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Antibiotic resistance integrons and extended-spectrum β-lactamases among Enterobacteriaceae isolates recovered from chickens and swine in Portugal

Elisabete Machado[1–3], Teresa M. Coque[3], Rafael Cantón[3], João Carlos Sousa[2] and Luísa Peixe[1]*

[1]REQUIMTE, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; [2]CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal; [3]Servicio de Microbiología, Hospital Universitario Rámon y Cajal, IMSALUD, Madrid, Spain

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***Objectives*: To investigate the diversity of integrons and extended-spectrum β-lactamases (ESBLs) among Enterobacteriaceae from chickens and swine in Portugal and analyse the clonal relationships between Portuguese ESBL-producing isolates of animal and human origin.**

***Methods*: We analysed samples from faeces of healthy swine (HSF, $n = 35$), from uncooked chicken carcasses (CM, $n = 20$) and from faeces of healthy chickens (HCF, $n = 20$). Samples were plated on MacConkey agar with and without ceftazidime (1 mg/L) or cefotaxime (1 mg/L). ESBLs were characterized by PCR and DNA sequencing. Bacterial identification, antibiotic susceptibility and conjugation assays were performed by standard procedures. Isolate clonal relatedness was established by PFGE and by RAPD for PFGE non-typeable isolates. *Escherichia coli* phylogenetic groups were identified by a multiplex PCR. Integron analysis was accomplished by PCR-RFLP and sequencing.**

***Results*: ESBL-producing Enterobacteriaceae were identified in 60% of CM, 10% of HCF and 5.7% of HSF samples, respectively, mostly corresponding to *E. coli* (phylogroups A, D and B1). TEM-52, SHV-2 and CTX-M-1 were detected from chicken and SHV-12 from swine samples. High clonal diversity was observed and most bla_{ESBL} genes were transferable (67%). Class 1 and/or class 2 integrons were identified in 80% of CM, 10% of HCF and 63% of HSF samples, with class 1 integrons more common than class 2 integrons (36% versus 12% of the isolates recovered, respectively). Ten class 1 integron types are described, *aadA1* and *dfrA1-aadA1* being the most frequently found. Two class 1 integron types (*aadA13-estX* and *dfrA14-aadA1-catB2*) and one class 2 integron (*aadA1*) are first reported here.**

***Conclusions*: This study is the first report of ESBLs and integrons from chickens and swine in Portugal and highlights the antibiotic-resistant bacteria and/or resistance genes that might be acquired by humans through the food chain.**

Keywords: CTX-M, TEM-52, SHV, ESBLs, animals

Introduction

Intestinal commensal Enterobacteriaceae of animals reared in high-population flocks are usually under different selective pressures by the use of antibiotics for treating infections, for metaphylaxis and for prophylaxis.[1] Several antimicrobial agents used in veterinary and human medicine belong to the same antibiotic families and hence different selective pressures exercised in distinct environments might contribute to the selection and dissemination of similar resistance genes.[2,3] Surveillance of antimicrobial resistance in commensal bacteria from food-producing animals is considered as one of the main priorities of the World Health Organization and the European Commission to better control the spread of antimicrobial resistance from food animal products to humans through the food chain.[4,5] Recent studies have alerted for the wide presence of extended-spectrum β-lactamases (ESBLs) and integrons in bacteria recovered from a diversity of animals and food products in different countries;[6–16] however, data on the occurrence of integrons and/or ESBLs among commensal Enterobacteriaceae from

*Corresponding author. Tel: +351-22-200-89-46; Fax: +351-91-209-33-77; E-mail: lpeixe@ff.up.pt

food-producing animals are very scarce in Portugal.[17] In this study, we analysed the occurrence and diversity of integrons and ESBLs among Enterobacteriaceae from faeces of healthy food-producing animals and raw chicken meat samples. Moreover, as ESBLs are widely disseminated in Portuguese hospitals,[18,19] we also investigated the clonal relationships between ESBL-producing Enterobacteriaceae from animal and human origin.

Materials and methods

Bacterial isolates

Samples from swine ($n = 35$) and chickens ($n = 40$) were analysed. Swine samples included faeces from healthy swine (HSF) freshly slaughtered at a slaughterhouse of the central region of Portugal ($n = 21$, 1998) or reared at two non-intensive-production farms in the north ($n = 14$, 2004). Chicken samples included uncooked chicken carcasses (CM) from two butcher shops corresponding to chickens from intensive-production farms of five different brands widely commercialized throughout Portugal ($n = 20$, 2003 and 2005) and faeces from healthy chickens (HCF) reared at two non-intensive-production farms ($n = 20$, 2005). The faecal samples collected in 2004 and 2005 were obtained from healthy animals of farms without records of antibiotic use in the 3 months preceding the sample recovery. Samples were processed immediately after collection. Rectal swabs were immersed in the transport medium and faeces were suspended in 1 mL of saline. Chicken carcasses (25 g) were pre-enriched in 500 mL of buffered peptone water for 18 h at 37°C. An aliquot of 0.2 mL from these suspensions was inoculated on MacConkey agar with and without ceftazidime (1 mg/L) or cefotaxime (1 mg/L). Presumptive Enterobacteriaceae were selected and identified by using the automated WIDER system (Fco. Soria Melguizo, Madrid, Spain) or API ID 32GN galleries (bioMérieux, Marcy l'Étoile, France). Representative isolates of ESBL-producing Enterobacteriaceae recovered from Portuguese hospitals were also included for establishing clonal relationships.

ESBL detection and antimicrobial susceptibility

Each different morphotype growing on MacConkey agar supplemented with ceftazidime or cefotaxime was screened for ESBL production by the standard double disc synergy test[18] and underwent susceptibility testing to additional non-β-lactam antibiotics using the standard CLSI disc diffusion method.[20] Non-β-lactam antibiotics tested were the following: gentamicin, tobramycin, amikacin, streptomycin, spectinomycin, netilmicin, neomycin, apramycin, kanamycin, sulphonamides, trimethoprim, tetracycline, chloramphenicol, ciprofloxacin and nalidixic acid. Morphotypes recovered from MacConkey agar with and without ceftazidime or cefotaxime, but identified as non-ESBL producers, underwent susceptibility testing to streptomycin, gentamicin, trimethoprim and sulphonamides using the standard CLSI disc diffusion method.[20] All resistant and intermediate-susceptible isolates were considered as non-susceptible.

Characterization of ESBLs

ESBL characterization was performed by PCR using specific primers for bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$ and further sequencing of both strands of each amplified *bla* gene using ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer, Foster City, CA, USA), as previously described.[21] The presence of IS*Ecp1* in the genetic environment of $bla_{\text{CTX-M}}$ was searched for by PCR.[22]

Clonal relationships among ESBL-producing isolates

These were established by PFGE, according to the Tenover *et al.* criteria,[23] using *Xba*I as restriction enzyme (Amersham, Life Sciences, Uppsala, Sweden) and the following electrophoresis conditions: 10–40 s for 24 h, 14°C, 6 V/cm^2.[18] As some isolates were not typeable by PFGE, randomly amplified polymorphic DNA analysis (RAPD) using primer M13 (5′-GAG GGT GGC GGT TCT-3′) and Ready-To-Go RAPD analysis beads (Amersham Biosciences, Amersham, Portugal) were also performed, as described.[24] Isolates with RAPD patterns showing two or more band differences were considered as unrelated.[25] The PFGE and RAPD patterns were visually analysed and were also compared with each clonal type/subtype of each ESBL-producing species identified in Portuguese hospitals.[18] The phylogenetic groups of ESBL-producing *Escherichia coli* isolates were determined by a multiplex PCR assay described by Clermont *et al.*[26]

Conjugation experiments

Transfer of β-lactam resistance was performed by the filter mating method using *E. coli* BM21R (nalidixic acid- and rifampicin-resistant, lactose fermentation positive and plasmid-free) as recipient.[21] Mating experiments were performed overnight at 37°C. Transconjugants were selected on MacConkey agar plates containing 100 mg/L rifampicin and 2 mg/L cefotaxime or ceftazidime.[21]

Analysis and characterization of integrons

Isolates resistant to streptomycin, gentamicin, trimethoprim and/or sulphonamides were screened for the presence of integrons, as these phenotypes are commonly associated with these genetic structures.[21] The PCR was used in the detection of class 1 and class 2 integrons using genomic DNA from wild-type and the corresponding transconjugant strains, and primers and conditions previously described for amplification of *intI1*, *intI2*, 5′CS-3′CS class 1 integron variable region and *attI2-orfX* variable region of class 2 integrons.[21] Typing of class 1 and class 2 integrons was performed by restriction fragment length polymorphism (RFLP) using *Alu*I and *Taq*I restriction endonucleases, respectively.[21] An amplified DNA fragment corresponding to the variable regions of each distinct class 1 or class 2 RFLP-type integron was sequenced using an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer, Foster City, CA, USA). Nucleotide sequences were compared with sequences in the GenBank and EMBL databases using the BLASTN local alignment search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Integrons were designated by roman numbers and a subindex indicates the class to which each integron belongs, as previously described.[21]

Results

Epidemiological background

Isolates with decreased susceptibility to expanded-spectrum cephalosporins were recovered from all chicken meat samples and from two HCF and three HSF samples. A total of 149 isolates representing different colony morphotypes and antibiotic susceptibility patterns were obtained from HSF ($n = 48$), HCF ($n = 33$) and CM ($n = 68$). Resistance to at least one antibiotic was observed in 113 isolates, being commonly resistant to streptomycin (65%), sulphonamides (51%) or trimethoprim (40%). Non-susceptibility rates for sulphonamides and trimethoprim

Table 1. Characteristics of ESBL-producing isolates recovered from healthy chickens, healthy swine and chicken meat products from Portugal

Isolate	Phylogenetic group	Date of isolation (mm/yy)	Origin	Brand[a]	ESBL[b]	PFGE type[c]	Non-β-lactam associated resistances[d]
C. freundii S205		Mar-04	swine faeces I	NA	SHV-12	L	STR, SPT, NAL, TET, KAN
C. freundii S210		Mar-04	swine faeces II	NA	SHV-12	L	STR, SPT, NAL, TET, NET, KAN
E. coli P215	A	Jun-05	poultry faeces M	NA	TEM-52	J	STR, SPT, NEO
E. coli P218	A	Jun-05	poultry faeces N	NA	TEM-52	K	STR, TET
E. coli P16	A	Jan-03	poultry meat A	1	TEM-52	A	STR, NAL, CIP, SUL, TMP, TET, APR, NEO
E. coli P35	D	Jan-03	poultry meat B	3	TEM-52	B	STR, SPT, NAL, CIP, SUL, TMP, TET
E. coli P69	A	Feb-03	poultry meat E	1	TEM-52	C	STR, NAL, SUL, TMP, TET, NEO, KAN
E. coli P110	D	Mar-03	poultry meat G	2	TEM-52	NT[e]	STR, SPT, NAL, CIP, SUL, TMP, TET
E. coli P121	A	Jun-05	poultry meat H	2	TEM-52	C	STR, SPT, NAL, SUL, TMP, TET, NEO, KAN
E. coli P123	A	Jun-05	poultry meat H	2	TEM-52	D	STR, SPT, NAL, SUL, TET
E. coli P142	D	Jun-05	poultry meat I	2	TEM-52	NT[e]	STR, SPT, NAL, SUL, TMP, TET, CHL
E. coli P144	A	Jun-05	poultry meat J	2	TEM-52	E	GEN, TOB, NAL, CIP, SUL, TMP, CHL, APR, NEO, KAN
E. coli P167	B1	Jun-05	poultry meat L	2	TEM-52	NT[f]	STR, SPT, NAL, CIP, SUL, TMP, TET, CHL, NEO, KAN
E. coli P155	D	Jun-05	poultry meat K	4	CTX-M-1	F	STR, SPT, NAL, SUL, TMP, TET, CHL, NEO
K. pneumoniae P54		Feb-03	poultry meat C	5	TEM-52	G	GEN, TOB, STR, SPT, NAL, CIP, SUL, TMP, TET, CHL, APR, NEO, NET, KAN
K. pneumoniae P62		Feb-03	poultry meat D	1	SHV-2	H	TET
K. pneumoniae P80		Feb-03	poultry meat F	1	SHV-2	I	STR, SPT, NAL, CIP, SUL, TMP, TET, CHL, NEO, KAN
K. pneumoniae P106		Mar-03	poultry meat G	2	SHV-2	H	TET

[a]NA, not applicable; 1–5 represent different Portuguese commercial chicken brands.
[b]ESBL transfer by conjugation is underlined.
[c]NT, non-typeable by PFGE.
[d]GEN, gentamicin; TOB, tobramycin; STR, streptomycin; SPT, spectinomycin; NET, netilmicin; NEO, neomycin; APR, apramycin; KAN, kanamycin; SUL, sulphonamides; TMP, trimethoprim; TET, tetracycline; CHL, chloramphenicol; CIP, ciprofloxacin; NAL, nalidixic acid. Patterns transferred by conjugation are underlined.
[e]Strains presented the same profile by RAPD typing: RAPD type D.
[f]Strain belonged to RAPD-type H.

were similar among isolates from chickens and swine (50% versus 54% for sulphonamides and 43% versus 35% for trimethoprim). Non-susceptibility rates for non-β-lactam antibiotics were more frequently found among ESBL producers than among non-ESBL-producing isolates: streptomycin (83% versus 63%), sulphonamides (67% versus 49%) and trimethoprim (61% versus 37%).

ESBL characterization

We identified 18 ESBL-producing Enterobacteriaceae corresponding to TEM-52 (11 *E. coli* and 1 *Klebsiella pneumoniae* from HCF or CM), SHV-2 (3 *K. pneumoniae* from CM), SHV-12 (2 *Citrobacter freundii* from HSF) and CTX-M-1 (1 *E. coli* from CM; $bla_{\text{CTX-M-1}}$ located downstream of IS*Ecp1*). These isolates were frequently resistant to aminoglycosides, sulphonamides, trimethoprim, tetracyclines, chloramphenicol or quinolones (Table 1). Genes encoding ESBLs were transferred from 67% of the isolates, often associated with genes encoding resistance to non-β-lactam antibiotics (Table 1). A great heterogeneity of PFGE patterns was observed, although some clones were persistently recovered, as TEM-52-producing *E. coli* PFGE type C and RAPD type D, isolated in 2003 and 2005, or SHV-2-producing *K. pneumoniae* PFGE type H isolated from different brands in 2003 (Table 1). When comparing these isolates with those previously published with ESBL from hospitalized patients in Portugal,[18] we did not find any relationship between PFGE or RAPD profiles. ESBL-producing *E. coli* isolates belonged to phylogenetic groups A ($n = 7/12$), D ($n = 4/12$) or B1 ($n = 1/12$).

Analysis of integrons

Isolates carrying class 1 and/or class 2 integrons were recovered from 80% of chicken meat, 10% of chicken faecal samples and 63% of swine faecal samples. Class 1 and/or class 2 integrons were detected in 40% ($n = 60/149$) of the isolates studied, being more common among isolates from swine than those from chickens: 48% versus 31% for class 1 integrons and 21% versus 8% for class 2 integrons (Table 2). Among ESBL-producing isolates, class 1 integrons were identified in isolates harbouring $bla_{\text{CTX-M-1}}$, $bla_{\text{SHV-2}}$ or $bla_{\text{TEM-52}}$. Co-transfer of integrons and *bla* genes was only observed for a CTX-M-1-producing *E. coli* and for an SHV-2-producing *K. pneumoniae*. Class 2 integrons were only detected among TEM-52-producing *E. coli* and were not transferred. Simultaneous presence of class 1 and class 2 integrons was observed in 8% of the isolates studied, all lacking bla_{ESBL} ($n = 12/149$). The presence of class 1 and/or class 2 integrons among non-ESBL-producing isolates was not a rare event (39%, 51/131), particularly in isolates recovered from swine and chicken meat samples. Ten different class 1 integron types were found, mostly corresponding to non-ESBL-producing isolates (Table 3). Gene cassettes coding for aminoglycoside (*aadA1*, *aadA1a*, *aadA2*, *aadA5*, *aadA13*) and/or trimethoprim (*dfrA1*, *dfrA12*, *dfrA14*, *dfrA17*) resistance were the most commonly identified. Integron types I_1 (*aadA1*) and II_1 (*dfrA1-aadA1*) were the most prevalent. Types I_1' (*aadA1a*), III_1 (*dfrA12-orfF-aadA2*), V_1 (*aadA2*) and XX_1 (*blaP1*) were confined to isolates from CM, whereas types $XXII_1$ (*aadA13-estX*) and $XXIII_1$ (*dfrA14-aadA1-catB2*) were only distributed among HSF. Three class 2 integron types were observed: type II_2, identical to that described for Tn*7*, and type III_2 (*estX-sat2-aadA1-orfX*) were the most widely distributed. Type IV_2 (*aadA1*) is first reported in this study. Gene cassettes were not identified for a number of non-ESBL-producing isolates harbouring *intI1* (15/149, 10%) or *intI2* (1/149, 0.7%).

Discussion

ESBLs frequently identified in humans associated with either hospitals or community settings in European countries are now being increasingly detected among animals.[8–10,16,27] The frequent recovery of Enterobacteriaceae producing ESBLs and/or carrying class 1 and/or class 2 integrons from faecal samples from food-producing swine and chickens, and from both different commercial brands and chickens reared at different farms from Portugal, confirms the role of animals as possible reservoirs for dissemination of resistance genes in the community. The presence of ESBLs among chicken samples from both different commercial brands and chickens reared at different farms might be caused by contamination of the few genetic lines of primary breeding flocks that are sold by the same producer, as also supported by previous studies on poultry enterococci populations.[28] The ESBLs were more frequently observed among *E. coli* mainly belonging to the phylogenetic group A (58.3%),

Table 2. Percentage of class 1 and class 2 integrons among Enterobacteriaceae recovered from swine and chickens from Portugal

			Class 1		Class 2			
Origin	ESBL	Number of isolates	*intI1*+	presence of gene cassettes as part of 5′CS-3′CS variable region	*intI2*+	presence of gene cassettes as part of *attI2-orfX* variable region	Class 1+2	Isolates integron+
Swine	+	2	0	0	0	0	0	0
	−	46	50	37	22	22	20	52
Chicken meat	+	14	43	43	21	21	0	64
	−	56	41	29	9	7	5	45
Chicken faeces	+	2	0	0	0	0	0	0
	−	29	7	0	0	0	0	7

Table 3. Class 1 and class 2 integron types found among Enterobacteriaceae from Portuguese chickens and swine

RFLP type	Length of variable region (bp)	Gene cassettes and order	Resistance phenotype[a]	Number of isolates	Chicken meat[b]		Swine	Isolation date
					ESBL+	ESBL−	ESBL−	
Class 1 integrons								
I_1	1000	*aadA1*	STR, SPT	10		1	9	1998–2005
I_1,	1000	*aadA1a*	STR, SPT	1		1		2003
II_1	1700	*dfrA1-aadA1*	TMP, STR, SPT	14	3 (2 EC TEM-52; 1 EC CTX-M-1)	9	2	2003–2005
III_1	2000	*dfrA12-orfF-aadA2*	TMP, unknown, STR, SPT	1	1 (KP SHV-2)			2003
V_1	1000	*aadA2*	STR, SPT	1	1 (KP TEM-52)			2003
VI_1	1700	*dfrA17-aadA5*	TMP, STR, SPT	4		3	1	2003–2005
XX_1	1000	*blaP1*	β-lactams	1		1		2005
XXI_1	1900	*estX-aadA1*	unknown, STR, SPT	2		1	1	2003–2004
$XXII_1$	1900	*aadA13-estX*	STR, SPT, unknown	3			3	1998–2004
$XXIII_1$	2300	*dfrA14-aadA1-catB2*	TMP, STR, SPT, CHL	1			1	2004
Class 2 integrons								
II_2	2000	*dfrA1-sat2-aadA1-orfX*	TMP, STH, STR, SPT	10	1 (EC TEM-52)	3	6	1998–2004
III_2	2300	*estX-sat2-aadA1-orfX*	unknown, STH, STR, SPT	6	2 (EC TEM-52)	1	3	1998–2005
IV_2	1000	*aadA1*	STR, SPT	1			1	1998

[a]STR, streptomycin; SPT, spectinomycin; TMP, trimethoprim; CHL, chloramphenicol; STH, streptothricin.
[b]For each integron-type observed, the Enterobacteriaceae species and the corresponding ESBL-type associated are indicated in parentheses. EC, *E. coli*; KP, *K. pneumoniae*.

which is associated with animal or human commensal *E. coli* strains, in agreement with other studies.[26] However, we also detected ESBL-producing *E. coli* of phylogenetic group D, which is associated with extra-intestinal human infection, mirroring an animal reservoir for these invasive strains.

All ESBL genes identified in this study have already been identified in Portuguese hospitals, and the most common ESBLs among animals, TEM-52 and SHV-12, are also the most frequent types among humans.[18] The $bla_{\text{TEM-52}}$ has also been detected among food-producing and wild animals, pets and healthy humans from Belgium, France, Greece, Portugal or Spain[8–10,29–31] and associated with epidemic conjugative plasmids among animal samples in areas with high prevalence of this ESBL type.[29] Our results increase the number of hosts of $bla_{\text{TEM-52}}$ in Portugal[9,10,18] and might reflect the successful spread of an epidemic plasmid of the IncI group.[32] This possibility is also supported by the absence of relationship between ESBL-producing isolates from animals and from patients at Portuguese hospitals located in the same regions studied.[18] The other ESBL genes found, $bla_{\text{CTX-M-1}}$, $bla_{\text{SHV-2}}$ or $bla_{\text{SHV-12}}$, are widely disseminated among Enterobacteriaceae from different continents by both clonal and plasmid spread and have recently been detected in Portugal.[8–10,18,27,29,33] The clonal diversity of isolates carrying these genes suggests that horizontal gene transmission may be responsible for the recent and fast spread of these variants in our country.

Most ESBL-producing isolates exhibited resistance to antibiotics used in intensive animal production, mainly streptomycin, sulphonamides and trimethoprim. In agreement with other studies, integrons were commonly identified and corresponded to a few integron types that have been mostly found among Enterobacteriaceae from food animals, hospitalized patients and/or healthy humans.[6,7,11,12,34–37] Some of them are globally disseminated as type I_1 (*aadA1*), type II_1 (*dfrA1-aadA1*), type III_1 (*dfrA12-orfF-aadA2*), type VI_1 (*dfrA17-aadA5*) or class 2 integron types II_2 and III_2, whereas others are first reported in this study as type $XXII_1$ (*aadA13-estX*), type $XXIII_1$ (*dfrA14-aadA1-catB2*) or type IV_2 (*aadA1*). Type XXI_1 (*estX-aadA1*) has been recently described to be associated with human community isolates.[38,39] Differences in integron dissemination might be explained by the association of specific integron types with particular globally disseminated plasmids and/or transposons potentially exchanged among humans and animals.[37] It is to be noted that the simultaneous presence of class 1 and class 2 integrons in 8% of the isolates and the finding of a new class 2 integron (type IV_2, *aadA1*), carrying one of the most common gene cassettes identified in class 1 integrons, highlight a possible variety of recombinatorial events among these genetic platforms.

In summary, we describe the presence and diversity of integrons and ESBLs in Enterobacteriaceae from chickens and swine in Portugal. The findings are worrisome as transmission to humans via the food chain of bacteria resistant to practically all antimicrobial classes cannot be dismissed.[13,40,41] Some of the ESBLs and integrons we found have already been detected among Enterobacteriaceae from different ecological niches in Portugal and other countries,[6,18,34,37] suggesting that the genetic mobile structures harbouring them are widespread. More strict veterinary antibiotic policies are needed in order to prevent emergence and dissemination of these strains among animals and humans, limiting future problems of therapy failure.

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References

1. Schwarz S, Kehrenberg C, Walsh TR. Use of antimicrobial agents in veterinary medicine and food animal production. *Int J Antimicrob Agents* 2001; **17**: 431–7.

2. Aarestrup FM. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. *Int J Antimicrob Agents* 1999; **12**: 279–85.

3. World Health Organization (WHO). *The Medical Impact of the Use of Antimicrobials in Food Animals*. Report of a WHO Meeting, Berlin, Germany, 13–17 October 1997. WHO/EMC/ZOO/97.4, 1997.

4. European Commission. *Opinion of the Scientific Steering Committee on Antimicrobial Resistance, DGXXIV, Consumer Policy and Consumer Health Protection.* 1999. http://europa.eu.int/comm/food/fs/sc/ssc/out50_en.pdf (29 June 2006, date last accessed).

5. World Health Organization. *WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food*. Geneva, Switzerland: WHO, 2000.

6. Antunes P, Machado J, Peixe L. Characterization of antimicrobial resistance and class 1 and class 2 integrons in *Salmonella enterica* isolates from different sources in Portugal. *J Antimicrob Chemother* 2006; **58**: 297–304.

7. Bass L, Liebert CA, Lee MD *et al.* Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. *Antimicrob Agents Chemother* 1999; **43**: 2925–9.

8. Briñas L, Moreno MA, Teshager T *et al.* Monitoring and characterization of extended-spectrum β-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain in 2003. *Antimicrob Agents Chemother* 2005; **49**: 1262–4.

9. Costa D, Poeta P, Briñas L *et al.* Detection of CTX-M-1 and TEM-52 β-lactamases in *Escherichia coli* strains from healthy pets in Portugal. *J Antimicrob Chemother* 2004; **54**: 960–1.

10. Costa D, Poeta P, Sáenz Y *et al.* Detection of *Escherichia coli* harbouring extended-spectrum β-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild animals in Portugal. *J Antimicrob Chemother* 2006; **58**: 1311–2.

11. Goldstein C, Lee MD, Sanchez S *et al.* Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock,

companion animals, and exotics. *Antimicrob Agents Chemother* 2001; **45**: 723–6.

12. Kang HY, Jeong YS, Oh JY *et al*. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. *J Antimicrob Chemother* 2005; **55**: 639–44.

13. Liebana E, Batchelor M, Hopkins KL *et al*. Longitudinal farm study of extended-spectrum β-lactamase-mediated resistance. *J Clin Microbiol* 2006; **44**: 1630–4.

14. Olesen I, Hasman H, Aarestrup FM. Prevalence of β-lactamases among ampicillin-resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. *Microb Drug Resist* 2004; **10**: 334–40.

15. Sunde M, Norström M. The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products. *J Antimicrob Chemother* 2006; **58**: 741–7.

16. Yang H, Chen S, White DG *et al*. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. *J Clin Microbiol* 2004; **42**: 3483–9.

17. European Food Safety Authority (EFSA). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005. *EFSA J* 2006; **94**: 2–288.

18. Machado E, Coque TM, Cantón R *et al*. High diversity of extended-spectrum β-lactamases (ESBL) among clinical isolates of Enterobacteriaceae from Portugal. *J Antimicrob Chemother* 2007; **60**: 1370–4.

19. Mendonça N, Leitão J, Manageiro V *et al*. Spread of clinical extended-spectrum β-lactamase (CTX-M)-producing *Escherichia coli* isolates in community and nosocomial environments in Portugal. *Antimicrob Agents Chemother* 2007; **51**: 1946–55.

20. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement M100-S17*. CLSI, Wayne, PA, USA, 2007.

21. Machado E, Cantón R, Baquero F *et al*. Integron content of extended-spectrum-β-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. *Antimicrob Agents Chemother* 2005; **49**: 1823–9.

22. Machado E, Coque TM, Cantón R *et al*. Emergence of CTX-M β-lactamase-producing Enterobacteriaceae in Portugal: report of an *Escherichia coli* isolate harbouring $bla_{CTX\text{-}M\text{-}14}$. *Clin Microbiol Infect* 2004; **10**: 755–7.

23. Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–9.

24. De Gheldre Y, Struelens MJ, Gloupczynski Y *et al*. National epidemiological surveys of *Enterobacter aerogenes* in Belgian hospitals from 1996 to 1998. *J Clin Microbiol* 2001; **39**: 889–96.

25. Gori A, Espinasse F, Deplano A *et al*. Comparison of pulsed-field gel electrophoresis and randomly amplified DNA polymorphism analysis for typing extended-spetcrum-β-lactamase-producing *Klebsiella pneumoniae*. *J Clin Microbiol* 1996; **34**: 2448–53.

26. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; **66**: 4555–8.

27. Briñas L, Moreno MA, Zarazaga M *et al*. Detection of CMY-2, CTX-M-14, and SHV-12 β-lactamases in *Escherichia coli* fecal-sample isolates from healthy chickens. *Antimicrob Agents Chemother* 2003; **47**: 2056–8.

28. Novais C, Coque TM, Costa MJ *et al*. High occurrence and persistence of antibiotic-resistant enterococci in poultry food samples in Portugal. *J Antimicrob Chemother* 2005; **56**: 1139–43.

29. Cloeckaert A, Praud K, Doublet B *et al*. Dissemination of an extended-spectrum β-lactamase $bla_{TEM\text{-}52}$ gene-carrying IncI1 plasmid in various *Salmonella enterica* serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. *Antimicrob Agents Chemother* 2007; **51**: 1872–5.

30. Politi L, Tassios PT, Lambiri M *et al*. Repeated occurrence of diverse extended-spectrum β-lactamases in minor serotypes of foodborne *Salmonella enterica* subsp enterica. *J Clin Microbiol* 2005; **43**: 3453–6.

31. Blanc V, Mesa R, Saco M *et al*. ESBL- and plamidic class C β-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Vet Microbiol* 2006; **118**: 299–304.

32. Pedrosa A, Novais A, Machado E *et al*. Recent dissemination of $bla_{TEM\text{-}52}$-producing *Enterobacteriaceae* in Portugal is caused by spread of IncI plasmids among *Escherichia coli* and *Klebsiella* clones. In: *Abstracts of the Eighteenth European Congress of Clinical Microbiology and Infectious Disease, Barcelona, 2008*. *Clin Microbial Infect* 2008; **14**: Abstract P2006.

33. Briñas L, Moreno MA, Teshager T *et al*. β-Lactamase characterization in *Escherichia coli* isolates with diminished susceptibility or resistance to extended-spectrum cephalosporins recovered from sick animals in Spain. *Microb Drug Resist* 2003; **9**: 201–9.

34. Box AT, Mevious DJ, Schellen P *et al*. Integrons in *Escherichia coli* from food-producing animals in The Netherlands. *Microb Drug Resist* 2005; **11**: 53–7.

35. Hsu SC, Chiu TH, Pang JC *et al*. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. *Int J Antimicrob Agents* 2006; **27**: 383–91.

36. Sunde M, Sorum H. Characterization of integrons in *Escherichia coli* of the normal intestinal flora of swine. *Microb Drug Resist* 1999; **5**: 279–87.

37. Machado E, Ferreira J, Novais A *et al*. Preservation of the integron types among Enterobacteriaceae producing extended-spectrum β-lactamases in a Spanish hospital over a 15-year period. *Antimicrob Agents Chemother* 2007; **51**: 2201–4.

38. Ahmed AM, Nakano H, Shimamoto T. Molecular characterization of integrons in non-typhoid *Salmonella* serovars isolated in Japan: description of an unusual class 2 integron. *J Antimicrob Chemother* 2005; **55**: 371–4.

39. Valverde A, Cantón R, Galán JC *et al*. In117, and unusual In0-like class 1 integron containing CR1 and $bla_{CTX\text{-}M\text{-}2}$ and associated with a Tn*21*-like element. *Antimicrob Agents Chemother* 2006; **50**: 799–802.

40. Mesa RJ, Blanc V, Blanch AR *et al*. Extended-spectrum β-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). *J Antimicrob Chemother* 2006; **58**: 211–5.

41. Sáenz Y, Zarazaga M, Briñas L *et al*. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. *Int J Antimicrob Agents* 2001; **18**: 353–8.