Original Article

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Fecal Carriage of Multi-drug Resistant *Escherichia coli* in Healthy Children: a Community-based Study

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ABSTRACT

Introduction: *Escherichia coli* is enteric Gram-negative bacilli, earlier recognized as non-invasive commensal. Invasive strains of *E. coli* have been identified and are associated with a variety of human infections. Increasing drug resistance among *E. coli* has been reported. Multidrug resistance is the ability of an organism to resist at least one agent in three or more antimicrobial categories. The aim of this study was to detect fecal carriage of MDR *E. coli* isolates among healthy school children.

Methods: A total of 139 stool samples were collected from Rastriya Secondary School, Purano Tudikhel, Pokhara-1. Isolation, identification, and Antibiotic susceptibility test pattern of *E. coli* isolates were done by standard microbiological techniques. Further Extended Spectrum Beta-Lactamase test was performed by Combined Disc Test and data analysis was done by using Microsoft Excel.

Results: A total of 106 *E. coli* were isolated from the stool samples. MDR *E. coli* were found to be 8 (7.55%) and 7 *E. coli* isolates were resistant to Ceftazidime which was further tested for ESBL and 5 isolates were found to be ESBL producers. The resistance of *E. coli* to Ampicillin, Nitrofurantoin, Ceftriaxone, Ceftazidime, Ciprofloxacin, and Gentamicin was 27.36%, 43%, 10.38%, 6.60%, 6.60%, and 2.83% respectively. Only one isolate was resistant to all antibiotics used except Imipenem.

Conclusion: *E. coli* is the main Gram-negative bacteria isolated from the stool sample and identified as the main carrier of antimicrobial resistance in species comparison of Enterobacteriaceae isolated from fecal samples. Therefore, quick surveillance is needed in order to effectively handle such MDR strains. The *E. coli* population susceptible to all antibiotics used was a much more diverse group than the resistant and ESBL-producing *E. coli*.

Keywords: *Escherichia coli, multidrug resistance, fecal carriage, children.*

INTRODUCTION

Enteric Gram-negative bacilli belonging to the Enterobacteriaceae family, *Escherichia coli* were first identified as non-invasive commensal. It is a very adaptable bacterial species, and its strains can either be commensal, living in symbiosis with pathogenic organisms in the human gut and providing resistance, or they can be pathogenic and cause disease in the intestinal and extraintestinal regions. Colonizers seldom infect immune-compromised hosts, whereas pathogenic strains of *E. coli* carry out the virulence components necessary for bacterial pathogenesis.[1,2] Antimicrobial resistance (AMR) has seriously threatened public health. It is difficult to underestimate the severity of the AMR problem and the challenges associated with implementing antimicrobial resistance control measures. Antimicrobial resistance has been recognized as a global health issue by the World Health Organization which has advised strengthening the knowledge and evidence base through AMR surveillance data.[3] Despite the fact that most research has been on pathogen antibiotic resistance, commensal flora can play a significant role in antimicrobial resistance. Even though many different bacterial species make up the intestinal flora, one of the main causes of antibiotic resistance is now understood to be *E. coli* among the Enterobacteriaceae family of bacteria isolated from feces.[4] Since *E. coli* is a widespread pathogen associated with illnesses in the community, it has been noted that in recent years, a variety of *E. coli* strains have emerged and spread widely, displaying resistance to a variety of antimicrobial treatments. As a result, communities are increasingly isolating multidrug-resistant E. col i.[5]

Antibiotic resistance is likely to increase at high rates if antibiotic prescription rates are high. Antibiotic resistance has increased globally, which has made treating bacterial

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illnesses more difficult. *E. coli*, a commensal flora that can serve as a significant antimicrobial resistance reservoir, was chosen as the focus of this investigation in order to identify its multi-drug resistance pattern. In Nepal, there are limited investigations of the multi-drug resistance pattern of *E. coli* in a fecal carriage. So, we were interested to perform this type of study.

The main objective of this study was to detect fecal carriage of MDR *E. coli* isolates amongst healthy school-going children. The specific goals were to first isolate *Escherichia coli* from a stool sample and perform antibiotic susceptibility testing. The prevalence of antibiotic-resistant *E. coli* in healthy children was obtained with further phenotypic detection of ESBL and AmpC.

METHODS

It was a cross-sectional study conducted within a community. The quantitative study was carried out to access microbiological analysis of *E. coli* in stool samples among healthy children with the approval of a proposal submitted to the School of Health and Allied Sciences, Pokhara University, Pokhara-30. This study was carried out from October 2018 to December 2018.

The sample size was done using Cochran's formula ($N= z^2pq/d^2$) where a prevalence (p) value of 0.9 was obtained for MDR *E. coli* in the fecal carriage of healthy children in a previous study with permissible error (d) of 5%.[6] The estimated sample size was 139. A total of 145 sample was collected from children of Rastriya Secondary School, Purano Tudikhel, Pokhara-1after obtaining approval and consent and the isolation and susceptibility patterns were studied in the Microbiology laboratory of the School of Health and Allied Sciences, Pokhara University. All the samples were collected in sterile vials. The inclusion and exclusion criteria for the sample selection are enlisted in Table 1 below.

Table 1: Inclusion and exclusion criteria of the study participants

Inclusion Criteria

- Both male and female subjects
- Children under 14 years.[7]

Exclusion Criteria

- Children under antimicrobial medications,
- Eligible samples of which the consent were not obtained and unlabeled ones.
- History of underlying disease.

Out of total collected samples 6 samples were excluded.

Laboratory diagnosis and Identification

From the healthy kids, a total of 139 stool samples were taken. All information was kept private, including the child's name, age, and parents' names. After obtaining the sample, they were transported to the lab using an ice bag and processed in accordance with SOPs. Inoculation of the sample was done in a culture plate using Mac-Conkey agar as culture media based on the streak plate method followed by Gram staining. The Sulphide Indole motility (SIM), Methyl Red, Voges-Proskauer (VP), Urease, Citrate Utilization, and Triple Sugar Iron (TSI) tests were carried out as part of the biochemical assay and the results were interpreted. According to CLSI recommendations, antibiotics were chosen based on the isolates.[8] The antibiotic susceptibility test was performed using the Modified Kirby Bauer Disc Diffusion Test. Antibiotics that were tested include Ampicillin, Ceftriaxone, Gentamicin, Ciprofloxacin, Nitrofurantoin Ceftazidime, and Imipenem. As per the CLSI guideline, antibiotics used were assigned as Sensitive Intermediate and Resistant.[8]

E. coli isolates resistant to Ceftazidime were further processed for the ESBL test (by Combined Disc Test).[9] *E. coli* resistant to Imipenem was further processed for the AmpC lactamase test (by Cefoxitin-EDTA Disc Diffusion Test).[10]

We worked extremely hard to maintain the quality of the results throughout our investigation. Quality Control activities were carried out to examine the media utilized, potential antibiotic contamination, and human error. In the sterile plastic container, the samples were gathered. The media was measured out accurately and then blended with distilled water. The media was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. The medium was cooled down to 40-50 °C before being poured into Petri dishes. The fridge was where the antibiotics were kept. The AST was performed in an aseptic setting after the plates had been incubated at 37°C for 18 to 24 hours.

Sample collection and the entire test panel were completed under extremely sterile conditions using the standard method in accordance with SOPs. The use of expired reagents was avoided. Using an ice bag, samples were delivered to the lab. Culture media was produced in a proper quality-controlled environment. The temperature for storage and incubation was confirmed. Under conventional CLSI recommendations, an antimicrobial susceptibility test was performed.[8] As a result, we can guarantee the validity and reliability of the study's findings.

Data analysis and compilation were done using Microsoft Excel and SPSS software and the results were interpreted. The significant association of dependent variables was assessed using Pearson's Chi-square test.

RESULTS

In our study, 139 stool samples were collected from healthy children. Among them 43 (30.93%) children were less than 8 years old; 74 (53.24%) children were from 8 to 10 years old and 22 (15.83%) children were more than 10 years old. As per sex categorization, 72 (51.8%) were male and 67 (48.2%) were female. Out of 139 stool samples collected, bacterial growth was observed in 133 samples and no growth was seen in 6 samples as shown in Figure 1.

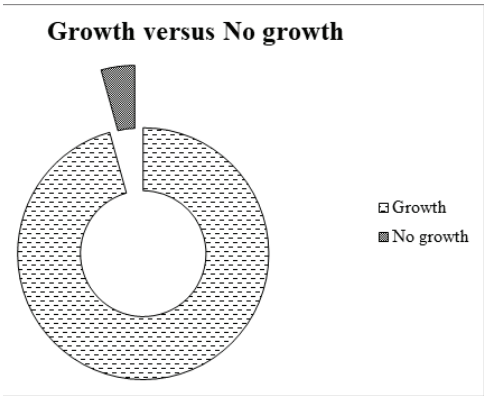

Figure 1: Growth versus No growth in collected samples

In our study, growth was obtained in 133 samples. Out of 133 bacterial isolates, 100 were only *E. coli*; 8 were only *Klebsiella spp.*; 6 were mixed isolates (both *E. coli* and *Klebsiella spp.*); 3 were only *Proteus spp.*; 5 were only Streptococcus spp.; 5 were only Gram-negative non-fermenters; and 6 were only Gram-positive bacilli. The bacterial categorization is shown in Table 2 below.

Table 2: Bacterial isolates categorization in the sample

Bacteria	Total number of samples	Percentage (%)
E. coli (only)	100	75.19
Klebsiella spp. (only)	8	6.01
Mixed isolates (both *E. coli* and *Klebsiella spp.*)	6	4.51
Proteus spp. (only)	3	2.26
Streptococcus spp. (only)	5	3.76
Gram negative non-fermenters (only)	5	3.76
Gram positive bacilli (only)	6	4.51%
Total	133	100%

E. coli isolates from the sample were also collected, and their antibiotic sensitivity pattern was displayed as followed in Table 3 below.

Table 3: Total number of *E. coli* isolates

Antibiotics used	Total number of *E. coli* isolates		
	Sensitive	Intermediate	Resistant
Beta-lactams			
Ampicillin (AMP)	75	2	29
Cephalosporins			
Ceftazidime (CAZ)	99	0	7
Ceftriaxone (CTR)	95	0	11
Aminoglycosides			
Gentamicin (GEN)	102	1	3
Quinolones			
Ciprofloxacin (CIP)	99	0	7
Nitrofuran			
Nitrofurantoin (NIT)	94	2	10
Carbapenems			
Imepenem (IPM)	106	0	0

Resistance rates were seen when observing *E. coli*'s antibiotic resistance patterns that show, for Ampicillin (27.36%), Ceftriaxone (10.38%), Nitrofurantoin (9.43%), Ceftazidime (6.60%), Ciprofloxacin (6.60%), and Gentamicin (2.83%). Resistance was not observed in the case of Imipenem. Among them, Ampicillin showed a higher resistance rate and Gentamicin showed a lower resistance rate. The age-wise comparison of antibiotic resistance patterns of drugs is given in the following Table 4:

Table 4: Age-wise antibiotic resistance pattern of antibiotics

Drugs	AST Pattern	Age (years)			Total	p-value
		<8	8-10	>10		
AMP	I	1	1	0	2	0.737
	R	10	12	7	29	
	S	20	30	25	75	
	Total	31	43	32	106	
CAZ	R	3	3	1	7	0.573
	S	28	40	31	99	
	Total	31	43	32	106	
CTR	R	3	6	2	11	0.551
	S	28	37	30	95	
	Total	31	43	32	106	

GEN	I	0	1	0	1	0.384
	R	2	0	1	3	
	S	29	42	31	102	
	Total	31	43	32	106	
CIP	R	2	3	2	7	0.991
	S	29	40	30	99	
	Total	31	43	32	106	
NIT	I	2	0	0	2	0.113
	R	3	6	1	10	
	S	26	37	31	94	
	Total	31	43	32	106	
IPM	S	31	43	32	106	<0.001
	Total	31	43	32	106	

Abbreviation: AMP:Ampicillin,CTR:Ceftriaxone, GEN: Gentamicin, CIP: Ciprofloxacin NIT:Nitrofurantoin, CAZ: Ceftazidime, IMP:Imepenem, S: Sensitive, R:Resistant, I:Intermediate

All the results showed insignificant output except for imipenem (p-value <0.05). The sex-wise observation of the antibiotic resistance pattern of antibiotics is shown in given Table 5 below.

Table 5: Sex-wise antibiotic sensitivity pattern of antibiotics

Drugs	AST Pattern	Sex		Total	p-value
		F	M		
AMP	I	1	1	2	
	R	16	13	29	0.739
	S	35	40	75	
	Total	52	54	106	
CAZ	R	4	3	7	
	S	48	51	99	0.658
	Total	52	54	106	
CTR	R	5	6	11	
	S	47	48	95	0.801
	Total	52	54	106	
GEN	I	1	0	1	
	R	1	2	3	0.513
	S	50	52	102	
	Total	52	54	106	
CIP	R	4	3	7	
	S	48	51	99	0.658
	Total	52	54	106	
NIT	I	1	1	2	
	R	5	5	10	0.998
	S	46	48	94	
	Total	52	54	106	
IPM	S	52	54	106	
	Total	52	54	106	<0.001

Abbreviation: AMP:Ampicillin,CTR:Ceftriaxone, GEN: Gentamicin, CIP: Ciprofloxacin NIT:Nitrofurantoin, CAZ: Ceftazidime, IMP:Imepenem, S: Sensitive, R:Resistant, I:Intermediate

Among sex-wise observation only the significant result was only seen in Imipenem (p-value <0.05). Out of 106 *E. coli* isolates, 19 isolates had just one antimicrobial agent resistance (1R); 9 isolates were resistant to two antimicrobial agents (2R); 4 isolates were resistant to three antimicrobial agents (3R) and 3 isolates were resistant to four antimicrobial agents (4R) and one isolate was resistant to six antimicrobial agents (6R) as illustrated in given Figure 2:

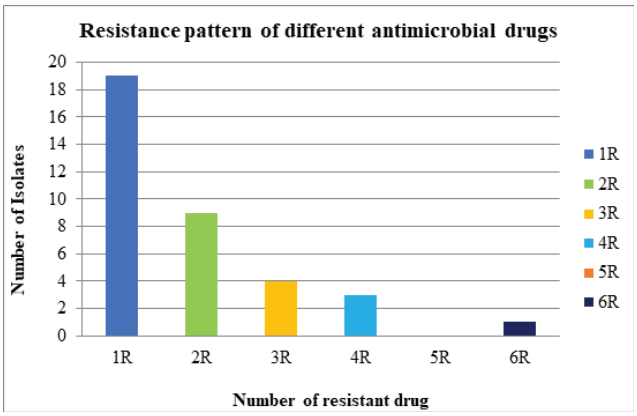

Figure 2: Resistance pattern of different antimicrobial drugs

Among 106 *E. coli* isolates secluded from the fecal specimens, 8 (7.55%) isolates showed multi-drug resistance. Among 106 isolates of *E. coli*, 7 isolates showed resistance to Ceftazidime. Out of them only 5 isolates were ESBL producers and 2 isolates were ESBL negative as shown in Figure 3.

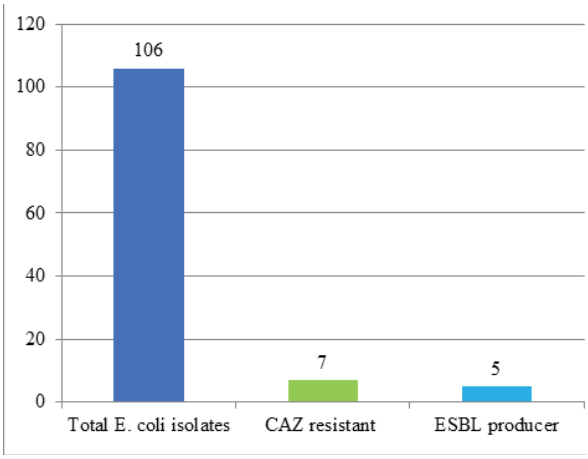

Figure 3: Detection of ESBL-producing *E. coli*

DISCUSSION

In feces, the Gram-negative bacillus *Escherichia coli* is released into the environment. In spite of the diversity of bacterial species that make up the gut flora, among

Enterobacteriaceae isolated from feces, *E. coli* has been identified as the main contributor to antibiotic resistance. To the best of our knowledge, in the scenario of Nepal, no research has tried to investigate the local status of antimicrobial resistance in the community focused on children because the majority of studies done in Nepal focus on the resistance patterns of microorganisms in the hospital context. So this work is a pioneer in the context of Nepal. Out of 139 stool samples collected from healthy children; 27.36% of isolates were resistant to Ampicillin but in a study done by Barreto A et al. 40.2% of the *E. coli* isolates Ampicillin resistant, out of 92 *E. coli* that have been isolated from stool specimens of 118 healthy children (1-14 years) in Portugal.[11] In our study, out of 139 stool samples; 4.71% of *E. coli* was ESBL producer. Similarly, a study done by Irajian G et al. in Iran showed that out of 109 stool samples, 17.45% of *E. coli* was ESBL producer.[12] Moreover, a total of 29 *E. coli* isolates were resistant to ciprofloxacin in the study done by Huang IF et al.in southern Taiwan but in our study, only 7 isolates were non-susceptible to Ciprofloxacin.[13] In our study, out of 106 *E. coli* isolates; 36 (33.96%) isolates were found to be resistant to at least one of the used antibiotics. 8 (7.55%) isolates displayed multidrug-resistant (MDR) and 5 (4.71%) isolates were found to be ESBL producers. A similar study by Singh AK et al. in Northeast India showed that among 550 *E. coli* isolates, 495 (90%) were resistant to at least one of the commonly used antibiotics (ADR). Multidrug resistance (MDR) was displayed by 226 (41%) isolates while only 17 (3%) of the isolates were found to be ESBL producers.[14] In our study, the *E. coli* isolates were not resistant to the Imepenem as all isolates were susceptible to it and a study done by Shakya P et al. in Ujjan, India also showed that the proportions of *E. coli* isolates resistant to Imepenem was 0%.[7] Out of 106, *E. coli* isolates in our study, the ESBL-producing *E. coli* were found to be 5 (4.71%). According to Shahandeh Z et al. study in Iran out of 259 isolated *E. coli*, the ESBL-producing *E. coli* were 137 (52.9%).[15]

In our study, out of 139 stool samples tested, 106 *E. coli* isolates were obtained; out of those isolates, 33.96% were carriage of resistance to one or more antibiotics and MDR was 7.55%. Resistance was most frequently observed to Ampicillin (27.36%) and a study in Tamil Nadu done by Seidman JC et al. showed that ≥1 antibiotic resistance rate of 63% and multiple drug resistance of 32%, respectively, in 119 *E. coli* colony pools (stool samples) analyzed.[16] Nalidixic acid (42%), ampicillin (39%), cotrimoxazole (37%) and tetracycline (35%) showed the highest rates of resistance.

The in-vitro antibiotic susceptibility pattern of 106 *E. coli* isolates among seven antimicrobial drugs showed that Imipenem was the most effective antibiotic out of the drugs examined which is similar to the study done by Baral BP et al.[17] Nonetheless, it shouldn't be used as the first line of treatment unless the infection is life-threatening because carbapenems are seen to be the last choice. Bacteria that are resistant to carbapenem may develop if this condition is not treated seriously and the medications are used improperly. Gentamicin is the most sensitive of the remaining six drugs followed by Ciprofloxacin, Ceftazidime, Ceftriaxone, Nitrofurantoin and Ampicillin.

LIMITATION

We became unable to perform further molecular and confirmatory tests due to constraining budget and time limitations.

CONCLUSION

The majority of the identified gram-negative bacteria from the stool sample are *E. coli* showing a rapid rise in multi-drug resistance representing as a primary carrier of antibiotic resistance. In our analysis, 106 *Escherichia coli* isolates were found in 139 samples. 33.96% of those *E. coli* isolates were resistant to a single drug only. The percentage of MDR *E. coli* was 7.5%. Further tests for ESBL revealed that 5 out of 7 isolates that were ceftazidime resistant also produced ESBL. Imepenem was effective against every *E. coli* strain that was discovered in the stool samples. So additional AmpC testing was not conducted. Only one isolate in our investigation was impervious to Imepenem and all other employed antibiotics. As a result, the population of *E. coli* in our study that was sensitive to all of the antibiotics utilized was significantly more varied than the resistant and ESBL-producing *E. coli*. It is urgently necessary to develop strategic policy measures to lower the prevalence of MDR as a result of the clinical threat posed by the increased prevalence of resistant organisms, which is causing serious treatment issues.

Abbreviation:

AMR	Antimicrobial resistance
AST	Antibiotic Sensitivity Pattern
CLSI	Clinical and Laboratory Standards Institute
ESBL	Extended Spectrum Beta-Lactamase
MDR	Multidrug Resistance
PURC	Pokhara University Research Centre
QC	Quality Control
SIM	Sulphide Indole motility
TSI	Triple Sugar Iron
VP	Voges Proskauer

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Competing interests

The authors state that there are no interests at odds with one another.

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