SURVEILLANCE OF GASTROINTESTINAL INFECTIONS IN INDIVIDUALS OVER THE AGE OF 5 YEARS IN SOUTH AFRICA

Siobhan Lindsay Johnstone

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Declaration

I, Siobhan Lindsay Johnstone, declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Siobhan Lindsay Johnstone

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I. **Johnstone SL**, Page NA, Thomas J, Madhi SA, Mutevedzi P, Myburgh N, Herrera C, Groome MJ. Diarrhoeal diseases in Soweto, South Africa, 2020: a cross-sectional community survey. BMC Public Health. 2021; **21**: 1431, https://doi.org/10.1186/s12889-021-11470-9

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Abstract

Gastrointestinal infections cause significant mortality and morbidity, especially in Africa. While children ≤5 years of age bear the brunt of diarrhoeal disease, there is a significant burden in older age groups. Limited data on aetiology in these older age groups limits appropriate interventions. Diarrhoeal surveillance is important for monitoring disease trends in a population and should inform testing and treatment guidelines, and interventions. This body of work evaluated the epidemiology of diarrhoea at each level of the surveillance pyramid to assist in interpretation of routine health data and identify gaps in surveillance.

A household survey was conducted in Soweto to estimate community diarrhoeal prevalence, associated risk factors and healthcare seeking behaviors. An analysis of diagnostic testing practices for diarrhoeal diseases was done, using a doctors' survey, at three public hospitals in South Africa. Routine diagnostic data and enhanced surveillance data were compared to evaluate patient-related factors associated with requests for diagnostic investigation, type of diagnostic testing offered and the efficiency of available tests. A hospital surveillance study investigated the infectious causes of diarrhoea in hospitalised patients >5 years.

Results indicated a high diarrhoeal burden across all age groups in South Africa (5.3% of respondents reported an episode in the preceding 2 weeks). While the majority of infections were mild, 40% required healthcare. Many of those requiring healthcare (34%), specifically adults, were unable to access the required care. Those that did access healthcare were treated empirically and seldom had stool samples collected for diagnostic investigations (approximately 10% of admitted cases). Available diagnostics in public health laboratories detected pathogens in only 13.7% of these submitted stools due to pre-analytical and analytical issues including not testing for all relevant pathogens. Diarrhoeal prevalence was particularly high among HIV-infected patients (67.5% of patients >5 years admitted for diarrhoea were HIV-infected) and these patients presented with a unique aetiology.

This research highlights the need for diarrhoeal testing and treatment guidelines based on local epidemiological data with a focus on HIV-infected patients. Current diagnostics require optimisation including specimen collection, standardisation, pathogens included in routine testing panels, turnaround time and methods of detection. This will guide decisions on future public health interventions including vaccines.

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Abbreviations

AMR	Antimicrobial resistance
ANDEMIA	African Network for improved Diagnostics, Epidemiology and Management of common Infectious Agents
aOR	adjusted odds ratio
ART	antiretroviral therapy
CDC	Centres for Disease Control and Prevention
CDW	Corporate Data Warehouse
CED	Centre for Enteric Diseases
CHAMPS	Child Health and Mortality Prevention Surveillance network
CI	confidence interval
CMV	cytomegalovirus
EAEC	Enteroaggregative *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
EPI	Expanded Program on Immunisation
ETEC	Enterotoxigenic *Escherichia coli*
FBD	Foodborne disease
GBD	Global Burden of Disease study
GEMS	Global Enteric Multicentre Study
GERMS-SA	Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa
HDSS	health and demographic surveillance site
HIV	human immunodeficiency virus
IMCI	Integrated Management of Childhood Illness
LMIC	low- and middle-income countries

MAL-ED	Malnutrition and Enteric Diseases Study
NHLS	National Health Laboratory Services
NICD	National Institute for Communicable Diseases
NMC	Notifiable Medical Condition
NP/OP	nasopharyngeal/oropharyngeal
OR	odds ratio
ORS	oral rehydration solution
PCR	polymerase chain reaction
SA	South Africa
SDG	Sustainable development goals
SSA	sub-Saharan Africa
STEC	Shiga toxin-producing *Escherichia coli*
USA	United States of America
WaSH	Water, sanitation and hygiene
WHO	World Health Organization

Preface

I started my career as a medical scientist working in the laboratory at a vaccines research unit based at Baragwanath Hospital in Soweto. This work exposed me to field of epidemiology and clinical research. I was particularly interested in epidemiology as a tool through which I could make changes and contributions to health systems and was drawn to the fusion of science, health, economics, statistics and social studies that this field offers.

My PhD was completed in the midst of the COVID-19 pandemic which provided a unique set of opportunities and challenges. As a full-time PhD student at the National Institute for Communicable Diseases (NICD), I was able to gain invaluable experience in terms of outbreak response, infectious diseases modelling, global health and health system preparedness. At the start of the pandemic, I volunteered at the Emergency Operations Centre to assist with case management and contact tracing for confirmed cases and answering questions on the NICD COVID-19 helpline. Following this, I worked with the Department of Health in the Free State to assist the field epidemiology team with identifying clusters, generating automated daily situational reports, setting up systems to maintain line lists, and planning for community testing and isolation. In early 2022, I started working with the South African COVID-19 Modelling Consortium (SACMC) to ensure data streams from the NICD for models and projections. As a junior on this team, I have been able to listen in on high-level meetings with some of the leading experts in the field and have been able to see first-hand how data is translated into policy decisions. Although this work on COVID-19 was beyond the scope of my PhD (and caused some delays since I was taken away from my PhD work), it has given me a broader understanding of epidemiology and infectious diseases at all levels and will always be instrumental to my training as an epidemiologist.

My PhD was funded by the German Federal Ministry for Education and Research through the African Network for Improved Diagnostics, Epidemiology and Management of common Infectious Agents (ANDEMIA). This funding allowed me to focus on my studies full-time, travel for workshops and conferences, and to network with students and leading academics from across the world. I am especially appreciative of the collaborations that were formed amongst the African partner countries and I hope these collaborations will continue into the future.

I am grateful for the opportunities I have been afforded in pursuing epidemiology as a career. I hope to use my research position to advocate for interventions against diseases of poverty which are often available, yet out of reach for so many.

Contents

List of Figures:

1. Introduction

1.1. Public health importance of diarrhoeal diseases

Diarrhoea is a common and usually self-limiting event experienced by most healthy individuals several times throughout their lifetime. Despite this, episodes may become chronic or persistent depending on the infecting pathogen and the host characteristics[1]. Diarrhoea is defined as the passage of a greater number of stools of decreased form than is usual for an individual, generally three or more loose stools per day[2]. Acute episodes are defined as lasting less than 14 days, persistent diarrhoea is defined as episodes lasting between 14 and 30 days and chronic diarrhoea as episodes lasting longer than 30 days[2]. Clinical presentation can be watery diarrhoea, dysentery (blood in the stool) or steatorrhea (fatty stool)[2]. Although there are a variety of physiological causes, the majority of diarrhoeal cases are due to infectious agents, specifically viruses, bacteria and protozoa[2]. Watery diarrhoea is generally associated with viral infection while dysentery is usually a result of bacterial infection[3]. Diarrhoea due to infectious agents is often accompanied by vomiting, nausea, abdominal pain, bloating and fever[4]. The most common cause of diarrhoea globally is estimated to be faecally contaminated water and food[5]. It is also commonly spread from person-to-person through the faecal-oral route due to poor hygiene practices[5].

Even in high-income settings, diarrhoea places a significant burden on healthcare systems, with estimates from the United States of America (USA) placing acute diarrhoea as a leading cause of outpatient visits, hospitalisations and lost quality of life[4]. It is estimated that 47.8 million cases occur annually in the USA amongst all ages, costing the country approximately US$150 million per year[4]. Economic effects of infections extend beyond those placed on healthcare systems, as they reduce economically active days for individuals. A USA telephone survey reported that 4% of respondents had suffered an episode of diarrhoea within the previous 4 weeks which had affected their ability to carry out their normal daily tasks[6]. Only 21% of those with a reported diarrhoeal episode sought medical care[6]. Chronic diarrhoea, specifically, leads to a poorer quality of life, with sufferers reporting being worried to leave the house to perform normal, everyday activities[7].

1.2. Prevention of diarrhoea

The acquisition and spread of diarrhoeal pathogens is largely due to lack of safe water, sanitation and hygiene (referred to as WaSH). Faecally contaminated water is a major concern for diarrhoeal diseases and those residing in low-income, specifically rural settings are at highest risk[8]. Meta-analysis data indicate that a quarter of diarrhoeal episodes may be prevented with point of use water disinfection such as filters or chemical disinfection[5], however, the drive is to provide all households with clean, potable water. The United Nations Sustainable Development Goal (SDG) 6 states that clean water and sanitation should be available to all[9]. Despite this, the World Health Organization

(WHO) estimates that one in three people still do not have access to safe drinking water and two fifths of the world's population do not have basic hand washing facilities[8]. The promotion of effective hand hygiene has been demonstrated to reduce diarrhoeal diseases by 30%, both in high-income childcare centres and in low-income community settings[10]. Individuals are encouraged to practice effective hand hygiene by ensuring they wash their hands with soap and water at critical times, namely; after using the toilet, after changing diapers/nappies, before preparing food, before eating and before feeding babies or young children. Other important times for hand hygiene include before and after caring for someone that is sick; before and after treating a wound; after blowing your nose; coughing or sneezing; after touching animals, animal feed, or animal waste; and after touching garbage[11]. Effective hand hygiene is particularly important in healthcare settings and in the food industry[12].

Despite improvements in WaSH over the past decades, specifically in high-income settings, a large proportion of diarrhoeal diseases are attributable to foodborne pathogens, including norovirus, *Campylobacter* spp. and *Salmonella* spp. [6,13]. Many high-income settings have sophisticated surveillance networks for foodborne pathogens, however, globally and in low- and middle-income countries (LMICs) estimates are lacking and the spectrum of pathogens is not well understood[13]. Food safety is of specific concern in these low-income settings as food production and distribution is often poorly regulated, especially since a large proportion of the population relies on the informal sector[14]. Increased globalisation means that food distribution networks are often large, with products traveling long distances between production and consumption[14]. The WHO has prepared communications around the 'five keys to safer food' to educate food handlers[15]. These five keys include: (1) 'keep clean', which refers to proper hand hygiene and sanitation of surfaces and equipment; (2) 'separate raw and cooked' referring specifically to raw meat, poultry and seafood and ensuring that separate equipment is used for preparing raw food; (3) 'cook thoroughly', specifically meat, poultry, eggs and seafood; (4) 'keep food at safe temperatures', which are either cold (below 5°C) or hot (above 60°C) rather than storing food at room temperature; (5) 'use safe water and raw materials', including safe or treated water, pasteurised milk, fresh foods and washing fruit and vegetables[15].

There are several vaccines available against important diarrhoeal pathogens. The most significant of these to date is the rotavirus vaccine which has been introduced into childhood immunization programs in over 100 countries since 2006, resulting in a 59% reduction in rotavirus hospitalisations, a 36% reduction in acute diarrhoeal hospitalisations and a 36% reduction in acute diarrhoeal deaths in children under the age of 5 years in these countries[16]. There are currently four pre-qualified oral, live, attenuated rotavirus vaccines available, namely RotaTeq™, Rotarix™, Rotavac™ and Rotasiil™ with the WHO recommending introduction of these vaccines for all countries[17]. Vaccines against

cholera and typhoid are available, however, administration is generally for emergency or outbreak situations, or to those travelling to areas with endemic transmission[18,19]. The typhoid conjugate vaccine (TCV) has been introduced into routine immunisation programmes in some high-burden countries[20]. *Shigella* and enterotoxigenic *Escherichia coli* (ETEC) have been identified as vaccine targets, and although there are promising candidate vaccines being evaluated in clinical trials, none are currently licensed for these pathogens[2,21,22]. Candidate vaccines against norovirus are currently being evaluated[23]. Norovirus has replaced rotavirus as the leading cause of diarrhoea amongst all ages in the USA[24]. One of the major barriers in advocacy for further development and efficacy testing of enteric vaccines is the lack of reliable burden estimates, specifically from low-income settings[25]. Burden estimates are required to inform vaccine investments and to provide baseline data for efficacy assessments[25]. Identifying high burden communities will also assist in ascertaining possible clinical trial sites once candidate vaccines achieve licensure[25].

1.3. Treatment of diarrhoea

Preventing dehydration is the cornerstone of treating diarrhoea. Severe cases may require intravenous rehydration but in the majority of cases, oral rehydration is sufficient[26]. Oral rehydration salts/solutions (ORS) are recommended by the WHO to prevent or correct dehydration[27]. ORS is a glucose-electrolyte solution that can be easily prepared at home and is given by mouth, making it an effective intervention for community and primary healthcare levels, as long as communities are well educated on when and how to use ORS[27]. Use has been shown to significantly improve survival for diarrhoeal cases[28] and estimates place reductions of child mortality due to the use of ORS in low-income countries at 50%[4]. Other diarrhoeal treatments include zinc, which is used to reduce severity and duration of disease, specifically in children, and antisecretory and antimotility drugs which are used to reduce the rate of stool production[4,26]. The use of antibiotics in the treatment of diarrhoea is dependent on the aetiology of the infection, however, since community-acquired diarrhoea is most likely to be viral in origin, the empiric use of antibiotics is not recommended in most settings[4]. Antibiotic treatment guidelines are often based on aetiological studies rather than diagnostics for individual cases[4]. Antimicrobial resistance (AMR) is becoming a progressively more important challenge facing healthcare systems globally. In LMICs, the high burden of bacterial infection means that AMR is an even greater risk, specifically in areas in with poor diagnostic capabilities, delayed presentation and poor access to second-line antibiotics[29]. Treatment guidelines and adherence to treatment guidelines are vital to ensure that antibiotics are not incorrectly prescribed or used[29]. Overuse of antibiotics has been documented to cause enteric disease or antibiotic-associated diarrhoea by reducing naturally-occurring microflora in the gut and hence allowing overgrowth with pathogenic species, such as *Clostridioides difficile* (*C. difficile*)[2]. Growing knowledge on the importance of gut health and the microbiome has led to the recognition of the use of probiotics for

both the treatment and prevention of diarrhoea. Probiotics are live bacteria, such as strains of *Lactobacillus*, which are found to be beneficial to the health of the human gut[26]. The mechanisms by which probiotics maintain a healthy gut environment are strain specific but generally involve the maintenance of pH conditions, prohibiting pathogen attachment and maintaining gut microflora[4]. Probiotics have been found to have some benefit in treating viral diarrhoea and are currently only used in high-income and private healthcare settings due to high costs[26].

1.4. Diagnosis of diarrhoea

Diagnostic methods available for diarrhoeal cases are heavily dependent on culture and microscopy in most settings, with molecular methods slowly becoming more widely available[2]. Although microscopy has the advantage of being inexpensive, it is known to be insensitive and requires substantial time, equipment and expertise[30]. Culture is also relatively inexpensive and is the only available method able to distinguish between viable and nonviable bacterial pathogens, however it is also limited by insensitivity, particularly in high antibiotic use settings[30]. The sensitivity of culture is particularly impacted by delays in getting to the laboratory and disruptions in cold chain, both of which are likely in LMICs. Although there are methods available to improve culture yields for particular bacterial pathogens, these methods generally limit the yields of other pathogens and a suspected diagnosis is required before testing[30]. Serology methods have limited relevance for diarrhoeal testing but are valuable for epidemiological studies in determining seroprevalence[30]. Molecular methods available for testing diarrhoeal stools are very sensitive and have the added benefit of being able to screen for multiple pathogens simultaneously. The drawback with molecular testing is that it may be difficult to distinguish between carriage and disease-causing organisms, particularly in settings where exposure to enteropathogens is common[30]. Asymptomatic carriage of enteropathogens is a particularly important area of research and further data are required to improve understanding[30]. Molecular testing may also be difficult to implement in low-resourced settings as it requires specialised equipment and laboratory expertise[31]. Rapid testing may offer an attractive solution for many settings since it requires minimal expertise or equipment and can be performed as a bedside test with a short turnaround time[31]. The major shortfall of rapid diagnostic tests is that each test is designed to detect only one pathogen and as such a suspected diagnosis is required before testing[31]. These tests may be helpful in quickly diagnosing or ruling out prevalent pathogens with relevance for treatment and are a growing field in diarrhoeal diagnostics. Currently there is no one-size-fits-all approach for diarrhoeal diagnostics and a combination of available methods may be required depending on setting, local epidemiology and available resources.

1.5. Global diarrhoeal disease burden

The Global Burden of Disease (GBD) study placed diarrhoea as the 8th leading cause of death amongst all ages in 2016, with an estimated 1 655 944 deaths globally[32]. In children under 5 years of age,

diarrhoea was found to be the 5th leading cause of death, with an estimated 446 000 deaths globally[32]. Although they found several differences in aetiology between different income settings, the GBD study attributed the majority of global diarrheal cases to 13 pathogens, namely rotavirus, *Shigella*, ETEC, non-typhoidal *Salmonella*, *Campylobacter* spp., adenovirus, norovirus, amoebiasis, *Cryptosporidium* spp., *Aeromonas*, enteropathogenic *Escherichia coli* (EPEC), *Vibrio cholerae* and *C. difficile*. They indicate that individuals >70 years have higher diarrhoeal mortality rates than children <5 years globally (171.7 deaths per 100 000 versus 70.6 deaths per 100 000), despite children <5 years having higher diarrhoeal incidence rates than individuals >70 years (1.75 episodes per person-year versus 0.90 episodes per person-year). This shows that although diarrhoeal disease is less common in the elderly compared to young children, it is often more severe. The disease incidence rate in the elderly has decreased from 1990 to 2016, however, the overall burden of disease in this age group has increased due to the aging global population[32]. The GBD estimates show that although diarrhoea affects all populations and regions, 90% of diarrhoeal deaths globally occur in south Asia and sub-Saharan Africa (SSA) and are due to lack of safe WaSH, poor access to healthcare and suboptimal case management[32].

Data from adults presenting to emergency departments with severe, acute diarrhoea in the US, indicated that pathogens could only be detected in 49% of stool samples[33]. Notably, this study found that viruses were the most common cause of severe, acute diarrhoea in adults in a high-income setting, with norovirus (26%) and rotavirus (18%) being the most commonly detected pathogens. Rotavirus was previously considered to be uncommon in adults[33]. This study also found a high proportion of pathogen-negative acute diarrhoeal cases, despite using available assays for all known pathogens, indicating these cases may be due to non-infectious causes or pathogens for which testing is not available.

1.6. Diarrhoeal diseases in low- and middle-income settings

Although there are several studies on diarrhoea in children in LMICs, two important studies namely, the Global Enteric Multicentre Study (GEMS) and Malnutrition and Enteric Diseases Study (MAL-ED), have helped inform data modelling globally in children under 5 years (including models described by the GBD study).

The GEMS study, which enrolled over 20 000 children from seven countries across Africa and Asia, was the largest study ever conducted on diarrhoeal diseases in low-income settings[34]. This study was done to determine the aetiology and population-based burden of disease in children under five years of age with moderate-to-severe diarrhoea seeking care at health centres using a matched case-control design over a 3-year period[34]. The study demonstrated the importance of including non-diarrhoeal controls in the collection of these data[35]. Although the aetiology of diarrhoeal infections

differed to some extent between sites, the majority of cases across all sites were due to four pathogens, namely, rotavirus, *Cryptosporidium*, ETEC producing heat-stable toxin and *Shigella*[36]. Estimates indicate that interventions targeting these four pathogens could prevent up to 40% of disease in the first two years of life. The study found that diarrhoeal infections with rotavirus, *Shigella*, *V. cholerae* O1 and adenovirus were more likely to be symptomatic and that the odds of death were 8.5 fold higher in cases as compared to matched community controls[36]. Controls were more likely to have greater access to improved water, belong to higher socioeconomic status groups and have mothers with higher education[36].

The MAL-ED study was a longitudinal birth cohort which followed 1 715 children from birth until 2 years, in seven low-income settings[37]. At least one pathogen was detected in almost two-thirds of diarrhoeal cases and diarrhoea due to viruses (36.4%) was more common than diarrhoea due to bacteria (25.0%) or parasites (3.5%). The most common pathogens found were *Shigella*, sapovirus, rotavirus, adenovirus, ETEC, norovirus, astrovirus, *Campylobacter jejuni* or *C. coli*, *Cryptosporidium*, and EPEC[38]. The study found that approximately half of the diarrhoeal cases were treated with antibiotics which, due to the high incidence of viral diarrhoea, was inappropriate in 91.7% of cases[38].

To date there have been no large scale, multicentre studies on diarrhoea in adults in LMICs. Current data available includes localised epidemiological studies, studies looking at specific pathogens and epidemic reports. A case-control study on the aetiology of acute diarrhoea in adults in south-western Nigeria found that enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and *Entamoeba histolytica* were significantly associated with symptomatic diarrhoea and that *E. histolytica* was the most commonly isolated pathogen[39]. This study only looked at bacteria and parasites and did not screen for viral infections. Since results from the MAL-ED study indicate that viral pathogens are the most common source of enteric disease in children, it is likely that this study did not show the full picture of enteric diseases in this adult population[38,39]. A retrospective review detected bacterial pathogens in 8.2% and parasites in 5.6% of diarrhoeal stool samples from patients of all ages analysed by the public diagnostic laboratory in Botswana[40]. *Shigella* spp., *Salmonella* spp., *Cystoisospora* spp. and *Cryptosporidium* spp. were the most commonly isolated pathogens. These results suggested that viral pathogens might be responsible for a large proportion of diarrhoeal diseases in this setting[40]. Botswana is a high HIV-burden setting and as such, it is possible that some of these pathogen-negative cases were due to HIV enteropathy[40].

1.7. Diarrhoeal diseases in South Africa

The South African Demographic and Health Survey (SADHS) conducted in 2016, reported that 10% of children under the age of 5 years had reported diarrhoea in the two weeks preceding the interview[41]. The same population reported a 3% prevalence of respiratory infections and 20% prevalence of

fever. Healthcare was sought more often for respiratory infections and fever than for diarrhoea (88% of respiratory cases and 68% of fever cases compared with 63% of diarrhoeal cases)[41]. In terms of treatment, ORS and zinc supplements were given in 51% and 37% of diarrhoeal cases, respectively[41]. Data on diarrhoeal diseases in adults were not collected. Findings from death notifications from Statistics South Africa (StatsSA) indicate that diarrhoea remains in the top 20 underlying causes of death amongst all ages in 2018 (13 245 deaths; 1.6%)[42]. Diarrhoea was responsible for a high proportion of deaths in infants (7.2%, ranked as the third highest underlying cause of death) and children between the ages of 1-14 years (6.0%, ranked as the second highest underlying cause of death). Surprisingly, diarrhoea was still amongst the top ten underlying causes of death for young adults between 15-24 years (1.1%). Diarrhoeal mortality was highest in the rural provinces of Mpumalanga and Limpopo[42].

The South African General Household Survey in 2018 indicated that household access to WaSH has improved significantly since 2002 with 83% of households having access to improved sanitation (defined as flush toilets or pit toilets with ventilation pipes) and 89% having access to clean drinking water[43]. In spite of this, over a third of households (37.6%) reported poor water service quality (including frequent water shortages and water that required treatment before consumption)[43]. Service delivery is unequally distributed in SA and is notoriously poor in rural areas. This is significant since 32.6% of the population lives in rural areas[44]. A community survey in rural Limpopo found that the majority of households (97.0%) stored water for drinking and cooking in containers due to frequent disruptions in water supply and since many rely on communal taps which are usually some distance away from the house or yard[45]. Water samples from the majority (60%) of these storage containers were contaminated with coliforms at levels exceeding safe drinking water quality, thereby posing a risk for diarrhoeal disease[45].

Much of the available diarrhoeal data in SA is for disease in young children, including extensive studies on rotavirus and the impact of rotavirus vaccination on incidence of hospitalisations. Before the introduction of the oral rotavirus vaccine into the South African Extended Program for Immunisation (SA-EPI) in 2009, diarrhoea was estimated to be the leading cause of hospitalisation in children under the age of 5 years and rotavirus was estimated to be responsible for 46% of diarrhoeal admissions[46,47]. This proportion decreased to 29% within two years of rotavirus vaccine introduction and it is estimated that 13 000 – 20 000 diarrhoeal hospitalisations in children under two years of age were prevented during this time period alone[47]. Other important diarrhoeal pathogens implicated in severe disease in young children are *Campylobacter* spp., ETEC and norovirus[48]. A study in school aged children in Cape Town found a high underlying prevalence (55.8%) of soil-transmitted helminths and that infection with such intestinal parasites may be a risk factor for acquisition of HIV and TB[49].

South Africa currently has the highest HIV burden in the world and is home to 19% of the global HIV-infected population[50]. Slim or wasting disease and chronic diarrhoea are recognised clinical features of HIV-infection with diarrhoea occurring in 60-90% of HIV-infected individuals not on antiretroviral treatment (ART)[51]. Literature indicates a shift in aetiology of diarrhoea with HIV progression and treatment[52]. Early stage disease is characterised by high CD4 cell counts and low viral loads with diarrhoeal episodes often linked to HIV seroconversion. With HIV progression comes decreased immune function and diarrhoea is often due to opportunistic pathogens including bacterial infections, such as *Mycobacteria*, parasitic infections, such as *Cystoisospora belli*, *Cyclospora*, S*trongyloides*, *Cryptosporidia*, *Microsporidia* and viral infections, such as cytomegalovirus (CMV)[52–54]. Studies have found a proportion of diarrhoea in HIV-infected patients to be pathogen negative and hypothesise that these may be due to HIV enteropathy and permanent damage to the gastrointestinal mucosa[55,56]. Other risk factors which increase diarrhoea in HIV-infected patients include changes in gut microbial populations which leads to dysbiosis and defects to cellular and humoral defence mechanisms in the gastrointestinal tract[57,58]. Patients with untreated HIV infection will continue to see decreases in CD4 cell count and increases in viral load and are at increased risk for chronic diarrhoea and polyparasitic infection[59,60]. Patients with well-treated HIV infection (with corresponding lower viral load and recovery of CD4 cell count) are at decreased risk for opportunistic infections and remaining diarrhoeal episodes are thought to be drug-related[55].

Studies done in SA and Mozambique have found HIV-infection to be a significant risk factor for infection with *C. difficile*, rotavirus, *Shigella* and *Cryptosporidium*[50,61]. Diarrhoea in HIV-infected children is known to be more severe, prolonged and result in more frequent hospitalisations with higher mortality rates[61]. Diarrhoea has been shown to be a major cause of morbidity and to be detrimental to quality of life in HIV-infected adults, with self-reported symptoms being associated with levels of psychological distress, independent of CD4 cell count and HIV viral load[7,51]. A case-control study amongst HIV-infected people in rural Limpopo found that being female (aOR: 2.02, 95% CI 1.10–3.73), being over the age of 45 years (aOR: 6.31, 95% CI 1.50–26.50), limited water access (aOR: 2.66, 95% CI 1.32–5.35) and not yet being initiated on ART (aOR: 5.87, 95% CI 3.05–11.27) were significant risk factors for diarrhoea[62].

An audit of clinical management found that 81% of patients presenting with diarrhoea to emergency departments in Kwa-Zulu Natal in 2013 were HIV-infected[63]. This study found that stool specimen diagnostics were requested in 47.2% of cases and that antibiotics were prescribed inconsistently[63]. The authors called specifically for more comprehensive guidelines for the management of diarrhoea in HIV-infected patients[63]. South African National Department of Health guidelines for the treatment of diarrhoea are currently included in the standard treatment guidelines and essential medicines list (hospital and primary healthcare levels)[64,65]. The primary healthcare guidelines specify that the

majority of chronic diarrhoea in adults is HIV-related and that HIV testing should be encouraged amongst these patients. Chronic diarrhoeal patients should have a specimen submitted for microscopy and should be treated empirically with metronidazole for giardiasis (which the guidelines mention is often difficult to diagnose on stool). HIV-associated diarrhoea is described in the guidelines as being persistent and associated with wasting. They recommend stool should be submitted for testing for ova, cysts and parasites, specifically cryptosporidium and that treatment should depend on laboratory results. Current treatment guidelines in SA for HIV-infected individuals include the use of the antibiotic prophylaxis; cotrimoxazole for opportunistic infections and isoniazid for prevention of TB infection[66]. The increased use of antibiotics suggests that antibiotic-associated diarrhoea may be prevalent amongst HIV-infected individuals. South Africa is also seeing an aging HIV epidemic due to improved ART and prophylaxis treatment[67]. The combined risk factors of HIV infection and aging are likely to result in epidemiological changes in opportunistic diarrhoeal infections.

1.8. Diarrhoeal diseases surveillance

Infectious disease surveillance is one of the most important epidemiological tools available in monitoring the health of a population and is considered an essential function of a public health system[68,69]. The International Health Regulations (2005) specify surveillance functions as a key competency for signatories, which includes SA[70,71]. The characteristics of a surveillance system depend largely on the disease under surveillance, resources and healthcare infrastructure available, frequency of information required and type of action or intervention available for the disease[72]. However, most surveillance systems are set up to achieve three overarching goals. These goals are to 1) describe the burden of the disease in the specified population; 2) to monitor disease trends in the population and; 3) to quickly identify outbreaks of infectious diseases as well as new pathogens of potential concern[68]. Although the three overarching goals for infectious diseases surveillance apply, the surveillance of diarrhoeal diseases should have specific aims and objectives given the characteristics of the disease and required data outputs necessary for public health response. In terms of the first goal, the main aim would be to quantify the burden of diarrhoeal disease in order to indicate the requirements for preventative interventions, such as improvements in WaSH or food safety practices, as well as medical resource allocation, such as vaccination programs and drug administration. The second goal (to monitor disease trends in the population) refers to epidemiological monitoring of diarrhoeal cases in terms of 'person, place and time' but also to monitoring of aetiological causes of diarrhoea. These data are also important for resource and intervention allocation as well as monitoring the effects of introduced interventions. The third goal (identification of outbreaks and new pathogens) is especially important for diarrhoeal surveillance as many diarrhoeal pathogens have strong epidemic potential. *Vibrio cholerae* is an example of such a

pathogen, and in most settings where cholera is not endemic, a single case is regarded as an outbreak and should activate public health investigation and response[73]. Other pathogens may be endemic to the population of interest and an outbreak is only declared once the detected number of cases has surpassed a predefined threshold. Surveillance is important not only to recognise an increase in these cases but also to describe the baseline incidence of enteric pathogens and define the alert threshold for outbreaks[74].

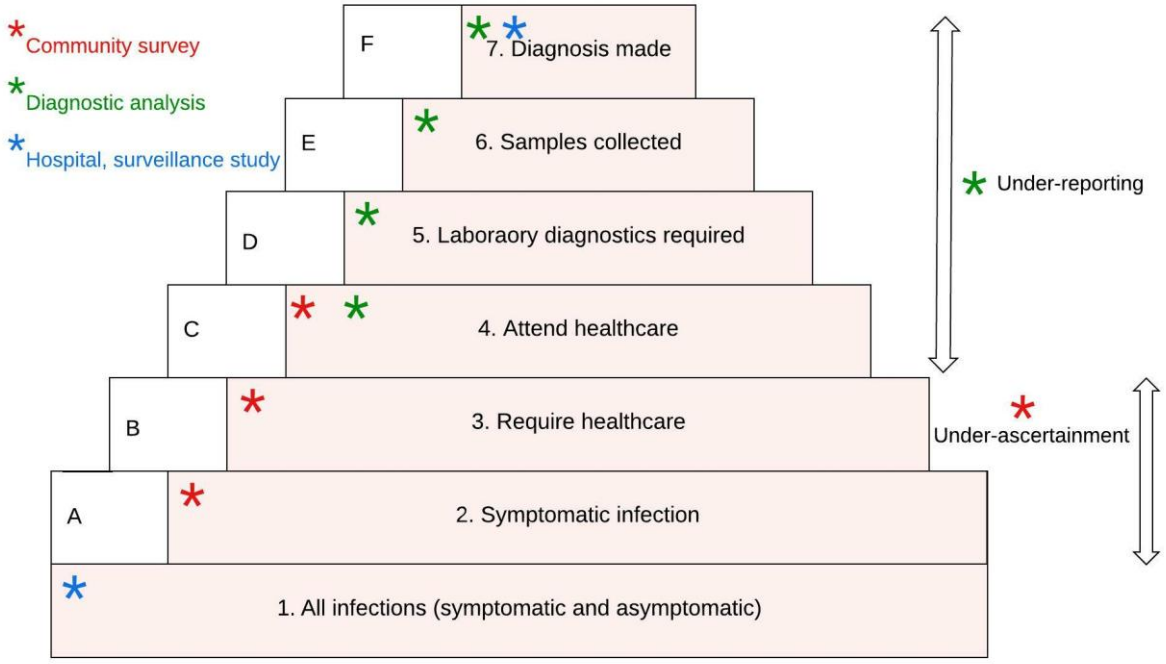
Perhaps one of the most extensive surveillance programs for diarrhoeal diseases is the Foodborne Diseases Active Surveillance Network (FoodNet), a collaborative program between the CDC, health departments, agricultural and food and drug departments as well as clinical laboratories across the USA which covers approximately 15% of this country's population[75]. FoodNet monitors burden and trends in specific foodborne pathogens using active clinical and laboratory surveillance as well as surveys amongst physicians and the general population and is a benchmark example of a surveillance program that aims to elucidate foodborne disease (FBD) pathogens at all levels of healthcare. It also provides the study infrastructure required to investigate specific questions regarding diarrhoeal diseases. In SA, there are several established surveillance programs that monitor diarrhoea at a facility-level as well as smaller, pathogen-specific research projects underway. Some of the main established surveillance programs coordinated by the National Institute for Communicable Diseases (NICD) include:

- The African Rotavirus Surveillance Network is a multinational system comprising 33 member states including SA[76]. It was initiated prior to the introduction of the rotavirus vaccine into the SA-EPI in 2009 and was conducted as a sentinel surveillance program at five hospitals across SA (Chris Hani Baragwanath Academic and Dr George Mukhari in Gauteng, Mapulaneng and Matikwana in Mpumalanga and Edendale in KwaZulu-Natal) with the objective to describe the epidemiology of rotavirus hospitalisation and monitor the effects of vaccination in children under the age of five years[77]. Since rotavirus hospitalisation has significantly decreased in children under five years of age, this surveillance has been extended to include other enteric viruses and is now referred to as the Diarrhoeal Diseases Syndromic Surveillance Network[76,78].
- The Group for Enteric, Respiratory and Meningeal Disease Surveillance in SA (GERMS-SA) is a laboratory based surveillance program which monitors several important bacterial pathogens with epidemic potential, including *Salmonella enterica*, *Shigella* spp., *Campylobacter* spp., and *Listeria* spp[78]. In 2020 there were approximately 222 public and private clinical microbiology laboratories contributing surveillance data and isolates to the GERMS-SA program, covering a large proportion of the South African population[78]. Bacterial isolates of interest are submitted to the NICD for further characterisation.

- The NICD has a Notifiable Medical Conditions (NMC) surveillance system, which aims to rapidly detect and respond to pathogens of concern. This passive surveillance system relies on healthcare workers to report diagnosed cases through an electronic notification system, which includes important clinical and epidemiological case information. Diseases that are classified as notifiable are those of public health concern due to their outbreak potential, have severe risk to human health, or diseases that have an available intervention which requires timeous implementation to be effective[79]. Some important enteric pathogens monitored through NMC are cholera, enteric fever (typhoid or paratyphoid fever), *Listeria*, *Salmonella* spp., *Shigella* spp. and Shiga toxin-producing *E. coli* (*STEC*)[79]. Foodborne disease outbreaks are classified as category 1 in the NMC system which requires reporting within 24 hours[79]. These notifications are received by the NICD in real time and relevant public health action is taken.
- The National Health Laboratory Services (NHLS) is the diagnostic pathology laboratory serving public health facilities which include over 80% of the SA population[80]. Results for all specimens submitted for diagnostic testing through the NHLS are stored in the Central Data Warehouse (CDW). The NICD utilises diagnostic laboratory data from the NHLS CDW as a routine health information system and surveillance is done through secondary data analysis[71]. Specimens submitted for individual patients' routine diagnostics therefore form an important part of surveillance. It is unclear which cases from the community are represented by those captured in the NHLS CDW routine health information system. Representativeness is an important consideration in surveillance and can be reduced if health seeking and reporting in the population differ by age, sex or geographical location[72]. Representativeness may also differ over time, for example during an outbreak the threshold for submission of stool specimens for diagnostic investigation may decrease due to increased awareness amongst physicians[81].
- The African Network for Improved Diagnostics, Epidemiology and Management of common Infectious Agents (ANDEMIA) is a collaborative, cross-country surveillance network conducted at 12 health facilities across four African countries, namely SA, Cote d'Ivoire, Burkina Faso and the Democratic Republic of the Congo. Detailed methods for this network are described in the methods section but in summary it is a prospective, syndromic, facility-based surveillance program[82]. The aim of the network is to generate surveillance data from patients admitted with common syndromes (respiratory tract infections, gastrointestinal infections and acute febrile diseases of unknown cause) for enhanced detection, control and treatment of these syndromes[82].

1.8.1. Surveillance pyramid framework

Figure 1 illustrates a surveillance pyramid for diarrhoeal diseases amended from literature[81,83]. It indicates cases captured in the NHLS CDW routine health information system and forms the main framework for this study. The first level includes all diarrhoeal infections, which may be symptomatic or asymptomatic, with the symptomatic fraction making up the second level. The total number of symptomatic infections in the community may not be relevant in terms of resource allocation, since many will be mild or self-limiting and will not require intervention or attendance in a healthcare setting but these cases may have economic impacts in terms of absence from work and decreased productivity. A proportion of symptomatic cases are severe enough, in terms of symptoms or duration of disease, for the patient to require healthcare; making up the third level of the pyramid. Personal or socioeconomic constraints may restrict healthcare access to many of those requiring intervention. Cases that do attend healthcare make up the fourth level of the pyramid. Diarrhoea is very often treated symptomatically or with empiric treatment and does not require diagnostic investigation[4]. The remaining fraction that do require diagnostic investigation constitutes the fifth level. The majority of these should then have a stool sample collected and sent for diagnostic investigation (making up the sixth level of the pyramid), however there may be cases where sample collection and submission is not possible due to personal or healthcare system restraints. Finally, samples submitted to the laboratory may yield a diagnostic result, which is captured in the NHLS CDW system (making up the final level of the pyramid).



A = Asymptomatic infections; B = mild disease not requiring healthcare; C = cases requiring but not able to access healthcare; D = laboratory diagnostics not required; E = laboratory diagnostics required but samples not collected; F = samples collected but no diagnosis made.

Figure 1: Surveillance pyramid framework for diarrhoeal diseases captured by the NHLS routine health information system

While the surveillance pyramid framework illustrates which cases are reflected in the routine health information system, it also illustrates which cases are lost to the system for various reasons. As previously mentioned, it is important that the design of each system is informed by the syndrome under investigation. As diarrhoea is largely a mild and self-limiting disease in healthy individuals, careful consideration should be given to which cases are important for detection in the system. It would be largely unhelpful for the system to attempt to capture diagnostic results for every diarrhoeal case of any severity in the community, and hence the majority of diarrhoeal cases will not, and should not, be included. However, the consideration and definition of which cases the system should ensure are ascertained is important, not only for the clinical treatment of these individual cases, but also to ensure that the core functions of the surveillance system are being met. Deciding on where in the surveillance pyramid to 'tighten the net' is a balance between available resources and the public health threat posed by losing cases at each level. Since surveillance data is often used for evidence-based decision making, policy, intervention and allocation of services, it is important to understand the limitations of such data, specifically in terms of cases that are not captured in the system[81]. Figure 1 indicates two main processes in which the surveillance system fails to capture diarrhoeal cases from the community. The first is under-ascertainment which occurs at a community-

level when those with symptomatic infections requiring healthcare do not access healthcare (A and B in Figure 1)[81]. This is specifically for a facility based or routine health information surveillance system, which does not attempt to investigate all cases in the community. The second is under-reporting (C-F in Figure 1) and pertains to cases presenting to healthcare that are still not captured in the system for several reasons which will be explored in more detail[81]. The following considerations should be made for cases not captured at each level of the surveillance pyramid framework (no attempt has been made to represent the fraction of cases not captured per level to scale in the figure):

A. Asymptomatic infections (denoted A in Figure 1) are particularly relevant in distinguishing between carriage and pathogenic infections, as has been done in important case-control studies of enteric pathogens[36]. Asymptomatic diarrhoeal infections generally do not require intervention or diagnostics and are not relevant for capture in the routine health information system. The exception to this would be cases of asymptomatic carriage in which there is still shedding and where the infected individual may act as a vector in transmitting the pathogen to others. An example is the infamous 'Typhoid Mary' or Mary Mallon, an Irish immigrant to the USA who worked as a cook for wealthy households in the early 1900s[84]. As a healthy carrier of *Salmonella typhi*, she was thought to have spread the bacteria to at least 122 people, resulting in at least 5 deaths. This asymptomatic spread is common for many enteric pathogens[85–87]. The detection of such asymptomatic cases might be done through reverse contact tracing but is not relevant for a routine health information system.
B. Those that do not require healthcare (denoted B in Figure 1) are also not relevant for capture in the surveillance system for reasons similar to those of the asymptomatic cases. As diarrhoea is largely a mild and self-limiting disease in most healthy individuals, it is expected that a large proportion of community cases will be lost at this level. The number of symptomatic cases in the community is important in determining true incidence and in indicating the fraction of cases that do require and seek healthcare[81]. Community based studies can be used to estimate the number of cases at this level and are helpful in determining barriers to accessing healthcare which may result in under-ascertainment of cases in the surveillance system[81]. Even mild cases (those that are self-limiting and do not require healthcare intervention) in the community may contribute to a loss of productivity with accompanying economic implications. Community level interventions to reduce even mild diarrhoeal diseases are therefore important.
C. Those that require healthcare but are unable to access it (denoted C in Figure 1) are of vital importance to the system and all efforts should be made to ensure that loss at this level is minimised. Cases not captured at this level may introduce bias into the surveillance system due to healthcare inequalities, such as variations in healthcare literacy or access to

healthcare. These may introduce differences in age- and sex-specific ascertainment rates, for example, younger children are generally more likely to seek healthcare for diarrhoea regardless of severity of symptoms and are likely to be overrepresented in surveillance systems[81]. There may be cultural, religious, legal, administrative or financial barriers to accessing healthcare which are generally more likely to affect marginalised groups[81].

D. Across settings, healthcare-attended diarrhoeal cases are often treated symptomatically or empirically without specific, directed laboratory investigations[4,88]. Again, this is a balance between available resources and clinical value offered to the patient. Diagnostic testing is likely to be more easily accessible in well-resourced healthcare settings[4]. While symptomatic and empiric treatment for diarrhoea is generally acceptable for the majority of cases and routine stool cultures are usually not recommended, this treatment should be evidence-based and requires a comprehensive understanding of the aetiology of diarrhoea in the specific population[88]. It is acceptable that a large proportion of cases will not be captured by the surveillance system since they do not require directed laboratory investigations (denoted D in Figure 1), however there should ideally be a good understanding of these cases (which may be supported by well-designed epidemiological studies, not necessarily the surveillance or routine health information system).

E. Specific diagnostic investigations are required for a proportion of cases. The specifics of these cases differ across settings and in some healthcare settings, guidelines are available to direct physicians as to which cases should be investigated. The American Academy of Family Physicians for example, indicates that laboratory investigations should be reserved for cases with 'severe dehydration or illness, persistent fever, bloody stool, or immunosuppression, and for cases of suspected nosocomial infection or outbreak', but these guidelines will differ by setting[88]. Healthcare workers may not always be able to request diagnostic investigations due to budget restrictions and other barriers or may not be aware of which tests should be performed[81]. The healthcare system should work to ensure that diagnostic testing is available for all cases for whom testing is specified or required, thereby ensuring cases not captured by the system (denoted as E in Figure 1) are minimised.

F. Stool diagnostics are subject to several constraints and hence pathogens are not always identified in samples sent for diagnostic investigation. Although this may be due to non-pathogenic causes of diarrhoea, it is often due to issues with sample collection, transport to the laboratory or laboratory detection methods used. The surveillance system should ensure that these issues are addressed in order to maximise yields for submitted samples and minimise samples in which no diagnosis is made (denoted F in Figure 1).

The stars on the diagram indicate the studies conducted as part of this research, that aim to elucidate each level of the pyramid. Each study will be discussed in more detail in the methods section.

2. Justification

The GBD 2016 study findings indicate that, although progress has been made towards reducing diarrheal disease globally, this reduction has not been uniform across all settings. Sub-Saharan Africa is one such area in which the burden of disease remains high and sources of data are limited[32]. This makes modelling difficult and results in estimates with high uncertainty. The GBD study specifically points out that data among populations older than 5 years of age are scarce[32]. Although the bulk of diarrhoeal morbidity and mortality exists in children under the age of 5 years, there is still significant burden in older age groups[32]. While this burden has implications for health systems, it may also have significant social and economic implications for families and communities due to absenteeism and loss of productivity[89]. Interventions to reduce disease in this group are fundamental to social and economic development[89]. Although there have been several studies on the epidemiology and aetiology of diarrhoea in children, there are still limited data in South African adults, specifically in relation to the high HIV burden. The studies that have been conducted have been within restricted populations or restricted to searches for specific pathogens[90]. As long as pathogen-specific causes of diarrhoea are unknown, required interventions remain unclear. Possible interventions such as household water connections and chlorination at point of delivery for water-borne pathogens or strategies of infection control, outbreak investigation, establishment of anti-microbial stewardship and, importantly, food safety policy could all be explored to reduce diarrhoeal morbidity and mortality[4,5]. Data to support and guide these interventions is required. Data on aetiology will also guide diagnostics in terms of which pathogens should be added to routine testing panels[91]. Candidate vaccines for ETEC, *Shigella* and norovirus are being developed, however, there has been some debate as to the requirements of this intervention due to poor or fluctuating epidemiological data in possible high burden settings[25,92]. More detailed data on these pathogens could help guide policy recommendations should the vaccines reach efficacy testing, as has been a longstanding WHO target for vaccine development specifically for ETEC and *Shigella*[89].

Unlike many LMICs, SA is in the fortunate position of having available routine health information systems for monitoring diarrhoeal pathogens. It is unclear however, what these data represent due to under-ascertainment and under-reporting of cases in the system. A review of the epidemiology of diarrhoea at each level of the surveillance pyramid will assist in interpreting these routine health data as well as identifying gaps in the system, ultimately strengthening the understanding of diarrhoeal diseases in individuals over 5 years of age and the ability to use this data for meaningful public health action.

3. Study Objectives

1. To describe diarrhoeal disease at a community level, by estimating the rate of and factors associated with diarrhoea across all age groups in Soweto and to determine the proportion of cases presenting to healthcare facilities. (PAPER I)
2. To describe the current diagnostic practices for diarrhoeal cases at ANDEMIA surveillance hospitals in SA using a doctors' survey as well as a comparison of routine diagnostic laboratory data (NHLS) and data from enhanced surveillance testing (ANDEMIA). (PAPER II)
3. To describe the epidemiology and aetiology of moderate to severe diarrhoeal cases over the age of 5 years presenting to ANDEMIA surveillance hospitals in SA between 2018 and 2021. (PAPER III)
4. To provide recommendations to improve the surveillance of diarrhoeal diseases and to decrease diarrhoeal morbidity and mortality in individuals over 5 years of age in South Africa. (PAPER IV)

4. Methods

4.1. Overview

This body of work comprised three studies, each designed and undertaken separately with the ultimate aim of allowing a comprehensive overview of diarrhoeal surveillance in individuals over the age of 5 years in SA. As previously introduced, Figure 1 indicates the framework for the routine health information system as a diarrhoeal surveillance system in SA and depicts how data collected through these three studies are used to contribute to an understanding of diarrhoeal disease at each level. Detailed methods are provided in the individual publications. A brief overview is given here with emphasis on integration of the data sources and subsequent analyses.

The first study, to address objective one, was a community survey (indicated by red stars in Figure 1) which was used to estimate under-ascertainment of community diarrhoeal cases. This was a house-to-house survey to estimate the two-week diarrhoeal prevalence at the community level in Soweto, giving an estimation for level two (symptomatic infections) in the pyramid. Severity of disease, requirements for healthcare interventions and barriers to accessing healthcare were investigated for individuals reporting a diarrhoeal episode in the past two weeks (levels 3 and 4 in the pyramid). Although population-based surveys can be considered a form of surveillance in their own right, this requires repetition on a regular basis and was not the purpose of this particular community survey[69]. This survey estimated symptomatic infections in the population at a single time point, in order to inform the framework in terms of detecting and describing burden of diarrhoeal disease.

The second study, to address objective two, was an analysis of diagnostic practices (indicated by green stars in Figure 1) with the aim to estimate under-reporting of diarrhoeal cases at a healthcare level. This analysis investigated diagnostic practices for patients admitted with diarrhoea by examining patient-related factors associated with requests for diagnostic investigation, diagnostic testing offered and the efficiency of the available tests (levels 4-7 in the pyramid).

The third study, to address objective three, was a hospital surveillance study (denoted with blue stars in Figure 1) which investigated the pathogenic causes of diarrhoea in hospitalised patients and included a case-control analysis (levels 1 and 7 in the pyramid). The methods for each objective and details of the data sources used are explained below.

Although this body of work focusses primarily on examining diarrhoea in patients over the age of 5 years, some aspects were expanded to include patients of all ages with a focus on older children and adults for comparison. This was due to the difficulty of limiting data collection to individuals over the age of 5 years for specific studies. In the pilot for the community survey, for example, asking respondents to answer questions on behalf of their households except for children under the age of 5 years resulted in some confusion amongst respondents. Similar issues were found in the doctors'

survey. Data were therefore collected for individuals of all ages and age-related factors considered during analysis.

4.2. Data sources

Secondary data from several different sources as well as primary data collection were used. Secondary data sources included the Child Health and Mortality Prevention Surveillance (CHAMPS) Soweto health and demographic surveillance site (HDSS) and NHLS CDW, while primary data sources include the ANDEMIA study, a community survey and a doctor's survey.

4.2.1. CHAMPS Soweto HDSS (secondary data)

Soweto is an urban settlement on the southwestern outskirts of Johannesburg and is home to approximately 1 271 628 people in 355 331 households[93]. Residents have relatively good access to services, with 93.1% of households having access to electricity for lighting, 91.3% with flush toilets connected to sewerage and 55% with piped water inside the household[93]. The CHAMPS network was established in seven countries across SSA and Asia to investigate causes of death in children under the age of 5 years with the ultimate aim to inform policy to prevent such deaths[94]. In SA, CHAMPS established the Soweto HDSS in 2017, covering eight clusters in low-income areas[94]. Two rounds of data collection are conducted per year in order to monitor population dynamics and community health[94]. An advantage of an established HDSS is the availability of a characterized sampling frame which can be used for sample selection for directed surveys[94]. Specific information on the Soweto HDSS has been published[94].

4.2.2. Community survey (primary data collection)

A cross-sectional community survey was undertaken to determine the 2-week prevalence of diarrhoeal diseases, of all severity and duration, at a community level. The purpose of the community survey was to estimate the community rate of and risk factors for diarrhoea across all age groups in Soweto and to determine the proportion of cases presenting to healthcare facilities. Data on handwashing practices, food preparation and storage practices, and ORS knowledge and use were collected. The survey used the established CHAMPS Soweto HDSS as a sampling framework. The survey data collection tool (Appendix B) was designed based on other similar surveys[6,95–97]. The data collection tool was tested in three stages, the first being a panel of epidemiology and clinical experts who provided review and feedback. The second stage was a pilot involving staff members from the NICD (including academic, laboratory, administrative and cleaning staff members). They were asked to complete the survey and to evaluate the confidence with which they could answer the questions on behalf of other members within their own households (including their children, spouse, parents and siblings). Finally, the tool was piloted in a small sample (n=10) of HDSS households which were

not included in the main study. This household pilot was also used as the practical field training for the fieldworkers.

A team of three fieldworkers and the investigator were available for data collection. For security, the team was split into two so that fieldworkers were in pairs at all times. Due to the small size of the team coupled with the need to complete data collection within the shortest period possible (due to the cross-sectional nature of the study), only four of the eight Soweto HDSS clusters were sampled. Probability proportional to size sampling was used to select four of the eight clusters and 125 households were randomly selected from each of the four selected clusters. Selected households were visited on two occasions and were considered as a non-response if not available at either visit. Written informed consent was obtained from an adult (≥18 years) representative of the household and the fieldworker administered the survey. All representatives were asked questions on the following:

- Handwashing practices including availability of handwashing facilities; what they used to wash their hands and; how often they washed their hands at critical times (before eating and feeding young children, after using the toilet and changing children's nappies/diapers)[11].
- Water supply, storage and treatment.
- Food practices, including where food is purchased; how it is stored and cooked (according to the WHOs five keys to safer food)[15].
- ORS knowledge and use.
- The occurrence of diarrhoeal episodes in the household (for all household members) in the past two weeks.

Only those that reported having had a self-reported diarrhoeal episode in the past two weeks (as indicated by response to the question 'have you or anyone in your household had diarrhoea (an upset stomach with 3 or more loose stools per day) in the last 2 weeks?') were asked additional questions on symptoms, duration of disease, treatment course and health seeking for the episode. Diarrhoeal episodes were categorised as mild if the respondent did not seek healthcare and did not feel that healthcare intervention was necessary due to the illness being self-limiting or not affecting their daily lives. Information on demographics, housing material, number of rooms, toilet and cooking facilities, and household assets were available for all HDSS households (from Soweto CHAMPS HDSS) and hence these data were not collected during the survey, but rather matched to households subsequent to enrolment. Households enrolled in the survey were matched to the HDSS data if the respondent's name, surname and age matched that recorded in the HDSS data. This was to prevent incorrect matching for households that may have relocated between the last round of

HDSS data collection and the current survey. The data were de-identified once matching was complete, and only the HDSS household number was used as a unique identifier.

A copy of the WHO 5 Keys to Safer Food and a recipe for homemade ORS was given and explained to the respondents at the end of the interview[15,98]. The study protocol was presented to the Soweto HDSS Community Advisory Board (CAB), which is made up of community members from each of the clusters. The CAB recommended that a member from each of the clusters accompany the fieldworkers on a daily basis for safety as well as to ensure acceptability within the community. The results of the analysis were presented to the CAB during their October 2020 meeting, following data collection and analysis.

4.2.3. ANDEMIA study (primary data collection)

This prospective, hospital-based surveillance study was undertaken at three hospitals (Kalafong, Mapulaneng and Matikwana) in SA between July 2018 and June 2022. Kalafong is a public, provincial, tertiary hospital which serves the generally low income communities residing in townships to the west of Pretoria while Mapulaneng (a 80 bed, district hospital) and Matikwana (a 250 bed, regional hospital) are smaller hospitals based in rural Mpumalanga[47,99]. Surveillance officers based at these hospitals did ward rounds on a daily basis to check for new admissions matching the inclusion criteria for the study. Consenting patients of all ages with diarrhoea (defined as the passage of three or more loose or liquid stools in the past 24 hours) were enrolled within 48 hours of admission (to exclude hospital-acquired infections). Diarrhoea of any duration, severity or type (dysentery, watery) was included as long as the case definition of three or more loose or liquid stools in the past 24 hours was met. A detailed case investigation form (including symptoms and medical history, current treatment, socioeconomic data, food and animal exposure, and travel history) was completed and a stool specimen collected. In cases where it was not possible to collect a stool specimen, a rectal swab was collected. Samples were sent to the Centre for Enteric Diseases (CED) at the NICD on ice and were tested for a panel of pathogens using the FastTrack Diagnostics stool viral gastroenteritis, stool bacterial gastroenteritis and stool parasite kits.

Hospital controls were enrolled between October 2019 and June 2022 from the surgical and orthopaedic wards as well as outpatient clinics (dental, eye, vaccination clinics) or visitors to the hospital. The aim of the nest case-control study was to allow for the adjustment of burden estimates for asymptomatic infections and for the identification of pathogens and risk factors associated with symptomatic infections. Deciding how to enrol controls was complex as the same protocol needed to be used across all ANDEMIA sites and therefore differences in settings, available resources and health systems needed to be considered. The choice between community or hospital controls was considered. The network decided to enrol hospital controls from the hospital wards and outpatient

clinics associated with the ANDEMIA hospital sites. This was more feasible than community controls since the same surveillance officers enrolling hospitalised cases were required to enrol controls and specimen collection in a hospital setting was preferable. Additionally, many of the ANDEMIA hospital sites serve large catchment areas and patients travel some distance to seek care. Selecting controls from the community surrounding the hospital may have resulted in a control population which is different to the case population, whereas, hospital controls minimized this selection bias since both cases and controls have taken the same route to care[100]. The use of hospital controls does however have limitations, such as over representing community prevalence of risk factors and may detect pathogens causing other symptoms[100]. The aim was to frequency match controls to cases by age group, site and month of enrolment (for seasonality) and to enroll 320 controls per country over a 12 month period. We enrolled pooled controls for all 3 syndromes, and as such controls had to be free of all symptoms relating to these syndromes for at least 3 weeks preceding enrolment. Each consenting control was requested to provide a nasopharyngeal/oropharyngeal (NP/OP) swab, a blood sample and a stool sample along with completion of the investigation form. Details of the ANDEMIA study method have been published[82].

4.2.4. Doctors' survey (primary data collection)

The doctors' survey was done at the three South African ANDEMIA surveillance hospitals (Kalafong, Mapulaneng and Matikwana hospitals). A survey tool was designed using Castor EDC, a cloud-based clinical data management system that allows surveys to be planned, sent out and the results captured. The survey consisted of three sections (Appendix C), the first for general information (including position at the facility, specialisation, ward placement and age group). The second section consisted of questions on specimen submission and treatment practices (including whether these decisions are governed by national guidelines, hospital guidelines, supervisor guidance or own clinical judgement and patient-related factors associated with requesting diagnostics) and the third section on barriers to stool specimen submission. The survey tool was tested by a panel of virology, epidemiology and clinical experts. Feedback was provided and the tool was amended accordingly. The finalised tool was tested by a group of ten academics at the NICD to ensure that online processes were working correctly and responses were correctly captured by the system. The pilot group also provided feedback to ensure that the survey questions made sense and had a logical flow.

The study and survey tool were presented at each of the three hospitals during departmental meetings (which are generally attended by all clinical staff). Doctors were given the option of completing the survey in a paper-based format, which was handed out during the presentation. Alternatively, they were asked to provide their email addresses and sent a link for the electronic Castor form. Email addresses were obtained for staff members not present in the departmental meeting. Paper forms were manually captured on the Castor system.

It was clearly explained to all participants (both verbally during the departmental meetings and written on the paper and electronic forms) that participation in the survey was entirely voluntary. No personal identifiers were collected and the electronic Castor survey did not link the responses to the respondent's email address. Results stratified by department, speciality or position within the hospital were presented at an aggregated level only so that individual respondents could not be identified. No written informed consent was obtained from participants as it was assumed that all participants taking part in the survey did so willingly. There was no compensation offered for participation. Results from this survey were presented in a departmental meeting after analysis.

4.2.5. NHLS CDW (secondary data)

Laboratory and diagnostic results generated through the NHLS are stored in the CDW repository and can be accessed on request from the NHLS Academic Affairs and Research Office. An application was made to the NHLS CDW for data on all stool specimens and rectal swabs submitted for diagnostic purposes from the three SA ANDEMIA surveillance hospitals between July 2018 and December 2019. The data request application was approved by the NHLS Academic Affairs and Research office. Data were provided without personal identifiers (except for date of birth, as age was an important variable for analysis). Fields included patient date of birth, sex, pathogens identified (including no identified pathogen), test type, sample registration date, hospital, and date of test.

4.3. Ethical permissions

The PhD proposal was approved by the Human Research Ethics Committee (Medical) (clearance certificate no. M190663 MED18-12-034) (Appendix A), which covered the secondary data analysis as well as primary data collection for the community survey and doctor's survey. The ANDEMIA study and CHAMPS Soweto HDSS were approved by the WHREC (Medical) (clearance certificate no. M170403 and M170216 respectively).

5. Analysis

5.1. Objective 1 (Paper I)

This paper used the data collected during the community survey. Data was specified as three-level, complex survey data to account for the cluster design of the study. Diarrhoeal rate was calculated as episodes per person-year. Factors associated with diarrhoeal episodes in the household and factors associated with seeking healthcare were determined using multivariate logistic regression. Further details can be found in Paper I.

5.2. Objective 2 (Paper II)

The doctors' survey was used to describe stool sample submission for diagnostic testing in terms of governance of these decisions, patient-related factors associated with submission and barriers to submitting stool specimens for diagnostic testing. Doctors' perceptions of which pathogens are routinely included in diagnostic tests (as reported in the doctors' survey) were compared to NHLS CDW data indicating which pathogens are routinely included in testing. In order to gauge the efficiency of stool diagnostics offered by the NHLS, the NHLS CDW data were compared to molecular results from the ANDEMIA study for the same hospitals over the same time period. As per the literature, the ANDEMIA molecular testing was considered to be notably more sensitive than culture and microscopy stool diagnostics offered by the NHLS and was used as a 'gold standard' in the comparison[30].

5.3. Objective 3 (Paper III)

An analysis of ANDEMIA cases indicated that the majority (67.5%) of patients over the age of 5 years admitted for diarrhoea are HIV-infected. The paper addressing objective 3, which was to describe the epidemiology and aetiology of moderate to severe diarrhoeal cases over the age of 5 years presenting to ANDEMIA surveillance hospitals, was therefore focussed on HIV-infected patients. This focus was supported by anecdotal evidence from the doctors' survey (doctors specifically requesting guidance on diagnostics and treatment for HIV-infected cases). Since the literature review indicated that there is data lacking specifically in this group and it has important implications for clinical guidelines and local epidemiology, the analysis was focussed on this group (but still included all patients over the age of 5 years including HIV-negative patients for comparison).

Pathogens could be detected in 82% of HIV-uninfected cases and only in 55% of HIV-infected cases using FastTrack Diagnostics. Therefore, additional testing was done for all cases and controls included in the analysis of the over 5 year olds using SeeGene kits for parasites as well as TaqMan array cards designed by the CED laboratory. The following targets were included: rotavirus, *Campylobacter jejuni/coli*, *Strongyloides*, *Salmonella Typhi* and *Salmonella Paratyphi* A, *Shigella flexneri* non6 and *Shigella sonnei*, *Shigella flexneri* 2 & 3, *Shigella flexneri* 5 and X, EAEC_aaiC & aatA,

ETEC_STh & STp, 18S, *E. bieneusi* & *E. intestinalis*, *Cryptosporidium*, *Giardia* and *Schistosoma*, *Campylobacter coli gyrA*, *Salmonella gyrA* 83, *Salmonella parC*, *Shigella/E.coli gyrA 83*, QnrA & gnrS, SHV_23840SESK, CTX-M, *Enterovirus*, RotaRix, sapovirus I, II, IV, norovirus GI.1 & GII.4, MS2, PhHV*, C. difficile*, *Plesiomonas*, *H. pylori*, *Yersinia*, *Shigella/EIEC*, *Salmonella Enteritidis*, *Salmonella Typhimurium*, *Shigella flexneri* 1 & 1C, *Shigella flexneri* 4 & 6, *V. cholerae*, *V. parahaemolyticus*, EPEC_bfpA & eae, ETEC_LT, *Cystoisospora*, STEC_stx1 & stx2, *E. histolytica* & O157, *C. hominis* & *C. parvum*, *Campylobacter parC*, *Campylobacter jejuni* gyrA, *Salmonella* gyrA 87, *Shigella/E.coli* parC, *Shigella/E.coli* gyrA 87, QnrB1, QnrB4, MOX_FOX, TEM.

Descriptive statistics (chi-squared/Fisher's exact test and t-tests) were used to compare characteristics, symptoms and pathogen detection between cases and controls for HIV-infected and HIV-uninfected cases. Details are presented in Paper III.

5.4. Objective 4 (Paper II, IV and policy brief)

The final objective of this project was to use the data generated in the previous three objectives to provide recommendations to improve the surveillance of diarrhoea and recommendations to decrease morbidity and mortality due to diarrhoea in this age group and setting.

Recommendations for the improvement of diarrhoeal surveillance, in terms of providing better guidelines and diagnostic tests, are provided in Paper II. The diarrhoeal diseases community survey highlighted the need for targeted communication regarding handwashing as a strategy for the prevention of diarrheal diseases. This finding was addressed by an in-depth analysis of handwashing and risk communication in this specific community, which is presented in Paper IV. Other suggested recommendations for public health intervention from findings generated in this study will be presented in the policy brief.

6. Results and Discussion

The WHO definition of surveillance as the 'ongoing systematic collection, analysis, and interpretation of outcome-specific data for use in planning, implementing and evaluating public health policies and practices', highlights that although surveillance largely involves data collection, considerations of how these data are used is a vital function of the system[72]. Figure 2 is a conceptual framework for surveillance and response amended from Nsubuga et al. 2006[69]. It shows how surveillance data collected are analysed in the 'data generation hemisphere'. These data should then be carefully interpreted and a response applied back into the health system as part of the 'data use hemisphere'. The data presented in the current body of work pertains to the 'data generation hemisphere' in the framework and helps to assist in answering the question of how to interpret diarrhoeal cases captured in the routine health information system (for example, how representative are these cases of diarrhoeal cases occurring in the community?). In terms of the 'data use hemisphere', there are two main questions that this project addresses. The first is what can be done to limit cases lost to the surveillance system at different levels? Addressing this question would feed into the health system and allow better data collection in the 'data generation hemisphere'. The second, more practical question in terms of public health action, is how can the data generated through this project be used to decrease diarrhoeal morbidity and mortality in this population? The main findings from the manuscripts generated through this research have been collated to answer these three questions.

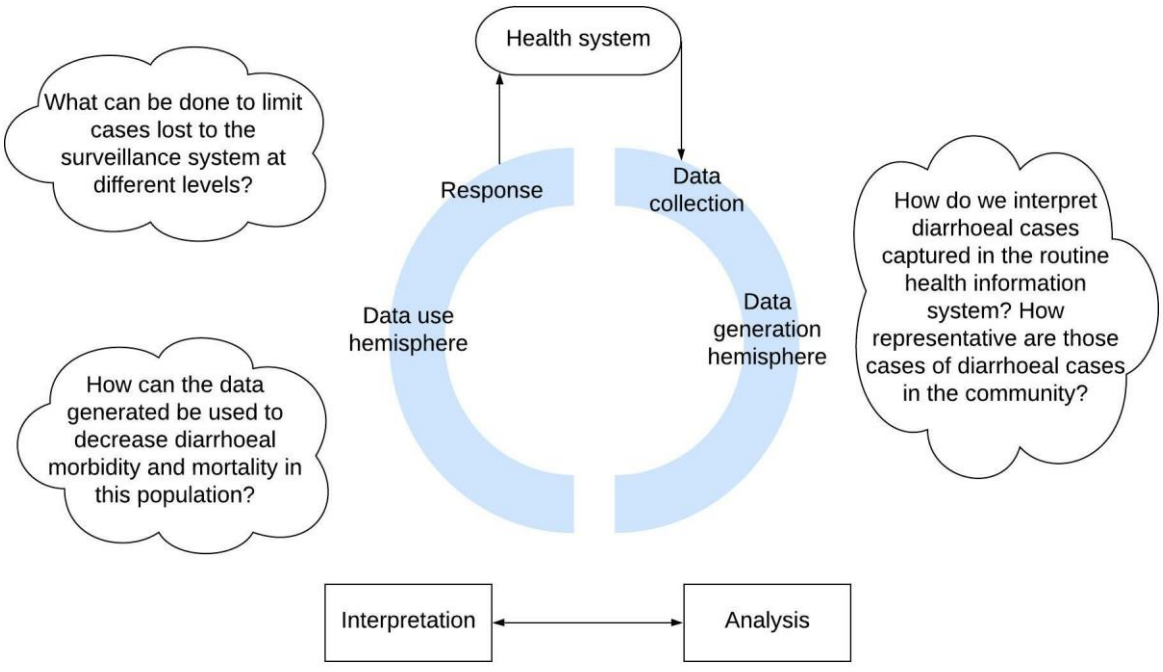


Figure 2: Surveillance and response conceptual framework (source: Nsubuga P, White ME, Thacker SB, et al. 2006[69])

6.1. Interpretation of diarrhoeal cases captured in the routine health information system

The community survey (Paper I) demonstrated a two-week diarrhoeal prevalence of 5.3% across all ages, which is relatively high compared to similar studies in high-income settings but lower than estimates reported from other African countries[6,96,101,102]. Interestingly, despite literature indicating that young children bear the brunt of diarrhoeal morbidity, we found similar prevalence across age groups. The majority of diarrhoeal episodes (58.6%) were mild (as the respondent indicated that the episode did not require healthcare), with **40%** of diarrhoeal sufferers indicating that their episode was severe enough to warrant healthcare. Of these requiring healthcare, only **66%** were able to access healthcare. Overall, few diarrhoeal sufferers (26%) sought healthcare, with the majority of these presenting to clinics (16%) followed by pharmacies (6%) and hospitals (5%). Children under the age of 5 years were significantly more likely to access healthcare than older age groups (OR = 5.9 (95% CI, 1.1–31.4), p = 0.039). This indicates that although there is a large burden of diarrhoea in older age groups, this burden is limited to the community and is unlikely to be detected through hospital surveillance. The doctors' survey (results included in Paper II) indicated that doctors submit stool specimens for approximately **10%** of diarrhoeal cases presenting to hospital. These cases with specimens submitted for diagnostics are likely to be chronic, cases of dysentery, suspected *C. difficile* infections, those with a travel history or suspected link to an outbreak. Stool samples are submitted for diagnostics more often at the urban site, meaning rural cases are likely underrepresented in routine health data. The analysis of routine diagnostic data (results included in Paper II) indicated that only **14%** of stool specimens submitted have a pathogen detected.

A summary of these multipliers (shown in bold) indicates that for every pathogen detected through routine diagnostic testing, there are approximately 270 symptomatic diarrhoeal cases in the community (using the estimates of 40% of diarrhoeal cases in the community require healthcare and 66% of these attend healthcare, of which 10% will have a stool specimen collected and 14% of these specimens will have a pathogen detected using routine methods). In an ideal surveillance and healthcare system, healthcare should be accessed by 100% of cases in which it is required and a pathogen should be detected in 65% of diarrhoeal stools tested (as per GEMS data using molecular methods)[38]. If we then assume that our estimate of 40% of diarrhoeal cases requiring healthcare is accurate and that doctors submit specimens in 10% of diarrhoeal cases attended, this number should be closer to 38 (i.e. the routine diagnostic system should detect one pathogen for every 38 cases of diarrhoea in the community). Although this requires more robust modelling, this estimate could serve in the interpretation of diarrhoeal cases captured in the routine health information system.

6.2. Limiting cases lost to the surveillance system

The community survey in Soweto indicated that 58.6% of diarrhoeal cases did not require healthcare as their disease was mild and self-limiting. As discussed previously, a diarrhoeal surveillance system should not attempt to capture diagnostic results for every diarrhoeal case of any severity (such as these mild cases, denoted B in Figure 1), especially in a resource-limited healthcare setting. It is however relevant to note that the burden of diarrhoeal diseases in the community is relatively high, and although not relevant to the healthcare system, these cases may contribute to a loss of productivity and are therefore of economic importance. Community level interventions to reduce community transmission of diarrhoeal diseases, including education on safe food preparation practices and good hand hygiene, are important and are discussed as outputs of this research.

Approximately one third of surveyed diarrhoeal cases that indicated their diarrhoeal episode was severe enough to warrant medical attention, reported being unable to access healthcare (denoted C in Figure 1). Reasons for not being able to access healthcare included personal issues (50.0%) such as not being able to take time off from work or home duties, and not having access to transport; as well as issues with the healthcare system (50.0%), such as being ill-treated at clinics in the past, long waiting times at clinics, clinics being closed on certain days. Losing cases at this level of the surveillance system should be prevented, not only for the representativeness of the surveillance system but also because medical attention should be available for cases when required. The community survey was conducted in an urban area with access to transport and several public healthcare facilities. The proportion of cases lost to the surveillance system at this level is estimated to be even higher in rural areas, with limited transport and long distances to clinics. A study in Bangladesh described how clinic attendance rates for diarrhoea decreased with increasing distance from the clinic[103].

Clinical guidelines are required for those that attend healthcare and are able to be treated without specific diagnostic investigations (denoted D in Figure 1). Guidance for the treatment and diagnosis of diarrhoea in SA is currently offered through the Integrated Management of Childhood Illness (IMCI) and Standard Treatment Guidelines and Essential Medicines List for children under the age of 5 years and through the Standard Treatment Guidelines and Essential Medicines List for SA primary care and hospital for adults[64,65,104,105]. If guidelines are relevant and supported by robust, local epidemiological data, healthcare workers should be able to use them to treat the majority of cases effectively without requiring expensive diagnostic investigations, allowing this proportion of cases (D in Figure 1) to be as large as possible. The diagnostics analysis (Paper II) indicated that this is currently not the case, since doctors do not feel the current guidelines are sufficient to assist them in clinical decision-making. Another local study showed that diarrhoeal treatment is often inappropriate since antibiotics are prescribed for a large proportion of diarrhoeal cases (60% of diarrhoeal cases

presenting to the emergency department), however only a fraction of these (35%) had a positive culture result[63].

Short-term surveillance studies can play an important role in providing local data for the strengthening of these guidelines. These studies should focus on specific populations of interest given the local context. We found through the ANDEMIA study, that 67.5% of patients over the age of 5 years admitted with diarrhoea were HIV-infected (Paper III). This estimate is lower than a study in Kwazulu-Natal in 2013 which indicated that 81% of diarrhoeal patients presenting to the emergency department were HIV-infected[63]. HIV-infected patients are a population of interest when looking at diarrhoeal diseases, specifically in older children and adults in SA. Currently the South African standard treatment guidelines recommend testing for ova, cysts and parasites in HIV-related diarrhoea and gives recommendations for *Cystoisospora* treatment, but do not offer specific guidance beyond these recommendations[65]. Our findings from the analysis of HIV-infected diarrhoeal patients (Paper III), show that aetiology of diarrhoea is very different in HIV-infected compared to HIV-uninfected patients and these differences should be considered for diagnostic and treatment guidelines. The most commonly detected pathogens amongst hospitalised, HIV-infected diarrhoeal patients were *Shigella* spp. (18.3%), *Cystoisospora* (17.7%), and *Cryptosporidium* spp. (15.9%) while *Shigella* spp. (36.7%) and *Salmonella* spp. (11.4%) were most commonly detected amongst HIV-uninfected cases. HIV status is an important consideration for the diagnosis and treatment of diarrhoea, for which consideration should be offered in the guidelines.

The American College of Gastroenterology has published a set of guidelines for the diagnosis and treatment of acute diarrhoeal infections in adults[4]. These guidelines include a flow diagram for the empiric therapy and diagnostic-directed management of acute diarrhoea, which takes epidemiology, and aetiology of infectious diarrhoea in this specific setting into consideration. These guidelines are considered in the public health context and are clear for clinicians to follow. The development of similar guidelines based on our local data and health system considerations should be considered in SA. The first step in developing these guidelines would be to convene a clinical working group to review available resources, data and determine process for development. The doctors' survey (Paper II) also pointed out issues in terms of doctors' knowledge on which pathogens are included in routine diagnostic testing. Part of these guidelines could be to standardize testing panels and to ensure doctors are well trained in terms of which tests are offered by laboratories.

Doctors report submitting specimens for approximately 10% of admitted diarrhoeal cases (Paper II). The majority (90.3%) of doctors agreed that diagnostic evaluations were helpful for clinical management, however they are often prevented from submitting samples for several reasons. Cases should not be lost at this level of the surveillance pyramid (denoted E in Figure 1) since diagnostics should be available in all cases in which the doctor feels they would be clinically beneficial. The most

common barriers to specimen submission included laboratory turnaround times being too long to assist with clinical decision-making (reported by 54.8% of respondents), and patients and nursing staff being unwilling to provide/collect stool specimens (reported by 35.5% and 32.3% of respondents respectively). Financial constraints on diagnostic testing were specifically reported by the rural hospitals (23.8% of respondents and the rural sites), and a large proportion of urban respondents specifically listed lack of confidence in laboratory results as a barrier to submission (40.0% of respondents at the urban site). Our analysis comparing routine diagnostic methods to molecular methods through the enhanced surveillance study, indicated that a large proportion of diarrhoeal cases are lost to the surveillance system as available diagnostics are unable to detect a pathogen (denoted F in Figure 1). Although it is acknowledged that a proportion of these cases may be non-infectious (34% of diarrhoeal cases in untreated HIV-infected individuals in a study in the Netherlands did not have a pathogen identified; GEMS investigators were unable to identify a pathogen in 35.1% of diarrhoeal stools using molecular methods), our diagnostic analysis suggests the majority of pathogen-negative results obtained through routine diagnostics are due to diagnostic error and insensitivity[38,56]. Although we were unable to match patients for the two different methods (referred to in limitations section), we compared the pathogen-detection rates for the same hospitals over the same period. Routine diagnostics were only able to detect a pathogen in 13.7% of samples tested (using culture methods), as compared to 73.3% through ANDEMIA using molecular methods ($p<0.001$).

The pathogen-specific differences have been discussed in detail in Paper II, indicating the differences between *Shigella* spp. (0.8% versus 22.7%, $p<0.001$), *Campylobacter* spp. (0.2% versus 11.8%, $p<0.001$) and viruses (2.6% versus 48.9%, $p<0.001$) were particularly noteworthy. Viruses are not routinely tested for (only 16.8% of routinely submitted stool specimens are tested for rotavirus, 15.7% for adenovirus and 3.1% for astrovirus, while no testing for norovirus was done routinely) despite ANDEMIA data indicating a high prevalence of these pathogens in our setting (detected in 15.6%, 25.6%, 5.9% and 14.2% of stools from total diarrhoeal cases enrolled respectively). An NICD report indicated that norovirus has not been detected in a FBD outbreak in over two years, despite it being one of the most common causes of foodborne outbreaks in other settings[106,107]. This is attributable to the lack of routine testing, rather than the pathogen not being present in the population. Routine testing for viruses is not always clinically relevant due to limited available treatment but it is important to rule out the requirement for antibiotics. The unnecessary use of antibiotics has implications for AMR and the use of antibiotics should be limited. The routine under diagnosis of these important pathogens leads not only to the incorrect treatment of patients, but also to their under reporting in the surveillance system, which is of public health concern.

As discussed previously, GERMS-SA uses isolates from clinical microbiology laboratories to monitor pathogens with epidemic potential, including *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. Our analysis indicates that these pathogens are significantly under-detected through clinical microbiology laboratories, which has implications for the GERMS-SA program amongst others. The diagnostics analysis (Paper II) concluded that improved guidelines, based on local epidemiological data, would minimise the number of patients requiring directed diagnostic investigations as doctors would be well informed on the underlying aetiology in the specific population. This would avail resources for the select cases where directed diagnostic investigations are required. The use of molecular and antigen-based diagnostics for prevalent pathogens whose detection has treatment and public health implications, such as *Shigella* spp., should be investigated. A re-analysis of GEMS samples using molecular methods increased pathogen detection to 64.9% of samples, compared to 32.8% using the original study methods[38]. Molecular methods would also allow a quicker turnaround time for results, which was the most commonly reported issue with diarrhoeal diagnostics in the doctors' survey, thereby improving clinical care. Molecular methods are significantly more expensive than culture techniques; however, this cost may be offset by shortened hospitalisations due to quicker resolution of symptoms, decreased requirements for antibiotics or use of directed antibiotics which would potentially decrease risk of AMR by promoting antibiotic stewardship. If costs for molecular tests are prohibitive, further work should be done to maximize yield and efficiency of current methods. The laboratory at CED is working on optimizing the yield of *Shigella* spp. using existing culture methods, looking at both pre-analytical and analytical processes. Currently there is no standard protocol for *Shigella* spp. culture at the NHLS. We aim to generate a testing algorithm for *Shigella* spp. detection using optimized methods which can then be implemented nationally in NHLS laboratories. We also plan to investigate the implementation of wipes and/or rectal swabs instead of stool sample collection in our setting. While laboratory work has already demonstrated wipes to be efficient for pathogen detection[108,109], research on the implementation of this sampling method in the public health system is required. This may address compliance issues since these samples are substantially easier to collect and patients may be more willing to agree to further diagnostic tests.

6.3. Decreasing diarrhoeal morbidity and mortality

Our survey found that in an urban community, respondents generally had a good understanding of safe food practices, with 97.1% storing perishable goods in cold storage, 96.8% indicating that they keep raw and cooked food separately and 65.2% indicating that they always ensure meat and eggs are cooked thoroughly before consumption. While the majority of respondents (96.0%) purchase meat products from formal distributers, many (53.7%) purchase fruit and vegetables from informal sellers (hawkers, markets) which may not have strict hygiene or cold chain procedures. The

community generally had good access to water with all surveyed households having a tap on the property. Despite this, 38.7% of surveyed households stored water in a container inside, 11.8% reported having at least one interruption to water supply in the past two weeks, and almost all respondents (93.0%) relied on municipal water to be potable, as they do not treat water before consumption. As presented in Paper I, we did not find any association between diarrhoeal episodes and food safety practice, food purchasing behaviour or water access and storage in this community. The lack of association is likely a product of the relatively small number of households surveyed, or because many of the FBD and waterborne outbreaks reported in SA are due to sporadic contamination and were not detected in the cross-sectional survey.

A well-documented example of a sporadic FBD outbreak is the 2017/2018 listeriosis outbreak which resulted in the death of at least 193 South Africans[110]. The source was contaminated polony produced at a single facility and distributed nationally. Since the listeriosis outbreak, a total of 337 foodborne outbreaks were reported to the NICD through the NMC system (between March 2018 and August 2020)[106]. These included 2932 cases, 319 hospital admissions and 20 deaths[106]. Household outbreaks were the most common (42%), followed by outbreaks in institutional settings (39%) and at social gatherings (7%). Non-typhoidal *Salmonella* (42%), *Shigella* spp. (19%) and *Salmonella typhi* (5%) were the most common pathogens isolated from stool samples however diagnostic investigations were not available for all reported outbreaks (available for 63% of those with complete data). This report highlighted the high proportion of cases coming from a household setting and recommended that education on food safety practices is required at a community level. It was noted that the majority of these household outbreaks came from informally-slaughtered food animals[106]. Considering this in context with the findings from our community survey, health education should likely focus particularly on food safety in terms of informally-slaughtered food animals but further research on current practices is required.

The community survey found inadequate handwashing to be associated with diarrhoeal episodes in the household, and that hand hygiene was poor across the surveyed population (only 42% of respondents reported adequate hand hygiene, defined as always washing their hands with soap and water at critical times). Detailed results are presented in the hand hygiene analysis (Paper IV), but in summary, although respondents reported good compliance with hand washing at critical times, the use of soap was limited. We found that respondents were less likely to use soap when washing their hands if they had a tap located outside the room housing the toilet (in the yard or in the kitchen only) compared to those with a tap in the bathroom/toilet (52.8% versus 74.6%, OR=0.38; 95% CI: 0.25-0.59; $p<0.001$). This is an important finding which may have relevance for handwashing communication in other settings, specifically in rural areas where outdoor taps and communal taps are common. Further qualitative research is required to explore the effects of mechanisms for

keeping soap at outside and communal taps as an intervention to improve hand hygiene. Following the publication of the manuscripts on the community survey and the handwashing communication (Paper I and IV), we were contacted by the Partnerships and Purpose Manager for Dettol (Reckitt Benckiser). This group provides hygiene education workshops to communities through schools and antenatal clinics as part of their community engagement and social impact project. Although this partnership has just started (our first meeting was on the 2nd of June 2022 and we presented research findings at the Dettol Global Handwashing Day on the 11th of October 2022), we hope that this will be the start of a partnership between the brand and the NICD to address gaps in hygiene practice. We have committed to assisting Dettol in identifying areas with high diarrhoeal transmission (through surveys and monitoring of routine data) which may benefit through community interventions for which they have the resources to provide. We will also advise them on interventions and resources required to improve the maintenance of hand hygiene in our setting, with one such intervention being the development of soap products to keep at outside and communal taps.

Oral rehydration solution is a simple, inexpensive intervention that can be administered by caregivers at a household level and is a proven, effective intervention for reducing diarrhoeal mortality[4]. Despite this, our community survey found that only half (51.0%) of respondents had some knowledge of ORS (knew the recipe or were able to name the ingredients), while only 17.9% could give the correct recipe. Being female and having children in the household was predictive of ORS knowledge, indicating that ORS knowledge is likely to come from antenatal, baby and childcare clinics as has been reported in Botswana[97]. This has highlighted a knowledge gap in males and households without children. The effective dissemination of information to all community members, specifically groups where this information has not previously been shared, should be further explored. A meta-analysis looking at strategies to increase ORS use at a household level indicated that there are limited data on the scaling up of use[111]. They were able to report on community interventions for co-promotion of zinc therapy with ORS and social marketing and mass media interventions, however limited data and heterogeneity of results prevented drawing of concrete conclusions. The authors suggest that strategies for the scaling up of ORS in communities requires investigation from multiple aspects including education of healthcare provides, and that baseline socio-cultural and economic factors of the specific population should be considered[111].

An example of a success story in terms of widespread community ORS use comes from Bangladesh, where an NGO used an innovative house-to-house campaign to educate mothers on the use and preparation of ORS[112]. The success of this program was largely attributed to the translation of the recipe using measurements and ingredients readily available to the majority of the community, the empowered education offered to mothers and the innovative methods used to scale up the

intervention. This program is credited to Bangladesh having the highest rate of ORS use in the world[112]. In order to introduce a similar program in SA, a better understanding of baseline use and habits in the community are required. While our community survey was a start, further qualitative research is required before designing implementation programs. This is an important area of future research and will have real implications for reducing diarrhoeal mortality, especially since we showed that few in the community seek healthcare for diarrhoea meaning effective household care has the capacity to reduce mortality.

Finally, in terms of interventions to prevent diarrhoeal morbidity and mortality, our data indicated a high burden of diarrhoea due to *Shigella* spp. in SA, supporting the introduction of a *Shigella* vaccine. Despite *Shigella* spp. being included in the diagnostic testing done for the vast majority (99.8%) of stool samples received by the NHLS, our analysis indicated that routine health information system data significantly underestimated *Shigella* spp. burden, with prevalence amongst routine samples of 0.8% compared to 22.7% in ANDEMIA molecular testing (p<0.001). *Shigella* spp. was the most prevalent pathogen detected in diarrhoeal cases over the age of 5 years (20.4%) (Paper III), with detection being particularly high in HIV-uninfected (36.3%) cases but still relatively high in HIV-infected (18.3%) cases. The detection of *Shigella* spp. was also strongly associated with diarrhoea in the case-control analysis, despite carriage in controls being relatively high (24.3% in cases and 10.9% in controls, p = 0.005). As discussed previously, there are several promising candidate vaccines against *Shigella* spp. and ETEC, however there is currently no licensed vaccine[22]. Once vaccines are licensed, an important step in advocating for introduction will be showing high baseline burden of disease. Robust surveillance and testing will then be needed to monitor changes in morbidity and mortality subsequent to introduction[25]. While there are some data on baseline mortality and morbidity due to *Shigella* spp. in young children in LMICs, there are limited data in older children and adults. The data presented here fills in some of the gaps in terms of burden of this specific pathogen in these older age groups. We are also working on a cross-network analysis of ANDEMIA data, which will include diarrhoeal cases from countries across Africa and will be an important step in demonstrating the burden of *Shigella* spp. across all age groups in LMICs. This analysis should be available by the end of 2022. The rise in antimicrobial resistant strains of *Shigella* spp. and ETEC also supports further development of vaccines[25]. Although we did not perform antimicrobial susceptibility testing for the ANDEMIA samples, we do plan to test these samples using TAC cards, which include detection of a number of antimicrobial resistance genes, and will report on these results separately.

6.4. Recommendations for diarrhoeal surveillance in SA

Data obtained from routine health information systems, such as NHLS diagnostic data, can be used as an effective tool in the surveillance of diarrhoea in specific groups in SA. It is important to understand the extent to which these data represent the true picture of disease in the community and to adjust

for the proportion not captured by the system, specifically when these data are used in policy decisions or for informing interventions[81]. Our research has shown that routine health information on diarrhoea in SA is prone to under-ascertainment (since the majority of diarrhoeal diseases are mild and there are issues of accessibility for those requiring healthcare) and underreporting (due to barriers to and inefficiency of diagnostic testing). By describing and quantifying this under-ascertainment and under-reporting, we have been able to identify weaknesses in the system which should be strengthened in the mid- to long-term. In the short-term understanding these issues will assist us in better analysis of currently available data. While complete ascertainment and reporting is not the aim of the system, it is important to ensure that cases of public health significance are identified. These are 1) those that require intervention and 2) those with outbreak potential[68]. Our research indicates that there is underestimation of diarrhoeal cases in both categories in the current system.

Diarrhoeal surveillance in SA relies on both facility-level syndromic and laboratory surveillance. Syndromic surveillance allows clusters of cases to be promptly detected and further investigated if required, while laboratory surveillance should monitor emerging antimicrobial resistance, as well as shifts in pathogen aetiology and serotype distribution[68]. This hybrid system is well suited to diarrhoeal surveillance and consideration should be given to how these systems work together to strengthen each other. Of particular importance is the notification and investigation of FBD outbreaks, which have been reported to be particularly poor in SA[113]. Electronic notification of FBD through the NMC system has streamlined the previously paper-based system, however the focus has been on case reporting (data generation hemisphere in Figure 2) while investigation and response activities (data use hemisphere in Figure 2) are lacking[113]. Our research shows that this system detects only a fraction of FBD pathogens, and while this should be improved as discussed, consideration of how these data are used is vital. Outbreaks of this nature are time sensitive and delays at any level of the surveillance pyramid can affect implementation of interventions, resulting in poorer outcomes[68].

Time delays were a common theme in this research and should be addressed at all levels. Mobile health applications have been suggested as a tool to monitor and manage FBD outbreaks in real-time in SA[113]. Web-based platforms, including the National Outbreak Reporting System (NORS) and Foodborne Disease Outbreak Surveillance System (FDOSS) have been used successfully for years in the USA and could guide the development of this technology in LMICs[114,115]. Such an application could combine outbreak verification, diagnostic and epidemiological investigations (both patient and environmental), as well as case management and interventions[113]. Suggestions have been made to include a decision support framework which will assist public health officials in terms of actions required in outbreak investigation, especially since qualitative data indicate fewer than half (42.6%)

of environmental health practitioners conducting FBD investigations feel they are adequately trained to do so and practices are inconsistent and often inadequate[113,116]. This technology would allow streamlining in both the data generation and data use hemispheres (Figure 2) and ensure that data are timeously collated and available in a format that is useful in public health decision making. Integration of laboratory data into this system is vital as the future of FBD surveillance is thought to be laboratory-based, provided this laboratory data is representative of 1) cases requiring intervention and 2) with outbreak potential[69].

The surveillance of diarrhoeal diseases in SA could perhaps be used as a model to assist other SSA countries to establish or improve existing surveillance. During the listeriosis outbreak in SA, there were no cases reported from any of the other 15 countries to which contaminated cold meat was exported[110]. This under-detection is likely due to insufficiencies at all levels of the surveillance pyramid and indicates the importance of extending established systems to low-income settings.

7. Limitations

An important limitation of this research is the generalisability of findings from the study sites to the rest of SA. Although it would have been beneficial to have several study sites across the country to increase the representativeness of the study population, we did feel that it was fairly representative due to the inclusion of both an urban and two rural hospital sites and the community survey in Soweto, which is representative of an urban community. The Bushbuckridge municipality (catchment area for Mapulaneng and Matikwana hospitals) consists of a population of approximately 548 760 (2016 community survey) separated into villages spread out over a large area[117,118]. In many respects, Bushbuckridge is typical of a rural population in SA. Atteridgeville (catchment area for Kalafong hospital) and Soweto are both large settlements with a mix of informal and formal housing and as such are representative of typical South African, mixed socioeconomic, urban populations[93,119]. Both Atteridgeville and Soweto have good access to water and sanitation including piped water inside dwellings (67.2% and 55% respectively compared to the national estimate of 45.2%) and access to flush toilets (99% and 91.6% compared to the national estimate of 64.8%)[93,120,121]. The age distribution in these settlements is comparable, with a large proportion of the population being of working age (72% and 71% between ages 15-64 years compared to 62.2% nationally), followed by young children between the ages of 0-14 years (22.6% and 24.7% compared to 28.8% nationally) and elderly over the age of 65 years (5.4% and 4.2% compared to 9% nationally)[93,120,122]. Average household size (3.7 and 3.4 respectively, compared to 3.34 nationally) and unemployment rates are also comparable (24.2% and 25% compared to 30.8% nationally) in Atteridgeville and Soweto[93,120,121]. These sociodemographic and living condition indicators are very different to those found in Bushbuckridge. Bushbuckridge has poor access to water and sanitation including piped water inside dwellings (only 11.9% of the population) and access to flush toilets (only 6.8% of the population)[123]. The age distribution in Bushbuckridge has proportionally more dependent individuals (37% young children between 0-14 years and 5.3% elderly over 65 years) with a smaller proportion of the population of working age (57.7% between 15-64 years) compared to the urban areas[123]. This is due to many of the working aged population being migrant workers to urban areas[118]. Although the average household size (4 individuals per household) is similar to the urban areas and national estimates, the unemployment rate is much higher in Bushbuckridge than in other study areas (52.1% compared to 24.2% for Atteridgeville, 25% for Soweto and 30.8% nationally)[123–126]. Extrapolating the findings from this research should be limited to rural and urban setting with similar sociodemographic and living conditions. Estimating burden of disease using epidemiological models and extrapolating data from a specific region to similar regions for which data are not available, is a recognized feature of infectious disease surveillance[68]. Acknowledging the limitations, our findings may be extrapolated to regions in SSA for which no diarrhoeal estimates exist, especially for sub-

populations such as those that are HIV-infected. The cross-network analysis of ANDEMIA data will also significantly contribute to diarrhoeal knowledge in African regions with sparse data.

Another limitation of this body of work was that the catchment population for the community survey (Soweto) was different to the catchment populations for the surveillance hospitals (Atteridgeville and Bushbuckridge). The community survey leveraged off the existing HDSS in Soweto as the sampling framework. Additional surveillance through the Diarrhoeal Diseases Surveillance Network was planned to start in 2020 for Chris Hani Baragwanath Academic Hospital, the main tertiary hospital in Soweto. The SARS-CoV-2 outbreak (which started in SA in March 2020), resulted in this surveillance network being halted. Since the catchment population for the community survey was different to that of the other studies, any extrapolation of these results and how they apply in the context of other levels of healthcare, are subject to some assumptions. Despite this, the urban population of Soweto is in many ways similar to that of Atteridgeville township (including similar access to potable water, sanitation, electricity, age distribution, population density, household crowding and dependency ratio)[93,120]. It is more difficult to extrapolate the findings from the community survey to the rural site. It would have been beneficial, had time and resources allowed, to repeat the survey in rural Mpumalanga (especially since Bushbuckridge also has an established HDSS). This should be considered for future study.

This research relied on quantitative data only and as such may have missed in-depth contextual components. Several aspects may have been enriched with the use of qualitative methods that may have provided a nuanced understanding of the research problems. These include possible qualitative analysis on health seeking behaviour, barriers to seeking care as well as social and cultures beliefs around diarrhoeal diseases, hand hygiene, ORS knowledge and use, and food safety knowledge for the community survey and on current practices, barriers to specimen submission and diagnostic knowledge for the diagnostic analysis. Since the aim of this work was largely exploratory and involved research into a broad field with limited existing data, the focus was not to define any of these topics in detail but rather to gain an understanding into the field as a whole, enabling clearer direction for future research.

Finally, this research project was done in the midst of the COVID-19 pandemic that offered a unique set of challenges. A second round of the community survey was planned, in which the same households would be visited and the questionnaire repeated during the winter months (June/July 2020) to provide seasonal estimates for diarrhoeal prevalence in the community. Unfortunately, this was not possible due to lockdown restrictions. Post-lockdown two years later, it was decided that repeating the survey would likely represent pre- and post-lockdown comparisons rather than providing estimates for changes in seasonality. The diarrhoeal diseases survey did however provide a good baseline for community prevalence estimates and, retrospectively, allowed a pre-pandemic

baseline description of community hand hygiene. Hand hygiene is since likely to have changed in the community due to mass messaging, and repeating the survey to capture these changes (or lack thereof) would be of interest. The pandemic and accompanying restrictions placed severe limitations on ANDEMIA enrolment. Case enrolment was halted for several weeks and thereafter restricted to specific wards and hours while control enrolment was halted for an extended period and remained slow until the end of the study due to restrictions on wards, lack of visitors to the hospitals and limited routine clinic visits. Low enrolment numbers limited additional analysis of ANDEMIA data.

The enrolment of pooled controls in general was a challenge in the ANDEMIA study, mainly due to the intensive sample collection required per patient. The surveillance officers reported patients being unwilling to give three specimens (blood, stool and NP/OP swabs), specifically when this sampling was not beneficial to their own clinical diagnosis or treatment. Enrolment was particularly difficult for young children, since parents were not willing to consent to unnecessary blood draws. It may have been more efficient to have targeted control enrolment. For exampling using collection of nappies from well-baby clinics for control stool specimens and excess routine bloods from other admissions or collecting additional blood from those that already required blood draws for clinical reasons would probably have been well accepted. This was unfortunately a limitation of the study as we did not have enough staff members to do targeted enrolment. The SA sites had dedicated surveillance officers, however many of the other ANDEMIA countries relied on clinical staff for enrolment. Different sampling collection methods could also have been explored (such as using dried blood spots), however this would have to have been applied to case enrolment as well for comparison. As previously described, the enrolment of hospital controls also had limitations in terms of comparability and generalizability. An indication of this is the HIV-prevalence of 31.5% among controls over the age of 5 years (reported in the HIV analysis in Paper III) which is higher than expected from the national prevalence (18.3% for adults aged 15-49 years[127]). This indicates that the use of hospital controls likely increased the proportion of enrolments with underlying conditions as compared to the general community. Enrolment of controls from the community would likely have resulted in a set of controls that are more representative of the population, however was not possible given the study framework, budget and logistics and must be acknowledged as a limitation of the study.

8. Conclusions

Surveillance plays an important role in the prioritisation of public health threats and in assessing the need for advocacy around required interventions[72]. Since little data are available on diarrhoeal diseases in older children and adults in SA, we rely on existing data systems and small, localised studies to describe the burden. We found that there is a high burden of diarrhoeal diseases across all age groups in SA. While the majority of these infections are mild, a proportion are severe enough to require healthcare. Many of those requiring healthcare, specifically adults, are not able to access it for personal reasons as well as the issues with the healthcare system. Those that do seek healthcare are treated empirically and seldom have stool samples collected for diagnostic investigations. Doctors are most likely to request diagnostic investigations at urban sites for chronic cases, case of dysentery, suspected *C. difficile* infection, cases with a travel history or links to suspected outbreaks. Currently available diagnostics in the public health laboratories detect a pathogen in a fraction of these submitted stool specimens. Although some cases maybe due to non-pathogenic causes, the majority of these pathogen-negative results are likely due to diagnostic error and insensitivity, which may stem from pre-analytical and analytical issues. This study estimates that for every pathogen detected in the NHLS routine health information system, there are approximately 270 diarrhoeal cases in the community. We estimate that 38 diarrhoeal cases per pathogen detected through the routine health information system is acceptable given that a diarrhoeal surveillance system should not aim to capture all cases due to the mild and usually self-limiting nature of the disease. Issues of under-ascertainment (specifically healthcare accessibility for moderate to severe cases) and under-reporting (specifically improving diagnostics) should be addressed. Addressing under-ascertainment is a question for the healthcare system as a whole but in terms of diarrhoeal diseases may include education on when to seek healthcare for diarrhoea. Under-reporting of diarrhoeal cases to the system needs to be considered by the NICD and the NHLS.

We recommend the development of diarrhoeal testing and treatment guidelines, based on local epidemiology and aetiology, specifically focussing on diarrhoea in HIV-infected patients, which was highlighted as an important group and presents with unique aetiology. These guidelines will assist in empiric treatment for the majority of diarrhoeal cases and will reserve diagnostic testing for cases in which it is required and recommended. Diagnostics currently available in public health laboratories require optimisation, specifically in terms of standardization, methods available and pathogens included in routine testing panels. Consideration should be given to improving turnaround time to ensure that results are available within a timeframe to ensure they are clinically relevant. A review of sample collection techniques, transport to the laboratory and training of medical staff on these issues is required. This research highlighted *Shigella* spp. as one of the main causative agents of

diarrhoea in SA and one that is significantly under-detected through routine data. This has implications for vaccine introduction once a licenced vaccine is available.

Poor hand hygiene is a problem in SA and is associated with an increased risk of diarrhoea. Further work on creating good hand hygiene habits in the community, given local context is required. An example is looking at how to ensure soap is available at outdoor and communal taps. We also found that ORS use and knowledge is limited and messaging should be scaled up, specifically for those in whom knowledge gaps were identified, including males and households without children.

Diarrhoeal surveillance in SA should aim to capture cases requiring intervention and those with outbreak potential through a combination of syndromic and laboratory surveillance. An important part of monitoring and evaluation of surveillance systems is to ensure that core functions of the surveillance system are being met and that surveillance data are used for decision making as required[72]. Efficient ways to collect, collate and action this data, such as the development of mobile health apps for FBD and other diarrhoeal outbreaks should be investigated. This work also highlighted the importance of well-designed, short-term epidemiological studies to collect data to inform gaps in current surveillance system. These studies are also a good way to monitor and assess the surveillance system.

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Appendices

Appendix A – Ethical clearance certificate

Appendix B – Diarrhoeal diseases community survey

Appendix C – Doctors' survey

Appendix D – Turnitin originality report

Paper I

Paper II

Paper III

Paper IV

Appendix A – Ethical clearance certificate

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 Mrs Siobhan Johnstone

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190663 MED18-12-034

NAME: (Principal Investigator)	Mrs Siobhan Johnstone
DEPARTMENT:	School of Public Health
PROJECT TITLE:	Epidemiology and aetiology of gastrointestinal infections in individuals over the age of 5 years in South Africa
DATE CONSIDERED:	28/06/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Nicola Page and Michelle Groome
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/11/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed June and will therefore be due in the month of June each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

18/12/2019
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B – Diarrhoeal diseases community survey

Diarrhoeal Illnesses – Community Survey

Background information:

Survey ID: _____

Household ID: _____

Survey participant (name and surname): _____
Age: _____

Female / Male

Study staff member conducting interview: _____

Number of people in household: _____

Children under 5 years: _____ Children between 5 and 15 years: _____ Adults>15 years: _____

Questionnaire

Handwashing practices -

1. Do you have a place to wash your hands in your house?
    - Yes, next to the toilet or in the same room as the toilet
    - Yes, in another room (not the same room as the toilet)
    - No
2. What do you normally use to wash your hands?
    - Water only
    - Soap and water
    - Other: _____
3. How often do you wash your hands?
    - After going to the toilet (always/sometimes/never)
    - Before preparing food (always/sometimes/never)
    - Before eating (always/sometimes/never)
    - Before feeding child (if applicable) (always/sometimes/never/NA)
    - After changing nappies (if applicable) (always/sometimes/never/NA)
4. Do you treat water before you drink it?
    - No
    - Yes with chlorine
    - Yes by boiling it first
    - Yes (other): _____

4b. Where do you store water in your house?

- Get it straight from the tap (no storage necessary)
- In an open container/bucket
- In a closed container
- Other: _____

5. In the past 2 weeks, how often have you had interruptions to your water supply?

   _____

   5a. How long do the interruptions to water supply usually last? _____

Food preparation practices -

6. In the past 2 weeks (*use highlighted calendar*), how often have you eaten the following food items:
   - Meat (including chicken) – at home
     (every day/every second day/once a week/one in 2 weeks/never)
   - Dairy
     (every day/every second day/once a week/one in 2 weeks/never)
   - Fresh fruit and vegetables
     (every day/every second day/once a week/one in 2 weeks/never)
   - Eggs
     (every day/every second day/once a week/one in 2 weeks/never)
   - Ready-to-eat meat products (such as ham or polony)
     (every day/every second day/once a week/one in 2 weeks/never)
   - Ready-to-eat meals or take-aways
     (every day/every second day/once a week/one in 2 weeks/never)
   - Eaten at a restaurant
     (every day/every second day/once a week/one in 2 weeks/never)
7. Where do you keep your food products such as meat and vegetables?
   - Refrigerator/freezer
   - Cupboard
   - On a counter
   - Other: _____
8. In the past 2 weeks, where have you purchased your fresh dairy and meat items from?
   - Commercial shop (such as Checkers, Pick'n Pay, Spar)
   - Informal shop/Spaza shop

- Informal traders on the side of the road/Hawker
- Other: _____

9. In the past 2 weeks, where have you purchased your fresh fruit and vegetable items from?
    - Commercial shop (such as Checkers, Pick'n Pay, Spar)
    - Informal shop/Spaza shop
    - Informal traders on the side of the road/Hawker
    - Other: _____
10. Do you keep raw meat and cooked food materials separately (can be separate containers in one fridge/freezer)?
    - Yes
    - No
    - Don't know
11. How often do you eat meat or eggs that are only partially cooked or raw?
    - Always
    - Sometimes
    - Never
    - Don't know

Diarrhoeal episodes in the household -

12. When you or anyone in your household has diarrhoea, do you take/give them a sugar and salt solution drink (or oral rehydration solution)?
    - Yes always.
    - Only if they are sick for more than 3 days.
    - No (know what it is but don't use it/give it).
    - No, I don't know what that is.

    11a. What sugar and salt solution drink do you give them or do you take?
    - Store/pharmacy bought (what kind) _____
    - Home made (how is it made)

    _____

    _____

13. Have you or anyone in your household had diarrhoea (an upset stomach with 3 or more loose stools per day) in the last 2 weeks?
    - Yes I have.

- Yes, someone else in my household has.
- No.

Skip pattern: If 'no' end questionnaire here, if 'yes I have' continue to question (16), if 'yes, someone else in my household has' continue to question (14).

Note: If several people in the household have had an episode, the questionnaire should be repeated for each person.

14. Who was the person in your household that was sick?
    Name and surname : _____
    Age: _____
15. If the person in the household that was sick is a child under 2 years, please indicate how they are currently fed:
    - Exclusively breastfed
    - Exclusively formula fed
    - Mixed breastfeeding and formula
    - Diversification (milk and solids)
16. How long were you sick for? **OR** How long was s/he sick for?
    Days _____ Weeks _____
17. Do you have a sugar and salt solution drink? OR Did s/he have a sugar and salt solution drink?
    - Yes
    - No
    - Don't know
18. Did you have any of the following symptoms? **OR** Did s/he have any of the following symptoms?
    - Cramps
    - Vomiting
    - Nausea
    - There was blood in the stool
    - Stool was very watery (completely clear liquid)
    - Stool was discoloured (green or yellow)
    - Headache
    - Respiratory symptoms (coughing, sneezing, sore throat)
    - Fever

- Body aches
- Loss of appetite/poor feeding in babies
- Stiff neck
- The illness lasted for an extended period of time

19. Did you visit a healthcare provider? **OR** Did s/he visit a healthcare provider?
    - Yes
    - No
    - Don't know

    Skip pattern: If 'Yes' proceed to (20), if 'No' skip to (23)

20. Where do you seek healthcare? **OR** Where did s/he seek healthcare?
    - CHBAH
    - Leratong hospital
    - Bheki Mlangeni District hospital
    - Local clinic
    - Private doctor
    - Private hospital
    - Faith healer
    - Traditional healer
    - Pharmacy
    - Other: _____
21. Were you admitted to hospital for this illness? **OR** Were they admitted to hospital for this illness?
    - Yes (if yes, please specify the hospital) _____
    - No
22. Did the healthcare provider ask you to give a stool specimen? **OR** Did the healthcare provider ask them to give a stool specimen?
    - No
    - Yes and I gave one.
    - Yes but I did not give one.
    - I can't remember/don't know.

Skip pattern: End questionnaire here for those that consulted a healthcare provider. If they answered 'no' to (19), ask question (23) and (24).

23. Did you want to consult a healthcare provider? **OR** Did s/he want to consult a healthcare provider?
    - No, the episode was mild and didn't affect daily life.
    - Yes, but wasn't able to go.

Skip pattern: if they answer 'no' end here; if they answer 'yes' continue to question (24)

24. Why were you not able to consult a healthcare provider? **OR** Why was s/he not able to consult a healthcare provider?
    - Could not take time off work.
    - Had duties to complete at home and wasn't able to leave.
    - Could not organise child care.
    - Did not have transport.
    - Did not have enough money.
    - The queues at the clinic are too long.
    - In the past, I have visited clinics and they did not treat me well.
    - In the past, I have visited clinics and they did not have the correct medication.
    - Was embarrassed to talk to someone about the problem.
    - Didn't know where to go or who to consult.
    - Other: _____

    Skip pattern: End here

Were there any additional members of the household that had diarrhoea?

Yes/No

Appendix C – Doctors' survey

Doctors' Survey – Diarrhoeal Diseases

Section 1 – General information

1. *Please indicate which facility/hospital you are based at*:

   ☐ Kalafong Hospital ☐Mapulaneng Hospital ☐Matikwana Hospital

2. *What is your position at the facility?*

   ☐ Intern ☐ Community service doctor ☐ Medical officer

   ☐ Registrar ☐ Consultant ☐ General practitioner

   ☐ Specialist ☐ Other

3. *If other, please specify:*

   _____

   _____

4. *Please indicate specialisation (if applicable):*

   ☐ General medicine ☐ Surgery ☐ Paediatrics

   ☐ OB/GYN ☐ Pathology ☐ Microbiology

   ☐ Other

5. *If other, please specify*:

   _____

   _____

6. *Which ward/s are you currently working in? (Please tick all that apply)*

   ☐ Paediatrics ☐ Adult medical ☐ Surgical

   ☐ ER ☐ Outpatients

   ☐ Obstetrics and gynaecology ☐ Other

7. *Please indicate your age group:*

   ☐ 21-29 years ☐ 30-39 years ☐ 40-49 years

   ☐ 50-59 years ☐ 60-69 years ☐ 70+ years

Section 2 – Specimen submission and treatment practices

1. *Approximately how many diarrhoeal cases do you see in a normal/average week (based on your experience pre COVID-19)?*

   _____

2. *In approximately what percentage of these diarrhoeal cases do you request a stool specimen for diagnostic purposes?*

   _____ %

3. *For which of the following cases would you be likely to request a stool specimen for diagnostic purposes? (Please tick all that apply)*
    - ☐ Known HIV infection
    - ☐ Suspected foodborne illness
    - ☐ History of travel
    - ☐ Presentation during a known/suspected outbreak
    - ☐ Suspected nosocomial infection
    - ☐ Suspected *Clostridioides difficile* infection (*Clostridium difficile/C. diff*)
    - ☐ Suspected rotavirus infection (during rotavirus season)
    - ☐ Chronic diarrhoea
    - ☐ Dysentery
    - ☐ Children under 5 years of age
    - ☐ Elderly patients
    - ☐ Other
4. *If other, please specify*.

    _____

    _____

    _____
5. *For which of the following symptoms would you be likely to request a stool specimen for diagnostic purposes? (Please tick all that apply)*
    - ☐ Prolonged diarrhoeal duration (>3 days)
    - ☐ Prolonged diarrhoeal duration (>2 weeks)
    - ☐ Prolonged diarrhoeal duration (>4 weeks)
    - ☐ Abdominal cramps
    - ☐ Vomiting
    - ☐ Nausea
    - ☐ Self-reported dysentery
    - ☐ Mucus in stool
    - ☐ Headache
    - ☐ Respiratory symptoms
    - ☐ Fever
    - ☐ Myalgia/arthralgia
    - ☐ Weight loss

- ☐ Loss of appetite/poor feeding
- ☐ Neurological symptoms
- ☐ Requiring intravenous rehydration
- ☐ None of the above

6. *Which of the following pathogens do you think are included in routine diagnostic stool culture and microscopy at your hospital laboratory? (Please tick all that apply)*
   - ☐ *Salmonella* spp.
   - ☐ *Campylobacter* spp.
   - ☐ *Shigella* spp.
   - ☐ All diarrhoeagenic *E. coli*
   - ☐ *E. coli* O157
   - ☐ All Shiga toxin-producing *E. coli* (STEC)
   - ☐ *Vibrio* spp.
   - ☐ *Aeromonas* spp.
   - ☐ *Clostridiodes difficile* (*Clostridium difficile/C. diff*)
   - ☐ *Yersinia enterocolitica*
   - ☐ *Entamoeba histolytica*
   - ☐ *Cryptosporidium* spp.
   - ☐ *Giardia lamblia*
   - ☐ *Cyclospora* spp.
   - ☐ *Isospora* spp.
   - ☐ *Microsporidia* spp.
   - ☐ Norovirus
   - ☐ Adenovirus
   - ☐ Rotavirus
7. *Which of the following cases would you consider treating with antibiotics? (Please tick all that apply)*
   - ☐ Known HIV infection
   - ☐ Suspected foodborne illness
   - ☐ History of travel
   - ☐ Presentation during a known/suspected outbreak
   - ☐ Suspected nosocomial infection
   - ☐ Suspected *Clostridioides difficile* infection (*Clostridium difficile/C. diff*)

- ☐ Suspected rotavirus infection (during rotavirus season)
- ☐ Chronic diarrhoea
- ☐ Dysentery
- ☐ Children under 5 years of age
- ☐ Elderly patients
- ☐ Other

8. *Which of the following cases would you consider treating with zinc? (Please tick all that apply)*
   - ☐ Known HIV infection
   - ☐ Suspected foodborne illness
   - ☐ History of travel
   - ☐ Presentation during a known/suspected outbreak
   - ☐ Suspected nosocomial infection
   - ☐ Suspected *Clostridioides difficile* infection (*Clostridium difficile/C. diff*)
   - ☐ Suspected rotavirus infection (during rotavirus season)
   - ☐ Chronic diarrhoea
   - ☐ Dysentery
   - ☐ Children under 5 years of age
   - ☐ Elderly patients
   - ☐ Other
9. *Which of the following cases would you consider treating with Loperamide? (Please tick all that apply)*
   - ☐ Known HIV infection
   - ☐ Suspected foodborne illness
   - ☐ History of travel
   - ☐ Presentation during a known/suspected outbreak
   - ☐ Suspected nosocomial infection
   - ☐ Suspected *Clostridioides difficile* infection (*Clostridium difficile/C. diff*)
   - ☐ Suspected rotavirus infection (during rotavirus season)
   - ☐ Chronic diarrhoea
   - ☐ Dysentery
   - ☐ Children under 5 years of age
   - ☐ Elderly patients

☐ Other

10. *How are your decisions on stool specimen collection practices governed? (Please tick most relevant)*
    - ☐ National guidelines
    - ☐ Hospital/facility policy
    - ☐ Senior clinician guidance
    - ☐ Own clinical judgement
11. *How are your decisions on antibiotic prescription practices governed? (Please tick most relevant)*
    - ☐ National guidelines
    - ☐ Hospital/facility policy
    - ☐ Senior clinician guidance
    - ☐ Own clinical judgement
12. *How are your decisions on treatment of diarrhoeal cases governed? (Please tick most relevant)*
    - ☐ National guidelines
    - ☐ Hospital/facility policy
    - ☐ Senior clinician guidance
    - ☐ Own clinical judgement

Section 3 – Barriers to specimen submission

1. *I am able to submit stool specimens for diagnostic testing when required.*
   ☐ Agree ☐ Disagree
2. *I would submit stool specimens for diagnostic purposes more often if I was able to do so.*
   ☐ Agree ☐ Disagree
3. *Diagnostic results from stool specimens are helpful in clinical management/decision making.*
   ☐ Agree ☐ Disagree
4. *Laboratory turnaround times for results are adequate to assist in clinical decision making.*
   ☐ Agree ☐ Disagree
5. *I have confidence in the results I receive from the laboratory.*
   ☐ Agree ☐ Disagree
6. *I am not always able to submit stool specimens for testing, even when they would be helpful, due to financial constraints on diagnostic testing in my department/hospital.*
   ☐ Agree ☐ Disagree

7. *Patients are often unwilling to provide stool specimens.*

   ☐ Agree ☐ Disagree

8. *Nursing staff are often reluctant to collect stool specimens.*

   ☐ Agree ☐ Disagree

9. *Please describe any other barriers/issues you experience when submitting stool specimens for testing (if applicable):*

   _____

   _____

   _____

   _____

   _____

   _____

Are there any additional comments you would like to make?

_____

_____

_____

_____

_____

_____

_____

Thank you very much for your time!

Appendix D – Turnitin Originality Report

SURVEILLANCE OF GASTROINTESTINAL INFECTIONS IN INDIVIDUALS OVER THE AGE OF 5 YEARS IN SOUTH AFRICA_Turnitin report.docx

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Diarrhoeal diseases in Soweto, South Africa, 2020: a cross-sectional community survey

Siobhan L. Johnstone[1,2*], Nicola A. Page[1,3], Juno Thomas[1], Shabir A. Madhi[4], Portia Mutevedzi[4], Nellie Myburgh[4], Carlos Herrera[4] and Michelle J. Groome[4]

Abstract

Background: In South Africa, there are limited data on the burden of diarrhoea at a community level, specifically in older children and adults. This community survey estimated rates of and factors associated with diarrhoea across all ages and determined the proportion of cases presenting to healthcare facilities.

Methods: Households were enrolled from an existing urban health and demographic surveillance site. A household representative was interviewed to determine associated factors and occurrence of diarrhoea in the household, for all household members, in the past 2 weeks (including symptoms and health seeking behaviour). Diarrhoeal rate of any severity was calculated for < 5 years, 5–15 years and > 15 years age groups. Factors associated with diarrhoea and health seeking behaviour were investigated using binomial logistic regression.

Results: Diarrhoeal rate among respondents (2.5 episodes/person-year (95% CI, 1.8–3.5)) was significantly higher than for other household members (1.0 episodes/person-year (95% CI, 0.8–1.4); IRR = 2.4 (95% CI, 1.5–3.7) $p < 0.001$). Diarrhoeal rates were similar between age groups, however younger children (< 5 years) were more likely to present to healthcare facilities than adults (OR = 5.9 (95% CI, 1.1–31.4), $p = 0.039$). Oral rehydration solution was used in 44.8% of cases. Having a child between 5 and 15 years in the household was associated with diarrhoea (OR = 2.3 (95% CI, 1.3–3.9), $p = 0.003$) and, while 26.4% of cases sought healthcare, only 4.6% were hospitalised and only 3.4% of cases had a stool specimen collected. While the majority of cases were mild, 13.8% of cases felt they required healthcare but were unable to access it.

Conclusion: Diarrhoeal rate was high across all age groups in this community; however, older children and adults were less likely to present to healthcare, and are therefore underrepresented through facility-based clinical surveillance. Current diarrhoeal surveillance represents a fraction of the overall cases occurring in the community.

Keywords: Diarrhoea, Community, Handwashing, Adults, Children, ORS

* Correspondence: siobhanjsa@gmail.com; siobhanj@nicd.ac.za
[1]Center for Enteric Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa
[2] School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
Full list of author information is available at the end of the article

Summary of article's main point

This study estimated diarrhoeal rate at a community level across all age groups in an urban township in South Africa. Factors associated with diarrhoea in the household, the proportion of cases presenting to healthcare, and factors associated with health seeking were determined.

Background

Although progress has been made towards improving water and sanitation globally, diarrhoea has remained in the top 10 causes of mortality and morbidity among all ages [1, 2]. In 2016, diarrhoea was the eighth leading cause of death among all ages (1,655,944 deaths) and the fifth leading cause of death among children under 5 years of age (446,000 deaths) [1]. Nutritional wasting in young children, unsafe water and poor sanitation were the leading factors associated with diarrhoeal morbidity and mortality [1, 3]. Overall, global childhood diarrhoeal diseases have decreased in the past 10 years, largely due to improved maternal education, access to rotavirus vaccination, and improvement of child growth due to better nutrition [1]. However, this decrease has not been uniform across age groups or across settings. Focussed attention on older populations (where there are large knowledge gaps in terms of aetiology and epidemiology) [1, 4] and low-income settings (which still bear the brunt of the burden of disease) [1] is required. Estimates show that the vast majority of global diarrhoeal deaths occur in south Asia and sub-Saharan Africa [1, 5].

Diarrhoeal pathogens are commonly transmitted through the faecal-oral route, due to poor hygiene and sanitation [6], and through ingestion of contaminated food and water, aided by poor food safety practices [7]. Diarrhoeal morbidity is therefore largely preventable through improved access to safe water and sanitation and by ensuring communities are well educated on good handwashing and safe food preparation practices. The WHO has defined five keys to safer food in order to simplify the messaging behind food safety [8]. Diarrhoeal deaths are also largely preventable if dehydration is properly managed [9]. Dehydration can be prevented through a simple, homemade, sugar and salt solution or oral rehydration solution (ORS) as recommended by the WHO [10, 11]. ORS is estimated to reduce diarrhoeal mortality by up to 93% at a healthcare level, although less is known about its impact and use at a community level [9]. Many of the interventions required to reduce diarrhoeal mortality and morbidity are relatively simple and can be addressed through community education. Diarrhoea is therefore one of the most tangible targets for reducing mortality and morbidity from preventable diseases.

Current diarrhoeal surveillance studies being conducted at several hospitals throughout South Africa enrol patients of all ages hospitalised for acute, moderate to severe diarrhoea. However, cases enrolled in these studies represent only a fraction of diarrhoea in the community and are biased towards people with better access to healthcare services. It is also important to understand the healthcare utilization patterns in the community when interpreting the data from hospital surveillance studies. This cross-sectional, questionnaire-based, community survey was undertaken to estimate the rate of and factors associated with diarrhoea in the community across all age groups in Soweto, South Africa and to determine the proportion of cases presenting to healthcare facilities. Secondary objectives were to investigate ORS knowledge and barriers to accessing healthcare in this community.

Methods

Study area and population

Soweto is a densely populated, urban township in Johannesburg, South Africa with an estimated population of 1.3 million people in 355,331 households (2011 census) [12]. According to the most recent census, 97% of residents have access to potable water provided by the municipality, with 55% having access to piped water inside the dwelling. Ninety two percent of residents use flush toilets [12]. Unemployment is high with 19% of households not receiving any set income and a dependency ratio of 40.8 [12]. The average household size is 3.4 people [12] with an average household income of R6500 ($455) per month [13]. Soweto is served by Chris Hani Baragwanath Academic Hospital, a large, secondary-tertiary care hospital, Bheki Mlangeni District Hospital, and several public clinics and private practitioners [13].

The Soweto health and demographic surveillance site (HDSS) was established in 2017 as part of the Child Health and Mortality Prevention Surveillance (CHAMPS) network [14] and currently tracks individuals from 20,778 households in eight clusters in Soweto through biannual data collection rounds. This diarrhoeal diseases survey used the Soweto HDSS as a sampling frame.

Sampling methods and data collection

Probability proportional to size sampling was used to select four of the eight Soweto clusters (due to limited resources and relative size of the clusters) from which households were then randomly sampled. Soweto HDSS data were used to verify that clusters were not significantly different in terms of socioeconomic status.

To obtain a representative sample of each of the four clusters with a 5% precision, 95% confidence level, using an estimated 2-week diarrhoeal prevalence of 6% [15] (amongst all ages), a survey size of 84 households was required per cluster with a total of 336 households in all four clusters. Non-response rate was estimated at 30%

hence 500 households were selected (125 in each of the four clusters). Fieldworkers visited the selected households, explained the study to an adult (≥18 years old) representative of the household, and obtained written informed consent. A questionnaire was administered in the preferred language of the respondent. The questionnaire included sections on handwashing practices; eating and food preparation practices (including where food is purchased, and how it is stored and prepared); water (source of drinking water, water treatment and storage); sanitation; and ORS use and knowledge. Respondents were also asked if any members of the household had experienced a diarrhoeal episode (defined as ≥3 loose or liquid stools in 24 h for any duration) in the past 2 weeks. Further questions on symptoms (as per other diarrhoeal community studies [16]) and health seeking behaviour were included for households with a reported diarrhoeal episode. Questions on diarrhoeal episodes were included for all members of the household in order to avoid selection bias resulting from limiting the survey to include only individuals found to be at home during the day. Households not available on the first visit were visited on a second occasion and considered a non-response if not available at either visit. Data collection was completed over a one-month period in February 2020.

Statistical analysis

The cluster design of the study was accounted for by specifying data as three-level, complex survey data (cluster, household and individuals within the household as the three levels). Demographic and socioeconomic information (including dwelling type, structure main material, home ownership, power source used for cooking, and toilet type) was obtained for enrolled households from HDSS data, using respondent name, surname and age, before being de-identified for the purposes of the analysis. The International Wealth Index (IWI) [17] was used as a composite measure of material wealth for each household. This measure combines assets, housing floor material, toilet facility, number of rooms, access to electricity and water source.

The number of individuals living with the respondent (as reported by the respondent) was used as the denominator for the two-week diarrhoeal rate. Respondents only answered questions pertaining to their household; individuals living in a separate dwelling on the same property were excluded as it was unlikely that the respondent would have been able to accurately answer questions pertaining to these individuals. Handwashing practices were considered adequate if the respondent reported always washing their hands with both soap and water (as opposed to water only) at critical times, including before eating and preparing food and feeding children, as well as after using the toilet and changing children's diapers.

Diarrhoeal rate was calculated as episodes per person-year (PY) using events per person over the 2-week period. Confidence intervals (95%) for diarrhoeal rates were calculated using the Poisson distribution. Incidence rate ratios (IRR) were calculated to compare the diarrhoeal rates among strata (including age groups; type of diarrhoea; and episodes reported for respondents versus other household members). Factors associated with at least one diarrhoeal episode being reported for a household were investigated using binomial logistic regression modelling. Health seeking (defined as seeking healthcare at a clinic, hospital, general practitioner or pharmacy) was investigated for reported diarrhoeal episodes, using binomial logistic regression modelling. Multivariate analysis included all variables significant at p-value< 0.15 in the univariate analysis and used backwards, stepwise selection (using likelihood-ratio test) to determine which variables to retain in the multivariate model. Households where the respondent could not be matched to the HDSS data (as they may have relocated between the most recent HDSS round and the current survey) were excluded from the multivariate analysis. Factors associated with ORS knowledge were investigated using X^2-test for categorical variables and t-test for continuous variables. Non-response rates were compared to ensure that there were no significant differences between clusters. Stata software (version 14) was used for all analyses.

Ethical considerations

This study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (approval number: M190663) and the CHAMPS Soweto HDSS Community Advisory Board.

Results

Enrolled households and respondents

A total of 374 households comprising 1640 individuals (77.2% of which were reported by proxy), were enrolled (Fig. 1). Respondents were majority female (67.4%) with a median age of 45 years (IQR: 24–59). A total of 355 (94.9%) respondents could be matched to the CHAMPS HDSS data.

Diarrhoeal rate

Of the 374 households surveyed, 78 (20.9%) reported at least one diarrhoeal episode in the past 2 weeks. Seventy-one (91.0%) of these had a single episode per household, six (7.7%) had two episodes, and one (1.3%) had four episodes. Hence, a total of 87 diarrhoeal episodes were reported, 36 (41.4%) of which were self-reported by the respondent and 51 (58.6%) were reported on behalf of someone else in the household.

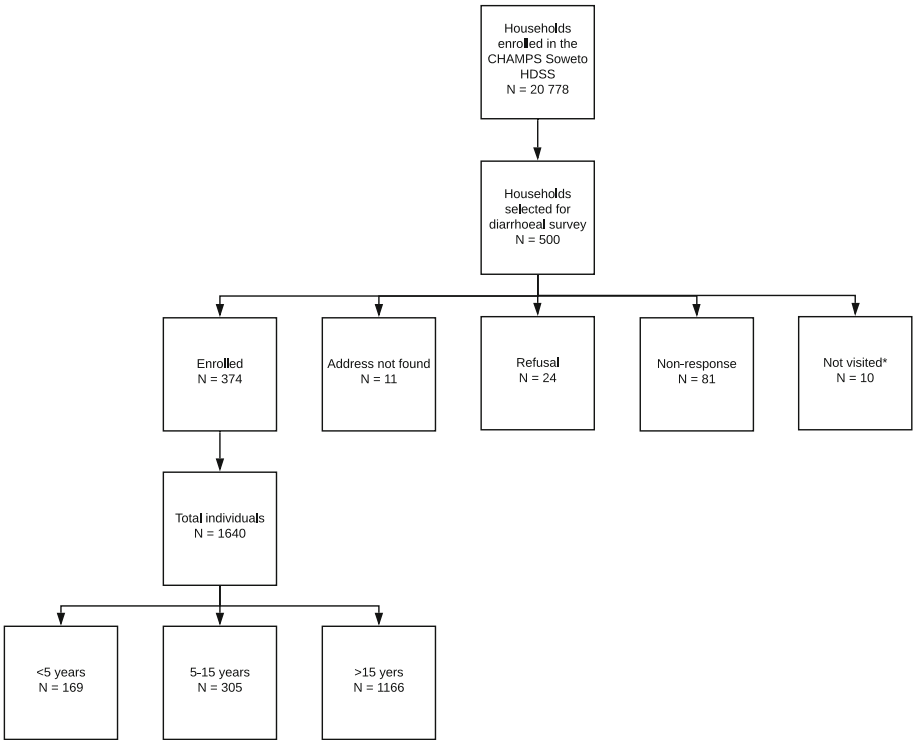

Fig. 1 Enrolment flow diagram. *10 households were not visited due to strike action which prevented fieldwork for several days. Despite this, the required sample size was reached

The overall 2-week diarrhoeal prevalence for the surveyed population was 5.3% which translates to a rate of 1.4 episodes/PY (95% CI, 1.1–1.7) (Table 1). Acute diarrhoea (< 14 days) was common (1.3 episodes/PY (95% CI, 1.0–1.6)) while persistent diarrhoea was rare (0.1 episodes/PY (95% CI, 0.0–0.2)). Reported 2-week prevalence for respondents was 9.6% (rate of 2.5 episodes/PY (95% CI, 1.8–3.5)) which was significantly higher than reported for other household members (2-week prevalence of 4.0% and rate of 1.0 episodes/PY (95% CI, 0.8–1.4)) as shown by the IRR of 2.4 (95% CI, 1.5–3.7, $p < 0.001$). Rates between age groups were similar (1.1 episodes/PY (95% CI, 0.4–2.2) in < 5 years; 1.3 episodes/PY (95% CI, 0.8–2.2) in 5–15 years; 1.4 episodes/PY (95% CI, 1.1–1.8) in > 15 years).

Factors associated with diarrhoeal episodes

Multivariate analysis found that having children aged 5–15 years in the household resulted in higher odds of having had a diarrhoeal episode in the household in the last 2 weeks (OR = 2.3 (95% CI, 1.3–3.9), $p = 0.002$), (Table 2). Inadequate handwashing (OR = 1.7 (95% CI, 1.0–3.0), $p = 0.064$) and having a flush toilet in the house compared to a flush toilet in the yard (OR = 1.7 (95% CI, 1.0–3.0), $p = 0.057$) were also associated with increased diarrhoeal episodes in the household; however, these did

Table 1 Rate of diarrhoeal disease reported for different groups

	Events (N)	Denominator (number of people)	2-week prevalence (%)	Person-years	Rate (episodes per person-year (95% CI))
Overall	87	1640	5.3	62.9	1.4 (1.1–1.7)
Acute (< 14 days)	81	1640	4.9	62.9	1.3 (1.0–1.6)
Persistent (≥14 days)	6	1640	0.4	62.9	0.1 (0.0–0.2)
Respondents	36	374	9.6	14.3	2.5 (1.8–3.5)
Other household members	51	1266	4.0	48.6	1.0 (0.8–1.4)
< 5 years	7	169	4.1	6.5	1.1 (0.4–2.2)
5–15 years	16	310	5.2	11.9	1.3 (0.8–2.2)
> 15 years	64	1171	5.5	44.9	1.4 (1.1–1.8)

Table 2 Factors associated with occurrence of diarrhoeal episodes in the household in the preceding two weeks

	Diarrhoeal episode in the household n/N (%) [c]	Univariate [d]		Multivariate [d, e]	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Dwelling type					
Formal	56/74 (75.7)	Referent	–	–	–
Informal	18/74 (24.3)	1.6 (0.9–3.0)	0.135		
Structure main material					
Brick	66/74 (89.2)	Referent	–	–	–
Metal sheets	8/74 (10.8)	1.1 (0.5–2.6)	0.765		
Home ownership					
Owned by residents	38/74 (51.4)	Referent	–	–	–
Rented	27/74 (36.5)	1.7 (1.0–3.0)	0.071		
Government issued	9/74 (12.2)	0.9 (0.4–1.9)	0.718		
Power source for cooking					
Electricity	74/74 (100.0)	Omitted		–	–
Paraffin	0/74 (0)				
Toilet type					
Flush toilet in yard	22/74 (29.7)	Referent	–	Referent	–
Flush toilet in house	52/74 (70.3)	1.8 (1.0–3.1)	**0.038**	1.7 (1.0–3.0)	0.057
Ventilated pit latrine	0/74 (0)	–	–	–	–
International Wealth Index					
Median (IQR)	85.6 (79.1–92.1)	1.0 (1.0–1.0)	0.827	–	–
Number of people living in the household					
Median (IQR)	5 (3-7)	1.1 (1.0–1.2)	**0.004**	–	–
Children < 5 years in the household					
No	42/78 (53.9)	Referent	–	–	–
Yes	36/78 (46.1)	2.0 (1.2–3.4)	**0.008**		
Children between 5 and 15 years in the household					
No	29/78 (37.2)	Referent	–	Referent	–
Yes	49/78 (62.8)	2.3 (1.4–3.9)	**0.002**	2.3 (1.3–3.9)	**0.002**
Handwashing [a]					
Adequate	24/78 (30.8)	Referent	–	Referent	–
Inadequate	54/78 (69.2)	1.8 (1.0–3.0)	**0.039**	1.7 (1.0–3.0)	0.064
Consume fresh fruit and vegetables [b]					
Never	3/77 (3.9)	Referent	–	–	–
Occasionally	24/77 (31.2)	0.5 (0.1–2.1)	0.307		
Often	50/77 (64.9)	0.4 (0.1–1.8)	0.236		
Consume meat [b]					
Never	3/78 (3.9)	Referent	–	–	–
Occasionally	28/78 (35.9)	1.4 (0.4–5.2)	0.617		
Often	47/78 (60.3)	1.2 (0.3–4.2)	0.815		
Consume dairy [b]					
Never	10/77 (13.0)	Referent	–	–	–
Occasionally	28/77 (36.4)	1.2 (0.5–2.8)	0.710		
Often	39/77 (50.7)	0.8 (0.3–1.7)	0.520		

Table 2 Factors associated with occurrence of diarrhoeal episodes in the household in the preceding two weeks *(Continued)*

	Diarrhoeal episode in the household n/N (%) [c]	Univariate [d]		Multivariate [d, e]	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Consume eggs [b]					
Never	16/77 (20.8)	Referent	–	–	–
Occasionally	26/77 (33.8)	0.7 (0.4–1.5)	0.420		
Often	35/77 (45.5)	0.7 (0.3–1.3)	0.224		
Consume ready-to-eat meat products [b]					
Never	21/77 (27.3)	Referent	–	–	–
Occasionally	28/77 (36.4)	1.4 (0.7–2.7)	0.341		
Often	28/77 (36.4)	1.4 (0.7–2.6)	0.391		
Consume take-aways [b]					
Never	28/77 (36.4)	Referent	–	–	–
Occasionally	39/77 (50.7)	1.3 (0.8–2.3)	0.313		
Often	10/77 (13.0)	1.8 (0.8–4.1)	0.182		
Eat at restaurants [b]					
Never	48/78 (61.5)	Referent	–	–	–
Occasionally	28/78 (35.9)	1.0 (0.6–1.7)	0.937		
Often	2/78 (2.6)	1.3 (0.2–6.5)	0.771		
Purchase meat					
Informal	2/78 (2.6)	Referent		–	–
Commercial	76/78 (97.4)	1.9 (0.4–8.5)	0.408		
Purchase fruit and vegetables					
Informal	39/76 (51.3)	Referent	–	–	–
Commercial	37/76 (48.7)	1.2 (0.7–2.0)	0.451		
Fridge/freezer storage					
No	2/78 (2.6)	Referent	–	–	–
Yes	76/78 (97.4)	1.2 (0.3–5.7)	0.822		
Separate raw and cooked food					
No	1/78 (1.3)	Referent	–	–	–
Yes	77/78 (98.7)	2.8 (0.4–22.0)	0.336		
Treat drinking water					
No	67/78 (85.9)	Referent	–	–	–
Yes	11/78 (14.1)	1.6 (0.7–3.5)	0.251		
Water storage					
None (straight from tap)	47/78 (60.3)	Referent	–	–	–
Closed container	29/78 (37.2)	1.1 (0.7–1.9)	0.658		
Open container	2/78 (2.6)	1.6 (0.3–8.3)	0.600		
Interruptions to water supply in the past 2 weeks					
No	69/76 (90.8)	Referent	–	–	–
Yes	7/76 (9.2)	0.7 (0.3–1.7)	0.393		

[a] Adequate defined as washing with soap and water at critical times (after using the toilet or changing diapers, and before preparing food, eating or feeding young children); [b] Occasionally defined as once/twice per week, and often defined as every day or every second day. [c] Denominator differs due to missing responses for some households (maximum of 78 households that experienced at least one episode of diarrhoea in the past two weeks); [d] Univariate and multivariate analysis using only the 355 households that could be matched to HDSS data. [e] The following variables were assessed in the multivariate model: number of people living in the household, dwelling type, home ownership, toilet type, children < 5 years in the household, children between 5 and 15 years in the household and handwashing. The following variables were retained in the model: toilet type, children between 5 and 15 years in the household and handwashing

not reach statistical significance at the multivariate level. Number of people in the household, dwelling type, IWI, eating habits, where food was purchased (formal or informal traders) and stored (availability of cold storage), knowledge on separation of raw and cooked food, and water treatment, interruptions and storage were not associated with increased diarrhoeal episodes.

Symptoms and health seeking behaviour

The median age for those with diarrhoea was 30 years (IQR: 13–53). Episodes lasted between a few hours to 28 days with a median of 2 days (IQR: 2–5). Abdominal cramps were the most commonly reported symptom (59.8%) followed by headache (31.0%), loss of appetite (31.0%) and fever (31.0%), (Table 3). Some cases (14.9%) experienced no symptoms additional to the diarrhoea.

Twenty-three of the 87 people with diarrhoea (26.4%) sought healthcare (Supplementary Figure S1). Fourteen (16.1%) visited a public clinic, while five (5.7%) visited a pharmacy and four (4.6%) were admitted to hospital. The admitted cases included a 4-month-old infant, two elderly adults (aged 65 and 77 years old) and a 23-year-old adult with dysentery, myalgia, abdominal cramps, fever, nausea and vomiting. Fifty-one (58.6%) cases did not seek healthcare as they felt their illness was mild, while 12 (13.8%) cases felt it was necessary but were unable to access healthcare. Reasons for not being able to access healthcare included personal issues (6/12, 50.0%) including not being able to take time off from work or home duties, and not having access to transport; as well as issues with the healthcare system (6/12, 50.0%), including being previously ill-treated at public clinics, long waiting times at the public clinic, and the public clinic being closed. In the multivariate analysis, children < 5 years and those with myalgia were significantly more likely to seek healthcare for diarrhoea compared with older children and adults and those without myalgia (OR = 5.9 (95% CI, 1.1–31.4), $p = 0.039$; OR = 3.4 (95% CI, 1.2–10.2), $p = 0.027$ respectively), (Table 3). The six cases that reported blood in the stool or prolonged symptoms all felt they required healthcare, although only three (50.0%) were able to access it. Stool specimens were collected in only three of the 87 cases (3.4%) or 16.7% of those that visited a public clinic or hospital (3/18) for their illness.

Only 44.8% ($n = 39$) of cases used ORS during the episode. Knowledge of ORS was poor in the surveyed population with only 51.0% ($n = 192$) of respondents having some knowledge of ORS (knew the recipe or were able to name the ingredients) and only 17.9% ($n = 67$) able to give the correct recipe. Females ($p < 0.001$), respondents with a child < 5 years old in the household ($p = 0.010$) or

Table 3 Frequency of concurrently reported symptoms amongst household members with diarrhoeal episodes and factors associated with seeking healthcare

	Frequency of symptoms (%)	Factors associated with seeking healthcare	
		Univariate analysis [a]	
		OR (95% CI)	*p*-value
Abdominal cramps	52 (59.8)	1.1 (0.4–2.9)	0.858
Headache	27 (31.0)	0.7 (0.2–2.1)	0.523
Loss of appetite	27 (31.0)	0.9 (0.3–2.7)	0.908
Fever	27 (31.0)	0.7 (0.2–2.1)	0.523
Myalgia	24 (27.6)	2.7 (1.0–7.4)	0.056
Respiratory symptoms [b]	17 (19.5)	0.8 (0.2–2.8)	0.738
Nausea	15 (17.2)	2.1 (0.7–6.8)	0.208
Watery stool	14 (16.1)	0.7 (0.2–2.8)	0.625
Vomiting	13 (14.9)	1.3 (0.4–4.6)	0.722
Blood in stool	4 (4.6)	2.9 (0.4–21.9)	0.301
Age group (years)			
< 5	–	3.6 (0.7–17.8)	0.115
5–15		0.4 (0.1–1.9)	0.239
> 15		Referent	–
Female	–	0.8 (0.3–2.3)	0.634
International Wealth Index	–		
		1.0 (1.0–1.1)	0.194

[a] The following variables were assessed and retained in the multivariate model: myalgia (OR = 3.4 (95% CI, 1.2–10.2), $p = 0.027$) and age group (OR = 5.9 (95% CI, 1.1–31.4), $p = 0.039$, for children < 5 years compared to those > 15 years). [b] Respiratory symptoms included cough, coryza and shortness of breath

children between the age of 5 to 15 years in the household ($p = 0.002$) were significantly more likely to have some knowledge of ORS compared with males and respondents without children in the household (Supplementary Table S1).

Discussion

The current perspective of diarrhoeal diseases in many low- and middle- income countries, including South Africa, is based largely on healthcare-level data focused on children under the age of 5 years [18, 19]. This study adds to the limited understanding of diarrhoeal diseases in all ages at a community level and assists in interpreting how representative healthcare and laboratory-level data are of these cases. This survey found a diarrhoeal rate of 2.5 episodes/PY (95% CI, 1.8–3.5) for respondents and 1.0 episodes/PY (95% CI, 0.8–1.4) for other household members (as reported by respondent as a proxy). Since respondents should not be different to other household members in terms of risk factors for diarrhoeal diseases, it is unlikely that this is a true difference and may be due to reporting bias (in which respondents underestimated episodes experienced by other household members) or, less likely, recall bias (in which respondents overestimate episodes experienced themselves). Since other studies using similar methods [3, 20] did not differentiate self-reported episodes to episodes reported on behalf of other household members, this difference cannot be compared to literature and requires further investigation. Both the self-reported rate and the rate for other household members were higher than those reported in high-income countries [15, 21]. This is expected, due to poorer living conditions and a higher burden of underlying conditions, including HIV and malnutrition, associated with increased diarrhoeal morbidity in our setting. The rates reported here are lower than those reported from other African countries, such as a household survey in Zambia which reported a rate of 1.7 episodes/PY for persistent diarrhoea in adults [22], and a household study in Ethiopia which found a diarrhoeal rate of 3.8 episodes/PY for children < 5 years of age [23]. Our reported prevalence for children < 5 years was similar to that previously reported in a community-based study from the same setting (4.0%) [13]. Rates for other household members are in keeping with the GBD estimates of 1.0 episodes/PY (95% CI, 1.0–1.1) for sub-Saharan Africa [1]. Since community-level data amongst all ages in sub-Saharan Africa are limited, it is possible that the higher self-reported estimates are accurate, and other estimates (based on healthcare-level data) are an underestimation. Unlike reports from studies in high income settings [15, 21], our survey found no significant difference between rates for different age groups. We did however find that healthcare was most likely to be sought for children < 5 years of age. This highlights that diarrhoeal cases in children < 5 years are seen disproportionally at a healthcare level in this community since many older children and adults do not seek healthcare for diarrhoeal episodes. The economic effects of diarrhoeal disease in the community therefore extend beyond healthcare system costs, and includes the reduction of economically active days for individuals of working age, causing social disturbance and lost economic opportunities [24].

The presence of children between 5 and 15 years in the household was significantly associated with episodes of diarrhoea. Since the diarrhoeal rate in this age group was similar to the rate for adults, it is likely that having a child of school-going age in the household is a risk factor for others in the household as these children may act as vectors. However, this is not previously reported in the literature and requires further investigation. Having a flush toilet in the house (as compared to in the yard) and inadequate handwashing were also associated with diarrhoea (although only marginally significant). Poor handwashing is a known risk factor for diarrhoeal diseases [7]; however, the association between diarrhoea and the location of the flush toilet requires further investigation and may be due to an unmeasured confounder. Food safety practice and food purchasing behaviour were not associated with diarrhoea in the current study.

Diarrhoeal episodes were relatively mild in the surveyed population, with a median duration of 2 days. The most common accompanying symptoms were abdominal cramps, headache, loss of appetite and fever. This is in agreement with systematic review data from low- and middle-income countries [25]. Only 21% of cases required healthcare intervention, which is similar to estimates of 21% from the United States [15]. Data from low- and middle-income countries estimate that 35.2% of diarrhoeal cases in children < 5 years [25] present to healthcare, but there are no data for rates in other age groups, which are expected to be lower. In the present study, of those that sought healthcare, the majority went to public clinics (61%), followed by pharmacies (22%) and public hospitals (17%). This differs to a community-based study in children < 5 years from the same community which found that 70% of cases sought healthcare at public clinics, 10% at a private practitioners, 10% at pharmacies and 5% at public hospitals [13]. This difference is probably because adults are more likely to seek healthcare at a pharmacy, rather than a public clinic, public hospital or private practitioner. This study was not powered to determine the difference in type of healthcare sought between age groups, however, no cases in children < 5 years sought healthcare at pharmacies. It is likely that diarrhoeal cases in children < 5 years were more severe, and that there was a lower threshold

for seeking healthcare at other facilities. The number of individuals seeking healthcare for their illness underrepresents the subjective severity of illness, since 33% (12/36) of those who felt they needed healthcare were unable to access it. Many of the barriers to accessing healthcare identified here were also highlighted in the previous Soweto study, including issues with the health system (such as deficiencies in healthcare delivery, dissatisfaction with services, medications being out of stock) and personal reasons (such as time, finance and transportation constraints) [13].

The data presented here shows that only 4.6% of diarrhoeal cases in the community would have been detected through hospital surveillance and 16.1% through clinic surveillance. Analysis of routine diagnostic laboratory data would represent only 3.4% of the cases, being limited to those that had stool specimens collected. These findings are important to consider when interpreting the representativeness of such data.

We found community ORS knowledge to be poor in the surveyed population. Women and respondents with children in the household were more likely to have some knowledge of ORS, indicating that this information is most likely disseminated through baby and childcare clinics, a finding which was also reported in rural Botswana [26]. There is a gap in information dissemination for men and households without children, which should be addressed.

This study was limited by the reliance on a single household member to answer questions on behalf of other household members. This may have introduced bias, as respondents were less likely to accurately respond to questions regarding diarrhoeal episodes experienced by others and may have inflated episodes experienced themselves. This method has been used in similar studies [3, 20] and was preferable to collecting data on respondents only, as this may have biased results towards those that stay at home during the day. The study could have been strengthened by interviewing all members of included households; however, this was impractical. We were also unable to investigate the association between HIV and diarrhoeal disease at an individual level as this data was unavailable and could not be requested from respondents for ethical reasons. Level of education is known to be associated with diarrhoea, however this was not investigated in the current study as this variable was missing for the majority of HDSS members. Our findings are generalizable to many similar communities in South Africa, but may not be applicable to rural settings where living conditions and health-seeking patterns are likely to differ substantially. Therefore, understanding of diarrhoeal diseases at a community level could be strengthened by expanding this study to a larger geographical area. Causal inference and predictors of diarrhoeal disease could not be determined due to the cross-sectional nature of the study.

Research on diarrhoeal disease focusses on children under the age of 5 years because this age group is particularly vulnerable to illness and are more likely to seek healthcare and therefore to be detected through healthcare and laboratory surveillance. Notably, this study shows that diarrhoeal rates in older age groups are high at a community level, but are missed through routine healthcare- or laboratory-based surveillance. It is therefore recommended that routine surveillance be extended to include public clinics and pharmacies. We recommend that handwashing practices in this community be further investigated in order to produce targeted health messaging. We also recommend that health education on ORS as a low cost, effective intervention for diarrhoeal diseases should be made widely available, and include men and households without young children as target groups for such health education.

Abbreviations

CHAMPS: Child Health and Mortality Prevention Surveillance network; CI: Confidence interval; GBD: Global Burden of Disease; HDSS: Health and demographic surveillance site; IQR: Interquartile range; IRR: Incidence rate ratio; IWI: International Wealth Index; ORS: Oral rehydration solution/salts; PY: Person-years; WHO: World Health Organisation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12889-021-11470-9.

Additional file 1: Figure S1. Health seeking for reported diarrhoeal episodes. **Table S1.** Factors associated with ORS knowledge.

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Authors' contributions

SLJ, NAP, JT, PM and MJG conceptualised and designed the study. S.L.J completed and managed fieldwork and data collection. SLJ, NAP, SAM, NM, CH and MJG acquired, analysed and interpreted the data. SLJ drafted the work. NAP, JT, SAM, PM, NM and MJG substantively revised the work. All authors reviewed the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used during the current study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (approval number: M190663) and the CHAMPS Soweto HDSS Community Advisory Board. Written informed consent was obtained from all included participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

None to declare.

Author details

[1]Center for Enteric Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa. [2] School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [3]Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Arcadia 0007, South Africa. [4]South African Medical Research Council: Vaccines and Infectious Diseases Analytics Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

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Publisher's Note

PAPER II

BMC Infectious Diseases

RESEARCH

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Diagnostic testing practices for diarrhoeal cases in South African public hospitals

Siobhan L. Johnstone[1,2*], Nicola A. Page[1,3], Michelle J. Groome[4,5], Nicolette M. du Plessis[6] and Juno Thomas[1]

Abstract

Background: Stool samples submitted for diagnostic testing represent a proportion of diarrhoeal cases seeking healthcare, and an even smaller proportion of diarrhoeal cases in the community. Despite this, surveillance relies heavily on these laboratory results. This study described diarrhoeal diagnostic practices and aetiological agents of diarrhoea in patients admitted to three South African public hospitals in order to understand biases in surveillance data, and inform guidelines, diagnostic and laboratory practices to improve clinical management.

Methods: A doctors' survey was conducted to determine sample submission, diarrhoeal treatment and barriers to submitting samples for testing. Results for all samples submitted for routine diagnostics were obtained from the NHLS Central Data Warehouse. An enhanced surveillance study enrolled patients with acute diarrhoea at the same hospitals over the same period. Differences between routine culture results and molecular testing from the surveillance study were described.

Results: Stool samples were seldom submitted for diagnostic testing (median of 10% of admitted cases). Current diagnostic guidelines were not useful, hence most doctors (75.1%) relied on their own clinical judgement or judgement of a senior clinician. Although most doctors (90.3%) agreed that diagnostics were helpful for clinical management, they reported patients being unwilling to provide samples and long laboratory turnaround times. Routine diagnostic data represent cases with chronic diarrhoea and dysentery since doctors are most likely to submit specimens for these cases. Pathogen yield (number of pathogens detected for samples tested for specific pathogens) was significantly higher in the surveillance study, which used molecular methods, than through routine diagnostic services (73.3% versus 8.2%, $p < 0.001$), including for viruses (48.9% versus 2.6%, $p < 0.001$), bacteria (40.1% versus 2.2%, $p < 0.001$) and parasites (16.2% versus 3.6%, $p < 0.001$). Despite viruses being commonly detected in the surveillance study, viral testing was seldom requested in routine diagnostic investigations.

Conclusions: Comprehensive diagnostic and treatment guidelines are required for diarrhoeal diseases. These guidelines should be informed by local epidemiological data, where diagnostic testing is reserved for cases most likely to benefit from specific treatment. Optimisation of current diagnostic processes and methods are required for these cases, specifically in terms of minimising turnaround times while maximising diagnostic acumen.

Keywords: Diarrhoea, Diagnostics, Aetiology, Low resource, Stool samples, Surveillance

Introduction

Diarrhoeal surveillance relies primarily on routine diagnostic laboratory data, even in settings with established surveillance systems. Analysis of routine diagnostic data is useful in describing trends for enteric pathogens, but such data represent a minor subset of diarrhoeal cases in the community. Only those cases consulting a healthcare

*Correspondence: siobhanj@nicd.ac.za; siobhanjsa@gmail.com

[1] Centre for Enteric Diseases, National Institute for Communicable Diseases, a division of the National Health Laboratory Service (NHLS), Johannesburg, South Africa
Full list of author information is available at the end of the article

professional and for whom the healthcare professional orders laboratory screening of a stool sample are included in this subset. In South Africa, the relevance of routine diagnostic data is not clear, since little is known about the factors influencing healthcare professionals' diagnostic choices at different healthcare levels. An understanding of diagnostic testing practices is important for the interpretation of routine diagnostic and laboratory-based surveillance data, specifically when determining how much these data underestimate the true burden of disease [1]. Knowledge of factors that influence doctors' diagnostic practices is required, not only to understand the strengths and weaknesses of routine diagnostic data [2], but also to highlight gaps in routine diagnostic services and guidelines [3] for improving patient management.

Diarrhoeal diagnostics should be limited to cases in which clinical management may benefit from knowledge of the aetiological agent, and tests should be limited to pathogens likely to be present in the population [3]. Guidelines should hence be based on local or regional epidemiological data, minimizing costs for healthcare and patients [3]. South African National Department of Health guidelines for the treatment of diarrhoea are included in the standard treatment guidelines and essential medicines list (hospital and primary healthcare levels) [4, 5]. An audit at a provincial hospital in KwaZulu-Natal found that patients with diarrhoea were managed inconsistently, specifically with regards to antibiotic treatment, which could result in an increased risk of antibiotic resistance and infection with *Clostridioides difficile (C. difficile)* [6]. The study reported that stool samples were submitted for routine microscopy (examination for ova and parasites) and culture in 47% of cases, yet 60% of cases were treated with antibiotics (only 35% of which had positive culture results). The authors called for clearer national guidelines for the management of diarrhoea, particularly for HIV-infected patients who comprised the majority (81%) of diarrhoeal cases presenting to the emergency department [6]. Robust epidemiological and aetiological data are needed to formulate nationally relevant guidelines.

When guidelines serve an advisory purpose and are not strictly prescriptive, they are not reliable predictors of diagnostic practice, and it is important to investigate other predictors. Studies in high-income countries have found dysentery [1, 2, 7], a diagnosis of HIV or immunosuppression from other causes [2, 7] and prolonged illness [1, 2, 7] to be strong predictors for requesting stool culture. In these settings, routine diagnostic data are likely to underestimate the pathogens that rarely cause dysentery while pathogens such as *Escherichia coli* 0157:H7 are likely to be overrepresented [2]. A strong association between requesting stool culture and the need for hospitalization, intravenous rehydration, further consultation and a diagnosis of HIV, means that surveillance data are biased towards patients who present with severe illness [2]. Other reported predictors include overseas travel and suspected links to an outbreak [1]. The rates of requests for stool culture differ between age groups, with studies from high-income settings indicating increased sample submissions for the young (< 5 years) and the elderly (≥ 60 years) [8], influencing the interpretation of surveillance data. The availability of laboratory services, pressure from laboratories to limit number of samples submitted, availability of sample collection kits, cost of tests, confidence in laboratory results, and turn-around-time for laboratory results are reported barriers preventing laboratory screening [7].

The aims of this study were to describe the diagnostic practice for diarrhoea at three hospitals in South Africa; to identify what informs these decisions; to understand clinicians' perceptions regarding laboratory tests; and to describe the aetiological agents of diarrhoea in patients admitted to these hospitals. The results will elucidate reasons for the bias in surveillance data, and inform recommendations for improving the management of diarrhoea in terms of guidelines, diagnostic practice and laboratory testing.

Methods

Study sites

The study was conducted at three hospitals (Kalafong, a provincial, tertiary hospital in an urban area in Gauteng Province; Matikwana, a regional hospital, and Mapulaneng, a district hospital, both in rural Mpumalanga Province) which are study sites for the African Network for Improved Diagnostics, Epidemiology and Management of common Infectious Agents (ANDEMIA), details of which have been described [9].

Attending doctors' survey

A survey was conducted among doctors attending patients in all wards (including paediatrics, adult medical, surgical, emergency, obstetrics and outpatient wards) at the three surveillance hospitals in October–November 2020. The questionnaire consisted of sections on general information (demographics, position in the hospital, speciality and ward); stool sample submission and diarrhoeal treatment practices; and barriers to submitting stool samples for testing. Virology, epidemiology and clinical experts revised the questionnaire before implementation. The survey was briefly presented to the doctors at departmental meetings and hard copy questionnaires completed during the meetings. An electronic version was emailed to those not present at the meetings using a

web-based platform (Castor EDC). Questionnaires were captured onto an electronic database.

Surveillance study

Prospective, hospital based, surveillance was conducted at the same study hospitals over an 18-month period (July 2018 to December 2019). Surveillance officers based at the hospitals enrolled patients of all ages presenting with acute diarrhoea (defined as three or more loose stools per day for 28 days or less). The study was explained to patients and those consenting were requested to provide a stool sample or rectal swab. Surveillance officers conducted interviews to determine demographic variables and risk factors. Stool samples were sent to the Centre for Enteric Diseases at the National Institute for Communicable Diseases (NICD) where real-time polymerase chain reaction (PCR) testing was performed using Fast Track Diagnostics assays for common viral, bacterial and parasitic causes of gastroenteritis. Detailed study methods are available [9].

Routine diagnostic test data

The tests routinely ordered for stool microbiology in the public health sector is colloquially termed stool 'MCS', and refers to stool microscopy for ova and parasites, culture, and antimicrobial susceptibility testing where applicable. Results for all stool and rectal swab samples submitted for routine microscopy for ova and parasites and culture as well as any additional microbiologic or virologic testing, to diagnostic laboratories at the study hospitals for the period July 2018 to December 2019 were extracted from the Central Data Warehouse (CDW) of the National Health Laboratory Services (NHLS). Data were de-identified with only patient sex and age included. Submission of samples for routine diagnostic testing by attending doctors was independent of enrolment in the surveillance study. The denominator for pathogen yield was the number of samples tested for a specific pathogen (as opposed to total samples collected since not all samples were tested for all pathogens).

Statistical analysis

Characteristics of survey respondents were described using proportions, medians and interquartile ranges (IQR). Responses for guidance of decision-making, factors associated with and barriers to sample submission were compared between urban and rural sites using Chi-squared test and Fisher's two-tailed exact test, as appropriate.

CDW data were used to determine proportions of submitted samples tested for specific pathogens. CDW data and surveillance study data for the same period were compared using Chi-squared/Fisher's two-tailed exact tests. Significant results were defined by $p \leq 0.05$. All data analysis was conducted using Stata software (version 14).

Ethics

The ANDEMIA surveillance study was approved by the Human Research Ethics Committee (Medical) at the University of the Witwatersrand (Approval number: M170403) and the University of Pretoria (Approval number: 101/2017). The doctors' survey and analysis of CDW data was approved by the Human Research Ethics Committee (Medical) at the University of the Witwatersrand (Approval number: M190663 MED18-12-034). Permission for the use of CDW data was obtained from the NHLS.

Results

Doctors' survey respondents

There were 32 responses to the doctors' survey, included medical officers (n = 17), interns (n = 8), registrars (n = 5), and specialists (n = 2). Respondents were based in paediatric (n = 14), outpatient (n = 8), obstetrics and gynaecology (n = 6), adult medical (n = 6), emergency medicine (n = 2) and surgical (n = 2) departments. The majority (67.7%) of respondents attended to ≤ 5 cases of diarrhoea per week, with a median of four cases per week (IQR 2–8 cases). Respondents at the urban site attended more cases per week than the rural sites (5.5 versus 3) although this difference was not significant ($p = 0.095$). The percentage of diarrhoeal cases for which a stool sample was requested varied widely between respondents (range 0–100%, median of 10% and IQR of 10–70%). Respondents at the urban site reported ordering stool screening for a higher number of cases than those at the rural sites (42.5% versus 10%), although this difference was not significant ($p = 0.054$).

Guidance for decision-making

There was poor agreement among the respondents as to how decisions for ordering stool testing, prescribing antibiotic therapy and the treatment of diarrhoea are governed (Table 1). The majority of respondents did not follow any guidelines for ordering stool tests, relying rather on their own clinical judgement (43.8%) or the guidance of senior clinicians (31.3%). This finding was consistent at both urban and rural sites. The majority of respondents followed treatment guidelines for the prescription of antibiotics (46.9% followed national guidelines and 15.6% followed hospital guidelines). Doctors at the rural sites were more likely to follow national guidelines than those at the urban site (59.1% versus 20.0%, $p = 0.060$), although this was not significant. National guidelines were often followed for treatment of

Table 1 Guidance for decision-making

	Total (n = 32)	Urban (n = 10)	Rural (n = 22)	p-value
Stool sample submission practices[a]				
National guidelines	9 (28.1%)	1 (10. 0%)	8 (36.4%)	0.210
Hospital policy	4 (12.5%)	3 (30.0%)	1 (4.6%)	0.079
Senior clinician guidance	10 (31.3%)	5 (50.0%)	5 (22.7%)	0.217
Own clinical judgement	14 (43.8%)	5 (50.0%)	9 (40.9%)	0.459
Antibiotic prescription practices[a]				
National guidelines	15 (46.9%)	2 (20.0%)	13 (59.1%)	0.060
Hospital policy	5 (15.6%)	1 (10.0%)	4 (18.2%)	> 0.999
Senior clinician guidance	8 (25.0%)	4 (40.0%)	4 (18.2%)	0.218
Own clinical judgement	8 (25.0%)	4 (40.0%)	4 (18.2%)	0.218
Treatment of diarrhoeal cases[a]				
National guidelines	19 (59.4%)	3 (30.0%)	16 (72.7%)	**0.049**
Hospital policy	3 (9.4%)	0 (0.0%)	3 (13.6%)	0.310
Senior clinician guidance	10 (31.3%)	4 (40.0%)	6 (27.3%)	0.683
Own clinical judgement	7 (21.9%)	4 (40.0%)	3 (13.6%)	0.165

P-values < 0.05 indicated in bold

[a] More than one answer could have been selected per respondent hence the total may exceed 100%

diarrhoeal cases (59.4%), specifically for the rural as compared to the rural sites (72.7% versus 30.0%, p = 0.049).

Patient-related factors associated with ordering stool tests

Respondents were likely to order stool tests for patients with chronic diarrhoea (81.3% of respondents would submit a sample), dysentery (65.6%), suspected *C. difficile* infection (62.5%), travel history (53.1%) or suspected link to an outbreak (53.1%) (Table 2). Patient-related factors associated with ordering stool screening were similar between urban and rural sites, except for HIV-related diarrhoea which was more likely to be associated with having a stool sample ordered at the urban site (70.0% versus 27.3%, p = 0.049).

Barriers to ordering stool tests

Most respondents (90.3%) reported that stool diagnostics assisted with the clinical management of diarrhoea (Table 3). More than half of the respondents (58.6%) indicated that they would order stool diagnostics more often in the absence of barriers, specifically at the rural sites (70.0% versus 33.3%, p = 0.064). The most frequently

Table 2 Patient-related factors influencing the likelihood of a doctor ordering stool tests

	Total (n = 32)	Urban (n = 10)	Rural (n = 22)	p-value
Case characteristic				
Chronic diarrhoea	26 (81.3%)	9 (90.0%)	17 (77.3%)	0.637
Dysentery	21 (65.6%)	6 (60.0%)	15 (68.2%)	0.703
Suspected *C. difficile* infection	20 (62.5%)	8 (80.0%)	12 (54.6%)	0.248
History of travel	17 (53.1%)	6 (60.0%)	11 (50.0%)	0.712
Suspected link to an outbreak	17 (53.1%)	6 (60.0%)	11 (50.0%)	0.712
Known HIV infection	13 (40.6%)	7 (70.0%)	6 (27.3%)	**0.049**
Suspected foodborne illness	12 (37.5%)	4 (40.0%)	8 (36.4%)	> 0.999
Suspected healthcare-associated infection	12 (37.5%)	3 (30.0%)	9 (40.9%)	0.703
Children under 5 years of age	11 (34.4%)	2 (20.0%)	9 (40.9%)	0.425
Suspected rotavirus infection (during rotavirus season)	7 (21.9%)	1 (10.0%)	6 (27.3%)	0.387
Adults ≥ 65 years of age	5 (15.6%)	2 (20.0%)	3 (13.6%)	0.506
Fever	7 (21.9%)	3 (30.0%)	4 (18.2%)	0.648
Dehydration requiring intravenous fluid replacement	6 (18.8%)	1 (10.0%)	5 (22.7%)	0.637
Vomiting	2 (6.3%)	0 (0.0%)	2 (9.1%)	> 0.999

P-values < 0.05 indicated in bold

Table 3 Reported barriers to ordering stool tests

	Total (n = 32)	Urban (n = 10)	Rural (n = 22)	p-value
Laboratory turnaround times too long to assist with clinical decision-making	17 (54.8%)	6 (60.0%)	11 (52.4%)	> 0.999
Patients unwilling to provide stool samples	11 (35.5%)	3 (30.0%)	8 (38.1%)	0.660
Nursing staff reluctant to collect stool samples	10 (32.3%)	5 (50.0%)	5 (23.8%)	0.222
Lack of confidence in laboratory results	6 (19.4%)	4 (40.0%)	2 (9.5%)	0.067
Financial constraints on diagnostic testing	5 (16.1%)	0 (0.0%)	5 (23.8%)	0.092
Diagnostic results are unhelpful in clinical management/ decision making	3 (9.7%)	1 (10.0%)	2 (9.5%)	> 0.999

reported barriers to ordering stool tests were laboratory turnaround times being too long to assist with clinical decision-making (reported by 54.8% of respondents) and patients being unwilling to provide stool samples (35.5%). Financial constraints on testing was a barrier reported by 23.8% of respondents at the rural sites, but by none at the urban site (p = 0.092). More respondents at the urban site reported a lack of confidence in laboratory results than the rural sites (40.0% versus 9.5%, p = 0.067). Other barriers reported by respondents included high patient numbers, the need to reserve testing for cases not responding to supportive treatment, and challenges with sample labelling, and transport to the laboratory.

Pathogens tested for in routine stool diagnostic tests

The majority of doctors believed that *Salmonella* spp. (84.4%), *Shigella* spp. (84.4%) and enterohaemorrhagic *Escherichia coli* (EHEC) (53.1%) were included in routine stool MCS (Table 4). Many respondents also thought that *Giardia lamblia* (50.0%), rotavirus (37.5%), adenovirus (37.5%) and norovirus (15.6%) were tested for in routine stool MCS.

Table 4 Pathogens doctors believed to be included in routine stool MCS investigations

	Total (n = 32)	Urban site (n = 10)	Rural sites (n = 22)	p-value
Bacteria				
Salmonella spp.	27 (84.4%)	10 (100.0%)	17 (77.3%)	0.155
Shigella spp.	27 (84.4%)	10 (100.0%)	17 (77.3%)	0.155
EHEC	17 (53.1%)	4 (40.0%)	13 (59.1%)	0.316
Campylobacter spp.	13 (40.6%)	4 (40.0%)	9 (40.9%)	> 0.999
All Shiga toxin-producing *E. coli* (STEC)	13 (40.6%)	5 (50.0%)	8 (36.4%)	0.699
C. difficile	11 (34.4%)	4 (40.0%)	7 (31.8%)	0.703
All diarrhoeagenic *E. coli*	9 (28.1%)	4 (40.0%)	5 (22.7%)	0.407
Vibrio spp.	4 (12.5%)	0 (0.0%)	4 (18.2%)	0.283
Yersinia enterocolitica	3 (9.4%)	1 (10.0%)	2 (9.1%)	> 0.999
Parasites				
Giardia lamblia	16 (50.0%)	4 (40.0%)	12 (54.6%)	0.704
Cryptosporidium spp.	10 (31.3%)	3 (30.0%)	7 (31.8%)	< 0.999
Cystoisospora spp.	9 (28.1%)	3 (30.0%)	6 (27.3%)	< 0.999
Entamoeba histolytica	9 (28.1%)	2 (20.0%)	7 (31.8%)	0.681
Microsporidia spp.	5 (15.6%)	2 (20.0%)	3 (13.6%)	0.637
Cyclospora spp.	2 (6.3%)	2 (20.0%)	0 (0.0%)	0.091
Aeromonas spp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	–
Viruses				
Rotavirus	12 (37.5%)	2 (20.0%)	10 (45.5%)	0.248
Adenovirus	12 (37.5%)	2 (20.0%)	10 (45.5%)	0.248
Norovirus	5 (15.6%)	1 (10.0%)	4 (18.2%)	> 0.999

'MCS' refers to microscopy for ova and parasites, culture, and antimicrobial susceptibility testing where applicable

Table 5 Pathogens tested for in stool samples submitted to diagnostic laboratories

	Total (n = 1 311)	Urban site (n = 1 222)	Rural sites (n = 89)	p-value
Salmonella spp.	1309 (99.9%)	1220 (99.8%)	89 (100.0%)	0.702
Shigella spp.	1308 (99.8%)	1219 (99.8%)	89 (100.0%)	0.640
Parasites	1210 (92.3%)	1133 (92.7%)	77 (86.5%)	**0.034**
Campylobacter spp.	873 (66.6%)	872 (71.4%)	1 (1.1%)	**< 0.001**
EHEC	677 (51.6%)	676 (55.3%)	1 (1.1%)	**< 0.001**
C. difficile	375 (28.6%)	374 (30.6%)	1 (1.1%)	**< 0.001**
Rotavirus	220 (16.8%)	220 (18.0%)	0 (0.0%)	**< 0.001**
Adenovirus	206 (15.7%)	206 (16.9%)	0 (0.0%)	**< 0.001**
Vibrio cholerae	93 (7.1%)	7 (0.6%)	86 (96.6%)	**< 0.001**
Vibrio spp.	90 (6.9%)	6 (0.5%)	84 (94.4%)	**< 0.001**
EPEC	64 (4.9%)	64 (5.2%)	0 (0.0%)	**0.027**
Astrovirus	40 (3.1%)	40 (3.3%)	0 (0.0%)	0.083
Norovirus	0 (0.00%)	0 (0.0%)	0 (0.0%)	–

P-values < 0.05 indicated in bold

CDW data indicate that most stool samples at both rural and urban sites were tested for *Salmonella* spp. (99.9%) and *Shigella* spp. (99.8%) (Table 5). Microscopy for ova and parasites was frequently included at rural and urban sites (86.5% and 92.7% respectively). Routine testing at the urban site frequently included culture for *Campylobacter* spp. (71.4%) and EHEC (55.3%) but culture for these pathogens was rarely performed at the rural sites (1.1% and 1.1% respectively). Testing for viruses was infrequent as specific requests are required for testing (rotavirus tested for in 16.8%, adenovirus in 15.7% and astrovirus in 3.1% of samples) and was exclusive to the urban site. Culture for *Vibrio cholerae* was done for the majority of samples at the rural sites (96.6%), but not for any samples from the urban site. There was a median of 4.6 days (IQR: 3.5–6.2) between sample collection and reporting of results for routine stool MCS.

Comparison of routine diagnostic data and surveillance study data

Over the study period, 591 stool samples were tested through the surveillance study and 1311 through routine diagnostic services (Table 6). The surveillance study included only patients admitted during regular working hours (Monday to Friday) who consented to study procedures, used a strict case definition of diarrhoea, and required samples to be collected within 48 h of admission to exclude possible healthcare-associated infections. Routine diagnostic services included patients seen in outpatient clinics and in casualty, accounting for the higher number of samples, specifically for the urban site, which has large outpatient clinics. Patients with stool samples submitted through routine diagnostic services were significantly older than those enrolled through the surveillance study (median of 34.0 years versus 1.0 year, $p < 0.001$), indicating doctors are less likely to request stool testing for admitted children. Proportionally more males were enrolled in the surveillance study compared to females (44.7% versus 50.8%, $p = 0.016$).

Pathogen yield was significantly higher in the surveillance study than through routine diagnostic services (73.3% versus 13.7%, $p < 0.001$). This was true for viruses (48.9% versus 2.6%, $p < 0.001$), bacteria (40.1% versus 8.0%, $p < 0.001$) and parasites (16.2% versus 3.6%, $p < 0.001$). Of specific concern was the difference in *Shigella* spp. detection rates (22.7% in the surveillance study and only 0.8% from CDW data). Pathogens most commonly detected through routine diagnostic services included rotavirus (13.2%), adenovirus (4.9%) and *Cryptosporidium* (3.0%), while the surveillance study most commonly detected adenovirus (25.6%), *Shigella* spp. (22.7%) and rotavirus (15.6%). Detection rates for rotavirus (15.6% versus 13.2%, $p = 0.394$) and astrovirus (5.9% versus 5.0%, $p = 0.814$) were similar between surveillance and CDW data. Antibiotics were prescribed for 386 (65.3%) of patients enrolled in the surveillance study, however, bacterial pathogens were only detected in 153 (39.6%) of these cases. The high yield of *C. difficile* through routine diagnostic testing (20.3% versus 5.8%, $p < 0.001$) was expected since only cases with clinically suspected *C. difficile* infection would be tested, whereas the surveillance study screened all samples for various enteric pathogens, regardless of suspected diagnosis.

Table 6 Comparison of samples submitted for routine diagnostic testing and samples tested through the surveillance study

	Routine diagnostic data (n = 1311)	Surveillance study (n = 591)	p-value
Samples per site			
Kalafong	1222 (93.2%)	311 (52.6%)	< 0.001
Mapulaneng	68 (5.2%)	103 (17.4%)	
Matikwana	21 (1.6%)	177 (29.9%)	
Age groups (years)[a]			
< 1	161 (13.0%)	211 (35.7%)	**< 0.001**
1–4	142 (11.5%)	183 (31.0%)	
5–17	69 (5.6%)	24 (4.1%)	
18–44	540 (43.6%)	98 (16.6%)	
45+	328 (26.5%)	75 (12.7%)	
Gender			
Male	581 (44.7%)	298 (50.8%)	**0.016**
Female	719 (55.3%)	289 (49.2%)	
Samples with pathogen detected	179/1311 (13.7%)	433 (73.3%)	**< 0.001**
Virus detected	34/1311 (2.6%)	289 (48.9%)	**< 0.001**
Adenovirus[b]	10/206 (4.9%)	151 (25.6%)	**< 0.001**
Rotavirus[b]	29/220(13.2%)	92 (15.6%)	0.394
Norovirus	NT	84 (14.2%)	–
Astrovirus[b]	2/40 (5.0%)	35 (5.9%)	0.814
Sapovirus	NT	21 (3.6%)	–
Bacteria detected	105/1311 (8.0%)	237 (40.1%)	**< 0.001**
Shigella spp.[b]	10/1308 (0.8%)	134 (22.7%)	**< 0.001**
Campylobacter[b]	2/873 (0.2%)	70 (11.8%)	**< 0.001**
Salmonella spp.[b]	17/1309 (1.3%)	21 (3.6%)	**0.001**
EHEC[b,c]	0/677 (0.0%)	9 (1.5%)	**0.001**
C. difficile	76/375 (20.3%)	34 (5.8%)	**< 0.001**
EPEC[b]	0/64 (0.0%)	NT	–
Vibrio spp.[b]	0/90 (0.0%)	NT	–
Vibrio cholerae[b]	0/93 (0.0%)	NT	–
Parasites detected	47/1311 (3.6%)	96 (16.2%)	**< 0.001**
Cryptosporidium spp.[b]	36/1210 (3.0%)	78 (13.2%)	**< 0.001**
Giardia lamblia[b]	0/1210 (0.0%)	22 (3.7%)	**< 0.001**
Cystoisosopora belli[b]	8/1210 (0.7%)	NT	–
Other[b,d]	3/1210 (0.2%)		–

NT not tested, *EHEC* enterohemorrhagic *Escherichia coli*, *EPEC* enteropathogenic *Escherichia coli*

P-values < 0.05 indicated in bold

[a] 71 CDW cases missing ages

[b] Denominators for CDW data refer to number tested for specific pathogen rather than total samples submitted

[c] Molecular toxin testing done for EHEC in the surveillance study and by culture for CDW data

[d] Other parasites include: *Endolimax nana*, *Schistosoma mansoni*, *Taenia* spp.

Discussion

The doctors' survey indicates that diarrhoeal cases in rural settings are likely to be underrepresented by routine diagnostic data as rural doctors reported attending fewer diarrhoeal cases and submitted stool samples for a smaller proportion of these cases as compared to their urban counterparts. Possible reasons for this include poorer health-seeking behaviour in rural communities [10], a decentralised public sector healthcare system which encourages patients to utilise local health clinics instead of seeking care directly at hospitals, and less budget for laboratory tests. Stool diagnostic practices at the surveyed sites were based on clinical judgement rather than national or facility guidelines. Doctors were most likely to order stool samples for patients with chronic diarrhoea, dysentery, suspected *C. difficile*

infection, a travel history or link to a known or suspected outbreak, all patient-related predictors similar to those reported in studies from high-income countries [1, 2, 7]. Doctors reported ordering stool testing more often for adults than for children even though more children are admitted for diarrhoea, which may introduce an age-related bias to routine diagnostic data. Doctors possibly suspect viral infections in children and, the availability of data on diarrhoea in children allows for empiric treatment.

As per studies in high-income settings [11], the majority of respondents reported that stool sample microbiology was useful in managing patients with diarrhoea and would order stool samples more often in the absence of current barriers. The major barrier to ordering samples was laboratory turnaround times being too long to guide clinical decision-making. Routine diagnostic data indicated an average of 4.6 days (IQR: 3.5–6.2) between sample collection and results, by which time most patients have been discharged. Other important barriers included patients being unwilling to provide samples, and reluctance of nursing staff to assist with sample collection. Other studies have identified laboratory turnaround time as a common barrier to ordering stool samples [7], but none have found reluctance of patients to provide samples or reluctance of healthcare workers to assist in sample collection to be important barriers. The resistance to stool sample collection in our setting could be influenced by many factors. Lack of basic knowledge or guidance on collection procedures and lack of equipment for sample collection may hinder compliance and patients seen in outpatient or casualty may be unable to provide stool samples during the consultation. Rectal swabs or wipes should be investigated to address compliance. Swabs are more convenient for both patients and clinicians and demonstrate decreased time to results [12]. Wipes are efficient in the detection of both bacterial and viral pathogens [13, 14], and collection without requiring nursing assistance, may address compliance issues.

Most doctors (84%) knew that *Salmonella* spp. and *Shigella* spp. were tested for in routine stool MCS. *Campylobacter* spp. and EHEC were tested for in 71.4% and 55.3% of samples respectively at the urban site but were seldom tested for at rural sites (1.1% of samples tested for each). Respondents at the urban site were all aware that *Vibrio cholerae* was not tested for in a routine stool MCS. In contrast, most (86.6%) samples submitted for routine stool MCS at the rural sites were tested for *V. cholerae*, but only 18% of respondents were aware of this. Routine testing for *V. cholerae* is common practice in several areas of South Africa which previously experienced cholera outbreaks and maintain a higher awareness for cholera, as is the case with both rural hospitals. Doctors were not always sure when testing for viruses was done. Many respondents (37.5%) believed that rotavirus and adenovirus were included in stool MCS, but $\leq$18% of samples at the urban site and no samples at the rural sites were tested for these viruses. Some respondents (15.6%) believed norovirus was included in routine testing but CDW data indicated testing was not done at any of the three sites. Overall, no samples from the rural sites and relatively few samples from the urban site were tested for viral pathogens, since clinicians should specifically request this testing. The majority of diagnostic laboratories in the public sector do not perform tests for enteric viral pathogens, and in cases where testing has been requested by the clinician, the samples are referred to an academic or reference laboratory able to perform the test. However, such tests are expensive, and transport to the reference laboratory can take several days, resulting in turnaround time being too long to assist with clinical decision-making.

Pathogen yield for stool samples tested at diagnostic laboratories (routine MCS as well as any additional microbiology or virology tests) was 13.7%, significantly lower than for samples collected in the enhanced surveillance study and tested with real-time PCR (73.3%). Likely reasons for the low yield of pathogens in routine diagnostic laboratory data include pre-analytic factors (timing of sample collection, sample quality, sample storage conditions, transport to laboratory, time from receipt to processing at the laboratory) and analytic factors (quality of testing, pathogen(s) tested for, sensitivity of test(s) performed). Routine diagnostic testing relies primarily on microscopy to detect parasites, and on culture to detect a variable range of bacterial pathogens. Microscopy for ova and parasites is labour intensive and highly dependent on the operator's skill and expertise [15]. Culture methods are known to deliver poor yields, which typically decrease even further in settings of high antibiotic use [15]. The advantages of multiplex PCR tests are well described; several studies have shown high sensitivity and specificity when used in symptomatic patients, and have shorter turnaround times than conventional culture-based tests [15]. Two landmark studies investigating the burden and aetiology of childhood diarrhoea (the Global Enteric Multicenter Study (GEMS) [16] and the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Children Health and Development (MAL-ED) [17] study) tested stool samples using conventional microbiologic methods (culture, enzyme immunoassay antigen (EIA) testing for rotavirus, adenovirus and protozoa) and compared results to multiplex real-time PCR testing. In the GEMS study, PCR increased the attributable incidence twofold for *Shigella*

spp. [18]. In the MAL-ED study, PCR showed the *Shigella* burden to be more than five times higher than estimated by culture [17]. The magnitude of the difference in *Shigella* spp. yield between surveillance samples (22.7%) and routine diagnostic samples (0.8%) in our study was particularly noteworthy. *Shigella*, *Campylobacter* and *Salmonella* are included in the World Health Organisation (WHO) priority pathogens list, a list of 12 bacteria identified to pose the greatest threat to human health due to rise in antibiotic resistance [19]. The underreporting and underdiagnoses of these pathogens is therefore of public health concern.

Adenovirus, rotavirus and norovirus were detected in 25.6%, 15.6% and 14.2% of surveillance study samples respectively. These high yields are likely due in part to the significantly younger study population (mean age of 1 year) as compared to patients with samples submitted through routine diagnostic services (mean 24 years). Interviewed doctors indicated that only 34.4% would consider submitting samples for paediatric patients, likely because these are assumed to be viral infections. However, when patients under the age of 5 years were excluded from the surveillance study data, the detection rates for adenovirus (19.2%), rotavirus (12.4%) and norovirus (11.8%) remained high, indicating that these viruses are also important causes of diarrhoea in older children and adults. Less than 20% of routine diagnostic samples were submitted for adenovirus and rotavirus testing, and none for norovirus. Routine testing for viruses may be considered to be of limited benefit, as detection has little significance for clinical management, however it does have implications for antibiotic use with surveillance study data indicating that the majority of patients receiving antibiotics (60.4%) were negative for bacterial pathogens. There may also be a lack of awareness of the importance of these viral pathogens in our setting.

This study had several limitations. Due to the small sample size of the doctors' survey, the results may not be generalizable. A more representative sample of hospitals should be drawn from a wider geographical area. This study did not investigate multifactorial associations with stool submission (a combination of patient-related factors may influence diagnostic investigation), which should be considered for future studies. Matching routine diagnostic and enhanced surveillance data sources at the patient-level was not possible as routine diagnostic data was de-identified. Despite these limitations, these data were sufficient to give an indication of trends and highlight shortfalls in routine diagnostic testing.

Conclusion

This study showed that diarrhoeal diagnostic testing is erratic, there are inconsistencies in terms of how decisions are guided and doctors are not well informed regarding pathogens included in routine testing. Current CDW data most likely represents chronic cases, cases with dysentery and those at urban sites. Inconsistencies are recognised by doctors, who requested further training and guidance. Robust, local epidemiological data should be available to inform treatment, reserving diagnostic resources for specific cases most likely to benefit from directed rather than empiric treatment. One respondent stated that 'if we are to practice evidence based medicine we have very little to argue for the routine diagnostic testing of stools in patients with diarrhoea. It very rarely changes clinical practice or patient outcome as it is presently practiced in our setting. Our practice has to be financially viable (feasible, sustainable), clinically meaningful, applicable and most likely in the future will be applied to a select few patients which could reap benefit from these investigations. Our studies should focus on which patients would benefit, how to identify these patients, as well as the specific therapies from which they gain improvement.' This statement summarises the need for guidelines that are well informed by local epidemiological data thereby removing the need for testing in the majority of cases. Low pathogen yields for CDW data indicates the need for optimization of laboratory methods, including improving turnaround times and yields, for those select cases requiring diagnostics. We recommend further research into the use of molecular and antigen-based diagnostics using rectal wipes or swabs instead of whole stool samples. The increased cost of more efficient diarrhoeal diagnostics may be offset by shortened hospitalisations due to quicker resolution of illness, and savings related to decreased requirements for antibiotics. In our setting, *Shigella* spp., *Campylobacter* spp. and *Salmonella* spp. specifically are routinely underdiagnosed and require further investigation in terms of sampling, transport to the laboratory and diagnostic methods used.

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Author contributions
SLJ, NAP, JT and MJG conceptualised and designed the study. SLJ, NAP, ND and JT completed and managed fieldwork and data collection. SLJ, NAP, MJG, ND and JT acquired, analysed and interpreted the data. SLJ drafted the work. JT substantively revised the work. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analysed during the current study are not publicly available as they include study data which has not yet been published are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Approval Number: M190663). Informed consent was obtained from all included survey participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Centre for Enteric Diseases, National Institute for Communicable Diseases, a division of the National Health Laboratory Service (NHLS), Johannesburg, South Africa. [2]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [3]Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Arcadia 0007, South Africa. [4]Division of Public Health Surveillance and Response, National Institute for Communicable Diseases, a division of the National Health Laboratory Service (NHLS), Johannesburg, South Africa. [5]School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [6]Department of Paediatrics, Kalafong Provincial Tertiary Hospital, Faculty of Health Sciences, University of Pretoria, Pretoria 0001, South Africa.

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Publisher's Note

PAPER III

Epidemiology and aetiology of moderate to severe diarrhoea in HIV-infected patients in South Africa, 2018-2021: a case-control analysis

Introduction

Diarrhoeal diseases pose a significant health burden across the globe, causing over 1.6 million deaths annually amongst all ages[1]. The majority of these deaths occur in low- and middle-income countries (LMICs), specifically in south Asia and Sub-Saharan Africa (SSA)[1]. Despite a decrease in global diarrhoeal mortality rates over the past two decades, decreases have not been uniform across age groups or settings and morbidity rates remain high[2]. Unsafe water, poor sanitation and hygiene (WaSH)[1], as well as malnourishment and compromised immunity[3] are the main risk factors associated with diarrhoeal morbidity and mortality. Other risk factors identified in LMICs include residence in a slum, use of communal toilets and exposure to animals[4]. Children under the age of 5 years are at the highest risk, however there is a significant burden in older age groups, specifically in LMICs[1,5]. Despite global improvements in WaSH[6], much of the remaining burden of diarrhoeal disease may be attributable to foodborne pathogens[7]. Food safety is of specific concern in LMICs where there is increased consumption of unsafe foods, use of effluent in agriculture and changes in food distribution networks with bulk production and increased distance between production and consumption as well as large, often poorly regulated, informal sectors[7].

Studies from high-income countries (HICs) have shown the majority of diarrhoeal diseases in adults seeking healthcare to be due to *Campylobacter* spp., *Salmonella* spp., norovirus and rotavirus[8,9]. Aetiology differs in SSA, with a meta-analysis amongst all ages identifying the main causative pathogens as *Escherichia coli*, *Cryptosporidium*, *Cyclospora*, *Entamoeba* and *Shigella* spp.[10] There are several reasons for these differences in aetiology including different exposures and risk factors associated with poorer living conditions and poor underlying health. One important consideration is the high HIV prevalence in SSA[11]. HIV is a known risk factor for diarrhoea, with episodes in HIV-infected individuals more likely to be severe, prolonged and result in hospitalisation with higher mortality rates than in HIV-uninfected individuals[12,13]. The aetiology of diarrhoea in HIV-infected individuals is known to shift with HIV disease progression and treatment[14]. During early disease stages, while CD4 counts are high and viral loads low, diarrhoea is often related to HIV seroconversion. As disease progresses, diarrhoea is frequently caused by opportunistic pathogens related to decreased immune function[14]. These pathogens include bacterial infections, such as *Mycobacteria*, parasitic infections, such as *Cystoisospora belli*, *Cyclospora*, S*trongyloides*, *Cryptosporidia*, *Microsporidia* and viral infections, such as CMV[14–16]. Patients with low CD4 cell counts are at increased risk for chronic diarrhoea[4] and polyparasitic infection[17]. The HIV infection

itself may be responsible for a proportion of the pathogen-negative cases, through HIV enteropathy and permanent damage to the gastrointestinal mucosa[18], as well as changes in gut microbial populations leading to dysbiosis[19]. Mucosa damage reduces small bowel villous surface area, causing diarrhoea through malabsorption[20] as well as defects to cellular and humoral defence mechanisms in the gastrointestinal tract[21]. A Dutch study found that 34% of diarrhoeal cases in untreated HIV-infected individuals could not be explained by a known causative agent, and hypothesized that these unexplained cases were due to the HIV-infection itself[22]. Initiation of antiretroviral treatment (ART), and accompanying increase in CD4 cell count, is associated with a significant shift in the aetiology of diarrhoea from opportunistic infectious causes to non-infectious causes[23]. Data from both HICs and SSA show that although patients initiated on ART have significantly reduced diarrhoeal incidence compared to those not on treatment, the overall diarrhoeal incidence remains high[18,24]. This remaining incidence in patients with well-controlled HIV may be drug-related[18].

There are limited data on HIV-related diarrhoeal diseases in SSA, with the majority of published studies focusing on children under the age of five, or limited by diagnostic technology[14]. A review of laboratory records in Botswana indicated that only 14% of stool specimens submitted for routine diagnostic testing for all ages had a pathogen detected, of which 8% were bacteria and 6% parasites[25]. *Shigella* spp. and *Salmonella* spp. were the most commonly detected bacteria while *Cystoisospora* spp. and *Cryptosporidium* spp. were the most commonly detected parasites. Although HIV status was not available for the participants in this analysis, Botswana is a high HIV prevalence setting with an estimated 23.9% of the population between 15-49 years old being HIV-infected[25]. Contrary to these findings, a longitudinal cohort study of Zambian adults with a 31% HIV sero-prevalence found that pathogens could be detected in 99% of stool samples collected (diarrhoeal and non-diarrhoeal patients combined), with *Cryptosporidium* spp., *Cystoisospora* spp. and *Citrobacter* spp. being significantly more common in HIV-infected individuals[5]. They found that HIV-infected adults in the pre-HAART era were 2.4 times as likely to suffer from diarrhoea than HIV-uninfected adults and that this increased risk lasted throughout the infection rather than being limited to those with low CD4 cell counts[5]. Meta-analysis data indicates a high burden of *Cryptosporidium*, microsporidia and *Cystoisospora* in SSA specifically[16]. Another recent meta-analysis, identified South Africa as having the highest global *Cryptosporidium* prevalence (57.0%, CI 95%: 24.4-84.5%), although they recognised that these estimates were based on limited data[26]. A study in rural South Africa found that 60% of patients suffering from chronic diarrhoea were HIV-infected and that the majority of these infections were due to *Campylobacter* spp. (20%), *Plesiomonas shigelloides* (17%), *Aeromonas* spp. (13%), *Shigella* spp. (10%), *Salmonella* spp. (10%) and *E. coli* spp. (10%)[27]. Testing did not include parasites or viruses and results were not stratified by

ART status. Other studies in African children have identified HIV-infection as a significant risk factor for rotavirus infection[13,28]. Very few studies in SSA in adults include screening for viruses, despite data from HICs showing that viruses (specifically norovirus and rotavirus) are responsible for as much as 44% of diarrhoea in adults presenting to emergency departments[9].

South Africa currently has the highest HIV burden in the world, including 19% of the global HIV-infected population[29]. It is estimated that 86% of HIV-infected adults in South Africa have been diagnosed, with 57% of these on ART (50.8% - 72.7% by province)[30]. Women, elderly patients, those with inadequate access to WaSH facilities and those not yet initiated on ART, have the highest risk for developing diarrhoea amongst HIV-infected South Africans[31]. The majority of data on HIV-related diarrhoea comes from low prevalence, HICs[14]. Since diarrhoeal aetiology is likely to vary between settings[32], there is a need for more targeted epidemiological studies[18], specifically in a population with high ART coverage. There are few studies in HIV populations which include a comprehensive range of pathogens or investigations of infections with multiple pathogens[19]. These data are important for informing public health interventions and updating diagnostic and treatment guidelines, especially since treatment is often based on guidelines rather than individual patient diagnostics[33]. This study aimed to determine the pathogenic causes for diarrhoeal admissions in HIV-infected patients compared to hospital controls at three South African hospitals between July 2018 and November 2021.

Methods

Case and control enrolment

Admitted diarrhoeal cases of all ages were enrolled at Kalafong, Mapulaneng and Matikwana hospitals through the African Network for improved Diagnostics, Epidemiology and Management of common Infectious Agents (ANDEMIA)[34]. Kalafong Hospital, is a public, provincial, tertiary hospital based on the western outskirts of Pretoria (Gauteng), serving the generally low income communities residing in urban townships to the west of Pretoria[35]. The rural sites were Mapulaneng, a 180 bed district hospital, and Matikwana, a 250 bed regional hospital[36], both based in Bushbuckridge district in Mpumalanga Province.

Cases were defined as patients admitted with diarrhoea (defined as the passage of three or more loose or liquid stools over a 24 hour period) for any duration. Surveillance Officers based at the hospitals performed daily ward rounds (Monday to Friday) to check admission logs for patients admitted with diarrhoea. Controls were defined as individuals presenting to the hospital or clinic for

reasons other than diarrhoea (vaccination clinic, orthopaedic or surgical ward) and had no gastrointestinal symptoms (vomiting or diarrhoea) in the past 3 weeks. The study was explained to patients matching the study definition and an information leaflet provided. Written informed consent was signed by patients ≥18 years of age and by the parents/guardians for patients <18 years. An assent form was signed for patients between 7-17 years. Surveillance Officers completed a detailed investigation form for both cases and controls by interviewing the patient or guardian and reviewing clinical notes and laboratory results where available. A stool specimen was collected for both cases and controls. Where a stool specimen could not be collected, a rectal swab (with visible stool material) was collected. Patients were enrolled within 48 hours of admission to exclude nosocomial infections. A one month follow up was performed telephonically to determine the patient's recovery status. Cases were enrolled from July 2018 to June 2021 and controls enrolled from October 2019 to November 2021. Cases and controls unable to provide a stool specimen or rectal swab were excluded from this analysis.

Laboratory testing

Stool specimens were transported to the National Institute for Communicable Diseases (NICD) Centre for Enteric Diseases (CED) where nucleic acid was extracted and pathogen screening performed on Fast-track Diagnostics (viral and bacterial gastroenteritis and stool parasite kits), Seegene Allplex (GI-Parasite and GI-Helminth(I) assays) as well as TaqMan Array Cards (TAC). Where there were discrepant results between testing platforms for a specific target, a singleplex PCR was run to determine final outcome.

Data management and statistical analysis

Results for CD4+ cell count were extracted from the NHLS routine diagnostic laboratory database (TrakCare) for HIV-infected cases. Only CD4+ count results within 12 months of the enrolment were included and where multiple results were available for a single patient, the results closest to the time of enrolment were used. Differences in risk factors, symptoms and pathogens between cases and controls were investigated using X^2-test and Fisher's exact for categorical variables and t-test for continuous variables. Stata software (version 14) was used for all analyses.

Ethical considerations

The ANDEMIA study and this specific sub-analysis was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (approval numbers M170403 and M190663 respectively).

Results

Enrolment and patient characteristics

A total of 989 diarrhoeal cases of all ages were enrolled. Cases without stool specimens (n=82) and unknown HIV status (n=82) were excluded from this analysis resulting in a total of 825 cases, 190 of which were HIV-infected. The age distributions of the HIV-infected versus HIV-uninfected cases differed, with the majority of HIV-uninfected cases (87.0%) being children under 5 years of age, while the majority (86.0%) of HIV-infected cases were over the age of 5 years. In order to exclude confounding by age, only cases over the age of 5 years were included in the current analysis, resulting in 164 HIV-infected case and 79 HIV-uninfected cases (Figure 1). A total of 101 controls over the age of 5 years were enrolled of which 23 (22.8%) were HIV-infected, 50 (49.5%) were HIV-uninfected and 28 (27.7%) had unknown HIV status.

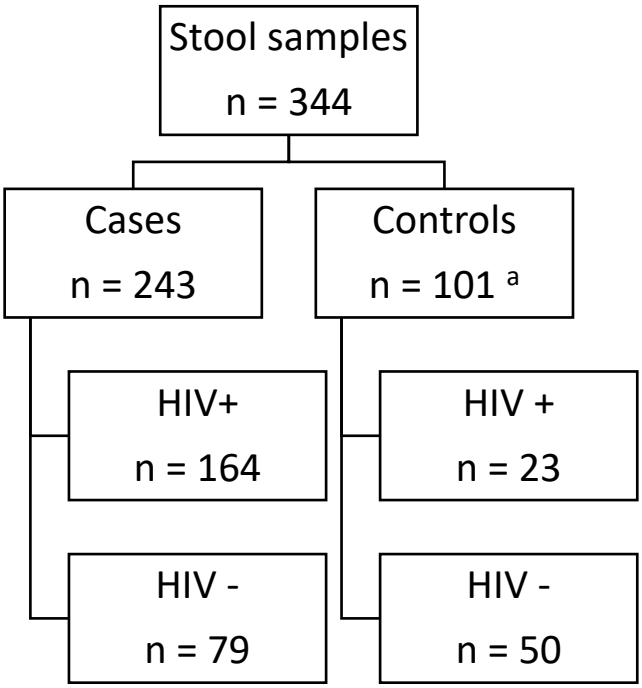

Figure 1: Stool samples analysed

[a]28 controls have unknown HIV status

The median age of included patients was 34 years (IQR of 23-47), with cases being slightly older (median of 36 and IQR of 26-51) than controls (median of 31 and IQR of 17-38). The majority of patients were female (59.4%) and from the rural sites (67.4%), with proportionally more controls coming from the rural site than cases (82.2% versus 61.3%, $p<0.001$). Cases and controls were similar with respect to household crowding and eating habits. Both cases and controls had good access to electric or gas cookers (86.1%), refrigerators (88.4%), improved WaSH (94.8% access to improved water source and 96.4% access to private latrines/flush toilets). Exposure to animals was higher

amongst controls than cases (26.5% versus 11.1%, *p*<0.001). CD4 cell count data (within 12 months of enrolment) was available for 99 of the 164 HIV-infected cases. Eight (8.1%) had counts >500 cells/µl, 21 (21.2%) were between 200-500 cells/µl and the majority (n=70, 70.7%) were <200 cells/µl. ART treatment details were available for 156 of the HIV-infected cases, of which 130 (83.3%) were on treatment. Mean CD4 cells counts were similar between those on ART (229.23 cells/µl) and those not on ART (169.7 cells/µl), (p = 0.418). Cotrimoxizole treatment was known for 151 of the HIV-infected patients, of which 63 (41.7%) were on treatment.

Table 1: Characteristics of samples included in the analysis (comparison of cases and controls)

	Total (n = 344)	**Cases (n = 243)**	**Controls (n = 101)**	***p*-value**
Age years – median (IQR)	34 (23-47)	36 (26-51)	31 (17-38)	<0.001
Male	129 (40.6%)	88 (36.2%)	41 (40.6%)	0.445
Rural	232 (67.4%)	149 (61.3%)	83 (82.2%)	<0.001
HIV-infected	187 (59.2%)	164 (67.5%)	23 (31.5%)	<0.001
Education [a]				
None	13 (3.9%)	10 (4.2%)	3 (3.0%)	0.002
<= 6 years	53 (15.7%)	47 (19.8%)	6 (6.0%)	
7-10 years	107 (31.8%)	78 (32.9%)	29 (29.0%)	
>10 years	164 (48.7%)	102 (43.0%)	62 (62.0%)	
Household crowding/pp per room – mean (std. dev)	2.0 (1.2)	2.0 (1.9)	2.0 (0.9)	0.733
Electric or gas cooker	296 (86.1%)	210 (86.4%)	86 (85.1%)	0.757
Refrigerator	289 (88.4%)	200 (86.6%)	89 (92.7%)	0.115
Improved water source [b]	326 (94.8%)	229 (94.2%)	97 (96.0%)	0.495
Treat drinking water [c]	45 (13.1%)	31 (12.8%)	14 (13.9%)	0.782
Toilet type				
Private flush	86 (25.5%)	62 (25.7%)	24 (25.0%)	0.746
Private latrine	239 (70.9%)	170 (70.5%)	69 (71.9%)	
Communal flush	8 (2.3%)	7 (2.9%)	1 (1.0%)	
Communal latrine	2 (0.6%)	1 (0.4%)	1 (1.0%)	
Other	2 (0.6%)	1 (0.4%)	1 (1.0%)	
Exposure to animals [d]	53 (15.6%)	27 (11.1%)	26 (26.5%)	<0.001
Dairy [d]	156 (45.4%)	107 (44.0%)	49 (48.5%)	0.447
Deli meat [d]	103 (29.9%)	79 (32.5%)	24 (23.8%)	0.107
Eggs [d]	183 (53.2%)	127 (52.3%)	56 (55.5%)	0.590

[a] highest level of education that the patient (or parent/caregiver if patient is <15 years) has obtained; [b] piped water to an inside or outside tap (as opposed to river, rain or well water or water from a truck); [c] by boiling or chemical treatment; [d] In the preceding 4 weeks

Symptoms amongst cases

HIV-infected cases were likely to experience symptoms for a longer duration before admission (median of 5 days versus 2 days, *p*<0.001) and were more likely to have chronic or persistent diarrhoea (8.5% versus 0.0%, *p*=0.006) than HIV-uninfected cases. The most common symptoms experienced by all cases were fatigue (182, 74.9%), weight loss (176, 72.4%), vomiting (172, 70.8%), fever (168, 69.1%), abdominal pain (166, 68.3%) and nausea (165, 67.9%). HIV-infected cases were more likely to suffer from weight loss (81.1% versus 54.4%, p<0.001) and nausea (72.0% versus 59.5%, *p*=0.051), while HIV-uninfected cases were more likely to suffer from dysentery (15.2% versus 4.3%, *p*=0.003). One month follow up data were available for 168 (69.1%) of included cases. Of these, 27 (16.1%) were deceased, 130 (77.4%) had recovered or were recovering and 11 (6.5%) were still ill or had deteriorated. Outcome at follow up was marginally worse for HIV-infected cases (19.1% had died compared to 8.6% of HIV-uninfected, *p*=0.074) although cause of death data was not available and hence deaths cannot be attributed to the diarrhoeal admission.

Table 2: Symptoms experienced by included cases

	Total (n=243)	**HIV-infected (n=164)**	**HIV-uninfected (n=79)**	***p*-value**
Duration of symptoms before admission – median (IQR)	4 (2-8)	5 (3-8)	2 (1-4)	**<0.001**
Chronic/persistent [a]	14 (5.8%)	14 (8.5%)	0 (0.0%)	**0.006**
Fatigue	182 (74.9%)	124 (75.6%)	58 (73.4%)	0.712
Weight loss	176 (72.4%)	133 (81.1%)	43 (54.4%)	**<0.001**
Vomiting	172 (70.8%)	118 (72.0%)	54 (68.4%)	0.564
Fever (current or history of fever in the past 10 days)	168 (69.1%)	113 (68.9%)	55 (69.6%)	0.910
Abdominal pain	166 (68.3%)	106 (64.6%)	60 (76.0%)	0.076
Nausea	165 (67.9%)	118 (72.0%)	47 (59.5%)	**0.051**
Respiratory symptoms	105 (43.2%)	77 (47.0%)	28 (35.4%)	0.090
Headache	100 (41.2%)	68 (41.5%)	32 (40.5%)	0.887
Chills	63 (25.9%)	48 (29.3%)	15 (19.0%)	0.087
Arthralgia	51 (21.0%)	38 (23.2%)	13 (16.5%)	0.229
Dermatological symptoms	31 (12.8%)	25 (15.2%)	6 (7.6%)	0.094
Myalgia	24 (10.0%)	16 (9.8%)	8 (10.1%)	0.928
Dysentery [b]	19 (7.8%)	7 (4.3%)	12 (15.2%)	**0.003**
Neurological symptoms	10 (4.1%)	6 (3.7%)	4 (5.1%)	0.732
Painful swollen glands	4 (1.7%)	3 (1.9%)	1 (1.3%)	>0.99
Outcome: Died [c]	26 (15.5%)	21 (19.1%)	5 (8.6%)	0.074

Outcome: Recovered completely from illness [c]	101 (72.1%)	59 (67.8%)	42 (79.3%)	0.143

[a] diarrhoea for 28 days or longer; [b] Dysentery defined as self-reported blood in the stool; [c] Outcome at one month follow-up phone call available for 168 patients and details of recovery available for 140 patients

Pathogen detection

Pathogens could be detected in 66.3% (228) of stool specimens tested, with significantly higher detection in cases compared to controls (72.8% versus 50.5%, p<0.001). Bacteria were more prevalent in cases compared to controls (50.2% versus 28.7%, p<0.001), specifically *Shigella* spp., *Salmonella* spp. and *Helicobacter pylori* (24.3% versus 10.9%, p=0.005; 8.6% versus 1.0%, p=0.007 and 8.6% versus 0.0%, p=0.001 respectively). Detection of viruses was significantly higher in cases compared to controls (21.8% versus 10.9%, p=0.018), specifically for adenovirus (11.0% versus 4.0%, p=0.038). Parasites were detected in 37.8% (130) of stool specimens tested, however detection was marginally significantly higher in cases than controls (40.7% versus 30.7%, p=0.080). Prevalence of *Cystoisospora*, *Cryptosporidium* spp. and *Enterocytozoon* spp. was significantly higher in cases than controls (11.9% versus 0.0%, p<0.00; 11.1% versus 3.0%, p=0.012; 4.5% versus 0.0%, p=0.038 respectively). *Blastocystis* and *Schistosoma* were more prevalent in controls than in cases (23.8% versus 10.3%, p=0.001; 6.9% versus 2.1%, p=0.025 respectively). Amongst HIV-infected participants, *Cystoisospora* spp. prevalence was significantly higher in cases than in controls (17.7% versus 0.0%, p=0.028), while *Schistosoma* was detected more often in controls compared to cases (17.4% versus 2.4%, p=0.009). Amongst the HIV-uninfected participants, *Shigella* spp., *Salmonella* spp. and *Helicobacter pylori* were more prevalent amongst cases than controls (36.7% versus 12.0%, p=0.002; 11.4% versus 0.0%, p=0.012; 10.1% versus 0.0%, p=*0*.023). Norovirus GII, *C. difficile*, *Campylobacter*, *Cystoisospora*, *Cryptosporidium* spp. and *Enterocytozoon* spp. were more prevalent amongst HIV-infected than HIV-uninfected participants (6.4% versus 1.6%, p=0.032; 7.0% versus 0.8%, p= 0.010; 6.4% versus 0.8%, p= 0.018; 15.5% versus 0.01%, p<0.001; 15.0% versus 1.6%, p<0.001; 5.4% versus 0.8%, p=0.031). *Shigella* spp. and *Blastocystis* were more prevalent amongst HIV-uninfected than HIV-infected participants (27.1% versus 17.1%, p=0.032; 25.6% versus 8.0%, p<0.001). *Shigella* spp. (18.3%) and *Cystoisospora* (17.7%) were the most prevalent pathogens detected in HIV-infected diarrhoeal cases, while *Shigella* spp. (36.7%) and *Salmonella* spp. (11.4%) were the most prevalent pathogens in HIV-uninfected diarrhoeal cases. Although *Blastocystis* was prevalent in HIV-uninfected cases, it was not associated with diarrhoea as prevalence was significantly higher in HIV-uninfected controls (40.0% versus 16.5%, p<0.001).

Table 3: Pathogen detection

	Overall – n[a] (%)				HIV-infected – n[a] (%)				HIV-uninfected – n[a] (%)				*p*-value [c]
	Total (n=344)	**Cases (n=243)**	**Controls (n=101)**	***p*-value [b]**	**Total (n=187)**	**Cases (n=164)**	**Controls (n=23)**	***p*-value [b]**	**Total (n=129)**	**Cases (n=79)**	**Controls (n=50)**	***p*-value [b]**	
Any pathogen	228 (66.3)	177 (72.8)	51 (50.5)	**<0.001**	187 (73.8)	124 (75.6)	14 (60.9)	0.132	84 (65.1)	53 (67.1)	31 (62.0)	0.555	0.097
Virus	64 (18.6)	53 (21.8)	11 (10.9)	**0.018**	43 (22.1)	40 (24.4)	3 (13.0)	0.296	20 (15.5)	13 (16.5)	7 (14.0)	0.707	0.101
Adenovirus	31 (9.0)	27 (11.1)	4 (4.0)	**0.038**	20 (10.7)	19 (11.6)	1 (4.4)	0.476	10 (7.8)	8 (10.1)	2 (4.0)	0.314	0.380
Adenovirus 40/41	3 (0.9)	3 (1.2)	0 (0.0)	0.558	2 (1.1)	2 (1.2)	0 (0.0)	>0.99	1 (0.8)	1 (1.3)	0 (0.0)	>0.99	0.637
Norovirus	18 (5.2)	14 (5.8)	4 (4.0)	0.603	13 (7.0)	13 (7.9)	0 (0.0)	0.374	5 (3.9)	1 (1.3)	4 (8.0)	0.074	0.246
Norovirus GI	4 (1.2)	2 (0.8)	2 (2.0)	0.584	1 (0.5)	1 (0.6)	0 (0.0)	>0.99	3 (2.3)	1 (1.3)	2 (4.0)	0.559	0.308
Norovirus GII	14 (4.1)	12 (4.9)	2 (2.0)	0.248	12 (6.4)	12 (7.3)	0 (0.0)	0.367	2 (1.6)	0 (0.0)	2 (4.0)	0.148	0.032
Enterovirus	16 (4.7)	12 (4.9)	4 (4.0)	0.787	12 (6.4)	10 (6.1)	2 (8.7)	0.645	4 (3.1)	2 (2.5)	2 (4.0)	0.641	0.296
CMV	3 (0.9)	3 (1.2)	0 (0.0)	0.558	3 (1.6)	3 (1.8)	0 (0.0)	>0.99	0 (0.0)	0 (0.0)	0 (0.0)	-	0.273
Astrovirus	5 (1.5)	4 (1.7)	1 (1.0)	>0.99	3 (1.6)	3 (1.8)	0 (0.0)	>0.99	2 (1.6)	1 (1.3)	1 (2.0)	>0.99	>0.99
Rotavirus	2 (0.6)	2 (0.8)	0 (0.0)	>0.99	1 (0.5)	1 (0.6)	0 (0.0)	>0.99	1 (0.8)	1 (1.3)	0 (0.0)	1.000	>0.99
Sapovirus	1 (0.3)	1 (0.4)	0 (0.0)	>0.99	1 (0.5)	1 (0.6)	0 (0.0)	>0.99	0 (0.0)	0 (0.0)	0 (0.0)	-	>0.99
>1 virus detected	9 (2.6)	7 (2.9)	2 (2.0)	>0.99	7 (3.7)	7 (4.3)	0 (0.0)	0.600	2 (1.6)	0 (0.0)	2 (4.0)	0.148	0.318
Bacteria	151 (48.9)	122 (50.2)	29 (28.7)	**<0.001**	87 (46.5)	76 (46.3)	11 (47.8)	0.894	61 (47.3)	46 (58.2)	15 (30.0)	**0.002**	0.894
***Shigella* spp.**	70 (20.4)	59 (24.3)	11 (10.9)	**0.005**	32 (17.1)	30 (18.3)	2 (8.7)	0.378	35 (27.1)	29 (36.7)	6 (12.0)	**0.002**	**0.032**
***Salmonella* spp.**	22 (6.4)	21 (8.6)	1 (1.0)	**0.007**	13 (7.0)	12 (7.3)	1 (4.4)	>0.99	9 (7.0)	9 (11.4)	0 (0.0)	**0.012**	0.993
C. difficile	14 (4.1)	13 (5.4)	1 (1.0)	0.074	13 (7.0)	12 (7.3)	1 (4.4)	>0.99	1 (0.8)	1 (1.3)	0 (0.0)	>0.99	**0.010**
Campylobacter	13 (3.8)	12 (4.9)	1 (1.0)	0.119	12 (6.4)	12 (7.3)	0 (0.0)	0.367	1 (0.8)	0 (0.0)	1 (2.0)	0.388	**0.018**
STEC	2 (0.6)	0 (0.0)	2 (2.0)	0.086	0 (0.0)	0 (0.0)	0 (0.0)	-	2 (1.6)	0 (0.0)	2 (4.0)	0.148	0.166
ETEC	11 (3.2)	9 (3.7)	2 (2.0)	0.519	7 (3.7)	5 (3.1)	2 (8.7)	0.207	4 (3.1)	4 (5.1)	0 (0.0)	0.157	>0.99
EPEC	21 (6.1)	14 (5.8)	7 (6.9)	0.631	15 (8.0)	11 (6.7)	4 (17.4)	0.094	6 (4.7)	3 (3.8)	3 (6.0)	0.676	0.237
EAEC	41 (11.9)	28 (11.5)	13 (12.9)	0.725	25 (13.4)	20 (12.2)	5 (21.7)	0.208	14 (10.9)	8 (10.1)	6 (12.0)	0.739	0.504
O157	4 (1.2)	4 (1.7)	0 (0.0)	0.325	3 (1.6)	3 (1.8)	0 (0.0)	>0.99	1 (0.8)	1 (1.3)	0 (0.0)	>0.99	0.648
Plesiomonas	1 (0.3)	1 (0.4)	0 (0.0)	>0.99	1 (0.5)	1 (0.6)	0 (0.0)	>0.99	0 (0.0)	0 (0.0)	0 (0.0)	-	>0.99
Helicobacter pylori	21 (6.1)	21 (8.6)	0 (0.0)	**0.001**	13 (7.0)	13 (7.9)	0 (0.0)	0.374	8 (6.2)	8 (10.1)	0 (0.0)	**0.023**	>0.99

>1 bacteria detected	45 (13.1)	37 (15.2)	8 (7.9)	0.067	23 (12.3)	20 (12.2)	3 (13.0)	>0.99	11 (8.5)	8 (10.1)	3 (6.0)	0.528	0.287
Parasite [d]		99 (40.7)			85 (45.5)	78 (47.6)	7 (30.4)	0.122	43 (33.3)	21 (26.6)	22 (44.0)	0.042	**0.031**
Cystoisospora	29 (8.4)	29 (11.9)	0 (0.0)	**<0.001**	29 (15.5)	20 (17.7)	0 (0.0)	**0.028**	0 (0.0)	0 (0.0)	0 (0.0)	-	**<0.001**
***Cryptosporidium* spp.**	30 (8.7)	27 (11.1)	3 (3.0)	**0.012**	28 (15.0)	26 (15.9)	2 (8.7)	0.537	2 (1.6)	1 (1.3)	1 (2.0)	>0.99	**<0.001**
Blastocystis	49 (14.2)	25 (10.3)	24 (23.8)	**0.001**	15 (8.0)	12 (7.3)	3 (13.0)	0.403	33 (25.6)	13 (16.5)	20 (40.0)	**0.003**	**<0.001**
***Giardia* spp.**	12 (3.5)	9 (3.7)	3 (3.0)	>0.99	7 (3.7)	7 (4.3)	0 (0.0)	0.600	4 (3.1)	2 (2.5)	2 (4.0)	0.641	>0.99
***Enterocytozoon* spp.**	11 (3.2)	11 (4.5)	0 (0.0)	**0.038**	10 (5.4)	10 (6.1)	0 (0.0)	0.614	1 (0.8)	1 (1.3)	0 (0.0)	>0.99	**0.031**
Schistosoma	12 (3.5)	5 (2.1)	7 (6.9)	**0.025**	8 (4.3)	4 (2.4)	4 (17.4)	**0.009**	4 (3.1)	1 (1.3)	3 (6.0)	0.298	0.767
Dientamoeba	2 (0.6)	1 (0.4)	1 (1.0)	0.502	0 (0.0)	0 (0.0)	0 (0.0)	-	2 (1.6)	1 (1.3)	1 (2.0)	>0.99	0.166
>1 parasite detected	19 (5.5)	13 (5.4)	6 (5.9)	0.827	24 (12.8)	22 (13.4)	2 (8.7)	0.744	10 (7.8)	6 (7.6)	4 (8.0)	>0.99	0.152
Mixed infections Virus-bacteria	40 (11.6)	34 (14.0)	6 (5.9)	**0.034**	25 (13.4)	23 (14.0)	2 (8.7)	0.744	14 (10.9)	10 (12.7)	4 (8.0)	0.564	0.504
Virus-parasite	35 (10.2)	29 (11.9)	6 (5.9)	0.094	25 (13.4)	23 (14.0)	2 (8.7)	0.744	11 (8.5)	7 (8.9)	4 (8.0)	>0.99	0.183
Bacteria-parasite	72 (20.9)	60 (24.7)	12 (11.9)	**0.008**	46 (24.6)	41 (25.0)	5 (21.7)	0.734	23 (17.8)	16 (20.3)	7 (14.0)	0.366	0.152

[a] Total is 344 for overall analysis and 316 for the HIV analysis due to 28 controls with unknown HIV-status being excluded from the HIV analysis; [b] *p*-value comparing pathogen detection in cases versus controls (for HIV-infected and HIV-uninfected groups respectively); [C] *p*-value comparing pathogen detection in HIV-infected versus HIV-uninfected persons (cases and controls combined); [d] The following parasites were screened for but not detected: *Vibrio cholerae*, *Yersinia enterocolitica*, *E. histolytica, Strongyloides* spp., *Cyclospora, Hymenolepsis, Ascaris, Taenia, Trichuris, Ancylostoma, Enterobius, Necator.*

Effect of HIV stage and treatment on symptoms and pathogen detection amongst HIV-infected cases

Presentation did not differ by CD4+ cell count for duration of symptoms, fever, vomiting, fatigue, nausea, respiratory symptoms, abdominal pain, dermatological symptoms, chills, headache, dysentery, arthralgia, myalgia, neurological symptoms or painful swollen glands. There was an increased proportion of cases reporting weight loss with lower CD4+ cell counts (91.4%, 81.0% and 62.5% for CD4+ cell counts of <200, 200-500, >500 cells/mm^3 respectively; p=0.032). Pathogens were detected more frequently in cases with low CD4+ cell counts (75.7%, 42.9% and 37.5% for CD4+ cell counts of <200, 200-500, >500 cells/mm^3 respectively; p=0.004). *Cryptosporidium* spp. specifically, was detected more often in those with low CD4+ cell counts (25.7%, 4.8% and 0.0% for CD4+ cell counts of <200, 200-500, >500 cells/mm^3 respectively; p=0.039).

Symptoms were similar amongst HIV-infected cases on ART and those not on ART. Those on cotrimoxazole prophylaxis were more likely to experience fatigue (88.9% versus 69.3%, p=0.005), weight loss (90.5% versus 76.1%, p=0.023) and chills (50.8% versus 15.9%, p<0.001) then those not on cotrimoxazole. Pathogen detection was similar amongst HIV-infected cases on ART and those not on ART. Parasites, specifically *Cystoisospora* spp. were more commonly detected amongst patients on cotrimoxazole as compared to those not on cotrimoxazole (27.0% versus 11.4%, p=0.014). Other pathogens were found in similar proportions across treatment groups.

Clostridioides difficile detection

There were 14 cases in which *C. difficile* was detected, 13 of which were in HIV-infected patients (92.9%). Symptoms were similar between cases in which *C. difficile* was detected and those without *C. difficile* detection, however those with *C. difficile* detected were more likely to have had a longer duration of illness before admission (6.5 (IQR 4-11) days versus 4 (IQR 2-7) days). As per the guidelines for diagnostic and clinical management of *C. difficile* from the South African Society for Clinical Microbiology, the case definition for infection includes onset of diarrhoea more than 48 hours after admission, or diarrhoea that continues for 3 days post admission and where there is no likely alternatively cause (no other pathogens detected or use of laxatives), or where the patient has had an admission or antibiotics use in the past 12 weeks.[37] Three of the 14 cases did not match this case definition as they did not have an admission in the past 12 weeks and had multiple pathogens detected on PCR. These three cases were however HIV-infected. Five of the cases had no other pathogens detected on PCR, all of which were HIV-infected. Eight of the cases did have a prior admission, however the majority of these (n=6, 75.0%) had multiple pathogens detected on PCR.

Discussion

Despite advances in HIV treatment, diarrhoea amongst HIV-infected patients remains a significant public health challenge[19]. Our study found that the majority (67.5%) of patients over the age of 5 years admitted with diarrhoea were HIV-infected, indicating the importance of HIV-related illnesses when describing the epidemiology of diarrhoea in South African adults. The Joint United Nations Programme on HIV/AIDS (UNAIDS) 90-90-90 treatment target aims to ensure that 90% of the HIV-infected population are diagnosed, 90% of those diagnosed are initiated on ART and 90% of those on ART should achieve viral suppression[38]. A recent South African analysis estimated that 70.7% of diagnosed HIV-infected individuals have initiated ART, and 87.4% of these are thought to be virally suppressed[38]. Proportions reported in the current analysis reflect these numbers, as 83.3% of those with known ART status were on ART, however a large proportion of HIV-infected patients included in this analysis had CD4 cell count <200 cells/µl (70/99, 70.7%) indicating that they are unlikely to be virally suppressed. Unfortunately viral load was not analysed here, since few recent results were available and hence recent CD4 cell count was used as a proxy. Mpumalanga (home province for the majority of the patients included in this analysis) is the only South African province in which the third-90 indicator value (the percentage of those on ART that are virally suppressed) is below 85%[38]. It is also likely that this study design is skewed towards detection of poorly suppressed HIV cases since they are most likely to be admitted for diarrhoea. Diarrhoea in patients with well-controlled HIV will likely be seen at a clinic level. We suggest further investigation at a clinic level in order to include the spectrum of cases. The clinical presentation of diarrhoea in HIV-infected cases differs to that of HIV-uninfected cases. HIV-infected cases were more likely to have symptoms for a longer duration before admission and to suffer from weight loss and nausea. HIV-uninfected cases were more likely to suffer from dysentery. This relates to the findings that dysenteric bacteria (specifically *Shigella* spp.) were more commonly detected in HIV-uninfected patients.

The use of molecular methods and the expanded panel of pathogens included in testing resulted in a high pathogen detection rate (66.3%), with pathogen detection being significantly higher in cases compared to controls (72.8% versus 50.5%, p>0.001). Although viruses were commonly detected (18.5% of all stools tested) and were more likely to be detected in cases as compared to controls (21.8% versus 10.9%, p=0.018), when stratified by HIV-status, viruses were not significantly associated with diarrhoea in this study. Although this lack of association may be in part due to asymptomatic carriage of viruses (specifically in HIV-uninfected patients), it is likely that the sample size in the current study was insufficient to determine significant differences between cases and controls within the HIV groups. Interestingly, we did find that norovirus GII was more prevalent in HIV-infected individuals than HIV-uninfected individuals and that detection in HIV-infected individuals was limited to cases. Previous findings have shown that HIV-infected children are more

likely to suffer poor outcomes related to norovirus infection[39], however detection rates were similar amongst HIV-infected and –uninfected children. This increased detection in HIV-infected adults has not been previously described. Bacteria was detected in 43.9% of stools tested and was significantly associated with diarrhoea overall (50.2% versus 28.7%, $p<0.001$) and amongst HIV-uninfected patients (58.2% versus 30.0%, $p=0.002$). Interestingly, prevalence of bacterial pathogens was high in both cases and controls amongst HIV-infected group (46.3% versus 47.8%, $p=0.894$). This was specifically due to the high prevalence of *E. coli* spp. (including ETEC, EPEC and EAEC) amongst HIV-infected controls. High *E. coli* spp. colonisation rates in HIV-infected patients have been documented in other settings[40]. We found *Shigella* spp. (24.3% amongst cases versus 10.9% amongst controls, $p=0.005$), *Salmonella* spp. (8.6% versus 1.0%, $p=0.007$) and *H. pylori* (8.6% versus 0.0%, $p=0.001$) to be significantly associated with diarrhoea in the current study, specifically amongst HIV-uninfected patients (36.7% versus 12.0%, $p=0.002$; 11.4% versus 0.0%, $p=0.012$; 6.2% versus 0.0%, $p=0.023$ respectively). It's likely that the sample size was insufficient to detect the difference in prevalence between cases and controls in the HIV-uninfected group. *Campylobacter* spp. and *C. difficile* were detected more often in HIV-infected patients as compared to HIV-uninfected patients but were not significantly associated with diarrhoea (likely due to small sample sizes).

As expected[14], parasite prevalence was significantly higher amongst HIV-infected compared to HIV-uninfected patients (45.5% versus 33.3%, $p=0.031$), specifically *Cystoisospora* (15.5% versus 0.0%, $p<0.001$), *Cryptosporidium* spp. (15.0% versus 1.6%, $p<0.001$) and *Enterocytozoon* spp. (5.4% versus 0.8%, $p=0.031$), although only *Cystoisospora* was significantly associated with diarrhoea amongst HIV-infected patients (17.7% versus 0.0%, $p=0.028$). The case-control analysis indicated that *Blastocystis* and *Schistosoma* are unlikely to be causative agents of diarrhoea since these pathogens were detected at higher rates in controls than in cases. Prevalence of *Schistosoma* among healthy individuals has been reported to be as high as 51.2% in the Democratic Republic of Congo and is likely to be higher in villages and rural areas where water is collected from rivers and dam[41]. *Blastocystis* has been noted as an important cause of diarrhoea amongst immunosuppressed patients[42], however this was not found in the current study.

Diarrhoeal aetiology differed with HIV status, with *Shigella* spp. (36.7%) and *Salmonella* spp. (11.4%) being prevalent amongst HIV-uninfected cases and *Shigella* spp. (18.3%), *Cystoisospora* (17.7%), and *Cryptosporidium* spp. (15.9%) being prevalent amongst HIV-infected cases. These differences should be considered for the development of diagnostic and treatment guidelines. This study highlights the importance of *Shigella* spp. in diarrhoeal morbidity amongst adults (regardless of HIV status). The detection of *Shigella* spp. in control stools demonstrates that carriage in our population is also relatively high. *Shigella* spp. were the leading cause of diarrhoeal deaths amongst individuals over

the age of 5 years and the second leading cause of death in young children according to the Global Burden of Disease Study (GBD)[1]. While there is currently no approved *Shigella* vaccine, there are several candidates which show promise for efficacy testing[43]. In order to introduce a candidate vaccine, burden estimates are required. Although a larger sample size is required, this analysis gives an indication of the high burden of *Shigella* spp. in our setting. Further work is required to determine if HIV-infected individuals should be considered as a risk group for possible target should *Shigella* vaccine become available.

Our results are in line with clinical literature which indicates a shift in diarrhoeal aetiology with CD4+ cell count amongst HIV-infected patients. Pathogen detection was high in those with CD4+ cell counts < 200 cells/µl, indicating increased opportunistic infections (specifically *Cryptosporidium* spp.) in this group. Pathogens could only be detected in just over a third of cases with high CD4+ cell count (>500 cells/µl), suggesting that these cases are possibly drug-related. This finding may be useful for treatment guidelines. It also highlights the importance of addressing diarrhoea in patients on ART especially since chronic diarrhoea has been highlighted as an major cause of dropout from ARV care services[44].

Detection of *C. difficile* in this study was almost exclusive to HIV-infected participants (13/14, 92.9%). Data from South African hospitals estimates that community acquired *C. difficile* infections represent only 1.3% of cases (with the remaining 98.7% being hospital acquired)[37]. Our study design excluded cases of hospital-acquired diarrhoea, as enrolment and sample collection was done within 48 hours of admission. The 5.4% *C. difficile* prevalence reported here is therefore lower than estimates of 15.8% amongst symptomatic patients from LMIC[37]. *C. difficile* was more commonly detected in HIV-infected cases than in –uninfected cases (7.3% versus 1.3%, p=0.050). While not considered an opportunistic infection, *C. difficile* it is associated with exposure to healthcare settings and with the use of antibiotics, both of which are increased amongst HIV-patients[45]. The increased management of HIV patients in an outpatient setting[46] may dispute the inclusion of a recent admission in the case definition. There is also growing evidence to suggest the possibility of *C. difficile* being an important community-acquired pathogen[46], and consideration of this should be given in populations with high HIV prevalence.

A major limitation of this study was the small sample size which restricted the analysis (attributable fractions and odds ratios could not be calculated as many categories had zero count). The ANDEMIA study was not specifically aimed at enrolling HIV-infected participants, therefore this analysis relied on incidental HIV admissions both for cases and controls. COVID-19 restrictions also affected enrolment, specifically of controls, since elective surgeries were postponed and hospital visitor

access was restricted. An extension of this analysis would benefit from enrolling controls from HIV clinics. Other limitations included CD4 cell counts not being available for all HIV-infected cases at the time of admission. Many of those without CD4 cell counts available were matched from the routine laboratory data system, however these tests were not always performed at the same time as the enrolment. We used a cut off period of 12 months on either side of enrolment for CD4 cell counts as this was deemed to be a reasonable estimation of the CD4 cell count at admission. In cases where there were significant changes in CD4 cell counts, these may have been incorrectly quantified. There were also limited data available for antibiotic use. Date of last dose was available, however start date for antibiotic course was not collected. This has limitations specifically for the *C. difficile* analysis, since we could not determine how long patients had been on antibiotics. Cause of death data was not available and hence diarrhoeal mortality cannot be estimated.

Author contributions:

S.L.J, N.A.P, M.J.G, L.E and J.T conceptualised and designed the study. S.L.J, N.A.P, N.D., T.A. and M.dV completed and managed fieldwork and data collection. S.L.J, N.A.P, M.J.G, L.E and J.T acquired, analysed and interpreted the data. S.L.J drafted the work. All authors reviewed the manuscript.

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Supplementary tables:

Table S1: Presentation by CD4+ cell counts for HIV-infected cases

	CD4+ cell count [a]			***p*-value**
	<200 cells/µl (n=70)	**200-500 cells/µl (n=21)**	**>500 cells/µl (n=8)**	
Duration of symptoms before admission – median (IQR)	6 (3-10)	7 (3.5-12)	5.5 (3-7)	0.822
Chronic/persistent	6 (8.6%)	2 (9.5%)	1 (12.5%)	0.855
Weight loss	64 (91.4%)	17 (81.0%)	5 (62.5%)	**0.032**
Fatigue	58 (82.9%)	16 (76.2%)	5 (62.5%)	0.293
Nausea	6 (75.0%)	15 (71.4%)	6 (75.0%)	>0.99
Vomiting	48 (68.6%)	14 (66.7%)	6 (75.0%)	>0.99
Fever (current or history)	46 (65.7%)	14 (66.7%)	5 (62.5%)	>0.99
Abdominal pain	41 (58.6%)	14 (66.7%)	4 (50.0%)	0.667
Respiratory symptoms	33 (47.1%)	12 (57.1%)	1 (12.5%)	0.097
Chills	32 (45.7%)	7 (33.3%)	3 (37.5%)	0.634
Headache	32 (45.7%)	7 (33.3%)	4 (50.0%)	0.635
Dermatological symptoms	13 (18.6%)	3 (14.3%)	0 (0.0%)	0.494
Arthralgia	11 (15.7%)	6 (28.6%)	0 (0.0%)	0.171
Myalgia	11 (15.7%)	1 (4.8)%	0 (0.0%)	0.334
Neurological symptoms	5 (7.1%)	0 (0.0%)	1 (12.5%)	0.347
Dysentery [b]	2 (2.9%)	0 (0.0%)	0 (0.0%)	>0.99
Painful swollen glands	2 (2.9%)	0 (0.0%)	0 (0.0%)	>0.99

[a] CD4+ count within 12 months of enrolment only known for 99 of the 164 HIV-infected patients included in molecular testing; [b] Dysentery defined as self-reported blood in the stool.

Table S2: Pathogen detection by CD4+ cell counts for HIV-infected cases

	CD4+ count [a] - n (%)			*p*-value
	>500 cells/µl (n=8)	200-500 cells/µl (n=21)	<200 cells/µl (n=70)	
Any pathogen	5 (62.5)	12 (57.1)	59 (84.3)	**0.022**
Virus	2 (25.0)	5 (23.8)	19 (27.1)	>0.99
Adenovirus	0 (0.0)	5 (23.8)	8 (11.4)	0.170
Adenovirus 40/41	0 (0.0)	1 (4.8)	0 (0.0)	0.293
Norovirus	0 (0.0)	1 (4.8)	6 (8.6)	>0.99
Norovirus GI	0 (0.0)	0 (0.0)	0 (0.0)	-
Norovirus GII	0 (0.0)	1 (4.8)	6 (8.6)	>0.99
Enterovirus	2 (25.0)	0 (0.0)	7 (10.0)	0.068
CMV	0 (0.0)	0 (0.0)	2 (2.9)	>0.99
Astrovirus	0 (0.0)	0 (0.0)	2 (2.9)	>0.99
Rotavirus	0 (0.0)	0 (0.0)	1 (1.4)	>0.99
Sapovirus	0 (0.0)	0 (0.0)	1 (1.4)	>0.99
>1 virus detected	0 (0.0)	1 (4.8)	5 (7.1)	>0.99
Bacteria	4 (50.0)	8 (38.1)	35 (50.0)	0.638
***Shigella* spp.**	1 (12.5)	1 (4.8)	12 (17.1)	0.389
***Salmonella* spp.**	0 (0.0)	0 (0.0)	7 (10.0)	0.322
C. difficile	0 (0.0)	1 (4.8)	9 (12.9)	0.450
Campylobacter	0 (0.0)	0 (0.0)	4 (5.7)	0.695
STEC	0 (0.0)	0 (0.0)	0 (0.0)	-
ETEC	0 (0.0)	0 (0.0)	2 (2.9)	>0.99
EPEC	0 (0.0)	0 (0.0)	6 (8.6)	0.464
EAEC	1 (12.5)	4 (19.1)	8 (11.4)	0.622
O157	0 (0.0)	0 (0.0)	2 (2.9)	>0.99
Plesiomonas	0 (0.0)	1 (4.8)	0 (0.0)	**0.293**
Helicobacter pylori	2 (25.0)	2 (9.5)	4 (5.7)	0.109
>1 bacteria detected	0 (0.0)	1 (4.8)	14 (20.0)	0.131
Parasite [b]	2 (25.0)	8 (38.1)	39 (55.7)	0.145
Cystoisospora	1 (12.5)	4 (19.1)	12 (17.1)	>0.99
***Cryptosporidium* spp.**	0 (0.0)	1 (4.8)	19 (27.1)	**0.028**
Blastocystis	1 (12.5)	2 (9.5)	5 (7.1)	0.563
***Giardia* spp.**	0 (0.0)	1 (4.8)	4 (5.7)	>0.99
***Enterocytozoon* spp.**	0 (0.0)	1 (4.8)	6 (8.6)	>0.99
Schistosoma	0 (0.0)	0 (0.0)	1 (1.4)	>0.99
>1 parasite detected	0 (0.0)	1 (4.8)	8 (11.4)	0.611
Mixed infections Virus-bacteria	1 (12.5)	4 (19.1)	9 (12.9)	0.793
Virus-parasite	1 (12.5)	3 (14.3)	12 (17.1)	>0.99
Bacteria-parasite	2 (25.0)	5 (23.8)	21 (30.0)	0.032

[a] CD4+ count within 12 months of enrolment only known for 99 of the 164 HIV-infected cases; [b] The following parasites were screened for but not detected: *Vibrio cholerae*, *Yersinia enterocolitica*, *E. histolytica, Strongyloides* spp., *Cyclospora, Hymenolepsis, Ascaris, Taenia, Trichuris, Ancylostoma, Enterobius, Necator, Dientamoeba.*

Table 3: Presentation by treatment for HIV-infected cases

	Antiretroviral treatment [a] - n (%)			Cotrimoxizole prophylaxis [a] - n (%)		
	Yes (n=130)	**No (n=26)**	***p*-value**	**Yes (n=63)**	**No (n=88)**	***p*-value**
Duration of symptoms before admission – median (IQR)	5 (3-8)	4.5 (2-10)	0.489	5 (3-9)	4 (3-8)	0.410
Chronic/persistent	10 (7.7)	3 (11.5)	0.456	6 (9.5)	7 (8.0)	0.774
Weight loss	108 (83.1)	19 (73.1)	0.231	57 (90.5)	67 (76.1)	**0.023**
Fatigue	98 (75.4)	19 (73.1)	0.804	56 (88.9)	61 (69.3)	**0.005**
Nausea	92 (70.8)	19 (73.1)	0.813	43 (68.3)	65 (73.9)	0.451
Fever (current or self-reported history in the past 10 days)	89 (68.5)	20 (76.9)	0.391	43 (68.3)	61 (69.3)	0.889
Vomiting	89 (68.5)	22 (84.6)	0.153	42 (66.7)	67 (76.1)	0.200
Abdominal pain	83 (63.9)	18 (69.2)	0.600	42 (66.7)	59 (67.1)	0.961
Respiratory symptoms	59 (45.4)	13 (50.0)	0.667	29 (46.0)	41 (46.6)	0.946
Headache	50 (38.5)	12 (46.2)	0.464	30 (47.6)	33 (37.5)	0.214
Chills	39 (30.0)	6 (23.1)	0.477	32 (50.8)	14 (15.9)	**<0.001**
Arthralgia	32 (24.6)	2 (7.7)	0.069	13 (20.6)	21 (23.9)	0.640
Dermatological symptoms	21 (16.2)	2 (11.5)	0.768	10 (15.9)	14 (15.9)	0.995
Myalgia	14 (10.8)	1 (3.9)	0.469	10 (15.9)	6 (6.8)	0.075
Dysentery [b]	6 (4.6)	1 (3.9)	>0.99	3 (4.8)	4 (4.6)	>0.99
Neurological symptoms	5 (3.9)	1 (3.9)	>0.99	4 (6.4)	2 (2.3)	0.236
Painful swollen glands	3 (2.3)	0 (0.0)	>0.99	2 (3.2)	1 (1.1)	0.571

[a] ART status known for 156 and cotrimoxazole use known for 151 of 164 HIV-infected cases; [b] Dysentery defined as self-reported blood in the stool.

Table S4: Pathogen detection by treatment for HIV-infected cases

	Antiretroviral treatment [a] - n (%)			Cotrimoxizole prophylaxis [a] - n (%)		
	No (n=26)	**Yes (n=130)**	***p*-value**	**No (n=88)**	**Yes (n=63)**	***p*-value**
Any pathogen	23 (88.5)	94 (72.3)	0.082	66 (75.0)	49 (77.8)	0.693
Virus	6 (23.1)	32 (24.6)	0.868	20 (22.7)	16 (25.4)	0.704
Adenovirus	3 (11.5)	14 (10.8)	>0.99	9 (10.2)	8 (12.7)	0.795
Adenovirus 40/41	0 (0.0)	1 (0.8)	>0.99	1(1.1)	0 (0.0)	>0.99
Norovirus	3 (11.5)	10 (7.7)	0.456	6 (6.8)	7 (11.1)	0.354
Norovirus GI	0 (0.0)	1 (0.8)	>0.99	1 (1.1)	0 (0.0)	>0.99
Norovirus GII	3 (11.5)	9 (6.9)	0.423	5 (5.7)	7 (11.1)	0.240
Enterovirus	1 (3.9)	8 (6.2)	>0.99	4 (4.6)	4 (6.4)	0.720
CMV	0 (0.0)	3 (2.3)	>0.99	2 (2.3)	1 (1.6)	>0.99
Astrovirus	0 (0.0)	3 (2.3)	>0.99	0 (0.0)	2 (3.2)	0.172
Rotavirus	0 (0.0)	1 (0.8)	>0.99	0 (0.0)	1 (1.6)	0.417
Sapovirus	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
>1 virus detected	1 (3.9)	5 (3.9)	>0.99	1 (1.1)	5 (7.9)	0.083
Bacteria	16 (61.5)	62 (47.7)	0.197	44 (50.0)	32 (50.8)	0.923
***Shigella* spp.**	5 (19.2)	24 (18.5)	>0.99	18 (20.5)	9 (14.3)	0.329
***Salmonella* spp.**	2 (7.7)	10 (7.7)	>0.99	6 (6.8)	5 (7.9)	>0.99
C. difficile	2 (7.7)	10 (7.7)	>0.99	6 (6.8)	6 (9.5)	0.544
Campylobacter	1 (3.9)	10 (7.7)	0.692	4 (4.6)	7 (11.1)	0.202
STEC	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
ETEC	1 (3.9)	3 (2.3)	0.522	3 (3.4)	1 (1.6)	0.641
EPEC	2 (7.7)	8 (6.2)	0.673	6 (6.8)	5 (7.9)	>0.99
EAEC	2 (7.7)	18 (13.9)	0.531	10 (11.4)	8 (12.7)	0.803
O157	0 (0.0)	3 (2.3)	>0.99	1 (1.1)	2 (3.2)	0.571
Plesiomonas	0 (0.0)	1 (0.8)	>0.99	0 (0.0)	1 (1.6)	0.417
Helicobacter pylori	4 (15.4)	8 (6.2)	0.117	6 (6.8)	6 (9.5)	0.544
>1 bacteria detected	2 (7.7)	24 (18.5)	0.252	11 (12.5)	13 (20.6)	0.178
Parasite [b]	12 (46.2)	59 (45.4)	>0.99	33 (37.5)	37 (58.7)	**0.010**
Cystoisospora	4 (15.4)	24 (18.5)	>0.99	10 (11.4)	17 (26.9)	**0.014**
***Cryptosporidium* spp.**	5 (19.2)	21 (16.2)	0.773	12 (13.6)	14 (22.2)	0.168
Blastocystis	4 (15.4)	6 (4.6)	0.063	8 (9.1)	2 (3.2)	0.195
***Giardia* spp.**	1 (3.9)	5 (3.9)	>0.99	4 (4.6)	2 (3.2)	>0.99
***Enterocytozoon* spp.**	0 (0.0)	10 (7.7)	0.215	4 (4.6)	6 (9.5)	0.321
Schistosoma	0 (0.0)	4 (3.1)	>0.99	3 (3.4)	1 (1.6)	0.641
>1 parasite detected	2 (7.7)	10 (7.7)	>0.99	7 (8.0)	5 (7.9)	>0.99
Mixed infections						
Virus-bacteria	3 (11.5)	20 (15.4)	0.768	11 (12.5)	11 (17.5)	0.394
Virus-parasite	2 (7.7)	19 (14.6)	0.531	8 (9.1)	11 (17.5)	0.126

Bacteria-parasite	8 (30.8)	34 (26.2)	0.633	19 (21.6)	22 (34.9)	0.069

[a] ART status known for 156 and cotrimoxazole use known for 151 of 164 HIV-infected cases; [b] The following parasites were screened for but not detected: *E. histolytica, Strongyloides* spp., *Cyclospora, Hymenolepsis, Ascaris, Taenia, Trichuris, Ancylostoma, Enterobius, Necator, Dientamoeba.*

PAPER IV

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Identifying gaps in hand hygiene practice to support tailored target audience messaging in Soweto: A cross-sectional community survey

Authors:
Siobhan L. Johnstone[1,2]
Nicola A. Page[1,3]
Michelle J. Groome[4,5]
Shabir A. Madhi[6]
Portia Mutevedzi[6]
Juno Thomas[1]

Affiliations:
[1]Centre for Enteric Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa

[2]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

[3]Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

[4]Division of Public Health Surveillance and Response, National Institute for Communicable Diseases, Johannesburg, South Africa

[5]School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

[6]South African Medical Research Council, Vaccines and Infectious Diseases Analytics Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Corresponding author:
Siobhan Johnstone,
siobhanjsa@gmail.com

Effective risk communication is essential for outbreak mitigation, as recently highlighted during the coronavirus disease 2019 (COVID-19) pandemic. Hand hygiene is one of the proposed public health interventions to protect against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) acquisition and transmission along with social distancing, improved ventilation, environmental cleaning, and wearing of masks. Improving hand hygiene practices in the community requires an understanding of the socio-behavioural context. This cross-sectional community survey in Soweto identified gaps in hand hygiene, which can inform appropriate messaging at the community level. Only 42% of survey respondents practiced adequate hand hygiene. Tailored educational messaging should be targeted at young adults in particular, and the importance of soap for hand hygiene must be emphasised for all age groups. Risk communication should expand to focus on preventing multiple infectious diseases during and beyond the COVID-19 pandemic.

Keywords: hand hygiene; risk communication; COVID-19; diarrhoeal diseases; community.

Background

Health communication has played a critical role during the coronavirus disease 2019 (COVID-19) pandemic, highlighting the importance of appropriate risk communication and community engagement (RCCE) in effectively mitigating or controlling outbreaks.[1,2] Hand hygiene is one component of the bundle of public health interventions[3] to decrease the risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and transmission. Furthermore, hand hygiene also prevents or reduces the risk of infection and transmission of many other putative pathogens.[4,5] Effective hand hygiene is estimated to reduce diarrhoeal episodes by 31% and respiratory illnesses by 21%.[5] It is estimated that approximately one million diarrhoeal deaths could be prevented globally each year with the universal adoption of adequate hand hygiene involving widely available regular soap products.[6] Hand sanitisers are shown to be as, if not more effective, at reducing pathogen transmission than regular soap and water.[7] Although often prohibitively expensive, waterless hand sanitisers are accepted as a feasible alternative in low-income communities with limited soap and water availability, especially during outbreaks.[8,9]

Much remains unknown about how best to encourage good hand hygiene practices at a community level, and specifically how best to sustain these habits in the long term.[4] Improving hand hygiene practices in settings with good access to sanitation facilities requires changing human behaviour,[10] which is subject to complexities including existing inequalities, cultural and socioeconomic factors.[3] Limited understanding of these practices and associated factors in the community is historically responsible for the limited success of promotion campaigns.[10] Data from South Africa show a significant decline in hand hygiene as a preventive behaviour between the first and second waves of COVID-19 as a result of pandemic fatigue, even amongst people at risk of severe disease.[11] Creating a culture of good hand hygiene should focus on all levels of society[10] with RCCE tailored to specific communities based on an understanding of socio-behavioural habits in these communities.[12] A sustained improvement in hand hygiene practices would have continued benefit

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during and beyond the COVID-19 pandemic, and health authorities should continue to leverage on the traction gained especially as pandemic fatigue sets in.[11]

We undertook a cross-sectional community survey in Soweto in February 2020, shortly before the first COVID-19 case in South Africa was reported on 05 March 2020.[13] This report highlights the key findings in terms of hand hygiene practices and factors affecting these practices and identifies gaps for tailored educational messaging to improve hand hygiene practices in this community.

Methods

Study area and population

Soweto is an urban township in the south of Johannesburg with an estimated 1.3 million people.[14] Residents generally have good access to piped water (96.8% have municipal water) and good sanitation (91.6% have flush toilets connected to a sewerage system).[14] Unemployment is high with 19.0% of households not receiving any set income.[14]

The Soweto Health and Demographic Surveillance Site (HDSS) was established in 2017 through the Child Health and Mortality Prevention Surveillance (CHAMPS) network and currently tracks individuals from 20 778 households in 8 clusters (or sub-places) in Soweto. The methods for this HDSS have been described elsewhere.[15] This hand hygiene survey was part of a larger survey on diarrhoeal diseases in the community, which used the Soweto HDSS as a sampling frame.[16]

Sampling methods and data collection

Five hundred households were randomly selected from four of the Soweto HDSS clusters (clusters selected using probability proportional to size sampling). Fieldworkers visited selected households and explained the study to an adult (≥ 18 years of age) representative of the household. Written informed consent was obtained and respondents were interviewed by fieldworkers, in the preferred language of the respondent.

Respondents were asked about water sources in the household, how they usually wash their hands (use of soap and water) and how often they wash their hands at the following critical times: after using the toilet, before preparing food, before eating, before feeding young children and after changing children's nappies or diapers (where applicable). Households were considered a non-response if they were not available after two visits on two separate days.

Statistical analysis

Demographic and socioeconomic information for consenting households was matched to the Soweto HDSS database before being de-identified. The International Wealth Index (IWI)[17] was used as a composite measure of material wealth, combining household assets, housing floor material, toilet facility, number of rooms, electricity access and water source, for each household. An aggregate variable describing hand hygiene as adequate or inadequate was generated from the responses to the questions on hand hygiene. A respondent was described as having adequate hand hygiene if they reported washing with soap and water (as opposed to water only) at all critical times (after using the toilet, before preparing food, before eating and for households with small children; before feeding young children and after changing children's nappies or diapers). Data were specified as three-level, complex survey data to account for the cluster design of the study. Logistic regression was used to determine risk factors for inadequate hand hygiene. Stata® software (version 14) was used for data analysis.

Ethical considerations

This study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (approval number: M190663) and the CHAMPS Soweto HDSS Community Advisory Board. Written informed consent was obtained from all included participants. All methods were carried out in accordance with relevant guidelines and regulations.

Results

A total of 374 households (including 1640 individuals) were enrolled. All respondents reported having a facility to wash

TABLE 1: Risk factors associated with inadequate hand hygiene.

Characteristics	*N*	*n*	%	OR	95% CI	*p*
Gender						
Male	122	76	62.3	Referent	Referent	-
Female	252	140	55.6	0.76	0.49–1.18	0.217
Children < 5 years old in the house						
No	247	139	56.3	Referent	Referent	-
Yes	127	77	60.6	1.20	0.77–1.85	0.420
Children between 5-15 years old in the house						
No	200	112	56.0	Referent	Referent	-
Yes	174	104	59.8	1.17	0.77–1.76	0.462
Dwelling						
Formal	290	165	56.9	Referent	Referent	-
Informal	65	42	64.6	1.38	0.79–2.42	0.255
Respondent age (years)						
< 30	88	65	73.9	Referent	Referent	-
30–39	58	34	58.6	0.49	0.23–1.02	0.056
40–49	49	25	51.0	0.37	0.17–0.81	0.012
50–59	60	37	61.7	0.60	0.29–1.25	0.172
60+	105	50	47.6	0.34	0.18–0.63	0.001
Diarrhoeal episode in the household in the past two weeks						
No	296	162	54.7	Referent	Referent	-
Yes	78	54	69.2	1.86	1.09–3.17	0.022
Basin/tap for hand washing						
Same room as toilet	193	103	53.4	Referent	Referent	-
Different room to toilet	181	113	62.4	1.47	0.97–2.23	0.066
International Wealth Index	-	-	-	1.00	0.98–1.02	0.912

Note: International Wealth Index: Median = 86.3, IQR = 79.1-92.1.
OR, odds ratio; CI, confidence interval; IQR, interquartile range.

their hands on the property, with 52% (n = 193) having a tap or basin in the room housing the toilet and 48% (n = 181) having a tap that was not in the room housing the toilet (either outside the dwelling or in the kitchen). The majority of respondents reported that they always washed their hands after going to the toilet (n = 331, 88.5%), before preparing food (n = 299, 80.6%), before eating (n = 293, 78.6%), before feeding babies and young children (71/92, 77.2%) and after changing children's nappies or diapers (62/78, 79.5%). Only 64.2% (n = 240) reported using soap and water to wash their hands whilst the rest (n = 134, 35.8%) used water only. Although specific questions on the use of waterless hand sanitiser were not included, respondents were asked if they use anything other than soap and water to wash their hands. No respondents reported using waterless hand sanitiser. The aggregate variable for adequate hand hygiene indicated that only 42% (n = 158) of respondents practiced adequate hand hygiene.

Gender, having children in the household, living in an informal dwelling and IWI did not affect hand hygiene practices. The odds of inadequate hand hygiene decreased significantly with increasing age of respondent (odds ratio [OR] = 0.49, 95% confidence interval [CI]: 0.23–1.02; OR = 0.37, 95% CI: 0.17–0.81; OR = 0.60, 95% CI: 0.29–1.25; OR = 0.34, 95% CI: 0.18–0.63; for age groups 30–39, 40–49, 50–59, ≥ 60 years compared with < 30 years, respectively). The odds of at least one diarrhoeal episode in the past two weeks were higher for households reporting inadequate hand hygiene (OR = 1.86, 95% CI: 1.09–3.17) and tended to be higher for households with a basin in a different room to the toilet (OR = 1.47, 95% CI: 0.97–2.23) (Table 1).

Although there was no difference in performance of hand hygiene at critical times between respondents with taps located inside or outside the room housing the toilet, respondents with taps located outside the room housing the toilet were less likely to use soap when washing hands (52.8% vs. 74.6%; OR = 0.38; 95% CI: 0.25–0.59; p = 0.000).

Discussion

Pre-COVID-19 hand hygiene practices in the surveyed population were poor, with only 42% of respondents reporting adequate hand hygiene (always washing their hands with soap and water at critical times).

Inadequate hand hygiene was specifically associated with not using soap for hand washing, although respondents reported good compliance in terms of washing at critical times. Waterless hand sanitiser was not used in this community pre-COVID-19, although the availability and use of such products may have since been introduced as part of pandemic mitigation. It is unlikely that the use of hand sanitiser would be a sustainable practice in low- and middle-income communities outside of pandemic conditions, especially in urban communities with good access to soap and piped water. Households reporting inadequate hand hygiene practices were more likely to have had a diarrhoeal episode in the past two weeks, in keeping with findings from previous studies.[5,6] This study may have been subject to response bias because of reliance on respondents to answer questions accurately rather than through observation. The importance of drying hands after washing could not be analysed as these data were not collected during the survey. Despite these limitations, we were able to highlight gaps regarding hand hygiene practices in this community.

Younger respondents (< 30 years) were more likely to practice inadequate hand hygiene than older respondents (> 30 years), a finding also made in a study on risk perception and preventive behaviour during the COVID-19 pandemic in South Africa.[11] All surveyed households had access to piped water on their property, but just under half had a tap located outside the room housing the toilet. Those who did not have a tap in the room housing the toilet were less likely to use soap when washing their hands. Tailored educational messaging should be targeted at young adults in particular, perhaps with programmes introduced at schools and learning centres, particularly through media relevant to a young audience. The importance of using soap for hand hygiene must be emphasised for all age groups. In particular, there is a need for messaging with low-cost, practical solutions (such as soap slings) to encourage keeping soap at taps located outside dwellings and at communal taps.

Studies have shown that direct, targeted communication is required to change risk mitigation behaviours, specifically during outbreaks.[12] Simple messaging around some of the findings from this study may support the maintenance of good hand hygiene in the long term. A variety of modalities (including peer health educators, multimedia, audiovisual and social media interventions) have been shown to be effective in delivering RCCE during virus outbreaks[12] and should be utilised for hand hygiene messaging. Effective RCCE for hand hygiene is especially important as COVID-19 pandemic fatigue lowers risk perceptions[2,12] and should expand to focus on preventing the spread of multiple infectious diseases.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Author's contributions

S.L.J., N.A.P., M.J.G. and J.T. conceptualised and designed the study. S.L.J. completed and managed fieldwork and data collection. S.L.J., N.A.P., M.J.G., S.A.M., P.M. and J.T. acquired, analysed and interpreted the data. S.L.J. drafted the work. N.A.P., M.J.G., S.A.M., P.M. and J.T. substantively revised the work. All authors reviewed the manuscript.

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Data availability

The data sets used during this study is available from the corresponding author, S.L.J., upon reasonable request.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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