

## **Research Article**

# **The Prevalence of *Salmonella typhi* Carriers in Calabar Municipality of Cross River State, Nigeria.**

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**Abstract:** This study was conducted to determine the prevalence of *Salmonella typhi* in Calabar Municipality. One thousand five hundred (1500) stool specimens were collected from food handlers, i.e. food-hawkers, canteen workers, restaurant workers and people working in drinking parlours. Eight hundred and twenty (54.7%) males and 680 (45.3%) females, aged between 5 and 55 years, participated in the study. Collection and processing of specimens lasted from February to August, 2010. *Salmonella typhi* was isolated from 40 stool specimens. The result represented a prevalence rate of 2.7%; with 10 positive cases (1.5%) among females and 30 positive cases (3.7%) among males. Antimicrobial sensitivity pattern showed that all 40 isolates were susceptible to Chloramphenicol, Cotrimoxazole, Amoxycillin and Erythromycin. Although this study shows a low prevalence rate for *S. typhi* carrier status in Calabar Municipality, there is need for public awareness of the routes of transmission of the bacilli and steps taken to maintain a high standard of hygiene.

**Keywords:** *Salmonella typhi*, Calabar Municipality, Food-hawkers, Canteen workers, Chloramphenicol.

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## **INTRODUCTION**

Salmonellosis is a term which refers to an infection by bacteria of the Genus *Salmonella*. The infection may result in a typhoid, paratyphoid or non-typhoid diarrhoeal disease, depending on the species of organism, site and severity of infection. Most people infected with *Salmonella* develop diarrhoea, fever, vomiting and abdominal cramps 12 to 72 hours after infection [1].

Typhoid fever is caused primarily by *Salmonella typhi*, which is a major cause of morbidity and mortality in developing countries. It is transmitted by the ingestion of food or water contaminated with the faeces of an infected person, which contains the bacterium *Salmonella enterica enterica*, serovar *typhi* [2].

Non-typhoid diarrhoeal diseases are caused by various serotypes of *Salmonella*, commonly *S. enteritidis* and *S. typhimurium*. Paratyphoid fever is caused by any of the three strains of *S. paratyphi*, viz.: *S. paratyphi* A, B, or C. Symptoms of paratyphoid fever resemble those of typhoid fever but are milder and have a shorter duration. Transmission occurs when the bacterium is passed from one person to another due to poor personal hygiene after using the toilet [3].

About 2% of persons with typhoid fever will become chronic carriers[4]. Carriers constitute a vehicle for the longevity and transmission of the *Salmonella* organism as they periodically excrete the pathogen via bile into their faeces. In this way, carriers pose a serious health hazard in restaurants, abattoirs, refectories and other food processing facilities. With the ever-increasing population in metropolitan cities, deterioration of social infrastructure and general decline in health standards, there is bound to be an upsurge in the prevalence of *Salmonella* and other food-borne pathogens.

## **MATERIALS AND METHODS**

A total of 1500 stool specimens were collected from people working in food-vending facilities in Calabar Municipality, the capital city of Cross River State, Nigeria. The workers were enlisted in the study after obtaining their personal consent and that of their Management. The stool samples were collected from food hawkers and those working in canteens, restaurants and drinking parlours.

### **Collection of Stool Specimens**

Fresh stool specimens were collected into clean, dry, leak-proof, chemical-free and screw-cap

universal containers. Only one stool specimen was collected from each subject. The samples were transported in dry cartons to the Parasitology Laboratory of the University of Calabar Teaching Hospital, where they were processed with minimum delay.

### Stool Culture

Approximately 1 gramme of well-mixed faeces was inoculated into 10 millilitres of Selenite F broth and incubated at 37°C overnight. After an overnight incubation, broth cultures were sub-cultured on to desoxycholate citrate agar (DCA) and incubated overnight at 37°C.

Non-lactose fermenting colonies on DCA were sub-cultured on to nutrient agar overnight at 37°C for purity. Pure cultures were incubated into Kligler's Iron Agar (KIA) and urea agar slopes.

### Tests for Identification of Isolates

All isolates were identified based on their motility, morphology, biochemical and serological reactions.

### Motility Test

Pure cultures from nutrient agar plates were sub-cultured in peptone water and incubated at 37°C for 6 hours. A ring of plasticine was made on a clean, grease-free slide. A drop of the well-mixed culture was placed on a clean cover-slip. The circular area of plasticine on the slide was inverted and super-imposed over the drop of culture on the cover-slip. Quickly, the arrangement was inverted, with the cover-slip facing upwards. Thus, the culture hanged down in the centre of the ring of plasticine. It was necessary to undergo this tedium in order to demonstrate bacterial motility to students from first principle.

The preparation was examined with x10 objective lens with reduced illumination. True motility was indicated by the tumbling movement of bacteria from one spot to another.

### Urease Test

This test was carried out to rule out *Proteus species* which hydrolyze urea to yield ammonia and carbon-dioxide. The presence of ammonia renders the medium alkaline and the indicator (Phenol red) changes colour to a reddish-pink.

The urea broth (OXOID) in test tubes was inoculated by shaking few colonies from pure cultures of the test organism on nutrient agar into the urea broth. All tubes were incubated at 37°C for 6 hours.

Controls: Positive: *Proteus vulgaris*

Negative: *Escherichia coli*

### Serological Typing

A thick saline suspension of an overnight agar culture of the isolate was made on one end of a clean,

grease-free slide. Using a flame-sterilized wire-loop, a drop of the bacterial suspension was mixed with a drop of O and H antisera.

A negative control was run by mixing a drop of the bacterial suspension with a drop of sterile normal saline at the other end of the same slide. The preparations were tilted on the slide several times and the result was read within 60 seconds.

A positive result was indicated by agglutination. Isolates were confirmed as *Salmonella typhi* by their agglutination with specific antisera, in conjunction with biochemical reactions on Kligler's Iron Agar (KIA).

### Test on KIA

A pure culture of the test organism on nutrient agar was inoculated on to KIA slope by using a straight wire to stab the butt and streak the slope. The tubes were plugged with sterile cotton wool and incubated at 37°C overnight.

### Specific Identification of Bacterial Isolates [5]

Bacterial cultures were identified as *Salmonella typhi* if they showed the following biochemical characteristics:

Lactose	-----	Negative
Mannitol	-----	Positive
Glucose	-----	Positive
Oxidase	-----	Negative
Motility	-----	Positive
Urease	-----	Negative
Growth on KIA --Slope:		(Red-pink-alkaline)
	--- Butt:	(Yellow- acid reaction)
	---- Gas production	(nil)
	--Hydrogen Sulphide production	(weak)

### Antibiotic Sensitivity Testing

In vitro antibiotic sensitivity testing of each isolate was determined using the disc diffusion technique by placing commercially prepared discs of Chloramphenicol, Co-trimoxazole, Amoxicillin, Erythromycin and Tetracycline on sensitivity test agar plates carrying pure cultures of the identified isolates.

### Preparation of inoculum

Pure cultures of *Salmonella typhi* were picked with flame-sterilized wire loop and inoculated into 10 millilitres of peptone water in McCartney bottles. The peptone water cultures were incubated at 37°C for 4 hours. The turbidity of the growth was compared with that of a barium sulphate standard corresponding to that of a previously prepared inoculum which yielded a dense but non-confluent growth on a sensitivity agar plate. Each inoculum was diluted to match the standard and used in flooding a sensitivity test plate.

### Method of antibiotic sensitivity test

The disc diffusion technique was used to determine the antibiotic sensitivity of each isolate. Commercial

discs of Chloramphenicol, Cotrimoxazole, Amoxicillin, Erythromycin and Tetracycline were placed on the surface of the sensitivity agar plates previously flooded with a broth culture of the test organism. All the plates were incubated overnight at 37°C.

A strain of *Escherichia coli* isolated in the laboratory was used as the control. Both test and control plates were incubated in the same atmosphere and temperature.

### Interpretation of Zones Of Inhibition

An isolate was regarded as sensitive to a certain drug if the diameter of the zone of inhibition around the disc was greater than or equal to that of the control organism (18mm) and resistant if the zone diameters were less than 18mm[6].

### RESULTS

A total of 1,500 subjects were included in the study. Eight hundred and twenty (54.7%) of the subjects were males while 680 (45.3%) were females. The average age of the subjects was 24.2 years and age range was from 15 to 55 years.

*Salmonella typhi* was isolated from 40 stool specimens, thus giving an overall prevalence rate of 2.7% among screened population. Out of the 40 positive cases, 10 (25%) came from food hawkers; 20 (50%) were obtained from canteen workers and 10 (25%) came from restaurant workers. Also, 10 positive cases were obtained from female while the remaining 30 cases were obtained from male subjects. These results showed a prevalence rate of 1.5% and 3.7% among female and male subjects, respectively.

Table 1 shows the percentage prevalence of *Salmonella typhi* carriers with respect to age-groups. Out of the 40 subjects who excreted *S. typhi*, 30 belonged to the (25-34) age-group while 10 belonged to the (35-44) age-group. These results showed a 7.7% prevalence rate within the age-group (25-34) and a 5.9% prevalence rate within the age-group (35-44).

**Table 1: The Prevalence of *S. typhi* carrier according to Age-groups**

Age group	No. of cases	No. (%) positive
25-34	390	30 (7.7)
35-44	170	10 (5.9)
Total	560	40 (7.1)

All the 40 isolates of *S. typhi* were tested for antimicrobial sensitivity against commercially prepared discs of Chloramphenicol, Cotrimoxazole, Amoxicillin, Erythromycin and Tetracycline. All the isolates were sensitive to Chloramphenicol, Cotrimoxazole, Amoxicillin and Erythromycin but resistant to Tetracycline.

### DISCUSSION

In this study, only 40 out of 1,500 stool samples yielded positive cultures of *S. typhi*. This shows a prevalence rate of 2.7%. This apparent low prevalence rate may be due to the fact that carriers of typhoid bacilli usually shed the organism intermittently in faeces and only one stool sample was collected from each subject. But in recent times, both the government and residents of Calabar metropolis have taken the issue of environmental sanitation very seriously. This practice has led to an increase in personal hygiene and reduction in indiscriminate dumping of faeces in the environments.

Female subjects had a lower prevalence of 10 (1.5%) compared to their male counterparts who recorded 30 (3.7%) out of the 40 positive cases. These results are consistent with the feeding habits of male and female subjects. Whereas females are more domestic and tend to eat mostly at home, the males are "pot-less" and more catholic in their feeding pattern; eating mostly away from their homes. This makes the latter more exposed to infection by water and food materials which might have been faecally contaminated. However, there were more males than females (820 v 680) in this study. There was no statistically significant difference in the prevalence rates between male and female subjects ( $\chi^2=1.98$ ;  $p>0.05$ ).

Out of the 40 positive carriers of *S. typhi*, 30 belonged to the (25-34) age-group while 10 belonged to the (35-44) age-group. These results showed a 7.7% prevalence rate within the age-group (25-34) and a 5.9% prevalence rate within age-group (35-44). These findings were very close to the results obtained by Ames and Robins who established that typhoid patients older than 30 years of age tend to progress to the carrier state more frequently than younger patients[7]. In this present study, subjects who shed *S. typhi* were considered as carriers because they shed the organism in their faeces without complaining of gastro-intestinal or systemic disorders usually associated with the acute disease.

All the 40 isolates were sensitive to Chloramphenicol, Cotrimoxazole, Amoxicillin and Erythromycin. This susceptibility pattern suggested that these drugs were not abused among the local population; at least in the treatment of typhoid and paratyphoid fevers.

### CONCLUSION

This study was carried out to determine the extent to which food handlers in Calabar Metropolis could face health risks to the community through the excretion of typhoid bacilli. This study has shown a low prevalence of salmonella typhoid carrier status in Calabar among food-handlers. Therefore, there is a very low risk of food and water contamination with faecal materials. However, this should not give room for complacency because various factors could account for non-isolation of typhoid bacilli from faeces. These

include intermittent shedding of the bacilli via faeces, single sample collection (as in this study), etc.

In order to control the incidence of *Salmonella*-associated infections, the following strategies should be employed:

- Health authorities should organize periodic seminars and symposia to enlighten the general public and food handlers, in particular, on proper hygiene concerning food-handling and processing.
- Refuse which accumulates after environmental sanitation exercise should be evacuated promptly.
- All suspected cases of typhoid fevers should be accurately diagnosed. Confirmed cases should be properly treated to curtail the incidence of carrier status.

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