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

Report to the Water Research Commission

by

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TABLE OF CONTENTS

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 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 V*ibrio 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 1.0×10^1 cfu.ml^{-1} and 1.3×10^6 cfu.ml^{-1} for heterotrophic plate counts, between 0 cfu.ml^{-1} and 1.0×10^6 cfu.ml^{-1} for total and faecal coliforms, between 0 cfu.ml^{-1} and 5.1×10^4 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: 1.0×10^2 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 stake holders

- 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 J Mulaudzi (2002) The incidence of diarrhoeal pathogens in stool samples from infants in Venda.

- 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.

- Miss MGF Mashau (2003) The use of coliphages in monitoring water quality and tracing sources of microbial contamination.

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

10. TECHNOLOGY TRANSFER

10.1. PUBLISHED ARTICLES

Obi CL, Potgieter N, Bessong PO and Matsaung G (2002) Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. *Water SA* **28** (3) 287-292.

Obi CL, Potgieter N, Bessong PO and Matsaung G (2003) Scope of potential bacterial agents of diarrhoea and microbial assessment of quality of river water sources in rural Venda communities in South Africa. *Water Science and Technology* **47** (3) 59-64.

Obi CL, Green E, Bessong PO, De Villiers B, Hoosen AA, Igumbor EO and Potgieter N (2004) Gene encoding virulence markers among *Escherichia coli* isolates from diarrhoic stool samples and river sources in rural Venda communities of South Africa. *Water SA* **30** (1) 37-42.

Obi CL, Potgieter N, Bessong PO, Igumbor EO and Green E (2004) Prevalence of pathogenic bacteria and rotaviruses in stools of patients presenting with diarrhoea from Venda rural communities, South Africa. *South African Journal of Science* (In press).

Obi CL, Bessong PO, Momba MNB, Potgieter N and Igumbor EO (2004) Profiles of antibiotic susceptibilities of water isolates and physico-chemical quality of water supply in rural venda communities, South Africa. *Water SA* (In press).

10.2. PAPERS IN PREPARATION FOR PUBLICATION

Obi CL, Potgieter N, Musie ME, Bessong PO, Sammi A and Venter SN (2004) Molecular epidemiology of *Salmonella* isolates from clinical and environmental samples. To be submitted to *Water Research*.

Potgieter N, Obi CL, Mashau MGF, Bessong PO, Sammi A and Igumbor EO (2004) The use of coliphages in assessing water quality and tracing sources of microbial contamination. To be submitted to *Water SA*.

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

Obi CL, Potgieter N, Maswime E, Green E, Sono R and Bessong PO (2001) Incidence of enteric bacterial pathogens in water sources and stools of residents in rural communities in the Venda region of South Africa. Poster presentation at the International Water Association, (IWA) 2nd World Water Congress, Berlin, Germany, 15 - 19 October, 2001.

Obi CL, Green E, Bessong PO, De Villiers B, Hoosen AA, Igumbor EO and Potgieter N (2003) Gene encoding virulence markers among *Escherichia coli* isolates from diarrhoic stool samples and river sources in rural Venda

communities of South Africa. Oral presentation at the International Water Association (IWA), World Water Congress, Cape Town, South Africa, 14 - 19 September, 2003.

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.

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Prof WOK Grabow	University of Pretoria
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LIST OF ABBREVIATIONS

A. hydrophilia	:	*Aeromonas hydrophilia*
AKC	:	Amikacin
AP	:	Ampicillin
APN	:	alkaline peptone water
C. jejuni	:	*Campylobacter jejuni*
C. coli	:	*Campylobacter coli*
CEF	:	Ceftriazone
CHL	:	Chloramphenicol
COT	:	Cotrimoxazole
CFU	:	Colony forming unit
DCA	:	Deoxycholate citrate agar
ds RNA	:	double stranded ribonucleic acid
DWAF	:	Department of Water Affairs and Forestry
E. coli	:	*Escherichia coli*
ELISA	:	Enzyme linked immunosorbent assay
ERY	:	Erythromycin
FC	:	Faecal coliforms
GM	:	Gentamicin
h	:	hour
HPC	:	Heterotrophic plate count
MAB	:	monoclonal antibody
MAX	:	maximum
MHA	:	Muellar – Hinton Agar
MIN	:	minimum
min	:	minute
NA	:	Nalidixic acid
NaCl	:	Sodium Chloride
ND	:	Not Done
Nl	:	Nitrofurantoin
P	:	Protease Sensitive Protein

PBS	:	Phosphate buffered saline
PCR	:	Polymerase Chain Reaction
PFU	:	Plaque forming unit
P. shigelloides	:	*Plesiomonas shigelloides*
RDP	:	Reconstruction and Development Programme
RT-PCR	:	Reverse Transcriptase Polymerase Chain Reaction
SD	:	Standard Deviation
SF	:	Selenite F-broth
SS Agar	:	Salmonella – Shigella agar
TC	:	Total coliforms
TCBS	:	Thiocitrate bile salt
TE	:	Tetracycline
VP4	:	Virion’s outer capsid spike protein
VP7	:	Virion’s outer capsid viral glycoprotein
WRC	:	Water Research Commission
XDCA	:	Xylose Deoxycholate Citrate agar.

LIST OF TABLES

LIST OF FIGURES

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 my 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 H_2S 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 37ºC. Colonies suggestive of *Shigella* were screened for negative reactions for motility, adonitol, citrate, DNase, gas from glucose, H_2S, 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 37ºC. A loopful from the surface of the APW culture was sub-cultured on Thiosulphate Citrate Bile Salt Sucrose agar (TCBS) and incubated overnight at 37ºC. 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 37ºC 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).

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.

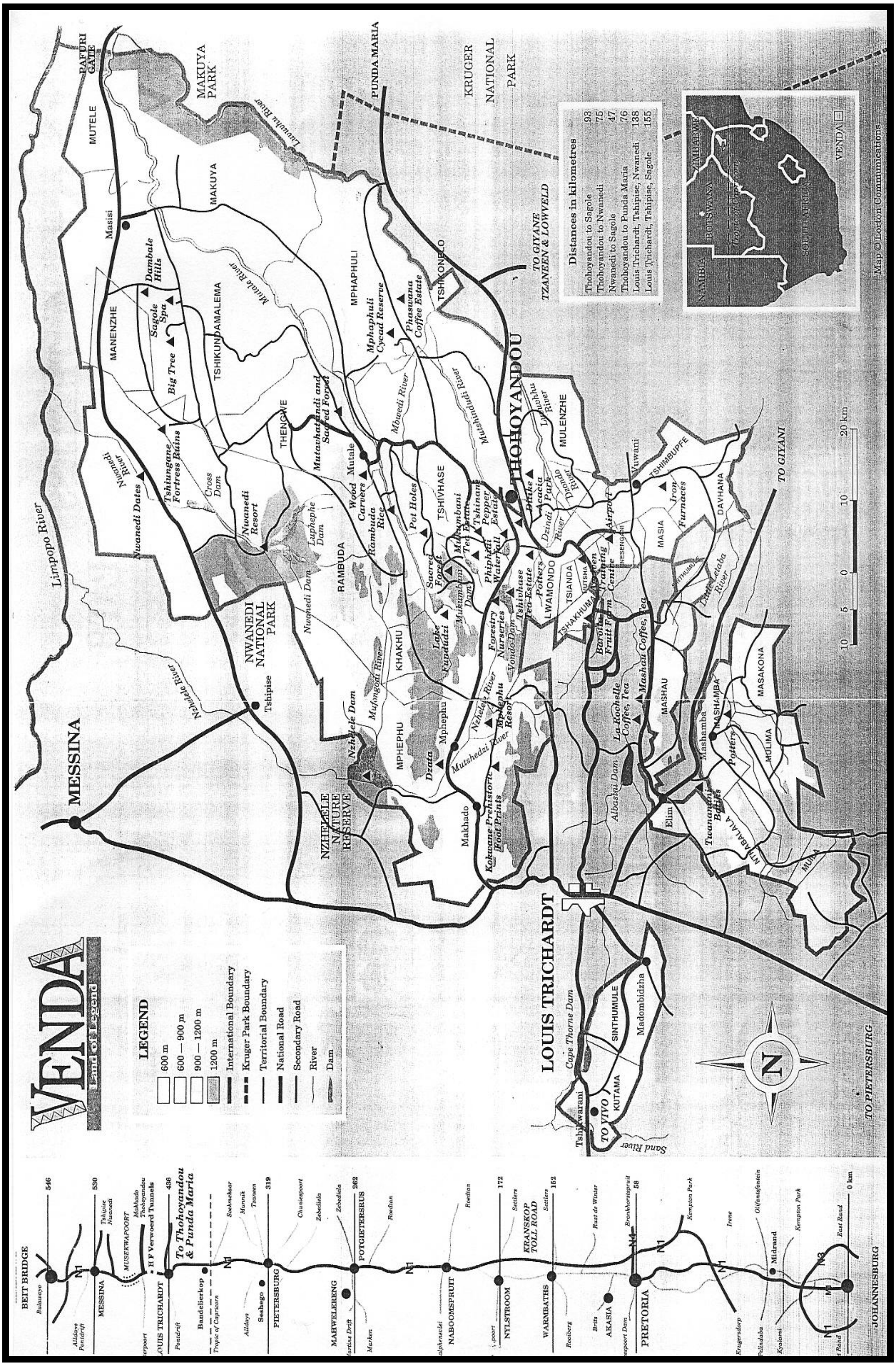

Figure 1.1: Map showing the Venda region in the Limpopo Province

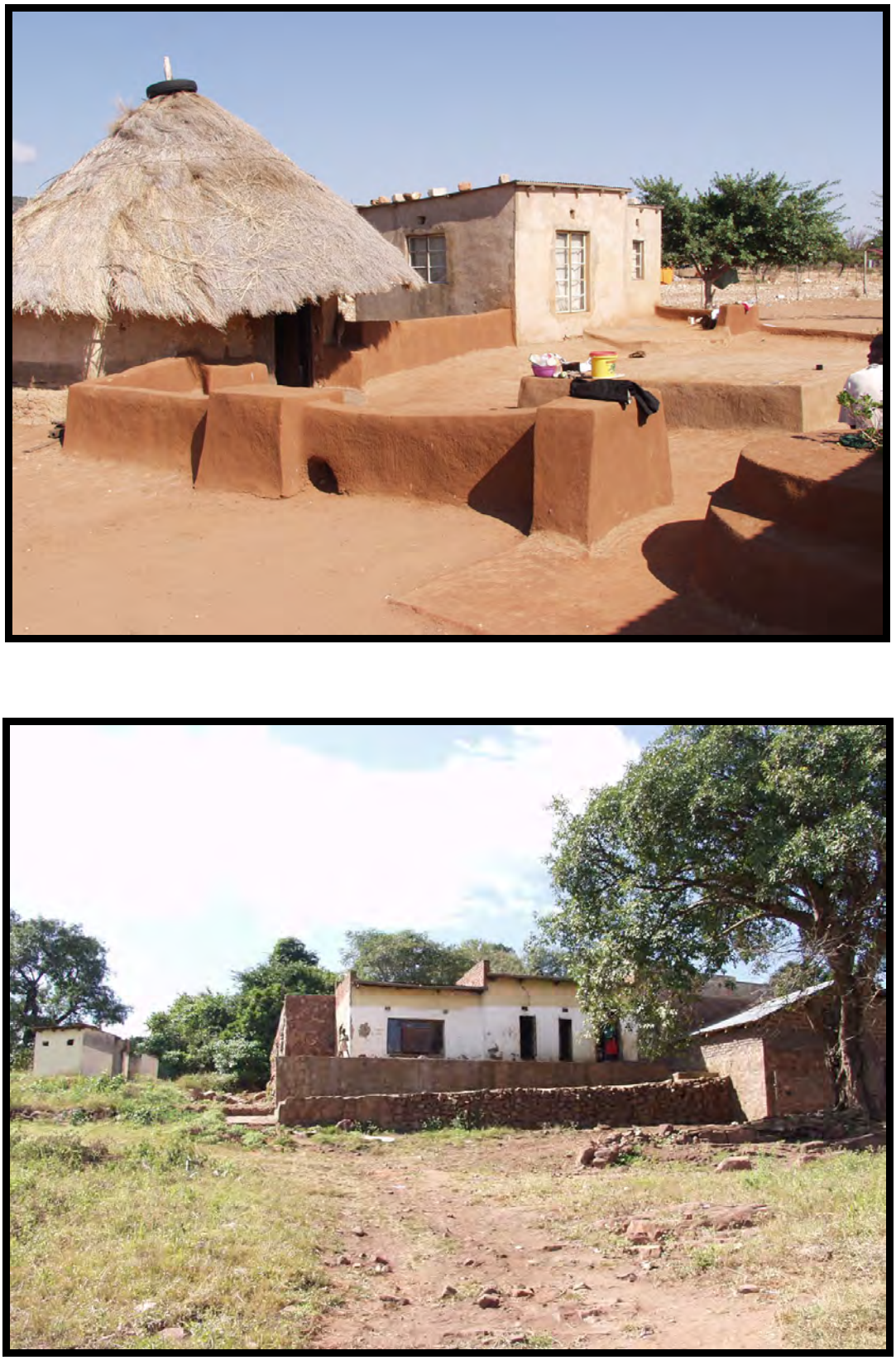

Figure 1.2: Typical households found in rural Venda communities

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 x 10^3 and 1.3 x 10^6 cfu.ml^{-1} for Masetoni point, between 5.0 x 10^3 and 3.1 x 10^4 cfu.ml^{-1} for Mhinga point, between 7.7 x 10^3 and 2.6 x 10^4 cfu.ml^{-1} for Dididi point, between 9.6 x 10^2 and 1.4 x 10^4 cfu.ml^{-1} for Tshikonelo point, between 1.8 x 10^2 and 2.0 x 10^3 cfu.ml^{-1} for Grootpad point, between 1.0 x 10^3 and 3.0 x 10^3 cfu.ml^{-1} for Mutoti point and between 7.0 x 10^3 and 2.7 x 10^5 cfu.ml^{-1} for the Vuwani point. For the other rivers, the counts ranged between 1.0 x 10^3 and 3.0 x 10^4 cfu.ml^{-1} for Mutale River, between 6.2 x 10^3 and 7.9 x 10^4 cfu.ml^{-1} for Ngwedi river, between 1.9 x 10^2 and 1.7 x 10^3 cfu.ml^{-1} for Tshinane River and between 1.9 x 10^2 and 1.7 x 10^3 cfu.ml^{-1} for Mudaswali River (Table 1.1.) The counts for the Mudaswali fountain ranged between 1.8 x 10^1 and 1.9 x 10^2 cfu.ml^{-1} and between 1.0 x 10^1 and 1.4 x 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 x 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 x 10^2 and 7.6 x 10^3 cfu.100ml^{-1} for Masetoni point, between 8.9 x 10^2 and 2.3 x 10^4 cfu.100ml^{-1} for Mhinga point, between 4.9 x 10^3 and 1.5 x10^4 cfu.100ml^{-1} for Dididi point, between1.1 x 10^3 and 1.8 x 10^3 cfu.100ml^{-1} for Tshikonelo point, between 1.3 x 10^4 and 2.1 x 10^4 cfu.100ml^{-1} for Grootpad point, between and 9.2 x 10^2 and 1.5 x 10^3 cfu.100ml^{-1} for Mutoti point and between 7.3 x 10^3 and 1.8 x 10^4 cfu.100ml^{-1} for Vuwani point. The minimum and maximum counts for the other rivers respectively ranged between 9.2 x 10^3 and 1.9 x 10^4 cfu.100ml^{-1} for Mutale River, between 2.0 x 10^4 and 3.4 x 10^4 cfu.100ml^{-1} for Tshinane River, between 2.8 x 10^3 and 3.7 x 10^4 cfu.100ml^{-1} for Ngwedi river and between 0.3 x 10^1 and 1.0 x 10^6 cfu.100ml^{-1} for Mudaswali river (Table 1.1). The counts for the Mudaswali fountain ranged between 0 and 1.24 x 10^4 cfu.100ml^{-1} and between 1.3 x 10^1

and 8.3 x 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 x 10^3 and 6.3 x 10^4 cfu.100 ml^{-1} for Masetoni point, between 5.2 x 10^3 and 1.72 x 10^4 cfu.100 ml^{-1} for Mhinga point, between 9.0 x 10^2 and 1.5 x 10^3 cfu.100 ml^{-1} for Tshikonelo point, between 6.1 x 10^3 and 1.2 x 10^4 cfu.100 ml^{-1} for Grootpad point, between 5.6 x 10^3 and 7.2 x 10^3 cfu.100ml^{-1} for Mutoti point, between 4.1 x 10^2 and 7.5 x 10^2 cfu.100 ml^{-1} for Dididi point, and between 2.9 x 10^2 and 1.1 x 10^4 cfu.100 ml^{-1} for Vuwani point. The minimum and maximum faecal coliform counts for the other rivers ranged between 5.6 x 10^3 and 2.0 x 10^3 cfu.100 ml^{-1} for Mutale River, between 7.4 x 10^2 and 3.9 x 10^3 cfu.100 ml^{-1} for Tshinane River, between 1.8 x 10^2 and 8.2 x 10^2 cfu.100 ml^{-1} for Ngwedi river and between 0.1 x 10^1 and 1.0 x 10^6 cfu.100 ml^{-1} for Mudaswali river (Table 1.1). The counts for the Mudaswali fountain ranged between 0 and 3.5 x 10^4 cfu.100ml^{-1} and between 0.4 x 10^1 and 7.4 x 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 x 10^3 and 5.5 x 10^3 cfu.100 ml^{-1} for Masetoni point, between 5.0 x 10^2 and 2.3 x 10^3 cfu.100 ml^{-1} for Mhinga point, between 1.2 x 10^3 and 3.1 x 10^3 cfu.100 ml^{-1} for Tshikonela point, between 1.0 x 10^3 and 1.0 x 10^4 cfu.100 ml^{-1} for Dididi point, between 4.0 x 10^3 and 2.1 x 10^4 cfu.100 ml^{-1} for Grootpad point, between 1.9 x 10^3 and 2.5 x 10^4 cfu.100 ml^{-1} for Mutoti point and between 1.0 x 10^1 and 5.1 x 10^2 cfu.100 ml^{-1} for Vuwani point. Enterococci counts for the other rivers ranged between 1.9 x 10^2 and 2.1 x 10^3 cfu.100 ml^{-1} for Mutale River, between 0 and 6.4 x 10^1 cfu.100 ml^{-1} for Mudaswali River, between 4.0 x 10^1 and 3.2 x 10^2 cfu.100 ml^{-1} for Tshinane River and between 6.6 x 10^3 and 2.2 x 10^4 cfu.100 ml^{-1}

for Ngwedi River (Table 1.1). Enterococci counts for the Mudaswali fountain ranged between 0 and 1.12 x 10^4 cfu.100 ml^{-1} and between 0 and 8.3 x 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, ceftriazone and gentamicin respectively and *Campylobacter* species with 62% susceptibility to ceftriazone.

Majority of bacterial isolates demonstrated multiple antibiotic resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphericol. 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.

Water Sources	Heterotrophic bacteria (cfu. ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	Somatic Coliphages (pfu.100 ml^{-1})
Levubu River : Masetoni point	Min : 6.0×10^3 Max : 1.3×10^6 Mean : 6.5×10^5 SD : 3.5×10^5	Min : 6.0×10^2 Max : 7.6×10^3 Mean : 3.2×10^3 SD : 8.0×10^2	Min : 1.5×10^3 Max : 6.3×10^4 Mean : 3.3×10^3 SD : 2.4×10^3	Min : 2.0×10^3 Max : 5.5×10^3 Mean : $3{,}75 \times 10^3$ SD : 1.5×10^2	Min : 0 Max : 7 Mean : 1.77 SD : 2.60
Levubu River : Mhinga point	Min : 5.0×10^3 Max : 3.1×10^4 Mean : 1.8×10^4 SD : 1.3×10^4	Min : 8.9×10^2 Max : 2.3×10^4 Mean : 1.6×10^3 SD : 7.2×10^2	Min : 5.2×10^3 Max : 1.72×10^4 Mean : 1.12×10^3 SD : 6.5×10^4	Min : 5.0×10^2 Max : 2.3×10^3 Mean : 1.4×10^3 SD : 9.0×10^2	Min : 0 Max : 154 Mean : 20.4 SD : 42.1
Levubu River : Dididi point	Min : 7.7×10^3 Max : 2.6×10^4 Mean : 1.7×10^4 SD : 1.0×10^4	Min :4.9×10^3 Max : 1.5×10^4 Mean : 9.95×10^3 SD : 5.0×10^2	Min : 4.1×10^2 Max : 7.5×10^2 Mean : 5.8×10^2 SD : 1.8×10^2	Min : 1.0×10^3 Max :1.0×10^4 Mean : 5.5×10^3 SD : 5.5×10^2	Min : 0 Max : 3 Mean : 0.88 SD : 1.05
Levubu River : Tshikonela point	Min : 9.6×10^2 Max : 1.4×10^4 Mean : 7.5×10^3 SD : 2.3×10^3	Min : 1.1×10^3 Max : 1.8×10^3 Mean : 1.5×10^3 SD : 4.3×10^2	Min : 9.0×10^2 Max : 1.5×10^3 Mean : 1.2×10^3 SD : 3.3×10^2	Min : 1.2×10^3 Max : 3.1×10^3 Mean : 2.2×10^3 SD : 1.0×10^2	Min : 0 Max : 7 Mean : 1.77 SD : 2.60
Levubu River : Grootpad point	Min : 1.8×10^2 Max : 2.0×10^3 Mean : 1.1×10^3 SD : 2.8×10^2	Min : 1.3×10^4 Max : 2.1×10^4 Mean : 1.7×10^4 SD : 4.3×10^2	Min : 6.1×10^3 Max : 1.2×10^4 Mean : 9.0×10^3 SD : 2.9×10^3	Min : 4.0×10^3 Max : 2.1×10^4 Mean : 1.3×10^4 SD : 8.6×10^3	Min : 0 Max : 12 Mean : 0.9 SD : 3.32
Levubu River : Mutoti point	Min : 1.0×10^3 Max : 3.0×10^3 Mean : 1.0×10^3 SD : 3.0×10^3	Min : 9.2×10^2 Max : 1.45×10^3 Mean : 5.0×10^3 SD : 1.91×10^4	Min : 5.6×10^3 Max : 7.2×10^3 Mean : 2.5×10^3 SD : 1.0×10^3	Min : 1.9×10^3 Max : 2.1×10^4 Mean : 3.4×10^3 SD : 2.5×10^4	Min : 0 Max : 3 Mean : 0.88 SD : 1.05
Levubu River: Vuwani point	Min : 7.0×10^3 Max : 2.7×10^5 Mean : 1.4×10^5 SD : 1.1×10^4	Min : 7.3×10^3 Max : 1.8×10^4 Mean : 1.3×10^4 SD : 6.4×10^3	Min : 2.9×10^2 Max : 1.1×10^4 Mean : 5.6×10^3 SD : 4.2×10^3	Min : 1.0×10^1 Max : 5.1×10^2 Mean : 2.6×10^1 SD : 2.5×10^1	Min : 0 Max : 5 Mean : 1.1 SD : 1.96
Mutale river	Min : 1.0×10^3 Max : 3.0×10^4 Mean : 1.6×10^4 SD : 1.4×10^3	Min : $9..2 \times 10^3$ Max : 1.9×10^4 Mean : 1.4×10^4 SD : 5.0×10^3	Min : 5.6×10^2 Max : 2.0×10^3 Mean : 1.3×10^3 SD : 2.5×10^2	Min : 1.9×10^2 Max : 2.5×10^3 Mean : 1.3×10^3 SD : 3.45×10^2	Min : 0 Max : 114 Mean : 20 SD : 37.2
Ngwedi River	Min : 6.2×10^3 Max : 7.9×10^4 Mean : $7.1x\ 10^4$ SD : 5.4×10^4	Min : 2.8×10^3 Max : 3.7×10^4 Mean : 2.0×10^4 SD : 2.1×10^3	Min : 1.8×10^1 Max : 8.2×10^2 Mean : 4.2×10^2 SD : 4.0×10^2	Min : 6.6×10^3 Max : 2.2×10^4 Mean : 1.4×10^4 SD : 1.9×10^3	Min : 0 Max : 154 Mean : 20.4 SD : 42.1
Tshinane River	Min : 1.9×10^2 Max : 1.7×10^3 Mean : 9.5×10^2 SD : 4.4×10^2	Min : 2.0×10^4 Max : 3.4×10^4 Mean : 2.7×10^4 SD : 2.6×10^3	Min : 7.4×10^2 Max : 3.9×10^3 Mean : 2.3×10^3 SD : 5.0×10^2	Min : 4.0×10^1 Max : 3.2×10^2 Mean : 1.8×10^2 SD : 1.5×10^2	Min : 0 Max : 210 Mean : 23.4 SD : 57.3
Mudaswali river	Min : 1.9×10^2 Max : 1.7×10^3 Mean : 9.5×10^2 SD : 4.4×10^2	Min : 0.3×10^1 Max : 1.0×10^6 Mean : 7.7×10^5 SD : 2.8×10^5	Min : $0.1x\ 10^1$ Max : 1.0×10^6 Mean : 7.7×10^5 SD : 2.8×10^5	Min : 0 Max : 6.4×10^1 Mean: 8.1×10^1 SD : 6.7×10^1	Min : 0 Max : 95 Mean : 13.8 SD : 31.9
Mudaswali fountain	Min : 1.8×10^1 Max : 1.9×10^2 Mean : 0.5×10^2 SD : 1.4×10^1	Min : 0 Max : 1.24×10^4 Mean : 2.47×10^3 SD : 2.64×10^1	Min : 0 Max : 3.7×10^4 Mean : 1.06×10^3 SD : 1.14×10^1	Min : 0 Max : 1.12×10^4 Mean : 1.58×10^3 SD : 2.48×10^1	Min : 0 Max : 12 Mean : 0.9 SD : 3.32
Makonde fountain	Min : 1.0×10^1 Max : 1.4×10^2 Mean : 1.0×10^2 SD : 0.8×10^1	Min : 1.3×10^1 Max : 8.3×10^1 Mean : 3.7×10^1 SD : 3.3×10^1	Min : 0.4×10^1 Max : 7.4×10^1 Mean : 2.6×10^1 SD : 2.5×10^1	Min : 0 Max : 8.3×10^1 Mean : 2.7×10^1 SD : 3.1×10^1	Min : 0 Max : 63 Mean : 5.4 SD : 17.35

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

Water sources	Enteric bacteria isolated from each source
Levubuvhu river: Vuwani point	*Escherichia coli* *Plesiomonas shigelloides* *Vibrio* isolates *Enterobacter cloacae* *Shigella* isolates *Salmonella* isolates
Levuvhu river: Tshikonela point	*Escherichia coli* *Enterobacter cloacae* *Vibrio* isolates
Levuvhu river: Masetoni point	*Escherichia coli* *Salmonella* isolates *Shigella* isolates *Enterobacter cloacae.*
Levuvhu river: Grootpad point	*Aeromonas hydrophila* *Aeromonas caviae* *Salmonella* isolates *Shigella* isolates *Vibrio* isolates
Levuvhu river: Dididi point	*Aeromonas hydrohila* *Aeromonas caviae* *Vibrio* isolates *Escherichia coli* *Shigella* isolates
Levuvhu river: Mhinga point	*Salmonella* isolates *Shigella* isolates *Aeromonas hydrophila* *Vibrio* isolates
Levuvhu river: Mutoti point	*Enterobacter cloacae* *Vibrio* isolates *Escherichia coli*
Mudaswali River	*Vibrio* isolates *Enterobacter cloacae* *Shigella* isolates *Salmonella* isolates
Mudaswali fountain	*Escherichia coli* *Shigella* isolates *Aeromonas caviae* *Vibrio* isolates
Tshinane River	*Escherichia coli* *Aeromonas* isolates *Campylobacter* isolates *Salmonella* isolates
Ngwedi River	*Escherichia coli* *Plesiomonas shigelloides* *Shigella* isolates *Campylobacter* isolates
Mutale River	*Shigella* isolates *Salmonella* isolates *Aeromonas* isolates
Makonde fountain	*Escherichia coli* *Shigella* isolates *Aeromonas hydrophila* *Aeromonas caviae* *Plesiomonas shigelloides* *Vibrio* isolates

Table 1.3 Antibiograms of enteric bacterial isolates from water sources in the Venda region, South Africa

ANTIBIOTIC SUSCEPTIBILITY NO (%)										
Bacteria	**NA**	**GM**	**COT**	**CIP**	**TE**	**AP**	**ERY**	**CHL**	**AKC**	**CEF**
***Campylobacter* isolates (n = 26)**	24 (92%)	23 (88%)	18 (69%)	25 (96%)	18 (69%)	15 (58%)	17 (65%)	16 (62%)	25(96%)	16 (62%)
***Escherichia coli* (n = 40)**	36 (90%)	37 (92.5%)	30 (75%)	38 (95%)	28 (70%)	20 (50%)	21 (52.5)	18 (45%)	39 (97.5%)	37 (92.5%)
***Salmonella* isolates (n = 30)**	28 (93%)	29 (96.6%)	20 (66.6%)	29 (96.6%)	14 (46.6%)	16 (53%)	24 (80%)	24 (80%)	30 (100%)	28 (93%)
***Shigella* isolates (n = 30)**	26 (86.6%)	28 (93%)	19 (63%)	28 (93%)	16 (53.3%)	15 (50%)	16 (53.5%)	18 (60%)	29 (96.6%)	28 (93%)
***Plesiomonas shigelloides* (n = 20)**	18 (90%)	18 (90%)	15 (75%)	19 (95%)	14 (70%)	13 (65%)	10 (50%)	11 (55%)	19 (95%)	18 (90%)
Aeromonas **(n = 20)**	18 (90%)	17 (85%)	14 (70%)	18 (90%)	15 (75%)	12 (60%)	9 (45%)	10 (50%)	18 (90%)	16 (80%)
Vibro cholerae **(n = 15)**	11 (73%)	8 (53%)	10 (66.6%)	14 (93.3%)	7 (47%)	8 (53%)	6 (40%)	9 (60%)	13 (87%)	9 (60%)
***Enterobacter* isolates (n = 20)**	17 (85%)	15 (75%)	16 (80%)	18 (90%)	12 (60%)	11 (55%)	10 (50%)	11 (55%)	17 (85%)	16 (80%)

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, Guillian-Barre syndrome which is a complication of *Campylobacter jejuni/coli* infection, haemolytic uraemic syndrome which is a sequelae of some *Eschericha 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

Acho-Chi C (2001) Management of water supply system in the Kano district of Nigeria: Problems and possibilities. AQUA **50** (4) 199-207.

Alabi SA and Odugbemi TO (1990) Occurrence of *Aeromonas* species and *Plesiomonas shigelloides* in patients with and without diarrhoea in Lagos, Nigeria. *Journal of Medical Microbiology* **32** 45-48.

Armon R, Araujo R, Koth Y, Lucena F and Jofre J (1997) Bacteriophages of enteric bacteria in drinking water. Comparison of their distribution in two countries. *Journal of Applied Microbiology* **83** 627-633.

Bailey IW and Archer L (2001) An assessment of the impact of RDP levels of water supply on community and environmental health. Water Research Commission Report No K5/925/0/1

Bauer AW, Kirby WMM, Sherris JC and Turck M (1996) Antibiotic susceptibility testing by a standardised single disk method. *American Journal of Clinical Pathology* **45** 413-496.

Black RE (1993) Persistent diarrhoea in children in developing countries. *Pediatrics Infectious Disease Journal* **12** 751-761.

Chunge RN, Karumba PN, Kaleli N, Wamwea M, Mutiso N *et al.,* (1992) Prevalence and frequency of *Giardia lamblia* in children aged 0-60 months with and without diarrhoea. *East African Medical Journal* **69** 311-313.

Coker AO and Dosunmu-Ogumbi O (1984) Isolation of *Campylobacter jejuni* in Lagos, Nigeria. A new bacterial agent of diarrhoea. *East African Medical Journal* **61** 52-55.

Coker AO and Adefoso AO (1994) The changing patterns of *Campylobacter jejuni/coli* in Lagos, Nigeria after ten years. *East African Medical Journal* **71** 437-440.

Department of Water Affairs and Forestry (DWAF) (1998) *South African Water Quality Guideline for Domestic Use* (2nd Edition), Pretoria.

Department of Water Affairs and Forestry (DWAF) (1994) White paper on Water and Sanitation.

Department of Water Affairs and Forestry (DWAF) (1995) Water supply and sanitation white paper. Pretoria.

Du Pont HL (1995) Diarrhoeal diseases in the developing world. *Infectious Disease Clinician, North America* **9** 313-324.

Echeverria P, Blacklow NR, Cukor S, Vibuldandhitkit S, Chang Chawalit S, Boonthai P (1983) Rotaviruses as a cause of severe gastroenteritis in adults. *Journal of Clinical Microbiology* **18** 663-667.

El-Assouli SM, Mohammed KA and Banjar ZM (1995) Human rotavirus genomic RNA electropherotypes in Jeddah, Saudi-Arabia from 1988-1992. *Annals of Tropical Paediatrics* **15** 45-53.

El-Sheikh SM and El-Assouli SM (2001) Prevalence of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Jeddah, Saudi-Arabia. *Journal of Health Population and Nutrition* **19** (1) 25-30.

Esrey SA, Potash J, Roberts L and Shiff C (1990) Health benefits from improvements in water supply and sanitation: Survey and analysis of the literature on selected diseases. WASH Report No 66.

Forch G and Bremann H (1998) Restoration of water supply schemes in the context of town development: The Bhaktapur project in Nepal. *Applied Geographical Development* **15** 54-61.

Ford-Jones EL, Minndorff CM, Gold R and Petric M (1990) The incidence of viral-associated diarrhoea after admission to a paediatric hospital. *Journal of Epidemiology* **131** 711-718.

Genthe B and Seager J (1996) The Effect of Water Supply, Handling and Usage on Water Quality in Relation to Health Indices in Developing Communities. Water Research Commission Report No 562/1/96.

Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK and Bhan MK (1992) Identification of group a rotavirus gene 4 types by polymerase chain reaction. *Journal of Clinical Microbiology* **30** 1365-1373.

Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B and Fang Z (1990) Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *Journal of Clinical Microbiology* **28** 276-282.

Gouvea V, De Castro L, Do Carmo Timenetsky M, Greenberg H and Santos N (1994) Rotavirus Serotype 95 associated with diarrhoea in Brazilian children. *Journal of Clinical Microbiology* **32** 1408-1409.

Grabow WOK (1991) Human viruses in water. *Water and Sewage* **11** 16-21.

Grabow WOK (1996) Waterborne diseases: Update on water quality assessment and control. *Water SA* **22** 193-210.

Grabow WOK, Coubrough P, Nupen EM and Bateman BW (1984) Evaluations of coliphages as indicators of the virological quality of sewage-polluted water. *Water SA* **10** (1) 7-14.

Grabow WOK, Taylor MB, Clay CG and De Villiers JC (2000) Molecular detection of viruses in drinking water: Implications for safety and distribution. Proc. 2nd Conference of the International *Life Sciecne Institute*. The safety of water disinfection: Balancing chemical and microbiological risks. Radisson Deauville Resort, Miami, Florida, USA. 15-17 November.

Grabow WOK (2001) Bacteriophages: Update on application as models for viruses in water. *Water SA* **27** (2) 251-268.

Hardina CM and Fujioka RS (1991) Soil: The environmental source of *Escherichia coli* and eteroccocci in Hawaii's streams. *Environtal Toxicology and Water Quality* **6** 185-195.

Hazen TC (1998) Faecal coliforms as indicators in tropical waters: A review. *Toxicology Assessment* **3** 461-477.

Joseph T (1987) The isolation rates of pathogenic bacteria from stools of gastro-enteritis patients. *Industrial Microbiology* **25** 189-193.

Klein JO, Feigin RD and McCracken Jr GH (1986) Report of the task force on diagnosis and management of meningitis. *Pediatrics* **78** 959-982.

Lacorchak JM, Klem DJ and Peck DV (1998) Environmental monitoring and assessement program – surface waters: field operations and methods for measuring the ecological condition of wadable streams. EPA/620/R – 94/004F. US Environmental Protection Agency, Washington DC.

Lehloesa LJ and Muyima NYO (2000) Evaluation of the impact of household treatment procedures on the quality of groundwater supplies in the rural community of the Victoria district, Eastern Cape. *Water SA* **26** (2) 285-290.

Levin MA, Fischer JR and Cabelli VJ (1975) Membrane filtration technique for enumeration of enterococci in marine waters. *Applied Environmental Microbiology* **30** 66-71.

Lourenco MH, Nicholas JC, Cohen J, Scherrer R and Bricout F (1981) Study of Human Rotavirus Genomen by electrophoresis: Attempt of Classification among strains isolated in France. *Annals in Virology (Paris)* **131** 161-173.

Matar GM, Adbo D, Khneisser I, Youssef M, Zouheiry H and Hakeh HS (2002) The multiplex-PCR based detection and genotyping of diarrhoeagenic *Escherichia coli* in diarrhoeic stools. *Annals in Tropical Medical Parasitology* **96** (3) 317-324.

Mermin JH, Townes JM, Gerber M, Dolan N, Mintz ED and Tauxe RV (1998). Typhoid fever in the United States, 1985-1994: Changing risks of international travel and increasing antimicrobial resistance. *Archives in Internal Medicine* **158** 633-638.

Mohammed KA, El-Assouli SM, Banjar ZM (1994) Human rotavirus subgroups and serotypes in Children with acute gastro-enteritis in Saudi Arabia from 1988 to 1992. *Journal of Medical Virology* **44** 237-242.

Nachamkin I (1999) *Campylobacter* and *Arcobacter.* In: Murray PR, Baron EJ, Faller MA, Tenover FC, Yolken RH (ed). *Manual of Clinical Microbiology*. 7th Ed. Washington, D C: American Society for Microbiology. 716-26pp.

Na'was TE and Abo-Shehada MN (1991) A study of the bacterial and parasitic causes of acute diarrhoea in Northern Jordan. *Journal of Diarrhoeal Disease Research* **9** 305-309.

Nakagomi T, Akatani K, Ikegami N, Katsushima N and Nakagomia O (1988) Occurrence of changes in human rotavirus serotypes with concurrent changes in genomic RNA electrophoresis. *Journal of Clinical Microbiology* **26** 2586-2592.

Nevondo TS and Cloete TE (1999) Bacterial and chemical quality of water supply in the Dertig villiage settelement. *Water SA* **25** (2) 215-220.

Obi CL, Coker AO, Epoke J and Ndip RN (1997) Enteric bacterial pathogens in stools of residents of urban and rural regions in Nigeria: A comparison of patients with diarrhoea and controls without diarrhoea. *Journal of Diarrhoeal Disease Research* **15** (4) 241-247.

Obi CL, Coker AO, Epoke J and Ndip RN (1995) *Aeromonas* and *Plesiomonas* species as bacterial agents of diarrhoea in urban and rural areas of Nigeria: Antibiogram of isolates. *Central African Journal of Medicine* **4** (12) 397-403.

Obi CL, Coker AO, Epoke J and Ndip RN (1998) Distributional pattern of bacterial diarrhoeagenic agents and antibiograms of isolates from diarrhoeic and non-diarrhoeic patients in urban and rural areas of Nigeria. *Central African Journal of Medicine* **44** (9) 223-229.

Obi CL, Potgieter N, Bessong PO and Matsaung G (2002) Assessment of the Microbial quality of river water sources in rural Venda communities in South Africa. *Water SA* **28** (3) 287-291.

Ogunsanya T, Rotimi VO and Adenuga A (1994) A study of the etiological agents of childhood diarrhoea in Lagos Nigeria. *Journal of Medical Microbiology* **40** 10-14.

Palupi K, Sumengen S, Inswiasri S, Augustina L. Nunik SA, Sunarya W and Quraisyn A (1995) River water quality study in the vicinity of Jakarta. *Water Science and Technology* **39** 17-25.

Parashar UD, Bresee JS and Glass RI (2003) The global burden of diarrhoeal disease in children. *Bulletin of the World Health Organisation*. **81** (4) 236-240.

Paul JH, Rose JB, Jiang S, Kellog C and Shinn E (1995) Occurrence of faecal indicator bacteria in surface water and the subsurface equiter in Key Largo, Florida. *Applied Environmental Microbiology* **61** (6) 2235-2241.

Payment P, Richardson L, Siemiatychi J, Dewar R, Edwardes M and Franco E (1991) A randomised trial to evaluate the risk of gastrointerstinal disease due to consumption of drinking water meeting current microbiological standards. *American Journal of Public Health* **81** (6) 703-308.

Prado V and O'Ryan ML (1994) Acute gastroenteritis in Latin America. *Infectious Disease Clinician North America* **8** 77-106.

Prasad KN, Nag VL, Dohle TN and Ayyagari A (2000) Identification of Enteric Pathogens in HIV-positive patients with diarrhoea in Northern India. *Journal of Health Population and Nutrition* **18** 23-26.

Rice EW, Covert TC, Wild DK, Berman D, Johnson SA and Johnson CH (1993) Comparative resistance of *Escherichia coli* and enterococci to chlorination. *Journal of Environmental Science and Health* **28** (1) 89-97.

Robins-Browne RM, Mackejee MKR, Bodasing MN and Coovadia HM (1983) Treatment of *Campylobacter* associated enterities with erythromycin. *American Journal of Diseases in Children* **137** 282-285.

Rowe B, Ward LR and Threlfau EJ (1997) Multidrug resistant *Salmonella typhi*: a worldwide epidemic. *Clinical Infectious Diseases* **24** (Suppl 1) 5106 – 5109.

Schalekamp M (1990) The UNO-drinking water decade 1990-1991: Problems and successes. Lecture Held on the Occasion of the 100th Anniversary of the Austrian Gas and Water Industry. Water Supply, Zürick, Industrial Corporations of the City of Zürick, Bombay.

Sethi SK and Khuffash F (1989) Bacterial and viral causes of acute diarrhoea in children in Kuwait. *Journal of Diarrhoeal Disease Research* **7** 85-88.

Simango C, Dindiwe J and Rukure G (1992) Bacterial contamination of food and household stored drinking water in a farm working community in Zimbabwe. *Central African Journal of Medicine* **38** (4) 143-148.

Snyder JD and Merson MH (1982) The magnitude of the global problem of acute diarrhoeal disease. A review of active surveillance data. *Bulletin of the World Health Organization* **60** 605-613.

Standard Methods for the Examination of Water and Waste Water (1998) Clesceri LS, Greenberg AE and Eaton AD (ed). American Public. Health Association, 20th Edition, Washington DC.

Tangermann RH, Gordon S, Wiesnar P and Kreckman L (1991) An outbreak of cryptosporidiosis in a day-care centre in Georgia. *American Journal of Epidemiology* **133** 471-476.

Taniguchi K, Urasaw T, Monta A, Greenberg HB and Urasawa S (1987) Direct Serotyping of Human Rotavirus in Stools by an Enzyme Linked immunosorbent

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

Theron J (2001) The challenge of identifying waterborne pathogens leads to new technology. *SA Waterbulletin* **27** (3) 10-13.

Thielman NM and Guerrant RL (1999) *Escherichia coli*. In: Yu VL, Merigan Jr TC, Barriere SL (eds). The Williams & Wilkins Company, Baltimore. 88-200 pp

Von Gravenitz A (1985) *Aeromonas* and *Plesiomonas.* In: Lenneth Ehm Balows A, Hansler WJ, Shadomy HJ (ed). Manual of Clinical Microbiology. Washington Society of Microbiology. 278-282 pp.

Wasfy MO, Momtaz O, Buhari A, John C, Tharwat IF and Leonard FP (2000) Isolation and antibiotic susceptibility of *Shigella, Salmonella* and *Campylobacter* from acute enteric infections in Egypt. *Journal for Health, Population and Nutrition* **18** 33-38.

Water Research Commission (1998) Quality of Domestic Water Supplies. Vol. 1-Assessment Guide. Water Research Commission Report No TT101/98.

Water Research commission (1993) Guidelines on the Cost Effectiveness of Rural Water Supply and Sanitation Projects. Water Research Commission Report No 231/1/93.

White L, Garcia D, Boher Y, Blanco M and Romer H (1991) Temporal distribution of human rotavirus serotypes 1,2,3 and 4 in Venezuelan children with gastroenteritis during 1979-1989. *Journal of Medical Virology* **34** 79-84.

WHO (1980) Scientific Working Group. Rotavirus and other viral diarrhoeas. *Bulletin of the World Health Organisation* **58** 183-198.

WHO (1993) *Guidelines for Drinking Water Quality*. Vol. 1. Recommendations (2nd Edition) World Health Organisation, Geneva, Switzerland.

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 calici-like viruses (Echeverria *et al.,* 1983; El-Assouli *et al*; 1995; El-Sheikh and El-

Assouli; 2001). Parasitic agents of diarrhoea, especially *Giardia lamblia* and *Cryptosporiduim 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 diarrhoic 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 37ºC 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 H_2S 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* 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 steaked 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). 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), Enterophathogenic *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 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 Xylose-lysine desoxycholate agar (XLDC) for 24h at 37ºC. Transparent colonies suggestive of *Shigella* were screened for negative reactions for motility, adonitol, citrate, DNase, gas from glucose, H_2S, 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 alkaline peptone water pH 8.6 (APW) and incubated at 37ºC. 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 37ºC 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 50ºC for 30 minutes. The supernatant was kept at 4ºC 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 *Eschericha coli* 81(20%). The pathogenic strains comprised of 20 (25%) necroxigenic *Escherichia coli* (NEC) 10 (12.5%) each of enterotoxigenic *E.coli* (ETEC), and Shiga-like toxin producing *Esherichia coli* (STEC); 30 (37%) of enteropathogenic *Esherichia coli* (EPEC) and 11 (13.6%) enteroaggregate *Esherichia 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. s*higelloides*, *Aeromonas* isolates and *Campylobacter jejuni/coli* to tetracycline, ampicillin, erythromycin and chloramphenicol were noted (Table 2.2).

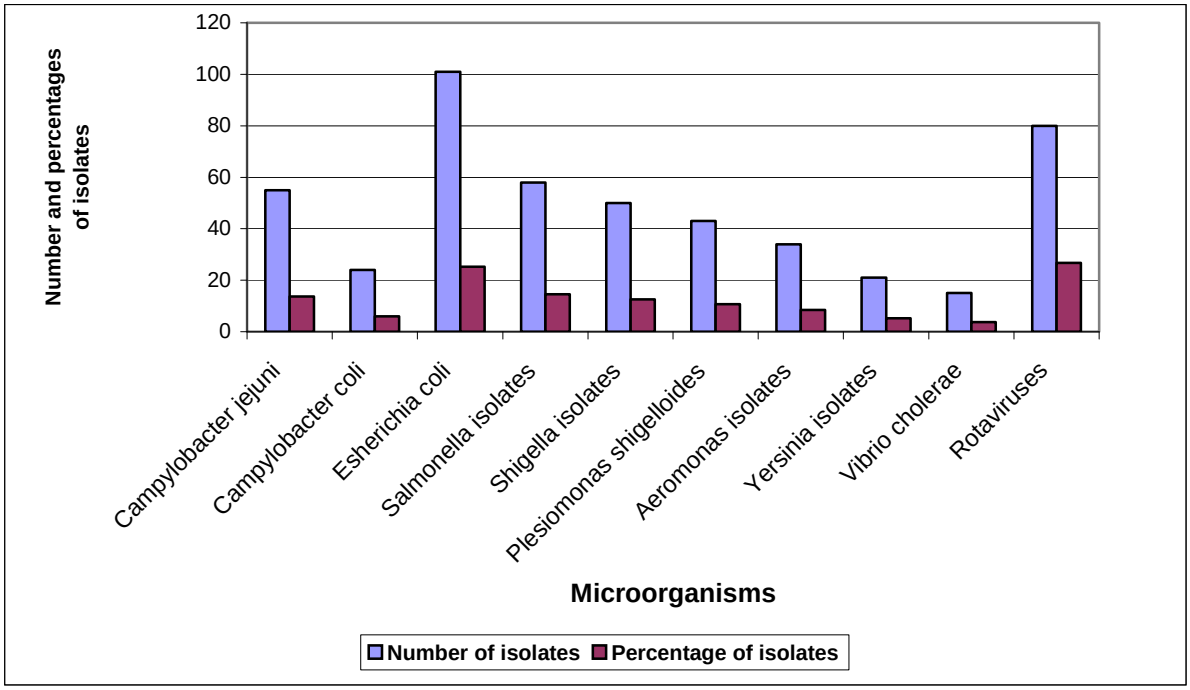

Figure 2.1: Frequency of isolation of potential enteric pathogens from stool specimens of patients with diarrhoea in the Venda region

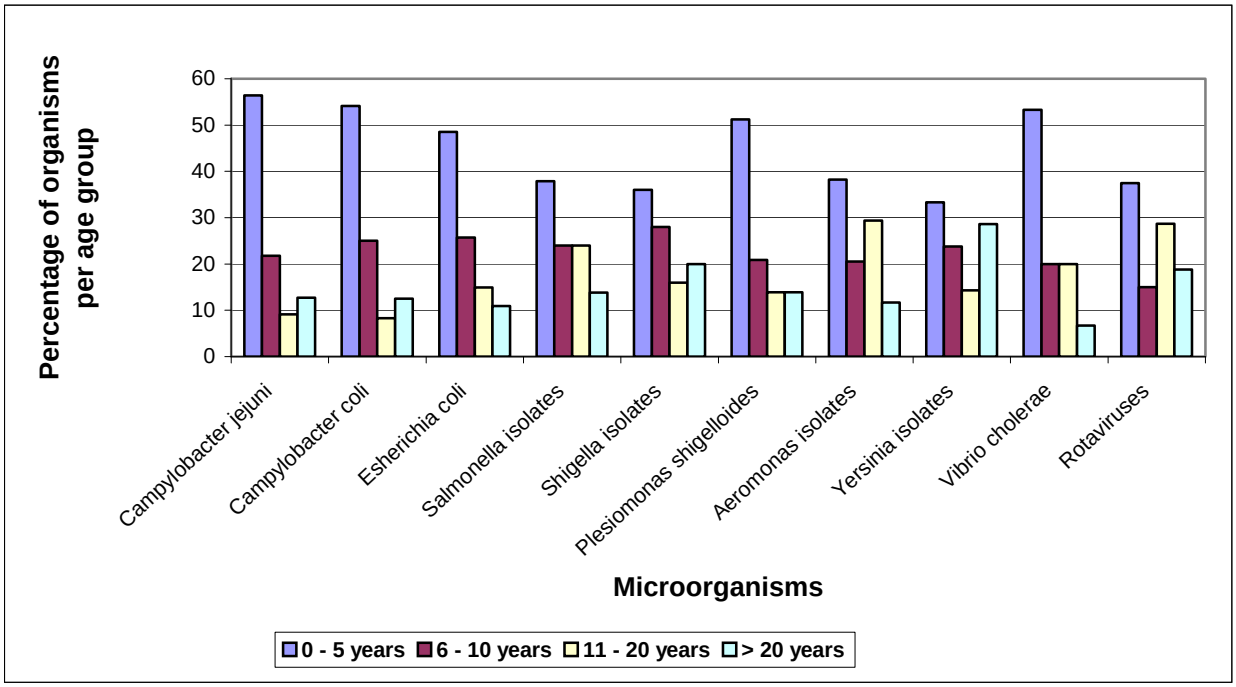

Figure 2.2: Age distribution of enteric organisms isolated from patients with diarrhoea in the Venda region

Table 2.1 Occurrence of Rotavirus VP4, VP7 and electropherotypes in diarrhoeal stool samples

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

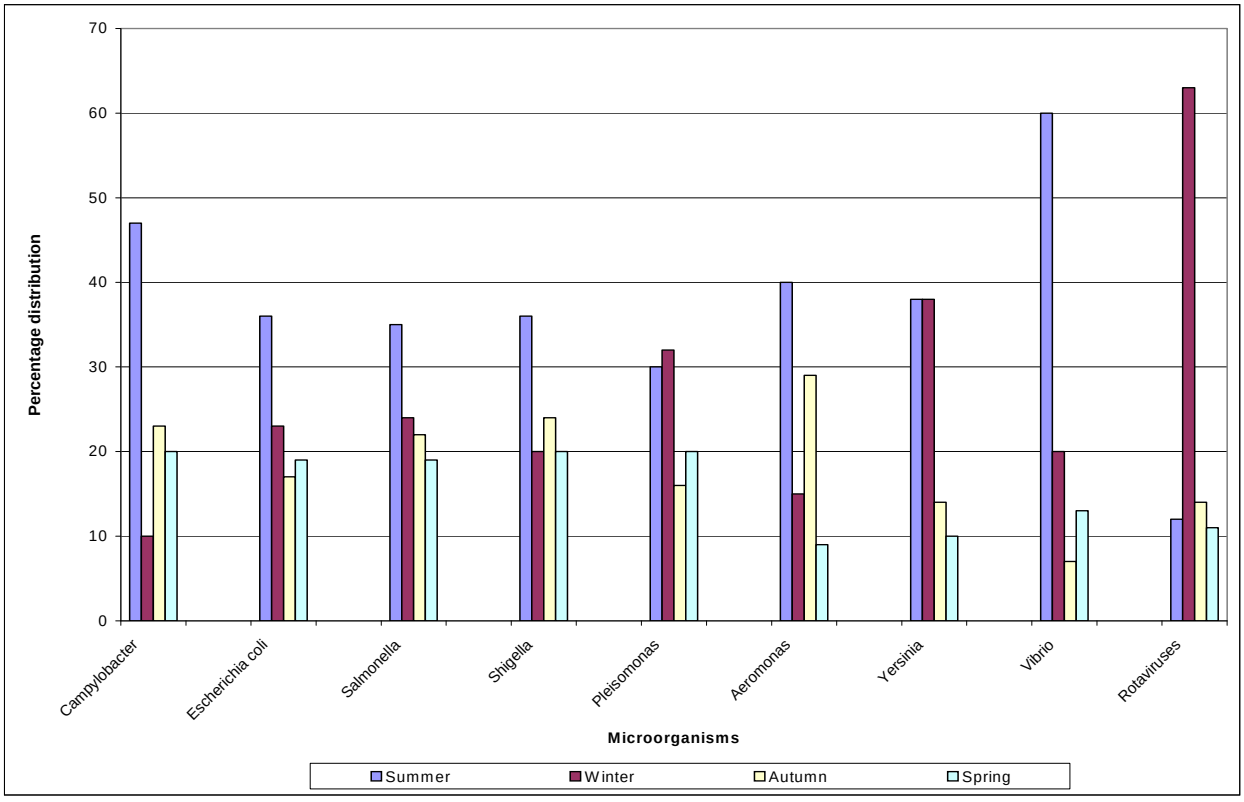

Figure 2.3: Seasonal distribution of enteropathogens isolated from diarrhoeal cases in the Venda Region

Table 2.2 Antibiotic susceptibilities of enteric bacterial pathogens from stool specimens of patients with diarrhoea

	Number of isolates sensitive to different antibiotics (% susceptibility)									
Bacteria	**NA**	**GM**	**COT**	**CIP**	**TE**	**AP**	**ERY**	**CHL**	**AKC**	**CEF**
Campylobacter jejuni (n=55)	51 (93%)	51 (93%)	41 (75%)	52 (95%)	40 (73%)	33 (60%)	38 (69%)	33 (60%)	54 (98%)	36 (65%)
Campylobacter coli (n=24)	18 (75%)	21 (88%)	16 (67%)	21 (88%)	15 (63%)	13 (54%)	14 (58%)	13 (54%)	22 (92%)	16 (67%)
Esherichia coli (n=101)	94 (93%)	95 (94%)	73 (72%)	97 (96%)	69 (68%)	44 (44%)	54 (53%)	43 (43%)	99 (98%)	91 (90%)
Salmonella isolates (n=58)	55 (95%)	58 (100%)	40 (68%)	56 (97%)	29 (50%)	29 (50%)	43 (74%)	45 (78%)	58 (100%)	55 (95%)
Shigella isolates (n=50)	42 (84%)	47 (94%)	31 (62%)	45 (90%)	25 (50%)	23 (46%)	ND	ND	49 (98%)	48 (96%)
Plesiomonas shigelloides (n=43)	40 (93%)	37 (86%)	30 (69%)	43 (100%)	29 (67.4%)	27 (63%)	18 (42%)	26 (60%)	42 (98%)	38 (88%)
Yersinia isolates (n=21)	17 (81%)	18 (86%)	11 (52%)	18 (86%)	13 (70%)	10 (48%)	ND	9 (43%)	18 (86%)	16 (76%)
Aeromonas isolates (n=34)	30 (88%)	30 (88%)	26 (76%)	32 (94%)	26 (76%)	22 (65%)	14 (41%)	18 (53%)	32 (94%)	28 (82%)
Vibrio cholerae (n=15)	10 (67%)	6 (40%)	11 (73%)	13 (87%)	7 (47%)	ND	10 (67%)	6 (40%)	13 (87%)	11 (73%)

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 (Household *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

Alabi SA and Odugbemi TO (1990) Occurrence of *Aeromonas* species and *Plesimonas shigelloides* in patients with and without diarrhoea in Lagos, Nigeria. *Journal of Medical Microbiology* **32** 45-48.

Bauer AW, Kirby WMM, Sherris JC and Turck M (1966) Antibiotic susceptibility testing by a standardised single disk method. *American Journal of Clinical Pathology* **45** 413-496.

Black RE (1993) Persistent diarrhoea in children in developing countries. *Pediatrics Infectious Disease Journal* **12** 751-761.

Chunge RN, Karumba PN, Kaleli N, Wamwea M, Mutiso N, Andala EO and Kinoti SN (1992) Prevalence and frequency of *Giardia lamblia* in children aged 0-60 months with and without diarrhoea. *East African Medical Journal* **69** 311-313.

Coker AO and Dosunmu-Ogumbi O (1984) Isolation of *Campylobacter jejuni* in Lagos, Nigeria. A new bacterial agent of diarrhoea. *East African Medical Journal* **61** 52-55.

Du Pont HL (1995) Diarrhoeal diseases in the developing world. *Infectious Disease Clinician North America* **9** 313-24.

DWAF (1995) Water supply and sanitation white paper. DWAF, Pretoria.

Echeverria P, Blacklow NR, Cukor S, Vibuldandhitkit S, Chang CS and Boonthai P (1983) Rotaviruses as a cause of severe gastroenteritis in adults. *Journal of Clinical Microbiology* **18** 663-667.

El-Assouli SM, Mohammed KA and Banjar ZM (1995) Human rotavirus genomic RNA electropherotypes in Jeddah, Saudi-Arabia from 1988-1992. *Annual Tropical Paediatrics* **15** 45-53.

El-Sheikh SM and El-Assouli SM (2001) Prevalence of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Jeddah, Saudi-Arabia. *Journal of Health Population and Nutrition* **19** (1) 25-30.

Enweani B, Obi CL and Jokpeyibo M (1994) Prevalence of Candida species in Nigerian Children with Diarrhoea. *Journal of Diarrhoeal Disease Research* **12** (2) 133-135.

Esrey SA, Potash J, Roberts L and Shiff C (1990) Health benefits from improvements in water supply and sanitation: Survey and analysis of the literature on selected diseases. *WASH Report* No 66.

Ford-Jones EL, Minndorff CM, Gold R and Petric M (1990) The incidence of viral-associated diarrhoea after admission to a paediatric hospital. *Journal of Epidemiology* **131** 711-718.

Galane PM and Le Roux M (2001) Molecular epidemiology of *Escherichia coli* isolated from young South African children with diarroeal diseases. *Journal of Health Population and Nutrition* **19** (1) 31-37.

Gentsch JR, Glass RL, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK and Bhan MK (1992) Identification of group a rotavirus gene 4 types by polymerase chain reaction. *Journal of Clinical Microbiology* **30** 1365-1373.

Gouvea V, Glass RL, Woods P, Taniguchi K, Clark H F, Forrester B and Fang Z (1990) Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *Journal of Clinical Microbiology* **28** 276-282.

Gouvea V, De Castro L, Do Carmo TM, Greenberg H and Santos N (1994) Rotavirus Serotype associated with diarrhoea in Brazilian children. *Journal of Clinical Microbiology* **32** 1408-1409.

Grabow WOK (1996) Waterborne diseases: Update on water quality assessment and control. *Water SA* **22** 193-202.

Ho BS, Wong T (1985) A one year survey of Campylobacter enterititis and other forms of bacterial diarrhoea in Hong Kong. *Journal of Hygiene* **94** (1) 55-60.

Household KC, Mann MD and Bowie MD (1988) Enteropathogens associated with acute infantile diarrhoea in Cape Town. *South African Medical Journal* **73** (2) 83-87.

Levine MM, Ferreccio C, Prado V, Cayazzo M, Abrego P, Martinez J, Maggi L, Baldini MM, Martin W and Maneval D (1993) Epidemiologic studies of Escherichia coli diarrhoeal infections in low socio-economic level peri-urban community in Santiago, Chile. *American Journal of Epidemiology* **138** (10) 849-869.

Lourenco MH, Nicholas JC, Cohen J, Scherrer R and Bricout F (1981) Study of Human Rotavirus Genome by electrophoresis: Attempt of classification among strains isolated in France. *Annals of Virology* **131** 161-173.

Matar GM, Adbo D, Khneisser I, Youssef M, Zouheiry H and Hakeh HS (2002) The multiplex-PCR based detection and genotyping of diarrhoeagenic *Escherichia coli* in diarrhoeic stools. *Annals of Tropical Medical Parasitology* **96** (3) 317-324.

Nachamkin I (1999) Campylobacter and Arcobacter. In: Murray PR, Baron EJ, Faller MA, Tenover FC and Yolken RH (ed). Manual of Clinical Microbiology. 7th ed. pp. 716-726. Washington, D C. American Society for Microbiology.

Nakagomi T, Akatani K, Ikegami N, Katsushima N, Nakagomia O (1988) Occurrence of changes in human rotavirus serotypes with concurrent changes in genomic RNA electrophoresis. *Journal of Clinical Microbiology* **26** 2586-2592.

Na'was TE and Abo-Shehada MN (1991) A study of the bacterial and parasitic causes of acute diarrhoea in Northern Jordan. *Journal of Diarrhoeal Disease Research* **9** 305-309.

Niyogi SK, Saha MR and De SP (1994) Enteropathogens associated with acute diarrhoel diseases. *Indian Journal of Public Health* **38** (2) 29-32.

Obi CL, Coker AO, Epoke J and Ndip RN (1995) *Aeromonas* and *Plesiomonas* species as bacterial agents of diarrhoea in urban and rural areas of Nigeria: Antibiogram of isolates. *Central African Journal of Medicine* **4** (12) 397-403.

Obi CL, Coker AO, Epoke J and Ndip RN (1997) Enteric bacterial pathogens in stools of residents of urban and rural regions in Nigeria: A comparison of patients with diarrhoea and controls without diarrhoea. *Journal of Diarrhoeal Disease Research* **15** (4) 241-247.

Obi CL, Coker AO, Epoke J and Ndip RN (1998) Distributional pattern of bacterial diarrhoeagenic agents and antibiograms of isolates from diarrhoeic and non-diarrhoeic patients in urban and rural areas of Nigeria. *Central African Journal of Medicine* **44** (9) 223-229.

Obi CL, Potgieter N, Bessong PO and Matsaung G (2002) Assessment of the Microbial quality of river water sources in rural Venda communities in South Africa. *Water SA* **28** (3) 287-291.

Ogunsanya T, Rotimi VO and Adenuga A (1994) A study of the etiological agents of childhood diarrhoea in Lagos Nigeria. *Journal of Medical Microbiology* **40** 10-14.

Ono K, Rai SK, Chikahira M, Fujimoto T, Shibata H, Wada Y, Tsuji H, Oda Y and Uga S (2001) Seasonal distribution of enteropathogens from diarrhoeal stool and water sources collected in Kathmandu, Nepal. *South East Asian Journal of Tropical Medicine and Public Health* **32** (3) 520-526.

Payment P, Richardson L, Siemiatychi J, Dewar R, Edwardes M and Franco E (1991) A randomised trial to evaluate the risk of gastrointerstinal disease due to consumption of drinking water meeting current microbiological standards. *American Journal of Public Health* **81** (6) 703-308.

Potgieter N, De Beer M, Grabow WOK, Taylor MB and Steel AD (2004) Diversity of unusual rotavirus genotypes in children with acute diarrhoea from rural areas in South Africa. Submitted.

Prasad KN, Nag VL, Dohle TN and Ayyagari A (2000) Identification of Enteric Pathogens in HIV-positive patients with diarrhoea in Northern India. *Journal of Health Population and Nutrition* **18** 23-26.

Prado V and O'Ryan ML (1994) Acute gastroenteritis in Latin America. *Infectious Disease Clinician North America* **8** 77-106.

Sethi SK and Khuffash F (1989) Bacterial and viral causes of acute diarrhoea in children in Kuwait. *Journal of Diarrhoeal Disease Research* **7** 85-88.

Simango C, Dindiwe J and Rukure G (1992) Bacterial contamination of food and household stored drinking water in a farm working community in Zimbabwe. *Central African Journal of Medicine* **38** (4) 143-148.

Snyder JD and Merson MH (1982) The magnitude of the global problem of acute diarrhoeal disease. A review of active surveillance data. *Bulletin of the World Health Organisation* **60** 605-613.

Standard Methods for the Examination of Water and Waste Water (1998) Clesceri LS, Greenberg AE and Eaton AD (ed). American Public. Health Association, 20th Edition, Washington DC.

Tangermann RH, Gordon S, Wiesnar P and Kreckman L (1991) An outbreak of cryptosporidiosis in a day-care centre in Georgia. *American Journal of Epidemiology* **133** 471-476.

Von Gravenitz A (1985) *Aeromonas* and *Plesiomonas*. In: Lenneth EHM, Balows A, Hansler WJ and Shadomy HJ (eds) Manual of clinical microbiology. Washington Society of Microbiology. 278-282

White L, Garcia D, Boher Y, Blanco M and Romer H (1991) Temporal distribution of human rotavirus serotypes 1,2,3 and 4 in Venezuelan children with gastroenteritis during 1979-1989. *Journal of Medical Virology* **34** 79-84.

WHO (1980) Scientific Working Group. Rotavirus and other viral diarrhoeas. *Bulletin of the World Health Organisation* **58** 183-198.

WHO (1993) *Guidelines for Drinking Water Quality*. Vol. 1. Recommendations (2nd Edition) World Health Organisation, Geneva, Switzerland.

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.