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Epidemiology & antibiograms of *Vibrio cholerae* isolates from a tertiary care hospital in Chandigarh, north India

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***Background & objectives*: Cholera is endemic in Chandigarh and its surrounding areas. This retrospective study was undertaken over a period of nine years (January 1999-December 2007) from a tertiary care hospital in north India to understand the changing epidemiology aspects and antibiotic resistance patterns in *Vibrio cholerae* isolates.**

***Methods*: A total of 277 isolates of *V. cholerae* were included in the study. *V. cholerae* was identified by standard microbiological procedures. Antibiotic sensitivity testing was performed by disc diffusion method and isolates phage typed.**

***Results*: All the isolates were identified as *V. cholerae* O1 biotype El Tor serotype Ogawa; phage 27 was the predominant type. Men were more commonly affected with maximum number in the age group 0-5 yr. Majority of the isolates were resistant to furazolidone but sensitive to gentamicin and cefotaxime. Resistance pattern to amoxycillin was variable. Three isolates were found to be resistant to ciprofloxacin. All the patients presented during June-October coinciding with the monsoon season and a majority were from suburbs.**

***Interpretation & conclusions*: The emergence of resistance amongst *V. cholerae* especially towards ciprofloxacin may significantly influence the control strategies in future outbreaks. Phage 27 remained the predominant type in all the years. Continuous surveillance with regard to drug resistance, early detection and a strong regional commitment may help contain the disease.**

Key words Cholera - drug resistance - epidemiology - Ogawa - phage type - *Vibrio cholerae*

Cholera outbreaks occur seasonally and are associated with monsoon season, warm temperature, heavy rain fall and increased plankton population. *Vibrio cholerae* has a unique ability to exist in an autochthonous state in riverine and brackish water estuaries and coastal waters and can exist in dwarfish forms in response to nutrient deprivation as a viable but non culturable form. It can exist in the gut and attached to the surface of both fresh water and marine copepods. In addition, the genetic assortments and reassortments that are going on in these isolates, equip them appropriately to survive better in the changing

environmental conditions. These also contribute to the increase in drug resistance amongst the *V. cholerae* strains[1,2].

The geographical distribution of cholera is changing and so is often considered as a re-emerging disease, in part because infections are appearing in novel communities or in communities where the disease had been absent for many years[3]. This may be due to the changes in the environment or climate, following the EI Nino phenomenon which has made conditions favourable for cholera worldwide[4]. The problem of global warming[5] and inland incursion of sea water covering more and more of the coastal stretches of land could lead us to the brink of a resurgent pandemic. Worldwide there has been increase in the number of cholera cases and outbreaks in the new communities and with changing profiles[3,6].

In the Asian region, the Indian subcontinent continues to harbour a major chunk (78%) of cholera cases[3]. Outbreaks of cholera including major epidemics have occurred from time to time at various places in India[6-14]. For the last nine years, cholera has been regularly resurfacing in Chandigarh, coinciding with onset of the monsoon season generally from June to September[12-14]. We studied the epidemiological profile and the changing trends in antimicrobial resistance among *V. cholerae* isolated during 1999 to 2007 in a tertiary care hospital in Chandigarh, north India.

Material & Methods

A total of 277 isolates of *V. cholerae* were obtained between 1999 and 2007 from suspected cases of cholera patients admitted to the Government Medical College Hospital (GMCH), Chandigarh. The stool samples of such patients were collected in a sterile container and enrichment was done in alkaline peptone water (APW, *p*H 8.0) for 6-8 h. Before the samples were plated, hanging drop preparation was made to confirm the typical darting motility of the *V. cholerae* if present, both directly from the sample and after enrichment in APW. The samples were plated on to MacConkey agar, blood agar and thiosulphate citrate bile salt sucrose (TCBS). The isolates obtained were identified as *V. cholerae* according to the recommended standard laboratory methods[15] and further confirmed by serology using antisera obtained from the Central Research Institute (CRI), Kasauli, India. Antimicrobial susceptibility was carried out as per the standard recommendations[16] using eight antimicrobial agents (µg/disc): amoxycillin (Am; 10), cefotaxime (Ce; 30), tetracycline (Te; 30), ciprofloxacin (Cf; 5) and furazolidone (Fz; 50), chloramphenicol (Ch; 30), gentamicin (G; 10) and co-trimoxazole (Co; 25). Standard strain of *Escherichia coli* ATCC 25922 was used as control strain. The culture media and antibiotic discs used were obtained from Hi-Media Ltd, Mumbai, India. All isolates of *V. cholerae* were sent to National Institute of Cholera and Enteric Diseases (NICED), Kolkata, for confirmation and phage typing[17,18].

Results & Discussion

During the period of nine years, 277 cases were confirmed microbiologically as cholera. A significant outbreak of cholera (56 cases) occurred in 1999 followed by only a few cases in 2000, and 29 in 2001. In July 2002 the city witnessed a major outbreak of cholera (61 cases) and was also reported in other studies[13,14]. Subsequent to this, a substantial number of cases occurred in 2003 and 2004, respectively. The last three years showed a steady number of cases, approximately 14, per season. The study of the epidemiology of cholera outbreaks showed that there was no readily discernible patterns (Fig). Males outnumbered the females by 1.6:1. Most of the cases *i.e.,* 87 (31.4%) were in the younger age group (0-5 yr) followed by 42 (15.1%) in the age group 6-10 yr and 36 (12.9%) in the age group 11-15 yr (Table I). Cholera affects all ages and both sexes. However, infection rate is increasingly reported in children[6,19,20]. In our study also, 165 (57%) cases were in age group less than 15 yr, with maximum cases in age group less than 5 yr (87, 29%).

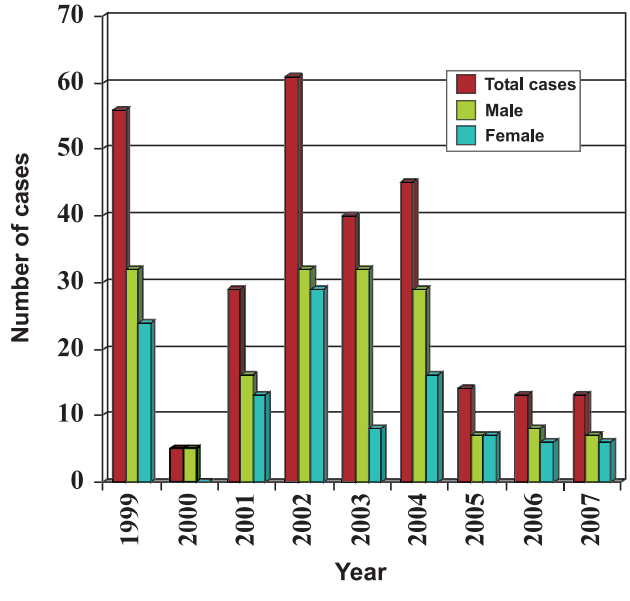

Fig. Year-wise and sex-wise distribution of the cases of cholera.

Table I. Distribution of cholera cases among different age groups

Year	Age group (yr)												
	0-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65
1999	11	5	5	8	7	6	3	3	3	3	0	2	0
2000	2	1	1	0	0	1	0	0	0	0	0	0	0
2001	12	6	3	2	0	1	1	2	2	0	0	0	0
2002	15	10	9	5	6	3	3	3	1	4	0	2	0
2003	7	8	12	2	4	2	4	0	0	0	0	0	1
2004	21	5	3	2	2	4	2	1	1	1	1	1	1
2005	5	1	2	1	2	0	0	0	0	1	0	1	1
2006	5	3	0	1	2	2	0	0	0	1	0	0	0
2007	9	3	1	0	0	0	0	0	0	0	0	0	0
Total	87	42	36	21	23	19	13	9	7	10	1	6	3

Table II. Phage typing of *V. cholerae* isolates

Years	Phage group (number of isolates)
2002	27 (57), 23 (1),13 (2), 7 (1)
2003	27 (26), 26 (5), 13 (3), 15 (2), 10, 17, 18, 20 (one each)
2004	27 (42), 26, 23, 15 (one each)
2005	27 (13), 26 (1)
2007	27(8), 26 (2), 24 (1), untypeable (2)

Isolates were not phage-typed during the years 1999, 2000, 2001, and 2006

Another important aspect of our study was that a number of cases were from slum dwellings or suburban areas in and around Chandigarh. *V. cholerae* is a known faeco-oral pathogen and indeed, infection rates were significantly higher in areas with poor sanitation. Mostly, the slums are occupied by migrant population where the hygienic conditions are quite often compromised.

Interestingly, the importance of weather and climate as having effect on water quality is being increasingly recognized[4]. In this study, all the cases presented during the months of June to October. The occurrence of cases correlated well with the onset of monsoon in this region. Rains increase the level of surface water and have been shown to be linked to leakage of water pumps and mixing of stagnant water through broken pipelines[12,14]. Such untreated water sources are used by people living in slum dwellings and suburban areas for bathing, cooking and drinking which enhances the chances of infection.

All the isolates were *V. cholerae* O1, biotype El Tor, serotype Ogawa. Phage typing was done for the isolates collected during 2002-2005 and 2007. Most of the isolates belonged to Phage T4 as per Basu-Mukerjee scheme[17]. According to the new scheme for phage typing developed by NICED[18], most of the isolates were of phage group 27 with a few exceptions (Table II). The percentage prevalence for phage 27 from the years 2001-2005 and 2007 was 93.4, 65, 93.5, 92.85 and 61.53 per cent respectively. The results of phage typing were consistent with the overall countrywide epidemiological data[21] which report type 27 to be the predominant one. The present findings corroborated well with previous studies[22]. This suggests that a particular clone of *V. cholerae* O1 strain is probably circulating all over India.

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[23].

The antibiogram over the period of nine years showed that isolates were consistently sensitive to gentamicin and cefotaxime (Table III). However, low level of resistance was seen to chloramphenicol (3.70-6.56%) and tetracycline (4.34-15.38%). These findings corroborated with other studies[6,13,14], which also reported isolates being sensitive to gentamicin and tetracycline. However, there are reports of tetracycline resistant *V. cholerae* strains responsible for major epidemics of cholera in Latin America, Tanzania, Bangladesh and Zaire[24]. The resistance profile of *V. cholerae* is known to show variations, depending on the local antibiotic over use/abuse at that period of time[25]. Hence even a low level of resistance (15.38%) is significant and requires monitoring.

Resistance to amoxycillin was found to be 20 per cent in 1999, with a peak of 100 per cent in the year 2002 and then decreasing to 14.28 in the year 2006.

Table III. Antibiograms (resistance percentages) of isolates of *V. cholerae*

Years	Am	Ce	Te	Ch	G	Cf	Fz	Co
1999	9/45	0/34	0/49	2/51	0/42	0/27	47/56	28/28
	(20)	(0)	(0)	(3.9)	(0)	(0)	(83.9)	(100)
2000	2/5	0/4	0/5	0/5	0/4	0/5	4/5	4/4
	(40)	(0)	(0)	(0)	(0)	(0)	(80)	(100)
2001	0/29	0/26	0/22	1/27	0/29	0/21	22/29	25/27
	(0)	(0)	(0)	(3.7)	(0)	(0)	(75.9)	(92.6)
2002	60/60	0/54	0/56	4/61	0/58	1/46	60/60	61/61
	(100)	(0)	(0)	(6.6)	(0)	(2.17)	(100)	(100)
2003	0/40	0/32	1/23	0/40	0/15	0/29	33/40	30/36
	(0)	(0)	(4.3)	(0)	(0)	(0)	(82.5)	(83.3)
2004	0/42	0/40	6/42	2/34	0/29	2/34	38/44	35/42
	(0)	(0)	(14.3)	(5.9)	(0)	(5.9)	(86.4)	(83.3)
2005	7/13	0/11	0/14	0/13	0/11	0/14	11/14	0/13
	(53.84)	(0)	(0)	(0)	(0)	(0)	(78.6)	(0)
2006	2/14	0/0	2/14	0/14	0/10	0/13	12/14	13/13
	(14.3)	(0)	(14.3)	(0)	(0)	(0)	(85.7)	(100)
2007	8/11	0/5	2/13	0/13	3/12	1/12	5/11	9/10
	(72.7)	(0)	(15.4)	(0)	(25)	(8.33)	(45.4)	(90)

Am, amoxicillin; Ce, cefotaxime; Te, tetracycline; Cf, ciprofloxacin; Fz, furazolidone; Ch, chloramphenicol; Co, co-trimoxazole; G, gentamicin

Overall, *V. cholerae* remained sensitive to amoxycillin except for the year 2002 when all isolates (100%) were found to be resistant. The resistance level to co-trimoxazole decreased from 100 to 83 per cent over the years 2003, 2004 and fell to 0 per cent resistance in 2005. However, during 2006 and 2007 there was 100 and 90 per cent resistance respectively (Table III). A study from Calcutta[26] reported an increase in resistance to ampicillin, co-trimoxazole, and nalidixic acid from the year 1994 onwards.

Resistance to furazolidone was consistently high (75-100%) from 1999-2006, however, it fell to 45.45 per cent in 2007. A similar trend has been reported earlier from other studies[6,13,14,26].

Our results showed that three isolates, two in 2004 (2/34, 5.9%) one in 2007 (1/12, 8.3%) were found to be resistant to ciprofloxacin. Isolation of ciprofloxacin resistant isolates of *V. cholerae* has also been reported earlier from India[27]. Extensive and injudicious use of this drug for the empirical treatment of diarrhoeal infections probably led to the emergence of ciprofloxacin resistant *V. cholerae* for the first time in Calcutta during 1992[27].

In conclusion, continuous monitoring of the changing trends in antimicrobial resistance patterns is a must, as in our study, three isolates were found to be ciprofloxacin resistant. 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.

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