SCVJ
Volume 5, Issue 1, 2024
DOI: http://doi.org/10.55989/SACX8774
Science View Journal
ISSN (Online): 2734-2638

Antibacterial Susceptibility Pattern of Salmonella spp from an Eastern Nigeria River

C. N. Okorie[1], D. A. Ihonde[1], S. C. Emencheta[1], E. B. Onuigbo[1], A. A. Attama[2]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

Email: chibundo.okorie@unn.edu.ng

Abstract:
Salmonella is ubiquitous bacteria commonly found in water, animals, humans, and the environment. The transmission is facilitated by human activities, including the irrigation of farm products using contaminated surface water, and it's a public health concern. This study was carried out to assess Salmonella contamination of the Ekulu River in the Enugu metropolis and determine their susceptibility to some selected antibiotics. Water samples were collected randomly in the region and processed for isolation and identification of Salmonella spp usingthe direct plating method. The antimicrobial susceptibility tests of the isolates against ciprofloxacin (5 µg), chloramphenicol (30 µg), amoxicillin (10 µg), and streptomycin (10 µg) were done using the disc-diffusion method. Twenty-six (26) (100%) of the isolates were resistant to amoxicillin antibiotics, and 42.31%, 23.07%, and 46.15% were resistant to ciprofloxacin, chloramphenicol, and streptomycin, respectively. This study shows the importance of establishing adequate measures to control contamination of surface water used for domestic activities in Enugu metropolis. Improved water quality increases health and reduces the transmission of diseases/(and)infections.

Keywords: Salmonella spp, Susceptibility pattern, Antibiotics, Surface water, Contamination.

I. Introduction

Water is an essential component of life. Water supply and accessibility is the sixth goalof the sustainable development goals (SDGs) and aims at ensuring environmental sustainability (Amenu et al., 2014). Historically, efforts to provide access to safe drinking and food processing water have focused on community-based water sources (Onyango et al., 2018). Children, women, immune-compromised individuals, and rural residents are at the highest risk of contracting waterborne pathogens. People can become infected by waterborne pathogenic agents if they consume contaminated water directly or indirectly through its use in food production, processing, or preparation (Onyango et al., 2018). Water that may be safe to drink could be infected with microorganisms that pose significant health risks. Therefore ensuring the safety of drinking water is a continual task.

The microbial examination of drinking water for indicator microorganisms is key to determining water quality and ensuring public health safety (Wen et. al., 2020). The presence of indicator microorganisms represents the faecal contamination of drinking water with pathogens and thus, the deterioration of the water quality. The microbiological assessment of drinking water quality is based on the relationship between indicator microorganisms and pathogens (Adentunde & Glover, 2011). In rural areas of most developing countries that use communal water sources, bacterial contamination of drinking water is a significant cause of waterborne disorders. They are also exposed to multiple faecal-oral transmission pathways in their neighborhood boundaries (Gwimbi et al., 2019). Waterborne diseases, including Cholera, Dracunculiasis, Typhoid fever, Diarrhea, Ulcers, Hepatitis, Respiratory tract infection, Kidney damage, and Endocrine damage are hazardous to the lives of organisms especially in humans; can lead ultimately to death (Onyango et al., 2018). Among the poor, especially in developing countries, diarrhoea is a significant killer due to limited access to good drinking water. 1n 1998, diarrhoea was estimated to have killed 2.2 million people, most of whom were under five years (Mishra et. al., 2022). Approximately 4 billion cases of diarrhoea are reported worldwide yearly.

Surfacewater is susceptible to faecal contamination as 892 million people worldwide practice open defecation (Belay et al., 2022). The people of Enugu State of Nigeria are prone to waterborne pathogenic disease due to their regular use of the river as their primary source of water for domestic activities and crop processing. The impact on child mortality rate is devastating, with more than 340,000 children under five dying annually (approximately 1000 per day) from diarrhoea disease due to poor sanitation, poor hygiene, or unsafe drinking water (Fischer et al., 2012). Salmonella present in irrigation waters has been regarded as one of the significant sources of freshwater contamination and has become a public health concern has drawn more attention from food safety regulatory agencies. Salmonella infection is still an important public health concern in the world. It contributes to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention, and treatment of the disease (Abdelmalek, 2019).

Detection of any species of this organism in a given water source indicates contamination. High incidence of childhood diarrhoea, helminthiasis, trachoma, typhoid fever, and overall

DOI: http://doi.org/10.55989/SACX8774

ISSN (Online): 2734-2638

high mortality rates are associated with poor environmental sanitation (Stella et al., 2018). Salmonella is a rod-shaped, gram-negative, non-spore-forming, predominantly motile Enterobacteria with diameters around 0.7 to 1.5µm, lengths from 2 to 5µm, and flagella that move in all directions. They are chemo organo-trophs and facultative anaerobes and, thus, obtain their energy from oxidation and reduction reactions using organic sources.

Antimicrobial susceptibility testing (AST) is a laboratory procedure done by medical technologists (clinical laboratory scientists) to identify which antimicrobial regimen is specifically effective for individual patients. On a larger scale, it aids in evaluating treatment services provided by hospitals, clinics, and national programs to control and prevent infectious diseases. Susceptibility testing of individual microbial isolates is necessary to ascertain species that may have acquired resistance mechanisms (e.g., members of the Enterobacteriaceae, Pseudomonas species, Staphylococcus species, Enterococcus species, and Streptococcus pneumonia (Reller et al., 2009). Methods of susceptibility testing such as Agar Disc-diffusion and Broth Dilution are official and routine methods used in many clinical microbiology laboratories for antimicrobial susceptibility testing. Thus, this study aims to isolate, identify, and determine the antibiotic susceptibility of Salmonella spp from the Ekulu River used for domestic activities and its health implications.

II. Materials and Methods

SAMPLE COLLECTION AND PROCESSING

Water samples were collected from Ekulu River at three (3) different locations in duplicates; upstream (NoA), point of contamination (NoB), and downstream (NoC), which serve as the major source of water for irrigation, and other domestic uses. The choice of water body was due to multiple sources of likely contamination with faecal matter from plants, animals, and humans in the area. The samples were collected aseptically from October to December (2021) during the dry season at a depth of 15 cm below the water using sterile 5 ml syringes.

TEST BACTERIAL ISOLATES

Using standard protocols and relevant media (Nutrient and Salmonella-shigella agar) prepared according to manufacturers' instructions, Salmonella spp were isolated from the water samples obtained from the different points of the Ekulu River in Enugu metropolis.

Briefly, a 100uL micro-pipette was used to withdraw 0.1ml and inoculate in triplicates on prepared Salmonella-shigella agar plates arranged in a sterile canister at the site. The plates were labelled appropriately. A glass spreader was used tospread the samples on the plates and were labelled appropriately and evenly. The plates were then transported to the Department of Pharmaceutical Microbiology, UNN, and incubated at 37°C for 24 hours. Three (3) – four (4) colonies from the initial culture were picked and subcultured into another prepared Salmonella-shigella agar plates to obtain discrete colonies. The subcultured colonies were then incubated at 37°C for 24 hours. After incubation, pure cultures were selected from the plates and stocked in prepared agar slants. The slants were then incubated at 37 °C for 24 hours, after which they were removed from the incubator and preserved in the refrigerator, pending further use.

IDENTIFICATION OF BACTERIAL ISOLATES

The bacterial isolates were identified by a combination of the standard protocols, including microscopy, morphological appearances on SSA, gram staining reactions, and the relevant biochemical tests, including motility, nitrate, indole, sulphur, and triple sugar iron Agar (TSIA) tests.

ANTIBIOTICS SUSCEPTIBILITY TESTING

The antibiotics susceptibility was tested by the disc-diffusion method as described by Kirby Bauer, 1966 using commercially available antibiotic-containing discs (OXOID) of ciprofloxacin (CIP 5µg), chloramphenicol (30µg), amoxicillin (10µg), and streptomycin (10µg). Bacteria suspensions of each test Salmonella spp isolate were prepared using sterile normal saline and adjusted to 0.5 McFarland turbidity standards. Sterile cotton swabs were used to spread the suspensions onto a sterile Mueller-Hinton agar plate's entire surface, thereby providing bacterial lawns with confluent growth. The inoculated plates were allowed to dry for approximately 10 minutes, and the antibiotics discs were placed on their surfaces, after which the plates were incubated aerobically at 35 oC for 16 – 18hours following the incubation period, the diameters of zones of inhibition (clear zones) that developed around each disk were measured and recorded. The breakpoints were classified according to Clinical & Laboratory Standards Institute (CLSI) guidelines described below.

DOI: http://doi.org/10.55989/SACX8774

ISSN (Online): 2734-2638

TABLE 1: STANDARD OPERATING PROCEDURE FOR ANTIBIOTICS ACCORDING TO CLSI.

Anti thmicrobial agent	Disc code (potency)	Resistant	Intermediate	Susceptible
Amoxicillin (Enterobacteriaceae)	AMC 20/10µg	≤13	14-17	≥18
Chloramphenicol (Enterobacteriaceae)	C 30µg	≤12	13-17	≥18
Ciprofloxacin (Enterobacteriaceae)	CIP 5µg	≤20	21-30	≥31
Streptomycin (Enterobacteriaceae)	S 10µg	≤11	12-14	≥15

III. RESULTS

BACTERIAL IDENTIFICATION AND CONFIRMATION

Forty (40) pure bacterial isolates were obtained from different points of the Ekulu River. The results of the identification tests were based on the cultural properties and microscopic properties. The isolates, when subcultured on the differential media, SSA, showed moderately large (2 - 4 mm), circular with smooth surfaces and transparent with black centres in colour after 24 hrs of incubation at 37 °C. The Salmonella spp were found to be Gram-negative short rods occurring singly and in groups. Biochemically, twenty-six (26) out of the forty (40) isolates were confirmed Salmonella spp.

Most of the isolates were positive for the motility, nitrate, sulphur, and TSIA tests, as shown in Table 2. Only a few normal isolates from points NOa, NOb, and NOc of the river were positive for the indole test. Nearly all the isolates were negative for the indole test.

Identification of Salmonella at specie level is done molecularly. All the salmonella isolates used for this work were suspected to be salmonella (based on cultural properties on selective media) and hence there seemed to be no need to put them in a table.

TABLE 2: RESULTS OF BIOCHEMICAL TESTS OF SALMONELLA ISOLATES FROM EKULU RIVER SOURCE.

S/N	Code	Motility	Nitrate	Indole	Sulphur Tests	TSIA
1	NOc A	+	+	+	+	+
2	B	+	+	+	+	+
3	C	+	+	_	+	+
4	D	+	+	_	+	+
5	NOb A	+	_	_	+	+
6	B	+	_	+	+	+
7	C	+	+	+	+	+
8	D	+	+	_	+	+
9	Noa B	+	+	+	+	_
10	Noa R A	_	+	+	+	+
11	B	+	+	_	+	+
12	C	+	+	_	+	+
13	D	+	+	_	+	+
14	Nob R A	+	+	_	+	+
15	B	+	_	_	+	+
16	C	+	+	_	+	+
17	D	+	+	_	+	+
18	Noc R A	+	_	_	+	+
19	B	+	+	_	+	+
20	C	+	+	_	+	+
21	D	+	+	_	+	+
22	B	+	+	_	+	+
23	C	+	+	_	+	+
24	D	+	+	_	+	+
25	D	+	+	_	+	+
26	Nob Ssa A	+	_	+	+	+
27	B	+	_	_	+	+
28	C	+	+	_	+	+
29	D	+	+	_	+	+
30	Noc Ssa B	+	_	_	+	+
31	C	+	+	_	+	+
32	Noa Sf C	+	+	_	+	+
33	D	+	+	_	+	+
34	Nob Sf A	+	+	_	+	+
35	C	+	+	_	+	+
36	D	+	+	_	+	+
37	Noc Sf A	+	+	+	+	+
38	B	+	+	_	+	+
39	C	+	+	_	+	+
40	D	+	+	_	+	+

Key: A, B, C, and D: points in the river; Noa: samples gotten from Ekulu river upstream; NOb: samples from Ekuluriver at the point of external contamination; NOc: samples from Ekulu river downstream; R: isolates from Rappaport-Vassiliadis Broth; Ssa: isolates from Salmonella Shigella agar; Sf:isolates from Selenite Broth; TSIA: Triple sugar iron agar; Size of antibiotic disk: 6mm.

Ekulu River against four (4) different antimicrobial agents is shown in Table 3. The isolates were most sensitive to chloramphenicol (69.23 %). Amoxicillin antimicrobial agent saw a 100 % resistance from the isolates. Streptomycin (46.15 %), ciprofloxacin (42.31 %) and chloramphenicol (23.07 %) followed suit in the resistance patterns, respectively.

DOI: http://doi.org/10.55989/SACX8774

TABLE 3: RESISTANCE PATTERN OF SALMONELLA SPP ISOLATES OF EKULURIVER.

Antimicrobial Agent	Resistant (%)	Intermediate (%)	Sensitive (%)
CIPRO	42.31	46.15	11.53
CHLORAM	23.07	2.0	69.23
AMOXIL	100.0	0	0
STREPT	46.15	38.46	15.38

Key: CIPRO: Ciprofloxacin; CHLORAM: Chloramphenicol; AMOXIL: Amoxicillin; STREPT: Streptomycin.

IV. Discussion

Diseases are caused by pathogenic organisms like bacteria, viruses, fungi, and parasites that are spread using tainted water. Various factors, such as anthropogenic activity, natural disasters, and animal contamination, can cause water pollution. Illnesses are transmitted when contaminated water is utilized for drinking, and other domestic purposes,including agricultural produce irrigation [Ahmad et al, 2020].

Numerous studies have identified the microorganisms that contribute to outbreaks brought on by tainted drinking water, including Vibrio cholerae, Shigella sp., S. typhi, Campylobacter, Escherichia coli, Cryptosporidium parvum, Entamoeba histolytica, Giardia sp., Balantidium coli, rotavirus, and hepatitis A virus [Robertine et al., 2017].

This present study assessed the biochemical properties and antimicrobial susceptibility patterns of Salmonella spp found in the Ekulu river. Forty (40) isolates of Salmonella spp were isolated from the Ekulu River. Biochemically, twenty-six (26) out of the forty (40) isolates were confirmed to be Salmonella spp, which gave a prevalence of 65%. Variations in the level of microorganisms in water bodies can be attributed to the indiscriminate discharge of human and animal faeces around and in water bodies [Adentunde et al., 2011]. The presence of Salmonella species anywhere in the dams, wells, rain, and tap water samples is worrying since most of the people and animals in Enugu State, Nigeria, and its environs drink from them. The World Health Organization [WHO, 2006] reported that good quality drinking water should be free from microbial contamination. The isolation of Salmonella spp from the water sample means that the direct consumption of such water without treatment may be hazardous. The genus is known to cause Salmonellosis. The S.typhi and S. paratyphi species cause typhoid fever which can be spread through contaminated water. This study, as expected, shows that there is high bacteria load in the river surface water sample, which can be associated with high mortality in rural areas due to no access to quality water and it's in agreement with those reported by [Kindhauser et al, 2019]. In a study conducted in Pakistan by Qamar, Farah Naz, et al.(2018), the presence of S. typhi was confirmed in water samples, especially samples collected near sewage lines. Similar findings were also reported by Osei, et al.(2021); and Akram, et al. 2020. A study conducted in Kaduna Metropolis, Nigeria, revealed the highest number of S. typhi in drinking water (Omotola et.al., 2020)

Since S. typhi is implicated in enteric fever, optimum antimicrobial treatment of patients with enteric fever depends on understanding local patterns of antimicrobial susceptibility of isolates. In the present study, the isolates showed the highest resistance to amoxicillin (100%), then streptomycin (46.15%), ciprofloxacin (42.31%), and chloramphenicol (23.07%). This result is consistent with reports by Admassu et al., 2019, in which Salmonella isolates showed higher resistance to ampicillin, chloramphenicol, and amoxicillin. Similar studies conducted in Kenya (Mengo et al., 2010) and Ethiopia (Dagnew et al., 2013) and a systematic review on antimicrobial susceptibility had similar findings (Ashley et al., 2011). These resistance patterns seen above could be due to the availability and use of these drugs from medicine stores and pharmacies and the lack of awareness of antimicrobial stewardship. The increasing resistance could lead to difficulty treating infections (Senthilkumar & Parabakaran(2005). Thus, routine screening of antimicrobial susceptibility before prescription to patients is important to reduce the spread/and development of resistant strains and improve the patient's prognosis.

V. Conclusion

The high level of resistance of Salmonella spp isolates from Ekulu River to selected antibiotics indicates a huge level of contamination of the River surface water and poor water quality, making it unsafe for domestic purposes. The variable resistance patterns in response to different antibiotics used in this research shows that the presence of Salmonella poses a public health risk. Thus, adequate hygienic measures should be ensured to reduce contamination of the Ekulu River, avoiding inappropriate use of antibiotics, which may impose resistance, a public health concern.

VI. RECOMMENDATION

Measures should be implemented to avoid the misuse or abuse of antibiotics to prevent or reduce the risk of resistance development. Enlightenment and proper education should be given (especially to people living in the riverine area). Measures should be implemented to ensure appropriate hygienic conditions, which will go a long way to preventing contamination and infection.

VII. CONFLICT OF INTEREST

The authors declare no conflict of interests.

VIII. ACKNOWLEDGEMENT

The authors wish to acknowledge the technical staff of the Department of Pharmaceutical Microbiology and Biotechnology of the University of Nigeria, Nsukka, for their technical support. We acknowledge TETFund, Nigeria for provision of funding through the Institution-Based Research (IBR) Intervention Scholarship (2022) granted to Chibundo N. Okorie.

IX. LIMITATION OF THE STUDY

This study has limitations due to its small sample size and lack of bacterial isolate serotyping and genotyping. Additionally, each isolate's minimum inhibitory concentration of an antibiotic was not tested.

DOI: http://doi.org/10.55989/SACX8774

X. References

Abdelmalek, S., Kadry, M., Elshafiee, E. A., Hamed, W., Moussa, I. M., Al-Maary, K. S., Mubarak, A. S., Hemeg, H. A., & Elbehiry, A. (2019). Occurrence of Salmonella infection and antimicrobial susceptibility for local Salmonella isolates from different sources in a cross-sectional study. Italian journal of food safety, 8(4), 8525. https://doi.org/10.4081/ijfs.2019.8525

Admassu Ayana, D., Marami, D., Asaminew, N., Teshome, B., & Teklemariam, Z., (2019). Salmonella and Shigella among patients with diarrhea at public health facilities in Adama, Ethiopia: Prevalence, antimicrobial susceptibility pattern, and associated factors. SAGE open medicine, 7, 2050312119846041.

Adentunde L. A, Glover R.L.(2011) Evaluation of bacteriological quality of drinking water used by selected secondary schools in Navorongo in Kassina-Nankana District in the Upper East Region of Ghana. Prim J Microbiol Res 2011;1:47-51.

Ahmad, M., Jamal, A., Tang, X. W., Al-Sughaiyer, M. A., Al-Ahmadi, H. M., & Ahmad, F. (2020). Assessing potable water quality and identifying areas of waterborne diarrheal and fluorosis health risks using spatial interpolation in Peshawar, Pakistan. Water, 12(8), 2163.

Akram, N. (2020). Consumption of safe drinking water in Pakistan: Its dimensions and determinants. Drinking Water Engineering and Science, 13(2), 43-50.

Extensively drug-resistant (XDR) typhoid: evolution, prevention, and its management. BioMed Research International, 2020. 2020.

Ali, S. A., & Ahmad, A. (2020). Analysing water-borne diseases susceptibility in Kolkata Municipal Corporation using WQI and GIS based Kriging interpolation. GeoJournal, 85(4), 1151-1174.

Corporation using WQI and GIS based Kriging interpolation. GeoJournal, 2020. 85(4): p. 1151- 1174.

Amenu, K., Spengler, M., Markemann, A., & Zárate, A. V. (2014). Microbial Quality of Water in Rural Households of Ethiopia : Implications for Milk Safety and Public Health. 32(2), 190–197.

Ashley, E. A., Lubell, Y., White, N. J., & Turner, P. (2011). Antimicrobial susceptibility of bacterial isolates from community acquired infections in Sub ‐ Saharan Africa and Asian low and middle income countries. Tropical Medicine & International Health, 16(9), 1167-1179.

Belay, D.G., Asratie, M.H., Aragaw, F.M. (2022). Open defecation practice and its determinants among households in sub-Saharan Africa: pooled prevalence and multilevel analysis of 33 sub-Saharan Africa countries demographic and health survey. Trop Med Health 50, 28 (2022). https://doi.org/10.1186/s41182-022-00416-5

Dagnew M, Yismaw G, Gizachew M, (2013) Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia. BMC Res Notes 2013; 6: 283.

Diarrhoeal disease [Internet]. World Health Organization. World Health Organization; [cited 2022Nov20]. Available from: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease

Fischer Walker, C.L., Perin, J., Aryee, M.J.(2012). et al. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. BMC Public Health 12, 220 (2012). https://doi.org/10.1186/1471-2458-12-220

Gwimbi, P., George, M., & Ramphalile, M. (2019). Bacterial contamination of drinking water sources in rural villages of Mohale Basin , Lesotho : exposures through neighbourhood sanitation and hygiene practices. 1–7.

Irfan, S., Hasan, Z., Qamar, F. N., Ghanchi, N., Ashraf, J., Kanji, A. & Hasan, R. (2023). Correction: Ceftriaxone resistant Salmonella enterica serovar Paratyphi A identified in a case of enteric fever: first case report from Pakistan (BMC Infectious Diseases,(2023), 23, 1,(267), 10.1186/s12879-023-08152-9). BMC Infectious Diseases, 23(1), 346.

Mishra, R., Acharya, A., Yadav, A. K., & Shreewastav, R. K. (2022). Diarrhoea among Children Under Five Years of Age Residing in a Village Development Committee: A Descriptive Cross-sectional Study. JNMA: Journal of the Nepal Medical Association, 60(249), 425.

Omotola, J., Ogbonna, I., & Iheukwumere, C. (2020). Prevalence of Typhoidal Salmonella Infections and Associated Risk factors in Kaduna Metropolis, Nigeria. Journal of Medical Microbiology and Infectious Diseases, 8(3), 84-92.

Onyango, A. E., Okoth, M. W., Kunyanga, C. N., & Aliwa, B. O. (2018). Microbiological quality and contamination level of water sources in Isiolo County in Kenya. Journal of Environmental and Public Health, 2018.

Osei, F. B., Boamah, V. E., Boakye, Y. D., Agyare, C., & Abaidoo, R. C. (2021). Antibiotic resistance of bacteria isolated from water supplies used in poultry production in Ashanti Region of Ghana. The Open Microbiology Journal, 15(1).

Overview of Commonly Used Susceptibility Testing Methods.
Irfan, S., Hasan, Z., Qamar, F. N., Ghanchi, N., Ashraf, J., Kanji, A. & Hasan, R. (2023). Correction: Ceftriaxone resistant Salmonella enterica serovar Paratyphi A identified in a case of enteric fever: first case report from Pakistan (BMC Infectious Diseases,(2023), 23, 1,(267), 10.1186/s12879-023-08152-9). BMC Infectious Diseases, 23(1), 346.
Reller, L. B., Weinstein, M., & Jorgensen, J. H. (2009). Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices

Rodier, G., & Kindhauser, M. K. (2009). Global health security: the who response to outbreaks past and future. Facing Global Environmental Change: Environmental, Human, Energy, Food, Health and Water Security Concepts, 529-540.

Robertine, L. F., Payne, V. K., Honorine, N. T., Mounchili, S., Saturine, M. M., Manjuh, B. R., & Roland, B. (2021). Trends of potential waterborne diseases at different health

ISSN (Online): 2734-2638
DOI: http://doi.org/10.55989/SACX8774

facilities in Bamboutos Division, West Region, Cameroon: a retrospective appraisal of routine data from 2013 to 2017. Journal of Water and Health, 19(4), 616-628.

Senthilkumar B and Prabakaran G.(2005). Multi-drug resistant Salmonella Typhi in asymptomatic typhoid carriers among food handlers in Namakkal District Tamil Nadu. Ind J Med Microbiol 2005; 23(2): 92–94.

Stella, E. I., M, E. I., Omtb, O., Mc, O., & Uf, O. (2018). Evaluation of Salmonella Species in Water Sources in Anambra State Evaluation of Salmonella Species in Water Sources in Two Local Government Areas of Anambra State. April.

Wen, X., Chen, F., Lin, Y., Zhu, H., Yuan, F., Kuang, D., ... & Yuan, Z. (2020). Microbial indicators and their use for monitoring drinking water quality—A review. Sustainability, 12(6), 2249.

World Health Organization.(2006) Microbial Aspect in Guideline for Drinking Water Quality. 3rd ed. Geneva: WHO; 2006

Wen, X., Chen, F., Lin, Y., Zhu, H., Yuan, F., Kuang, D., & Yuan, Z. (2020). Microbial indicators and their use for monitoring drinking water quality—A review. Sustainability, 12(6), 2249.