High Incidence of Enteroaggregative *Escherichia coli* Among Food Handlers in Three Areas of Kenya: A Possible Transmission Route of Travelers' Diarrhea

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Background. Contaminated food and water are acknowledged vehicles for the transmission of travelers' diarrhea (TD). Importance of food handlers as reservoirs of enteroaggregative *Escherichia coli* (EAEC), enteropathogenic *E coli* (EPEC), and Shiga toxin–producing *E coli* (STEC) causing TD has not been clearly demonstrated.

Methods. We undertook a 1-year prospective study to determine the presence and selected risk factors of carriage of EAEC, EPEC, and STEC by 1,399 food handlers working in tourist hotels in three popular tourist destinations of Kenya. Enterotoxigenic *E coli* (ETEC) was not sought in this study.

Results. During the period April 2003 to May 2004, EAEC harboring the *aggR* gene were detected from 29 (2.1%) subjects and EPEC harboring the *eaeA* gene and STEC harboring the *stx2* gene were detected from 11 (0.8%) and 2 (0.1%) of the study subjects, respectively. Mean age of subjects with EAEC was significantly lower (24.6 y) than the rest of the study population (28.2 y) ($p < 0.05$). Pit latrines usage was significantly associated with the isolation of EAEC (<0.001) but not with EPEC and STEC. Four of the 29 EAEC isolates were sensitive to all antibiotics tested, and 19 (65.5%) were multidrug resistant (MDR). Antibiotic resistance varied from 6.9% for cefuroxime to 72.4% for co-trimoxazole. Six EPEC isolates (6/13, 46.2%) showed multidrug resistance. Cluster analysis of the pulsed-field gel electrophoresis (PFGE) profiles showed that the EAEC isolates belonged to two clonally unrelated genotypes.

Conclusions. We conclude that food handlers working in tourist hotels are important carriers of EAEC that could cause TD and a high proportion of the EAEC are MDR. The isolation of MDR EAEC from food handlers working in tourist hotels is of potential public health importance. There is a need for a study employing molecular methods including PFGE to examine carriage of similar pathogens in food handlers, processed foods, and travelers consuming the food who develop diarrhea.

Infectious diarrheal diseases continue to be important causes of morbidity and mortality, especially in developing countries.[1–3] Among enteric pathogens, diarrhegenic *Escherichia coli* (DEC) belong to the most common bacteria causing intestinal infections in both the temperate and the tropical areas of the world. In Kenya, bacterial diarrhea has been reported to account for up to 30% of all cases of infantile diarrhea[4] and is the most common cause of travelers' diarrhea (TD).[5–7] Contaminated water and food have been shown to be the most important vehicles for the transmission of TD.[6,8] The importance of food handlers as reservoirs and a possible transmission route of enteroaggregative *E coli* (EAEC) and other causative agents of TD has not been clearly addressed. Most studies have concentrated on TD in tourists and its impact without a concomitant investigation of the food handlers

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working in the tourist hotels. The current study was designed to investigate the epidemiology, etiology, and sanitary factors of carriage of EAEC, enteropathogenic *E coli* (EPEC), and Shiga toxin–producing *E coli* (STEC), which is also referred to as enterohemorrhagic *E coli* (EHEC), isolated from food handlers working in tourist hotels in three popular tourist destinations in Kenya.

Materials and Methods

Study Sites

The study was carried out in Nairobi, Malindi, and Diani, which are some of the main tourist destinations in Kenya. Nairobi, with a population of about 3 million people, is the capital city and has the main international airport used for the arrival of the majority of tourists coming to Kenya.[9] Malindi, whose main economic activity is tourism, is a small, northern coastal town in Kenya with a population of about 53,805 and is one of the major tourist destinations in Kenya.[10] Diani, with a population of about 50,000 people in the south coast of Kenya, has a high concentration of tourist facilities, especially along the beach.

Study Population

The study subjects were all food handlers working in tourist hotels in the three study areas. Inclusion criteria for this study included all food handlers who were defined as any person involved in any way with handling, processing, and serving of food such as waiters, cooks, chefs, barmen, butchers, and delivery people. Excluded from the study were food handlers who refused consent, were away from work during the stool collection period, or workers who were not food handlers.

Stool Samples Collection and Initial Processing

The study subjects were asked to provide stool samples in sterile wide-mouth containers after obtaining written informed consent from them. The form of stool was documented and presence of diarrhea established by asking the study subjects. The study was approved by the Scientific Steering Committee of the Kenya Medical Research Institute (KEMRI) and the National Ethical Committee. Socioeconomic and demographic data on age, sex, water source, sanitation disposal, residence, and antibiotic use in the preceding 2 weeks were also obtained from each individual. The stool samples from workers from Nairobi hotels were transported to the laboratory in chilled boxes. These were processed within 1 hour of collection. For hotels in Malindi and Diani, the stool samples were collected in containers as before and transported chilled to the KEMRI laboratories in Malindi and Kwale for initial processing.

Microbiological Procedures

Isolation, Real-Time Polymerase Chain Reaction for Identification and Serotyping of EAEC, EPEC, and STEC

Stool samples were inoculated onto McConkey agar and incubated aerobically at 37°C for 18 to 24 hours. Five pink/red lactose-fermenting colonies were picked at random from each plate and preserved in Trypticase Soy Broth with 15% glycerol until analyzed. Real-time polymerase chain reaction (PCR) was done by the method described previously.[11] The primers and probes used are shown in Table 1. The specimen was considered to be positive when both pathogen-specific target sequences were amplified and when C_T value was less than 35. In each PCR run, Milli-Q water and bacterial DNA obtained from each of the pathogens served as negative and positive controls, respectively. Commercial *E coli* agglutinating antisera (Denka Seiken, Tokyo, Japan) was used to serotype the EAEC, EPEC, and STEC isolates. The stool samples were also cultured for other enteric bacterial pathogens such as *Salmonella*, *Shigella*, and *Vibrio* species and examined for enteric parasites using standard culture media and routine microbiological techniques.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed on the isolates using the Kirby–Bauer disc diffusion technique.[12] Sensitivity and/or resistance of the isolates were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS).[13] The antibiotics used included 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). These antibiotics were chosen on the basis of their use in the management of enteric bacterial infections. *Escherichia coli* ATCC 25922 was used as a control for drug potency and growth.

Determination of Antibiotic Minimum Inhibitory Concentrations

The minimum inhibitory concentration (MIC) of antibiotics against the test isolates was determined by the agar dilution technique as described by the American Society for Microbiology and revised by the NCCLS.[13,14]

Table 1 Primers and fluorogenic probe sequences for detection of STEC, EPEC, and EAEC

Bacterium	Gene	Sequence (5′–3′)	T_m (°C)
STEC	*stx1*	F: TCTCGACTGCAAAGACGTATGTAGA	59
		R: TCCTGATGAAATAGTCTGTAATGGAGTAC	58
		P: r-TCGCTGAATGTCATTCGCTCTGCAATA-q	68
	stx2	F: ACCCCACCGGGCAGTT	58
		R: GGTCAAAACGCGCCTGATA	58
		P: r-TTTTGCTGTGGATATACGAGGGCTTGATGT-q	68
EPEC	*eaeA*	F: TGTTGCTTTGTTTAATTC(T/C)GATAAGC	57/61
		R: GGAATCGGAGTATAGTTTACACCAA	57
		P: r-AGTCGAATCCTGGTGCGGC-q	62
EAEC	*aggR*	F: ATGCCCTGATGATAATATACGGAATAT	58
		R: TCAGCATCAGCTACAATTATTCCTTT	58
		P: r-AAAAGTAGATGCTTGCAGTTGTCCGAATTGG-q	68

F = forward primer; R = reverse primer; P = Probe; r = reporter; d = dye; q = quencher dye.

The MIC was defined as the lowest concentration of the antibiotic to prevent visible growth of the bacteria. 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.[13] The concentrations to be tested were determined by the interpretative breakpoints as provided by NCCLS. *Escherichia coli* ATCC 25922 was used to control for drug potency and growth.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed using the CHEF-III electrophoresis apparatus (Bio-Rad, Richmond, CA, USA) according to the procedures described earlier[15,16] in 1% pulsed-field certified agarose in 0.5× Tris Borate EDTA buffer (TBE) buffer for 40 hours at 200 V at a temperature of 14°C with the following modified pulse times: 1 to 10 seconds for 10 hours, 3 to 28 seconds for 10 hours, 3 to 35 seconds for 5 hours, and 5 to 70 seconds for 15 hours, a gradient of 6.0 V/s, and a 120 degrees switch angle. The gels were then stained with ethidium bromide, destained, and photographed under ultraviolet illumination on GELDOC 2000(Bio-Rad) system. The DNA size standards used were the bacteriophage lambda ladder (Bio-Rad) ranging from 50 to 1,000 kb.

Results

Demographic Data of the Study Subjects

In this study conducted from April 2003 to May 2004, a total of 1,399 food handlers working in the three designated study areas were recruited and their stool samples examined after obtaining individual informed consent. The hotels sampled varied from low-budget backpackers' hotels to deluxe five-star hotels. There were a total of 19 hotels sampled from Nairobi and 16 from Malindi and 7 from Diani. The demographic data and other characteristics of the study subjects are shown in Table 2. The age of the subjects from whom EAEC was isolated ranged from 15 to 63 years, with the mean age varying between 20 years in Malindi and 30.4 years in Nairobi ($p = 0.108$). It was, however, lower (24.6 y) than for the general study population (28.2 y) ($p > 0.05$). The male-to-female ratio was also different: 7.8:0.3 and 9.3:0.7, respectively. This difference was not statistically significant ($p > 0.05$). The ratio of males to females varied between 7:3 in Nairobi and 8:2 in Malindi ($\chi^2 = 3.97$, $p < 0.05$) to 9:1 in Diani. There were significantly more males than females in Diani study population than for either Nairobi or Malindi ($p < 0.05$). The median age between the three population samples was similar with only slight variations.

Only nine subjects responded to the questions on antibiotic use, water source, and sanitation disposal. There was no difference between the number of subjects who admitted to having used any form of antibiotic in the last 14 days and those who did not ($\chi^2 = 0.80$, $p = 0.368$).

The use of pit latrine as a means of sanitation disposal was significantly associated with the isolation of EAEC ($\chi^2 = 88.04$, $p < 0.001$). The subjects from whom EAEC were isolated were mostly male and were significantly much younger than the rest of the population studied ($\chi^2 = 3.97$, $p < 0.05$).

Incidence of EAEC in the Stools of the Food Handlers Sampled

In total, 29 EAEC isolates were detected. No enteroparasites were detected from any of the subjects from whom an EAEC was isolated.

Table 2 Demographic data and other characteristics of the study subjects

Parameter	No pathogen isolated ($N = 1,316$)	EAEC ($n = 29$), $N = 1,399$	p Value
Age (y)			
Range	15.0–63.0	21.0–51.0	$p = 0.05$
Mean	32.5	24.6	
SD	8.1	16.42	
Sex			
Males	1,021 (77.6%)	27/29 (93.1%)	$\chi^2 = 3.97$, $p < 0.05$
Females	295 (22.4%)	2/29 (6.9%)	
Stool form			
Formed	563 (62.4%)	11	$\chi^2 = 21.95$, $p < 0.0001$
Loose*	82 (9.0%)	10	
Mucoid	27 (3.0%)	0	
Semiformed	222 (24.7%)	8	
Watery*	1 (0.1%)	0	
Mucoid/semiformed	1 (0.1%)	0	
Loose/watery*	5 (0.5%)	0	
Loose/mucoid/blood stained*	1 (0.1%)	0	
Not indicated	1 (0.1%)	0	
Antibiotic use in the last 14 d			
Yes	62/1,316 (4.7%)	1/9	$\chi^2 = 0.80$, $p = 0.368$
No	1,254/1,316 (95.3%)	8/9	
Water source			
Rainwater/borehole	0 (0.0%)	0	$\chi^2 = 0.83$, $p = 0.0001$
Tap water	447 (80.8%)	5/9	
Borehole	84 (14.5%)	1/9	
Well water	5 (0.9%)	3/9	
Sanitation disposal			
Pit latrine	282 (49.6%)	9/9	$\chi^2 = 88.04$, $p < 0.001$
Flush toilet	262 (46.1%)	0	
Other	25 (4.4%)	—	
Parasites	31/1,316 (2.4%)	No O/C seen	0.403

EAEC = enteroaggregative *Escherichia coli*; O/C = Ova/Cysts.
*Indicates diarrhea stool samples.

The isolation rates of EAEC were 1.2% (3/262) in Diani compared to 4% EAEC (10/253) in Malindi and 1.8% EAEC (16/885) in Nairobi. This gave an overall incidence rate of 29/1,399 (2.1%). All the EAEC harbored the *aggR* toxin gene. Only 7 of the 29 of the EAEC isolates were serotypable, and the serotypes were O8, O44, O119, O126, O128 (two isolates), and O127. A total of 42 non-typhi *Salmonella* species were isolated from 262 subjects sampled in Diani, giving an incidence of 16.0%. All the subjects from whom the salmonella were isolated were males. The stools were mostly diarrheic (26/42, 61.9%) with the rest being from semiformed stools. No other enteropathogens were isolated.

Antibiotic Susceptibility Profiles of the Isolates

The isolates had varied antibiotic resistance levels from ciprofloxacin 0%, cefotaxime 3.5%, augmentin 6.0%, gentamicin 6%, cefuroxime 6.9%, chloramphenicol 27.6%, ampicillin 37.9%, co-trimoxazole 72.4%, and tetracycline 79.3%. The two isolates that showed resistance to amoxicillin-clavulanate were tested for extended-spectrum beta lactamase (ESBL) production by the *E*-test strip method, and no ESBL production was detected. Multidrug resistance (resistance to three or more antibiotics) was seen in 19 of the 29 (65.5%) isolates. The most common resistance patterns seen were resistance to ampicillin, co-trimoxazole, and tetracycline by three isolates (10.3%). Six isolates were resistant to one or two antibiotics. No resistance was detected in 4 of the 29 (30.1%) isolates.

Only one subject admitted to having taken an antibiotic in the last 14 days before sampling.

The MIC for 13 of the 29 EAEC was tested using the *E*-test method. Very high concentrations were required to achieve MIC90 for tetracycline (>64 μg/mL) and ampicillin (>256 μg/mL). No resistance to ciprofloxacin, cefuroxime, cefotaxime, coamoxyclav, and gentamicin was detected by the MIC method (Table 3).

Table 3 MIC resistance range, MIC_{50}, and MIC_{90} of the EAEC isolates

Antibiotic tested	No. (%) resistant	MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
Chloramphenicol	1 (7.8)	4 to >256	8	12
Amoxicillin-clavulanate	0 (0.0)	2 to 8	3	6
Gentamicin	0 (0.0)	0.75 to 1.5	1	1.5
Co-trimoxazole	11 (84.6)	1 to >32	>32	>32
Ciprofloxacin	0 (0.0)	0.012 to 0.75	0.016	0.023
Ampicillin	4 (30.8)	3 to >256	6	>256
Cefuroxime	0 (0.0)	3 to 8	4	8
Tetracycline	24 (89.9)	1 to >64	>64	>64
Cefotaxime	0 (0.0)	0.047 to 0.125	0.094	0.125

EAEC = enteroaggregative *Escherichia coli*; MIC = minimum inhibitory concentration.

Clonal Relationships of the Isolated EAEC

The clonal relatedness of the EAEC isolates from food handlers was determined by the use of pulsed-field electrophoresis method. The PFGE analysis revealed that 13 EAEC strains produced 11 distinct DNA fragment profiles. Each PFGE profile displayed several bands from below 50 to about 600 kb within the 50 to 1,000 kb range (Figure 1). The cluster analysis of the PFGE profiles revealed that the EAEC tested belonged to two major genetic groups. Cluster I comprised eight isolates, whereas cluster II consisted of five isolates. There was a 100% similarity between isolates UT134, UT140, and IC02. These were in turn closely related to WGC9. There were two subclusters in Cluster I with very close genetic distance from each other. In Cluster II, there were two subclusters with one subcluster consisting of one isolate (TP299).

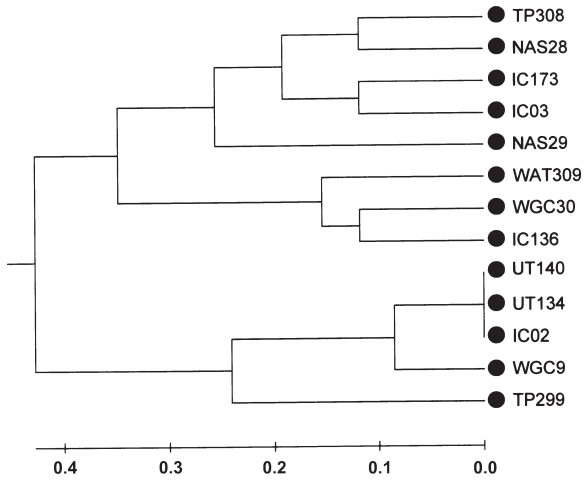

Figure 1 The dendrogram outlining the clonal relationship of the EAEC isolates. The geneticdistance between isolates is shown on the scale. Isolates TP308, WAT309, and TP299 were from Malindi. The rest of the isolates were all from Nairobi.

Discussion

Our study observed an incidence of EAEC of 29/1,399 (2.1%). All the EAEC 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 chromosome. The *aggR* gene–expressing EAEC are termed typical EAEC.[17] In a study on DEC in Tanzania, a prevalence of 18.8% of EAEC was found.[18] The carriage found in our study could be an underestimation as the study population may not represent true proportion in the community. HEp-2 cell assay was not done due to limitation of resources, and this is a limitation as this may result in an underestimate of the true incidence of EAEC. Multiple groups have found asymptomatic infection in international travelers, and it is now known what makes the EAEC strains virulent for humans and finding organisms in stool does not mean they are infected with a disease-producing organism. Significantly more males (27/29) than females (2/29) had EAEC, which was approximately 9:1 ($p < 0.05$). This closely mirrored the study population, which also had a ratio of males to females of 7.8:2.2. EAEC was isolated from 19/29 formed/semiformed stools compared with 10/29 loose stools ($p < 0.0001$). Keskimaki and colleague found a ratio of 35 and 26% DEC in diarrheic and nondiarrheic stools, respectively.[6]

EAEC, formerly called enteroadherent *E coli*, was first recognized as a pathogen causing diarrhea in infants and HIV patients and in some adults in 1987.[19] Currently, EAEC are recognized as important causative agents of TD[20,21] and also as causative agents of other diarrheal illnesses in both developing and developed countries.[22] The identification of this bacterial pathogen is mainly by PCR, HEp-2 cell culture, and DNA probes, all of which are expensive and out of reach of clinical laboratories, especially in resource-poor countries. The testing of the strains for virulence factors is important and

simpler; rapid test needs to be developed, especially for resource-poor countries.

In an earlier study, EAEC was isolated from asymptomatic adults in the tropics.[6] The EAEC isolated in this study, however, carried *aggR* virulence genes, and no other bacterial pathogen was isolated from these subjects, about half of whom also had diarrhegenic stools. It therefore demonstrates a distinct possibility of the transmission of these pathotypes to tourists by food handlers when the food handlers unhygienically handle foods, especially salads and cold cuts. ETEC and EAEC were found to be the most common causes of TD in a study conducted in three different geographical regions, namely Goa in India, Ocho Rios in Jamaica, and Guadalajara in Mexico.[21] In that study, EAEC was isolated in 25, 30, and 38% in each of the three sites, respectively. Even though the overall carriage rate in the current study (2.1%) may appear low, it should be a cause of concern that the food handlers could easily disseminate these pathogens to the tourists and the rest of the community.

Antimicrobial-resistant bacterial diarrhea is a significant public health problem throughout the developing world.[23] In our study, the finding of high rates of resistance and especially multidrug resistance (19/29, 65.5%) to commonly used antibiotics in food handlers is of major public health importance as this could be a source of spread of resistance to clients and family members in close contact with hotel workers. In this study, high antibiotic resistance rates to tetracycline 79.3%, ampicillin 62.1%, chloramphenicol 27.6%, and amoxicillin-clavulanate 31.0% should be viewed with a lot of concern. These are inexpensive, widely available antibiotics that may be bought over the counter without prescription in many drug outlets in Kenya.

These findings are in agreement with the work done in children in southwest Nigeria where they found similarly high rates of resistance of 80.9% for ampicillin, 95.4% for tetracycline, and 46.5% for chloramphenicol.[24] Multidrug resistant (MDR) EAEC were also isolated from children in a study in Kenya.[25] This resistance has been demonstrated to be transferable and linked to virulence of EAEC and is carried on the same plasmid.[24] These findings also mirror what was found from the feces of healthy volunteers from eight countries where they found resistance to ciprofloxacin ranging from 1% to 63%, >20% of resistance for gentamicin, and similarly high levels of resistance to ampicillin, oxytetracycline, and trimethoprim.[26] In Tanzania, a resistance rate of 83.1% for ampicillin, 57% for chloramphenicol, 87.7% for tetracycline, and 90.8% for co-trimoxazole was recorded.[18] These figures are much higher than what has been found in our current study.

Non-typhi *Salmonella* species were isolated from 42/1,400 (3.0%) and more significantly from only one of the three study sites and only from male food handlers (42/262, 16.0%). The male-to-female ratio in Diani was 9:1, but this does not explain why the salmonellae were isolated only from the males. The isolation of a large number of *Salmonella* from one establishment, 31/103 (30.1%), and 11/35 (31.4%) in another establishment in one of the study areas indicates very strongly that there was a possible salmonella outbreak, and this shows unacceptably high numbers of carriage in food handlers. In a study on etiology of TD in three locations,[5] *Salmonella* species was isolated from 3% of the tourists sampled in Mombasa, Kenya.

It has been estimated that about 80% of residents of developing countries have no sanitary facilities for sewage disposal.[27] We found that over 90% of the respondents to our question on toilet type and sanitation disposal used pit latrines ($p = 0.003$). Several factors such as urban migration with crowding and improper sewage disposal have been associated with the spread of antibiotic-resistant organisms between people and the exchange of resistance genes among bacteria, thereby increasing the prevalence of resistant bacteria.[27] All the aforementioned conditions were found in this study and could possibly explain the finding of such high levels of antibiotic-resistant EAEC.

PFGE is widely used as a molecular subtyping method of *E coli* strains due to its high discriminatory power and good reproducibility.[28] In this study, the EAEC were all isolated from food handlers working in tourist hotels in three areas of Kenya. Our isolates were largely unrelated and hence not likely to be clonal in origin. This would imply that several genotypes of EAEC exist in the study population and possibly in the community.[29] However, the 100% similarity of the three isolates UT134, UT140, and IC02 raises the possibility of a common source of infection for these three study subjects. In resource-poor settings especially, it is very difficult to routinely test for the DEC and there is a scarcity of data from Sub-Saharan Africa. The use of basic isolation media, antibiotic susceptibility testing, real-time PCR, and PFGE profiling has enabled the establishment of the status of carriage of EAEC by food handlers in Kenya. It has been suggested that EAEC strains comprise a heterogenous set of pathogens that share certain chromosomal and plasmid-borne genes.[30] This study has also

established the heterogeneity of the EAEC as evidenced by the PFGE dendrogram profile.

In conclusion, we found that food handlers working in tourist hotels are important carriers of clonally unrelated, MDR *aggR*-expressing EAEC strains that could cause TD and food poisoning and that a large proportion of these isolates are MDR. There is a need for a study employing molecular methods including PFGE to examine carriage of pathogens in food handlers and presence of the same pathogens in processed foods and in travelers consuming the food who develop diarrhea.

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Declaration of Interests

The authors state that they have no conflicts of interest.

References

1. Oundo JO, Kariuki S, Maghenda JK, Lowe BS. Antibiotic susceptibility and serotypes of non-typhi Salmonella isolates from children in Kilifi on the Kenya coast. Trans R Soc Trop Med Hyg 2000; 94:212–215.
2. Taneja N, Mohan B, Khurana S, Sharama M. Antimicrobial resistance in selected bacterial enteropathogens in north India. Indian J Med Res 2004; 120:39–43.
3. Prestel E, Nadrchal R, Wolf D, et al. Enteroaggregative and enterotoxigenic *Escherichia coli* among isolates from patients with diarrhoea in Austria. Eur J Microbiol Infect Dis 1999; 18:209–212.
4. Saidi SM, Iijima Y, Sang WK, et al. Epidemiological study on infectious diarrhoeal diseases in children in a coastal rural area in Kenya. Microbiol Immunol 1997; 41:773–778.
5. Jiang ZD, Lowe B, Verenkar MP, et al. Prevalence of enteric pathogens among international travellers with diarrhoea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). J Infect Dis 2002; 185:497–502.
6. Keskimaki M, Mattila L, Peltola H, Shitonen A. Prevalence of diarrhoegenic *Escherichia coli* in Finns with or without diarrhoea during a round the world trip. J Clin Microbiol 2000; 38:4425–4429.
7. Steffen R. Chemoprophylaxis for travellers' diarrhoea: consensus. J Travel Med 1994; 1:224–225.
8. Johnson JR, Kuskowski MA, Smith K, et al. Antimicrobial resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. J Infect Dis 2005; 191:1040–1049.
9. Kenya facts and figures. Available at: http://www.cbs.go.ke/census/1999.html. (Accessed 2006 Jun)
10. City Population Kenya. Available at: http://www.citypopulation.de/kenya.html. (Accessed 2006 Jun)
11. Iijima Y, Asako NT, Aihara M Hayashi K. Improvement in the detection rate of diarrhoegenic bacteria in human stool specimens by a rapid real-time PCR assay. J Med Microbiol 2004; 53:617–622.
12. Bauer A, Kirby WM, Sherris JC, Turk, M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966; 45: 493–496.
13. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. Villanova, PA: National Committee for Clinical Laboratory Standards, 2002.
14. Isenberg HD. Clinical microbiology procedures handbook. Washington, DC: American Society for Microbiology, 1992.
15. Albert MJ, Bhuiyan NA, Talukder KA, et al. Phenotypic and genotypic changes in *Vibrio cholerae* 0139 Bengal. J Clin Microbiol 1997; 35:2588–2592.
16. Talukder KA, Dutta DK, Albert MJ. Evaluation of pulsed-field gel electrophoresis for typing of *Shigella dysenteriae* type 1. J Med Microbiol 1999; 48:781–784.
17. Nataro JP. Enteroaggregative *Escherichia coli* pathogenesis. Curr Opin Gastroenterol 2005; 21:4–8.
18. Vila J, Vargas M, Casalas C, et al. Antimicrobial resistance of diarrhoegenic *Escherichia coli* isolated from children under the age of five years from Ifakara, Tanzania. Antimicrob Agents Chemother 1999; 43:3022–3024.
19. Nataro JP, Kaper JB, Robins-Browne R, et al. Patterns of adherence of diarrheagenic *Escherichia coli* to HEP-2 cells. Pediatr Infect Dis J 1987; 6:829–831.
20. Mathewson JJ, Johnson PC, DuPont HL, et al. A newly recognised cause of travellers' diarrhoea: enteroadherent *Escherichia coli*. J Infect Dis 1985; 151:471–475.
21. Adachi JA, Zhi-Dong J, Mathewson JJ, et al. Enteroaggregative *Escherichia coli* as a major etiologic agent in travellers' diarrhea in 3 regions of the world. Clin Infect Dis 2001; 32:1706–1709.
22. Teng LJ, Hsueh PR, Liaw SJ, et al. Genetic detection of diarrhoegenic *Escherichia coli* isolated from

children with sporadic diarrhoea. J Microbiol Immunol Infect 2004; 37:327–334.
23. Shapiro RL, Kumar L, Phillips-Howard P, et al. Antimicrobial resistant bacterial diarrhoea in rural western Kenya. J Infect Dis 2001; 183: 1701–1704.
24. Okeke IN, Lamikanra A, Czeczulin J, et al. Heterogenous virulence of enteroaggregative *Escherichia coli* strains isolated from children in southwest Nigeria. J Infect Dis 2000; 181:252–260.
25. Sang WK, Oundo JO, Mwituria JK, et al. Multidrug resistant enteroaggregative *Escherichia coli* associated with persistent diarrhoea in Kenyan children. Emerg Infect Dis 1997; 3:373–374.
26. Nys S, Okeke IN, Kariuki S, et al. Antibiotic resistance of faecal *Escherichia coli* from healthy volunteers from eight developing countries. J Antimicrob Chemother 2004; 54:952–955.
27. Okeke IN, Laminkanra A, Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis 1999; 5:18–27.
28. Weiner M, Dacko J, Osek J. Molecular analysis of enterotoxigenic, shiga toxin-producing and enteroaggregative *Escherichia coli* strains isolated from suckling piglets with diarrhoea by the use of pulsed-field electrophoresis. Bull Vet Inst Pulawy 2004; 48:225–231.
29. Peruski LF Jr, Kay BA, El-yazeed RA, et al. Phenotypic diversity of enterotoxigenic *Escherichia coli* strains from a community-based study of pediatric diarrhoea in periurban Egypt. J Clin Microbiol 1999; 37:2974–2978.
30. Czeczulin JR, Whitman TS, Henderson IR, et al. Phylogenetic analysis of enteroaggregative and diffusely adherent *Escherichia coli*. Infect Immun 1999; 67:2692–2699.