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Characteristics of antibiotic resistance of *Escherichia coli* strains in people suffering from gastroenteritis in the Republic of Guinea

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This study aims to assess the spectrum of antibiotic resistance and to determine the antibiotic resistance genes of *Escherichia coli* strains isolated from patients suffering from diarrhea. It was carried out from 2019 to 2022, alternating between in-patients at Kindia Regional Hospital. A coproculture was performed, strains were identified using the API 20E kit and antibiograms were performed using the Kirby bauer method. PCR identified resistance genes. From 724 stool samples analyzed, 53 (7.32%) were positive for *E. coli* strains and 52 (98.1%) were resistant to antibiotics. The resistance of *E. coli* strains to β-lactams was 47 (88.7%) for ampicillin, 14 (26.4%) for III-IVth generation cephalosporins, 18 (34%) for quinolones (nalidixic acid), 1 (1.9%) for fluoroquinolones (ciprofloxacin), 35 (66%) for tetracycline, 38 (71.7%) for trimethoprim/sulfamethoxazole, 9 (17.0%) for gentamicin, 7 (13.2%) for tobramycin and 2 (3.8%) for chloramphenicol. However, of 15 antibiotics tested, no *E. coli* strains resistant to Meropenem, Amikacin, or Nitrofurantoin were detected. Analysis of genetic determinants revealed 22 antibiotic resistance genotypes. The most frequent resistance phenotype (AMP+TET+TSM) was identified in 24 (46.15%) of the strains.

Key words: *Escherichia coli*, antibiotic susceptibility, Kindia, whole genome sequencing.

INTRODUCTION

Antimicrobial resistance (AMR) is one of the greatest threats to global public health in the 21st century, particularly in developing countries (Nadeem et al., 2020).

In 2022, AMR caused 127 million deaths worldwide, with a high rate in western sub-Saharan Africa (Murray et al.,

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2022). The World Health Organization (WHO) global report on antimicrobial resistance showed that bacterial resistance to common antimicrobials has reached alarming levels in many parts of the world, suggesting that many available treatments for common infections are becoming ineffective in some areas (WHO, 2022). In human health, bacterial infections are mainly caused by Gram-negative bacteria, particularly those belonging to the enterobacterales (Ngalani et al., 2019). The rapid growth of multi-resistant *Escherichia coli* has been reported not only in the ecological environment and human medicine but also widely in animal husbandry, in particular with an increasing prevalence of extended-spectrum beta-lactamases (ESBLs), which greatly compromises the effectiveness of treatment and increases morbidity and mortality (Ferraresso et al., 2022; Shi et al., 2021).

The three main categories of β-lactamases are plasmid-mediated extended-spectrum β-lactamases (ESBLs), AmpC cephalosporinases, and carbapenemases. *E. coli* is one of the main strains of ESBL-producing enterobacterales associated with resistance genes encoded by bla_{TEM}, $bla_{SHV,}$ and bla_{CTX-M} (Bush and Bradford, 2020; Poirel et al., 2008). Currently, more than 187 TEM, 141 SHV, and 260 CTX-M variants have been identified and grouped into five subfamilies (CTX-M-1, CTX-M-2, CTX-M- 8, CTX-M-9, and CTX-M-25) based on their amino acid identity (Prendergast et al., 2022; Hoek et al., 2011). Enzymes belonging to the CTX-M-1 and CTX-M-9 subfamilies are widespread and frequently reported. Among them, CTX-M-55, which is a variant of CTX-M-15, belongs to the CTX-M-1 group and contains an amino acid substitution (Ala-80-Val) that enhances activity against ceftazidime (Lupo et al., 2018; Hoek et al., 2011). They are often colocated with other resistance genes, such as fosA3 alleles mediating fosfomycin resistance, plasmid-mediated quinolone resistance genes, and 16S rRNA methyltransferase genes, including the rmtB gene conferring pan-resistance to aminoglycosides is the most widespread (Giske, 2015).

In all these documents, in addition to a series of measures aimed at optimizing the use of antimicrobial agents, a great deal of attention is paid to studying the patterns of development and spread of drug resistance within populations of micro-organisms. The main thrust of the fight against antibiotic resistance is to set up a system for monitoring the circulation of drug-resistant micro-organisms and the genes that determine antimicrobial drug resistance (AMD). A single, standardized methodology for determining the susceptibility of micro-organisms to AMDs and common interpretation criteria based on modern knowledge of resistance mechanisms and the pharmacokinetics of AMDs will make it possible to improve the quality of research and conduct effective surveillance at the regional or national level (Murray et al., 2022; WHO, 2020).

The difficulties encountered to date in studying the burden of AMD resistance in various diseases include the lack of data on infections caused by antibiotic-resistant bacteria in the population and in medical organizations, as well as on the incidence of these infections and the burden they represent. The WHO lacks quality data characterizing community-acquired infections and the situation in middle- and low-income countries. Major gaps in epidemiological data (with the exception of tuberculosis) are evident, particularly in the African and Eastern Mediterranean regions (Kouassi, 2020). Gaps in prevalence estimates are due not only to dysfunctional epidemiological surveillance systems but also to the scarcity or poor quality of diagnostic tools, a current problem in many low-income countries (Mouiche et al., 2019). It is therefore possible to provide much more comprehensive epidemiological surveillance covering all four sectors on the basis of a single interconnected and integrated healthcare system. There is an urgent need for more standardized approaches to the prevention and control of infectious diseases. The treatment of patients according to the specificities of their disease is still complicated by the lack of microbiological laboratories in low- and middle-income countries (Founou et al., 2018; Manhique-Coutinho et al., 2022).

In Guinea, studies focused on the multidrug resistance profile of a strain of *E. coli* isolated from diarrhoeal stools at the China-Guinea Friendship Hospital in Kipé, Conakry. This strain of *E. coli* was resistant to all beta-lactams except carbapenems and was resistant to gentamicin, tobramycin, and quinolone (Makanéra et al., 2022). However, there is little data on the genes encoding resistance in *E. coli* strains in Guinea. The objective of this study is to evaluate the level and spectrum of antimicrobial resistance and to determine the genes coding for the resistance of strains of *E. coli* collected from the diarrheal stools of inhabitants of the Republic of Guinea.

MATERIALS AND METHODS

Study environment

This study was carried out in the prefecture of Kindia. It covers an area of 9,115 km^2. It is bordered to the east by the prefecture of Coyah, to the North by the prefecture of Télimélé, to the south by Sierra Leone, and to the west by the prefecture of Mamou. In 2016, the population numbered 469,446, with an average density of 52 inhabitants per km^2 and an estimated growth rate of 34% (‘Kindia Prefecture’, 2024).

Study framework

The laboratory of the Russian-Guinean Research Centre for Epidemiology and Prevention of Infectious Diseases managed by Rospotrebnadzor (Kindia (IRBAG)/Guinea), as well as the laboratory of intestinal infections of the Pasteur Institute of Epidemiology and Microbiology of Saint Petersburg (Saint Petersburg, Russian Federation) served as a framework for this

work.

Sample collection method

This is a cross-sectional study, which lasted 4 years from 2019 to 2022 in alternation. The work involved 724 stool samples taken from outpatients and inpatients at the Alpha Oumar Diallo Kindia Regional Hospital (emergency department and other departments) suffering from diarrhoeal syndrome. Samples were collected using containers containing Cary-Blair transport medium. Unique study identification numbers were assigned to specimens and transported to the Laboratory within two hours of collection.

Culture and purification of stool samples

Samples were directly streaked onto MacConkey agar (MAC) (Oxoid), Eosin Methylene Blue (EMB), and XLD agar (Xylose-Lysine-Deoxycholate) incubated for 18 to 24 h at 37ºC for selective isolation of *E. coli* species. Phenotyping showed that *E. coli* produced bright metallic green colonies on EMB agar, yellow colonies on XLD agar, and red lactose fermentation colonies on MAC. Preliminary identification of bacteria was made using Gram stain, catalase test, and oxidase test (Humphries and Linscott, 2015).

Identification and confirmation of the bacterial isolate using the BioMerieux API 20E kit

Biochemical tests were carried out on the various isolates: O-nitrophenyl-β-Dgalactosidase, lysine, arginine dihydrolase and ornithine decarboxylase, citrate, hydrogen sulfide, indole, urease, tryptophan deaminase, Voges-Proskauer, gelatin liquefaction, etc. Fermentation of mannitol, glucose, inositol, rhamnose, sucrose, melibiose, amygdalin, and arabinose, reduction of nitrates, and production of nitrogen gas.

Antimicrobial susceptibility testing

Antibacterial susceptibility tests were determined using the Kirby Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK). A MacFarland suspension of 0.5 pure *E. coli* colonies was prepared in normal saline (BioMérieux). The most commonly used antibiotics were tested: Ampicillin (10 ug), Cefotaxime (30 ug), Cefepime (30 µg), Cefuroxime (30 ug), Gentamycin (10 ug), Trimethoprim-Sulfamethoxazole (1.25+23.75 µg), Nalidixic acid (30 ug), Ciprofloxacin (5 ug), Chloramphenicol (30 ug), Nitrofurantoin (300 µg), Tetracycline (30 ug), Tobramycin (30 ug), Amikacin (30 ug), Imipenem (10 ug), and Meropenem (10 ug). The results obtained were interpreted according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM 2018-2021). There was a multidrug resistance (MDR) phenotype resistance to three or more classes of antimicrobials (Magiorakos et al., 2012; Oli et al., 2019).

DNA extraction

Colonies were suspended in nutrient agar and incubated at 37°C for 24 h to extract DNA. 300 µl of sterile 0.25X TE (Tris-EDTA) buffer was added to 1.5 ml microcentrifuge tubes, vortexed for 10 s, and centrifuged at 13,000 rpm for 2 min. The supernatant was then removed to preserve the bacterial pellet. The DNA was purified using the QIAamp DNA mini kit (Qiagen). DNA purifications using the QIAamp DNA mini kit (Qiagen) are carried out using optimized buffers and enzymes that facilitate the lysis of biological samples, stabilize nucleic acids, and improve the selective adsorption of DNA onto the QIAamp membrane. Alcohol is then added and the lysates are loaded onto the QIAamp centrifugation column. Wash buffers are used to remove impurities, and the pure, ready-to-use DNA is then eluted in a buffer supplied with the kit.

Polymerase chain reaction (PCR)

Real-time PCR was performed using Rotorgene 6000 machines (Corbett Research, Australia) for the detection of diarrhoeogenic *E. coli* (DEC) from five pathogroups EPEC, EHEC, ETEC, EIEC/ *Shigella* species, and EAEC.

The reported analytical sensitivity for diarrhoeogenic *E. coli* DNA isolated from the fecal extract is up to 103 copies/mL.

Stool samples positive for EIEC/ *Shigella* spp. were cultured for identification. *Shigella* spp. strains were not isolated.

Whole genome sequencing analysis of antimicrobial-resistant *E. coli*

Genome-wide DNA sequencing was performed using the MiSeq (Illumina, USA) and DNBSEQ-G50 (MGI, China) sequencing platforms. For sequencing on the Illumina platform, 200 ng of total DNA was collected, ultrasonically fragmented on a Covaris M220 (Covaris, USA), and converted into a DNA library using the TruSeq DNA Nano LP kit (Illumina, USA) according to the manufacturer's protocol. Two-sided dimensional selection of DNA libraries was performed on the basis of the median value of the DNA insert with a size of 500-550 bp. Sequencing with the DNBSEQ-G50 (MGI, China) was performed with a read length of 2 × 100 using the DNBSEQ-G50RS High Throughput Sequencing Kit (FCL PE100/FCS PE150) (MGI, China).

Processing quality control was performed using FastQC software (v. 0.11.9). Genomes were assembled de novo using SPAdes assembly software (v. 3.13.1) (Prjibelski et al., 2020). The assembly results were evaluated in QUAST (v. 5.2.0) (Gurevich et al., 2013). The search for genetic factors of resistance to AMD was carried out in ResFinder (Zankari et al., 2012).

Statistical analysis

The data were saved in Excel and analyzed using R software. The chi-square test and Fisher's exact test were used to assess the association between the CTA distribution and different variables using Stata 14.0. A p-value < 0.05 was considered statistically significant.

RESULTS

Out of the 724 samples analyzed, 374 (51.7%) produced *E. coli* isolates and 53/374 (14.17%) (Table 1) were identified as CDE by culture and PCR. All culture-positive samples were confirmed positive by PCR.

Genome analysis showed resistance genes to β-lactams, tetracycline, chloramphenicol, aminoglycosides, trimethoprim/sulfamethoxazole, as well as single nucleotide substitutions and mutations in the *gyrA, parC,* and *parE* genes that cause resistance to quinolones and fluoroquinolones (Table 2).

Table 1. Distribution of *E. coli* pathotypes by sex and age group

Pathotypes	DEC	EAEC	EPEC	ETEC	EIEC	STEC	p-value
Number total (%)	53/374 (14.2) CI 10.9-18.2	33/53 (62.2) CI 30.1-57.6	10/53 (18.9) CI 9.9-32.4	08/53 (15.1) CI 7.9-28.1	01/53 (1.9) CI 0.1-11.4	01/53 (1.9) CI 0.1-11.4	<0.001
Male (%)	23/53 (43.4) CI 30.1-57.6	12/53 (22.6) CI 12.7-36.6	2/53 (3.8) CI 0.7-14.1	7/53 (13.2) CI 5.9-26.0	1/53 (1.9) CI 0.1-11.4	1/53 (1.9) CI 0.1-11.4	<0.001
Female (%)	30/53 (56.6) CI 42.4-69.9	21/53 (39.6) CI 26.8-54.0	8/53 (15.1) CI 7.9-28.1	1/53 (1.9) CI 0.1-11.4	0 CI 0-8.4	0 CI 0-8.4	<0.001
Age range (years)							
0-5 (%)	23/66 (34.8) CI 23.8-47.7	11/23 (47.8) CI 27.4-68.9	8/23 (34.8) CI 17.2-57.2	4/23 (17.4) CI 5.7-39.6	0 CI 0-17.8	0 CI 0-17.8	<0.001
6-17 (%)	6/69 (8.7) CI 3.6-18.6	5/6 (83.3) CI 36.5-99.1	0 CI 0-48.3	1/6 (16.7) CI 0.9-63.5	0 CI 0-48.3	0 CI 0-48.3	<0.001
>18 (%)	24/239 (10.1) CI 6.7-14.7	17/24 (70.8) CI 48.8-86.6	2/24 (8.3) CI 1.5-28.5	3/24 (12.5) CI 3.3-33.5	1/24 (4.2) CI 1.5-28.5	1/24 (4.2) CI 1.5-28.5	<0.001

CI: Confidence interval

DISCUSSION

This study determined the resistance characteristics of *E. coli* pathotypes in diarrhea patients. Diarrhoeogenic *E. coli* (DEC) pathotypes are known to cause acute episodes of diarrhea, particularly in children under 5 years of age (Khairy et al., 2020; Mokomane et al., 2018; Ndjangangoye et al., 2023; Yeda et al., 2024). Studies carried out in many African countries have reported this state of affairs, particularly in South Africa and Nigeria (Heine et al., 2024; Ogunbiyi et al., 2023). Numerous studies report cases of resistance of *E. coli* to antibiotics (Kalule et al., 2024; Thakur et al., 2018). This phenomenon of resistance could be linked to the overuse of antibiotics. In recent years, antibiotic resistance has become a worldwide public health concern (Mkuhlu et al., 2020; WHO, 2019). This is why it is so important, particularly in developing countries, to develop a strict policy on the use of antibiotics in communities.

The overall prevalence of diarrhoeogenic *E. coli* observed during our research was 14.1% (Table 1). An almost identical prevalence was reported in Ethiopia (14.8%) (Abey et al., 2024) while in Ghana a similar study reports a 62.3% prevalence rate (Dela et al., 2022). In general, prevalence varies according to the size of the sample studied, the diagnostic techniques used, and the study area (Kalule et al., 2024; Yu et al., 2018). The prevalence was significantly higher in females (56.6%) than in males (43.4%). A high prevalence of *E. coli* in females compared to males has been reported in Congo by Mfoutou Mapanguy et al. (2021) with rates of 61 and 39% respectively. The strain responsible, *E. coli*, remains as an external reservoir and causes urinary tract infections (Kaba et al., 2024).

E. coli infections in this study were dominated by the EAEC pathotype (62.2%), followed by EPEC (18.9%) and ETEC (15.1%) (Table 1). The EIEC and STEC pathotypes were poorly represented, with identical frequencies of 1.9% each. The results that were reported in this study on the distribution of pathotypes are in agreement with a similar study carried out in South Africa which reported a predominance of EAEC (47%), EPEC (40%), and ETEC (23%) pathotypes (Lääveri et al., 2018). However, Wolde et al. (2024b) noted a predominance of EPEC (29.2%),

Table 2. Genetic determinants of AMD resistance in *E. coli* strains in the Republic of Guinea.

Antimicrobial drug	Determinants for resistance		
	Gene	n	Percentage
β-lactams (Ampicillin III-IV generation cephalosporins)	bla_{TEM-1}	48	90.6
	bla_{OXA-1}	3	5.7
	$bla_{CTX-M-15}$	7	13.2
	$bla_{CTX-M-27}$	7	13.2
	$bla_{CTX-M-42}$	1	1.9
Nalidixic acid	**gyrA* p. S83A	1	1.9
	**gyrA* p. S83L	16	30.2
	**parE* p. I529L	7	13.2
Ciprofloxacin	**gyrA* p. D87N	1	1.9
	**parC* p. S57T	1	1.9
	**parC* p. S80T	1	1.9
	**parE* p. S458A	1	1.9
Aminoglycosides (Gentamicin, Tobramycin)	*aac(3)-IId*	8	15.1
Tetracycline	*tetA*	21	39.6
	tetB	12	22.6
	tetD	5	9.4
Chloramphenicol	*catA*	2	3.8
Trimethoprim/ Sulfamethoxazole	*dfrA*	38	71.7
	sul1	11	20.8
	sul2	33	62.3

Gene mutations/substitutions

EAEC (12.5%), and ETEC (16.7%) pathotypes.

The distribution of prevalence according to age group was observed at 34.8% among children aged 0 to 5 years. Other studies also report a high prevalence among children in Egypt (20.6%) (Khairy et al., 2020) and in Nigeria (25.9%) (Ogunbiyi et al., 2023). It was reported that the prevalence of DEC was 10.1% in adults while in Senegal Ouaddane et al. (2024) found a prevalence of 31.4%. In adults, it was obtained that the respective frequencies of EAEC and EPEC pathotypes were 70.8 and 8.3% in Senegal while Ouaddane et al. (2024) found the respective frequencies of 19.7 and 7.2%.

In this study, all positive isolates were tested for antibiotic susceptibility. In total, a panel of fifteen antibiotics (Figure 1) commonly used for the treatment of diarrhea and other infectious diseases were evaluated (Ramatla et al., 2023).

Resistance to the antibiotics tested was detected in 52 (98.1%) of the *E. coli* strains. Literature data from previous studies also report high frequencies of resistance (Kalule et al., 2024; Tapia-Pastrana et al., 2024). Thus it was noted that the frequency was 54.1% in Ethiopia (Wolde et al., 2024a), 67.2% in Zambia (Mwansa et al., 2023) and 39.7% in Nigeria (Aworh et al., 2019).

Beta-lactams are antimicrobial agents widely used for the treatment of infectious diseases including gastroenteritis. The resistance profile of the isolates in the present study indicates high resistance to ampicillin (88.7%), which is higher than other studies conducted in Ethiopia (52.7%) (Wolde et al., 2024a), in Zambia (46.8%) (Mwansa et al., 2023), in Benin (22.3%) (Sintondji et al., 2023), and Ghana (13.2%) (Dela et al., 2022).

High frequencies of resistance were noted for antibiotics such as sulfamethoxazole/trimethoprim (71.7%), tetracycline (66.0%), and nalidixic acid (34.0%). Frequencies lower than those that were reported were found with respective rates of 48.3, 61.7, and 19.0% for sulfamethoxazole/trimethoprim, tetracycline, and nalidixic acid (Mwansa et al., 2023).

The percentages of *E. coli* resistant to gentamicin (17.0%) were higher than those reported in Ethiopia (1.9%) (Wolde et al., 2024a) and South Africa (2%) (Mkuhlu et al., 2020).

The strains isolated in this study showed sensitivity to Meropenem (carbapenem group), Amikacin (aminoglycoside group), and Nitrofurantoin (nitrofuran

Table 3. Phenotype and genomic characterization of AMD resistance in *E. coli* strains in the Republic of Guinea

Phenotype	n	Genotype	n
AMP	5	*bla*$_{TEM-1}$	4
NA	1	gyrA p.S83L	1
TET	1	*tetA*	1
AMP+TET+SXT	24	*bla*$_{TEM-1}$+*tetD*+*dfrA*+*sul2*	1
		bla$_{TEM-1}$+*tetA*+*dfrA*+ *sul1*	1
		bla$_{TEM-1}$+*tetA*+*dfrA*+ *sul2*	6
		bla$_{TEM-1}$+*tetB*+*dfrA*+ *sul2*	5
		bla$_{TEM-1}$+*tetA*+*dfrA*+ *sul1*+*sul2*	9
		bla$_{TEM-1}$+*tetA*+*tetB*+*dfrA*+ *sul1*	1
		bla$_{TEM-1}$+*tetD*+*dfrA*	1
AMP+NA+SXT	2	*bla*$_{TEM-1}$+*gyrA* p.S83L+ *dfrA*+*sul2*	2
AMP+3rd and thGC+NA+SXT	2	*bla*$_{TEM-1}$+*bla*$_{CTX-M-15}$+*bla*$_{CTX-M-42}$+ *gyrA* p.S83A +*dfrA*	2
AMP+3rd and thGC +TET+SXT	3	*bla*$_{TEM-1}$+*bla*$_{CTX-M-15}$+*tetA*+*dfrA*+*sul2*	2
		bla$_{TEM-1}$+*bla*$_{CTX-M-15}$+*tetB*+*dfrA*+*sul2*	1
AMP+NA+TET+SXT	2	*bla*$_{TEM-1}$+*gyrA* p. S83L+*tetA*+*dfrA*+*sul2*	1
		bla$_{TEM-1}$+*gyrA* p. S83L+*tetB*+*dfrA*+*sul2*	1
NA+TET+SXT+ GM	1	*gyrA* p.S83L+*tetA*+*dfrA*+sul2+*aac(3)-IId*	1
AMP+3rd and thGC +NA+ GM + NN	7	*bla*$_{TEM-1}$+*bla*$_{CTX-M-27}$+*gyrA* p. S83L+*parE* p. I529L+*aac(3)-IId*	7
AMP+3rd and thGC +TET+SXT + GM	1	*bla*$_{TEM-1}$+*bla*$_{CTX-M-15}$+*tetB*+*dfrA*+*sul2*+*aac(3)-IId*	1
AMP+NA+TET+SXT+ C	2	*bla*$_{TEM-1}$+*bla*$_{OXA-1}$+*gyr*A p. S83L+*tetB*+*dfrA*+ *sul2*+*catA*	2
AMP+3rd and thGC+NA+CIP+TET+ SXT	1	*bla*$_{TEM-1}$+*bla*$_{OXA-1}$+ *bla*$_{CTX-M-15}$+*gyrA* p.S83L+ *gyrA* p. D87N+*parC* p. S57T+*parC* p. S80T *parE* p. S458A+*tetD*+*dfrA*+*sul2*	1
Total	13	Total	22

AMP: Ampicillin, 3rd and 4th GC: 3rd and 4th Generation Cephalosporin, NA: Nalidixic acid, CIP: Ciprofloxacin, GM: Gentamicin, NN: Tobramycin, TET: Tetracycline, C: Chloramphenicol, SXT: Trimethoprim/Sulfamethoxazole.

group). These results differ from those reported by Ouaddane et al. (2024) in Senegal, which showed sensitivity to carbapenems but resistance to Amikacin and Nitrofurantoin.

The multidrug-resistant (MDR) phenotype in this study was 88.7% of *E. coli*, which is higher than other studies conducted in Ethiopia (19.2%) (Wolde et al., 2024a), in Zambia (29.3%) (Mwansa et al., 2023), in South Korea (45.3%) (Park et al., 2022), in South Africa (71%) (Mkuhlu et al., 2020). However, these results are lower than those reported by Aworh et al. (2023) who indicate a frequency of 96.3% in Nigeria.

These results therefore suggest that these antibiotics should not be considered as an empirical treatment for DEC infections, as they could further fuel the spread of antibiotic resistance in the population.

Antimicrobial resistance determinants were screened on the basis of observed phenotypic resistance (Table 2). This study showed that β-lactamase production genes carried at least one ESBL-encoding gene, of which *TEM-1* was the most common (90.6%), followed by *CTX-M-15* (13.2%), *CTX-M-27* (13.2%) and *CTX-M-42* (1.9%), *OXA* (5.7%) and *SHV* (0%). These results differ from the previous study by Jafari et al. (2020) of which *CTX-M9* was the most common (68.2%), followed by *TEM* (54.5%), *SHV* (45.4%) and *OXA* (40.9%) (Table 3).

Trimethoprim-sulfamethoxazole resistance was due to the production of efflux genes encoded by the combination of *sul1* (20.8%) and *sul2* (62.3%) sulfonamide resistance genes and *dfrA* (71.7%) trimethoprim resistance genes. Lower frequencies than those reported here were identified, with rates of 8.3, 33.3, and 8.3% respectively for *sul1, sul2* and *dfrA* genes (Wolde et al., 2024b).

It should be noted that the sul genes showed significant variability in expression within *E. coli*, which is probably correlated with the variability observed in cotrimoxazole resistance in this study (Zhou et al., 2021).

In *E. coli* strains phenotypically resistant to the quinolone/fluoroquinolone group, chromosomal mutations and single nucleotide substitutions were found in the *gyrA, parC, and parE* genes, while the plasmid-mediated

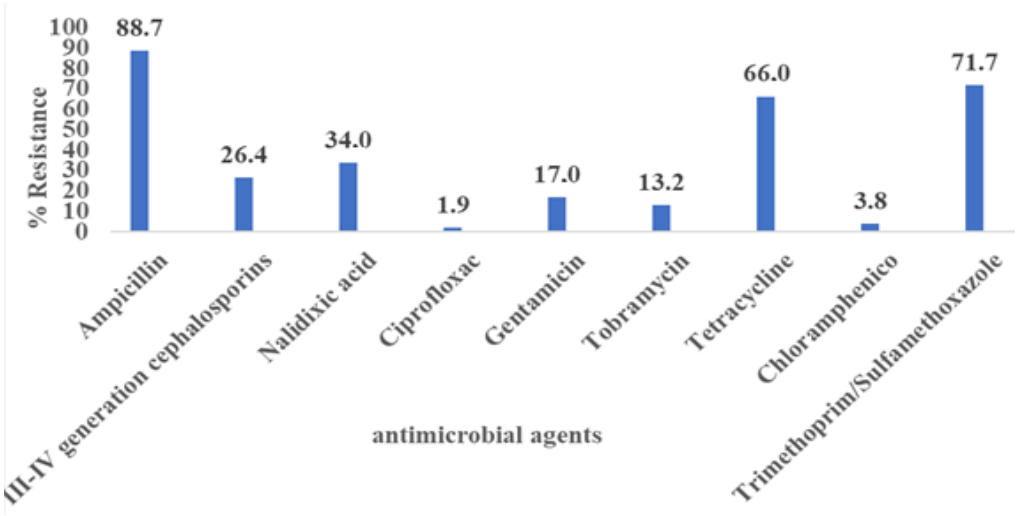

Figure 1. Frequency (%) of antimicrobial resistance in *E. coli* strains isolated from diarrhoeal stool.

qnrS1 gene was not found. In parallel, five *E. coli* strains phenotypically sensitive to nalidixic acid revealed the *qnrS1* gene, which determines weak resistance to quinolones. This is inconsistent with the results of different studies carried out in Nigeria, where the qnrS1 gene was the most widespread (Aworh et al., 2023). This study showed that *gyrA* gene mutants carrying the S83L mutation, play a major role in quinolone resistance and are significantly higher than other quinolone resistance mechanisms. This is consistent with the results of similar studies carried out in the Czech Republic, where the *gyrA* gene *p. S83L* was the most widespread (Röderova et al., 2017).

High frequencies of resistance were noted for tetracycline resistance genes such as *tetA* (39.6%), *tetB* (22.6%), and *tetD* (9.4%). Frequencies lower than those reported were found with respective rates of 16.7, 12.5, and 0% for *tetA, tetB,* and *tetD* genes (Wolde et al., 2024b).

Conclusion

EAEC and EPEC infections were the main etiological agents of diarrhoea in patients with DEC pathotypes. Analysis of genetic determinants revealed a large number of genotypes, indicating considerable heterogeneity in the antimicrobial resistance phenotypes of DEC strains. The results of this study support further research into the identification of phenotypic and genotypic profiles of a larger number of CDE pathotypes in various clinical samples.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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