

BACTERIAL CONTAMINANTS IN LETTUCE, TOMATOES, BEEF AND GOAT MEAT FROM THE ACCRA METROPOLIS

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SUMMARY

A survey on the role of lettuce, tomatoes, beef and goat meat in the transmission of food-borne bacteria in Accra was conducted by evaluating their microbial quality.

All 60 market lettuce samples had significantly higher faecal bacteria compared to 8(12%) from the farms (5.0 ± 0.9 vs. 4.3 ± 0.9 , $p < 0.001$). *Salmonella* Groups D and G, *Shigella dysenteriae*, *flexneri* and *boydii* were detected in both farm and market samples. Irrigation with tap water instead of drain water significantly reduced the level of faecal coliforms (4.4 ± 0.9 vs. 3.8 ± 0.7 , $p < 0.001$). Market (16) and farm (8) samples of tomatoes had similar levels of coliforms and faecal coliforms but rotten tomatoes had more coliforms (6.8 ± 0.37 vs. 4.6 ± 0.65 , $p < 0.001$) and faecal coliforms (4.7 ± 0.29 vs. 3.5 ± 0.81 , $p < 0.001$) than whole tomatoes. *Salmonella* Group B was isolated from one farm sample. Mesophilic counts of beef from the abattoir were within acceptable limits of less than $7.0 \log_{10}$ cfu/g. Market samples had significantly higher coliforms (5.0 ± 0.62 vs. 3.5 ± 1.8 , $p < 0.05$) than the abattoir samples. *Salmonella* Group B and *Shigella dysenteriae* were isolated from beef from the market. Coliforms and faecal coliforms were numerically higher in the market and supermarket samples of goat meat. *Shigella flexneri* was isolated from only the supermarket samples.

Vegetables and meat available in the Accra Metropolis could be sources of food-borne bacteria. Predisposing factors and strategies to improve their hygienic quality are discussed.

Keywords: Food-borne illness, enteropathogens, fresh produce, meat, contamination.

INTRODUCTION

The public health impact of food-borne illness has increased worldwide as a result of changes in food

consumption habits. There is a growing tendency to eat outside the home with increases in outbreaks of food-borne illness in the United States¹. The consumption of fresh produce has also increased in popularity. Although fresh produce is susceptible to contamination by human or animal excreta, disinfection is not adequately carried out prior to preparation². As a result green onions, fresh squeezed orange juice, lettuce and sliced tomatoes have been associated with major disease outbreaks³. Animal products have also been implicated in several outbreaks internationally³.

We have in an earlier study showed unacceptable levels of bacteria in most street foods, especially, salads, fresh vegetable sauces and some meat dishes⁴. A bird's eye view shows an increase in the patronage of ready-to-eat foods with a resultant growth in fast-food joints and salad bars in the Accra Metropolis. In order to avert food-borne disease outbreaks, vendors of such foods are often given food hygiene education. Improved food safety does not rest on the processing stage alone as virtually every step from production to consumption can impact dramatically on the final product⁵.

We therefore conducted this study on the microbial contaminants in lettuce, tomatoes, beef and goat meat to assess the role played by these agricultural produce in the transmission of diarrhoea pathogens.

MATERIALS AND METHODS

Samples

Samples of lettuce and tomatoes were purchased from farms and open markets in the Greater Accra Metropolis. In order to assess the impact of the use of different types of water for irrigation on the hygienic quality of vegetables, samples of lettuce were also collected from farmers that used chlorinated pipe-borne water, water from drains and well water.

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Beef samples were collected from the same open market as above and the Accra Abattoir. Goat meat was collected from the open market and from a supermarket, which used refrigeration during display of goat meat.

From May to August 1998, 250 g to 500 g of vegetables or meat was purchased at random and placed into sterile stomacher bags for transportation on ice to the laboratory. The specimens were stored at 4°C and examined within 24 h of collection at the Bacteriology Unit of the Noguchi Memorial Institute for Medical Research.

Pre-treatment of samples

The surface rinse method was used in the removal of bacteria from the samples⁶. In this 100 g to 250 g of sample was weighed into a sterile stomacher bag and an equivalent volume of Phosphate Buffered Saline (PBS) ('Dulbecco A' BR 14a UNIPATH (Oxoid) Basingstoke, UK.) was added. The samples were gently massaged, shaken at intervals and allowed to drip before removal from the PBS.

10 ml of the rinse was used to prepare 1 in 10 serial dilutions for the enumeration of bacteria in the pour plate method. The remaining rinses were centrifuged at 11,500 x g for 30 min at 4°C using an automatic high-speed refrigerated centrifuge (Hitachi 20 PR-520, Japan). The supernatant fluid was discarded and the resultant pellet examined for members of the *Enterobacteriaceae* as described below.

Enumeration of Bacteria

One ml of the 1:10 dilutions of the rinse from the samples were inoculated into Plate Count Agar (PCA) (Oxoid CM7, UNIPATH (Oxoid), Basingstoke, UK) for enumeration of total counts (mesophilic bacteria), MacConkey agar (Oxoid CM7) incubated at 37°C for total coliforms and MacConkey agar at 44°C for faecal coliforms.

Agar plates showing 30 to 300 colonies were selected and counted using a colony counter (Gallenkamp, UK). The microbial concentration in the original sample was estimated by multiplying the count with the dilution factor.

Isolation and identification

Three to four loopfuls of the pellet was streaked onto salmonella/shigella (SS) agar (Difco Laboratories, UK), xylose lysine deoxycholate (XLD) agar (Oxoid CM 469) and MacConkey agar (Ox-

oid CM7) for *Salmonella*, *Shigella*, *E. coli* and other *Enterobacteriaceae*.

Selective enrichment for *Salmonella* and *Shigella* was achieved by inoculating portions of the pellet into selenite-lactose broth (SB) (42001 Eiken, Japan). After incubation for about 18 h, the SB was streaked onto SS and XLD agars for isolated colonies.

All isolates were further investigated for *Salmonella*, *Shigella*, *E. coli* and other gram-negative bacilli using standard methods^{7,8,9}. API 20E kits (bioMerieux SA, Marcy-l'Etoile, France) was used as a confirmatory identification test. Specific antisera: *Salmonella* antisera O-grouping and Vi sera (No. 21041 Denka Seiken, Tokyo, Japan) and *Shigella* antisera (III) (No. 1453, Denka Seiken) and *E. coli* antisera (I) (No. 24506, Denka Seiken) were used for typing of these bacteria.

Data analysis

The data was analysed using Sigma plot software (SigmaStat Statistical Analysis System, ver 1.02, Jandel Corporation). The values obtained for number of colony forming units per gram (cfu/g) of food was transformed into log₁₀ values. Cross tabulations of the levels of the various bacteria tested, the type of water and the location of the samples were made. Analysis of variance was conducted and where the variances were different, chi square for percentage of samples contaminated and Student t-test for numbers of bacteria were performed on the groups. Counts of bacteria from goat meat, beef, and tomatoes violated the parametric test so the Mann-Whitney rank sign test was used instead.

RESULTS

Lettuce from the farms and market had unacceptable levels of coliforms and faecal coliforms. All the market samples had faecal coliforms, with significantly higher levels, but only 8(12%) farm samples were positive (Table 1).

Table 1 Level bacterial contaminants in lettuce by location.

Location	No.	Type of bacteria (Mean Log ₁₀ cfu/g)		
		Mesophiles	Total coliforms	Faecal coliforms
Farm	66	*4.8(1.0)	**4.3(0.9)	***3.3(1.6)
Market	60	*5.2(1.2)	**5.0(0.9)	***4.7(0.8)

Notes: ANOVA Standard deviations in parenthesis

* p<0.05

** p<0.001

*** p>0.05

Table 2 shows that lettuce that was watered with chlorinated pipe-borne water had the least contamination. Interestingly the only farm that used well water had most contamination. It is worth noting that the well was a shallow dug out which could be polluted by surface water. *Salmonella* Groups D and G. and *Shigella dysenteriae*, *flexneri* and *boydii* were detected in both market and farm samples (Table 3). They were also present in samples irrespective of the source of water (Table 4).

Table 2 Level of contamination by irrigation water source

		Type of bacteria (Mean Log ₁₀ cfu/g±SD)		
Water	No.	*Mesophiles	**Total coliforms	***Faecal coliforms
Drain	45	4.9±1.1	4.4±0.9	3.6±1.0
Tap	17	4.1±0.4	3.8±0.7	2.1±1.5
Well	1	6.7	6.9	5.6
Stream	3	5.6±0.3	4.6±0.3	4.5±0.7

* p<0.01
 ** p<0.001
 *** p>0.05

Table 3 Prevalence of enteropathogens on lettuce by location

Location	Bacteria No. (%)			
	None	Salmonella	Shigella	Total
Farm	61(92.4)	3(4.5)	2(3.1)	66
Open Market	54(90.0)	1(1.7)	5(8.3)	60
Total	112(91.3)	4(3.2)	7(5.6)	126

Table 4 Prévalence of enteric pathogens by irrigation water source

Water Source	Bacteria No. (%)			
	None	Salmonella	Shigella	Total
Drain	41(93.2)	2(4.5)	1(2.3)	44
Tap	15(88.2)	1(5.9)	1(5.9)	17
Well	1	0	0	1
Stream	4	0	0	4
Total	61(92.4)	3(4.5)	2(3.0)	66

Whole tomatoes from the farms were more contaminated with mesophilic bacteria and coliforms than the market samples but the figures were not significant statistically. Faecal coliform counts were similar (Table 5). Rotten tomatoes were significantly more contaminated than whole tomatoes from the market (Table 5). Several bacteria be-

longing to the *Enterobacteriaceae* were detected but of significance are *E. coli* from rotten market tomatoes and *Salmonella* Group B from farm samples (Table 6).

Table 5 Bacterial contaminations in whole and rotten tomatoes by location

		Type of bacteria (Mean Log ₁₀ cfu/g±SD)		
Location	No	Mesophiles	Total coliforms	Faecal coliforms
Market				
Whole	8	*4.4 ± 0.45	**4.6±0.65	***3.5±0.81
Rotten	8	*5.1 ± 0.39	**6.8±0.37	***4.7±0.29
Farm				
Whole	8	5.3 ± 1.25	5.2 ± 1.24	3.5±1.87

* p=0.003
 ** p<0.001
 *** p<0.001

Table 6 Microbial flora of tomatoes by location

Bacteria	Location		
	Open Market No. (%)		Farm No. (%)
	Whole	Rotten	Whole
<i>Salmonella</i> sp.	0	0	1(5.9)
<i>E. coli</i>	0	1(5.9)	5(29.4)
<i>Staphylococcus aureus</i>	1(5.6)	2(11.9)	0
<i>Alcaligenes</i> sp	2(11.1)	1(5.9)	0
<i>Pseudomonas</i>			
<i>Flourescens</i>	8(44.4)	5(29.4)	1(5.9)
<i>Enterobacter sakazaki</i>	1(5.6)	1(5.9)	0
<i>Klebsiella pneumoniae</i>	1(5.6)	0	1(5.9)
<i>Citrobacter freundii</i>	1(5.6)	1(5.9)	2(11.9)
<i>Providencia rettgeri</i>	1(5.6)	1(5.9)	0
<i>Providencia alcalifacens</i>	1(5.6)	1(5.9)	0
<i>Serratia ficaria</i>	0	2(11.9)	0
<i>Enterobacter cloacae</i>	2(11.1)	2(11.9)	7(41.2)
Total	18	17	17

Table 7 Bacterial contaminants in beef by location

		Type of bacteria (Mean Log ₁₀ cfu/g±SD)		
Location	No.	Mesophiles	Total coliforms	Faecal coliforms
Market				
	10	*6.0 ± 0.63	**5.0 ± 0.62	***4.9 ± 0.76
Abattoir				
	10	*4.2 ± 1.32	**3.5 ± 1.84	***2.5 ± 1.78

* p<0.05
 ** p<0.05
 *** p<0.05

Beef samples from the abattoir were significantly less contaminated than those from the market (Table 7). *Salmonella* Group B and *Shigella dysenteriae* were present in only the market samples but *E. coli* was present in samples from both sites (Table 8).

Table 8 Microbial flora of beef by location

Bacteria	Location of sample	
	Open Market No. (%)	Abattoir No. (%)
<i>Shigella dysenteriae</i>	4(8.5)	0
<i>Salmonella</i> sp	2(4.3)	0
<i>E. coli</i>	8(17.0)	3(11.5)
<i>Proteus</i> sp	1(2.1)	3(11.5)
<i>Klebsiella oxytoca</i>	3(6.4)	1(3.8)
<i>Citrobacter freundii</i>	2(4.3)	1(3.8)
<i>Pseudomonas</i> spp.	4(8.5)	1(3.8)
<i>Serratia</i> spp.	1(2.1)	3(11.5)
<i>Yersinia</i> spp.	3(6.4)	3(11.5)
<i>Morgenella morganii</i>	0	1(11.5)
<i>Staphylococcus aureus</i>	1(1.1)	1(3.9)
<i>Coagulase negative</i>		
<i>Staphylococcus</i>	8(17.0)	5(19.2)
<i>Hafnia alvei</i>	1(2.1)	0
<i>Aeromonas</i> spp.	9(19.1)	5(19.2)
Total	47	26

Goat meat from the open market was more contaminated than the supermarket samples but only total counts were statistically significant (Table 9). Surprisingly, *Shigella flexneri* was isolated from supermarket samples but not from the open market (Table 10). *E. coli* was present in samples from both sources.

Table 9 Bacterial contaminants in goat meat by location

		Type of bacteria (Mean Log ₁₀ cfu/g±SD)		
Location	No.	Mesophiles	Total coliforms	Faecal coliforms
Market	12	*6.6 ± 0.99	**6.06±1.0	***4.9±0.88
Supermarket	6	*5.5 ± 0.60	**5.6±0.74	***4.8±0.76

* p=0.028

** p=0.31

*** p=0.81

Table 10 Microbial flora of goat meat by location

Bacteria	Location of sample	
	Open Market No. (%)	Supermarket No. (%)
<i>Shigella flexneri</i>	0	2(5.6)
<i>Escherichia coli</i>	5(19.4)	2(5.6)
<i>Proteus</i> spp.	12(25.0)	6(16.7)
<i>Yersinia</i>		
<i>Pseudotuberculosis</i>	0	4(11.1)
<i>Klebsiella</i> spp	2(4.2)	0
<i>Coagulase negative</i>		
<i>Staphylococcus</i>	5(10.4)	6(16.7)
<i>Streptococcus</i> spp.	3(6.3)	6(16.7)
<i>Citrobacter</i> spp.	12(25.0)	6(16.7)
<i>Enterobacter</i> spp.	4(8.3)	2(5.6)
<i>Aeromonas</i> spp.	4(8.3)	0
<i>Pseudomonas</i> spp.	0	2(5.6)
<i>Tatumella ptyseos</i>	1(2.1)	0
Total	48	36

DISCUSSION

There is a shift from traditional consumption of homemade food towards ready-to-eat foods in most parts Ghana. In addition there is an increase in the number of tourists visiting the country, a result of which is an increase in the number of fast food joints and salad bars where cold and under-cooked meals are often served. There are therefore new routes as well as new hosts to facilitate food-borne disease transmission. This study was therefore conducted to evaluate the role of some of the common food ingredients in disease transmission.

Lettuce and tomatoes had unacceptable levels of faecal coliforms (more than 3.0 cfu/g) and could therefore be sources of enteropathogenic bacteria. The presence of *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Salmonella* Group D and G in lettuce as well as *Salmonella* Groups B and *E. coli* in tomatoes confirms this.

The responsibility of ensuring food safety does not rest on the processor alone, as events occurring, before the crop is planted are equally important. The growing location and history of the land are the initial factors to be considered. Fields that have been grazed by livestock and wild animals often have enteric pathogens¹⁰. Certain bacteria, for example *Salmonella* and *Listeria monocytogenes* could survive for prolonged periods in sewage sludge commonly applied to agricultural soil.

Our results make clear that while tap water irrigation decreased the level of contamination of vegetables at the farm gate, the prevalence of specific pathogens was not affected. Animal manure could be the source of these pathogens. We observed that farmers applied cow dung and chicken droppings as manure. Work from our laboratory isolated *Salmonella*, *Campylobacter* and *Escherichia coli* from the cloacal contents of live birds¹¹. Humans remain the sole reservoir for *Shigella* spp., which implies that contamination with human excreta is possible on these farms and could be a result of the absence of toilet facilities on these farms. In addition wastewater from open gutters that has all manner of waste is commonly used for irrigating vegetables. It has been revealed that the application of wastewater before harvest, rather than during the early stages of the production cycle could be of greater concern¹². Flood waters, also become polluted with human and animal excreta and may contaminate farmlands and crops.

At the market vegetables are handled at ground level. In addition large quantities of vegetables are

washed in limited quantities of water to remove soil debris. There is no sanitization of vegetables at the market level. Although washing and sanitising vegetables for the purpose of removing microflora have been shown to be inefficient², washing of Brussel sprouts in 200 µg ml⁻¹ (PPM) of hypochlorite reduced the population of *L monocytogenes* by more than eight logs. The use of only water, however, gave a reduction of two logs¹³. This implies that if clean water is used to wash vegetables, for example, lettuce at our markets, the bacterial load could be reduced by at least two logs.

There are also problems with packaging and display of fresh produce at our markets and this requires some attention. Indeed the microbial quality of fresh produce depends on a number of factors and legislation to ensure safety must be multifactorial and not at the level of the producer or the processor alone.

Beef and goat meat were equally contaminated with unacceptable levels of bacteria. Meat and meat products have been implicated in a number of food borne outbreaks world-wide^{14,15,16}. Several authors have isolated *Salmonella*, *Shigella*, *Campylobacter* and *E coli* from beef and other meats^{17,18}. The environment in which the animal is taken through the different stages of slaughter is an important factor that could affect the hygienic quality of meat. Other factors are; equipment for processing, for example, grinders used in the preparation of ground beef and the personal hygiene of the staff^{19,20}. We have evaluated some of these factors at the old Accra slaughterhouse, the Accra abattoir and a typical traditional slaughter slab. The findings from these studies support this assertion.

The use of car boots and other unauthorised vans for carting meat to the markets are unacceptable as the meat is exposed to dust and flies and there is no refrigeration during transportation. Cold storage vans designated for this purpose must be used. On arrival at the market meat is carried on shoulders of untrained attendants to the stalls.

Conditions prevailing at the markets are also ideal for bacterial contamination and proliferation. Open display of meat at the markets expose them to flies, contaminated air and dust. Flies are known mechanical vectors of enteropathogenic bacteria²¹. There are no refrigeration facilities at the markets and the high ambient temperature allows bacteria to multiply.

The detection of *Shigella flexneri* in samples from the supermarket is a grave finding indeed. These establishments are supposed to use more advanced technology and display their meat under refrigeration. It is noteworthy that *Shigella flexneri* have been isolated from chicken from the same supermarket in our laboratory¹¹. The original source of the meat is not known but there appears to be cross contamination of meat in the butchery of this supermarket. A butcher who is carrier of *Shigella flexneri* could also contaminate the meat during handling, especially, if the individual has poor personal hygiene.

Handling of meat in the Accra metropolis requires urgent attention and should involve all sectors of the food chain. Food handlers and consumers should be educated on the hazards associated with poor food hygiene. Consumers should also be advised to maintain the traditional method of cooking meat till it becomes tender as this process kills most vegetative bacteria and reduces the risk of their transmission via food.

There are a number of methods for reducing the bacterial load on beef and other meat products and these include: immersion in 2% acetic acid, immersion in 2% lactic acid, hot water spraying and spraying and immersion for 10 s using 12% trisodium phosphate²². Food irradiation appears to be effective and safe²³. But, all these methods require careful validation before they can be adopted in Ghana.

Fresh lettuce, tomatoes, goat meat and beef on sale in the Accra Metropolis could be sources of food borne bacteria. Efforts to avert major food-borne disease outbreaks should include improving the hygienic quality of these ingredients.

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