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Cross-Sectional Study of Major Strains of *Salmonella enterica* Subspecies Enterica Serovar Typhi among Borehole Used in Uli Community

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Abstract

Salmonella enterica subspecies *enterica* serovar Typhi; a dominant species found in borehole water, has been receiving drastic attention not only in causing human infections but also for its involvement in antibiotic resistance, of which 80% of these resistant genes are encoded in the plasmid. This study was undertaken to evaluate the cross-sectional study of enteric *Salmonella* species in borehole water used in Uli community. Samples were randomly collected from different boreholes from different locations in Uli community using standard microbiological techniques. The prevalence of the different strains encountered in the samples were also determined. The study revealed the presence of *Salmonella enterica* subspecies *enterica* serovar Typhi strain CMCST (STCM), *Salmonella enterica* subspecies *enterica* serovar Typhi strain WG51146 (STWG) and *Salmonella enterica* subspecies *enterica* serovar Typhi strain R192829 (STR1)of which 58.00% were positive to *Salmonella* ser. Typhi, and the occurrences of STCM, STR1 and STWG were 31.03 %, 24.14 % and 44.83 % respectively. From the above study, different strains of *Salmonella enterica* ser. Typhi were isolated from borehole water used at Uli community, of which isolate STWG was most predominant strain in the study samples.

Keywords: *Salmonella enterica serovar Typhi, Borehole water contamination, Antibiotic resistance, Plasmid-encoded resistance genes, Uli community, Prevalence of Salmonella strains*

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1. Introduction

Salmonella is a well-known bacterial pathogen responsible for a significant proportion of foodborne illnesses and waterborne infections worldwide (Centers for Disease Control and Prevention, 2020; World Health Organization, 2018). It belongs to the family Enterobacteriaceae and comprises numerous serotypes, each capable of causing various clinical symptoms in humans (Grimont and Weill, 2007). Salmonella infections can lead to gastroenteritis, characterized by symptoms such as diarrhea, abdominal pain, fever, and nausea (Hohmann, 2001). In severe cases, Salmonella infections can result in hospitalization and, in rare instances, even fatalities (Jones et al., 2008).

Waterborne diseases pose a major public health concern, with contaminated water being a significant source of transmission (WHO, 2019). Water sources contaminated with pathogens, including Salmonella, can have severe implications for communities, particularly those relying on these sources for drinking, cooking, and other household activities (Levin et al., 2002). Inadequate water treatment, improper sanitation practices, and poor hygiene contribute to the spread of waterborne diseases, making it crucial to understand and mitigate the risks associated with pathogen contamination (Prüss-Ustün et al., 2008).

In many communities, boreholes serve as a vital source of water supply, offering a seemingly reliable alternative to surface water sources (Howard et al., 2003). Boreholes are deep wells drilled into the ground to tap into underground water reserves. However, the safety and quality of borehole water cannot be taken for granted, as it can still be susceptible to various forms of contamination, including microbial pathogens like Salmonella (Pedley et al., 2006).

The Uli community relies heavily on boreholes for their daily water needs. While these boreholes have provided a convenient water source, the potential presence of *Salmonella* species remains a significant concern for public health (Hunter et al., 2009). The ingestion of Salmonella-contaminated water poses a considerable risk of infection and subsequent transmission within the community.

Salmonella species are a common cause of foodborne illness. They can have serious health consequences, particularly for vulnerable populations such as young children, the elderly, and those with weakened immune systems (Gupta et al., 2018).
A comprehensive understanding of the prevalence and distribution of Salmonella species among boreholes in the Uli community is essential to assess the potential health risks and implement appropriate interventions.

2. Methodology

Study Area: The study was carried out in Ihiala L.G.A Anambra State. Ihiala is situated at Latitude 5.85ºN and Longitude 6.86ºE, with an elevation of 144 m above the sea level. It is located 48 Km North of Owerri and 40 Km south of Onitsha. It covers an area of 304SqKm and is bounded by Ogbaru (in Ogbaru L.G.A, Anambra State) on the West, Ozubulu (in Ekwusigo L.G.A, Anambra State), Ukpor and Osumenyi (in Nnewi South L.G.A, Anambra State) in the North and in the South by Egbuoma, Ohakpu, Ozara and Oguta in Egbema/Oguta L.G.A of Imo State. Ihiala has tropical climate (rainy and dry seasons) with double maximal rainfall. The rainy season is between April and October, and the dry season is between November and March. The annual rainfall ranges from 1800 mm to 2000 mm. The major anthropological activities are farming/agriculture and trading, of which pig farming is one of the major farming practices. In this study, samples were collected from the major towns in Ihiala L.G.A. which included Amorka, Azia, Lilu, Okija, Mbosi, Isseke, Orsumoghu, Ubuluisiuzor and Uli.

Study Design: Cross-sectional study was conducted to investigate the prevalence of S*almonella enterica* ser. Typhi among boreholes used in Uli community. A cross-sectional study is a type of research design in which data is collected at a single point in time from many different individuals or groups. This design was selected because it allowed us to collect data that can be used to calculate the prevalence of S*almonella* species among boreholes used in Uli community at a specific point in time.

Study Population: The study population for this research includes boreholes in Uli community in the month of December 2022.

Inclusion Criteria
- Boreholes in Uli community
- Boreholes which the owners gave consent

Exclusion Criteria
- Boreholes outside Uli community
- Boreholes which the owner did not give consent

Sample Size: A total of 200 samples were taken for this research, 40 (each in triplicate) samples were taken from boreholes located in each village, for total of five villages in the community

Sample Collection: The samples used for this study were drawn from boreholes. The samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, then rinsed with 70% ethanol and finally rinsed three times with distilled water, after which the containers were placed in a UV chamber and allowed for 60 minutes. The borehole tap was turned on and allowed to flow for 2 minutes after which the sample was collected using the sterile container. This sample was covered immediately and kept in a cooler containing ice block. This procedure was repeated for the collection of the remaining samples. The samples were transported to the laboratory for immediate analysis (Iheukwumere *et al.*, 2020).

Transportation of the Samples: The samples were placed into a cooler containing ice blocks wrapped in a sterile polythene bag and was used for the sample transportation. The temperature of the cooler was checked and adjusted to 28ºC-30ºC by reducing the quantity of the ice inside the cooler in order to reduce or prevent microbial shock. The samples were carefully and aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was then covered and the drain plug was securely taped with packing tape to prevent accidental opening of the cooler. The cooler was then safely carried to the Laboratory for analysis within 2 h of sample collection. The same procedure was repeated for other collection times (Wolking and Daris, 2013).

Isolation of Organisms: One milliliter of the diluted sample (10^{-1} and 10^{-2}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Deoxycholate Agar (DCA/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h.

Characterization and Identification of the Isolates: The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere et al. (2018), biochemical reactions as described in the study published by Iheukwumere et al. (2020) and molecular characterization as described in the study published by Gabriela et al. (2014). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Prevalence and Distribution of The Isolates in the Borehole Samples: The number each bacterial isolate in each sampling area were enumerated, and these were calculated in percentage of the occurrences. The bacterial that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere et al (2021).

Statistical Analysis: The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level as described in the study published by Iheukwumere et al (2018)

3. Results

The occurrence of the isolate in the sample is shown in table one. The study revealed that 34% of the samples were positive for *Salmonella enterica* ser. Typhi. The water samples collected from community D showed the highest occurrences of the test organism whereas, community C recorded the lowest occurrence (Table 1). The test bacterial isolates exhibited similar appearances on Deoxycholate citrate agar (DCA) and Nutrient Agar (NA) plates as they showed colourless and dark centered and greyish white colours respectively (Table 2). They exhibited circular and entire colonies on DCA and NA with smooth surfaces. They varied in colony sizes. The isolates were Gram negative rods, arranged singly/pairs with peritichous flagella The biochemical characteristics of the isolates revealed that the isolates were catalase,hydrogen sulphide production and methyl red positive, and oxidase, citrate, indole, Urease and Vogels prokauer's negative as shown in Table 3. The isolates differ in their variation in utilization of sugars. They were all glucose, maltose, and tetrahalose positive but differ in their abilities to utilize galactose, xylose, Surbitol, inositol and dulcitol.The Nucleic acids extracted from the isolates showed the ratio of their absorbance at wavelength of 260 nm and 280 nm using Nanodrop was at the range of 1.80-1.90, and this confirmed that the nucleic acids were DNA as shown in Table 4. The molecular identities of the isolates revealed that isolate A,B and C were *salmonella enterica* subspecies *enterica serovar typhi* strain CMCST (STCM), *salmonella enterica* subspecies *enterica serovar typhi* strian R192829 (STR1) and *salmonella enterica serovar typhi* strain WGS1146 (STWG) as shown in Table 5. The study also revealed that STWG showed the highest occurrence in the studied borehole samples whereas STR1 recorded the least occurrence as shown in Table 6.

Table 1: Occurrences of the isolate

Community	Number collected from site	P%	N%
A	40	16 (40.00)	24 (60.00)
B	40	14 (35.00)	26 (65.00)
C	40	8 (20.00)	32 (80.00)
D	40	18 (45.00)	22 (55.00)
E	40	12 (30.00)	28 (70.00)
TOTAL	200	68 (34.00)	132 (66.00)

Table 2: Cultural and morphological characteristics of the isolate

Parameter	Isolates A	Isolates B	Isolates C
Appearance	Colorless and dark centered in DCA	Colorless and dark centered in DCA	Colorless and dark centered in DCA
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Surface	Smooth	Smooth	Smooth
Gram Reaction	-	-	-
Cell Morphology	Rods	Rods	Rods
Endospore	-	-	-
Position of the spore	-	-	-
Bulging	-	-	-
Motility	+	+	+

Table 3: Biochemical characteristics of the isolates

Parameter	A	B	C
Catalase	+	+	+
Oxidase	-	-	-
Citrate	-	-	-
Indole	-	-	-
Urease	-	-	-
Methyl red	+	+	+
Voges prokauer	-	-	-
H_2S	+	+	+
Glucose	+	+	+
Maltose	+	+	+
Galactose	+/-	-	+/-
Xylose	+	+/-	+/-
Sorbitol	+/-	+/-	+
Inositol	+	+/-	+
Dulcitol	-	+/-	-
Tetrahalose	+	+	+

Table 4: The Nucleic acids extracted from the isolates

Isolate code	Conc. (mg/ml)	280nm	260nm	260/280
A	102.40	1.6802	3.0580	1.82
B	108.10	1.6940	3.0661	1.81
C	120.20	1.7002	3.1284	1.84

Table 5: The molecular identities of the isolates

PARAMETER	A	B	C
Max score	7239	13573	6593
Total score	7239	13573	6593
Query cover (%)	100	100	100
E–value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession length	4861882	4812688	4813117
Accession number	CP053702	CP046429	CP046575
Description	*salmonella enterica* Subspecies *enterica* *Serovar typhi* Strain CMCST (STCM)	*salmonella enterica* Subspecies *enterica* *Serovar typhi* Strain R192829 (STR1)	*salmonella enterica* Subspecies *enterica* *Serovar typhi* Strain WGS1146 (STWG)

Table 6: Occurrences of the isolates in the sample

Isolates	Number	Percentage %
STCM	18	31.03
STR1	14	24.14
STWG	26	44.83
TOTAL	58	100.00

4. Discussion

The presence of enteric bacteria in the studied water samples could be traced from the management practices, and transportation of the water, poor handling and sanitary conditions attributed to the water samples. Similar findings were reported by many researchers (Mengistie *et al*., 2012; Usman *et al*., 2018; Alemeshet Asefa *et al*., 2021). Researchers had shown that transportation of equipment's can also harbour enteric bacteria and this contributes to the contamination of borehole water if you have as you just have and how has it has nothing to (Martinez-Sanctos *et al*., 2020). Ashbolt, (2004), also stated that the high prevalence and high populations of enteric bacteria in borehole water was evidence that poor sanitation could be a principal source of enteric pathogens to individuals. Borehole water contaminated by enteric bacteria pathogenic to humans can contribute to human water-borne illness through drinking. This shows that the digging of boreholes requires microbiological safety regulations to escape microbial contamination of the water. Similar deduction was drawn by different researchers (Gurein, 2014; Duru *et al.*, 2017; Nwandkor and Ifeanyi, 2017).

The variation of enteric bacteria from different boreholes studied could be attributed to the nature, texture and composition of the borehole materials. Ashbolt (2004) reported that variation in microbial counts in different borehole samples depends on the water activity, oxygen tension, and pH of the borehole water.

The presence of *Salmonella enterica* subspecies *enterica serovar Typhi* strain CMCST (STCM), *Salmonella enterica* subspecies *enterica serovar Typhi* strain WG51146 (STWG) and *Salmonella enterica* subspecies *enterica serovar Typhi* strain R192829 (STR1), of which STR1 was mostly encountered in the borehole sample. Traditionally, the laboratory detection of *Salmonella* species has relied on non-selective and/or selective enrichment and subsequent culture on selective media. The introduction of molecular techniques provides a more sensitive and rapid technique for detecting these bacteria .

The highest counts of enteric bacteria recorded among different boreholes could be attributed to the poor handling, poor sanitation and series of distribution channels involved before reaching the individuals. Similar findings were stated by many researchers (Palamuleni and Akoth, 2015; Christine *et al.*, 2018).

5. Conclusion

The study has revealed the presence of *Salmonella enterica* subspecies *enterica serovar Typhi* strain CMCST (STCM), *Salmonella enterica* subspecies *enterica serovar Typhi* strain WG51146 (STWG) and *Salmonella enterica* subspecies *enterica serovar Typhi* strain R192829 (STR1), of which STR1 was mostly encountered in the borehole samples. The present study recommends personal hygiene, community education and thorough boiling of water before use as a better means of controlling the transmission of *Salmonella* species.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Ethical approval

Not applicable

Authors Contributions

All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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