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Detection of virulence genes in bacteria associated with microplastics from selected Rivers in Ado-Ekiti, Nigeria

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Abstract

This study investigated the detection of virulence genes in bacteria associated with microplastics in selected rivers in Ado-Ekiti, Nigeria. Water samples were collected biweekly over a period of 24 months from the rivers (Ureje, Emirin, Ogbese, Odo-Ayo and Elemi). Isolation of bacteria, antibiotics susceptibility testing of the isolates and detection of virulence genes were carried out using standard methods. The bacteria isolated include *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae, Bacillus subtilis, B. licheniformis*, *Pseudomonas aeruginosa, Proteus mirabilis*, *Micrococcus luteus*, *Enterobacter aerogenes, B. cereus*, *Citrobacter freundii, Aeromonas* spp*, Proteus vulgaris. Staphylococcus aureus and Staphylococcus epidermidis.* The antibiotic susceptibility profile of the isolates showed that both Gram negative and Gram positive bacterial isolates exhibited multiple antibiotic resistance. Of the 423 bacterial isolates, 226 were positive to biofilm formation, 78 to heamolysin formation, 87 to phospholipase and 76 to gelatinase production. Virulence genes *stn*, *spvC* and *invA* were detected in *Salmonella typhi* (EM22), *fimH* and *sxt1*genes were detected in *E. coli* (Urj.23) and *Shigella dysenteriae* (EM6), *fimH*, *rmpA* and *entB* genes were detected in *Klebsiella pneumoniae* (Ogb4). In *Bacillus cereus* (EM4), *nheA*, *hblC* and *hblD* genes were detected while *oprL*, *exoS* and *oprI* genes were detected in *Pseudomonas aeruginosa* (ELE12). The findings of this study suggests that water from the rivers are of poor microbial qualities, the bacterial isolates were multiple antibiotic resistant with various virulence genes. Therefore, water from the rivers must be adequately treated before use.

Keywords: Antibiotic resistance; Bacteria; Microplastics; Rivers and Virulence genes

1. Introduction

Microplastics otherwise known as microscopic plastics are fragments of any type of plastics that are less than 5 mm or 0.2 inches in length of either primary or secondary manufacturing origin (NOAA, 2023). Microplastics are one of the most concerning groups of contaminants that pollute the environment most especially water bodies. These microplastics materials are too numerous in the environment, this had led to a period of plasticene. As a result of this, during rainy season, plastic wastes such as plastic bottles, plastics bags and utensils enter the water bodies and become fragmented as a result of degradation processes into microplastics (Haram *et al*., 2020). Beside this, discarded plastics released into the environment as a result of human activity can degrade into microplastics due to various factors, such as sunlight, seawater flow and temperature (Lamchhane *et al*., 2023).

Microplastics have been found to be widely distributed in rivers, oceans, beaches and deep seas because they can be transported through long distances by the ocean currents and wind depending on the density of the particles (Concetta

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et al., 2021). They serve as surfaces for the attachment of microorganisms, some microplastic surfaces has been shown to exhibit selective enrichment of bacterial pathogens which enable them to serve as carriers of dangerous hitchhikers that are disperse over long distances (Xinyi *et al*., 2023). This ability of microplastics to serve as carriers for dangerous hitchhikers poses threats to aquatic ecosystems and human health.

In addition, microplastics act as surfaces for the attachment of various biomolecules (such as LPS, allergens, and antibiotics) (Concetta *et al*., 2021). Microorganisms begin to attach and colonize the microplastics surfaces to form biofilm immediately after the material find its way to aquatic ecosystem (Huan *et al*., 2023). Natural organic matter referred to as eco-corona rapidly forms on microplastics surfaces when they enter aquatic environment (Huan *et al*., 2023). This eco-corona provides primary nutrients for microbial growth and serves as a foundation for biofilm development. Biofilms on the surface of microplastics act as a shield, enabling microorganisms, including pathogens, to withstand adverse environmental conditions and disperse them to new niches. This area surrounding the microplastics surfaces is known as plastiphere and many species of microorganisms that are found in the plastisphere may be potentially pathogenic, toxigenic or harmful and may also be antibiotic resistant. (Marathe and Bank, 2022). The plastisphere environment on microplastics may promote horizontal gene transfer where the bacteria can exchange genetic material such as genes for antibiotic resistance and virulence. This suggests that bacteria in the plastisphere can acquire new characters including the ability to cause disease and resist antibiotics (Vlaanderen *et al*., 2023).

Researches have investigated the isolated of different types of bacteria from the plastisphere of microplastics from marine environment, but limited researches are available on bacteria associated with microplastics found in fresh water bodies such as rivers and their virulence. Olena *et al*. (2021) found evidence that members of the phylum Proteobacteria (31–45%) which may include the pathogenic species, potentially pathogenic *Vibrio* spp., Gram-negative bacteria, facultative anaerobes and several species of microorganisms that may cause waterborne and foodborne diseases in animals and humans, were present in the plastisphere of microplastics.. Different types of microorganisms including bacteria such as *Aeromonas*, *Rhodococcus*, *Pseudomonas*, *Enterobacter*, *Halomonas*, *Mycobacterium*, *Photobacterium*, *Shigella* and fungi have been found on microplastics surfaces (Wenjie *et al*., 2023). Kruglova *et al*. (2022) also found out that bacteria found in marine waters especially those that can cause gastroenteritis in humans such as *Vibrio* spp, *Campylobacter* spp and Enterobacteriaceae were isolated on microplastic surfaces. Thus, the continuous accumulation of microplastics in the environment, most especially in water bodies poses ecological threats and has been an increasing problem worldwide because of the potential effects on living organisms (Wang *et al*., 2021).This may facilitates the transfer of contaminants along the food chain, with potentially grave consequences for human health (Concetta *et al*., 2021). It is therefore pertinent to assess bacteria associated with microplastics and their virulence genes in fresh water bodies in Ado- Ekiti, Nigeria.

2. Materials and Methods

2.1. The study area

The study area is Ado- Ekiti, the capital of Ekiti- state in southwest Nigeria. It is located between latitude 7 .62^0 north and longitude 5.22^0 east of the Greenwich meridian with an estimated population of 535,916 as at 2024 (WPR, 2024). Five water bodies were selected for this study; these include Ureje, Emirin, Ogbese, Odo-Ayo and Elemi rivers, these rivers flow across the town of Ado- Ekiti and there are intense human activities along their banks.

2.2. Collection of water samples

Water samples were collected biweekly over a period of 24 months from the rivers by using composite sampling method was adopted to collect 191. The samples were placed in a cool box with icepacks and transported to the laboratory for analysis.

2.3. Isolation of bacteria from microplastics in water samples from the rivers

Six (6) litters of each water sample was filtered using (mesh 60) of pore size 0.25mm to obtain the microplastics particles. The residues obtained were flushed into clean beakers and 1ml was taken to prepare serial dilution. Isolation of bacteria was carried out by pour plate method as described by Olutiola *et al*., 2000. About 1ml was taken from the last dilution into each sterile petri dish and molten agar was poured on it, mixed gently and allowed to solidify. The cultured plates were incubated at 37^{o}C for 24hrs and observed for the presence of growth. .

2.4. Identification of bacterial isolates

The identification of the bacterial isolates was carried out by cultural, morphological and biochemical characteristics according to Bergey's manual of determinative bacteriology (Bergey and Holt 1994).

2.5. Determination of antibiotic sensitivity pattern of the bacterial isolates

Antibiotics sensitivity of the isolates was carried out using disk diffusion method according to the method used by Zeeshan *et al*. (2019). Broth cultures of the isolated bacteria was inoculated on the solidified agar plate, gram positive and gram negative antibiotics disc were placed separately on the agar plates. The plates were incubated at 37 °C for 24 hrs and observed for the presence of zones of inhibition. The diameter of the zones of inhibition were measured and compared with zone diameter interpretative standard chart of Clinical Laboratory Standard Institute CLSI, (2021) to determine resistant (R), intermediate (I) and Susceptible (S) of the isolates.

2.6. Screening of the bacterial isolates for virulence factors

The screening was carried out using the method of Quingping and Juah, (2018). Bacterial isolates with high multiple antibiotics resistant index (MAR) were selected and screened for the presence of virulence factors. These factors include biofilm production, gelatinase production, heamolysin formation and phospholipase production.

2.7. Biofilm production

Biofilm producing potentials of the isolates was carried out according to the method used by Harika *et al*. (2020) by using Congo red agar. The plates were inoculated and incubated for 24hrs at 37 °C and observed for the presence of growth. Black colonies with dry crystalline morphology indicated positive result while pink colonies indicated negative result.

2.8. Phospholipase or lecithnase activity

The isolates were screened for phospholipase activity by using egg yolk agar according to the method used by Gaanpret *et al*. (2017) with little modifications. The isolates were inoculated by streaking on egg-yolk agar plates and incubated at 37 °C for 24hrs. The plates were observed for the presence of clear zone around the colonies, this indicates phospholipase positive bacteria.

2.9. Gelatinase activity

Gelatinase activity was carried out using gelatin agar according to the method of Vaish *et al*. (2016) The gelatin agar plates were inoculated with *the isolates* and incubated at 37 °C for 24 hours. After incubation, the plates were flooded with mercuric chloride solution. Presence of zone of opacity around the colonies indicated positive result.

2.10. Hemolysin production

Production of hemolysin was carried out by using blood agar to which 5% red blood cells has been added. The isolates were streaked on the agar plates and incubated at 37 °C for 24 hours. The presence of clear zone around the colonies indicated hemolysin production.

2.11. Detection of virulence genes of the bacterial isolates.

Virulence genes of the isolates were detected according to the method of Adenipekun *et al*. (2022), this involves DNA extraction, PCR amplification of the virulence genes using specific primers and visualization of the amplified products by gel electrophoresis.

2.12. Extraction of bacterial DNA

Overnight broth culture of each isolate (*Salmonella typhi* (EM22), *E. coli* (Urj. 23), *Shigella dysenteriae* (EM6), *Klebsiella pneumoniae* (OGB4), *Bacillus cereus* (EM4) and *Pseudomonas aeruginosa* (ELE12) was centrifuged and the supernatants were discarded. The Pellets were washed and suspended in 1 mL of nuclease free water (Invitrogen, Paisley, UK). The suspension was boiled at 98 °C for 10 minutes in a thermal block and then centrifuged at 17,310 rpm for 5 minutes to remove the cellular debris. After centrifugation, the supernatant was used as template DNA for the amplification of the virulent genes.

2.13. PCR amplification of virulent genes of the isolates

Molecular investigation of virulence genes in the selected bacteria was carried out by simple PCR on the extracted DNA using gene specific primers. Primer sequences were as earlier documented. Reaction cocktail used for all PCR per primer set included (Reagent Volume µl) - 5X PCR SYBR green buffer (2.5), MgCl2 (0.75), 10pM DNTP (0.25), 10pM of each forward and backwards primer (0.25), 8000U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair. The electrophoresis was run at 100 V for 1 hour with 1000 bp DNA ladder (Roche Diagnostics, Mannheim, Germany), followed by visualization and sizing of the DNA bands under UV light. The sizes of the DNA fragments of the respective amplicons were presented.

Tables 1-6 below show the primer sequence and PCR profile used in amplifying each fragment.

Table 1 Primer pairs used for the detection of virulence genes in *Salmonella typhi* (EM22).

Targeted virulence genes	Primer sequence	Amplicon size (Bp)	Profile
invA	F:GTGAAATTATCGCCACGTTCGGGCAA	284	An initial denaturing 5minutes at 94ºC, then 35 cycles of 94 ºC for 30s, 60 ºC for 20s 72 ºC for 20s and terminated at 72 ºC for 10minutes
	R:TCATCGCACCGTCAAAGGAACC		
spvR	F:CAGGTTCCTTCAGTATCGCA	310	An initial denaturing 5minutes at 94 ºC, then 35 cycles of 94 ºC for 30secs, 52ºC for 30secs 72ºC for 30secs and terminated at 72 ºC for 10minutes
	R:TTTGGCCGGAAATGGTCAGT		
spvC	F:ACTCCTTGCACAACCAAATGCGGA	571	An initial denaturing 5min at 94 ºC, then 35 cycles of 94ºC for 30s, 50ºC for 30s 72ºC for 30s and terminate at 72 ºC for 10min
	R:TGTCTTCTGCATTTCGCCACCATCA		
fimA	F:CCTTTCTCCATCGTCCTGAA	85	An initial denaturing 5min at 94 ºC, then 35 cycles of 94 ºC for 30s, 50 ºC for 20s 72 ºC for 20s and terminate at 72 ºC for 10min
	R:TGGTGTTATCTGCCTGACCA		
Stn	F:CTTTGGTCGTAAAATAAGGCG	260	An initial denaturing 5min at 94ºC, then 35 cycles of 94ºC for 30s, 48ºC for 30s, terminated at 72ºC for 10 minutes
	R:TGCCCAAAGCAGAGAGATTC		

Table 2 Primer pairs used for the detection of virulence genes in *E coli* (Urj23).

Targeted virulence genes	Primer sequence	Amplicon Size (Bp)	Profile
eaeA	F: GACCCGGCACAAGCATAAGC	384	An initial denaturing 5min at 94 ºC, then 35 cycles of 94 ºC for 30s, 50 ºC for 30s 72 ºC for 60s and terminated at 72 ºC for 10min
	R: CCACCTGCAGCAACAAGAGG		
stx2	F: GGCACTGTCTGAAACTGCTCC	255	
	R: TCGCCAGTTATCTGACATTCTG		
stx1	F: ATAAATCGCCTATCGTTGACTAC	180	

	R: AGAACGCCCACTGAGATCATC		
fimH	F: TGCAGAACGGATAAGCCGTGG	508	
	R: GCAGTCACCTGCCCTCCGGTA		
papAH	F: ATGGCAGTGGTGTCTTTTGGTG	720	
	R: CGTCCCACCATACGTGCTCTTC		

Table 3 Primer pairs used for the detection of virulence genes in *Shigella dysenteriae* (EM6)

Targeted genes	**Primer sequence**	**Size (BP)**	**Profile**
eaeA	CTGGATGGTATGGTGAGG	320	An initial denaturing 5min at 94ºC, then 35 cycles of 94ºC for 30s, 50 ºC for 30s 72 ºC for 60s and terminate at 72 ºC for 10min
	GGAGGCCAACAATTATTTCC		
stx2	GGCACTGTCTGAAACTGCTCC	255	
	TCGCCAGTTATCTGACATTCTG		
stx1	ATAAATCGCCTATCGTTGACTAC	180	
	AGAACGCCCACTGAGATCATC		
fimH	TGCAGAACGGATAAGCCGTGG	508	
	GCAGTCACCTGCCCTCCGGTA		
Sen	ATGTGCCTGCTATTATTTAT	799	An initial denaturing 5min at 94ºC, then 35 cycles of 94ºC for 30s, 50ºC for 30s 72ºC for 60s and terminate at 72ºC for 10min
	CATAATAATAAGCGGTCAGC		

Table 4 Primer pairs used for the detection of virulence genes in *Klebsiella pneumoniae* (OGB4).

Targeted Gene	**Primer sequence**	**Size (Bp)**	**Profile**
roN	AAGTCAAAGCAGGGGTTGCCCG	665	An initial denaturing 5minutes at 94 ºC, then 35 cycles of 94 ºC for 30secs, 50 ºC for 30secs 72 ºC for 40s and terminate at 72 ºC for 10minutes
	TGACGCCGACATTAAGACGCAG		
rmpA	ACTGGGCTACCTCTGCTTCA	516	An initial denaturing 5minutes at 94 ºC, then 35 cycles of 94 ºC for 30secs, 48 ºC for 30s 72 ºC for 30s and terminated at 72 ºC for 10min
	CTTGCATGAGCCATCTTTCA		
wcaG	GGTTGGGTCAGCAATCGTA	169	An initial denaturing 5min at 94ºC, then 35 cycles of 94 ºC for 30s, 50 ºC for 30s 72 ºC for 20s and terminated at 72 ºC for 10mins
	ACTATTCCGCCAACTTTTGC		
fimH	TGCTGCTGGGCTGGTCGATG	506	An initial denaturing 5min at 94 ºC, then 35 cycles of 94 ºC for 30s, 55 ºC for 30s 72 ºC for 50s and terminate at 72 ºC for 10mins
	GGGAGGGTGACGGTGACATC		
entB	GTCAACTGGGCCTTTGAGCCGGTC	400	
	TATGGGCGTAAACGCCGGTGAT		

Table 5 Primer pairs used for the detection of virulence genes in *Bacillus cereus* (EM4).

Targeted gene	Primer sequence	Length (Bp)	Profile
nheA	TACGCTAAGGAGGGGCA	480	An initial denaturing 5min at 94 ºC, then 35 cycles of 94 ºC for 30s, 55 ºC for 30s 72 ºC for 60s and terminate at 72 ºC for 10mins
	GTTTTTATTGCTTCATCGGCT		
nheB	CTATCAGCACTTATGGCAG	754	
	ACTCCTAGCGGTGTTCC		
hblA	GTGCAGATGTTGATGCCGAT	301	
	ATGCCACTGCGTGGACATAT		
hblC	AATGGTCATCGGAACTCTAT	731	
	CTCGCTGTTCTGCTGTTAAT		
hblD	AATCAAGAGCTGTCACGAAT	411	
	CACCAATTGACCATGCTAAT		

Table 6 Primer pairs used for the detection of virulence genes in *Pseudomonas aeruginosa* (ELE12)

Targeted gene	Primer sequence	Lngth (Bp)	Profile
oprI	ATGAACAACGTTCTGAAATTCTCTGCT	250	An initial denaturing 5mins at 94 ºC, then 35 cycles of 94 ºC for 30secs, 50 ºC for 30secs 72 ºC for 60secs and terminated at 72 ºC for 10mins
	CTTGCGGCTGGCTTTTTCCAG		
oprL	ATGGAAATGCTGAAATTCGGC	500	
	CTTCTTCAGCTCGACGCGACG		
LasB	GGAATGAACGAAGCGTTCTC	300	
	GGTCCAGTAGTAGCGGTTGG		
toxA	GGTAACCAGCTCAGCCACAT	352	
	TGATGTCCAGGTCATGCTTC		
exoS	CTTGAAGGGACTCGACAAGG	504	
	TTCAGGTCCGCGTAGTGAAT		
nan1	AGGATGAATACTTATTTTGAT	316	
	TCACTAAATCCATCTCTGACCCGATA		

2.14. Statistical analysis

Data were analyzed by descriptive statistical methods and one-way analysis of variance (ANOVA) using SPSS version 22 at 95% confidence level.

3. Results and Discussion

A total of 423 bacterial isolates belonging to 16 genera were isolated from the water samples of the selected rivers (Ureje, Emirin, Ogbese, Odo-Ayo and Elemi) (Figures 1-5). These were *Escherichia coli*, *Bacillus subtilis, Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa, Shigella dysenteriae, Enterobacter aerogenes, Proteus vulgaris,*

Bacillus cereus, Aeromonas spp, *Citrobacter freundii, Staphylococcus aureus, Salmonella typhi*, *Bacillus licheniformis, Staphylococcus epidermidis* and *Micrococcus luteus.* Ninety eight (98) bacterial isolates were isolated from the water samples of Ureje River, one hundred and two (102) bacterial isolates were obtained from the water samples from Emirin River, seventy nine (79) bacterial isolates were isolated from the water samples of Ogbese River, seventy three (73) bacterial isolates were obtained from the water samples of Elemi River and a total of seventy four (74) bacterial isolates were isolated from the water samples from Odo-Ayo River. *E. coli* and *B. subtilis* had the highest frequency of occurrence while *Bacillus cereus* had the least frequency. The presence of these bacterial isolates implies contamination and poor microbial quality of the water samples from the rivers. This may be as a result of improper methods of refuse disposal in the environment, practice of open defeacation by the people in and around the rivers or other anthropogenic activities such as washing of motor cycles, rugs or cloths. This can result to significant health risks in the form of water borne diseases to the users of the water from the rivers. This result corroborates the work of Hernandez-sanchez *et al*. (2023) who reported the presence of *Staphylococcus aureus*, *E. coli*, *Vibrio* spp and *Enterococci* on microplastics found in the bathing areas of oceanic island, Tenerife, Canary Islands.

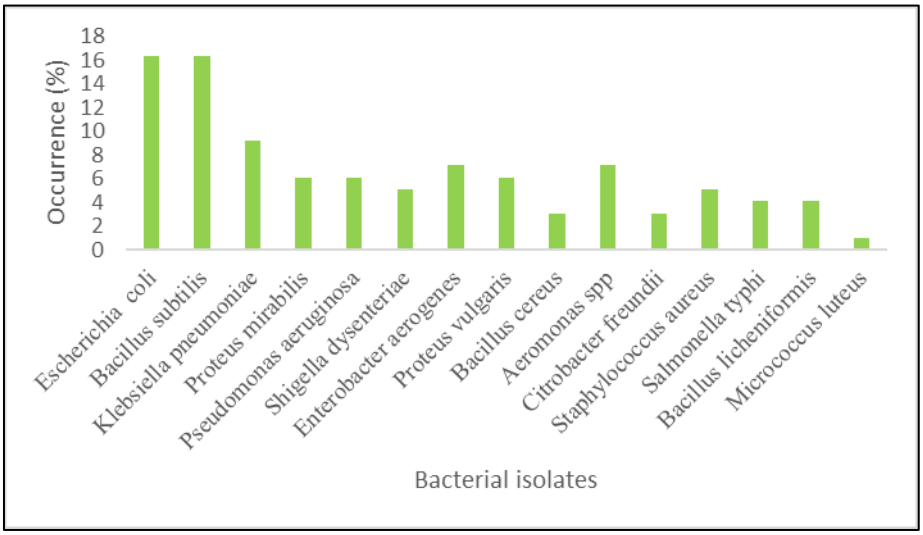

Figure 1 Occurrence of bacterial isolates in the water samples from Ureje River

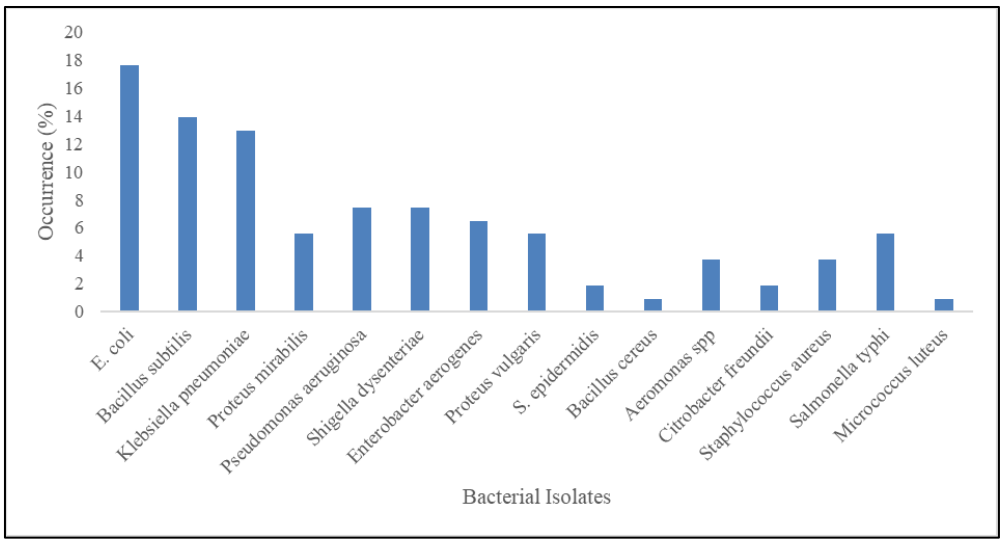

Figure 2 Occurrence of bacteria isolates in the water samples from Emirin River

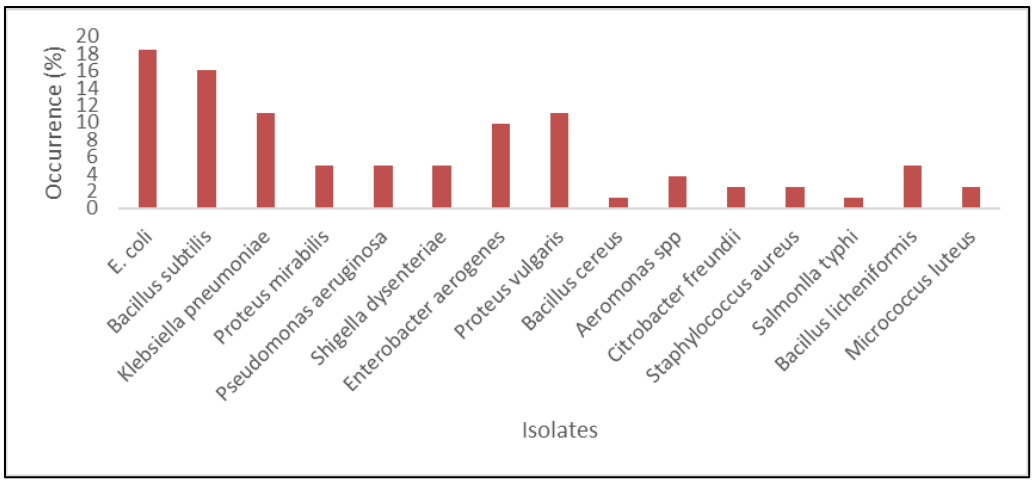

Figure 3 Occurrence of bacteria isolates in the water samples from Ogbese River

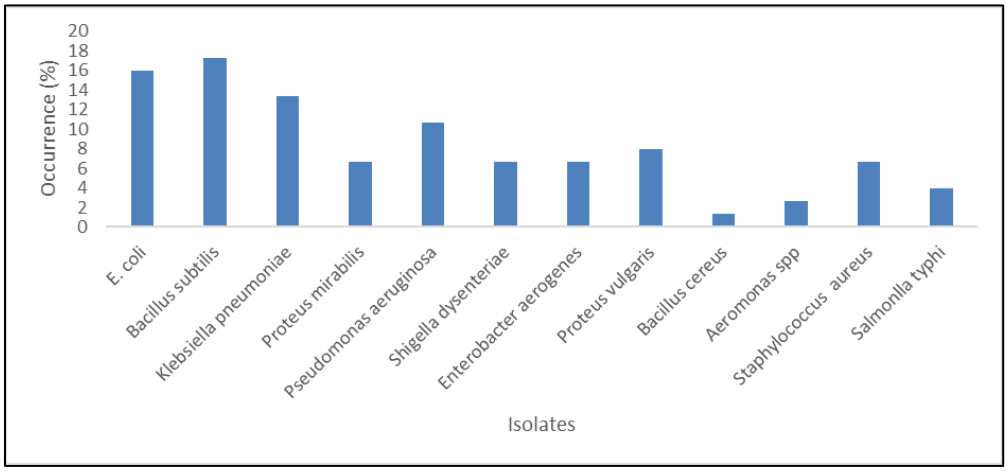

Figure 4 Occurrence of bacteria isolates in the water samples from Elemi River

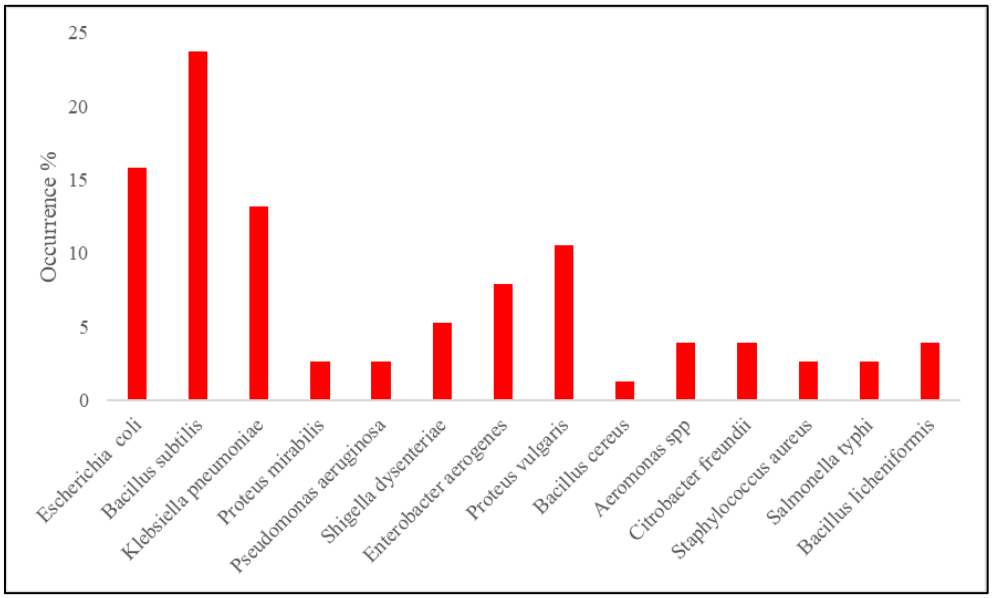

Figure 5 Occurrence of bacterial isolates in the water samples from Odo-Ayo River

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Figure 6 and 7 showed the antibiotic sensitivity profile of both Gram negative and Gram positive bacterial isolates, these isolates exhibited multiple antibiotic resistance. They were resistant to augumentin, ciproflox, septrin, streptomycin, erythromycin, rifampicin, amoxil and pefloxacin. This implies the presence of multiple antibiotic resistant bacteria in the selected rivers, and this may subsequently lead to proliferation of multiple antibiotic resistant bacteria in the environment that may pose health risks to human and aquatic animals. This result corroborates the work of Bunduki *et al.*(2023) where the authors observed high resistance of aminopencillins, tetracyclins, cotrimoxazole, nalidixic acid and cephalopsporins in uropathogenic *E. coli.*

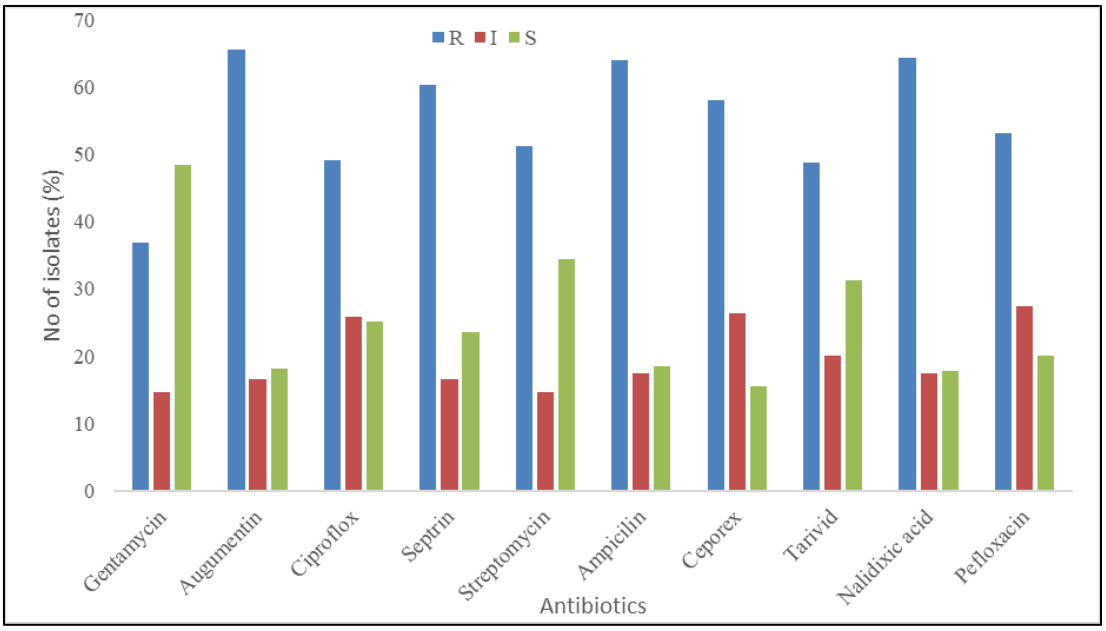

Figure 6 Antibiotic sensitivity profile of Gram negative bacterial isolates

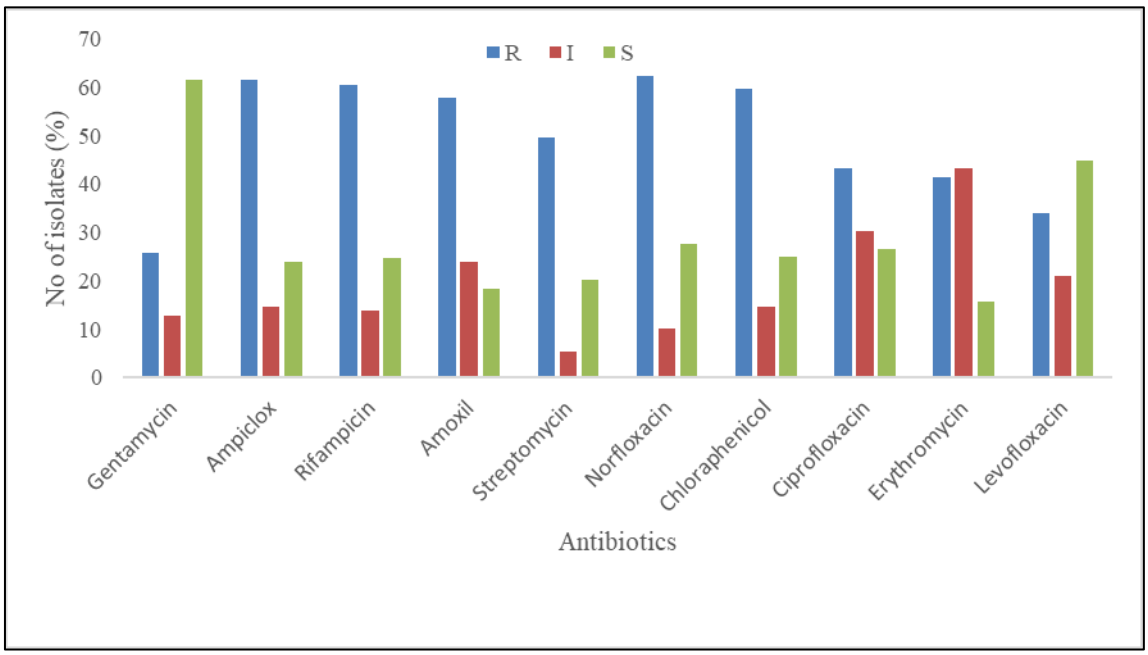

Figure 7 Antibiotic sensitivity profile of Gram positive bacterial isolates

Of the 423 bacterial isolates, 266 were positive to biofilm formation, 78 were positive to heamolysin formation, 87 were positive to phospholipase production and 76 were positive to gelatinase production (Table 7). Bacterial isolates like *Pseudomonas. Aeruginosa, Salmonella typhi, Aeromonas* spp and *Proteus vulgaris* were positive to biofilm formation, *E. coli* and *Staphylococcus aureus* were positive for heamolysin formation. It was also observed that *E. coli, P. aeruginosa*

B. cereus and K. pneumoniae were positive to phospholipase production while *E. coli*, *Salmonella typhi, P. aeruginosa and Aeromonas* spp were able to produce gelatinase. Findings from this study revealed that majority of the bacterial isolates possess one or two virulence factors, this implies their highly pathogenic nature, since virulence factors are molecules produced by bacteria or other pathogens that contribute to their ability to cause diseases and each of the virulence factors codes for a particular virulence genes (D'Onofrio *et al*., 2023). The presence of these virulence factors may be as a result of the possession of fimbriae, capsule and secretion systems in the screened isolates which may provide strong attachment of pathogens to the host bodies, it may also enable the transmission or spread of the pathogenic bacterial isolates in the environment. Virulence factor like biofilm formation may protect the bacteria from antibiotics and immune system aiding in chronic infections, the ability of the bacteria to form biofilm also help in the establishment and maintenance of the bacterial community (Bunduki *et al*., 2021). This result is in line with Vaish *et al*. (2016), where the authors observed the presence of gelatinase and heamolysin production in *Escherichia coli* isolated from extraintestinal infections.

Table 7 Screening of isolates for virulence factors

Virulence factors	**Ureje (n = 92)**	**Emirin (n = 98)**	**Ogbese (n = 88)**	**Elemi (n= 69)**	**Odo-Ayo (n= 76)**	**Total**
Biofilm formation	62	61	56	46	41	266
Heamolysin formation	16	20	15	15	12	78
Phospholipase production	20	18	20	14	15	87
Gelatinase production	18	16	15	14	13	76

Figures 8-12 show the virulence genes in (*Salmonella typhi* (EM22), *E.coli* (Urj. 23), *Shigella dysenteriae* (EM6), *Klebsiella pneumoniae* (OGB4), *Bacillus cereus* (EM4) and *Pseudomonas aeruginosa* (ELE12). In *Salmonella typhi* (EM22), the primers for *invA*, *spvR spvC*, *fimA* and *stn* genes were used, the gel image indicates a positive amplification for *stn*, *spvC* and *invA* genes. Each of these virulence genes encodes a specific virulence factor and has specific roles in virulence, for instance, *stn* virulence gene in *Salmonella typhi* EM22 encodes Salmonella enterotoxin whose function is to enhance the pathogenicity of the bacterium by contributing to the gastrointestinal symptoms of the infection (Yan *et al*., 2022). *spvC* is *Salmonella* virulence gene C which encodes an effector protein that is translocated into host cells through the Type III Secretion System (T3SS), the function is to promote systemic infection and survival inside macrophages. Invasion gene A (*invA*) gene encodes Salmonella Pathogenicity Island 1 (SPI-1) and the function is important for the initial stage of infection especially entry into host cells via membrane ruffling and endocytosis (Kang *et al*., 2024)

In *E. coli* (Urj. 23) and *Shigella dysenteriae* (EM6), the primers for *eaeA*, *stx2*, *stx1*, *fimH* and *papAH* genes were used. The gel image indicates a positive amplification for *eaeA*, *stx1* and *fimH* genes in *E. coli* and a positive amplification for *stx1* and *fimH* genes in shigella (EM6). The *eaeA* virulence gene (*E. coli* attaching and effacing gene A) encodes intimin which an outer membrane protein that mediates intimate adhension of *E. coli* to intestinal epithelial cells, *stx1* encodes shiga toxin that prevent proteins synthesis in the host cells. The *fimH* (fimbrial adhesion H gene) encodes the adhesion tip protein of type 1 fimbriae (pili) that are important in urinary tract infections (it aids colonization and persistence of the bacterium in the urinary tract despite flushing by urine) and also aids biofilm formation (Shahrivar *et al*., 2025). This study is in line with Shahrivar *et al*. (2025) where the authors observed a high prevalence of key virulence genes: *stx1*, *stx2* and *eaeA* from enterohemorrhagic *E. coli* strain isolated from traditional ice cream and juice samples in sales centres.

In *Klebsiella pneumoniae* (OGB4), the gel image indicates a positive amplification for *fimH*, *entB* and *rmpA* genes. The virulence gene *entB* (enterobactin biosynthesis gene B) encodes an enzyme involved in the biosynthesis of enterobactin an iron- chelating molecule, this gene helps in acquiring iron from the host which is essential for growth and survival during infections (Holden and Bachman, 2015). The *rmpA* virulence gene (regulator of mucoid phenotype A) encodes a transcriptional activator of capsule polysaccharide synthesis genes whose function is to increase capsule production and contribute to the hypermucoviscosity and promotes virulence in the host (Yu *et al*., 2008).

In *Bacillus cereus* (EM4), the gel image indicates positive amplification for *nheA*, *hblC* and *hblD* virulence genes, the presence of the ***nheA***, ***hblC***, and ***hblD*** genes indicates the organism's potential to produce two major enterotoxin complexes (the non-hemolytic enterotoxin and hemolytic BL enterotoxin) involved in foodborne illness. The ***nheA*** gene encodes a component of the **non-hemolytic enterotoxin (Nhe)** complex, which contributes to **pore formation** and **cytotoxic effects** in epithelial cells, particularly in the human intestine (Jeßberger *et al*., 2015). The ***hblC*** (encodes component L1) and ***hblD*** (component L2) are part of the **hemolysin BL *(Hbl)*** enterotoxin complex. Hbl is a tripartite toxin (consist of B, L1, and L2 components) whose function is to form pores in host cell membranes which result to **hemolysis**, **fluid secretion**, and **tissue damage**, thus playing a key role in diarrheal pathogenesis (Böhm *et al*., 2016).

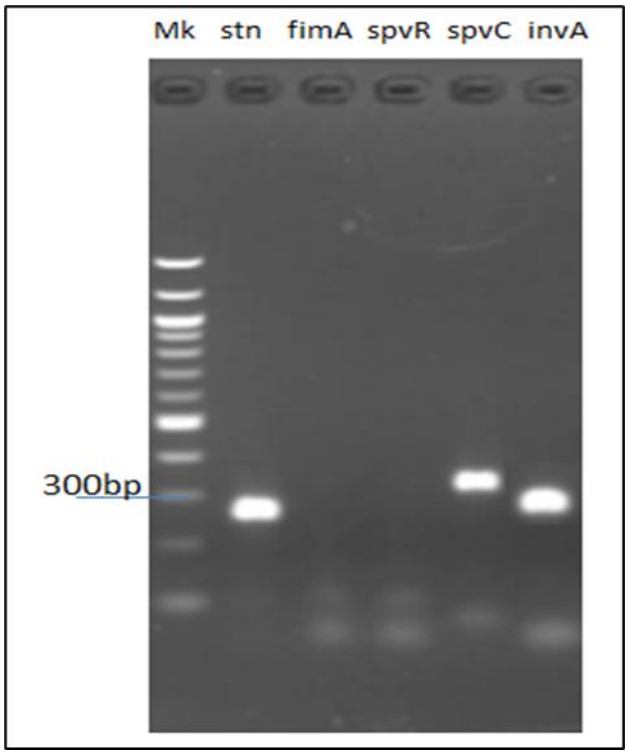

Figure 8 Virulence genes in *Salmonella typhi* (EM22)

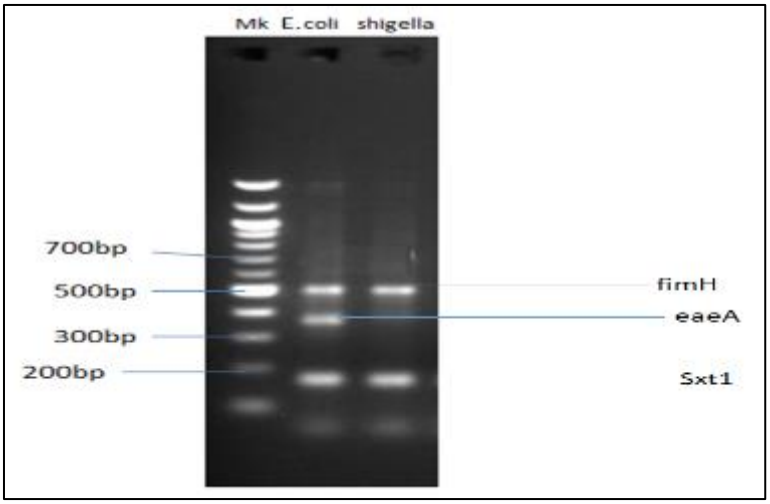

Figure 9 Virulence genes in *E.coli* (Urj.23) and Shigella (EM6)

In *Pseudomonas aeruginosa* ELE 12, the gel image indicates a positive amplification for *oprL*, *exoS* and *oprI* genes. *Oprl* and *oprI* encode outer membrane lipoprotein I and L respectively, they support virulence by enhancing bacterial

survival under stress , *exoS* virulence genes encodes exoenzyme S whose function is to disrupts host cells, immune evasion and cytotoxity (Hauser, 2019). The presence of virulence genes in all the isolates implies that all the bacterial isolates are highly pathogenic and they can use the different types of virulence genes to invade the host body and establish infections in the hosts. When these types of bacteria are found in water bodies, it may pose significant health risks in the form of water borne diseases to the users. This result is in line with Wilkie *et al*. (2024) where the authors detected *fimH, toxA,* virulence genes in Gram- negative isolates including *Klebsiella pneumoniae* isolated from daycare centres in Ile-Ife, Nigeria. A study by Adenipekun *et al*. (2023) also detected *oprL oprI and esoS* virulence gene in *Pseudomonas aeruginosa* in clinical isolates from a public hospital in Lagos, Nigeria.

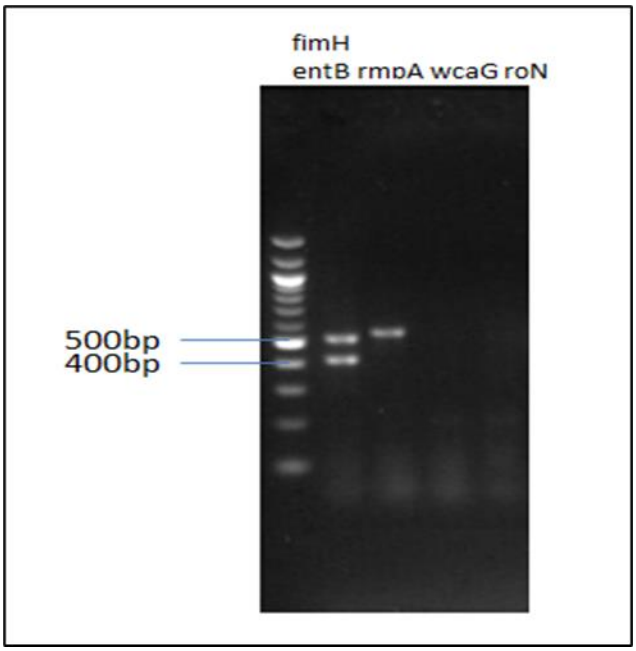

Figure 10 Virulence genes in *Klebsiella pneumoniae* (OGB4)

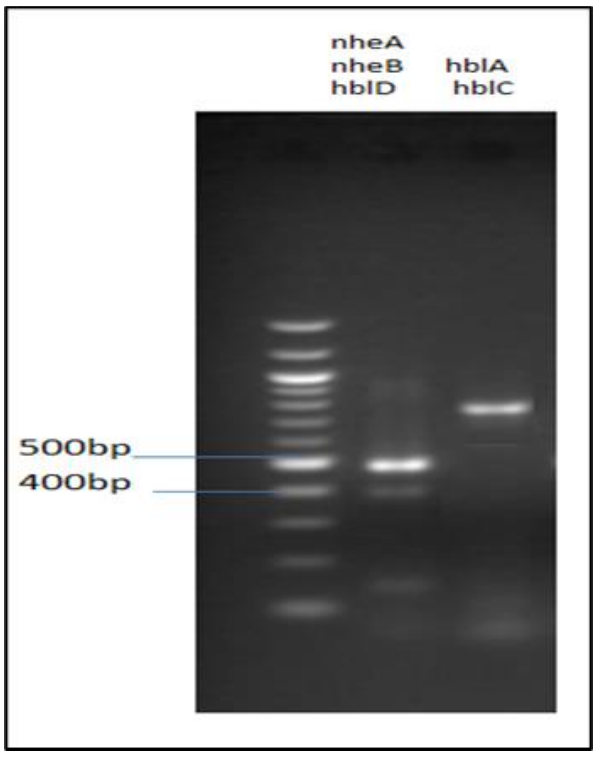

Figure 11 Virulence genes in *Bacillus cereus* (EM4)

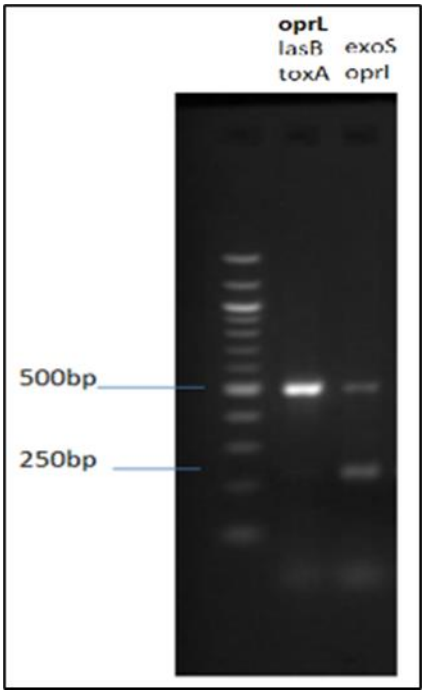

Figure 12 Virulence genes in *Pseudomonas aeruginosa* (ELE12)

4. Conclusion and Recommendation

Klebsiella pneumoniae, *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis Bacillus substilis, Bacillus licheniformis*, *Micrococcus luteus*, *Pseudomonas aeruginosa, Proteus mirabilis*, *Enterobacter aerogenes, Bacillus cereus*, *Citrobacter freundii, Aeromonas spp* and *Proteus vulgaris* were isolated in the plastisphere of microplastics detected in the selected rivers in Ado-Ekiti, Nigeria. The bacterial isolates were resistant to multiple antibiotics and poses virulences factors that encode numerous virulence genes including *rmpA*, *stn*, *spvC*, *invA, eaeA*, *stx1*, *fimH* and *entB* genes The rivers have poor microbiological qualities and must be adequately treated before use.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Adenipekun, E. O., Akinleye, E. F., Tewogbade, O. A., & Iwalokun, B. A. (2023). Detection of virulence genes and multidrug resistance in Pseudomonas aeruginosa clinical isolates from a public hospital in Lagos, Nigeria. *Scientific African, 22*, 195-205.

[2] Böhm, M. E., Krey, V. M., Jeßberger, N., Frenzel, E. and Scherer, S. (2016). Comparative bioinformatics and experimental analysis of the Hbl, Nhe, and CytK toxins of *Bacillus cereus*. *Frontiers in Microbiology*, *7*, 718.

[3] Bunduki, G. K., Heinz, E., Phiri, V.S. (2021). Virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) isolated from urinary tract infections: a systematic review and meta-analysis. *BMC Infect Dis* **21**, 753

[4] Concetta, P., Maria, R., Oriana, M., Yienia, M., Antonio, P., & Luigi, M. (2021). Microplastics in the environment: intake thrrough the food web, Human exposure and Toxicological effects. *Toxics, 9*(9), 224.

[5] D'Onofrio, V., Cartuyvels, R., A., M. P., Ivan, B., & Gyssens, I. C. (2023). Virulence factors genes in nvasive Eschericial coli are associated with clinical outcomes and disease severity in patients with sepsis: A prospective observation cohort study. *Microorganisms, 11*, 1827.

[6] Gaanpret, K., Soni, S. K., & Rupinder, T. (2017). Optimization of Phospholipase A1 (PLA1) production from a soil isolate Bacillus subtilis subsp inaquosorum via solid state fermentation. *Journal of pure and aplied Microbiology, 11*(2), 829-836.

[7] Haram, p. E., Carton, J. T., Ruiz, G. M., & Maximenko, N. A. (2020, January). A plasticene lexicon. *Marine Pollution Bulletin*, pp. 1100-1107.

[8] Holden, V. I. and Bachman, M. A. (2015). Diverging roles of bacterial siderophores during infection. *Metallomics*, *7*(6), 986–995.

[9] Jeßberger, N., Krey, V. M., Rademacher, C., Böhm, M. E., Mohr, A., Ehling-Schulz, M. and Scherer, S. (2015). From genome to toxicity: a combinatory approach highlights the complexity of enterotoxin production in *Bacillus cereus*. *Frontiers in Microbiology*, *6*, 560.

[10] Kang L, You J, Li Y, Huang R, Wu S. (2024) Effects and mechanisms of Salmonella plasmid virulence gene spv on host-regulated cell death. *Curr Microbiol*. 81(3): 86

[11] Lamchhane, G., Acharya, A., & Maracatha, R. (2023). Microplastics in environment global concern, challenges and controlling measures. *International journal of Environmental Science, 20*, 4672-4694.

[12] Mächtel, R., Jeßberger, N., Frenzel, E., and Ehling-Schulz, M. (2020). NheA is an essential subunit of the Nhe toxin complex for pore formation and cytotoxicity. *Frontiers in Microbiology*, *11*, 573288.

[13] Marathe, N. P., & Bank, M. (2022). The Microplastics-Antibiotic Resistance connection in Microplastics in the Environment: Pattern and Process, Environmental contamination Remediation and Management. *Springer Cham.*, 311-322.

[14] NOAA. (2023). *Microplastics/ Marine debtis program.* Retrieved from National oceanic and atmospheric administration service: https//marinedebris noaa gov/facts/microplasticshtml

[15] Olena, S., Viktor, S., Andry, M., Maris, K., Linards, K., & Ashok, V. (2021). Microbial life on the surface of Microplastics in Natural Waters. *Applied Sciences, 11*, 11692.

[16] Quinging, W. V., & Juah, W. (2018). Prevalence, virulence genes, Antimicrobial susceptibilty and genetic diversity of Bacillus cereus isolated from pasteurized milk in China. *Frontier Microbiology, 9*, 1419-1428.

[17] Shahrivar E, Rahimi E, Khamesipour F. (2025) Prevalence, identification of virulence genes, and antibiotic resistance properties of Shiga-toxin producing *Escherichia coli* (STEC) strains isolated from ice cream and juice in sales centers. *BMC Infect Dis*,25(1):581.

[18] Viaanderen, E. J., Ghaly, T. M., Moore, L. R., Focardi, A., Paulsen, I. T. and Tetu, G. S. (2023). Plastic leachate exposure drives antibiotic resistance and virulence in marine bacterial communities, *Environmental pollution*, 327, 1432-1440

[19] Wang, J., Guo, X., & Xue, J. (2021). Biofilm-Developed Microplastics as vectors of pollutants in aquatic environment. *Environ Science Technology, 55*(19), 12780-12790.

[20] Wenjie, Y., Yang, L., & Diana, B. (2023). Association between Microrganisms and Microplastics: How does it change the Host-Pathogen interaction and subsequnt immune response. *International Journal of molecular Science, 24*(4), 4065.

[21] Wilkie, E. D., Alao, J. O., Sotala, T. T. and Oluduro, A. (2024). Molecular characterisation of virulence genes in bacterial pathogens from daycare centres in Ile-Ife, Nigeria: Implication for infections control, *BMC Infectious diseases* 24: 1196

[22] WPR. (2023). *Location of Ekiti-State.* Retrieved from World population review: https//www.world population review.com

[23] Yan S, Liu X, Li C, Jiang Z, Li D, Zhu L. (2022). Genomic virulence genes profile analysis of *Salmonella enterica* isolates from animal and human in China from 2004 to 2019. *Microb Pathog*. ;173.

[24] Yu, W. L., Ko, W. C., Cheng, K. C., Lee, H. C., Ke, D. S., Lee, C. C. and Chuang, Y. C. (2008). Association between rmpA and magA genes and clinical syndromes caused by *Klebsiella pneumoniae* in Taiwan. *Clinical Infectious Diseases*, *47*(5), 642–650.