankel, G. (2005) Enteropathogenic *Escherichia coli*: Unravelling Pathogenesis. *FEMS Microbiology Reviews*, **29**, 83-98. <https://doi.org/10.1016/j.femsre.2004.07.002>
- [37] Kotloff, K.L., *et al.* (2013) Burden and Aetiology of Diarrhoeal Disease in Infants and Young Children in Developing Countries (the Global Enteric Multicenter Study, GEMS): A Prospective, Case-Control Study. *Lancet (London, England)*, **382**, 209-222. [https://doi.org/10.1016/S0140-6736\(13\)60844-2](https://doi.org/10.1016/S0140-6736(13)60844-2)
- [38] Nataro, P.J. and Kaper, B.J. (2010) Diarrheogenic *Escherichia coli*. *Journal of Clinical Microbiology*, **68**, 203-207.
- [39] Omondi, E. (2018) Prevalence, Virulence Factors and Drug Resistance of Enteraggregative *Escherichia coli* Isolated from Children under Five Years Old in Kiambu County Hospital. Master's Thesis, Kenyatta University, Nairobi.

- [40] Kariuki, S., *et al.* (2019) Multidrug-Resistant Nontyphoidal Salmonella Hotspots as Targets for Vaccine Use in Management of Infections in Endemic Settings. *Clinical Infectious Diseases*, **68**, S10-S15. <https://doi.org/10.1093/cid/ciy898>
- [41] Ministry of Health Republic of Kenya (2016) Basic Pediatric Protocol. 4th Edition, 25.
- [42] Kariuki, S., Wairimu, C. and Mbae, C. (2021) Antimicrobial Resistance in Endemic Enteric Infections in Kenya and the Region, and Efforts towards Addressing the Challenges. *The Journal of Infectious Diseases*, **224**, S883-S889. <https://doi.org/10.1093/infdis/jiab457>
- [43] Okeke, I.N., Lamikanra, A. and Edelman, R. (1999) Socioeconomic and Behavioral Factors Leading to Acquired Bacterial Resistance to Antibiotics in Developing Countries. *Emerging Infectious Diseases*, **5**, 18-27. <https://doi.org/10.3201/eid0501.990103>
- [44] Mukokinya, M.A., Opanga, S., Oluca, M. and Godman, B. (2018) Dispensing of Antimicrobials in Kenya: A Cross-Sectional Pilot Study and Its Implications. *Journal of Pharmacy Practice and Research*, **7**, 77-82. https://doi.org/10.4103/jrpp.JRPP_17_88
- [45] Omulo, S., *et al.* (2021) Carriage of Antimicrobial-Resistant Bacteria in a High-Density Informal Settlement in Kenya is Associated with Environmental Risk-Factors. *Antimicrobial Resistance & Infection Control*, **10**, Article No. 18. <https://doi.org/10.1186/s13756-021-00886-y>
- [46] Konaté, A., *et al.* (2017) Epidemiology and Antibiotic Resistance Phenotypes of Diarrheagenic *Escherichia coli* Responsible for Infantile Gastroenteritis in Ouagadougou, Burkina Faso. *European Journal of Microbiology and Immunology*, **7**, 168-175. <https://doi.org/10.1556/1886.2017.00014>
- [47] Fulgenzio, C. *et al.* (2021) Impact of Prior Antibiotic Use in Primary Care on *Escherichia coli* Resistance to Third Generation Cephalosporins: A Case-Control Study. *Antibiotics*, **10**, Article 451. <https://doi.org/10.3390/antibiotics10040451>
- [48] County, T., *et al.* (2016) Epidemiology of Antimicrobial Resistance among *Escherichia coli* Strains in Trans-Nzoia County, Kenya. *Journal of Microbiology and Infectious Diseases*, **6**, 107-112.
- [49] Abbasi, E., Mondanizadeh, M., van Belkum, A. and Ghaznavi-Rad, E. (2020) Multi-Drug-Resistant Diarrheagenic *Escherichia coli* Pathotypes in Pediatric Patients with Gastroenteritis from Central Iran. *Infection and Drug Resistance*, **13**, 1387-1396. <https://doi.org/10.2147/IDR.S247732>
- [50] Konaté, A., *et al.* (2017) Molecular Characterization of Diarrheagenic *Escherichia coli* in Children Less than 5 Years of Age with Diarrhea in Ouagadougou, Burkina Faso. *European Journal of Microbiology and Immunology*, **7**, 220-228. <https://doi.org/10.1556/1886.2017.00011>