Preliminary study on the effectiveness of detergent and sanitiser products on Arcobacter butzleri strains

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Preliminary study on the effectiveness of detergent and sanitizer products on *Arcobacter butzleri* strains

Elisabetta Chiarini[1], Davide Buzzanca[1], Kurt Houf[2], Valentina Alessandria[1]

[1] Department of Agricultural, Forest and Food Sciences, University of Torino, Italy
[2] Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Belgium

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BACKGROUND

Arcobacter butzleri is a zoonotic pathogen mainly isolated from the poultry industry.

Despite disinfection procedures, numerous studies have documented the prolonged presence of *A. butzleri* in food processing environments.

In 2021, Botta et al., **sampled the equipment of a poultry slaughterhouse following sanitization procedures and isolated strains of *A. butzleri*** (*Figure 1*).
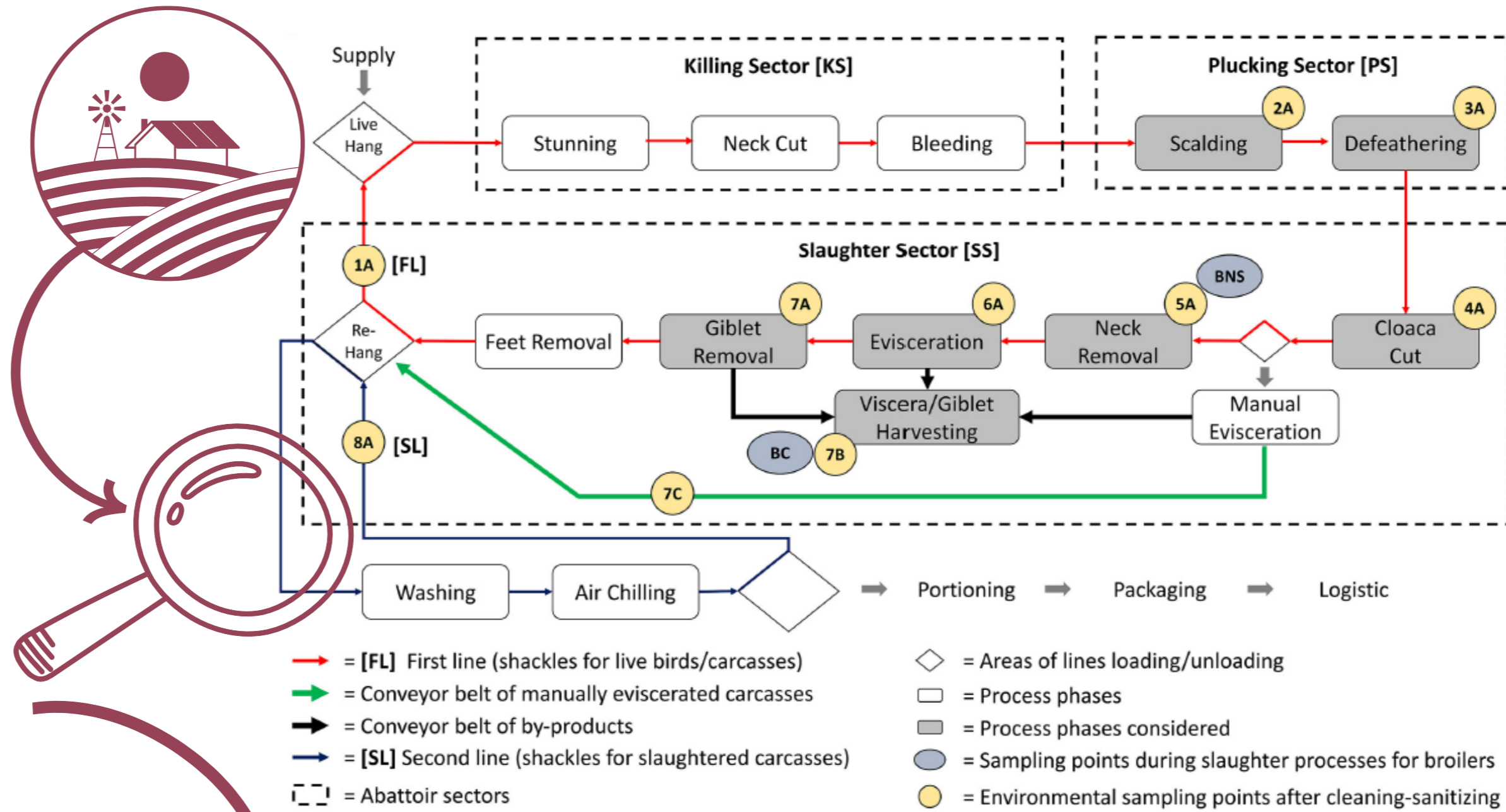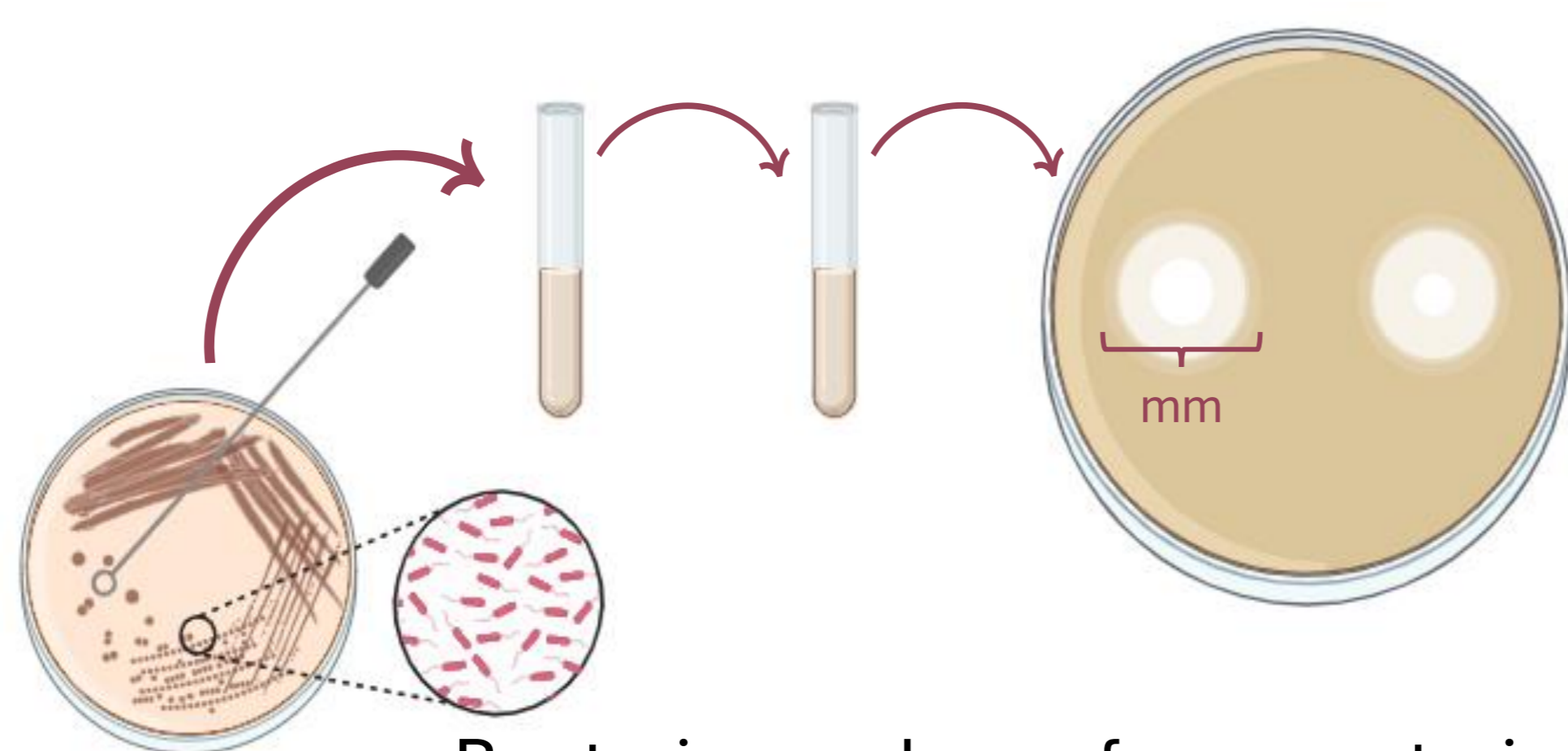
OBJECTIVES

Considering the high persistence of this pathogen, this study aims to investigate the susceptibility of *A. butzleri* isolated from poultry carcasses and slaughtering equipment to the most commonly used commercial disinfectants and detergents in food processing plants for surface disinfection and organic matter removal.

Figure 1.
Schematic representation of the slaughter process with sampled equipment surfaces (alphanumeric code) after routine cleaning-sanitization.
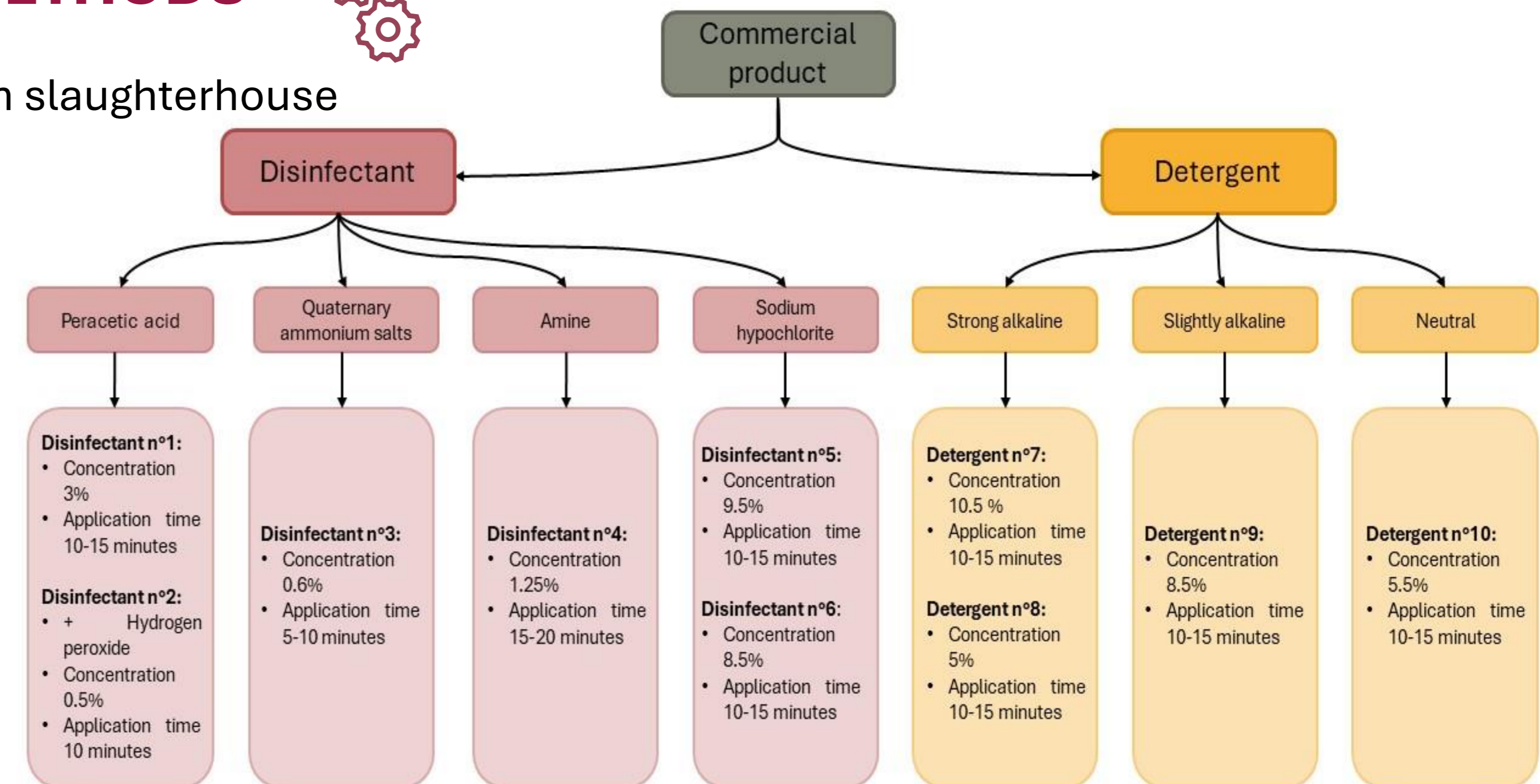

MATERIALS & METHODS

The agar disk diffusion method was used, adapting the proposed EUCAST standardized guidelines for antibiotic resistance.

150 µl of broth culture grown overnight were inoculated on MH plates, following the 0.5 McFarland standardization. Selected commercial products were inoculated onto each disk.

The inhibition halo was measured in millimeters.

- 31 strains of *A. butzleri* isolated from the chicken slaughterhouse

Bacteria used us reference strains:
- *A. butzleri* LMG10828[T]
- *Escherichia coli* ATCC35150
- *Staphylococcus aureus* ATCC25923

Commercial product → Disinfectant / Detergent

Disinfectant: Peracetic acid · Quaternary ammonium salts · Amine · Sodium hypochlorite

Detergent: Strong alkaline · Slightly alkaline · Neutral

Disinfectant n°1:
- Concentration 3%
- Application time 10-15 minutes

Disinfectant n°2:
- + Hydrogen peroxide
- Concentration 0.5%
- Application time 10 minutes

Disinfectant n°3:
- Concentration 0.6%
- Application time 5-10 minutes

Disinfectant n°4:
- Concentration 1.25%
- Application time 15-20 minutes

Disinfectant n°5:
- Concentration 9.5%
- Application time 10-15 minutes

Disinfectant n°6:
- Concentration 8.5%
- Application time 10-15 minutes

Detergent n°7:
- Concentration 10.5 %
- Application time 10-15 minutes

Detergent n°8:
- Concentration 5%
- Application time 10-15 minutes

Detergent n°9:
- Concentration 8.5%
- Application time 10-15 minutes

Detergent n°10:
- Concentration 5.5%
- Application time 10-15 minutes

RESULTS
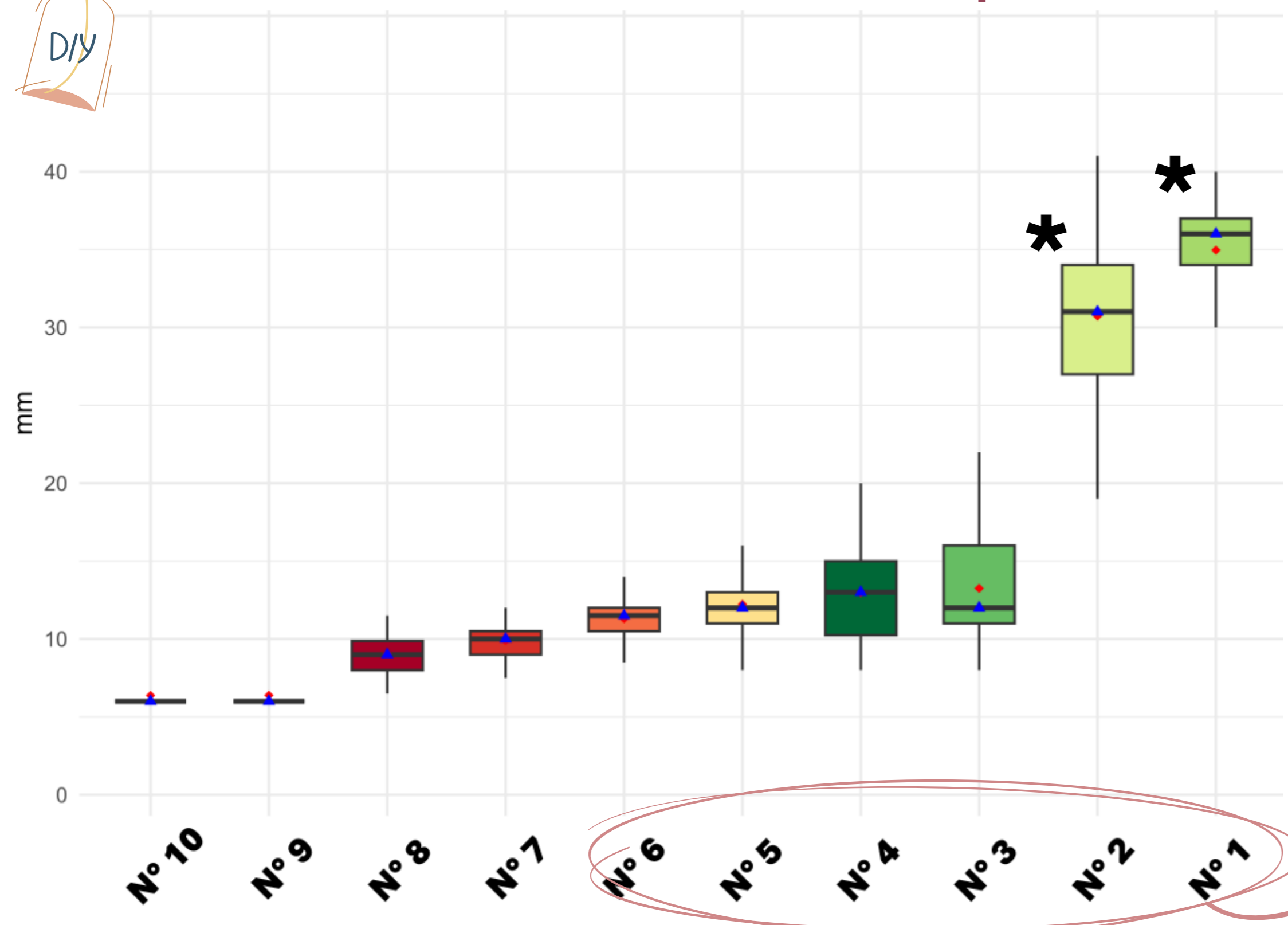
Resistance to commercial product

Figure 2. Box plot of the data showing the distribution of inhibition zones (in millimeters) produced by different formulation. The blue triangle represent the median, and the red dot indicates the mean. From N°1 to N°6 are the millimeters produced by the inhibition of disinfectants, from N°7 to N°10 the millimeters produced by detergents (Dunn's test, Bonferroni corrected p-value < 0.05).
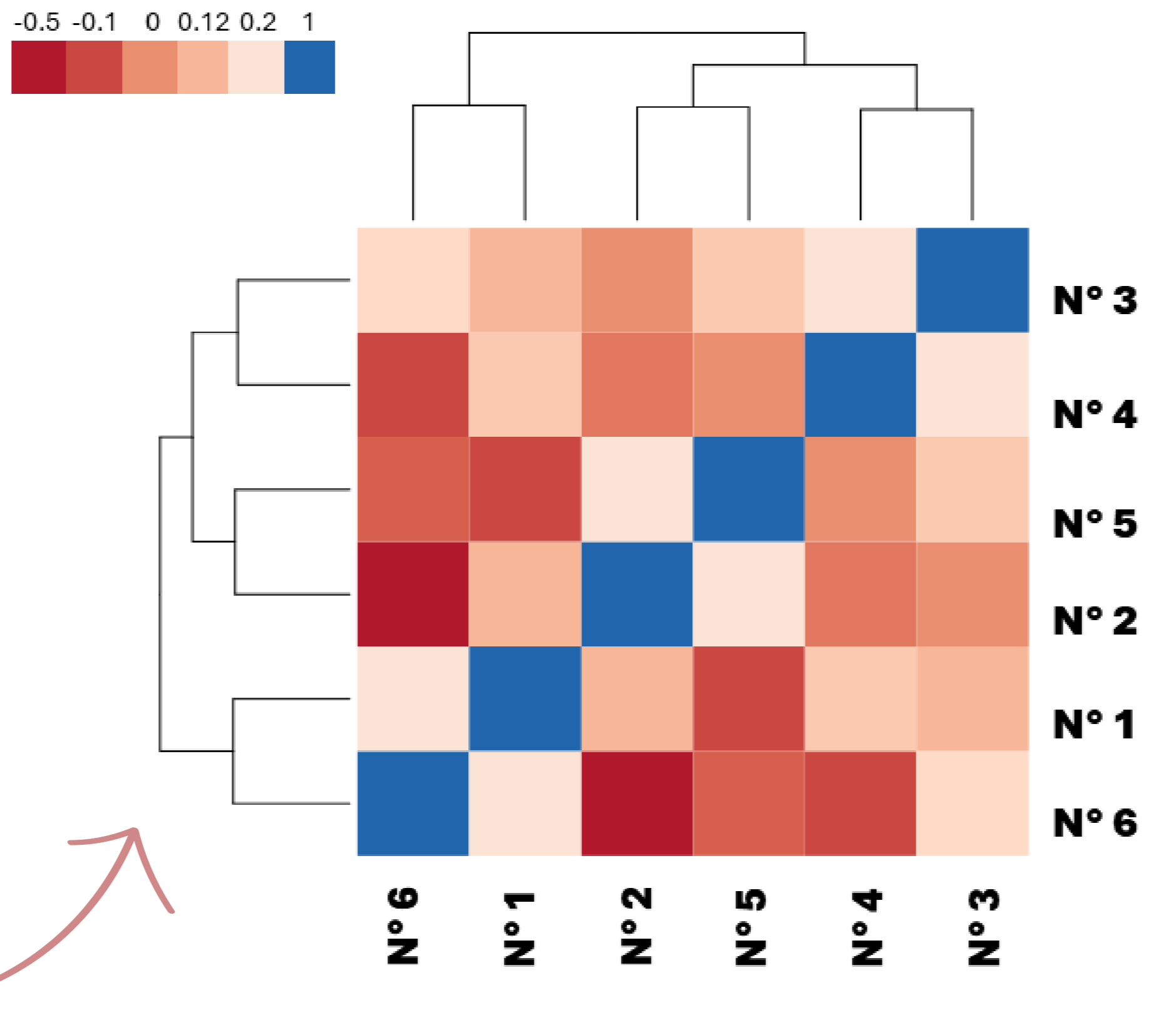

Figure 4. Heatmap with significant correlation between disinfectants (Spearman method). The colour scale ranges from blue (maximum positive correlation) to red (negative correlation). Each cell in the heatmap compares the respective row and column samples.

Considering the susceptibility of *A. butzleri*, the results show that peracetic **acid disinfectants (N°1-2) produce the greatest inhibition** (*Figure 2*).

In contrast, **the disinfectants to which they are most resistant are sodium hypochlorite-based** (N°5-6). No inhibition halo is produced with the neutral detergent (N°10).

Statistically significant results were identified on the disinfectants used (*Figure 2*). In comparison, no significant difference in resistance was found between the strains tested (*Figure 3*). There is a correlation between the inhibition caused by disinfectants with different components (*Figure 4*).

Figure 3. Box plot showing the distribution of inhibition zones (in millimeters) produced by various products against different strains. The blue triangle represent the median, and the red dot indicates the mean. Analysis of variance (ANOVA) was performed, and no significant differences were found between the groups (p > 0.05).

A. butzleri from broiler's gut · *A. butzleri* from broiler's neck skin · *A. butzleri* from slaughterhouse equipment · Reference strains

CONCLUSIONS

This study shows how different formulations can have different efficacy against the strains examined. Nevertheless, **the survival of bacteria after cleaning and disinfection procedures suggests the existence of broad-spectrum resistance mechanisms to multiple classes of molecules.**
It is assumed that differentially expressed genes are in common between disinfectant and antibiotic resistance.

Next steps…

To better understand the mechanisms of resistance, further tests will be performed in the future:
1. Evaluation of the **metabolic activity** of living cells;
2. **RNA sequencing** will be conducted to understand the genes and pathways involved. Genes in common between disinfectant and antibiotic resistance will be searched for.
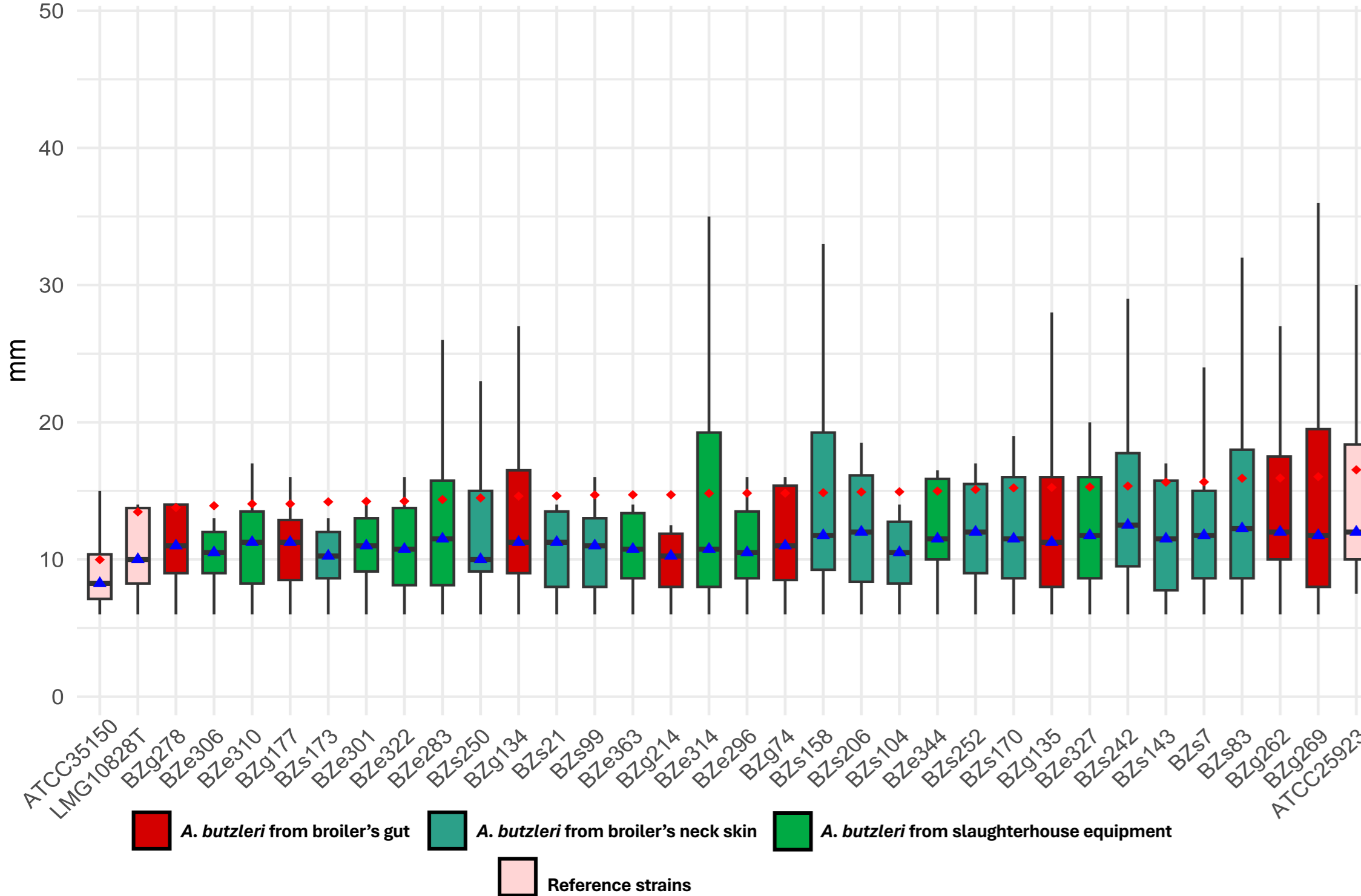
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Contact: elisabetta.chiarini@unito.it

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