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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *Vibro* species ISOLATED FROM DIARRHOIEC STOOL OF PATIENTS ATTENDING UMARU SANDA GENERAL HOSPITAL, BIDA NIGER STATE, NIGERIA

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ABSTRACT

The study was carried out to determine the antibiotic susceptibility pattern of *Vibrio* species isolated from diarrhoeic stool of patients attending Umaru Sanda General Hospital, Bida Niger State, Nigeria between February and March 2018. A total of one hundred and twenty (120) samples were collected from diarrhoeic patients. The diarrhoeic stool samples were analyzed using Thiosulphate citrate bile salt sucrose (TCBS) agar and Muellar-Hinton agar. The isolates were Gram-stained, identified and characterized using biochemical tests. Antibiotic susceptibility test was conducted using the disc diffusion method. The diarrhoeagenic *Vibrio* species isolated from 120 diarrhoeic stool samples included *Vibrio cholerae* 16 (59.26%), *Vibrio parahaemolyticus* 08 (29.63%) and *Vibrio vulnificus* 03 (11.11%). All the isolates were susceptible to Gentamycin and Ofloxacin but highly resistant to Amoxyclav, Cotrimaxole and Neticillin. The present study revealed that Gentamycin and Ofloxacin could be used as drugs of choice for empirical treatment of diseases associated with *Vibrio* infection, such as cholera. The bacteria could be called as Amoxyclav-Cotrimaxole-Neticillin resistance *Vibrio*.

Keywords: Antibiotic, Diarrhea, Patient, Stool, *Vibrio*, Infection**.**

INTRODUCTION

Diarrhoea can be defined as the passing out of three or more loose watery stools or one bloody loose stools for a period of 24-hours (Galadima and Kolo, 2014). Diarrhoeal diseases are a globally important public health concern due to high morbidity and mortality rates among all age groups that originate from this disease (Nutan *et al*., 2018). Diarrhoeagenic *Vibrio* is that type of *Vibrio* bacterium isolated from diarrhoea thus called diarrhoeagenic *Vibrio*. *Vibrio* species is a Gram-negative, facultative anaerobic motile asporogenous rod or curved rod-shaped bacterium. It has a single polar

flagellum which colonizes small intestine of human beings (Ryan and Ray, 2004). Several *Vibrio* species are pathogenic and are mostly associated with gastroenteritis, such as *Vibrio cholerae, Vibrio paraheamolyticus,* and *Vibrio vulnificus* (Udoh and Itah, 2012).

Vibrio species are the common pathogens responsible for watery diarrhoea in infected humans and belong to the *Vibrionaceae* family. Faecal-oral transmission from person to person in areas with poor sanitation and contaminated or non-chlorinated water are the source of diarrhoea (Muhammad *et al*., 2020). Globally, *Vibrio* is known to cause approximately 3 million cases per annum (Ali *et al*., 2012). Infections caused by *Vibrio* are mainly categorized into *Vibrio* cholera and non-cholera *Vibrio* infections. *Vibrio* species causes profuse watery diarrhoea and are known for global epidemics and pandemics (Nair *et al.*, 1994). Symptom of *Vibrio*-mediated gastroenteritis includes watery rice stool, severe dehydration, fever and vomiting.

Vibrio cholerae produces potent enterotoxin called Choleragen which is responsible for cholera symptoms (Nester *et al.*, 1995; Sack *et al*., 2004). The choleragen is heat-labile and its protein molecule is composed of two parts: A and B. The B- fragment has toxic activity, while fragment A has no toxicity activity, causing the activation of the enzyme adenylate cyclase, which converts ATP to cyclic Adenosine Monophosphate (CAmp). Accumulation of (CAmp) in the cell causes a markedly increased secretion of water and electrolytes. The massive form of it can amount to two liters per day, and because of its appearance, the watery diarrhoeal fluid has been described as rice stool (Nutan *et al.*, 2018). The abrupt on-set of this disease is massive, painless followed by vomiting, severe dehydration and shock, which may lead to death if prompt medical attention is not given. The present study aims to isolate and determine antibiotic susceptibility pattern of *Vibrio* species isolated from diarrhoiec stool of patients attending Umaru Sanda General Hospital, Bida Niger State.

MATERIALS AND METHODS

Study Area

The study was carried out at Etsu Umaru Sanda General Hospital, Bida Niger State, Nigeria between February and May 2018. The Hospital is one of the hospitals sited in Bida town, while Bida is the Bida local Government headquarter. Niger State is located in the central part of Nigeria (middle belt) and is the largest state in the country. The state lies on latitude $08^0$to $11^{0.}30^1$ North and longitude $03^0.30^1$ to $07^0.40^1$ east. It has a land-mass of seventy-six thousand three hundred- and sixty-three-kilometer square ($76{,}363km^2$) with a population of four million eighty-two thousand five hundred and fifty-eight (4,082,558). Below are the maps showing the sampling site (Figure 1).

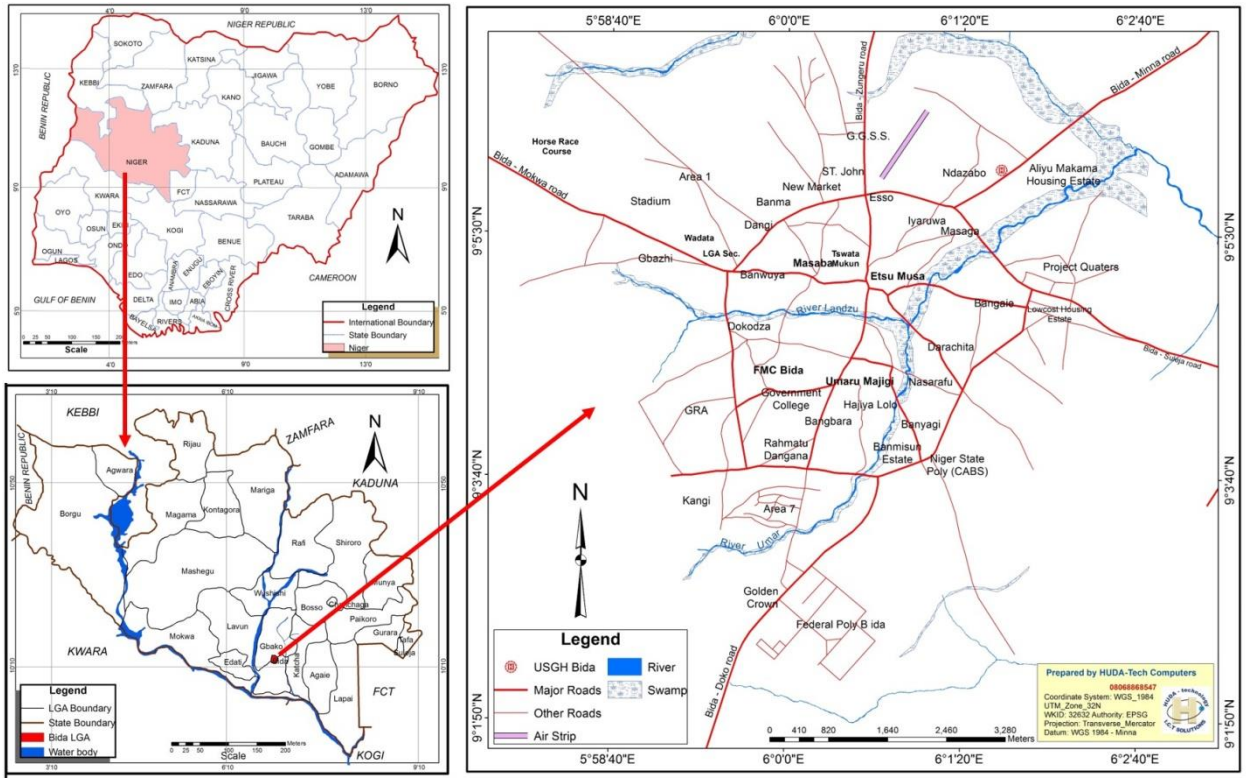

Figure 1: Map of Nigeria showing Niger State and map of Bida showing Umaru Sanda General Hospital, Bida (Sampling site).

Stool Sample Collection

A total of one hundred and twenty (120) stool specimens were collected into wide mouth sterile screw cap bottles from Etsu Umaru Sanda General Hospital, Bida. Each sample was clearly labeled and registered with the patient's sex, age and sample appearance. All stool samples were initially stored at Microbiology Laboratory of Etsu Umaru Sanda General Hospital, Bida before transporting them to Ibrahim Badamasi Babangida University's multi-purpose laboratory Lapai for further analysis.

Stool Analysis

Each sample was homogenized with a swab stick, and about 0.5ml was introduced onto Thiosulphate citrate bile salt sucrose (TCBS) agar and then incubated at 37°C for 24 hours (Chessbrough, 2002). Pure cultures were stocked in a Nutrient agar slant at 4°C for further studies.

Antibiotic Assay

A method of Baba *et al*. (2016) was adopted in which Muellar-Hinton agar was prepared, dispensed into sterile Petri dishes and was allowed to solidify. The isolates, which were diluted to a standard of 0.5McFarland's standard (approximately $1x10^8$ -$2x\ 10^8$ colony forming units per mile), were then streaked on the surface of the solidified Mueller-Hinton agar using sterile swab sticks. Commercially prepared antibiotics

discs, each was picked using sterile forceps and placed on the surface of the agar already inoculated with the test isolate and then lightly pressed with forceps. The plates were incubated at 37^0C for 24 hours, in which the zone of inhibition was measured to the nearest millimeters (mm) (Chessbrough, 2002). However, the percentage susceptibility was determined by dividing the isolates' number and then multiplied by one hundred. The interpretation of the zones of inhibition was done using the chart adapted from the clinical laboratory standard institute (CLSI, 2009).

RESULTS

The result of the distribution of *Vibrio* species among diarrhoeagenic patients attending Umaru Sanda General Hospital Bida, Niger State, is presented in Table 2. Different *Vibrio* species were isolated from the diarrhoeic stool of patients attending Umaru Sanda General Hospital Bida, Niger State. The species included 16(59.26%) *Vibrio cholera*e, 8(29.63%) *Vibrio parahaemolyticus* and 3(11.11%) *Vibrio vulnificus*.

Table 1: Distribution of *Vibrio* species among patients' 1-50 Years attending Umaru Sanda General Hospital Bida, Niger State

Vibrio species	Total number	Percentage
Vibrio cholerae	16	59.26
Vibrio parahaemolyticus	08	29.63
Vibrio vulnificus	03	11.11
Total	27	100.00

The result of the susceptibility pattern of *Vibrio* species to some tested antibiotics are presented in Table 2. From the results,the susceptibility pattern of *Vibrio* species showed that 87.50% (14) of *Vibrio cholerae* were susceptible to Gentamycin, followed by 70% (12) of *V. cholera*e that were susceptible to Ofloxacin. Also, 50% (8) and 6.25% (1) of *Vibrio cholerae* were susceptible to Ceftriaxone and Levofloxacin respectively. However, 100% of *Vibrio cholerae* were entirely resistant for Amoxyclav,

Cotrimaxole, Neticillin, and Tetracycline, respectively. As for the *Vibrio parahaemolyticus*, 100% (8) of the isolates were susceptible to Ofloxacin and 50% (4) of the isolates were also susceptible to Gentamycin, while 25% (2) of *Vibrio parahaemolyticus* showed their susceptibility to Levofloxacin. However, 100% *Vibrio parahaemolyticus* indicated their resistivity to Amoxyclav, Cotrimaxole, Ceftriaxone, Neticillin and Tetracycline. On the other hand, 100% (3) of *Vibrio vulnificus* were susceptible to Tetracycline and 66.67% (2) isolates were susceptible to Gentamacin and Ofloxacin respectively, while 33.33% (1) isolates showed their susceptibility to only Levofloxacin. *Vibrio vulnificus* were completely resistant to Amoxyclav, Cotrimaxole, Ceftriaxone and Neticillin respectively.

Table 2: The percentage susceptibility pattern of *Vibrio* species to antibiotics

S/n	Antibiotic	Potency of disc (ug)	*Vibrio cholerae* n=16 (%)	*Vibrio parahaemolyticus* n=8(%)	*Vibrio vulnificus* n=3(%)
1	Amoxyclav (AMC)	30	0 (00)	0 (00)	0 (00)
2	Cotrimaxole (COT)	25	0 (00)	0 (00)	0 (00)
3	Ceftriaxone (CTR)	30	8 (50)	0 (00)	0 (00)
4	Gentamacin (GEN)	10	14 (87.50)	4 (50)	2 (66.67)
5	Levofloxacin (LEV)	5	1 (6.25)	2 (25)	1 (33.33)
6	Netillin (NET)	3	0 (00)	0 (00)	0 (00)
7	Ofloxacin (OFX)	5	12 (70)	8 (100)	2 (66.67)
8	Tetracycline (TET)	30	0 (00)	0 (00)	3 (100)

Key: n=Number of isolates; %=Percentage; ug=Concentration; s/n=Serial number

DISCUSSION

Diarrhea may be contracted by ingestion of contaminated food or drinking unwanted water (Muhammad *et al*., 2020). These contaminants are mostly Bacteria, Viruses and Protozoa and are capable of causing inflammation of gut resulting in diarrhoea, among other symptoms of gastrointestinal illness (Muhammad *et al*., 2020).

The occurrence of *Vibrio* species in this study showed that; 59.26% *Vibrio cholereae* was the most prevalent etiology of gastroenteritis

in this area. This was followed by *Vibrio parahaemolyticus,* with the prevalence of 29.63%, while *Vibrio vulnificus* had a prevalence of 11.11%.

This study indicated that *Vibrio cholereae* had a prevalence of 59.26%. The reason may be attributed to geographical location and season of sample collection (dry season). This finding is contrary to the report of Udoh and Itah, (2012). Theydocumented a prevalence of 76.50% in a study carried out in Akwa Ibom of South-South Region, Nigeria, and 36.60% prevalence reported in Ikom, Cross River State, Nigeria (Chigbu and Iroegbu, 2010).

According to our findings, the total prevalence of 29.63% of *Vibrio parahaemolyticus* was recorded. It is in line with the finding of Udoh and Itah (2012), who documented a prevalence of 28.59% for *Vibrio parahaemolyticus* in Akwa Ibom, South-South Region, and Nigeria. The finding is contrary to the studies conducted in Ikom, Cross River State, Nigeria, with the prevalence of 48.80% for *Vibrio parahaemolyticus* (Chigbu and Iroegbu, 2010). However, contrary to our findings, Adejo *et al*. (2016) reported the lowest prevalence of 10.06% in Minna on the study conducted on the prevalence and antibiotic susceptibility pattern of bacteria associated with gastroenteritis in Niger State.

Based on our findings, *Vibrio vulnificus* had the lowest prevalence of 11.11% compared to the other species recovered. This finding differs from the studies conducted by Udoh and Itah (2012) in Akwa Ibom State and by Chigbu and Iroegbu, (2010) in Cross River State, Nigeria, in which *Vibrio vulnificus* was not isolated at all.

The susceptibility profile of 50% and 6.25% of *Vibrio cholerae* to Ceftriaxone and Levofloxacin, respectively, is in line with findings of Nutan *et al*. (2018). The study showed that 100% Vibrio cholerae were completely resistant to Amoxyclav, Cotrimaxole, Neticillin, and Tetracycline, respectively. This may be attributed to spontaneous mutation, acquisition of plasmids, and misused of antibiotics. To support this, previous studies reported a very higher level of resistance against Niticillin, Cotrimaxole, Amoxyclav, Tetracycline, Nalidixic acid, Ceftazidine and Ceftriaxone (Kumar and Obero, 2014 ; Mala *et al*., 2014 ; Baron *et al*., 2016).

Vibrio parahaemolyticus showed 100% susceptibility to Ofloxacin, 50% sensitive to Gentamycin and 25% susceptible to Levofloxacin. The high susceptibility of Ofloxacin and moderate sensitivity of Gentamycin is comparable to data obtained in a study conducted by Chijoke *et al*. (2014), who documented 100% and 59.5% of *V. parahaemolyticus* susceptibility to Ofloxacin and Gentamycin, respectively. However, this finding contrasts with the data obtained by Adejo *et al*. (2016), who reported 72.73% and 60.61% susceptibility to Ofloxacin and Gentamycin, respectively. Our findings in this study indicated that 100% of *Vibrio parahaemolyticus* were resistant to Amoxyclav, Cotrimaxole, Ceftriaxole, Neticillin and Tetracycline. This may be attributed to the study area's prescription pattern, which might have forced the isolates to undergo mutation. This finding is similar

to the study conducted in India by Nutan *et al*. (2018), but contrary to results reported by Chijoke *et al*. (2014), who documented 78.9%, 63.2%, 89.5%, 84.4% and 89.5% to Cotrimaxole, Amoxyclav, Ceftriaxole, Niticillin and Tetracycline respectively.

Vibrio vulnificus showed the greatest sensitivity levels against Tetracycline (100%), followed by Gentamycin and Ofloxacin (66.67%), respectively, and while 33.33% were susceptible to Levofloxacin. Similar findings have also been reported in India by Nutan *et al*. (2018), who documented 80% Tetracycline, 60% Gentamycin, and 65% to Levofloxacin on the estimation of *Vibrio* species incidences and antibiotic resistance in diarrhoeal patients. The result is also similar to Chijioke et al. (2014), who reported a sensitivity of 100% to Gentamycin, Levofloxacin, and Ofloxacin, respectively Tetracycline recorded 50%.

Our findings showed 100% resistance to Amoxyclav, Ceftriaxole, Cotrimaxole, and Neticillin. The resistance to these antibiotics may be attributed to the production beta-lactamase, an enzyme that inactivate beta lactam ring in beta lactam antibiotics. To support this finding, previous authors have reported very higher levels of resistance against cell wall synthesis inhibitors such as beta-lactam drugs (Udoh and Itah, 2012 ; Nutan *et al*., 2018).

CONCLUSION

Conclusion: Twenty-seven isolates of *Vibrio* species were obtained from the study giving a prevalence of 22.50%. The *Vibrio* species detected from the diarrhoeic stool of patients in this study area included *Vibrio cholerae* 16(59.26%), *Vibrio parahaemolyticus* 8(29.63%) and *Vibrio vulnificus Vibrio vulnificus* 3(11.11%). All *Vibrio* species detected in this study were susceptible to Gentamycin, Ofloxacin and Levofloxacin but highly resistantresistant to Amoxyclav, Cotrimaxole and Neticillin. Therefore, the present finding has shown that Gentamycin, Ofloxacin and Levofloxacin may be required to supplement supportive anti-dehydration treatment, which is the cornerstone of acute diarrhoea therapy.

Recommendation:

It is therefore recommended that:

1. Public awareness should be embarked upon, particularly on personal hygiene and adequate sewage disposal.
2. Proper treatment of drinking water should be encouraged, especially among rural dwellers with limited access to chlorinated pipe-borne water.
3. The high resistance of *Vibrio* species to Amoxyclav, Cotrimaxole and Neticillin in the study area calls for -scale detailed research that works thorough large-scale research on antibiotic resistance pattern *Vibrio* species in the locality. In addition, misuse or un-prescribed antibiotics by Doctors should not be taken by patients (self-medication).

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