



# Fecal Carriage Rate of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Among Apparently Health Food Handlers in Dilla University Student Cafeteria

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**Background:** The rapid spread of extended-spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae among food handlers is a public health concern and has become a serious world threat. *Klebsiella pneumoniae* and *Escherichia coli* are the predominant ESBL-producing Enterobacteriaceae. This study aimed to assess the magnitude of ESBL producing *K. pneumoniae* and *E. coli* isolated from the stool of food handlers in the Dilla University Student Cafeteria.

**Methods:** A cross-sectional study was conducted from November to September 2018/2019. A total of 220 stool samples were cultured on MacConkey agar and a series of biochemical tests were performed for the identification of bacterial species. Double-disk synergy method was used for the detection of ESBL-producing strains.

**Results:** The rate of fecal carriage of *E. coli* and *K. pneumoniae* among the food handlers of the current study was 66.4%, of which 81.5% was *E. coli*, while 18.5% was *K. pneumoniae*. Of the total bacterial isolates, 25.3% were confirmed to be positive for ESBL production, of which 19.9% was *E. coli*, while 5.5% was *K. pneumoniae*. ESBL-producing fecal carriage showed high resistance to aztreonam, cefotaxime, ceftriaxone, cefuroxime, and ceftazidime, with resistance rates ranging from 88% to 100%, while the lowest resistance rate was observed against imipenem accounted <26.3%. In this study, 91.9% of ESBL-producers were co-resistant to  $\beta$ -lactams antibiotics plus at least one of the non- $\beta$ -lactam antibiotics. ESBL carriers were more common among food handlers who used antibiotics repeatedly, drunk unpasteurized milk, and ate raw meat.

**Conclusion:** The magnitude of ESBL-producing *E. coli* and *K. pneumoniae* fecal carriage is alarmingly high and a threat to human health. MDR to third-generation cephalosporins and non- $\beta$ -lactam antibiotics is more common among ESBL producers. Detecting ESBL-producing organisms has a remarkable importance in clinical decision-making. Hence, a coordinated effort should be implemented for the prevention and control of the disease.

**Keywords:** extended-spectrum  $\beta$ -lactamase, Enterobacteriaceae, antibiotic resistance, *Klebsiella pneumoniae*, *Escherichia coli*, Ethiopia

## Background

Enterobacteriaceae are a substantial, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of human beings and animals.<sup>1</sup> These microorganisms have emerged as one of the most important reasons for nosocomial

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and community-obtained infections.<sup>2–5</sup> Enterobacteriaceae are typically associated with a range of infections,<sup>6</sup> among which urinary tract infections, bloodstream infections, health facility associated pneumonia, and some intra-abdominal infections are the most crucial.<sup>7,8</sup> Studies conducted in different underdeveloped countries indicate a high case-fatality rate associated with bloodstream infection due to Enterobacteriaceae.<sup>9,10</sup>

Antibiotics play a vital role in decreasing the load of communicable diseases worldwide.<sup>11</sup> Microbial resistance to antimicrobial agents is remarkably rising globally.<sup>12–14</sup> This rapid spread of resistance among pathogenic microorganisms is a serious problem globally,<sup>2,4</sup> because it limits the drug treatment alternative against infections.<sup>15</sup> Antimicrobial resistance has presently been recognized as one of the most important problems facing human health by the World Health Organization (WHO).<sup>15,16</sup> Frequent isolation of multidrug resistant (MDR) pathogens in both hospital and community acquired infections further intensified the problem of antimicrobial resistance.<sup>5</sup>

Nowadays, extended-spectrum  $\beta$ -lactamases (ESBLs) producing *Klebsiella pneumoniae* and *Escherichia coli* are a serious public health issue globally.<sup>1</sup> They become resistant to beta-lactam antibiotics via the production of beta-lactamase enzymes that inactivate beta-lactam antibiotics and this continues to be the prominent cause of  $\beta$ -lactam antibiotic resistance among Enterobacteriaceae.<sup>17,18</sup> It can rapidly develop resistance against a range of important broad-spectrum antimicrobials.<sup>19,20</sup> Inappropriate and irrational use of antimicrobial drugs, poor sanitation, and infection control practices in developing countries play a critical role in an increased prevalence of resistant bacteria in a community providing favorable conditions for resistant microorganisms to emerge and spread.<sup>21,22</sup> This can lead to a proliferation of organisms with broad-spectrum  $\beta$ -lactamase activity that threatens the future of the  $\beta$ -lactam class in clinical care.<sup>21</sup>

The increasing rate of human infections caused by antimicrobial resistance strains of Enterobacteriaceae makes clinical management more difficult by prolonging the illness and compromising the treatment.<sup>5</sup> This can have a potentially serious impact on human health. The situation is more common in developing countries where there is widespread and uncontrolled use of antibiotics.<sup>16</sup> Data on extended-spectrum  $\beta$ -lactamases producing Enterobacteriaceae in Ethiopia are limited and there is no data associated with this. Therefore, this study aimed to assess the magnitude of ESBL producing *Klebsiella pneumoniae* and *Escherichia coli* isolated from

the stool of apparently healthy food handlers in the Dilla University Student Cafeteria.

## Methods

### Study Design and Period

An institutional-based cross-sectional study was carried out among food handlers to evaluate the magnitude and antimicrobial resistance pattern of ESBL-producing *E. coli* and *K. pneumoniae* from stools of apparently healthy food handlers in Dilla University Student Cafeteria, Southern Ethiopia from November 2018 to September 2019. Three sample selection sites were identified prior to data collection. To select representative participants, the final sample size was proportionally allocated to each stratum, and food handlers were selected using a systematic random sampling technique. Finally, a total of 220 were included in the study based on the single population proportion formula.

### Data Collection and Analysis

Data related to sociodemographic characteristics and personal hygiene practices were collected via face-to-face interviewing of the patient or guardian of the patient using a well-structured questionnaire before laboratory sample collection. One environmental health and four medical laboratory professionals were recruited for data collection, supervision, and microbiological analysis. The data collectors were trained for 2 days by the principal investigator on observational data collection and specimen collection procedures.

### Laboratory Data Collection and Analysis

After interviewing, all respondents were asked to give a fresh stool specimen in a sterile, clean, wide-mouthed plastic and leak proof container by a clean wooden applicator stick for microbiological analysis. The isolation and characterization of *E. coli* and *K. pneumoniae* were performed based on the standard procedure. Briefly, a mixture of a stool sample (1 mL) was transferred to the selective media, Hicrome ESBL agar base, to assess ESBL production in 2 hours (Oxoid, Ltd, UK). An inoculum from Hicrome ESBL agar base was cultured onto MacConkey agar (Oxoid, Ltd, UK). After overnight incubation at 37°C the growth of *E. coli* and *K. pneumoniae* were differentiated by their colony characteristics, pigment production (pink to colorless flat or mucoid colonies), motility, and Gram-staining techniques (Gram-negative rods, non-sporing, and non-capsulated).

A list of biochemical tests conducted for further identification of isolates were made by conformation of motility and other relevant biochemical tests. An isolate was considered as *E. coli* when it was indole positive (dark pink ring) and methyl-red positive, citrate negative (no change or remained green) and urea negative, gas and acid producer, and motile and was considered as *K. pneumoniae* when it is indole and methyl-red negative, citrate positive, urea slow producing, and non-motile. In case of delay, the isolated bacteria were kept at 2–8°C in the nutrient broth for no more than 24 hours until the antimicrobial sensitivity test was done.

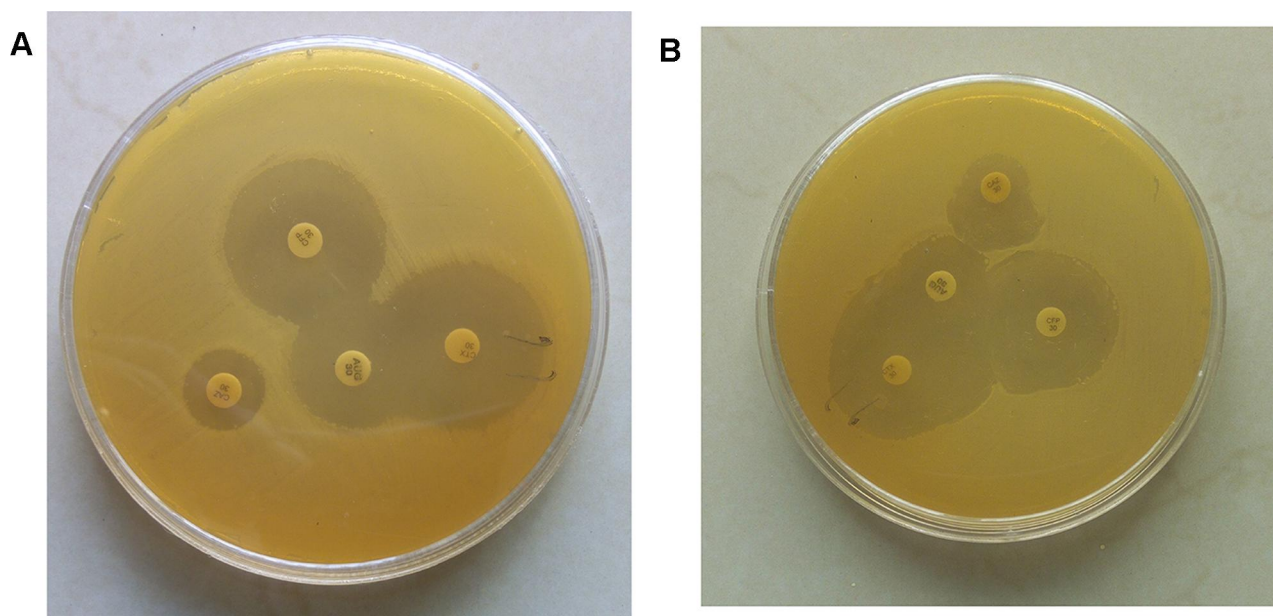
#### Detection Method of ESBL-Producing *E. coli* and *K. pneumoniae*

The disk-diffusion method was used to screen ESBL producers using ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg) (Oxoid, UK) as per CLSI criteria. A suspension of pure colony was wrapped on Mueller–Hinton agar (MHA) (Oxoid, UK) with a sterile cotton swab, and antibiotic discs were placed at a distance of 20 mm from each other and incubated at 35±2°C for 16–18 hours. The isolates with diminished susceptibility with breakpoints of ≤22 mm for ceftazidime, ≤25 mm for ceftriaxone, and ≤27 mm for cefotaxime were suspected for ESBL production.<sup>15</sup> Phenotypic confirmation of ESBL producers was performed using double disc synergy between the indicator cephalosporin and clavulanic acid, according to EUCAST guidelines.<sup>23</sup> A disc of amoxicillin/

clavulanic acid (20/10 µg) was placed in the center of the MHA plate, and then cefotaxime (30 µg), ceftazidime (30 µg), and aztreonam (30 µg) were placed at a distance of 20 mm edge to edge from the amoxicillin/clavulanic acid disk. After 24 hours incubation, the elevation of ≥5 mm zone inhibition between either of the cephalosporin disks and their respective amoxicillin/clavulanic acid disks was interpreted as an ESBL producer<sup>24</sup> (Figure 1A and B).

#### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed for both Enterobacteriaceae by using the disc diffusion technique on Mueller–Hinton agar based on EUCAST guidelines<sup>23</sup> for the listed antibiotics: Ampicillin (10 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg) and imipenem (30 µg) (all from Oxoid, UK). The resistance and sensitivity results were interpreted according to the EUCAST guidelines.<sup>23</sup> MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. The quality and performance of culture media, biochemical tests, and antimicrobial susceptibility discs were checked using a control strain (*E. coli* ATCC 25,922 and *K. pneumoniae* ATCC 700,603) obtained from the Ethiopian Public Health Institute, Addis Ababa.



**Figure 1 (A and B)** The phenotypic confirmation test of ESBL producers performed using double disc synergy between amoxicillin-clavulanic acid and cefotaxime, ceftazidime, and aztreonam on Mueller-Hinton agar plate.

## Data Analysis

Descriptive statistical analysis was performed using SPSS version 24 software. The prevalence of ESBL producers and non-ESBL-producing isolates were categorized by personal hygiene related factors of food handlers and presented by calculating frequencies and percentages. The Chi-square test was used with appropriate correction for the observation. Multivariate logistic regression analysis was used to identify risk factors for colonization by ESBL.  $P$ -values  $< 0.05$  were considered statistically significant.

## Ethical Consideration

Ethical clearance was obtained from the Ethical Review Committee of Dilla University Medical and Health Science College. Written informed consent was obtained from each study participant. Strict confidentiality was maintained during the interview process as well as anonymity was maintained during data processing and report writing. Food handlers who were found to be positive for enteric pathogens (parasite and bacterial) were referred to their respective staff medical center for appropriate antiparasitic and antimicrobial treatments.

## Results

### Socio Demographic Characteristics of Study Participant

A total of 220 food handlers were included in this study. Most participants ( $>90\%$ ) were females which explains high prevalence of ESBLs in females. The majority of the study participants were between the age group of 20 and 40 years (84.5%) with a mean age of 31.2 (standard deviation =  $\pm 8.8$  years). Of the total respondents, 51.4% reported that they always perform periodic medical checkups, 81.8% always trimmed their finger weekly, and 63.2% always wear a clean apron. Similarly, 69.1% wear a hair garment regularly in their working area, and 79.5% reported that they always use soap and water for hand washing after visiting the toilet. The finding from observation also supported that personal hygiene was practiced by the food handlers. From the total, 97.3% use pipe water for drinking. About 86.8% of the study participants drunk unpasteurized milk, while 42.3% of the study participants ate raw meat (Table 1).

### ESBL Production Fecal Carriage Rate of *E. coli* and *K. pneumoniae* from Stool Culture

In the present study, The rate of fecal carriage of *E. coli* and *K. pneumoniae* among the food handlers of the current

study was found to be 146 (66.4%), of which 119 (81.5%) were *E. coli*, while 27 (18.5%) were *K. pneumoniae*. From the total bacterial isolates, 37 (25.3%) were confirmed to be positive for ESBL production. 29 (19.9%) of ESBL producing isolates were *E. coli*, while eight (5.5%) were *K. pneumoniae* (Figure 2).

In this study, ESBL-producers and non ESBL producers isolates were predominantly reported in females with a prevalence rates of 35 (24.0%) and 97 (66.4%). *E. coli* and *K. pneumoniae* producing ESBL were predominantly reported among the age group of 20–40 years with a prevalence of 31 (21.2%). Most ESBL producers were reported in food handlers who did not attend a regular medical checkup with a prevalence of 25 (17.1%). The majority of *E. coli* and *K. pneumoniae* producing ESBL were reported among food handlers who did not wash their hands with soap, with the prevalence of 24 (16.4%). In the present study, the majority of the demographic characteristics study participants have no association with *E. coli* and *K. pneumoniae* producing ESBL. Only food handlers who used antibiotics in the last 3 months had a strong association with the ESBL carriage with  $P$ -values of 0.001 (Table 1).

### Antimicrobial Resistance Pattern of ESBL-Producing and Non-ESBL-Producing *E. coli* and *K. pneumoniae*

In the current study, ESBL-producing isolates showed significantly higher resistance not only towards the third generation cephalosporins but also towards other antimicrobial agents tested ( $P > 0.001$ ). Both *E. coli* and *K. pneumoniae* ESBL producers were highly resistant to Aztreonam, cefotaxime, ceftriaxone, cefuroxime, and ceftazidime, with resistance rates ranging from 87–100%. Almost all ESBL-producing and non-ESBL-producing isolates were highly resistant to ampicillin and tetracycline, with a resistance rate of 89.5%. Higher resistance rates of ESBL producing isolate were also reported among ciprofloxacin, trimethoprim-sulfamethoxazole, and nitrofurantoin with resistance rates ranged from 67–75%. In the current study, ESBL producing isolates were the least resistant to imipenem with a resistance rate of 13.8% (Table 2).

### Antibiogram Pattern of Multidrug-Resistant *E. coli* and *K. pneumoniae*

In this study, around 34 (91.9%) ESBL-producing isolates were resistant to both  $\beta$ -lactam groups and tetracycline,

**Table 1** Characteristics of Food Handlers and Fecal Carriage Rate of ESBLs-Producing and Non-ESBLs-Producing *E. coli* and *K. pneumoniae* in Dilla University Students' Cafeteria, Southern Ethiopia (n=220)

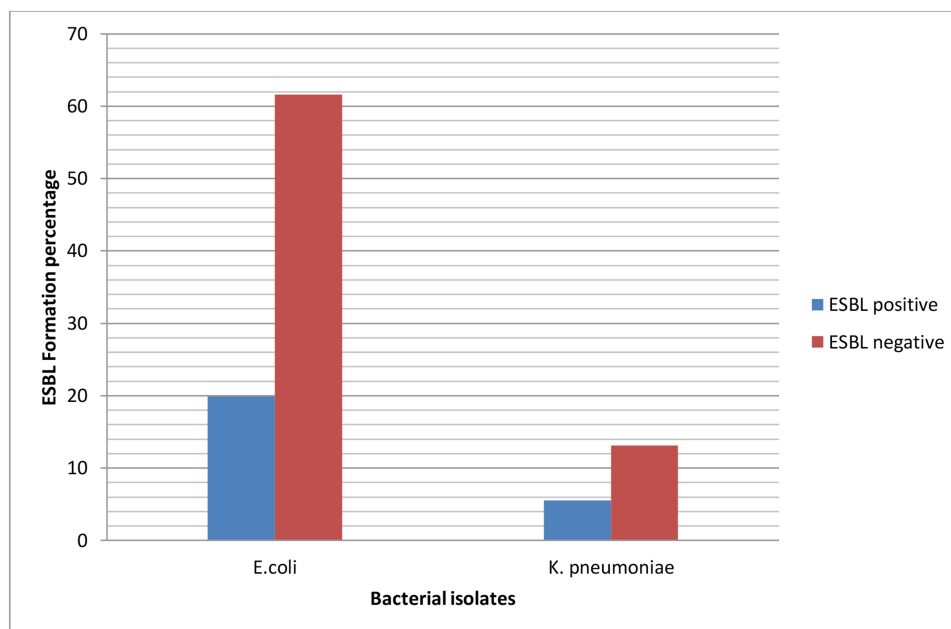
Variables	Category	Number Tested	Proportion of <i>E. coli</i> (n=119)		Proportion of <i>K. pneumoniae</i> (n=27)		P-value
			ESBLs-Positive (n=29)	ESBLs-Negative (n=90)	ESBLs-Positive (n=8)	ESBLs-Negative (n=19)	
Sex	Male	20	1 (0.84%)	5 (4.2%)	1 (3.7%)	7 (25.9%)	1 0.213
	Female	200	28 (23.5%)	85 (71.4%)	7 (25.9%)	12 (44.4%)	
Age in years	<20	10	1 (0.84%)	28 (23.5%)	1 (3.7%)	4 (14.8%)	1 0.121 0.301
	20–40	186	26 (21.8%)	61 (51.3%)	5 (18.5%)	9 (33.3%)	
	>40	24	2 (1.7%)	18 (15.1%)	2 (7.4%)	6 (22.2%)	
Service years	1–5 year	38	2 (1.7%)	28 (23.5%)	3 (11.1%)	6 (22.2%)	1 0.513
	>5 year	170	27 (22.7%)	62 (52.1%)	5 (18.5%)	13 (48.1%)	
Medical checkups	Yes	113	9 (7.6%)	31 (26.1%)	3 (11.1%)	8 (29.6%)	1 0.08
	No	107	20 (16.8%)	59 (49.6%)	5 (18.5%)	11 (40.7%)	
Hands washing habit	Yes	216	28 (23.5%)	83 (69.7%)	6 (22.2%)	15 (55.6%)	1 0.612
	No	4	1 (0.84%)	7 (5.9%)	2 (7.4%)	4 (14.8%)	
Finger nail status	Trimmed	180	24 (20.2%)	60 (50.4%)	5 (18.5%)	13 (48.1%)	1 0.341
	Not trimmed	40	5 (4.2%)	30 (25.2%)	3 (11.1%)	6 (22.2%)	
Antibiotics used in the last 3 months	Yes	139	27 (22.7%)	58 (48.7%)	7 (25.9%)	12 (44.4%)	0.001* 1
	No	81	2 (1.7%)	32 (26.9%)	1 (3.7%)	7 (25.9%)	
Wear hair garment	Yes	152	22 (18.5%)	48 (40.3%)	6 (22.2%)	14 (51.9%)	0.53 1
	No	68	7 (5.9%)	42 (35.3%)	2 (7.4%)	5 (18.5%)	
Hands wash with soap	Only with water	45	18 (15.1%)	15 (12.6%)	6 (22.2%)	3 (11.1%)	0.154 1
	With soap	175	11 (9.2%)	75 (63.1%)	2 (7.4%)	16 (59.3%)	
Source of water for drinking	Pipe	214	28 (23.5%)	78 (65.5%)	5 (18.5%)	12 (44.4%)	1 0.301 0.462
	Hand dug well	25	1 (0.84%)	9 (7.5%)	2 (7.4%)	5 (18.5%)	
	Other	195	28 (23.5%)	3 (2.5%)	1 (3.7%)	2 (7.4%)	
Unpasteurized milk	Yes	29	1 (0.84%)	9 (7.6%)	2 (7.4%)	5 (18.5%)	1 0.211
	No	191	28 (23.5%)	81 (68.1%)	6 (22.2%)	14 (51.9%)	
Eating raw meat	Yes	93	11 (9.2%)	40 (33.6%)	3 (11.1%)	6 (22.2%)	1 0.412
	No	127	18 (15.1%)	50 (42.1%)	5 (18.5%)	13 (48.1%)	

**Note:** \*Indicates ESBL producers isolates were significantly associated with food handlers who used antibiotics repeatedly in the last 3 months.

while 26 (70.3%) ESBL-producing isolates were Co-resistant to  $\beta$ -lactams groups and ciprofloxacin and 20 (54.1%) ESBL producers were resistant to both  $\beta$ -lactams groups and trimethoprim sulfamethoxazole. Other ESBL-producing isolates were co-resistant to  $\beta$ -lactams groups, and three, four, and five types of non- $\beta$  lactam antibiotics with resistance rates of 17 (45.9%), 12 (32.4%), and 8 (21.6%), respectively (Table 3).

## Discussion

The emergence and rapid spread of multidrug resistance strains of ESBL producing Enterobacteriaceae is a serious public health issue worldwide. Rapid expansion of ESBLs highly affects the activity of broad-spectrum antibiotics, creating major therapeutic difficulties with a significant impact on the outcomes for patients.<sup>25</sup> Few data are available on the prevalence of fecal carriage of ESBL



**Figure 2** The distribution of ESBL-producing *E. coli* and *K. pneumoniae* isolated from the stool of apparently healthy food handlers.

producing Enterobacteriaceae, and little attention was given to it in Africa. In this study, we examined the fecal carriage rate of ESBL producers *E. coli* or *K. pneumoniae* in food handlers working at the Dilla University student cafeteria.

The phenotypic information obtained in the current study indicates a significant prevalence of ESBL producers. The overall magnitude of fecal carriage of ESBL producing *E. coli* or *K. pneumoniae* among apparently health food handlers was 25.3%. This indicates a remarkably high number of ESBL productions among food handlers working in food preparation. Our finding is consistent with studies conducted in India,<sup>26</sup> Italy,<sup>27</sup> Egypt,<sup>28</sup> and in Turkey,<sup>29</sup> with prevalence ranging from 15–33%. However, it is lower than the study conducted in Central Africa,<sup>30</sup> Tanzania,<sup>31</sup> Nigeria,<sup>32</sup> Burkina Faso,<sup>33</sup> and Ghana,<sup>34</sup> with prevalence ranging from 40–59%. The variation might be explained by methodological differences, differences in study area, quality of media used, or type of specimen collected. The use of low quality, inappropriate use of antibiotics, and weak infection prevention measures may additionally make a contribution to the high magnitude of ESBL production.

In the present study, *E. coli* was the leading fecal carriage of ESBL producing Enterobacteriaceae with a prevalence of 19.9%, followed by *K. pneumoniae* (5.5%). Our finding agrees with studies conducted in India,<sup>26,27</sup> Central Africa,<sup>30</sup> Central India,<sup>35</sup> and Burkina Faso,<sup>33</sup> where *E. coli* was the predominant ESBL producer than *K. pneumoniae*.

However, studies conducted in Uganda,<sup>36</sup> Gambia,<sup>37</sup> and elsewhere<sup>38</sup> reported a higher prevalence of *K. pneumoniae* than *E. coli*. Those bacteria can result in the transfer of the resistance genes to other strains of *E. coli* and *K. pneumoniae* within the gastrointestinal tract that can result in fatal consequences. In addition, when those carriers are admitted to hospitals they can easily transmit the infection to other hospitalized patients. Quick adaptation of those strains to the harsh environment, upregulation of intrinsic resistance mechanisms, and rapid acquisition and transferring of drug resistance genes through mobile genetic elements could be a possible explanation for an elevated overall drug resistance prevalence rate against different categories of drugs.

In the current study, the fecal carriage of ESBLs among food-handlers working in the cafeteria was significantly associated with food handlers who used antibiotics repeatedly in the last 3 months and those food handlers who did not attend regular medical check studies.<sup>39,40</sup> In most parts of developing countries including Ethiopia, antibiotics were used without prescription, which can lead to overuse or misuse of antibiotics. This can contribute to the emergence and spread of antimicrobial-resistant strains. Moreover, in developing countries, poor personal hygiene practices may increase the prevalence of bacterial gastrointestinal infections which may play a role in rising antimicrobial resistance. This increases the risk of treatment failure with potentially serious consequences. This can be overcome by performing bacterial culture and performing an antimicrobial susceptibility test

**Table 2** Antimicrobial Resistance Patterns of ESBLs-Positive and ESBLs-Negative *E. coli* and *K. pneumoniae* Isolated from Food Handlers in Dilla University Student Cafeteria, Southern Ethiopia (n=220)

Antibiotics	Pattern	Antimicrobial Resistance Pattern of <i>E. coli</i> (n=119)		Antimicrobial Resistance Pattern of <i>K. pneumoniae</i> (n=27)	
		ESBLs-Positive (n=29)	ESBLs-Negative (n=90)	ESBLs-Positive (n=8)	ESBLs-Negative (n=19)
Ampicillin	S	0 (0.0%)	20 (22.2%)	0 (0.0%)	2 (10.5%)
	R	29 (100%)	70 (77.8%)	8 (100%)	17 (89.5%)
Aztreonam	S	1 (3.4%)	6 (6.7%)	1 (12.5%)	2 (10.5%)
	R	28 (96.6%)	84 (93.7%)	7 (87.5%)	17 (89.5%)
Cefotaxime	S	1 (3.4%)	7 (7.8%)	0 (0.0%)	2 (10.5%)
	R	28 (96.6%)	83 (92.2%)	8 (100%)	17 (89.5%)
Cefuroxime	S	2 (6.9%)	6 (6.7%)	1 (12.5%)	2 (10.5%)
	R	27 (93.1%)	84 (93.7%)	7 (87.5%)	17 (89.5%)
Ceftazidime	S	0 (0.0%)	6 (6.7%)	0 (0.0%)	2 (10.5%)
	R	29 (100%)	84 (93.3%)	8 (100%)	17 (89.5%)
Ceftriaxone	S	1 (3.4%)	13 (14.4%)	1 (12.5%)	6 (31.6%)
	R	28 (96.6%)	77 (85.6%)	7 (87.5%)	13 (68.4%)
Ciprofloxacin	S	9 (31.0%)	29 (32.2%)	2 (25.0%)	5 (26.3%)
	R	20 (69.0%)	61 (67.8%)	6 (75.0%)	14 (73.7%)
Imipenem	S	25 (86.2%)	68 (75.6%)	7 (87.5%)	14 (73.7%)
	R	4 (13.8%)	22 (24.4%)	1 (12.5%)	5 (26.3%)
Nitrofurantoin	S	9 (31.0%)	29 (32.2%)	2 (25.0%)	5 (26.3%)
	R	20 (69.0%)	61 (67.8%)	6 (75.0%)	14 (73.7%)
Tetracycline	S	1 (3.4%)	5 (5.6%)	0 (0.0%)	2 (10.5%)
	R	28 (96.6%)	85 (94.4%)	8 (100%)	17 (89.5%)
Trimethoprim sulfamethoxazole	S	9 (31.0%)	29 (32.2%)	2 (25.0%)	5 (26.3%)
	R	20 (69.0%)	61 (67.8%)	6 (75.0%)	14 (73.7%)

to select the most effective antibiotics for suspected cases of gastrointestinal infected patients.

In the present study, ESBL-producing *E. coli* and *K. pneumoniae* were highly resistant to ampicillin, cefotaxime, aztreonam, ceftazidime, and ceftriaxone, with resistance rates ranging from 88–100%, while the isolates were highly susceptible to imipenem rating 86.2%. Our finding agrees with studies conducted in Gambia,<sup>41</sup> Burkina Faso,<sup>33</sup> Ghana,<sup>34</sup> Saudi Arabia,<sup>42</sup> Israel,<sup>43</sup> Poland,<sup>44</sup> Sierra Leone,<sup>45</sup> and Lebanon.<sup>46</sup> This indicates ESBL-producing Enterobacteriaceae were rapidly emerging in developing and developed countries. In this study, ESBL-producing *E. coli* and *K. pneumoniae* were not only resistant to third-generation cephalosporins but also to other non- $\beta$  lactams groups of antibiotics. The finding of this study showed that imipenem had a better performance against ESBL-producing

Enterobacteriaceae than other antibiotics including the cephalosporin group. This is inconsistent with a study conducted by Moges et al,<sup>47,48</sup> who reported that imipenem had higher performance for the treatment of ESBL-producing Enterobacteriaceae.

Our findings showed that greater than 90% of the ESBL producing isolates were MDR. The MDR nature of ESBL-producing *E. coli* and *K. pneumoniae* may be explained by the fact that they are plasmid-mediated enzymes which are carrying multi-resistant genes by plasmid, transposon, and integron and they are readily transferred to other bacteria through conjugation, transduction, or transformation. The carrier of those bacteria can easily disseminate to the community and hospitalized patients, which can result in remarkable consequences. As a result, nowadays bacteria with multiple resistance to antibiotics

**Table 3** Multidrug Resistance Pattern of *E. coli* and *K. pneumoniae* Fecal Carriage Isolated from Food Handlers in Dilla University Student Cafeteria, Southern Ethiopia (n=220)

Antibiotics Categories	<i>E. coli</i> (n=29)	<i>K. pneumoniae</i> (n=8)	Total MDR Rate (n=37)
β-lactams+TET	27 (93.1%)	7 (87.5%)	34 (91.9%)
β-lactams+TET, CIP	20 (69.0%)	6 (75.0%)	26 (70.3%)
β-lactams+TET, SXT	15 (51.7%)	5 (62.5%)	20 (54.1%)
β-lactams+TET, SXT, CIP	13 (44.8%)	4 (50.0%)	17 (45.9%)
β-lactams+TET, SXT, NIT, CIP	9 (31.0%)	3 (37.5%)	12 (32.4%)
β-lactams+CIP, IM, NIT, TET, SXT	6 (20.7%)	2 (25.0%)	8 (21.6%)

**Abbreviations:** Beta-lactams groups, ampicillin, cefotaxime, cefuroxime, ceftazidime, ceftriaxone; CIP, ciprofloxacin; IM, imipenem; NIT, nitrofurantoin; TET, tetracycline; SXT, trimethoprim sulfamethoxazole.

are widely distributed in hospitals and communities and became a serious problem throughout the world.<sup>49</sup> This is the most challenging and alarming condition in the management of infectious diseases associated with ESBL-producing *Enterobacteriaceae*.

## Limitations of the Study

The limitations of this study were the lack of a molecular characterization using PCR. Most of the participants were unable to provide the previous prescribed antibiotics to show association with the fecal carriage of ESBLs (recall bias). Most of the study participants were female, which tends to show the gender bias.

## Conclusion

In this study, the magnitude of fecal carriage of ESBL-producing *E. coli* and *K. pneumoniae* is alarmingly high and a threat to human health. ESBL-producing fecal carriage showed high resistance to aztreonam, cefotaxime, ceftriaxone, cefuroxime, and ceftazidime. The better option for the treatment of ESBL-producing *E. coli* and *K. pneumoniae* in this study is imipenem. The occurrence of multidrug resistance to third-generation cephalosporins and non-β lactam antibiotics is more common among ESBL producers. In the present study, ESBL-producing isolates were co-resistant to β-lactams antibiotics plus at least one of the non β-lactam antibiotics. Only food handlers who used antibiotics repeatedly in the last 3 months were significantly associated with the ESBL fecal carriage. The rise of ESBL-producing *Enterobacteriaceae* requires strict infection prevention and control strategies and strengthening of the diagnostic capacity of laboratory professionals for the detection and surveillance of antibiotic resistance that have a significant role in clinical decision-making. Hence a coordinated effort should be implemented. Molecular

confirmation and characterization using PCR of the ESBL producers to reveal the mobile genetic elements, resistance, and virulence of the detected isolates is also recommended as a future direction in the study area.

## Data Sharing Statement

The data set used for analysis during the current study are available from the corresponding author on reasonable request.

## Ethics Statement

A protocol for patient recruitment and participation in the study followed the principles of the Declaration of Helsinki and was approved by the Dilla University Health Research Ethics Review Committee under protocol unique number 005/2019-01, date 10-01-2019. During data collection, each child's parent/guardian was informed about the aim of the study. Written consent was obtained from the parent/legal guardian for each patient before the start of data collection. The clinical samples were specifically collected for this research. Samples with positive culture results were communicated to physicians in order for patients to get treatment according to the drug susceptibility results of the isolates.

## Additional Information

All data relevant to the study are included in the article.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no conflicts of interest.

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