ACM/892

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

ACMSF Working Group on Newly Emerging Pathogens
Report on CTX-M ESBL-producing *E. coli*

Issue

1. To update the Committee on work carried out by the ACMSF Working Group on Newly Emerging Pathogens to consider the risk to human health from CTX-M Extended-Spectrum beta-lactamase (ESBL) producing *E. coli* in the food chain.

Background

2. ESBL-producing *E. coli* have been of concern in human medicine for a number of years. ESBLs confer on bacteria the ability to resist a wide range of beta-lactam antibiotics (notably including third generation cephalosporins as well as penicillins).

3. Data from across the world show increasing levels of ESBL producers in human infections caused by members of the Enterobacteriaceae (including *E. coli*). Throughout the 1980s and 1990s ESBLS of the TEM and SHV types were generally the most common ESBLs reported in the UK. Despite being found in (mostly) *Klebsiella* spp. causing relatively large hospital-associated outbreaks, TEM and SHV ESBLs did not become widespread in the community. Rather, they were most often found in patients in intensive care or other specialist settings. However, since the late 1990s there has been displacement of TEM and SHV ESBLs by another family, the CTX-M ESBLs.

4. CTX-M ESBLS are commonly found in *E. coli* and are found in both hospitalised and community patients. The appearance of CTX-M ESBL-producing *E. coli* in recent years as an apparently community-acquired infection has led to the suggestion that healthy humans could become colonised with the organism through the food chain. The discovery of CTX-M ESBL-producing *E. coli* in cattle and their detection in raw chicken samples at retail sale added weight to this hypothesis.

5. Following a request from the Epidemiology of Foodborne Infections group the ACMSF agreed to refer the issue of ESBL-producing *E. coli* in the food chain for consideration by the Working Group on Newly Emerging Pathogens in March 2007.

6. The Group were asked to consider the following questions:

   a) Is there evidence to suggest food is the primary source of CTX-M ESBL-producing *E. coli*?
   b) Is the frequency of these organisms in food different to that in humans?
   c) Could human/food transmission be cyclical?
   d) Should *E. coli* remain the focus of surveillance for ESBLs?
   e) Would a holistic approach to the problem be worthwhile?

   The issues considered by the Group and background information summarising research into ESBL-producing *E. coli* can be found in paper ACM 892a. Membership details for the Working Group can be found in Annex 1.

7. The Working Group met on two occasions: 26th July and 16th November 2007. They considered the issues surrounding and evidence available regarding CTX-M ESBL-producing *E. coli* in relation to disease in people, their occurrence in animals and food and the possibility of humans becoming colonised with the organism via food. As they considered the wider transmission pathways these organisms could traverse they also explored whether a holistic approach to this problem would be worthwhile undertaking.

Outcomes

8. The Group considered the potential transmission pathways for ESBLs. They noted that the CTX-M group of genes are on highly transmissible plasmids which easily move to other bacteria. A number of potential transmission pathways were identified including radiation outwards from healthcare settings and person to person transmission, direct transmission via animal contact and transmission via the food chain. The role of the environment and the potential for cycling of bacteria and genes through different sectors i.e. food, agriculture and sewage was also discussed.

9. While there is well documented nosocomial spread of CTX-M ESBL-producing *E. coli* community based transmission is less well-defined. Members acknowledged that the spread of these organisms in humans in the community may be partly as a result of the changes in demography and more elderly people regularly visiting hospitals and living in care homes.

10. A particular strain of CTX-M ESBL-producing *E. coli*, ST131 carrying the CTX-M-15 ESBL gene is particularly widespread in human infections and

has been found in a number of countries. It is common in the UK and CTX-M-15 is the most common CTX-M ESBL type seen in the UK.

11. In the veterinary sector CTX-M ESBL-producing *E. coli* were first identified in 2004 in the UK in cattle on a Welsh dairy farm. An in-depth investigation of this farm and follow-up over a number of years showed persistence of the CTX-M ESBL in *E. coli* on the farm. Since this initial description and in the period to the end of September 2007, the Veterinary Laboratories Agency (VLA) has identified a further 29 farms where CTX-M ESBL-producing *E. coli* are present in cattle. The majority of isolates from cattle carry the CTX-M-15 ESBL gene, but CTX-M-14, CTX-M-1 and CTX-M-3 have also been found. Only one of the ESBL-producing *E. coli* from cattle examined so far has been serotype O25 (the serotype which has been associated with many human infections) and this isolate was not identical when examined by Pulsed-Field Gel Electrophoresis to isolates previously found in humans. However, sequence typing has not been carried out. The origin or source of the ESBL-producing *E. coli* in cattle on these farms has not been definitively identified.

12. The Group felt that a more in depth comparison of the *E. coli* isolates and the plasmids carrying the CTX-M ESBL genes from cattle to those seen in humans would be helpful.

13. Consideration of the role that food might play in transmission of CTX-M ESBL-producing *E. coli* focussed on foods that might be important in transmission and interventions which could reduce the likelihood of this occurring. Members noted that in a recent small survey of raw chicken in the UK CTX-M ESBL-producing *E. coli* were found. Members suggested that in addition to raw meats foods such as salads and vegetables might also be important. The Group noted that cooking is a key intervention and would minimise any risk of transmission. However, they highlighted that cross-contamination could also be important and correct handling prior to cooking was important in minimising disease. The group agreed there was a need to build on these existing interventions.

14. The group highlighted that there was a lack of data pertaining to the prevalence of CTX-M ESBL-producing *E. coli* in foods, both home produced and imported. However, members felt that isolated surveillance of food commodities without concomitant work on other potential reservoirs and transmission pathways would not be helpful in elucidating the primary pathways responsible for the spread of these organisms.

15. Currently available evidence is insufficient to determine if food is the primary source of CTX-M ESBL producing *E. coli*. Similarly there is a lack of appropriate studies to allow a robust comparison of the frequency of

these organisms in humans and foods and to determine if cyclical transmission is prevalent.

16. As there are a number of potential transmission routes for ESBL-producing *E. coli* and a lack of evidence that would currently allow the relative importance of these pathways to be determined the Group felt that a holistic approach to the problem was required. The Defra Antimicrobial Resistance Co-ordination Group (DARC) and the Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) Committee are considering the problem of ESBL-producing *E. coli* and the Group feel that a cross governmental approach to consideration of this problem would be helpful.

17. ARHAI has recently set-up their Working Group on ESBL-producing *E. coli*. The terms of reference for this group are wide and as well as considering the problem in human medicine the Group also has a remit to examine the role that the veterinary sector may have as a reservoir for ESBL-producing *E. coli* and food may have as a transmission vehicle. Members of the ACMSF Working Group felt it important that the ARHAI subgroup contained representation from a food microbiologist.

18. The Group also briefly considered the evidence regarding the spread of CTX-M ESBLs into other members of the Enterobacteriaceae. The primary concern would be the spread of these genes into *Salmonella* of animal origin. It was reported that the VLA had been screening all Salmonella isolates recovered from animals since 2001 for ESBLs and none had been detected. This programme is ongoing. A recent study conducted by the Health Protection Agency and the VLA examined cephalosporin resistant *Salmonella* isolated between 1992 and 2003. Of 278,308 non typhoidal salmonellas 106 were resistant and 14 of these carried CTX-M ESBL genes. All isolates were from cases of human infection and three were associated with foreign travel. The other 11 cases were domestically acquired but the source of infection could not be determined. To date there are no published reports of CTX-M ESBLs being identified in *Salmonella* spp. of animal origin from the UK; the situation is different on the continent of Europe, where CTX-M ESBLs have been detected in a number of *Salmonella* serotypes, including *S.* Enteritidis and *S.* Virchow. Screening of *Salmonella* from food surveys and human cases for cephalosporin resistance is ongoing and all cephalosporin resistant isolates will be investigated for the presence of ESBLs.

In summary

1) The Group identified the need for a holistic approach as key to defining and understanding the problem. The Group recommended cross-

government working to address the holistic context. It was noted that both DARC and ARHAI are considering ESBL-producing *E. coli,* with ARHAI having a specific working group on the topic.

2) As the Group felt that the new ARHAI subgroup was best placed to co-ordinate a cross-governmental approach on this subject they recommended that ARHAI subgroup on ESBL-producing *E. coli* take this forward.
3) The Group identified the need for information on the presence and relevance of ESBL-producing *E. coli* in food to determine if infection is linked to food and how important this may be as a transmission route.
4) Surveillance undertaken needs to be across a variety of sectors as isolated surveillance of food commodities would not be beneficial. Human, sewage and livestock carriage of ESBL-producing *E. coli* also required investigation.
5) The Group recommended exploring ways to use available surveillance/routine sampling to help identify ESBL-producing *E. coli*.
6) The Group recommended work to establish the prevalence of ESBL-producing *E. coli* in non-health care settings (the wider community).
7) The Group recognised that current interventions promoted through the FSA Foodborne Disease Strategy would reduce the likelihood of transmission of ESBL-producing *E. coli* through a foodborne route and recommended that the FSA continue to build upon these. The 4C's principles (cooking, cleaning, chilling and avoiding cross-contamination) form part of the food hygiene campaign to reduce food poisoning and are key in controlling transmission of microorganisms found in foods. The Group recognised that cooking and pasteurisation will destroy *E. coli* irrespective of whether the organisms are resistant to antibiotics or not. However, the Group also highlighted avoiding cross-contamination as a key intervention.

Secretariat
March 2008

Annex 1:

Members of the Working Group on Newly Emerging Pathogens

Chair

Professor Paul Hunter

Members

Professor Sarah O'Brien
Dr Rick Holliman
Dr David Brown
Mr Alec Kyriakides
Dr David Livermore
Professor Peter Hawkey
Mr Chris Teale

Assessor

Mr Stephen Wyllie

Secretariat

Dr Lucy Foster
Ms Gael O'Neill
Mr Adekunle Adeoye
Miss Sarah Butler

Terms of reference

The terms of reference of the Newly-Emerging Pathogens Working Group are to :-

- gather intelligence on a continuous basis in order to facilitate the rapid identification of potential threats to UK consumers from exposure to newly-emerging or re-emerging pathogens through food chain exposure pathways;

- assess the significance of newly-emerging or re-emerging foodborne pathogens; and

- undertake any risk assessment work on newly-emerging or re-emerging pathogens referred to it by the Advisory Committee on the Microbiological Safety of Food;

in each case consulting other experts as necessary.

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

NEWLY-EMERGING PATHOGENS WORKING GROUP

CTX-M ESBL-producing *E. coli*

This paper was considered by the Group in July 2007

Introduction

1. Bacterial resistance to antimicrobial drugs is an ongoing problem and the situation is ever-changing. A newly emergent cause of concern in the UK is resistance to b-lactam antibiotics through the production of Extended Spectrum b-lactamase enzymes (ESBLs) by members of the Enterobacteriaceae, particularly *E. coli*. ESBLs have been known since the 1980s but *E. coli* producing the CTX-M type ESBL have become particularly problematic in causing human disease in the UK in the last few years. The problem of human infection with organisms resistant to antibiotics is thought to be largely due to antibiotic usage in human medicine. However, the recent appearance of CTX-M ESBL-producing *E. coli* as a community-acquired infection has led to the suggestion that this could be due to healthy humans becoming colonised with the organism through the food chain.

CTX-M ESBL-producing *E. coli* in people

2. In September 2005 the HPA published a report looking at the increasing problem of infections caused by multi-drug resistant ESBL-producing *E. coli* in England (HPA, 2005). They reported that since 2003, new highly resistant strains of *E. coli* had become widespread in England and parts of Northern Ireland. These strains of *E. coli* were able to destroy a large number of common antibiotics, making the infections they cause very difficult to treat. Although ESBL-producing *E. coli* were first recognised in the 1980s, the new strains seen in the UK from 2000 onwards produce a particular type of ESBL, the CTX-M type, which is able to break down a wider range of antibiotics (see Annex 1 for more detail). These strains were unrecorded in the UK prior to 2000 but have spread rapidly, particularly since 2003, causing infections such as urinary tract infections in hospital patients as well as those treated in the community (See Annex 2, Section 1 for more detail).

3. A key development is that these strains are being found in cases that appear to have arisen in the community whereas, in the past, they tended to be found in people who had been hospitalised over lengthy periods (See Annex 2, Section 1

for more detail). It is worth noting that the robustness of the definition of "community cases" is open to question and some of these may have had prior inpatient healthcare exposure that could have led to colonisation by CTX-M ESBL producing *E. coli*. However, the HPA report recommended that, if carriage of CTX-M ESBL-producing *E. coli* is found to be common in the community, food (including imported food) should be investigated as a potential source.

4. The present situation in the community is not known. We are not aware of any studies in the UK where carriage of CTX-M ESBL-producing *E. coli* in the healthy population has been assessed and know of only a single study assessing the presence of these in diarrhoeic stools obtained from individuals in the community - 1.9% of stool specimens were found to be positive. However, there have been studies of healthy individuals in Spain where carriage of CTX-M ESBL-producing *E. coli* has been demonstrated (See Annex 2, Section 2 for more detail).

5. A number of studies have shown that prior antibiotic therapy is the most commonly identified risk factor for disease with ESBL producing Enterobacteriaceae and among community patients prior hospitalisation was also an important risk factor. No studies have investigated food as a risk factor and risk factors for carriage only (no disease) have also not been investigated (See Annex 2, Section 3 for more detail).

CTX-M ESBL-producing *E. coli* in food animals

6. CTX-M ESBL-producing *E. coli* have been found in various food animals in different parts of the world. In the UK the first report of CTX-M ESBL-producing *E. coli* from animals was in cattle in North Wales in the autumn of 2004. In this case, the CTX-M type was one that is seen less commonly in clinical cases in the UK (CTX-M-14) and the type of *E. coli* it was found in was different from human clinical isolates. The second case in UK livestock to date was discovered in the south of England in July 2006 and also involved cattle. In this case the CTX-M type was one commonly seen in human clinical cases (CTX-M-15), although again the type of *E. coli* it was found in is not one that is similar to those from humans (See Annex 2, Section 4 for more detail).

7. The VLA began screening veterinary isolates of *E. coli* for CTX-M ESBLs in 2006. The last update given (April 2007) indicated that CTX-M ESBL producing isolates had been found in cattle on 18 farms in regions across the UK: Cheshire, Wiltshire, Somerset, Hertfordshire, Dorset and Wales.

CTX-M ESBL-producing *E. coli* in food

8. There has been little work assessing whether CTX-M ESBL-producing *E. coli* are present in food. However, in late 2006 the presence of CTX-M ESBL-producing *E. coli* in food was reported in Spain. A small unpublished survey of raw chicken carried out by the Royal Shrewsbury Hospital and the HPA laboratory at Birmingham Heartlands Hospital has found CTX-M ESBL-producing

E. coli in 17/129 (13.2%) samples of fresh and frozen chicken fillets from supermarket chains. No large-scale surveys for the presence of these bacteria in meat or milk in the UK have been carried out yet although screening for the organisms has recently been included in the ongoing FSA survey B18020: UK-wide survey of raw red meat at retail sale (See Annex 2, Section 5 for more detail).

We ask the ACMSF *ad hoc* group on Emerging Pathogens to consider the following:

1. Is there evidence that food is a primary source of CTX-M ESBL producing *E. coli*? How do we address any lack of evidence in this field?
2. Is the frequency or occurrence of CTX-M ESBL-producing *E. coli* in food at a level that is different to the levels in humans?
3. Could transmission be cyclical?
4. Should *E. coli* remain the focus of investigation of ESBLs in the food chain or should other Enterobacteriaceae also be included?

Annex 1: Classification, Evolution and Spread of β-lactamases

β-lactamases are classified into four groups, Ambler classes A-D (Jacoby & Munoz-Price, 2005). Prior to the introduction of third generation cephalosporins the majority of resistance to β-lactam antibiotics by organisms belonging to the family Enterobacteriaceae was due to TEM and SHV enzymes (Class A). The spectrum of activity of these early enzymes included amoxicillin and other penicillins as well as early generation cephalosporins. They did not confer resistance to third generation cephalosporins.

After introduction of the third generation cephalosporins in the 1980s new "extended spectrum" β-lactamases were quickly discovered in the Enterobacteriaceae. When sequence data was obtained it became evident that most of these were mutations of the classical TEM and SHV enzymes (Livermore & Hawkey, 2005). However, others, such as the CTX-M enzymes, appeared to be completely new. Since the initial discovery of β-lactamases in Gram negative bacteria hundreds of different variants have been characterised (Jacoby, 2006).

TEM & SHV ESBLs

The extension of the activity of TEM and SHV β-lactamases to include the third generation cephalosporins marked the first appearance of the ESBL phenotype. Throughout the 1980s and 1990s these were generally the most common ESBLs reported in the UK. Despite causing rare, relatively large hospital associated outbreaks, TEM and SHV ESBLs did not become widespread in the community. Rather, they could be viewed as an occasional nuisance, most often found in nosocomial *Klebsiella* spp. from patients in intensive care or other specialist settings (Livermore & Hawkey, 2005).

CTX-M ESBLs

The CTX-M ESBLs represented a previously unseen type of □-lactamase. These were first described in the late 1980s in Japan and Germany and seen in the explosive spread of human infection caused by cephalosporin resistant *Salmonella* in South America. During the 1990s and 2000s these have spread rapidly across the world (Bonnet, 2004). Investigations have shown these enzymes to be almost identical to a chromosomally encoded β-lactamase found in *Kluyvera* spp - a commensal or environmental member of the Enterobacteriaceae. It appears that mobilisation of these genes from the chromosome of *Kluyvera* spp. onto transmissable plasmids may be responsible for the appearance and spread of these genes among other Enterobacteriaceae (Olsen *et al*., 2005; Rodriguez *et al*., 2004). Phylogenetic studies of the relationship between CTX-M enzymes has clearly shown 5 major clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25) defined on the basis of differences in the nucleic acid sequence of the gene. Members of each cluster

will only differ by small numbers of nucleotide differences - in many cases only by a single nucleotide (http://www.lahey.org/studies/other.asp).

Group	1	2	8	9	25
Members (CTX-M-)	-1; -3; -10; -11; -12; -15; -22; -23; -28; -29; -30; -32; -33; -36; -54; UOE-1	-2; -4; -5; -6; -7; -20; -31; -44 (TOHO-1); FEC-1	-8; -40	-9; -13;-14 (-18); -16; -17; -19; 21; -24; -27; -45 (TOHO-2); -46; -47; -48; -49; -50;	-25; -26; -39; -41
Potential Origin	*Kluyvera ascorbata*	*Kluyvera ascorbata*	*Kluyvera georgiana*	*Kluyvera georgiana*	Unknown

(Canton & Coque, 2006; http://www.lahey.org/studies/other.asp)

Multi-resistant strains

For an organism to be classed as multi-resistant it should display resistance to at least 3 different classes of antimicrobial drugs and the majority of isolates of CTX-M ESBL-producing *E. coli* meet this definition. In the UK, these organisms are only reliably susceptible to parenteral carbapenems (e.g. imipenem which is administered intravenously rather than orally), nitrofurantoin (use of this drug is limited to uncomplicated urinary tract infections) and fosfomycin (which is not readily available in the UK) (Livermore & Hawkey, 2005). Not all strains are resistant to all drugs – resistance profiles are variable with the most common resistances associated with ESBL production being to the aminoglycosides and the quinolones.

Spread of ESBL genes

In the majority of isolates examined the genes for ESBL production are encoded on plasmids and are often found embedded in Type I integrons or other highly transferable genetic elements. Recent work suggests that the plasmids encoding CTX-M genes belong to the same incompatibility groups as those of early antibiotic resistance plasmids. These may encode resistances to some or all of a range of other antibiotics including the aminoglycosides, tetracycline, sulphonamides or fluoroquinolones and this probably helps to facilitate the dissemination of the CTX-M genes because of co-selection processes (Canton & Coque, 2006). Also, in many cases these plasmids are broad host range and self transmissible which means they are often transferable across closely related species or genera. There are many examples in the literature of these types of plasmids transferring between *E. coli*, *Enterobacter, Citrobacter*, *Klebsiella*, *Salmonella* and *Shigella*. The review by Canton & Coque (2006) gives a detailed summary of the current understanding of the molecular mechanisms driving the spread and evolution of the CTX-M genes.

Annex 2: CTX-M ESBLs in humans, animals and the foodchain

1. Human Infections with CTX-M ESBL producing Enterobacteriaceae

The majority of publications relating to infections with ESBL producing Enterobacteriaceae relate to infections seen in hospital patients. In this population reports from across the world describing infection with Enterobacteriaceae show a similar picture - increasing levels of ESBL producers and increasing frequency of CTX-M genes. All show broadly the same picture – since the late 1990s there has been displacement of TEM and SHV ESBL genes by CTX-M genes and the resistances have moved out from primarily nosocomial pathogens such as *Klebsiella* into *E. coli*. There are differences across the world with different CTX-M types being predominant in different areas. In South America CTX-M-2 dominates. In the Far East a range of types are seen with CTX-M-2 and CTX-M-3 dominant in Japan (although CTX-M-14 and CTX-M-15 have also been isolated). China, Taiwan and Korea all report CTX-M-3 as dominant although CTX-M-9, CTX-M-13 and CTX-M-14 are also seen. CTX-M-14 has become increasingly dominant in China recently with reported prevalence rates of up to 10%. CTX-M-15 was reported from India in 2000 and is very common there. In Eastern Europe, CTX-M-3 was dominant in Poland although more recently CTX-M-15 has been seen. Reports from Latvia indicate CTX-M-2 and CTX-M-5 are most common and CTX-M-4 has been seen in Russia. In all areas these genes are being seen more frequently in *E. coli* isolated from patients with serious infections – often Gram-negative septicaemia (Bonnet, 2004; Canton & Coque, 2006; Livermore *et al*., 2007).

<u>Recent experiences in Europe</u>

Data collected from Southern Spain between 1995 and 2003 indicated an overall prevalence rate of 1.7% for ESBL production among *E. coli* but this increased significantly from <0.36% before 1999 to 4.8% in 2003 (Romero *et al*., 2005). Various studies have found CTX-M-9 and CTX-M-14 to be the predominant types in Spain (Valverde *et al*., 2004; Hernandez *et al*., 2005; Miro *et al*., 2005; Solozano *et al*., 2006). Data collected from ten laboratories distributed throughout Italy in 1999 showed a prevalence rate of 1.9% for ESBL production among *E. coli* from hospital inpatients and none detected from outpatients. A similar study conducted in 2003 showed a prevalence of 4.4% among *E. coli* isolates from inpatients and 1.9% in outpatients. In 1999 the majority of isolates carried TEM or SHV genes but by 2003 55% carried a CTX-M gene. All CTX-M determinants were of group 1, with CTX-M-15 and CTX-M-1 being the most prevalent variants (Luzarro *et al*., 2006).

In the UK the first description of a CTX-M ESBL producer was in 2000 (Alobwede *et al*., 2003). By mid-2003 there were numerous reports of ESBL producing *E. coli*, many from UTIs in community patients. The majority of these carried the CTX-M-15 gene but CTX-M-14 and CTX-M-9 were also found. A systematic

survey undertaken in 2004 in South East England examined consecutive cephalosporin-resistant Enterobacteriaceae from 16 laboratories. This study showed that *E. coli* with CTX-M enzymes were now the most frequent cephalosporin-resistant Enterobacteriaceae in this region, despite the fact that such strains had not been seen in the UK before 2000 (HPA, 2005).

Emergence of CTX-M ESBL-producing *E. coli* in the community

The major factor that is driving the suggestion of food as a possible source of ESBL-producing *E. coli* infections in humans is the rise of community cases. A number of reports have detailed UTIs and other (more serious) infections that have arisen in the community and are caused by ESBL producing *E. coli*, particularly those carrying the CTX-M enzymes. Reports on this subject have been published from the Middle East, Canada, Europe and the UK among others (Pitou *et al*., 2005). Unlike hospital patients, who could easily become infected nosocomially with *E. coli*, community patients with *E. coli* infections are usually infected from their own resident gut flora. Although a proportion of community onset patients may have had recent healthcare exposure where they could have acquired ESBL-producing *E. coli* evidence is emerging that at least some proportion of these patients have no recent inpatient healthcare exposure (Woodford *et al*., 2006). This suggests that there may be a non-nosocomial transmission route or routes for ESBL producing *E. coli*.

2. Human carriage of CTX-M ESBL producing *E. coli*

There have been few studies that directly aimed to determine asymptomatic gut carriage of ESBLs. A study carried out in Spain in 2003 (Valverde, 2004) was the only one to actually examine stool specimens from healthy volunteers. 108 specimens were examined, 4 (3.7%) of these individuals were found to be carrying ESBL-producing *E. coli* and two of these carried CTX-M genes. Other studies have screened diarrhoeal stool specimens for their presence. This is a surrogate measure for asymptomatic carriage as these organisms are not known to cause diarrhoeal disease. During another study in Spain carried out in 2001/2002 (Miro *et al*., 2005), 1321 stool specimens were examined and a prevalence rate of 3.3% was found - 42/44 isolates were *E. coli* and 75% of isolates produced CTX-M enzymes. Another Spanish study carried out in 2003 (Mesa *et al*., 2006) is probably the most comprehensive look at this issue. They examined stool specimens from 948 individuals presenting to the A&E department of a major hospital and found an overall prevalence rate of 6.6% - none of these individuals came from a nursing home of healthcare centre or was involved in outbreak of foodborne disease. It is not clear whether they were suffering from diarrhoeal disease. They also examined stool specimens from 544 patients involved in 61 outbreaks of foodborne disease and found ESBL carriers present in 19 outbreaks (31.1%). In the outbreaks where ESBL carriage was detected between 4.4% - 66.6% of individuals were colonised. Five samples of human sewage were analysed from 2 separate sewage treatment works and

all five were positive for ESBL producing *E. coli*. A UK study carried out in York (Munday *et al*., 2003) examined 1000 consecutive stool specimens submitted for diagnosis of diarrhoeal disease. 565 of these specimens were submitted from general practice (community) patients. The overall prevalence of ESBLs was 1.9% with a prevalence of 1.6% in community patients and 2.2% in hospital patients. All the community isolates were *E. coli* and CTX-M-9, -14 and -15 were found.

3. Risk factors for infection and /or carriage with CTX-M ESBL producing *E. coli*

A number of studies have been conducted and show prior antibiotic therapy is the most commonly identified risk factor for disease with ESBL producing Enterobacteriaceae – among community patients prior hospitalisation was also an important risk factor (Pena *et al*., 2005; Brigante *et al*., 2005) . No studies have investigated food as a risk factor and risk factors for carriage only (no disease) have also not been investigated.

4. Carriage of CTX-M ESBL-producing *E. coli* by Food Animals

A small number of reports have appeared in the literature over the past 3-4 years detailing carriage of CTX-M ESBL-producing *E. coli* by animals. The frequency of these reports is increasing with a number published during 2006.

The earliest report, from Spain in 2003, concentrated on isolation of these organisms from pet animals (Brinas *et al.*, 2003a). This group also reported isolating these organisms from healthy chickens (Brinas *et al.*, 2003b). These reports were quickly followed by a Japanese report in 2004 detailing isolation of these organisms from cattle (Shiraki *et al.*, 2004). *E. coli* strains producing CTX-M-2 were isolated from 6 (1.5%) of 396 cattle faecal samples and 2 (0.7%) of 270 surface swabs of cattle carcasses.

The Spanish have been most active in this area. In a further report in 2005, 459 *E. coli* isolates obtained from sick animals were tested for the presence of CTX-M ESBLs – 7 were positive (1.5%) (Brinas *et al.*, 2005). The CTX-M positive isolates were obtained from pigs, poultry and cattle and CTX-M-1, CTX-M-9, CTX-M-14 and CTX-M-32 enzymes were found. In addition, 60 isolates of *E. coli* obtained from chickens at slaughter yielded 5 (8.3%) carrying CTX-M genes – predominantly CTX-M-14. Two reports in 2006 detail the isolation of ESBL-producing *E. coli* from farmed rabbits, poultry and pigs. ESBL-producing *E. coli* were isolated from samples from 2/10 rabbit farms, 8/10 pig farms and 10/10 poultry farms (Mesa *et al*., 2006). The ESBL types were predominantly CTX-M-9 and CTX-M-14 from the poultry farms, CTX-M-1 from the pig farms and CTX-M-9 from the rabbit farms (Blanc *et al.*, 2006).

A recent study conducted in Hong Kong isolated ESBL-producing *E. coli* from 1.4% of 734 rectal or cloacal swabs from cattle, ducks, pigs, chickens, ducks, geese and pigeons. Isolates were obtained from pigs, cattle and a pigeon. The CTX-M types were CTX-M-3, CTX-M-13, CTX-M-14 and CTX-M-24 (Duan *et al*., 2006).

In the UK, the first report on this subject was in 2004 (Teale *et al.*, 2005; Liebana *et al*., 2006). Two isolates of ESBL-producing *E. coli* were recovered from diarrhoeic calves that were being reared on a dairy farm in Wales. These isolates carried the CTX-M-14 gene and a recent update (Oct 2006) from the VLA indicates these organisms have persisted and spread. In 2005 24% of cattle on the farm were colonised in 2006 this has risen to 56%. It should be noted that prevalence on this farm has increased despite selection pressure (i.e. antibiotic use) decreasing. The second case in UK livestock to date was discovered in the south of England in July 2006 and involved the death of several calves. The situation however differed to that of the first case as these had been part of a group of calves, sourced from 4 different premises and housed together at a veterinary referral centre, rather than a working farm. This *E. coli* isolate was found to possess a gene encoding CTX-M-15. In 2006 the VLA has rolled out screening of isolates of *E. coli* obtained from clinical samples for the presence of ESBLs. CTX-M ESBL producing isolates had been found in cattle on 18 farms in regions across the UK: Cheshire, Wiltshire, Somerset, Hertfordshire, Dorset and Wales. Not all the isolates have yet been fully characterised but isolates from 7 farms are carrying the CTX-M-15 gene and isolates from 2 farms are carrying the CTX-M-14 gene.

5. CTX-M ESBL-producing *E. coli* in Foods

There is little information in the published literature regarding the presence of ESBL-producing *E. coli* in food. In a very recently published Spanish study 3/738 (0.4%) foods were positive (Mesa *et al*., 2006). It should be noted that 80% of the foods in this study were cooked and the positives were from two salad samples and a sample of cooked chicken. In addition, there has been a small unpublished study in the UK examining chicken portions at retail sale. This study examined 129 chicken portions and overall 17 (13.2%) were positive for ESBL producing *E. coli*. Of the samples 65 were definitely of British/Irish origin, 40 samples were of unknown origin, 4 were from either Brazil, Poland or France, 10 were from Brazil, 2 from the Netherlands and 4 from Poland. Only 1 sample of British/Irish origin was positive for an ESBL-producing *E. coli* – this carried the CTX-M-1 gene. 6 of the unknown origin samples were positive and were carrying CTX-M-1 (1), CTX-M-2 (1), CTX-M-8 (1) and CTX-M-14 (4). Half of the Brazilian samples were positive and all carried CTX-M-2 as did both the Dutch samples and 3/4 samples from Brazil/Poland/France (Ensor *et al*., 2007).

There has been more work examining *Salmonella* isolates obtained from foods for the presence of CTX-M ESBLs. Studies from Denmark (Aerstrup *et al*., 2005) the Netherlands (Hasman *et al*., 2005) and Greece (Politi *et al*., 2005) have all recorded CTX-M genes is Salmonella isolates obtained from poultry products.

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