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# Escalating emergence of Fluoroquinolone-Resistant Strains of *Vibrio cholerae* O1 and O139 Among Hospitalized Patients with Cholera in Orissa, India

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**Eight hundred forty *Vibrio cholerae* O1 and 171 O139 strains isolated from diarrhoea patients from 1995-2007 in Orissa, India were analyzed to determine the changing trends of fluoroquinolone susceptibility pattern. Emergence of ciprofloxacin, 17.4% and norfloxacin, 13% resistant *V. cholerae* O1 was found in 1995 and peaked to 64. 8% and 63.2% respectively in the year 2007. Similarly emergence of ciprofloxacin, 13.7% and norfloxacin, 10.3% resistant *V. cholerae* O139 was found in 1995 and both peaked to 62.2% in the year 2001. Nalidixic acid resistant *V. cholerae* O1 was observed throughout the study period where as the emergence of nalidixic acid resistant *V. cholerae* O139 was found for the first time in 1999. Selective pressure exerted by nalidixic acid and disproportionate use of fluoroquinolones may be the predeterminant of the genesis of fluoroquinolone resistant *V. cholerae* O1 and O139 serogroup which must be closely monitored.**

**Key words:** *Vibrio cholerae*, Antibiotics, Resistance & Mutation.

Cholera is a major public health problem causing large morbidity and mortality in developing countries where ciprofloxacin and norfloxacin are the drug of choice possessing excellent activity against the clinical strains of *V. cholerae* O1 and O139 serogroups[1]. Clinical studies have shown that ciprofloxacin and norfloxacin are effective in treatment of cholera in adults and children[2]. Emergence of fluoroquinolone resistant O1 and O139 strains has appeared as a great threat in theuraptic practice in the world and was observed in different corner of India during the past one decade, although few in numbers[3]. Orissa an eastern state of Indian subcontinent, experiences cholera in each year due to *V. cholerae* O1 and O139[4-5]. For the last 12 years we have been monitoring the incidence of antimicrobial susceptibility and genotyping in *V. cholerae* isolates from cholera patients admitted to different hospitals of Orissa. Here we report

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the emergence of fluoroquinolone resistant *V. cholerae* O1 El Tor biotype and O139 serogroup at a higher rate isolated from diarrhoea patients in Orissa.

## MATERIAL AND METHODS

Thiosulphate-citrate-bile salt sucrose agar (TCBS, Eiken, Tokyo, Japan) was used as the selective medium for the isolation of *V. cholerae*. Individual rectal swabs collected from diarrhoea patients were inoculated on TCBS plate and streaked for colony isolation. The inoculated plates were incubated at 37°C for 18-24 h and subsequently examined for growth of *V. cholerae.* A multitest medium was used for presumptive identification of *V. cholerae*[6]. Serogroup of *V. cholerae* was done using growth from the multitest medium with polyvalent O1 and monospecific Inaba and Ogawa antisera (Difco, USA). *V. cholerae* strains which did not agglutinate with the O1 antiserum were checked with monoclonal O139 antiserum developed at NICED, Kolkata[7].

All the 1017 *V. cholerae* strains used in this study were of clinical origin were isolated during the period from June 1999 to November 2003 from hospitalized diarrhea patients. Isolation of *V. cholerae* O139 was decreased from 2001 and disappeared in 2003. Of the total strains, 840 and 171 were *V. cholerae* O1 and O139 respectively. All the *V. cholerae* O1 and O139 strains were included in this study for the analysis of drug resistance patterns.

Antimicrobial susceptibility analysis of *V. cholerae* strains was performed by disc diffusion method[8] on Muller-Hinton agar (Difco, Detroid, Mich) with commercial disc (Himedia, Mumbai, India). The antimicrobials used were ciprofloxacin, 5 µg; nalidixic acid, 30 µg; norfloxacin 10 µg and tetracycline, 30µg. Characterization of strains as susceptible or resistant was based on the size of the inhibition zone around each disc according to manufacturer's instruction which matched interpretive criteria recommended by WHO[9]. These zone size interpretive criteria for susceptibility corresponded to MICs of 0.25, 0.06 and 0.06µg/ml for nalidixic acid, ciprofloxacin and norfloxacin respectively. Strains showing intermediate zone of growth inhibition were interpreted as resistant to that drug on the basis of previous MIC studies conducted with *V. cholerae*[10].

## RESULTS AND DISCUSSION

We report the fluoroquinolone resistant *V. cholerae* O1 from the beginning of the study

**Table 1.** Resistance to quinolone among *V. cholerae* O1 and O139 isolates from cholera patients.

| | No. of resistant strains (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No of strains | | Nalidixic acid | | Ciprofloxacin | | Norfloxacin | |
| | O1 | O139 | O1 | O139 | O1 | O139 | O1 | O139 |
| 1995 | 23 | 3 | 23(100) | 0 (0.0) | 4(17.4) | 0 (0.0) | 3(13) | 0 (0.0) |
| 1996 | 7 | 0 | 7(100) | 0 (0.0) | 1(14.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| 1997 | 17 | 0 | 16(94.1) | 0 (0.0) | 1(5.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| 1998 | 11 | 4 | 11(100) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| 1999 | 54 | 29 | 53(98.1) | 5(17.2) | 34(62.9) | 4(13.7) | 9(16.6) | 3(10.3) |
| 2000 | 78 | 72 | 73((93.5) | 72(100) | 34(43.5) | 28(38.8) | 12(15.3) | 12(16.6) |
| 2001 | 56 | 32 | 56(100) | 32(100) | 23(41) | 20(62.2) | 7(12.5) | 20(62.2) |
| 2002 | 71 | 23 | 67(94.3) | 23(100) | 6(8.4) | 5(21.7) | 3(4.2) | 0 (0.0) |
| 2003 | 213 | 0 | 213(100) | 0 (0.0) | 47(22) | 0 (0.0) | 47(22) | 0 (0.0) |
| 2004 | 47 | 0 | 47(100) | 0 (0.0) | 12(25.5) | 0 (0.0) | 15(31.9) | 0 (0.0) |
| 2005 | 83 | 0 | 82(98.8) | 0 (0.0) | 3(3.6) | 0 (0.0) | 4(4.8) | 0 (0.0) |
| 2006 | 55 | 8 | 53(96.3) | 5(62.5) | 8(14.5) | 0 (0.0) | 3(5.4) | 2(25) |
| 2007 | 125 | 0 | 107(85.6) | 0 (0.0) | 81(64.8) | 0 (0.0) | 79(63.2) | 0 (0.0) |

in 1995 besides the nalidixic acid resistance. In 1999 and 2007 we have recorded highest occurrences of ciprofloxacin and norfloxacin resistant *V. cholerae* O1 through wavering trend (Table 1). It is noteworthy to mention that equally 61.3% ciprofloxacin and norfloxacin resistant *V. cholerae* O1 were isolated in a recent cholera outbreak in September, 2007 affecting more than 104872 populations and 159 deaths (Data not published). To our knowledge this is the first report on such a high incidence of fluoroquinolone resistance among toxigenic *V. cholerae* strains. The MICs of ciprofloxacin, norfloxacin and nalidixic acid for ciprofloxacin, norfloxacin and nalidixic acid resistant *V. cholerae* strains ranged between 0.5 and 2 mg/L, 0.5 and 5 mg/L and 8 and e" 240 mg/L respectively; when tested with Hi-comb test strips (Hi-media, Mumbai, India) on Muller-Hinton agar. The incidence of nalidixic acid resistance among *V. cholerae* O1 was almost 100% in each year where as O139 was completely sensitive before 1999 and peaked during subsequent years as shown in Table-1. Possibly ciprofloxacin and norfloxacin resistance might have emerged in direct response to the selective pressure exerted by nalidixic acid and disproportionate use of fluoroquinolones in the clinical settings. This portend is exhibited in the present study with the high (100%) incidence of nalidixic acid resistant strains of *V. cholerae* O1 (probably with a single mutation in *gyrA* and/or other related genes). Similarly the emergence of quinolone and fluoroquinolone resistant O139 in 1999 was observed contrast to the incidence during and before 1998. One possible explanation is that emergence of nalidixic acid resistance could be the prerequisite for the development of fluoroquinolone resistance in O139.

From 1995, emergence of fluoroquinolone resistant *V. cholerae* O1 passed through wavering trend and peaked in 2007 almost equally. The possible explanation for this may be probably excessive use of ciprofloxacin and norfloxacin initiated resistance causing treatment failure. Condition of high selective pressure would have forced the mutant *V. cholerae* to multiply, the progeny of which might have outnumbered the susceptible strains to acquire high peak. The clonal nature of *V. cholerae* strains determined by molecular analysis[11] and dendrogram[12] constructed from phylogenetic tree provides evidence that fluroquinolone resistance may have developed due to closely related strains. However additional continuous study is required to acknowledge the probable spectrum of mechanism involved in the evolution of fluoroquinolone resistant *V. cholerae* O1and O139 strains.

Emergence of fluoroquinolone resistance in *V.cholerae* has appeared as a great threat in the therapeutic field and must be carefully monitored. Fortunately, *V. cholerae* O1 and O139 strains are susceptible to tetracycline which is an effective drug for treatment of cholera in the state of Orissa and used as the best panacea in the recent cholera epidemic and saved many lives. However, until a best alternative drug is identified for use in children and adults the problems in treating patients remain serious. Therefore methods to control the spread of bacterial resistance, rationale use of fluoroquinolones, change to newer antimicrobial, rotational use of drugs and constant surveillance should be adopted.

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