

## **Emerging Impact of Biofilms on the Environment of Care.**

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Recently, several published studies have highlighted the role of biofilm on dry environmental surfaces in harboring and protecting multidrug resistant bacteria in healthcare establishments. Vickery *et al.* demonstrated that biofilms on dry hospital surfaces are far more widespread than previously recognized (1). A following study by Hu *et al.* found bacteria dwelling in biofilm on over 90% of surfaces tested from an Intensive Care Unit (ICU), after two terminal cleanings (2). Furthermore, over 50% of these contained multidrug-resistant (MDR) bacteria (2). This work suggests that many commonly used disinfectants are not effective against bacteria within biofilms and that unless we consider the potential impact of these biofilms we are unlikely to attain the goal of reduced infection rates.

Mature biofilms are complex, multi-species structures that could contain bacteria, fungi, algae, yeasts and protozoa. No two biofilms are the same. Viruses can also become encased and protected by biofilms produced by other species. Biofilms are the most common form of growth for microorganisms and are a key survival factor. Biofilms provide protection against desiccation, Ultra Violet (UV) light, antimicrobials (including disinfectants and biocides) and provide an environment for microorganisms to access nutrients and share genetic material, including drug-resistance genes (3–6). The formation of biofilms in damp and aquatic environments has been well documented for some time. Briefly, the microorganisms loosely attach to the surface, followed by strong adhesion. Subsequently the microorganisms excrete extracellular polymeric substances (EPS), which eventually makes up 75 – 90% of the biofilm and acts as a protective barrier (7). As the population within the biofilm grow and multiply, the complexity of the microbial population within the biofilm increases and the size (area and depth) of the biofilm also increases.

Included in the identified activities within biofilm is the sharing of genetic material such as plasmids that may contain antibiotic resistance genes (8, 9). By sharing plasmids, it is possible for a previously antibiotic susceptible bacterial population within a biofilm to become resistant by acquiring these mobile genetic elements, thus increasing the spread of Multi Drug Resistant (MDR) bacteria.

A further defense mechanism for biofilms are specialized survivor cells called “persisters”, a small dormant sub-population of the overall population (10). When the biofilm is disturbed, such as through the action of disinfectants or mechanical cleaning, the persister cells can survive and rapidly repopulate the biofilm. This could even increase the thickness of the biofilm to provide enhanced protection against future attacks

Most of this is nothing particularly new to biofilm researchers as the interaction of organisms and survival mechanisms have been studied for decades. To date, much of research work has been focused on wet or damp environments, however, the recent work by Vickery *et al.* revealed that there are extensive biofilms found on “dry” surfaces in the healthcare environment (1). This significantly changes how we should think about cleaning and disinfection. Firstly, these biofilms are typically not large, often only a few microns thick, fitting into microscopic crevices found on most surfaces, the method of detection being destructive sampling and then scanning electron microscopy. Secondly, many of the commonly used disinfectants are not effective against biofilms. Almatroudi *et al.* cultivated dry biofilms of *Staphylococcus aureus* in

*vitro* and exposed them to bleach at concentrations up to 20,000 ppm (11). Live cells were still detectable after exposure and reformed the biofilm in a number of days (11). Another study found that up to 11% of MRSA and 80% of *Pseudomonas aeruginosa* cells in biofilm survived after treatment containing either benzalkonium chloride, chlorohexidine or triclosan (12).

The biofilm provides a reservoir where bacteria can survive on dry surfaces for prolonged periods without dehydration. There is some debate as to how biofilms form on otherwise dry surfaces, as the majority of microorganisms have an optimal water activity of 0.95 – 0.98, and a minimum for most bacteria of 0.88 - 0.91 (13, 14). This measure is relative to pure distilled water which has a water activity of 1. Vickery *et al.* hypothesized that in the hospital setting, these dry surface biofilms may form when a surface is temporarily wet due to incidental spills, condensation or high humidity (1). After initial biofilm formation, the EPS would then protect the microorganisms from desiccation (1). Work published by Otter *et al.* demonstrated that bacteria were detectable on surfaces for longer than would typically be considered possible with planktonic bacteria (15). Many Gram negative bacteria such as *Klebsiella* and *Staphylococcus* were shown to survive for 6 weeks on dry surfaces, where classically significant declines in population within a few days would be expected (15).

Biofilms have been shown to be >1500 times more resistant to biocides than planktonic cells (1, 12). Oxidizing chemistries are more effective than a range of other chemicals, including alcohols, phenols and quaternary ammonium compounds (16). This may be due to the oxidizing agents targeting multiple cell and biofilm targets (16–19). Commodity bleach even at 2 to 4 times typical doses is not fully effective (11). For products that use a two-step process of clean then disinfect, it is important that the disinfectant be applied in a relatively short time frame after the cleaning phase, as treatment with cleaning agents alone will not achieve total biofilm inactivation and could lead to rapid regrowth (20).

Users should also be aware of the implications for UV “robot” cleaners. Biofilm provides significant protection for bacteria from the impact of UV irradiation. One researcher indicates that the dose of UV required to provide a four log kill of bacteria protected by biofilm is four to five times greater than if there is no biofilm present (21). In effect, to attain the same level of kill as demonstrated on test strips one would need to run the device for four to five times as long. Biofilm is nature's very own SPF 30. All surfaces should be cleaned and disinfected prior to the application of a UV system, once again it is important that no surfaces are missed and that any use of UV occurs within a short time frame of the manual clean and disinfection.

Bacteria in dry biofilms are not isolated from patient populations, recent publications by Chowdhury *et al.* showed that a single touch of an ungloved finger was capable of transferring *Staphylococcus aureus* to 19 agar plates (22). A subsequent update on that study demonstrated that a gloved hand can also transfer bacteria from a single touch of dry biofilm. However, while nitrile gloves produced similar results to an ungloved finger, latex gloves resulted in approximately 50% less transmission.

Cotton bed sheets do not prevent the transmission of bacteria in dry surface biofilm on vinyl mattresses to the patient (23). Chowdhury *et al.* reported that although sheets with a higher fiber count reduced the level of transmission, even high quality bed linens allowed transmission of bacteria from the dry surface

biofilm through the sheet. In addition, when the sheets were moistened, the rate of transmission was significantly increased (23). It is clear there are multiple routes of transmission for bacteria surviving in dry surface biofilm that could impact the patient, either through direct contact with a surface or through the vector of healthcare workers hands, both gloved and ungloved.

To protect your patient from the risk associated with dry surface biofilm we need to take steps to eliminate the bacteria found on these surfaces. The EPA recently introduced the first and only registered test protocol to determine the efficacy of disinfectants against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in biofilms. The test method is based on the ASTM method E2871 and allow disinfectant manufacturers to make public health claims for their products in regard to their efficacy against bacteria in biofilms.

To date there is only one product on the market in the US that has an EPA registered claim to kill *Pseudomonas aeruginosa* and *Staphylococcus aureus* in biofilm. That product is a novel sporicidal chemistry in the form of a fast dissolving tablet based on a blend of Sodium dichloro isocyanurate (NaDCC) and surfactant. The chemistry also has an EPA registered claim for *Clostridium difficile* spores in 4 min and is sold under the brand name Defender.

To protect patients and to ensure a safe working environment for our staff and visitors it is vital that environmental cleaning practices address the root cause of pathogen reservoirs in patient care areas. Unless we use products that are effective in eliminating bacteria living within biofilm we will not be in position to truly reduce risk.

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