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Presence of pathogenic *E. coli* in ready-to-be-eaten salad food from vendors in the Kumasi Metropolis, Ghana

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This study was carried out to assess the distribution of *E. coli* O157:H7 in salad foods from restaurants and street food vendors within the Kumasi Metropolis from January to April, 2013. A detailed and well-structured questionnaire was first administered to 500 vendors with emphasis on knowledge of personal hygiene and salad food preparation. A total of 270 salad foods were aseptically sampled from vendors and transported on ice to the laboratory to determine the presence of total coliforms and *E. coli* *(E. coli* O157:H7*)* using standard microbiological methods. Out of the total samples analysed, all the samples were found to contain some counts of total coliforms and *E. coli*. Mean logcfu/g of total coliforms and *E. coli* were found to be 6.35 ± 0.09 and 5.1 ± 0.1, respectively. Three (3) samples showed positive to *E. coli* O157:H7 giving a prevalence of 1.1%. The low prevalence still suggests that *E. coli* O157:H7 is still a public health concern especially ready to be eaten salad foods since a relatively low infectious dose could be fatal. Although, street food vending has a positive impact on food supply and livelihoods of the ordinary people by providing cheap and affordable means of green-leafy or vegetable food in the Kumasi Metropolis, it poses a health risk which can result in serious health implications for consumers.

Key words: *E. coli* O157:H7, hygiene, restaurant, vendors, salad food.

INTRODUCTION

Public announcements and awareness of the intake of vegetables have increased massively (Chadrasekhar et al., 2003). While there are many reasons to include these nutritious foods into our daily diets, their accessibility and portability cannot be underplayed. Eating a diet rich in vegetables and fruits as part of an overall healthy diet may reduce risk for heart disease (He et al., 2007), including heart attack and stroke (He et al., 2006), as well as reduce certain types of cancers (World Cancer Research Fund, 2007). Outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables have increased in recent

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years (Mensah et al., 2002). Food-borne diseases are increasing and becoming wide spread, therefore, food safety has been considered as an important global health issue (Bernstein et al., 2007). One of the vehicles of food-borne diseases is through the consumption of raw vegetables such as salad foods (Mensah et al., 2002). Research has shown that vegetables are increasingly eaten as salad foods by the urban population in Ghana and estimated that, each day; about 800,000 people in the cities of the country consume wastewater irrigated salad foods (Seidu and Drechsel, 2010). The street food industry plays a very important role in meeting food requirements of commuters and urban dwellers in many cities and towns of developing countries, as it feeds thousands of people daily with a large range of food that are relatively cheap and easily accessible (Tambekar et al., 2008). Studies conducted in Accra revealed that salad foods had a high level of microbial contamination (Mensah et al., 2002) which suggests a high risk of microbial infection. Many studies have been carried out on other microbial pathogens but little is known about the occurrence of Entero-haemorragic *Ecsherichia coli*, especially in salad food especially in Ghana. In Ghana, diarrhoea has been recognized as one of the major causes of hospital attendance and 16% of deaths in African children younger than five years are directly attributable to diarrhoeal diseases (Bruce et al., 2005). Despite the commitment and dedication of various agencies to improved food safety systems, it has not been widely implemented which raises much concerns about the probable role street and other vending food play in food poisoning (Kosek et al., 2003; Soyiri et al, 2008)

The aim of this study was thus to assess the level of contamination of ready to-be-eaten salad foods by Total coliforms, the normal *E. coli* strain and entericpathogenic *E. coli* 0157:H7*,* from various food vendors and the relationship between salad consumption and the prevalence of microbial contamination by *E. coli* and total coliform within the Kumasi Metropolis.

MATERIALS AND METHODS

Kumasi was selected as the study area, which is located in the forest zone and is about 270 km north of the national capital, Accra. Its location is between latitude 6.35° to 6.40° and longitude 1.30° to 1.35°, at elevation which ranges between 250-300 m above sea level with an area of about 254 km^2 (KMA Development Plan, 2006-2009). Kumasi is divided into ten (10) sub-metropolitan areas namely Bantama, Subin, Manhyia, Oforikrom, Tafo, Nhyiaeso, Kwadaso, Suame, Asokwa and Asawase.

The study area is unique and centrally placed which makes it possible for people from all parts of the country to converge for trading and engage in other equally important activities. The metropolis falls within the wet sub-equatorial type with an average minimum temperature of about 21.5°C and a maximum average temperature of 30.7°C. The average humidity is about 84% at 0900 GMT and 60% at 1500 GMT (KMA Development Plan, 2006).

Urban agriculture is the main activity practiced by farmers in the metropolis with about 90% of farmers using wastewater for irrigating their crops throughout the year due to the lack of potable water for irrigation purposes (Ghana Statistical Service, 2010).

The farmers cultivate vegetable crops such as lettuce, cabbage, spring onions, green pepper, carrots among others.

Study population

About 500 salad food vendors were included in the study from all the 10 sub-metros within the metropolis and 270 salad food samples were collected. The study was carried out from January to April, 2013; where salad food vendors were interviewed using a detailed questionnaire where information such as the educational level, training on salad food preparation, knowledge of personal hygiene among others were captured while some observations were made. The vendors included restaurants/cafeteria managers and street food sellers.

Sampling and analysis

The inclusion criteria were food vendors who serve salad in addition to the food and are located within the Kumasi metropolis. In most cases, salad consisted of slices of lettuce, onion- spring onion or round bulb-like onion, cabbage, cucumber and carrot. After a month of questionnaire administration, triplicate samples of salad foods were collected in sterile plastic bags kept on ice and transported to the laboratory for analysis.

Triplicates samples of salad food ready for consumption were thoroughly mixed together to form a composite and then 1 g of the composite sample was weighed and added to 9 ml of saline. The content was then pulsified for sixty seconds (60 s) to wash away the surface contents of the samples and thus form a stock solution or diluent. Diluent was used to inoculate Eosine Methylene Blue Agar (EMBA) plates and incubated at 44°C for 24 h. Plates showing growth of *E. coli* colonies (blue black with metallic sheen appearance) were sub cultured onto Sorbitol MacConkey agar (SMA) plates and incubated at 44°C for 24 h. Colonies that showed colourless appearance were purified (Baron et al., 1994). Pure colonies were then serologically confirmed with *E. coli* prolex^{TM} latex agglutination test kit from Oxoid, England. One drop of *E. coli O157:H7* antisera were dispensed onto a reaction card and a loop full of the pure colony of the test organism was then added. This was then carefully emulsified into a smooth suspension. The card was carefully rocked in a circular motion. If agglutination occurred within 1 min then the test organisms were considered positive for *E. coli O157:H7*. A similar reaction was carried out as a control using normal saline instead of the *E. coli O157:H7* antisera. Quantifications were based on colony forming units (CFU) (Oxoid Limited, 2012).

The specificity and sensitivity of this protocol was 100 and 99%, respectively.

Sample of questionnaire

Food vendors' questionnaire on salad preparation and handling within the Kumasi Metropolis is shown below:

Date of interview:

Interview start:
End:

Name of interviewer Tel. No.

Suburb:

Type of eating place: A. Hotel [ ] B. Restaurant [ ] C.Cafeteria [ ] D. Street food [ ]

Do they serve salad? A. Yes [ ] B.No [ ]

(Inclusion criteria is facilities where salad is served)

Name of respondent
Tel. No.

1. Age of respondent:

2. Gender: A. Male [ ] B. Female [ ]

3. Marital status: 1. Single [ ] 2. Married [ ] 3. Divorced [ ] 4. Widowed [ ]

4. Educational level: Can you read and write? Yes ( ) No ( )

A. Primary [ ] B. Secondary [ ] C. Tertiary [ ] D. No answer [ ]

5. How long have you been working as a food vendor? A. <1 yr ( ) B. 1-2 year [ ] C. 3-5 years [ ] D. 6-10 years [ ] E. More than10 years

6. Are vegetables good for our health? 1. Yes [ ] 2. No [ ]

If yes, why? .....

If No, why?.....

7. What type(s) of vegetable(s) do you use to prepare your salad?
A. Cabbage [ ] B. lettuce [ ] C. green pepper [ ] D. Carrots [ ] E. Other [ ] F. cabbage and lettuce G. cabbage, lettuce and carrots [ ]

If other, Specify……

8. Where do you buy raw vegetables? A. Farm [ ] B.Wholesale [ ] C. Retail Market [ ] D. Other source

If other source specify……

If farm, where is it located? ……

If a wholesale market, where is it located?.....

If a retail market, where is it located?.....

Do you always obtain your vegetables from the same place?

A. Yes [ ] B. No [ ]

9. Where do you store the raw vegetables before salad preparation?

A. On the floor [ ] B. Refrigerator [ ] C. On table [ ] D. Sack [ ]

10. How do you wash your raw vegetables?

A. Water only [ ] B. Water and salt only [ ] C. Water and vinegar only [ ] D. Water with Salt and vinegar solution [ ] E. Chlorine tablets [ ] F. Potassium permanganate [ ] G. Do not wash [ ] H. Other

If other specify…….

11. How long do you soak the vegetables? A. Less than 1 minute [ ] B. 1–2 minutes [ ] C. 3–4 minute [ ] D. 5–10 minutes [ ] More than 10 minutes [ ] E. Do not know [ ]

12. Have you had any training on salad preparation? A. Yes [ ] B. No [ ]

If yes where? A. School [ ] B. Friends [ ] C. Relatives [ ] D. Other [ ]

If other, specify……..

13. Do you use running tap water or a bowl of water? A. Running [ ] B. Bowl [ ]

14. Where do you cut your vegetables? A. Chopping board [ ] B. Ordinary table [ ] C. any plane surface [ ] D. Other [ ]

If other, specify……

15. How often do you clean your Cutting surface? 1. Everyday [ ] 2. Every Two days [ ] E. Once a week [ ] F. Other [ ]

If other, specify……

16. How often do you change you cutting surface? A. 1-3 months [ ] B. 4- 6months [ ] C. 7-12months [ ] D. More than 12months

17. Do you wash your hands before food preparation? A. [ ] Yes [ ] B. No

If yes, how? A. With water only [ ] B. With soap and water [ ]

19. Do you wash your hands after visiting the toilet? A. Yes [ ] B. No [ ]

If yes, how? A. With water only [ ] B. With soap and water [ ]

21. How do you preserve left over vegetable salad?
A. Refrigerator [ ] B. Leave it uncovered [ ] C. Kitchen cupboard [ ] [ ]D. other

If other, Specify……..

Observational checklist

Observational checklist used is shown in Table 1.

RESULTS AND DISCUSSION

Most outbreaks of food borne diseases have been implicated with consumption of unrestricted wastewater irrigated vegetables (Gould et al., 2013). Two hundred and seventy (270) salad food samples prepared from wastewater irrigated vegetables were analyzed for *E. coli* O157:H7, total coliforms and faecal indicator *E. coli* from both restaurants/cafeteria and street food vendors in Kumasi. The overall prevalence of *E. coli* O157:H7 was found to be 1.1% with mean log cfu/g of 1.1 ± 0.3 and within the sub-metro where *E. coli* O157:H7 occurred, the prevalence was 13.0% which confirms the study reporting that wastewater irrigated lettuce is contributing to the

Table 1. Observational checklist.

Observation	Yes (1)	No (2)	Remarks
Near a toilet facility			
Near a refuse dump			
Near a storm water drain/gutter			
Water used for cleaning utensils looks dirty			
Flies can be seen around facility			
Cutting board for vegetables is dirty			
Unprocessed vegetables are lying on the floor			

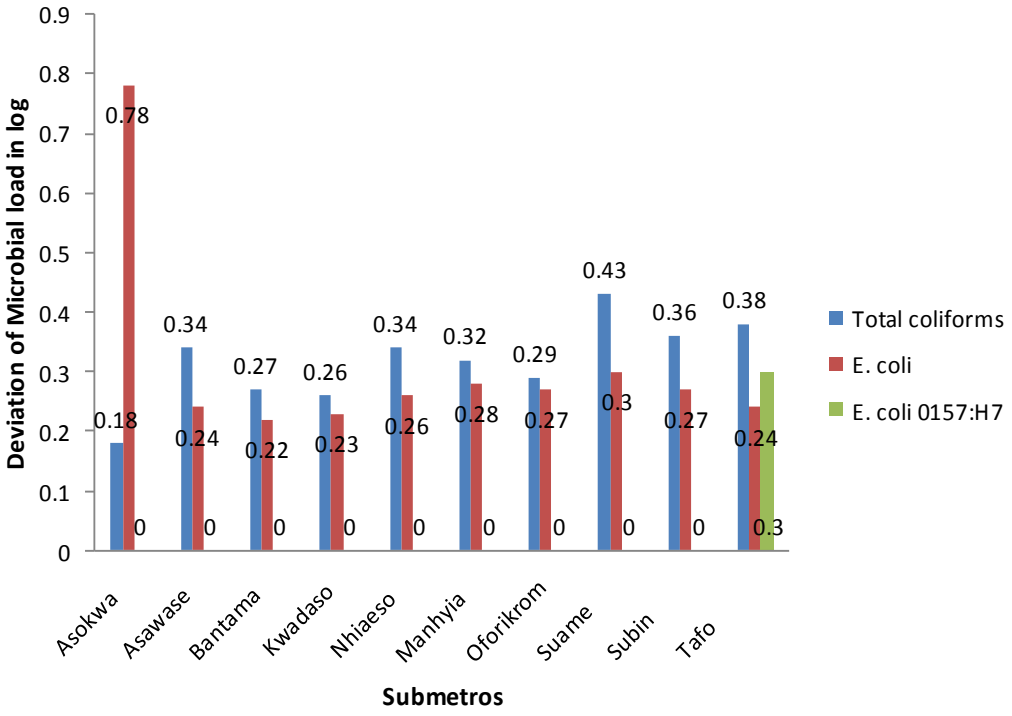

Figure 1. Mean $\log_{10}$ counts of total coliforms and *E. coli* and *E. coli 0157:H7* in ready-to-be-eaten salad food from vendors in the Kumasi Metropolis.

transmission of *E. coli* O157:H7 in Kumasi, Ghana (Seidu et al., 2014). Among the 270 food samples analyzed, 42 were sampled from restaurants and cafeterias which were all negative for *E. coli* O157:H7 which thus imply a null prevalence rate. This study therefore noted that hygiene practices among restaurants and cafeteria food vendors' rates higher compared with street food vendors. However, all the samples had some levels of total coliforms and faecal indicator *E. coli* which also indicates that salad foods sold in Kumasi contains microbial pathogens (Seidu et al., 2014). The mean log cfu/g of total coliforms and faecal indicator *E. coli* from both restaurants/cafeteria and street foods were found to be 2.9±0.34, 2.4±0.30 and 2.8 ± 0.25, respectively. The sub metro that recorded the highest total coliform result was Oforikrom (Figure 1) with mean log cfu/g of 6.8± 0.9 which happens to be one of the sub metros with quiet a number of farmers growing vegetables like lettuce, cabbage, green pepper, carrot and spring onion among others with the main source of water for irrigation been untreated wastewater from drains which have been noted by other studies as possible source of *E. coli* O157:H7 (Tanaro et al., 2010). From Table 2, Total coliform and E. coli have significant values as 0.999 and 0.991 respectively which are all greater than the alpha value (0.05) we conclude that the test is not significant. This implies that the mean log/cfu of Total coliform and E. coli present in salad foods is the same throughout the ten sub-metros.

On the other hand, since the significant value (0.011) of

Table 2. Chi-Square Test of significance of microorganisms between sub-metros.

Microorganism	Degrees of freedom	Chi-Square Value	Significant value	Alpha value
Total Coliform	8	0.800	0.999	0.05
E. coli	8	1.200	0.991	0.05
E. coli 0157:H7	8	6.400	0.011	.005

Table 3. ANOVA table of significant difference between means.

Medium of microbes	Sum of squares	Degrees of freedom	Mean square	P-Value	Alpha value
Within means	52.104	3	17.368	0.093	0.05
Between means	30.396	6	5.066		
Total	82.500	9			

E. coli O157:H7 is less than alpha value (0.05) we conclude that the test is significant. This implies that the mean log/cfu of *E. coli* O157:H7 is not the same throughout the ten sub-metros.

From Table 3, since the p-value (0.093) is greater than the alpha value (0.05) we have enough evidence to reject the null hypothesis Ho:u1=u2=u3 hence we conclude that the test is not significant. This implies that the mean log/cfu of the three different microorganisms in the salad foods is the same.

Farmers from this study mostly use manure from livestock which serves as a reservoir of pathogen and therefore stands as a route for pathogen transmission (Oliveira et al., 2012). During the study faeces of livestock grazing around irrigation fields were also common to sight, which get washed into these irrigation water bodies when there is a fall increasing the pathogen contamination to a lager extent (Karesh et al., 2012). It was also noted during interactions with the food vendors that, most of the vegetables cultivated by these farmers are the main source of vegetables salad food vendors close-by and around the vicinity use for salad food preparations. About 90.4% of the vendors buy their raw vegetables from the retail market within the metropolis and 6.4% obtain theirs directly from the farms which they claim is the cheapest means of acquiring their raw materials for salad food preparation. From this study, it was observed that majority of the salad food vendors (SFV) fall within the ages 20-30 recording a percentage of 32.6 as against 26-45 years recorded by Monney et al. (2014) and the smallest group is within the age range 51-55 giving a percentage of 1.0 and about 70.4% of the SFV are female which is a little lower than 95% reported by Monney et al., (2014) and this is attributed to the fact that in the Ghanaian culture, food preparation is considered as the female's job. It was again noted that about 48.4% of the SFV have had secondary education which puts them in a better position to be taken through training of salad food preparation and made it less difficult to deal with in explaining the bases of the study to them. During the study it was also recorded that about 61.4% of the respondents have had training for the job they were engaged, from vocational training schools and friends who are into the salad food vending business. 63.1% of the vendors stated that during their training they were taught to use water and salt to wash their vegetable before preparing, which has been the routine practice; 18.7% use vinegar and water only, 0.2% use potassium permanganate and 9.85% blended water, salt and vinegar for washing. It has been noted from this study that not only disinfection can reduce pathogen load from fresh vegetables but rather pathogen load reduction involves several other factors (Bermudez- Aguirre and Barbosa-Canovas, 2013). Microbial contamination of vegetables can occur through various factors which include soil, irrigation water, various farming practices such as manure application (Amoah et al., 2005), poor sanitary conditions on farm, poor post-harvest handling of vegetables by market women and unhygienic salad food preparation (Amoah et al., 2007). Poor conditions of vending sites could also serve as major vehicles of microbial contaminations because it was observed from this study that, with the restaurant operators who observed a bit of standard level of hygienic conditions, there were lower level of microbial loads recorded compared to the street food vendors.

The study realized from the pattern of response that, 8.2% SFV use ordinary water for washing the vegetables with the idea of removing some levels of microbial load and these are people without any form of training in the job they are handling as well as other contributing factors like weak and inconsistent local bye-laws and logistical constraints (Monney et al., 2014).

Conclusion

Regardless public education and other training programmes organised occasionally by the Food and Drugs Authority and concerns by municipal authorities

such the Kumasi Metropolitan Assembly, salad contamination by food-borne pathogens remains a major public health issue. Almost all the vegetables used for salad food preparations within the city of Kumasi are obtained for wastewater irrigated farms either directly from the farmers or the retail centers. As such, these salad foods contain high levels of microbial pathogens which were isolated in this study. Effort should therefore be made to improving our irrigation systems and adopt new technologies practiced elsewhere since most of the vegetables produced in Kumasi are irrigated with wastewater from the drains and streams. As the structured questionnaire of the study showed, an important element will be to raise public health awareness on options to reduce health risks through proper and effective vegetable washing at the various restaurants or cafeteria as well as street food vendors, which is necessary in and as part of the efforts of addressing salad contamination by food-borne pathogens in the Kumasi Metropolis. Eventually, regular laboratory examinations of the microbial quality of salad foods prepared and sold in restaurants and streets has to remain the target, notwithstanding public awareness on the need to insist on salad foods of good quality as well as improving agricultural practices such as wastewater irrigation by treating these them well before irrigation.

Conflict of interests

The authors did not declare any conflict of interest.

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