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Microbiological quality and antimicrobial resistance of Bacteria species recovered from ready-to-eat food, water samples, and palm swabs of food vendors in Accra, Ghana

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ABSTRACT

This study sought to investigate microbial quality and antimicrobial resistance of bacteria species from Ready-to-Eat (RTE) food, water, and vendor palm swab samples.

Between 2019 and 2020, RTE food, water and vendor palm swab samples were collected from food vending sites in Accra, Ghana. Samples were cultured and confirmed using the Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). Antimicrobial susceptibility testing (AST) was conducted using disk diffusion method. Beta-lactamase and Diarrheagenic *Escherichia coli* (DEC) genes were determined using Polymerase Chain Reaction (PCR). Total plate count (TPC) and Total coliform count (TCC) were performed on food and water samples.

In total, 179 RTE food, 72 water and 10 vendor palm swab samples were collected. *Enterobacter* spp. (16.8 %), *Citrobacter* spp. (10.1 %), *Enterococcus faecalis* (7.8 %), *Pseudomonas* spp. (6.7 %) and *Klebsiella pneumoniae* (4.0 %) occurred in food. Isolates from water and palm were *Klebsiella pneumoniae* (20.8 %), *Aeromonas* spp. (16.7 %) and *Enterobacter cloacae* (11.1 %). Resistance to Amoxicillin-clavulanate, Tetracycline, Azithromycin, Sulfamethoxazole-trimethoprim, and Nitrofurantoin were common among Enterobacterales. High mean TPC and TCC showed in some RTE food and different water types used in vending depicting their unsafe condition for consumption and usage. The bla_{SHV} and bla_{TEM} genes were present in some Enterobacterales from food and water. The *lt* gene was identified in two food samples.

AMR organisms associated with nosocomial infections in the samples investigated, calls for continuous surveillance in the food industry in Ghana. Also, the unsafe outcome of RTE food and water depicts the need for the enforcement of Ghana's food safety laws.

1. Introduction

Ready-To-Eat (RTE) food is a major dependency of urban and *peri* urban dwellers in the world due to its popularity, lucrativeness, economic importance and accessibility (Rane, 2011). RTE foods are readily available and affordable meals with dietary traditions and nutritional needs (Abakari et al., 2018; Mosupye and Von Holy, 1999). It serves as a side attraction for tourism (Choudhury et al., 2011) and has created jobs for the informal sector in worldwide (Ababio and Lovatt, 2015; Choudhury et al., 2011; Rane, 2011).

Despite their significance in the food industry, RTE food is one of the vehicles for the spread of foodborne pathogens (FBP) (Mosupye and Von Holy, 1999; Rane, 2011). Personal hygiene of street food vending (SFV) aside unsanitary conditions have been the major concern of many FBP studies worldwide (Amare et al., 2019; Asamoah et al., 2016; Barro et al., 2006; Nyenje et al., 2012; Rane, 2011; Yeleliere et al., 2017).

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Lack of proper handwashing of food vendors and contaminated water for cooking, improper dishwashing practices and improper ways of handling money are some of the gaps in the spread of FBPs (Barro et al., 2006; Mosupye and Von Holy, 1999; Rane, 2011). From an estimated 600 million people, one out of ten people fall ill after the consumption of contaminated food, resulting in the 33 million loss of healthy life years (DALYs) which exerts pressure on the health care system and other associated harm to tourism and national economies (WHO, 2020).

Major bacterial FBPs that have been recovered from SFV sources in Ghana includes *Enterobacter* spp., *Escherichia* spp., *Staphylococcus* spp. *Pseudomonas* spp., *Bacillus* spp., *Klebsiella* spp., *Citrobacter* spp., and *Aeromonas* spp. (Ababio and Lovatt, 2015; Addo et al., 2007; Feglo and Sakyi, 2012; Mensah et al., 2002). *Staphylococcus aureus, S. epidermidis,*

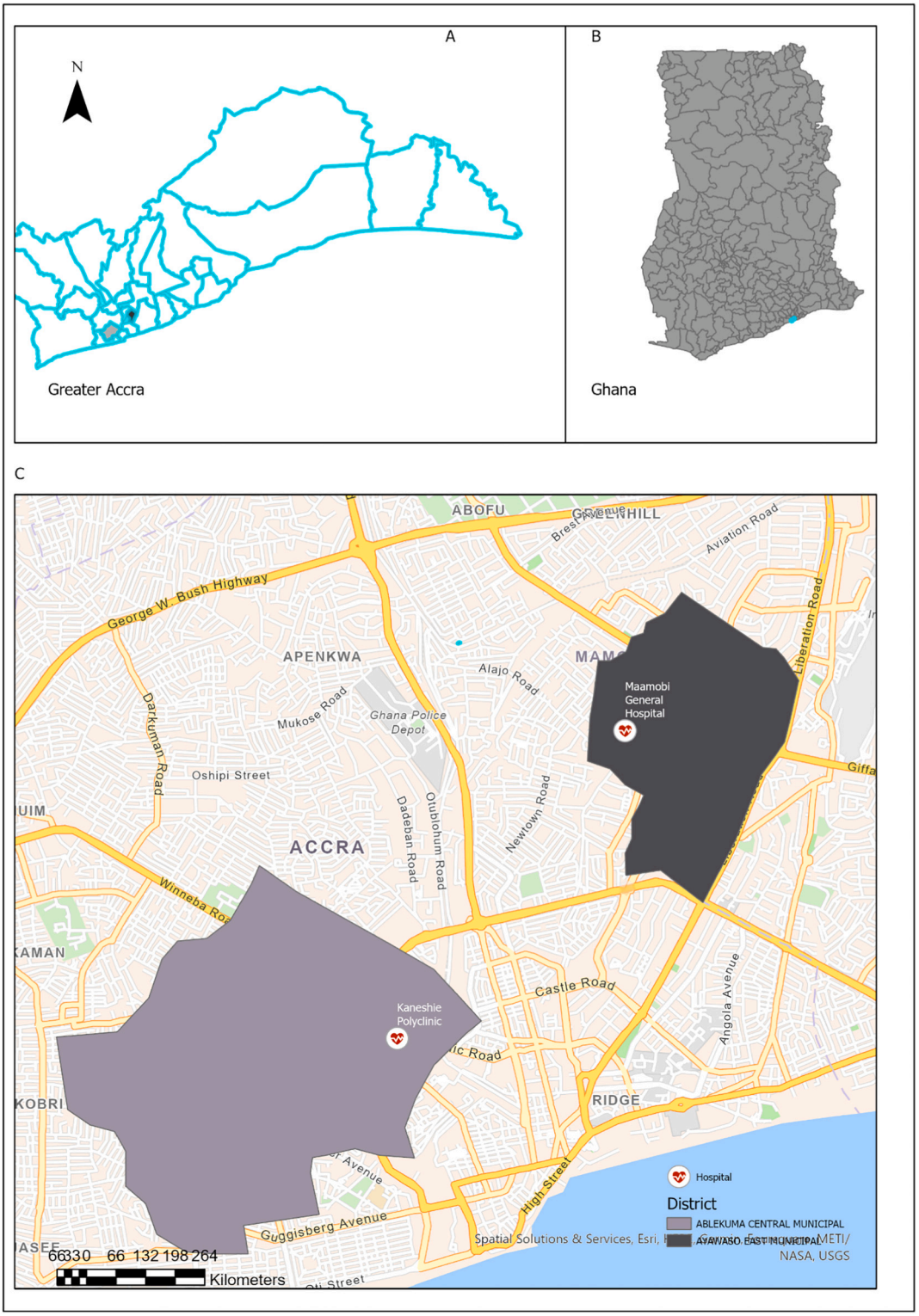

Fig. 1. Map showing the location of food vending sites; A) Maamobi General Hospital and Kaneshie Polyclinic located in the Greater Accra Region; B)The map of Ghana C) Detailed map showing study sites.

E. coli, P. aeruginosa, Streptococcus spp., *Salmonella* spp. and *Shigella* spp. have been recovered from the palms of food vendors in some parts of Nigeria (Barro et al., 2006; Okareh and Erhahon, 2015). Microbial counts implicating reduced quality and safety of RTE food have been reported in Ghana and other countries (Amare et al., 2019; Eromo et al., 2016; Feglo and Sakyi, 2012; Mensah et al., 2002; Yeboah-Manu et al., 2010).

Diarrhoeagenic *E. coli* (DEC) consisting of six pathotypes known to cause diarrhoea include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC). However, Enterotoxigenic *E. coli* (ETEC) and Enteropathogenic *E. coli* (EPEC) have been reported to be primarily transported via contaminated food and water in South America, Africa and from some RTE food in Ghana (Lima et al., 2017; Mensah et al., 2002; Okeke, 2009).

Studies conducted in Ghana and other countries have shown the resistance of bacteria to Ampicillin, Chloramphenicol and Amoxicillin in RTE food (Abas et al., 2019; Amare et al., 2019; Eromo et al., 2016), hands (Hassan et al., 2018; Lues and Van Tonder, 2007) and water (Duedu et al., 2017; Karikari et al., 2016; Mosupye and Von Holy, 1999; Praveenkumarreddy et al., 2020). Also, antimicrobial resistance (AMR) associated with DEC posing risk to treatment of diarrhoea have been investigated among humans and RTE food (Natarajan et al., 2018; Opintan et al., 2010).

Through the lens of the One Health System, there might be transfer of AMR bacteria through food (Ghana Ministry of Health, Ministry of Food and Agriculture, Ministry of Environment, Science, T. and I. and M. of F. and A. D., 2018; Havelaar et al., 2015; WHO, 2017) as Extended Spectrum Beta Lactamase (ESBLs) have been detected in food from Ghana and other parts of the world (Dsani et al., 2020; Falgenhauer et al., 2019; Ye et al., 2018).

In this study, the microbial quality of RTE food and water were determined coupled with the collection of swab samples from palms of food vendors for investigations in the environs of Maamobi General Hospital and Kaneshie polyclinic in Ghana. Using phenotypic and molecular tools, occurrence of bacteria and their AMR were determined.

2. Material and methods

2.1. Study design

This cross-sectional study was conducted between January 2019 to March 2020 in two main areas of Accra (Maamobi General Hospital and Kaneshie polyclinic) with high population of settlers and commercial activities, thus enhancing the SFV activity. As in Fig. 1, the map showing the location of food vending was generated using the ArcGIS Pro 2.8.0.

2.2. Sample collection and microbial analyses

A total of 179 RTE food, 72 water and 10 palm samples from 93 vendors were collected after receiving informed consent. Food samples were collected aseptically using sterile zip lock bags. Palm samples were collected using sterile cotton swabs, dampened in 0.85 % saline and rolled in between fingers depending on whether the vendors were right or left-handed before washing hands. Cooking water ($n = 13$), storage water ($n = 29$) and washing water ($n = 30$) were collected from 65 food vendors. All samples were transported at 4 °C to the Noguchi Memorial Institute for Medical Research (NMIMR) for further testing.

Specified quantities of food samples (10 g/ml liquid, 25 g dry/solid) were weighed, added to buffered peptone water and homogenized in a stomacher blender. One in ten serial dilutions of up to 10^4, prepared in Phosphate-buffered salt (PBS) of each sample were transferred into petri dishes for TPC and TCC using Plate count agar (PCA) and MacConkey agar (MCA) (Oxoid Ltd., Basingstoke, Hamspshire, England) (Amare et al., 2019; Feng et al., 2020). PCA plates were incubated at 24–48 h at 37 °C and MacConkey/Coliform selective agar plates for 24 h at 37 °C.

Ten milliliters of water and palm samples were processed using a threefold dilution and one in ten serial dilution up to 10^3 prior to TPC and TCC analysis (Addo et al., 2007).

Growth on plates after overnight incubation having colonies between 30 and 300 were counted using colony counter, model CL-570 (Sibata scientific technology Ltd./Sibata); whiles those above and below range were not counted and recorded as either "Too Numerous to Count" (TNC) or not viable for counting. Results of the average bacterial colony counts for all samples were interpreted using their specific sample type standard guidelines (Center for Food Safety, 2014; Feglo and Sakyi, 2012; GSA, 2013). TPC for RTE food (CFU)/g: Satisfactory $<10^3$; Borderline 10^3- $<10^5$; Unsatisfactory $\geq 10^5$ while TCC for RTE food for Enterobacterales (CFU)/g: Satisfactory $<10^2$; Borderline 10^2- $<10^4$; Unsatisfactory $>10^4$ were the ranges used in interpreting the acceptable nature of food for consumption or not (Center for Food Safety, 2014; GSA, 2013). On the other hand, water TCC must not exceed 500 CFU/ml to be considered safe for consumption and use. TPC in water (CFU)/ml: Low: 1–10 CFU/ 100 ml, Intermediate: 11-100 CFU/100 ml, High: >100 CFU/100 ml were the standard ranges used in the interpretation of water quality (GSA, 2013; WHO, 2011).

Specified quantities of homogenate (food + buffered peptone water) were transferred into 10 ml of Tryptic Soy Broth (TSB), Alkaline Peptone Water (APW), Selenite cystine broth (SCB) and incubated at 35° C for 24 h. Enrichment media with inoculum (10 µl) were transferred onto Baird Parker, Thiosulphate citrate bile-salt sucrose (TCBS), Salmonella-Shigella and MacConkey agars and incubated at 35° C-37° C for 24 h for the recovery of *S. aureus*, *V. cholerae*, *Salmonella* and *E. coli* where present. Further confirmation of identified isolates and unidentified isolates were confirmed using the MALDI-TOF-MS (MALDI TOF) (Bruker, Daltonics, Germany). Colonies of fresh overnight cultures were spotted on the MALDITOF-MS target plated. Formic acid-70 % (1 µl) was added and allowed to dry for 15 min, followed by the addition of a 1 µl matrix preparation (HCCA matrix- a-Cyano-4-hydroxycinnamic acid) placed on each sample with another 15 min drying time. The MALDI-TOF MS was used to confirm different species of bacteria by generating ionization peaks that were matched against its integrated reference library while providing a matching score known as logarithmic score value. Scores of >2 were considered as very good matches for species identification while score values between 1.7 and 2.0 were sufficient for identification of the genus. Score values below 1.7 demonstrate an outcome of no identification of isolate.

Bacteria identification in water involved incubation and centrifugation of 10mls of water at 10,000 rpm for 30 mins after which the supernatant was decanted. A loop-full of the pellet was inoculated into SCB for selective enrichment of *Salmonella/ Shigella* and transferred unto MCA for the detection of *E. coli* and other Enterobacteriaceae. Two loop-fulls from the SCB was subcultured on SSA and incubated further to enable the detection of *Salmonella* and *Shigella* (Addo et al., 2007). The possible recovery of *S. aureus* and *V. cholerae* recovery involved the use of enrichment in Tryptic soya broth (TSB) and Alkaline Peptone water, followed by transfer unto Baird Parker and Thiosulphate citrate bile-salt sucrose (TCBS) agars. Confirmation of the various bacteria were confirmed using the MALDI-TOF-MS.

Saline solutions containing palm swabs of food vendors were subcultured using MacConkey and 5 % sheep blood agar to recover Enterobacteriaceae, including *E. coli*. Additionally, Mannitol salt agar was employed to enable the detection of *S. aureus*. The cultures were incubated at 35–37 °C for 24–48 h to determine coliform counts and bacterial growth. Following this, the isolates were transferred onto Nutrient Agar and incubated overnight at 35–37 °C. To confirm the identity of the isolates, MALDI-TOF-MS was used, along with presumptive tests such as gram staining, catalase, and coagulase.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out using the Kirby Bauer disk diffusion by the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2017).

The following antibiotics were used in the testing of Enterobacterales: Ceftriaxone (30 µg), Sulfamethoxazole-trimethoprim (25 µg), Piperacillin-tazobactam (110 µg), Ticarcillin-clavulanate (85 µg), Tetracycline (30 µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Meropenem (10 µg), Azithromycin (15 µg), Chloramphenicol (30 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg), Ceftazidime (30 µg) and Amoxicillin-clavulanate (30 µg) (Oxoid, Basingstoke, Hants, UK) described by Dela et al. (2022) were adopted in addition to Vancomycin (30 µg), Erythromycin (15 µg), Rifampin (5 µg), Penicillin (10 units) and Linezolid (30 µg) as additional antibiotics that were used to test *Staphylococcus spp* and *Enterococcus* spp.

2.4. Phenotypic detection of ESBL

Enterobacterales resistant to Ceftriaxone and Ceftazidime were tested further by the double-disk synergy test (MAST, Merseyside, UK) using both cefotaxime (30 µg) and ceftazidime (30 µg) alone and in combination with Clavulanic acid (i.e.: Ceftazidime, Ceftazidime/ Clavulanic acid and Cefotaxime, Cefotaxime/ Clavulanic acid) to test for the presence of ESBLs. Quality control testing was ensured using *Klebsiella pneumoniae*, ATCC 700603 and *Escherichia coli*, ATCC 25922.

2.5. Detection of ESBL and DEC genes

DNA was extracted for ESBL and DEC PCR detection. Detection of ESBL genes (Table 1) consisted of 10 µM of each primer; $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{TEM}, multiplex PCR master mix (13 µl) (QIAGEN), PCR grade water (10 µl) and 2 µl DNA template, in a total volume of 25 µl. Using previously described protocol (Sharma, 2013), amplification with cycling conditions; 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 2 min, and a final extension lap at 72 °C for 10 min followed by gel electrophoresis.

To determine DEC in food, each 25 µl of reaction mixture contained 0.5 µl of each primer (Table 1) and deoxynucleoside triphosphates, 5 µl $MgCl_2$, 0.25 µl of Taq DNA polymerase and 3 µl of template DNA. PCR was performed based on a few modifications from Vidal et al. (2005) at 1.5 min at 94 °C for denaturation, 1.5 min at 60 °C for primer annealing, amplification for 35 cycles, and 1.5 min at 72 °C for strand elongation followed by gel electrophoresis.

2.6. Statistical analysis

Data was entered in Microsoft Excel and exported to STATA version 13 for analysis. Descriptive statistics such as frequencies, percentages and bar graph described the isolated pathogen distribution and antimicrobial resistance profiles in food and water samples. Mean and Standard deviation were used to calculate TPC and TCC.

3. Results

3.1. Characteristics of participants (vendors) and RTE foods

A total of 90 food vendors participated in the study with majority being females with >50 % below 40 years in age. With 6.7 % reporting having no formal education, over 92 % received some form of formal education ranging from primary (21.1 %), secondary (44.4 %) to tertiary (5.56 %).

Most of the RTE food were prepared offsite and sold at ambient temperature (Table 2) with food such as rice, stew, 'Waakye' and 'Hausa kooko' being frequently collected (Table 3).

The most recovered bacteria from the food samples obtained was *Enterobacter* spp. (16.8 %) (Fig. 2). Two *E. coli* isolates were recovered from a rice and 'Hausa kooko' respectively (Table 4)

Klebsiella pneumoniae (20.8 %) was frequently recovered pathogen from water and palm samples (Fig. 3).

Table 1
DEC and ESBL primer sequence details.

Gene	Primer sequence (5′-3′)	Target	Expected product size (bp)
Stx_1	CAG TTA ATG TGG TGG CGA AGG	shiga toxin-1 gene of STEC	348
	CAC CAG ACA ATG TAA CCG CTG		
Stx_2	ATC CTA TTC CCG GGA GTT TAC G	shiga toxin-2 gene of STEC	584
	GCG TCA TCG TAT ACA CAG GAG C		
eae	TCA ATG CAG TTC CGT TAT CAG TT	attaching and effacing lesion of EPEC	482
	GTA AAG TCC GTT ACC CCA ACC TG		
lt	GCA CAC GGA GCT CCT CAG TC	heat-labile gene of ETEC	218
	TCC TTC ATC CTT TCA ATG GCT TT		
stII	AAA GGA GAG CTT CGT CAC ATT TT	heat stable gene of ETEC	129
	AAT GTC CGT CTT GCG TTA GGA C		
VirF	AGC TCA GGC AAT GAA ACT TTG AC	gene responsible for transcription of virulent factors of EIEC	618
	TGG GCT TGA TAT TCC GAT AAG TC		
ipaH	CTC GGC ACG TTT TAA TAG TCT GG	invasion plasmid antigen H of EIEC	933
	GTG GAG AGC TGA AGT TTC TCT GC		
daaE	GAA CGT TGG TTA ATG TGG GGT AA	fimbrial adhesin gene of DAEC	542
	TAT TCA CCG GTC GGT TAT CAG T		
aafII	CAC AGG CAA CTG AAA TAA GTC TGG	aggregative adherence fimbrae- II genes of EAEC	378
	ATT CCC ATG ATG TCA AGC ACT TC		
DEC Primer sequences adopted from Vidal et al. (2005)			
$bla_{CTX\text{-}M}$	GAA GGT CAT CAA GAA GGT GCG	cefotaximase (CTX-M) beta-lactamase	560
	GCA TTG CCA CGC TTT TCA TAG		
bla_{SHV}	GTC AGC GAA AAA CAC CTT GCC	sulfhydryl (SHV) beta-lactamase	383
	GTC TTA TCG GCG ATA AAC CAG		
bla_{TEM}	GAG ACA ATA ACC CTG GTA AAT	temoneira (TEM) beta-lactamase	459
	AGA AGT AAG TTG GCA GCA GTG		

ESBL Primer sequences adopted from Sharma, 2013.

3.2. Microbial evaluation of RTE foods, water, and palm of vendors

Bread (3.1×10^3 CFU/g) had the lowest mean plate count, interpreted as satisfactory while stew (1.9×10^5 CFU/g) showed the highest mean plate count (unsatisfactory). Stews had the highest number of bacteria species isolated (eleven pathogens). 'Hausa kooko' (porridge), banku, and rice had mean plate counts of unsatisfactory interpretation (Table 3).

The highest mean coliform count showing unsatisfactory/ unacceptable results in RTE foods exceeding $>10^4$ CFU/g was shown in stew and 'waakye' (1.8×10^5 CFU/g and 1.8×10^5CFU/g). Fish/meat and bread recorded the lowest mean coliform counts and were interpreted satisfactory (6.7×10^2 CFU/g and 2.5 x 10^3CFU/g).

Table 2
Ready to eat foods encountered in the study.

Food items	Ingredients	Description/cooking method/storage
Ampesi	Yam, plantain, salt	Boiling/Ambient
Banku	Maize and cassava dough, salt	Fermented maize mixed with cassava, boiling/Ambient
Beans	Beans, salt, red oil	Boiling and mixed with oil/ With heating
Bofloat/ Koose/ Pinkaso	Beans, onion, pepper, ginger, salt, vegetable oil, plain flour, bonnet pepper	Frying/Ambient
Bread	Plain flour, yeast, salt, margarine	Baking/Ambient
Fried egg	Egg, salt, oil, vegetables	Frying/ Ambient
Fried fish/ meat	Fish, meat, salt, plain flour, vegetable oil	Frying/Ambient
Fried pepper ("Shitor")	Pepper, onion, dried fish and shrimps, oil, salt, and spices	Frying/Ambient
Fried rice	Rice, salt, vegetables, eggs, spices	Boiling and stir frying/ Ambient
Gari	Cassava grits	Stirring in hot frying tray/ Ambient
Grinded pepper	Red/green pepper, onion, tomatoes, salt	Grinding in earthenware/ Ambient
Hausa kooko (porridge)	Millet, ginger, cloves, pepper, salt	Fermented, Boiling/Ambient
Indomie/ spaghetti	Macaroni, fish/meat, salt, vegetables, oil	Boiling, frying/Ambient
Jollof	Rice, salt, vegetables, spices	Boiling, rice mixed with tomato sauce and boiled/ Ambient
Kenkey	Maize dough, salt	Fermented, wrapped in corn husk, and boiled/Ambient
Rice	Rice, salt	Boiling/Ambient
Salad	Cabbage, carrot, onion, lettuce, cucumber, mayonnaise	Cut and mixed/Ambient
Soup	Okro, palm nut, peanut butter, salt, fish, meat, spices	Boiling/With heating
Stew	Tomatoes, onions, pepper, vegetable oil, garlic, ginger, salt	Frying/Ambient
Waakye	Rice, salt, beans, millet leaves	Boiling/Ambient

More than half of cooking water were considered unsatisfactory for TPC and TCC by national and international standards. Also, more than half of storage and washing water were unsatisfactory by TPC but satisfactory by TCC. All palm samples from ten food vendors were satisfactory after TPC and TCC analysis (Table 5).

3.3. Antibiotic susceptibility pattern of isolated strains

Majority of the *E. cloacae* were resistant to Nitrofurantoin (76.7 %), Amoxicillin-clavulanate (60.0 %), Azithromycin (36.7 %) and Tetracycline (30.3 %). Over 50 % resistance to Sulfamethoxazole-trimethoprim (77.8 %), Tetracycline (66.7 %) and Amoxicillin-clavulanate (66.7 %) was detected in *Citrobacter* spp. food isolates (Table 6). All three *E. coli* were susceptible to all antibiotics apart from Tetracycline (33.3 %) and Nalidixic acid (66.7 %).

Staphylococcus spp. from food was resistant to Erythromycin (33.3 %) and Chloramphenicol (66.7 %). Out of a total of fourteen *E. faecalis* isolates, only ten were resistant to Rifampin (71.4 %) and five resistant to Vancomycin (35.7 %).

Although few isolates were recovered from water and palm samples, some showed 50 % resistance to Sulfamethoxazole-trimethoprim, Tetracycline, Nitrofurantoin/ Azithromycin, and Amoxicillin-clavulanate (Table 7).

E. faecalis, *Staphylococcus* spp. and *Pseudomonas* spp. were some of the non-Enterobacterales recovered from water (Fig. 3). A hundred percent resistance to Linezolid, Penicillin, Erythromycin and Rifampin among all three *Staphylococcus* spp. isolates occurred while 25 % of *E. faecalis* were resistant to Linezolid, Tetracycline, Penicillin and Erythromycin.

3.4. DEC and ESBL occurrence

The bla_{TEM} gene was found in two *Citrobacter freundii* isolates (Fig. 4) from Indomie/ spaghetti and 'kenkey' while the *Lt* gene of ETEC was detected in strains isolated from Porridge and rice.

Although *E. coli* was not recovered in water and palms of vendors, bla_{SHV} and bla_{TEM} genes were found in *C. freundii* and *K. pneumoniae* from storage water.

Table 3
Mean TPC and TCC of RTE food.

Food items	Total number of food items	Total plate count Standard Deviation CFU/g	Total plate count Mean CFU/g	Total coliform count Standard Deviation CFU/g	Total coliform count Mean CFU/g
Ampesi	7	1.8×10^4	1.7×10^4	1.5×10^4	5.7×10^4
Banku	15	$*8.3 \times 10^5$	$*1.6 \times 10^5$	2.9×10^4	1.6×10^4
Beans	6	4.1×10^4	2.9×10^4	4.1×10^4	1.7×10^4
Bofloat/ Koose/ Pinkaso	7	5.8×10^4	3.7×10^4	9.5×10^4	5.1×10^4
Bread	2	4.4×10^4	3.1×10^3	3.5×10^3	2.5×10^3
Fried egg	3	2.0×10^4	1.4×10^4	1.2×10^4	1.2×10^4
Fried fish/ meat	6	1.7×10^4	1.5×10^4	1.6×10^3	6.7×10^2
Fried pepper (Shitor)	2	3.1×10^4	2.2×10^4	–	–
Fried rice	1	–	3.2×10^4	–	4.0×10^4
Gari	1	–	3.2×10^4	–	–
Grinded pepper	11	3.8×10^4	3.4×10^4	1.7×10^4	9.5×10^4
Hausa kooko (porridge)	18	$*1.3 \times 10^5$	$*1.0 \times 10^5$	5.2×10^4	3.0×10^4
Indomie/ spaghetti	10	3.6×10^4	1.8×10^4	2.2×10^4	1.0×10^4
Jollof	8	8.4×10^4	5.9×10^4	3.0×10^4	1.2×10^4
Kenkey	8	2.6×10^4	3.2×10^4	3.5×10^4	3.7×10^4
Rice	21	$*1.5 \times 10^5$	$*1.0 \times 10^5$	6.7×10^4	4.1×10^4
Salad	1	–	6.9×10^4	–	–
Soup	9	3.1×10^4	3.2×10^4	1.6×10^4	8.9×10^4
Stew	26	$*1.5 \times 10^5$	$*1.9 \times 10^5$	$*2.9 \times 10^5$	$*1.8 \times 10^5$
Waakye	17	1.1×10^4	7.3×10^4	$*2.5 \times 10^5$	$*1.8 \times 10^5$

TPC for RTE food (cfu)/g: Satisfactory $<10^3$; Borderline 10^3- $<10^5$; Unsatisfactory $\geq 10^5$.
TCC for RTE food for Enterobacteriaceae (cfu)/g: Satisfactory $<10^2$; Borderline 10^2- $<10^4$; Unsatisfactory $>10^4$.
*Counts that are unsatisfactory by interpretation.
– not detected.

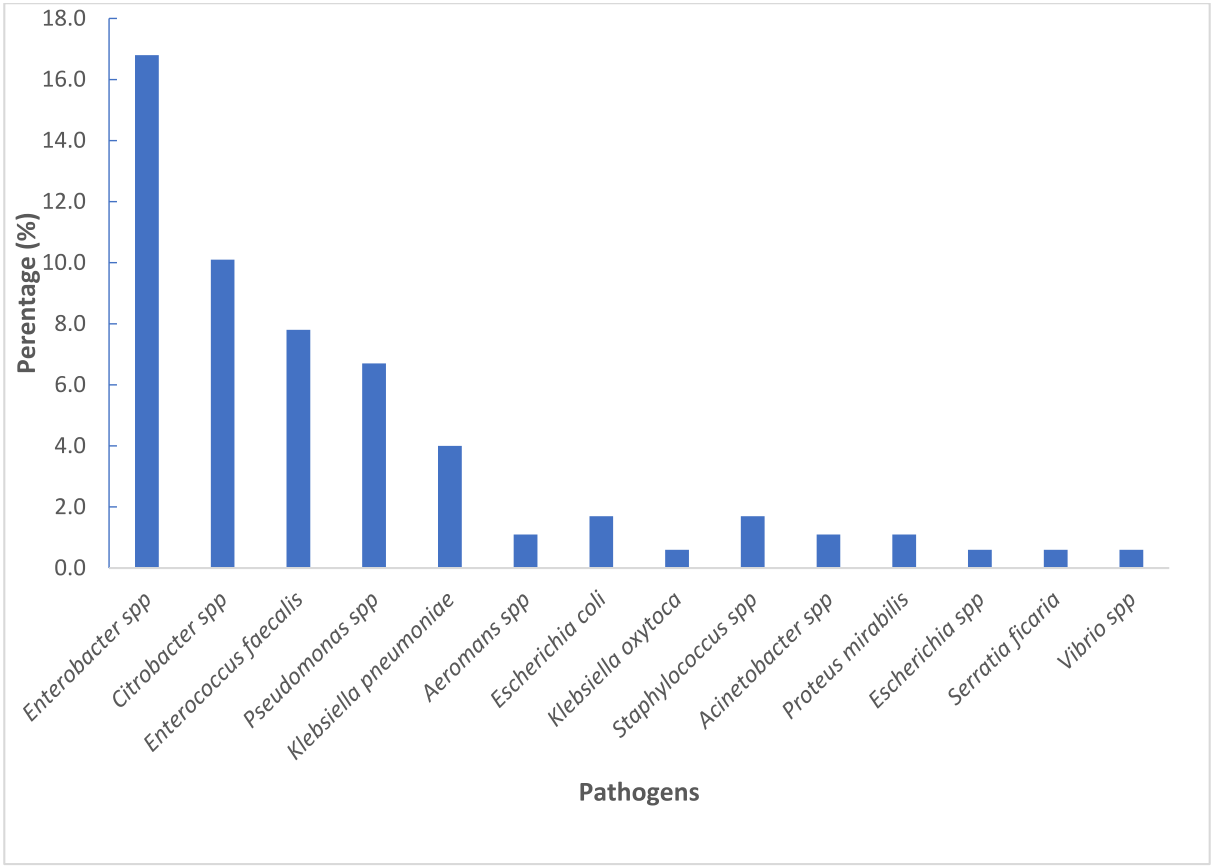

Fig. 2. The total percentages of all the bacteria organisms isolated in the RTE foods.
(recovered from the food vending areas near the Mamobi General Hospital and Kaneshie Polyclinic)

Table 4
Species of bacteria isolated from RTE food samples.

Ready-to-Eat Food item	Bacteria isolated
Ampesi	*Acinetobacter baumanii, Staphylococcus sciuri, Enterobacter asburiae*
Banku	*Enterobacter cloacae, Citrobacter freundii*
Beans	*Enterobacter cloacae*
Fried egg	*Enterobacter cloacae, Enterococcus faecalis*
Gari	*Enterobacter cloacae*
Grinded pepper	*Aeromonas caviae, Citrobacter braakii, Enterobacter cloacae, Enterococcus faecalis*
Indomie/ spaghetti	*Acinetobacter baumannii, Citrobacter freundii* (ESBL-bla_{TEM}), *Enterococcus faecalis, Pseudomonas aeruginosa*
Jollof	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Escherichia* spp., *Proteus mirabilis, Pseudomonas aeruginosa, Serratia ficaria, Pseudomonas mendoncina, Citrobacter braakii*
Kenkey	*Citrobacter freundii* (ESBL-bla_{TEM})
Hausa kooko	*Citrobacter koseri, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* (ETEC-*Lt* gene), *Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas plecoglossicida*
Rice	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* (ETEC-*Lt* gene), *Staphylococcus epidermidis, Pseudomonas aeruginosa, Pseudomonas mendoncina*
Salad	*Proteus mirabilis*
Soup	*Enterobacter cloacae*
Stew	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Escherichia hermanii, Proteus mirabilis, Pseudomonas aeruginosa, Serratia ficaria, Pseudomonas mendoncina, Citrobacter braakii, Pseudomonas putida*
Waakye	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus* spp., *Vibrio fluvialis*

4. Discussion

High mean TPC of $\geq 10^5$ was classified as above the acceptable limits and therefore considered unsafe for consumption, likewise high mean TCC of $>10^4$ (Center for Food Safety, 2014). More than half of the RTE food were at borderline by TPC whereas the possibility of fecal contamination in them were minimal by TCC and considered microbiologically safe for consumption (Table 3). Mensah and colleagues in a street food study conducted in 2002 concluded that the street foods investigated could be considered microbiologically safe but other studies that conducted in later years considered most RTE food unsafe for consumption (Abakari et al., 2018; Marras and Agbendech, 2016; Mensah et al., 2002; Yeboah-Manu et al., 2010; Yeleliere et al., 2017). Targeted focus on food safety and hygiene education is required to improve the quality of RTE food.

Stews showed the highest mean TPC and TCC results (1.9 x 10^5CFU/g; 1.8 $\times$ 10^5CFU/g) consistent with Yeleliere et al. (2017) and Mensah et al. (2002) which was unexpected because of the mode of preparation of stews in Ghana that involves rigorous boiling of ingredients for prolonged periods making it difficult for coliforms to thrive (Newman, 2005). Post exposure factors like storage (ambient), serving, could be the reasons for the presence of coliforms in stew. Rice also showed TPC above acceptable limits (1.0 x 10^5CFU/g); unsafe for consumption. 'Waakye,' also showed high levels of TCC (1.8 $\times$ 10^5 CFU/g) above acceptable limits. Some RTE studies have reported rice dishes to show high levels of contamination in those transferred into storage containers as compared to types directly sold from cooking pots (Mensah et al., 2002; Nyenje et al., 2012; Yeleliere et al., 2017).

Another food unsafe for consumption was 'Hausa kooko' (1.0 x 10^5CFU/g), with observed unrenewal of water for vending similar to Mensah et al.'s study (Mensah et al., 2002). Also, 'banku', showed high

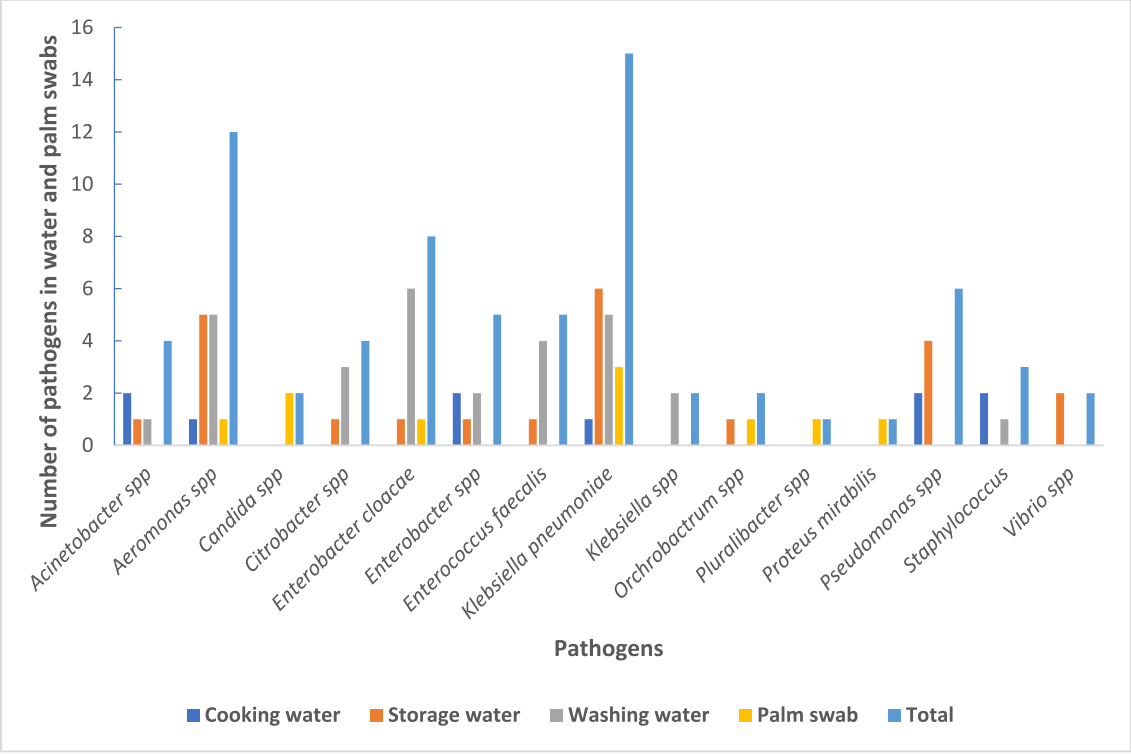

Fig. 3. Pathogens detected in the different types of water and palm swab samples of SFV from vending sites where RTE food were collected(all pathogens were confirmed using the MALDITOF-MS equipment).

Table 5
TPC and TCC of water and palm swab samples of food vendors.

	TPC	TCC
Cooking water		
Unsatisfactory n(%)	9(69.23)	7(53.85)
Satisfactory n(%)	4(30.77)	6(46.15)
Storage water		
Unsatisfactory n(%)	15(51.72)	14(48.28)
Satisfactory n(%)	14(48.28)	15(51.72)
Washing water		
Unsatisfactory n(%)	17(56.67)	17(56.67)
Satisfactory n(%)	13(43.33)	13(43.33)
Palm swab		
Unsatisfactory n(%)	0(0.00)	0(0.00)
Satisfactory n(%)	10(100.00)	10(100.00)

levels of TPC which could be a result of continuous exposure to bare hands and the probability of contaminated materials during processing (Mensah et al., 2002).

Like several RTE studies conducted in Ghana (Ababio and Lovatt, 2015; Feglo and Sakyi, 2012; Yeleliere et al., 2017), grinded pepper (3.4×10^4 CFU/g; 9.5×10^4 CFU/g), being at borderline was considered unsafe by interpretation. There is possible contamination of this food and the need for education on proper handling of vegetables (Abakari et al., 2018; Hartantyo et al., 2020; Karikari et al., 2017; McMahon and Wilson, 2001; Nyenje et al., 2012; Stratev and Odeyemi, 2016). Grinded pepper is known to be prepared with fresh vegetables, processed in earthenware pots/ blenders, and served to consumers as accompaniments for most Ghanaian meals. Lack of cooking before consumption renders them prone to contamination from various equipment and ingredients during preparation.

Fried meat/ fish and bread were the least contaminated which could be due to less handling, unlike high mean aerobic counts in the local breads ('kita- 6.1 X 10^5 CFU/g and 'Ambasha'- 3.0×10^5 CFU/g) in Ethiopia (Eromo et al., 2016).

Although all ten palm samples showed no coliforms and were considered satisfactory during TPC and TCC analysis, several practices including handling money without proper handwashing prior to serving food, and lack of potable water were observed. However, other SFV studies who identified high TPC and TCC in palms of vendors suggested possible transfer of pathogens to food (Amegah et al., 2020; Barro et al., 2006; Hassan et al., 2018; Lues and Van Tonder, 2007; Tadesse et al., 2019).

Over 50 % of dishwashing water were unsatisfactory by both TPC and TCC analysis similar to Okere and colleagues' SFV study in Ouagadougou, attributed to mainly unrenewal of washing water at vending sites (Barro et al., 2006). The observation of infrequent dishwashing water renewal and their misuse as mediums of handwash could have accounted to the presence of mesophilic bacteria and reduced bacteriological quality of food in this study (Barro et al., 2006; Mensah et al., 2002). Majority of the cooking water collected in the study were unsatisfactory (WHO, 2011; WRC, 2015). Reasons for their contamination although not established in the study could have been due to pollution from source, transportation mediums to vending sites, inadequate and prolonged storage.

One of the most common fecal-orally transmitted pathogens mostly recovered from RTE studies in Ghana is *Enterobacter cloacae* (Mensah et al., 2002; Yeboah-Manu et al., 2010; Yeleliere et al., 2017). In this study, *E. cloacae* was frequently detected in majority RTE food, especially in stew, similar to the findings of Nyenje and colleagues in South Africa who isolated 24 % of *E. cloacae* from beef stew (Nyenje et al., 2012). *C. freundii*, the second common pathogen isolated from most foods like *E. cloacae* is a gastrointestinal commensal known to be an opportunistic pathogen involved in causing nosocomial infections such as UTIs, septicemia, meningitis, and wound infections aside diarrhoea transmission in humans (Liu et al., 2017). *E. faecalis* is known to be

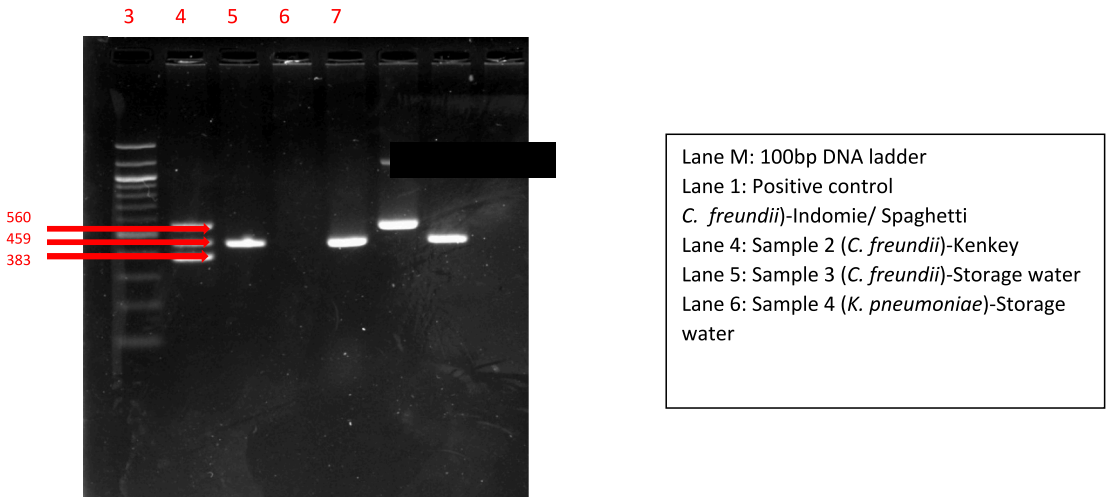

Fig. 4. Multiplex PCR assay for detecting bla_{SHV} and bla_{TEM} genes from phenotypically positive isolates.

involved in food spoilage and fermentations (Franz et al., 2011). Being commensals in the gut of humans and animals, their presence in food could lead to gastroenteritis, especially among immunocompromised individuals (Pesavento et al., 2014; Yeboah-Manu et al., 2010). *E. faecalis* was isolated in majority of the RTE foods implicating widespread occurrence and tendency to contribute to food spoilage, especially in the absence of refrigeration at majority of the vending outlets.

E. coli was isolated from two of the food samples, known to cause foodborne outbreaks. Their presence is an indication of fecal contamination which could be due to raw materials and unhygienic conditions during food preparation as well as an indicator of the presence of other enteric pathogens (Dewey-Mattia et al., 2018; Lima et al., 2017). The presence of *K. pneumoniae, A. baumannii* and *Aeromonas* spp. although of minimal recovery are pathogenic and of public health importance when enumerated from RTE food (Abakari et al., 2018; Anning et al., 2019; Hartantyo et al., 2020; Hassan et al., 2018; Mensah et al., 2002; Nyenje et al., 2012; O'Neill, 2016; Rane, 2011; Tellevik et al., 2016; Yeboah-Manu et al., 2010).

Occurrence of the bla_{TEM} gene in *Citrobacter freundii* from two food items ('kenkey' and spaghetti) concurs with other RTE studies' findings showing the frequent detection of bla_{TEM} among Enterobacterales from food (Dsani et al., 2020; Liu et al., 2017; Ye et al., 2018). The monitoring of ESBLs among FBPs should be encouraged based on their AMR transmission potential from RTE food. Detection of the two ESBL gene types (bla_{SHV} and bla_{TEM}) in *C. freundii* and *K. pneumoniae*, isolated from storage water is an indicator of a community exposure when transmitted.

The presence of the *Lt* gene in ETEC among two of the *E. coli* isolates from food shows their possible transfer to humans which could lead to diverse forms of diarrhoea, including traveler's diarrhoea and diarrhoea in children under five (Lima et al., 2017; Qadri et al., 2005). Stool samples from diarrhoea patients in a study conducted by Dela and colleagues established the *Lt* gene of ETEC as the only DEC present (Dela et al., 2022). Occurrence in two *E. coli* isolates from food could be an indication of being the dominant strain in Ghana.

In this study, *K. pneumoniae* (20.8 %), *Aeromonas* spp. (16.67 %), *E. cloacae* (11.11 %) found in water could be attributed to fecal contamination as they considered commensals in soil or groundwater (WHO, 2011). Reuse of water without frequent changing coupled with lack of proper hand hygiene and the improper handling of money have been associated with the recovery of such FBP in Ghana and other countries (Barro et al., 2006; Bintsis, 2017; Hassan et al., 2018; Mensah et al., 2002; Rane, 2011).

Based in similarities of AMR in humans, animals and environment, monitoring the trend in RTE food is important since it could lead to difficult to treat enteric infections upon transmission (WHO/Europe, 2011). Over 50 % of the most recovered Enterobacterales in RTE food (*E. cloacae*, *C. freundii*) showed resistance to some of the most frequently used antibiotics in Ghana (Table 6). Studies carried out in Ethiopia and Bangladesh showed resistance to Sulfamethoxazole-trimethoprim, Tetracycline and Amoxicillin-clavulanate similar to this study's resistance panel of an emerging AMR trend and consistent to the a six-month nationwide surveillance in Ghana, carried out by Newman and Opintan (Amare et al., 2019; Banik et al., 2019; Eromo et al., 2016; Newman and Opintan, 2015). The low AMR to Rifampin (70 %) and Vancomycin (40 %) in *E. faecalis* and *Staphylococcus* spp. in this study was inconsistent with high percentages of resistance among non-Enterobacterales recorded in an RTE study in Italy (Pesavento et al., 2014). The presence of Vancomycin resistant *Enterococci* (VRE) in the study is an indication of complicated diarrhoea treatment upon transmission. The possibility of AMR transfer to non-pathogenic bacteria is another characteristic of resistant strains of *Enterococcus* spp., thus leading to spread and persistence (Pesavento et al., 2014).

Water plays a role during SFV and as such, routine investigations are recommended in securing the appropriate treatment algorithms during waterborne outbreaks. Unfortunately, there is limited information of AMR profile involved in SFV operations unlike sachet and bottled water studies that have been carried out in Ghana (Addo et al., 2009; Mosi et al., 2019). This study showed a 50 % resistance to some of the most commonly used antibiotics (Opintan et al., 2015) such as tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol for the treatment of gastroenteritis in Ghana, similar to a study conducted among stored drinking water showed resistance to Cephalosporins, Fluoroquinolones, Tetracycline and Penicillins among some Enterobacterales (Amoah et al., 2006). AMR in *Staphylococcus* spp. and *E. faecalis* to some of the recommended antibiotics of treatment is an indication of difficult to treat gastroenteritis of water origin which could be as a result of possible skin (hand) and fecal-oral transmission respectively (Bintsis, 2017; Rane, 2011; WHO, 2011).

In conclusion, the presence of common pathogens such as *E. cloacae*, *K. pneumoniae* and *E. faecalis* in RTE food and vending water shows their misplacement and a possible occurrence of nosocomial diseases. Similarities of pathogens outlined in this study to show resistance to frequently used antibiotics that could lead to difficult to treat diarrhoea upon transmission and persistence in the environment. The occurrence of *Lt* gene of ETEC in two food samples is an indication of possible epidemiology of DEC in Ghana. Although the overall microbiological quality of RTE food was considered safe despite borderline interpretations, some of the individual food items revealed their unhygienic and unsafe conditions. Targeted education of food vendors and

Table 6
Antibiotic resistance profiles of Enterobacterales isolates from food samples.

Pathogens	Antibiotics												
	SXT(n)	TE(n)	F(n)	CN(n)	NA(n)	AZM (n)	AK(n)	C(n)	MEM(n)	CIP(n)	CRO(n)	CAZ(n)	AMC(n)
Enterobacter cloacae (n = 30)	4	10	23	1	1	11	2	2	0	1	0	0	18
Citrobacter spp (*n* = 18)	14	12	8	1	3	4	1	1	1	0	1	1	12
Klebsiella pneumoniae (*n* = 7)	0	0	3	0	0	2	1	0	0	0	0	0	1
Aeromonas spp (n = 2)	0	0	1	0	1	1	0	0	0	0	0	0	1
Escherichia coli (n = 3)	0	1	0	0	2	0	0	0	0	0	0	0	0
Klebsiella oxytoca (n = 1)	0	0	0	0	0	1	0	0	0	0	0	0	0
Proteus mirabilis (n = 1)	0	1	1	0	0	0	0	0	0	0	0	0	0
Escherichia spp (n = 1)	1	1	1	0	0	0	0	0	0	0	0	0	1
Serratia ficaria (n = 1)	0	1	0	0	0	0	0	0	0	0	0	0	0

Key: SXT- Sulfamethoxazole-trimethoprim; TIM- Ticarcillin-clavulanate; TE-Tetracycline; F-Nitrofurantoin; CN-Gentamicin; NA- Nalidixic acid; TZP- Piperacillin-tazobactam; AZM-Azithromycin; AK-Amikacin; C- Chloramphenicol; MEM-Meropenem; CIP- Ciprofloxacin; CRO- Ceftriaxone; CAZ- Ceftazidime; AMC- Amoxicillin-clavulanate.

Table 7
Antibiotic resistance profiles of Enterobacterales isolates from water and palm swab samples.

Pathogens	Antibiotics							
	SXT (n)	TE (n)	F (n)	NA (n)	AZM (n)	C (n)	CRO (n)	AMC (n)
Enterobacter cloacae (*n* = 8)	3	2	0	0	2	0	0	6
Enterobacter spp (*n* = 5)	0	3	1	1	2	1	0	3
Citrobacter spp (*n* = 4)	3	3	2	1	2	1	1	3
Klebsiella pneumoniae (*n* = 15)	1	5	8	1	7	2	1	2
Aeromonas spp (*n* = 12)	2	1	1	0	0	0	0	0

Key: SXT- Sulfamethoxazole-trimethoprim; TE-Tetracycline; F-Nitrofurantoin; NA- Nalidixic acid; AZM- Azithromycin; C- Chloramphenicol; MEM-Meropenem; CRO- Ceftriaxone; CAZ- Ceftazidime; AMC- Amoxicillin-clavulanate.

regular inspection of vending sites could improve the microbiology quality of RTE food and water.

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Ethical approval statement

Ethical approval was obtained from the Noguchi Memorial Institute for Medical Research (NMIMR) and the Ghana Health Service (GHS) Institutional Review Boards (IRB) with the approval numbers NMIMR-IRB CPN 097/17–18 and GHS-ERC001/08/18 respectively. Street food vendors who participated in the study were administered written consent after detailed explanation in the presence of witnesses who gave written authorization as well.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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