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Isolation, Identification and Antibiotic Susceptibility Profiles of Diarrheagenic Bacteria Associated with Food Handlers in Kericho Town, Kenya

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Abstract
Background: Diarrhea as a result of food contamination from food handlers continues to pose serious public health concern. Food handlers as reservoirs of diarrheagenic bacteria are a constant source of infection to clientele visiting food establishments within both urban and rural settings. Isolating diarrheagenic bacteria from food handlers is necessary for accurate prediction on the frequency of these pathogens and potential changes in antibiotic resistance patterns.**Methods:** A cross sectional study was conducted in Kericho town to determine the burden of diarrheagenic bacteria among food handlers. A total of 384 stool samples were collected from January 2015 to March 2015.Bacterial pathogens were identified by conventional microbiological methods; antibiotic susceptibility of bacterial isolates was ascertained using the disk diffusion and agar dilution methods.**Results:** Significant isolates were the *Escherichia coli* (Enteroinvasive *Escherichia coli* 5.7%, Enteroaggregative *Escherichia coli* 2.1%, Enteropathogenic *Escherichia coli* 2.1%, and Enterotoxigenic *Escherichia coli* 1.6%), *Salmonella* isolates 3.1% and *Shigella* isolates 1.0%. **Conclusions**: The study findings emphasize the importance of food handlers as potential sources of infections and suggest appropriate hygienic and sanitary control measures. Accurate epidemiologic information on diarrheagenic bacteria associated with food handlers in Kericho town will be critical for augmenting existing diarrhea management policies in terms of treatment and to strengthen future awareness and health promotion programs.
Keywords: Diarrheagenic bacteria, antimicrobial resistance, food handlers, Kericho town.

Introduction

Diarrhea due to bacteria is a worldwide public health problem causing high morbidities and mortalities, particularly in areas of poor sanitation and hygiene[27]. Prevalence ranges from 20 to 50% with geographical variation[37].Diarrheal diseases, mostly caused by food borne or waterborne microbial pathogens, are leading causes of illness and deaths in developing countries, killing an estimated 1.9 million people annually globally[24].In developed countries, an estimated one-third of the population is affected by microbiological food borne diseases each year [38]. In Kenya, diarrhea is the second most common cause of death[22]. However, the prevalence of bacterial diarrheal diseases varies from one area to another, depending on the level of personal and community hygiene[4]. Globally, the most frequently identified diarrheagenic bacteria are *Escherichia coli, Vibrio* species, *Salmonella, Shigella*, and *Campylobacter*[16]. Factors determining their presence in any particular region include sanitation infrastructure, social culture and personal hygiene practices.

Despite diarrheal diseases being largely associated with overcrowding, poor hygiene, contaminated water and food, there is another silent group “the food handlers” that have a potential of becoming vehicles of transmission of diarrheal pathogens[20]. Food-handlers with poor personal hygiene could be potential sources of infections of many enteropathogenic bacteria, intestinal helminthes, and protozoa[20]. Food-handlers who harbor and excrete enteropathogenic bacteria and intestinal parasites may contaminate foods from their feces via their fingers, then to food processing, and finally to healthy individuals. Such individuals may be either asymptomatic or exhibiting clinical disease. A number of outbreaks of bacterial diarrhea have been shown to have been initiated by infected food handlers[33]. The role of food handlers in the transmission of diarheagenic bacteria is often ignored, with most attention being given to casualties of diarrheal disease without considering the possibility of victims having eaten food contaminated by unhygienic food handlers working in the food establishments.

It can be expected that a large number of illnesses remain under-reported as only the most serious cases get attention. Lack of effective diagnostic facilities often leads to empirical diagnosis of enteropathogens by healthcare providers without laboratory confirmation and this often results in missed diagnosis or prescription of inappropriate treatment. In addition, many food borne illnesses share common symptoms and cannot be distinguished by the symptoms alone. Diagnosis of a food borne illness can only be made after considering the recent food-consumption history of a patient and performing proper laboratory tests for disease-producing parasites, bacteria, and bacterial toxins[25]. Diagnosis of causative agents of diarrheal disease right from the source is crucial for the development of the effective treatment strategies and control measures[28].

Treatment of bacterial diarrhea is by use of antibiotics, but antibiotic resistance is increasing in clinical practice as a result of abuse and improper prescription due to lack of confirmatory diagnosis[31]. Due to lack of antibiotic drug susceptibility profiles, the status of antibiotic resistance to the conventionally used antibiotics is unknown in Kericho town, Kenya. Studies done in other parts have highlighted important geographical variations in the distribution of diarrheagenic bacteria and variations in the prevalence of resistance[30]. Therefore, this study was aimed at isolation, identification and antibiotic susceptibility tests of diarrheagenic bacteria from stool samples gotten from the food handlers in the selected food establishments of Kericho town, Kericho County, Kenya so as to address the lack of updated data for the region.

Methods

Study site and population

The study targeted selected food establishments within Kericho town, Kenya where workers involved in handling food were the key participants. The town is located to the south west of Nairobi, the Kenya's capital city. It lies within the highlands west of the Great Rift Valley. It is located 35° East and longitude 0° , 30° south, with an altitude of 2096 meters above sea level. The prevailing conditions in this region are moderate temperatures, moderate humidity, inadequate clean water supplies coupled with poor living conditions especially in slums set up. These are the places where safety of food was of a major concern.

Data collection

The study design was observational cross sectional with an experimental component involving use of questionnaires to determine the socio-demographics parameters of the study participants.

Persons involved either in handling, processing or serving of food in the selected food establishments and consented to participate in the study were included and excluded Persons who did not consent or were unavailable during a time of sample collection.

Experimental procedure

We collected data on social and demographic factors using a structured questionnaire. A minimum sample size of 384 study participants was calculated and enrolled in the study. Stool samples were transported to the Kericho District Hospital microbiology Laboratory in ice boxes and processed within an hour of collection. Identification of bacterial pathogens and parasites was conducted using conventional microbiological methods and the antimicrobial susceptibility was ascertained using the disk diffusion susceptibility method[3] and agar dilution technique.

For PCR analysis, five lactose-fermenting colonies of similar morphology were isolated from MacConkey plates and bacterial DNA was extracted by resuspending the colonies in 50µl of sterile water, boiling the suspension for 5 minutes and centrifuging at 10000g for 1.5 minutes. The resulting supernatant was used as the DNA template for the PCR reaction

Sub-species identification for Enteroinvasive *Escherichia coli* (EIEC), Enteroaggregative *Escherichia coli* (EAEC), Enteropathogenic *Escherichia* coli (EPEC) and Enterotoxigenic *Escherichia coli* (ETEC) was done by a multiplex PCR as developed by Hayden M.J.,*et al.*[14] Isolates of *Salmonella* and *Shigella* were sub cultured to sheep blood agar before carrying out serotyping using latex antisera kit (Wellcolex, USA).

Statistical analysis

The data collected in the study was entered in a computer using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, USA). To establish which of the bacteria strains was highly inhibited by the tested antibiotics; a one-way ANOVA was done, followed by Tukeys test post-hoc. A p value of <0.05 was considered statistically significant. Selected practices were presented using bar charts and pie charts. Descriptive statistics was used to analyze on the socio-demographic parameters of the study participants.

Results

Majority of the respondents, 58.0% were in the age of 20 – 50 years. 61.5% were male. Most respondents had secondary level of education 41.7%, 15.1% tertiary education, 22.4% primary education and 20.8% had no formal education. The food handlers sampled were mainly waiters 57.8%, cooks 23.4%, chefs 8.6%, butcher 7.6% and others 2.6%.

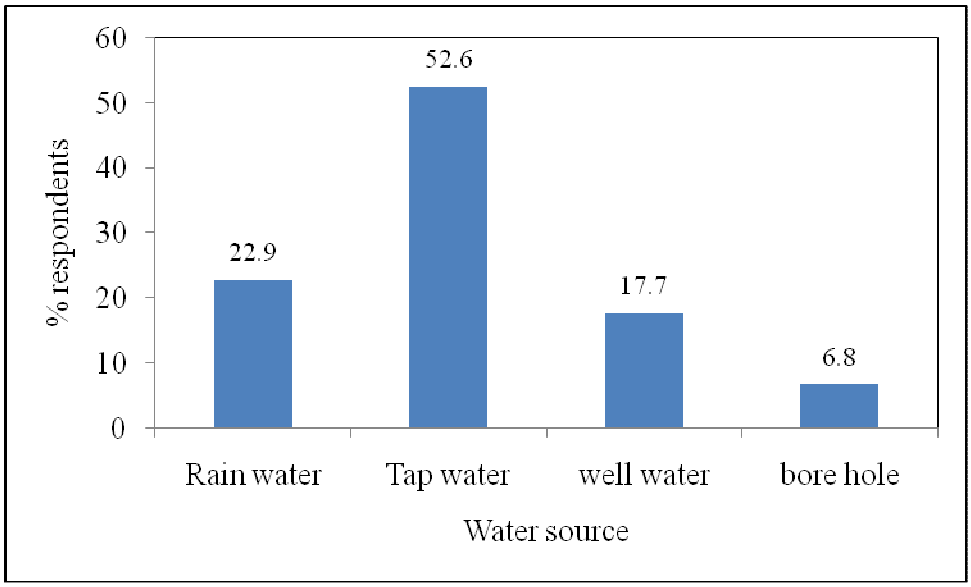

Figure 1: Sources of water used by the respondents in food establishments in Kericho town

A significant number of the respondents, 51.50% used pit latrines, 41.70% used flush toilets and 6.80% used other means.78.6% of respondents wash hands after visiting the toilets with a significant number, 78.6% using soap.52.6 % of the respondents used Piped water present within food establishment and 35.6% boil their drinking water always. It was observed that 26.6% of the respondents used clean pit latrine, 23.4% used dirty pit latrine, 1.6% had no pit latrine and 48.4%were non committal.

Table 1: Bacteria isolated and identified from stool samples from the respondents in Kericho town, Kenya

Bacteria isolated*	**Frequency (n = 384)**	**Percent**
Salmonella isolates	12	3.1
Shigella isolates	4	1.0
EIEC	22	5.7
EAEC-*aa1c*	16	4.2
EAEC-*aatA*	8	2.1
EPEC	6	1.6
ETEC-*st*	6	1.6
EPEC-*eae*	8	2.1
None	302	78.6
Total	384	100

*Abbreviation: st=heat stable toxin; stx=shiga toxin, eae=enteropatognic attachment and effacement;

Some parasites were also identified with the most prevalent being *E. histolytica* at 8.3%,*Ascaris lumbricoides* 6.3%, *Taenia species* 2.1%, *G. lamblia* 1.6% and *S. stecoralis, T. trichuris, S. mansoni* at 0.5% each.

Table 2: Antibiotic Susceptibility profiles of the isolated pathogenic bacteria from food handlers in Kericho town, Kenya

	Salmonella sp.			*Shigella* species			*Escherichia coli*		
Antibiotic tested	**R. f**	**%**	**mic**	**R. f**	**%**	**mic**	**R.f**	**%**	**mic**
Amoxicillin-clavulinic acid	0	0	≤8/4	0	0	≤8/4	2	4.5	16/8
Ampicillin-Sulbactam	0	0	≤8/4	0	0	≤8/4	4	9.1	16/8
Ampicillin	2	40	≤8	3	75	>16	8	18.2	>16
Aztreonam	0	0	≤1	0	0	≤1	0	0	>8
Cefepime	0	0	≤1	0	0	≤1	2	4.5	>8
Cefotaxime	0	0	≤1	0	0	≤1	0	0	>16
Ceftazidime	0	0	≤1	0	0	≤1	0	0	>16
Ciprofloxacin	0	0	≤1	0	0	≤1	4	9.1	>2
Imipenem	0	0	≤1	0	0	≤1	0	0	≤1
Levofloxacin	0	0	≤2	0	0	≤2	4	9.1	>4
Meropenem	0	0	≤1	0	0	≤1	0	0	≤1
Piperacillin/Tazobactam	0	0	≤16	0	0	≤16	0	0	≤16
Tigecycline	0	0	≤1	0	0	≤1	0	0	≤1
Trimethoprim-Sulfamethoxaz	3	60	8	1	25	8	4	9.1	8
Amikacin	-	-	-	-	-	-	6	13.6	32
Colistin	-	-	-	-	-	-	0	0	≤2
Gentamicin	-	-	-	-	-	-	6	13.6	>8
Tobramycin	-	-	-	-	-	-	2	4.5	>8
Cefuroxime	-	-	-	-	-	-	2	4.5	>16

Key: MIC - Minimum inhibitory concentration
RF - Resistant frequency; indicating number of resistant bacteria isolates
% – is the percentage of the resistant isolates.

Table 3: ANOVA result for the MIC values for the three bacteria isolates from food handlers in Kericho town, Kenya

ANOVA

MIC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	592.752	2	296.376	5.003	.011
Within Groups	2665.914	45	59.243		
Total	3258.667	47			

Mean Separation of the MIC values for the three bacteria isolates

Multiple Comparisons

MIC
TUKEY'S HSD

					95% Confidence Interval	
(I) bacteria	(J) bacteria	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Salmonella sp	Shigella sp.	-1.00000	2.90916	.937	-8.0507	6.0507
	E. coli	-7.58571*	2.68211	.019	-14.0861	-1.0853
Shigella sp.	Salmonella sp	1.00000	2.90916	.937	-6.0507	8.0507
	E. coli	-6.58571*	2.68211	.046	-13.0861	-.0853
E. coli	Salmonella sp	7.58571*	2.68211	.019	1.0853	14.0861
	Shigella sp.	6.58571*	2.68211	.046	.0853	13.0861

*. The mean difference is statistically significant at $p < 0.05$

Table 4: Mean groups of the MIC values for the three bacteria isolates from food handlers in Kericho town, Kenya.

Tukey's HSD

bacteria	N	Subset for alpha = 0.05	
		1	2
Salmonella sp	14	3.7143	
Shigella sp.	14	4.7143	4.7143
E. coli	20		11.3000
Sig.		.930	.054

Means for groups in homogeneous subsets displayed.

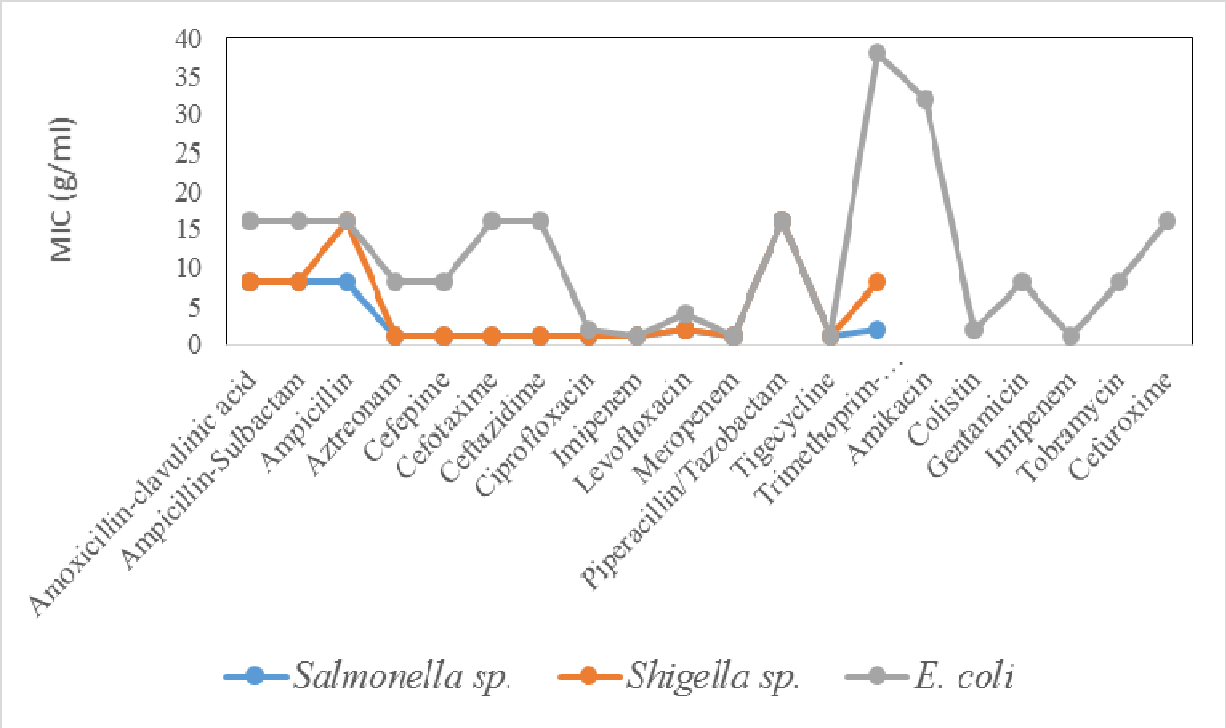

Figure 2: MIC of the bacteria isolates.

Discussion

This study established that the dominant type of bacteria in the 5.7% of the samples was EIEC,*Salmonella* isolates 3.1%, and *Shigella* isolates 1.0%. The presence of bacterial isolates was varied from one food establishment to the other and handlers to handlers. These enteropathogens can lead to significant microbiological threat to the consumers causing food poisoning. Prevalent intestinal parasite isolated and identified in the samples was *Entamoeba histolytica* at 8.3%, *Ascaris lumbricoides* 6.3% and the least prevalent were *S. stecoralis, T. trichuris* and *S. mansoni* at 0.5% each. These findings agree with what has been established by past researchers[9].It is postulated that this is largely due to poor personal hygiene practices and environmental sanitation, lack of supply of safe water, poverty, ignorance of health-promotion practices, and impoverished health services. These findings therefore demonstrates a distinct possibility of the transmission of these diarrheagenic bacteria and intestinal parasites to clientele visiting food premises when those tasked with food handling ,unhygienically handle food.

This study found out that *Salmonella* isolates were resistant to both Ampicillin at 40% and to Trimethoprim - Sulfamethoxazole at 60 % and sensitive to other antibiotics tested. *Shigella* isolates detected were resistant to both Ampicillin 75% and Trimethoprim-Sulfamethoxazole at 25% resistant.*E. coli* bacteria showed resistance to Ampicillin, Gentamicin, Amikacin,Ampicillin-Sulbactam, Levofloxacin, Ciprofloxacin, Trimethoprim-Sulfamethoxazole, Amoxicillin-clavulinic acid, Cefepime, Cefuroxime and Tobramycin. These

commonly used antibiotics is a significant public health concern as this could be a source of spread of resistance to clients visiting food establishments and the family members in close contact with these food handlers. This raises a serious concern and is comparable to the antibiotic resistance observed in previous studies[29-30]. Most of these are antibiotics that walk-in clients buy over the counter without prescriptions, widely available in drugs outlets in Kericho town and are inexpensive. The emergence of multidrug resistant bacteria is an ongoing problem in Kenya, as a result of ready access to the drugs, unwarranted use of the drugs in clinical settings without proper laboratory diagnosis, and the ease of genetic exchange of resistance genes among the bacterial pathogens[40].WHO guidelines state that antibiotic use is warranted only in cases of shigellosis[40].Empiric diagnosis of the cause of acute diarrhea could result in unnecessary prescription of antibiotics. From this study, it is evident that food handlers are an important reservoir of diarrhoegenic bacteria and which are also multi-drug resistant (MDR). The possibilities of the spread of these virulent MDR organisms into the food chain are real. There is also the danger of therapy failure in cases of infections with these MDR organisms. The resistance seen with these organisms will influence the treatment of diarrhea.

The minimum inhibitory concentration (MIC) of the antibiotics on the three bacteria strains were established as shown in Figure 2.To establish which of the bacteria isolates was highly inhibited by the tested antibiotics, a one-way ANOVA was used and the means were tabulated using Tukey's test. The result showed that there was a significant difference in inhibition ($F = 5.003$, $P = 0.011$) of the antibiotics tested on the bacterial isolates. Higher concentrations were required to inhibit *E. coli* (mean 11.3 ± 2.29) than to inhibit *Salmonella* species (mean 3.714 ±1.22) and *Shigella* species (mean 4.714 ± 1.49).The mean difference was significant at the $p< 0.05$ level for the three bacteria isolates. Most of the bacterial isolates were susceptible to aztreonam, cefotaxime, ceftazidine, imipenem, meropenem, piperacillin/Tazobactam and Tigecycline.

If antibiotics are to be prescribed then aztreonam, cefotaxime, ceftazidine, imipenem, meropenem, piperacillin/Tazobactam and Tigecycline should be the drug of choice as most bacterial isolates were susceptible.

Adoption of practices that may predispose an individual to infectious agents can promote the spread of diarrheagenic bacteria and intestinal parasites among humans. This research identified such practices to be common with food establishments in Kericho town and they correspond to increased prevalence of some diarheagenic bacteria and intestinal parasites among the study participants. These practices and their prevalence include; not boiling water(36.5%);using water that was likely to be contaminated for instance unprotected tank water that can come in contact with droppings of terrestrial animals (12.2%) ;water from rivers that have a high possibility to be contaminated with fecal matter from surface runoffs after downpour; inadequate and inappropriate washing of hands after using latrines(21.36%),washing hand in a basin of water(8.33%) instead of running tap water; dirty latrines with flies and in close proximity with dining halls(23.4%) and in some cases absence of latrines(1.6%);inadequate and inappropriate use of toiletries in the latrines hence increasing the chance of fecal contamination(22.4%);and failure to use soap when washing hands after utilizing a latrine(78.6%).

These unhygienic practices among the food handlers working in the selected food establishments in the study area can lead to fecal contamination of the hands hence predisposing their clients to infections and are comparable to what has been observed by other previous researchers[9]. It was observed that, there was no hand sanitizer on wash basins for the personnel to wash their hands after using toilets or handling foods or raw ingredients. Education on proper hand washing and improved sanitation and hygiene should be emphasized on the food handlers. Asymptomatic carrier and infected handlers also play an important role since they shed the pathogenic organisms. It was reported that, many food handlers continue to work while they were suffering from diarrhea, vomiting or pyrexia. More than half of the people globally carry around them an 'intestinal zoo' of diarrheagenic infectious agents, majority of which are transmitted to human orally by swallowing them in contaminated food, water or through contamination of the hands by infected feces or feacally contaminated soil[36] and is consistent with the findings of this study.

Conclusion

The results of this work demonstrated that food handlers working in the selected food establishments harbor diarrheagenic bacteria and the intestinal parasites and they remain to be a major source of exposure to clientele visiting these food establishments. Multidrug resistances to the most common antibiotics were evident in Kericho. This exposure is likely to continue to be a public health problem until food handlers are trained and sensitized on safe practices in food processing and handling .Chronic healthy carriers of typhoid and paratyphoid fever are important sources of infection and their identification was necessary for epidemiological and public health purposes. Cultural examination has proved to be of paramount help in identifying this group of chronic healthy carriers for epidemiological and public health purposes. Control of food borne diseases requires adoption of hygienic practices and appropriate practices which can be made popular through health education programs in the study area. In future, it will be necessary for the food handlers to adhere to food safety practices, observe high standards of hygiene in the latrines so as to thwart any transfer of pathogens to areas where food is

processed. Use of toiletries and proper hand washing after visiting latrines using soap and running tap water should be advocated for by all and frequent screening of food handlers by relevant authorities.

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Competing interests: None declared.
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