ABSTRACT

Introduction - Cholera is an acute diarrhoeal disease caused by Vibrio cholerae. Of the 206 serogroups of V.cholerae only O1 and O139 which produce cholera toxin (CTX) are known to cause epidemics. The present study was conducted to determine biotypes, serotypes, phage types of V.cholerae prevalent in Hyderabad and to determine the antibiotic susceptibility pattern of isolates. Materials & Methods: A prospective study was conducted during the period January 2012 to July 2012 at a Tertiary Care Hospital, Hyderabad. During this study period 200 clinically suspected cases of cholera were included. STOOL Specimens were cultured directly on Nutrient agar, Blood agar, MacConkey’s agar, Thiosulfate citrate bile salt sucrose agar (TCBS), Xylose lysine deoxycholate agar (XLD). Antibiotic susceptibility testing was performed by Kirby Bauer’s Method. Results: Out of 200 samples collected, 62 isolates were of Vibrio cholerae. The predominant age group affected were 15 – 30 years. Males were more susceptible than Females. All the 62 isolates of Vibrio cholerae belonged to Serogroup O1, Biotype ElTor and Serotype Ogawa. All the strains showed 100% resistance to Nalidixic acid, Furazolidine and 99% resistance to Cotrimaxozole. Conclusion: The isolation rate of Vibrio cholerae in our study was 31%. All the 62 Vibrio cholerae isolates belongs to Serogroup O1, Biotype ElTor, and Serotype Ogawa. 80% have mild and moderate symptoms and 20% have severe dehydration can leads to death if untreated. T2 was the predominant phage according to Basu and Mukherjee...
Method. T27 was the predominant phage according to New Typing Scheme. All the isolates showed uniform sensitivity to Amikacin, Gentamycin, Ceftriaxone, Cefotaxime, Doxycycline. All the isolates showed high degree of resistance to Nalidixic acid, Furazolidine, Cotrimoxazole.

**KEY WORDS:** V. cholerae, Serotype, Biotype, Phage type, CTX.

**INTRODUCTION**
Cholera is an acute diarrhoeal infection caused by ingestion of food or water contaminated with the bacterium Vibrio cholerae. (1) Cholera is responsible for 10-20% diarrhoeal cases.\(^1\)

According to W.H.O – 2012, 3 – 5 million cholera cases and 1,00,000 – 1,20,000 deaths were reported every year. (1) Of the 206 serogroups of V. cholerae only O1 and O139 which produce CTX are known to cause epidemics.\(^2\) Six pandemics of Cholera were caused by V. cholerae O1 classical biotype.\(^3\)

The seventh pandemic is the most extensive of the pandemics in geographic spread and in duration, and the causative agent is V. cholerae O1 of the El Tor biotype. The pandemic, which began in 1961 on the island of Sulawesi in Indonesia, spread to other islands, by the end of 1962.\(^4\) The seventh pandemic is ongoing, and it continues to cause seasonal outbreaks in many developing countries, especially Bangladesh and India. However, in 1992, V. cholerae belonging to a non-O1 serogroup (now referred to as O139) caused large epidemics of cholera in India and Bangladesh and spread to some other countries, this may represent the beginning of the eighth pandemic.\(^5\)

**MATERIALS & METHODS**
A prospective study was conducted during the period January 2012 to July 2012 at a Tertiary Care Hospital, Hyderabad. During this study period 200 clinically suspected cases of cholera were included.

Stool specimens were collected on admission in a clean, wide mouthed, leak proof container preferably before starting antibiotics and transported immediately to the laboratory for processing.

Specimens were cultured directly on Nutrient agar, Blood agar, MacConkey’s agar, Thiosulfate citrate bilesalt sucrose agar (TCBS), Xylose lysine deoxycholate agar (XLD).
In addition to direct plating specimens were subcultured on MacConkey’s agar, Thiosulfate citrate bile salt sucrose agar (TCBS) after enrichment in Alkaline peptone water. Plates were examined after overnight incubation at 37°C.

Colonies suggestive of Vibrio cholerae were identified by standard biochemical tests. The isolated strains were confirmed by serotyping using high titre antisera obtained from King Institute of Preventive Medicine, Guindy, Chennai. O385 (classical) and N16961 (ElTor) were used as control strains.

Biotyping was done by Conventional Methods such as Chick cell agglutination, VP test, Polymyxin-B sensitivity, Sheep RBC hemolysis.

Phage typing was done by Old and New methods and susceptibility to phage IV and V for biotyping was done at Vibrio Phage Reference Lab, National institute of Cholera and Enteric Diseases (NICED), Kolkata.

Antibiotic susceptibility testing was performed by Kirby Bauer’s Method. The following commercial (Himedia) antibiotic discs were used Cefotaxime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Tetracycline (30µg), Doxycycline (30µg), Gentamicin (10µg), Amikacin (30µg), Azithromycin (15µg), Nalidixic acid (30µg), Furazolidine (100µg), Cotrimaxozole (25µg). The plates were read after 16 – 18 hours of incubation at 37°C. The zone of inhibition for each antibiotic was interpreted as per Clinical and Laboratory Standard Institute (CLSI) guidelines.

RESULTS

Two hundred stool samples were collected from clinically suspected cases of Cholera admitted in Tertiary care hospital during the period January 2012 to July 2012.

The predominant age group affected were 15 – 30 years. Males were more susceptible than Females.
The maximum number of Vibrio cholerae isolates were seen in the month of May followed by April. The isolation rate of Vibrio cholerae in the present study was 31%. (Table 1)

**Table 1 - Isolation rate of Vibrio cholerae in the present study**

<table>
<thead>
<tr>
<th>Total No. of stool samples collected</th>
<th>No. of V. cholerae isolates</th>
<th>Percentage of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>62</td>
<td>31%</td>
</tr>
</tbody>
</table>

All the 62 isolates of Vibrio cholerae belonged to Serogroup O1, Biotype ElTor and Serotype Ogawa.

Among people who develop symptoms, 80% have mild or moderate symptoms, while around 20% develop acute watery diarrhoea with severe dehydration. This can lead to death if untreated Phage typing was done at Vibrio Phage Reference Lab, National institute of...
Cholera and Enteric Diseases (NICED), Kolkata. A total of 62 strains were phage typed. Phage typing was done by Basu and Mukherjee method and New Phage Typing Method.

According to Basu and Mukherjee method T2 was the most prevalent phage type and 12.9% of the strains were untypable. According to New Typing Scheme T27 was the most predominant phage type and all the strains were typable by this method. (Table 2)

Table 2 – Phage typing of Vibrio cholerae O1 isolates

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Method</th>
<th>Phage type</th>
<th>No. of Isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Basu and Mukerjee</td>
<td>T2</td>
<td>54</td>
<td>87.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UT</td>
<td>08</td>
<td>12.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>62</td>
<td>100%</td>
</tr>
<tr>
<td>2.</td>
<td>New Typing Scheme</td>
<td>T27</td>
<td>40</td>
<td>64.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T26</td>
<td>06</td>
<td>9.67%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T7</td>
<td>04</td>
<td>6.45%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T13</td>
<td>06</td>
<td>9.67%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T12</td>
<td>06</td>
<td>9.67%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>62</td>
<td>100%</td>
</tr>
</tbody>
</table>

All the strains showed 100% resistance to Nalidixic acid, Furazolidine and 99% resistance to Cotrimaxozole. All the strains showed uniform sensitivity to Amikacin, Gentamicin, Ceftriaxone, Cefotaxime, Doxycycline. (fig.3)

Figure 3 - Antibiogram of the Vibrio cholerae O1 isolates

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.

The isolation rate of Vibrio cholerae in our study was 31% is in correlation with this study.\(^5\) In this study all the 61 Vibrio cholerae isolates belonged to Serogroup O1, Biotype ElTor and Serotype Ogawa is in correlation with other studies.\(^6,7,8\)

ElTor biotype was reported for the first time from Andhra Pradesh in Vishakapatnam in the year 1965 by Bhaskaran and spread to other parts of the state and completely replaced classical biotype in the latter half of 1965.\(^9\)

O139 was reported for the first time in Chennai and spread to various other places. However O139 was reported from Hyderabad in 1993. It is continued to coexist with ElTor till 2004 and in 2005, ElTor completely replaced O139. In our study O139 was not detected.\(^9\)

Among people who develop symptoms, 80% have mild or moderate symptoms, while around 20% develop acute watery diarrhoea with severe dehydration and this can leads to death if untreated. The present study goes in correlation with W.H.O studies on cholera in 2012.\(^1\)

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, 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. 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.\(^8\)

Among O1 vibrios, all the isolates belonged to Biotype ElTor. Out of these, 96.01% were of serotype Ogawa. Serotype Inaba was rare till the Year 1998 (1990 and 1992-one isolate each) but became the main serotype in the year 1999, when 11 out of the total 15 (73.3%)
V. cholerae isolates were Inaba and reappeared in the year 2005 (4 out of 30 isolates, 13.33%).[11]

Phage typing for Vibrio cholerae is one of the best established tool and marker for epidemiological characterization of the isolates. Phage typing was done by Old and New methods. According to Basu and Mukherjee method our study showed T2 as the Predominant Phage type which correlates with various other studies. Some studies conducted in India reported T4 as predominant phage type. While few studies from South India reported T2 and T4 phage types.[10,11,12]

According to New Typing Method 100% strains are typable. With the New Phage Typing Method our study showed T27 was the predominant phage type which correlated with various other studies.[10,11,12]

In our study other phage types are T26, T7, T13, T12 were seen which was in contrast to other studies which reported T26, T23, T21, T25, T13 phage types.[10,11,12]

All the strains showed uniform sensitivity to Amikacin, Gentamicin, Ceftriaxone, Cefotaxime, Doxycycline and 100% resistance to Nalidixic acid, Furazolidine and 99% resistance to Cotrimoxazole in correlation with other studies.[13,14,15]

CONCLUSION
The isolation rate of Vibrio cholerae in our study was 31%. All the 62 Vibrio cholerae isolates belongs to Serogroup O1, Biotype ElTor, and Serotype Ogawa. 80% had mild and moderate symptoms and 20% had severe dehydration which can lead to death if untreated. T2 was the predominant phage according to Basu and Mukherjee Method. T27 was the predominant phage according to New Typing Scheme.

All the isolates showed uniform sensitivity to Amikacin, Gentamycin, Ceftriaxone, Cefotaxime, Doxycycline. All the isolates showed high degree of resistance to Nalidixic acid, Furazolidine, Cotrimoxazole.

With the dismal scenario of an alarming increase in the drug resistance of various infectious pathogens, continuous surveillance becomes imperative to understand the pathogens and their changing drug resistance profiles for an effective treatment.
REFERENCES


