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Antibiotic Susceptibility Patterns of *Vibrio cholera* O_1 Isolated During Cholera Outbreak in Uzebba (Edo State)

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Abstract

A survey was carried out to determine the presence and antibiotic susceptibility of *vibrio cholerae* O_1 from stool samples during cholera outbreak in Uzebba, Edo – State. Strains of *Vibrio cholerae* O_1 isolated from patient stool samples using Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar during cholera were identified using standard biochemical tests and serotypes using polyvalent sera. Disc diffusion method of Kirby-Bauer was used to test the susceptibility of *Vibrio cholerae* O_1 to ten antimicrobial agents. All isolates were (100%) susceptible to ciprofloxacin, pefloxacin and sparfloxacin. Some strains showed moderate sensitivity to gentamicin, streptomycin and augumentin. *Vibrio cholerae* O_1 recorded the least susceptibility pattern of 15.8% to tetracycline (19%). From our findings, quinolones (Ciprofloxacin, Pefloxacin and Sparfloxacin) are still useful for the treatment of cholera, while the least sensitivity to tetracycline still generate questions and concern, especially in regions where patients can not afford the cost of quinolones.

Keywords

Vibrio cholerae, Cholera Outbreak, Antibiotic Susceptibility, Uzebba (Edo – State)

1. Introduction

Vibrio cholerae O_1 the causative agent of cholera was first described by Robert Koch in 1883. *Vibrio* organisms are free living widely distributed highly motile Gram-negative curved or comma-shaped rods with a single polar flagellum and most species are oxidase positive (Lee *et al.,* 2004). *Vibrio cholerae* is spread by eating food or drinking water that has been contaminated with cholera bacteria. Contamination usually occurs when human feces from a person who has the disease seeps into a community water supply (Schild*et al.,* 2005).Cholera epidemic outbreaks have killed millions of people and continue to be a major public health concern world wide (Faraque *et al.,* 2000). When infected with *Vibrio cholerae*, 90% cases develop uncomplicated vomiting and diarrhea (Fisher, 2000). Fewer than 10% of cases develop the severe form of disease, which if not treated can be rapidly life threatening due to dehydration and shock. Severe cases of diarrhea can be very profuse. The loss of fluid can make the disease so serious (Dobson and Gasper, 1999).Dehydration is essential for fluid and electrolyte replacement for the treatment of cholera (Deb *et al.,* 2002). *Vibrio cholerae* O_1 as other bacteria of medical significance is continuously developing more resistance to newer antibiotics and has been reported in various places, especially in the developing countries. Poverty, inappropriate prescribing methods, counterfeit and substandard drugs, poor laboratory support and surveillance, lack of antibiotic combination strategies or treatment methods and non-adherence to laid down drug policies have been identified as the major causes of resistance in the developing countries. Spread of cholera epidemic worldwide has been associated also with the emergence of multiple drug resistance among a large number of *Vibrio cholerae* O_1 strains (Albert, 1994; Mhalu *et al*., 1979; Finch *et al*., 1988). This study was aimed to determine the antibiotic susceptibility patterns of Vibrio cholera O1 responsible for the outbreak in Uzebba (Edo State)

between September – November, 2004.

2. Materials and Methods

2.1. Study Samples

The study samples were made up of V*ibrio cholerae* O_1 strains; obtained from stool samples of patients hospitalized in Uzebba General Hospital (Edo State) during cholera outbreak between September and November 2004. The strains were stored on Nutrient agar slants at 4°C, with three monthly subcultures and subsequently sub cultured on Meuller Hinton agar for susceptibility testing.

2.2. Bacteriological Analysis

Stool samples were inoculated onto Thiosulphate Citrate–Bile–Salt Sucrose (TCBS) agar and incubated at 37°c for 24 hours. Suspected *Vibrio cholerae* were purified by sub-culturing single colonies onto Nutrient agar. Following standard morphological and biochemical tests according to Buchanan and Gibbons (1974), characteristic colonies grown on the selective agar were then confirmed for identification. The series of biochemical tests commonly used to identify *Vibrio cholerae* include:

Oxidase test, L-analrinose test, methyl red test, and Voges Proskauer reaction, motility, Gram staining and agglutination with specific *Vibrio cholerae* polyvalent O_1 and *Vibrio cholerae* serotype Ogawa and Inaba antisera.

Table 1. *Antimicrobial Susceptibility patterns of Vibrio cholerae O_1 Isolated during Cholera Outbreak in Uzebba.*

Antibiotic	Total Number of Isolates	Number of Isolates Sensitive (%)
Ciprofloxacin(10µg)	63	63 (100)
Gentamicin(10µg)	63	53 (84.1)
Pefloxacin(10µg)	63	63 (100)
Colistin(10µg)	63	12 (19)
Sparfloxacin(10µg)	63	63 (100)
Amoxicillin(10µg)	63	21 (33.3)
Augmentin(30µg)	63	29 (46)
Streptomycin(10µg)	63	38 (60.3)
Chloramphenicol(30µg)	63	23 (36.5)
Tetracycline(30µg)	63	10 (15.8)
Septrin(30µg)	63	19 (30.1)

2.3. Antibiotic Sensitivity Testing

This was performed by disc diffusion method using guidelines established by Bauer *et al.,* 1966 and recommended by the Clinical and Laboratory Standard Institute (2002) using commercial antimicrobial discs. A total of eleven antibiotic discs (Oxoid) which include ciprofloxacin (10µg), gentamicin (10µg), colistin (10µg), sparfloxacin (10µg) amoxicillin (10µg), augmentin (30µg), streptomycin (10µg), chloramphenicol (30µg), tetracycline (30µg), septrin (30µg) and pefloxacin (10µg) were used.

By standard method of inoculation, a single colony of the isolate was picked with a sterile wire loop and inoculated into 2ml of sterile Mueller Hinton Broth.The broth was then incubated at 37°c for 4 hours to obtain the young growth culture. The turbidity of actively growing growth culture was then adjusted to 0.5 Mcfarland standard and then a sterile cotton swab was dipped into the adjusted suspension for 2 minutes and excess broth was poured by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the agar plate to obtain uniform inoculum. The plates were then allowed to dry for 5 minutes. Antibiotic impregnated discs were then applied to the surface of the inoculated plates with sterile forcep. Each disc was gently pressed down onto the agar to ensure complete contact with agar surface. Evenly distribution of disc and minimum distance of 24mm from center to center were ensured, control disc was placed on each Petri dish. Within 15 minutes of the application of the discs, the plates were inverted and placed in an incubator at 37°c. After 18 – 24 hours of incubation, the plates were examined and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured using a transparent plastic ruler and compared with those of the control organism which was tested when ever a batch of *Vibrio cholerae* O_1 strain was tested. The sensitivity pattern was scored simply as either sensitive or resistant.

Antibiotic susceptibility patterns was carried out on those isolates as shown in table 1, where *Vibrio cholerae* O_1 had 100% susceptibility to ciprofloxacin, pefloxacin and sparfloxacin; followed with 84.1%, 60.3% and 46% susceptibility to gentamicin, streptomycin and augmentin respectively. *Vibrio cholerae* O_1 recorded the least susceptibility of 15.8% and 19% to tetracycline and colistin respectively.

3. Discussion

Cholera is a disease of history that remains a major public Health problem in many parts of Africa, Asia and Latine America. Cholera continues to be an important public health problem among many vulnerable communities despite the detailed understanding of the bacteriology, epidemiology and public health aspects for more than a century (Shears, 2001; Hlady and Klontz, 2008; Okoh and Igbinosa, 2010).

Cholera is caused by a Gram negative bacterium known as *Vibrio cholerae*. Annual outbreaks of children are regular feature in some parts of Nigeria and other African countries. Effective antibiotic treatment can reduce the volume and duration of diarrhea and period of pathogen excretion in cholera cases. The emergence of multiple-drug resistance strains of *Vibrio cholerae* is becoming a global Public Health concern. This may be due to the fact that in contrast to the *Vibrio cholerae* O_1 strains causing outbreaks in South-east Asia, which have been extensively characterized little information is available on the characteristic of the recent epidemic strains implicated in cholera outbreaks African countries (WHO, 1998).

Despite the facts that only 63 *Vibrio cholerae* O_1 strains were studied for the susceptibility patterns, our finding will be of interest as just little information is available concerning case of outbreaks in Edo North.

All isolates were 100% sensitive to ciprofloxacin, pefloxacine and sparfloxacin, which suggested that some of these mentioned quinolones were very effective for the management of cholera outbreaks. These findings were in accordance with the work of Urassa *et al.,* (2000), reported 98% and 100% *Vibrio cholerae* O_1 isolated from patient stool samples in Tanzania were susceptible to ciprofloxacin, which also agree with the work of Neelam *et al.,* (2005) that *Vibrio cholerae* O_1 had 100% susceptibility to ciprofloxacin. 2008, Shukla *et al., (2008)* reported that 100% *Vibrio cholerae* O_1 were resistant to ciprofloxacin, which disagrees with our findings. These isolates had 84.1% and 60.3% susceptibility to gentamicin and streptomycin respectively, which can be justified by the work of Razvykh *et al.,* (1990) that vibrios were highly sensitive to gentamicin and sensitive to streptomycin. The findings during the study showed also that *Vibrio cholerae* O_1 had 46%, 36.5%, 33.3% and 30.1% susceptibility to augumentin, chloramphenicol, amoxicillin and septrin respectively which is a serious matter of concern as these may be responsible for serious resistance problem in future. Therefore, there is need to determine the minimum inhibitory concentration (MIC) of those antibiotics that will be able to give no visible growth of *Vibrio cholerae* O_1 strains. Tetracycline that was recognized by Word Health Organization as one drug of choice for cholera outbreak recorded the least sensitivity, with *Vibrio cholerae* O_1 having 15.8% susceptibility to tetracycline. This was supported by the work of Ehara *et al.,* (1993) who carried out studies in Kenya and reported that majority of *Vibrio cholerae* were resistant to tetracycline. The least susceptibility 15.8% recorded by *Vibrio cholerae* O_1 to tetracycline was not in agreement with the work of Urassa *et al.,*(2000) who reported that 93.6% of *Vibrio cholerae* O_1 strains isolated in Tanzania were susceptible to tetracycline. Our findings also contradicted the report of Muhummad *et al.,* (2008) that 100% *Vibrio cholerae* were susceptible to tetracycline. During this study, most isolates showed multiple drug-resistances to tetracycline, colistin, septrin, amoxicillin and chloramphenicol, which could also be responsible for their spread. The association between the development of resistance to tetracycline, chloramphenicol and septrin with large-scale use of antibiotics for treatment and prophylaxis of cholera is well recognized, (Tabtieng *et al.,* 1989; Finch *et al.,* 1988; Glass *et al.,* 1983). Still, our demonstration of multiple drug resistant *Vibrio cholerae* O_1 isolates showing resistance to almost all the antibiotics traditionally used to treat cholera is touching and still generating questions on the management and treatment of cholera cases in Nigeria and other developing countries that can be affected by cholera outbreaks. Knowing fully that high mortality rates experienced during cholera outbreaks in African countries could be associated with multiple-drug resistant *Vibrio cholerae* O_1 isolates, harboring resistant genes located in SXT elements and class intergrons (Dalsgard *et al.,* 2000).Antibiotics should be judiciously used in the management of diarrheal disease and in epidemics and at the same time restrict their use. It important to closely monitor the spread of tetracycline and other traditionally used antibiotics resistant in the region as they have vital role in the management of cholera.

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