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Multidrug resistant Salmonella spp. and Escherichia coli from a popular Ready-to-Eat local food (Fufu) from commercial food vendors in Ghana

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ABSTRACT

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Microbial contamination of vended foods are of public health importance due to the potential of becoming a reservoir of foodborne pathogens and resistant strains of bacteria. This study looked at the presence of pathogenic bacteria in a popular Ready-To-Eat (RTE) traditional food, Fufu in Ghana. Sixty (60) Fufu samples were obtained from various food joints categorized as Opened, Semi-closed and Closed or Restaurants. Samples were processed and analyzed using standard bacteriological methods. The susceptibility profiles of the isolates were obtained by using the Kirby-Bauer disk diffusion method with the EUCAST guidelines with the five antibiotics. Prevalence of E. coli was 85% and Salmonella species was 68%. Microbial count of isolated E. *coli* ranged from 0 to 3×10^6 cfu/ml. There were no significant differences (p>0.05) among the different modes of operations. Fufu samples from Opened, Semi-closed and Closed joints were respectively contaminated with E. coli and Salmonella species as follows: 92%, 76%; 80%, 60% and 80%, 65%. The Salmonella species showed highest resistance to erythromycin (58.5%) and E. coli species were commonly resistant to Ceftazidime (88.2%) and Ceftriaxone (94.1%). All isolates were susceptible to nitrofurantoin. Multidrug resistance was detected among 27.5% of E. coli strains and 14.6% of Salmonella species. Fufu from the different eating joints in the Tamale Metropolis were substantially contaminated with multidrug resistant pathogens. The study recommends surveillance studies of resistant pathogens in foods, increased education and training of food vendors on sanitation, food handling and safety practices in the region.

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1. Introduction

Foodborne diseases are of global public health interest and constitute a spectrum of illnesses responsible for a significant proportion of morbidities and mortalities (1). Gastroenteritis with diarrhea as a common symptom, causes high rate of morbidity and mortality in infants and young children, especially in developing countries (2,3). Most incidence of gastroenteritis are caused by foodborne pathogens (3,4).

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These pathogens are easily spread periodically or epidemiologically through large scale cooking where food passes through several hands and processes thereby increasing the risk of microbial contamination (5). Street food vending has over the years played a major role in food supply chain, becoming a viable, crucial informal sector industry (6). The food sold by street vendors are often referred to as ready-to-eat (RTE) foods and receive high patronage from the general public due to its affordability, nutritional value, uniqueness of taste, availability and convenience (7). One of such foods is Fufu, which is popular and mostly found in Western and Central Africa. Its preparation slightly differs from country to country. In Ghana,

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pieces of boiled cassava and plantain or yam and cassava or cocoyam are pounded together in a giant wooden mortar using a wooden pestle. As pounding progresses, the mixture is turned by the bare hand and little amount of water is intermittently added till it becomes slurry and sticky. Street food vending has over the years played a major role in food supply chain, becoming a viable, crucial informal sector industry (6). The food sold by street vendors are often referred to as ready-to-eat (RTE) foods and receive high patronage from the general public due to its affordability, nutritional value, uniqueness of taste, availability and convenience (7). One of such foods is Fufu, which is popular and mostly found in Western and central Africa. Its preparation slightly differs from country to country. In Ghana, pieces of boiled cassava and plantain or yam and cassava or cocoyam are pounded together in a giant wooden mortar using a wooden pestle. As pounding progresses, the mixture is turned by the bare hand and little amount of water is intermittently added till it becomes slurry and sticky. Numerous studies worldwide have attributed outbreaks of food poisoning to street foods (11-14). Annor and Baiden (15) have indicated that about 70% of all bacterial food poisoning in Ghana is through the activities of food vendors. Nonetheless, surveillance studies on microbial quality of street and restaurant foods are inadequate and the few available data described the situation in the southern part of Ghana. It has also been reported that microbial food contamination in Ghana is alarming and most microbial researches carried out on food in Ghana did perform susceptibility test of isolated not microorganisms leaving us with very little information on resistant pattern of such foodborne organisms (16). Therefore, this study reports the prevalence and resistance profile of E. coli and Salmonella spp. in the northern region of the country.

2. Materials and Methods

2.1. Study area

The research was carried out in the Tamale Metropolis in the northern region of Ghana. It is the only Metropolis and the most populous in the northern half of the country. Geographically the Metropolis lies between latitude 09°16 and 09°34 north and longitude 00°57 west.

2.2. Sample collection

Sixty samples of Fufu were collected from street vendors and restaurants in the Tamale Metropolis based on mode of operation (opened, closed and semiclosed) from November 2016 to January, 2016. Open vendors were those operating in the open space without sheds, closed vendors were those operating in an enclosed room and semi-closed were those operating under sheds. Vendors operating in the closed mode were classified under restaurants while those operating in the opened and semi closed were put under street food vendors. Samples were collected in sterilized bags, kept on ice packs and transported immediately in a container to the laboratory for processing. Samples were taken purposively and covered almost 80 percent of Fufu vendors in the Metropolis.

2.3. Processing, isolation and confirmation of *Salmonella* spp.

Samples were processed according to the ISO 6579, 2002 guidelines. Twenty-five (25 g) of each Fufu sample was weighed and transferred into a sterile stomacher bag containing 225 ml of buffered peptone water (BPW). The samples were then homogenized manually with a mallet in the BPW followed by incubation at 42 °C for 18-24 h. After incubation, 1 ml of the preenrichment broth was transferred onto prepared plates containing 10-15 ml of Modified Semi-Solid Rappaport-Vassiliadis (MSRV) media (BioMérieux, France) each in three different locations of the media. Inoculated MSRV plates were incubated at 42°C for 18-24 h. The selected suspected Salmonella colonies on MSRV, that is white, motile Salmonella strains spread through the semi-solid agar were streaked onto Xylose Lysine Deoxycholate (XLD) agar (Oxoid Limited, Basingstoke, UK) and incubated for 24 h at 37°C. Red colonies with or without black centre were selected as suspected Salmonella. Salmonella spp. were confirmed by inoculating typical suspected colonies on slanted test tubes of Simmons Citrate agar (Oxoid Limited, Basingstoke, UK). The inoculated test tubes were then incubated for 24 to 48 h at 37°C. Confirmed Salmonella spp. were then streaked on nutrient agar and pure isolates stored in freezing media (1 ml of peptone, 0.25 ml of Glycerin 0.25 ml of distilled water) and frozen for future use.

2.4. Processing, isolation and confirmation of *E. coli*

Twenty-five (25 g) of the Fufu samples were weighted into 225 ml of BPW and homogenized manually with a mallet. 0.1 ml of the homogenized samples in BPW were pipetted onto CHROMagar *E. coli* agar plates (www.chromagar.com), spread evenly with glass beads (spreader) and incubated at 37°C for 24 h. CHROMagar *E. coli* agar is a chromogenic agar and the presence of *E. coil* is indicated by bluish colonies on the agar. After incubation *E. coli* produced blue color. Colony counters were used to count all the colonies and the cfu/ml calculated. Colonies of *E. coli* were streaked on Nutrient agar and stored in freezing media (1 ml of peptone, 0.25 ml of Glycerin 0.25 ml of distilled water) and frozen for future use.

2.5. Antibiotic susceptibility test

Antibiotic susceptibility tests of *Salmonella* species and *E. coli* were performed by the Kirby-Bauer disc diffusion method, following the EUCAST 2018 guidelines. The Antibiotics tested included Ceftriaxone (30 µg), Ceftazidime (30 µg), Nitrofurantoin (100 µg), Gentamicin (10 µg), Erythromycin (15 µg) and Ciprofloxacin (5 µg) (Oxoid Limited, Basingstoke, UK). The inhibition zones were recorded and results interpreted according to EUCAST breakpoints for *E. coli* and *Salmonella*. Multidrug resistance was defined as resistance to three or more antibiotics.

2.6. Data analysis

Data was analysed using Statistical Package for the Social Sciences (SPSS) software version 16 and results presented in percentages with tables. Fufu samples were as satisfactory (<20 cfu/g), borderline ($20 \le 10^2$ cfu/g) and unsatisfactory ($>10^2$ cfu/g) using the guideline on the interpretation of results for hygienic indicator organisms in ready-to-eat foods (17).

3. Results

3.1. Salmonella spp. isolated from Fufu samples

Of the 60 samples taken, 41 (68%) were positive for *Salmonella* spp. Under the opened mode of operation, 19 (76%) out of the 25 samples were positive for *Salmonella* spp. The closed mode of operations had 13 (65%) out of 20 samples while the semi closed had 9 (60%) of the 15 samples taken (Table 1). The opened mode recorded the highest contamination rate of *Salmonella* spp. on Fufu.

3.2. E. coli isolated from Fufu samples

Majority of the vendors 25/60 (41.6%) were operating in the opened category followed by closed 20/60 (33.3%) and semi closed 15/60 (25%). E. coli count ranged from 0 to 3×10^6 cfu/ml. There was no significant difference (p>0.05) in the total viable count of *E. coli* among the different modes of operations that is opened, semi closed and closed. However, there were significant differences (p<0.05) within the different mode of operations. Of the 60 samples taken, 51 samples were contaminated with E. coli, representing 85%. Only nine samples (9/60) representing 15% were not contaminated with E. coli. From the study, sixteen (16) samples out of 20 from the closed sites were positive for E. coil representing 80% of the total samples, whereas 12 out of fifteen (15) samples from semi-closed were positive for *E. coli* representing 80%. Finally, 23 of the 25 samples from opened joints were positive for *E. coli* representing over 92% (Table 1). Even though equal number of samples were not taken for the different mode of operations, again the number of positive samples from the opened vendors were the most contaminated.

According to the Center of Food Safety guideline (17) 28 (46.7%) of the *E. coli* positive samples are said to be satisfactory <20 cfu/g, 10 (16.67%) of the samples are at the borderline $20 \le 10^2$ cfu/g while 22 (36.67%) of samples are unsatisfactory >10² cfu/g for human consumption. Of the 22 unsatisfactory samples, 10 (45.4%) were from the opened mode of operation, 8 (36.4%) from the closed mode and 4 (18.2%) from the semi closed samples. The opened mode of operation again recorded the highest number of unsatisfactory samples.

3.3. E. coli or/and Salmonella isolates in samples

Of the 60 samples taken, both *E. coli* and *Salmonella* were present together in 32 (53.3%) samples taken from the vendors of which 9 samples were from the closed vendors, 6 samples from the semi closed vendors and 17 samples were from the opened vendors (Table 1). All the 60 samples were contaminated by either *E. coli* or *Salmonella*. All the 9 samples that were negative for *E. coli* were positive for *Salmonella*. All the 19 samples without *Salmonella* were positive for *E. coli*.

No. of	Mode of Operation			Preval
Positive				ence
Sample				(%)
s	Close	Semi-	Ope	
	d (20)	closed	ned	
		(15)	(25)	
51/60	16	12	23	85
41/60	13	9	19	68
32/60	9	6	17	53
	Positive Sample s 51/60 41/60	Positive Sample s Close d (20) 51/60 16 41/60 13	Positive Sample s Close Semi- d (20) closed (15) 51/60 16 12 41/60 13 9	Positive Sample s Close Semi- Ope d (20) closed ned (15) (25) 51/60 16 12 23 41/60 13 9 19

Table 1. Contamination rates of Fufu with *Salmonella* and *E. coli* from various food joints in Tamale

3.4. Antibiotic resistance pattern of E. coli

E. coli isolates showed high resistance of 94.1% and 88.2% to Ceftriaxone and Ceftazidime respectively and least (0%) to Gentamicin and Nitrofurantoin. Resistance was 27.5% to Erythromycin and 17.6% to Ciprofloxacin (Table 2). Multidrug resistance was observed among 14 (27.5%) of the isolates.

Table 2. Susceptibility profile of 51 E. coli species isolated from Fufu
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Antibiotic	Resista nt	Intermedia te	Sensitiv e
	(%)	(%)	(%)
Ciprofloxacin			
(5µg)	9 (17.6)	1 (2)	41(80.4)
Gentamic (10µg	0 (0)	2 (5 0)	40(04.1)
in) Erythromycin	0 (0) 14	3 (5.9)	48(94.1)
(15µg)	(27.5)	24 (47)	13(25.5)
(10)	48	()	
Ceftriaxone (30 µg	(94.1)	0 (0)	3(5.9)
Ceftazidime	45		
(10µg)	(88.2)	3(5.9)	3(5.9)
Nitrofurantoin (100µg)	0 (0)	0 (0)	51(100)

3.5. Antibiotic resistance pattern among Salmonella spp.

All the *Salmonella* species were sensitive to Nitrofurantoin, but showed highest resistance to Erythromycin (58.5%), followed by Ceftriaxone (51.2%). Resistance of below 50% was observed against Ceftazidime, Ciprofloxacin and Gentamicin (Table 3). Multidrug resistance of 14.6% was recorded.

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Antibiotic	Resistant	Intermediate	Sensitive
	(%)	(%)	(%)
Ciprofloxacin (5µg)	9 (22)	1 (2.4)	31 (75.6)
Gentamicin (10µg)	2 (4.9)	19 (46.3)	20 (48.8)
Erythromycin (15µg)	24 (58.5)	8 (19.5)	9 (22)
Ceftriaxone (30 µg	21 (51.2)	3 (7.3)	17 (41.5)
Ceftazidime (10 µg)	18 (43.9)	5 (12.2)	18 (43.9)
Nitrofurantoin (100µg)	0	0	41(100)

Table 3. Susceptibility pattern of 41 Species of Salmonella isolated from Fufu

4. Discussion

More than two thirds (36.67%) of the Fufu samples were classified as unsatisfactory (>10² cfu/g) but comparable to studies in Nigeria (18) and similar to the report of Mensah et al. (7) on street Fufu samples in Accra, Ghana. But a related study in the Central region of the country revealed low bacterial count on street Fufu samples (19). The high bacterial load was expected considering the preparation process of Fufu which involves pounding in a mortar and turning with bare hands couple with the poor hygiene exhibited by most street food vendors. It has been established that the hands of food handlers are the most important vehicle for the transfer of organisms from faeces, nose, and skin to the food (20). Vendors can be carriers of pathogens such as S. aureus, E. coli, Salmonella which may finally be transferred to consumers.

The study isolated *E. coli* (85%) and *Salmonella* species (68%) which have also been identified in street foods in Mexico, Ghana, Nigeria and Ethiopia (13, 21-23). Our rate was, however, higher than findings of Temesgen et al. (24) who recovered 29.6%. *E. coli* and 12.7% *Salmonella* spp. in street foods but comparable to the work of Kibret and Tadesse (23).

Recovery of coliform bacteria from RTE foods is an indication of faecal contmination arising from unsanitary processing and handling. The source of faecal coliform contamination in our study could have come from polluted water for turning the Fufu, hands of the vendors, flies and dust. The water that is mixed with the Fufu are not regularly changed and if so, different people dip their hands in it. It was generally observed that most of the joints where sampling took place, lacked running tap water and depended on water stored in gallons and open pans for cooking and washing. These water sources could easily become contaminated from dust, insects and garbage nearby. Sanitation was also a major issue at the sites as unavailable drainage system for liquid flow, poor wastewater and garbage disposal system prevailed, which advanced throwing of wastes onto nearby streets and gutters. These places serve as homes for rodents, breeding sites for flies and media for microorganisms to thrive which ultimately create public health risk (6).

The relatively equal rate of food contamination from the different sampling sites was surprising. It was anticipated that Fufu from the Closed Joints should have been significantly less contaminated than the rest, but rates of 84% (Opened), 70% (Semi closed) and 72.5% (Closed/restaurants) were observed. This finding corroborates the fact that surroundings alone do not contribute to food contamination but other factors such as water which is a critical raw material, cooking equipment, personal hygiene of vendors are key sources of microbial contamination of foods. In recent years, foodborne pathogens resistant to commonly prescribed antibiotics are increasingly being reported (9, 25). The E. coli species isolated exhibited high resistance to the Cephalosporins; Ceftazidime (88.2%) and Ceftriaxone (94.1%) which may be an indication of β -lactamase producing strains which are difficult to manage clinically when implicated in infections. The observed resistance to the Cephalosporins were more than documented rates on RTE foods in Ethiopia (24). The Salmonella strains, however, showed highest resistance to erythromycin (58.5%) which was lower than 86.9% described by Kibret and Tadesse (23). All Salmonella and E. coli strains were sensitive to nitrofurantoin (Table 2 and 3) and showed resistance range of 0-5% to Gentamicin which is comparable to reported rates in Italy, Turkey and Ethiopia (26-28). This result probably reflects the infrequent usage of these drugs in the management of foodborne pathogens in this region. Multidrug resistance was detected among 27.5% of E. coli strains and 14.6% of Salmonella species which is lower than recorded rates in Ethiopia (23). The unacceptable levels of microbial contamination on sampled Fufu in Tamale Metropolis, coupled with the presence of multidrug resistant strains of *Salmonella* and *E. coli* leave much to be desired since these pathogens have been implicated in major outbreaks of food poisoning and also for causing life threatening infections in humans.

5. Conclusions

This study demonstrated that Fufu sold in restaurants and streets of Tamale were unsatisfactory in terms of microbial contamination. The multidrug resistant foodborne pathogens recovered could be a common source of bacterial food poisoning as well as a reservoir for exchange and spread of resistant strains to consumers. Therefore, pragmatic education for vendors on food safety and sanitary engagement is necessary to assure quality and safe food. Consumers will be protected when frequent monitoring of food trade practices are carried out to enhance compliance to food safety standards.

Conflict of interest

The authors declare to have no conflict of interest.

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