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SHORT REPORT

Distribution of *Zonula occludens* Toxin (*zot*) Gene Among Clinical Isolates of *Vibrio cholerae* O1 from Bangladesh and Africa

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ABSTRACT

Seventy-two clinical isolates of *Vibrio cholerae* O1 from Bangladesh, and 12 and 9 isolates respectively from Tanzania and Nigeria were screened for sequences homologous to zonula occludens toxin (*zot*) and cholera toxin (*ctx*) genes. As observed previously, all isolates in the present study also possessed sequences for both toxins which suggested that *zot* does not occur independent of *ctx*. It appears that along with the virulence genes located in the "virulence cassette" region of the bacterial chromosome, *zot* may play a role in the pathogenesis of cholera.

Key words: *V. cholerae* O1, Zonula occludens toxin gene, Cholera toxin gene

INTRODUCTION

Cholera caused by intestinal infection with *Vibrio cholerae* O1 is an acute dehydrating diarrhoea. The copious diarrhoea characteristic of cholera is principally produced by the potent enterotoxin, cholera toxin (CT) elaborated by these organisms in the small intestine of patients. The CT molecule is composed of the enzymatically active single A subunit which stimulates adenylate cyclase activity and five identical B subunits, which are responsible for the binding of CT to the GM1 ganglioside receptor on the intestinal mucosa (1,2). A few live oral vaccine strains have been constructed by deleting the major part of the gene encoding the A subunit of the CT (*ctx* A) resulting in A^-B^+ strains (phenotypically CT^-) (3,4). However, volunteer studies have shown that these non-CT producing strains still provoke mild to moderate diarrhoea in some individuals (5). Search for additional toxin(s) which would explain this reactogenicity resulted in the discovery of two more toxins: zonula occludens toxin (Zot) (6) and accessory cholera enterotoxin (Ace) (7). In Ussing chamber (UC) assay with rabbit ileal tissue, Zot altered the permeability of the ileal mucosa which was associated with a decrease in electrical resistance of the intestinal tissue due to disruption of the intercellular tight junction, zonula occludens. Ace, on the other hand, increased short-circuit current (Isc) in rabbit ileal tissue in UC by increasing the potential difference (PD). It has been hypothesized that Zot may be associated with constitutional symptoms, but Ace with other classic enterotoxins may cause diarrhoea (7).

The genes encoding the three enterotoxins (*ace*, *zot* and *ctx*) and core-encoded pilin (*cep*, a gene for a colonization factor) (8), flanked by RS1 elements are located on a 4.5 kb "core region" of the chromosome which can be perceived as a "virulence cassette" of *V. cholerae* (7). The *zot* gene has been cloned and sequenced (9). It consists of a 1.3 kb open reading frame, which could potentially encode a 44.8-kDa polypeptide. The *zot* gene is located immediately upstream of the *ctx* gene (9). We undertook the present study to obtain information about the relative occurrence of *zot* and *ctx* genes in a collection of clinical isolates of *V. cholerae* O1 from Bangladesh and Africa.

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MATERIALS AND METHODS

V. cholerae O1

Bangladesh isolates consisting of 72 strains belonging to both biotypes and serotypes were obtained from patients who attended the treatment centres of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) located in Dhaka and Matlab. These isolates were stored either in lyophilized form or in sealed deep nutrient agar at room temperature in our culture collection. The African strains consisted of 12 El Tor strains of either serotype from Tanzania (courtesy: F. Mhlau, Muhimbili Medical Centre, Dar-es-Salaam) and 9 El Tor strains of Ogawa serotype from Nigeria (courtesy: H. van Vliet, World Health Organization, Lagos). The African strains also originated from patients with cholera and were isolated during 1992. Before use, the cultures were reconfirmed as *V. cholerae* O1 by biochemical reaction and serology.

Probes and hybridization

The *ctx* gene probe was a 0.5 kb *Eco*RI fragment of the recombinant plasmid pCVD27, which had been constructed by cloning a 0.5 kb *Xba*I-*Cla*I fragment corresponding to the A subunit of CT into pBR325 using *Eco*RI linkers (10). The *zot* gene probe was a PCR generated probe. An 840 bp region internal to the *zot* gene was amplified from the recombinant plasmid pBB241 (9) using the two primers: primer I: 5'-GAACGCATAGCTAAGTAC-3', and primer II: 5'-CCTGTCGCCCATAGACCA-3'. The primers were annealed at 55°C and the PCR was continued through 30 cycles of amplification. The resulting 840 bp product was the only band from the PCR, and this band was gel-purified. The *ctx* gene probe and the PCR-generated *zot* probe used for hybridization, were labelled either by random priming (11) using a random primers DNA labelling kit (BRL) and {^{32}P}-deoxycytidine triphosphate (3000 Ci/mmol, Amersham International plc, Ayelesbury, UK) or with digoxigenin (Boehringer Mannheim) according to manufacturer's specifications. Colony blots were prepared, processed and hybridized under stringent conditions as described previously (12).

For Southern blot hybridization, chromosomal DNA was prepared by standard methods, digested with *Pst*I, and electrophoresed in a 0.7% agarose gel. The DNA was transferred to Magnagraph (MSI, Westboro, MA,USA) and baked for 2h at 80°C. The blot was prehybridized for 2h at 42°C and hybridized overnight with the digoxigenin-labelled *zot* probe at 42°C in the recommended hybridization solution containing 50% formamide. The blots were washed and processed according to manufacturer's recommendations.

RESULTS

All 72 *V. cholerae* O1 isolates from Bangladesh and 21 isolates from Africa were hybridized with both the *ctx* probe and the PCR-generated *zot* probe in colony hybridization. The presence of *zot* gene sequences were further confirmed in 8 selected isolates (4 Bangladeshi, 3 Tanzanian and 1 Nigerian) in Southern blot hybridization, and all of them showed signals consistent with *zot* gene.

DISCUSSION

There have been two previous studies on the distribution of *zot* and *ctx* genes (13,14). In one of these studies, Karasawa *et al.* (13) found simultaneous occurrence of both toxin genes among the majority of their clinical isolates of *V. cholerae* O1. In the study of Karasawa *et al.* (13), only one strain was found to be *ctx*$^-$ but *zot*$^+$. Furthermore, of the 98 isolates of *V. cholerae* O1 studied from food and environmental sources, 62 were positive for both toxins and 36 were positive for *zot* only. In the second study carried out by Johnson *et al.* (14), all 59 clinical isolates of *V. cholerae* O1 possessed genes for both toxins.

The results of the present study with Bangladeshi strains and African strains essentially supported the findings of the above two studies and suggests that *zot* gene does not occur independently from the *ctx* gene in clinical isolates of *V. cholerae* O1. It is conceivable why this inter-relationship between *zot* gene and *ctx* gene is so, because both genes are located on the "virulence cassette" region of the chromosome side by side (7). This suggests that in clinical isolates of *V. cholerae* O1, *zot* is not an independent virulence gene, but in conjunction with other virulence genes located in the "virulence cassette", it may play a role in the pathogenesis of cholera.

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