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

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P1591

**INTRODUCTION AND PURPOSE**

- Non-typhoidal *Salmonella* causes frequent foodborne infections mainly associated with the consumption of egg/poultry products¹, being the foodborne pathogen targeted by European Union actions (*Salmonella* control or monitoring programs)²⁴. 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 regions²⁴.
- The effectiveness of control practices (e.g., organic acids in feed/bioceods) on the elimination of *Salmonella*, and particularly of EU targeted serotypes (S. Enteritidis, S. Typhimurium and S. 4,5,12:i-,l), 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 (20 samples corresponding to each of the 15 carcasses of the same batch) and 56 egg samples belonging to 28 chickens (2 per chicken), and corresponding to 14 chicken producers, were collected in 2018 during spring and summer periods. All the samples were collected in sterile plastic bags, transported refrigerated and processed the same day at the laboratory.

**Detection of *Salmonella* by the standard cultural method**. *Salmonella* spp were pre-enriched (PET) in Buffered Peptone Water (BPW) for 1-2.5 hours at 37°C. A 1 mL loop was used to transfer the sample to a NNN broth enrichment (10% of the sample) and incubated at 37°C for up to 18-24 hours. Thoroughly shaking of the broth (every 3 hours) was performed. S. Enteritidis, S. Typhimurium and S. Virchows were identified by using biochemical tests (entero, citrulline, oxidase and methyl red) and susceptibility to nalidixic acid. The typical colonies on XLD agar were confirmed by the standard cultural method.

**Detection of *Salmonella* by a molecular method.** A PCR targeting 16S rRNA gene was applied directly to the pre-enrichment broth samples using the *Salmonella* Multiplex PCR Kit (Idaho Technology, USA). DNA was extracted using 1 mL of each enrichment by a boiling protocol: i) centrifugation (13,000 g, 5 min) and resuspension of the pellet in 200 µL of saline ii) centrifugation (13,000 g, 5 min) and resuspension of the pellet in 200 µL of saline. DNA extraction from the pre-enrichment broths was then performed using a boiling protocol: i) centrifugation (13,000 g, 5 min) and resuspension of the pellet in 200 µL of saline ii) centrifugation (13,000 g, 5 min) and resuspension of the pellet in 200 µL of saline. DNA extraction from the pre-enrichment broths was then performed using a boiling protocol: i) centrifugation (13,000 g, 5 min) and resuspension of the pellet in 200 µL of saline ii) centrifugation (13,000 g, 5 min) and resuspension of the pellet in 200 µL of saline.

**Detection and characterization of *Salmonella* clinically-relevant serotypes.** Isolates (n=3) of *Salmonella* Enteritidis, S. Typhimurium and S. Virchows were confirmed by both cultural and molecular approaches, suggesting successful control practices in avian production.

**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 with only total DNA obtained from the selective enrichments.
- All the isolates (n=6) from the spring sample belonged to a non-H₅-S-producing S. 1,4,[5],12:i-; which presented the typical antibiotic (blaTEM⁺, strR⁺, strS⁺, sul₂⁻, tetB⁺) and metal (pcd⁺, sul⁺, arsB/sul₁smX₄⁺) resistance features of clones currently circulating in the European Union, the widespread clinically-relevant "European clone"²⁹. The isolates (n=3) of *Salmonella* obtained from the summer sample were confirmed belonging to S. Enteritidis. Both detected serotypes are currently covered by the EU food Regulation 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 60–70 mg/L below the recommended level for disinfection in food/feed industry (100-1000 mg/L for Product-Type PT 4: PT4d and PT4e)²⁹.

**REFERENCES**


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

<table>
<thead>
<tr>
<th>Serotype (no. Isolates)</th>
<th>No. Samples</th>
<th>No. Poultry-producers</th>
<th>Season</th>
<th>Antibiotic resistance phenotype / genotype</th>
<th>Metal tolerance gene(s)</th>
<th>Minimum Growth pH</th>
<th>Minimum Survival pH</th>
<th>PAA-MIC (mg/L)</th>
<th>PAA-MBC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella Enteritidis</em></td>
<td>1 sample / Poultry-producer / Spring</td>
<td>1</td>
<td>1</td>
<td>Aminoglycosides (araB, aac(6′)-Ib-cr), macrolides (ermA), tetracyclines (tetA⁺), sulphonamides (sul₂⁻)</td>
<td>pcd⁺</td>
<td>4.0</td>
<td>6.0</td>
<td>50–60</td>
<td>60–70</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>1 sample / Poultry-producer / Summer</td>
<td>1</td>
<td>1</td>
<td>Susceptible</td>
<td></td>
<td>4.5–6.5</td>
<td>6.0</td>
<td>50–60</td>
<td>60–70</td>
</tr>
</tbody>
</table>

**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.