

## Cholera Outbreak Due to *V. cholerae* O1 El Tor Variant in Odisha, India 2011: At the Edge of the Eighth Pandemic

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Currently hyper virulent *V. cholerae* O1 El Tor variant carrying cholera toxin B subunit of classical strain have been reported spreading most part of the world. An outbreak of cholera that occurred during January 2011 was investigated in Puri district, Odisha India. A total of 15 rectal swabs and 6 water samples were collected from hospitalized diarrhoea patients and environmental sources respectively. Samples were bacteriologically analyzed to isolate bacterial enteropathogens, antibiogram profiles and detection of various toxic genes. Four *V. cholerae* O1 El Tor biotype was isolated and confirmed as El Tor variant carrying *ctxB* gene of classical. The investigation revealed that the resident *V. cholerae* O1 El Tor biotype was replaced by El Tor variant in Puri area and its spread in the coastal district should be closely monitored.

**Key words:** El Tor variant, *Vibrio cholerae*, Odisha.

Cholera continues to be one of the major threats for public health problems particularly in developing countries caused by *V. cholerae* O1 and O139 serogroups<sup>1</sup>. *V. cholerae* O1 is further classified into two biotypes, classical and El Tor based on a battery of biochemical tests<sup>1</sup>. Clinical manifestations of cholera are caused by cholera toxin (CT), the principal virulence factor encoded by the *ctxAB* of *V. cholerae* located on the CTX prophage integrated on its chromosome (1). Cholera toxin-B subunit encoded by *ctxB* belongs to its biotype, i.e. a biotype classical strain has classical type *ctxB* and a biotype El Tor strain has El Tor type *ctxB*. *V. cholerae* O1 El Tor biotype is the causative agent of seventh pandemic while classical biotype caused the earlier pandemics.

Over the decades, *V. cholerae* O1 the causative agent of cholera has been witnessed to pass through different evolution probably to accommodate adverse condition for its survival. The stepwise evolution of *V. cholerae* O1 from classical through El Tor appeared as El Tor variant with classical CT probably since 1992<sup>2</sup>. *V. cholerae* O1 El Tor variant with its more virulence (3) gradually spread most part of the cholera prone region in the world leading to large morbidity and mortality<sup>2</sup>. Cholera has been reported in Odisha, caused by *V. cholerae* O1 and O139 serogroups<sup>4,5,6</sup> and endemic in coastal district<sup>7</sup>. Recently a large cholera outbreak was occurred due to *V. cholerae* O1 El Tor variant<sup>6</sup> in 2007 followed by spread of this strain in the state causing outbreaks in 2008-09. This study reported a cholera outbreak in Chandanpur villages in Puri district in January 2011 caused by *V. cholerae* O1 El Tor variant.

### MATERIALS AND METHODS

Chandanpur is situated 10 km away from Puri, on the coast of Bay of Bengal. Puri is a holy city where national and international tourists come

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to visit throughout the year. Puri town is the district headquarter of Puri district constituting 26 peripheral villages including Chandanpur known as Puri Sadar. The entire sadar area is surrounded by estuarine and brackish water. A local outbreak of diarrhoea occurred between 1st- 12<sup>th</sup> January 2011 in Chandanpur one of the major village of Puri Sadar. It is a semi-urban village situated at the side of main road from Puri to Bhubaneswar the capital city of the state. A branch river, Bhargabi carrying water from Mohanadi (the main river of the state) passes adjacent to Chandanpur.

Diarrhoea continues to be one of the important health problems in this locality. Considering water as the important vehicle of transmission of diarrhoea causing agents, government has provided supply of safe drinking water to Chandanpur and neighboring villages from Bhargabi river through network of pipe.

Diarrhoea outbreak in Chandanpur was reported on 1<sup>st</sup> January 2011 and continued up to 12th January 2011. A case of diarrhea was defined as the occurrence of more than three watery stools a day among residents of the Chandanpur area. Anticipating extensive and explosive nature of the outbreak of diarrhoea, local health authorities, doctors and paramedical workers visited the patients with clinical symptom of diarrhoea in Chandanpur area. All the diarrhoea cases had high frequency of loose stools more than three times with rice water appearance. Diarrhoea patients were admitted in community health center (CHC), Chandanpur and infectious disease hospital (IDH), Puri. The patients having uncontrolled severe diarrhoea were referred from Chandanpur CHC, to the cholera ward in IDH, Puri. On recommendation of the health authorities, immediately all suspected water sources and reservoir of supply water at pump house in diarrhoea affected area were disinfected.

An investigating team from the Microbiology department, Regional Medical Research Centre (RMRC), Bhubaneswar collected clinical and epidemiological data from the hospitalized diarrhoea cases with simultaneous collection of rectal swabs admitted to IDH, Puri and CHC, Chandanpur and these cases were included in this study. Patients were interviewed following WHO guidelines and appropriate management was advised. There were no fatal cases reported during the study period. The

epidemic curve was constructed to describe the beginning and progression of the outbreak over time and cases were identified in the affected area to plan action for control of the outbreak.

Fifteen rectal swabs were collected non-randomly from untreated hospitalized patients in Cary-Blair transport medium (CBT, Difco, USA). Water was suspected as the vehicle of transmission and mode of spread of this localized outbreak. Seven water samples were collected non-randomly in one-liter sterile bottles from different water sources like one from reservoir of supply water, two tube wells, two-piped water and two-stored drinking water.

All rectal swabs were processed at the Microbiology laboratory, RMRC for detection of diarrhoeagenic pathogens using standard technique<sup>8</sup>. Water samples were analyzed to find out the source of contamination following previously described method<sup>9</sup>. Antimicrobial susceptibility test was done following the modified Kirby-Bauer disk diffusion technique<sup>10</sup> with commercially available discs (Difco, USA) ampicillin (10µg), chloramphenicol (30µg), cotrimoxazole (25µg), ciprofloxacin (5µg), furazolidone (100µg), gentamicin (10µg), neomycin (30µg), nalidixic acid (30µg), norfloxacin (10µg), streptomycin (10µg) and tetracycline (30µg). Characterization of strains as being susceptible or resistant was based on the size of inhibition zone around each disc according to manufacturer's instruction, which matched interpretive criteria recommended by the WHO<sup>11</sup>. Strains with an intermediate inhibition zone were interpreted as being resistant on the basis of minimum inhibition concentration (MIC) studies conducted with *V. cholerae*<sup>12</sup>. The presence of virulent genes *ctxA*, *tcpA*, *zot*, and *ace* were determined using PCR assay as described elsewhere<sup>13, 14</sup> and MAMA PCR for detection of *ctxB* classical {(*ctxB<sup>C</sup>*)<sup>15</sup>}.

## RESULTS

One diarrhoea case was reported at 11 PM on 1<sup>st</sup> January 2011 and within few hours, at least 15 people were admitted in the hospital with severe to moderate dehydration on 2.1.2011 in the morning. Total affected population in the study area was about 6255 from 2684 families. A total of 136 diarrhoea cases representing all age groups

were reported from the affected Chandanpur and neighboring villages. Most cases presented with profuse watery diarrhoea and vomiting and 92 cases were admitted in IDH hospital and CHC, Chandanpur. The index case was a 70-year-old man with severe dehydration admitted in the CHC, Chandanpur on 1.1.2011 belonged to Thentapur, a neighboring village of Chandanpur. He did not visit any cholera outbreak area before the disease appeared. The outbreak was peaked on 3<sup>rd</sup> January, 2011 (Figure-2). By the time, awareness on early hospitalization was spread among people and patients having watery diarrhoea, immediately reported to the health facilities. No new additional

case was reported after 12<sup>th</sup> January 2011. Out of 92 cases, 88 (95.6%) and 4 (4.3%) were adults and pediatrics respectively.

Microbiological analysis of 15 rectal swabs collected during the investigation revealed 4 (26.6%) were positive for *V. cholerae* O1 Ogawa, El Tor biotype. No other enteropathogens were isolated. The strains were uniformly (100%) susceptible to azithromycin, ampicillin, gentamicin, chloramphenicol, ciprofloxacin, norfloxacin, ofloxacin, neomycin and tetracycline and were uniformly (100%) resistant to co-trimoxazole, furazolidone, nalidixic acid and streptomycin. All the 4 *V. cholerae* O1 strains carried *ctxA*, *tcpA*, (El

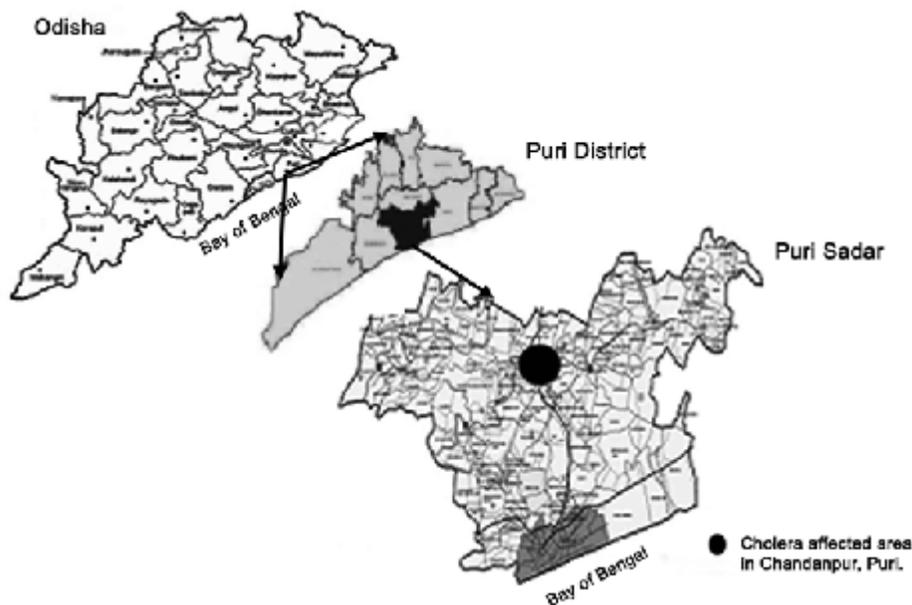


Fig. 1. Diarrhoea-affected Chandanpur area, Puri district (1<sup>st</sup> - 12<sup>th</sup> January, 2011)

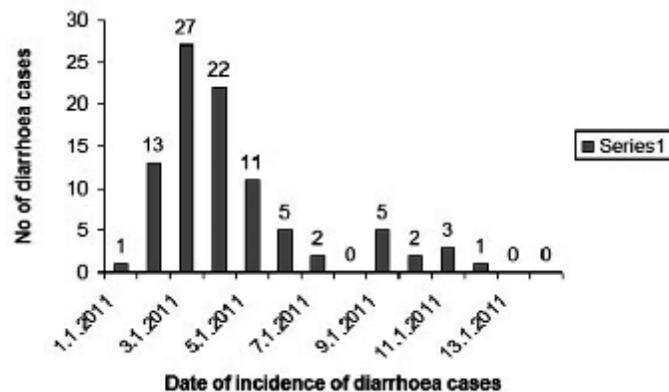


Fig. 2. Number of cases of diarrhoea by date, Chandanpur area (1<sup>st</sup> - 12<sup>th</sup> January, 2011)

Tor biotype) *ace* and *zot* genes, which verified genetically their toxin producing capacity and epidemic potential. All these strains carried *ctx B<sup>C</sup>* and showed conventional El Tor phenotype implying that these were El Tor variant as per the revised scheme<sup>17</sup>. This indicates that El Tor variant has replaced the resident prototype El Tor vibrio.

The network of pipelines is passed along the roads, through waterlogged area and beside the drains in the village. During the investigation, we found one leakage in pipe merged in estuarine water that may have contaminated the drinking water due to reverse pressure. The leakage was repaired after two days and Local board authorities did super chlorination of water sources before acquiring the samples, to prevent further transmission among the population. So the test result of water samples came negative and fit for drinking.

### DISCUSSION

More commonly, people in Puri Sadar use pond water for cooking and some time drink as hard water is coming in the tube well and dug well. Large numbers of diarrhoea cases admit to different local hospitals including IDH every year from Puri Sadar. A ten-year diarrhoea surveillance report by RMRC Bhubaneswar revealed *V. cholerae* was the important causative agent of the diarrhoea in this locality<sup>7</sup>. Khuntia *et al*<sup>5</sup> reported environmental *V. cholerae* O139 was the progenitor of a local outbreak in Rusipada village under Chandanpur. People in Chandanpur area mostly use supply water from river as their main source of drinking water and domestic uses. Present cholera outbreak in Chandanpur may have occurred because of contaminated drinking water by *V. cholerae* through leakage found in the water pipe. The second part of the peak of the epidemic curve had 12% patients were due to secondary person-to-person transmission.

Currently, the dissemination of highly virulent El Tor variant has been encountered replacing the prototype El Tor round the world<sup>18</sup>. Pal *et al*,<sup>6</sup> reported a large outbreak of severe cholera in Odisha, India due to the El Tor variant. In another study Goel *et al*,<sup>19</sup> reported the severe cholera outbreak in Odisha was due to the mutant of El tor variant in 2007. Retrospective analysis of

the *V. cholerae* strains from sporadic outbreaks in 2000 were El Tor variant. Recently the epidemic occurred in Haiti was due to *V. cholerae* O1 El Tor Variant<sup>20</sup>. Due to genetic homology between Haiti and Odisha strains 2007, it was hypothesized that *V. cholerae* O1 El Tor Variant spread from Odisha to Haiti through human activity<sup>20</sup>. The present study revealed a cholera outbreak due to El Tor variant replacing the resident prototype *V. cholerae* O1. This suggested that the isolates of *V. cholerae* that caused the present outbreak were recently introduced and caused the present outbreak, which should be monitored to document its spread along east coast of India.

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