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2 **Short Research Article**

3 **Survival of *Salmonella typhi* in Brewed**

4 **Pito Retailed in Accra**

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8 **ABSTRACT**

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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: *Salmonella typhi* was introduced into pito samples and subsequently sub-cultured onto 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.

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11 **Keywords:** Pito, *Salmonella typhi*, survival, antimicrobial, lactic acid bacteria.

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13 1. INTRODUCTION

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

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

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

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

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

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

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2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1. Sample Site, Design and Size

The traditional processing of pito last 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

A loopful of *Salmonella* isolate was suspended in 2ml of peptone water and 1ml inoculated into 9ml of fermented and unfermented pito respectively. Control samples (un-inoculated pito samples) were incubated alongside the test samples for an hour after which a loopful of each specimen was subcultured unto Salmonella-Shigella agar for 24 hours. This procedure was repeated on the pito samples 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) and SSA were used as culture media. *S. typhi* (0.1ml) was inoculated (via spread plate technique) on both media using non-absorbent cotton swabs. The Kirby Bauer agar diffusion method [9] 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.

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3. RESULTS AND DISCUSSION

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

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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

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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)

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94 **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)

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97 Culture results of *Salmonella typhi* in pito yielded no bacteria growth. However, upon
 98 carrying out susceptibility test on the isolate using pito as the antimicrobial agent, pito was
 99 found to have no inhibitory effect on *Salmonella typhi*. Several factors could have accounted
 100 for this.

101 Firstly, Sorghum has probiotic properties. Probiotic foods contain a single or mixed culture of
 102 probiotic microbes that improves the health of the host by improving intestinal microbial
 103 balance [10]. A study conducted by Kunchala *et al* in 2016 confirmed that probiotic properties
 104 of bacteria isolated from flour and batter samples of sorghum and pearl millet inhibited the
 105 growth of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*.

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

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4. CONCLUSION

Salmonella 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.'"

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