Removing biofilms from endoscopes: the importance of the cleaning chemistry

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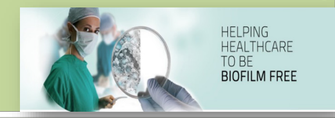

Introduction

Several studies have associated the use of endoscopes with several outbreaks of infection in various clinical settings, including gastrointestinal, thoracic, urological, and other endoscopic procedures. The ability of bacteria to form biofilms is a major cause for endoscopes contamination. *Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* are the most likely germs to cause these contaminations. We compared the biofilm dispersion activity, against bacterial species mentioned above, of one enzymatic cleaner developed by OneLIFE Sa (enziQure®) and 10 cleaners commonly used for the manual cleaning of endoscopes.

Materials and Methods

We used 5 reference strains and 10 clinical isolates (C1-C10; infections on medical devices and tissues). Biofilms were grown for 24 h in 96-wells plates. biofilms were exposed for 1h at 40°C to concentrations recommended by manufactures (0.5%-1%) of cleaners prepared in standardized water (WSH :1.25 mM $MgCl_2$, 2.5 mM $CaCl_2$, 3.33 mM $NaHCO_3$). The ability of cleaners to remove biofilm biomass was determined using crystal violet assay as previously described (1). Tridimensional images were performed using confocal laser scanning microscopy (CLSM). Biofilms were grown on coverslips, washed with distilled water; after which coverslips were transferred into a fresh well and incubated during 30 min at room temperature with LIVE/DEAD kit (1). Image stacks were acquired at a resolution of 700x500 pixels, recorded using Z-Stack module for acquisition of image series from different focus planes, and used to construct three-dimensional images with AxioVision software.

References

1- Siala, W. et al. The antifungal caspofungin increases fluoroquinolone activity against Staphylococcus aureus biofilms by inhibiting N-acetylglucosamine transferase. Nat Commun 2016; 7:13286.

Acknowledgements

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Results

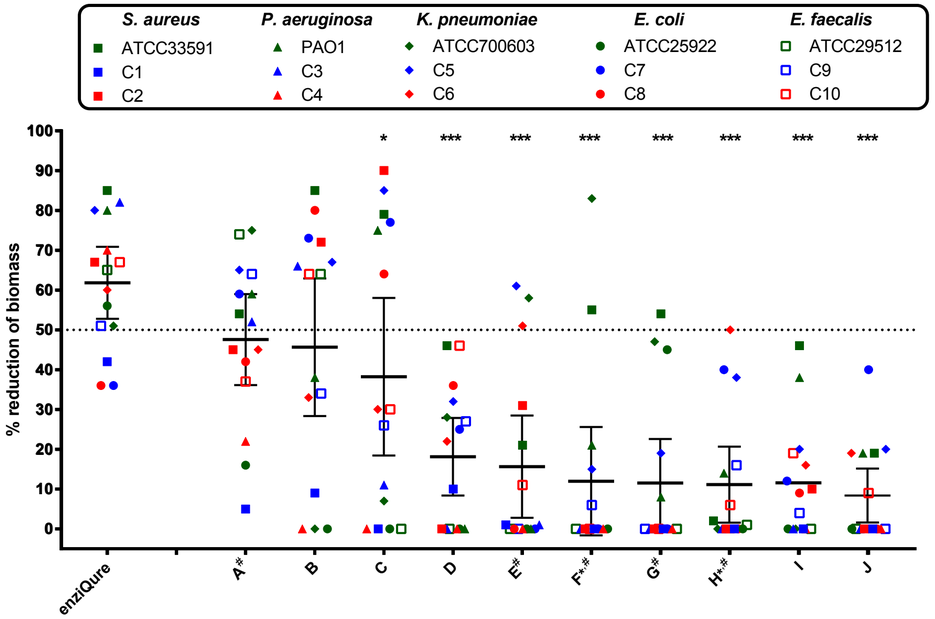

Figure 1 – Percentage of biomass reduction for 15 biofilms (formed by 3 isolates from 5 different species) after exposure to enziQure® and 10 other cleaners in the absence of mechanical action (* : no enzyme; # : germicidal activity). The dotted line shows the 50 % limit. Dots show the data for individual strains, with mean and 95% confidence intervals shown in black for each cleaner. Means (% ± SD): enziQure®: 62 ± 16; A: 48 ± 20; B: 45.7 ± 31; C: 38 ± 35; D: 18 ± 17; E: 16 ± 23; F: 12 ± 24; G: 12 ± 19; H: 11 ± 17; I: 12 ± 14; J: 8± 12. Statistical analysis: one-way ANOVA with Dunnett's multiple comparison test (cleaner vs enziQure®): *: $p < 0.05$; ***: $p < 0.001$

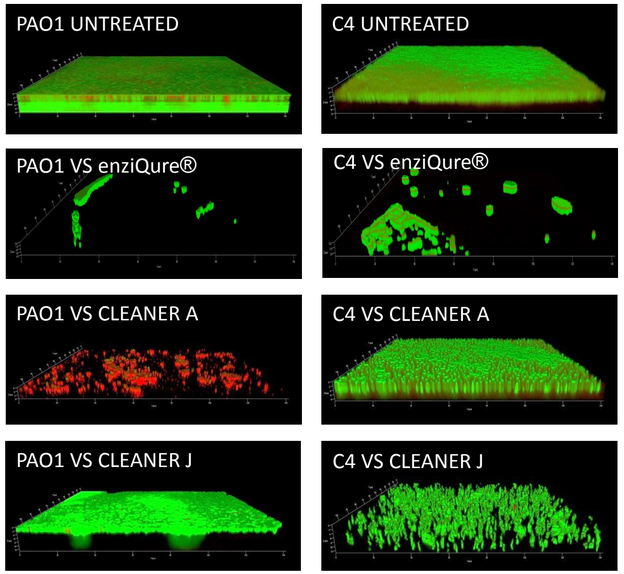

Figure 2 – Tridimensional images in confocal laser scanning microscopy of PAO1 and clinical isolate (C4) biofilms. Biofilms were incubated during 1h at 40°C with 1% enziQure®; 0.5% cleaner A or 0.5% cleaner J and labelled with live/dead staining (red: dead; green: live). PAO1 biofilms are shown on the left, C4 biofilms are shown on the right.

Conclusions

While most of the current cleaning solutions are poorly active on biofilms, the enzymatic formulation of enziQure® allows marked (> 50 %) biofilm dispersion for 80% of tested strains. This effect nevertheless remains strain-dependent, probably related to the nature of the produced biofilm matrix.