To Clean or Not to Clean:
Exploring the Ability of Biocidal Compounds to Induce Physiological Changes in Bacteria that may Increase the Spread of Antibiotic Resistance Genes

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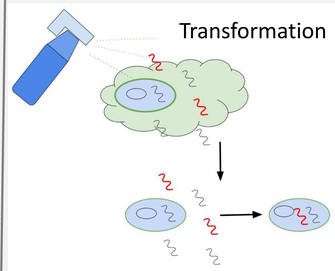
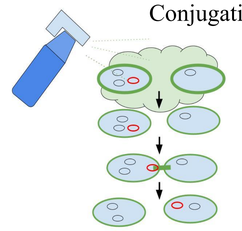
Abstract and Background

The prevalence of antibiotic resistance genes in non-clinical settings is increasing, and so are cleaning measures, especially over the past few years in relation to increased COVID-19 pandemic cleaning guidelines. This may increase the likelihood of a changed chemical environment created by the degradation of cleaning compounds on surfaces. The physiological changes to surface-associated bacteria caused by cleaning solutions may disrupt membrane integrity and the action of efflux pumps as well as allow for an increased presence of extracellular genetic material on surfaces. The presence of bacterial DNA and antibiotic resistance genes on surfaces was quantified using qPCR and assessed using gel electrophoresis. If membrane disruption occurs in conjunction with the presence of extracellular genetic information, there may be a greater potential for the uptake and subsequent spread of antibiotic resistance genes if the environment creates selective pressure for this potential advantage. The potential for membrane disruption was characterized via an adapted version of Lehrer's assay and the Hoechst assay. If cleaning solutions are found to create a selective pressure at low concentrations, cleaning practices may need to be updated to prevent the surface level concentration of cleaning solution from becoming problematic.

Objective



Transformation, a type of horizontal gene transfer, involves bacteria picking up and internalizing genetic information from surfaces.

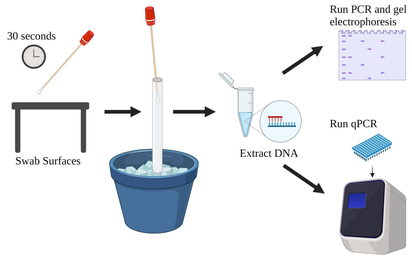


Conjugation, a type of horizontal gene transfer, involves bacteria transferring genetic information that may provide a survival advantage to another organism without division via extension of a part of the membrane.

Biocidal compounds at nonlethal concentrations may affect the bacteria on surfaces via changes in membrane permeability and an increased presence of extracellular genetic information. Can membrane disruption be characterized? Can this affect horizontal gene transfer?

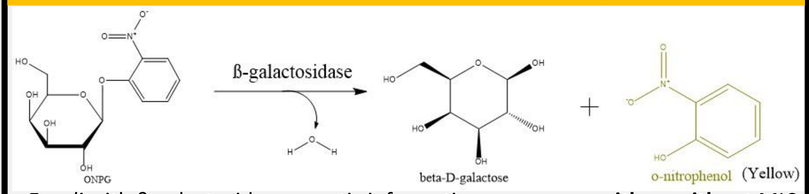
Methods

Protocol for swabbing commonly-cleaned surfaces for presence of 16s rDNA and BlaTEM genetic information



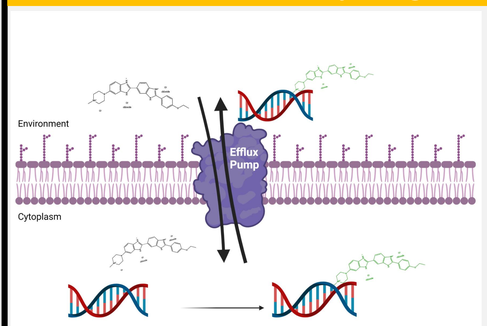
Four regularly cleaned surfaces were swabbed.
DNA was extracted from each sample.
Each sample was amplified via PCR.
The extracted DNA was run on gel electrophoresis to check for off target amplifications.
Each sample was roughly quantified using qPCR.

Adapted Lehrer's assay: ONPG is broken down to yellow pigment o-nitrophenol in conjunction with β-galactosidase.



E. coli with β-galactosidase genetic information was grown **with or without** MIC of cleaning solution
ONPG was added.
Yellow pigment production (o-nitrophenol) was measured via change in absorbance at 420 nm over a 20-minute time period.

Efflux accumulation assay using fluorescent Hoechst reagent.



Various organisms were grown with or without MIC of cleaning solution.
Fluorescent Hoechst reagent was added to each well.
The change in fluorescence was measured over a 180-cycle period at emission filter 460 nm and excitation filter 355 nm.

Results

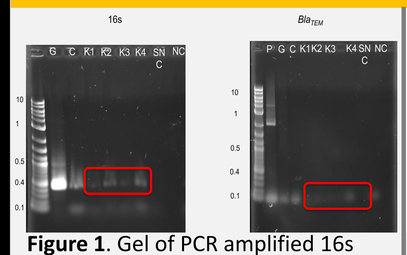


Figure 1. Gel of PCR amplified 16s rDNA and *bla*$_{TEM}$ primers with swab samples (red boxes).

- The expected band size for 16S rDNA is 466 bp
- The expected band size for *bla*$_{TEM}$ is 85 bp
- The first lane after the molecular weight marker in the 16s rDNA goose positive control, while the first lane in the *bla*$_{TEM}$ gel is the pbluescriptsk+ positive control for *bla*$_{TEM}$. K1-K4 in each gel corresponds to samples swabbed from different locations in Knowlton Café, SNC corresponds to the swab negative control, and NC corresponds to the PCR negative control

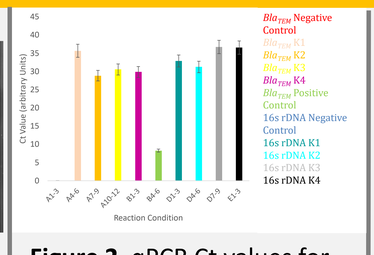


Figure 2. qPCR Ct values for each PCR reaction.

- **Ct Value:** The number of amplification cycles it took for the total quantity of product to exceed a set baseline of background fluorescence.
- Ct values **below 29 indicate a high amount** of genetic information.
- Ct values **above 38 indicate a low amount** of genetic information.
- The exact copy number is not known as no amplification cuve was developed.

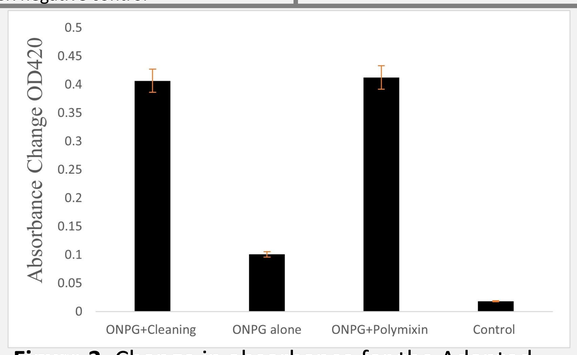


Figure 3. Change in absorbance for the Adapted Lehrer's assay to observe membrane disruption.

- *K12* strain *E. Coli* were used for the intrinsic β-galactosidase genetic information
- *E. coli* in the presence of cleaning solution demonstrated similar levels of change over time to E. coli in the presence of polymyxin, a known membrane disrupting antibiotic.
- *E. coli* not in presence of cleaning solution has significantly less disruption.

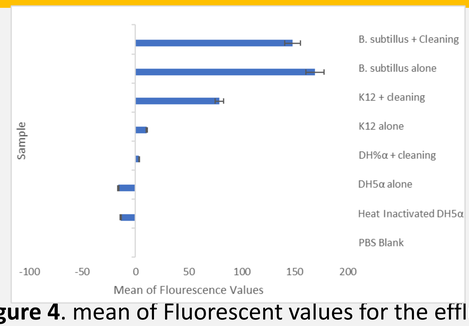


Figure 4. mean of Fluorescent values for the efflux accumulation assay using Hoechst reagent. Efflux was thought to be affected by cleaning solution at sub-Minimal inhibitory concentrations.

- For *E. coli* species, more efflux activity was seen in the presence of cleaning solutions. For *B. Subtilis*, more efflux was seen in the strain itself without cleaning solution.
- Different organisms have different efflux pump families which use different energy sources
  - Perhaps why there is a difference in efflux accumulation between species in the same conditions
- Specific components of cleaning solutions may interfere with specific types of efflux pumps

Conclusions

If there is extracellular DNA on surfaces and sub–Minimal Inhibitory Concentration levels of cleaning compounds on surfaces, this combination may affect membrane permeability and efflux, which could affect the success of transformation and conjugation events.

Future Work

- Assays that can more precisely identify and quantify the underlying mechanism of the effects of subMIC levels of cleaning solution
- Measure the actual concentrations of cleaning compound components on surfaces
- Observe how natural transformation and conjugation events are affected by subMIC levels of cleaning solution

Sources

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Acknowledgements

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