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CTX-M: changing the face of ESBLs in the UK

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The UK has experienced a sudden rise in extended-spectrum β-lactamase (ESBL) rates, largely due to the appearance and spread of *Escherichia coli* producing CTX-M-15 type β-lactamase. The British Society for Antimicrobial Chemotherapy organized two update meetings during 2004 to report and discuss the recognition, clinical diagnosis, treatment and control of bacteria producing these β-lactamases. This paper reports the data and reviews made by contributors to the conferences. The historical distribution and emergence of ESBLs was reviewed along with the emergence of plasmid-mediated CTX-M ESBLs following their mobilization from the chromosome of *Kluyvera* spp. The first significant outbreak of CTX-M producers in the UK occurred in 2001 and involved *Klebsiella pneumoniae* with CTX-M-26 at one site, but by 2003, cloned and diverse *E. coli* with CTX-M-15 were widespread, with Shropshire one of the most affected regions. The specific experience in Shropshire was reported on and a comprehensive review made of the level of awareness of the need for ESBL detection in laboratories in England and Wales, together with a description of the variety of methods that may be applied, with recommendations for optimal methodology. The increased mortality associated with inappropriate treatment of infections caused by ESBL-producing strains was highlighted, together with discussion on potential control of cross-infection. The meeting concluded that the CTX-M genes have now become widespread in not only *E. coli* but other Enterobacteriaceae in the UK and this will represent a substantial threat to both the treatment of infections caused by these bacteria in the community and within hospitals.

Keywords: extended-spectrum β-lactamases, ESBLs, BSAC

Extended-spectrum β-lactamases (ESBLs) are an increasingly important cause of multidrug resistance in Gram-negative bacteria throughout the world.[1,2] Enterobacteriaceae with ESBLs have climbed the agenda of UK microbiologists in the last year or so, largely because of the rapid spread of CTX-M β-lactamases. The changing prevalence and epidemiology of these problem bacteria prompted the BSAC to organize two update meetings during 2004, covering ESBL recognition, clinical diagnosis, treatment and control. This article stems from the second of these meetings, held at the Royal College of Physicians in London on 17 September 2004. Since it includes speakers' unpublished data (which are available from www.bsac.org.uk) it is written as reportage, with attribution.

The meeting started with Professor Hawkey reviewing the history and epidemiology of ESBLs, noting how the short paper by Kliebe *et al.*[3], describing transferable resistance to extended-spectrum cephalosporins, proved to be the harbinger of a major event in the history of antibiotic resistance. The authors had found *Klebsiella ozaenae* isolates with plasmid-mediated resistance to extended-spectrum cephalosporins, and noted that these compounds should be 'handled carefully' with the development of resistance 'monitored by microbiological means'. These were telling words since, very soon, outbreaks of infection due to Enterobacteriaceae with transferable cephalosporin resistance were being reported also in France.[4] Although these original descriptions lacked DNA sequence data, it was soon apparent that the enzymes were mutants of the classical TEM and SHV penicillinases, which were already widespread. The original enzyme found by Kliebe *et al.*[3] was an SHV variant (SHV-2), with a single amino acid substitution at position 238, from glycine to serine, enabling hydrolysis of extended-spectrum cephalosporins.

During the next decade, SHV- and TEM-derived ESBLs became recognized worldwide, with over 100 different mutations described as conferring some degree of activity against extended-spectrum cephalosporins.[4] The heavy and increasing use of oxyimino-β-lactams such as ceftazidime, cefotaxime and ceftriaxone undoubtedly drove the selection of these mutant enzymes, which were termed 'extended-spectrum β-lactamase' by Philippon *et al.*[5] in 1989.

In a review of ESBLs in 1995, there was a very brief mention of two class A β-lactamases, MEN-1 (now CTX-M-2) and CTX-M-3,

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which were said *not* to be closely related to the TEM or SHV penicillinases, and were then thought to be derived from the chromosomally-encoded K1 enzyme of *Klebsiella oxytoca.*[4] These enzymes seemed to be curiosities, along with other exotica such as PER-1[6] and OXA-10 mutants[7,8]—which were then being reported from *Pseudomonas aeruginosa* collected in Turkey. During the 1990s however, CTX-M enzymes became the most common ESBL types in Argentina.[9] More recently, they have become widespread in Europe and Asia, although they still appear uncommon in North America.[10] In Asia, early reports of ESBLs mostly concerned SHV enzymes, particularly SHV-6 and SHV-12. However, work in the early 2000s revealed that CTX-M enzymes are now the predominant ESBLs in most of East Asia,[11] and a similar replacement now seems to be occurring in Europe. The view that CTX-M ESBLs had evolved from K1 enzyme proved to be incorrect, and there is good evidence that at least three of the five DNA homology groups now recognized within the family have been mobilized, by insertion elements, from the chromosomes of *Kluyvera* spp.[12]

In the UK, there were many reports of TEM and SHV ESBLs during the 1990s[13–15] but—despite rare large outbreaks—producers did not become common. Rather, these enzymes could be viewed as an occasional nuisance, most often found in nosocomial *Klebsiella* spp. from patients in intensive care or other specialist settings. The first CTX-M ESBL in the UK was found as recently as 2000, in a solitary isolate of *K. oxytoca,*[16] while the first outbreak—caused by *Klebsiella pneumoniae* producing the new enzyme CTX-M-26—was recorded in Birmingham in 2001.[17] These reports caused concern in view of how CTX-M enzymes had spread in South America and we sought, unsuccessfully, to fund a snapshot prevalence survey in 2002. By mid-2003 these plans were overtaken by events, as well-scattered laboratories began to advise the Health Protection Agency that they were encountering numerous *Escherichia coli* with ESBLs, many of them stated to be from urinary tract infections in community patients. Subsequent investigation has often revealed that these patients were elderly, with underlying medical problems, and had a history of recent hospitalization or other healthcare contact.

Dr N. Woodford (Health Protection Agency, Colindale) described reference laboratory investigation of these isolates, while Dr G. Harvey (Royal Shrewsbury Hospital) described the experiences of one heavily affected NHS Trust. The former work is now published[18] and the latter is available as an ECCMID poster (http://www.hpa.org.uk/srmd/div_nsi_armrl/armrl_posters.htm). As Dr Woodford outlined, the Agency's reference laboratories examined over 600 *E. coli* referred from over 70 UK laboratories as possible ESBL producers during the period May 2003–September 2004, with these representing only a fraction of all producers isolated. Almost all proved to carry $bla_{\text{CTX-M}}$ variants. About 25% of ESBL-positive *E. coli* received by the reference laboratory belonged to one major strain, designated A, with a characteristic DNA fingerprint, and another 40% belonged to four other strains, B–E, all of which may share a common ancestor. The remaining producers are diverse. Strains A–E, and most of the diverse organisms, produce group 1 CTX-M enzymes, with CTX-M-15 predominant, although a few of the diverse strains instead have CTX-M-9 or -14. Most producers are multiresistant, and are consistently susceptible only to parenteral carbapenems (imipenem, meropenem and ertapenem), nitrofurantoin (use limited to uncomplicated urinary tract infections) and fosfomycin (not readily available in the UK). Strain A is also susceptible to gentamicin and is less resistant to cephalosporins (particularly ceftazidime) than other producers, probably owing to an IS*26* element between $bla_{\text{CTX-M}}$ and its usual promoter.[18] Strain A is predominant (*inter alia*) in Shropshire, Ulster and parts of southern England. Other early epicentres of the ESBL problem, notably SW London/NE Surrey, had clonally-diverse CTX-M-15 producers. The reasons for these epidemiological differences are unclear.

Dr Harvey described the clinical experience in Shropshire, where the problem with CTX-M producers had come to light following up-grading of species identification and susceptibility testing for urinary isolates late in 2002. He noted that, among the first 105 patients found to be infected with CTX-M-15-producing *E. coli*, there had been 28 deaths, a point subsequently highlighted in the national press following a coroner's inquest. The relationship between mortality and infection remains under analysis and further comment would be premature. Nevertheless the situation had led to revisions in antibiotic policy in Shropshire, with restriction of quinolones and third-generation cephalosporins, balanced by increased use of carbapenems, particularly ertapenem, in infections where ESBL producers were likely or proven. General practitioners were advised to continue to use trimethoprim first line in community-acquired urinary infections, but to substitute nitrofurantoin in high-risk patients, including those with prior hospitalization; they were also advised urgently to submit urines from any patient responding poorly to treatment for urinary infection. Dr Harvey also stressed the incidence of faecal carriage by CTX-M-15-producing *E. coli*, with the organism recovered from 2.6 and 4.6% of diarrhoeal in- and outpatients respectively. The faecal strains were diverse by PFGE, but included strain A and another locally prevalent strain, D. The implications of gut carriage—now reported in a study in York undertaken by Munday *et al.*[19] and in Spain by Valverde *et al.*[20]—is that multiple CTX-M-producing *E. coli* strains have spread perhaps via the food chain, producing a reservoir of multiresistant gut colonists that may be subsequent agents of urinary infection in vulnerable patients.

The emergence of CTX-M ESBLs raises issues of clinical and laboratory awareness. The former aspects were addressed by Dr Andrew Pearson (CDSC, Health Protection Agency) and the latter by Dr David Livermore (Health Protection Agency, Colindale). Dr Pearson described a survey of UK laboratories' methods of detecting ESBLs and an analysis of bacteraemia reports to the Health Protection Agency. The methods survey, undertaken during January–March 2004, found that the ability to detect, report and investigate ESBL-producing *E. coli* was patchy, with several centres not then having appropriate methods in place. Nevertheless, this survey revealed that 32 laboratories had investigated incidents or outbreaks of ESBL-producing *E. coli* in the preceding year and that half of these had submitted isolates for reference laboratory characterization. Only two had reported these events as 'Serious Untoward Incidents' to their Strategic Health Authorities. This situation prompted issue of guidelines on ESBL detection by the Health Protection Agency (http://www.hpa.org.uk/srmd/div_nsi_armrl/ESBL_advice_June_2004.pdf). A further survey, in early January 2005 found that approximately 70% of responding laboratories had adopted the guidelines; approximately 70% of all laboratories having responded.

Although most CTX-M β-lactamase producers submitted to the reference laboratories were from urinary infections, a minority were from bacteraemias, a setting where the Health Protection Agency has extensive voluntary reporting in place.[21] This reporting indicates a year-on-year increase in resistance to cefotaxime

and ceftazidime among *E. coli*, from 1.8% in 2001 to 3.5% in 2003 and 5% in the first quarter of 2004 in England, perhaps reflecting the spread of ESBL producers. The most rapid change has occurred in the London Region, from under 2% resistance in 2001 to 7.5% in the first half of 2004. Data from the BSAC Bacteraemia study in 2002 shows that 8/210 isolates of *E. coli*, 10/154 *K. pneumoniae* and 18/179 *Enterobacter* spp. from the UK were ESBL producers, suggesting a developing problem (www.bsacsurv.org). A systematic survey of ESBL prevalence in London and SE England has now been initiated by the Health Protection Agency and, while still under analysis, it is already apparent that ESBL-producing *E. coli* are now the most prevalent group of cephalosporin-resistant Enterobacteriaceae in the two regions. Although it might be suggested that the more mobile population in these regions has contributed to the rise in rates, the earliest epicentres were in Shropshire and Northern Ireland, areas with rather stable populations.

Dr Livermore concentrated his presentation on the new Health Protection Agency guidelines for ESBL detection, noting that screening with ceftazidime alone can no longer be recommended with the advent of CTX-M enzymes. Rather, laboratories should screen *all* Enterobacteriaceae isolates either with cefpodoxime (the best general substrate for *all* ESBLs) or with *both* cefotaxime and ceftazidime. Isolates found resistant to any of these cephalosporins should then be tested for cephalosporin/clavulanate synergy to distinguish ESBL producers (synergy positive) from strains with cephalosporin resistance mediated via other enzymic (e.g. AmpC) or non-enzymic mechanisms (synergy negative). Various methods can be used for the synergy tests: traditional 'Jarlier' double disc tests[22] are convenient, but the spacing of the discs is critical, meaning that some ESBL producers may be missed. Better are combination discs,[23,24] when the diameters of zones of inhibition around cephalosporin discs are compared with those around discs containing the same cephalosporin plus clavulanate. Alternatively, Etest ESBL detection strips can be used: these are sensitive and specific,[25] but are more expensive than combination discs. Fuller identification of CTX-M enzymes involves the use of PCR and sequencing.

There has been much debate over the clinical significance of ESBLs, especially where producers appear susceptible to cephalosporins *in vitro*, and Dr R. A. Howe (Southmead Hospital Bristol) reviewed the current state of the literature. He drew attention first to the widely different susceptible breakpoints for third-generation cephalosporins between the NCCLS (generally ≤8 mg/L), compared with the BSAC and European breakpoints of ≤2 mg/L for ceftazidime and ≤1 mg/L for cefotaxime. This differential can lead to some confusion, as reports of 'cephalosporin-susceptible' ESBL producers that failed to be eradicated by cephalosporin therapy often concern isolates that would have been graded resistant by BSAC criteria. Dr Howe rehearsed the arguments both for and against the presence of an ESBL having a negative affect on antimicrobial treatment with third-generation cephalosporins, drawing attention to the studies of Du *et al.*[26]and Lautenbach *et al.*,[27] which showed identical failure rates for infections caused by ESBL-positive and -negative isolates. By contrast, a report by Paterson *et al.*[28] did show an association between ESBL production and failure of cephalosporin therapy when the isolates appeared susceptible on NCCLS criteria; however, outcomes were mostly favourable for those producers that would be defined as susceptible on BSAC criteria. One of the most convincing studies was that of Kim *et al.*,[29] who retrospectively analysed 142 episodes of bacteraemia in children. After correcting for underlying disease and other confounding variables, patients treated with a third-generation cephalosporin for infection with ESBL-positive strains had a much poorer outcome then those given alternative therapies.

Dr Howe drew attention to the anomaly whereby ESBL-producing strains can appear to be susceptible to cephalosporins in agar dilution MIC tests when a standard low inoculum (10^4–10^5 cfu) is used, but where the MIC rises above clinically achievable levels if the inoculum is raised to 10^7 cfu. Animal models also provide insight into the potential problems of treating infections due to ESBL-producing bacteria, Dr Howe added. Both the work of Rice *et al.*[30] and that of Szabo *et al.*[31] showed that clinical failure was frequent in infections due to ESBL-producing bacteria when third-generation cephalosporins were used. Both sets of authors concluded that strains with MICs ≥ 1 mg/L were associated with clinical failure, a criterion roughly matching the BSAC breakpoint. Dr Howe went on to explore the therapeutic options with other agents and concluded that carbapenems are the treatment of choice for severe infections due to ESBL producers. Non-β-lactams might be used but resistance to aminoglycosides and quinolones is very common, as with the current CTX-M producers in the UK. Finally, he reinforced the view that the efficacy of β-lactamase inhibitor combinations is unclear, with some patients responding and others failing. Many of the current *E. coli* with CTX-M-15 enzyme are resistant to inhibitor combinations (such as piperacillin/tazobactam and amoxicillin/clavulanate), owing to concurrent production of OXA-1-like enzymes, which are inhibitor-resistant penicillinases (Karasik, Woodford & Livermore, unpublished); moreover, except for members of strain A, which may appear ceftazidime-susceptible, most CTX-M-15-producing *E. coli* are very obviously resistant to third-generation cephalosporins.

Control of antibiotic-resistant bacteria is often accomplished by improving infection control practices in hospitals, and there have been many accounts of ESBL-producing Enterobacteriaceae spreading from patient to patient. Dr E. Davies [The National Public Health Service for Wales (NPHS) Microbiology, Cardiff] therefore asked whether ESBL producers are the 'new MRSA'. She doubted whether an ESBL-specific infection control policy was warranted, favouring a generic approach towards control of cross-infection by multiresistant Gram-negative bacteria. She then went on to highlight the confusion that exists because the spread of *E. coli* with CTX-M enzymes on the hospital/community interface may be fundamentally different from the earlier and more localized nosocomial spread of *Klebsiella* spp. with TEM and SHV ESBLs mutants.

One of the most useful interventions probably is the restriction of third-generation cephalosporins, and possibly also of other unrelated antibiotics that may co-select for ESBL producers, such as fluoroquinolones, trimethoprim and non-oxyimino cephalosporins, e.g. cefalexin. Dr Davies went on to review current guidelines, emphasizing the importance of identification of patients and vigorous application of contact precautions, although these seem most relevant in the case of nosocomially spread organisms such as *Klebsiella* spp. Their role is less clear where, as with *E. coli*, the endogenous faecal flora is a substantial reservoir for infections. As already noted, recent work from York, Shropshire and Spain suggests that several different CTX-M ESBLs are established among Enterobacteriaceae in the community faecal flora.[19,20] Dr Davies concluded her review by noting generalized screening would be difficult if community gut carriage of ESBL producers continued to rise.

In conclusion, the meeting described a rapidly evolving—and disturbing—situation, with CTX-M-β-lactamase-producing *E. coli*. The evidence suggests (but does not confirm) a significant new element of antibiotic resistance emerging in *E. coli*, reducing the range of treatment options and exerting an adverse clinical impact. There is at least the potential that serious community-acquired, as well as hospital-acquired, infections will require the use of parenteral antibiotics, principally carbapenems. Delay in starting appropriate antibiotic therapy for urinary tract infections that do not respond to normal treatment may increase morbidity or mortality. It is troubling that CTX-M-producing *E. coli* were not detected by routine local or regional surveillance systems but were found first by a laboratory that unilaterally introduced new methodology. There is evidence of faecal carriage of CTX-M producers, but the clinical relevance and opportunities for intervention are not clear. Should $bla_{\text{CTX-M}}$ genes become widespread in *E. coli*—as ampicillin, trimethoprim and fluoroquinolone resistances did previously—the consequences for treatment, costs and complexity of antibiotic choice could be substantial.

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