Changing trends in the epidemiology of *Vibrio cholerae* in an outbreak of 2013 in Solapur, Maharashtra

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Abstract

Background: Cholera, one of the Notifiable Diseases is known to cause serious epidemics and pandemics in developing countries like India. An outbreak had occurred in 2013 in Solapur and the isolates revealed certain change in the trends of the epidemiological markers.

Methods: A total of 30 cases presented with gastroenteritis during the months of July and August of 2013. 30 stool specimens were cultured on Blood agar, Mac Conkey agar and TCBS medium and enriched on alkaline peptone water broth. Identification of the isolates was done by standard Biochemical and Serological Agglutination tests. Antibiotic susceptibility testing was done using Kirby Bauer disc diffusion method, according to CLSI guidelines. Phage typing was done at the National Institute of Cholera and Enteric Diseases, Kolkata.

Results: Out of the 30 stool specimen cultured, 14 strains of *V.cholerae* biotype El Tor serotype Ogawa was isolated. Phage Typing could be done on 6 isolates. All the strains identified belonged to T-2 phage type, according to Basu and Mukherjee scheme. According to the new scheme, two isolates belonged to T-27 phage type, two belonged to T-3 phage type, while one each belonged to T-6 and T-12 phage types. Most of the strains isolated in 2013 epidemic were sensitive to tetracycline, cotrimoxazole, and ciprofloxacin and resistant to ampicillin and gentamicin.

Interpretation and Conclusion: Due to the changing epidemiological markers of *V.cholerae* such as Phage type and Antibiogram, all the epidemics must be thoroughly investigated and the isolates must be subjected to complete identification and Antibiotic Susceptibility Testing.

Keywords: Antibiogram, Basu and Mukherjee scheme, epidemiological markers, phage typing, *Vibrio cholerae*.

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Introduction

Vibrio cholerae, the etiological agent of cholera, has both epidemic and pandemic causing potential.(1) It has appeared in epidemic proportions in many developing countries and is endemic in regions of Asia, Africa and South America.(2)

The pathogenic and epidemic potential of the different strains of *V.cholerae* vary. These strains are classified into 139 serogroups based on their somatic O antigen on the cell wall, which also forms the basis for their serotyping scheme. The O1 serogroup is further divided into 3 serotypes, namely, Inaba, Ogawa and Hikojima. The epidemic strains of O1 serogroup can be classified into two biotypes, classical and El Tor. El Tor is actively haemolytic biotype and was named so, since it was first isolated in El Tor quarantine of Egypt.(1) Classical biotype is more toxigenic as compared to El Tor biotype, while El Tor biotype comparatively has better adaptability.(3)

The El Tor biotype resulted into seventh and the most recent pandemic, which began in 1961 in Indonesia. All the earlier pandemics were caused by classical biotype. Classical biotype was believed to be extinct, but recently its re-emergence has been reported in Bangladesh.(1)

V.cholerae O139 was first reported during 1992-93, causing large epidemics in Bangladesh, India and other neighbouring countries, replacing the existing O1 strains. Later, during the year 1994-95, O139 strain was largely replaced by new clone of *V.cholerae* O1, the El Tor biotype.(4) Many cases of *V.cholerae* O1 biotype El Tor have also been reported during the sporadic diarrhoeal outbreaks after the super cyclone in 1996 and also in 2005.(2)

V.cholerae possesses many virulence and regulatory genes which are culprits in the pathogenesis. These include A) Cholera toxin (CT), encoded by *ctx*AB gene, present on a filamentous CTX bacteriophage (CTXΦ). Each biotype has unique gene sequence coding for B subunit of cholera toxin. B) Toxin co-regulated pilus (TCP) – a receptor for CTX bacteriophage. C) Zonula occludens toxin (*zot*). D) Accessory cholera enterotoxin (*ace*). E) Outer membrane protein (*omp*U). F) Central regulatory

protein (*tox*R). G) *hly* gene coding for hemolysin. H) RTX toxin gene cluster coding for presumptive cytotoxin (*rtx*A), an acyltransferase (*rtx*C) and an associated ATP binding cassette transport system.[5]

Solapur, being a small city with poor water sanitation, frequently comes across patients suffering from severe diarrhoea due to *V.cholerae* infection. The last epidemic was witnessed in 2010-2011. The present study highlights on the cholera outbreak that occurred in 2013 due to El Tor biotype of *V.cholerae.*

Material and Methods

A total of 30 cases presenting with gastroenteritis and moderate to severe dehydration were admitted to medicine ward in the months of July and August of 2013. Stool samples collected from these patient were immediately processed as per the standard guidelines – Hanging drop preparation, culture on Blood agar, MacConkey agar and TCBS medium (Hi Media, Mumbai). For enrichment, samples were also inoculated into alkaline peptone water broth and further cultured on solid media after 4 hours of incubation. The colonies grown were identified using standard biochemical tests[1] (Hi Media, Mumbai) and then by serological agglutination with Poly O antisera of *V.cholerae*. On confirmation, further serotyping was done using specific antisera for Ogawa and Inaba[1] (Anand Brothers, Denka Seiken Co. Ltd Japan).

Antibiotic susceptibility testing was done using Kirby Bauer disc diffusion method[6] and CLSI guidelines[7], against gentamicin, tetracycline, ciprofloxacin, doxycycline and ampicillin.

The strains isolated, were sent to National Institute of Cholera and Enteric Diseases, Kolkata, for phage typing.

Results

Out of 30 stool samples collected from clinically suspected cholera patients, 14 were identified to have acquired *V.cholerae* infection. Amongst them, 8 patients were of paediatric age group while 6 were adults.

All the 14 isolates obtained after culturing the stool samples belonged to serotype Ogawa and biotype El Tor. The antibiotic susceptibility pattern is as shown in Table 1. Out of the 14 isolates sent to National Institute of Cholera and Enteric Diseases, Kolkata for phage typing, 8 samples got contaminated during the transport, thus phage typing was done only of the remaining 6 samples. The Phage typing was done according to two different schemes: Basu and Mukherjee scheme[8] and New scheme.[9] All the strains identified belonged to T-2 phage type, according to Basu and Mukherjee scheme. According to the New scheme, two isolates belonged to T-27 phage type, two belonged to T-3 phage type, while one each belonged to T-6 and T-12 phage types.

Table 1: Antibiotic Susceptibility Pattern of Isolates of *V.cholerae*

Drug	Sensitive (%)	Resistant (%)
Ampicillin	5 (35.71%)	9 (64.28%)
Tetracycline	10 (71.42%)	4 (28.57%)
Cotrimoxazole	8 (57.14%)	6 (42.85%)
Ciprofloxacin	9 (64.28%)	5 (35.71%)
Gentamicin	3 (21.42%)	11 (78.57%)

Discussion

Cholera is an acute diarrhoeal disease which is caused by toxigenic strains of V.*cholerae* belonging to serogroups O1 and O139. Currently the El Tor biotype of V.*cholerae* is most common serogroup in India. From 2004-2008, a total of 838315 cases have been reported to World Health Organisation, which showed 24% increase when compared with data obtained during a period of 2000-2004.[10]

Six pandemics have occurred since 1817 and the seventh one began in 1961, which is still in progress affecting almost the entire world. It has also greatly affected the Indian subcontinent resulting into endemicity in the country and frequent epidemics in different parts of the country. Cholera outbreaks usually occur during dry (March-April) and rainy (September-October) seasons in India. A high population density, lack of safe drinking water, poor sanitation and low socioeconomic conditions, which are seen in Indian population, facilitates the faeco-oral route of transmission of cholera.[3]

Cholera is changing epidemiologically. In addition to this, there are constant changes occurring in the toxigenic strains of *V.cholerae* which has resulted in survival advantage of the bacteria. There are evidences which suggest that the *V.cholerae* O1 strains have the ability to undergo antigenic variation and serotype switching between Ogawa and Inaba.[3] Recently hybrid strains have been reported which seeks for emergency attention.[10] Three variants of El Tor biotype of *V.cholerae* O1 have been described recently: A) Matlab variants - show combined characteristics of both classical and El Tor strains, described in 2002, B) Mozambique variants – they have typical El Tor genome and a tandem repeat of classical prophage located in a small chromosome, described in 2004, C) Altered El Tor strains – They have an El Tor CTX prophage, but produce cholera toxin of classical type.[2]

In the study conducted in Orissa (India), all the strains of V.cholerae biotype El Tor were resistant to ciprofloxacin, ampicillin and cotrimoxazole, while they were sensitive to gentamicin and tetracycline.[2] While another study shows that the isolates of Ogawa strain, were sensitive to chloramphenical, while being resistant to ampicillin and cotrimoxazole. [11] In present study, most of the *V.cholerae* biotype El Tor strains was sensitive to tetracycline, cotrimoxazole and

ciprofloxacin while they were resistant to ampicillin and gentamicin.

From 1992-1996, seven *V.cholerae* O139 strains were isolated, belonging to ribotypes B-I,B-II, B-IV and B-V.[4] In a study conducted in the state of Orissa, *V.cholerae* O1 strains were isolated, out of which almost all strains (48 out of 49) were of Ogawa serotype while only one strain belonged to Inaba serotype.[2] In another study conducted on cholera outbreak at Solapur during 2010-2011, all the strains isolated were *V.cholerae* O1 biotype Ogawa. Similar findings were observed in our study.

The phage types of the strains isolated earlier from Solapur area during 2010-2011 were T-7, T-13, T-26 and T-27, according to New scheme,[5] while the strains isolated during 2013 outbreak in Solapur area were of phage types (New scheme) T-3, T-6, T-12, T-27. This shows the changing patterns in the toxigenic strains of *V.cholerae* evidenced every time the outbreak occurs, even in one particular area. Thus, it is very important to keep vigilance on the epidemiological markers of *V.cholerae* as change in the markers causes change in the antigenic structure, as had happened with the evolution of O 139 that is known to cause cholera epidemics.

Recently, strains of *V.cholerae* with multiple antibiotic resistances have emerged, which are limiting the treatment strategies.[10] Emergence of drug resistant strains has been attributed to presence of R-plasmid.[3] The study done on the outbreak of 2010-2011, reported sensitivity to ampicillin and gentamicin and resistance to nalidixic acid and cotrimoxazole whereas most of the strains isolated in 2013 epidemic were resistant to ampicillin and gentamicin. This change in the sensitivity pattern necessitates the constant application of susceptibility testing.

For long term effects, improvement in water sanitation form the mainstay of intervention, while short term effect for immediate response can be obtained through oral cholera vaccines, now licensed in India.[10]

Conclusion

Due to changing trends and evolution in the toxigenic strains of *V.cholerae*, it is very difficult to eradicate cholera. Not to forget, it also forms a part of normal flora and is a normal inhabitant of surface water. This seeks for constant monitoring and surveillance of cholera epidemics, the changing patterns of antibiotic susceptibility among different strains of *V.cholerae* and vigilance of R-plasmid, so that proper management can be initiated.

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