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Original Research Article

Molecular Characterization and Resistance Profiling of Multidrug-Resistance *Salmonella* Species Isolated from Southeast Nigerian River

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ABSTRACT

Water, which aids in the sustainability of life, also contributes to diseases, including *Salmonella*-implicated diseases, attributable to human anthropogenic activities leading to cross-contamination and transfer of multi-drug-resistant pathogens. There is however need for constant surveillance of public water. This study is aimed at molecular identification and resistance profiling of multidrug-resistant *Salmonella* spp. isolated from a Southeast, Nigerian River. Using water samples obtained from sites 1–8, the microbiological water quality and faecal counts were determined using the membrane filtration technique. *Salmonella* spp. from the sample was isolated and biochemically and molecularly characterized and the resistance profile to ciprofloxacin (5µg), chloramphenicol (30µg), amoxicillin (30µg), and streptomycin (10µg) assessed using the Kirby-Bauer sensitivity technique. The mean faecal counts according to the sampling months ranged from 35,000–45,000CFU/100ml and according to the sites, ranged from 37233.3–41900CFU/ml. The pre-identification of the isolates was done using isolates with negative results to catalase, coagulase, Voges-Proskauer, urease, oxidase and gram-stain, positive results to citrate, indole, methyl red, and alkaline/acid result to H_2S. The molecular identification confirmed seven *Salmonella enterica* (coded M1 to M7)of different strains and subspecies. The strains showed the highest sensitivity to chloramphenicol (80%), followed by ciprofloxacin (16%). The highest resistance was recorded with amoxicillin (98%), followed by streptomycin (48%). Detected fluoroquinolone genes included *QnrA, QnrSM, ParC,* and *gyr*in some or all the isolates. The study revealed the presence of multi-drug resistant *S. enterica* strains in the study site, further exposing the heavy contamination in the site and informing policymaking.

***Keywords*:**Resistance profiling, Molecular characterization, Multidrug *Salmonella* species, River

Introduction

With 75 % of the earth's surface covered with water, it is indispensable to life.[1] It is a crucial component of human nutrition, consumed/used directly and/or indirectly as components of foods and in diverse daily applications, including for domestic purposes and fresh produce irrigation. Aside from the positives, it also serves as one of the vehicles for transmitting disease and infection in both developed and developing countries.[1]

Surface water pollution often results from diversified routes.[2] Specifically, poor environmental sanitation continues to harm health and leads to microbial transmission and diseases including diarrhoea and typhoid consequently promoting high mortality rates.[3]

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Surface water quality degradation and composition is significantly affected by human anthropogenic activities. These include open defecation, industrial, and residential effluents/wastewater discharges, agricultural activities, including the use of fertilizers and pesticides, and abattoir effluents which influence both the physical, chemical, and microbial quality and profile of surface water. Globally, 844 million people still lack access to basic drinking water, especially in the rural areas of low- and middle-income countries (LMICs) and about 10 % of the Global Disease Burden could be prevented by improving water access, sanitation, hygiene and management.[4] Specifically, about 66 million people do not have adequate access to drinking water.[5]

Coliforms are known to be associated with water contamination and many are implicated in severe health challenges, leading to co-morbidities and mortality in humans. Gastrointestinal diseases, UTI, skin and respiratory diseases, and food poisoning are just a few examples of associated disease conditions, with *Salmonella* spp often implicated, especially the first mentioned.

The presence of *Salmonella* like other pathogenic microorganismsin surface water bodies, via cross contamination contributes to increasing morbidity and mortality rates and has become a major public health concern.[6] It has been identified as a major causative organism of bacteremia and gastroenteritis worldwide. Many species cause Salmonellosis of which *Salmonella typhi* and *Salmonella paratyphi* are the major species implicated in man.[7] Thus, demanding proper and adequate assessment of these surface water bodies would inform intervention policies.

Infections involving invasive serotypes are often life-threatening, due to the need for effective and comprehensive antibiotic therapy. The

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efficacy of antibiotic treatment has declined since the emergence of multi-drug resistance (MDR) *Salmonella* consequently adding to treatment and economic burden. The maintenance of good food hygiene, proper dieting, water sanitation and other measures as reduction in the use of antibiotics in food animals are the major preventive measures.[7]

Salmonella infections remain one of the most serious public health issues worldwide, contributing to the economic imbalance and destabilization of both industrialized and underdeveloped countries due to the cost of resources invested for surveillance, prevention and treatment of the associated diseases.[8] *Salmonella* belongs to the family *Enterobacteriaceae* of which 2600 serotypes have been identified using the Standard Kauffman White Scheme and most of the serotypes adapt easily within animal hosts, including humans.[9]

Salmonella enterica is a leading cause of community-acquired bloodstream infections in both Africa and Asia.[10] *S. enterica* serovars Typhi, Paratyphi A, Paratyphi B, and Paratyphi C may all be referred to as typhoidal *Salmonella*, while other serovars are placed as nontyphoidal *Salmonella* (NTS). Typhoidal *Salmonella* strains are human host-restricted organisms that cause typhoid and paratyphoid fever, together referred to as enteric fever. NTS strains may be host generalists, infecting or colonizing a broad range of vertebrate animals, or may be adapted or restricted to nonhuman animal species.[11]

Ekulu River, a river in the city of Enugu, Nigeria, offers an alternative source of water to the residents for drinking, recreation, domestic use, and fresh produce irrigation. Anthropogenic activities, including open defecation and the indiscriminate mismanagement of refuse dumps, human wastes, treated, and untreated domestic sewage, abattoir effluents, piggery effluents and industrial effluents lead to the discharge along the river. Thus, the river potentially serves as a reservoir for pathogenic bacteria through these human effects and necessitates the need for regular surveillance which would inform interventional approaches. These include the definite identification of pathogenic bacterial isolates present in the surface water and the resistance profiling including the testing against conventional antibiotics and the tracking of specific resistance genes. Thus, this study aimed to examine the molecular characterization and resistance profiling of multidrug-resistant *Salmonella* spp. isolated from the river.

Materials and Methods

Study site

Ekulu River is a 25 km (16 miles) long river in the Enugu city of Enugu State, located within Latitude $6^0$24'35.1 (6.40980) North and Longitude 7º36'38.2 (7.6106) East as shown in Figure 1. The river originated from the foot of Udi Hills in the State. The overflow of the river along its watercourse has other tributaries and feed streams including Ohune, Inyi, and Nnadiri. The river plays key roles in the community, including serving both the Enugu and Ebonyi State Water Corporation as the major source of water for their activities. Though often treated, it also serves surrounding institutions, including industries, universities, hospitals, small businesses such as car washing business, block moulding, and abattoirs and agricultural purposes including irrigation of farm produce. Some of these activities usually done close to the riverbank produce and inject contaminants into the river resulting in the pollution of the water body and making them a source of infection.[12]

Sample collection

Duplicate composite samples were collected aseptically for a period of ten (10) months, starting from September 2021 to June 2022, between 8 a.m. and 1 p.m. (5 hours) using 2 litres of screw-capped Roux bottles and were done once monthly at an approximate depth of 15 cm below the water surface. The samples were placed in a lightproof insulated box containing ice packs and transported to the laboratory for analysis. Upon arrival, the samples were stored at -4ºC and analyzed within 8 h.

Determination of the microbiological water quality and faecal coliforms (FC) count

The microbiological water quality and FC counts were determined using the membrane filtration technique.[13] All samples were analyzed in triplicates and were used to determine the faecal coliform counts of the water sample. The culture plates for the assays were prepared accordingly using salmonella-shigella agar (Oxoid), and chromo-cult coliform agar (Merck. Wadeville, South Africa) and as directed by the manufacturers.

For the FC counts, the water samples were processed by making tenfold serial dilution with 100 ml of each composite and filtering 100 ml of choice dilution through membrane filters (47 mm diameter, 0.45 µm pore size; Millipore, County Cork, Ireland) attached to a vacuum pump. Thereafter, the Millipore filter papers were placed on chromocult coliform agar (Merck Wadesville, South Africa) and incubated at 37 °C for 24 h. The FC counts were reported as colony-forming units per 100 ml.[14]

Detection, purification, and biochemical test of Salmonella species

Pre-enrichment of the samples was done by transferring 1 ml of the sample to 9 ml of buffered peptone water and was incubated for 18 h at 37 °C. Following incubation, 0.1 ml was transferred to 10 ml of Rappaport-Vasiliadis broth (RV) (Oxoid)) and incubated for 24 - 48 hrs at 42 °C,[6] after which a loopful of the broth was plated onto salmonella-shigella agar (SSA) (Oxoid) and incubated. Plates with distinct colonies were sub-cultured on fresh labelled nutrient agar plates until pure isolates were obtained. Colony morphology and microscopic examination were used to pre-characterize the isolates. Discreet colonies were passed through necessary biochemical tests, including gram staining, catalase, oxidase, coagulase, blood haemolysis, Voges-Proskauer, citrate, glucose fermentation, sucrose, mannitol, lactose, xylose, urease, and IMVIC test. Also, bacterial isolate identifications were aided by comparison with known taxa as described in Bergy's Manual of Determinative Bacteriology.[15]Presumptively identified *Salmonella* isolates were stored in 15 % glycerol-based agar slants, at -4 °C pending further analysis.

Molecular identification and resistance gene elucidation of isolates

The pure isolates were molecularly elucidated and involved standard protocols as demonstrated by Saitou and Nei,[16] Tamura et al.,[17] and Kumar et al.[18]for the DNA extraction, PCR amplification, agarose gel electrophoresis, 16S rRNA sequencing, and sequence analysis.

Antibiotics susceptibility test

Kirby-Bauer sensitivity techniques were employed for the antibiotic sensitivity tests, involving ciprofloxacin (5 µg), chloramphenicol (30 µg), amoxicillin (30 µg), and streptomycin (10 µg). A loopful of the isolate was inoculated onto nutrient broth and incubated for 24 hours. Approximately, 0.2 ml of the 24 h culture of the test organisms was added to 20 ml of sterile nutrient broth incubated for 3–5 hours and adjusted to 0.5 McFarland standards (10^6 CFU/ml) before use.[19] 0.1 ml of the standardized culture was aseptically inoculated into a 20 ml molten Mueller-Hinton agar and gently swirled to effect mixing.

The medium was poured aseptically into sterile Petri dishes and allowed to solidify. The plates were labelled appropriately. This was repeated for the rest of the isolated culture. After solidifying, a sterile forceps was used to implant the commercial antibiotics discs onto the surface of the medium" aseptically. This culture medium was incubated at 37 °C for 24 hours. Petri dishes were examined and the inhibition zones across the various antibiotics were determined.

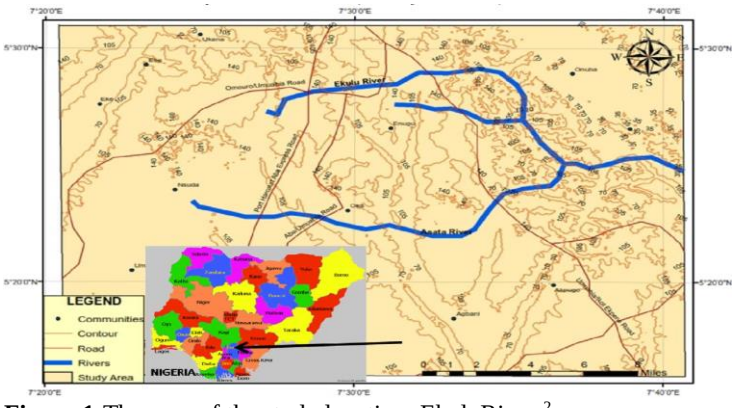

Figure 1: The map of the study location: EkuluRiver.[2]

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Statistical analysis
The study data was descriptively and statistically analysed using the SPSS software, Version 17, employing descriptive statistics for the aggregation of the data and the generation of graphs.

Results and Discussion

Mean fecal count
The mean Fecal count (Figure 2) from the month of September to June was evaluated. The faecal count ranged from 35,000 – 45,000 CFU/100ml, with the highest being observed in June and the least in April and December.

The mean faecal counts of different sites were also evaluated. The faecal count ranged from 37233.3 – 41900 CFU/100ml, with the highest count observed in site 8 and the least in site 2 (Figure 3).
The study results, though unsurprising, highlight the heavy bio-load of *Salmonella* spp. in the study site, a river sourced for drinking, bathing, recreational activities, food preparation, or other purposes, without pretreatment. Thus, shows the potential implications, including the public risks of developing bacteremia, meningitis, diarrhoea, and other water-borne illnesses.[20] This is attributable to the continuous inflow of effluent from sewage pipes and the direct discharge of highly contaminated human sewage due to deteriorating septic tanks.[21]
The result showed the occurrence of *Enterobacteriaceae* in the collected river water samples assessed for bacteriological quality. The detection of *Salmonella* spp. is a confirmation of faecal contamination and the possible presence of other pathogens. The possible causes of the varying percentage of occurrence may be due to the nature of the surroundings from which such water is collected, and the presence/absence of minerals required by individual organisms for their survival and reproduction.[22]
River water, however, has been demonstrated to be one of the largest reservoirs of viable *Salmonella* and is often linked to animal ancestors. Rainwater runoff may directly carry this pathogen from the excretions or exudates of wild animals to irrigation ponds or rivers.[23] Also, cross-contamination can occur using river water as the source of irrigation supply for agricultural purposes[23] *Salmonella* in manure is estimated to last for up to 231 days, and eventually contaminate farm produce through splashing from rainwater or surface irrigation water.

Pre-identification and biochemical test results
Following the morphological identification, characteristic isolates with results corresponding to Table 1, indicative of typical *Salmonella* spp were selected.

Molecular identification of Salmonella species
The Accession number, Sequence, E-value and percentage similarity were considered during identification (Table 2), with the gel image showing amplification of the 16SrRNA gene presented in Figure 4. The molecular characterization of the *S. enterica* strains showed a similarity index ranging from 85.63 - 99.10 % similarity.

Susceptibility to the used antibiotics
From the analysis, chloramphenicol showed a high sensitivity of 80 % to the isolates, ciprofloxacin showed 16 %, and streptomycin showed 4 %. While amoxicillin showed 98 % resistance (Table 3). The high resistance observed in some of the isolates against the corresponding commonly prescribed antibiotics may be attributed to drug misuse and abuse, leading to the development and transmission of antibiotic-resistant genes.[22]

Table 1: Biochemical Characterization and Identification of *Salmonella* sp isolated from Ekulu River

Biochemical test	Result
Catalase	Negative
Coagulase	Negative
VP	Negative
Citrate	Positive
TSIA	Alkaline/Acid with H_2S
Indole	Positive
Urease	Negative
Oxidase	Negative
Methyl red	Positive
Gram stain	Gram-negative rods

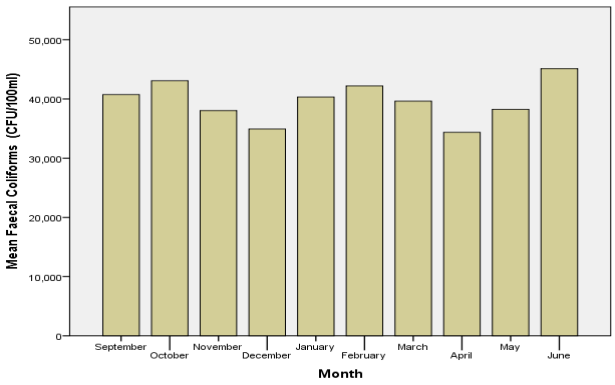

Figure 2: The mean Fecal count of Ekulu River.

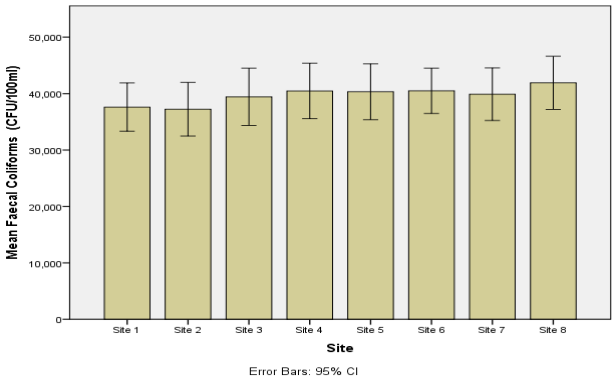

Figure 3: Mean faecal count of water samples from different sites

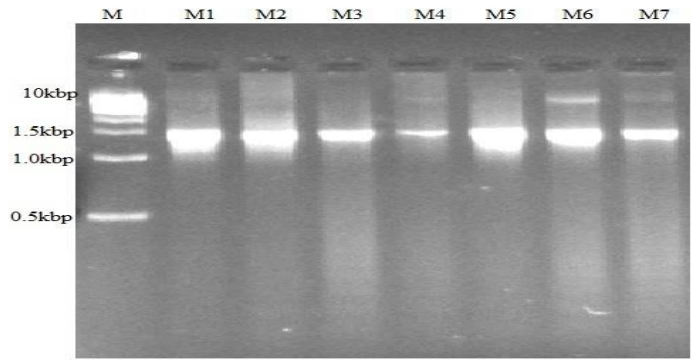

Figure 4: Gel image showing amplification of 16SrRNA gene of *Salmonella* sp

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Table 2: Molecular identification of *Salmonella* species

S/N	Sample code	Accession Number	Sequence	E-value	% Similarity	Probable Isolate
1	M1	MH352205	CMO1	0	85.63	*S. enterica* subsp. Enterica serovar Enteritidis strain CP018642
2	M2	CP03O209	CMO2	0	99.02	*S. enterica* strain SA20044414
3	M3	CP018219	CMO8	0	99.10	*S. enterica* subsp. Enterica strain LSP 389/97
4	M4	CPO32444	CMO4	0	98.70	*S. enterica* subsp. Enterica serovar Freshnostrain USMARC-69835
5	M5	JQ694241	CMO5	0	95.30	*S. enterica* subsp. Enterica serovar Bareillystrain 170
6	M6	CP032815	CMO6	0	97.60	*S. enterica* subsp. Enterica strain 11TTU1615b
7.	M7	CP030211	CM07	0	98.7	*S. enterica* strain SA20051528

Table 3: Profile of *Salmonella* isolates to antibiotics

Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
Ciprofloxacin (5 µg)	36	48	16
Chloramphenicol (30 µg)	16	4	80
Amoxicillin (30 µg)	98	2	0
Streptomycin (10 µg)	48	48	4

The resistance fluoroquinolone genes in *Salmonella* sp isolated were evaluated. Genes such as *QnrA, QnrSM, Par C*, and *GyrA* were analyzed (Figures 5 - 8).

Percentage of fluoroquinolone genes in the isolates

The Percentage of Fluoroquinolone genes in the isolates was evaluated. Fluoroquinolones genes, *QnrA, QnrSM, ParC,* and *GyrA* genes (Table 4) were present in the isolates with *QnrA*as the most dominant, followed by *QnrSM, GyrA,* and *ParC,* respectively. The percentage rate of isolates carrying the *QnrA*gene among quinolone-resistant *salmonella* was 100 %. Thus, while all the isolates possessed *QnrA*, four (4) but M2, M4, and possessed *ParC*, six (6) but M2 possessed*QnrSM*, and four (4) but M1, M4, and M5 possessed *GyrA*.Falodun& Oladimeji[21] and Mustapha & Imir[22] reported a significant rate of multi-drug resistance among some *Enterobacteriaceae* strains from clinical, environmental, and hospital food sources, and showed without a doubt, the creation of enzymes encoding for multidrug-resistant genes carried on multiple plasmids, as evidenced by the gene present in *Salmonella*, is the main mechanism of the reported multidrug resistance to antimicrobials and resulting from the overuse of antibiotics which would lead to strong selection pressure for resistant bacteria in the study area.[24]

Conclusion

Of public health concern, the study highlighted the presence of potentially pathogenic *Salmonella* spp. in a commonly used surface river in Enugu state, Nigeria. The study revealed the ranging resistance of the isolates to the test antibiotics and the corresponding occurrence of resistance genes. And further underlines the effect of the anthropogenic activities around the study site and leading to the cross-contamination of pathogenic bacterial strains. Thus, there is a need for adequate concern, including more elaborate surveillance, involving other key bacterial genera and species and the development of effective intervention policies, regulations, and appropriate enforcement.

Conflict of Interest

The authors declare no conflict of interest.

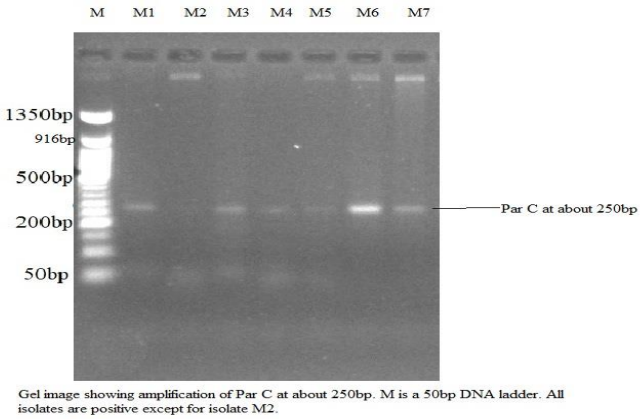

Figure 5: Gel image showing amplification of ParC of *Salmonella* sp. at about 250bp

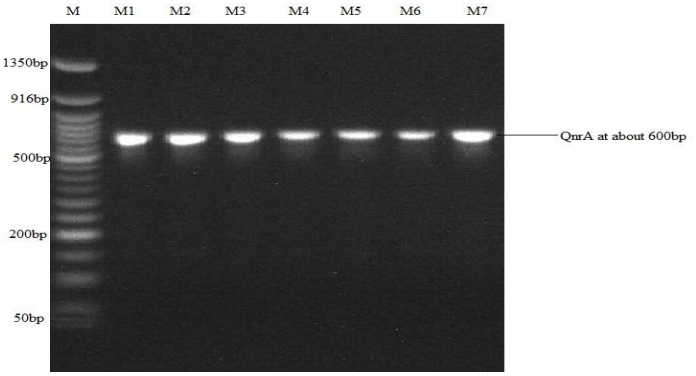

Figure 6: Gel image showing amplification of QnrA of *Salmonella* sp. at about 600bp

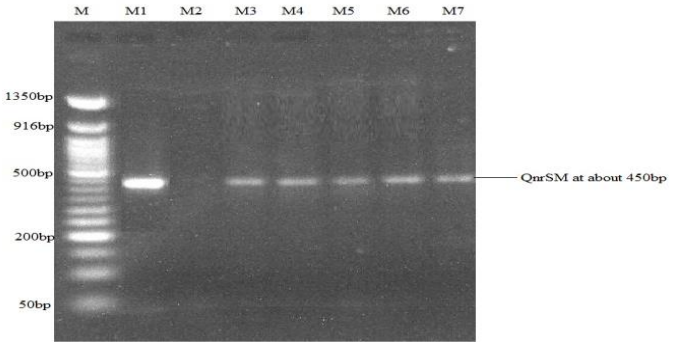

Figure 7: Gel image showing amplification of QnrSM of *Salmonella* sp. at about 450bp

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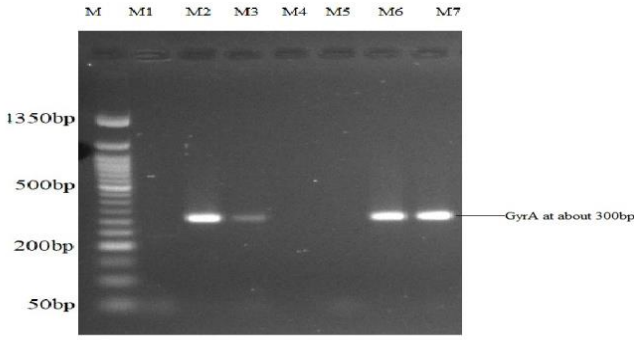
Table 4: Percentage of fluoroquinolone-resistant genes in *Salmonella* isolates

Gene	*Salmonella*	Primer	Sequences	Amplicon size
QnrA	7(100 %)	QnrA-F,	5'AGAGGATTTCTCACGCCAGG'3	600bp
		QnrA-R	5'TACCAGGCACAGATCTTGAC'3	
QnrSm	6(85.7 %)	QnrSmF	GCAAGTTCATTGAACAGGGT'3	450bp
		QnrSmR	TCTAAACCGTCGAGTTCGGCG'3	
GyrA	4(57.14 %)	GyrA-F	5'CGTTGGTGACGTAATCGG'3	300bp
		GyrA-R	5'CCGTACCGTCATAGTTAT'3	
ParC	4(57.14 %)	ParCF	5'CTATGCGATGTCAGAGCTGG'3	250bp
		ParCR	5'TAACAGCAGCTCGCGTATT'3	



Gel image showing amplification of GyrA at about 300bp. M is a 50bp DNA ladder. isolates M2, M3, M6 and M7 are positive while isolates M1, M4 and M5 are negative..

Figure 8: Gel image showing amplification of G*yrA* of *Salmonella* sp. at about 300bp

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Kacholi DS, Sahu M. Levels and health risk assessment of heavy metals in soil, water, and vegetables of Dar es Salaam. Tanzania J. of Chem. 2018; 1-9.
2. Karim B, Samiha K, Houzi G, Hefdhallah A, Mohamed F. Physicochemical Properties of Water Used for Irrigation of Rice (*Oryza sativa*) in the Gharb Plain, Morocco. Trop J. Nat Prod Res. 2022; 6(5):721-727.
3. Ken-Onukuba DN, Okeke OC, Amadi CC, Akaolisa CCZ, Okonkwo SI, Offoh JI, Nwachukwu HGO. Water quality assessment of Ekulu and Asata rivers in Enugu area, Southeastern Nigeria, using physico-chemical and bacteriological parameters. J. of Geog Envi and Earth Sci Intern. 2022; 11(4):70-92.
4. Gomez M, Perdiguero J, Sanz A. Socioeconomic factors affecting water access in rural areas of low and middle income countries. Water. 2019; 11(2):202.
5. Adamu I, Andrade FCD, Singleton CR. Availability of drinking water source and the prevalence of diarrhea among nigerian households. Am J. Trop Med Hyg. 2022; 6;107(4):893-897. Doi: 10.4269/ajtmh.21-0901
6. Adiele G, Ojeaga K, Ekhator OC, Akhionbare SO. Effects of abattoir waste discharge on the quality of the Trans-Amadi creek, Port Harcourt, Rivers State, Nigeria. Trop J. Nat Prod Res. 2019; 3(4):132-137. Doi: 10.26538/tjnpr/v3i4.5
7. Caterina L, Lucia B, Rossella B, Elisabeth G, Simon T, Valter T. *Salmonell*a in surface and drinking water: occurrence and water-mediated transmission, Food Res Internat.2012; 45(2): 587-602. Doi: 10.1016/j.foodres.2011.06.037
8. Pal M, Merera O, Abera F, Rahman MT, Hazarika RA. Salmonellosis: a major foodborne disease of global significance. Bever and Food World. 2015; 42(12): 21-24.
9. Jajere SM. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Veterin World. 2019; 12(4): 504.
10. Deen J, Von Seidlein L, Andersen F, Elle N, White NJ, Lubell Y. Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review. The Lan Infect Dis. 2012; 12(6):480-487.
11. Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon, MA. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. The Lan. 2013; 379(9835): 2489-2499.
12. Ezenwaji EE, Eduputa BM, Uwadiegwu BO. Pollution of Ekulu River in Enugu: A case of negative human impact on the environment. J. of Envi Sci, Toxicolo and Food Tech. 2014; 8(10):83-92.
13. Rompré A, Servais P, Baudart J, de-Roubin MR, Laurent P. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J. Microbiol Method. 2002; 49(1):31-54. Doi: 10.1016/s0167-7012(01)00351-7.
14. Buchanan RE, Gibbons NE, Cowan ST. Bergey's manual of determinative bacteriology. 1974.
15. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Ameri Soci for Micro. 2009; 15:55-63.
16. Dahunsi SO, Owamah HI, Ayandiran TA, Oranusi SU. Drinking water quality and public health of selected towns in South Western Nigeria. Wat Qual Expos Heal. 2014; 6:143-153.
17. Ruby T, McLaughlin L, Gopinath S, Monack D. *Salmonella*'s longterm relationship with its host. FEMS Microbiol Rev. 2012; 36:600-615.
18. Oludairo OO, Aiyedun JO. Contamination of commercially packaged sachet water and the public health implications: an overview. Bang J. of Vet Medi. 2015; 13(2):73-81.

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19. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mole Biol and Evolut. 1987; 4:406-425.
20. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceed of the Nat Acad of Sci (USA). 2004; 101:11030-11035.
21. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol and Evol. 2018; 35:1547-1549.
22. Andino A, Hanning I. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. Sci World J. 2015; 52:1-79.
23. Chuah LO, Effarizah ME, Goni AM, Rusul G. Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. Curr Environ Healt Rep. 2016; 3:118-127.
24. Falodun OI, Oladimeji OS. Extended Spectrum Beta Lactamase (ESBL) producing enteric bacteria from hospital wastewater, Ibadan, Nigeria. World News of Nat Sci. 2019; (22):62-74.
25. Mustapha A, Imir T. Detection of multidrug-resistance gram-negative bacteria from hospital sewage in North East, Nigeria. Fron Environ Microbiol. 2019; 5(1):1-7
26. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. J. of Cel. 2017; 128(6):1037-1050.

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