OPEN ACCESS International Journal of Applied Biology

ISSN : 2580-2410
eISSN : 2580-2119

Multidrug Resistant *Vibrio* Species Isolated From Abattoir and Aquaculture Environment in Ebonyi State, Nigeria

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Abstract

Vibrio species in abattoir and aquaculture environment are of human health significance and may be increasing in pathogenicity and abundance. This study was aimed at determining the multidrug resistance of *Vibrio* species isolated from abattoir and aquaculture environment in Ebonyi state. Effluent samples were obtained from different abattoir sites within Ebonyi State from the butchers' table, drainage, and wash water, while aquaculture sample were collected from different pond sites within the study area. *Vibrio* isolates were detected using Thiosulphate Citrate Bile Salt (TCBS) agar and identified based on the morphology and biochemical characterization. The results revealed a total of 50 isolates from 20 waste water samples. Waste water samples from different points of drainage water from abattoir had the highest microbial load of $3.85\pm0.35x10^6$ CFU/mL. While, samples from butcher's table and wash water from abattoir had high microbial load of $3.30\pm0.14x10^6$and 3.25 ± 0.14 $x10^6$ CFU/mL. respectively when compared to aquaculture samples which had the least microbial load of 0.6 ± 3.9 $x10^6$ CFU/mL. Percentage antibiotic susceptibility profile showed *Vibrio* isolates were highly resistant to Tobramycin 40(80%), Cefoxitin 40(80%), amoxicillin/clavulanic acid 40(80%), Meropenem 30(52%), Cefepime28(56). Abattoir effluents and aquaculture are important reservoirs of multidrug resistant *Vibrio* pathogens. This implies that abattoir effluents could be important contributors to the episodes of epidemic cholera, non-*Vibrio* cholera and *Vibrio* causing illnesses in the study area

Article History
Received November 18, 2021
Accepted June 14, 2022

Keyword
Aquaculture;
Abattoir;
Vibrio species;
Ebonyi State

Introduction

Vibrio is a genus of Gram-negative, curved-rod bacteria, some species may cause food-borne infection which are typically associated with eating undercooked seafood. Usually, *Vibrio* species are found in salt water and are facultative anaerobes that test positive for oxidase and do not form spores (Thompson *et al.,* 2015).Various *Vibrio* species are pathogens. Gastroenteritis is associated with some disease-causing strains, but they can also infect open wounds and cause sepsis. Many marine animals may carry them, such as

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crabs or prawns, and during exposure they are known to cause fatal infections in humans. The specific pathogenic species of *Vibrio* are *V. cholerae, V. parahaemolyticus and V. vulnificus* (Quilici and Robert-Pillot, 2011).

An abattoir is a special facility designed and licensed for receiving, holding, slaughtering, and inspecting meat animals and meat products before release to the public (Witus and Vun, 2016). Abattoir inspection of live animals (ante-mortem) and carcasses (post-mortem) is critical to surveillance for animal diseases and zoonoses (Amini *et al.,* 2011).

Aquaculture, also known as aquafarming, is the farming of fish, crustaceans, molluscs, aquatic plants, algae, and other organisms (Nordstrom *et al.,* 2009). Nigeria's aquaculture and abattoir industries are primarily related to their economic gains from supplying domestic and foreign demands and producing steady income for farmers. Fish and meat are among the most common protein products that consumers from African countries consume on a daily basis. Approximately 75% of global fish and meat production is primarily for human consumption (Teh, 2012). Fish consumption in Nigeria has risen since 1970 and now exceeds 40 kg / capita / year (Hajeb et al., 2009). These products have a healthy diet, such as high protein levels, omega-3 fatty acids (n-3), essential vitamins and minerals that an individual requires. The growth and strength of these industries (aquaculture and abattoir) has contributed to immune systems suppression and increases the vulnerability of fish and meat to bacterial infections (Finlay and Falkow, 2010).

Antibiotic-resistant infection has become more difficult to treat with existing antibiotics, leading to infections that lead to increased morbidity and mortality, resulting in huge societal costs. Some human pathogens, including *Vibrio* species, are involved in this increasing resistance. Multidrug-resistant (MDR) bacteria have been a major health issue in medically used antibiotics and a major challenge to drug discovery programs around the world. It is well documented that there are antibiotic resistance traits in both clinical and environmental strains of *Vibrio* (Koelle *et al.,* 2016).

Vibrio species will serve as a reservoir of antibiotic resistance genes for the environment. *Vibrio* species, independent of sero-group, has been found to have a plastic genome and a long history of active plasmid interaction. In other words, *Vibrio* species may be able to acquire and exchange genes through either integrons or integrative and conjugative elements (ICE) such as the SXT element, due to its genetic characteristics.(Ceccarelli *et al.,*2013).

The problem of antimicrobial resistance among the agent continues to be alarming. There is a growing global occurrence of *Vibrio* spp in aquaculture and abattoir and the strains has been known to be resistant to various antibiotics. Despite some studies on diarrhoeal diseases in Nigeria, there is lack of adequate information on bacterial enteric pathogens and their antimicrobial resistance trend within the study area. This study will determine the best suitable antibiotics for the treatment of *Vibrio* spp especially within the study area.

Materials and Methods

Sample Collection

Samples used in this study were obtained from different selected abattoir sites in Ebonyi State viz: Ezzamgbo, Effium, Ngbo, New market, Kpirikpiri and Meat market all in Ebonyi State. The samples were obtained from the butchers' table, drainage sites and

washwaters, while, the Aquaculture samples were collected from selected aquaculture pond sites in different localities in Ebonyi State such as Josel fish pond Nkaliki, Matthew pond in Izzi, Onuebonyi Abakaliki, Obodo pond in Ezzamgbo, and Ifeanyi pond in Ngbo. Samples were collected using sterilized bottles and were preserved in peptone water for 24 hours for enrichment before analysis.

Sample Processing

Five (5 ml) of freshly prepared peptone water was dispensed into test tubes and were sterilized at 121 ℃ 15 psi for 15 minutes. Thereafter, one (1 ml) each of the samples were aseptically weighed into the sterile peptone water and were incubated for 24 hours for enrichment of the whole sample.

Isolation of *Vibrio* Species

One (1 ml) each of the enriched samples was aseptically measured into test tubes containing 9 ml of sterile distilled water and shaken thoroughly for even distribution of organisms to make a stock. Thereafter, ten-fold serial dilution of the samples were carried out and subsequently inoculated on a freshly prepared nutrient agar plates and were incubated at 37 ℃ for 24 hours. After 24 hours of incubation, some colonies were transferred to Thiosulphate Citrate Bile Salt (TCBS) agar plates based on their colour and shape under aseptic condition with the aid of a wire loop and incubated at 37 ℃ for 24 hours. After 24 hours, colonies of *Vibrio* in Thiosulphate Citrate Bile Salt agar showed yellowish colour. The colonies were picked, sub-cultured and subsequently identified using standard microbiological procedure.

Antibiotics Sensitivity Testing

Susceptibility test was done on Muller Hinton Agar (Oxoid, UK) plates by standard disk diffusion method in conformity to the recommended standard of Clinical and Laboratory Standard Institute (2005). The antibiotic disks used include; imipenem (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), cefeprime (30 µg), meropenem (10 µg), tobramycin (10 µg) ceftazidime (30 µg) and amoxicillin clavulanic acid (30 µg). All the antibiotics disk were procured from Oxoid limited (Oxoid, UK). These antibiotics were chosen either because they are used in both medicine and human veterinary practice or as a result of previous studies with reports of microbial resistance to them. Colonies of confirmed *Vibrio* isolates were collected using wire loop and were dispensed into test tubes containing 5 ml distilled water. The cell concentration was adjusted to 0.5 MacFarland standard. Sterile swap stick was used to collect the organisms and these were streaked on freshly prepared Mueller-Hinton agar plates. The plates were allowed to stand for 15 minutes so that the cells will adapt to the environment of the medium. After this, the standard antibiotic disks were placed 15 mm apart and was incubated at 30^{o}C for 24 hours and the zones of inhibition diameter was measured according to CLSI criteria (2005).

Determination of Multiple Antibiotic Resistance Index (MAR)

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR = a/b, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility. MARI of relative ratio >1 is shown to represent potential risk source of resistant strain from the environment. If MAR index value

is between 0.200 and 0.250 it becomes a very risky case where there are equal chances that MAR may fall in the high risk and low risk phases (Raiz *et al*, 2011).

Statistical Analysis

Experimental data was presented as mean±standard deviation, while one way ANOVA procedure will be used to analyze statistical difference in the data generated.

Results

Microbial Load of The Isolated *Vibrio* Species from Different Aquaculture and Abattoir Samples

The result as represented on Table 1 revealed that a total of 50 isolates of *Vibrio* were isolated from 20 waste water samples. Samples from drainage water from abattoir had the highest microbial load of $3.85\pm0.35x10^6$, followed by butchers table, $3.30\pm0.14x10^6$, while wash water from Hartchery had the least microbial load of $1.21\pm0.33\ x10^6$. For aquaculture; samples from concrete pond had highest microbial load of $2.2\pm3.4x10^6$ from Ebonyi farm when compared to earthen pond which had the least microbial load of $1.0\pm3.0\ x10^6$ from Chiboy farm. Hence in comparison of the waste water samples, abattoir samples from drainage water from Ezzamgbo had the highest microbial load of 3.85±0.35x106, while aquaculture samples from earthen pond had the least microbial load of$1.21\pm0.33\ x10^6$.

Table1. Microbial load of *Vibrio* species obtained from aquaculture and abattoir samples

Aquaculture Fish Pond	CHIBOY	OBODO	CHUKS	JOSEL	EBONYI	CHALI
Concrete	2.0±1.4	1.5±4.2	1.6±4.0	0.6±3.9	2.2±3.4	12.0±0.96
Earthen	1.0±3.0	1.5±0.6	1.7±04.1	1.5±0.6	1.22±2.9	19.9±0.65

Abattoir	WW	BT	DW
New market	2.10±1.55	3.30±0.14	2.12±0.03
Kpiripkiri	3.25±0.14	1.97±0.41	2.46±0.23
Meat market	2.72±0.70	1.85±0.64	2.36±0.90
Ezzamgbo	2.30±0.12	2.13±1.23	3.85±0.35
Jemenny	2.60± 0.22	2.10±1.30	2.22±0.10
Ishieke market	2.85±0.35	1.90±0.14	2.40±0.42
Amike-Aba	2.87±0.21	2.13±1.65	2.46±0.48
Eke-Aba	2.87±0.21	1.48±0.64	2.67±0.40
Abofia market	2.70±0.42	1.75±0.43	2.32±0.38
Azugwu market	2.70±0.22	2.12±1.12	2.56±0.26

Umuoghara	2.22±0.10	2.20±1.31	2.40±1.13
Ogoja road	2.87±0.21	1.34±0.11	2.34±1.12
Hartchery	1.21±0.33	2.22±0.10	2.40±1.13
Ngboagbaja market	2.72±0.70	1.47±0.21	2.52±1.03

KEY: BT = Butcher's table, WW = Wash water, DW = Drainage water, CFU = Colony forming unit, mL = milliliter

Distribution of *Vibrio* Species Isolated from Samples from Abattoir and Aquaculture Farm

The result of occurrence and distribution of *Vibrio* species is represented in (Table 2). The results reveal that waste water samples from butchers table showed highest percentage distribution 15(50.00%) from different locations than drainage water 8(26.68%), while wash water had the least percentage distribution 7(23.33%).

Aquaculture samples showed highest percentage distribution 12(60%) among *Vibrio* species from earthen pond while concrete pond showed least percentage distribution 8(40%).

Table 2. Distribution of the isolates from samples from abattoirs and aquaculture farms

Abattoir	New market	Kpirikpiri	Ezzamgbo	Jemenny	Meat market	**Total (%)**
Drainage	2 (6.67)	0 (0)	2 (6.67)	2 (6.67)	2 (6.67)	8 (26.68)
Washwater	2 (6.67)	2 (6.67)	1(3.33)	1(3.33)	2 (6.67)	7(23.33)
Butchers table	3 (10.0)	4 (13.33)	5 (16.67)	0 (0)	3 (10)	15(50.0)
Total	6 (20)	3 (10)	6 (20)	7 (23.33)	8 (26.67)	**30 (100)**

Fish pond	**Chiboy**	**Obodo**	**Chucks**	**Jossel**	**Hartchery**	**Ebonyi**	**Total**
Concrete	1 (5)	0(0)	1 (5)	2 (10)	1 (5)	1 (5)	8 (40)
Earthen	2 (10)	2 (10)	1 (5)	3 (15)	2 (10)	1 (5)	12 (60%)
Total	3 (15)	2 (10)	2 (10)	5 (25)	3 (15)	2 (10)	**20 (100)**

KEY: WW = Wash water, DW = Drainage water, BT= Butcher's table, % = Percentage, CP = Concrete pond, EP = Earthen pond, % = Percentage.

Antibiotics susceptibility pattern of the *Vibrio* species to the commonly used antibiotics

Table 3 shows the result of the antibiotics susceptibility of *Vibrio* species to the respective antibiotics used. The results reveal that the isolates were highly resistant to FOX, TOB, AMC, 40(80%) respectively. Some of the isolates showed slight resistance to FEP and MEM, 28(56%) and 30(52%) respectively, while lesser percentage of the isolates showed least resistance to IPM and CAZ at 12(24%) respectively. Meanwhile, the susceptibility pattern revealed that the isolates had highest susceptibility to IPM and CAZ,38(76%) respectively while few were slightly susceptible to AMC, TOB, FOX, 10(20%) respectively.

Table 3. Antibiotics susceptibility pattern of the isolates to the used antibiotics.

Antibiotics	Resistance(%)	Intermediate(%)	Susceptibility(%)	Total (%)
IPM	12(24)	0(0)	38(76)	50 (100%)
FOX	40(80)	0(0)	10(20)	50 (100%)
CTX	10(20)	5(10)	35(70)	50 (100%)
FEP	28(56)	10(20)	12(24)	50 (100%)
TOB	40(80)	0(0)	10(20)	50 (100%)
MEM	30(52)	5(10)	15(30)	50 (100%)
CAZ	12(24)	0(0)	38(76)	50 (100%)
AMC	40(80)	0(0)	10(20)	50 (100%)

KEY: IPM = Imipenem, FOX = Cefoxitin, CTX = Cefotaxime, FEP = Cefepime, TOB = Tobramycin, MEM = Meropenem, CAZ = Ceftazidime, AMC = Amoxicillin/Clavulanic acid, % = Percentage.

Multiple Antibiotics Resistance of *Vibrio* Species

Table 4 reveals the results of the respective drugs resistances of the isolated *Vibrio* species with respect to their different samples collection sites. Multiple antibiotics resistance (MAR) index is a tool that reveals the tendency of an organism to form resistance to more than two antibiotics.

From the results, *Vibrio* species obtained from drainage water had the least MAR indices of 0.1 and 0.2, while butcher's table and wash water had the highest MAR indices of 0.4, 0.5 and 0.6 respectively when compared to aquaculture samples which were highest with multiple antibiotics resistance indices of 0.6 and 0.7. The isolates had high multiple resistance to IPM, FOX, TOB, MEM, CAZ and FEP at a MAR index of 0.7, while they showed lower multiple resistance to AMC at a MAR index of 0.1.

Table 3. Multiple antibiotic resistance index of the isolates from abattoir and aquaculture.

Sample point	Mari (n/n)	Antibiotics
Chiboy Fish farm	0.6	FOX CTX TOB MEM AMC
Ogbodo fish farm	0.6	FOX CTX TOB CAZ AMC
Chucks Fish farm	0.6	FOX CTX TOB CAZ AMC
Jossel Fish farm	0.7	IPM FOX TOB MEM CAZ AMC
Ebonyi Fish farm	0.6	FOX FEP TOB MEM AMC

Jemenny abattoir	0.4	FOX TOB AMC
Kpirikpiri market	0.5	FOX TOB MEM AMC
Meat market abattoir	0.6	FOX FEP TOB MEM AMC
New market abattoir	0.4	FOX FEP AMC
Hartchery abattoir	0.2	FOX AMC
Amike-Aba abattoir	0.2	IPM CTX
Umuoghara abattoir	0.1	AMC
Eke-Aba abattoir	0.2	IPM CTX
Ogoja Road abattoir	0.2	IPM CTX
Hill-top abattoir	0.4	FOX FEP AMC
Azu-ugwu abattoir	0.5	FOX TOB MEM AMC
Ezzamgbo abattoir	0.5	FOX CTX FEO MEM
Ngboagbaja abattoir	0.4	FEP TOB MEM

KEY: IPM = Imipenem, FOX = Cefoxitin, CTX = Cefotaxime, FEP = Cefepime, MEM = Meropenem, CAZ = Ceftazidime, AMC = Amoxicillin/Clavulanic acid, MARI = Multiple antibiotics resistance index, n = Number of the antibiotics that the isolates were resistant to, N = Total number of all the tested antibiotics.

Discussion

Vibrio species in abattoir and aquaculture environment are of human health significance and may be increasing in pathogenicity and abundance. *Vibrio* illness originating from dermal contact with *Vibrio* laden waters or through ingestion of seafood originating from such waters and abattoir effluent can cause deleterious health effects, particularly if the strains involved are resistant to clinically important antibiotics. The purpose of this study was to evaluate antimicrobial resistance pattern among these pathogens.

The results revealed that a total of 50 different isolates of *Vibrio* species were gotten from aquaculture and abattoir samples within the study area. The result of the microbial load revealed that *Vibrio* species were highest in microbial load among the waste water samples from drainage, while those samples from butchers table and wash water had lower microbial load when compared. Although samples from aquaculture showed slight difference from those abattoir samples, the result revealed that abattoir and aquaculture is a reservoir for *Vibrio* species which collaborated the work of Baumann and Schubert (2005) Who have advance hypothesis that aquaculture settings serve as foci or reservoir for pathogenic *Vibrio* strains, during certain period of the year. Pathogenic *Vibrio* would withstand environmental conditions within aquaculture settings and when favourable environmental conditions established, *Vibrio* would be able to cause disease in wild animal (Deepanjali *et al*., 2005).

Also, the result revealed that samples from aquaculture had lower percentage distribution while samples from abattoir effluent had higher percentage distribution which is in agreement with the report of (Costa *et al*., 2015), which suggested that the detection

of virulence and invasive *Vibrio* isolates in abattoir effluent indicates that abattoir effluent is an important repository of pathogenic *Vibrio* species; and could be a considerable contributor to the recurrent episodes of epidemic cholera and other non-*Vibrio* cholerae outbreaks in certain parts of Nigeria localities.

A total of 50 *Vibrio* species (N=50) were tested against eight commonly used antimicrobials in this study. The isolates exhibited remarkable sensitivity to Imipenem, cefepime, meropenem and ceftazidime (Table 3), in agreement with the report of Chiang and Chuang (2003) who observed that imipenem and the cephalosporins, including ceftazidime were effective against *Vibrio* infections. However, contrary to the submission of Chiang and Chuang (2003), some species in this study exhibited reduced sensitivity and resistance to cefotaxime (20%). It is noteworthy that aquaculture and abattoirs environment within Ebonyi State has shown to be a reservoir of antibiotics resistant bacteria. A study conducted by Onuoha *et al*, (2016a, 2016b, 2017) in both abattoir and aquaculture environment in Ebonyi State showed high multi-drug resistance which is widespread among majority of the bacteria studied. Also, Igbinosa *et al*., 2009 reported considerable resistance of *Vibrio* isolates from municipal wastewater against ampicillin, trimethoprim, and trimethoprim/sulphamethoxazole in South Africa; while Marin *et al*., 2013 documented resistance against trimethoprim and trimethoprim/sulphamethoxazole amongst clinical *Vibrio* strains isolated from different parts of Nigeria. Strains of *Vibrio* tested in this study (except few) were generally resistant to tobramycin, amoxicillin and cefoxitin (80%) in agreement with reports from Tanzania and Rwanda, but contrary to reports from Kenya, South Sudan, South Africa and Somalia, Materu *et al*., (1997). This might be due to the use of this antibiotic for long period of time in the community because it is relatively cheap and easily availability.

More so, of the 50 isolates tested for antibiogram against 8 different antibiotics, the highest multiple antibiotics resistance index of the isolated *Vibrio* spp. was shown by the *Vibrio* species from aquaculture samples from earthen pond (0.7) which is in contrast with what was obtained by Igbinosa *et al.,* (2019). While butcher's table and wash water had lower MAR index when compared to aquaculture samples. Consistent with the observation of this study, Onuoha *et al,* 2016b reported that bacteria isolated from their study were completely resistant to tetracycline, cephalothin, Penicillin G, and erythromycin with MARI values above 0.20 with the highest values in the range of 0.90, which was higher than what was observed in this study. Also, Igbinosa *et al,* 2019, reported that the percentage of isolates exhibiting MAR was (10 - 20%) which was relatively higher than that of (0.7) as observed in this study. The isolates had high multiple resistance to imipenem, cefoxitin, tobramycin, meropenem, ceftazidime and cefepime, while they showed lower multiple resistance to amoxicillin. Hence, aquaculture and abattoir effluents are considered to be one of such high-risk sources of contamination since they are associated with waste from livestock, which are often feed containing antibiotic additives. Residual antibiotics that enter the environment with abattoir waste effluent have been reported (Kümmerer, 2003) to exert selective pressure on microbial populations contained therein, thereby enhancing MAR, as observed in this study.

Conclusions

Although abattoir and aquaculture effluents have been reported to be important environmental reservoirs for *Vibrio* species, there is a dearth of information in the literature on antibiotic susceptibility patterns of *Vibrio* species isolated from aquaculture and abattoir

effluents in Abakaliki, Ebonyi state. Isolates of the current study exhibited remarkable sensitivity to imipenem, meropenem, cefotaxime and cefepime. The MAR index from this work indicated that *Vibrio* species were highly resistant to most of the commonly used antibiotics, which is a clear indication that these microorganisms are rapidly developing resistance to the commonly used antibiotics.

Funding Information

The authors received no specific funding for this work.

Author Contribution

OSC.; Conceptualization, Methodology, Formal Analysis, Resources, Writing (Reviewing & Editing), Supervision, OFN; Project Administration. OCO; Formal Analysis and Investigation, OKN; Resources and Visualization.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

We wish to acknowledge the owners of the various fish bonds and abattoir workers for granting us accesses to collect samples for the work.

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