

this study, it is concluded that a single 1 g dose of ciprofloxacin is as effective as a single 300 mg dose of doxycycline in terms of clinical response, and that ciprofloxacin is more efficient in eradicating *V. cholerae* from faeces.



## Inhibition of Cholera Toxin-Induced Salt and Water Secretion By Short-Chain Fatty Acids in Vivo

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**Objective:** Determine the effect of SCFAs on cholera toxin-induced colonic secretion. Short-chain fatty acids (acetate, propionate, butyrate) produced by the fermentation of unabsorbed carbohydrates by the colonic bacteria have been shown to stimulate Sodium chloride absorption in the isolated colonic epithelium *in vitro*.

**Methods:** The effects of SCFAs on cholera toxin (CT)-induced colonic ion and water secretion in adult rabbit have been determined in this study using a perfusion technique with polyethylene glycol as a non-absorbable marker. Facilities of the ICDDR,B's Dhaka-based hospital, the Clinical Research and Service Centre, and its Animal Resources Branch, were used for this study.

**Results:** The study indicates that an 18-hour exposure to purified cholera toxin (5-100 µg) resulted in colonic water and electrolyte secretion in a dose-dependent manner. Perfusion with different SCFAs significantly ( $p < 0.001$ ) inhibited net colonic water secretion; the rates ( $\mu\text{l}/\text{min}^{-1}.\text{cm}^{-1}$ ) of inhibition being 99%, 94%, and 86% for butyrate (30 mM), propionate (60 mM), and acetate (90 mM) respectively. The rates of net sodium secretion were also significantly less ( $p < 0.01$ ) in the SCFA-treated colon than those treated with SCFA-free solution ( $\text{Na}^+$ , mean  $\pm$  SD,  $\mu\text{M}/\text{min}^{-1}.\text{cm}^{-1}$ :  $5.17 \pm 0.95$ ,  $7.31 \pm 0.65$ ,  $12.7 \pm 0.8$  for butyrate, propionate, and acetate respectively; and  $80.2 \pm 20.6$  for controls). Butyrate (30 mM) induced the highest inhibition of  $\text{Na}^+$  and water secretion followed by propionate and acetate. All 3 SCFAs significantly ( $p < 0.01$ ) inhibited  $\text{Cl}^-$  secretion, whereas only butyrate and propionate inhibited  $\text{K}^+$  secretion. There was no significant alteration of the colonic  $\text{HCO}_3^-$  secretion by the SCFAs, and none was able to reverse colonic secretion into net absorption.

**Conclusions:** SCFAs stimulate salt and water absorption from CT-stimulated colon and may be useful as absorption-promoting agents in oral rehydration solutions.



## Oxidative Stress in Patients With Severe Cholera

MA Khaled and GH Rabbani

**Objective:** Determine the adverse metabolic effects of oxidative stress in cholera. Oxidative stress is an adverse metabolic condition induced by the Reactive Oxygen Species (ROS). These ROS are produced and catabolized by specific enzymes during the normal course of metabolism. Lipid peroxidation due to ROS occurs during infection and malnutrition leading to oxidative stress and chemical injuries to the tissues. However, nothing is known about the adverse metabolic effects of oxidative stress in cholera.

**Methods:** To assess the degree of oxidative stress and lipid peroxidation in patients with severe cholera, the present investigators determined the faecal contents of thiobarbituric acid-reacting substances (TBARS), an index of lipid peroxidation, in 6 adults with severe dehydrating diarrhoea due to *Vibrio cholerae* infections and in 5 healthy adult volunteers. These volunteers were drawn each year from the 100,000 diarrhoea patients attending the ICDDR,B's Dhaka-based hospital, the Clinical Research and Service Centre.

**Results:** The preliminary results showed that the patients with acute cholera had significantly higher faecal concentrations of TBARS than had the healthy volunteers ( $9.56 \pm 4.41 \mu\text{mol/l}$  for cholera patients vs.  $4.03 \pm 1.86 \mu\text{mol/l}$  for the controls). This observation indicates that patients with active cholera may be associated with varying degrees of oxidative stress probably due to toxin-induced alteration of mucosal metabolism involving xanthine production leading to loss of fluid and electrolytes in the diarrhoeal stool.

**Conclusions:** Further studies will be required to characterize the metabolic abnormalities in cholera patients which may have important therapeutic implications.



## Survival Potential of Non-Culturable *Vibrio Cholerae* O1 by Laboratory Microcosms Using Polymerase Chain Reaction and Fluorescent Antibody Methods

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**Objective:** Assess the survival potential of *V. cholerae* O1 using conventional cultural, fluorescent antibody and recently developed polymerase chain reaction techniques. In Bangladesh, cholera is endemic in certain areas and flares into seasonal epidemics. *V. cholerae* O1 is one of the causative agents of cholera. The survival potential of *V. cholerae* O1 was carried out in various environmental samples by using conventional techniques.

**Methods:** The strain *V. cholerae* O1 biotype El Tor serotype Ogawa was used in this study carried out at the ICDDR,B's laboratories in Dhaka, Bangladesh. A measured inoculum of about  $10^5$  *V. cholerae* O1 per ml was added to 100 ml autoclaved pond water in a 500-ml conical flask, mixed and stored at room temperature. Culturable cells were counted on gelatin agar (GA) and taurocholate tellurite gelatin agar at various time intervals until the bacteria were no longer culturable.

**Results:** The non-culturable *V. cholerae* O1 was detected by fluorescent antibody and PCR techniques. The culturable *V. cholerae* O1 was isolated up to 44 days from the pond water microcosms. The non-culturable *V. cholerae* O1 was detected up to 7 weeks by FA and PCR techniques after they lost their culturability.

**Conclusions:** The non-culturable stage reported here for *V. cholerae* O1 is significant for understanding the epidemiology of cholera because the non-culturable state of *V. cholerae* O1 may pose health problems. Volunteer studies have shown that non-culturable *V. cholerae* O1 became culturable in volunteers intestine. This study demonstrated the survival of non-culturable *V. cholerae* O1 in surface water which may be important from the view point of public health.



## Cholera Toxin Stimulates Absorption of D-Glucose from the Adult Rabbit Small Intestine in Vivo

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**Objective:** Determine the effects of purified cholera toxin (CT) on intestinal absorption of glucose. Glucose is known to stimulate intestinal sodium absorption which provides the basis for the glucose-containing oral rehydration solution for the treatment of diarrhoea. Although this physiologic mechanism is well-preserved during severe cholera, the effects of purified cholera toxin on intestinal absorption of glucose itself has not been evaluated.