29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

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

Sofia Ribeiro[1], Joana Mourão[1], Andreia Rebelo[1,2], Carla Novais[1], Luísa Peixe[1], Patrícia Antunes[1,3]

[1] UCIBIO/REQUIMTE. Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Portugal; [2] Escola Superior de Saúde, Instituto Politécnico do Porto, Portugal; [3] Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Portugal

* patriciaantunes@fcna.up.pt

P1591

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:i:-), 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 (37ºC/16-18h) in Buffered Petpone Water (BPW) following ISO 6579-1:2017 standard[6]. A PCR-assay targeting *invA* gene[7] 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 enrichments broths (RVS broth and MKTTn 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 ºC, 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:i:-) and their antibiotic and metal resistance markers were performed by PCR applied to isolates recovered from positive samples, as described[8-10]. Minimum growth pH was assessed by broth-microdilution (pH_{int}=2.0-6.5/16h-20h±2h/37ºC) and minimum survival pH by plating wells without growth (Muller-Hinton-II/24h-48h±2h/37ºC). 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.

REFERENCES

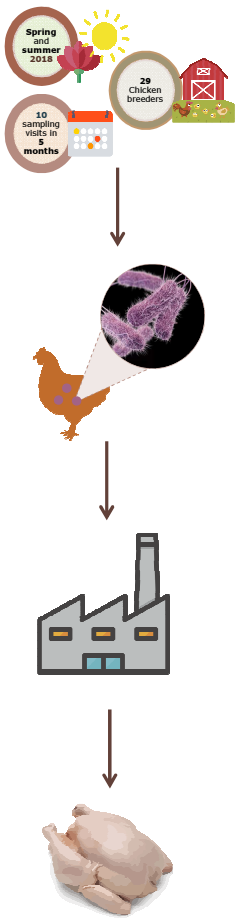
1. EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 2018;16(12):5500, 262 pp. https://doi.org/10.2903/j.efsa.2018.5500
2. Antunes P, Mourao J, Campos J, Peixe L. Salmonellosis: the role of poultry meat. CMI Themed Issue Poultry and human infections. *Clin Microbiol Infect* 2016; 22(2): 110-21. Review.
3. Commission Regulation (EU) No 1086/2011 of 27 October 2011 amending Annex II to Regulation (EC) No 2160/2003 of the European Parliament and of the Council and Annex I to Commission Regulation (EC) No 2073/2005 as regards *salmonella* in fresh poultry meat. Official Journal of the European Union 281.
4. Commission Regulation (EC) No 200/2012 of 8 March 2012 concerning a Union target for the reduction of *Salmonella* enteritidis and *Salmonella* typhimurium in flocks of broilers, as provided for in Regulation (EC) No 2160/2003 of the European Parliament and of the Council. OJ L 71, 9.3.2012, p. 31–36.
5. Regulation (EC) No 2160/2003 of the European Parliament and of the Council and Regulation of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents. OJ L 325, 12.12.2003, p. 1–15.
6. International Organization for Standardization, ISO 6579-1:2017. Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of *Salmonella* -- Part 1: Detection of *Salmonella* spp.
7. Pritchett, L.C., Konkel, M.E., Gay, J.M., Besser, T. E. Identification of DT104 and U302 phage types among *Salmonella enterica* serotype Typhimurium isolates by PCR. *J. Clin Microbiol.* 2000; 38: 3484-3488.
8. Tennant SM, Diallo S, Levy H, Livio S, Sow SO, Tapia M, Fields PI, Mikoleit M, Tamboura B, Kotloff KL, Nataro JP,Galen JE, Levine MM. Identification by PCR of non-typhoidal *Salmonella enterica* Serovars associated with invasive infections among febrile patients in Mali. *PLoS Negl Trop Dis* 2010; 4:e621.
9. Mourão J, Novais C, Machado J, Peixe L & Antunes P. Metal tolerance in emerging clinically relevant multidrug-resistant Salmonella enterica serotype 4,[5],12:i:- clones circulating in Europe. Int J Antimicrob Agents 2015; 45: 610-6.
10. Campos J, Cristino L, Peixe L, Antunes P. MCR-1 in multidrug-resistant and copper-tolerant clinically relevant *Salmonella* 1,4,[5],12:i:- and *S.* Rissen clones in Portugal, 2011 to 2015. Euro Surveill. 2016 Jun 30;21(26).
11. Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products Evaluation of active substances Assessment Report: Peracetic acid. Finland. 2015.

AKNOWLEDGMENTS

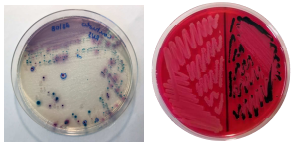
This work was supported by **Investigação Jovem da Universidade do Porto** – Projeto PP-IJUP2017-SOJA DE PORTUGAL-27 and by the **Applied Molecular Biosciences Unit- UCIBIO** which is financed by national funds from FCT/MCTES (**UID/Multi/04378/2019**) and NORTE-01-0145-FEDER-000011 – *Qualidade e Segurança Alimentar — uma abordagem (nano) tecnológica*.

Sofia Ribeiro was supported by a fellowship from Fundação Amadeu Dias. Joana Mourão was supported by a post-doc fellowship from project NORTE-01-0145-FEDER-000024. Andreia Rebelo was supported by a PhD fellowship (grant SFRH/BD/137100/2018) from Fundação para a Ciência e a Tecnologia (FCT).

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-H_2S-producing *S.* 1,4,[5],12:i:-, which presented the typical antibiotic (bla_{TEM}+*strA-strB*+*sul2*±*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 Regulation[3] 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].



A B

FIGURE 1:

A) *Salmonella* colonies on CHROMagar™ Salmonella plus

B) *Salmonella* colonies on XLD agar: atypical non-H_2S-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:i:- (n=6)	1 sample / 1 poultry-producer /Spring	ASSu(T) / bla_{TEM}, *strA-strB*, *sul2*, [*tet(B)*]	*pcoD*, *silA*, *arsB*, (*merA*)	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.**