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Occurrence of Extended Spectrum Beta-Lactamase Producing *E. coli* in some Ready-to-Eat Foods sold in Akure, Ondo State, Nigeria

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Abstract

This study investigated bacterial contamination and antibiotic resistance in ready-to-eat (RTE) foods sold by local vendors in Akure, Ondo State, Nigeria. A total of 416 food samples, including rice, beans, meat pies, and snacks, were analyzed for pathogenic bacteria. Escherichia coli (E. coli), including strains like Enterotoxigenic E. coli (ETEC) and Shiga-toxin-producing E. coli O157:H7, was among the most isolated contaminants, particularly from meat samples. Across all samples, 100% bacterial contamination was observed, with additional pathogens such as Staphylococcus aureus, Bacillus spp., and Streptococcus spp. playing a significant role. Notably, 68.42% of the E. coli isolates were found to produce Extended Spectrum Beta-Lactamase (ESBL), conferring significant resistance to beta-lactam antibiotics. Resistance to other antibiotics such as clotrimazole, tetracycline, and amoxicillin was widespread, though isolates remained sensitive to ofloxacin and nalidixic acid. These findings underscore the persistent public health risk of foodborne illnesses, driven by poor hygiene practices and rising antimicrobial resistance. The study emphasizes the need for molecular characterization, advanced biochemical systems like API and VITEK for accurate pathogen identification, stricter food safety regulations, and responsible antibiotic use to curb the threat of antimicrobial resistance in foodborne pathogens.

Keywords: Antibiotics, Resistance, Escherichia coli, Extended Spectrum Beta-Lactamase (ESBL), Ready-to-Eat (RTE) foods, Foodborne illness

INTRODUCTION

"Ready-to-eat" (RTE) food, commonly known as street food, includes a wide variety of foods, snacks, and beverages sold in public spaces and often prepared on-site. These foods are popular in urban areas due to their affordability,

accessibility, and convenience (Ibrahim *et al.*, 2020). While RTE foods are consumed immediately or taken away, consumers often overlook their safety and hygiene (Oseyemi, 2023). This negligence raises significant concerns as street foods have been associated with foodborne diseases caused by pathogens introduced through improper food handling and preparation (Andrade *et al.*, 2023). A primary contamination route is through hands contaminated with fecal matter, leading to the presence of fecal coliforms like *Escherichia coli* (*E. coli*) in the food. *E. coli*, a Gram-negative, rod-shaped bacterium, is usually harmless and resides in the intestines of warm-blooded animals. However, certain *E. coli* strains can cause severe foodborne illnesses in humans (Ramos *et al.*, 2020). *E. coli's* genetic diversity, driven by its highly adaptable genome, allows it to act as a reservoir for genes related to antibiotic resistance and virulence, making it a critical focus in food safety and public health studies (Horesh *et al.*, 2021). This genetic flexibility contributes to the spread of antimicrobial resistance (AMR), which is a growing global threat. *E. coli's* high levels of antibiotic resistance have led the WHO to designate it as a priority infection (Vounba *et al.*, 2019). Addressing the evolution of novel bacterial infections that threaten the health of humans and animals requires an understanding of its function in AMR. Foodborne illnesses, including those caused by *E. coli*, remain a global health issue, particularly in developing countries where foodborne diseases are a leading cause of death (Havelaar *et al.*, 2015). Challenges in ensuring food safety vary between industrialized and developing nations, with the latter frequently encountering challenges, including conventional food processing, inadequate storage conditions, and poor cleanliness among food workers, all contributing to the spread of foodborne illnesses (Makinde *et al.*, 2020). Outbreaks caused by enteropathogenic bacteria such as Salmonella, Vibrio cholerae, and *Staphylococcus aureus*, among others, are common worldwide (Igbinosa *et al.*, 2021).

Detecting *E. coli* and other common bacterial contaminants in food has been a key area of research for many years, mainly due to the serious health risks these pathogens pose. Although *E. coli* contamination is well-researched, the emergence of antibiotic-resistant strains and virulent pathotypes like ETEC and Shiga-toxin-producing *E. coli* O157:H7 is a serious risk to public health. The prevalence of foodborne illnesses worldwide emphasizes the necessity of continued study to address the growing problem of antimicrobial resistance (AMR) and the changing characteristics of pathogen profiles, particularly in countries with lax regulatory frameworks (Ramos *et al.*, 2020). Recent studies emphasize the importance of in-depth characterization of *E. coli* strains isolated from food, including their resistance patterns and strain-specific virulence factors (Horesh *et al.*, 2021). Pathotypes like ETEC, responsible for severe diarrhea, and *E. coli* O157:H7, a leading cause of hemolytic-uremic syndrome, reinforce the critical need for continued research and vigilance in food safety.

The treatment of infectious diseases has greatly improved with the discovery and application of antibiotics. However, antibiotic resistance has increased due to the overuse and abuse of antibiotics, especially in veterinary care, human medicine, and agriculture (Odeyemi, 2016). Factors contributing to this include microbial adaptation, international travel, environmental changes, and inadequate public health strategies (Lammie and Hughes, 2016). As bacteria develop resistance through various mechanisms, prolonged or improper use of antibiotics accelerates the emergence of resistant strains, some of which produce beta-lactamase enzymes, making them resistant to beta-lactam antibiotics like penicillin (Naveed *et al.*, 2020). This results in the production of Extended Spectrum Beta-Lactamase (ESBL), an enzyme that enables bacteria to survive a wider variety of antibiotics. Infections caused by ESBL-producing *E. coli* present significant clinical challenges due to limited treatment options and poor patient outcomes. Controlling the spread of such bacteria requires careful antibiotic use and stringent infection control measures, including barrier precautions (Vázquez-López *et al.*, 2023). ESBL-producing *E. coli* can enter the environment through sources like manure and sewage, contaminating fresh produce and posing risks to animals and humans (Singh *et al.*, 2020). The persistence of these bacteria on food surfaces and their potential to penetrate produce underscore the serious threat they pose to public health and food safety (Beshiru *et al.*, 2023). This study focused on identifying bacterial contamination in RTE food samples from local vendors in Akure, Ondo State. It also examined the antibiotic sensitivity patterns of isolated *E. coli* strains and screened for beta-lactamase production in antibiotic-resistant *E. coli*.

MATERIALS AND METHODS

Sample Collection

The study targeted ready-to-eat (RTE) foods from eight locations in Akure, Ondo State, Nigeria, including Off-Campus (FUTA), FUTA Campus, Cathedral, Oja Oba, Old Garage, FUTA Junction, Road Block, and FUTA Express. Food samples were purposively selected based on their popularity and high consumption rates. The selected food types included rice, beans, meat pies, doughnuts, spaghetti, and moinmoin.

A total of 416 food samples were analyzed. The sample size was determined based on previous studies on foodborne pathogens, diversity of food types to ensure broad representation, geographical spread for better generalizability, microbiological study standards for food safety assessments, and statistical validity to ensure reliable conclusions. Samples were collected aseptically in sterile Whirl-Pak bags, labeled, and transported within 30–45 minutes to the Microbiology Laboratory at FUTA for analysis to maintain sample integrity.

Isolation and Identification of Microorganisms

The food sample underwent serial dilution, and specimens were inoculated on nutrient agar, Eosin Methylene Blue (EMB) agar, MacConkey agar, and Mueller-Hinton agar using both pour plate and spread plate methods. Plates were incubated at 37°C for 24 hours, after which emerging colonies were counted and subcultured onto fresh nutrient agar plates. Morphological characteristics were observed on petri dishes, followed by microscopic examination, as described by Cheesbrough (2002). Various biochemical tests were performed for preliminary bacterial identification, including motility, catalase, oxidase, carbohydrate fermentation, and indole tests (Jimeta *et al.*, 2022). To enhance accuracy and depth in pathogen identification, modern biochemical systems such as API 20E and VITEK 2 Compact were subsequently employed, adhering to the guidelines of the National Committee for Clinical Laboratory Standards (Yu *et al.*, 2022). These systems enabled the precise identification of *E. coli* and other gram-negative bacteria with higher reproducibility and reduced turnaround times, improving conventional culture methods used earlier in the analysis.

In addition to biochemical testing, molecular techniques such as polymerase chain reaction (PCR) were employed to detect virulence genes associated with *E. coli* pathotypes. Primers targeting genes specific to ETEC and *E. coli* O157:H7 were used to determine the presence of these strains in the food samples (Horesh *et al.*, 2021). Molecular confirmation of ESBL production was performed by detecting the blaCTX-M gene responsible for beta-lactamase enzyme production.

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method was used to investigate antibiotic susceptibility on Mueller-Hinton agar (Sidek *et al.*, 2018). A wide range of medications, such as nitrofurantoin (200 mg), tetracycline (25 mg), amoxicillin (30 mg), ofloxacin (5 mg), gentamicin (10 mg), nalidixic acid (30 mg), clotrimazole (25 mg), and Augmentin (30 mg), were tested against the bacterial isolates. The zones of inhibition on the plates were examined to assess the level of sensitivity (Sader *et al.*, 2016).

Detection of ESBL production

Double disc synergy tests (DDST) were used for this, as described by Garba *et al.* (2018). Test organisms were inoculated onto Mueller-Hinton agar plates, and third-generation cephalosporins (in µg) were used: Amoxicillin/clavulanic acid, ceftriaxone, and cefoxitin. The Amoxicillin/clavulanic acid (20µg/10µg) disk was placed on the Mueller-Hinton agar plates containing the antibiotic-resistant *E. coli*, positioned 30mm from the plate's center, with the other discs arranged 15mm away from the clavulanate disk in a triangular pattern. The National Committee for Clinical Laboratory Standards (NCCLS, 2004) interprets an obvious extension of the inhibition zone's border towards the clavulanate disk as synergy, indicating the existence of an ESBL.

RESULTS

The analysis of samples collected from various locations revealed a 100% contamination rate across all areas. *Staphylococcus aureus* was isolated at a rate of 100% from Off Campus, School Area, and Cathedral, while Express showed the lowest isolation rate at 71.43% (Table 1). The highest occurrence of *Bacillus* spp. was recorded Off Campus (98.33%), followed by the School Area (97.26%), with the lowest percentage observed at Express (57.14%). For *E. coli*, the highest isolation rate of 17.95% was from Old Garage, followed by Express and Road Block, both at 14.29%. The lowest rate was 2.56% from Cathedral. *Streptococcus* spp. showed the highest isolation rate from the School Area at 85.62%, followed by Old Garage (79.49%), Oja Oba (78.26%), and Off Campus (75%), with the lowest percentage from FUTA Junction (39.29%) (Table 2). Morphological and biochemical tests identified the presence of *E. coli*, *Bacillus* spp., *Staphylococcus aureus*, and *Streptococcus* spp. in the samples (Table 3).

Antibiotic sensitivity testing on the isolated gram-negative *E. coli* was performed using a broad-spectrum antibiotic disk, including Ofloxacin (OFL), Augmentin (AUG), Tetracycline (TET), Amoxicillin (AMX), Clotrimazole (COT), Nitrofurantoin (NIT), Nalidixic Acid (NAL), and Gentamycin (GEN). Additional tests for ESBL detection used Ceftriaxone, Amoxicillin-Clavulanic Acid, and Cefoxitin (Figures 1 and 2). ESBL-producing *E. coli* exhibited resistance to Cefoxitin but were susceptible to Ceftriaxone and Amoxicillin-Clavulanic Acid (Figure 3).

Meat samples had the greatest occurrence of *E. coli* (44.44%), followed by rice (21.05%), beans (13.16%), moinmoin (10.53%), meat pie (7.89%), and spaghetti (2.6%). A 100% contamination rate was observed across all sampled locations, with a high prevalence of *E. coli*, *Staphylococcus aureus*, *Bacillus* spp., and *Streptococcus* spp. Pathogenic *E. coli* strains such as O157:H7 and ETEC were isolated, particularly from meat samples. Meat samples showed a 44.44% occurrence of *E. coli*, while rice and beans had 21.05% and 13.16%, respectively (Figure 4). The antibiotic resistance distribution among gram-negative *E. coli* isolated from meat samples exhibited varied levels of resistance (R), slight resistance (R.s), susceptibility (S.S), and average susceptibility (S.s) to antibiotics such as GEN, NIT, NAL, COT, AMX, TET, AUG, and OFL (Table 4). *E. coli* isolated from rice samples from Off Campus and School Area showed similar antibiotic resistance patterns, but no *E. coli* was isolated from rice samples collected at Old Garage, Oja Oba, Cathedral, Road Block, or FUTA Junction (Table 5).

All *E. coli* isolates exhibited high resistance to commonly used antibiotics such as tetracycline, amoxicillin, and nitrofurantoin. ESBL production was confirmed in 68.42% of the *E. coli* isolates, with high resistance to cephalosporins and fluoroquinolones. However, susceptibility to ofloxacin and amoxicillin-clavulanic acid was observed. Antibiotic resistance trends were detected among *E. coli* isolated from Off Campus, School Area, and Old Garage bean samples. However, no *E. coli* was found in beans samples from Oja Oba, Cathedral, or FUTA Junction (Table 6). In moinmoin samples from Off Campus and School Areas, *E. coli* exhibited varying degrees of resistance, while no isolates were found in samples from Old Garage, Oja Oba, Cathedral, Road Block, and FUTA Junction (Table 7).

E. coli isolated from meat pie samples from the School Area and Oja Oba also showed varying antibiotic resistance patterns. No isolates were detected in Off Campus, Old Garage, Cathedral, Road Block, and FUTA Junction (Table 8). Similarly, *E. coli* isolated from spaghetti samples from Oja Oba showed resistance, but none were found in samples from other locations (Table 9).

Gram-negative *E. coli* isolated from moinmoin, meat pie, and spaghetti samples demonstrated the highest susceptibility to TET and AUG, while those isolated from beans had the lowest susceptibility to NAL. *E. coli* from spaghetti, meat pie, and beans exhibited the highest resistance to OFL, with moinmoin isolates showing resistance to AUG, while the lowest resistance was observed with GEN (Figures 5 and 6).

Beans and spaghetti samples showed a 100% occurrence of ESBL-producing *E. coli*, followed by rice (80%) and moinmoin (75%). Meat samples had the lowest occurrence at 64.71%. The isolates of *E. coli* that produced ESBLs had a high level of resistance to Cefoxitin and were highly sensitive to Amoxicillin-Clavulanic Acid, with intermediate resistance observed in all the antibiotics (Figures 7 and 8).

The PCR study showed that 30% of *E. coli* O157:H7 isolates have the stx1 and stx2 genes, while elt and est genes were found in 25% of ETEC isolates. The prevalence of these virulence genes highlights the potential for severe foodborne illnesses. ESBL genes (bla_CTX-M and bla_TEM) were identified in 55% of the isolates, indicating a high potential for multidrug resistance.

Table 1: Percentage of bacterial contamination of ready-to-eat food collected

Locations	Number of samples analysed (Cfu/ml)	Number of samples with bacterial growth (Cfu/ml)	Contamination (%)
Off Campus	120	120	100
School Area	146	146	100
Old Garage	39	39	100
Oja Oba	23	23	100
Cathedral	39	39	100
Express	14	14	100
Road Block	7	7	100
FUTA Junction	28	28	100
TOTAL	**416**	**416**	**100**

Key: % - Percentage, cfu – Colony forming Unit, ml-millilitre

Table 2: Frequency distribution of the isolated bacteria from the examined meat samples

Bacteria isolated	*Staphylococcus aureus*	*Bacillus* spp.	*Escherichia coli*	*Streptococcus* spp.
Off Campus	120 (100%)	118 (98.33%)	9 (7.5%)	90 (75%)
School Area	146 (100%)	142 (97.26%)	18 (12.33%)	125 (85.62%)
Old Garage	38 (97.44%)	36 (92.31%)	7 (17.95%)	31 (79.49%)
Oja Oba	22 (95.65%)	20 (86.96%)	3 (13.04%)	18 (78.26%)
Cathedral	39 (100%)	34 (87.14%)	1 (2.56%)	28 (71.79%)
Express	10 (71.43%)	8 (57.14%)	2 (14.29%)	5 (35.71%)
Road Block	6 (85.71%)	5 (71.42%)	1 (14.29%)	3 (42.86%)
FUTA Junction	25 (89.29%)	20 (71.43%)	1 (3.57%)	11 (39.29%)
Average	**406 (98.32%)**	**383 (92.07%)**	**38 (9.13%)**	**311 (74.76%)**

Table 3: Biochemical tests for identification of the isolated organisms

System	Test	Result	Probable organism
API 20E	ONPG	Positive (Yellow)	*Escherichia coli*
	ADH (Arginine Dihydrolase)	Negative	
	LDC (Lysine Decarboxylase)	Positive	
	ODC (Ornithine Decarboxylase)	Positive	
	CIT (Citrate Utilization)	Negative	
	H_2S (Hydrogen Sulfide)	Negative	
	Urease	Negative	
	IND (Indole)	Positive	
	GLU (Glucose Fermentation)	Positive	
VITEK 2	ß-Galactosidase (ONPG)	Positive	
	Indole Production	Positive	
	Ornithine Decarboxylase	Positive	
	Glucose Fermentation	Positive	
	Urease	Negative	
	Citrate Utilization	Negative	
VITEK 2	Catalase	Positive	*Bacillus* spp.
	Oxidase	Negative	
	Nitrate Reduction	Positive	
	Urease	Negative	
	Motility	Positive	
VITEK 2	Catalase	Positive	*Staphylococcus* aureus
	Coagulase	Positive	
	Mannitol Fermentation	Positive	
	DNase	Positive	
VITEK 2	Catalase	Negative	*Streptococcus* spp.
	Bile Esculin	Positive	
	PYR	Positive	
MORPHOLOGY		PC on MAC	*Escherichia coli*
		GMS on EMB	
		MSC on NA	*Bacillus* spp.
		TMC on NA	*Staphylococcus aureus*
		LMC on NA	*Streptococcus* spp.
GRAM REACTION		-ve rods in clusters	*Escherichia coli*
		+ve rods in chains	*Bacillus* spp.
		+ve cocci in clusters	*Staphylococcus aureus*
		+ve cocci in chains	*Streptococcus* spp.

Key: GMS-Green Metallic Sheen, PC-Pink Colony, NA-Nutrient agar, LMC-Light Milkish Colony, TMC-Tiny Milkish colony, MSC- Medium size colony, (+) Positive, (-) Negative

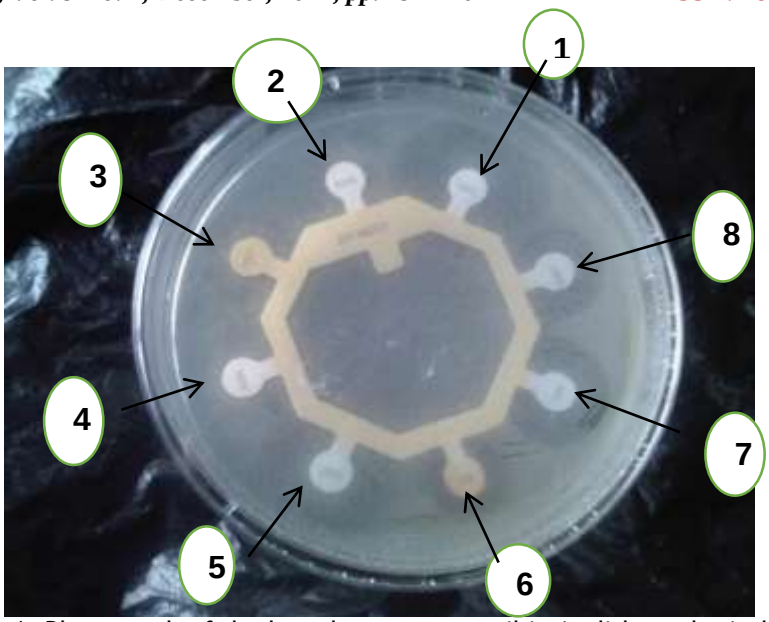

Figure 1: Photograph of the broad-spectrum antibiotic disk on the isolated gram-negative *E. coli*
Key: 1 - Ofloxacin, 2 - Augmentin, 3 - Tetracycline, 4 - Amoxycillin, 5 - Clotrimazole, 6 - Nitrofurantoin, 7 - Nalidixic acid, 8 - Gentamycin.

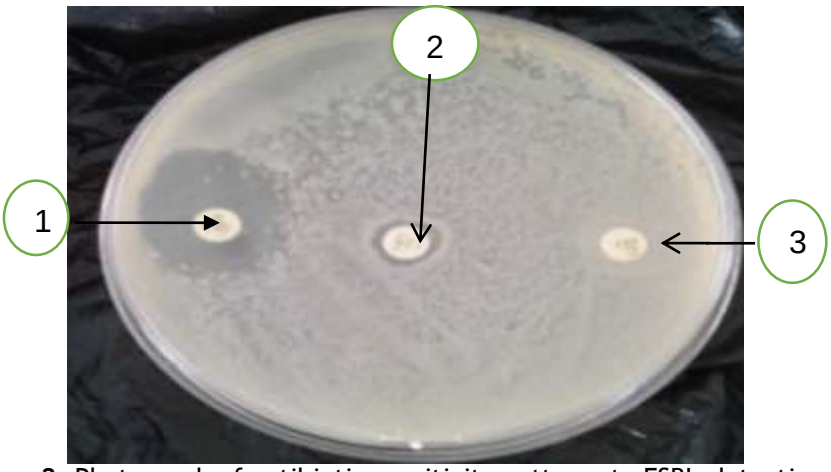

Figure 2: Photograph of antibiotic sensitivity patterns to ESBL-detecting antibiotics
Key: ESBL-Extended spectrum beta-lactamase, 1 = Ceftriaxone, 2 = Amoxycillin-clavulanic acid, 3 = Cefoxitin

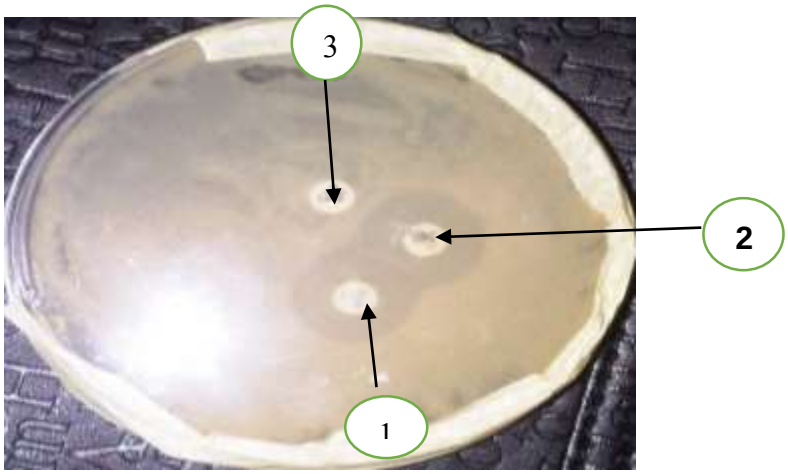

Figure 3: Photograph of an ESBL-producing *E. coli* conferring resistance to cefoxitin and susceptible to ceftriaxone and Amoxycillin-clavulanic acid, forming synergy.
Key: 1 = Ceftriaxone, 2 = Amoxycillin-clavulanic acid, 3 = Cefoxitin

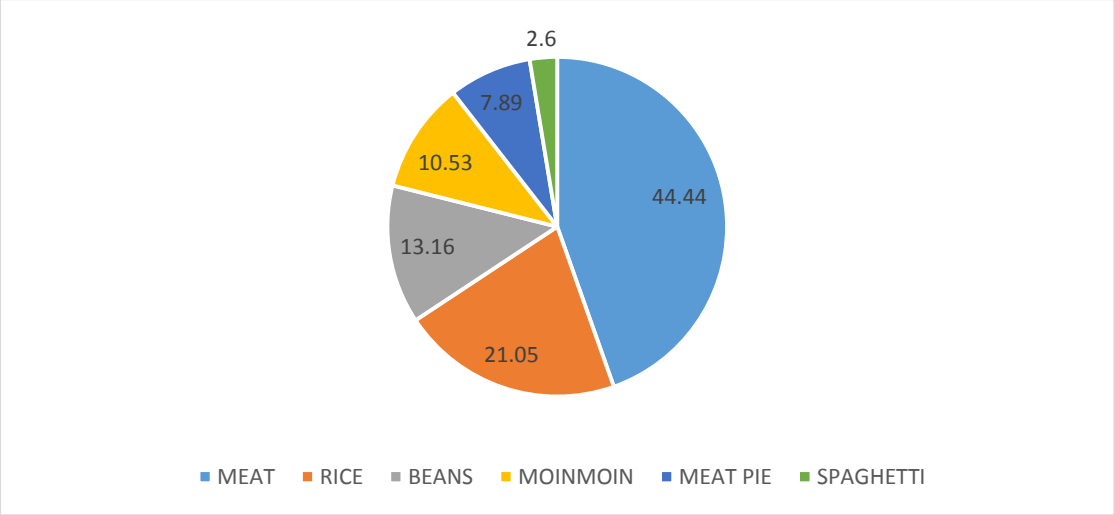

Figure 4: Occurrence of the isolated gram-negative *E. coli* in different food samples

Tables 4, 5, 6, 7, 8, & 9 show the distribution and percentage distribution of the antibiotic sensitivity patterns carried out on the *E. coli* isolates from the different food samples, which are meat, rice, beans, moinmoin, meat pie, and spaghetti, respectively.

Table 4: Distribution of antibiotic resistance among gram-negative *E. coli* isolated from meat.

LOCATION	GEN	NAL	NIT	COT	AMX	TET	AUG	OFL
Off Campus	20(S.S)	18(S.S)	19(S_S)	05(R)	06(R)	07(R)	10(R)	21(S.S)
School Area	18(S_S)	17(S.S)	16(S_S)	06(R)	07(R)	08(R)	11(R)	20(S.S)
Old Garage	15(R)	14(S.S)	13(R_S)	07(R)	08(R)	09(R)	12(R_S)	19(S.S)
Oja Oba	16(R)	15(S.S)	14(R_S)	08(R)	07(R_S)	09(R)	10(S.S)	18(S.S)
Cathedral	17(R)	16(S.S)	15(S.S)	06(R)	08(R)	09(R)	10(R)	17(S.S)
Road Block	14(R)	13(S_S)	12(S.S)	07(R)	08(R)	09(R)	10(R)	16(S.S)
FUTA Junction	15(R)	14(S.S)	13(R_S)	08(R)	09(R)	10(R)	11(S.S)	18(S.S)

Key: R - Resistant, R_S - Slightly resistant, S.S - Susceptible, S_S - Averagely susceptible, **GEN** - Gentamycin, **NIT** - Nitrofurantoin, **NAL** - Nalidixic acid, **COT** - Clotrimazole, **AMX** - Amoxycillin, **TET** - Tetracycline, **AUG** - Augmentin, **OFL** - Ofloxacin

Table 5: Distribution of antibiotic resistance among gram-negative *E. coli* isolated from Rice

LOCATION	GEN	NAL	NIT	COT	AMX	TET	AUG	OFL
Off Campus	18(S.S)	16(S.S)	15(S_S)	06(R)	07(R)	08(R)	09(S_S)	19(S.S)
School Area	16(R)	14(S_S)	13(S.S)	07(R)	08(R)	09(R_S)	10(R)	18(S.S)
Old Garage	-	-	-	-	-	-	-	-
Oja Oba	-	-	-	-	-	-	-	-
Cathedral	-	-	-	-	-	-	-	-
Road Block	-	-	-	-	-	-	-	-
FUTA Junction	-	-	-	-	-	-	-	-

Key: - = No *E. coli* found in the sample collected, R - Resistant, R_S - Slightly resistant, S.S - Susceptible, S_S - Averagely susceptible, **GEN** - Gentamycin, **NIT** - Nitrofurantoin, **NAL** - Nalidixic acid, **COT** - Clotrimazole, **AMX** - Amoxycillin, **TET** - Tetracycline, **AUG** - Augmentin, **OFL** - Ofloxacin

Table 6: Distribution of antibiotic resistance among gram-negative *E. coli* isolated from beans

LOCATION	GEN	NAL	NIT	COT	AMX	TET	AUG	OFL
Off Campus	17(S.S)	15(S.S)	14(R_S)	08(R)	09(R)	10(R)	11(R_S)	20(S.S)
School Area	15(R_S)	13(S.S)	12(S.S)	06(R)	07(R)	08(R)	09(R)	19(S.S)
Old Garage	18(S_S)	16(S.S)	15(S.S)	07(R)	08(R)	09(R)	10(R)	18(S.S)
Oja Oba	-	-	-	-	-	-	-	-
Cathedral	-	-	-	-	-	-	-	-
Road Block	-	-	-	-	-	-	-	-
FUTA Junction	-	-	-	-	-	-	-	-

Key: - = No *E. coli* found in the sample collected, R - Resistant, R_S - Slightly resistant, S.S - Susceptible, S_S - Averagely susceptible, **GEN** - Gentamycin, **NIT** - Nitrofurantoin, **NAL** - Nalidixic acid, **COT** - Clotrimazole, **AMX** - Amoxycillin, **TET** - Tetracycline, **AUG** - Augmentin, **OFL** - Ofloxacin

Table 7: Distribution of antibiotic resistance among gram-negative *E. coli* isolated from Moinmoin

LOCATION	GEN	NAL	NIT	COT	AMX	TET	AUG	OFL
Off Campus	14(R)	13(S.S)	12(S_S)	06(S_S)	07(R)	08(R_S)	09(S_S)	17(S.S)
School Area	16(R_S)	14(S.S)	13(R)	07(R_S)	08(R)	09(R)	10(S.S)	18(S.S)
Old Garage	-	-	-	-	-	-	-	-
Oja Oba	-	-	-	-	-	-	-	-
Cathedral	-	-	-	-	-	-	-	-
Road Block	-	-	-	-	-	-	-	-
FUTA Junction	-	-	-	-	-	-	-	-

Key: - = No *E. coli* found in the sample collected, R - Resistant, R_S - Slightly resistant, S.S - Susceptible, S_S - Averagely susceptible, **GEN** - Gentamycin, **NIT** - Nitrofurantoin, **NAL** - Nalidixic acid, **COT** - Clotrimazole, **AMX** - Amoxycillin, **TET** - Tetracycline, **AUG** - Augmentin, **OFL** - Ofloxacin

Table 8: Distribution of antibiotic resistance among gram-negative *E. coli* isolated from meat pie

LOCATION	GEN	NAL	NIT	COT	AMX	TET	AUG	OFL
Off Campus	-	-	-	-	-	-	-	-
School Area	15(R)	13(S.S)	12(S_S)	06(R)	07(R)	08(R)	09(R)	17(S.S)
Old Garage	-	-	-	-	-	-	-	-
Oja Oba	16(S.S)	14(S.S)	13(S.S)	07(R)	08(R)	09(R)	10(S_S)	18(S.S)
Cathedral	-	-	-	-	-	-	-	-
Road Block	-	-	-	-	-	-	-	-
FUTA Junction	-	-	-	-	-	-	-	-

Key: - = No *E. coli* found in the sample collected, R - Resistant, R_S - Slightly resistant, S.S - Susceptible, S_S - Averagely susceptible, **GEN** - Gentamycin, **NIT** - Nitrofurantoin, **NAL** - Nalidixic acid, **COT** - Clotrimazole, **AMX** - Amoxycillin, **TET** - Tetracycline, **AUG** - Augmentin, **OFL** - Ofloxacin

Table 9: Distribution of antibiotic resistance among gram-negative *E. coli* isolated from Spaghetti

Location	GEN	NAL	NIT	COT	AMX	TET	AUG	OFL
Off Campus	-	-	-	-	-	-	-	-
School Area	-	-	-	-	-	-	-	-
Old Garage	-	-	-	-	-	-	-	-
Oja Oba	14(S_S)	12(S_S)	11(R_S)	06(R)	07(R)	08(R)	09(R)	16(S.S)
Cathedral	-	-	-	-	-	-	-	-
Road Block	-	-	-	-	-	-	-	-
FUTA Junction	-	-	-	-	-	-	-	-

Key: - = No *E. coli* found in the sample collected, R - Resistant, R_S - Slightly resistant, S.S - Susceptible, S_S - Averagely susceptible, **GEN** - Gentamycin, **NIT** - Nitrofurantoin, **NAL** - Nalidixic acid, **COT** - Clotrimazole, **AMX** - Amoxycillin, **TET** - Tetracycline, **AUG** - Augmentin, **OFL** - Ofloxacin

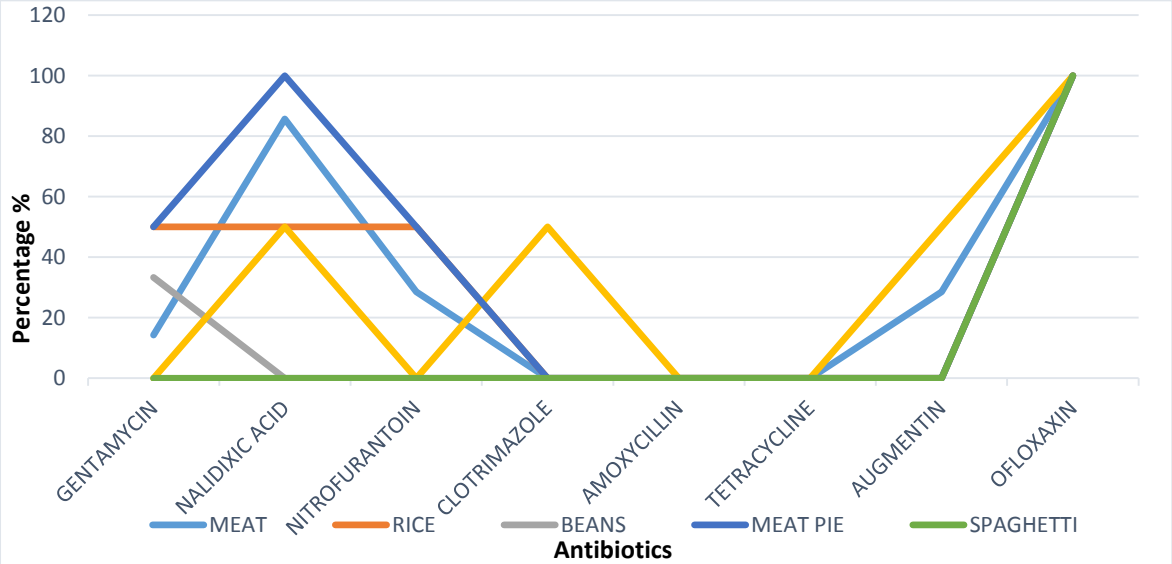

Figure 5: Percentage distribution of antibiotic susceptibility among *E. coli* isolates obtained from ready-to-eat (RTE) food samples

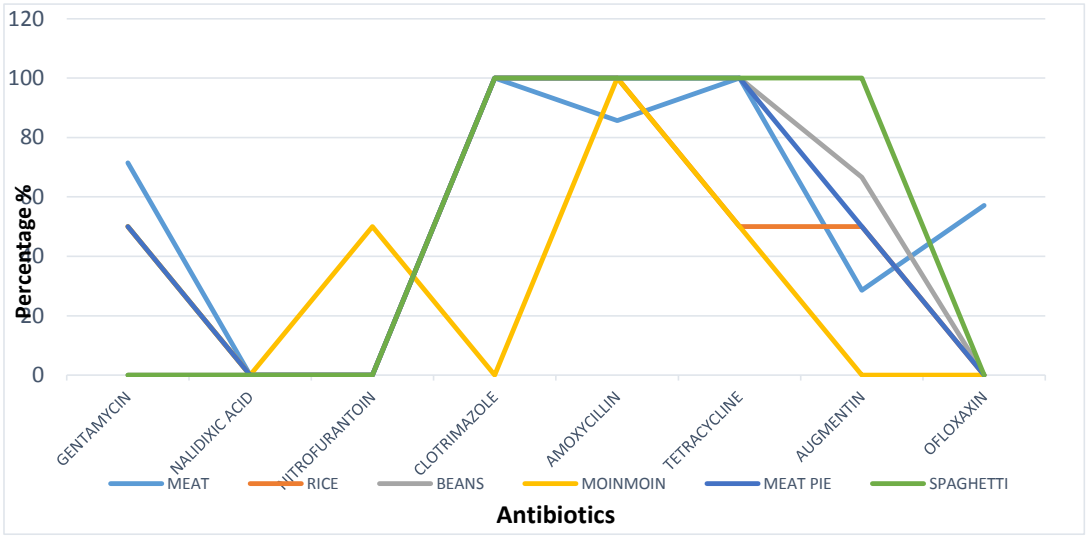

Figure 6: Percentage distribution of antibiotic resistance among *E. coli* isolates obtained from ready-to-eat (RTE) food samples

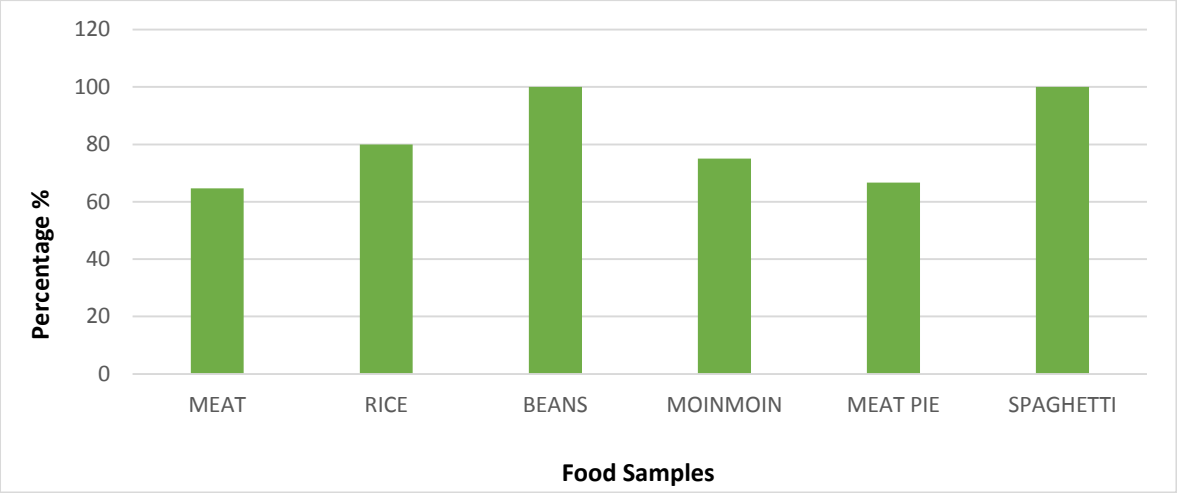

Figure 7: Distribution of Extended Spectrum Beta-Lactamase (ESBL)-producing *E. coli* across various ready-to-eat (RTE) food samples analyzed in the study
Key: ESBL - Extended Spectrum Beta-Lactamase

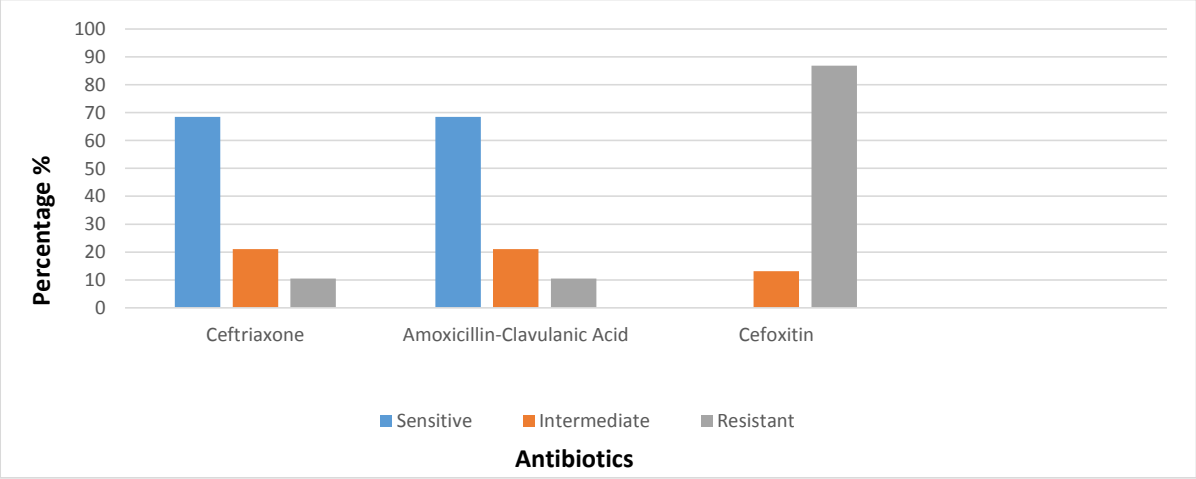

Figure 8: Percentage occurrence of ESBL-producing *E. coli* in various ready-to-eat (RTE) food samples in Akure

DISCUSSION

The prevalence of antimicrobial-resistant *E. coli* in foods such as moinmoin, meat pie, spaghetti, and beans raises serious public health concerns, especially regarding ESBL production and multidrug resistance (MDR). The varying resistance patterns across food types and locations emphasize the need for ongoing monitoring of street-vending foods. In certain areas, like Old Garage, Oja Oba, and FUTA Junction, the absence of *E. coli* could indicate improved food hygiene, while in others, such as the School Area and Oja Oba, the presence of resistant *E. coli* in meat pie and spaghetti reflects poor handling and cooking practices, as seen in other studies (Conway *et al.*, 2023). The susceptibility of *E. coli* isolates to TET and AUG is encouraging, suggesting these antibiotics may still be effective in treating infections. However, the growing resistance to fluoroquinolones, particularly OFL, is concerning, with misuse of antibiotics in both healthcare and agriculture contributing to this issue (Caneschi *et al.*, 2023). Fluoroquinolones are critical antibiotics used to treat bacterial infections, and increasing resistance reduces their efficacy, leading to prolonged illnesses, higher medical costs, and increased mortality. The misuse of antibiotics in healthcare and agriculture is a major factor contributing to this issue. In agriculture, excessive and unregulated use of antibiotics in livestock and poultry farming promotes the emergence of antimicrobial-resistant bacteria, which can spread to humans through direct contact, the food chain, or environmental pathways. Several studies have highlighted the link between antibiotic use in agriculture and the rise of antimicrobial resistance. Manyi-Loh *et al.* (2018) reviewed how intensive farming practices, driven by the growing demand for animal protein, have led to the widespread misuse of antibiotics. Their study emphasized that antibiotic residues in animal-derived food products and the environment contribute to the development of multidrug-resistant bacteria, posing significant public health risks. Similarly, a recent study by Ndahi *et al.* (2023) investigating antimicrobial use in commercial poultry farms in Plateau and Oyo States, Nigeria, found that farmers frequently administered multiple antibiotic classes, including those critical for human medicine, often without veterinary guidance. This practice accelerates the emergence of resistant bacterial strains, which may be transmitted to humans through poultry consumption or environmental exposure. These findings underscore the urgent need for prudent antibiotic use in agriculture, stricter regulatory enforcement, and comprehensive antimicrobial stewardship programs. Implementing surveillance systems to monitor antibiotic resistance patterns and promoting responsible antibiotic usage are critical steps to mitigate the growing threat of antimicrobial resistance, ensuring the continued efficacy of fluoroquinolones and other essential antibiotics in human and veterinary medicine. The resistance of moinmoin isolates to AUG may be related to ß-lactamase production, complicating treatment options further (Dela *et al.*, 2023). The detection of 100% ESBL-producing *E. coli* in beans and spaghetti, and their high prevalence in rice and moinmoin, supports global concerns about the rise of ESBLs in food sources (Giri *et al.*, 2021). These organisms are known for their resistance to cephalosporins and other ß-lactam antibiotics, posing significant treatment challenges (Bush and Bradford, 2020). While ESBL-producing *E. coli* showed sensitivity to Amoxicillin-Clavulanic Acid, the observed intermediate resistance to multiple antibiotics signals the potential emergence of multidrug-resistant strains (Band and Weiss, 2014).

Antibiotic resistance continues to pose a significant challenge in veterinary medicine, animal husbandry, livestock management, and human healthcare (Adeluwoye *et al.*, 2021). This issue is particularly concerning as ESBL (Extended-Spectrum Beta-Lactamase) producers are resistant to a broad range of antibiotics, limiting treatment options (Azekhueme *et al.*, 2015). This study found that food samples that were supplied to consumers were tainted with harmful bacteria, posing a potential health risk and leading to foodborne illnesses. The research assessed the bacterial quality of ready-to-eat foods sold in the Akure metropolitan area. Of the 416 food samples analyzed, 100% were contaminated with various microorganisms, including *Streptococcus* spp. (74.76%), *Bacillus* spp. (92.07%), *Escherichia coli* (9.13%), and *Staphylococcus aureus* (98.32%). Among the analyzed samples, meat showed the highest occurrence of *E. coli* (44.44%). The high prevalence of ESBL-producing *E. coli* in this study is not surprising, as contamination is often linked to poor hygiene practices, particularly the transmission of bacteria through fecal contact with food or water when food handlers fail to wash their hands properly (Munekata *et al.*, 2020). Numerous studies worldwide, including Kinsella *et al.* (2009), have reported bacterial contamination in meat. Similarly, extensive research has highlighted the presence of harmful bacteria in ready-to-eat (RTE) foods, posing significant public health risks. A systematic review in developing countries found *Escherichia coli* in 23.8% of RTE food samples, while

Salmonella was detected in 17.4%. Other bacteria, including Enterobacter (11.3%) and Klebsiella (9.1%), were also identified (Mengistu *et al.*, 2022). Similarly, another meta-analysis reported higher pooled prevalence rates of *E. coli* (33.8%), Salmonella (26.0%), and *Staphylococcus aureus* (46.3%) in RTE foods (Mengistu and Tolera, 2020). In a multisite survey conducted in Nanjing, China, researchers found RTE meat products contaminated with coliforms, Salmonella spp., and *Staphylococcus aureus*. The study noted that bacterial levels increased over time during processing and selling, indicating multiple contamination sources (Wang *et al.*, 2020). Another investigation into the bacteriological quality of RTE foods sold in public places linked microbial contamination to improper food handling and unhygienic preparation practices, underscoring the need for stringent quality control measures (Emmanuel, 2015). These findings confirm the widespread presence of various bacteria in RTE foods, including *E. coli*, Salmonella, Staphylococcus aureus, Enterobacter, and Klebsiella species. Such contamination presents serious health risks, highlighting the need for proper food handling, strict hygiene practices, and regular monitoring to maintain food safety. The study's findings on the prevalence of ESBL are particularly concerning for public health, as they pose a significant threat to consumers in the area. Contaminated meat, especially when undercooked, heightens the risk of infection from ESBL-producing *E. coli*. The study also found high susceptibility to tetracycline (TET) and augmentin (AUG) in *E. coli* isolates from foods like moinmoin, meat pie, and spaghetti, suggesting that these antibiotics remain relatively effective against foodborne pathogens in some regions, consistent with findings from developing countries where AUG is frequently used in clinical settings (Kimera *et al.*, 2020). However, low susceptibility to nalidixic acid (NAL) in bean isolates reflects growing resistance to older quinolones (Andersson and Hughes, 2017). The widespread resistance to ofloxacin (OFL) across multiple food types is alarming, as this fluoroquinolone is a key antibiotic in human medicine, indicating a troubling trend (Ventola, 2015). The resistance of moinmoin isolates to AUG may signal overuse or misuse in food preparation practices (Klees *et al.*, 2020). Meanwhile, the low resistance to gentamycin (GEN) suggests its continued efficacy, though increasing reports of gentamicin resistance in *E. coli* raise concerns for the future (Perovic *et al.*, 2018).

CONCLUSION

This study underscores the critical need for enhanced food safety regulations, stricter hygiene practices among street vendors, and continuous surveillance of ready-to-eat (RTE) foods in Nigeria. The high prevalence of pathogenic and antibiotic-resistant *Escherichia coli*, particularly extended-spectrum beta-lactamase (ESBL)-producing strains, necessitates comprehensive monitoring and targeted intervention strategies. Additionally, the responsible use of antibiotics in both medical and agricultural settings is imperative to mitigate the emergence and spread of multidrug-resistant (MDR) pathogens. Further research into the molecular mechanisms underlying antimicrobial resistance in foodborne bacteria is essential to inform evidence-based public health policies and address the root causes of resistance, especially in regions with inadequate food safety measures.

RECOMMENDATION

Based on the findings of this study, the following recommendations are proposed:

1. Food Safety Education – Training programs should be implemented to educate food handlers and consumers on proper hygiene, food handling, and storage practices to reduce foodborne illnesses.
2. Enhanced Detection and Monitoring – Advanced techniques should be employed to detect and track foodborne pathogens. Regular monitoring of ready-to-eat food is essential to identify microbial contamination, particularly antibiotic-resistant bacteria.
3. Regulatory Enforcement – Strict enforcement of food safety regulations, including licensing of street vendors and routine inspection of food establishments, should be prioritized to ensure compliance with hygiene standards.
4. Promotion of Voluntary Compliance – Food vendors should be encouraged to adhere to safety regulations through training programs, certification, and incentive-based approaches.
5. Control of Antibiotic Resistance – Efforts should focus on reducing unnecessary antibiotic use in food production, veterinary practices, and human medicine to mitigate the spread of antibiotic-resistant bacteria.
6. Public Awareness Campaigns – Educational initiatives should be conducted to inform the public about food safety, proper food handling, and the risks associated with foodborne diseases.
7. Improved Sanitation in Food Vending Areas – Infrastructure improvements such as access to clean water, waste disposal facilities, and proper sanitation should be ensured in food vending locations to minimize contamination risks.

8. Research and Policy Development – Scientific research on foodborne illnesses and antibiotic resistance should be promoted to guide evidence-based policy-making and improve food safety interventions.

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