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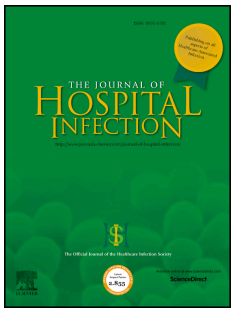

How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence

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

Running title: Microbiological sampling of hospital surfaces

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Structured Summary

Background: 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 patchy and there is no guidance or legislation in place on how to do this.

Aim: The aim of this review was to assess current literature on surface sampling methodologies, including the devices used, processing methods, the environmental and biological factors that might influence results.

Methods: 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.

Findings: 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, multi-drug 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 was incomplete and incomparable

Conclusion: 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.

Keywords: healthcare; environment; surfaces; sampling; HCAIs; infection prevention and control

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 figures from low income countries indicate that prevalence rates are in the region of 15-19% [2]. In Europe, HCAIs are attributed to approximately 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 ITU, 40-60% are due to endogenous flora, 20-40% are due to the contaminated hands of healthcare workers (HCW), 20-25% are due to antibiotic driven change and 20% potentially due to environmental contamination [4].

The hospital surface environment is an important factor in infection risk as it can act as a reservoir for nosocomial pathogens. Prior room occupants shed microorganisms 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 colonised 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 [9] on general monitoring of the hospital environment, in which surfaces are assessed by visible audit. 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, however, this guidance does not provide readers with the microbiological protocols required [11].

77 In light of the changing awareness of the risk the surface environment poses, more hospitals are
78 considering instigating routine monitoring of their environments, either to assess cleaning or as part
79 of a continuous risk assessment. This review will investigate what microorganisms have been
80 isolated from hospital surfaces, how those samples were taken and processed, in order to build a
81 clearer picture of the contaminants in the hospital surface environment and to prepare evidence for
82 the development of an optimised 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 were listed in Supplementary Table I. Search terms are listed in Supplementary Table II. A systematic review was not possible due to current evidence, 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 speciality 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 provides an overview of 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 points the reader to the different sections of the review and their survival until the sample processing or analytical phase. In this review, the term recovery is defined as the percentage of cells that are viable and therefore can be detected successfully from the original number of cells inoculated onto or present in a sampling device or from a surface. Thirty-three studies were reviewed exploring methods of surface sampling: 7 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 agar plates that can be directly pressed onto a surface to take a quantitative sample. Contact plates can be made with selective or non-selective agar, with or without a neutralising agent, all of which lead to differences in recovery of the target organism. The main

advantage of contact plates is the production of semi-quantitative data in the form of a colony counts, which can help elucidate trends [12].

Recovery of organisms ranged between 23-56% depending on the plate and organism [13]. Contact plates were found to be better than swabs for recovery from 100% cotton fabric [14]. Methicillin-containing contact plates recovered methicillin-resistant *Staphyloccocus aureus* (MRSA) best from stainless steel, outperforming dipslides and swabs [15, 16]. Contact plates were also found to be best for recovering *Staphyloccocus 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 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 could be considered a better option due to their flexibility; unlike contact plates, and can sample uneven surfaces without the additional processing losses faced by non-direct contact methods. 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 to TSA contact plates[19]. Violet red blood glucose (VRBG) dipslides (77% total positive samples) and TSA and VRBG dipslides were best for faecal indicator spp (66% total positive samples) compared with TSA contact plates and MAC dipslides [19]. The same study reported that dipslides, with the addition of neutralisers, performed significantly better than those without [19].

155

156 Swabs

157 Swabs are indirect sampling devices that can be made of various materials, including, cotton, rayon,
158 polyester, calcium alginate or macrofoam and can be flocked by design with numerous processing
159 options. Swabs can be manipulated around difficult or uneven surfaces, such as door handles, bed
160 rails and around sinks and taps. From the available literature, they were the most commonly used
161 sampling method (Figure 3). This is potentially due to their simplicity, affordability, and availability in
162 the hospital environment.

163

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

174

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

179

180 Sponges

Sponges are an indirect sample device they 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 ($P < .0001$) better for *C. 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 [18] and so may be beneficial for sampling fabric surfaces. Handling during the sampling process can 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].

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 40.5-98.3% [26]. Electrostatic wipes were found to give

207 better recoveries for *S. aureus* on stainless steel plates, outperforming swabs and contact plates
208 [27].

209

210

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 neutralises quaternary ammonium compounds [21]. It is also possible to use a wide variety of transport media and neutralisers. When choosing a neutraliser, 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 hours or stored in transport media at 4 °C for no more than 24 hours [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 [28] where 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 *E. 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 *Burkholdaria thailandensis* recovery [21]. Cotton tipped swabs in ¼ 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 Neutralisers

Transport medium, such as anaerobic universal transport medium, aerobic Amies medium and neutralising 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]. Neutralising broths help to keep microbial cells intact while also neutralising 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 hours, 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 hours, 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 onto 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, Butterfields'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 than can 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 premoistening and the use of vortexing, recovery with swabs can vary between <0.01-43.6% [22]. An optimum time of two minutes vortexing was shown to be superior over 12 minutes 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. A commonly used broth is brain-heart infusion broth [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]. While enrichment allows recovery of stressed or injured cells, it is important to note 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 18-48 hrs, or non-specific "overnight" [13, 14, 22]. Twenty-three studies used incubations at 37 °C for 24-48 hours and seven studies reviewed incubated at 35 °C for 24-48 hours. Choice of incubation temperature can 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°C to 45°C [34].

Molecular Biology Processing

Molecular methods are extremely valuable for analysing the microbiological contaminants of the hospital surface environment. While, historically, organisms were identified using culture methods, not all clinically relevant organisms are culturable, such as norovirus, where 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 utilising next generation sequencing, such as metagenomic approaches and 16S rDNA gene sequencing, which support the capturing 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 utilised (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], while 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 which has been adopted in many clinical laboratories is matrix-assisted laser desorption ionization time-of-flight mass spectrometry, or 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 gives an overview of 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 while 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 give positive results at the lowest levels of MRSA concentration [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 Microorganisms

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]. While all studies report some differences between sampling method, many of these are to no statistical significance, such as *Acinetobacter baumannii*

in real hospital environment, where there was no statistical difference between sponge and swab recoveries [47].

Injured Microorganisms

Sponges were found to be superior to swabs for the recovery of uninjured *L. monocytogenes* [45]. While no statistical significance could be reported between swabs and sponges for recovering injured and uninjured *L. monocytogenes* from test steel surfaces, results show sponges 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 (Truetech Inc) 73.5%, and cotton swabs (Fisher Scientific) 68.6% 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].

S. 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 gave the poorest recoveries when tested against petrifilms and contact plates [16]. *S. aureus* repeatedly gives higher recoveries, regardless of sampling method, in comparison to *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 (MRSA)

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 gave the best sensitivity for MRSA compared to MRSA contact plates, neutralising 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 beta-lactamase producing (ESBL) *E. coli* from surfaces [16]. However, TSA contact plates were best for *Acinetobacter* and *Pseudomonas spp.* recovery (83%) compared to a range of dipslides [19]. For *Enterobacteriaceae,* MAC dipslides gave greater recoveries compared to 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

406 Macrofoam swabs were better than contact plates overall when recovering from stainless steel,
407 tested against *Candida albicans*, *Aspergillus niger*, *B. subtilis*, *Micrococcus luteus* and *Brevibacillus*
408 *parabrevis* [16]. Rayon and flocked swabs gave poorest recoveries when tested against petrifilms
409 and contact plates [16]. Macrofoam swabs, pre-moistened and vortexed for two minutes during
410 processing also gave the best percentage recovery for *B. anthracis* on stainless steel surfaces [22].
411 Flocked swabs were better than standard cotton swabs [16, 50]. Cotton swabs had the highest
412 sampling losses (7.2%) compared to swab kit (2.1%) and sponge, (0.12%) and failed to detect *B.*
413 *anthracis* when concentrations were low [51]. For norovirus, macrofoam swabs appeared more
414 effective than cotton, rayon or polyester for recovery [22, 24].
415

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 can 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 [52, 53]. Percentage of surfaces reporting contamination will vary depending on surfaces chosen for each experiment, in combination with target organism. Certain combinations of target surface and organism will give positive results, such as looking for CoNS on patient charts, which will be handled by personnel without gloves, which gave up to 100% contamination [54, 55]. In contrast, looking for Gram-negative organisms, which are found significantly less frequently ($P<0.0001$) in the hospital environment than Gram-positive organisms, will undoubtedly reflected lower recoveries [52].

Findings of Hospital Surface Studies

Simple colony forming unit (CFU) numbers per cm^2 provided by total viable counts (TVC's) 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 multi-drug resistant organisms (MDRO's) which are of greater clinical concern [5, 56]. TVC sampling is frequently undertaken in order to monitor cleaning, rather than as a risk assessment [57]. 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 (Supplementary 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 specialities and geographical locations.

Importantly, despite overall contamination being reported as low, MDROs and clinically significant pathogens could be 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), methicillin-resistant *Staphylococcus aureus* (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 [52], possibly due to method bias towards Gram-positive bacteria.

In this review, fifty-five studies sampled for bacterial contaminants, 2 for fungi, 5 for DNA viruses and 4 for RNA viruses. MRSA had the longest reporting timeframe, 1997-2019 [6, 58, 59]. Other

456 species were only targeted in more recent publications, such as carbapenem-resistant *A. baumannii*
457 [7] with only one study in 2016. Publications targeting *C. difficile* had erratic publication dates,
458 ranging from 2001 [60] to 2015 [46].

459

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 which exist in the real hospital environment.

This review has aimed to synthesise conclusions from the variety of literature available on the microbiological sampling of healthcare environment surfaces. Although it has been difficult to draw firm conclusions, there are recommendations can be made, supported by multiple publications and results (Figure 3). However, some recommendations formed on the basis on just a few publications and further studies are needed.

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, studies choosing only to look for a select organism or pathogen, and the wide range of sampling methods, results analysis and unit presentation of results (e.g. few studies give 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 assessing all sampling methods and variabilities with different nosocomial pathogens.
- Most studies are lab 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 disinfects 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 utilised stainless steel coupons. General conclusions can be made about the best sampling methods, though correct application of these methods according to surface circumstances can allow increased significance and sensitivity.
- Some environmental monitoring methods are popular within other industries, but have yet to be explored fully for clinical use, such as dipslides and petrifilms.
- A single study has yet to explore the recovery efficacy for a range of clinical organisms under a single variable.

To conclude, multi-drug resistant organisms (MDRO's) are being isolated from the hospital surface environment, and this review has reported a wide range of organisms that have been recovered. For high-risk patients (e.g. immunocompromised patients; 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 state and 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. Although an attempt to synthesise some guidance using information from the current literature, this publication highlights the need for more evidence-based sampling assessment under different and specific conditions in order to truly draw conclusions about the best sampling methods for different surfaces and microorganisms.

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784 **Figure legends**

785

786 **Figure 1.** Flow diagram outlining review findings and the process of designing a sampling protocol.

787

788 **Figure 2.** Devices most commonly used for the collection of microbiological samples from surfaces:

789 a) contact plate; b) dipslide; c) petrifilm; d) swab; e) sponge; and f) wipe/gauze. The pie chart shows

790 how commonly each device was used in the publications included in this review.

791

792 **Figure 3.** Summary of conclusions.

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

	Contact Plate	Dipslide	Petrifilm	Swab	Sponge
Wet Surface			+	+*	
Dry Surface	+		+		
Flat Surface	+	+			+
Uneven Surface	-	+	+	+	+
High Bioburden	-			+	
Low Bioburden	+	+	+		+
Injured Cells				+	+
S. aureus & MRSA	+		+		
C. difficile					+
Gram negative bacteria				+	
Viruses	-	-	-	+	-

*cotton, rayon, polyester or macrofoam. Brush-textured swabs perform poorly on wet surfaces. Empty cells indicate lack of data.

Table II. Factors causing variation in sampling efficiencies and recoveries.

Factors Affecting Organism Recovery	Details	References Hospital-based studies underlined
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, 25, 26, 45, 49, 51, 61]
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, 44, 46, 51]
Wet/dry surface	Cotton swabs removed significantly more colonies than other swabs from a wet surface. Brush textured swabs performed poorly. 3M Enviroswabs gave better at recovery on some surface types.	[21, 44, 62]
Adsorption of cells	Adsorbed cells are best recovered with direct contract methods such as contact plates and dipslides.	[13, 15, 24, 27, 44, 63]
Pressure and contact time	Insufficient pressure will not recover all organisms from the surface, and contact time of 10 seconds must be adhered to for maximum recovery.	[13, 23, 28, 46, 62]
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, 51, 62, 63]
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]
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, 44, 48, 49]
Brand	Cherwell contact plates were shown to give better recoveries than Oxoid or Biomerieux, 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, 63, 64]
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, 46, 49]
Number of samples	Time of processing may make some methods less suitable.	[52, 53]
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 requires 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, 45, 47, 65]
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, 44, 46, 51, 61]
Hospital or ward speciality	There is a difference in contamination found between wards and ward type (general or specialist) Rooms with infected or colonised patients show increased recovery of the same organism.	[49, 52, 66, 67]

Figure 1

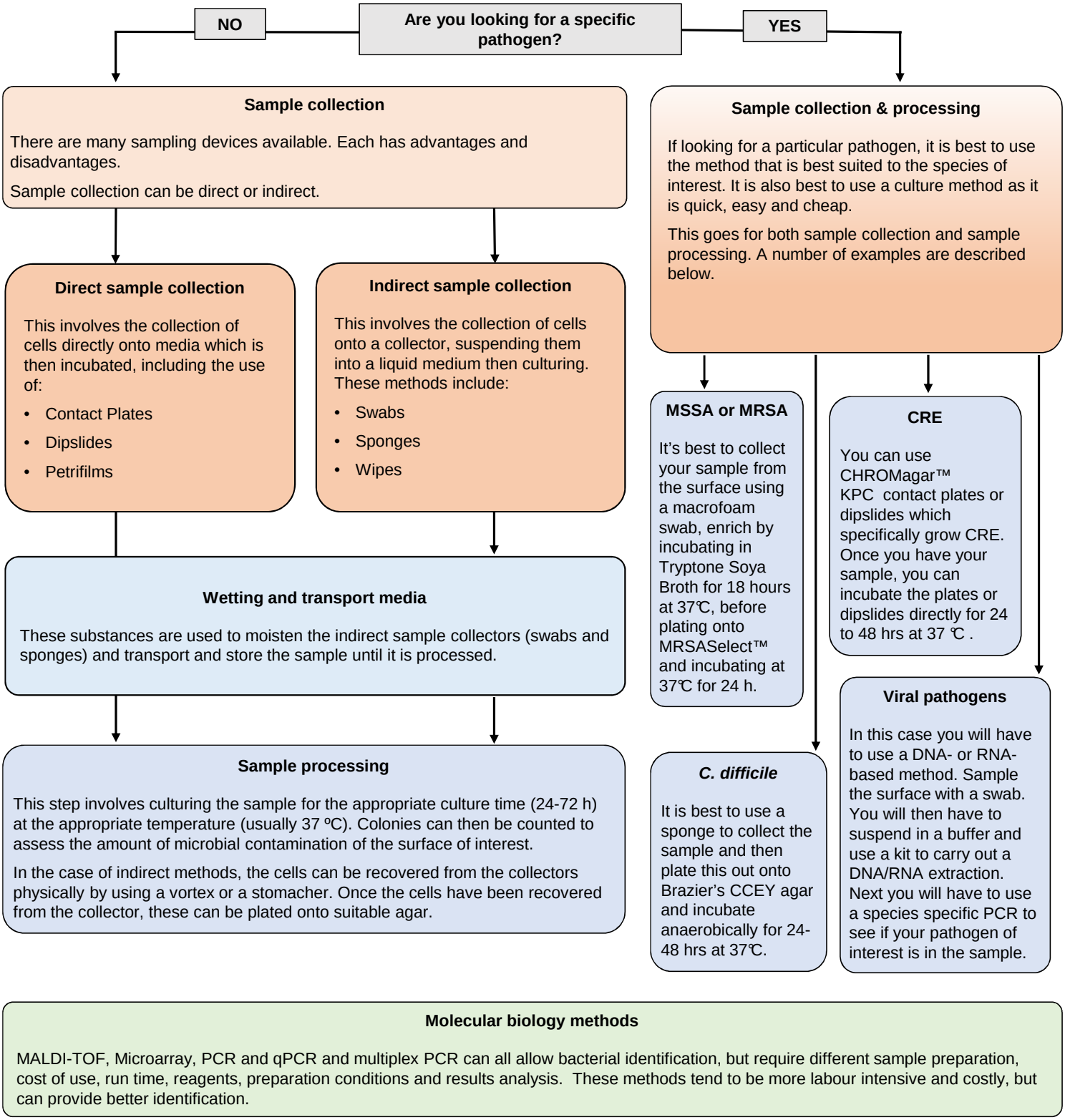
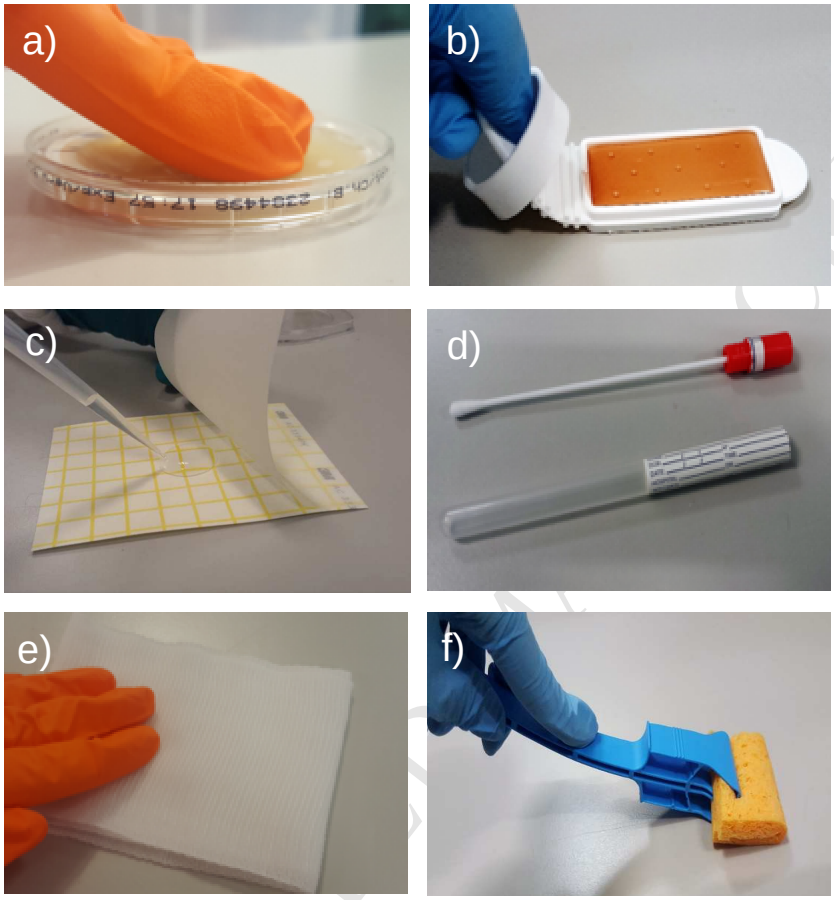

Are you looking for a specific pathogen?
NO
YES
Sample collection
There are many sampling devices available. Each has advantages and disadvantages.
Sample collection can be direct or indirect.
Direct sample collection
This involves the collection of cells directly onto media which is then incubated, including the use of:
• Contact Plates
• Dipslides
• Petrifilms
Indirect sample collection
This involves the collection of cells onto a collector, suspending them into a liquid medium then culturing. These methods include:
• Swabs
• Sponges
• Wipes
Wetting and transport media
These substances are used to moisten the indirect sample collectors (swabs and sponges) and transport and store the sample until it is processed.
Sample processing
This step involves culturing the sample for the appropriate culture time (24-72 h) at the appropriate temperature (usually 37 ºC). Colonies can then be counted to assess the amount of microbial contamination of the surface of interest.
In the case of indirect methods, the cells can be recovered from the collectors physically by using a vortex or a stomacher. Once the cells have been recovered from the collector, these can be plated onto suitable agar.
Sample collection & processing
If looking for a particular pathogen, it is best to use the method that is best suited to the species of interest. It is also best to use a culture method as it is quick, easy and cheap.
This goes for both sample collection and sample processing. A number of examples are described below.
MSSA or MRSA
It's best to collect your sample from the surface using a macrofoam swab, enrich by incubating in Tryptone Soya Broth for 18 hours at 37℃, before plating onto MRSASelect™ and incubating at 37℃ for 24 h.
CRE
You can use CHROMagar™ KPC contact plates or dipslides which specifically grow CRE. Once you have your sample, you can incubate the plates or dipslides directly for 24 to 48 hrs at 37 ℃ .
C. difficile
It is best to use a sponge to collect the sample and then plate this out onto Brazier's CCEY agar and incubate anaerobically for 24-48 hrs at 37℃.
Viral pathogens
In this case you will have to use a DNA- or RNA-based method. Sample the surface with a swab. You will then have to suspend in a buffer and use a kit to carry out a DNA/RNA extraction. Next you will have to use a species specific PCR to see if your pathogen of interest is in the sample.
Molecular biology methods
MALDI-TOF, Microarray, PCR and qPCR and multiplex PCR can all allow bacterial identification, but require different sample preparation, cost of use, run time, reagents, preparation conditions and results analysis. These methods tend to be more labour intensive and costly, but can provide better identification.

Figure 2. Devices most commonly used for the collection of microbiological samples from surfaces: a) contact plate; b) dipslide; c) petrifilm; d) swab; e) sponge; and f) wipe/gauze. The pie chart shows how commonly each device was used in the publications included in this review.



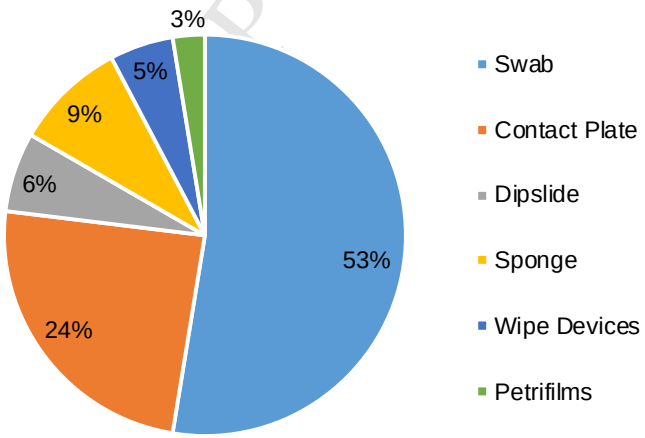
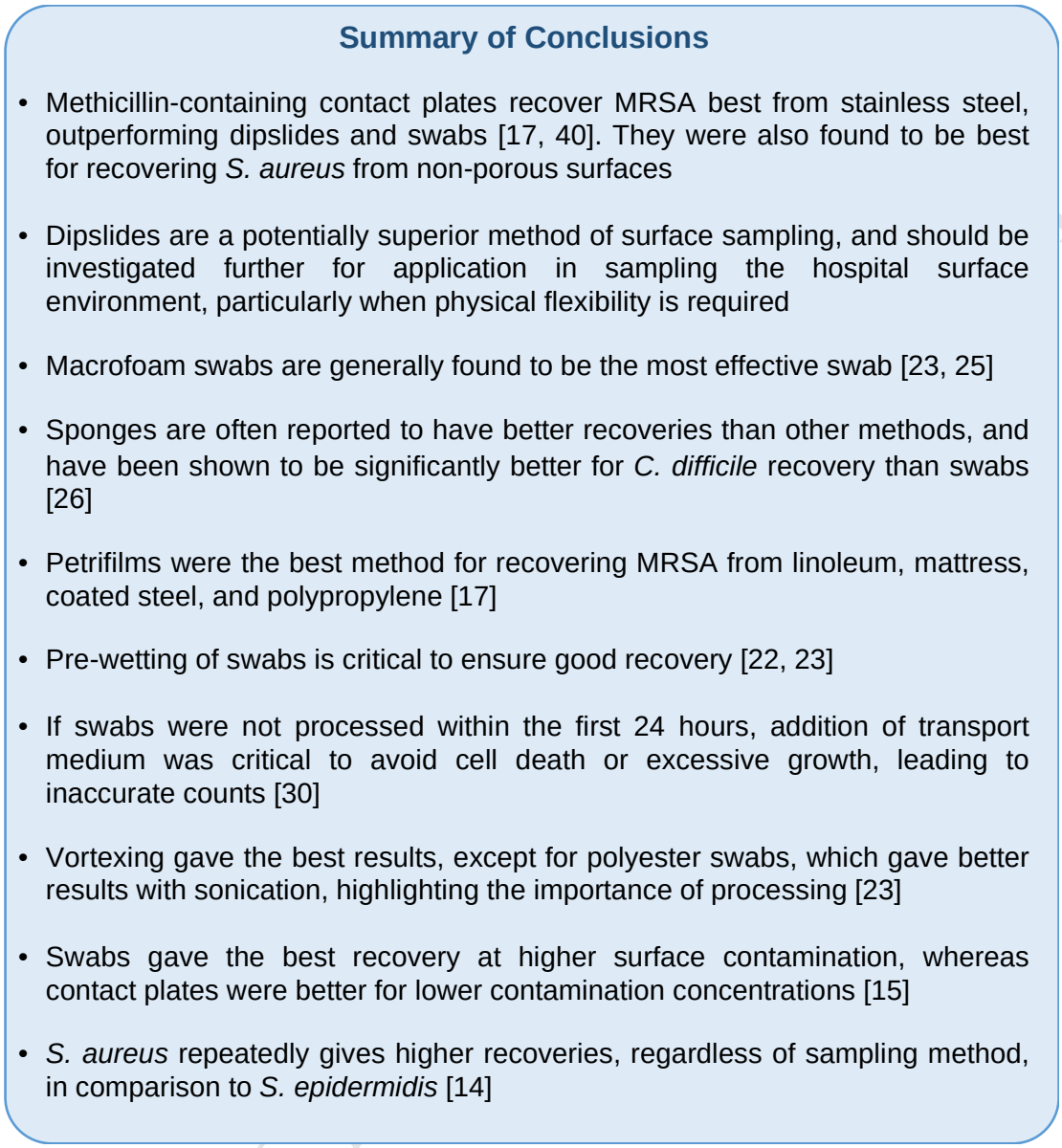


Figure 3


Summary of Conclusions
• Methicillin-containing contact plates recover MRSA 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 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 MRSA from linoleum, mattress, coated steel, and polypropylene [17]
• Pre-wetting of swabs is critical to ensure good recovery [22, 23]
• If swabs were not processed within the first 24 hours, addition of transport medium was critical to avoid cell death or excessive growth, leading to inaccurate counts [30]
• Vortexing gave the best results, except for polyester swabs, which gave better results with sonication, highlighting the importance of processing [23]
• Swabs gave 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, in comparison to S. epidermidis [14]