

# Mismatch Amplification Mutation Assay (MAMA) PCR Reveals Altered El Tor *Vibrio Cholerae* O1 Circulating in Delhi, India

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**ABSTRACT-** The acute diarrheal disease cholera is caused by *Vibrio cholerae*, a major public health problem in Asia, Africa and Latin America. *V. cholerae* has more than 200 known serotypes but not all the strains are pathogenic. In recent years, it has been seen that the emergence of new variants of *V. cholerae* O1 have carried characteristics of both classical and El Tor biotypes and these variants of *V. cholerae* O1 are called as 'atypical El Tor'. These strains might have evolved from El Tor variants that acquired certain characteristics from classical genome. 30 *V. cholerae* O1 isolates (Table 1) were revived from stock cultures maintained at Laboratory Department in Maharishi Valmiki Infectious Diseases Hospital (MVIDH), Delhi during 2012-2014. Mismatch amplification mutation assay was used for classifying the strains into prototype El Tor, hybrid, or El Tor variant biotype based on their *ctxB* gene. All isolates were biochemically identified as *V. cholerae* biotype El Tor and serologically O1 Ogawa. All isolates were amplified with classical specific primers. Cholera continues to be endemic in large number of states in the eastern, western, northern and southern parts of India and there were 38 cholera outbreak reports published in India during 2007 to 2013. These Indian outbreaks have been associated with new El Tor variant.

**Key-words-** *V. cholerae* O1, El Tor, MAMA PCR, *ctxB*

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## INTRODUCTION

The acute diarrheal disease cholera is caused by *Vibrio cholerae*, a major public health problem in Asia, Africa and Latin America<sup>1</sup>. According to the World Health Organisation (WHO) estimates there were 3-5 million cholera cases and 1,00,000- 1,20,000 deaths each year throughout the world<sup>2</sup>. *V. cholerae* has more than 200 known serogroups but not all the strains are pathogenic. Among them, O1 and O139 antigens are highly pathogenic and acknowledged to cause epidemic and pandemic disease<sup>3</sup>. The symptom of the acute cholera is rice water stools.

Clinical manifestations of the disease range from mild symptoms such as abdominal cramps, nausea and vomiting, to more severe symptoms such as dehydration, shock and death<sup>4</sup>. A devastating cholera outbreak that had occurred in Haiti since October 2010, involved 6, 98, 893 cases and 8,540 deaths<sup>5</sup>. *V. cholerae* serogroup O1 is classified into two biotypes, which are termed 'classical' and 'El Tor'<sup>6</sup>. The current seventh pandemic of cholera is caused by the El Tor biotype was originated in 1961 from Celebes Islands in Indonesia<sup>7</sup>. Spread of this biotype was recorded for the first time during 1964 in India and it made its first appearance in Delhi during June, 1965<sup>8</sup>. In recent years, it has been seen that the emergence of new variants of *V. cholerae* O1 have carried characteristics of both classical and El Tor biotypes and these variants of *V. cholerae* O1 are called as 'atypical El Tor'. These strains might have evolved from El Tor variants that acquired certain characteristics from classical genome<sup>9</sup>. In this study, we focused on type of CT genotype existing among *V. cholerae*

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O1 isolated from the patients who were admitted in MVIDH, Delhi during 2012-2014.

**MATERIALS AND METHODS**

**Collection and processing of samples**

30 *V. cholerae* O1 isolates were revived from stock cultures were maintained at Laboratory Department in MVIDH, Delhi during 2012-2014. These samples were revived and transferred to alkaline peptone water (APW, pH-8.6) and incubated at 37°C for 4-6 hrs. After incubation, the enriched cultures were further inoculated to the thiosulphate-citrate-bile-salts-sucrose (TCBS) agar and bile salt agar (BSA, pH-8.6) (Hi-Media, Mumbai)<sup>10</sup>.

**Confirmation of *V. cholerae***

Typical colonies appearing on TCBS/BSA were confirmed by standard biochemical tests<sup>10</sup>. These isolates were further tested serologically<sup>11</sup> with commercially available *V. cholerae* O1 polyvalent and monovalent and O139 antiserum (BD, USA and Denka Seiken, Ltd. Japan).

**Genomic DNA preparation**

Genomic DNA from each isolate was extracted using protocol described previously<sup>12</sup>.

**PCR assays for detection of *ctxB* genotype**

Mismatch amplification mutation assay was used for classifying the strains into prototype El Tor, hybrid, or El Tor variant biotype based on their *ctxB* gene<sup>13</sup>. In this PCR, the common forward primer for both classical and El Tor alleles was used. Two allele specific primers Re-Cla and Re-Elt were used respectively. The PCR conditions and cycles were described previously<sup>13</sup>. *V. cholerae* O1 strains 569B (classical) and N16961 (El Tor) were used as control strains. The primers used were synthesized by Invitrogen, India and dNTPs, Taq polymerase, 10X Taq buffer used in this study were obtained from Genei, Bangalore. The primer sequences were used in this PCR is given in Table 2.

**RESULTS**

**Characterisation of isolates**

A total of 30 *V. cholerae* O1 isolates were revived from maintained isolates during 2012-2014 (Table 1). All isolates were biochemically confirmed as *V. cholerae* biotype El Tor and serologically confirmed with polyvalent O1 and then agglutination with monovalent Ogawa.

**Table 1:** Showing types of *ctxB* in Delhi isolates during 2012-2014

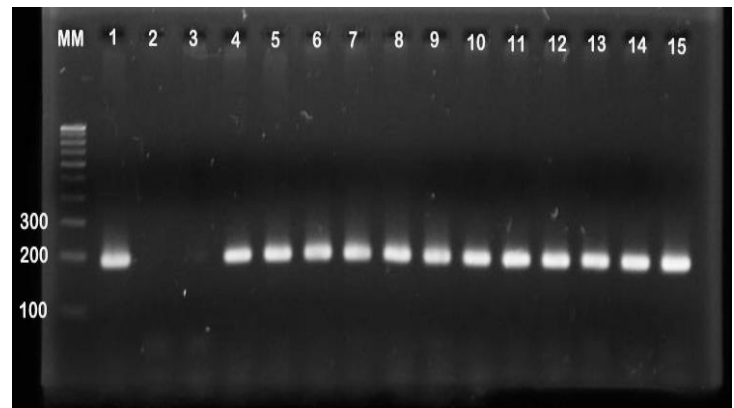
| No. of isolates | Year | Serogroup/Serotype | <i>ctxB</i> |
|-----------------|------|--------------------|-------------|
| 10              | 2012 | O1/Ogawa           | Classical   |
| 10              | 2013 | O1/Ogawa           | Classical   |
| 10              | 2014 | O1/Ogawa           | Classical   |

**Table 2:** Primer sequences were used in MAMA PCR

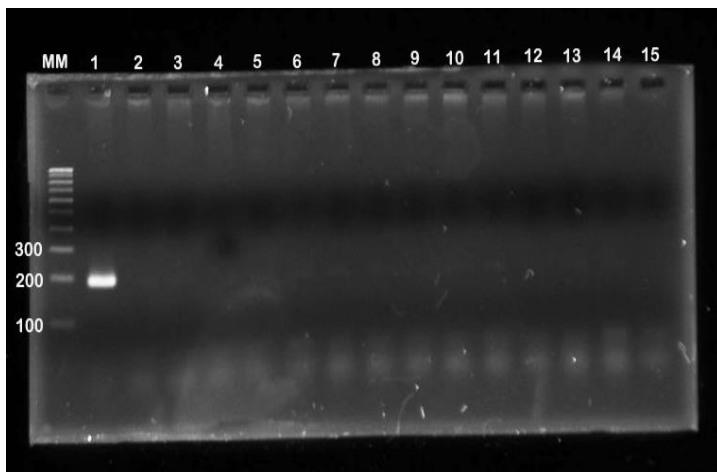
| Gene (S)       | Primer Sequence           | Amplicon Size (bp) | Ref. |
|----------------|---------------------------|--------------------|------|
| <i>ctxB</i> FW | ACTATCTTCAGCAT-ATGCACATGG |                    | 13   |
| Re-EI          | CTGGTACTTCTACTTGAAACA     | 186bp              | 13   |
| Re-Cla         | CTGGTACTTCTACTTGAAACG     | 186bp              | 13   |

**Confirmation of *ctxB* gene by MAMA PCR**

All 30 isolates were screened for *ctxB* gene by MAMA PCR with two allele specific primers, one for Classical and other for El Tor. All isolates were amplified with classical specific primers (Fig. 1). Only control strain N16961 were amplified with El Tor specific primers (Fig. 2).



**Fig. 1:** MAMA PCR assay of agarose gel electrophoresis of *ctxB* yielded 186bp amplicon size fragment when using primer pair FW-Com with Re-Cla. Lane M, 100bp molecular weight marker, lane 1-569B Positive Control, lane 2- N16961 Control strain for El Tor, lane 3- Negative Control and lane 4-15 test strains shown positive band



**Fig. 2:** MAMA PCR assay of agarose gel electrophoresis of *ctxB* yielded 186bp amplicon size fragment when using primer pair FW-Com with Re-Elt. Lane M, 100bp molecular weight marker, lane1-N16961 Positive Control, lane 2- 569B Control strain for Classical, lane 3- Negative Control and 4-15 test strains shown negative band

## DISCUSSION

Cholera continues to be endemic in large number of states in the eastern, western, northern and southern parts of India and there were 38 cholera outbreak reports published in India during 2007 to 2013<sup>14</sup>. These Indian outbreaks have been associated with new El Tor variant<sup>15</sup>. Classical and El Tor biotypes differ in not only their phenotypic and genotypic characteristics, but also their pathogenic potentials, survivalness and diseases causing ability in their human hosts<sup>16</sup>. El tor strains are associated more frequently with asymptomatic infections and lower fatalities rate, better survival in the environment and in the human hosts and more efficient host-to-host transmission as compared with classical strains where as classical strains is more severe than El Tor biotype and fatality rate is high as compared to El tor biotype<sup>16-17</sup>. In 2006, first naturally occurring atypical El Tor variants observed by Nair<sup>18</sup> among stool samples of hospitalized patients strains of *V. cholerae* O1 isolated between 1991 and 1994 in Matlab, Bangladesh. In the present study, we found all the isolates carried *ctxB* gene of classical biotype i.e. *ctxB<sup>Cl</sup>* (*ctxB<sup>I</sup>*). It revealed that atypical El Tor is circulating in Delhi since 2008<sup>12</sup>.

## CONCLUSIONS

In the present study, all isolates are phenotypically El Tor but carried *ctxB* gene of classical biotype and known as altered El Tor. Altered El Tor is more pathogenic than El Tor because it harboured *ctxB* gene of classical biotype and


better survival in the environment. More molecular study is needed to understand the changing epidemiology of *V. cholerae* O1 infections and better planning to control cholera disease in this part of the country.

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