CODA-CERVA

Report on susceptibility of Salmonella serotypes in Belgium 2014.

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1 Introduction

Salmonella is one of the most important bacterial zoonotic agents. Though in general, infections in humans do not require treatment, in some cases, it is lifesaving. Therefore, surveillance for resistance in animals is of importance since these zoonotic bacteria may finally end up in humans. Next to this direct resistance transfer, indirect resistance transfer may happen: resistance genes on mobile genetic elements may be transferred from *Salmonella* spp. to commensal human bacteria and eventually to human pathogens. Surveillances in commensal bacteria from animals have a similar significance and surveillances in animal pathogens are aiming more at guidance for veterinarians in the treatment of infections caused by these bacteria. However, it should be taken into account that animal pathogenic bacteria, that are often more resistant than commensals, are part of the transferable resistance gene pool. *Salmonella* spp. may cause also health problems in animals. This is very evident in bovines, and likewise, most bovine strains originate from clinically ill animals.

2 Materials and Methods

2.1 Susceptibility testing

Salmonella spp. strains were serotyped by the Salmonella reference laboratory for animals, CODA-CERVA, Brussels. Care was taken to include only one strain per farm/animal/other per sampling point. An official epidemiological surveillance program was only established for poultry in 2014. Strains were transferred to the national reference laboratory for antimicrobial resistance (CODA-CERVA) for susceptibility testing after serotyping. Upon arrival, the strains were plated on Columbia agar with 5% sheep blood and susceptibility was tested using a micro broth dilution method (Trek Diagnostics). To this end, 1 to 3 colonies were suspended in sterile physiological water to an optical density of 0.5 McFarland. Ten microliter of this suspension was inoculated in 11ml cation adjusted Mueller Hinton broth with TES buffer. Fifty microliter of the Mueller-Hinton broth with bacteria was brought on a microtiter plate with the antimicrobials lyophilised, the EUVSEC plate (antibiotics tested are presented in table 1) as produced by Trek Diagnostics, using the auto-inoculating system of Trek Diagnostics. Plates were incubated 18-24 hours at 35°C and read. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected. MICs were semiautomatically recorded by the Trek Vision system using the SWIN software. Results were automatically exported to an Excel file.

2.2 Analysis of data

Data from the Excel file generated by the software of the semi-automated susceptibility equipment (sensivision, Trek Diagnostics) and merged to the administrative date from the LIMS system at CODA-CERVA. These files were validated for consistency. The excel file was then imported into an Access file in which the number of strains having a MIC for a certain antibiotic was calculated. These data were set in a table that was subsequently exported to an Excel file. The data were interpreted for susceptibility using breakpoints based on the EUCAST ECOFFs or as defined by the EU reference laboratory on antimicrobial resistance (DTU, DK) are indicated. The number of resistant strains was counted and resistance percentages were calculated. Exact confidence intervals for the binomial distribution were calculated using a visual basic application in Excel. A 95% symmetrical two-sided confidence interval was used with p=0.025. The lower and upper bound of confidence interval for the population proportion was calculated. Based on the Pearsons chi-square test, and where appropriate the Fischer exact test, significance of the differences were calculated. Multi-resistance was determined by transforming the MIC data into resistant (R) and susceptible (S). Number of antimicrobials to which a strain was resistant to was counted and cumulative percentages were calculated.

3 Results

Results are shown in tables 2 and 3 and in figure 1. Only the results of strains tested in the framework of official monitoring are presented. Table 2 gives a general overview of all strains tested, followed by the presentation of the resistances by serotype (table 3) and finally in figure 1 some specific resistances are highlighted. It should be noted that no EUCAST breakpoint (ECOFF) for tigecycline, sulphamethoxazolone and azithromycin are available for *Salmonella* spp., so resistance against these antibiotics is therefore not discussed in this report.
In total 218 strains were tested for antimicrobial susceptibility with the first panel. Based on the obtained results 7 *Salmonella* strains were tested with a confirmatory test for ESBL production. It should be noted that the results largely depend on the proportion of the different *Salmonella* serotypes. Some serotypes do not seem to acquire resistances easily, while others do.

Table 2 shows that highest resistance is seen against trimethoprim (82.1%), followed by ampicillin (53%), ciprofloxacin (45%) and nalidixic acid (42%). Two serotypes, *S*. Livingstone and *S*. Mbandaka were only resistant toward trimethoprim and to no other antibiotic tested. Resistance towards trimethoprim was found in high percentages of these serotypes (90.5% and 54.5% respectively) and these serotypes are normally associated with pigs. Also all *S*. Paratyphi B and *S*. Minnesota strains were 100% resistant towards trimethoprim (Table 3). Resistance against colistin was slightly higher than 20%. The resistance of *Salmonella* spp. towards colistin is significant higher compared to colistin resistance of commensal *E. coli* (almost absent in *E. coli*) however, it should be noted that for example *S*. Enteritidis and *S*. Dublin have a natural lower susceptibility towards this antibiotic. The higher the proportion of these serotypes the higher the global resistance percentage against this antibiotic. In this dataset 35 *S*. Enteritidis strains were included. The others serotypes that were resistant towards colistin were *S*. Dublin (as indicated above) and *S*. Chester.
Tetracycline resistance was 14%, which is quite low compared to what is found in *E. coli* (45.6%) from animals and was seen especially in *S*. Typhimurium and *S*. Infantis (Table 3).
Less than 3.2% of the strains were resistant to extended spectrum cephalosporins (cefotaxime and ceftazidime) and none of them were resistant towards meropenem. All ceftazidime resistant strains (5) were resistant to cefotaxime, as expected, while two strains were cefotaxime resistant and showed not ceftazidime resistance. It should be noted that the 5 strains that showed resistance toward cefotaxime and ceftazidime, were multiresistant towards 6 antibiotics (figure 1) and all of them had the same antibiotic resistance profile. 4 out of 5 were *S*. Paratyphi B. The seven strains were confirmed to be ESBL with a second panel (EUVSEC2 plates). Three strains were presumptive ESBL, one was presumptive ESBL + Ampc and three strains were presumptive pAmpC.
Ciprofloxacin and nalidixic acid are both antimicrobials from a same class and likewise, resistance is frequently observed towards both molecules. High prevalence of fluoroquinolone resistance can be seen in *Salmonella* spp. (20.6% and 19.3%) but is significant lower than in *E. coli* isolated from poultry (69.6% and 63.3% respectively). Slightly more resistance against ciprofloxacin can be noted. Discrepancies can be explained by the presence of plasmid mediated quinolone resistance, which was seen in as much as 3 strains. Frequently, the plasmids on which these genes are located are carrying multiple resistance genes. Plasmid mediated quinolone resistance can be due to the *qnr* genes (protection of DNA gyrase or topoisomerase enzymes), the *aac(6')-Ib-cr* (acetylation of ciprofloxacin) or *oqxAB* and *qepA* genes (efflux pumps).
Resistance towards chloramphenicol and gentamicin were as low as 1.4%.

4 Conclusions

The highest resistance was seen against trimethoprim, ampicillin, ciprofloxacin and nalidixic acid. Tetracycline resistance was significant lower compared to what was found in *E. coli* (45.6%) in 2014. Less than 3.2% of the strains were resistant towards extended spectrum cephalosporins (cefotaxime and ceftazidime) and none of them were resistant towards meropenem. High prevalence of fluoroquinolone resistance can be seen in *Salmonella* spp. but again significant lower than in *E. coli* isolated from poultry. The most resistance serotype by far was *S*. Paratyphi B followed by *S*. Typhimurium.

5 References

Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. (2009) Plasmid-mediated quinolone resistance: a multifaceted threat. Clinical Microbiology Reviews (4), 664-689.

Table 1. List of abbreviations

Abbreviation	
AMP	Ampicillin
AZI	Azithromycin
CHL	Chloramphenicol
CIP	Ciprofloxacin
COL	Colistin
FOT	Cefotaxime
GEN	Gentamicin
MERO	Meropenem
NAL	Nalidixic acid
SMX	Sulphamethoxazole
TAZ	Ceftazidime
TET	Tetracycline
TGC	Tigecycline
TMP	Trimethoprim

Table 2. Antibiotic resistance in *Salmonella* spp.

Concentration	AMP	FOT	TAZ	MERO	NAL	CIP	TET	TGC	SMX	TMP	CHL	GEN	COL	AZI
<=0.008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.015	0	0	0	0	0	75	0	0	0	0	0	0	0	0
0.03	0	0	0	209	0	98	0	0	0	0	0	0	0	0
0.06	0	0	0	9	0	0	0	0	0	0	0	0	0	0
0.12	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0.25	0	209	0	0	0	11	0	131	0	18	0	0	0	0
0.5	0	2	207	0	0	16	0	67	0	21	0	203	0	0
1	99	0	4	0	0	16	0	17	0	0	0	11	137	0
2	58	3	2	0	0	1	187	3	0	0	0	1	60	5
4	8	0	1	0	169	0	17	0	0	0	0	0	13	98
8	0	4	0	0	5	0	0	0	2	0	189	0	8	99
16	0	0	4	0	2	0	1	0	6	0	26	2	0	15
32	2	0	0	0	1	0	0	0	36	0	0	1	0	1
64	0	0	0	0	0	0	1	0	75	179	0	0	0	0
128	51	0	0	0	2	0	12	0	47	0	0	0	0	0
256	0	0	0	0	39	0	0	0	7	0	3	0	0	0
512	0	0	0	0	0	0	0	0	3	0	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	0	0	0	0	0
>=2048	0	0	0	0	0	0	0	0	42	0	0	0	0	0

N	218	218	218	218	218	218	218	218	218	218	218	218	218	218
NR	53	7	5	0	42	45	14	ND	ND	179	3	3	21	ND
%R	24,3	3,2	2,3	0,0	19,3	20,6	6,4			82,1	1,4	1,4	9,6	
CI	18,8-31	1,3-7	0,7-5	0-1	14,3-25	15,5-27	3,6-11			76,1-87	0,3-4	0,3-4	6,1-14	

Line: breakpoint, N: Number, NR: Number resistant, %R percent resistant, CI: confidence interval, ND: not determined

Table 3. Antibiotic resistance in different *Salmonella* spp. serotypes.

		AMP	FOT	TAZ	MERO	NAL	CIP	TET	TGC	SMX	TMP	CHL	GEN	COL	AZI
***S*. Enteritidis**	N	35	35	35	35	35	35	35	35	35	35	35	35	35	35
	NR	0	0	0	0	0	0	0	ND	ND	25	0	0	17	ND
	%R	0,0	0,0	0,0	0,0	0,0	0,0	0,0			71,4	0,0	0,0	48,6	
	CI	0-8	0-8	0-8	0-8	0-8	0-8	0-8			53,7-85	0-8	0-8	31,4-66	
***S*. Typhimurium**	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
	NR	16	1	1	0	3	3	7	ND	ND	20	2	0	0	ND
	%R	66,7	4,2	4,2	0,0	12,5	12,5	29,2			83,3	8,3	0,0	0,0	
	CI	44,7-84	0,1-21	0,1-21	0-12	2,7-32	2,7-32	12,6-51			62,6-95	1-27	0-12	0-12	
***S*. Livingstone**	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	NR	0	0	0	0	0	0	0	ND	ND	19	0	0	0	ND
	%R	0,0	0,0	0,0	0,0	0,0	0,0	0,0			90,5	0,0	0,0	0,0	
	CI	0-13	0-13	0-13	0-13	0-13	0-13	0-13			69,6-99	0-13	0-13	0-13	
***S*. Paratyphi B variant Java**	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	NR	19	3	3	0	20	20	0	ND	ND	21	0	1	0	ND
	%R	90,5	14,3	14,3	0,0	95,2	95,2	0,0			100,0	0,0	4,8	0,0	
	CI	69,6-99	3-36	3-36	0-13	76,2-100	76,2-100	0-13			83-9-100	0-13	0,1-24	0-13	
***S*. Infantis**	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	NR	0	0	0	0	7	7	5	ND	ND	15	0	0	0	ND
	%R	0,0	0,0	0,0	0,0	35,0	35,0	25,0			75,0	0,0	0,0	0,0	
	CI	0-14	0-14	0-14	0-14	15,4-59	15,4-59	8,7-49			50,9-90	0-14	0-14	0-14	
S. Agona	N	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	NR	1	0	0	0	0	1	0	ND	ND	10	0	1	0	ND
	%R	6,7	0,0	0,0	0,0	0,0	6,7	0,0			66,7	0,0	6,7	0,0	
	CI	0,2-32	0-18	0-18	0-18	0-18	0,2-32	0-18				0-18	0,2-32	0-18	
***S*. Minnesota**	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	NR	5	0	0	0	0	0	0	ND	ND	12	0	0	0	ND
	%R	41,7	0,0	0,0	0,0	0,0	0,0	0,0			100,0	0,0	0,0	0,0	
	CI	15,2-72	0-22	0-22	0-22	0-22	0-22	0-22			73,5-100	0-22	0-22	0-22	
***S*. Mbandaka**	N	11	11	11	11	11	11	11	11	11	11	11	11	11	11
	NR	0	0	0	0	0	0	0	ND	ND	6	0	0	0	ND
	%R	0,0	0,0	0,0	0,0	0,0	0,0	0,0			54,5	0,0	0,0	0,0	
	CI	0-24	0-24	0-24	0-24	0-24	0-24	0-24			23,4-83	0-24	0-24	0-24	
Others	N	75	75	75	75	75	75	75	75	75	75	75	75	75	75
	NR	14	3	1	0	13	16	2	ND	ND	62	1	2	4	ND
	%R	18,7	4,0	1,3	0,0	17,3	21,3	2,7			82,7	1,3	2,7	5,3	
	CI	10,6-29	0,8-11	0-7	0-4	9,6-28	12,7-32	0,3-9			72,7-90	0-7	0,3-9	1,5-13	

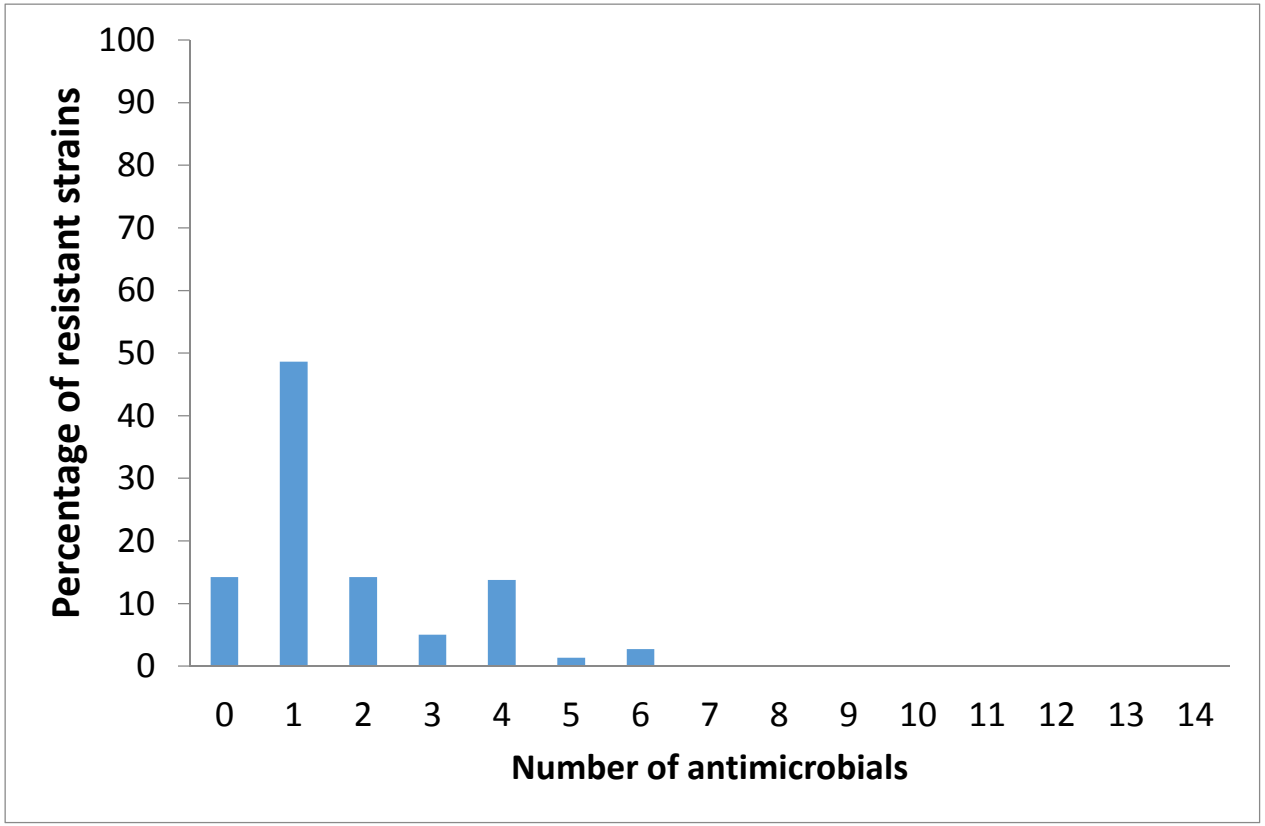

Figure 1. Multiresistance amount *Salmonella* spp. isolates