The Mhlathuze river catchment: Bacterial contamination and antibiotic resistance patterns of the isolates

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Abstract

The present study reports on the fecal contamination in Mhlathuze river catchment and the incidence of antibiotic resistance amongst the bacterial isolates from water sources as well as from diarrhoea patients living in the area. The Mhlathuze river catchment (Northern KwaZulu-Natal, RSA) is home to an extensive rural population that is dependent on water from this river for all domestic purposes. An increase in indicator fecal coliform bacteria was observed between 1998 and 1999. Increased surface water temperature during this period (1998 – 1999) appeared to be one of the factors affecting the increased bacterial counts. Bacteria isolated from the river also included *E. coli*, *Pseudomonas-**, Enterobacter*-. (detected frequently), *Serratia*-, *Klebsiella* spp., and *Aeromonas hydrophila* (detected less frequently).

Pathogens isolated from stool samples of diarrhoea patients of the area and their drinking water included *Salmonella*-, *Shigella* spp. and *E. coli* O157:H7. More than 90% of the specimens of the *Shigella*-, *Salmonella* spp. were resistant to ampicillin and penicillin. Multiple (three or more) antibiotic resistance amongst isolated pathogenic and non-pathogenic bacteria was a common occurrence.

1 Introduction

Increase of fecal pollution in source water is a problem of developing as well as developed countries [3]. Waterborne bacterial pathogens such as *E.coli* 0157, *Salmonella*-, *Shigella* spp. and *Vibrio cholera* can lead to diarrhoea outbreaks that
may have serious medical and economic implications [14,15]. This problem is enhanced by incidence of antibiotic resistance bacterial flora in water systems.

Distribution of microorganisms are influenced by a number of factors, amongst others temperature, light, rainfall, available nutrients and environmental pollutants [12]. The elevation, decrease or disappearance of certain bacterial groups within an aquatic habitat is a function of changing biological, chemical, physical and hydroclimato logical conditions. Exposure to environmental pollutants and changes in nutrient composition may lead to selection pressures favouring certain organisms or genotypes. Pollutants present in agricultural run-off, sewerage and industrial effluent include substances that favour selection for antibiotic and heavy metal resistance. Recent studies demonstrated positive correlations between industrial [5,9] pollution and the spatial distribution of antibiotic resistance. Medical and veterinary use of antibiotics and other drugs [1,13] as well as domestic and agricultural use of pesticides and related compounds [1], contribute extensively towards incidence of antibiotic resistance among bacteria.

The Mhlathuze river system (Figure 1) is situated in a subtropical region of southern Africa and is utilized for domestic, recreational, agricultural and industrial purposes. Although the Mhlathuze estuary is protected area, it is adjacent to the Richardbay coal terminal, a coal export harbour. Industrial activities are concentrated along the area close to estuary and include aluminium smelters, sugar mills, paper and pulp factories, fertilizer manufacturing and mineral mines. The major agricultural crops in the fertile soil of Mhlathuze river system are sugar cane and timber. Small-scale commercial and informal production of live stock and other crops also exist. The Empangeni-Richardsbay area is thus a prospering industrial and agricultural hub in Northern KwaZulu-Natal. It provides employment opportunities for people from the area, those from other major industrialized zones as well as individuals from more remote areas. A large cross-section of the population live in rural areas and due to industrial developments, the urbanized population is increasing. Water need in this area is thus steadily increasing. Several rural communities are directly dependent on the river for all their water needs and retrieve untreated water from the river, boreholes and natural springs. Sanitation facilities in many communities are poor and non-existent. The residents from the rural area in the Mhlathuze river catchment are thus prone to diarrhoeal disease. The present (2000-2001) *Vibrio cholera* epidemic affecting the Mhlathuze river catchment and other areas of KwaZulu-Natal, was preceded by a diarrhoea outbreak (May to August 2000) in the Mangeza area (Figure 1).

The aims of our study were to (i) determine trends of fecal coliform changes in the Mhlathuze river during 1998 - 1999; (ii) determine if a correlation exist between antibiotic resistance patterns of bacteria isolated from water samples and those from stool samples of diarrhoea patients from the Mhlathuze area.
2 Materials and methods

2.1 Study area and sampling

2.1.1 Study area

Figure 1. Map of the eastern region of the Mhlathuze catchment area indicating the sampling sites (site 1-KwaDlangezwa, site 2-KwaDlangubo, site 3-Mhlathuze estuary, site 4-Mhlathuze pump station and site 5-Felixton bridge). The solid dark areas indicate lakes (Lake Mzingazi and Lake Nseleni) other inland water bodies and estuaries. Developed residential areas to which treated pipeline waters are supplied are also indicated. (UZ = University of Zululand).

2.1.2 Water samples

All water samples were collected biweekly in sterile Schott bottles, at five different locations along the Mhlathuze river (KwaDlangezwa-site 1, KwaDlangubo-site 2, Mhlathuze estuary-site 3, Mhlathuze pump station-site 4 and Felixton bridge-site 5) (Figure 1). Once collected, the samples were immediately stored on ice in a dark cooler box and transported to the laboratory. The samples were stored at 4°C and analyzed within 6 hours of collection.

2.1.3 Stool samples

All diarrheal stool samples were provided by the Khandisa primary health care clinic, KwaDlangezwa. The samples were stored at 4°C and immediately analyzed for bacterial pathogens.
2.2 Microbiological analysis

2.2.1 Water samples
A series of ten-fold dilutions of the water samples were used (where necessary) for the enumeration of bacterial counts using plate count and membrane filtration (Millipore, HANG 47 mm) methods. Nutrient agar (NA), mEndo Les, mFc and S-S agars (Merck) were used to determine heterotrophic bacterial, total coliform, fecal coliform and Salmonella and Shigella spp. respectively. Plates were incubated at 35 °C for 24 hours except that mFc plates were incubated at 44.5°C for 24 h. Experiments were performed in duplicate.

2.2.2 Stool samples
Sterile swabs were used to transfer stool specimens to media plates. S-S agar was used for enumeration of Shigellas and Salmonellas. MacConkey and XLD agars were used to isolate lactose fermenters.

2.3 Identification of bacteria
Several single colonies from each plate were randomly isolated based on the morphology. The isolates were then identified by Gram stain and biochemical reactions, specific agglutinating serums (Mast Diagnostics, United Kingdom) and confirmed by API 20E strips used according to the manufacturer’s instructions (bioMerieux, France).

2.4 Antibiotic sensitivity tests
The disc diffusion method was used to determine antibiotic sensitivity of the isolates. Overnight broth cultures were spread on Hilton-Mueller agar plates. The plates were dried at room temperature for 2 hours. Antibiotic discs were placed at equi-distances.

2.5 Temperature and pH analysis
Temperature was measured in situ using a mercury bulb thermometer (Brannan, England) and pH measurements were taken in the laboratory at 25°C (sample temperature), using a PHI 34 pH meter (Beckman, USA).

2.6 Rainfall data
Rainfall data for the sampling region (South African Weather Bureau number 0304823) was supplied by the Computing Centre for Water Research (CCWR) at the University of Natal, Pietermaritzburg campus, Republic of South Africa.

2.7 Statistical analysis
Arithmetic mean values of microbiological, rainfall, temperature and pH analysis data were used to present monthly values for these factors. Pearson correlation
efficient \((r)\) was used to show correlation between microbiological data on the one hand and rainfall and temperature on the other.

3 Results

Figure 2 depicts the changes in fecal coliform bacteria detected in the Mhlathuze river over a twenty one month period, extending two cold periods (May 1998 - August/September 1998 and May 1999 - August 1999) and one warm period (September/October 1998 - March/April 1999). Cyclic increases and decreases are clearly shown in the graphs in Figure 2. These graphs show that the Felixton sampling site was the most contaminated throughout the study period. During the warm period (November 1998 - March 1999) very large numbers of fecal coliform bacteria (between \(1.5 \times 10^3\) to \(9 \times 10^3\) cfu/ml) were detected at this site.

The graphs in Figure 2 also show that there was an increase in numbers of the fecal coliform in 1999, when compared to the 1998 values. This increase in observed colony forming units (cfu's) were also observed for heterotrophic plate count bacteria and total coliform bacteria (results not shown).

![Figure 2. The mean fecal coliform bacteria in the Mhlathuze River as detected per site during the study period (March 1998 to November 1999). For February 1999 samples could not be collected at sampling site closest to the University of Zululand, due to inaccessibility of the sample area.](image-url)
These changes may be associated with environmental conditions such as changes in temperature and rainfall. In Figure 3 a comparison is demonstrated between surface water temperature and fecal coliforms detected. The trend of elevated fecal coliform levels is also supported by the graphs of the mean values of all five sites as shown in Figure 3.

The fecal coliform graphs (1998 and 1999) and surface water temperature (1998 and 1999) show similar topologies. For both parameters the periods month of April and the months July to November 1999 show greater values than the corresponding values in 1998. The observed changes in the fecal coliform counts can thus be explained in terms of surface water temperature. Similar topologies were also demonstrated by the total coliform bacteria-surface water temperature graph over the same period (1998-1999; graph not shown).

![Mean Fecal Coliform bacteria Detected and Mean Surface Water Temperature Measured in the Mhlathuze Microbiological (1998 and 1999)](image)

**Figure 3:** Comparison of the mean monthly fecal coliform (FC) bacteria detected at all five sites and surface water temperature measured in the Mhlathuze River during the study period (March 1998 to November 1999).

A similar comparison of rainfall data to fecal coliform and total coliform data also indicated a similar trend as the above-mentioned (results not shown). Pearson correlation coefficient ($r$) analysis showed that there was a positive correlation between increase in surface water temperature and increase in bacterial counts ($r = 0.805$ for total coliform and $r = 0.678$ for fecal coliform). Similar analysis for rainfall figure changes and bacterial count changes also showed a positive relationship ($r = 0.646$ for total coliform and $r = 0.622$ for fecal coliform).
During 2000 no further elevation in bacterial counts were observed in the water from the Mhlathuze river. A study was then conducted to determine the extent to which bacterial pathogens contributed to the diarrhoea problem in one of the areas (Mangeza) of the Mhlathuze catchment. Comparative examination of stool samples of diarrhoea patients from the area and their water sources showed that *Salmonella* and *Shigella* spp. were amongst the transmitters of the diarrhoea. These pathogens isolated from the two different sources (i.e. diarrhoeal patients as well as drinking water sources) also showed similar antibiotic susceptibility/resistance patterns confirming that the cause of the outbreak was waterborne. *E.coli* O157:H7 was also isolated from only one of stool samples but was not detected in any of the water samples.

The resistance profiles of the various antibiotics tested on bacteria isolated from stool samples of diarrhoea patients and those isolated from water samples collected in 2001 are presented in Table 1. A positive correlation \( r = 0.865 \) was demonstrated for the observed similarity in antibiotic resistance/susceptibility profiles of the isolates from stool samples when compared to those from recently collected water samples.

**Table 1:** Shows percentage of bacterial isolates that were resistant to the various antibiotics tested. The stool samples included pathogens as well as *E. coli* isolated from diarrhoea patients. Water samples were collected and tested in 2001 and were from the various collection sites along Mhlathuze river.

<table>
<thead>
<tr>
<th>Antibiotics Tested and Concentrations</th>
<th>% Bacteria Resistance</th>
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<tbody>
<tr>
<td></td>
<td>Stool Samples (n= 71)</td>
</tr>
<tr>
<td>Penicillin (10 μg/ml)</td>
<td>80.28</td>
</tr>
<tr>
<td>Ampicillin (10 μg/ml)</td>
<td>74.64</td>
</tr>
<tr>
<td>Nalidixic acid (10 μg/ml)</td>
<td>18.31</td>
</tr>
<tr>
<td>Tetracycline (30 μg/ml)</td>
<td>16.9</td>
</tr>
<tr>
<td>Streptomycin (10 μg/ml)</td>
<td>16.9</td>
</tr>
<tr>
<td>Cefuroxim (30 μg/ml)</td>
<td>16.9</td>
</tr>
<tr>
<td>Chloramphenicol (30 μg/ml)</td>
<td>4.2</td>
</tr>
<tr>
<td>Ciproflaxacin (5 μg/ml)</td>
<td>2.8</td>
</tr>
<tr>
<td>Gentamycin (10 μg/ml)</td>
<td>1.4</td>
</tr>
<tr>
<td>Cefotaxim (30 μg/ml)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Pseudomonas*-, *Enterobacter*-, *Serratia*-, *Klebsiella*- and *Aeromonas* spp. were frequently detected in the water samples from the Mlhathuze river (1998 – 2001).
The presence of these bacteria (particularly the coliforms) in the water is indicative of the contamination of the water source.

4 Discussion

The results from the various focus areas of this study is relevant to industrial and agricultural development as well as medical management in Northern KwaZulu-Natal. It shows that, although contamination of the river is generally constant over time, there are periods when very large increases in bacterial numbers are experienced. The following factors influenced the observed elevated levels of fecal and total coliform bacteria. (i) Activities (domestic, recreational, agricultural, industrial) along the river increase during the summer period. At Felixton, for example, a sugar-mill and paper factory is operating at full capacity and informal agricultural activities also increase. (ii) The river catchment is situated in a summer rainfall area and increased run-off is thus experienced during September to February/March annually. (iii) Surface water temperatures of between 20°C and 30°C were regularly detected during the summer period September 1998 to April 1999. Fecal coliform counts peaked at $8.3 \times 10^3$ cfu/ml. This type of contamination may pose (health) risks if used for any domestic, agricultural or recreational purposes and is cause for concern. Recent studies showed similar elevated bacterial counts were due to changes in environmental factors [2,11,12]. Evidence from these and other studies [5] have highlighted criticisms against the use of fecal coliform bacteria as indicators of fecal pollution in tropical and subtropical waters. The results from the present study contribute to the views by other authors [2,5] that fecal coliform observations should be considered within a wider context and that other more applicable indicators should be considered for subtropical waters.

The elevation of bacterial levels from 1998 to 1999 was originally disturbing, suggesting possible increase in bacterial contamination levels. However, subsequent continued monitoring did not show any further rises in bacterial numbers. As expected, a strong positive correlation was demonstrated between surface water temperature and rainfall data on the one hand and the elevated bacteria levels on the other hand, indicating that these two mentioned parameters had a major influence on the observed elevated bacterial levels.

Diarrhoea is one of the serious medical problems of the area and could be attributed to poor sanitation levels. A study by Pretorius [11] illustrated how the levels of sanitation in a developing urban area affected the quality of surface water of river. In the present study, antibiotic profiles of the *Samonella* and *Shigella* spp. isolated from boreholes and those from stool samples were identical, demonstrating that the possible cause of diarrhoea perpetuated by the sanitation practices. A recent cholera outbreak in KwaZulu Natal in general, and the Mhlathuze river catchment area in particular (August 2000- May 2001) has highlighted the plight of these communities [7, 10]. Presently, programmes aimed
at educating the rural communities on household water treatment procedures and sanitation [7]. Water is also supplied to needy communities by tank trucks [10].

The high levels of multiple antibiotic resistance amongst the isolated bacteria is a major cause for concern and may have serious medical disease management as well as economic implications [8,13]. This is also a major problem in developed communities with superior healthcare facilities [3]. In the case of poor rural communities that are reliant on primary health care facilities and traditional medicines, this problem may be further augmented. It is thus imperative that, the determination of antibiotic susceptibility/resistance patterns of isolated microbes is a part of the microbial monitoring process. The observations should be closely correlated with disease management practices and other possible factors that may contribute to antibiotic resistance [6].

A study is underway to determine the effect of medical, veterinary, agricultural and industrial practices on the incidence of antibiotic resistance in allochthonous as well as autochthonous bacteria of the Mhlathuze river system. The intention of the study is also to: (i) generate data of the spatial and temporal distribution of the Mhlathuze river microbial population as well as antibiotic resistance and (ii) to test the observed microbial distribution patterns and antibiotic distribution patterns to theoretical distribution models of organisms along river systems.

Acknowledgements

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References


