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PHENOTYPIC AND GENOTYPIC DETECTION OF ANTIBIOTIC RESISTANCE AMONG *Plesiomonas shigelloides* ISOLATED FROM EBUTE RIVER, SOUTHWEST NIGERIA

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Abstract

Plesiomonas shigelloides, is an emerging enteric pathogen found in water with high resistance ability against commonly used antibiotics. This study evaluated the incidence of *Plesiomonas shigelloides* in ebute river, Okitipupa, Nigeria and their antibiogram fingerprints. Confirmed isolates were evaluated for their antibiogram profiles against a panel of 15 antibiotics using disc diffusion method and further screened for antibiotic-resistant determinant. About 64 presumptive *Plesiomonas shigelloides* isolates was recovered from the river out of which 30 (46.9%) were confirmed positive using polymerase chain reaction techniques. Results showed that, 93% of the isolates were resistant against erythromycin and ceftazidime, 90%, 83%, 80%, 73%, 63%, 60%, 57% and 53% of the isolates exhibited resistance against cefotaxime and ampicillin, cephalothin, streptomycin, amoxicillin, tetracycline, Trimethoprim and chloramphenicol respectively. Similarly, resistance against the other antibiotics followed the order: Meropenem (33%), trimethoprim + sulphamethoxazole (27%) and Ciprofloxacin (23%) with others less than 20%. Ninety four percent of *Plesiomonas shigelloides* that exhibited resistant against tetracycline possesses tetA genes, 50% harboured tetE genes. About 67% of the isolates that showed resistant to ampicillin harboured ampC genes. Sixty three percent of the isolates that resisted the effect of chloramphenicol possessed cmlA1 while 43% harboured cat gene. dfr1 and dfr18 was examined in isolates that resisted the effect of sulfonamides, and 57% possessed dfr1 while 40% possessed dfr18. strA genes was also found in 42% of isolates that was resistant to streptomycin. This study concludes that ebute river is a reservoir of antibiotic-resistant enteric pathogen, and a potential public health threat.

Keywords: *Plesiomonas shigelloides*, Ebute river, Antibiotics, Resistant genes, Public health

Introduction

Plesiomonas shigelloides is a bacterium that has been classified as one of the emerging pathogens for more than three (3) decades (Ekundayo and Okoh, 2018). *Plesiomonas shigelloides* is a rod-shaped, oxidase-positive, Gram-negative bacterium that causes intestinal infections linked to eating seafood, eating raw food, and drinking tainted water. One of the main risk factors for *Plesiomonas* in humans is traveling abroad (Janda *et al.*, 2016). *Plesiomonas shigelloides* has been shown to have a negative impact on human health, including extra-intestinal illnesses and gastroenteritis (Bowman *et al.*, 2016). Numerous nations have reported the

presence and recovery of *Plesiomonas shigelloides* from a variety of sources, including water, animals, food, and people (Nwokocha and Onyemelukwe, 2014; Bowman *et al.*, 2016*).* The symptoms of *Plesiomonas shigelloides* infection include diarrhea, abdominal pain, nausea, and body aches (Milivoje *et al.*, 2017). The bacteria had been primarily linked to aquatic settings and aquatic animals (Alexander *et al.*, 2016).

One of the bacteria with zoonotic significance that is present in a wide range of environments is *Plesiomonas shigelloides*. This microbe has been identified as an animal pathogen, particularly in fish, and has been described as a component of the microbiota of waterfowl and ectothermic animals (Kim *et al.*, 2015; Janda *et al.*, 2016). *Aeromonas* species and *Plesiomonas shigelloides* are significant foodborne and waterborne pathogens that can cause acute diarrhea or extraintestinal infections that impact the skin, soft tissues, and several internal organs in people. Aquaculture workers and food handlers are more frequently reported to have these infections (Parker and Shaw, 2011; Janda *et al.*, 2016). *Plesiomonas shigelloides* is now recognized as a human enteric infection cause and has grown in importance since it produces cholera-like diarrhea in the majority of infected individuals (Alexander *et al.*, 2016), particularly after consuming tainted water and raw seafood. Additionally, this bacterium causes a number of extraintestinal disorders, including as pneumonia, septicemia, and meningitis, which can result in a high death rate, particularly in individuals with impaired immune systems (Klatte *et al.*, 2012; Miyoshi and Stappenbeck, 2013). It has been linked to diarrhea outbreaks and occasional occurrences all over the world (Bodhidatta *et al.*, 2010; Bowman *et al.*, 2016). *Plesiomonas shigelloides* has become a known cause of enteric infections in human and has gained increased significance as it causes cholera-like diarrhea in most infected persons (Alexander *et al.,* 2016) especially following consumption of contaminated water and raw sea foods.

The existence of several virulence factors has been ascribed to the prospective pathogens' capacity to cause infections. According to reports, *Plesiomonas shigelloides* possesses 14 virulence factors, including effector, efflux pump, protease, and adhesion (Yin *et al.*, 2020). Additionally, biofilm development, adhesion, eukaryotic host cell invasion, and serum complement resistance (Aquilini *et al.*, 2013). The formation of cytotoxic lipolysaccharide complex, invasion factors, cytolysin, and histamine are among the virulence components of *Plesiomonas* that have been the subject of numerous reports (Bjornsdottir-Butler *et al.*, 2011). *Plesiomonas shigelloides* attaches to and invades host cells in vitro and has enterotoxicity and cytotoxicity (Aquilini *et al.*, 2013).

As drug resistance increases around the world, antibiotics are losing their effectiveness, making it harder to cure infections and avoid fatalities (WHO, 2020). In freshwater environments, *Plesiomonas shigelloides* is a new and important pathogen that may exhibit strong resistance to widely used antibiotics (Nwokocha and Onyemelukwe, 2014; Adesiyan *et al.*, 2019). *Plesiomonas* isolates with varied resistances to numerous tested antibiotics have been described by many authors (Bonatti *et al.*, 2012; Chen *et al.*, 2013; Abdelhamed *et al.*, 2018). *Plesiomonas shigelloides* had a high level of resistance to ampicillin, trimetroprim + sulphamethoxazole, and tetracycline, but a lower level of resistance to imipenem, aztreneonam, and cefotaxime, according to a study by Nwokocha and Onyemelukwe

(2014). While several studies have documented resistance against tetracycline, streptomycin, erythromycin, neomycin, and sulphamethoxazole, other countries have reported that *Plesiomonas shigelloides* resisted the effects of antibiotics such as aminoglycosides and penicillin (González-Rey *et al.*, 2004; Adesiyan *et al.*, 2019; Agboola *et al.*, 2024). Therefore, it is essential to assess water bodies that are important to people in riverine areas for the presence of potential pathogens and antibiotics resistant determinant as part of our surveillance study on the reservoirs of antimicrobial resistance pathogen that could cause epidemic in our environment. This is to provide information on the prevalence of the potential pathogen and possible effective antibiotics that could be utilized to treat infection that might arise in future by this organism.

Materials and Methods

Study Area

This study was conducted in Okitipupa using Ebute River (surface water) located in Ondo State, Nigeria. The coordinate of the sample site is N 6° 51'37.1'', E 4 ° 79' 03.2'' (Ebute surface water). The water was selected based on the fact that it is a key freshwater source in Okitipupa meeting the municipal, domestic and economic needs of the people.

Sample collection

Water samples were collected aseptically at three different points along the river bank (Ebute river in Okitipupa) over a period of 3 months. The sampling point chosen was as a result of prominent anthropogenic activities with the water source. The water Samples were collected from Ebute River once in a week using sterile bottles and transported to the laboratory using a box containing ice packs, processing of the samples was done within 6 hours of sample collection. The procedure recommended by America Public Health Association (APHA, 2012) was followed.

Presumptive isolation of *Plesiomonas shigelloides*

The choice of sampling points was based on the evidence of human-water contact activities around the area. Processing of samples was done within 6 h of collection, following the procedure recommended by American Public Health Association (APHA, 2012). Water samples were enriched in peptone-water (pH 8.6) and incubated at 37 °C for 24 h after which they were serially diluted. After dilution, 0.1 ml each of the dilution was placed at the centre of well labelled dried plates of Inositol Brilliant Green Bile agar (Conda Pronadisa, Spain). Immediately, a sterile glass spreader was used to evenly spread sample all over the agar plate surface. The plates were then incubated at 37 °C in an inverted position for 24 h. Pinkish colonies were selected as presumptive *P. shigelloides* on each plate. Gram staining and oxidase test were further carried out and only Gram-negative, oxidase positive isolates were selected and sub-cultured on purity plate before being stored on 25% glycerol for further analysis.

Extraction of DNA

Extraction of DNA was done using the boiling method (Agboola *et al.*, 2024). Presumptive *P. shigelloides* colonies were selected and subcultured on non-selective agar plates for 18–24 h at 37 °C. Pure and distinct colonies selected were placed in 200 µl sterile distilled water, vortexed, boiled for 15 min at 100 °C and centrifuged for 10 min at 15,000 rpm. The resultant supernatants were stored in Eppendorf tube at −80 °C until ready for use.

PCR Confirmation of *Plesiomonas shigelloides* isolates

The presumptive *P. shigelloides* identity were confirmed by 23SrRNA gene using

polymerase chain reaction technique and using primer sets PS23FW3/PS23RV3 developed by González-Rey *et al.* (2000) which amplifies a 284 bp sequence of the 23SrRNA gene [(PS23FW3: 5′-CTCCGAATACCGTAGAGTGCTATCC-3) and (PS23RV3: 5′-CTCCCCTAGCCCAATAACACCTAAA-3)] of *P. shigelloides*. The PCR conditions used were as described by González-Rey *et al.* (2000) with slight modifications. The 25 µl total reaction volume reaction consisted of PCR master mix (Biolabs, United Kingdom), 12.5 µl, oligonucleotide primer 1 µl each, DNA template 5 µl and nuclease free water 5.5 µl (González-Rey *et al.*, 2000). Protocols used for the PCR reaction included; the initial denaturation for 5 min at 95 °C, then denaturation, annealing and extension steps, at 94 °C for 1 min; 68 °C for 1 min; 72 °C for 2 min respectively and final extension step at 72 °C for 10 min at the end of 35 cycles. Electrophoresis was done by loading 5 µl aliquots of the amplicons on 1.5% agarose gel in which 5 µl ethidium bromide stain has been added. PCR products were visualized with gel documentation equipment (UVsave, Applied Bioscience, UK). DNA ladder of 100-bp was used for molecular size calibration on the gel and the electrophoresis running condition was 100 V for 50 min. *P. shigelloides* DSMZ 8224 was used as a reference strain.

Antibiotic susceptibility testing

Antibiotic susceptibility test was performed using the disc diffusion method (Kirby *et al.*, 1966). Distinct colonies were picked from an 18 h culture of the test organism and placed in tubes in which 3 ml of 0.85% physiological saline has been added. Turbidity of resultant mixture was compared to 0.5 McFarland standard solutions (equivalent to 1.5 × 108 CFU). Using a sterile swab, 0.1 ml of the standardized suspension was spread on plates of Mueller Hinton agar and allowed to dry before placing antibiotics disc on them. A total of 15 antibiotics (Mast Diagnostics, UK) selected for the test included, amikacin (30 µg), streptomycin (30 µg), trimethoprim (5 µg), gentamycin (10 µg), cephalothin (30 µg), cefotaxine (30 µg), ciprofloxacin (5 µg), meropenem (10 µg), ceftazidime (30 µg), erythromycin (15 µg), chloramphenicol (30 µg), tetracycline (30 µg), trimethoprim + sulphamethoxazole (25 µg), amoxicillin (25 µg) and ampicillin (10 µg). Antibiotic discs were placed on Mueller Hinton agar plates and subsequently incubated at 37 °C for 24 h. The zones of inhibition width were calculated and recorded as susceptible (S), resistant (R) or intermediate (I) as recommended by Clinical and Laboratory Standards Institute (CLSI, 2015) for zone diameter interpretation.

Detection of antimicrobial resistance genes

Plesiomonas shigelloides isolates that exhibited resistance phenotypically were assayed for detection of relevant antibiotic resistance genes. The genes assayed for included: tetA, tetE, (which encodes tetracycline resistance) catII and cmlA1 (chloramphenicol resistance), strB (aminoglycoside resistance), dfr1 and dfr (18) (trimethoprim resistance), and β-lactamases-encoding gene (ampC). The primers sequences and the targeted amplicon sizes are as shown in Table 1. The PCR reaction mixture contained 12.5 µl of PCR Master Mix (Biolabs, United Kingdom), 1 µl each of oligonucleotide primer (Inqaba Biotech, SA), 5 µl of DNA template and 5.5 µl of nuclease free water to constitute a total reaction volume of 25 µl. Gel electrophoresis of the PCR product was carried out with 5 µl of the amplicons in 1.5% (w/v) agarose for 45 min at 100 v and visualized with UV transillumination gel documentation system (UVsave, Applied

Bioscience, UK). (Cd), and mercury (Hg), pose serious threats to both human health and ecological systems (Singh et al., 2018). These pollutants can originate from various sources, including improper waste disposal, vehicle emissions, leachate from decomposing refuse, industrial activities, and agricultural practices (Alloway, 2013). According to Das et al. (2022) Industrialization and large scale human activities have significantly contributed to environmental pollution. Emissions of dust, smoke, fumes, and toxic gasses are common such as highly polluted industries, coal mines, cement, sponge iron, steel and ferroalloys plants as well as petroleum, and chemicals industries release. In addition, these industries release substantial

Table 1: Primers used for PCR amplification of antibiotics resistance genes.

Antibiotic group	Primer	PCR primer sequence (53′)	Amplicon size (bp)
Sulfonamides	dfr1	F: CGAAGAATGGAGTTATCGGG	372
		R: TGCTGGGGATTTCAGGAAAG	
	dfr(18)	F: TGGGTAAGACACTCGTCATGGG	389
		R: ACTGCCGTTTTCGATAATGTGG	
Tetracycline	TetA	F: GCTACATCCTGCTTGCCTTC	209
		R: ATAGATCGCCGTGAAGAGG	
	TetE	F: GCGCTNTATGCGTTGATGCA	246
		R: ATGTGTCCTGGATTCCT	
Phenicol	cmlA1	F: CACCAATCATGACCAAG	116
		R: GGCATCACTCGGCATGGACATG	
	CatII	F: ACACTTTGCCCTTTATCGTC	543
		R: TGAAAGCCATCACATACTGC	
Beta-Lactam	AmpC	F: TTCTATCAAMACTGGCARCC	550
		R: CCYTTTTATGTACCCAYGA	
	StrA	F: CTTGGTGATAACGGCAATTC	548
		R: CCAATCGCAGATAGAAGGC	

(Adesiyan *et al.*, 2019)

Results

Confirmation of *P. shigelloides*

In total, 64 presumptive isolates were selected for molecular confirmation of which 30 (46.9%) were confirmed to be *P. Shigelloides*. *P. shigelloides* confirmed by PCR amplification of the 23rSRNA is also shown in Plate 1.

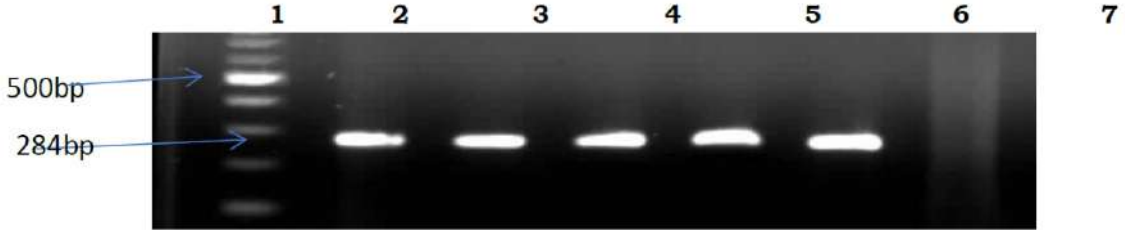

Plate 1: Gel picture of *Plesiomonas shigelloides PS23* gene confirmation
Lane 1*:* Molecular weight marker (100bp); lane 2-5: *Plesiomonas shigelloides* isolates; Lane 6: positive control; Lane 7*:* negative control.

Antibiotic Susceptibility Profiles

All the 30 confirmed *P. shigelloides* isolates were assessed for phenotypic resistance against a panel of 15 different antibiotics selected across 9 classes of antibiotics. Results showed that, 93% of the isolates were resistant against erythromycin and ceftazidime, whereas 90%, 83%, 80%, 73%, 63%, 60%, 57% and 53% of the isolates exhibited resistance against cefotaxime and ampicillin, cephalothin, streptomycin, amoxicillin, tetracycline, Trimethoprim and chloramphenicol respectively (Figure 1). Similarly, resistance against the other antibiotics followed the order: (57%), Meropenem (33%), trimethoprim + sulphamethoxazole (27%) and Ciprofloxacin (23%) while others were less than 20% (Table 2). On the other hand, isolates were susceptible to other antibiotics in the following order: gentamicin (90%), amikacin (83%), ciprofloxacin (77%), Trimethoprim + sulphamethoxazole (73%), meropenem (67%), trimethoprim (43%). The antibiogram of *P. shigelloides* isolates from each selected rivers is as shown in Table 2.

Detection of Antibiotics Resistant Genes

Screening for certain antibiotics resistant determinants via PCR revealed that 94% of *Plesiomonas shigelloides* that exhibited resistant against tetracycline possess *tetA* genes while 50% harboured *tetE* genes. About 67% of the isolates that showed resistant to ampicillin harboured *ampC* genes that confer resistance. Sixty three percent of the isolates that resisted the effect of chloramphenicol possessed cmlA1 while 43% harboured cat gene that had been shown to confer resistance in bacteria. For the isolates that resisted the effect of sulfonamides, dfr1 and dfr18 were examined and it was discovered that 57% possess dfr1 while 40% possess dfr18. strA genes was also screened for in isolates that were resistant to streptomycin and it was discovered that 42 % were positive for this gene conferring resistant to streptomycin (Plate 2).

Table 2: Antibiogram of the confirmed *Plesiomonas shigelloides*

Antibiotic group	Antibiotic agent	Disc Code	P (µg)	Susceptible (%)	Intermediate (%)	Resistant (%)
Aminoglycosides	Amikacin	AK	30	25 (83)	1 (3)	4 (14)
	Gentamicin	G	10	27 (90)	3 (10)	0 (0)
	Streptomycin	S	10	6 (20)	5 (17)	19 (63)
β-Lactams	Ampicillin	AP	10	3 (10)	5 (17)	22 (73)
	Amoxicillin	AMC	25	8 (27)	7 (23)	15 (50)
Cephems	Cefotaxime	CTX	30	3(10)	1(3)	26(87)
	Cephalotin	KF	30	5 (17)	2 (7)	23 (76)
	Ceftazidime	CAZ	30	2 (7)	7 (23)	21 (70)
Carbapenems	Meropenem	MEM	10	20 (67)	2 (7)	8 (27)
Fluoroquinolones	Ciprofloxacin	CIP	5	23 (77)	1 (3)	6 (20)
Sulfonamides	Trimethoprim	TM	5	13 (43)	8 (27)	9 (30)
	Trimethoprim + sulphamethoxazole	TS	25	22 (73)	2 (7)	6 (20)
Tetracyclines	Tetracycline	T	30	12 (40)	8 (27)	10 (33)
Phenicols	Chloramphenicol	C	30	14 (47)	3 (10)	13 (43)
Macrolides	Erythromycin	E	15	2 (7)	0 (0)	28 (93)

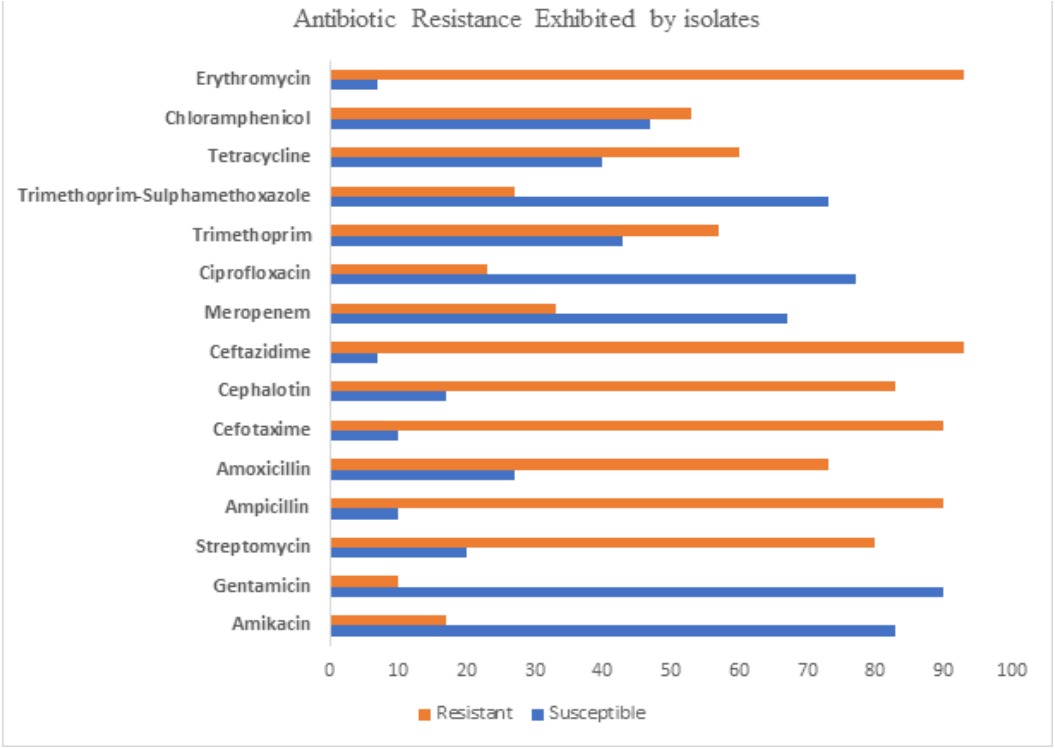

Figure 1: Percentage resistance exhibited by the isolates against the selected antibiotics

Table 3: Prevalence of Antibiotics Resistant Genes among the Isolates

Antibiotic group	Antibiotics	Antibiotic resistance gene	% of isolates that are positive
Beta -Lactams	Ampicillin (n = 27)	ampC	67% (18)
Sulfonamides	Trimethoprim + sulphamethoxazole (n= 8)	dfr1	52% (13)
	Trimethoprim (n = 17)	dfr(18)	40% (10)
Tetracyclines	Tetracyclines (n = 18)	tetA	94% (17)
		tetE	50% (9)
Phenicols	Chloramphenicol (n = 16)	cmlA1	63% (10)
		Cat	43% (7)
Aminoglycosides	Streptomycin (n = 24)	strA	42% (10)

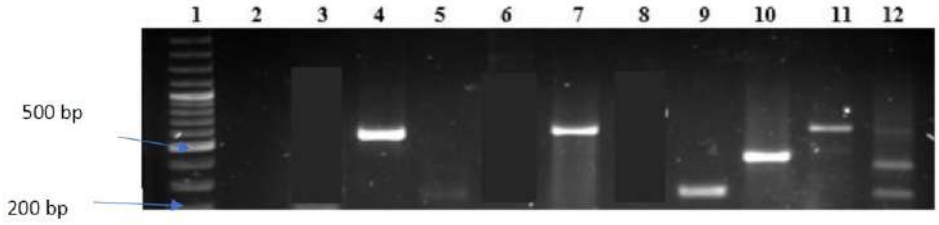

Plate 2. A representative Gel Electrophoresis Profile of Antibiotic-Resistant Genes of *P. shigelloides* Isolates.
Lanes 1: Molecular weight marker (100 bp DNA ladder), lane 2: negative control, lane 4: *cat11* (543 bp), lane 5: *tetE* (246 bp), lane 7: *strA* (548 bp), lane 9: *tetA* (209 bp), lane 10: *dfrI* (372 bp), lane 11: *ampC* (550 bp), lane 12: *dfr(18)* (389 bp).

Discussion

According to Singh *et al.* (2017), water is a renewable resource that is necessary for the production of food, the maintenance of all living forms, and economic growth for overall well-being. According to Kim *et al.* (2015), water bodies represent a significant means of pathogen transmission. Additionally, it contributes to the spread of antimicrobial medications in the environment (Wellington *et al.*, 2013). The incidence and pattern of antibiotic susceptibility of *Plesiomonas shigelloides* in Okitipupa surface water (Ebute River), Ondo State, are examined in this study. This body of water is frequently utilized for commercial, agricultural, and residential reasons. The antibiotic-resistant determinant of *P. shigelloides* isolated from the water body was also investigated in this study. Using a species-specific PCR assay, the presumed *P. shigelloides* isolates were verified by amplifying the 23SrRNA gene's nucleotide regions C-906 and G-1189. This organism's discovery in the water body suggests that the contamination may be the result of human activity near the water and the discharge of effluents into the river or their washing into it during runoff. Among the activities seen near the riverbank are fishing, traveling, bathing, washing, fish markets, and cow rearing. Animal and human excreta litters were also observed near the surface water, suggesting that the isolates might have come from animal and human excrement (Hacloglu and Tosunoglu, 2014; Titilawo *et al.*, 2015). This study also confirms the findings of diverse researchers who reported the presence of this pathogen in water and aquatic animals in diverse countries (Hacioglu and Tosunoglu, 2014; Kim *et al.*, 2015; Adesiyan *et al.*, 2019; Agboola *et al.*, 2024).

Plesiomonas shigelloides is thought to be a prevalent cause of human gastroenteritis and has been linked to food poisoning epidemics (Kusumawaty *et al.*, 2023). In humans, the bacterium has also been demonstrated to cause keratitis, osteomyelitis, pneumonia, sepsis, meningitis, bacteremia, and other non-diarrheal illnesses (Jiang *et al.*, 2021; Bruage *et al.*, 2022). *Plesiomonas shigelloides* is a common cause of significant financial losses for the global fish farming sector. A disease epidemic in the wetland's H. molitrix (silver carp) occurred in May 2016. 60% silver carp mortality was noted during the illness investigation period and through passive data collecting. *Plesiomonas shigelloides* was also identified from fish clinical cases during the mass death of *Orechromis nilotica* and *Ctenopharyngodo nidellus,* and it was found to be extremely harmful to these farmed fishes (Adesiyan *et al.*, 2019). The management of infections brought on by this bacterium exacerbates the issue of antibiotic resistance in the environment, particularly in aquatic environments.

It is well recognized that water sources have a role in the environmental distribution of antibiotics, particularly those often employed in aquaculture and agriculture, which leads to the emergence and spread of resistance bacterial strains (Wellington *et al.*, 2013). Multiple antibiotic resistance was observed in isolates of *Plesiomonas shigelloides*. Although the organisms used in this study came from aquatic animals, the level of resistance shown by the isolates against trimethoprim, erythromycin, tetracycline ampicillin, chloramphenicol, amoxicillin, and streptomycin is consistent with findings from other researchers (Chen *et al.*, 2013; Adesiyan *et al.*, 2019; Agboola *et al.*, 2024, Odey *et al.*, 2025). Contrary to the findings of some researchers who reported reduced susceptibility to gentamicin in some environmental and clinical isolates, the isolates obtained from this study exhibited

high susceptibility to the antibiotics, which may be one of the differences between clinical isolates and environmental strains. It has been noted that *Plesiomonas shigelloides'* resistance may be due to the possibility that its outer membrane prevents antibiotics from entering its cell wall. On the other hand, isolates were varyingly susceptible to amikacin, gentamicin, ciprofloxacin, and meropenem, which is consistent with similar studies. This suggests that the prospective infections' susceptibility to aminoglycosides may differ based on their sources and the activities they engage in near the chosen bodies of water. Numerous studies have highlighted the role of wastewater effluent in the spread of enteric bacteria that are resistant to multiple antibiotics, carrying resistant plasmids, and serving as a major site for horizontal gene transfer because of the high concentration of microorganisms and the abundance of nutrients (Akhter *et al.*, 2014; Adesiyan *et al.*, 2019). Therefore, surface runoff from many locations that the river receives may be the cause of the resistance to various antibiotics displayed by the potential pathogen identified in this study.

Additionally, water is the primary means by which antibiotic-resistant genes spread throughout the environment and contribute to their development as pollutants (Stalder *et al.*, 2012; Stange *et al.*, 2016). Genes that encode resistance to aminoglycosides, chloramphenicol, β-lactams, sulfonamides, tetracycline, and trimethoprim are among the numerous antibiotic-resistant determinants that have been found in a variety of environmental tests (Adesiyan *et al.*, 2019). In this investigation, *P. shigelloides* isolates were screened for antibiotic resistance determinants. Since beta-lactams are the most widely used antibiotics for treating a variety of infections and are frequently found in the environment, they represent a serious risk to public health. 67% of the isolates in this investigation tested positive for the *ampC* gene. It is also feasible that the remaining isolates may carry other beta-lactams resistance genes not assayed for in this study as hypothesized by another researcher (Adesiyan *et al.*, 2019) It has been shown in previous studies, that, diverse groups of beta-lactamases are present in environmental samples of *P. shigelloides* showing that the acquisition of these beta-lactamase genes has not emerged entirely from selective pressure of beta-lactamase therapy (Avison *et al.*, 2000). Similar studies have reported high incidence in gram-negative bacteria recovered from aquatic environments (Hamelin *et al.*, 2006; Titilawo *et al.*, 2015; Alexander *et al.*, 2015), and minimal β-lactam drug resistance is also known to be naturally present in various gram-negative bacteria from the environment (Esiobu *et al.*, 2002).

A high frequency of the dfr1 gene (52%) was found (O1-specific trimethoprim resistance) when two trimethoprim genes (dfr1 and 18) were investigated. The frequency of cholera-encoding gene cassettes Since these medications are typically used in conjunction with sulfonamide as a therapy for cholera, O1-specific trimethoprim resistance in the isolates is concerning. One of the signs of an infection brought on by *P. Shigelloides* is cholera-like diarrhea, which is also known to produce cholera-like toxin. Bacteria isolated from aquatic environments have also been found to possess these genes (Igbinosa, 2010; Šeputienė *et al.*, 2010; Stange *et al.*, 2016). Similarly, tetA and tetE were found in this study at different percentages, with tetA (94%) having the highest frequency, which is consistent with the results of Adesiyan *et al.* (2019). The most commonly found resistant gene in aquatic bacteria (Wang *et al.*, 2013; Lin *et al.*, 2015; Xu *et al.*, 2017) is the ribosomal efflux gene tetA, which has a broad

host range and is easily carried by various kinds of bacteria (Zhang *et al.*, 2009). However, Xu *et al.* (2016) also noted that tetA and tetE were the most abundant ARGs in terms of absolute abundance in Beijing, China's urban waterways, while Usui *et al.* (2016) revealed that the tetE gene was the most commonly seen ARG in Thailand's aquatic environment. However, out of the eleven tetracycline genes evaluated in non-urban sewage plants, this gene was shown to be low in frequency (Xu *et al.*, 2017).

About 63% of the isolates contained cmlA1 (a gene that resists chloramphenicol), and 43% had catII. It was also discovered that there are a number of different types of cat and cml genes that are known to have environmental origins (Dang *et al.*, 2008; Adesiyan *et al.*, 2019). However, comparable research has shown that bacteria from aqueous environments have low levels of the cmlA1 and catII genes (Fischbach and Walsh, 2009; He *et al.*, 2016). A class of antibiotics known as aminoglycosides has gained importance in the management and prevention of severe invasive bacterial infections in both human and veterinary medicine. In contrast to a prior discovery that 67% of isolates carried the gene, the strA (42%) that confers streptomycin phosphoryltransferase resistance was found in this investigation (Adesiyan *et al.*, 2019). However, in a study conducted in Haiti by Baron *et al.* (2016), strA was identified and consistently found in conjunction with strB, whereas the strB gene was found in 11 additional isolates that were streptomycin-susceptible. However, it is possible that the isolates include additional streptomycin resistance genes because other streptomycin gene variants were not searched for.

Different resistance mechanisms, including horizontal gene transmission of antibiotic resistance genes between bacteria, selective pressure of antibiotic substances and heavy metals (Seiler and Berendonk, 2012), inclinations for genetic modifications and recombination events (Alexander *et al.*, 2015), and the diffusion of antibiotic-resistant bacteria from human and animal medicine (Cantas *et al.*, 2013), are responsible for the occurrence and persistence of antibiotic resistance in the environment. These processes support the pattern of antibiotic resistance that has been extensively documented in aquatic environments and assist the microbe in adapting to its changing surroundings.

Although PCR-based techniques utilized in this study provide a specific approach to identifying *Plesiomonas shigelloides* and its antibiotic resistance determinants, several limitations affect the overall findings. Firstly, PCR is inherently limited to the detection of known genetic markers, meaning that only resistance genes for which primers were designed can be identified while novel or mutated resistance genes would remain undetected which could potentially underestimate the resistance burden in the ecosystem as stated by van Hoek *et al.* (2011). Another important limitation is the fact that conventional PCR only detects the presence of a gene and does not indicate whether the gene is actively expressed. Thus, there may be discrepancies between genotypic detection and phenotypic resistance expression as seen in this study that it's not all the isolates that showed resistance to antibiotics phenotypically harboured its corresponding resistance determinants (Munita and Arias, 2016). This could be because environmental isolates' gene expression may be influenced by diverse environmental conditions or horizontal gene transfer.

This study's geographic limitation to a single river system—Ebute River—restricts the generalizability of the findings. Seasonal

fluctuations and localized pollution sources (e.g., sewage, industrial discharge) could significantly influence microbial and resistance gene diversity but may not be captured in a few-time sampling event. Therefore, future study should include other water bodies in the environment, seasonal study and quantifying gene copy abundance to help in understanding the ecological and epidemiological significance of the detected genes. This makes techniques such as qPCR or metagenomic sequencing very vital in providing more nuanced insights.

Conclusion

The incidence of antibiotic-resistant bacteria in ebute river is of major concern due to daily contact of people with the aquatic environment. Each isolates' genotypic expression did not match the phenotypic resistance expression found in the antibiotic susceptibility test. The corresponding genes were not detected by PCR in several of the isolates that showed phenotypic antibiotic resistance. The current study's frequency of resistance genes deviates from findings previously published from a different location in southwest Nigeria, suggesting that the distribution of resistance genes appears to be influenced by the population of bacteria, the geographic source, and human activity. This highlights the importance of incorporating antibiotic resistance gene surveillance into epidemiological studies. Unregulated use of antibiotics in industry, farms, and homes with subsequent discharge of industrial, agricultural, hospital and domestic waste into the aquatic environment will continue to produce a reservoir of resistance genes in the rivers. In order to lessen the likelihood that antibiotic-resistant bacterial strains and their genetic components would arise and proliferate, the results of this study point to the necessity of measures that will prevent the spread of antibiotic residues in the environment.

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