J Antimicrob Chemother 2012; **67**: 638–651
doi:10.1093/jac/dkr539 Advance Access publication 29 December 2011

Journal of Antimicrobial Chemotherapy

Pan-European monitoring of susceptibility to human-use antimicrobial agents in enteric bacteria isolated from healthy food-producing animals

Anno de Jong[1,2]*, Valérie Thomas[1,3], Shabbir Simjee[1,4], Kevin Godinho[1,5], Brigitte Schiessl[1,6], Ulrich Klein[1,6], Pascal Butty[1,7], Michel Vallé[1,8], Hervé Marion[9] and Thomas R. Shryock[10]

[1]EASSA Study Group, c/o CEESA, 1 Rue Defacqz, Brussels, Belgium; [2]Bayer HealthCare AG, Animal Health GmbH, Leverkusen, Germany; [3]MSD Animal Health, Boxmeer, The Netherlands; [4]Elanco Animal Health, Basingstoke, UK; [5]Pfizer Animal Health, Sandwich, UK; [6]Novartis Animal Health, Basel, Switzerland; [7]Ceva Santé Animale, Libourne, France; [8]Vetoquinol S.A., Lure, France; [9]European Animal Health Study Centre (CEESA), Brussels, Belgium; [10]Elanco Animal Health, Greenfield, IN, USA

*Corresponding author. Tel: +49-2173-384475; Fax: +49-2173-383502; E-mail: anno.jong@bayer.com

Received 14 October 2011; returned 7 November 2011; revised 23 November 2011; accepted 25 November 2011

Objectives: To determine the antimicrobial susceptibility of *Escherichia coli*, *Salmonella*, *Campylobacter* and *Enterococcus* from cattle, pigs and chickens across the European Union (EU) using uniform methodology.

Methods: Intestinal samples (1624) were taken at slaughter across five EU countries. Bacteria were isolated in national laboratories, whilst MICs were determined in a central laboratory for key antimicrobials used in human medicine. Clinical resistance was based on CLSI breakpoints and decreased susceptibility based on European Food Safety Authority (EFSA)/EUCAST epidemiological cut-off values.

Results: Isolation rates were high for *E. coli* ($n=1540$), low for *Salmonella* ($n=201$) and intermediate for *Campylobacter* ($n=940$) and *Enterococcus* ($n=786$). For *E. coli* and *Salmonella*, clinical resistance to newer compounds (cefepime, cefotaxime and ciprofloxacin) was absent or low, but decreased susceptibility was apparent, particularly in chicken strains. Resistance to older compounds (except gentamicin) was variable and higher. Colistin resistance was absent for *E. coli*, but apparent for *Salmonella*. For *Campylobacter jejuni*, ciprofloxacin resistance was markedly prevalent for chickens, whereas clinical resistance and decreased susceptibility to erythromycin was absent or very low. For *Campylobacter coli*, resistance was notably higher. None of the *Enterococcus faecium* strains was resistant to linezolid, but some were resistant to ampicillin or vancomycin. Resistance to quinupristin/dalfopristin was frequent.

Conclusions: Resistance patterns varied widely depending on bacterial species, antibiotics, hosts and region. Resistance varied among countries, particularly for older antimicrobials, but clinical resistance to newer antibiotics used to treat foodborne disease in humans was generally very low. In the absence of resistance to newer compounds in *E. coli* and *Salmonella*, the apparent decreased susceptibility should be monitored.

Keywords: antimicrobial resistance, *E. coli*, *Salmonella*, *Campylobacter*, *Enterococcus*, surveillance

Introduction

The potential for transmission of antimicrobial-resistant enteric zoonotic bacteria from food animals to humans via contaminated food has been a public health concern for several decades. Bacteria, carrying antimicrobial resistance genes, found in the intestinal tract of food animals could contaminate carcasses and food products, which may lead to foodborne disease in consumers that may not respond well to antimicrobial treatment. Programmes to monitor zoonotic bacterial resistance are therefore essential to assist in risk management interventions that are guided by risk assessment. Various European countries have implemented national monitoring programmes [e.g. Denmark (DANMAP), France (FARM), Netherlands (MARAN), Norway (NORM-VET), Sweden (SVARM) and Spain (VAV)] to assess susceptibility to antibiotics among enteric bacteria isolated from healthy food animals.[1–6] However, results of these surveys are difficult to compare with one another since there are differences between the national programmes (e.g. point of sample collection, bacterial isolation/laboratory methodology and interpretive criteria). Multinational monitoring studies are best carried out using standardized and uniform methods of sample collection, organism identification, antimicrobial susceptibility testing and interpretive criteria,[7] preferably by a single

laboratory.[8–10] Moreover, many national surveys use different interpretive criteria and some have changed the interpretive criteria over time.[11] In this respect, it is important to note that MIC interpretive criteria appropriate for predicting clinical efficacy (clinical breakpoints) might differ from those criteria used for surveillance purposes (epidemiological cut-off values).[12,13] An isolate might, through spontaneous mutations or acquisition of resistance genes, exhibit decreased susceptibility to a given antimicrobial, but still have a sufficiently low MIC value to allow successful therapy. Thus, for monitoring purposes the isolate might be considered microbiologically resistant, but clinically susceptible. For major enteric species, epidemiological cut-off values have been defined by EUCAST and the European Food Safety Authority (EFSA).[13–15]

The present surveillance study was part of the European Antimicrobial Susceptibility Surveillance in Animals (EASSA), which is coordinated by the European Animal Health Study Centre (CEESA). Sampling periods 1999–2001 and 2002–03 have been reported previously.[16,17] The EASSA programme collects bacteria from healthy food animals and employs a protocol with common methods of sampling and bacterial isolation, together with a single central laboratory for determination of the MICs of a panel of antimicrobials commonly used in human medicine. The zoonotic organisms of interest are *Salmonella* and *Campylobacter* species, and commensal *Escherichia coli* and *Enterococcus* species as indicator organisms. Faecal or caecal isolates were collected from each of the major food animal species for three production categories: beef cattle, slaughter pigs and broiler chickens. Both epidemiological cut-off values and clinical breakpoints were applied to determine the susceptibility of the organisms.

Methods

Sampling procedures

Countries included in the programme were representative of major areas of cattle, pig and chicken production in the European Union (EU) from Scandinavia in the north to Spain and Italy in the south. Five countries were selected for each animal species (see Table 1). A common protocol for sampling was used across countries and species, as described previously.[17] The slaughterhouses (per country: 4–9 for cattle, 4–14 for pigs and 4–10 for chickens) were selected based on animal throughput and geographical distribution within the countries. Staggered sampling began in 2003 or 2004 and continued for a 1 year period in each country, resulting in a sampling period from 2003 to 2005. The targeted number of samples was 100 per country and per host, with few exceptions. A single animal was selected at random as being representative of a flock or herd. As the prevalence of *Salmonella enterica* was particularly low, efforts were made to supplement the numbers by adding isolates from the French and Spanish national collections that fulfilled the selection criteria. The final number of isolates per country and per animal species, as well as the total numbers per host, are shown in Tables 1–5.

Microbiological isolation and identification

Organisms were isolated by a single microbiology laboratory in each country using standard microbiological procedures. The only exceptions to this were Germany and France (two laboratories each) and Italy (four laboratories), where isolation was performed in laboratories located in the regions of sampling. One randomly selected isolate for each bacterial species was retained from each sample. Isolates obtained at national microbiology laboratories were sent to the central laboratory (Charles River, Scotland), which was the repository for the CEESA culture collection. Cultures were stored at −70°C suspended in growth medium with glycerol as cryopreservative until antimicrobial susceptibility testing was performed.

E. coli, *Salmonella*, *Campylobacter* and *Enterococcus* were recovered and identified as described previously.[16–18] *Salmonella* isolates were serotyped according to the Kauffmann–White scheme. If applicable, phage typing was conducted. Identification of *Campylobacter jejuni* and *Campylobacter coli* isolates was based on the ability to hydrolyse sodium hippurate and indoxyl acetate, and also susceptibility to cephalothin. Isolates showing unusual MIC patterns (e.g. resistance to nalidixic acid, yet full susceptibility to ciprofloxacin) were re-examined by real-time PCR for identification of C. *jejuni* or *C. coli*. Recovery and identification of *Enterococcus faecium* and *Enterococcus faecalis* isolates were conducted by standard phenotypic methods, as described previously,[17,18] and in a few countries by PCR. The primary focus with respect to public health is on *E. faecium* and *E. faecalis*. Other *Enterococcus* species were not included as they have less relevance as indicator organisms.

Antimicrobial susceptibility testing

All MIC testing was performed at the central laboratory. *E. coli*, *Salmonella* and *Enterococcus* were tested by standard agar dilution methods according to the recommendations of the CLSI (M31-A3).[19] *Campylobacter* were tested according to M45-A2.[20] Reference strains were tested concurrently: for tests with *E. coli*, *Salmonella* and *Enterococcus* the quality control strains were *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212. For tests with campylobacter, *C. jejuni* ATCC 33560 was used.

Antibiotics selected for MIC testing are in accordance with the EFSA recommendations,[13,14] including the antibiotics critically important for human medicine. MICs of the following nine antibiotics were determined against *E. coli* isolates: ampicillin, cefepime, cefotaxime, ciprofloxacin, chloramphenicol, colistin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole. For *Salmonella* isolates, nalidixic acid, streptomycin and sulfisoxazole were added to this panel of antibiotics. MICs of ciprofloxacin, erythromycin, gentamicin, nalidixic acid and tetracycline were determined for *Campylobacter*. For enterococci the susceptibility to the following five antibiotics/antibiotic combinations was determined: ampicillin, gentamicin, linezolid, quinupristin/dalfopristin and vancomycin. All antimicrobials were obtained from Sigma Chemical Co. except cefepime (Bristol Myers Squibb, Syracuse, NY, USA), ciprofloxacin (Bayer HealthCare AG, Leverkusen, Germany), linezolid (Pfizer Ltd, Sandwich, UK) and quinupristin/dalfopristin (King Pharmaceuticals, Bristol, TN, USA). For each of the antimicrobial agents, 12–15 concentrations in 2-fold dilution series were tested.[17] *E. coli* and *Salmonella* isolates exhibiting cefotaxime MICs exceeding 0.5 and 1 mg/L, respectively, were tested phenotypically for the presence of extended-spectrum β-lactamases (ESBLs) according to the CLSI recommendations.

MIC_{50} and MIC_{90} values, as well as percentages of clinical resistance, were calculated. Since no noticeable differences were determined between sampling sites within a given country, results were summarized by country. Clinical resistance for Enterobacteriaceae and enterococci was determined for each drug, organism, animal source and country according to CLSI guidelines as defined by M100-S20[21] and for *Campylobacter* as defined by M45-A2 (ciprofloxacin, erythromycin, tetracycline)[20] or M100-S20 (gentamicin, nalidixic acid).[21] In the absence of CLSI breakpoints (streptomycin, colistin), the breakpoints adopted by the US National Antimicrobial Resistance Monitoring System (NARMS)[22] (streptomycin) and recommended by EUCAST[15] (colistin) for Enterobacteriaceae were applied. For the purpose of this study, isolates with intermediate susceptibility were categorized as susceptible. Additionally, any detected

decreased susceptibility was based on epidemiological cut-off values (ECVs) as defined by EFSA.[13,14] The number of isolates with MIC values exceeding the wild-type MIC distribution (above ECVs) but not deemed to be clinically resistant (below clinical breakpoint) was used to calculate the percentage of decreased susceptibility. For some compounds the decreased susceptibility refers to a range of dilution steps (e.g. ciprofloxacin for *Salmonella*: 0.12–2 mg/L); it may also comprise only one dilution difference (e.g. ciprofloxacin for *Campylobacter* or quinupristin/dalfopristin for *E. faecium*: 2 mg/L).

Two-sided χ^2 tests were used for an overall comparison and, in the case of a significant difference, for pairwise comparisons of resistance and decreased susceptibility prevalence between countries and between animal species. Countries or animal species with <10 isolates were not considered in the statistical analysis. A *P* value of ≤0.05 was considered significant.

Results

In total, 1624 samples were investigated and 3465 isolates were available for testing. The total numbers of *E. coli*, *Salmonella*, *C. jejuni*, *C. coli*, *E. faecium* and *E. faecalis* isolates were 1540, 201, 305, 602, 634 and 67, respectively. Their antibiotic susceptibility profiles are presented as $MIC_{50/90}$ and percentage of resistance in Tables 1–5; decreased susceptibility is summarized in Table 6. Non-*C. jejuni*/*coli* (33), as well as non-*E. faecium*/*faecalis* (85) isolates, were excluded from further analyses.

E. coli

The isolation rate for *E. coli* approached 100% in all hosts and countries with the exception of Denmark (pigs) and Germany (chickens). The total number of isolates for each host just exceeded 500 (Table 1).

Generally, the prevalence of resistance was markedly lower among cattle isolates than among pig and chicken isolates. The prevalence of resistance was highest against ampicillin, tetracycline and trimethoprim/sulfamethoxazole, ranging on average from 10.4% to 50.4%, 17.1% to 70.6% and 9.0% to 43.1%, respectively, across all hosts. Marked country differences were noted for all three antibiotics. For instance, in chickens, ampicillin resistance was significantly lower in France than in the Netherlands, Spain or the UK; prevalence of resistance was the highest in Germany (75%). In pigs, isolates from Spain showed the highest prevalence of ampicillin resistance (66%). Resistance was much lower in cattle than in pigs and chickens (20%–23% in France and Italy and 0%–5% in Germany, Ireland and the UK). Similarly, in samples from pigs there was a range of tetracycline resistance from 36% (Denmark) to 94% (Spain). The proportion of isolates resistant to trimethoprim/sulfamethoxazole from chickens in France and Spain was significantly lower than in the other countries sampled. Resistance was almost absent for colistin and low for gentamicin (on average 1%–4%) in all hosts. In chickens and pigs resistance to chloramphenicol—banned from use in food animals for many years in the EU—was on average 13% and 20%, respectively.

Of the cephalosporins, cefotaxime MIC_{50} and MIC_{90} for *E. coli* were 0.06 and 0.06–0.12 mg/L, respectively; clinical resistance was absent in cattle and only encountered for one isolate from pigs in Denmark (Table 1). For chickens, resistance was only found in the Netherlands (2.6%) and Spain (6%). Eight of these *E. coli* isolates, all of chicken origin, were phenotypical ESBLs. The ciprofloxacin MIC_{90} was generally one dilution higher than the MIC_{50} (i.e. 0.008 mg/L) for cattle and pigs, but in chickens increased MIC_{90} values of 0.06–8 mg/L (Spain) were observed. The prevalence of clinical resistance was similar among four countries in chickens (1%–2%), but for the fifth country (Spain) resistance was markedly higher (22%). In contrast, clinical resistance was absent among *E. coli* isolates from cattle and pigs, with the exception of Italy and France (cattle 4%) and Germany (pigs 1%).

Multiple drug resistance was defined as resistance to at least four antimicrobials of different classes tested, with trimethoprim plus sulfamethoxazole considered as one unit since the testing was in combination. Overall multiple drug resistance amounted to 8.2% (cattle 4.6%; pigs 11.5%; chickens 8.5%). The most frequent phenotype was represented by resistance to ampicillin, chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole (cattle 1.2%; pigs 11.0%; chickens 5.8%). Overall, twelve isolates (0.8%) were resistant to five compounds (the most frequent phenotype was ampicillin, chloramphenicol, ciprofloxacin, tetracycline, trimethoprim/sulfamethoxazole) and five isolates (0.3%) to six compounds (in all cases but one ampicillin, chloramphenicol, ciprofloxacin, gentamicin, tetracycline, trimethoprim/sulfamethoxazole).

Salmonella spp.

The overall isolation percentage was low (mean 3.5%; range 0%–12%); *Salmonella* prevalence was 2%, 6% and 3% for cattle, pigs and chickens, respectively. Where practicable, the number was supplemented by adding isolates from the national collections (see above), but nevertheless the limited number of isolates makes comparison between countries difficult. In the case of chickens (France, the Netherlands and UK), pigs (Denmark and Germany) and cattle (all five countries), the small numbers precluded proper evaluation of antimicrobial susceptibility. Hence, overall figures are presented for each host species (pigs, $n=143$; chickens, $n=49$) and for countries from which >15 *Salmonella* isolates were recovered (Table 2). Results from cattle are not presented as only nine isolates were recovered. In all, 201 isolates were serotyped, with at least one member of 30 *Salmonella* serotypes represented. The main serotypes found (excluding cattle since numbers were too small) were *Salmonella* Typhimurium (22% of the total, only pigs), *Salmonella* Derby (18%, only pigs), *Salmonella* Indiana (12%, only chickens), *Salmonella* Rissen (13%, only pigs), *Salmonella* Bredeney (4%, only pigs) and *Salmonella* Enteritidis (12%, virtually only chickens). Other serotypes (26%) were present only in small numbers ($n=1–5$).

The picture for *Salmonella* differs from that for *E. coli*, with the prevalence of resistance being most notable for the porcine isolates (Table 2). The overall prevalence of ampicillin resistance in pigs (32%) was markedly higher than for cattle (11%; data not shown) and chickens (14%). For the frequently detected individual serotypes, 64% of *Salmonella* Typhimurium ($n=29$) were resistant to ampicillin, compared with 6% for *Salmonella* Derby ($n=2$) and 0% for *Salmonella* Indiana. Resistance among pig isolates to sulfisoxazole and tetracycline was 50% and 72%, respectively, whereas it varied from 22% to 35% for the other older molecules chloramphenicol, streptomycin and

Table 1. Summary of antimicrobial susceptibility of *E. coli* isolates[a]

	Cattle						Pigs						Chickens					
	France, $n=102$	Germany, $n=99$	Italy, $n=96$	Ireland, $n=111$	UK, $n=94$	total[b], $n=502$	Denmark, $n=75$	France, $n=101$	Germany, $n=104$	Netherlands, $n=140$	Spain, $n=100$	total[b], $n=520$	France, $n=102$	Germany, $n=59$	Netherlands, $n=154$	Spain, $n=100$	UK, $n=103$	total[b], $n=518$
Ampicillin																		
MIC_{50}	2	2	2	2	2	2	2	2	2	2	>128	2	2	>128	8	32	>128	32
MIC_{90}	>128	4	>128	2	4	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
% R	22.5^{v}	5.1^{w}	19.8^{v}	0^{x}	5.3^{w}	10.4^{V}	24.0^{v}	24.8^{v}	33.7^{v}	25.7^{v}	66.0^{w}	34.6^{W}	32.4^{v}	74.6^{w}	50.0^{x}	52.0^{x}	53.4^{x}	50.4^{X}
Cefepime																		
MIC_{50}	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.06	0.03	0.03
MIC_{90}	0.06	0.06	0.06	0.03	0.03	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.12	0.06	0.06
% R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cefotaxime																		
MIC_{50}	0.06	0.06	0.06	0.06	0.06	0.06	0.03	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
MIC_{90}	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.12	0.12	0.12	0.5	0.12	0.12
% R	0	0	0	0	0	0^{V}	1.3^{v}	0^{v}	0^{v}	0^{v}	0^{v}	0.2^{V}	0^{v}	0^{v,w}	2.6^{v,w}	6.0^{w}	0^{v}	1.9^{W}
Ciprofloxacin																		
MIC_{50}	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.016	0.016	0.25	0.008	0.008
MIC_{90}	0.016	0.016	0.12	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.12	0.016	0.12	0.25	0.25	8	0.06	0.25
% R	3.9^{v}	0^{v}	4.2^{v,w}	0^{v,x}	0^{v}	1.6^{V}	0^{v}	0^{v}	1.0^{v}	0^{v}	0^{v}	0.2^{W}	1.0^{v}	1.7^{v}	1.3^{v}	22.0^{w}	1.0^{v}	5.2^{X}
Chloramphenicol																		
MIC_{50}	4	8	4	4	4	4	4	8	4	8	8	8	4	8	8	4	4	4
MIC_{90}	128	8	8	8	8	8	8	64	128	64	128	64	8	>128	128	64	8	128
% R	11.8^{v}	2.0^{w,x}	6.3^{v,x,y}	0^{w}	1.1^{w,y}	4.2^{V}	6.7^{v}	20.8^{w}	13.5^{v,w}	14.3^{v,w}	42.0^{x}	19.6^{W}	6.9^{v}	16.9^{v,w}	16.9^{w}	18.0^{w}	7.8^{v}	13.3^{X}
Colistin																		
MIC_{50}	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25
MIC_{90}	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5
% R	0	0	0	0	0	0^{V}	0^{v}	0^{v}	0^{v}	0^{v}	1.0^{v}	0.2^{V}	0	0	0	0	0	0^{V}
Gentamicin																		
MIC_{50}	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MIC_{90}	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
% R	5.9^{v}	0^{w}	7.3^{v}	0^{w}	0^{w}	2.6^{V}	0^{v,w}	2.0^{v,w}	0^{v}	0^{v}	5.0^{w}	1.4$^{v;W}$	5.9^{v}	3.4^{v}	3.2^{v}	8.0^{v}	1.0^{v}	4.2^{V}
Tetracycline																		
MIC_{50}	1	1	1	1	4	1	1	128	128	128	128	128	128	128	128	128	128	128
MIC_{90}	>128	2	>128	2	4	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
% R	35.3^{v}	8.1^{w}	33.3^{v}	0.9^{x}	9.6^{w}	17.1^{V}	36.0^{v}	83.2^{x}	64.4^{w}	67.9^{w}	94.0^{y}	70.6^{W}	72.5^{v}	61.0^{w}	63.6^{w}	82.0^{v}	65.0^{w}	68.9^{W}
Trimethoprim/sulfamethoxazole[c]																		
MIC_{50}	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.5	0.06	0.25	>128	0.25	0.06	>128	0.5	0.25	>128	0.5
MIC_{90}	>128	0.125	>128	0.06	0.125	0. 5	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
% R	17.6^{v}	3.0^{w}	21.9^{v}	0^{w}	3.2^{w}	9.0^{V}	14.7^{v}	43.6^{w}	33.7^{w}	42.1^{w}	66.0^{x}	41.3^{W}	23.5^{v}	57.6^{w}	46.8^{w,x}	34.0^{v,x}	57.3^{w}	43.1^{W}

[a]Resistance (R) breakpoints: ampicillin, ≥32 mg/L; cefepime, ≥32 mg/L; cefotaxime, ≥4 mg/L; ciprofloxacin, ≥4 mg/L; chloramphenicol, ≥32 mg/L; colistin, ≥4 mg/L; gentamicin, ≥16 mg/L; tetracycline, ≥16 mg/L; trimethoprim/sulfamethoxazole, ≥4/76 mg/L. Superscripts within the same host and the same line showing different lower case letters indicate significant differences between countries; letters that are the same indicate no significant difference between countries for the same host.

[b]$MIC_{50/90}$ (mg/L) and resistance rates are based on the summed isolate numbers for each host animal. Different upper case letters indicate statistically significant differences among host species.

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

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

	Pigs				Chickens		
	France, $n=34$	Netherlands, $n=18$	Spain, $n=86$	total[b], $n=143$	Germany, $n=18$	Spain, $n=26$	total[b], $n=49$
Ampicillin							
MIC_{50}	2	2	2	2	1	2	2
MIC_{90}	>128	>128	>128	>128	>128	2	>128
% R	14.7[v]	27.8[v,w]	38.4[w]	31.5[V]	22.2[v]	3.8[v]	14.3[W]
Cefepime							
MIC_{50}	0.06	0.06	0.12	0.12	0.06	0.12	0.12
MIC_{90}	0.25	0.25	1	0. 5	0.25	0.12	0.25
% R	0	0	0	0	0	0	0
Cefotaxime							
MIC_{50}	0.12	0.12	0.25	0.12	0.12	0.12	0.12
MIC_{90}	0.25	0.25	0.5	0.5	0.5	0.25	0.25
% R	0[v]	0[v]	1.2[v]	0.7[V]	0	0	0[V]
Ciprofloxacin							
MIC_{50}	0.016	0.016	0.016	0.016	0.03	0.12	0.03
MIC_{90}	0.03	0.03	0.03	0.03	0.5	0.25	0.25
% R	0	0	0	0	0	0	0
Chloramphenicol							
MIC_{50}	8	8	8	8	4	4	4
MIC_{90}	>128	8	>128	>128	16	8	8
% R	14.7[v]	5.6[v]	34.9[w]	26.6[V]	5.6[v]	0[v]	2.0[W]
Colistin							
MIC_{50}	0.5	0.5	0.5	0.5	0.5	8	0.5
MIC_{90}	1	2	2	2	1	8	8
% R	0[v]	5.6[v]	9.3[v]	6.3[V]	5.6[v]	76.9[w]	42.9[W]
Gentamicin							
MIC_{50}	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MIC_{90}	1	1	2	1	1	1	1
% R	0[v]	0[v]	8.1[v]	4.9[V]	5.6[v]	3.8[v]	4.1[V]
Nalidixic acid							
MIC_{50}	4	4	4	4	4	>128	4
MIC_{90}	4	4	8	4	>128	>128	>128
% R	0[v]	0[v]	2.3[v]	1.4[V]	22.2[v]	73.1[w]	49.0[W]
Streptomycin							
MIC_{50}	8	8	32	16	16	2	8
MIC_{90}	128	>128	128	128	32	16	32
% R	35.3[v]	22.2[v]	36.0[v]	34.3[V]	5.6[v]	3.8[v]	8.2[W]
Sulfisoxazole							
MIC_{50}	64	128	>2048	64	64	64	64
MIC_{90}	>2048	>2048	>2048	>2048	>2048	64	64
% R	44.1[v]	38.9[v]	55.8[v]	50.3[V]	11.1[v]	0[v]	8.2[W]
Tetracycline							
MIC_{50}	32	1	128	128	1	1	1
MIC_{90}	>128	>128	>128	>128	>128	1	32
% R	64.7[v]	38.9[v]	83.7[w]	72.0[V]	22.2[v]	0[w]	10.2[W]

Continued

Table 2. *Continued*

	Pigs				Chickens		
	France, $n=34$	Netherlands, $n=18$	Spain, $n=86$	total[b], $n=143$	Germany, $n=18$	Spain, $n=26$	total[b], $n=49$
Trimethoprim/sulfamethoxazole[c]							
MIC_{50}	0.06	0.06	0.12	0.06	0.06	0.12	0.06
MIC_{90}	>128	>128	>128	>128	0.25	0.12	0.25
% R	11.8[v]	22.2[v]	26.7[v]	21.7[V]	0	0	4.1[W]

[a]Resistance (R) breakpoints: ampicillin, ≥32 mg/L; cefepime, ≥32 mg/L; cefotaxime, ≥4 mg/L; ciprofloxacin, ≥4 mg/L; chloramphenicol, ≥32 mg/L; colistin, ≥4 mg/L; gentamicin, ≥16 mg/L; nalidixic acid, ≥32 mg/L; streptomycin, ≥64 mg/L; sulfisoxazole, ≥512 mg/L; tetracycline, ≥16 mg/L; trimethoprim/sulfamethoxazole, ≥4/76 mg/L. Countries with <10 isolates were not included in the table and not analysed statistically. Superscripts within the same host showing different lower case letters indicate significant differences; letters that are the same indicate no significant difference between countries for the same host.
[b]$MIC_{50/90}$ (mg/L) and resistance rates are based on the summed isolate numbers per host animal. Even though countries with <10 isolates are not included in the table, they are included in the totals. Hence totals will in some cases exceed the sum of isolates from the countries listed. Different upper case letters indicate statistically significant differences among the host species.
[c]$MIC_{50/90}$ figures refer to trimethoprim concentrations only.

trimethoprim/sulfamethoxazole. In contrast, resistance in cattle and chicken isolates was usually <10% for these antibiotics. In Spain, tetracycline resistance varied from 0% (chickens) to 84% (pigs). Similarly, the prevalence of resistance to chloramphenicol varied from 0% among isolates from chickens to 35% among isolates from pigs. Of the Spanish chloramphenicol-resistant isolates, 75% were *Salmonella* Typhimurium. For all hosts, low prevalence of resistance to gentamicin (mean 4%–5%) was noted. Colistin resistance, usually at a very low prevalence, amounted surprisingly to 43% for all chicken isolates, which was attributed to 21 *Salmonella* Enteritidis strains comprising four differing phage types (PT1, PT3, PT22, RDNC); these strains showed colistin MICs of 4–8 mg/L. These *Salmonella* Enteritidis isolates also exhibited nalidixic acid resistance and decreased susceptibility to ciprofloxacin except for five isolates that were PT22, PT21 and RDNC. It is to be noted that all 21 *Salmonella* Enteritidis isolates were fully susceptible to cephalosporins, chloramphenicol, gentamicin, streptomycin, tetracycline and trimethoprim/sulfamethoxazole.

Clinical resistance to cefepime was absent; resistance to cefotaxime was detected in only one pig isolate from Spain. This strain was determined phenotypically to be an ESBL producer. No clinical resistance to ciprofloxacin was seen in any of the isolates recovered from any of the countries.

Multiple drug resistance was studied for the drugs with clinical relevance; the marker compounds nalidixic acid and streptomycin were excluded. Resistance to four or more drugs of different classes (21.9%) was seen in varying combinations; the most frequent combination was ampicillin/chloramphenicol/sulfisoxazole/tetracycline (11.9%). Resistance to compounds of five classes was observed in 2.5% of isolates; most frequently observed was the above pattern and the trimethoprim/sulfamethoxazole combination. Four isolates (2.0%; all from pigs) were resistant to compounds of six classes (ampicillin, chloramphenicol, sulfisoxazole, tetracycline, trimethoprim/sulfamethoxazole and either gentamicin or colistin); finally, one pig isolate was resistant to seven compounds (ampicillin, cefotaxime, chloramphenicol, colistin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole).

C. jejuni and C. coli

The isolation percentage was variable for both for *C. jejuni* and *C. coli* from all three host species. In cattle, 66% and 23% of the isolates were identified as *C. jejuni* and *C. coli*, respectively; another 11% of the isolates were identified as *Campylobacter fetus* ($n=16$) or *Campylobacter hyointestinalis* ($n=15$). In contrast, 99% of the isolates from pigs were identified as *C. coli* and 1% as *C. jejuni*. In broilers, the proportions of *C. jejuni* and *C. coli* were similar. The antimicrobial susceptibility patterns presented (Tables 3 and 4) are limited to the major human pathogens, *C. jejuni* and *C. coli*.

None of the *C. jejuni* strains displayed resistance to gentamicin and clinical resistance to erythromycin was <1% (Table 3). The highest prevalence of resistance in *C. jejuni* was found for ciprofloxacin, nalidixic acid and tetracycline. Dutch strains of chickens displayed the highest prevalence of resistance, with 47% of isolates resistant to ciprofloxacin and 53% to nalidixic acid; UK strains showed the lowest prevalence (12%). Conversely, the prevalence of tetracycline resistance in chicken isolates was 59% the UK, 67% in France, 42% in the Netherlands and 30% in Germany. In cattle, *C. jejuni* isolates from the UK had the lowest overall prevalence of resistance with the exception of nalidixic acid, whereas France and Italy had a higher overall prevalence.

For *C. coli* the prevalence of resistance for all tested antibiotics was notably higher than for *C. jejuni* (Table 4). Overall, the prevalence of ciprofloxacin resistance varied from 35% in cattle and 36% in pigs to 55% in chickens. Differences between countries were noted; for instance, the prevalence of resistance to ciprofloxacin and nalidixic acid in porcine *C. coli* isolates ranged from 5% in the Netherlands to 93% in Spain. Erythromycin resistance was most notable in *C. coli* (33%) recovered from pigs, with country variation from 11% to 63%. Resistance to gentamicin was minimal, but the prevalence of resistance to tetracycline exceeded 64% in *C. coli* for all three hosts.

Multiple drug resistance for clinically relevant drugs (ciprofloxacin, erythromycin, gentamicin, tetracycline) was found at

Table 3. Antimicrobial susceptibility of *C. jejuni* isolates[a]

	Cattle					Chickens				
	France, $n=101$	Germany, $n=14$	Italy, $n=22$	UK, $n=31$	total, $n=170$	France, $n=46$	Germany, $n=10$	Netherlands, $n=38$	UK, $n=34$	total, $n=132$
Ciprofloxacin										
MIC_{50}	0.12	0.12	0.12	0.12	0.12	0.25	0.5	1	0.25	0.25
MIC_{90}	16	0.5	8	0.25	16	32	64	32	8	32
% R	24.8^{v}	$0^{w,x}$	$18.2^{v,x}$	0^{w}	17.1^{V}	17.4^{v}	$30.0^{v,w}$	47.4^{w}	11.8^{v}	28.0^{W}
Nalidixic acid										
MIC_{50}	4	4	4	4	4	4	4	32	8	8
MIC_{90}	>128	8	>128	128	>128	>128	>128	>128	>128	>128
% R	24.8^{v}	$0^{w,x}$	$18.2^{v,x}$	38.7^{w}	24.1^{V}	17.4^{v}	$30.0^{v,w}$	52.6^{w}	11.8^{v}	28.0^{V}
Erythromycin										
MIC_{50}	1	1	1	2	1	1	2	2	1	1
MIC_{90}	4	2	2	4	4	2	4	4	2	2
% R	1.0^{v}	0^{v}	0^{v}	0^{v}	0.6^{V}	0	0	0	0	0^{V}
Gentamicin										
MIC_{50}	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
MIC_{90}	0.25	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5
% R	0	0	0	0	0	0	0	0	0	0
Tetracycline										
MIC_{50}	2	0.12	0.25	0.12	0.25	128	0.5	4	32	32
MIC_{90}	128	1	64	0.5	128	>128	64	128	>128	>128
% R	50.5^{v}	$7.1^{w,x}$	$27.3^{v,x}$	0^{w}	34.1^{V}	67.4^{v}	30.0^{w}	42.1^{w}	$58.8^{v,w}$	55.3^{W}

[a]MICs are in mg/L. Resistance (R) breakpoints: ciprofloxacin, ≥4 mg/L; nalidixic acid, ≥32 mg/L; erythromycin, ≥32 mg/L; gentamicin, ≥16 mg/L; tetracycline, ≥16 mg/L. Countries with <10 isolates were not included in the table and not analysed statistically. Even though countries with <10 isolates are not included in the table, they are included in the totals. Hence totals will in some cases exceed the sum of isolates from the countries listed. Superscripts within the same host showing different lower case letters indicate significant differences; letters that are the same indicate no significant difference between countries for the same host. Different upper case letters indicate statistically significant differences among host species.

variable percentages. Resistance to multiple drugs was observed for one *C. jejuni* isolate and amounted to 15.6% of *C. coli* isolates, almost exclusively in porcine strains recovered from Spain. Similarly, resistance to the combination ciprofloxacin/erythromycin/tetracycline was found in porcine *C. coli* ($n=122$; 20.2%), as was the combination ciprofloxacin/erythromycin ($n=6$; 1.7%). The latter two resistance phenotypes were absent for *C. jejuni*.

E. faecium and E. faecalis

Among the enterococci, *E. faecium* was by far the dominant species in all three hosts ($n=634$); *E. faecalis* recovery was variable and the total number collected ($n=67$) precluded any comparisons among countries. Total numbers of other enterococcal species also varied, but were usually less than those of *E. faecalis*. The antimicrobial susceptibilities discussed below are limited to *E. faecium* and *E. faecalis*, the two major human pathogens.

Little or no resistance to ampicillin or gentamicin was observed for *E. faecium* strains (Table 5). The overall prevalence of resistance to ampicillin in cattle, pigs and chickens was 1.0%–2.6%. None of the *E. faecalis* isolates exhibited resistance to ampicillin (data not shown). For gentamicin, six *E. faecium* isolates and four *E. faecalis* isolates displayed high-level resistance. The prevalence of resistance of *E. faecium* to quinupristin/dalfopristin was much higher, on average 40% for the three species, with marked country differences once again noted. Whereas the prevalence of resistance to quinupristin/dalfopristin among pig isolates was 10% in the Netherlands, in Spain 63% of the *E. faecium* isolates displayed resistance. With regard to chickens, isolates from France showed the lowest prevalence (17%) whilst Germany had the highest (64%) prevalence of quinupristin/dalfopristin resistance. As expected, resistance of *E. faecalis* to quinupristin/dalfopristin was close to 100% in isolates from all animal species (data not shown). None of the *E. faecium* or *E. faecalis* strains was resistant to linezolid, but eight *E. faecium* isolates displayed vancomycin resistance in (VRE; 1.3%). For *E. faecalis*, no vancomycin resistance was observed. *E. faecium* and *E. faecalis* showed a high level of susceptibility to four of the five compounds tested, and hence multiple drug resistance was absent.

Decreased susceptibility

The above reported percentages of resistance are principally based on clinical breakpoints. However, it is important to consider the population of isolates with decreased susceptibility. In particular, this applies to antimicrobial agents essential for

Table 4. Antimicrobial susceptibility of *C. coli* isolates[a]

	Cattle				Pigs						Chickens			
	France, $n=29$	Germany, $n=11$	UK, $n=11$	total, $n=58$	Denmark, $n=58$	France, $n=97$	Germany, $n=91$	Netherlands, $n=65$	Spain, $n=96$	total, $n=407$	France, $n=60$	Spain, $n=29$	UK, $n=40$	total, $n=137$
Ciprofloxacin														
MIC_{50}	0.5	0.12	0.25	0.25	0.12	0.25	0.25	0.25	16	0.25	0.25	16	4	8
MIC_{90}	32	16	8	32	8	16	16	0.5	32	16	16	32	64	32
% R	41.4^{v}	18.2^{v}	18.2^{v}	36.2^{V}	13.8^{v,w}	23.7^{v}	19.8^{v}	4.6^{w}	92.7^{x}	34.6^{V}	40.0^{v}	89.7^{w}	55.0^{v}	54.7^{W}
Nalidixic acid														
MIC_{50}	16	4	8	8	4	8	8	8	>128	8	8	64	128	64
MIC_{90}	>128	>128	>128	>128	128	>128	128	16	>128	>128	>128	>128	>128	>128
% R	41.4^{v}	18.2^{v}	54.5^{v}	43.1^{V}	13.8^{v,x}	27.8^{w}	19.8^{v,w}	4.6^{x}	92.7^{y}	35.6^{V,X}	40.0^{v}	93.1^{w}	60.0^{x}	56.9^{V,W}
Erythromycin														
MIC_{50}	2	2	2	2	2	8	4	4	>128	4	4	4	1	4
MIC_{90}	>128	4	4	>128	>128	>128	>128	128	>128	>128	>128	>128	128	>128
% R	20.7^{v}	0^{v}	9.1^{v}	15.5^{V}	25.9^{v}	44.3^{w}	11.0^{x}	10.8^{x}	62.5^{y}	33.2^{W}	13.3^{v}	24.1^{v}	15.0^{v}	17.5^{V}
Gentamicin														
MIC_{50}	0.5	0.25	0.25	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	0.25	1	0.5	0.5	0.5	1	1	128	1	0.5	0.5	0.5	1
% R	3.4^{v}	0^{v}	0^{v}	3.4^{V}	0^{v}	0^{v}	0^{v}	0^{v}	16.7^{w}	3.9^{V}	0^{v}	6.9^{w}	0^{v,w}	1.5^{V}
Tetracycline														
MIC_{50}	>128	0.12	0.12	128	0.25	64	64	128	>128	64	128	>128	0.25	128
MIC_{90}	>128	64	0.5	>128	0.5	>128	>128	>128	>128	>128	>128	>128	>128	>128
% R	100.0^{v}	36.4^{w}	0^{x}	63.8^{V}	0^{v}	91.8^{w}	75.8^{x}	81.5^{b,c}	100.0^{d}	75.4^{V}	96.7^{v}	93.1^{v}	40.0^{w}	78.8^{V}

[a]MICs are in mg/L. Resistance (R) breakpoints: ciprofloxacin, ≥4 mg/L; nalidixic acid, ≥32 mg/L; erythromycin, ≥32 mg/L; gentamicin, ≥16 mg/L; and tetracycline, ≥16 mg/L. Countries with <10 isolates were not included in the table and not analysed statistically. Even though countries with <10 isolates are not included in the table, they are included in the totals. Hence totals will in some cases exceed the sum of isolates from the countries listed. Superscripts within the same host showing different lower case letters indicate significant differences; letters that are the same indicate no significant difference between the countries of the same host. Different upper case letters indicate statistically significant differences among host species.

Table 5. Antimicrobial susceptibility of *E. faecium*[a]

	Cattle					Pigs					Chickens					
	France, $n=45$	Germany, $n=16$	Ireland, $n=10$	Italy, $n=28$	total, $n=99$	Denmark, $n=29$	France, $n=59$	Netherlands, $n=71$	Spain, $n=100$	total, $n=266$	France, $n=48$	Germany, $n=14$	Netherlands, $n=80$	Spain, $n=90$	UK, $n=29$	total, $n=269$
Ampicillin																
MIC_{50}	1	1	0.5	1	1	1	2	2	2	2	2	1	2	2	2	2
MIC_{90}	4	1	2	4	2	2	4	4	4	4	2	2	4	4	4	4
% R	0^{v}	0^{v}	0^{v}	3.4^{v}	1.0^{V}	0^{v}	1.7^{v}	0^{v}	3.0^{v}	1.5^{V}	$2.1^{v,w}$	7.1^{v}	0^{w}	$4.5^{v,w}$	$3.4^{v,w}$	2.6^{V}
Gentamicin																
MIC_{50}	4	8	8	4	4	8	4	4	4	4	4	8	8	8	8	8
MIC_{90}	8	16	32	>1024	16	16	8	8	8	8	8	8	8	16	16	8
% R	0^{v}	$0^{v,w}$	$0^{v,w}$	10.3^{w}	3.0^{V}	0^{v}	0^{v}	0^{v}	1.0^{v}	0.4^{V}	0^{v}	0^{v}	1.3^{v}	2.0^{v}	0^{v}	1.1^{V}
Linezolid																
MIC_{50}	2	2	2	2	2	2	2	2	1	2	2	2	1	2	2	2
MIC_{90}	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
% R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quinupristin/dalfopristin																
MIC_{50}	2	4	4	4	4	2	2	2	4	2	2	4	2	2	2	2
MIC_{90}	4	4	>32	8	8	8	8	4	8	8	4	8	32	32	4	16
% R	23.2^{v}	62.5^{w}	60.0^{w}	69.0^{w}	45.5^{V}	28.6^{v}	44.1^{v}	9.9^{w}	62.6^{x}	40.4^{V}	17.4^{v}	64.2^{w}	$41.8^{w,x}$	$43.8^{w,x}$	$25.9^{v,x}$	37.2^{V}
Vancomycin																
MIC_{50}	1	0.5	2	1	1	1	0.5	0.5	1	1	1	1	1	1	1	1
MIC_{90}	2	1	512	4	4	2	4	1	2	2	1	4	2	2	2	2
% R	0	0	0	0	0^{V}	$3.6^{v,w}$	6.8^{v}	$1.4^{v,w}$	$1.0^{v,w}$	2.6^{V}	0^{v}	0^{v}	1.3^{v}	0^{v}	0^{v}	0.4^{V}

[a]MICs are in mg/L. Resistance (R) breakpoints: ampicillin, ≥16 mg/L; gentamicin, >500 mg/L; linezolid, ≥8 mg/L; quinupristin/dalfopristin, ≥4 mg/L; vancomycin, ≥32 mg/L. Countries with <10 isolates were not included in the table and not analysed statistically. Even though countries with <10 isolates are not included in the table, they are included in the totals. Hence totals will in some cases exceed the sum of isolates from the countries listed. Superscripts within the same host showing different lower case letters indicate significant differences; letters that are the same indicate no significant difference between countries for the same host. Different upper case letters indicate statistically significant differences among host species.

Table 6. Decreased susceptibility of *E. coli*, *Salmonella*, *C. jejuni* and *E. faecium* to critically important antimicrobials[a]

	E. coli			*Salmonella* spp.		*C. jejuni*		*E. faecium*		
	cattle, $n=502$	pigs, $n=520$	chickens, $n=518$	pigs, $n=143$	chickens, $n=49$	cattle, $n=170$	chickens, $n=132$	cattle, $n=99$	pigs, $n=266$	chickens, $n=269$
Ampicillin	0	0	0	1.4^{v}	0^{v}	—	—	0^{v}	0.4^{v}	1.2^{v}
Cefotaxime	0.4^{v,w}	0.2^{v}	1.5^{w}	4.2^{v}	0^{v}	—	—	—	—	—
Ciprofloxacin	3.0^{v}	3.8^{v}	29.7^{w}	1.4^{v}	46.9^{v}	0.6^{v}	0^{v}	—	—	—
Erythromycin	—	—	—	—	—	4.1^{v}	1.5^{v}	—	—	—
Gentamicin	0^{v}	0.8^{v}	0.4^{v}	0	0	0.6^{v}	0.8^{v}	0^{v}	0^{v}	0.8^{v}
Tetracycline	—	—	—	—	—	0^{v}	3.8^{w}	—	—	—
Quinupristin/dalfopristin	—	—	—	—	—	—	—	28.3^{v}	33.6^{v}	27.9^{v}
Vancomycin	—	—	—	—	—	—	—	4.0^{v}	0.4^{v}	1.1^{v}

[a]Decreased susceptibility is defined as the percentage of isolates between the epidemiological cut-off value and the clinical breakpoint and is based on the epidemiological cut-off values (mg/L) defined by EFSA: ampicillin, >8 (*E. coli*) and >4 (*Salmonella* and *E. faecium*); cefotaxime, >0.25 (*E. coli*) and >0.5 (*Salmonella*); ciprofloxacin, >0.03 (*E. coli*), >0.06 (*Salmonella*) and >1 (*C. jejuni*); erythromycin, >4; gentamicin, >2 (*E. coli* and *Salmonella*), >1 (*C. jejuni*) and >32 (*E. faecium*); tetracycline, >2 (*C. jejuni*); quinupristin/dalfopristin, >1 (*E. faecium*); and vancomycin >4 mg/L (*E. faecium*). Superscripts within the same bacterial species showing different letters indicate significant differences; letters that are the same indicate no significant difference between host species for the same bacterial species.

human and veterinary medicine, such as fluoroquinolones, cephalosporins and macrolides. Recently, EUCAST and EFSA set ECV values for various antimicrobials appropriate for foodborne bacteria.[13–15] For these organisms we determined the percentage of decreased susceptibility (Table 6), excepting *C. coli* (because erythromycin breakpoints and cut-offs are identical) and *E. faecalis* (because of the low numbers of isolates). For most antimicrobials the ECV is identical to the clinical breakpoint and hence calculation of decreased susceptibility is not applicable.

In the case of *E. coli* and *Salmonella*, a particularly high prevalence of decreased susceptibility to ciprofloxacin was observed in chickens in all five collection countries. Forty-seven percent of the avian *Salmonella* isolates exhibited decreased susceptibility, expressed by MICs of 0.12–0.5 mg/L. The *Salmonella* isolates showing decreased susceptibility to ciprofloxacin were also resistant to colistin and nalidixic acid (MICs >128 mg/L). Decreased ciprofloxacin susceptibility of cattle and pig *Salmonella* isolates was ∼1%. For cefotaxime, decreased susceptibility in *E. coli* was very low (0.2%–1.5%); decreased susceptibility (MICs 1–2 mg/L) in *Salmonella* accounted for 4.2% in pig isolates only. For ampicillin and gentamicin, the prevalence of decreased susceptibilities was very low and a relation to clinical resistance was absent. Similarly, for *C. jejuni* and *E. faecium* an absence or low prevalence of decreased susceptibility was usually observed. Interestingly, decreased susceptibility to quinupristin/dalfopristin was noted and accounted for ∼30% of the isolates in each of the three host species.

Discussion

The present monitoring study was based on bacteria isolated from healthy animals at slaughter, and employed uniform methods of sampling and bacterial isolation and a single central laboratory for determination of the MICs of a panel of antimicrobials commonly used in human medicine. Public health concern is primarily with resistance in human pathogens, including those bacteria that are foodborne, so the antibiotics tested were compounds considered important in treating human patients rather than antibiotics of importance in veterinary medicine. The procedures followed the general recommendations of the OIE (World Organisation for Animal Health) guidelines,[7] and specifically those of CLSI.[19] Comparability of results between isolates from different sources is crucial, and is made possible by the use of a central testing laboratory.[9,23] The main organisms of interest were *Salmonella* and *Campylobacter* species as zoonotic organisms, and commensal *E. coli* and *Enterococcus* species as indicator organisms for Gram-negative and Gram-positive bacteria, respectively. Slaughter is considered to be the most likely time for contamination of carcasses and subsequently meat products with intestinal bacteria, and therefore it is the most relevant timepoint to obtain isolates for antibiotic susceptibility testing. Additionally, the prevalence of antibiotic-resistant bacteria represents the final outcome (of cumulative exposure to, and selection by, any antibiotic used during the lifespan of the animal) that may be present and antibiotic-resistant bacteria could therefore potentially enter the food chain. Faecal or caecal isolates as appropriate were collected immediately post-slaughter from each of the major food animal species for three production categories: beef cattle, slaughter pigs and broiler chickens. Sampling of carcasses was omitted because considerable cross-contamination in abattoirs has been described, which would bias the determination of prevalence of the organisms on the individual farms and not reflect the susceptibility of isolates recovered from the individual animals.

Resistance among bacteria isolated from food animals is a potential hazard in that the resistance may occur in zoonotic pathogens and so potentially reduce the effectiveness of antimicrobial treatment of foodborne disease if contracted by humans.[7,24] The degree of risk posed by this hazard is difficult to estimate, partly because there is a shortage of information regarding the prevalence of resistance among bacterial isolates

from food animals, coupled with inadequate information on both the international distribution and trends of antimicrobial resistance. Although national resistance monitoring programmes have been introduced in many countries, differences in methodology make it difficult to compare results from the different programmes.[11] It is important to know both the prevalence of and trends in antibiotic resistance as such information, together with comparisons of antibiotic use practices between countries, may indicate the best approach to control resistance. The EASSA programme described here, by being international and longitudinal in its surveillance of bacteria from healthy farm animals, is exceptional in being the only programme collecting international samples and employing uniform methodology and interpretation criteria.

The predictably high recovery frequency of *E. coli* allowed comparisons to be made between host species, countries and antimicrobial agents. Comparing different antimicrobial agents, the prevalence of resistance was higher among older compounds, notably ampicillin, tetracycline and trimethoprim/sulfamethoxazole, while a much lower prevalence of resistance was observed for the newer compounds cefepime, cefotaxime and ciprofloxacin. The occurrence of cephalosporin resistance in *E. coli* from livestock was absent apart from one Danish porcine isolate; the percentage of decreased susceptibility was very low (Table 6). More frequent resistance including ESBLs were recovered from chickens. Further molecular analysis of *E. coli* isolates with increased cefotaxime MICs revealed that the majority of the isolates with MICs between 1 and 4 mg/L possessed AmpC cephalosporinases, whereas for MICs >4 mg/L ESBLs were identified (data not shown). Colistin (polymyxin E), although by no means a modern compound, was nevertheless almost universally efficacious against the *E. coli* isolates tested. As observed in the previous studies,[16,17] the prevalence of resistance to chloramphenicol was relatively high in pig and chicken isolates. In view of the fact that chloramphenicol had been discontinued as a therapeutic agent many years ago, it seems difficult to explain the persistence of chloramphenicol resistance, especially because the use of the related compound florfenicol was not yet approved for use in pigs and poultry at the time of this study. Co-selection with unrelated compound(s) appears to be the most likely explanation. For cattle, isolates from France and Italy tended to show a higher prevalence of resistance to several compounds than those from Germany, Ireland or the UK. Pig isolates from Spain generally showed higher prevalences of resistance than those from Denmark, Germany, France or the Netherlands. In our earlier report,[16] Spain was also identified as having a higher prevalence of resistance than the countries with which it was compared. In *E. coli* isolates from chickens, there was, apart from ciprofloxacin (Spain), no obvious and consistent difference between the different countries sampled.

Resistance among *Salmonella* isolates showed a pattern of variation between countries similar to that seen for *E. coli*, but the low rate of recovery limited the ability to make comparisons. Nalidixic acid was included as an indicator of a shift in ciprofloxacin MIC away from the fully susceptible wild-type population. For chickens, isolates from Germany displayed as a rule the highest prevalence of resistance, whereas those from Spain showed the most marked reduction in susceptibility among pig isolates. An exception was the high levels of resistance to colistin and nalidixic acid present in Spanish chicken isolates. All isolates exhibited an identical resistance pattern to the drugs tested and all belonged to *Salmonella* Enteritidis. It is possible that these findings are related to a clonal spread of this serovar in Spain. The presence of nalidixic acid resistance among *Salmonella* strains may suggest that fluoroquinolone resistance, although largely absent based on the clinical end-point, is a potential emerging issue. However, the prevalence of clinical resistance to fluoroquinolones remains very low,[25–27] presumably due to prohibitive fitness costs in the resistant organisms.[28,29] Even in Spain, a country with a relatively high selection pressure resulting in a high prevalence of fluoroquinolone resistance in *E. coli* strains and high levels of decreased susceptibility to fluoroquinolones in *Salmonella*, this was not translated into clinical resistance of *Salmonella* to fluoroquinolones.[17] This further illustrates the contrast between the ECVs and the clinical breakpoints,[30,31] where the former can indicate an emerging decrease in susceptibility while the latter allows informed antibiotic choices to be made and clinical efficacy to be predicted. Clearly, for *Salmonella*, at least, decreased susceptibility to fluoroquinolones does not lead to clinical resistance and ECVs do not act as an early warning for clinical resistance.

Campylobacter has continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the EU for the past several years, and the prevalence of reported confirmed human campylobacteriosis cases has been fairly constant over the past 5 years.[32] Usually, infections are self-limiting. Medication is infrequently needed, but, when prescribed, macrolides are typically the first choice in EU countries.[33,34] Human foodborne disease is primarily associated with *C. jejuni* and C. *coli*. In *C. jejuni* (isolates were available only for cattle and chickens), resistance varied between countries for both nalidixic acid and ciprofloxacin, and, as expected, nalidixic acid resistance corresponded quite closely to that for ciprofloxacin. The exception was the UK, with several bovine isolates characterized by ciprofloxacin MICs <2 mg/L (0% resistance), whilst nalidixic acid MICs were >64 mg/L (39% resistance). In contrast, among the UK chicken isolates 12% were resistant to nalidixic acid and to ciprofloxacin. However, isolates from chickens in the Netherlands showed 53% resistance to nalidixic acid and 47% to ciprofloxacin. Erythromycin and gentamicin resistances were near zero or absent in *C. jejuni* isolates from both cattle and chickens, while tetracycline resistance was variable (34%–55%). For *C. jejuni*, the percentage of isolates exhibiting decreased susceptibility (Table 6) was low for erythromycin (1.5%–4.1%) and very low for ciprofloxacin (0%–0.6%). For ciprofloxacin, a relation with clinical resistance was absent, which can be expected due to the minimal difference between the ciprofloxacin ECV and the clinical breakpoint (one dilution step) and based on the ciprofloxacin MIC distribution. Hence, 'resistance' as based on ECVs seems highly unlikely to act as an early warning signal for clinical resistance.

Generally, *C. coli*, associated with <10% of human campylobacter infections, was more frequently resistant than *C. jejuni* regardless of animal source. *C. coli* isolates were recovered from cattle, pigs and chickens, but only for pigs in sufficient numbers to allow comparisons. They appeared to exhibit resistance to a greater extent than for *C. jejuni*, particularly to erythromycin. However, it should be recognized that the prevalence of *C. coli* in pork meat is extremely low,[35,36] and thus retail pork is

not considered to be a significant source of human campylobacteriosis. In the USA, NARMS has discontinued testing for *Campylobacter* isolates from retail pork chops and ground beef due to very low recoveries.[36] European surveys have experienced similarly low recoveries of *C. coli* from porcine carcasses and retail meat.[1,3]

In contrast to the previous EASSA study,[16] enterococci were sampled in this study, because human enterococcal infections, especially *E. faecium*, continue to be of importance,[37,38] but are not considered a foodborne pathogen. *E. faecium* was the species recovered most frequently, while *E. faecalis* was isolated markedly less frequently. Only a few *E. faecium* and none of the *E. faecalis* isolates displayed resistance to ampicillin. High-level resistance to gentamicin was infrequent. None of the *E. faecium* and *E. faecalis* isolates was resistant to linezolid, as might be expected since no related compound has been used in animals. Resistance of *E. faecium* to quinupristin/dalfopristin was approximately 40%, even though no related streptogramin is now permitted for animal use in the EU (although prior to 1999 another streptogramin, virginiamycin, was used as an antimicrobial growth promoter in food animals). The overall prevalence of resistance was higher in pigs and chickens than in cattle. *E. faecalis* showed high resistance to quinupristin/dalfopristin, but this was expected since this organism is intrinsically resistant to streptogramins. The overall rate of resistance to vancomycin was 1%–3% and had only declined slightly since the earlier sampling,[18] although the use of the related compound avoparcin had been banned from use in animals some 8 years earlier.

CLSI clinical breakpoints were primarily used in the present study rather than ECVs. However, it is important to consider the population of isolates with decreased susceptibility. This mainly applies to fluoroquinolones, cephalosporins and macrolides. Application of only clinical breakpoints can mask important shifts in MICs towards a less susceptible population. EFSA[13,14] has recently introduced the stand-alone use of ECVs, not including clinical breakpoints, thus missing the opportunity to characterize and separate both populations (isolates with MICs between the ECV and the clinical breakpoint versus the population of isolates with MICs at or above the clinical breakpoint).[39] Similarly, some national monitoring surveys also used only ECVs as interpretive criteria.[1] This approach can cause confusion, particularly among clinicians—the potential target audience of such surveys—who are likely to interpret the term 'resistant' as 'clinically resistant', and not, as may be the case, 'less susceptible but nevertheless susceptible to the prescribed treatment at registered doses'.[40] This illustrates the relevance of applying both ECVs and clinical breakpoints in monitoring programmes such as the present one.

In our work ECVs recommended by EFSA were applied, but this is not always the case in the EU national monitoring surveys. For instance, NORM-VET 2008, the Norwegian survey,[4] ignored the recommended values of EFSA/EUCAST for ciprofloxacin and porcine *E. coli* as the distribution of MICs obtained were one or two dilution steps higher than those of the EUCAST distribution. This approach was used, despite EUCAST values being available, because the recommended EUCAST ECV (>0.03 mg/L) cuts through the wild-type distributions of MICs in NORM-VET in a manner not in agreement with the concept of wild-type distributions. This would cause an erroneously high frequency of 23% 'resistance' with an ECV of >0.03 mg/L versus 0% with an ECV of >0.06 mg/L. Likewise, this is true for MARAN 2007,[3] which, like NORM-VET, used an ECV of >0.06 mg/L for ciprofloxacin and porcine *E. coli*, resulting in 2.4% resistance versus 69% 'resistance' with an ECV >0.03 mg/L. There is therefore a situation where NORM-VET and MARAN calculated ciprofloxacin resistance in *E. coli* as >0.06 mg/L, whereas DANMAP and the current EASSA study uses >0.03 mg/L.[1] From these examples, it is clear that harmonizing the way in which wild-type distributions are determined is essential.

Taking the results together, for all four bacterial species there was considerable variation between countries and hosts in the prevalence of resistance. It is tempting to ascribe such variation to differences in use practices and amounts of antimicrobial products used. Several national resistance monitoring surveys include antibiotic consumption data[1–5] or report consumption data as such.[41,42] Generally, compounds most commonly used in veterinary medicine for therapy in food animals are tetracyclines, penicillins, sulphonamides and macrolides, which matched the present results, in which resistance to these classes 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. In this respect, the initiative of the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) to record in a uniform manner national antibiotic consumption in veterinary medicine is applauded because availability of national consumption data for all EU countries, as is the case in human medicine (ESAC), is needed.[43,44] A comparison of consumption for each animal species would also be very helpful, but is challenging in the short term because figures are rarely broken down to species level. Although differences in antimicrobial consumption may explain part of the findings from this study, anomalies remain. Observations like those for chloramphenicol (see above) and the relatively high prevalence of ciprofloxacin resistance (up to 30%) among porcine *Campylobacter* in Sweden in 2000[5] and bovine *Campylobacter* in Denmark in 2005[1] demonstrate at least some disconnect between antimicrobial resistance and veterinary use of the same class of antimicrobial drug. Similarly, clonal spread of quinolone-resistant strains of *Salmonella* is a more likely explanation for reduced susceptibility than a link with fluoroquinolone use.

This pan-European survey used a uniform methodology to demonstrate that there are differences in the prevalence of antimicrobial-resistant bacteria isolated from different host species, and different EU countries, 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. Further studies are needed to shed light on any apparent differences in antimicrobial resistance between countries within the EU. However, these studies should adhere to a common protocol and the appropriate use of interpretive criteria to maximize the value of the data, which may be useful in risk assessments and further risk management interventions, including responsible antibiotic use practices.

Acknowledgements

Portions of the above data were presented at the Forty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009 (Abstracts C2-1534 and C2-1950), the Nineteenth European Congress of Clinical Microbiology and Infectious Diseases, Helsinki, 2009 (Abstracts P646 and P647) and the Fifteenth International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms, Niigata, 2009 (Abstract O3-1).

We thank the following national sampling and laboratory coordinators: Dr Frank Aarestrup, Dr Antonio Battisti, Dr Anne Brisabois, Dr Sophie Granier, Dr Paul Todd, Dr Florence Humbert, Dr Isabel Kempf, Dr Nolan Leonard, Dr Jean-Yves Madec, Professor Dik Mevius, Dr Miguel Moreno, Dr Kirsten Müller, Dr Pascal Sanders and Dr Reinhard Weiss. Mrs Eva Zschiesche is gratefully thanked for performing the statistical analysis.

Funding

This work was supported by Alpharma, Bayer, Ceva, Elanco, Intervet, Novartis, Pfizer, Schering-Plough and Vetoquinol, and did not receive other external funding.

Transparency declarations

All authors apart from H. M. and M. V. are full-time employees of the sponsoring companies. H. M. is employee of CEESA and M. V. is a full-time consultant to the veterinary pharmaceutical industry.

References

1 DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark*. 2009. http://www.danmap.org/ (9 March 2011, date last accessed).

2 FARM. *French Antimicrobial Resistance Monitoring Program in Bacteria of Animal Origin*. 2007–08. http://www.anses.fr/Documents/SANT-Ra-FARM.pdf (30 January 2011, date last accessed).

3 MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2006/2007*. 2007. http://www.cidc-lelystad.nl (9 September 2010, date last accessed).

4 NORM/NORM-VET. *Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway*. 2008. http://www.vetinst.no (3 November 2011, date last accessed).

5 SVARM. *Swedish Veterinary Antimicrobial Resistance Monitoring*. 2010. The National Veterinary Institute (SVA), Uppsala, Sweden. http://www.sva.se (14 April 2011, date last accessed).

6 VAV. Veterinary Monitoring of Antimicrobial Resistance in Spain. Twelfth Report. 2005. http://www.vigilanciasanitaria.es/data/VAV2005.pdf (14 November 2011, date last accessed).

7 Franklin A, Acar J, Anthony F *et al*. Antimicrobial resistance: harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. *Rev Sci Tech* 2001; **20**: 859–70.

8 White DG, Acar J, Anthony F *et al*. Antimicrobial resistance: standardisation and harmonisation of laboratory methodologies for the detection and quantification of antimicrobial resistance. *Rev Sci Tech* 2001; **20**: 849–58.

9 Schwarz S, Simjee S, Woodford N *et al*. Assessing the antimicrobial susceptibility of bacteria obtained from animals. *Vet Microbiol* 2010; **141**: 1–4.

10 Giske CG, Cornaglia G, on behalf of EDGARS. Supranational surveillance of antimicrobial resistance: the legacy of the last decade and proposals for the future. *Drug Resist Updat* 2010; **13**: 93–8.

11 Silley P, de Jong A, Simjee S *et al*. Harmonisation of resistance monitoring programmes in veterinary medicine: an urgent need in the EU? *Int J Antimicrob Agents* 2011; **37**: 504–12.

12 Aarestrup FM, McDermott PF, Kahlmeter G. Antimicrobial susceptibility testing clinical breakpoints and epidemiological cut-off values. Newsletter CRL-AR-No. 2 November 2007.

13 Report from the task force on zoonoses data collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal *Escherichia coli* and *Enterococcus* spp. from food animals. *EFSA J* 2008; **141**: 1–44.

14 Report of the task force on zoonoses data collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers. *EFSA J* 2007; **96**: 1–46.

15 EUCAST. www.srga.org/EUCASTwt/eucast.htm (11 July 2011, date last accessed).

16 Bywater R, Deluyker H, Deroover E *et al*. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *J Antimicrob Chemother* 2004; **54**: 744–54.

17 de Jong A, Bywater R, Butty P *et al*. A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. *J Antimicrob Chemother* 2009; **63**: 733–44.

18 Bywater R, McConville M, Phillips I *et al*. The susceptibility to growth-promoting antibiotics of *Enterococcus faecium* isolates from pigs and chickens in Europe. *J Antimicrob Chemother* 2005; **56**: 538–43.

19 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals—Third Edition: Approved Standard M31-A3*. CLSI, Wayne, PA, USA, 2008.

20 Clinical and Laboratory Standards Institute. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria—Second Edition: Approved Guideline M45-A2*. CLSI, Wayne, PA, USA, 2010.

21 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA, 2010.

22 NARMS. NARMS 2009 Executive Report. 2009. http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm268951.htm (9 September 2011, date last accessed).

23 Kahlmeter G, Brown DFJ. Resistance surveillance studies—comparability of results and quality assurance of methods. *J Antimicrob Chemother* 2002; **50**: 775–7.

24 Schwartz S, Kehrenberg C, Walsh TR. Use of antimicrobial agents in veterinary medicine and food animal production. *Int J Antimicrob Agents* 2001; **17**: 432–7.

25 Hakanen A, Kotilainen P, Huovinen P *et al*. Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from Southeast Asia. *Emerg Infect Dis* 2001; **7**: 996–1002.

26 Friederichs SM, de Jong A, Greife HA. Prevalence of antimicrobial resistance and detection of Qnr in avian *Salmonellae* in two EU regions, 1998–2006. *Clin Microbiol Infect* 2008; **14** Suppl 7: P2092.

27 de Jong A, Ludwig C, Stephan B. Twelve years of resistance monitoring of *Salmonella* from healthy chickens in two EU regions. *Clin Microbiol Infect* 2011; **17** Suppl S4: P1100.

28 Giraud E, Baucheron S, Cloeckaert A. Resistance to fluoroquinolones in *Salmonella*: emerging mechanisms and resistance prevention strategies. *Microbes Infect* 2006; **8**: 1937–44.

29 O'Regan E, Quinn T, Frye JG *et al*. Fitness costs and stability of a high-level ciprofloxacin resistance phenotype in *Salmonella enterica* serotype enteritidis: reduced infectivity associated with decreased expression of *Salmonella* pathogenicity island 1 genes. *Antimicrob Agents Chemother* 2010; **54**: 367–74.

30 Bywater R, Silley P, Simjee S. Antimicrobial breakpoints—definitions and conflicting requirements. *Vet Microbiol* 2006; **118**: 158–9.

31 Kahlmeter G, Brown DFJ, Goldstein FW *et al*. Harmonisation of MIC breakpoints for antimicrobial testing of bacteria. *J Antimicrob Chemother* 2003; **52**: 145–8.

32 EFSA/ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. *EFSA J* 2011; **9**: 2090.

33 Kist M. Impact and management of *Campylobacter* in human medicine—European perspective. *Int J Infect Dis* 2002; **6** Suppl 3: S44–8.

34 Wassenaar TM, Kist M, de Jong A. Re-analysis of the risks attributed to ciprofloxacin-resistant *Campylobacter jejuni* infections. *Int J Antimicrob Agents* 2007; **30**: 195–201.

35 Zhao S, Young SR, Tong E *et al*. Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. *Appl Environ Microbiol* 2010; **76**: 7949–56.

36 NARMS. Retail Meat Report 2009. http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM257587.pdf (19 November 2011, date last accessed).

37 Murray BE. The life and times of the enterococcus. *Clin Microbiol Rev* 1990; **3**: 46–65.

38 Top J, Willems R, Bonten M. Emergence of CC17 *Enterococcus faecium*: from commensal to hospital-adapted pathogen. *FEMS Immunol Med Microbiol* 2008; **52**: 297–308.

39 EFSA/ECDC. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2009. *EFSA J* 2011; **9**: 2154.

40 Simjee S, Silley P, Werling H-O *et al*. Potential confusion regarding the term 'resistance' in epidemiological surveys. *J Antimicrob Chemother* 2008; **61**: 228–9.

41 Belgian Veterinary Surveillance of Antimicrobial Consumption. National Consumption Report 2007–2008–2009. www.belvetsac.ugent.be (19 July 2011, date last accessed).

42 *Sales of Antimicrobial Products Authorized for Use as Veterinary Medicines, Antiprotozoals, Antifungals and Coccidiostats in the UK in 2009*. http://www.vmd.gov.uk/Publications/Antibiotic/salesanti09.pdf (4 October 2011, date last accessed).

43 European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Trends in the sales of veterinary antimicrobial agents in nine European countries. Reporting period: 2005–2009. EMA/238630/2011.

44 Grave K, Torren-Edo J, Mackay D. Comparison of the sales of veterinary antibacterial agents between 10 European countries. *J Antimicrob Chemother* 2010; **65**: 2037–40.