Original Article

Retrospective analysis to study trend of biotype, serotype and phage type of *Vibrio cholerae* isolates in South Gujarat over last 17 years

Abstract

Introduction: Cholera is a major infectious disease with epidemic potential, especially among communities living in congested urban slums and vast rural areas without proper sanitary facilities. Epidemiology of cholera showed remarkable changes soon after the identification of *El Tor* biotype of *Vibrio cholerae* in India in 1964. The classical biotype reported in earlier years was believed to have become extinct in the recent past. Presently, *V. cholerae* O1 belonging to the *El Tor* biotype is most common in India. **Materials and Methods:** A retrospective study was carried out in Department of Microbiology to study the epidemiological pattern of *V. cholerae* in South Gujarat. *V. cholerae* isolates were confirmed by various biochemical tests. Agglutination was done with antisera. All the isolates were sent to the National Institute of Cholera and Enteric Diseases, Kolkata for phage typing. Antibiotic susceptibility was carried out by Kirby Bauer disc diffusion technique. **Result:** 98% of the isolates were of *El Tor* biotype and 2% of non O1 non O139. 98.8% of the isolates were of Ogawa serotype, only 1.2% of Inaba. According to Basu and Mukerjee classification, T-2 phage was found in 76% isolates. T-4 phage was seen during 1995-1996 and 2002-2004 only. According to the new scheme classification, T-27 phage type was found in 74% isolates followed by T-25 (4%), T-19 (3.7%) and T-25 (3%). **Conclusion:** T-2 is predominant serotype found in South Gujarat region and was constant since 2005 onwards. *El Tor* biotype was found commonly and no isolation of classical biotype was seen. Data analysis will help for future studies.

Key words: Antibiotic susceptibility, cholera, El tor, phage type, serotype

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INTRODUCTION

Cholera is a major infectious disease with epidemic potential, especially among communities living in congested urban slums and vast rural areas without proper sanitary facilities.[1] Cholera has re-emerged as a major infectious disease in the recent past, with a global increase in its incidence.[2] Serogroup O1 is classified into two biotypes, classical and *El Tor*. The seventh and most recent pandemic of cholera was caused by the *El Tor* biotype.[1] The epidemiology of cholera showed remarkable changes soon after the introduction of *El Tor* biotype of *Vibrio cholerae* in India in 1964.[3] At present, *V. cholerae* O1 belonging to the *El Tor* biotype is most common in India. The frequency of biotype O139 has declined considerably over the past few years, which was identified for time in 1992 and was responsible for many local outbreaks in India.[2] The classical biotype reported in earlier years was believed to have become extinct in the recent past. Although rapid replacement of fluid and electrolyte using oral rehydration therapy or intravenous fluids and the subsequent maintenance of hydration remains the mainstay of management in cholera patients, specific antimicrobials are given to reduce the duration and severity of diarrhea as well as to speed up the clearance of organisms from the stools.[4]

The emergence of multiple antibiotic resistant isolates of *V. cholerae* and the changing pattern of antibiogram has increased concerns of treating cholera with antibiotics and reconsidering the role of antibiotics in cholera epidemics. As there was no data available for the South Gujarat indicating prevalent biotype, serotype and phage type of *V. cholerae*, the present study was planned out to study the epidemiology and antibiotic susceptibility pattern of *V. cholerae*. Hence that any change in the epidemiology could be picked up if baseline data are available.

MATERIALS AND METHODS

A retrospective study was carried out in Department of Microbiology, Government Medical College, Surat. Analysis of stool samples received during last 17 years, from 1995 to 2012 in the laboratory was carried out and total isolates of *V. cholerae* were calculated. Ethical permission was taken from IRB. Stool samples received in the laboratory were cultured on Mac Conkey agar, nutrient agar and thiosulfate citrate sucrose bile salt (TCBS) agar. Small amount of the sample was enriched in alkaline peptone water and subculture was done after 4 h on TCBS agar. All the plates were incubated at 37°C for 24 h. Next day colonies of *V. cholerae* were identified using colony morphology, Gram stain from colony smear, various biochemical tests such as oxidase test, catalase test and string test. Further confirmation was done by putting all the batteries of biochemical tests including cholera red test.

For serotyping of isolates, Vibrio cholera polyvalent, Vibrio cholera Ogawa and Vibrio cholera Inaba antisera were used for agglutination (Denka Seiken Co., Ltd., Japan). Antibiotic susceptibility analysis of last 2 year isolates was done using ampicillin, ciprofloxacin, norfloxacin, tetracycline, doxycycline, co-trimoxazole, ceftriaxone, nalidixic acid, gentamycin and chloramphenicol by Kirby Bauer disc diffusion technique (Biogram, Microexpress-Goa). All the isolates were sent to the National Institute of Cholera and Enteric Diseases (NICED), Kolkata in nutrient agar vials in duplicate for biotyping and phage typing.

RESULT

404 isolates of *V. cholerae* were analyzed in the present study, which were isolated from stool samples. 399 isolates (98.8%) showed agglutination with Ogawa antisera, only 5 isolates (1.2%) showed agglutination with Inaba antisera. These Inaba isolates were found during year 2006 (4) and 2007 (1) only. Rest of the years Ogawa was the predominant isolates. No discordance was found for serotyping done in the laboratory and results provided by NICED, Kolkata. Though scattered isolates of *V. cholerae* were seen throughout the year, 74.6% of isolates were in summer and rainy seasons (April-August).

Among the isolates, 397 isolates (98%) were of *El Tor* biotype and 7 isolates (2%) were of non O1 non O139 biotype. Out of the 7 non O1 non O139 isolates, 2 were isolated in year 2003 and 5 in 2009. 307 isolates (76%) belonged to T-2 phage and 87 isolates (21.5%) were from T-4 phage according to Basu and Mukerjee classification. 10 isolates (2.5%) of *V. cholerae* were untypable. T-4 phage type was observed during years 1995-1996, 2000 and 2002-2004. Rest of the years, T-2 was the predominant phage type in the area. According to the new scheme classification which was introduced after 1995, T-27 (74%) was the predominant phage type throughout the years studied, followed by T-25 (4%), T-19 (3.7%) and T-21, T-26 (3% each). Other commonly isolated phage type according to the new scheme classification were T-7, T-13,

T-23, T-4, T-14, T-3, T-6, T-2, T-11, T-16, T-24 and T-10. Graphic representations showing the distribution of biotype, serotype and phage type according to Basu and Mukerjee are shown in below Figures 1-3 respectively.

Phage type distribution according to the new scheme classification is shown in Figure 4.

Antibiotic susceptibility was performed for the 2011-12 isolates by Kirby Bauer disc diffusion method. Isolates showed 70% sensitivity to ampicillin and 94% to chloramphenicol. All the isolates were sensitive to ciprofloxacin, norfloxacin, tetracycline and gentamycin. Only 19% sensitivity was recorded to co-trimoxazole, whereas all the isolates were resistant to nalidixic acid.
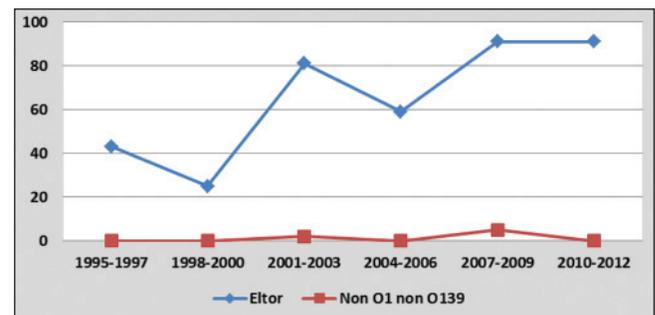

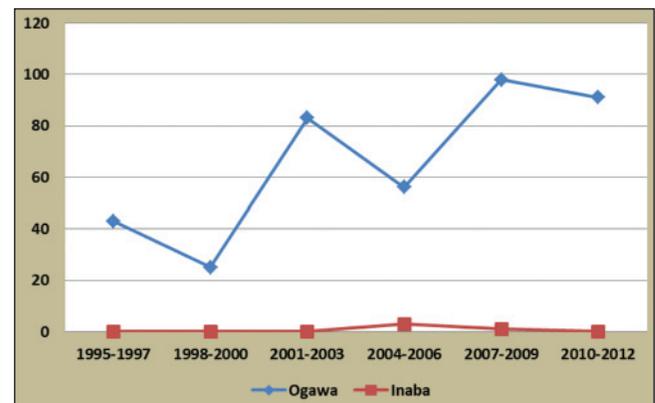**Figure 1:** Distribution of *Vibrio cholera* biotype

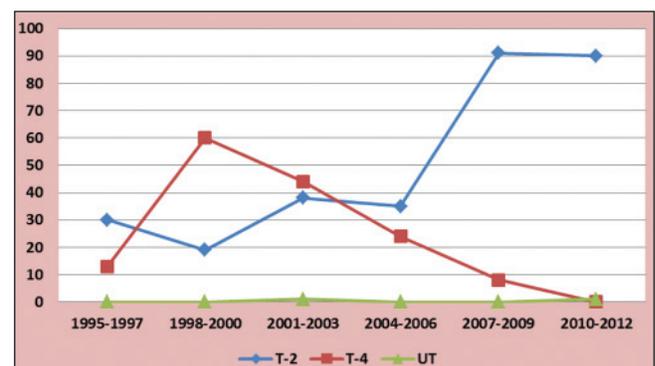**Figure 2:** Distribution of *Vibrio cholera* serotype

Figure 3: Distribution of *Vibrio cholerae* phage type according to Basu and Mukerjee classification
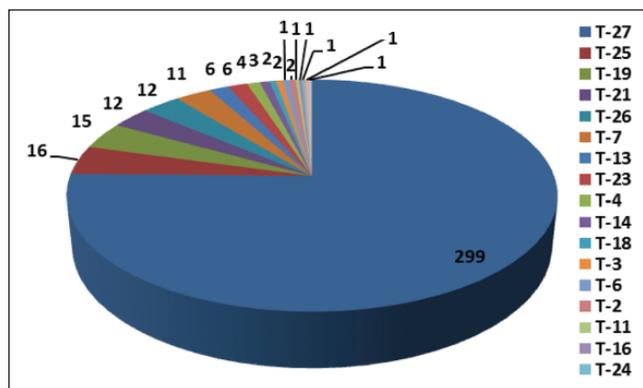
Figure 4: Distribution of isolates according to the new scheme classification

DISCUSSION

Change in epidemiology is noted with cholera also as like with other diseases. In the present study, 98% isolates were of *El Tor* biotype. Kanugo *et al.*,[5] in their study has also reported that *V. cholerae* O1 belonging to the *El Tor* is the most common biotype in India and frequency of O139 biotype has been declined considerably over the past few years. Narang *et al.*[2], Barve *et al.*[15] and Wong *et al.*[16] had also reported the same. Although Ogawa serotype was prevalent throughout the years, 5 Inaba serotype isolates were reported during 2006-2007 in the present study. Narang *et al.*[2] has also reported such a shift in 1999 and 2005. Pal *et al.*[6] has reported a cholera outbreak in Orissa during 2005 with *V. cholerae El Tor* serotype Inaba. This may be due to flooding in Orissa during that period. The present study area also had flood in 2006, during which Inaba serotype was reported. Many other studies from different part of the world have also reported association of increased isolation of Inaba serovars during floods. Any change in prevalent serotype should not be neglected and proper surveillance should be carried to rule out major outbreaks in the community.

Phage typing is one of the best tools to study the epidemiological aspect of isolates.[5] Sarkar *et al.*,[7] in a study of different isolates of *V. cholerae* from different parts of the India has reported T-4 phage type as a predominant phage type during years 1999-2000 and 2003-2004. The finding was quite similar with the present study, where also peak of T-4 phage type was seen during the same period. The Sarkar *et al.*[7] study has reported T-27 phage type of new scheme classification as a predominant phage type throughout the years in all parts of the country. The present study has also noticed the same finding during last 17 years. A study by Bhowmick *et al.*[8] has reported that T27 and T25 in particular were predominant among strains isolated from different geographical regions of India and from both Bangladesh and Brazil. The study has also reported that phage type variation was associated with tetracycline resistance in strains isolated from Kolkata. Thus, monitoring of phage type variation will help to study change in antibiotic resistance pattern in *V. cholerae* isolates. A study by Das *et al.*[4] from New Delhi, has reported 87.2% isolates of *El Tor* and

10% of Non O1 non O139 biotype, T 27 and T 23 phage type according to the new scheme classification. The findings were correlated with the present study of western India. Narang *et al.*[2] has also reported the same finding showing *El Tor* biotype, Ogawa serotype and T-27 being the most common phage by the new scheme classification. Turbadkar *et al.*[9] has reported an outbreak of cholera in Mumbai, by biotype *El Tor*, serotype Ogawa, phage type 4 of Basu and Mukerjee and type 27 (97.5%) and type 23 (2.4%) of the new scheme classification.

Antibiotic resistance is a major problem in recent scenario in every organisms and *Vibrio cholera* is also not far from being a multidrug resistant organism. A study by Narang *et al.*[2] has reported 48% sensitivity to chloramphenicol and 68% to nalidixic acid, whereas that was 94% to chloramphenicol in the present study. All the isolates tested in the present study were resistant to nalidixic acid. As chloramphenicol is not being used commonly in routine since a long time, selection pressure is decreasing and increased susceptibility is recorded by isolates. Ciprofloxacin susceptibility (100%) seen in the present study was similar to other studies by Mandal *et al.*[10] (97%) and Shrestha *et al.*[11] (100%) carried out in Puducherry and Nepal respectively. An antibiotic susceptibility result reported by Chander *et al.*[12] was quite similar to the present study. No association between the phage type and antimicrobial susceptibility pattern was observed in the present study.

Major drawback of the present study was that antibiotic susceptibility was studied for last 2 years only. As it was a retrospective analysis, socio-economic status, clinical presentation, clinical outcome of patients and case fatality rate were not studied. Continuous monitoring of antibiotic susceptibility should be carried out as resistance to tetracycline has been reported from some of the parts of the country.[13,14] The study will help to compare the prevalent serotype, biotype and phage type in other areas of Gujarat state. Molecular studies should be taken up to study the presence of the virulence-associated genes such as ctxA, zot, ace, tcpI and toxR to establish a correlation with biotype and virulence factors.

CONCLUSION

El Tor and Ogawa were most commonly prevalent biotype and serotype in the region, quite similar to other states of India and other neighboring countries. T-27 phage type was prevalent throughout the years. T-2 is the most common phage throughout the years except few peaks of T-4. Improved surveillance of the disease will be useful in assessing the actual burden of cholera in the country and in planning interventions appropriately. Co-trimoxazole, ampicillin and nalidixic acid were the drugs, which showed a high degree of resistance, while chloramphenicol, gentamycin, ciprofloxacin and tetracycline showed good sensitivity in the area studied. The quick shift in resistance pattern indicates that all isolates should be subjected to susceptibility and resistance pattern should be monitored. Periodic epidemiological surveys should be carried out to study the trend of serotype, biotype

and phage type in the community. All academic institutes must prepare and maintain a database of isolates so that any deviation in epidemiology can be picked up early. The isolates should be sent to a reference laboratory routinely to identify phage type and biotype. Policy-makers should target control interventions in high-risk areas including vaccines, along with improved access to safe water and adequate sanitation.

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