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Multiple Antibiotic Resistance Quotients (RQs) of *Plesiomonas shigelloides* Recovered from some Rivers in Southwest Nigeria

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ABSTRACT

The freshwater environment represents a habitat of diverse microorganisms and have also been identified as a contributor to the continuous spread of waterborne diseases in addition to antibiotic resistance determinants. This study, evaluated antibiotic resistance quotients of *Plesiomonas shigelloides* isolates recovered from selected freshwaters in Osun and Oyo states, Southwest Nigeria. Water samples, collected over 12 months, were analysed using standard procedures and subsequently confirmed by means of polymerase chain reaction (PCR) technique using PS gene. Confirmed *Plesiomonas shigelloides* (44%) were screened for *in-vitro* antibiotic resistance profile against a panel of 20 commonly used antibiotics. Resistance by all *P. shigelloides* from the selected sampling sites were frequently observed against sulphamethoxazole (100%), erythromycin (87-93%) ampicilin (79-90%), cephalotin (63-82%), streptomycin (55- 64%), and chloramphenicol (48 -58%) though susceptibility against netilmicin (100%), meropenem (91-94%), gentamicin (83-88%), imipenem (72-79%) amikacin (65-70%), ciprofloxacin (68-70%) norfloxacin (54-59%) were similarly observed across all sampled sites. Multiple antibiotic resistance indexes among *P. shigelloides* across the sampled rivers ranged between 0.14 – 0.62 with highest MAR index of 0.62 recorded in some isolates from SR2 (Dandaru river) and SR4 (Eleyele river). These findings indicated high prevalence of antibiotic resistance *P. shigelloides* in the freshwater, which may well be attributed to undue antimicrobial usage around the selected rivers, thereby necessitating recommendation of good hygiene and proper sanitation as well as enforcing legislation against indiscriminate use and disposal of antimicrobials in the study communities.

KEYWORDS: Antibiotics; Multidrug Resistance*; Plesiomonas shigelloides;* Public Health; Rivers.

1. Introduction

The freshwater environment represents habitat of diverse microorganisms together with *Plesiomonas shigelloides*, considered as etiologic agent of infectious diseases to animals and humans. *Plesiomonas shigelloides* (previously named *Aeromonas shigelloides*) is biochemically characterised as, oxidase positive, facultatively anaerobic Gram negative, polarly flagellated, rod-shaped bacteria. Molecular characterisation has revealed the relatedness of this organism to some members of the Enterobacteriaceae family and hence, its placement into the family where it stands as the only oxidase-positive member. The name *Plesiomonass higelloides* resulted because many strains cross react antigenically with shigella especially *Shigella sonnei* (Janda, 2005). This bacterium is principally an aquatic organism found in fresh as well as estuarine waters and ocean water particularly in humid and subtropical climates however its abundance in freshwater (rivers, streams, ponds, lakes) environmentsin temperate and cold climates has been documented (Krovacek *et al*., 2000; Pasquale and Krovacek, 2001). This organism has also been isolated in fish, shrimp, crabs, oysters and mussels(Oxley *et al*., 2002; Huber *et al*., 2004; Gu *et al*., 2006).In recent years *P. shigelloides* studies has gained cognizance as causative agent of gastrointestinal and colitis with mild cholera-like diarrhoea illness ranging between mild to severe illnesses, especially in the aged, children and immune-compromised individuals, likewise being associated with travel related diarrhoea. Most reported gastroenteritis cases and outbreaks are linked with ingestion of polluted water, raw fish, shellfish and crustaceans (CDC, 1999; Shigematsu *et al.*, 2000; Kirov, 2001) and mostly presenting as an epidemic.

Antibiotics development is no doubt one of the significant accomplishments of the 20th century with millions of lives saved through it. However, resistance to antibiotic by microorganisms has now become an issue of concern to global public health because infectious organisms are increasingly becoming resistant to all available antibiotics usually prescribed for treatments (Cosgrove, 2006). But of utmost concern is that rate of development of resistance keeps increasing, from common resistance to a single antibiotics class to resistance to multiple classes of antibiotics in addition to extreme drug resistance (Walsh, 2013). There is an alarming increase of antibiotics resistance in several bacteria that are spread in hospitals and communities causing various serious diseases (Bisi-Johnson *et al*., 2011). Hence, the environment has emerged as a reservoir of antibiotics resistant bacteria with their antibiotic resistant determinant genes after they are released from humans and animals and accumulates in diverse parts of the environment (Vaz-Moreira *et al*., 2014; Lin *et al*., 2015). A major force contributing to the spread antibiotics' resistance has been the use of antibiotics which has increased greatly in recent times with main contributions from developing countries (Van Boeckel *et al*., 2014; Berendonk*et al*., 2015). Larger portion of these antibiotics, as well as bacteria from animal and human origin from different sources contaminate fresh waters (Devarajan et al., 2016). Varying resistance to antibiotics has been reported in *P. shigelloides.* Most *P. shigelloides* strains, like other members of the family Enterobacteriaceae, are resistant to a wide range of penicillin (Stock and Wiedemann, 2001). Resistance to co-trimoxazole and chloramphenicol, aminoglycosides have also been documented (Ekundayo *et al*., 2020). However, sensitivity to second- and third- generation cephalosporins have also been recorded (Adesiyan et al., 2019). Therefore, we evaluate the Multiple Antibiotic resistance quotients (RQs) of *P. shigelloides* recovered from some Rivers in Southwest Nigeria in order to determine its fitness for human use.

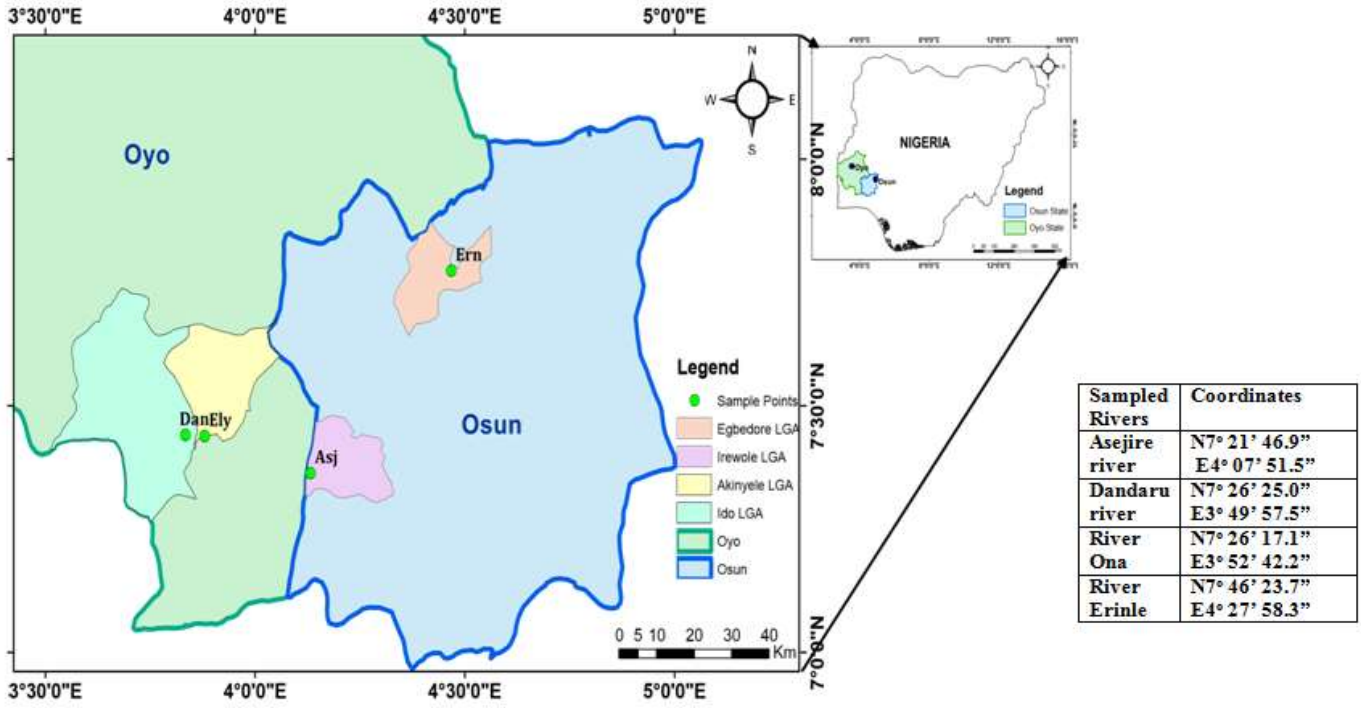
2. Materials and Methods

2.1 Study area Description

Water samples were sourced from selected rivers (Asejire, Erinle, Ona and Dandaru) across two state; Oyo and Osun in Southwest Nigeria. The rivers were selected based on noticeable anthropogenic activities around the river and its

usage which includes: agricultural irrigation, fishing, livestock drinking water, and domestic purposes in addition to waste sewer for industries for Asejire river; domestic purpose, washing, religious bathing, irrigation as well as waste drains for industries like hotels, hospitals and small-scale industries in Dandaru river. Likewise, notable usage of Ona river includes local transportation to adjoining communities, fishing, irrigation, livestock drinking water, spiritual bathing and domestic purposes while animal rearing, agricultural farming and domestic activities were observable usage of the river. Also, the river is abstracted for municipal water supply by government owned Water Corporation at Ede, Osun States.

The climate in Southwest Nigeria is tropical, vegetation typically semi-tropical having an average annual rainfall of ~ 1600mm as well as temperature of 32^{o}C straddling the two distinct seasons; the rainy and dry season. Sampled rivers labelled as follows SR1; Asejire: SR2; Dandaru: SR3; Erinle: SR4; Eleyele are illustrated on the map below (Figure. 1)



Sampled Rivers	Coordinates
Asejire river	N7° 21' 46.9" E4° 07' 51.5"
Dandaru river	N7° 26' 25.0" E3° 49' 57.5"
River Ona	N7° 26' 17.1" E3° 52' 42.2"
River Erinle	N7° 46' 23.7" E4° 27' 58.3"

Figure 1: Map of the Study Area

2.2 Presumptive *P. shigelloides* Sampling and Identification

Three different sampling points were selected for aseptic sampling of water across the four rivers monthly for a period of 12 months.Samples collected in 1L sterile bottles were processed within 6 hours according to the American Public Health Association recommended procedure (APHA, 2005). Water samples were enriched in sterile peptone water (pH 8.6), incubated for 18-24h at 37^{o}C and thereafter serially diluted. Then, 0.1 mL each of suitable dilution were pipetted onto a dried and well labelled Inositol Brilliant Green Bile agar plates (Conda Pronadisa, Spain) and spread evenly with a glass spreader. Plates were immediately position invertedly and incubated at 35°C for 24 h. Pinkish colonies were selected on each plate as presumptive *P.shigelloides*and sub-cultured on non-selective agar before storage on glycerol for subsequent analysis.

2.3. Extraction of DNA

The method for DNA extraction was as earlier described by Adesiyan *et al.*, (2019). Pure, distinct colonies of 18-24 h old *Plesiomonas*

shigelloides subculture on non-selective agar at 37°Cwere selected and placed in 200µl sterile distilled water. The mixture was vortexed, boiled at 100 °C for 15 min and centrifuged at 15,000 rpm for 10 min. The resulting supernatant was stored at −80 °C pending subsequent use.

2.4 Molecular Confirmation of *P. shigelloides*

Polymerase chain reaction technique was employed for confirmation of presumptive *P. shigelloides.*The 23SrRNA gene sequencing was used by employing the primer sets PS23FW3 / PS23RV3 established by González-Rey (2000) that amplifies at 284bp sequence of the 23SrRNA gene [(PS23FW3: 5´-CTCCGAATACCGTAGAGTGCTATCC-3 and (PS23RV3: 5´-CTCCCCTAGCCCAATAACACCTAAA-3)] of *P. shigelloides*. The PCR running conditions used in this study were as documented in our previous study (Adesiyan *et al.*, 2019) but with slight modifications. The reaction blend consists of PCR master mix (12.5 µl, Thermo Scientific, EU, Lithuania), oligonucleotide primer(1 µl each), DNA template (5 µl) and nuclease free water (5.5 µl) which made up a total of 25 µl reaction cocktail volume (González-Rey, 2000).The PCR reaction protocols used is; first 5 min denaturation at 95 °C, followed by 35 cycles steps of 1 min denaturation at 94°C, 1 min annealing at 68 °C and 2 min extension at 72 °C respectively and final extension for 10min at 72 °C after the completion of the 35 cycles. For the electrophoresis, 5 µl aliquot of the amplicons were loaded on 2 % agarose gel containing 5 µl ethidium bromide stain. Gel documentation equipment (Alliance 4.7, France) was used to visualize the PCR products. 100-bp DNA ladder was used for molecular size calibration on the gel and the running condition was set at 100v for 45 minutes.*P. shigelloides* DSMZ 8224 was used as positive

2.5 Antibiotic susceptibility assay

Disc diffusion assay was used for antibiotic susceptibility test on *P. shigelloides* (Kirby-Bauer *et al*. 1966). Distinct colonies from an 18 h culture of *P. shigelloides* were placed in tubes containing 3 ml of 0.85 % physiological saline. The Turbidity of mixture was compared to 0.5 McFarland standard solution (equivalent to 1.5×10^8 CFU) after which a sterile swab was used to spread 0.1 ml of the standardized suspension on Mueller Hinton agar plates. The surface of the seeded Mueller Hinton agar plates was allowed to dry and antibiotic discs were placed on them using disc dispenser. The plates were subsequently incubated at 37 °C at an inverted position for 24 h. In total, 20 antibiotics (Mast Diagnostics, UK) were selected for this test and these includes; streptomycin (300 µg), neomycin (10 µg),amikacin (30 µg),, gentamycin (10 µg), Trimethoprim (5 µg), cephalothin (30 µg), Netilmicin (30 µg), cefotaxine (30 µg), meropenem (10 µg), imipenem (10 µg), sulphamethoxazole (25 µg), ceftazidine (30 µg), neomycin (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), chloramphenicol (30 µg), Trimethoprim + Sulphamethoxazole (25 µg), tetracycline (30 µg), amoxycillin (25 µg) and ampicillin (10 µg). The width of the inhibition zone was calculated and recorded as either resistant (R), intermediate (I) or susceptible (S), according to the recommendation of the clinical and Laboratory Standards Institute (CLSI, 2018) for zone diameter interpretation.

2.6 *P. shigelloides* antibiotic resistance pattern abundance (ARPA) and resistance quotients (RQs) determination

Antibiotic resistance pattern abundance (ARPA) was calculated (Deng *et al.* 2020)

$$ARPA = {}^{RT}/_{TS} \qquad (1)$$

RT = the number of resistance type

TS = the total number of strains assay

Likewise, the resistance quotient (RQ) was also calculated. This is used to determine probable changes in antibiotics l resistant phenotypes of ***P. shigelloides*** species across the different antibiotics tested. This is to evaluate the possible ecological risk conferred by the antibiotics on ***P. shigelloides*** in the freshwater environment. The equation 2 below was used for RQs calculation (Amos *et al.* 2018).

$$Resistant\ quituent = \frac{Number\ of\ resistant\ bacteria}{Total\ number\ of\ bacteria\ tested} \times 100 \quad (2)$$

2.7 Statistical analysis

Data obtained were analysed with Statistical Package for Social Sciences [(SPSS) IBM version 22 software

3. Results

3.1 Molecular identification and prevalence of *P. shigelloides* isolates

Sixty-six (66: 44.6%) were eventually confirmed to be *P. shigelloides* isolates.The representative gel electrophoresis images of the amplified PS23 gene (284 bp) of some of the confirmed *P. shigelloides* are as shown in Figure 2.

3.2 Antibiotic resistance and susceptibility profile

The antibiotics susceptibility test (AST) profile of the *P. shigelloides* were evaluated for its phenotypic resistance against a board of 20 antibiotics spanning 9 different antimicrobial families. The AST result of the 66 PCR confirmed isolates shows that all the isolates were 100% sulphamethoxazole resistant. Likewise, varied degree of resistance was recorded against erythromycin (93%), ampicillin (90%) cephalothin (82%), streptomycin (64%) and chloramphenicol (58%) respectively. Resistance against other antibiotics was as follow: Trimethoprim + Sulphamethoxazole (36%), amoxicillin (53%), tetracycline (49%), neomycin (38%) and cefotaxime (50%)(Table 3). Also, all tested isolates were 100% netilmicin susceptible, while susceptibilities to other antibiotics was as follows: meropenem (94%), gentamicin (88%), imipenem (79%), amikacin (70%), ciprofloxacin (70%), Norfloxacin (59%), Trimethoprim (56%), and ceftazidime (56%). (Figure. 3). The Antibiogram profile of the *P. shigelloides* isolates are shown in Table 3.

3.3 Multiple Antibiotic Resistance Phenotypes(MARPs)

The patterns of multiple antibiotic resistance phenotypes (MARPs)of *P. shigelloides* isolates, the numbers of antibiotics, resistance patterns and frequencies obtained in all the sampling sites is as shown in Table 4. The MARPs demonstrated by all *P. shigelloides* isolates in this study ranges from 3-8 antibiotics with multidrug resistance recorded in all isolates against 3 or more tested antibiotics as follows: resistance to 3 - 8 classes of antibiotics were recorded at Asejire (SS1); 5 - 8 classes at Dandaru (SS2); 4 - 8 classes, Eleyele (SS3), and isolates from Erinle river (SS4) recorded resistance to 3 - 7 classes of antibiotics respectively.

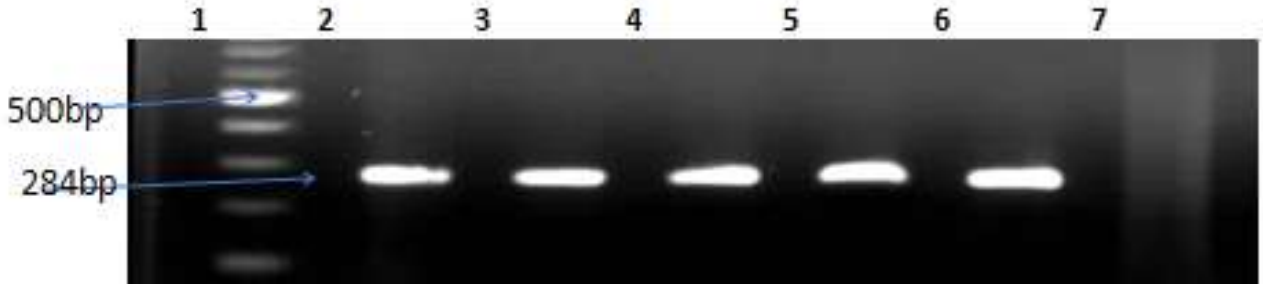


Figure 2: Lane 1*:* Molecular marker (100bp); lane 2-5: *Plesiomonas shigelloides* isolates; Lane 6: positive control; Lane 7*:* negative control.

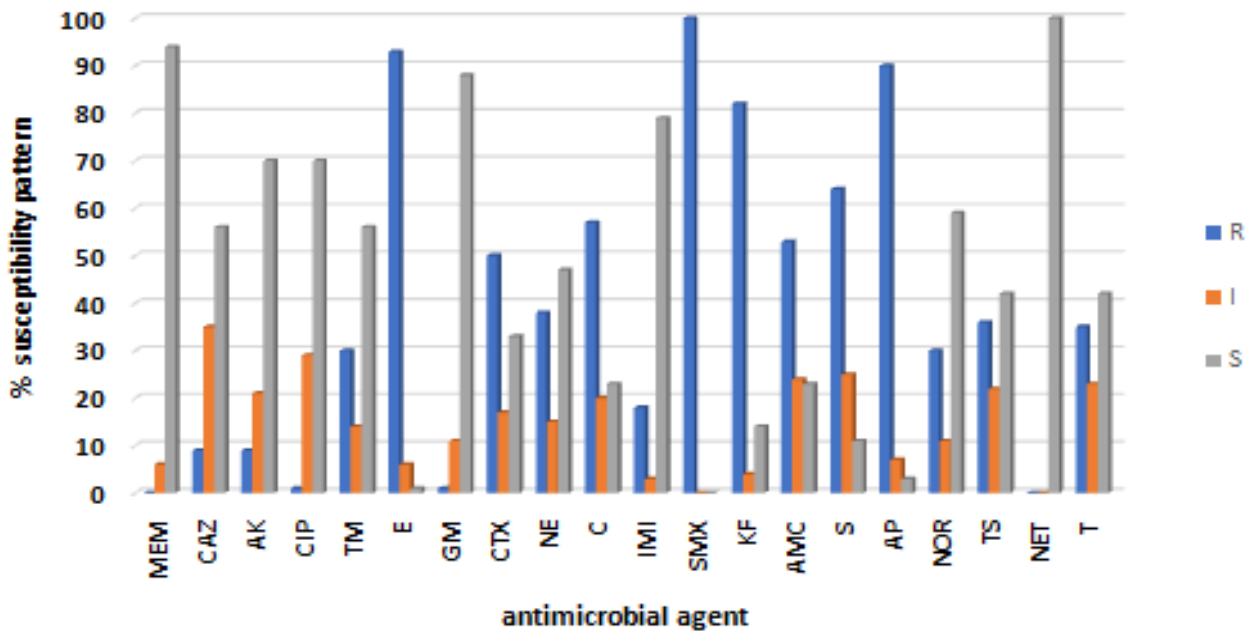


Fig. 3: Susceptibility profile of *P. shigelloides* isolates against selected antibiotics in different classes. *MEM* Meropenem, *CAZ* ceftazidine, *AK* Amikacin, *CIP* Ciprofloxacin, *TM* Trimetroprim, *E* erythromycin, GM Gentamycin, *CTX* Cefotaxime, *NE* Neomycin, *C* Chloramphenicol *IMI* Imipenem *SMX* Sulphamethoxazole, *KF* Cephalothin, *AMC* Amoxicillin, *S* Streptomycin, *AP* Ampicillin, *NOR* Norfloxacin, *TS* Trimetroprim- Sulphamethoxazole, *NET* Netilmicin, *T* Tetracycline

Table 2: Antibiogram profile of *Plesiomonas shigelloides* isolates from selected rivers

Antibiotic family	**Antibiotic agent**	**Disc code**	**Potency (µg)**	**Total Antibiotic Resistant Isolates SR1**	**SR2**	**SR3**	**SR4**	**Total (%)**
Sulfonamides	Trimethoprim	TM	5	4	7	3	6	20 (30)
	Sulphamethoxazole	SMX	25	18	13	22	13	66 (100)
	Trimethoprim + Sulphamethoxazole	TMP+SMX	25	5	8	4	7	24 (36)
Aminoglycosides	Amikacin	AK	30	0	2	1	2	5 (8)
	Gentamicin	G	10	0	1	0	0	1 (2)
	Netilmicin	NET	0	0	0	0	0	0 (0)
	Neomycin	NE	30	12	5	4	4	25 (38)
	Streptomycin	S	10	14	7	10	11	42 (64)
β-lactams	Ampicillin	AP	10	16	12	19	12	59 (89)
	Amoxycillin	AMC	25	7	10	13	5	35 (53)
Cephems	Cefotaxime	CTX	30	12	5	9	7	31 (47)
	Cephalotin	KF	30	17	11	15	11	54 (82)
	Ceftazidine	CAZ	30	0	0	4	2	6 (9)
Carbapenems	Meropenem	MEM	10	0	0	0	0	0 (0)
	Imipenem	IMI	10	4	1	1	6	12 (18)
Fluoroquinolones	Ciprofloxacin	CIP	5	0	1	0	0	1 (2)
	Norfloxacin	NOR	30	9	6	1	4	20 (30)
Tetracycline	Tetracycline	T	30	6	4	7	6	23 (35)
Phenicols	Chloramphenicol	C	30	10	8	11	9	38 (58)
Macrolides	Erythromycin	E	15	16	12	20	13	61 (92)

In general, the MAR indexof *P. shigelloides* ranges between 0.14 – 0.62 across all the river. Isolates with MARI below the 0.2 threshold was recorded in SR1 (n=1; 0.16) and SR3 (n=5; 0.14, 0.14, 0.14, 0.18, 0.18) while the highest MARI of 0.62 were recorded in SR2 (n=1), and SR4 (n=2) having displayed resistance to 8 out of 13 antibiotics tested. Range of MARI across the selected rivers were 0.16 - 0.44 (SR1), 0.38-0.62 (SR2), 0.14 – 0.32 (SR3), and 0.31 – 0.62 (SR4) respectively. Nevertheless, resistance against 5-7 classes of antibiotics were observed to be common to all *P.shigelloides* isolates. Antibiotic Resistance Pattern Abundance (ARPA) was highest at SR4 (0.38). ARPA in other rivers were 0.33 at SR1, 0.31 SR2, and the lowest, 0.23 recorded for SR3 respectively (Table 2).

Table 3: Patterns of multiple antibiotic resistance phenotypes (MARPs) of *Plesiomonas shigelloides*

No of antimicrobials	Resistance pattern	Frequency of pattern	MARI	ARPA
	Sampling site=SR1(N=18)			0.33
3	SUL-BLA-CTX	1	0.16	
4	SUL-QUI-C-TET	1	0.22	
5	MAC-SUL-BLA-CTX-TET	2	0.27	
	MAC-SUL-BLA-AMG-CTX	1	0.27	
	MAC-SUL-BLA-AMG-CTX	1	0.27	
6	MAC-SUL-AMG-CTX-CAB-QUI	2	0.33	
	MAC-SUL-BLA-AMG-CTX-QUI	1	0.33	
	MAC-SUL-BLA-AMG-CTX-C	1	0.33	
	MAC-SUL-BLA-AMG-CTX-QUI	1	0.33	
7	MAC-SUL-BLA-AMG-CTX-C-TET	1	0.38	
	MAC-BLA-AMG-CTX-C-QUI-TET	1	0.38	
	MAC-SUL-BLA-AMG-CTX-QUI-TET	1	0.38	
	MAC-SUL-BLA-AMG-CAB-CTX-C	1	0.38	
	MAC-SUL-AP-AMG-CTX-QUI-TET	1	0.38	
	MAC-SUL-BLA-AMG-CTX-C-QUI	1	0.38	
8	MAC-SUL-BLA-AMG-CTX-C-QUI-TET	1	0.44	
	Sampling site=SR2 (N=13)			0.31
5	MAC-SUL-BLA-CTX-TET	2	0.38	
	MAC-SUL-BLA-AMG-QUI	1	0.38	
6	MAC-SUL-BLA-AMG-CTX-C	3	0.46	
	MAC-SUL-BLA-AMG-CTX-TET	1	0.46	
	MAC-SUL-BLA-CTX-C-TET	1	0.46	
	MAC-SUL-BLA-AMG-CTX-QUI	1	0.46	
7	MAC-SUL-BLA-CTX-C-QUI-TET	1	0.54	
	MAC-SUL-BLA-AMG-CTX-C-QUI	2	0.54	
8	MAC-SUL-BLA-AMG-CAB-CTX-C-QUI	1	0.62	
	Sampling site=SR3 (N=22)			0.23
3	SUL-BLA-AMG	1	0.14	
	MAC-SUL-BLA	1	0.14	
	SUL-BLA-CTX	1	0.14	
4	MAC-SUL-BLA-TET	1	0.18	
	MAC-SUL-BLA-CTX	3	0.18	
5	MAC-SUL-BLA-CTX-C	3	0.23	
	MAC-SUL-CTX-C-TET	1	0.23	
	MAC-SUL-BLA-CTX-TET	2	0.23	
	MAC-SUL-BLA-AMG-CTX	2	0.23	
6	MAC-SUL-BLA-CTX-C-TET	1	0.27	
	MAC-SUL-BLA-AMG-CTX-C	3	0.27	
7	MAC-SUL-BLA-CTX-AMG-C-TET	1	0.32	
	MAC-SUL-BLA-AMG-CTX-QUI-TET	1	0.32	
	MAC-SUL-BLA-AMG-CAB-CTX-C	1	0.32	

	Sampling site=SR4 (N=13)		0.38
4	MAC-SUL-AMG-CTX	1	0.31
5	MAC-SUL-BLA-CTX-TET	1	0.38
	MAC-SUL-BLA-CAB-CTX	1	0.38
	MAC-SUL-AMG-QUI-TET	1	0.38
6	MAC-SUL-BLA-AMG-CTX-TET	1	0.46
	MAC-SUL-BLA-AMG-CAB-CTX	1	0.46
	MAC-SUL-BLA-AMG-CTX-C	1	0.46
7	MAC-SUL-BLA-CAB-CTX-C-TET	1	0.54
	MAC-SUL-BLA-AMG-CTX-C-TET	1	0.54
	MAC-SUL-BLA-AMG-CAB-CTX-C	2	0.54
8	MAC-SUL-BLA-AMG-CTX-C-QUI-TET	1	0.62
	MAC-SUL-BLA-AMG-CAB-CTX-C-QUI	1	0.62

Keys:MAC-Macrolides (Erythromycin), SUL-Sulfonamide (Trimethoprim, Sulphamethoxazole, Trimethoprim-Sulphamethoxazole), BLA-Beta-Lactam (Ampicillin, Amoxicillin), AMG- Aminoglycosides (Amikacin, Netilmicin, Neomycin, Gentamicin, Streptomycin), CTX-Cephems (Cefotaxime, ceftazidine, Cephalothin), CAB-Carbapenems (Meropenem, Imipenem), QUI-Quinolones (Norfloxacin, Ciprofloxacin), TET-Tetracycline (Tetracycline), C-Phenicol (Chloramphenicol).

Ecological risk of *P. shigelloides* as determined by resistance quotient (RQ)

The calculated resistance quotients of *P.shigelloides* isolates from selected rivers are as shown in Table 5. All the *P. shigelloides* isolates had 0% RQs against Meropenem and Netilmicin. Likewise, all isolates of *P. shigelloides* also had low RQs against ceftazidime, Amikacin, Ciprofloxacin, Gentamycin andImipenem. On the other hand, significantly high RQ were recorded for Erythromycin, Sulphamethoxazole, Cephalothin and Ampicillin (Table 5).

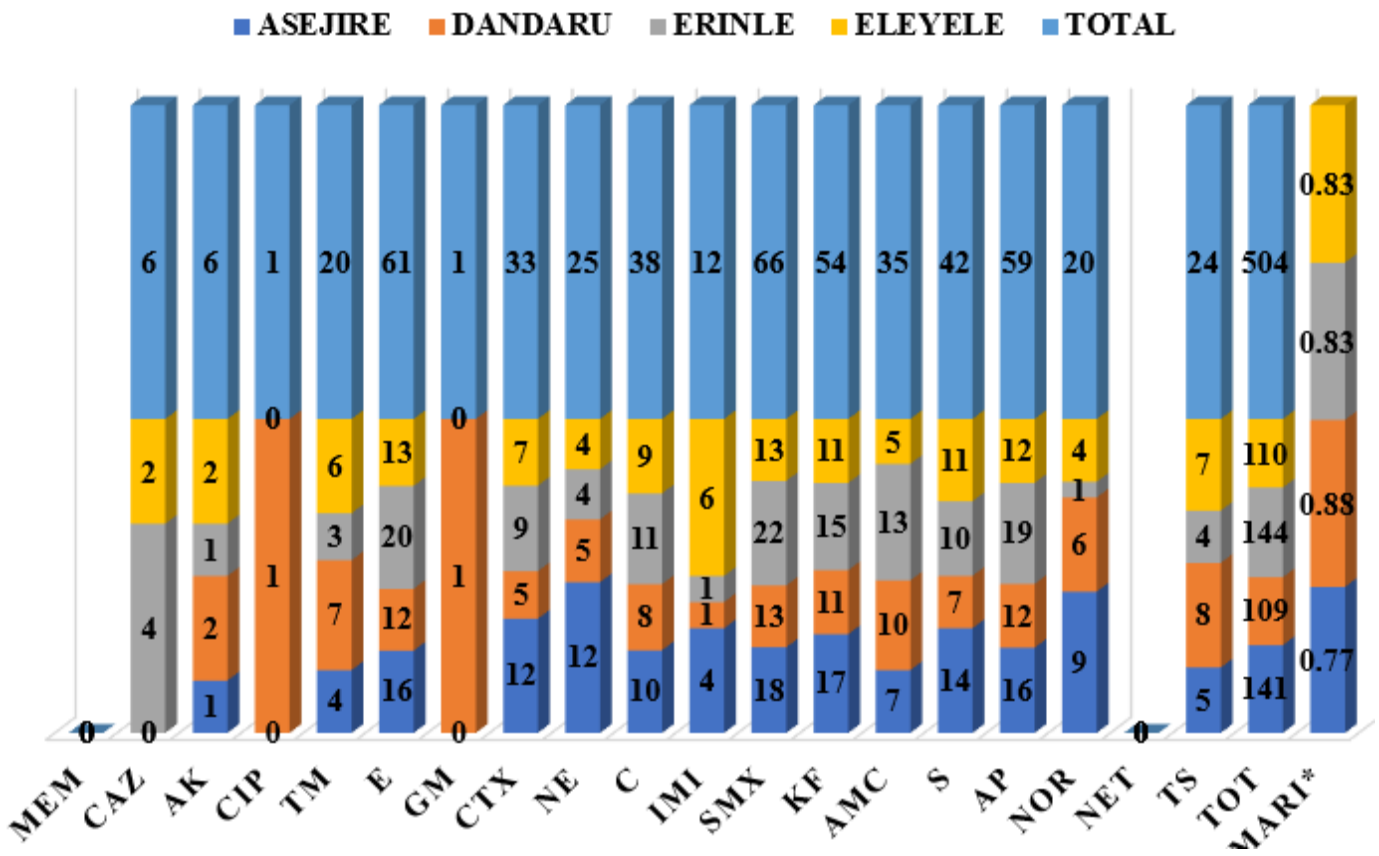


Figure 4: **Keys:** MARI* multiple antibiotic resistance across selected rivers. *MEM* Meropenem, *CAZ* ceftazidine, *AK* Amikacin, *CIP* Ciprofloxacin, *TM* Trimetroprim, *E* erythromycin, *GM* Gentamycin, *CTX* Cefotaxime, *NE* Neomycin, *C* Chloramphenicol

IMI Imipenem *SMX* Sulphamethoxazole, *KF* Cephalothin, *AMC* Amoxicillin, *S* Streptomycin, *AP* Ampicillin, *NOR* Norfloxacin, *TS* Trimetroprim- Sulphamethoxazole, *NET* Netilmicin, *T* Tetracycline.

Table 5: Antibiotic resistance quotients (RQs) of *P. shigelloides* isolates across selected rivers

LOCATION	MEM	CAZ	AK	CIP	TM	E	GM	CTX	NE	C	IMI	SMX	KF	AMC	S	AP	NOR	NET	TS	MARI*
ASEJIRE	0	0	1	0	4	16	0	12	12	10	4	18	17	7	14	16	9	0	5	0.77
DANDARU	0	0	2	1	7	12	1	5	5	8	1	13	11	10	7	12	6	0	8	0.88
ERINLE	0	4	1	0	3	20	0	9	4	11	1	22	15	13	10	19	1	0	4	0.83
ELEYELE	0	2	2	0	6	13	0	7	4	9	6	13	11	5	11	12	4	0	7	0.83
TOTAL	0	6	6	1	20	61	1	33	25	38	12	66	54	35	42	59	20	0	24	

Keys: MARI* multiple antibiotic resistance across selected rivers; *MEM* Meropenem, *CAZ* ceftazidine, *AK* Amikacin, *CIP* Ciprofloxacin, *TM* Trimetroprim, *E* erythromycin, *GM*Gentamycin, *CTX* Cefotaxime, *NE* Neomycin, *C* Chloramphenicol *IMI* Imipenem*SMX* Sulphamethoxazole, *KF*Cephalothin, *AMC* Amoxicillin, *S* Streptomycin, *AP* Ampicillin, *NOR* Norfloxacin, *TS* Trimetroprim- Sulphamethoxazole, *NET* Netilmicin, *T* Tetracycline.

4. Discussion and Conclusion

P. shigelloides, frequently linked to freshwater milieu is known as human pathogen and causative agent of many opportunistic infections (Adesiyan et al., 2019). This study examined the Antibiotic resistance quotients (RQs) of *P. shigelloides* recovered from some Rivers in Southwest Nigeria. Species-specific simplex PCR assay was used to confirm presumptive *P. shigelloides* isolates through the amplification of nucleotides C-906 and G-1189 within the 23SrRNA gene region (Gonzalez-Rey *et al*., 2000). Detection of *P. shigelloides* from the selected rivers points to the impact of anthropogenic activities around these waters and discharge of effluent into rivers as well. The results of this study support previous studies (Kim *et al.,* 2015; Adesiyan *et al.,* 2019) that this organism is well associated with freshwater environment. Isolation from rivers in middle and northern Europe have also been described (Krovacek *et al.,* 2000). The report of the isolation of this organism in stool samples of patients diagnosed with diarrhoea and acute gastroenteritis further indicates its easy transmission through water (Chen *et al.*, 2013; Novoa-Farías *et al*., 2015).

The dissemination of antimicrobial drugs in the environment has been intensified by agricultural practices that relies on water sources and aquatic ecosystem such as aquaculture thus contributing to spread and development of antibiotics resistant bacteria strains; a global public health concern. (Wellington *et al*., 2013). The antibiotics susceptibility profile of the *P. shigelloides* isolate suggested high resistance against multiple, commonly prescribed antibiotics. The result of multiple antibiotic resistance observed in this isolate is consistent with that of other studies which reported resistance against all antibiotics tested (Stock and Wiedemann 2001; Hacioglu and Tosunoglu, 2014; Nwokocha and Onyemelukwe, 2014). The high resistance rate of the bacterial isolates against erythromycin, ampicillin, Sulphamethoxazole, tetracycline streptomycin chloramphenicol, Neomycin and amoxicillin, agrees with other findings where resistance against these antibiotics were reported in varying degrees (González-Rey *et al*., 2004; Chen *et al*., 2013, Adesiyan *et al*., 2019). However, low susceptibility to gentamicin is well reported in clinical isolates compared to environmental isolates in many studies with the submission that sources of isolates may contributes to the varied susceptibility to aminoglycosides as observed in this organism (Chen *et al*., 2013; Lee *et al*., 2016; Pence, 2016) Nevertheless, Maia *et al*. (2016) also reported resistance to gentamicin in four strains of *P. shigelloides* isolated from a zoo environment. The high resistance to commonly used antibiotics in all sampled locations maybe attributed to the various antibiotics associated anthropogenic activities being carried out around the rivers thereby leading

to the spread and dissemination of the residues in the waters.

The development of resistance against antibiotics that are used as first-line oral agents for gastrointestinal and diarrhoea illnesses when antimicrobial therapy is indicated is of serious concerns to public health as this can result to treatment failures in clinical settings.

To assess the degree of exposure of the freshwater resources to contamination by antibiotics and evaluate the risk of multiple antibiotic resistance, MAR index was calculated. According to Krumperman (1983), MAR > 0.2 signifies that the water resources constitute high risk of antimicrobial contamination. The highest MAR index of 0.62 recorded at SR2 and SR4, was more than the 0.2 threshold value which is the set value for distinguishing low- and high-risk contamination (Krumperman, 1983). This is not unexpected as the two sites were observed to be highly impacted by human activities which includes farming, bathing, fishing, animal rearing, washing etc. Moreover, antibiotics selective pressure has been reported to differ according to the nature of the environment. Low MARindex of 0.14/SR3 and 0.16/SR1 recorded is similar to MAR index 0.106 obtained from *P. shigelloides* isolates from Tilapia fish in Indian.

Wastewater discharge into freshwater has been implicated in the environmental dissemination of enteric bacteria resistance to multiple antibiotics and containing resistant plasmid (Akhter *et al*., 2014).

The 0% RQs of *P. shigelloides* against Meropenem and Netilmicin may indicate that there is currently low or none selection pressure for resistance to these two antibiotics in the selected freshwater sources. Nevertheless, significantly high RQ recorded for erythromycin, sulphamethoxazole, cephalothin and ampicillin shows that *P. shigelloides* isolates are more reactive to the presence of selection pressure for the antibiotics in the freshwater environment. According to Amos *et al.* (2018) high RQs implies that sampled sources are greatly impacted by antimicrobial pollution and wastewater effluent. The observed resistance of *P. shigelloides* to multiple antibiotics have serious consequences on clinical outcomes. The recorded variation in resistance or sensitivity profile of the isolates to panel of antibiotics belonging to the same class could be associated with point mutation accumulation or acquisition of diverse copies of resistance genes. Hence, a single isolate might therefore acquire more than one resistance mechanisms thereby accounting for its wide-ranging resistance to more than one antibiotic belonging to the same antimicrobial class.

5. Conclusion

The study revealed the high prevalence of *P.shigelloides* isolates with resistance to multiple antibiotics highest among isolates recovered from SR2 and SR4 (Dandaru and Eleyele) respectively. This observation portends possible abuse of antimicrobial usage around or close to the study environment and release of waste products containing antimicrobial residue with resistance conferring properties into the water bodies. Resistance of *P. shigelloides* to some antibiotics that are commonly prescribe in clinical settings may threatens the effectiveness of these antibiotics in the treatment of *P. shigelloides* associated infections. Accordingly, monitoring, surveillance, and evaluation of the prevalence of antimicrobial resistance pathogens in this studied area is very critical in order to determine region specific mitigation strategies that will assist in mitigating the impact of antibiotic resistance dissemination in this environment. Likewise, there is need for proper monitoring and regulation of antibiotic usage in human, agricultural and aquaculture settings in order to stem it's spread and dissemination in the environment. This study therefore concludes that the rivers examined in this present study are unfit for human and animal usage without proper monitoring.

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