Population and resistance patterns of *Salmonella Typhimurium* and *Staphylococcus aureus* biofilms exposed to sublethal chemical disinfection under mono- and dual-species multi-strain conditions

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Materials & Methods

**A. Inoculation**
- ST: 3 strains *Salmonella Typhimurium* or
- SA: 3 strains *Staphylococcus aureus* or
- AT: Mixture of ST and SA

**B. Disinfection step**
- Double rinsing with 10 ml of 1/4 Ringer solution (each time onto each coupon)
- SS coupons immerged in disinfectant* for 6 min

* BC: benzalkonium chloride (50ppm), PA: paracetic acid (10ppm), SH: sodium hypochlorite (10ppm), W: water or NO: no use of disinfectant

**C. Recovery of biofilm cells from coupons**
- SS coupon immerged in 6 ml of 1/4 Ringer solution & 10 sterile glass beads
- Vortexing for 2 min

**D. Microbiological analysis**
- Biofilm cells
- Serial Dilutions
- Counts
- Recovery of representative isolates
- Pulsed Field Gel Electrophoresis (PFGE)

Results & Discussion

- Dual-species conditions seem to lead to a reduction in the number of sessile cells (0.6 and 1.1 log cfu/cm²) for ST and SA, respectively, compared to mono-species conditions
- BC was found to be more effective in both mono- and dual-species biofilm communities compared to the other two disinfectants (PA, SH)

- PA was more effective against ST biofilm cells under either mono or dual-species conditions
- SH was the least effective in all cases studied
- ST multi-drug resistance human epidemic strain, was recovered in higher percentages in all cases, except after disinfection with PA of dual species conditions.
- In the case of SA, strain isolated from human lesion and COL strain were recovered in higher percentages in mono and dual culture conditions, respectively.

**Conclusion**
- The presence of ST strongly decreased the resistance of SA biofilm to PA, while ST resistance remained stable
- Different strains here employed behaved differently with regard to both biofilm formation and antimicrobial resistance
- Such research expands our knowledge on the physiology of multi-species pathogenic bacterial biofilms and may facilitate the development of efficient control methods to be used in the food industry

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*Introduction*: Following inadequate sanitation, the remaining microflora on food industry surfaces may contain multiple bacterial species which -under permissible surrounding conditions- can eventually grow and form biofilms. Cell-to-cell interactions within such sessile communities may influence both cell physiology and population dynamics.

*Purpose*: To evaluate the possible influence of bacterial interactions encountered in mono- and dual-species multi-strain biofilms of *Salmonella* Typhimurium (ST) and *Staphylococcus aureus* (SA) on: (i) the ability of strains to develop biofilm, and (ii) their subsequent resistance to sublethal chemical disinfection.

*Methods*: 3 strains per species were left to develop biofilms on stainless steel (SS) coupons submerged in TSB for 144 h at 20°C, under either mono- or dual-species conditions. Following biofilm formation and the removal of loosely attached cells, SS coupons were exposed for 6 min to benzalkonium chloride (BC, 50ppm), peracetic acid (PA, 10ppm) and sodium hypochlorite (SH, 10ppm). The dominance of each strain in the sessile communities both before and after disinfection was monitored using a promising PFGE approach.

*Results*: Dual-species conditions led to a reduction in the number of SA sessile cells (1.1 log cfu/cm\(^2\)), compared to mono-species ones, while ST sessile population remained rather unaffected. Regarding disinfection resistance and under both conditions, BC was found to be the most effective disinfectant, while SH was the least effective in all cases studied. Interestingly, the presence of ST strongly decreased the resistance of SA biofilm cells to PA. PFGE analysis revealed that the different strains here employed behaved differently with regard to both biofilm formation and antimicrobial resistance.

*Significance*: Such research expands our knowledge on the physiology of multi-species pathogenic bacterial biofilms and may hopefully facilitate the development of efficient control methods to be used in the food industry.
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