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ANTIBIOTIC RESISTANCE OF ESBL-PRODUCING *E. COLI* AND OTHER GRAM-NEGATIVE BACTERIA ISOLATED FROM STREET-VENDED FOODS IN BANGLADESH

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ABSTRACT

The prevalence and impact of antibiotic-resistant pathogens transmitted through food, particularly street-vended foods, is becoming a major public health concern. Although a significant proportion of the urban population in developing countries consumes street-vended foods, the role of these foods in spreading antibiotic resistance has been rarely investigated. In this study, 50 bacterial isolates were obtained from 25 samples representing five categories of street-vended foods: Phuchka, Chatpati, Sausage, Bun, and Salad. The IMVIC test revealed a notably high occurrence of *Escherichia coli* (n=32) within the collected samples. Three representative isolates were selected for molecular identification using DNA sequencing of 16S rDNA. They were identified as *Klebsiella oxytoca*, *Burkholderia fungorum*, and *Serratia nematodiphila*. The antibiotic susceptibility of the identified isolates (n=35) was investigated using twelve antibiotics following the Kirby-Bauer disk diffusion method. Around 65.63% of the *E. coli* isolates (n=21) exhibited multidrug resistance. Double Disc Synergy Test (DDST) and Phenotypic Confirmatory Disk Diffusion Test (PCDDT) confirmed ESBL production of Eight multidrug-resistant *E. coli* isolates (38.09%). The multiple antibiotic resistance (MAR) index showed that 22 *E. coli* isolates had MAR above 0.2, with resistance mostly against oxacillin, ampicillin, and cefuroxime. The *Klebsiella oxytoca* isolate showed multidrug resistance viz., ampicillin, oxacillin, cefuroxime, and kanamycin. The *Burkholderia fungorum* isolate showed no distinct inhibition zone against ampicillin and chloramphenicol. Additionally, the *Serratia nematodiphila* isolate showed no distinct inhibition zone against three antibiotics, including ampicillin, oxacillin, and cefuroxime. These findings might contribute to the knowledge of emerging antibiotic-resistant foodborne pathogens and raise concerns about the safety of street-vended foods in Bangladesh.

Keywords: Antibiotic-resistant pathogens, public health, street-vended foods, *E. coli*, *Klebsiella oxytoca*, *Burkholderia fungorum*, *Serratia nematodiphila*

INTRODUCTION

Nowadays, the dissemination of antibiotic resistance has become an undeniable concern worldwide and the resistant strains of foodborne pathogens may lead to dire consequences. Antibiotic-resistant (AR) bacterial infections might become the leading cause of mortality worldwide by 2050 **(Cerqueira et al., 2019)**. A rising threat to food safety is connected to how food might expose people to AR bacteria that are either commensal, zoonotic, or originate from environmental origins **(Aerts et al., 2019; Koutsoumanis et al., 2021)**. The AR bacteria are transmitted to humans through the food chain due to the uncontrolled use of antibiotics to promote growth in livestock, aquaculture, and apiculture **(Caniça, Manageiro, Abriouel, Moran-Gilad, & Franz, 2019; Thapa, Shrestha, & Anal, 2020)**. Antibiotic resistance is spreading at an alarming rate throughout the world, and the pattern of resistance varies based on geographical region **(O'Neill, 2016)**. Moreover, climate change and global warming are also related to antibiotic resistance **(Kaba, Kuhlmann, & Scheithauer, 2020; Reverter et al., 2020)**. Among all the World Health Organization (WHO) regions, Southeast Asian countries, including Bangladesh, have the highest risk of antibiotic resistance **(Hoque et al., 2020)**.

According to recent research, bacteria resistant to antibiotics may be more virulent than their counterparts vulnerable to antimicrobials **(Guillard, Pons, Roux, Pier, & Skurnik, 2016)**. Gram-negative bacteria have developed resistance to β-lactamase stable cephalosporins due to the extensive use of second and third generation cephalosporins. Extended-spectrum β-lactamases (ESBL) are considered to be responsible for this resistance. These plasmid-mediated enzymes provide resistance to oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime, etc.) and monobactams (e.g., aztreonam), but they are inactive against cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., meropenem or carbapenem) **(Bush & Bradford, 2020; Eiamphungporn, Schaduangrat, Malik, & Nantasenamat, 2018)**. The majority of ESBL-producing organisms are *Escherichia coli* and *Klebsiella* spp. (Barrios et al., 2017; Padmini, Ajilda, Sivakumar, & Selvakumar, 2017). *Escherichia coli* has evolved into a threatening foodborne pathogen that is responsible for outbreaks of gastroenteritis in Europe, North America, Africa, and Asia **(Ekici & Dümen, 2019)**. Many strains of *E. coli* have been repeatedly linked to undercooked foods, tainted ground beef, raw milk, unpasteurized apple and cider juice, bean sprouts, and fresh leafy vegetables like lettuce and spinach **(Beauchamp & Sofos, 2009)**. The risk of causing the emergence of antibiotic resistance is high as well. Furthermore, antibiotic resistance of *Klebsiella pneumoniae* has been reported over time in several community sources, such as ready-to-eat (RTE) foods and raw vegetables, and *K. pneumoniae* has acquired resistance to carbapenems, the last-line antibiotic as well **(Grundmann et al., 2017; Indrajith et al., 2021; Nishida & Ono, 2020)**.

Street-vended food (SVF) is extremely popular among city dwellers as it is readily available, convenient, and inexpensive. SVF provides employment to a significant proportion of the population in many developing countries. However, street food vendors generally lack basic hygienic practices and infrastructure, as well as have limited factual knowledge and poor understanding of food safety procedures **(Jahan et al., 2018)**. RTE meats and salads have long been considered to be hidden carriers of pathogenic microorganisms that cause food poisoning, like *Enterobacteriaceae* **(Castellano, Pérez Ibarreche, Blanco Massani, Fontana, & Vignolo, 2017; Gourama, 2020)**. Foodborne bacterial pathogens in SVF cause outbreaks of foodborne diseases like cholera, diarrhea, food poisoning, and typhoid fever **(Jahan et al., 2018)**. In Bangladesh, foodborne diseases affect over 30 million individuals annually **(Hasan et al., 2021)**. The easy access to medicines in pharmacies, pill-by-pill sales, improper use and overuse of antibiotics, and reluctance to complete prescribed antibiotic courses once the patient feels fine are factors contributing to the rise in antibiotic resistance in Bangladesh **(Wellington et al., 2013)**. Furthermore, AR bacteria present in various foods are a significant factor in foodborne disease transmission, making the prevention of foodborne diseases quite challenging. Although some studies in Bangladesh determined the antibiotic resistance levels in SVF, the information gathered in those studies was insufficient to understand the seriousness of the situation in Bangladesh **(Sunzid Ahmed, Tasnim, Pervin, & Islam, 2014; Ali, Khan, & Saha, 2011; Mrityunjoy et al., 2013; Sarker et al., 2013; Tabashsum et al., 2013)**. More information is required to understand better the risk of exposure to AR bacteria through food items, particularly SVFs. Thus, in this study, we assessed the antibiotic

susceptibility of Gram-negative bacteria isolated from five different SVFs in Bangladesh.

MATERIAL AND METHODS

Sample collection and isolation of bacteria

In this study, 25 samples of five categories of SVFs (phuchka, chatpati, sausage, bun, and salad) were collected from street vendors. Approximately 200–250 g of food sample was collected in sterile bags, transported immediately to the laboratory, and stored at 4 °C until they were analyzed; samples were not stored for more than 12 h. Approximately 10 g of the samples were weighed in a sterile beaker. Then, 90 ml of sterile saline solution was used to blend the samples in a sterile condition and prepare a homogenized dilution of 10^{-1}. Later, a 10-fold dilution was obtained. All samples were then inoculated on MacConkey agar plate and incubated at 37 °C for 24h. Two suspected colonies of gram-negative bacteria with typical morphological characteristics and size were selected from each sample and isolated by the streak plate method. Furthermore, the Eosin Methylene Blue (EMB) agar medium was used to study the morphological characteristics of the suspected coliform bacteria, and the IMViC test was performed for biochemical identification. The experimental design of this study is shown in Figure 1.

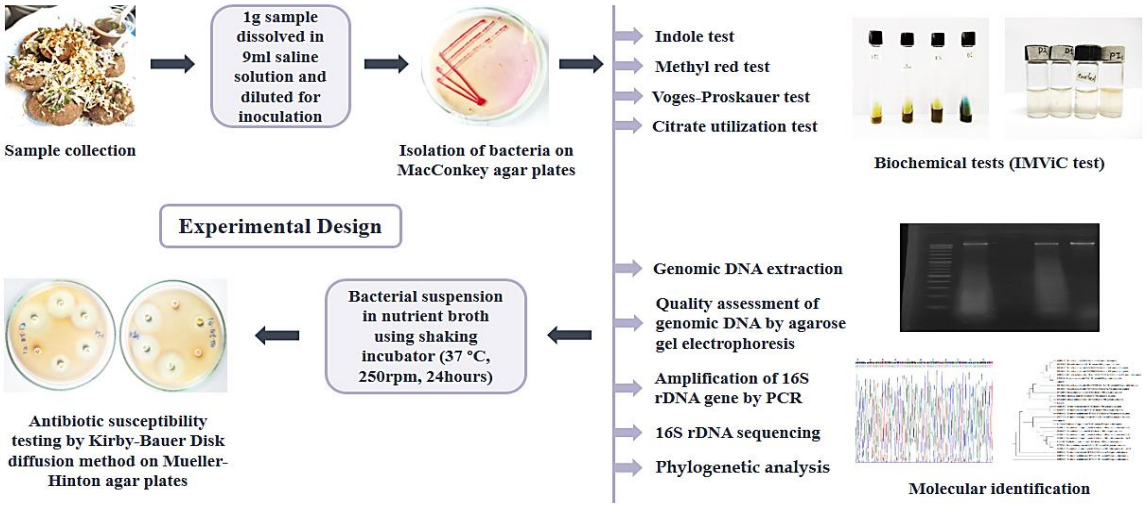

Figure 1 Experimental design

Molecular identification

Three distinct and representative isolates, F38 (*Klebsiella oxytoca*), F42 (*Serratia nematodiphila*), and F45 (*Burkholderia fungorum*) from the remaining isolates with two or more 0 mm inhibition zones in the disk diffusion test, were selected for molecular identification by DNA sequencing of the 16S rDNA. The genomic DNA of the gram-negative bacteria was extracted using the Monarch DNA Gel Extraction Kit (New England Biolabs). After isolation of the genomic DNA, electrophoresis was performed in a horizontal gel apparatus (MyRun Cosmo Bio Co. Ltd., IMR-201). Electrophoresis was conducted in horizontal agarose gel (0.7%) to visually confirm the integrity of the genomic DNA (Figure 2) **(Forcic et al., 2005)**. PCR amplification of the bacterial 16S rDNA gene was performed using the forward primer 16S 8F and the reverse primer 16S 1492R (Table 1) **(Iqbal et al., 2018)**. In a thermal cycler (SimpliAmp™ Thermal Cycler, Applied Biosystem®, USA), PCR was performed using a 25 µL reaction mixture (Table 2). The cycling parameters were 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 3 min, with a final extension at 72 °C for 10 min for each primer pair. PCR products were visualized through electrophoresis onto 1.2% (w/v) agarose gels, stained with ethidium bromide and visualized under UV light (Figure 2) **(Armstrong & Schulz, 2015)**. The molecular size of PCR products was determined by comparing them with a 1kb DNA ladder (GeneRuler 1kb DNA ladder, Thermo Fisher Scientific, USA). The concentration of the DNA was maintained at 10-50 ng/µL by measuring with Nanodrop. After confirmation of PCR, the PCR products were purified from agarose gel by an extraction kit (FavorPrep™ Gel/PCR Purification Mini Kit, Favorgen® Biotech Corp., Taiwan). The purified PCR products were sequenced with respective primers by the di-deoxynucleotide method, which involves base-specific termination of the enzymatic extension of DNA chains by dideoxy analogues **(Chen, 2014)**. 16S rDNA sequencing was performed using a DNA Sequencer (Model 3130, Applied Biosystem® Automated Genetic Analyzer), and the sequences were submitted to the National Centre for Biotechnology Information (NCBI), GenBank. The 16S rDNA sequences were aligned and paralleled with the sequences of the corresponding bacteria in NCBI, GenBank by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search. The sequences were aligned with similar sequences using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/), and a phylogenetic tree was constructed using the MEGA 6.1 software (Figure 3) **(X. Wang, Liu, & Sun, 2021)**.

Table 1 List of primers for 16S rDNA gene amplification

Primers name	Sequences	Amplicon size
Forward primer 16S 8F	5′- AGA GTT TGA TCC TGG CTC AG-3′	1500 bp
Reverse primer 16S 1492R	5′- CGG TTA CCT TGT TAC GAC TT- 3′	1500 bp

Table 2 Concentration of PCR reaction mixture

SL. No.	Components	Final concentration	Final volume (25 µL)
1	Forward primer	50 µM	1 µL
2	Reverse primer	50 µM	1 µL
3	DNA template	≤ 250 ng	2.5 µL
4	PCR Master Mix, 2X GoTaq® G2 Hot Start Colorless Master Mix (Promega)	1X	12.5 µL
	- Taq DNA polymerase	25 U/ml	
	- dNTPs	200 µM	
	- $MgCl_2$	1.5 mM	
5	Nuclease free water	N/A	8.5 µL

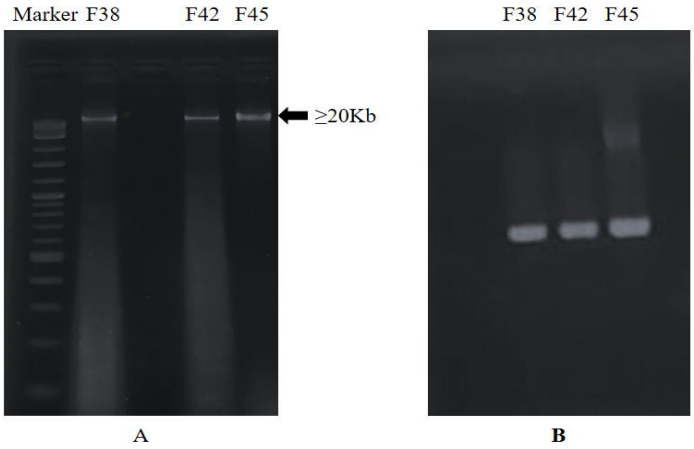

Figure 2 (A) Agarose gel electrophoresis of genomic DNA of Isolate F38 (*Klebsiella oxytoca*), F42 (*Serratia nematodiphila*) and F45 (*Burkholderia fungorum*) and **(B)** Agarose gel electrophoresis of PCR products obtained from the genomic DNA of the bacterial isolates F38 (*Klebsiella oxytoca*), F42 (*Serratia nematodiphila*) and F45 (*Burkholderia fungorum*) respectively

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was used to test antibiotic susceptibility after culturing the bacteria on Mueller Hinton agar (HiMedia; **(Mrityunjoy et al., 2013)**). The isolates were inoculated in nutrient broth and the suspension was vortexed at 37°C and 250 rpm to create a smooth suspension. The turbidity of this suspension was adjusted to a 0.5 McFarland standard **(Hombach, Maurer, Pfiffner, Böttger, & Furrer, 2015)**. We applied 12 antibiotic agents (HiMedia), including Ampicillin (25 µg), Aztreonam (30 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Chloramphenicol (30 µg), Cotrimoxazole or Trimethoprim/sulfamethoxazole (25 µg), Enrofloxacin (5 µg), Gentamicin (10 µg), Kanamycin (30 µg), Nalidixic Acid (30 µg), Oxacillin (1 µg), and Tetracycline (30 µg). Following the guidelines of the Clinical and Laboratory Standards Institute (CLSI), the inhibition zones were assessed and classified as sensitive or resistant **(CLSI, 2022)**. Multidrug-resistant (MDR) isolates were those resistant to at least one antimicrobial agent in three or more categories **(Magiorakos et al., 2012)**. The MAR (multiple antibiotic resistance) index was calculated as the ratio of total antibiotics used to the number of antibiotics to which the bacterial isolate was resistant **(Titilawo, Sibanda, Obi, & Okoh, 2015)**.

Screening for ESBL isolates by Double Disc Synergy Test (DDST)

The ESBL-producing MDR *E. coli* isolates were identified following the Double Disc Synergy Test (DDST) using commercially available antibiotic disks viz., Amoxicillin/clavulanic acid 20/10µg (Oxoid), Cefotaxime 30µg (Oxoid), Ceftazidime 30µg (Oxoid), and Aztreonam 30µg (Oxoid) **(CLSI, 2022)**. Mueller Hinton agar plates were prepared and inoculated with standardized inoculum (0.5 McFarland standard). Each antibiotic disk was positioned on the agar at a distance of 15 mm center to center from the amoxicillin/clavulanic acid 20/10µg disk. The negative control was *E. coli* ATCC 25922. When the inhibitory zone extended towards the amoxicillin/clavulanic acid (20/10µg) disk from the test antibiotic disk, it indicated that ESBL was developed.

Phenotypic Confirmatory Disk Diffusion Test (PCDDT)

The PCDDT was conducted using Cefotaxime 30µg and Ceftazidime 30µg disks alone as well as in combination with clavulanic acid 10µg **(CLSI, 2022)**. Disks of Cefotaxime and Ceftazidime with Clavulanic acid (30µg/10µg) were prepared using a stock solution of Clavulanic acid at 1000 µg/mL, which was stored at -70°C before use. Before applying to the plates, 10µL of the clavulanic acid solution was added to the disks. An increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid was observed compared to its zone when tested alone. If the zone diameter for ceftazidime and cefotaxime increased ≥ 5 mm, then the isolate was considered an ESBL producer.

RESULTS

Identification of isolates

Among 50 isolates, 32 isolates (64%) showed green metallic sheen on EMB agar plates, indicating *E. coli*, which was confirmed through biochemical tests. The IMViC test revealed the presence of *E. coli* through the following indicators: Indol-positive and motile by showing indole ring and inverted spruce formation on the Sulfide-Indole-Motility (SIM) test, MR-positive by showing red color change on the methyl red (MR) test, VP-negative by showing yellow color on the Voges–Proskauer (VP) test, and Citrate-negative by showing green color on the citrate test **(Farizqi et al., 2023)**. The phylogenetic tree constructed with similar sequences showed that the isolates F38, F42, and F45 were closely related to the *Klebsiella oxytoca* strain PF 42 (Accession number: KY614353.1), the *Serratia nematodiphila* strain XM7 (MT023384), and the *Burkholderia fungorum* strain TN (KJ933410), respectively (Figure 3).

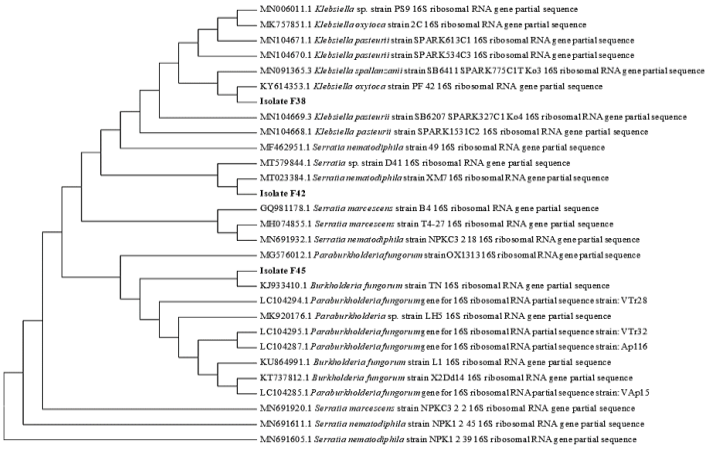

Figure 3 Phylogenetic tree for F38 (*Klebsiella oxytoca*), F42 (*Serratia nematodiphila*) and F45 (*Burkholderia fungorum*)

Antibiotic susceptibility pattern and MAR index of *E. coli* isolates

The antibiotic susceptibility pattern showed that most of the *E. coli* isolates (65.63%) were multidrug-resistant (MDR), with high sensitivity to aztreonam (93.75%), enrofloxacin (93.75%), gentamicin (84.38%), and kanamycin (84.38%), whereas high resistance was observed for the other antibiotics tested (Table 3). Two isolates (F12 and F19) showed resistance to all the antibiotics (MAR index 1.0). Furthermore, among the MDR isolates, 100% were resistant to oxacillin, 95.23% were resistant to ampicillin, and 80.95% were resistant to cefuroxime. Twenty-two isolates (68.75%) had a MAR index greater than 0.2, and 10 (31.25%) isolates had a MAR index less than 0.2 (Table 4). An MAR index greater than 0.2 denotes a high-risk source of contamination where the frequent use of antibiotics is observed **(Mir, Salari, Najimi, Rashki, & Science, 2022)**.

Table 3 Antibiotic susceptibility pattern of *E. coli* isolates

Antibiotic agents	Total number of isolates, n = 32		MDR isolates, n = 21 (65.63%)	
	Resistant	Sensitive	Resistant	Sensitive
Ampicillin (25 µg)	28(87.5%)	4(12.5%)	20(95.23%)	1(4.76%)
Aztreonam (30 µg)	2(7.14%)	30(93.75%)	1(4.76%)	20(95.23%)
Ceftriaxone (30 µg)	11(34.38%)	21(65.62%)	7(33.33%)	14(66.66%)
Cefuroxime (30 µg)	21(65.62%)	11(34.38%)	17(80.95%)	4(19.05%)
Chloramphenicol (30 µg)	9(28.13%)	23(71.86%)	5(23.81%)	16(76.19%)
Cotrimoxazole or Trimethoprim/sulfamethoxazole (25 µg)	9(28.13%)	23(71.86%)	7(33.33%)	14(66.66%)
Enrofloxacin (5 µg)	2(7.14%)	30(93.75%)	1(4.76%)	20(95.23%)
Gentamicin (10 µg)	5(15.63%)	27(84.38%)	3(14.29%)	18(85.71%)
Kanamycin (30 µg)	5(15.63%)	27(84.38%)	4(19.05%)	17(80.95%)
Nalidixic Acid (30 µg)	7(21.86)	25(78.13%)	3(14.29%)	18(85.71%)
Oxacillin (1 µg)	29(90.65%)	3(9.37%)	21(100%)	00
Tetracycline (30 µg)	7(21.86)	25(78.13%)	4(19.05%)	17(80.95%)

Table 4 MAR indices of *E. coli* isolates (n=32)

MAR index	Number
00	3(9.38%)
0.1	4(12.5%)
0.2	3(9.38%)
0.3	6(18.75%)
0.4	5(15.63%)
0.5	4(12.5%)
0.6	1(3.13%)
0.7	3(9.38%)
0.8	00
0.9	1(3.13%)
1.0	2(7.14%)
Total number of isolates with MARI>0.2	22(68.75%)
Total number of isolates with MARI≤0.2	10(31.25%)

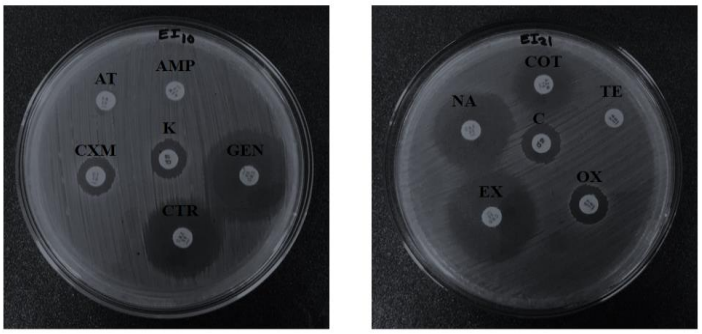

Figure 4 Representative antibiogram of *Escherichia coli* isolate

ESBL production of MDR *E. coli* isolates

MDR *E. coli* isolates (n=21) were studied for ESBL production by Phenotypic Confirmatory Disk Diffusion Test (PCDDT) and Double Disk Synergy Test (DDST). Eight (38.09%) ESBL-producing isolates were obtained by DDST using cefotaxime, ceftazidime, and aztreonam. The PCDDT detected ESBLs in 6 (75%) of the 8 ESBL-producing isolates. The ESBL-producing MDR isolates showed diverse resistance phenotypes, presented in Table 5.

Table 5 Resistance phenotype of ESBL producing MDR *E. coli* isolates

Isolate no.	Resistance phenotype
F5	AMP, COT, CTR, CXM, OX
F8	AMP, C, CXM, K, OX
F11	AMP, COT, CXM, OX, TE
F15	AMP, GEN, NA, OX
F23	AMP, AT, CTR, K, OX
F29	AMP, CTR, EX, OX
F35	COT, CXM, GEN, OX
F48	AMP, C, NA, OX, TE

AMP=Ampicillin, AT=Aztreonam, C=Chloramphenicol, COT=Cotrimoxazole, CTR=Ceftriaxone, CXM=Cefuroxime, EX=Enrofloxacin, GEN=Gentamicin, K=Kanamycin, NA=Nalidixic acid, OX=Oxacillin, and TE=Tetracycline

Among the street-vended food samples, Phuchka comprises the highest number of both MDR and ESBL-producing MDR *E. coli.* No ESBL-producing MDR *E. coli* were found in Sausage and Bun samples (Figure 5).

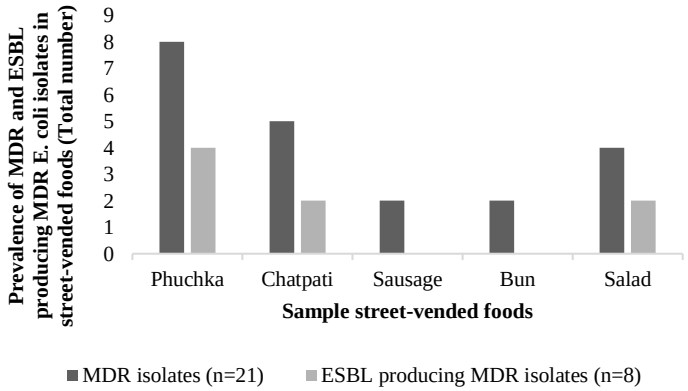

Figure 5 Prevalence of MDR and ESBL-producing MDR *E. coli* isolates in street-vended foods

Antibiotic susceptibility pattern of *Klebsiella oxytoca, Serratia nematodiphila,* and *Burkholderia fungorum* isolates

In our study, *Klebsiella oxytoca* isolate showed resistance against ampicillin, oxacillin, cefuroxime, and kanamycin. *Serratia nematodiphila* isolate did not show a clear inhibition zone against ampicillin, oxacillin, and cefuroxime. *Burkholderia fungorum* isolate did not show a clear inhibition zone against ampicillin and chloramphenicol (Table 6).

Table 6 The diameter of the disk diffusion inhibition zone

Antibiotic agents	Inhibition zone diameter (mm)		
	***Klebsiella oxytoca* (Isolate F38)**	***Serratia nematodiphila* (Isolate F42)**	***Burkholderia fungorum* (Isolate F45)**
Ampicillin (25 µg)	00 (R)	00	00
Aztreonam (30 µg)	21 (S)	25	22
Ceftriaxone (30 µg)	22 (S)	22	24
Cefuroxime (30 µg)	12 (R)	00	11
Chloramphenicol (30 µg)	23 (S)	28	00
Cotrimoxazole or Trimethoprim/sulfamethoxazole (25 µg)	20 (S)	21	28
Enrofloxacin (5 µg)	25 (S)	28	27
Gentamicin (10 µg)	16 (S)	20	11
Kanamycin (30 µg)	11 (R)	11	20
Nalidixic Acid (30 µg)	22 (S)	28	29
Oxacillin (1 µg)	00 (R)	00	10
Tetracycline (30 µg)	16 (S)	09	19

R =Resistant and S =Susceptible as per CLSI guidelines **(CLSI, 2022)**.

DISCUSSION

According to our knowledge, this is the first report on the prevalence of antibiotic-resistant *Klebsiella oxytoca* species in street food, as well as *Serratia nematodiphila*, and *Burkholderia fungorum* species in food items. All three isolates showed resistance against two or more antibiotics, including ampicillin. Similar results were found in a study on *Klebsiella oxytoca* isolated from animal-based food items and manured soil, which can be potential sources of contamination **(Abebe, 2020; L. Wang et al., 2019)**. In a study on RTE foods (soft cheese and salami) producing facilities, all *Klebsiella oxytoca* isolates showed resistance against ampicillin and susceptibility against azithromycin **(Crippa et al., 2023)**. In Bangladesh, the prevalence of *Klebsiella oxytoca* in street foods has not been reported. However, a study on hospitalized patients in Bangladesh reported that *K. oxytoca* showed 100% resistance toward ampicillin, along with resistance to amoxicillin, ceftriaxone, ciprofloxacin, cotrimoxazole, and gentamicin by 75%, 25%, 50%, 50%, and 0%, respectively. The study found that gentamicin was the most potent drug **(Chakraborty et al., 2016)**. The CLSI guidelines for determining antibiotic susceptibility using the disk diffusion method are currently unavailable for *Serratia nematodiphila* and *Burkholderia fungorum*. Very few studies have investigated the antibiotic resistance of *Serratia nematodiphila* and *Burkholderia fungorum*. A study on *Serratia* spp. isolated from sediments in Northeast India reported antibiotic resistance against five antibiotics **(Sarma, Acharya, & Joshi, 2016)**. Another study on isolates from the western Amazon soil reported that certain *Burkholderia fungorum* strains exhibited resistance against at least four antibiotics **(de Oliveira-Longatti et al., 2014)**.

Our study further highlighted the prevalence of multidrug-resistant *E. coli* in street foods of Bangladesh, considering that 64% of the isolates were identified as *E. coli*. The unhygienic food handling behaviors of street vendors might be the cause of fecal contamination, indicated by the presence of *E. coli* **(Giri, Kudva, Shetty, & Shetty, 2021; Rane, 2011; Rheinländer et al., 2008; Sarter & Sarter, 2012)**.

Additionally, 22 isolates had a MAR index >0.2, which indicated the overuse of antibiotics and the possibility of contamination from high-risk sources during production, handling of raw materials, manufacturing, and transportation **(H. A. Ahmed et al., 2018; Ateba, Tabi, Fri, Bissong, & Bezuidenhout, 2020; Maloo, Fulke, Mulani, Sukumaran, & Ram, 2017; Saeed et al., 2022)**. The prevalence of ESBL-producing MDR isolates (n=8) raises serious public health concerns, indicating that the resistance to last-resort antibiotic carbapenems proliferates **(Bush & Bradford, 2020)**. Prevalence of ESBL-producing *E. coli* has been previously reported in wild and migratory birds, aquatic environments and frozen meat in Bangladesh **(Haque, Yoshizumi, Saga, Ishii, & Tateda, 2014; Islam et al., 2022; Lina et al., 2014; Mahmud et al., 2020; Parvin et al., 2020; Rashid, Rakib, & Hasan, 2015)**. The multidrug resistance (viz. cefotaxime, aztreonam, and ceftazidime) of ESBL-producing *E. coli* isolated from the household water supply in Bangladesh was reported by **Talukdar *et al.* (2013),** and our study shows similar results for the isolates from street foods **(Talukdar et al., 2013)**. In our study, the highest prevalence of MDR and ESBL-producing isolates (n=8 and n=4, respectively) were found in Phuchka, followed by Chatpati (n=5 and n=2, respectively), which are popularly consumed street foods in Bangladesh **(Al Mamun, Rahman, & Turin, 2013)**. The presence of *E. coli* strains in Phuchka samples is usually caused by the lack of vendor personal hygiene, use of contaminated water to wash utensils and salads, storage of food in unsanitary places, and lack of other facilities related to food safety **(Rane, 2011)**. Several studies conducted on street foods in Taiwan, the Philippines, Portugal, Mexico, and Ecuador reported resistance of *E. coli* isolates to ampicillin, chloramphenicol, tetracycline, sulfamethoxazole/trimethoprim, nalidixic acid, and gentamicin which support the results of our study **(Campos, Gil, Mourao, Peixe, & Antunes, 2015; Estrada-Garcia et al., 2004; Lin et al., 2017; Manguiat & Fang, 2013; Zurita, Yánez, Sevillano, Ortega-Paredes, & Paz y Miño, 2020)**. A study on RTE Chutney samples served at street food stalls in Nepal reported that ESBL production of *E.* coli isolates was significantly affected by water sources, hygiene practices, and literacy rate of street food vendors. Ampicillin was found to be the most ineffective drug as all *E. coli* resisted this drug, which aligns with the findings of our study **(Adhikari et al., 2023)**. Similar results were reported in another study on the ineffectiveness of ampicillin against *E. coli* isolated from RTE street foods sold in the city of Maputo, Mozambique **(Salamandane, Alves, Chambel, Malfeito-Ferreira, & Brito, 2022)**. A study on *E. coli* strains isolated from food and environmental sources reported that almost 53% of isolates were resistant to nalidixic acid **(Pormohammad, Nasiri, & Azimi, 2019)**. However, resistance against nalidixic acid was observed lower (22%) in our study. According to our findings, *E. coli* isolates showed high sensitivity towards aztreonam, enrofloxacin, gentamicin, and kanamycin. Another study reported gentamicin to be the most effective antibiotics against *E. coli* strains, which supports our findings **(Pajohi Alamoti et al., 2022)**.

Quantitative risk assessment (QMRA) can be used to evaluate the likelihood that consumers will be exposed to MDR and ESBL-producing bacteria from street food **(Noman et al., 2021)**. Data on the incidence, concentrations, and potential for survival of *E. coli* on street foods are needed for that purpose. A comparison with the impacts of other potential transmission channels, such as direct contact with human and animal carriers, would enable such analysis to shed light on the overall role of street foods in the transmission of MDR and ESBL-producing *E. coli* to the community **(Sarfraz Ahmed et al., 2020)**. Therefore, to effectively restrict the emergence of community-associated resistant strains of *E. coli*, it is required to map the relative contributions of all possible transmission pathways, including transmission through various street foods. To combat antibiotic resistance, the WHO has provided a list of priority pathogens, and members of *Enterobacteriaceae* (including *Klebsiella*, *E. coli*, *Serratia*, and *Proteus*) have been included in the most critical category **(WHO, 2019)**. Although our study had a limited number of isolates, the results suggested the prevalence and spread of lesser-known species of resistant bacteria throughout Bangladesh.

CONCLUSION

Antibiotic-resistant microorganisms can spread through the food supply chain and adversely affect the ability to combat the persistent threat of infectious diseases. In this study, most of the *E. coli* isolates (90.63%) collected from street foods showed resistance against the applied antibiotics. ESBL-producing MDR isolates were also prevalent, which is alarming. Additionally, the presence of resistant strains of *Klebsiella oxytoca*, *Serratia nematodiphila*, and *Burkholderia fungorum* indicated potential health risks locally and globally. Future studies should cover a wider geographic area and focus on the horizontal transmission of multi-drug-resistant genes. Adequate measures, such as avoiding the use of unrestrained antibiotics and making street-vended foods more hygienic, should be taken to prevent the spread of resistant strains of pathogens. This study provides information regarding the emerging global issue of antibiotic-resistant bacteria. It emphasizes the need for developing standardized food safety measures and training street food vendors regularly.

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