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Assessment of Antibiotic Resistance, Virulence, and Toxigenic Genes of Bacteria Isolated from Ox-Bow Lake in Bayelsa State

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ABSTRACT

Multidrug resistant (MDR) bacteria are of global concern due to the rapid horizontal transfer of resistance genes within microbial populations and with challenges in quick treatment interventions. This study investigated virulence factors, antibiotic sensitivity of bacteria isolated from a sand mining (dredging) site of Ox-Bow Lake, in Yenagoa, Bayelsa State, Nigeria. Multidrug resistant (MDR) isolates were further investigated by molecular studies to determine the presence or antibiotic resistance genes, toxigenic genes and virulence genes. Bacteria previously isolated from the sand mining site which were; *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Vibrio cholerae*, *Chromobacterium violaceum*, *Staphylococcus aureus*, *Staphylococcus cohnii*, *Kluyvera ascorbata*, *Bacillus tequilensis*, *Enterobacter aerogenes*, *Bacillus myoides*, *Yersinia intermedia*, *Citrobacter koseri*, *Salmonella typhi*, and *Bacillus cereus* were employed for this study. Results revealed that all the isolates possess multiple virulence traits and widespread antibiotic resistance. Several isolates exhibited multidrug resistance (MDR) to fluoroquinolones, β-lactams, and other antibiotic classes. Notably, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Vibrio cholerae*, *Chromobacterium violaceum*, *Salmonella typhi*, and *Bacillus cereus* showed resistance to multiple antibiotics, while species, such as *Kluyvera ascorbata* and *Enterobacter aerogenes*, retained susceptibility to aminoglycosides. Molecular analysis revealed presence of blaCTX-M resistance gene in *Klebsiella pneumoniae* strain DSM 30/04 and *Chromobacterium violaceum* strain JCM 1249, and presence of qnrA resistance gene in *Staphylococcus aureus* Subsp. aureus strain MRSA 252. The stx1 toxigenic gene was present in *Klebsiella oxytoca* strain ATCC 13182 and *Klebsiella pneumoniae* strain DSM 30/04, while *Staphylococcus aureus* strain ATCC12600 and *Staphylococcus aureus* Subsp. aureus strain MRSA 252 possess the entFM toxigenic gene. *Klebsiella oxytoca* strain ATCC 13182 possess both fimH and spvC virulence genes while *Staphylococcus aureus* strain ATCC12600 and *Staphylococcus aureus* Subsp. aureus strain MRSA 252 possess the spvC virulence genes. This study demonstrates that Ox-Bow Lake harbors multidrug-resistant, virulent and toxigenic bacteria, representing a potential source of public health hazards through water use, seafood consumption, and direct human contact. These findings emphasize the importance of sustained environmental monitoring, effective waste management, and strengthened antibiotic stewardship to reduce the risks associated with antimicrobial resistance in aquatic ecosystems.

Keywords: Ox-Bow Lake, multidrug resistance, virulence traits, antibiotic resistance genes, toxigenic genes public health.

Introduction

Water bodies such as Ox-Bow Lake represent complex and dynamic ecosystems that support a vast diversity of life forms. Among the most essential inhabitants of these environments are microorganisms, including bacteria, fungi, archaea, and protozoa. These microbial communities play pivotal roles in maintaining ecological balance through processes such as nutrient cycling, organic matter decomposition, detoxification of pollutants, and primary productivity enhancement (Gartside *et al.*, 2020). Acting as the biological engine of aquatic ecosystems, these microbes facilitate key biogeochemical transformations that underpin the survival of higher trophic levels, thus ensuring the sustainability of aquatic food webs. Microbial communities also contribute significantly to water purification through natural bioremediation processes, helping to mitigate the impact of pollutants by breaking down or immobilizing harmful substances (Rivett *et al.*, 2018).

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In this capacity, they serve as a biological buffer against environmental contamination, helping to preserve water quality and safeguard the health of both aquatic organisms and human populations reliant on these water bodies. However, these beneficial functions are increasingly being undermined by anthropogenic pressures. Industrial discharge, agricultural runoff, improper waste disposal, urban encroachment, and dredging activities are all altering the physical and chemical properties of water bodies, with cascading effects on microbial structure and function (Jenkins *et al.,* 2021; Aleruchi and Obire, 2018). These disturbances often result in shifts in microbial diversity and dominance patterns, leading to a decline in the ecosystem's resilience and self-purifying capacity. For example, nutrient enrichment commonly caused by fertilizer runoff can trigger eutrophication, promoting the proliferation of opportunistic or harmful microorganisms such as cyanobacteria, while reducing overall biodiversity (Smith *et al.,* 2021). This not only threatens aquatic organisms but also diminishes the water body's ecological and economic value. A particularly pressing concern within aquatic microbial ecology is the emergence and spread of antibiotic-resistant bacteria. Aquatic environments often act as reservoirs and transmission pathways for antibiotic resistance genes (ARGs), especially in regions where antibiotics are heavily used in agriculture, aquaculture, and human medicine without proper regulation (Martinez, 2009).

Once introduced into the environment, resistant strains may proliferate and exchange resistance genes via horizontal gene transfer, thereby amplifying the threat of antimicrobial resistance (AMR) (Graham *et al.,* 2016). The presence of such resistant strains in natural water bodies poses a serious public health risk, particularly in communities that rely on untreated surface water for domestic use or recreational activities. Notably, the World Health Organization (WHO) has identified antimicrobial resistance as one of the top ten global public health threats. Compounding these challenges is the role of climate change, which is introducing new stressors into aquatic systems. Changes in temperature, precipitation, and hydrology affect microbial metabolism, community composition, and the mobility and toxicity of pollutants (Doney *et al.,* 2012). Warming waters can enhance microbial growth rates but may disrupt the balance between microbial groups, enabling pathogenic or resistant strains to dominate. As such, climate variability may exacerbate the ecological impacts of pollution and accelerate the spread of AMR in aquatic habitats. Considering these growing concerns, it is crucial to monitor and assess the antibiotic susceptibility profiles of bacteria in aquatic environments, particularly those experiencing intensive anthropogenic activities such as sand mining. Sand mining while often necessary for construction, navigation and flood control, can disturb sediment layers that harbor dormant microbial communities, altering microbial ecology.

Multidrug resistant (MDR) bacteria are of global concern due to the rapid horizontal transfer of resistance genes within microbial populations and with challenges in quick treatment interventions. This study therefore, aims to conduct a comprehensive investigaton of the virulence factors, and antibiotic sensitivity of bacteria isolated from a sand mining (dredging) site of Ox-Bow Lake, in Yenagoa, Bayelsa State, Nigeria. The study will also investigate isolates which are most resistant to several antibiotics by molecular studies to determine the presence or possession of antibiotic resistance genes, toxigenic genes and virulence genes. The study seeks to ascertain the presence of virulence traits, resistance patterns, and the presence of antibiotic resistance genes, toxigenic genes and virulence genes in the isolates from Ox-Bow Lake. The study seeks rapid response treatment interventions to organisms implicated in waterborne infections and to safeguard public health.

Materials and Methods

Study area and bacteria isolates used for the study

The study area is a sand mining (dredging) site in the Ox-Bow Lake with coordinates - $04^{0}54'16{\cdot}90"$N, $006^{0}16'45.30"$E) located in Yenagoa Metropolis, Bayelsa State, Nigeria. The bacteria used for this study were the bacteria that were previously isolated from the sand mining site and identified by the authors (Iyeritei *et al*., 2025).

The bacteria were *Klebsiella pneumoniae, Klebsiella oxytoca, Vibrio cholereae, Kluyvera ascorbata, Salmonella typhi, Chromobacterium violaceum, Bacillus cereus, Staphylococcus aureus, Stapylococcus cohnii, Bacillus tequilensis, Klebsiella aerogenes, Bacillus myoides, Citrobacter koseri,* and *yersinia intermedia*.

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Microbiological Analysis

Determination of Virulence Factor of the Bacteria

Biofilm Production

To assess biofilm production, the medium was prepared according to the manufacturer's specifications. Thirty-seven grams of Brain Heart Infusion Agar, 5 grams of sucrose, 15 grams of Agar Agar, and 0.8 grams of Congo red were dissolved in 1 liter of distilled water. Congo red was prepared as an aqueous solution, autoclaved at 121°C for 15 minutes, and then added to the autoclaved Brain Heart Infusion Agar mixture containing sucrose at 55°C. The medium was allowed to cool to approximately 45°C and then dispensed aseptically into sterile Petri dishes. The plates were dried in a hot air oven before use (Marthur *et al.*, 2018). The bacterial isolates were streaked onto the prepared Congo Red Agar plates and incubated at 37°C for 24–48 hours. Biofilm production was indicated by the appearance of black colonies with a dry crystalline consistency (positive result, +), while non-biofilm producers exhibited red or pink colonies with a smooth consistency (negative result, −).

Haemolytic Activity

Haemolytic activities of the bacterial isolates were assayed to determine the organism's capacity to digest red blood cells. The assay was done by streaking representative colonies of the isolates onto freshly prepared blood agar according to the method of Sagar, (2015), isolates were incubated at 37^0C for 24-48hours. Results were recorded according to the organism's reaction around the streak (α-greenish color (+), γ- no coloration (-), β- clear transparent (+).

Lecithinase Test

Bacterial isolates were assayed for their ability to hydrolyze or breakdown lecithin using the Lecithinase test, egg yolk media was prepared by dissolving 50.41g in 900ml of water and heated to dissolve and sterilized at 121^0C for 15 minutes at 15psi. The medium was allowed to cool to 50^0C- 55^0C and then aseptically add 10ml of sterile egg yolk emulsion, and then dispensed into Petri dish. The isolates were streak onto egg yolk agar and incubated at 37^0C for 24-48hours (Noura, *et al.*, 2013). The results were reported considering if there were clear zones (+) or no clear zone (-) around the streak.

Protease Test

Bacterial isolates from the samples were assayed for their ability to bring about proteolysis (proteolyic exoenzymes called proteinase or caseinase using the protease test. Milk agar was prepared by dissolving 51.5g in 1000ml of water and sterilized at 121^0C for 15 minutes at 15psi. The medium was allowed to cool to 45^0C- 50^0C and then dispensed into Petri dish. The isolates were streak onto Milk agar and incubated at 37^0C for 24-48hours (Vijayaraghan & Vincent, 2013). The results were reported considering if there were clear zones (+) or no clear zone (-) around the streak.

Hydrogen Sulfide Test

This is used for the identification of microorganisms that can produce hydrogen sulphide (H_2S) and ferment Glucose, Lactose and Sucrose with or without gas. Triple sugar iron agar was prepared by dissolving 65g in 1000ml of water, and 10ml of the medium was dispensed into test tubes and sterilized at 121^0C for 15 minutes at 15psi. The medium was allowed to cool in a slanting position; the slant was inoculated with the isolates by streaking on the slant and stabbing, and then incubated at 37^0C for 24-48hours. Blackening of the medium indicated production of hydrogen sulphide, yellow color indicated acid production while gas production was shown by a crack or split within the medium due to tension produced by the gas (Nevena & Joy, 2014).

Antibiotic Susceptibility Testing

The antimicrobial susceptibility of each isolate was tested using the conventional disc diffusion method, following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2017). Commercial antibiotic discs were used, including Gentamicin (10 µg), Ampicillin (30 µg), Chloramphenicol (25 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Cefuroxime (30 µg), Amoxicillin (30 µg), Cloxacillin (5 µg), Amoxicillin-Clavulanic Acid (Augmentin) (30 µg), Clotrimazole (25 µg), and Erythromycin (10 µg).

These antibiotics were selected due to their common use in treating bacterial infections affecting both Gram-positive and Gram-negative bacteria. A turbid suspension of the isolates was prepared in distilled water using the 0.5 McFarland Standards as a comparator.

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A sterile swab was dipped into the bacterial suspension, excess liquid was removed by pressing the swab against the side of the container, and the swab was then used to evenly streak the surface of Mueller-Hinton agar plates. Sterile forceps were employed to place the antibiotic discs in a circular pattern on the media. This process was repeated for all identified isolates, and the plates were incubated at 37°C for 24 hours.

After incubation, the zones of inhibition around each antibiotic disc were measured in millimeters (mm) using a transparent ruler. The readings were judged according to the interpretative criteria provided by the NCCLS (2017), which categorized the isolates as sensitive (S), intermediate (I), or resistant (R) based on the diameter of the inhibition zones compared to standardized breakpoints for each antibiotic.

Molecular Identification

Five (5) pure cultures of bacterial isolate that were most resistance to six antibiotics and above were selected for molecular identification.

Extraction of DNA

Extraction of DNA is a phenomenon by which DNA is separated from proteins, membranes and other cellular materials contained in the cell. Boiling method was used for the extraction process. A 24 hours old pure culture of the isolates was put in Luria-Bertani (LB) Broth and incubated at 37ºC. About 0.5ml of an overnight broth culture of the isolates in Luria Bertani (LB) was put into properly labeled Eppendorf tubes and filling to mark with normal saline and was centrifuged at 14000rpm for 3 min and the supernatant was decanted leaving the DNA at the base. This process was repeated 3 times. The cells were re-suspended in 500ul of normal saline and heated at 95ºC for 20 min. The heated bacterial suspension was cooled on ice (About 10minutes) and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro-centrifuge tube and stored at -20ºC for other down-stream reactions.

DNA Quantification

Extracted DNA genomics were quantified using the Nanodrop 1000 spectrophotometer. Beer Lambert's principle which is used to evaluate the quality and quantity of the genomic DNA was used by the Nanodrop spectrophotometer. The Nanodrop spectrophotometer was connected to a computer with Nanodrop software installed. The software of the equipment was launched by double clicking on the Nanodrop icon. The sample pedestals were properly cleaned. The equipment was initialized using 2µl of sterile distilled water and blanked using 2µl of Normal saline. About 2µl of the extracted DNA of the individual isolates of fungi and bacteria was loaded onto the lower pedestal to measure the concentration of the sample, and the upper pedestal was brought down to contact the DNA on the lower pedestal. Then, DNA concentration was measured by clicking the measure button displayed on the computer screen (Olsen and Morrow, 2012).

16S rRNA Amplification

Amplification of the 16S rRNA was carried out using an ABI 9700 Applied Biosystems Thermal Cycler and method described by Srinivasan *et al*. (2015). The 16S rRNA region of the rRNA gene of the bacterial isolates were amplified using the forward primer; 27F:5'-AGAGTTTGATCMTGGCTCAG-3'and Reverseprimer;1492R:5'CGGTTACCTTGTTACGAC TT-3'primers at a final volume of 40 micro litres for 35 cycles. The PCR mix includes: (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template, Buffer 1X and water. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light trans-illuminator for a 1500bp amplicons (Srinivasan *et al*., 2017).

DNA Sequencing

Sequencing of the amplified product was carried out using the Big-Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ulBigDye® terminator v1.1/v3.1, 2.25ul of 5 x Big Dye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows; 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4minutes (Srinivasan *et al*., 2017).

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Phylogenetic Analysis

Sequences with similar characteristics were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN prior to the edition of the obtained sequences using the bioinformatics algorithm Trace edit. MAFFT were used to align these sequences. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

Amplification bla$_{CTX-M}$ (Resistance) and entFM and stx1 (Toxigenic) genes of Bacterial Isolates

Bla$_{CTX-M,}$ QnrA, entFM and stx1 genes from bacterial isolates were amplified using the blaCTX-mF: 5′-ATGTGCAGYACCAGTAARGT-3´and blaCTX-mR:5′-TGGGTRAAR TARGTSACCAGA-3´; QnrAF: 5′-GATCGTGAAAGCCAGAAAGG-3´ and QnrAR: 5′-CGATGCCTGGTAGTTGTCC-3´; entFMF: 5′- AAAGAAATAA TGGA CAAA CTCAAACTCA-3´ and entFMR: 5′-GTATGTAGCTGGGCCTGTACGT-3´ sxt1F: 5′-CAGTTAATGTGGTGGCGAAG-3´ and sxt1 R: 5′-CTGTCACAGTAACAACCGT-3´ primers on ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 micro-litres for 35 cycles. The PCR mix included: The X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 60ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 58ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV trans-illuminator for a 1484bp and 372bp product size (Bell *et al.*, 1998).

Amplification FimH and spvC (Virulent) genes of Bacterial Isolates

FimH genes from bacterial isolates were amplified using the FimHF: 5′- ATGAACGCCTGGTCC TTTGC-3´and FimHR:5′- GCTGAACGCCTATCCC CTGC-3´; spvCF: 5′- ACCAGAGACATTGCCTTCC-3´ and spvCR: 5′- TTCTGAT CGCCGC TATTCG-3´ primers on ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 micro-litres for 35 cycles. The PCR mix included: The X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 60ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 58ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV trans-illuminator for a 1484bp and 372bp product size.

Results

Table 1 presents the results for virulence factors of the bacterial which were *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Vibrio cholerae*, *Kluyvera ascorbata*, *Salmonella typhi*, *Chromobacterium violaceum*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus cohnii*, *Enterobacter aerogenes*, *Bacillus tequilensis*, *Klebsiella aerogenes*, *Bacillus mycoides*, and *Yersinia intermedia* isolated from the sand mining site at Ox-Bow Lake. All the isolates exhibited multiple virulence traits.

Table 2 presents the antibiotic susceptibility profile of the bacteria isolates and it shows considerable variation in resistance patterns among species. Several isolates, including *Klebsiella pneumoniae*, *Vibrio cholerae*, *Chromobacterium violaceum*, *Salmonella typhi*, and *Bacillus cereus*, exhibited resistance to many of the tested antibiotics, suggesting the presence of multidrug-resistant (MDR) strains. *Staphylococcus aureus*, *Kluyvera ascorbata*, and *Yersinia intermedia* were largely susceptible to most antibiotics tested, indicating lower resistance potential in these isolates. *Bacillus tequilensis* and *Enterobacter aerogenes* also displayed high susceptibility, though with occasional intermediate responses.

Notably, resistance to commonly used antibiotics such as Pefloxacin (PEF), Tarivid (OFX), and Amoxacillin (AM) was widespread across multiple isolates, while susceptibility was more frequently observed with Streptomycin (S), Chloramphenicol (CH), and Gentamycin (CN) in certain species.

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Table 1: Virulence Factor Test Result of Isolates from Dredging Site in Ox-Bow Lake

Bacterial Isolate	Haemolysis	Hydrogen Sulphide	Lecithinase production	Casein hydrolysis	Biofilm production
Klebsiella pneumoniae	-	-	+	+	+
Klebsiella oxytoca	+	+	+	+	+
Vibrio cholereae	-	+	+	+	+
Chromobacterium violaceum	-	-	+	+	+
Staphylococcus aureus	+	+	+	+	+
Staphylococcus cohnii	+	-	+	-	+
Kluyvera ascorbata	-	-	+	+	+
Bacillus tequilensis	-	+	+	+	+
Enterobacter aerogenes	-	+	+	+	+
Bacillus myoides	+	-	+	+	-
Yersinia intermedia	+	+	+	+	-
Citrobacter koseri	+	-	-	+	+
Salmonella typhii	-	-	-	+	+
Bacillus cereus	+	-	-	+	+

Key: + = Positive; - = Negative

Table 2: Antibiotic Susceptibility of Bacteria Isolated from Dredging site (Ox-Bow Lake)

Bacterial Isolate	PEF	OFX	S	SXT	CH	SP	CPX	AM	AU	CN
Klebsiella pneumoniae	S	R	R	R	R	R	R	R	I	R
Klebsiella oxytoca	R	R	S	R	S	R	R	R	R	R
Vibrio cholerae	R	R	I	R	R	R	S	R	R	R
Chromobacterium violaceum	R	R	R	I	R	R	S	S	R	I
Staphylococcus aureus	S	S	S	S	S	S	R	I	I	S
Staphylococcus cohnii	I	I	S	S	S	S	R	R	R	S
Kluyvera ascorbata	S	S	S	R	S	S	S	S	S	R
Bacillus tequilensis	S	S	S	S	S	S	R	I	S	I
Enterobacter aerogenes	S	S	S	S	I	I	I	I	S	S
Bacillus myoides	S	S	S	I	R	R	R	R	R	R
Yersinia intermedia	S	S	S	S	S	S	R	I	I	S
Citrobacter koseri	R	S	S	R	S	S	S	S	S	S
Salmonella typhii	R	R	I	R	R	R	S	S	I	R
Bacillus cereus	S	R	S	R	S	R	R	R	R	R

Key: PEF; Pefloxacin 30µg, OFX; Tarivid 10µg, S; Streptomycin 30µg, SXT; Septrin 30µg, CH; Chloramphenicol 30µg, SP; Sparfloxacin 10µg, CPX; Ciprofloxacin 30µg, AM; Amoxacillin 30µg, AU; Augumentin10µg, CN; Gentamycin 30µg, , R; Resistant, I; Intermediate and S; Susceptible

The agarose gel electrophoresis of the 16S rRNA gene from molecular characterization of antibiotic-resistant bacteria is presented in Plate 1, confirmed the successful amplification of bacterial DNA of the isolates from Ox-Bow Lake. The gel displayed distinct bands at 1500 bp for all 5 selected bacterial isolates, indicating the expected fragment size for 16S rRNA gene amplification. Lane M represents the 100 bp molecular ladder, which served as a reference for determining the sizes of the DNA fragments. The bacterial isolates identified in each lane include 1; *Staphylococcus* sp, 2; *Citrobacter* sp, 3; *Klebsiella* sp, 4, *Stapylococcus* sp, and 5; *Klebsiella* sp. The presence of clear and distinct bands suggests high-quality DNA extraction and successful PCR amplification. These results confirm that the isolates are suitable for further molecular identification and phylogenetic analysis.

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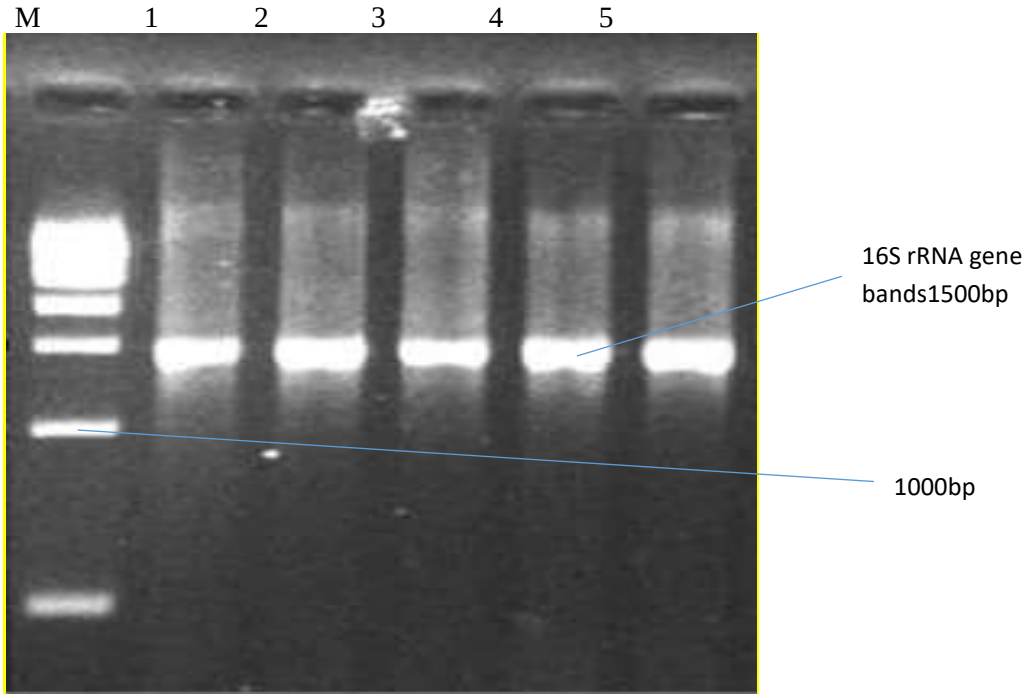

Plate 1: Agarose gel electrophoresis showing the 16SrRNA bands. Lane 1-18 showing the 16SrRNA bands at 1500bp while lane M represents the 100bp molecular ladder

The 16S rRNA of all the bacteria isolated from Ox-Bow Lake showed percentage similarities to their genBank relatives and to other species at 100% similarity. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolate D/S2.3, DLS2.3, D/S3.3, D/S1.3, and D/S1 were closely related to *Staphylococcus aureus strain ATCC 12600, Staphylococcus aureus subsp. aureus strain MRSA252, Chromobacterium violaceum strain JCM 1249, Klebsiella oxytoca strain ATCC 13182,* and *Klebsiella pneumoniae strain DSM 30104,* respectively as shown in the phylogenetic tree in Figure 1.

Table 3 presents the molecular identification the antibiotic resistant bacteria, percentage relatedness to GeneBank reference strains. Isolates identified were *Klebsiella* sp (100% related to *Klebsiella oxytoca* strain ATCC 13182) - ascension number NR041749; *Staphylococcus* sp (100% related to *Staphylococcus aureus* strain ATCC12600) - ascension number BX571856; *Klebsiella* sp. (100% related to *Klebsiella pneumoniae* strain DSM 30/04) - ascension number NR036794; *Proteus* sp. (100% related to *Chromobacterium violaceum* strain JCM 1249) with ascension number AB594767 and *Staphylococcus* sp. (100% related to *Staphylococcus aureus* Subsp. aureus strain MRSA 252) with ascension number NR118997.

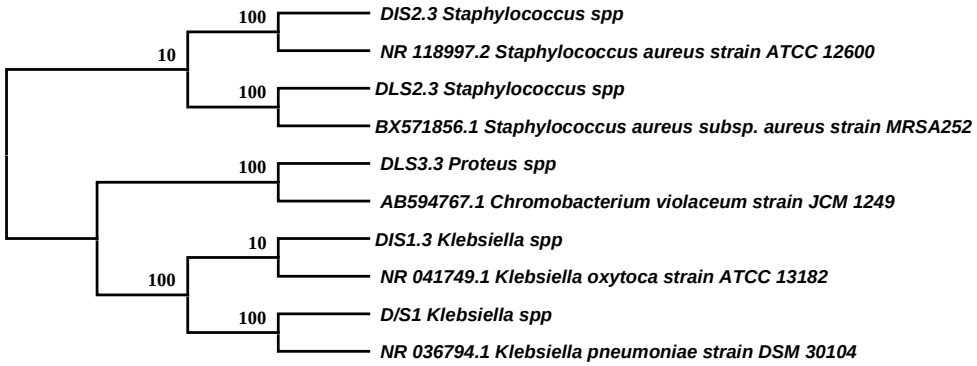

Figure 1: Phylogenetic Tree showing evolutionary distance between bacterial Isolates

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Table 3: Molecular Identification of Antibiotic-Resistant Bacteria from Ox-Bow Lake and Percentage Relatedness with Gene Bank Relatives and their Ascension Numbers

Isolate code	Physiological Identification	Molecular Identification /Gene Bank Relative	Percentage (%) of Relatedness	Ascension Number
DIS1.3	*Klebsiella* sp	*Klebsiella oxytoca* strain ATCC 13182	100	NR041749
DIS2.3	*Staphylococcus* sp	*Staphylococcus aureus* strain ATCC12600	100	BX571856
DIS1	*Klebsiella* sp	*Klebsiella pneumoniae* strain DSM 30/04	100	NR036794
DIS3.3	*Proteus* sp	*Chromobacterium violaceum* strain JCM 1249	100	AB594767
DLS2.3	*Staphylococcus* sp	*Staphylococcus aureus* Subsp. aureus strain MRSA 252	100	NR118997

The results of the molecular profiling of antibiotic resistance genes, toxigenic genes and virulence genes of the isolates are presented in Plates 2 to 7. The gel electrophoresis results in Plate 2 show the amplification of the blaCTX-M gene, which is associated with extended-spectrum beta-lactamase (ESBL) production and resistance to third-generation cephalosporins. Lanes 1 and 3 contains DNA from different bacterial isolates *Klebsiella pneumonia,* and *Chromobacterium violaceum*. The presence of distinct bands at 500 bp in each lane confirms the successful amplification of the blaCTX-M gene in these bacterial species. The 1000 bp molecular ladder serves as a size reference. The comparison confirms that the observed bands align with the expected 500 bp fragment size of the blaCTX-M gene.

The gel electrophoresis results in Plate 3 shows the presence of the stx1 gene, a known toxigenic gene, was detected in Lane 1 - 2 represent the stx1 gene bands at 180bp of *Klebsiella oxytoca*, *Klebsiella pneumoniae*. The results confirmed distinct bands at 180bp, indicating the successful amplification of the stx1 gene in these bacterial isolates.

Plate 4 shows the presence of the spvC gene, a known virulence factor, was confirmed in the spvC gene bands at 500bp of *Staphylococcus aureus* strain ATCC12600 *Staphylococcus aureus* Subsp. aureus strain MRSA 252, and *Klebsiella oxytoca*, through agarose gel electrophoresis. The results, as illustrated in Plate 4, showed distinct bands at 400bp, indicating the successful amplification of the SpvC gene in these bacterial isolates.

The agarose gel electrophoresis results in Plate 5 show the amplification of the entFM gene, which is associated with bacterial virulence and biofilm formation. Lane 2 represents the entFM gene bands at 1300bp of *Staphylococcus aureus*. The presence of clear bands at 1300 bp suggests the successful amplification of the entFM gene in both bacterial species. The agarose gel electrophoresis results in Plate 6 show the amplification of the FimH gene, which encodes type 1 fimbrial adhesin, a key virulence factor involved in bacterial adhesion and biofilm formation. The fimH gene bands at 508bp of *Klebsiella oxytoca* while M represents the 100-2000bp molecular ladder. The presence of distinct bands at 508 bp in all lanes indicates the successful amplification of the FimH gene in these bacterial species. The 100-2000 bp molecular ladder serves as a reference for estimating the DNA fragment sizes. The alignment of the bands confirms that the observed FimH gene bands match the expected 508 bp fragment size. The agarose gel electrophoresis results in Plate 7 indicate the presence of the qnrA gene in *Staphylococcus aureus* at approximately 600 bp. Lane 1 contain DNA samples from *S. aureus*. The presence of distinct bands at 600 bp suggests successful amplification of the qnrA gene, which is associated with quinolone resistance. The 100 bp molecular ladder serves as a reference to estimate the sizes of the amplified DNA fragments. The banding pattern confirms that the observed qnrA gene bands correspond to the expected 600 bp size.

Table 4 presents the antibiotic resistance genes, toxigenic, and virulence gene profile of bacteria.

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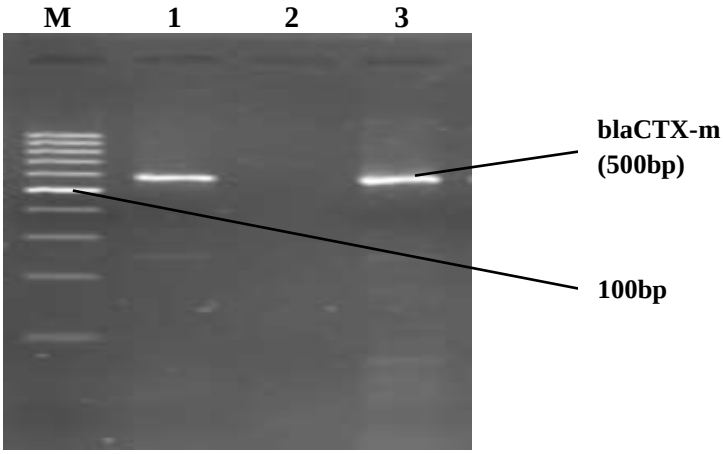

Plate 2: Lane 1-3 represents the blaCTX-M gene bands at 500bp of *Klebsiella pneumonia,* and *Chromobacterium violaceum* while M represents the 100bp molecular ladder.

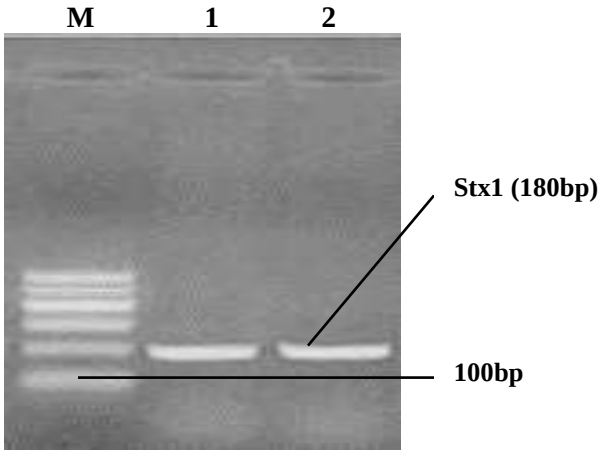

Plate 3: Lane 1- 2 represent the stx1 gene bands at 180bp of *Klebsiella oxytoca*, *Klebsiella pneumoniae*.

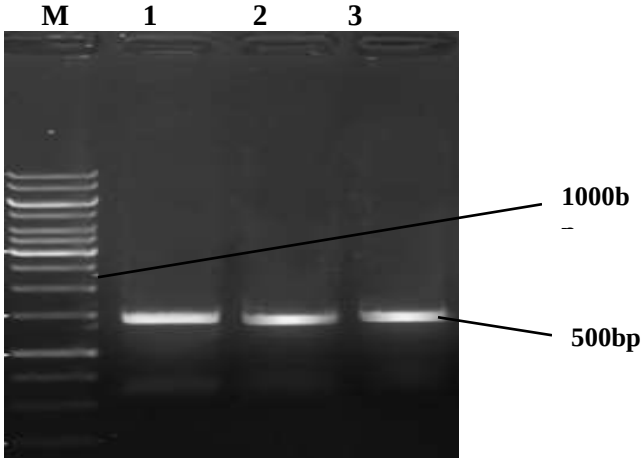

Plate 4: The spvC gene bands at 500bp of *Staphylococcus aureus*, *Staphylococcus aureus*, and *Klebsiella oxytoca*,

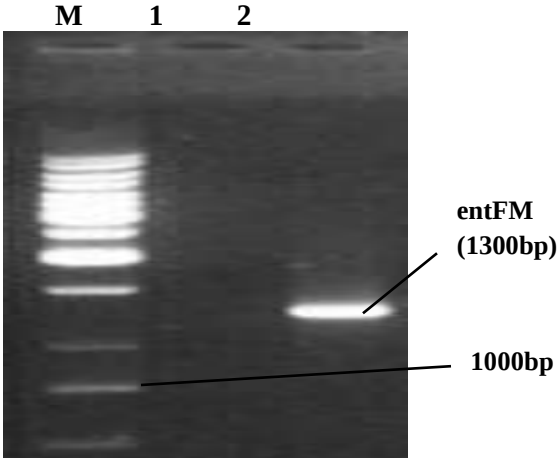

Plate 5: Lane 2 represent the entFM gene bands at 1300bp of *Staphylococcus aureus*

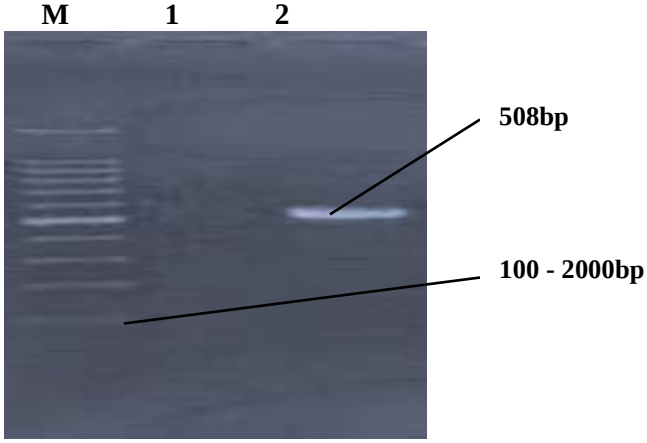

Plate 6: The fimH gene bands at 508bp of *Klebsiella oxytoca* while M represents the 100-2000bp molecular ladder.

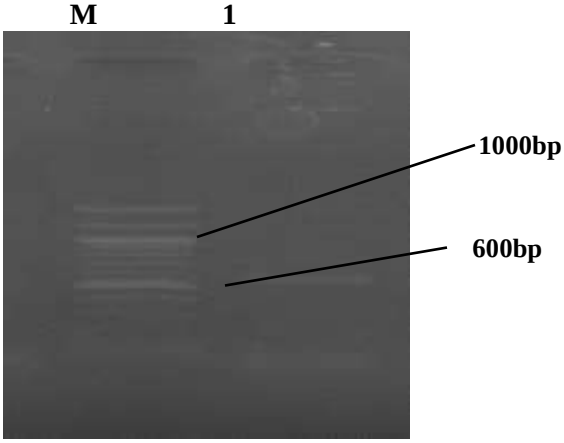

Plate 7: The qnrA gene bands at 600bp of the *Staphylococcus aureus*

Plates showing Agarose gel electrophoresis of the Antibiotic resistance, toxigenic, and virulence genes
Note: Lane M represents the 100bp - 1000bp molecular ladder

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Table 4: Antibiotic resistance, toxigenic, and virulence gene profile of bacteria isolated from Ox-Bow Lake

Isolate code	Molecular Identification /Gene Bank Relative	Number of Base pairs	Antibiotic Resistance Gene	Toxigenic Gene	Virulence Gene(s)
DIS1.3	*Klebsiella oxytoca* strain ATCC 13182	1434	Negative	stx1	fimH and spvC
DIS2.3	*Staphylococcus aureus* strain ATCC12600	1494	Negative	entFM	spvC
DIS1	*Klebsiella pneumoniae* strain DSM 30/04	1224	blaCTX-m	stx1	Negative
DIS3.3	*Chromobacterium violaceum* strain JCM 1249	1441	blaCTX-m	Negative	Negative
DLS2.3	*Staphylococcus aureus* Subsp. aureus strain MRSA 252	1110	qnrA	entFM	spvC

Discussion

This present study has revealed the presence of virulence traits in bacteria that were isolated from the sand mining (dredging) site of the Ox-Bow Lake in Yenagoa, Bayelsa State, Nigeria. The bacterial community identified from the dredging site at Ox-Bow Lake illustrates the complex and dynamic nature of microbial ecology in freshwater environments. Several key bacterial taxa detected which include *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Vibrio cholerae*, *Chromobacterium violaceum*, *Staphylococcus aureus*, *Staphylococcus cohnii*, *Kluyvera ascorbata*, *Bacillus tequilensis*, *Enterobacter aerogenes*, *Bacillus myoides*, *Yersinia intermedia*, *Citrobacter koseri*, *Salmonella typhi*, and *Bacillus cereus* highlight the mixture of human-associated and environmental organisms in the lake. The diversity of isolates reflects the coexistence of environmental microorganisms, opportunistic pathogens, and recognized causative agents of serious human diseases. This pattern is consistent with previous research indicating that aquatic ecosystems act as reservoirs for both environmental and pathogenic bacteria, many of which exhibit antimicrobial resistance (Igbinosa et al., 2017; Odonkor & Ampofo, 2013). The presence of *Klebsiella* species is of particular concern. These organisms are associated with hospital-acquired infections such as pneumonia, urinary tract infections, and septicemia and are well known for their ability to develop multidrug resistance, particularly via extended-spectrum β-lactamase (ESBL) production. Similar findings have been reported by Igbinosa *et al.* (2017) in other Niger Delta water bodies, suggesting sewage or wastewater contamination as a probable source.

The detection of *V. cholerae* signals potential public health risks, as it is the etiological agent of cholera, a severe diarrheal disease linked to water contamination. Its occurrence aligns with reports from Okoh *et al.* (2010) in South Africa, where *V. cholerae* was linked to poor sanitation and runoff from surrounding communities. Likewise, the presence of *S. typhi* which is the causative agent of typhoid fever indicates fecal contamination and mirrors observations by Odonkor and Ampofo (2013) in Ghana. Less common but clinically relevant bacteria such as *K. ascorbata* and *C. violaceum* were also isolated.

While often environmentally, these species can cause severe opportunistic infections, particularly in immuno-compromised individuals. The Gram-positive isolates (*B. cereus*, *B. tequilensis*, *B. mycoides*, *S. aureus*, *S. cohnii*) reflect the influence of both natural environmental presence and potential contamination from human activity, given *S. aureus*'s known association with skin and soft tissue infections, including MRSA strains. The detection of *Y. intermedia*, though primarily environmental, is notable due to its genus's inclusion of serious human pathogens such as *Y. pestis* and *Y. enterocolitica*.

Environmental factors such as seasonal rainfall, runoff, temperature fluctuations, and dissolved oxygen levels—are known to influence microbial load and diversity (Chigor *et al.*, 2012; Ayandele & Adebayo, 2012). Increased runoff during wet seasons can transport nutrients, organic matter, and contaminants into the aquatic environment, creating conditions favorable for bacterial proliferation, including resistant strains.

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The antibiotic susceptibility profile of Ox-Bow Lake isolates revealed that *K. pneumoniae*, *V. cholerae*, *C. violaceum*, *S. typhi*, and *B. cereus* exhibited multidrug resistance (MDR), with resistance to fluoroquinolones (Pefloxacin, Tarivid, Ciprofloxacin), β-lactams (Amoxicillin, Augmentin), and in some cases, even broad-spectrum agents like Chloramphenicol. The presence of MDR bacteria in aquatic environments is alarming because it reduces therapeutic options and increases the risk of untreatable infections (Martínez, 2009; WHO, 2022). These findings are consistent with Akinbowale *et al.* (2006), who reported that aquaculture environments often harbor MDR bacteria due to sublethal antibiotic exposure from agricultural and aquacultural activities. Similarly, Okeke *et al.* (2005) described untreated sewage as a significant reservoir for MDR pathogens. The resistance profiles of *K. pneumoniae* and *S. typhi* align with Chanda *et al.* (2019), who documented widespread β-lactam and fluoroquinolone resistance in aquatic bacterial isolates. In contrast, *S. aureus*, *K. ascorbata*, *Y. intermedia*, *B. tequilensis*, and *E. aerogenes* exhibited higher susceptibility to aminoglycosides (Gentamycin) and older agents such as Streptomycin and Chloramphenicol. Adelowo *et al.* (2014) suggested that the continued susceptibility of some environmental bacteria to aminoglycosides may be due to their limited use in aquatic contexts.

However, intermediate susceptibility in these isolates signals the potential for resistance to emerge over time if selection pressures persist (Andersson & Hughes, 2014). When compared to other aquatic systems, Ox-Bow Lake shows MDR patterns similar to those observed in Lagos Lagoon, Nigeria (Igbinosa *et al.*, 2017), where Enterobacteriaceae with high resistance levels were prevalent. In contrast, Lake Naivasha in Kenya, a less industrially impacted ecosystem, displayed lower MDR prevalence (Kariuki *et al.*, 2020), underscoring the role of environmental contamination in shaping bacterial resistance patterns. The detection of MDR pathogens in a freshwater body that may be used for fishing, recreation, or domestic purposes has direct implications for human health. Such bacteria can enter human populations via contaminated seafood, direct water contact, or through horizontal gene transfer to other pathogens (Baquero *et al.*, 2008). Mobile genetic elements such as plasmids, integrons, and transposons facilitate the spread of resistance genes from environmental bacteria to clinically significant strains, creating health challenge. Communities relying on untreated or inadequately treated water sources are particularly vulnerable to waterborne disease outbreaks, which may be harder to control when caused by MDR organisms. Additionally, the contamination of fish and other aquatic food sources serves as another pathway for resistance transmission to humans. The molecular characterization of bacterial isolates from Ox-Bow Lake demonstrated that all sequences obtained through 16S rRNA gene analysis exhibited 100% similarity with GenBank reference strains, including *Klebsiella oxytoca*, *K. pneumoniae*, *Staphylococcus aureus*, *Chromobacterium violaceum*, and *Proteus*. This high level of sequence homology provides reliable taxonomic identification and corroborates earlier reports of the persistence of opportunistic pathogens in aquatic ecosystems impacted by human activities (Igbinosa et al., 2017; Adekanmbi et al., 2020). The phylogenetic clustering of these isolates with their closest evolutionary relatives further validates the robustness of the identification process. Molecular profiling revealed the widespread distribution of clinically relevant antibiotic resistance genes among the isolates. The plasmid-mediated qnrA gene (~600 bp) was detected in *Staphylococcus aureus* Subsp. aureus strain MRSA 252, indicating fluoroquinolone resistance. Fluoroquinolones are critically important antimicrobials, and the occurrence of qnrA in aquatic isolates suggests that the lake may serve as an environmental reservoir for resistance dissemination (Robicsek et al., 2006). Additionally, amplification of the blaCTX-M gene (~500 bp) in *Klebsiella pneumonia*, and *Chromobacterium violaceum* highlights the prevalence of extended-spectrum β-lactamase (ESBL)-producing strains. ESBLs confer resistance to third-generation cephalosporins and penicillins, thus posing therapeutic challenges and reducing available treatment options (Paterson & Bonomo, 2005). These findings are consistent with earlier studies linking ESBL occurrence in aquatic ecosystems to anthropogenic inputs such as untreated sewage, aquaculture effluents, and hospital wastewater (Igbinosa *et al.*, 2017). In addition to resistance determinants, toxigenic genes were also widely distributed among the isolates. The Shiga toxin gene (stx1, ~180 bp) was detected in *Klebsiella oxytoca*, *Klebsiella pneumoniae*. The stx1 is strongly associated with hemorrhagic colitis and hemolytic uremic syndrome, indicating a significant risk of gastrointestinal infections from these aquatic bacteria (Scheutz & Strockbine, 2005).

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Similarly, the entFM gene (~1300 bp) was detected in *Staphylococcus aureus*. This gene encodes an enterotoxin implicated in foodborne intoxications and gastrointestinal illness (Fisher & Thorne, 2012), emphasizing the potential for public health hazards if these bacteria contaminate food chains. Virulence gene analysis revealed widespread distribution of the FimH adhesin gene (~508 bp) in the isolate *Klebsiella oxytoca* strain ATCC 13182. FimH plays a critical role in adhesion, biofilm formation, and host colonization, particularly in urinary tract and gastrointestinal infections (Johnson & Russo, 2005). The spvC gene (~400 bp) was also detected in *Staphylococcus aureus* strain ATCC12600 *Staphylococcus aureus* Subsp. aureus strain MRSA 252, and *Klebsiella oxytoca*. The spvC gene enhances intracellular survival and systemic dissemination of pathogens, contributing to severe infections (Fàbrega & Vila, 2013). The coexistence of these virulence determinants with resistance genes underscores the multifactorial pathogenic potential of the bacterial community in Ox-Bow Lake. Notably, the isolate such as *Chromobacterium violaceum* did not harbor detectable virulence determinants, suggesting variability in pathogenic potential within the microbial population. However, the dynamic nature of aquatic environments increases the likelihood of horizontal gene transfer, which could enable these organisms to acquire resistance and virulence traits over time. Collectively, the findings of this study demonstrate that Ox-Bow Lake serves as a reservoir of multidrug-resistance, virulence, and toxigenic bacteria, with the simultaneous presence of resistance genes (blaCTX-M, qnrA), toxigenic determinants (stx1, entFM), and virulence factors (FimH, spvC). This convergence of resistance and pathogenicity poses a dual threat to public health and aquaculture, emphasizing the urgent need for environmental monitoring, prudent antimicrobial use in agriculture and clinical practice, and effective wastewater treatment to limit the dissemination of resistance and virulence determinants in aquatic ecosystems.

Conclusion

Ox-Bow Lake represents a significant environmental reservoir of multidrug-resistant and potentially pathogenic bacteria, including *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Salmonella typhi*. The coexistence of antibiotic resistance, virulence, and toxigenic determinants among the bacteria from the Ox-Bow Lake reflects strong selective pressures driven by anthropogenic inputs such as sewage discharge, agricultural runoff, and aquaculture effluents. These findings raise critical concerns for both public health and ecosystem integrity. Addressing this threat will require regular microbial surveillance of aquatic environments, stricter regulation of waste disposal, rational antibiotic use, and improved sanitation infrastructure to reduce the dissemination of antimicrobial resistance.

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