# The use of microbiology in the study of hygiene behaviour

E. C. Kaltenthaler[1]*, B. S. Drasar[2], and C. W. Potter[3]

[1]Health Research Institute, Sheffield Hallam University, Collegiate Crescent Campus, Sheffield S10 2BP, Great Britain, [2]Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, and [3]Institute for Cancer Studies, University of Sheffield Medical School.

*Key words:* faecal coliforms, hygiene behaviour, faecal indicator bacteria

## Abstract

Faecal indicator bacteria have been used to measure levels of hygiene in a variety of settings. This paper describes a study in northern Botswana which used the isolation of faecal indicator bacteria in combination with other quantitative and qualitative techniques to gain information regarding hygiene behaviour. The microbiological samples included, samples from stored drinking water and water sources; eating plates; infant feeding bottles; dishcloths and the fingertips of carers and children. Water was usually clean at source but contaminated after storage. Presumptive faecal coliforms contaminated 31% of the eating plates, 29% of the dishcloths and 40% of the infant feeding bottles. Many of the presumptive faecal coliform isolates were not identified as *Escherichia coli*, indicating the need for further research into methodologies appropriate for isolating *E. coli* in tropical climates.

## Introduction

Hygiene behaviour is defined as actions which promote health, and particularly those associated with the prevention of water and sanitation related infectious diseases (Boot and Cairncross, 1993). Microbiology provides a useful tool when studying the relationship between hygiene behaviour and health as it gives an objective measure of faecal contamination. Using microbiology gives evidence of links between specific hygiene behaviour and health. Through the use of microbiological techniques, a considerable amount of information can be obtained regarding behaviour which results in faecal contamination and therefore poses a health risk.

The majority of investigations which have used microbiological techniques to assess hygiene behaviour have measured levels of faecal contamination using faecal indicator bacteria rather than pathogenic bacteria (Kaltenthaler *et al.*, 1991; Pinfold *et al.*, 1988). Most commonly used are faecal coliforms and faecal streptococci. However, some studies have isolated pathogens from the environment (Vadivelu *et al.*, 1989). Microbiological techniques can be applied to a variety of surfaces including water, food, human hands, and working surfaces. The faecal indicator bacteria which were isolated provide a useful alternative to the isolation of pathogens since they were present in greater numbers, survived for longer periods and the methods

associated with their isolation were easier and more economical to use (PHLS, 1984). The isolation of faecal indicator bacteria can be used as a proxy for monitoring diarrhoea morbidity and mortality which can be very time consuming and costly. If faecal indicator bacteria are isolated there is the potential for diarrhoea resulting in other micro-organisms being present.

This paper describes a study from northern Botswana which used a variety of simple, low-cost microbiological techniques to gain information regarding possible faecal-oral transmission routes. The following samples were collected for bacteriological analysis as part of a hygiene behaviour study: stored drinking water, water sources, eating plates, dishcloths, impressions from fingertips of carers and children under 6 years old. The information obtained in this work provided the basis for hygiene education programmes as well as identifying possible areas warranting further research.

## Materials and methods

Two villages were included in the investigation with 55 households randomly chosen from each of these giving a total of 110 households. The following samples were collected from each household: stored drinking water, water from the source, a swab from a plate used for eating, a swab from an infant feeding bottle if available, a swab from a dishcloth, and fingertip impressions from the carers and any children present under 6 years old.

### Water samples

The samples were collected in sterile, screw-capped bottles. The carer in each household was asked to provide the cup usually used for dipping into the drinking water container. This cup was dipped into the water which was then poured into sterile 100 ml bottles. Samples from the water sources were collected by directly placing the bottle under the tap or into the stream. The samples were analysed using the membrane filtration method (PHLS, 1984). The membranes were placed on sterile absorbant paper pads saturated with membrane lauryl sulphate broth (Oxoid). The plates were left at room temperature (~30°C) for 3 to 4 h in order to resuscitate stressed micro-organisms. The plates were then incubated at 44°C in sealed plastic bags to prevent the pads from drying out. After 15 h incubation all the bright yellow colonies with colonial morphology characteristic of lactose fermenting faecal coliforms were counted (convex, at least 1 mm in diameter). Large, mucoid colonies, presumably *Klebsiella* were not counted as faecal coliforms. The counts were expressed per 100 ml and counts over 1,000 were expressed as 1,000/100 ml. Confirmatory

tests were conducted on all faecal coliform colonies where it was possible to isolate a single colony.

#### Plates for eating, infant feeding bottles and dishcloths

A sterile cotton swab dipped in quarter strength Ringer's solution was swabbed over a defined area of 5 $cm^2$ for 5 s over the eating plate. The swab was then spread over an entire plate of MacConkey agar number 3. It was also noted whether or not the eating plate was washed or unwashed. For bottles a swab was spread over the teat for 5 s and then onto a plate of MacConkey agar number 3. All the plates were incubated for 24 h at 37°C, and all faecal coliform colonies were subcultured for confirmatory tests. The methods used were as described by Ekanem *et al.* (1983). The procedure for dishcloths was essentially the same as for eating plates. It was also noted when the cloth was damp or dry.

#### Fingertips

Each carer and all children in the study were asked to gently place each fingertip onto a plate of MacConkey agar number 3. The top of each plate was marked and the fingers placed in a pattern so that it was possible to identify the imprints after incubation. The plates were incubated at 37°C for 24 h. Again, all non-mucoid faecal coliform colonies were subcultured for confirmatory tests.

## Results

#### Water samples

The mean presumptive coliform counts for stored drinking water in the two villages are shown in Table 1. With regard to water sources, most families had access to public standpipes. In village A all 20 standpipes were tested (mean = 0.5/100 ml, sd = 1.24, n = 20). In village B all nine standpipes were tested (mean = 111.67/100 ml, sd = 333.13, n = 9). One standpipe in this village had a count of 1,000/100 ml while the other eight were between 0–4/100 ml. The private standpipes in the two villages were also tested (mean = 0.25/100 ml, sd = 0.71, n = 8). The unprotected sources included two large ponds, a spring and a river. Both ponds were contaminated: village A 220/100 ml and village B 1,000/100 ml. The spring and river were less contaminated with the former having a count of 10/100 ml and the latter a count of 0/100 ml.

These results show that water was usually clean at source, except for the ponds which were heavily used by animals. However, upon storage water often became contaminated emphasising the need for hygiene

**Table 1** Mean presumptive faecal coliform counts in stored drinking water

| Counts per 100 ml: Village A | Village B | p |
|---|---|---|
| 52.87 ± 194.53 | 95.30 ± 272.36 | 0.74 |
| n = 52 | n = 50 | |

education with regard to water storage. Interestingly the spring and river were not heavily contaminated. Both were protected from animals in that the spring was surrounded by branches and the river was a dry, sandy river bed where women dug a hole to facilitate collection from the resulting pool. Water from standpipes was usually stated as the preferred source for drinking and domestic use but this was not always the case.

**Table 2** Plate and cloth counts: presence of presumptive faecal coliforms by condition

| | n | Presumptive faecal coliforms: Negative | Positive |
|---|---|---|---|
| CLOTHS | | | |
| Wet | 27 | 13 (48%) | 14 (52%) |
| Damp | 24 | 21 (88%) | 3 (13%) |
| Dry | 47 | 36 (77%) | 11 (23%) |
| PLATES | | | |
| Washed | 34 | 24 (71%) | 10 (30%) |
| Unwashed | 75 | 51 (68%) | 24 (32%) |

For cloths: $\chi^2 = 10.82$; df = 2; p = 0.004. For plates: $\chi^2 = 0.0$; p = 0.96.

Even small amounts of faecal contamination in drinking water pose a public health threat as there is always the possibility of pathogenic bacteria also being present. The infective dose of *E. coli* able to initiate infection can be as low as 100 organisms (Drasar and Barrow, 1985).

**Plates for eating, infant feeding bottles and dishcloths**

In Table 2 the results of the plate and cloth samples are recorded, and there did not appear to be any difference in counts between washed and unwashed plates. Dishes were often washed in dirty, previously used water without soap. For the cloths, those which were wet were more likely to have positive counts than those that were dry. This result was not surprising as wet cloths would enable bacteria to survive longer than dry cloths.

Infant feeding bottles were found in fourteen households giving a total of fifteen bottles tested. Only the bottles seen in the compound were tested. No questions were asked about the existence of bottles if none were seen. Of the fifteen bottles, five had no growth (33.3%), six had faecal coliforms (40%) and four had *Klebsiella* colonies (26.7%). Although this is a very small sample size, many studies have pointed to problems with contamination of bottles, even in developed countries (Anderson and Gatherer, 1970; Philips *et al.*, 1969). This is an area requiring further research to determine the extent of the problem.

**Fingertips**

Table 3 shows the results of the fingertip microbiology assessment. Fingertip impressions were made of 278 carers and children. There did not appear to be a difference in counts between carers and children. There was also no appreciable difference in counts with regard to activity. This is in contrast to other studies which have found a relationship between counts and activity prior to testing (Kaltenthaler *et al.*, 1991; Pinfold, 1990). Low isolation rates of faecal bacteria on fingertips has been reported in other studies (Kaltenthaler *et al.*, 1995). The skin provides an unfavourable environment for bacteria and in laboratory tests 99% of inoculated *E. coli* died within 10 min (Pinfold, 1990).

**Confirmatory test results**

The confirmatory tests for *E. coli* were conducted in the Department of Medical Microbiology, University of Sheffield Medical School. Not all colonies were tested as some were obviously mucoid or there was confluent growth making it impossible to isolate single colonies. The isolates were stored on nutrient agar slopes before confirmatory tests were carried out. Some isolates did not appear to survive on the slopes. A total of 71 colonies were tested. Of the water samples 32% were confirmed as *E. coli*, and 14% from the hands and 24% from

**Table 3** Fingertip impression plate counts

| | n | Presumptive faecal coliforms: Positive | Negative |
|---|---|---|---|
| SOURCE | | | |
| Carers | 108 | 19 (17.6) | 89 |
| Children | 170 | 34 (20.0) | 136 |
| Total* | | 53 | 225 |
| (chi square=0.12, p value=0.73) | | | |
| ACTIVITIES | | | |
| Sitting/sleeping | 76 | 10 (13) | 66 |
| Playing | 89 | 16 (18) | 73 |
| Eating/food preparation | 60 | 14 (23) | 46 |
| Washing dishes/clothes/body | 18 | 5 (28) | 13 |
| Housework, sweeping, Building, tobacco grinding | 14 | 3 (21) | 11 |
| Other activities | 21 | 5 (24) | 16 |

*$\chi^2 = 0.12$; $p = 0.73$.
Activities: $\chi^2 = 3.74$; df = 5; $p = 0.59$. Percentages shown in parentheses.

environmental samples were also confirmed as *E. coli*. The results of the confirmatory tests are shown in Table 4. Other studies have indicated that a large percentage of presumptive faecal coliforms were not *E. coli*, particularly in tropical climates (PHLS, 1984; Feachem *et al.*, 1983).

## Discussion

This study identifies some of the problems associated with isolating *E. coli* from environmental samples in tropical climates. Of particular importance was that after storage several isolates of presumptive faecal coliforms were no longer viable. They may have been damaged by the filtration process or growth on membrane lauryl sulphate broth. It is intended that confirmatory tests will be carried out as soon as possible.

**Table 4** Results of confirmatory tests for faecal coliforms

| Source | n | Number of samples positive |
|---|---|---|
| Water | 19 | |
| *Enterobacter* | | 8 |
| *E. coli* | | 6 |
| *Klebsiella* | | 1 |
| *Citrobacter* | | 3 |
| Unknown | | 1 |
| Hands | 7 | |
| *Acinebacter* | | 1 |
| *Citrobacter* | | 1 |
| *Enterococcus* | | 1 |
| *Enterobacter* | | 1 |
| *E. coli* | | 1 |
| Unknown | | 2 |
| Environmental | 45 | |
| *Enterobacter* | | 8 |
| *Escherichia sp.* | | 4 |
| *E. coli* | | 11 |
| *Citrobacter* | | 6 |
| *Enterococcus* | | 1 |
| *Klebsiella* | | 1 |
| *Serratia* | | 2 |
| *Aeromonas* | | 5 |
| *Hafnia* | | 1 |
| Unknown | | 6 |

A solution to the problem of thermotolerant faecal coliforms may be the use of faecal streptococci as an indicator of faecal contamination particularly for fingertip microbiology as they survive longer on the skin (Kaltenthaler and Pinfold, 1995).

Applications for these methods have included community studies and hospital investigations as well as their use in developing countries. The community day care centres have been the focus of much attention. Ekanem *et al.* (1983) found that during outbreaks of diarrhoea, faecal coliforms were recovered more frequently from hands

and classroom objects. Laborde *et al.* (1993) showed that in day care centres faecal contamination of hands, sinks and taps were predictors of diarrhoeal risk. In primary schools in Leeds, England, faecal streptococci were isolated from children's hands and those children with poor hygiene training tended to have more faecal contamination on their hands than those children with better hygiene knowledge (Kaltenthaler *et al.*, 1995). In hospitals, faecal coliforms have been used to assess the spread of hospital infections (Sanderson and Weissler, 1992).

Hygiene indices can be developed by combining faecal bacteria isolation from various sites to form an overall indicator of hygiene status. These indices can then be used to determine whether children from families with poor hygiene have more or less diarrhoea than those families with better hygiene standards. As part of this work, a hygiene index was developed for each family combining bacteriological counts for plate, dishcloths and fingertips; distance to water source; housing conditions; animals in the kitchen; distance to water source, toilet facilities and faeces in the soil. Children from families with poor hygiene conditions had more diarrhoea than children from families with good levels of hygiene (Kaltenthaler and Drasar, 1996).

Several low cost methods were utilised which were appropriate for studying hygiene behaviour in developing countries using minimum expertise. By isolating faecal indicator bacteria it is possible to quantify hygiene behaviour and thus facilitate measuring changes which may be brought about through health education intervention. Indeed, quantifying hygiene behaviour facilitates measuring variations between communities.

## References


ANDERSON J.A. D. and Gatherer A. 1970. Hygiene of infant-feeding utensils, practices and standards in the home. *Br. Med. J.* **2** 20–3.

BOOT M. T. and Cairncross S. (Editors). 1993. Actions speak: the study of hygiene behaviour in water and sanitation projects. IRC International Water and Sanitation Centre and London School of Hygiene and Tropical Medicine, The Hague, The Netherlands.

DRASAR B. S. and Barrow P. A. 1985. *Intestinal Microbiology.* Van Nostrand Reinhold Co. Ltd, Wokingham, England.

EKANEM E. E., Dupont H. L., Pickering L. K., Selwyn B. J., and Hawkins C. M. 1983. Transmission dynamics of enteric bacteria in day-care centers. *Am. J. Epidemiol.* **118** 562–72.

FEACHEM R. G., Bradley D. J., Garelick H. and Mara D. 1983. Detection, survival and removal of pathogens in the environment. *In* Sanitation and Disease: Health Aspects of Wastewater Management. J. Wiley, Chichester, England.

KALTENTHALER E., Waterman R. and Cross P. 1991. Faecal indicator bacteria on the hands and the effectiveness of hand-washing in Zimbabwe. *J. Trop. Med. Hyg.* **94** 358–63.

KALTENTHALER E. C. and Pinfold J. V. 1995. Microbiological methods for assessing handwashing practice in hygiene behaviour studies. *J. Trop. Med. Hyg.* **98** 101–6.

KALTENTHALER E. C., Elsworth A. M., Schweiger M. S., Mara D. D. and Braunholtz D. A. 1995. Faecal contamination on childrens' hands and environmental surfaces in primary schools in Leeds. *Epidemiol. Infect.* **115** 527–34.

KALTENTHALER E. A. and Drasar B. 1996. The study of hygiene behaviour in Botswana: a combination of qualitative and quantitative methods. *Trop. Med. Intern. Hlth* In press.

LABORDE D. J., Weigle K. A., Weber D. J. and Kotch J. B. 1993. Effect of faecal contamination on diarrheal illness rates in day-care centers. *Am. J. Epidemiol.* **138** 243–55.

PHILLIPS I., Lwanga S. K., Lore W. and Wasswa D. 1969. Methods and hygiene of infant feeding in an urban area of Uganda. *J. Trop. Pediat.* **15** 167–71.

PINFOLD J., Horan N. and Mara D. 1988. The faecal coliform fingertip count: a potential method for evaluating the effectiveness of low cost water supply and sanitation initiatives. *J. Trop. Med. Hyg.* **91** 67–70.

PINFOLD J. V. 1990. Faecal contamination of water and fingertip rinses as a method for evaluating the effect of low-cost water supply and sanitation activities on faecal-oral disease transmission. 1. A case study in rural northeast Thailand. *Epidemiol. Infect.* **105** 363–75.

PUBLIC Health Laboratory Service (PHLS). 1984. *The bacteriological examination of water supplies.* HMSO Report 71, London.

SANDERSON P. J. and Weissler S. 1992. Recovery of coliforms from the hands of nurses and patients: activities leading to contamination. *J. Hosp. Infect.* **21** 85–93.

VADIVELU J., Feachem R. G., Drasar B. S., Harrison T. J., Parasakthi N., Thambypillai V. and Puthucheary S. D. 1989. Enterotoxigenic *Escherichia coli* in the domestic environment of a Malaysian village. *Epidemiol. Infect.* **103** 497–511.

Accepted 20 December 1996