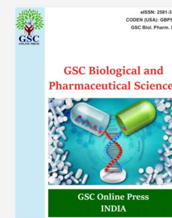
GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/



(Research Article)

Resistance genes and plasmid profile of bacteria Isolated from selected water bodies in Owo Metropolis

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GSC Biological and Pharmaceutical Sciences, 2023, 23(01), 033–045

Publication history: Received on 21 February 2023; revised on 01 April 2023; accepted on 04 April 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.1.0132

Abstract

Aquatic samples from three water bodies were collected following laid down procedures. The bacteria were isolated using, Eosin Methylene Blue agar (EMB), Mannitol salt agar (MSA), Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar, Muller Hinton (MH). The isolates were subjected to colonial, morphological and biochemical characterization. Pathogenicity test was carried out on the isolates using blood agar to determine their hemolytic activities. Antibiotic resistance status was also investigated against standard antibiotics using the Kirby Bauer disk diffusion method and the multiple antibiotic resistance indices of the isolates were also determined. The plasmid profiles were determined and the resistance genes were identified using molecular means. The bacteria species isolated were *Veillonella* Spp, *Vibrio orientalis* and *Micrococcus luteus*. *Veillonella* Spp showed Beta hemolysis while *Micrococcus luteus* showed alpha hemolysis and *Vibrio orientalis* showed gamma hemolysis. Antibiotic susceptibility test showed all isolates to be antibiotic resistant to varying degrees and multiple antibiotic resistance indices (MARi) depending on their level of resistance. *Veillonella*. spp. had MARi of 0.3 while *M. luteus* and *V. orietalis* had MARi of 0.5. Plasmid profiling showed all isolates to have plasmids with bandwidth of 10,000 bp. *Vibrio* spp and *Veillonella* Spp were positive for beta lactamase genes (*bla SHV*). Only *Vibrio* was positive for resistance to aminoglycosides (*aac(3)-IV*). *Vibrio orientalis* and *Micrococcus luteus* were found to have the resistance genes for quinolones (*qnrA gene*) while all the isolates possess genes against the sulphonamides (*sul1* gene) used. The result of this work establishes the antibiotic resistance status of bacteria isolated from aquatic habitat and suggests their important role in the spread of antimicrobial resistance AMR, and antibiotic resistance genes (ARGs)

Keywords: Antibiotic resistance genes; Water bodies, Hemolysis; Plasmids profiles; Aminoglycosides (*aac(3*); Quinolones (*qnrA* gene); Sulphonamides (*sul1* gene); Integrons; Primers

1. Introduction

The presence of antimicrobials, antimicrobial resistant bacteria (ARB), and antimicrobial resistance genes (ARGs) in the aquatic environment is becoming a cause of great concern as the possibility of development of antibiotic-resistant pathogens, even superbugs, is increasingly posing problems to the environment and human health (Singh *et al.*, 2022). The widespread use and abuse of antibiotic therapy has evolutionary and ecological consequences. One well known consequence is the fixation of mutations and lateral gene transfer (LGT) events that confer antibiotic resistance (Gillings, 2013).

The presence of antibiotic resistant bacteria in water bodies can be linked to the practices of integrated agriculture–aquaculture farming system, where the aquaculture is being sustained by livestock and human wastes. This inadvertently increases the risk of exposure of humans, animals, and environment to antibiotics used in agricultural practices (Kim *et al.*, 2013). Gillings (2013) reported that environmental contamination by antibiotics can be through human waste, streams, and wastes from veterinary use and livestock farming. Most of the administered antibiotics are

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not fully metabolized, and are released unchanged into the environment, i.e., water, manure or soils. As a result, water bodies and waste streams will contain both the antibiotics and resistance genes which are both considered as pollutants (Martinez, 2009; Zhao *et al*., 2010).

Antibiotics and their metabolites stockpiled in animal manure may seep through to surface and groundwater, and also into the soil. This is especially so for antibiotics with high water affinity or which are water soluble, thus making their spread and ecotoxicity in the environment faster, and widely with the aid of water fluidity (Du and Liu, 2002).

Aquatic environments are ideal setting for acquisition and dissemination of antibiotic resistance (Amarasiri *et al*., 2019) and human exposure to this environment may pose an additional health risk. Apart from through drinking, humans can be exposed to ARB and ARGs through activities like bathing, aquatic sports, occupational exposure during agricultural irrigation, and consumption of food produce irrigated with reclaimed water (Leonard *et al*., 2018; Leonard *et al*., 2015).

Kohanski, *et al.,* (2010) observed that bacteria can become antibiotic resistant through mutations at very low antibiotics concentrations in natural aquatic habitat. Several authors reported also that antibiotic resistance genes (ARGs) in aquatic environments can be transferred through mobile genetic elements (MGEs) and can later be assimilated by the environmental bacteria to become antibiotic resistant (Pruden *et al*., 2006; Tao *et al*., 2016; Park *et al*.,2018). Davies and Davies, (2010) and Sharma *et al.,* (2016) have also documented that antibiotic resistance of environmental bacteria can be intrinsic, acquired via spontaneous mutations (de novo) or horizontal gene transfer (HGT).

The aquatic environment can serve both as a natural reservoir of antibiotic resistance and a conduit for the spread of clinical resistance (Michael *et al*., 2013). Aquatic environments are critical for studying the development and spread of antibiotic resistance worldwide because of their role as endpoint of wastewater effluent from treatment plants or direct contamination by sewage from human or animal origin (Zheng *et al*., 2021; Miłobedzka *et al.,* 2022), and other anthropogenic factors that contribute to the propagation of antimicrobial resistance determinants. The aim and objective of this work is to determine the antibiotic resistance profile of bacteria isolated from water bodies and to investigate their plasmid profile.

2. Material and methods

2.1. Study Area

Owo is a Local Government Area in Ondo State, Nigeria. The local government has a population of 222,262, based on 2006 population census. Owo is situated in South-Western Nigeria, at the southern edge of the Yoruba Hills, and at the intersection of roads from Akure, Kabba, Benin City and Siluko. Owo is situated halfway between the towns of Ile Ife and Benin City.

2.2. Preparation of Samples

Water samples were collected from three locations known as Obukele, Ijasan and Ogbara streams flowing around the Owo metropolis. These water bodies were chosen because of their close proximity to human habitation and are constantly visited by humans and foraging animals. The water samples were collected following standard procedure. Briefly, the three streams water (Obukele, Ijasan and Ogbara-) were collected into different sterile conical flask covered with aluminum foil. Aseptically, the cap cover of each flask was removed with the mouth facing upstream (i.e., towards the flow of the water). The neck was immersed downwards about 30 cm below the water surface and filled completely before carefully replacing the cap cover. Each bottle was labeled with the sample code number. The samples were then quickly taken to the laboratory for bacteriological examination.

2.3. Culture media

The culture media were prepared following the manufacturer's direction. The culture media used for isolation, stocking and for biochemical characterization of the bacteria isolates were eosin methylene blue agar (EMB), Nutrient agar, MacConkey agar, Blood, agar, Mannitol salt agar (MSA) Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar, Muller Hinton (MH) and Nutrient broth.

2.4. Isolation Procedure (Preparation of Stock Culture) from water samples

The water samples collected from the three rivers were filtered using Whatmann no 1 filter paper. The filter paper was allowed to dry and the particles collected from the filter paper were used to inoculate peptone water as a stock culture.

The stock solution was prepared by dissolving 1gm of the particles into 9 ml of sterile water and shaken gently to disperse the particles. Serial dilution was carried out as in 2.3 above using the stock solution prepared.

2.4.1. Standardization of Inocula (Preparation of $BaSO_4$ (McFarland's constant).

A.0.5 McFarland's constant was prepared by mixing 0.5 ml of 1.1175% of Barium chloride ($BaCl_2.2H_2O$) with 9.95ml of 1% sulphuric acid (H_2SO_4). This stock as prepared in 2.4. Above was placed in the spectrophotometer and was compared with the McFarland's constant at 500nm. Peptone water was gradually added to the stock culture until a constant was attained that tallied with the $BaSO_4$ solution.

2.5. Isolation of Organisms

The stock standardized culture was used to carry out serial dilution and the inoculum was taken from the test tube containing 10-6 dilution. The inoculation was done by the pour plate method. The plates were then incubated at 37ºC for 24 hours after which the plates were observed for growth. Plate count was carried out using the J-3colony counter, China. Isolates from water bodies were cultured on EMB (Eosine- methylene blue) agar, TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) agar and MHA (Mueller Hinton) agar.

2.5.1. Preparation of Pure Culture

After observing the plates distinct colonies from the previously incubated plates were lifted using a sterile wire loop and used to streak a freshly prepared but gelled agar plate. The freshly streaked plates were allowed to incubate at 37ºC for 24 hours after which plates were observed for pure culture. Subsequently, pure culture of each isolates was subjected to colonial, morphological and biochemical analysis for characterization.

2.6. Gram Staining

Pure isolates were subjected to Gram staining to determine their Gram status following the laid down procedure.

2.7. Colonial and Morphological Characterization of Isolates

Colonial identification of isolates was based on criteria like morphology, growth parameter such as size, color, texture, elevation, appearance and shape.

2.8. Biochemical characterization

The following biochemical tests were carried out to identify isolates following Standard methods. Sugar fermentation, Citrate utilization Test, Oxidase Test, Indole Test, Urease Test, Methyl red Test & Voges Proskauer Test as described by Fawole and Oso (1988).

2.9. Pathogenicity Test

In-vitro pathogenicity test of isolates was done by streaking pure culture of the isolated bacteria on blood agar plates using the modified method described by Buxton, (2005). The plates were incubated at 30±2ºC for 7days. The pathogenicity test was confirmed by observing α, β and ɣ heamolytic zones of the growing bacteria on the plate.

2.10. Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of the isolates were determined using Kirby-Bauer (1966) method on Mueller-Hilton agar according to CLSI guidelines for antimicrobial susceptibility testing (CLSI, 2017). The following standard antibiotics (Celtech diagnostics, Belgium Inc.) were used are, Chloramphenicol (C) 30µg, Nalixidic acid (NAL) 25µg, Ciprofloxacin (CIP) 5µg, Ampicillin (AMP) 30µg, Gentamycin (CN) 10µg, Vancomycin (VAN) 10µg Penicillin (PEN) 25µg.

2.11. Multiple Antibiotic Resistance Index

The multiple antibiotic resistance index for the resistant bacteria isolates, was determined according to the procedure described by Krumperman (1983). This is essentially to determine the degree of bacterial resistance to antibiotics. These indices were determined by dividing the numbers of antibiotics to which the organisms were resistant to (a) by the numbers of antibiotics tested. (b) Resistance to three or more antibiotics is taken as multiple antibiotics resistance and MAR greater than 0.2 is considered a health risk.

2.12. Plasmid profiling of isolates

Plasmids were isolated using the QIAGEN Plasmid Purification mini kit.

2.12.1. Gel Integrity

The integrity of the extracted plasmid was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60 °C and stained with 3µl of 5 mg/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 µl) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 10µl of each PCR product and loaded into the wells after the 100bp-10kbp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the plasmid was estimated by comparison with the mobility of the molecular weight ladder that was ran alongside experimental samples in the gel. Extracted plasmid was then used as the template for PCR amplification.

2.13. Molecular identification

Primer sequences were as earlier documented by Barghouthi (2011). 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 Gene Amp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

3. Results and discussion

3.1. Total microbial count of samples

The total count of the samples are as shown in table 1. The total plate count for each sample is indicated. Water samples were cultured on EMB, MHA and TCBS agar. The count from Ijasan river cultured on EMB was highest with value of 2.5 $x10^{-6}$ Cfu/ml, followed by Obukele with a count of 1.9 x $10.^{-6}$ cfu/ml and Ogbara river had a count of 1.2 x 10^{-6} cfu/ml. Cultures on MHA showed the highest count of 5.0 $x10^{-6}$ cfu/ml. Obukele river produced 3.0. x 10^{-6} cfu/ml on TCB agar while Ogbara produced 1.2 x 10^{-6} cfu/ml and Ijasan produced 2.1 x 10^{-6} cfu/ml. The result obtained from this study is in agreement with the work of Umeoduagu *et al*., (2016) who recorded the same range of count for water samples from Ukpo Town. Similarly Adesakin, *et al* (2020) recorded a variation of microbial count but with count within the same numerical values. The variation in count is expected because the water bodies are not located within the same location. Further, the high bacterial count in virtually all the samples is an indication that water bodies are rich in aquatic microbes. Orazurike *et al*. (2008) opined that such water bodies would be dangerous if they enter into human body through any means such as bathing, domestic activities or even drinking, although, it is not expected that such water should be consumed without proper treatment. Banwo (2006) also reported that the presence of bushes and shrubs attracts smaller mammals that go to the water bodies to drink water and possibly pass out faeces into the water which inadvertently contaminates the water.

Table 1 The bacterial count of different samples from Obukele, Ijasan and Ogbara rivers

	Water samples		
Media/ samples (Cfu/ml)($x10^{-6}$)	**Obukele**	**Ijasan**	**Ogbara**
EMB	1.9 x 10^{-6}	2.5 $x10^{-6}$	1.2 x 10^{-6}
MHA	5.0 $x10^{-6}$	2.3 x 10^{-6}	1.6 $x10^{-6}$
TCBS	3.0. x 10^{-6}	1.2 x 10^{-6}	2.1 x 10^{-6}

Table 2 shows the biochemical characterization and the organisms identified based on the characterization aided by the Bergey's manual of determinative bacteriology. Organisms identified from water bodies were *Veillonella* spp, *Micrococcus luteus*, and *Vibrio orientalis*. The isolation of these organisms does not delimit the presence of other microorganisms in the water bodies as reported by Umeoduagu *et al.,* (2016). Egberongbe *et al*., (2012) isolated *Escherichia coli, Campylobacter* species, *Salmonella* species*, Klebsiella* species, and *Proteus* species amongst other

bacteria strains. Several authors also reported the presence of diverse bacteria species in water bodies including coliforms and enterobacteriaceae (Adesakin, *et al*., 2020; Al-Bayatti *et al*., 2010).

3.2. Pathogenicity properties of isolates

The pathogenicity test carried out on the isolates showed that they were all pathogenic with different degrees of heamolysis. *Vibrio orientalis* had Beta (complete) hemolysis by completely lysing the blood cells presenting clear zones around the colonies after incubation *Micrococcus luteus* showed alpha heamolysis with evidence of green patches around the colonies signifying partial heamolysis while *Veillonella* presented gamma heamolysis by not showing lysing of blood (Fig 1-3 while fig 4 shows the control). Hemolysins, considered an important virulence factor, are compounds produced by a variety of bacterial species. These compounds are responsible for membrane damage, cell lysis and destruction of neighboring cells and tissues in order to provide nutrients, mainly iron, for the toxin-producing bacteria (Bullen *et al.,* 2005).

Mogrovejo-Arias *et al*., (2020) pointed out that bacteria can exhibit three different types of hemolytic activity (Buxton 2005): Beta (β) hemolysis, when the toxin causes the complete lysis of the red blood cells. Often referred to as true lysis, it manifests as a clear, transparent area in the blood agar cultures; alpha (α) hemolysis, when lysis does not occur but the hemoglobin of the red blood cells is reduced to methemoglobin and a brown/green colored area can be observed in blood agar cultures; gamma (γ) hemolysis, or non-hemolysis, when no damage to the cells is caused and no change in the agar plate is observed. Indeed many of the coliforms comprise entero-pathogenic serogroups and have been found to be hemolytic and verocytotoxigenic (Pelczar *et al*.,1986; Welch *et al*., 2000). All isolates are pathogens of human, animals and aquatic organisms.

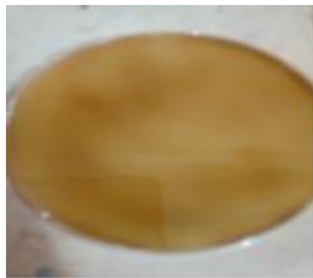

Figure 1 Beta heamolysis of *Vibrio* spp

Figure 2 Alpha heamolysis of *M. luteus*

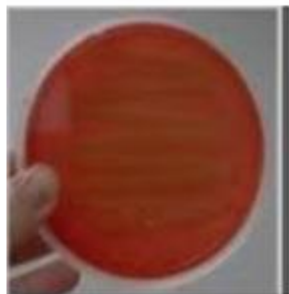

Figure 3 Gamma heamolysis of *V. spp*

Figure 4 Control (Blood agar)

Veillonella spp are non-motile Gram negative cocci, usually a normal flora of the intestinal tract is implicated in oral infections, head, neck and various soft tissue infections. They can also be implied in sinuses, lung and heart infections (Jeffery, 2012), *Micrococcus luteus* are also normal inhabitants of the human body but occurs in wide range of environments including water and soil. *M. luteus* are opportunistic pathogens often causing infections in immunocompromised individuals, they may be involved in diseases such as bacteremia, septic shock, meningitis and cavitating pneumonia (Greenblat *et al*., 2004). *Vibrio orientalis* is a commensal of shrimps and can cause food borne infections usually associated with eating undercooked seafood leading to a variety of vibriosis diseases. Vibriosis can pose threat to growing food demands and global food security (Valente and Wan, 2021).

3.3. Antibiotic sensitivity of isolates

The antibiotic resistance profile of the isolates showed that the organisms were resistant to some of the antibiotics used in different degrees (Table 3). 10 antibiotics belonging to five classes of antibiotics were used in this study. *Veillinella* Spp was susceptible to 70% of the antibiotics used in this work. Some *in -vitro* studies suggest that *Veillonella* Spp. remain susceptible to a wide range of commonly used antibiotics (Yagihashi and Arakaki, 2012; Marchandin et al., 2001). *Micrococcus luteus* was resistant to 50% of the antibiotics used. Rind and Khan (2000) recorded activity of sulphamethoxazole / trimethoprim, chloramphenicol, tetracycline and ampicillin against strains of *Micrococuus* Spp. Other authors also reported the antibiotic resistance profile of *Micrococcus luteus.* In their work, Munawar *et al*., (2021) reported the sensitivity of *M. luteus* to Ciprofloxacin which is contrary to what was observe in this work, the disparity in the result could be as a result of horizontal transfer of resistance plasmid in the water body. Fresh water environment is susceptible to contamination by residual antibiotics that are released from sources such as human, animal wastes and agricultural runoffs (Nnadozie and Odume, 2019). The result of this work agrees with the reports of several authors who recoded widespread resistance in aquatic bacteria (Thakur *et al*., 2003; Bughe *et al*., 2016; Mishra *et al*, 2010; Nakamura *et al.,* 2012; Shirin and Micheal, 2020; Girlich *et al*., 2020).

Vibrio Orientalis showed 50% resistance to the antibiotics used (Table 3). The pathogenicity of *Vibrio* Spp had been a source of debate amongst researchers and thus a hot topic of research,

Table 2 Morphological and biochemical characteristics of isolates

Isolate	morphology	Gram	Sugar fermentation						oxidase	catalase	VP	indole	Methyl red	Citrate	Suspected org.
			Sucrose	fructose	lactose	glucose	galactose	maltose							
1	cocci	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	*Veillonella Spp*
2	cocci	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	*Micrococcus luteus*
3	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	*Vibrio orientalis*

(Schroeder *et al.,* 2017). The *Vibrio* Spp cause systemic infections resulting in the death of fish and other aquatic animals and even human disease (Md Yasin *et al.,* 2019). The high level of antibiotic resistance in *Vibrio* Spp may not be unconnected with the indiscriminate use and uncontrolled access to antibiotics used in aquaculture as well as in animal farming. The use and discharge of antibiotics into water bodies is not currently regulated (Wang *et al.,* 2014). Gxalo *et al.,* (2021) in their work recorded high level of resistance of *Vibrio* Spp to seven classes of antibiotics used, and discovered that *Vibrio* isolates from municipal water showed 95% antibiotic resistance to the tested antibiotics.

Generally, there was a high level of resistance to Beta lactam antibiotics. This same opinion was expressed by Bush and Bradford (2016) in their own study. Tooke *et al*., (2019) reported the wide distribution of beta lactamases in plasmid. The acquisition resistance gene in *Vibrio* isolates in our study may have been through plasmid mediation in the aquatic environment through horizontal transfer from other bacteria.

Table 3 Antibiotic resistance profile of isolates

Antibiotics	V. orientalis	M. varians	Veillonella
	Zones of inhibition in mm		
Ciprofloxacin	15	R	18
Sparfloxacin	17	R	R
Augmentin	R	11	R
Ampicillin	R	12	10
Pefloxacin	R	R	22
Streptomycin	R	13	14
Gentamycin	21	10	16
Chloramphenicol	24	15	16
Septrin	R	R	R
Ciprofloxacin	21	R	14

Legend: R- Resistant

3.4. Multiple Antibiotic Resistance Index (MARi)

Multiple antibiotics resistance index is used to determine the risk level of antibiotic resistant organisms. The result of this work showed that *Vibrio orientalis* and *Micrococcus luteus* each had MARi value of 0.5 while *Veillonella* Spp had 0.3 (Table 4). Multiple antibiotic resistance (MAR) analysis was introduced to discern bacteria from sources using antimicrobial agents regularly approved for human therapy. MAR is most frequently associated with presence of plasmids which harbours single of multiple resistance genes (Ruppe *et al*., 2015). It is recorded that MARi above 0.2. is an evidence of high risk source of contamination (Osundiya *et al*., 2013).

The MAR index of *Vibrio orientalis* was 0.5. *M, luteus* had MAR index of 0.5 while *Veillonella* had MAR index of 0.3. The MAR indices of all isolates show that they are high risk bacteria both to humans during causing severe infections or to animals as well as to aquatic organisms. Baquero *et al*., (2008) posited that any MARi greater than 0.2 constitute public health risk. High prevalence of multidrug resistance indicates a serious need for broad based antimicrobial resistance surveillance and the resultant planning of intervention to reduce multidrug resistance in pathogens (Olayinka *et al*., 2004).

Table 4 Multiple Antibiotic Resistance Index (MARi)

Organisms	MAR (a/b)	indices
Vibrio orientalis	5/10	0.5
Micrococcus luteu	5/10	0.5
S veillonella	3/10	0.3

3.5. Plasmid profile of isolates and gene expression of resistance using antibiotic primers.

Figure 5 present the plasmid profiles of isolates from water bodies studied in this work. The results show that all the isolates have plasmids mediated resistance genes with bandwidth of 10,000 base pairs and above. The large plasmid observed in the isolates is an indication of resistance to multiple antibiotics. This type of plasmids belongs to the class 1 integron because it has resistance to a variety of antibiotics.

Class 1 integrons are central players in the problems of antibiotic resistance because they can capture and express diverse resistance genes and are often embedded in promiscuous plasmid and transposons (Gillings *et al*., 2008). Gillings also reported the presence of class 1 integron plasmid from Betaproteobacteria isolated from freshwater. Generally, conjugative plasmids are contributors to horizontal gene transfer and carry a wide variety of accessory genetic elements (Frost *et al*., 2005). The role of plasmid in the dissemination of antibiotic resistance is becoming

worrisome for human and animal health. Several authors have documented the presence of plasmids in *Veillonella* spp (Reig *et al*., 1997). Although it appears that plasmids occur naturally in some bacterial strains, but incidence of plasmid is probably higher in countries where antibiotics are readily available and are used indiscriminately as it is in Nigeria (Akanbi *et al*., 2004).

Figure 6 presents the molecular fingerprints of the isolates showing positive result to the primers used in detecting the resistance genes. The figure shows showed that *Vibrio* spp and *Veillonella* were also positive to beta lactamase genes (bla SHV). The primer sequence used to identify *SHV* F (5'-3') gene was CTTTATCGGCCCTCACTCAA and that of *SHV R* was AGGTGCTCATCATGGGAAAG.

Figure 7 shows positive for resistance to aminoglycosides (aac(3)-IV). The primer sequence used for the identification of *aac (3)-IV F* (5'-3') gene was CTTCAGGATGGCAAGTTGGT and for

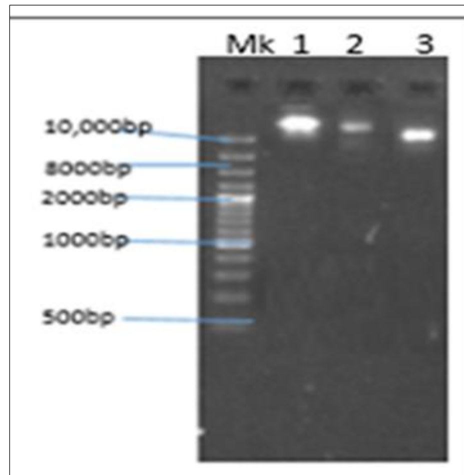

Figure 5 Agarose gel electrophoresis showing the profiling of the plasmid extracted from Vibrio orientalis (1), Micrococcus varians (2) Veillonella (3).

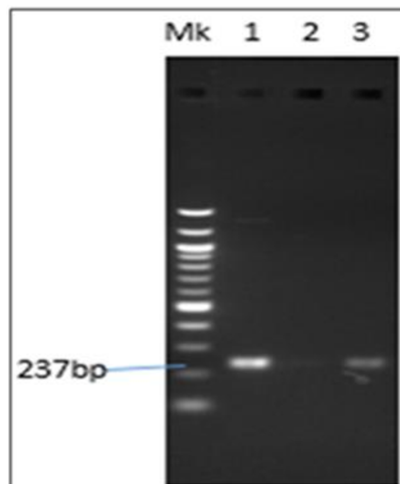

Figure 6 Agarose gel electrophoresis of the PCR bla SHV gene fragment amplified from selected bacteria isolates. Band size approximately 237bp indicates positive amplification. Gel image indicates a positive amplification in samples 1 and 3 indicating the presence of ESBL- Bla SHV gene

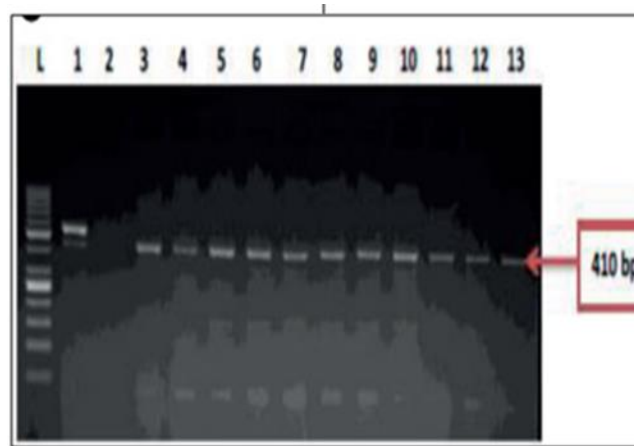

Figure 7 Agarose gel electrophoresis of the PCR aac(3)-IV gene fragment amplified from selected bacteria isolates. Band size approximately 410bp indicates positive amplification. Gel image indicates a positive amplification in only Vibrio spp indicating the presence of aac(3)-IV gene

aac(3)-IVR was TCATCTCGTTCTCCGCTCAT. Only Vibrio was positive for resistance to aminoglycosides.

Figure 8 showed positive result for the presence of resistance gene to quinolones (*qnrA* gene). Vibrio orientalis and Micrococcus luteus had resistance genes for quinolones (*qnrA* gene). The primer sequence for the identification of *qnrA* F (5'-3') primer was ATTTCTCACGCCAGGATTTG and for qnrA R was GATCGGCAAAGGTTAGGTCA. In the same manner,

Figure 9 shows the resistance pattern of the isolates to the sulphonamides *(sul1* gene). All the isolates possess genes against the sulphonamides used. The primer sequence (5'-3') for the identification of *Sul1* F gene was CGGCGTGGGCTACCTGAACG and *Sul1* R was GCCGATCGCGTGAAGTTCCG.

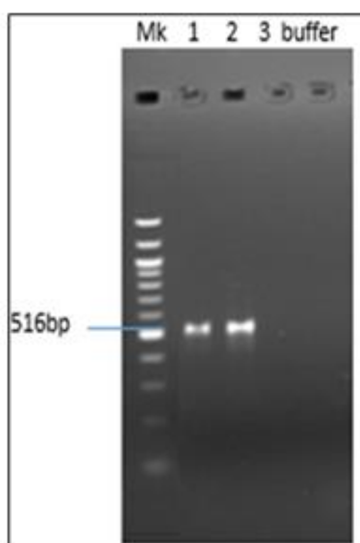

Figure 8 Agarose gel electrophoresis of the PCR qnrA gene fragment amplified from selected bacteria isolates. Band size approximately 516bp indicates positive amplification. *Vibrio orientalis* and *Micrococcus varians* showed the presence of resistance gene for qnrA gene

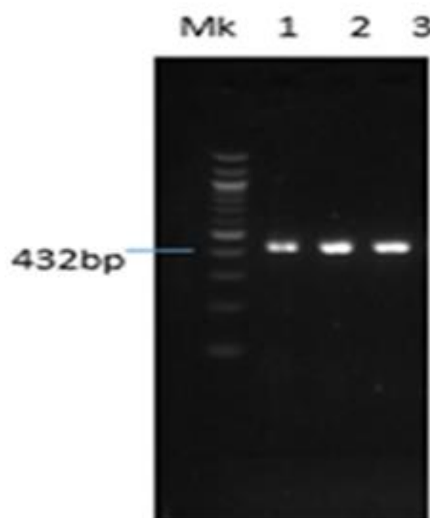

Figure 9 Agarose gel electrophoresis of the PCR sul1 gene fragment amplified from selected bacteria isolates. Band size approximately 432bp indicates positive amplification. Gel image indicates a positive amplification in all samples indicating the presence of sul gene.

The resistance of enterobactericeae rods to aminoglycosides has been reported by several authors (Fernandez-Martinez, *et al*., 2015). Odjana *et al*., (2018) documented that this resistance development reduces therapeutic options for infections caused by multi drug resistant organisms because of the changing epidemiology of extended spectrum beta lactamase and resistance to aminoglycosides. Resistance of enterobacteriacea to fluoroquinolones and cephalosporin have been documented by several authors. Fluoroquinolone resistance occur primarily through mutation in the DNA gyrase (A and B) and topoisomerase 4 (Nakato *et al.,* 2019).

In his work Beshiru *et al*. (2019) showed that only two strains of the *Vibrio* isolated carried the gene for fluoroquinolones. About 70% of his isolates carried resistance genes for aminoglycosides and about 29% carried gene for Beta lactamases. The result of this study agrees with the work of various authors who confirmed the presence of resistance genes in *Vibrio* isolates (Beshiru *et al.,* 2019).

Micrococcus luteus is usually not a pathogenic organism, they are normal inhabitants of the human saliva. It can however become opportunistic pathogens in immune compromised individuals (Kloss *et al.,* 2006). The presence of this organism in water bodies signifies contamination form human sources as a result of wash off from soil from domestic environment or even domestic activities of humans. Hussain *et al*., (2016) observed high level of sensitivity of *Micrococcus* spp isolated form bovine semen. Other authors as Fazlani *et al*., (2011); Rind and Khan (2000) share the same observation.

Class 1 integrons are central players in the problems of antibiotic resistance because they can capture and express diverse resistance genes and are often embedded in promiscuous plasmid and transposons (Gillings *et al.,* 2008). Gillings also reported the presence of class 1 integron plasmid from Betaproteobacteria isolated from freshwater.

Veillonella spp is a non-motile Gram-negative diplococcus. It is also a normal flora of humans especially in the gastrointestinal tract. The presence of this organism in water bodies sampled is an indication of feacal contamination from humans. The organism is often regarded as contaminants and implicated in oral and various soft tissue infections (Actor, 2012).

4. Conclusion

Conclusively, conjugative plasmids are distributors to horizontal gene transfer and carry a wide variety of accessory genetic elements. The role of plasmid in the dissemination of antibiotic resistance is becoming worrisome for human and animal health. Plasmids are very important vehicles for the communication of genetic information between bacteria. The exchange of plasmids transmits pathogenically and environmentally relevant trait. The fact that most of the antibiotics used in this study are of major therapeutic importance in human medicine, this present public health

concern. This study has been able to identify some common aquatic bacteria and their level of resistance to some common classes of antibiotics. It has also established the fact that microorganisms can acquire plasmid mediated resistance to antimicrobials irrespective of the ecosystem and this is a cause for concern as most ecosystem are interconnected and interrelated. Human and animal activities also contribute to the transfer of organisms into different habitat which is an undeniable factor in the transfer of antimicrobial resistance to previously non- resistant strains. It is evident that there is a need to devote resources to studying antibiotic resistance in different ecosystems. It is no gain saying that the use of antibiotics in farm animals is one of the major contributions to the development of antibiotic resistant organisms involved in life threatening human infections. It leads to the host bacteria promoting their rapid evolution and adaptation to various environment. Also, Plasmodomes of water bodies are known to have fluctuating microbial community along with lower cell count which accommodates the presence of human normal flora in aquatic habitat.

Compliance with ethical standards

Acknowledgments

We hereby acknowledge the management and staff of Biological Sciences of Achievers University for the use of the laboratory facilities for this research.

Disclosure of conflict of interest

There is no conflict of interest in this work.

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