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A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals

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***Objective*: To study antimicrobial resistance in zoonotic bacteria isolated from food animals in different countries using uniform methodology.**

***Methods*: Samples were taken at slaughter from chickens, pigs and cattle in four EU countries per host. *Escherichia coli* (indicator organism; $n = 2118$), *Salmonella* spp. ($n = 271$) and *Campylobacter* spp. ($n = 1325$) were isolated in national laboratories and MICs tested in a central laboratory against, where appropriate, ampicillin, cefepime, cefotaxime, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, nalidixic acid, streptomycin, tetracycline and trimethoprim/sulfamethoxazole.**

***Results*: Isolation rates were high for *E. coli*, low for *Salmonella* and intermediate for *Campylobacter*. MIC results showed resistance prevalence varied among compounds, hosts and countries. For *E. coli* and *Salmonella*, resistance to newer compounds (cefepime, cefotaxime, ciprofloxacin) was absent or low, but to older compounds (except gentamicin), resistance was variable and higher. *E. coli* isolates from Sweden showed low resistance, whereas among isolates from Spain (pigs), resistance to ampicillin, chloramphenicol, streptomycin, tetracycline and trimethoprim/sulfamethoxazole was higher; the UK, France, the Netherlands, Germany, Italy and Denmark were intermediate. For *Campylobacter* spp. isolates from chickens, nalidixic acid and ciprofloxacin resistance was >30% in France and the Netherlands, >6% in the UK and zero in Sweden. Nalidixic acid resistance was high in cattle (20%–64%), whereas ciprofloxacin resistance was markedly lower in cattle, variable in pigs (3%–21%) and highest in Sweden. Generally, *Campylobacter coli* was more resistant than *Campylobacter jejuni*.**

***Conclusion*: Antimicrobial resistance among enteric organisms in food animals varied among countries, particularly for older antimicrobials, but resistance to newer compounds used to treat disease in humans was generally low.**

Keywords: zoonotic, enteric organisms, food animals, antibiotics, resistance, surveillance

Introduction

The potential for transfer of antimicrobial resistance from enteric zoonotic bacteria of food animals to the human population is a cause of concern. Programmes to monitor resistance are, therefore, essential. A number of countries have national surveillance programmes to assess bacterial susceptibility to antibiotics among enteric bacteria isolated from healthy animals.[1–5] Results of such surveys are difficult to compare with one another since there are differences in sample collection, bacterial isolation and laboratory methodology. Surveillance studies in different countries are best carried out using standardized methods of

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sample collection, organism identification and susceptibility testing,[6] preferably by a single laboratory.[7]

The present European Antimicrobial Susceptibility Surveillance in Animals (EASSA) was coordinated by the European Animal Health Study Centre, Brussels (CEESA). The study was based on bacteria from healthy animals, and employed uniform methods of sampling and isolation, together with a single central laboratory for MIC determination to a panel of antimicrobials commonly used in human medicine. The procedures followed the recommendations of the OIE (World Organization for Animal Health) guidelines[6] and the target organisms were *Salmonella* and *Campylobacter* species as zoonotic organisms, and commensal *Escherichia coli* as an indicator organism. Slaughter is potentially the most important stage for bacterial contamination of meat products, and therefore it is the most relevant point from which to obtain bacterial isolates for susceptibility testing. Faecal or caecal isolates as appropriate were collected from each of the major food animal species: beef cattle, slaughter pigs and broiler chickens.

Materials and methods

Selection of countries and sample sites

Countries included in the programme (Table 1) were representative of major areas of farm animal production in the EU, from Scandinavia in the north to Spain in the south. Responsibility for identification of appropriate animal slaughter sites to provide samples for isolation of target organisms was allocated to a single individual based within each participating country, in collaboration with members of the national meat hygiene services. The national coordinators arranged for samples to be taken by standard procedures and transported to national microbiology laboratories for bacterial isolation. The slaughterhouse sites (if possible at least four per country) were chosen as representative of animal production within individual countries, in terms of animal throughput and geographical location. The numbers of slaughterhouses per country were for chickens 4–16, for pigs 5–15 and for cattle 4–9 (except France: 1 abattoir).

Animal sampling procedures

Pigs and broiler chickens were sampled from Q4 1999 to Q4 2000, cattle were sampled in Q4 2000 to Q4 2001. For broiler chickens, entire caeca were removed at slaughter and dispatched to microbiology laboratories, where their contents were removed. For pigs and cattle, *ca*. 5 g of content was aseptically removed from the large bowel after incision with a scalpel. All samples were taken, where possible, within 10 min of slaughter and held in sterile containers. For chickens and pigs, a single bird or animal was selected at random as being representative of a flock or herd. As the prevalence of *Salmonella* in cattle appears to be particularly low, faecal samples of cattle were taken from 2–5 animals per herd and pooled prior to bacterial isolation to increase the chance of isolating *Salmonella* spp.

The target numbers to be tested were 200 isolates per country per host for *E. coli* and *Campylobacter* spp. in pigs and poultry, and 100 isolates per country per host for *Salmonella* spp. and also for cattle isolates of *E. coli* and *Campylobacter* spp. The low isolation rate found for *Salmonella* spp. was countered by supplementation from the Danish national collection ($n = 95$), which fulfilled the selection criteria, including the time period of collection or by increasing the number of samples tested (France, Netherlands,

Table 1. Numbers of isolates available for MIC testing (percent recovery is indicated in parentheses)

	Chickens			Cattle			Pigs		
	E. coli	*Salmonella* spp.	*Campylobacter* spp.	*E. coli*	*Salmonella* spp.	*Campylobacter* spp.	*E. coli*	*Salmonella* spp.	*Campylobacter* spp.
Denmark[a]	–	–	–	–	–	–	200 (100%)	100 (4%)	196 (50%)
France	199 (99%)	75 (13%)	213 (50%)	21 (100%)	0	0	–	–	–
Germany	–	–	–	355 (92%)	0	126 (66%)	–	–	–
Italy	–	–	–	189 (98%)	0	27 (14%)	–	–	–
Netherlands	204 (100%)	2 (1%)	116 (26%)	–	–	–	200 (99%)	31 (8%)	182 (42%)
Spain	–	–	–	–	–	–	48 (7%)	15 (4%)	4
Sweden	199 (99%)	0	16 (3%)	–	–	–	204 (100%)	1 (0.5%)	209 (35%)
UK	200 (93%)	43 (6%)	200 (70%)	99 (100%)	4 (4%)	36 (50%)	–	–	–
Totals	802	120	545	664	4	189	652	147	591

[a]Percentage recovery of *Salmonella* excluding any supplementation of isolates from the Danish collection.

Spain, UK). The actual sample size per country and per animal species can be calculated from Table 1.

Microbiological isolation and identification

Organisms were isolated at single microbiology laboratories in the same countries as the samples originated, under the supervision of one individual using standard microbiological procedures. An exception was Italy, where isolation was performed at four laboratories local to the region of sampling. One isolate for each bacterial species was retained from each sample.

E. coli. Sample material was plated on MacConkey agar and typical large, pink-to-rose coloured colonies were isolated. Isolates testing positive for indole production were presumed to be *E. coli*. Identification was subsequently confirmed using Fluorocult LMX Broth, where *E. coli* cleaves the fluorogenic substrate.

Salmonella spp. Sample material was pre-enriched in buffered peptone water (20 h at 35°C) and selectively enriched by inoculation on Modified Semisolid Rappaport Vassiliadis Medium at 41.5°C for 24 h. Bacteria were plated on Rambach or other selective media to obtain *Salmonella* spp. Isolates were presumed to be *Salmonella* spp. after a typical colour reaction and production of H_2S (black) and CO_2 on Triple Sugar Iron or Kligler–Hajna medium, and positive production of lysine decarboxylase.

Campylobacter spp. Sample material was diluted in peptone water and plated on *Campylobacter* Blood Free Selective Medium (Modified CCDA-Improved) containing cefoperazone and amphotericin, incubated under an atmosphere of 5% O_2, 10% CO_2 and 85% N_2 at 42°C for 48 h. A preliminary enrichment step was included for material from cattle, as the numbers of campylobacters were assumed to be lower than in broiler chickens and pigs. Campylobacter Enrichment Broth with selective supplement containing cefoperazone, vancomycin, trimethoprim and cycloheximide was used as a medium for the cattle samples. Cultures were incubated at 37°C for 48 h and then plated on selective agar medium, as described above. Small, curved, oxidase-positive Gram-negative bacilli were presumed to be *Campylobacter* spp. Identification to species level was subsequently based on the ability to hydrolyse sodium hippurate and indoxyl acetate, and also susceptibility to cefalothin. Isolates showing unusual MIC patterns (i.e. resistance to nalidixic acid, yet fully susceptible to ciprofloxacin) were identified by multiplex PCR, as described by Inglis & Kalischuk.[8]

Isolates obtained at national microbiology laboratories were sent to the central laboratory (Inveresk Research, Scotland), which was the repository for the culture collection. Isolates were usually sent on dry ice, although some campylobacters were shipped on charcoal transport medium swabs at ambient temperature. Cultures were held at −70°C and suspended in growth medium with glycerol as cryopreservative, until susceptibility testing was performed.

MIC testing

All MIC testing was performed at the central laboratory. *E. coli* and *Salmonella* were tested by standard agar dilution methods: NCCLS M31-A2.[9] To test the susceptibility of *Campylobacter*, Brucella agar was used supplemented with 5% defibrinated sheep's blood containing test agent. Bacterial suspensions were applied to plates by multipoint inoculation at 10^5 cfu per spot. They were then scored for the presence or absence of growth after 48 h incubation at 37°C in an atmosphere of 5% O_2, 7% CO_2 and 88% N_2 using a microaerophilic work station (Fred Baker Scientific, Runcorn, UK). For all MIC tests, the presence or absence of growth was assessed using a 'Domino' image analysis system (Perceptive Instruments Ltd, Steeple Bumpstead, UK) running dedicated MIC test evaluation software.

MICs for the following antibiotics were determined against *E. coli* and salmonella isolates: ampicillin, cefepime, cefotaxime, ciprofloxacin, chloramphenicol, gentamicin, streptomycin, tetracycline and trimethoprim/sulfamethoxazole. MICs for the following antibiotics were determined against campylobacter isolates: ciprofloxacin, erythromycin, gentamicin, nalidixic acid and tetracycline. All antimicrobials were obtained from Sigma Chemical Co. except ciprofloxacin (Bayer HealthCare AG, Leverkusen, Germany) and cefepime (Bristol Myers Squibb, Syracuse, NY, USA). Antibiotics were generally tested in a two-fold concentration series over the range 0.06–128 mg/L. Exceptions were cefepime (0.03 to 16 mg/L), cefotaxime (0.008 to 16 mg/L) and ciprofloxacin (0.002 to 16 mg/L).

Reference standard bacterial strains were tested concurrently as controls, including the following: for tests with *E. coli* and salmonella the quality control strains were *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. More than 98% of the MIC values for these three control strains were within the MIC ranges included in M100-S14.[10] For tests with campylobacter, *Campylobacter jejuni* ATCC 33560 was used.

MIC_{50} and MIC_{90} values, as well as rates of resistances, were calculated and presented. Since there existed no clear differences among slaughter sites within a given country, data were summarized by country. Resistance was defined according to breakpoints published by the NCCLS.[10] Where there were no NCCLS breakpoints published for a particular compound (streptomycin), those adopted by the US National Antimicrobial Resistance Monitoring System (NARMS) were used.[5] Fisher's exact test for pair-wise comparisons was used to determine the significance of differences of resistance prevalence among countries and within animal species. A value of $P \leq 0.05$ was considered significant.

Results

In total, 3714 isolates were available for testing. Numbers of isolates obtained per animal species and the rate of recovery are shown in Table 1; susceptibility findings are reported in Tables 2, 3, 4 and 5.

E. coli (Table 2)

The isolation rate for *E. coli* approached 100% in all hosts and countries, with the exception of Spain, where the rate of recovery of *E. coli* from pigs was <10%.

Ampicillin. The distribution of ampicillin MICs varied considerably between countries. In chickens, resistance was significantly lower in Sweden than in France, the Netherlands or the UK. There was a tendency for resistance to be lower in cattle than in chickens; in France, Germany and the UK, resistance among cattle isolates was even undetectable or close to zero. In pigs, there was also considerable variation in resistance between countries.

Cefepime and cefotaxime. $MIC_{50/90}$ varied from 0.03–0.12 mg/L, but MICs from Spain for cefepime were slightly lower. Resistance was not encountered for either cephalosporin.

Table 2. Summary of antimicrobial susceptibility of *E. coli* isolates[a]

		Chickens				Cattle				Pigs			
		France $n=199$	Netherlands $n=204$	Sweden $n=199$	UK $n=200$	France $n=21$	Germany $n=355$	Italy $n=189$	UK $n=99$	Denmark $n=200$	Netherlands $n=200$	Spain $n=48$	Sweden $n=204$
Ampicillin	MIC_{50}	>128	2	2	4	1	2	2	2	2	2	128	2
	MIC_{90}	>128	>128	4	>128	1	2	>128	4	128	>128	>128	4
	% R	51.3[b]	36.8[c]	5[d]	43.0[b,c]	0	1.7[b]	14.3[c]	1[b]	10.5[b]	17.0[b]	52.1[d]	3.4[c]
Cefepime	MIC_{50}	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.008	0.032
	MIC_{90}	0.063	0.063	0.063	0.063	0.032	0.032	0.032	0.032	0.032	0.063	0.016	0.063
	% R	0	0	0	0	0	0	0	0	0	0	0	0
Cefotaxime	MIC_{50}	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.032	0.063
	MIC_{90}	0.125	0.125	0.125	0.125	0.125	0.125	0.063	0.063	0.063	0.063	0.125	0.125
	% R	0	0	0	0	0	0	0	0	0	0	0	0
Chloramphenicol	MIC_{50}	8	8	8	4	4	8	8	4	8	8	128	4
	MIC_{90}	>128	8	8	8	4	16	16	8	8	32	>128	8
	% R	16.6[b]	3.9[c]	0.5[c]	2[c]	0	2[b,c]	5.3[c]	0[b]	3.5[b]	11.0[c]	52.1[d]	0.5[b]
Ciprofloxacin	MIC_{50}	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.008	0.032	0.016
	MIC_{90}	0.25	0.25	0.016	0.125	0.016	0.016	0.016	0.016	0.016	0.016	0.125	0.016
	% R	4[b]	2.9[b,c]	0[c]	0.5[b,c]	0	0[b]	2.1[b]	0[b]	0	0	0	0
Gentamicin	MIC_{50}	1	1	1	1	0.25	0.5	0.5	0.5	0.5	1	1	1
	MIC_{90}	2	1	1	1	0.5	1	1	0.5	1	1	1	1
	% R	4.5[b]	3.9[b]	0[c]	3.0[b,c]	0	0.3[b]	2.1[b]	0[b]	0	0	0	0
Streptomycin	MIC_{50}	16	32	4	32	4	4	4	4	4	32	64	4
	MIC_{90}	>128	>128	16	>128	8	8	128	8	128	>128	128	64
	% R	46.7[b]	38.2[b]	6.5[c]	41.5[b]	0	3.7[b]	15.3[c]	6.1[b]	31.0[b]	43.5[c]	54.2[d]	10.7[e]
Tetracycline	MIC_{50}	128	128	2	64	1	1	2	1	2	64	2	1
	MIC_{90}	>128	>128	8	>128	1	2	128	2	128	>128	>128	16
	% R	85.4[b]	61.3[c]	6.5[d]	53.5[c]	0	6.5[b]	19.6[c]	9.1[b]	28[b]	59[c]	43.8[c]	10.3[d]
Trimethoprim/ sulfamethoxazole[f]	MIC_{50}	4	0.25	0.063	1	0.063	0.063	0.125	0.063	0.063	0.125	>128	0.063
	MIC_{90}	>128	>128	0.25	>128	0.125	0.125	1	0.125	1	>128	>128	0.125
	% R	50.2[b]	38.2[b]	2[c]	46.5[b]	0	0.8[b]	8.5[c]	2[b,c]	7.5[b]	36.0[c]	52.1[d]	2.5[b]

[a]Resistance (R) breakpoints: ampicillin, ≥32 mg/L; cefepime, ≥32 mg/L; cefotaxime, ≥64 mg/L; chloramphenicol, ≥32 mg/L; ciprofloxacin, ≥4 mg/L; gentamicin, ≥16 mg/L; streptomycin, ≥64 mg/L; tetracycline, ≥16 mg/L; and trimethoprim/sulfamethoxazole, ≥4/76 mg/L.

[b–e]Superscripts within the same host showing different letters indicate significant differences ($P \leq 0.05$, Fisher's exact test). Countries where $n < 40$ were not analysed statistically.

[f]$MIC_{50/90}$ figures refer to trimethoprim concentrations only.

Table 3. Summary of antimicrobial susceptibility of *Salmonella* isolates[a]

		Chicken		Pig		
		France $n=75$	UK $n=43$	Denmark $n=100$	Netherlands $n=31$	Spain $n=15$
Ampicillin	MIC_{50}	>128	1	1	1	1
	MIC_{90}	>128	>128	>128	>128	>128
	% R	54.7	25.6	27.0	16.1	40.0
Cefepime	MIC_{50}	0.063	0.032	0.063	0.032	0.032
	MIC_{90}	0.125	0.125	0.125	0.125	0.063
	% R	0	0	0	0	0
Cefotaxime	MIC_{50}	0.125	0.125	0.125	0.125	0.125
	MIC_{90}	0.125	0.125	0.125	0.125	0.25
	% R	0	0	0	0	0
Chloramphenicol	MIC_{50}	8	8	8	8	8
	MIC_{90}	8	>128	>128	8	>128
	% R	6.7	14.0	21.0	9.7	40.0
Ciprofloxacin	MIC_{50}	0.032	0.032	0.032	0.016	0.032
	MIC_{90}	0.25	0.032	0.032	0.032	0.032
	% R	0	0	0	0	0
Gentamicin	MIC_{50}	0.5	0.5	0.5	0.5	1
	MIC_{90}	1	0.5	1	0.5	1
	% R	5.4	0	0	0	0
Streptomycin	MIC_{50}	32	8	8	8	4
	MIC_{90}	128	128	>128	128	16
	% R	44.0	14.0	32.0	25.8	0
Tetracycline	MIC_{50}	32	2	2	1	>128
	MIC_{90}	128	64	>128	>128	>128
	% R	52.0	34.9	37.0	38.7	100
Trimethoprim/[b] sulfamethoxazole	MIC_{50}	0.063	0.063	0.063	0.063	>128
	MIC_{90}	>128	>128	0.25	>128	>128
	% R	24.0	25.6	9.0	19.4	100

[a]Resistance (R) breakpoints: ampicillin, ≥32 mg/L; cefepime, ≥32 mg/L; cefotaxime, ≥64 mg/L; chloramphenicol, ≥32 mg/L; ciprofloxacin, ≥4 mg/L; gentamicin, ≥16 mg/L; streptomycin, ≥64 mg/L; tetracycline, ≥16 mg/L; and trimethoprim/sulfamethoxazole, ≥4/76 mg/L. Countries with <10 isolates were not included in the table.
[b]$MIC_{50/90}$ figures refer to trimethoprim concentrations only.

Table 4. Percentages of antimicrobial-resistant *Campylobacter* isolates per species[a]

	Chicken		Cattle			Pigs		
	C. jejuni $n=390$	*C. coli* $n=154$	*C. jejuni* $n=141$	*C. coli* $n=17$	*C.* spp. $n=31$	*C. jejuni* $n=122$	*C. coli* $n=418$	*C.* spp. $n=47$
Ciprofloxacin	14.9	39.6	13.5	23.5	3.2	4.9	14.1	2.1
Nalidixic acid	15.1	39.6	17.7	23.5	100	7.4	14.8	78.7
Erythromycin	1.0	13.6	4.3	0	3.2	15.6	32.1	29.8
Gentamicin	0	0	0.7	0	0	0.	0	0
Tetracycline	35.4	58.4	19.9	41.2	0	15.6	19.4	48.9

[a]*Campylobacter* spp. from cattle and pigs comprised *C. fetus*, *C. hyointestinalis*, *C. lanienae* or an as yet unidentified species; *Campylobacter* spp. from chicken ($n=1$) is not included in the table.

Chloramphenicol. In chickens, resistance varied from 0.5% in Sweden to 16.6% in France. In cattle, resistance was undetectable or low, whereas in pigs resistance varied from 0.5% in Sweden to 52.1% in Spain.

Ciprofloxacin. Resistance was absent among *E. coli* with the exception of France, the Netherlands and the UK (chickens) and Italy (cattle). The incidence of resistance did not exceed 4% in any country. MIC_{90} was usually identical to MIC_{50}, but for

Table 5. Summary of antimicrobial susceptibility of *Campylobacter* isolates[a]

		Chickens				Cattle			Pigs		
		France $n=213$	Netherlands $n=116$	Sweden $n=16$	UK $n=200$	Germany $n=126$	Italy $n=27$	UK $n=36$	Denmark $n=196$	Netherlands $n=182$	Sweden $n=209$
Ciprofloxacin	MIC_{50}	0.5	0.25	0.125	0.125	0.25	0.25	0.25	0.125	0.25	0.125
	MIC_{90}	>16	>16	0.25	0.5	16	0.5	1	1	0.25	16
	% R[b]	31.5[c]	34.5[c]	0	6[d]	16.7	3.7	5.6	8.2[c]	3.3[c]	21.1[d]
Nalidixic acid	MIC_{50}	8	8	4	4	4	8	128	4	8	8
	MIC_{90}	>128	>128	4	8	>128	>128	>128	32	32	>128
	% R	31.9[c]	34.5[c]	0	6.5[d]	19.8	44.4	63.9	11.7[c]	20.3[d]	23.0[d]
Erythromycin	MIC_{50}	2	1	1	1	2	2	2	2	4	2
	MIC_{90}	4	2	1	2	4	2	4	>128	>128	4
	% R	8[c]	3.4[c,d]	0	2[d]	3.2	3.7	5.6	36.7[c]	41.8[c]	9.1[d]
Gentamicin	MIC_{50}	0.25	0.25	0.25	0.25	0.25	0.125	0.5	0.5	0.5	0.5
	MIC_{90}	0.5	0.5	0.25	0.25	0.5	0.5	1	1	1	1
	% R	0	0	0	0	0	3.7	0	0	0	0
Tetracycline	MIC_{50}	64	1	0.25	0.5	0.5	0.5	0.5	0.5	64	0.5
	MIC_{90}	>128	128	2	128	128	8	1	2	>128	2
	% R	58.2[c]	30.2[d]	0	35[d]	26.2	7.4	0	1.5[c]	63.7[d]	1.9[c]

[a]Resistance (R) breakpoints: ciprofloxacin, ≥4 mg/L; nalidixic acid, ≥32 mg/L; erythromycin, ≥8 mg/L; gentamicin, ≥16 mg/L; and tetracycline, ≥16 mg/L. Countries with <10 isolates were not included in the table.

[b–d]Superscripts within the same host showing different letters indicate significant differences ($P \leq 0.05$, Fisher's exact test). Countries where $n < 40$ were not analysed statistically.

Spain (pigs), France, the Netherlands and the UK (all chickens) increased values of 0.12 or 0.25 mg/L were observed.

Gentamicin. Resistance was either absent or very low in *E. coli* isolates from all animal species and countries. In poultry, there was low but detectable resistance in *E. coli* among isolates from France, the Netherlands and the UK, although not from Sweden. In cattle samples from Italy, ~2% were found to be resistant. No resistance was detected in samples from pigs in any of the four countries sampled.

Streptomycin. Resistance in *E. coli* isolates from chickens was close to 50%, with the exception of Swedish isolates, where only 6.5% showed resistance. *E. coli* from cattle showed resistance, varying from zero in France (although the number of isolates was limited) to 15.3% in Italy. Isolates from pigs showed resistance rates as high as 54.2% (Spain), but resistance was only 10.7% in isolates from Sweden.

Tetracycline. Among *E. coli* isolates from chickens, resistance was >50%, except in those from Sweden where the resistance rate was only 6.5%. In isolates from cattle, the resistance rate was lower than in chickens, whereas in samples from pigs there was a variable rate from 10.3% (Sweden) to 59% (Netherlands).

Trimethoprim/sulfamethoxazole. The proportion of resistant isolates of *E. coli* from chickens was low in samples from Sweden, and was significantly higher in the other countries sampled. In isolates from cattle, resistance was lower than in those from chickens, with the highest being in those from Italy (8.5%). In *E. coli* isolates from pigs, those from Sweden showed the lowest incidence of resistance (2.5%), those from Spain the highest (52.1%).

Multiple resistance. Multiple resistance was defined as simultaneous resistance to at least four antimicrobials tested, with trimethoprim plus sulfamethoxazole considered as one unit since the testing was in combination. Taking all isolates of *E. coli* collected from the different countries into account, the most frequent phenotypes were ampicillin/streptomycin/tetracycline /trimethoprim plus sulfamethoxazole (in 9.5% of chicken isolates, 2.5% of pig isolates and 1% of cattle isolates).

Salmonella spp. (Table 3)

The overall isolation rate was low (mean 4.9%; ranging from 0%–13%; Table 1); *Salmonella* prevalence per species amounted to 7.1%, 4.5% and ≤0.6% for chickens, pigs and cattle, respectively. Where practicable, the number was supplemented by increasing the number of samples examined, or from the Danish national collection (see above), but even so the low numbers made comparisons difficult. In the case of cattle, chickens (the Netherlands, Sweden) and pigs (Sweden) the small numbers precluded comment on susceptibility to antimicrobials. Following isolation from chickens ($n = 120$), cattle ($n = 4$) and pigs ($n = 147$), 271 isolates were serotyped, with at least one member of 31 salmonella species represented in the total. The main species found (excluding cattle since numbers were too small) were *S.* Typhimurium (40% of the total, mostly pigs), *S.* Heidelberg (9.3%, chickens), *S.* Hadar (6.4%, chickens), *S.* Derby (5.3%), and *S.* Newport (3.6%). Other species were present only in small numbers.

Ampicillin. The incidence of resistance in the isolates overall varied from 16.1% (the Netherlands, pigs) to 54.7% (chickens, France). Other countries where there were significant numbers of isolates recovered had an intermediate resistance. For the individual salmonella species, 31% of the *S.* Typhimurium were resistant to ampicillin ($n = 112$), compared with 83% for *S.* Hadar ($n = 18$), 53% for *S.* Heidelberg ($n = 26$) and 7% for *S.* Derby ($n = 15$).

Cefepime and cefotaxime. Neither resistance nor decreased susceptibility was seen in any of the isolates recovered from any of the countries.

Chloramphenicol. Resistance varied from 40% among isolates from pigs in Spain (although the number of isolates was small) to 6.7% among isolates from chickens in France. Of the resistant isolates, 71% were *S.* Typhimurium, and it is notable that the 15 isolates from Spain were made up of 10 *S.* Tilburg and five *S.* Ohio. *S.* Tilburg was not identified from elsewhere.

Ciprofloxacin. No resistance was seen in any of the isolates recovered from any of the countries, although the *S.* Hadar had a higher MIC_{90} (0.25 mg/L) than the other isolates ($MIC_{90} \leq 0.03$ mg/L).

Gentamicin. The only isolates showing an incidence of resistance were chicken isolates from France (5.4%), which consisted of *S.* Heidelberg ($n = 2$) and *S.* Enteritidis ($n = 1$).

Streptomycin. Resistance overall varied from 0% (pig isolates, Spain, $n = 15$) to 44% (chicken isolates, France). Most of the resistant isolates were *S.* Typhimurium.

Tetracycline. Resistance varied from 35% (chicken isolates, UK) to 100% (pig isolates, Spain, $n = 15$). *S.* Hadar isolates were particularly resistant (33%, $n = 18$), and originated from France.

Trimethoprim/sulfamethoxazole. Resistance varied from 7% (pig isolates, Denmark) to ~25% (chicken isolates, France and UK) to 100% (pig isolates, Spain), and was not linked to particular salmonella serotypes.

Multiple resistance. Resistance to four or more drugs was seen in varying combinations. In chickens, the most common phenotype (5.8%) was the combination of ampicillin /chloramphenicol/streptomycin/tetracycline/trimethoprim plus sulfamethoxazole. Among pig isolates, the most common (15%) was ampicillin/chloramphenicol/streptomycin/trimethoprim plus sulfamethoxazole.

Campylobacter spp. (Tables 4 and 5)

The isolation rate was variable (Table 1), with the lowest isolation rate being from Sweden (3% in chickens) and the highest in the UK (70% in chickens). A few campylobacters were also isolated in Spain, but there were technical difficulties which suggested that this figure may be unreliable and the results have therefore been omitted. In broilers (Table 4), the most frequently isolated species was *C. jejuni* (72%) and 154 isolates (28%) were identified as *Campylobacter coli*. In cattle, 75% and 9% of the isolates were determined as *C. jejuni* and *C. coli*, respectively, but another 16% of the isolates were identified as *Campylobacter fetus* ($n = 18$), *Campylobacter hyointestinalis* ($n = 11$) or *Campylobacter lanienae* ($n = 7$). In contrast, 71% of the isolates

from pigs were identified as *C. coli*, 21% as *C. jejuni*, and 8% as *Campylobacter* spp., mainly comprising a species of *Campylobacter*-like organism ($n = 41$) not yet identified. For the five antibiotics tested, resistance was higher among *C. coli* than *C. jejuni*, except for resistance to gentamicin. Antimicrobial patterns among the non-*C. jejuni*, non-*C. coli* species were similar.

Ciprofloxacin. The highest rates of resistance of *Campylobacter* spp. were among chicken isolates from the Netherlands (34.5%) and France (31.5%). Resistance was low among chicken isolates from the UK (6%) and absent in those from Sweden, but numbers were low ($n = 16$). Swedish pig isolates, all but two being *C. coli* and *C. jejuni*, showed a 21.1% incidence of resistance, and cattle isolates from Germany 16.7%. The incidence of resistance in other countries varied from 3.3%–8.2%.

Nalidixic acid. Resistance was absent among chicken isolates from Sweden, and was low in chicken isolates from the UK (6.5%). Generally, resistance to nalidixic acid resembled the resistance to ciprofloxacin, but cattle isolates from Italy and the UK showed markedly higher incidences of resistance (44.4% and 63.9%, respectively) due to increased numbers of campylobacters other than *C. jejuni* and *C. coli*. Nalidixic acid resistance in porcine isolates from the Netherlands was 20.3%.

Erythromycin. Resistance was highest in pig isolates from Denmark (36.7%) and the Netherlands (41.8%). Resistance was low in strains from chickens (0%–8%) and cattle (3.2%–5.6%) in all countries sampled and in isolates from pigs from Sweden (9%).

Gentamicin. The only resistance encountered was one cattle isolate from Italy (3.7%).

Tetracycline. Tetracycline resistance was highly variable, ranging from 0% in chicken isolates from Sweden and cattle isolates from the UK, to 63.7% among pig isolates from the Netherlands.

Multiple resistance. Multiple resistance was found at low rates in the *Campylobacter* spp. The most frequent was ciprofloxacin/nalidixic acid/erythromycin/tetracycline (1.8% chicken, 0.3% pig). No quadra-resistance was found in cattle isolates. Multiresistant isolates always remained susceptible to gentamicin.

Discussion

Resistance in bacteria isolated from food has been seen as a potential source of resistance in human pathogens.[11,12] Where resistance is present among zoonotic organisms, such as *Salmonella* or *Campylobacter* species, then it is by definition possible for resistant bacteria from animals to be transmitted to a human subject. Surveillance of the resistance rates among pathogens of animals is clearly important in risk assessment and management.[6]

Some European countries, including Denmark,[1] Sweden,[2] the UK[3] and Spain,[4] have resistance surveillance programmes for bacteria isolated from farm animals, particularly in relation to the zoonotic organisms. However, such studies use different methods for sampling, selection or testing of isolates, so any differences seen between countries may result from differences in technical procedure rather than real variation between countries. Comparability of results between isolates from different sources is crucial,[7] and is assisted by use of a central testing laboratory.[13,14] The present study used common methodology and a central testing laboratory to overcome these shortcomings.

For practical reasons, only four countries were tested per host species, but countries were chosen to reflect north/south Europe differences and regional differences in animal husbandry, which may also imply differences in usage of antibacterial compounds. In particular, it was of interest to compare the traditionally conservative approach to antibacterial use in Sweden with countries elsewhere in Europe. Samples were taken immediately post-slaughter since this is the stage where meat is most likely to become contaminated with bacteria of animal origin.

The organisms isolated were *Salmonella* spp., *Campylobacter* spp. and *E. coli* (the last of which, with the exception of strain 0157:H7, is not normally classed as zoonotic but was included as an indicator organism). The host species sampled were chickens, pigs and cattle. The first two species are usually housed under relatively intensive conditions, whereas cattle in the EU are generally less intensively reared. Intensive housing may be associated with greater disease potential and therefore a greater tendency for antibiotic use to control disease. Since concern is primarily with resistance in human pathogens, the antibiotics tested were compounds considered important for treating human patients rather than antibiotics of importance in veterinary medicine.

The antibacterial susceptibility was determined by agar dilution MIC determination and allocation of breakpoints (NCCLS where available). In the case of campylobacters, there are no internationally agreed breakpoints, so in this study we used the NCCLS interpretive criteria for other bacteria species to analyse data for *Campylobacter*. It must, however, be emphasized that due to the lack of validated breakpoints for campylobacters, resistance *in vivo* remains undefined. For *Campylobacter*, several methods, including disc diffusion, broth microdilution, Etest and agar dilution have been used to determine the *in vitro* susceptibility. However, until recently none of these methods has been validated and standardized, which complicates interpretation of different studies. In 2002, the NCCLS Subcommittee on Veterinary Antimicrobial Susceptibility Testing (NCCLS-VAST) approved the agar dilution test as a validated, standardized test to determine antimicrobial susceptibility, and *C. jejuni* ATCC 33560 was validated as a quality-control organism. We conducted our study before the NCCLS method[9] finally became available. A retrospective comparison of the compounds with quality-control MIC ranges included in NCCLS M100-S14 (ciprofloxacin, erythromycin, gentamicin) showed that the majority of the MIC values for *C. jejuni* ATCC 33560 were within the acceptable ranges, even though there were minor differences in methodology.

It was expected that the isolation rate for *E. coli* in faecal samples would be high, and indeed with the exception of isolates from pigs in Spain, this was the case. Samples from Spanish pigs showed an unexpectedly low isolation rate of *E. coli* (7%). The reason for this remains unclear, but might have reflected differences in diet or the inclusion of antimicrobial compounds in the feed. Isolation rates for salmonellae were low, often preventing comparison between countries and host species. Supplementation of isolates from additional screening and from the Danish collection was used in some cases to overcome

the absence of isolates from the available faecal samples. *Campylobacter* isolation rates varied between countries and host species, and in the case of Spain, technical problems in the country laboratory prevented use of these isolates.

Overall comparison of resistance rates suggested that, where the numbers of isolates for testing allowed, comparison of results from different countries revealed interesting differences. Isolates from Sweden tended to show a lower incidence of resistance than those from the other countries sampled, whereas isolates from Spain tended to show a higher incidence of resistance than elsewhere. Other countries (UK, France, Netherlands and Denmark) were roughly intermediate. For *E. coli* and *Salmonella* spp., the incidence of resistance to the older compounds such as ampicillin and tetracycline was rather high, although variable. Conversely, resistance to newer compounds such as ciprofloxacin, cefotaxime and cefepime was low or absent. Gentamicin, although a relatively old compound, has had little use in animals, and as expected, resistance in most cases was absent, although in France the incidence was 4.5% (*E. coli*) and 5.4% (salmonellae). Isolates of *E. coli* from cattle had generally lower levels of antimicrobial resistance than did isolates from the other two species sampled. Whereas this may reflect lower usage of antimicrobials, it may also be explained by the greater maturity, since adult cattle have been shown to harbour less resistance than calves.[15] Multiple resistance was found to some degree in all host species, as has been reported in national surveys.[1–3]

For salmonellae, the low isolation rate (even with some supplementation) limited the comparisons that could be made with France and the UK (chickens) and Denmark, the Netherlands and Spain (pigs). A wide variety of species of salmonella were found, although there were host links; *S.* Typhimurium, Derby and Tilburg to pigs, *S.* Heidelberg, Hadar, Enteritidis and Newport to chickens and *S.* Derby to cattle.

Among *Campylobacter* isolates, ciprofloxacin resistance was present in >30% of chicken isolates from France and the Netherlands. In cattle isolates, the ciprofloxacin resistance was lower, although in the case of isolates from Italy and the UK this resistance was associated with an unexpectedly high frequency of resistance to nalidixic acid. This may have been the result of some of the cattle isolates being *C. fetus*, *C. hyointestinalis and C. lanienae*, which are intrinsically less sensitive to nalidixic acid than *C. jejuni* and *C. coli.*[16] This outcome seems compatible with an Italian study,[17] where a high percentage of thermophilic cattle strains, not identified as *C. jejuni* and *C. coli*, were detected; the majority were assessed to be *C. hyointestinalis* based on a biochemical identification test. Similarly, Canadian workers detected various *Campylobacter* species in bovine faeces, among them *C. fetus*, *C. hyointestinalis* and *C. lanienae*.[8] There was an unusually high incidence of resistance to ciprofloxacin (21%) among pig isolates from Sweden, although a similarly high frequency of fluoroquinolone resistance among campylobacter isolates from pigs in Sweden (30%) has been previously reported.[2]

Macrolide resistance was present in *Campylobacter* isolates from pigs (predominantly *C. coli*) of the Netherlands and Denmark, but resistance was lower in isolates from other animal species. *Campylobacter* resistance to tetracycline varied from 0% (Swedish chickens and UK cattle) to 64% (Netherlands pigs). Resistance to macrolides among *C. jejuni*, which is the causative agent responsible for over 90%–95% of human campylobacteriosis, was low and has been reported as changing little over time.[18] Our findings are compatible with the literature and confirm macrolides as treatment of first choice in EU countries.[19,20]

Taken together, for all three bacterial species there was considerable variation between countries and hosts in the incidence of resistance. It is tempting to ascribe such variation to differences in consumption of antimicrobials.[1] In 1999, the European Medicines Evaluation Agency published figures[21] (obtained from the animal health industry association and based on sales in 1997) which showed that the compounds most commonly used for therapy in animals were tetracyclines, macrolides and penicillins, namely those where the present results showed resistance among zoonotic bacteria was highest. However, these compounds are also those with the longest history of use, so both exposure volume and duration may influence the resistance seen. Conversely, those newer compounds with lower usage, such as fluoroquinolones and newer cephalosporins, were associated with little or no resistance. The countries using the greatest quantities were predictably those with the largest agricultural industry (the UK, France, Germany and Spain). Sweden has a comparatively limited animal production and a low animal population density, with a moderate (but by no means negligible) consumption of therapeutic antibacterial compounds.[2] A comparison of consumption per animal species would be helpful but is unrealistic because figures are rarely broken down to species level.

Although antimicrobial consumption differences may explain some of the findings from this study, anomalies remain. Chloramphenicol has been banned from use in farm animals in the EU for many years, and the related compound florfenicol, although used in cattle, was not used in pigs or chickens at the time of this study. Therefore, the relatively high level of chloramphenicol resistance among *E. coli* and *Salmonella* isolates from chickens and pigs in general, and compared with isolates from cattle, is not explained by phenicol use in veterinary medicine. Other researchers have also reported chloramphenicol resistance among *E. coli* and *Salmonella* spp. isolates from chickens and pigs in the absence of phenicol use in these animal species for many years.[1,2,5,22] Co-resistance with other unrelated compounds appears the likely explanation.[23,24]

Other anomalies were found, such as the relatively high incidence of ciprofloxacin resistance among *Campylobacter* spp. isolates from pigs in Sweden, referred to above. This was unexpected since no fluoroquinolones are authorized for group treatment of pigs in Sweden. Moreover, the total consumption of fluoroquinolones in Sweden in 2000 (all species) was 157 kg[2] compared with 150 kg in Denmark for the same year,[1] but for a much larger pig production. Conversely, quinolone resistance, as measured by disc diffusion, declined among *Salmonella* Typhimurium isolated from cattle in Belgium during 1991–1998, during a period when there was no apparent fall in consumption.[25] Similar observations were made in Germany.[26] Clonal spread of quinolone-sensitive strains was a more likely explanation for the reduced resistance than a link with consumption.[27] Such observations, like those for chloramphenicol (see above), demonstrate at least some disconnection between antimicrobial resistance and veterinary use of the same class of antimicrobial drug. Interestingly, a high resistance prevalence to antimicrobial compounds was found in isolates from humans living in a remote rural Bolivian community, with virtually no exposure to antimicrobials either in humans or animals.[28] Similarly, high levels of antimicrobial resistance were found among

small wild rodents living in woodland separated from animal and human contact.[29] It therefore appears that detailed information on antimicrobial consumption by farm animals, though no doubt interesting and informative, may fail to explain all of the complexities associated with the epidemiology of antimicrobial resistance.

This survey, across the EU and based on uniform methodology, therefore demonstrates that there are differences in incidence of antimicrobial resistance between different host species, different EU countries and different bacterial species, which are not attributable to technical differences in sampling or testing. The multifactorial complexity of antimicrobial resistance highlights the value of a pan-European susceptibility surveillance programme that allows direct comparison across animal species and individual countries. It is desirable that such studies be repeated in future years to study temporal trends and to determine whether the generally low level of resistance to newer antibiotics can be maintained.

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References

1. DANMAP. (2000). *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark*. Danish Veterinary Laboratory, Copenhagen, Denmark.

2. SVARM. (2001). Swedish Veterinary Antimicrobial Resistance Monitoring. National Veterinary Institute, Uppsala, Sweden.

3. Veterinary Laboratories Agency. (2001). Antimicrobial Sensitivity Report 1999. Department for Environment, Food and Rural Affairs U.K. [Online.] http://www.defra.gov.uk/animalh/diseases/zoonoses/zoonoses_reports/report.PDF (August 2004, date last accessed).

4. Moreno, M. A., Dominguez, L., Teshager, T. *et al.* (2000). Antibiotic resistance monitoring: the Spanish programme. The VAV Network. Red de Vigilancia de Resistencias Antibioticas en Bacterias de Origen Veterinario. *International Journal of Antimicrobial Agents* **14**, 285–90.

5. NARMS. (2001). FDA/USDA/CDC national antimicrobial resistance monitoring system-enteric bacteria (NARMS-EB) Veterinary Isolates Preliminary Report 2001. [Online.] http://www.arru.saa.ars.usda.gov/narms/narms_2001/narms_toc01.htm (August 2004, date last accessed).

6. Franklin, A., Acar, J., Anthony, F. *et al.* (2000). Antimicrobial resistance: harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. *Scientific and Technical Revue, Office International des Epizootiques (O.I.E.)* **20**, 859–70.

7. White, D. G., Acar, J., Anthony, F. *et al.* (2001). Antimicrobial resistance: standardisation and harmonisation of laboratory methodologies for the detection and quantification of antimicrobial resistance. *Scientific and Technical Revue, Office International des Epizootiques (O.I.E.)* **20**, 849–58.

8. Inglis, G. D. & Kalischuk, L. D. (2003). Use of PCR for direct detection of Campylobacter species in bovine feces. *Applied Environmental Microbiology* **69**, 3435–47.

9. National Committee for Clinical Laboratory Standards. (2002). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals—Second Edition: Approved Standard M31-A2*. NCCLS, Wayne, PA, USA.

10. National Committee for Clinical Laboratory Standards. (2004). *Performance Standards for Antimicrobial Susceptibility Testing: Fourteenth Informational Supplement M100-S14*. NCCLS, Wayne, PA, USA.

11. Schwartz, S., Kehrenberg, C. & Walsh, T. R. (2001). Use of antimicrobial agents in veterinary medicine and food animal production. *International Journal of Antimicrobial Agents* **17**, 432–7.

12. Wise, R. & Soulsby, E. J. L. (2002). Antibiotic resistance—an evolving problem. *Veterinary Record* **151**, 371–2.

13. Bax, R., Bywater, R., Cornaglia, G. *et al.* (2001). Surveillance of antimicrobial resistance—what, how and whither? *Clinical Microbiology and Infection* **7**, 316–25.

14. Kahlmeter, G. & Brown, D. F. J. (2002). Resistance surveillance studies—comparability of results and quality assurance of methods. *Journal of Antimicrobial Chemotherapy* **50**, 775–7.

15. Khachatryan, A. R., Hancock, D., Besser, T. E. *et al.* (2004). Role of calf-adapted Escherichia coli in maintenance of antimicrobial resistance in dairy calves. *Applied Environmental Microbiology* **70**, 752–7.

16. Taylor, D. E., Ng, L.-K. & Lior, H. (1985). Susceptibility of Campylobacter species to nalidixic acid, enoxacin and other DNA gyrase inhibitors. *Antimicrobial Agents and Chemotherapy* **28**, 708–10.

17. Pezzotti, G., Serafin, A., Luzzi, I. *et al.* (2003). Occurrence and resistance to antibiotics of Campylobacter jejuni and Campylobacter coli in animals and meat in northeastern Italy. *International Journal of Food Microbiology* **82**, 281–7.

18. Nachamkin, I., Ung, H. & Li, M. (2002). Increasing fluoroquinolone resistance in Campylobacter jejuni, Pennsylvania, USA, 1982–2001. *Emerging Infectious Diseases* **8**, 1501–3.

19. Kist, M. (2002). Impact and management of Campylobacter in human medicine—European perspective. *International Journal of Infectious Diseases* **6**, Suppl. 3, 44–8.

20. Wagner, J., Jabbusch, M., Eisenblätter, M. *et al.* (2003). Susceptibilities of *Campylobacter jejuni* isolates from Germany to ciprofloxacin, moxifloxacin, erythromycin, clindamycin, and tetracycline. *Antimicrobial Agents and Chemotherapy* **47**, 2358–61.

21. European Medicines Evaluation Agency. (1999). Report: Antibiotic resistance in the European Union associated with therapeutic use of veterinary medicines, EMEA/CVMP/342/99-Final.

22. Keyes, K., Hudson, C., Maurer, J. J. *et al.* (2000). Detection of florfenicol resistance genes in Escherichia coli isolated from sick chickens. *Antimicrobial Agents and Chemotherapy* **44**, 421–4.

23. Chiew, Y.-F., Yeo, S.-F., Hall, M. C. *et al.* (1998). Can susceptibility to an antimicrobial be restored by halting its use? The case of streptomycin versus Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy* **41**, 247–51.

24. Mircovich, C., Chauvin, P., Sanders, M.-H. *et al.* (2004). Modes d'elevage des porcs et prévalence de E. coli résistants aux antimicrobiens. *Journées Recherche Porcine* **36**, 365–70.

25. Imberechts, H., D'Hooghe, I., Bouchet, H. *et al.* (2000). Apparent loss of enrofloxacin resistance in bovine Salmonella

typhimurium strains isolated in Belgium 1991–1998. *Veterinary Record* **147**, 76–7.

26. Malorny, B., Schroeter, A. & Helmuth, R. (1999). Incidence of quinolone resistance over the period 1986 to 1998 in veterinary Salmonella isolates from Germany. *Antimicrobial Agents and Chemotherapy* **43**, 2278–82.

27. Hancock, D., Besser, T. Gay, J., *et al.* (2000). The global epidemiology of multiresistant Salmonella enterica serovar Typhimurium DT104. In *Emerging Diseases of Animals* (Brown, C. & Bolin, C. Eds), pp. 217–43. ASM Press, Washington, DC, USA.

28. Bartelloni, A., Bartelsi, F., Mantella, A. *et al.* (2004). High prevalence of acquired antimicrobial resistance unrelated to heavy antimicrobial consumption. *Journal of Infectious Diseases* **189**, 1291–4.

29. Gilliver, M., Bennet, M., Begon, M. *et al.* (1999). Enterobacteria: antibiotic resistance found in wild rodents. *Nature* **401**, 233–4.