Journal of Pollution Monitoring, Evaluation Studies and Control
ISSN: 3115-5723
J. Pollut. Monit. Eval. Stud. Control, 5(1): 176-184 (2026)
DOI: https://doi.org/10.54117/141e8k97

Health Risks of Shigella-Contaminated Borehole Water in Awka Metropolis

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Abstract

Shigella species are a significant cause of diarrheal diseases worldwide, particularly in developing countries. The rise of antibiotic-resistant strains has necessitated the search for alternative antibacterial agents and understanding of the epidemiology of Shigella infections. This study aimed to isolate and characterize Shigella species from stool samples and determine their molecular identities. Stool samples were collected and cultured on Deoxychocolate citrate agar (DCA) for isolation of Shigella species. The isolates were characterized using cultural, morphological, and biochemical tests. Molecular identification was performed using 16S rRNA gene sequencing. The study revealed that 42% of the samples were positive for Shigella species, with sample O showing the highest occurrence (70%). The isolates exhibited characteristic cultural, morphological, and biochemical features, and were identified as *Shigella dysenteriae* strains SD53, SD07, and SDBU. SD53 was the most prevalent strain (55.36%). The study provides insights into the epidemiology of *Shigella* infections and highlights the importance of molecular characterization of isolates for understanding the spread of antibiotic-resistant strains. This study generates data on the occurrence and molecular characteristics of *Shigella* species in stool samples, which can inform public health strategies and antibiotic stewardship.

Keywords: *Shigella dysenteriae*, molecular characterization, antibiotic resistance, diarrheal diseases, epidemiology.

Article History
Received: 28 Dec 2025
Accepted: 10 Feb 2026
Published: 16 Feb 2026

Scan QR code to view♦

How to cite this paper: Unaeze, B. C., Iheukwumere, I. H., Iheukwumere, C. M., & Nnagbo, P. A. (2026). Health risks of *Shigella*-contaminated borehole water in Awka metropolis. *Journal of Pollution Monitoring, Evaluation Studies and Control, 5*(1), 176–184. https://doi.org/10.54117/141e8k97

Introduction

Access to safe drinking water remains a critical public health challenge, particularly in rapidly urbanizing areas of low- and middle-income countries. In the Awka Metropolis, borehole water serves as a primary source of domestic water for a significant proportion of the population due to inconsistent municipal supply. However, groundwater sources are increasingly vulnerable to fecal contamination from inadequate sanitation, improper waste disposal, and deteriorating infrastructure, elevating the risk of waterborne pathogen transmission (Eze *et al.*, 2013).

Among the pathogens of concern, *Shigella* spp. are a leading cause of bacillary dysentery (shigellosis), responsible for an estimated 188 million global cases annually, with the highest burden in sub-Saharan Africa (Khalil *et al.*, 2018). Infections are characterized by severe, often bloody diarrhea, abdominal cramps, and fever, with higher morbidity and mortality rates in children under five, the elderly, and immunocompromised individuals. The public health impact is compounded by the emergence of multidrug-resistant (MDR) *Shigella* strains, with plasmid-mediated resistance genes enabling rapid spread and limiting effective treatment options (Baker & The, 2018).

Contamination of borehole water with *Shigella* represents a critical but often overlooked transmission route. Despite its health significance, there is limited surveillance data on the prevalence and resistance profiles of *Shigella* in borehole water within the Awka Metropolis. Such data are essential for risk assessment, informing targeted interventions, and developing evidence-based water safety plans. Therefore, this study aims to determine the prevalence, distribution, and antibiotic resistance patterns of *Shigella* species in borehole water samples across the Awka Metropolis. The findings will

provide crucial evidence to guide public health action, water resource management, and policies aimed at reducing the burden of waterborne shigellosis in the region.

Materials and Methods

Isolation and Characterization of *Shigella* Species

Sample collection, handling and transportation: The samples used for this study were drawn from the rivers. A total of 100 freshwater samples were collected from five different streams used in Awka metropolis. Samples were taken from twenty different sites, each site in triplicates. The stream samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and final rinsed three times with distilled water. The containers were placed inverted in order to drain the water inside them. The container was inverted and lowered 5 cm below the river water sample, then placed vertically for the water sample to refill the sample container. This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis. This was done using the method described in work published by Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Egbe *et al*. (2025a).

Isolation of organisms

One gram (1.0 g) of the sample was aseptically transferred into a sterile test tube (Pyrex), then 3 ml of diluent (sterile normal saline) was added and then made up to 10 ml, and from this, ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Deoxycholate agar medium (DCA/Biotech) using the pour plate method. All the plates in triplicate were incubated inverted at 37±2˚C for 24-48 h (Egbe *et al*., 2025b; Egbe *et al*., 2025c; Iheukwumere *et al*., 2025d; Iheukwumere *et al*., 2025e).

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an 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*. (2018b), Iheukwumere *et al*. (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al*. (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekesiobi *et al*. (2025), Ekechukwu *et al*. (2025a), Ekechukwu *et al*. (2025b), Ezedianafo *et al*. (2025a), and Ezedianafo *et al*. (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2020b), Idigo *et al*. (2025a), Idigo *et al*. (2025b), Idigo *et al*. (2025c), Idigo *et al*. (2025d), and Ezedianafo *et al*. (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al*., 2017; Iheukwumere *et al*., 2017a; Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2023a; Iheukwumere *et al*., 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2018c) Ike *et al*. (2025a), Iheukwumere *et al*. (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2022b), Iheukwumere *et al*. (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al*. (2025b) Ike *et al*. (2025c) Iheukwumere *et al*. (2022d), Idigo *et al*. (2025e), Obiefuna *et al*. (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al*. (2017), Obianom *et al*. (2024), Ekechukwu *et al*. (2025c), Obiefuna *et al*. (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al*. (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), Idigo *et al*. (2025e), Ezedianafo *et al*. (2025d), Ezedianafo *et al*. (2025e) and Iheukwumere *et al*. (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol

and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025d), Ike *et al*. (2025e), Idigo *et al*. (2025f), Idigo *et al*. (2025g) and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al*. (2017), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), and Idigo *et al*. (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025h), Idigo *et al*. (2025i), Iheukwumere *et al*. (2025j) and Idigo *et al*. (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Idigo *et al*. (2025k), Idigo *et al*. (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of *α*-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), and Idigo *et al*. (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025l), Iheukwumere *et al*. (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2025n), and Iheukwumere *et al*. (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025p; Iheukwumere *et al*., 2025q; Chude *et al*., 2020).

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2025r; Iheukwumere *et al*., 2025s; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2025t; Nwike *et al*., 2017).

Prevalence and Distribution of the Isolates in the water Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated as a percentage of the occurrences. The bacteria that appeared in each sample

location were detected and recorded as described in the study published by Iheukwumere *et al* (2025u), Iheukwumere *et al*. (2025v).

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. Pair wise comparison of mean was done by Student "t" test sa described in the study published by Iheukwumere *et al*. (2017e), Manasseh *et al*. (2025), Idigo *et al*. (2025n), Idigo *et al*. (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Ugwu *et al*. (2025a) and Ugwu *et al*. (2025b).

Results

The occurrences of the Isolates in the sample is showed in Table 1. The study revealed that 42% of the samples were positive for S*higella* species. Sample O showed highest occurrences of the Test Organism whereas sample P recorded the lowest occurrences

The cultural and morphological characteristics of the Isolates is shown in Table 2. The study revealed that the Isolates exhibited different appearances on Deoxychocolate citrate agar and similar elevation, Edge and surface. And also similar morphological characteristics on Gram reaction, cell morphology, Endospores and motile nature. The biochemical characteristics of the Isolates revealed that the Isolates were Voges prokaver, I dole, Citrate, Hydrogen sulphide production, Urease, Dulcitol and Sucrose negative as shown in Table 3. The Isolates differ in their variation in utilization of sugars. They were all catalase and Glucose positive but differ in their abilities to utilize Lactose, Mannitol and Inositol. The nucleic acid extracted from the Isolates showed the ratio of their absorbance at wave length of 260 nm and 280 nm. using Nano drop 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 E, F and G were *Shigella dysenteriae* strain 53—3937(SD53), *Shigella dysenteriae* strain 07—3308(SD07) and *Shigella dysenteriae* strain BU53W(SDBU) as shown in Table 5.

The study also revealed that SD53 showed highest occurrences in the studied sample whereas SD07 recorded the least occurrences as shown in Table 6.

Table 1: Occurrences of the Isolates in the studied samples

Sample	Number	P(%)	N(%)
M	20	9(45.00)	11 (55.00)
N	20	6(30.00)	14(70.00)
O	20	14(70.00)	6(40.00)
P	20	5(25.00)	15(75.00)
Q	20	8(40.00)	12(60.00)
Total	100	42 (42.00)	58(58.00)

Table 2: Cultural and morphological characteristics of the Isolates

Parameter	G	H	I
Appearance on DCA	Colourless/pale	Pale	Colourless
Elevation	Convex	Convex	Convex
Edge	Smooth	Smooth	Smooth
Surface	Smooth	Smooth	Smooth
Gram reaction	____	____	____
Cell morphology	Rods	Rods	Rods
Endospore	____	____	____
Motility	____	____	____

Table 3: Biochemical characteristics of the Isolates

Parameter	G	H	I
Catalase	+	+	+
Voges prokaver	_	_	_
Indole	_	_	_
Citrate	_	_	_
H_2S	_	_	_
Urease	_	_	_
Glucose	+	+	+
Lactose	+/-	_	+/-
Mannitol	+/-	+/-	+
Dulcitol	_	_	_
Sucrose	_	_	_
Inositol	_	+/-	_

Table 4: Molecular characterization of the isolates

Parameter	G	H	I
Max Score	6076	6076	7239
Total score	6076	6076	15503
Query score (%)	100	100	100
E-value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession Number	4382743	4382687	184894
Description	*Shigella dysenteriae* strain 53-3937 (SD53)	*Shigella dysenteriae* strain 07-3308 (SD07)	*Shigella dysenteriae* strain BU53W (SDBU)

Table 5: Occurrence of the isolates

Isolate	Number	Percentage (%)
SD53	31	55.36
SD07	9	16.07
SDBU	16	28.57
Total	56	100

Discussion

The presence of *Shigella* spp. in borehole water samples from the Awka Metropolis indicates significant fecal contamination, which can primarily be attributed to inadequate management, poor sanitation infrastructure, and unsafe handling practices within the community. This finding aligns with established research linking waterborne pathogen prevalence to compromised sanitation and unhygienic conditions in vulnerable settings (Immerseel *et al.*, 2014; Alshawabkeh, 2016). Contamination is further exacerbated by anthropogenic activities such as improper waste disposal, pit latrine seepage, and surface runoff in proximity to boreholes, factors known to introduce enteric pathogens into groundwater (Iheukwumere *et al.*, 2018a; Kupryś-Caruk *et al.*, 2018). The high prevalence and concentration of *Shigella* observed underscore that untreated borehole water can serve as a principal reservoir and transmission route for waterborne shigellosis, thereby necessitating urgent microbiological safety regulations and point-of-use treatment.

Variation in *Shigella* contamination across borehole sites likely reflects differences in local environmental pressures, including proximity to contamination sources, hydrogeological characteristics, and population density. Site-specific risks, such as inadequate casing, cracked seals, or flooding, can further influence pathogen ingress into groundwater systems.

The identification of specific strains SD53, SD07, and SDBU confirms the presence of clinically significant *Shigella dysenteriae* isolates in drinking water sources and this was n ne with the findings of many researchers (Davies and Wales, 2010; Ali *et al.*, 2014). This is particularly concerning given the virulence and antibiotic resistance potential of *S. dysenteriae*, which is associated with severe dysentery and outbreaks.

CONCLUSION

This study confirms the presence of *Shigella dysenteriae* strains SD53, SD07, and SDBU in borehole water from the Awka Metropolis, with SD53 being the most predominant isolate. These findings highlight a direct and significant public health risk, as contaminated borehole water serves as a potential vehicle for shigellosis transmission in the community. To mitigate this risk, it is imperative to implement targeted public health interventions, including community education on safe water handling, promotion of point-of-use water treatment methods, and regular microbiological monitoring of borehole water quality. Strengthening sanitation infrastructure and enforcing protective zoning around boreholes are also essential to prevent fecal contamination and safeguard community health.

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