DATA ARTICLE

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Microbial profile of common spices and spice blends used in Tamale, Ghana



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Abstract

Background: The main purpose of using spice to grill meat is to add aroma, colour, flavour, taste and pungency. However, the purpose is sometime befitted when spice is contaminated with pathogenic bacteria that result in foodborne illnesses and toxicological effect.

Results: The study was necessitated by paucity information on handling practices and microbial load common spices used for grilling meat, Ghana. A total of twenty spice samples were collected from five popular and widely patronised joints in the Tamale in Ghana. Detection and identification of potential pathogens was carried out following standard procedures. *E. coli* count ranged from 0 to 3.14 log₁₀ cfu/ ml with a mean of $1.17 \pm 1.07 \log_{10}$ cfu/ ml. Contamination level for *Salmonella* spp ranged between 0 and 0.9 log₁₀ cfu/ ml with a mean of $0.38 \pm 0.31 \log_{10}$ cfu/ ml. Coliform bacteria were present in almost all the spices sampled. Faecal coliform and *E. coli* presence was an indication of contamination by fresh faecal matter. The possible sources of spice contamination include storage equipment, handling, unhygienic surroundings, vehicular transmission, atmospheric particles and air-microbes.

Conclusions: There is a possible risk to public health associated with consumption of spicy meat from the selected joints. There is the need to maintain good sanitary practice and hygienic quality during production stages of spice in order to avoid or reduce prevalence of food borne illnesses in Tamale and Ghana as a whole.

Keywords: Contamination, foodborne illness, pathogenic bacteria, spice, Ghana

Background

Street food is sold in every nook and cranny of major towns and cities in both developed and developing countries. Street foods are mostly prepared in open-air public spaces either stationary or itinerant, either on foot or from mobile outlets, removable outlets, and fixed outlets without enclosed space by food vendors to accommodate consumers (Food and Agriculture Organization of the United Nations 2016). Street food vending and consumption have recently flourished for some decades in Africa largely due to increase in urban population and wide spread of urban boundaries and urban sprawl (Food and Agriculture Organization of the United Nations 2016). Food safety is a concern for the general public as almost everybody for the past year has ever suffered from foodborne illnesses at least once (WHO 2015). Diarrhoea has been one of the major causes of hospital attendance in Ghana, and 16% deaths of children younger than five years in African (Bruce et al. 2005). Food-borne disease outbreaks due to importation were reported to have increased between 2009 and 2010 in which fish and spice were the most common sources (Centre for Disease Control CDC 2012).

Most of these street foods are sold to consumers with spices that are traditionally or locally prepared. Spices are group of food additives utilised to enhance sensory quality of some foods by making those foods more palatable (Debs-Louka et al. 2013). Sodium and fat content is very low in spice causing the increased in their consumption (Srinivasan 2005). However, conditions under which they are produced and basic operations such as drying, harvesting, threshing, transport and storage are potential sources of contamination with bacteria, fungi and insects (Alam-Khan and Abrahem 2010; Sádecká 2007). This can lead to an increase in certain



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foodborne illnesses and intoxications (Buckenhuskes and Rendlen 2004) and food spoilage (Ahene et al. 2011).

Spice has low moisture content making it safe, but when in contact with some food products that has high water, microbial populations could be stimulated because of increase in water activity (Menlove and Sainsbury 2002). The interventions in production process are mostly based on reduction or elimination of some contamination by means of heat treatment (Zwietering et al. 2016).

Generally, in Ghana, mix-powdered spice is usually used in grilling meat such as chevon, chicken, guinea fowl, suya (dried smoke meat), pork and many more which are processed manually and sold to general public at various lorry terminals, by roadside or itinerant vendors (Mensah et al. 2002). A study by Addo (2005) reported fungal count of 3.08 \log_{10} cfu/g to 2.40 \log_{10} cfu/g in the ginger samples. Also, the total aerobic bacteria count in the spice ranged from 3.6 \log_{10} cfu/g in ginger sample to 3.7 \log_{10} cfu/g sample (in mixture of ginger and garlic) bacteria species which collectively contaminated the spice were Enterobacter spp, Aeromonas salmonicida and Salmonella spp. Sagoo et al. (2009) and Shamsuddeen (2009) also reported potential public health risk of spices and herbs after isolating high counts of *Bacillus cereus*, *Clostridium perfrigens* and Escherichia coli. Spices are usually sold as loose or packed in the local markets and consumed in cities, towns and countryside of Ghana. The study was necessitated by paucity information on handling practices and microbial load on common spices used for grilling meat, Ghana.

Methods

Study area

The research was carried out in the central part of Tamale. Tamale is the capital city of the Northern Region of Ghana and lies in latitude 9° 15′ and 9° 05′ N and in longitude 0° 45′ and 1° 0′ W at an altitude of 185 m above sea level. It is third most populous settlement in Ghana with 537,986 inhabitants according to 2010 census (Ghana Statistical Service 2012). As a fast growing city, a lot of people migrate to Tamale to do businesses (Abankwa et al. 2009). It is a home to both locals and internationals.

Sample collection and preparation

A total of twenty spice samples were collected from five popular and widely patronised selected sites in Tamale. Four (4) samples were collected from each vendor on weekly basis for a period of one month (February to March, 2014). A small plastic container cleaned with methylated spirit was used to collect samples from the vendors. The samples were stored in a sterile box containing ice cubes and taken to the CSIR- Water Research Institute laboratory (Tamale) for analysis. Strict standard practices and aseptic conditions were observed to avoid possible contamination during sample collection and transportation, prior to analysis. Vendors of spicy meat were interviewed to ascertain possible sources of bacteria contaminant.

Using a sterilise spatula and weighing boat, five-grams of the spice samples were transferred into a sterile falcon tube with 45 ml of sterile Phosphate Buffer Saline $10 \times$ water and vortexes for about 2 min. It was then carefully swirled few times to ensure a homogenous mixture.

Microbial of determination

Microbiological analysis was done in accordance with American Public Health Association (APHA) (2008) standard procedures for analysis. A 3-fold serial dilution was performed for the enumeration of *Salmonella* spp, *E. coli*, total coliform, faecal coliform and *Clostridium perfringens* bacteria from spice samples. Hi-crome, MFC agar, M-Endo agar and Salmonella-Shigalla (SS) agar were used for detection of *Escherichia coli*, faecal coliform, total coliform and *Salmonella* spp, respectively whereas nutrient agar was used for *Clostridium perfringens* detection using pour plate method.

Faecal coliform was determined by filtering 60 ml of sample through a 0.45 um filter onto a Petri dish containing MFC and incubated at 44 ± 2 °C for 18–24 h and colonies were then counted. For detection for total coliform and *Escherichia coli*, sample was plated in M-Endo and Hichrome and incubated at 37 ± 2 °C for 18–24 h, respectively. Colonies of total coliform and *E. coli* were counted.

For *Salmonella* spp detection, 10 ml of the supernatant was filtered after priming membrane filter with 10 ml spice dissolved in water. Another 5 ml of spice dissolved in water was further used to rinse inner sides of funnel. Membrane filter was then transferred onto Salmonella-Shigalla agar for detection of the target microbes with the aid of a sterilised forceps. In *Clostridium perfringens* detection, 1 ml of the supernatant derived from the wash of spice samples were aliquot into a test tube after being heated in a water bath for about 15 min at about $50 \pm 2^{\circ}$ C. Nutrient agars of about 20 ml were poured into the Petri dish, and then swirled both clockwise and anticlockwise. Colonies were counted with a colony counter.

Statistical analysis

Analysis of variance (ANOVA) was deployed to determine significant difference between microbial loads from five popular meat grilling joints. Pearson's correlation matrix was also conducted before changing the microbial count values to a logarithmic scale.

Results and Discussion

To prevent occurrence of food borne illnesses, it is important to ensure foods safety and hygiene at every stage of food processing and selling. The present study recorded faecal coliform count that ranged between 0 and 3.4 \log_{10} cfu/ ml with a mean of $1.17 \pm 1.07 \log_{10}$ cfu/ ml (Table 1).

Note: All values are units of \log_{10} cfu/ml: A-D: Samples sites and 1–4 means first to fourth sample

TC Total coliform, FC Faecal coliform, E. coli: Escherichia coli, SS Salmonella spp., C. perfringens Clostridium perfringens

Total coliform count ranged from 0 to 1.7 \log_{10} cfu/ ml with a mean of $0.54 \pm 0.55 \log_{10}$ cfu/ ml (Table 1). *E. coli* count ranged between 0 and 3.14 \log_{10} cfu/ ml with a mean of $1.17 \pm 1.07 \log_{10}$ cfu/ ml (Table 1).

Coliform bacteria were present in almost all the spice sampled. The faecal coliform and *E. coli* detected in the samples was an indication of contamination by fresh faecal matter. This might be due to inadequate hand washing by vendors and absence of good personal hygiene. This according to FDA (2011) can cause vibrio cholera, bloody diarrhoea, and kidney failure in children or people with weak immune system.

Most food poisoning is commonly cause by *Salmonellae* that are pathogens. Salmonellosis is principally a food borne disease (European Union EU 2004). Contamination level for *Salmonella* spp ranged from 0 to 0.9 \log_{10} cfu/ml with a mean of $0.38 \pm 0.31 \log_{10}$ cfu/ml (Table 1).

Salmonella spp was detected in few samples. The contamination might be due to poor hygienic conditions, presence in food can cause abdominal clamps, fever and vomiting (Mankee et al. 2003). Salmonella presence is of concern in spices as the commodity is added in sufficient quantities to grilled meat for public consumption.

Clostridium perfringens can be found in dust, soils, vegetation among others environment media. *Clostridium perfringens* were not detected in any of the 20 samples. This might be attributable to poor growth parameter like favourable temperature. Since, its ability to grow in food is largely dependent on storage temperature (European Food Safety Authority 2004). This finding collaborate with that of Gardenguides (2002) that reported limited or no *Clostridium perfringens* count in spice in Australia. This seems to pose no risk to public health, however at slightly conducive temperature spore germination and cell multiplication can occur.

Similar studies conducted on spice used for selling local meat (kilichi) in Northern Nigeria revealed that the spices were greatly contaminated with E. coli, Salmonella spp and Clostridium spp (Shamsuddeen 2009). Sagoo et al. (2009) also isolated high counts of Clostridium perfringens and E. *coli* from spice and herb in United Kingdom which have potential public health risk. Spices have been associated in large scale outbreaks of food borne illnesses (Gustavsen and Breen 1984), the impact of pathogenic contaminated spice on incidence of food borne illnesses in Ghana cannot be override. The contamination of spices can be attributed to many factors. Since, Banerjee et al. (2003) suggested that spices such as curry powder, thyme, white pepper, paprika are subjected to microbial contamination at various stages of preparation. Equipment and food handlers have also been associated with contamination of food with various types of etiologic agent (Moro et al. 2001).

Possible sources of contaminants in spices

The study revealed that most vendors buy spices such as pepper, ginger, curry powder, paprika, nutmeg, chilli pepper (all in powdered form) from market and are then mixed in a plastic container. It was observed that vendors mixed spices with their bare hands without wearing hand groves and the prepared spices are left uncovered in the plastic container at the site. This exposes spice to contaminant such as dust, atmospheric particles and airborne microbes.

The study revealed that only one vendor out of five vendors had obtained permits from the Ghana Standard Board and Ghana Tourism Authority. Some reasons for not having permits were due to lack of funds and some vendors not aware that they needed to work with permits. Four out of five vendors had no formal education this might have influenced vendors' consciousness on personal hygiene, food handling and processing techniques. This according to

 Table 1 Microbial load of spices used for grilling meat in the Tamale

Sample Sites	TC	FC	E. coli	SS	C. perfringens	
A1	0	2.7	1.81	1.81 0.03 0		
B1	0.9	1.63	0	0.44	0	
C1	0	0	2	0.34	0	
D1	0.31	0	1.76	0 0		
E1	0.9	1.95	0.68 0.2 0		0	
A2	0	2.13	1.43 0 0		0	
B2	0	1.84	0 0 0		0	
C2	1.66	0.8	2.32	2.32 0.46 0		
D2	0.22	1.31	0	0.9 0		
E2	0.9	0	0	0.47 0		
A3	0.23	0.77	3.14	0.3 0		
B3	1.7	2.4	0.54	54 0.3 0		
C3	0	1.7	0	0.64	0	
D3	0.69	0	1.9	1.9 0.5 0		
E3	0.4	0.62	2.2	0	0	
A4	1.04	0.32	2.6	0.9	0	
B4	0.36	0	0.66	0.72	0	
C4	0	0	1.51	0.4	0	
D4	0.3	1.2	0	0.8	0	
E4	0.5	0	0	0	0	
Min	0	0	0	0	0	
Max	1.7	2.7	3.14	0.9	0	
Mean	0.54	0.96	1.17	0.38	0	
SD	0.55	0.94	1.07	0.31	0	
GSB (2006)	0	0	0	0	0	
WHO Limits (2011)	0	0	0	0	0	
Note: All values are un	its of log	cfu /ml	A Di Camali	oc citoc or	d 1 4 maans	

Barro et al. (2006), Mensah et al. (2002) and Bryan et al. (1997) are possible means of transmitting pathogens into foods. Pesewu et al. (2014) and Amponsah-Doku et al. (2010) attested that substantial persistence and proliferation of bacteria from food production/ processing to consumption is a reflection of poor sanitary and food handling practices, and conditions at various stages of the chain.

Spice handlers should adopt and practice good handling processes to ensure food safety for consumers. The levels of some pathogenic bacteria are not acceptable as the pose serious health implication. This is similar to earlier report by Addo (2005) that there is serious health implication in the consumption of spices on the market.

In Tamale, it is evident that most food vendors are challenged with exogenous factors such as lack of public infrastructures for food vendors to comply with standard hygienic practices (such as clean water sources, public toilets), inadequate waste disposal service, poor sanitary conditions at vending sites (for example dust from dirt roads, open-air sewages, traffic fumes), poor storage conditions, contaminated inputs from farmers and market sellers, and unclean transportation conditions. These findings are echoed by FAO (2016). Tomlins and Johnson (2004) reported that street food pollution can partly originate from raw materials from rural small holders. Also, poor management of markets environment in Ghana has been linked as major cause of food contamination by Soriyi et al. (2008). Most fresh foods especially fermented food like beef and fish are highly vulnerable to pathogenic bacteria invasion and food poisoning.

Statistically, pathogenic bacteria count from the various selected sites is not significant difference (Table 2). The correlation matrix of bacteria count in spices demonstrates no interrelationships between pathogenic bacteria study. Hence, source of contamination of the pathogens is different, affirming the multiple sources of contamination of the spices observed.

Table 2 Comparative analysis of bacter
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ANOVA		Sum of Squares	df	Mean Square	F	Sig.
TC	Between Groups	467.3	4	116.83	0.52	0.72
	Within Groups	3369.5	15	224.63		
FC	Between Groups	68853.3	4	17213.33	1.23	0.34
	Within Groups	210109.5	15	14007.3		
EC	Between Groups	607037.8	4	151759.5	1.83	0.18
	Within Groups	1246760	15	83117.33		
SS	Between Groups	18.22	4	4.56	0.67	0.63
	Within Groups	102.64	15	6.84		

Conclusion

The study profiled some pathogenic bacteria on common spices used for grilling meat in Tamale. People have the right to consume safe and suitable food. The study showed that mixtures of spices were contaminated with some pathogenic bacteria. Salmonella spp and Escherichia coli are potential enteric pathogens. There is a possible risk to public health associated with consumption of spicy meat from the selected joints. The possible sources of spice contamination include storage equipment, handling, unhygienic surroundings, vehicular transmission, atmospheric particles and airmicrobes. The consumption of meat spiced with these contaminated spices can lead to health related problems such as cholera, diarrhoea, stomach cramps, typhoid fever and dysentery. It is recommended that periodic sanitary inspection and certification of vendors by regulatory body is absolutely necessary to ensure consistency.

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Authors' contributions

NB, ASA, ABD have contributed in the study conception and design, data acquisition, and analysis and interpretation of data. SJC and SM participated in intellectual helping in different stages of the study. NB, ABD, SJC and SM participated in drafting of manuscript and preparation of final version. All Authors have read the manuscript and have agreed to submit it in its current form for consideration for publication. All read and approved the final manuscript.

Competing interests

The authors of this research paper have no competing interests.

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