

Antimicrobial resistance, adhesion and biofilm formation ability of *Salmonella* strains isolated from the French pig and pork industry

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Introduction

Salmonella remains the most frequently detected causative agent in the food-borne outbreaks reported in 2013 in European Union (22.5 % of total outbreaks) (EFSA, 2015). Pork and products thereof are commonly implicated in *Salmonella* outbreaks. *Salmonella* Derby and *Salmonella* Typhimurium are the two main prevalent serovars in the French pig and pork industry.

Objective

This study aims at characterizing a collection of *S. Typhimurium* and *S. Derby* isolated along the pork meat production chain in France for various phenotypic traits i.e. antimicrobial resistance patterns, motility, adhesion and biofilm formation on abiotic surfaces.

Material & Methods

Bacterial strains

- 16 *S. Typhimurium* (including the ATCC[®] 14028 strain) and 15 *S. Derby* strains, isolated along the pork meat production chain in France

Motility tests

- Twitching, swarming and swimming motilities were evaluated using nutritive media with 1%, 0.5% (plus 0.5% glucose) and 0.25% agar concentration, respectively.

Adhesion and biofilm formation

- Adhesion ability to two abiotic surfaces, i.e. stainless steel (SS) and high-density polyethylene (HDPE), was evaluated after 3h-incubation in physiological salt solution at 37°C (ACTIA, 2015).
- Biofilm formation was determined by growing the bacterial cultures for 48 h in 96-well microtiter plates followed by confocal laser scanning microscopy observations.

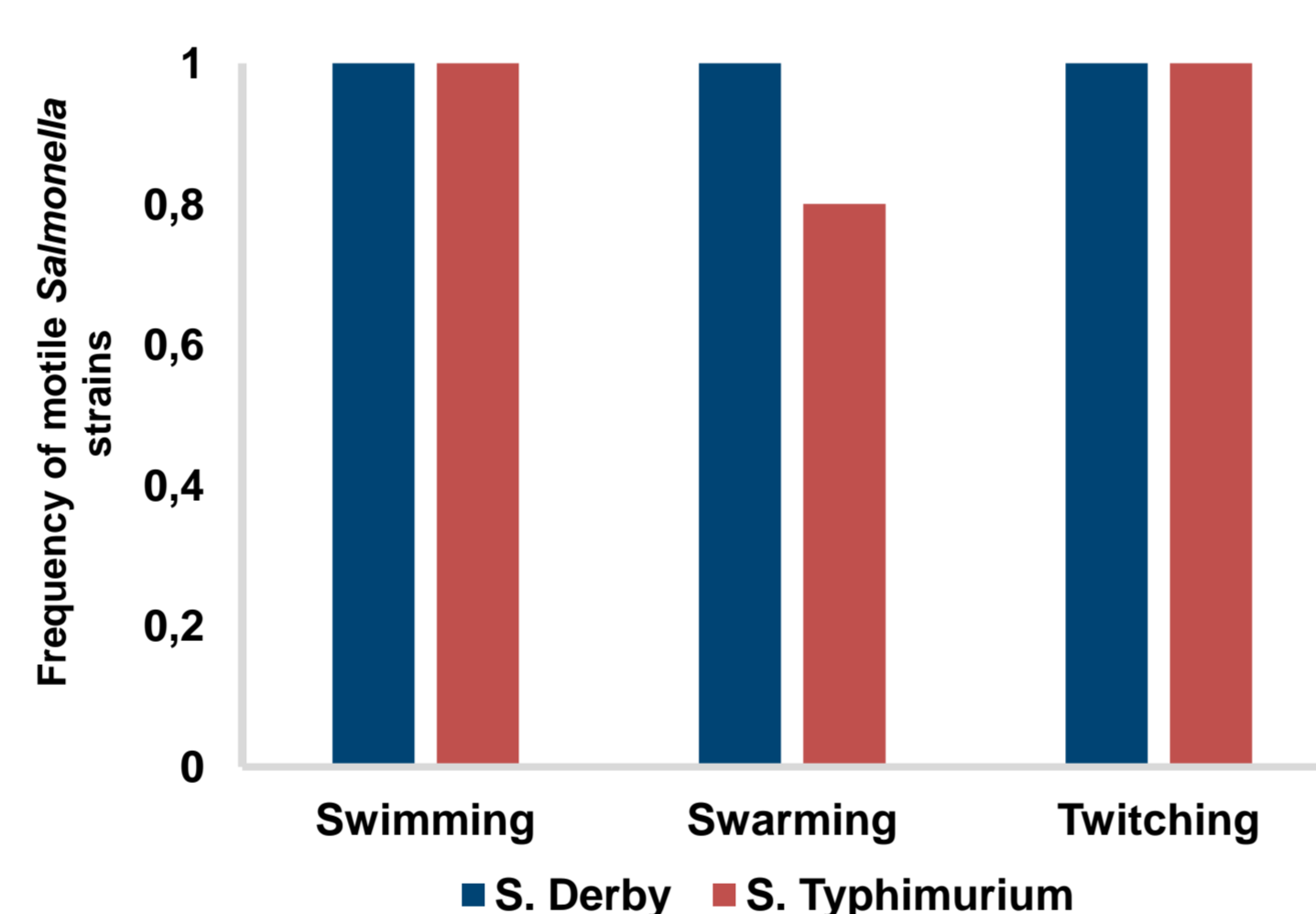
Antimicrobial resistance patterns

- Antibiotic susceptibility tests were performed using a standard microdilution method with the Sensititre[®] system on EUMVS-2 microtiter plates (Trek Diagnostic Systems, England).
- Susceptibility to biocides commonly found in disinfectant formulations in the French pork industry, was evaluated using a standard microdilution method (Soumet *et al.*, 2005).

Results

The majority of *Salmonella* strains displayed efficient motility

Figure 1 : Frequency of *S. Typhimurium* and *S. Derby* strains displaying active swimming, swarming and twitching motility



High ability to adhere and form biofilm on abiotic surfaces

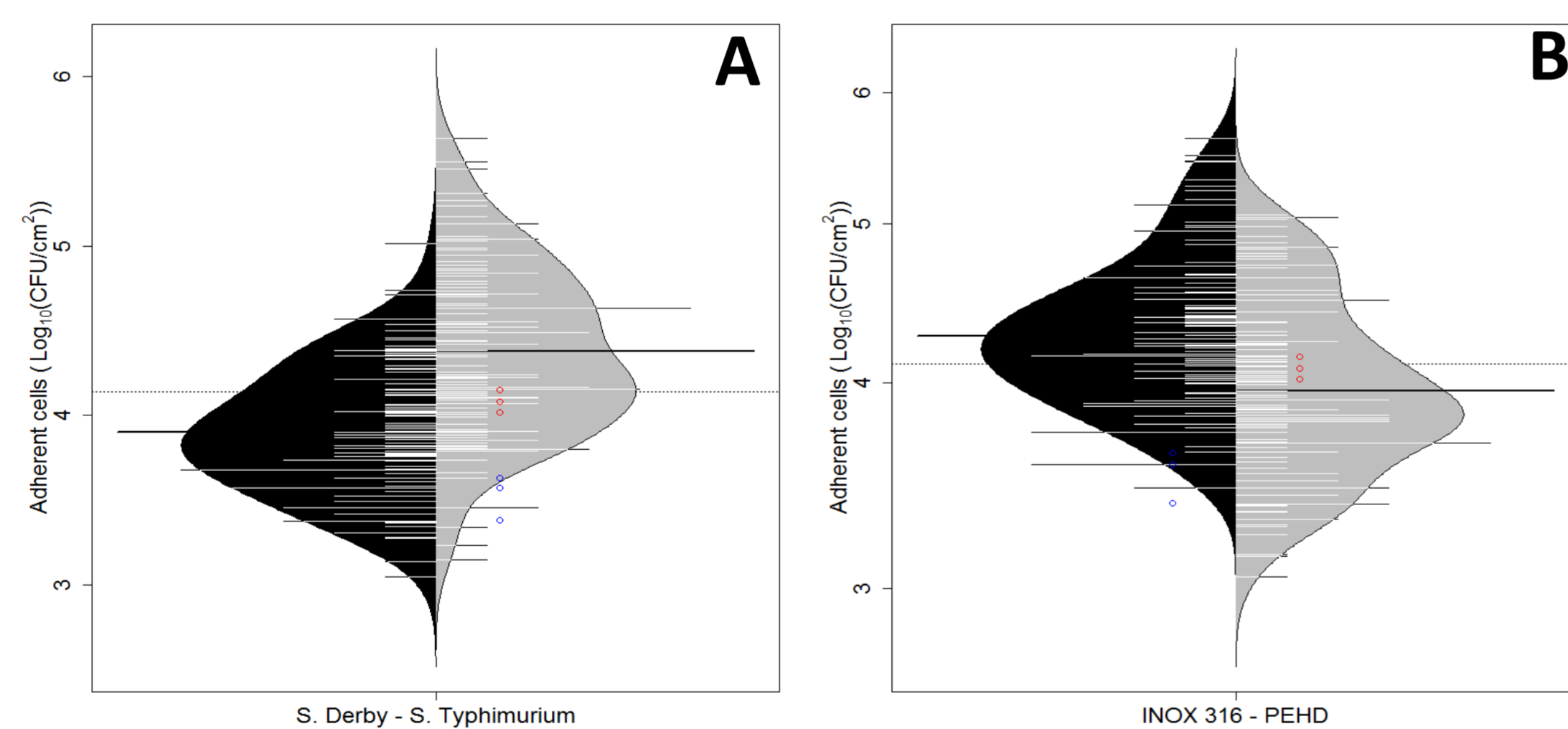


Figure 2 : Levels of adherent *Salmonella* cells according to (A) the serovar Derby and Typhimurium; (B) the type of surfaces SS and PEHD (non-polar surfaces with $\theta_{\text{water}} = 95^\circ$). Levels obtained for the ATCC[®] 14028 are displayed in red and blue for SS and PEHD, respectively.

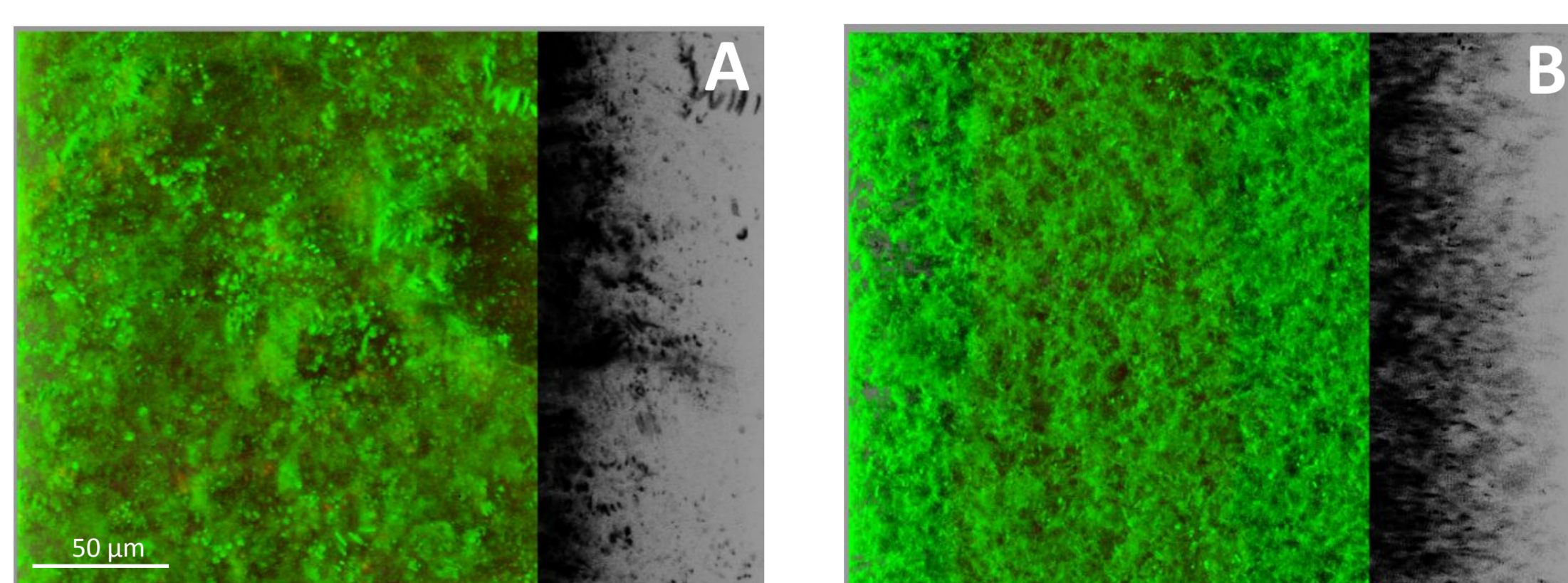


Figure 3 : Confocal laser scanning microscopy observations of *Salmonella* biofilms on 96-well microplate (A: *S. Typhimurium* strain ; B : *S. Derby* strain)

Salmonella strains exhibit frequent multidrug resistances

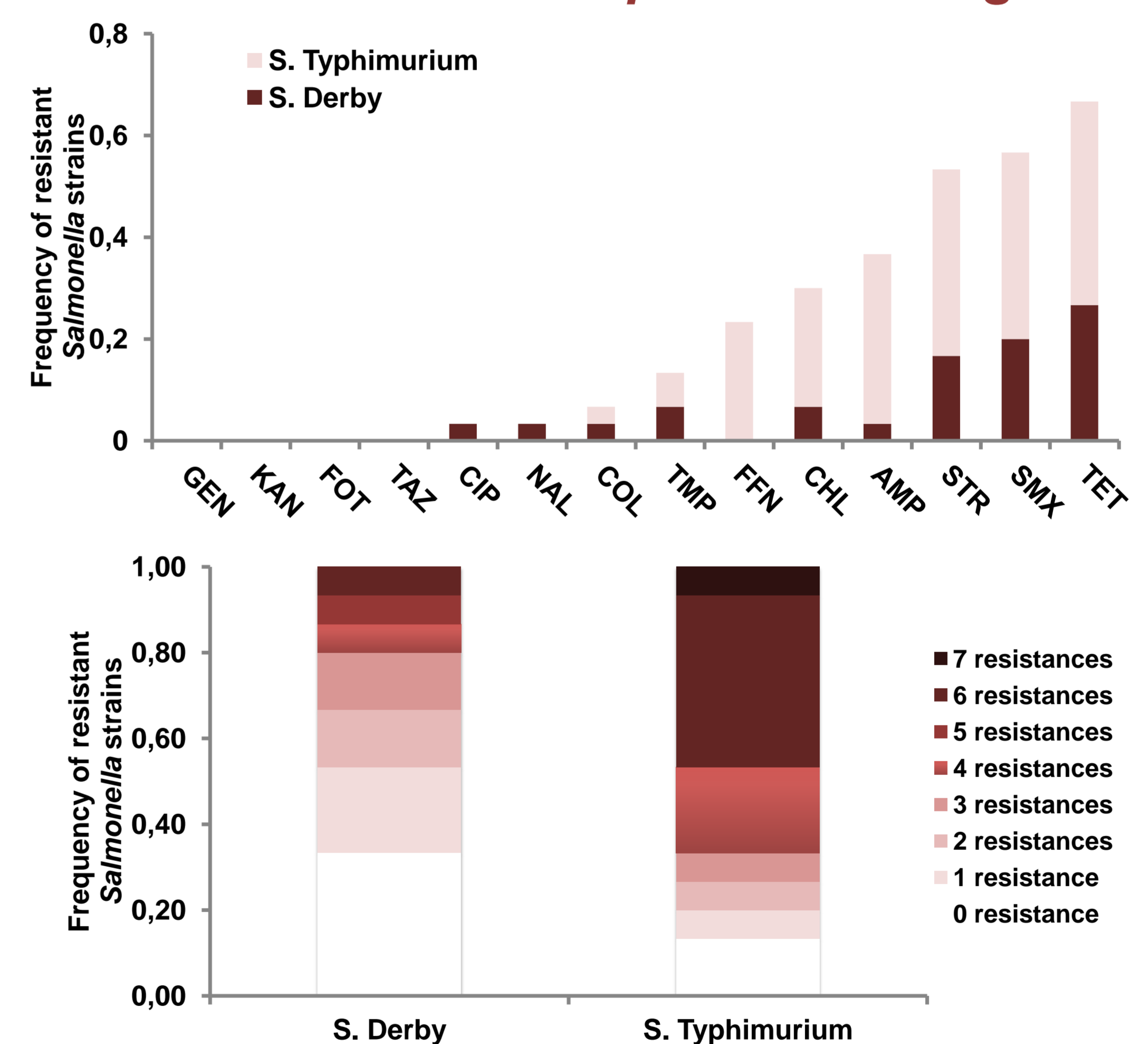


Figure 4 : Frequency of antibiotic resistances in *S. Typhimurium* and *S. Derby* strains
GEN : gentamycin, KAN : kanamycin, FOT : cefotaxime, TAZ : ceftazidime, CIP : ciprofloxacin, NAL : nalidixic acid, COL : colistin, TMP : triméthoprim, FFN : florfenicol, CHL : chloramphenicol, AMP : ampicilline, STR : streptomycine, SMX : sulfaméthoxazole, TET : tetracycline

Table 1 : Biocide susceptibility of *S. Typhimurium* and *S. Derby* strains

Didecyl Dimethyl Ammonium Chloride (DDAC), benzalkonium chloride (BC), hydrogen peroxide (H_2O_2), sodium hypochlorite (NaClO), peracetic acid ($\text{C}_2\text{H}_4\text{O}_3$), Galox Horizon (end-use formulation with DDAC and glutaraldehyde)

Tolerance thresholds ($\mu\text{g/ml}$) : DDAC > 6, Galox Horizon > 4, BC > 32, H_2O_2 > 40, NaClO > 600, $\text{C}_2\text{H}_4\text{O}_3$ > 0.015

| <i>Salmonella</i> strains (n = 30) | DDAC | Galox Horizon | BC | H_2O_2 | NaClO | Peracetic acid |
|-------------------------------------|------|---------------|-------|------------------------|---------|----------------|
| CMI ($\mu\text{g/ml}$) | 4-8 | 3-6 | 24-64 | 30-40 | 300-600 | 0.015 |
| nTolerant/nTotal | 6/30 | 11/30 | 1/30 | 0/30 | 0/30 | 0/30 |
| ATCC 14028 CMI ($\mu\text{g/ml}$) | 1.5 | 1.5 | 12 | 30 | 400 | 0.015 |

Conclusion

- S. Derby* and *S. Typhimurium* strains isolated from the French pig and pork industry showed great potential to persist in the food factory environment
- Other studies are necessary to increase our understanding of the determinants and mechanisms involved in the predominance and persistence of these two specific serovars in pigs



Références

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