



Detection of *Salmonella* serotypes adapted to diverse stresses in poultry meat at the processing level in Portugal

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INTRODUCTION AND PURPOSE

- Non-typhoidal *Salmonella* causes frequently foodborne infections mainly associated with the consumption of eggs/poultry products^{1,2}, being the foodborne pathogen targeted by European Union actions (*Salmonella* control or monitoring programs)³⁻⁵.
- A decreasing trend in human salmonellosis, particularly associated with *Salmonella* Enteritidis, have been observed in European Union (EU) due to successful control programs at the avian production level. Nevertheless, expansion of less frequent serotypes and/or certain well-adapted clones has been reported in diverse geographical regions^{1,2}.
- The effectiveness of control practices (e.g. organic acids in feed/biocides) on the elimination of *Salmonella*, and particularly of EU targeted serotypes (*S. Enteritidis*, *S. Typhimurium* and *S. 4,5,12:-*), in poultry has been scarcely explored.
- In this study, we investigated the presence of *Salmonella*, using conventional and molecular approaches, and characterized their clinically-relevant serotypes, among fresh chicken-meat samples at poultry processing level in Portugal.**

METHODS

Sampling strategy. Fifty-three pooled chicken-meat samples (each sample corresponding to neck skin from 10 carcasses of the same batch) obtained after slaughter and chilling, corresponding to 29 Portuguese producers, were collected in 2018 during spring and summer periods. All the samples were collected in sterile plastic bags, transported refrigerated and processed in the same day at the laboratory.

Detection of *Salmonella* by the standard cultural method. Samples (25g) were pre-enriched (37°C/16-18h) in Buffered Petone Water (BPW) following ISO 6579-1:2017 standard⁶. A PCR assay targeting *invA* gene⁷ confirmed the suspected *Salmonella* colonies.

Detection of *Salmonella* by a molecular method. A PCR targeting *Salmonella invA* gene was applied directly in the pre-enrichment and enrichment broths (BPW broth and MKTn broth). DNA was extracted using 1 mL of each enrichment by a boiling protocol: i) centrifugation (13000 g, 5 min) and resuspension of the pellet in 200 µL of saline ii) centrifugation (13000 g, 5 min) and resuspension of the pellet in 200 µL of Triton X-100 (1%) and then repeat the step with saline i); iii) centrifugation (13000 g, 5 min) and resuspension of the pellet in 100 µL of ultrapure water; iv) boiling (100 °C, 20 min) and final centrifugation (13000 g, 5 min). The final supernatant contained the DNA.

Detection and characterization of *Salmonella* clinically-relevant serotypes. Search of EU targeted serotypes (*Enteritidis*/typhimurium/4,5,12:-) and their antibiotic and metal resistance markers were performed by PCR applied to isolates recovered from positive samples, as described⁸⁻¹⁰. Minimum growth pH was assessed by broth-microdilution ($\text{pH}_{\text{min}}=2.0-6.5/16\text{h}-20\text{h}/2\text{h}/37^\circ\text{C}$) and minimum survival pH by plating wells without growth (Muller-Hinton-II/24h-48h+2h/37°C). Determination of minimum inhibitory concentration (MIC) and bactericidal (MBC) to Peracetic Acid (PAA; 5-90 mg/L; adjusted pH=4.5) were also evaluated by broth-microdilution.

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RESULTS

- Salmonella* was detected in two samples of fresh chicken-meat (4%) obtained from different poultry farms in both seasons, using the standard cultural approach (**Figure 1**). In addition, the presence of *Salmonella* was confirmed by the PCR-assay, but only with total DNA obtained from the selective enrichments.
- All the isolates (n=6) from the spring sample belonged to a non-H₂S-producing *S. 1,4,[5],12:-*, which presented the typical antibiotic (*bla*_{TEM}+*strA*-*strB*+*suI2*+*tetB*) and metal (*pcoD*+*silA*+*arsB1*+*merA*) resistance features of clones currently circulating in Europe, the widespread clinically-relevant “European clone”^{9,10}. The isolates (n=3) of *Salmonella* obtained from the summer sample were confirmed as belonging to *S. Enteritidis*. Both detected serotypes are currently covered by the EU food Regulation³ as a food safety microbiological criterion for fresh poultry meat. Most of the isolates grew at minimal pH=4 and survived until pH=3.5 and showed a MIC to PAA between 50–60 mg/L and a MBC between 60–70mg/L, below the recommended level for disinfection in food/feed industry (100–1000mg/L for Product-Type PT 4: PT4d and PT4e)¹¹.

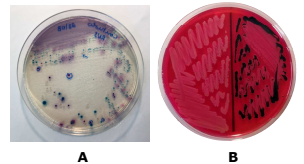


FIGURE 1:

A) *Salmonella* colonies on CHROMagar™ *Salmonella* plus
B) *Salmonella* colonies on XLD agar: atypical non-H₂S-producing (left)

TABLE 1. Characterization of *Salmonella* isolates recovered from chicken meat samples, Portugal

Serotype (no. isolates)	No. Samples / No. Poultry-producers / Season	Antibiotic resistance phenotype / genotype ^a	Metal tolerance genes ^b	Minimum Growth pH	Minimum Survival pH	PAA-MIC (mg/L)	PAA-MBC (mg/L)
1,4,[5],12:- (n=6)	1 sample / 1 poultry-producer / Spring	ASSu(T) / <i>bla</i> _{TEM} , <i>strA</i> - <i>strB</i> , <i>suI2</i> , [tet(B)]	<i>pcoD</i> , <i>silA</i> , <i>arsB</i> , (<i>merA</i>)	4,01	3,53 - 4,01	50 - 60	60 - 70
Enteritidis (n=3)	1 sample / 1 poultry-producer / Summer	Susceptible		4,01 - 4,55	4,01	50 - 60	60 - 70

AMP: ampicillin; STR: streptomycin; SUL: sulfamethoxazole; TET: tetracycline; PAA: peracetic acid
^a Variable antibiotic resistance phenotypes and genotypes are presented between brackets. ^b Metal tolerance genes that were not observed in all the isolates are presented between brackets.

CONCLUSIONS

- This study showed a low occurrence of *Salmonella* in raw chicken carcasses in a poultry production facility, confirmed by both cultural and molecular approaches, suggesting successful control practices in avian production.
- Molecular detection methods as PCR could be alternative to laborious and slower conventional approaches, with the possibility for further improvements in sensitivity at pre-enrichment step.
- The detection of two serotypes of public health significance with ability to grow under diverse stresses alerts for the need to evaluate current biosafety measures to prevent the spread of these pathogens in the poultry production through the final consumer.