THE EPIDEMIOLOGY AND COST OF TREATING DIARRHOEA IN SOUTH AFRICA

Volume 2: Prevalence and antibiotic profiles of diarrheagenic pathogens in children under the age of 5 years – A case of Vhembe District, Limpopo Province

N Potgieter, TG Barnard, LS Mudau and AN Traore

WATER RESEARCH COMMISSION

TT 761/18

THE EPIDEMIOLOGY AND COST OF TREATING DIARRHOEA IN SOUTH AFRICA

Volume 2

Prevalence and antibiotic profiles of diarrheagenic pathogens in children under the age of 5 years – A case of Vhembe District, Limpopo Province

Report
to the Water Research Commission

by

N Potgieter [1], TG Barnard [2], LS Mudau [3] and AN Traore [1]

[1] University of Venda, [2] University of Johannesburg and
[3] Tshwane University of Technology

WRC Report No. TT 761/18

ISBN 978-0-6392-0027-9

September 2018

Obtainable from:

Water Research Commission
Private Bag X03
Gezina 0031
South Africa
orders@wrc.org.za or download from www.wrc.org.za

This report emanates from the Water Research Commission project, titled: *Epidemiological and economic implications of diarrhoea in water sources from rural and peri-urban communities in the Limpopo Province, South Africa* (K5/7150). The outputs of this research project are presented in three separate publications:

- **Volume I: Prevalence of diarrheagenic pathogens in water sources in the Vhembe District of the Limpopo Province (TT 760/18)**
- **Volume II: Prevalence and antibiotic profiles of diarrheagenic pathogens in children under the age of 5 years – A case of Vhembe District of the Limpopo Province. (This report)**
- **Volume III: The cost of treating diarrhoea in children under the age of 5 years in rural and peri-urban communities – A case study of Vhembe District of the Limpopo Province. (TT 762/18)**

Printed in the Republic of South Africa

EXECUTIVE SUMMARY

BACKGROUND

Diarrhoeal diseases remain an important global health concern. Diarrhoea is commonly caused by viruses (e.g. norovirus, rotavirus), bacteria (e.g. *Escherichia coli*, *Clostridium difficule*, *Shigella*) and parasites (e.g. *Cryptosporidium, Giardia*), and is often the result of contaminated water/food and poor sanitation. In South Africa, about 1.5 billion diarrhoea episodes and 10.2% deaths are reported per year (Department of Health, 2016). An important element of a diarrhoea control programme aimed at reducing diarrhoea mortality involves continuous epidemiological and prevalence assessment of diarrhoeagenic pathogens in communities. Epidemiological surveys provide vital information to health statisticians regarding the prevalent strains circulating in these communities. This data can be used to design/select vaccine programmes and to inform on the effectiveness of other treatment interventions.

The emergence of resistant pathogens, which is typically associated with genetic changes in microorganisms (e.g. acquisition of resistance genes; mutations in elements relevant for the activity of the antibiotic) and in some situations, achieved without any genetic alteration (e.g. phenotypic resistance), adds a further complexity to the diarrhoea problem (Corona & Martinez, 2013). Antimicrobial resistance is a major threat to the effective treatment of infections, especially in vulnerable patients, resulting in prolonged illness and increased mortality. Information on epidemiology and antimicrobial susceptibility patterns is limited. Although several studies in South Africa have reported on antimicrobial-resistant patterns of isolated diarrhoeal pathogens, several studies have been done in isolation and the protocols used are not standard and uniform; therefore, the data is not always reliable. More studies are needed, especially in resource-poor areas, to understand the treatment of diarrhoea and the use/misuse of antibiotics.

AIM OF THE STUDY

Assess the prevalence, incidence and genetic diversity of pathogenic bacteria, viruses and parasitic organisms among children under the age of 5 years old suffering from diarrhoea in the Vhembe District, Limpopo Province, and to determine the antimicrobial resistance of diarrhoea-causing bacteria isolated from stool samples.

OBJECTIVES

The objectives of the study were as follows:

- Collect stool samples from children under the age of 5 years old suffering from diarrhoea from rural and peri-urban communities in the Vhembe District of the Limpopo Province.
- Determine genetic diversity of pathogenic bacteria, viruses and parasitic organisms in stool samples using a qualitative multiplexed nucleic acid-based in vitro diagnostic test.
- Isolate aerobic and micro-aerophilic bacteria associated with diarrhoeal disease.
- Identify Gram-positive and Gram-negative bacteria using VITEK 2™ cards.
- Determine the antimicrobial susceptibility of potential pathogenic, and selected commensal, bacterial isolates.
- Interpret the antimicrobial resistance data in terms of the National Department of Health and World Health Organization guidelines.
- Make recommendations in relation to disease occurrence and prevention that could impact policies.
- Describe how the results obtained influence current antimicrobial resistance monitoring strategies.

METHOD

The study was conducted in the Vhembe District of the Limpopo Province, South Africa. Stool samples were collected from primary health care clinics for 12 months between 2015 and 2016 from children under the age of 5 years who suffered from diarrhoea. In addition, stool samples were also collected over a period of 4 months in 2016 from three hospitals where children under the age of 5 years were treated for severe cases of diarrhoea. All stool specimens were tested for the presence of diarrhoea-causing bacteria, viruses and parasites using the BioFire® FilmArray® Gastrointestinal (GI) Panel, which is a qualitative multiplexed nucleic acid-based in vitro diagnostic test that simultaneously detects 22 bacteria, viruses and parasites in one stool sample.

Diarrhoea-causing bacteria collected from stool samples were analysed for antibiotic susceptibility. The project aim was to target aerobic and micro-aerophilic diarrhoea-associated bacteria using standard microbiological methods to isolate *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Streptococcus* spp. and *Campylobacter* spp., as well as known coliforms and specifically *E. coli* O157:H7. All typical isolates were identified as Gram-negative or Gram-positive species using VITEK 2™ cards. A subset of the identified bacterial strains was tested further for their antimicrobial susceptibility. Results obtained were interpreted and corrected using the 2017 Clinical Laboratory Standards Institutes “Performance Standards for Antimicrobial Susceptibility Testing” and phenol-typical database.

No stool samples were collected from asymptomatic children. Some of the stool samples were frozen, which could have influenced the survival and recovery of the bacteria present in the samples. Due to the high number of isolates, limited funding and low detection with the GI Panel, anaerobic bacteria were not included in the study to test for antimicrobial resistance.

MAIN FINDINGS

The BioFire® FilmArray® tool was used to analyse 275 samples (184 from primary health care clinics and 91 from hospitals) for pathogenic bacteria, viruses and parasites. The most isolated bacteria pathogens were enteroaggregative *E. coli* [EAEC], enteropathogenic *E. coli* [EPEC], enterotoxigenic *E. coli* [ETEC], *Giardia*, *Cryptosporidium*, adenovirus F40/41, norovirus and rotavirus. Single symptoms were seen in 33% of patients while multiple symptoms were seen in 67% of patients. A total of 55% of the patients had liquid stool; 15% had soft/mushy stools and 23% had formed stools. A total of 472 bacterial strains were isolated for antimicrobial susceptibility patterns, of which 433 was identified using the VITEK 2™ system. Of these, 81.8% were Gram-negative and included both potential pathogenic and commensal species.

A selection of the bacterial strains isolated were tested for their antimicrobial resistance profiles and included the ESKAPE pathogens (*Enterococcus faecium/faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *E. coli* species) as outlined by the NDOH (2017). Of these, only *Enterococcus faecium/faecalis, K. pneumoniae* and *E. coli* strains could be isolated and are reported.

The species were tested against a panel of antibiotics, which included the recommended antibiotics for each of the species. As expected, the 115 *E. coli* strains showed a variety of wild type and acquired antimicrobial resistance, with some strains showing resistance against multiple classes of antibiotic. Of these, 56% (n = 64) had extended spectrum beta-lactamase resistance. Similarly, the *K. pneumoniae* strains showed wild type resistance against several antibiotic classes. Approximately 36.4% of *Entero-coccus* strains showed resistance to vancomycin, with again a wide range of wild type resistance reported. Despite this, there were strains that showed multiple resistance against various combinations of the recommended antibiotics tested.

CONCLUSION

The BioFire® FilmArray® was a good tool to use for fast results in severe diarrhoeal cases. Although some stool samples did not show any organisms, this could be attributed to the vast number of new emerging organisms causing diarrhoea and not to the failure of the tool itself. It was clear that most of the severe cases in hospitals were caused by viral infections; however, more studies need to be done as confirmation. The fact that *E. coli* was the major bacterial isolate shows the potential transmission routes of faecal contamination through water, and poor hygiene and sanitation practices.

Antimicrobials should not be used routinely to treat children. The high level of antimicrobial resistance observed in this study raises a broader discussion about the indiscriminate use of antimicrobials and the risks (fatal side effects) of empirical antibiotics in the therapy in children of a very young age. There are multiple resistant strains of *E. coli, K. pneumoniae* and *Enterococcus faecium/faecalis* isolated from the faeces of children under the age of 5 years living in rural and peri-urban communities. There were also wide varieties of other bacterial strains (e.g. *Aeromonas hydrophila, Sphingomonas paucimobilis* and *Stenotrophomonas maltophilia*) that showed antimicrobial resistance that does not have to be reported, but that could contribute to the spread of antimicrobial resistance via a variety of processes.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Continued assessment of clinical samples to assess the prevalence of diarrhoea-causing bacteria, viruses and parasites.
- Continued assessment of fast, reliable and easy-to-use isolation and identification methods of causative diarrhoea agents to assist in the treatment of diarrhoea.
- Increased social behaviour education (including interventions) to rural household members, especially mothers with young children regarding water, sanitation and hygiene aspects.
- Reassessment of treatment schedules used in hospitals and primary healthcare clinics to lower antibiotic resistance in pathogenic bacteria.
- The Group for Enteric, Respiratory and Meningeal Surveillance in South Africa (GERMS-SA) annually reports on the costs and limited staff resources available to do continuous monitoring of bacterial and fungal pathogens, along with their susceptibility/resistance characteristics. By linking with academics, this problem could be addressed using student projects to gather bacterial strains or antimicrobial resistance information. This would require inputs from the various stakeholders to determine which method and guidelines to use to achieve this and be able to compare results.
- Further testing is required to characterise the *E. coli* strains pathogenicity (polymerase chain reaction testing) and to confirm colistin susceptibility or resistance.

CAPACITY BUILDING

Student name	Degree (University)	Title of study	Supervisors	Degree awarded
Lee Heine	DTech (University of Johannesburg)	Incidence and antimicrobial resistance of diarrhoeagenic bacteria in Vhembe region of South Africa	Prof. TG Barnard Prof. N Potgieter	In final stage
Tshikani Basil Minyuku	MTech (Tshwane University of Technology)	Cost implications of diarrhoea treatment related to water, sanitation and hygiene of children under 5 years in public health clinics of Vhembe District, Limpopo Province, South Africa	Dr LS Mudau Prof. MI Mokgobu	In final stage
Jean Pierre Kabue Ngandu	PhD (University of Venda)	Molecular characterization of norovirus strains circulating in rural communities of the Limpopo Province of South Africa	Prof. N Potgieter Dr E Meader Prof. PR Hunter	Graduated 2018
Ellen Ledwaba	MSc (University of Venda)	Prevalence of selected bacterial and viral entero-pathogens in children less than 5 years of age in Limpopo Province of South Africa	Prof. N Potgieter Prof. AN Traore	Graduated 2016
Simbarashe Karambwe	MSc (University of Venda)	Prevalence of diarrhoea-causing bacteria, viruses and parasites in water sources in the rural communities in the Vhembe District	Prof. N Potgieter Prof. AN Traore	Graduated 2017

LIST OF RESEARCH OUTPUTS

Published journal articles:

- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2016). Human norovirus prevalence in Africa: A review of studies from 1990 to 2013. *Tropical Medicine and International Health* 21(1): 2–17.
- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2016). Norovirus prevalence and estimated viral load in symptomatic and asymptomatic children from rural communities of Vhembe District, South Africa. *Journal of Clinical Virology* 84:12–18.
- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2017). Genetic characterisation of norovirus strains in outpatient children from rural communities of Vhembe District, South Africa: 2014–2015. *Journal of Clinical Virology* 94:100–106.

Conference attendance:

- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2016). Molecular characterization of norovirus strains circulating in rural communities of the Limpopo Province of South Africa. Oral presentation. 8th International Water and Health seminar. June 27–29. Cannes, France.
- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2018). Genetic diversity of norovirus strains in outpatient children from rural communities of Vhembe District, South Africa, 2014-2015. Oral presentation. 20th International conference on Infectious Diseases. January 29–30. Sydney, Australia. *Presentation won best presentation award.*

ACKNOWLEDGEMENTS

The project team wishes to thank the Water Research Commission for funding this project, as well as the following people for their contributions to the project.

Name	Affiliation
Dr Jennifer Molwantwa	(former) Water Research Commission
Dr Eunice Ubomba-Jaswa	Water Research Commission
Dr Nonhlanhla Kalebaila	Water Research Commission
Dr Nicky Page	National Institute for Communicable Diseases
Dr Janet Mans	University of Pretoria
Mr Vusi Ntema	Magalies Water
Prof. Moses Mbewe	University of Mpumalanga

The following people made the work easier and the authors would also like to thank them for assisting in the project:

Name	Organisation	Role
R Pieterse	University of Johannesburg	In-service Trainee
N Ramaru	Tshwane University of Technology	Intern – Data Quality Management
L Baloyi	Tshwane University of Technology	Data capturer
M Ratshivhadelo	Tshwane University of Technology	Data capturer
M Makhado	Management Sciences for Health	Data analysis
N Zitha	University of Venda	Sample collection
T Banda	University of Venda	Intern – Administrative Assistant
P Mukhuvha	University of Venda	Administrative assistant

CONTENTS

EXECUTIVE SUMMARY iii
CAPACITY BUILDING vi
LIST OF RESEARCH OUTPUTS vii
ACKNOWLEDGEMENTS viii
CONTENTS ix
LIST OF FIGURES xi
LIST OF TABLES xii
ACRONYMS AND ABBREVIATIONS xiii
CHAPTER 1: INTRODUCTION 1
1.1 Problem Statement 1
1.2 Aim of the Study 2
1.3 Objectives 2
1.4 Limitations of the Study 2
1.5 Ethical Approval 2
CHAPTER 2: AETIOLOGY AND EPIDEMIOLOGY OF DIARRHOEA – A REVIEW 3
2.1 Introduction 3
2.2 Common Diarrhoeagenic Pathogens 3
2.2.1 Bacterial pathogens 3
2.2.1.1 Escherichia coli 3
2.2.1.2 Vibrio cholerae 4
2.2.1.3 Salmonella 4
2.2.1.4 Shigella 4
2.2.2 Parasitic pathogens 4
2.2.2.1 Cryptosporidium 5
2.2.2.2 Giardia 5
2.2.2.3 Entamoeba histolytica 5
2.2.3 Viral pathogens 5
2.2.3.1 Rotaviruses 5
2.2.3.2 Noroviruses 6
2.2.3.3 Sapoviruses 6
2.3 Molecular Diagnosis of Diarrhoea 7
2.4 Treatment of Diarrhoea 7
2.5 Antimicrobial Resistance of Diarrhoeagenic Pathogens 7
2.5.1 What is antimicrobial resistance? 7
2.5.2 Antimicrobial resistance surveillance 9
CHAPTER 3: METHODS 12
3.1 Study Area 12
3.2 Stool Sample Collection 13
3.3 Stool Sample Analysis 14
3.4 Antibiotic Resistance Profiles of Bacterial Pathogens 15
3.4.1 Isolation of bacterial pathogens 15
3.4.1.1 Vibrio species 16
3.4.1.2 Salmonella and Shigella species 16
3.4.1.3 Coliforms and intestinal pathogens 16
3.4.1.4 Escherichia coli O157:H7 16
3.4.1.5 Staphylococci species 16

3.4.1.6 Campylobacter species 16
3.4.2 Isolate identification 16
3.4.3 Antimicrobial susceptibility testing 17
3.5 Statistical Analysis 17
CHAPTER 4: RESULTS AND DISCUSSION 18
4.1 Overview of the Study Population 18
4.1.1 Gender distribution 18
4.1.2 Age distribution 19
4.1.3 Clinical symptoms displayed by children before treatment 20
4.1.4 Stool type reported for children with diarrhoea 21
4.2 Pathogen Frequency and Distribution in Stool Samples 21
4.3 Antibiotic Susceptibility of Isolates 25
4.3.1 Bacterial isolation 26
4.3.2 Bacterial antimicrobial resistance 28
4.3.2.1 Gram-negative AST 28
4.3.2.2 Gram-positive AST 34
CHAPTER 5: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS 36
5.1 Summary of Findings and Conclusions 36
5.2 Recommendations 37
REFERENCES 38

LIST OF FIGURES

Figure 1: Map of Vhembe District Municipality 12
Figure 2: The BioFire® FilmArray® procedure 14
Figure 3: Illustration of the protocol followed to isolate, identify and characterise bacteria 15
Figure 4: Gender distribution of children in study cohort 18
Figure 5: Age distribution of children in study cohort 19
Figure 6: Distribution of clinical symptoms displayed by children in study cohort 20
Figure 7: Stool type distribution and frequency per health facility 21
Figure 8: Prevalence of selected parasite pathogens in stool samples of children 22
Figure 9: Prevalence of selected viral pathogens in stool samples in children 23
Figure 10: Bacterial prevalence in stool samples from children 24

LIST OF TABLES

Table 1: Summary of antimicrobial resistance mechanisms 8

Table 2: Summary of the target of antibiotics, their classification, examples and development 11

Table 3: List of primary health care clinics as per hospital referred 13

Table 4: Summary on BioFire® FilmArray® stool data from study cohort 22

Table 5: Summary of BioFire® FilmArray® results of the fresh and frozen stool samples 25

Table 6: Breakdown of number of samples analysed for bacterial isolation and identification 27

Table 7: Summary of the type of bacterial species isolated 27

Table 8: Summary of the AST results obtained for the *E. coli* strains (n = 115) tested and reported 29

Table 9: Summary of the AST phenotypes obtained for the *E. coli, Klebsiella* and *Salmonella* strains tested 31

Table 10: Summary of the antimicrobial resistance results obtained for the *K. pneumonia* strains (n = 7) tested and reported 32

Table 11: Summary of the antimicrobial resistance results obtained for the *Salmonella* strains (n = 5) tested and reported 32

Table 12: Summary of AST phenotypes obtained for the *Citrobacter* and *Enterobacter* strains tested 33

Table 13: Summary of the antimicrobial resistance results obtained for the *E. faecium/faecalis* strains (n = 22) tested and reported 34

Table 14: Summary of the AST phenotypes obtained for the *Enterococci* strains tested 34

Table 15: Summary of the antimicrobial resistance results obtained for the *Staphylococcus* strain (n = 1) tested and reported 35

ACRONYMS AND ABBREVIATIONS

AMR	–	Antimicrobial Resistance
APW	–	Alkaline Peptone Water
AST	–	Antimicrobial Susceptibility Testing
BHI	–	Brain Heart Infusion
BPW	–	Buffered Peptone Water
DNA	–	Deoxyribonucleic Acid
CNS	–	Coagulase Negative *Staphylococcus*
DAEC	–	Diffusely Adherent *E. coli*
EAEC	–	Enteroaggregative *E. coli*
EHEC	–	Enterohaemorrhagic *E. coli*
EIEC	–	Enteroinvasive *E. coli*
EPEC	–	Enteropathogenic *E. coli*
ESBL	–	Extended Spectrum Beta-lactamase
ESKAPE	–	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species
ETEC	–	Enterotoxigenic *E. coli*
GEMS	–	Global Enteric Multicentre Study
GERMS-SA	–	Group for Enteric, Respiratory and Meningeal Surveillance in South Africa
GI	–	Gastrointestinal
GLASS	–	Global Antimicrobial Resistance Surveillance System
IQR	–	Interquartile Range
KPC	–	*K. pneumoniae* carbapenemase
MAC-AMR	–	Ministerial Advisory Committee on Antimicrobial Resistance
MRSA	–	Methicillin-resistant *Staphylococcus aureus*
NDOH	–	National Department of Health
PCR	–	Polymerase Chain Reaction
RNA	–	Ribonucleic Acid
spp.	–	species
STEC	–	Shiga Toxin-producing *Escherichia coli*
TCBS	–	Thiosulfate-citrate Bile Salts Sucrose Agar
UK	–	United Kingdom
US	–	United States
WHO	–	World Health Organization

Page left blank intentionally

CHAPTER 1: INTRODUCTION

1.1 Problem Statement

Diarrhoeal diseases remain an important global health concern. The World Health Organization (WHO) estimates that almost 1.7 billion cases are reported each year resulting in more than 750 000 deaths in children younger than 5 years of age (WHO, 2015). In South Africa, it is estimated that 1.5 billion episodes of diarrhoea are reported per year and that diarrhoea is responsible for up to 10.2% of deaths per annum (Department of Health, 2016). A study conducted by Catherine et al. (2012) explained that the number of deaths from intestinal infectious diseases in South Africa rose from 14 276 in 2000 to 37 398 in 2007. This indicates an increase of 61.8% in the rate of deaths due to the intestinal infectious diseases. Diarrhoea has been reported as the leading cause of death (17.6%) in children under the age of 5 years (Bradshaw et al., 2003; Stats SA, 2014). The 2010 General Household Survey, a nationally representative inquiry into the livelihood of South Africans, showed that there were over 60 000 cases of childhood diarrhoea per month and approximately 9000 child diarrhoeal deaths in the same year (Chola et al., 2015). Infections caused by pathogenic enteric bacteria, parasites and viruses in the intestinal tract can result in diarrhoea (Bushen & Guerrant, 2003). Viruses (such as norovirus and rotavirus); bacteria (such as *Escherichia coli*, *Clostridium difficule*, *Shigella*) and parasites (such as *Cryptosporidium*, *Giardia*) are common pathogens that cause diarrhoea, which are often transmitted through contaminated water/food, and through poor sanitation (Binnicker, 2015).

Reliable field data from epidemiological studies is required to study diarrhoea epidemiology and the effect of interventions. In addition, rapid and accurate detection of diarrhoea-causing pathogens is important so that appropriate therapy can be introduced and suitable infection control and epidemiological interventions can be carried out to help stop or reduce the disease from spreading (Hatchette & Farina, 2011). The lack of confirmatory diagnosis for diarrhoea organisms is responsible for the abuse and improper prescription of antimicrobials. Antimicrobial resistance is a major threat to the effective treatment of infections, especially in vulnerable patients, resulting in prolonged illness and increased mortality.

The development of antibiotic resistance is typically associated with genetic changes in microorganisms (e.g. acquisition of resistance genes; mutations in elements relevant for the activity of the antibiotic) and in some situations, resistance can be achieved without any genetic alteration (e.g. phenotypic resistance) (Corona & Martinez, 2013). The impact of antimicrobial resistance on human health and on costs for the health care sector and the wider societal impact thereof are still largely unknown (Huttner et al., 2013).

When studying human infections, the WHO requires that data on the prevalence of *Acinetobacter* spp., *E. coli*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae* is reported. In South Africa, the National Department of Health, through its antimicrobial resistance stewardship programme, monitors only the ESKAPE group of pathogens (bacteria that are the main causes of nosocomial infections and usually highly resistant to antibiotics), which include *E. coli, K. pneumoniae, Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterococcus faecalis* and *E. faecium*; in addition, *Candida* spp. isolated from blood samples are also included (Navidinia, 2016).

Information on epidemiology and antimicrobial susceptibility patterns are limited. Although several studies in South Africa have reported on antimicrobial-resistant patterns of isolated diarrhoeal pathogens, several studies have been done in isolation and the protocols used are not standard and uniform; therefore, data is not always reliable. Recent developments of multiplex molecular assays for the detection and identification of diarrhoea-causing pathogens have allowed health care staff to achieve a fast diagnosis, which may be important in critically ill patients and vulnerable individuals.

1.2 Aim of the Study

Assess the prevalence, incidence and genetic diversity of pathogenic bacteria, viruses and parasitic organisms among children under the age of 5 years old suffering from diarrhoea in the Vhembe District, Limpopo Province, and to determine the antimicrobial resistance of diarrhoea-causing bacteria isolated from stool samples.

1.3 Objectives

The objectives of the study were as follows:

- Collect stool samples from children under the age of 5 years old suffering from diarrhoea from rural and peri-urban communities in the Vhembe District of the Limpopo Province.
- Determine genetic diversity of pathogenic bacteria, viruses and parasitic organisms in stool samples using a qualitative multiplexed nucleic acid-based in vitro diagnostic test.
- Isolate aerobic and micro-aerophilic bacteria associated with diarrhoeal disease.
- Identify Gram-positive and Gram-negative bacteria using VITEK 2™ cards.
- Determine the antimicrobial susceptibility of potential pathogenic, and selected commensal, bacterial isolates.
- Interpret the antimicrobial resistance data in terms of the National Department of Health and WHO guidelines.
- Make recommendations in relation to disease occurrence and prevention that could impact policies.
- Describe how the results obtained influence current antimicrobial resistance monitoring strategies.

1.4 Limitations of the Study

- No stool samples were collected from asymptomatic children.
- Some of the stool samples were frozen, which could have influenced the survival and recovery of the bacteria present in the samples.
- Due to the high number of isolates, limited funding and low detection with the BioFire® FilmArray® Gastrointestinal (GI) Panel, anaerobic bacteria were not included in the study to test for antimicrobial resistance.

1.5 Ethical Approval

Ethical approval was obtained from the Higher Degree Ethics Committee from the University of Venda (SMNS/16/MBY/07/2904). Permission to undertake the study was approved by:

- The Limpopo Provincial Department of Health.
- The Vhembe District Department of Health.
- The chief executive officers from the respective hospitals, namely, Tshilidzini Hospital, Louis Trichardt Memorial Hospital and Donald Fraser Hospital.

CHAPTER 2: AETIOLOGY AND EPIDEMIOLOGY OF DIARRHOEA – A REVIEW

2.1 Introduction

Diarrhoeal infections are estimated to be the cause of 1.3 million deaths annually in children younger than 5 years of age (Black et al., 2010; Walker et al., 2013). The prevalence of acute infectious diarrhoea differs regarding the time of year, geographical location and population studied (Dennehy, 2005). The frequency of mortality and morbidity among children younger than 5 years is a recurring and a dangerous problem in both emerging and industrialised countries (Gracey & King, 2009). The prevalence of diarrhoea is related to low household and community socio-economic status: approximately 88% of deaths associated with diarrhoea are caused by unsafe water, poor sanitation and unhygienic conditions (Magnani et al., 1993: Ahiadeke, 2000; Ezzati et al., 2002).

Diarrhoea is characterised by loose and watery stools occurring more than three times a day (Mwambete et al., 2010). Diarrhoea can last for several days and, in certain cases, can lead to serious complications such as dehydration that requires hospitalisation. A variety of bacterial, viral and parasitic organisms can cause diarrhoea. The occurrence of these pathogens varies among industrialised and emerging world settings. According to a systematic literature review done in 2013 by Lanata and co-workers on deaths due to diarrhoeal diseases, it was estimated that 70% of deaths are attributable to 13 pathogens. The review further showed that rotavirus, enteropathogenic *E. coli* (EPEC), entero-toxigenic *E. coli* (ETEC), calicivirus and *Shigella* were the most important pathogens. The Global Enteric Multicentre Study (GERMS-SA, 2015) agrees with this; however, GEMS also includes *Cryptosporidium* spp. as an important pathogen. Therefore, it is recommended that diarrhoea prevention and treatment should be a combination of the reports by Lanata et al. (2013) and Kotloff et al. (2013): it should concentrate on pathogens such as ETEC and EPEC*, Shigella* spp., rotaviruses, norovirus spp. and *Cryptosporidium* spp.

This section reviews the common causative agents, symptoms, diagnosis and aspects related to the treatment of diarrhoea, including cases involving resistant microorganisms.

2.2 Common Diarrhoeagenic Pathogens

2.2.1 Bacterial pathogens

2.2.1.1 Escherichia coli

E. coli bacteria are the leading etiologic agents of urinary tract and pyogenic infections, encompassing a highly heterogeneous group of strains (Khalili et al., 2012). *E. coli* is known to be able to withstand highly acidic environments and can survive at pH ranges as low as 3.3–4.2 (Johnson et al., 2012). *E. coli* strains that cause disease outside the intestine are known as extra-intestinal pathogenic *E. coli* of humans, which is why urine and stool consist of these strains (Johnson et al., 2012). Pathogenic strains of *E. coli* can be classified into six categories based on its virulence properties, namely (Newell, 2010):

- ETEC.
- EPEC.
- Enteroinvasive *E. coli* (EIEC).
- Enterohaemorrhagic *E. coli* (EHEC).
- Enteroaggregative *E. coli* (EAEC).
- Diffusely adherent *E. coli* (DAEC).

These strains have evolved from non-pathogenic pathogenic strains by acquiring mobile genetic elements such as colonising factors, enterotoxins, cytotoxins, haemolysis and invasins (Faruque, 2012).

2.2.1.2 Vibrio cholerae

Cholera, caused by *Vibrio cholerae*, is a severe epidemic diarrhoeal disease, which continues to devastate many developing countries where socio-economic conditions are poor, sanitary systems and public hygiene are not well developed, and safe drinking water is not available (Chen et al., 2007). Toxigenic strains of *V. cholerae* serogroups O1 and O139 are causative agents of cholera, which are spread by contaminated water and food (Charles & Ryan, 2011). *V. cholerae* have been isolated from surface water (Fraga et al., 2007), and the occurrence of *V. cholerae* in water sources can be linked to faecal pollution (Cox et al., 2005).

In November 2008, one of the largest African outbreaks of cholera occurred in Zimbabwe (Islam et al., 2011). Within the first 5 months of the outbreak, more than 73 000 cases and 3500 deaths had been reported (Mintz & Guerrant, 2009). This cholera outbreak spread to neighbouring Zambia and South Africa, causing thousands of additional cases (Mintz & Guerrant, 2009). In South Africa, the outbreak was first identified in Musina in the Limpopo Province (NICD, 2012). Between 15 November 2008 and 30 April 2009, a total number of 12 706 cases of cholera were reported by the National Department of Health (NICD, 2012). Of the given total number of cases, 1114 (9.0%) were laboratory-confirmed cases, and 65 deaths (case fatality rate of 0.5%) were recorded (NICD, 2012).

All nine provinces within South Africa were affected, with most cases being reported by the Mpumalanga (54%) and Limpopo (43%) provinces. The causative organism was a multidrug-resistant strain of *V. cholerae* O1 (NICD, 2012). Cholera cases have also been reported in Mozambique where people travelled through the Beitbridge area in Zimbabwe (Department of Health, 2008). An inadequate supply of clean drinking water and poor levels of hygiene have been implicated as the reasons for these outbreaks (WHO, 2008).

2.2.1.3 Salmonella

The genus *Salmonella* belongs to the family of *Enterobacteriaceae*. Compared with *E. coli*, *Salmonella* appears to withstand a wider variety of environmental fluctuations and may persist in various water environments for extended periods (Winfield & Groisman, 2003). Biofilms of potable water distribution systems have the potential to harbour *Salmonella.* Low numbers (15–100 colony-forming units) of *Salmonella* in water may pose a public health risk (Jyoti et al., 2009). In the aquatic environment, this pathogen has been detected repeatedly in various types of natural waters such as rivers, lakes, coastal waters, estuaries as well as in contaminated groundwater (Moganedi et al., 2007; Haley et al., 2009; Wilkes et al., 2009; Levantesi et al., 2010). *Salmonella* presence has been attributed to run-off from fields with animal husbandry in addition to untreated sewage from nearby communities in natural water resources (Moganedi et al., 2007; Jenkins et al., 2008).

2.2.1.4 Shigella

The genus *Shigella* consists of four species, which include *Shigella dysenteriae, S. flexneri, S. boydii* and *S. sonnei* (Kingombe et al., 2005). *Shigella* species are Gram-negative, non-motile, non-encapsulated, non-lactose fermenting, facultative anaerobe bacteria that are pathogenic to human beings (Penatti et al., 2007). *Shigella* bacteria cause an acute intestinal illness called shigellosis. Their mode of transmission is usually through the ingestion of contaminated food and water, as well as person-to-person contact. In 2009, the outbreak in South Africa had 1812 cases of invasive and non-invasive shigellosis, predominantly in children younger than 5 years (Keddy, 2010).

2.2.2 Parasitic pathogens

Parasitic diarrhoeal diseases have surfaced in recent years, which have highlighted the importance of protozoan parasites in causing diarrhoea, particularly *Giardia* spp., *Cryptosporidium* spp. and *Entamoeba* spp. (Obi et al., 2002; Hunter & Thompson, 2005; Samie et al., 2006). *Giardia* and *Cryptosporidium* are responsible for waterborne outbreaks of gastroenteritis via contaminated drinking

water as well as recreational waters. These species cause diarrhoeal diseases in humans and animals (Lalancette et al., 2010). They infect the small or large intestine, or both. Giardiasis and cryptosporidiosis are important causes of diarrhoea in children; cryptosporidiosis is particularly associated with growth failure and malnutrition.

2.2.2.1 Cryptosporidium

Cryptosporidium species, which are coccidian protozoan parasites, play an important role in the epidemiology of cryptosporidiosis. The species of concern are *Cryptosporidium hominis* (which exclusively infect humans) and *C. parvum* (which infect humans, ruminants and other animals) (Samie et al., 2006). According to Dallingham et al. (2002), *Cryptosporidium* have shown to dominate in diarrhoeic stools relative to non-diarrhoeic stools. The coccidian parasite is implicated in sporadic, often water-related outbreaks of self-limiting diarrhoea in otherwise healthy persons; in chronic, life-threatening illness in immunocompromised patients, most notably those with HIV/AIDS; and in diarrhoea and malnutrition in young children in developing countries (Mor & Tzipor, 2008). *Cryptosporidium* has been considered as one of the most frequently identified etiological agents, which is associated with 23.7% of drinking-waterborne illness outbreaks worldwide (Lalancette et al., 2010). Prevalence of cryptosporidiosis during childhood in developing countries culminates to as many as 45% of children experiencing the disease before the age of 2 years.

2.2.2.2 Giardia

Giardia is a flagellated protozoan parasite that is luminal; its trophozoites reside predominantly in the proximal small intestine. Giardiasis causes diarrhoea of varying severity and malabsorption in some patients. According to Wright (2005), *Giardia* is more common in areas where personal and public sanitation are inadequate and, in these areas, it mainly infects children. Transmission is through contaminated food or water, person-to-person contact during sexual activity or among children. *Giardia* cysts are an important mode of transmission because the cysts remain viable in the environment for days to weeks when conditions are cool and moist.

2.2.2.3 Entamoeba histolytica

Amoebiasis is mainly a disease of the tropics and sub-tropics where sanitation is commonly inadequate. It affects adults more often than children, and males more often than females, in particular in the case of amoebic abscesses (Wright, 2005). *Entamoeba histolytica* is a micro-aerophilic protozoan parasite that exhibits two clinical forms, namely, cysts and trophozoites. Trophozoites are motile and are found in the large intestines of humans. They encyst and form resistant cysts, which are excreted in faeces (about 2–4% of asymptomatic individuals), which are the source of infection as they contaminate the environment, food and water. Following ingestion of food or water contaminated by faeces, excystation occurs in the intestines and trophozoites invade the large bowel. Necrosis is the trademark of invasive amoebiasis, which produces flask-shaped, undermined ulcers with amoeba in the base and advancing margins. Amoebic liver abscesses manifest. In some instances, metastasis from the liver to the brain is inevitable; hence, brain abscess can occur.

2.2.3 Viral pathogens

2.2.3.1 Rotaviruses

Rotaviruses form a genus *Rotavirus* within the *Reoviridae* family (Estes & Kapikian, 2007). They can be divided into seven groups, namely, Group A to Group G, with most human infections caused by viruses of Group A (Adlhoch et al., 2011). Rotaviruses are non-enveloped ribonucleic acid (RNA) viruses (Van Zyl et al., 2006). Group A rotaviruses are the most important cause of acute viral gastro-enteritis in infants and young children (Van Zyl et al., 2006). Rotaviruses replicate within the host in the gastrointestinal tract from where they are shed in large quantities. They may be disseminated widely into environmental waters such as groundwater (Post et al., 2011), surface water (Grassi et al., 2010),

drinking water (He et al., 2009), and waste water (Van Zyl et al., 2006). Group A rotaviruses have been detected in untreated and treated drinking water samples in southern Africa (Van Zyl et al., 2006).

2.2.3.2 Noroviruses

Noroviruses belong to the genus *Norovirus*. They are members of the *Caliciviridae* family, which comprises non-enveloped, single-stranded RNA viruses. Noroviruses have been found in humans, pigs, cattle, sheep and mice (Wolf et al., 2010). Noroviruses are currently classified into five genogroups (Patel et al., 2009). Three of these genogroups, namely, GI, GII and GIV occur in human infections (Green, 2007); however, most noroviruses affecting humans belong to GI or GII (Burton-MacLeod, 2004).

In a major review of norovirus classification, Zheng et al. (2006) examined 164 amino acid sequences from the capsid region of both human and animal noroviruses. Zheng et al. (2006) identified five genogroups comprising 29 genotypes, with eight genotypes in GI; 17 genotypes in GII; two genotypes in GIII; and one genotype each in GIV and GV. More recently, Patel et al. (2009) identified 32 genotypes among the five genogroups.

The most common cause of human norovirus outbreaks worldwide is the GII.4 norovirus. Genetic research focuses significantly on GII.4. The strains of GII.4 frequently undergo genetic change and these altered forms are sometimes termed “variants” (Siebenga et al., 2008) or “sub-types” (Motomura et al., 2010). There is some evidence that the emergence of new GII.4 variants correlates with the occurrence of norovirus outbreak epidemics (John & Leesa, 2011). Human noroviruses are the most common etiological agent for gastroenteritis outbreaks as well as the leading cause of non-bacterial gastroenteritis in all age groups (Siebenga et al., 2008; Gentry et al., 2009).

In South Africa, norovirus-associated gastroenteritis outbreaks were first reported in 1993, where the Norwalk (GI.1) and Hawaii (GII.1) strains were each identified as causative agents in outbreaks (Taylor et al., 1993). In 2008, noroviruses were characterised from paediatric patients hospitalised with gastroenteritis in the Pretoria region of South Africa. Noroviruses were detected in 14% of stool specimens; the characterised strains included three GI and eight GII genotypes with GII.4 being predominant (Mans et al., 2014).

2.2.3.3 Sapoviruses

Together with the norovirus, the sapovirus belongs to the family *Caliciviridae.* The sapoviruses are the only medical pathogens in this family. Five genogroups (GI–GV) have been described to date. They appear to correlate with antigenic differences in the VP1 protein. Genotypes I, II, IV and V are considered as known groups that are able to infect humans (Roman et al., 2012). Illnesses due to sapovirus tends to occur predominantly in young children: by the age of 5, virtually all children would have had experienced an infection by sapovirus.

As one of the global causes of viral gastroenteritis, sapovirus is also associated with sporadic cases and outbreaks of gastroenteritis worldwide. Sapovirus prevalence was shown to range from 0.3% to 9.3%, and is usually much lower than norovirus (Hansman, 2014). However, in most cases, sapovirus accounts for about 5% of cases of infantile diarrhoea. Chhabra et al. (2013) detected sapovirus in the stool specimens of 5.4% of patients with acute gastroenteritis and in 4.2% of healthy controls in children younger than 5 years of age in the United States. In a study done in Australia following outbreaks of acute gastroenteritis, White et al. (2012) revealed that the prevalence of human sapovirus in the stool specimens of children presenting with sporadic gastroenteritis at the hospital was less than 5% in samples from the patients examined.

Studies in Japan have detected sapovirus in clams, oysters and water, which implies that environmental samples might be a source of sapovirus transmission. However, the environmental source of human sapovirus transmission is not completely understood (White et al., 2012). In South Africa, norovirus and

sapovirus have been detected in hospitalised paediatric patients in the Gauteng Province. However, norovirus was more prevalent than sapovirus and the detection was most frequently after rotavirus (Mans et al., 2010). In a study by Murray et al. (2013) on human calicivirus diversity in waste water in South Africa, 72.5% of the samples tested were positive for sapovirus.

2.3 Molecular Diagnosis of Diarrhoea

Conventional methods such as culture and antigen detection are time-consuming, laborious and not always specific (Binnicker, 2015; Huang et al., 2016). The objective is to have a test that is fast, reliable, accurate, not expensive, specific, approved by relevant authorities, and able to detect more than one organism simultaneously. This will bring about more accurate diagnosis and more effective treatment for patients.

Currently, multiplex polymerase chain reaction (PCR) assays to detect and identify infectious microorganisms are commercially available (Khare et al., 2014; Stockmann et al., 2015; Spina et al., 2015; Stockmann et al., 2017; Murphy et al., 2017). One such test is the BioFire® FilmArray® test, which simultaneously detects 22 organisms (bacteria, viruses and parasites) in a stool sample within one hour. Studies have shown that the sensitivity and specificity of the gastrointestinal panel were 94.5–100% and 97.1% respectively (Buss et al., 2015).

2.4 Treatment of Diarrhoea

Diarrhoea in children is usually treated after their immune systems fail to clear the infection, which causes diarrhoea that lasts for more than a few days. Failure to treat diarrhoea at home leads to children visiting primary health care facilities where treatments given to children with acute diarrhoea usually include an oral antibiotic such as ceftriaxone, as well as sodium chloride, oral rehydration therapy and zinc. In cases of severe dehydration caused by diarrhoea, the child is either admitted to hospital or treated with doxycycline, tetracycline, erythromycin, ciprofloxacin, metronidazole or vancomycin antibiotics (Caramia et al., 2015). Antibiotics administered in severe cases have helped intensely in curing children infected with enteric bacterial pathogens. However, antimicrobial resistance in bacteria due to misuse and overuse of antibiotics threatens to undermine the control of diarrhoea caused by bacterial infections (Sang et al., 2012; Ventola, 2015). As with the antibiotics, discussing antimicrobial resistance is a hefty task influenced by the type of mechanism used, the organisms discussed and the stage of adaptation.

2.5 Antimicrobial Resistance of Diarrhoeagenic Pathogens

2.5.1 What is antimicrobial resistance?

Antimicrobial resistance is the ability of a microorganism (such as bacteria, viruses and some parasites) to stop an antimicrobial treatment (such as an antibiotic, antiviral or anti-malarial medication) from working against it (WHO, 2018). Table 1 summarises the various antimicrobial resistance approaches used by bacteria. As a result of these approaches, standard treatments become ineffective, infections persist and may even spread to others (Michael et al., 2014; WHO, 2018).

Antimicrobial resistance is a growing threat to the effective treatment of infections, especially vulnerable patients, resulting in prolonged illness and increased mortality. The development of antibiotic resistance is typically associated with genetic changes in microorganisms – either through the acquisition of resistance genes, or to mutations in elements relevant to the activity of the antibiotic. However, in some situations, resistance can be achieved without any genetic alteration. This is called phenotypic resistance (Corona & Martinez, 2013).

Table 1: Summary of antimicrobial resistance mechanisms as described by Munita and Arias (2016)

Antimicrobial resistance method	Explanation and examples
A) Genetic basis of antimicrobial resistance	
1) Mutational resistance	Mutations in gene(s) often associated with the mechanism of action of the compound.
2) Horizontal gene transfer	Acquisition of foreign deoxynucleic acid (DNA) coding for resistance determinants through horizontal gene transfer.
I. Transformation	Incorporation of naked DNA.
II. Transduction	Phage mediated.
III. Conjugation	Gene transfer that involves cell-to-cell contact.
B) Mechanistic basis	
1) Modify antibiotic molecule	Produce enzymes that inactivate the drug by adding specific chemical moieties to the compound or that destroy the molecule itself.
I. Chemical alterations	Biochemical reactions include: i) acetylation (aminoglycosides, chloramphenicol, streptogramins), ii) phosphorylation (aminoglycosides, chloramphenicol), and iii) adenylation (aminoglycosides, lincosamides).
II. Destruction of antibiotic molecule	Example is beta-lactam resistance by producing beta-lactamase to destroy beta-lactam ring.
2) Decrease antibiotic penetration and efflux	
I. Decrease permeability	Decreasing uptake of the antimicrobial molecule to prevent the antibiotic from reaching its intracellular or periplasmic target.
II. Efflux pumps	Production of bacterial machineries to extrude a toxic compound from the cell.
III. Change in target sites	Protection of the target (avoiding the antibiotic to reach its binding site) and modifications of the target site that result in decreased affinity for the antibiotic molecule.
a) Target protection	Example target protection mechanism like tetracycline resistance determinants Tet(M) and Tet(O).
b) Modification of target site	
I. Mutations of target site	Example of mutational resistance is the development of rifampin resistance.
II. Enzymatic alteration of target site	Example methylation of the ribosome catalysed by an enzyme encoded by the erm genes (erythromycin ribosomal methylation), which results in macrolide resistance.
III. Replacement of bypass of target site	Examples include methicillin resistance in *Staphylococcus aureus* due to the acquisition of an exogenous PBP (PBP2a) and vancomycin resistance in *Enterococci* through modifications of the peptidoglycan structure mediated by the van gene clusters.
c) Resistance due to global cell adaptations	Examples are development of resistance to daptomycin and vancomycin (low-level in *Staphylococcus aureus*) resistance phenotypes that are the result of a global cell adaptive response to the antibacterial attack.

2.5.2 Antimicrobial resistance surveillance

One of the first obstacles a researcher working on antimicrobial resistance faces is exactly what to test for. The WHO's Global Action Plan for antimicrobial resistance recommends that in order to study antimicrobial resistance in terms of the One Health approach, an integrated approach must be taken to monitor the spread of antimicrobial resistance. WHO recommends testing different bacteria (with some overlap) from different sources and for different combinations of antibiotics (refer to Table 1-1 in WHO, 2017). Although the WHO and all its collaborators support the requirement for a more combined monitoring approach, the question driving the research often dictates the selected organisms.

When studying actual human infections, the WHO requests, as part of its early implementation phase (2015–2019), data on *Acinetobacter* spp., *E. coli, K. pneumoniae, N. gonorrhoeae, Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae*. The data is collected through a case-finding surveillance system that collects results from blood, urine, stool, as well as cervical and urethral specimens. It should be noted that only *Salmonella* and *Shigella* species are collected from stool samples. *E. coli*, for instance, is limited to urine and blood samples.

When looking at the interaction between animals and humans, the WHO, via their food surveillance programme, recommends testing for *Salmonella, E. coli, Campylobacter, Enterococcus* and *Staphylococcus* species (WHO, 2017). A similar recommendation is made for pathogens from agriculture and animals. It is even recommended in the sampling approach that human stool samples be tested and not necessarily extra-intestinal samples (example blood cultures) only. As an example, the WHO states in its 2014 report that "Infections with *E. coli* usually originate from the person affected (auto-infection), but strains with a particular resistance or disease-causing properties can also be transmitted from animals, through the food chain or between individuals" (WHO, 2014).

South Africa pledged its commitment to the World Health Assembly resolution EB134/37 "Combating Antimicrobial Resistance Including Antibiotic Resistance", with the subsequent development of the Antimicrobial Resistance National Strategic Framework, 2014–2024 (Antimicrobial Resistance Strategic Framework). This framework relies on the commitment of most of the key stakeholders within the One Health triad (human and animal health; agriculture) as well as science and technology sectors to combat antimicrobial resistance in the country (NDOH, 2015).

The primary national governance structure for antimicrobial resistance is the Ministerial Advisory Committee on Antimicrobial Resistance (MAC-AMR). This is a multi-disciplinary committee within the National Department of Health, which includes multiple intersectoral members to optimise the national One Health response to antimicrobial resistance. Included in the MAC work plan is structured national surveillance to determine antibiotic consumption (from multiple sectors) and antibiotic resistance (public and private sector) and to document the observed trends in bacterial resistance to antibiotics (NDOH, 2017). Current surveillance is mainly reported on by the Group for Enteric, Respiratory and Meningeal Surveillance in South Africa (GERMS-SA), which is a network of clinical microbiology laboratories (in the public and private sector) that participate in an active laboratory-based surveillance programme for pathogens of public health importance (GERMS-SA, 2015). Access to funding and staff who can continuously do surveillance affect the number of samples and organisms tested, but they still deliver a much-needed service.

The first contribution to the WHO Global Antimicrobial Resistance Surveillance System (GLASS) report, covering data submitted for 2016–2017, submits mainly data on *Staphylococcus aureus* and *Streptococcus pneumoniae* (WHO, 2018). None other organisms were reported, which highlights the need for more collaboration between all stakeholders to gather trustworthy data. The National Department of Health in South Africa, through it antimicrobial resistance stewardship programme, monitors only for the so-called ESKAPE pathogens, which refer to bacteria that are the main causes of nosocomial infections that are usually highly resistant to antibiotics (Navidinia, 2016). The ESKAPE

pathogens include *E. coli, K. pneumoniae, Staphylococcus aureus, A. baumannii, P. aeruginosa, E. faecalis* and *E. faecium*. In addition, *Candida* spp. isolated from blood samples are also included. Depending on the source, *Enterobacter* rather than *E. coli* isolates may be included, which will be discussed later.

These multiple recommendations, not to mention the numerous standards for interpreting antibiotic minimum inhibitory concentrations and breakpoints, create a confusing environment for researchers working in One Health sectors. It may be necessary that the research community starts looking at which pathogens need to be sampled, isolated and tested for (guided by medical professionals). This will assist in creating proper selected workgroups that work towards the common goal of protecting our communities and the most vulnerable, and not simply one group or small isolated groups of people who gather information that is analysed using the most convenient standards available.

A bit more daunting and confusing task is selecting antibiotics to be included in the experiments when disc diffusion or tube dilution methods are used. The task seems enormous simply based on the number of antibiotics available, the need to understand the way they are administered, their target organisms etc. Table 2 summarises the antibiotic target, as well as the classes and examples. It takes a while to understand that this is not necessarily such a daunting task if you compare antimicrobial resistance and water testing methods. Take for instance testing water for bacteria. It is not feasible to test every sample for every bacterium and it becomes easier to simply test for an indicator of what you are looking. For example, *E. coli* is typically used as indicator of faecal pollution. Similarly, there are ways of testing for specific groups of antibiotics if you know the so-called “game rules”. Different types of antibiotic are grouped into classes per their chemical and pharmacological properties: those with similar properties fall into their own individual antibiotic class. Antibiotics within the same class regularly have similar configurations of effectiveness, toxicity and allergic potential, which result in their capability of being able to kill the same or related bacteria (Walsh, 2003). While some antibiotics can completely kill bacteria, some are only able to inhibit bacterial growth. Those that kill bacteria are termed bactericidal while those that inhibit bacterial growth are termed bacteriostatic (Walsh, 2003).

Although antibiotic generally refers to antibacterial, it must be remembered that antibiotic compounds are differentiated as antibacterial, antifungals and antivirals to reflect the group of microorganisms they antagonise (Brooks et al., 2004; Russell, 2004). Antibiotics target and inhibit specific functions related to cell wall synthesis, protein synthesis, DNA and RNA synthesis, and mycolic acid and folic acid synthesis (Etebu & Arikekpar, 2016). These form the basis for the classification of the antibiotics (Table 2). The exact mechanisms and adaptation of current, or even development of new antibiotics is a broad field that cannot be covered in detail in this report. As much as there is a need to control bacterial, viral and fungal infection from a human perspective, there is a need for survival from the microorganism’s perspective. In a review done by Munita and Arias (2016) this is perfectly summarised by stating:

> “*The bacterial response to the antibiotic “attack” is the prime example of bacterial adaptation and the pinnacle of evolution. “Survival of the fittest” is a consequence of an immense genetic plasticity of bacterial pathogens that trigger specific responses that result in mutational adaptations, acquisition of genetic material or alteration of gene expression producing resistance to virtually all antibiotics currently available in clinical practice.*”

Table 2: Summary of the target of antibiotics, their classification, examples and development

Antibiotic Target		Antibiotic Group	Antibacterial Class		Antibiotics			
Cell wall synthesis	Beta-lactams	Penicillins	Penicillinase Sensitive	Natural Penicillins	Penicillin G	Penicillin V		
				Aminopenicillins	Ampicillin	Amoxicillin		
			Penicillinase Resistant		Oxacillin	Dicloxacillin	Methicillin	
					Nafcillin	Cloxacillin		
			Anti-pseudomonal	Carboxypenicillins	Ticarcillin	Carbenicillin		
				Ureidopenicillins	Azlocillin	Mezlocillin	Piperacillin	
		Cephalosporins	1st Generation		Cefazolin	Cephalexin	Cephapirin	Cefadroxil
					Cephadrine	Cephalotin		
			2nd Generation		Cefuroxime	Cefoxitin	Cefotetan	Cefamandole
					Cefprozil	Cefmetazole	Cefonicid	Cefaclor
			3rd Generation		Cefoperazone	Ceftriaxone	Ceftazidime	Cefpodoxime
					Cefdinir	Ceftibuten	Cefixime	Cefditoren
					Ceftizoxime	Cefotaxime		
			4th Generation		Cefepime	Cefpirome		
			5th Generation		Ceftaroline			
		Carbapenems			Ertapenem	Doripenem	Imipenem	Meropenem
		Monobactams			Aztreonam			
		Beta-lactamase inhibitors			Sulbactam	Tazobactam	Clavulanic acid	
	No lactam	Glycopeptides			Vancomycin	Teicoplanin	Polymyxin B	Polymyxin E
					Ramoplanin	Decaplanin	Bacitracin	Telavancin
Protein synthesis	Anti-30S ribosomal sub-unit	Aminoglycoside			Gentamycin	Neomycin	Streptomycin	Amikacin
					Tobramycin			
		Tetracyclines			Doxycycline	Demeclocycline	Minocycline	Tigecycline
					Tetracycline			
		Oxazolidinones			Linezolid			
	Anti-50S ribosomal sub-unit	Streptogramins			Quinupristin	Dalfopristin		
		Chloramphenicol			Chloramphenicol			
		Macrolides			Erythromycin	Azithromycin	Clarithromycin	
		Lincosamides			Clindamycin	Lincomycin		
DNA synthesis inhibitors		Fluoroquinolones	1st Generation		Nalidixic acid			
			2nd Generation		Norfloxacin	Levofloxacin	Ofloxacin	Enoxacin
					Ciprofloxacin			
			3rd Generation		Gatifloxacin			
			4th Generation		Moxifloxacin	Gemifloxacin		
		Metronidazole			Metronidazole			
Folic acid synthesis		Trimethoprim/ Sulphonamides			Trimethoprim/ Sulfamethoxazole	Sulfadiazine	Sulfisoxazole	
		DHFR inhibitors			Trimethoprim	Pyrimethamine		
RNA synthesis		Rifampin			Rifampin			

CHAPTER 3: METHODS

3.1 Study Area

The study was carried out in the northern part of the Limpopo Province (South Africa), which has five district municipalities. This study focused specifically on the Vhembe District Municipality, which comprise four local municipalities, namely, Musina, Mutale, Thulamela and Makhado (Figure 1). The Vhembe District is bounded in the north by the Limpopo River, in the west by the Sand River, and in the south and east by the Luvuvhu River (Samie et al., 2011).

The Vhembe District is largely a rural area faced with infrastructural backlogs for water, sanitation and electricity, which affect the health of the communities negatively. The rural and peri-urban communities share their water sources with animals, which leads to contamination. Due to a lack of household waste (rubbish) removal, communities contaminate water sources. Due to water shortages, communities collect water in various containers and store these. In addition, many rural households still use fresh cow dung to smear and draw patterns on their floors. Sanitation in the rural and peri-urban areas is still basic and many households use the same dishcloth for several purposes; a practice that can add to the transmission of pathogens.

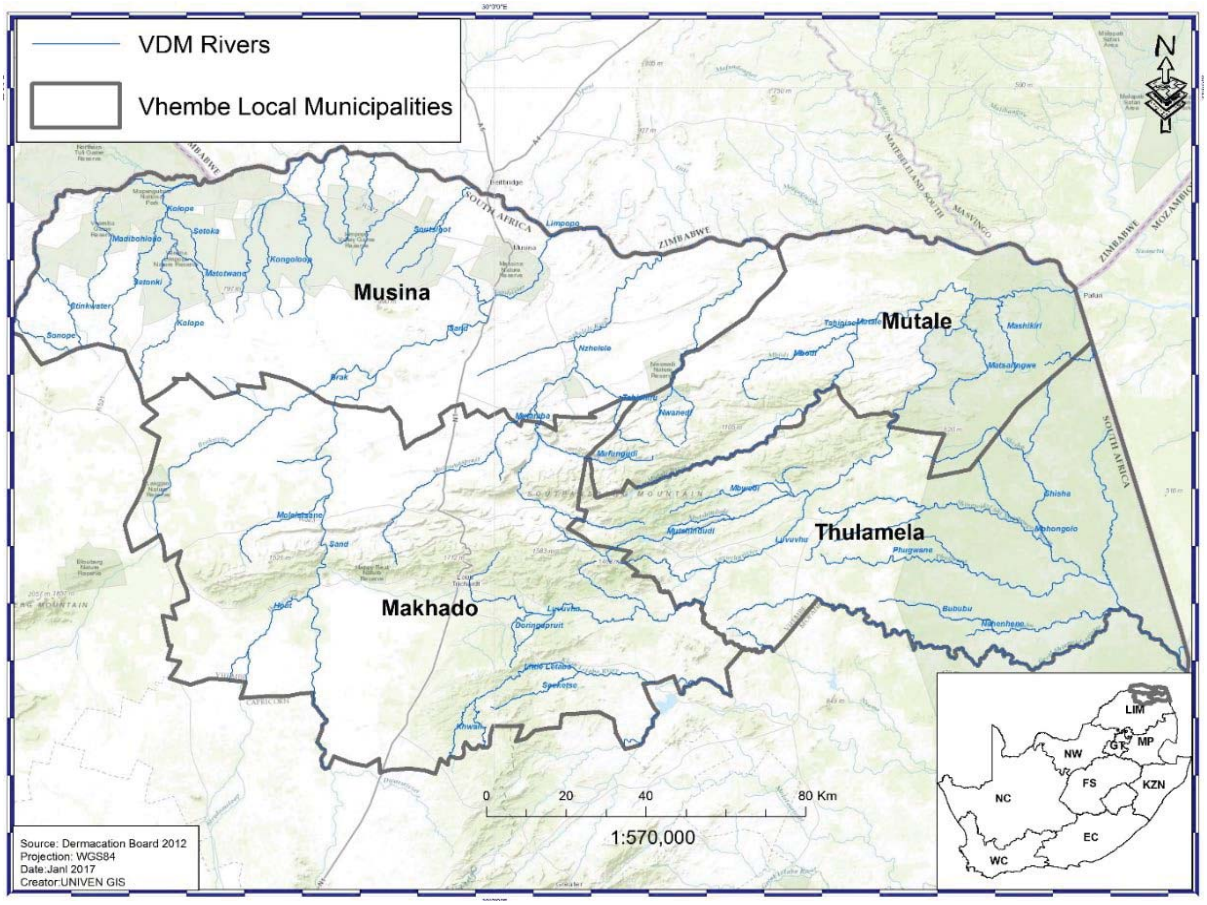


Figure 1: Map of Vhembe District Municipality

3.2 Stool Sample Collection

Two district hospitals from rural and urban settings as well as one regional hospital were included in the study. The regional hospital included Tshilidzini Hospital, which is situated in a rural area. The two district hospitals included Louis Trichardt Memorial Hospital situated in an urban area and the Donald Fraser Hospital situated in a rural area. Tshilidzini Hospital is the referral centre for all district hospitals in Vhembe District Municipality. The primary health care clinics where stool specimens were collected all served under these hospitals in the Vhembe District (Table 3).

A total of 184 diarrhoeal stool samples from children under the age of 5 years were collected from primary health care clinics in the Vhembe District for a period of 12 months from June 2015 to June 2016. Clinic nurses assisted with collecting diarrhoeal samples from clinics patients. The stool specimens were kept at 4°C until collection whereafter the samples were delivered to the laboratory where they were processed further.

A total of 91 stool samples were collected (severe cases of diarrhoea) from hospitals for a period of 4 months from September to December 2016. After obtaining consent from caregivers and gathering relevant information, the data capturer collected the stool samples directly at the hospital using a stool specimen swab with Cary Blair transport media. The stool swabs were kept at 4°C until collected and delivered it to the laboratory where the samples were processed further.

Table 3: List of primary health care clinics as per hospital referred

Donald Fraser Hospital	Tshilidzini Hospital	Louis Trichardt Hospital
Matavhela Clinic	Davhana Clinic	Kutama Clinic
Thengwe Clinic	Ha Mutsha Clinic	Madombidza Clinic
Shakadza Clinic	Levubu Clinic	Louis Trichardt PHC
Tshiungani Clinic	Manavhela Clinic	Tshakuma Clinic
Tshikundamalema Clinic	Tshino Clinic	Tshilwavusiku CHC
Mutake CHC	Tshimbupfe Clinic	Midoroni Clinic
Tshipise Cinic	Shayandima Clinic	
Masisi Clinic	Mulenzhe Clinic	
Mulala Clinic	Magwedzha Clinic	
Makuya Clinic	Tswinga Clinic	
Manenzhe Clinic	Tshisaula Clinic	
Tsaulu Clinic	Muledani Clinic	
Vhuru-Vhuru Clinic	Lwamondo Clinic	
Sambandou Clinic	Dzwerani Clinic	
Lambani Clinic	Tshilidzi Gateway	
Duvhuledza Clinic	Sibasa Clinic	
Tshifudi Clinic	Tshififi Clinic	
Tshiombo Clinic	Mbilwi Clinic	
Makonde Clinic	Pfanani Clinic	
Damani Clinic	Phiphidi Clinic	
Mukula Clinic	Thohoyandou CHC	
Sterstroom Clinic		
Thondo Tshivhase Clinic		
Gondeni Clinic		
William Eddie CHC		
Vhufulu-Tshitereke		
Rambuda Clinic		

PHC: primary health clinic; CHC: community health centre

3.3 Stool Sample Analysis

All stool samples were analysed using the BioFire® FilmArray® GI Panel, which is a qualitative multiplexed nucleic acid-based in vitro diagnostic test, intended for use with FilmArray® systems. The FilmArray® GI Panel can simultaneously detect and identify nucleic acids from multiple bacteria, viruses and parasites directly from stool samples in Cary Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. A total of 22 organisms that included the following were analysed in each diarrhoea sample:

- Parasites: *Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*.
- Bacteria: *Campylobacter*, *C. difficule* toxin A/B, *Plesiomonas shigelloides*, *Salmonella*, *Vibrio*, *V. cholera*, *Yersinia enterocolitica*, EAEC, EPEC, ETEC, Shiga toxin-producing *E. coli* (STEC), *E. coli* O157, Shigella-EIEC.
- Viruses: adenovirus F40/41, astrovirus, norovirus GI/GII, rotavirus A, sapovirus.

The principle of the test is shown in Figure 2.

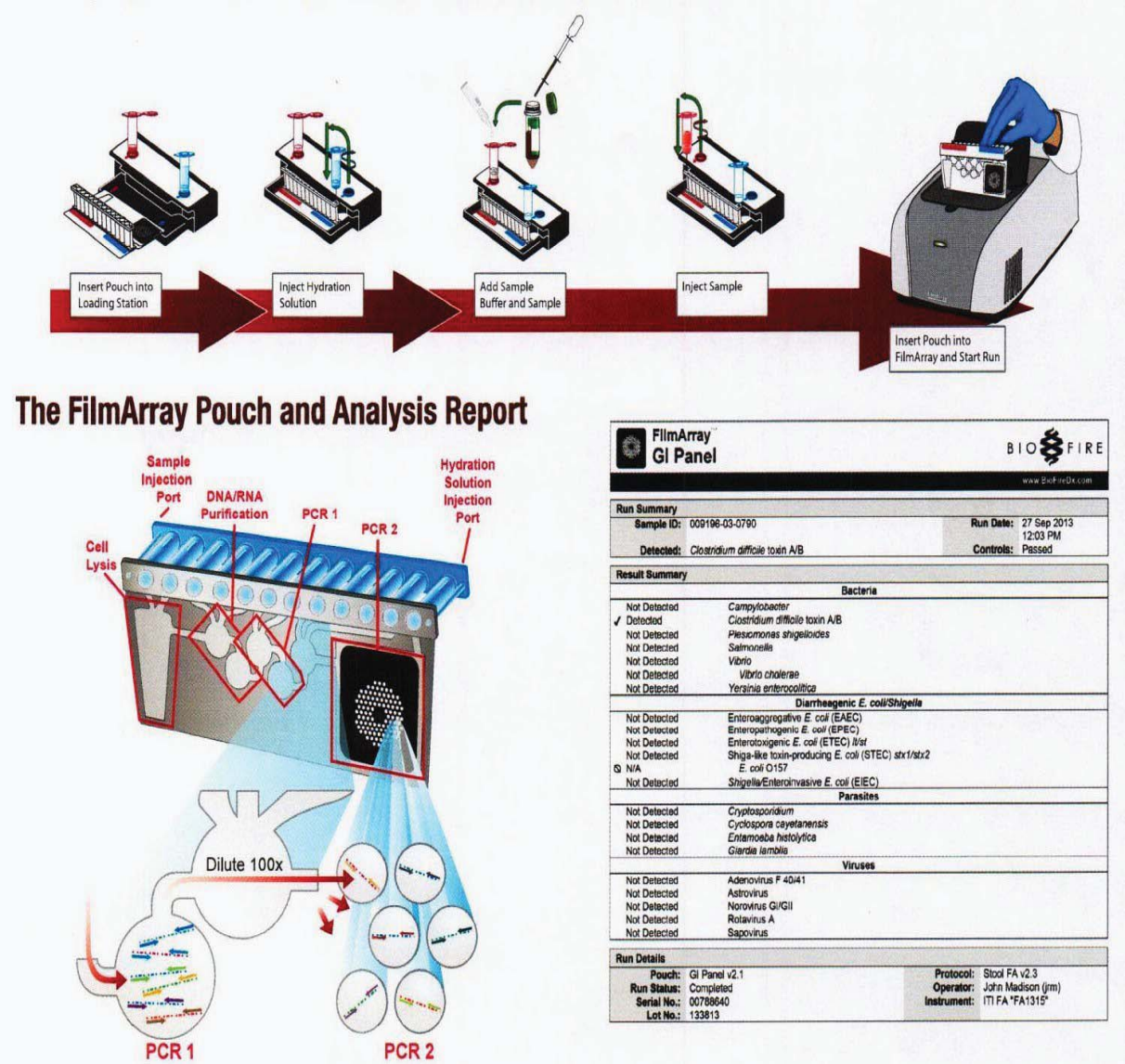


Figure 2: The BioFire® FilmArray® procedure

3.4 Antibiotic Resistance Profiles of Bacterial Pathogens

3.4.1 Isolation of bacterial pathogens

The frozen and fresh stool samples were analysed as outlined in Figure 3. The frozen samples were subjected to an additional initial enrichment step to increase the revival and recovery of bacteria from the samples. This isolation protocol was chosen to isolate aerobic and micro-aerophilic bacteria associated or responsible for diarrhoea, as outlined in the following sections.

Fresh and frozen stool samples were enriched in either alkaline peptone water (APW; Oxoid; Cat no. CM1028) or buffered peptone water (BPW; Oxoid; Cat no. CM0509) to enhance the likelihood of isolating *Vibrio* species (APW) or *Salmonella* and *Shigella* species (BPW) from stool samples. The APW and BPW were inoculated with a loopful of the sample and incubated for 24 hours at 35°C. Following incubation, an inoculation loopful of the sub-cultured sample was plated onto the different agar media (Figure 3). For frozen samples, an inoculation loopful of stool sample was also enriched in brain heart infusion broth (BHI; OXOID; Cat no. CM1135) at 35°C for 24 hours. Following incubation, the enriched sample was treated as a fresh stool sample and analysed as such.

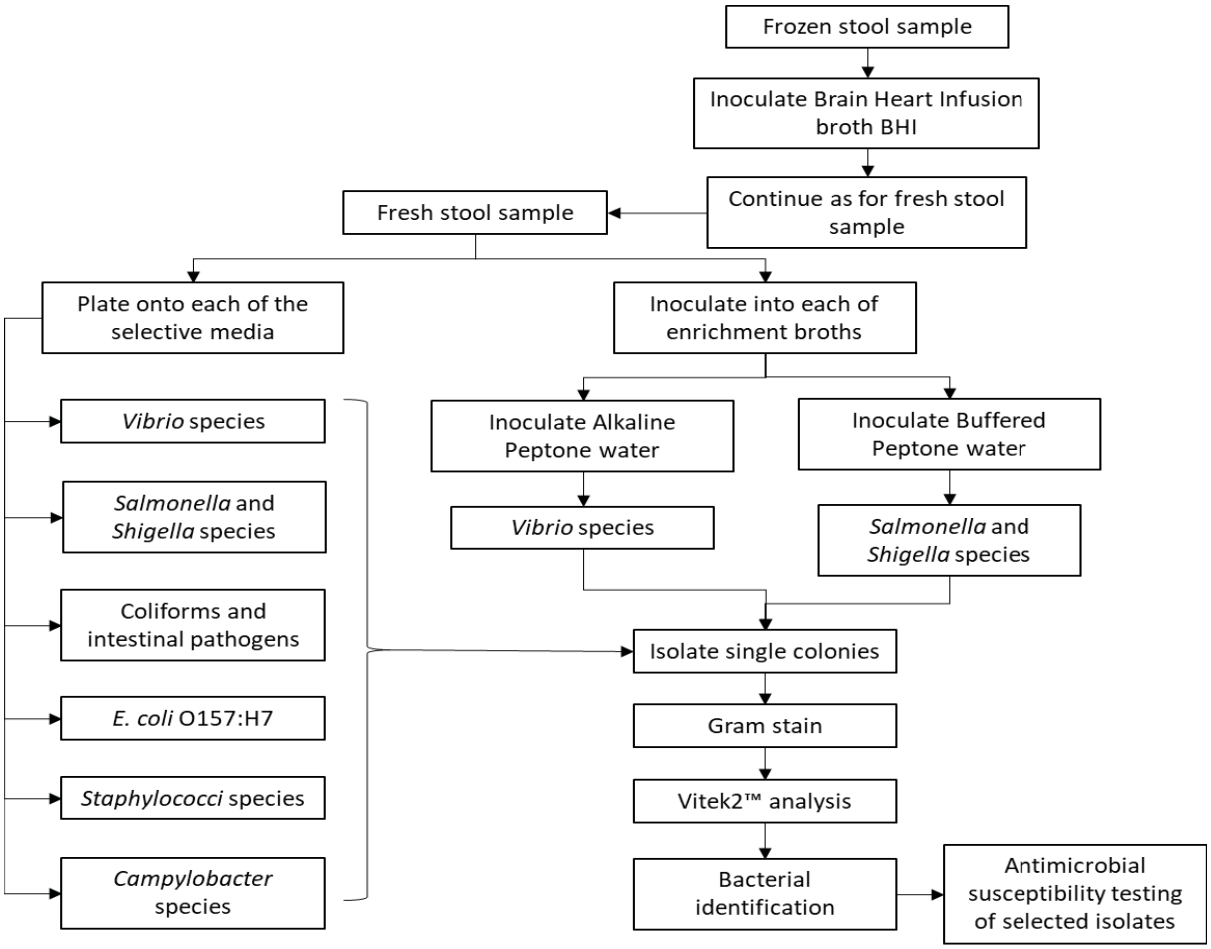


Figure 3: Illustration of the protocol followed to isolate, identify and characterise bacteria from the collected stool samples (fresh and frozen)

3.4.1.1 Vibrio species

Samples were analysed for the presence of *Vibrio* species using Oxoid thiosulfate-citrate bile salts sucrose agar (TCBS; Cat no. CM0333). An inoculation loopful of either the stool sample or sample sub-culture in APW was plated onto TCBS plates and incubated aerobically at 35°C for 24–48 hours. Presumptive colonies (from either 24- or 48-hour incubation) were sub-cultured onto TCBS plates (to confirm single colonies) or Müeller–Hinton agar (Oxoid; Cat no. CM0337) for VITEK 2™ analysis.

3.4.1.2 Salmonella and Shigella species

Samples were analysed for the presence of *Salmonella* and *Shigella* species by plating an inoculation loopful of faecal sample or sample sub-cultured in BPW onto deoxycholate citrate agar (Oxid, Cat no. CM0227) plates. Plates were incubated at 35°C for 48 hours with presumptive colonies sub-cultured for single colonies on deoxycholate citrate agar (to confirm single colonies) or on Müeller–Hinton agar (Oxoid; Cat no. CM0337) for VITEK 2™ identification.

3.4.1.3 Coliforms and intestinal pathogens

Samples were analysed for the presence of coliforms and other intestinal bacteria (*E. coli, Enterococcus* species, *Aeromonas aerogenes, Staphylococcus* species and *P. aeruginosa*) using MacConkey agar (Oxoid; Cat no. CM0007). An inoculation loopful of faecal sample was plated onto the media and incubated for 48 hours at 35°C with presumptive colonies sub-cultured for single colonies on MacConkey agar (to confirm single colonies) or on Müeller–Hinton agar (Oxoid; Cat no. CM0337) for VITEK 2™ identification.

3.4.1.4 Escherichia coli O157:H7

Samples were tested for the presence of *E. coli* O157:H7 using sorbitol MacConkey agar (Oxoid; Cat no. CM0813). An inoculation loopful of faecal sample was plated onto the media and incubated for 48 hours at 35°C with presumptive colonies sub-cultured for single colonies on sorbitol MacConkey agar or on Müeller–Hinton agar (Oxoid; Cat no. CM0337) for VITEK 2™ identification.

3.4.1.5 Staphylococci species

Samples were tested for the presence of pathogenic *Staphylococci* strains using mannitol salt agar (Oxoid; Cat no. CM0085). An inoculation loopful of faecal sample was plated onto the media and incubated for 48 hours at 35°C with presumptive colonies sub-cultured for single colonies on mannitol salt agar or on Müeller–Hinton agar (Oxoid; Cat no. CM0337) for VITEK 2™ identification.

3.4.1.6 Campylobacter species

An inoculation loopful of the sample was plated onto two *Campylobacter* blood-free selective media plates (Oxoid; Cat no. CM0739) supplemented with charcoal cefoperazone deoxycholate agar (CCDA) selective supplement (Oxoid; Cat no. SR0155) and incubated at 42°C in aerobic and micro-aerophilic (candled jar) atmospheres. Plates were incubated for 48 hours with growth monitored and presumptive colonies sub-cultured after 24 and 48 hours of incubation. Single colonies were isolated on 10% sheep blood agar plates obtained from the National Health Laboratory Service (Cat no. DMPA0115) and incubated in micro-aerophilic atmosphere for 24 hours. Single colonies from these plates were used for bacterial identification and antimicrobial sensitivity testing with the VITEK 2™ system.

3.4.2 Isolate identification

A representation of all presumptive bacterial pathogens and other intestinal bacteria was selected for testing using the VITEK 2™ system. Bacterial isolates were grown on Müeller–Hinton agar plates as described above. Each colony was Gram-stained for classification as either Gram-negative or Gram-positive to select the appropriate identification and susceptibility testing card.

A single colony was collected from the plate and suspended in sterile saline solution (bioMérieux South Africa; Cat no. V1204) in sterile 5 ml plastic tubes (bioMérieux South Africa; Cat no. 69285) and compared to the VITEK 2™ DensiCHEK™ Plus standards (bioMérieux South Africa; Cat no. 21255) to obtain a test solution with a turbidity that compares to a 0.5 McFarland standard. Isolates were identified using the Gram-negative (bioMérieux South Africa; Cat no. 21341) and Gram-positive (bioMérieux South Africa; Cat no. 21342) specific testing cards.

3.4.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed for Gram-negative isolates using AST-N256 cards (bioMérieux South Africa; Cat no. 413725). AST was performed for Gram-positive isolates with AST-P645 cards (bioMérieux South Africa; Cat no. 419602). Minimum inhibitory concentration results obtained were interpreted and corrected for using the 2017 Clinical Laboratory Standards Institute's "Performance Standards for Antimicrobial Susceptibility Testing" breakpoints and phenol-typical database. The AST data is presented only for the pathogens required by the NDOH (2017) and WHO (2017) irrespective of the source of the isolate, i.e. faecal isolates data was interpreted as if they were isolated from blood and food samples. The results are typically reported as percentage susceptible versus percentage non-susceptible organisms per antibiotic (WHO, 2014) but the decision was taken to include the "Intermediate" category separately for this report.

3.5 Statistical Analysis

All data was imported to an Excel™ spreadsheet and analysed with Strata 14 statistical package.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Overview of the Study Population

All patients are treated as outpatients at primary health care clinics. The Donald Fraser and Tshilidzini hospitals also have clinics, such as primary health care clinics, that treat patients. Primary health care clinics only refer severe cases of children with diarrhoea to hospitals. The children are then treated as inpatients.

In this study, the hospital and clinic samples were not collected during the same time period, and therefore no statistical analysis was done to compare the data between the hospital and clinic samples. The primary objective of the study was only to determine the diversity of the pathogenic diarrhoea-causing bacteria, viruses and parasites in children under the age of 5 years suffering from diarrhoea in order to give an overview of what is circulating in the rural and peri-urban communities of the Vhembe District.

4.1.1 Gender distribution

No information on gender was found in seven of the stool samples. Figure 4 shows the distribution of the gender in the study cohort per health facility.

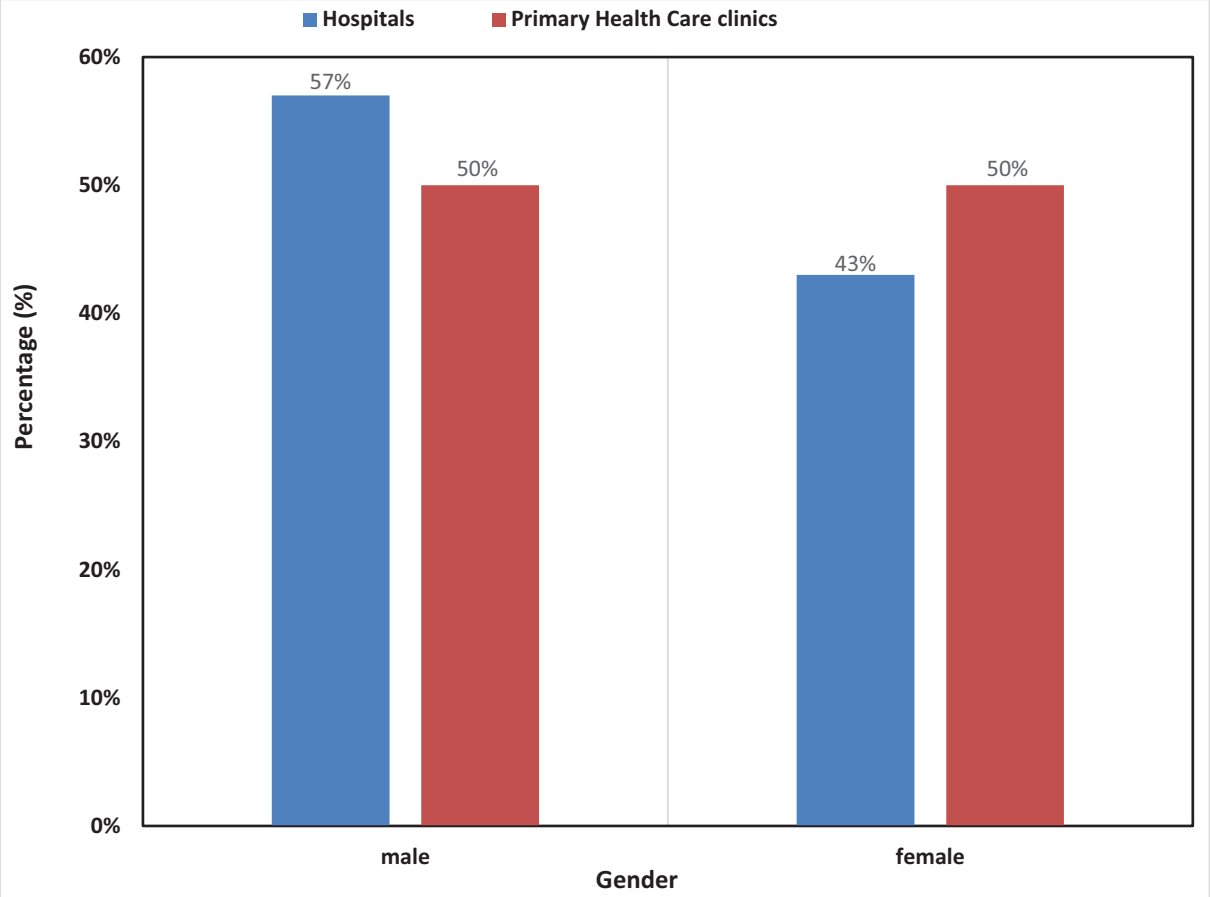


Figure 4: Gender distribution of children in study cohort

4.1.2 Age distribution

Of the 275 stool samples, only 265 stool samples (96.4%) had data regarding the ages of patients. The outstanding data (n = 10) was from samples collected at the primary health care clinics. Figure 5 provides a breakdown of the age distribution and frequency of stools received per age group and per health facility. The majority of stool samples positive for diarrhoea pathogens were from children in the age group 0–24 months, which is in line with global studies (Rogawski et al., 2017).

- The median age distribution overall for the study cohort (hospital and primary health care clinic samples) was 10 months with an interquartile range (IQR) of 6 to 18 months.
- The median age distribution for the hospital cohort was 12 months with an IQR of 9 to 17 months.
- The median age distribution for the primary health care clinic cohort was 9 months with an IQR of 4 to 18 months.

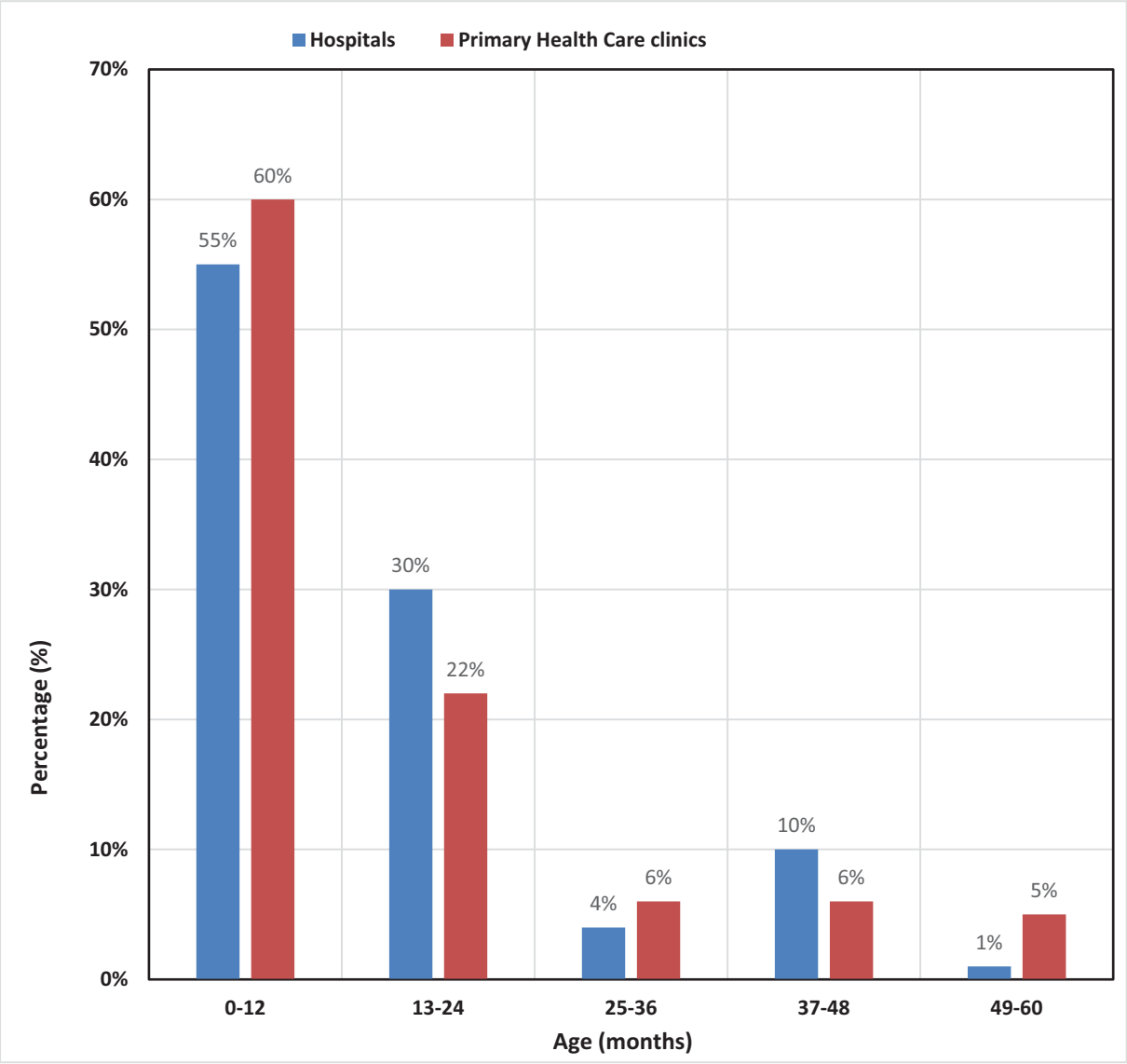


Figure 5: Age distribution of children in study cohort

4.1.3 Clinical symptoms displayed by children before treatment

Figure 6 provides an overview of the symptoms recorded for the children suffering from diarrhoea. All (100%) children in the study cohort had diarrhoea and came for treatment at the health facilities specifically for diarrhoea. Of the hospital patients, 24% (22/91) had single symptoms and 66% (60/91) had multiple symptoms. Of the primary health care clinic patients, 24% (43/184) had single symptoms and 55% (95/184) had multiple symptoms.

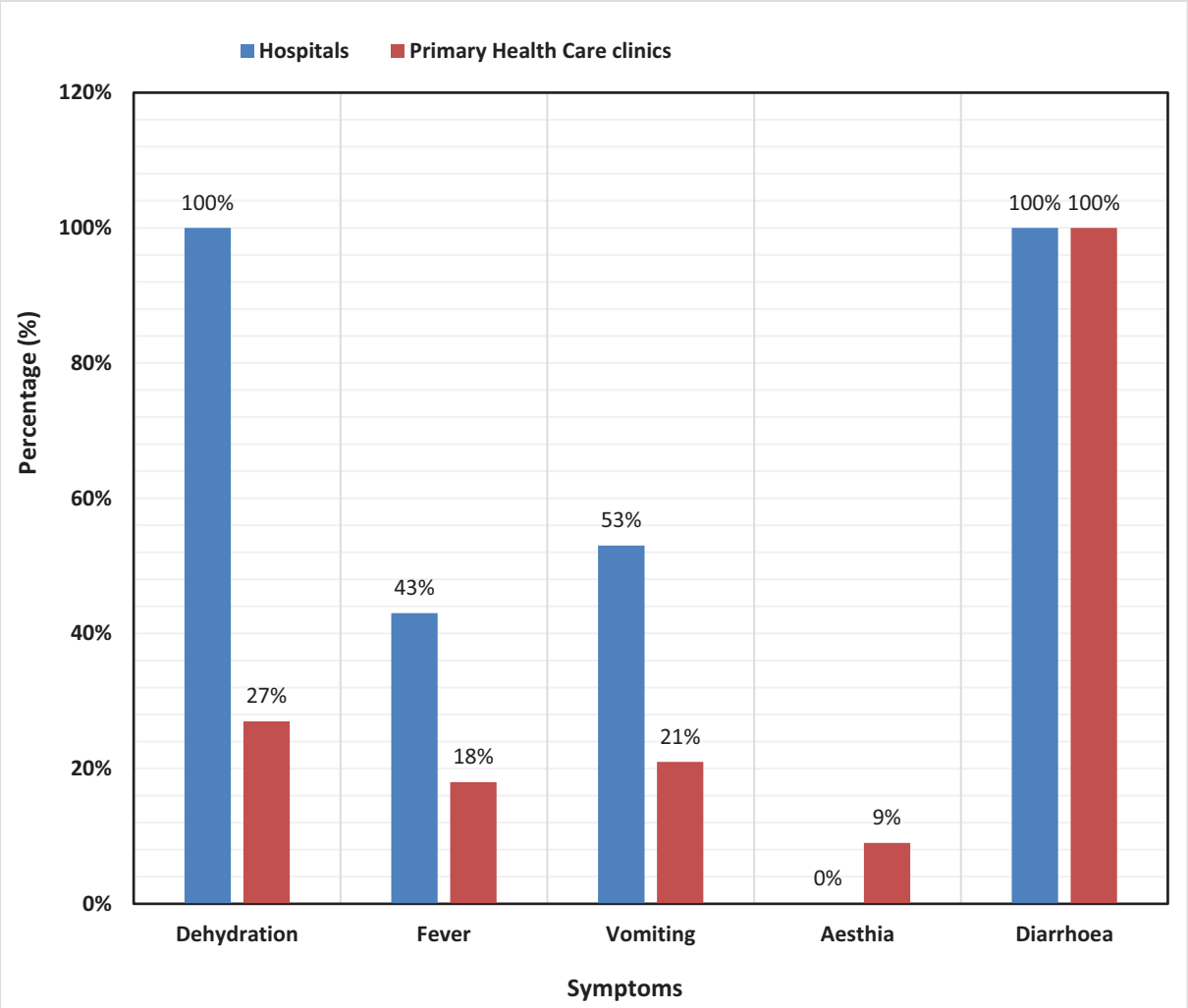


Figure 6: Distribution of clinical symptoms displayed by children in study cohort

4.1.4 Stool type reported for children with diarrhoea

A total of 14 (5%) stool samples had no information concerning the stool condition. In the study cohort, 15% of patients with soft stools, 32% with formed stools and 11% with liquid stools were negative for the pathogenic organisms tested. In the study cohort, single pathogen infections were seen in 21% of patients with soft stools, 32% with formed stools and 24% with liquid stools. In the study cohort, multiple pathogen infections were seen in 65% of patients with soft stools, 43% with formed stools and 65% with liquid stools. Figure 7 provides an overview of the stool type recorded for each child per health facility.

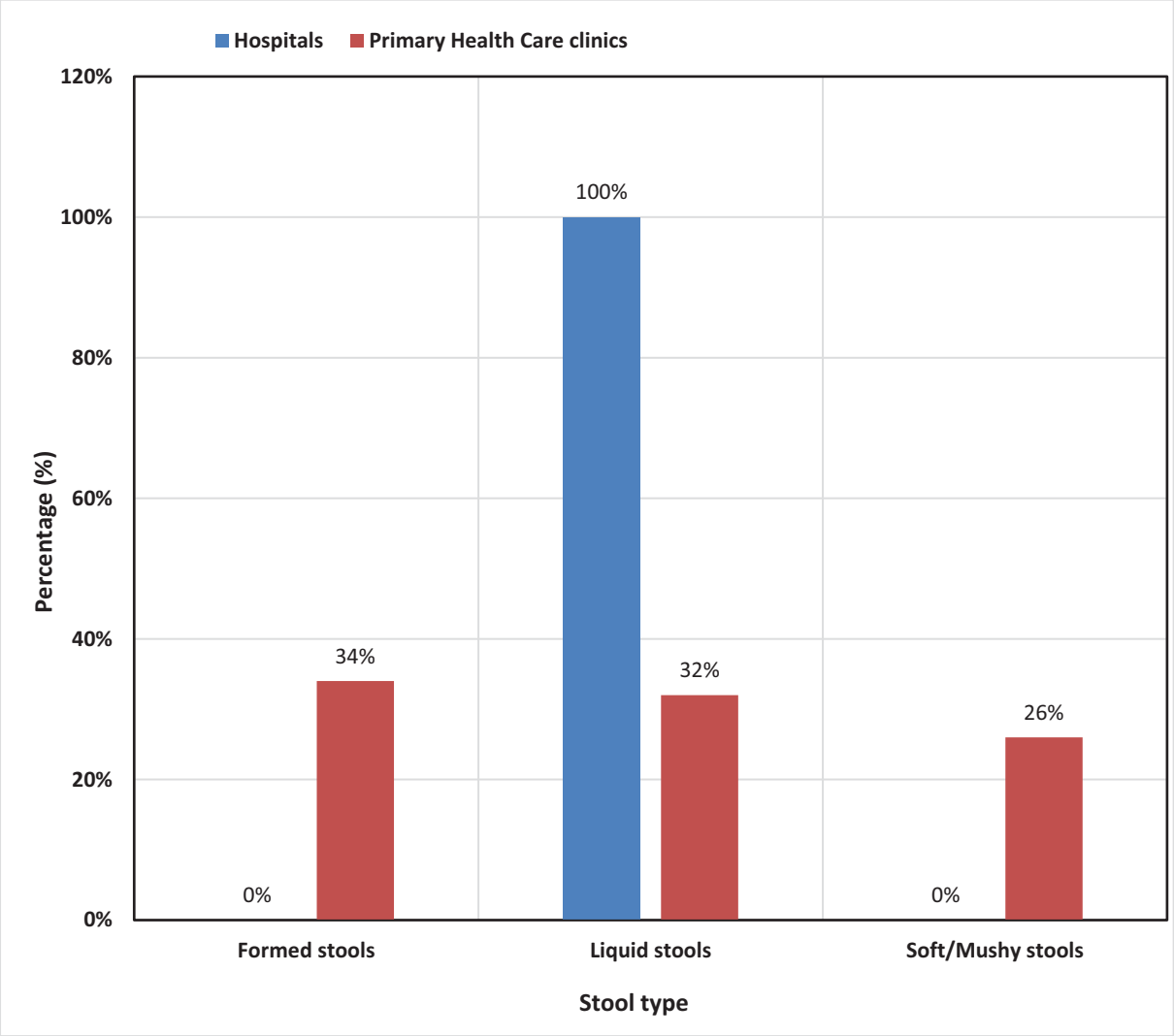


Figure 7: Stool type distribution and frequency per health facility

4.2 Pathogen Frequency and Distribution in Stool Samples

Using a BioFire® FilmArray® GI Panel, 17 of the 22 (77%) diarrhoeal organisms in the test were detected in the study cohort. The five organisms not detected included *Vibrio*, *V. cholera, Y. enterocolitica, C. cayetanensis* and *Entamoeba histolytica*. Table 4 summarises the stool samples from the study cohort. Figure 8 to Figure 10 indicate the isolation of specific diarrhoeal pathogens from the health care centres. Overall, a total of 18% (49/275) stool samples did not test positive for any of the 22 diarrhoeal pathogens using the BioFire® FilmArray® test. The diarrhoea may be caused by yet undiscovered pathogens or non-infectious agents of diarrhoea (Lanata et al., 2013). Of the remaining stool samples, 24% (65/275) of the stool specimens had single pathogens while 59% (161/275) of the stool specimens had multiple pathogens.

Table 4: Summary on BioFire® FilmArray® stool data from study cohort

Stool Sample Details	Health Facility	
	Hospital Patients	Primary Health Care Clinic Patients
Infections:	**184 (100%)**	**91 (100%)**
Negative for pathogens in panel	40 (22%)	9 (10%)
Positive for pathogens in panel	144 (78%)	82 (90%)
Single infections:	**43 (23%)**	**22 (24%)**
Bacterial infections	30	8
Virus infections	12	13
Parasite infections	1	1
Multiple infections:	**101 (55%)**	**60 (66%)**

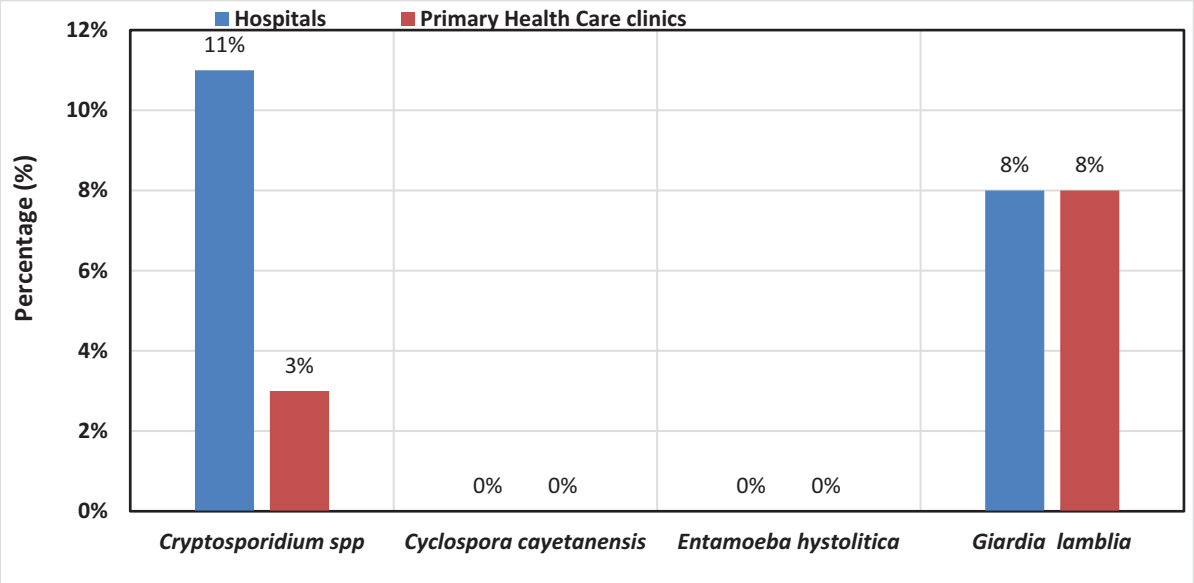


Figure 8: Prevalence of selected parasite pathogens in stool samples of children in the study cohort

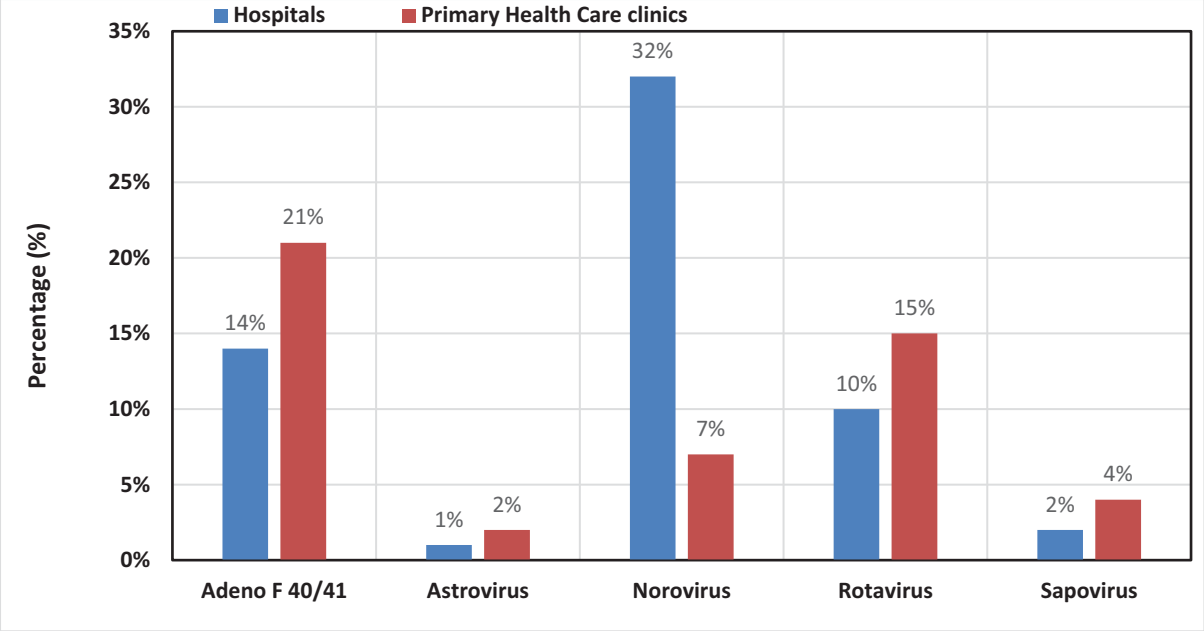


Figure 9: Prevalence of selected viral pathogens in stool samples in children in the study cohort

Generally, the most isolated bacterial pathogen was EAEC (42%), followed by EPEC (32%), ETEC (22%) and *Shigella*-EIEC (22%); *Cryptosporidium* and *Giardia* were recovered at 6% and 8% respectively; and adenovirus F40/41 was the most isolated viral pathogen at 19% followed by norovirus (16%) and rotavirus (13%). The results agree with the reports by Lanata et al. (2013) and Kotloff et al. (2013) on the prevalence of pathogens detected in stool samples.

EAEC has been linked with persistent diarrhoea in children living in areas where EAEC is endemic (Wanke et al., 1991). Contaminated food appears to be the main source of EAEC infection and has been implicated in several foodborne outbreaks of diarrhoea (Hedbeg et al., 1997; Itoh et al., 1997; Rogawski et al., 2017).

Epidemiological studies have indicated that atypical EPEC is more prevalent than typical EPEC in both developed and developing countries (Ochoa et al., 2008). Atypical EPEC is important in paediatric endemic diarrhoea and diarrhoea outbreaks (Ochoa & Contreras, 2011) and has been reported in association with prolonged diarrhoea (Afset et al., 2004). According to Nguyen et al. (2006), diarrhoea caused by atypical EPEC is usually mild and generally not associated with dehydration; its importance lies in its association with prolonged diarrhoea, which is a major contributor to childhood illness in developing countries.

ETEC is a multivalent pathogen responsible for repeated infections that may adversely affect the nutritional status of children younger than 2 years and the susceptibility of infants and young children due to poor public health and hygiene conditions (Qadri et al., 2000; Rao et al., 2003; Qadri et al., 2005).

Since the introduction of the rotavirus vaccine in South Africa, other viruses such as the norovirus and human adenovirus F40/41 have become more prevalent in children suffering from diarrhoea (Mans et al., 2017).

Parasitic infections, causing illnesses such as cryptosporidiosis, are an important cause of childhood diarrhoea in Africa and have a serious impact on child growth and development (Aldeyarbi et al., 2016).

An interesting discovery was that patients in hospital had more single infections from viruses (especially norovirus and adenovirus F40/41) than patients treated in primary health care clinics, who had more single infections from bacteria (Table 4). This is, however, not conclusive due to the small number of stool samples collected. More studies need to be done in order to be able to make valid conclusions.

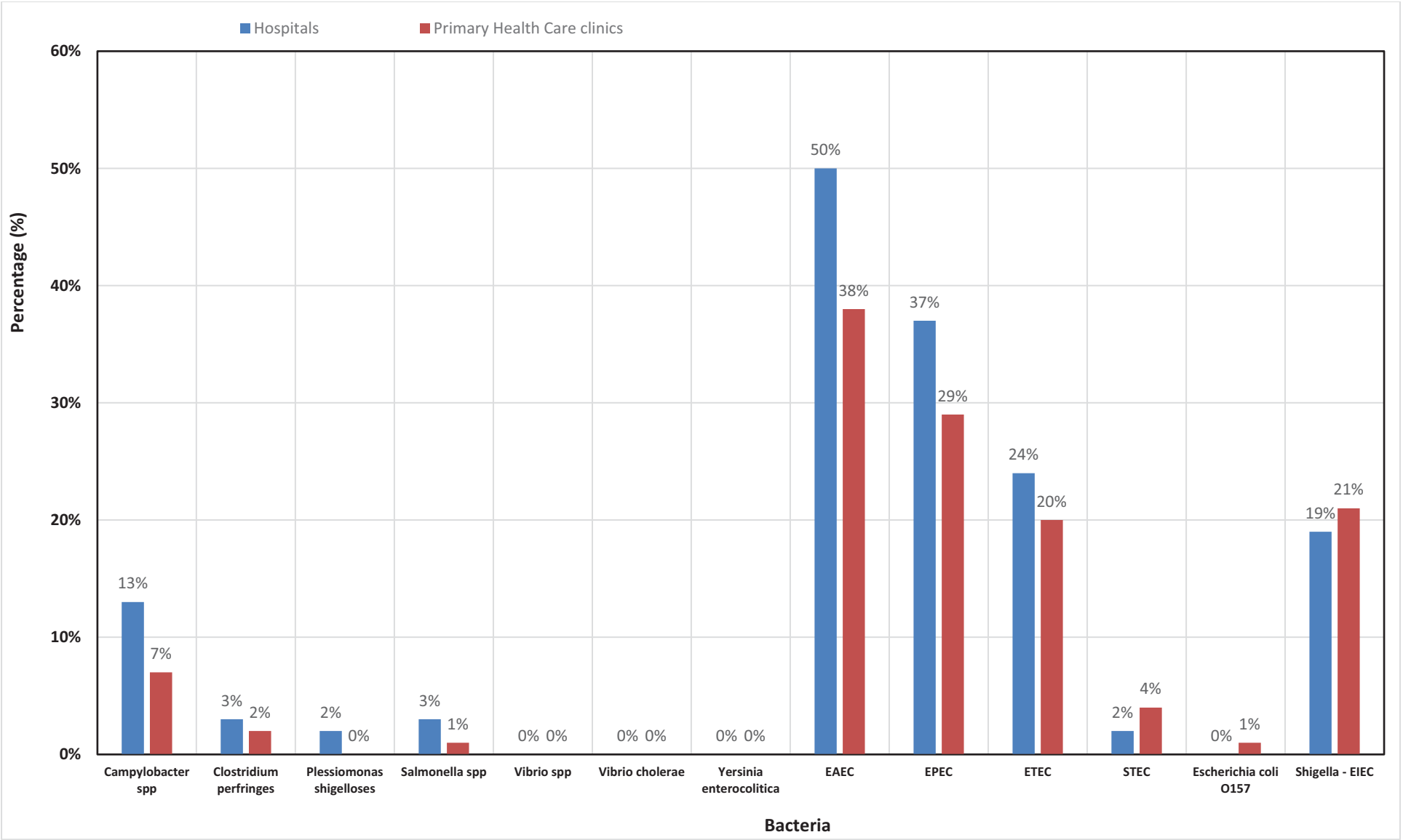


Figure 10: Bacterial prevalence in stool samples from children in the study cohort

4.3 Antibiotic Susceptibility of Isolates

Table 5 gives an overall summary of the results and was used as the basis for the isolation and identification of bacterial species. The decision was taken to focus on the aerobic and micro-aerophilic bacteria reported in Table 5. Although *Vibrio* species were not detected, it was decided to still include the testing for these species in case the dose in the stool was below the detection limit of the equipment. The media used were chosen in such a way that they could potentially isolate other organisms that have been associated with diarrhoea from emerging pathogens as well as food-related pathogens.

Table 5: Summary of BioFire® FilmArray® results of the fresh and frozen stool samples

Organism		n
Bacteria	*Campylobacter*	28 (5%)
	C. difficule toxin A/B	7 (1.2%)
	P. shigelloides	2 (0.4%)
	Salmonella	5 (0.9%)
	Vibrio	0
	V. cholerae	0
	Y. enterocolitica	0
	EAEC	118 (21%)
	EPEC	89 (15.8%)
	ETEC	60 (10.6%)
	STEC	10 (1.8%)
	E. coli O157	2 (0.4%)
	Shigella/EIEC	56 (10%)
Viruses	Adenovirus F40/41	51 (9.1%)
	Astrovirus	7 (1.2%)
	Norovirus GI/GII	42 (7.5%)
	Rotavirus A	37 (6.6%)
	Sapovirus	12 (2.1%)
Parasites	*Cryptosporidium*	16 (2.8%)
	C. cayetanensis	0
	C. cayetanensis	0
	Entamoeba histolytica	0
	G. lamblia	22 (3.9%)

4.3.1 Bacterial isolation

It must be mentioned that access to BioFire® FilmArray® was only arranged midway through the project, which expanded the potential of the data that could be generated from the samples. Up to then, samples were collected, and an initial isolation and test was performed and frozen for further testing. The team was unsure if the pathogens in the frozen samples would still be detected with the BioFire® FilmArray® GI Panel (the supplier does not recommend it). However, with the success of the experiments, it was decided to analyse the frozen samples again to isolate and characterise bacterial pathogens not originally included in the project.

The 91 fresh stool samples were collected, stored and transported according to the manufacturer's recommendations: we were confident that we would be successful with the bacterial isolations from these samples. As expected, there was no growth observed for aerobic and micro-aerophilic organisms in 39 (14.2%) of the samples (A report by Qamar & co-workers (2016) reported that as part of the GEMS in South Asia and in sub-Sharan Africa, dysentery-like symptoms in children younger than 24 months are likely to be related to *Aeromonas* infections. A similar report by Shah & co-workers (2016) showed that they found *Aeromonas* in 5.5% of the samples. Since *Aeromonas* is a common organism in water and soil, it could be recommended that researchers should start screening for *Aeromonas* in diarrhoeal samples from children younger than 5 years old. In the mix of pathogens isolated were four microorganisms that fall within the group of the ESKAPE pathogens (*E. faecium, Staphylococcus aureus, K. pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* species), which are recommended to be studied, although more from clinical samples. The presence of these organisms does support the One Health approach to study antimicrobial resistance due to the ease with which certain plasmid-borne genetic traits can be transferred to other organisms (Robinson, 2016). It is important to make mention of two strains from Table 7 not dealt with:

- The first is the *Sphingomonas paucimobilis* strains that rarely cause infection but that have been linked to biofilm formation in pipes (Gulati & Ghosh, 2017). The organism isolated and tested in this study was chlorine-resistant and could participate in biofilm formation (containers are well-known to contain biofilms) that can protect other pathogens (Nishiuchi et al., 2017). If this organism is from the water source, it could show how the water influences the child's health by possibly supporting pathogenic microorganism's survival.
- The second is *Stenotrophomonas maltophilia*, which has been reported as an "archetypal environmental opportunistic bacterium responsible for health care-associated infections", possibly from animal sources (Jayol et al., 2018). This organism has been reported as the cause of an extensively drug resistant *S. maltophilia* strain outbreak in a burn unit in a tertiary hospital, supporting the importance of not overlooking opportunistic pathogens (Ali et al., 2017).

Table 6) and most of the isolates turned out to be Gram-negative. A summary of the bacterial strains identified using the VITEK 2™ cards is shown in Table 7. When compared with the results obtained with the BioFire® FilmArray® GI Panel, it is clear why most samples were *E. coli* strains. Not only is *E. coli* commensal in human intestines, but the pathogenic strains made up the bulk of the pathogens detected. Since *E. coli* can easily exchange genetic traits, it was decided to test as many of the *E. coli* isolates as possible for their antimicrobial susceptibility. Like the GI Panel results, no *Vibrio* species were isolated, and we were not successful in isolating any *Campylobacter* or *Shigella* species. It should be noted that the GI Panel did identify the *Shigella* isolates as either *Shigella* or EIEC, so these strains may be represented by the large *E. coli* population isolated. The isolation of the *Aeromonas* strains correlates well with the newest literature linking *Aeromonas hydrophila* and *A. caviae* to diarrhoea in children.

A report by Qamar & co-workers (2016) reported that as part of the GEMS in South Asia and in sub-Sharan Africa, dysentery-like symptoms in children younger than 24 months are likely to be related to

Aeromonas infections. A similar report by Shah & co-workers (2016) showed that they found *Aeromonas* in 5.5% of the samples. Since *Aeromonas* is a common organism in water and soil, it could be recommended that researchers should start screening for *Aeromonas* in diarrhoeal samples from children younger than 5 years old. In the mix of pathogens isolated were four microorganisms that fall within the group of the ESKAPE pathogens (*E. faecium, Staphylococcus aureus, K. pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* species), which are recommended to be studied, although more from clinical samples. The presence of these organisms does support the One Health approach to study antimicrobial resistance due to the ease with which certain plasmid-borne genetic traits can be transferred to other organisms (Robinson, 2016). It is important to make mention of two strains from Table 7 not dealt with:

- The first is the *Sphingomonas paucimobilis* strains that rarely cause infection but that have been linked to biofilm formation in pipes (Gulati & Ghosh, 2017). The organism isolated and tested in this study was chlorine-resistant and could participate in biofilm formation (containers are well-known to contain biofilms) that can protect other pathogens (Nishiuchi et al., 2017). If this organism is from the water source, it could show how the water influences the child's health by possibly supporting pathogenic microorganism's survival.
- The second is *Stenotrophomonas maltophilia*, which has been reported as an "archetypal environmental opportunistic bacterium responsible for health care-associated infections", possibly from animal sources (Jayol et al., 2018). This organism has been reported as the cause of an extensively drug resistant *S. maltophilia* strain outbreak in a burn unit in a tertiary hospital, supporting the importance of not overlooking opportunistic pathogens (Ali et al., 2017).

Table 6: Breakdown of number of samples analysed for bacterial isolation and identification

Description	n	Percentage (%)
Total number of samples	**275**	**100.0**
Samples with bacterial growth	236	85.8
Samples with no bacterial growth	39	14.2
Number of bacterial isolates from samples	**472**	**100.0**
Number of isolates identified using VITEK 2™	**433**	**91.7**
Gram stain results		
Gram-negative	354	81.8
Gram-positive	79	18.2

Table 7: Summary of the type of bacterial species isolated

Gram-negative species	n		Gram-positive species	n
Aeromonas	8		***Enterococcus***	64
Aeromonas hydrophila	1		*Enterococcus avium*	1
Aeromonas hydrophila/ caviae	3		*Enterococcus casseliflavus*	4
Aeromonas salmonicida	1		*Enterococcus durans*	1
Aeromonas sobria	3		*Enterococcus faecalis*	31
Citrobacter	30		*Enterococcus faecium*	23
Citrobacter amalonaticus	3		*Enterococcus gallinarum*	3
Citrobacter braakii	10		*Enterococcus hirae*	1
Citrobacter farmer	3		***Micrococcus***	1
Citrobacter freundii	13		*Micrococcus luteus*	1

Gram-negative species	n		Gram-positive species	n
Citrobacter youngae	1		***Staphylococcus***	3
Delftia acidovorans	2		*Staphylococcus epidermidis*	1
Enterobacter	19		*Staphylococcus vitulinus*	1
Enterobacter asburiae	3		*Staphylococcus warneri*	1
Enterobacter cloacae complex	4		***Streptococcus***	2
Enterobacter cloacae spp. cloacae	12		*Streptococcus sobrinus*	1
Escherichia coli	139		*Streptococcus thoraltensis*	1
Klebsiella	57			
Klebsiella oxytoca	14			
Klebsiella pneumoniae spp. ozaenae	1			
Klebsiella pneumoniae spp. pneumoniae	42			
Morganella morganii spp. morganii	4			
Ochrobactrum anthropi	2			
Pseudomonas fluorescens	1			
Raoultella planticola	53			
Serratia marcescens	4			
Salmonella group	2			
Sphingomonas paucimobilis	1			
Stenotrophomonas maltophilia	1			

4.3.2 Bacterial antimicrobial resistance

The selection of bacterial isolates to be tested for their antimicrobial selection was based on the organisms that matched those detected with the BioFire® FilmArray® GI Panel results (Table 5), organisms that the NDOH (2017) and WHO (2017) propose be reported (even if not from requested sources such as blood) and the ESKAPE pathogens isolated. Although the samples may not be from blood cultures, infections can occur from the gut of the person affected (auto-infection), can be transmitted from animals, can be via food and spread between individuals (WHO, 2017). The strains tested using the VITEK 2™ AST cards are tested for a predefined set of antibiotics, although only selected single antibiotics are reported as requested by the NDOH (2017) and WHO (2017) to make the data comparable to other reports. The antimicrobial sensitivity testing phenotypes of the organisms are also provided in case the reader needs a broader overview of the AST and will be reported on in separate academic publications as well.

4.3.2.1 Gram-negative AST

The strains tested for AST included *E. coli, Klebsiella, Salmonella, Citrobacter* and *Enterobacter* strains isolated (Table 7). Table 8 shows the results for the *E. coli* AST. Table 9 shows the intrinsic resistance and phenotypes. Using the phenotypical characteristics, significant wild type resistance was noted, with a wide variety of strains with multiple antimicrobial resistance. Similar reports in literature show an increase in human- and animal-related *E. coli* with multiple antimicrobial resistance (Tadesse et al., 2012). More importantly, 56% (n = 64) of the strains showed extended spectrum beta-lactamase resistance, which is concerning for hospital- and community-related infections (Shaikh et al., 2015). A study by Chrichton et al. (2018) showed that 100% of the *E. coli* isolated from blood stream infections in children in Cape Town (South Africa) showed extended spectrum beta-lactamase resistance, which highlights the need to better understand AST related to *E. coli.* Comparing the results with that obtained from the same region from children aged 4-12 months showed that *E. coli* strains from this study showed more resistance to the antibiotic than DeFrancesco et al. (2017). Strains from that study showed no resistance to gentamycin and ciprofloxacin whereas the strains from this study showed more resistance.

Table 8: Summary of the AST results obtained for the *E. coli* strains (n = 115) tested and reported using the antibiotics recommended by the NDOH (2017) and WHO (2017)

Antibiotic	NDOH (2017)			WHO (2017)		
	% R	% I	% S	% R	% I	% S
Ampicillin	98.3		1.7	98.3		1.7
Amoxicillin/Clavulanic Acid	47.8	26.1	26.1	47.8	26.1	26.1
Piperacillin/Tazobactam				57.1	28.6	14.3
Cefoxitin	38.3		61.7			
Cefotaxime	98.3		1.7	98.3		1.7
Ceftazidime	99.1		0.9	99.1		0.9
Cefepime	98.3		1.7	99.1		0.9
Ertapenem				3.5	60.9	35.7
Imipenem	0.9	45.2	53.9	0.9	45.2	53.9
Meropenem	14.8	65.2		16.5	19.1	64.3
Amikacin				2.6	59.1	38.3
Gentamicin	38.3	37.4	24.3	57.1	28.6	14.3
Ciprofloxacin	15.7	0.9	83.5	15.7	0.9	83.5
Tigecycline			100.0			
Trimethoprim/Sulfamethoxazole	6.5	0.0	23.5			
Colistin						94.8

R = Resistant; I = Intermediate; S = Sensitive

The exceptions were for imipenem where all or almost all strains in both studies showed complete sensitivity to the compound. The reason for the difference may be that the samples analysed were from children that had previous diarrhoeal cases but may not have had a current infection. *K pneumoniae* are also multidrug-resistant organisms that have been identified as an urgent threat to human health by various organisations, such as the WHO, the US Centers for Disease Control and Prevention and the UK Department of Health. *K. pneumoniae* strains carrying the *K. pneumoniae* carbapenemase (KPC) gene, as well as numerous other acquired antimicrobial resistance determinants, have been responsible for outbreaks on several continents (Paczosa & Mecsas, 2016).

Of importance to this study is that *K. pneumoniae* infections are particularly problematic among neonates within the healthcare setting, as well as in community-acquired infections (Paczosa & Mecsas, 2016; Quan et al., 2016). The results in Table 10 show that multidrug-resistant *K. pneumoniae* strains were isolated although only 14.3% showed carbapenem resistance (against meropenem). Reports of colistin resistance is on the increase, which is often seen as the last treatment option for KPC-producing *K. pneumoniae* (Nation et al., 2015). In this study, all strains were sensitive to colistin although follow-up tests would be recommended to confirm this.

The testing of *Salmonella* strains is recommended by the WHO for foodborne isolates, and as such the five strains were included in this report (Table 11). It is reported that the emergence of multi-drug resistant non-typhoidal *Salmonella enterica* represents an additional challenge for public health authorities. Animals raised for food production are considered a major reservoir and potential source of foodborne salmonellosis, hence its inclusion in the WHO requirements (Hong et al., 2016; WHO, 2017). The results obtained for the five strains showed that these strains also contained multidrug-resistant with extended spectrum beta-lactamase resistance in three of the strains, among some of the resistance traits. More strains would need to be isolated from the community and animals to better understand the occurrence of the organism, but it could have contributed to the diarrhoea in the children in this study.

The AST phenotype information for the *Citrobacter* and *Enterobacter* isolates is shown in Table 12. Enterobacter species are part of the so-called ESKAPE pathogens (consisting of *E. faecium*,

Staphylococcus aureus, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species) and are the leading cause of nosocomial infections throughout the world. The ESKAPE strains are defined as “capable of escaping the biocidal action of antibiotics and mutually representing new paradigms in pathogenesis, transmission and resistance” (Pendleton et al., 2013). The ESKAPE strains typically show multiple drug resistance, ranked among the top three threats to global public health (Santajit & Indrawattana, 2016). Like other reports, the strains isolated and tested as part of this study showed multiple drug resistance supporting the inclusion of the organisms in the ESKAPE pathogen group.

Table 9: Summary of the AST phenotypes obtained for the *E. coli, Klebsiella* and *Salmonella* strains tested

Antibiotic family	Phenotypes	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*Salmonella*
Beta-lactams	Acquired penicillinase		1		
	Carbapenemase [+ or − extended spectrum beta-lactamase (ESBL)]	17		4	
	Extended spectrum beta-lactamase	64	2	3	5
	Extended spectrum beta-lactamase, SHV1 hyperproduction	6	1		
	Impermeability CARBA (+ESBL or +HL AmpC), carbapenemase (+ or −ESBL)	26	3		
Aminoglyco-sides	Resistant GEN (AAC(3)-I), resistant GEN TOB NET (AAC(3)-IV)	17			
	Resistant GEN TOB (ANT(2")), resistant GEN TOB NET (AAC(3)-II), Resistant GEN TOB NET AMI		1		2
	Resistant GEN TOB NET (AAC(3)-IV, resistant GEN TOB (ANT(2"))	1			
	Resistant GEN TOB NET (AAC(3)-IV, resistant GEN TOB (ANT(2"), resistant GEN TOB NET (AAC(3)-II))	1			
	Resistant GEN TOB NET (AAC(3)-IV), resistant Gen TOB NET AMI, resistant GEN TOB (ANT(2"))	21			
	Resistant GEN TOB NET (AAC(3)-IV), resistant Gen TOB NET AMI, resistant GEN TOB (ANT(2")), resistant GEN TOB NET (AAC(3)-II)	1			
	Resistant GEN TOB NET AMI	48	1		2
	Resistant GEN TOB NET AMI (AAC(6'))	1	1		1
	Wild	23	4	5	
	Wild, resistant TOB NET AMI (AAC(6'))			2	
Quinolones	Decreased susceptibility	4	4	3	
	Decreased susceptibility, wild	91	2	4	
	Partially resistant				1
	Resistant	9	1		
	Resistant, decreased susceptibility	9			
	Wild				4
Tetracyclines	Resistant, wild	113	7	7	5
Polypeptides	Resistant, wild		1		
	Wild	108	6	7	3
Trimethoprim/ sulphonamides	Resistant	88	3	5	
	Trimethoprim resistant, wild	25	4	2	5

Table 10: Summary of the antimicrobial resistance results obtained for the *K. pneumonia* strains (n = 7) tested and reported using the antibiotics recommended by the NDOH (2017) for clinical bacteria

Antibiotic	% Resistant	% Intermediate	% Sensitive	% No Results
Amoxicillin/Clavulanic Acid	57.1	28.6	14.3	
Piperacillin/Tazobactam	42.9		42.9	14.3
Cefotaxime	85.7		14.3	
Ceftazidime	85.7		14.3	
Cefepime	85.7		14.3	
Ertapenem		42.9	57.1	
Imipenem		71.4	28.6	
Meropenem	14.3	28.6	57.1	
Amikacin		42.9	57.1	
Gentamicin	14.3	14.3	71.4	
Ciprofloxacin	14.3		85.7	
Colistin			100.0	
Amoxicillin/Clavulanic Acid	57.1	28.6	14.3	

Table 11: Summary of the antimicrobial resistance results obtained for the *Salmonella* strains (n = 5) tested and reported using the antibiotics recommended by the WHO (2017) for foodborne bacteria

Antibiotic	% Resistant	% Intermediate	% Sensitive
Ampicillin	100.0		
Amoxicillin/Clavulanic Acid		60.0	40.0
Cefoxitin	100.0		
Cefotaxime	60.0	0.0	40.0
Ceftazidime	80.0		20.0
Cefepime	60.0		40.0
Imipenem	0.0	80.0	20.0
Meropenem			100.0
Gentamicin	100.0		
Ciprofloxacin	20.0		80.0
Tigecycline			100.0
Trimethoprim/Sulfamethoxazole			100.0

Table 12: Summary of AST phenotypes obtained for the *Citrobacter* and *Enterobacter* strains tested

Antibiotic family	Phenotypes	*C. braakii*	*C. freundii*	*E. cloacae*	*E. cloacae complex*	*E. cloacae* spp. *cloacae*
Beta-lactams	Carbapenemase (+ or −ESBL)	3				
	Extended spectrum beta-lactamase		1	9	1	1
	High level case (AmpC), extended spectrum beta-lactamase	3	1			
	Impermeability CARBA (+ESBL or +HL AmpC)			12		
Aminoglyco-sides	Resistant GEN TOB (ANT(2")), resistant GEN TOB NET AMI	1				
	Resistant GEN TOB (ANT(2")), resistant GEN TOB NET AMI, resistant GEN TOB NET AMI (AAC(6'))	1				
	Resistant GEN TOB (ANT(2")), resistant GEN TOB NET (AAC(3)-II), Resistant GEN TOB NET AMI			2		
	Resistant GEN TOB NET (AAC(3)-II, resistant GEN TOB (ANT(2"))			1		
	Resistant GEN TOB NET AMI	2		3		
	Resistant GEN TOB NET AMI (AAC(6'))			7		
	Wild	1	1	3		1
	Wild, resistant TOB NET AMI (AAC(6'))		1	5	1	
	Wild, Resistant GEN TOB (ANT(2")), resistant GEN TOB NET AMI, resistant GEN (AAC(3)-I), resistant TOB NET AMI (AAC(6'))	1				
Quinolones	Decreased susceptibility			10		1
	Decreased susceptibility, wild	6	2	9	1	
	Resistant			1		
	Resistant, decreased susceptibility			1		
	Resistant, wild	6	2	21	1	1
Polypeptides	Resistant, wild			3		
	Wild	6	2	18	1	1
Trimethoprim/ sulphonamides	Resistant		1	11		
	Trimethoprim resistant, wild	6	1	10	1	1

4.3.2.2 Gram-positive AST

Enterococcus faecalis and *E. faecium*, typically associated with the intestinal flora, have become some of the important nosocomial pathogens and a growing clinical challenge. The organisms have used a diverse number of genetic strategies to develop resistance to virtually all antimicrobials currently used in clinical practice. Multidrug-resistant *Enterococci* can become the dominant flora in the intestine under antibiotic pressure, predisposing the severely ill and immunocompromised patient to invasive infections. (Hollenbeck & Rice, 2012; Miller et al., 2014).

Table 13 and Table 14 summarise the results obtained from *E. faecium/faecalis* strains. The obtained results are concerning as *Enterococci* have been reported to "likely function as a reservoir of drug resistance determinants and can serve as the springboard for the spread of these genes to other Gram-positive pathogens" (Miller et al., 2014).

Table 13: Summary of the antimicrobial resistance results obtained for the *E. faecium/faecalis* strains (n = 22) tested and reported using the antibiotics recommended by the NDOH (2017) for clinical bacteria and WHO (2017) for foodborne bacteria

Antibiotic	NDOH (2017)				WHO (2017)			
	% R	% I	% S	% N	% R	% I	% S	% N
Gentamicin					54.5		31.8	13.6
Ciprofloxacin					81.8	18.2		
Erythromycin					100.0			
Daptomycin	15.7	0.9	83.5		15.7	0.9	83.5	
Teicoplanin	40.9		59.1		40.9		59.1	
Vancomycin	36.4		50.0	13.6	36.4		50.0	13.6
Tetracycline					15.7	0.9	83.5	
Nitrofurantoin					72.7	13.6	13.6	
Linezolid	36.4	0.0	50.0	13.6				

R = Resistant; I = Intermediate; S = Sensitive; N = No results

Table 14: Summary of the AST phenotypes obtained for the *Enterococci* strains tested

Antibiotic Family	Phenotype	*E. casseliflavus*	*E. faecalis*	*E. faecium*	*E. gallinarum*
Aminoglyco-sides	High level resistant STR + GEN, high level resistant gentamicin		3	8	
	Wild, high level resistant kanamycin, high level resistant STR +KAN, high level resistant streptomycin	3	1	7	1
Quinolones	Resistant	1	2		
	Resistant, wild	2		5	2
	Wild		5	8	
Macrolides/ lincosamides/ strepto-gramins	Resistant			4	
	Resistant (MLSB)	1	1		
	Wild	2	6	10	2
	Resistant		1		
	Wild	3	6	14	2

Antibiotic Family	Phenotype	*E. casseliflavus*	*E. faecalis*	*E. faecium*	*E. gallinarum*
Glyco-peptides	Resistant (VAN A like)	3	7	2	1
	Resistant (VAN B like)			2	
	Resistant (VAN C)				1
	Wild			10	
Tetracyclines	Resistant		4	5	
	Wild	3	3	9	2
Furanes	Resistant	3	1	11	1
	Resistant, wild		3	3	

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics; almost everyone has heard of methicillin-resistant *Staphylococcus aureus* (MRSA) (Chambers & DeLeo, 2009). Up to date, no *Staphylococcus aureus* strain has been isolated from the sample but *S. warneri* has been isolated, which is worth mentioning. *S. warneri* is reported as an emerging pathogen and a coagulase-negative *Staphylococcus* (CNS) commonly present in the flora of human epithelia and mucosal membranes. The organism can cause serious infections usually in association with the presence of implant materials, but, at times, even in the absence of a foreign body and in patients considered immunocompetent (Campoccia et al., 2010). Orthopaedic infections were found to involve *S. warneri* strains with low antibiotic resistance potential, differing in this respect from the strains isolated at neonatal intensive care units, where this species has been described to figure among the principal causative agents and exhibit an alarming profile of antibiotic resistance (Campoccia et al., 2010). The *S. warneri* strain isolated as part of this study showed resistance to multiple antibiotics (Table 15) but more strains would need to be isolated and studied before any conclusions can be drawn.

Table 15: Summary of the antimicrobial resistance results obtained for the *Staphylococcus* strain (n = 1) tested and reported using the antibiotics recommended by the WHO (2017) for foodborne bacteria

Antibiotic	% Resistant	% Intermediate	% Sensitive	% No Results
Cefoxitin Screen				100
Oxacillin	100			
Gentamicin	100			
Ciprofloxacin	100			
Erythromycin	100			
Clindamycin				100
Vancomycin	100			
Tetracycline	100			
Rifampicin	100			
Trimethoprim/ Sulfamethoxazole	100			

CHAPTER 5: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of Findings and Conclusions

The aim of this study was to assess the prevalence, incidence and genetic diversity of pathogenic bacteria, viruses and parasitic organisms among children under the age of 5 years who suffer from diarrhoea. This was done to determine the antimicrobial resistance of diarrhoea-causing bacteria isolated from stool samples in the Vhembe District, Limpopo Province. The study also evaluated the use of the BioFire® FilmArray® GI Panel, which is a qualitative multiplexed nucleic acid-based in vitro diagnostic test that simultaneously detect 22 bacteria, viruses and parasites in one stool sample. The BioFire® FilmArray® GI Panel proved to be a useful tool in the rapid diagnosis (one-hour turnaround time for one stool sample) of gastrointestinal/diarrhoeal pathogens. The increased detection rate and wide spectrum of diarrhoeal pathogens detected by the BioFire® FilmArray® panel will also assist with patient treatment/management, especially in outbreak situations or in critically ill patients. More studies are needed to test the cost-effectiveness of multiplex gastrointestinal panels in the South African health care system.

The results from this study can be used to generate up-to-date valid conclusions on the prevalence of pathogenic organisms causing gastroenteritis in children younger than 5 years living in rural and peri-urban communities. However, no definitive conclusion can be made whether the detection of a specific pathogen or co-infections with multiple diarrhoeal pathogens was associated with disease, although all children came for diarrhoea treatment at the health facility. The outcomes from this study highlight the wide spectrum of possible enteric pathogens in children under the age of 5 years with diarrhoea. With the exception of five organisms, namely, *Vibrio, V. cholera, Y. enterocolitica, C. cayetanensis* and *Entamoeba histolytica*, all other 17 pathogenic organisms in the BioFire® FilmArray® panel were detected at least once.

The findings further showed that antimicrobial-resistant bacteria cause a high percentage of infections in rural and peri-urban communities in the Vhembe District. Many of the organisms showed a variety of wild type and acquired antimicrobial resistance, with some strains showing resistance against multiple classes of antibiotics. Most bacterial strains isolated from the hospital and primary health care clinics were *E. coli*, which were the most detected pathogens with the BioFire® FilmArray® GI Panel. The study was successful in isolating *Citrobacter, Enterobacter* and *Enterococci* species that form part of the ESKAPE pathogens.

The detection of resistance is a warning appeal for closer surveillance studies, identification and understanding of the epidemiology of the resistance with a view to engage with different stakeholders and to introduce preventative strategies that can minimise or stop the emergence and spread of resistance to the antibiotic collection currently in use in South Africa. Consequently, the required laboratory infrastructure and protocols for surveillance must be established, monitored, evaluated and sustained.

GERMS-SA report annually on the costs and limited staff resources available to do continuous monitoring of bacterial and fungal pathogens, along with their susceptibility/resistance characteristics. By linking with academics, this problem could be addressed using student projects to gather bacterial strains or antimicrobial resistance information. This would need inputs from the various stakeholders to determine which method and guidelines to use to achieve a common objective and to have comparable results to use in preventative strategies.

5.2 Recommendations

The following is recommended:

- Continued assessment of clinical samples to assess the prevalence of diarrhoea-causing bacteria, viruses and parasites in children presenting with diarrhoea as well as asymptomatic children should be investigated to establish possible carrier status and determine causative agents of diarrhoea.
- Continued assessment of fast, reliable and easy-to-use isolation and identification methods of causative diarrhoea agents to assist in the treatment of diarrhoea is needed to establish cost-effectiveness, reliability, quality of the test and time to perform the test to isolate and identify potential pathogens.
- Increased social behaviour education must be done by the relevant authorities to rural household members, especially mothers and caregivers with young children on water, sanitation and hygiene aspects, and preventative strategies should be put in place. These mothers/caregivers must be educated on what to do in specific circumstances to stop the spread of the disease and improve the health of the children.
- Reassess treatment schedules used in hospitals and primary healthcare clinics to lower antibiotic resistance in pathogenic bacteria.
- Further testing is required to characterise the *E. coli* strains pathogenicity (PCR testing) and to confirm colistin susceptibility or resistance.
- Various stakeholders involved in studying antimicrobial resistance in South Africa, irrespective of their field of study, come together and work on a focused way to systematically study antimicrobial resistance in humans, animals and the environment.

REFERENCES

ADLHOCH C et al. 2011. Highly sensitive detection of the group A Rotaviruses using Apolipoprotein H-coated ELISA plates compared to quantitative real-time PCR. Virol J 8(63):1-7.

AHIADEKE C. 2000. Breastfeeding, diarrhoea and sanitation as components of infant and child health: A study of large scale survey data from Ghana and Nigeria. J Biosoc Sci 32:47-61.

ALDEYARBI HM, ABU EL-EZZ NMT and KARANIS P. 2016. Cryptosporidium and cryptosporidiosis: The African perspective. Environ Sci Pollut Res 23(14): 13811-13821. DOI: 10.1007/s11356-016-6746-6

ALI U, ABBASI SA, KALEEM F, AFTAB I and BUTT T. 2017. Outbreak of extensively drug resistant Stenotrophomonas maltophilia in burn unit. J Ayub Med Coll Abbottabad 29(4):686-688.

BINNICKER MJ. 2015. Multiplex molecular panels for diagnosis of gastrointestinal infections: Performance, result interpretation, and cost-effectiveness. JCM 53(12):3723-3728.

BLACK RE et al., 2010. Global, regional and national causes of child mortality in 2008: A systematic analysis. Lancet 375:1969-1987.

BRADSHAW D et al. 2003. Initial burden of disease estimates for South Africa, 2000. Cape Town: Medical Research Council of South Africa 93(9):68-687.

BROOKS GF, BUTEL JS and MORSE SA. 2004. Jawetz, Melnick and Adelberg's Medical Microbiology, 23rd ed. McGraw Hill Companies, Singapore.

BURTON-MACLEOD JA, KANE EM, BEARD RS, HADLEY LA, GLASS RI and ANDO T. 2004. Evaluation and comparison of two commercial enzyme-linked immunosorbent assay kits for detection of antigenically diverse human noroviruses in stool samples. J Clin Microbiol 42:2587-2595.

BUSS SN et al. 2015. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol 53:915-923.

CAMPOCCIA D et al. 2010. Characterization of 26 Staphylococcus warneri isolates from orthopedic infections. Intern J Artificial Organs 33(9):575-581.

CARAMIA G, SILVI S, VERDENELLI MC and COMAN MM. 2015. Treatment of acute diarrhoea: Past and now. Int J Enteric Pathog 3(4):e28612.

CHAMBERS HF and DELEO FR. 2009. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature Rev Microbiol 7(9):629-641. DOI: 10.1038/nrmicro2200

CHARLES RC and RYAN ET. 2011. Cholera in the 21st century. Curr Opin Infect Dis 24:472-477.

CHEN JC et al. 2007. Inhibition of Escherichia coli heat-labile enterotoxin-induced diarrhea by Chaenomeles speciosa. J Ethnopharma 113(2):233-239.

CHHABRA P et al. 2013. Etiology of viral gastroenteritis in children <5 years of age in the United States, 2008-2009. J Infect Dis 1-9.

CHOLA L, MICHA LOW J, TUGENDHAFT A and HOFFMAN K. 2015. Reducing diarrhoea deaths in South Africa: Costs and effects of scaling up essential interventions to prevent and treat diarrhoea in under-five children. BMC Public Health 15(1):1.

CHOLA L and ROBBERSTAD B. 2009. Estimating average inpatient and outpatient costs and childhood pneumonia and diarrhoea treatment costs in an urban health centre in Zambia. Cost Effectiveness and Resource Allocation 7:16.

CORONA F and MARTINEZ JL. 2013. Phenotypic resistance to antibiotics. Antibiotics 2(2):237-255. DOI: 10.3390/antibiotics2020237

COX P, GRIFFITH M, ANGLES M, DEERE D and FERGUSON C. 2005. Concentrations of pathogens and indicators in animal feces in the Sydney watershed. Appl Environm Microb 71:5929-5934.

DEFRANCESCO AS, TANIH NF, SAMIE A, GUERRANT RL and BESSONG PO. 2017. Antibiotic resistance patterns and beta-lactamase identification in Escherichia coli isolated from young children in rural Limpopo Province, South Africa: The MAL-ED cohort. SA Med J 107(3):205-214.

DENNEHY PH. 2005. Rotavirus Vaccines: An Update. Curr Opin Ped 17:88-92.

DEPARTMENT OF HEALTH. 2008. Cholera outbreak in Zimbabwe and South Africa. Statement by Minister of Health Barbara Hogan on the outbreak of cholera in Zimbabwe and South Africa. 26 November 2008.

ESTES M and KAPIKIAN A. 2007. Rotaviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizmzn B (Eds) Fields Virology, 5th ed. Kluver Health/Lippincott Williams & Wilkins, PA. pp. 1917-1974.

ETEBU E and ARIKEKPAR I. 2016. Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. Intern J Appl Microbiol Biotech Res 4:90-101.

EZZATI M, LOPEZ AD, RODGERS A, VAN DER HOORN S, MURRAY CJ and COMPARATIVE RISK ASSESSMENT COLLABORATING GROUP. 2002. Selected major risk factors and global and regional burden of disease. The Lancet 360(9343):1347-1360.

FARUQUE SM. 2012. Foodborne and waterborne bacterial pathogens. Epidemiology, evolution and molecular biology, Caister Academic Press, Norfolk. 318 pp.

FRAGA SG et al. 2007. Environment and virulence factors of Vibrio cholerae strains isolated in Argentina. J Appl Microbiol 103(6):2448-2456.

GENTRY J, VINJE J and LIPP EK. 2009. A rapid and efficient method for quantitation of genogroups I and II norovirus from oysters and application in other complex environmental samples. J Virol Meth 156:59-65.

GERMS-SA. 2015. Annual Report. GERMS-SA, Johannesburg. Available online: http://www.nicd.ac.za/assets/files/GERMS-SA%20AR%202015-1.pdf

GRACEY M and KING M. 2009. Indigenous health part 1: Determinants and disease pattern, Lancet 65-75.

GRASSI T, BAGORDO F, IDOLO A, LUGOLI F, GABUTTI G and DE DONNO A. 2010. Rotavirus detection in environmental water samples by tangential flow ultrafiltration and RT-nested PCR. Environ Monit Assess 164(1-4):199-205.

GREEN K. 2007. Caliciviridae: The noroviruses. In: Knipe DM, et al., eds. Fields Virology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 949-979.

GULATI P and GHOSH M. 2017. Biofilm forming ability of Sphingomonas paucimobilis isolated from community drinking water systems on plumbing materials used in water distribution. J Wat Health 15(6):942-954. DOI: 10.2166/wh.2017.294

HALEY BJ, COLE DJ and LIPP EK. 2009. Distribution, diversity and seasonality of waterborne Salmonella in a rural watershed. Appl Environ Microbiol 75:1248-1255.

HANSMAN G et al. 2004. Detection of norovirus and sapovirus infection among children with gastroenteritis in Ho Chi Minh City, Vietnam. Arch Virol 149:1673-1688.

HATCHETTE TF and FARINA D. 2011. Infectious diarrhea: When to test and when to treat. CMAJ, 183:339-344. DOI: 10.1503/cmaj.091495

HE XQ et al. 2009. First molecular detection of group A rotaviruses in drinking water sources in Beijing China. Bull Environ Contam Toxicol 83(1):120-124.

HOLLENBECK BL and RICE LB. 2012. Intrinsic and acquired resistance mechanisms in Enterococcus. Virulence 3(5):421-569. DOI:10.4161/viru.21282

HONG S et al. 2016. Serotypes and antimicrobial resistance in Salmonella enterica recovered from clinical samples from cattle and swine in Minnesota, 2006 to 2015. PLOS One. DOI: 10.1371/journal.pone.0168016

HUANG RS, JOHNSON CL, PRITCHARD L, HEPLER R, TON TT and DUNN JJ. 2016. Performance of the Verigene enteric pathogens test, BioFire FilmArray gastrointestinal panel and Luminex xTAG gastrointestinal panel for detection of common enteric pathogens. Diagn Microbiol Infect Dis 86(4):336-339.

HUNTER PR and THOMPSON RC. 2005. The zoonotic transmission of Giardia and Cryptosporidium. Intern J Paras 35:1181-1190.

HUTTNER A et al. 2013. Antimicrobial resistance: A global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrobial Resist Infect Contr 2:31. DOI: 10.1186/2047-2994-2-31

ISLAM MS et al. 2011. Phenotypic, genotypic, and antibiotic sensitivity patterns of strains isolated from the cholera epidemic in Zimbabwe. J Clin Microbiol 49:2325-2327.

ITOH Y, NAGANO I, KUNISHIMA M and EZAKI T. 1997. Laboratory investigation of enteroaggregative Escherichia coli O untypeable: H10 associated with a massive outbreak of gastrointestinal illness. J Clin Microbiol 35:2546-2550.

JAYOL A et al. 2018. Are animals a source of Stenotrophomonas maltophilia in human infections? Contributions of a nationwide molecular study. Europ J Clin Microbiol Infect Dis [Epub ahead of print]. DOI: 10.1007/s10096-018-3203-0

JENKINS MB, ENDALE DM and FISHER DS. 2008. Most probable number methodology for quantifying dilute concentrations and fluxes of Salmonella in surface waters. J Appl Microbiol 104:1562-1568.

JOHNSON TJ, WANNEMUEHLER Y, KARIYAWASAM S, JOHNSON JR, LOGUE CM and NOLAN LK. 2012. Prevalence of avian-pathogenic Escherichia coli strain O1 genomic islands among extraintestinal and commensal E. coli isolates. Bacteriol 194(11):2846-2853.

KEDDY K. 2010. The group for enteric, respiratory and meningeal disease surveillance in South Africa. Communic Dis Surveil Bull 8(2):25-27.

KHALILI H, SOLTANI R, SAFHAMI S, DASHTI-KHAVIDAKI S and ALIJANI B. 2012. Antimicrobial resistance pattern of gram-negative bacteria of nosocomial origin at a teaching hospital in the Islamic Republic of Iran. East Mediterr Health J 18(2):172-177.

KHARE R et al. 2014. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. J Clin Microbiol 52:3667-3673.

KINGOMBE CI, CERQUEIRA-CAMPOS ML and FARBER JM. 2005. Molecular strategies for the detection, identification, and differentiation between enteroinvasive Escherichia coli and Shigella species. J Food Prot 68:7549-7553.

KOTLOFF et al., 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. Lancet 382:209-222.

LALANCETTE C, DI GIOVANNI GD and PREVOST M. 2010. Improved risk analysis by dual direct detection of total and infectious Cryptosporidium oocysts on cell culture in combination with immunofluorescence assay. Appl Environm Microbiol 76:566-577.

LANATA CF et al. 2013. Global causes of diarrheal disease mortality in children <5 years of age: A systematic review. PLOS One 8(9):e72799.

LEVANTESI C, LAMANTIA R, MASCIOPINTO C, BÖCKELMANN U, AYUSO-GABELLA MN and SALGOT M. 2010. Quantification of pathogenic microorganisms and microbial indicators in three wastewater reclamation and managed aquifer recharge facilities. Sci Total Environ 408(21): 4923-4930.

MAGNANI RJ, MOCK NB, BERTRAND WE and CLAY DC. 1993. Breast- feeding, water and sanitation, and childhood malnutrition in the Philippines. J Biosoc Sci 25:195-211.

MANS J, ARMAH GE, STEELE AD and TAYLOR MB. 2017. Norovirus epidemiology in Africa: A review. PLOS One 11(4):e0146280.

MANS J, DE VILLIERS JC, DU PLESSIS NM, AVENANT T and TAYLOR MB. 2010. Emerging norovirus GII.3 2008 variant detected in hospitalized paediatric patients in South Africa. J Clin Virol 49: 258-264.

MANS J, MURRAY TY and TAYLOR MB. 2014. Novel norovirus recombinants detected in South Africa. Virol J 11:168.

MICHAEL CA, DOMINEY-HOWES D and LABBATE M. 2014. The antimicrobial resistance crisis: Causes, consequences, and management. Front Public Health 2:145. DOI: 10.3389/fpubh.2014.00145

MILLER WR, MUNITA JM and ARIAS CA. 2014. Mechanisms of antibiotic resistance in Enterococci. Expert Rev Anti-infective Therapy 12(10):1221-1236. DOI: 10.1586/14787210.2014.956092

MINTZ ED and GUERRANT RL. 2009. A lion in our village: The unconscionable tragedy of cholera in Africa. N Engl J Med 360:1060-1063.

MOGANEDI KLM, GOYVAERTS EMA, VENTER SN and SIBARA MM. 2007. Optimisation of the PCR-invA primers for the detection of Salmonella in drinking and surface waters following a pre-cultivative step. Water SA 33:195-201.

MOR SM and TZIPOR S. 2008. Cryptosporidiosis in children in sub-Saharan Africa: A lingering challenge. Clin Infect Dis 47:915-921.

MOTOMURA K et al. 2010. The Norovirus Surveillance Group of Japan. Divergent evolution of norovirus GII/4 by genome recombination from May 2006 to February 2009 in Japan J Virol 84:8085-8097.

MUNITA JM and ARIAS CA. 2016. Mechanisms of antibiotic resistance. Microbiol Spectr 4(2):1-37. DOI: 10.1128/microbiolspec.VMBF-0016-2015

MURPHY CN, FOWLER RC, IWEN PC and FEY PD. 2017. Evaluation of the BioFire FilmArray gastrointestinal panel in a Midwestern Academic Hospital. Eur J Clin Microbiol Infect Dis 36:747-754.

MURRAY TY, MANS J and TAYLOR MB. 2013. Human Calicivirus diversity in wastewater in South Africa. Department of Medical Virology, University of Pretoria, p 1-15.

MWAMBETE KD and JOSEPH R. 2010. Knowledge and perception of mothers and caregivers on childhood diarrhoea and its management in Temeke Municipality, Tanzania. J Health Res 12(1):1-9.

NATION RL et al. 2015. Framework for optimisation of the clinical use of colistin and polymyxin B: The Prato polymyxin consensus. Lancet Infect Dis 15: 225-234.

NATIONAL DEPARTMENT OF HEALTH (NDOH). 2015. Implementation plan for the antimicrobial resistance strategy framework in South Africa: 2014-2019. National Department of Health, Pretoria.

NATIONAL DEPARTMENT OF HEALTH (NDOH). 2017 Guidelines on implementation of the antimicrobial strategy in South Africa: One health approach and governance. National Department of Health, Pretoria.

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASE (NICD) BULLETIN. 2008 Cholera outbreak in South Africa: Preliminary descriptive epidemiology on laboratory- confirmed cases, 15 November 2008 to April 2009. Available online: http://www.nicd.ac. za/assets/files/CommDisBullMay09_Vol0702

NAVIDINIA M. 2016. The clinical importance of emerging ESKAPE pathogens in nosocomial infections. J Paramed Sci 7(3):43-57.

NISHIUCHI Y, IWAMOTO T and MARUYAMA F. 2017. Infection sources of a common non-tuberculous Mycobacterial pathogen, Mycobacterium avium complex. Front Med 4:27. DOI: 10.3389/fmed. 2017.00027

OBI CL, POTGIETER N, BESSONG PO and MASTAUNG G. 2002. Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. Water SA 28(3): 87-291.

OCHOA TJ, BARLETTA F, CONTRERAS C and MERCADO E. 2008. New insights into the epidemiology of enteropathogenic Escherichia coli infection. Trans R Soc Med Hyg, 102(9):852-856.

PACZOSA MK and MECSAS J. 2016. Klebsiella pneumoniae: Going on the offense with a strong defense. Microbiol Molec Biol Rev 80:629-661.

PATEL MM, HALL AJ, VINJE J and PARASHAR UD. 2009. Noroviruses: A comprehensive review. J Clin Virol 44:1-8.

PENATTI MPA et al. 2007. Epidemiological characterization of resistance and PCR typing of Shigella flexneri and Shigella sonnei strains isolated from bacillary dysentery cases in Southeast Brazil. Braz J Med Biol Res 40(2):249-258.

PENDLETON JN, GORMAN SP and GIMORE BF. 2013. Clinical relevance of the ESKAPE pathogens. Exp Rev Anti-infective Ther 11(3):297-308.

POST GB, ATHERHOLT TB and COHN PD. 2011. Health and aesthetic aspects of drinking water. In Water Quality and Treatment. 6th ed. Edited by Edzwald JK. American Water Works Association, Denver, CO, pp. 1-100.

QADRI F, SCVENNERHOLM A, FARUQUE ASG and SACK. 2005. Enteroxigenic Escherichia coli in developing countries: Epidemiology, microbiology, clinical features, treatment and prevention. Clin Microbiol Rev 18:465-483.

QAMAR FN et al. 2016. Aeromonas-associated diarrhea in children under 5 years: The GEMS experience. Am J Trop Med Hyg 95(4):774-780. DOI: 10.4269/ajtmh.16-0321

QUAN TP et al. 2016. Increasing burden of community-acquired pneumonia leading to hospitalisation, 1998-2014. Thorax 71:535-542.

RAO MR et al. 2003. High disease burden of diarrhea due to enterotoxigenic Escherichia coli among rural Egyptian infants and young children. J Clin Microbiol 41(10):4862-4864.

ROBINSON TP et al. 2016. Antibiotic resistance is the quintessential One Health issue. Trans Royal Soc Trop Med Hyg 110(7):377-380. DOI: 10.1093/trstmh/trw048

ROGAWSKI ET al. 2017. Epidemiology of enteroaggregative Escherichia coli infections and associated outcomes in the MAL-ED birth cohort. PLOS Negl Trop Dis 11(7):e0005798.

ROMAN S, AZIMZADEH P, MOHEBBI SR, BOZORGI SM, ZALI N and JADALI F. 2012. Prevalence of sapovirus infection among infant and adult patients with acute gastroenteritis in Tehran, Iran. Gastroenterol Bed Bench 5(1):43-48.

RUSSELL AD. 2004. Types of antibiotics and synthetic antimicrobial gents. In: Denyer S. P., Hodges N. A. & German S. P. (eds.) Hugo and Russell's Pharmaceutical Microbiology. 7th ed. Blackwell Science, UK. Pp. 152-186.

SAMIE A et al. 2006. Cryptosporidium species: Preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. Exp Parasitol 1-9.

SAMIE A, MAKONTO TE, ODIYO J, OUABOI-EGBENNI PO, MOJAPELO P and BESSONG OP. 2011. Microbial quality, diversity and antibiotic susceptibility profiles of bacterial isolates from borehole water used by schools in Greater Giyani Municipality, Mopani District, South Africa. Afr J Microbiol Res 5(3): 198-210.

SANG WK, OUNDO V and SCHNABEL D. 2012. Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhoea in four provinces of Kenya. J Infect Dev Count 6(07): 572-578.

SANTAJIT S and INDRAWATTANA N. 2016. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Internat. DOI: 10.1155/2016/2475067

SHAH M et al. 2016. Prevalence, seasonal variation, and antibiotic resistance pattern of enteric bacterial pathogens among hospitalized diarrheic children in suburban regions of central Kenya. Trop Med Health 44:39. DOI: 10.1186/s41182-016-0038-1

SHAIKH S, FATIMA J, SHAKIL S, RIZVI SMD and KAMAL MA. 2015. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci 22(1): 90-101. DOI: 10.1016/j.sjbs.2014.08.002

SIEBENGA J, KRONEMAN A, VENNEMA H, DUIZER E and KOOPMANS M. 2008. Food-borne viruses in Europe network report: the norovirus GII.4 2006b (for US named Minerva-like, for Japan Kobe034-like, for UK v6) variant now dominant in early seasonal surveillance. Euro Surveill 13.

SPINA A et al. 2015. Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicentral study of community azured gastroenteritis. Clin Microbiol Infec 21:719-728.

STATISTICS SOUTH AFRICA (STATS SA). 2014. Mortality and leading causes of death in South Africa 2013. Findings from death notifications. Available online: http://www.statssa.gov.za/publications/P03093/P030932013.pdf

STOCKMANN C et al. 2015. How well does physician selection of microbiological tests identify Clostridium difficule and other pathogens in pediatric diarrhea? Insights using multiplex PCR-based detection. Clin Micribiol Infect 21:179.e9-179.e.15.

STOCKMANN C et al. 2017. Detection of 23 gastrointestinal pathogens among children who present with diarrhoea. J Ped Infect Did Soc 6:231-238.

TADESSE DA et al. 2012. Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002. Emerg Infect Dis Res 18(5):741-749.

TAYLOR MB et al. 1993. Two successive outbreaks of SRSV-associated gastroenteritis in South Africa. J Med Virol 41:18-23.

VAN ZYL WB, PAGE NA, GRABOW WOK, STEELE AD and TAYLOR MB. 2006. Molecular epidemiology of group A rotaviruses in water sources and selected raw vegetables in southern Africa. Appl Environ Microbiol 72(7):4554-4560.

VENTOLA CL. 2015. The antibiotic resistance crisis: Part 1: Causes and threats. Pharm Ther 40(4): 277-283.

WALKER CLF et al. 2013. Global burden of childhood pneumonia and diarrhoea. The Lancet 381(9875):1405-1416.

WALSH C. (2003). Antibiotics: Actions, Origins, Resistance. 1st ed. ASM Press, Washington, DC. 345 pp.

WANKE CA, SCHORLING JB, BARRETT LJ, DESOUZA MA and GUERRANT RL. 1991. Potential role of adherence traits of Escherichia coli in persistent diarrhea in an urban Brazilian slum. Pediatr Infect Dis J 10:746-751.

WHITE PA, EDEN JS and HANSMAN GS. 2012. Molecular epidemiology of noroviruses and sapoviruses and their role in Australian outbreaks of acute gastroenteritis. Microbiolog Aust.

WILKES G et al. 2009. Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape. Water Res 43:2209-2223.

WOLF S, HEWITT J and GREENING GE. 2010. Viral multiplex quantitative PCR assays for tracking sources of faecal contamination. Appl Environ Microbiol 76:1388-1394.

WORLD HEALTH ORGANIZATION (WHO). 2008. Health system problems aggravate cholera outbreak in Zimbabwe. WHO, Geneva, Switzerland

WORLD HEALTH ORGANIZATION (WHO). 2014. Antimicrobial resistance: Global Report on Surveillance. WHO, Geneva, Switzerland

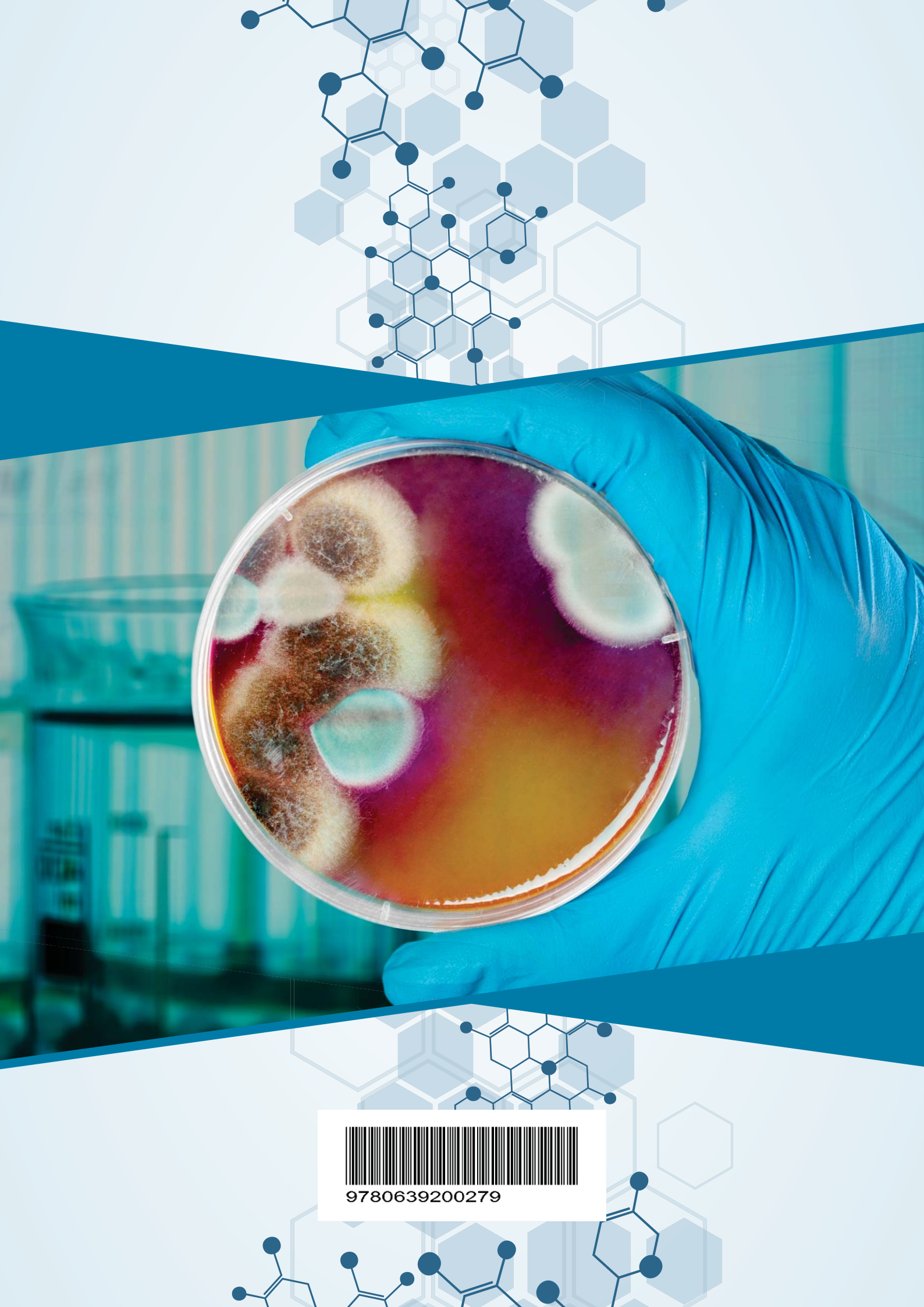
WORLD HEALTH ORGANIZATION (WHO). 2015. Diarrheal disease. Available online: http://www.who.int/mediacentre/factsheets/fs330/en/ [Accessed July 2017].

WORLD HEALTH ORGANIZATION (WHO). 2017. Integrated surveillance of antimicrobial resistance in foodborne bacteria: Application of a One Health Approach. WHO, Geneva, Switzerland

WORLD HEALTH ORGANIZATION (WHO). 2018. Integrated surveillance of antimicrobial resistance in foodborne bacteria: Early implementation 2016-2017. WHO, Geneva, Switzerland

WRIGHT S. (2005) Amoebiasis and giardiasis. Medicine 33(8):47-50.

ZHENG DP, ANDO T, FANKHAUSER RL, BEARD RS, GLASS RI and MONROE SS. 2006. Norovirus classification and proposed strain nomenclature. Virol 346:312-323.



9780639200279