

# Fecal Carriage of Extended-Spectrum beta-Lactamase Producing *Escherichia coli* among Health Science Students

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## ABSTRACT

**Background:** The incidence of antibiotic resistance in commensal bacteria is increasing with the production of extended-spectrum beta-lactamase. Therefore, this study was conducted to understand the status of fecal carriage of such enzyme producing *Escherichia coli* among health science students of seven different faculties of Institute of Medicine, Tribhuvan University.

**Methods:** This was a cross-sectional study conducted over six months among the health science students. One stool sample collected from each student was cultured and *Escherichia coli* isolates were identified, antibiotic sensitivity profile was produced, and extended-spectrum beta-lactamase production was detected following Clinical and Laboratory Standards Institute guidelines.

**Results:** A total of 156 students participated in the study, and *Escherichia coli* was isolated from all. Out of the total 156 *Escherichia coli* isolates, 11.5% were extended-spectrum beta-lactamase-producers and 14.7% were multidrug-resistant. The highest rate of fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* was found among Bachelor of Medicine and Bachelor of Surgery students (17.5%) and Bachelor of Science in Medical Imaging Technology (16.7%) students. Such enzyme producing *Escherichia coli* was found in the range of 6.9% to 25.0% among second- to fifth-year students. A significant number of extended-spectrum beta-lactamase-producing isolates were resistant to ciprofloxacin and gentamicin, apart from other extended-spectrum beta-lactamase substrate antibiotics, when compared with non-producers.

**Conclusions:** A high rate of extended-spectrum beta-lactamase-producing *Escherichia coli* was detected from the gut of healthy health science students which indicates their possible dissemination throughout the wider community resulting in potential outbreak of infections caused by such organisms.

**Keywords:** *Escherichia coli*; fecal carriage; health science students; multidrug-resistant.

## INTRODUCTION

Multidrug-resistant (MDR) intestinal commensals including *Escherichia coli* have been documented years ago from healthy individuals, and the incidence of antibiotic resistance in these bacteria is continuously rising.<sup>1,2</sup> Third-generation cephalosporin resistance in *E. coli* is a major public health problem to be addressed.<sup>3</sup> The production of extended-spectrum beta-lactamase (ESBL) is the primary mechanism for this resistance.<sup>4,5</sup>

Our concern is that the Health Science(HS) students are

exposed to many patients who have been colonized or infected by MDR bacteria including ESBL-producers. As a result, these students are expected to spread such bugs in hospitals and communities. However, there are limited published reports on the fecal carriage of ESBL-producing *E. coli* among HS students from Nepal.<sup>4</sup>

This study aimed to determine the status of fecal carriage of ESBL-producing *E. coli* among HS students of Maharajgunj Medical Campus (MMC) and Maharajgunj Nursing Campus (MNC), Institute of Medicine (IOM), Tribhuvan University (TU), Nepal.

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## METHODS

This descriptive cross-sectional study was carried out over six months period from May to October 2019 among the HS students of MMC and MNC, IOM, Tribhuvan University, and microbiological procedures were carried out at the clinical microbiology department of Tribhuvan University Teaching Hospital, Nepal. The ethical approval for the study was obtained from Nepal Health Research Council (Ref. No. 2241). Any healthy HS students willing to participate in the study and gave consent were included in the study. The students who were under antibiotic medications or with intestinal diseases were excluded from the study. The calculated sample size was 156 and the same number of HS students were enrolled in the study.

One stool sample was collected from each student in a clean, sterile, wide-mouthed container with a lid. The collected samples were cultured onto MacConkey agar plates and incubated aerobically at 37°C for overnight.<sup>6</sup> The colony characteristics of bacterial growth were observed and *E. coli* isolates were identified by Gram's staining, motility test, and different biochemical reactions according to standard methods of the American Society for Microbiology (ASM).<sup>7</sup>

The antibiotic susceptibility profile of *E. coli* isolates was determined by the Kirby-Bauer disk diffusion method according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), M100S Document, 26<sup>th</sup> Edition (2016) on Mueller-Hinton agar (MHA). The bacterial isolates were tested against ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), and gentamicin (10 µg). For the validity of the antibiotic susceptibility test, *E. coli* ATCC 25922 was used as the control organisms.<sup>8</sup> The *E. coli* isolates were identified as MDR strains when they were resistant to at least one agent in three or more antimicrobial classes according to Magiorakos et al.<sup>9</sup>

For the screening of ESBL-producing *E. coli*, ceftriaxone (CTR, 30 µg) and ceftazidime (CAZ, 30 µg) disks were used. When the zone of inhibition (ZOI) was either  $\leq 25$  mm for CTR or  $\leq 22$  mm for CAZ, the isolates were suspected of being potential ESBL-producers. The isolates that were suspected as ESBL-producers by the screening test were further confirmed by the combination disk (CD) method according to CLSI guidelines. The CD test was performed on MHA where CTR and CAZ alone and in combination with clavulanic acid (CTR/CA, 30/10 µg and CAZ/CA, 30/10 µg) were used. When an increase in ZOI of  $\geq 5$  mm for either CTR/CA or CAZ/CA than CTR or CAZ was observed after an incubation of 16-18 hours at 35±2°C,

the isolates were confirmed as ESBL-producer. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls respectively for the validation of the ESBL detection test.<sup>8</sup>

Data were processed and analyzed using Statistical Package for Social Sciences version 16.0 and interpreted according to frequency distribution and percentage. Variables were compared using Pearson's chi-square test and data with the p-value <0.05 were considered significant.

## RESULTS

The total number of HS students who participated in the study was 156 and were from seven different academic programs viz. Bachelor of Science in Medical Laboratory Technology (B.Sc. MLT) (n=42, 26.9%), Bachelor in Medicine and Bachelor in Surgery (MBBS) (n=40, 25.6%), Bachelor in Pharmacy (B. Pharmacy) (n=22, 14.1%), Bachelor in Sciences Nursing (B.Sc. Nursing) (n=21, 13.5%), Bachelor in Public Health (BPH) (n=14, 9.0%), Bachelor in Optometry (B. Optometry) (n=11, 7.1%), and Bachelor of Science in Medical Imaging Technology (B.Sc. MIT) (n=6, 3.8%). Among the total 156 students, 93 (59.6%) were male and 63 (40.4%) were female. The mean age of the study participants was 23.12 years (SD±2.87) ranging from 19 to 34 years. From 156 stool samples, a total of 156 *E. coli* isolates were grown on culture, i.e., one isolate from each sample. Out of total *E. coli* isolates, 11.5% (n=18) were ESBL-producers and 14.7% (n=23) were MDR. The highest rate of fecal carriage of ESBL-producing *E. coli* was found among MBBS students followed by B.Sc. MIT, B. Pharmacy, B.Sc. MLT, B.Sc. Nursing and B. Optometry students. The highest rate of fecal carriage of MDR *E. coli* was found among MBBS students followed by B.Sc. Nursing, B. Pharmacy, B.Sc. MIT, B.Sc. MLT, and B. Optometry students (Table 1).

The students who participated in this study belonged to the four years HS programs and the fifth and sixth-year students were the Junior Intern and Senior Intern of MBBS faculty. ESBL-producing *E. coli* was not detected among the stool samples of first-year students however it increased among the second, third, fourth, and fifth-year students (6.9% to 25.0%). Similarly, the fecal carriage of MDR *E. coli* was only 5.0% among first-year students and it increased among the second, third, fourth, and fifth-year students (6.9% to 29.4%) (Table 2 and Figure 1).

The antibiotic susceptibility profile of commensal *E. coli* isolates is shown in Table 3. Most of the isolates were found susceptible to amoxicillin-clavulanate,

ciprofloxacin, and gentamicin with relative resistance against ampicillin, cotrimoxazole, ceftriaxone, and ceftazidime. When comparing to ESBL non-producers, a significant number of ESBL-producing isolates were resistant to ampicillin, ceftriaxone, ceftazidime, ciprofloxacin, and gentamicin (Table 4).

Table 1. Distribution of fecal carriage of ESBL-producing and MDR *E. coli* according to the study program.

Study Program	Number of <i>E. coli</i> isolates	Number (%)			
		ESBL-producers	ESBL-non-producers	MDR	Non-MDR
B.Sc. MLT	42	4 (9.5)	38 (90.5)	4 (9.5)	38 (90.5)
MBBS	40	7 (17.5)	33 (82.5)	9 (22.5)	31 (77.5)
B. Pharmacy	22	3 (13.6)	19 (86.4)	4 (18.2)	18 (81.8)
B.Sc. Nursing	21	2 (9.5)	19 (90.5)	4 (19.0)	17 (81.0)
BPH	14	0 (0)	14 (100)	0 (0)	14 (100)
B. Optometry	11	1 (9.1)	10 (90.9)	1 (9.1)	10 (90.9)
B.Sc. MIT	6	1 (16.7)	5 (83.3)	1 (16.7)	5 (83.3)
Total	156	18 (11.5)	138 (88.5)	23 (14.7)	133 (85.3)

Table 2. Distribution of fecal carriage of ESBL-producing and MDR *E. coli* according to study year.

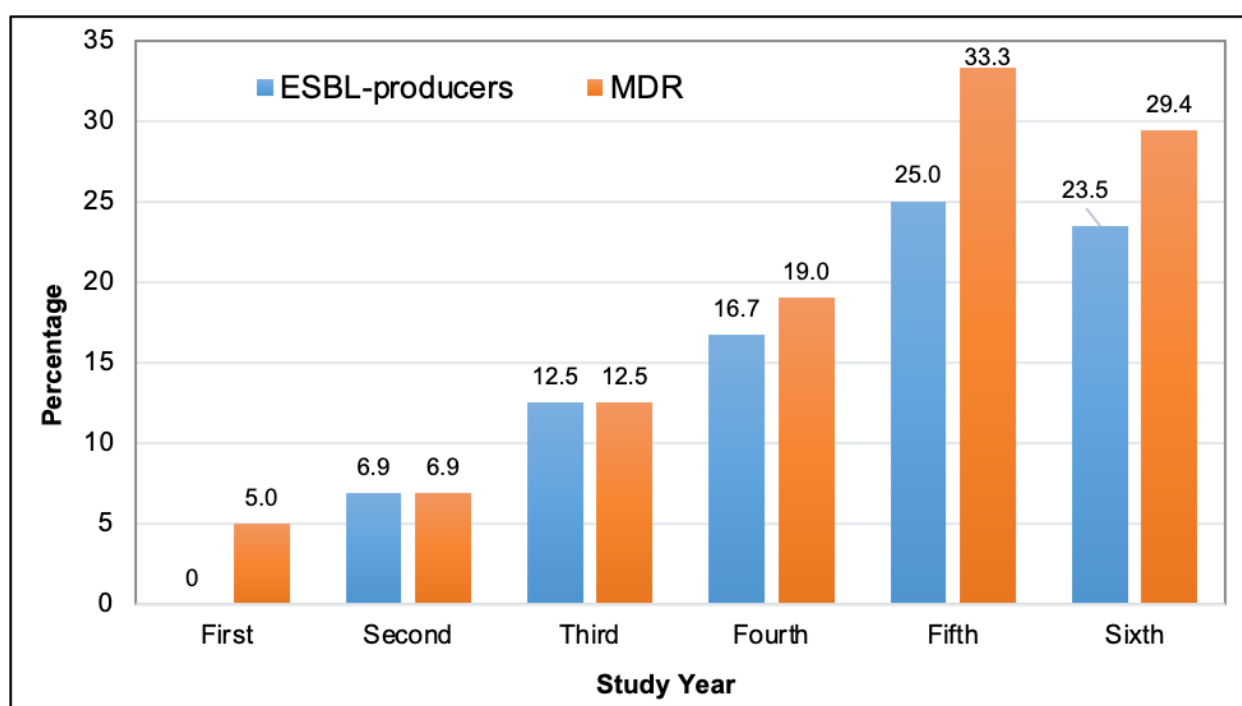
Study Year	Number of <i>E. coli</i> isolates	Number (%)			
		ESBL-producers	ESBL-non-producers	MDR	Non-MDR
First	40	0 (0)	40 (100)	2 (5.0)	38 (95.0)
Second	29	2 (6.9)	27 (93.1)	2 (6.9)	27 (93.1)
Third	16	2 (12.5)	14 (87.5)	2 (12.5)	14 (87.5)
Fourth	42	7 (16.7)	35 (83.3)	8 (19.0)	34 (81.0)
Fifth	12	3 (25.0)	9 (75.0)	4 (33.3)	8 (66.7)
Sixth	17	4 (23.5)	13 (76.5)	5 (29.4)	12 (70.6)
Total	156	18 (11.5)	138 (88.5)	23 (14.7)	133 (85.3)

Table 3. Antibiotic susceptibility profile of commensal *E. coli* isolates.

Antibiotics	Number (%)	
	Sensitive	Resistant
Ampicillin	113 (72.4)	43 (27.6)
Amoxicillin-clavulanate	148 (94.9)	8 (5.1)
Ciprofloxacin	145 (92.9)	11 (7.1)
Cotrimoxazole	132 (84.6)	24 (15.4)
Gentamicin	151 (96.7)	5 (3.2)
Ceftriaxone	133 (85.3)	23 (14.7)
Ceftazidime	133 (85.3)	23 (14.7)

Table 4. Antimicrobial resistance rate of ESBL-producing and non-producing *E. coli* isolates.

Antibiotics	ESBL-producer (n=18)	ESBL-non-producer (n=138)	p-value
Ampicillin	18 (100%)	25 (18.1%)	<0.001
Amoxicillin-clavulanate	1 (5.6%)	7 (5.1%)	0.931
Ciprofloxacin	4 (22.2%)	7 (5.1%)	0.007
Cotrimoxazole	5 (27.8%)	19 (13.8%)	0.123
Gentamicin	4 (22.2%)	1 (0.7%)	<0.001
Ceftriaxone	18 (100%)	5 (3.6%)	<0.001
Ceftazidime	18 (100%)	5 (3.6%)	<0.001

Figure 1. Percentage of fecal carriage of ESBL-producing and MDR *E. coli* according to study year.

## DISCUSSION

Antibiotic resistance is a global threat to human health that is constantly evolving. *Escherichia coli* may be a source of antibiotic resistance genes and may be responsible for a number of endogenous infections.<sup>10</sup> Human gastrointestinal tract has been well described as an epicenter for antibiotic-resistant bacteria.<sup>11</sup> Intestinal colonization with MDR and ESBL-producing bacteria can result in nosocomial infections, cross-transmission to other people, including outbreaks in hospitalized patients.<sup>2</sup> Various studies have been carried out in different parts of the world and have shown an elevated prevalence and dissemination of

ESBL-producing bacterial isolates in fecal samples of healthy people.<sup>12</sup> Health science students could be unnoticed reservoirs of ESBL-producing *E. coli*, resulting in the spread of antibiotic-resistant bacteria to patients in hospitals as these groups of students undergo hospital posting during the study period. Thus, finding of probable healthy carrier populations, here HS students, harboring antibiotic-resistant and ESBL-producing bacteria is becoming extremely relevant. In this study, we have investigated the status of fecal carriage of ESBL-producing *E. coli* among the students of Maharajgunj Medical Campus, Kathmandu, Nepal.

According to our study, the fecal carriage rate of

ESBL-producing *E. coli* among HS students was 11.5%. Another study carried out by Maharjan et al in 2016 reported nearly a similar rate (12.5%) of ESBL-producing commensal *E. coli* among healthy adult volunteers of a health science college in Nepal.<sup>2</sup> From previous studies, a higher rate of fecal carriage of ESBL-producing *E. coli* was reported by Sapkota et al from Nepal (46.5%) and Bassyouni et al from Egypt (62.7%) among HS students and healthcare workers, respectively.<sup>4,10</sup> However, a lower rates of ESBL-producing *E. coli* carriage have been previously documented among healthy individuals from Tunisia (7.3%), France (6%), and UK (5.95%).<sup>5,13,14</sup> These variations in the rate of intestinal colonization with ESBL-producing *E. coli* are likely to have been produced by the different extent of exposure to antibiotics or the bacterial strains in the different study participants.<sup>4</sup> The existence of ESBL-producing *E. coli* within the gastrointestinal tract of healthy people may result in the dissemination of antibiotic-resistance genes to other strains of *E. coli* and to other organisms within the gut. Furthermore, their presence raises the risk that other people will become carriers as a result of human-to-human or environmental transfer of resistant bacteria<sup>15</sup> as fecal carriage of ESBL-producing *E. coli* has been found to last for more than four months in a study in China among medical students.<sup>16</sup>

In this study, we have also investigated the trend of fecal carriage of ESBL-producing and MDR *E. coli* among HS students from different educational programs and study years. MBBS and B.Sc. MIT students had the highest rate of ESBL-producing *E. coli* in their stool samples (17.5% and 16.7%, respectively), followed by B. Pharmacy (13.6%), B.Sc. Nursing (9.5%), B.Sc. MLT (9.5%), and B. Optometry students (9.1%) while none of the students of BPH were found to be the carrier of ESBL-producing *E. coli*. Similarly, MBBS students also had the greatest rate of fecal carriage of MDR *E. coli* (22.5%), followed by B.Sc. Nursing (19.0%), B. Pharmacy (18.2%), B.Sc. MIT (16.7%), B.Sc. MLT (9.5%), and B. Optometry students (9.1%). When looking at study year, the rate of ESBL-producing and MDR *E. coli* colonization increased from second to fifth-year students, almost in parallel with fifth and sixth-year students. The increased hospital posting during the study period, which increases the exposure to antibiotic-resistant bacteria from patients and the hospital environment, could be the reason for the higher rate of ESBL-producing and MDR *E. coli* carriage among MBBS, B.Sc. MIT, B.Sc. Nursing students. Sapkota et al also reported that HS students had a greater rate of fecal carriage of ESBL-producing *E. coli* than non-HS students and B.Sc. Nursing students had a higher carriage rate than B.Sc. MLT students.<sup>4</sup>

Multidrug resistance is a common phenomenon, as

evidenced by our findings among commensal *E. coli* isolates. The fecal carriage rate of MDR *E. coli* among HS students was 14.7%, which is comparatively lower than the rate documented by Maharjan et al (21.3%) from Nepal,<sup>2</sup> Mathai et al (50.0%) from India<sup>17</sup>, and Royden et al (32.1%) from UK<sup>5</sup> among healthy populations. The commensal *E. coli* isolates showed resistance towards ampicillin (27.6%), cotrimoxazole (15.4%), and ceftriaxone/ceftazidime (14.7%). Besides these, a higher degree of resistance was observed among ESBL-producing *E. coli* isolates as compared to ESBL-non-producers towards ampicillin (100% vs 18.1%,  $p < 0.001$ ), ceftriaxone/ceftazidime (100% vs 3.6%,  $p < 0.001$ ), ciprofloxacin (22.2% vs. 5.1%,  $p < 0.007$ ), cotrimoxazole (27.8% vs 13.8%,  $p < 0.123$ ) and gentamicin (22.2% vs 0.7%,  $p < 0.001$ ). ESBL-producing isolates were also found to have a higher rate of resistance to quinolones, cotrimoxazole, and gentamicin in other studies from Nepal, India, and Japan.<sup>2,17,18</sup>

## CONCLUSIONS

An alarming rate of ESBL-producing and MDR *E. coli* was detected from the gut of healthy HS students. Healthcare providers should be aware that HS students are a high-risk population for carriage of MDR and ESBL-producing bacteria, which could lead to the dissemination of resistant bacteria throughout the healthcare and community settings. A broader and more detailed study should be carried out in different parts of the country to determine the status of fecal carriage of antibiotic-resistant bacteria among various medical and HS students, as well as there is a need for the development and implementation of policies that minimize their carrier rate.

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## CONFLICT OF INTEREST

None

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