Vibrio cholerae O1 Ogawa strains carrying the ctxB7 allele caused large cholera outbreak during 2014 in the tribal areas of Odisha, India

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Running Head: Haitian variant V. cholerae in Odisha
SUMMARY: The large outbreak of cholera reported during July to Sept, 2014 in Narla block of Kalahandi district was investigated to find out the causative organism. The rectal swabs collected from diarrhoea patients and environmental water samples were sub-cultured following standard techniques. The causative organism was *V. cholerae* O1 Ogawa biotype El Tor while DMAMA-PCR assay confirmed that all strains were *ctxB*7 of Haitian variants *V. cholerae* O1. The water samples were negative for *V. cholerae*. The *V. cholerae* O1 strains were sensitive to tetracycline, ciprofloxacin, norfloxacin, ofloxacin, doxycycline, azithromycin, but were resistant to erythromycin, gentamicin, chloramphenicol, furazolidone, neomycin, cotrimoxazole, nalidixic acid and ampicillin. The early reporting of the pathogen enabled the government authorities to implement adequate control measures in time to check the spread of the disease. This is the second large cholera outbreak due to Haitian variants of *V. cholerae* O1 after the Haiti cholera outbreak during 2010 reported from Odisha, India and also from the globe. Active surveillance is required to track the spread of this strain in this region.
INTRODUCTION

Cholera an ancient water borne disease continues to be devastating disease globally with high prevalence in developing countries. The disease cholera caused by *V. cholerae* having more than 200 sero-groups of which O1 and O139 sero-groups are named as pathogenic/epidemic strains. The *V.cholerae* O1 sero-group has two biotypes: Classical and El Tor. The classical biotype is believed to be extinct where as El Tor biotype is continuing as 7th pandemic strain. The devastating cholera epidemic during 2010 in Haiti after a century placed this ancient scourge in the forefront of the global public health agenda (1) and WHO in May 2011 documented the reemergence of cholera as a substantial global public health problem and asked for the execution of an integrated and inclusive global approach to control the cholera (2). Meanwhile several reports were published regarding evolution of altered El Tor variants *V.cholerae* O1 causing the cholera outbreaks /epidemics from Asia, Africa as well as from different countries from globe. The several ctxB alleles were identified in *V.cholerae* strains of different biotypes and serogroups, where these alleles differ in a few point mutations (non- random). *V. cholerae* O1 has three ctxB alleles as ctxB1 to ctxB3 where as *V.cholerae* O139 has three ctxB genotypes as ctxB4 to ctxB6. A new ctxB genotype similar to ctxB1 has been reported in O1 strain named as ctxB7 genotype. The above mentioned different ctxB alleles differ each other in their variable nucleotides and the corresponding amino acid position of different alleles.(3) In recent years the novel pathogenic variants of ctxB alleles of *V.cholerae* O1 have been emerged and documented throughout the world (4,5,6,7). Many published reports demonstrated that Haitian cholera toxin (HCT) variants *V.cholerae* O1 were predominant throughout the World, but they were highlighted after the disastrous Haitian cholera outbreaks. Presence of ctxB and tcpA ctrs alleB were reported
from the cholera outbreaks in 2009 and 2010 from Nigeria (8). Appearance and gradual dissemination of ctxB7 variant allele in Kolkata from 2006 onwards was published by Naha et al, 2012 (6). El Tor variant strains of V.cholerae O1 classical CT have completely replaced the El Tor CT producing strain in Kolkata since 1995 (6). El Tor type ctxB was also replaced by the classical allele in Bangladesh since 2001(9) and presence of classical CT producing El Tor strain from Gulf coast was also reported by Olsvik et al, 1993 (10). New ctxB genotypes were also observed in Zambia and Mexico during 2013 which were due to ctxB7 of HCT variants V.cholerae (11,12). The ctxB7 variant V.cholerae O1 first reported from the tribal areas of Odisha during 2007 (6,13). These HCT variant strains of V. cholerae O1 were also reported from Maharashtra (14) and Bihar during 2012 (15). The present study was envisaged to document the causative agent of large cholera outbreak during July to September, 2014 from Narla block of Kalahandi district of Odisha.

MATERIALS AND METHODS

Study area: The microbiologist along with medical officer of Narla Community Health Centre (CHC), Kalahandi district visited the diarrhoea-affected villages (16.9.14 to 20.9.14) of Narla block to get the information on the date-wise line listing of all diarrhoea cases, index case, clinical signs and symptoms of the severe diarrhoea patients reported from the diarrhoea- affected villages. The index case was traced back from the hospital record. The source of drinking water, chlorination of drinking water sources and sanitary conditions prevailing in those villages were also checked.
**Isolation and identification of *V.cholerae*:** The informed consent of participating patients / their attendants were taken before collection of samples. Rectal swabs were collected from the diarrhoea patients from the villages and also from the Narla CHC, transported in Cary-Blair transport medium, enriched in APW and further streaked on TCBS agar. Sucrose fermenting yellow colonies were selected and tested using standard biochemical tests. The sucrose fermenting colonies from TCBS agar plates were sub-cultured in triple sugar iron agar medium (A/A reaction without acid and gas production and oxidase positivity).

Serological conformation was done using *V.cholerae* specific polyvalent O1 and monovalent Ogawa and Inaba antisera. The sensitivity and the resistance patterns of *V. cholerae* O1 strains were tested with antibiotic-impregnated commercial disks (Hi-Media, Mumbai, India) (16).

**DMAMA-PCR Assay:** DMAMA(Mismatch Amplification Mutation Assay) PCR assay was used to detect the type of *ctxB* allele in all *Vibrio cholerae* O1 strains isolated from this outbreak along with 30 *V. cholerae* strains isolated between 1999 to 2007 (laboratory stocks) using primers (*ctxB*- F3/ Rv-cla) for the Haitain *ctxB* allele and (*ctxB*- F4/ Rv-cla) from the classical *ctxB* allele. These allele specific primers each carry specific nucleotides A and C for the Haitain and classical allele respectively, at the 3’ end as described by Naha et. al.,2012 (6). Template DNA for PCR assay was prepared from *V. cholerae* overnight culture grown with Luria-Bertani broth at 37°C. PCR mixture (25 μl) contained 5 μl of template DNA after extraction by boiling method. PCR conditions were as follows: initial denaturation at 96°C for 2 min, 25 cycles of denaturation at 96°C for 10 sec, annealing at 60°C for 10 sec and extension at 72°C for 30 sec and final extension at 72°C for 2 min. The amplified fragments were detected by agarose gel electrophoresis after staining with ethidium bromide (6).
RESULTS

Narla block is located in Kalahandi district of western part of Odisha. The block is having 1,27,043 population, total population affected-46236, villages affected-57, total cases reported- 321 and deaths were 3. The index case occurred on 28/07/14 in Bankel village and gradually the cases increased. As per the hospital record the index case was a 62 year male (goat keeper) from Bairpada sahi of Bankel village. The village is situated at the bottom of the mountain adjacent to a water reservoir. He went to the mountain for goat grazing on 28.07.14 early morning, felt severe thirst, drank water from the water reservoir at 11am and returned home in the afternoon, suffered from severe diarrhoea at 7pm on that night. He had profuse rice water stool, vomiting associated with severe dehydration, muscular pain and abdominal cramping. He was admitted to the hospital on 29.7.14 morning, treated and cured. The diarrhoea clothes were cleaned in the field adjacent to his house. At that time there was continuous heavy rain fall for 7-10 days. The rain water with fecal materials might have mixed and flowed downward, mixed with small nala and water of “Sandul” river. Gradually the cases were reported from the nearby villages of that river along the down flow of water. The diarrhoea cases were reported from Narla, Sargiguda villages which were worst diarrhoea affected and spread to other villages also. The line listing of all severe diarrhoea cases were analyzed from the available hospital records of that CHC. The index case was reported on 28.7.2014, the highest number of severe diarrhoea cases (27) were reported on 11/09/14 and last cases were reported on 16.9.14 (Fig-1). The date of occurrence of first and last diarrhoea cases in each village were noted from the hospital record. Considering the occurrence of these diarrhoea cases and smallest distance between the villages; the probable spread of the disease from village to village was drawn Fig. 2(a). The most cholera affected
villages were Narla, Bankel, Asurgada, Sargiguda etc. The cholera epidemic continued from July to September, 2014 and more number of cases and villages were affected during the month of August and September, 2014 (Fig. 2(b)).

Out of 17 rectal swabs 11 samples were positive for *V. cholerae* O1 Ogawa biotype El Tor and 5 were E. coli and one was culture negative for any bacterial enteropathogens. The *V.cholerae* strains were sensitive to ciprofloxacin, norfloxacin, tetracycline, doxycycline, ofloxacin, azithromycin; but were resistant to ampicillin, gentamicin, furazolidone, nalidixic acid, erythromycin, neomycin, co-trimoxazole and chloramphenicol. The DMAMA-PCR results revealed that all eleven *V.cholerae* O1 stains were positive for ctxB7 of Haitian variant (Fig: 3). Again 8 out of 30 *V. cholerae* strains isolated during 1999 to 2007 [1999-2/5, 2000-3/10, 2003-0/5, 2007-3/10] were positive for HCT variant *V. cholerae* O1 Ogawa.

**DISCUSSION**

Group discussions were held among the people in different villages during field visits. It was found that some people went to the cotton fields for working and drank water from the nearby water reservoir, took bath and returned home in the afternoon. The people reported diarrhoea with rice water stool in the night associated with vomiting and abdominal cramping. Gradually the patients developed severe dehydration in the night, hospitalized on the next day morning, treated and cured. This clearly indicated that the source of infection was the large water reservoir. The cases gradually increased due to unhygienic condition in the house, person to person contact in the family, unsafe disposal of fecal materials, cleaning of diarrhoea clothes in nearby water reservoir and migration of people from one village to other villages after attending their relatives who suffered from diarrhoea. The infection spread to other family in the nearby house in the same village and also to other villages. It was learned
from the old persons in those cholera affected villages that there was no cholera outbreak reported in that block since last thirty years. The hospital records of last five years also indicated that there was no diarrhoeal outbreak reported in that block. Contamination of drinking water sources was the main cause of transmission of cholera. In the rainy season people mainly go to paddy field for farming, drink water from nala, small water reservoir, river and steam, gets infection in this way. The water samples collected during 2007 cholera outbreak in the tribal areas were positive for \textit{V. cholerae} O1 Ogawa (16). The diarrhoea patients mostly belonged to schedule tribe and schedule caste followed by general caste and the patients were above 20 years age. This group of people were mostly illiterate and had poor knowledge on acquiring and spread of cholera infection.

Though the index case was from Bankel village, the cases increased from nearby villages of the “Sandul” river at the down flow of water stream of the river. Early reporting enabled the state health authorities to implement adequate control measures in time, like chlorination of drinking water sources, putting sand bags mixed with bleaching powder near the upstream of the water flow near the ghats of the river/nala/water reservoir (everyday), safe burial of fecal matters and vomits etc. These control measures checked the spread of the disease into unaffected areas of this block.

The \textit{V. cholerae} O1 Ogawa biotype El Tor strains were sensitive to: ciprofloxacin, azithromycin, norfloxacin, ofloxacin, doxycycline, and tetracycline; but were resistant to ampicillin, nalidixic acid, neomycin, furazolidone, erythromycin, chloramphenicol, gentamicin, and co-trimoxazole. Similar types of antibiogram profile were reported during 2010 cholera epidemic in Rayagada district where tetracycline which was resistant at that
Kumar et al, 2014 (17) reported that the HCT variant *V. cholerae* O1 (*ctx*B7) first reported from Odisha during 2007, after which this strain caused a devastating cholera epidemic in Haiti during 2010. Similarly the HCT variants *V. cholerae* O1 Ogawa were isolated after a cholera outbreak in Yavatmal district in Maharashtra (14) from South India during 2012-2014 (18). Several studies indicated that there were certain changes in *ctx*B gene located in *ctx* Q element of *V. cholerae* strains. The HCT variant *ctx*B *V. cholerae* strains carries a mutation at 58th nucleotide corresponding to 20th nucleotide corresponding to 20th amino acid (His 20 in classical and El-Tor Asn in Haitian allele) (8).This mutation has been rarely reported globally and recently being reported in few outbreaks in India (19). To find out its origin some representative strains from previous years *V. cholerae* O1 stocks were tested using DMAMA-PCR assay. It was found that HCT variant *V. cholerae* strains originated during post cyclone of 1999 from the coastal district of Odisha. Further molecular studies are warranted to investigate the spread of this HCT variant *V. cholerae* O1 strains in coastal and tribal areas of Odisha.

The altered biotype in *V. cholerae* El Tor (classical CT) strains have been reported from different geographical regions (19). The hybrid property (classical CT in altered El Tor biotype) enhances the infection potential of the bacterium and such strains are associated with more fluid loss and higher case fatality rate. These strains were reported to cause outbreaks in different parts of India including Odisha, Chennai, Hyderabad, Solapur and Assam (20, 21, 22). The HCT variants *V. cholerae* were reported for the first time from cholera outbreak in Odisha during 2007, later from Haiti during 2010 and subsequently from Asia and Africa (15,
There are few reports available which describe that HCT variant *V. cholerae* O1 were either isolated or caused small outbreaks from West Bengal, Bihar, South India (6, 13, 14). Similar reports on HCT variant *V. cholerae* were also published from Nigeria (11) and Mexico cholera outbreak during 2013 (12). The present investigation reports the multiple drug resistant HCT variant *V. cholerae* O1 causing large cholera outbreak in the tribal areas for the first time from Odisha, India. This may be the second large cholera epidemic due to HCT variants *V. cholerae* O1 reported from globe after the cholera epidemic of Haiti in 2010. Continuous surveillance is highly required to trace its origin and spread in different parts of Odisha as the future outbreaks/epidemics may happen with this new HCT variant *V. cholerae* strains.

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**Conflicts of interest:** None to declare

**REFERENCES**


Fig 1: Date wise incidence of cholera cases in Norla block
Fig 2: Date wise (a) and month wise (b) spread of cholera cases in Narla Block

Fig-3: DMAMA PCR Assay showing ctxB7 of Haitian Variants *V.cholerae* O1 Ogawa strains (lane1- 1 Kb Ladder, lane 2: Positive control Haitian Variant, lane 3 to 13: *V.cholerae* O1 Ogawa strains, lane 14: Negative control)