BSFM 22nd Conference on Food Microbiology, 21st and 22nd September 2017

Comparison between swabbing devices in order to analyse the microbial flora found on surfaces of community kitchens by classical microbiology and amplicon sequencing

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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 sampling 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 raw **chicken meat** (+/- 10^2 CFU/mL)

Sterile smooth surface — 1 mL spread with L-spreader, Air-dry for 10 min — Sterile rough surface

1 mL of each of those 3 bacteria (in droplets):
- ***Bacillus cereus*** ATCC 13061 (Bc)
- ***Escherichia coli*** (Ec)
- ***Salmonella*** Enteritidis (Sa)

3 concentrations:
A: 10^4 CFU/mL of Bc, 10^4 CFU/mL of Ec, 10^4 CFU/mL of Sa
B: 10^3 CFU/mL of Bc, 10^3 CFU/mL of Ec, 10^3 CFU/mL of Sa
C: 10^2 CFU/mL of Bc, 10^2 CFU/mL of Ec, 10^2 CFU/mL of Sa
D: Only chicken

Air-dry for 10 min

Sterile swabbing devices + Maximum Recovery Diluent *Oxoid CM0733*


Cotton pads *Colruyt*

Gauze pads *Mercurochrome*


Sponges (with Neutralising Buffer) *3M™ HS10NB2G*

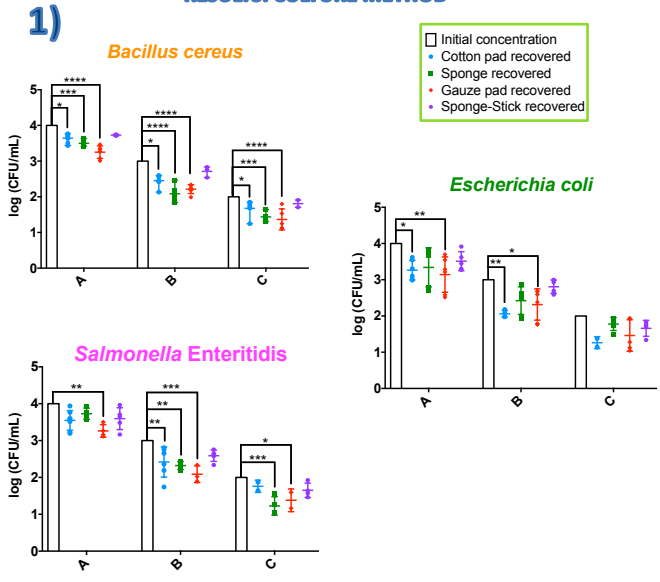

Sponge-Sticks *3M™ SSL100*

1) Culture method:
- PCA *Bio-Rad 356-4475*
- MYP *Oxoid CM0929 + SR0047+ SR0099*
- Rapid'E.coli 2 *Bio-Rad 355-5299*
- Rapid'Salmonella *Bio-Rad 356-3961*

2) V1-V3 16S rRNA amplicon sequencing:
DNA extraction *Qiagen Blood & Tissue kit, Cat.No/ID: 69506*
Illumina MiSeq
Analysis by mothur

RESULTS: CULTURE METHOD

1)



Figures 1, 2 and 3. Comparison between inoculation dosis and recovery numbers of *Bacillus cereus*, *Escherichia coli* and *Salmonella* Enteritidis, respectively, by the use of different swabs and different concentrations. Differences between recovery numbers from smooth and rough surfaces were not statistically significant (data not shown).

(A: concentration A (10^4 CFU/mL) on the surface; B: concentration B (10^3 CFU/mL) on the surface; C: concentration C (10^2 CFU/mL) on the surface; CFU/mL: colony-forming unit/milliliter; * = 0.0332; ** = 0.0021; *** = 0.0002; **** < 0.0001;
†: Each sample was realized at least in duplicate; *2-way ANOVA and Tukey's multiple comparisons performed with Prism 7*)

RESULTS: 16S rRNA AMPLICON SEQUENCING

2)

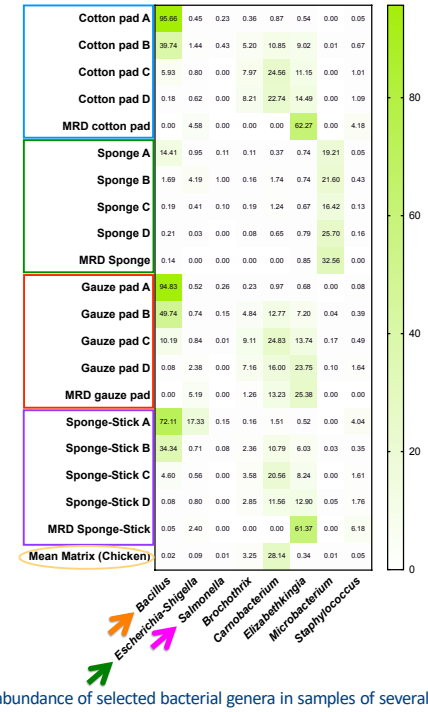


Figure 4. Relative abundance of selected bacterial genera in samples of several concentrations on surfaces recovered with different swabs.

(†: 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.
First of all, the difference of recovery from smooth or rough surfaces was not statistically significant (data not shown). 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 allowed to conclude that sponge samples were loaded with *Microbacterium* genus (from Neutralising 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 amounts of DNA in controls lead to the emergence of free DNA contaminants like *Elizabethkingia* population, which can be considered as a bias. In the end, these results attest the similarity of population diversity and good recovery numbers in cotton pad and Sponge-Stick samples, leaving the final choice to the operator.