An evaluation of sorbitol-fermenting bifidobacteria as specific indicators of human faecal pollution of environmental water

P Jagals[1]* and WOK Grabow[2]

[1]*Department of Environmental Sciences, Technikon Free State, Private Bag X20539, Bloemfontein 9300, South Africa*

[2]*Department of Medical Virology, University of Pretoria, PO Box 2034, Pretoria 0001, South Africa*

Abstract

The value of selected indicators for the assessment of faecal pollution, as well as to distinguish whether the pollution is of animal or human origin, was investigated. Faecal coliform bacteria, faecal streptococci and sorbitol-fermenting bifidobacteria were included as indicator organisms. Comparative tests were carried out on samples collected from a stream and river exposed to predominantly faecal pollution of domestic animal origin. Water from the same stream and river was also tested after downstream exposure to runoff from a low socio-economic informal settlement with limited sanitation. Samples were collected from perennial flow during the dry season and from storm-water runoff after thunder showers. Sorbitol-fermenting bifidobacteria were found to be reliable indicators of human faecal pollution. The ratio of faecal coliforms to faecal streptococci was in the order of 3.5 to 4.7 immediately after heavy exposure to faecal pollution of human origin. This ratio may distinguish between pollution of human and animal origin under certain conditions but is not a reliable indication of pollution origin. The results show that runoff from the informal settlement constituted a major source of human faecal pollution for a river used as a downstream source of water for human consumption. It further showed that faecal pollution of human and animal origin can reliably be distinguished by means of appropriate combinations of indicators which may include sorbitol-fermenting bifidobacteria.

Introduction

Urban settlements contribute to pollution of aquatic environments (Quereshi and Dutka, 1979). Human activities within urban settlements create both point and non-point sources of inorganic and organic pollutants that find their way into rivers and streams. Surface runoff from informal settlements and residential areas with inadequate sanitary facilities adversely affects the quality of receiving waters (Jagals, 1994).

Indicator micro-organisms can be used to determine the level of faecal pollution in water. Bacteria from the faecal coliform group are popular to use (*Standard Methods*, 1992), and are a realistic indication of faecal pollution of water (Geldreich, 1976).

However, faecal coliforms have certain drawbacks as indicators, one of which is the specific indication of the levels of human faecal pollution (Geldreich and Kenner, 1969; Grabow, 1983).

An increasing requirement for microbiological indicators is to distinguish between faecal pollution of human and animal origin. The distinction between human and animal pollution may be very useful in epidemiological studies or tracing the source of faecal contamination of water (Mara and Oragui, 1983). A further value of such distinction is the development of sanitary education programmes for developing communities. A confirmed pollution source is useful information for focused sanitary programme design. This may help community development workers to obtain community participation to address the problem. It is, therefore, valuable to develop techniques using highly specific bacterial indicators (Jagals, 1994).

The Modder River (Fig. 1), is a major source of potable water for the city of Bloemfontein, South Africa. Surface runoff from a large low socio-economic rural urban development some 60 km east of Bloemfontein reaches a tributary of the Modder River. The impact of this surface runoff on the sanitary quality of Modder River water was investigated. Due to poor sanitation standards existing in the settlement, special emphasis was placed on testing the health-related microbial quality of the river water.

Keeping domestic and other farming-related livestock within city limits is customary in such developing regions. These usually substantial concentrations of animals also contribute to faecal pollution of the environment. However, the risk of infection to humans due to human faecal pollution is higher than risks due to faecal pollution of animal origin (Jagals et al., 1994). A mechanism to distinguish between animal and human faecal pollution may be a valuable tool to assess such a risk.

In the past, one of the methods recommended to distinguish between human and animal faecal pollution was to make use of the ratio between faecal coliforms and faecal streptococci (FC/FS ratio) (Geldreich, 1976). This method is unreliable (Jagals, 1994; Mara and Oragui, 1985; *Standard Methods*, 1992).

Various factors can give rise to inaccurate interpretation of results from such ratios (Mara and Oragui, 1985). These include differential die-off of these organism groups and inconsistent occurrence of these organisms in the intestines of humans and warm-blooded animals in various parts of the world. Other indicator methods are therefore required. During this study an alternative method was investigated to give a more reliable indication of the source of faecal pollution. Sorbitol-fermenting bifidobacteria were previously reported to specifically indicate human faecal pollution (Mara and Oragui, 1985). The occurrence of these organisms was investigated in the same pollution environment as the more generally used indicator organisms such as faecal coliforms and streptococci.

This study deals with an evaluation of sorbitol-fermenting bifidobacteria as indicators of faecal water pollution from a human source, and also assesses the impact of surface runoff from a high-density low socio-economic settlement with limited sanitation, on the microbiological quality of water in a river system used downstream for domestic and recreational purposes.

* To whom all correspondence should be addressed.

☎ (051) 407-3130; fax (051) 407-3199; e-mail jagals@studm.tofs.ac.za

Received 30 August 1995; accepted in revised form 23 February 1996.

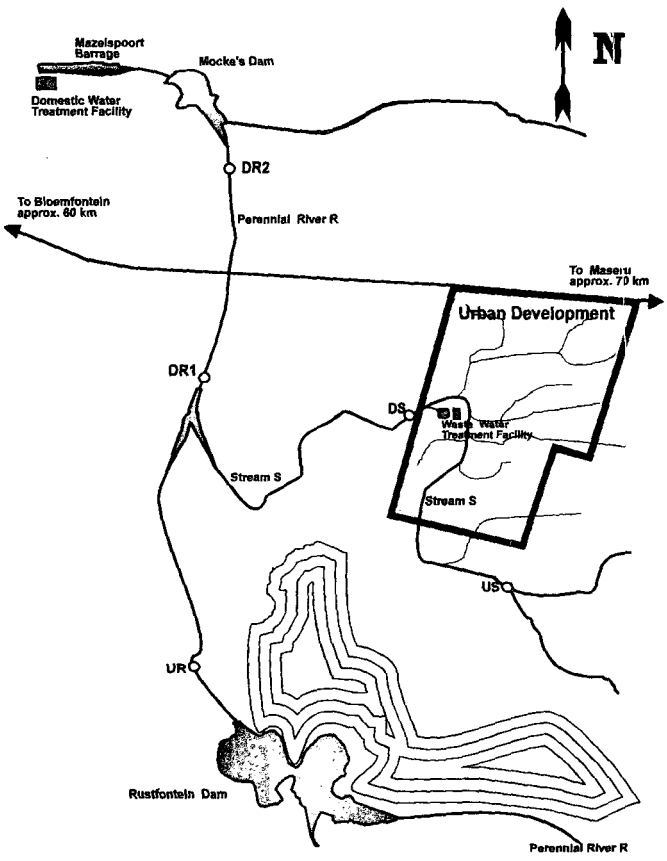

Figure 1
Target section of the Modder River indicating the sampling sites

Materials and methods

Study area (Fig. 1)

A high-density low socio-economic informal settlement, with approximately 200 000 inhabitants and limited sanitary facilities, was selected. Sanitation consisted mainly of pit latrines. A smaller portion of the residences were provided with bucket latrines. Waterborne sewerage was available only to a minor part of the settlement.

Sampling (Fig. 1)

Samples were collected during the dry season of the year, the rainy season and immediately after thunder-showers from a non-perennial stream (S) which runs through the settlement, and a perennial river (R), into which the stream drains. Sampling sites (Fig. 1) were on stream S upstream (US) and downstream (DS) of the settlement, and from river R upstream (UR), immediately downstream (DR1) and some 20 km downstream (DR2) of the inflow of stream S.

Upstream sampling sites US and UR represented points where water in the stream and river was not exposed to known faecal pollution of human origin. Water in the stream at DS was exposed predominantly to human faecal pollution from the settlement, as was water in the river at DR1. Samples from water in the river at DR1 and DR2 were analysed in order to assess the survival of organisms in the stream and river environment over a distance of some 20 km from the point source of urban pollution.

TABLE 1
COUNTS OF MICRO-ORGANISMS PER 100 mℓ IN A STREAM AND RIVER EXPOSED TO FAECAL POLLUTION FROM URBAN EFFLUENTS DURING THE DRY SEASON AND STORM-WATER RUNOFF DURING THE RAINY SEASON

	Sorbitol-fermenting bifidobacteria		Faecal coliforms		Faecal streptococci	
	Dry	Wet	Dry	Wet	Dry	Wet
US	*n* = 10 0	*n* = 11 21 (0 - 85)	*n* = 10 141 (2 - 930)	*n* = 11 1 679 (227 - 10 500)	*n* = 10 105 (8 - 467)	*n* = 11 1 479 (110 - 8 533)
DS	*n* = 16) 200 (1 - 3 600	*n* = 20 3 691 (60 - 92 000)	*n* = 16 550 (0 - 4 900)	*n* = 20 11 588 (960 - 840 000)	*n* = 16 158 (1 - 2 800)	*n* = 20 2 440 (92 - 65 000)
UR	*n* = 15 0	*n* = 13 0	*n* = 15 110 (5 - 1 600)	*n* = 13 234 (30 - 2 433)	*n* = 15 71 (1 - 880)	*n* = 13 272 (18 - 2 667)
DR1	*n* = 16 66 (1 - 470)	*n* = 17 871 (130 - 21 667)	*n* = 16 76 (4 - 780)	*n* = 17 4 120 (18 - 108 000)	*n* = 16 91 (8 - 628)	*n* = 17 2 454 (21 - 37 000)
DR2	*n* = 6 29 (3 - 270)	*n* = 7 66 (3 - 273)	*n* = 6 129 (15 - 250)	*n* = 7 668 (260 - 3 000)	*n* = 6 102 (8 - 260)	*n* = 7 537 (110 - 6033)

Stream S upstream (US) and directly downstream (DS) from low socio-economic urban settlement. River R upstream (UR) and immediately downstream (DR1) and 20 km downstream (DR2) of the inflow of stream S. Counts = geometric means on middle line and ranges on lower line. *n* Values on upper line = number of samples analysed.

Microbiological analyses

Samples were collected in sterile nalgene bottles, transported at 4 to 10°C and analysed within 5 to 8 h of collection. Tests were carried out in triplicate according to generally accepted basic laboratory procedures (*Standard Methods,* 1992; Grabow et al., 1993; Mara and Oragui, 1983) using the following methods:

Faecal coliforms

Faecal coliforms were enumerated by means of the membrane filter technique using m-FC agar (Difco) in triplicate at 44.5°C ± 0.2°C for 24 ± 2 h. Faecal coliform colonies appeared in various shades of blue.

Faecal streptococci

Faecal streptococci were enumerated by means of the membrane filter technique using m-Enterococcus agar (Difco) in triplicate. Membranes were placed on the agar and the plates were left to stand for 30 min before incubation at 35°C ± 0.5°C for 48 h. Faecal streptococci appeared as pink to red colonies.

Sorbitol-fermenting bifidobacteria

Sorbitol-fermenting strains of bifidobacteria were enumerated by means of the membrane filter technique using Human Bifid Sorbitol Agar (HBSA) according to the methods of Mara and Oragui (1983). Enumeration was done in triplicate. Plates were inverted and incubated anaerobically at 37°C ± 0.2°C for 48 h. Sorbitol-fermenting human bifidobacteria appeared as deep yellow, domed, mucoid colonies (Mara and Oragui, 1983).

The colonies were well cohered and stayed intact if touched by an inoculation needle-eye. They could also be readily moved about as a whole on the membrane, also corresponding to characteristics such as described by Mara and Oragui (1983). Microscopic confirmation of the organisms from the typical colonies showed configurations of bacterial strings resembling Arabic writing (sometimes referred to as Chinese writing), which are typical of *Bifidobacterium brevé* and *Bifidobacterium adolescensis,* the sorbitol-fermenting strains of bifidobacteria.

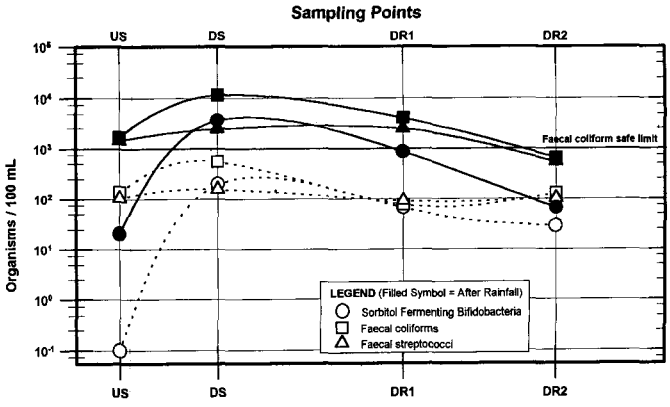

Figure 2
Sampling points on the stream and river downstream from confluence DR1
(Faecal coliform safe limit = SA Water Quality Guidelines (1993) Recreational use @ 1 000 org./100 mℓ)

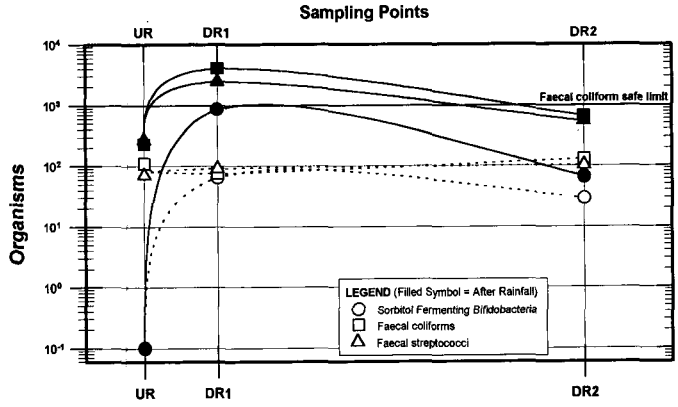

Figure 3
Sampling points on the river including the confluence point DR1
(Faecal coliform safe limit = SA Water Quality Guidelines (1993) Recreational use @ 1 000 org./100 mℓ)

Results

Features of counts in Table 1 that are important to the subject of this paper are illustrated in Figs. 2 and 3 and may be summarised as follows:

- During dry weather, the mean values for faecal coliforms and faecal streptococci in stream S obtained at all the sampling points (except DS) were within the same order. DS tested the highest mean values and ratio difference.
- During the rainy season levels of faecal coliforms and faecal streptococci at all sampling points increased. The most noticeable increase was at DS. Faecal streptococci were present at DR1 with levels similar to DS.
- After rain activity, relatively high counts of sorbitol-fermenting bifidobacteria were detected predominantly in downstream parts of the stream (DS) and river (DR1) exposed to pollution from the settlement. No sorbitol-fermenting bifidobacteria were isolated at US and UR during dry conditions but some numbers were found at US after rain activity (but still no presence at UR). At DR2, the numbers declined at a quicker rate than faecal streptococci and faecal coliforms.

Discussion

Bifidobacteria have been considered as the "ideal indicator" for faecal pollution because of their natural habitat in the faeces of man and a few warm-blooded animals (Mara and Oragui, 1983). All the members of the bifidobacteria group are, however, not specific enough to the faeces of man (Resnic and Levin, 1981). Due to the occurrence of bifidobacteria in animal faeces, this group could as a whole not suit the purpose of distinction. However, Mara and Oragui (1983), consistently isolated sorbitol-fermenting bifidobacteria (on the highly selective HBSA growth medium of Mara and Oragui (1983)) almost exclusively from the faeces of humans. Sorbitol-fermenting bifidobacteria (mainly *Bifidobacterium adolescensis,* and *Bifidobacterium brevé*) constitute about 93% of total bifidobacteria found in human faeces. With regard to the distinction between faecal pollution of human and animal origin, the results in Table 1 show that sorbitol-fermenting

bifidobacteria are highly specific indicators for faecal pollution of human origin. These organisms were found in the target stream and river wherever human faecal pollution was evident.

The presence of sorbitol-fermenting bifidobacteria in the upstream sampling point of the stream (US) during wet conditions, while totally absent in the up-river samples during rainy spells, was due to human activity (laundering, watering livestock, swimming) at point US which was much closer to the settlement than UR. Constant deposition of fresh human faeces on land in the vicinity of the sampling point became evident after subsequent investigation. These substances flushed into the stream during rain, emphasising the indicator value of sorbitol-fermenting bifidobacteria as specific for human faecal pollution. Without including these organisms in a combination of indicators, it would not have been possible to establish human faeces as part of the pollution source at this point. The FC/FS ratios for US were 1.3 and 1.1 for the dry and rainy season respectively and would not have been meaningful in this regard.

The ratio of faecal coliforms to faecal streptococci in the stream immediately downstream of the settlement at DS was 3.5 during the dry season and 4.7 after thunder-showers, which was considerably higher than ratios upstream of the settlement and further downstream which varied from 0.8 to 1.7 (Table 1). This would seem to agree somewhat with findings elsewhere (Geldreich, 1976; Clausen et al., 1977; *Standard Methods*, 1992) according to which a ratio of less than 0.7 would indicate faecal pollution of predominantly animal origin. The river environment downstream from point DS to DR1 was subjected to intensive agricultural activities including livestock farming. Judged by the suggested FC/FS ratio, this could have led to the conclusion that pollution in this vicinity could be from animal origin, had it not been for the presence of the bifidobacteria.

Furthermore, the ratio only seemed to have meaning after marked thunderstorm activity, showing that the indicator value of FC/FS ratios was limited to heavy storm-water runoff situations. Sorbitol-fermenting bifidobacteria had in all these instances, provided a good indication of pollution origin.

Numbers of sorbitol-fermenting bifidobacteria were generally lower than those of faecal coliforms in the stream and river immediately downstream of the human settlement during both dry and wet seasons (Table 1). However, the ratio of faecal coliforms to sorbitol-fermenting bifidobacteria increased in the river from DR1 to DR2 some 20 km downstream, suggesting that the bifidobacteria are less resistant to conditions in the river environment than faecal coliforms. This agrees with findings of Resnic and Levin (1981), that bifidobacteria are short-lived in the environment. The presence of sorbitol-fermenting bifidobacteria would, therefore, indicate recent faecal pollution of human origin.

Notwithstanding the fact that downstream die-off of the bifidobacteria limited the indicator value of these organisms to that of recent pollution, these organisms showed significant and unexpected resilience during flow of polluted water downstream. Because of varying volumes of stream flow, it was not possible to estimate the time it would take for a quantity of water to reach this downstream point from the settlement. However, a general estimate of flow judged by flow figures obtained from the Department of Water Affairs and Forestry indicated that it a time-span of between 6 h to 3 d would be applicable. The presence of these organisms so far downstream from the point source of pollution, after such a possible time lapse, indicated some preservation mechanism that is at present being further investigated.

Conclusion

Sorbitol-fermenting bifidobacteria are reliable indicators of faecal water pollution of human origin. This organism group can be used with more conventional indicator organisms to confirm pollution origin, especially as ratios between faecal coliforms and faecal streptococci cannot reliably distinguish between human and animal faecal pollution of water. Due to the anaerobic nature of sorbitol-fermenting bifidobacteria, this group has specific value as indicators of recent human faecal pollution of water. However, indications are that these organisms can persist under certain environmental conditions, which, if the reasons for this can be ascertained, should enhance their indicator value.

Acknowledgements

Financial support for this project by the Foundation For Research Development is gratefully acknowledged.

References

CLAUSEN EM, GREEN BL and LITSKY W (1977) Faecal streptococci: Indicators of pollution. In: Hoadley AW and Dutka BJ (eds.) *Bacterial Indicators/Health Hazards Associated With Water*, ASTM STP 635. American Society for Testing and Materials, Philadelphia. 247-264.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (1993) *South African Water Quality Guidelines.* Volumes **1&2**, Pretoria.

FEACHEM RG (1983) *Sanitation and Disease: Health Aspects of Excreta and Wastewater Management.* John Wiley & Sons, New York.

GELDREICH EE and KENNER BA (1969) Concepts of faecal streptococci in stream pollution. *J. of the Water Pollut. Control Fed.* **41** R336-R352.

GELDREICH EE (1976) Faecal coliform and faecal streptococcus density relationships in waste discharges and receiving waters. *CRC Critical Rev. on Environ. Control* 349-369

GRABOW WOK (1983) *Tentative Safety Guidelines for Highest Acceptable Counts of Micro-organisms in Various Waters.* National Institute for Water Research, CSIR, Pretoria, South Africa.

GRABOW WOK, HOLTZHAUZEN CS and DE VILLIERS CJ (1993) Research on Bacteriophages as Indicators of Water Quality, 1990-1992. Water Research Commission, Pretoria.

JAGALS P (1994) The Effect of Diffuse Effluents on the Quality of Water in the Modder River. M Dissertation, Technikon Free State, Bloemfontein.

JAGALS P, GRABOW WOK and DE VILLIERS CJ (1995) Evaluation of indicators for assessment of human and animal faecal pollution of surface runoff. *Health Related Water Microbiol.: Water Sci. and Technol.* **31** (5-6) 235 - 241.

MARA DD and ORAGUI JI (1983) Sorbitol-fermenting bifidobacteria as specific indicators of human faecal pollution. *J. Appl. Bacteriol.* **55** 349-357.

MARA DD and ORAGUI JI (1885) Bacteriological methods of distinguishing between human and animal faecal pollution of water: Results of fieldwork in Nigeria and Zimbabwe. *Bull. World Health Organ.* **63** 773 - 783.

ORAGUI JI and MARA DD (1983) Investigation of the survival characteristics of *Rhodococcus coprophilus* and certain faecal indicator bacteria. *Appl. Environ. Microbiol.* **46** 356-360.

QUERESHI AA and DUTKA BJ (1979) Storm runoff microbiology adds to concerns. *Water Sewage WKS* (March) 86-88.

RESNICK IG and LEVIN MA (1981) Assessment of bifidobacteria as indicators of human faecal pollution. *Appl. Environ. Microbiol.* **42/3** 433-438.

STANDARD METHODS (1992) *Standard Methods for the Examination of Water and Wastewater* (18th edn.). Clesceri LS, AE Greenberg and AD Eaton (eds.) APHA, Washington DC.