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Molecular Characterization and Virulence Profiling of *Yersinia ruckeri* and Associated Enteric Bacteria in a Tropical River System: Implications for Consumer Health in Edo State, Nigeria

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Abstract: Anthropogenic alterations in aquatic ecosystems have made natural water bodies carriers of waterborne pathogens and associated public health risks. This study evaluated the molecular and virulence characteristics and antibiotic profiles of *Yersinia ruckeri* and associated enteric bacteria in the Ossiomo River system. Triplicate water samples were collected at monthly intervals over 12 months (July–Dec, 2019 and Jan–June, 2021) across station 1 (Upstream), station 2 (Midstream), and station 3 (Downstream) for bacteriological analyses. Enumeration and isolation of total heterotrophic bacteria (THB), total coliforms (TC), and *Yersinia* species were performed using standard bacteriological techniques. Phenotypic virulence traits were assessed using standard hemolytic assays, and antibiotic susceptibility profiles were determined by the Kirby-Bauer disk diffusion method. Molecular characterization was conducted using standard molecular procedures. *Yersinia* spp. counts ranged from 0.0±0.0×103 cfu/mL (upstream) - 5.0±3.5×103 cfu/mL (midstream), THB counts ranged from 1.1±0.0×103 cfu/mL (upstream) - 17.8±4.9×103 cfu/mL (midstream), while the mean coliform load ranged from 14 MPN/100 mL upstream - 201 MPN/100 mL midstream. Taxonomic identification confirmed the presence of *Yersinia ruckeri* at 100% identity (MK548507.1), *Shigella flexneri* at 91.57% (MK918539.1), and *Providencia alcalifaciens* at 88.57% (HQ407250.1). Hemolytic assay demonstrated distinct virulence, with *Y. ruckeri* exhibiting alpha (α) hemolysis and *Escherichia coli* beta (β) hemolysis. *Y. ruckeri* and other enteric bacteria were susceptible to levofloxacin, ciprofloxacin, ceftazidime, gentamicin, cefuroxime, ceftriaxone, and ofloxacin. The presence of these virulent, hemolytically active strains in Ossiomo River serves as a reservoir for potent human pathogens, mandating stringent, pre-consumption purification interventions to mitigate public health risks.

1. Introduction

Globally, the anthropogenic actions and environmental contamination of tropical river systems have become critical ecological and public health concerns. The uncontrolled discharge of hazardous effluents has continuously threatened the aquatic physicochemical matrices from essential water resources into vehicle for the transmission of human waterborne illnesses (Sener *et al.* (2017), (Kumar and Singh, 2018).

In developing nations including Nigeria, rapid demographic shifts have accelerated the settlement of populations along coastal areas. These communities are profoundly dependent on rivers as well as streams for drinking and their basic domestic water requirements, without essential pre-consumption purification processes (Servais *et al.* (2007). Consequently, these untreated water resources are reservoirs of uncultivated, unidentified, and highly diverse pathogenic microorganisms (WHO, 2003). Surface waters heavily polluted by sewage materials encourage dense, heterogeneous microbial consortia such as viruses, bacteria, fungi, and protozoans (WHO, 2003), (Servais *et al.* (2007). A large proportion of these contaminants, which are of fecal origin, are introduced into natural aquatic habitats via open defecation and specific point sources, including poorly managed municipal treatment facilities, urban drainage systems, and effluents originating from abattoirs and livestock operations (Okoh *et al.,* 2007), (Igbinosa and Okoh, 2009), (Boutebib *et al.,* 2023), (Errich *et al.,* 2023). Among these microbial populations, *Yersinia ruckeri* and other enteric bacteria are members of the family Enterobacteriaceae, which constitutes one of the most prominent bacterial groups isolated from polluted aquatic ecosystems. Historically, *Y. ruckeri* has been responsible for Enteric Redmouth Disease (ERM) in salmonids, as well as infections in non-salmonid species such as sturgeon, catfish, carp, perch, and burbot (Kumar *et al.* (2015). However, a shift in epidemiological perspectives, notably in recent clinical investigations, has documented the isolation of *Y. ruckeri* from infected human wounds (De keukeleire *et al.,* 2014), as well as from commercial food matrices such as milk, cheese, poultry, and minced meat (Ozdemir and Arslan, 2015).

Waterborne diseases primarily mediated by the ingestion of water containing fecal coliforms and associated enteric pathogens demand the tracking of the emerging threat of *Y. ruckeri* (Adeyinka *et al.,* 2014). Hence, this study aimed to evaluate the molecular and virulence characteristics of *Yersinia ruckeri* and related enteric bacteria, and the potential disease burden on populations depending on this tropical river system in Edo State, Nigeria.

2. Materials and Methods

2.1. Study area

This study was carried out in Ossiomo River (Latitudes $6^0$30'- $6^0$32'0''N; Longitudes $5^0$39'-$5^0$40'30''E), a tributary of Benin River located in Abudu community, Orhionmwon Local Government Area, Edo State, Southern Nigeria. Ossiomo River stretches over 250 km within Edo and Delta States, Nigeria. Samples were collected along the stretch of the river from three (3) different sampling locations, namely Upstream, Midstream, and Downstream.

2.2 Sample collection

Water samples were collected in triplicate at monthly intervals from July 2019 – June 2021, covering three sampling locations, across station 1 (Upstream), station 2 (Midstream), and station 3 (Downstream). Samples were collected in sterile 1 L screw-cap glass bottles and thereafter transported to the laboratory for bacteriological analyses (APHA, 2005).

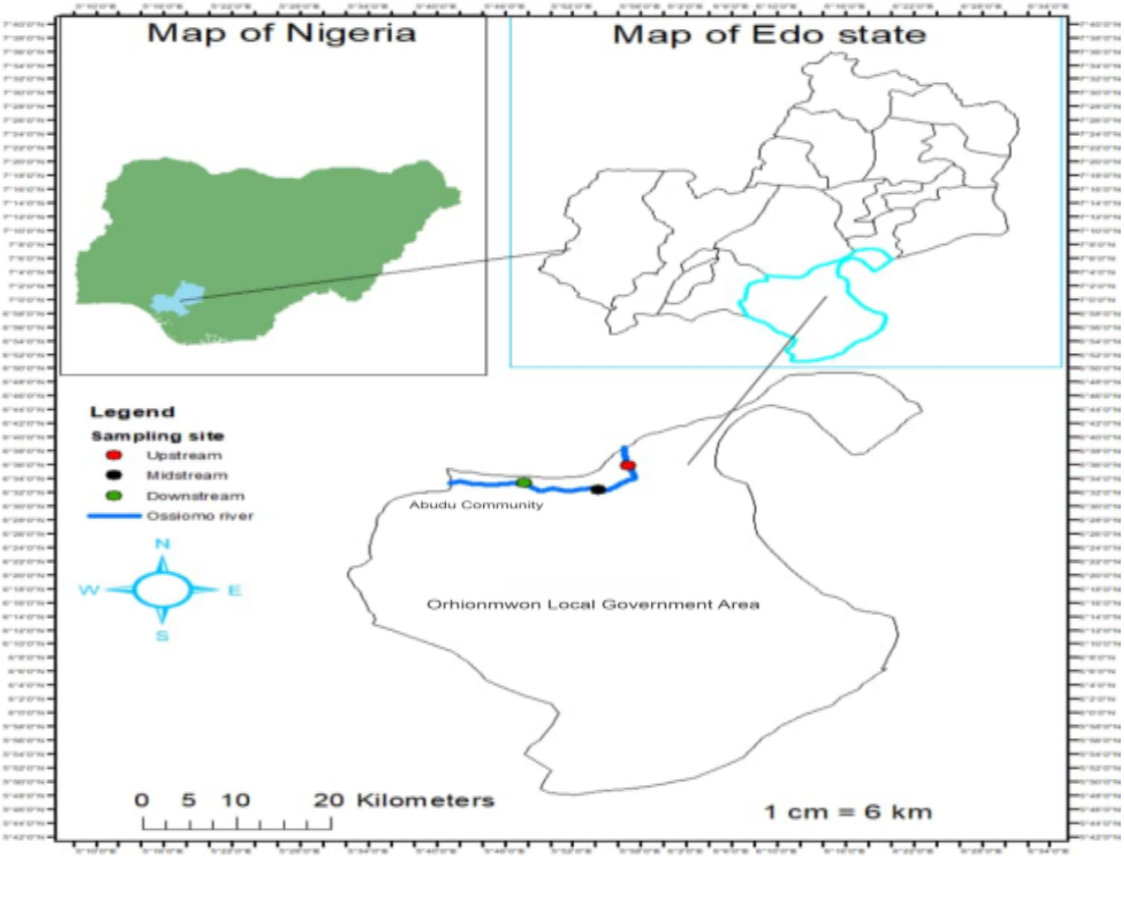

Figure 1: Map of Edo State showing the sampling sites along Ossiomo River stretch.

1.1 *Bacteriological analyses of sampled water*

Isolation and enumeration of total viable bacterial isolates were cultured using nutrient agar, MacConkey agar, and eosin methylene blue agar as suitable media for isolation of total heterotrophic bacteria using pour plate methods, while total coliforms were determined using Most Probable Number (MPN) according to Cheesebrough, (2000).

2.4 Enumeration of total heterotrophic bacteria

The population of bacteria in the samples was enumerated using the serial dilution method according to Cheesebrough, (2000). One (1) ml of the water sample was serially diluted in sterile distilled

water, and the diluents were aseptically plated into nutrient agar. The agar plates were inverted and incubated at 37°C for 24 hours to enumerate the aerobic bacteria. The colonies on plates were counted using a colony counting machine, and results were expressed as colony forming unite cfu/mL of the samples. Plates were incubated at 37 ^{0}C for 24 hours, and discrete colonies were further subcultured onto Nutrient agar to obtain pure isolates. Pure culture isolates were stored in Nutrient agar slant at 4 ^{0}C according to methods (Cheesebrough, (2006).

2.5 Most Probable Number

2.5.1 Presumptive test

This test was carried out using 15 sets of test tubes containing lactose fermentation broth. Each tube contained 10 mL of fermentation broth and was inoculated with the water sample in a sequential order of 10 mL in five of each lactose fermentation broth, 1 mL in five of each lactose fermentation broth, and 0.1 mL in five of each lactose fermentation broth. All the test tubes were incorporated with Durham tubes for detection of gas fermentation by Gram-negative coliform bacteria. The test tubes were capped and incubated at 37°C for 24 to 48 hours. The presence of Gram-negative bacteria was indicated by gas formation, indicated by downward displacement of the broth in the Durham tubes and colour change.

2.5.2 Confirmed test

Positive samples that produced gas in the lactose fermentation broth were selected for the confirmatory test procedure to detect the indicator bacteria of fecal origin. Another 15 sets of test tubes containing 10 mL each of lactose fermentation broth were inoculated with positive tubes in sequential order of 10, 1, and 0.1mL in five of each containing a Durham tube and incubated for 24 hours at 37 °C to reconfirm the positive lactose fermentation.

2.5.3 Completed test

The complete test for coliform bacteria was performed using eosin methylene blue agar (EMB), which differentiated Escherichia coli from other Gram-negative coliform bacteria. A loopful of the sample from the positive test tubes was streaked onto EMB culture plates and incubated at 37°C for 48 hours. The production of a green metallic sheen indicated the presence of E. coli.

2.6 Isolation and enumeration of Yersinia spp.

Buffered peptone water (BPW) was used as a cold enrichment medium for isolating *Yersinia* spp. A 100 ml volume of water sample was filtered through a 0.45µm filter, which was transferred to 20 ml of BPW on the same day, after 7 days, and 21 days incubation at 4 ^{0}C. 0.1 ml of the BPW was streaked out onto 75258 *Yersinia* selective supplement medium (Cefsulodin 7.50 mg/500 ml medium, Triclosa 2.0 mg/ 500 ml medium, Novobiocin 1.25 mg/ 500 ml medium) agar (CIN-agar, Difco) and incubated overnight at 25° and 37°C according to Sahota *et al.,* (2014). Distinct convex colonies with red centres and white to translucent periphery, characteristic bull's eye morphology that were 2 to 3 mm in diameter were considered presumptive *Yersinia* colonies. Positive *Yersinia*

isolates were further identified based on Gram staining, motility (at 25ºC), morphology under the microscope, and biochemically based on urease, indole, catalase, methyl red (MR), Voges-Proskauer's (VP), triple sugar iron (TSI), citrate utilization, nitrate reduction, mannitol, and oxidase test according to (Cheesebrough, (2006). The culture was stored at 4ºC and maintained on *Yersinia* selective agar slant. For each experiment, cultures were taken from the frozen stocks and subcultured before use to curb changes in virulence.

2.7 Phenotypic and biochemical identification of isolates

The phenotypic identification of bacterial isolates was carried out with a focus on Gram staining and biochemical reactions using standard techniques according to the methods of (Cheesebrough, (2000). Gram staining technique was carried out to determine the shape, arrangement, and classification of the bacterial isolates, and conventional biochemical tests were conducted for further identification according to (Cheesebrough, (2000). Suspected colonies were subjected to different biochemical tests, including oxidase, indole, urease, citrate, fructose, galactose, lactose, maltose, mannitol, mannose, nitrate reduction, triple sugar iron (TSI), and sucrose.

2.8 Bacterial DNA extraction

Bacterial DNA was extracted according to the lysozyme-based cell lysis and DNA precipitation procedure for bacteria (Klindworth *et al.* (2013). The DNA obtained was spectrophotometrically quantified using the NanoDrop ® Technologies (USA) at 260nm and the purity level of DNA samples was determined at a ratio of absorbance (A260/A280). The purity ratio ranging from 1.8 to 2.2 was used as a DNA template for Polymerase Chain Reaction (PCR) (Lee *et al*. (2003), (Fietto *et al*. (2004).

2.9 Amplification of the 16S rRNA genes

The 16S rRNA gene from total bacterial isolates and genomic DNA, respectively, was amplified by Polymerase Chain Reaction (PCR) using universal bacterial primers (27F-AGAGTTGATCCTGGCTCAG and 1492R-GGTTACCTTGTTACGACTT). The PCR amplification was carried out in a Techne TC-412 Thermal Cycler (Model FTC41H2D, Bibby Scientific Ltd, UK) in a 50 µl reactions containing 25 µl of 2 × PCR Master Mix (Norgen Biotek, Canada), 1.5 µl of template DNA (0.5 µg), 1 µl of both forward and reverse primers (2.5µM of each) and 21.5µl of nuclease free water in a PCR tube added in that order. PCR was carried out at an initial denaturation step at 94ºC for 2 min, followed by 30 cycles at 94ºC for 30 sec, 52ºC for 30 sec, and 72ºC for 2 min, and a final extension step at 72ºC for 5 min. PCR products (amplicons) were separated by electrophoresis on a 1% agarose TAE gel containing ethidium bromide and visualized by UV trans illumination (Foto/UV 15, Model3-3017, Fotodyne, USA) according to (Klindworth *et al.* (2013).

2.10 Determination of hemolytic properties of bacterial isolates

The methods of (Mulamttathil *et al*. (2014) were used to test for isolates' hemolytic activity on Nutrient agar (Biolab, Merck, SA) supplemented with 5% (v/v) sheep blood. Bacterial isolates were

streaked onto blood agar plates and incubated at 37°C for 24 and 48 hours. After incubation overnight, the medium was checked for signs of alpha, beta, or gamma-hemolysis, which were reported according to methods (Mulamttathil *et al*. (2014) and (Delor *et al*. (1990). The β- hemolytic zones developed by bacterial isolates were wider (13.7±0.7 cm), α- hemolytic zones (9.3±0.1 cm), and δ-hemolytic zone was not observed. Positive control showed transparent zones around colonies, while negative control showed no hemolytic activity.

2.11 Antibiotic susceptibility test of bacterial isolates

The antibiotic susceptibility test for each isolate was performed on freshly prepared, dry-surface Mueller-Hinton agar (Oxoid) using the Kirby-Bauer disk susceptibility method according to the Clinical Laboratory Standards Institute (CLSI, 2008). A total of Eleven (11) tested antibiotics disc (Oxiom) were employed, namely augmentin (30 µg), ceftriaxone (30 µg), nitrofurantoin (300 µg), ofloxacin (5 µg), azithromycin (15 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), pefloxacin (5 µg), and ceftazidime (30 µg). The ranges of the diameter were measured in millimeters (mm) of each antibiotic disc and interpreted as susceptible, intermediate, or resistant compared with the performance standards of (CLSI, (2008).

2.12 Statistical analysis

Raw data were cleaned on a Microsoft Excel sheet; mean and standard deviation were extrapolated. Graphs were also plotted. Data generated were analyzed by one-way analysis of variance (ANOVA) to determine the significant differences in group results. SPSS version 21 and GraphPad Prism version 6.0 were used (Ogbeibu, 2005).

2. Results and Discussion

Presented in **Table 1** are values obtained for *Yersinia* spp. across the monitored stations and periods ranged from 0.0±0.0 - 5.0±3.5×10^3 cfu/mL. A distinct value was observed at the upstream station, which consistently yielded zero counts 0.0±0.0×10^3 cfu/mL during the dry season months of January, February, and March, whereas downstream samples maintained consistently low counts across both seasons. Conversely, the midstream station exhibited the highest counts of 5.0±3.5× 10^3 cfu/mL. The proliferation of *Yersinia* spp. within the midstream and downstream stations can be attributed to the elevated index of anthropogenic activities characterizing these specific river segments. Notably, the bacterium exhibited a significantly higher frequency of isolation during the wet season months (July – September) compared to the dry season periods (November – May). This seasonal trend aligns with the findings of (Coquet *et al.* (2002) regarding the environmental occurrence and phenotypic characterization of *Yersinia ruckeri* strains exhibiting biofilm-forming capacities within aquaculture systems. Historically, human yersiniosis linked to the consumption of contaminated water has been underreported, a phenomenon often determined by virulence characteristics of the agent (Sahota *et al.* (2014). Different animal species serve as the primary zoonotic reservoir for this pathogen, shedding the bacterium via fecal matter into the surrounding environment. During rainy season, agricultural runoff and soil erosion facilitate the transportation of these terrestrial pathogens into

nearby waterbodies, establishing a primary transmission vector to human consumers (Romalde *et al.* (2003). While *Y. ruckeri* is widely responsible for diverse pathologies across multiple fish species including carp, catfish, sturgeon, perch, and burbot (Kumar *et al.* (2015), it remains a threat within the aquaculture industry as the etiological agent of Enteric Redmouth Disease (ERM) in salmonids, inducing severe economic losses (Guijaro *et al.* (2018). However, its role as an emerging zoonotic threat is increasingly documented. (De keukelire *et al.* (2014) reported the isolation of *Y. ruckeri* from a human wound infection acquired via river water exposure, while (Ozdemir and Arslan (2015) confirmed its prevalence as a contaminant in commercial food matrices, including milk, cheese, chicken, and minced meat.

Table 2 presents the total heterotrophic bacterial counts, ranging from 1.1±0.0 - 17.8±4.9×10^3 cfu/mL across the sampling stations and periods. The highest bacterial counts were recorded at the midstream station during August, 17.8±4.9×10^3 cfu/mL, followed by the downstream station, 13.6±2.4×10^3 cfu/mL. However, the lowest count of 1.1±0.0×10^3 cfu/mL was reported in upstream in May. The significant differences ($P < 0.05$) observed among the upstream, midstream, and downstream stations during the wet season indicate high bacterial load. A condition influenced by nutrient-rich agricultural runoffs and industrial effluents containing heavy organic pollutants that are largely absent or diluted during the dry season is introduced into the river systems by rain. This finding conforms with the reports of (Fagorite *et al.* (2019) on the Otamiri River in Owerri, which similarly correlated elevated wet-season bacterial loads with intense organic pollution. The mean total coliform counts shown in **Table 3** further validate the impact of location and seasonal variations on bacterial proliferation in aquatic systems. The upstream station revealed the lowest bacterial counts, with values ranging from 17 to 126 MPN/100 mL, while the midstream station revealed the highest coliform counts, ranging between 31 and 201 MPN/100 mL. The elevated coliform counts reported in the midstream station are attributed to high anthropogenic pressures and point-source contamination. This finding corresponds with the work of (Aieze *et al.* (2016) who noted that high bacterial density serves as a reliable proxy for the extent of organic matter enrichment in surface waters. According to (Mulamattahil *et al.* (2014), surface water contaminants extensively degrade water quality, rendering it unfit for domestic uses and direct human consumption without prior treatment.

Table 4 revealed the results of the molecular identification of bacterial isolates, percentage identity and accession number of *Yersinia ruckeri* 100 % (MK548507.1), *Providencia rettgeri* 99.62 % (MT276161.1), *Klebsiella variicola* 99.38 % (MT000001.1), *Shigella sonnie* 99.14 % (MF399269.1), *Pseudomonas otitidis* 98.78 % (OK035334.1), *Providencia stuartii* 98.54 % (MF671921.1), *Proteus cubarius* 98. 15 % (MN922513.1), *Salmonella enterica* 98.03 % (MH352201.1), *Proteus mirabilis* 97.36 % (JF784035.1), *Klebsiella pneumonia* 96.67 % (MG201994.1), *Serratia marcescens* 96.31 % (MW467370.1), *Escherichia coli* 95.87 % (MF754146.1), *Ralstonia mannitolilytica* 95.28 % (MN022574.1), *Pseudomonas aeruginosa* 94.75 % (MK157268.1), *Shigella flexneri* 91.57 % (MK918539.1) and *Providencia alcalifaciencs* 88.57 % (HQ407250.1) respectively. According to Drancourt and Raoult (2005), bacterial isolates would belong to different species if the similarity in the 16S rRNA gene sequence between them were > 97% identity.

Table 1. Total *Yersinia* spp count of Ossiomo River during the study period

	2019								2021			
Sampling stations	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**March**	**Apr**	**May**	**June**
Upstream	0.3±0.6	2.3±1.2	3.7±2.9	1.3±1.5	1.0±1.0	0.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.6±1.2	0.7±1.2
Midstream	1.7±0.6	3.7±2.1	5.0±3.5	2.7±2.1	2.0±1.7	1.7±1.5	1.0±1.0	1.0±1.0	1.0±1.0	1.0±1.0	1.7±1.2	2.3±1.5
Downstream	1.7±0.6	3.7±2.1	3.7±1.2	1.3±1.5	0.3±0.6	0.3±0.6	0.3±0.6	0.0±0.0	0.3±0.6	0.3±0.6	0.7±1.2	1.0±1.0

Key: Value expressed as mean triplicates determination ±SD. Values are x10^3 cfu/mL. World Health Organization permissible limits for portable water=0

Table 2. Total heterotrophic bacteria count of Ossiomo River during the study period

	2019								2021			
Sampling stations	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**March**	**Apr**	**May**	**June**
Upstream	7.4±1.7	10.7±1.0	9.1±1.7	2.4±1.1	3.2±0.2	4.5±3.1	4.6±1.0	3.1±1.2	3.0±1.1	2.0±0.0	1.1±0.0	4.0±1.2
Midstream	15.9±7.0	17.8±4.9	16.0±1.8	9.2±6.7	5.4±2.1	4.7±2.4	5.0±2.2	4.2±1.2	4.0±1.2	3.1±0.1	3.1±0.1	9.1±2.1
Downstream	8.2±6.3	13.6±2.4	12.4±2.6	6.8±4.3	3.6±1.2	4.7±2.8	4.9±1.8	3.2±1.3	3.1±1.1	3.0±1.1	4.1±2.9	6.2±2.1

Key: Value expressed as mean of triplicates determination ±SD. Values are x10^3 cfu/mL. World Health Organization Permissible Limits for portable water= 0

Table 3. Mean total coliform count of Ossiomo River during the study period

	2019								2021			
Sampling stations	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**March**	**Apr**	**May**	**June**
Upstream	53	75	126	65	23	27	37	32	21	17	14	51
Midstream	83	201	189	121	76	79	66	53	43	31	23	62
Downstream	65	97	152	78	34	35	32	27	32	31	34	56

Key: Values expressed as mean of triplicate determinations. Values are MPN/100 mL. World Health Organization permissible limits for portable water=0

Conversely, two bacterial isolates would not belong to the same species if the similarity in 16S rRNA gene sequence between them is < 3 % identity (Drancourt *et al*., 2004). These data imply that an original 16S rRNA gene sequence exhibiting <97 % similarity with its closest relative would create the basis for the description of a new bacterial taxon as reported in the phylogenetic tree of bacterial isolates.

Table 4. Molecular identification, percentage identity and accession number of bacterial isolated from Ossiomo River

Bacterial isolates	% identity	Accession number
Yersinia ruckeri	100	MK548507.1
Serratia marcescens	96.31	MW467370.1
Klebsiella pneumoniae	96.67	MG201994.1
Pseudomonas aeruginosa	94.75	MK157268.1
Shigella sonnie	99.14	MF399269.1
Escherichia coli	95.87	MF754146.1
Klebsiella variicola	99.38	MT000001.1
Pseudomonas otitidis	98.79	OK035334.1
Salmonella enterica	98.03	MH352201.1
Shigella flexneri	91.57	MK918539.1
Providencia alcalifaciencs	88.57	HQ407250.1
Ralstonia mannitolilytica	95.28	MN022574.1
Proteus cibarius	98.15	MN922513.1
Proteus mirabilis	97.36	JF784035.1
Providencia rettgeri	99.62	MT276161.1
Providencia stuartii	98.54	MF671921.1

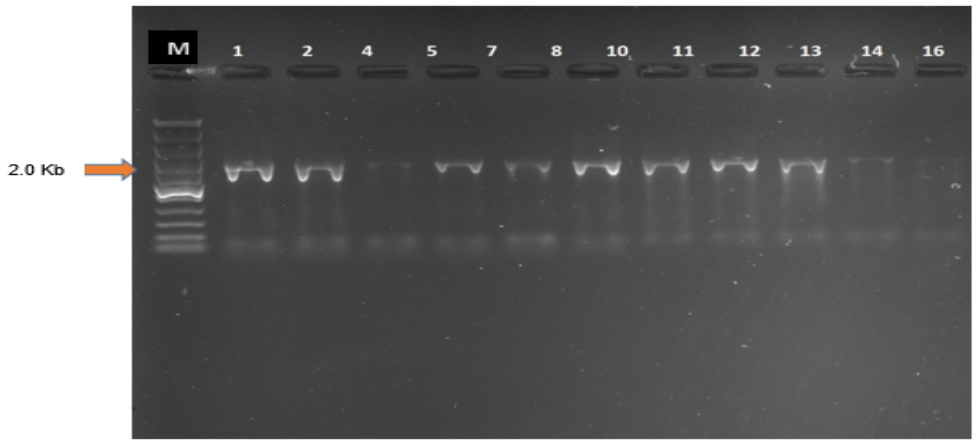

Plate 1. Agarose gel electrophoresis image of bacterial isolates from Ossiomo River. Lane M – molecular maker; Lane 1 - *Yersinia ruckeri*; Lane 2 - *Serratia marcescens;* Lane 3 - *Klebsiella pneumonia;* Lane 4 - *Pseudomonas aeruginosa;* Lane 5 - *Shigella sonnie;* Lane 6 - *Escherichia coli;* Lane 7 - *Klebsiella variicola;* Lane 8 - *Pseudomonas otitidis;* Lane 9 - *Salmonella enterica;* Lane 10 - *Shigella flexneri;* Lane 11 - *Providencia alcalifaciencs;* Lane 12 - *Ralstonia mannitolilytica;* Lane 13 - *Proteus cibarius;* Lane 14 - *Proteus mirabilis;* Lane 15 -*Providencia rettgeri*andLane 16 - *Providencia stuartii*

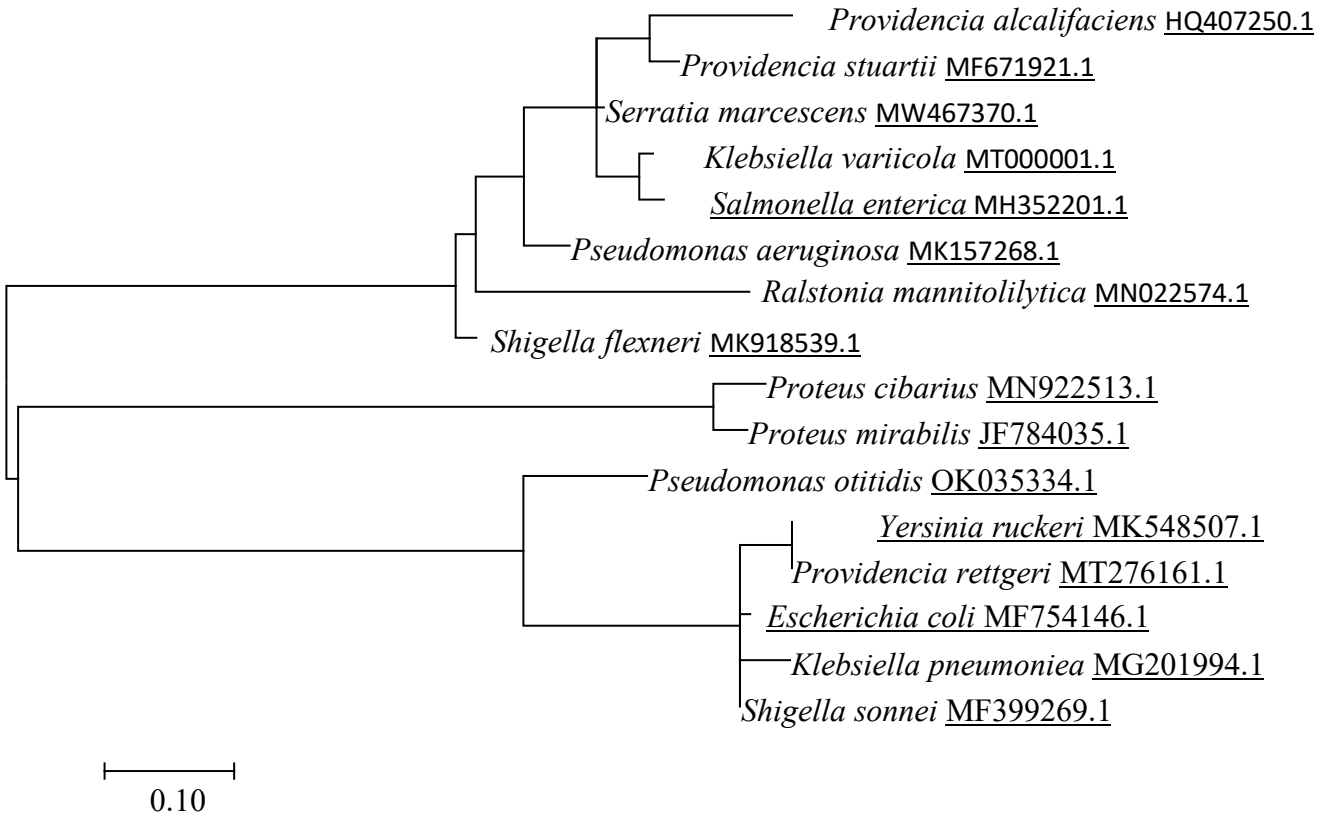

Figure 2. Phylogenetic tree of *Yersinia ruckeri* and other bacteria isolated from Ossiomo river water constructed using MEGA 11 with the Neigboring-Joining method-distance Kmura 2, for a 1500 bp fragment of the 16S rRNA coding region of the bacteria.

The percentage frequencies of occurrence of bacterial isolates from Ossiomo River are shown in **Table 5,** with *Escherichia coli* having the highest percentage frequency of occurrence (13.8 %), while the lowest was observed for *Yersinia ruckeri* (2.3 %).

Table 5. Percentage frequency of occurrence of bacterial isolates in Ossiomo River during the Study Period

	2019						2021						
Bacterial isolates	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Mean
Serratia sp.	6 (6)	10 (6.3)	10 (6.9)	6 (5.9)	5 (7.9)	5 (8.6)	8 (11)	6 (11)	5 (5)	5 (7)	6 (10.7)	11 (13.8)	6(7)
Shigella sp.	8(8)	18 (11.4)	16 (11.0)	16 (15.7)	5 (7.9)	4 (6.7)	10 (13.5)	9 (12.2)	8 (9.8)	9 (14.1)	9 (16.1)	10 (12.5)	10(11.5)
Ralstonia sp.	10 (10)	16 (10.1)	20 (13.7)	14 (13.7)	7 (11.11)	4 (6.9)	7 (9.5)	7 (5.5)	6 (8.4)	5 (7.8)	5 (8.9)	9 (11.3)	9(10.3)
Proteus sp.	13 (13)	18 (11.4)	17 (11.7)	10 (9.8)	8 (12.7)	5 (8.6)	7 (9.5)	7 (9.5)	14(19.7)	11 (17.2)	4 (7.1)	7 (8.8)	10(11.5)
Klebsiella sp.	12 (12)	20 (13)	17 (11.7)	14 (13.7)	7 (11.11)	6 (10.3)	8 (11)	7 (9.4)	8 (11.3)	7 (11)	7 (12.5)	10 (8)	10(11.5)
Escherichia sp.	15 (15)	32(20.3)	37 (25.5)	16 (15.7)	10 (15.9)	8 (13.8)	3 (4)	3 (2.3)	4 (5.6)	5 (7.8)	4 (7.1)	10 (12.5)	12(13.8)
Yersinia sp.	4 (4)	7 (4.4)	5 (3.4)	3(2.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	3 (3.8)	2(2.3)
Pseudomonas sp.	10 (10)	16(10.1)	12 (8.3)	7 (6.9)	6 (9.5)	17 (29.3)	8 (11)	16(21.6)	7 (9.9)	6 (9.4)	3 (5.4)	8 (10)	10(11.5)
Salmonella sp.	14(14)	21 (13.3)	11 (7.6)	9 (8.8)	15 (23.8)	9 (15.5)	9 (12.2)	9 (12.1)	7 (9.9)	5 (7.8)	13 (23.2)	13 (16.3)	11(12.6)
Providencia sp.	8(8)	9 (5.7)	12 (8.3)	11 (10.8)	9 (14.3)	7 (12.1)	15 (20.3)	5 (6.7)	5 (7)	5 (7.8)	7 (12.5)	6 (12.5)	8(9.2)
Total	100	158	145	102	63	58	74	74	71	64	56	80	87

Table 6 revealed the hemolysin production activities of all bacterial isolates on blood agar plates. *Yersinia ruckeri* showed (α) hemolysin, *Serratia marcescens* (α), *Klebsiella pneumoniae* (α), *Pseudomonas aeruginosa* (β), *Shigella sonnei* (α), *E. coli* (β), *Klebsiella varricola* (β), *Pseudomonas otitidis* (α), *Salmonella enterica* (α), *Shigella flexneri* (α), *Providencia alcalifaciens* (α), *Ralstonia mannitolilytica* (α), *Proteus cibarius* (α), *Proteus mirabilis* (α), *Providencia rettgeri* (α) and *Providencia stuartti* (α) respectively. According to Ramachandran, (2013), alpha (α) and beta (β) hemolytic activities are considered as virulence-associated determinants of clinical importance when assessing the significance of the hemolytic environmental bacterial species or strains.

Table 6. Hemolytic activity of bacteria isolated from Ossiomo River

	Hemolytic activity		
Bacterial isolates	**Beta (β)**	**Alpha (α)**	**Gamma (γ)**
Yersinia ruckeri	-	+	-
Serratia marcescens	-	+	-
Klebsiella pneumoniae	-	+	-
Pseudomonas aeruginosa	+	-	-
Shigella sonnei	-	+	-
Escherichia coli	+	-	-
Klebsiella variicola	+	-	-
Pseudomonas otitidis	-	+	-
Salmonella enterica	-	+	-
Shigella flexneri	-	+	-
Providencia alcalifaciens	-	+	-
Ralstonia mannitolilytica	-	+	-
Proteus cibarius	-	+	-
Proteus mirabilis	-	+	-
Providencia rettgeri	-	+	-
Prodencia stuartii	-	+	-

Shown on **Table 7** is the antibiotic susceptibility test of bacterial isolates. Levofloxacin, cefloxacin, ciprofloxacin, gentamicin, azithromycin, ceftriaxone, pefloxacin, ceftazidime, and cefuroxime were (100 %) and ofloxacin (75 %) effective drugs for the treatment of *Yersinia* and other enteric bacterial infections while augmentin (0 %) and nitrofurantoin (0 %) were less effective drugs.

Table 7. Antibiotic susceptibility pattern of bacterial isolated from Ossiomo River

		Antibiotics										
Bacterial Isolates	**No. of isolates**	**LEV 5 µg**	**PEF 5 µg**	**CRO 30 µg**	**GEN 10 µg**	**CIP 5 µg**	**CXM 30 µg**	**AZM 5 µg**	**OFL 5 µg**	**CAZ 30 µg**	**AUG 30 µg**	**NIT 300 µg**
Y. ruckeri	16	100	50	100	90	100	50	90	75	50	0	0
E. coli	85	73	50	75	100	100	50	50	75	50	0	0
P. otitidi	65	93	50	75	100	100	50	50	75	50	0	0
P. aeroginosa	61	57	95	100	100	100	65	50	62	50	0	0
K. pneumonia	80	68	67	0	0	61	70	50	60	62	0	0
K. variicola	55	68	66	50	0	60	70	61	60	60	0	0
S. sonnie	62	85	100	100	65	100	93	65	65	100	0	0
S. flexneri	70	80	85	96	50	100	93	74	75	84	0	0
S. marcescens	59	100	50	50	90	90	65	80	75	50	0	0
S. enterica	80	87	50	50	0	100	94	80	76	60	0	0
R. mannitolilytica	75	95	90	0	75	70	96	75	71	95	0	0
P. alcalifaciens	35	100	66	87	79	80	60	0	81	65	0	0
P. rettgeri	30	100	60	75	68	89	60	0	78	65	0	0
P. stuatii	42	97	65	70	69	80	72	0	72	63	0	0
P. mirabilis	75	75	60	89	59	100	100	70	70	100	0	50
P. cibarius	56	79	50	87	60	100	99	60	80	99	0	50

Key: *Yersinia ruckeri, Escherichia coli, Pseudomonas otitidis, Pseudomonas aeroginosa, Klebsiella pneumonia, Klebsiella variicola, Shigella sonnie, Shigella flexneri*. LEV=Levofloxacin, PEF=Pefloxacin, CRO=Ceftriaxon, GEN=Gentamicin, CIP=Ciprofloxacin, CXM=Cefuroxime, OFL=Ofloxacin, CAZ=Ceftazidine, AUG=Augmentin,NIT=Nitrofurantoin and AZM= Azythromycin. Sensitivity= 91-100 %, Intermediate=51-90 %, Resistance1-50 %

Conclusion

The findings of this study established the presence of *Yersinia ruckeri* within the Ossiomo River system, located in the Orhionmwon Local Government Area of Edo State, Nigeria. While this pathogen is traditionally recognized as the primary etiological agent of systemic bacteremia and Enteric Redmouth Disease (ERM) in salmonids and other fish species, its public health concern cannot be overlooked. In this study, the human pathogenic potential of the aquatic isolates was phenotypically demonstrated by their expression of alpha (α) hemolysis on blood agar. The pathogenic virulence of *Y. ruckeri* is multifactorial, predicated by an intricate interplay between structural virulence genes and fluctuating environmental variables, notably ambient water temperature. Given the widespread documentation of *Y. ruckeri* as a contaminant in both dietary foods and river systems, it poses a huge threat of infection to human populations who consume poorly prepared food or raw, untreated surface waters. Ultimately, the genomic and phenotypic hemolytic datasets generated in this study provide a critical baseline that explains the precise mechanisms by which *Y. ruckeri* manifests virulence in waterborne and foodborne human diseases.

Further Studies: The findings established in this study suggest that further studies should examine amplification and sequencing of specific virulence-associated genes (such as *yrpA*, *rovA*, and *inv*) to map the exact genetic determinants responsible for the observed alpha-hemolytic activity. Furthermore, given the temperature-dependent nature of *Y. ruckeri* virulence, *in vitro* expression studies (e.g., via RT-qPCR) should be conducted across a gradient of tropical water temperatures to determine how climatic variations affect toxin production and pathogenicity.

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