Gene encoding virulence markers among *Escherichia coli* isolates from diarrhoeic stool samples and river sources in rural Venda communities of South Africa

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Abstract

River water sources and diarrhoeic stools of residents in the Venda Region, Limpopo Province of South Africa were analysed for the prevalence of *Escherichia coli* (*E. coli*) and the presence of virulence genes among the isolates. A control group of 100 non-diarrhoeic stool samples was included. *Escherichia coli* was isolated and identified by standard cultural and biochemical methods. Pathogenicity of environmental and human isolates was determined by amplification of genes associated with virulence of *E. coli,* using specific primers.

Of a total of 228 water and river sediment samples screened, *E. coli* was recovered from 200 (87.7%), and 135 (67.5%) of these had one or more genes associated with pathogenicity. The highest frequency of isolation of pathogenic strains was found in Ritavi River water and sediment (80.6%), followed by Lotanyanda River (76.9%), and the least (45.8%) in Nzhelele River 2. *Escherichia coli* was recovered from all of the 252 diarrhoeic stools tested (100%), and 119 (47.28%) of these had one or more genes associated with pathogenicity. The frequency of isolation of potential pathogenic *E. coli* from humans was highly significant ($t = 6.3$; $pd \bullet 0.01$) in comparison to water isolates. Cytotoxic necrotizing Factor 1 (*cnf1*) and cytotoxic necrotising Factor 2 (*cnf2*) coding for necrotoxigenic *E. coli* (NEC); bundle-forming pilus (*bfpA*) and enteropathogenic attachment and effacement (*eaeA*) coding for enteropathogenic *E. coli* (EPEC), occurred in 35% and 34% respectively of river isolates. Heat-stable (*ST*) and heat-labile (*LT*) toxin genes coding for enterotoxigenic (ETEC) and Shiga-like toxin 1 (*Stx1*) and Shiga-like toxin 2 (*Stx2*) coding for Shiga-like toxin-producing *E. coli* (STEC) were not encountered in the river isolates. Isolates from stool samples had 21.8% and 12.6% of EPEC and NEC strains respectively; while enterotoxigenic (ETEC), Shiga-like toxin-producing (STEC) and enteroaggregative *E. coli* (EAEC) had a prevalence of 5%, 5.8% and 5.8% respectively. One human isolate possessed *stx2* and *eaeA* indicating *E. coli* O157: H7. No genes associated with pathogenicity were observed in human non-diarrhoeic stool isolates. Results have revealed a possibility of a recycling of pathogenic *E. coli* strains, particularly the EPEC and NEC strains, between the water sources and the local population.

Keywords: *Escherichia coli*, virulence markers, water, stool, Venda, South Africa

Introduction

Over 500 x 10^6 cases of acute diarrhoea have been reported to occur yearly in children aged less than 5 years across the globe (Snyder and Merson, 1982). Diarrhoeal diseases are responsible for a huge proportion of morbidity and mortality in developing countries, particularly among children (Snyder and Merson, 1982; DuPont, 1995a). Apart from protozoans such as *Giardia lamblia*, *Entamoeba histolytica, Cryptosporidium parvum*, *Isospora belli*, and viruses such as Rotavirus, and Norwalk-like virus, implicated in cases of diarrhoea, frequently isolated bacterial diarrhoeagenic agents include *Escherichia coli*, *Campylobacter jejuni/coli, Salmonella*, *Shigella,* and *Aeromonas* species (Obi et al., 1995, 1997; Lainson and Silva, 1999; Coker et al., 2002; Oyofo et al., 2002). *Escherichia coli* is, however, the focus of this study. Although a normal flora of animals and humans, some *E. coli* strains are pathogenic and may account for life-threatening infections. Such infections include urinary tract infections (Falagas and Gorbach, 1995), haemolytic colitis, neonatal meningitis, nosocomial septicaemia, haemolytic uremic syndrome and surgical site infections (Klein et al., 1986; Thielman and Guerrant, 1999). Diarrhoeal diseases due to the virulent strains have been extensively reported and account for a substantial degree of morbidity and mortality in different age groups (El-Sheikh and El-Assouli, 2001; Galane and Le Roux, 2001). Virulent strains of *E. coli* include enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), and enterotoxigenic *E. coli* (ETEC) (Giron et al., 1991; Falbo et al., 1992; Blanco et al., 1992; Chan et al., 1994; Levine, 1987). The clinico-epidemiological patterns of the virulent strains vary, and association with travellers' diarrhoea, extra-intestinal infections, acute, chronic or persistent diarrhoea are known (Falbo et al., 1992; Chan et al., 1994). Indices of pathogenicity among *E. coli* strains include pili, k-antigen, haemolysin, adhesive factor, enterotoxins, cytotoxins, effacement factors and cytotoxic necrotic factors (Galane and Le Roux, 2001).

Animals, humans and the environment including water sources serve as natural habitats of virulent strains of *E. coli* (DuPont, 1995; Nataro et al., 1998; Griffin, 1999; Kuhnert et al., 2000; Stephan and Schumacher, 2001). Infection with pathogenic *E. coli* strains is closely linked to poor sanitation and personal hygiene. In developing countries infection could be due to the consumption of contaminated water from wells, rivers, and other surface waters (Grasso et al., 2000; Welch et al., 2000; Tumwine et al., 2002). In many rural areas of South Africa, clean potable water and sanitation are

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