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ORIGINAL RESEARCH

Commonly Consumed Vegetables as a Potential Source of Multidrug-Resistant and β-Lactamase-Producing Bacteria in Debre Berhan Town, Ethiopia

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Background: Recently, antibiotic resistance of bacteria contained in foods such as vegetables has become a public health problem. In Ethiopia, the diversity of bacterial contamination and level of antibiotic resistance in vegetables are poorly understood. Local analysis of vegetable contamination and its contribution to the spread of antibiotic resistance are therefore essential for One Health interventions. Therefore, the aim of this study was to investigate the level of bacterial contamination of commonly consumed vegetables and their antimicrobial resistance patterns.

Methods: A cross-sectional research was conducted in Debre Berhan town from February to August 2022. Questionnaires were used to collect data on sociodemographic variables, hygiene practices, and market hygiene. Six carefully selected vegetables (30 each, 180 in total) were purchased at a local market. Bacterial isolation and identification, multidrug-resistant (MDR) screening and confirmation, extended-spectrum β-lactamase (ESBL) screening and confirmation, and antibiotic susceptibility tests were performed using standard operating procedure. The data were analysed statistically using SPSS software version 25.

Results: The contamination rate of vegetables was 119 (66.1%). Of the 176 bacteria isolates, *E. coli* (26.1%; 46/176), *S. aureus* (18.8%; 176), *S. epidermidis* (10.8%; 19/176), *Klebsiella* spp. (9.1%; 16/179) and *Acinetobacter* spp. (6.8%; 12/176) were the most frequently detected isolates. Of the 180 samples tested, (66.1%; 119/180) were contaminated with at least one type of bacteria. Lettuce (22.7%; 40/176), spinach (18.6%; 33/176), and cabbage (19.2%; 32/176) were the most contaminated vegetables. Of the 176 bacteria isolates, (64.8%; 114/176) were MDR, and (18.5%; 23/124) isolates were ESBL producers. The kind of vegetables, vendor/seller finger-nail status, medium of display, market type, and not cleaned before to display were all significantly associated with bacterial contamination.

Conclusion: This study found that commonly consumed vegetables are contaminated with antibiotic-resistant bacteria. Vegetables were also notable for the incidence of multidrug-resistant, extended β-lactamase-resistant, and methicillin-resistant bacterial isolates. Therefore, we urge local health authorities to develop and implement effective control strategies to reduce vegetable contamination.

Keywords: foodborne bacteria, multidrug-resistant, extended β-lactamase-resistant, risk factors, vegetables

Introduction

In many countries, including Ethiopia, the frequency of food-borne illnesses related to vegetables is rising as well.[1–3] Contamination can occur in all vegetables sold in public markets, supermarkets, and even on the sides of the road, causing significant health problems for consumers.[2] In developing countries like Ethiopia, the burden is significantly higher due to poverty and a lack of public health awareness.

With an estimated 1.9 million fatalities per year worldwide,[4,5] food-borne diseases are a significant public health problem. Every year, nearly one-third of the population, even in developed countries, contracts a food-borne illness.[3] Due to poor food handling hygiene standards, this issue is particularly severe in resource limited countries.[6,7] Vegetable

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contamination and related diseases can be caused by parasites, viruses, and fungi, but most foodborne outbreaks are caused by bacteria.[8,9] Post-harvest handling,[10,11] preparation areas,[12] and consumer movement[13] result in a high risk of bacterial contamination. Nowadays, it is widely acknowledged that eating vegetables increases the risk of contracting intestinal infections.[14]

Because of the advent and fast spread of antibiotic resistance in humans, animals, and the environment, pathogenic bacteria that regularly contaminate vegetables are recognized as a worldwide health problem.[15] There are worries regarding the spread of antibiotic-resistant microorganisms from fresh vegetables to the general population. Nevertheless, research in low-income countries that evaluate foodborne pathogens and their antibiotic resistance patterns in humans, animals, food, and the environment are sparse.[4,5,16] There are no past data from Ethiopia on methicillin-resistant staphylococci, beta-lactamase-producing Enterobacteriaceae, or multidrug-resistant bacteria in regularly consumed vegetables. Furthermore, there is no market-level microbiological quality standard for vegetables commonly consumed in Ethiopia. Therefore, this study was designed to assess the hygiene practices of vegetable vendors, the hygiene conditions of local markets, the level of bacterial contamination and patterns of antimicrobial resistance in commonly consumed market vegetables in the town of Debre Berhan, Ethiopia.

Methods

Study Design and Area

A cross-sectional research was undertaken in the town of Debre Berhan, 130 kilometers northeast of Addis Ababa, from February to August 2022. Horticulture, agro-industrial processing, urban agriculture, and other service industries are the primary economic sectors of the town and adjacent settlements. Vegetables are widely accessible at the town's local market, and the majority are consumed raw. Most vegetables were purchased directly from farmers, dealers, or middlemen at local marketplaces.

Data and Sample Collection

Data on socio-demographic variables, hygiene practices, and market sanitary conditions were collected using a pretested structured questionnaire (Supplementary Table 1). In the local market, six varieties of vegetables were purchased: lettuce, cabbage, spinach, carrots, tomatoes, and green peppers. The selection of vegetables in this study was supported by data from observational studies of local markets and the municipality of the town of Debre Berhan. The samples were collected in the morning (8 AM) and afternoon (3 PM) once in every week. A total of 180 samples (30 of each) were collected. Fresh vegetables were bought, packaged in sterile stomacher bags, labelled, and brought to the Debre Berhan University Microbiology Laboratory in cold chain boxes at the time of collection.

Sample Preparation

The initial analysis of the sample was started within 24 hours after collection. 200 g of each vegetable sample was measured using an electronic balance (Model: electronic balance, HC.c, 4002, China) then minced and washed for 20 minutes in a beaker containing 500 mL of peptone-buffered water (3M company, St. Paul, MN 55144–1000, USA), followed by 5 minutes of agitation on a shaker for appropriate washing. The washed-water was used for laboratory analysis.[2]

Isolation and Identification of Bacteria

From each serial dilution a volume of 0.1 mL aliquot was aseptically taken and inoculated onto solidified MacConkey Agar (Oxoid Ltd., Basingstoke and Hampshire, UK), and Mannitol Salt Agar (Oxoid Ltd., Basingstoke and Hampshire, UK) using the pour plate technique.[2]

After obtaining pure colonies and recording key characteristics, Gram-negative bacteria (*Enterobacteriaceae*) were identified based on colonial morphology and pigmentation, oxidase test, carbohydrate fermentation, H_2S production, citrate utilization, motility, indole formation, lysine decarboxylase and lysine deaminizes production, and urea hydrolysis.

Gram-positive isolates (*Staphylococci*) were also differentiated by colonial characteristics, catalase test coagulase tests, and novobiocin susceptibility test.

Antimicrobial Susceptibility Testing

The CLSI-recommended Kirby-Bauer disk diffusion technique was performed to evaluate the antibiotic susceptibility profiles of the isolates. The Clinical Laboratory Standard Institute (CLSI) susceptibility breakpoints were used for interpretation.[17] All antibiotics were obtained from Oxoid Ltd, UK. The antibiotics were selected because they are widely prescribed in Ethiopia (Supplementary Table 2).

Screaming of Methicillin-Resistant *Staphylococcus* Species

All isolates of *S. aureus* and *S. epidermidis* were screened for methicillin-resistance using cefoxitin disc (30 µg) using Kirby-Bauer disk diffusion technique. Isolates with a zone of inhibition ≤21mm around the cefoxitin disc were identified as methicillin-resistant isolates.[17]

Screening of MDR Isolates

MDR isolates were those that were resistant to one or more drugs from three or more antimicrobial classes.[18]

Screening and Confirmation of ESBLs Producing Bacteria

Each *Enterobacteriaceae* isolate with reduced susceptibility for cefotaxime and/or ceftazidime was included as a potential ESBL producer. Isolates with an inhibition zone size of ≤22 mm for ceftazidime (30 µg) and/or ≤27 mm for cefotaxime (30 µg) were considered potential ESBL producers.[17] Discs of ceftazidime (30 µg) and cefotaxime (30 µg) alone and in combination with clavulanic acid (30 µg/10 µg) were placed at 25 mm distance on Mueller-Hinton agar plates with bacterial suspension prepared in accordance with 0.5 McFarland turbidity standards and incubate overnight (18–24 h) at 37°C. Bacterial isolates were identified as ESBL producers that showed an increased zone of inhibition diameter ≥5 mm in combination discs compared to ceftazidime or cefotaxime.[17]

Quality Assurance

Standard operating procedures were used to conduct laboratory analyses. Reagents were tested and handled in accordance with established protocols before use. Quality control strains such as *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were utilized as quality control organisms throughout the antimicrobial susceptibility testing. ESBLs positive *K. pneumoniae* (ATCC 700603) and ESBLs negative *E. coli* (ATCC 25922) control strains were also utilized for the ESBL confirmatory test. Each quality control strain was obtained from Ethiopian Public Health Institute (EPHI).

Statistical Analysis

SPSS Statistics for Windows, Version 25.0 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp) was used to summarized and analyzed data. Statistical tables were used to display quantitative values. The socio-demographic and sanitary risk variables for bacterial contamination of vegetables were assessed using a bivariate logistic regression analysis. The factors with a P-value of 0.25 were further investigated using multivariable logistic regression. Adjusted odds ratio (AOR), 95% CI and P-value ≤.05 was used to identify the significant association.

Results

Socio-Demographic and Hygienic Practice of Vegetables Vendors

From a total of 180 vegetable vendors, (35.6%; 64/180) were male and (64.4%; 116/180) were female. Most of the vendors (28.8%; 52/180) had college level education. Most vendors' (62.8%; 113/180) finger nails were trimmed. The majority of the vendors sourced their produces from middlemen (35.0%; 63/180) and farmers (33.3%; 60/180). Most vendors wash their items in tap water and wash them before displaying them. The majority of the vegetable samples (60%; 108/180) were obtained from supermarket (Table 1).

Table 1 Socio-Demographic Characteristics and Hygienic Practice of Vegetables Vendor, Debre Berhan Town, Ethiopia, 2022

Variables		**Frequency**	**Percent**
Sex	Male	64	35.6
	Female	116	64.4
Educational Level of Vendors	Unable to read and write	39	21.7
	Primary education	44	24.4
	Secondary education	45	25.0
	College and above	52	28.8
Vendors Figure Nail Status	Trimmed	113	62.8
	Untrimmed	67	37.2
Kinds of Produce	Lettuce	30	16.7
	Cabbage	30	16.7
	Spinach	30	16.7
	Carrot	30	16.7
	Tomato	30	16.7
	Green pepper	30	16.7
Source of Produce	Farmers	60	33.3
	Middle men	63	35.0
	Merchant	57	31.7
Means of Transportations	By human	47	26.1
	By cart	56	31.1
	By car	77	42.8
Market Type	Supermarket	108	60.0
	Open market	72	40.0
Washed before Display	Yes	126	70.0
	No	54	30.0
Water Source for Washing	Pipe water	123	97.6
	Well water	1	0.8
	River water	2	1.6
Means of Display	On the floor	64	35.6
	In bucket	36	20.0
	On the shelf	80	44.4
Sampling Time	Morning	90	50.0
	Afternoon	90	50.0

Bacterial Contamination of Vegetables

The contamination rate of vegetables was (66.1%; 119/180) (95% CI: 2.136–5.245, P = 0.029). Of the 176 bacteria isolates, (29.5%; 52/176) were Gram-positive and (70.5%; 124/176) were Gram-negative. *Escherichia coli* (26.1%; 46/176), *S. aureus* (18.8%; 33/176), *S. epidermidis* (10.8%; 19/176), *Klebsiella* spp. (9.1%; 16/176), *Acinetobacter* spp. (6.8%; 12/176), *Enterobacter* spp. (6.25%; 11/176), *Shigella* spp. (6.25%; 11/176), and *Salmonella* spp. (4.6%; 8/176) were the most frequently detected isolates [Table 2].

Distribution of Bacterial Contamination in Different Vegetables

The most contaminated vegetables were lettuce (22.7%; 40/176), spinach (18.6%; 33/176), and cabbage (19.2%; 32/176). Among Gram-positive bacteria isolates, *S. aureus* was the highest contamination of spinach with (21.2%; 7/33), cabbage with (18.2%; 6/33) and green paper with (18.2%; 6/33). From enteric bacteria isolates, *E. coli* was found to be the most contaminant for lettuce (21.7%; 10/46) and cabbage (21.7%; 10/46). *Klebsiella* spp were also isolated mostly in spinach (31.3%; 5/16) and lettuce (25.0%; 4/16). *Shigella* spp. was isolated from lettuce (27.3%; 3/11) and green pepper (27.3%; 3/11) (Table 3).

Antimicrobial Resistance of Bacterial Isolates

The highest level of resistance was observed with amoxicillin (81.1%; 137/169), ampicillin (79.3%; 134/169) and penicillin (73.1%; 38/52). Gram-positive bacteria showed the highest resistance to penicillin at (73.1%; 38/52) and methicillin at (38.4%; 20/52) (Table 4).

Relatively high rates of resistance to Ethiopia's most commonly prescribed antibiotics were observed in all isolates. For example, an alarming resistance rate was observed for erythromycin (50.3%; 85/169), cotrimoxazole (45.0%; 76/169), doxycycline (40.8%; 69/169), ceftriaxone (40.2%; 68/169), gentamycin (39.8%; 70/176) and chloramphenicol

Table 2 Frequency of Bacterial Contamination in Commonly Consumed Vegetables Sold in Debre Berhan Town, Ethiopia, 2022

Isolates	Frequency	Percent
S. aureus	33	18.8
S. epidermidis	19	10.8
E. coli	46	26.1
Klebsiella Spp.	16	9.1
Enterobacter Spp.	11	6.25
Shigella Spp.	11	6.25
Salmonella Spp.	8	4.6
Citrobacter Spp.	4	2.3
P. aeruginosa	7	4
Acinetobacter Spp.	12	6.8
Serratia Spp.	7	4
Proteus Spp	2	1.1
Total	176	100

Table 3 Distribution of Bacterial Contamination Among Commonly Consumed Vegetables Sold in Debre Berhan Town, Ethiopia, 2022. The Values are Provided as Numbers (n) and Percentage (%), n (%)

Isolates (n)	Lettuce	Cabbage	Spinach	Carrot	Tomato	Green Pepper
S. aureus (33)	5 (15.2)	6 (18.2)	7 (21.2)	6 (18.2)	3 (9.1)	6 (18.2)
S. epidermidis (19)	4 (21.1)	3 (15.8)	2 (10.5)	3 (15.8)	3 (15.8)	4 (21.1)
E. coli (46)	10 (21.7)	10 (21.7)	9 (19.6)	5 (10.9)	8 (17.4)	4 (8.7)
Klebsiella spp (16)	4 (25.0)	2 (12.5)	5 (31.3)	1 (6.2)	2 (12.5)	2 (12.5)
Enterobacter spp. (11)	3 (27.3)	2 (18.2)	2 (18.2)	2 (18.2)	0 (0.0)	2 (18.2)
Shigella spp. (11)	3 (27.3)	2 (18.2)	2 (18.2)	1 (9.1)	0 (0.0)	3 (27.3)
Salmonella spp. (8)	3 (37.5)	2 (25.0)	1 (12.5)	0 (0.0)	1 (12.5)	1 (12.5)
Citrobacter spp. (4)	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
P. aeruginosa (7)	3 (42.9)	1 (14.3)	1 (14.3)	0 (0.0)	1 (14.3)	1 (14.3)
Acinetobacter spp. (12)	2 (16.7)	3 (25.0)	3 (25.0)	2 (16.7)	1 (8.3)	1 (8.3)
Serratia Spp. (7)	0 (0.0)	1 (14.3)	1 (14.3)	0 (0.0)	2 (28.6)	3 (42.9)
Proteus Spp (2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)
Total (176)	40 (22.7)	32 (18.2)	33 (18.8)	21 (11.9)	21 (11.9)	29 (16.5)

(39.6%; 67/169). However, lower resistance was observed for recently introduced antibiotics to Ethiopia like meropenem (13.7%; 17/176), imipenem (17.7%; 22/176), ceftazidime (21.0%; 37/176), and cefotaxime (24.4%; 43/176) (Table 4).

Of the bacteria isolates, (15.3%; 27/169) were resistant to eight or more antibiotics, while (7.9%; 14/176) isolates were not resistant to any of the antibiotics tested. There were also resistant bacteria for one (11.4%; 20/176), two (10.8%; 19/176), three (11.4%; 20/176), four (13.6%; 24/176), five (13.1%; 23/176), six (8.0%; 14/169) and seven (8.5%; 15/169) antibiotics (Table 5).

Prevalence of MDR and ESBL Confirmed Isolates

Of the 176 bacterial isolates, 64.8% (114/176) were multidrug resistant (MDR), (43.5%; 54/124) were screened for ESBL production and 18.5% (23/124) isolates were confirmed to be ESBL producers. Among the isolates, *Enterobacter* spp. and *Citrobacter* spp. were 100% MDR followed by *Klebsiella* spp. (75.0%; 12/16), *S. aureus* (69.7%; 23/33), *S. epidermidis* (68.4%; 13/19) and *E. coli* (63.0%; 29/46). *Citrobacter* spp. (50.0%; 2/4), *Acinetobacter* spp. (25.0%; 3/12), *Klebsiella* spp. (25.0%; 4/16), *Salmonella* spp. (25.0%; 2/8), *Enterobacter* spp. (18.2%; 2/11) and *Shigella* spp. (18.2%; 2/11) were among the isolates confirmed to be ESBL producer (Table 6).

Factors Associated with Bacterial Contamination of Vegetables

Vegetables sold by vendors who did not cut their nails were most likely to be contaminated (AOR = 1345; 95% CI: 0.171–0.852, p = 0.019) compared to the vendor's with trimmed nails. In addition, leafy vegetables (lettuce, cabbage, and spinach) had a higher risk of contamination than non-leaf vegetables (carrots, tomatoes and green peppers) (AOR = 1459; 95% CI: 0.164–0.527, p = 0.001). Vegetables not washed before display were more likely to be contaminated than vegetables washed before display (AOR = 1.724; 95% CI: 1.629–3558, p = 0.003). In addition, vegetables sold in the market on the floor were more likely to be contaminated than vegetables displayed on the table/shelf (AOR=3.001; 95% CI: 1.641–6.982, p = 0.012) [Table 7].

Table 4 Antibiotics Resistance Patterns of Bacterial Isolates in Selected Fruit and Vegetables Sold in Selected Market at Debre Berhan Town, Ethiopia, 2022. The Values are Provided as Numbers (n) and Percentage (%), n (%)

Isolates	AMC (30μg)	AMP (30μg)	PEN (30μg)	SXT (30μg)	CIP (5μg)	CAF (30μg)	GEN (10μg)	ERY (15μg)	TET (30μg)	DXT (30μg)	FOX (30μg)	CRO (30μg)	IMI (10μg)	MRP (10μg)	CTX (30μg)	CAZ (30μg)
S. aureus (33)	26 (78.8)	24 (72.7)	25 (75.8)	10 (30.3)	7(21.2)	12 (36.4)	7 (21.2)	11 (33.3)	6 (18.2)	6 (19.2)	12 (36.4)	11 (33.3)	NT	NT	6 (19.2)	6 (19.2)
S. epidermidis (19)	16 (84.2)	15 (78.9)	13 (68.4)	9 (47.4)	5(26.3)	8 (42.1)	5 (26.3)	10(52.6)	5 (26.3)	6 (31.6)	8 (42.1)	6 (31.6)	NT	NT	5 (26.3)	4 (21.1)
E. coli (46)	31 (67.4)	34 (73.9)	NT	14 (30.4)	11 (23.9)	12 (26.1)	13 (28.3)	20 (43.5)	11 (23.9)	13 (28.3)	10 (21.7)	14 (30.4)	6 (13.0)	5 (10.9)	8 (17.4)	7 (15.2)
Klebsiella spp (16)	16 (100.0)	15 (93.8)	NT	8 (50.0)	6 (37.5)	9 (56.3)	8 (50.0)	9 (56.3)	9 (56.3)	9 (56.3)	8 (50.0)	9 (56.3)	3 (18.8)	3 (18.8)	7 (43.8)	6 (37.5)
Enterobacter spp. (11)	11 (100)	11 (100.0)	NT	7 (63.6)	5 (45.5)	5 (45.5)	6 (54.5)	7 (63.6)	6 (54.5)	6 (54.5)	5 (45.5)	6 (54.5)	3 (27.3)	2 (18.2)	4 (36.4)	4 (36.4)
Shigella spp. (11)	9 (81.8)	8 (72.7)	NT	6 (54.5)	5 (45.5)	5 (45.5)	6 (54.5)	7 (63.6)	6 (54.5)	7 (63.6)	5 (45.5)	5 (45.5)	2 (18.2)	1 (9.1)	4 (36.4)	2 (18.2)
Salmonella spp. (8)	7 (87.5)	7 (87.5)	NT	7 (87.5)	4 (50.0)	5 (62.5)	5 (62.5)	6 (75.0)	5 (62.5)	5 (62.5)	4 (50.0)	4 (50.0)	2 (25.0)	0 (0.0)	2 (25.0)	1 (12.5)
Citrobacter spp. (4)	4 (100.0)	4 (100.0)	NT	4 (100.0)	3 (75.0)	3 (75.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	3 (75.0)	3 (75.0)	2 (50.0)	2 (50.0)	2(50.0)	2 (50.0)
P. aeruginosa (7)	NT	NT	NT	NT	3 (75.0)	NT	4 (57.1)	NT	NT	NT	NT	NT	1 (14.3)	1 (14.3)	1 (14.3)	1 (14.3)
Acinetobacter spp. (12)	10 (83.3)	10 (83.3)	NT	7 (58.3)	5 (41.7)	5 (41.7)	6 (50.0)	7 (58.3)	8 (66.7)	8 (66.7)	6 (50.0)	6 (50.0)	3 (25.0)	3 (25.0)	4 (33.3)	3 (25.0)
Serratia Spp. (7)	5 (71.4)	5 (71.4)	NT	4 (57.1)	3 (42.8)	3 (42.8)	5 (71.4)	4 (57.1)	5 (71.4)	5 (71.4)	2 (28.6)	4 (57.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)
Proteus Spp (2)	2 (100.0)	1 (50.0)	NT	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total (176)	137 (81.1)	134 (79.3)	38 (73.1)	76 (45.0)	57 (32.4)	67 (39.6)	70 (39.8)	85 (50.3)	65 (38.5)	69 (40.8)	63 (37.3)	68 (40.2)	22 (17.7)	17 (13.7)	43 (24.4)	37 (21.0)

Abbreviations: AMC, Amoxicillin; AMP, Ampicillin; PEN, Penicillin; SXT, Cotrimoxazole; CIP, Ciprofloxacin; CAF, Chloramphenicol; GEN, Gentamicin; ERY, Erythromycin; TET, Tetracycline; DXT, Doxycycline; FOX, Cefoxitin; CRO, Ceftriaxone; IMP, Imipenem; MRP, Meropenem; CTX, Cefotaxime; CAZ, Ceftazidime; NT, Not Tested.

Table 5 Multiple-Drug Resistance Patterns of Bacterial Isolates in Selected Vegetables Sold in Selected Market at Debre Berhan Town, Ethiopia, 2022. N (%)

Isolates	R0	R1	R2	R3	R4	R5	R6	R7	≥ R8
S. aureus (33)	4(12.1)	4(12.1)	2(6.1)	3(9.1)	3(9.1)	4(12.1)	4(12.1)	5(15.2)	4(12.1)
S. epidermidis (19)	1(5.2)	1 (5.2)	2(10.5)	3(15.8)	3(15.8)	3(15.8)	2(10.5)	2(10.5)	2(10.5)
E. coli (46)	3(6.5)	7 (15.2)	5(10.9)	4(8.7)	11(23.9)	6(13.0)	3(6.5)	2(4.3)	5(10.9)
Klebsiella spp (16)	0(0.0)	0(0.0)	2(12.5)	2(12.5)	2(12.5)	2(12.5)	0(0.0)	2(12.5)	6(37.5)
Enterobacter spp. (11)	0(0.0)	0(0.0)	0(0.0)	1(9.1)	1(9.1)	3(27.3)	1(9.1)	1(9.1)	4(36.4)
Shigella spp. (11)	1(9.1)	1(9.1)	2(18.2)	1(9.1)	1(9.1)	2(18.2)	1(9.1)	1(9.1)	1(9.1)
Salmonella spp. (8)	1(12.5)	1(12.5)	2(25.0)	1(12.5)	0(0.0)	0(0.0)	1(12.5)	1(12.5)	1(12.5)
Citrobacter spp. (4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	1(25.0)	1(25.0)	0(0.0)
P. aeruginosa (7)	1(14.3)	3(42.9)	1(14.3)	2(28.6)	0(0.0)	0(0.0)	NT	NT	NT
Acinetobacter spp. (12)	1(8.3)	2(16.7)	1(8.3)	1(8.3)	2(16.7)	0(0.0)	1(8.3)	0(0.0)	4(33.3)
Serratia Spp. (7)	2(28.6)	1(14.3)	1(14.3)	1(28.6)	0(0.0)	2(28.6)	0(0.0)	0(0.0)	0(0.0)
Proteus Spp (2)	0(0.0)	0(0.0)	1(50.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total (176)	14 (7.9)	20(11.4)	19(10.8)	20(11.4)	24(13.6)	23(13.1)	14(8.3)	15(8.9)	27(15.9)

Abbreviations: R0, Not resistant to any antibiotics; R1, Resistant to one antibiotic; R2, Resistant to two antibiotics; R3, Resistant to three antibiotics; R4, Resistant to four antibiotics; R5, Resistant to five antibiotics; R6, Resistant to six or more antibiotics; R7, Resistant to seven antibiotics; ≥ R8, Resistant to eight and more antibiotics; NT, Not Tested.

Table 6 Distribution of MDR and ESBL Confirmed Bacterial Contamination in Vegetables Sold at Debre Berhan Town, Ethiopia, 2022

Isolates	MDR	Screened for ESBL	ESBL Confirmed
S. aureus (33)	23(69.7)	Not Tested	Not Tested
S. epidermidis (19)	13(68.4)	Not Tested	Not Tested
E. coli (46)	29(63.0)	14(30.4)	7(15.2)
Klebsiella spp (16)	12(75.0)	12(75.0)	4(25.0)
Enterobacter spp. (11)	11(100.0)	8(72.7)	2(18.2)
Shigella spp. (11)	6(54.5)	6(54.5)	2(18.2)
Salmonella spp. (8)	4(50.0)	4(50.0)	2(25.0)
Citrobacter spp. (4)	4(100.0)	2(50.0)	2(50.0)
P. aeruginosa (7)	2(28.6)	3(42.9)	1(14.3)
Acinetobacter spp. (12)	7(58.3)	5(41.6)	3(25.0)
Serratia Spp. (7)	3(42.9)	0(0.0)	0(0.0)
Proteus Spp (2)	0(0.0)	0(0.0)	0(0.0)
Total (176)	114(64.8)	54(43.5)	23(18.5)

Table 7 Factors Associated with Bacterial Contamination in Vegetables Sold at Debre Berhan Town, Ethiopia, 2022

Variables		Frequency (N (%))	Positive Result (N (%))	COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Vendors Figure Nail Status							
Trimmed		113(62.8)	64(56.6)	1			
Untrimmed		67(37.2)	55(82.1)	0.28 (0.138–0.590)	0.001*	1.345 (0.171–0.852)	0.019*
Kinds of Produce							
Leafy	Lettuce	30 (16.7)	23(76.7)	1.022 (1.420–1.982)	0.007*	1.459 (0.164–0.527)	0.001*
	Cabbage	30 (16.7)	23(76.7)				
	Spinach	30 (16.7)	21(67.7)				
Non-leafy	Carrot	30 (16.7)	15(51.7)	1			
	Tomato	30 (16.7)	18(58.1)				
	Green P.	30 (16.7)	19(65.5)				
Market Type							
Supermarket		108 (60.0)	63(58.3)	1			
Open market		72(40.0)	56(77.8)	0.432 (0.204–0.785)	0.008*	1.209 (1.344–3.958)	0.004*
Washed before Display							
Yes		126 (70.0)	76(60.3)	1			
No		54(30.0)	43(79.6)	1.552 (0.183–0.825)	0.014*	1.724 (1.629–3.558)	0.003*
Means of Display							
On the floor		64(35.6)	24(66.7)	1.233 (0.978–2.274)	0.053*	3.001 (1.641–6.982)	0.012*
In bucket		36(20.0)	51(79.7)	0.784 (0.334–1.194)	0.884		
On the shelf		80(44.4)	44(55.0)	1			

Note: *Significantly associated value.
Abbreviations: N, Number; %: Percent; COR, Crude Odds Ratio; CI, Confidence Interval; AOR, Adjusted Odds Ratio.

Discussion

Despite the potential benefits, questions regarding the safety and quality of vegetables have increased. Fresh vegetable associated outbreaks have brought to light gaps in our knowledge of the ecology of pathogenic bacteria outside of human and animal hosts.[19] The incidence of antibiotic-resistant bacterial contamination in a range of fresh vegetables accessible in Ethiopian urban marketplaces has not before been studied.

In this study, the vegetable contamination rate was (66.1%; 119/180) (95% CI: 2.136–5.245, P = 0.029). Among bacterial isolates, (29.5%; 52/176) were Gram-positive and (70.5%; 124/176) were Gram-negative. It could be due to a different reason. For example, in this study area, markets are congested with dust-emitting vehicles, vegetables are transported long distances, unhygienically harvested, and stored openly in locations exposed to multiple contaminants, which can be a source of various bacterial contamination.

According to the findings of this study, fresh vegetables widely purchased in Ethiopian marketplaces are a source of potentially harmful bacteria, posing a public health risk. For example, *S. aureus* (18.8%; 33/176) and *S. epidermidis* (10.8%; 19/176) are among the Gram-positive bacteria commonly isolated from vegetables. The same study performed in Arba Minch, Ethiopia,[2] Nigeria,[20] Sudan,[21] Bangladesh,[22] and India[23] indicated that *S. aureus* is the predominant isolate in commercially available vegetables. This might be due to cross-contamination from the vegetable handlers' hands, as staphylococci can survive on the hand and surface for a long time after initial contact.[24]

In this study, enteric bacteria including *E. coli* (26.1%; 46/176), *Klebsiella* spp. (9.1%; 16/176), *Acinetobacter* spp. (6.8%; 12/176), *Enterobacter* spp. (6.25%; 11/176), *Shigella* spp. (6.25%; 11/176), and *Salmonella* spp. (4.6%; 8/176) were isolated. Although the scope of the studies varied, other studies have also reported different types of enteric bacteria such as *E. coli* and *Salmonella* spp. from fresh vegetables.[2,20–23] All of these differences might be attributed to the number of bacteria in the environment, the culture technique utilized, and the period of data collection. In general, the prevalence of enteric bacteria might be attributed to coliform bacteria, which are generally discharged with faeces and are obviously prevalent in environments where open defecation is widespread. Moreover, farmers sometimes utilize human and animal manure as a natural fertilizer, which contributes to the contamination of vegetables cultivated on these farms.[25,26]

In this study, Gram-positive bacteria had the highest rate of methicillin resistance (38.4%; 20/52). Methicillin resistance varied between species, with S. aureus resistance (36.4%; 12/33) and S. epidermidis resistance (42.1%; 8/19). The existence of methicillin-resistant *Staphylococcus* spp. in vegetables has yet to be documented in Ethiopia, but it has been reported in other countries, including China.[27] Methicillin-resistant *Staphylococcus* spp. is now recognized as the largest cause of nosocomial infections across the world and has been identified as an emerging pathogen outside of the healthcare context.[28] In general, this finding revealed the presence of *Staphylococcus* spp. in Ethiopian vegetables bearing non-intrinsic resistance determinants, such as methicillin resistance in fresh vegetables.[29] These types of vegetables analysed in this study are always eaten raw, suggesting that consumers may be at risk of *Staphylococcus* infection.

In this study, from the 176 isolates, (64.8%; 114/176) were multidrug-resistant pathogens (MDR). There is worry about the possible spread of antibiotic-resistant microorganisms from crops to humans. Nevertheless, few researches have been conducted to date on the growth of antibiotic-resistant bacteria in fresh vegetables sold in supermarkets.[26,30–33] Both *Staphylococcus* spp. and *Enterobacteriaceae* were shown to have a significant frequency of MDR in this study. Many additional investigations have found a reasonably high frequency of MDR in *Enterobacteriaceae* such as *Salmonella* spp. and *E. coli*. According to a research conducted on a big market in Chittagong, Bangladesh, all *Salmonella* and *E. coli* isolates from green lettuce were MDR.[34] Another study conducted in the same city of Chittagong found that 48.2% of the isolates were MDR.[35] In a study of raw salads served in hotels and restaurants in Bhaktapur, Nepal,[36] 9 (13.6%) MDR *Salmonella* isolates and 4 (13.8%) MDR *E. coli* isolates were found. These studies revealed that vegetables are potential conduits, although rare, for a wide range of beta-lactamase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Bacterial-strain resistance rates varied widely between studies. This might be due to varied geographical locations, variable characteristics of fresh vegetable samples, and different antibiotic selections and breakpoint.

In this study, the prevalence of ESBL-producing *Enterobacteriaceae* was (18.5%; 23/124), suggesting that raw vegetables might be a source of resistance genes. These findings are consistent with recent research pointing to vegetables as potential channels for resistance gene dissemination in the population.[31,37,38] Another study[36] demonstrated that ESBLs produced *Salmonella* 5 (7.57%) and *E. coli* 4 (13.8%) from raw salad supplied to restaurants. Raphael et al[39] and Bezanson et al[40] discovered 2.3% ESBL producers among bacterial strains from spinach and 1.9% among lettuce, respectively. The percentage of ESBL producers in all three investigations was lower than the current study. Beta-lactam antibiotic overuse and anarchic usage may have contributed to the emergence of ESBL producers.

Vegetables with leafy surfaces were more susceptible to contamination than those with non-leafy surfaces. This could be because of how the vegetables are; whereas non-leafy vegetables are less likely to harbour contaminating bacteria, leafy vegetables appear to sustain a lot of bacteria.[41] For example, in this study, from bacterial isolates, (22.7%; 40/176), (18.8%; 33/176), and (18.2%; 32/176) were found in lettuce, spinach and cabbage, respectively. Although certain bacteria preferentially attach to cut edges and can enter leaf tissue, bacterial populations may change due to chemical variations between different vegetable kinds.[42] Pathogens that internalize are known to be more resistant to chemical and physical washing.[43] Compared to individuals who had trimmed nails, vegetables purchased from persons who had untrimmed nails were more likely to be contaminated. This is clear evidence of the risk of bacterial spread through cross-contamination by food handlers. The likelihood of contamination is higher for vegetables that were not washed before presentation in the market than for ones that were. Also, vegetables on the floor in an open market are more likely to be contaminated than those on a table or shelf. As a result, using appropriate vegetable hygiene practices will significantly lower the chance of bacterial spread from food handlers and floors.

Limitation of the Study

E. coli strains that cause diarrhoea, *S. aureus* strains that cause enteritis and the species of most enteric bacteria were not identified. Due to a shortage of lab space, it was not possible to do a molecular analysis of the isolates' genes for virulence and antibiotic resistance. The molecular characterisation of the MDR and ESBL-positive isolates as well as the clonal relatedness of the isolates was not done. The common combinations of resistant antibiotics were not computed. Factors associated with MDR bacterial contamination of vegetables have not been reported.

Conclusion and Recommendations

The results of this study showed that a significant proportion of vegetables were contaminated with foodborne bacteria. The isolated bacteria also showed high levels of resistance to commonly prescribed antibiotics in Ethiopia. In addition, a significant number of bacterial strains have shown resistance to multiple drug and extended-spectrum-β-lactamase. The findings also pointed to possible risk factors for vegetable contamination throughout marketing and indicate generally inadequate handling and vegetable safety procedures used by vegetable vendors. In order to assure acceptable quality and safety in the production, transportation, storage, and sale of fresh vegetables, vendors and distributors should reduce the possibility of antibiotic-resistant bacteria contaminating the produce. Therefore, it is necessary to guarantee that regulated parties are overseeing vegetable safety and to encourage safe vegetable handling and manufacturing across the whole vegetable production chain.

Ethical Approval and Consent to Participate

The Institutional Review Board at Debre Berhan University provided ethical approval [protocol number: IRB-003], and the head department of Debre Berhan town's North Shoa Zonal Office provided formal authorization. All participants were made aware of the study's goal. Lastly, each vegetable handler provided written informed consent.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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