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Original Research Article

Haitian ctxB producing *Vibrio cholerae* in Karnataka, India: Emphasis on molecular epidemiology and laboratory networking system

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Abstract

Cholera is a primeval waterborne, severe dehydrating diarrheal disease, caused by *V. cholerae*, with a considerable health burden especially in developing countries. Due to lack of laboratory based evidences of an etiological agent many outbreaks are recorded as diarrhea outbreaks and cholera as well as *V. cholerae* El Tor biotype may remain unrecognized. The present report is an effort to trace the circulating strain in the community with the support of laboratory networking system and the aid of molecular techniques in epidemiology.

Under Integrated Disease Surveillance Programme [IDSP], Dept of Microbiology Bangalore Medical College and Research Institute is recognized as a state referral laboratory for investigation of outbreaks in linked districts. In the month of July 2016 a total of 06 representative stool samples randomly collected in Cary Blair media from suspected cases of cholera were received for isolation and identification of etiological agent. All stool specimens were processed according to standard protocol and *V. cholerae* isolates were sent to National Institute for Cholera and Enteric Diseases [NICED] for molecular characterization.

V. cholerae was isolated from 05 out of 06 samples tested. Serological tests revealed that all 05 isolates belonged to *V. cholerae* strain O1 serotype Ogawa. PCR based study from NICED confirmed all the *V. cholerae* strains contain Haitian ctxB.

This molecular epidemiological study under the laboratory networking system revealed the circulation of Haitian variants of *V. cholerae* in some regions of Karnataka. Since the mortality and morbidity is high with Haitian genotypes than the El tor biotype coordinated and vigilant monitoring of *V. cholerae* is essential.

Keywords: Haitian ctxB, *V. cholerae*, Laboratory networking system, India.

1. Introduction

Cholera, a primeval waterborne, severe dehydrating diarrheal disease, caused by *V. cholerae*, globally still continuing with a considerable health burden especially in developing countries due to poor socio economic status and sanitation facilities.[1]

World health Organization in 2015 reported 1,72454 cases and 1,304 deaths of cholera. According to National health profile (2015) of India, 116,73018, cases

and 1,323 deaths of acute diarrhea were reported among these 969 cases and 05 deaths were due to cholera .[2,3]

More than 200 serogroups of *V. cholerae* were identified. Epidemics and pandemics were caused by O1 and O139 serogroups.

El Tor biotype, the causative agent of ongoing seventh pandemic is more resistant to environmental factors and has genetic plasticity which leads to emergence and

persistence of more virulent and drug resistant hybrid strains.

In recent years, an El Tor biotype variant strain has emerged with a novel *ctxB* and disseminated throughout the world. It was highlighted as Haitian *ctxB* only after the catastrophic Haitian cholera outbreak (2010). Such strains are associated with drug resistance, more fluid loss and a higher case fatality rate. [1,4]

Because of lack of laboratory based evidences of an etiological agent many outbreaks are recorded as diarrhea outbreaks and cholera as well as *V. cholerae* El Tor biotype may remain unrecognized.[5] The present report is an effort to trace the circulating strain in the community with the support of laboratory networking system and the aid of molecular techniques .

2. Material and Method

2.1 Study area demography

Virupapura is a small village of 275 houses with 1,271 population in Magadi Taluq, Ramanagara Dist, Karnataka, South India. It is 40 km away from Ramanagara main town. Open defecation is more common in this area. Drinking water has been supplied through pipes from a common source. During the month of July 2016 an outbreak of acute diarrhea was noticed at Virupapura by public health authorities.

2.1.1 Case definition

According to Integrated Disease Surveillance Programme [IDSP], suspected case is defined as acute watery diarrhea, with or without vomiting, in any patient. A suspected case on laboratory confirmation is considered as confirmed case. Laboratory criteria for diagnosis is isolation of *V. cholerae* O1 or O139 from stool sample in any patient with diarrhea.

2.1.2 Outbreak data and immediate control measures

An outbreak of diarrhea was suspected in Virupapura on July 13th night when 06 patients visited the primary health centre for the treatment of diarrhea and vomiting. Clinical symptoms were suggestive of cholera and patients were put on rehydration therapy and treated empirically. The outbreak affected only a small portion of people in Virupapura and lasted for 09 days. A total of 83 cases were reported, 06 cases were reported first day and increased every day for the next four days and after institution of proper control measures and treatment cases gradually decreased up to 9th day and 10th day onwards no cases were reported. According to the first information report of District surveillance unit Ramanagara there was breakage in the water pipes installed near the drainage in three streets of the village and contaminated water on the ground may have entered back into the water pipes as control valves are installed below the ground level. An

immediate action was taken by concerned authorities to replace the broken pipes with new pipes and control valves were installed above the ground level to prevent the reverse flow of contaminated water in to the water pipes.

2.1.3 Clinical samples

Department of Microbiology, Bangalore Medical College and research Institute [BMCRI] is recognized as a state referral laboratory under IDSP for investigation of outbreaks in linked districts. On July 14th and 15th 2016, a total of 06 representative stool samples randomly collected in Cary Blair media were received for culture and sensitivity. The stool samples were collected from suspected cases of cholera during the first two days of acute diarrhea and severe dehydration in Virupapura.

2.1.4 Isolation and identification of etiological agent

All stool samples received in the laboratory were subjected for wet mount and hanging drop technique to screen for parasites and bacterial motility respectively. Simultaneously samples were inoculated onto MacConkey agar, Blood agar, Thiosulphate citrate bile salt sucrose agar [TCBS] and Alkaline peptone water [APW] for enrichment and identification of enteric pathogens. Sub culture from APW was done after 06 hrs on to MacConkey agar, blood agar and TCBS agar. colonies on MacConkey agar and TCBS with the characteristic appearance of *V. cholerae* were screened by Hanging drop technique and confirmed by biochemical tests according to WHO guidelines.[6] Antibiotic susceptibility test was done by Kirby Bauer disk diffusion method using Muller-Hinton agar plates following Clinical and Laboratory Standards Institutes (CLSI 2007).[7] Antibiotic disks (Hi-Media, Mumbai) used were ampicillin (10µg), norfloxacin (10µg), cotrimoxazole (25µg), tetracycline (30µg), ceftriaxone (30µg) and chloramphenicol (30µg).

Isolates identified as *V. cholerae* by biochemical method were further subjected to serotyping with polyvalent O1, and mono-specific Ogawa and Inaba antisera (Denka Seiken, Japan). Serologically confirmed *V. cholerae* isolates were sent to NICED for further biotyping and molecular characterization.

3. Result

An Outbreak of Cholera in Virupapura was notified by District Health authorities of Ramanagara after reporting of 06 cases with vomiting and diarrhea clinically suggestive of cholera. There was no history of travel of these patients to any place and close contact of any case with diarrhea. A total of 83 cases were reported within a span of 09 days and all cases were clustered in Virupapura village and all age groups were affected. Outbreak was subsided after 9th day with one death.

V. cholerae was isolated from 05 out of 06 samples tested. Biochemical and serological tests revealed that all 05 isolates belong to *V. cholerae* strain O1 serotype Ogawa. All isolates were resistant to Tetracycline and Cotrimoxazole, among these 03 isolates were also resistant to Ampicillin. All were sensitive to Chloramphenicol, Norfloxacin and Ceftriaxone.

PCR based study from NICED confirmed that isolates belonged to *V. cholerae* strain O1 serotype Ogawa and all the *V. Cholerae* strains contain Haitian *ctxB*, in other words 20th amino acid of the *ctxB* possesses asparagine instead of histidine which is located in the signal region of the *ctxB*.

4. Discussion

Laboratory investigation at BMCRI confirmed that the etiological agent of diarrhea outbreak in Virupapura was *V.cholerae* O1 ogawa possessing Haitian *ctxB*. It was the sole etiological agent isolated from 05 out of 06 samples received and no pathogen was isolated from one sample which may be attributed to delay in transportation of the sample. The number of stool samples tested were less because of lack of facilities for transportation of sample from the secluded village to referral lab and may be a limitation for our study.

As this cholera outbreak occurred during the month of July corresponding to pre monsoon period, acute shortage of water, poor environmental hygiene coupled with probable contamination of drinking water supplied through pipes attributed to poor maintenance may be the probable cause for the outbreak.

The cholera toxin responsible for most of the manifestations of the illness is encoded by *ctxAB*. Subunit B of the principle toxin of Cholera is encoded by *ctxB*. There are 11 different genotypes of *ctxB* distributed among diverse serogroups of *V. cholerae*. Serogroup O1 strains are found with genotypes 1, 2, 3, 7, 10, and 11, in serogroup O139, genotypes 3, 4, 5, 6 are found, and genotypes 8 and 9 are found only in serogroups O27 and O37. All *V. cholerae* strains isolated during the outbreak in Virupapura possess genotype 7 of *ctxB*. [8, 9]

Classical strains associated with genotype 1 are allocated worldwide and along USA gulf coast, El Tor biotype strains of pre-seventh pandemic from Australia harbored genotype 2 and El Tor biotype strains from the seventh pandemic and the Latin American epidemic are found to be associated with genotype 3. [10]

In Classical strains *ctxB* is more conserved but in El Tor strains it is subjected to many variations. Currently there are 3 reported variants of El Tor biotype in circulation - Matlab variants, Mozambique variants and Hybrid El Tor variants.

Matlab strains were isolated from acute diarrhea cases in Matlab, Bangladesh between 1991 -1994. These strains possess the classical allele of *tcpA* and *ctxB* gene and other El tor specific genetic elements. They exhibit mixed phenotypic characteristics of both Classical and El Tor biotypes and hence could not be biotyped.

Mozambique variants have typical El Tor genome and classical type CTX prophage. Except for classical *ctxB* allele these strains show all El Tor specific phenotypic and genetic characteristics. These strains were reported in 2004, isolated from cholera treatment center in Beira, Mozambique.

The hybrid El Tor variant, has a typical El Tor biotype but the *ctxB* of this new variant was found with single nucleotide polymorphism (C→A) at position 58 that leads to replacement of histidine at position 20 with asparagine in contrast to classical *ctxB*. This new *ctxB* allele was referred to as genotype 7 and was first reported during cholera outbreak in Orissa, Eastern India in 2007 and later noticed in cholera outbreaks in Haiti and Nigeria in 2010. [8, 9,11]

All *V. cholerae* strains isolated during the present outbreak in Virupapura possess genotype 7 of *ctxB*. Kutar *et al* reported that 46.2% *V. cholerae* isolated during the 2009 outbreak in Kolkata had Haitian *ctxB* allele.[12] Kumar *et al* reported that *ctxB7* genotype was found in *V. Cholerae* serotype O1 ogawa El tor biotype isolated in May 2012 cholera outbreak at Yavatmal district of Maharashtra India.[13] Sharma *et al* in 2015 reported multidrug resistant including tetracycline *Vibrio cholerae* isolates possessing *ctxB7* Haitian genotype isolated from the suspected cholera cases during the year 2012 from different parts of Haryana [14], in our study also all isolates were found to be resistant to Tetracycline and Cotrimoxazole. Akinsinde Kehinde Adewale reported that 103 out of the 115 epidemic isolates of the 2007–2013 cholera outbreaks in Nigeria were *V. cholerae* O1 and majority of them carried the atypical El Tor *ctxB7* allele.[15] Michel A. Marin reported the presence of *ctxB7* genotype in the *V. cholerae* isolated during the 2010 outbreaks in Nigeria.[16] Sameer M Dixit, Fatema –Tuz Johura in their study found, *ctxB7* in all 28 *V. cholerae* O1 strains isolated in 2012 from three districts of Nepal.[9] In Bangladesh El Tor strains isolated between 2008 and 2011 harbored *ctxB7* or Haitian *ctxB* and Shah M. Rashed *et al* stated that 2008-2010 was the time of genetic conversion of *ctxB* genotype 1 to genotype 7 and both were coexisted during 2009 in Bangladesh[10]. The emergence of Haitian variant of *V. cholerae* O1 ogawa strains in Karnataka was first reported by Bhattacharya *et al* in April 2016 from the outbreaks and sporadic cases of cholera during 2010-2014 in Belgaum, Karnataka South India.[17]

This molecular epidemiological study under the laboratory networking system revealed the circulation of Haitian variants of *V. cholerae* in some regions of Karnataka. Such studies help in tracking the origin, dissemination and evolution of new variants of the noxious pathogens that may cause outbreaks, epidemics and pandemics. Since the mortality and morbidity is high with Haitian genotypes than the El tor biotype coordinated and vigilant monitoring of *V. cholerae* is essential to know about the emergence of novel strains and antibiotic sensitivity pattern of the isolates circulating in the region for proper institution of empirical therapy to treat outbreaks and epidemics of Cholera.

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References

- [1]. Ghosh P, Naha A, Pazhani GP, Ramamurthy T, Mukhopadhyay AK, et al. Genetic traits of *Vibrio cholerae* O1 Haitian isolates that are absent in contemporary strains from Kolkata, India. *PLoS ONE* 2014;9(11):e112973.doi:10.1371/journal.pone.0112973.
- [2]. World health Organization. Fact sheet. 2015
- [3]. Central Bureau of Health Intelligence, WHO collaborating centre on family of international classification. National Health Profile -2015. New Delhi: Ministry of Health and Family Welfare, Government of India; 2015.
- [4]. Kumar P, Mishra DK, Deshmukh DG, Jain M, Zade AM, Ingole KV, Goel AK, Yadava PK. *Vibrio cholerae* O1 Ogawa El Tor strains with the ctxB7 allele driving cholera outbreaks in south-western India in 2012. *Infection, Genetics and Evolution* 2014; 25: 93–96.
- [5]. Verma R, Singh B, Bhalla K, Kamal M. Epidemiological Lesson Learnt: Diarrhea Outbreak Investigation In A Remote Village Of District Rewari (Haryana), India. *International Journal of Basic and Applied Medical Sciences* 2015; 5 (1): 103-107.
- [6]. WHO (1987). Manual for Laboratory Investigations of Acute Enteric Infections. CDD/83.3. Geneva, Switzerland: World Health Organization.
- [7]. CLSI (2007). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard M2-A10. Wayne, PA: Clinical and Laboratory Standards Institute.
- [8]. Dixit SM, Johura F, Manandhar S, Sadique A, Rajbhandari RM, Mannan SB, et al. Cholera outbreaks (2012) in three districts of Nepal Reveal clonal transmission of multi-drug resistant *Vibrio cholerae* O1. *BMC Infectious Diseases* 2014; 14:392.
- [9]. Zaw MT, Emran NA, Naing DKS, Lin Z. Atypical El Tor: new *Vibrio cholerae* O1 biotype causing epidemic cholera. *Borneo Journal of Medical Sciences* 2016; 10(1):36-46.
- [10]. Rashed SM, Mannan SB, Johura F, Islam MT, Sadique A, Watanabe H, et al. Genetic characteristics of drug-resistant *Vibrio cholerae* O1 causing endemic cholera in Dhaka, 2006–2011. *Journal of Medical Microbiology* 2012; 61: 1736–1745.
- [11]. Taneja N, Mishra A, Sangar G, Singh G, Sharma M. Outbreaks caused by new variants of *Vibrio cholerae* O1 El Tor, India [Letter] *Emerging Infectious Diseases* 2009;15(2): 352.
- [12]. Kutar BMRNS, Rajpara N, Upadhya H, Ramamurthy T, Bhardwaj AK. Clinical Isolates of *Vibrio cholerae* O1 El Tor Ogawa of 2009 from Kolkata, India: Preponderance of SXT Element and Presence of Haitian ctxB Variant. *PLoS ONE* 2013; 8(2): e56477 doi: 10.1371/journal.pone.0056477.
- [13]. Kumar P, Mishra DK, Deshmukh DG, Jain M, Zade AM, Ingole KV, Yadava PK. Haitian variant ctxB producing *Vibrio cholerae* O1 with reduced susceptibility to ciprofloxacin is persistent in Yavatmal, Maharashtra, India, after causing a cholera outbreak.[Research note] *Clinical Microbiology and Infection* 2014; 20: O292–O293.
- [14]. Sharma NC, Kumar D, Sinha P, Seth DK, Gupta SK. Tetracycline Resistant *Vibrio cholerae* O1 Ogawa With Ctxb7 Allele (Haitian) is Prevalent in Haryana, India. *Indian Journal of Applied Research* 2015; 5(3): 476-79.
- [15]. Adewale AK, Pazhani GP, Abiodun IB, Afolabi O, Kolawole OD, Mukhopadhyay AK, et al. Unique Clones of *Vibrio cholerae* O1 El Tor with Haitian Type ctxB Allele Implicated in the Recent Cholera Epidemics from Nigeria, Africa. *PLoS ONE* 2016); 11(8): e0159794. doi:10.1371/journal.pone.0159794.
- [16]. Marin MA, Thompson CC, Freitas FS, Fonseca EL, Aboderin AO, et al. Cholera Outbreaks in Nigeria Are Associated with Multidrug Resistant Atypical El Tor and Non-O1/Non-O139 *Vibrio cholerae*. *PLoS Negl Trop Dis* 2013; 7(2): e2049. Doi: 10.1371/journal.pntd.0002049.
- [17]. Bhattacharya D, Dey S, Pazhani GP, Ramamurthy T, Parande MV, Kholkute SD et al. *Vibrio cholerae* O1 El Tor variant and emergence of Haitian ctxB variant in the strains isolated from South India. *Med Microbiol Immunol* 2016; 205(2):195-200.