

**MULTIDRUG RESISTANT *SALMONELLA* SPECIES ISOLATED FROM
FUFU GRINDING MACHINES IN GHANA****Suglo P^{1,2}, Kpordze WS² and CKS Saba^{2*}****Courage Kosi Setsoafia Saba**

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ABSTRACT

Multidrug resistant *Salmonella* infection has become one of the most dangerous health concerns in Sub-Saharan Africa. Most previous research shows that food and water are the sources of the human *Salmonella* infection in Ghana. This article examines *Salmonella* contamination of fufu, a thick paste prepared from pounded boiled tubers, traditionally prepared using pestle and mortar, a common food in West and Central Africa. The fufu grinding machine, a new technology for grinding fufu, is gaining root in many parts of Sub-Saharan Africa, particularly in the urban areas where most people are inclined to use machines to minimize drudgery, leaving behind the traditional way which involves the use of a wooden mortar and pestle. To investigate the sources of these contaminations, 100 samples were collected from 50 randomly sampled fufu grinding machines in the Tamale Metropolis to examine the prevalence and antibiotic susceptibility of *Salmonella* species. Fufu samples (SA) and fufu wash-out samples (SB) were collected from each grinding machine as described in ISO 6579:2002 protocol for the detection of *Salmonella* in food. Of the total 100 samples, 27% were confirmed *Salmonella* positive, of which 16 were fufu samples while 11 were fufu wash-out samples. Forty-eight percent (48%) of the 50 machines were contaminated with *Salmonella*. Contamination of wooden machines (85.7%) was higher as compared with the metallic machines (41.9%). The resistance levels of the isolates to the various antibiotics used were as follows: gentamicin (7.1%), nitrofurantoin (18.5%), ciprofloxacin (22.2%), erythromycin (81.5%), ceftazidime (85.2%) and ceftriaxone (88.9%). More of the isolates were resistant to three or more antibiotics (81.5% multidrug resistance). From this research, it can be concluded that there is high prevalence of *Salmonella* isolated from fufu grinding machines in the Tamale metropolis. Measures must be taken by the regulatory authorities to ensure that fufu prepared in grinding machines is safer. Also, awareness creation on antibiotic resistance and strict enforcement of laws on self-prescriptions of drugs would help avert multidrug resistance.

Key words: Fufu, fufu machine, *Salmonella*, antibiotic resistance, Tamale-Ghana



INTRODUCTION

Foodborne diseases are important global health concern, especially in developing countries [1, 2, 3]. The most common mode of infection of humans is by the ingestion of contaminated food and water [1]. *Salmonella* is a facultative anaerobic, gram-negative and rod-like bacterium, which is one of the leading causes of foodborne diseases [2]. *Salmonella* species are pathogens of water and food that have been recognized to cause illnesses, sometimes leading to death, especially in Africa [3]. Tackling *Salmonella* infection is challenging in most parts of Africa, for instance the northern part of Ghana, due to insufficient research data on its prevalence [4]. Most data available in Ghana are focused on human-borne *Salmonella* rather than foodborne *Salmonella*, which makes it more complicated to eradicate the disease, since food is a common source of human *Salmonella* infection in Ghana.

Salmonella contaminates different food samples due to improper cooking methods and poor hygiene such as ineffective hand washing before handling of foods [5, 6]. Food contamination by *Salmonella* spp. is common during processing and preparation stages because these demand high human contact. This is exacerbated when processing equipment is contaminated [7]. Residues of food stuck to the surfaces of processing equipment harbor food microbes like *Salmonella* and provide needed nutrients for their growth and survival [8]. Cross-contamination or contamination of end-product by food pathogens are also known to be through raw material, air in the processing plant, processing surfaces and factory personnel [9, 10]. Unsafe handling of foods such as tomato, pepper and fufu by plant operators may lead to recontamination during and after processing of foods [11].

A serious development is that antibiotic resistant strains of food pathogens have been identified. Antibiotic resistance is a significant threat to humans [12]. In spite of the various global interventions, resistance of foodborne pathogens to various antibiotics has not yet been addressed [13]. Over the past decades, the incidence of antibiotic resistance in Africa has increased, undoubtedly due to unregulated use of antibiotics with limited data on their resistance levels [14]. The level of resistance of *Salmonella* species to various antibiotics vary from one country to another depending on the pathogen's exposure, the choice of the antibiotics or the cross-country transmission levels [15]. Controlling antibiotic resistant in developing countries is more difficult. Especially, now that most patients' choices of antibiotics usually depend on their cost, availability and with few who depend on effectiveness [16, 17]. It is clear that the treatment of *Salmonella* infections become complicated when they develop resistance to most of the accessible antibiotics without reliable data on their resistance levels [7].

Fufu is a traditional food in western and central Africa which is believed to have been originated from the Akan tribe in Ghana [19]. Currently, there are two main methods of fufu preparation in Ghana; the traditional method which makes use of a wooden pestle and mortar to pound cooked yam, cocoyam, plantain or cassava (Figure 1), and the mechanized system which uses electricity or fuel to generate power for the grinding of the cooked tubers in a metal machine [20]. The traditional preparation of fufu requires physical energy and it is time consuming. This led to the invention of the mechanized

system for easy grinding of fufu in Ghana and some other African countries. The fufu machine is made up of shaft, electric motor, trough, propellers (yam beater), pulleys and the frame with vents for adequate cooling of the machine during operation [21]. However, according to Puoza *et al.* [20], the machine operates with the joint functioning of hopper, throat, pounding chamber, discharge outlet, main frame, an electric motor seat, shaft and pulleys.



Figure 1 a: Traditional method of fufu preparation



Figure 1 b: Mechanized system of fufu preparation

The nature of the design of the machine has made it difficult to easily clean the internal parts, which may serve as hiding place for foodborne pathogens. Failure to decontaminate and improper cleaning of the internal parts may lead to cross-contamination of freshly pounded fufu. Contaminated fufu is risky as it is not heated before eating site. This technology has gained root in most urban areas in Ghana, and most people are inclined to use the new technology, leaving behind the traditional method of pestle and mortar. Despite the boom in use of the technology, related issues of public health must not be compromised. The purpose of this study was to determine the prevalence of *Salmonella* spp. in the fufu grinding machine and their resistance pattern to common antibiotics used in the Tamale metropolis.

MATERIALS AND METHODS

Location and period of study

The study was carried out on various fufu grinding machines in the Ghanaian metropolis of Tamale. It was carried out from January to April of 2018. The isolation and identification were done at the Spanish Laboratory Complex on Nyankpala campus of the University for Development Studies, which is 16 km away from the Tamale Metropolis.

Sample collection, handling and transportation

One hundred samples were collected from 50 fufu grinding machines in the Tamale metropolis. Two samples were collected from each grinding machine. Fufu samples (which were labeled; S1A, S2A, S3A... and S50A) and fufu wash-outs samples

(labeled; S1B, S2B, S3B... and S50B) were collected from each fufu grinding machine. The fufu samples are the solid portion of the fufu taken right after grinding while the fufu wash-outs samples are liquid samples taken after rinsing the internal part of the machine. Sterilized water from the laboratory was used in the collection of the fufu wash-outs. All samples were collected in 500 ml plastic sterile ziplock bags and kept in the ice chest containing ice cubes to keep the temperature at 4 °C. Samples were transported to the Spanish laboratory for isolation and analysis of *Salmonella* within 1 hour of collection.

Salmonella detection

The ISO 6579:2002 protocol for the detection of *Salmonella* in the food chain with slight modification was employed [22]. From each fufu sample, 25 g was weighed into a sterile bag whilst 25 ml of each fufu wash-out sample was measured. A volume of 225 ml of prepared peptone water (Oxoid, Basingstoke, UK) was added to each of the measured samples in the sterile bag. Samples were then homogenized manually and incubated at 37 °C for 18-24 hours. The incubated samples in the peptone water were inoculated (100 µl) on a selective enrichment medium, Modified Semi-Solid Rappaport Vassiliadis (MSRV) (Oxoid, Basingstoke, UK). Inoculated MSRV plates were incubated at 42 °C for 18-24 hours. The presence of an opaque halo centered on the point of inoculation was a presumption of suspected *Salmonella* growth. Plates showing suspected *Salmonella* growth were further identified on Xylose Lysine Deoxycholate agar (XLD) (Oxoid, Basingstoke, UK) by streaking. Streaked plates of XLD were incubated at 37 °C for 18-24 hours. A black center with a pink-red zone surrounding a colony was identified as *Salmonella*.

Identified *Salmonella* colonies on the XLD were biochemically confirmed with a citrate utilization test. Slants of Simmons citrate agar (Oxoid, Basingstoke, UK) in test tubes were inoculated by stabbing the butt and streaking the slant with the suspected isolates. Tubes were incubated at 37 °C for 18-24 hours. *Salmonella* showed growth with intense blue colour formation in citrate medium. Positive *Salmonella* isolates were sub-cultured on nutrient agar medium and stored in eppendorf tubes containing 1.5 ml of 20% glycerol with Brain Heart Infusion storage medium. Stored isolates were kept under -20 °C for antibiotic susceptibility tests and future research.

Procedure for antibiotic susceptibility testing

Disk diffusion method was used for the antibiotic susceptibility testing of the *Salmonella* isolates while following the EUCAST [23, 24] guidelines and interpretations for breakpoints. A 24-hour culture of isolates on nutrient agar was used. Following an antimicrobial testing manual [24], prepared agar plates of Mueller-Hinton and normal saline solution were also used. Sterile tubes containing 2 ml each of normal saline solution were labelled with codes of respective isolated. A reasonable quantity of *Salmonella* (usually 3-5 pure colonies) on the nutrient agar was picked up with a sterilized loop and dissolved in tubes with saline solution. The turbidity of the resulting suspension was then compared with the 0.5 McFarland standard using a densitometer-DEN 1B (Grant Instruments Ltd, England) [24]. The suspension's turbidity was adjusted by adding some saline solution or more bacterial culture, when above or below the 0.5 McFarland, respectively. Obtained suspensions were inoculated by dipping in a



sterile cotton swab (Copan, Italy), pressed gently against the wall of tubes to remove excess water, and streaked or spread uniformly on surfaces of labelled Mueller-Hinton agar plates. Inoculated plates were allowed to dry for about 5 minutes. Using a pair of sterile forceps, antibiotic disks were placed on the inoculated agar and gently pressed to firmly fix them on the media surfaces. Antibiotics used in this study were gentamicin (10 µg), nitrofurantoin (100 µg), ciprofloxacin (5 µg), erythromycin (15 µg), ceftazidime (10 µg) and ceftriaxone (30 µg). Agar plates were then incubated at 37°C for 18-24 hours. After incubation, the zones of inhibition were measured in millimeters for each of the antibiotics and the results were compared with European Committee on Antibiotic Susceptibility Testing (EUCAST) break points [23]. Multidrug resistant isolates were classified as those resistant to 3 or more antibiotics. Results were interpreted as susceptible, intermediate and resistant.

Data analysis

The data obtained were entered into an excel sheet and presented with graphs and tables with descriptive statistics.

RESULTS AND DISCUSSION

Prevalence rate of *Salmonella* in fufu grinding machines

To reduce unnecessary human contacts with foods, which is one of the major causes of food contamination, the use of food processing machines such as fufu grinding machines can be encouraged. However, these machines must be used in a hygienic way. The prevalence rate of *Salmonella* found by this study in food processing machine in the northern region of Ghana is not different from any other part of developing countries [14]. This recorded rate of contamination is mostly attributed to poor sanitation, lack of potable drinking water, and improper handling of food during preparation and processing [5]. From the 100 samples that were collected from fufu grinding machines in Tamale metropolis, 27 were contaminated with *Salmonella*; thus, 48% of the 50 fufu grinding machines surveyed were contaminated with *Salmonella*. In addition, 3 machines had both their fufu samples (SA) and fufu wash-out samples (SB) contaminated. This result correlates with that of Saba *et al.* [25] which reported 72% contamination of *Salmonella* in fufu sampled from various vendors and restaurants in the Tamale metropolis. From the 27 contaminated samples, 59.3% were from SA and 40.7% in SB. It was revealed by the operators that using soap to wash the internal part of the machines make fufu smell like soap and hence, they only use water. Moreover, the complexity of the machines makes it impossible to wash their internal parts which may serve as hiding places for *Salmonella* pathogens.

Salmonella isolation rate in machine types: wooden machines (WM) versus metallic machines (MM)

The number of wooden machines (WM) and metallic machines (MM) sampled were 7 and 43, respectively. From the wooden machines, 85.7% were contaminated with *Salmonella* while 41.9% was recorded for metallic machines (Figure 2). This indicates that the wooden machines have higher potential of *Salmonella* contaminations compared to metallic machines, probably due to the more absorbent nature of woods



than metals. It might have also resulted from the more complex nature of wooden machines, hence making the cleaning of their internal parts very difficult.

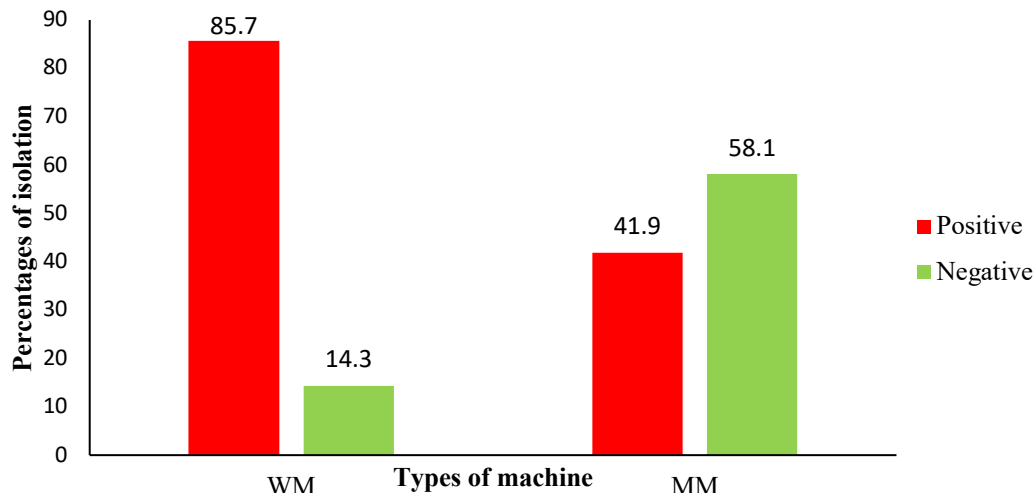


Figure 2: Prevalence rate of *Salmonella* in wooden machines (WM) and metallic machines (MM)

***Salmonella* contamination based on how customers are served**

From the sampled fufu grinding machines, 6 of the machines were used for commercial purposes only, 26 were for chop bar purposes only, and 18 both for chop bar and commercial purposes, respectively. *Salmonella* prevalence rates found among these machines for various purposes were 50% in chop bar only, 30.8% in only commercial and 72.2% in machines used for both commercial and chop bars purposes (Table 1). These observed findings could be due to the environment and diverse ways employed by operators in machines for different purposes. The study found that most of the chop bar operators wash their machines once a week. Also, unlike the commercial operators, most of the chop bar machines were found in enclosed places to prevent public criticisms. However, per our interaction with operators, it was realized that their knowledge about contamination was limited. They only checked whether visible dirt was present around their machines, and were not concerned about the hygienic nature of their machines in terms of microbial contaminations. The contamination of the machines used for commercial purposes is attributed to different fufu materials from different households with unknown contamination backgrounds being placed in the same machine. Thus, if the first fufu ground is contaminated, it may indirectly contaminate any other food that would be ground subsequently. This can only be avoided if the grinding machine is decontaminated between each use.

***Salmonella* contaminations from covered and uncovered machines after operation**

Considering the prevalence rate of *Salmonella* based on whether they covered the machine after operation or not, the study recorded 43.2% and 61.5% of covered and uncovered machines been contaminated, respectively (Table 2). From the survey, it was discovered that most of the customers do not cover their cooked tubers before bringing them to the machine (figure 3). They usually expose their cooked tubers to the air to

allow them to cool before grinding. This prevents the fufu from being very soft after grinding. This act of exposure by customers may serve as a source of contamination since airborne microbes can easily contaminate exposed foods [26]. It was also realized that customers were served with bare hands, and the same water was used by the operators throughout their operations without changing. These acts by operators while delivering services to customers could cause cross-contamination among foods. The cross-contamination of food occurs not only when operators use contaminated hands to serve different foods or when contaminated processing machines or equipment are used, but also when pathogens are directly transferred of from a contaminated food source to an uncontaminated food source during processing [10]. In this way, pathogen-free boiled tubers can be contaminated with pathogens from the fufu grinding machines.



Figure 3: How customers were served

Antibiotic susceptibility profile of isolated *Salmonella* from fufu grinding machines

Of the 27 *Salmonella* isolates from fufu grinding machines in Tamale metropolis, 7.4%, 18.5%, 22.2%, 81.5%, 85.2%, 88.9% were resistant to gentamicin, nitrofurantoin, ciprofloxacin, erythromycin, ceftazidime and ceftriaxone, respectively (Figure 4). Furthermore, almost all the isolates (81.5%) were resistant to three or more of the antibiotics used and hence classified as multidrug resistant. From the multidrug resistant *Salmonella* isolates, 68.2% were resistant to three antibiotics, 27.3% to four antibiotics, 4.5% to five antibiotics and none was resistant to all the 6 antibiotics (Figure 5).

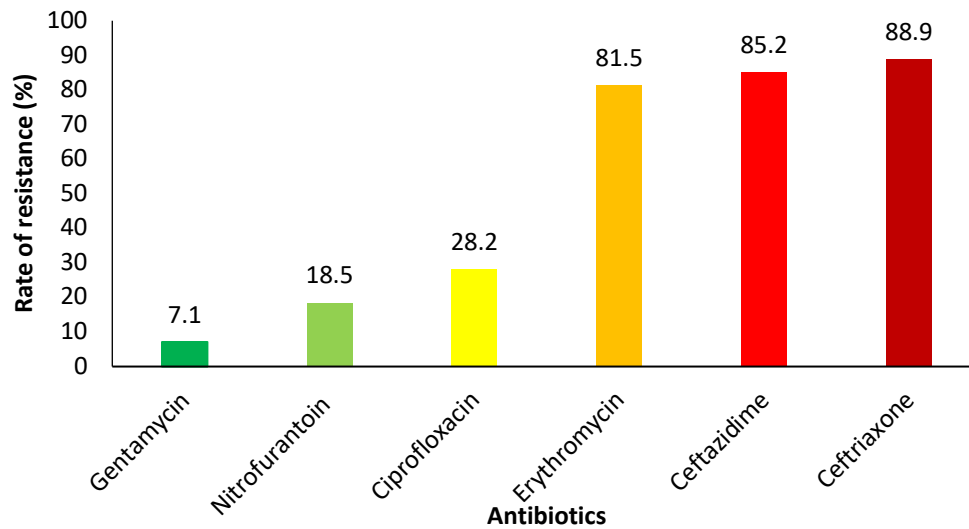


Figure 4: Antibiotic resistant pattern of isolated *Salmonella* from fufu grinding machines

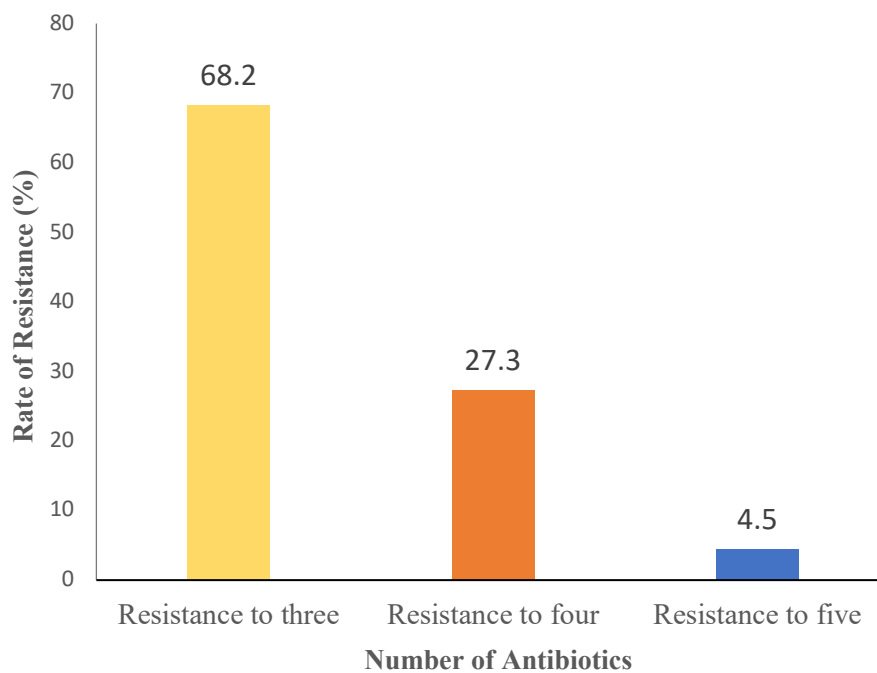


Figure 5: Multidrug resistant pattern of *Salmonella* isolates

The observed increase in resistance rates of *Salmonella* spp. in this study to third generation cephalosporins, which were known to be effective in treating invasive *Salmonella* infections, have also been reported elsewhere [16]. The misuse of antibiotics by the populace may have caused the increased resistant strains of *Salmonella* spp. that ended up in the food chain as seen in the study. It could also come from the misuse of antibiotics in animal production. In addition, studies confirm that third generation cephalosporins and fluoroquinolones have been the most prescribed antibiotics to *Salmonella* infections after the increased in the ineffectiveness of

ampicillin and cotrimoxazole which use to be the first line of prescription to invasive *Salmonella* infections in Africa [17, 18]. Some previous reports from Ghana and other African countries have raised concern about the gradually increasing resistance rate of *Salmonella* spp. to the third generation cephalosporins and fluoroquinolones [27, 28]. The higher resistance rates recorded in this current study are not different from the previous reports. Thung *et al.* [29] reported 27% and 100% resistance of *Salmonella* to ceftazidime and ciprofloxacin, respectively, in foods sampled in Malaysia. While our study revealed higher resistance rates towards erythromycin, ceftazidime and gentamicin, a study by Labi *et al.* [27] showed a lower rate of 10% resistance to ceftazidime, and 0% each to gentamicin and erythromycin, respectively. Also, *Salmonella* isolated from food vendors in Jirapa in the Upper West region of Ghana by Gyansah and Feglo [30] showed 77.3% of resistance to gentamicin which was higher than our study, and 0% each to ceftazidime, ceftriaxone and ciprofloxacin, respectively.

CONCLUSION

Findings from this research revealed high prevalence of *Salmonella* spp. (48%) in fufu grinding machines (48%) in the Tamale metropolis. More contamination was seen in machines made of wood than machines made with metal, and could be due to the nature of the raw materials, as woods are known to be more absorbent than metals. The operators of fufu grinding machines find it difficult to clean the internal and hidden parts of the machines and find it hard to spend part of their productive time on cleaning their machines. Many of the customers may be less informed about food pathogens and hence do not care about unhygienic nature of the machines or complain about how they are being served, hence the use of bare hands by most operators. A higher percentage of the isolated *Salmonella* were multidrug resistant, and were generally resistant towards the third-generation cephalosporins (ceftazidime and ceftriaxone) as well as macrolide (erythromycin). Based on the research findings, it is, therefore, recommended that the general public and operators be educated on the microbiological safety of the fufu grinding machines.



Table 1: Prevalence of *Salmonella* contamination base on purpose of the machine

| Purpose of the machine | Number of machines sampled | Number of machines contaminated | Percentage of machines contaminated (%) |
|--------------------------------------|----------------------------|---------------------------------|---|
| Chop bars only | 6 | 3 | 50.0 |
| Commercial purpose only | 26 | 8 | 30.8 |
| Both chop bar and commercial purpose | 18 | 13 | 72.2 |
| Total | 50 | 24 | 48 |

Table 2: *Salmonella* contamination based on how the machines were managed after operation

| Management of machines after operation | Number of machines sampled | Number of machines contaminated | Percentage of machines contaminated (%) |
|--|----------------------------|---------------------------------|---|
| Covered | 37 | 16 | 43.2 |
| Not covered | 13 | 8 | 61.5 |
| Total | 50 | 24 | 48 |

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