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Trend of antibiotic resistance of *Vibrio cholerae* strains from East Delhi

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***Background & objective*: Epidemics of cholera caused by toxigenic *Vibrio cholerae* O1 and O139 (Bengal strain) represent a major public health problem in most developing countries. In view of the reported shift in epidemiology and pattern of antibiotic resistance in this was study carried out to assess the development of resistance to essential drugs like fluroquinolones during treatment of cholera and cholera like cases in Delhi.**

***Methods*: Faecal specimens collected from 1184 patients with cholera and cholera like illness between 2001-2006 admitted to Guru Teg Bahadur hospital, East Delhi were subjected to culture isolation. Antimicrobial susceptibility testing of *V. cholerae* isolates was done by disc diffusion method.**

***Results*: Of the 1184 faecal samples examined, 670 (56.6%) were positive for *V. cholera* from 2001-2006. *V. cholerae* El Tor Ogawa (54.6%) was more common than serotype Inaba (32.5%). During 2004-2006 *V. cholerae* Inaba emerged as the predominant serotype. Resistance to nalidixic acid, furazolidone and co-trimoxazole was constantly high (100%). Multiple antibiotic resistance (MAR) *V. cholerae* O1 Inaba isolates exhibited increased resistance to ciprofloxacin with MIC >4 µg/ml, but largely all remained susceptible to other antibiotics like, gentamicin, tetracycline and chloramphenicol.**

***Interpretation & conclusion*: *V. cholerae* have a permanent existence in the environment and during the quiescent period, their survival in water bodies allows dissipation of resistance patterns to different serotypes or strains of *V. cholerae* O1 and therefore there is need for constant observation.**

Key words Antibiotic resistance - cholera - Inaba serotype

The epidemic spread of cholera from the Indian subcontinent to rest of the world attributed to the ease of travel and increasing number of travelers, contributing to the spread of cholera[1,2]. It was after 1961, the El Tor biotype apparently spread out of Indonesia to other Asian countries resulting in the cholera pandemic and remained for a decade globally. In 1992, an unprecedented event occurred in the history of this disease - the emergence of *Vibrio cholerae* O139 Bengal[3]. After an explosive expansion in 1992 to 1994,

especially in children and young adults, it remained in this country and continued to cause sporadic diarrhoea in the following years[4]. However, during this period, the El Tor biotype of *V. cholerae* O1 reappeared in Kolkata and other parts of the Indian subcontinent once again assuming a dominant serogroup causing cholera[5]. *V. cholerae* O1 biotype El Tor mostly belonged to serotype Ogawa. Except for a few scattered reports of cholera due to *V. cholerae* O1 Inaba in 1998 and 1999[6], *V. cholerae* O1 Ogawa has always been the predominant serovar causing outbreaks. During 2004 and 2006, the frequency of isolation of *V. cholerae* O1 Inaba strains steadily increased in our hospital. The emergence of multiple antibiotic resistant isolates of *V. cholerae* (MAR)[7] and the changing pattern of antibiograms has increased concerns of treating cholera with antibiotics or reconsidering the role of antibiotics in cholera epidemics.

The present study was thus undertaken to understand the changing patterns of *V. cholerae* isolations in and around Delhi and the development of antibiotic resistance in these organisms.

Material & Methods

A total of 670 isolates of *V. cholerae* were obtained between January 2001 to December 2006, from 1184 stool samples, collected from all consecutive cholera and cholera like patients admitted to Guru Teg Bahadur hospital, East Delhi. *V. cholerae* were identified by standard laboratory methods[8,9] and confirmed by serotyping[10] using specific antisera (Difco, USA; NICED Kolkata). Hence, the number of isolates to be studied were selected on the basis of their culture isolation and serotyping. Antimicrobial susceptibility analysis was performed by disc diffusion technique with commercially available discs (Hi-Media, Mumbai, India) and MIC of Ciprofloxacin was determined as per standard guidelines[11]. The following antibiotics were used: ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5µg), co-trimoxazole (25µg), furazolidone (50 µg), gentamicin (10µg), nalidixic acid (30µg) and tetracycline (30µg). Isolates were characterized as susceptible, intermediate resistant or resistant based on size of the inhibition zones according to the standard guidelines. Phage typing was done at Vibrio phage reference laboratory National Institute of Cholera and Enteric Disease (NICED), Kolkata, following the conventional Basu and Mukherjee's method and the new phage-typing scheme[12,13].

Statistical analysis: Simple linear regression analysis was done taking time as independent and antibiotics (ciprofloxacin and co-trimoxazole) as dependent variables to predict the change with respect to years.

Results

Of the 1184 faecal specimens 670 (56.6%) were positive for *V. cholerae*. *V. cholerae* O1 El Tor was isolated in 584 (87.2%) cases which was more common than *V. cholerae* O139 re-covered in 19 (2.8%) cases and *V. cholerae* non O1 non O139 in 67 (10%).

Until 2003, *V. cholerae* O1 Ogawa was the predominant isolate. However, from 2004 to 2006 *V. cholerae* O1 Inaba was isolated in large numbers. (Fig. 1). The occurrence of non O1 non O139 was fairly constant, occurring sporadically in low numbers. All isolates during the year 2001 to 2006 depicted a constant increase in resistance to co-trimoxazole. The resistance to ampicillin and furazolidone has remained constant throughout these years (Table I). The gradual increase in resistance to nalidixic acid in the past several years[14] has eventually led to its 100 per cent resistance in the present study period. In the year 2004, 10 per cent isolates of *V. cholerae* O1 inaba showed resistance to ciprofloxacin and in the subsequent years, the number increased to 21 and 30.4 per cent (Fig. 2). The minimum inhibitory concentration (MIC) of ciprofloxacin was >" 4 µg/ml by agar dilution method. Antimicrobial resistance towards ciprofloxacin (Cip-12869.41+6.429 X years) and Co-trimoxazole (Cot-12123.65+6.094 X years) reflected a significant linear regression trend for

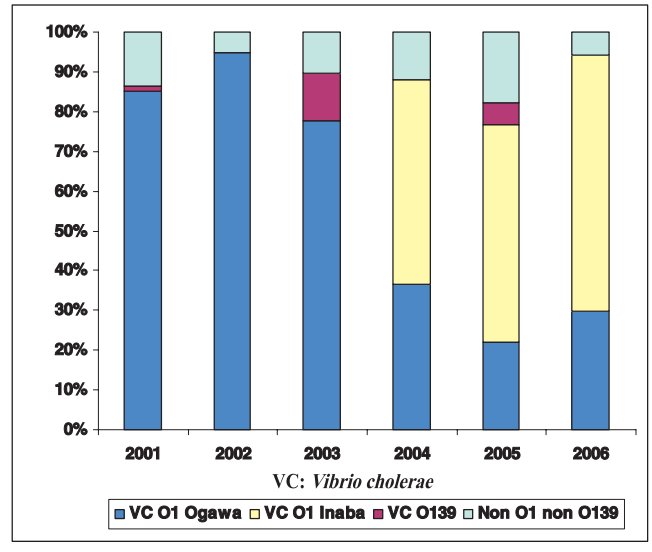

Fig. 1. *V. cholerae* isolates from 2001 - 2006.

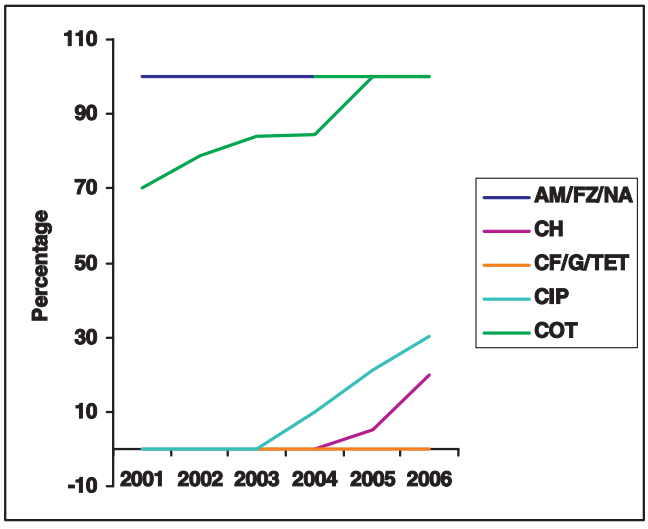

Fig. 2. Year-wise trend of drug resistance pattern of *V. cholerae*. AM: Ampicillin; FZ: Furazolidone; NA: Nalidixic acid; CH: Chloramphenicol; CF: Cefotaxime; G: Gentamicin; TET: Tetracycline; CIP: Ciprofloxacin; COT: Co-trimoxazole. (Cip- $P<0.007$; Cot- $P<0.002$).

the years 2001 to 2006 ($P<.05$). The MICs of ciprofloxacin resistant *V. cholerae* O1 for the years 2001-2006 are depicted in Table II.

The phage typing pattern (Basu and Mukherjee scheme) of *V. cholerae* O1 isolates showed a dominance of phage type 2 and 4 throughout the study period. With the new typing scheme, most were distributed among phage T 27 and T 23.

Discussion

Annual outbreaks of cholera are a regular feature in our country. A high population density along with open drains and poor sanitation provides an optimal niche for survival, sustenance and transmission of *V. cholerae*. Our data clearly demonstrate that the dynamics of *V. cholerae* transmission is complex with different serogroups predominating at different times. The seasonal outbreaks of cholera are a reminder of the endemicity of the illness and its emergence as an important pathogen of acute watery diarrhoea.

This study was an attempt to highlight the divergence of *V. cholerae* O1 isolates and subsequent emergence of drug resistance during different time period (January 2001 – December 2006) from hospitalized patients with cholera.

From 2001 to 2006 there has been a reduction in the number of diarrhoeal cases probably attributing to enhanced general awareness and local sanitation facilities made available to the slum areas. *Vibrio cholerae* O1 isolates predominantly belonged to serotype Ogawa. However, in the year 2004, there was an evident change in the serotype to Inaba - an emergence after an interval of several years. Sporadic reports of *V. cholerae* O1 Inaba were reported earlier in 1998-1999 from Delhi[6], but never exceeded to large numbers. *V. cholerae* O1 El Tor Inaba has been isolated for the first time in 2003 in our hospital. This serotype was quiescent for a long time since 1985, and reappeared in Warangal and some parts of Delhi in 1998-1999[15]. Such a serotype conversion from Ogawa to Inaba during an infection is simply a mutant enrichment produced with antibodies to Ogawa. Such pre-existing Ogawa antibodies or perhaps antimicrobial selection pressure may be responsible for these sequential changes.

The frequency of conversion of Ogawa to Inaba is approximately 10^5 whereas the conversions of Inaba to Ogawa are rare and may be strain dependent[16,17]. The genes responsible for O1 antigen biosynthesis wbe (rfb) are localized on a 21.6 kb sac I fragment of DNA[16]. This ability of wbe mutants to persist and amplify in environment may lead to epidemics in the subsequent season.

The use of antimicrobial agents is generally accepted as a method of reducing the duration and volume of diarrhoea as well as decreasing the period of *V. cholerae* excretion in stool. Multiple antibiotic resistant (MAR) *V. cholerae* with epidemic outbreaks (both classical and El Tor biotypes) have been reported in Bangladesh[7,18].

Even though the reservations about the use of ciprofloxacin as a first line of treatment in such cases of MAR cholera has been expressed in developing countries[19]; the high level resistance to nalidixic acid has led to the use of ciprofloxacin in paediatric cases in our hospital in the year 2004 to 2005. Subsequent reports of relapses and treatment failure led to the determination of MIC of ciprofloxacin, which was found to be high. This was responsible for the emergence of ciprofloxacin resistance in *V. cholerae* O1 Inaba in our hospital[20]. Such resistance can be due to spontaneous mutation in *V. cholerae* or transfer of resistance from other co-inhabiting microbes, which are fluroquinolone resistant. The profiles of major MAR *V. cholerae* as documented in Kolkata and other parts of India and Bangladesh are: AFZ (Ampicilllin, Furazolidone), AFZN (Ampicillin, Furuzolidone, Neomycin), AFZ NS

Table I. Resistance pattern (%) of *V. cholerae* against various antimicrobials during 2001- 2006

Drugs	2001(n=74)	2002 (n=114)	2003 (n=107)	2004 (n=109)	2005 (n=95)	2006 (n=171)
Ampicillin	100	100	100	100	100	100
Chloramphenicol	0	0	0	0	5	20
Cefotaxime	0	0	0	0	0	0
Ciprofloxacin	0	0	0	10	21	30.4
Gentamicin	0	0	0	0	0	0
Furazolidone	100	100	100	100	100	100
Co-trimoxazole	70	79	84.1	84.4	100	100
Nalidixic acid	100	100	100	100	100	100
Tetracycline	0	0	0	0	0	0

Table II. MIC of ciprofloxacin of *V. cholerae* isolates from 2001-2006

Year	MIC of ciprofloxacin(µg/ml)								
	<1	1	2	4	8	16	32	64	128
2001(n=74)	74	-	-	-	-	-	-	-	-
2002(n=114)	114	-	-	-	-	-	-	-	-
2003(n=107)	107	-	-	-	-	-	-	-	-
2004(n=109)	98	-	4	6	1	-	-	-	-
2005(n=95)	75	-	-	5	6	-	-	9	-
2006(n=171)	119	-	-	-	-	9	-	30	13

(Ampicillin, Furazolidone, Neomycin, Streptomycin)[21]. The antibiotic resistance pattern of epidemic strains have changed frequently with the emergence of different *V. cholerae* O1 or O139 strains. Therefore selection of such drug resistant clones can lead to seasonal epidemics of cholera with emergence of new clones replacing the existing clones.

The knowledge of nalidixic acid resistance leading to low level resistance to ciprofloxacin is well documented in other enteric pathogens which could transfer these plasmids to other organisms[19,22]. Synchronized monitoring of such clinical as well as environmental strains thus becomes essential in order to understand the clonal spread of these novel MAR strains in the cholera endemic areas.

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