



# Antibiotic Resistance of Typhoidal *Salmonella* Isolated from Humans in Kaduna and its Associated Factors

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## Research Article

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## Abstract

**Background:** Pathogenic *Salmonella* species cause various types of infections ranging from mild gastroenteritis to life-threatening typhoid fever caused by *Salmonella typhi*, or paratyphoid fever caused by *S. paratyphi* A, B or C; of which *S. paratyphi* A is prevalent. Currently, treatment of typhoid infection is being hampered by the emergence of multidrug-resistant strains, while vaccines provide only temporal or partial protection.

**Objective:** The aim of the study was to determine antibiotic resistance of typhoid causing *Salmonella* isolated from human stool.

**Methodology:** A cross-sectional descriptive study was carried out in the capital city of Kaduna State, Nigeria. Questionnaires were administered to 300 consenting individuals; comprising 250 patients and 50 volunteering healthy individuals, to obtain relevant socio demographic and clinical information. Stool specimens were equally collected in sampling containers for isolation of typhoidal *Salmonella* on *Salmonella Shigella* Agar. Suspected typhoidal *Salmonella* colonies were confirmed by biochemical tests as *S. typhi* or *S. paratyphi* A, before antimicrobial susceptibility test was done using Kirby-Bauer disk diffusion method. Diameters of zone of inhibitions were recorded and interpreted according to Clinical Laboratory Standards Institute guidelines. The data and results obtained were represented on tables and charts. Chi-square analysis was also used to determine statistical association among parameters and p-values  $\leq 0.050$  were considered significant.

**Results:** Thirty-two (32) typhoidal *Salmonella* were isolated; 25 were *S. typhi* and seven *S. paratyphi* A. Thirty isolates came from patients while two were isolated among healthy individuals. High antibiotic resistance was observed with Augmentin (100%), Ceftazidime (87.5%), Cefuroxime (84.4%), Cefixime (84.4%) and Gentamicin (40.6%), while Ciprofloxacin (12.5%), Nitrofurantoin (12.5%) and Ofloxacin (18.8%) showed a lower resistance. Age, marital status and state of origin were socio demographic factors found to be associated with the antibiotic resistance. Also, frequency of infection, presenting symptoms and sampling duration were statistically associated with the antibiotic resistance.

**Conclusion:** Multi-drug resistance, high antibiotic resistance of typhoidal *Salmonella* and factors associated with it in this study, should awaken public health concerns in curbing the excesses of typhoidal *Salmonella*.

**Keywords:** Antibiotic Resistance; Typhoidal *Salmonella*; Isolates; Typhoid Infection

**Abbreviations:** KIA: Kligler Iron Agar; MRVP: Methyl Red-Voges Proskauer; HMA: Mueller-Hinton Agar; NIT: Nitrofurantoin; GEN: Gentamicin; CXM: Cefixime; CPR: Ciprofloxacin; OFL: Ofloxacin; CAZ: Ceftazidime; AUG: Augmentin; CRX: Cefuroxime; SSA: *Salmonella Shigella* Agar; SPSS: Statistical Package for the Social Sciences; CLSI: Clinical Laboratory Standards Institute.

## Introduction

In recent times, research on *Salmonella* has attracted the attention of clinical microbiologists, epidemiologists, geneticists and even molecular biologists [1]; more particularly because *Salmonella typhi* is host restricted to humans [2] and has the ability to bypass host immune defences [3]. Host restrictions to humans speculate that it might be transmitted by sex occasionally [4]. *Salmonella enterica* serovar Typhi otherwise known as *Salmonella typhi*, cause typhoid fever, while a similar paratyphoid fever is caused by *S. enterica* serotype Paratyphi A, B or C [5], of which *Salmonella paratyphi* A is most prevalent [6]. Although some *Salmonella* infections resolve without treatment, resistant infections like typhoid fever may not respond to first-line drugs, thus are commonly associated with longer illness and more severe outcomes [7]. *Salmonella* are Gram-negative bacteria which are particularly resistant to antibiotics because they possess two membranes that protect them from harmful substances [8]. Typhoid infections are becoming increasingly difficult to treat with antibiotics, forcing researchers to look for alternatives to control these pathogens [8]. Multiple drug resistance transmitted genetically by plasmids among enteric bacteria is also a key problem in treating typhoid infections [9].

Despite availability of more recent data on typhoid fevers, it remains a significant health burden [10]. Therefore, understanding antibiotic resistance in individuals, especially in areas where high antibiotic resistance strains are likely to hamper treatment [10], will aid an optimal and timely antimicrobial treatment of patients, which is also important for reducing the high infection and mortality rate associated with typhoid fevers in developing countries like Nigeria [11]. The present work determined the antibiotic resistance of typhoidal *Salmonella* isolated from stool of patients and healthy individuals in Kaduna.

## Methods

### Study Area and Design

This was a cross-sectional descriptive study carried out in the capital city of Kaduna State, Nigeria (Figure 1), between July 2018 and February 2019. Ethical approval/clearance was obtained from the Ministry of Health and

Human resources and Barau Dikko Teaching Hospital Health Research and Ethical Committee in Kaduna State. Consent was duly sought and obtained from participants (>15 years) enrolled into the study or from guardians (for participants ≤ 15 years).

### Study Population

The study population comprised of 50 healthy volunteering individuals and 250 patients who visited any of four selected general hospitals in the metropolis; General Hospital Sabon Tasha, Gwamna Awan General Hospital Kakuri, Barau Dikko Teaching Hospital Ungwan-Rimi and Yusuf Dantosh Memorial Hospital Tudun Wada, with suspected cases of typhoid/paratyphoid fever by clinical diagnosis, within the study duration.

### Sample and Data Collection

Stool specimens were obtained from participants in stool sampling bottles and immediately transported to the microbiology laboratory for isolation of *Salmonella typhi* or *paratyphi* A on *Salmonella Shigella* Agar (SSA), using standard bacteriological procedures. Well-structured questionnaires were also used to collect relevant socio-demographic data and clinical information.



Figure 1: Map of Nigeria Showing the Study Area [12].

### Isolation of Typhoidal *Salmonella*

A pinch of each stool sample was enriched in Selenite F broth (HIMEDIA Laboratories Ltd., India) and incubated at 37°C for 6-12 hours, and using aseptic laboratory procedures, a loop full of the enriched broth was cultured by streaking onto prepared plates of SSA (Lab M Limited, Heywood Lancashire, United Kingdom). The plates were incubated aerobically at 37°C for 18-24 hours, after which they were observed for characteristic culture/colony formations of

typhoidal *Salmonella*; smooth, circular, convex, colourless, transparent, flat colonies with a clear or black centre [13,14].

### Biochemical Confirmation of Typhoidal *Salmonella*

Suspected colonies of typhoidal *Salmonella* on SSA were confirmed with biochemical tests on appropriate media and performed with standard operational procedures. The biochemical tests included; Kligler Iron Agar (KIA), urease (Titan Biotech Ltd., Rajasthan, India), motility, indole, Methyl Red – Voges Proskauer (MRVP), citrate utilization, lysine decarboxylase (HiMedia Laboratories Pvt. Ltd., India), Gram staining, catalase and oxidase tests.

### Antibiotic Susceptibility Test

Confirmed *Salmonella* isolates were further subjected to antibiotic susceptibility test - Kirby-Bauer disk diffusion method, using a Gram negative disk (Abtek Biologicals Limited, Liverpool, UK; sensitivity rings NZ53N), which contained the following antibiotics; Ceftazidime (CAZ) (30µg), Cefuroxime (CRX) (30µg), Gentamicin (GEN) (10µg), Cefixime (CFM) (5µg), Ofloxacin (OFL) (5µg), Augmentin (AUG) (30µg),

Nitrofurantoin (NIT) (300µg) and Ciprofloxacin (CPR) (5µg). The disk was placed firmly on Mueller-Hinton Agar (HMA) plates (TM MEDIA, Titan Biotech Limited, India), streaked with identified colonies of *Salmonella typhi* or *paratyphi A*. The plates were incubated at 37°C for 24 hours, after which diameters of zone of inhibitions were measured to the nearest millimeter with a transparent ruler and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI) [15].

### Analysis of Data

Data generated both from laboratory experiments and questionnaires were presented as frequency tables and charts. Chi-square analysis was done on IBM SPSS version 20 and p-values  $\leq 0.050$  at 95% confidence level was considered significant for parameters examined.

### Results

Thirty-two (32) typhoidal *Salmonella* isolates were recorded; twenty-five (25) were *S. typhi* isolates and seven (7) were *S. paratyphi A*. Thirty (30) isolates came from patients while two (2) were isolated among healthy volunteering individuals as shown on Table 1.

Category of Participants	HV	BD	GA	GS	YM	Total
Number of participants	50	50	93	57	50	300
No Typhoidal <i>Salmonella</i> Isolated	48	44	80	51	45	268
<i>S. typhi</i> Isolated	1	4	11	5	4	25
<i>S. paratyphi A</i> Isolated	1	2	2	1	1	7
Total Number of Isolates	2	6	13	6	5	32

**Table 1:** Distribution of Isolated Typhoidal *Salmonella* According to Categories of Participants.

Healthy Volunteers (HV), Barau Dikko Teaching Hospital (BD), Gwamna Awan General Hospital (GA), General Hospital Sabon Tasha (GS) and Yusuf Dantosh Memorial Hospital (YM).

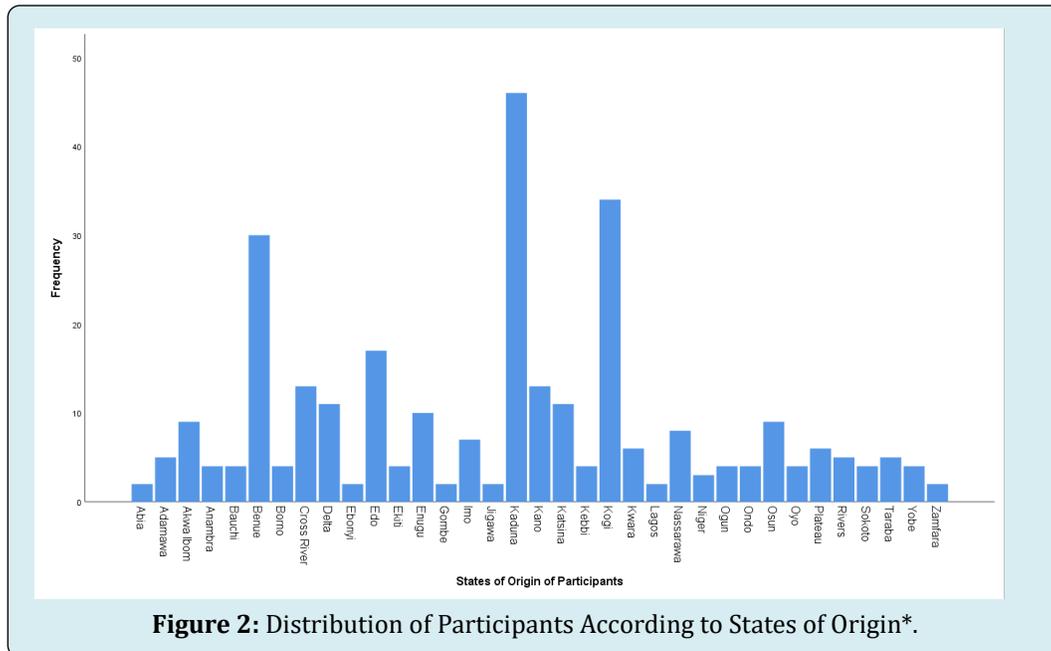
Ages of participants ranged from a two-week old baby to a 72-year-old man. A frequency distribution of isolates by age grouping is shown on Table 2. Age group 20-29 recorded most of the isolates (10/32), closely followed by age group  $\leq 9$  years (8/32).

Age*Group (Years)	Total Number of Participants	Nil Typhoidal <i>Salmonella</i>	<i>S. paratyphi A</i> Isolated	<i>S. typhi</i> Isolated	Total Number of Isolates
$\leq 9$	44	36	2	6	8
10-19	46	41	1	4	5
20-29	97	87	2	8	10
30-39	66	62	0	4	4
40-49	35	33	1	1	2
50-59	9	6	1	2	3
$\geq 60$	3	3	0	0	0
<b>Total</b>	<b>300</b>	<b>268</b>	<b>7</b>	<b>25</b>	<b>32</b>

**Table 2:** Distribution of Typhoidal *Salmonella* Isolates According to Age\*Group.

Participants in this study represented 35 of the 36 states in Nigeria. Majority of participants (46/300) hailed from Kaduna State probably because the research was carried out in the capital city, followed by Kogi (34/300) and Benue (30/300) States (Figure 2). Similarly, the highest number of isolates was recorded by Kaduna (5/32) and Benue (5/32) followed by Kogi (4/32). Sixty-two (62) spoken languages

or tribes were represented, with Hausa (36/300), Yoruba (33/300) and Ibo (25/300) topping the list and yielding 3, 5 and 2 isolates respectively. Other tribes of significance were Fulani (14/300), Idoma (14/300), Igala (13/300), Tiv (12/300), Bajju (10/300) and Kabba (10/300) yielding 1, 3, 1, 1, 2 and 2 isolate/s respectively. The other 53 tribes (133/300) resulted in the remaining 12 isolates.



**Figure 2:** Distribution of Participants According to States of Origin\*.

Table 3 shows the socio demographic information of the participants and distribution of typhoidal *Salmonella*. Females (17/32), singles (22/32) and unemployed

participants (20/32) recorded more typhoidal *Salmonella* isolates.

Socio Demographic Information		Total Number of Participants	Nil Typhoidal <i>Salmonella</i>	<i>S. paratyphi</i> A Isolated	<i>S. typhi</i> Isolated	Total Number of Isolates
Gender	Male	163	148	3	12	15
	Female	137	120	4	13	17
Marital Status*	Single	194	172	5	17	22
	Married	98	90	1	7	8
	Divorced	3	2	1	0	1
	Widowed	5	4	0	1	1
Employment Status	Unemployed	162	142	4	16	20
	Self Employed	69	62	2	5	7
	Employed	69	64	1	4	5
	<b>Total</b>	<b>300</b>	<b>268</b>	<b>7</b>	<b>25</b>	<b>32</b>

**Table 3:** Typhoidal *Salmonella* Isolated According to Socio Demographic Data.

Table 4 shows the frequency distribution of typhoidal *Salmonella* isolates according to the clinical information of

the participants.

Other Relevant Information	Categories	Total Number of Participants	Nil Typhoidal <i>Salmonella</i>	<i>S. paratyphi</i> A Isolated	<i>S. typhi</i> Isolated	Total Number of Isolates
Frequency of Infection*	Monthly	1	-	-	1	1
	Quarterly	36	27	2	7	9
	Twice a year	89	80	3	6	9
	Once a year	129	120	2	7	9
	Once in many years	1	1	-	-	-
	First time	4	-	-	4	4
	Never	40	40	-	-	-
Type of treatment	Hospital based treatment	173	153	2	18	20
	Self-Medication	25	22	1	2	3
	Chemist Prescription	32	28	3	1	4
	Herbal treatment	27	25	1	1	2
	Not applicable	43	40	-	3	3
	<b>Total</b>	<b>300</b>	<b>268</b>	<b>7</b>	<b>25</b>	<b>32</b>

**Table 4:** Typhoidal *Salmonella* Isolated According to Clinical Information Obtained from Participants.

The CLSI 2017 guidelines was the standard for interpreting diameters of zone of inhibitions of antibiotics

tested and the result is summarized on Table 5.

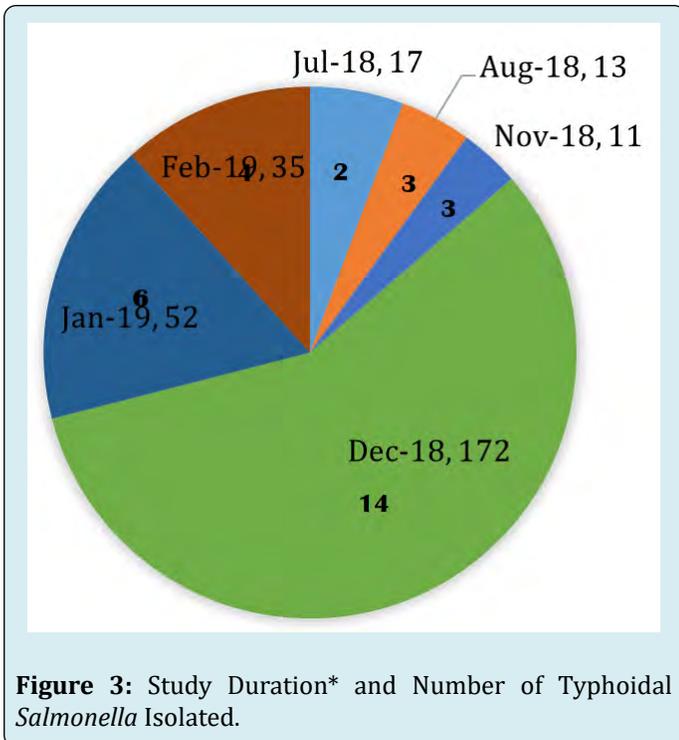
Antibiotics	CPR			NIT			OFL			GEN			CXM			CRX			CAZ			AUG		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
<i>S. paratyphi</i> A	5	2	-	3	2	2	6	-	1	4	1	2	-	1	6	-	1	6	-	1	6	-	-	7
<i>S. typhi</i>	16	5	4	19	4	2	18	2	5	14	-	11	1	3	21	-	4	21	-	3	22	-	-	25
<b>Total</b>	<b>21</b>	<b>7</b>	<b>4</b>	<b>22</b>	<b>6</b>	<b>4</b>	<b>24</b>	<b>2</b>	<b>6</b>	<b>18</b>	<b>1</b>	<b>13</b>	<b>1</b>	<b>4</b>	<b>27</b>	<b>-</b>	<b>5</b>	<b>27</b>	<b>-</b>	<b>4</b>	<b>28</b>	<b>-</b>	<b>-</b>	<b>32</b>
<b>Percentage Resistance</b>	12.5			12.5			18.8			40.6			84.4			84.4			87.5			100		

**Table 5:** Summary of Antibiotic Susceptibility Test of Typhoidal *Salmonella* Isolated.

Ciprofloxacin (CPR) Nitrofurantoin (NIT) Ofloxacin (OFL) Gentamicin (GEN) Cefixime (CXM) Cefuroxime (CRX) Ceftazidime (CAZ) Augmentin (AUG) Susceptible to antibiotic (S) Intermediate level susceptibility to antibiotic (I) Resistant to antibiotic (R). All 32 isolates were resistant to Augmentin, a combination of amoxicillin and clavulanic acid (100% resistance). Twenty-eight (28) isolates (22 *S. typhi* and six *S. paratyphi* A) were resistant to Ceftazidime (87.5% resistance), 27 isolates (21 *S. typhi* and six *S. paratyphi* A) were resistant to both Cefuroxime and Cefixime (84.4% resistance), 13 isolates (11 *S. typhi* and two *S. paratyphi* A) were resistant to Gentamicin (40.6% resistance), six (6) isolates (five *S. typhi* and one *S. paratyphi* A) were resistant to Ofloxacin (18.8% resistance) and four (4) isolates were resistant to both Nitrofurantoin and Ciprofloxacin (12.5% resistance). Interestingly, the four isolates resistant to

Ciprofloxacin were *S. typhi* isolates only, as compared to Nitrofurantoin having two *S. typhi* and two *S. paratyphi* A isolates resistant to it (Table 5).

A summary of the order of presenting signs and symptoms documented by participants were; Headache (137/300) > Weakness of the body (110/300) > High temperature (105/300) > Vomiting (77/300) > Abdominal pain (73/300) > Fever (71/300) > Body pain (55/300) > Cold (51/300) > Dizziness (11/300) > Joint pain (7/300) > Loss of appetite (5/300) > Cough (4/300) > Pus on the mouth (3/300) > Rashes (2/300) > Drowsiness (1/300), Pains (1/300), Chest pain (1/300) and Diarrhoea (1/300). The number of typhoidal *Salmonella* isolated during the study duration is shown on Figure 3.



## Discussion

Antimicrobial resistance of typhoidal *Salmonella* isolates to first-generation quinolones and antimicrobial agents like nalidixic acid, amoxicillin, ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole; and even third-generation cephalosporins like ceftriazone, have been reported by previous studies [9,16,17], therefore, a set of gram negative antibiotics expected to be effective against typhoidal *Salmonella* isolates was investigated in this study. A strong relationship existed between antibiotic resistance and the typhoidal *Salmonella* isolates in this study ( $p = 0.000$ ). High antibiotic resistance was observed especially with Augmentin showing 100% resistance, Cefotaxime showing 87.5% resistance, Cefuroxime and Cefixime showing 84.4% resistance and Gentamicin showing 40.6% resistance. Ofloxacin showing 18.8% resistance, Nitrofurantoin and Ciprofloxacin both showing 12.5% resistance, did not show very high resistance. Nevertheless, multi-drug resistance was established in this study. Ciprofloxacin was found to be the most effective antibiotic tested, as it was effective against all isolates of *S. paratyphi* A while four *S. typhi* isolates showed resistance. This was followed by Nitrofurantoin which had two *S. paratyphi* A and two *S. typhi* isolates resistant to it (Table 5). Augmentin was the least effective antibiotic tested as all 32 isolates were resistant to it.

It was observed in this study that individuals may show high antibiotic resistance to typhoidal *Salmonella* infections as a result of high exposure to the infection and

the continual use of antibiotics, as a total of 27 isolates were recovered from participants who claimed to be infected either quarterly (9), twice a year (9) or at least once every year (9) (Table 4). This was revealed in an association between antibiotic resistance and frequency of infection ( $p = 0.001$ ) in this study. Similarly, the presenting symptoms recorded in this study had a strong relationship with antibiotic resistance ( $p = 0.001$ ) and the summarized order of presenting signs and symptoms in this study shared similarities with the reports of previous studies [16,20]. Clinical manifestations of typhoid infections are similar to common febrile ailments like malaria and dengue fever [17], therefore, careful diagnostic measures by health care providers before administering antibiotic treatments should be priority, to curb the multi-drug/high antibiotic resistance of typhoidal *Salmonella*.

One hundred and seventy-three participants agreed to a hospital based treatment in the event of a typhoid infection and recorded 20 of the 32 isolates. Other isolates were found among those who agreed to chemist prescription (4/32), self-medication (3/32), and herbal treatment (2/32) (Table 4). This finding most likely suggest the high antibiotic resistance observed in the present study; which could be results of over prescription of antibiotics by health personnel, or instituted treatment without thorough confirmatory diagnosis. Hence, there is a need for proper diagnosis and management of typhoid infections. This should include providing proper health care services, properly trained laboratory and health care personnel to diagnose and manage typhoid infections respectively, provision of appropriate diagnostic laboratories and materials, and public health enlightenment on the dangers of not undergoing optimal and timely treatment of typhoid infections, as also suggested by previous studies [18,19]. Diagnosis should be done preferably by culturing causative *Salmonella* at least from stool, other than just the popular Widal test; and antibiotic susceptibility test should also be done before prescribing antibiotic treatment [20]. This will limit high antibiotic resistance and multi-drug resistance of typhoidal *Salmonella* [20].

There was a statistical association between antibiotic resistance and age of participants ( $p = 0.034$ ) as well as marital status ( $p = 0.001$ ), suggesting that these sociodemographic factors contributed to the antibiotic resistance of typhoidal *Salmonella* observed in this study. In like manner, the close association of antibiotic resistance and states of origin ( $p = 0.001$ ), also suggest that, there might be environmental or genetic factors common to individuals of a locality, tribe or area, that influences antibiotic resistance of typhoidal *Salmonella*. The study duration was also seen to be associated with antibiotic resistance ( $p = 0.002$ ), showing that times or seasons of the year could influence antibiotic resistance of typhoidal *Salmonella*.

## Conclusion

The strong relationship observed with typhoidal *Salmonella* isolates and antibiotic resistance, as well as high antibiotic resistance observed with antibiotics including Augmentin, Cefotaxime, Cefuroxime, Cefixime and Gentamicin, suggest a need for intensified efforts in the control and prevention of antibiotic resistant typhoid infection among the populace. Therefore, beyond looking at better ways to treat typhoidal *Salmonella* infections by overcoming their virulent characteristics with antimicrobials, public health measures such as provision of adequate healthcare and promotion of sanitary living conditions of persons residing in underdeveloped or poorly developed areas is relevant. This will reduce frequency of exposure to typhoid infection and curb the spread of high or multi-drug resistant typhoidal *Salmonella*.

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