Prevalence of pathogenic bacteria and rotaviruses in stools of patients presenting with diarrhoea from rural communities in Venda, South Africa

C.L. Obi1,†, N. Potgieter†, P.O. Bessong∗, E.O. Igumbor∗ and E. Green∗

We report the prevalence of bacterial enteropathogens and rotaviruses in diarrhoeic cases of rural residents in the Venda region of South Africa and provide baseline data on antibiotic susceptibility profiles of bacterial isolates. The prevalence of Aeromonas, Campylobacter, Escherichia coli, Salmonella, Shigella, Plesiomonas, Vibrio cholerae, Yersinia enterocolitica and rotaviruses from stools of patients attending clinics was determined using standard microbiological methods. Antibiotic susceptibility profiles of bacterial isolates were determined using the Kirby-Bauer disc diffusion method. Escherichia coli was the most common bacterium isolated (20% of samples) followed by Campylobacter jejuni/coli (20%), Salmonella (14.5%), Shigella (12.5%), Plesiomonas shigelloides (11%) and Vibrio cholerae (3.7%). Rotaviruses were detected in 27% of specimens. The prevalence of bacteria and rotaviruses was higher in children aged less than five years than in older age groups (6–20 + years). All rotavirus-positive samples were of the G1 (VP7) variant belonging to serotype 1, whereas 44% each were of the P6 and P8 (VP4) serotypes and 12.5% were mixed strains (P6 + P8). PAGE analysis indicated that 78 (97.5%) and 2 (2.5%) were of the long and short electropherotypes, respectively. Over 85% of bacterial isolates were susceptible to ciprofloxacin, gentamicin, amikacin and nalidixic acid. All Salmonella isolates were susceptible to gentamicin and amikacin and all Plesiomonas isolates were susceptible to ciprofloxacin. Multi-antibiotic resistance of virtually all isolates to tetracycline, ampicillin, erythromycin and chloramphenicol was observed. Enteric bacterial pathogens and rotaviruses are therefore potentially serious threats to the health of the study population.

Introduction

More than 800 million cases of diarrhoea occur annually in developing countries, particularly in rural areas, accounting for about 4.5 million deaths.‡ South Africa is no exception to this hazard. Children below the age of five, especially those in areas without access to a potable water supply and sanitation, are prone to the devastating effect of diarrhoea, the agents of which may be transmitted by contaminated water.†

The incidence of morbidity and mortality due to diarrhoea among children under five years is significantly higher where water supply and sanitation fall below the standards stipulated by Department of Water Affairs and Forestry§ than in children in formal urban residential areas with in-house connections.¶

The Venda region of Limpopo province, South Africa, is predominantly rural. Most of the rural communities have very limited access to good roads, electricity, clean water and sanitation. Drinking water is usually taken from rivers and ponds and is untreated.‖ It is thus a potential source of diarrhoeal diseases. There are few data on the prevalence of enteric pathogens in diarrhoeic stool samples from the local population. Several pathogens cause diarrhoea and include bacteria such as Campylobacter, E. coli, Salmonella, Shigella, Plesiomonas shigelloides, Aeromonas, Vibrio cholerae and Yersinia enterocolitica. Viral agents responsible consist of rotaviruses, norwalk viruses, and calici-like viruses; rotaviruses are the main agents of diarrhoea in children and adults worldwide.¹¹–¹³ Parasitic agents of diarrhoea, especially Giardia lamblia and Cryptosporidium parvum¹⁴,¹⁵ and fungal agents such as Candida,¹⁶ are also common.

Management of diarrhoea and other complicating factors may involve the use of antibiotics because they can shorten the duration of disease, reduce stool output and abrogate some of their side effects.¹⁷ The increasing resistance of bacteria to antibiotics is well documented.¹⁰,¹⁷ There are few data on antibiotic resistance of potential bacterial pathogens isolated from diarrhoeic stools of residents in rural communities in Venda. The aim of this study was to report on the prevalence of bacterial and rotavirus agents of diarrhoea in Venda’s rural communities and to determine the antibiotic susceptibilities of the bacterial isolates.

Materials and methods

Stool samples were collected from the following clinics in Venda: Mphethe, Vuvu, Hlutsane, Pshana, Makonde, Tshaulu, Mutale, Phiphi, William Eddie, and Tshiamo. Ethical approval for the study was obtained from the Research Committee, Department of Health and Social Welfare, Polokwane, Limpopo. Signed informed consent was obtained from each participant in the study before sample collection. Four hundred and one diarrhoeic stool samples were collected on a weekly basis from children (100 specimens) and adults attending the above clinics between June 1999 and September 2002. Specimens were deposited into sterile plastic containers and transported to the laboratories at the Department of Microbiology, University of Venda, and the Diarrhoeal Pathogens Research Unit, Medical University of Southern Africa. Microbiological investigations were conducted within 6–8 h of sample collection. diarrhoea was defined as the passage of 3–4 watery stools per day for not less than 3 days.

Isolation and identification of bacteria

Aeromonas and Plesiomonas species. Specimens were inoculated onto xylene deoxycholate citrate agar (XDA), incubated at 37°C for 24 h. Non-xylene fermenting colonies on XDA were screened for oxidase production.¹⁸ Oxidase-positive colonies were further confirmed as belonging to Aeromonas or Plesiomonas shigelloides. Aeromonas species give positive reactions for ornithine decarboxylase, DNAse tests and resistance to vibriostatic agent O/129, while Plesiomonas shigelloides produces neither gas nor H₂S on triple sugar iron agar.¹⁹

Campylobacter species. Campylobacter was isolated from stools by culturing on Skirrow’s and Butzler’s media as previously described.¹⁵,²⁰ Briefly, the plates were incubated at 42°C under microaerophilic conditions for 72 h. Organisms were considered to be Campylobacter if they were S-shaped, Gram-negative, motile, oxidase-positive, grew at 42°C but not at 25°C and sensitive to nalidixic acid. C. jejuni and C. coli were differentiated on the basis of hippurate and indoxyl acetate hydrolysis. C. jejuni is positive for both tests whereas C. coli is positive for indoxyl acetate hydrolysis only.²¹

Escherichia coli. Samples were streaked on eosin methylene blue agar (EMB) (Merck) and incubated aerobically for 24 h at 37°C. Colonies with
a blue-purple or metallic green sheen indicative of E. coli were confirmed by positive reactions for indole, p-nitrophenyl-β-D-galactopyranoside (ONPG), xylose, citrate utilization and negative reactions for oxidase, DNase, KCN, phenylalanine deaminase and Voges-Proskauer tests. Pathogenic E. coli isolates were identified by amplifying the corresponding virulence gene markers. Genes coding for necrotogenic E. coli (NEC), enterotoxigenic E. coli (ETEC), shiga-like toxin producing E. coli (STEC), enteropathogenic E. coli (EPEC) and enterohaggregative E. coli (EAEC) were sought as previously described.  

Salmonella species. Stool specimens were streaked on bismuth sulphite agar and incubated for 48 h at 37°C. Black colonies with a metallic silver sheen suggestive of Salmonella were confirmed by positive reactions for motility, fermentation of mannitol and sorbitol, and negative reactions for DNase, indole, phenylalanine deaminase, urease, Voges-Proskauer, growth in KCN, ONPG and fermentation of adonitol, sucrose, lactose, raffinose and salicin.  

Shigella species. Samples were cultured on xylose-lysine deoxycholate agar (XLD) (Merck) for 24 h at 37°C. Transparent colonies suggestive of Shigella were screened for negative reactions for motility, adonitol, citrate, DNase, gas from glucose, H₂S, lysine, phenylalanine, sucrose, urease, Voges-Proskauer, inositol, KCN, lactose, malonate, salicin and xylose.  

Vibrio cholerae. About 2 ml of faeces was inoculated in 20 ml of alkaline peptone water (APW) at pH 8.6 and incubated for 8 h at 37°C. A loopful from the surface of the APW culture was sub-cultured on thiosulphate citrate bile salt sucrose agar (TCBS) and incubated overnight. Yellow colonies suggestive of Vibrio growth were sought and screened with V. cholerae O1 antiserum (Wellcome Reagents, Wellcome Research Laboratories, Beckenham, Kent).  

Yersinia enterocolitica. Y. enterocolitica was enriched with Yersinia selective supplement SR109 (Oxoid) and subsequently subcultured onto Yersinia agar medium (Oxoid). All inoculated media (enrichment and subculture) were incubated at 37°C for 24 h. Presumptive colonies of Y. enterocolitica with dark red centres and transparent peripheries were characterized by positive tests for ornithine carbamylase and sucrose fermentation, and negative reactions for raffinose, rhizomos and melibiose fermentation.  

Identification and genotyping of rotaviruses  

Three hundred stool specimens comprising 100 from children of five years and younger were screened for the presence of rotavirus. Faecal specimens were diluted (10%) in distilled water and kept at 4°C. A commercial ELISA (Dako, Denmark) was used to screen stool specimens for the detection of rotaviruses, as recommended by the manufacturer and as previously described.  

RNA was extracted from specimens positive for rotaviruses using phenol-chloroform mixture followed by ethanol precipitation. Genomic double-stranded RNAs were electrophoresed on 10% polyacrylamide slab gels with a 3.5% stacking gel to enhance resolution. Electrophoresis was performed overnight at 100 V and silver-stained to visualize the RNA bands.  

VP4 and VG7 genotyping of rotavirus RNA. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on the specimens using methods described previously. VP4 (gene 4) typing was carried out using the con2 and con3 primers. VP7 (gene 9) typing was carried out using Beg9 and End9 primers. Type-specific primers were used for the G (VP7) and P (VP4) typing. PAGE RNA profiles (electropherotypes) were analysed by gel electrophoresis in 2% agarose gels (Seakem, Flowgen) with 5 µg ml ethidium bromide at 100 V. A 100-bp DNA ladder (G2101, Promega, England) served as the marker.  

Antibiogram determination  

Bacterial isolates were tested for antibiotic susceptibility on Mueller-Hinton agar using the Kirby-Bauer disk diffusion method. The antibiotics investigated were ampicillin (10 µg), amikacin (30 µg), gentamicin (10µg), tetracycline (30µg), ciprofloxacin (10µg), ceftazzone (30µg), chloramphenicol (30µg), erythromycin (15µg) and nalidixic acid (30µg).  

Table 1. Prevalence of potential enteric pathogens in the stools of patients with diarrhoea in Venda rural communities.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Prevalence of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Aeromonas spp.</td>
<td>34 (8%)</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>24 (6%)</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>55 (14%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>81 (20%)</td>
</tr>
<tr>
<td>Enterotoxigenic E. coli</td>
<td>20 (5%)</td>
</tr>
<tr>
<td>Enterotoxigenic E. coli</td>
<td>10 (13%)</td>
</tr>
<tr>
<td>Shiga-like toxin producing E. coli</td>
<td>10 (13%)</td>
</tr>
<tr>
<td>Enteropathogenic E. coli</td>
<td>30 (37%)</td>
</tr>
<tr>
<td>Enterohaggregative E. coli</td>
<td>11 (14%)</td>
</tr>
<tr>
<td>Plesiomonas shigelloides</td>
<td>43 (11%)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>58 (15%)</td>
</tr>
<tr>
<td>Shigella</td>
<td>50 (13%)</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>15 (4%)</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>21 (5%)</td>
</tr>
<tr>
<td><strong>Rotaviruses</strong></td>
<td></td>
</tr>
<tr>
<td>(n = 300)</td>
<td>80 (27%)</td>
</tr>
</tbody>
</table>

*n is the number of stool samples examined.

Results  

Isolation rates of pathogens  

The incidence of potentially pathogenic organisms isolated from stools of diarrhoeic patients are presented in Table 1. The most common bacterial isolates were pathogenic E. coli (20%), whose strains comprised necrotogenic E. coli, 25%; enterotoxigenic E. coli, and Shiga-like toxin producing E. coli, 15% each; enteropathogenic E. coli, 37% and enterohaggregative E. coli, 14%. Other isolates comprised C. jejuni/coli (20%), Salmonella (15%), Shigella (13%), P. shigelloides (11%) The lowest incidence was recorded for V. cholerae (4%). Rotavirus was detected in 80 (27%) of the 300 stool samples analysed (Table 1).

Distribution of pathogens according to age of subject  

There was a greater prevalence of enteric bacteria and rotaviruses in the 0–5-year age group than in older age groups (11–20 years and >20 years) (Table 2). About 50% of the isolation rates of C. jejuni, C. coli, P. shigelloides, E. coli and V. cholerae were recorded in the 0–5-year age group whereas the isolation in the older patients ranged from 7% to 25% (Table 2) and this was statistically significant (P < 0.05).

Of the 80 stool specimens in which rotaviruses were present, 30 (38%) were detected in the 0–5-year age group, whereas 12 (15%), 23 (29%) and 15 (19%) were detected in the age groups 6–10, 11–20 and >20 years, respectively (Table 2).

Genotypes and electropherotypes of rotaviruses  

The occurrence of human rotaviruses VP4, VP7 and electropherotypes among the 80 positive samples are presented in Table 3. For the VP4, 35 (44%) were of the P6 and P8 strains each and 10 (13%) contained mixed strains (P6 + P8). For the VP7 rotaviruses, all were of the G1 genotype. There were no mixed and no untypeable serotypes. All positive samples were electropherotyped and 78 (98%) were represented by long electropherotypes and only 2 (3%) by short electropherotypes (Table 3).

Antibiotic susceptibility profiles  

Antibiotic susceptibility profiles of the various bacterial isolates from infected stools presented in Table 4 showed that over 85% of all the enteric bacterial isolates were sensitive to gentamicin, ciprofloxacin and amikacin. In addition, over 90% of C. jejuni, E. coli, Salmonella species and P. shigelloides were sensitive to nalidixic acid. All Salmonella isolates were sensitive to
The presence of these bacterial pathogens in diarrhoeal cases is consistent with other reports. The presence of rotaviruses was detected in 27% of stool samples screened, which is consistent with previous reports. Escherichia coli accounted for 13% of diarrhoeal cases in Saudi Arabia whereas Salmonella isolation rates ranged from 2–18%. Shigella and Campylobacter isolates constituted 17–30% of isolates from patients with diarrhoea in Kuwait, Jordan and Egypt.

Other bacterial diarrheagenic agents identified in this study were P. shigelloides, Aeromonas species, and Escherichia coli. Aeromonas and P. shigelloides were reportedly incriminated in cases of diarrhoea, with a greater prevalence in rural communities. In a study in Nigeria, prevalence rates of 5% for Shigella, and E. coli were sensitive to ceftriazone. Isolates of E. coli, V. cholerae, P. shigelloides, Aeromonas species and Campylobacter jejuni coli were resistant to tetracycline, ampicillin, erythromycin and chloramphenicol; an indication of multi-drug resistance (Table 4).

### Discussion and conclusions

The most common bacterial pathogens isolated were variants of E. coli (20%), comprising NEC, EPEC, STEC, ETEC and EAEC, followed by Campylobacter species (C. jejuni and C. coli), constituting 20%; Salmonella species (14.5%) and Shigella species (12.5%). The presence of these bacterial pathogens in diarrhoeal cases is consistent with other reports. Escherichia coli accounted for 13% of diarrhoeal cases in Saudi Arabia whereas Salmonella isolation rates ranged from 2–18%. Shigella and Campylobacter isolates constituted 17–30% of isolates from patients with diarrhoea in Venda rural communities.

Other bacterial diarrheagenic agents identified in this study were P. shigelloides, Aeromonas species, Yersinia species and V. cholerae. Aeromonas and P. shigelloides were reportedly incriminated in cases of diarrhoea, with a greater prevalence in rural communities. In a study in Nigeria, prevalence rates of 5% for P. shigelloides and 9.9% for Aeromonas species were reported. All the enteropathogens studied were isolated from the different age groups but with higher rates among children less than five years, a pattern consistent with previous studies. The presence of rotaviruses was detected in 27% of stool samples screened, which is consistent with previous reports incriminating rotaviruses as major causes of diarrhoea, particularly in children. The predominance of rotaviruses in children less than five years of age is also in harmony with hospital-based data across the globe. Among 80 rotavirus strains detected, 78 (97.5%) and 2 (2.5%) were of the long and short electropherotypes, respectively. The predominance of long electropherotypes over short ones has been reported by other investigators. Serotype analysis of the VP7 revealed that all the rotavirus isolates were of the G1 strain and were all typeable. G strains have been shown to be common worldwide and cause rotavirus outbreaks. The incrimination of the wide range of bacterial pathogens and rotaviruses in diarrhoeal cases constitutes a significant threat to health in the Venda region. Although the organisms identified could be transmitted by contaminated food and by personal contact, the lack of potable water supplies would compound the problem. The typical water sources are usually faecally contaminated and untreated. Obi et al. reported that local rivers harboured diarrheagenic pathogens, were unsafe for consumption, microbiologically unacceptable and likely to be potential sources of transmission of water-borne diseases to humans.

Our results suggest that the broad spectrum of activity of the

### Table 4. Susceptibilities of enteric bacterial isolates, from stools of patients with diarrhoea in Venda rural communities, to different antibiotics.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>NA</th>
<th>GM</th>
<th>COT</th>
<th>CIP</th>
<th>TE</th>
<th>AP</th>
<th>ERY</th>
<th>CHL</th>
<th>AKC</th>
<th>CEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas spp. (n = 34)</td>
<td>30%</td>
<td>30%</td>
<td>26%</td>
<td>32%</td>
<td>26%</td>
<td>22%</td>
<td>14%</td>
<td>18%</td>
<td>32%</td>
<td>28%</td>
</tr>
<tr>
<td>C. coli (n = 24)</td>
<td>18%</td>
<td>21%</td>
<td>16%</td>
<td>6%</td>
<td>15%</td>
<td>13%</td>
<td>14%</td>
<td>13%</td>
<td>22%</td>
<td>16%</td>
</tr>
<tr>
<td>C. jejuni (n = 55)</td>
<td>51%</td>
<td>51%</td>
<td>41%</td>
<td>52%</td>
<td>40%</td>
<td>33%</td>
<td>38%</td>
<td>33%</td>
<td>54%</td>
<td>36%</td>
</tr>
<tr>
<td>E. coli (n = 81)</td>
<td>74%</td>
<td>75%</td>
<td>53%</td>
<td>77%</td>
<td>49%</td>
<td>24%</td>
<td>48%</td>
<td>40%</td>
<td>79%</td>
<td>71%</td>
</tr>
<tr>
<td>P. shigelloides (n = 43)</td>
<td>40%</td>
<td>37%</td>
<td>30%</td>
<td>43%</td>
<td>29%</td>
<td>27%</td>
<td>18%</td>
<td>26%</td>
<td>42%</td>
<td>38%</td>
</tr>
<tr>
<td>Salmonella spp. (n = 58)</td>
<td>55%</td>
<td>58%</td>
<td>56%</td>
<td>97%</td>
<td>29%</td>
<td>29%</td>
<td>43%</td>
<td>45%</td>
<td>58%</td>
<td>55%</td>
</tr>
<tr>
<td>Shigella spp. (n = 50)</td>
<td>42%</td>
<td>47%</td>
<td>31%</td>
<td>45%</td>
<td>25%</td>
<td>23%</td>
<td>ND</td>
<td>ND</td>
<td>49%</td>
<td>48%</td>
</tr>
<tr>
<td>V. cholerae (n = 15)</td>
<td>10%</td>
<td>6%</td>
<td>11%</td>
<td>13%</td>
<td>10%</td>
<td>10%</td>
<td>6%</td>
<td>13%</td>
<td>11%</td>
<td>7%</td>
</tr>
<tr>
<td>Y. enterocolitica (n = 21)</td>
<td>17%</td>
<td>18%</td>
<td>11%</td>
<td>12%</td>
<td>13%</td>
<td>10%</td>
<td>ND</td>
<td>9%</td>
<td>18%</td>
<td>16%</td>
</tr>
</tbody>
</table>

NA, nalidixic acid; TE, tetracycline; CIP, ciprofloxacin; GM, gentamicin; ERY, erythromycin; AKC, amikacin; COT, cotrimoxazole; CHL, chloramphenicol; AP, ampicillin; CEF, ceftriaxone. ND = not done.
antibiotics ciprofloxacin, gentamicin, amikacin and nalidixic acid indicates their potential usefulness in the management of diarrhoea requiring antibiotic therapy. The susceptibility of bacterial pathogens to these antibiotics reflects previous reports.

Periodic surveys of antibiograms of bacterial agents of diarrhoea are recommended in order to unravel trends in antibiotic resistance and provide updated guidelines for the management of diarrhoea. Furthermore, the identification of organisms such as *Vibrio cholerae*, capable of causing epidemics, is a signal that improving the microbiological quality of the water sources of these areas is an urgent issue that has to be redressed now to avoid an epidemic.

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