Research Article

Endemic Cholera in Kolar Region, 2001-2014: Seasonality, Serotypes, and Reversal of Resistance to Antibiotics among *Vibrio cholerae* Isolates

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Abstract

Background: Cholera is endemic in many parts of India. Though epidemics of cholera are frequently reported, there is a paucity of reports on endemic cholera. Biotypes, serotypes, and antibiogram of *Vibrio cholerae (V. cholerae)* isolated from patients admitted to a tertiary care hospital in Kolar have been analyzed.

Methods: Stool samples or rectal swabs from patients suspected of cholera from 2001 to 2014 were cultured and the isolates were identified by standard biochemical tests; serotyping was done by slide agglutination test. The antibiogram was determined by the Kirby-Bauer disc diffusion method.

Results: V. cholerae was isolated from 122 hospitalized patients of Kolar region, Karnataka, during the 14 years of study. The age of the patients ranged from 8 months to 80 years (mean 34 years). Fourteen percent of the patients were less than 10 years of age. But for a lone isolate of O139 in the year 2001, all the isolates belonged to *V. cholerae* serogroup O1 El Tor biotype; 90.1% were of serotype Ogawa and 9.9% were of serotype Inaba. Cholera occurred mainly during rainy season but a few cases were also seen during winter.

V.cholerae isolates, resistant to cotrimoxazole and ampicillin, were encountered throughout the study period. The strains were sensitive to ciprofloxacin and tetracycline initially. Resistance to ciprofloxacin was first recorded in 2005 and to tetracycline in 2007. However, the resistance to these two antibiotics saw a reversal. All the strains isolated after 2008 were sensitive to ciprofloxacin and those isolated after 2010 were sensitive to tetracycline also.

Conclusion: In this hospital-based laboratory surveillance for cholera conducted over a period of 14 years, it is found that Kolar region is endemic for cholera and cases occurred during monsoon as well as in winter. *V. cholerae* serogroup O1 biotype El Tor serotype Ogawa was found to be a major pathogen accounting for 90.1% and *V. cholerae* serogroup O1 biotype El Tor serotype Inaba accounted for 9.9%. Resistance to ciprofloxacin and tetracycline observed in the last decade has reversed; recently-isolated strains of *V. cholerae* are sensitive to both the drugs.

Keywords: *V. cholerae*, Biotypes, Serotypes, Reversal of resistance.

Introduction

Cholera is an acute diarrheal illness endemic in many parts of India. Outbreaks of cholera have also been reported from time to time.[1] However, cholera is thought to be under-reported due to lack of clear guidelines for reporting endemic cholera.[2] Recently, the endemicity of cholera has been defined as 'the occurrence of faecal culture-confirmed cholera diarrhea in a population in at least 3 of the past 5 years'.[3]

In this article, we report the endemicity of cholera in Kolar region over a period of 14 years from 2001 to 2014 as per the above definition and also present the biotypes, serotypes and antibiogram of the isolates.

Materials and Methods

During the years, from 2001 to 2014, stool samples or rectal swabs were collected from patients with severe gastroenteritis and clinically diagnosed as cholera admitted to R. L. Jalappa Hospital, Kolar. The samples were inoculated on blood agar, MacConkey agar and Thiosulphate citrate bile salt sucrose medium (TCBS) for the isolation of *V. cholerae*.[4] In addition, samples were also inoculated into alkaline peptone water and later subcultured on MacConkey agar and TCBS medium. Colonies suggestive of *V. cholerae* on these media were identified by standard biochemical tests and confirmed serologically.[4]

Serotyping was done by slide agglutination test with specific antisera (Denka Seiken Company Limited, Japan) and biotyping was done preliminarily by testing for sensitivity to Polymyxin B (50U). The biotypes and serotypes were further confirmed at the National Institute of Cholera and Enteric Diseases (NICED), Kolkata. Non-agglutinating strains were confirmed as O139 at NICED, Kolkata. Antibiotic sensitivity testing was done by Kirby-Bauer disk diffusion method for the following antibiotics with disks obtained from Hi-Media, Mumbai: Ampicillin (10 mcg), Ciprofloxacin (5 mcg), Tetracycline (30 mcg) and Cotrimoxazole (1.25/23.75 mcg). In the absence of CLSI guidelines on inhibitory zone diameters for interpreting sensitivity for *V. cholerae*, the sensitivity of the isolates was determined as per the interpretational zone diameters for the members of *Enterobacteriaceae*.

Results

During the years, from 2001 to 2014, *V. cholerae* was isolated from stool samples of 122 hospitalized patients who were residents of Kolar region, Karnataka. Age distribution of patients is presented in Table 1. The age of the patients ranged from 8 months to 80 years (mean age was 34 years). Seventeen (14%) of the patients were less than 10 years of age. There were 73 (60%) males and 49 (40%) female patients (sex ratio was 1:0.7).

Table 1.Age and Sex Distribution of the Cholera Cases over the Years 2001 to 2014

Age (years)	Number of patients (%)	Male (%)	Female (%)
<1	1 (0.8)	1 (100)	0 (0)
1-10	16 (13.1)	10 (62.5)	6 (37.5)
11-20	17 (14)	8 (47.05)	9 (52.95)
21-30	29 (23.8)	16 (55.17)	13 (44.83)
31-40	13 (10.65)	8 (61.54)	5 (38.46)
41-50	18 (14.75)	11 (61.1)	7 (38.9)
51-60	18 (14.75)	11 (61.1)	7 (38.9)
61-70	9 (7.38)	7 (77.8)	2 (22.2)
71-80	1 (0.8)	1 (100)	0 (0)
	122 (100)	73 (60%)	49 (40%)

The isolations of *V. cholerae* in different years along with serotypes and biotypes during the study period are presented in Fig. 1. *V. cholerae* was isolated in all the years excepting 2002 and 2014. But for a lone isolate of O139 in the year 2001, all the other isolates belonged to *V. cholerae* serogroup O1 biotype El Tor. Among the *V. cholerae* serogroup O1 biotype El Tor isolates, 109 (90.1%) belonged to the serotype Ogawa and 12 (9.9%) were of the serotype Inaba.

The cumulative distribution of cholera cases diagnosed by corresponding months over the years along with their serotypes is shown in Fig. 2. A bimodal distribution of cholera cases was observed, with a peak in August, during monsoon and another peak in November, during winter. Both Ogawa and Inaba serotypes were isolated from the cases that occurred during the monsoon. However, only Ogawa serotype was isolated from the cases that occurred during winter.

The resistance pattern of the isolates is presented in Fig. 3. Strains resistant to cotrimoxazole and ampicillin were encountered throughout the study. Overall, 82.5% strains were resistant to cotrimoxazole and 47.5% of the strains were resistant to ampicillin. The sensitivity pattern for ciprofloxacin and tetracycline showed conspicuous changes over the years with development of resistance to these drugs and again reversal to sensitivity. The *V. cholerae* strains isolated before 2005 were sensitive to ciprofloxacin and tetracycline. However, strains resistant to ciprofloxacin appeared in 2005 and those resistant to tetracycline appeared from

2007 onwards. Again, all the strains isolated since 2009 were sensitive to ciprofloxacin and to tetracycline from 2011 onwards. Thus, 38 strains of *V. cholerae* isolated after 2008 were all sensitive to ciprofloxacin and 16 strains isolated after 2010 were sensitive to tetracycline as well as to ciprofloxacin.

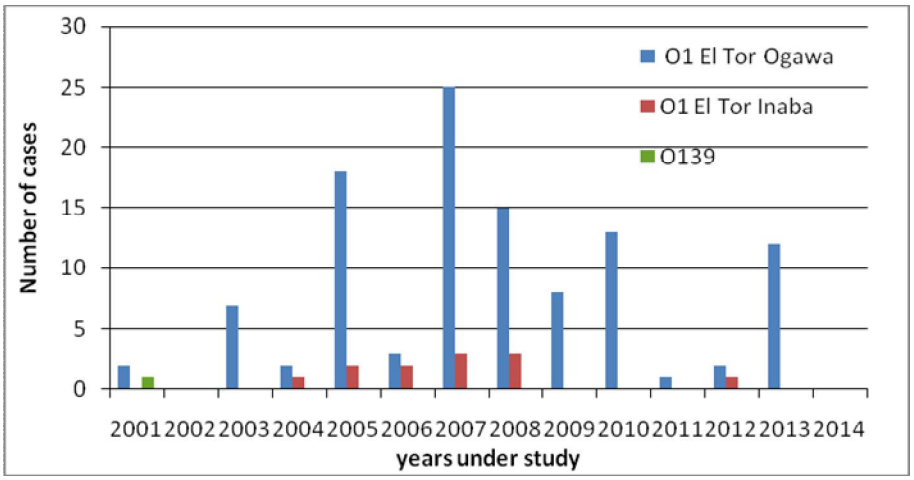


Figure 1.Serogroup, Biotype and Serotype of *V. cholerae* Isolates at Kolar between 2001 and 2014

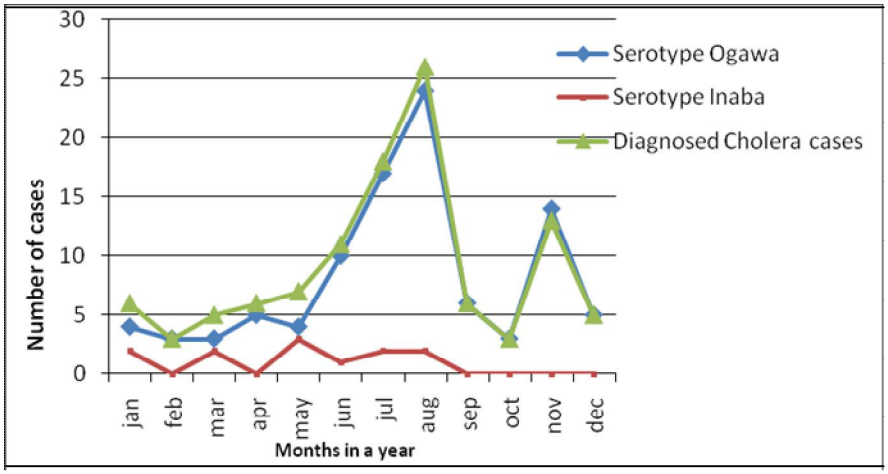
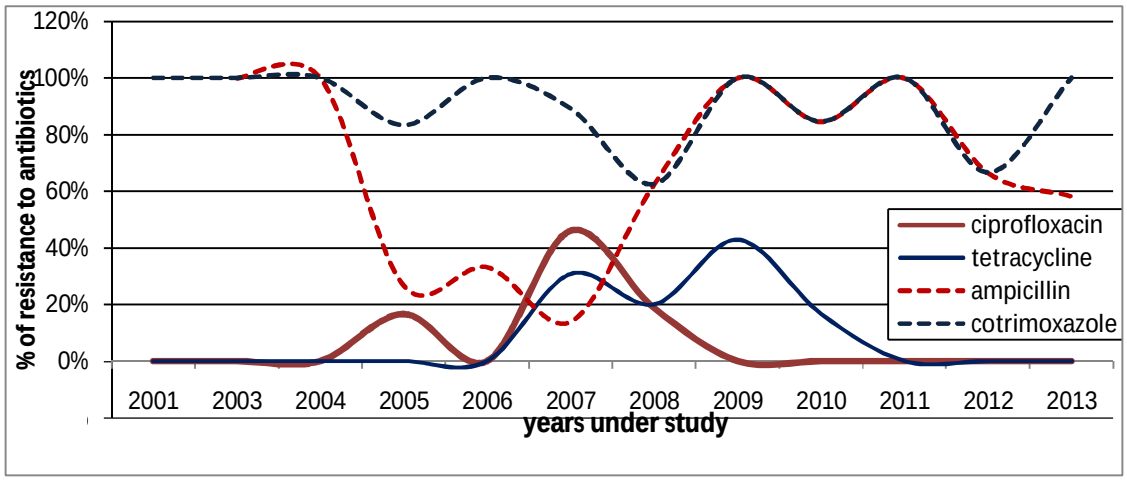


Figure 2.The Cumulative Distribution of Cholera Cases Diagnosed by each Corresponding Month along with Their Serotypes during the Years



There were no isolations of *V. cholerae* in the years 2002 and 2014

Figure 3.Antibiotic Sensitivity Pattern of *V. cholerae* Isolates

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Discussion

In the study spanning 14 years presented here, the laboratory confirmed cholera cases from Kolar region were documented in almost all the years excepting 2002 and 2014. As there was no lull in cholera cases admitted to the hospital for three years in any span of five consecutive years as per definition, the data presented here confirms the endemicity of cholera in Kolar region, Karnataka. On this endemic status, epidemics were superimposed in some of the years.[5,6] The patients with severe cholera reportedly came from villages and small towns in Kolar district. The populations in these areas depend on lake or bore well water carried through pipes which at times leak and provide opportunities for faecal contamination of water and spread of cholera.[5,6] The seasonality of the disease corresponds to the monsoon months as reported from other parts of India.[7] However, we also observed that a few cases of cholera occurred during winter. The seasonality seen in Kolar region is reminiscent of that reported from Vellore, a place nearby.[8]

In the last decade, cholera in India was mainly caused by *V. cholerae* biotype El Tor serotype Ogawa and *V. cholerae* biotype El Tor serotype Inaba has accounted for a small proportion of cases.[9] Similar trend was also reflected among the endemic cholera cases in Kolar region: 90.1% of the *V. cholerae* isolates were *V. cholerae* biotype El Tor serotype Ogawa and 9.9% were *V. cholerae* biotype El Tor serotype Inaba. Inaba and Ogawa serotypes are however interconvertible.[10] In an endemic area, the emergence of Inaba strains are thought to be due to the development of herd immunity to major Ogawa serotype in the population.[11] Though the Inaba serotype of *V. cholerae* constitutes only about 10%, in the recent times, it has emerged as a pathogen responsible for many outbreaks.[12,13]

Antibiotic therapy is considered as an important adjunct to other measures in the treatment of cholera: ciprofloxacin and doxycycline were recommended for this purpose.[14] However, development of resistance to these antibiotics in *V. cholerae* strains has caused concern.[15-17] Macrolides are recommended to counter this problem.[18] In Kolar region, strains resistant to ciprofloxacin were detected for the first time in 2005 and to tetracycline in 2007. The ciprofloxacin and tetracycline resistance among the strains has, however, shown a trend towards reversal in the recent years which is noteworthy. Studies from Bangladesh and Delhi have also reported a similar reversal of resistance and return to sensitivity to tetracycline among the *V. cholerae* strains in recent years.[19,20]

In addition to tetracycline, here we report that there was a reversal of resistance to ciprofloxacin also. To the best of our knowledge, reversal of resistance to ciprofloxacin is being reported for the first time. These changes in sensitivity pattern in *V. cholerae* isolates call for regular antibiotic sensitivity testing on case-to-case basis.

In endemic areas, cholera has been shown to occur mainly among children as per the population-based sentinel studies. However, in our study, only 14% of the patients were children less than 10 years of age. This could be due to the hospital-based data, which represents only the severe end of the spectrum.

We recommend that cholera should be suspected in all patients with severe gastroenteritis, especially occurring during rainy season and winter in Kolar region. The diagnosis should be confirmed bacteriologically and antibiotic sensitivity pattern should be ascertained. Stool samples especially from children irrespective of severity of diarrhea should be screened bacteriologically for *V. cholerae*. These efforts would give a proper picture of endemicity of cholera in the region. Such studies are important as they alert the health professionals in the region to expect cholera during the season and be prepared to institute proper therapeutic and control measures. They also help to evaluate the outcome of the efforts directed against prevention.

Conclusion

Our study confirms endemicity of cholera in kolar region. Cholera cases occur mainly during monsoon and winter months. *V. cholerae* biotype El Tor serotype Ogawa was the main serotype causing cholera. The trends in antibiotic resistance observed over the last 14 years was similar to that reported from other parts of the country. However, we report the reversal of resistance to tetracycline and ciprofloxacin; the recent isolates from Kolar region are sensitive to both these drugs.

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Conflict of Interest: None

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