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Screening for Salmonella spp. in Food, Water, and Hands of Households in Rumuwoji, Port Harcourt, Nigeria

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Abstract
Faecal-oral chain transmission of *Salmonella* spp. was investigated in some homes of Port Harcourt to determine the possible route of typhoid fever and other infections of *Salmonella* spp. diagonized in recent times as reported. *Salmonella* spp. infection is becoming alarming with no detailed trace of its emergence in the house even after heavy hygiene practices have been observed. Considering the importance of food and water for human growth and development, a total of sixty (60) homes volunteered their household food, water and occupants' palms (hand) for microbiological examination. The study involved carrying out standard microbiological procedure that employed the use of Salmonella/Shigella growth media to screen for *Salmonella* spp. in food items namely: processed cassava products (garri and foo-foo), soup, palm oil, table salt, pepper, sachet water and in manually refilled bottle water and hands of occupants of the twenty homes. Results showed no visible growth of *Salmonella* spp. colonies, thus indicating the absence of *Salmonella* spp. in food and water materials in the home. Hence, the study showed evidently, good health conditions amongst households and the general public at large. The result therefore calls for further screening of other foods purchased from the market and transferred to the house.

Keywords: *Salmonella* spp.; Food; Water; Hand; Homes; Fecal-oral transmission

Introduction

Efficient and effective hygienic practices have not comfortably gained ground amongst most homes, leading to an increased proportion of health issues associated with *Salmonella* spp. spread in water and food in recent times (Ferreira et al., 2022). According to Ferreira et al. (2022), this ineffective hygiene practice is due to several factors interfering with the adoption of good hygiene practices in Bahia, Brazil. The traditional food market allegedly plays a huge role in the chain of microbial infestation, specifically *Salmonella* spp. (Gizaw, 2019). Gizaw (2019) noted public health risks from the food market, which is due to a lack of knowledge of food origin before it enters the market. Food can be contaminated through cross-contamination, environmental contamination or by unwashed hands of food handlers. The food purchased is then transported to the home for further preparation and consumption. Water which is also an element of food could also be a route of *Salmonella* spp. spread to consumers. Hence, water sourced outside the home for drinking if untreated could harbour *Salmonella* spp. invariably portrays danger to consumers' health with associated diseases. Consequently, the faecal-oral transmission of *Salmonella typhii*, a bacterium implicated in typhoid fever disease is an issue of concern in some homes. Food, water and palm contaminated with *Salmonella* spp. are largely considered as means of spreading the disease (Liu et al., 2018). The presence and persistence of *Salmonella* in water and food have always been reported (Liu et al.., 2018). Food and water are consumed for the growth, repair, and maintenance of body tissues and the regulation of vital processes. Thus, water consumption plays an important and huge role in food digestion.

According to Popkin et al. (2011), there can be no life on earth without water, consequently, 60 percent of the body is notably, made up of water (Popkin et al., 2011). Water consumed, helps regulate body temperature, transport nutrients into cells, eliminate waste products and maintain other bodily functions. The hand which is noted as part of the body is a well-known route for pathogen transmission (Edmods-Wilson et al., 2015). According to Edmods-Wilson et al. (2015), the hand plays a crucial role in the process of eating, which involves the transfer of food to the

mouth, by grasping, manipulating, scooping and partitioning. These roles of the hand/palm make it an indispensable tool for eating, enabling humans to interact with food effectively, with cutlery handling, to pick and manipulate various food items (Edmods-Wilson et al., 2015). The hand/palm which serves as a faecal-oral route for transmission of infectious pathogens occurs when microorganisms are excreted in faeces, and the faeces come in contact with the hand and during ingestion, without proper hand hygiene, the microbe (*Salmonella* spp.) is transmitted to the mouth (WHO 2009). *Salmonella* infection (Salmonellosis) is a common bacterial disease that affects the intestinal tract (Kim et al., 2013). However, *Salmonella* spp. naturally inhabit the intestines of warm-blooded animals. Humans become infected mostly through the consumption of contaminated water or food and thereafter, fall with diarrhoea, fever and stomach (abdominal) cramps. Efficient screening methods for the detection of *Salmonella* spp. in various sources of food, water, and palm, are crucial to prevent outbreaks and ensure public safety (Enabulele & Awunor, 2016). The study aims at checkmating disease outbreaks and thus would go a long way in protecting public health/interest as, *Salmonella* spp. contamination in food products poses a significant threat to home safety. The study would give a guide to homes, as early detection allows for timely interventions to avert widespread outbreaks. Hence, the study seeks to screen food, water and hands for the presence of *Salmonella* spp. in some households in Port Harcourt.

Materials and Methods

The study area is the Rumuwoji community in Port Harcourt City Local Government Area of Rivers State, Nigeria. Rumuwoji home residents are largely dependent on raw food and public water. The Rumuwoji market, otherwise called the Mile One market is located in the heart of Port Harcourt with a clustering of people. The market pulls people from other communities, who come and buy foodstuff. Raw foods are occasionally sold in open markets in Rumuwoji, where diverse unhygienic activities are practised within the neighbourhood such as indiscriminate waste disposal practice and open urination (Brown et al., 2015), all of which could contaminate food, water and fingers with microbes and thereafter, result to *Salmonella* infection (Enabulele & Awunor, 2016).

Twenty (20) different samples of food, water and finger swabs were obtained from some homes in three (3) different batches. In obtaining the palm/finger swab, a swab stick was moistened in sterile normal saline and used to swab on the palm/finger of the household participants. Thereafter, the stick was introduced into an already prepared sterile peptone water (broth culture) in a test tube, and the test tube was incubated for 18 hours for the growth of viable cells. Food samples collected were composed of processed cassava products (garri & foo-foo), soup, palm oil, table salt and pepper. For water samples collection; only the refilled bottled water for drinking was considered and collected. All collected samples were taken to the Biology laboratory of Ignatius Ajuru University of Education, Port Harcourt for microbiological analysis. Media preparation involved weighing the required amount of Nutrient agar and Salmonella/ Shigella agar needed (Sigma, 2023). The media were dissolved in water, and sterilized in an autoclave for Nutrient agar preparation while for Salmonella/ Shigella agar preparation, the media was boiled as instructed by the manufacturer before use. Fifteen gram (15g) of Salmonella/ Shigella agar were dissolved in 250 ml distilled water, boiled and dispensed into petri dishes for isolation and confirmation of *Salmonella* spp. and *Shigella* spp. bacteria (Sigma, 2023).

Enumeration of *Salmonella* spp. load entailed aseptically, inoculating the swabbed palm components, food and water samples into the freshly, prepared nutrient agar and Salmonella/ Shigella agar media with a sterile pipette. A 0.1 inoculum volume was introduced into the media. The media after inoculation, was incubated at an ambient temperature for 24 hours. Isolation of pure cultures of *Salmonella* spp. and *Shigella* spp. after 24 hours was done based on the appearance of black colonies on the media background (plate) and further isolation of heterotrophic bacteria (Malorny et al.,2008)

Colonial characterization involved the macroscopic description of the colony appearance of *Salmonella* spp. concerning colour, size, elevation, edge, opacity and shape. Gram staining procedures were done as adopted by Malorny et al. (2008) aimed at characterizing the isolates morphologically. The characterization processes involved; heat fixing of the isolate on a sterile macroscopic slide via flame side by side. Thereafter, the slide was stained with crystal violet, iodine, ethanol and safranin at different time intervals with each phase, the reagent was washed off with water simultaneously. In viewing the image on the slide, a Light microscope was used adopted by Malorny et al. (2008), and the images were reported as Gram-positive, for purple backgrounded images or Gram-negative for pink/red backgrounded images.

The bacterial isolates recovered were further identified biochemically using the identification scheme of Malorny et al. (2008), where the isolates were inoculated into commercially prepared reagents such as Sodium Citrate, Hydrogen Peroxide etc. and the composition incubated appropriately as recommended. Isolates in which the medium changed colour and gas produced were considered and further identified on Salmonella/Shigella agar. Thus, the following biochemical tests were carried out accordingly; Catalase test, Motility test, Capsule test, Voges-Proskauer test, Citrate utilization test and Indole test.

The isolate was screened for its ability to break down Hydrogen Peroxide. In carrying out the test, a loop full of the bacterium was inoculated into a clean slide and thereafter the catalase reagent (Hydrogen Peroxide) was inoculated on the slide and observed. Visible effervescent gas bubbles indicated catalase utilization while an absence indicated poor or no effervescent (Malorny et al., 2008). The isolate was tested for its motile property. In carrying out the test, a freshly prepared semi-molten Nutrient agar was dispensed into the test tube. Following these, the test bacteria were inoculated aseptically, into the medium by stabbing. Thereafter, the medium was incubated at 37 degrees centigrade for 24 - 48 hours. An inference of growth in a diffused form, from the line of stab indicated a positive result, whereas growth only along the line of stab indicated a negative result (Malorny et al., 2008).

The presence of a capsule, an outer covering that surrounds the cell was checked. The procedure for the capsule test as carried out by Malorny et al. (2008) entailed adding a few drops of crystal violet reagent onto the test bacteria on a clean glass slide. Thereafter, the component was stirred and viewed under a light microscope. An inference of a light blue appearance from the objective lens indicated an encapsulated cell, while the reverse indicated the cell was not capsulated (Malorny et al., 2008). Voges-Proskauer test was done to determine if the test bacteria could ferment glucose to yield acetone, a by-product. In carrying out the test aseptically, a loopful of the bacterium was inoculated into freshly prepared Voges-Proskaurer broth medium in a test tube and the medium was incubated at 37degrees centigrade for 48 hours. Following the incubation, 0.6ml of 5 percent – naphthol and 0.2ml of 40 percent Potassium Hydroxide reagent were introduced to the medium. A change in the medium colour to red colouration indicated Voges-Proskauer positive while a negative Voges-Proskauer showed no change in colour (Malorny et al., 2008). The utilization of citrate by the test bacterium as its source of carbon and inorganic ammonium salt was determined as carried out by Malorny et al. (2008). The test involved the preparation and dispensing of a citrate medium into a test tube for sterilization and subsequent aseptic inoculation of the test bacteria with the aid of a sterile wire loop. The medium was then incubated at 37 degrees centigrade for 24 hours, following which a change in colour from green to blue indicated positive citrate utilization whereas no change in colour indicated negative citrate usage (Malorny et al., 2008). The test bacteria were examined for their ability to break down the amino acid tryptophan in the presence of tryptophanase to produce pyruvic acid, ammonia and indole. The test involved inoculating a loopful of the bacterium into a sterile peptone water and the component was incubated at 37 degrees centigrade for 48 hours. Following this, 0.3 - 0.5 ml of Kovac's reagent was added with the aid of a Pasteur pipette. Thus, a red ring on the medium indicated an indole utilization while a yellow ring indicated the absence of indole (Malorny et al., 2008).

Results

Mean Spatial Distribution of Bacteria Load in Water, Food and Palm (Hand) Samples

Table 1, below shows the mean load of bacteria isolated from water, food and palm (hand) samples. The load of heterotrophic bacteria recovered from the samples varied. The water samples obtained, showed a mean heterotrophic bacteria load of 6.6 x 10^2CFU/ml whereas no growth of *Salmonella* sp. and *Shigella* sp were detected. Similarly, no *Salmonella* spp. and *Shigella* spp. growth was detected for food and palm (hand) samples. However, heterotrophic bacteria recorded a mean growth of 7.3 x 10^2CFU/ml and 4.7 x 10^2CFU/ml for food and palm (hand) samples respectively.

Table 1: Mean Bacteria Load in Water, Food and Finger Samples

Bacteria	Water (x 10^2CFU/ml)	Food (x 10^2CFU/ml)	Palm/Finger (x 10^2CFU/ml)
Heterotrophic Bacteria	6.6	7.3	4.7
Salmonella spp.	-	-	-
Shigella spp.	-	-	-

Key: CFU (Coliform Forming Unit)/ Mill

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Morphological Characteristics of the Heterotrophs

Colonial classification of the heterotrophic bacteria isolated as seen in Table 2, showed appearance in terms of colour, size, elevation, edge, opacity and shape of the isolates as well as the isolate reaction to Gram stains. Isolates that reacted to Gram's reaction, showed a light yellowish colour, with an opaque view/display and large-sized colonies with curved edges. However, some other bacteria showed cream colour and reacted negatively to Gram stain with the following colonial features; circular shape, small size and translucent texture.

Table: 2 Morphological Characteristics of the Heterotrophs

Bacteria	Colour	Size	Elevation	Edge	Opacity	Shape	Gram React.
Heterotroph	Yellow	Large	High	Curve	Opaque	Irregular	+
Heterotroph	Cream	Large	High	Curve	Opaque	Round	-

Note + = Positive - = Negative

Biochemical Characterization of the Heterotrophs Recovered

The biochemical screening of the heterotrophs as reported in Table 3did not reveal the presence of *Salmonella* spp. the heterotroph isolated had a positive Gram reaction and a negative Gram reaction. This was followed by; positive coagulase and catalase reactions. Sugar, motility and citrate indicated positive also.

Table 3 Biochemical Characterization of the Isolates Recovered

Bacteria	Catalase	Motility	Indole	Citrate	Voges-Proskauer	Capsule
Heterotroph	-	+	-	+	+	+
Heterotroph	+	+	-	+		+

Discussion

The absence of *Salmonella* in water, food and hands of occupants of a home in this study as observed has never been considered by literature. However, in several findings, high loads of *Salmonella* spp. have been reported in water, food and hand (Ehuwa et al., 2021). According to Dekke et al. (2015), *Salmonella* spp. has been isolated from dug wells in rural Ghana. In the foregoing, Ajemikalajah (2018) has been able to project bacteria load in fried rice prepared in five different restaurants, with no recorded load of *Salmonella* spp. or *Shigella* spp. Following this, the survival of *Salmonella* spp. on fingertips and subsequent transfer to food have been noted by Fracis et al. (2012). Despite the absence of *Salmonella* in the finger/hands in this study, Fracis et al. (2012), further noted the presence of *Salmonella* spp. in the hand during egg dish preparation. In all these reports, the implication of *Salmonella* spp. in faecal-oral transmission of diseases remains a threat (Graaf et al., 2016). Heterotrophic bacteria as recovered, having been noted in the hand, could transfer bacteria even after hand washing with soap and water to the mouth (Zapka et al., 2011). The presence of this permissible count of heterotrophs in this investigative sample is not questionable; this is because most heterotrophs require food sources. Thus, the investigative food sample provided all the needed nutrients for heterotroph growth (Zabel & Morrell, 2020). Heterotrophic bacteria count in water was permissible and less than 100 CFU/ml. However, this study showed a heterotrophic bacteria load lower in water according to the World Health Organization (WHO) standards. By the counts recovered in this study, the water is said to be potable for drinking purposes. According to Agada et al. (2014), *Salmonella* spp. are characterized using a biochemical test method, following the test, *Salmonella* spp. were not identified with black centre colonies and translucent features on Salmonella/Shigella agar media. Similarly, in studies carried out by Jiang et al. (2018) *Salmonella* spp. were characterized as brown, grey with black centre without metallic sheen; blue or blue-green with or without black centres (Estrade-Gacia et al.,2004).

Conclusion

The study noted the absence of *Salmonella* in all investigative food, water and hand samples under study. Hence, the health and safety condition of the households /consumers is guaranteed of *Salmonella* spp. infections.

Recommendation

The study calls for further in-depth analysis of other types of food purchased from the market and transferred to the house.

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