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Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods

A. Samie [a,b,*], C.L. Obi [d], L.J. Barrett [b], S.M. Powell [c], R.L. Guerrant [b]

[a] *Department of Microbiology, University of Venda, Thohoyandou, 0950, Limpopo, South Africa*
[b] *Center for Global Health, Division of Infectious Diseases and International Medicine, Department of Medicine, University of Virginia, Charlottesville, VA 22908, USA*
[c] *Department of Pathology, University of Virginia Health system, Charlottesville, VA 22908, USA*
[d] *College of Agriculture and Environmental Sciences, School of Agriculture and Life Sciences, Department of Life and Consumer Sciences, University of South Africa, Pretoria, South Africa*

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Summary *Objectives:* This study determined the prevalence of *Campylobacter* spp., *Helicobacter pylori* and *Arcobacter* spp. in stool samples from Venda in relation to diarrhea, intestinal inflammation and HIV status using specific molecular methods.
Methods: Stool samples were collected from hospital patients (255) and primary school children (67). Genomic DNA was extracted from the stools and molecular methods including PCR, PCR followed by restriction analysis and multiplex PCR were used to test for the different organisms. The lactoferrin content of the stools was determined using commercial kits from TechLab (Blacksburg, VA, USA).
Results: The prevalence of the different organisms was 50.6% for *H. pylori*, 10.2% for *C. jejuni*, 6.2% for *A. butzleri*, 6.5% for *C. coli*, 3.1% for *C. concisus*, 2.8% for *A. cryaerophilus* and 1.9% for *A. skirrowii*. Of all the organisms, only *C. jejuni* was significantly associated with diarrhea (84.8%) ($\chi^2 = 21.025$, $P < 0.001$) and elevated levels of lactoferrin (78.8%) ($\chi^2 = 16.919$, $P < 0.005$) and was an important pathogen associated with diarrhea among HIV positive individuals (22.8%).

* Corresponding author. Center for Global Health, Division of Infectious Diseases and International Health, University of Virginia, MR4, Lane Road, Room 3146, Charlottesville, VA 22908, USA. Tel.: +1 434 924 5242; fax: +1 434 977 5323.
E-mail addresses: samieamidou@yahoo.com, as8sd@virginia.edu (A. Samie).

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Conclusions: Campylobacter infections are common causes of gastroenteritis in Venda. Non-*C. jejuni/coli* Campylobacters such as *C. concisus* as well as *A. butzleri* and *H. pylori* may be involved in gastrointestinal diseases in the region but further studies are needed to confirm this hypothesis.

Introduction

First described in 1886 by Theodor Escherish, what are known today as Campylobacters were first observed in the colons of infants who died of a disease he named ''cholera infantum.''[1] However, due to difficulties in culturing these organisms they remained overlooked until the first successful isolation from human feces in 1972.[2] For several years Campylobacters were referred to as ''Vibrio like organisms'' until 1963 when Sebald and Veron gave the name *Campylobacter* to the genus based on their shape, low DNA base composition, their microaerophilic growth requirement, and their non-fermentive metabolisms.[3] Later development of selective media initiated the routine detection of Campylobacters in clinical laboratories and subsequent establishment of surveillance programs in the United States and in the United Kingdom revealed their importance as intestinal and septicemic pathogens.[4,5] However such programs do not exist in developing countries including South Africa, thus information about infection rates in these countries is lacking.

As a consequence of Campylobacter infections, accumulated losses due to clinical cost and lost working hours amounts to US $1.3–6.2 billions in the USA. These losses do not take into consideration the fact that Campylobacter infections are under-reported since the true incidence of Campylobacteriosis, is estimated to be up to 10 times higher than documented case numbers.[6] The incidence of Campylobacter infections in African countries is not clearly defined and losses will probably be higher due to the high prevalence of Campylobacter in chickens, combined with the weakened immune system of HIV infected individuals.[7,8] Studies in Cape Town have indicated a prevalence of up to 22% of all Campylobacters when the filter method is used for isolation.[9] In Venda, the infection level by *Campylobacter* spp. was found to be around the same level (20%) amongst HIV infected individuals.[10] However, the isolates were not ascertained by the use of molecular methods and very few studies have determined the genetic variability of Campylobacters in Africa.

Traditional methods for the detection, identification, and differentiation of *Campylobacter*, *Arcobacter* and *Helicobacter* spp. include culture followed by a battery of biochemical tests, serotyping including the Lior and Penner systems, and phenotyping methods.[11] These methods are reliable, but are time consuming, typically requiring 3–7 days to obtain results.[12] DNA based molecular techniques including real-time Polymerase Chain Reaction (PCR) and fingerprinting methods Terminal Restriction Fragment Length Polymorphisms (T-RFLP) and Ligase Detection Reaction (LDR) have been described and are used for the rapid detection and identification of *Campylobacter*, *Arcobacter* and *Helicobacter* spp.[13,14] In the present study we determined the prevalence of different *Campylobacter* spp., *Helicobacter pylori* and *Arcobacter* spp. in stool samples obtained from public hospitals and primary schools in the Vhembe district, Limpopo Province, South Africa, using different specific PCR protocols.

Material and methods

Ethical clearance

The study was approved by the Research and Ethical Committee of the University of Venda, the Department of Health and Welfare and the Department of Education in Polokwane, Limpopo Province, South Africa, before the initiation of the study.

Study site and sample collection

Stool samples were collected from the three major hospitals (Tshilidzini, Elim and Donald Frazer) serving local populations of the Venda region and two primary schools between November 2004 and May 2005 and preserved in the freezer at −80 °C until they were sent to the University of Virginia (Charlottesville, VA, USA) where all the molecular analysis were completed. In total, 322 stool samples were collected of which 255 were from the hospitals and 67 from the schools. At the hospitals, samples were collected at the hospital laboratory from patients with gastrointestinal complaints or with diarrhea according to the health center's guidelines. At the primary schools, the objectives of the study were explained to the parents in a meeting with the authority of the schools who then distributed the collection bottles to the pupils whose parents had agreed to the study and signed a consent form. The pupils then brought the collection bottles home and with the help of their parents collected stools in the bottles. The samples were collected the following morning at the schools and transported within 2 h to the laboratory of Microbiology, University of Venda. All the samples were further aliquoted in a 1.5 ml Eppendorf tube without dilution for diarrheal (unformed: soft to liquid) samples or diluted in sterile saline for non-diarrheal (formed) stools. Demographic information such as age and sex as well as HIV status was also collected.

Bacterial culture and maintenance

Reference strains used in this study included *Campylobacter jejuni* subsp. jejuni (ATCC 33291), *C. coli* (ATCC 33559), *C. concisus* (ATCC 33237), *C. fetus* subsp. fetus (ATCC 27374), *C. hyointestinalis* (ATCC 35217), *C. upsaliensis* (ATCC 43954), *Helicobacter pylori* (ATCC 43504), *Arcobacter*