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Drug Resistance Pattern of *Vibrio Cholerae* Isolated from Acute Diarroheal Disease Outbreak

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ABSTRACT

Vibrio cholerae has been recognised as one of the common causes of bacterial diarrhoea throughout developing world. Cholera is a self-limiting illness. However it is changing epidemiologically. In recent cholera outbreaks from various places, severity of the disease appears to be intensifying and have run a more protracted course. Multiple antibiotic resistant strains of *V. cholerae* have emerged. The emergence of these has added a new dimension to the variability in pathogenicity and potential virulence in precipitating diarrhoeal illness. To isolate and identify *Vibrio cholerae* from stool specimens and to know their antibiotic susceptibility pattern. Faecal specimens collected from 240 patients with acute diarrhoeal disease were subjected to culture & sensitivity as per standard protocol. *Vibrio cholerae* was isolated from 72/240 (30%) stool samples. All isolates belonged to biotype El Tor. Serotype Ogawa was seen in 66(91.67%) and Hikojima 6(8.33%). Resistance pattern of Ogawa was nalidixic acid (100%), co-trimoxazole(97%), Ampicillin(79%), tetracycline(29%), ciprofloxacin(25%), gentamycin(18%). Resistant pattern of Hikojima was nalidixic acid (100%), co-trimoxazole(100%), Ampicillin & ciprofloxacin(67%), gentamycin(33%), tetracycline(16%). All isolates were sensitive to Amikacin. Tetracycline is still an effective drug. Hikojima serotype was more resistant than Ogawa so susceptibility pattern for vibrio is essential to avoid emergence of resistant strains by empirical treatment.

Keywords: *Vibrio cholerae*, drug resistance, ogawa, hikojima

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INTRODUCTION

Vibrio cholerae has been recognized as one of the common causes of bacterial diarrhoea throughout the developing world. In Asian region, the Indian subcontinent continues to harbour a major chunk (78%) of cholera cases. Outbreaks of cholera including major epidemics have occurred from time to time at various places in India.[1] Epidemics of cholera caused by toxigenic *V. cholerae* O1 and *V. cholerae* O139 represent a major public health problem.[2] Presently, *V. cholera* O1 belonging to the El Tor biotype is the most common sero group in India, while the frequency of sero group O139 has declined considerably over the past few years.[3] Cholera is also changing epidemiologically.[4] In recent cholera outbreaks from various places, severity of the disease appears to be intensifying and have run a more protracted course.[3]

Though fluid and electrolyte replacement either by oral rehydration or intravenous fluid therapy is the treatment of choice for acute diarrhoea, antibacterial agents are indicated as useful adjuncts for the treatment of cholera as these shorten the duration of hospital stay, stop excretion of vibrios in the stool and also minimize the requirement for fluid. [5]

The emergence of multiple antibiotic-resistant (MAR) isolates of *V. cholerae* has added a new dimension to the variability in pathogenicity and potential virulence in precipitating diarrhoeal illness.[2] Antimicrobial resistance is therefore a global public health problem. The increase in the magnitude of bacterial species resistant to multiple antimicrobial agents have shown linear relationship with the amount of antimicrobial agents dispensed in a particular hospital or community for treatment or prophylaxis[6]. The emergence of such resistance amongst *V. cholerae* may significantly influence the control strategies in future outbreaks. Strong regional commitment to surveillance and preparedness for outbreaks should be maintained and timely information should be given to the health authorities as well as to the public.[1] Notification and surveillance systems of infectious diseases including that caused by *V.* cho*lerae* 01 in most developing countries is lacking, but available epidemiological and clinical evidence show that cholera is a public health problem.[6]

This study was undertaken to isolate and identify *Vibrio cholerae* from stool specimens and to know their antibiotic susceptibility pattern.

MATERIALS AND METHOD

The present study was conducted in the Department of Microbiology, Mysore Medical College and Research Institute, Mysore. Faecal specimens were collected form 240 patients with acute diarrhoeal disease. The specimen was inoculated into Alkaline peptone water for enrichment and

direct plating of samples were done on MacConkey, blood agar, and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates and incubated overnight at 37^{o}C. Subcultures were made from alkaline peptone water after 6 hours of incubation onto TCBS agar. The isolates of *V. cholerae* were identified morphologically and biochemically and biotyping was performed using standard protocol.[7] Confirmation of isolates were done by sero-agglutination using Vibrio polyvalent O1, monospecific Ogawa, Inaba antisera. (King Institute of Preventive Medicine, Guindy).

Antibiotic susceptibility testing of *V. cholerae* isolates were done on Mueller Hinton agar by Kirby & Bauer disc diffusion method. The antibiotic discs that were used are tetracyclin(30 µg), ciprofloxacin(5 µg), gentamicin(30 µg), ampicillin(10 µg),), nalidixic acid (30 µg) and trimethoprim/sulphamethoxazole (1.25/23.75µg) (HI-Media, Mumbai).

RESULTS AND DISCUSSION:-

It is well known that the incidence of cholera depends not only on the presence of the causative organism but also on the opportunities available for transmitting it and the immune status or herd immunity of the population.[8] *V.cholerae* was isolated from 72/240 (30%) stool samples**.** Cholera affects all ages and both sexes. However, infection rate is increasingly reported in children.[1,9] It was isolated from age group of less than 1 year to up to 70 years most of the cases *i.e.,* 33(45%) were in the younger age group (1-10yr). Probably because this age group have the easy accessibility to the contaminated food and also immunity of this group is comparatively lower than adults. Table 1

Table 1-Showing the distribution of *V.cholerae* in different age groups

Age in years	Number (%)
< 1	3(4)
1-10	33(45)
11-20	8(11)
21-30	12(18)
31-40	8(11)
41-50	1(1)
51-60	4(5)
61-70	3(4)
Total	72

The reporting of cholera cases in India is incomplete and the available data are sparse and heterogeneous as cholera notification is highly deficient and the methods used to keep statistics on cholera incidence are inadequate.[3] Biotype El Tor *V.cholerae* have replaced their classic counterpart over the last few decades. Many recent reports of cholera outbreak in various parts of Indian subcontinent have been due to El Tor *V. cholerae*.[10] In the present study all isolates of *V.*

cholerae were biotype El Tor. The biotypes were further serotyped. Even though ogawa is the major serotype isolated, there is 8.33% of hikojima serotype which are epidemiologically important, as these are known to be very unstable, and has been demonstrated to interconvert and undergo serotype switching.[11] The distribution of serotypes shown in Table2

Table 2-Distribution of various serotypes

Serotype	Number(%)
V.cholerae Ogawa	66(91.67%)
V.cholerae Inaba	0
V.cholerae Hikojima	6(8.33%)

Majority of cholera patients are usually treated by replacement of fluids and electrolytes, and only a small proportion with severe disease require antibiotic treatment.[6] In India, cholera patients are treated with antibiotics along with a correction of dehydration status. Therefore, it becomes important to analyze the trends of antibiotic resistance among the clinical strains of *V. cholerae*. However, unlike other bacterial infections, little is reported about resistance patterns of *V. cholerae,* as laboratories do not routinely test susceptibility to different classes of antimicrobial agents.[2] The antibiotic sensitivity profile showed that resistance was observed in both serotypes to most of the routinely used drugs. All isolates were sensitive to Amikacin. Tetracycline is still an effective drug. The resistance pattern is shown in table 3

Table 3- Antibiotic resistance pattern of the serotypes

Serotype	Ampicillin No. (%)	Gentamicin No. (%)	Ciprofloxacin No. (%)	Cotrimoxazole No. (%)	Tetracycline No. (%)	Nalidixic acid No. (%)
Ogawa	52(79)	12(18%)	13(25%)	64(97%)	19(20%)	66(100%)
Hikojima	4(67%)	2(33%)	4(67%)	6(100%)	1(16%)	6(100%)

Literature on the antibiotic susceptibility of cholera organisms from most developing countries is patchy. Worldwide, *V. cholerae* 01 strains resistant to tetracycline, trimethoprim/ sulpha methoxazole and ampicillin are common. In many of these studies, the main reasons for the rapid rise in antimicrobial resistance have been extensive antimicrobial prophylaxis, unauthorized dispensing and use of these agents in animal husbandry.[6]

The antibiotic resistance patterns of epidemic strains isolated from Bangladesh have documented reduced susceptibility to ampicillin, furazolidone, neomycin, streptomycin.[12] High-level resistance to choramphenicol, ampicillin, ciprofloxacin, neomycin, nalidixic acid and norfloxacin has been reported from Kolkata.[13]

Study by C Chandralekha et al. reported100% resistance to cotrimoxazole, nalidixic acid and a variable sensitivity to ampicillin in 13 Hikojima isolates. [14]

In the present study, significant increase in resistant strains was observed against nalidixic acid, co-trimoxazole and ampicillin. This correlated with earlier studies that reported resistance of *V.cholerae* isolates to these antibiotics. [1,14,15,16]

Tetracycline which is generally used for treatment of cholera still remains susceptible to a large extent in our study. To avoid emergence of resistance to this drug discourage using this for prophylaxis in contacts of cholera patients, as there can be a significant increase in the proportion of *V. Cholerae* 01 developing resistance to tetracycline which could be attributed to antibiotic pressure. Rotational use of anti-cholera antibiotics may lead to emergence of fully susceptible strains over time, which may allow for extension of use of the most effective therapies such as tetracycline.[6]

CONCLUSION

The present outbreak was due to *V. cholerae* El Tor biotype with Ogawa as the main serotype few Hikojima strains were also isolated. A significant proportion of *V. cholerae* 01 isolates were resistant to commonly used antimicrobial agents. Hikojima serotype was more resistant than Ogawa in the present study. The study reflects the importance of close monitoring and surveillance of all cholera outbreaks to know the prevalent serotype. Susceptibility pattern for vibrio is essential to avoid emergence of resistant strains by empirical treatment. Of utmost importance is the need to establish regular nationwide antibiotic susceptibility surveillance of *V. cholerae* 01 in different parts of the country in order to provide guidance on the best options in different situations.

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