

New Data Confirm Biofilms on Majority of Dry Surfaces



Evidence of biofilm and bacteria embedded in thick extracellular protein substances on 41 of 44 dry surfaces tested.

Bacteria embedded in biofilms can be up to 1000 times more resistant to disinfectants.

Biofilms have grown in disinfecting concentrations of quaternary disinfecting solutions.

Cleaning with surfactant based detergents/ disinfectants deposits organic residues which some bacteria are able to metabolize as a nutrient source for growth in biofilms.

Routine cleaning and disinfection practices used currently are not effective at removing biofilms.

When using quaternary disinfectants or surfactant based detergents (surface active ingredients) chemical dispensers can become contaminated with biofilms containing highly resistant pathogenic bacteria.

Bacterial populations found in the health care environment are often more resistant to antibiotics and surface disinfectants than the ATCC strains used for testing disinfectants.

Use of disinfectants on environmental surfaces in health care facilities has not achieved the desired eradication of target pathogens.

High concentrations of oxidizers have the greatest potential for removing biofilm matrix.

New cleaning and disinfecting processes are required in order to prevent the accumulation of organic soils and bacteria on frequently cleaned surfaces.

Look for NEW PCS Oxidizing Cleaning and Disinfecting Processes to prevent and remove accumulated dried organic soils.

Biofilms in Hospitals Prevalent on Dry Environmental Surfaces, May Contribute to Infection

Biofilms harboring multi-antibiotic-resistant organisms are found at unexpected levels on dry hospital surfaces and could contribute to the risk of infection transmission, according to the results of a new study.

"This emphasizes how adaptable bacteria are," lead researcher Karen Vickery, PhD, from Macquarie University in Sydney, Australia, told Medscape Medical News.

"Biofilms are forming on many hospital surfaces because they aren't cleaned frequently enough. The bacteria have a chance to attach and excrete extracellular polymeric substances, or slime, which makes them more resistant to removal and tolerant to disinfectants,"

In their study, Dr Vickery and her team aseptically obtained hard surface sections of additional furnishings and equipment from an intensive care unit after terminal cleaning.

They used aerobic culture and real-time quantitative polymerase chain reaction (PCR) to determine bacterial presence including the 16S rRNA gene and *Staphylococcus aureus*.

They found evidence of biofilm and bacteria embedded in thick extracellular polymeric substances on 41 of 44 items (93%), which was visually confirmed with scanning electron microscopy.

It was a surprise to see the high number of items with biofilm on them.

Biofilms in Hospitals Prevalent, May Contribute to Infection

Medscape November 27th 2014 Nancy A Melville

Presence of biofilm containing viable multi-resistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit be a source of infection

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Surface-attached cells, biofilms and biocide susceptibility: implications for hospital cleaning and disinfection

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Microbes tend to attach to available surfaces and readily form biofilms, which is problematic in healthcare settings. Biofilms are traditionally associated with wet or damp surfaces such as indwelling medical devices and tubing on medical equipment. However, microbes can survive for extended periods in a desiccated state on dry hospital surfaces, and biofilms have recently been discovered on dry hospital surfaces. Microbes attached to surfaces and in biofilms are less susceptible to biocides, antibiotics and physical stress.

Thus, surface attachment and/or biofilm formation may explain how vegetative bacteria can survive on surfaces for weeks to months (or more), interfere with attempts to recover microbes through environmental sampling, and provide a mixed bacterial population for the horizontal transfer of resistance genes.

The capacity of existing detergent formulations and disinfectants to disrupt biofilms may have an important and previously unrecognized role in determining their effectiveness in the field, which should be reflected in testing standards. There is a need for further research to elucidate the nature and physiology of microbes on dry hospital surfaces, specifically the prevalence and composition of biofilms.

Resistance and reduced susceptibility Biofilms constitute a protected mode of growth, allowing bacteria to survive in hostile environments.

Biocide susceptibility Many studies have evaluated the impact of established biofilms on biocide susceptibility,

For example, clinical isolates of MRSA and *P. aeruginosa* were grown as biofilms on discs of common materials in the hospital environment, and treated with three commonly used hospital biocides: benzalkonium chloride (1% w/v), chlorhexidine gluconate (4% w/v) and triclosan (1% w/v).⁷ The MBCs of all biocides for planktonic cultures of both organisms were considerably less than the concentrations recommended for use by the manufacturer. However, when isolates were grown as biofilms, the biocides were ineffective at killing bacteria at the concentrations recommended for use. The MBCs of all three biocides were found to be 10- 1000-fold higher than the same isolates grown in planktonic culture for MRSA and *P. aeruginosa*

Another study evaluated the susceptibility of four *Candida* spp. and two *Escherichia coli* strains to sodium hypochlorite, ethanol, hydrogen peroxide and iodine.²⁰ Strains were tested in planktonic culture, as attached cells and as biofilms in microtitre plates. Whilst susceptibility varied by organism and biocide, biofilms were less susceptible than attached cells, which were less susceptible than planktonic cells.

Some biocides are more effective than others at inactivating bacteria in biofilms, although conflicting data have been reported, which may be explained by differences in experimental conditions.^{20,24,35,49,50} In one study, susceptibility varied by phase, organism and biocide.²⁰ In another study, the oxidizing agents sodium hypochlorite and peroxygens were more effective than a range of other chemicals (including alcohols, biguanides, halogens, phenols and quaternary ammonium compounds) for inactivating *P. aeruginosa* and *S. aureus* biofilms.³⁵ In other studies, sodium

hypochlorite was more effective than chlorhexidine for inactivating *Enterococcus faecium* and MRSA in biofilms,

Comparing biocides may be further confounded by the 'dose response' type relationship that has been shown between biofilm susceptibility and biocide concentration.^{35,51,52}

For example, one study showed that 10% hydrogen peroxide was considerably more effective for inactivating bacteria in biofilms compared with 6% hydrogen peroxide.

One study showed that only sodium hypochlorite and hydrogen peroxide damaged both the bacteria within the biofilm and the biofilm matrix itself.

Persistence

Vegetative bacteria dried on to surfaces can survive for weeks to months (or more) in vitro, despite the lack of a nutrient source or water (aside from ambient humidity).^{8,9} Biofilms may explain this surprising propensity of vegetative bacteria.^{8e10} This is supported by a recent study which found that biofilm-forming strains of *Acinetobacter baumannii* survived for longer on dry surfaces than non-biofilm-forming strains (36 vs 15 days; $P < 0.001$).⁶⁴ In-vitro studies evaluating the persistence of dried inocula did not supply any water or nutrients.^{8e10,14,65} However, in the hospital environment, daily and terminal cleaning or disinfection does provide a supply of water, and some bacteria may be able to metabolize some constituent parts of detergents and even disinfectants, providing a nutrient source for the growth in biofilms.

Tackling surface-attached cells and biofilms Surface-attached cells, especially established biofilms, present a difficult challenge to hospital cleaning and disinfection, combining protection from physical removal with reduced susceptibility to biocides (Table 1).^{31,57} A number of different approaches are available to tackle surface-attached cells and biofilms. Using physical methods to dislodge detached bacteria, which can be aided by the use of a detergent, can be effective in removing established biofilms and preventing the development of biofilms.^{5,56,73} However, detergent cleaning alone may not be sufficient to remove biofilms.^{5,15,31,56,61,73} Tackling the microbes in the biofilm alone (e.g. using some disinfectants or attempts to interfere with quorum sensing) can be effective, but may not reach microbes protected deep in the biofilm matrix. Tackling the biofilm matrix alone (e.g. using enzymatic digestion) will help to reach microbes protected within the biofilm matrix and interrupt persistence of the biofilm, but will not necessarily have direct microbicidal activity.

Thus, tackling both the microbes in the

biofilm and the biofilm matrix simultaneously (using oxidizing disinfectants or combination approaches) offers the potential to reach microbes protected deep in the matrix and interrupt the persistence of the biofilm.

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Safety and Cleaning of Medical Materials and Devices

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Abstract: A study was undertaken to evaluate different procedures to safely remove microorganisms, protein, and mammalian cells from materials and provide a suitable method for cleaning and assessing effectiveness of cleaning medical devices for reuse or for analysis of failure.

Cleaning Protocol. The cleaning agent, 100 mL, was added to each well in 3 or 4 columns (24-32 wells) of the plate. Water was used in 3 or 4 columns as control. The plates were left to sit at room temperature for various time periods, washed with water again, and then assessed for efficacy of cleaning. There was no agitation or scrubbing of the wells, because this was a static test.

Allowing the biofilm to dry first made cleaning very difficult.

Only the NaOCl bleach could subsequently remove the dried or aldehyde fixed organisms

Hydrogen peroxide-based bleaches did not clean effectively. However diluted 5.25% sodium hypochlorite (NaOCl) cleaned very effectively. The recommendations on the bleach bottle to disinfect surfaces are equivalent to a 1/10 dilution. Therefore, studies were done with different dilutions of NaOCl in water. Dilutions from 1/10 to 1/50 were effective in removing the organisms. The 1/100 dilution was not effective. The contact time with the biofilm was also studied. Cleaning with the 1/10 dilution sodium hypochlorite bleach (0.525% NaOCl) was effective in the shortest time period used, which was 15 min at room temperature.

Additional Studies with Bleach. NaOCl based bleach was then demonstrated to clean the wells under all conditions: it cleaned wells with adhered bacteria that were allowed to dry, that had been fixed first in formalin or liquid disinfectants, and those that had been cleaned, effectively or ineffectively, with detergents. Because blood is often a contaminant of the medical devices being cleaned for reuse, studies were done with *S. epidermidis* RP62A incubated in TSB with 10% sheep blood.

The data obtained indicated that the organisms in TSB/blood did not adhere as much to the polystyrene compared to the bacteria that were incubated in TSB alone. A 10% NaOCl based bleach for 1 h at room temperature cleaned the plates of *S. epidermidis* with and without sheep blood. Microscopic analysis did not reveal any blood cells left after this bleach treatment. Both the detergents containing enzymes and the 10% NaOCl bleach were effective in removing the blood. Analysis for residual protein using Bradford reagent, brilliant blue R, and naphthol blue black stains indicated that NaOCl

This study confirmed that used medical devices, contaminated with microorganisms, protein, and/or mammalian cells, should not be allowed to dry before cleaning and that a thorough cleaning procedure should precede sterilization or disinfection (with the exception of NaOCl bleach which also cleans). © 2000 John Wiley & Sons, Inc. *J Biomed Mater Res (Appl Biomater)* 53: 131-136, 2000

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Current and emergent strategies for disinfection of hospital environments Review

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A significant number of hospital-acquired infections occur due to inefficient disinfection of hospital surfaces, instruments and rooms. The emergence and wide spread of multi resistant forms of several microorganisms has led to a situation where few compounds are able to inhibit or kill the infectious agents. Several strategies to disinfect both clinical equipment and the environment are available, often involving the use of antimicrobial chemicals. More recently, investigations into gas plasma, antimicrobial surfaces and vapour systems have gained interest as promising alternatives to conventional disinfectants. This review provides updated information on the current and emergent disinfection strategies for clinical environments.

HAIs are among the major causes of death and increased morbidity among hospitalized patients, with a minimum of 175000 deaths every year in industrialized countries.

Main hospital pathogens

The increase in HAI is associated with the higher capacity of bacteria to resist and adapt to harsh environmental conditions,

agents. Deadly pathogens can survive for long periods of time on hospital surfaces, making the environment a continuous reservoir of infectious agents. The adhesion of pathogens to a surface followed by biofilm formation in, 24 h is a critical microbiological problem for healthcare services.

In fact, the concentration of disinfectants required to kill sessile bacteria may be 1000-fold higher than that required to kill planktonic bacteria of the same strain.

Cleaning is related to the clearance of foreign material from a surface or equipment, allowing the removal of some organic material and microorganisms by detergents.^{11,33} However, this process does not kill bacteria, which, under favourable conditions, can redeposit elsewhere and form biofilms.

A disinfectant is almost never 100% effective due to the resistance of some bacteria to specific compounds and due to inefficient cleaning protocols.

Chlorine-releasing agents can oxidize membrane proteins and are very effective in removing biofilms from surfaces, requiring short exposure times for growth inhibition.

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Poorly processed reusable surface disinfection tissue dispensers may be a source of infection

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Neglecting adequate processing of surface disinfectant dispensers has contributed to frequent and heavy contamination of use-solutions based on surface active ingredients.

Background

The emergence of multi-resistant Gram-negative bacteria in healthcare associated infections has led to an increased awareness for prevention of transmission [1], e.g. by hand disinfection or targeted surface disinfection [2]. Especially surfaces in the immediate proximity of patients and those with frequent hand contacts should be wiped regularly with a surface disinfectant which may contain quaternary ammonium compounds (QAC), amines, glucoprotamin or amphotensides (all summarized as "surface-active ingredients")

Results: 66 dispensers containing disinfectant solutions with surface-active ingredients were collected in 15 healthcare facilities. 28 dispensers from nine healthcare facilities were contaminated with approximately 107 cells per mL of *Achromobacter* species 3 (9 hospitals), *Achromobacter xylosoxidans* or *Serratia*

In none of the hospitals dispenser processing had been adequately performed. Isolates regained susceptibility to the disinfectants after five passages without selection pressure but were still able to multiply in different formulations from different manufacturers at room temperature within 7 days.

Conclusions: Neglecting adequate processing of surface disinfectant dispensers has contributed to frequent and heavy contamination of use-solutions based on surface active ingredients. Tissue dispenser processing should be taken seriously in clinical practice.

A heavy contamination with 106 to 107 cells per mL was found in 28 of the solutions with surface-active ingredients (42.4%) whereas the disinfectants containing also aldehydes or alcohol did not reveal any relevant contamination.

Whenever a contamination was detected the healthcare facility was immediately informed to allow instant removal of other dispensers.

In polypropylene microtiter plates biofilm formation was found within a few hours in all three surface disinfectant solutions contaminated with *Achromobacter* species 3 or *Serratia marcescens*.

Adaption to BAC has a potentially harmful consequence. It may substantially enhance biofilm production by non-BAC-resistant cells in the post-adaption period as a response to the antimicrobial stress

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Reference Documents

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