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Review

How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence

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SUMMARY

There is increasing evidence that the hospital surface environment contributes to the spread of pathogens. However, evidence on how best to sample these surfaces is inconsistent and there is no guidance or legislation in place on how to do this. The aim of this review was to assess current literature on surface sampling methodologies, including the devices used, processing methods, and the environmental and biological factors that might influence results. Studies published prior to March 2019 were selected using relevant keywords from ScienceDirect, Web of Science, and PubMed. Abstracts were reviewed and all data-based studies in peer-reviewed journals in the English language were included. Microbiological air and water sampling in the hospital environment were not included. Although the numbers of cells or virions recovered from hospital surface environments were generally low, the majority of surfaces sampled were microbiologically contaminated. Of the organisms detected, multidrug-resistant organisms and clinically significant pathogens were frequently isolated and could, therefore, present a risk to vulnerable patients. Great variation was found between methods and the available data were incomplete and incomparable. Available literature on sampling methods demonstrated deficits with potential improvements for future research. Many of the studies included in the review were laboratory-based and not undertaken in the real hospital environment where sampling recoveries could be affected by the many variables present in a clinical environment. It was therefore difficult to draw overall conclusions; however, some recommendations for the design of routine protocols for surface sampling of healthcare environments can be made.

Introduction

Healthcare-associated infections (HCAIs) lead to poor clinical outcomes and death [1]. In high-income countries HCAIs affect approximately 5–15% of patients, whereas in low-income countries prevalence rates are in the region of 15–19% [2]. In Europe, HCAIs are attributed to approximately

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37,000 deaths per year and 25,000 people per year die from antibiotic-resistant HCAIs [3]. It is estimated that, of the HCAIs that develop within the intensive therapy unit, 40–60% are due to endogenous flora, 20–40% are due to the contaminated hands of healthcare workers (HCWs), 20–25% are due to antibiotic-driven change, and 20% are potentially due to environmental contamination [4].

The hospital surface environment is an important factor in infection risk as it may act as a reservoir for nosocomial pathogens. Prior room occupants shed micro-organisms into their environment, posing a risk to the next patient if terminal cleaning is not effective with, on average, patients being 73% (28.8–87.5%) more likely to acquire HCAIs if a previous room occupant was colonized or infected [5–8]. Within the UK, under the Health and Social Care Act, there is a requirement for clinical environments to be safe. Currently, there is some guidance available from National Specifications for Cleanliness in the UK, National Health Service on general monitoring of the hospital environment, in which surfaces are assessed by visible audit [9]. However, no microbiological screening is indicated.

Generally, hospital environments are only sampled in response to an outbreak. Routine sampling is not usually indicated for healthcare environments. Guidelines are provided by Public Health England for monitoring during an outbreak or for evaluating cleaning efficacy, using both swabs and contact plates [10]. Guidance suggests that environmental monitoring can be undertaken, but this guidance does not contain the microbiological protocols required [11].

In light of the changing awareness of the risk posed by the surface environment, more hospitals are considering instigating routine monitoring of their environments, either to assess cleaning or as part of a continuous risk assessment. This review will investigate what micro-organisms have been isolated from hospital surfaces, how those samples were taken and processed, in order to build a clearer picture of the contaminants in the hospital surface environment and to prepare evidence for the development of an optimized evidence-based sampling protocol.

Methods

Studies were selected using ScienceDirect, Web of Science and Medline (PubMed). Abstracts were reviewed and all data-based studies in peer-reviewed journals in the English language were included. Keywords were as follows: hospital, environment, sampling, surface, monitoring, contamination, swab, sponge, petrifilm, and contact plate. This review focuses on the development of routine sampling methodologies, which led to the exclusion of outbreak and intervention studies. This exclusion was due to the higher levels of contamination frequently found in outbreaks and the requirement for increased test sensitivity outside of the outbreak setting. Bacterial, viral, and fungal contaminants were included. Only surface samples were included and other samples such as hand, water, and air samples were not considered. These studies were excluded due to the focus of this review being on how to undertake surface sampling within the healthcare setting. Studies were included up until March 2019. Inclusion criteria for this review are listed in Supplementary Table I. Search terms are listed in Supplementary Table II. A systematic review was not possible due to current evidence, therefore a structured narrative review was produced as per the criteria outlined.

All types of hospital, regardless of sampling technique chosen, target organism, geographical location or specialty were included. All organisms were included in the study to capture the level of variation present. As many of the comprehensive sampling experiments come from the food industry, these were also included.

Results

A total of 98 studies looking at both the surface bioburden and sampling methodologies were included. Seventy-three studies were selected for consideration of the hospital surface contaminants. Thirty-three studies were selected for consideration of sampling methodology, to critically analyse and compare methods for surface sampling. Figure 1 summarizes the review findings.

Sampling devices

There are both direct and indirect methods of sampling. Direct methods, such as contact plates, are self-enclosed and require no further processing. Indirect methods, such as swabs, require an extraction step to remove the sample from the sampling device. Pre-analytical techniques affect the recovery of organisms from the environment and point the reader to the different sections of the review and their survival until the sample processing or analytical phase. In this review, 'recovery' is defined as the percentage of cells that are viable and therefore can be detected successfully from the original number of cells inoculated on to, or present in, a sampling device or from a surface. Thirty-three studies were reviewed exploring methods of surface sampling: seven sampled the real hospital environment and 26 were laboratory-based studies using surrogate surfaces such as stainless steel coupons. The sampling devices considered in this review and the frequency of their use in the studies included are shown in Figure 2. The sampling devices best suited to different surfaces, conditions, and pathogens are shown in Table I and described below.

Contact plates

Contact plates are convex agar plates that can be directly pressed on to a surface to take a quantitative sample. Contact plates can be made with selective or non-selective agar, with or without a neutralizing agent, all of which lead to differences in recovery of the target organism. The main advantage of contact plates is the production of semiquantitative data in the form of colony counts, which can help elucidate trends [12].

Recovery of organisms ranged between 23% and 56% depending on the plate and organism [13]. Contact plates were found to be better than swabs for recovery from 100% cotton fabric [14]. Meticillin-containing contact plates recovered meticillin-resistant *Staphylococcus aureus* (MRSA) best from stainless steel, outperforming dipslides and swabs [15,16]. Contact plates were also found to be best for recovering *Staphylococcus aureus* from non-porous surfaces [17].

Dipslides

Dipslides are a direct contact method, similar to contact plates, held inside a plastic container which reduces contamination risk and agar drying. Dipslides have a paddle formation with two separate sides, which can contain two different selective or non-selective agars. The two sides can be

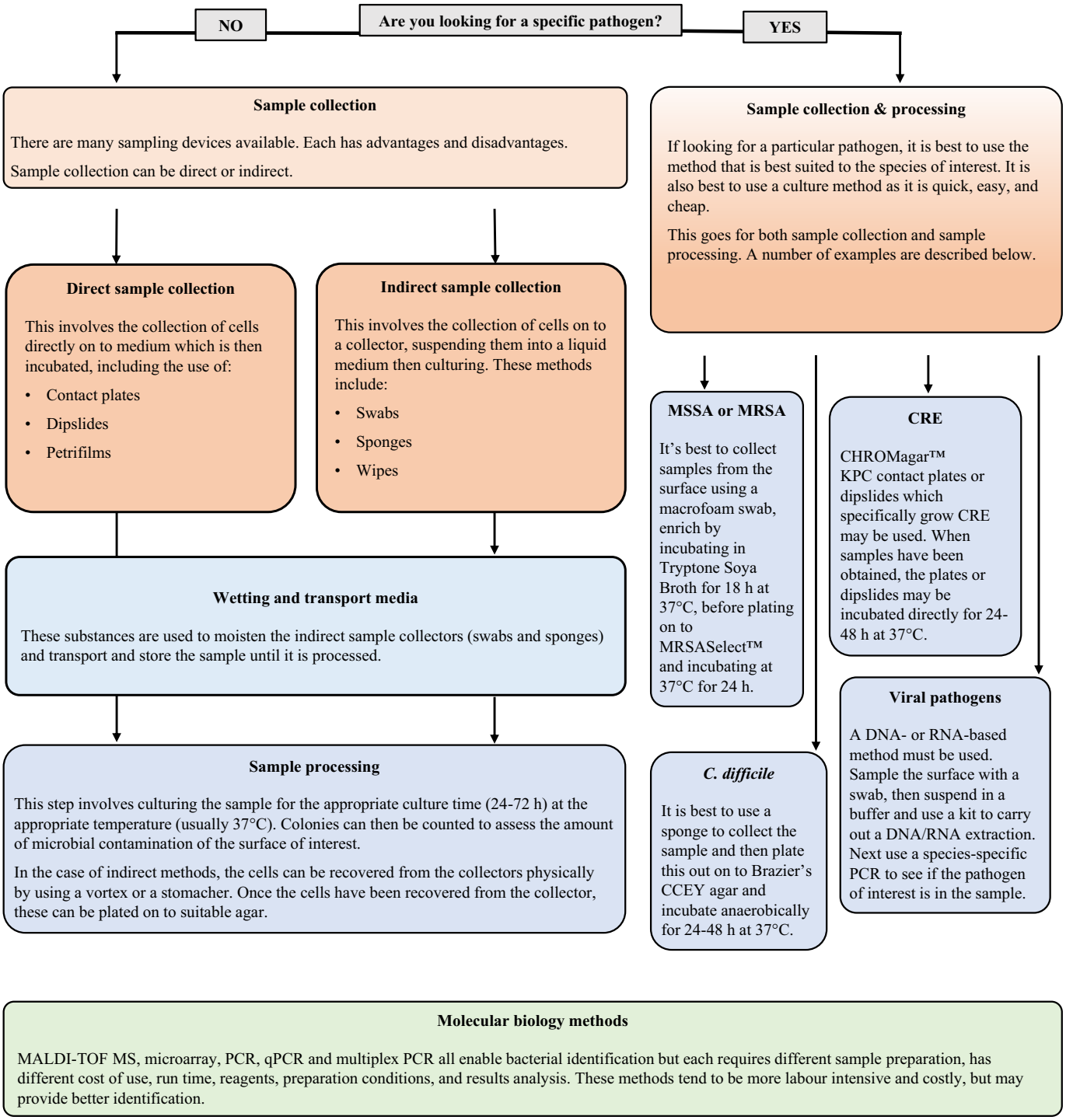

Figure 1. Flow diagram outlining review findings and the process of designing a sampling protocol. CRE, carbapenem-resistant Enterobacteriaceae; KPC, *Klebsiella pneumoniae* carbapenemase; CCEY, cycloserine–cefoxitin–egg yolk; PCR, polymerase chain reaction; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

used to take two samples with different media, or to take two separate samples using the same media. Most commonly, dipslides will have one side with a selective agar and one side with a non-selective agar. Dipslides might be considered a better option due to their flexibility; unlike contact plates, they can sample uneven surfaces without the additional processing losses faced by non-direct contact methods such as swabs. Most losses occur during processing, such as vortexing [18]. Direct contact methods such as dipslides and contact plates can eliminate these extra losses.

Dipslides with Tryptic Soy Agar (TSA) and MacConkey agar (MAC) were found to be best for recovering Enterobacteriaceae when compared with TSA contact plates [19]. Violet Red Blood Glucose (VRBG) dipslides (77% total positive samples) and TSA

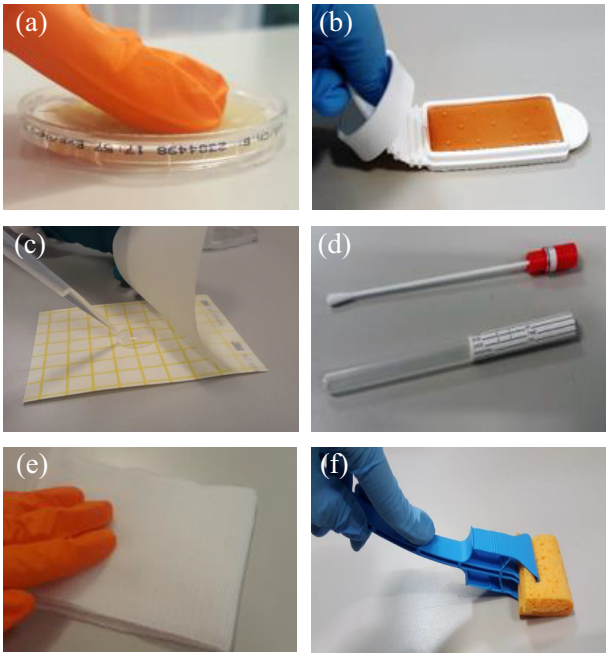

Figure 2. Devices most commonly used for the collection of microbiological samples from surfaces in the publications included in this review: (a) contact plate, 24%; (b) dipslide, 6%; (c) petrifilm, 3%; (d) swab, 53%; (e) sponge, 9%; and (f) wipe/gauze, 5%.

and VRBG dipslides were best for faecal indicator species (66% total positive samples) compared with TSA contact plates and MAC dipslides [19]. The same study reported that dipslides, with the addition of neutralizers, performed significantly better than those without [19].

Swabs

Swabs are indirect sampling devices made of various materials, including cotton, rayon, polyester, calcium alginate, or macrofoam, and they may be flocked by design with numerous processing options. Swabs can be manipulated around difficult or uneven surfaces, such as door handles, bed rails, and around sinks and taps. According to the available literature, they were the most frequently used sampling method (Figure 2). This is perhaps due to their simplicity, affordability, and availability in the hospital environment.

Flocked swabs have a nylon fibre coating added in a flocking process. This coating allows better sample adsorption through capillary action [20]. Rayon- and polyester-tipped swabs are manufactured similarly to cotton swabs, though the bud material is different. Brush-textured swabs are produced by spraying nylon flock on to a plastic spatula or swab bud [21]. Handles are made of plastic, wood, or metal. Under some experimental conditions, some studies report cotton swabs to be more effective than swabs made of other materials [21], or just as comparable [22], and that two sequential swabs per sample site are better than one [23]. It was found that cotton swabs recovered significantly more colonies than other swabs from a wet surface [21]. These results emphasize the need to understand the surfaces that will be sampled to optimize swab choice. Across the literature, macrofoam swabs are generally found to be the most effective type of swab [22,24].

However, despite popularity, the use of swabs is difficult to standardize. Variation in results is not only explained by difference in device, target organism, and surface state, but by the difficulty in standardizing sampling pressure, size of sampling area, angle of swab, and pattern while sampling. This can cause variation in recoveries between 22% and 58% for *S. aureus* [23].

Sponges

Sponges are an indirect sample device which can be manipulated around uneven surfaces, can sample a wider surface area with ease, and some pressure can be exerted during sampling. As such, sponges are often reported to have better recoveries than other methods, and have been shown to be significantly ($P < 0.0001$) better for *Clostridioides difficile* recovery than swabs (28.0% versus 1.5%, respectively) [25]. When considering surface material, the literature reports better recovery efficiency with sponges for *Pantoea agglomerans* (previously *Enterobacter agglomerans* or *Erwinia herbicola*) from nylon cushions, vinyl tiles and plastic seats, than the 3M swab or foam spatula and so may be beneficial for sampling fabric surfaces [18]. Handling during the sampling process may lead to increased risk of contamination if not handled appropriately.

Petrifilms

Petrifilms are more often used in the food industry, though they should not be overlooked for use in clinical environments. They are fast, simple to use, and have a wide variety of applications. Petrifilms can be inoculated with a swab, or can be used as a direct contact method for both surface sampling and finger dabs. Once the surface of the petrifilm paper has been wetted, the paper is pressed against the surface for testing, the film closed, and incubated. A plate count can be read directly from the petrifilm. They are available impregnated with either selective or non-selective media for colony counts or specific pathogen detection. Petrifilms have an advantage over contact plates as they are flexible and can adapt to the topography of a surface [16]. Petrifilms were the best method for recovering MRSA from linoleum, mattress, coated steel, and polypropylene [16].

Table I
Suitability of sampling method for different surface condition and target organism

	Contact plate	Dipslide	Petrifilm	Swab	Sponge
Wet surface			+	+[a]	
Dry surface	+		+		
Flat surface	+	+			+
Uneven surface	−	+	+	+	+
High bioburden	−			+	
Low bioburden	+	+	+		+
Injured cells				+	+
S. aureus and MRSA	+		+		
C. difficile					+
Gram negative bacteria				+	
Viruses	−	−	−	+	−

MRSA, meticillin-resistant *Staphylococcus aureus*.
[a] Cotton, rayon, polyester or macrofoam. Brush-textured swabs perform poorly on wet surfaces. Empty cells indicate lack of data.

Wipe devices

Wipe methods involve the use of a sterile cloth or gauze to wipe a surface and collect a sample. This method requires excellent aseptic technique to avoid contamination of the sample. The wipe is placed into a sterile container or stomacher bag for further processing. Wipe methods were shown to give a wide range of recoveries, between 40.5% and 98.3% [26]. Electrostatic wipes were found to give better recoveries for *S. aureus* on stainless steel plates, outperforming swabs and contact plates [27].

Pre-analytical sampling choices: sample device wetting, transport, and storage

Different methods and additional processing steps and options to improve recovery are available. Swabs, sponges, and wipe methods can be enhanced by pre-wetting prior to surface sampling. Wetting solutions and diluents can either aid or hinder recovery, depending on the target organism. There are many wetting agents available, ranging from sterile saline [28], buffered peptone water, various strengths of Ringer solution and letheen broth, which neutralizes quaternary ammonium compounds [21]. It is also possible to use a wide variety of transport media and neutralizers. When choosing a neutralizer, it is important to consider the potential presence of chemical residue on the surface. When selecting transport medium, time between sampling and processing must be determined in advance. Samples were generally processed immediately, within 4 h or stored in transport media at 4°C for no more than 24 h [21].

Wetting agents

Microbial recovery from surfaces was significantly improved by pre-moistening for all swab types [21,22]. A dry cotton swab gave 8.0% recovery and pre-moistening improved recovery to 41.7% [22]. This is further supported by another study in which all swab recoveries were improved by pre-moistening, taking recovery rates from 57.5% dry positive rate, to 83.4% moistened positive rate [28].

The Cyto-brush textured swab in Copan rinse formula was best for *S. aureus* recovery [21]. Wetting solutions with letheen broth and solutions with buffered peptone water significantly increased recovery rates of *S. aureus* and *Escherichia coli* at room temperature [21]. Phosphate-buffered saline was optimal for *E. coli* and *Bacillus cereus*, whereas phosphate-buffered saline with Tween was better for *Burkholderia thailandensis* recovery [21]. Cotton-tipped swabs in one-quarter-strength Ringer solution were best for *E. coli* recovery alone [21]. However, one of the buffers tested, Butterfield's buffer, had a marked reduction in recovery if used with *E. coli*, from 60.6% to just 40.5% [26].

Transport media and neutralizers

Transport medium, such as anaerobic universal transport medium, aerobic Amies medium and neutralizing buffer, is the solution used for sample storage before processing. Choice of transport medium is important, and the choice should vary depending on the target organism, time taken to transport to the laboratory, and post-test storage conditions and storage time [29,30]. Neutralizing broths help to keep microbial cells intact while also neutralizing any chemical cleaning substances that may have been collected along with the microbiological sample [31,32]. Some transport media allow inhibition of growth to enable more accurate estimation of counts [29].

Polyurethane swabs without transport medium gave the highest recoveries if tested within 2 h, and viscose swabs with aerobic Amies transport medium were second best, giving 90.7% and 25.7% recoveries respectively [29]. Viscose swabs with no transport medium had the lowest recoveries overall at just 8.4% [29]. However, if swabs were not processed within the first 24 h, addition of transport medium was critical to avoid cell death or excessive growth, leading to inaccurate counts [29]. It was shown that bacteria that adhere to dry fibres can become desiccated, allowing only 3–5% recovery [29].

Sample processing

If using an indirect sampling method, following sampling, direct plating on to agar, enrichment or molecular processing are the available options. The choice of processing method is dependent on the organisms being investigated, cost, and time available.

Culture analytical processing options

Sample extraction. Swab, sponge, and wipe samples require extraction (i.e. removal of the target from the swab) in order to undergo further processing. Extraction solutions include: phosphate-buffered saline, Butterfield's buffer, Butterfield's buffer and Tween, and maximum recovery diluent [26]. After target organism, choice of extraction solution was found to have the next biggest impact on extraction efficiency [27].

Ensuring optimum extraction of the sample is important in the reduction of associated losses. Vortexing, agitation, or sonication of the swab or sponge are three methods that may increase recovery. Vortexing improved recovery from flocked swabs from 60% to 76%, but not from rayon swabs [20]. Overall, vortexing gave the best results, except for polyester swabs, which gave better results with sonication, highlighting the importance of processing [22]. Furthermore, depending on pre-moistening and the use of vortexing, recovery with swabs can vary between $<$0.01% and 43.6% [22]. An optimum time of 2 min vortexing was shown to be superior over 12 min of sonication, followed by agitation to remove *Bacillus anthracis* spores from a swab [22].

Sample enrichment. Enrichment involves placing the sample directly into a broth and incubating, providing time to grow in favourable conditions. It can be useful for slower-growing organisms, cells that have become stressed, or to select the target organism from a swab or non-selective sample. Following incubation, aliquots are then subcultured and plated out onto various selective or non-selective media. Brain–heart infusion broth is widely used [29]. Thirty-one studies in this review used subculturing. Broth composition and incubation time and temperature vary depending on the organism of interest. One study found that enrichment in tryptone soya broth improves detection rate of *S. aureus* from 61.3% to 80% [28]. Whereas enrichment allows recovery of stressed or injured cells, it is important to note that this step produces a presence or absence result and is not accurately quantitative [33]. When sampling in healthcare settings with predicted low levels of contamination, adding an amplification step (such as enrichment) may provide a viable alternative due to the losses

from other processing techniques such as those requiring sample extraction.

Incubation conditions. Incubation times and temperatures varied in the literature, ranging from 18 to 48 h, or non-specific 'overnight' [13,14,22]. Twenty-three studies used incubations at 37°C for 24–48 h and seven studies reviewed incubation at 35°C for 24–48 h. Choice of incubation temperature may have an impact on growth or recovery of an organism, as temperatures required to grow one organism may inhibit another. For clinical pathogens, temperatures required a range of between 25 and 45°C [34].

Molecular biology processing

Molecular methods are extremely valuable for analysing the microbiological contaminants of the hospital surface environment. Whereas historically organisms were identified using culture methods, not all clinically relevant organisms are culturable, such as norovirus, for which polymerase chain reaction (PCR) methods based on nucleic acid detection must be used [35,36]. Studies which investigated the presence of other viruses on surfaces also used PCR methods. As such, molecular methods using next generation sequencing, such as metagenomic approaches and 16S rDNA gene sequencing, which support the capture of total bacterial or organism diversity, should be considered in order to provide a true picture of the contaminants in the hospital environment. To ensure that diversity is accurately assessed, consideration should be given to targets within the 16S rDNA gene. As with all detection methods, these can also be affected by primer design and inhibition due to contaminants such as cleaning agents and sample processing bias.

For the majority of studies focusing on bacteria in this review, only traditional microbiological culture methods were used ($N = 43$). Molecular methods were generally only used for comparisons of environmental and patient strains ($N = 6$) or to further identify specific pathogens after performing phenotypic tests ($N = 7$). Only two studies used high-throughput sequencing to investigate the entire collection of isolates further identified using molecular methods to give a comprehensive reflection of the microbiome: one of these looked at the hospital microbiome [37], the other examined the microbiome of surfaces on the International Space Station [38]. For studies focusing on viral contamination, molecular methods were the only way of assessing presence, absence, and species identification [35,36,39–41].

Another molecular identification method that has been adopted in many clinical laboratories is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) [42]. This method is able to identify a range of bacteria, mycobacteria and fungi by looking at their protein fingerprint, based on the charge and size of the proteins. A number of the studies included in this review used MALDI-TOF to confirm species identification after using selective media and phenotypic tests [7,31,43].

Environmental and biological factors to consider

Environmental factors, such as surface state, are a major cause of variability in method efficacies, and the effect on recovery when the cells are dried or adsorbed to a surface is variable. For example, dry surfaces consistently have lower recovery rates than wet surfaces [44]. Table I lists the appropriate methods when considering environmental and biological factors. Furthermore, the choice of target organism causes variance in the effectiveness of each method [13,15,16,19,20,23], and, regardless of method chosen, recoveries vary between species and strain [26,45].

High versus low predicted contamination levels

Surface bioburden is an important consideration [46]. For highly contaminated surfaces, sponges were significantly better for recovering *C. difficile* ($P < 0.05$) than contact plates. Sponges can detect *C. difficile* at <10 cfu spores, with a recovery of 94.4% on polypropylene work surfaces, 94.4% on stainless steel, and 83.3% from a bed rail, whereas contact plates had no recovery on all surfaces during the same experiment [46]. Macrofoam swabs were more sensitive than contact plates or other swabs, as they can yield positive results at the lowest MRSA concentrations [30]. Foam swabs were described as being more abrasive against the surfaces, giving better recovery of organism [30]. Swabs gave the best recovery at higher surface contamination, whereas contact plates were better for lower contamination concentrations [14].

Adsorbed micro-organisms

Adsorption occurs when the organism adheres to a surface. Significant differences in sensitivities for direct swab methods were found when sampling adsorbed and non-adsorbed cells. Direct contact methods gave higher recoveries when sampling non-adsorbed MRSA than swabbing [15]. Dipslides were the most sensitive for adsorbed cells [15]. Although all studies report some differences between sampling method, many of these are to no statistical significance, such as *Acinetobacter baumannii* in the real hospital environment, where there was no statistical difference between sponge and swab recoveries [47].

Injured micro-organisms

Sponges were found to be superior to swabs for the recovery of uninjured *Listeria monocytogenes* [45]. No statistical significance was reported between swabs and sponges for recovering injured and uninjured *L. monocytogenes* from test steel surfaces, but sponges were found to have a slightly higher percentage recovery: a mean of 96.7% for sponges for uninjured, versus 92.05% for swabs. For injured *L. monocytogenes*, the mean recovery for sponges was 76.05% versus 75.25% for swabs [45]. Sponges, at 74.3%, had better observed mean efficiency over a swab kit (73.5%; Truetech) and cotton swabs (68.6%; Fisher Scientific) at recovering *B. subtilis* spores from glass surfaces, though to no statistical significance [48].

Target organism

Target organism causes variance in the effectiveness of each sampling method [13,14,16,19,20,23], and, regardless of the method chosen, recoveries naturally vary between species and strain [26,45].

Staphylococcus aureus and coagulase-negative staphylococci (CoNS). TSA contact plates were best for recovering *S. aureus* and CoNS (99%) when compared to a range of dipslides [19]. However, overall macrofoam swabs were better than contact plates when recovering from stainless steel, tested with *S. aureus* [16]. Rayon and flocked swabs yielded the poorest recoveries when tested against petrifilms and contact plates [16]. *S. aureus* repeatedly gives higher recoveries, regardless

Table II
Factors causing variation in sampling efficiencies and recoveries

Factors affecting organism recovery	Details	References
Target organism and strain	Different sampling techniques recover different species with varying success. Different strains of the same organism can recover differently, even with the same technique.	[13,16], [19] [a], [25,26,45], [49] [a], [51,52]
Level of contamination	Some sampling techniques are not appropriate for surfaces with a high bioburden. For highly contaminated surfaces, sponges were significantly better for recovering *C. difficile* ($P < 0.05$) than contact plates. Contact plates may also show confluent growth leading to inaccurate counts.	[23], [30] [a], [44], [46] [a], [51]
Wet/dry surface	Cotton swabs recovered significantly more colonies than other swabs from a wet surface. Brush textured swabs performed poorly. 3M Enviroswabs gave better recovery on some surface types.	[21,44,53]
Adsorption of cells	Adsorbed cells are best recovered with direct contact methods such as contact plates and dipslides.	[13,15,24,27,44,54]
Pressure and contact time	Insufficient pressure will not recover all organisms from the surface, and contact time of 10 s must be adhered to for maximum recovery.	[13,23,28], [46] [a], [53]
Surface material and topography	Smoother surfaces are generally easiest to recover from. Some sampling devices are inappropriate for uneven or rough surfaces, such as contact plates. Some methods are more suitable for smaller and uneven areas such as swabs.	[13,14,16,18,22], [30] [a], [51,53,54]
Media	Different types of media recover different organisms and can inhibit growth of others. Target organism and potential surface bioburden must be considered before selection.	[15], [19] [a]
Pre-wetting, enrichment, transport medium and post-test processing	Wetting solutions and diluents can either aid or hinder recovery, depending on the target organism. Choice of transport medium is important [73] and the choice should vary between the target organism, time taken to transport to the lab, and post-test storage conditions and storage time. Most losses occur during processing, such as vortexing.	[17,21,22,24,26], [28–30[a]], [44,48], [49] [a]
Brand	Cherwell contact plates were shown to give better recoveries than Oxoid or bioMérieux, with significantly better recovery for *S. epidermidis*	[13]
Cell injury and environmental stressors	Uninjured cells recover better than injured or stressed cells. Sponges were shown to potentially recover injured *L. monocytogenes* from a steel surface, though to no statistical significance.	[15,17,45,54,55]
Size of surface sampled	If a large surface area is to be sampled, the method choice should reflect this. Sponges and roller-devices can easily sample large surface areas.	[24,25], [30] [a], [46] [a], [49] [a]
No. of samples	Time of processing may make some methods less suitable.	[56] [a], [57] [a]
Technician time and skill	Some methods, such as contact plates, allow fast sampling and easy interpretation, and require less training. Other techniques, such as swabs, can have variability in method between technician and require some skill to allow proper sample recovery.	[26]
Cost	Some sampling techniques, while giving better recoveries, may not be used in favour for sampling equipment that is cheaper or more readily available in the clinical environment.	[17], [30] [a], [45], [47] [a], [58]
Sensitivity	More sensitive methods will give truer results. Macrofoam swabs gave the best sensitivity for MRSA over contact plates and swabs, needing the lowest concentration to give a positive result. Dipslides were the most sensitive for adsorbed cells.	[14,15], [30] [a], [44], [46] [a], [51,52]
Hospital or ward speciality	There is a difference in contamination found between wards and ward type (general or specialist). Rooms with infected or colonized patients show increased recovery of the same organism.	[49] [a], [56] [a], [59], [60] [a]

[a] Hospital-based studies.

of sampling method, compared with *S. epidermidis* [13]. Once the samples are collected, enrichment may be appropriate (e.g. *S. aureus* recovery benefits from enrichment in Tryptic Soy Broth), followed by culture on the appropriate culture media.

Meticillin-resistant Staphyloccocus aureus. Compared to contact plates, flocked swabs, rayon swabs, and petrifilms allow better recovery of MRSA from surfaces [16]. Of the most commonly used techniques, macrofoam swabs showed the best sensitivity for MRSA compared with MRSA contact plates,

Table III
Sampling and processing methods used in hospital surface contamination studies

Method	Colony counting and phenotypic identification	Molecular biology methods used for identification	Total
Contact pate	9 studies [5,8,56,61,60,65–68]	0 studies	9 studies
Dipslide	4 studies [68–71]	0 studies	2 studies
Petrifilm and wipe	3 studies [72,70,73]	1 study [37]	3 studies
Swab	36 studies [6,12,31,32,39,43,56,74,75,60,66,67,76–99]	16 studies [6,31,35–37,39–41,43,79,83,89,92–94,100]	52 studies
Sponge	5 studies [7,31,32,63,101]	2 studies [7,31]	7 studies
Total	57 studies	19 studies	

neutralizing swabs, saline swabs, and sweep plates, needing the lowest concentration to give a positive result for 1.0×10^{2} MRSA cells/cm^2 on a mattress and 3.9×10^{-1} MRSA cells/cm^2 on a bench [30]. Flocked swabs were found to be superior compared to rayon, demonstrating 60% versus 20% recovery, respectively [20] as the flocculation allows enhanced recovery of organisms from microscopic undulations on the surfaces and better release into collection medium [30].

C. difficile. Sponges were shown to be significantly ($P = 0.006$) better at recovering *C. difficile* from inoculated hospital surface environments; sponges gave 52% recovery whereas swabs recovered 0% [49].

Gram-negative bacteria. Results show that swabs are better than contact plates for recovery of Gram-negative rods [30] with flocked or rayon swabs and petrifilms allowing better recovery of extended-spectrum β-lactamase-producing (ESBL) *E. coli* from surfaces [16]. However, TSA contact plates were best for *Acinetobacter* and *Pseudomonas* spp. recovery (83%) compared with a range of dipslides [19]. For Enterobacteriaceae, MAC dipslides gave greater recoveries compared with a range of others and VRBG were best for faecal indicators [19]. For *P. aeruginosa* and *Salmonella abony*, macrofoam swabs were better than contact plates overall when recovering from stainless steel [16].

Other bacteria, fungi, and viruses. Macrofoam swabs were better than contact plates overall when recovering from stainless steel, tested against *Candida albicans*, *Aspergillus niger*, *B. subtilis*, *Micrococcus luteus*, and *Brevibacillus parabrevis* [16]. Rayon and flocked swabs gave poorest recoveries when tested against petrifilms and contact plates [16]. Macrofoam swabs, pre-moistened and vortexed for 2 min during processing, also yielded the best percentage recovery for *B. anthracis* on stainless steel surfaces [22]. Flocked swabs were better than standard cotton swabs [16,50]. Cotton swabs had the highest sampling losses (7.2%) compared with swab kit (2.1%) and sponge (0.12%) and failed to detect *B. anthracis* when concentrations were low [51]. For norovirus, macrofoam swabs appeared more effective than cotton, rayon or polyester for recovery [22,24].

Sampling bias

When trying to draw conclusions and make comparisons in the literature, it is important to consider a wide range of potential sampling bias. In addition, there are other factors that may introduce bias (Table II).

Sampling sites and number of samples taken vary considerably between studies. The number of samples taken ranged between 24 and 2532 [56,57]. Percentage of surfaces reported as contaminated will vary depending on surfaces chosen for each experiment, in combination with target organism. Certain combinations of target surface and organism will yield positive results, such as looking for CoNS on patient charts handled by personnel without gloves, which yielded up to 100% contamination [61,62]. By contrast, looking for Gram-negative organisms, which are found significantly less frequently ($P < 0.0001$) in the hospital environment than Gram-positive organisms, undoubtedly produces lower recoveries [56].

Findings of hospital surface studies

Simple colony-forming unit (cfu) numbers per cm^2 provided by total viable counts (TVCs) often do not reflect the true risk to the patient, as studies show that surfaces with the highest bioburden are not always the surfaces with the most multidrug-resistant organisms (MDROs), which are of greater clinical concern [5,63]. TVC sampling is frequently undertaken in order to monitor cleaning, rather than as a risk assessment [64]. Seventy-three studies sampling the hospital environment were reviewed with varying contamination of surfaces (0–100%) likely due to studies using different sampling methodologies, processing methods and targeting different organisms on different surfaces (Table III). Swabs are the most popular sampling device used in combination with cfu counts on selective media and phenotypic tests. Additionally, a range of sampling surfaces were chosen, and samples were taken at varying times of year, in different ward specialties and geographical locations.

Importantly, despite overall contamination being reported as low, MDROs and clinically significant pathogens have been isolated from the near-patient environments and other high-touch surfaces. Among the studies selected for this review, a wide range of organisms, including those of clinical concern such as vancomycin-resistant enterococci ($N = 9$), MRSA ($N = 28$), and *Klebsiella pneumoniae* ($N = 9$), were shown to be isolated from surfaces.

When evaluating the contamination of the surface environment, one study reported isolation of Gram-positive organisms isolated significantly ($P < 0.0001$) more frequently than Gram-negative organisms; reported as 24.7% environmental detection rate in comparison to just 4.9%, respectively, possibly due to method bias towards Gram-positive bacteria [52].

In this review, 55 studies sampled for bacterial contaminants, two for fungi, five for DNA viruses, and four for RNA viruses. MRSA had the longest reporting timeframe, 1997–2019

Box 1
Summary of conclusions

- Meticillin-containing plates recover best from stainless steel, outperforming dipslides and swabs [17,40]. They were also found to be best for recovering *S. aureus* from non-porous surfaces.
- Dipslides are a potentially superior method of surface sampling, and should be investigated further for application in sampling the hospital surface environment, particularly when physical flexibility is required.
- Macrofoam swabs are generally found to be the most effective type of swab [23,25].
- Sponges are often reported to have better recoveries than other methods, and have been shown to be significantly better for *C. difficile* recovery than swabs [26].
- Petrifilms were the best method for recovering meticillin-resistant *S. aureus* from linoleum, mattress, coated steel, and polypropylene [17].
- Pre-wetting of swabs is essential to ensure good recovery [22,23].
- If swabs were not processed within the first 24 h, addition of transport medium was critical to avoid cell death or excessive growth, leading to inaccurate counts [30].
- Vortexing produced the best results, except for polyester swabs, which yielded better results with sonication, highlighting the importance of processing [23].
- Swabs produced the best recovery at higher surface contamination, whereas contact plates were better for lower contamination concentrations [15].
- *S. aureus* repeatedly gives higher recoveries, regardless of sampling method, compared with *S. epidermidis* [14].

[6,58,59]. Other species were only targeted in more recent publications, such as carbapenem-resistant *A. baumannii* with only one study in 2016 [7]. Publications targeting *C. difficile* had erratic publication dates, ranging from 2001 to 2015 [46,60].

Conclusions

Background environmental monitoring of the hospital surface environment is not enforced by law or legislation and hospitals are under no obligation to monitor surfaces. Hospitals that choose to sample may use in-house guidelines or guidelines from the food or pharmaceutical industry. There are no comprehensive guidelines available for hospital sampling and there is little evidence-based literature on efficacies of sampling methods under different conditions that exist in the real hospital environment.

This review has aimed to synthesize conclusions from the variety of literature available on the microbiological sampling of healthcare environment surfaces. Although it has been difficult to draw firm conclusions, some recommendations can be made, supported by multiple publications and results (Box 1). However, some recommendations based on a few publications require further study and evaluation.

This review has identified gaps in the literature and it is impossible to form a picture of the entire hospital surface microbiome due to a lack of studies sampling the general environment under non-outbreak situations, due to studies choosing only to look for a select organism or pathogen, and due to the wide range of sampling methods, results analysis and unit presentation of results (e.g. few studies show results in cfu/cm^2), making comparison between the literature challenging.

- Many studies looking into recovery efficacies of sampling methods from surfaces are based on the food industry, using *L. monocytogenes* as their target organism. Further research is needed to assess all sampling methods and variabilities with different nosocomial pathogens.
- Most studies are laboratory-based, with only 22% undertaken in a real hospital environment. Representative results of sampling efficacy on hospital surfaces with residual organic compounds, dust, detergents, and disinfectants in any possible combination have not been replicated in the laboratory environment.
- Some studies have sought to replicate the hospital surface environment by including representative surfaces, though many have used stainless steel coupons. General conclusions can be made about the best sampling methods, though correct application of these methods according to surface circumstances may allow better statistical evaluation and sensitivity.
- Some environmental monitoring methods, such as dipslides and petrifilms, are popular within other industries but have yet to be explored fully for clinical use.
- A single study has yet to explore the recovery efficacy for a range of clinical organisms under a single variable.

To conclude, MDROs are being isolated from the hospital surface environment, and this review has reported a wide range of organisms recovered. For high-risk patients (e.g. immunocompromised patients, or patients with open wounds) the environmental surface bioburden and the clinically significant pathogens which reside there should be of great concern. Recovery of each sampling method varies and the suitability of a chosen method can change depending on target organism, surface material, and on the available resources. As such, there is no one sampling method that fits all circumstances and the specific sampling situation and motivation needs to be evaluated before the most suitable method is selected. This review highlights the need for more evidence-based sampling assessment under different and specific conditions in order to draw better conclusions about the best sampling methods for different surfaces and micro-organisms.

Conflict of interest statement
None declared.

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Appendix A. Supplementary data

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