

## Transmission of Foodborne Illnesses: The Role of Serving Utensil - Dependent Transmission of Enteric Pathogens from Street - Food Vending Outlets at the University of Cape Coast Community in Ghana

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**Received:** September 11, 2015; **Published:** October 12, 2015

### Abstract

Foodborne illnesses pose serious public health threat globally, but requisite data to inform proper planning and prioritizing food safety interventions especially in developing countries is lacking. This study investigated microbiological quality of serving utensils (serving plates or bowls) for enteric pathogens from food vending and serving outlets within the University of Cape Coast campus and surrounding villages in the Central Region of Ghana. A total of 120 swab samples were collected from serving utensils after they were cleansed by food servers at the food vending outlets. Each swab sample was inoculated in an equal volume of bacteriological peptone water and incubated at 37°C for 24 hours. Serial dilutions and aerobic colony counting was performed by spread plating on plate count agar (PCA) for each sample. Isolated bacteria colonies from the PCA plates were sub-cultured on blood and MacConkey agars. Subsequently, bacteria species were identified by standard biochemical methods and modified Kirby - Bauer disc diffusion technique was used to investigate antibiotic susceptibility patterns of each bacteria species. All the swab samples produced bacteria counts > 10<sup>5</sup> CFU/ml at mean count of 1.066 × 10<sup>10</sup> CFU/ml. Nine (*Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter* spp, coagulase-negative *Staphylococci*, *Proteus mirabilis*, and *Micrococcus* spp) bacteria species were isolated with *Bacillus cereus* (21.59%) being the predominant bacteria and *Proteus mirabilis* and *Micrococcus* spp (0.85 %) being the least. The Gram - positive bacteria species were susceptible to gentamycin but resistant to ampicillin, while the Gram - negative bacteria species were susceptible to cotrimoxazole and amikacin but resistant to ciprofloxacin and tetracycline. The serving utensils at the vending outlets were highly contaminated with enterotoxigenic bacteria and this poses a serious risk to food consumers within the study area. Importantly, this finding provides a rationale for public health authorities to increase education of food vendors as well as strictly enforcing sanitation codes.

**Keywords:** Antibiotic susceptibility patterns; *Bacillus cereus*; *E. coli*; Foodborne illnesses; Food safety, Enterotoxigenic pathogen; Ghana

### Introduction

Globally foodborne illnesses have increased [1] leading to not only high episodes of foodborne illnesses but also increased hospitalization, and deaths [2]. In view of this, foodborne illnesses have become a serious public health threat in both developed and developing countries of the world. Indeed, with urbanization and its attendant economic pressures many people the world over rely on 'street food'

**Citation:** Alex Boye, et al. "Transmission of Foodborne Illnesses: The Role of Serving Utensil - Dependent Transmission of Enteric Pathogens from Street - Food Vending Outlets at the University of Cape Coast Community in Ghana". *EC Nutrition* 2.4 (2015): 386-395.

or 'street - vended foods' [3] because of convenience and time constraints, but in most cases these 'street - vended foods' are operated under sub - standard hygienic conditions therefore pose serious risk to food consumers. Notably, it was estimated that annually 1.6 - 2.4 million episodes of foodborne illnesses are reported and the underlying causes were attributed to many transmission routes (food, environment, water, persons, and zoonotic) with foodborne transmission being the major cause [4]. Similarly, 9.4 million episodes of foodborne illnesses due to 31 major pathogens were reported to have resulted in 55,961 hospitalizations and 1,351 deaths [2]. The major enteric pathogens commonly involved in foodborne illnesses includes *Bacillus cereus* [5,6] *Clostridium perfringens* [7], *Cyclospora cayentanensis*, *Trichinellaspp*, *Vibrio spp*, *Yersinia enterocolitica*, *Campylobacter spp*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp*, *Staphylococcus aureus* [1]. The threat posed by enteric pathogens to the food industry [1] as well as the consuming public is enormous partly due to their ability to survive disinfection and food processing methods and their multi - drug resistance ability [8,9]. A typical example, is demonstrated by *Bacillus cereus* and *Clostridium perfringens* arguably two most important enteropathogens of clinical importance [6,10] were shown to have become psychotropic growing at temperatures as low as 4 - 6°C [11] while surviving temperatures as high as 105 - 110°C [5,12] as well as their ability to produce diverse enterotoxins [6].

In spite of all these public health threats posed by foodborne illnesses, relevant data on foodborne illnesses still remains a challenge, especially in developing countries like Ghana. In developed countries including USA, Canada, Australia and others, surveillance systems, 'Expert elicitation' as well as use of advanced simulation systems are used to estimate the epidemiological dynamics of foodborne illnesses to afford proper public health planning and prioritization of food safety interventions, but sadly, this is not the case in Ghana as lack of relevant data remains a challenge. It is therefore important to increase efforts in developing countries to make available relevant data to inform food safety measures as well as prioritize public health interventions to ensure food safety among students and the general public. Earlier, a report on the level of observance of sanitary and hygienic practices at some fast - food joints and street food vending points on the University of Cape Coast campus and the surrounding communities in the Central Region of Ghana had raised concerns about declining sanitary and hygienic practices among food vendors, subsequently, an annual report from the Cape Coast District Community Health Center (CCDCHC, 2004 - 2006), also indicated that there have been an annual increase in the number of reported foodborne illnesses and the report attributed the situation to consumption of 'street - vended foods, however, the report was silent on the enteropathogens probably involved in the cases reported. It is on the basis of these earlier reports coupled with the need to bridge the existing data gaps, that this study investigated the common enteropathogens transmitted from serving utensils to food consumers on the University of Cape Coast Campus and the surrounding communities.

## **Materials and Method**

### **Study Area**

The study was conducted at the University of Cape Coast campus within the Cape Coast Metropolis of the Central Region of Ghana. Cape Coast Metropolis lies approximately between latitudes 5°.07' to 5°.20' north of the equator and between longitudes 1°.11' to 1°.41' west of the Greenwich Meridian. It has common boundaries with TwifoHeman Lower Denkyira District in the north, Gulf of Guinea in the south, AburaAsebu-Kwamankese District in the east and Komenda - Edina - Eguafo-Abrem (K.E.E.A) District in the west. It has 84 communities including Efutu, Adisadel, Apewosika, Nkanfoa, Abura, Pedu and Nyinesin. The total population of Cape Coast Metropolis is 118,106 out of which 57,365 are males and 60,741 females. Farmers, fishermen as well as those into agriculture-related activities form about 60% of the population (Projection from the 2000 Housing and Population Census, Ghana Statistical Service). The people are of different educational backgrounds ranging from illiterates to University graduates who are involved in occupations such as trading, artisanship, farming, teaching, health services, fishing, transport, government employment, construction, financing, tourism and religious activities.

### **Study population**

Street - food vendors operating within the University of Cape Coast campus and surrounding communities were randomly recruited after stratification of the study area into three zones, namely: new site (University science market), old site (Oguaa Hall market and Atlantic Hall market), and the community (Amamoma and Apewosika). The study lasted from January, 2013 to April, 2013.

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## Materials

Some of the chemicals and other materials used in this study included antibiotic - impregnated paper discs (Medical wire and Equipment Co. Ltd., Potley Corsham, England), Nuvu NF 200 centrifuge (NuveSanayi Taalzemeleri Imalat A.S., Turkey), IPF400 precision incubator (Mettler, Germany), peptone water (Sigma-Aldrich), and sterile swab sticks (Biolife, China), Blood agar (Thermo Scientific), plate count agar (PCA) (OXOID CM0325), Quebec colony counter (RE-3325, Reichert Inc).

## Sample Size/Swabbing Procedure

A total of 120 swab samples from the inside of serving utensils (serving plates or bowls) at vending points were collected in batches. By using sterile dry cotton swab sticks, the inside of each sampled serving utensils was swabbed from the edges to the center and placed in sterile containers and then transported to the laboratory, within an hour after collection. The samples were then taken through series of bacteriological analyses.

## Peptone Water Inoculation and Incubation

The swab samples were each inoculated in 10 ml sterile peptone water dispensed in universal tubes by aseptically cutting off the swab sticks. This was done to enhance the growth of microbes present. The tubes containing the inoculated peptone water were labeled, closed tightly and shaken to ensure uniform mixing of the sample, and then incubated for 24 hours at 37°C.

## Serial Dilution and Inoculation on Plate Count Agar (PCA)

The serial dilution was done by aseptically pipetting 1 ml of the overnight broth culture into a clean dry test tube containing 9 ml of sterile saline to obtain a volume of 10 ml. The 1:10 dilution of the broth and saline was thoroughly mixed and further foldsof dilution were made by aseptically transferring 1ml of the mixture into 9 ml of the diluent from 1:10 to 1:10<sup>10</sup>. The spread plate method was used to inoculate the serially diluted samples on the PCA (OXOID CM0325) plate by transferring a volume of 0.1ml of the 7<sup>th</sup> dilutions. Using sterilized L-shaped glass rod the inoculum was spread to ensure even distribution on the medium. The inoculated PCA plates were inverted to prevent the condensation of moisture on the media and incubated at 37°C overnight.

## Bacteria Enumeration

Bacterial enumeration was performed to determine the bacterial load of each swab sample. The Quebec colony counter was used to count the colony forming units on PCA for each sample. Bacterial load per milliliter of each sample was calculated by using the formula below:

$$\text{Bacteria/mL} = \frac{\text{Number of Colony Forming Unit (CFU)}}{\text{Volume plated (mL)} \times \text{Dilution factor}}$$

The number of bacteria per ml of the serially diluted samples was determined and the plates with viable counts (30-300 colonies) were chosen for sub-culturing.

## Sub - culturing and Isolation of Bacteria

Isolates from PCA plates with viable counts of 30-300 were sub- cultured by streaking on blood agar (OXOID CM0271) and MacConkey agar (OXOID CM0007) to obtain pure bacteria isolates. The plates were labeled, inverted and incubated for 18-24 hours at 37°C.

## Identification of Bacteria Isolates

Pure bacteria isolates from the sub - culture were identified, using both conventional and standard biochemical methods. The conventional method of identification used Gram staining to establish Gram reactivity of each bacteria isolate based on the bacteria cell wall composition, wet preparation for motility, odor, colonial morphology and pigmentation. The standard biochemical methods used for definitive identification of bacteria isolates included catalase test to differentiate *staphylococci spp* from *streptococci spp*; coagulase test to identify *staphylococcus aureus*; triple sugar iron agar (TSI) for lactose and glucose fermentation; H<sub>2</sub>S and gas production, and citrate utilization to identify and differentiate enterobacteria; urease test to aid identification of *Proteus spp*, *Yersinia enterocolitica*; and indole test to differentiate Gram-negative rods, particularly *Escherichia coli* as previously described [13].

**Antimicrobial Susceptibility Testing**

The modified Kirby-Bauer disc diffusion technique was used. Briefly sterile Mueller Hinton (LAB-39) agar plates were prepared and inoculated with cell suspensions from the various pure bacteria cultures prepared by obtaining turbidity equivalent to 0.5 McFarland standards, using sterile swab sticks. Antibiotic multi-disk, depending on the Gram reactivity of each bacteria species was aseptically placed on the freshly inoculated Mueller Hinton agar plates and incubated aerobically for 24 hours at 37°C. The antibiotic - impregnated disc used for Gram - positive bacteria were ampicillin (AMP), cotrimoxazole(COT), tetracycline (TET), cefotaxime (CTX), ciprofloxacin (CPF), gentamycin (GEN), cloxacillin (CXC), and that for the Gram - negative bacteria species were ampicillin (AMP), cotrimoxazole (COT), tetracycline (TET), cefotaxime (CTX), ciprofloxacin (CPF), gentamycin (GEN), chloramphenicol (CHL) and amikacin (AMK). The diameters of the zone of inhibition after 24 hr incubation were measured and cross-referenced to performance standards for antimicrobial disc susceptibility tests to determine the susceptibility, intermediate and resistance of each bacteria species (National Committee for Clinical Laboratory Standards, 2003).

**Statistical Analysis**

Data obtained were expressed as mean ± SEM. Significant differences in measured parameter were done using one-way analysis of variance (ANOVA) with Bonferroni’s Multiple Comparison test (*post hoc* test) in Graph-Pad Prism for Windows Version 5.0 (Graph-Pad Software, San Diego, CA, USA). P ≤ 0.05 was regarded as statistically significant.

**Results**

The swab samples from each of the three zonal areas produced significant mean bacteria counts > 10<sup>9</sup> (Table 1). A total of 9 bacteria species were identified from all serving utensils from the three zonal areas with *Bacillus cereus* being the predominant bacteria isolate while *Proteus mirabilis* was the least isolated bacteria species (Figure 1). All the 9 identified bacteria species were distributed across the three zonal areas though the frequency of distribution was not uniform (Figure 2). At all the zonal areas more pathogenic bacteria species were identified compared to non - pathogenic bacteria species (Figure 3).

Descriptive statistics	Street-Food Vending Outlet		
	New Site	Old Site	Community
Number of samples	40 <sup>a</sup>	40 <sup>b</sup>	40 <sup>c</sup>
Minimum count	3.9×10 <sup>9</sup>	3.8×10 <sup>9</sup>	4.8 ×10 <sup>9</sup>
Maximum count	2.41×10 <sup>10</sup>	2.01×10 <sup>10</sup>	1.98 ×10 <sup>10</sup>
Mean count	1.066 ×10 <sup>10</sup>	1.081 ×10 <sup>10</sup>	1.274 ×10 <sup>10</sup>
Std. Deviation	4.577 ×10 <sup>9</sup>	4.785 ×10 <sup>9</sup>	4.192 ×10 <sup>9</sup>
Std. Error	7.238 ×10 <sup>8</sup>	7.566 ×10 <sup>8</sup>	6.628 ×10 <sup>8</sup>

**Table 1:** Descriptive statistics of the microbial counts (CFU/ ml) of total bacteria isolates from each street - food vending outlets.

<sup>a</sup>(Mean count of new site), <sup>b</sup>(Mean count of old site), and <sup>c</sup>(Mean count of community)<sup>a</sup>v<sup>b</sup>, P > 0.05; <sup>a</sup>v<sup>c</sup>, P < 0.05; <sup>b</sup>v<sup>c</sup>, P < 0.05

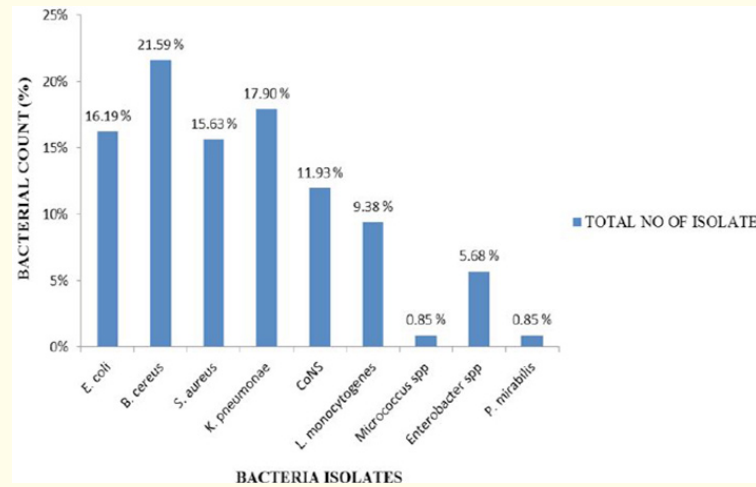


Figure 1: Each bar shows the total isolates of each bacteria species from the three street food vending outlets.

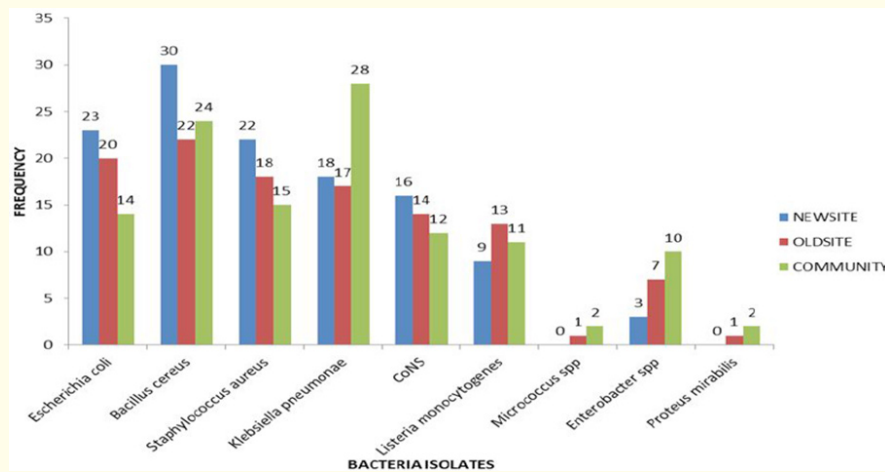


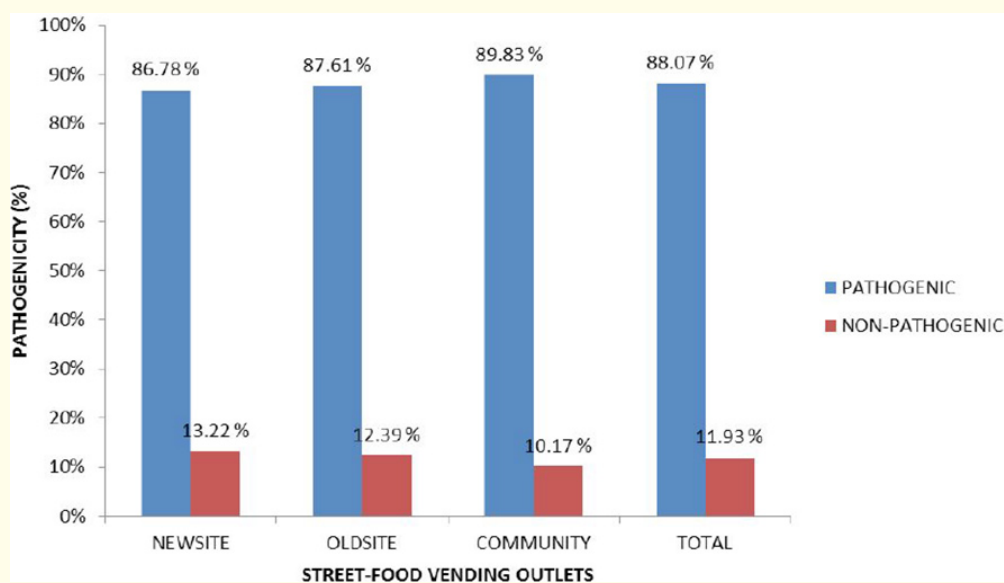
Figure 2: Each bar shows the frequency of each bacteria species across the three street-food vending outlets.

Among the Gram - positive bacteria isolates L. monocytogenes was the most susceptible to all the antibiotics while S. aureus was the least susceptible. Also, Micrococcus spp showed the highest antibiotic resistance with L. monocytogenes showing the least antibiotic resistance. All the bacteria isolates were resistant to ampicillin and cloxacillin but sensitive to gentamycin (Table 2).

	Bacteria Isolates														
	S. aureus (N = 4)			B. cereus (N = 4)			CoNS (N = 3)			L.monocytogenes (N = 4)			Micrococcus spp (N = 2)		
Antibiotics	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
AMP(20 mg)	4	0	0	4	0	0	3	0	0	4	0	0	2	0	0
COT(25 mg)	0	0	4	0	2	2	0	0	3	0	1	3	0	0	2
TET(30 mg)	3	1	0	0	0	4	0	1	2	1	0	3	2	0	0
CTX(30 mg)	0	4	0	0	2	2	0	3	0	0	0	4	0	2	0
CPF(5 mg)	4	0	0	0	2	2	1	2	0	0	2	2	2	0	0
GEN(10 mg)	0	1	3	0	0	4	0	0	3	0	1	3	0	0	2
CXC(5 mg)	4	0	0	4	0	0	3	0	0	3	2	0	2	0	0
Susceptible(S)	0.25			0.5			0.38			0.5172			0.286		
Intermediate(I)	0.2143			0.2143			0.29			0.2069			0.143		
Resistant(R)	0.5357			0.2857			0.33			0.2759			0.571		

**Table 2:** Antibiotic susceptibility patterns of Gram - positive bacteria isolates.

AMP: Ampicillin; COT: Cotrimoxazole; TET: Tetracycline; CTX: Cefotaxime; CPF: Ciprofloxacin; GEN: Gentamycin; CXC: Cloxacillin; N: Number of bacteria isolates; Coagulase negative Staphylococci



**Figure 3:** Distribution of pathogenic and non-pathogenic bacteria isolates from the three street-food vending outlets.

Also, among the Gram - negative bacteria isolates, *Enterobacter spp* produced the highest susceptibility (least antibiotic resistance) while *Proteus mirabilis* showed the least susceptibility (high antibiotic resistance). The entire Gram - negative bacteria isolates showed no sensitivity to ciprofloxacin and tetracycline but were all susceptible to cotrimoxazole and amikacin (Table 3).



	Bacteria Isolates											
	<i>E. coli</i> (N = 4)			<i>K. pneumoniae</i> (N = 3)			<i>Enterobacter spp</i> (N = 3)			<i>P. mirabilis</i> (N = 3)		
Antibiotics	R	I	S	R	I	S	R	I	S	R	I	S
AMP (20 mg)	4	0	0	3	0	0	0	1	2	3	0	0
COT (25 mg)	0	0	4	0	0	3	0	0	3	0	0	3
CTX (30 mg)	3	1	0	1	0	2	3	0	0	3	0	0
CHL (30 mg)	0	2	2	2	1	0	0	0	3	3	0	0
CPF (5 mg)	1	3	0	3	0	0	1	2	0	2	1	0
TET (30 mg)	3	1	0	3	0	0	2	1	0	3	0	0
GEN (10 mg)	0	0	4	3	0	0	0	0	3	3	0	0
AMK (30 mg)	0	0	4	0	0	3	0	0	3	0	0	3
Susceptible (S)		0.4375			0.3333			0.5833			0.25	
Intermediate (I)		0.2187			0.0417			0.1667			0.0417	
Resistant (R)		0.3438			0.625			0.25			0.7083	

**Table 3:** Antibiotic susceptibility pattern of Gram - negative bacteria isolates.

AMP: Ampicillin; COT: Cotrimoxazole; TET: Tetracycline; CTX: Cefotaxime; CPF: Ciprofloxacin; GEN: Gentamycin; CHL: Chloramphenicol; AMK: Amikacin; N: Number of bacteria isolates

## Discussion

This study investigated the bacteriological quality of serving utensils used by street - food vendors to serve customers at three zonal vending outlets within the University of Cape Coast campus and surrounding communities. Results from the study show high levels of contamination (>105 CFU/ml) of the serving utensils as shown by the mean bacterial counts ( $1.066 \times 10^{10}$  CFU/ml) from each of the vending outlets (Table 1). Indeed, bacteria count of  $1.066 \times 10^{10}$  CFU/ml recorded in the present study is even higher than  $10^7$  cfu/g measured from some contaminated foods [14]. The reasons for this high level of contamination of serving utensils may not be different from earlier observations [3] which attributed food contamination to poor sanitary and hygienic practices among vendors. For example, it is a common practice among most vendors to use one pan full of soap solution to wash all used serving bowls without replacing the soap solution, only to rinse the bowls in another pan containing water which is also not replaced, which in many instances lead to visibly dirty water. It is highly probable that the dirty rinsing water might be the source of contamination, perhaps serving as a reservoir or an 'inoculum' for cross - contamination. It was not surprising that a total of 9 different bacteria species were identified from the contaminated serving utensils (Figure 1) with majority of them being enterotoxigenic pathogens (Figure 3). What is even worrying was the observation that all the serving utensils from the three vending outlets were contaminated with all the nine bacteria species identified, indirectly emphasizing a general decline in the observance of hygienic practices by vendors within the study zones. This has the potential to expose students, University workers as well as inhabitants of the communities to risk of foodborne illnesses. Of serious concern was the identification of *Bacillus cereus* as the most predominant bacteria isolate from the serving utensils. *Bacillus cereus* is one of the most reported pathogenic bacteria implicated in foodborne illnesses [1,6,7,15] after *Clostridium perfringens* [7,12] and *Vibrio spp* [1] in view of its ubiquitous nature coupled with its ability to produce diverse enterotoxins, as well as survival under extreme cold [11] and high temperatures [6,12]. It is possible that serving food at ground level as well as improper washing of serving utensils could account for the source of *Bacillus cereus* contamination of serving utensils. Also, identification of *E. coli* from the serving utensils sampled from the three vending outlets gives a strong indication of fecal contamination, in view of the fact that *E. coli* is a fecal coliform [9]. This may be so in view of the unavailability of toilet facilities as well as water supply at some vending outlets, a situation which has the potential to compel some vendors to engage in all manner of unhygienic practices such as eating at obscure places close to vending sites. The present observation with regard to *E. coli* is not different from reports from independent studies that determined

high contamination from foods [16-20]. The most important source of *Staphylococcus aureus* is from body surface and fluids with the principal reservoir being the nose [14,21]. Between 30-40 % of healthy people carry *Staphylococcus aureus* in their nasal cavity, on their hands and on other parts of the body [22]. Also, *Staphylococcus aureus* may be found in fresh water, dust, air, and on plants and on hair [23]. Contamination of the serving utensils with *Staphylococcus aureus* as observed in this study may possibly be attributed to vendors' use of their hands on their body yet displaying poor hand washing attitudes and unprotected coughing and sneezing while washing serving utensils. Indeed earlier reports have attributed *Staphylococcus aureus* contamination to poor personal hygiene among food handlers [21,24]. Indeed, *Staphylococcus aureus* contamination has the potential to cause fatal diseases including septicemia, pneumonia, osteomyelitis and endocarditis [25,26]. Also, *Micrococcus spp* and *Staphylococcus spp* dependent contamination of serving utensils give a clear indication of cross - contamination as these two bacteria species thrive on surfaces which might have been touched by the hands of vendors. Notably, food vendors wash their dirty hands in water they have used to wash and rinse serving utensils and this might be the source of contamination of serving utensils by *Micrococcus spp* and *Staphylococcus spp*. Further, *Listeria monocytogenes* which is one of the most virulent food borne pathogens [27,28] were identified on the serving utensils. *Listeria monocytogenes* dependent foodborne illnesses may cause gastrointestinal discomfort, nervous system infections, sepsis, and local abscesses [29]. Contamination of serving utensils with *L. monocytogenes* could explain possible transfer of serving utensils which accidentally drop on the ground into rinsing water as this is often the case at most vending sites. Importantly, all the other bacteria species identified from the serving utensils in this study including *Klebsiella pneumonia* [30], *Proteus mirabilis* [31,32], *Enterobacter spp* [33] and *coagulate negative Staphylococci* [34] all have the potential to cause foodborne illnesses as well as to produce biofilms adhering to food contact surfaces [35,36] and could also survive washing and cooking temperatures [37]. These observations call for much attention to the quality of serving utensils used by food vendors in the study area. Clearly, contaminated serving utensils represent a major source of food contamination with enterotoxigenic bacteria possibly through cross -contamination. This has the potential to increase risk of foodborne illnesses among unsuspecting food consumers in the study area.

Knowledge of susceptibility patterns of enteropathogens is crucial for management and treatment of enteropathogenic foodborne illnesses, especially at a time when antibiotic resistance has become a major threat to treatment of infectious diseases in general [8,9]. Results of this study showed that all the Gram - positive bacteria species were susceptible to cotrimoxazole and amikacin while they were all resistant to ciprofloxacin and tetracycline indicating that prescription of antibiotics to Gram - positive - dependent foodborne illnesses must reflect the sensitivity pattern of the underlying enteropathogens. Similarly, Gram - positive bacteria species identified in this study were susceptible to gentamycin but resistant to ampicillin and cloxacillin. Importantly, Gram - reactivity test must precede the choice of antibiotic for any bacteria contaminated foodborne illness to increase treatment success.

## Conclusion

Put together, the present study unveils serving utensils as a major reservoir of enterotoxigenic bacteria increasing the risk of transmission of foodborne illnesses among food consumers. Importantly, our results provide a strong rationale not only for further studies on the topic but a wakeup call on public health authorities to revamp public health education of key stakeholders in the food industry including food vendors and consumers while at the same time increase supervision and enforcement of food sanitation by laws to protect the general public in the study area.

## Acknowledgement

We thank the laboratory staff of the Department of Medical laboratory, UCC and the biomedical scientists at MDS - Lancet Laboratories Gh. Ltd, Accra, Ghana.

## Conflict of Interest

None declared.



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**Volume 2 Issue 4 October 2015**

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