

Spatial Distribution of Surface Water *Escherichia coli* Biotypes across on Informal Settlement



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Abstract

- **Background**
- In surface water management faecal indicator bacteria play a crucial role in determining water quality. These bacteria represent enteric microorganisms that may be voided with pathogens. Indicator organisms such as *Escherichia coli* that are strictly associated with humans may be more desirable in flagging potential hazard to water downstream users. *E. coli* is ubiquitous in the environment from plants, soil and animals. Awareness of differences in *E. coli* biotypes before and after an informal settlement (pieces of land that are occupied without the authority of municipalities concerned) could suggest anthropogenic pollution.
- **Objectives**
- This objective of this study was to assess the impact of the settlement on water quality and anthropogenic activity by investigating *E. coli* biotypical variation before and after the river enters the settlement.
- **Materials and methods.**
- Water samples were collected from two sampling sites (before the settlement and after the river passes the settlement) and filtered through a Satorious cellulose nitrate filter, pore size 0.45µm. The filter was then placed on Chromocult agar for isolating *E. coli* and isolates with the Analytical Profiling Index API 20 E kit. One hundred biotypical differences were investigated.
- **Results and conclusion**
- Anthropogenic pollution was clearly visible with deposits of faeces on the river banks as the river passes the settlement. The two sampling sites reflected different EC API profiles. Sucrose and sorbitol fermentation showed significant differences across the settlement with 100 ± 34.64 , 2 ± 34.64 and 99 ± 9.19 , 73 ± 45.12 ; respectively. The phenotypical differences of the 2 populations suggested adaptive genetic variation across the two sites, suggestive of different sources of pollution. It also raised concern that upon pooling together of the two populations downstream the diversity of population in the biofilm would be more pronounced. This could lead to sharing of foreign genetic material with deleterious effect to humans. Further studies should be done across informal settlements in order evaluate if this is not a singular event peculiar to this study. If this is a norm, sorbitol and sucrose fermentation assays in *Escherichia coli* could be used as a bacterial source tracking tool.
- This study was funded by the National Research Foundation of South Africa.

Background

- Informal settlements often are pieces of land that are occupied without the authority of municipalities concerned
 - As a result have inadequate infrastructure
 - Often with high population density in shacks
- Khayamnandi was founded in the 1950s.
- It is predominantly an informal settlement with a hostel built in 1966 for male migrant labourers.
- Erhart (2000) reported that public latrines that were built for the hostels had to cater for the township resident at large at a ratio of 1:136 (latrine: individual) in 1994.
- He deems the situation had improved by 2001 to 1 latrine to 75 residents.
- In 2001 the population of the township was estimated above 22 000 people living on an area just over 1 square kilometre with 208 public toilets¹.
- The households in shacks were served with water by a single tap by the roadside. It is estimated that this tap served at least 6 households.
- The hostels which were better served in this regard had no baths or shower facilities. Refuse for the settlement is collected once a week¹.
- The impact of this settlement was assessed across three sites.
- These three sampling sites are indicated on the map (next slide).

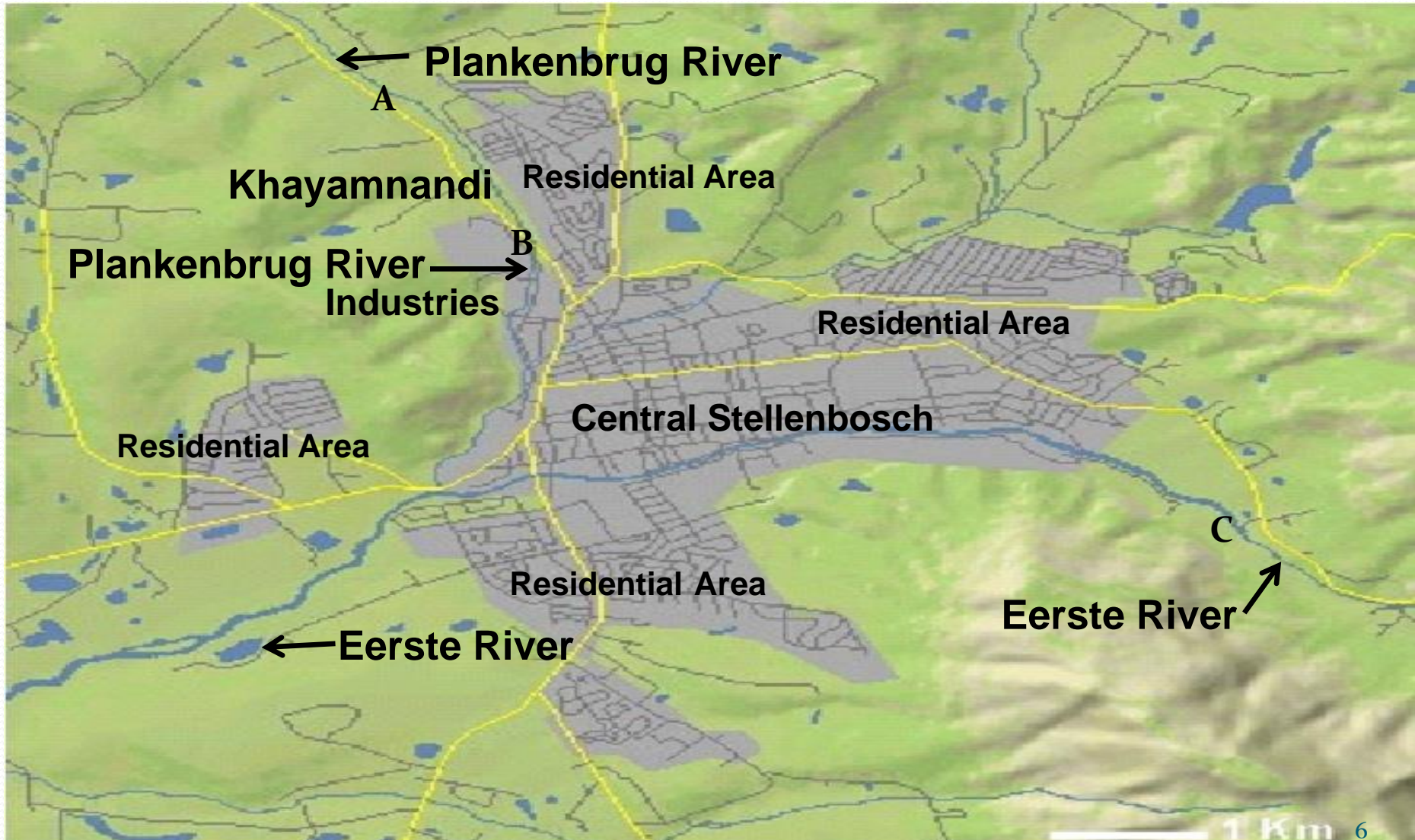
Introduction

- It is unquestionable that the presence of unplanned or improperly planned settlements on riverbanks poses serious negative impact on both surface and underground water quality.
- The lack of sanitation and infrastructure tend to lead to faecal contamination and other pollutants of nearby rivers.
- Population density compounds the circumstances.
- However, it is also important to look beyond the apparent source of pollution, especially after several attempts to correct the situation constantly seem to fail.
- It could happen that the apparent source is but a contributory factor in the situation although significant.
- Upstream evaluation of water quality can shed light to ambient conditions before the settlement, thus enabling alternative measures to curb the pollution.

The study questions

- Is it worthwhile focusing resources into the settlement to improve the nearby river water quality?
- Is Khayamnandi the only source of faecal pollution on Plankenbrug River?
- If not, to what extent does Khayamnandi contribute to the problem?
- In order to evaluate toxicity of water after Khayamnandi, the following questions are asked:
 - What is the toxicity potential of the water in terms of faecal indicator organisms after the settlement? To what extent has the settlement affected this potential? Is there a vast difference in the distribution of biotypes before and after the settlement?
- In order to answer these questions quantitative analysis of spatial distribution of faecal indicators and the presence of certain biotypes was investigated.

Site Layout



Test site

(Post informal settlement)

once annual river
now
perennial



Reference site

Eerste River from the mountains predominantly impacted by feral animals



The Importance of Plankenbrug River

- Plankenbrug River is one of the tributaries constituting the Eerste-Kuils River drainage basin ^{2, 3}.
- This river system drains Jonkershoek Mountains, the Bottelery Mountains and the eastern part of the Cape Flats.
- It then proceeds from the basin to open into False Bay at Macassar beach
- The Eerste-Kuils River drainage basin supplies fresh water to farms for irrigation².
- Therefore, its integrity is of high importance.
- Moreover, it impacts on the quality of marine waters too as it eventually drains into the ocean.
- This water occasionally has above 1000 CFU FC/100ml⁵.

- Amongst the tributaries of Eerste River , Plankenbrug River is the only river trversing an informal settlement before discharging into the catchment area⁶.

- The informal settlements are notorious for affecting the integrity of surface waters^{7, 8, 9}.
- Furthermore, the Plankenbrug River has consistently been found with high faecal pollution^{2, 3, 10}.
- The Eerste River supplements water supply to the town of Stellenbosch ¹¹. Thus needs protection as much as possible.

- In order to protect this water the understanding of conditions in its tributaries is essential.

Recent Developments

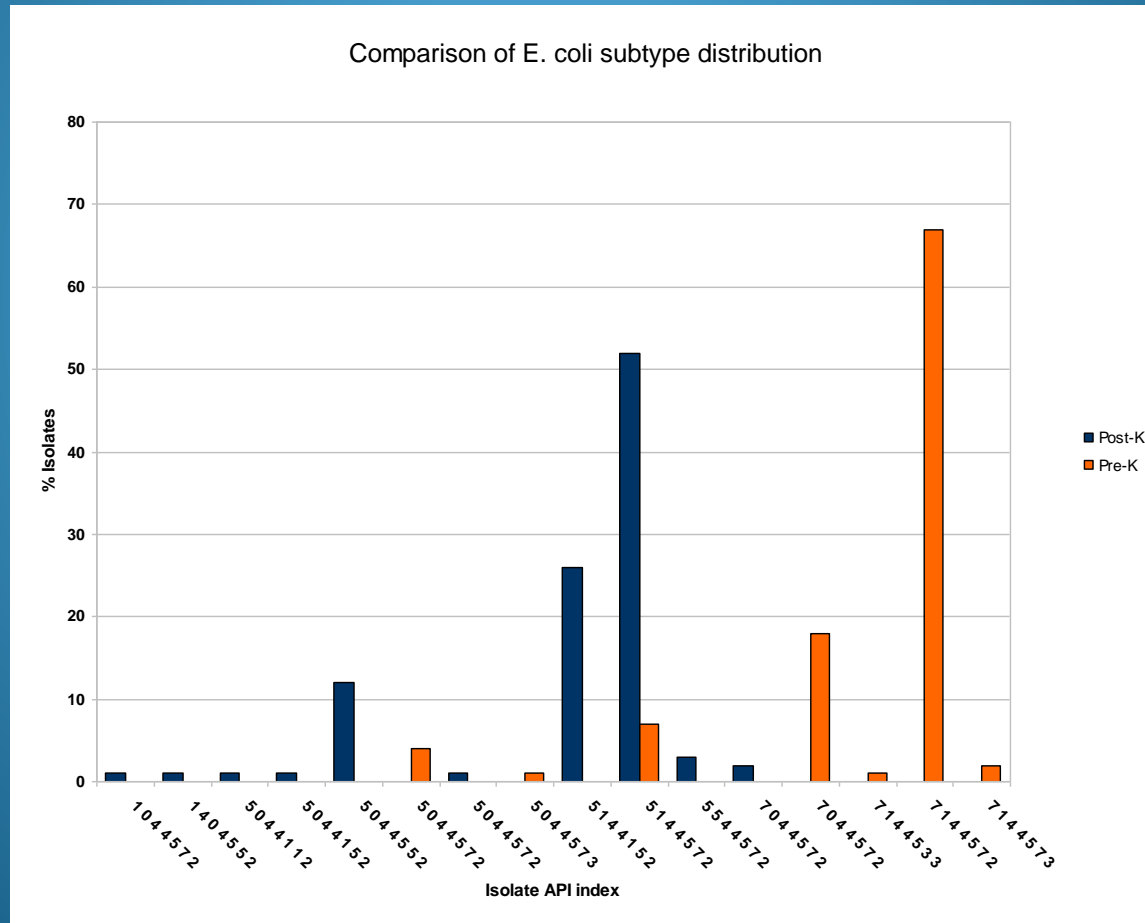
- Recently, there have been infrastructural developments in the township, but these have not translated to river water quality improvement^{12, 13, 14}.
- The river itself has been transformed. It now flows throughout the year whereas earlier it used to flow only in winter¹⁰.
- These above two points seem to indicate that there could be forces outside Khayamnandi that could be adding to the problem at hand.
- Only when such extra forces have been elucidated and dealt with together with the Khayamnandi problem can water downstream be improved, that is, *only if they exist*.
- Thus, there is a need to find out whether such sources do exist or not.
- Informal settlements generally are underserviced with respect to effluent infrastructure.
- This could be due to unpredictable demographics and it leads to excessive demand on the meagre existing amenities¹⁵.
- Erection of structures on its own tends to flood the existing drainage systems rather than allowing natural percolation of rain water into the soil, thus lend to stressing drainage systems¹⁶.
- Intermittent water supply has is also closely implicated in water contamination to flooding of sewage drainage facilities¹⁷.

Characterization of *E. coli* with API

- The Analytical Profile Index (API 20E)
 - is a biochemical methods for characterization of bacteria.
 - diagnostic kit with a miniaturized panel of biochemical tests
 - useful for identification of groups *Enterobacteriaceae*.
- API 20 E exploits that organisms of the same species often utilize limited combinations of metabolic and enzymatic activities for identification of an unknown isolate.
- The number of test that are performed on each test strip enables one to discern variations in these activities demonstrating phenotypic expressions of genes.
- The index generated from the test has wide enough limits to allow identification of biotypes within a species (Instruction Manual version E #2012).
- When this diversity is pronounced across two sites and particularly when samples show spatial diversity, it can be used to discriminate between two sources.
- The API system enables this diversity and thus can be used as a potential bacterial source-tracking tool^{18, 19}.
- Literature has reported some of the API 20E test as potential indicators of bacterial source tracking mechanisms. This is afforded by distribution of positive and negative results for these tests in different animal species.
- Sorbitol and sucrose fermentation are examples of such tests^{18, 19}.

Biotype Distribution

(before and after the informal settlement)



API biotypes

- Graph (previous slide) represents % distribution of *E. coli* subtypes across the informal settlement
- Pre-Khayamnandi isolates were dispersed with one prominent group (accounting for about 70%), while those of Post-Khayamnandi seemed clustered in two prominent groups.
- After entering the 7 digit codes all isolates were identified as *E. coli* 1 with confidence ranging from 86.2 to 99.9 % except for two isolates from Post-Khayamnandi.
- Exceptions
 - One with profile index of 1 404 552 and identified as *Citrobacter freundii* with the second best identification as *E. coli* 1.
 - The second had a profile of 5 044 112 and identified as *E. coli* 2, with second best identification as *E. coli* 1.

Comparison of biotype distribution

- Isolates from Pre-Khayamnandi site gave 7 distinct analytical profiles, while Post-Khayamnandi yielded 10 profiles.
- The API's for each site clustered in contrast.
- Pre-Khayamnandi peaks stood out at the opposite end of the range to those that characterized Post-Khayamnandi isolates.
- One common profile index, i.e. 5144572
- 5144572 accounted for > 50% of Post-Khayamnandi isolates (and < 10 % Pre-Khayamnandi isolates)

API 20E individual tests

| Percentage positives of isolates | | | Significant difference across sites |
|----------------------------------|-------|--------|-------------------------------------|
| TEST | Pre-k | Post-k | |
| ONPG | 100 | 99 | None |
| ADH | 95 | 1 | Yes |
| LDC | 100 | 97 | None |
| ODC | 78 | 81 | None |
| CIT | 3 | 2 | None |
| H ₂ S | 0 | 4 | Yes |
| MAN | 100 | 99 | None |
| INO | 2 | 0 | None |
| SOR | 100 | 73 | Yes |
| RHA | 100 | 99 | None |
| SAC | 100 | 59 | Yes |
| MEL | 99 | 97 | None |
| AMY | 4 | 0 | None |

API 20 E biochemical activity

| Site | | Pre - Khayamnandi | Post- Khayamnandi |
|--------------------------|-----------------------|----------------------|----------------------|
| Prevalence(%) ± Stdev | Sorbitol fermentation | 99±9.19 | 73±45.12 |
| | Sucrose fermentation | 100±34.64 | 2±34.64 |

Sorbitol fermenters

- Sorbitol fermentation is traditionally used to discriminate serotypes of *E. coli* where O157:H7 does not ferment²⁰.
- It has been reported that these traditionally non-sorbitol fermenting serotype can mutate to sorbitol positive variants²¹.
- Sorbitol fermenting *Stx* strain of *E. coli* O157 (STEC) has been implicated in haemolytic uremic syndrome outbreaks in southern Germany^{22, 23, 24,25}.
- Beef was implicated as the source ^{22, 23}.
- Interconversion between various *E. coli* pathotypes involving *stx2* gene has been demonstrated^{24, 26}.
 - These bidirectional interchanges result in ephemeral changes in the pathotypes during an infection.
 - These changes are mediated by a *stx2* encoding phage.
- Sorbitol fermenting EHEC O157:H⁻ strains and non-sorbitol fermenting EHEC O157:H have distinct plasmids²⁶.
 - suggesting that sorbitol fermentation is related to the plasmid.
- Sorbitol fermenting EC biotypes have been isolated in water²⁷.
- The presence of sorbitol fermenting *E. coli* O157 in livestock in South Africa has been suggested²⁸.
- Our study indicated that the prevalence of *E. coli* after the informal settlement is merely augmented with anthropogenic species.
- Different distribution of EC biotypes across the two sites was demonstrated.
- The high prevalence of sorbitol fermenting species in one site could be used to purport possible introduction of new species as the water passes the settlement.
- Different distribution of sorbitol fermenting biotypes across the two sites suggest different sources of pollution.
- The affirmation of these two assertions would assist in assessing the distribution of biotypes and the probable toxicity of the water at each site.
- It might further point a direction to the development of a bacterial source tracking tool for contamination of surface water ^{18, 19}.

Sucrose fermenters

- Sucrose is also used to discriminate different biotype of EC. This sugar is amongst the most highly water soluble components in plant tissue²⁹.
- Hence it may be readily available for microbial uptake. However, some EC biotypes are known for inability to ferment it³⁰.
- In some EC biotypes this ability is masked by competing genes and it can be realized by knocking off these genes^{31, 32}.
- Otherwise plasmids confer sucrose fermentation to EC via the expression of the *scr* regulon^{33, 34}.
- Sucrose fermenting is common in herbivores and avian population^{35, 36}. In this study the distribution of sucrose fermenters and those that do not, the non-sucrose fermenters, is interrogated if it can be used as a predictive tool for indicating source of river toxicity. This may also be used as a bacterial source tracking mechanism^{18, 19}.
- A skew distribution toward either Post- or Pre-Khayamnandi served this purpose.
- Several studies have shown that the distribution of phylogenetic subgroups of bacteria is not random^{37, 38, 39, 40}.
- Different groups of animals harbour diverse subgroups of the same organism. With respect to EC omnivores (including humans) demonstrate more diverse population in comparison to herbivores³⁷.

Concerns

- Directing remedial measures effectively.
- The general message is that there is danger in intermixing human biotype with animal/environmental biotypes.
 - Confer drug resistance
 - Altered chemical basis for variation in phenotype = change in genotype
 - Via mutations
 - Bacteriophages
 - Temperate phages may act as transposons due to physiological state of bacteria
 - In nutrient depleted medium
 - May be involved in transduction
 - Plasmids
 - Some plasmids have genes linked with acquisition and rearrangement of DNA.
 - May be involved in conjugation

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