

1
2
3
4
5
6
7
8
9
10

11
12
13

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

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.
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.

14 1. INTRODUCTION

15

16 In developing countries, typhoid fever is one of the commonest diseases that affect humans.
17 It is still an important cause of global morbidity and mortality [1]. Global estimates in the year
18 2000, showed that typhoid fever accounted for 21,000,000 illnesses and 216,000 deaths [2].
19 Typhoid is caused by a bacterium called *S. Typhi*. The *Salmonella* spp. was first identified in
20 pigs in 1886 by an American pathologist called Dr. Daniel Salmon, and thus the name
21 “*Salmonella*” [3]. *S. Typhi* is transmitted through food or water contaminated with faeces or
22 urine from infected persons, persistent excretors or from chronic asymptomatic carriers who
23 handle food [4].

24 Food and food products provide the much needed carbohydrates, proteins, lipids, dietary
25 fibers, and essential elements required by humans. Sorghum, a cereal rich in carbohydrate,
26 constitutes a staple food for people in the tropical regions of Africa and Asia. In West Africa,
27 sorghum grains are malted and used to produce traditional beers like pito common in
28 Ghana, Togo and Nigeria. Pito is a popular food drink in West Africa, produced and sold by
29 women with important socio-economic implications [5].

30 Food borne diseases remain a major public health problem across the globe. The problem is
31 severe in developing countries due to difficulties in securing optimal hygienic food handling
32 practices [6]. *Salmonellae* are not fastidious and thrive in many foods and drinks; both
33 freshly prepared and stored food, whether hot or cold in temperature. Healthy chronic
34 carriers, especially food and drink handlers are very important in the transmission;
35 particularly in places with low environmental sanitation where many people eat and drink
36 outside their homes [7]. In endemic areas, identified risk factors for the disease include
37 eating food prepared outside the home by street vendors, drinking contaminated substance,
38 close contact with an infected person, poor housing with inadequate facilities for personal
39 hygiene, and the recent use of antimicrobial drugs [4].

40 It is estimated that 78% of the rural population in the third world are without clean water
41 supply and 85% are without adequate sewage and other excreta disposal facilities [3].
42 Ghana is not exempted. There is seasonality in occurrence and frequency of *Salmonella*
43 infections in Ghana. *S. Typhi* infections are seen to follow the rainfall pattern with the peak in
44 July. Water used for brewing pito may not be potable or hygienically handled prior to usage.
45 Pito could be exposed to microorganisms such as *Salmonella* spp. during the brewing
46 process. After brewing, the pots are kept to cool down in these environments and the
47 chances of flies perching on the drink cannot be eliminated. It is consumed at events like
48 funerals, weddings, birthday parties etc. It is usually served in open calabash bowls. Women
49 that brew pito could be asymptomatic carriers of *S. Typhi* and could be transmitting it to
50 consumers, hence causing typhoid.

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

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

56

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

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 [8]. This is done in an open environment (figure 1 here).



Figure 1. Pito in a calabash bowl

Samples of pito were taken from Madina in the La Nkwantanang Municipality and sent to the Microbiology Laboratory of Radford University College. 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 9ml of pito while 10ml of pito was used as 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™ 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 1µl loop was used to introduce *Salmonella* isolate into 2ml of peptone water and incubated for 24 hours at 37°C after which 1ml each of the suspended isolate was inoculated into 9ml of fermented and unfermented pito respectively and incubated for 5 days

79 alongside the control samples (un-inoculated pito samples).After an hour of incubation, a 1µl
80 loopful of both test and control specimens were subcultured separately unto Salmonella-
81 Shigella agar (Oxoid, England) for 24 hours at 37⁰C for growth observation. This procedure
82 was repeated each day on the pito samples (both test and control) for 5 consecutive days.

83

84 2.3. Susceptibility Test Using Pito

85 Pito was tested to see its potency as a herbal medicine against S. Typhi and its
86 effectiveness was determined. Mueller Hinton Agar (MHA) (Rapid Labs, UK) and SSA
87 (Oxoid, England) were used as culture media. S. Typhi (0.1ml) was inoculated (via spread
88 plate technique) on both media using non-absorbent cotton swabs (Puritan 6" Sterile Cotton
89 Swab). The Kirby Bauer agar diffusion method [9] was used on the petri dishes as micro-
90 wells were created in the media and 0.1ml each of the pito, Ciprofloxacin was dispensed into
91 the various wells respectively. Ciprofloxacin served as an antibiotic control.

92

93

94 3. RESULTS AND DISCUSSION

95

96 Table 1 shows the culture results. No growth of S. Typhi was noticed from pito after 124
97 hours.

98 **Table 1:** Showing results of individual cultures per day

DAYS	CULTURE RESULTS
After 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

99

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

102 **TABLE 2:** Showing antimicrobial effect of pito on *S. Typhi*

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

103

104

105 Culture results of *S. Typhi* in pito yielded no bacteria growth. However, upon carrying out
 106 susceptibility test on the isolate using pito as the antimicrobial agent, pito was found to have
 107 no inhibitory effect on *S. Typhi*. Several factors could have accounted for this.

108 Firstly, Sorghum has probiotic properties. Probiotic foods contain a single or mixed culture of
 109 probiotic microbes that improves the health of the host by improving intestinal microbial
 110 balance [10]. This study conducted by Kunchala *et al* in 2016 confirmed that probiotic
 111 properties of bacteria isolated from flour and batter samples of sorghum and pearl millet
 112 inhibited the growth of *Escherichia coli*, *Staphylococcus aureus* and *S. Typhi*.

113 In addition, pito gets fermented by the addition of *Lactobacillus fermentum* (Lactic acid
 114 bacteria [LAB]) to it [11]. The production of organic acids reduces the pH to below 4.0
 115 making it difficult for some spoilage organisms that are present in cereals to survive. The
 116 antimicrobial effect is believed to result from the action of the acids in the bacterial
 117 cytoplasmic membrane, which interferes with the maintenance of the membrane potential
 118 and inhibits the active transport. Apart from their ability to produce organic acids, the LAB
 119 possesses the ability to produce hydrogen peroxide through the oxidation of reduced nicotin-
 120 amide adenine dinucleotide (NADH) by flavin nucleotides, which react rapidly with oxygen.
 121 As LAB lack true catalase to break down the hydrogen peroxide generated, it can
 122 accumulate and be inhibitory to some microorganisms [12] including *S. Typhi*. *L. fermentum*
 123 strain ME-3 DSM-14241, is a unique strain of *Lactobacillus* species that has antimicrobial
 124 and physiologically effective antioxidative properties and expresses health-promoting
 125 characteristics if consumed [13]. This could be a cause of *S. Typhi* survival inhibition in pito.
 126 The combined treatment of *L. fermentum* ME-3 and ofloxacin, eradicates *S. Typhi* from
 127 blood, ileum and liver, decreases the number of animals with liver and spleen granulomas
 128 and reduces the value of lipid peroxides in the ileum mucosa [14].

129

130

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.

CONSENT

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

REFERENCES

- [1] Shu-Kee Eng, Priyia Pusparajah, Nurul-Syakima Ab Mutalib, Hooi-Leng Ser, Kok-Gan Chan & Learn-Han Lee. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance, *Frontiers in Life Science*. 2015; 8:3, 284-293, DOI: [10.1080/21553769.2015.1051243](https://doi.org/10.1080/21553769.2015.1051243)
- [2] Labi A. K., Obeng-Nkrumah N., Addison N. O. and Donkor E. S. Salmonella Blood Stream Infections in a Tertiary Care Setting in Ghana. *BioMed Central Infectious Diseases*. 2014; 14 (3857): 1-10.
- [3] Myron Schultz. Theobald Smith. *Emerg Infect Dis*. 2008; 14(12) 1940–1942. doi: 10.3201/eid1412.081188
- [4] Smith S. I., Alao F., Goodluck H. T., Fowora M., Bamidele M., Omonigbehin E. and Coker A. O. Prevalence of S. Typhi Among Food Handlers from Bukkas in Nigeria. *Br J Biomed Sci*. 2008; 65:158-60.
- [5] Sawadogo-Lingani H., Diawara B., Traore´ A. S. and Jakobsen M. Technological Properties of Lactobacillus fermentum Involved in the Processing of Dolo and Pito, West African Sorghum Beers, for the Selection of Starter Cultures. The Society for Applied Microbiology, *Journal of Applied Microbiology*. 2007^a; 104: 873–882.
- [6] Eileen R. Choffnes, David A. Relman, LeighAnne Olsen, Rebekah Hutton, and Alison Mack. Improving food safety through a one health approach. The National Academies Press, Washington DC. 2012
- [7] Ogah JO, Adekunle OC, Adegoke AA. Prevalence of Salmonellosis among Food Handlers and the Health Implications on the Food Consumers in Lagos State, Nigeria. *J Med Microb Diagn*. 2015; 4:187. doi: 10.4172/21610703.1000187

- 174 [8] Sawadogo-Lingani H., Lei V., Diawara B., Nielsen D. S., Møller P. L., Traore´ A. S. and
175 Jakobsen M. The Biodiversity of Predominant Lactic Acid Bacteria in Dolo and Pito Wort
176 for the Production of Sorghum Beer. *The Society for Applied Microbiology, Journal of*
177 *Applied Microbiology*. 2007; 103: 765–777.
- 178 [9] James J. Biemer. Antimicrobial Susceptibility Testing by the Kirby-Bauer Disc Diffusion
179 Method. *Annals of clinical laboratory science*. 1973; vol. 3 , no. 2
- 180 [10] Kunchala R., Banerjee R., Mazumdar S. D., Durgalla P., Srinivas V. and
181 Gopalakrishnan S. Characterization of Potential Probiotic Bacteria Isolated From
182 Sorghum and Pearl Millet Of The Semi-Arid Tropics. *African Journal of Biotechnology*.
183 2016; Vol. 15(16), pp. 613-621.
- 184 [11] Soro-Yao AA, Brou K, Amani G, Thonart P, Djè KM. The Use of Lactic Acid Bacteria
185 Starter Cultures during the Processing of Fermented Cereal-based Foods in West
186 Africa: A Review. *Tropical Life Sciences Research*. 2014; 25(2):81-100.
- 187
188 [12] Blandino A., Al-Aseeri M. E., Pandiella S. S., Cantero D. and Webb C. Cereal-Based
189 Fermented Foods and Beverages. *Food Research International*. 2002; 36 (2003) 527–
190 543.
- 191 [13] Mikelsaar M. and Zilmer M. *Lactobacillus fermentum* ME-3 An Antimicrobial and
192 Antioxidative Probiotic. *Microbial Ecology in Health and Disease*. 2009; 21: 1-27.
- 193 [14] Truusalu K., Mikelsaar R. H., Naaber P., Karki T., Kullisaar T., Zilmer M. and Mikelsaar
194 M. Eradication of *S. Typhimurium* Infection in a Murine Model of Typhoid Fever with the
195 Combination of Probiotic *Lactobacillus fermentum* ME-3 and Ofloxacin. *BMC*
196 *Microbiology* 2008, 8:132.