

# MICROBIOLOGY AND FOOD SAFETY

## Monitoring antimicrobial resistance trends in *Salmonella* spp. from poultry in Eastern Spain

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**ABSTRACT** *Salmonella* spp. is one of the most important zoonotic pathogens with economic impact in public health worldwide. The relevance of *Salmonella* increases with the appearance of resistant strains. The aim of this study was to determine the level of antimicrobial resistance in 332 *Salmonella* isolates selected from 3 different poultry productive orientations in Eastern Spain during 3 yr (2015–2017). Antimicrobial susceptibility was evaluated by broth microdilution method using 14 antibiotics. Epidemiological cut-off values (**ECOFF**) were used to evaluate the microbiological resistance to antibiotics. The rates of *Salmonella* resistance at least to one antibiotic were 96, 98, and 56% in broilers, turkeys, and layers, respectively. Regarding multidrug resistance, all productive orientations seem to present a decreasing trend along the study, being the mean rates 80% in turkeys followed by broilers (40%) and layers (6%). Throughout the study, the highest percentage of resistance was found to sulfamethoxazole in all productive orientations. Strains from broilers showed the highest resistance

rates to sulfamethoxazole (73%), gentamicin (57%), ciprofloxacin (50%), nalidixic acid (29%), and tetracycline (24%). Relative to turkeys the highest resistance rates were to sulfamethoxazole (76%), ciprofloxacin (69%), tetracycline (75%), nalidixic acid (63%), and ampicillin (63%). Layers presented the most elevated resistance rates to sulfamethoxazole (39%) and tetracycline (13%). Regarding serovars the most MDR common serovars to the 3 productive orientations were *S. Kentucky* and *S. Hadar*. In the other hand, high MDR rates were found in other serovars like *S. Infantis* and *S. Typhimurium* in broilers and turkeys. Results shown in the present study suggest that the reduction in the use of antibiotics begins to be reflected in the reduction of the number of MDRs, especially in layers, with no MDR *Salmonella* strains in the last period. However, the level of resistances found in this study suggests the necessity of continuing working on the limitation of the use of antimicrobials in poultry to achieve (as in layers) the control of MDRs.

**Key words:** antimicrobial resistance, multiresistance, *Salmonella*, poultry, Eastern Spain

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## INTRODUCTION

*Salmonella* spp. is one of the most important zoonotic pathogens with economic impact in public health worldwide. The latest results reported by the European Safety Authority (EFSA) during 2019 showed that 17.9% of foodborne cases were caused by *Salmonella*, with a total of 87,923 confirmed human cases in the European Union (EU). Salmonellosis has become the second most often reported zoonotic disease in humans (EFSA, 2021b).

Although a decreasing trend has been observed between the years 2008 to 2016, during the last 5 yr (2012–2016) the trend has remained stable without a significant increase or decrease (EFSA, 2021b). Food of animal origin, specifically poultry products (meat, eggs, and egg products), are indicated by the EFSA (2021) as one of the main routes of infection in humans. Taking into account the importance of poultry products as a source of *Salmonella* human infection, National Control Programmes (PNCS) have been compiled in Spain, for the surveillance of certain serovars as *Salmonella* Enteritidis (*S. Enteritidis*) and *Salmonella* Typhimurium (*S. Typhimurium*) in layers of *Gallus gallus* species since 2008, broilers of *Gallus gallus* species since 2009, as well as for turkeys since 2010.

The relevance of *Salmonella* increases with the appearance of antimicrobial resistant strains. In

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veterinary medicine, antibiotics have been widely used therapeutically, prophylactically, and as growth promoters in the past (Usera et al., 2002). Although most cases of salmonellosis in humans are self-limiting and usually resolve without the need of treatment, in severe cases or immunocompromised patients, antibiotic therapy may be necessary with ciprofloxacin in adults and ceftriaxone in children (Berrang et al., 2009).

The emergence and development of antimicrobial resistance (AMR) is a worldwide health concern that involves both veterinary medicine and public health. In the EU, it is mandatory for the Member States to monitor and report *Salmonella* AMR (EFSA, 2021a). In this regard, in 2014 the National Antibiotic Resistance Plan (PRAN) was implanted in Spain for the period of 2014–2018, and recently extended until 2021 (MAPA, 2018). Therefore, to provide useful information on the influence of the PRAN implementation in the poultry sector it is essential to monitor the AMR trends of *Salmonella* spp. in the field. Hence, the main objectives of this study were to investigate the dynamics of *Salmonella* resistance to antibiotics in 3 different poultry productive orientations in Eastern Spain throughout the period of 3 yr (2015–2017) and to assess the resistance patterns to antibiotics currently used in veterinary and human therapy.

## MATERIALS AND METHODS

### Collection of *Salmonella* Isolates

In order to know the antibiotic resistance of *Salmonella*, 332 strains were selected belonging from positive samples of PNCSs in Eastern Spain collected from 2015 to 2017. All the samples were analyzed at Centro de Calidad Avícola y Alimentación Animal de la Comunidad Valenciana (CECAV). More than 95% of the poultry farms located in this region participated in the study.

### Isolation and Identification Procedure

*Salmonella* isolation procedure was made according to the ISO 6579:2002/Amd 1:2007 method (ISO, 2007). All strains isolated were serotyped according with the

Kauffman-White-Le Minor technique. The isolated *Salmonella* strains were frozen at  $-80^{\circ}\text{C}$  after being resuspended in sterile distilled water with 20% (v/v) double-distilled 99.5% glycerol (VWR, Leuven, Belgium).

### Antimicrobial Susceptibility Test

Antimicrobial susceptibility profiles were determined using broth microdilution, according to ISO 20776–1:2006 (ISO, 2006), by commercially available microtitre plates Sensititre EUVSEC (Thermo Scientific, East Grinstead, United Kingdom). These plates are a micro version of the classic antibiogram method based on dilution in broth. The antibiotics selected and their concentrations were those set forth in Decision 2013/653 (European Union, 2013) including 2 quinolones: ciprofloxacin (CIP) and nalidixic acid (NAL); 3 b-lactams: ampicillin (AMP), cefotaxime (FOT), and ceftazidime (TAZ); one phenicol: chloramphenicol (CHL); one sulfonamide: sulfamethoxazole (SMX); one polymyxin: colistin (COL); one macrolide: azithromycin (AZI); one glycolcycline: tigecycline (TGC); one aminoglycoside: gentamicin (GEN); one tetracycline: tetracycline (TET); one carbapenem: meropenem (MERO) and one pyrimidine: trimethoprim (TMP). The antibiotics panel at different concentrations as has been described in Table 1.

For the recovery of the frozen strains, 10  $\mu\text{L}$  of frozen suspension was sown on solid nutritive agar (Biokar, France). Subsequently, they were incubated at  $37 \pm 1^{\circ}\text{C}$  for  $24 \pm 3$  h. After the strains' growth, the microtitre plates Sensititre EUVSEC (Thermo Scientific) were inoculated and interpreted following the manufacturer's instructions. Epidemiological cutoff values (ECOFF) were taken to determine resistance against the antibiotics analyzed. These values were established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and those set forth in Decision 2013/653 (EU, 2013). The values not included in this legislation (AZI and STX) were assessed following National Committee for clinical Laboratory Standards (NCCLS) criteria (CLSI, 2017). The growth of the *Salmonella* CECT 4300 strain was used as a positive quality

**Table 1.** Sensititre antimicrobial test panel concentrations, after the addition of 50  $\mu\text{L}$  of inoculated broth and ECCOFFs.

Antimicrobial agent	Abbreviation	Panel range concentrations ( $\mu\text{g}/\text{mL}$ )	ECCOFF <i>Salmonella</i> spp. ( $\mu\text{g}/\text{mL}$ )
Sulfamethoxazole	SMX	8–1024	$\geq 76$
Trimethoprim	TMP	0.25–32	$> 2$
Ciprofloxacin	CIP	0.06–8	$> 0.064$
Tetracycline	TET	2–64	$> 8$
Meropenem	MERO	0.12–16	$> 0.125$
Azithromycin	AZI	2–64	$\geq 32$
Nalidixic acid	NAL	4–128	$> 16$
Cefotaxime	FOT	0.25–1	$> 0.5$
Chloramphenicol	CHL	8–128	$> 16$
Tigecycline	TGC	0.25–8	$> 1$
Ceftazidime	TAZ	0.5–8	$> 2$
Colistin	COL	1–16	$> 2$
Ampicillin	AMP	1–64	$> 8$
Gentamicin	GEN	0.5–32	$> 2$

control. Strains resistant to 3 or more antibiotics were considered multiresistant (EFSA, 2021a).

### Statistical Analysis

A generalized linear model was used to compare the AMR rates of each antibiotic within the same poultry production throughout the years (2015, 2016, and 2017), and to compare the global results between each poultry production. This model was also used to compare results within the same year. A *P*-value of  $\leq 0.05$  was considered to indicate a statistically significant difference. Analyses were carried out using a commercially available software application (Statgraphics Centurion XVI 16.2.04 software package; Statgraphics Technologies, Inc., The Plains, VA, 2021).

## RESULTS

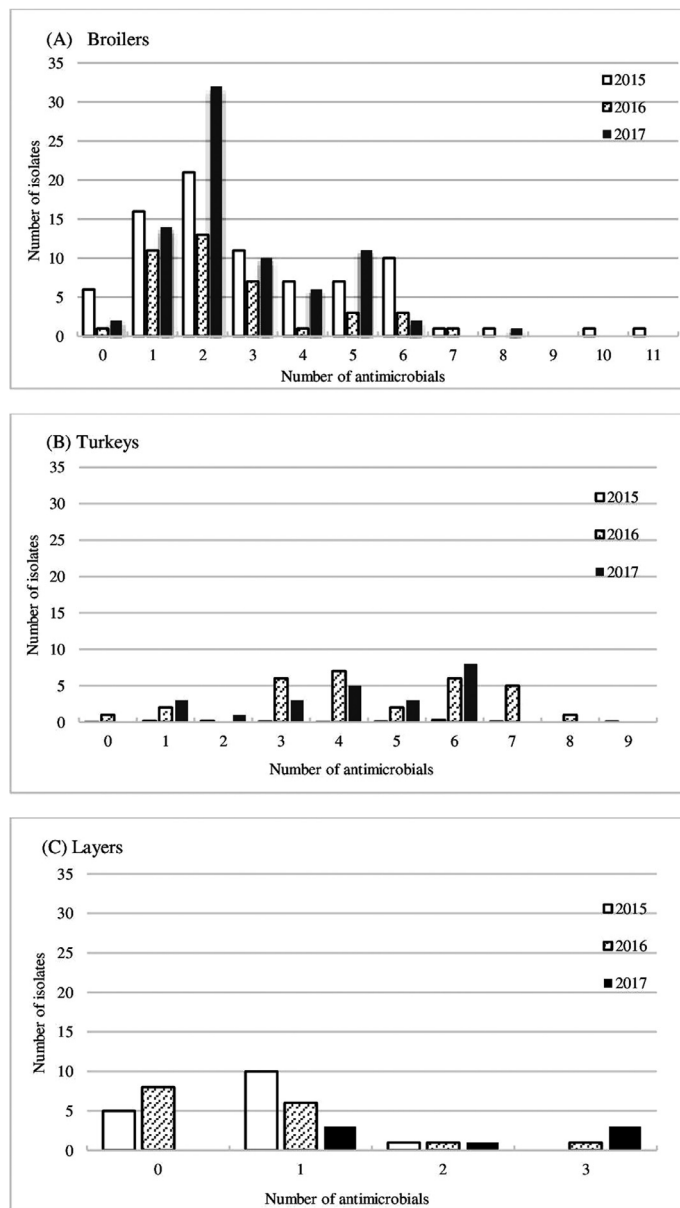
In this study, 332 strains were selected (200, 86, 46 from broilers, turkeys, and layers, respectively).

Antimicrobial susceptibility of *Salmonella* field strains isolated from broilers farms was determined (82 in 2015; 40 in 2016; 78 in 2017). A total of 93% (72/82) in 2015, 97% (39/40) in 2016, and 97% (76/78) in 2017 of *Salmonella* strains presented AMR to at least one antibiotic. Regarding multidrug resistances (MDR), a total of 47.5% (39/82) in 2015, 37.5% (15/40) in 2016 and 38.5% (30/78) in 2017 of *Salmonella* strains were found (Table 2). The *Salmonella* strains' distribution in relation to the number of antibiotics to which was resistant in all production orientations is shown in Figure 1.

As shown in Table 3, resistances to SMX, CIP and GEN were the most commonly obtained, and were observed in 73, 50, and 57% of the isolates, respectively. Regarding to SMX and TMP, the mean resistance levels presented a statistically significant increase (*P*-value  $\leq 0.05$ ; Table 3).

In the period 2015-2017, 20 different patterns of resistance were observed (Table 4). The patterns: SMX-CIP-GEN and SMX-CIP-TET-NAL-AMP-GEN, were repeated along the 3 yr.

The MDR rates according to the different serotypes are listed in Table 5. The serotypes that presented MDR during this period were *Salmonella* Kentucky (*S. Kentucky*), *Salmonella* Mikawasima (*S. Mikawasima*), *Salmonella* Senftenberg (*S. Senftenberg*), and *Salmonella* Virchow (*S. Virchow*). Regarding *Salmonella* Typhimurium (*S. Typhimurium*) presented MDR rates of 75% in 2015 and 100% in 2017. MDR was not



**Figure 1.** Distribution of *Salmonella* spp. strains isolated from different poultry production (broilers, turkeys, and layers) according to the number of antibiotics to which they were resistant. X-axis represents the number of antimicrobials to which strains are resistant.

observed in the *Salmonella* Enteritidis (*S. Enteritidis*) isolate which was only resistant to CIP and NAL.

From turkeys AMR of 86 *Salmonella* spp. strains isolated was determined (33 in 2015; 30 in 2016; 23 in 2017). A total of 97% (32/33) in 2015, 97% (29/30) in 2016, and 100% (23/23) in 2017 of *Salmonella* isolates were resistant to at least one of the antimicrobial agents tested. Regarding MDR, a 66.7% (22/33) was found in

**Table 2.** Summary of resistance, multi-resistance and maximum number of antibiotics to which *Salmonella* is resistant for broilers, turkeys, and layers during 2015–2017.

Resistance rates	Broilers			Turkeys			Layers		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Resistance (%)	93	97	97	97	97	100	50	50	69
Multidrug resistance (%)	44.5	37.5	38.5	66.7	90	82.6	14	6	0
Maximum number of antibiotics	11	7	8	9	8	6	3	3	2

**Table 3.** Antibiotic resistance rates of *Salmonella* isolated from different poultry production.

Drugs	Broilers			Turkeys			Layers		
	2015% (n = 82)	2016% (n = 40)	2017% (n = 78)	2015% (n = 33)	2016% (n = 30)	2017% (n = 23)	2015% (n = 14)	2016% (n = 16)	2017% (n = 16)
SMX	65 <sup>ab</sup>	65 <sup>ab</sup>	87 <sup>bB</sup>	64 <sup>ab</sup>	83 <sup>abB</sup>	83 <sup>bB</sup>	29 <sup>A</sup>	38 <sup>A</sup>	50 <sup>A</sup>
$\bar{x}$		73 <sup>b</sup>			76 <sup>b</sup>			39 <sup>a</sup>	
TMP	3.7 <sup>aA</sup>	2.5 <sup>aA</sup>	19 <sup>b</sup>	30 <sup>B</sup>	20 <sup>B</sup>	13	0 <sup>A</sup>	0 <sup>A</sup>	0
$\bar{x}$		9 <sup>a</sup>			22 <sup>b</sup>			0 <sup>a</sup>	
CIP	59 <sup>bB</sup>	53 <sup>abB</sup>	40 <sup>ab</sup>	61 <sup>ab</sup>	70 <sup>abB</sup>	83 <sup>bC</sup>	14 <sup>A</sup>	13 <sup>A</sup>	0 <sup>A</sup>
$\bar{x}$		50 <sup>b</sup>			69 <sup>c</sup>			9 <sup>a</sup>	
TET	29 <sup>bA</sup>	10.0 <sup>aA</sup>	24 <sup>abA</sup>	70 <sup>B</sup>	83 <sup>B</sup>	74 <sup>B</sup>	21 <sup>A</sup>	6 <sup>A</sup>	13 <sup>A</sup>
$\bar{x}$		24 <sup>a</sup>			75 <sup>b</sup>			13 <sup>a</sup>	
MERO	1.2	3	1	0	0	0	0	0	0
$\bar{x}$		1.5			0			0	
AZI	3.7	0	1	3	7	0	0	0	0
$\bar{x}$		2			3			0	
NAL	35 <sup>A</sup>	23 <sup>A</sup>	26 <sup>B</sup>	58 <sup>B</sup>	63 <sup>B</sup>	70 <sup>C</sup>	14 <sup>A</sup>	13 <sup>A</sup>	0 <sup>A</sup>
$\bar{x}$		29 <sup>b</sup>			63 <sup>c</sup>			8.6 <sup>a</sup>	
FOT	3.7 <sup>A</sup>	2.5 <sup>A</sup>	1 <sup>A</sup>	27 <sup>B</sup>	23 <sup>B</sup>	13 <sup>B</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
$\bar{x}$		2 <sup>a</sup>			22 <sup>b</sup>			0 <sup>a</sup>	
CHL	0 <sup>A</sup>	3	0	12 <sup>bB</sup>	13 <sup>a</sup>	0	0 <sup>A</sup>	0	0
$\bar{x}$		0.5 <sup>a</sup>			9 <sup>b</sup>			0 <sup>a</sup>	
TGC	1.2	0	0	0	0	0	0	0	0
$\bar{x}$		0.5			0			0	
TAZ	6.1 <sup>A</sup>	5	1	18 <sup>bB</sup>	10 <sup>ab</sup>	0 <sup>a</sup>	0 <sup>A</sup>	0	0
$\bar{x}$		4 <sup>a</sup>			10 <sup>b</sup>			0 <sup>a</sup>	
COL	2.4	0	1	0	3	0	0	0	6
$\bar{x}$		1.5			1.2			2.1	
AMP	35.4 <sup>bB</sup>	20 <sup>abA</sup>	15 <sup>aA</sup>	70 <sup>C</sup>	57 <sup>B</sup>	61 <sup>B</sup>	0 <sup>A</sup>	0 <sup>A</sup>	6 <sup>A</sup>
$\bar{x}$		24.5 <sup>b</sup>			63 <sup>c</sup>			2.1 <sup>a</sup>	
GEN	60 <sup>abB</sup>	70 <sup>bB</sup>	47 <sup>ab</sup>	18 <sup>A</sup>	23 <sup>A</sup>	22 <sup>AB</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
$\bar{x}$		57 <sup>c</sup>			22 <sup>b</sup>			0 <sup>a</sup>	

$\bar{x}$ : period mean.

For each drug, the values within the same poultry production with different superscript lowercase letters in a row are significantly different ( $P < 0.05$ ).

<sup>AB</sup>For each drug, the values within the same year with different superscript capital letters in a row are significantly different ( $P < 0.05$ ).

<sup>ab</sup>For each drug, the mean values of each poultry production with different superscript lowercase letters in a row are significantly different ( $P < 0.05$ ).

**Table 4.** Multidrug resistance patterns of *Salmonella* spp. strains.

Drugs patterns	Broilers			Turkeys			Layers		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
SMX-CIP-GEN	5	4	4	-	-	-	-	-	-
SMX-CIP-NAL	-	-	-	-	-	-	1	1	-
SMX-NAL-GEN	1	-	-	-	-	-	-	-	-
SMX-TET-AMP	1	-	-	2	-	-	-	-	-
SMX-TET-CHL	-	-	-	-	1	-	-	-	-
CIP-TET-AMP	1	-	-	-	-	-	-	-	-
CIP-TET-NAL	1	-	-	-	-	1	1	-	-
CIP-NAL-GEN	1	-	-	-	-	-	-	-	-
CIP-AMP-GEN	1	-	-	-	-	-	-	-	-
NAL-TAZ-GEN	-	-	-	-	-	-	-	-	-
SMX-CIP-NAL-GEN	5	-	-	-	-	-	-	-	-
SMX-CIP-NAL-TAZ	1	-	-	-	-	-	-	-	-
SMX-CIP-TET-AMP	1	-	-	-	-	-	-	-	-
SMX-TET-AZI-AMP	-	-	1	-	-	-	-	-	-
CIP-TET-NAL-AMP	-	-	-	1	-	1	-	-	-
CIP-TET-NAL-COL	-	-	-	-	1	-	-	-	-
SMX-CIP-NAL-AMP-GEN	9	-	-	-	-	-	-	-	-
SMX-CIP-MERO-TET-GEN	-	-	-	-	-	-	-	-	-
SMX-CIP-TET-CHL-AMP	-	-	-	1	1	-	-	-	-
SMX-CIP-TET-NAL-AMP	-	-	-	-	-	2	-	-	-
SMX-TMP-CIP-TET-NAL	-	-	9	-	-	-	-	-	-
TMP-CIP-AZI-FOT-TAZ-AMP	1	-	-	-	-	-	-	-	-
SMX-CIP-TET-NAL-AMP-GEN	3	3	2	-	-	-	-	-	-
SMX-TMP-CIP-TET-FOT-AMP	-	-	-	2	-	1	-	-	-
CIP-TET-NAL-FOT-TAZ-AMP	-	-	-	-	1	-	-	-	-
SMX-CIP-TET-NAL-TGC-AMP-GEN	1	-	-	-	-	-	-	-	-
SMX-TMP-CIP-TET-FOT-TGC-AMP	-	1	-	-	-	-	-	-	-
SMX-TMP-CIP-TET-AZI-FOT-AMP	-	-	-	-	1	-	-	-	-
SMX-TMP-CIP-TET-NAL-TAZ-GEN	-	-	1	-	-	-	-	-	-
SMX-TMP-CIP-TET-NAL-FOT-AMP	-	-	-	-	1	-	-	-	-
SMX-CIP-TET-NAL-FOT-CHL-TAZ-AMP-GEN	-	-	-	1	-	-	-	-	-
SMX-TMP-CIP-TET-AZI-NAL-FOT-TAZ-AMP-GEN	1	-	-	-	-	-	-	-	-
SMX-TMP-CIP-TET-MERO-NAL-FOT-TAZ-COL-AMP-GEN	1	-	-	-	-	-	-	-	-

2015, 90% (27/30) in 2016 and 82.6% (19/23) in 2017 (Table 2).

The antibiotics with higher resistances rates in this period were SMX (76%), TET (75%), CIP (69%), NAL (63%), and AMP (63%; Table 3). Concerning the resistances development in the period (2015–2017) a significant statistical increase of AMR has been detected to SMX (64, 83, and 83%) and CIP (61, 70, and 83) ( $P$  value  $\leq 0.05$ ). In addition, a statistically significant decrease was found in AMR rates of CHL (12, 13, and 0%) and TAZ (18, 10, and 0%;  $P$ -value  $\leq 0.05$ ).

Twelve patterns of resistance were observed (Table 4). The patterns CIP-TET-NAL-AMP; SMX-CIP-TET-CHL-AMP, and SMX-TMP-CIP-TET-FOT-AMP were repeated during 2 yr.

Regarding MDR according to serotypes, *S. Kentucky*, *Salmonella Agona* (*S. Agona*), *Salmonella Bredeney* (*S. Bredeney*) and *S. Hadar* presented MDR in all the period. *S. Typhimurium* presented MDR rates of 66% in 2015 and 50% in 2016 (Table 5).

Antimicrobial susceptibility of 46 *Salmonella* strains isolated from layers was analyzed (14 in 2015; 16 in 2016; 16 in 2017). A total of 50% (7/14) in 2015, 50% (8/16) in 2016, and 69% (11/16) in 2017 of *Salmonella* isolates were resistant to at least one antibiotic (Table 2). Regarding MDR, a 14% (2/14) was found in 2015, 6% (1/16) in 2016 and 0% (0/16) in 2017.

As shown in Table 3, the most resistant antibiotic was SMX (39%). Small levels of resistance to TET (13%),

CIP (9%), NAL (8.6%), and AMP (2.1%) was also detected.

In the period 2015–2017, two different patterns of resistance were observed (Table 4). The pattern SMX-CIP-NAL was repeated during 2 yr.

*S. Hadar* and *S. Kentucky* presented MDR in layers during 2015–2016. There were no MDR strains in 2017. Concerning *S. Enteritidis*, MDR was not observed in the 5 isolates.

## DISCUSSION

The widespread use of antibiotics in both human and veterinary medicine in the last years is well known (Anjum et al., 2011). In the past, the use of antibiotic in intensive farming was justified to prevent further extension of infections, and consequently this fact caused an increase in MDR *Salmonella* strains development (Usera et al., 2002). In Eastern Spain, there are no previous studies regarding antimicrobial resistance of *Salmonella* isolates recovered from commercial flocks of broilers, turkeys, and layers.

This study revealed a high *Salmonella* resistance rate in poultry farms to at least one antibiotic (97, 100, and 69%, in broiler, turkeys, and layers, respectively). In line with our findings, Usera et al. (2002) reported in Spain a resistance prevalence of 81.5% in broiler samples. Also, Carramiñana et al. (2004) and Álvarez-Fernández

**Table 5.** Resistance and multiresistance distribution of *Salmonella* serotypes.

Source	Year	Agona	Bredeney	Enteritidis	Hadar	Infantis	Kentucky	Mikawasima	Ohio	Senftenberg	Typhimurium	Virchow	Others	
<b>Broilers</b>	2015	R <sub>1</sub>	100	-	-	100	-	100	100	50	96	100	100	73
		R <sub>3</sub>	-	-	-	100	-	100	30	50	31	75	87	18
	2016	R <sub>1</sub>	-	-	100	100	-	100	100	-	100	-	100	88
		R <sub>3</sub>	-	-	-	-	-	66	50	-	32	-	100	33
	2017	R <sub>1</sub>	100	-	-	-	100	100	100	-	100	100	100	85
		R <sub>3</sub>	-	-	-	-	86	100	14	-	13	100	100	38
<b>Turkeys</b>	2015	R <sub>1</sub>	83	100	-	100	-	100	100	-	-	100	-	100
		R <sub>3</sub>	66	100	-	20	-	100	-	-	-	66	-	80
	2016	R <sub>1</sub>	100	90	-	100	100	100	-	-	-	100	-	100
		R <sub>3</sub>	80	80	-	100	100	100	-	-	-	50	-	100
	2017	R <sub>1</sub>	100	100	-	100	-	100	-	-	-	-	-	100
		R <sub>3</sub>	100	50	-	100	-	100	-	-	-	-	-	-
<b>Layers</b>	2015	R <sub>1</sub>	-	-	-	100	100	100	-	50	-	-	-	50
		R <sub>3</sub>	-	-	-	100	-	100	-	-	-	-	-	-
	2016	R <sub>1</sub>	-	-	50	-	33	100	-	40	-	-	100	50
		R <sub>3</sub>	-	-	-	-	-	100	-	-	-	-	-	-
	2017	R <sub>1</sub>	-	-	-	-	50	-	-	50	100	400	-	75
		R <sub>3</sub>	-	-	-	-	-	-	-	-	-	-	-	-

Data are presented as percentage.

R<sub>1</sub>, resistant to one or more antibiotics; R<sub>3</sub>, resistant to 3 or more antibiotics (multiresistant).

(2012) studies showed rates of 100% both resistance and MDR in samples of *Salmonella* isolated from poultry sources. In addition, the last rates reported by the EFSA showed a resistance of 51.8 and 80.6% from broilers and turkeys, respectively, in *Salmonella* spp. isolates recovered from animals/carcasses during 2018 in Spain (EFSA, 2021a).

The present study showed that layers were the ones with the lowest resistance rates, possibly due to the existence of restrictions in the use of antibiotics. In line with these results, Musgrove et al. (2006) showed a 66.9% of resistance in *Salmonella* isolates from commercial shell eggs. However, other research groups find a lower degree of antimicrobial resistance in the majority of *Salmonella* isolates, as Pande et al. (2015) in Australia (8.28%) and Snow et al. 2007 in UK (24%). The EFSA report showed a lower resistance rates (17.8%) from layers in Spain during 2018 (EFSA, 2021a).

Regarding MDR, a high resistance rates were observed in turkeys (80%) followed by broilers (40%), although both presented a decreasing trend in all the period. These findings are in agreement with other researchers in Spain who reported high MDR rates in poultry of 65.4% (Carramiñana et al., 2004) and 100% (Álvarez-Fernández et al., 2012). The MDR rates reported by the EFSA in the UE in 2018 were lower in turkeys (38.8%) and similar to broilers (38.2%). It is important to highlight that in this study no MDR were obtained from layers in the last period which is in line with the lower rates (6.5%) reported by the EFSA in 2018 (EFSA, 2021a).

Concerning the maximum number of antibiotics to which the strains were MDR, broilers presented a higher rate, but a decreasing trend, followed by the stable trend of MDR in and the absence in layers for 2017. This reduction or stabilization suggests a decrease in the use of some families of antimicrobials. The large number of antibiotics to which the strains were resistant in the present study is consistent with the findings of

Yang et al. (2010) who reported that 28% of *Salmonella* isolates presented MDR to 9 antibiotics. Also, Álvarez-Fernández et al. (2012) showed in Spain a resistance to 13 antibiotics (22.5%).

In the present study, resistance to SMX was the most common in broilers (73%), turkeys (76%), and layers (39%). These results were expected, since SMX has been widely used for many years in veterinary medicine to treat infections in production animals (EFSA, 2021a). The rates found in this study are higher than the data collected by EFSA in Spain during 2018, who presented resistance rates of 32.9, 54.1, and 7.7% from broilers, turkeys and layers, respectively. Other research groups in Spain, Usera et al. (2002) found in Spain a 13.7% of resistance against the family of sulphonamides.

Regarding CIP, a recognised first-line drug for the treatment of invasive salmonellosis in adults (Threlfall et al., 1999), a high resistance rate was observed in turkeys and broilers. This reduced susceptibility can be associated with the overuse of CIP or use of enrofloxacin in food-producing animals, due the similar structure and antibiotic spectrum (Lai et al., 2014). EFSA reported similar rates in 2018 period from broilers (45.3%). Concerning turkeys, the rates were lower (55.3%) and similar to layers (8.9%). Other authors reported in Spain (0%) to this antibiotic in poultry during previous years (Carramiñana et al., 2004; Usera et al., 2002).

On the other hand, AMP and CHL have been for decades the drugs of choice in the treatment of human salmonellosis. Thus, an increasing of resistance to these antimicrobials have been observed since the use of fluoroquinolones and cephalosporins became common (Miranda et al., 2009). In our research, we found some strains that were resistant to both antibiotics. This pattern of resistance suggests that these strains could compromise the treatment of human *Salmonella* infection. However, a low resistance rate to CHL has been observed in all production orientations. It could be

attributed to its non-use in animal production (Álvarez-Fernández et al., 2012). The rates from AMP were statistically higher for turkeys (63%) than broilers (24.5%) and layers (2.1%) ( $P$  value <0.05).

TET has been one of the most commonly used antibiotics in animal production (Antunes et al., 2003). Our study showed higher levels of resistance in (75%) than those reported by the EFSA in 2021 (54.1%). Statistical lower levels were found in broilers (24%) and layers (13%) in line with the results reported by the EFSA (32.4% in broilers and 6.5% in layers) (EFSA, 2021a). Carramiñana et al. (2004) reported similar rates in broilers (21.8%) in Spain.

Low resistance rates were found to COL during the last period (1.5% broilers, 1.2% turkeys, and 2.1% layers), in line with those reported by the EFSA (2021a) (4.1% broilers, 0% turkeys, and 6.5% layers).

Antimicrobial resistance associated with specific *Salmonella* serovars has been described in previous studies (Musgrove et al., 2006; Aslam et al., 2012). In the present study the most prevalent MDR serovars were *S. Kentucky* (95%) and *S. Hadar* (91%). High MDR rates were found in *S. Infantis* (86% in broilers and 100% in turkeys) and *S. Typhimurium* (88% in broilers and 58% in turkeys) serovars. *S. Typhimurium* is particularly resistant (Usera et al., 2002). The high average of MDR in this study is consistent with most surveys (Capita et al., 2007; Berrang et al., 2009). EFSA reported rates of MDR of less than 20% in *S. Typhimurium* and of 75% in monophasic (EFSA, 2021a). *S. Typhimurium* in broilers while in turkeys showed rates of 40% and 80% respectively. *S. Typhimurium* causes more serious consequences on human health than others *Salmonella* serovars (Álvarez-Fernández et al., 2012). Relative to *S. Infantis*, the rates obtained by the EFSA (2021a) were also high in broilers (80%) and turkeys (around 80%). Regarding *S. Enteritidis*, MDR was not observed in any of the isolated strains analyzed. This result is in accordance with those published by Capita et al. 2007 and Álvarez-Fernández et al. 2012, which demonstrated that *S. Enteritidis* was less prone to developing resistances than other serovars. In addition, results reported by the EU in 2018 (EFSA, 2021a) showed a low level of MDR in *S. Enteritidis* (<5% in broilers and layers).

Results shown in the present study suggest that the reduction in the use of antibiotics, since the implementation of the PRAN and the good practices of Spanish poultry producers, begins to be reflected with the reduction in the number of MDRs found with a decreasing trend especially in layers. However, the level of resistances found in this study suggests the need of continuing working on the limitation of the use of antimicrobials in poultry to achieve (as in layers) the disappearance of MDR. This way, the Spanish broiler sector has voluntarily agree to adhere to REDUCE national programme in 2020 (MAPA, 2019). The main objective of this agreement is the rational use of antibiotics and to propose preventive health plans thanks to the development of new, more effective therapeutic measures that allow the

reduction of the use of antibiotics. Finally, the best antibiotics for each pathology will be determined knowing the resistances and avoiding the use of ineffective ones.

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## DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

- Álvarez-Fernández, E., C. Alonso-Calleja, C. García-Fernández, and R. Capita. 2012. Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from poultry in Spain: comparison between 1993 and 2006. *Int. J. Food Microbiol* 153:281–287.
- Anjum, M. F., S. Choudhary, V. Morrison, L. C. Snow, M. Mafura, P. Slickers, R. Ehricht, and M. J. Woodward. 2011. Identifying antimicrobial resistance genes of human clinical relevance within *Salmonella* isolated from food animals in Great Britain. *J. Antimicrob. Chemother.* 66:550–559.
- Antunes, P., C. Réu, J. C. Sousa, L. Peixe, and N. Pestana. 2003. Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *Int. J. Food Microbiol* 82:97–103.
- Aslam, M., S. Checkley, B. Avery, G. Chalmers, V. Bohaychuk, G. Gensler, R. Reid-Smith, and P. Boerlin. 2012. Phenotypic genetic characterization of antimicrobial resistance in *Salmonella* serovars isolated from retail meats in Alberta. *Can. Food Microbiol* 32:110–117.
- Berrang, M. E., J. C. Bailey, S. F. Altekruise, W. K. Shaw, B. L. Patel, R. J. Mainermann, and P. J. Fedorka-Cray. 2009. Prevalence, serotype, and antimicrobial resistance of *Salmonella* on broiler carcasses postpick and postchill in 20 U.S. processing plants. *J. Food Protec* 72:1610–1615.
- Capita, R., C. Alonso-Calleja, and M. Prieto. 2007. Prevalence of *Salmonella enterica* serovars and genovars from chicken carcasses from slaughterhouses in Spain. *J. Appl Microbiol* 103:1366–1375.
- Carramiñana, J. J., C. Rota, I. Agustín, and A. Herrera. 2004. High prevalence of multiple resistance to antibiotics in *Salmonella* serovars isolated from a poultry slaughterhouse in Spain. *Vet. Microbiol* 104:133–139.
- CLSI. (Clinical and Laboratory Standards Institute). 2017. Performance Standards for Antimicrobial Susceptibility Testing. M100. . 27th ed. CLSI, Wayne, Pennsylvania, EE.UU.
- EFSA. European Food Safety Authority and European Centre for Disease Prevention and Control. 2021a. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. *EFSA J.* 19:6490.
- EFSA. European Food Safety Authority and European Centre for Disease Prevention and Control. 2021b. The European Union one health 2019 zoonoses report. *EFSA J.* 19:6406.
- European Union Commission. 2013. Commission implementing decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. *Off. J. Eur. Union* 303:26–39.
- ISO 20776-1:2006. 2006. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 1: broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. International Organization for Standardization. Ginebra, Suiza.

- ISO 6579:2002/Amd 1:2007. 2007. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp. — Amendment 1: Annex D: detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. International Organization for Standardization. Ginebra, Suiza.
- Lai, J., C. Wu, C. Wu, J. Qi, Y. Wang, H. Wang, Y. Liu, and J. Shen. 2014. Serotype distribution and antibiotic resistance of *Salmonella* in food-producing animals in Shandong province of China, 2009 and 2012. *Int. J. Food Microbiol* 180:30–38.
- MAPA (Ministry of Agriculture, Fishing and Food. 2018. Informe JIACRA España. Primer análisis integrado del consumo de antibióticos y su relación con la aparición de resistencia. Accessed May 2021. <http://www.resistenciaantibioticos.es/es/publicaciones/informe-jia-cra-espana>
- MAPA (Ministry of Agriculture, Fishing and Food. 2019. Acuerdo para el desarrollo del Programa de Prescripción y Uso Razonable de Antibióticos en POLLOS BROILER. Accessed Oct.2021. <https://www.resistenciaantibioticos.es/es/programa-reduce-pollos-broiler>.
- Miranda, J. M., A. C. Mondragón, B. Martínez, M. Guarddon, and J. A. Rodríguez. 2009. Prevalence and antimicrobial resistance patterns of *Salmonella* from different raw foods in Mexico. *J. Food Prote* 72:966–971.
- Musgrove, M. T., D. R. Jones, J. K. Northcutt, N. A. Cox, M. A. Harrion, P. J. Fedorka-Cray, and S. R. Ladely. 2006. Antimicrobial resistance in *Salmonella* and *Escherichia coli* isolated from commercial shell eggs. *Poult. Sci.* 85:1665–1669.
- Pande, V. V., V. C. Gole, A. R. McWhorter, S. Abraham, and K. K. Chousalkar. 2015. Antimicrobial resistance of non-typhoidal *Salmonella* isolates from egg layer flocks and egg shells. *Int. J. Food Microbiol* 203:23–26.
- Snow, L. C., R. H. Davies, K. H. Christiansen, J. J. Carrique-Mas, A. D. Wales, J. L. O'Connor, A. J. C. Cook, and S. J. Evans. 2007. Survey of prevalence of *Salmonella* species on commercial laying fars in the United Kingdom. *Vet. Rec.* 161:471–476.
- Threlfall, E. J., L. R. Ward, and B. Rowe. 1999. Resistance to ciprofloxacin in non-typhoidal salmonellas from humans in England and Wales- the current situation. *Clin. Microbiol. Infect.* 5:130–134.
- Usera, M. A., A. Aladueña, R. Gonzalez, M. De la Fuente, J. García-Peña, N. Frías, and M. A. Echeita. 2002. Antibiotic resistance of *Salmonella* spp. from animal sources in Spain in 1996 and 2000. *J. Food Protec* 65:768–773.
- Yang, B., D. Qu, X. Zhang, J. Shen, S. Cui, Y. Shi, M. Xi, M. Sheng, S. Zhi, and J. Meng. 2010. Prevalence and characterization of *Salmonella* serovars in retail metas of marketplace in Shaanxi, China. *Int. J. Food Microbiol.* 141:63–72.