ENTERIC PATHOGENS
IN WATER SOURCES AND STOOLS
OF RESIDENTS IN THE VENDA REGION
OF THE LIMPOPO PROVINCE

Report to the Water Research Commission

by

Chikwelu Larry Obi, Natasha Potgieter and Pascal Obong Bessong
Department of Microbiology
University of Venda for Science and Technology
Private Bag X5050, Thohoyandou, 0950, South Africa

WRC REPORT NO. 1126/1/05

JUNE 2005
Disclaimer

This report emanates from a project financed by the Water Research Commission (WRC) and is approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the WRC or the members of the project steering committee, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.
TABLE OF CONTENTS

Executive Summary 3
Acknowledgement 14
List of Abbreviations 16
List of Tables 18
List of Figures 19

CHAPTER 1 ASSESSMENT OF THE MICROBIAL QUALITY OF WATER SOURCES

1.1. INTRODUCTION 21
1.2. MATERIALS AND METHODS 22
   1.2.1. Study areas 22
   1.2.2. Sample collection 22
   1.2.3. Microbiological analysis 23
      1.2.3.1. Indicator organisms 23
      1.2.3.2. Enteropathogenic bacteria 23
   1.2.4. Antibiogram determination 25
   1.2.5. Statistical analysis 26
1.3. RESULTS 34
   1.3.1. Heterotrophic plate counts 34
   1.3.2. Total coliforms 34
   1.3.3. Faecal coliforms 35
   1.3.4. Faecal enterococci 35
   1.3.5. Somatic coliphages 36
   1.3.6. Pathogenic bacteria 36
   1.3.7. Antibiogram determination 36
1.4. DISCUSSION 41
1.5. REFERENCES 43
CHAPTER 2  BACTERIAL ENTEROPATHOGENS AND ROTAVIRUSES IN DIARRHOEAL CASES

2.1.  INTRODUCTION 52

2.2.  MATERIAL AND METHODS 53

2.2.1.  Study sites 53

2.2.2.  Ethical approval 53

2.2.3.  Sample collection 54

2.2.4.  Bacteriological methods 54

2.2.5.  Identification of rotaviruses 56

2.2.5.1.  Extraction of rotavirus RNA 57

2.2.5.2.  VP4 and VP7 tying of rotavirus RNA 57

2.2.6.  Antibiogram determination 57

2.3.  RESULTS 58

2.3.1.  Isolation rates of pathogens 58

2.3.2.  Age distribution of pathogens 58

2.3.3.  Seasonal variations in the isolation of pathogens 59

2.3.4.  Serotypes and electropherotypes of rotaviruses 59

2.3.5.  Antibiotic susceptibility profiles 59

2.4.  DISCUSSION 63

2.5.  REFERENCES 65

CHAPTER 3  CONCLUSIONS

3.1.  ASSESSMENT OF THE MICROBIAL QUALITY OF RIVER WATER SOURCES 71

3.2.  BACTERIAL ENTEROPATHOGENS AND ROTAVIRUSES IN DIARRHOEAL CASES 71
EXECUTIVE SUMMARY

1. BACKGROUND TO THE STUDY AND PROBLEM STATEMENT

In developing countries, the majority of rural communities are poverty-stricken, lack access to potable water supplies and rely mainly on water sources such as rivers, streams, ponds, fountains and boreholes for their daily water needs (Nevondo and Cloete, 1991; Obi et al., 2002). Water from these sources are used directly by the inhabitants and the water sources from most rural communities are faecally contaminated and devoid of treatment. Consequently, a significant proportion of residents in rural communities in South Africa are exposed to water-borne diseases and their complications (Schalekamp, 1990). These diseases include various bacterial, fungal, viral and parasitic infections that cause crippling, devastating and debilitating effects on rural residents and further exacerbates the already strained health burden and facilities in the country (Genthe and Seager, 1996; Grabow, 1996). It is therefore imperative to monitor the microbial quality of water supply in rural areas in order to highlight the poor quality of water supplies and to provide the impetus for sustained government intervention.

The Venda Region of the Limpopo Province is mostly rural and most of the communities have limited or no access to good roads, electricity, water and sanitation (Census, 2000). Currently, a paucity of data exists on the prevalence of enteric pathogens in diarrhoeic stool samples obtained in the region. Several pathogens are known to cause diarrhoea and include bacterial pathogens such as Campylobacter, Salmonella, Escherichia coli, Aeromonas and Plesiomonas (Obi et al., 1995, 1997, 1998; El-Sheikh and El-Assouli, 2002) and viruses such as rotaviruses, astro viruses and Noro viruses (E’cheverria et al., 1983; El Assouli et al., 1995; El-Sheikh and El-Assouli, 2001). Rotaviruses are globally recognised as major pathogens of diarrhoea in children and adults (El-Sheikh and El-Assouli, 2002).
Children younger than the age of five years, especially those in areas devoid of access to potable water supplies and sanitation are extremely prone to the devastating effect of diarrhoea since diarrhoea may be transmitted by poor water quality (Esrey et al., 1990, Parashar et al 2003). Incidence of morbidity and mortality due to diarrhoea among children younger that five years of age are significantly higher in such settlements where water supply and sanitation falls below the level equivalent to those stipulated by the Department of Water Affairs and Forestry (DWAF) (1995) for the Reconstruction and Development Programme (RDP) in comparison to children in formal urban residential areas with in-house connections (Payment et al., 1991; Tonglet et al., 1992).

Although increasing resistance of bacteria to antibiotics is well documented, the management of diarrhoea and other complications may involve the use of antibiotics. Antibiotics are known to shorten the duration of diarrhoea, decrease stool output and abrogate some of the complications caused by diarrhoea (Black, 1993; Obi et al., 1998). Antibiograms against bacterial pathogens are known to vary from place to place and also with time. Therefore it is necessary for periodic updates in order to uncover resistance patterns that may develop in different regions. There are no baseline data on the antibiograms of potential bacterial pathogens of diarrhoea isolated from water sources and diarrhoeic stool specimens in rural communities in the Venda Region.

2. PROJECT OBJECTIVES.

i. To determine the prevalence of enteric pathogens in water sources and stool samples of residents in the Venda region of the Limpopo Province.

ii. To ascertain the extent to which enteric bacterial infections influence the incidence of diarrhoea among infants and adults in urban and rural areas in the Venda region of the Limpopo Province.

iii. To determine the antibiograms of bacterial isolates from human and environmental sources in order to provide updated information on their susceptibility patterns.
iv. To explore the use of bacteriophages (viruses) and bacteria as indicators of water quality.

v. To determine seasonal variations of enteropathogens isolated.

vi. To provide feedback to communities on findings and implications regarding microbiological water quality.

3. ACCOMPLISHMENT OF OBJECTIVES

All the objectives stated above have been accomplished. The microbial quality of various water sources in the Venda region, Limpopo province has now been documented. The prevalence of various enteropathogens in water sources and stool samples as well as antibiograms of isolates were determined. The frequency or extent of isolation of enteric bacterial pathogens in diarrhoeal cases among infants and adults was also established. Seasonal distributions of the various enteropathogens are reported. The use of bacteriophages (Viruses) and bacteria as indicators of water quality was accomplished. Feedback to communities on findings and implications regarding microbiological quality has been initiated and is on-going. Accomplishment in terms of capacity building included training of several postgraduate students at BSc (Honours), MSc and PhD levels.

4. METHODOLOGY USED

The microbial quality of various water sources, used by rural communities in the Venda region of South Africa, was assessed over a period of two years to highlight the possible occurrence of water-borne diseases. The water sources studied included the Levubu, Mutale, Ngwedi, Tshinane, Makonde and Mudaswali rivers and the Makonde and Mudaswali fountains. Indicator organisms such as total and faecal coliforms, faecal enterococci and somatic coliphage counts were used to determine the microbiological quality of the water sources. The prevalence of bacterial enteropathogens such as
Salmonella, Shigella, Campylobacter, Plesiomonas, Escherichia coli, Yersinia, Aeromonas and Vibrio cholerae in water and stool samples was determined using standard biochemical tests. Rotaviruses in stool specimens were determined using a commercial Enzyme Link Immuno-sorbant Assay (ELISA) test. Rotavirus serotypes were characterised using RT-PCR and the virulence factors of Escherichia coli isolates were determined using the polymerase chain reaction with specific primers. Antibiotic susceptibility profiles of both water and clinical bacterial isolates were determined using the Kirby-Bauer disc diffusion method.

5. SUMMARY OF MAJOR FINDINGS

Prevalence of enteric pathogens in water sources and stool samples of residents in the Venda region of the Limpopo Province.

Water sources:
Results obtained showed that the minimum and maximum counts of all the sampling points investigated with regard to indicator organisms ranged between $1.0 \times 10^1 \text{ cfu.mL}^{-1}$ and $1.3 \times 10^6 \text{ cfu.mL}^{-1}$ for heterotrophic plate counts, between $0 \text{ cfu.mL}^{-1}$ and $1.0 \times 10^6 \text{ cfu.mL}^{-1}$ for total and faecal coliforms, between $0 \text{ cfu.mL}^{-1}$ and $5.1 \times 10^4 \text{ cfu.mL}^{-1}$ for faecal enterococci and for somatic coliphages the counts ranged between 0 and 154 pfu.100 ml$^{-1}$. The results for the indicator organisms were higher than the guideline values for safe drinking water stipulated by the Department of Water Affairs and Forestry of South Africa. According to these guidelines, the maximum values are as follows: $1.0 \times 10^2 \text{ cfu.mL}^{-1}$for heterotrophic plate count, 5 cfu.100 ml$^{-1}$ for total coliforms, 0 cfu.100 ml$^{-1}$ for faecal coliforms, 0 cfu.ml$^{-1}$ for faecal enterococci and 1 pfu.100 ml$^{-1}$ for somatic coliphages. The microbial quality of the water sources could be unsafe for human consumption. Enteric pathogens isolated from the studied water sources included Escherichia coli, Plesiomonas shigelloides, Vibrio, Enterobacter cloacae, Shigella, Salmonella, Aeromonas hydrophila, Aeromonas caviae and Campylobacter. A high percentage of the Escherichia coli isolates from water (87.7%) and sediment (67.5%) samples contained one or more of the genes responsible for pathogenicity.
Stool samples:
Results indicated *Escherichia coli* as the most prevalent bacterial isolate (20%), followed by *Campylobacter jejuni/coli* (19.7%), *Salmonella* species (14.5%), *Shigella* (12.5%), *Plesiomonas shigelloides* (10.7%) and the least (3.7%) was recorded for *Vibrio cholerae*. Rotaviruses were detected at a frequency of 26.7%. Isolation rates of enteric bacteria and rotaviruses were statistically higher in children aged less than five years than in older age groups (6 – 10, 11 – 20 and > 20 years). Detection of bacterial enteropathogens was seen throughout the year but showed a slightly higher tendency in the summer months in comparison with observations for other months (p<0.05). The detection of rotaviruses was significantly higher in the winter season (p<0.05). This finding may be of importance in the administration of rotavirus vaccines. All rotavirus positive samples (100%) were of the G1 serotype (serotype 1) with 78 (97.5%) of the isolates having long electropherotypes and 2 (2.5%) of the isolates having short electropherotypes. Enteric bacterial pathogens and rotaviruses were noted to be important agents of diarrhoea among residents in the rural communities studied.

**Antibiograms of bacterial isolates from human and environmental sources.**

Water sources:
Antibiotic susceptibility results of water isolates revealed marked susceptibilities (over 90%) of *Campylobacter*, *Salmonella* and *Escherichia coli* to nalidixic acid, ciprofloxacin and amikacin. All of the *Salmonella* isolates (100%) from water sources were sensitive to amikacin. Multi-resistance patterns of various isolates to tetracycline, ampicillin, erythromycin and chloramphenicol were noted.

Human sources:
Antibiotic susceptibility profiles of bacterial isolates from human sources showed that the majority of isolates (over 85%) were sensitive to ciprofloxacin, gentamicin amikacin and nalidixic acid. All of the human *Salmonella* isolates (100%) were sensitive to gentamicin and amikacin and similarly 100% of the human *P. shigelloides* isolates were sensitive to ciprofloxacin. Multi resistance
patterns of virtually all the human isolates to tetracycline, ampicillin, erythromycin and chloramphenicol were observed.

**The use of bacteriophages (viruses) and bacteria as indicators of water quality.**

In this study somatic coliphages were used as indicators for the presence of enteric viruses in the water sources. The prevalence of somatic coliphages in all the sampling points could indicate the presence of faecal contamination and hence potential viral contamination.

**Seasonal variations of isolated enteropathogens.**

Detection of bacterial enteropathogens was recorded throughout the year but showed a higher tendency in the months of summer in comparison with data for the other seasons (p<0.05). The detection of rotaviruses was noted to be significantly higher in the winter season (p<0.05).

**Feedback to communities on findings and implications regarding microbiological water quality.**

This is an ongoing process. This report will be sent to the Department of Health, Polokwane and also to the hospitals and primary health clinics in the Venda region.

6. **SUMMARY OF CONCLUSIONS REACHED**

The untreated water sources used for drinking and other domestic purposes and the multi antibiotic resistance profiles of the enteric bacteria from these sources are potential threats to the health of residents and therefore calls for urgent intervention strategies by government, the community and other relevant role players. The detection of rotaviruses was significantly higher in the winter season and this finding may be strategic for vaccination against rotaviruses. The study further indicated that ciprofloxacin, amikacin and gentamicin are the
recommended antibiotics to be used for the treatment of diarrhoea cases requiring antibiotic therapy.

7. RECOMMENDATIONS FOR INTERVENTION STUDIES

- Interventions to improve the quality of drinking water at the household level must include sanitation and hygiene education

- Education of rural communities must be based on the biology of water-borne diseases and how to prevent these diseases through basic hygiene and sanitation interventions

- Sustainability of water quality intervention strategies must be monitored periodically and will be achieved only with the active participation of Water Affair Officers, Health Officers, the community residents and other stakeholders

- Intervention measures should include point-of-use treatment of contaminated water sources and safe storage.

8. RECOMMENDATIONS FOR FUTURE RESEARCH

In order to build on the thrust of this project, the following future research studies are recommended:

- Conduct molecular epidemiological studies of diarrhoeal agents from water sources and stool samples in order to link the water pathogens and stool pathogens.

- Conduct periodic monitoring of antibiotic susceptibility profiles of bacteria from environmental and clinical samples in order to provide updated data on resistance patterns.

- Determine the factors influencing household water quality and incidence of diarrhoeal diseases in rural Venda communities.
• Assess the virulence characteristics of *Campylobacter* and *Salmonella* from water and stool samples.

• Conduct studies on *E. coli* and *Salmonella* using molecular and serological typing for virulence to link the water pathogens to the stool pathogens.

9. **CAPACITY BUILDING**

9.1. **BSc Honours Microbiology**

• Mr KE Maswime (2001)  Prevalence of *Campylobacter, Escherichia, Salmonella, Shigella and Clostridium* and their antibiograms in stools of residents of Venda, South Africa.

• Mr E Green (2001)  Antibiotic susceptibility profiles of potential enteric pathogens isolated from water sources in the Venda region of the Northern Province of South Africa.

• Mr R Sono (2001)  The prevalence of *Aeromonas, Plesiomonas* and *Vibrio* species in stools of patients with diarrhoea in rural communities in the Venda region and the antibiograms of isolates.

• Miss LP Mamathunsha (2001)  The effect of different storage containers on water quality.

• Mr T Mudau (2001)  Incidence of enteric pathogens and diarrhoea cases in the Venda region of the Northern Province of South Africa.

• Miss G Matsaung (2002)  Isolation and antibiotic susceptibility patterns of potential enteric bacterial pathogens from water sources in the Venda region.

• Miss MM Moraba (2003) Assessment of raw water sources using somatic and F-RNA coliphages as indicators of water quality in the Limpopo Province of South Africa.

• Miss RL Ramulumo (2003) Antibiotic susceptibility patterns of *Clostridium perfringens* and *Campylobacter jejuni* isolated from water and stool samples in the Venda region of South Africa.

9.2. MSc Microbiology

• Mr E Green (2003) Molecular characterization of *Escherichia coli* isolates from water and stool samples in the Venda Region of South Africa.


• Mr E Musie (2003) Molecular epidemiology of *Salmonella* isolates from clinical, food, sediment and water samples.

10. TECHNOLOGY TRANSFER

10.1. PUBLISHED ARTICLES


**10.2. PAPERS IN PREPARATION FOR PUBLICATION**


Potgieter N, Obi CL, Bessong PO, Igumbor EO, Sammi A and Ramulumo RI (2004) Antibiotic susceptibility patterns of *Clostridium perfringens* and *Campylobacter jejuni* isolated from water and stool samples in the Limpopo Province of South Africa. To be submitted to *Water Research*.

**10.3. CONFERENCE PRESENTATION**


10.4. PROPOSAL FOR ARCHIVING OF DATA

Data obtained on the various aspects of this study will be kept at the Department of Microbiology, University of Venda for Science and Technology, Thohoyandou, South Africa.

10.5. TECHNOLOGY TRANSFER TO COMMUNITIES AND CLINICS

In order to sustain water quality intervention strategies, technology transfer to local water affairs officers, health and environmental officers and other stakeholders are part of ongoing research projects.
ACKNOWLEDGMENTS

The Water Research Commission Project Steering committee members responsible for this project:

Mrs APM Moolman  Water Research Commission (Chairperson)
Prof WOK Grabow  University of Pretoria
Prof MNB Momba  University of Fort Hare
Prof VP Onyango-Otieno  University of Venda
Dr JM Barnes  University of Stellenbosch
Dr ML van der Walt  Medical Research Council, Pretoria
Mrs B Genthe  CSIR (Stellenbosch)
Mr M Vumbi  Thohoyandou Local Government
Mr MW Mandlguzi  Health and Welfare, Giyani
Mr S Mosai  DWAF, Pretoria
Mr L Moisela  Resource Quality Services

This project was only possible with the cooperation of many individuals and institutions. The authors therefore wish to record their sincere thanks to the following individuals:

Prof AA Hooseen  Department of Microbiology, Medical University of Southern Africa.
Prof AD Steele  MRC Diarrhoeal Research Unit, Medical University of Southern Africa.
Prof TE Cloete  Department of Microbiology and Plant Pathology, University of Pretoria.
Prof GM Nkondo  Vice-Chancellor and Principal, University of Venda for Science and Technology
Dr SN Venter  Department of Microbiology and Plant Pathology, University of Pretoria.
Dr S Gundry  AQUAPOL Project, Bristol University, UK.
Mr B De Villiers  Department of Microbiology,
Medical University of Southern Africa.
Ms P Malangi  Research and Publications Office,
University of Venda for Science and Technology.
Mrs R Khakhu  Committee section,
University of Venda for Science and Technology.
Mr S Mukoma  Finance section,
University of Venda for Science and Technology.
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hydrophilia</td>
<td><em>Aeromonas hydrophilia</em></td>
</tr>
<tr>
<td>AKC</td>
<td>Amikacin</td>
</tr>
<tr>
<td>AP</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>APN</td>
<td>Alkaline peptone water</td>
</tr>
<tr>
<td>C. jejuni</td>
<td><em>Campylobacter jejuni</em></td>
</tr>
<tr>
<td>C. coli</td>
<td><em>Campylobacter coli</em></td>
</tr>
<tr>
<td>CEF</td>
<td>Ceftriazone</td>
</tr>
<tr>
<td>CHL</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>COT</td>
<td>Cotrimoxazole</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>DCA</td>
<td>Deoxycholate citrate agar</td>
</tr>
<tr>
<td>ds RNA</td>
<td>Double stranded ribonucleic acid</td>
</tr>
<tr>
<td>DWAF</td>
<td>Department of Water Affairs and Forestry</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>ERY</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>FC</td>
<td>Faecal coliforms</td>
</tr>
<tr>
<td>GM</td>
<td>Gentamicin</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HPC</td>
<td>Heterotrophic plate count</td>
</tr>
<tr>
<td>MAB</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>MAX</td>
<td>Maximum</td>
</tr>
<tr>
<td>MHA</td>
<td>Muellar – Hinton Agar</td>
</tr>
<tr>
<td>MIN</td>
<td>Minimum</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>NA</td>
<td>Nalidixic acid</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>ND</td>
<td>Not Done</td>
</tr>
<tr>
<td>NI</td>
<td>Nitrofurantoin</td>
</tr>
<tr>
<td>P</td>
<td>Protease Sensitive Protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PFU</td>
<td>Plaque forming unit</td>
</tr>
<tr>
<td>P. shigelloides</td>
<td>Plesiomonas shigelloides</td>
</tr>
<tr>
<td>RDP</td>
<td>Reconstruction and Development Programme</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse Transcriptase Polymerase Chain Reaction</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SF</td>
<td>Selenite F-broth</td>
</tr>
<tr>
<td>SS Agar</td>
<td>Salmonella – Shigella agar</td>
</tr>
<tr>
<td>TC</td>
<td>Total coliforms</td>
</tr>
<tr>
<td>TCBS</td>
<td>Thiocitrate bile salt</td>
</tr>
<tr>
<td>TE</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>VP4</td>
<td>Virion’s outer capsid spike protein</td>
</tr>
<tr>
<td>VP7</td>
<td>Virion’s outer capsid viral glycoprotein</td>
</tr>
<tr>
<td>WRC</td>
<td>Water Research Commission</td>
</tr>
<tr>
<td>XDCA</td>
<td>Xylose Deoxycholate Citrate agar.</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table 1.1 Microbiological assessment of water quality from various water sources used for drinking purposes in rural Venda communities</th>
<th>38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.2 Pathogenic bacteria isolated from water sources in the Venda region of South Africa</td>
<td>39</td>
</tr>
<tr>
<td>Table 1.3 Antibiograms of bacterial isolates from water sources in the Venda region, South Africa</td>
<td>40</td>
</tr>
<tr>
<td>Table 2.1 Occurrence of Rotavirus VP4, VP7 and electropherotypes in diarrhoeal stool samples</td>
<td>62</td>
</tr>
<tr>
<td>Table 2.2 Antibiotic susceptibilities of enteric bacterial pathogens from stool specimens of patients with diarrhoea</td>
<td>64</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>The Venda region in the Limpopo Province</td>
<td>27</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Typical households found in rural Venda communities</td>
<td>28</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Two different types of water sources used by the rural communities as drinking water sources</td>
<td>29</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Various activities taking place at the water sources</td>
<td>30</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Types of containers used in the collection and storage of water</td>
<td>31</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Transportation of drinking water to the household</td>
<td>32</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Storage of water in the household</td>
<td>33</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Frequency of isolation of potential enteric pathogens from stool specimens of patients with diarrhoea in the Venda region</td>
<td>61</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Age distribution of enteric organisms isolated from patients with diarrhoea in the Venda region</td>
<td>61</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Seasonal distribution of enteropathogens isolated from diarrhoeal cases in the Venda region</td>
<td>62</td>
</tr>
</tbody>
</table>
CHAPTER 1

ASSESSMENT OF THE MICROBIAL QUALITY OF WATER SOURCES

1.1. INTRODUCTION

Rural communities in developing countries such as the Venda region of South Africa are poverty-stricken, lack access to potable water supplies and rely mainly on river, stream, fountain and borehole water sources for their daily water needs (Nevondo and Cloete, 1991) (Figures 1.1, 1.2). Consequently, the water sources used by most rural communities are faecally contaminated and devoid of treatment which pose a significant health risk because of the danger of waterborne diseases and their complications (Schalekamp 1990, Bailey and Archer, 2001). Water-borne diseases which may be caused by bacterial, fungal, viral and parasitic pathogens are responsible for crippling, devastating and debilitating effects on rural residents and further exacerbate the already strained health burden and facilities in the country (Genthe and Seager, 1996; Grabow, 1996). Therefore it is important to critically monitor the microbial quality of water supply in rural areas in order to provide the impetus for sustained government intervention (Acho-Chi, 2001).

Although government has made some efforts to ensure access to potable water supply to rural communities in South Africa, these projects have been fraught with financial and human resource constraints, making it unlikely that high-quality water will be made available to the bulk of rural residents in the future (Nevondo and Cloete, 1999). In areas where potable water supplies have been provided, these supplies are unreliable and insufficient, forcing residents to revert to contaminated river sources (WRC, 1993; Nevondo and Cloete, 1999). Figures 1.3 and 1.4

The major health risk associated with these drinking water sources is contamination by human or animal faeces (Lehloesa and Muyima, 2000). Since
it is impractical to test water supply for all pathogens related to water-borne diseases due to the complexity of the testing, time and cost (Lehloesa and Muyima, 2000), indicator organisms are used (Hazen, 1988; Grabow, 2001). However, no simple indicator that complies with all the criteria is available, hence more than one indicator organism is employed (Genthe and Seager, 1996).

In spite of the problem of poor water quality in rural areas, little data exists on the bacterial quality of water supply in these settings, since most studies approach the problem by focusing on urban communities (Nevondo and Cloete, 1999). In this study indicators of pollution (heterotrophic plate counts, total coliforms, faecal coliforms, faecal enterococci and somatic coliphages) were used to determine the microbial quality of water sources of rural communities in the Venda region and these results were compared with guideline values described by the Department of Water Affairs and Forestry (DWAF, 1998).

1.2. MATERIALS AND METHODS

1.2.1. Study areas

The study sites included various rural communities in the Venda Region of the Limpopo Province, South Africa (Figures 1.1 and 1.2). The main water sources used by the people in these communities were identified and sampled (Figures 1.3 and 1.4). They comprised: Mutshindudi, Tshinane, Ngwedi, Mutale, Mudaswali and Levuvhu rivers and the Makonde and Mudaswali fountains.

1.2.2. Sample collection

The collection of water samples from the various water sources were carried out on a weekly basis from August 2000 to July 2002. Water samples were collected aseptically into 1litre Nalgene containers and transported on ice to the base laboratories in the Department of Microbiology, University of Venda for Science and Technology, South Africa. Microbiological investigations were performed within 8 h after collection.
1.2.3. Microbiological analysis

1.2.3.1. Indicator organisms

Microbiological analyses of water samples were performed as previously described (Standard Methods, 1998). Briefly, for heterotrophic bacteria, the pour plate method was carried out using Plate Count agar (Merck) and plates were incubated at 37ºC for 48h. Bacterial indicator organisms were detected and enumerated using the membrane filtration method. Total coliforms were assessed on m-Endo agar (Merck) after 24h with an incubation temperature of 37ºC. Faecal coliforms were enumerated on m-Fc medium and incubated at 44.5ºC for 24h. The m-Enterococcus agar (Merck) was used for the isolation of faecal enterococci after an incubation at 37ºC for 48h. For somatic coliphage counts, the double agar layer plaque assay on phage agar described by Grabow et al (1996, 2000, 2001) at 37ºC for 24h was used. The nalidixic acid resistant *Escherichia coli* WG5 strain was used as host.

1.2.3.2. Enteropathogenic bacteria

Standard methods (1998) were employed for the isolation and identification of *Campylobacter*, *Aeromonas*, *Plesiomonas*, *Salmonella*, *Shigella*, *Vibrio* and *Yersinia* species. Media, supplements and chemicals were obtained from Merck, South Africa and Oxoid, South Africa.

*Aeromonas* and *Plesiomonas* isolation and identification

Specimens were inoculated onto Xylose Deoxycholate Citrate Agar (XDCA), incubated at 37ºC for 24h. Non-xylose fermenting colonies on XDCA were screened for oxidase production (Alabi and Odugbeni, 1990). Oxidase-positive colonies were further confirmed as belonging to *Aeromonas* or *Plesiomonas shigelloides*. *Aeromonas spp* give positive reactions for ornithine decarboxylase, DNase tests and resistance to vibrostatic agent O/129, while *Plesiomonas shigelloides* produces neither gas nor H₂S on Triple sugar iron agar (Von Gravenitz, 1985).
**Campylobacter** isolation and identification

Isolation of *Campylobacter* from stool was done on Skirrow’s and Butzler’s media as previously described (Coker and Dosunmu-Ogumbi, 1984; Alabi and Odugbemi, 1990; Obi et al., 1998). Briefly, the plates were incubated at 42ºC under microaerophilic conditions for 72h. Organisms were considered to be *Campylobacter* if they were S-shaped, Gram negative, motile, oxidase-positive, grew at 42ºC but not at 25ºC and sensitive to nalidixic acid. *C. jejuni* and *C. coli* isolates were differentiated on the basis of hippurate and indoxyl acetate hydrolysis. *C. jejuni* is positive for both tests while *C. coli* is positive for indoxyl acetate hydrolysis only (Nachamkin, 1999; Prasad et al., 2000).

**Escherichia coli** isolation and identification

Samples were streaked on Eosin Methylene Blue agar (EMB) and incubated aerobically for 24h at 37ºC. Blue-purple or metallic green sheen colonies indicative of *E. coli* were confirmed by positive reactions for indole, o-nitrophenyl-β-D-galactopyranoside (ONPG), xylose, citrate utilization and negative reactions for oxidase, DNase, KCN, phenylalanine deaminase and Voges-Proskauer tests (Ogunsanya et al., 1994).

**Salmonella** isolation and identification

Stool specimen was streaked on Bismuth sulphite agar and incubated for 48h at 37ºC. Black colonies with 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 Potassium cyanide (KCN), ONPG and fermentation of adonitol, sucrose, lactose, raffinose and salicin (Simango et al., 1992).
**Shigella isolation and identification**

Samples were cultured on Salmonella-Shigella agar (SS) for 24h at 37°C. 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 (Obi et al., 1998; Nachamkim, 1999).

**Vibrio isolation and identification**

For each sample 1 ml of water was inoculated in 10 ml of alkaline peptone water, pH 8.6 (APW) and incubated for 24h 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 at 37°C. Yellow colonies suggestive of *Vibrio* growth were sought and screened using *V. cholerae* O1 antiserum (Wellcome Reagents, Wellcome Research Laboratories, Beckenham) (Alabi and Odugbeni, 1990; Ogunsanya et al., 1994).

**Yersinia enterocolitica isolation and identification**

Samples were cultured on Yersinia agar enriched with *Yersinia* selective supplement SR109 (Oxoid) (Simango et al., 1992) and incubated at 37°C for 24h. Presumptive colonies of *Y. enterocolitica* were characterized by positive tests for ornithine carbamoylase and sucrose fermentation, and negative reactions for raffinose, rhamnose and melibiose fermentation (Alabi and Odugbeni, 1990).

**1.2.4. Antibiogram determination**

Antibiotic susceptibility testing of bacterial isolates was determined on Mueller-Hinton agar using the Kirby – Bauer disk diffusion method (Bauer et al., 1966; Obi et al., 1998). Antibiotics employed were commercially obtained from Davies Diagnostics (South Africa) and included ampicillin (10ug), gentamicin (10ug),
penicillin (10 ug), carbenicillin (30 ug), tetracycline (30ug), chloramphenicol (30ug), erythromycin (15ug) and cephalosporin (30ug).

1.2.5. Statistical analysis

Excel statistical package was employed for statistical analysis.
Figure 1.1: Map showing the Venda region in the Limpopo Province
Figure 1.2: Typical households found in rural Venda communities
Figure 1.3: Two different types of water sources used by rural communities as drinking water sources
Figure 1.4: Various activities taking place at river water sources
Figure 1.5: Types of containers used in the collection and storage of water
Figure 1.6: Transportation of drinking water to the household
Figure 1.7: Storage of drinking water in the household
1.3. RESULTS

1.3.1. Heterotrophic plate counts

Heterotrophic bacterial counts in the Levubu river were in the range of between $6.0 \times 10^3$ and $1.3 \times 10^6$ cfu.ml$^{-1}$ for Masetoni point, between $5.0 \times 10^3$ and $3.1 \times 10^4$ cfu.ml$^{-1}$ for Mhinga point, between $7.7 \times 10^3$ and $2.6 \times 10^4$ cfu.ml$^{-1}$ for Dididi point, between $9.6 \times 10^2$ and $1.4 \times 10^5$ cfu.ml$^{-1}$ for Tshikonelo point, between $1.8 \times 10^2$ and $2.0 \times 10^3$ cfu.ml$^{-1}$ for Grootpad point, between $1.0 \times 10^3$ and $3.0 \times 10^3$ cfu.ml$^{-1}$ for Mutoti point and between $7.0 \times 10^3$ and $2.7 \times 10^5$ cfu.ml$^{-1}$ for the Vuwani point. For the other rivers, the counts ranged between $1.0 \times 10^3$ and $3.0 \times 10^4$ cfu.ml$^{-1}$ for Mutale River, between $6.2 \times 10^3$ and $7.9 \times 10^4$ cfu.ml$^{-1}$ for Ngwedi river, between $1.9 \times 10^2$ and $1.7 \times 10^3$ cfu.ml$^{-1}$ for Tshinane River and between $1.9 \times 10^2$ and $1.7 \times 10^3$ cfu.ml$^{-1}$ for Mudaswali River (Table 1.1.) The counts for the Mudaswali fountain ranged between $1.8 \times 10^1$ and $1.9 \times 10^2$ cfu.ml$^{-1}$ and between $1.0 \times 10^1$ and $1.4 \times 10^2$ cfu.ml$^{-1}$ for Makonde fountain (Table 1.1). The recommended limit for no risk in terms of heterotrophic bacterial count is $1.0 \times 10^2$ cfu. ml$^{-1}$ (DWAF, 1998, WRC, 1998).

1.3.2. Total coliforms

The minimum and maximum total coliform counts were in the following ranges for the Levubu river points: between $6.0 \times 10^2$ and $7.6 \times 10^3$ cfu.100ml$^{-1}$ for Masetoni point, between $8.9 \times 10^2$ and $2.3 \times 10^4$ cfu.100ml$^{-1}$ for Mhinga point, between $4.9 \times 10^3$ and $1.5 \times 10^4$ cfu.100ml$^{-1}$ for Dididi point, between $1.1 \times 10^3$ and $1.8 \times 10^3$ cfu.100ml$^{-1}$ for Tshikonelo point, between $1.3 \times 10^4$ and $2.1 \times 10^4$ cfu.100ml$^{-1}$ for Grootpad point, between $9.2 \times 10^2$ and $1.5 \times 10^3$ cfu.100ml$^{-1}$ for Mutoti point and between $7.3 \times 10^3$ and $1.8 \times 10^4$ cfu.100ml$^{-1}$ for Vuwani point. The minimum and maximum counts for the other rivers respectively ranged between $9.2 \times 10^3$ and $1.9 \times 10^4$ cfu.100ml$^{-1}$ for Mutale River, between $2.0 \times 10^4$ and $3.4 \times 10^4$ cfu.100ml$^{-1}$ for Tshinane River, between $2.8 \times 10^3$ and $3.7 \times 10^4$ cfu.100ml$^{-1}$ for Ngwedi river and between $0.3 \times 10^1$ and $1.0 \times 10^6$ cfu.100ml$^{-1}$ for Mudaswali river (Table 1.1). The counts for the Mudaswali fountain ranged between 0 and $1.24 \times 10^4$ cfu.100ml$^{-1}$ and between $1.3 \times 10^1$ and $1.9 \times 10^2$ cfu.100ml$^{-1}$.
and $8.3 \times 10^1$ cfu.100 ml$^{-1}$ for Makonde fountain (Table 1.1). The counts exceeded the 5 cfu. 100 ml$^{-1}$, which is the recommended limit for no risk (DWAF, 1998, WRC, 1998)

1.3.3. Faecal coliforms

The minimum and maximum faecal coliform counts for the various sites were as follows in the Levubu river: between $1.5 \times 10^3$ and $6.3 \times 10^4$ cfu.100 ml$^{-1}$ for Masetoni point, between $5.2 \times 10^3$ and $1.72 \times 10^4$ cfu.100 ml$^{-1}$ for Mhinga point, between $9.0 \times 10^2$ and $1.5 \times 10^3$ cfu.100 ml$^{-1}$ for Tshikonelo point, between $6.1 \times 10^3$ and $1.2 \times 10^4$ cfu.100 ml$^{-1}$ for Grootpad point, between $5.6 \times 10^3$ and $7.2 \times 10^3$ cfu.100ml$^{-1}$ for Mutoti point, between $4.1 \times 10^2$ and $7.5 \times 10^2$ cfu.100 ml$^{-1}$ for Dididi point, and between $2.9 \times 10^2$ and $1.1 \times 10^4$ cfu.100 ml$^{-1}$ for Vuwani point. The minimum and maximum faecal coliform counts for the other rivers ranged between $5.6 \times 10^3$ and $2.0 \times 10^3$ cfu.100 ml$^{-1}$ for Mutale River, between $7.4 \times 10^2$ and $3.9 \times 10^3$ cfu.100 ml$^{-1}$ for Tshinane River, between $1.8 \times 10^2$ and $8.2 \times 10^2$ cfu.100 ml$^{-1}$ for Ngwedi river and between $0.1 \times 10^1$ and $1.0 \times 10^6$ cfu.100 ml$^{-1}$ for Mudaswali river (Table 1.1). The counts for the Mudaswali fountain ranged between 0 and $3.5 \times 10^4$ cfu.100ml$^{-1}$ and between $0.4 \times 10^1$ and $7.4 \times 10^1$ cfu.100 ml$^{-1}$ for Makonde fountain (Table 1.1). The recommended limit for no risk of faecal coliforms is 0 cfu.100 ml$^{-1}$.

1.3.4. Faecal enterococci

Enterococci counts for the Levubu river points ranged between $2.0 \times 10^3$ and $5.5 \times 10^3$ cfu.100 ml$^{-1}$ for Masetoni point, between $5.0 \times 10^2$ and $2.3 \times 10^3$ cfu.100 ml$^{-1}$ for Mhinga point, between $1.2 \times 10^3$ and $3.1 \times 10^3$ cfu.100 ml$^{-1}$ for Tshikonela point, between $1.0 \times 10^3$ and $1.0 \times 10^4$ cfu.100 ml$^{-1}$ for Dididi point, between $4.0 \times 10^3$ and $2.1 \times 10^4$ cfu.100 ml$^{-1}$ for Grootpad point, between $1.9 \times 10^3$ and $2.5 \times 10^4$ cfu.100 ml$^{-1}$ for Mutoti point and between $1.0 \times 10^1$ and $5.1 \times 10^2$ cfu.100 ml$^{-1}$ for Vuwani point. Enterococci counts for the other rivers ranged between $1.9 \times 10^2$ and $2.1 \times 10^3$ cfu.100 ml$^{-1}$ for Mutale River, between 0 and $6.4 \times 10^1$ cfu.100 ml$^{-1}$ for Mudaswali River, between $4.0 \times 10^1$ and $3.2 \times 10^2$ cfu.100 ml$^{-1}$ for Tshinane River and between $6.6 \times 10^3$ and $2.2 \times 10^4$ cfu.100 ml$^{-1}$
for Ngwedi River (Table 1.1). Enterococci counts for the Mudaswali fountain ranged between 0 and $1.12 \times 10^4$ cfu.100 ml$^{-1}$ and between 0 and $8.3 \times 10^1$ cfu.100 ml$^{-1}$ for Makonde fountain (Table 1.1). The recommended limit for no risk of faecal enterococci is 5 cfu.100ml$^{-1}$ (DWAF 1998).

1.3.5. Somatic coliphages

Somatic coliphage counts obtained from the various water sources are presented in Table 1.1. Briefly, coliphage counts for all the water sampling points ranged between 0 and 210 pfu.100 ml$^{-1}$. The recommended limit for no risk of somatic coliphages is 1 pfu.100 ml$^{-1}$.

1.3.6. Pathogenic bacteria

Bacteria such as *Escherichia coli*, *Vibrio cholerae*, *Aeromonas hydrophila*, *Aeromonas caviae*, *Shigella*, *Plesiomonas shigelloides*, *Enterobacter cloacae*, *Campylobacter* and *Salmonella* were isolated and identified from the various water sources. The results are indicated in Table 1.2.

1.3.7. Antibiogram determination

Antibiogram results of bacterial enteropathogens presented in Table 1.3 revealed marked susceptibilities (over 90%) of *Campylobacter*, *Salmonella* and *Escherichia coli* to nalidixic acid, ciprofloxacin and amikacin. All 100% of *Salmonella* species were susceptible to amikacin. Eighty-eight percent of *Campylobacter* species and over 90% of *Escherichia coli*, *Salmonella*, *Plesiomonas* and *Shigella* species were susceptible to gentamicin. *Aeromonas*, *Enterobacter* and *Vibrio cholerae* demonstrated over 90% susceptibilities to ciprofloxacin and over 85% of the three isolates were in addition susceptible to amikacin. In general, over 80% of the bacterial isolates were susceptible to nalidixic, gentamicin, ciprofloxacin, amikacin and ceftiriazone with the exception of *Vibrio cholerae* with susceptibility rates of 73%, 60%, 53% to nalidixic acid, ceftiriazone and gentamicin respectively and *Campylobacter* species with 62% susceptibility to ceftiriazone.
Majority of bacterial isolates demonstrated multiple antibiotic resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphenicol. A total of 35% of the *Campylobacter* isolates were resistant to erythromycin whereas 20% - 30% of *Campylobacter, Salmonella, Shigella, Plesiomonas, Aeromonas* and *Enterobacter* isolates showed resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphenicol.
Table 1.1  Microbiological assessment of water quality from various water sources used for drinking purposes in rural Venda communities.

<table>
<thead>
<tr>
<th>Water Sources</th>
<th>Heterotrophic bacteria (cfu. ml⁻¹)</th>
<th>Total coliforms (cfu.100 ml⁻¹)</th>
<th>Faecal coliforms (cfu.100 ml⁻¹)</th>
<th>Faecal enterococci (cfu.100 ml⁻¹)</th>
<th>Somatic Coliphages (cfu.100 ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levubu River: Masetoni point</td>
<td>Min : 6.0 x 10⁴</td>
<td>Max : 7.6 x 10⁴</td>
<td>Mean : 5.3 x 10⁴</td>
<td>SD : 1.2 x 10⁴</td>
<td>Min : 2.0 x 10⁴</td>
</tr>
<tr>
<td>Levubu River: Mhindga point</td>
<td>Min : 8.9 x 10⁴</td>
<td>Max : 2.3 x 10⁵</td>
<td>Mean : 1.1 x 10⁵</td>
<td>SD : 6.5 x 10⁴</td>
<td>Min : 5.0 x 10⁴</td>
</tr>
<tr>
<td>Levubu River: Dididi point</td>
<td>Min : 4.9 x 10⁴</td>
<td>Max : 1.5 x 10⁵</td>
<td>Mean : 9.95 x 10⁴</td>
<td>SD : 5.0 x 10⁵</td>
<td>Min : 1.0 x 10⁴</td>
</tr>
<tr>
<td>Levubu River: Tshikonela point</td>
<td>Min : 9.6 x 10⁴</td>
<td>Max : 1.4 x 10⁵</td>
<td>Mean : 7.5 x 10⁴</td>
<td>SD : 4.3 x 10⁵</td>
<td>Min : 1.0 x 10⁴</td>
</tr>
<tr>
<td>Levubu River: Grootpaad point</td>
<td>Min : 1.3 x 10⁵</td>
<td>Max : 2.1 x 10⁵</td>
<td>Mean : 1.7 x 10⁵</td>
<td>SD : 2.9 x 10⁵</td>
<td>Min : 1.2 x 10⁵</td>
</tr>
<tr>
<td>Levubu River: Mutoli point</td>
<td>Min : 9.2 x 10⁴</td>
<td>Max : 1.4 x 10⁵</td>
<td>Mean : 5.0 x 10⁵</td>
<td>SD : 1.0 x 10⁵</td>
<td>Min : 1.0 x 10⁴</td>
</tr>
<tr>
<td>Levubu River: Vuwani point</td>
<td>Min : 7.3 x 10⁴</td>
<td>Max : 1.3 x 10⁵</td>
<td>Mean : 6.4 x 10⁵</td>
<td>SD : 4.2 x 10⁵</td>
<td>Min : 1.0 x 10⁴</td>
</tr>
<tr>
<td>Mutale river</td>
<td>Min : 9.2 x 10⁴</td>
<td>Max : 1.9 x 10⁵</td>
<td>Mean : 1.4 x 10⁵</td>
<td>SD : 5.0 x 10⁵</td>
<td>Min : 1.9 x 10⁴</td>
</tr>
<tr>
<td>Ngwedi River</td>
<td>Min : 2.8 x 10⁴</td>
<td>Max : 3.7 x 10⁴</td>
<td>Mean : 2.0 x 10⁴</td>
<td>SD : 4.2 x 10⁴</td>
<td>Min : 1.8 x 10⁴</td>
</tr>
<tr>
<td>Tshinanane River</td>
<td>Min : 3.4 x 10⁴</td>
<td>Max : 2.7 x 10⁴</td>
<td>Mean : 5.0 x 10⁴</td>
<td>SD : 2.3 x 10⁴</td>
<td>Min : 1.0 x 10⁴</td>
</tr>
<tr>
<td>Mudawali river</td>
<td>Min : 0.3 x 10⁴</td>
<td>Max : 1.0 x 10⁴</td>
<td>Mean : 7.7 x 10⁴</td>
<td>SD : 2.8 x 10⁴</td>
<td>Min : 0.1 x 10⁴</td>
</tr>
<tr>
<td>Mudawali fountain</td>
<td>Min : 0</td>
<td>Max : 1.24 x 10⁴</td>
<td>Mean : 2.47 x 10⁴</td>
<td>SD : 2.64 x 10⁴</td>
<td>Min : 0</td>
</tr>
<tr>
<td>Makonde fountain</td>
<td>Min : 1.3 x 10⁴</td>
<td>Max : 8.3 x 10⁴</td>
<td>Mean : 7.4 x 10⁴</td>
<td>SD : 2.6 x 10⁴</td>
<td>Min : 0</td>
</tr>
</tbody>
</table>

cfu = colony forming unit  
ml = millilitres  
min = minimum  
max = maximum  
SD = standard deviation  
pfu = plaque forming unit
Table 1.2  Pathogenic bacteria isolated from water sources in the Venda region of South Africa

<table>
<thead>
<tr>
<th>Water sources</th>
<th>Enteric bacteria isolated from each source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levubuvhu river:</td>
<td></td>
</tr>
<tr>
<td>Vuwani point</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Plesiomonas shigelloides</em></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella isolates</em></td>
</tr>
<tr>
<td>Levuvhu river:</td>
<td></td>
</tr>
<tr>
<td>Tshikonela point</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio isolates</em></td>
</tr>
<tr>
<td>Levuvhu river:</td>
<td></td>
</tr>
<tr>
<td>Masetoni point</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter cloacae.</em></td>
</tr>
<tr>
<td>Levuvhu river:</td>
<td></td>
</tr>
<tr>
<td>Grootpad point</td>
<td><em>Aeromonas hydrophila</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas caviae</em></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio isolates</em></td>
</tr>
<tr>
<td>Levuvhu river:</td>
<td></td>
</tr>
<tr>
<td>Dididi point</td>
<td><em>Aeromonas hydrophila</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas caviae</em></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td>Levuvhu river:</td>
<td></td>
</tr>
<tr>
<td>Mhinga point</td>
<td><em>Salmonella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas hydrophila</em></td>
</tr>
<tr>
<td>Levuvhu river:</td>
<td></td>
</tr>
<tr>
<td>Mutoti point</td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Mudaswali River</td>
<td><em>Vibrio isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella isolates</em></td>
</tr>
<tr>
<td>Mudaswali fountain</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas caviae</em></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio isolates</em></td>
</tr>
<tr>
<td>Tshinane River</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Campylobacter isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella isolates</em></td>
</tr>
<tr>
<td>Ngwedi River</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Plesiomonas shigelloides</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Campylobacter isolates</em></td>
</tr>
<tr>
<td>Mutale River</td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas isolates</em></td>
</tr>
<tr>
<td>Makonde fountain</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Shigella isolates</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas hydrophila</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas caviae</em></td>
</tr>
<tr>
<td></td>
<td><em>Plesiomonas shigelloides</em></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio isolates</em></td>
</tr>
</tbody>
</table>
Table 1.3  Antibiograms of enteric bacterial isolates from water sources in the Venda region, South Africa

<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>Campylobacter isolates (n = 26)</td>
<td>24 (92%)</td>
<td>23 (88%)</td>
<td>18 (69%)</td>
<td>25 (96%)</td>
<td>18 (69%)</td>
<td>15 (58%)</td>
<td>17 (65%)</td>
<td>16 (62%)</td>
<td>25 (96%)</td>
<td>16 (62%)</td>
</tr>
<tr>
<td>Escherichia coli (n = 40)</td>
<td>36 (90%)</td>
<td>37 (92.5%)</td>
<td>30 (75%)</td>
<td>38 (95%)</td>
<td>28 (70%)</td>
<td>20 (50%)</td>
<td>21 (52.5)</td>
<td>18 (45%)</td>
<td>39 (97.5%)</td>
<td>37 (92.5%)</td>
</tr>
<tr>
<td>Salmonella isolates (n = 30)</td>
<td>28 (93%)</td>
<td>29 (96.6%)</td>
<td>20 (66.6%)</td>
<td>29 (96.6%)</td>
<td>14 (46.6%)</td>
<td>16 (53%)</td>
<td>24 (80%)</td>
<td>24 (80%)</td>
<td>30 (100%)</td>
<td>28 (93%)</td>
</tr>
<tr>
<td>Shigella isolates (n = 30)</td>
<td>26 (86.6%)</td>
<td>28 (93%)</td>
<td>19 (63%)</td>
<td>28 (93%)</td>
<td>16 (53.3%)</td>
<td>15 (50%)</td>
<td>16 (53.5%)</td>
<td>18 (60%)</td>
<td>29 (96.6%)</td>
<td>28 (93%)</td>
</tr>
<tr>
<td>Plesiomonas shigelloides (n = 20)</td>
<td>18 (90%)</td>
<td>18 (90%)</td>
<td>15 (75%)</td>
<td>19 (95%)</td>
<td>14 (70%)</td>
<td>13 (65%)</td>
<td>10 (50%)</td>
<td>11 (55%)</td>
<td>19 (95%)</td>
<td>18 (90%)</td>
</tr>
<tr>
<td>Aeromonas (n = 20)</td>
<td>18 (90%)</td>
<td>17 (85%)</td>
<td>14 (70%)</td>
<td>18 (90%)</td>
<td>15 (75%)</td>
<td>12 (60%)</td>
<td>9 (45%)</td>
<td>10 (50%)</td>
<td>18 (90%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>Vibro cholerae (n = 15)</td>
<td>11 (73%)</td>
<td>8 (53%)</td>
<td>10 (66.6%)</td>
<td>14 (93.3%)</td>
<td>7 (47%)</td>
<td>8 (53%)</td>
<td>6 (40%)</td>
<td>9 (60%)</td>
<td>13 (87%)</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>Enterobacter isolates (n = 20)</td>
<td>17 (85%)</td>
<td>15 (75%)</td>
<td>16 (80%)</td>
<td>18 (90%)</td>
<td>12 (60%)</td>
<td>11 (55%)</td>
<td>10 (50%)</td>
<td>11 (55%)</td>
<td>17 (85%)</td>
<td>16 (80%)</td>
</tr>
</tbody>
</table>

NA = Nalidixic acid  TE = Tetracycline  GM = Gentamicin  ERY = Erythromycin  COT = Cotrimoxazole  AP = Ampicillin  CIP = Ciprofloxacin  AKC = Amikacin  CEF = Ceftriazone  CHL = Chloramphenicol
1.4. DISCUSSION

The high counts of indicator organisms revealed that the microbiological quality of the water sources were above the recommended safety guidelines for drinking water as stipulated by DWAF (1998). This is in agreement with findings by other researchers who conducted similar studies in rural areas (Palupi et al., 1995; Nevondo and Cloete, 1999).

The detection of somatic phages in the water sources could indicate faecal contamination and hence potential viral infections (Grabow, 2001). However, there is controversy since some findings have indicated that viruses were not detected although coliphages were detected and in other cases viruses were detected while coliphages were not. Coliphages can therefore only serve as indicators or as possible models to indicate potential presence of viruses (Grabow et al., 1984; Armon et al., 1997; Grabow et al., 2000; Grabow, 2001).

Potential pathogenic enteric bacteria including *Escherichia coli*, *Vibrio cholerae*, *Aeromonas hydrophila*, *Shigella*, *Plesiomonas* and *Campylobacter* species were isolated from the various water sources. The presence of these pathogens in river water sources is in agreement with previous reports (Nevondo and Cloete, 1999; Theron, 2001). These enteric bacteria are reportedly causative agents of various diseases and their complications (Grabow, 1996). Such diseases include dysentery caused mainly by *Shigella* species, Guillain-Barre syndrome which is a complication of *Campylobacter jejuni/coli* infection, haemolytic uraemic syndrome which is a sequelae of some *Escherichia coli* species, and *Vibrio cholerae* which could cause manifestations such as hypovalaemic shock, acidosis and haemoconcentration (Klein et al., 1986; Thielman and Guerrant, 1999).

Possible sources of contamination of the water sources might include human and animal faeces. However, birds and insects could also be factors that contribute to the contamination of the drinking water sources, especially during storage (Figure 1.7) (Paul et al., 1995; Nevondo and Cloete, 1999; Lehloesa and Muyima, 2000). Most of the river sources are reportedly prone to higher
bacterial levels due to heightened ecological activities, and may therefore not be suitable for human consumption (Lazorchak et al., 1998). These multiple sources of contamination are compounded by limited environmental awareness in rural areas (Figures 1.3 and 1.4) (Lehloesa and Muyima, 2000).

It should however be noted that the presence of faecal coliforms in the water sources may not be conclusive of a faecal origin of the bacteria (Paul et al., 1995). Investigators have reported the presence of faecal coliforms in tropical environments in the absence of any source of faecal contamination (Hardina and Fujioka, 1991, Hazen, 1998). For this reason, we employed an additional faecal indicator, faecal enterococci, which may be a better indicator of human faecal pollution in water (Levin et al., 1975; Rice et al., 1993).

Results presented showed multiple antibiotic resistance of all bacteria isolates to ampicillin, erythromycin, tetracycline, chloramphenicol and cotrimoxazole. Multiple antibiotic resistance refers to resistance to two or more classes of antibiotics. The multiple antibiotic resistance of Salmonella, Shigella, Campylobacter, Aeromonas and Plesiomonas demonstrated in this study accords with other findings (Robins-Browne et al., 1983; Coker and Adefeso, 1994; Obi et al., 1997, 1998). Erythromycin used to be the drug of choice for Campylobacteriosis but increasing resistance of Campylobacter to erythromycin is well known (Coker and Adefeso, 1994). In this study, 35% of Campylobacter isolates were resistant to erythromycin. Strains of Salmonella, particularly Salmonella typhi, accounted for several outbreaks in the United States and worldwide, partly due to resistance to chloramphenicol, ampicillin and trimethoprim (Rowe et al., 1997; Mermin et al., 1998). This resistance pattern simulates the 20% and 47% resistance rates of Salmonella obtained in this study to chloramphenicol and ampicillin respectively.

Antibiotic susceptibility profiles showed that all enteric bacterial isolates were markedly sensitive to nalidixic acid, gentamicin, ciprofloxacin, amikacin and ceftriazone. These drugs may therefore be of value for the treatment of enteric infections requiring emperic antibiotic therapy. These reported susceptibilities are in harmony with reports of other investigators (Wasfy et al., 2000). It should
be noted that susceptibility of bacteria to antibiotics is not static and resistance may be due to antibiotic abuse, antibiotic overuse or may be chromosomally or plasmid mediated (Obi et al., 1998). Antibiotic usage must therefore be carefully regulated and monitored.

This study and other studies on domestic consumption of water in rural communities in the developing world showed the challenges for health and water resources in South Africa and other developing countries (Palupi et al., 1995; Nevondo and Cloete, 1999; Acho-chi, 2001; Lehloesa and Muyima, 2001). The provision of safe potable water supply in rural communities is necessary in order to satisfy basic needs and is seen as important for assessing social development in developing countries (Forch and Biemann, 1998; Acho-chi, 2001). According to the results it can be concluded that the microbiological quality of the water sources investigated in this study suggest a potential risk of infection for consumers and necessitates calls for prompt interventions to mitigate the socio-economic and health impact of water-borne diseases in these rural communities.

1.5. REFERENCES


assay using serotypes 1,2,3 and 4 specific monoclonal antibodies. *Journal of Infectious Disease* **155** 1159-1166.


CHAPTER 2

BACTERIAL ENTEROPATHOGENS AND ROTAVIRUSES IN DIARRHOEAL CASES

2.1. INTRODUCTION

In developing countries more than 800 million cases of diarrhoea are reported annually and most cases occur in rural areas, which accounts for about 4.5 million deaths (Snyder and Pherson, 1982; Esrey et al., 1990; Prado and O'Ryan, 1994; Du Pont, 1995). Children below the age of five, especially those in areas devoid of access to potable water supply and sanitation are prone to the devastating effect of diarrhoea (Esrey et al., 1990). The incidence of morbidity and mortality due to diarrhoea among children younger than five years of age are significantly higher in communities where water supply and sanitation falls below the level equivalent to those stipulated by DWAF (1995) for the Reconstruction and Development Programme (RDP), in comparison to children in formal urban residential areas with in-house water connections (Payment et al., 1991).

The Venda Region is mostly a rural area and the majority of the communities have no access to good roads, electricity, water and sanitation. Water sources for drinking are usually from different rivers, boreholes and fountains and devoid of treatment (Obi et al., 2002). Thus, they are potential sources for the transmission of diarrhoeal diseases. Inspite of this, very little data is available on the prevalence of enteric pathogens in diarrhoeic stool samples obtained in the region. Several pathogens are known to cause diarrhoea and they include bacteria such as Campylobacter, Escherichia coli, Salmonella, Shigella, Plesiomonas shigelloides, Aeromonas, Vibrio cholerae and Yersinia enterocolitica (Obi et al., 1995, 1997, 1998; El-Sheikh and El-Assouli, 2001), and viral agents such as rotaviruses, norwalk viruses, adenoviruses and calicivirus (Echeverria et al., 1983; El-Assouli et al; 1995; El-Sheikh and El-
Parasitic agents of diarrhoea, especially *Giardia lamblia* and *Cryptosporidium parvum* (Tangermann *et al.*, 1991; Chunge *et al.*, 1992, El-Sheikh and El-Assouli, 2001) and fungal agents such as *Candida* (Enweani, *et al.*, 1994) are also common.

Antibiotic treatment which shortens the duration of diarrhoea, decrease stool output and abrogate some of the complications of diarrhoea, is one of the methods used in the management of diarrhoea and its complications (Black, 1993). However, the increasing resistance of bacteria to antibiotics is well documented (Black, 1993; Obi *et al.*, 1998). Although antibiograms are known to vary from place to place and with time, necessitating the need for periodic updates in order to uncover resistance patterns, there are no baseline data on antibiograms of potential bacterial pathogens of diarrhoea isolated from diarrhoeic stool specimens in rural communities in the Venda region. An urgent need, therefore, exists to ascertain the incidence of enteric pathogens in diarrhoeic stools, as well as antibiograms of these bacterial isolates. This study reports the prevalence of rotaviruses, bacterial agents of diarrhoea and antibiograms of bacterial isolates in rural communities in the Venda region.

2.2. MATERIALS AND METHODS

2.2.1. Study sites

Stool samples were collected from the following primary health care clinics in the Venda Region of South Africa: Mphephu, Vuvha, Itsani, Pfapfanani, Mukula, Makonde, Tshaulu, Mutale, Phiphidi, William Eddie and Tshiombo Clinics.

2.2.2. Ethical approval

Ethical approval for the study was obtained from the Research Committee, Department of Health and Social Welfare, Limpopo Province, Polokwane, South Africa. Signed informed consent was obtained from each of the study subjects before sample collection.
2.2.3. Sample collection

Diarrhoeic stool samples were collected on a weekly basis from children and adults attending the mentioned hospitals and primary health care clinics between June 1999 and September 2002. Stool samples were collected into sterile plastic stool sample containers (Merck, South Africa) and transported to the laboratories at the Department of Microbiology, University of Venda and the Diarrhoeal Pathogens Research Unit at the Medical University of South Africa. Microbiological investigations were done within 6-8 hours after sample collection. Diarrhoea was defined as the passage of 3-4 watery stools per day for not less than 3 days. A total of 401 stool specimens were collected of which only 300 were assayed for the presence of human group A rotaviruses.

2.2.4. Bacteriological methods

Standard methods (1998) were employed for the isolation and identification of *Campylobacter, Aeromonas, Plesiomonas, Salmonella, Shigella, Vibrio* and *Yersinia* species. All media, supplements and chemicals were obtained from Merck and Oxoid, South Africa.

*Aeromonas* and *Plesiomonas* isolation and identification

Specimens were inoculated onto Xylose Deoxycholate Citrate Agar (XDCA), incubated at 37°C for 24h. Non-xylose fermenting colonies on XDCA were screened for oxidase production (Alabi and Odugbemi, 1990). Oxidase-positive colonies were further confirmed as belonging to *Aeromonas* or *Plesiomonas shigelloides*. *Aeromonas spp* give positive reactions for ornithine decarboxylase, DNase tests and resistance to vibrostatic agent O/129, while *Plesiomonas shigelloides* produces neither gas nor H₂S on Triple sugar iron agar (Von Gravenitz, 1985).
Campylobacter isolation and identification

Isolation of Campylobacter from stool was done on Skirrow’s and Butzler’s media as previously described (Coker and Dosunmu-Ogumbi, 1984; Alabi and Odugbemi, 1990; Obi et al., 1998). Briefly, the plates were incubated at 42°C under microaerophilic conditions for 72h. 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 while *C. coli* is positive for indoxyl acetate hydrolysis only (Nachamkin, 1999; Prasad et al., 2000).

Escherichia coli isolation and identification

Samples were streaked on Eosin Methylene Blue agar (EMB) and incubated aerobically for 24h at 37°C. Blue-purple or metallic green sheen colonies indicative of *E. coli* were confirmed by positive reactions for indole, o-nitrophenyl-β-D-galactopyranoside (ONPG), xylose, citrate utilization and negative reactions for oxidase, DNase, KCN, phenylalanine deaminase and Voges-Proskauer tests (Ogunsanya et al., 1994). The determination of pathogenic *E coli* isolates was done by amplifying the corresponding virulence gene markers. Genes coding for Necrotoxigenic *E. coli* (NEC), Enterotoxigenic *E. coli* (ETEC), shiga-like toxin producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC) and Enteroaggregative *E. coli* (EAEC) were sought as previously described (Matar et al., 2002).

Salmonella isolation and identification

Stool specimen was streaked on Bismuth sulphite agar and incubated for 48h at 37°C. Black colonies with 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 Potassium cyanide (KCN), ONPG and
fermentation of adonitol, sucrose, lactose, raffinose and salicin (Simango et al., 1992).

Shigella isolation and identification

Samples were cultured on Xylose-lysine desoxycholate agar (XLDC) for 24h at 37°C. Transparent colonies suggestive of Shigella were screened for negative reactions for motility, adonitol, citrate, DNase, gas from glucose, \( \text{H}_2\text{S} \), lysine, phenylalanine, sucrose, urease, Voges-Proskauer, inositol, KCN, lactose, malonate, salicin and xylose (Obi et al., 1998; Nachamkim, 1999).

Vibrio cholerae isolation and identification

About 2 ml of faeces was inoculated in 20 ml of alkaliine peptone water pH 8.6 (APW) and incubated 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) (Alabi and Odugbemi, 1990; Ogunsanya et al., 1994).

Yersinia enterocolitica isolation and identification

Samples were cultured on Yersinia agar enriched with Yersinia selective supplement SR109 (Oxoid) (Simango et al., 1992) and incubated at 37°C for 24h. Presumptive colonies of Y. enterocolitica were characterized by positive tests for ornithine carboxylase and sucrose fermentation, and negative reactions for raffinose, rhamnose and melibiose fermentation (Alabi and Odugbeni, 1990).

2.2.5. Identification of rotaviruses

A commercially available ELISA kit (Dako, Denmark) was employed for the screening of stool specimens for the detection of the presence of group A
human rotaviruses, as recommended by the manufacturer and as previously described (El-Sheikh and El-Assouli, 2001). Briefly, each of the faecal specimens was diluted (10%) in distilled water and centrifuged at 50°C for 30 minutes. The supernatant was kept at 4°C and used for further tests.

2.2.5.1. Extraction of rotavirus RNA

RNA was extracted from specimens positive for rotaviruses as previously reported (EL-Assouli et al., 1995; El-sheikh and El-Assouli, 2001). Briefly, the rotavirus double stranded RNA (dsRNA) was extracted from the faecal suspension using phenol-chloroform mixture followed by ethanol precipitation. Genomic double stranded RNAs were subjected to electrophoresis on a 10% polyacrylamide slab gels with a 3.5% stacking gel to enhance resolution of the gel. Electrophoresis was performed overnight at 100 volts and silver stained in order to visualize the RNA bands.

2.2.5.2. VP4 and VP7 typing of rotavirus RNA

Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on the specimens using con2 and con3 primers for VP4 (gene4) and Beg9 and End9 primers for VP7 (gene 9) while type specific primers were used for typing G and P. Amplified products were analysed by gel electrophoresis in 2% agarose gels (Seakem) with ethidium bromide at 100 volts. A 100 bp DNA ladder (G2101, Promega, England) was employed as the marker (Gentsch et al., 1992; Gouvea et al., 1990, 1994).

2.2.6. Antibiogram determination

Antibiotic susceptibility testing of bacterial isolates was determined on Mueller-Hinton agar using the Kirby – Bauer disk diffusion method (Bauer et al., 1966; Obi et al., 1998). Antibiotics employed were commercially obtained from Davies Diagnostics (South Africa) and included ampicillin (10ug), amikacin (30ug), gentamicin (10ug), tetracycline (30ug), ciprofloxacin (10ug), ceftriazone (30ug), chloramphenicol (30ug), erythromycin (15ug) and nalidixic acid (30ug).
2.3. RESULTS

2.3.1. Isolation rates of pathogens

The rates of isolation of potentially pathogenic organisms from stools of patients with diarrhoea are presented in Figure 2.1. The most prevalent bacterial isolates from diarrhoeic patients were *Escherichia coli* 81 (20%). The pathogenic strains comprised of 20 (25%) necrooxigenic *Escherichia coli* (NEC) 10 (12.5%) each of enterotoxigenic *E.coli* (ETEC), and Shiga-like toxin producing *Escherichia coli* (STEC); 30 (37%) of enteropathogenic *Escherichia coli* (EPEC) and 11 (13.6%) enteroaggregate *Escherichia coli* (EAEC). Other isolates comprised *Campylobacter jejuni* 55 (13.7%), *Campylobacter coli* 24 (6.0%), *Salmonella* 58 (14.5%), *Shigella* 50 (12.5%), *Plesiomonas shigelloides* 43 (10.7%) whereas the least isolation rate was recorded for *Vibrio cholerae* 15 (3.7%). Rotavirus was detected in 80 (26.7%) of the 300 stools specimens analysed.

2.3.2. Age distribution of pathogens

Age distribution of isolates revealed greater prevalence of enteric bacteria and rotaviruses in the 0-5 years age group when compared with the patterns in older age groups (11-20 years and >20 years) (Figure 2.2). A value of more than 50% of the isolation rates of *Campylobacter jejuni, Campylobacter coli, Plesiomonas shigelloides* and *Vibrio cholerae* were recorded in the 0-5 years age group whereas the isolation in the older age groups (6-10, 11-20 and >20 years) ranged from 6.7% to 25% and this was of statistical significance (p<0.05). Out of a total of 300 stool specimens examined for the presence of rotaviruses, 30 (37.5%) of the positive samples were detected in the age 0-5 years age group whereas 12 (15%), 23 (28.7%) and 15 (18.8%) were detected in the age groups 6-10, 11-20 and >20 years respectively (Figure 2.2).
2.3.3. Seasonal variations in the isolation of pathogens

Detection of bacterial enteropathogens was seen throughout the year but showed a higher tendency in the months of summer in comparison with observations for other seasons (p<0.05). The detection of rotaviruses was noted to be significantly higher in the winter season (p<0.05)(Figure 2.3).

2.3.4. Serotypes and electropherotypes of rotaviruses

The occurrence of human rotaviruses VP4, VP7 and electropherotypes among the 80 positive samples are presented in Table 2.1. For the VP4, 35 (43.75%) were of the P6 and P8 genotypes respectively and 10 (12.5%) were dual P6 + P8 infections. The VP7 serotyping indicated that all positive rotavirus samples (100%) belonged to the G1 group. All positive samples, (100%) were electropherotyped and 78 (97.5%) were identified as long electropherotypes whereas only 2 (2.5%) were short electropherotypes (Table 2.1).

2.3.5. Antibiotic susceptibility profiles

Antibiotic susceptibility profiles of the various bacterial isolates from stool specimens presented in Table 2.2 showed that the majority of all the enteric bacterial isolates (over 85%) were sensitive to gentamicin, ciprofloxacin and amikacin. In addition, over 90% of C. jejuni, E. coli, Salmonella isolates and P. shigelloides were sensitive to nalidixic acid. All (100%) of Salmonella isolates were sensitive to gentamicin and amikacin and all (100%) of the Plesiomonas shigelloides isolates were sensitive to ciprofloxacin. At least 88% of Plesiomonas shigelloides, Shigella isolates, Salmonella isolates and E. coli isolates were sensitive to ceftriazone. Multi-resistance of E. coli, Vibrio cholerae, P. shigelloides, Aeromonas isolates and Campylobacter jejuni/coli to tetracycline, ampicillin, erythromycin and chloramphenicol were noted (Table 2.2).
Figure 2.1: Frequency of isolation of potential enteric pathogens from stool specimens of patients with diarrhoea in the Venda region

Figure 2.2: Age distribution of enteric organisms isolated from patients with diarrhoea in the Venda region
Table 2.1 Occurrence of Rotavirus VP4, VP7 and electropherotypes in diarrhoeal stool samples

<table>
<thead>
<tr>
<th>Stool samples</th>
<th>Specimen distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of stool specimens</td>
<td>Positive = 80 (26.7%)</td>
</tr>
<tr>
<td></td>
<td>Negative = 220 (73.3%)</td>
</tr>
<tr>
<td></td>
<td>n = 300</td>
</tr>
<tr>
<td>P-genotypes (VP4)</td>
<td>P6 = 35 (43.75%)</td>
</tr>
<tr>
<td></td>
<td>P8 = 35 (43.75%)</td>
</tr>
<tr>
<td></td>
<td>P6 + P8 (mixed) = 10 (12.5%)</td>
</tr>
<tr>
<td>G-serotypes (VP7)</td>
<td>G1 = 80 (100%)</td>
</tr>
<tr>
<td>Electrophoretypes</td>
<td>Long electrophoretypes = 78 (97.5%)</td>
</tr>
<tr>
<td></td>
<td>Short electrophoretypes = 2 (2.5%)</td>
</tr>
</tbody>
</table>

Figure 2.3: Seasonal distribution of enteropathogens isolated from diarrhoeal cases in the Venda Region
Table 2.2  Antibiotic susceptibilities of enteric bacterial pathogens from stool specimens of patients with diarrhoea

<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>(n=55) Campylobacter jejuni</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></td>
<td>(93%)</td>
<td>(93%)</td>
<td>(75%)</td>
<td>(95%)</td>
<td>(73%)</td>
<td>(60%)</td>
<td>(69%)</td>
<td>(60%)</td>
<td>(98%)</td>
<td>(65%)</td>
</tr>
<tr>
<td>(n=24) Campylobacter coli</td>
<td>18</td>
<td>21</td>
<td>16</td>
<td>21</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>13</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>(75%)</td>
<td>(88%)</td>
<td>(67%)</td>
<td>(88%)</td>
<td>(63%)</td>
<td>(54%)</td>
<td>(58%)</td>
<td>(54%)</td>
<td>(92%)</td>
<td>(67%)</td>
</tr>
<tr>
<td>(n=101) Esherichia coli</td>
<td>94</td>
<td>95</td>
<td>73</td>
<td>97</td>
<td>69</td>
<td>44</td>
<td>54</td>
<td>43</td>
<td>99</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>(93%)</td>
<td>(94%)</td>
<td>(72%)</td>
<td>(96%)</td>
<td>(68%)</td>
<td>(44%)</td>
<td>(53%)</td>
<td>(43%)</td>
<td>(98%)</td>
<td>(90%)</td>
</tr>
<tr>
<td>(n=58) Salmonella isolates</td>
<td>55</td>
<td>58</td>
<td>40</td>
<td>56</td>
<td>29</td>
<td>29</td>
<td>43</td>
<td>45</td>
<td>58</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>(95%)</td>
<td>(100%)</td>
<td>(68%)</td>
<td>(97%)</td>
<td>(50%)</td>
<td>(50%)</td>
<td>(74%)</td>
<td>(78%)</td>
<td>(100%)</td>
<td>(95%)</td>
</tr>
<tr>
<td>(n=50) Shigella isolates</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></td>
<td>(84%)</td>
<td>(94%)</td>
<td>(62%)</td>
<td>(90%)</td>
<td>(50%)</td>
<td>(46%)</td>
<td>ND</td>
<td>ND</td>
<td>(98%)</td>
<td>(96%)</td>
</tr>
<tr>
<td>(n=43) Plesiomonas shigelloides</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></td>
<td>(93%)</td>
<td>(86%)</td>
<td>(69%)</td>
<td>(100%)</td>
<td>(63%)</td>
<td>(42%)</td>
<td>(60%)</td>
<td>(98%)</td>
<td>(96%)</td>
<td>(88%)</td>
</tr>
<tr>
<td>(n=21) Yersinia isolates</td>
<td>17</td>
<td>18</td>
<td>11</td>
<td>18</td>
<td>13</td>
<td>10</td>
<td>ND</td>
<td>9</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>(81%)</td>
<td>(86%)</td>
<td>(52%)</td>
<td>(86%)</td>
<td>(70%)</td>
<td>(48%)</td>
<td>ND</td>
<td>9</td>
<td>(43%)</td>
<td>(86%)</td>
</tr>
<tr>
<td>(n=34) Aeromonas isolates</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></td>
<td>(88%)</td>
<td>(88%)</td>
<td>(76%)</td>
<td>(94%)</td>
<td>(76%)</td>
<td>(65%)</td>
<td>(41%)</td>
<td>(53%)</td>
<td>(94%)</td>
<td>(82%)</td>
</tr>
<tr>
<td>(n=15) Vibrio cholerae</td>
<td>10</td>
<td>6</td>
<td>11</td>
<td>13</td>
<td>7</td>
<td>ND</td>
<td>10</td>
<td>6</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>(67%)</td>
<td>(40%)</td>
<td>(73%)</td>
<td>(87%)</td>
<td>(47%)</td>
<td>ND</td>
<td>(67%)</td>
<td>(40%)</td>
<td>(87%)</td>
<td>(73%)</td>
</tr>
</tbody>
</table>

NA = Nalidixic acid  GM = Gentamicin  ERY = Erythromycin  TE = Tetracycline  COT = Cotrimoxazole  CHL = Chloramphenicol
CIP = Ciprofloxacin  AKC = Amikacin  AP = Ampicillin  CEF = Ceftriazone  ND = not done
C. jejuni = Campylobacter jejuni  C. coli = Campylobacter coli
P. shigelloides = Plesiomonas shigelloides  E. coli = Escherichia coli
2.4. DISCUSSION

This study was undertaken to ascertain the prevalence of enteric bacterial pathogens and rotavirus strains in diarrhoeal cases in the Venda region. In addition, antibiograms of bacterial isolates were determined to serve as a guide to clinicians for emperic use of antibiotics in the management of diarrhoea cases requiring antibiotic therapy.

The most common bacterial pathogen isolated was *Escherichia coli* (20.2%), comprising NEC, EPEC, STEC, ETEC and EAEC, followed by *Campylobacter* species (*C. jejuni* and *C. coli*), together constituting 19.7%, *Salmonella* isolates (14.5%) and *Shigella* isolates (12.5%). The substantiality of the roles of these bacterial pathogens in diarrhoeal cases is consistent with reports of other investigators (Sethi and Khuffash, 1989; Na'was and Aboshehada, 1991; Obi et al., 1997; El-Sheikh and El-Assouli, 2001). *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 (Sethi and Khuffash, 1989; Na'was and Abo-Shehada 1991; El-Sheikh and El-Assouli, 2001).

Other bacterial diarrhoeagenic agents identified in this study were *Plesiomonas shigelloides*, *Aeromonas*, *Yersinia* and *Vibrio cholerae*. *Aeromonas* and *Plesiomonas shigelloides* were reportedly incriminated in cases of diarrhoea, with a greater prevalence in rural communities (Obi et al., 1995). In a previous study in Nigeria, prevalence rates of 5% for *P. shigelloides* and 9.9% for *Aeromonas* species were reported (Obi et al., 1995). 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 reports (Prado and O’Ryan, 1994; Du Pont, 1995). Detection of bacterial enteropathogens occurred more frequently in summer compared to other seasons (p<0.05). This observation is consistent with previous reports in Nepal (Ono et al., 2001), India (Niyogi et al., 1994), Chile (Levine et al., 1993), South Africa (Househam et al., 1998), and Hong Kong (Ho and Wang, 1985).
Besides bacterial agents of diarrhoea, rotaviruses were detected in 80 (26.7%) of 300 diarrhoeic stool samples screened. This trend is consistent with previous reports incriminating rotaviruses as a major cause of diarrhoea, particularly in young children (Ford-Jones et al., 1990; El-Sheikh and El-Assouli, 2001). The predominance of rotaviruses in children less than 5 years of age is also in harmony with hospital based data across the globe (WHO, 1980, Grabow, 1996). The detection of rotavirus was noted to be significantly higher in the winter season (p<0.05). Ono et al., (2001) and Gomes et al., (1991) also reported that detection of rotaviruses was significantly higher in the winter season. 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 the short ones has been reported by other studies (Lourenco et al., 1981; Nakagomi et al., 1988). Serotype analysis of the VP7 revealed that all the rotavirus isolates were G1 serotypes which is consistent with other studies carried out in South Africa (Potgieter et al., 2004). The results also confirm other studies on the prevalence of diverse rotavirus strains present in different regions of the world (Nakagomi et al., 1988; White et al, 1991; Potgieter et al., 2004).

The prevalence of a wide range of bacterial pathogens and rotaviruses in diarrhoeal cases remains a significant threat to the health of communities in the Venda region. The health threat is further compounded by lack of potable water supply, poverty, poor sanitary and hygienic practices. In this region, most of the rural households rely mainly on river, stream, fountain and borehole water sources for their daily water needs and these water sources are usually faecally contaminated and devoid of treatment (WHO, 1993). A previous study carried out by Obi et al., (2002), reported that several river water sources harboured diarrhoeagenic pathogens, were unsafe for consumption, microbiologically unacceptable and likely to be potential sources of transmission of water-borne diseases to humans.

One method that is employed in the management of patients with bacterial water-borne or diarrhoeal diseases is by the administration of antibiotics (Black, 1993). The results of this study suggest that the broad spectrum of activity of
ciprofloxacin, gentamicin, amikacin and nalidixic acid may indicate their usefulness in the management of diarrhoea requiring antibiotic therapy. The susceptibility of bacterial pathogens to the aforementioned antibiotics is in harmony with previous reports. (Obi et al., 1995; Galane and Le Roux, 2001).

Periodic surveys of antibiograms of bacterial agents of diarrhoea is recommended in order to unravel trends in antibiotic resistance and to provide updated guidelines for the management of diarrhoea requiring antibiotics.

2.5. REFERENCES


CHAPTER 3

CONCLUSIONS

3.1. Assessment of the microbial quality of water sources

- The minimum and maximum counts with regard to all the sampling points investigated for bacterial indicators (heterotrophic plate counts, total coliforms, faecal coliforms, faecal enterococci) and somatic coliphages exceeded the acceptable recommended targets of up to two orders of magnitude for drinking water prescribed by the Department of Water Affairs and Forestry (DWAF, 1998).

- The high prevalence of indicator organisms of faecal pollution and pathogenic bacteria in the water sources used by rural communities in the Venda region, indicated a potential health risk to consumers.

3.2. Bacterial enteropathogens and rotaviruses in diarrhoeal cases

- Results of this study revealed *Escherichia coli* as the most common enteric bacteria isolated from stool specimens of patients with diarrhoea whereas the least isolation rate was recorded for *Vibrio cholerae*.

- Results also revealed a large percentage of rotaviruses in diarrhoea stool samples, among the study population. Thirty (37.5%) of the positive samples were detected in the age 0-5 years age group whereas 12 (15%), 23 (28.7%) and 15 (18.8%) were detected in the age groups 6-10, 11-20 and >20 years respectively.

- Bacterial agents of diarrhoea and rotaviruses were more frequently isolated from children less than 5 years than in the older age groups (6-10, 11-20 and >20 years) and this was of statistical significance (p<0.05).
• Bacterial enteropathogens were more prevalent during the summer season whereas detection of rotaviruses was higher in the winter season. The significant detection of rotaviruses in winter is of importance in vaccination strategies.

• This investigation also indicated that all rotavirus isolates were G1 serotype specific for VP7. This is important for future vaccine development.

• The long electrophoretypes were significantly more predominant than the short electropherotypes among rotaviruses detected in this study. This is consistent with the G1 serotype found.

• Antibiotic profiles showed that the majority of enteric bacterial isolates were sensitive to gentamicin, ciprofloxacin and amikacin.

• Strains of *Escherichia coli*, *Vibrio cholerae*, *Campylobacter jejuni/coli*, *Plesiomonas shigelloides* and *Aeromonas* were resistant to tetracycline, ampicillin, erythromycin and chloramphenicol.