Our evolving understanding of biofilm in healthcare environment.
T'ES FRANÇAIS !?

ET TOI QUEBECOIS ?!
• Biofilm basics
• Biofilm in Healthcare
• Eliminating Biofilms
Biofilm basics
Why should we be interested in biofilm?

“Our microbes find more and more subtle parades: after the appearance of multi-resistance genes to antibiotics, comes the time biofilms resistant to the penetration of antibodies and antibiotics.”

Professeur Luc Montagnier: codécouvreur du virus du SIDA
Prix Nobel de médecine et de physiologie 2008

The next big challenge in Infection Prevention!
Globally, biofilm is the dominant lifestyle of bacteria

Two lifestyles

Planktonic bacteria

Bacteria in biofilm (99%)
What is a biofilm?

“Biofilms are a community of microorganisms attached to a surface by polysaccharides, proteins and nucleic acids.”

(Sauer et al., 2007)
Picture: Steven Lower et al., PNAS
What is a biofilm?

- Biofilms are made up of bacteria living in a layer of organic material made from sugars (polysaccharides), fats, protein, and DNA. The organic material becomes a shield to protect the bacteria.
- The layer of sugar is called EPS or extracellular polymeric substances.
Which microorganisms are present in a biofilm?

- Bacterial biofilms typically have many species of bacteria – they are generally not a single species.
- Even within the same bacterial species, the ability of biofilm formation can vary among different strains.
- Biofilms with Acinetobacter baumannii showed in one study that there are high biofilm forming substrains and low biofilm forming substrains. There are also MDR and non-MDR substrains. The MDR high biofilm forming substrains were 50% more resistant to drying out than non-MDR low biofilm forming substrains (Greene, 2016).

- **Fungi**
  - Yeasts
  - Mold
- **Yeast**
- **Algae**
- **Protozoa**
Biofilms can be found in wet and dry environments.

Wet biofilms are typically 300-500 um in thickness and visible to the naked eye. They can grow to be several cm thick.

Dry surface biofilms are 10-50 um in thickness and are invisible to the naked eye (a key issue for Diversey).
Wet versus dry surface surface biofilms

- Wet biofilms are ~90% water and are typically found in showers, floor drains, sinks/drains or in/on certain medical equipment.
  - Biofilms are often found in unhygienic environments with the organic soil contributing to the growth of the colony.
  - Solids, such as dust, skin cells, and other organic material that lands on the biofilm can become trapped in the biofilm.

- Newer research suggests that biofilms are common on dry surfaces as well.
  - The humidity from the air or occasional contact with moisture is adequate to keep the colony alive.
  - Skin cells and other organic matter provide the food source.
  - Minerals from water supply also contribute to food source.
  - Dry surface biofilms are less robust than wet biofilms, but can be much harder to detect.
Understanding the biofilm development cycle

1. **Initial attachment**: adhesion takes place in some of the cells on the surface.

2. **Irreversible adhesion** caused by excreting polymers that consolidate the adhesion.

3. **Maturation 1.**
   - Microcolonies forming.
   - Transport of nutrients into the biofilm.
   - Growth-replication and production of extracellular polymers.

Understanding the biofilm development cycle

1. Initiation: the process begins with the attachment of bacterial cells to a surface.

2. Maturation 1: as the biofilm grows, it starts to form a gel-like matrix.

3. Maturation 2: the biofilm matures into a three-dimensional structure containing clusters or groups of cells.

4. Maturation 3: further maturation results in a complex network of cells.

5. Dispersal: when a concentration of cells is reached and access to nutrients becomes difficult, cells are removed and dispersed.

Not all of a biofilm has the same activity

- The bacteria closest to the surface typically have the least activity, but killing surface bacteria does not kill the entire biofilm because the depth provides shielding for those layers with the lowest activity.
- The visual below shows how slow the attack be disinfection can be due to the shielding of the biofilm.
Advantages of living in a biofilm for microorganisms

- Cooperation with different skilled organisms
- Increased resistance
- Attached to a wide range of environmental surfaces.
- Proximity favors horizontal gene transfer
The biofilm protects the bacteria from death and drying out, which helps the bacteria survive.

Biofilms increase their survival by slowing growth rates for the bacteria and becoming dormant when needed.
What makes biofilms special?

- Key characteristics of a biofilm:
  - Standard disinfection not adequate to kill the bacteria or remove the biofilm. Quats are especially weak.
  - The bacteria can secrete acids, which can damage the surface.
  - In response to stresses, the biofilm will slough off portions of the biofilm.
  - Cleaning may remove some of the biofilm, but may have little impact due to the toughness of the matrix. Cleaning may actually spread biofilms if it removes and redeposits portions of the matrix.
  - Microbial biofilms are naturally tolerant of antibiotics and can survive doses 1000 times greater than doses that kill planktonic bacteria.
  - Bacteria in a biofilm “signal” each other by releasing chemicals to help create coordinated behaviors.
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How biofilm affects the biocidal efficacy?

- Concentration gradient of active principles prevents minimum biocidal concentration
- Partial neutralization of active principles
- Genetic material exchange
- Favors metabolic and physiological changes of bacteria cells
Where are biofilms a problem in Healthcare?

- Biofilm formation is inevitable where there are:
  - A surface
  - Microorganisms
  - Water
  - Nutrients

- Environmental surfaces that are frequently wet (Rogers, 1994):
  - Water supply lines, drain, and drain piping, shower heads, faucets, and other water discharge devices
  - Inside certain equipment associated with water/liquid
    - Ice machines
    - Cooling towers

Where are biofilms a problem in Healthcare?

- Medical devices
  - Shavers and other devices with moving pieces that need to be taken apart to be cleaned
  - Endoscopes and other devices with interior channels that need to be flushed to be cleaned
  - Indwelling devices, such as catheters and pumps.

- 60% of human infection arise from biofilm formation (mainly in wounds)

Biofilms can be found in wet and dry environments.

Lab study illustrating the potential for dry surface biofilms to harbour bacteria that can then be transferred via the hands of healthcare workers

- 95% of 61 surfaces tested from three UK hospital groups had dry surface biofilms, and 58% of these samples grew MRSA

Findings:

- The structure of the biofilms was consistent across the hospitals.
- The microbial composition of the biofilms was very different in one of the hospitals, containing mainly Bacillus species compared with Staphylococcus predominating in the other two hospitals. It suggests that differences in local cleaning and disinfection strategy will influence the composition of dry surface biofilm.

K. Ledwocha et al., “Beware biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; a multi-centre study.” JHI, 2018 e47-e56
Vickery (2012) cut up environmental surfaces from an ICU following terminal cleaning and tested for the presence of biofilms and specifically for MRSA and VRE. Biofilms were detected on multiple samples and MRSA was identified from the venetian cord blind and the curtain.

Hu (2015) cut up environmental surfaces from an ICU that had been disinfected twice with 500 ppm of chlorine and stored for 12 months and found that 90% of the surfaces contained viable bacteria in a biofilm including some with MRSA.

In short, it is likely that biofilms are present widely on environmental surfaces. Removing the biofilm is difficult and strong cleaning is needed, which is why AHP works well on biofilms.

Biofilm in Healthcare

Biofilms issues

- Endoscope
- Catheters
- Handwashing sink

This study examines the wastewater of 31 patient rooms of various German clinics for possible residues of antibiotics, as well as the wastewater of five private households as a reference.

This study shows for the first time that in hospitals with high antibiotic consumption rates, residues of these drugs can be regularly detected in toilets, sink siphons and shower drains. After complete flushing of the wastewater siphons, antibiotics are no longer detectable, but after temporal stagnation, the concentration of the active substances in the water phases of respective siphons increases again, suggesting that antibiotics persist through the washing process in biofilms.

Biofilms may help explain how antibiotic resistance develops

- Bacteria in a biofilm may have some protection from antibiotics.
- Antibiotics rely on a lock (bacteria) and key (antibiotic) approach to kill bacteria.
- Over time the bacteria evolve stronger resistance. They change the shape of the lock and the key no longer fits.
- When these antibiotic resistant bacteria live on healthcare surfaces, all patients are at risk of exposure.
- Hand hygiene and surface disinfection are required to prevent transmission, but are often done inconsistently.

![Antibiotic Timeline Diagram]

Note: Some of the dates are estimates only.
Biofilm resistance against immune system?

- Chronic infection associated to biofilms: ostéomyélite
- (S. aureus) (1/675 admission à l'hôpital. 50,000 cas aux USA/an.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3322633/#SD1
EPA and Montana State University collaborated on a development program for biofilm testing methods.

- ASTM method E2562-12 (EPA SOP MB-19-02) was developed using the CDC biofilm reactor procedure to grow reproducible biofilms.
- ASTM method E2871-12 was developed to test the efficacy of a disinfectant against a biofilm. *Pseudomonas aeruginosa* is the test organism in the method (also called EPA SOP MB-20-01).
- The efficacy standard is the same 6 log reduction required in other EPA disinfectant test methods.
- Additional methods are in development by EPA and will likely become available in the coming years. This is a large new area to regulate for EPA.
- There are currently no EN methods for use in Europe, so the US-EPA method is being used in some research.
Eliminating Biofilms
The amount of EPS in the biofilm can dilute or inactive some of the active ingredient, making it hard to disinfect a surface with a biofilm. The EPS itself acts as a protective barrier, shielding the bacteria from damage.

Chlorine is a strong disinfectant, but appears to have mixed effectiveness on biofilms.

Almatroudi reported that 10 min exposure resulted in a 7 log reduction in bacteria and removed >95% of the biofilm. However even after exposure to 20,000 ppm of chlorine, S. aureus cells were still viable and regrew.

Days required for recovery of biofilm bacteria, release of planktonic bacteria, and development of turbidity in broth following treatment with sodium hypochlorite solution when incubated at 25C (blue bars) and 37C (orange bars). Four coupons/treatment.

What chemistries are more/less effective?

- Machado (2012) exposed Pseudomonas and E. Coli to quats and found that the size of the biofilm increased as a result, indicating relatively poor efficacy by the quat and a strong adaptive response by the biofilm.

- Perumal (2014) found that unformulated hydrogen peroxide had a low ability to kill bacteria in biofilms when compared to testing against planktonic bacteria, where it performed better.

- We know from testing that AHP performs well and we have a study to show it.

There is some evidence that disinfectants that are partially effective against biofilms can cause more problems than if they had little effect.

As the biofilm detects the disinfectant, it is believed to be capable of making some of the bacteria enter a dormant state, which is much more resistant to disinfection (Flemming, 2016)

Killing a significant portion of the bacteria in the biofilm, but not removing the biofilm, can make the biofilm more susceptible to secondary colonization by more pathogenic organisms, which ultimately makes to more dangerous to people.

Example of Pseudomonas biofilm

- Culotti (2014, 2015) showed that Pseudomonas in a biofilm enhanced the colonization of E. coli and Campylobacter.
- In both cases, the Pseudomonas appears to affect the biofilm in ways that makes colonization by other species more favorable. This may occur through holes bored through the biofilm or regulating the level of oxygen or some other mechanisms.

Strategies to address bacterial biofilms

Current Biofilm related research

Prevention
- Antibacterial coating
- Surface modification
- Cleaning and disinfection
- Biofilm disruption

Diagnosis
- Biosensors for early detection
- Advanced imaging
- Nanoparticles

Treatment
- Dissolution of EPS
- Antimicrobial PDT
- Electric and magnetic fields

Adapted from Khatoon et al., “Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention” Heliyon 4 .2018
Diversey products and biofilm efficacy

- Purdue tested 5 AHP products (Oxivir Tb, Oxivir One, Oxivir Five 16, Oxivir Excel (Europe), and Oxivir Sporicide (Europe)), Avert, Virex II 256 and Virex Plus using the EPA testing method.
- All of the AHP products and Avert passed the testing, with the Oxivir Five 16 showing a bit less efficacy than the other AHP products. We are in the process of adding biofilm claims to several of our products.
- Neither quat product performed well, especially against Pseudomonas. None of our quat products will carry a biofilm claim.

Lineback et al, “Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against Staphylococcus aureus and Pseudomonas aeruginosa biofilms than quaternary ammonium compounds”, ARIC, 2018; 7:154.
Thank you for your attention

Questions?