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New technique to take samples from environmental surfaces using flocked nylon swabs

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SUMMARY

Environmental surfaces near infected and/or colonised patients in hospitals are commonly contaminated with potentially pathogenic micro-organisms. At present, however, there is no standardised method for taking samples from surfaces in order to perform quantitative cultures. Usually contact plates or swabs are used, but these methods may give different results. The recovery rate of traditional swabbing, e.g. cotton or rayon, is poor. With a new type of swab utilising flocked nylon, the recovery may be enhanced up to three times compared with a rayon swab. In this study, we inoculated reference strains of *Staphylococcus aureus* and *Enterococcus hirae* onto a bedside table and took samples 1 h later when inocula were dry. Sequential samples were taken from the same surface. A new sampling technique using two sequential nylon swabs for each sample was validated. The efficiency of the sampling, percentage recovery of the inoculum and the variation of culture results obtained from repeated experiments are described. Enhanced efficiency and higher recovery of inoculum were demonstrated using two sequential flocked nylon swabs for sampling.

Introduction

Many of the micro-organisms associated with hospital-acquired infection commonly contaminate inanimate surfaces near the patients.[1–3] To date there has been no standardised method for taking surface samples in order to perform quantitative cultures. Usually contact plates or swabs are used, but these methods may give different results.[4] There is also no standardised method to evaluate the antibacterial effect of a surface disinfectant when used in a hospital ward, a so-called field test under practical conditions.

The recovery rate of traditional swabbing is often poor, generally <25% in some studies.[5] A problem with cotton as well as rayon swabs is that the release of bacteria from the swab is incomplete when it is immersed into a solution after sampling. Bacteria become trapped within the fibre matrix. The release of bacteria from a flocked nylon swab is greater, with the recovery being up to three times higher than that of a rayon swab.[6] In another study a flocked nylon swab was shown to be more efficient than a rayon swab, but the difference was statistically significant only when samples were taken from a wet surface.[5]

The aim of the present study was to investigate a new technique for surface sampling, using two sequential flocked nylon swabs to take each sample.

Methods

Preparation of test surfaces

The surface of an EVAB bedside table with plastic laminated MDF-board-surface (Proton Caretec AB, Skillingaryd, Sweden) was cleaned and disinfected by the use of an alcohol-based disinfectant with a non-ionic detergent (Dax ytdesinfekion plus, Opus Health Care AB, Malmö, Sweden). The surface was allowed to air-dry for about 1 h before application of the test bacteria.

Application of test bacteria

Reference strains of *E. hirae* ATCC 10541 (CCUG 32258) and *S. aureus* ATCC 6538 (CCUG 10778) were inoculated on to blood agar (6.5% defibrinated horse blood, Columbia blood agar base, Acumedia, Lansing, MI, USA). After incubation at 36 °C for one day, a few colonies were suspended in phosphate-buffered saline (PBS), pH 7.4, and the turbidity was visually adjusted to McFarland 0.5. A viable count was performed by serial 10-fold dilutions in PBS

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followed by culture of 100 μL from each dilution on blood agar. A rayon-tipped swab was moistened by immersion into the undiluted bacterial suspension, McFarland 0.5. The precise weight of the whole swab was then determined, using a balance. A square cardboard frame 5 × 5 cm was used to define each test area. The rayon swab was rotated and rubbed in a zig–zag pattern over the whole surface and this process was repeated at an angle of 90° to the first rub. The weight of the swab was determined once again. By comparing the weight before and after inoculation (1 mg corresponding to 1 μL) and by knowing the viable count [colony-forming units (cfu)/mL] it was possible to calculate the number of viable bacteria applied on the test surface. In some of the experiments another method was used for inoculation of the test surfaces. Using a pipette, 5 × 10 μL of the bacterial suspension was applied within a cardboard frame, 5 × 5 cm. Each 10 μL spot was spread with the same plastic applicator (1 mm wide at the end) in a circle about 1 cm^2 in size. The surfaces within six frames were inoculated in the same way at the same time to prepare for a study of the variation of bacterial counts obtained by samples taken with the same method.

Surface sampling

After application the bacterial suspension was allowed to dry at ambient temperature. Sampling was started exactly 1 h after the application. Different sampling methods were used: two different swabs, two different sampling solutions, and two different techniques (one or two swabs per sample).

Swabs

Flocked nylon swabs manufactured by Copan Flock Technologies (Brescia, Italy), product number CP502CS01 (Nordic Biolabs AB, Täby, Sweden). Rayon-tipped swabs were made by Copan (Brescia, Italy), product number CP167KS01 (Nordic Biolabs AB).

Sampling solutions

The sampling solution used was PBS or neutraliser (polysorbate 80, 30 g/L; saponin, 30 g/L; lecithin, 3 g/L; pH 7.0).

One swab per sample

The tip of a swab was immersed into sampling solution, and then pressed against the wall of the tube to remove excess solution. The swab was rotated and rubbed in a zig–zag pattern over the whole surface and this process was repeated at an angle of 90° to the first rub. The swab was then put in a tube with 1 mL sampling solution, pressed against the wall of the tube and shaken to dislodge bacteria. The swab was left in the tube for 5 min and then, after vortexing the tube, discarded. Serial 10-fold dilutions of the sampling solution were prepared in PBS, and 100 μL from each dilution was inoculated on to blood agar plates. After incubation at 36 °C for 24 h the numbers of cfu on each plate were counted. Counts in the range 15–300 cfu were used for further computation. If suitable counts were obtained from two adjacent dilution steps, the weighted arithmetic mean of both was calculated.

Two swabs per sample

Two sequential swabs were used to take each sample: first, a wet swab, as above, thereafter an initially dry swab which was moistened during sampling by the solution that remained on the surface after completion of the first swab. The second swab was gently applied to the surface to absorb as much solution as possible, and then put into the same tube with sampling solution as the first swab. The process thereafter was the same as when only one swab was used.

With each method, using one or two swabs to take each sample, as described above, sequential sampling from the same surface site was performed no less than six times (three times in the repeated experiments where variation of results was investigated). As soon as the surface appeared to be dry the next sampling took place.

Series of experiments

Experiments were performed on different days, and in each experiment two different variants of the sampling method were compared, for example a flocked nylon swab versus a rayon-tipped swab, etc. For each experiment a new suspension of test bacteria was prepared as described above. Repeated experiments to study the distribution of results and the coefficient of variation were performed with the sampling method with two flocked nylon swabs per sample with neutraliser as sampling solution.

Computation of results

The efficiency of each sampling method was determined as proposed by Whyte *et al.*[7] Samples were taken repeatedly from the same site and the decreasing numbers of bacteria found each time by culture were used for computations, using the Microsoft Excel software. The log number of bacteria found by culture was plotted as a function of the serial sample number (log/linear graph). The correlation coefficient was calculated. The efficiency was derived from the correlation coefficient and defined by the number of bacteria sampled divided by number of bacteria present immediately before sampling.[7] In addition, the ratio between the number of bacteria found in the first sample and the number of bacteria inoculated on the surface 1 h before sampling was calculated. This ratio was expressed as the percentage recovery. In the repeated experiments the mean, standard deviation and coefficient of variation were calculated.

Results

As expected, the yield was always higher when two swabs instead of one were used to take a sample, and this was true for both types of swab. Examples of log–linear graphs are shown for the results from two of the experiments with *E. hirae* (Figure 1). Results from the experiments where two sequential swabs were used to take each sample are shown in Table I. The correlation coefficient was high for all the sampling methods, varying from 0.94 to 1.00. The flocked nylon swab showed the highest efficiency (Table I).

The percentage recovery, i.e. the ratio between the number of bacteria found by culture of the first sample and the number of bacteria inoculated on the surface 1 h before sampling, is also shown in Table I. The recovery was higher when flocked nylon swabs were used compared to when rayon-tipped swabs were used. The recovery was further enhanced, especially for *S. aureus*, when neutraliser instead of PBS was used as sampling solution.

The results of the sampling method with two flocked nylon swabs per sample with neutraliser as sampling solution are shown in Table II.

Discussion

The problem with traditional swab methods for surface sampling is the relatively low yield. The yield can be enhanced if a flocked nylon swab is used instead of a rayon swab.[5,6] In addition, we demonstrated higher recovery of the inoculum when two sequential flocked nylon swabs were used to take a sample instead of one. When a moistened swab is used to rub a surface a small amount of fluid always remains on the surface after sampling, meaning that some bacteria also remain on the surface. Some of the

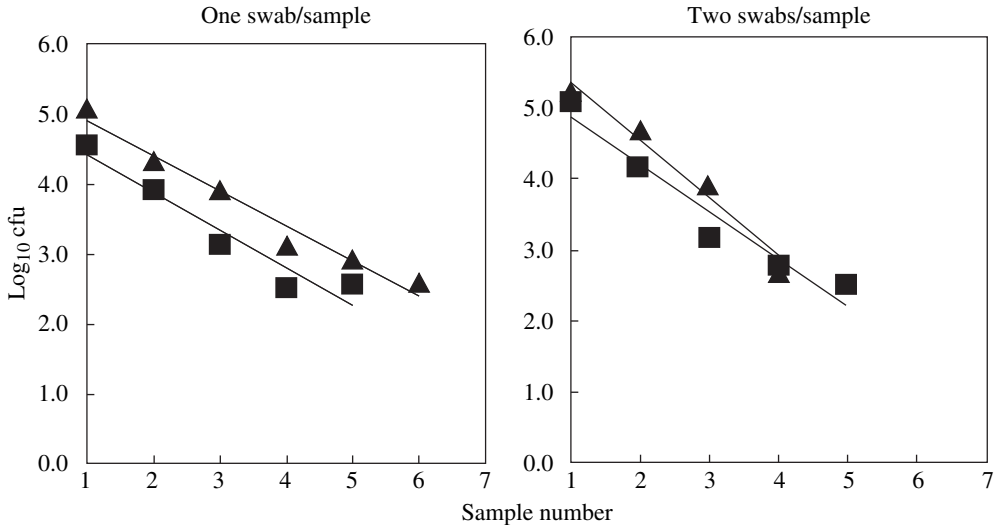

Figure 1. *Enterococcus hirae* was inoculated on a 25 cm^2 surface of a bedside table. Sampling was started 1 h later and was repeated six times from the same surface site. The constantly decreasing numbers of bacteria found by culture are shown. ▲, flocked nylon swab, ■, rayon-tipped swab.

remaining fluid can be absorbed if a second dry swab is gently applied to the surface. The two sequential swabs are placed in the same tube with sampling solution.

Repeated samples were taken from the same surface, commencing 1 h after inoculation. The samples in the series were taken as soon as the surface appeared to be dry after the previous sampling. The sampling efficiency was calculated from the decreasing counts obtained in the series.[7] Percentage recovery was calculated from the ratio between the number of bacteria found by culture of the first sample in the series and the number of bacteria inoculated on the surface 1 h earlier. The percentage recovery was lower than the efficiency, probably due to loss of bacterial viability during the hour of drying. Recovery was further increased when neutraliser instead of PBS was used as sampling solution. This increase was highest for *S. aureus*. The higher recovery with neutraliser may be explained by polysorbate 80, which breaks up clumps of cells, an effect which has been observed in other studies.[5]

Distribution of results as well as the coefficient of variation was investigated for the sampling method which seemed to be the most efficient, i.e. the method with two sequential flocked nylon swabs per sample and with neutraliser as sampling solution. As seen in Table II, correlation coefficients and efficiencies which were calculated from the three series of samples taken from the same surface were high. The number of bacteria found by culture of the first sample in the series from the six surfaces varied between 0.53 and 3.57×10^6 cfu for *E. hirae* and between 0.64 and 2.32×10^6 cfu for *S. aureus*. The coefficients of variation were 63% and 52%. The percentage recovery of the first sample varied between 13% and 85% with a mean of 39% for *E. hirae* and between 22% and 58% with a mean of 35% for *S. aureus*. This variation means that the higher recovery for nylon swabs in comparison with rayon swabs may have been a coincidence, because the comparison experiments were single. Repeated experiments might have given another result. Our superior results with the nylon swab, however, agree with the results from other studies.[5,6] In our study the loss of bacterial viability due to drying is an unknown factor which is included in the observed variation.

The degree of precision needed can be discussed when the number of bacteria on a surface is determined. For example, in studies concerning the antibacterial effect of a disinfectant, a reduction in bacterial counts $>10^5$ cfu is often required for a product to pass the test. The variation that we observed, where all the counts varied around 10^6 cfu, would be acceptable in that context.

In our study the maximum recovery of *S. aureus* in a single experiment was 58%, whereas the mean recovery in repeated experiments was 35%. Dalmaso *et al.* reported 42% recovery of *S. aureus* when one flocked nylon swab was used.[6] In their study, however, samples were taken as soon as 3 min after the surfaces had been inoculated with bacteria, and the loss of bacterial viability because of drying was minimal.

The inocula in our study were high, about 10^5–10^6 cfu/50 cm^2. Heavy contamination of the environment occurs from spillage of human secretions and excretions. Environmental contamination can also be low grade, and this includes contamination of surfaces from hands and by the airborne route. The swab rinse method is

Table I
Sampling with two sequential swabs

Experiment	Test strain	Sampling fluid	Type of swab	Correlation coefficient	Efficiency (%)	No. of cfu $\times 10^6$ applied (A)	No. of cfu $\times 10^6$ obtained by culture of first sample (S)	Ratio S/A (% recovery)
A	*E. hirae*	PBS	Nylon	0.96	86	1.02	0.17	17
		PBS	Rayon	0.94	78	0.95	0.12	13
B	*S. aureus*	PBS	Nylon	0.99	96	0.41	0.14	34
		PBS	Rayon	1.00	93	0.44	0.07	16
C	*E. hirae*	PBS	Nylon	0.99	74	1.04	0.27	26
		Neutraliser	Nylon	1.00	81	0.98	0.33	34
D	*S. aureus*	PBS	Nylon	0.95	87	1.26	0.35	28
		Neutraliser	Nylon	0.98	82	0.87	0.49	56

cfu, colony-forming units; PBS, phosphate-buffered saline.

Table II
Two flocked nylon swabs per sample and with neutraliser as sampling solution

Test strain	Correlation coefficient	Efficiency (%)	No. of cfu $\times 10^6$ applied, A	No. of cfu $\times 10^6$ obtained by culture of first sample S	Ratio S/A (% recovery)
E. hirae	100	86	4.22	1.75	41
	89	77	4.22	0.53	13
	95	84	4.22	3.57	85
	94	71	4.22	1.32	31
	90	84	4.22	1.29	31
	97	71	4.22	1.34	32
Mean	94	79		1.63	39
SD	4.2	6.8		1.03	25
CV (%)	4.4	8.6		63	64
S. aureus	98	80	3.97	2.27	57
	97	65	3.97	0.64	16
	98	71	3.97	1.15	29
	98	62	3.97	0.86	22
	98	85	3.97	1.14	29
	99	84	3.97	2.32	58
Mean	98	74.5		1.39	35
SD	0.6	9.9		0.72	18
CV (%)	0.6	13		52	51

SD, standard deviation; CV, coefficient of variation.

suitable for sampling of surfaces that are rather heavily contaminated, whereas contact plate methods are considered to be more suitable for surfaces with low numbers of bacteria.[8,9] In another recent study, we compared different methods to take samples from bedside tables with a low level of bacterial contamination, $<$100 cfu/50 cm^2. We found that the total aerobic bacterial counts that were obtained by using the contact plate method were not significantly different from the counts obtained by using our new swab rinse method.[10]

There is no legislation, rules or standards concerning the level of surface bacterial contamination allowed in a hospital. A total aerobic count of $<$5 cfu/cm^2 on different hand contact surfaces has been proposed in the UK.[11] In light of the current problems with spread of multiresistant bacteria such as meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) in hospitals, it is hard to say which level of surface contamination is critical for the spread of micro-organisms. We think that quantitative surface cultures from the hospital environment are still needed, at least for research. Based on the results from previous studies, where recovery rates have been poorer than in our study, the usefulness of taking swab samples from a surface in a hospital for quantitative culture has been questioned.[5] The contact plate method is not suitable for sampling when the level of contamination is high, but our new swab rinse method permits quantitative cultures at all levels of contamination.[8,9] Another advantage of the swab rinse method is that it can easily be modified for quantitative culture of specific bacteria such as MRSA, VRE and *Clostridium difficile*. This is done by subculture of the sampling solution on appropriate selective media. The presence of *S. aureus*/MRSA at specific environmental sites was not related to total bacterial counts in a recent study, and this may also be true for other specific bacteria.[12]

Only if a standardised sampling method is used will it be possible to adequately describe and compare the amount of bacterial contamination on surfaces in hospitals and to study the effect of surface disinfectants. Some aspects of swab sampling are difficult to standardise, such as the degree of pressure applied to the swab during use. The use of two sequential flocked nylon swabs for taking surface samples is proposed as part of a standard methodology.

Conflict of interest statement
None declared.

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