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)
- 1 mL spread with L-spreader
- Air-dry for 10 min
- 1 mL of each of those 3 bacteria (in droplets):
  - Bacillus cereus ATCC 13061 (Bc)
  - Escherichia coli (Ec)
  - Salmonella Enteritidis (Sa)
- Air-dry for 10 min
- Sterile swabbing devices + Maximum Recovery Diluent Oxoid CM0733
- Cotton pads Gilray
- Gauze pads Mercurochrome
- Sponges (with Neutralising Buffer) JM™ HS10N820
- Sponge-Sticks JM™ SS100

RESULTS: CULTURE METHOD

1) Initial concentration
- Culture on Bio-Rad 356-4475
- MYP Oxoid CM0929 + SR0047+ SR0099
- Rapid™ E.coli 2 Bio-Rad 355-5299
- Rapid™Salmonella Bio-Rad 356-3961

2) Comparison between swabbing devices in order to analyse the surface

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

RESULTS: 16S rRNA AMPLICON SEQUENCING

1) DNA extraction Qiagen Blood & Tissue kit, Cat. No/ID: 69506
- Illumina MiSeq
- Analysis by mothur

2) V1-V3 16S rRNA amplicon sequencing:

- Comparison of bacterial genera in samples of several concentrations on surfaces recovered with different swabs.
- Figures 1, 2 and 3. Comparison between inoculation dose 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).
- Figure 4. Relative abundance of selected bacterial genera in samples of several concentrations on surfaces recovered with different swabs.

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 dose and recovered number of bacteria, p > 0.1234). The 16S rRNA amplicom 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.