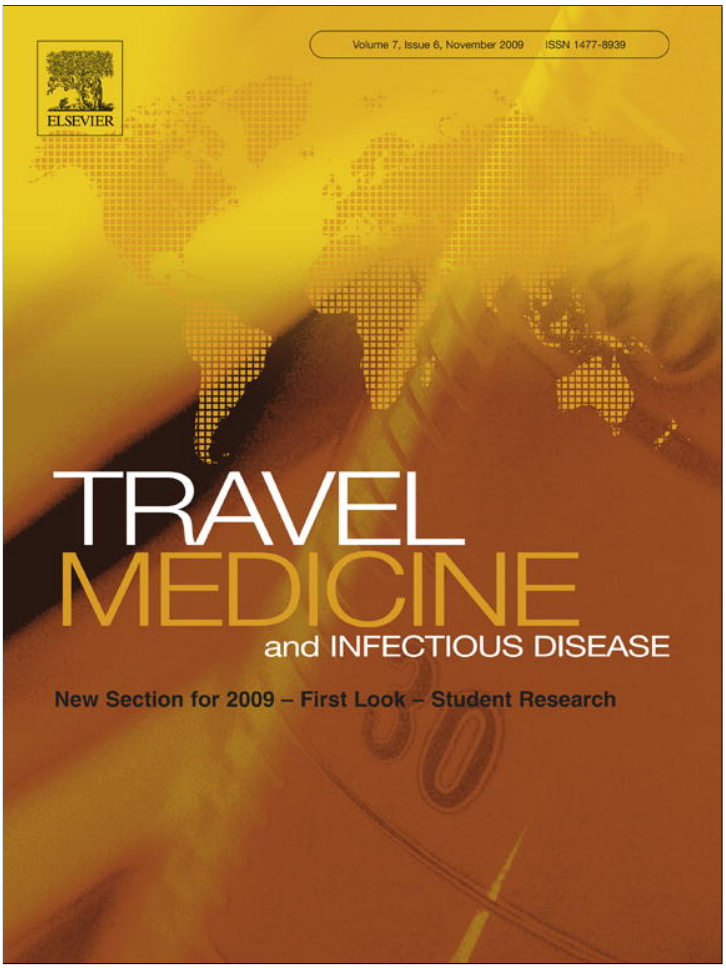

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Pathogenic *Escherichia coli* and food handlers in luxury hotels in Nairobi, Kenya

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Summary *Background:* The epidemiology and virulence properties of pathogenic *Escherichia coli* among food handlers in tourist destination hotels in Kenya are largely uncharacterized.
Method: This cross-sectional study among consenting 885 food handlers working in nine luxurious tourist hotels in Nairobi, Kenya determined the epidemiology, virulence properties, antibiotics susceptibility profiles and conjugation abilities of pathogenic *Escherichia coli*.
Result: Pathogenic *Escherichia coli* was detected among 39 (4.4%) subjects, including 1.8% enteroaggregative *Escherichia coli* (EAEC) harboring *aggR* genes, 1.2% enterotoxigenic *Escherichia coli* (ETEC) expressing both *LT* and *STp* toxins, 1.1% enteropathogenic *Escherichia coli* (EPEC) and 0.2% Shiga-like *Escherichia coli* (EHEC) both harboring *eaeA* and *stx2* genes respectively. All the pathotypes had increased surface hydrophobicity. Using multivariate analyses, food handlers with loose stools were more likely to be infected with pathogenic *Escherichia coli*. Majority 53.8% of the pathotypes were resistant to tetracycline with 40.2% being multi-drug resistant. About 85.7% pathotypes trans-conjugated with *Escherichia coli* K12 F^- NA^r LA.
Conclusion: The carriage of multi-drug resistant, toxin expressing pathogenic *Escherichia coli* by this population is of public health concern because exposure to low doses can result in infection. Screening food handlers and implementing public awareness programs is recommended as an intervention to control transmission of enteric pathogens.

Introduction

Kenya is one of the world's great tourism destinations, known for its remarkable diversity of landscapes, wildlife and culture.[1] Currently, this industry faces threats of decline because among other factors food borne diseases

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are a major threat.[2] About 50% of international travelers including tourists experience diarrhea as a result of ingesting water and or food contaminated with feces.[3] Consequently, food borne illnesses are very costly for tourism industry, health services, and society as a whole.

The transmission of enteric pathogens including helminths, protozoa, and enteropathogenic bacteria occurs directly or indirectly by food, water, nails, and fingers contaminated with feces indicating the importance of fecal–oral person-to-person transmission.[4] Consequently, food handlers with poor personal hygiene working in food serving establishments are potential sources of infection with enteric pathogens.[5] Food handlers harboring and excreting enteric pathogens may contaminate foods with fecal material via their fingers to food processing and eventually to healthy individuals.[6]

Escherichia coli causing diarrhea is among enteric pathogens that cause intestinal infections in both the temperate and the tropical areas of the world. In Kenya, 30% of all cases of infantile diarrhea are caused by bacterial diarrhea[7] and it is also the most common cause of travelers' diarrhea.[8,9] Most of these pathogenic *Escherichia coli* isolates exhibit multidrug resistance to common antibiotics available in Kenya through prescriptions to the public.[7]

The involvement of food handlers working in tourist destination hotels in the transmission of pathogenic *Escherichia coli* associated with travel diarrhea has not been studied. The aim of the study was to determine the epidemiology and the virulent properties of pathogenic *Escherichia coli* among food handlers in selected tourist hotels in Nairobi, Kenya.

Materials and methods

Design and study population

The supply of tourism enterprises in Nairobi province includes about 28 tourist hotels, 50 tour operators and 13 curio shops. The number of tourists visiting many Kenya's national parks and reserves are concentrated mainly in Nairobi.[1]

As a public health requirement in Kenya, food handlers in food industries are routinely screened for intestinal infections including protozoa and helminths in their stool, salmonella infection in their blood and urinary tract infection in their urine twice a year as a strategy to control transmission of food borne diseases. The Center for Microbiology Research of Kenya Medical Research Institute (CMR-KEMRI), has been contributing in this service since the year 2000 under the Hospitality Industry Support Program (HISP) with a clientele of about 148 food serving hotels and industries including 19 luxury tourist hotels.

This cross-sectional study was designed to determine the characteristics of pathogenic *Escherichia coli* among random samples of 885 food handlers from conveniently selected nine tourist hotels in Nairobi under the CMR-KEMRI HISP. Food handlers including waiters, cooks, chefs, barmen, butchers and delivery personnel working in the selected luxurious tourist hotels in Nairobi for at least past six months, were at least 18 years old, handled food and water at least once in the past two weeks and were able to understand and give informed consent were enrolled in the study. Stool samples were collected from each of the food handlers in small sample tubes at the hotels and transported to CMR-KEMRI, Nairobi Kenya for culture and further assays. This study was approved by the ethical review committee of the Kenya Medical Research Institute.

Laboratory assays

Isolation and identification of organisms

One swab from each stool samples was inoculated aseptically onto MacConkey agar and incubated aerobically at 37 °C for 18–24 h. The pink/red lactose-fermenting colonies from each subject from each plate were picked and preserved in one trypticase soy broth with 15% glycerol until tested. The colonies were inoculated into Triple Sugar Iron (TSI), Motility Indole Ornithine (MIO), Simon's citrate agar and urea agar for identification based on Biochemical chart for *Enterobacteriaceae*, *Aeromonas* and *Plesiomonas*.[10]

Antibiotic susceptibility testing

Antibiotics used for management of enteric bacterial infections including ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), co-trimoxazole (25 μg), cefuroxime (30 μg), ciprofloxacin (1 μg), cefotaxime (10 μg), amoxicillin-clavulanate (30 μg), and tetracycline (10 μg), were used for sensitivity testing using the Kirby–Bauer disc diffusion method of Bauer.[11] Sensitivity and/or resistance of isolates were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS).[12] The *Escherichia coli* ATCC 25922 was used as a control for drug sensitivity and growth.

Determination of antibiotic minimum inhibitory concentrations

The minimum inhibitory concentration (MIC) defined as the lowest concentration of the antibiotic to prevent visible growth of the isolated *Escherichia coli* was determined by the agar dilution technique as described by the American Society for Microbiology and revised by the NCCLS.[12,13] Pure antibiotic powders of ampicillin, chloramphenicol, gentamicin, cefuroxime, ciprofloxacin, tetracycline, and amoxicillin-clavulanate acid were used to prepare doubling dilutions of the antibiotics in Mueller–Hinton agar.[12,13] The concentrations to be tested were determined by the interpretative breakpoints as provided by NCCLS using *Escherichia coli* ATCC 25922 as control.

Detection of enterotoxin among pathogenic *Escherichia coli*

An enterotoxin assay was carried out using DNA hybridization test as described by Tamatsukuri et al.[14] The salting out method for the quantitation of the hydrophobic surface properties of bacteria was undertaken.[15] Briefly, the bacteria were grown at 37 °C for 18 h on nutrient agar slopes and suspended in 0.002 M sodium phosphate buffer (pH 6.8) at a concentration of about 5×10^9 bacteria per ml. About 25 μl of the bacterial suspension was mixed with an equal volume of varying concentrations of ammonium sulphate in 0.002 M sodium phosphate (pH 6.8).

The transconjugation assays

Plasmid DNA extraction and profiling were carried out for all the pathogenic *Escherichia coli* isolates according to the method described by Birnboimh and Doly.[16] The *in vitro* conjugation tests on transferable antimicrobial resistance to recipient *Escherichia coli* K12 (F^- Na^r Lac^+) strain were conducted according to the method of Walia et al.[17]

Statistical analyses

The overall and type-specific pathogenic *Escherichia coli* prevalence was determined for all the food handlers. In bivariate analyses, prevalence ratios (PR) and 95% confidence intervals (CI) for the association between pathogenic *Escherichia coli* infection and demographic or behavioral characteristics was calculated using Poisson regression. In multivariate analyses, a manual backward elimination approach was used to reach the most parsimonious model including factors that were associated with pathogenic *Escherichia coli* infection at the significance level of $p \leq 0.05$. All statistical analyses were performed using STATA v 9.2 (StataCorp LP, Texas USA).

Results

Characteristics of the study population

A total of 885 food handlers working in 9 conveniently selected luxurious tourist hotels in Nairobi were enrolled. Of these 885 food handlers enrolled, their mean age was 30 years (range: 19—63 years) while majority 73.7% were men. About 43.2% were aged 21—30 years while 41.7% had formed stools.

Prevalence of intestinal infection

The prevalence of pathogenic *Escherichia coli* isolated from the food handlers was 39/885 (4.4%), which included 1.8% enteroaggregative *Escherichia coli* (EAEC), 1.2% enterotoxigenic *Escherichia coli* (ETEC), 1.1% enteropathogenic *Escherichia coli* (EPEC) and 0.2% Shiga-like *Escherichia coli* (EHEC). Approximately 58.3% of these pathotypes were isolated from loose stools. Stool samples from 1.5% of food handlers had other intestinal infections including protozoa such as 0.8% *Entamoeba coli* and 0.1% *Entamoeba histolytica*/*Entamoeba*dispar and helminths including; 0.2% hookworm, 0.2% *Trichuris trichiura* and 0.1% *Ascaris lumbricoides* (Fig. 1).

Overall, of the 4.4% pathogenic *Escherichia coli* isolated; 5.2% were isolated from men, 7.8% were isolated from food handlers aged 31—40 years of age, 63.6% had loose stools, while 15.3% were isolated among food handlers sampled from hotel 2. Mixed infection with different *Escherichia coli* pathotypes or a co-infection between pathogenic *Escherichia coli* and other intestinal parasites was not found in any individual (Table 1).

Demographic characteristics associated with pathogenic *Escherichia coli* infection

Table 1 shows the characteristic-specific prevalence of pathogenic *Escherichia coli* infection and the corresponding bivariate and multivariate PRs and 95% CIs. In bivariate analyses, food handlers who had semi-formed stool (PR 18.57, 95% CI 1.9—178.5), loose stool (PR 26.4, 95% CI 35.5—195.4) and loose mucoid stool (PR 27.85, 95% CI 2.5—307.2) were more likely to be infected with pathogenic *Escherichia coli* than those food handlers who had formed stools.

In multivariate analyses, semi-formed (PR 26.67, 95% CI 2.37—300.08), loose (PR 27.8, 95% CI 32.41—238.19) and loose mucoid stools (PR 31.10, 95% CI 2.34—414.04) remained the only factors associated with pathogenic *Escherichia coli* infection.

The characteristics of pathogenic *Escherichia coli* isolated

Table 2 shows the pathogenic *Escherichia coli* toxin and serotypes isolated from the food handlers and their corresponding stool type. Overall, 11/39 (28.2%) of the isolated pathogenic *Escherichia coli* were typed. The serotypes indentified included; 2/39 (5.1%) each of the O157 and O128. Other serotypes identified included 1/39 (2.6%) each of O18, K88, O115, O119, O126, O159 and O63.

The isolated pathogenic *Escherichia coli* among these food handlers were carriers of different toxins. All the 10/39 (25.6%) EPEC isolated from food handlers with loose stool were carriers of *eaeA* toxin. Out of the 11/39 (28.2%) ETEC isolated, 6/39 (15.4%) and 5/39 (12.8%) had toxin types *STp* and *LT* respectively. About 36.4% of these isolates were from loose stools. About 15/39 (38.5%) of EAEC had *aggR* toxin type

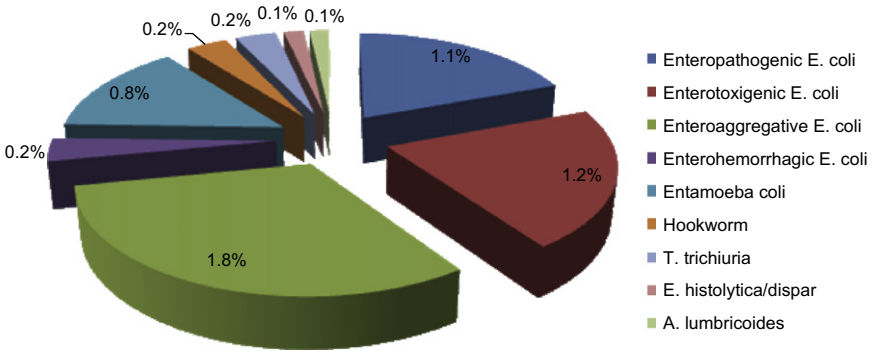

Figure 1 Percent distribution of the intestinal infection among food handlers.

Table 1 Characteristic-specific prevalence of pathogenic *Escherichia coli* infection and the corresponding bivariate and multivariate PRs and 95% CIs.

Characteristic	Sample size	Food handlers infected with enteropathogenic *Escherichia coli*		Bivariate	Multivariate
		No.	%	PR (95% CI)	PR (95% CI)
Sex					
Male	652	34	5.21	Referent	Referent
Female	233	5	2.15	0.41 (0.16, 1.05)	0.76 (0.29, 2.04)
Age in years					
18–20	5	0	0	Referent	Referent
21–30	383	14	3.66	0.79 (0.39, 1.58)	0.55 (0.23, 1.35)
31–40	365	19	5.21	1.43 (0.76, 2.72)	1.27 (0.59, 2.68)
41–50	64	5	7.81	1.55 (0.58, 4.18)	0.95 (0.33, 2.78)
>51	30	1	3.33	0.65 (0.08, 4.87)	0.72 (0.09, 5.91)
Stool type					
Formed	369	12	3.25	Referent	Referent
Semi-formed	63	3	4.76	18.57 (1.9, 178.5)	26.67 (2.37, 300.08)
Loose	33	21	63.63	26.4 (35.5, 195.4)	27.8 (32.41, 238.19)
Loose mucoid	33	3	9.09	27.85 (2.5, 307.2)	31.10 (2.34, 414.04)
Other intestinal infections					
Entamoeba coli	7	0	0		
A. lumbricoides	1	0	0		
Hookworm	2	0	0	NS	NS
E. histolytica	2	0	0		
T. trichuria	2	0	0		
Destination hotels					
Hotel 1	53	3	5.66		
Hotel 2	72	11	15.28		
Hotel 3	60	0	0		
Hotel 4	185	7	3.78		
Hotel 5	126	9	7.14	NS	NS
Hotel 6	53	0	0		
Hotel 7	82	4	4.88		
Hotel 8	45	1	2.22		
Hotel 9	209	4	1.91		

NS — not significant; PR — prevalence ratio; CI — confidence interval; No. — number; % — percentage.

while 1/39 (2.6%) had both *LT* and *aggR* toxin type and 20.5% of the EAEC were isolated from loose stools. All the 2/39 (5.1%) EHEC had toxin type *stx2* and were isolated from loose stools.

The lowest molar dilution of ammonium sulphate resulting in auto-agglutination for the isolated 39 *Escherichia coli* pathotypes (median 0.6 M) was significantly lower ($p < 0.001$) than that of control isolates (median 2 M).

The distribution of resistant types among *Escherichia coli* pathotypes

Table 3 shows the pathogenic *Escherichia coli* antibiotics susceptibility profile. The isolates had varied antibiotic resistance levels ranging from cefotaxime 2.6%, gentamicin 5.1%, ciprofloxacin 7.7%, cefuroxime 10.3%, chloramphenicol 10.3%, amoxicillin-clavulanic acid 15.4%, ampicillin 46.2%, sulphamethoxazol/trimethroprim 51.3% and tetracycline 53.8% (Table 3). The pathotypes were significantly resistant to tetracycline than the other antibiotics ($\chi^2 = 3.172$, df = 8, $P = 0.013$). Multi-drug resistance (resistance to three or more antibiotics) was found in 16/39 (40.15%) isolates. The most common multi-drug resistance was to amoxicillin-clavulanic, sulphamethoxazol/trimethroprim and tetracycline combined. One isolate each was resistant to five different antibiotics including one isolate to chloramphenicol, amoxicillin-clavulanic, ampicillin, sulphamethoxazol/trimethroprim and tetracycline and another isolate to chloramphenicol, gentamicin, ampicillin, sulphamethoxazol/trimethroprim and tetracycline.

The MIC for all the 39 *Escherichia coli* was tested using the *E*-test method. Very high concentrations were required to achieve MIC_{90} for tetracycline (>64 μg/mL) and sulphamethoxazol/trimethroprim (>1024 μg/mL).

Conjugation among pathogenic *Escherichia coli*

Table 4 shows frequencies of plasmids among the pathogenic *Escherichia coli* isolates and the corresponding *Escherichia coli* K12 F^- NA^r LA conjugates. The majority (89.7%) of the isolated pathogenic *Escherichia coli* had different plasmid types ranging in fragment size from 6 to

Table 2 The characteristics of *Escherichia coli* pathotypes and other associated data.

Pathotype	Hotel	Stool type	Toxin	Serotypes
Enteropathogenic *Escherichia coli* (EPEC)				
	Hotel 2	Loose	*eaeA*	UT
	Hotel 2	Loose	*eaeA*	UT
	Hotel 2	Loose	*eaeA*	O63
	Hotel 5	Loose	*eaeA*	O157
	Hotel 9	Loose	*eaeA*	UT
	Hotel 9	Loose	*eaeA*	O157
	Hotel 4	Loose	*eaeA*	UT
	Hotel 4	Loose	*eaeA*	UT
	Hotel 4	Loose	None	UT
	Hotel 4	Loose	*eaeA*	UT
Enterotoxigenic *Escherichia coli* (ETEC)				
	Hotel 2	Loose mucoid	*LT*	UT
	Hotel 2	Formed	*LT*	UT
	Hotel 2	Formed	*STp*	UT
	Hotel 5	Formed	*LT*	UT
	Hotel 5	Formed	*LT*	UT
	Hotel 1	Loose	*STp*	UT
	Hotel 7	Formed	*STp*	UT
	Hotel 7	Formed	*LT*	UT
	Hotel 7	Formed	*STp*	UT
	Hotel 7	Loose	*STp*	K88
	Hotel 8	Loose	*STp*	18
Enteroaggregative *Escherichia coli* (EAEC)				
	Hotel 2	Formed	*aggR*	UT
	Hotel 2	Formed	*aggR*	UT
	Hotel 2	Loose	*aggR*	UT
	Hotel 2	Loose	*aggR*	UT
	Hotel 5	Formed	*aggR*	UT
	Hotel 5	Semi-formed	*aggR*	UT
	Hotel 5	Formed	*LT* and *aggR*	UT
	Hotel 5	Semi-formed	*aggR*	UT
	Hotel 5	Semi-formed	*aggR*	O126
	Hotel 9	Loose	*aggR*	O128
	Hotel 9	Loose	*aggR*	O128
	Hotel 4	Loose	*aggR*	UT
	Hotel 4	Loose	*aggR*	UT
	Hotel 4	Loose	*aggR*	UT
	Hotel 1	Formed	None	UT
	Hotel 1	Loose	*aggR*	O119
Enterohemorrhagic *Escherichia coli* (EHEC)				
	Hotel 2	Loose mucoid	*stx2*	O159
	Hotel 5	Loose	*stx2*	UT

UT — untyped.

>100 Mega Dalton (MDa). Plasmid of fragment size 56 MDA was the majority (12.8%) type. About 30/35 (85.7%) of pathogenic *Escherichia coli* conjugated with the standard strain of *Escherichia coli* K12 F^- NA^r LA. The plasmid of fragment sizes ranging from 4.5 to 58 MDA were isolated from the transconjugant *Escherichia coli* K12 F^- NA^r LA with 15.4% with a plasmid of fragment size 49.6 MDA.

Discussion

The results are among the first comprehensive studies on the epidemiology and virulence characteristics of pathogenic *Escherichia coli* isolated from food handlers in tourist hotels in Nairobi. The prevalence of infection with pathogenic *Escherichia coli* among the food handlers in the tourism industry was 4.4%. EAEC was the most common pathotype while EHEC was the least common. A food handler co-infected with more than one *Escherichia coli* pathotypes or a co-infection between *Escherichia coli* pathotypes and other identified intestinal parasites was not identified. A study among food handlers in Kenya identified 2.1% EAEC and 0.9% EPEC infection[7] while in Ethiopia; a prevalence of 3.1% of pathogenic *Escherichia coli* was found.[18] Several studies have stressed the importance of food handlers in the transmissions of parasitic and bacterial

Table 3 MIC resistance range, MIC_{50}, and MIC_{90} of the *Escherichia coli* isolates.

Antibiotics	No. (%) resistant	MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
Chloramphenicol	4 (10.4)	4 to >128	4	64
Amoxicillin-clavulanate	6 (15.4)	4 to 16	4	16
Gentamicin	2 (5.1)	1 to 4	2	4
Ciprofloxacin	3 (7.7)	<0.125	<0.125	<0.125
Ampicillin	18 (46.2)	2 to >128	4	>128
Sulphamethaxazol/trimethroprim	20 (51.3)	128 to >1024	>128	>1024
Cefuroxime	4 (10.3)	2 to >128	4	16
Tetracycline	21 (53.8)	1 to >64	>64	>64
Cefotaxime	1 (2.6)	2 to 128	4	64

MIC — minimum inhibitory concentration.

diseases.[18–23] Evidence suggests that the infectious dose for pathogenic *Escherichia coli* is very low, as few as 10 organisms[24]; hence the potential for person-to-person transmission among sporadic pathogenic *Escherichia coli* cases might be expected to be far greater than among sporadic cases of other common gastrointestinal pathogens.[25] It is recognized that the prerequisite for the control and prevention of intestinal parasitosis and diseases due to enteropathogenic bacteria is a clear understanding of their epidemiology as the information guides the design of the most practical and economic control and preventive measures.

Independent risk factors for pathogenic *Escherichia coli* infection consistent with previous reports, and some unique factors were identified. Food handlers who had loose stools remained the only factor associated with pathogenic *Escherichia coli* infection. Reports indicate that individuals with diarrhea are both at risk of transmitting and being infected probably secondary to a relative lack of immunity and increased fecal—oral contamination.[3] In the current study not only were these pathogenic *Escherichia coli* isolated from loose stools, a significant number were isolated from diarrheal asymptomatic food handlers suggesting a clear likelihood for the food handlers to transmit these pathotypes to tourists when they handle food. No association between gender and pathogenic *Escherichia coli* infection was found. This is consistent with the observation of Evans et al.[26] indicating gender as having no influence on the incidence of diarrhea in travelers.

Separate studies have identified other independent factors associated with traveler's diarrhea which were either not measured or found to be significant in the current study, including age.[27] The highest incidence was reported among young adults aged 21—29 years probably due to a lack of vigilance in avoiding contaminated food combined with a more adventurous lifestyle.[28] Hotel type,[29] dining in expensive restaurants or luxury hotels have been associated with several outbreaks. Studies of food handlers reported the destination as the single most important risk factor for developing traveler's diarrhea. Developing countries of Latin America, Africa, Asia, and parts of the Middle East, considered as high-risk regions have reported attack rates for traveler's diarrhea ranging between 20% and 75%.[3,30,31] The cross-sectional nature of this study and inadequate assessment of other key hygienic and behavior factors, could explain the observed lack of association between pathogenic *Escherichia coli* infection and the independent factors listed above.

Our study isolated *Escherichia coli* O157 serotypes more commonly. This serotype has been linked to most outbreaks, suggesting that it is a more virulent serotype. Nevertheless, other serotypes of Stx-producing *Escherichia coli* have been implicated in both sporadic disease and outbreaks.[32]

All the isolated pathogenic *Escherichia coli* had different toxin genes; including either *STp* or *LT* or both combined from ETEC. Generally, ETEC producing *STa* alone or both *STa* and *LT* are associated with more severe symptoms than those producing only *LT*.[33] ETEC producing *LT*, *STa*, or both toxins have been isolated from travelers with diarrhea.[8,34] The *LT* and ST toxins act by inducing extrusion of chloride ions and water into the intestine, inhibit sodium and chloride absorption and interacts with the enteric nervous system enhancing the intestinal secretory cascade.[35] Majority of the EAEC isolated harbored the *aggR* gene. The *aggR* gene controls the expression of adherence factors, a dispersin protein, and a large cluster of genes encoded on the EAEC

Table 4 The plasmid types of the *Escherichia coli* isolates and corresponding *Escherichia coli* K12 F^- NA^r LA conjugates.

Enteropathogenic *Escherichia coli*		*Escherichia coli* K12 F^- NA^r LA	
Size (MDA)	No. (%)	Size (MDA)	No. (%)
6	4 (10.3)	4.5, 4.9	3 (8.6)
7	3 (7.7)	4.9, 5.3	2 (5.7)
7.2, 6	2 (5.1)	46	4 (11.4)
46	4 (10.3)	47	1 (2.9)
51, 7.2	2 (5.1)	47, 6	2 (5.7)
51, 42, 7.2	3 (7.7)	48	3 (8.6)
51, 44, 17	1 (2.6)	49, 6	6 (17.1)
51, >100	4 (10.3)	51	5 (14.3)
56	5 (12.8)	51, 4.6	2 (5.7)
58, 47	1 (2.6)	54	3 (8.6)
>100, 41	3 (7.7)	56, 6	1 (2.9)
>100, 46	3 (7.7)	58	2 (5.1)
None	4 (10.3)	None	5 (14.3)
Total	**39**		**35**

MDa — Mega Dalton.

chromosome.[36] The EPEC, linked to infant diarrhea in the developing world in our study all expressed *eaeA* toxins. The hallmark of infections due to EPEC is characterized by effacement of microvilli and intimate adherence between the bacterium and the epithelial cell membrane encoded for by the eae genes, observed in intestinal biopsy.[37] The major virulence factor, and a defining characteristic of EHEC, is Stx; a potential cytotoxin and is the factor that leads to death. The two EHEC isolated from our study expressed the sxt2 toxin. One possible mechanism for fluid secretion in response to Stx involves the selective destruction of absorptive villus tip on intestinal epithelial cells by Stx.[37]

Pathogenic *Escherichia coli* have adhesive properties which are mirrored by an increase in surface hydrophobicity.[38] All the isolated *Escherichia coli* pathotypes required the lowest concentration of ammonium sulphate to cause auto-agglutination than the controls meaning that they had increased surface hydrophobicity. The *Escherichia coli* which cause diarrhea in man[39] and animals[40] possess an adhesive property which is a virulence factor, without it, toxin producing and enteropathogenic organisms lose their pathogenic potential.

We identified 48.7%, 53.8% and 56.4% *Escherichia coli* isolates resistant to ampicillin, sulphamethoxazol/trimethroprim and tetracycline respectively. More than half, 61.5%, of these isolates were resistant to more than two different drug regimens. In Nigeria, resistance pattern of 80.9% ampicillin, 95.4% tetracycline and 46.5% chloramphenicol were observed[41] while in Tanzania resistance rates of 83.1% to ampicillin, 57% chloramphenicol, 87.7% tetracycline and 90.8% co-trimoxazole were found.[42] Of major concern now is that while all drugs which were more resistant are cheap, inexpensive and available and could have been abused, drugs such as ciprofloxacin and cefotaxime which are reserve antibiotics in Kenya, showed considerable increasing resistance. This indicates misuse of these and other classes of antibiotics which would have major implications in the treatment of *Escherichia coli* causing diarrhea and drug policies in Kenya and other developing and developed countries.

Antibiotic resistance has been shown to be plasmid mediated.[43] As demonstrated in this study, there is a strong possibility that some of the drug resistance conferred by *Escherichia coli* causing diarrhea was plasmid mediated as demonstrated by the ease of transfer of resistance to *Escherichia coli* K12 F^- NA^r LA by conjugation. Mckeon et al.[44] and Adams et al.[45] found strong evidence of plasmid conferring multi-drug resistance in the *enterobactericiae*.

The limitation of the current study included lack of additional data from the subjects such as personal hygiene information including hand-washing and sanitation disposal, samples from other sources such as the fingers and contact tracing of people reporting diarrhea after visiting hotels where these pathogenic *Escherichia coli* were isolated. These data could have demonstrated the actual involvement of the food handlers in the transmission dynamics of pathogenic *Escherichia coli*.

Conclusion

The presence of multi-drug resistant, toxin expressing with ease to trans-conjugate pathogenic *Escherichia coli* among food handlers in Kenya's luxury tourist hotels is of major public health concern. A reliable surveillance system needs to be established to determine the presence of pathogens in food handlers, in processed foods and in travelers consuming the food who develop diarrhea.

Conflict of Interest

The authors state that they have no conflicts of interest.

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