

Microbiological methods for assessing handwashing practice in hygiene behaviour studies

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SUMMARY

Personal hygiene, especially handwashing, is frequently mentioned as an important aspect of diarrhoeal disease prevention in water and sanitation programmes. Handwashing practice is difficult to assess but the microbiological analysis of hands shows promise as an indicator of this behaviour. Most methods for isolating bacteria from the hands have been developed for use in hospitals in order to investigate the spread of nosocomial infections. However, reliable and inexpensive methods which need only limited expertise are needed for use in developing countries where diarrhoeal diseases remain a major health risk. Techniques for sampling hands and bacteriological analysis methods are discussed with special emphasis on practical considerations for conducting tests in developing countries. Several studies have used these methods successfully and have investigated hygiene behaviour and how living conditions affect behaviour and the role of hands in diarrhoeal disease transmission. We recommend the use of impression plates for isolating faecal indicator bacteria from the hands and also recommend faecal streptococci as an indicator of faecal contamination.

Keywords: handwashing, hygiene behaviour, faecal bacteria

INTRODUCTION

Diarrhoeal disease continues to be a major cause of morbidity and mortality among children in developing countries. Pathogens causing diarrhoea are spread via the faecal-oral route (Feachem 1984) and infection may be caused by drinking contaminated water. There are also a variety of other ways by which diarrhoea is transmitted almost all of which, either directly or indirectly, involve the hands. In many settings improving water quality alone has had little impact on diarrhoeal incidence, whereas overall improvements to water supply and sanitation activities have been far more effective (Esrey *et al.* 1985). Hygiene behaviour plays an important part in low-cost water supply and sanitation

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programmes but it is an area often overlooked because of the difficulties in studying behaviour. Recently, however, handwashing has provoked special attention because of its important role in preventing cross-contamination especially at the oral end of faecal-oral transmission.

Several studies in a variety of settings have shown a relation between promoting handwashing practices and diarrhoeal disease with reductions of between 26 and 48% in diarrhoeal morbidity being achieved (Khan 1982; Black *et al.* 1991; Stanton & Clemens 1987; Han & Hlaing 1989; Alam *et al.* 1989). Although this relation appears clear, the very nature of handwashing makes it difficult to measure; it is not easy to observe and there are no visible signs that illustrate the effectiveness of handwashing methods. One study in Bangladesh used continuous observation of hygiene

Table 1. Methods for isolating bacteria from the hands

Technique	Description of method	Representative studies
Glove	Hand placed in glove or bag with measured volume of sterile solution	Salzman <i>et al.</i> (1967); Casewell and Phillips (1977); Laborde <i>et al.</i> (1993)
Rinse	Hand or fingertips rinsed and rubbed in sterile solution for specific length of time	Price (1938); Tebbutt (1984); Tebbutt & Southwell (1989); Hoque and Briend (1991); Pinfold <i>et al.</i> (1988); Pinfold <i>et al.</i> (1990a); Kaltenthaler <i>et al.</i> (1991)
Swab	Swab dipped in sterile solution and used to sample parts of the hands	Hart <i>et al.</i> (1981); Evans & Stevens (1976); Han <i>et al.</i> (1986)
Impression	Fingertips pressed directly onto selective agar	Coates <i>et al.</i> (1987); Ekanem <i>et al.</i> (1983); Henry & Rahim (1990); Echeverria <i>et al.</i> (1987); Vadivelu <i>et al.</i> (1989); Van <i>et al.</i> (1991); Pinfold (1993); Kaltenthaler <i>et al.</i> (1994)

practices in order to assess risk factors of diarrhoea and demonstrate whether behaviour change occurred from the intervention (Stanton & Clemens 1987). When quantitative data are required such methods are time consuming, require considerable numbers of trained personnel and are difficult to standardize. It is not clear from observation alone how effective handwashing is, nor how this effectiveness changes with intervention.

With increased emphasis on the study of hygiene behaviours as a component of low-cost water supply and sanitation programmes, simple indicators need to be developed. One promising indicator involves isolating faecal bacteria from hands. This paper reviews methods for sampling hands under different settings and discusses how information on hand contamination has been useful in hygiene behaviour studies. A more comprehensive document concerning qualitative and quantitative methods for studying human behaviour in relation to water and sanitation has been published (Boot & Cairncross 1993).

METHODS OF SAMPLING HANDS

Before investigating various microbiological methods used to analyse bacteria from hands, it is important to

understand the environment hands provide for bacteria. Hands contain bacteria similar to other skin sites on the body, namely coagulase-negative staphylococci and coryneform bacteria (Larson 1985). The greatest concentrations of hand bacteria are under and around the fingernails (Rayan & Flournoy 1987). These are known as resident bacteria, which are defined as those bacteria that survive and multiply on the skin and can be cultured repeatedly. In contrast, transient organisms which are acquired by contact with a contaminated object are easily removed and do not multiply or survive long (Steere & Mallison 1975). Only transient bacteria are important in faecal-oral disease transmission.

Until recently, most methods for detecting bacteria on the hands were designed in hospital settings to investigate the spread of nosocomial infections and to determine the effectiveness of antimicrobial agents. Four basic methods are currently being used to both quantify and qualify bacteria on the hands and these are the glove, rinse, swab and impression methods (Table 1).

The glove technique is used for quantifying bacteria and can provide either total bacterial counts or counts of specific bacteria, when a selective medium is used. It is expensive and requires trained staff and a well

equipped laboratory. The rinse technique is also a quantifiable method and standard membrane filtration methods used in water microbiology can be applied to analyse the samples (DHSS 1982; APHA 1980). With the swab technique only small areas of the hands can be sampled at a given time and bacteria may cling to the swab. The impression plate method is the quickest to use as well as the least expensive. It does however have the disadvantage of not being as quantitative as the other methods.

The authors recommend the use of impression plates in hygiene behaviour studies as they are inexpensive, quick and technically simple to use. They also reduce the problem of administering sterile techniques in the field. Many samples may need to be taken due to daily variation in individuals' fingertips and the use of impression plates facilitates this.

Adopting these methods in developing countries

Before choosing a method it is essential to determine the availability of laboratory facilities and level of expertise. Water microbiology, however rudimentary, is fairly widespread, even in developing countries, and it seems sensible to 'borrow' from this field. Faecal indicator bacteria are used to demonstrate whether water has been contaminated and whether pathogens may also be present. No one bacterium fulfils the conditions of an 'ideal' faecal indicator but those often used are faecal coliforms, faecal streptococci and less commonly *Clostridium perfringens* (Feachem *et al.* 1983).

It is important to remember that hands provide a radically different environment to water. Faecal indicator bacteria all survive much longer in water than on skin. Survival of bacteria depends on factors such as humidity, pH, the presence of anti-bacterial substances in the skin and the presence of competitive organisms. In general, Gram-negative bacilli (including coliforms and bacterial pathogens) are more susceptible to desiccation than Gram-positive cocci (e.g. streptococci) and most of them do not survive long on the skin. Laboratory tests on clean hands have shown 99% of inoculated *E. coli* died within 10 minutes whereas faecal streptococci survived much longer with 10–30% surviving for 2–4 hours (Pinfold 1990a). Other experiments indicate that *Klebsiella*, *Enterobacter*, *Salmonella* and *Shigella* all survive longer on the skin than *E. coli* (Hart *et al.* 1981; Pether & Gilber 1971; Hutchinson 1956).

BACTERIOLOGICAL MATERIALS AND METHODS

With the impression technique, fingertips are pressed directly onto a selective media. The studies reviewed have used MacConkey agar (Oxoid) for isolating faecal coliforms. MacConkey agar no. 3 gives better differentiation between coliforms and non-lactose fermenting organisms than does ordinary MacConkey agar (Oxoid *Manual* 1982). Slanetz and Bartley agar (Oxoid) and KF Streptococcus agar (Oxoid) may be used for isolating faecal streptococci. The incubation period for Slanetz and Bartley agar is 4 hours at 37°C and 44 hours at 44°C; 48 hours at 37°C for KF Streptococcus agar. Plates on this medium may also be incubated at 44°C (Oragui & Mara 1981) and this will help to prevent the growth of staphylococci and non-faecal streptococci which are not completely inhibited on KF agar.

If a rinse method is chosen, there are a variety of media used for isolating faecal coliforms, the selection of which is generally dictated by availability. The studies reviewed preferred membrane lauryl sulphate broth (Oxoid) for the isolation of faecal coliforms by membrane filtration with an incubation period of 18 hours at 44°C. One study recommends the addition of penicillin B (50 mg l⁻¹) to this medium to inhibit the growth of *Staphylococcus epidermidis* which is part of the normal skin flora (Pinfold 1990a). Slanetz and Bartley agar (Oxoid) and KF Streptococcus agar (Oxoid) have also been used to isolate faecal streptococci from rinse samples. Solutions may also be tested for pathogenic bacteria if media and expertise are available.

Isolation of faecal coliforms and streptococci using both of these methods are presumptive only. A study of surface water in Nigeria found that the majority of presumptive faecal coliforms were not *E. coli* (Mara & Oragui 1985). In Thailand the proportion of *E. coli* to presumptive faecal coliforms was found to be less in fingertip rinses than in water samples; this may be due to different survival time on the skin (Pinfold 1990b). Colonies morphologically suggestive of *E. coli* can be confirmed by their ability to produce indole from tryptophan and gas from lactose with an incubation period of 24 hours at 44°C (Senior 1989). For the isolation of faecal streptococci, care must be taken when counting colonies as some can be very small. A study by Pinfold (1993) has found that some species of staphylococci are not completely inhibited and are

sometimes morphologically similar to streptococci colonies. These have been distinguished by the ability of staphylococci to produce gas from hydrogen peroxide (catalase test). This has been successfully applied without replating individual colonies. However, non-faecal streptococci are commonly found on the skin and produce a negative catalase reaction (Kaltenthaler *et al.* 1994). Therefore, colonies may need to be subcultured onto bile aesculin agar for confirmation of faecal streptococci; incubation is for 24 hours at 44°C.

The authors recommend the isolation of faecal streptococci due to their longer survival time on the skin. Faecal streptococci are also preferred because fewer technical problems are associated with their isolation. Low numbers of both faecal coliforms and faecal streptococci can be expected and therefore a large sample size may be necessary depending on the objective of the study being undertaken. Repeat sampling may also be necessary as results may vary due to a variety of factors such as activity before sampling and the relative humidity at the time of sampling.

PRACTICAL CONSIDERATIONS

Hospitals, universities and public health departments responsible for food and water microbiology may have the necessary facilities and personnel but some of these institutions may be overutilized. It is important to consider whether breakages can be readily repaired or replaced and to allow time for ordering media. Fundamental requirements are running water, incubator, refrigerator, bench space, hotplates, autoclave, flame and scales as well as various glassware and sampling equipment. Reliable transportation is also necessary. None of the bacteria discussed produce spores and therefore sterilizing by boiling equipment in water should be sufficient in most cases. Rinse techniques require a lot of glassware and samples may have to be transported back to the laboratory for subsequent analysis. Membrane filtration is possible in the field which greatly increases the number of samples that can be processed in one day. Acetone has been used to sterilize equipment, but more care is required to ensure sterile conditions (Pinfold 1990a). The impression technique is the simplest method and normally requires least equipment. Incubators may well be set at 37°C for routine microbiology and this would favour the selection of faecal streptococci. Personnel trained in water

microbiology would require little extra training for these methods. However, the isolation of pathogenic bacteria would generally be much more complicated.

REVIEW OF STUDIES APPLYING THESE TECHNIQUES

Using microbiological methods to assess handwashing behaviour can provide much useful information regarding the spread of faecal indicator bacteria and therefore the potential spread of faecal pathogens. Hand contamination has been used in public health studies to investigate the following:

- (1) Identification of behaviours which result in faecal contamination on the hands. In Zimbabwe and Thailand these methods have helped to identify behaviours resulting in faecal contamination which could be remedied by improving hygiene practices (Pinfold 1990a; Kaltenthaler *et al.* 1991). This type of investigation has proved particularly useful when combined with other methods of studying behaviour. Handwashing methods have also been evaluated in Bangladesh (Hoque & Briend 1991) and in Burma (Han *et al.* 1986).
- (2) Monitoring the effectiveness of hygiene interventions that involve handwashing. This has successfully been applied in Thailand (Pinfold 1990b).
- (3) Identifying risk groups. Many studies have investigated hand contamination of different groups of people such as food handlers and hospital staff and primary school children (Casewell & Phillips 1977; Tebbutt 1984; Tebbutt & Southwell 1989; Kaltenthaler *et al.* 1994; Hart *et al.* 1981). Studies in Lesotho and Thailand have shown that people living in homes with multiple water points have less hand contamination than people who have to carry water to their homes (Pinfold *et al.* 1988; Pinfold 1990a). In Zimbabwe, living on a commercial farm and having an infant in the family were found to be risk factors for having faecal contamination on the hands (Kaltenthaler *et al.* 1991).
- (4) Investigating the relationship between hand contamination and diarrhoeal incidence. In Bangladesh, diarrhoeal incidence was significantly correlated with the degree of hand contamination in the two study areas (Henry & Rahim 1990). Other studies in Malaysia and Thailand have concentrated on transmission of diarrhoea caused by enterotoxigenic *E. coli* (ETEC) (Vadivelu *et al.* 1989). Other studies have investigated

outbreaks of infant diarrhoea in day care centres (Ekanem *et al.* 1983; Laborde *et al.* 1993; Van *et al.* 1991). Because of the methodological problems and expense of full health impact evaluation studies, investigations are under way to assess whether hand contamination is a useful indicator of diarrhoea.

(5) Investigating the seasonal effects of disease transmission. Studies in both Zimbabwe and Thailand have shown hand contamination to be positively correlated with relative humidity and this has helped to shed some light on the seasonal patterns of diarrhoea (Kaltenthaler *et al.* 1991; Pinfold *et al.* 1991). Although many other factors will affect seasonal patterns, diarrhoea in Thailand was also found to correlate with humidity early in the rainy season.

These examples show the variety of possible applications of measuring faecal contamination on the hands. The methods are most useful when combined with other methods for studying hygiene behaviour and can be used to prioritize interventions in hygiene education programmes (Boot & Cairncross 1993). Although the spread of diarrhoeal diseases is not actually measured with these methods, they do provide an objective measure of the spread of faecal bacteria via the hands.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Sandy Cairncross for originally suggesting the idea for this paper as well as Dr Nigel Horan and Ms Marieke Boot for commenting on earlier drafts.

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