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Presence of carbapenem resistance in hybrid *Escherichia coli* pathovars from ready-to-eat fresh-cut fruits in Accra, Ghana

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Abstract

Aim: This study reports the presence of carbapenem-resistant *Escherichia coli* hybrid pathovars and its prevalence in 200 fresh-cut fruits from Accra.

Methods and results: Standard culture methods were used to quantify microbial indicators and *E. coli* on fresh-cut fruits retailed in formal and informal outlets in Accra. The Kirby–Bauer disc diffusion method was used to determine the antibiotic resistance profile of *E. coli*, while multiplex PCR was employed to identify the virulence and carbapenem-resistance genes. *Escherichia coli* prevalence in cut fruits was 17%, with pawpaw, watermelon, and mixed fruit having higher prevalence than pineapple. Of the 34 *E. coli* isolates from fresh-cut fruits, 44% showed broad resistance to beta-lactam antibiotics, while 5.9% showed carbapenem resistance. The study identified virulence genes associated with all *E. coli* isolates, including *stx1*, *stx2*, *escV*, and *ipaH*, of which 97% were hybrid pathovars bearing genes for Shiga toxin-producing *E. coli*/enteropathogenic *E. coli*/enteroinvasive *E. coli*. The carbapenemase gene, bla_{IMP}, was associated with both carbapenem-resistant *E. coli* phenotypes identified.

Conclusion: Despite a low-carbapenem-resistance prevalence observed among *E. coli* isolates, hypervirulent hybrid strains of *E. coli* is present in fresh-cut fruits in the sampling area, posing a potential public health risk to fresh-cut fruit consumers.

Impact Statement

Consuming fresh-cut fruits contaminated with carbapenem-resistant *Escherichia coli* pathovars can lead to a serious and often untreatable infections. Our findings call for increased monitoring of antibiotic-resistance profiles and the presence of *E. coli* pathovars as adulterants in fresh-cut fruits.

Keywords: *E. coli*; fresh-cut fruits; carbapenem resistance; shiga toxin; hybrid pathovars

Introduction

The World Health Organization recently published an updated list of the bacteria priority pathogens of public health importance (WHO 2024). In that list, carbapenem-resistant Enterobacterales are in the critical group of priority pathogens. The critical group refers to antibiotic-resistant bacterial pathogens that present the gravest public health threat because they have the highest disease burden, limited treatment options, and few or no alternative antibiotic candidates in the pipeline. Carbapenem-resistant *Escherichia coli*, which was ranked number 10 in the 2017 Bacteria Priority Pathogen List, moved to rank 5 in the updated list, indicating an increased public health risk of carbapenem-resistant *E. coli* over the years (WHO 2017, WHO 2024).

Escherichia coli naturally colonizes the lower tract of warm-blooded animals, including humans (Jang et al. 2017). Most *E. coli* in the human intestines are harmless; however, some are pathogenic and can cause bloody and non-bloody diarrhoea due to the presence of virulent genetic elements (Gomes et al. 2016). Pathogenic *E. coli* have been classified into various distinct pathovars based on the variety of diseases they cause (Aijuka and Buys 2019). These pathovars include Enterohaemorrhagic *E. coli*, Uropathogenic *E. coli*, Shiga toxin *E. coli*, Enteroinvasive *E. coli*, Enterotoxigenic *E. coli*, Enteroaggregative *E. coli*, and Enteropathogenic *E. coli* (Aijuka and Buys 2019).

Escherichia coli is historically associated with animal-source food (Jakobsen et al. 2012), but they are becoming increasingly relevant for plant-based foods such as fresh-cut fruits (Benjamin et al. 2018). Fresh-cut fruits have been identified as a significant transmission pathway for pathogenic *E. coli* as they are normally consumed raw or minimally processed (Berger et al. 2010). Due to the numerous nutritional benefits of fruit consumption, it is often recommended to the general populace and particularly for vulnerable groups such as young, old, pregnant women, and immunocompromised individuals (YOPI) as part of a normal healthy diet (Laupland et al. 2008). While anyone can experience *E. coli* infections (Simonne 2023), the YOPI group is at greater risk of *E. coli* infections through food exposure.

Furthermore, the risk of antibiotic resistance associated with *E. coli* is a threat to human health. Globally, carbapenem-resistant enteropathogens are emerging in clinical infections and in animal-source foods, but little evidence exists about

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their prevalence in plant-based foods. Recently, reports by Majlander et al. (2021), Bai et al. (2023), and Oliveira et al. (2020) have indicated the co-occurrence of beta-lactam resistance genes associated with carbapenem-resistant Enterobacteriaceae, indicating the need for antibiotic surveillance in all food systems. Additionally, in Ghana, carbapenem-resistance detection in pathogens mostly features clinical isolates with a limited record of their incidence in food. Since carbapenem-resistance strains in food are an important risk factor for human health, it is necessary to determine their occurrence and their epidemiological significance. With ongoing work on free trade on the African continent, the harmonization of food standards by the African Organization for Standardization to champion free food trade and the epidemiological relevance of carbapenem-resistant *E. coli* pathovars and other hybrid *E. coli* pathovars in fruits consumed raw extend beyond the borders of Ghana. This study provides evidence of the microbiological quality of fresh-cut fruits and carbapenem-resistant *E. coli* pathovars vended in Accra, the major commerce hub in Ghana.

Materials and methods

Study area and study design

The study was conducted within 10 districts in the Greater Accra Region, namely Accra Metropolitan Assembly, Ayawaso West Municipal Assembly, Ga Central Municipal Assembly, Ga West Municipal Assembly, Ga East Municipal Assembly, La Dade Kotopon Municipal Assembly, La Nkwantanag Municipal Assembly, Okaikwe North Municipal Assembly, Tema Metropolitan Assembly, and Weija Gbawe Municipal Assembly. These areas were chosen randomly from the 29 districts in the Greater Accra Region for this study. A descriptive cross-sectional study design was employed in this research to determine the prevalence and carbapenem resistance of *E. coli* pathovars in the fresh-cut fruit samples.

Sampling technique and sample collection

A total of 200 fresh-cut fruits were sampled. Out of the total samples, 180 comprised watermelon ($n = 60$), pawpaw ($n = 60$), and pineapple ($n = 60$), and 20 mixed cut fruits from supermarkets and hawkers. The watermelon, pawpaw, and pineapple samples were purchased from fruit vendors (hawkers) within the 10 districts in Greater Accra using a multistage sampling technique. These fresh-cut fruits are often sold in the open at high ambient temperatures to customers in vehicles and pedestrians at heavy traffic zones by fresh-cut fruits hawkers. Watermelons are sliced without peeling and arranged on round trays covered with a transparent polyethylene film and carried on a head pan for selling. When a consumer decides to buy, it is then peeled, chopped, and packaged into transparent polyethene bags. However, pawpaw and pineapples are often peeled, sliced, and/or chopped and packaged in transparent polyethene bags and displayed on round trays for retail.

From each district, three communities with heavy vehicle traffic zones and marketing activities were selected by purposive sampling, and within each community, two fresh-cut fruit vendors selling all three of the fresh-cut fruits of interest (watermelon, pawpaw, and pineapple) were selected by purposive sampling. One sample of each of the three selected fresh-cut fruits, watermelon, pawpaw, and pineapple, was purchased from each vendor. In addition, 20 samples of mixed-fruit salad were sampled from one of the selected districts (Ayawaso West Municipality) since they were not available in the other nine selected districts. A total of 15 samples of the mixed-fruit salad were sampled from hawkers on the streets in heavy traffic zones and the remaining 5 samples were sampled from supermarkets. All the fresh-cut fruit samples were obtained between 6:00 a.m. and 10:00 a.m. and transported at 4°C to the Microbiology Research Laboratory for immediate microbial analysis.

Microbiological assessment and prevalence of *E. coli* on fresh-cut fruits

To determine the microbiological quality of fresh-cut fruits, the following enumeration tests were conducted on the samples: aerobic plate count with plate count agar (Oxoid), Enterobacteriaceae with violet red bile glucose agar, *E. coli* with eosin methylene blue (EMB) and Brilliance UTI agars (Oxoid), *Staphylococcus aureus* with mannitol salt agar (Oxoid), and yeast and moulds with dichloran Rose Bengal chloramphenicol (DRBC, Oxoid) agar. Enumeration tests were conducted by surface plating appropriate serial dilutions of fresh-cut fruit homogenates on prepared agar plates. Homogenates were prepared by adding 90 ml of phosphate buffered saline (PBS, Fisher Chemical) to 10 g of samples and blended with a stomacher (Seward Stomacher 400 Circulator). All inoculated plates were incubated at 37°C for 24–48 h except for EMB incubated at 44°C for 24–48 h and DRBC incubated at 25°C for 3–5 days.

Identification of virulence genes associated with the various *E. coli* isolates

Genomic DNA extraction and amplification of *E. coli* virulence genes

Escherichia coli isolates that passed biochemical tests were cultured in nutrient broth for 24 h. Fresh cultures were harvested by centrifugation, washed twice with PBS, and resuspended in 200 µl of PBS to achieve a concentration between 8 and 9 log CFU ml^{-1}. About 200 µl of the suspended *E. coli* cells was used for each extraction. Genomic DNA extraction was done using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research). The extraction was done according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) optimization was done for multiplex and single-plex PCR protocols as depicted in Table 1.

Table 2 shows the virulence genes that were assessed in this study. The amplification reaction was performed by multiplex PCR in a 25 µl reaction mixture consisting of 12.5 µl master mix (One Taq Quick-Load 2X Master Mix, New England Biolabs), multiplex primers (0.2 µmol l^{-1}) that were designed using the PrimerQuest tool (Abd-Elsalam 2003) and synthesized by inqaba biotech, 4 µl of DNA, and nuclease-free water. The amplifications were performed in the iCycler (Bio-Rad). The amplification program included an initial denaturation at 95°C for 3 min, 35 cycles of 1 min denaturation at 95°C, 45 s of annealing, and 1 min elongation at 72°C, followed by a final extension for 5 min at 72°C. The amplified products were held at 4°C. The amplification products were separated on 1.2% agarose gel with ethidium bromide, 0.5 mg ml^{-1},

Table 1. Primer groups and their optimum annealing temperatures.

Primer groups for multiplex PCR	Primers	T_m (°C)	T_a (°C)	Concentration (μM)
Multiplex A	*stx1* fwd	51		
	stx1 rev	52	48	0.2
	aggR fwd	52		
	aggR rev	50		
	astA fwd	51		
	astA rev	51		
Multiplex B	*elt* fwd	50		
	elt rev	54	47.5	0.2
	eae fwd	50		
	eae rev	54		
	ent fwd	51		
	ent rev	54		
	ipaH fwd	55		
	ipaH rev	50		
Multiplex C	*escV* fwd	53		
	escV rev	48	45.5	0.2
	invE fwd	49		
	invE rev	53		
	pic fwd	51		
	pic rev	49		
Single-plex D	*stx2* fwd	48		
	stx2 rev	47	43.5	0.2

T_m: melting temperature; T_a: annealing temperature.

Table 2. *Escherichia coli* pathovars and corresponding target virulence genes for identification.

Pathovar group	Target genes
ETEC	*est*, *elt*
STEC	*stx1*, *stx2*,
EHEC	*eae*, *stx1*, *stx2*
EAEC	*aggR*, *pic*
EIEC	*invE*, *ipaH*
EPEC	*ent*, *escV*

ETEC: enterotoxigenic *E. coli*, STEC: Shiga toxin-producing *E. coli*, EHEC: enterohaemorrhagic *E. coli*, EAEC: enteroaggregative *E. coli*, EPEC: enteropathogenic *E. coli*, EIEC: enteroinvasive *E. coli*.

and 100 bp molecular weight marker (New England Biolabs).

Phenotypic characterization of carbapenem-resistance *E. coli* isolates

Determination of antibiotic-resistance profile

The Kirby–Bauer disc diffusion method (1966) was employed for antibiotic-resistance profiling of *E. coli* isolates. All 34 *E. coli* isolates were assessed against extended spectrum beta-lactam inhibitor cefpodoxime (10 μg), combined cefpodoxime/clavulanic acid (10 μg/1 μg), and carbapenem (meropenem, 10 μg), according to the Clinical and Laboratory Standards Institute (CLSI 2020) guidelines. Overnight pure cultures of *E. coli* with turbidity adjusted to 0.5 McFarland standard were swabbed on pre-poured Mueller–Hinton agar (Oxoid) plates. The various antibiotic discs were placed on the agar plates and incubated at 37°C for 24 h. After the incubation period, inhibition zones were measured using the millimetre rule and the results were interpreted as Susceptible (S), Intermediate resistant (I), or Resistant (R) (CLSI 2020). *Escherichia coli* isolates were classified as ESBL positive if the difference in the inhibition zone diameter of cefpodoxime combined with clavulanic acid and the diameter of the inhibition zone for the cefpodoxime only disc was ≥5 mm (Dsani et al. 2020). The ESBL-positive isolates were expressed as frequencies of occurrence.

Genotypic characterization of carbapenem-resistance *E. coli* isolates

Detection of antimicrobial-resistance genes

The *E. coli* isolates that showed phenotypic resistance to carbapenem were selected for antimicrobial resistance gene analysis. Multiplex PCR was performed using four primer sets to detect antibiotic-resistance genes. The targeted antibiotic-resistance genes include carbapenemase genes (bla_{KPC}, bla_{OXA}, bla_{SPM}, bla_{IMP}) as depicted in Table 3. The set of primers was designed using the PrimerQuest tool (Abd-Elsalam 2003) and synthesized by inqaba biotech.

Reactions were performed in a total volume of 25 μl, including 12.5 μl One Taq Quick-Load Master Mix, and 4 μl of DNA sample. Multiplex primer sets were performed with 2 μmol l^{-1} of each primer pair. The amplification cycles were initial denaturation at 95°C for 3 min, 35 cycles of 1 min denaturation at 95°C, 45 s annealing at 51.1°C, and 1 min elongation at 72°C, followed by a final extension for 5 min at 72°C. The amplified products were separated and viewed as previously described.

Data analysis

One way Analysis of Variance (ANOVA) was used to determine differences between the means of microbial counts among cut fruit categories. Tukey's post hoc test was used to determine where the differences lie. For statistical significance, α was set at 0.05. Descriptive statistics were used to determine prevalence as percentages of *E. coli* isolates. Using the formula the number of *E. coli* isolates resistant to specific antibiotics/the total number of *E. coli* isolates obtained,

Table 3. Primer groups for carbapenemase producers and their optimum annealing temperatures.

Primers for multiplex PCR		T_m (°C)	T_a (°C)	Concentration (μM)
Multiplex A	bla_{IMP} fwd	50		
	bla_{IMP} rev	50	51.1	0.2
	bla_{KPC} fwd	54		
	bla_{KPC} rev	52		
	bla_{OXA-24} fwd	52		
	bla_{OXA-24} rev	51		
	bla_{SPM} fwd	51		
	bla_{SPM} rev	52		

T_m: melting temperature; T_a: annealing temperature.

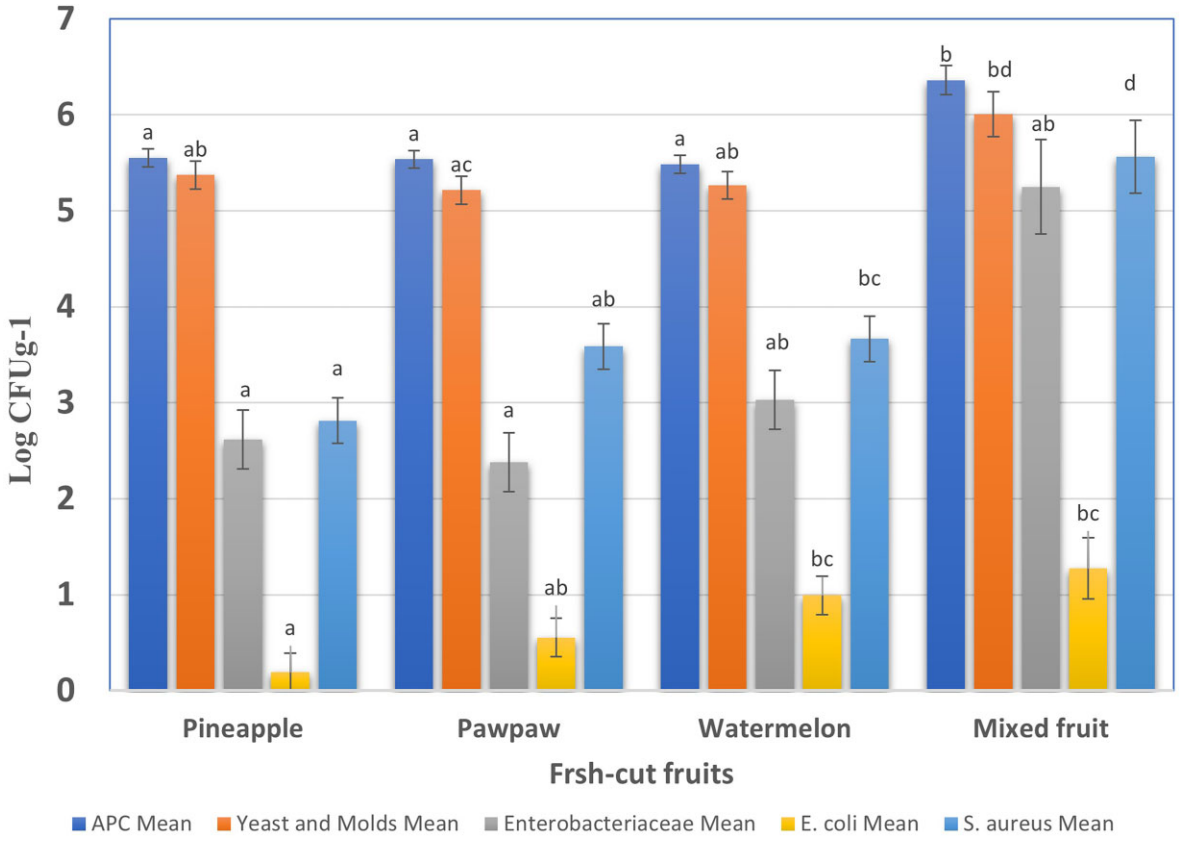

Figure 1. Microbiological quality and *E. coli* prevalence in fresh-cut fruits vended in Accra.

the prevalence of carbapenem-resistant *E. coli*, and ESBL-producing *E. coli* was determined and expressed as percentages.

Results

Microbial quality of fresh-cut fruits

Microbiological counts of quality and safety indicators are presented in Fig. 1. The aerobic bacteria, yeast and moulds, Enterobacteriaceae, and *S. aureus* counts on pineapple, pawpaw, and watermelon were similar but statistically different from the mixed fruits ($P = 0.04$). The average *S. aureus* count was above the statutory requirement of 2 log CFU g^{-1} for cut fruits (Ghana Standard GS955: 2019). *Escherichia coli* mean counts from the various fruits were within the acceptable microbiological limit (≥2.0, Ghana Standard GS955: 2019) as presented in Table 4. However, samples that recorded *E. coli* counts had counts >4 Log CFU g^{-1}.

Prevalence of *E. coli* associated with the various fresh-cut fruit samples

Fresh-cut pineapple samples recorded the least *E. coli* prevalence, while watermelon samples recorded the highest prevalence among the three different fresh-cut fruits (Table 4). Of the mixed-fruit salad, the samples obtained from hawkers recorded the highest prevalence (40%) of *E. coli* compared to those that were sampled from supermarkets (20%). The overall prevalence of *E. coli* was 17% (34/200).

Table 4. *Escherichia coli* counts from fresh-cut fruits obtained in Greater Accra, Ghana.

Samples	*Escherichia coli* counts (log CFU g^{-1}) Range	Mean ± SD	*Escherichia coli* prevalence (%)
Pineapple (H)	0.0–4.3	0.2 ± 0.9	6.7
Pawpaw (H)	0.0–5.3	0.6 ± 1.6	13.3
Watermelon (H)	0.0–5.4	0.9 ± 1.8	25.0
Mix fruits (H)	0.0–5.3	1.3 ± 2.1	40.0
Mix fruits (S)	0.0–4.7	0.8 ± 1.7	20.0
Standard (GS955: 2019)		≤2.0	

S: supermarkets; H: hawkers.

Table 5. Detection and distribution of *E. coli* virulence genes in *E. coli* isolated from fresh-cut fruits in Greater Accra.

			Fresh-cut fruits from which *E. coli* were isolated				
Escherichia coli pathovar	Virulence genes	Number of isolates (*N* = 34)	Pineapple (*N* = 60)	Pawpaw (*N* = 60)	Watermelon (*N* = 60)	Mixed-fruit (supermarket) (*N* = 5)	Mixed-fruit (hawkers) (*N* = 15)
STEC	*stx1*	33 (97%)	4 (6.7%)	10 (16.7%)	15 (25%)	1 (20%)	3 (20%)
STEC	*stx2*	34 (100%)	4 (6.7%)	10 (16.7%)	15 (25%)	1 (20%)	4 (26.7%)
EIEC	*ipaH*	32 (94%)	4 (6.7%)	10 (16.7%)	13 (21%)	1 (20%)	4 (26.7%)
EPEC	*escV*	11 (32%)	1 (1.7%)	6 (10%)	3 (5%)	1 (20%)	ND
EHEC	*eae*	ND	ND	ND	ND	ND	ND
ETEC	*elt*, *est*	ND	ND	ND	ND	ND	ND
EAEC	*aggR*, *pic*	ND	ND	ND	ND	ND	ND

ND: not detected.

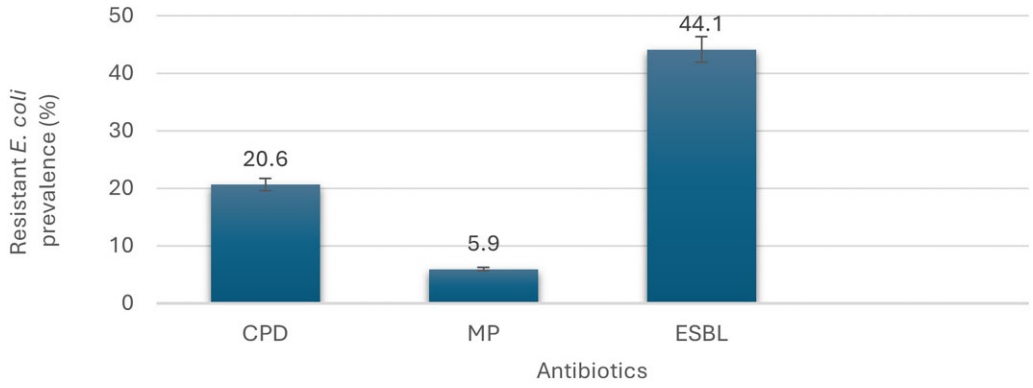

Figure 2. Prevalence of antibiotic-resistant *E. coli* phenotypes associated with fresh-cut fruits

Virulence genes associated with the various *E. coli* isolates

This study revealed that all the 34 *E. coli* isolates from the fresh-cut fruits had at least one virulent gene, indicating that all the *E. coli* isolates were pathogenic. Four virulent genes (Table 5) were found to be associated with the various *E. coli* isolates. The Shiga toxin 1 (*stx1*) gene was detected in 33 *E. coli* isolates, while the Shiga toxin 2 (*stx2*) gene was detected in all the *E. coli* isolates. In addition, the invasion plasmid antigen H (*IpaH*) gene was also detected in 32 of the *E. coli* isolates and the export apparatus of the type III secretion system gene (*escV*) was detected in 11 of the *E. coli* isolates. The remaining virulent genes (*est*, *elt*, *ent*, *eaeA*, *invE*, *pic*, *aggR*) were not detected in any *E. coli* isolates.

Our study detected hybrid pathovars among the isolates with at least two genes from different pathovar groups (Table 6). Among the *E. coli* isolates, 11 isolates were found to harbour the *stx1*, *stx2*, *escV*, and *ipaH* genes associated with STEC, EPEC, and EIEC pathovar groups respectively, while 20 isolates were found to harbour the *stx1*, *stx2*, and *ipaH* genes. Also, one isolate contained the *stx1* and *stx2* genes, one isolate had only the *stx1* gene, and one isolate contained the *stx2* and *ipaH* genes.

Phenotypic and genotypic characterization of carbapenem resistance among *E. coli* isolates

Two isolates, both resistant to carbapenem and cefpodoxime, were identified as ESBL producers. Among the *E. coli* isolates, 44.1% exhibited phenotypic resistance to cefpodoxime and combined cefpodoxime/clavulanic acid, and of these, two isolates showed phenotypic resistance to meropenem (Fig. 2). The two carbapenem-resistant *E. coli* observed in this study were found to carry the bla_{IMP} carbapenem-resistant gene (Table 7). These *E. coli* isolates were obtained from mix fruits sampled from hawkers. These two isolates were also found to be ESBL producers and carried virulent genes (*stx* and *ipaH*).

Table 6. Hybrid and non-hybrid *E. coli* pathovars obtained from fresh-cut pineapple, pawpaw, watermelon, and mixed-fruit salad in Greater Accra.

			Fresh-cut fruits associated with the pathovars			
Pathovars	Virulence gene combination	Number of isolates ($n = 34$)	Pineapple ($n = 60$)	Pawpaw ($n = 60$)	Watermelon ($n = 60$)	Mixed-fruit salad ($n = 20$)
STEC/EPEC/EIEC	*stx1/stx2/escV/ipaH*	11 (32%)	1 (1.7%)	5 (8.3%)	3 (5%)	2 (10%)
STEC/EIEC	*stx1/stx2/ipaH*	20 (59%)	3 (5%)	5 (8.3%)	10 (16.7%)	2 (10%)
STEC	*stx1/stx2*	1 (3%)	0	0	1 (1.7%)	0
STEC	*stx2*	1 (3%)	0	0	1 (1.7%)	0
STEC/EIEC	*stx2/ipaH*	1 (3%)	0	0	0	1 (5%)

Table 7. Amplified carbapenemase genes from the phenotypic resistant *E. coli* isolated from fresh-cut fruit.

	Carbapenemase genes				
Isolates	bla_{KPC}	bla_{OXA}	bla_{SPM}	bla_{IMP} (100%, 2/2)	Fresh-cut fruits associated with the carbapenem resistant *E. coli*
F41				+	Mix fruit (H)
F42				+	Mix fruit (H)

Carbapenemase genes: bla_{KPC} (*Klebsiella pneumoniae* carbapenemase), bla_{SPM} (Sao Paulo metallo-β-lactamase), bla_{OXA} (oxacillinases), and bla_{IMP} (imipenem-resistant *Pseudomonas*), and mix fruit (H): mix fruit from hawkers.

Discussion

The microbial quality of fresh fruits is important to establish their hygienic status and microbiological safety. They are indicators for commerce and public health protection. The levels of aerobic plate count and yeast and moulds (>5 log CFU g^{-1}) recorded from fruit samples might be due to the handling conditions along the supply chain and their high ambient storage during retail (Balali et al. 2020). High levels of yeast and moulds may indicate the onset of spoilage, development of off flavours, scabs, or rotting (Vimont et al. 2019). While yeasts and moulds cause deterioration of foods, some species of moulds such as *Aspergillus flavus* can produce toxins that can impact food safety (Vimont et al. 2019). High concentrations of yeasts and moulds on fruits can result in substantial economic losses to producers, processors, and consumers (Romanazzi et al. 2016). Enterobacteriaceae is a hygiene indicator used to assess the efficacy of prerequisite measures in a food safety system. High levels are reflective of sub-optimal hygienic conditions of the cut fruits. Also, high levels of *S. aureus* observed from this study indicate poor hand hygiene and/or no use of gloves. Staphylococci are natural inhabitants of the skin. Their presence on the fresh-cut fruits in this study reflects the poor-handling practices among the fresh-cut fruit vendors and the potential for production of heat-stable staphylococcal enterotoxins when concentrations reach 5 log CFU g^{-1} as was the case in the mixed-fruit samples (Fig. 1).

The low prevalence of *E. coli* observed in the pineapple samples might be due to an intrinsic property of the pineapple such as low pH (averaging 3.5), which is inhibitory to *E. coli*. The highest prevalence of *E. coli* observed in the watermelon samples might be due to their neutral pH, high moisture, and ambient storage temperature that likely promoted their growth. While the mean concentrations of *E. coli* in the cut fruits were <2 log CFU g^{-1}, because most of the samples had undetectable levels of *E. coli*, products that were positive for *E. coli* had counts >4 log CFU g^{-1}, which is alarmingly high as all isolated *E. coli* carried virulence genes (Table 5). Factors that contribute to *E. coli* cross-contamination events include the use of contaminated water to wash the fruits and contact surfaces, and processing and handling operations such as cutting, chopping, and peeling fruits (Yu et al. 2018). Our data support other previously published reports on the microbial quality of fruits and vegetables that reported a wide range of bacteria, including *E. coli* on cut fruits (Olu-Taiwo et al. 2021). *Escherichia coli* pathovars have been linked to foodborne disease outbreaks that involved fruits, vegetables, and other food commodities (Saxena et al. 2015, de Oliveira Elias et al. 2018, Irvin et al. 2021, Minary et al. 2022, Brown et al. 2023). *Escherichia coli* pathovars may cause diarrhoea, haemorrhagic colitis, haemolytic uremic syndrome (HUS), sepsis, neonatal meningitis, inflammatory bowel diseases, and urinary tract infections.

STEC prevalence was high among the *E. coli* isolates (Tables 5 and 6). STEC is a zoonotic pathovar linked to gastrointestinal disease and HUS in humans (Masters et al. 2011). The potential transmission route of STEC on fresh-cut fruits could be the unregulated use of animal manure to fertilize plants on the field and the use of contaminated surface water or wastewater for operational activities, or poor hand hygiene (Steele and Odumeru 2004). Other studies, conducted in Mexico, Alexandria (Egypt), and Czech Republic have also detected *stx* genes associated with *E. coli* obtained from fruits and vegetables (Skočková et al. 2013, Gómez-Aldapa et al. 2016, Khalil and Gomaa 2016).

EIEC are associated with the human host, and they contaminate food through the faecal-oral route (Enciso-Martínez et al. 2022). EIEC harbours the plasmid-mediated *ipaH* gene (Ashida and Sasakawa 2016), which encodes effector proteins for epithelial cell, intracellular survival, multiplication, maintenance of infection, and evasion of host immune responses (Masters et al. 2011). EIEC infection causes dysentery (Escher et al. 2014) and has been implicated in the severe foodborne outbreak in Italy, where several employees were admitted to the hospital with severe diarrhoea after consuming cooked vegetables (Escher et al. 2014). Additionally, the *escV* gene associated with EPEC pathovars codes an integral membrane protein that forms the largest part of the export apparatus of the type III secretion system, and functions as a membrane anchor to facilitate adhesion to the host cells (Mitrović et al. 2021). Previous studies have reported the association of the

escV gene with STEC (Sun et al. 2022), suggesting that it aids in the secretion of effectors during colonization to enhance its pathogenicity.

Other reports by Gomes et al. (2016) and Santos et al. (2020) have demonstrated the occurrence of hybrid *E. coli* pathovars is more common than previously believed since the 2011 German outbreak, which was caused by one *E. coli* strain carrying the virulence genes of both EAEC and STEC (Bielaszewska et al. 2011, Santos et al. 2020). Hybrid *E. coli* pathovars have also been reported in South Africa (Bolukaoto et al. 2021), Norway (Lindstedt et al. 2018), India (Dutta et al. 2015), and South Korea (Lee et al. 2023). Horizontal gene transfer and gene transfer by conjugation are popular reasons for the emergence of diarrhoeagenic hybrids (Nyholm et al. 2015, Yang et al. 2020). It is worth noting that the standard diagnostics for *E. coli* infections may be challenged by the emergence and spread of these hybrid *E. coli* strains, which could worsen the public health risk associated with them.

Carbapenem antibiotics are used to treat severe bacterial infections caused by multidrug-resistant bacteria, often as a last-resort treatment option. However, the emergence of carbapenem-resistant Enterobacterales is of a global health concern (Mazumder et al. 2020, WHO 2024). Two isolates, both resistant to carbapenem and cefpodoxime, were identified as ESBL and carbapenemase producers, indicating their potential to establish successful infection. The two carbapenem-resistant *E. coli* observed in this study were found to carry the bla_{IMP} carbapenem-resistant gene and virulence genes *stx* and *ipaH*. They were obtained from mixed-fruits sampled from street hawkers. The ESBL *E. coli* prevalence (44.1%, Fig. 2) observed in this study is higher compared to other studies (Day et al. 2019, Song et al. 2020, Kurittu et al. 2021), while carbapenem resistance (5.9%, 2/34) was higher than other studies in the United Kingdom and Ghana that reported zero prevalence of carbapenem-resistant *E. coli* (Randall et al. 2017, Karikari et al. 2022). Nonetheless, higher prevalence of carbapenem-resistant *E. coli* has been reported in other ready-to-eat foods (vegetable salad and fufu) and animal-source foods (Karikari et al. 2022, Huang et al. 2023).

Research suggests that horizontal gene transfer of antimicrobial-resistant genes, selection pressure due to inappropriate use of broad-spectrum antibiotics in food-producing animals, and misuse of broad-spectrum antibiotics in both hospital and community settings are the common mechanisms that encourage bacteria to acquire resistance genes (Burmeister 2015). There is an urgent need for food safety regulators to monitor the activities of fresh-cut fruit handlers to ensure stringent adherence to hygiene and food safety protocols. Also, source tracking is crucial to identify transmission routes to enable targeted risk mitigation decisions.

Conclusion

We reported relatively high microbial counts on fresh-cut pineapples, pawpaw, watermelon, and mixed fruits vended as ready-to-eat foods in Accra. *Escherichia coli* was present at a prevalence rate of 17%, all of which bore virulence genes associated with different pathogroups. Of the 34 *E. coli* isolates, 100% had *stx* gene, 94% had the *ipaH* gene, and 32% had the *escV* gene. All *E.* coli isolates except one were hybrid pathovars. Carbapenem-resistant and ESBL-producing *E. coli* were detected, with bla_{IMP} identified as the carbapenem-resistance gene. Adopting cutting-edge postharvest practices such as good hygienic practices, Hazard Analysis and Critical Control Point (HACCP), and food safety education initiatives is crucial to protect consumers from the public health risks that *E. coli* and *S. aureus* pose. Also, revision of the existing regulatory limits for *E. coli* can be an important risk management step. For example, instead of a regulatory limit for generic *E. coli*, STEC, and other prevalent *E. coli* pathovars can be treated as adulterants in fresh-cut fruits. The study recommends enhanced antibiotic-resistance surveillance for early detection of resistant pathogenic bacteria in fresh-cut fruits and immediate risk management efforts to prevent and control the potential spread of the hypervirulent strains.

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Author contributions

Agnes Nketiah (Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft), Joycelyn K. Quansah (Investigation, Project administration, Supervision, Validation, Writing – review & editing), and Angela Pary-Hanson Kunadu (Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing)

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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