

Effectiveness of three disinfectants on the removal of *Listeria monocytogenes* biofilms

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Introduction and Objective

Listeria monocytogenes is a foodborne pathogen that can cause severe invasive human illness (listeriosis) in susceptible individuals. There is a special concern in the food industry due to *Listeria* spp. ability to form biofilm and to persist in the processing environment. When in biofilms, cells are more resistant to disinfectants and this led to an inefficient disinfection of surfaces and equipment. Disinfectants are classified by their chemical nature and each class has unique characteristics, hazards, toxicities and efficacy against various microorganisms. Quaternary ammonium, oxidizing agents and alcohol compounds are some of the most usual disinfectants used in the food industry. The aim of this study was to evaluate the efficiency of three disinfectants applied over a biofilm produced by *Listeria monocytogenes* isolated from a food processing area.

Material and Methods

Listeria monocytogenes strain

- ✓ *L. monocytogenes* was isolated from food processing environment



Biofilm

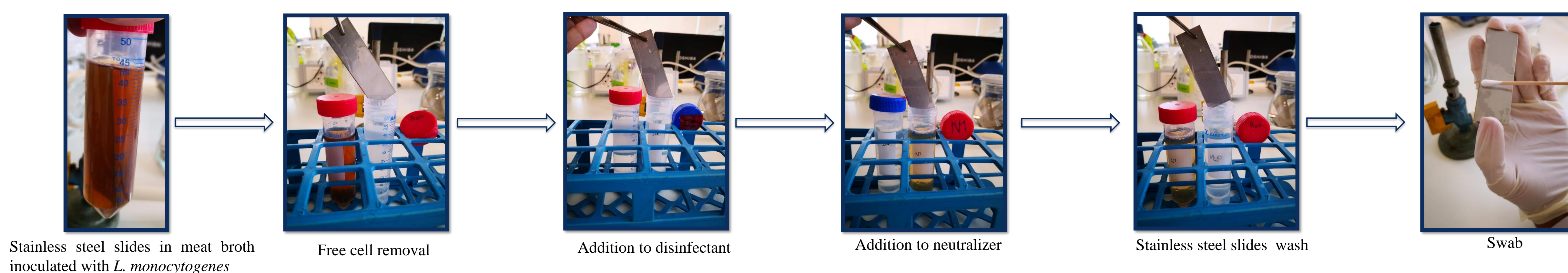
- ✓ Biofilms were produced on stainless steel slides incubated in meat broth inoculated with *L. monocytogenes* during 24h to let the surface to be covered by a biofilm layer (4.2×10^8 CFU/cm²).

Microbiological analysis

- ✓ The slides were washed with sterilized tap water and the disinfectants efficacy were analysed in accordance with EN 1276 European - Standard for evaluation of bactericidal efficacy of disinfecting liquids.

Disinfectants (D) and neutralizer (N)

- ✓ D1: ethanol:isopropanol:benzilic alcohol (46:27:1)
- ✓ D2: hydrogen peroxide
- ✓ D3: benzalkonium chloride (0.5 e 2%)
- ✓ N1: polysorbate 80 (30 g/L), lecitin (3 g/L), saponin (30 g/L)
- ✓ N2: thiosulfate (10 g/L), polysorbate (50 g/L) and lecitin (3 g/L)



- ✓ The slides were dipped in three disinfectants followed by neutralizer during 1, 15, 30 and 60 minutes in each solution and washed again prior to swab.
- ✓ The procedure was done to D1-N1, D2-N2, D3 (0.5%)-N1 and D3 (2%)-N1.
- ✓ Each collected sample from swabs was serially diluted in Ringer solution and plated on TSA-YE in duplicate, by the drop count technique (Miles & Misra, 1938). After incubation at 37°C for 48h the colonies were counted and the CFU/cm² calculated. Experiments were performed in triplicate.

Results

- ✓ The results evidence that after 1 minute the average rates reduction were 6.03 log to D1, >8.55 log to D2, 7.29 log to D3 (0.5%) and >8.55 log to D3 (2%).
- ✓ It was not possible to observed microbial growth after 15, 30 and 60 min in contact with the tested disinfectants.

Conclusion

- ✓ Once the tested concentration were those recommended by disinfectants producers, we can conclude that it is necessary to keep these products in contact with the surfaces at least 1 minute to reach an appropriate microbial reduction.

Acknowledgements

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References

- EN 1276 European (2009) Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2, step 1). European Standard.
- Miles, A.A., Misra, S. S. 1938. The estimation of the bactericidal power of the blood. Journal of Hygiene 38, 732-749.