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CTX-M: changing the face of ESBLs in Europe

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Since around 2000—earlier in Poland and Spain and later in France and the UK—dramatic shifts have occurred in the prevalence and types of extended-spectrum β-lactamases (ESBLs) in Europe. Before this watershed, most producers were nosocomial isolates, often *Klebsiella* spp. or *Enterobacter* spp. from specialist care units, and had mutant TEM or SHV ESBLs. Subsequently, CTX-M ESBLs have become dominant, with much greater penetration into *Escherichia coli*, and with many infections in 'complicated community' patients, usually with underlying disease, recent antibiotic usage, or healthcare contact. The degree of clonality among producers varies with the country, as does the enzyme type produced, with group 9 (CTX-M-9 and -14) enzymes dominant in Spain and group 1 enzymes (particularly CTX-M-3 and -15) dominant elsewhere. Irrespective of the particular enzyme, most producers are multiresistant. These changing patterns present major therapeutic and infection control challenges, with the public health intervention points unclear.

Keywords: extended-spectrum β-lactamases, antibiotic resistance, β-lactamases, CTX-M β-lactamases

Introduction

Until the late 1990s, European surveys of extended-spectrum β-lactamases (ESBLs) almost exclusively found TEM and SHV enzyme variants, often SHV-2 and SHV-5, and largely found these in *Klebsiella* spp. Many ESBL-producing outbreak strains of *Klebsiella pneumoniae* were described, with a few 'epidemic' clones affecting multiple hospitals, notably serotype K25 lineages with SHV-4 enzyme that spread in Belgium and France,[1,2] and also an *Enterobacter aerogenes* clone with TEM-24 enzyme that became (and remains) widespread in Belgium and France.[3–6] Affected patients were mostly in specialist units, and European surveys in 1994 and 1997–98 found ESBLs in 23–25% of all *Klebsiella* spp. from ICUs.[7,8] CTX-M ESBLs were recorded rarely, although there were large outbreaks of *Salmonella* Typhimurium with CTX-M-4 and -5 enzymes in Latvia,[9] Russia and Belarus[6] in the mid-1990s.

These patterns have now changed dramatically, with CTX-M enzymes replacing TEM and SHV mutants as the predominant ESBLs in many European countries, with *Escherichia coli* joining *K. pneumoniae* as a major host, and with producers increasingly isolated from community patients. CTX-M enzymes are supplanting TEM and SHV variants in East Asia too,[10,11] just as one of them (CTX-M-2) did in the early 1990s in Argentina.[12] Only in North America are TEM and SHV mutants still dominant, though substantial outbreaks of *E. coli* with CTX-M-15 β-lactamase have occurred in Canada[13,14] and a scatter of ICAAC abstracts describe CTX-M producers in the USA.

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Antibiotics by Broadening the Knowledge on Molecular Mechanisms behind Resistance to Inhibitors of Cell Wall Synthesis) Consortium summarizes the current European situation by country. It does not aim to be a comprehensive account of the biochemistry, genetics or evolution of CTX-M β-lactamases; others have reviewed these topics recently and well.[15,16] It should however be said that over 50 CTX-M β-lactamases are recognized and that they divide into five clusters on sequence homology, namely the CTX-M-1, -M-2, -M-8, -M-9 and -M-25 groups (http://www.lahey.org/studies/webt.stm). Group 1 and 2 types evolved by the escape of chromosomal genes from *Kluyvera ascorbata*,[17] whereas group 8 and 9 enzymes evolved via similar escapes from *Kluyvera georgiana*.[18] Once mobilized, $bla_{\text{CTX-M}}$ genes can be hosted by many elements, but most often by large multiresistance plasmids. IS*Ecp1* insertion sequence elements were often involved in the initial mobilization events[13,19] but this is not universally true, particularly for group 2 and 9 enzymes,[16,20] and analysis of a 12 kb region surrounding $bla_{\text{CTX-M-10}}$ from different isolates revealed links to a phage-related element, which may have facilitated the initial escape of $bla_{\text{CTX-M-10}}$ from *Kluyvera* spp. Expression is often from the promoter in IS*Ecp1*, but may be from those in unusual class 1 integrons.[21]

CTX-M enzymes are more active against cefotaxime and ceftriaxone than ceftazidime, but point mutations can increase activity against ceftazidime; thus CTX-M-15 and -32 differ from CTX-M-3 and -1, respectively, solely by Asp-240→Gly substitutions, but are 100-fold more active against ceftazidime.[22,23] Cephamycins, temocillin and carbapenems are not hydrolysed by CTX-M enzymes; clavulanate, tazobactam and sulbactam inhibit activity, but producers are often resistant to β-lactamase inhibitor combinations because of concurrent production of inhibitor-resistant penicillinase, (e.g. OXA-1), sometimes encoded by the same plasmids.[13,24,25]

CTX-M enzymes, by country

The United Kingdom

No isolates with CTX-M enzymes were reported in the UK before 2000, when a single *Klebsiella oxytoca* with CTX-M-9 was found.[26] This was followed, in 2001, by a clonal outbreak in a Birmingham hospital, involving 30 patients and caused by a *K. pneumoniae* strain with CTX-M-26 β-lactamase.[27] Also in 2001, a survey examined over 900 *E. coli* from 28 hospitals in the UK and Ireland and recorded four isolates with CTX-M-15 enzyme.[28] These were unique by PFGE, were from three hospitals, and were a harbinger of what was to follow. Unnoticed strains with CTX-M enzymes were present earlier, as revealed by retrospective detection of CTX-M group 9 enzymes in *Salmonella enterica* serotype Virchow isolates from the 1990s, several of them from patients with a history of foreign travel.[29]

In mid-2003 the Health Protection Agency began to receive requests to investigate 'outbreaks' (in the loosest sense) of ESBL-producing *E. coli* from community as well as hospital patients. On receipt, over 90% of these isolates proved to have CTX-M-15 enzyme or, much more rarely, CTX-M-3 or -9.[30] In the subsequent 24 months the Agency received over 1200 producers, from over half of all the clinical microbiology laboratories in the UK. These represent only a small fraction of all producers isolated. A prospective survey was undertaken late in 2004, covering 16 laboratories in south-east England, and seeking up to 100 consecutive cephalosporin-resistant Enterobacteriaceae per site. This yielded 1127 isolates with confirmed cephalosporin resistance.[31] Among those with substantive mechanisms (ESBLs, AmpC or hyperproduced K1 enzyme), 51% were *E. coli*, and the largest group, comprising 292 isolates, were *E. coli* with CTX-M enzymes. CTX-M types also were the dominant ESBLs in *Klebsiella* spp. (Table 1). Just 4 years previously the dominant cephalosporin resistance types had been AmpC-derepressed *Enterobacter* spp. and *Klebsiella* spp. with TEM and SHV ESBLs, and only four *E. coli* with CTX-M enzymes were found.[28,32]

Table 1. Mechanisms found among consecutive cefpodoxime-, cefotaxime- or ceftazidime- resistant *E. coli*, *Klebsiella* spp. or *Enterobacter* spp. collected at 16 laboratories in London and south-east England from August to October 2004[31]

	E. coli (n = 574)	*Klebsiella* spp. (n = 224)	*Enterobacter* spp. (n = 157)
CTX-M ESBL	292	190	6
Non-CTX-M ESBL	88	25	20
AmpC—chromosomal or plasmid-mediated	41	1	72
Hyperproduction of K1 chromosomal β-lactamase	0	8	0
No substantive mechanism defined; low-level resistance, mostly to cefpodoxime only	153	0	59

UK *E. coli* isolates with CTX-M-15 β-lactamase include five major serotype O25 clones, designated A–E, along with a great diversity of non-clonal producers.[30] The major clones had 78% similarity by PFGE and may share a common ancestor. The most prevalent of them, A, is less resistant to cephalosporins than the others, because of an IS*26* element between $bla_{\text{CTX-M-15}}$ and its normal promoter. It is dominant in several parts of the UK (Figure 1), whilst other areas have the more localized B–E clones, or predominantly have non-clonal producers. Around Preston (north-west England; light grey circle in Figure 1), some strain A *E. coli* additionally have a plasmidic AmpC β-lactamase, CMY-23.[33] A minority (7%) of *E. coli* with CTX-M enzymes have group 9 (CTX-M-9 or -14) enzymes; these are non-clonal or form small local clusters.[34] Less work has been done on *Klebsiella* spp. with CTX-M enzymes but, again, CTX-M-15 is dominant.[31]

Regardless of species or enzyme type, most producers are multiresistant to aminoglycosides (except gentamicin for *E. coli* strain A), classical tetracyclines, quinolones, trimethoprim and sulphonamides. β-Lactamase/inhibitor combinations have variable activity: most isolates with CTX-M-15 enzyme are resistant because of concurrent production of OXA-1 penicillinases, but sporadic isolates with group 9 enzymes mostly are susceptible.[30] Drugs generally remaining active include carbapenems, nitrofurantoin, fosfomycin, temocillin and tigecycline. A few *K. pneumoniae* isolates with CTX-M-15 enzyme are carbapenem-resistant because of porin loss, with ertapenem more compromised than imipenem or meropenem.[35] These are widely scattered, but nowhere frequent.

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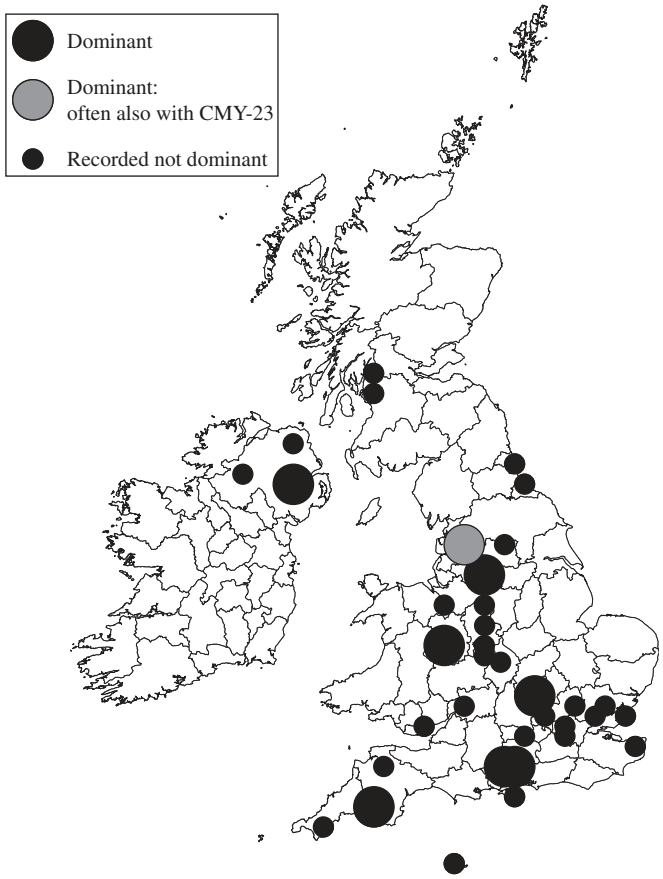

Figure 1. Occurrence of the epidemic *E. coli* strain A with CTX-M-15 β-lactamase in the UK, 2003–5.

Most (77%) producers are from urinary infections,[31] with minorities from bacteraemias or other sites. Many (45% in the 2004 survey[31]) are indicated as coming from community patients, but diligent enquiry often reveals that these individuals are elderly, have multiple underlying health problems (e.g. diabetes or dementia), and have been hospitalized in the preceding 1–3 years.

In Shropshire, which was an early epicentre of infections with *E. coli* strain A, there were 25 deaths among the first 108 cases, with infection deemed contributory to death in 10 of the first 54 deaths, and possibly contributory in a further four.[24] Stool cultures detected ESBL producers, including strain A, in 4.5% and 2.5% of diarrhoeal in- and outpatients, respectively in Shropshire.[24] Isolates with CTX-M enzymes were also found in 2% of diarrhoeal patients around York,[36] although these mostly had group 9 enzymes, as did those from diarrhoeal calves in Wales,[37] implying little link to the major clinical problem. There is no suggestion that the CTX-M$^+$ *E. coli* were related to the diarrhoea; diarrhoeal faeces were screened solely because of laboratory availability. Nevertheless, gut carriage is potentially significant since most urinary and intra-abdominal infections with *E. coli* arise endogenously.

France

French ESBL surveys during the 1990s showed that an *E. aerogenes* clone with TEM-24 β-lactamase and several *K. pneumoniae* clones with SHV-4 enzyme had become widespread.[2–4,38,39] The *E. aerogenes* clone is also widespread in Belgium, and has been found in Italy, Spain and Portugal, with the earliest representatives dating from 1988.[40]

One of the first CTX-M enzymes described, CTX-M-1, was discovered in France in 1989.[41] A decade later CTX-M-3 was found in an *Enterobacter cloacae* isolate recovered in the suburbs of Paris, from a patient without overseas travel.[42] Shortly afterwards, Dutour *et al.*[43] reported Enterobacteriaceae with CTX-M-1, -3 and -14 enzymes from several Parisian hospitals, and demonstrated that the encoding plasmids were related to elements identified a decade earlier. Retrospectively examining enterobacteria recovered between 1989 and 2000 in three Parisian hospitals, Saladin *et al.*[44] identified nine *E. coli* and *Proteus mirabilis* that produced CTX-M-1, -2, -9, -14, -20 or -21 enzymes. Despite these early discoveries, producers of CTX-M enzymes remained rare during the 1990s. A survey in Aquitaine (south-west France) in 1999 found ESBLs in only 1.5% of Enterobacteriaceae from private laboratories serving community and healthcare centre patients;[45] the TEM-24$^+$ clone of *E. aerogenes* was widely represented, but only one CTX-M enzyme producer was identified, an *E. coli* isolate with CTX-M-1.

As elsewhere in Europe, CTX-M enzymes are accumulating with the new century, especially in the north of France. An *E. coli* strain with CTX-M-15 β-lactamase caused an outbreak from October 2001 to March 2003 in a 35-bed long-term care facility in a suburb of Paris,[46] involving 26/47 residents. By 2002–2003 *E. coli* was the main host species for ESBLs at multiple hospitals in and around Paris, with many of the producers clonally related, at least within sites, and with CTX-M-15 the dominant type.[47–50] Other CTX-M types (CTX-M-3, -10 and -14) continued to be seen, as did non-CTX-M types, predominantly TEM-3 and, in *E. aerogenes*, TEM-24. Many of the isolates belonged to phylogroup B2, which is associated with extra-intestinal infections; they were variably resistant to aminoglycosides and tetracyclines. By contrast to these studies in northern France, surveys in 2002–2003 in the Auvergne (south-east France)[51] and southern France[52] found that *E. aerogenes* remained the most frequent ESBL producer, with TEM-3 and TEM-24 accounting for 90% of all ESBLs. This north–south divide may now be breaking down, and a 2004 study identified CTX-M enzymes in southern France, including in hospitals at Perpignan, Toulouse and Montpellier[53] whereas further work shows that CTX-M-15 is widespread around Reims in the east (C. De Champs and P. Nordmann, unpublished data).

Whilst most work has concentrated on producer strains from hospitals and long-term care facilities, the community spread of *E. coli* with CTX-M-15 enzyme is illustrated by two reports, one of a *Salmonella* Typhimurium strain with CTX-M-1 enzyme and the other of a *Shigella sonnei* strain with CTX-M-15;[48] neither patient had travelled abroad. *S. enterica* Virchow with CTX-M-9 enzyme has been identified in France from poultry as well as humans.[54]

Spain and Portugal

Reports of ESBL-producing Enterobacteriaceae in Spain date from 1988[55] and, as in other European countries, early publications concerned bla_{SHV} or bla_{TEM} variants.[55–57] CTX-M β-lactamases were first reported in 2000, with the publication of papers describing *E. coli* and *Salmonella* with CTX-M-9 recovered in

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Barcelona and Murcia in 1996–98[58,59] and an *E. coli* isolate with CTX-M-10 enzyme recovered in Madrid in 1997.[60] Subsequent re-examination of ESBL-producing *K. pneumoniae* and *Enterobacter* collected in Madrid from 1988 onwards revealed that CTX-M enzymes had emerged in Spain at least by the early 1990s,[5,61] only a few years after their first discovery in Germany, Japan and Argentina. The earliest producers found were *K. pneumoniae* from 1990—one with CTX-M-10 enzyme from an outpatient urine sample,[61] the other, with CTX-M-9, from a gastroenterology inpatient; also an *Enterobacter gergoviae* with CTX-M-10 was recovered from a catheter urine in 1991.[5] CTX-M-10 β-lactamase was also found in clinical *E. coli* isolates and in faecal *Citrobacter freundii* and *K. pneumoniae* isolates from outpatients from 1991.[62,63]

Despite these early examples, CTX-M β-lactamases did not become prevalent in Spain in the 1990s. A study in Barcelona between 1994 and 1996 found that only 7% of *E. coli* isolates with decreased susceptibilities to broad-spectrum cephalosporins had CTX-M-9-like enzymes[64] and a long-term (1995–2003) surveillance of *K. pneumoniae* and *E. coli* in Seville first recorded CTX-M producers only from 1998.[65] As in other European countries, this pattern has since changed dramatically, with multiple reports of CTX-M β-lactamase-producing isolates, most of them from community patients.[62,65–70] One multicentre study examined all *E. coli* and *K. pneumoniae* isolates with an ESBL phenotype from March to June 2000 from 40 hospitals across Spain.[67,68] ESBLs were found in 0.5% and 2.7% of *E. coli* and *K. pneumoniae*, respectively and it was notable that 90% of participating centres recovered producers. The most prevalent ESBLs in *E. coli* were CTX-M-9 (27.3%), SHV-12 (23.9%) and CTX-M-14 (16.7%). CTX-M-10 enzyme, though longer established, was present in only 4.5% of producer isolates. In the case of *K. pneumoniae*, CTX-M-10 (12.5%) was the only CTX-M enzyme type found, whilst TEM-4 (25%) and TEM-3 (16.7%) were the most common ESBLs. *E. coli* isolates with CTX-M-9 and -14 enzymes were encountered widely, whereas CTX-M-10 was concentrated in the central region, with just a few producers from the north.[68,71] Fifty-one per cent of *E. coli* with ESBLs (but only 7% of the *K. pneumoniae*) were from non-hospitalized patients. There was little clonality among isolates with CTX-M enzymes,[71] although a few strains broke this pattern, as in the case of an *E. coli* lineage with a unique CTX-M-9-encoding plasmid, observed in faecal samples of multiple attendees at a summer camp in 2002.[72]

The high prevalence of CTX-M-9 enzyme found in these large-scale surveys is corroborated by local studies of clinical and faecal *E. coli, K. pneumoniae* and *Salmonella* spp. isolates[73] from hospitalized and community patients, and from healthy and sick chickens.[74] CTX-M-14 enzyme, which also belongs to CTX-M group 9, also occurs widely,[67] including from *Salmonella* spp.[75] and in faecal *E. coli* from healthy volunteers with little or no hospital contact;[75] it was the most frequent ESBL in a long-term study from Seville.[65] Most recently, group 1 enzymes, including CTX-M-1, -3, -15, -28 and -32 have been encountered.[23,70,74,76] As in the UK, Italy and France, CTX-M-15 seems to be spreading rapidly[76] with producers also reported from Portugal;[25] once again it is genetically linked with $bla_{\text{OXA-1}}$. CTX-M-1 and -32 enzymes were also recovered from healthy and sick animals in 2003.[74] Group 2 enzymes, which are widely distributed in South America and Israel, have recently been detected in clinical *E. coli* isolates from Barcelona[76] and in a faecal *E. coli* isolate from a healthy volunteer in Madrid;[62] enzymes belonging to the CTX-M-8 and -25 groups have not yet been found in Spain.

Among hospitalized patients, prior oxyimino-β-lactam use, diabetes, and underlying diseases were independent risk factors for infection or colonization with non-clonal *E. coli* that had CTX-M enzymes, whereas previous fluoroquinolone use was associated with infection or colonization with those with SHV and TEM enzymes.[77] Among non-hospitalized patients, most ESBL-positive *E. coli* isolates (64%) had $bla_{\text{CTX-M-9}}$ and were from patients with high scores for Charlson's co-morbidity index for severity of underlying disease.[66] Many were old and reported fluoroquinolone use in the preceding 2 months. Most infections were of the urinary tract and almost half of the cases appeared truly community-acquired, as the patients lacked recent hospitalization and were not in nursing homes. Similar risk factors were reported for infections with ESBL producers outside the hospital in Israel, where CTX-M-2 predominates, although more patients had recent hospitalization.[78]

Dissemination of $bla_{\text{CTX-M-9}}$ is associated with a few large conjugative plasmids of long-established incompatibility groups; the gene is located within class 1 integrons with a low, but increasing, diversity of resistance cassettes[79,80] often also conferring resistance to trimethoprim and aminoglycosides. Fluoroquinolone resistance is common among isolates with CTX-M-9 and -14, but is not associated with *qnr* (P. Nordmann and L. Poirel, unpublished data). Dissemination of epidemic plasmids carrying $bla_{\text{CTX-M-14}}$ or $bla_{\text{CTX-M-32}}$ among *E. coli* has been described,[73] as was transmission of a plasmid coding for CTX-M-10 between *E. cloacae* and various *E. coli* and *K. pneumoniae* strains.[5,61,81]

Italy

CTX-M-type enzymes were not sought in the first nationwide Italian survey of ESBL production, carried out in 1999.[82] However, only 8% of collected Enterobacteriaceae isolates with ESBL phenotypes lacked TEM- and/or SHV-ESBLs, implying that any diffusion of CTX-M-type enzymes was limited.

Reports of Italian isolates with CTX-M ESBLs only began to appear in 2003, and then included: (i) several *E. coli, E. aerogenes* and *C. freundii* isolates with CTX-M-1 enzyme, but with no information on sources or clonal relationships;[83] (ii) a few isolates with CTX-M enzymes (one *K. pneumoniae* with CTX-M-15; two related *Proteus vulgaris* isolates with CTX-M-2, and three unrelated *E. coli* isolates with CTX-M-1) from a hospital in northern Italy.[84] In 2001–2002, only 2.6% of all ESBL producers had CTX-M phenotypes, with cefotaxime MIC > ceftazidime MIC.[84]

A second nationwide ESBL survey, in 2003, sought CTX-M enzymes specifically, and found them to be widespread[85] Producers—mostly *E. coli* and, in lower numbers, *K. pneumoniae*—were detected in 10/11 participating centres and accounted for ∼20% of all ESBL-producing isolates, though this proportion varied from <2% to ∼50%. All the CTX-M enzymes were of group 1, with CTX-M-1 and -15 predominant and CTX-M-32 rarer. Producers were detected from inpatients and outpatients at similar rates. Genotyping revealed multiple lineages of *E. coli* and *K. pneumoniae* irrespective of whether the enzyme present was CTX-M-1 or -15. This diversity, and the ability to transfer ESBL determinants conjugatively, was greater among *E. coli* isolates with CTX-M-1 enzyme than among those with CTX-M-15.[86]

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The rapid and recent dissemination of CTX-M-β-lactamase-producing *E. coli* was further documented by a longitudinal surveillance examining 20 000 isolates collected over 5 years in northern Italy.[87] The prevalence of ESBLs increased steadily from 0.2% in 1999 to 1.6% in 2003, with this change largely attributable to CTX-M-β-lactamase-positive isolates, which comprised 38% of all ESBL producers in 2003 compared with 12% in 1999. Most had CTX-M-1 enzyme, which was also found in *Citrobacter amalonaticus* and *Morganella morganii*—unusual hosts for ESBLs. In both instances the isolates were from patients co-infected with CTX-M-1$^+$ *E. coli*, suggesting *in vivo* transfer.[88]

CTX-M-1 β-lactamase was present in 76% of ESBL-positive *E. coli* isolates from sick and healthy companion or stray dogs and cats, based on a survey carried out in Rome from 2001 to 2003. Perhaps even more strikingly this study found ESBL producers in specimens (mostly faeces) from 21 of the 298 animals examined.[89]

Poland

It is impossible to determine when CTX-M β-lactamase producers first appeared in Poland and whether they first emerged in nosocomial pathogens or in the community because, until the mid-1990s, Polish laboratories did not screen for ESBLs and there were no epidemiological surveys of ESBLs. ESBL surveys only commenced in 1996, when the then Sera and Vaccines Central Research Laboratory also began to promulgate the value and methodology of ESBL detection to clinical laboratories.

Among the first ESBL producers detected and analysed there were four clonal *C. freundii* isolates and one *E. coli* isolate from the Praski Hospital in Warsaw, one of the first centres to introduce routine ESBL detection.[90] A then novel CTX-M variant, CTX-M-3, was identified in these isolates, and proved similar to CTX-M-1, previously observed in Germany and France.[91,92] $bla_{\text{CTX-M-3}}$ was located on a readily conjugative plasmid. Subsequent examination, from November 1996 to February 1997, found that CTX-M-3 was the predominant ESBL in the Praski Hospital,[93] with producers recovered in many wards. Their prevalence reflected clonal spread of *K. pneumoniae*, *C. freundii* and *Serratia marcescens* producers, along with intense plasmid dissemination. The same $bla_{\text{CTX-M-3}}$-encoding plasmid, originally identified in the *C. freundii* isolates from July 1996 proved widespread, and was designated plasmid A1, later pCTX-M3.[90] Almost all the other encoding plasmids found were its derivatives. Hosts of pCTX-M3 family plasmids included 16 RAPD types of seven enterobacterial species (*E. coli*, *K. pneumoniae*, *K. oxytoca*, *C. freundii*, *E. cloacae*, *S. marcescens* and *M. morganii*). Several of the *E. coli* and *K. pneumoniae* isolates were from outpatients, though they may have had earlier hospitalization.

The first multicentre survey on ESBLs among Enterobacteriaceae in Polish hospitals was conducted in Spring 1998 and covered seven institutions (M. Gniadkowski, A. Barnaiak, J. Fiett and W. Hryniewicz, unpublished data). Isolates with CTX-M enzymes were found at six centres, and accounted for 19% of all ESBL producers collected. This frequency was below that of SHV ESBLs (60.4%, mainly SHV-5), but comparable to that of TEM ESBLs (20.8%). All the survey isolates with CTX-M enzymes, together with those from 11 other hospitals up to the end of 2000 (89 isolates altogether), were subjected to detailed analysis.[94–96] Eighty-six had CTX-M-3 β-lactamase whereas three had its variant, CTX-M-15. In the vast majority of cases these enzymes were coded by conjugative plasmids of the pCTX-M3 family.[97,98] A more recent (2003) survey covering 13 regional Polish hospitals further underscored the rise of CTX-M enzymes, which were found in 82% of all 264 ESBL-producing isolates (M. Gniadkowski, J. Empel, A. Barnaiak, J. Fiett, E. Literacka, A. Mrówka and W. Hryniewicz, unpublished data). Once again, the enzymes found were mostly CTX-M-3 or, less often, CTX-M-15 types and producers were strongly represented at all the participating centres, varying from 62.5% to 100% of all ESBL producers. CTX-M enzymes were found in even more species than previously, including *E. aerogenes*, *P. mirabilis* and *Providencia rettgeri*.

pCTX-M3 is an IncL/M, conjugative, broad-host-range plasmid (I. Kern-Zdanowicz, M. Gołębiewski, M. Zienkiewicz, M. Adamczyk, M. Gniadkowski and P. Ceglowski, unpublished data) with a molecular mass of 89 468 bp (GenBank accession number AF550415). Apart from $bla_{\text{CTX-M-3}}$, it carries $bla_{\text{TEM-1}}$, *dfr* (dihydrofolate reductase), *aac(3)-II* and *aadA2* (aminoglycoside-modifying enzyme) genes; *dfr* and *aadA2* lie within a class 1 integron, which may be part of a composite transposon formed by two IS*26* elements. $bla_{\text{CTX-M-3}}$ is accompanied by an IS*Ecp1* element, 128 bp upstream from the coding region (ref. 96 and M. Gniadkowski and A. Baraniak, unpublished data), which seems likely to have been involved in the initial mobilization.[19] Recently, a 1377 bp fragment of pCTX-M3, starting just behind IS*Ecp1* and encompassing $bla_{\text{CTX-M-3}}$ along with most of *orf477*, was found to be very similar to a chromosomal DNA fragment from a *K. ascorbata* strain,[17] suggesting a possible origin. $bla_{\text{CTX-M-15}}$ genes from Polish isolates were similarly spaced from IS*Ecp1* as for $bla_{\text{CTX-M-3}}$ and had the same flanking sequences;[96] moreover, they are located in pCTX-M3-type plasmids, implying that they had emerged by point mutation of $bla_{\text{CTX-M-3}}$, not via separate gene escapes.

In summary, the ESBL epidemiology in Poland is dominated by CTX-M producers, which have occurred for at least 10 years and are now widespread in hospitals and among bacterial species. The pool of $bla_{\text{CTX-M}}$ variants is very homogeneous, with $bla_{\text{CTX-M-3}}$ dominant and, although a few isolates have $bla_{\text{CTX-M-15}}$, these probably are direct descendants of those with $bla_{\text{CTX-M-3}}$. Almost all the $bla_{\text{CTX-M-3}}$ genes seem to have arisen from a single mobilization event, with subsequent intense dissemination of plasmid pCTX-M3 and its derivatives. Recent unpublished data suggest spread in the community, though studies are limited.

Elsewhere in Europe

This article has concentrated on France, Italy, Poland, Spain and the UK, but the rise of CTX-M enzymes has occurred more widely. National spread of a *K. pneumoniae* clone with CTX-M-15 has recently been reported in Hungary[99] whilst there is good evidence that *E. coli* and *K. pneumoniae* with CTX-M enzymes are becoming widely scattered, if not frequent, in many other European countries (Figure 2).[25,99–105] It is notable that *E. coli* with these enzymes are causing concern even in Scandinavian countries with an exemplary record for infection control.[106] CTX-M-15 is the most-reported CTX-M type across most of the further countries for which data are available, with CTX-M-1 and -3 also widely reported (Figure 2). Exceptionally, one Belgian

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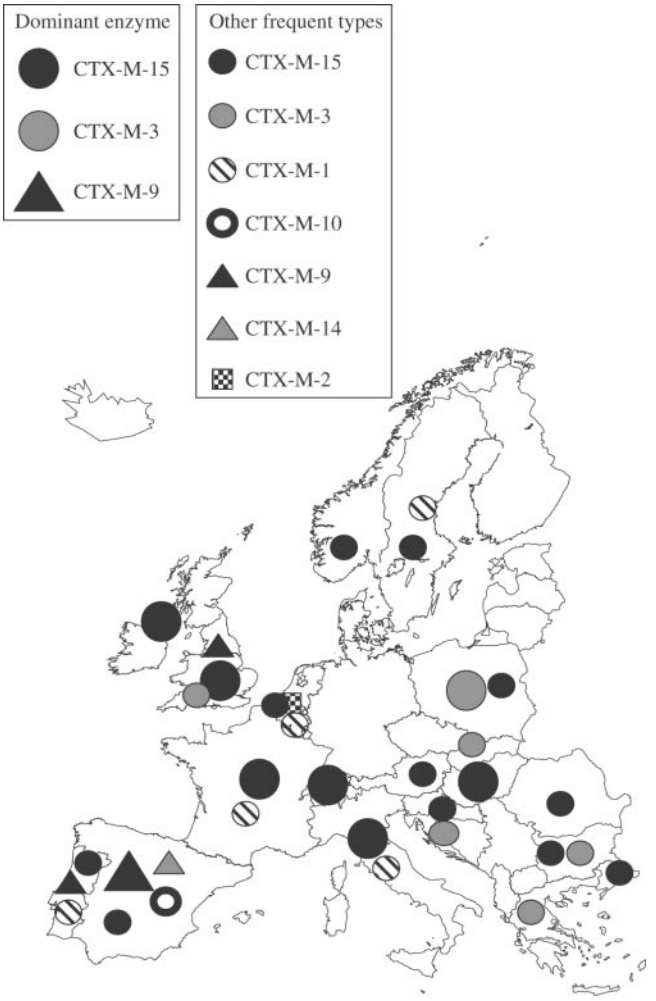

Figure 2. Dominant and frequent CTX-M β-lactamase types in different European countries. Data for France, Italy, Poland, Spain and the UK are based on the references cited in the text. Those for other countries are based on further reports, [25,99–105] also, for Croatia, B. Bedenic and M. Gniadkowski., unpublished data and, for Switzerland, A. Wenger and P. Nordmann, unpublished data, with national dominance only inferred based on large-scale surveys. Many other CTX-M enzymes have been isolated on a few occasions or (as with CTX-M-5-producing *Salmonella* Typhimurium in Latvia) have caused sizeable but time-defined outbreaks before apparently declining. CTX-M-type enzymes are emerging rapidly also in Greece but reports that stress prevalence do not identify the particular enzyme types.

study found that CTX-M-2 accounted for 14% of all CTX-M types.[104]

The best continent-wide resistance surveillance (http://www.earss.rivm.nl) does not distinguish ESBL producers from those with other modes of cephalosporin resistance, and has limited data for Enterobacteriaceae other than *E. coli*. Nevertheless, it shows widespread rises in cephalosporin resistance in *E. coli* from 2001 to 2005 (Figure 3); along with even more dramatic increases in fluoroquinolone resistance (see website).

Routes of spread

Relationships among strains with CTX-M enzymes from different countries remain largely unclear although, *prima facie*, it is likely that the continent-wide rise of CTX-M ESBLs reflects a series of independent events.

It seems that the pCTX-M3-type plasmids, encoding CTX-M-3, began to spread in Poland a little before related enzymes, predominantly CTX-M-15, became prevalent in western Europe. It therefore is reasonable to ask, whether increased movement between east and west Europe has allowed this 'Polish' plasmid to spread westwards. Some insight here can be gained by examining the length of the spacer region between IS*Ecp1* and $bla_{\text{CTX-M-3/15}}$. On this basis it seems most likely that $bla_{\text{CTX-M-15}}$ genes in isolates from the UK (E. Karisik, D.M. Livermore and N. Woodford, unpublished data), Boulogne[49] and Lisbon[107]—also New Delhi,[108] Istanbul,[109] Toronto[13] and Yaounde (Cameroon)[110]—emerged independently from those observed in Poland, since the IS*Ecp1* elements are located much closer (48 bp) to $bla_{\text{CTX-M}}$. On the other hand, the IS*Ecp1*-associated $bla_{\text{CTX-M-3}}$ gene from an *E. cloacae* from Versailles (France) was located on a pCTX-M3-type plasmid.[43] Similarity was also suggested between CTX-M-3 producers from Poland and Taiwan.[111] A representative of the UK *E. coli* strain A was found in Austria, but without any obvious epidemiological link to the UK.[103]

It is plausible too that the *E. coli* clones with CTX-M-15 enzyme, now spreading in the UK and France, originated in other countries with which Europe has extensive and growing population exchanges, perhaps in India, where CTX-M-15 was first described,[22] or North Africa.[112] Clonal relationships have recently been noted between *E. coli* with CTX-M-15 enzymes from Paris, Tunis and, to a lesser extent, the Central African Republic.[113] Such data do not, however, prove the direction of spread and, in the UK, the early spread of CTX-M-15 was not centred in conurbations with large immigrant populations.[30] Moreover—if migration is proposed as a route of spread—it remains unclear why group 9 enzymes (mainly CTX-M-9 itself) currently predominate in Spain whereas the rest of Europe largely has group 1 (CTX-M-3 and -15) types (Figure 3). Group 9 types (CTX-M-9 or -14) are otherwise dominant in East Asia, notably China, whereas South America—with substantial population flows to and from Spain—predominantly has CTX-M-2 type enzymes, at least in its Southern 'cone'. Within Eurasia, CTX-M-2 appears frequent only in Israel[114] a country with fewer obvious cultural and migrational links than Spain to South America.

Food is another possible vector of spread and this article has repeatedly noted the detection of *E. coli* with CTX-M enzyme in food animals. Nevertheless, there is no proven link between this carriage and human infections. In the UK, at least, the enzyme types found in both food animals and in foodstuff belong to groups 2 and 9 (as in Spain[74]) whereas the huge majority of clinical producers have CTX-M-15.

Routes of transmission of *Klebsiella* spp. with ESBLs are well understood in the context of nosocomial outbreaks and whilst nosocomial transmission of *E. coli* clones is less common it can occur. What is less clear is the route by which community infections arise. On one hand, many patients with 'community' infections with CTX-M-β-lactamase-producing *E. coli* have a history of recent hospitalization,[78] where they may have been colonized. In support of this view the distribution of enzyme producers among urinary infections is biased towards complex cases and older age groups,[66] whereas a more random distribution, with more producers from uncomplicated cystitis, would be predicted if most acquisition was via the food chain in the community. On the other hand, not all colonized individuals have

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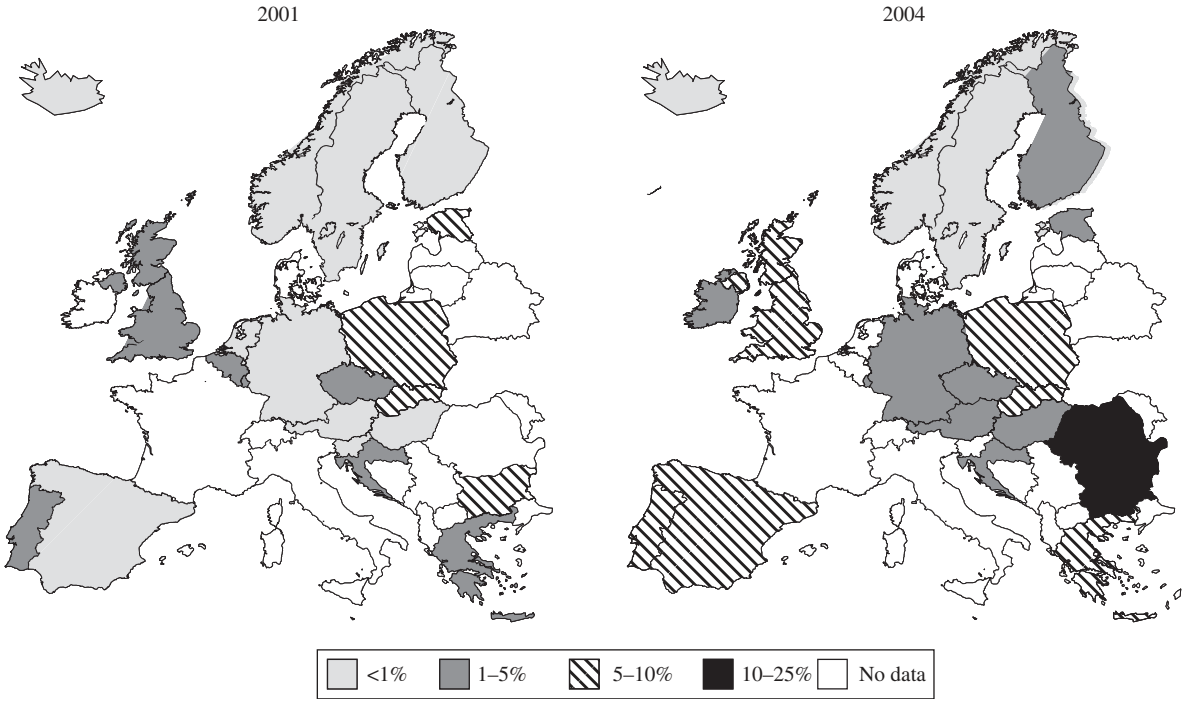

Figure 3. Prevalence of resistance to extended-spectrum cephalosporins among *E. coli* isolates from bacteraemias, as reported under the European Antimicrobial Resistance Surveillance System (http://www.earss.rivm.nl) in 2001 and 2004, supplemented with UK data based on laboratories' reports to the Health Protection Agency.

a history of hospitalization and it may be that low-level gut colonization occurs in the community, via the food chain, perhaps with plasmid transfer to resident *E. coli*, and that the proportion of resistant *E. coli* with CTX-M enzymes tends to be enriched during healthcare contacts, owing to frequent antimicrobial exposure. Screening populations for faecal or rectal carriage would be the obvious way to resolve these issues, but has not yet been undertaken on any wide scale.

Whatever the precise mode of spread (and spread through plasmid dissemination, hospital cross infection, down the food chain, and via human migration are not mutually exclusive), it is clear that Europe is moving to a situation where ESBLs are more common and less confined to hospitals, as well as one where *E. coli* is a major host species. If so, the opportunities for control are disturbingly small. Old data show that ampicillin resistance, largely due to TEM-1 enzyme, rose first in *E. coli* from hospital-acquired infections and only later among those from community-acquired cases.[115] History may be repeating itself with CTX-M ESBLs.

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Transparency declarations

Conflicts of interest: D. M. L., grants from AstraZeneca, Merck and Wyeth; speakers' bureaux for AstraZeneca, Johnson & Johnson, Merck and Wyeth; shareholdings in AstraZeneca GlaxoSmithkline, Pfizer and Schering Plough. G. M. R., grants from Wyeth; consultancies from Wyeth, Janssen-Cilag and Nabi Pharmaceuticals; speakers' bureaux for Wyeth and Merck; R. C., grants from Sanofi-Aventis and Wyeth. Other authors: no conflicts to declare.

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