

Disinfection in Healthcare: Pitfalls Challenges and Getting it Right

The Impact of Biofilms on Patient Infection and Environmental Disinfection
“If you are Disinfecting for Biofilm you are not Disinfecting.”

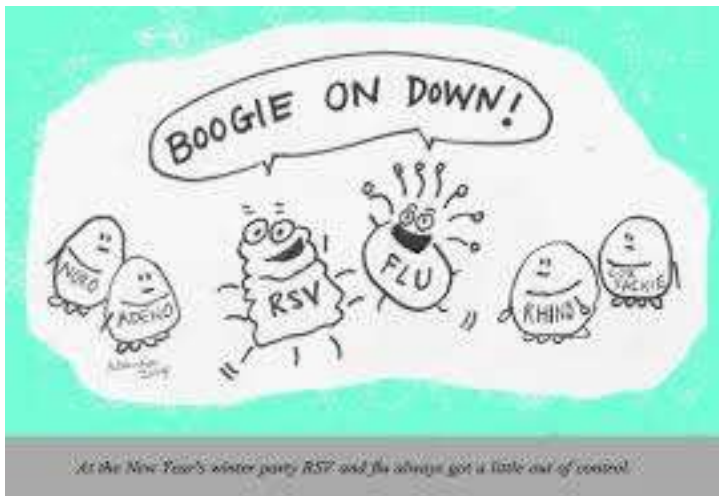
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Objectives



1. Explain how biofilms develop
2. Describe the function of persister cells
3. Explain how dry biofilms differ from traditional biofilms
4. Discuss the risk that dry biofilms pose in the hospital environment
5. Describe the best antibacterial chemistries to address biofilms

From The Director General Of The World Health Organization

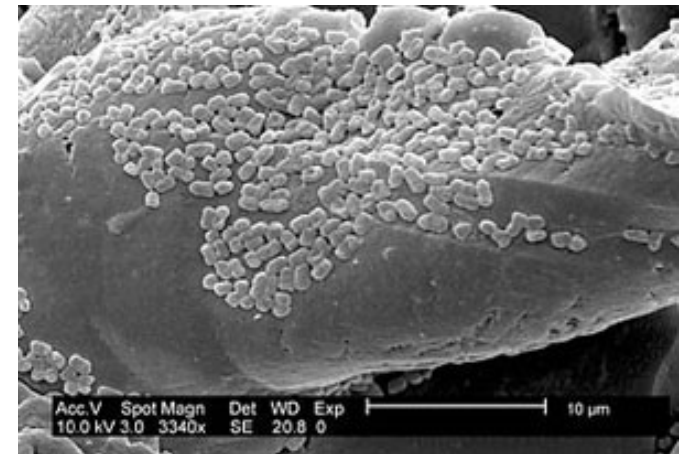
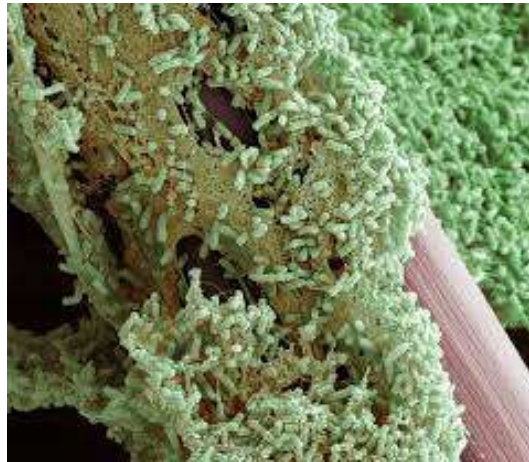
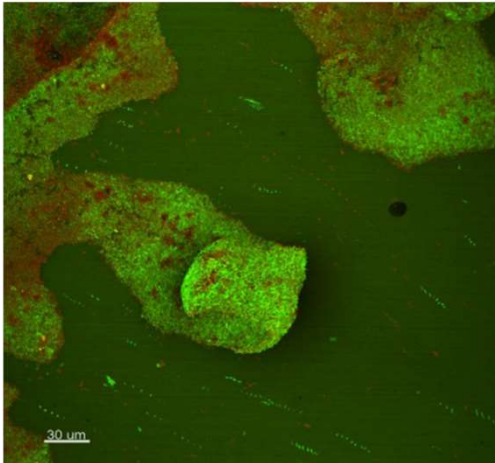
- **“The emergence and spread of drug-resistant pathogens has accelerated. More and more essential medicines are failing. The therapeutic arsenal is shrinking. The speed with which these drugs are being lost far outpaces the development of replacement drugs. In fact, the R&D pipeline for new antimicrobials has practically run dry”**
- **So disinfection is last line of defense**
- **We can predict the probability of Hospital Acquired Infection from a specific pathogen based on the status of the prior room occupant. We are not eliminating fomites as a source of infection.** ¹

1. Huang *et al.* 2006 Risk of Acquiring Antibiotic-Resistant Bacteria From Prior Room Occupants *Arch Intern Med* 2006; 166: 1945-51.



Biofilm

The next big challenge in Infection Prevention

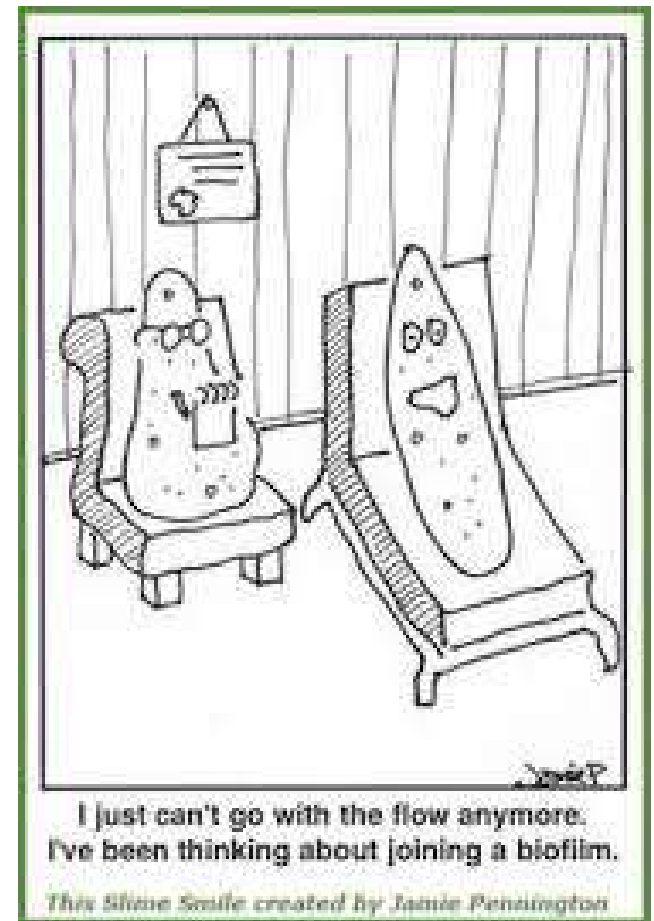


Part 1: What, Where, How, and, Why

Biofilms in a Healthcare Setting

We study bacteria on a culture plate.
In a liquid suspension, or in
isolation.

That is not how they behave and
interact in the real world.



Biofilms

- 60% of human infection arise from pathogens in biofilm
(mainly in wounds and implants)
- Biofilms can act as a reservoir of pathogens in the hospital and offer favorable environment for pathogens to persist over extended periods.
- The biofilm structure protects embedded pathogens against biocides
- Biofilms increase patient exposure and provide higher infectious dose
- Typically we look at biofilms in a wet or damp environment (prosthetics, catheters, tracheotomy tubes)

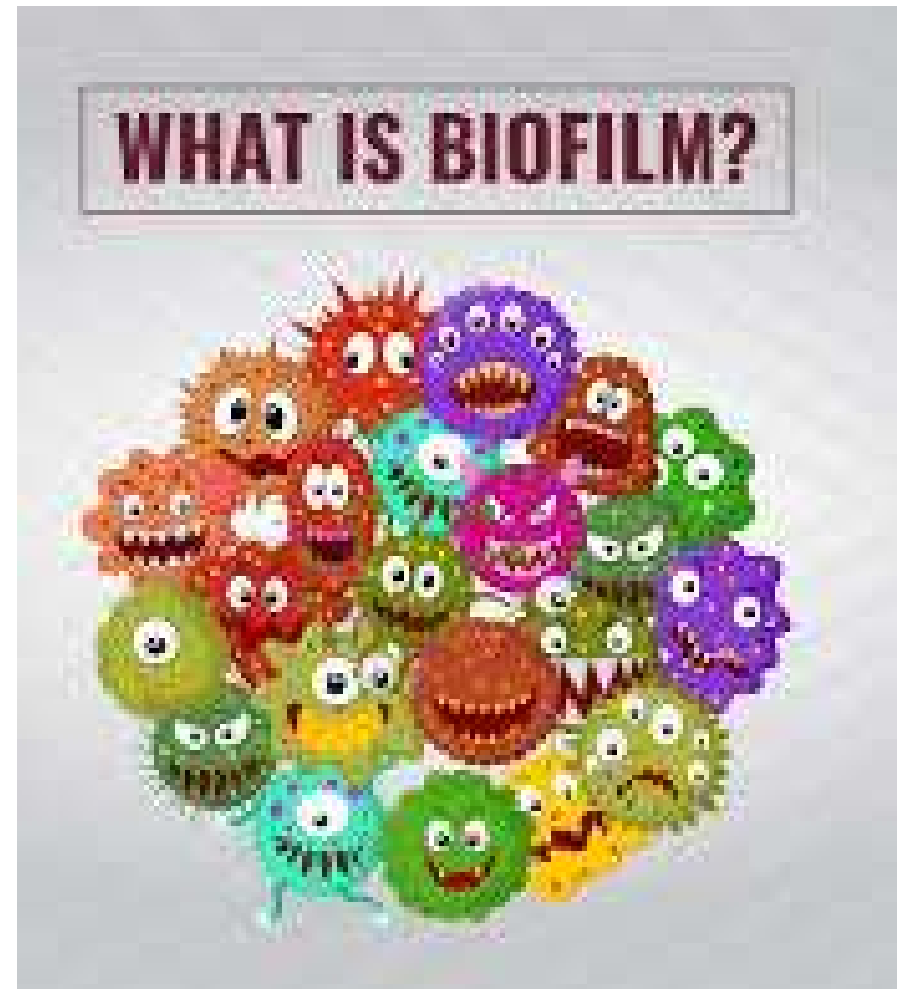


Biofilm?

- A biofilm is a community of microorganisms attached to a substrate which produce

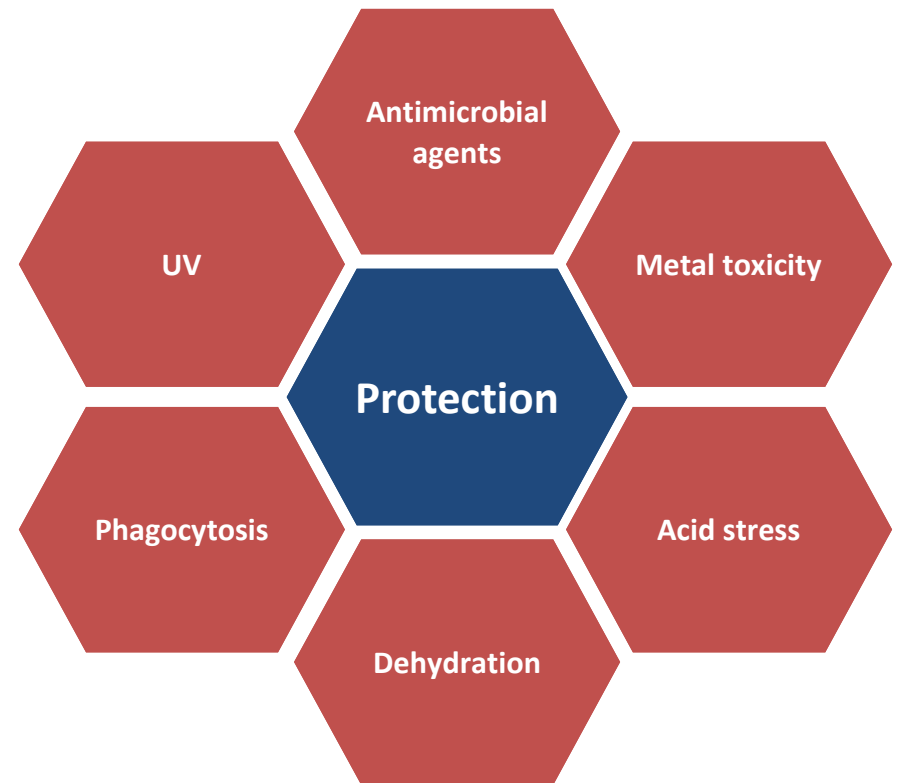
Extracellular Polymeric Substances (EPS)

- EPS includes proteins, genetic material, polysaccharides (not required)
 - Bacteria
 - Fungi
 - Algae
 - Yeasts
 - Protozoa
- One or typically more species in a biofilm
- Most common form of growth for microorganisms
- Protected form of growth : KEY SURVIVAL FACTOR



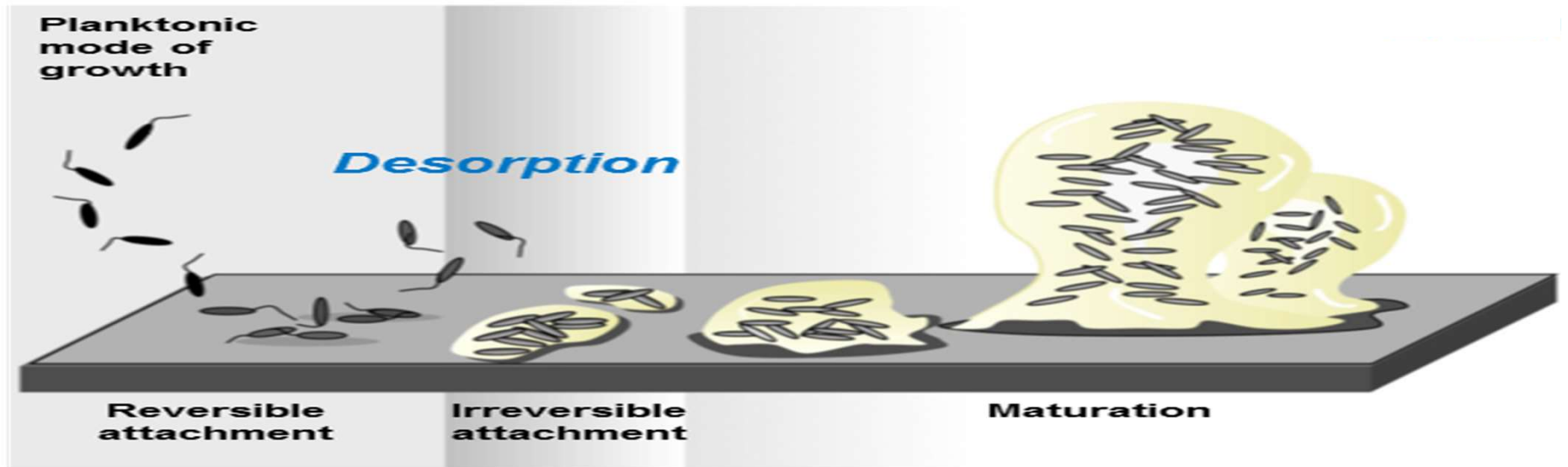
Why do microorganisms form biofilms?

- Biofilm provides a protective layer from environmental stress
(e.g. can be >1500 less susceptible to certain biocides)
- Allows interaction opportunities for microorganisms
- Access to nutrients
- Protects from Amoebae/Neutrophils



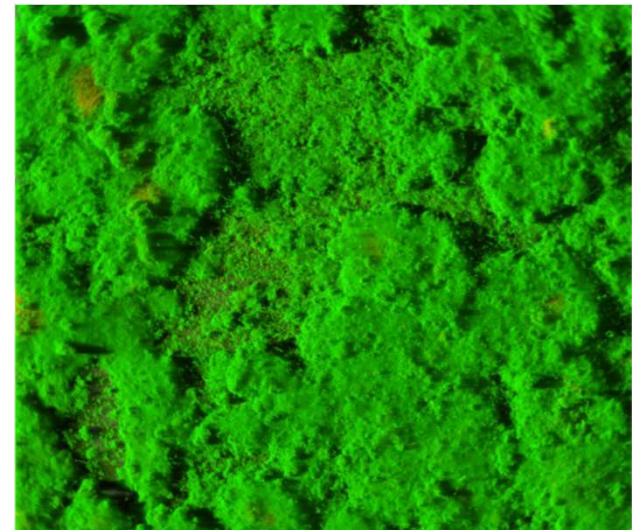
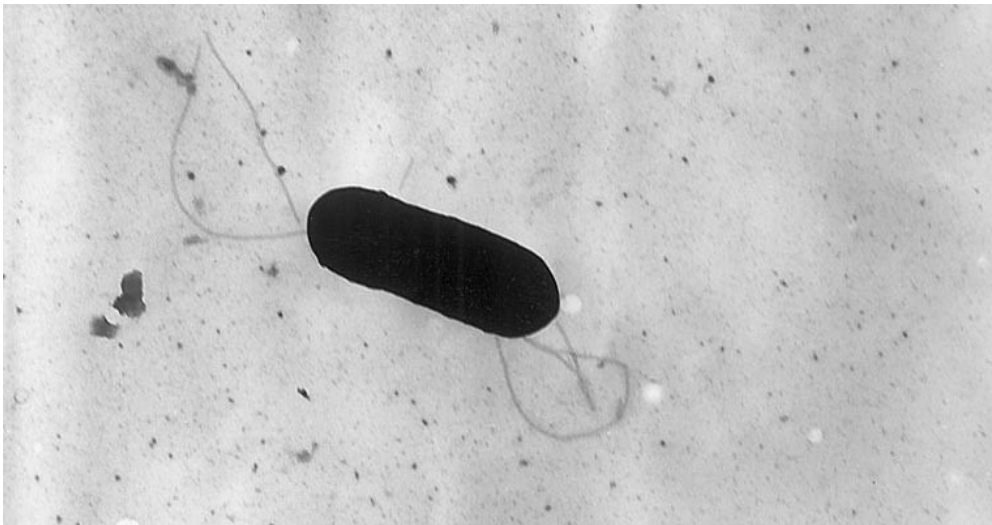
Formation of Biofilm

- Initial attachment improved by presence of flagella (for some species, *Listeria*, this makes formation temperature dependent)
- Initial growth rate dependent on available nutrient and temperature
- Once you get to 10^5 to 10^6 cfu you have a strong biofilm that will demonstrate resistance factors



There are variations

- The common morphology of *Listeria* is
- Under environmental stress the pathogen can change to a filamentous bacteria and form a free floating biofilm matrix
- Remove the environmental stress and the bacteria reverts to typical morphology.



Types of biofilms found in Healthcare

Hydrated biofilms

One of the most commonly studied types of biofilm

Formed in wet environments (pipes, water cooling towers, sewage treatment)

Over 90% water, bacteria are growing and multiplying

The present EPA test protocol for disinfectants is based on wet biofilm



Build up biofilm

Occurs on areas which may be regularly exposed to a cycle of wet/dry conditions

Examples: faucets, drains, waste pipes

Considered to be hydrated due to their high moisture content



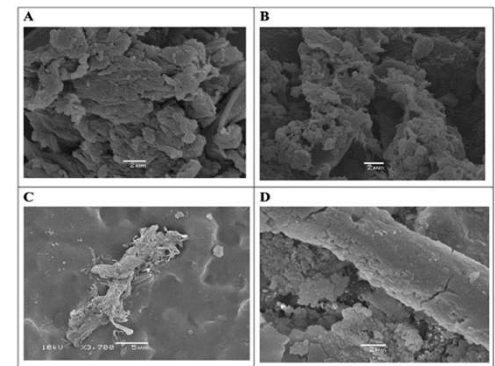
Dry surface biofilm

Exist on **normally** dry surfaces (handrails, floors, privacy curtains)

Contain less than 61% water, these guys are dormant¹

Present on >90% of surfaces in ICU²

The Biofilm will attain equilibrium relative humidity (A_w) with the surrounding air.



Hu, H. et al. 2015. J. Hosp. Infect. 91:35-44

¹Almatroudi, A et al. 2018. J. Hosp. Infect. 98:161-167. ²Hu, H et al. 2015. J. Hosp. Infect. 91:35-44

Interesting things happen in Biofilm

Plasmids and DNA Exchange In Biofilm



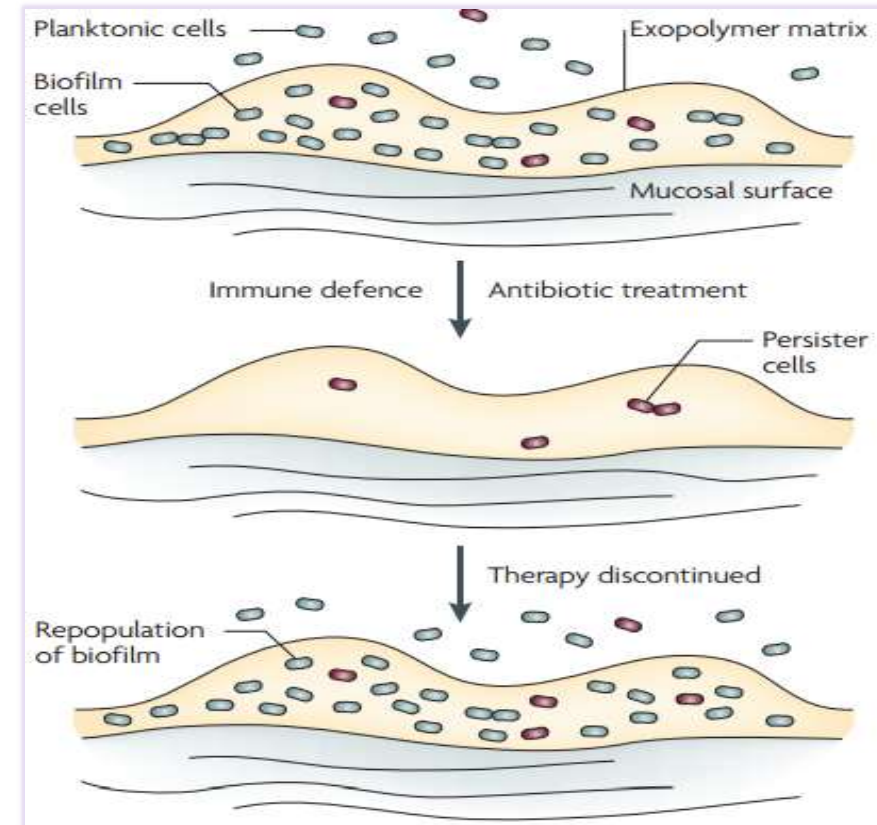
Bacteria swap plasmids in Biofilm, including the CRE plasmid

It may not have been a resistant organism when it joined the community but it will be soon

Protection and Stress Resistance

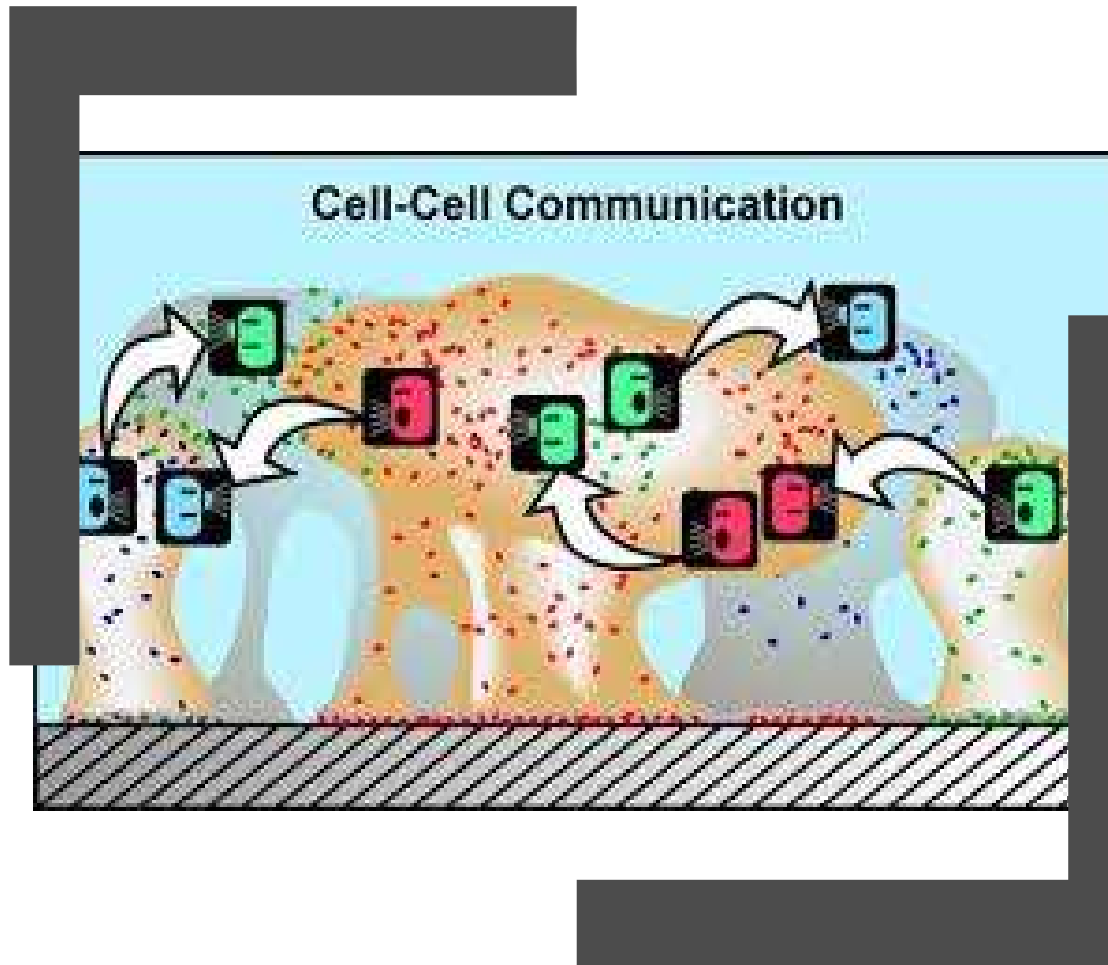
Persister cells

- Small dormant sub-population of biofilm which can remain viable after treatment with an antimicrobial
- Lose tolerance upon re-growth
- Specialized survivor cells, completely dormant so no cellular membrane transport
- Allows quick regrowth of biofilms making them extremely difficult to eradicate, biofilm can reform in as little as 40 min, up to twice as thick.



Lewis, K. (2007) *Nature. Rev. Microbiol.* 5:48-56

Bacteria Communicate in Biofilm



Quorum Sensing: Bacteria communicates to one another through chemical signals

Use of chemical signals to indicate:

- Flee
- Eat
- Become dormant
- Multiply
- Die

And probably a bunch of stuff we have not yet identified

Special Circumstances

A fully hydrated Biofilm in Every Patient Room

Deadly drains...

New research is beginning to highlight the risk of infection from sink drains

- Adding a nutrient source (blood, foodstuffs, beverages, surplus medication) down the sink drain could allow biofilm to grow up the drain at a rate of 2.54 cm per day¹
- Disruption of these sink/drain biofilms can transfer viable organisms up to 1 m to surrounding area²
 - Dispersal of organisms from the tap had been attributed to previous outbreaks^{3,4}
- *Pseudomonas aeruginosa* and Carbapenem resistant Enterobacteriaceae (CRE) are frequently associated with hospital sinks
 - Disinfection of sinks has been shown to dramatically decrease level of CRE colonization⁵
 - Resistant pathogens have been shown to colonize drains for over 6 years

¹Kotay, S. et al. 2017. Appl. Environ. Microbiol. 83:1-12.

²Aranega-Bou, P. et al 2019 J. Hosp. Infect 102:63-69.

³Kotsanas, D. 2013, Med. J. Aus. 198:267-269.

⁴Leitner, E. 2015. Antimicrob. Agents Chemother 59:714-716.

⁵Smolders, D. 2019 J. Hosp. Infect 102:82-88.

Example of a Sink Disseminating Bacteria



Placing a Handwashing sink inside an ICU room increases the IRR of HAI by 1.24, and IRR for Hospital Acquired Respiratory Infection from *Pseudomonas* by 1.44.

G-B. Fucini, C. Geffers, F. Schwab, W. Sunder J. Moellmann, P. Gastmeier: Sinks in patient rooms in ICUs are associated with higher rates of hospital-acquired infection: a retrospective analysis of 552 ICUs, VOLUME 139, P99-105

SHEA/CDC/APIC

Recently Published Guidelines on Hand Hygiene

- Focus on the risk of hand contamination during hand hygiene; pathogens in the drain do not stay in the drain.
- Risk is identified as contaminated drain-lines with biofilm in p-traps harboring resistant organisms
- Recommends disinfectants that have passed the US EPA biofilm test method present best solution⁶
- Applied to the drain line in a foam

⁶Glowicz et al Infection Control & Hospital Epidemiology (2023), 44, 355–376

Disinfecting Drain Lines

- Make sure your disinfectant has an EPA registered claim as effective against bacteria in biofilm.
- Use a foaming device to displace the water in the P-Trap with foamed disinfectant.
- Do not use the sink for the required contact time of the disinfectant.
- Make sure the disinfectant has a pH greater than 5 – otherwise it is illegal to discharge to sanitary sewer under 40CFR403.



Foaming a P-Trap - Video



A Case History

- Level III trauma center in California
- Outbreak focused on radiology suite
- 18 month duration
- 12 patients infected including 6 with pulmonary infections
- *Stenotrophomonas maltophilia* (Trimethoprim-sulfamethoxazole sensitive)
- Lots of investigation including whole genome sequencing and the CDC dismantling the plumbing system.
- Drain lines in hand washing sinks treated with foaming device over a two-week period, no infections for the past 9 months

More Impact From Biofilms

Addressing *Clostridioides*
difficile and other
Pathogens Hidden in
Toilets



Pathogens Hidden in Toilets

- Contaminated toilets are a silent threat, serving as a breeding ground for recurring surface contamination that significantly contribute to HAI's.
- Commercial toilets generate an upward jet air carrying droplets up to 5 feet above the bowl within 8 seconds.*
- Only ~ 50% of hospital toilets are cleaned and disinfected



Credit: John Crimaldi, Professor of Civil, Environmental and Architectural Engineering, University of Colorado Boulder

* <https://wgno.com/news/nmw/toilets-spew-invisible-aerosol-plumes-with-every-flush-heres-the-proof-captured-by-high-powered-lasers/>

Toilet plume aerosol generation rate and environmental contamination following bowl water inoculation with *Clostridium difficile* spores

- Explored toilet contamination persistence and environmental contamination produced over a series of flushes after contamination.
- Spores were present in bowl water even after **24 flushes**.
- Toilets contaminated with *C difficile* spores are a persistent source of environmental contamination over an extended number of flushes.



[https://www.ajicjournal.org/article/S0196-6553\(18\)31098-8/fulltext](https://www.ajicjournal.org/article/S0196-6553(18)31098-8/fulltext)

Issues with Commercial Toilet Bowl Cleaners



- Current IFU requires full purging of the water in the bowl to effectively disinfect – by purging the toilet you further aerosolize pathogens in the area
 - No one purges water (time & messy), so some companies recommend 6 ounces instead of 1-2 ounces on label
 - RTU products should not be further diluted
- Old chemistry, not sporicidal, quats have 10 minute contact times
- Not biodegradable (quats, other, end up in blood stream of fish)
- Not effective against bacteria in Biofilms

Tablet Toilet Bowl Solutions

Daily disinfection *C. diff* patient (CDC recommendation*)

Discharge disinfection everyone

One (1) 13.1g tablet



- Drop the tablet(s) in the bowl when you first enter the bathroom.
- Let it fizz & dissolve whilst cleaning the bathroom.
- Scrub bowl with a brush using the treated solution in the bowl.
- Flush before use.

*<https://www.cdc.gov/cdiff/clinicians/cdi-prevention-strategies.html>

Focus on...

- Terminal Cleans
- *C. diff*
- Isolation

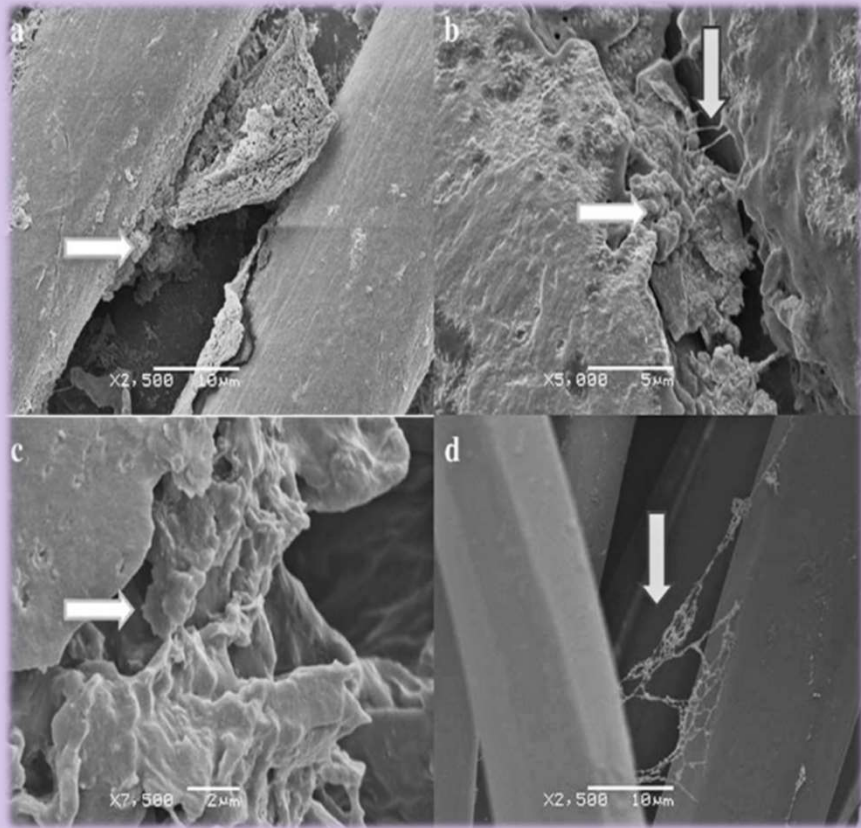


Clostridioides difficile infection rates are not dropping despite introduction of new technologies such as UV. This is an opportunity to address a potential source of infection at minimal cost, replacing ineffective products that are typically applied off label and are not effective. This is one area we have the advantage over RTU liquids, we should exploit the advantage.

Biofilms on Normally Dry Surfaces

The unexpected challenge

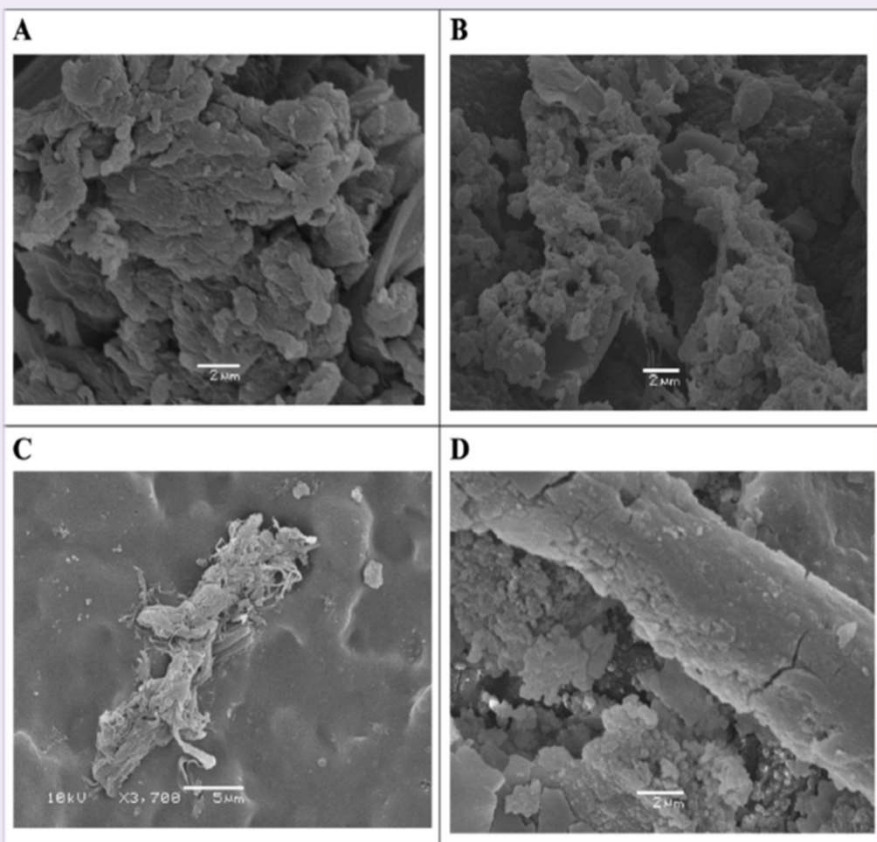
Biofilms – New Research



- First characterized by Vickery *et al.* after destructive sampling of an ICU
- Evidence of biofilms on:
 - a. Blind chord
 - b. Opaque ward door
 - c. Reagent box
 - d. Privacy Curtains
- Hypothesis: surface condensation or high relative humidity allowed biofilms to form. Or just the wrong disinfectant incorrectly applied. Once formed EPS protected the microbes from desiccation & made them difficult to remove

K. Vickery et al. 2012. Presence of biofilm containing viable multi resistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. J. Hos. Infect. 80:52-55. <https://www.journalofhospitalinfection.com/article/S0195-6701%2811%2900319-7/abstract>

Biofilms – New Research



Hu *et al.* also found dry surface biofilms on 93% of surfaces in an ICU, 52% had MDROs

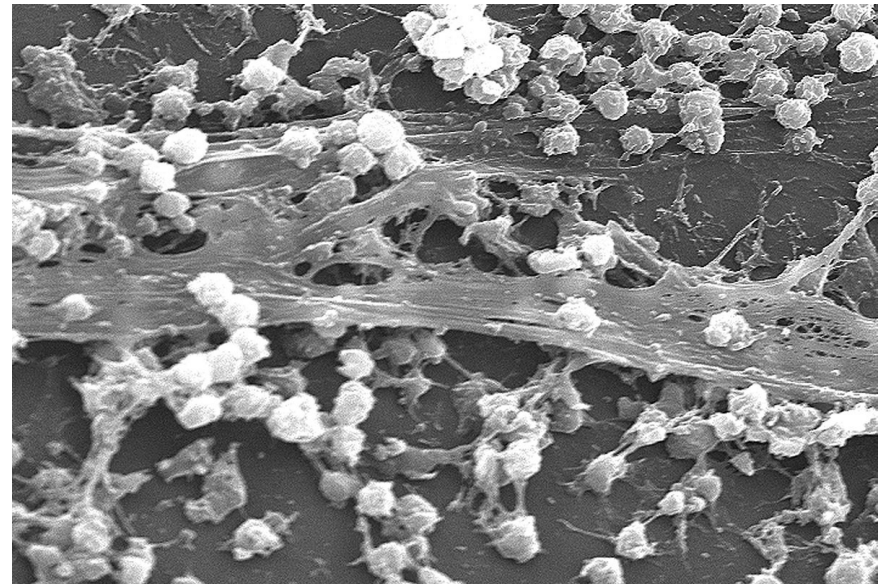
- a. Privacy curtain**
- b. Ward entry door**
- c. Mattress**
- d. Wire note clip**

All these biofilms were found after 2 rounds of terminal cleaning!

Are the cleaning processes in place truly effective?

Biofilms

- Hu *et al.* investigated what species were present in these biofilms
- All were multi-species
- *Staphylococcus aureus* present in 50% of biofilms
- Most common species overall:
 - *Faecalibacterium prausnitzii*
 - *Massilia timonae*
 - *Staphylococcus aureus*
 - Coagulase-negative staphylococci
 - *Pseudomonas* species
 - *Propionibacterium acnes*



Hu, H. *et al.* 2015. Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: combined results of conventional culture, pyrosequencing, scanning electron microscopy, and confocal laser microscopy. *J. Hos. Infect.* 91:35-44

<https://www.ncbi.nlm.nih.gov/pubmed/26187533>

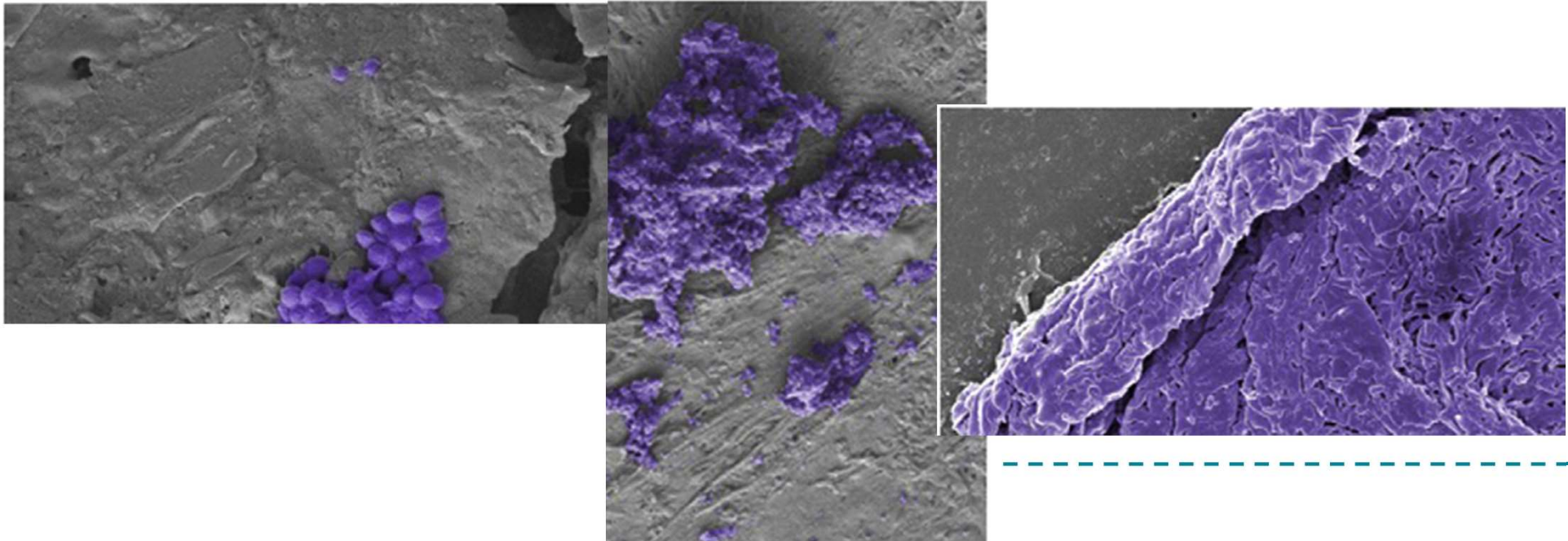
Its Not Just Australia

Others have repeated the studies and found the same thing

Otter JA, et al., Surface-attached cells, biofilms and biocide susceptibility: implications for hospital cleaning and disinfection, Journal of Hospital Infection (2014), <http://dx.doi.org/10.1016/j.jhin.2014.09.008>

K. Ledwoch et al. / Journal of Hospital Infection 100 (2018) e47ee56

- 65 Samples from 3 UK hospitals
- Multi-species dry biofilms were recovered from 95% of 61 samples.
- Abundance and complexity of dry biofilms were confirmed by SEM.
- All biofilms harbored Gram positive bacteria including pathogens associated with HCAI;
- 58% of samples grew methicillin-resistant *Staphylococcus aureus*.
- Dry biofilms had similar physical composition regardless of the type of items sampled or the ward from which the samples originated.



Wet-Dry what is the difference?

The extent of biofilm growth on “Normally Dry” surfaces was Unexpected when first observed.¹

All biofilms initially grow as hydrated biofilm, in the lab it takes 24 to 48 hours to dehydrate a wet biofilm.²

A dry biofilm starts to rehydrate after 4 to 8 min and can be completely rehydrated after 36 to 40 min.³

Why would a “dry” surface grow biofilm

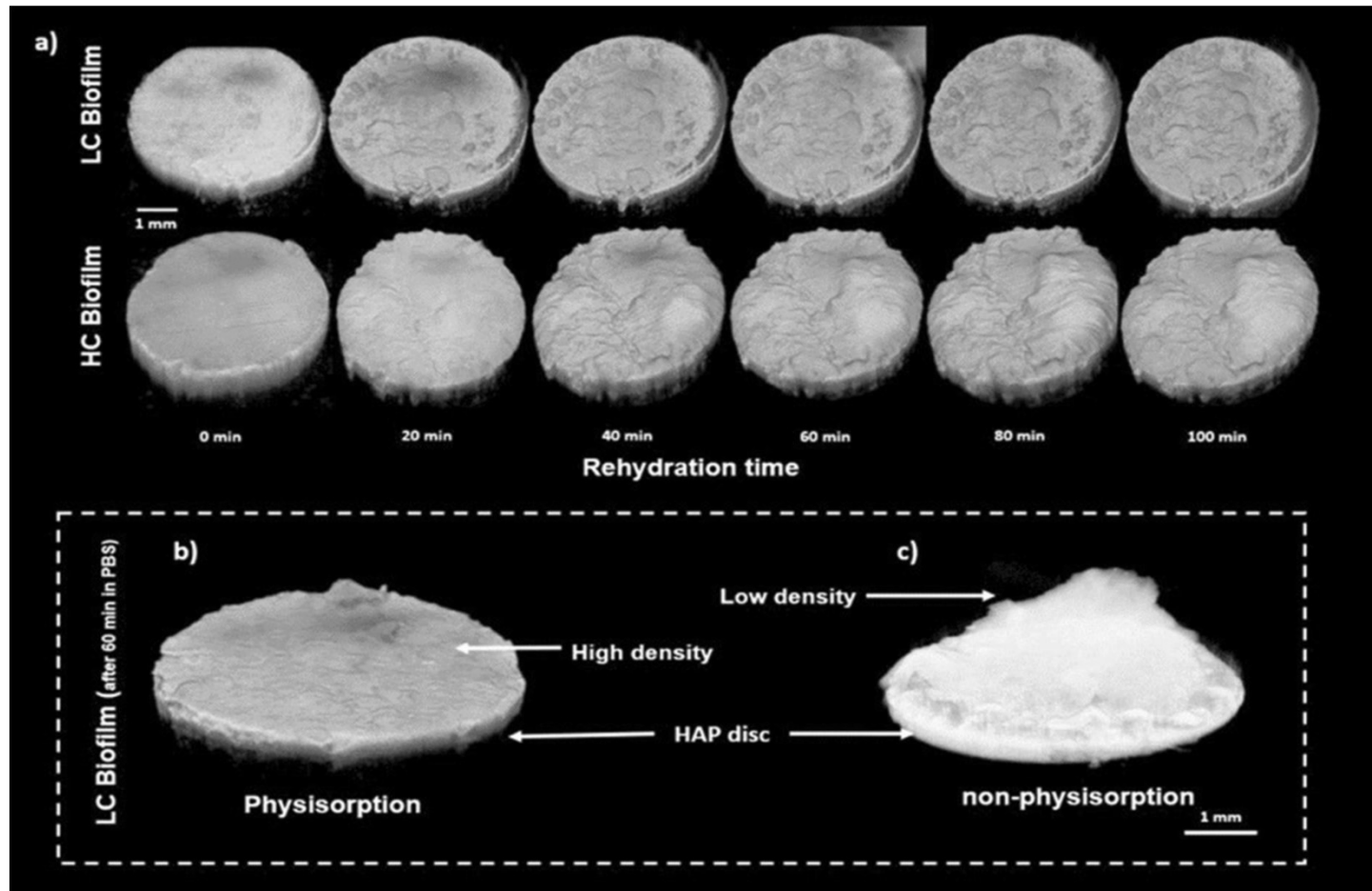
- High humidity (>63 % for fungi, >95 % for bacteria)
- Localized high humidity (location of vents, lack of insulation)
- Incidental spills
- Condensation
- Cleaning and disinfection
 - What if our disinfectant is the cause of biofilm growth?
 - If you apply a 10 min quat with a wiper then you just added water to the surface to keep the surface wet for 10 min.

Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. Vickery, K.; Deva, A.; Jacombs, A.; Allan, J.; Valente, P.; Gosbell, I.B. J. Hosp. Infect. **2012**, 80, 52–55.

A rapid model for developing dry surface biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* for in vitro disinfectant efficacy testing Carine A. Nkemngong¹, Maxwell G. Voorn¹, Xiaobao Li², Peter J. Teska² and Haley F. Oliver^{1*}Antimicrobial Resistance and Infection Control (2020) 9:134

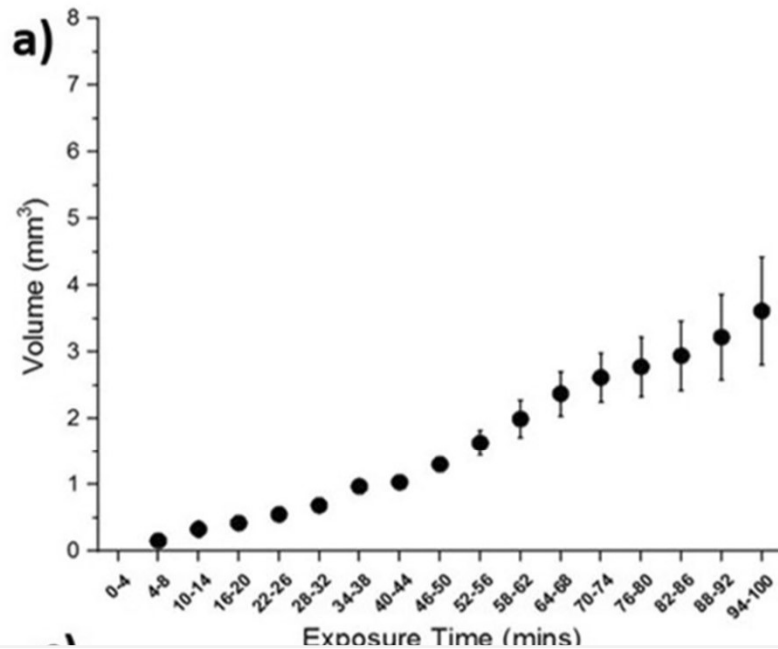
³Dependency of hydration and growth conditions on the mechanical properties of oral biofilms J. Pattem^{1,6,7*}, M. Davrandi^{2,7}, S. Aguayo³, B. Slak⁴, R. Maev⁴, E. Allan², D. Spratt² & L. Bozec^{1,5} Scientific Reports (2021) 11:16234

This is a dynamic process, biofilms are constantly changing

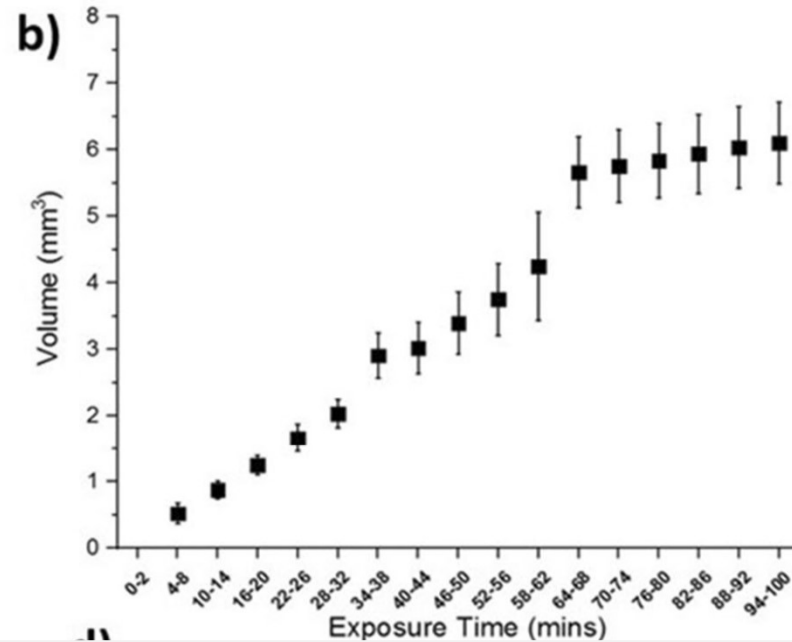


- LC Low carbon substrate
- HC high carbon substrate
- High Carbon substrate does absorb moisture and rehydrate faster

How quickly do biofilms rehydrate?



Low Carbon Substrate



High Carbon Substrate

Addition of moisture from either free water or elevated humidity will rehydrate Biofilm rapidly
Removal of moisture source results in slow drying
Repeated hydration and dehydration constantly change the nature of the biofilm

Bacteria in Biofilm Transfers to Healthcare Workers Hands

- Biofilm grown on test strips and allowed to dry
- Biofilm touched with index finger and transferred to agar.
- 5.5 and 6.6% of the bacteria were transferred to hands with one touch
- 20% were then transferred to agar with one touch
- Overall transfer rate of 1.04 to 1.26%.
- Large numbers of bacteria were transferred by bare hands to multiple fomites
- Sufficient bacteria to cause infection were transmitted up to 19 times following one touch of the Dry Surface Biofilm.



Chowdhury D, et al., Effect of disinfectant formulation and organic soil on the efficacy of oxidizing disinfectants against biofilms, *Journal of Hospital Infection* (2018), <https://doi.org/10.1016/j.jhin.2018.10.019D>

The Sheet is Not a Barrier to Transmission

- Cotton sheets act as vehicles of transmission from dry surface biofilm on mattresses.
- Between 100 - 1000 bacterial colonies transferred for up to 20 touches.
- Thicker sheets transferred less bacteria than thin sheets with less than 100 colonies transferred/touch.
- Wetting the Dry Surface Biofilm increased the number of colonies transmitted to ≥ 1000 bacteria/touch.

Chowdhury D, et al., Effect of disinfectant formulation and organic soil on the efficacy of oxidizing disinfectants against biofilms, *Journal of Hospital Infection* (2018), <https://doi.org/10.1016/j.jhin.2018.10.019D>



Registering Disinfectants and Making Efficacy Claims

What works and what does not

Remember : “It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Read the entire label and use strictly in accordance with precautionary statements and directions.”

US EPA testing based on ASTM E2871

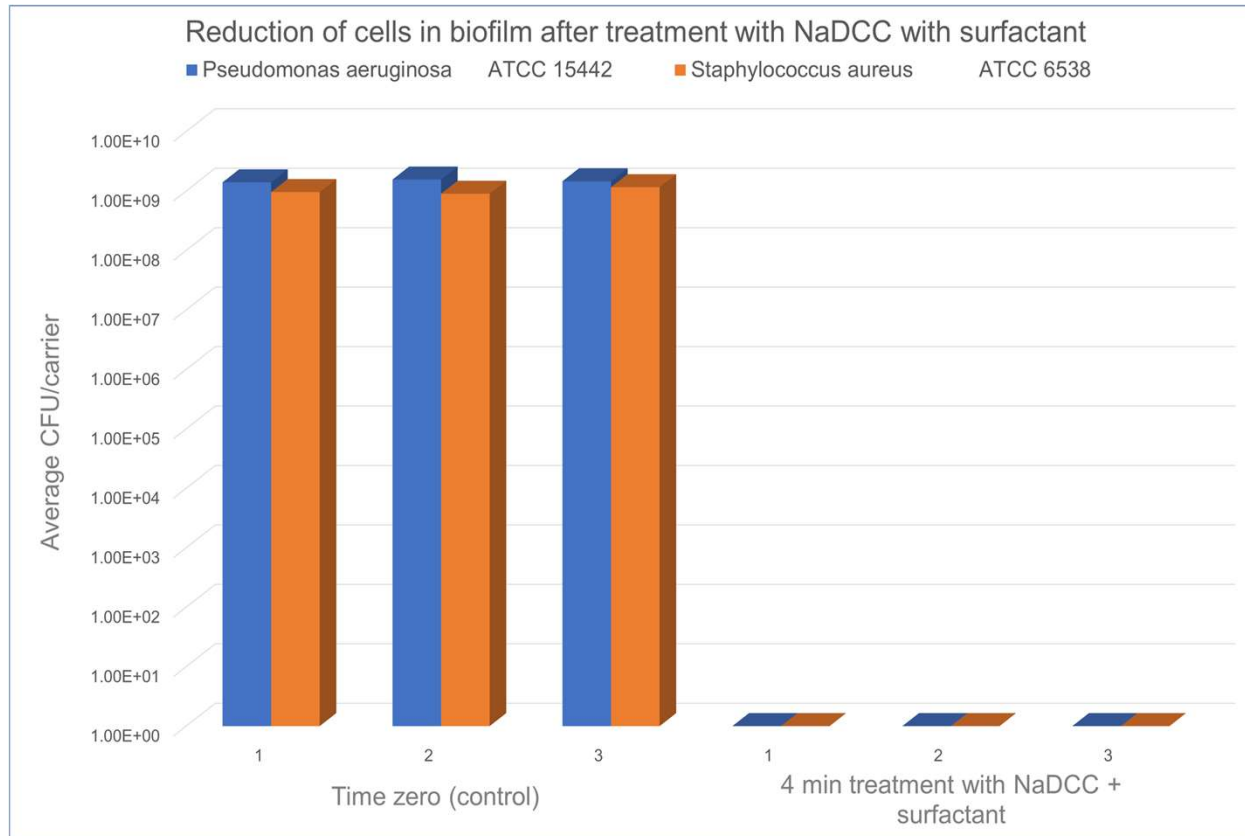
- ASTM E2871 “Evaluating Disinfectant Efficacy Against *P. aeruginosa* Biofilm Grown in CDC Biofilm Reactor Using Single Tube Method”
- Two clinically relevant species
 - *Staphylococcus aureus*
 - *Pseudomonas aeruginosa*
- Emphasis on Repeatability & Reproducibility
- Biofilms grown in low nutrient and high shear in a CDC Bioreactor (seen here) to ensure strong biofilms
- To qualify: product will need to be a hospital grade disinfectant, and will need to demonstrate efficacy against both *S. aureus* and *P. aeruginosa*
- The level of efficacy: ≥ 6 Log₁₀ Reduction



Not many disinfectants have passed the EPA Biofilm test to date (October 2023)
The EPA has not yet published a convenient list of products

- A ready to use Peroxy acetic acid (PAA) based product that contains four percent hydrogen peroxide (pH 2.5 to 3.0)
 - A concentrate PAA/H₂O₂ blend used at 4 oz per gallon for a 10 min kill claim (diluted pH 3.0)
 - A ready to use 1.4 percent hydrogen peroxide product, spray apply only (pH 2.5)
 - A ready to use Bleach based product that contains 13,000 ppm of bleach, ~2.5 times the typical 5,250 ppm strength (pH 12.0).
 - A liquid concentrate that is a combination of quaternary ammonium compounds and hydrogen peroxide, a two part product that is mixed on site generating a final concentration of hydrogen peroxide of 3.1 percent and a quaternary ammonium concentration of 3 percent
 - A tablet form of sodium dichloroisocyanurate (NaDCC) that contains a surfactant, when dissolved in water the tablet produces a solution of 4306 ppm of hypochlorous acid (pH 6.0 to 7.0).
- Products that have not been registered in accordance with the EPA test:
 - Bleach at 10:1 dilution (ie 5,250ppm)
 - Quaternary ammonium compounds (Quats) concentrates
 - Quat Alcohol products
 - Alcohol
 - Thymoil
 - Citric acid products
 - Chlorine dioxide
 - NaDCC without surfactant
 - Phenolic based products

NaDCC plus 2% Surfactant EPA Testing Against Biofilm



Pseudomonas aeruginosa: >8.97
Log₁₀ reduction

Staphylococcus aureus:
>7.79 Log₁₀ reduction

Testing against biofilm 4300 ppm NaDCC + 2% surfactant

Test microorganism	Contact time	Test	Average Log ₁₀ CFU/carrier ¹	Average % reduction	Average Log ₁₀ reduction
<i>Pseudomonas aeruginosa</i> ATCC 15442	Time zero	Control	9.27	N/A	
	4 minutes	NaDCC + surfactant	<0.3	>99.999986%	>8.97
<i>Staphylococcus aureus</i> ATCC 6538	Time zero	Control	8.25	N/A	
	4 minutes	NaDCC + surfactant	<0.46	>99.99999%	>7.79

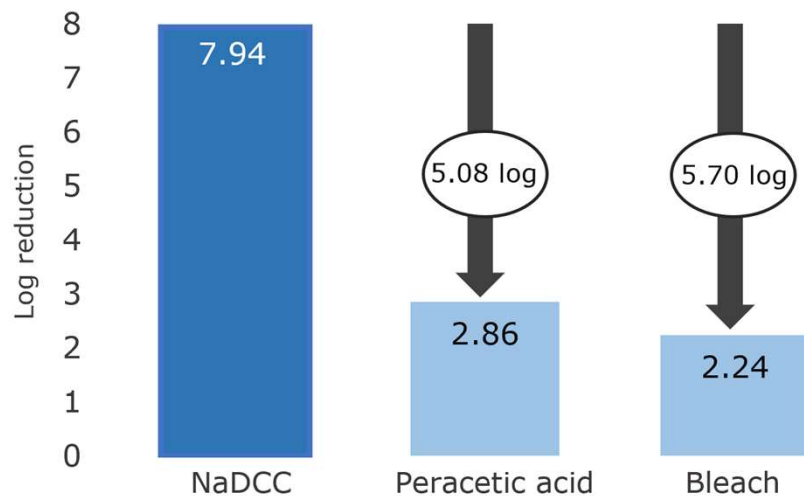
Dry-surface biofilms and the EPA

- No standardised test method available for the evaluation of disinfectants against dry biofilms.
- The EPA test method looks at Hydrated Biofilm.
- Chowdhury *et al* evaluated disinfectants against simulated DSB (article in press)
 - At 1000 ppm NaDCC nearly 3 Log₁₀ reduction
 - At 5000 ppm hydrogen peroxide, <1 Log₁₀ reduction

Its harder to kill bacteria in dry surface biofilm than in hydrated biofilm (the bacteria are not active), if you can't pass the EPA test for hydrated biofilm the product will not be effective on dehydrated biofilm

This is not a static situation, add water and dry biofilm becomes hydrated biofilm, we do that once a day in most hospitals.

Comparison of Chemical Efficacy Dry Biofilm



- At one min NaDCC with surfactant is more effective than 5000 ppm bleach or PAA/H₂O₂.

Initial Inoculum of *Pseudomonas aeruginosa* grown in Bioreactor in accordance with ASTM E2562-22 then dried for 48 hours. Dried sample tested to show the presence of 7.94 log, Disinfectants prepped in accordance with EPA registration One min exposure. then neutralized and remaining bacteria counted.

Special Circumstances

It is not only bacteria we need to be concerned about.

2019 Novel Coronavirus (2019-nCoV).

Von Borowski RG, Trentin DS. 2021. Biofilms and coronavirus reservoirs: a perspective review. Appl Environ Microbiol 87: e00859-21.

<https://doi.org/10.1128/AEM.00859-21>

- Viruses colonize biofilms, phages will attack bacteria and grow, human viruses simply survive within the biofilm.
- SARS CoV-2 survival on a dry surfaces increases from 3 to at least 30 days
- Biocides and UV are less effective against viruses Including SARS CoV in biofilms
- Biofilms present a reservoir of viral particle that can result in patient infection for a period of time after the final case has resolved.

SARS CoV -2 can survive protected in Biofilms for prolonged periods of time > 30 days, protected from environmental stressors and remaining infectious.

Candida auris and biofilm

- *C. auris* produces biofilm on hard surfaces.
- Experiment
 - Grow *C. auris* biofilm in bioreactor
 - Dehydrate biofilm 24 hours
 - Regrow hydrated biofilm 3 times
 - Produce a 7 to 8 log dry biofilm
 - Expose dry biofilm to 1000 ppm of bleach , neutralize after 5 min exposure
 - Regrow biofilm
 - Expose dry biofilm to 1000 ppm of bleach neutralize after 5 min exposure
 - Regrow biofilm
 - Expose dry biofilm to 1000 ppm of bleach neutralize after 5 min exposure.
- When the biofilm is first exposed to 1000 ppm of bleach a 5 to 6 log reduction is obtained
- The second exposure to 1000 ppm of bleach a 3 to 4 log reduction is obtained
- When the same biofilm is treated with 1000 ppm of bleach the third time it produces only a 1 to 2 log reduction *C. auris* concentration.
- Your disinfectant may have an EPA claim against *C. auris* but unless it also has an efficacy claim against biofilm you will not address the outbreak.

Summary

- Prevalent in the hospital environment (Even “Dry” Surfaces)
- More resistant to biocides than planktonic cells
- Current cleaning and disinfection procedures may not be sufficient to remove/kill dry-surface biofilms
- Source of multiple drug resistant organisms
- Dry-surface biofilms adds a level of difficulty to risk management of environmental contamination that was not previously recognized
- Bacteria in Dry Surface Biofilm can transfer to patients via healthcare workers hands
- Dynamic conditions: a dry biofilm can become a hydrated biofilm, just add water

If your disinfectant does not work on bacteria in biofilms you are not Disinfecting

Additional References

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Thank You

