Serotype distribution and antimicrobial resistance of clinical *Salmonella enterica* isolated in Portugal between 2014 and 2017

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INTRODUCTION

Food-borne salmonellosis is an important public health concern worldwide.

The objective of this study was to characterize the epidemiological patterns and antimicrobial resistance profile of clinical *Salmonella enterica* isolates sent to the National Reference Laboratory for Gastrointestinal Infections of the National Institute of Health (INSA), between January 2014 and December 2017.

MATERIAL and METHODS

Serotyping

- 1124 clinical isolates
- Kauffmann-White-Le Minor scheme

Antimicrobial susceptibility

- 969 clinical isolates (2015-2017)
- Disk diffusion according to EUCAST guidelines

Multiple-Locus Variable number tandem repeat Analysis (MLVA) and Whole Genome Sequencing (WGS) was performed for outbreak identification

RESULTS

- ✓ 13.6% of the strains were received in 2014, 22.8% in 2015, 29.5% in 2016, and 34.1% in 2017.
- ✓ 75.4% were isolated in faeces, 10.1% in blood, 1.1% in faeces and blood, and 3.6% in other biological products; 9.8% isolates had no information.
- ✓ 16.3% (166/1014) of the *Salmonella* isolates were associated with invasive infections.
- ✓ Salmonellosis was more frequent in the 0-5 years age group (37.5%), and males (54.8%).

Salmonella serotype diversity

- ✓ Overall, 68 different serotypes were identified between 2014 and 2017.
- ✓ The most frequent serotypes were *S.*4,5:i:-, *S.*Enteritidis, *S.*Typhimurium, *S.*Rissen, *S.*Typhi, and *S.*Stanley (Fig. 1) and accounted for 87.2% of the isolates received.
- ✓ 12.8% cases (n=144) were associated to infection by uncommon serotypes (Fig. 1).
- ✓ There was a significantly increase of *S.*Enteritidis from 2014 to 2017 (15.7% in 2014 to 37.6% in 2017) and a reduction of *S.*4,5:i:- (38.6% in 2014 to 26.6% in 2017) (Fig. 2).
- ✓ 88.6% (147/166) invasive infections were associated to non-typhoidal serotypes.
- ✓ All the 25 cases of *S.*Typhi were acquired in endemic zones.
- ✓ MLVA and NGS allowed the identification of several outbreaks of *S.*Enteritidis, particularly during 2016 and 2017.

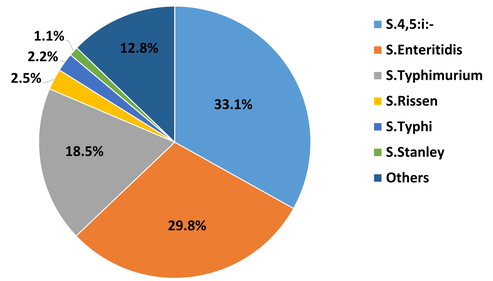

Figura 1: ***Salmonella*** **serotypes identified in 1124 clinical isolates.**

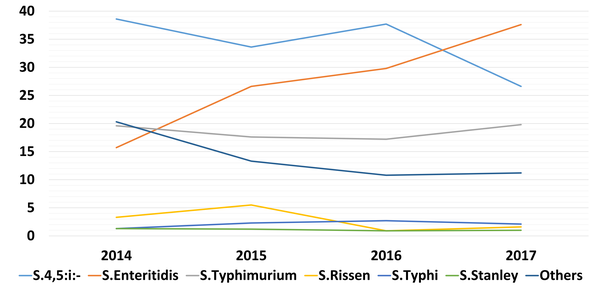

Figura 2: Trend of ***Salmonella*** **serotypes identified between 2014-2017.**

Antimicrobial susceptibility

- ✓ Overall, 71.9% of the 969 isolates showed resistance to at least one antimicrobial agent and 39.9% showed multiple antimicrobial resistance (resistance to ≥3 classes of antimicrobials).
- ✓ The highest resistance was observed in response to sulfamethoxazole, ampicillin and tetracycline, while moderate resistance was observed in response to fluoroquinolones and trimethoprim. Response to cephalosporins and to carbapenems showed a very low-level of resistance (Table 1).
- ✓ Among the most frequent serotypes, *S.*4,5:i:- and *S.*Rissen showed the highest antimicrobial resistance (>95%) (Table 2).
- ✓ Multiple antimicrobial resistances occur in either the most frequent serotypes (81.1% in *S.*4,5:i:-:, 51.1% in *S.*Typhimurium) and in the less common (100% in *S.*Saintpaul , *S.*Ohio, *S.*Blockley and *S.*Essen) (Table 2).
- ✓ The most common antibiotic resistance pattern was AMP-TET-SMX.

Table 1. Antimicrobial resistance of *Salmonella* isolated in Portugal between 2015-2017

Class of antimicrobials	Antimicrobials	No. of resistant isolates (%) (n=969)
Penicilins	AMP	435 (44.6)
	AMC	125 (12.9)
Cephalosporins	FOX	2 (0.2)
	CAZ	3 (0.3)
	CTX	3 (0.3)
	FEP	1 (0.1)
	CRO	3 (0.3)
Carbapenems	MEM	1 (0.1)
Fluoroquinolones	NAL	127 (13.1)
	PEF	130 (13.4)
Tetracyclines	TET	425 (43.9)
	TGC	2 (0.2)
Aminoglycosides	GEN	15 (1.5)
Miscellaneous agents	CHL	79 (7.8)
	TMP	117 (12.1)
	SMX	585 (60.4)
Macrolides	AZM	15 (1.7)

AMP-Ampicillin; AMP-Amoxicillin- clavulanic acid; FOX-Cefoxitin; CAZ-Ceftazidime; CTX-Cefotaxime; FEP-Cefepime; CRO-Ceftriaxone; MEM-Meropenem; NAL-Nalidix acid; PEF-Pefloxacin; TET-Tetracycline; TGC-Tigecycline; GEN-Gentamicin; AZM-Azithromycin; CHL-Chloramphenicol; TMP-Trimethoprim; SMX-Sulfamethoxazole.

Table 2. Antimicrobial resistance of *Salmonella* serotypes isolated in Portugal between 2015-2017

Serotypes	No.	% of resistant isolates	% of Multiple antimicrobial resistances
*S.*4,5:i:-	312	96.8	81.1
*S.*Enteritidis	310	47.7	0.3
*S.*Typhimurium	178	76.4	51.1
*S.*Rissen	23	95.7	65.2
*S.*Typhi	23	56.5	4.3
*S.*Stanley	10	50.0	10.0
Others	113	61.9	20.4
Less common serotypes*			
*S.*Infantis	4	75.0	25.0
*S.*Saintpaul	3	100	100
*S.*Schwerzengrund	3	100	66.7
*S.*London	3	66.7	33.3
*S.*Ohio	2	100	100
*S.*Blockley	1	100	100
*S.*Essen	1	100	100

*, Only some serotypes were showed.

CONCLUSIONS

- Although overall incidence rates of *Salmonela* did not change over time, trends and epidemiological factors differed remarkably by serotype. Indeed, there was a significantly increase of *S.*Enteritidis from 2014 to 2017 related to multi-country European outbreaks.
- Continued surveillance of antimicrobial susceptibility is important due to the risk of resistance, particularly to fluoroquinolones and third-generation cephalosporins, and the high-level of multiple antimicrobial resistance.
- A better understanding of *Salmonella* epidemiology will assist in responding to this disease and in planning and implementing prevention activities.