Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe

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Extended-spectrum beta-lactamases (ESBLs) have been increasingly reported in Europe since their first description in 1983. During the 1990s, they were described mainly as members of the TEM- and SHV-beta-lactamase families in *Klebsiella pneumoniae* causing nosocomial outbreaks. Nowadays, they are mostly found in *Escherichia coli* that cause community-acquired infections and with increasing frequency contain CTX-M enzymes. Dissemination of specific clones or clonal groups and epidemic plasmids in community and nosocomial settings has been the main reason for the increase in most of the widespread ESBLs belonging to the TEM (TEM-24, TEM-4, TEM-52), SHV (SHV-5, SHV-12) and CTX-M (CTX-M-9, CTX-M-3, CTX-M-14 or CTX-M-15) families in Europe. Co-selection with other resistances, especially to fluoroquinolones, aminoglycosides and sulfonamides, seems to have contributed to the problem. The emergence of epidemic clones harbouring several beta-lactamases simultaneously (ESBLs, metallo-beta-lactamases or cephamycinases) and of new mechanisms of resistance to fluoroquinolones and aminoglycosides warrants future surveillance studies.

Introduction

Enterobacteriaceae have become one of the most important causes of nosocomial and community acquired infections. Beta-lactams (mainly extended-spectrum cephalosporins and carbapenems) and fluoroquinolones constitute the main therapeutic choices to treat infections caused by these microorganisms. However, resistance to these compounds has been reported more and more frequently in Europe in the past years [1-5].

Acquired resistance to beta-lactams is mainly mediated by extended-spectrum beta-lactamases (ESBLs) that confer bacterial resistance to all beta-lactams except carbapenems and cephamycins, which are inhibited by other beta-lactamase inhibitors such as clavulanic acid. A shift in the distribution of different ESBLs has recently occurred in Europe, with a dramatic increase of CTX-M enzymes over TEM and SHV variants. Other non-TEM, non-SHV enzymes, such as PER, GES, IBC or certain OXA types, have also been found in some European countries [1]. Although ESBLs still constitute the first cause of resistance to beta-lactams among Enterobacteriaceae, other "new beta-lactamases" conferring resistance to carbapenems, such as metallo-beta-lactamases

Table 1

Global surveillance studies covering Europe and including ESBL-producing bacterial isolates

Surveillance Study	Date (Year)	Countries (no.)	Centres (no.)	Sample Origin	Overall frequency (%)	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*P. mirabilis*	*Enterobacter* spp.	Reference
SENTRY	1997-98	15	25	Blood, urine, respiratory tract, wounds,	4.9	1.3	18.4	12.6	5.3	n.a.	[3]
SMART	2004	9	31	Intra-abdominal		6.4	8.8	n.a.	n.a.	11.8	[4]
TEST	2004-06	19	62	Blood, urine, respiratory tract, wounds, sterile fluids		7.6	13.3	n.a.	n.a.	n.a.	[5]
MYSTIC	2006	12	40	Blood culture, urine, sputum, sterile fluids, wounds	5.6	8.2	9.8	n.a.	1.4	n.a.	[6]
EARSS	2006	31	ca. 800	Blood		<1-41	0-91	n.a.	n.a.	n.a.	-

ESBL: Extended-spectrum beta-lactamases; SMART: Study for Monitoring Antimicrobial Resistance Trends; TEST: Tigecycline Evaluation and Surveillance Trial; MYSTIC: Meropenem Yearly Susceptibility Test Information Collection; EARSS: European Antibiotic Resistance Surveillance System (http://www.rivm.nl./earss/).
n.a.: not available.

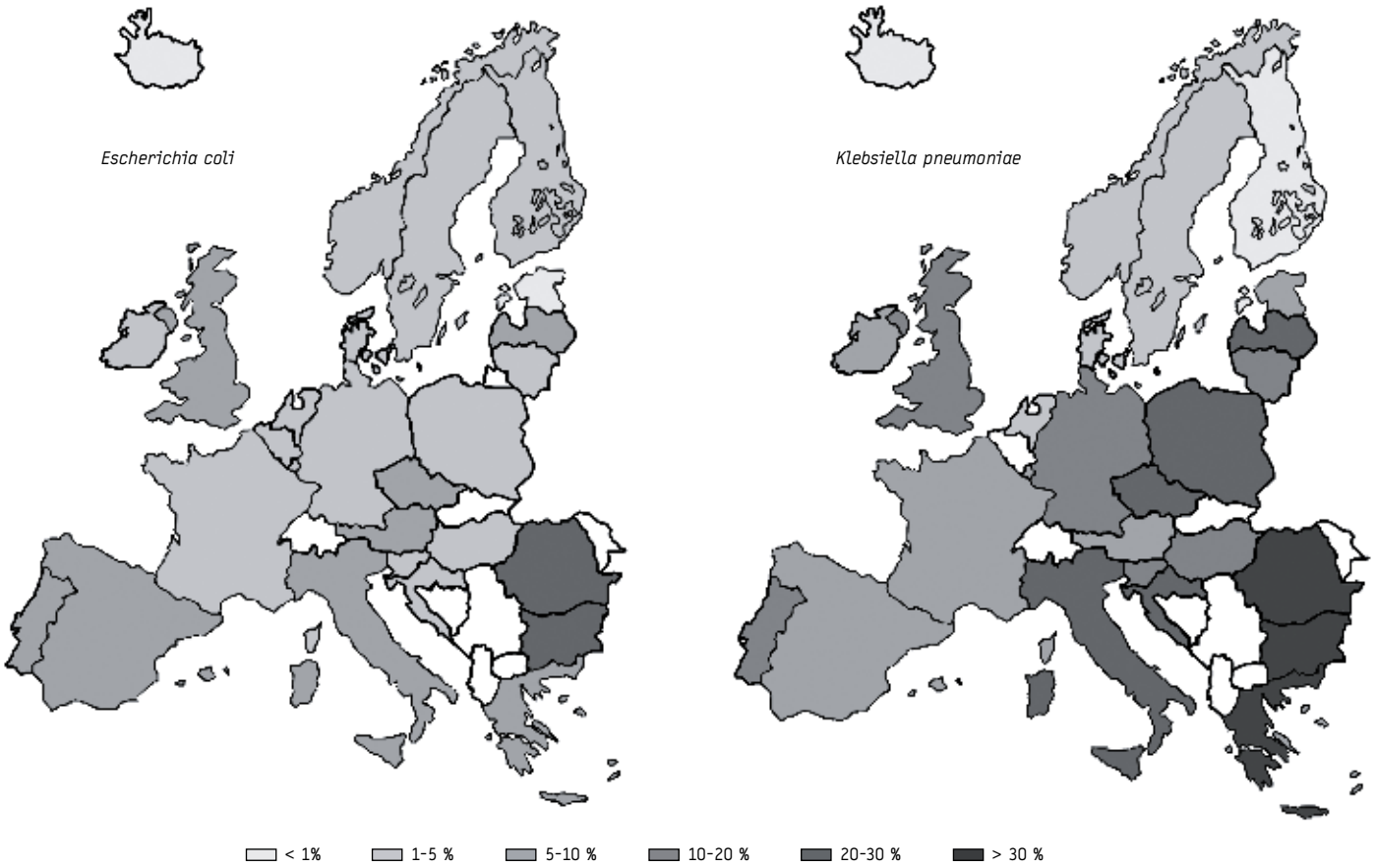
(MBL) and KPC carbapenemases, or to cephamycins, such as CMY enzymes, have more recently emerged and are often associated with ESBLs (see section *Epidemiology of ESBL in Europe*).

Overall data on resistance to third generation cephalosporins, mainly due to ESBL, in Europe have been provided by the European Antibiotic Resistance Surveillance System (EARSS; http://www.rivm.nl/earss/) and other international surveillance systems (Table 1). In addition to a large number of detailed molecular analyses on particular ESBL types, multicentre studies performed in hospitals, farms, or slaughterhouses, using different surveillance systems in each country, have contributed to a better understanding of the epidemiology of these enzymes at local, national and international level. The current increase in ESBL-producing bacteria in inpatients as well as outpatients at the time of hospital admission points towards a continent-wide rise, mainly in *Escherichia coli*, with great variations in the occurrence and distribution of different ESBLs among countries (see section *Epidemiology of ESBL in Europe*). A community-origin explaining this rise has been highlighted in many surveys, but the prevalence of ESBLs in this setting is difficult to ascertain accurately, as faecal colonisation surveys among humans without direct or indirect hospital exposure are scarce (see section *Faecal colonisation surveillance studies*).

Antibiotic overuse in humans and animals, hospital cross-infection, the food chain, trade and human migration seem to have contributed to the recent dissemination of ESBLs outside hospitals, although the role of these factors is variable and linked to particular epidemiological situations (see sections *Epidemiology of ESBL in Europe and ESBLs in non-humans hosts*). Recent studies have demonstrated the clonal expansion of certain enterobacterial clones that are able to acquire multiple ESBL plasmids (see section *Clonal expansion of ESBL-producing Enterobacteriaceae*). These successful clones seem to have favoured the expansion of ESBLs on our continent, as exemplified by the highly virulent *E. coli* O25:H4-ST131, a strain that is thought to be responsible for the pandemic dissemination of the CTX-M-15 enzyme. The origin of widespread *E. coli* clonal complexes is still unknown, although it is likely that the resistance they exhibit against trimetoprim-sulfamethoxazole or fluoroquinolones is due to a strong selection pressure prior to ESBL acquisition (see section *Clonal expansion of ESBL-producing Enterobacteriaceae*). Plasmid dissemination also plays a critical role in the wide spread of ESBL in Europe (see section *The impact of plasmid transfer on ESBL-producing Enterobacteriaceae*). The increasing description of isolates simultaneously containing ESBLs, carbapenemases, CMY or new mechanisms of resistance to fluoroquinolones and aminoglycosides is of concern (see section *Multi-resistance profiles*

Figure 1

Proportion of invasive *Escherichia coli* and *Klebsiella pneumoniae* isolates resistant to third generation cephalosporins in 2006 (EARSS study)



EARRS: European Antibiotic Resistance Surveillance System

in ESBL producing isolates). In this review, we summarise the more recent findings on ESBL epidemiology in Europe in order to understand the recent increase in hospitals and in the community, and to implement appropriate intervention strategies to avoid their pandemic dissemination as has happened with certain Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus faecium*.

Epidemiology of ESBL in Europe

General surveillance studies

European and intercontinental surveillance studies have collected data on ESBL-producing Enterobacteriaceae in Europe, all of which consistently show a variable proportion among different geographic locations, enterobacterial species and isolates from different sources (Table 1, Figure 1). Some of them allow comparison with non-European geographic areas, such as the TEST (Tigecycline Evaluation and Surveillance Trial) or SMART (Study for Monitoring Antimicrobial Resistance Trends) [4], which showed that ESBL were far less frequent in Europe than in Latin America and Asia/Pacific regions but more common than in North America (Figure 2). However, these studies have not addressed potential differences between hospital and community isolates.

A recent multicentre European study performed in 2005 in settings with a high antibiotic selection pressure such as intensive care units (ICU) gave results similar to those collected by EARSS [7]. That study had been designed to monitor the association between specific antibiotic consumption and antimicrobial resistance, but no clear correlation was found between the two. This was probably due to differences in the prevalence of patients who were colonised with resistant pathogens at admission, and to the different efforts put in place in different ICUs to avoid cross-transmission of these bacteria.

To date, there have not been any specific European multicentre studies addressing the prevalence of ESBL among community isolates, although there have been different efforts at national and local levels. A study performed in Turkey showed a prevalence of 21% ESBL producers among *E. coli* causing community-acquired urinary tract infection (UTI) during 2004 and 2005 [8]. This percentage was higher than the 5.2% observed in a Spanish multicentre study covering 15 microbiology laboratories in 2006 [9]. Moreover, the rate of community-acquired bacteraemias caused by ESBL-producing *E. coli* was 6.5% in Spain, whereas it ranged from 12.9% to 26.8% for *K. pneumoniae* in studies performed in Spain and the United Kingdom (UK) [10-12].

Faecal colonisation surveillance studies

There are no multicentre studies to address faecal colonisation rates with ESBL-producing isolates in Europe, although this is a common practice in the hospital setting for implementing epidemiological measures to curtail or control their spread. Nevertheless, the rate of inpatients, outpatients and healthy volunteers colonised by ESBL producers has been addressed in a few national studies and provided interesting observations. A Spanish analysis demonstrated that the frequency of faecal carriers had increased from under 1% to 5% among outpatients and from under 1% to 12% among hospitalised patients between 1991 and 2003, with a prevalence of 4% in healthy volunteers during 2004 [13]. It is of interest to note that the ESBL characterised among isolates obtained from faecal carriers was similar to the one obtained in the clinical setting in Spain at the time these studies were performed. This could prove useful for monitoring ESBL trends [14,15]. Nevertheless, these proportions are in contrast with what was found in a study performed among 322 healthy volunteers in the Paris area that did not detect any carriers of ESBLs. However, the same study frequently observed colonisation with prevalent clones that are associated with particular ESBLs but did not actually contain these enzymes [16].

Two other Spanish studies showed that the faecal carriage rate of ESBL-producing *E. coli* in community patients who had UTIs caused by this pathogen was around 70%, which is much higher than that of individuals with infections not associated with ESBLs [17,18]. Interestingly, faecal carriage in the household contacts of infected patients with ESBL-producing *E. coli* ranged from 16.7% to 27.4% in these two studies. This led to the suggestion that faecal colonisation with ESBL-producing bacteria is a risk factor for acquisition of UTI caused by these pathogens and a potential source for transmission among households.

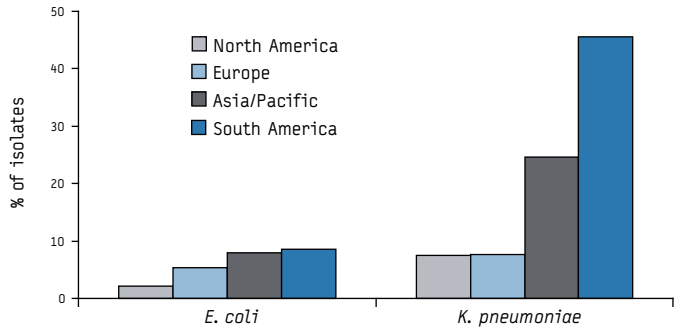
Geographic differences and ESBL types circulating in European hospitals

The last EARSS report from 2006, covering over 800 laboratories from 31 countries, showed a continuous increase since 2000 in invasive *E. coli* and *K. pneumoniae* isolates resistant to third generation cephalosporins, with prevalences higher than 10% for half of the enrolled countries (Figure 1). In addition, it shows important geographical differences, ranging from a percentage of under 1% (Estonia) to 41% (Romania) for *E. coli* and from 0% (Iceland) to 91% (Romania) for *K. pneumoniae*. Although these proportions are generally associated with the production of ESBL, they might be somewhat overestimated due to the inclusion of isolates with a greater susceptibility to beta-lactams when EUCAST breakpoints are used, or due to isolates overproducing AmpCs which represent about 1-2% of isolates resistant to third generation cepholosporins.

All published studies have confirmed that in most northern European countries, the prevalence of ESBL isolates is still low compared to southern and eastern European countries. Unfortunately, not all publications indicate precise frequency rates, since most

Figure 2

Frequency of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates reported in the TEST surveillance study (2004-2006) in different geographic areas [27]



ESBL: extended-spectrum beta-lactamases; TEST: Tigecycline Evaluation and Surveillance Trial.

of them were designed to establish the molecular epidemiology of circulating ESBLs, but not to ascertain the prevalence of these isolates.

Northern European countries

In Denmark (www.danmap.org), Norway (www.antibiotikareistens.no) and Sweden (www.strama.se), yearly national surveillance and published studies show continuous rising trends of ESBLs. In the Copenhagen area of Denmark, the occurrence of ESBL producers was below 1% in isolates received at a national reference laboratory, with dominance of CTX-M and SHV enzymes [19]. In Norway, a prospective survey of clinical *E. coli* isolates with reduced susceptibility to oxyimino-cephalosporins demonstrated the dominance of CTX-M-15 (46%) and CTX-M-9-like (30%) enzymes among ESBL-positive *E. coli* and of SHV-5 (47.4%) and SHV-2 (21.0%) among ESBL-positive *K. pneumoniae* isolates [20]. This ESBL distribution is similar to that encountered in Sweden during the period from 2001 to 2006, when 92% of consecutive non-duplicate ESBL-positive *E. coli* isolates expressed a CTX-M-type enzyme, CTX-M-1 being the predominant group [21]. Similar results were found in multicenter studies performed between 2002 and 2004 in Finland [22]. More recently, clonal outbreaks caused by CTX-M-15 *K. pneumoniae* have been reported in Scandinavia [23].

Southern countries

The prevalence of ESBL producers in Spain and Portugal has increased over time, with a predominance of CTX-M-producing *E. coli* causing community acquired UTIs [14,24-26]. In Spain, a shift in the proportion of ESBL-producing *Klebsiella* isolates recovered from outpatients (7% to 31%) and ICU patients (41% to 25%) was observed between the periods 1989 to 2000 and 2001 to 2004 [27]. Although a high diversity of ESBLs are reported in most Spanish studies, high local prevalence of CTX-M-9, CTX-M-14, CTX-M-10 and TEM-4 enzymes is observed among inpatients, outpatients and healthy individuals [13,15,17]. In Portugal, nationwide surveys are not available. Studies of individual hospitals reflect a common spread of CTX-M-14, TEM-52, and GES [24,26]. TEM-24, CTX-M-15, CTX-M-32 and SHV-12 are frequently detected in both Spain and Portugal [15,24].

In Italy, the prevalence of ESBL producers among clinical isolates has also increased over the past ten years [28]. The most prevalent ESBL-positive species are *E. coli* among hospitalised patients and *Proteus mirabilis* among outpatients. A predominance of TEM enzymes (45.4%), SHV-12, and the emergence of non-TEM, non-SHV enzymes (CTX-M-type in *E. coli* and *K. pneumoniae*, and PER-type in *P. mirabilis*) has been described. More recent studies performed in single institutions showed the frequent recovery of CTX-M-15-producing *E. coli* and other variants from this group such as CTX-M-1 and CTX-M-32 [29-31].

In France, the prevalence of ESBL production in Enterobacteriaceae reported in different multicentre studies is under 1%, with a progressive increase in the occurrence of CTX-M enzymes linked to *E. coli* expansion [32]. The frequency of certain ESBL producers in 2005 was far lower than reported in previous years including *P. mirabilis* (3.7% versus 1.3%), *Enterobacter aerogenes* (53.5% versus 21.4%) and *K. pneumoniae* (9.4% versus 3.71%), but had increased for *E. coli* (0.2% versus 2%). In addition, ESBLs have frequently been observed in the community setting, linked to nosocomial acquisition [33]. CTX-M-variants were predominant and belonged primarily to the CTX-M-1 (85%) and CTX-M-9 (11.3%). A variety of TEM enzymes has been identified both in hospitals and in the community, although TEM-3 and TEM-24 remain the more common types, they have persistently been recovered since the late 1990s and have often been associated with clonal outbreaks [32,33].

United Kingdom

A recent dramatic increase in ESBL-producing organisms is being observed both in hospitals and in the community, mainly caused by the CTX-M-15 enzyme [2]. This enzyme, first reported in the UK in 2003, initially co-existed with CTX-M-9, CTX-M-14, SHV-variants (mainly SHV-12), and to a lesser extent with TEM derivatives both in the hospital and in the community. It has now become the most prevalent enzyme in both settings [2,34].

Eastern countries

The occurrence and distribution of ESBLs in this area differs from that in other countries. The prevalence of ESBLs is over 10% in Hungary, Poland, Romania, Russia and Turkey. *K. pneumoniae* is the most frequent ESBL-producing species in Hungary and Russia, and an increase in the percentage of ESBL producers among *K. pneumoniae* isolates has been reported from Poland, Turkey, Bulgaria, and Romania [35-40]. CTX-M-3, SHV-2 and SHV-5 are usually widely spread in eastern European countries.

In Poland, the proportion of ESBL producers in hospitals (11.1%) varied for different species from 2.5% for *E. coli*, 40.4% for *K. pneumoniae* and 70.8% for *Serratia marcescens*, the latter two having a higher prevalence due to outbreak situations. ESBL types were dominated by CTX-Ms (82%, CTX-M-3) and SHV types (17%, SHV-2, SHV-5, and SHV-12), while TEM-like enzymes (<1%, TEM-19 and TEM-48) were found only sporadically. In contrast to other countries, CTX-M-15 was rarely recovered in Poland [35]. The current scenario in Poland differs from that in the late 1990s, when there was a dominance of TEM ESBLs and spread of CTX-M-3 producers all over the country [41,42].

In Bulgaria, hospital outbreaks caused by CTX-M-3, CTX-M-15 and SHV-12 are described, often with an involvement of *S. marcescens* in addition to *K. pneumoniae* [40]. In Hungary, a recent eruptive and extensive spread of highly ciprofloxacin-resistant CTX-M-15 *K. pneumoniae* epidemic clones has been detected [36]. Nosocomial outbreaks involving SHV-2a-producing *K. pneumoniae* are also frequent [38]. In Turkey, CTX-M-15 is widely distributed [8,39], and epidemic strains of *K. pneumoniae* isolates producing the carbapenemase OXA-48 and the ESBLs SHV-12 or CTX-M-15 have emerged [43].

Predominant ESBLs circulating in Europe

The emergence and wide spread of the CTX-M-15 enzyme in most European countries, including those with previous low rates of ESBLs, is one of the most relevant findings associated with the current epidemiology of ESBL in Europe [8,14,23,36,44,45]. This enzyme is increasingly being associated with isolates from the community setting, including healthcare centres, as documented in studies from France, Spain, Turkey and the UK, [2,8,14,32,46, see also section *Clonal expansion of ESBL-producing Enterobacteriaceae*].

Other CTX-M variants are amplified locally, such as CTX-M-9 and -10 in Spain [15,25], CTX-M-14 in Portugal and Spain [15,24,47],

CTX-M-3 in eastern countries [35,40] and CTX-M-5 in Belarus and Russia [37]. The SHV-12 enzyme is one of the most prevalent enzymes associated with nosocomial *K. pneumoniae* isolates in Italian, Polish and Spanish hospitals and is also increasingly reported in *E. coli* isolates from community patients [13,31,48]. SHV-5, widely disseminated in Europe, is especially abundant in Bosnia and Herzegovina, Croatia, Greece, Hungary and Poland [35,38,48,49,50].

In addition, particular TEM types deserve special attention as they were traditionally associated with the ICU setting, TEM-3 and TEM-4, are associated with epidemic clones of *K. pneumoniae* in France and Spain, while TEM-24 is associated with epidemic *E. aerogenes* strains in Belgium, France, Portugal and Spain [24,32,33,51]. Nowadays, these enzymes have been also characterised in *E. coli* and *P. mirabilis* recovered in the community [24,33,51]. Finally, TEM-52, first identified in *Salmonella* spp. isolates from animal origin, is currently found among different Enterobactereriaceae species involved in human infections [24,33].

Co-production of different ESBLs is increasingly reported in European countries. Clinical isolates expressing SHV (SHV-5 or SHV-12) or TEM-24 and also other ESBL (CTX-M-9 or CTX-M-14) or carbapenemases (KPC, OXA, or VIM) have been described, sometimes associated with clonal outbreaks [43,49,52-54].

ESBLs in non-humans hosts

ESBL-producing E.coli and non-typhoidal *Salmonella* species have been isolated from farm animals, wild animals, food, pets and from environmental samples in different European countries [55-59]. The variability in the date of emergence and in the proportion of ESBL producers among animals seem to be due to differences between European countries in cephalosporin usage, and detection method, and to the importation of resistant strains through travellers or trade [59-62].

Different national surveys performed in Italy [63], France [64], the UK [http://www.defra.gov.uk/], Denmark [60], Norway [65] and Spain [57,66] demonstrated that the resistance to broad-spectrum cephalosporins is still low among zoonotic pathogens. However, a recent study performed in Denmark showed that veterinary beta-lactams (amoxicillin, ceftiofur, cefquinome) select for indigenous ESBL-producing *E. coli* in the intestinal flora of pigs and favour the emergence of strains that acquire ESBL genes by horizontal transfer. This selective effect persists for a period longer than the withdrawal time required for these antimicrobials [67]. Although the transmission of ESBL-producing bacteria through the food

Table 2

Plasmids involved in the wide dissemination of specific ESBLs in European countries

ESBL	Country	Year	Inc Group	Origin	Species	Reference
CTX-M-1[a]	France (10 slaughterhouses, 5 districts)	2005	IncI1	Animals	*E. coli*	[64]
CTX-M-2	Belgium, France	2000-2003	IncHI2	Poultry flocks, poultry meat, humans	*S. enterica* serovar.Virchow	[68, 98]
CTX-M-3[b]	Poland	1996-2005	IncL/M	Hospitals	*K. pneumoniae, Serratia marcescens, E. coli*	[35, 41, 99]
	Bulgaria, Poland, France		IncL/M	Hospitals	*Different species*	[94]
CTX-M-9	Spain, UK[c]	1996-2006	IncHI2	Hospitals	*E. coli, Salmonella*	[73, 95, 98]
	Spain	1998-2003	IncP1-α	Hospitals	*E. coli*	[86, 95]
	France	2003	IncHI2	Poultry	*S. enterica* serovar.Virchow	[69, 98]
CTX-M-14	Spain UK	1996-2006 2004-2005	IncK IncK	Hospitals Poultry	*E. coli* *E. coli*	[47] [75]
CTX-M-15[d]	Spain, Portugal, Italy, Turkey, Switzerland, France, Norway, Canada, Kuwait, India	2000-2007	IncFII	Hospitals	*E. coli, Klebsiella*	[30, 73, 78, 88]
CTX-M-32	Spain, Portugal, UK	2000-2006	IncN	Hospitals	*E. coli*	[86, 87]
TEM-24	Spain, Portugal, France, Belgium		IncA/C_2	Hospitals	*Enterobacter aerogenes, Proteus mirabilis, K.oxytoca*	[51]
TEM-52[e]	Spain, Portugal, France, The Netherlands, Belgium	2001-05	IncI1	Hospitals, animals	*E. coli, Salmonella*	[65, 70, 76]
SHV-5	Poland	1996-	IncFII	Hospitals	*E. coli*	[100]
	Hungary	1998-2003	Not determined	Hospitals	*K.pneumoniae*	[38]
SHV-12	Italy	2005	IncI1	Poultry	*E. coli*	[89]
	Spain	2005	IncI1	Humans	*E. coli, Klebsiella*	[Valverde, unpublished]

ESBL: Extended-spectrum beta-lactamases.
(a)The $bla_{CTX-M-1}$ gene has been located on plasmids of incompatibility groups N (among *E. coli* from humans and swine in Spain and Denmark, respectively) and A/C (from Spanish inpatients) [86,98].
(b) Relationship among these two plasmids has not been published.
(c) Associated with travel to Spain [73].
(d) CTX-M-15 plasmids of the group IncI1 have been described among human *Salmonella* Typhimurium isolates in the UK, although their distribution is unknown [73].
(e) This IncI plasmid has also been associated with bla_{TEM-20} in *E. coli* from Norway and *Salmonella* Paratyphi B dT from the Netherlands [65].

chain or direct contact between humans and animals has seldom been proven [66-68], animals should be considered as an important reservoir of ESBL-strains and highly transmissible plasmids.

ESBLs isolated from animals include different variants belonging to the CTX-M (-1,-2,-3,-8, -9,-13,-14,-15,-24,-28,-32), SHV (-2,-5,-12), and TEM (-52,-106,-116) families. CTX-M-1, TEM-52 and SHV-12 are the ones most commonly found to date. Their dissemination among non-human hosts seems to have been facilitated mainly by mobile conjugative elements [55; Table 2]. The epidemiology of the most prevalent variants in European countries exemplifies different transmission routes and is therefore briefly revised in this section.

The CTX-M-1-like-enzymes (CTX-M-1, -15 and -32) are widely distributed among animals from western European countries and mainly associated with epidemic plasmid spread among clonally unrelated *E. coli* [57,58,62,64,67]. CTX-M-1 is widespread among healthy and sick farm animals (poultry, swine) and pets in Belgium, Denmark, France, Italy, the Netherlands, Portugal and Spain [56-58,62,64,67,71]. It was also the most frequent ESBL in a Belgium survey, representing 27.4% of ESBL producers, some of which were also producing CMY-2 [62]. CTX-M-32 has been detected among healthy and sick animals in Greece, Portugal and Spain [57,58,72]. CTX-M-15, frequently recovered among clinical isolates, has been sporadically identified from pets and farm animals in different countries in the European Union (EU), although it is associated with different strains and plasmids than the ones that are responsible for the wide distribution of this ESBL in hospitals [73].

The CTX-M-9-like enzymes (CTX-M-9 and CTX-M-14) have been linked directly or indirectly with animals in different countries. CTX-M-9 producers have been detected among healthy and sick animals in Spain since 1997 [57,66]. In France, it was found in unrelated poultry isolates of *Salmonella enterica* serotype Virchow collected by the Agence Française de Sécurité Sanitaire des Aliments network in 2003 in a single hatchery located in the southwest of France that supplied different farms with chicks [69]. CTX-M-9 producers have also been linked to food-borne disease outbreaks or colonisation of food handlers in Spain, travellers returning to the UK from Spain and quails imported by Denmark from France [55,67,74]. CTX-M-14-producing *E. coli* or *Salmonella* on the other hand were identified from different slaughter animals in Belgium, Denmark, France, Spain and the UK. It was also linked to travellers returning to the UK from Thailand and to imported chickens in the UK [59,62,67,75].

Epidemic strains of *S. enterica* serotype Virchow producing CTX-M-2 have been isolated from poultry and poultry products in Belgium, France, and the Netherlands since 2000 [61,62,68]. The recent recovery in the UK of *E. coli* producing CTX-M-2 from imported raw chicken meat from Brazil suggests a transmission route from areas where this enzyme is endemic [59].

TEM-52-producing *E. coli* and *Salmonella* isolates have been detected in sick and healthy farm animals, pets, and beef meat food in, Belgium, Denmark, France, Greece, the Netherlands, Spain and the UK [61,70,72]. In Portugal, TEM-52 was widely disseminated among different enterobacterial species recovered from humans, pets, wild animals and livestock [56,58]. In Belgium and France, TEM-52 producers have frequently been isolated from *Salmonella* isolates of different serovars recovered from poultry and humans [70]. It is noteworthy that multidrug-resistant isolates of the serovars Agona (widely distributed in Belgian poultry) and Typhimurium phagotype DT104 (disseminated globally) have been detected which carry both SGI1 and a plasmid-borne ESBL [70]. Not only has clonal transmission involving *Salmonella* Blockey and Hadar been demonstrated within the Netherlands [61], but the joint spread of two epidemic plasmids between countries has been shown in two different studies [70,76]. Importation of animals or meat was the potential source of $bla_{\text{TEM-52}}$ in some areas in the EU [61,77].

SHV-12 producers in animals were detected in Italy during 2005 and 2006, and they were genetically related clones of *Salmonella* Livingstone, scattered on different farms in the northeast of the country, the main region for poultry production [http://www.istat.it; 63]. In Spain, the Netherlands and the UK, SHV-12-positive *Salmonella* and/or *E. coli* isolates have been identified from faecal samples from poultry and pigs [35,57,61,66]. Surprisingly, SHV-12 from animal origin has rarely been described in other European countries.

Clonal expansion of ESBL-producing Enterobacteriaceae

One of the major factors involved in the current prevalence of ESBL-producing Enterobacteriaceae is clonal spread. The most representative example linked to ESBL-producing Enterobacteriaceae is the recent and fast global dissemination of the highly virulent ciprofloxacin-resistant clone B2-*E. coli* O25:H4-ST131 that causes UTI and is associated with the CTX-M-15 pandaemia. This clone has been detected in the majority of European countries, e.g. France, Greece, Italy, Norway, Portugal, Spain, Switzerland, Turkey, and the UK [8,22,44,45,78]. Interestingly, B2-*E. coli* ST131 is able to acquire multiple resistance mechanisms, and this strain was identified repeatedly, harbouring different CTX-Ms, AmpC or SHV-12 recovered in recent British (2004-2005) and Spanish (2004) multicentre hospital surveys [44, Oteo *et al.*, personal communication]. It was also frequently identified among quinolone-resistant non-ESBL UTI-causing *E. coli* strains in clinical isolates from 10 different countries included in the last ARESC study (2004-2005) as well as in healthy volunteers in the Paris area (2007) [16,46,79]. Other widely distributed quinolone-resistant *E. coli* clones in the EU are responsible for the spread of specific ESBLs, such as A-*E. coli* ST10 or B1-*E. coli*-ST359, ST155, which are mainly identified among CTX-M-14 producers in the central area of Spain [16,47]. These findings suggest that the acquisition of ESBL plasmids by widespread continental fluoroquinolone-resistant *E. coli* clones may have contributed to the dissemination, amplification and persistence of ESBL on our continent.

Nationwide dissemination of particular multidrug-producing *K. pneumoniae* clones has been observed in several countries. In Greece, an endemic SHV-5-producing strain that emerged in the 1990s has recently acquired plasmid-borne VIM-1. This clone is currently spread among Greek hospitals and has also been identified in France [49,80]. Clonal outbreaks caused by *K. pneumoniae* producing SHV-5 and VIM-1 have also been detected in Italy, although a possible link with the Greek clone has not been investigated [54]. A predominance of SHV-type (SHV-5 and SHV-2a)-producing *K. pneumoniae* susceptible to ciprofloxacin is responsible for major clonal outbreaks in Hungarian neonatal ICUs, but endemic or inter-hospital dissemination of these local epidemic clones has not been addressed [38]. Dissemination of

ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-producing *K. pneumoniae* clones has recently been reported from the ICUs of 35 hospitals in 13 counties across Hungary, representing 97% of all CTX-M producers in this country [36,38]. The ST15 *K. pneumoniae* clone has also been identified in ESBL-producing isolates from France, Poland and Portugal, although the real dissemination impact of this clone in these countries is unknown [51]. Long-term persistence (>2 years) of ESBL-producing *K. pneumoniae* has been documented in single institutions in France (TEM-24), Greece (SHV-5), Hungary (SHV-2a), Portugal (GES-1) and Spain (TEM-4, SHV-12) [27,38,81,82]. Only a few sporadic cases of international exchange of epidemic *K. pneumoniae* clones are reported in the literature [80].

Representative examples of clonal expansion in other enterobacterial species include a multidrug-resistant *E. aerogenes* strain widely disseminated in EU hospitals since the 1990s, which is responsible for the spread of TEM-24 in Belgium, France, Portugal and Spain [24,51,83]. This clone can simultaneously carry $bla_{\text{TEM-24}}$ and plasmids encoding different ESBLs ($bla_{\text{SHV-12}}$, $bla_{\text{SHV-5}}$, $bla_{\text{TEM-20}}$) and MBLs ($bla_{\text{IMP-1}}$, $bla_{\text{VIM-2}}$) [84]. An aminoglycoside-resistant *Enterobacter cloacae* clone containing a conjugative plasmid carrying the *qnrA1*, $bla_{\text{CTX-M-9}}$, and *aad*B genes has been detected in 11 of 15 Dutch hospitals and has caused outbreaks in at least four of them [85]. ESBL-producing *P. mirabilis* (TEM-24), *Shigella sonnei*, *S. marcescens* and *Klebsiella oxytoca* have caused clonal outbreaks in different EU countries, although it remains to be elucidated whether they are of more than local significance [24,51,62].

The increasingly frequent description of endemic bacterial strains that are able to acquire genes coding for ESBLs, carbapenemases (VIM, OXA), and AmpC highlights the need to identify and successfully follow up the clones occurring in Europe [43,44,49,53,80,83].

The impact of plasmid transfer on ESBL-producing Enterobacteriaceae

Currently, the high prevalence of all blaESBL genes in different European regions is caused by horizontal transfer of plasmids among clonally unrelated clones and also among local or international epidemic clones. Plasmid transmission has played a significant role in the persistence of CTX-M-3 in Poland from the late 1990s until today [35,41], the persistence of TEM-4, CTX-M-10, CTX-M-9 and CTX-M-14 in Spanish hospitals since the first description of each enzyme [27,86], and the spread of SHV-5 in hospitals in Greece, Hungary and Poland [38]. Spread of plasmids between countries has been reported for CTX-M-2 (Belgium and France), CTX-M-15 (10 countries), CTX-M-32 (Mediterranean area), TEM-24 and TEM-52 (Belgium, France, Portugal and Spain) [51,68,70,76,78,87,88]. Plasmid-mediated horizontal transfer of $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-9}}$ genes has been demonstrated between poultry and human *S. enterica* and *E. coli* strains isolated in very different geographical regions [67,68,89]. The predominant plasmids circulating in Europe in both hospitals and the community are listed in Table 2.

The emergence of epidemic strains that simultaneously carry several plasmids encoding distinct ESBLs, AmpC and MBLs is of concern and deserves further follow-up (see above, section *Clonal expansion of ESBL-producing Enterobacteriaceae*).

Multidrug-resistance profiles in ESBL-producing isolates

ESBL producers are commonly resistant to different antibiotic families including – besides beta-lactams – fluoroquinolones, aminoglycosides and trimetoprim-sulfametoxazole, which contribute to the selection and persistence of multidrug-resistant ESBL strains and plasmids in both clinical and community settings [1,91]. The proportion of ESBL-producing isolates resistant to fluoroquinolones has increased over time, initially in *K. pneumoniae* and later also in *E. coli* [1,89,90]. This increase has apparently occurred in parallel to the increase in plasmid-mediated resistance mechanisms including *Qnr* proteins (*qnrA*, *qnrB* or *qnrS*), acetylases that can affect the action of certain fluroquinolones (*aac(6')-Ib-cr*) or systems pumping fluoroquinolones out of the bacteria (qepA) [92,93].

Very recent studies indicate that the *aac(6')-Ib-cr* gene seems to be confined to *E. coli* ST131 and thus has mainly been linked to CTX-M-15 isolates in different surveys, whereas *qnr* genes are mostly associated with enzymes from the CTX-M-9 or CTX-M-1 groups, which reflects the fact that genes coding for resistance to beta-lactams and quinolones are located on the same plasmid and thus passed on together among different enterobacterial species [79,92].

A high level of fluoroquinolone resistance is often due to additional loss of outer membrane proteins or efflux pump overexpression in clones that already contain *gyrA* and *parC* chromosomal mutations and plasmid-mediated mechanisms [79]. Genes that encode resistance to aminoglycosides (different modifying enzymes and ArmA methylase), trimetoprim or sulfonamides and are located on a wide range of genetic elements such as class 1, 2 and 3 integrons or transposable elements have been associated with different multidrug-resistant ESBL plasmids from human and animal origin [93-96; Curiao *et al.*, unpublished results].

Finally, the recent recovery of plasmids coding for ESBLs that express a low level of resistance to beta-lactams [65] or contain multiple silenced antibiotic resistance genes [97] is of particular concern, as they may serve as reservoirs of antibiotic resistance determinants in bacteria that we are unaware of and that cannot be detected by phenotype.

Concluding Remarks

Increased prevalence of Enterobacteriaceae resistant to extended spectrum beta-lactamases has been reported all over Europe, albeit with a great variability in the occurrence and distribution of ESBL enzymes among different geographic areas. Nordic European countries still show the lowest rates of ESBL prevalence in clinical isolates and have not reported any isolates in animals, while southern and eastern countries present high and increasing frequencies of ESBL-producing strains in both nosocomial and community settings. However, some general epidemiological features such as:

1. the wide representation of CTX-M enzymes, particularly among *E. coli* isolates that cause community-acquired infections,
2. the wide spread of particular successful clones and multidrug-resistant plasmids,
3. and the increasing number of Enterobacteriaceae with ESBLs that also contain MBLs or AmpCs and other new mechanisms of resistance to fluoroquinolones or aminoglycosides indicate that the recent increase of ESBL producers in Europe constitutes a complex multifactorial problem of high public health significance that deserves a deep analysis and the implementation of specific interventions at different levels.

Firstly, the use of broad spectrum cephalosporins and fluoroquinolones in humans and animals should be urgently limited to cases in which other therapeutic alternatives according to evidence-based guidelines are not possible. Limiting antimicrobial use may curtail the selection and persistence of predominant ESBL clones and the probable dissemination of conjugative plasmids among strains, thus decreasing not only the number of potential ESBL donors but also the accumulation of antibiotic resistance genes on common genetic elements.

Secondly, and in accordance with the former recommendation, methods should be improved to efficiently detect and track those bacterial clones and plasmids that constitute the major vehicles for the spread of ESBL-mediated resistance. Ideally, such methods of detection should be accessible to medium-level diagnostic microbiology laboratories, to assure the possibility of performing interventions in real time.

Thirdly, the importation of ESBL-producing bacterial strains through food animals and pets has the potential to cause the wide dissemination of antibiotic resistance among countries and their spread to humans. It highlights the need for national and supra-national public health efforts to implement surveillance, epidemiologic, environmental health, and policy-making components.

Fourth, the implementation of ecological surveillance of ESBL-producing organisms, including environmental (particularly water environments, as sewage) and faecal colonisation surveillance studies in community-based individuals and animals is urgently needed to address the "colonisation pressure" outside hospitals, to detect circulation of highly epidemic clones and to monitor ESBL trends. These ecological studies could be useful as biosensors of modifications in the ESBL landscape.

Fifth, an improvement is needed in the methods for detecting multidrug-resistant ESBL producers that express a low level of resistance to beta-lactams or might contain silenced antibiotic resistance genes not detectable by standard phenotype. Also strongly suggested is a standardisation of beta-lactam breakpoints recommended by the different agencies and committees.

Finally, the scientific and public health community should be aware that the potential interventions directed to control the world-wide spread of ESBL-producing organisms have a limited time-window for effective action. Once a number of thresholds were crossed (critical absolute number of ESBL-genes in the microbial world, critical associations of these genes with wide-spread genetic platforms, critical dissemination of ESBLs among different bacterial species and clones), the control will be simply impossible by applying the standard measures. We should act now, and be prepared for the uncertain future, by promoting innovative ways of controlling ESBL-producing organisms.

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