A bacteriological survey of washrooms and toilets

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SUMMARY

A survey of the bacterial flora present at various positions in 130 male and female washrooms and toilets is reported. Several bacteria of faecal origin were found in large numbers: the areas likely to be the most important sources of cross-infection from faecal contamination are indicated. The results are used to assess priorities for disinfection.

INTRODUCTION

Upon notification of a disease outbreak it has become usual to conduct a bacteriological survey of the environment of the infected patients in order to establish the aetiology of the infection. Several reported studies of this type have traced the source of infection to toilets. Outbreaks of Sonne dysentery have been studied by Hutchinson (1956) and more recently by Thomas & Tillett (1973) and surfaces such as toilet seats have been shown to be contaminated with *Shigella sonnei*. McCullagh's suggestion (1953) that the main cause of *Trichomonas vaginalis* infection was the water-closet seat and the later work of Whittington (1957) on the survival of this protozoon were confirmed by the studies of Burgess (1963). The flushing of toilets is known to produce bacteria-laden aerosols (Darlow & Bale, 1959; Bound & Atkinson, 1966) in which the particle size is small enough to cause respiratory tract infection.

Williams, Blowers, Garrod & Shooter (1966), in their review of cross-infection risks in hospitals, indicated that toilets presented a significant risk, particularly when used by persons suffering from gastro-intestinal or staphylococcal infections. However, their statements were not supported by bacteriological evidence and a recent survey of hospital toilets by Newsom (1972) showed that for well-maintained toilets cleaned daily the numbers of faecal bacteria were low. A similar conclusion was reached by Maurer, Efstratiou & Watson (1972). Newsom concluded from his results that there was little risk of cross-infection from toilets within a hospital unless the surfaces were heavily contaminated with faeces.

The controversy about the risks to health in washrooms and toilets and the lack of extensive survey data led us to carry out the present study. The aim was to provide information about types of bacteria and their populations occurring at various positions within a large random sample of washrooms and toilets from a range of premises. Barnard (1972) has shown the value of a bacteriological survey in devising the best method of disinfection and a further aim of our work was to use the results to indicate priorities for disinfection at various parts of the washrooms and toilets where necessary.

MATERIALS AND METHODS

The 130 sites chosen for this study were selected at random from buildings which included shops, offices, factories, railway premises, schools and hospitals.

Samples were taken by swabbing various positions in the washroom and toilet with Calgiswabs (Wilson Diagnostics Inc.) moistened with sterile Ringer's solution (quarter strength). For flat surfaces a 25 cm^2 template was used to keep the sampling area constant. For irregular surfaces such as tap and door handles the complete item was swabbed and the area calculated. Immediately after swabbing a surface the Calgiswab was sealed in a Bijou bottle (5 ml.) containing 5 ml. of sterile Ringer's solution (quarter strength) and stored in a cold box at $6-8^{\circ}$ C. to prevent further growth; all samples were mechanically shaken to disperse bacteria and examined within 24 hr. The main sampling positions used in the survey are listed in Table 1. Where results were likely to differ with changes in position (e.g. on the toilet seat) several samples were taken and the results averaged.

Total colony counts were determined by serial dilution of the samples in nutrient agar at 45° C. (Sharpe & Kilsby, 1971). Replicate 0.1 ml. amounts of the dilution were placed on sterile plastic Petri dishes using a Colworth Droplette Dispenser. The plates were incubated for 18-24 hr. at 37° C. Colony counts were made with the aid of the magnification screen which is part of the Droplette Dispenser.

Bacteria were identified by standard biochemical and morphological studies modified from those given by Cowan & Steel (1965). Samples were streaked on agar plates. The agar media were blood, McConkey, brilliant green, desoxycholate citrate and XLD (BBL). All plates were incubated for 18-24 hr. at 37° C. A replicate set of blood agar plates was incubated, anaerobically, under the same conditions using a Gaspack (BBL). Lactose-fermenting bacteria were identified from the McConkey agar plates. Colonies of Staphylococcus spp. were tested in citrated plasma for coagulase activity. Coagulase-positive results indicated Staph. aureus; coagulase-negative, Staph. albus. Non-lactose fermenters were inoculated into dextrose tubes and streaked on agar plates. For dextrose-negative cultures, a negative test with an Oxidase taxo disk (BBL) indicated Acinetobacter lwoffi. Growth from Oxidase-positive cultures streaked onto Pseudosel agar (Cetrimide agar, BBL) indicated Pseudomonas aeruginosa; no growth indicated Alkaligenes faecalis. The dextrose-positive cultures were tested in lactose, urea and sucrose. Lactose-positive cultures indicated Escherichia coli; sucrose-positive, Paracolon spp., and urea-positive, Proteus spp. Sugar tubes containing maltose and mannitol together with a tube of peptone water (for the Indol test) were used to confirm Proteus spp. Lactose- and urea-positive tests indicated Klebsiella spp., lactose- and sucrose-positive, Esch. coli. Lactose-negative cultures were identified using Enterotubes (Roche); this method being particularly useful for Salmonella spp. and Shigella spp. Confirmation of identification was carried out using standard procedures.



Fig. 1. The species of bacteria found during the survey, their relative frequency and probable origin.

1.	Escherichia coli	
2.	Streptococcus faecalis	
3.	Paracolon species	(47.9) of foodal amigin (F)
4.	Alkaligenes faecalis	(47%) of factor origin (F)
5.	Clostridium welchi	
6.	Klebsiella aerogenes)
7.	Proteus species	4 % from urinary tract (U)
8.	Staphylococcus albus	$\frac{1}{32.0}$ from skin (S)
9.	Pseudomonas aeruginosa	$\int \frac{\partial z}{\partial t} = \int \frac{\partial z}{\partial t$
10.	A cine to bacter lwoffi	13 % air-borne (A)
11.	Others, including:	
	Citrobacter spp.	
	Staphhlococcus aureus	
	Diphtheroids	angle 4 % from F, S and A
	Micrococcus	
	Bacillus spp.)

RESULTS AND DISCUSSION

Bacterial isolations

Frequency of occurrence

The species of bacteria found during the survey, their relative frequency and probable origin (Cruickshank, 1968) are shown in Fig. 1. Several are known to cause infection. Previous, less detailed results (Newsom, 1972), are confirmed.

Human faeces always contain *Esch. coli*, *Strep. faecalis*, *Bacteroides* spp. and *Bifidobacterium* spp. Other bacteria such as *Proteus* spp., *Clostridium* spp. and *Klebsiella pneumoniae* occur in about one third of the population (Ketyi & Barna, 1964). Nearly all of these bacteria were isolated in this survey; those not isolated are anaerobic and non-spore forming.

No salmonellas or shigellas were found. *Proteus morgani* can occur as a concomitant of shigellas; the isolation of this species could indicate previous contamination with dysentery bacilli. The survey results (Fig. 1) indicate that persons with

						Bacteria				:	
Sampling position	Esch. coli	Strep. faecalis	Paracolon spp.	Alkali- genes faecalis	Clos. welchii	Klebs. aerogenes	Proteus spp.	Staph. albus	Pseudo- monas aeru- ginosa	Acineto- bacter lwoff	Others
Cubicle door inside		(-) +		1	[]	1	(+) +	+ +	1 1	+ +	(+) -
Cubicle door lock	(+ + +	⊦ +	(+)	1 1	11	ł (⊦ +		⊦ ≁	(+)
Wall	+	+	1	() +	I	ſ	(-) +	(-) +	I	+	ļ
Flush handle	÷	+	ł	ļ	I	1	+	+	+	+	ł
Outside handle of	+	+	I	+	I	ſ	ł	+	I	+	+
entrance door Inside handle of	(-)+	+	(+	(-) +	I	(-) +	(-) +	(+) + +	ł	+	+
entrance door Water in W.C.	(+) + +	+		+	I	(+) -	(-) +	+	+	+	+
pedestal Ten handles		4	H	-	I		-	(+) + + +	H	-4	-1
Wash-basin overflows	++	- +	⊦∔	⊦ ∔	il	-+-	() +	- - - +	++	- +	- +
Under flushing rim	(+) + +	+	+	+	+	+	, , +	+	+	+	+
Floor in cubicle	++	+	Ŧ	+	+	(<u>-</u>) +	+	++	Ŧ	+	+
W.C. seat	+ +	+ + +	+	÷	+ +	() +	+	+ + +	+	(++)+	+
Urinals	+ + +	+ + +	+	÷	I	+	+	+ +	++++++	+ +	+
-, No isolations; + The results given ar	, bacteria is e for the m	olated in 1 ale rooms	-25% of was. Where the	shrooms an results for	d toilets; • the fem	+ +, 25–50 ale rooms w	%; and + ere differen	+ +, over 5 t, this is in	0%. dicated 1	oy a second	result, in

parentheses.

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Table 1. Percentage of occasions on which bacteria were isolated from various sampling positions

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Fig. 2. Total colony counts. The number of times (%) none, up to 10^3 and more than 10^3 bacteria/cm² were found.

Salmonella typhimurium and Shigella sonnei infections could be expected to contaminate areas in a washroom or toilet.

The relative occurrence of the bacterial strains varied with sampling positions (Table 1). The bacteria found in male and female washrooms and toilets were similar. However, *Staph. aureus* was isolated from one female but three male washrooms and toilets; diphtheroid bacteria were only found in the latter.

Bacterial populations

The number of bacteria at the sampling positions varied considerably. The number of occasions on which no bacteria, and populations up to, and above, 10^3 bacteria/cm² were found is expressed as a percentage in Fig. 2.

Wash-basin overflows showed the highest frequency of occurrence of bacteria; 90% of these were contaminated. Toilet seats are often contaminated but mostly with relatively low numbers of bacteria. The incidence of bacterial counts over 10^{3} /cm² is about 20% on toilet seats – a similar value is recorded for tap handles.

Table 2. Frequency (%) with which surfaces were contaminatedby faecal bacteria

Sampling point	Male	Female
Cubicle door lock	5	4
Cubicle door handles	5	6
Flush handle	6	5
Entrance door handle, outside	12	8
Entrance door handle, inside	23	20
Tap handles	25	29
Water in W.C. pedestal	38	24
Floor in cubicle	39	46
Under flushing rim	53	38
Wash-basin overflow	55	59
W.C. seat	68	58
Urinals	89	

Faecal contamination

Areas contaminated with faecal bacteria are a risk to health; this risk increases with greater contamination. Table 2 gives the number of times (expressed as a percentage) faecal bacteria were found at different positions in the washrooms and toilets surveyed.

Some surfaces (cubicle door locks and handles and flush handles) were rarely contaminated. These surfaces are normally dry and bacteria cannot be expected to survive well.

In contrast, the inside handles of the entrance door and tap handles showed an alarming degree of contamination – particularly so since these are normally touched after washing the hands. No doubt moisture from the hands aids bacterial survival.

The toilet seat and water in the pedestal, the area under the flushing rim, and the floor in front of the toilet are often contaminated, as would be expected. Over 70 % of toilet seats were contaminated with faecal bacteria; this result conflicts with the findings of Newsom (1972). Newsom used *Esch. coli* alone as an indicator of faecal contamination. The present study detected *Strep. faecalis* and other probable faecal bacteria when *Esch. coli* was absent, probably because of different survival times. Total colony counts (such as we report) of all bacteria of probable faecal origin should be used when assessing faecal contamination.

Faecal contamination of male and female toilets is noticeably different for certain positions (Table 2). Contamination underneath the flushing rim and water in the pedestal is lower for female toilets. This is undoubtedly because female toilets are used for both defaecation and urination and are flushed more often than male toilets. Flushing a toilet is known to dilute a bacterial population by 10³ (Darlow & Bale, 1959). Floors in cubicles are probably contaminated by splashing and, to a lesser extent, by the aerosol effect of flushing.

Faecal bacteria occurred most often in wash-basin overflows and in urinals. This is to be expected since these areas provide good conditions for bacterial growth. Urinals become contaminated with bacteria from the genital region and possibly. from aerosols as discussed previously.

Disease transmission

Infection by direct ingestion of bacteria has been reported (Hutchinson, 1956) but a high degree of bacterial contamination is required. The ingestion of 10^6 pseudomonads, 10^4 *Esch. coli*, 10^5 - 10^6 of the food-poisoning salmonellas and 10^3 *Sal. typhi* is required to cause infection (Newsom, 1972). Counts of 10^6 and greater have been recorded in this study in areas likely to be touched by the hands (e.g. wash-basins, toilet seats). Cross-infection with the protozoon *Trichomonas vaginalis* from splashing during defaectation has been reported (Burgess, 1963). Bacterial infection by this route could similarly occur.

Transmission of disease via the respiratory route cannot be neglected (Darlow & Bale, 1959; Bound & Atkinson, 1966; Jessen, 1955). Newsom has indicated that 10^{11} organisms are required in the whole volume of the water in the pedestal; counts up to 10^{12} were recorded in this work. Bound & Atkinson (1966) discussed possible aerosol formation by urinals. The high faecal contamination found in the present work suggests the possibility of a cross-infection risk from this source.

Disinfection priorities

Priority for disinfection can be assigned to areas depending upon the comparative risk to health. The extent of faecal contamination (Table 2) cannot be used directly to give these priorities since cross-infection will also depend on the likelihood of contact.

The most important areas are the toilet seat, wash-basin overflow, tap handles and the inside handle of the entrance door; these provide routes for cross-infection via the body and hands. Of moderate importance are the flush handles, cubicle door handles and lock, under the flushing rim, and water in the pedestal. Urinals, floors and walls are not normally touched and present a lower risk to health. Although not touched, some of these areas should be cleaned for other reasons: bacteria under the flushing rim contribute to the contamination of the water in the pedestal; those in urinals contribute to malodour.

During the survey it was noted that most washrooms and toilets were cleaned daily. Our results show that this is clearly inadequate. It is likely that in order to maintain low bacterial populations, daily cleaning of contact surfaces should be effected and a regular more extensive maintenance and disinfection programme (hygiene service) employed to reduce contamination in all areas, including those not covered by daily cleaning.

CONCLUSIONS

Faecal bacteria occur in large numbers on surfaces which users of washrooms and toilets readily contact. Pathogens, if present, can similarly be transmitted.

Daily cleaning and disinfection in conjunction with a regular hygiene service are recommended to reduce cross-infection risks in washrooms and toilets.

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