Evaluation of Antibiogram Profile of *Vibrio cholerae* Isolates from Sea Foods and water samples from Cross River State, Nigeria

ABSTRACT

Aim: To evaluate the antibiogram profile of *Vibrio cholerae*[*V. cholerae]* strains isolated from Cross River State environment.
Study design: The cross-sectional study was a completely Randomized design.
Place and Duration of Study:This was conducted in the Department of Microbiology University of Cross River State Calabar, Nigeria, between 2022 and 2024.
Methodology: A total of 1,155 *V. cholerae* strains were isolated from water samples and sea foods from the North, Central *and* Southern geographical zones of C RS.A total of 30samples were assessed from three different locations in each geographical zone,for the presence of *V. cholerae* strains using conventional culture methods and isolates identified bio/serologically with commercial polyvalent and monovalent antisera. The Clinical and Laboratory Standards Institute (CLSI) protocols, were implored, for testing the susceptibility of the isolates to 10 commercially used antibiotics.
Results:*A total of 529±9.90 (45.8%) of the isolates showed resistance to Augmentin 30µgand 83±2.92 (7.17%) showed resistance to Gentamycin 10 µg. The overall percentage mean resistance by source, showed that the lowest resistance was from Cray fish (44.26±18.422%), and the highest was from Periwinkle (2.17±6.10%). It was also observed that the isolates from Ikom were the most resistant, with, 31.93±25.41%, followed by Calabar with 24.54± 19.43% and Obanlikwu, the least with 9.07±18.80%. Statistically, significant differences were observed in the resistance pattern of the isolates from the different sources and locations, with significant values of .00 respectively (P=.05)*
Conclusion: *There are great Chances that human infections, caused by these environmental* V. cholerae *strains can arise from contacts with these environmental sources. There is therefore, a need to carry out a surveillance on these MDR V. cholerae strains to help curb any eventual case of cholera outbreak of in the state.*

Keywords: Antibiogram profile, V. cholerae, CRS environment.

1. INTRODUCTION

Antibiotic resistance is no longer a misnomer as the reports of multiple drug-resistance [MDR] due to circulating virulent strains of bacteria are continually recorded globally. Since life is not a constant, bacteria also continue to portray some changes in their chemical compositions, due to the acquisition of novel/foreign characteristics [including resistance genes] through lateral and horizontal gene transfer.

The evolution of these new stains with novel characteristics, has promoted the quest for bioprospecting and development of new alternative solutions to curb infections and diseases originating from MDR strains of microorganisms. However, this still, has not proffer the solution to MDR, because, it is seemingly observed that resistant strain development is increasing with the increase in the new drugs produced. For instance; Salversan and Penicillin which were

the first drugs to be implored as remedies for Syphilis and *Staphylococcus aureus* infections, respectively [1], have long ago been replaced by many new drugs for the same purpose. These new alternatives also, have witnessed increasing resistance at some level of the other [2]. This is serious global challenge, which is antecedent to some of the great knots (treatment failures) encountered by the health care management of cholera cases [1, 3, 4, 5].

In this trouble of multidrug resistance, *strains* of *V. cholerae* isolated from sea foods, have not been left out. There are many documented evidences from several parts of the world attesting to this fact. The pointers are tilting towards the selective pressure from antibiotics in the environment (from Farmlands, pesticides, insecticides, animal feeds etc) and from excessive use and misuse of antibiotics [3, 4, 5, 6,7].

The inhabitants of Cross River State (CRS), are sustained primarily by the products from the water bodies that surround the Senatorial Districts. These water bodies that serve the diverse populace are prone to pollution by the same populace. Yet, microbiologically, little is known about them.

The aim of this research therefore, was to evaluate the antibiogram profile of *Vibrio cholerae* (*V. cholerae)* strains isolatedfrom CRS environment.

2. MATERIALS AND METHODS

2.1 Study Area and Bacterial Strains

The three geographical zones of CRS (North, Central and Southern Zones were the sample collection locations and

the Department of Microbiology in the University of Calabar, CRSN, was the evaluation center where the research was done.

2.2 Sample Collection and Processing

The protocol described by Dixit *et al.* [8], was used for this study. A total of 30 samples each, were collected from three different local Government areas in the different geographical zones of CRS. Live and smoked seafood samples (crabs, crayfish, lobsters, fish etc were purchased from different markets and beaches in the North, Central and Southern geographical zones of CRS in to sterile closed caped containers.They were dissected with sterile knife to remove the digestive tracts and gills, which were then homogenized in a sterile mortar. Then 45 ml of Alkaline Peptone Water (APW+ 1MNaOH pH-8.4) was added to the homogenate and mixed thoroughly before incubating for about 6–8 h, at 37^{o}C. After this, the samples were ready for further tests.

The water samples were collected from rivers, streams, and waste waters from the areas of study using clean sterile syringes into sterile screw caped bottles and transported to the laboratory in ice bags.

2.3 Determination of Viable Counts and Isolation of Vibrio Strains

About 1ml of the previously incubated homogenized sea food and water samples were then added to 9 ml of sterile Alkaline Peptone Water (APW) + 1MNaOH pH-8.4, and diluted ten-fold with the same solvent (APW). Then about 0.1 ml of each diluted sample from each test tube was transferred onto duplicate plates. Then already previously sterilized Thiosulphate Citrate Bile Salt Agar (TCBS) agar, cooled to about 45-50^{o}C was added int the samples in the plates and swirled for even distribution. They were then incubated overnight at 37^{o}C and checked for the appearance of green / or yellow colonies, which were presumed to be other species of vibrio / or *V. cholerae.* Any emergent discrete colonies were immediately isolated and sub-cultured for purification of the strain. Stock cultures were then prepared from the pure isolates using nutrient agar slants and store in the refrigerator for further identification [8].

2.4 Identification and Characterization of *V. cholerae* Strains from the Environment

Culturally, physiologically morphologically and biochemically, the pure isolates from the stock culture bottles were identified and characterized. Cultural feature of each isolate such as the size and shape of the colony, its elevation and colour were noted. The morphological appearance on gram-stained slides and the motility in distilled water were also recorded. The biochemical tests that were used for identification and characterization of isolates include, catalase, sugar utilization citrate utilization, starch hydrolysis, hydrogen sulphide, indole and urease production as well as the Voges Proskauer and salt tolerance test at 0, 3, 6, 8 and 10% concentration.

2.4.1 Serological identification

The presumptively identified isolates were Sero-grouped with specific polyvalent antisera for *V. cholerae* O1 and O139 (ANTEC, UK) and further screened with specific monoclonal antibody for both O1 and O139 serogroups. Then the anti-Ogawa, and anti-Inaba polyvalent antisera (BioRad, USA), was also used for the characterization of the positive isolates into the different serotypes. The slide agglutination method described by Eyisi*et al*. (9), was employed in this test. Already identified *V. cholerae* O1 Ogawa strain and *Escherichia coli* strains from Professor C.U. Iroegbu's Microbiology Research laboratory in Cross River University of technology Calabar were used as positive and negative controls for internal quality control.

2.4.2 Bio-typing of *V. cholerae* Strains from the environment

2.4.2.1 Plate hemolysis

Approximately, 5% to 10% of sheep erythrocytes were used to prepare blood agar in nutrient are base which was used to inoculate the presumptively identified *V. cholerae* O1 colonies from and overnight growth, and incubate at 35^{o} to 37^{o}C for 18 to 24 hours. The appearance of any observed clear zones of hemolysis around the colonies was indicative of the presence of *V. cholerae* O1[10].

2.4.2.2 The direct hemagglutination

For three times, approximately 25 ml of 0.01 M, phosphate-buffered saline (PBS), pH 6.8-7.2 was used to wash 20 ml of chicken erythrocytes before they were resuspended in normal saline, (2.5% vol/vol). Thereafter, the test bacteria from a 24-hour growth culture were added to a loopful of the red cell suspension and mixed, on a microscope slide. Any observed agglutination of the red cells within 30 to 60 seconds was then noted as *V. cholerae* O1 El Tor strain present. If no hemagglutination was observed within the said period of time, this was indicative of the presence classical strain of *V. cholerae* O1. Known control *V. cholerae* strains were always included with every new suspension of red cells in every batch of test performed [10].

2.5 Polymyxin B, O/129 (vibrio-static compound) sensitivity and Antibiotic Susceptibility Testing of *Vibrio cholerae* Isolates by Disk Method:

The standard susceptibility testing protocol described by the Clinical and Laboratory Standards Institute [11,12], was implored. Polymyxin B, O/129 (vibrio-static compound) and ten commercial antibiotic discs were used.

The test was carried out on Mueller Hinton Agar according to the manufacturer's instructions. Prepared agar was poured into sterile Petri dishes and allowed to solidify. The plates were kept to dry and checked for sterility the next day. Each isolate was aseptically transferred with an inoculating wire loop in to 5 ml of Normal saline. This was mixed vigorously and allowed to stay for 15 seconds and compared to 0.5 MacFarlane Standard. Then a sterile cotton swab stick was immersed into the standardized inoculum and excess removed by gently pressing the soaked swab against the wall of the tube.

The swab was then used to aseptically streak the entire surface of the plate containing the sterile Mueller Hinton agar for few seconds. The inoculated plates were allowed to stand on the bench for a few minutes. Thereafter, Gram negative antibiotic disk containing Septrin 30µg (SXT), Chloramphenicol 30µg (CH), Pefloxacin 10 µg (SP), Ciprofloxacin 10 µg (CPX), Amoxycillin 30µg (AM), Augmentin 30µg (AU), Gentamycin 10 µg (CN), Pefloxacin 30µg (PEF), Ofloxacin 10 µg (OFX), Streptomycin 30µg (S),were placed on the culture plate using a sterile forceps. The Polymyxin B and O/129 (vibrio-static compound) discs were also included in to separately prepare and seeded plates. Then all the plates were incubated at 37^{o}C for 24 hours. The zones of inhibition were observed and measured using a graduated ruler. The susceptibility profiles were interpreted by measuring the inhibition zone diameters and comparing them with a standard chart to determine the sensitivity of each of the isolates to each antibiotic. The test was carried out in duplicates and the mean zone diameters were recorded. Positive and negative controls were included with each batch of the test.

3. RESULTS AND DISCUSSION

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3.1 Results

3.1.1 The total number of *V. cholerae* isolates that showed susceptibility to different antibiotics

The total of 1,155 *V. cholerae* isolates from the different sources in CRS, were subjected to ten different commercial antibiotics. 1,072±16.70 (92.81%) showed susceptibility to CN, 1,056±16.49(91.43%) to OFX, 885±12.82(76.62%) to PEF, 876±12.83(75.84%) to AM, 865±14.01 (74.89%) to SXT, 857±13.06 (74.19%) to CPX, 830±11.83(71.86%) to S, 764±10.87 (66.15%) to SP, 725±11.50 (62.78%) to CH, and 626±9.74(54.19%) showed susceptibility to AU. On the whole, the results showed that the effects of the antibiotics on *V. cholerae* isolates were such that CN>OFX> PEF > AM > SXT > CPX > S >SP>CH>AU (Table 1).

3.1.2 Overall percentage of *V. cholerae* isolates resistant from different antibiotic agents tested

The total number of *V. cholerae* isolates that showed resistance to AU were 529±9.90 (45.80%), 430±7.78 (37.23%) to CH,391±8.49 (33.85%) to SP, 325±7.87 (28.14%) to S, 298±6.01 (25.80%) to CPX, 290±5.69 (25.11%) to SXT, 279±5.87 (24.16%) to AM, 270±6.06 (23.38%) to FEF, 99±3.13(8.57%) to OFX and, 83±2.92 (7.17%) showed resistance to CN. (Table: 2).

Table 1: The Total Number of *V. cholerae* Isolates that showed Susceptibility to Different Antibiotics

Treatment	Mean	N	Std. Deviation	Sum
SXT	10.68	81	14.01	865.00
CH	8.95	81	11.50	725.00
SP	9.43	81	10.87	764.00

CPX	10.58	81	13.06	857.00
AM	10.81	81	12.83	876.00
AU	7.73	81	9.74	626.00
CN	13.23	81	16.72	1072.00
PEF	10.93	81	12.82	885.00
OFX	13.04	81	16.49	1056.00
S	10.25	81	11.84	830.00
Total	10.56	810	13.18	8556.00

SXT= Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin10 µg, AM= Amoxycillin 30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg

Table 2: Overall Percentage Means of *V. cholerae* Isolates Resistant to Different Antibiotic Agents Tested

Treatment	Mean No of isolates	Std. Deviation	Sum
SXT	3.58	5.69	290.00
CH	5.31	7.78	430.00
SP	4.83	8.49	391.00
CPX	3.68	6.01	298.00
AM	3.44	5.87	279.00
AU	6.53	9.90	529.00
CN	1.02	2.92	83.00
PEF	3.33	6.06	270.00
OFX	1.22	3.13	99.00
S	4.01	7.87	325.00
Total	3.69	6.86	2994.00

SXT=Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin 10 µg, AM= Amoxycillin30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg

3.1.3 Antibiotic susceptibility pattern of *V. Cholerae* isolates from different sources

Out of the 236 *V. cholerae* isolates from Cray fish, 169(71.61%) were susceptible to AM, 156 (66.10) to CN and OFX, 144(61.02%) to PEF, 143(60.59%) to CPX, 132(55.93%) to SXT and S respectively, 114(48.3) to SP, 96 (40.68%) to CH and the least (72)30.51% were susceptible to AU. For the isolates from the apple Snails 72(53.73%) out of 134 were susceptible to AU and 134(100 %) to CN.

The isolates from Periwinkle showed a susceptibility pattern ranging from 122(93.855%) out of 130, for SP and AM each, -130(100%) for CN and OFX each.

The Blue crab had 82 isolates from which 38(46.34%) were susceptible to AU, while all 82(100%) showed susceptibility to CN and OFX each.

Lobster isolates showed a range from 58(50%) for AU-116(100%) for CN and OFX each. For those form Fish, the range was from 93(66.91%) for AU-100.00% for OFX and CN respectively. For the River/stream Water isolates 53(60.23%) were susceptible to CH and 88 (100.00%) were susceptible to OFX. The range for the isolates from the gutter water was from 33(54.09%), for CH- 60(98.36%) for CN. Lastly Isolates from Sea water showed a range from 76 (44.97%) for SP -169(100%) for CN and OFX (Table 3).

Comparing the effect of the treatment on the isolates, it was observed that there were significant differences in the response of the isolates from the different sources with a significant F- Calculated value of 51.96, significant at .000 (P<.05). The treatment effect on the various isolates was also significantly different with an F-Calculated value of 4.82, significant at .00 (P<.05) The interaction Between the Source and Treatment was not significant F- Cal= .47, sig value of 1.00>.05

Table 3: The Number/ Percentage of *V. Cholerae* Isolates from Different sources in Cross River State Susceptibility to different Antibiotic Pattern

Sample	TNI	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Crayfish	236	132/55.93	96/40.68	114/48.3	143/60.59	169/71.61	72//30.51	156/66.10	144/61.02	156/66.10	132/55.93
Apple snail	134	102/76.12	78/58.21	90/67.16	95/70.89	93/69.40	72/53.73	134/100	102/76.12	120/89.55	108/80.59

Periwinkle	130	127/97.69	125/96.15	122/93.85	125/96.15	122/93.85	124/95.38	130/100	128/98.46	130/100	128/98.46
Blue crab	82	66/80.49	70/85.37	60/73.17	56/68.29	60/73.17	38/46.34	82/100	70/85.37	82/100	70/85.37
Red lobster	116	94/81.03	72/62.06	83/71.55	78/67.24	89/76.72	58/50	116/100	106/91.38	116/100	94/81.03
Fish	139	116/83.45	104/74.82	98/70.50	108/77.69	90/64.75	93/66.91	139/100	119/85.61	139/100	102/73.38
Sea Water	169	123/72.78	94/55.62	76/44.97	118/69.82	111/65.68	69/40.83	169/100	107/63.31	169/100	80/47.34
River/stream Water	88	67/76.14	53/60.23	65/73.86	79/89.77	81/92.05	53/60.23	86/97.72	60/68.18	88/100	76/86.36
Gutter Water	61	38/62.29	33/54.09	56/91.80	55/90	61/100	47/77.05	60/98.36	49/80.33	56/91.80	40/65.57
Total	1155	865/74.89	725/62.77	764/66.15	857/74.19	876/75.84	626/54.19	1072/92.81	885/76.62	1056/91.43	830/60.43

TNI= Total Number of Isolates, SXT=Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin10 µg, AM= Amoxycillin30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg

3.1.4 Antibiotic resistance pattern of *V. Cholerae* isolates from different sources in cross river state

Following the resistance pattern of the *V. cholerae* isolates, it was observed that AU was the most resisted antibiotic agent with a total resistance of 529 (45.80%); the highest resistance was from the cray fish isolates 164(69.49%), from Seawater 100(59.17%), from the apple snail, 62(46.29) from lobsters 58(50%), from fish 46(33.09%), from blue crab 44(53.66%) and the least 6(4.62%) form Periwinkle.

The second most resisted drug was CH, with a total resistance of 430(37.23%); of which 140(59.2%) were cray fish isolates, 75(44.38) Sea Water, 56(41.79%) Apple snail, 44(37.93%) lobster, and the least number 5(3.85%) from periwinkle.

The third, was SP with 391(33.85%); 122(51.69%) cray fishisolates, 93(55.03%) sea water, 44(32.84%) apple snail, 41(29.49%) fish, and gutter water 5(8.18%) as the lowest. This was closely followed by S with 325(28.14%) as the total resistance; 104 (44.04%) for cray fish isolates, 89(52.66%) for Sea water, 37(26.62%) for fish, and 2(1.54%) for periwinkle.

Furthermore, CPX was resisted by 298(25.80%) isolates; 93(54.24%) for cray fish isolates, 51(41.42%) for sea water, 39(55.23%) for apple snail, 38(56.89%) for Lobster, and the least number 5(3.85%) for periwinkle isolates. The total for SXT was 290(25.11%); the highest number 104(44.07%) for cray fish isolates, and the lowest number 3(2.31%) was seen with the gutter water isolates. CN was recorded as the least resisted drug with a total of 83(7.19%); 80(33.88%) for cray fish, 2(2.27%) for river/streamwater, 1(1.14%) for gutter water isolates and 0(0.00%) for blue crab, apple snail, periwinkle, lobster, fish, and Sea water isolates respectively (Table:4).

3.1.5 Overall percentage of *V. cholerae* isolates resistant from different sources

The overall percentage mean of the isolates from cray fish that showed resistance to the different antibiotics was 44.26±18.422%, from fish 20.76±17.78%, river/stream water 16.42±18.51%, gutter water 16.01±19.46%, Blue Crab 15.79±22.9%, lobsters 13.68 ±21.21%, Apple Snail 13.09±21.21%, Sea Water 7.65±17.78% and Periwinkle 2.17±6.10% (Table V).

The cumulative resistance shown to the antibiotics was as follows: AU 28.73±28.72% CH 25.86±24.42% SP 20.16±22.05%, SXT 19.35±19.99%, S 18.41±21.59%, CPX15.68±19.74%, PEF14.75±17.89 %, AM 14.39±18.69%, OFX 5.06 ±12.46, and CN 4.07±11.66% (Table 5)

Statistically, significant differences were observed in the resistance pattern between the different sources, the treatments, as well as in the interactions between the treatments and the sources. P=.00<.05.

Table 4: Number/Percentage of V. *Cholerae* Isolates from Different sources in Cross River State Resistant to Antibiotic

Sample	TNI	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Crayfish	236	104/44.07	140/59.2	122/51.69	93/54.24	67/64.41	164/69.49	80/33.88	92/38.98	80/33.88	104 /44.04
Apple snail	134	32/23.88	56/41.79	44/32.84	3955.23	41/41.79	62/46.29	0/0.00	32/23.88	14/10.45	26/19.40
Periwinkle	130	3/2.31	5/3.85	8/6.15	5/3.85	8/6.15	6/4.62	0/0.00	2/1.54	0/0.00	2/1.54
Blue crab	82	16/19.51	12/14.63	22/26.83	26/53.66	22/26.83	44/53.66	0/0.00	12/14.63	0/0.00	12/14.63
Red lobster	116	22/18.97	44/37.93	33/28.45	38/56.89	27/37.93	58/50	0/0.00	10/8.62	0/0.00	22/18.96
Fish	139	23/16.55	35/25.18	41/29.49	31/22.30	49/35.25	46/33.09	0/0.0	2/14.59	0/0.00	37/26.62
Sea Water	169	46/27.22	75/44.38	93/55.03	51/41.42	58/36.69	100/59.17	0/0.00	62/36.69	0/0.00	89/52.66
River/stream Water	88	21/23.81	35/39.77	23/26.14	9/10.23	7/7.95	35/39.77	2/2.27	28/31.82	0/0.00	12/13.64

Gutter Water	61	23/37.72	28/45.90	5/8.18	6/26.23	0/0.00	14/22.95	1/1.14	12/19.67	5/8.19	21/34.43
Total	1155	290/25.11	430/37.23	391/33.85	298/25.80	279/24.16	529/45.80	83/7.19	270/23.38	99/8.57	325/28.14

SXT=Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin10 µg, AM= Amoxycillin30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg

Table 5: Overall Percentage of *V. cholerae* Isolates Resistant from Different sources

Source	Overall Mean (%)	Std. Deviation
Cray fish	44.26	18.42
Fish	20.76	17.78
River/stream Water	16.42	18.51
Gutter Water	16.01	19.46
Blue Crab	15.79	22.98
Periwinkle	2.17	6.10
Apple Snail	13.09	21.21
Lobsters	13.68	19.97

Sea Water	7.65	17.78
Treatment		
SXT	19.35	19.99
CH	25.86	24.42
SP	20.16	22.05
CPX	15.68	19.74
AM	14.39	18.69
AU	28.73	28.72
CN	4.07	11.66
PEF	14.75	17.89
OFX	5.06	12.46
S	18.41	21.59
Total	16.65	21.53

SXT=Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin10 µg, AM= Amoxycillin 30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg.

3.1.6 The effects of the commercial antibiotics on *V. cholerae* isolates by locations.

The Effects of the various antibiotics tested against *V. cholerae* isolates from the different locations are shown in figure 1. CN and OFX with a mean-values of 39.39% each, exerted the best effects on the *V. cholerae* isolates from Ogoja. This was followed by AM with 18.43%, CPX and PEF with a mean-values of 33.74%, 32.73% and 31.51% respectively. The least effects were from S and AU (26.41% and 26.017% respectively), in Ogoja.

The best effects observed on the isolates from Obudu, were from S (43.65%), OFX/CN (39.09% each), CPX (37.28%), AM (35.47%) and SP with 33.69% and 18.15%. The least effect observed were from AU (26.29%) and CH (26.08%) respectively. From Obanlikwu, CN and OFX with a mean-values of 40.74% each, also exerted the best effects on the *V. cholerae* isolates.

This was followed by S, CPX, PEF, AM with and with a mean-values of 37.83%, 36.77%, 36.51% and 35.19% respectively. The least effects were from CH (27.65%). Furthermore, from Boki 39.39% each was recorded for CN and OFX, 35.08% for CPX, 34.63% for AM, 33.93% for S, and the least effect 25.62% was recorded for AU.

From Ikom, CN, OFX, and PEF took the lead with a mean-values of 83.95%, 77.68%, 64.19% respectively, while the least effect 44.73% was recorded for CH. For Etung, the effect seen on the isolates was as follows; CN and OFX 74.24% each, PEF 71.49% CPX 63.71% S 63.17% AM 63.01% SP 60.20% and AU 50.88%.

In the same trend as above, the effect seen on the isolates from Akamkpa was as follows; CN and OFX 86.81% each, S 83.59% PEF 80.07% CPX 76.79% AM 76.05% SP 69.18% and AU 57.68%. From Calabar, CN took the lead position with 96.13%, followed by OFX with 93.45%, AM 76.75%, SXT 75.65%, PEF 74.62%, S 73.17%, CPX 71.72% and the least AU 55.91%. The drug effect on the isolates from Akpabuyo was such that CN and OFX showed 96.97% each, AM 89.10%, CPX 88.57%S %, PEF 82.59%, SXT 79.89%, SP 79.05%, CH 68.99% and AU 62.22% (Fig 1)

3.1.7 Cumulative susceptibility of the isolates from different locations

The total mean values obtained, showed that the effect of the drugs on the *V. cholerae* isolatesincreased from North to South, with isolates from Ogoja showing the least mean susceptibility of 30.97±38.99%, while those from Akpabuyo showed the highest mean susceptibility of 82.79 ±18.34% (Table 6).

However, the drug effects also showed that CN was the overall most active antibacterial agent evaluated with 66.30±44.75%, while Au was the least active with 54.10±39.42% (Table 6).

The effects of the drugs on the isolates form various locations compared, were significantly different from each other (F-Cal = 4.11, for Treatments and 32.069, for Location, Sig=.000 <.05) respectively

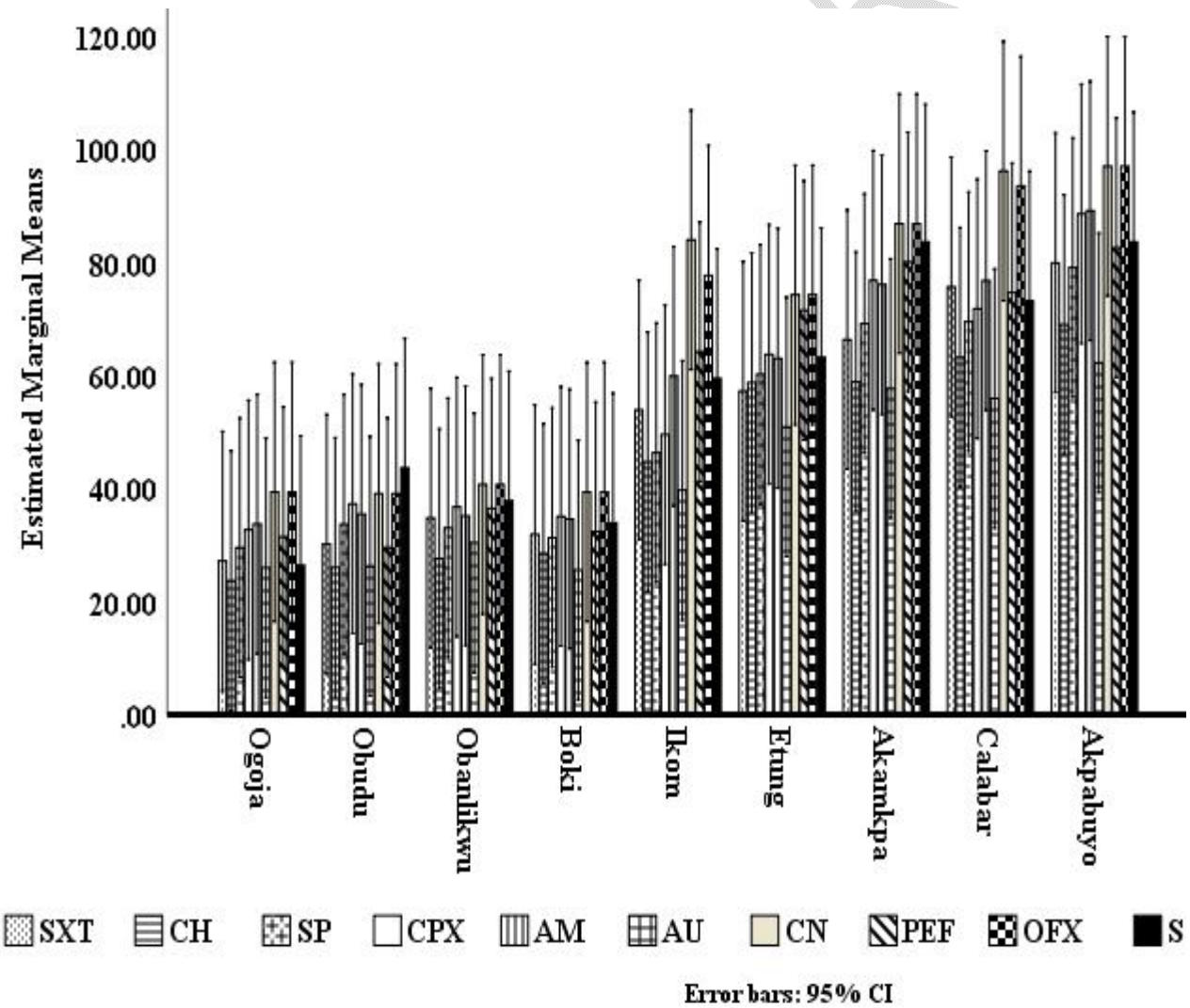

Figure 1: Descriptive Statistics of the Susceptibility of *V. cholerae* Isolates by Locations Examined

SXT=Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin10 µg, AM= Amoxycillin30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg

. Table 6: Cumulative Susceptibility of the Isolates from Different Locations

Location	Total Percentage Mean	Total Std. Deviation
Ogoja	30.97	38.99
Obudu	34.05	38.84
Obanlikwu	35.37	42.79
Boki	33.22	39.97
Ikom	57.94	30.89
Etung	63.69	38.99
Akamkpa	74.11	30.83
Calabar	75.01	19.23
Akpabuyo	82.79	18.34
Treatment		
CN	66.30	44.75
OFX	65.31	44.30
AM	55.98	39.96
PEF	55.88	39.41
S	55.75	37.15
CPX	54.69	39.49
SXT	50.79	36.51
SP	50.22	37.25
CH	44.51	33.99
AU	41.64	34.81
Total	54.10	39.42

3.1.8 Descriptive statistics for the antibiotic resistance of isolates from the different locations

The isolates from Ogoja, Obudu, and Obanlikwu resisted CH than all other antibiotic agent tested (20.69%, 18.36% and 16.79% respectively). This was closely followed by AU with (18.43%, 18.15%, and 14.02% respectively), SXT (17.23%, 14.24%), and S (17.02% and 16.0%) for Ogoja and Obudu.

In Boki Ikom Etung, Akamkpa, Calabar, and Akpabuyo, AU was most resisted than all other drugs tested, with 18.82%, 49.18%, 26.89%, 31.20%, %44.09% and 37.7% respectively. This was followed by CH with 15.91%, 44.16%, 19.04%, 30.01%, 36.79%, and 31.01% respectively.

In the order of least drug resistance, the isolates from Ogoja, Obudu, Obanlikwu and Boki, resisted OFX and CN (5.05%, 5.35%, 3.70% and 5.05% respectively) less than all other drugs, while those from Ikom, Etung, Akamkpa, Calabar and Akpabuyo resisted CN less, followed by OFX respectively (Fig 2).

3.1.9 Overall representation of the percentage resistance of isolates by their various locations

Categorizing the drug resistance pattern by Location, it was observed that the isolates from Ikom were the most resistant, with, 31.93±25.41%, followed Calabar with 24.54± 19.43%, then Akpabuyo with 17.04±18.43%, Akamkpa 16.33±19.29%, Etung 14.38±20.09 %, Ogoja 13.37±22.93%, Obudu 11.92±20.15%, Boki 11.22±19.02%, and Obanlikwu 9.07±18.80%(Table 7).

In the overall evaluation, it was observed that AU was the most resisted drug (28.72±28.72%), followed by CH, SP, SXT, S, CPX, PEF, AM, OFX and then CN with 25.86 ±24.42%, 20.16± 22.05%, 19.35 ±19.99%, 18.41 ±21.59%, 15.68± 19.74%, 14.75± 17.89%, 14.39 ±18.6%, 5.06 ±12.46%, and then 4.07± 11.6% respectively (Table 7).

The Analysis of Variance for the Resistance of isolates by Locations revealed that there were statistically significant differences between the locations evaluated and the treatments used on the isolates (P<.05). The interactions between the Locations and the treatments were not statistically significant (F- Cal=.521and sig=1.00, P>.05).

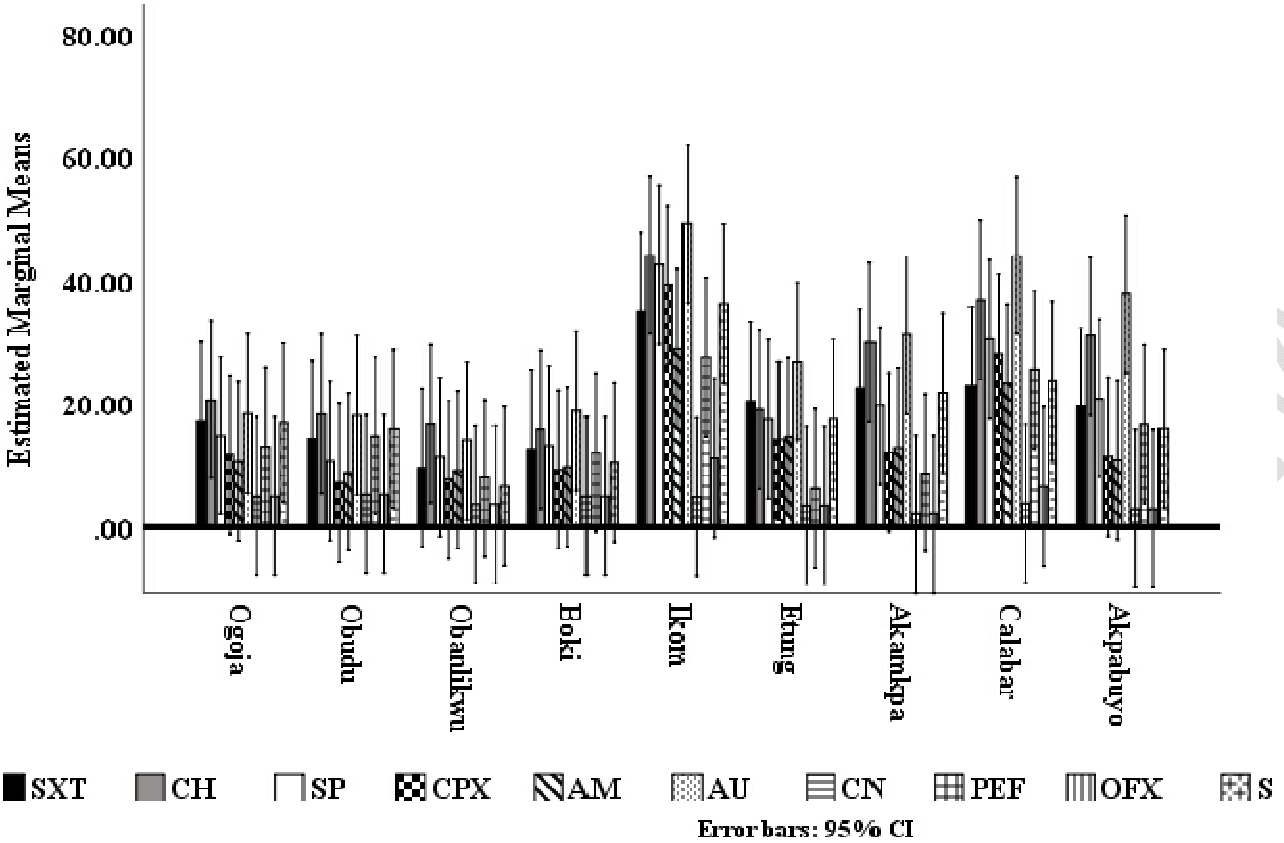

Figure 2: Mean percentage Resistance of Isolates from Different Locations

SXT=Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin10 µg, AM= Amoxycillin30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg

Table 7: Overall Representation of the Percentage Resistance of Isolates by their Various Locations

Total	Location	Mean (%)	Std. Deviation
	Ogoja	13.37	22.93
	Obudu	11.92	20.15
	Obanlikwu	9.07	18.80
	Boki	11.22	19.02
	Ikom	31.93	25.41
	Etung	14.39	20.09
	Akamkpa	16.33	19.29
	Calabar	24.54	19.43
	Akpabuyo	17.04	18.43
	Treatment		
	SXT	19.35	19.99
	CH	25.86	24.42
	SP	20.16	22.05
	CPX	15.68	19.74
	AM	14.39	18.69
	AU	28.72	28.72
	CN	4.07	11.659
	PEF	14.75	17.89
	OFX	5.06	12.46
	S	18.41	21.59
	Total	16.65	21.53

SXT=Septrin 30µg, CH = Chloramphenicol 30µg, SP = Sparfloxacin 10 µg, CPX = Ciprofloxacin10 µg, AM= Amoxycillin30µg, AU = Augmentin 30µg, CN = Gentamycin10 µg, PEF = Pefloxacin 30µg, OFX = Tarivid/Ofloxacin 10 µg, S = Streptomycin 30µg.

3.1.10 Comparing the effects of the commercial antibiotics on *V. cholerae* isolates by source and locations.

The effects of the antibiotics were also compared based on sources and locations. It was observed that Akpabuyo-crayfish were 73.64% susceptible, Akamkpa- cray fish 61.8%, Obanlikwu- cray fish 60.00%, Etung-cray fish 59.09%, Calabar-cray fish 57.07%, Boki-cray fish 50.00%, Ikom-cray fish 49.26%, Obudu -cray fish 46.67% and lastly Ogoja - cray fish 43.18%.

For the Isolates from the fish, Akamkpa/Etung – fish were 89.33% each susceptible, Calabar- fish 85.33%, Akpabuyo -fish 82.78%, Ikom- fish 82.14%, Obudu-fish 82%, Boki-fish 81.60% Obanlikwu- fish 63.33%, and Ogoja- fish 57.33%.

The results from the River/stream Water sources showed that Obanlikwu-River/stream Water 100.00%, Boki 91.67%, Ogoja/Obudu -River/stream Water 85.71% each, Etung-River/stream Water 83.33%, Akamkpa-River/stream Water 82.73, Akpabuyo-River/stream Water 80.00%, Calabar-River/stream Water 75.41% and Ikom-River/stream Water 67.69%.

For those from the Gutter water, the mean susceptibilities were as follows; Etung-gutter water 100%, Obanlikwu-gutter water 95.00%, Ogoja-gutter water 92.50%, Akamkpa-gutter water 88.00%, Calabar-gutter water 80.00%, Obudu-gutter water 78.33%, Akpabuyo-gutter Water 78.00%, Boki- gutter Water, 75.71%, and Ikom-gutter Water isolates 68.33%, as the least susceptible

The Blue Crab, Periwinkle, Apple Snail, Lobsters and Sea Water, were not Evaluated in Ogoja, Obudu, Obanlikwu and Boki, but the Akpabuyo- Blue Crab isolates showed 86.49% susceptibility, Calabar- Blue Crab 79.14%, Akamkpa/ Etung -Blue Crab 70%, and Ikom- Blue Crab 54%. From periwinkle, those from Etung/ Akamkpa showed 100% susceptibility, Akpabuyo- Periwinkle 99.29%, Calabar- Periwinkle 97.21%, and Ikom- Periwinkle 84%as the least. From Apple Snail, Akpabuyo- Apple Snail 85.13%, Akamkpa- Apple Snail 80.57, Calabar- Apple Snail 67.23%, Ikom- Apple Snail 49.23% (Figure 3).

Akpabuyo-Lobsters 88.57%, Akamkpa- Lobsters 80.57%, Calabar- Lobsters 77.74%, Ikom- Lobsters 59.99%Etung- Lobsters68.75%.Sea-Water was only evaluated in Calabar and Akpabuyo. The Akpabuyo- Sea Water isolates were 72.44% susceptible while Calabar isolates were only -58.73% susceptible.

The overall susceptibility pattern was as follows: Akpabuyo 83.1634 ± 17.89186 %> Calabar (73.9960 ± 19.24875%) >Akamkpa (73.2927 ± 31.60332%) > Etung (63.6905 ± 38.88237%) >Ikom (57.7900±30.66702%) >Obanlikwu (35.3704 ± 42.66949%) > Boki (33.2202 ± 39.85496%) <Obudu (31.8201 ± 38.93745%) >Ogoja (30.5839 ± 38.58972%) (Table 8).

The effects of the drugs on the isolates form various sources and locations were significantly different from each other (F-Cal = 535.164, and 399.486, Sig=.000 <.05) respectively. The interaction effect between sources and locations were also significantly different from each other (F-Cal=56.765 sig=.000<.05)

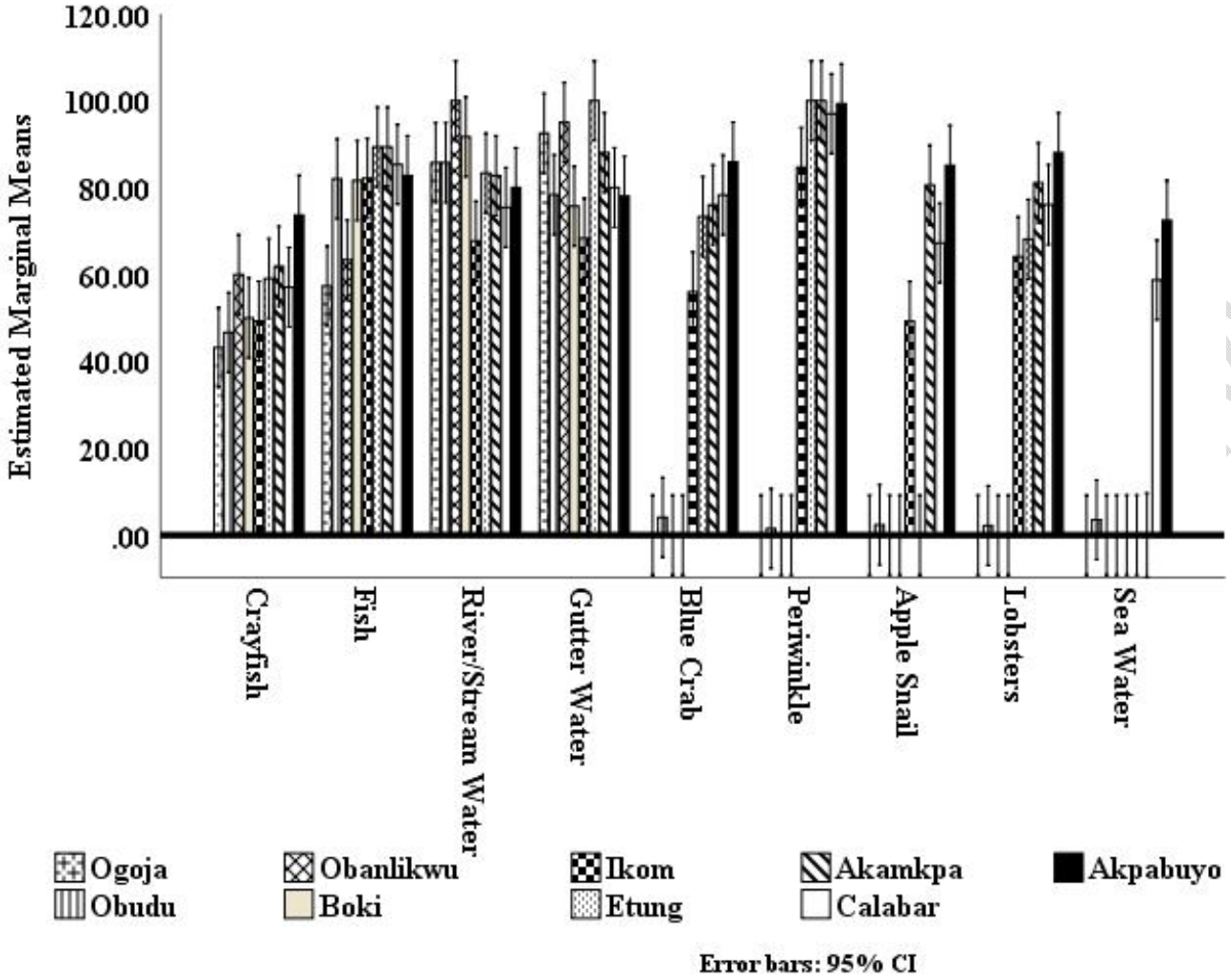

Figure 3: Comparative Evaluation of the Susceptibility of *V. cholerae* Isolates from Different Sources and Locations

Table 8: Overall Descriptive Statistics of the Percentage Susceptibility of the Isolates by Locations and Sources

Total	Location	Mean	Std. Deviation
	Ogoja	30.97	38.99
	Obudu	32.52	39.54

Obanlikwu	35.37	42.79
Boki	33.22	39.97
Ikom	57.18	30.54
Etung	63.39	38.79
Akamkpa	72.56	31.69
Calabar	75.32	19.373
Akpabuyo	82.93	18.41
Source		
Crayfish	55.64	18.58
Fish	79.24	17.78
River/stream Water	83.59	18.51
Gutter Water	83.99	19.460
Blue Crab	39.96	40.20
Periwinkle	53.39	48.37
Apple Snail	31.35	38.42
Lobsters	41.74	40.67
Sea Water	14.58	29.34
Total	53.72	39.54

3.1.11 Antibiotic resistance pattern of *V. cholerae* isolates from the different sources compared and locations

Looking at the resistance pattern of the isolates from crayfish, it was noted that those from Ogoja showed the highest resistance to all the antibiotics (55.91%). This was closely followed by those from Obuducrayfish (53.33%), Ikom 50.74%, Boki 50.00%, Calabar 42% etc. The least resistance was from the Isolates from Akpabuyo (26.36%).

The resistance pattern observed with the *V. cholerae* isolates from fish showed that those from Ogoja were more resistant to all the antibiotics tested (42.67%), followed by those from Obanlikwu with 36.67%, then by Boki 18.40%. The least resistance observe was from the isolates from Etung and Akamkpa10.6667% each. The highest resistance observed from the River/stream isolates was from Ikom 32.31%.

This was seconded by those from Calabar with 24.53%, then thirdly by those from AKpabuyo with 20.0%. The least resistance was from the vibrio isolates from Obanlikwu with 0.00%. From the Gutters the resistance pattern observed was as follows: Ikom 31.67%, Boki 24.29%, Akpabuyo22%, Obudu 21.67%, Calabar 20%, Akamkpa12% Ogoja 7% Obanlikwu 5% and Etung the least with 0.00% The resistance observed with Ikom isolates from the Blue crab, was 48%, Etung and Akamkpa 30% each, Calabar 20.86% and Akpabuyo 13.24%. Blue crab, Periwinkle, Apple snail, Lobsters and Sea water were not evaluated in Ogoja, obudu, Obanlikwu and Boki. Thus, no data was generated for these locations.

For the Ikom Periwinkle isolates, 16% showed resistance to all the antibiotics tested, Calabar isolates 2.79%, Akpabuyo 0.71%, and Etung and Akamkpa 0.00% each. The trend from the Apple snail was as follows: Ikom 50%, Calabar 32.77%, Akamkpa 19.43% and Akpabuyo 14.87%.

The vibrio isolated from Lobster sources in Ikom showed 40% resistance, Etung 31.25%, Calabar 21% Akamkpa 19.44% and Akpabuyo 11.43%. Sea water sources were only evaluated in Akpabuyo and Calabar and the resistance observed from the *v. cholerae* isolates showed that those from Calabar were 41.27% resistant to the antibiotics tested while those from Akpabuyo showed 27.56% resistance (Fig 4).

3.1.12 Overall representation of the percentage resistance of isolates by their various sources and locations

The cumulative percentage resistance pattern was such that Cray fish showed 44.26±18.42%, Fish 20.76±17.78%, River/stream Water 16.42±18.51%, Gutter Water 16.01±19.46%, Blue Crab 15.79±22.98%, Lobsters 13.68±19.97%, Apple Snail 13.09±21.21%, Sea Water 7.65±17.76% and Periwinkle 2.17± 6.10% (Table 9).

Categorizing the drug resistance pattern by Source and Location, it was observed that the isolates from Ikom showed, 31.93±25.41% drug resistance, followed Calabar with 24.54± 19.43%, then Akpabuyo with 17.04±18.43%, Akamkpa 16.33±19.29%, Etung 14.38±20.09 %, Ogoja 13.37±22.93%, Obudu 11.92±20.15%, Boki 11.22±19.02%, and Obanlikwu 9.07±18.80% (Table 9).

The Resistance of isolates by their sources and Locations were significantly different (P<.05). The interactions between the Locations and the sources were also statistically significant (F- Cal= 6.44 and sig at .00, P<.05).

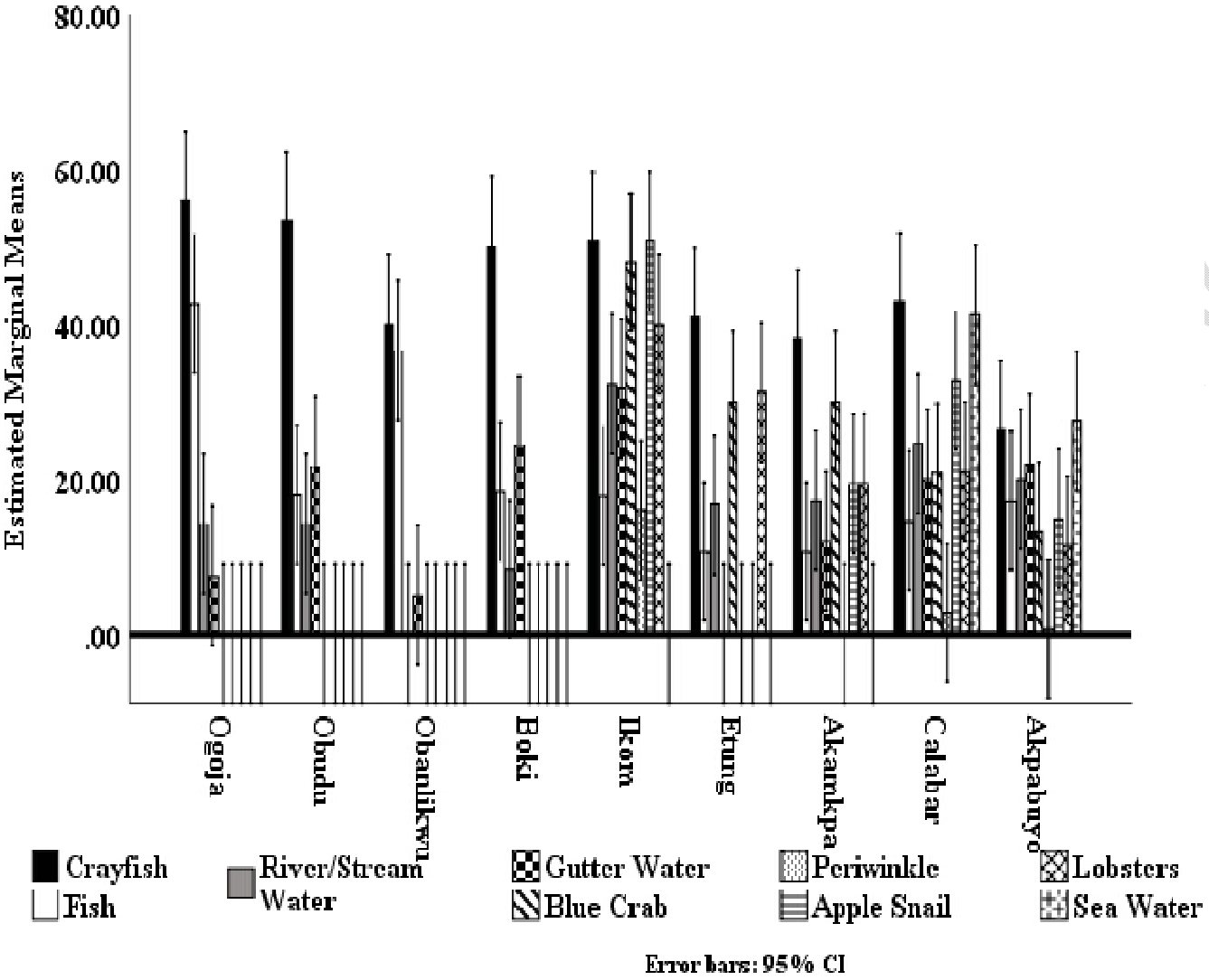

Figure 4: Descriptive Statistics of the Antibiotic Resistance of *V. cholerae* Isolates from the Different Sources Compared and Locations

Table 9: Overall Representation of the Percentage Resistance of Isolates by their Various Sources and Locations

Source	Mean [%)	Std. Deviation
Cray fish	44.26	18.42
Fish	20.76	17.78
River/stream Water	16.42	18.51
Gutter Water	16.01	19.46
Blue Crab	15.79	22.98
Periwinkle	2.17	6.10
Apple Snail	13.09	21.21
Lobsters	13.68	19.97
Sea Water	7.65	17.78
Location		
Ogoja	13.37	22.93
Obudu	11.92	20.15
Obanlikwu	9.07	18.80
Boki	11.22	19.02
Ikom	31.93	25.41
Etung	14.39	20.09
Akamkpa	16.33	19.29
Calabar	24.54	19.43
Akpabuyo	17.044	18.43
Total	16.65	21.53

3.2. Discussion

In this study, gentamycin was the least resisted of all the antibiotics tested with [7.17%] resistance. This is in agreement with similar reports by [13, 14,115, 16, 17, 18]. Also, a 25% gentamycin resistance was reported by Chatterjee *et al*. [19].

Chloramphenicol resistance was also evident in this work, and these has only confirmed the fact that chloramphenicol resistance has long ago in the 1970s [20] and in 1990 [21]been reported in *V. cholerae.*

Multi Drug-Resistance [MDR] was demonstrated by all the *V. cholerae* isolated from CRS environment though this was at varying degrees. The resistance recorded in the present study against SP, CPX, FEF], and OFX, is in line with the report given by Krishna *et al*. [22], who also noted that *V. cholerae* isolates showed resistance against quinolones like Nalidixic, norfloxacin, Gatifloxacin, Moxifloxacin as well as gentamicin; a macrolide whose resistance has not been reported so easily.

Also, MDR was observed in this study against Septrin [SXT] and Hydroxy-ampicillin [AM], streptomycin [S]. This agrees with the claims by Chatterjee *et al*. [19], in their study titled "Mapping cholera outbreaks and antibiotic resistant *V. cholerae*in India: An assessment of existing data and a scoping review of the Literature", which stated that records of cotrimoxazole resistance have risen above 75% since 1997, while resistance against streptomycin, has remained between the range of 75–100%.

This statement about the high streptomycin resistance was refuted by the results obtained in this study, which showed that 28.14% resistance was recorded against Streptomycin, and that given by Ceccarelli *et al*.[23], who also reported 8.2% resistance to streptomycin.

Information from current literature searches revealed that species of Vibrio that express antibiotic resistance are capable of habiting shallow water surfaces than the sensitive species **[**24]. This phenomenon was observed in the present study, although, with some slight deviations; because susceptible species were also encountered in this study. For instance, sensitivity to Ciprofloxacin in this study was [74.19%] and similar works in India and New Guinea, also showed 96.8% and 99%, respectively, to this same drug [25 26].

In this study, both the *V. cholerae* O1 and NON-O1 strains isolated from the same ecological niches showed great variations in their susceptibility patterns to the antibiotics tested. This is an indication that resistance to antimicrobial agents could have been transferred between the strains while within their hosts [27].

Antibiotic resistance was observed in all the locations evaluated, though at varying degrees. Ikom which had the highest percentage resistance is noted for cocoa farming and these crops are always treated with herbicides, fungicides and bactericides to prevent infection and promote increased yield. Also, Calabar had many resistant strains which placed it second to Ikom. The indiscriminate use and mis use of drugs as well as the dumping of left over and expired antibiotics in the environment, the over use of pesticides, animal and human wastes as manures during farming activities could have actually contributed to this resistance, since the entire state is sustained through subsistent farming.

The self-modification ability of the bacteria themselves as well as the changing characteristics of the environment itself, could have led to disparity in the resistance pattern shown by the *V. cholerae* strains [28]. For instance, a continuous flow of sewage, waste/expired drugs into the river/streams, gutters, sea or changing environmental conditions like from cold to hot climates due to global warming [29] could have a role to play in this.

Quilici *et al*. [30], observed that a substitution mutation in Ser-83-Ile in gyrA and Ser-85-Leu in parC [the enzyme topoisomerase II and IV genes], and accumulated point mutations in the gyrA and parC genes that encode for topoisomerase II and IV [31], decrease the killing effect of quinolones like ciprofloxacin on *V. cholerae* [32]. Also, from spontaneous mutations in the DNA gyrase, topoisomerase, b-subunit of RNA polymerase (RpoB) and small subunit ribosomal protein 12; target receptors of the antibiotic agents are affected [32].

Verma *et al*. [33], further reports that one *V. cholerae* isolate has the ability to carry approximately 40 different genes that are capable of conferring resistance against 22 antibiotics from about nine unique classes of antimicrobial agents. These MDR and Extensive drug resistant (XDR) genes in *V. cholerae* have been traced to mobile genetic elements (MGEs) [34, 35], self-transmissible, autonomously replicating plasmids or integrative IMGEs, Integrating Conjugative Elements (ICEs), Insertion Sequences (IS) and transposable genetic elements [33]. ICEs encode for resistance against trimethoprim (dfrA1), sulfamethoxazole(sul2), streptomycin (strAB) and chloramphenicol (floR) [36]. Insertion sequences (IS), transposons, conjugative plasmids; have some link with mobile integron genes, that encode resistance against trimethoprim, b-lactams, aminoglycosides, erythromycin, chloramphenicol, rifampicin, fosfomycin, quinolones, etc [37, 38]. Thet are known for their ability to transfer resistance against AM, S, CN, tetracycline, CH and SXT [39].

Wang *et al*. [40] and Das *et al*. [41], stated that plasmids also, can carry the aac [3]-IIa, blaCMY-2, blaCTX-M-2, blaTEM-1, blaNDM, shble, dfrA15, mphA, arr3, aadA16, sul1, strAB, floR and tetA (genetic determinants of MDR), at times be connected to IS. They also noted that the SXT-ICE transferred qnrVC genes (qnrVC1 and qnrVC3) can confer quinolone resistance in *V. cholerae.*

Aminoglycosides and lincosamide antibiotics have been associated with the nucleotidylylation, O-glycosylation, O-ribosylation by enzymes [42]. O- and N-acetylation, acetylation also inactivates chloramphenicol, fluoroquinolone, streptothricin etc, while hydroxylation/ sequestration [41, 44], plasmid/transposon mediated enzymes [44], the indiscriminate/or misuse of antibiotics [45], *V. cholerae* expression of the AlmG enzyme [for peptide-bound antibiotic resistance] [46], reorganization of its Lipid-A portion of the extracellular lipopolysaccharides [46,47] are other ways by which MDR can develop.

The presence of these MDR *V. cholerae* in the environment, subsequently, may become a public health threat. This is because these MDR genes could be laterally and/ or horizontally transferred to potential pathogenic strains, which might eventually become out of control [48, 49,5051,52]. Therefore, prompt identification of such emergent resistant strains with unusual characteristics would give a clue towards future epidemiologic preparedness in case of any outbreak of disease [53].

4. CONCLUSION

Although all the *V. cholerae* isolates showed some level of Multi Drug Resistance against the antibiotic agents tested, with Gentamycin featuring as the most active drug, while Augmentin was the most resisted drug, the isolates from Cray fish showed the highest percentage of MDR, while the others followed in this order; fish, River/stream water, Gutter Water, Blue Crab, Lobsters, Apple Snail, Sea Water and Periwinkles. It can therefore, be inferred that there is high level of faecal pollution of the environment, since evidence from literature search reveal that highly polluted environments favor the development of resistance in bacteria through HGT. This therefore is an indication that the observed MDR resistance in this study, could have been transferred between the strains, while within their hosts in these environments. The presence of these MDRstrainsmight eventually spread resistant genotypes to other areas of the country with the flow of these contaminated bodies of water. There is therefore, need for more research in to the development of alternative therapeutics that can curb such emerging strains in case of future crises. Moreover, a continuous assessment of the antibiotic profile of these environmental strains will give the epidemiologist and health authorities an insight to the formulation of policies, taking of regulatory decisions, planning and implementation of disease control programmes, surveillance, monitoring and control strategies, in case there is an outbreak of cholera due to these emerging MDR resistant strains.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

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