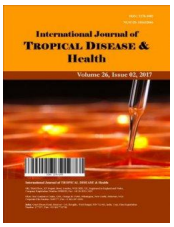

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Survival of *Salmonella enterica* ssp. *enterica* ser. Typhi in Brewed Pito Retailed in Accra

Osisiogu Udochukwu Emmanuel[1*], Chudi-Oji Ifeoma Onyedika[2], Akanlu A. Servacious[1] and Verner N. Orish[3]

[1]Department of Science Laboratory Technology, Wa Polytechnic, Wa, Ghana.
[2]Department of Medical Laboratory Technology, Faculty of Allied Health Sciences, Radford University College, Accra, Ghana.
[3]Department of Microbiology and Immunology, University of Health and Allied Health Sciences Ho, Volta Region, Ghana.

Authors' contributions

This work was carried out in collaboration between all authors. Authors OUE and COIO designed the study and performed the laboratory analysis. Authors AAS and VNO wrote the protocol and edited the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at ascertaining the survival ability of *S. typhi* in both fermented and unfermented pito.
Study Design: The study followed an experimental design pattern.
Place and Duration of Study: The study was carried out at the Microbiology Laboratory of Radford University College, East Legon.
Methodology: *S. typhi* was introduced into pito samples and subsequently sub-cultured unto Salmonella-Shigella agar for 24 hours and the process repeated for five (5) consecutive days. The antimicrobial potential of pito against *S. typhi* was also investigated.
Results: Culture yielded no bacterial growth and pito had no significant antimicrobial effect on isolate.

**Corresponding author: Email: osisioguemmanuel@yahoo.com*

Conclusion: The study, therefore, concluded that the alcoholic and nonalcoholic forms of pito could not be a route of transmission of *S. typhi*. We recommend that the phytochemical compositions in sorghum should be extracted and its antimicrobial properties, especially against *S. typhi*, investigated.

Keywords: Pito; S. Typhi, survival; antimicrobial; lactic acid bacteria.

1. INTRODUCTION

The genus *Salmonella* is comprised of two species, *S. enterica* and *S. bongori*, with *S. enterica* being divided into six subgroups (*enterica*, *salamae*, *arizonae*, *diarizonae*, *indicia*, and *houtenae*). *S. enterica* subspecies *enterica* strains primarily infect warm-blooded hosts and are responsible for >95% of human infections, while the remaining five subspecies and *S. bongori* primarily infect cold-blooded hosts [1,2]. In developing countries, typhoid fever is one of the commonest diseases that affect humans. It is still an important cause of global morbidity and mortality [3]. Global estimates in the year 2000, showed that typhoid fever accounted for 21,000,000 illnesses and 216,000 deaths [4]. Typhoid is caused by a bacterium called *S.* Typhi. The *Salmonella* spp. was first identified in pigs in 1886 by an American pathologist called Dr. Daniel Salmon, and thus the name "Salmonella" [5]. *S. typhi* is transmitted through food or water contaminated with faeces or urine from infected persons, persistent excretors or from chronic asymptomatic carriers who handle food [6].

Food and food products provide the much-needed carbohydrates, proteins, lipids, dietary fibres, and essential elements required by humans. Sorghum, a cereal rich in carbohydrate, constitutes a staple food for people in the tropical regions of Africa and Asia. In West Africa, sorghum grains are malted and used to produce traditional beers like pito common in Ghana, Togo and Nigeria. Pito is a popular food drink in West Africa, produced and sold by women with important socio-economic implications [7].

Foodborne diseases remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices [8]. *Salmonellae* are not fastidious and thrive in many foods and drinks; both freshly prepared and stored food, whether hot or cold in temperature. Healthy chronic carriers, especially food and drink handlers are very important in the transmission; particularly in places with low environmental sanitation where many people eat and drink outside their homes [9]. In endemic areas, identified risk factors for the disease include eating food prepared outside the home by street vendors, drinking contaminated substance, close contact with an infected person, poor housing with inadequate facilities for personal hygiene, and the recent use of antimicrobial drugs [6].

It is estimated that 78% of the rural population in the third world are without clean water supply and 85% are without adequate sewage and other excreta disposal facilities [5]. Ghana is not exempted. There is seasonality in occurrence and frequency of *Salmonella* infections in Ghana. *S. typhi* infections are seen to follow the rainfall pattern with the peak in July. Water used for brewing pito may not be potable or hygienically handled prior to usage. Pito could be exposed to microorganisms such as *Salmonella* spp. during the brewing process. After brewing, the pots are kept to cool down in these environments and the chances of flies perching on the drink cannot be eliminated. It is consumed at events like funerals, weddings, birthday parties etc. It is usually served in open calabash bowls. Women that brew pito could be asymptomatic carriers of *S. typhi* and could be transmitting it to consumers, hence causing typhoid.

Many are ignorant of the virulence of *Salmonella* spp. and its various modes of transmission. Pito is commonly brewed and drank by those who live in rural areas with little knowledge on the possibility of it being a route of transmission for *S. typhi.*

This research was therefore aimed at investigating the ability of *S. typhi* to survive in brewed pito and the extent to which it can.

2. MATERIALS AND METHODS

2.1 Sample Site, Design and Size

The traditional processing of pito lasts two days and involves milling of malted sorghum, mashing, acidification, cooking, cooling and the alcoholic fermentation of the final wort by dried yeast taken

from a previous fermentation [10]. This is done in an open environment (Fig. 1. here).

Fig. 1. Pito in a calabash bow

Samples of pito were taken from Madina in the La Nkwantanang Municipality and sent to the Microbiology Laboratory of Radford University College. The experimental design was used to determine if *S. typhi* could survive in pito. *S. typhi* which was obtained from the Centre for Scientific Research into Plant Medicine (Akropong) was administered into 9 ml of pito while 10 ml of pito was used as a control. A total of 10 pito samples (5 bottles each of the fermented and unfermented pito) were obtained for the study.

2.2 Inoculation and Culture

The Salmonella isolate was first inoculated onto Nutrient Agar (Accumix^{TM} Belgium) as well as Salmonella Shigella Agar (SSA) (Oxoid, England) and incubated at 37°C for 24 hours after which growth was observed and colonies confirmed as *Salmonella* spp. via standard biochemical tests. A 1 µl loop was used to introduce *Salmonella* isolate into 2 ml of peptone water and incubated for 24 hours at 37°C after which 1ml each of the suspended isolates was inoculated into 9ml of fermented and unfermented pito respectively and incubated for 5 days alongside the control samples (un-inoculated pito samples). After an hour of incubation, a 1 µl loopful of both test and control specimens were subcultured separately unto Salmonella-Shigella agar (Oxoid, England) for 24 hours at 37°C for growth observation. This procedure was repeated each day on the pito samples (both test and control) for 5 consecutive days.

2.3 Susceptibility Test Using Pito

Pito was tested to see its potency as a herbal medicine against *S. typhi* and its effectiveness was determined. Mueller Hinton Agar (MHA) (Rapid Labs, UK) and SSA (Oxoid, England) were used as culture media. *S. typhi* (0.1 ml) was inoculated (via spread plate technique) on both media using non-absorbent cotton swabs (Puritan 6"Sterile Cotton Swab). The Kirby Bauer agar diffusion method [11] was used on the petri dishes as micro-wells were created in the media and 0.1ml each of the pito, Ciprofloxacin was dispensed into the various wells respectively. Ciprofloxacin served as an antibiotic control.

3. RESULTS AND DISCUSSION

Table 1 shows the culture results. No growth of *S. typhi* was noticed from pito after 124 hours.

Table 1. Showing results of individual cultures per day

Days	Culture results
After the first hour of incubation (1 hour)	No bacteria growth
Day one (24 Hours)	No bacteria growth
Day two (48 Hours)	No bacteria growth
Day three (72 Hours)	No bacteria growth
Day four (96 Hours)	No bacteria growth
Day five (124 Hours)	No bacteria growth

The antimicrobial effect of pito on *S. typhi* was monitored using the Kirby Bauer susceptibility test method but pito had no significant effect on the isolate (Table 2).

Table 2. Showing the antimicrobial effect of pito on *S. typhi*

	Mueller Hinton Agar containing *S. typhi*	Salmonella/Shigella Agar containing *S. typhi*
Ciprofloxacin (control)	29 mm (Diameter of inhibition)	30 mm (Diameter of inhibition)
Fermented Pito (Test sample)	0 mm (Diameter of inhibition)	0 mm (Diameter of inhibition)
Unfermented Pito (Test sample)	0 mm (Diameter of inhibition)	0 mm (Diameter of inhibition)

Salmonella serovars are the most common causes of hospitalization and death among foodborne pathogens that are tracked by the Foodborne Diseases Active Surveillance Network (FoodNet) [12] with most cases of the human disease being caused by serovars of *S. enterica* subsp. *enterica*. Numerous serovars can cause gastroenteritis, and they are collectively referred to as nontyphoidal Salmonellae (NTS). The most common NTS serovars worldwide are Typhimurium and Enteritidis [13]. Serovars Typhi and Paratyphi A may cause bloodstream invasion in the absence of gastroenteritis and are referred to as Invasive Salmonellae; the disease they cause is usually classified as typhoid fever. This study focused on *S. typhi.*

Salmonella spp. are known to survive in non-host environments [14], but the mechanisms of persistence are not well understood. For example, the well-characterized acid tolerance response [15] is usually presumed to be a pathogenesis adaptation to ensure smooth passage of *Salmonella* through the mammalian stomach. Several studies involving the analysis of *Salmonella* persistence in poultry houses and other food processing environments have reported that vectors (*i.e.*, rodents, insects) represent a main environmental reservoir of *Salmonella* spp. [16,17]. More recently, there is evidence of biofilm formation, a multicellular behaviour that may enable *Salmonella* spp. to survive long-term in the environment without requiring an animal reservoir. However, Culture results of *S. typhi* in pito yielded no bacteria growth during this study. However, upon carrying out susceptibility test on the isolate using pito as the antimicrobial agent, pito was found to have no inhibitory effect on *S.* Typhi. Several factors could have accounted for this.

Firstly, Sorghum has probiotic properties. Probiotic foods contain a single or mixed culture of probiotic microbes that improve the health of the host by improving intestinal microbial balance [18]. This study conducted by Kunchala et al in 2016 confirmed that probiotic properties of bacteria isolated from flour and batter samples of sorghum and pearl millet inhibited the growth of *Escherichia coli*, *Staphylococcus aureus* and *S. typhi*.

In addition, pito gets fermented by the addition of *Lactobacillus fermentum* (Lactic acid bacteria [LAB]) to it [19]. The production of organic acids reduces the pH to below 4.0 making it difficult for some spoilage organisms that are present in cereals to survive. The antimicrobial effect is believed to result from the action of the acids in the bacterial cytoplasmic membrane, which interferes with the maintenance of the membrane potential and inhibits the active transport. Apart from their ability to produce organic acids, the LAB possesses the ability to produce hydrogen peroxide through the oxidation of reduced nicotinamide adenine dinucleotide (NADH) by flavin nucleotides, which react rapidly with oxygen. As LAB lack true catalase to break down the hydrogen peroxide generated, it can accumulate and be inhibitory to some microorganisms [20] including *S. typhi*. *L. fermentum* strain ME-3 DSM-14241 is a unique strain of *Lactobacillus* species that has antimicrobial and physiologically effective antioxidative properties and expresses health-promoting characteristics if consumed [21]. This could be a cause of *S. typhi* survival inhibition in pito.

4. CONCLUSION

S. typhi, having been inoculated in pito (fermented and unfermented) and cultured, could not survive in pito. The organism was inoculated into pito for five (5) days and still yielded no bacterial growth. Therefore, pito cannot serve as a route of transmission of *S. typhi* nor cause typhoid fever. Also, the antimicrobial susceptibility testing of pito showed that pito was not effective as a herbal medicine for *S. typhi.*

Based on the findings of this study, we recommend that the phytochemical compositions in sorghum should be extracted and its antimicrobial properties, especially against S. Typhi, investigated. We also recommend that molecular analysis of Pito samples containing *S.* Typhi should be conducted to determine whether the organism persisted in a viable but non-culturable form. In addition, there is a continued need for increased epidemiological surveillance to identify reservoirs in the environment as *Salmonella* has adapted remarkably well to diverse environments and increasing our knowledge about transmission would help to minimize its worldwide impact.

NOTE OF THE AUTHORS

"All authors declare that 'written informed consent was obtained from the Pito Bar operator for publication of this experimental report and accompanying images."

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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