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MICROBIAL PROFILE OF DAWADAWA SOLD IN TAMALE CENTRAL MARKET

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Abstract
Dawadawa is a common spice used in most rural homes across West Africa to spike the protein source in meals. This study assessed the handling practices of vendors and the microbial load of dawadawa vended in the Tamale Central Market. Samples were collected from five sections of the market using the random stratified sampling technique. Eight of the samples (53.33%) had Salmonella spp, one sample (6.67%) had E. coli and all the samples (100%) had Staphylococcus aureus. Dawadawa that were covered on display recorded no count of Salmonella spp *and E. coli but high counts for Staphylococcus aureus. The pH and temperature of the samples ranged 6.91 to 8.02 and 26.90 to 27.60, respectively. Factors accounting for the high prevalence of Staphylococcus aureus might be cross-contamination associated with improper handling practices as the human skin is known to be a reservoir of the bacterium. There is the need for vendors to package dawadawa in neat disposal packs to reduce direct contact.*

Keywords: Dawadawa, *E. coli*, Handling Practices, *Salmonella* spp, *Staphylococcus aureus*

Introduction

In recent times, consumers have become inclined towards nutritious and hygienic natural products containing herbs or spices. Spice is an edible part of a plant that is either whole, powdered, or crushed and are used for flavouring and preservation of food (Bedada et al., 2019). It can be gotten from plant bark, seed or roots. Besides food, some spices are used traditionally to speed-up the recovery process, enhance uterine contraction after childbirth as well as relieve pain such as rheumatic pains. Dawadawa is a local spice made from *Parkia biglobosa* also known as Africa locust bean tree. It is used in the seasoning of soups, stews, and other dishes. Dawadawa is widely known to enhance the meatiness of dishes and is considered to be a good source of protein especially for the poor (Esenwah & Ikenebomeh, 2008). However, spices can be contaminated through numerous means with a host of toxic and pathogenic bacteria (Székács, Wilkinson, Mader, & Appel, 2018).

Worldwide, it is estimated that about 93.8 million people suffer from illness associated with gastroenteritis, with 155,000 deaths and a mean occurrence of 1.14 episodes per 100,000 persons spp (Harb, O'Dea, Abraham, & Habib, 2019). These statistics are associated with infection from *Salmonella* spp (Harb et al., 2019). The contributory factors associated with food contamination could be chemical or biological. *Vibrio cholera*, *Shigella* spp and *Salmonella* spp are examples of biological contaminants (Hussain, 2016). Among the

hurdles that the health sector faces, food contamination issues that primarily arise from poor hygienic conditions are top on the list. Cooking equipment and utensils may be means of pathogen transfer to food (Akanele, Mgbo, Chukwu, & Ahudie, 2016). Dawadawa from the production site, salespersons through to the consumers goes through various handling and storage processes that can cause microbial contamination. Hence, the present study characterised the occurrence of pathogenic bacteria (i.e., *Salmonella* spp., *E. coli* and *Staphylococcus aureus*), which are of public health concern in this product.

Materials and Methods

Study Area

The study was conducted at the Tamale Central Market, which is the main market located in the heart of the Central Business District of the Tamale Metropolis in the Northern Region of Ghana. Tamale is estimated to have a land size of about 646.90180 km^2, at longitude 0^0 36 and 0^0 57 West and latitude 9^0 16 and 9^0 34 North (Ghana Statistical Service, 2014). The Metropolis shares boundaries with Central-Gonja District to the South-West District, Sagnarigu West District and Sagnarigu North District, Mion District to the East and East-Gonja District to the South (Ghana Statistical Service, 2014).

Sampling Technique and Data Collection

Stratified random sampling was used to collect the dawadawa samples. The central market was sectioned into five to ensure representativeness of the samples in the area. A total of 15 samples (3 samples from each section) were collected into sterile bags which were well labelled.

All samples were taken inside the Tamale central market. A1: Located close to the entrance gate of the market from the NIB bank's Aboabo branch. A2: Located close to a butcher at entrance gate of the Tamale Central Market butcher's house. A3: Positioned close to cosmetics store located in the market. B1: Right-turn from A3 near the bend, B1 (B1 offers a variety of things on display, including peanut paste) is located close to a garden egg and turkey berry vendor. B2: Just a few metres from B1, B2 is located close to a butcher. B3 who sells beside a corn and cassava dough vendor. C1: is located close to a public toilet. C2: is also located close to a restroom entrance, onion vendors and baby care store. C3: Directly behind C2 which is located close to groundnut oil vendors with C3 beside them but near to the public toilet. D1: is located from the NEDCO vendor gate of the market just at the end of the first storey building which shares same space with a butcher. D2: is located directly opposite a food vendor who sits beside a shop that sells bakery items (flour, yeast, margarine). D3: D3 is directly opposite D2. Beside D3 is a palm oil and vegetable vendor. E1: Was not far from D3. E2: Adject E2. E3: E3 is directly opposite E2. Beside E3 is a corn vendor.

The samples were placed in a thermo-flask containing ice cubes and transported to the Council for Scientific and Industrial Research – Water Research Institute, Tamale laboratory for analysis.

A questionnaire was administered to 15 vendors from whom the samples were bought, to find out if the vendors produced the product themselves and the duration it stays in the market. Personal observations such as the mode of product display, whether covered or uncovered were also taken into consideration.

Peptone Water Preparation

The peptone water was prepared according to the manufacturer`s protocol. In brief, peptone powder of 67.50 g was measured using an electric scale and transferred into a beaker (American Public Health Association (APHA), 2017). Distilled water 3375 mL was poured into a beaker containing the peptone powder and stirred continually to dissolve suspended particles. The peptone solution was then

poured into glass bottles and sterilised at a pressure of 103.40 Kpa, cooled and stored in the refrigerator (APHA, 2017).

Sample Preparation

After the collection of samples, they were taken to the laboratory for analysis. Each sample was grounded into a powdery form using a mortar and pestle in the laboratory and weighed using an electric balance. A mass of 25g of each sample was transferred into sterile zip lock bags and a peptone water of 225 mL was added to each sample under the Ultraviolet (UV) light chamber in accordance with APHA (2017) procedure. The mixture was manually massaged until homogenous solution was achieved.

Preparation of Salmonella-Shigella Agar, Chromo Cult Agar and Mannitol Salt Agar

Salmonella-Shigella (SS) Agar and Chromo cult agar of 63g and 17.25g were respectively measured into a 500 mL beaker each. Distilled water of 1000 mL was poured into the beaker containing each of the measured agar and stirred. The mixture was heated to completely dissolve suspended particles using a hot plate with stirrer and then kept to cool to room temperature in the lamina flow hood disinfected with 70% alcohol in accordance with APHA (2017) procedure. The media was poured into sterilised petri-dishes and allowed to solidify.

Mannitol salt agar was weighed into a beaker and 100 mL of distilled water added and stirred. The mixture was allowed to boil with continual stirring to dissolve properly. The homogenised media was then sterilised at 102.40 Kpa at a temperature of 121°C using the pressure heat steriliser in accordance with APHA (2017) procedure. The solution was cooled in a lamina hood to about 48 °C.

Bacteriological Analysis

After the collection of samples, they were taken from the thermo-flask containing ice and sent to the working area and arranged in an ascending order. Each sample was grounded into a powdery form and weighed using an electric balance to a mass of 25g and 225 mL of peptone water was added to each sample in sterile zip lock bags . The mixture was manually massaged until homogenisation was achieved. The viable cell count of samples was determined by carrying out serial dilution of each stock solution of each formulation sample to obtain dilutions of 10^{-1} - 10^{-5} five levels. Ten (10) mL of each formulation sample was measured and 90 mL of distilled water transferred into the sample to form the stock solution in homogenisation. The stock (0.1 mL) and distilled water solutions were picked using a pipette and transferred into the plates containing the media prepared. With the aid of sterile cotton swaps the mixture was spread evenly on the plates and incubated at 37 °C for 18 – 24 h. The growth of suspected microbes was counted and was calculated using the formula:

$$\text{Cfu g}^{-1} = \frac{\text{Total number of colonies counted} \times \text{dilition factor}}{\text{Volume plated}}$$

Determination of Temperature and pH

Each sample was grounded into powder and 5g of each weighed into a beaker using an electric scale. 500 mL of distilled water was added to the sample in the beaker and then mixed with a spatula to form a paste. The pH and temperatures were measured using Jenway 3510 pH meter prior to the culturing. The pH and temperatures of standard buffer solutions; 4, 7 and 10 were pre-checked before samples reading was taken as part control and assurance pf results of samples. Distilled water was used to rinse thoroughly the probes after reading each sample.

Statistical Analysis

Analysis of variance (ANOVA) was done using version 16 of the microsoft excel at a significance level of $P < 0.05$ for the microbes, location and sources and mode of packaging.

Results and Discussion

Occurrence of Microbial Isolates in Dawadawa

The occurrence of *Salmonella* spp, *E. coli* and *Staphylococcus aureus* are shown in **Table 1**. The detection of these bacteria may be as a result of poor food handling practices and storage procedures that are mostly observed in small-scale processing units in developing countries (Oloo, Daisy, & Oniang'o, 2018). Developing countries are faced with the struggle of poor, disintegrated, and uncoordinated food safety systems that pose immense danger to the health of consumers. This menace is on the rise as a result of weak implementation of food safety regulation (Oloo et al., 2018).

Bacterial prevalence varied based on sample collection points. Bacterial counts obtained from all the 15 samples collected showed a greater percentage of *Staphylococcus aureus* i.e. 100%, than *Salmonella* spp (53.33%) and *E. coli (*6.67%). Samples collected around highly polluted environments like samples from B3, C1, C2, and D1 recorded the presence of *Salmonella* spp whilst A2, A3, B1, B2, B3 and D3 had higher counts of *Staphylococcus aureus*. *E. coli* isolates were from samples collected from location (C3) near to the public toilet. *E. coli* presence indicated a fresh faecal matter contamination of the dawadawa. *E. coli* is known to be associated with faecal matter contaminations (FAO, 2016).

Table 1: Bacterial count of Dawadawa Samples from Various Sections of the Market

Site	Sample	Ss (cfu/g)	*E. coli* (cfu/g)	Sa (cfu/g)	*Ss*	*E. coli*	*Sa*
					OCCURRENCE		
Section 1	A1	0	0	26	–	–	+
Section 1	A2	0	0	108	–	–	+
Section 1	A3	0	0	141	–	–	+
Section 2	B1	14	0	101	+	–	+
Section 2	B2	2	0	141	+	–	+
Section 2	B3	221	0	132	+	–	+
Section 3	C1	151	0	30	+	–	+
Section 3	C2	207	0	55	+	–	+
Section 3	C3	4	18	14	+	+	+
Section 4	D1	209	0	98	+	–	+
Section 4	D2	0	0	98	–	–	+
Section 4	D3	0	0	110	–	–	+
Section 5	E1	0	0	91	–	–	+
Section 5	E2	11	0	48	+	–	+
Section 5	E3	0	0	74	–	–	+

Note: Ss: Salmonella spp count (*P* value of 0.034), *E. coli; E. coli* count (*P* value of 0.336), and *Sa: Staphylococcus aureus* count (*P* value of 0.001)

Bacteria Count Based on Duration on Market

According to key informants, dawadawa products remain on the market within a space of one week after production. From data collected, 10 (66.67%) vendors asserted to the sales of their product below a week (daily) whilst five (5) (33.33%) asserted that it takes a maximum of a week (weekly) for a batch to be sold. The presence of bacteria in the dawadawa product could be attributed to the exposure to the airborne within the environment as the days go by. The duration of products on market increases the risk of contamination if not stored properly (Svobodová & Tůmová, 2015). The more a product is exposed to environmental factors the more highly it is prone to microbial contamination (Temitope & Igbokwe 2014). *Staphylococcus aureus* has the highest occurrence in all time durations whilst *E. coli* had the least (**Figure 1**).

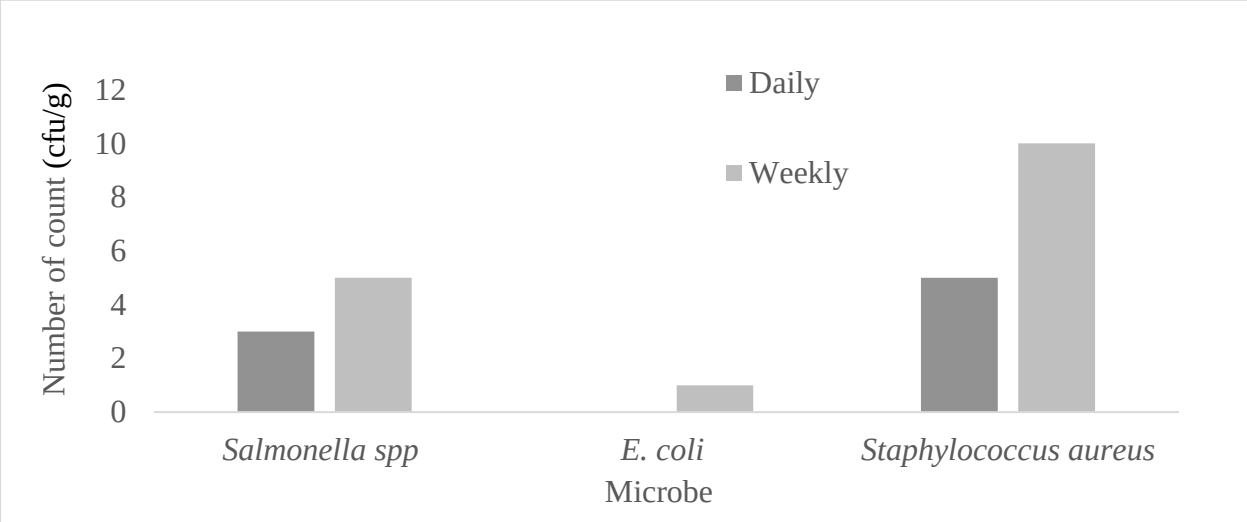

Figure 1: Positive bacteria count in dawadawa samples based on duration on the market

Bacteria Isolation Based on Mode of Packaging of Dawadawa for Sales

This distribution was based on the state of packaging of the dawadawa samples from the market. Covered samples recorded no count of *Salmonella* spp and *E. coli* but higher counts of *Staphylococcus aureus*. Covered products recorded a low count for microbial presence in all isolates due to reduced contact with houseflies and the atmosphere. The higher counts of isolates for uncovered samples could be associated with exposure to environmental factors (microbial spores in the air). Microbial spores are present in the atmosphere and can multiply when attached to favourable surfaces/ substances/ media (Majeed, 2017).

Distribution of bacteria based on the wholesalers or producers and retailers

The classification along the product chain, from processors to wholesale and/or retailers showed bacterial contamination resulting from their activities (**Figure 2**). The wholesale packaging (moulding into balls) and remoulding by retailers and repackaging to consumers; with hands, containers, water used in sprinkling to mould are major sources of bacterial contact with the processed dawadawa. All these chain processes of handling could contribute to the varying bacterial counts of all the three isolates. Observation from the study indicated that samples obtained from producers were relatively high in the three isolates especially *Staphylococcus aureus* due to compromise in food safety standards. This

result is similar to reports on prevalence of microbes associated with production, environment and persons involved with production of food (Adu-Gyamfi, Torgby-Tetteh & Appiah, 2012; Biranjia-Hurdoyal & Latouche, 2016). Most food processing sites are set up in backyards or squalid areas. Some are managed by persons with no expertise in food technology hence their inability to adhere to food safety standards (Oloo et al., 2018). Dawadawa preparation goes through various processes of preparation before it reaches the consumer. These processes, if not handled according to safety standards will expose the product to microbial contamination (Ajayi, 2014).

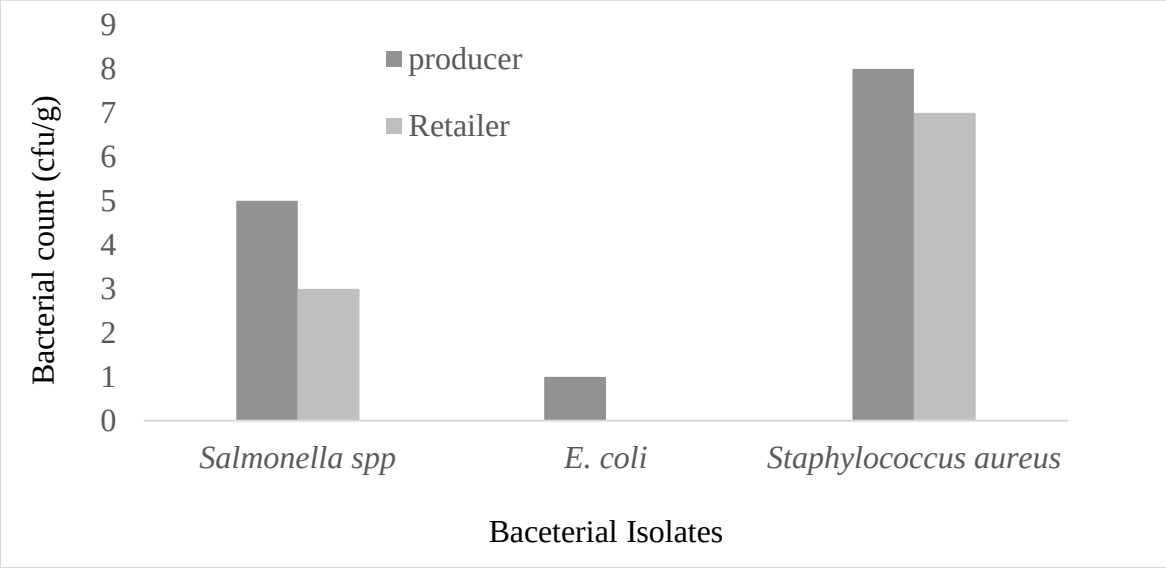

Figure 2: Distribution of bacteria isolates base on the wholesalers or producers and retailers

Dawadawa is a common alkaline fermented spice or condiment. It is dried in an open area under the sun with the intension of reducing microbial load and sold with no further treatment done to them. It comes as no surprise as studies revealed the addition of micro flora species in spices hence the isolation of *Salmonella* spp, *E. coli* and *Staphylococcus aureus* (Banerjee & Sarkar, 2003).

Bacterial count variation observed in the spices could be as a result of factors such as mode of presentation on the market, duration on the market, and source of product. Generally, all the processes involved in the preparation of dawadawa pose a threat of potential food contamination; bare feet, sand, mortar and pestle are employed in the de-hulling process, water used in the preparation process, fermentation, drying and moulding and finally plastic bags employed in packaging could all be sources of contact contamination of the product (Ajayi, 2014).

pH and Temperature

pH and temperature for the dawadawa samples ranged from 6.91 to 8.02 and 26.90 to 27.60 °C, respectively (**Table 2**). The pH of dawadawa samples collected has the potency to favour microbial growth and multiplication if food safety measures are not adhered. As a fermented product, the pH of dawadawa was reported to be needful to assess the survival ability of the bacteria during storage (Cabello-Olmo, Oneca, Torre, Díaz, Encio, Barajas & Araña, 2020). Microbes are known to be specific to the environment and pH is one of its major factors. The pH ranged from 4.0 - 9.8,

4.4 - 10 and 5 - 7 for *Staphylococcus* spp, *E. coli* and *Salmonella* spp, respectively (Medveďová, Valík & Studeničová, 2010; Gullian-Klanian & Sánchez-Solis, 2018).

Table 2: pH and temperature of the dawadawa

Sample ID	pH	Temperature (°C)
A1	7.44	27.60
A2	7.90	27.00
A3	7.41	27.20
B1	6.91	27.40
B2	7.01	27.20
B3	7.49	27.40
C1	7.04	26.90
C2	7.31	27.30
C3	7.18	27.20
D1	8.02	27.60
D2	7.15	27.20
D3	7.90	27.00
E1	7.90	27.00
E2	7.45	27.30
E3	7.38	27.40
Mean	7.43	27.25
Min	6.91	26.90
Max	8.02	27.60

Conclusion

From the study, all samples tested positive for *Staphylococcus aureus*. Factors accounting for the prevalence of *Staphylococcus aureus* might be cross-contamination associated with improper handling practices as the human skin is noted to be the largest reservoir for *Staphylococcus aureus*. The microbial count for *E. coli* may be associated with fresh faecal contamination. Bacterial count variation was attributed to factors such as mode of presentation on the market, duration on market, and source of product. Generally, the processes involved in the preparation of dawadawa is also a potential source of contamination and that could be investigated in further studies.

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Conflict of Interest

Authors have no conflicting interest.

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