IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 *Volume 3 issue 2 May, 2024* Research Article

Antibiotic Sensitivity Pattern and Resistance Genes of *Campylobacter* and *Vibrio* Species Associated with Sea Foods Sold in Port Harcourt Metropolis

Inana, M. E[1]*., Ogbonna, D. N[2]. , Sokari, T.G[2] and Odu, N. N[2].
[1]Nigerian Stored Products Research Institute, Port Harcourt
[2]Department of Microbiology, Rivers State University,
Nkpolu-Oroworukwo, PMB 5080, Port Harcourt, Nigeria.
***Corresponding Author:** *mandudu2000@gmail.com*

ABSTRACT

Campylobacter and *Vibrio* species are common foodborne pathogens associated with seafood consumption and their antibiotic resistance is a growing concern worldwide. This study investigated the antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with Oysters (*Crassostrea gasar*), Shrimps (*Penaeid Shrimps*) and Prawns (*Penaeus monodon*). Seafood samples (360) were purchased from vendors in three major markets in Port Harcourt over a period of one year. The antibiogram of *Campylobacter* and *Vibrio* species isolated were determined using the disc diffusion method while Resistance genes were detected using the Polymerase chain reaction (PCR) and DNA sequencing. The result indicates that most of the *Campylobacter* and *Vibrio species* isolated from Prawns, Shrimps and Oysters were 100% resistant to Ceftazidime (CAZ), Cefuroxime (CRX) and Cloxacillin (CXM), the isolates showed 11– 100% sensitivity to Augmentin, 70-100% to Gentamycin, 73-100% to Ceftriaxone, 76-100% to Nitrofurantoin and 100% to Ofloxacin. Except *Campylobacter jejuni* and *Vibrio mimicus* that does not possess CTX-M gene and *Campylobacter coli* and *Vibrio alginolyticus* which does not possess *blaTem gene*, all the *Campylobacter* and *Vibrio* isolated possessed antibiotic-resistance genes such as *blaTem*, SHV and CTX-M highlighting the potential risk associated with the consumption of sea foods contaminated with these bacteria. This study therefore emphasizes the need for stringent hygienic practices during handling, processing, storage, and in distribution of seafood as to prevent the risk of foodborne illnesses associated with *Campylobacter* and *Vibrio* species. The fact that the transfer of resistance gene in these isolates between the aquatic environments and humans through handling and consumption of seafood pose a serious hazard to public health, continuous surveillance and adherence to food safety standards/regulations to safeguard consumers' health must be sustained.

Keywords: *Campylobacter, Vibrio*, Antibiotic resistance, resistance genes, Sea foods, food safety standards/regulations.

Introduction

Campylobacter and *Vibrio* species are common foodborne pathogens associated with seafood consumption. Antibiotic resistance in these pathogens is a growing concern worldwide. According to Inana *et al.* (2024), *Campylobacter* and *Vibrio* species are significant foodborne pathogens associated with oysters, shrimps, and prawns. Their prevalence in these sea foods poses a major public health concern, and effective measures are necessary to prevent and control their occurrence. Proper handling, storage, cooking, and processing practices are essential to reduce the risk of foodborne illnesses associated with these pathogens (Inana *et al.*, 2024). In addition to sea foods being a vital component of the global food chain, sea foods provide nourishment to millions of people worldwide.

Consuming tainted sea foods present serious public health risks because it has been connected to several bacterial illnesses (Iwamoto *et al.*, 2010). Seafood products that are improperly handled, processed, stored and distributed to consumers may all result in the contamination of fish, shellfish and crustaceans by a range of bacterial species (Daniels *et al.*, 2000).

Among the most often reported bacterial species connected to seafood contamination are *Vibrio* species, including *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* (Efiuvwevwere and Amadi, 2015). These bacteria are naturally present in marine environments and can thrive on a range of sea foods. Li *et al.* (2020) discovered that *Vibrio parahaemolyticus* and *Vibrio vulnificus* are known to cause foodborne illnesses and can considerably reduce the shelf-life of seafood products.

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

These findings emphasize the importance of effective measures to control bacterial contamination during seafood processing and storage. Similarly, Zangoei-Fard *et al*. (2020) that *Vibrio* species, particularly *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi*, are considered imperative foodborne pathogens associated with seafood consumption. The members of the *Vibrio* genus are spread worldwide, being autochthonous in marine, coastal and riverine environments. Some *Vibrio* species are potentially pathogenic and lives freely in the surface waters and have been reported as the etiologic agent of cholera outbreaks.

Campylobacter genomes are relatively unstable; several mechanisms that may lead to this genetic instability have been proposed, including bacteriophage activity, DNA recombination and transformation (FDA, 2012). Antibiotics resistance however, refers to the mechanism by which microorganisms become resistant to an antibiotic which include degrading of the antimicrobial substance, modification of the chemical structure of the antibiotic, over secretion of the target enzyme, obtaining alternate pathways to those drugs that can inhibit or cause changes in the bacterial cell permeability thus restricting the access of the antimicrobial agent to the target site, active removal of the antibiotic from the bacterial cell and remodelling of the target for the antibiotic (VanHoek *et al*., 2011; Azuonwu and Ogbonna, 2019). Genes associated with microbial antibiotic resistance has been found in different environments at quantities higher than those present before antibiotics were mass-produced (Knapp *et al.,* 2010). These antibiotic resistance genes (ARGs) are unique contaminants in that, they are of biological origin and can be transferred through genetic processes into different types of organisms regardless of distance.

Although antibiotic resistant β-lactamases only became popular when clinical resistance surfaced, spontaneous mutation alone cannot be implicated in the prevalence and spread of microbial resistance to modern antibiotics (Keen and Patrick, 2013; Marinescu and Lazar, 2013). Spread of resistance genes through horizontal gene transfer to human pathogens may occur, thereby complicating antibacterial therapy when infection occurs (Garcia-Graells *et al.*, 2019). According to Lin *et al.* (2015) multiple mechanisms for antibiotic resistance exists which can be coded for by either single or by multiple genes.

These mechanisms start first with random mutation in an organism which later spreads to other organisms through the process of gene transfer.

Horizontal gene transfer (HGT) occurs through transduction, transformation and conjugation (Snyder *et al.,* 2014). Conjugation takes place when DNA is transferred to a cell through direct cell contact or through a multi-protein conjugative complex (Bellanger *et al.,* 2014). Transformation occurs by the take up of exogenous DNA by an organism while bacterial transduction takes place when a bacteriophage injects a DNA into a bacterial cell (Colomer-Lluch *et al.,* 2011; Fard *et al.,* 2021). Either of these mechanisms aid gene transfer from the environmental gene pool consisting of genetic information that can be reached by more than one species of bacteria (Grohmann, 2011). This study was thus aimed at determining the presence of some resistant genes in microorganisms isolated from seafood in Port Harcourt as there exist paucity of data on the incidence of *Vibrio spp*. And *Campylobacter* species.

Materials and Methods

Materials and Methods Study Area; The study was carried out in three different markets; the Creek Road market, Mile One market, and Rumuokoro market all located within Port Harcourt metropolis. These markets were selected because of the high population density, catchment areas for consumers and its easy accessibility to the purchase of seafood. Seafood samples were purchased randomly from Ten (10) different vendors from each of the three (3) markets. Samples were purchased and aseptically collected using appropriate aseptic methods. Samples were collected in the months of January, May, September and December in the year 2022**.**

Seafood Sample Collection

Seafood samples were purchased from different markets within the Port Harcourt metropolis in sterile bags, labelled and transported in an ice-chest box aseptically to the Department of Microbiology Laboratory Rivers State University for bacteriological analysis. The seafood samples namely Oyster (*Crassostrea gasar*), Prawn (*Penaeus monodon*) and Shrimps (*Caridea*) were identified by Dr. Chidinma Amuzie of the Department of Animal and Environmental Biology Rivers State University, Port Harcourt.

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

Antibiotic Sensitivity Testing
Preparation of Standard Bacterial Suspension

A 24-hour old pure culture of bacterial isolates were emulsified in a sterile nutrient broth in test tube and adjusted to 0.5 McFarland Turbidity Standard and the bacterial suspension was used for the susceptibility test (Cheesbrough, 2005).

Preparation of 0.5 McFarland Turbidity Standard

One percent (1% v/v) Solution of Sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and properly mixed, 0.5g of dehydrated Barium Chloride ($Bacl_2$ $.2H_2O$) was dissolved in 50ml of distilled water to prepare 1% w/v of Barium Chloride Solution (CLSI, 2017). 0.05ml of 1.175% Barium Chloride solution was added to 9.95ml of 1% sulphuric acid solution and properly mixed. A prepared turbid solution was transferred to a capped tube and kept in well-sealed container in the dark at room temperature (25-28ºC).

Agar Disc Diffusion Method (Kirby Bauer Disc Diffusion)

A sterile swab stick was dipped into the tube containing the bacterial suspension and its turbidity equivalent to 0.5 McFarland Turbidity Standard and the swab was used to swab the surface of the petri dish evenly which contain already prepared Mueller Hinton agar and rotating the plates to about 60ºC to ensure even distribution of the organism. The agar was allowed to dry for about 3-5minutes. With Sterile forceps, the impregnated antibiotic discs were placed evenly on the surface of the inoculated plate and the disc was placed 15mm away from the edge of the plate. The head of the forcep was used to Press down each disc slightly to make contact with the agar. After applying the discs, the plates were incubated in an inverted position aerobically at 35ºC for 16-18h. After incubation, the test plates were examined to ensure confluence growth or near confluence. The diameter of each zone of inhibition was measured in mm using a ruler on the underside of the plate and recorded (CLSI, 2017).

Detection of Resistance Genes

Using the SHV F: 5' CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' primers, SHV genes from the isolates were amplified on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 µl for 35 cycles.

The resultant product was resolved on a 1 % agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 281 bp product size. The same procedure was carried out but CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR: 5'-ACCGCGATATCGTTGGT-3' primers were used for CTX-M gene and TEMF: 5'-ATGAGTATTCAACA TTTCCGTG-3' and TEMR: 5'-TTACCAATGCTTA ATCAGTGAG-3' primers for TEM gene. Sizes of resolved products were 281 bp, 560 bp and 960 bp for SHV, CTX-M and TEM, respectively.

Statistical Analysis of Data

The data obtained was analyzed using analysis of variance (ANOVA) to test for significance and where differences occur Duncan multiple range test was used to separate the means using the Statistical Package for Social Science (SPSS) version 22 (Bewick *et al.*, 2004).

Results

In this study the frequency and percentage occurrence of *Vibrio* and *Campylobacter* species in respected to seafood samples is presented in Tables 1. Table 2 shows the frequencies of the *Vibrio* and *Campylobacter* species in respect to samples location 2. The *Vibrio* and *Camphlobacter* species which include *Vibrio parahaemolyticus, Vibrio alginolyticus Ariake-S2 , Vibrio fluvialis, Vibrio mimicus, Vibrio cholera, Vibrio vulnificus E4010, Vibrio cholerae NSTH36* and *Vibrio parahaemolyticus* VP35/2 isolated from seafood (Prawn ,Oyster and Shrimps) across the three markets.

The antibiotic susceptibility pattern of *Vibrio* and *Campylobacter* species isolated from seafoods is presented in Table 3. The *Vibrio* and *Campylobacter* were subjected to different antibiotics such as Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cloxacillin (5µg), Ofloxacin (5µg), Augumentin (30µg), Ceftriaxone (30µg), Nitrofurantoin (30µg).

The percentage resistance of *Vibrio* and *Campylobacter species* isolated from seafood in Mile one market , Creek road market and Rumuokoro market markets were 100% resistant to Ceftazidime (CAZ), Cefuroxime (CRX) and Cloxacillin (CXM), the isolates showed 11– 100% sensitivity to Augmentin, 70-100% to Gentamycin, 73-100% to Ceftriaxone, 76-100% to Nitrofurantoin and 100% to Ofloxacin (Table 3).

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

Table 1: Percentage Occurrence of *Vibrio* and *Campylobacter* Species in Prawn, Oyster and Shrimp

Isolates	Sea food		
	Prawn	**Oyster**	**Shrimp**
Vibrio alginolyticus Ariake-S2	9(5.0)	7(3.9)	3(1.7)
Vibrio parahaemolyticus	6(3.4)	4(2.2)	7(3.9)
Vibrio fluvialis	7(3.9)	1(0.5)	2(1.1)
Vibrio mimicus	7(3.9)	4(2.2)	9(5.3)
Vibrio cholerae	11(6.1)	6(3.4)	4(2.2)
Vibrio vulnificus E4010	8(4.5)	6(3.4)	3(1.7)
Vibrio cholerae NSTH36	6(3.4)	3(1.7)	10(5.6)
Vibrio parahaemolyticus VP35/2	4(2.2)	0(0)	2(1.1)
Campylobacter jejuni G-149-05-1	5(2.8)	3(1.7)	2(1.1)
Campylobacter lari subsp	5(2.8)	3(1.7)	4(2.2)
Campylobacter coli	4(2.2)	2(1.1)	2(1.1)
Campylobacter jejuni subsp	8(4.5)	4(2.2)	1(0.5)
Campylobacter lari	4(2.2)	3(1.7)	0(0)

Table 2: The Percentage Occurrence of *Vibrio* and *Campylobacter* Species in Respect to the Sea Foods Prawn, Oyster and Shrimp

Isolates	Market Locations			Total Frequency	% Frequency
	Mile 1	**Town**	**Rumuokoro**		
Vibrio alginolyticus Ariake-S2	5	8	6	19	10.6
Vibrio parahaemolyticus	3	6	8	17	9.5
Vibrio fluvialis	2	5	3	10	5.6
Vibrio mimicus	7	8	4	20	11.2
Vibrio cholera	5	10	6	21	11.7
Vibrio vulnificus E4010	4	7	6	17	9.5
Vibrio cholerae NSTH36	4	8	7	19	10.6
Vibrio parahaemolyticus VP35/2	1	2	3	6	3.4
Campylobacter jejuni G-149-05-1	2	5	3	10	5.6
Campylobacter lari subsp	4	6	2	12	6.7
Campylobacter coli	1	4	3	8	4.5
Campylobacter jejuni subsp	4	6	3	13	7.3
Campylobacter lari	2	2	3	7	3.8
Total	44	77	57	179	100

Table 3: Antibiotic Susceptibility Pattern of *Vibrio* and *Campylobacter* Species Isolated from Seafoods

ISOLATES	ANTIBIOTIC							
	n	**CAZ**	**CRX**	**GEN**	**CXM**	**OFL**	**AUG**	**CPR**	**NIT**
Vibrio alginolyticus Ariake-S2	19	0(0.00)	0(0.00)	14(73.7)	0(0.00)	19(100)	0(0.00)	14(73.7)	19(100)
Vibrio parahaemolyticus	17	0(0.00)	0(0.00)	14(82.4)	0(0.00)	17(100)	0(0.00)	14(82.4)	15(88.2)
Vibrio fluvialis	10	0(0.00)	0(0.00)	7(70)	0(0.00)	9(90)	0(0.00)	8(80)	10(100)
Vibrio mimicus	20	0(0.00)	0(0.00)	15(78.9)	0(0.00)	19(100)	0(0.00)	17(84.5)	14(73.7)
Vibrio cholera	21	0(0.00)	0(0.00)	19(90.5)	0(0.00)	21(100)	1(4.76)	18(85.7)	20(95.2)
Vibrio vulnificus E4010	17	0(0.00)	0(0.00)	13(76.5)	0(0.00)	17(100)	2(11.8)	15(88.2)	14(82.4)

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

Vibrio cholerae NSTH36	19	0(0.00)	0(0.00)	15(78.9)	0(0.00)	19(100)	1(5.3)	14(73.9)	18(94.7)
Vibrio parahaemolyticus VP35/2	6	0(0.00)	0(0.00)	6(100)	0(0.00)	6(100)	0(0.00)	5(83.3)	5(83.3)
Campylobacter jejuni G-149-05-1	10	0(0.00)	0(0.00)	8(80)	0(0.00)	10(100)	0(0.00)	9(90)	9(90)
Campylobacter lari subsp	12	0(0.00)	0(0.00)	9(75)	0(0.00)	12(100)	2(16.7)	11(91.7)	8(66.7)
Campylobacter coli	8	0(0.00)	0(0.00)	7(87.5)	0(0.00)	8(100)	8(100)	7(87.5)	8(100)
Campylobacter jejuni subsp	13	0(0.00)	0(0.00)	13(100)	0(0.00)	13(100)	0(0.00)	13(100)	10(76.9)
Campylobacter lari	7	0(0.00)	0(0.00)	4(57.1)	0(0.00)	7(100)	0(0.00)	6(85.7)	7(100)

Key: CAZ= Ceftazidime (30µg) CRX= Cefuroxime (30µg), GEN= Gentamicin (10µg), CXM = Cloxacillin (5µg), OFL= Ofloxacin (5µg), AUG= Augmentin (30µg), CPR= Ceftriaxone (30µg) NIT= Nitrofurantoin (30µg).

The result of antibiotic resistance genes carried out in this study for *BlaTem*, CTX-M and SHV genes confirms the presence of these genes segments in the chromosomes in most of the isolates analyzed. The results of the antibiotic resistance gene are shown in Plates 1, 2 and 3. Agarose Gel Electrophoresis image showing amplification of *BlaTem* gene are shown in Plate 1. All isolates showed positive amplification meaning that the gene is present in the isolates except isolate OV4 and SC2 (*Vibrio alginolyticus* and *Campylobacter coli*,). Plate 2 shows the amplification of CTX-M gene of the bacterial isolates. Lane 1-13 represents the CTX-M gene bands (550bp). Lane M represents the 1000bp Molecular ladder *Vibrio parahaemolyticus VP35/2*; *Vibrio mimicu and Campylobacter coli*, showed negative amplification meaning that the gene is not present in those isolates. Plate 3 is an agarose gel electrophoresis showing the Amplified SHV Genes Lane 1-13 represent the SHV Gene Bands at 200bp While Lane L Represents to 100bp Molecular Ladder. All isolates showed positive amplification meaning that the gene is present in the isolates.

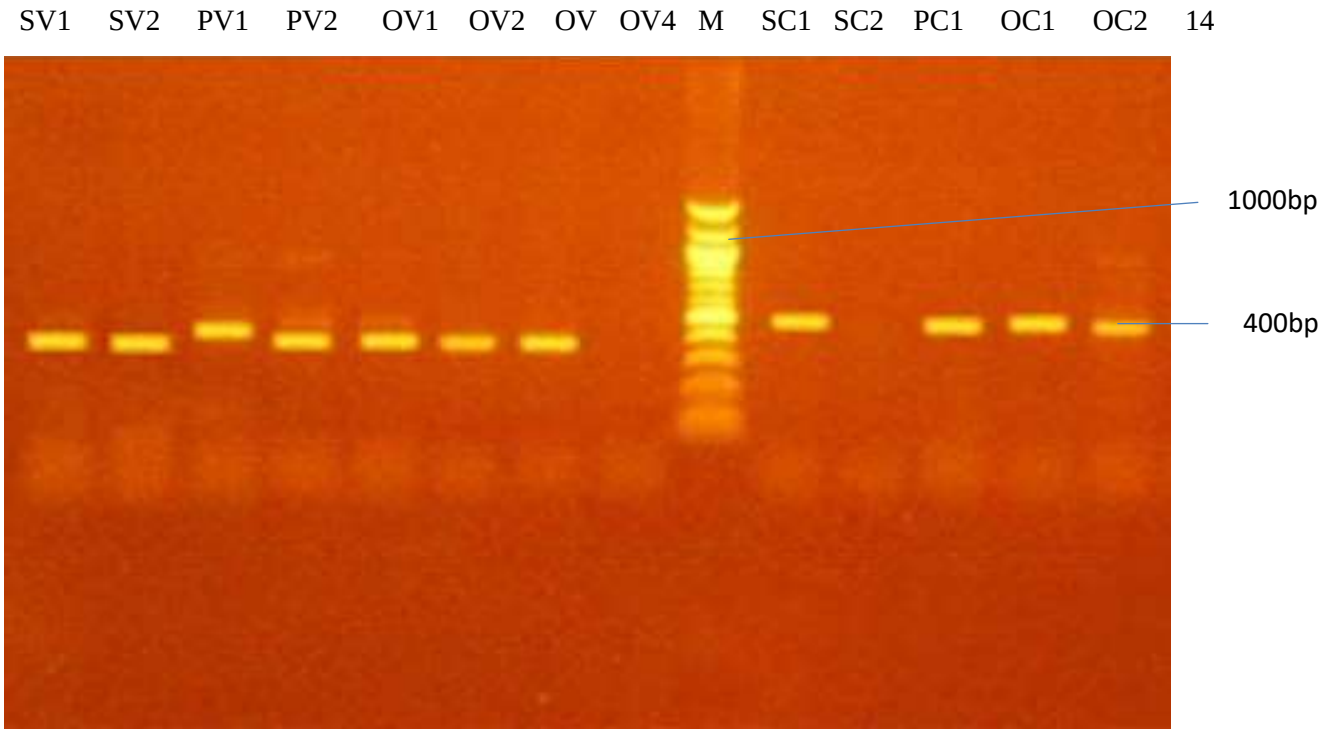

Plate 1: Agarose Gel Electrophoresis of *BlaTem* Gene of some Selected Isolates. Lane 1-13 Represents the *blaTem* Gene Bands (400bp). Lane M Represents the 1000bp

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

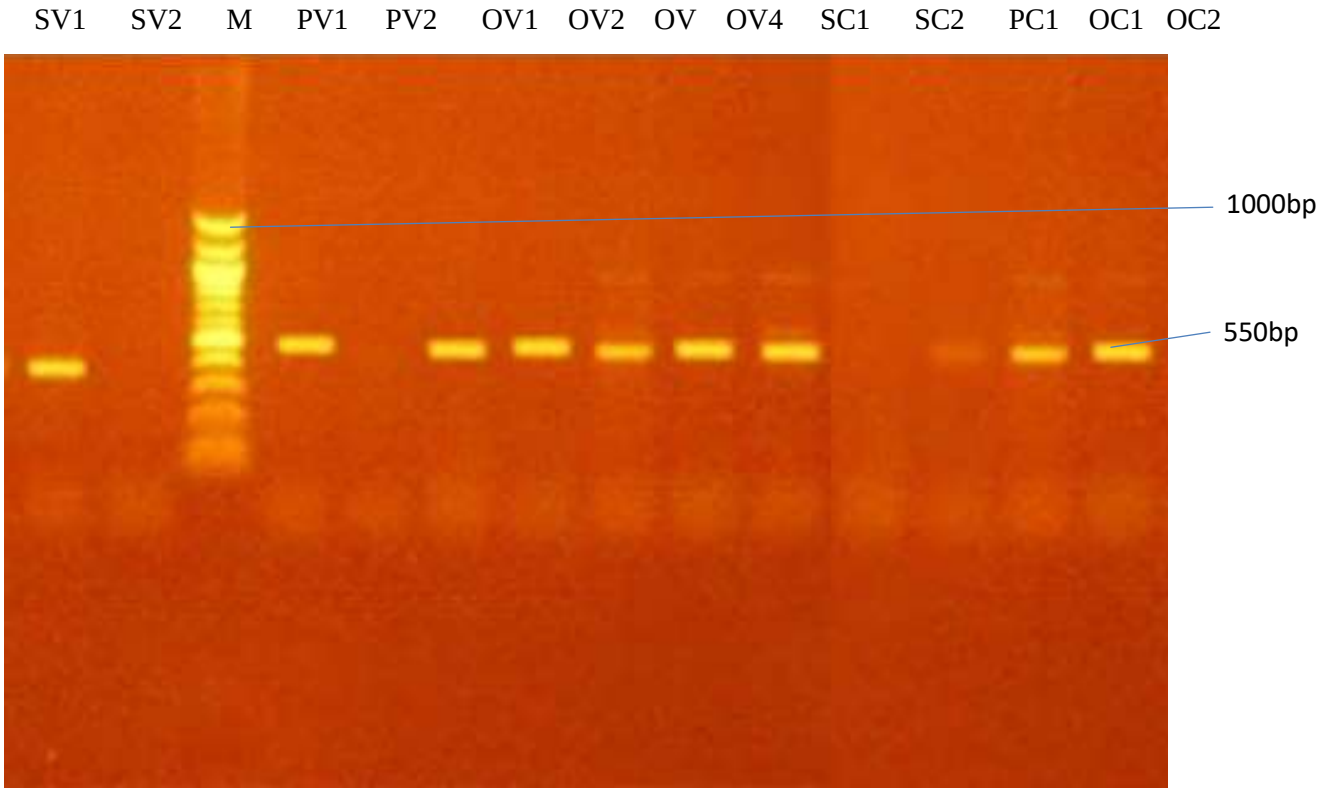

Plate 2: Agarose Gel Electrophoresis of CTX-M Gene of the Bacterial Isolates. Lane 1-3 Represents the CTX- M Gene Bands (550bp). Lane M Represents the 1000bp Molecular Ladder

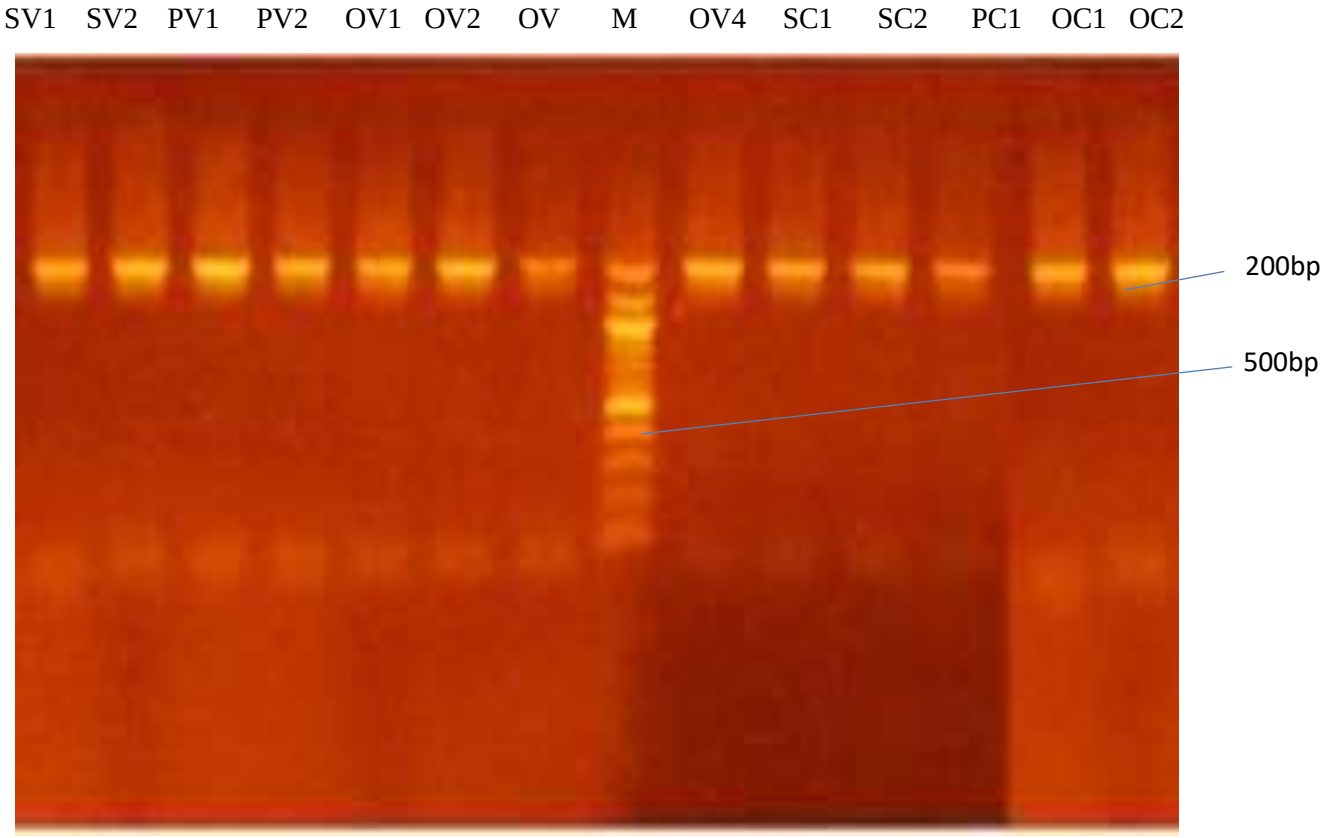

Plate 3: Agarose Gel Electrophoresis Showing the Amplified SHV Genes. Lane 1-3 Represent the SHV Gene Bands at 200bp While Lane L Represents to 100bp Molecular Ladder

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

Discussion

The global concern has been the emergence of antibiotic resistance in food- and water-borne bacteria. Antimicrobial agents and the emergence of resistant genes are now widely acknowledged (Abdollahzadeh *et al*., 2016), However, since it may reveal the degree to which anthropogenic activities have altered water ecosystems, research on antibiotic resistance in bacterial pathogens isolated from seafood is crucial (Alonso *et al*., 2001). Antibiotics have over the decades been used for both human and animal disease treatment. However, the extensive and indiscriminate use of antibiotics, especially the B-lactamase therapeutic and sub therapeutic doses for treatment of infections, growth promotion and prophylaxis has led to increased rate of antibiotics resistance among bacteria species (Waters *et al.*, 2011; Threedeach *et al.*, 2012).

Campylobacter species such as *Campylobacter lari Ca917, Campylobacter jejuni, Campylobacter coli, Campylobacter lari subsp, Campylobacter jejuni* and *Vibrio species* belonging to the genera *Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio fluvialis, Vibrio mimicus Vibrio cholera RC782, Vibrio cholera, Vibrio parahaemolyticus VP35/2* and *Vibrio vulnificus E4010* isolated and identified in this study exhibited high levels of susceptibility to Ofloxacin, ceftriaxone and Nitrofurantoin and exhibited high resistance to Augumentin, Ceftazidime, and Cefuroxime, according to the susceptibility pattern of the bacteria from seafoods. *Campylobacter* and *Vibrio* species sensitivity to ofloxacin and Nitrofuratoin is consistent with Shakoor *et al.* (2012) findings, although it deviates from their 100% Nitrofuratoin sensitivity reported. In the present study 100% Nitrofuratoin sensitivity (Table 3), was not recorded for all the species and also there were differences in the percentage of sensitivity across the isolates from different sources (markets). When comparing the antimicrobial susceptibility pattern found in this study to some other studies conducted previously, there may have been differences in the environmental conditions.

These conditions may have included the organism being exposed to frequently used antibiotics and changes in the genome brought about by harsh and chemical environments, which allowed the organisms to transform into strains capable of resisting antibiotics to which they would normally be susceptible. The reason behind the high level of resistance to beta-lactam antibiotics can be attributed to their widespread and unregulated usage. It is also as a result of their affordability and ability to transfer the genes encoding the extent-ended-spectrum β-lactamases (Gourmelon *et al.*, 2006). The bacteria could be mediated by keeping the medications from entering the ribosomes, which is where they are intended to act. This is typically accomplished in two (2) ways either by changing the cell envelope to prevent drug uptake, or by modifying the drug by deactivating enzymes, which runs counter to the findings of the current study. Similar findings were reported by Abdissa *et al* (2017), who showed Ofloxacine susceptibility to Gram negative bacteria isolated from seafood in Abia State (Onuoha, 2018). This medication, Ofloxacine, attaches itself to the cell and prevents the synthesis of proteins and the organisms' acquisition of the aac gene (Vakulenko and Mobashery, 2003).

The high percentage of resistance to commonly used antibiotics recorded in this study may be caused by mechanisms such as the synthesis of low affrinity-β-lactams binding proteins, the production of penicillinase, and transferable genetic elements which included plasmids that may contain different resistant genes and the ability of the isolates to produce biofilms which increase the opportunity for gene transfer among bacteria (Ogbonna and Azuonwu, 2019). In addition to having structural barriers, biofilm-forming bacteria can undergo physiological changes such as slow growth rate and producing persistent cells. In these occasions, antibiotics cannot inhibit, kill, or eradicate these slow-growing and persistent cells which are found inside the biofilm matrix (Okafor *et al.*, 2005). Biofilms promote genetic exchange among bacterial cells through horizontal gene transfer, allowing the transfer of antibiotic resistance genes between different species or strains of bacteria. This genetic exchange can further enhance antibiotic resistance within the biofilm and potentially spread it to other bacteria in the surrounding environment. The high percentage of susceptibility of the isolates to Ceftriazone, Nitrofurantoin, and Oxfloxacin is in consonance with a study by Ogbonna and Inana (2018). This study recorded resistance of Gram negative isolates to Cloxacillin, Ceftazidine, Cefuroxime, and Augumentin.

The bacterial drug resistance could lead to the emergence of resistant bacteria that may be transferred to consumers, leading to difficulty to treat infections (Onuoha, 2018). This in turn leads to increased cost of treatment, mortality and morbidity rates (Igbinosa and Obuekwe, 2014).

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

The high percentage of susceptibility of the isolates to Ceftriazone, Nitrofurantoin, and Oxfloxacin is in consonance with a study by Ogbonna and Inana (2018) who carried out antibiotic sensitivity tests on microorganisms isolated from ponds. This study recorded resistance of Gram negative isolates to Cloxacillin, Ceftazidine, Cefuroxime, and Augmentin. The bacterial drug resistance could lead to the emergence of resistant bacteria that may be transferred to consumers, leading to difficulty to treat infections (Onuoha, 2018).

The Antibiotic resistance genes carried out in this study showed the presence of *BlaTem*, CTX-M and SHV genes. *BlaTem*, genes are known to confer a low-level resistance to fluoroquinolone in *Enterobacteriaceae*. They are often found on the same resistance plasmids as extended spectrum β-lactamase (ESBL) and constitute the most common antibiotic resistance mechanism (Saboohi *et al.*, 2012). All isolates showed positive amplification meaning that the gene is present in the isolates except isolate *Staphylococcus aureus*. This is in disagreement with previous study by Saboohi *et al* (2012), who reported a 25.8% occurrence of the *BlaTem*, genes in *Salmonella* species. However, this study agrees with Salah *et al*. (2019) who reported a very high presence of *BlaTem*, genes of 69% in *E. coli* in Sokoto. This study also corroborates with other researchers (Guessennd *et al.*, 2008, Moumouni, *et al.*, 2017; Bouchakour *et al.*, 2010) who also reported various prevalence of *BlaTem*, genes in *E. coli* in their studies. Chromosomal and plasmid mediated quinolone resistance may facilitate the spread and increase in frequency of quinolone resistant bacterial strains (Moumouni *et al.*, 2017).

The presence of *BlaTem*, genes has clinical implications as acquisition of the gene by quinolone susceptible ESBL producing strains could lead to selection of Ciprofloxacin and Cephalosporin resistant strains (Saboohi *et al.*, 2012) and increase the Mutant Prevention Concentration (MPC) (Guessennd *et al.*, 2008). More so, the presence of ESBL and some of the quinolone resistance genes in some mobile genetic elements could explain the co-resistance to beta-lactamase and flouroquinolones (Saboohi *et al.*, 2012). β-lactamase has been found to be the primary cause of resistance to β-lactams among members of the family enterobacteriaeceae. SHV gene emergence in enterobacteriaceae has been observed to be related to infections in different epidemiological settings including humans, animals and the environment.

In this study, 100% of the *Campylobacter* and *Vibrio* species including *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari subsp*, *Campylobacter jejuni* and *Vibrio species* belonging to the genera *Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio fluvialis, Vibrio mimicus Vibrio cholera RC782, Vibrio cholera, Vibrio parahaemolyticus VP35/2* and *Vibrio vulnificus E4010* harboured segments containing SHV resistant genes of 200bps in their genome.

This study agrees with Eman and Mushtak (2018), who reported the presence of SHV resistant genes in *Vibrio* species. However, this study didn't detect or record the presence of the SHV genes. The differences observed in this study may be as a result of differences in scope of the studies and environmental availability of these resistant genes to be taken up by transformation in the locality studied. Also, CTX-M resistant gene profiling in our study reviewed that the genes was present in *most of the species of Campylobacter and Vibrio species.*

In conclusion, this study shows that high level of antibiotic resistant pattern of *Campylobacter* and *Vibrio* species most commonly uses antibiotics such as Cefuroxime, Cloxacillin, Ceftazidime, Gentamycin and Augmentin which is a major concern as they have the potential to seriously compromise public health. The findings suggest that the best medications for treating foodborne illnesses as a result *Campylobacter* and *Vibrio* species linked to the consumption of seafood are Ofloxacin and Nitrofurantoin, which are associated with the *Campylobacter* and *Vibrio* species. This study has discovered that the presence of resistant genes (blaTEM, CTX, and blaSHV) in the bacterial isolates which can confer resistance to promote or enhance the degree of pathogenesis in *Campylobacter* and *Vibrio* species. Therefore, effective monitoring and regulation of antibiotic use in aquaculture and food handling practices are essential to combat antibiotic resistance.

References

Abdissa-Haile, W., Fite, A., Beyi, A., Agga, G., Edao, B., Tadesse, F., Korsa, M., Beyene, T., De Zutter, L. Cox, E. & Goddeeris, B. (2017). Prevalence of *Escherichia coli* O157:H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. *Biomedical Journal of Infection and Disease, 17*, 277-311.

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

Abdollahzadeh, E., Ojagh, S. M., Hosseini, H., Irajian, G. & Ghaemi, E. A. (2016). Prevalence and molecular characterization of *Salmonella* spp. and *Listeria monocytogenes* isolated from fish, shrimp, and cooked ready-to-eat (RTE) aquatic products in Iran. *LWT-Food Science and Technology, 73*, 205-211.

Alonso, A., Sánchez, P. & Martínez, J. L. (2001). Environmental selection of antibiotic resistance genes. *Environmental Microbiology, 3(1),* 1-9.

Azuonwu, T.C. & Ogbonna D.N (2019). Resistant Genes of Microbes Associated with Abattoir Wastes. *Journal of Advances in Medical and Pharmaceutical Sciences, 21(2),* 1-11.

Bellanger, X., Guilloteau, H., Bonot, S., Merlin, C. Demonstrating plasmid-based horizontal gene transfer in complex environmental matrices: A practical approach for a critical review. *Sci. Total Environ, 493*, 872– 882.

Bewick, V., Cheek, L. & Ball, J. (2004). Statistics Review 9: One-way Analysis of Variance (ANOVA). *Critical Care, (2),* 130–136.

Cheesbrough, M. (2005) District Laboratory in Tropical Countries, Part 1. 2nd Edition, Cambridge University Press, Cambridge.

Clinical and Laboratory Standards Institute (2017). Supplement M100. In Performance Standards for Antimicrobial Susceptibility Testing, 27th ed. *Clinical and Laboratory Standards Institute, Wayne, PA*, USA.

Colomer-Lluch, M. Jofre, J. & Muniesa, M. (2011). Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. *PLoS ONE, 6, e17549*.

Daniels, N. A., MacKinnon, L.C., Bishop, R., Altekruse, S., Ray, B., Hammond, R.H., Thompson, S., Wilson, S., Bean, N.H., Griffin, P.M. and Slutsker, L. (2000). *Vibrio parahaemolyticus* infections in the United States, 1973-1998. *Journal of Infectious Disease, 181*, 1661-1666.

Efiuvwevwere, B. J. O. & Amadi, L. O. (2015). Effects of preservatives on the bacteriological, chemical and sensory qualities of mangrove oyster (C, gasar). *British Journal of Applied Sciences &Technology, 5(1),* 76-84.

Eman, A. & Mushtak, T. S. (2018). Molecular detection and sequencing of SHV gene encoding for extended-spectrum β-lactamases produced by Multidrug resistance some of the Gram-negative bacteria. *International Journal of Green Pharmacy, 4 (2),* 4-10.

Fard, R.M.N, Barton M.D. & Heuzenroeder, M.W. (2021) Bacteriophage-mediated transduction of antibiotic resistance in enterococci. *Lett. Appl. Microbiology, 52*, 559–564.

FDA. (2012), Bad Bug Book Foodborne Pathogenic Microorganisms and Natural Toxins. Second Edition

Françoise, L. (2010). Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology, 27(6),* 698-709.

Garcia-Graells, C, Bottleldoorn, N and Dierick K. (2019). Microbial surveillance of ESBL *E. coli* in poultry meat, a possible vehicle for transfer of antimicrobial resistance to humans, *20(6),* 12-25.

Gourmelon, M. Montet, M. P., Lozach, S. Le Mennec, C. Pommepuy, M. Beutin, L. & Vernozy-Rozand, C. (2006). First isolation of Shiga toxin 1d producing *Escherichia coli* variant strains in shellfish from coastal areas in France. *Journal Applied Microbiology, 100*, 85-97.

Grohmann, E. (2011), Horizontal gene transfers between bacteria under natural conditions. *In: Microbes and Microbial Technology: Agricultural and Environmental Applications*. *Springer*, 8, 163–187.

Guessennd, N. Bremont S, Gbonon V, Kacou-Ndouba A, Ekaza E, & Lambert T, (2008). Qnr-type quinolone resistance in extended-spectrum beta-lactamase producing enterobacteria in Abidjan, Ivory Coast. *Pathologie-biologie, 56 (7–8),* 439–46.

Igbinosa, E.O. & Obuekwe, I.S. (2014). Evaluation of antibiogram resistant gene in abattoir environment. *Journal of Applied Sciences and Environmental Management, 18(2),* 5-9.

Inana, M. E., Ogbonna, D. N., Odu, N. N. and Amadi, L. O. (2024). Prevalence of *Campylobacter* and *Vibrio* species Associated with selected Seafoods; Oyster (Crassostra gasar), Shrimps (Caridea) and Prawn (Penaeus monodon) *International Journal of Microbiology and Applied Sciences, 3(2),* 54 – 65.

Inana, M.E., Ogbonna, D.N., and Douglas, S.I. (2019). Microbiological Quality and Antibiotic Susceptibility Profile of Microorganisms Associated with Stored Vegetables in Port Harcourt. *Microbiology Research Journal International, 29(2),* 1-10.

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.

Keen, P.L, and Patrick, D.M. (2013). Tracking change: A look at the ecological footprint of antibiotics and antimicrobial resistance. *Antibiotics*, *2*, 191–205.

Knapp, C.W, Dolfing, J, Elert PA, and Graham, D.W (2010). Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. *Environmental Science and Technology, 44(2),* 580-587.

Li, Y., Xie, T., Pang, R., Wu, Q., Zhang, J., Lei, T., Xue, L., Wu, H., Wang, J., Ding, Y., Chen, M., Wu, S., Zeng, H., Zhang, Y. & Wei, X. (2020). Food-Borne *Vibrio parahaemolyticus* in China: Prevalence, Antibiotic Susceptibility, and Genetic Characterization. *Frontier in. Microbiology*, *11*, 1670.

Lin, J. Nishino, K, Roberts, K.C, Tolmasky, M, Aminov, R.I. & Zhang, L. (2015). Mechanisms of antibiotic resistance. *Frontiers in Microbiology, 6(34),* 1-3.

Marinescu, F. and Lazar, V. (2013). Origins, transfer and accumulation of antibiotic resistance genes in the aquatic environment. *Biointerface Res. Appl. Chem*. *3*, 588–598.

Moumouni, A, Diagbouga S, Nadembèga C, Metuor Dabire A, Ouattara K, & Zohoncon T, (2017). Quinolone Resistance (qnr) genes in fecal carriage of extended Spectrum beta-lactamases producing Enterobacteria isolated from children in Niger. *Curr Res Microbiol Biotechnol*, *5(1),* 953–7.

Ogbonna, D. N. & Inana, M. E. (2018). Characterization and multiple antibiotic resistance of bacterial isolates associated with fish aquaculture in ponds and rivers in Port Harcourt, Nigeria. *Journal of Advances in Microbiology*, *10(4),* 1-14.

Ogbonna, D.N and Azuonwu, T C (2019). Plasmid Profile and Antibiotic Resistance Pattern of Bacteria from Abattoirs in Port Harcourt City, Nigeria. *International Journal of Pathogen Research*, 2(2), 1-11.

Okafor, N. (2005). Aquatic and Waste Microbiology, Enugu, Nigeria: Fourth dimension Publishing, 8-78

Onohuean, H., Okoh, A. I., & Nwodo, U. U. (2021). Epidemiologic potentials and correlational analysis of *Vibrio* species and virulence toxins from water sources in greater Bushenyi districts, Uganda. *Scientific Reports*, 17.

Onuoha, O. (2018). Distribution and Antibiogram of bacterial species in effluents from abattoir in Nigeria . *Journal of Environmental and Occupational Sciences, 77(1),* 1-8).

Saboohi, M.I., Anderson, W.B. & Huck, P.M. (2012). An evaluation of methods for the isolation of *Yersinia enterocolitica* from surface waters in the Grand River watershed. *Journal of Water and Health*, *7(3),* 392-403.

Shakoor, A., Muhammad, S. A., Kashif, M., Rehman, Z. U., Hussain, A. & Hameed, M. R. (2012). Effects of Thuja Occidentalis as an alternative remedy in the treatment of Papillomatosis in Cattle, *Veterinary World, 5(2*), 118-120.

Snyder, L. & Champness, W. (2014). Molecular genetics of bacteria third edition. Washington, D.C. ASM Press.

Threedeach, S. Chiemchaisri, W. Watanabe, T. Chiemchaisri, C. Honda, R. & Yamamoto, K. (2012). Antibiotic resistance of Escherichia coli in leachates from municipal solid waste landfills: comparison between semi-aerobic and anaerobic operations. *Bioresource Technology, 1(13),* 253–258.

Vakulenko, S.B. & Mobashery, S. (2003). Versatility of Aminoglycosides and Prospects for Their Future. *Clinical Microbiology Reviews, 16(3),* 430 – 450.

Vandamme M., Ayers, T., Mahon, B. E. & Swerdlow, D. L. (2008). Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews, 23(2),* 399–411.

VanHoek, A.A.M, Mevius, D, Guerra, B. Mullany, P, Roberts, A.P. & Henk, J. M. (2011) Acquired antibiotic resistance genes: An overview. *Frontiers in Microbiology*. *2(203),* 1-24.

Waters, A. E., Contente-Cuomo, T., Buchhagen, J., Liu, C. M., Watson, L. & Pearce, K. (2011). Multidrug-resistant *Staphylococcus aureus* in US Meat and Poultry. *Clin infect Dis, 52*, 1227-1230.

Zangoei-Fard, S., Rahimi, E. & Shakerian, A. (2020). Incidence and Phenotypic Pattern of Antibiotic Resistance of *Vibrio* Species Isolated from Seafood Samples Caught from the Persian Gulf. *European Journal of Vascular and Endovascular Surgery, 51*, 337-347.

Citation: Inana *et al*. (2024). Antibiotic sensitivity pattern and resistance genes of *Campylobacter* and *Vibrio* species associated with sea foods sold in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 78 – 87.