

## Comparison between swabbing devices in order to analyze the microbial flora found on surfaces of community kitchens by classical microbiology and 16S rRNA amplicon sequencing



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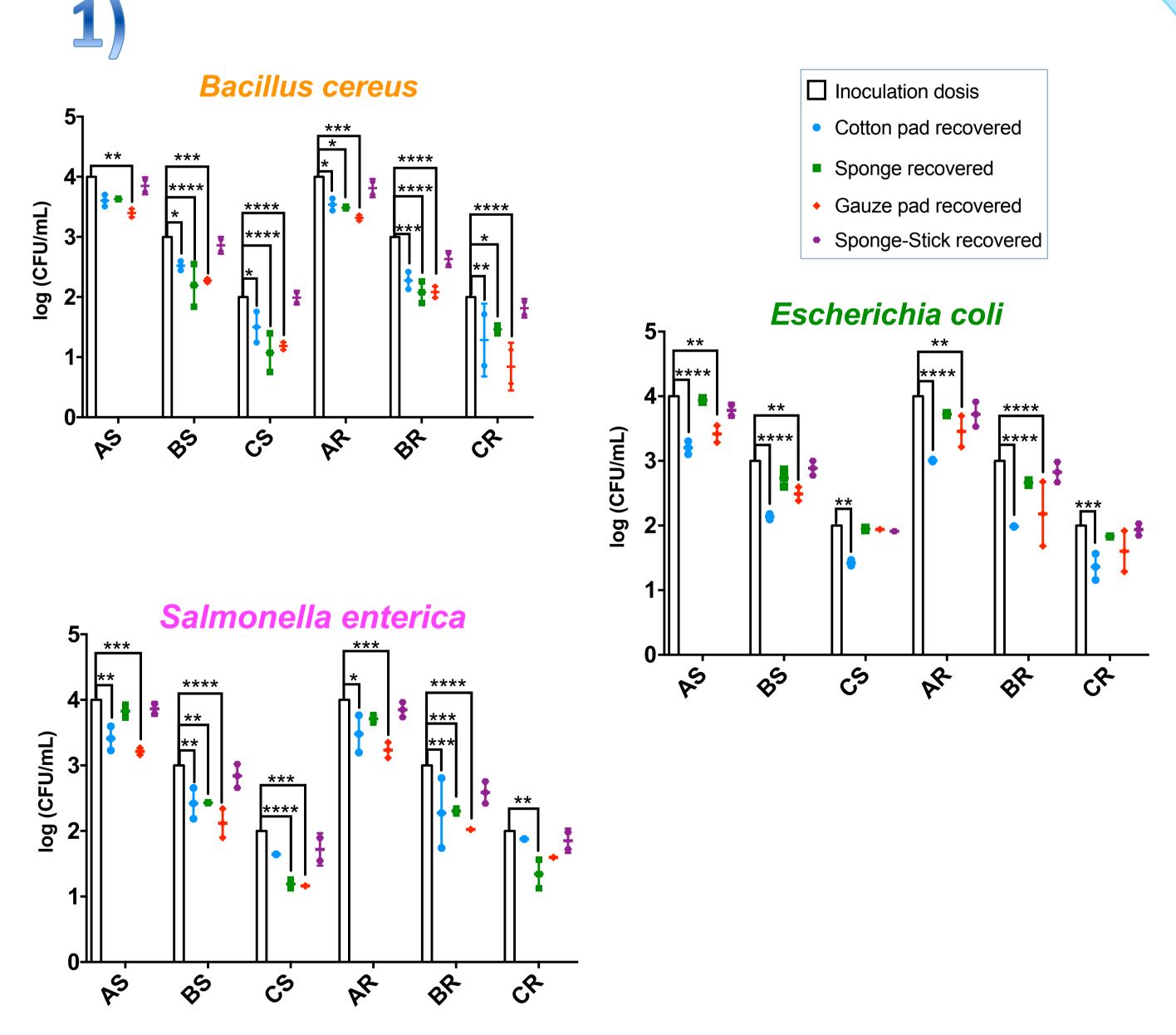
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INTRODUCTION: The work in community kitchens involves several manual steps bearing the risk of transmitting pathogenic microorganisms. This fact justifies accurate control of surfaces to prevent foodborne illnesses. In this study, the efficiency of different swabbing devices was tested in terms of recovery of microorganisms by culture methods and

## culture-independent 16S rRNA amplicon sequencing. **MATERIAL & METHODS:** Solution of 2-times diluted **chicken** (+/- 10<sup>2</sup> CFU/mL) 1 mL spread with L-spreader Left to dry for 10 min Sterile stainless steel (SS, smooth) Sterile polypropylene cutting board (PP, rough) At 3 concentrations: A: 10<sup>4</sup> CFU/mL of Bc, 10<sup>4</sup> CFU/mL of Ec, 10<sup>4</sup> CFU/mL of Sa 1mL of each of those 3 bacteria (in droplets): B: 10<sup>3</sup> CFU/mL of Bc, 10<sup>3</sup> CFU/mL of Ec, 10<sup>3</sup> CFU/mL of Sa **Bacillus cereus** ATCC 13061 (Bc) C: 10<sup>2</sup> CFU/mL of Bc, 10<sup>2</sup> CFU/mL of Ec, 10<sup>2</sup> CFU/mL of Sa Escherichia coli (Ec) D: Only chicken Salmonella enterica ssp. enterica serovar Enteriditis (Sa) Left to dry for 10 min + Maximum Recovery Diluent Oxoid CM0733 4 sterile swabbing devices: Mercurochrom Sponge-Sticks 3M™ SSL100 Cotton pads *colruyt* Gauze pads Mercurochrome **Sponges** (with Neutralizing Buffer) *3M™ HS10NB2G* 1) Culture method: 2) V1-V3 16S rRNA amplicon sequencing: PCA Bio-Rad 356-4475 DNA extraction Qiagen Blood & Tissue kit, Cat.No/ID: 69506 MYP Oxoid CM0929 + SR0047+ SR0099 Illumina MiSeq Rapid'E.coli 2 Bio-Rad 355-5299 Analysis by mothur Rapid'Salmonella Bio-Rad 356-3961 **RESULTS: CULTURE METHOD RESULTS: 16S rRNA AMPLICON SEQUENCING**



Figures 1, 2 and 3. Comparison between inoculation dosis and recovery numbers of Bacillus cereus, Escherichia coli and Salmonella enterica, respectively, by the use of different swabs and different concentrations.

(AS/AR: concentration A (10<sup>4</sup> CFU/mL) on the smooth/rough surface; BS/BR: concentration B (10<sup>3</sup> CFU/mL) on the smooth/rough surface; CS/CR: concentration C (10<sup>2</sup> CFU/mL) on the smooth/rough surface; CFU/mL: colony-forming unit/milliliter; \* = 0.0332; \*\* = 0.0021; \*\*\* = 0.0002; \*\*\*\* < 0.0001; †: Each sample was realized in duplicate; 2-way ANOVA and Tukey's multiple comparisons performed with Prism 7)

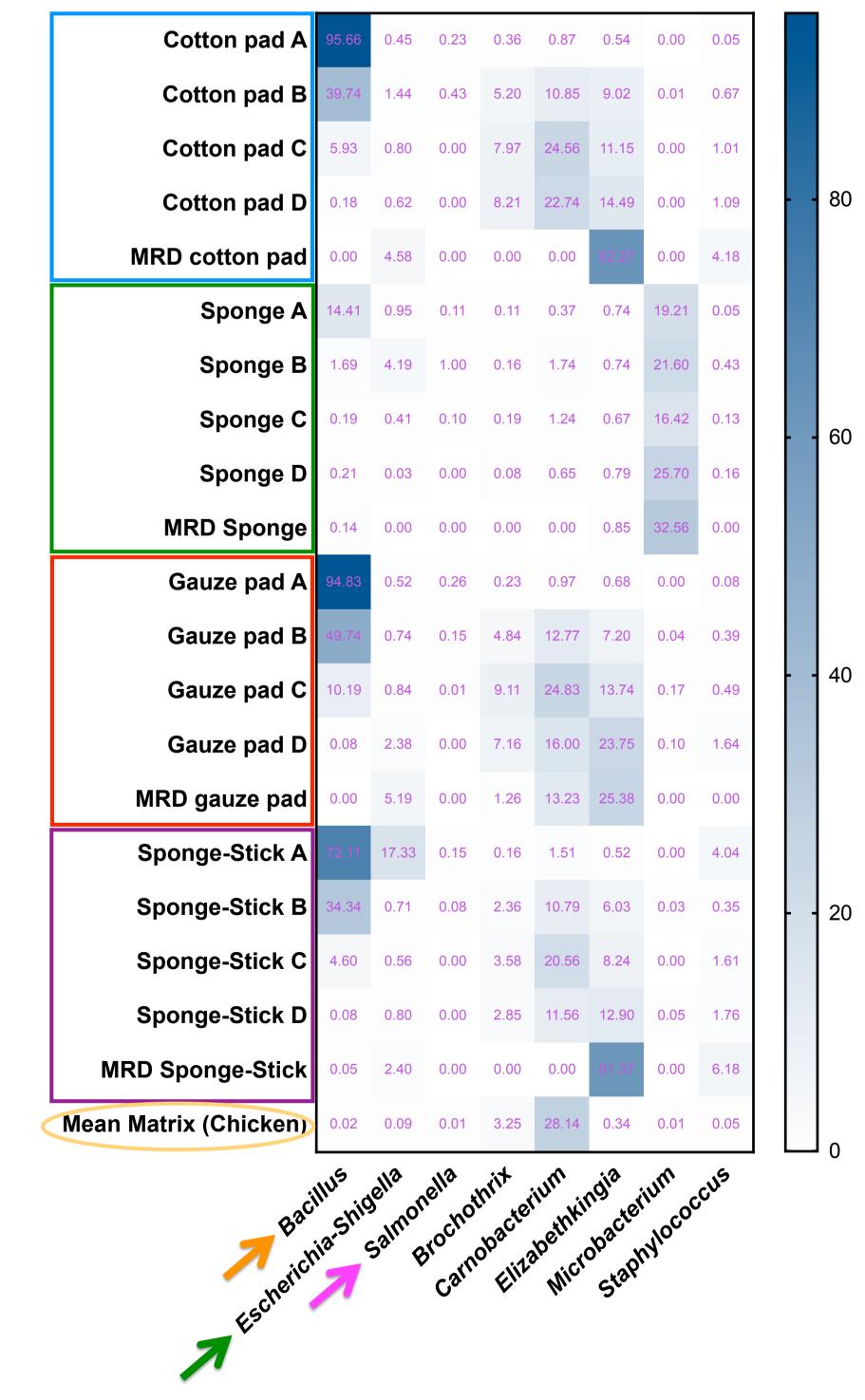


Figure 4. Relative abundance of selected bacterial genera in samples of several concentrations on surfaces recovered with different swabs.

(†: Each result was composed by the mean of 4 samples; *Heatmap realized with Prism 7*)

## **CONCLUSIONS:**

The perfect swab for kitchen analyses should recover the highest number of viable bacteria with a high population diversity from the surfaces.

Classical culture method showed best recovery numbers for the 3 inoculated bacteria with Sponge-Sticks (no significant difference between inoculation dosis and recovered number of bacteria, p > 0.1234). The 16S rRNA amplicon sequencing allows to conclude that sponge samples were loaded with Microbacterium genus (from Neutralizing buffer). Furthermore, a high relative abundance of Bacillus genus was found in cotton pad, gauze pad and Sponge-Stick samples. Salmonella genus was detected in only low proportions, whereas Escherichia genus are problematic as their DNA can be contaminants of reagents used during library creation. However, differences in recovery or enumeration with each method must be considered, as they can induce estimation bias on the initial concentration or recovered CFU/mL. Finally, low amount of DNA in controls lead to the emergence of free DNA contaminants like *Elizabethkingia* population, which can be considered as a bias.

Further studies will be performed to assess the recovery of bacteria after longer drying times in order to approach real kitchen conditions and make the final swab decision.