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Enterotoxigenicity Profile of Salmonella Enterica Serovar Typhimurium in Suckling Albino Mice

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Abstract
This study was carried out to investigate the enterotoxigenic potentials of *Salmonella enterica* serovar Typhimurium from different food samples in suckling albino mice. A total of 27 food samples comprising sample A (9), sample B(9) and Sample C(9) were collected randomly from different markets in Uli community and screened for the presence of *Salmonella enterica* serovar Typhimurium using pour plate technique. The isolates obtained were characterized and identified using their colonial descriptions, morphological and biochemical characteristics. Intragastric method in suckling mice was used to evaluate the enterotoxigenicity potententials of the isolates using gut/body ratio (G/B) and fluid accumulation ratio (FAR). *Salmonella enterica* serovar Typhimurium (ST1,ST2,ST3,ST4,ST5,ST6) was detected in 14(51.85) food samples out of 27 food samples screened, of which the isolate was discovered mostly in sample B. ST5 was significant ($P<0.05$) for enterotoxin after 4 h exposure and ST2, ST3, ST5 and ST6 were significant ($P<0.05$) after 24 h. It was observed that most of the strains of *Salmonella enterica* serovar Typhimurium tested, were positive to enterotoxin mainly after 24 h, of which ST5 proved to be most enterotoxigenic after 4 h and 24 h.

INTRODUCTION

Salmonella constitutes a major health problem in many countries (Slender *et al*., 2012). An infection can only begin after living *Salmonellae* (not merely *Salmonella*-produced toxins) reach the gastrointestinal tract. Some of the bacteria are killed in the stomach, while the surviving ones enter the small intestine and multiply in tissues. Gastric acidity is responsible for the destruction of the majority of ingested bacteria, but *Salmonellae* has evolved a degree of tolerance to acidic environments that allows a subset of ingested bacteria to survive. By the end of the incubation period, the nearby host cells are poisoned by endotoxins released from the *Salmonellae*. The local response to the endotoxins is enteritis and gastrointestinal disorder (Heymann *et al*. 2006).

Globally, food borne infection and intoxications have been estimated that one billion cases of acute diarrhea occur annually in children under the age of 5years in African, Asia and Latin America, approximately 5 million of these cases were proved fatal (Cummings *et al*., 2010). Poultry has been widely acknowledged to be a reservoir for *Salmonella*. Egg contents may be contaminated with *Salmonella* by trans-ovarian (vertical transmission) which occurs when *Salmonellae* are introduced from infected reproductive tissues to eggs prior to shell formation. Ready to eat food samples which include roasted meat, smoked fish and even cooked food also serve as reservoir for *Salmonella*. They are contaminated through horizontal transmission or cross contamination by unhygienic handling, storage and preparation of this food items thus when ingested they cause food poisoning/intoxication resulting from *Salmonellae* produced endotoxins.

Several studies focused on the study of the enterotoxigenicity of Salmonella species isolated from other sources (Asiton *et al.*, 2012; Prince and Berry, 2012; Lawnon *et al*., 2013) but little or no report has been documented on the enterotoxigenicity of *Salmonella enterica* serovar Typhimurium isolated from food samples. Hence, *Salmonella* serovar Typhimurium remains among the primary causes of reported Gastroenteritis and food borne infections in the developing countries majorly in Ihiala Local Government Area, Anambra State, Nigeria. This study was undertaken to evaluate the enterotoxigenicity profile of *Salmonella enterica* serovar Typhimurium isolated from different food water samples used in Uli community in Ihiala L.G.A.

MATERIALS AND METHODS

Sample collection: The food samples used for this study were raw eggs, roasted meats and smoked ice fishes. The egg samples were collected randomly from different egg sellers in Uli town. The roasted meats were aseptically collected using sterile aluminium foil, from different point where they were sold in Uli town whereas the smoked fishes were aseptically collected using sterile aluminium foil, from different fish sellers in Uli town. The samples were done in triplicate. After collection, the samples were placed in a cooler to maintain the temperature during transportation for laboratory analysis. All samples were analyzed within 4 h of collection and were analysis were to be delayed, the samples were refrigerated at 4ºC.

Isolation of organism: Different strains of the organism used for this work were isolated from raw eggs,

smoked ice fish and roasted meat. The egg was disinfected using 70% ethanol and aseptically separated the albumin (white part) from the yolk (yellow part). 1ml of each separated albumin was aseptically collected and plated on Salmonella-Shigella Agar (S-S A) (Biotech). The smoke ice fish and roasted meat was separately pulverized using electric grinder and aseptically weighed 10g into 100 ml of sterile peptone water. The preparation was homogenized and 1ml sample of each preparation was aseptically plated into S-S Agar using pour plate method. This was done in triplicate and incubated at 37ºC for 24-48 h (Arora and Arora, 2008).

Isolation, characterization and identification of the organism: Different strains of the organism used for this work were isolated from food samples in Uli community. This was carried out by aseptically inoculating 1.0 ml of the food samples on *Salmonella-Shigella* agar using the pour plate method and incubate at 37ºC for 24-48 h. After 48 h of incubation, the grown colonies were subcultured, characterized and identified using the colony descriptions, microscopic and biochemical characteristics (Arora and Arora, 2008).

Procurement of albino mice: A total of 28 suckling mice of mixed sex were obtained from an animal keeping house in Nnewi, Anambra State were used for the study. They were housed in thoroughly cleaned and disinfected metal cages and provided with feeds and water prior to infection.

Inoculum preparation: The isolate was first cultured on a nutrient agar (NA) and incubated at 37ºC for 24h. The organism was subcultured into nutrient broth (NB) and incubated at 37ºC for 24h. After incubation, the culture was centrifuged at 3000rpm for 5 minutes and the supernatant was decanted. The sediment was washed two times using phosphate buffer saline and re-suspended in normal saline (0.85% NaCl). The turbidity of the suspended cells was adjusted to match the turbidity of 0.5 McFarland solutions which was prepared by mixing 0.6ml of 1% $Bacl_2.2H_2O$ and 99.4ml of 1% concentrated H_2SO_4. The turbidity was standardized using spectrophotometer at 660nm which was equivalent to approximately 10^8cells/ml (Iheukwumere and Umedum, 2013).

Suckling mice test for enterotoxigenicity: This was carried out according to Asiton *et al*. (2012). The suckling mice (1-3 days old) were separated from their mothers shortly before the test and place in 7 groups containing four (4) suckling mice each. The mice were intragastrically inoculated with 0.1ml of the prepared cells using syringes without needle. All the inoculated mice were kept at room temperature (25ºC) for 4 and 24 hours respectively, after which they were killed with chloroform. The gut (intestine) was surgically removed from the body and each weighed separately. The fluid accumulation (FA) ratio (weight of entire intestine/ total body weight–weight of intestine) of each animal was calculated. The gut/body ratio was also calculated.

Statistical analysis: The results of the data generated in this study were expressed as mean. The statistical analysis of data generated from this enterotoxigenic study was carried out using chi-square at 95% confidence limit. (Iheukwumere and Umedum, 2013).

RESULTS

A total of 27 samples comprising of sample A (9), sample B (9) and sample C (9) were collected of which 14(51.85%) samples were positive for the isolate (Table 1). The isolate was mostly detected in sample B while sample A showed least occurrence. The colonial description, morphological and biochemical charcteristics of the isolates is shown in Table 2. The enterotoxigenicity of the isolates using the suckling albino mice is shown in Tables 3 and 4. When the gut/body weight (G/B) ratio is greater than 0.065, the isolate is significant for enterotoxin. Also, when the fluid accumulation is a standard (FAR) is greater than 0.070, the isolate is also significant for enterotoxin. The present study showed that ST5 was significant ($P<0.05$) for enterotoxin after 4 h (Table 3). Also ST2, ST3, ST5 AND ST6 were significant ($P<0.05$) for enterotoxin after 24 h exposure (Table 4). ST1 and ST4 were non-significant to enterotoxins after 4 h and 24 h exposure (Tables 3 and 4).

Table 1: Occurrence of *Salmonella enterica* serovar Typhimurium in the studied food samples

Food sample	Positive sample	Negative sample	Total (%)
A	3(11.11)	6(22.22)	9(33.33)
B	6(22.22)	3(11.11)	9(33.33)
C	5(18.52)	4(14.81)	9(33.33)
Total	14(51.85)	13.48.14)	27(99.99)

N-total no of food samples
A - Raw eggs
B - Roasted meat
C - Smoked fish

Table 2: Characteristics and identity of *Salmonella enterica* serovar Typhimurium

Parameter	ST1	ST2	ST3	ST4	ST5	ST6
Appearance on S.S agar	Colourless with dark centers	Colourless with dark centers	Colourless with dark centers	Colorless with dark centers	Colourless with dark centers	Colourless with dark centers
Elevation Edge	Raised entire	Raised entire	Raised entire	Raised entire	Raised entire	Raised entire
Gram reaction	–	–	–	–	–	–
Morphology	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
H_2S production	+	+	+	+	+	+
Coagulase	+	–	–	–	–	–
Indole	–	–	+	–	–	–
Citrate	+	+	+	+	+	+
V.P	+	–	+	+	+	+
M.R	+	+	+	+	+	+
Oxidase	–	–	–	–	–	–
Lactose	–	–	–	–	–	–
Mucate	+	+	+	+	+	+
Galactose	+	+	+	+	–	+
Inositol	+	+	+	+	+	+
Xylitol	+/–	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Dulcitol	_	–	–	–	–	–
Sorbitol	+	+	–	+	+	–
Maltose	+	+	+	+	+	+

ST – *Salmonella enterica* serovar Typhimurium + - Positive
M.R - Methyl Red – - Negative
VP - Voges Prokauer
H_2S - Hydrogen sulphide
S-S agar - Salmonella Shigella agar

Table 3: Enterotoxigenicity study of *Salmonella enterica* serovar Typhimurium after 4h

Isolatecode	BW (g)	GW (g)	FAR	G/B	Result
ST1	4.800	0.280	0.061	0.058	–
ST2	5.100	0.310	0.064	0.061	–
ST3	5.180	0.320	0.066	0.062	–
ST4	4.910	0.290	0.062	0.059	–
ST5	5.200	0.350	0.072	0.067	+
ST6	5.190	0.330	0.065	0.06	–
Control	4.900	0.280	0.061	0.057	–

ST – *Salmonella enterica* Serovar Typhimurium + - Positive
BW - Mean body weight – - Negative
GW - Mean gut weight
FAR - Fluid accumulation ratio= GW/BW-GW
G/B - Gut weight/body weight
If G/B >0.065, it is significant for enterotoxigenicity

Table 4: Enterotoxigenicity study of *Salmonella enterica* serovar Typhimurium after 24h

Isolate code	BW (g)	GW (g)	FAR	G/B	Result
ST1	5.100	0.310	0.065	0.061	–
ST2	5.230	0.350	0.072	0.067	+
ST3	5.300	0.360	0.073	0.068	+
ST4	5.190	0.330	0.068	0.064	–
ST5	5.500	0.390	0.076	0.071	+
ST6	5.320	0.370	0.075	0.070	+
Control	4.900	0.280	0.061	0.057	–

ST – *Salmonella enterica* serovar Typhimurium
BW - Mean body weight
GW - Mean gut weight
FAR - Fluid accumulation ration= GW/BW-GW
G/B - Gut weight/body weight
– - Negative
+ - Positive
If G/B >0.065, it is significant for enterotoxigenicity

DISCUSSION

The presence of *Salmonella enterica* serovar Typhimurium in the food samples could be traced from cross contamination, unhygienic and improper handling and preparation of the food samples. The significant enterotoxin production by *Salmonella enterica* serovar Tyhimurium ST5 after 4h exposure to the suckling mice corroborated with the findings of many researchers (Murray, 2006; Balaban and Rasooyl, 2009; Asiton *et al*., 2012). This shows that the organism has the potential of producing heat stable enterotoxin (Arora and Arora, 2008). The heat stable enterotoxin is not destroyed by heating at 100^0C for 30 minutes, and it activates guanylate cyclase causing the increased production of cyclic guanosine monophosphate (CGMP) and subsequent hypersecretion (Arora and Arora, 2008). Also for isolates ST5 to produce heat stable enterotoxin intragastrically in a suckling mice, signifies that the heat stable enterotoxin is truly type 1 (ST-1) (Arora and Arora, 2008).

The significant production of enterotoxin by isolates ST2, ST3, ST5 and ST6 after 24 h intragastric exposure to albino suckling mice, agrees with the finding of Balaban and Rasooyl (2009). The ability of these isolates to produce toxins after 24 h could be attributed to the multiplication of the isolates in the intestinal loop of the mice and production of more colonization factors and toxins that might alter the host-bacterium interaction (Murray, 2006). The inability of isolate ST1 and ST4 to produce toxins after 4h and 24h exposure could be attributed to the difference in the stability of the toxin in solution (Asiton *et al*., 2012). Also, it could be that mechanisms other than production of enterotoxin are responsible for their pathogenic activity, although the isolate are from food samples which were contaminated either by environmental factors (Asiton *et al*., 2012).

CONCLUSION

This study has revealed the presence of *Salmonella enterica* serovar Typhimurium in the food samples, of which *Salmonella* serovar Typhimurium was recorded in 51.85% of the studied samples. Most of the studied isolates exhibited significant enterotoxigenicity potentials among the infected suckling albino mice of which ST5 proved to be most enterotoxigenic after 4 h and 24 h.

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