Effects of HWTS on rural people living with HIV/AIDS
OBJECTIVES

• To improve the health-related microbial quality of drinking water at point of use in rural households by selected home treatment interventions.

• To measure the effects of improved water quality on rural people living with HIV/AIDS.
SPECIFIC OBJECTIVES

• Recruit, in collaboration with the primary health care clinics and VCT councillors, people living with HIV/AIDS (PLWHA) to participate in this study (these households will serve as the case households) - Blood samples will be used to determine the baseline CD4 count;

• Randomly recruit control households from the same areas as the PLWHA (HIV/AIDS status of households not known - to serve as control households)

• Obtain baseline (before intervention) data on household demographics, health, environment, water and social aspects related to the study;

• Collect drinking water from all the recruited households (case and controls) and analyse for baseline health-related water quality;

• Implement the Potters for Peace ceramic intervention in the participating households;

• Collect drinking water from all the recruited households (case and controls) and analyse for real-time health-related water quality after 3 months;

• During the real-time data period, collect diarrhoeagenic stool samples from affected persons in all study households as well as non diarrhoeagenic stool samples in the same households;

• Compare the various sets of data to determine any possible shifts in the parameters for disease incidence, immune status, pathogenicity and water quality measured in households using improved water quality versus the control groups (no intervention).
WATER QUALITY MEASUREMENTS

- Before providing Potters for Peace ceramic filters to households, a two-week period commences during which baseline data are collected on health (diarrhoea), immune status (CD4 counts), household demographics, water and environmental aspects related to the study;
- Potters for Peace ceramic filters will be allocated to the intervention group at the end of the two-week period;
- Water samples from traditional water storage containers will be collected from households at the implementation of the household into the study (beginning of the active surveillance period (17 weeks);
- A sample of the water from the ceramic water filter will be collected at the end of active surveillance period;
- Health-related microbiological water quality tests will be performed to identify indicator as well as pathogenic organisms;
- Results obtained from these tests will be used to determine the exposure level of household members to pathogenic microorganisms in contaminated water as well as of those not exposed (the intervention group).
**HEALTH END-POINT MEASUREMENTS**

- All the households in the study will receive self-reporting diaries (symptom diaries) for recording incidences of diarrhoea. A record of diarrhoea episodes will be kept for each member of every household by the respondent on diary pages, which will be collected weekly by symptom diarists.

- Each household (intervention and non-intervention) will be monitored on a weekly basis for a period of 17 weeks (two weeks baseline and 15 weeks real-time) for diarrhoea incidence.

- Incidence of diarrhoea in each household will be measured by active surveillance (symptom diaries) every week;

- CD4 counts are a reliable indicator of the immune status of HIV-positive cases. CD4 counts will be taken at the implementation of the household; at the end of the active surveillance period and 3 months after the active surveillance period. Shifts in CD4 counts will be measured to determine the effect of filtered water consumption on HIV-positive cases.

- The two groups that will be dealt with in this study are the Cases (HIV-positive) and the Controls (HIV-negative);

- Within those groups there will be the intervention and non-intervention households;

- Comparisons will be made between HIV-positive intervention households and HIV-positive non-intervention households to determine the effect of the intervention on the immune status of PLWHA;

- A similar comparison will be made for the HIV-negative group to determine the effect of the intervention on the incidence of diarrhoea;
Study Household selection
Collection and transport of water from source
Storage of water at household
Human/animal interaction
Hygiene
Road

Gate

Shack

Stored water

27 paces to pit latrine
No toilet at HH 8

Stored water

Road

Gate

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In total: 1315 people were taking part in the study from these 248 households.
Age and gender spread

The age distribution of the people living with HIV/AIDS was not even.

The women consisted of 79.4% of all the people living with HIV/AIDS in the study and the men only consisted of 20.6%.

The biggest age group is 30-39 years (34.8%). This group mostly consists of females.

The second largest group is 40-49 years (25.4%) and mostly consists of males.

The third largest age group is 20-29 years (17.5%) and mostly consists of females.

The fourth largest group is an older group of 50-59 years (14.3%). - primarily females
The following problems were encountered during the study with the enrolment of HIV/AIDS households in the Nwanedi catchment:

- VCT councilors at clinics did not want to assist in recruitment of HIV/AIDS clients
- Primary Health Care clinics did not want to assist in recruitment of HIV/AIDS clients
- ARV’s were not always available to the HIV/AIDS patients when they went to the clinics
- Households whom had children identified by the VCT or clinic sister as being positive – did not want the child to take part in the study
- Many male HIV/AIDS positive clients refused to be part of the study due to stigmatization
- Many of the HIV/AIDS clients identified to be part of the study was migrant laborers and thus excluded
- HIV/AIDS clients refused to be part of the study due to stigmatization and they did not want their family to know they were positive
- Some HIV/AIDS clients died before the study could be implemented in their households
- Some HIV/AIDS clients died during the study
- Some HIV/AIDS clients were intimidated to not take part in the study by VCT councilors and clinic sisters
- Some people did not want to give blood or any other type of bodily fluids due to the fact of witchcraft
Of all the names collected at the clinics 10 names could not be used.

Six (6) of these people were migrant labourers. Migrant labourers were not included in the study (although noted) because they do not use the water for four or more days a week. It will also be difficult to determine if the filter had any health effects if they did not use drink the filter water on a regular basis.

Two (2) people passed away after agreeing to participate in the study but before they were actually actively part of it.

One (1) person did agree to participate but when the research group wanted to include her in a group she decided to withdraw because she did not believe that she was infected anymore.

The last person was a minor child. Although the grand mother (who is the legal guardian) agreed to include him and signed to consent forms, his father was not aware of his status and due to fear of exposing the child’s status it was agreed not to include this child.

Of the PLWHA that were actively part of the study, two people passed away during their active time in the study.
The impact of the intervention on CD4 counts change in count from month 1 to month 6 (absolute change) showed the following:

Non-Intervention group: Mean 34.88 (std dev 212.38)
Intervention group: Mean 80.12 (std dev 2.17)

This indicated no change (p=0.344) between the non-intervention and the intervention groups with regards to the CD4 counts.

However, this may be a call to do a bigger study to determine the effect of interventions on the health of HIV/AIDS patients.

The impact of ARV treatment on CD4 counts change in count from month 1 to month 6 (absolute change) showed the following:

No ARV therapy: mean 3.29 (std dev 241.9),
ARV therapy: mean 95.9 (std dev 186.9)

Looking at the use of ARV’s on the CD4 counts, indicated a significant change (p=0.054) which showed that ARV treatment did improve the CD4 counts in infected patients.
Diarrhoeal assessment
Stool specimen assessment:
Stool samples collected from members of households when diarrhea was reported and assessed for same organisms as in water assessment - Biowipes

Diarrhoea incidence was monitored for the duration of the time the household was part of project (all members of household) using diarrhoeal diaries.

Also collected stool samples from household members in the study - get an idea of circulating strains in community

![Fig. 1. The pictorial diary used for recording frequency and consistency of stool in the study](image)
Current collection methods may negatively impact identification and isolation of causative pathogens

Social resistance

Dirty job

This factor was overcome using alternative collection method

The Bio-wipe kit consists of white absorbent material with an orange plastic backing, which is used in the same manner as toilet tissue paper.
Use of a Biowipe-Kit to collect stool specimen
Collection of Biowipes

It is a less intrusive and more user friendly collection method that appeared to be more accepted by the villagers than conventional methods. Although the assumption is made that all the participants were honest with the reporting of diarrhoea the question remains whether they might have preferred not to report diarrhoea out of fear of supplying a stool specimen. This is a feasible concern when working with cultures that believe that any off their body parts or excretions can be used for magic. A more detailed study is needed to determine if the new collection technique is acceptable to the villagers.

• Despite training of the participants on the correct use of the Biowipes various Biowipe pack were received that were incorrectly used. This hindered the recovery of the faecal matter from the Biowipe resulting in too little material to perform all the planned tests in the laboratory.

• There is a need for a better marking system for the Biowipes since Biowipes were received in the laboratory that did not have all the information needed that had an influence on the statistical analysis of the results.

• From a scientific point of view the most worrying part was whether the Biowipes would still give reliable results after storage in the field and transport to the University of Johannesburg before they were analysed. Studies done in the laboratory confirmed that reliable results would still be obtainable and was published by Mieta et al. (2010)
Optimisation of methods for the collection and detection of bacterial pathogens from diarrhoeal human faecal samples using a novel stool collection kit

SIK Mietz1, N Polgieter3, MD Soobray and TG Bamard3

1 Water and Health Research Unit, University of the Western Cape, Private Bag X1701, Bellville 7535, South Africa
2 Department of Microbiology, University of the Witwatersrand, Private Bag X3505, Johannesburg, 2050, Gauteng Province, South Africa
3 University of North Carolina, CB#7431, Roseau Hall B, Room 148, Chapel Hill, NC 27599-7431, USA

Abstract

Bacterial pathogens such as Escherichia coli, Salmonella, Shigella, and Vibrio species are known to be causative agents for diarrhoeal disease in humans. This study aimed to develop a culture-independent PCR assay for the detection of bacterial pathogens present in human faecal samples using a novel stool collection technique. The Bio-nips kit (Bio-nips, PCRDNA extraction kit) was developed for targeting the E. coli pathogenic, the Salmonella spp., the Shigella spp., and the Vibrio spp. present in enteric-virulent E. coli and Shigella spp. The influence of the Bio-nips extraction method and specimen type on the detection of the bacterial pathogens was investigated. A qualitative multiplex PCR extraction method used in laboratory-prepared samples could successfully extract DNA from 91% of the samples analyzed. Both E. coli and Vibrio species could be clearly identified and differentiated between the various pathogens tested and were specific for the single pathogens. Faecal matter was successfully recovered from all Bio-nips samples and the bacterial DNA could be detected from these samples. A see-through Bio-nips filter, containing inert Bio-nips, along with the Bio-nips kit, can be used for collection and detection of bacterial pathogens during clinical and/or research settings.

Keywords: Bio-nips kit, bacterial pathogens, faecal matter, PCR, DNA extraction

Introduction

The human intestinal tract is home to a complex community of microbial species which serve as markers of enteric intestinal health (Zhang et al., 2006). The occurrence and distribution of bacterial pathogens causing diarrheal diseases in humans has been shown in various studies (Brenz, 2000; Biondo et al., 2009). A high incidence of causative diarrheal disease is associated with specific enteropathogens, E. coli (EPEC), in addition, data published by Khan et al. (2006) indicated that Vibrio cholera, Salmonella, and Shigella spp. are commonly associated with enteric diarrheal disease in Bangladesh.

The app is used commonly used for the identification and detection of these pathogens based on either bacterial isolate characterisation or the prevalence of pathogens in a sample population (Defriz, 2009; Muchira et al., 2009; Sidrak, 2009). The first problem encountered with isolate characterisation is the need to identify the causative agent due to the typical small size of bacterial colonies tested per sample (Garbidge et al., 2009; Litt et al., 2009; Fagmag et al., 2009). A second problem is the inaccessibility to laboratories outside the clinic, preventing the efficient isolation and detection of bacterial pathogens.
PCR results obtained for the detection of pathogenic *E. coli*, *Shigella flexneri* and *Salmonella* species in the collected Bio-wipes. The percentages are given in brackets.

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<td>HIV status of participants from whom Bio-wipes was received</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV positive</td>
<td>31</td>
<td>2 (6.45)</td>
<td>2 (6.45)</td>
<td>2 (6.45)</td>
<td>18 (58.06)</td>
<td>0 (0)</td>
<td>1 (3.33)</td>
<td>29 (93.55)</td>
<td>1 (3.23)</td>
</tr>
<tr>
<td>HIV negative</td>
<td>120</td>
<td>17 (14.17)</td>
<td>12 (10)</td>
<td>6 (5)</td>
<td>39 (32.5)</td>
<td>3 (2.5)</td>
<td>3 (2.54)</td>
<td>110 (91.67)</td>
<td>2 (1.67)</td>
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<td>Unknown</td>
<td>60</td>
<td>15 (25)</td>
<td>11 (18.33)</td>
<td>5 (8.33)</td>
<td>32 (53.33)</td>
<td>4 (6.67)</td>
<td>2 (3.39)</td>
<td>54 (90)</td>
<td>1 (1.67)</td>
</tr>
</tbody>
</table>
Data analysis was done with STATA™ version 10.

Comparison of illness rates between intervention and non-intervention communities were done using multilevel mixed effects Poisson regression to handle possible clustering within village and household and repeat sampling from individual participants.

New cases were recorded for every diarrhoea day when there had been three consecutive previous days without diarrhoea.

Crude diarrhoea incidence rates in all volunteers are:
0.485 (95% CI 0.387 – 0.599) per person year in control population and
0.459 (95% CI 0.366– 0.567) in intervention group
Incidence rate ratio 0.955 95%CI= 0.639 – 1.425, z=-0.23 p=0.820
So a very small reduction in diarrhoea incidence but not significant.

Crude diarrhoea incidence rates in HIV positive volunteers:
0.615 (95% CI 0.282 – 1.168) per person year in control population and
0.918 (95% CI 0.458– 1.643) in intervention group
IRR= 1.53 95%CI =0.564 – 4.159, p=0.403
So more diarrhoea in HIV positive people using the filter but not significant.
• Influence of “prolonged shedding” status of individuals

• Correlate occurrence of pathogens with age / sex / intervention groups

• Unanswered questions
  ▪ Are the bacteria viable / infective?
  ▪ What is the causative agent?
  ▪ How can we link Biowipe results with water results?

• Biowipes was well received in rural areas

• The Bio-wipe kit is an easy-to-use and cost effective technique

• The Bio-wipe can successfully be used for routine collection of faecal matter from individuals and during outbreaks for rapid identification of diarrhoeal causative agents.
Water quality assessment
Source water collection

The collection of the source water samples presented various problems due to the diverse nature and distribution of the sources. The main problems encountered were:

• One of the main problems encountered was the availability of the water in the piped systems services certain areas of the study areas. It would often take several attempts and visits to these points in order for the team to successfully collect a water sample.

• The collection of water from the sources were challenging due to the distance between the various sources, especially the rivers and springs that required the members to walk long, and sometimes difficult, routes to collect the water samples. This did however a new respect to the villagers who do this on a regular basis to collect enough water to meet the household water needs on a daily basis.

• Collecting water from these samples during the rainy season proved to be impossible for several of the sources due to the increased levels of the rivers and the wetness making several of the routes extremely dangerous.
Household water collection

Collecting water from households, especially from the ceramic filters, presented the following problems:

• The first problem was the actual collection of the water samples from the households. On numerous occasions it was a struggle to get the participants at home so that water could be collected. This was problematic since the team only went to the field for one week a month resulting in some household being visited for three months before a sample could be collected.

• Finding people at home was more difficult on “Grant” days which meant that most of the villagers went to collect their grant money after which they did shopping at the various “flea markets”.

• On more than one occasion the team was met with empty containers and had to return to the households on a later stage to collect the water. This was especially the case with the ceramic water filter that sometimes only had enough water to cover the bottom of the filter housing container.

• In many cases samples could not be taken since no one of legal age was at home to assist with the questions asked when the water was collected.

• Similar to the collection of the source samples the distance travelled between houses also lead to fewer samples taken per day. This was due to the staggered design of the project so that the households participating per month being scattered all over the study area.
### Intervention households

<table>
<thead>
<tr>
<th></th>
<th>EPEC</th>
<th>EHEC</th>
<th>ETEC</th>
<th>EAEC</th>
<th>EIEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 (120)</td>
<td>2.42</td>
<td>1.61</td>
<td>4.84</td>
<td>0.81</td>
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</tr>
<tr>
<td>M3 (116)</td>
<td>6.45</td>
<td>3.23</td>
<td>2.42</td>
<td>9.68</td>
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</tr>
<tr>
<td>F (120)</td>
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<td>0</td>
<td>1.61</td>
<td>0.81</td>
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</table>

### Non-intervention households

<table>
<thead>
<tr>
<th></th>
<th>EPEC</th>
<th>EHEC</th>
<th>ETEC</th>
<th>EAEC</th>
<th>EIEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 (124)</td>
<td>4.84</td>
<td>4.03</td>
<td>5.65</td>
<td>2.42</td>
<td>0.81</td>
</tr>
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<td>M3 (124)</td>
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<td>4.84</td>
<td>4.03</td>
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<td>HIV positive households</td>
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<td>EHEC</td>
<td>ETEC</td>
<td>EAEC</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Intervention</td>
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<td>2.22</td>
<td>4.44</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>M3 (45)</td>
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<td>6.67</td>
<td>2.22</td>
<td>8.89</td>
</tr>
<tr>
<td></td>
<td>F (45)</td>
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<td>0</td>
<td>0</td>
<td>2.22</td>
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<tr>
<td>Non-intervention</td>
<td>M1 (79)</td>
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<td>4.44</td>
<td>6.67</td>
<td>2.22</td>
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<tr>
<td></td>
<td>M3 (79)</td>
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<td>2.22</td>
<td>8.89</td>
<td>8.89</td>
</tr>
<tr>
<td>HIV negative households</td>
<td>Intervention</td>
<td>M1 (79)</td>
<td>2.53</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Non-intervention</td>
<td>M1 (79)</td>
<td>5.06</td>
<td>3.8</td>
<td>5.06</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>M3 (79)</td>
<td>6.33</td>
<td>6.33</td>
<td>1.27</td>
<td>3.8</td>
</tr>
</tbody>
</table>
HWTS acceptance by study households
<table>
<thead>
<tr>
<th>Field research overview</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of houses visited</td>
<td>221</td>
</tr>
<tr>
<td>Total number of household interviews/observations</td>
<td>167</td>
</tr>
<tr>
<td><strong>Of all houses visited, interviews were conducted at 77% of the houses, (23% of the houses were visited without interviews/observations being able to be conducted)</strong></td>
<td>76%</td>
</tr>
<tr>
<td>Total number of villages visited</td>
<td>26</td>
</tr>
<tr>
<td>Total number of villages in which interviews were conducted</td>
<td>26</td>
</tr>
</tbody>
</table>
Assessment of the use of the Potpaz filter

- Yes: 107 (69%)
- No: 32 (20%)
- Possibly - some water in bottom though: 8 (5%)
- No, filter is no longer used for filtering: 4 (3%)
- No, neglected: 5 (3%)

Legend:
- Green: Yes
- Red: No
- Yellow: Possibly - some water in bottom though
- Brown: No, neglected
On what surface is the filter placed?
Where is the Potpaz filter located in relation to stored water?

- **Separate rooms, 60, 47%**
- **Directly next to water, 30, 23%**
- **Same room, not directly next to filter, 22, 17%**
- **Water stored outside house, 17, 13%**
How do you fill the ceramic filter? (scooping from drum, pouring from large bottle, etc.)

- Buckets: 47%, 33%
- Pour from 20/25l container: 31%, 21%
- Scoop into filter using a small jug: 59%, 41%
- Decant using a small bowl: 7%, 5%

(scooping from drum, pouring from large bottle, etc.)
How many times do you fill the filter per day?

- Once: 93%, 69%
- 2 times per day: 25%, 19%
- Once every 3 days: 9%, 7%
- Once every 2 days: 4%, 3%
- 3 times per day: 3%, 2%
CONCLUSIONS

• Too many aspects impacting people living with HIV/AIDS - not only water ......put people in glass houses and study different aspects

• Bio-wipe kit - easy-to-use and cost effective technique and must be developed further

• Diarrhoeal incidence as proof of effectiveness of POU device??? - what about long term shedders?

• Look at consumer behaviour - what do they like and what will make them take ownership?