FOOD SAFETY INVITED ARTICLE

Patricia M. Griffin, Section Editor

Public Health Risks of Enterobacterial Isolates Producing Extended-Spectrum β-Lactamases or AmpC β-Lactamases in Food and Food-Producing Animals: An EU Perspective of Epidemiology, Analytical Methods, Risk Factors, and Control Options

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The bla_{ESBL} and bla_{AmpC} genes in Enterobacteriaceae are spread by plasmid-mediated integrons, insertion sequences, and transposons, some of which are homologous in bacteria from food animals, foods, and humans. These genes have been frequently identified in *Escherichia coli* and *Salmonella* from food animals, the most common being $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CMY\text{-}2}$. Identification of risk factors for their occurrence in food animals is complex. In addition to generic antimicrobial use, cephalosporin usage is an important risk factor for selection and spread of these genes. Extensive international trade of animals is a further risk factor. There are no data on the effectiveness of individual control options in reducing public health risks. A highly effective option would be to stop or restrict cephalosporin usage in food animals. Decreasing total antimicrobial use is also of high priority. Implementation of measures to limit strain dissemination (increasing farm biosecurity, controls in animal trade, and other general postharvest controls) are also important.

Keywords. resistance; ESBLs; AmpC; occurrence; transmission; control.

Received 15 June 2012; accepted 30 November 2012; electronically published 14 December 2012.

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Clinical Infectious Diseases 2013;56(7):1030–7

DOI: 10.1093/cid/cis1043

In the last decade a variety of plasmid-mediated ß-lactamases have emerged in gram-negative bacteria. These included both extended-spectrum ß-lactamases (ESBLs) and AmpC ß-lactamases (AmpC).

ESBLs confer resistance to a variety of ß-lactams, including penicillins, first-, second-, third-, and fourth-generation cephalosporins, and monobactams, but usually not to the carbapenems or the cephamycins

Table 1. Main Hydrolytic Characteristics of Extended-Spectrum β-Lactamases and AmpC

Antimicrobial Classes	Examples of Antimicrobials	ESBL Hydrolytic Activity	AmpC Hydrolytic Activity
Penicillins	Penicillin G, amoxicillin, ampicillin, ticarcillin, piperacillin	+++	+++ (exception is ticarcillin: no hydrolysis)
Penicillin/β-lactam inhibitor	Amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin-tazobactam	Inhibitor usually neutralizes ESBL activity	AmpC activity is not neutralized by the inhibitor
First-gen cephalosporins	Cefazolin, cefalexin, cefalotin	+++	+++
Second-gen cephalosporins	Cefaclor, cefuroxime	++	++
Third-gen cephalosporins	Cefotaxime, ceftazidime, ceftriaxone	++	+ (if produced at a basal level)
			+++ (if overproduced)
Fourth-gen cephalosporins	Cefepime	++	+/–
Monobactams	Aztreonam	++	++
Cephamycins	Cefoxitin, cefotetan	–	+++
Carbapenems	Imipenem, meropenem, ertapenem, doripenem	–	– or +/– (depending on the type of ß-lactamase)

Abbreviations: +++, high level of hydrolysis; ++, medium level of hydrolysis; +, weak level of hydrolysis; +/–, none or very weak hydrolysis which can contribute to and influence the final susceptibility results, usually only if combined with additional resistance mechanisms; –, no hydrolysis; ESBL, extended-spectrum β-lactamase; gen, generation.

(Table 1). The most frequent ESBLs in Enterobacteriaceae belong to the TEM, SHV, and CTX-M families. There are also some class D ß-lactamases (so-called oxacillinases) that might be considered "extended spectrum" enzymes.

Before 2000, SHV and TEM types of ESBLs were the predominant variants found in *Klebsiella* species and *Escherichia coli*, mainly causing human nosocomial infections. The corresponding genes were often located on transmissible plasmids, facilitating their efficient spread. During the last decade, CTX-M–type enzymes spread worldwide, being now the most prevalent ESBLs in human Enterobacteriaceae. Whereas ESBLs used to be produced mainly by nosocomial *Klebsiella pneumoniae*, community-acquired *E. coli* producing CTX-M enzymes (specifically CTX-M-15) has been increasingly reported.

AmpC β-lactamases are intrinsic cephalosporinases found on the chromosome of many gram-negative bacteria. These enzymes confer resistance to penicillins and to first-, second-, and third-generation cephalosporins, including β-lactam/inhibitor combinations and cephamycins, but usually not to fourth-generation cephalosporins and carbapenems. A growing number of AmpC enzymes have now "escaped" onto plasmids (termed "acquired" or "plasmidic" AmpCs). Such enzymes fall into 6 phylogenetic groups, with CMY-2 being the most common.

Significance and Public Health Threat of Human Infections With ESBL/AmpC β Lactamase–Producing Bacteria

Community- and healthcare-associated infections with ESBL/AmpC β-lactamase–producing bacteria have been increasingly reported worldwide [1]. Reports from the European Antibiotic Resistance Surveillance System show that in hospitals in Europe, rates of invasive *E. coli* and *K. pneumoniae* isolates resistant to cefotaxime and ceftazidime have been increasing since 2000 [2]. This is recognized as a public health threat because resistance to β-lactams and coresistance to other antimicrobial classes (eg, the fluoroquinolones) limit the choice of effective antimicrobial agents available for treatment and commonly used as first-line therapy [1]. Moreover, infections with ESBL/AmpC β-lactamase–producing bacteria may result in delays in the initiation of timely and adequate antimicrobial therapy [3], increased morbidity and mortality, longer hospital stays, and higher costs [3, 4].

Of the many risk factors found associated with patient colonization or infection with ESBL or AmpC ß-lactamase–producing bacteria [5, 6], prior antibiotic use, especially with the oxyimino-ß-lactams (cefuroxime, cefotaxime, ceftriaxone, ceftazidime, or aztreonam) [7, 8], fluoroquinolones [9] and ß-lactam–ß-lactamase inhibitor combinations [9], has been consistently reported.

Possible Reservoirs of ESBL/AmpC β-Lactamase–Producing Bacteria

Transmission of ESBL-producing Enterobacteriaceae from person to person has been demonstrated in hospital, community, and household settings, the evidence strongly suggesting that human intestinal colonization with these resistant organisms serves as a reservoir for spread [10–12]. The primary reservoirs, however, of such organisms are still contentious. ESBL/AmpC-producing *E. coli* and *Salmonella* have been isolated from food animals in many European countries, particularly poultry and cattle, and farm animals are now recognized as important carriers [13]. Similarly, there have been an increasing number of

reports of isolations from foods of animal origin [14]. These reports raise questions about the possible role of animal- and food-related reservoirs in the spread of ESBL/AmpC-producing microorganisms. This paper reviews the public health risks of bacterial strains producing ESBL or AmpC enzymes in food and food-producing animals, with particular reference to the situation in the European Union (EU). Methods for their detection and possible control options to limit their spread are discussed.

ESBL- AND AmpC-PRODUCING BACTERIAL STRAINS AND GENES RELEVANT FOR PUBLIC HEALTH AND LINKED TO FOOD-PRODUCING ANIMALS OR FOODBORNE TRANSMISSION

Occurrence and Epidemiology of Acquired Resistance to Broad-Spectrum Cephalosporins in Food-Producing Animals and Food

Official harmonized monitoring of resistance to third-generation cephalosporins in EU member states is based on cefotaxime susceptibility patterns. In those EU countries reporting such resistance, the occurrence of cefotaxime resistance was low among *E. coli* and *Salmonella* isolates recovered from nonselective plates. Total prevalences in different animal species and meat in 2009 ranged from 0.4% to 5% for *Salmonella*, and from 2% to 9% for *E. coli* [15].

In studies targeted to detect ESBL or AmpC genes, the percentages of samples of food-producing animals or food in which ESBL-carrying *E. coli* were detected varied from 0.2% to 40%. Percentages of occurrence ranged from 10% to 40% in Portugal, the Netherlands, and France, with slightly lower percentages in other countries [15]. In a pilot study carried out in the Netherlands, 100% of broiler farms and >80% of animals were positive for ESBL *E. coli* [16]. The prevalence of ESBLs among *Salmonella* isolates was much lower than for *E. coli*, with percentages of <1% found in 2 studies in Germany and Spain, respectively [17, 18]. The methodology used in the different studies is heterogeneous, and comparisons are therefore difficult.

ESBL-producing (eg, TEM, SHV, CTX-M, PER) and AmpC-producing (eg, CMY, DHA-1, ACT-1) organisms have been detected in food-producing animals (poultry, swine, bovines, horses, rabbits, ostriches, wild boar), marine aquaculture systems, and foods of animal origin [15]. The most common ESBL genes are those encoding CTX-M enzymes. In food-producing animals and food in EU countries, 11 different ESBL subtypes of the CTX-M type have been described, with CTX-M-1 being most common [15]. Although CTX-M-15 has spread in a pandemic fashion in humans, the enzyme type has been only recently been reported in food-producing animals or food, and then only in very few EU countries [19–21]. SHV ESBLs, in particular SHV-12 and SHV-2, have been also frequently detected throughout the European Union. The most frequently detected TEM ESBL has been TEM-52, whereas TEM-20 has been less common. The type of AmpC β-lactamase detected was almost always the CMY-2 variant [15]. ESBL producers have been mostly found in Europe, whereas AmpC producers have been particularly common in North America, mirroring the trends for human isolates. ESBL and AmpC genes have most frequently been detected in *E. coli* and nontyphoidal *Salmonella* in isolates from terrestrial food-producing animals; and in *Aeromonas*, *Vibrio*, and *Edwardsiella* species from fish [15].

Transmission of ESBL/AmpC genes in both humans and animals is mainly driven by the IncF, IncI, IncN, IncA/C, IncL/M, and IncK plasmid families [22]. Most of these plasmid types can only replicate within Enterobacteriaceae, and show a narrow host range (ie, IncF, IncI, IncK). Plasmids of other families (ie, IncA/C) can replicate within the species of many genera and even families, and are defined as broad-host-range plasmids. IncA/C plasmids have been associated with the spread of $bla_{\text{CMY-2}}$ in both the United States and the United Kingdom [23] and IncN, IncI, and IncL/M plasmids with the spread of $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, and $bla_{\text{TEM-52}}$ in Europe [24]. IncK plasmids carrying the $bla_{\text{CTX-M-14}}$ gene have become diffused in both Spain and the United Kingdom [25].

Clonal expansion of ESBL/AmpC producers has also been documented. Specific clones of *E. coli* such as B2–*E. coli* O25b:H4-sequence type (ST) 131, D–*E. coli* O25a-ST648, and D–*E. coli* ST69 and ST393 have been increasingly detected among humans and animals [20, 26–28]. Other clones of *E. coli* (ST57, ST156, ST371) are widespread among poultry in some EU countries and in Japan [29–31]. Recent reports documenting outbreaks of ESBL producers of Shiga toxin–producing *E. coli* clones O111:H8 and O104:H4 linked to food or food-producing animals are of concern [32, 33]. A range of *Salmonella* serovars (*Salmonella* Agona, *Salmonella* Virchow, *Salmonella* Infantis, *Salmonella* Typhimurium) have been associated with the dissemination of ESBLs in poultry, cattle, and pigs. AmpC producers of *Salmonella* Heidelberg and *Salmonella* Newport have been reported, mostly in cattle in North America [15]. The diversity of genetic contexts and clonal or plasmid backgrounds is comprehensively tabulated elsewhere [15].

Transmission of ESBL/AmpC-Resistant Bacterial Strains or Resistance Genes to Humans by Consumption or Handling of Contaminated Food or Through the Food-Animal Production Environment

There are few studies that provide evidence of the existence of common clones of ESBL- or AmpC-producing *E. coli* in foods of animal origin and in humans [19, 21, 34]. Transmission of ESBL/AmpC-producing *Salmonella* from different animal species, including poultry, to humans throughout the food chain has been suggested in different studies [15], although the evidence with regard to the possibility of spread of such organisms to humans via direct contact with animals, or indirectly via the environment, is limited [35]. People working

with poultry have been demonstrated to have a higher risk for intestinal carriage of ESBL/AmpC-producing bacteria [36].

The occurrence of Enterobacteriaceae resistant to carbapenems is a growing threat in human medicine. The presence of such resistance in bacteria from animals is largely unknown, although *E. coli* producing VIM-1 carbapenemase resistance has been recently recorded in pigs in Germany [37]. As yet, there are no indications of the zoonotic transfer of such resistance to humans.

METHODS (PHENOTYPIC AND GENOTYPIC), AND THE INTERPRETIVE CRITERIA CURRENTLY USED FOR DETECTION (ISOLATION AND IDENTIFICATION) AND CHARACTERIZATION OF ESBL- OR AmpC-PRODUCING BACTERIAL STRAINS, ESBL- OR AmpC-ENCODING GENES, AND ASSOCIATED MOBILE ELEMENTS

Isolation from animal feces or foods is optimally performed with selective growth media preceded by selective enrichment in broth, using low concentrations of cefotaxime or ceftriaxone as the selective agent. Suspected isolates are identified and confirmed as ESBL or AmpC producers by phenotypic confirmation tests using harmonized interpretive criteria such as those proposed by the European Committee on Antimicrobial Testing of the Clinical and Laboratory Standards Institute). Final identification is performed by molecular characterization of the genes conferring resistance.

Molecular identification of ESBL or AmpC genes is performed by screening assays using polymerase chain reaction (PCR) or microarray, and subsequent sequence analysis. Lists of primers for the most important β-lactamases in Enterobacteriaceae have been published [38]. Several commercial microarrays have been developed for rapid and specific detection of β-lactamase genes [39, 40]. Identification of the subtype of the ESBL or AmpC genes detected by PCR or microarray is normally conducted by sequence analysis of PCR fragments.

Plasmid isolation and electrophoresis in agarose gels provides information on the number and mass/size of plasmids present in one isolate. Transfer of plasmids to well-characterized recipients by conjugation or electroporation facilitates the typing of individual plasmids. Categorization of plasmids in incompatibility groups can be performed by PCR-based replicon typing method, targeting the major plasmid families of Enterobacteriaceae [41]. Novel plasmid families can be recognized by complete DNA sequencing, also using the relaxase gene as a phylogenetic marker [42]. Further characterization of plasmids belonging to groups I, F, N, HI2, and HI can be performed by plasmid multilocus sequence typing (MLST) [43] (http://pubmlst.org/plasmid/).

The purpose of molecular typing is to determine genetic relatedness of isolates to allow source tracking/attribution. The discriminatory power varies among methods, and influences the conclusions that can be drawn from the results. The choice of method is determined by the goal of the work. Pulsed-field gel electrophoresis or multiple loci variable number of tandem repeats analysis are often used to identify clonal clusters of isolates that are related to a certain "outbreak" in a restricted time frame. MLST is often the method used to identify the relatedness of isolates of the same species from different backgrounds (eg, animal vs human).

RISK FACTORS CONTRIBUTING TO THE OCCURRENCE, EMERGENCE, AND SPREAD OF ESBL- OR AmpC-PRODUCING BACTERIAL STRAINS IN FOOD-PRODUCING ANIMALS AND FOOD

A variety of farm management factors may facilitate the introduction and spread of ESBL- and AmpC-producing bacteria (eg, animal exposure to contaminated water or feed; absence of water acidification in poultry production) [44, 45]. The establishment of risk factors for occurrence of ESBL/AmpC-producing bacteria is complicated by scarcity or lack of accuracy of reliable data. Further research is needed to understand more about the driving forces that have led to the rapid spread of these resistant bacteria in many countries worldwide.

Most ESBL- and AmpC-producing strains may carry additional resistances such as to sulfonamides and other commonly used veterinary drugs. Therefore, persistence and dissemination of ESBL/AmpC-producing bacteria can be selected for by the use in food-producing animals, not only of cephalosporins, but also of other compounds such as amoxicillin, sulphonamides, trimethoprim, fluoroquinolones, and aminoglycosides [44, 45]. The most efficient selection pressure will be driven by systemic use of third- and fourth-generation cephalosporins. A strong correlation between a reduction in ceftiofur-resistant *Salmonella* Heidelberg and ceftiofur-resistant *E. coli* (both producing AmpC) from both human infections and retail poultry in different regions of Canada and withdrawal of ceftiofur for disease prophylaxis in hatcheries has been reported [46]. In the European Union, approved systemic use of third- and fourth-generation cephalosporins is limited to cases that are expected to respond poorly to other antimicrobials; no use is approved for poultry. Off-label use of cephalosporins also occurs. For instance, ceftiofur has been used prophylactically in 1-day-old piglets [47], and there are also indications of its widespread off-label use of in poultry (eg, in ovo use, or use as spray or by subcutaneous injection in hatcheries). Moreover, illegal use has likely increased owing to simplified access through the Internet.

Table 2. Main Measures to Control the Selection and Dissemination of Extended-Spectrum β-Lactamase/AmpC-Producing Organisms in Food-Producing Animals (Based on Best Available Evidence and Expert Opinion)

Control Measure	Options
Stop/reduce the use of cephalosporins in food animals	• Provided adequate compliance, the measure would be more effective the more comprehensive the restrictions. • The restrictions could range from stopping all uses of cephalosporins/systemically active third- and fourth-generation cephalosporins, to more or less strict restriction of their use, allowing use only under specific circumstances.
Increase compliance with existing legislation	• Off-label use of veterinary medicinal products, including cephalosporins, is restricted according to articles 10 and 11 of Directive 2001/82/EC as amended. Such use should be limited to use by way of exception, under the veterinarian's direct personal responsibility and in particular to avoid causing unacceptable suffering.
Decrease the total antimicrobial use in animal production in the European Union	• To implement systems to monitor and control antimicrobial usage at MS and EU level • To implement measures to ensure transparency in antimicrobial usage (at farm and prescriber level) • To promote more tailored treatments by implementation of adequate diagnostic tools • To launch information campaigns on prudent use principles targeting farmers and responsible veterinarians • To stop antimicrobial use at hatcheries
Implement measures to control dissemination	• To promote closed production systems with high biosecurity standards • To introduce EU monitoring systems to control trade of ESBL/AmpC-contaminated food-producing animals in production pyramids • To improve hygiene throughout the food chain
Control antibiotic use and dissemination in the poultry production pyramid	• To restrict antimicrobial use in poultry production • To implement infection control measures to prevent vertical transmission from the top of the poultry production pyramid • To improve hygienic measures to prevent local recirculation within subsequent flocks

Abbreviations: ESBL, extended-spectrum β-lactamase; EU, European Union; MS, member state.

An extensive trade of animals occurs in the European Union, with few countries leading the production and the export, and with a small number of companies producing pure-line grandparent stock. How widespread are ESBL-carrying bacteria in food-producing animals is generally unknown, although few reports suggest that ESBL/AmpC are not uncommon in the top of some production pyramids. In Sweden, transmission of resistant bacteria from imported breeding chickens was documented by findings of *E. coli* carrying genes in environmental samples from hatcheries rearing production animals or breeding stock (parent animals) [48]. Recent data from the Netherlands indicate that ESBL- or AmpC-producing *E. coli* are introduced in the Dutch poultry production chain through imported day-old grandparent chickens. Moreover, the data indicate that the occurrence of these organisms in the different levels layers of the Dutch poultry production chain is the result of vertical transmission, local recirculation, and selection [36, 49].

Contamination of meat products with resistant bacteria may contribute to further spread within the human population. This has been shown to be particularly applicable to ESBLs, where genes might rapidly transfer from foodborne commensals to human pathogens [34, 50].

Contamination of food of plant origin with bacteria producing ESBLs or AmpC has also been recognized, as demonstrated by the recent large outbreak caused by CTX-M-15–producing *E. coli* O104:H4 linked to the consumption of contaminated sprouts [51].

Recommendations for further research are (1) to undertake risk assessments to quantify relationships (if any) between the occurrence of ESBL/AmpC-producing bacteria in food-producing animals and antimicrobial consumption (both of cephalosporins and all antimicrobials overall) and (2) to perform cross-sectoral studies to assess and quantify factors contributing to the emergence and dissemination of ESBL/AmpC resistance in different food animal species.

POSSIBLE CONTROL OPTIONS TO REDUCE THE PUBLIC HEALTH RISK CAUSED BY ESBL- OR AmpC-PRODUCING BACTERIAL STRAINS TRANSMITTED VIA THE FOOD CHAIN OR THE FOOD-ANIMAL PRODUCTION ENVIRONMENT

The effect of reducing the prevalence of ESBL- or AmpC-producing bacteria in animals or food on public health risks is difficult to assess. Although there is evidence of the contribution of resistant microorganisms transmitted via food-producing animals and food to public health risks, it is difficult to quantify that risk. Moreover, the magnitude of the contribution from

animals and foods to public health risks, and the perception of the risks for humans, will be greatly influenced by the local epidemiology of ESBL- or AmpC-producing organisms in healthcare settings and in the community. This epidemiology may vary greatly by country. In countries where human associated ESBL- or AmpC-producing clones have spread endemically in healthcare settings and the community, contaminated animals and foods will have a minor relative importance compared to countries where these organisms occur only incidentally in healthcare settings and the community.

Public health risks caused by ESBL- or AmpC-producing bacteria are primarily determined by (1) the frequency of the occurrence (prevalence) and the quantity of these organisms in food-producing animals and food, (2) the genetic characteristics of the β-lactamase genes involved, and (3) the transmission from animals or food to humans. Mitigation measures should therefore aim to reduce the prevalence in animals and food, and to reduce transmission from contaminated animals and foods to humans.

The prevalence of resistant organisms in a certain niche (animals or foods) is determined by 2 basic mechanisms: (1) selection by antibiotic usage and (2) dissemination within farms and animal production chains.

There are no data on the comparative efficiency of individual control options in reducing public health risks caused by ESBL- or AmpC-producing bacteria related to food-producing animals. Prioritization is complex, and the effectiveness of measures discussed is based on the best available evidence and expert opinion (Table 2). As such, a highly effective control option to reduce selection of ESBL/AmpC-producing bacteria at an EU level would be to stop all uses of cephalosporins or systemically active third- and fourth-generation cephalosporins, or to restrict their use (ie, only allowed under specific circumstances). The more comprehensive the restriction, the more prominent would be the effect on selection pressure, although a very restrictive policy might have unintended consequences on animal health and welfare if effective antimicrobials are not available for treatment.

Efforts should be directed to the implementation of measures intended to minimize off-label use of cephalosporins. Because coresistance is an important issue, decreasing the total antimicrobial use in animal production in the European Union is also of high priority. Also of importance (more so after the ESBL/AmpC-producing microorganisms have emerged) are the measures to control their dissemination, such as by implementing increased farm biosecurity and controls on animal trade (of ESBL/AmpC-carriers), or by improving hygiene throughout the food chain (implementing general postharvest controls for foodborne pathogens). Because most evidence is available for high prevalence of ESBL/AmpC-producing bacteria in the poultry production pyramid [16, 36, 48, 49], and their consequent involvement in public health [34, 50], it is of high priority to reduce selection pressure imposed by the use of antimicrobials, to prevent vertical transmission from the top of the poultry production pyramid and to prevent local recirculation within flocks.

CONCLUSIONS

Because of the ubiquity of ESBL- and AmpC-producing bacterial strains and of the associated genetic determinants, it is unlikely that any single control measure will be sufficient to limit their transmission through the food chain. Nevertheless, it is of paramount public health importance that the potential contribution of food-producing animals or foods to public health is minimized. There is little doubt that the use of cephalosporins and related compounds has been one of the driving forces in the spread of organisms exhibiting resistance to such antimicrobials. Stringent controls are therefore necessary to limit the use of cephalosporins in food animals. Owing to coresistance, the general use of antimicrobials is also a risk factor for emergence and spread of clones and/or plasmids carrying these resistance genes. As the most problematic area is the high prevalence of ESBL/AmpC-producing bacteria in the poultry production pyramid and their consequent involvement of such organisms in public health, in the first instance controls should be targeted at poultry production. As an example, a strong correlation between a reduction in ceftiofur-resistant *Salmonella* and *E. coli* from human infections and retail poultry and withdrawal of ceftiofur for prophylaxis in hatcheries has been reported in Canada. Of importance are also measures to control dissemination, for example, by implementing increased farm biosecurity and controls on animal trade (of ESBL/AmpC carriers), by improving hygiene throughout the food chain, and by implementing other general postharvest controls for foodborne pathogens. The effectiveness of any control measures should be monitored on a regular basis by targeted surveys of food animals and foods for cephalosporin-resistant bacteria, using selective isolation methods and preenrichment of samples as necessary.

Notes

Acknowledgments. This work is dedicated to the memory of Professor J. D. (Dan) Collins, late Emeritus Professor, University College Dublin, Chair of the European Food Safety Authority (EFSA) BIOHAZ panel, 2006–2011, who passed away in April 2012. The authors thank the members of the Biological Hazards Panel that adopted the EFSA Opinion on "The public health risks of bacterial strains producing ESBLs or AmpC β-lactamases in food and food-producing animals" (EFSA Journal 2011; 9:2322). This review paper is largely based on the mentioned Opinion prepared under the auspices of EFSA.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis **2008**; 8:159–66.
2. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2010. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Vol. 2011. Stockholm, Sweden: ECDC, **2011**.
3. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum β-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J Antimicrob Chemother **2007**; 60:913–20.
4. de Kraker MEA, Wolkewitz M, Davey PG, et al. Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to *Escherichia coli* resistant to third-generation cephalosporins. J Antimicrob Chemother **2011**; 66:398–407.
5. Harris AD, McGregor JC, Johnson JA, et al. Risk factors for colonization with extended-spectrum β-lactamase-producing bacteria and intensive care unit admission. Emerg Infect Dis **2007**; 13:1144–9.
6. Ben-Ami R, Rodríguez-Baño J, Arslan H, et al. A multinational survey of risk factors for infection with extended-spectrum β-lactamase-producing Enterobacteriaceae in nonhospitalized patients. Clin Infect Dis **2009**; 49:682–90.
7. Zaoutis TE, Goyal M, Chu JH, et al. Risk factors for and outcomes of bloodstream infection caused by extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species in children. Pediatrics **2005**; 115:942–9.
8. Park YS, Yoo S, Seo M-R, Kim JY, Cho YK, Pai H. Risk factors and clinical features of infections caused by plasmid-mediated AmpC β-lactamase-producing Enterobacteriaceae. Int J Antimicrob Agents **2009**; 34:38–43.
9. Wener KM, Schechner V, Gold HS, Wright SB, Carmeli Y. Treatment with fluoroquinolones or with β–lactam-β-lactamase inhibitor combinations is a risk factor for isolation of extended-spectrum-β-lactamase-producing *Klebsiella* species in hospitalized patients. Antimicrob Agents Chemother **2010**; 54:2010–6.
10. Hilty M, Betsch BY, Bogli-Stuber K, et al. Transmission dynamics of extended-spectrum β-lactamase-producing Enterobacteriaceae in the tertiary care hospital and the household setting. Clin Infect Dis **2012**; 55:967–75.
11. Valverde A, Grill F, Coque TM, et al. High rate of intestinal colonization with extended-spectrum-β-lactamase-producing organisms in household contacts of infected community patients. J Clin Microbiol **2008**; 46:2796–9.
12. Decre D, Gachot B, Lucet JC, Arlet G, Bergogne-Berezin E, Regnier B. Clinical and bacteriologic epidemiology of extended-spectrum β-lactamase-producing strains of *Klebsiella pneumoniae* in a medical intensive care unit. Clin Infect Dis **1998**; 27:834–44.
13. Carattoli A. Animal reservoirs for extended spectrum β-lactamase producers. Clin Microbiol Infect **2008**; 14(suppl 1):117–23.
14. Bergenholtz RD, Jorgensen MS, Hansen LH, Jensen LB, Hasman H. Characterization of genetic determinants of extended-spectrum cephalosporinases (ESCs) in *Escherichia coli* isolates from Danish and imported poultry meat. J Antimicrob Chemother **2009**; 64:207–9.
15. European Food Safety Authority. The public health risks of bacterial strains producing ESBLs and/or AmpC β-lactamases in food and food-producing animals. EFSA Journal **2011**; 9:2322.
16. Dierikx CM, Fabri T, van der Goot JA, et al. Prevalence of extended-spectrum- β-lactamase producing *E. coli* isolates on broiler farms in the Netherlands. Dutch J Med Microbiol **2010**; S28–9.
17. Rodriguez I, Barownick W, Helmuth R, et al. Extended-spectrum β-lactamases and AmpC β-lactamases in ceftiofur-resistant *Salmonella enterica* isolates from food and livestock obtained in Germany during 2003–07. J Antimicrob Chemother **2009**; 64:301–9.
18. Riano I, Moreno MA, Teshager T, Saenz Y, Dominguez L, Torres C. Detection and characterization of extended-spectrum β-lactamases in *Salmonella enterica* strains of healthy food animals in Spain. J Antimicrob Chemother **2006**; 58:844–7.
19. Lopez-Cerero L, Egea P, Serrano L, et al. Characterisation of clinical and food animal *Escherichia coli* isolates producing CTX-M-15 extended-spectrum β-lactamase belonging to ST410 phylogroup A. Int J Antimicrob Agents **2011**; 37:365–7.
20. Platell JL, Johnson JR, Cobbold RN, Trott DJ. Multidrug-resistant extraintestinal pathogenic *Escherichia coli* of sequence type ST131 in animals and foods. Vet Microbiol **2011**; 153:99–108.
21. Mora A, Herrera A, Mamani R, et al. Recent emergence of clonal group O25b:K1:H4-B2-ST131 *ibe*A strains among *Escherichia coli* poultry isolates, including CTX-M-9-producing strains, and comparison with clinical human isolates. Appl Environ Microbiol **2010**; 76:6991–7.
22. Carattoli A. Resistance plasmid families in Enterobacteriaceae. Antimicrob Agents Chemother **2009**; 53:2227–38.
23. Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A. Replicon typing of plasmids carrying CTX-M or CMY beta-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. Antimicrob Agents Chemother **2006**; 50:3203–6.
24. Cloeckaert A, Praud K, Doublet B, et al. Dissemination of an extended-spectrum- β-lactamase $bla_{\text{TEM-52}}$ 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.
25. Cottell JL, Webber MA, Coldham NG, et al. Complete sequence and molecular epidemiology of IncK epidemic plasmid encoding $bla_{\text{CTX-M-14}}$. Emerg Infect Dis **2011**; 17:645–52.
26. Cortes P, Blanc V, Mora A, et al. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl Environ Microbiol **2010**; 76:2799–805.
27. Mora A, Blanco M, Lopez C, et al. Emergence of clonal groups O1:HNM-D-ST59, O15:H1-D-ST393, O20:H34/HNM-D-ST354, O25b:H4-B2-ST131 and ONT:H21,42-B1-ST101 among CTX-M-14-producing *Escherichia coli* clinical isolates in Galicia, northwest Spain. Int J Antimicrob Agents **2011**; 37:16–21.
28. Vincent C, Boerlin P, Daignault D, et al. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis **2010**; 16:88–95.
29. Bonnedahl J, Drobni P, Johansson A, et al. Characterization, and comparison, of human clinical and black-headed gull (*Larus ridibundus*) extended-spectrum β-lactamase-producing bacterial isolates from Kalmar, on the southeast coast of Sweden. J Antimicrob Chemother **2010**; 65:1939–44.
30. Randall LP, Clouting C, Horton RA, et al. Prevalence of *Escherichia coli* carrying extended-spectrum beta-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. J Antimicrob Chemother **2011**; 66:86–95.
31. Simoes RR, Poirel L, Da Costa PM, Nordmann P. Seagulls and beaches as reservoirs for multidrug-resistant *Escherichia coli*. Emerg Infect Dis **2010**; 16:110–2.
32. Valat C, Haenni M, Saras E, et al. CTX-M-15 extended-spectrum β-lactamase in a Shiga toxin-producing *Escherichia coli* isolate of serotype O111:H8. Appl Environ Microbiol **2012**; 78:1308–9.
33. King LA, Nogareda F, Weill FX, et al. Outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 associated with organic fenugreek sprouts, France, June 2011. Clin Infect Dis **2012**; 54:1588–94.
34. Lavilla S, Gonzalez-Lopez JJ, Miro E, et al. Dissemination of extended-spectrum β-lactamase-producing bacteria: the food-borne outbreak lesson. J Antimicrob Chemother **2008**; 61:1244–51.
35. Moodley A, Guardabassi L. Transmission of IncN plasmids carrying $bla_{\text{CTX-M-1}}$ between commensal *Escherichia coli* in pigs and farm workers. Antimicrob Agents Chemother **2009**; 53:1709–11.
36. Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D. Increased detection of extended spectrum β-lactamase producing

Salmonella enterica and *Escherichia coli* isolates from poultry. Vet Microbiol **2010**; 145:273–8.
37. Fischer J, Rodriguez I, Schmoger S, et al. *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. J Antimicrob Chemother **2012**; 67:1793–5.
38. Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother **2010**; 65:490–5.
39. Batchelor M, Hopkins KL, Liebana E, et al. Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in gram-negative bacteria. Int J Antimicrob Agents **2008**; 31:440–51.
40. Cohen Stuart J, Dierikx C, Al Naiemi N, et al. Rapid detection of TEM, SHV and CTX-M extended-spectrum β-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. J Antimicrob Chemother **2010**; 65:1377–81.
41. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods **2005**; 63:219–28.
42. Garcillan-Barcia MP, Francia MV, de la Cruz F. The diversity of conjugative relaxases and its application in plasmid classification. FEMS Microbiol Rev **2009**; 33:657–87.
43. Carattoli A. Plasmids in gram negatives: molecular typing of resistance plasmids. Int J Med Microbiol **2011**; 301:654–8.
44. Persoons D, Haesebrouck F, Smet A, et al. Risk factors for ceftiofur resistance in *Escherichia coli* from Belgian broilers. Epidemiol Infect **2010**; 139:765–71.
45. Smet A, Martel A, Persoons D, et al. Diversity of extended-spectrum β-lactamases and class C β-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. Antimicrob Agents Chemother **2008**; 52:1238–43.
46. Dutil L, Irwin R, Finley R, et al. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis **2010**; 16:48–54.
47. Jorgensen CJ, Cavaco LM, Hasman H, Emborg HD, Guardabassi L. Occurrence of CTX-M-1-producing *Escherichia coli* in pigs treated with ceftiofur. J Antimicrob Chemother **2007**; 59:1040–2.
48. SVARM. Swedish veterinary antimicrobial resistance monitoring. Uppsala, Sweden: SVR, **2011**. Available at: www.sva.se. Accessed 21 September 2012.
49. Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2009. Available at: http://edepot.wur.nl/165958. Accessed 17 December 2012.
50. 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.
51. Buchholz U, Bernard H, Werber D, et al. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. N Engl J Med **2011**; 365: 1763–70.