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What is the best method? Recovery of methicillin-resistant Staphylococcus aureus and extended-spectrum beta-lactamase producing Escherichia coli from inanimate hospital surfaces

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ABSTRACT

Narrative abstract (50 words max)

Environmental sampling in hospitals, when required, needs to be reliable. We evaluated different methods of sampling methicillin-resistant Staphylococcus aureus and extended-spectrum beta-lactamase producing Escherichia coli on five materials of the hospital setting. Petrifims and contact-plates were superior to swabs for all of the surfaces studied.

Keywords: MRSA, ESBL-E. coli, environmental contamination, hospital surfaces, swabs, contact-plates, petrifilms.

Contamination of hospital surfaces by nosocomial microorganisms has long been recognized.[1] Transmission of these microorganisms occurs directly or indirectly, e.g. via contaminated healthcare workers' gloves.[2] Inadequate cleaning has also been associated with the transmission of healthcare-associated infections (HCAI).[3] Furthermore, the use of novel decontamination methods such as gaseous plasma requires reliable methods to monitor their microbiological effectiveness. The Center for Disease Control provide recommendations for evaluating hospital environmental cleaning however, these are not standard and they do not consider specific outbreak scenarios or other research driven investigations.[4] Sampling of the healthcare environment has been inconsistently reported using a variety of swabs (cotton, rayon or nylon flocked) or contact-plates.[5,6] Although common in the food industry, petrifilms have rarely been used in assessing environmental contamination within the healthcare setting.[7]

We compared the recovery and limit of detection (LoD) of two important HCAI pathogens, i.e. methicillin-resistant Staphylococcus aureus (MRSA) and extended spectrum β-lactamase producing Escherichia coli (ESBL-E), from different materials commonly found in hospitals using swabs, contact-plates and petrifilms.

METHODS

Clinical strain 31(ST22-MRSA-IV) and ESBL-E clinical strain (CL2), both from our collection were used in this study.[8,9] Columbia blood agar (CBA) and ESBL Brilliance agar (Oxoid Ltd, UK) were used for MRSA and ESBL-E, respectively.

Cultures were grown aerobically overnight at 37°C with rotation in tryptic-soy broth (TSB) supplemented with 5% NaCl and Mueller-Hinton (MH) broth (Sigma Aldrich, Ireland) for MRSA and ESBL-E, respectively. Cultures were centrifuged for 10min at 15,500g and washed three times in phosphate buffered saline (PBS). The culture was adjusted to the density of a 0.5McFarland standard (approximately $1x10^8$ MRSA and $3.2x10^7$ ESBL-E CFU/mL) in PBS using a DensiChek™ colorimeter (Vitek). Serial dilutions (10^{-1} to 10^{-6}) were prepared in PBS and 50µl of each (including the original suspension) were applied to the sterile test surfaces. These were sections ($25cm^2$) of; linoleum flooring (Forbo Flooring, Ireland), polyurethane mattress fabric (Meditec Medical, Ireland) provided by the Maintenance Department, Beaumont Hospital, Dublin, polypropylene (GoodFellow Cambridge Ltd., UK), powder-coated mild steel (Watermark Engineering, Ireland) and stainless steel. Test sections were washed for 30min (1% virkon solution for linoleum and mattress and 70% ethanol for powder coated mild steel, polypropylene and stainless steel) before placing in Petri-dishes under UV light for 30min before bacterial inoculation.

Inoculated sections were air dried in a laminar flow cabinet over 1h before recovery. The recovery of bacteria from surfaces was assessed using rayon and nylon flocked eSwabs (Copan, Italy), contact-plates - MRSA Chromagar (Cruinn Diagnostics Ltd, Ireland) selective for MRSA, Brilliance UTI agar (Oxoid Ltd, UK) selective for ESBL-E and 3M™Petrifilms; Staph Express Count for MRSA and Enterobacteriaceae Count for ESBL-E (3M Petrifilm Trafalgar Scientific, UK). Swabs were pre-moistened in PBS and the entire section then targeted. Swabs

were placed in 3mL of PBS in round-bottomed tubes and briefly vortex mixed. Serial dilutions were prepared to confirm the total viable count (TVC). Aliquots (10µl,100µl) of each suspension were spread onto CBA for MRSA and ESBL Brilliance agar for ESBL-E. Sterile contact-plates (65mm x 15mm) (VWR®) were poured with either MRSA Chromoagar (MRSA selective) or UTI brilliance agar (E. coli selective), dried and applied to the inoculated sections for 20 to 30s, ensuring firm contact with the surface. Petrifilms were prepared according to the supplier instructions. Briefly 1mL of sterile water was added to each petrifilm before storage at 4°C for 2h before use. Petrifilms were applied ensuring that the entire material section was covered. Sub-cultured plates from swabs, contact-plates and petrifilms were incubated at 37°C overnight. Colony enumeration was performed macroscopically the following day. The limit of detection (LoD) was defined as the lowest concentration of bacteria applied that was detected by a specific method.

Statistical data analysis was carried out using GraphPad Prism 5.00 software. The means of the $\log_{10}$(CFU/mL) recovered between methods or between materials was compared by one-way analysis of variance (ANOVA). When significant, i.e. $p<.05$, further analysis on the variance of the means between methods was carried out by Tukey's multiple comparison test. Comparison of the recoveries between microorganisms was analysed by t-test.

RESULTS

Figure 1 shows the recovery of MRSA from five materials, using four methods, at

different applied inocula (8 to 1 log_{10}CFU/mL). Petrifilms were best in recovering MRSA from all materials except stainless steel, when contact-plates were better. After petrifilms, contact-plates were best for recovering MRSA from linoleum, powder-coated mild steel and polypropylene. The second best method for stainless steel was the petrifilm and for mattress the flocked swabs. For all surfaces, rayon swabs had the lowest recovery of MRSA, compared to petrifilms (**P<.01). The LoD for MRSA was of $1x10^2$ CFU/ml for all materials except linoleum (LoD of $1x10^4$ CFU/ml).

Figure 2 shows the recovery of ESBL-E from five materials, using four methods at different inocula concentrations (7.5 to 1.5 log_{10}CFU/mL). Petrifilms were best in recovering ESBL-E from all the materials followed by contact-plates. The lowest recovery of ESBL-E from all materials was with rayon and flocked swabs. Petrifilms and contact-plates were significantly better than either swab (***P<.001). The LoD for ESBL-E from petrifims was $3.2x10^1$ CFU/ml for powder-coated mild steel, polypropylene and stainless steel, and $3.2x10^2$ CFU/ml and $3.2x10^3$ CFU/ml from mattress and linoleum, respectively.

The poorest recovery of MRSA and ESBL-E was with swabs. However, the recovery of MRSA using rayon and flocked swabs was significantly higher compared with ESBL-E from all surfaces (**P<.01 and ***P<.001, respectively). The recovery of MRSA from powder-coated mild steel, polypropylene and stainless steel was greater than from the mattress and linoleum but this difference was not statistically significant. Similarly, ESBL-E recovery was not significantly different among the different materials.

DISCUSSION

To our knowledge, this is the first study to evaluate petrifilms for assessing hospital surface contamination and compare it with other commonly used methods. Like contact-plates, petrifilms are a direct contact method advantageous over the aforementioned on its flexibility to adjust to non-flat surfaces (e.g. door handles). Interestingly, from those evaluated here, the petrifilm was the overall best method to recover both MRSA and ESBL-E from all surfaces tested followed by contact-plates. A previous study showed that Gram-positive contamination of the environment adjacent to 54 patients with Gram-positive infections versus 136 with Gram-negative infections was more heavily contaminated by Gram-positives (24.7%) than Gram-negatives (4.9%).[10] Our findings suggest that contamination by Gram-negatives may be underestimated in studies where direct contact methods were not used. We show that the recovery of MRSA and ESBL-E differ significantly according to the method used and the type of surface being screened. Particularly, for MRSA, recovery was lower from linoleum and mattress compared to other surfaces, possibly due to the high porosity of these materials allowing bacteria to penetrate and being harder to culture.

This study limitation includes not evaluating the materials characteristics (e.g. porosity, roughness, hydrophobicity), bacteria were enumerated per volume rather than per area, and surfaces were free of protein unlike often in practice.

In conclusion, notwithstanding financial considerations, we suggest that direct contact methods, i.e. petrifilms and contact-plates and not swabs, are best

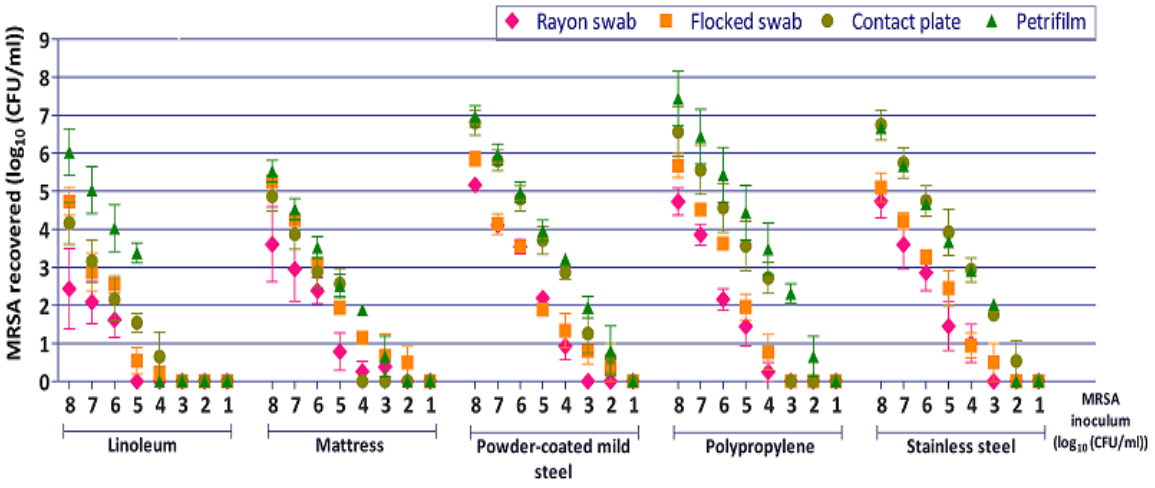
for the detection of MRSA and ESBL-E in the healthcare environment. They are more rapidly processed than swabs and can be used as appropriate to the surface type. Additional work is needed to confirm these findings in the actual hospital environment.

ACKNOWLEDGEMENTS

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Potential conflicts of interest. H.H. has recent research collaborations with Steris Corporation, Inov8 Science, Pfizer & Cepheid and has also received lecture & other fees from Novartis, AstraZenca & Astellas. All other authors declare no potential conflict of interest.

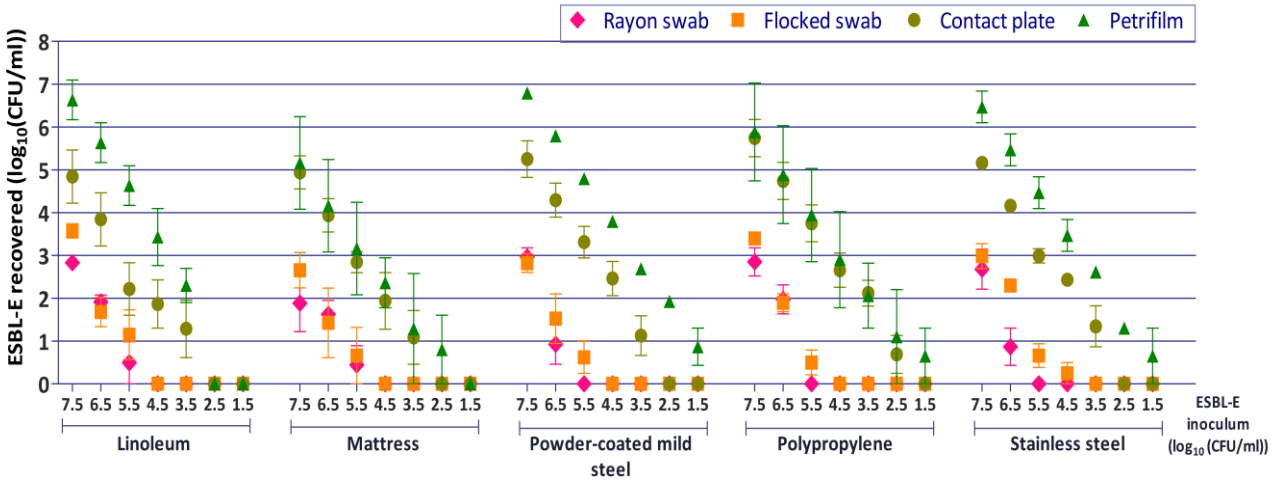
176 Figure 1



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179 Figure 2



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222 FIGURE LEGENDS

223

224 Figure 1 – Numbers of MRSA inoculated and recovered using rayon and flocked
225 swabs, contact-plates and petrifilm from surfaces common to the hospital
226 environment. Each data point represents the mean of at least three individual
227 assays n≥3, error bars represent the standard error of the mean (SEM).

228

229 Figure 2 – Numbers of ESBL E. coli inoculated and recovered using rayon and
230 flocked swabs, contact-plates and petrifilm from surfaces common to the hospital
231 environment. Each data point represents the mean of at least three individual
232 assays n≥3, error bars represent the standard error of the mean (SEM).