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Antimicrobial susceptibility of zoonotic *Salmonella enterica* from cattle, pigs and poultry isolated in 8 European countries over a four year period (2002-2006) (EASSA program)

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H. Moyaert, A. de Jong, P. Butty, U. Klein, H. Marion, S. Simjee, V. Thomas and M. Vallé

EASSA Study Group, CEESA, Brussels, Belgium

Contact:
Hilde.Moyaert@Pfizer.com

EASSA

- The EASSA project is a European resistance monitoring system for zoonotic and commensal bacteria originating from healthy food-producing animals at processing. To achieve this purpose, an extensive culture collection has been set up. To date, the collection contains more than 25,000 isolates.

- EASSA is an initiative of, and is co-ordinated by, the European Animal Health Study Center (CEESA) in Brussels. It has been operational since 1998. CEESA's membership is composed of international pharmaceutical companies researching and producing veterinary medicinal products.

- Sponsors of the current EASSA II project were the following 10 companies:

  Alpharma BVBA, Belgium; Bayer HealthCare AG, Animal Health Division, Germany; CEVA Sante Animale, France; Elanco Animal Health, UK; Fort Dodge Animal Health, Belgium; Intervet International MSD Animal Health, The Netherlands; Novartis Animal Health Inc., Switzerland; Pfizer Animal Health, Belgium; Schering-Plough Animal Health, UK; Vetoquinol S.A., France.

- National co-ordinators of the monitoring network for cattle, pigs or chickens were:

  Denmark: F. Aarestrup and A. Sörensen
  France: P. Sanders, M. Bruneau, S. Granier, I. Kempf and J.-Y. Madec
  Germany: K. Müller and R. Weiss
  Ireland: N. Leonard
  Italy: A. Battisti and A. Franco
  Netherlands: D. Mevius and K. Veldman
  Spain: Moreno, C. Porrero and L. Dominguez
  United Kingdom: P. Heath and P. Todd

- The culture collection of EASSA II has been organized by a contract research organisation serving human and animal health, Charles River, Edinburgh, UK. All MIC determinations were performed by Charles River.

- The CEESA project leader is Hervé Marion, CEESA, Rue Defacqz 1, Bte 8, B-1000 Brussels, Belgium.

  For correspondence:
  Tel : +32 2 543 7572
  Fax : +32 2 543 7585
  e-mail: ceesa@ceesa.eu

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We thank the national co-ordinators (for names see above) and the national microbiology laboratories involved in the sampling and isolation procedures.

Conclusions

This pan-European survey demonstrates high variability in antimicrobial susceptibility among zoonotic *S. enterica* isolates from healthy food producing animals at slaughter. Prevalence of *Salmonella* was particularly low. For older molecules such as sulfisoxazole and tetracycline, prevalence of clinical resistance was notably high, but most isolates displayed high susceptibility to newer compounds, critically important in human medicine.

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Revised Abstract

Background: The European Antimicrobial Susceptibility Surveillance in Animals (EASSA) is the first ongoing program monitoring antimicrobial susceptibility relevant for human medicine of zoonotic and commensal bacteria from healthy food-producing animals at slaughter across Europe. Susceptibility results of *S. enterica (Se)* isolated from 2002-2006 are presented here.

Methods: Colon or caecal content from healthy beef cattle, fattening pigs and broiler chickens was randomly sampled in 8 EU countries (5 countries/host; 4 slaughterhouses/country; 1 sample per herd/flock). Salmonella isolation, identification and serotyping were performed by standard methods. MICs of 11 antimicrobials were determined by agar dilution (CLSI, M31-A3) in a central laboratory. Results were interpreted using clinical breakpoints (CLSI M100-S21, except colistin: EUCAST) and Epidemiological Cut-off Values (ECVs) as defined by EFSA (2007). Decreased Susceptibility (DS), i.e. MIC values exceeding the wild-type MIC distribution (> ECV) but not the clinical breakpoint, was determined for 4 antimicrobials.

Results: In total, 659 Se strains (48 different serotypes) were identified: 57 from cattle, 420 from pigs and 182 from chickens. Following serotypes were predominantly recovered: Typhimurium (18), Dublin (12), Enteritidis (9) and Montevideo (7) from cattle; Typhimurium (135), Derby (110) and Rissen (53) from pigs and Enteritidis (61), Indiana (43) and Typhimurium (17) from chickens. Overall, mean resistance percentage for each respective animal host was: ampicillin 19.3, 33.1, 12.6; chloramphenicol 17.5, 25.2, 4.4; colistin 10.5, 3.1, 27.5; gentamicin 0.0, 3.6, 1.6; nalidixic acid 3.5, 3.6, 36.3; sulfisoxazole 21.8, 53.0, 12.2; tetracycline 22.8, 73.8, 13.2 and trimethoprim/sulfamethoxazole 5.3, 23.6, 4.9. Except for three cefotaxime-resistant strains, clinical resistance against cefepime, cefotaxime and ciprofloxacin was absent. DS was particularly apparent for ciprofloxacin: 3.5% for cattle, 2.9% for pigs and 35.2% for chickens, whereas the corresponding figures for cefotaxime were 0, 1.7 and 0%. For ampicillin and gentamicin, DS was negligible (0.5% or less).

Conclusion: This pan-European survey demonstrates high variability in antimicrobial susceptibility among zoonotic *Salmonella enterica* isolates from healthy food producing animals at slaughter. For older molecules, prevalence of clinical resistance was notably high, but most isolates displayed high susceptibility to newer compounds, critically important in human medicine.

Background

In 1998, the animal health industry through the European Animal Health Study Center (CEESA) established the surveillance of the antimicrobial susceptibility of zoonotic and commensal bacteria from healthy food-producing animals at slaughter across Europe. The monitoring scheme comprises *Salmonella enterica* and *Campylobacter* spp. as representative of zoonotic bacterial pathogens as well as *Escherichia coli* and *Enterococcus* spp. as representative of commensal Gram-negative and Gram-positive bacteria, respectively. All isolates are obtained from the major host species cattle, pigs and chickens and are tested against a range of antimicrobial classes relevant to human medicine.

Several individual European countries have national surveys to monitor the antimicrobial susceptibility of enteric bacteria in healthy animals, but due to differences in sample collection, isolation techniques, laboratory MIC methodology and data analyses, prudence is called for when comparing their results. The European Antimicrobial Susceptibility Surveillance in Animals (EASSA) is the first pan-European monitoring program using uniform sampling and bacterial isolation procedures, and performing MIC determination in a central laboratory. Susceptibility results of *S. enterica* isolated from 2002-2006 (EASSA II) are presented below.

Materials and Methods

Colon or caecal content from healthy beef cattle, fattening pigs and broiler chickens was randomly sampled in 8 EU countries (5 countries/host; 4 slaughterhouses/country; 1 sample per herd/flock). *Salmonella* isolation, identification and serotyping were performed by standard methods. As the prevalence was particularly low, numbers were supplemented by adding isolates from national collections that fulfilled the selection criteria. MICs of 11 antimicrobials were determined by agar dilution (CLSI, M31-A3) in a central laboratory (Charles River). Results were interpreted using clinical breakpoints (CLSI M100-S21, except colistin: EUCAST) and Epidemiological Cut-off Values (ECVs) as defined by EFSA (2007). Decreased Susceptibility, i.e. MIC values exceeding the wild-type MIC distribution (> ECV) but not the clinical breakpoint, was determined for 4 antimicrobials. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S.* Typhimurium NCTC 74 were used as quality control strains.

Results

In total, 659 *S. enterica* strains (48 different serotypes) were identified: 57 from cattle, 420 from pigs and 182 from chickens. The major serotypes of each host species are presented in Table 1. Prevalence of *Salmonella* appeared to be low for all three host species. Despite adding isolates from national collections, the small numbers per country preclude comparisons.

Table 1. Numbers of *Salmonella* serotypes

Cattle		Pigs		Chicken	
Serotype	*n*	*Serotype*	*n*	*Serotype*	*n*
Typhimurium	18	Typhimurium	135	Enteritidis	61
Dublin	12	Derby	110	Indiana	43
Enteritidis	9	Rissen	53	Typhimurium	17
Montevideo	7	Bredeney	19	Infantis	5
Mbandaka	3	Wien	14	Kottbus	5
Anatum	3	Anatum	12	Others < 5	50
Others < 3	15	Others < 12	77		

Antimicrobial susceptibility results per country, including MIC_{50} and MIC_{90}, are presented in Table 2. Overall, mean resistance percentage for each respective animal host (cattle, pigs and chickens) was: ampicillin 19.3, 33.1, 12.6; chloramphenicol 17.5, 25.2, 4.4; colistin 10.5, 3.1, 27.5; gentamicin 0.0, 3.6, 1.6; nalidixic acid 3.5, 3.6, 36.3; sulfisoxazole 21.8, 53.0, 12.2; tetracycline 22.8, 73.8, 13.2 and trimethoprim/sulfamethoxazole 5.3, 23.6, 4.9. Colistin resistance was surprisingly high for chicken isolates due to 48 *S.* Enteritidis strains. All 48 strains were fully susceptible to cephalosporins, chloramphenicol, gentamicin, tetracycline and trimethoprim/sulfamethoxazole. However, they all exhibited nalidixic acid resistance and decreased susceptibility to ciprofloxacin.

Table 2. In vitro antimicrobial activity (MIC in mg/L; resistance (R) and decreased susceptibility (DS) in %) of various antimicrobials against *Salmonella enterica*.

		Cattle						Pigs						Chickens					
Antimicrobial	**Interpretation***	**GE 2**	**FR 42**	**IR 6**	**IT 4**	**UK 3**	**Total** 57**	**GE 8**	**SP 237**	**FR 110**	**NL 59**	**DK 6**	**Total** 420**	**GE 62**	**SP 64**	**FR 30**	**NL 5**	**UK 21**	**Total** 182**
Ampicillin	MIC_{50}	-	1	-	-	-	1	-	1	1	1	-	1	1	1	1	-	1	1
	MIC_{90}	-	-	-	-	-	>128	-	>128	>128	>128	-	>128	>128	4	>128	-	2	>128
	R (≥ 32)	0	19.0	0	50.0	0	19.3	37.5	39.7	24.5	25.4	0	33.1	12.9	0.08	23.3	40.0	0.05	12.6
	DS (8-16)	0	0	0	0	0	0	0	0.8	0	0	0	0.5	0	0	0	0	0	0
Cefepime	MIC_{50}	-	0.06	-	-	-	0.06	-	0.06	0.06	0.06	-	0.06	0.06	0.06	0.06	-	0.06	0.06
	MIC_{90}	-	0.12	-	-	-	0.12	-	0.25	0.025	0.12	-	0.25	0.12	0.12	0. 12	-	0.25	0.12
	R (≥ 32)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cefotaxime	MIC_{50}	-	0.12	-	-	-	0.12	-	0.12	0.12	0.12	-	0.12	0.12	012	0.12	-	0.2	0.12
	MIC_{90}	-	0.12	-	-	-	0.12	-	0.25	0.25	0.12	-	0.25	0.25	0.25	0.12	-	0.25	0.25
	R (≥ 4)	0	0	0	0	0	0	0	0.9	0	0	0	0.2	0	3.1	0	0	0	1.1
	DS (1-2)	1.0	0	0	0	0	0	0	3.0	0	0	0	1.7	0	0	0	0	0	0
Chloramphenicol	MIC_{50}	-	4	-	-	-	4	-	8	8	8	-	8	4	4	4	-	4	4
	MIC_{90}	-	128	-	-	-	128	-	>128	>128	128	-	>128	8	8	8	-	8	8
	R (≥ 32)	0	21.4	0	25.0	0	17.5	37.5	30.8	20.0	13.6	0	25.2	8.1	0	6.6	0	4.8	4.4
Ciprofloxacin	MIC_{50}	-	0.03	-	-	-	0.03	-	0.016	0.016	0.016	-	0.016	0.03	0.12	0.03	-	0.016	0.03
	MIC_{90}	-	0.03	-	-	-	0.03	-	0.03	0.03	0.03	-	0.03	0.06	0.25	0.25	-	0.03	0.25
	R (≥ 4)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	DS (0.12-2)	0	4.8	0	0	0	3.5	0	4.2	0.9	1.7	0	2.9	6.5	82.8	23.3	0	0	35.2
Colistin	MIC_{50}	-	0.25	-	-	-	0.25	-	0.25	0.25	0.25	-	0.25	0.5	4	0.25	-	0.25	0.5
	MIC_{90}	-	0.5	-	-	-	4	-	0.5	1	0.5	-	0.5	1	8	0.5	-	2	8
	R (≥ 4)	50.0	2.4	50.0	0	33.3	10.5	0	3.8	3.6	1.7	0	3.1	9.7	68.8	0	0	0	27.5
Gentamicin	MIC_{50}	-	0.25	-	-	-	0.25	-	0.5	0.5	0.5	-	0.5	0.5	0.25	0.25	-	0.5	0.5
	MIC_{90}	-	0.5	-	-	-	0.5	-	1	1	1	-	1	1	0.5	1	-	1	1
	R (≥ 16)	0	0	0	0	0	0	0	5.5	1.8	0	0	3.6	0	1.6	3.3	0	0	1.6
	DS (4-8)	0	0	0	0	0	0	0	0.8	0	0	0	0.05	0	0	0	0	0	0
Nalidixic Acid	MIC_{50}	-	4	-	-	-	4	-	4	4	4	-	4	4	>128	4	-	4	4
	MIC_{90}	-	8	-	-	-	8	-	8	8	8	-	8	8	>128	>128	-	8	>128
	R (≥ 32)	0	4.8	0	0	0	3.5	0	5.5	0.9	1.7	0	3.6	8.1	82.8	26.6	0	0	36.3
Sulfisoxazole	MIC_{50}	-	64	-	-	-	64	-	2048	>2048	64	-	2048	64	64	64	-	64	64
	MIC_{90}	-	>2048	-	-	-	>2048	-	>2048	>2048	>2048	-	>2048	>2048	64	>2048	-	1024	2048
	R (≥ 512)	0	23.8	0	100	0	21.8	75.0	56.1	56.1	37.3	0	53.0	14.5	0.05	16.7	40.0	15.0	12.2
Tetracycline	MIC_{50}	-	1	-	-	-	1	-	128	32	2	-	128	1	1	1	-	1	1
	MIC_{90}	-	64	-	-	-	64	-	>128	128	128	-	>128	64	2	128	-	128	64
	R (≥ 16)	0	26.2	0	50.0	0	22.8	37.5	86.0	73.6	35.6	16.7	73.8	12.9	4.7	26.7	0	19.0	13.2
Trimethoprim/ sulfamethoxazole	MIC_{50}	-	0.06	-	-	-	0.06	-	0.12	0.25	0.06	-	0.12	0.06	0.12	0.06	-	0.06	0.06
	MIC_{90}	-	0.25	-	-	-	0.25	-	>128	>128	>128	-	>128	0.25	0.12	0.25	-	0.12	0.25
	R (≥ 4/76)	50.0	4.8	0	0	0	5.3	25.0	30.8	11.8	18.6	0	23.6	1.6	3.1	6.7	60.0	4.8	4.9

*The clinical breakpoints and MIC values of decreased susceptibility are indicated in parentheses. Countries included were Denmark (DK), Germany (GE), France (FR), Italy (IT), Ireland (IR), The Netherlands (NL), Spain (SP) and United Kingdom (UK).

**$MIC_{50/90}$, % resistance and % decreased susceptibility are based on the summed isolate numbers per host animal.

One pig strain and two chicken isolates (all originating from Spain) were clinically resistant to cefotaxime (0.45% of total number). The three isolates were ESBLs (twice *S.* Virchow, CTX-M-9 and one S. Rissen, SHV-12), as described by Nordmann *et al.* (2009). Clinical resistance against cefepime and ciprofloxacin was absent. Decreased Susceptibility was particularly apparent for ciprofloxacin in chicken isolates (35.2%), whereas this was only 3.5% for cattle and 2.9% for pigs. For ampicillin, cefotaxime and gentamicin, Decreased Susceptibility was negligible (1.7% or less).