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Antimicrobial Susceptibility Patterns of Escherichia coli and Shigella Isolated from Stool Samples from Adults and Children in Zambia

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Abstract

Introduction

Antimicrobial resistance (AMR) is a growing public health problem in low-to-middle income countries which have a high burden of infectious diseases. Poor antimicrobial stewardship in these regions has resulted in a rise in reported cases of AMR creating a need for country specific data to inform policy on the strategies of combating AMR. Here we show antimicrobial susceptibility patterns of *Shigella,* and *E. coli* isolated from stools of children under 5 years of age and adults.

Methods

The study was nested under an enterotoxigenic *Escherichia coli* (ETEC) vaccine clinical trial and diarrhoea surveillance. Stool samples were collected at baseline, during scheduled visits and whenever the participants presented with diarrhoea as per study design. Following microbiological techniques for culture and microorganism identification, pure colonies were run on the BD Phoenix™ 100 for identification and antimicrobial susceptibility. For ETEC identification, colony PCR was done on all *E. coli* positive samples using heat-labile toxin and stable toxin specific primers, respectively.

Results

Among the 211 samples analysed, 52.5% were from individuals with diarrhoea. Un-typed *E. coli* were the most common organism isolated (63.6%), followed by ETEC (12.7%) and 4.8% were *Shigella* sp. Majority of the organisms isolated were either susceptible or intermedial (80-100%) to all tested antibiotics except for Trimethoprim/Sulfamethoxazole which showed a high resistance of 82 – 93%. We also observed some multi-drug resistance (3.5%) among all organisms tested to the different antibiotics.

Conclusions

The observed high prevalence of co-trimoxazole resistance and intermedial susceptibility to fluoroquinolones among ETEC, *Shigella* and other un-typed *E. coli* isolates, is critical for informing policy on the urgent need for antimicrobial stewardship and strengthening of AMR surveillance systems in Zambia.

Background

Antimicrobial agents have been used to control and treat bacterial infections since their discovery in the early 20th century resulting in better disease outcomes and improved well-being for many people [1, 2]. Unfortunately, these gains are threatened by the emergence of multi-drug resistant organisms and the lack of effective antimicrobials to combat them [3]. An increase in bacterial resistance to several antimicrobials has been seen in recent years with an estimated 700, 000 people reported to die every year from antibiotic-resistant infections globally [1, 2, 4, 5].

Although antimicrobial resistance (AMR) occurs naturally due to genetic mutations or acquisition of genetic material from other microbes, [6] the use of antibiotics accelerates the selection for the presence of AMR genes in microorganisms [7]. Its rise in the recent past has been attributed to several factors including, excessive and unregulated use of antibiotics in healthcare and agriculture, increased international travel, lack of adequate diagnosis, lack of effective vaccines, lack of access to quality-assured antimicrobials, as well as irrational prescription of medication are all among causes of AMR [1, 2, 8–10]. AMR has been shown to affect all regions regardless of income level and is likely to pose a higher financial burden on Low and middle-income countries as they have the highest burden of infectious diseases requiring both preventive and therapeutic antimicrobials [3, 11].

Enteric bacteria, which reside as normal flora such as *E.coli*, [5, 12–14] are an important cause of extraintestinal infections such as urinary tract infections (UTIs), bacteremia, or septicemia which are important causes of morbidity [3, 14, 15]. External factors, including antibiotic use and invasion with outside pathogenic organisms, facilitate the development and spread of AMR among enteric bacteria [9] making the gut a channel for the genesis and environmental/community spread of drug-resistant bacteria [3].

Diarrheagenic *E. coli's* such as *ETEC* along with *Shigella* are major contributors to the diarrhoea burden in developing countries [16–18]. These along with other *Enterobacteriaceae* (gram-negative enteric bacteria) have been reported to have acquired several resistant genes in the recent past and have growing resistance to third-generation cephalosporins as they carry extended-spectrum-beta-lactamases (ESBLs) [3, 6]. Resistance to carbapenems and quinolones are also on the rise [3] and has been reported in *Enterobacteriaceae*, such as *Escherichia coli (E. coli), Klebsiella* sp., and *Enterobacter* sp. which are frequent causes of UTIs and bloodstream infections [3, 14]. *Shigella* has shown resistance to ceftriaxone, azithromycin, and ciprofloxacin in Asia [19].

Emergence of quinolone/fluoroquinolone resistance among enteric bacteria has also been reported in several African countries including Mozambique [11] Central African Republic, Ghana, Kenya and South Africa [20]. In a study conducted to identify enteric pathogens among under five children at a tertiary level hospital in Zambia, multiple drug resistant strains were detected and all *E. coli* isolates were extended spectrum beta lactamase producers (for a list of antibiotics used, please refer to Chiyangi et al. 2017) [21].

The real burden of AMR in LMICs, including Zambia, has been difficult to estimate due to challenges related to AMR surveillance and inadequate diagnostic facilities [2, 5]. A review of AMR in Africa published in 2017 bemoaned the lack of data on AMR, particularly from Southern Africa [5]. Although, the recent years have seen an increase in efforts targeted at improving antimicrobial stewardship and surveillance activities in Zambia, with a few studies reporting on AMR in Zambia, more studies are needed to understand the true extent of the burden of AMR in Zambia and address the urgent need for country-specific data given that AMR patterns vary across regions [22].

Our study set out to document AMR patterns for *Shigella*, and *E. coli* isolated from adults and children participating in a clinical trial and a diarrhea surveillance study in Lusaka, Zambia. Given the scarcity of information on the extent of community spread of AMR in Zambia, it is expected that these data will be useful for informing policy on key strategies for strengthening AMR surveillance and pharmacovigilance activities.

Materials And Methods

Study design

This study utilized stool samples collected in a clinical trial assessing an oral vaccine's safety and immunogenicity against *ETEC* and from a community surveillance of diarrhoea infections. The clinical trial was conducted between September 2019 and December 2020, while the diarrhoea surveillance began in September 2020 and was still ongoing at the time of writing this manuscript.

Study sites

Participants of the study were recruited from peri-urban areas of Lusaka including Matero, George, and Chainda compounds. These are informal housing settlements with, poor sanitation, and whose water is supplied from mainly communal boreholes and local taps. They are also high-density communities with high unemployment rates and generally increased incidence of infectious diseases.

Participants and sampling

All participants were required to submit a stool sample for screening purposes during the clinical trial and diarrhoea surveillance. The clinical trial included adult participants aged 18-45 years and children aged 6-24 months that presented to the health facility with no diarrhoeal symptoms. The surveillance included children aged 0-59 months recruited into the study cohort presenting to either Matero, George, or Chainda health facilities with diarrhoea.

Data collection

Socio-demographic and clinical data (age, water source, HIV status, sex, location) were collected using the study-specific case report form (CRF). This information was stored in an encrypted electronic database *District Health Information Software 2* (DHIS2).

Stool sample collection and processing

As part of the routine screening before enrolment into the studies, participants were required to submit a stool sample for microbiology analysis. Stool samples were collected from enrolled patients within 7 days of the screening. Stool samples were also collected whenever a participant presented with diarrhoea (defined as the passage of 3 or more loose stools in 24 hours). Stool specimens were stored at 2–8 °C at the study sites and transported in sterile containers within 12 hours of sample collection to the Center for Infectious Disease Research in Zambia (CIDRZ) central laboratory. The samples were transported at the same temperature using a cooler box with ice packs and were cultured upon reception at CIDRZ central laboratory.

Bacteriological identification of enteric pathogens

Aseptic techniques were applied in the processing of stool samples. Primary cultures were obtained by inoculation of stool samples on MacConkey agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India) upon arrival to the laboratory and incubated overnight at 37˚C. Single lactose fermenting and non-lactose fermenting colonies suggestive of *E. coli* and *Shigella*, respectively were sub-cultured on MacConkey agar and incubated overnight at 37˚C to obtain pure isolates.

Biochemical tests such as Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), and Sulfide, Indole, Motility (SIM) (HiMedia Laboratories Pvt. Ltd. Mumbai, India) were performed on these colonies for confirmatory identification of suspected ETEC and *Shigella*-like colonies.

Furthermore, the colonies that resulted in *Shigella*-like biochemical properties were grown on Hektoen-Enteric Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India) and sero-grouped using polyvalent MAST® ASSURE *Shigella* Agglutinating Antisera (Mast Group Limited., Merseyside, U.K) targeting the serotypes; *Shigella boydii, Shigella sonnei, Shigella flexneri and Shigella dysenteriae.*

A loop full of all pure isolated lactose and non-lactose fermenting colonies were stored in 15% glycerol at -80˚C for further screening using colony PCR, automated identification and antibiotic susceptibility testing using the BD Phoenix™ 100 machine (Becton Dickinson, Oxford, UK).

Molecular identification of ETEC and *Shigella* spp.

Frozen isolates were revived on MacConkey agar and incubated overnight at 37˚C. Deoxyribonucleic acid (DNA) was extracted using a heat lysis protocol previously described [23]. Briefly, a half loopful of bacterial culture was inoculated in 100 ul of molecular grade water and boiled for 10 minutes at 100˚C on a heating block. The bacterial cells were immediately centrifuged for 2 minutes at 10,000 x g and 100 ul of the supernatant was transferred to a sterile 1.5 ul microcentrifuge tube to serve as DNA template for the PCR reactions.

The ETEC colony multiplex PCR was run targeting the toxin genes LT, STh, and STp with gene-specific primers previously described [24, 25] (refer to S Table 1). The PCR was carried out in a 20 µl final volume using 10 µl of ReadyMix™ (x2) (PCR kit KAPA 2G Fast HS (KAPA BIOSYSTEMS cat KM 5610), 0.5 µl of each primer set targeting the LT (10mM) and STp (10mM), and 1µl of primer STh (10mM), 0.4ul of $MgCl_2$ (25mM), 4.6 µl of molecular grade water and 1 µl of the DNA template (rapid boil extract).

The PCR was run on an ABI Gene Amp 9700 thermal cycler (AB Applied Biosystems, Foster City, CA, USA) with the following cycling conditions: Initial denaturation at 95˚C for 2 min, and 30 cycles of denaturation at 95˚C for 15 s, primer annealing at 52˚C for 8 s and extension at 72˚C for 10 s, followed by a final extension at 72˚C for 2 minutes.

The *Shigella* colony multiplex PCR was run with primers previously described [26]. The PCR was performed in a total reaction volume of 25 µl as follows: 12.5 µl of OneTaq^{TM} Quick Load 2X MM with standard buffer (New England Biolabs, UK), 3.75 µl of the primer multiplex mix (0.5 µl of each the listed primers 1-6 (0.2 mM) and each of primers 7 and 8 (0.15 mM) at 0.375 µl each), 4 µl DNA template and 4.6 µl molecular grade water. The PCR was run on an ABI Gene Amp 9700 thermal cycler (AB Applied Biosystems, Foster City, CA, USA) with the following cycling conditions: Initial denaturation at 94˚C for 2 min, and 35 cycles of denaturation at 94˚C for 30 s, primer annealing at 65˚C for 30 s and extension at 68˚C for 30 s, followed by a final extension at 68˚C for 5 minutes.

All PCR amplicons were run and analysed on a 1.5% agarose gel electrophoresis alongside a 100 bp DNA Mass Ladder (Thermo Scientific, USA).

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was done using the BD Phoenix™ 100 automated identification system (Becton Dickinson, Oxford, UK) following the manufacturer's instructions. Stored bacterial isolates were revived and inoculated on MacConkey agar and incubated at 37˚C for 18-24 hours. One pure colony from each sample was used for identification and antibiotic susceptibility testing (ID/AST). Briefly, an 18-24-hour old pure colony was picked and inoculated in the ID broth, then mixed to prepare a 0.5-0.6 McFarland standard suspension measured using a PheonixSpec™ nephelometer (Becton Dickinson, Oxford, UK). A suspension of 25µl was used for antibiotic susceptibility testing, and results were ready within 24 hours. Common drugs used for the treatment of suspected gram-negative bacterial infections in Zambia were tested. A total of 20 drugs used included: Ampicillin, Amoxicillin/Clavunate, Colistin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem/Relebactam, Meropenem/Nacubactam, Piperacillin/Tazobactam, Amikacin/Fosfomycin, Imipenem, Gentamycin, Ciprofloxacin, Levofloxacin, Tetracycline, Nitrofurantoin, Trimethoprim/Sulfamethoxazole and Tigecycline (refer to the BD phoenix NMIC/ID panel package insert for antibiotic concentration). Multi-drug resistance was defined as one isolate being resistant to three or more classes of antibiotics tested [27]. The AST results obtained were interpreted using the Clinical and Laboratory Standards Institute (CLSI) guideline [28].

Quality control

Reference strains of *Escherichia coli* ATCC-25922 were used for quality control of the identification and drug susceptibility testing as recommended by the manufacturer.

Data analysis

The results generated were entered into the WHONET database, and the AST data was interpreted using the 2020 CLSI guidelines. Microsoft excel® was also used to collate data and generate frequency tables.

Results

Participant characteristics

Socio-demographic features of the participants from whom the stool samples were obtained are summarized in Table 1. We had 117 males and 94 females. Of these, 31 where adults while 180 were children below the age of five. Of the children, 17 (9.4%) were HIV positive. 98 were classified as asymptomatic while 113 were symptomatic and had diarrhea at the time of stool collection as shown in Table 1.

Table 1: Socio-demographic and clinical characteristics of the study participants

	Symptomatic.		**Asymptomatic**
Characteristics	**Children**	**Children**	**Adults**
Gender			
Female	58 (51%)	27 (40%)	9 (29%)
Male	55 (49%)	40 (60%)	22 (71%)
Source of Water			
Protected source	0 (0%)	64 (96%)	28 (90%)
Unprotected source	0 (0%)	3 (4%)	3 (10%)
HIV Status			
Negative	98 (87%)	61 (91%)	31 (100%)
Positive	11 (10%)	6 (9%)	0 (0%)
Not consented	4 (3%)	0 (0%)	0 (0%)
Total	113 (100%)	67 (100)	31 (100)

Isolation and identification of enteric bacteria

354 enteric bacteria isolates were obtained from the stool specimens collected from the study participants. 186 (52.5%) isolates were from symptomatic and 168 (47.5%) from asymptomatic individuals respectively (Figure1). The enteric bacteria isolated included 45 (12.7%) ETEC strains, 225 (63.6%) *E. coli (untyped)* and 17 (4.8%) *Shigella* species, and 67 (18.9%) belonged to other genera of bacteria as shown in Table 2. *Shigella sonnei* was the most predominant serotype isolated among *Shigella* species.

Table 2: Prevalence of enteric bacteria isolated from stool specimens of patients with and without diarrhoea (Heathy) (N=354).

		Source[a]	
Bacteria	**Frequency**	**Symptomatic**	**Asymptomatic**
ETEC	45	31 (68.9)	14 (31.1)
Untyped E. coli	225	118 (52.4)	107 (47.6)
Shigella boydii	1	1 (100)	0(0)
Shigella dysenteriae	2	2 (100)	0(0)
Shigella flexineri	4	3 (75)	1(25)
Shigella sonnei	10	10 (100)	0(0)
Escherichia fergasonii	4	4 (100)	0 (0)
Proteus mirabilis	2	1 (50)	1(50)
Enterobacter cloacae	22	3(33.6)	19 (86.4)
Enterobacter asburiae	1	1 (100)	0 (0)
Citrobacter freundii	3	1 (33.3)	2 (66.7)
Klebsiella pneumoniae	18	3 (16.7)	15 (83.3)
Kluyvera ascorbata	4	2 (50)	2(50)
Aeromonas caviae	2	2 (100)	0 (0)
Providencia alcalifaciens	3	3(100)	0 (100)
Enterobacter agglomerans	1	1 (100)	0 (0)
Klebsilla pneumoniae ozaenae	1	0(0)	1 (100)
Klebsiella Oxytoca	3	0(0)	3 (100)
Citrobacter Koseri (diversus)	1	0(0)	1(100)
Citrobacter farmeri	1	0(0)	1(100)
Citrobacter amatonaticus	1	0(0)	1(100)
Total	354	186 (52.5)	168 (47.5)

[a]Data is represented in N (%), *E. coli*: *Escherichia coli*, ETEC: enterotoxigenic *E. coli.*

Antimicrobial susceptibility patterns of the bacterial isolates

As summarized in Table 3 below, the antibiotic susceptibility results showed that susceptibility of *E. coli* was maximum (100%) to most of the antibiotics, such as Amoxicillin/Clavulanic acid, Ceftazidime and Cefepime. In contrast, a high percentage of ETEC strains were intermediately susceptible to Ampicillin 82.2% and Levofloxacin (97.8) while 74% and 51.1% of un-typed *E. coli* were intermediate susceptible to Ampicillin and Levofloxacin, respectively.

Majority of the *ETEC*, un-typed *E. coli* and *Shigella* species isolates were resistant to trimethoprim-sulfamethoxazole 84.4%, 91.6% and 82.4% respectively.

Overall results showed that 10 (3.7%) *E. coli* isolates tested were MDR with 5 different patterns as shown in Table 4.

The most common resistance pattern was trimethoprim/Sulfamethoxazole-ciprofloxacin-levofloxacin (4.1%), followed by trimethoprim/Sulfamethoxazole-ciprofloxacin-levofloxacin- ceftriaxone (1.1%).

For the *Shigella* isolates, Ciprofloxacin (100%), Levofloxacin (100%) showed intermediate susceptibility while 82.4% were resistant to Trimethoprim/Sulfamethoxazole. Among the *Shigella* species, all the *S. flexneri*, *S. dysenteriae* and *S. sonnei* were resistant to trimethoprim/sulfamethoxazole (100%) but showed intermediate susceptibility to ciprofloxacin Levofloxacin (100% for both) while *S. bodii* was 100% intermediate susceptible to Ciprofloxacin and Levofloxacin (Supplementary Table 1).

Table 3: Antimicrobial susceptibility of *Shigella* spp and *Escherichia coli* isolated from isolated stool specimens.

Antimicrobial categories	Antimicrobial	*ETEC (n=45)*			*Un-typed E.coli* (n=225)			*Shigella* spp (n=17)		
		S	I	R	S	I	R	S	I	R
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N
Aminoglysides	Gentamicin	45 (100)	0 (0.0)	0(0.0)	211(93.8)	14 (6.2)	0 (0.0)	17 (100)	0 (0.0)	0 (0
Carbapenems	Ertapenem	45 (100)	0 (0.0)	0(0.0)	225 (100)	0 (0.0)	0 (0.0)	17 (100)	0 (0.0)	0 (0
β-lactamase inhibitor combinations	Imipenem/Relebactam	44 (97.8)	0 (0.0)	1(2.2)	223 (99.1)	2 (0.9)	0(0.0)	17 (100)	0 (0.0)	0 (0
	Meropenem/Nacubactam	45 (100)	0 (0.0)	0(0.0)	225 (100)	0 (0.0)	0 (0.0)	17 (100)	0 (0.0)	0 (0
	Piperacillin/Tazobactam	45 (100)	0 (0.0)	0(0.0)	225 (100)	0 (0.0)	0 (0.0)	17 (100)	0 (0.0)	0 (0
2nd generation cephalosporins	Cefuroxime	36 (80)	9 (20)	0(0.0)	184 (81.8)	41 (18.2)	0 (0.0)	17 (100)	0 (0.0)	0 (0
Extended Spectrum cephalosporins; 3rd and 4th generation cephalosporins	Ceftazidime	45(100)	0 (0.0)	0(0.0)	222 (98.7)	0 (0.0)	3 (1.3)	17 (100)	0 (0.0)	0 (0
	Ceftriaxone	42(93.3)	0 (0.0)	3(6.7)	206 (91.6)	0 (0.0)	19 (8.4)	17 (100)	0 (0.0)	0 (0
	Cefepime	44(97.8)	0 (0.0)	1(2.2)	223 (99.1)	0 (0.0)	2 (0.9)	17 (100)	0 (0.0)	0 (0
Penicillins	Ampicillin	8 (17.8)	37(82.2)	0(0.0)	58(25.8)	167(74.2)	0 (0.0)	12 (70.6)	5 (0.4)	0 (0
β-lactamase inhibitors	Amoxicillin/Clavulanic acid	43 (95.6)	2 (4.4)	0(0.0)	221(98.2)	4 (1.8)	0 (0.0)	17 (100)	0 (0.0)	0 (0
Folate pathway inhibitor	Trimethoprim/Sulfamethoxazole	6 (13.3)	1(2.2)	38 (84.4)	19 (8.4)	0 (0.0)	206 (91.6)	3 (17.6)	0 (0.0)	14 (8
Quinolones/Fluoquinolones	Ciprofloxacin	0 (0.0)	43 (95.6)	2(2.2)	205 (91.1)	0 (0.0)	20 (8.9)	0 (0.0)	17 (100)	0 (0
	Levofloxacin	0 (0.0)	44 (97.8)	1(2.2)	89 (39.6)	115 (51.1)	21 (9.3)	0 (0.0)	17 (100)	0 (0
Glycylcycline	Tigecycline	45(100)	0 (0.0)	0(0.0)	225 (100)	0(0.0)	0 (0.0)	17 (100)	0 (0.0)	0 (0

S: Susceptible, I: Intermediate and R: Resistant

Table 4: Antimicrobial Resistance Pattern of *E. coli* isolates (n=270)

Antimicrobial resistance patterns	Resistant n	(%)
CIP-LVX	1	(0.4)
CRO-SXT	12	(4.4)
COL-NIT	1	(0.4)
CIP	1	(0.4)
CRO-CAZ	1	(0.4)
SXT-COL	1	(0.4)
SXT-LVX	1	(0.4)
SXT-CIP-LVX	11	(4.1)
SXT-LVX-CRO	1	(0.4)
SXT-COL-IPM	1	(0.4)
SXT-CIP-LVX- CRO	3	(1.1)
SXT-CIP-LVX- CRO- CAZ	2	(0.7)
SXT-CIP-LVX- CRO-FEP	3	(1.1)
TOTAL MDR	10	(3.7)

SXT: Trimethoprim/Sulfamethoxazole, CIP: Ciprofloxacin, LVX: Levofloxacin, CRO: Ceftriaxone,

CAZ: Ceftazidime and FEP: Cefepime, COL: Colistin, CAZ: Ceftazidime and NIT: Nitrofurantoin and MDR: multidrug resistant.

Discussion

Shigella and ETEC are ubiquitous in low-to-middle income countries. According to a study on the aetiology of diarrhoea in the region, the two were reported to be among the major causes of diarrhoea in Zambian infants [18].

Our study shows *E. coli* as the most frequently isolated organism in stool samples compared to *Shigella* (Table 2). This has been attributed to the fastidious nature of *Shigella*, which is not as easy to isolate as *E. coli* thus, isolation rates are largely dependent on the technique employed [29].

All but one (94%) of the *Shigella* isolates were from individuals with symptomatic infection (diarrhoea) suggesting that *Shigella* is virulent. Not all *Shigella* and ETEC isolates identified in this study were susceptible to Quinolones/ Fluoroquinolones; this could have been attributed to the wide use of these as broad-spectrum antibiotics in the region. Although there have been numerous reports of increasing *Shigella* resistance to ciprofloxacin globally [30, 31], none of our isolated *Shigella* spp showed resistance to Quinolones/ Fluoroquinolones. Studies have reported quinolone and fluoroquinolone resistance among enteric pathogens in Africa [20, 32–34]. Fluoroquinolone resistance among *E. coli* and other enteric pathogens is flagged as a principal public health threat by the World Health Organization (WHO) [35].

Our study observed that most isolates were resistant to Trimethoprim/Sulfamethoxazole (co-trimoxazole), with more than 80% of both *Shigella* and *E. coli* reporting resistance. Our finding concurs with an earlier study conducted in Lusaka in 2002 (nearly two decades ago), for which co-trimoxazole resistance was observed [35]. While the authors are concerned that resistance may compromise the usefulness of co-trimoxazole in preventing bacterial infections in HIV-infected individuals, they still conclude that any effect in preventing *Pneumocystis carinii* pneumonia or isosporiasis would still be valuable [36]. This is comparable to similar reports from studies in Madagascar and Uganda, with 80% and 74% resistance to co-trimoxazole, respectively [22, 37]. co-trimoxazole resistance is a matter of global concern as the major prophylactive drug used in HIV-infected individuals to prevent opportunistic infections. Thus, there are ongoing debates regarding co-trimoxazole as prophylaxis in HIV-exposed (uninfected infants) in low malaria prevalence areas [38].

We classified 10 *E. coli* isolates (3.7%) as MDR based on the CDC definition. Though this is a small number, it calls for concern as *E.coli*, being a commensal organism, can be a key reservoir for AMR [3] that could pass on resistance to other enteric bacteria. As earlier stated, pathogenic *E. coli* strains are important causes of extra-intestinal disease and therefore have the danger of becoming a true superbug [3]

Two colistin-resistant *E. coli* isolates were also observed. This is a source of concern given that this is an antibiotic of last resort [3]. In addition, studies have reported the emergence of colistin resistance in patients, most of whom had previously received colistin therapy or with acquisition via nosocomial transmission. However, there are reports of colistin resistance in humans who have not received the drug previously or without nosocomial transmission [39]. This may be the case in our study, given that we have *E. coli* isolates from asymptomatic individuals.

Our study highlights antimicrobial resistance as an emerging problem for Zambia. We have shown enteric organisms resistant to common antibiotics both in clinical (symptomatic) and community (asymptomatic) isolates. This suggests that there could be community spread of AMR occurring in our population and calls for concerted efforts towards preventing the further development of AMR among enteric organisms. We have also noted the high resistance to co-trimoxazole; therefore, given Zambia's endemicity to many infectious diseases including HIV, there may be a need to consider alternative treatment prophylaxis

against opportunistic infections [36]. This study is however limited by lack of data on the use of antibiotics from our adult and children's cohorts mainly because the parent studies for which this study was nested did not focus on AMR.

Conclusion

The high prevalence of co-trimoxazole resistance among *E. coli* and *Shigella* isolates from symptomatic and asymptomatic individuals and the presence of multi-drug resistant *E. coli* are of great clinical importance in Zambia. Unfortunately, this calls for the consideration of more expensive antimicrobial agents for the treatment of *E. coli* and *Shigella* infections. Therefore, making effective, affordable parenteral antimicrobial agents for efficient treatment and prophylaxis to septicaemia infection in hospitals and health centres ever more complex. There is need for strengthened AMR surveillance systems to better understand AMR patterns observed in Zambia at the national level to inform policy on the appropriate drugs of choice.

Abbreviations

AMR	Antimicrobial resistance
BD	Becton Dickinson
BSIs	Bloodstream infections
CDC	Center for Disease Control and Prevention
CIDRZ	Center for Infectious Disease Research in Zambia
CLSI	Clinical and Laboratory Standards Institute
CRF	Case Report Form
ESBLs	extended-spectrum-beta-lactamases
ETEC	enterotoxigenic *E. coli*
HIV	Human immunodeficiency virus
ID/AST	identification and antibiotic susceptibility testing
LIA	Lysine Iron Agar
LMICs	Low-middle income countries
MDR	Multi drug resistance
PCR	Polymerase Chain Reaction
SIM	sulfide, indole, motility
TSI	Triple Sugar Iron
UNZABREC	University of Zambia Biomedical Research Ethics Committee
UTIs	urinary tract infections
WHO	World Health Organization

Declarations

Ethics approval and consent to participate.

Ethical clearance was secured from University of Zambia Biomedical Research Ethics Community (UNZABREC) reference number 005-01-19 (for the clinical trial) and reference number 696-2020 for the *Shigella* and ETEC surveillance studies respectively for which **all experiments were performed in accordance with relevant guidelines and regulations**. Final approval was obtained from the National Health Research Authority (NHRA). Written informed consent was obtained from the parents/guardians of children before proceeding to data collection. Information obtained during the study was kept confidential.

Consent for publication

Not Applicable

Availability of data and materials

All data supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Figures

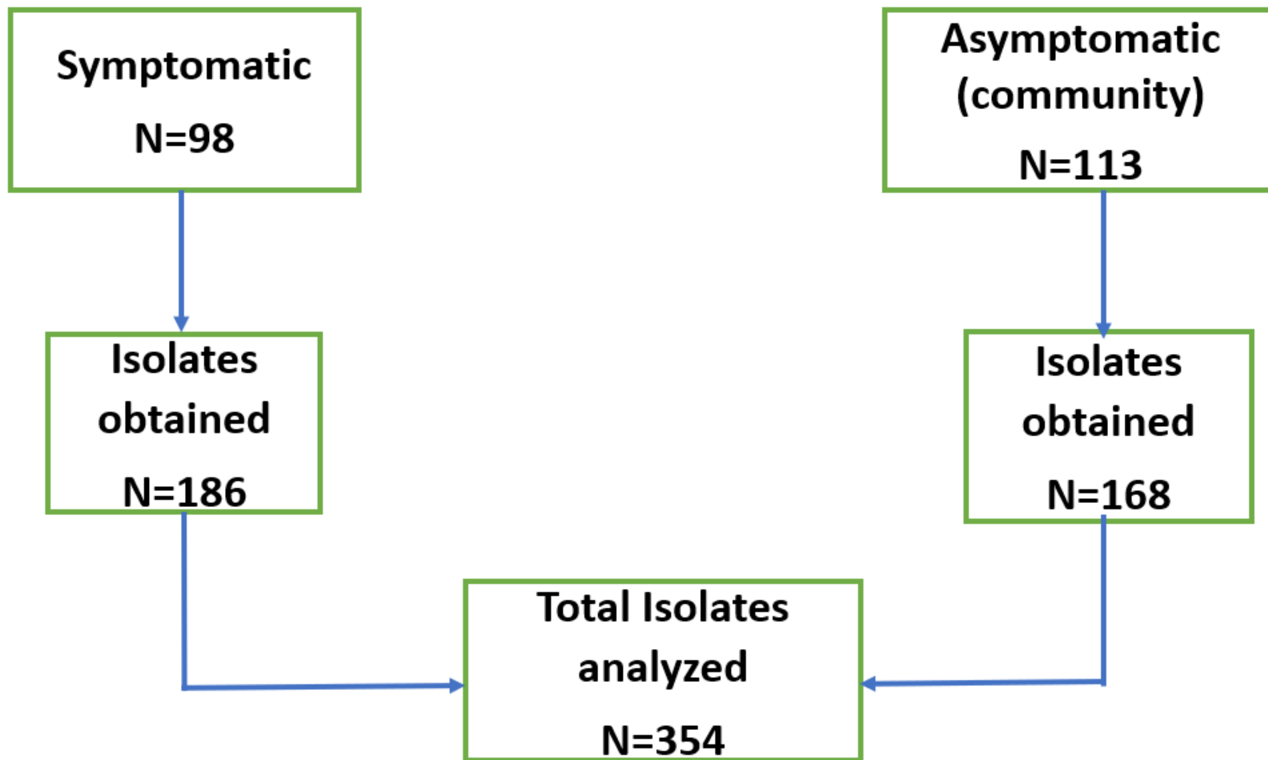

Figure 1

Flow diagram of isolates used in the study.

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