Epidemiological studies of cholera outbreaks in Western and South India during 2009-2010 reveal involvement of multidrug resistant *Vibrio cholerae* with altered El Tor biotype

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Introduction

Vibrio cholerae, a Gram negative bacterium has been responsible for several cholera pandemics affecting millions of people over the globe. This is one disease in modern time that is endemic, epidemic and pandemic in nature. Several developing countries are still plagued by recurrence cholera outbreaks times to time. This disease is mainly localized in those areas where primary health and sanitation is still a challenge. In urban areas, generally it erupts due to accidental contamination of drinking water supply. Two distinctive epidemiological features of cholera are its tendency to appear in explosive outbreaks, often starting in several distinct foci simultaneously, and its propensity to cause true epidemics that progressively affect many countries in multiple continents over the course of many years (Kaper *et al*., 1995). World has already faced seven cholera pandemics in the past two centuries. Among the two biotypes of *V. cholerae*, El Tor strains are supposed to have better adaptability to survive in the environment and in the human host than classical biotype. Strains of classical biotype are suggested to be more toxigenic than El Tor strains (Huq *et al*., 1993). However, after 2001, hybrid strain of classical and El Tor biotypes were reported from different parts of India and Bangladesh (Nair *et al*., 2006). Recently, a new variant of this hybrid strain was reported from India (Goel *et al*., 2008, Goel and Jiang, 2009). These hybrid strains are reported to be more toxigenic having the potential to produce large amount of cholera toxin. In this study, we have investigated the epidemiological features of *V. cholerae* isolates from cholera outbreaks in India during 2009-2010.

Materials and methods

V. cholerae O1 strains were isolated from the patients during cholera outbreaks in India during 2009-2010. Serological identification of the isolates was done by slide agglutination using commercially available polyvalent antiserum against *V. cholerae* O1 and O139 (Difco, USA). Two multiplex PCR assays were employed for detection of various toxigenic and pathogenic genes encoding outer membrane protein (*ompW*), cholera toxin (*ctxB*), somatic antigen (*rfbO1*), toxin co-regulated pilus (*tcp*), zonula occuludens toxin (*zot*), repeat in toxin (*rtxC*), accessory cholera enterotoxin (*ace*), haemolysin (*hlyA*), outer membrane protein (*ompU*) and toxin regulator (*toxR*) as described earlier (Kumar *et al*., 2009).

The antimicrobial susceptibility of the *V. cholerae* isolates was determined by the disc diffusion method on Mueller Hinton agar. Presence of class 1 integrons and SXT constins was detected by PCR as described earlier (Hochhut *et al*., 2001). PCR product of cholera toxin B (*ctxB*) gene was amplified and sequenced and were aligned with the earlier reported El Tor and classical strains.

Results and discussion

A total of 64 *V. cholerae* strains were isolated from the affected patients from different cholera outbreaks in India during 2009-10. All the isolates were biochemically identified as *V. cholerae* and serologically confirmed as O1 Ogawa. The isolates were PCR positive for *ompW, ctxAB, rfbO1, tcp, zot, rtxC, ace, hlyA, ompU* and *toxR* genes. The presence of *rfbO1* gene confirmed the O1 serogroup of all the isolates. All the strains harboured *ctxB* and *tcp*

genes, which are involved in cholera pathogenesis of *V. cholera.* The pilus colonization factor TCP acts as a receptor for CTXϕ, which can infect non-toxigenic *V. cholerae,* leading to the emergence of new toxigenic strains. All the strains were found PCR positive for *ctx*, *zot* and *ace* besides *hly*, *ompU* and *toxR* gene, suggesting the presence of intact core toxin region in all isolates. These genes are found together and represent the genome of filamentous bacteriophage, CTXϕ. The presence of *rtxC* gene in isolates helps to differentiate the biotypes in *V. cholerae* O1 serogroup. Repeat in toxin (*rtxC*) gene was present in all the isolates indicating the El Tor biotype of strains.

All the isolates were susceptible to ampicillin, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, norfloxacin and tetracycline. However, the isolates exhibited high rate of resistance towards, co-trimoxazole, nalidixic acid, nitrofurantoin, polymyxin-B, spectinomycin, streptomycin, sulphamethizole, sulphamethoxazole and trimethoprim. Multi-drug resistance among *V. cholerae* strains limits the therapeutic potential of these drugs and presents additional challenges to disease management.

PCR results showed positive amplification of class I integron and SXT constins in all the isolates. These elements are not autonomously mobile but are able to capture, integrate and express resistance gene cassettes in their variable region. PCR results also confirmed the presence of *aadA2* gene cassettes within the integron which encode aminoglycoside adenylyltransferases inactivating streptomycin and spectinomycin. These gene cassettes are among the most prevalent gene cassettes in class 1 and class 2 integrons.

SXT constin is an important element for horizontal dissemination of antibiotic resistant genes in bacteria. In SXT constins, the antibiotic resistance genes are clustered within a composite transposon-like structure found near the 5′ end of SXT which confer resistance to chloramphenicol, sulphamethoxazole, streptomycin and trimethoprim (Beaber *et al*., 2002).

The alignment of sequences from different outbreak strains with O1 El Tor and O1 classical reference strains revealed that the *ctxB* gene sequences from all the outbreak strains were identical and were aligned with that of the classical biotype of *ctxB*. The deduced amino acid sequences differ from that of the El Tor strain by a histidine at position 39 and a threonine at position 68. Similar results were reported earlier from the Bangladesh *V. cholerae* isolates (Nair *et al.*, 2006). Classical biotype strains were replaced by El Tor biotype in seventh and current pandemic of cholera. Both biotypes of *V. cholerae* O1 are closely related in their O-antigen biosynthetic genes. However, the genomic structure of the CTXΦ, in which the cholera toxin genes are contained, differs between the classical and El Tor biotypes (Ansaruzzaman *et al*., 2004). The results from India (this study) and Bangladesh showed that classical CT producing El Tor strains have now replaced the seventh pandemic El Tor strains.

Thus, *V. cholerae* strains from cholera outbreaks in India during 2009-10 were resistant to several antibiotics and possessed various toxigenic and pathogenic genes along with class 1 integron and SXT elements. The study also showed that now, traditional El Tor biotype strains have been replaced by altered El Tor biotype having *ctxB* gene sequence of classical biotype strains.

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