This publication was withdrawn on 15 February 2023 because it's out of date.
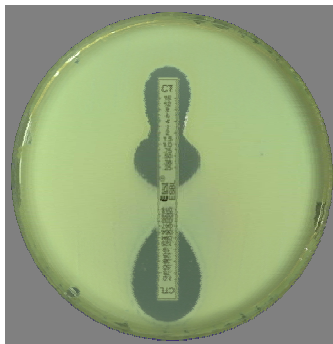
ESBLs – A threat to human and animal health?

Etest for ESBLs

Cefotaxime

Cefotaxime + clavulanate

CTX-M-32
CTX-M-15
CTX-M-12
CTX-M-3

2 3 4 7

Report by the Joint Working Group of DARC and ARHAI

CONTENTS

SCOPE AND PURPOSE

The use in human medicine of third generation cephalosporins (3GCs, e.g. cefotaxime, ceftazidime, ceftriaxone) is generally believed to have been a major selective force in the emergence of extended-spectrum beta (β)-lactamases (ESBLs). Whilst initially confined to enterobacteriaceae causing hospital acquired infection, the emergence and spread particularly in the community of *Escherichia coli* (*E. coli*) strains producing CTX-M ESBLs is a very serious challenge to effective therapy of infections caused by all Gram negative bacteria.

The small but gradually increasing use of 3GCs and quinolones in food animal production (for details see VMD, 2010) may be linked to the recent emergence of ESBLs in bacteria associated with cattle, poultry and pigs.

A joint working group of the Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) and the Defra Antimicrobial Resistance Coordination (DARC) Group was set up to address these concerns and has produced the following report which:

- reviews the current state of knowledge with regard to the occurrence, distribution, identification and ecology of ESBL-producing bacteria;

- considers the causation and development of the problem;

- assesses the impact on human and animal health;

- identifies the areas in which collaborative working and research could lead to a greater understanding, a reduction or a slowing of the rate of increase in the occurrence of ESBL Producing Coliforms (ESBLPCs); and

- provides a range of recommendations for public health and animal health.

CONCLUSION

Antibiotic resistance has been identified as a priority area by ARHAI and DARC as it affects our ability to treat infections. Our knowledge and understanding of the complex nature of the issues involved continues to improve.

Local, regional, national, and international epidemiology has been examined but data are incomplete and changes can occur rapidly. Thuis advice has to be modified as evidence develops in the future.

ARHAI and DARC will continue to monitor new national and international developments in this area and provide advice to the Department of Health on this on-going challenge.

LAY SUMMARY

Introduction

The Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) and the Defra Antimicrobial Resistance Coordination (DARC) Group commissioned a joint working group to report on the growing problem and concerns related to the development of bacterial resistance to 3rd generation cephalosporins (3GCs) by ESBLPCs (extended spectrum beta (β) lactamase producing coliforms. ESBLs are enzymes that destroy penicillin's and cephalosporins). These antibiotics are used in the treatment of severe infections in humans and, to a lesser extent, in animals.

The working group produced the following report which:

- reviewed the current state of knowledge with regard to the occurrence, distribution, identification and ecology of ESBL-producing bacteria;

- considered the causation and development of the problem;

- assessesed the impact on human and animal health;

- identified the areas in which collaborative working and research could lead to a greater understanding, a reduction or at best a slowing of the rate of growth of the occurrence of ESBL Producing Coliforms (ESBLPCs); and

- provided a range of recommendations for public health and animal health.

Background

Antibiotics kill or interfere with the growth of micro organisms, especially bacteria, and are used to treat and prevent infections in man and animals. However, resistance to antibiotics is becoming more common and the risk to both human and animal health posed by ESBLPCs is of great concern to those involved with human and animal health.

Penicillins and cephalosporins (the β–lactams) are one of the most commonly used groups of antibiotic used in human medicine. The heavy use of these antibiotics is believed to have been a selective force in the emergence of resistance. The emergence and spread of resistance particularly into *E. coli* (a common cause of gut and urine infection) is a very serious challenge.

The small but gradually increasing use of 3GCs in food animal production may be linked to the recent emergence of ESBLs in bacteria associated with cattle, poultry and pigs. It is thought that emergence of ESBL bacteria in food producing animals may present a risk of resistant strains being transmitted to humans through the food chain.

Antibiotic resistance is more common in some countries than others (usually those where use of antibiotics is less strictly controlled). ESBLs have now been found in all parts of the world in a variety of coliforms, but until recently, they were relatively uncommon in *E. coli*. Increased international travel means that individuals colonised or infected with resistant bacteria in one country can spread them to another country very quickly.

In practice, an infection caused by bacteria that are resistant to antibiotics usually used to treat that infection, may fail to respond to treatment. This can result in a longer illness and a greater risk of death.

Findings and recommendations

The report has identified the key importance of characterisation of these bacteria; the transfer pathways of spread; the surveillance both medical and veterinary; the therapy in medical and veterinary practice; the control options and clear measurements of outcomes and research as being crucial to understanding and controlling the problem. The following recommendations have been made:

Characterisation - Clinical (medical and veterinary) laboratories have a key role in identifying and reporting prevalence of these organisms. Reference laboratories are critical for typing and epidemiological purposes.

Transfer pathways - These bacteria spread rapidly among humans and there is evidence of spread among animal populations. A greater understanding of the size of potential reservoirs, the drivers of transmission and the transmission pathways by which they spread (including the relative importance of each pathway) is crucial. Further work is required in these areas.

Surveillance - Human and animal surveillance of these organisms has identified that there is an on-going increase in resistance and spread. At present, there is no national established system to collect and publish the data on the use of antibiotics in hospitals, community and veterinary practice. Further work is required on molecular characterisation.

Therapy - Appropriate national guidelines on antimicrobial prescribing, and good antibiotic stewardship should be followed and should include education and training of staff. Critically important agents used for treating human infections (e.g. carbapenems) should not be licensed for use in animals.

Control options - Clear guidance on good infection control practices, to include recognition and management of an outbreak of infection, should be based on national guidance and be appropriate for the settings and should include education and training of staff and appropriate written information for patients and the public.

Outcome measures - There should be agreed, clear outcome measures, which should include infection rates and monitoring of antibiotic prescribing in medical and veterinary settings.

Research gaps - Research should be carried out into methodologies for control. These included surveillance of organisms in gut carriage in humans, domestic animals and imported foods. It also recommended that work should be carried out on rapid testing and detection of these organisms. The effectiveness of novel therapies, use of antibiotics, routes of transmission between human and animal cycling, including waste should be evaluated.
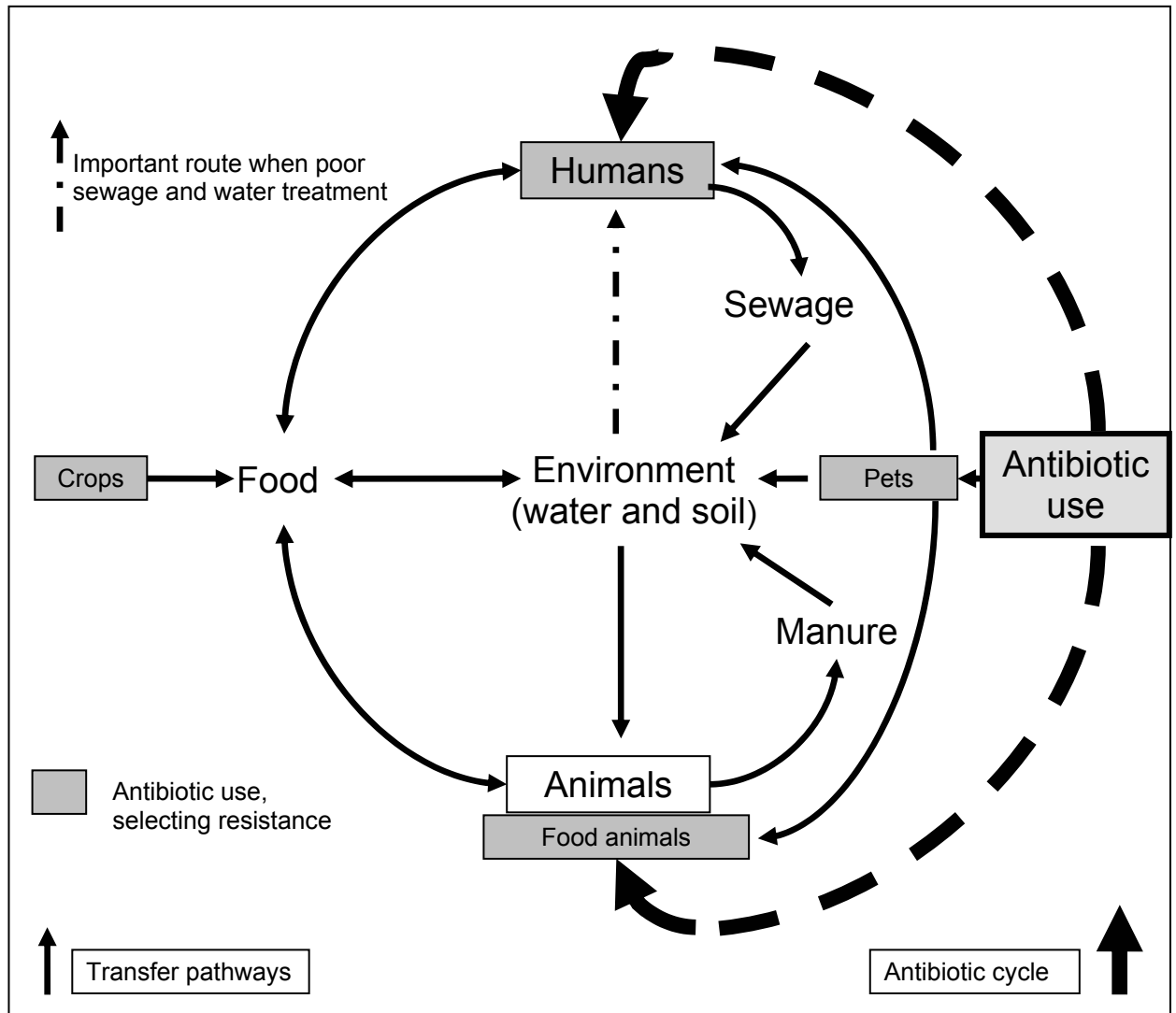
Principal routes outlining the transfer pathways for antibiotic resistance genes between humans, animals, food and the environment.

INTRODUCTION

Development and use of third generation cephalosporins

Third generation cephalosporins (3GCs) (e.g. cefotaxime, ceftazidime, ceftriaxone) were developed in the 1970s and introduced into human medicine in the early 1980s. They represented a huge therapeutic advance for the treatment of infections caused by multi-resistant Gram-negative bacteria such as *Klebsiella* spp*., Escherichia coli* and *Enterobacter* spp. They were developed to be resistant to hydrolysis by the highly prevalent (≥40% in *E. coli*) TEM-1 β-lactamase and rapidly became the treatment of choice for serious infections caused by Gram-negative bacteria. Because of their low toxicity, high specific activity and ease of production, they were used intensively worldwide for conditions such as community-acquired pneumonia where the Gram-negative activity was not an essential feature of their therapeutic activity.

Emergence of plasmid mediated resistance to 3GC (TEM/SHV ESBLs)

Shortly after the introduction of cefotaxime, transferable plasmid-mediated resistance was noted, initially in Germany, with the identification of a mutant β-lactamase SHV-2 that conferred high-level resistance to all of these agents (Knothe et al. 1983). The following year, in France, a similar mutated variant of TEM β-lactamase (TEM-3) was observed (Philippon et al. 1989) and this led to the adoption of the term extended-spectrum β-lactamase (ESBL). Some variants of TEM- and SHV-ESBLs (e.g. SHV-5, SHV-12, TEM-26, TEM-12, etc.) became globally distributed, but these were largely confined to *Klebsiella* spp. in nosocomial hospital settings, especially intensive care units. TEM- and SHV-derived ESBLs continue to occur throughout the world but at a markedly lower prevalence than CTX-M ESBLs, which emerged in the 1990s.

Rise in importance of CTX-M ESBL

In the early 1990s, a different type of ESBL gene (bla_{CTX-M}) was identified amongst isolates of ESBL-producing coliforms (ESBLPCs) from Europe. This has become distributed on plasmids in enterobacteriaceae as a result of mobilisation from the environmental bacterium *Kluyvera* spp. (Poirel et al. 2002). Following the first reports of CTX-M ESBL in France and Germany, these genes were found to be common in Argentina and Israel and subsequently in China (Chanawong et al. 2002) and India (Ensor et al. 2006). The resistance rate to 3GCs in *E. coli* is a broad indicator of the occurrence of ESBLs.

Most European countries show marked variation and have comparable or higher rates of resistance compared to the UK (Diagram1.1).

Diagram 1.1. Resistance to third generation cephalosporins in *Escherichia coli* in different European countries in 2006. Data from EARSS.
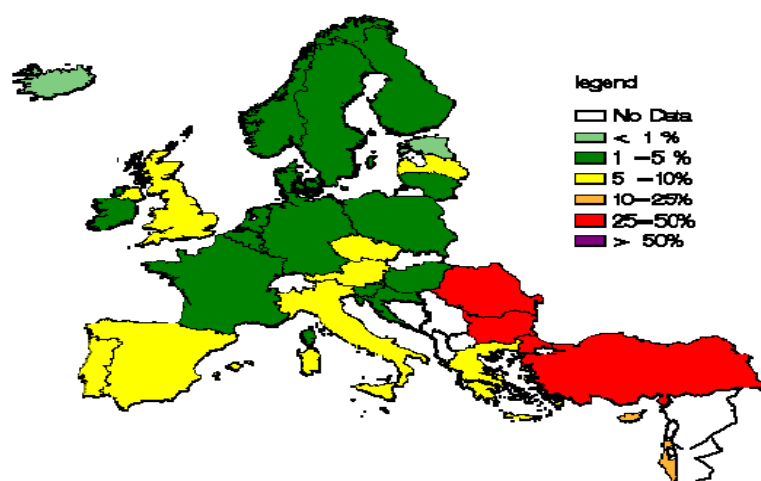
Distribution of genotypes of CTX-M ESBL

Five groups of genotypes have been recognised amongst CTX-M, which probably derived from separate genetic mobilisations from different species of *Kluyvera* followed by further mutations within the group. CTX-M-15 was originally dominant in India (Ensor et al. 2006) and CTX-M-14 in China (Chanawong et al. 2002). Two specific genotypes from groups 1 and 9 have become very widely distributed in the world with CTX-M-2 being the dominant genotype in South America. In the UK, following sporadic reports in 2002 to the Health Protection Agency (HPA) national reference laboratory, multiple reports of CTX-M-15-producing *E. coli* were identified in NHS diagnostic laboratories across the country from 2003. The number of isolates has progressively increased and this is reflected in reports from the BSAC bacteraemia survey (Diagram 1.2). The wide distribution of $bla_{CTX-M-15}$ is thought to be linked to the successful clone of *E. coli*, ST-131 (Clermont et al. 2009). This lineage serotype O25:H4 is widely distributed, from Japan and Korea, across India and the Middle East to Europe, North Africa and Canada, with occasional representatives – particularly in Northern Ireland – having other ESBL types and plasmids.
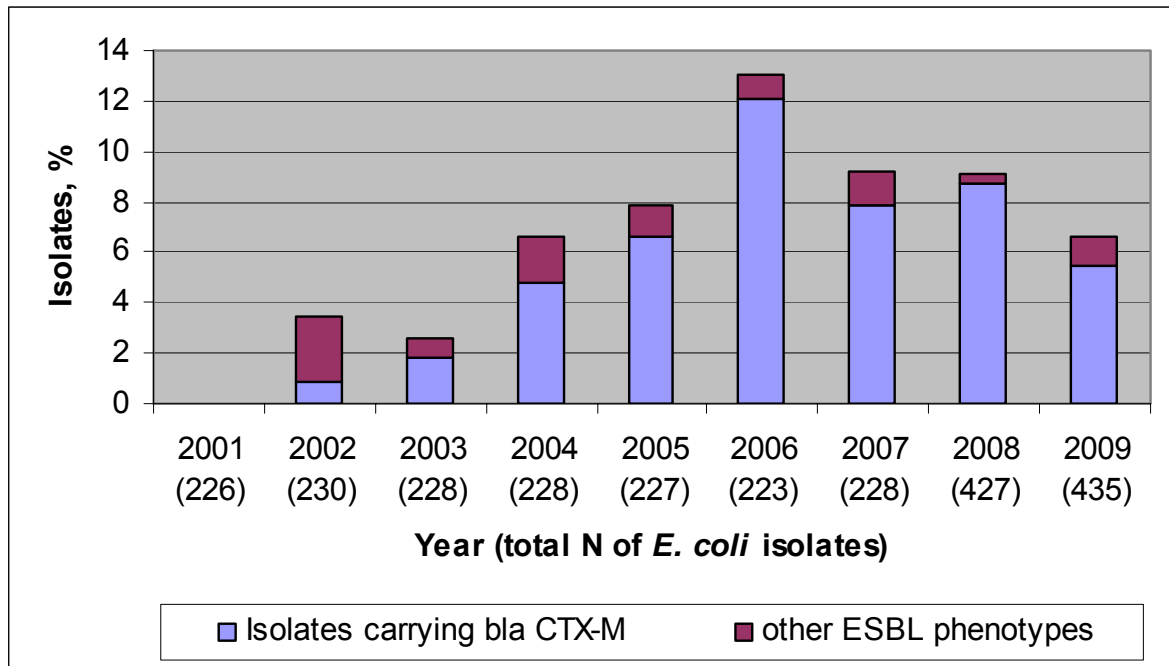
Diagram 1.2 BSAC UK bacteraemia susceptibility survey data for *E. coli* exhibiting an ESBL phenotype (www.BSAC.org)

In addition, it has been found that travellers to countries with high rates of ESBLPC (e.g. Egypt, India) readily acquire asymptomatic faecal carriage (Tham et al. 2010). More recently CTX-M-14 has emerged as the second most frequently encountered genotype worldwide and this is the same pattern as has been observed in both Canada and the USA. India, however, has a highly restricted genotype distribution of only CTX-M-15 (Diagram 1.3).
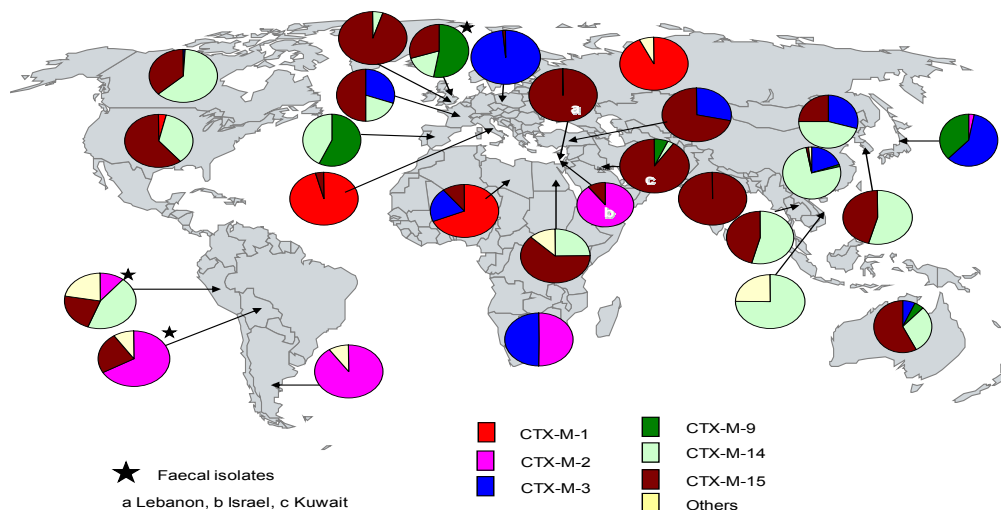
Diagram 1.3 Global distribution of CTX-M genotypes (Hawkey & Jones 2009)

CTX-M ESBLs in humans and animals

The epidemiology of nosocomial infection by *Klebsiella* spp. was elucidated in the late 1970s for gentamicin-resistant strains (Casewell et al. 1977). The patient's gastrointestinal tract was the source and spread was via touch contact and secondary environmental contamination with faeces. The appearance and spread of TEM/SHV ESBL-producing *Klebsiella* spp. followed the same pattern (Chanawong et al. 2001; Hibbert-Rogers et al. 1995). More recently, CTX-M producing *E. coli* have become the most frequently encountered ESBLPCs in human medicine and CTX-M has become the dominant ESBL type in *Klebsiella* and other members of enterobacteriaceae.

ESBL-producing *E. coli* have been late to appear in veterinary medicine but in the UK it is evident that both CTX-M-14 and CTX-M-15 are established in some dairy herds and both surveys and sporadic reports have identified them in other food animals. Although the majority of cases in humans have some connection either with hospitals or care homes (Rooney et al. 2009), CTX-M-producing *E. coli* are encountered in some patients who have had no previous contact with healthcare facilities. This observation has led to concern that the bla_{CTX-M} genes are becoming rapidly established in commensal *E. coli* in the UK and indeed CTX-M-15 has been identified to be frequently located on *Inc*FII plasmids which are both transmissible and frequently carried by members of enterobacteriaceae (Hawkey & Jones 2009).

ESBLs and the environment

There have also been concerns expressed that humans colonised with CTX-M-producing *E. coli and Klebsiella* spp. will release large quantities of these bacteria into the environment which will then enter into the biosphere via sewage, soil and water, leading to colonisation of food animals, see Diagram 1.4 (Gaze et al. 2008). The appearance of CTX-M-14 and CTX-M-15 ESBLPCs in food animals in the UK may have arisen from exposure to human sources of these bacteria as they were first noted in human medicine (Teale 2010). This process can be seen as a 'reverse zoonosis'.

The diversity of CTX-M types, together with some of the molecular features of the ESBL plasmids seen in cattle support the notion of exposure of these animals to diverse environmental sources of ESBL-producing *E. coli,* which may have ultimately been of human origin. The situation is perhaps different in pigs and poultry where CTX-M-1 has been the predominant ESBL, possibly reflecting spread within animal production systems and pyramids following the initial introduction of CTX-M-1 from an unknown source.
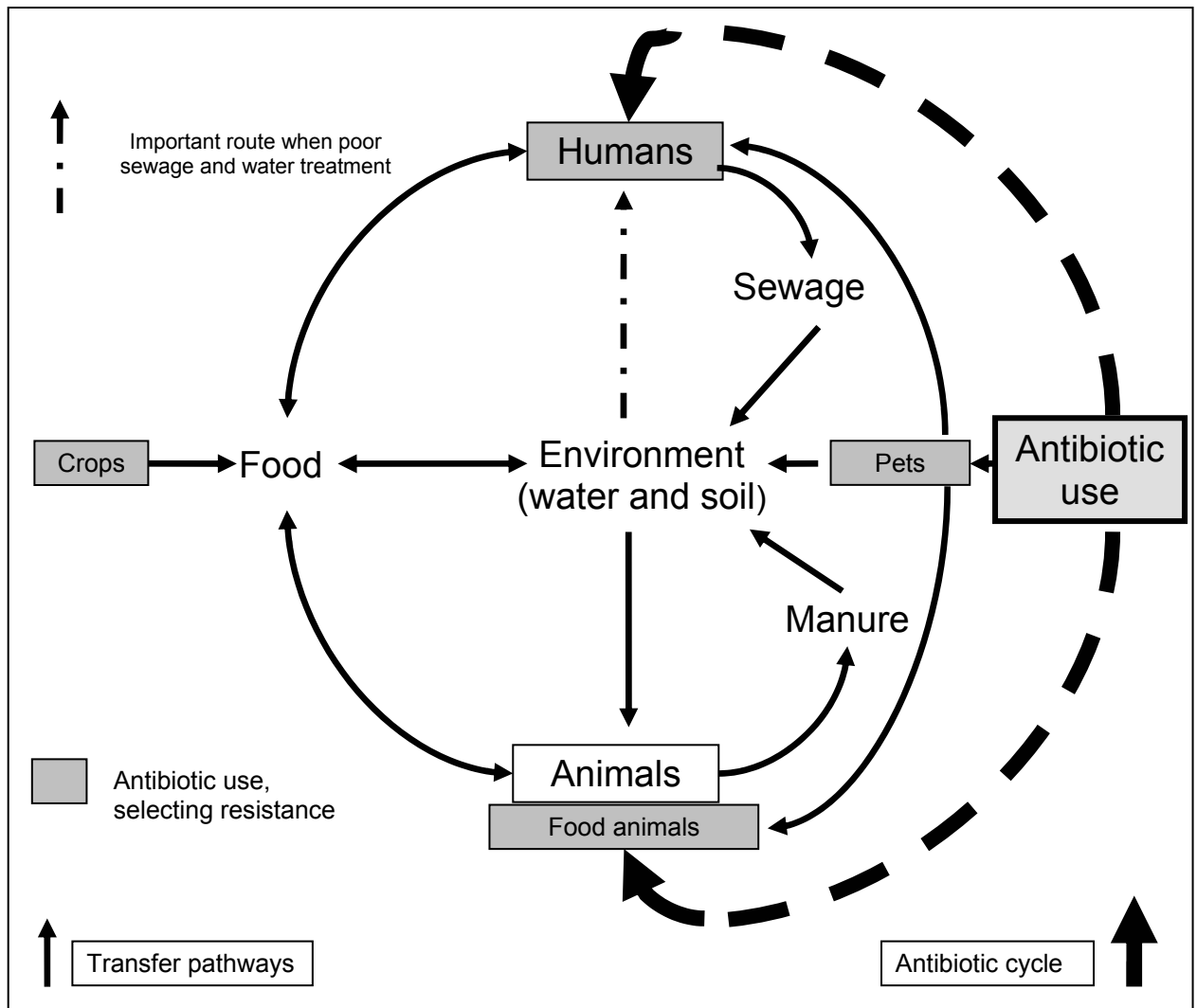
Diagram 1.4 Principal routes outlining the transfer pathways for antibiotic resistance genes between humans, animals, food and the environment

Selection of ESBLs by antibiotics

The influence of selective antibiotics is considerable and much work in human medicine has shown that 3GCs and ciprofloxacin (many of the clones of *E. coli* carrying CTX-M are resistant to ciprofloxacin) select for ESBL-producing Enterobacteriaceae (Rodriguez-Bano et al. 2010; Wener et al. 2010). The role of antibiotic selection in veterinary medicine, now that these genes have been introduced into food animals, is of concern (Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use European Medicines Agency (EMEA) London UK 2009). The recent rise in the use of 3GCs such as ceftiofur to treat mastitis in cattle has been suggested to be one possible driver for the increase in prevalence in bovines (Grove-White & Murray 2009). Ceftiofur is also sometimes administered in combination with Marek's disease vaccine to young chicks (not broilers) in some sectors of the poultry industry to counter problems of septicaemia. This practice and the administration of ceftiofur in conjunction with *in ovo* vaccines is reported to occur in other European countries and in the Netherlands it is thought that this has

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contributed to the selection and vertical transmission of ESBLs in the poultry production pyramid (MARAN 2008, 2010).

The emergence of carbapenemases in Enterobacteriaceae

The spread of extended-spectrum β-lactamases (ESBLs) and quinolone resistance in *E. coli* and *Klebsiella pneumoniae* has increased reliance on carbapenems in severe infections. They are used as definitive therapy – i.e. once the pathogen has been identified as an ESBL producer and are used as immediate empirical treatment in settings and cases where ESBL producers are likely based on the local epidemiology and the individual patient's risk factors. Such use is justifiable, in severely ill patients, in whom the risk of death roughly doubles if the empirical antibiotics prove to be ineffective owing to resistance. Most ESBL producers are broadly resistant to antibiotics other than carbapenems, meaning that there are few alternatives to this strategy.

Any emergence of carbapenem resistance in Enterobacteriaceae is therefore extremely disturbing. Until recently such resistance was extremely rare and most of the few cases that were seen involved ESBL producers that had became impermeable through porin loss, restricting entry of the antibiotic molecules into the bacteria. Whilst troublesome in individual patients these organisms failed to spread, probably because their impermeability impaired their fitness. Carbapenem-destroying β-lactamases, called 'carbapenemases' were seen in non-fermenters, principally a few *Pseudomonas aeruginosa* isolates and widespread clones of *Acinetobacter* spp., but showed little tendency to spread from these organisms into Enterobacteriaceae (Diagram 1.5).
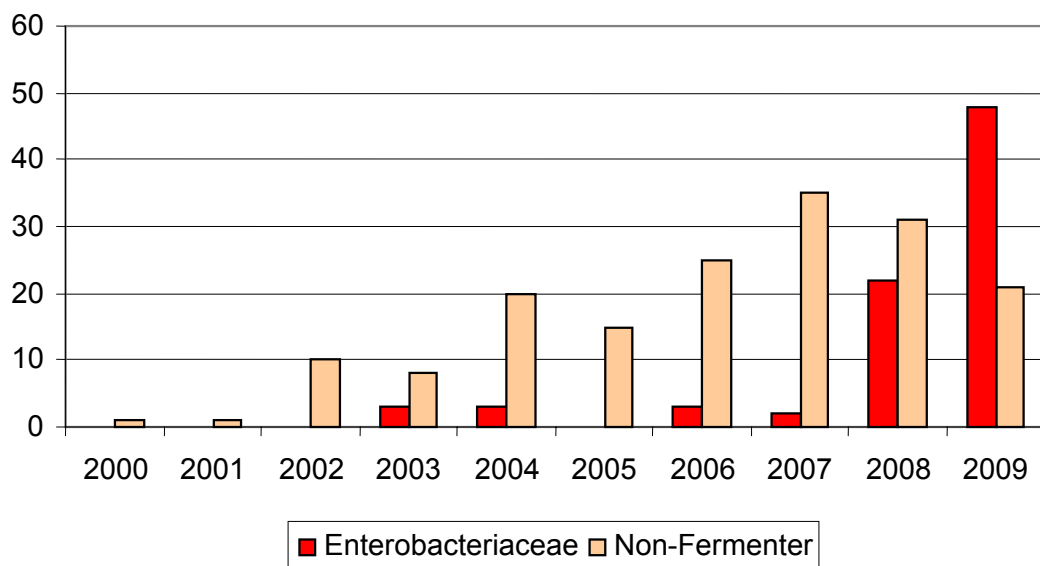
Diagram 1.5 Carbapenemase producers: ARMRL referrals, HPA

Now, however, carbapenemases are starting to appear in Enterobacteriaceae, especially *K. pneumoniae*. A variety of types are circulating internationally becoming prevalent in some countries and are starting to be seen at low frequency in the UK (Diagram 1.6). Some UK source patients have been

previously hospitalised in countries where producers are prevalent, others have not.
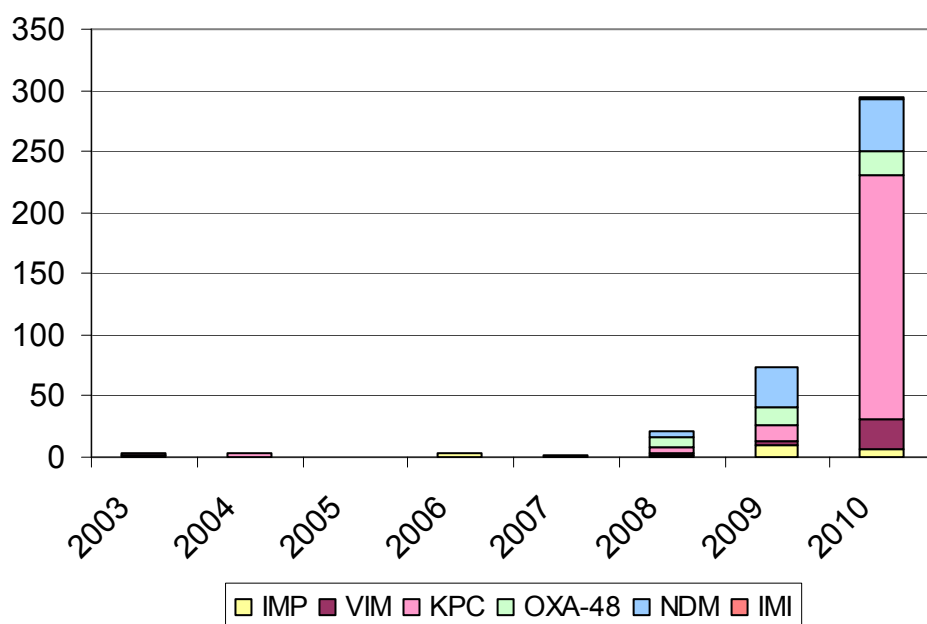
Diagram 1.6 Carbapenemase-producing Enterobacteriaceae: ARMRL referrals from UK labs, HPA 2010

Two types – KPC and NDM – deserve special mention. Clonal sequence type ST258 *K. pneumoniae* with KPC carbapenemase have spread across the US, Israel and, latterly, Greece. Several producers have been referred to the Health Protection Agency (HPA) from UK laboratories. They include three isolates from patients around Glasgow with no obvious overseas link along with several from patients hospitalised in the countries of the eastern Mediterranean. NDM-1 is a much more recent discovery, first recorded in 2008 from *E. coli* and *K. pneumoniae* from a patient transferred from India to Sweden. During 2009, however, NDM has become the commonest acquired carbapenemase among isolates referred to the HPA's Antibiotic Resistance Monitoring and Reference Laboratory. It is plasmid-mediated, and recorded in multiple species. Around half the UK source patients have a history of medical exposure in India or Pakistan, where the enzyme is circulating widely. The nature of the exposure ranged from cosmetic surgery to renal transplantation.

The worldwide increase in the use of carbapenems would be expected to drive resistance and this has begun to occur in the USA for KPC carbapenemase (Hawkey & Jones 2009) and in India for NDM metallo-carbapenemases (Hammerum et al 2010).

Most carbapenemase producers are extremely multiresistant, including to all β-lactams, ciprofloxacin and most, sometimes all, aminoglycosides. Polymyxins and tigecycline generally retain activity. The HPA is urging hospitals to be extremely vigilant for carbapenemase producers, especially among patients with prior medical exposure in the eastern Mediterranean or the Indian Subcontinent. Laboratories are urged to refer suspected producers to the HPA's Antibiotic

Resistance Monitoring and Reference Laboratory (ARMRL) for investigation and to take the utmost care to prevent the spread among patients.

Aside from confirming mechanisms and typing producers, the HPA is urgently reviewing the activity of old, discarded antibiotics against producers and is liaising with pharmaceutical companies to investigate the activity of novel agents.

Carbapenems are not licensed for veterinary use, and we are unaware of producers from animal sources.

1 CHARACTERISATION

1.1 Introduction

Because of their clinical importance, both clinical and veterinary diagnostic laboratories should be able to recognise ESBL producers and strains with high-level AmpC β−lactamases.

- The standard strategy to identify the presence of ESBLs and other potent β−lactamases (Figure 1.1) is to screen first for resistance to one or more indicator oxyimino cephalosporins. This test should be applied to *all* Enterobacteriaceae ('coliform') isolates of clinical significance and those collected for surveillance purposes.

- Isolates found resistant to oxyimino cephalosporins should then have synergy tests done to confirm whether an ESBL, AmpC enzyme, or other mechanism is present. It may be appropriate to proceed to molecular characterisation of specific β-lactamase genes for epidemiological purposes.

All purified, clinically significant, enterobacteriaceae isolates

↓

Susceptibility test with cefotaxime *and* ceftazidime, or with cefpodoxime

↓	↓
Resistant to ANY tested cephalosporin	**Susceptible to ALL tested**
↓	↓
Do synergy tests to identify mechanism	No further action
↓	
Synergy between cephalosporin and clavulanate ⟶	ESBL-positive-avoid cephalosporin therapy
Synergy between cephalosporin and cloxacillin or boronic acid ⟶	High level AmpC-avoid cephalosporins except 4th generation agents
Resistant also to carbapenems investigation ⟶	Send for reference lab

Figure 1.1 Flow diagram for detection of isolates with ESBLs or high-level AmpC activity

These screening and confirmatory methods are described in detail below. It should be noted that this approach involves a 72-hour lag between the clinical specimen being taken and the mechanism being identified. If the patient is on

inappropriate therapy in the interim and the infection is serious then the mortality risk is increased (Schwaber & Carmeli 2007). The rapid methods, described later in this section, give a result in 24–48h post-specimen and, though less used, may represent a significant improvement. The adoption of lower breakpoints by EUCAST and CLSI facilitate rapid recognition of the potential presence of an ESBL for the clinical management of the patient. It is important that suspect isolates are also *all* tested using an ESBL screening test for confirmation (Kahlmeter 2008).

1.2 Screening tests

Ideally, *all* Enterobacteriaceae isolates should be tested with *both* ceftazidime and cefotaxime as this achieves the best sensitivity and specificity in ESBL detection. (http://www.hpa-standardmethods.org.uk/ documents/qsop/pdf/ qsop51.pdf). If only a single cephalosporin can be accommodated in the testing scheme, then the best choice is cefpodoxime, which has good sensitivity for detection of ESBL producers but poorer specificity than testing both cefotaxime and ceftazidime. Many clinical laboratories test cefotaxime and ceftazidime against isolates from hospitalised patients but test cefpodoxime against those from community-acquired urine samples. Isolates resistant to *any* screening cephalosporin should then continue to the confirmatory tests, outlined below. It is also desirable to test cefoxitin, and either cefepime or cefpirome, since AmpC hyperproducers are resistant to cefoxitin, but are intermediate or susceptible to these fourth-generation cephalosporins, whereas ESBL producers show the converse pattern (Livermore et al. 2001).

BSAC/EUCAST breakpoints should be followed (see http://www.bsac.org.uk or http://www.eucast.org). Care on these aspects is needed when automated systems are used as these often default to CLSI criteria which until recently used higher breakpoints; users should ensure that they are set to follow EUCAST breakpoint values see Table1.1.

	MIC breakpoint (mg/L)			Disc load μg	Zone Breakpoint (mm)		
	R >	I	S <=		R >=	I	S >=
Cefepime	8	2–8	1	30	26	27–31	32
Cefotaxime	2	2	1	30	29	–	30
Cefpirome	1		1	30	24		25
Cefpodoxime[a]	1		1	10	19		20
Ceftazidime	8	2–8	1	30	17	18–29	30

[a]Organisms with cefpodoxime zone diameters of <20 mm have a substantive mechanism of resistance. Organisms with zone diameters of 21–25 mm are uncommonly ESBL producers and may require further investigation. From http://www.bsac.org.uk

Table 1.1 Breakpoints for cephalosporins vs Enterobacteriaceae

A number of chromogenic selective media (Brilliance ESBL, ChromID ESBL and CHROMagar CTX) are now commercially available to detect ESBLPC which exhibit excellent sensitivity and specificity as well as a high negative predictive value (Huang et al. 2010) CHROMagar CTX has the advantage of incorporating an inhibitor of AmpC and it has been evaluated using veterinary specimens

(Randall et al. 2009). It is not recommended that all clinical specimens are plated but the media be used for outbreak investigation (e.g. screening faeces for ESBLPC carriage) and surveillance.

1.3 Confirmatory phenotypic tests

1.3.1 Confirming ESBLs

Confirmation of ESBL production is based on synergy between oxyimino cephalosporin(s) and clavulanic acid. For *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* it is best to perform these tests using whichever cephalosporin(s) the isolate proved resistant to in the screening test. For *Enterobacter* spp. *Citrobacter freundii*, *Serratia* spp. and *Morganella morganii* it is best to use cefepime or cefpirome, as these are least affected by any chromosomal AmpC activity potentially induced by clavulanate. This activity can mask the inhibition of any ESBL (http://www.hpa-standardmethods.org.uk/documents/ qsop/pdf/qsop51).

Cephalosporin/clavulanate synergy tests can be performed by the double disc method, with a cephalosporin 30 μg disc 20–30 mm apart from an amoxicillin-clavulanate 20+10 μg disc (Figure 1.3a). It is convenient to flank an amoxicillin-clavulanate disc with ceftazidime and cefotaxime which also allows the addition of a cefepime disc to detect ESBL in the presence of AmpC. But this is prone to false-negative results if the disc separation is suboptimal; unfortunately, the definition of 'optimal' varies with the test strain. Better alternatives are to:

- compare the zones of 'combination' discs (presently available from Mast Group or Becton Dickinson, with similar 'tablets' available from Rosco) containing the cephalosporin (30 μg) with and without clavulanic acid (10 μg) (Figure 1.3b). A zone diameter >5 mm in the presence of the inhibitor indicates ESBL production; or

- use Etest® strips (bioMerieux) with a cephalosporin at one end and the same cephalosporin plus clavulanic acid at the other, taking an MIC reduction of >8-fold by the clavulanate to indicate ESBL (Figure 1.3c).

Combination discs are less costly but care must be taken not to mix those from old and new batches, and to run controls. The following are available from the NCTC:

- *E. coli* NCTC13351 TEM-3 positive- broad-spectrum ESBL phenotype.
- *E. coli* NCTC13352 TEM-10 positive- ceftazidimase phenotype.
- *E. coli* NCTC13353 TEM-3 positive- cefotaximase phenotype.

These should give accurate results. Additionally, *E. coli* NCTC10418 or ATCC25922 should serve as a negative control, with equal zones regardless of the clavulanate. Whilst all the CTX-M β-lactamases hydrolyse cefotaxime only, some genotypes can hydrolyse ceftazidime on account of possessing an Asp 240 Gly amino acid substitution as in CTX-M-15 (Poirel et al. 2003) and CTX-M-25 (Munday et al. 2004a), whereas CTX-M-14, for instance, lacks such activity.

This can give an indication as to the genotype seen but is invalidated if for instance a plasmidic AmpC is also present which itself confers resistance to ceftazidime. Performing the cephalosporin/clavulanate synergy test with cefepime will permit the ready identification of an ESBL in the presence of AmpC. Adding a cefoxitin disk also confirms the likely presence of AmpC β-lactamase and can be combined in a 'four-disc synergy test' (Figure 1.3d).

1.3.2 Confirming AmpC (high-level chromosomal or plasmid-mediated).

High-level AmpC should be suspected in isolates resistant to cefotaxime, ceftazidime, cefpodoxime and cefoxitin but *not* cefepime and cefpirome. Confirmatory tests are less developed than for ESBLs and are marketed as 'for investigational purposes only'.
They include:

- combination discs (MastGroup) of cefotaxime 10 μg with and without cloxacillin 200 μg, with a zone expansion of >5 mm by cloxacillin indicating AmpC;

- Etests (bioMerieux) with cefotaxime at one end and cefotaxime + cloxacillin at the other, with a $\geq$8-fold reduction in cefotaxime MIC by the cloxacillin taken to indicate an AmpC enzyme;

- tablets (Rosco) containing either cloxacillin 500 μg or boronic acid (unspecified content). These are placed 20–30 mm from a cephalosporin disc and significant expansion of the cephalosporin zone is taken to indicate AmpC activity.

1.3.3 Isolates giving anomalous phenotypic tests

The above tests allow the accurate categorisation of most oxyimino-cephalosporin-resistant Enterobacteriaceae but the following caveats should be noted.

- A few isolates owe resistance to other mechanisms, e.g. some *Klebsiella. oxytoca* (never *K. pneumoniae*) hyperproduce their chromosomal K1 β-lactamase (Livermore, Winstanley & Shannon 2001). These are resistant to cefuroxime, piperacillin, tazobactam and aztreonam, borderline to cefepime and cefotaxime and susceptible to ceftazidime. Little synergy is seen between cephalosporins and clavulanate or in boronic acid-based tests and none with cloxacillin.

- Some isolates give negative synergy tests because they are impermeable. These also test resistant to ertapenem and, in extreme cases, other carbapenems (Woodford et al. 2007). Such isolates account for most carbapenem resistance among clinical Enterobacteriaceae but a growing minority of carbapenem-resistant Enterobacteriaceae isolates (mostly *Klebsiella* spp.) have true carbapenemase including KPC, metallo (IMP, VIM or NDM) or OXA-48 types (Poirel et al. 2007). Since they are of major public health concern, carbapenem-resistant Enterobacteriaceae from clinical

material should be submitted to the HPA's Antibiotic Resistance Monitoring and Reference Laboratory.

- Even using cefepime or cefpirome, ESBL tests have poor sensitivity (but good specificity) for *Enterobacter* spp., especially if AmpC is concurrently hyperproduced. Some producers are only revealed by molecular testing.
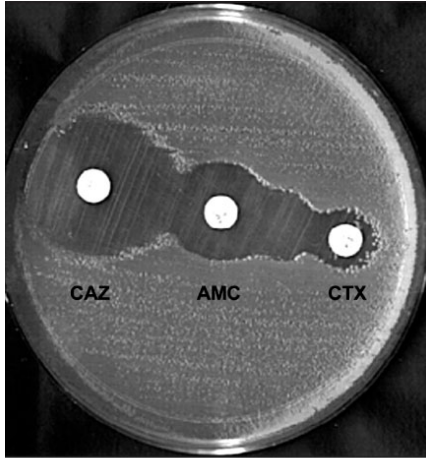
(a) double disc synergy
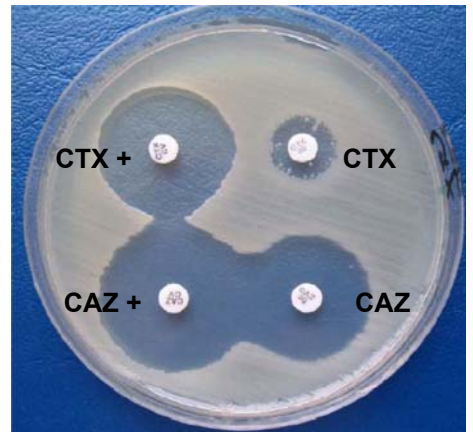
(b) combination disc method
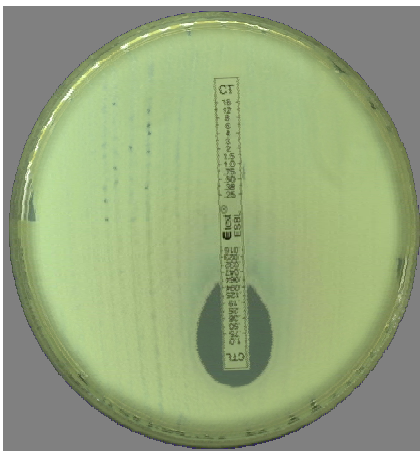
(c) Etest
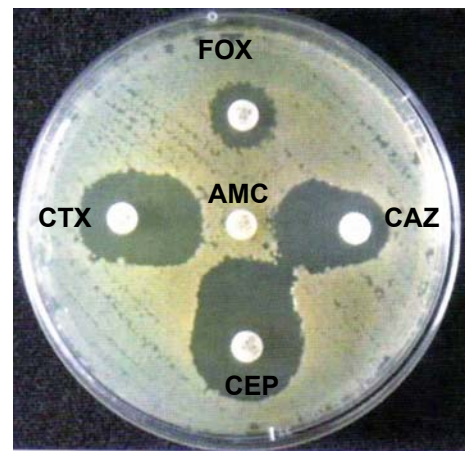
d) four-disc synergy test

Figure 1.3 ESBL detection by: (a) double disc synergy, (b) combination disc method applied to a strain producing CTX-M-14 genotype with poor ceftazidimase activity, (c) Etest cefotaxime/cefotaxime+clavulanate, (d) four-disc synergy test for ESBLs in an AmpC background.

Cefotaxime (CTX); ceftazime (CAZ); co-amoxiclav (AMC); cefoxitin (FOX); cefepime (CEP); Cefotaxime + clavulanate (CTX+); ceftazime + clavulanate (CAZ+).

1.4   Rapid ESBL detection

Various strategies can be used to identify ESBL producers and AmpC hyperproducers more rapidly than the two-step procedure outlined above. Some of the methods that are appropriate for routine diagnostic laboratories are outlined below.

1.4.1   Automated systems

Screening and confirmation are run in parallel on automated systems such as the Vitek (bioMerieux), Phoenix (Becton Dickinson) or Microscan Walkaway (Siemens). These either incorporate a specific ESBL test on their card or interpret the presence of an ESBL or AmpC enzyme based on overall phenotype. Numerous analyses suggest good performance (Afzal-Shah et al. 2001; Livermore et al. 2002; Robin et al. 2008; Snyder et al. 2008; Thomson et al. 2007) though this can vary with the particular card composition and in relation to the behaviour of locally-prevalent clones (Farber et al. 2008). Great care should be taken to perform validation assays with known resistance types and controls if custom cards are to be used.

1.4.2   Specific media

Selective agar for ESBL producers (e.g. ChromID ESBL, bioMerieux) can be used, particularly for identifying faecal carriage. An evaluation of this medium suggested that it had 94% sensitivity, though a minority of AmpC hyperproducer strains and *Pseudomonas aeruginosa* isolates also grew, giving false positive results (Glupczynski et al. 2007; Reglier-Poupet et al. 2008). There is now an alternative medium available (Brilliance ESBL agar, Oxoid) which gives comparable results (Huang et al. 2010). CHROMagar CTX has the advantage of incorporating an inhibitor of AmpC and has been evaluated using veterinary specimens (Randall et al. 2009).There is no comparable medium available for AmpC producers.

1.4.3   Colorimetric tests

The Cica βTest, marketed by MastGroup, comprises a chromogenic oxyimino cephalosporin, HMRZ-86, which is applied to paper strips variously impregnated with inhibitors of AmpC enzymes (boronic acid), metallo-β–lactamases (mercaptoacetic acid) and ESBLs (clavulanate). The cephalosporin turns from yellow to red on hydrolysis. If this colour change is seen without any inhibitor, the inhibitor combinations are tested in turn, first seeking MBLs, then ESBLs and, lastly, AmpC types, with the first positive result for inhibition being counted. Used alone, as would be the case, if working with the purified cultures available 24h after a clinical specimen is taken, and tested blind, the method correctly identified 85%, 77% and 72% of ESBL, MBL and AmpC producers, respectively (Livermore et al. 2007). These proportions would be increased if the method was used together with phenotypic data.

1.5 Quality control

Appropriate control strains should be used to control all tests. External quality assurance data from UKNEQAS suggests that ESBLPCs with MICs of 3GCs close to the breakpoint are not reliably detected by all laboratories. When a *K. pneumoniae* strain producing SHV-3 was distributed only 94% of laboratories reported it resistant to cefotaxime despite a reference MIC of 8–16 mg/L which falls in the resistant range for both BSAC and EUCAST. When applying an ESBL test 99.4% of laboratories reported a positive result demonstrating the value of such tests. In a distribution of *Enterobacter cloacae* producing a CTX-M ESBL, only 92% reported a positive ESBL test demonstrating the difficulty of ESBL detection in an AmpC producer background.

1.6 Molecular characterisation

Definitive identification of β-lactamases is the role of reference and academic rather than general diagnostic laboratories. CTX-M types are now the most prevalent ESBLs and, for these, there is: (i) a generic PCR to detect the presence of bla_{CTX-M}, (Saladin et al. 2002); (ii) PCR to identify the five major CTX-M groups (Woodford et al. 2006; Xu et al. 2005); (iii) a reverse-line blot methodology that allows discrimination between some of the commoner genotypes within groups, for example between CTX-M-3 and M-15 (Group 1) and between M-9 and M-14 (Group 9) (Ensor et al. 2007); and (iv) dHPLC heteroduplex analysis which allows potential identification of all individual genotypes (Xu et al. 2007). Definitive identification requires sequencing.

The identification of TEM and SHV variants is more difficult than that of bla_{CTX-M} genotypes because: (i) there are over 100 closely related variants in each of these families and (ii) isolates often concurrently harbour both classical and ESBL variants for example all *K. pneumoniae* have a chromosomal SHV enzyme as well as an ESBL variant and different plasmid copies in the same cell may encode different gene variants. Cloning as well as sequencing is needed for definitive identification. However, recently, a commercially available ligation-mediated amplification method combined with microarray analysis has become available (Cohen et al. 2010). A non-commercial high density spotted micro-array with greater discrimination has also recently been described (Leinberger et al. 2010).

Chromosomal AmpC types are normally identified in terms of the producer species, though sequence variation exists even within species. Plasmid-mediated AmpC types represent chromosomal gene escapes (Table 1.2.). They can be grouped by multiplex PCR (Perez-Perez & Hanson 2002) though, once again, definitive identification requires sequencing.

Bacterial source	Plasmid-mediated AmpC genes
C. freundii	CMY-2 to -7; LAT-1,3,4; BIL-1
Enterobacter spp.	ACT-1; MIR-1
Aeromonas spp.	FOX-1 to -5
Aeromonas spp.	MOX-1, -2; CMY-1, -8 to -11
M. morganii	DHA-1, -2
H. alvei	ACC-1

Table 1.2 Bacterial chromosomal source of plasmid-mediated AmpC β-lactamases

As noted elsewhere in this report, many of the human isolates of *E. coli* with CTX-M-15 enzyme in the UK belong to the international ST131 clone (Coque et al. 2008b; Nicolas-Chanoine et al. 2008). Its UK-prevalent members include five major strains, A–E. along with further minor variants (Woodford et al. 2004; Lau et al. 2008). Among these, strain A is the most prevalent. There is a PCR assay to identify members of the ST131 clone (Pitout et al. 2009) as well as the *IS*26 *bla*CTX-M-15 link that is characteristic of strain A, though no longer exclusive to it (Woodford et al. 2004).

1.7 Recognition of carbapenemases producers

Carbapenems (imipenem, meropenem, ertapenem and doripenem) are invaluable for the treatment of infections due to multi-resistant gram-negative bacteria, including those with extended-spectrum β-lactamases. Carbapenem-resistant Enterobacteriaceae remain rare but are emerging. Their transmission characteristics and pathogenesis resemble those of more sensitive Enterobacteriaceae, but the infections are much more difficult to treat.

Carbapenem resistance in Enterobacteriaceae can involve:

Combinations of ESBL or AmpC and porin loss: Porin loss is often unstable and may impose a fitness cost, meaning that these strains rarely spread. Ertapenem is particularly affected.

Acquired carbapenemases: These are the more serious risk and are beginning to spread in Enterobacteriaceae already resistant to multiple antibiotics. Several types occur (Table 1.3), some with close geographic associations. They belong to three molecular classes: IMP, VIM and NDM types are metallo enzymes, with zinc at the active site; whereas KPC and OXA-48 belong to separate non-metallo families. Other carbapenemases (SME, IMI, SPM) occur, but are very rare.

	Geographic distribution	**Molecular epidemiology**
NDM	Widespread in Enterobacteriaceae (esp. *K. pneumoniae* and *E. coli* in India and Pakistan. Imported to UK via patients with travel/ hospitalisation/ dialysis in India and Pakistan.	Diverse strain types in UK. Plasmid spread among strains and species is more important than clonal spread among patients. Nevertheless, there have been a few cases of cross-infection in the UK.
VIM	Scattered globally, endemic in Greece; mostly *K. pneumoniae*. Sometimes imported to UK via patients previously hospitalised in Greece.	Plasmid spread among strains is more important than clonal spread of producer strains.
IMP	Scattered worldwide; no clear associations.	Mostly plasmid spread.
KPC	USA since 1999. Prevalent also Israel, and Greece; outbreaks elsewhere in Europe. Some UK cases imported via patient transfers, but local spread in NW England.	Some plasmid spread: mostly among *K. pneumoniae,* occasionally to other Entero-bacteriaceae. Also clonal spread, including global *K. pneumoniae* ST258 lineage.
OXA-48	Widespread *K. pneumoniae* in Turkey; Mid-East and N. Africa. Some import to UK and an outbreak in one London renal unit 2008-9.	Mixture of plasmid and clone spread.
IMI, SME, SPM	SPM common in *P. aeruginosa* in Brazil; others extremely rare, SME in *Serratia* and IMI in *Enterobacter*, fewer than 20 recorded cases over 20 years.	National distribution of a *P. aeruginosa* clone with SPM Brazil; other coded by chromosomal inserts.
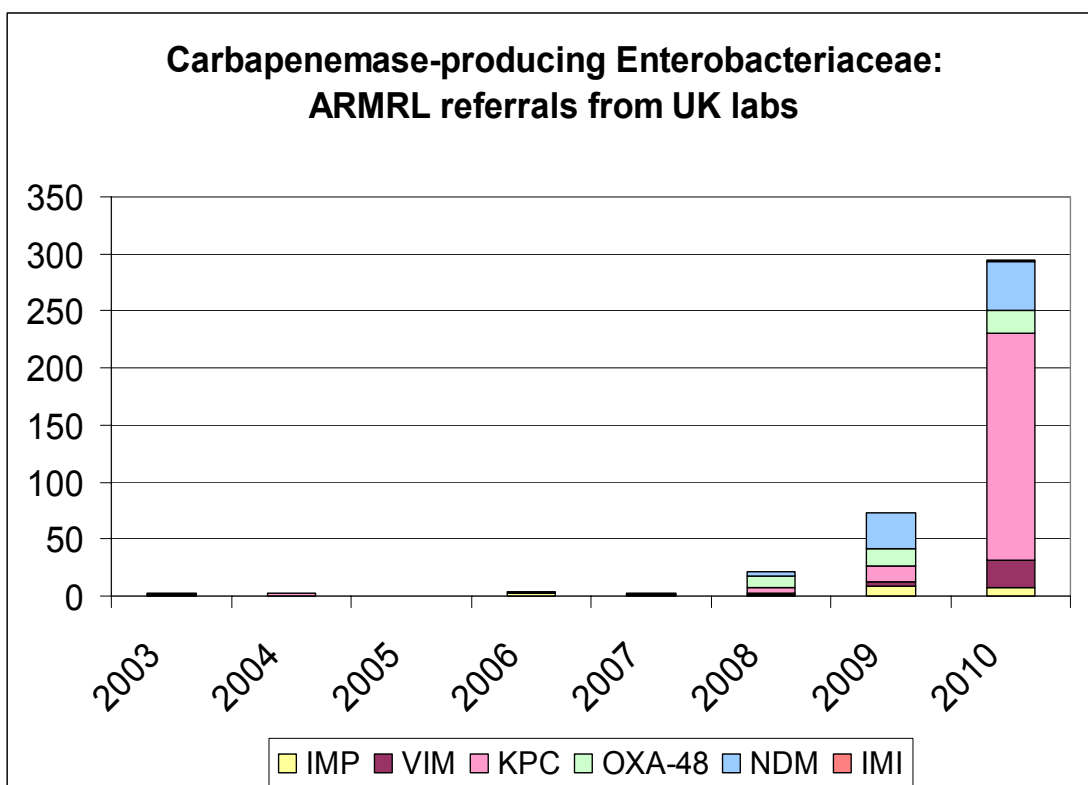
Table 1.3: Main Carbapenemases: distribution and molecular epidemiology

1.8 Detection of carbapenemases producers

Enterobacteriaceae with carbapenemases may only have small reductions in carbapenem susceptibility, meaning that laboratories should have a high index of suspicion about isolates with borderline sensitivity. Most producers are broadly resistant to β-lactams, but those with OXA-48 may remain susceptible to cephalosporins, and this can create problems for automated systems. Laboratories should participate in NEQAS, which will distribute carbapenemase producers in quality assurance exercises during 2011.

Suspect isolates should be sent for confirmation to ARMRL and Laboratory of Healthcare Associated Infection to (i) confirm the antibiogram, (ii) seek to identify any carbapenemase, (iii) undertake typing to identify outbreaks, and (iv) track disseminated clones where relevant.

Screening of faeces, rectal swabs, urine or skin trauma/ catheter sites can be accomplished by plating onto CLED or MacConkey agar and placing a meropenem or ertapenem disc on the primary streak. When clearing patients from isolation a more sensitive method is advisable particularly for rectal swabs which can be placed in 5-10 ml of broth to which an imipenem disc has been added, incubated or 18h and sub cultured as above.

Exceptions, not requiring referral for carbapenemase investigation are:

- Proteeae resistant to imipenem only; these species have inherently low susceptibility.

- *Enterobacter* spp. with cephalosporin and low-level ertapenem resistance but susceptibility to imipenem and meropenem – these generally have combinations of AmpC and impermeability.

- Carbapenem-resistant Acinetobacter or *P. aeruginosa*, unless these have exceptional levels of resistance (grow up to carbapenem discs) or give a positive EDTA-imipenem synergy test implying metallo-enzyme presence. Carbapenemases have not been found in cystic fibrosis isolates.

Laboratories wishing to undertake carbapenemase detection may find the following tests useful but none has clear interpretive standards so suspect Enterobacteriaceae should be referred.

Cloverleaf ('Hodge') test

Agar is spread with *E. coli* NCTC10418 (or ATCC25922), as for a disc test. The test strain is then inoculated, as 3 arms, 120° apart, cut into or streaked heavily on the agar from the plate centre. Imipenem, meropenem and ertapenem 10 μg discs are put at the end of these arms.
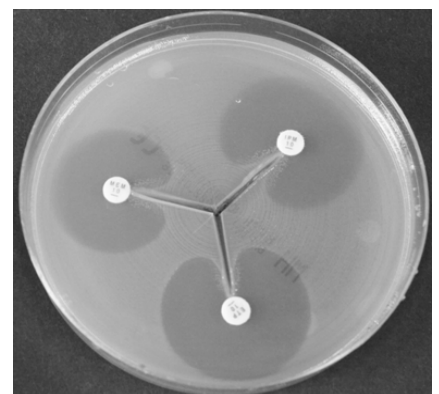
Indentation of the inhibition zone(s) indicates that the test strain attacks carbapenems. Caveats are that reading is subjective and that AmpC enzymes give weak false positive results.

Synergy tests

Metallo carbapenemases (IMP, NDM, VIM) are inhibited by EDTA or dipicolinic acid. Synergy between carbapenems and EDTA, indicating MBL productions, can be detected with Etests (see below) or using double disc tests (with EDTA discs). Caveats are that false-positive results are common with *P. aeruginosa* and *A. baumannii*; though rare with Enterobacteriaceae. KPC carbapenemases are inhibited by boronic acids, and synergy between boronic acid discs and imipenem indicates their presence.
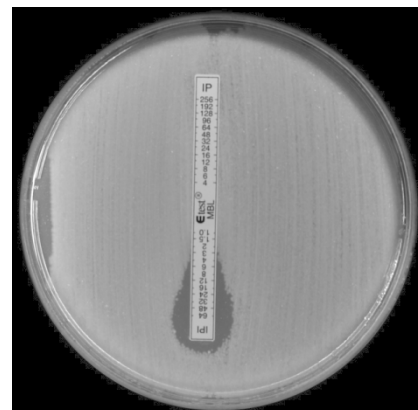
1.9 Veterinary considerations

The types of β-lactamase resistance that are currently being detected in veterinary bacteria in the UK have all been described in the medical literature. There are currently no recognised β-lactamases in the UK found exclusively in the veterinary field. The considerations that apply to the testing of bacterial isolates recovered from human patients can, therefore, be generally applied to veterinary isolates. National guidelines have been published for veterinary laboratories by the Veterinary and Public Health Test Standardisation Group (Teale 2005). A feature of some veterinary samples can be the relatively high proportion of *E. coli* isolates which have an AmpC phenotype often through promoter mutations affecting their endogenous AmpC enzyme. A selective medium has been developed for the isolation of ESBL *E. coli* from veterinary samples where they may occur together with AmpC *E. coli* (Randall et al. 2009).

1.10 Recommendations

All clinical laboratories should identify coliforms to species level and use reliable tests for detection of ESBLPCs adhering to EUCAST breakpoints whilst testing all presumptive ESBL- producing isolates for the presence of ESBLs.

All clinical laboratories should report overall prevalence of ESBL phenotypes on coliforms and they should increase the usage of rapid ESBL tests to ensure patients are given optimal treatment.

Protocols for appropriate use of reference laboratories should be in place. These should include any Enterobacteriaceae isolate suspected of being carbapenem-resistant must be sent to the HPA reference laboratory for confirmation and typing. All reference laboratories should be capable of providing genotyping of both ESBL genes (e.g. CTX-M) and producer strains to elucidate epidemiology and support surveillance for new successful clones.

2 TRANSFER PATHWAYS

2.1 Introduction

ESBL-producing *E. coli* and other Enterobacteriaceae, particularly those producing CTX-M, have spread rapidly among humans and there is evidence of spread among animal populations. To attempt to control and contain the spread of ESBLPC an understanding of the size of potential reservoirs, the drivers of transmission and the transmission pathways by which they spread (including the relative importance of each pathway) is crucial.

2.2 Drivers and mechanisms for the spread of ESBL-producing bacteria

Antimicrobial usage is the primary driver of the development and dissemination of antimicrobial-resistant bacteria. There is evidence that usage of the primary substrates for ESBLs – a wide range of β-lactam antibiotics including 3GCs – selects for, and can drive, the spread of ESBL-producing Enterobacteriaceae among both human and animal populations (Cavaco et al. 2008; Lautenbach et al. 2001). However, during 2009 in the UK, the combined total quantity of third and fourth generation cephalosporins authorised as veterinary medicines was 0.78 tonnes, representing just 0.2% of the 402 tonnes of all veterinary antimicrobials sold during the same period.There are clearly other factors that influence how resistance genes, as well as resistant bacteria, spread. These are discussed further.

2.2.1 Co-selection for resistance genes

It is commonly assumed that in the absence of antibiotic selection, mobile resistance genes will be lost and the host bacterium will return to a sensitive phenotype. Co-selection is one mechanism whereby other resistance genes carried on the same genetic element produce selection for an entire mobile genetic element.

The most obvious example of co-selection is if resistance to other non-β-lactam antibiotics is located on the same plasmid as ESBL genes. Analysis of the plasmids carrying ESBL genes from both human and animal sources show these elements frequently carry a wide range of resistance genes. They often encode resistance to aminoglycosides, trimethoprim, tetracyclines and low-level resistance to fluoroquinolones (Mshana et al. 2009). Use of any one of these classes of drugs may select for ESBL-producing Enterobacteriaceae.

There are other agents that can also be co-selective, particularly in the environment. Human activity produces emissions of complex mixtures of xenobiotics, bacterial pathogens and antibiotic resistance genes into the environment in the form of industrial and domestic effluent and human and animal waste. Industrial and domestic pollutants such as quaternary ammonium compounds (QACs) have also been shown to exert an extremely strong selective pressure for class 1 integrons (see below) which are a major

mechanism for dissemination of antibiotic resistance (Gaze et al. 2005), including ESBLs.

2.2.2 Class 1 integrons and generic mobilisation of ESBL genes

The role of class 1 integrons in conferring antibiotic resistance to clinical isolates of many bacterial strains is well documented (Briggs & Fratamico 1999; Leverstein-van Hall et al. 2003; Segal et al. 2003; White et al. 2000). This has also been documented for bacterial isolates of animal origin (Kadlec & Schwarz 2008). Fluit & Schmitz (2004) summarised the gene diversity seen within the gene cassettes carried by class 1 integrons. The TEM ESBL genes are carried and mobilised on the classical Tn1 & 3 type transposons SHV EBSLs are disseminated on replicons forming a defective compound transposon (Poirel et al. 2008). The situation with regards to CTX-M ESBLs is more complex with two types now identified. For CTX-M-9,2 and 1 this involves class 1 integrons but the gene is not part of a cassette of genes (Su et al. 2008). In the case of CTX-M-15 and -14 the genes are mobilised differently (Poirel et al. 2008).

As well as the presence of class 1 integrons in clinically significant bacteria, unpublished work by Gaze, Wellington and Hawkey has shown a high prevalence of class 1 integrons in environmental bacteria, particularly in those exposed to detergents and antibiotic residues, e.g. in fully digested sewage sludge and animal slurries. Preliminary work has also shown elevated prevalence of class 1 integrons downstream of sewage treatment plant outfalls. Whilst this evidence does not show transfer of ESBLs it does show dissemination into the wider environment of genetic elements that are known to carry ESBLs and can subsequently integrate ESBL gene cassettes once present in a bacterium.

2.3 Transmission pathways

Transmission pathways for antibiotic resistant bacteria, such as ESBL-producing *E. coli*, are outlined in the introduction. Within this outline the transmission pathways are varied and complex, and as yet we have little direct evidence of the relative importance of many of these routes in transmitting bacteria. This leads to inherent uncertainties when attempting to develop detailed risk assessments and risk management strategies.

2.4 Infection, carriage and transmission of ESBL-producing bacteria in humans

2.4.1 Infections caused by ESBL-producing bacteria

In the UK, outbreaks caused by ESBL-producing Enterobacteriaceae were rarely recorded in the 1980s and 1990s, with the exception of several well-documented hospital-based outbreaks largely involving *Klebsiella* (Hobson et al. 1996) and plasmid spread among different species of Enterobacteriaceae caused by TEM and SHV ESBLs (Hibbert-Rogers et al. 1995). This observation was supported by a large-scale survey of resistance to 3GCs in 43 hospitals in the UK in 1990/1 when only 1% of unselected isolates of Enterobacteriaceae were found to

produce ESBLs (mainly of the TEM and SHV type) (Piddock et al. 1997). Further characterisation of those isolates 10 years later failed to identify any CTX-M producing strains (P. Hawkey, unpublished data).

The first UK nosocomial outbreak of ESBL-producing coliforms (ESBPLC which carried CTX-M type β-lactamase was of a CTX-M-26 producing clone of *Klebsiella pneumoniae* which although largely hospital based also involved some patients in the community (Brenwald et al. 2003). There then followed in 2003/4 a sudden and dramatic increase in *E. coli* producing CTX-M exclusively of the CTX-M-15 type (Livermore & Hawkey 2005; Woodford et al. 2004). Analysis of these strains suggested that one particular strain referred to as epidemic strain A, was particularly common in some locations in the UK and subsequent investigation has shown that strain A and a number of other related clones identified by Pulsed Field Gel Electrophoresis (PFGE) belong to the internationally dispersed sequence type ST131 (Lau et al. 2008).

The most common infection caused by ESBL-producing bacteria is urinary tract infection (UTI). A Spanish study of community-acquired ESBL *E. coli* infections (122 cases) showed that 93% of the patients had UTI, 6% of those patients were bacteraemic and 10% required hospitalisation (Rodriguez-Bano et al. 2008a). The meropenem yearly susceptibility test information collection (MYSTIC) antibiotic surveillance programme reported that the percentage of Enterobacteriaceae producing ESBLs in the UK rose from 4.8% in 1997 to 7.4% in 2002; it should be noted that these were only from tertiary hospitals and intensive care units (Masterton & Turner 2006). Data from the British Society of Antimicrobial Chemotherapy (BSAC) recorded an increase in bacteraemia cases caused by CTX-M-producing *K. pneumoniae* from 0.9% in 2002 to 11.8% in 2007 and for *E. coli* an increase from 0.9% to 8.3% over the same period (www.bsacsurv.org/mrsweb/bacteraemia). In the UK, CTX-M-15 ESBL genes were first identified in *E. coli* and then spread into *Klebsiella* as shown in isolates from a large study from 16 London hospitals (Potz et al. 2006; Ensor et al. 2007).

2.4.2 Risk factors for the acquisition of ESBLs by humans

A number of risk factors for acquiring ESBL-producing bacteria have been identified in hospitalised patients, most of which also apply to other multiresistant Gram-negative bacilli. These risk factors include: prolonged hospital stay; prior hospitalisation; previous use of 3GCs, aminoglycosides and quinolones; presence of medical devises (i.v. lines/ urinary catheters); and mechanical ventilation (Khurana et al. 2002; Paterson & Bonomo 2005; Rodriguez-Bano et al. 2006b; Sturenburg & Mack 2003). In the case of community acquired ESBL infections, older age, female gender, recurrent UTIs/ prior invasive procedures (e.g. catheterisation), known faecal carriage, contact with healthcare facilities/ residents in care homes and previous antimicrobial treatment are all well described risk factors (Moor et al. 2008; Pena et al. 1998; Pitout et al. 2004; Rodriguez-Bano et al. 2008a)

2.4.3 Carriage of ESBL-producing bacteria

The first study to assess faecal carriage of ESBLs in the UK was undertaken in York in 2003 and detected a carriage rate of 2% of CTX-M producing Enterobacteriaceae amongst faeces samples from general patients with diarrhoeal illness. Although *E. coli* producing CTX-M-15 were identified, a number of *E. coli* and *Klebsiella* isolates producing CTX-M-14 and CTX-M-9 were also detected (Munday et al. 2004b). At that time there were no reports of infections caused by CTX-M-14 or CTX-M-9 in the UK but subsequently CTX-M-14 was identified in a large London survey as the second most common CTX-M genotype in the UK (Ensor et al. 2007).

The carriage rate in faeces in different parts of the world varies tremendously; with high rates recorded in countries such as India and China. A study in Spain in 2005/6 found a prevalence of 67.9% amongst patients with a UTI compared to a rate of 7.4% in unrelated members of the general population (Rodriguez-Bano et al. 2008a). The rate of 7.4% was similar to that recorded in a recent study of elderly Chinese individuals with no contact with healthcare or long-term care facilities such as nursing homes (Tian et al. 2008). Another factor which almost certainly influences carriage of ESBLs by individuals is foreign travel, as has been recorded in people who have travelled to areas outside the USA (Sannes et al. 2008). Travellers to areas of the world such as India where very high rates of ESBL are present, have been noted to become readily colonised, asymptomatically, with CTX-M-producing ESBL strains (Tham et al. 2010).

2.4.4 Transmission

The transmission dynamics of Enterobacteriaceae within the healthcare setting was worked out in the 1970s when gentamicin resistant *Klebsiella* was first noted in UK hospitals (Casewell et al. 1977). Detailed analysis of likely sources identified asymptomatic colonisation of the gastrointestinal tract by *K. pneumoniae* with contamination of skin surfaces leading to contamination of hands of staff. In addition those patients with UTIs disseminated bacteria widely into the environment: contaminating bedpans, urinals and sluice areas (Curie et al. 1978). Introduction of barrier nursing, hand washing with chlorhexidine compounds and the use of disposable aprons contained both of the early outbreaks in the UK (Casewell et al.1977; Curie et al. 1978). These studies set the standard for understanding the epidemiology of transmission of Enterobacteriaceae in a hospital environment and formed the basis of current interventions to prevent cross infection in hospital by Gram-negative bacteria. A recent study (Laurent et al. 2008) describing a nosocomial outbreak caused by CTX-M-15-producing *K. pneumoniae* demonstrated the importance of rigid adherence to rectal screening, hand washing and antimicrobial control. *Escherichia coli* is not traditionally thought of as a common nosocomial pathogen but an investigation of an outbreak in 2004/5 in Cornwall demonstrated significant transmission in the ward setting following the introduction into the hospital of patients from the community with UTIs caused by CTX-M-15- and CTX-M-9-producing *E. coli* (Woodford et al. 2007).

Transmission in the community is probably quite complicated. Individuals in long-term care homes where high carriage rates of CTX-M producing Enterobacteriaceae have been observed (Rooney et al. 2009) may then spread

strains to other non care-home residents. The evidence for a significant spread amongst household contacts has recently been presented in a Spanish study which showed that 70% of index cases of patients with ESBL-producing strains causing UTI in the community had positive contacts in 16.7% of their household members. 66% of the ESBL-producing bacteria from the index cases were indistinguishable by PFGE from isolates in their household contacts. (Valverde et al. 2008).

In summary, transmission of CTX-M-producing Enterobacteriaceae relies on reservoirs which currently appear to be in faeces of individuals either receiving healthcare or in a long term residential care setting. Those individuals experience a much higher rate of infection with ESBL-producing strains than individuals in the general community. In the UK, however, there is also a rising proportion of individuals in the community who acquire CTX-M-producing ESBL infections from asymptomatic faecal carriage of those organisms despite no exposure to health care or to antibiotics. There are no current estimates of that reservoir but this may well be increasing in size, as evidenced by the increasing numbers of ESBL-*E. coli* in bacteraemia cases, many of which originate in the community. Such a reservoir would fuel further increases in clinically significant infections in the human population.

2.5 Carriage and transmission of ESBL-producing bacteria in farm and companion animals

2.5.1 Colonisation as a source

Animal populations have the potential to act as reservoirs for a number of zoonotic infections, including pathogenic ESBL-producing Enterobacteriaceae. ESBLs may also be carried by the commensal *E. coli* flora of animals. In Europe, a very wide range of ESBL genotypes have been reported from animals: CTX-M (-1,-2,-3,-8, -9,-13,-14,-15,-24,-28,-32), SHV (-2,-5,-12), and TEM (-52,-106,-116). Across Europe CTX-M-1, TEM-52 and SHV-12 have been most commonly found in animals to date (Coque et al. 2008a). Some clear associations with particular country/genotypes have also been observed. For example, in Spain CTX-M-14 and the closely related CTX-M-9 have been repeatedly reported from surveys of *E. coli* in animals (Blanc et al. 2006;Brinas et al. 2005). These genotypes are also the most commonly encountered in humans (CTX-M-9 was first identified in Spain in 2000) both in infections and asymptomatic carriage in children and adults (Rodriguez-Bano et al. 2006a). The first of these genotypes to be identified in *E. coli* from animlas was isolated in 2003. Most of these animal isolations have been from *E. coli* in farm animals, including poultry.

In the UK, the majority of animal isolates of ESBL-producing *E. coli* originate from cattle; the ESBLs involved include CTX-M 14, 15 and less frequently CTX-M -1, -3, -20 and -32. In addition, unpublished work has identified ESBL *E. coli* carrying CTX-M-2 and -14 in sheep and CTX-M-14 in horses at a farm in the UK. ESBL-producing *E. coli* have also been identified in pigs in the UK on three separate and apparently unrelated premises and all cases involved the ESBL CTX-M-1. One of these premises had supplied pigs to four other farms and each of these farms was also positive for ESBL *E. coli* producing CTX-M-1, indicating

that animal movements are likely to be important in the dissemination of ESBL *E. coli* in livestock. ESBLs appear to occur less frequently in other bacteria isolated from livestock but this may be largely through lack of surveillance other than for *E. coli* and *Salmonella*.

Some European countries have reported ESBLs in *Salmonella* isolates, including CTX-M-2, -9, -14, TEM-52 (Coque et al. 2008a). In 2008 the first UK isolation from animals of a *Salmonella* which had acquired ESBL resistance was made by the Veterinary Laboratories Agency (VLA). This involved *Salmonella* Kedougou and the ESBL CTX-M-1, isolated from a pig farm. A visit to the farm in 2009 identified that *E. coli* carrying the ESBL CTX-M-1 were present in both the pigs and their environment and were widespread on the farm; *Salmonella* Kedougou carrying CTX-M-1 was much less prevalent. Neither of these bacteria appeared to have caused a disease problem, but the VLA liaised with the farm's veterinary surgeon so that the management of other ongoing diseases that were affecting the herd was re-assessed to minimise the use of antibiotics that may have selected for the spread of this particular ESBL on this farm. A particular focus was that the cephalosporin antimicrobial ceftiofur was being routinely used on the affected farm to control and treat *Streptococcus suis* infection in piglets. *Salmonella* Kedougou is a rare cause of salmonellosis in humans in the UK, and the ESBL enzyme CTX-M-1 is not one of the common ESBLs occurring in human bacteria in the UK. The Health Protection Agency (HPA) confirmed that at that time there were no reported human cases of infection with *Salmonella* Kedougou resistant to 3GCs (VLA 2009).

In 2009 in England and Wales, 2% of 485 *Salmonella* isolates tested from pigs were found to be resistant to cefotaxime; these isolates belonged to the monophasic *Salmonella* serotypes 4,12:i:-, 4,5,12:i:- and to Bovismorbificans. All the isolates recovered were epidemiologically linked to a single index case premises (VLA 2010). The *Salmonella* isolates possessed the ESBL CTX-M-1, which was also detected in *E. coli* isolates from pigs on the affected premises.

Companion animals (e.g. horses, dogs, cats, etc.) also present a potential reservoir for transmission to humans. CTX-M genes have been identified in dogs in the UK (Steen & Webb 2007) but the extent to which ESBLs are present in other companion animals or their potential as reservoirs for human infection is not clear. Defra are currently funding studies to determine the prevalence of ESBL *E. coli* in dogs and horses including risk assessments on the potential for transfer to humans. Although the VLA deals principally with food-producing animals, they also receive some samples, for serotyping, from companion animals, including *Salmonella* isolates from dogs, cats and other pets. One such case in 2009 involved *Salmonella* Java resistant to both ciprofloxacin and 3GCs that had been isolated from a dog which had been fed raw chicken. The isolate possessed the ESBL CTX-M-16. *Salmonella* isolates belonging to this serotype with similar resistance have been reported from poultry in Europe (excluding the UK) and it seems likely that this may have been the source in this case.

In the UK most of the recorded occurrences of ESBLs in animals documented so far result from scanning surveillance and not through targeted approaches. The VLA began screening veterinary isolates of *E. coli* for CTX-M ESBLs in 2006. The data to date indicate that CTX-M ESBL-producing isolates were found in cattle on at least 70 farms in regions across England and Wales. However, since the presence of ESBL *E. coli* on cattle farms rarely results in any clinical disease in the animals, this figure is likely to underestimate the actual number of farms on which ESBL *E. coli* are present.

Testing of samples from structured national surveys of broilers and turkeys has recently been completed and provides prevalence estimates for ESBLs in these species (see below). A more accurate picture of how widespread ESBLs are in animals, and the dominant types present, will be important in determining the significance of animal reservoirs as potential sources of transmission to humans. A study in France in which samples from healthy cattle and diseased cattle were screened for ESBLs found ESBL *E. coli* in both groups of animals at 4.1% and 2.6% respectively (Madec et al. 2008).

A recent longitudinal study by VLA on a dairy farm which has been studied in detail showed that calves were colonised by ESBL *E. coli* shortly after birth and remained colonised until approximately the age of weaning (VLA, studies in progress).

2.5.2   Transmission pathways to/from animals

Data on transmission between animals and the environment and other animals are limited. Most published reports are cross-sectional prevalence studies establishing different animal species as possible reservoirs, but the origin or dynamics of ESBL carriage in animals remains largely unknown. ESBL-producing bacteria have been isolated from dogs, horses, poultry (and other birds), pigs, cattle and rabbits (Carattoli 2008; Coque et al. 2008a). Several authors noted the low genetic homology of strains even within farms (Girlich et al. 2007; Hasman et al. 2005; Machado et al. 2007).   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 (Teale et al. 2005). In this case, the CTX-M type was one that is seen less commonly (second most after CTX-M-15) in clinical cases in the UK (CTX-M-14) and the type of *E. coli* in which it was found was different from the majority of human clinical isolates.   The second case in UK livestock identified by the VLA e was discovered in the south of England in July 2006 and also involved cattle (Liebana et al. 2006). 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* was different to the ST131 lineage that dominates among human CTX-M-15-positive *E. coli*.

Molecular typing data of livestock isolates from Portugal indicated that they were not related to strains circulating in hospitals (Machado *et al.* 2007). Human, animal and environmental strains from pig farms in Denmark displayed high genetic diversity but harboured indistinguishable or closely related *Inc*N plasmids carrying $bla_{CTX-M-1}$ suggesting transmission of these plasmids between pigs and farm workers (Moodley & Guardabassi 2009).

There is some evidence for the spread of these organisms between animals and potentially into humans. For example, clonal spread of ESBL-producing *S. enterica* serovar Virchow in poultry was described in Belgium and France from 2000 to 2003 with a first isolation of the same strain from humans in 2003 (Bertrand et al. 2006). The chronology of the findings suggested a spread of the strain throughout the poultry production chain subsequently reaching humans. A similar clonal spread was reported for ESBL-producing *Salmonella* Livingstone (Chiaretto et al. 2008). This may indicate the significance of spread through animal trade.

European Food Standards Agency (EFSA) recently reviewed the extent to which humans may be exposed to ESBL resistance (EFSA 2009) and noted that ESBL resistance has recently been detected in many countries worldwide in various serotypes of Salmonella. Strains exhibiting such resistance have been detected in both humans and animals in Europe (Bertrand et al., 2006; EMEA, 2008). In Belgium and France, a cephalosporin-resistant *Salmonella* Virchow clone (carrying CTX-M-2) was found in poultry, poultry products and humans in 2000–2003. Two human patients who contracted this clone were initially treated unsuccessfully with extended-spectrum cephalosporins, confirming the clinical significance of 3GC resistance. All isolates belonging to this clone of *Salmonella* Virchow also displayed decreased susceptibility to ciprofloxacin. The chronology of isolation suggested that the isolates had been transmitted to humans by the food chain, probably by poultry meat. A similar spread was demonstrated for a clone of cephalosporin-resistant *Salmonella* Infantis in poultry and humans in Belgium and France over the period 2001–2005. In this case, ESBL resistance (TEM-52) was located on a conjugative plasmid which also spread to some other serotypes, including Java and Typhimurium (Bertrand et al., 2006; Cloeckaert et al., 2007). The authors comment that human infections with cephalosporin-resistant *Salmonella* Infantis were probably related to ingestion of undercooked poultry products.

Investigations related to the detection of ESBL-producing *E. coli* in the UK indicated both horizontal plasmid transfer between strains, horizontal gene transfer between plasmids and transfer between animals reaching almost 100% prevalence over seven months (Liebana et al., 2006). Further on-farm studies are currently underway to investigate the within-farm epidemiology of ESBLs.

2.6 Food

2.6.1 Presence of ESBL-producing bacteria in foods

There is a small but increasing body of information in the published literature regarding the presence of ESBL-producing *E. coli* in foods. There is little available information available from the UK but, as a significant proportion of the food we consume is sourced elsewhere the presence of these organisms in foodstuffs from other countries is of relevance.

In a Spanish study 3/738 (0.4%) foods that were tested were positive (Mesa et al. 2006) for ESBL-producing *E. coli*. 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. A further study by this group (Lavilla et al., 2008) also found ESBL-producing bacteria in 35/131 (26%) raw meat samples purchased at retail; 27/47 (57%) chicken samples, 7/12 (58%) rabbit, and 1/20 (5%) lamb samples were positive. A study of foods from Tunisia found 10/38 (26%) samples contained ESBL-producing *E. coli*, with nine foods containing a CTX-M producer. Varieties of foods of animal origin (beef, chicken and turkey) were found to contain the CTX-M producers.

There has only been a single published study of foods at retail sale in the UK. Warren et al. (2008) examined 129 chicken breast samples purchased from retail outlets. Overall, 16/129 (12%) samples contained a CTX-M ESBL-producing *E. coli* but there was a marked difference between prevalence in UK produced and imported chicken. Only 1/62 UK-reared chicken samples carried *E. coli* producing a CTX-M enzyme, whereas 10/27 samples reared overseas had *E. coli* with CTX-M enzymes, the genotypes of which were the most commonly encountered genotype in humans in Brazil and the Netherlands. Specifically, 4/10 Brazilian, 3/4 Brazilian/Polish/French, and 2/2 Dutch samples had *E. coli* with CTX-M-2 enzymes. Six of 40 samples for which the country of rearing was not known had producers of CTX-M enzymes, five of them with CTX-M-14, a genotype common in the Far East and Spain.

The presence in food of *Salmonella* producing CTX-M enzymes has also been described. Studies from Denmark (Aarestrup et al. 2005) the Netherlands (Hasman et al. 2005), Portugal (Machado et al. 2008) and Greece (Politi et al. 2005) have all recorded CTX-M genes in S*almonella* isolates from poultry products.

2.6.2 Transmission via food

Given that there appears to be transmission of ESBL-producing *E. coli* in the community amongst humans, the hypothesis that consumption of some food products contaminated with *E. coli* producing CTX-M ESBLs could be a significant source has arisen. There are some studies that provide circumstantial evidence that food may result in transmission of ESBL-producing Enterobacteriaceae amongst the general population. For example, a Spanish study in 2003–2004 in Barcelona involved the examination of stool samples from 905 people involved in 132 acute gastroenteritis outbreaks and 226 food handlers related to the outbreaks. In 31 of the 132 outbreaks, 58 individuals were found to carry one or more ESBL-producing bacteria. In 10 of those outbreaks, two or more diners shared the same strain of ESBL-producing Enterobacteriaceae and in four of those a strain was also shared with food handlers who were identified in the retail premises (Lavilla et al. 2008).

Further support for this hypothesis comes from an observation made in the study of Rodriguez-Bano et al. (2008b). In this study, in addition to looking at carriage amongst those individuals with community acquired UTI with CTX-M producing, ESBLs from household contacts and non-household relatives were also examined. It was that found that those who had eaten their main meal outside their own home ≥ 15 days during the previous month were highly significantly

less likely to carry CTX-M producing ESBLs. This supports the concept that food prepared within the household of a carrier may represent a significant route of transmission amongst family groups in a household setting.

2.7 Wider environment

The environment also has an important role to play as both a source and a reservoir of antibiotic resistance genes and bacteria. Naturally occurring bacteria found in soil and water are important sources of antibiotic resistance genes and the genetic elements (such as type 1 integrons). Additionally, contamination of both land and water with Enterobacteriaceae can occur via human and animal faeces from sewage outfalls and from spreading of human waste and animal slurry on agricultural land.

2.7.1 Environmental origins of resistance CTX-M enzymes

The identification of environmental progenitors of ESBL CTX-M enzymes responsible for resistance to 3GCs in bacteria of the genus *Kluyvera* clearly indicates the significance of the environment in the evolution of emerging antibiotic resistance determinants (Bonnet 2004; Rodriguez et al. 2004; Smith et al. 2005). *Kluyvera* spp. are rare human pathogens and are more often found associated with plants. Sequence similarity between the genes suggests that the natural β-lactamases of *K. ascorbata* and *K. georgiana* are the progenitors of the CTX-M-2 and CTX-M-8 enzyme groups respectively (Bonnet, 2004). Evidence suggests that the process of gene transfer from the chromosome of *Kluyvera* to other clinically important bacteria has occurred several times involving different mobile elements, such as the IS-10-like element found upstream of both KLUG-1 and CTX-M-8 and ISEcp1 found upstream of KLUA-1 and members of the CTX-M-2 group (Poirel et al., 2008).

2.7.2 Sewage

About 347,000 km of sewers collect over 11 billion litres of wastewater every day in the UK. This is treated at about 9,000 sewage treatment works before being discharged to inland waters, estuaries and the sea. Defra's statistics on sewage sludge indicates that approximately 1.5 million tonnes of dry solids per annum are produced each year, the bulk of which is disposed of to land. This amount may rise as a greater proportion of sewage is treated and higher treatment standards are applied under the phased implementation of the Urban Waste Water Treatment Directive (UWWTD) 91/271/EEC (Defra, 2002). After application to land, pathogens may be transported overland and by subsurface flow into watercourses. UK regulations for pathogen removal are becoming more stringent, but the processes used to reduce bacterial indicator species numbers may have a quite different effect on resistance gene numbers and this is an area which has not been previously studied. Beta-lactam and aminoglycoside resistance genes have been isolated by exogenous isolation from activated sewage in Germany, illustrating that final stage sludge is a source of antibiotic resistance genes (Tennstedt et al. 2005). A Spanish study (Mesa et al. 2006) detected the presence of ESBL-producing *E. coli* in all five sewage samples

examined. However, there have been no studies examining sewage sludge or animal slurries for the presence of these organisms.

The dissemination of antibiotic resistance genes and bacteria from the sources described above is a plausible route by which both domestic and wild animals may be exposed and colonised. Investigations of farms in the UK where ESBL-producing *E. coli* in livestock have shown the presence of these organisms in wild animals (unpublished VLA findings) which may provide evidence for the importance of the environment in transmitting these organisms.

Crucially, although sewage and sewage sludge has been demonstrated to contain antibiotic resistance genes and pathogenic bacteria, the extent of this problem and the potential for transfer of resistance to aquatic bacteria and ultimately its effect on the human and animal populations is unknown.

There are many potential and actual reservoirs for ESBL-producing Enterobacteriaceae in humans, animals and the environment. However, transmission pathways are clearly highly complex, with resistance genes and bacteria flowing in both directions between and within animal and human populations directly or via the environment. Although transmission pathways are relatively well understood in some limited settings, e.g. within hospitals, there are many gaps in our understanding of this process and the key drivers within it.

2.8   Recommendations

Further work should be carried out to understand what happens to bacteria with antimicrobial resistance genes in human and animal waste during storage and associated processes before it is applied to land.

The carriage rate of ESBLPCs in the healthy human population and in travellers to high prevalence areas should be determined as denominator data from which to compare rates of ESBLPCs in patients with exposure to healthcare facilities.

The prevalence of ESBL-carrying organisms or resistance determinants in retail food samples, environmental samples and all categories of food handlers should be determined to elucidate the resistance gene cycle.

3 SURVEILLANCE

3.1 Medical

3.1.1 Emergence of Enterobacteriaceae with ESBLs in the UK

Production of CTX-M ESBLs in the UK was first recorded in *Klebsiella oxytoca* in 2000 and in *K. pneumoniae* causing a hospital outbreak in Birmingham in 2001 (Brenwald et al. 2003; PHLS 2003).

In 2003, CTX-M-producing *E. coli* began to be reported widely in the UK, with major clonal outbreaks in the West Midlands and South East, together with numerous cases involving non-clonal strains around London and South East England and affecting both community and hospital patients (Pearson et al. 2005). In 2003, 291 CTX-M-producing *E. coli* isolates were studied by the Health Protection Agency (HPA) from 42 UK centres; 70 (24%) were reportedly from community patients, many of whom had only limited recent hospital contact (Woodford et al. 2004). Community isolates were referred by 12 centres. Almost all (95.9%) ESBL producers contained genes encoding group 1 CTX-M enzymes, although 12 contained $bla_{CTX-M-9}$-like alleles. An epidemic CTX-M-15-producing strain 'A' was identified, with 110 community and inpatient isolates referred from six centres. Representatives of four other major strains, B–E, were related to A and also produced CTX-M-15 enzyme, as did several sporadic isolates examined. Most producers were multi-resistant to fluoroquinolones, trimethoprim, tetracycline and aminoglycosides as well as to non-carbapenem β-lactams. It has since become apparent that strains A–E all belong to ST131, a globally disseminated lineage (see Figure 3.1).

3.1.2 National surveillance

In order to understand the national epidemiology of ESBLPCs and to identify strategies for national control, the Department of Health (DH) should support prospective national surveillance of ESBLPCs particularly in bacteraemia and urinary tract infection (UTI) including molecular characterisation of selected strains. These data and their interpretation should be fed back in a timely manner to the NHS for information and action. The data should be reviewed by appropriate expert committees (ARHAI in England) for advice and commentary. The DH will need to support national and local actions to reduce rates of infection.

Similarly, for animals, nationally derived information is vital. Therefore the VLA, SAC and DARDNI should work together to ensure that UK-wide information is available. These data will be reviewed by the Defra Antimicrobial Resistance Coordination (DARC) Group, which includes representatives from England, Northern Ireland, Scotland and Wales, and may also be referred on from DARC to other expert committees such as ARHAI and the Welsh Antimicrobial Resistance group.

3.1.3   Regional surveillance

In 2007, *E. coli* was found to be the most common cause of bacteraemia in England, Wales and Northern Ireland (HPA 2008a). Since then the number of reports of bacteraemia caused by *E. coli* have continued to increase (Figure 3.1). In 2009, there were 25,532 voluntary reports made to the HPA. This is a 7% increase in the number of reports made to the HPA in 2008 (23, 971) and a 15% increase on 2007 (22, 128 reports).
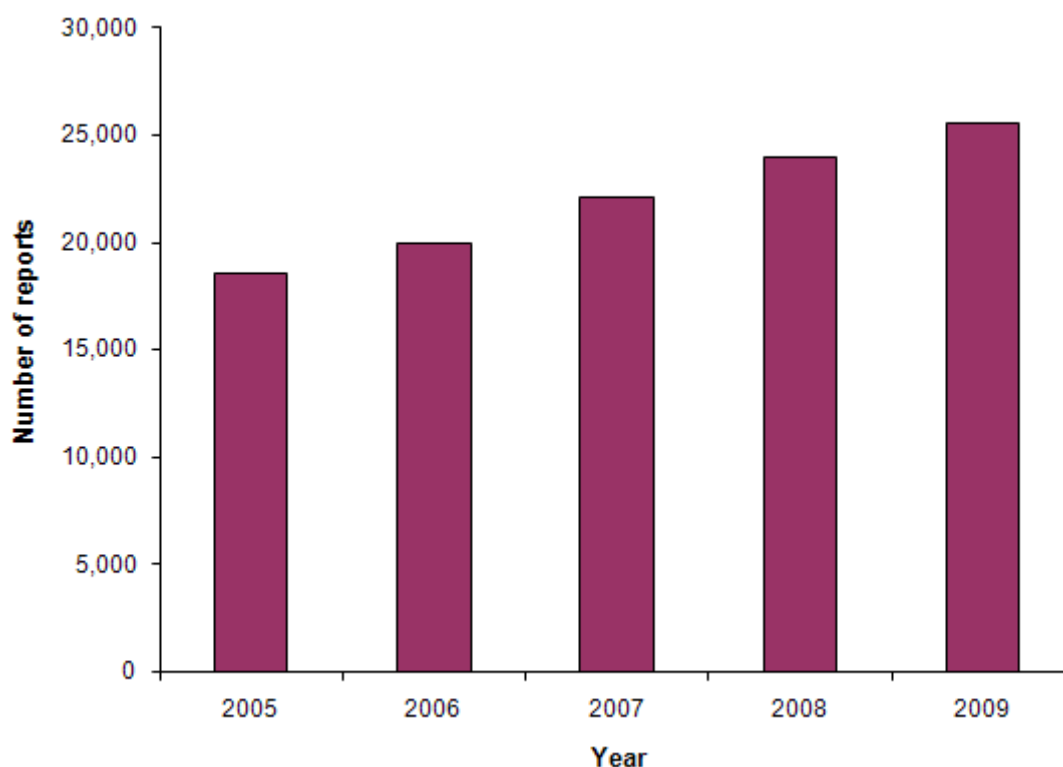
Figure 3.1 *E. coli* bacteraemia reports, England, Wales and Northern Ireland: 2005 to 2009

Resistance to oxyimino cephalosporins (cefotaxime and ceftazidime) had increased year-on-year from about 2% in 2001 (it was also low in the 1990s), to about 12% for both agents in 2007 (Figure 3.2). HPA surveillance in the West Midlands region of all ESBLPCs isolated in 13 hospitals in April and May 2006, revealed marked variation in the occurrence of the UK dominant strain A and ST131 as well as substantial increases in resistance to gentamicin ((Xu et al. 2010).

It should be noted that only ~1 in 20 of infections with an ESBL *E. coli* is a bacteraemia (Potz et al. 2006), so the above data are only the tip of an iceberg of infected individuals. Far more infections involve the urinary tract, but there is no national surveillance of these nor of the (probably far greater) number with benign gut carriage.
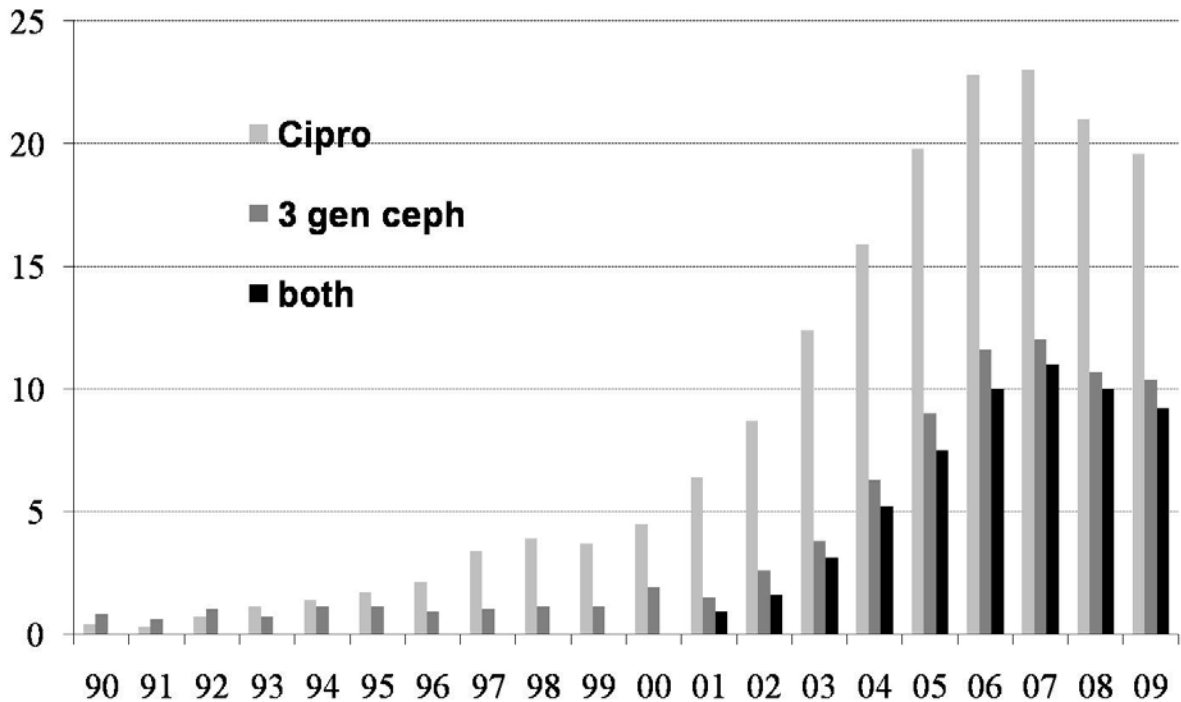
Figure 3.2 Antibiotic resistance rates among *E. coli* from cases of bacteraemia in England, Wales and Northern Ireland between 1990–2009. (Cipro: ciprofloxacin; 3GSc.)

It is likely that, whilst some of the increase in resistance to oxyimino cephalosporins in *E. coli* since 2003 reflects increased ascertainment, most of it is due to the emergence and spread of strains of *E. coli* producing CTX-M type ESBLs (particularly CTX-M-15, although other types of resistance mechanisms, such as AmpC β-lactamase, are also observed) (HPA 2006; Potz et al 2005). This is supported by centralised testing of isolates collected under the ambit of the BSAC Bacteraemia Surveillance. Based on isolates from a network of 25 UK laboratories, between 2002 and 2006, it was found that 78% of *E. coli* resistant to oxyimino cephalosporins produced CTX-M type ESBLs (Reynolds *et al.* 2007). Data from this network were further analysed for this report. Between 2001 and 2007, 97 out of 1734 (6%) *E. coli* isolates were ESBL producers and 84% of these were also resistant to ciprofloxacin, 39% to gentamicin and 34% to both. The corresponding data for ESBL-producing *Klebsiella* spp. (181 of 1679 referred: 11% of total referred resistant) were 77%, 67% and 57% and *Enterobacter* spp. (130 of 1415 referred: 9% of total referred resistant) 51%, 73% and 40%, respectively. Far more *Enterobacter* spp. were resistant to oxyimino cephalosporins owing to the hyperproduction of chromosomal AmpC enzymes.

Many of the human ESBL *E. coli* with CTX-M-15 enzymes belong to ST131 and are of serotype O25:H4. The experiences of ARMRL/LHCAI are that the patterns of multi-resistance in *E. coli* data vary in different hospitals or locales depending on the predominant local strains. Strain A, for example, an ST131/CTX-M-15+ variant dominant in parts of Hampshire, Lancashire, Shropshire and Northern Ireland is usually sensitive to gentamicin and resistant to ciprofloxacin, whereas other ST131/CTX-M-15+ variants generally are gentamicin resistant as are many non-ST131 ESBL producers.

In 2004, Potz and co–workers conducted a prospective study in London and South East England, where 16 hospital microbiology laboratories each submitted up to 100 consecutive clinically significant cephalosporin-resistant Enterobacteriaceae isolates over a 12-week period. The predominant mechanism of cephalosporin resistance in isolates from both hospital and community settings was the production of CTX-M-type ESBLs, the commonest being CTX-M-producing *E. coli,* although other major mechanisms of cephalosporin resistance included production of non-CTX-M ESBLs and AmpC β-lactamases (Potz et al. 2006).

3.2   Local surveillance

Hospitals should implement the practice of 'alert organism surveillance' (i.e. daily laboratory produced lists for the Infection Control Teams of locally agreed important organisms isolated from submitted samples). Where an outbreak is suspected then further clinical and screening samples will be taken. To elucidate the epidemiology of an outbreak, typing will often be necessary and isolates will be referred to the Reference Laboratories, who also can perform enhanced studies. Data required to inform such surveillance/outbreak investigations will include admission date, date of specimen taking and the various wards visited. Urinary tract and bacteraemia data will predominate, but other sources such as pressure sores and surgical wounds will be encountered, particularly as an organism spreads. Valid definitions of infections (e.g. cystitis, pyelonephritis) should be used. Faecal samples will also be needed during outbreak investigations to detect colonised subjects. Where there is clearly a community problem investigations will have to extend to long-term care facilities. At this time there are no common standards or recommendations for local surveillance and action to be taken following a significant rise in cases.

A study in Belfast found that the faeces in 119/294 residents (40.5%) from 18 care homes contained ESBL-producing *E. coli.* Importantly, half of these were ST131 Strain A with CTX-M-15 enzyme; most of the remainder were also ST 131, some with CTX-M-3 (which is only one amino acid different from CTX-M-15) and some non strain A but also with CTX-M-15, each carried on a range of different plasmids (Rooney et al. 2009).

3.3   Veterinary

Veterinary surveillance for ESBL-producing bacteria in most countries, including the UK, has focused on *Salmonella* and *E. coli.* Surveillance can be divided into two broad categories – surveillance of carriage by healthy animals and surveillance of diagnostic samples from animals with disease. Surveillance of healthy animals may be carried out on farms or at abattoirs. Such surveillance provides information on the faecal flora which may contaminate products entering the food chain. Surveillance of this type is usually statistically-based, providing representative information relating to the population sampled. Surveillance of veterinary clinical diagnostic samples (from animals with disease) provides complementary information from animals both before starting treatment or having failed to respond to an antimicrobial treatment and includes animals

such as young calves. Antimicrobial resistance is usually much more prevalent in bacteria from clinical diagnostic samples than in samples collected from healthy animals. Surveillance of clinical samples can provide the first indications of new resistances occurring in bacteria in animals, although the coverage of species/locations is highly variable. *Salmonella* surveillance in animals in the UK is done under statute (the Zoonoses Order, 1989).

3.3.1 Surveillance of clinical veterinary diagnostic material

Veterinary diagnostic laboratories receive samples from animals for examination for bacterial pathogens, as well as for other purposes. In the UK, susceptibility tests performed on Enterobacteriaceae from food-producing animals by the laboratories of the VLA (England and Wales), SAC (Scotland) and DARD (Northern Ireland) include the relevant screening of cephalosporins for optimal detection of ESBL-producers (ceftazidime and cefotaxime or cefpodoxime). Results from VLA for 2006/2008 have been published by VMD in the 'Overview of antimicrobial usage and bacterial resistance in selected human and animal pathogens in the UK: 2007' and are given at Table 3.3.

	3/2006	4/2006	1/2007	3/2007	4/2007	1/2008
Confirmed ESBL of any type	8	6	7	4	9	13
CTX-M-1	1	-	-	1	2	1
CTX-M-3	-	-	-	-	-	-
CTX-M-14	3	2	3[a]	1[a]	2 + 1[a]	4 + 3[a]
CTX-M-15	4	3	4	1	3	5
CTX-M-20	-	-	-	1	-	-
CTX-M-32	-	-	-	-	1	-
Total *E. coli* examined from diagnostic samples (% ESBL-producers)	272 (3)	621 (1)	802 (0.9)	355 (1)	463 (2)	509 (3)

[a]CTX-M-14 variant - Figures are provisional and reflect the latest available data.

Table 3.3 ESBL-producing *Escherichia coli* recovered by quarter from bovine diagnostic samples in 2006/2008 in England and Wales by Veterinary Laboratories Agency

The majority of ESBL-producing *E. coli* recorded from surveillance of veterinary clinical diagnostic material have been detected in cattle, particularly calves. However, the numbers of clinical submissions received varies markedly by species and some species (for example cattle) are much better represented than others in diagnostic surveillance.

Most veterinary clinical diagnostic work in companion animals in the UK is undertaken by private laboratories or at the university veterinary schools. There

is a published report of ESBL detection in *E. coli* from dogs by one laboratory, although molecular characterisation was not performed (Steen & Webb 2007). Liverpool University have also reported the presence of ESBLs in companion animals at a BSAC Research Meeting in Birmingham 2008. There are also reports from other European countries of ESBL *E. coli* in companion animals (Carattoli et al. 2005). SAC undertake surveillance of bacteria from companion animals in Scotland. Defra are funding surveillance of companion animals for ESBLs (see appendix 3) and this includes a study performed by Liverpool University (Ahmed et al 2010).

Surveillance of samples from pigs has recently identified the ESBL CTX-M-1 in *E. coli* and *Salmonella* and this has been discussed in the section on colonisation of animals.

Salmonella isolates recovered from food-producing animals must be referred to the VLA under statute (the Zoonoses Order). Isolates from new incidents are tested for their antimicrobial susceptibility, and surveillance of *Salmonella* is therefore considered to be reasonably comprehensive. Currently, amongst food-producing animals in England and Wales, ESBLs have only been detected in a limited number of *Salmonella* serotypes from pigs.

3.3.2   Abattoir surveillance

Abattoir-based surveillance was undertaken of cattle, pigs and sheep at slaughter in the UK in 1999/ 2000 and again in 2003. ESBL-producing Enterobacteriaceae (*E. coli* and Salmonella) were not detected in those surveys, although they did not use selective culture techniques for ESBL-producers. There are a number of reports of surveillance for ESBL *E. coli* in food-producing animals in Europe, including one where isolation from healthy cattle at abattoirs (*n*=607) was compared with diseased cattle on farms (*n*=657); ESBL *E. coli* prevalence was 4.1% and 2.6% respectively (Madec et al. 2008). Twelve *E. coli* isolates from healthy poultry in France, recovered from a sample of 112 caeca from slaughtered birds, were found to carry CTX-M-1 (Girlich et al. 2007).

Defra-funded targeted surveillance studies of broiler chickens and turkeys for ESBL *E. coli* have recently been completed by VLA. The studies were performed on a statistical sample of the population and should therefore be representative of the broiler and turkey populations within the area sampled. The broiler study ran from January 2008 to January 2009 and was performed at 23 abattoirs across Great Britain. It included chickens from 21 different companies. Caecal samples (388) were examined for ESBL *E. coli* using ESBL selective media; 3.6% of broiler caecal samples were positive for *E. coli* producing ESBLs belonging to the CTX-M family of enzymes. The percentage of abattoirs from which ESBL *E. coli* were isolated was 52% and broiler chickens originating from 12/21 companies were positive. The predominant CTX-M types were 1 (accounting for 78% of CTX-M isolates), -3 and
-15. No *E. coli* serotype O25 isolates (the predominant human strain) carrying ESBLs were identified and no isolates possessed transferable fluoroquinolone resistance genes (Randall et al 2011, VLA 2010).

The sampling for CTX-M-producing *E. coli* in turkey flocks was carried out during an EU Baseline Survey for *Salmonella* in turkey flocks. Boot swabs (i.e. swabs of litter collected on boots) comprising five samples per flock were taken from 442 turkey flocks including 125 breeder and 317 meat flocks from 337 different farms throughout Great Britain in 2006 and 2007. Selective media were used for ESBL isolation and 5.2% of farms with birds producing meat (*n*=308 farms) and 6.9% of farms with breeding birds (*n*=29 farms) were positive for CTX-M-producing *E. coli*. The CTX-M types identified were -1, -14, -15, -55 and of these CTX-M-14 was predominant and the only CTX-M ESBL detected on breeding farms (Randall et al 2011, VLA 2010).

3.3.3   Targeted farm visits to ESBL-positive premises

In England and Wales, visits are made where possible by VLA to farm premises on which ESBLPCs have been detected. Visits are not compulsory and have several objectives, including collecting epidemiological information, looking at the prevalence of ESBL-producing *E. coli* in different groups of animals and assessing potential sources of introduction. Advice on disease control and antimicrobial usage may also be given. Where such investigations have been undertakn on cattle farms after an initial isolation from a sample submitted for diagnostic reasons, ESBL *E. coli* are most frequently detected in calves.

3.3.4   Molecular comparison between medical and veterinary isolates

ESBL-producing *E. coli* from animals and humans can be compared at several levels, including the genotype of ESBL, plasmid and host bacterium. Epidemic strains of ESBL *E. coli* have been detected in humans, though isolates from animals tend to be relatively diverse (Girlich et al. 2007; Liebana et al. 2006). A number of techniques may be used to characterise isolates, though currently multi-locus sequence typing (MLST) is often favoured. Coque et al 2008a reviewed the incompatibility group distribution of plasmids involved in the dissemination of specific ESBLs in different European countries. Plasmid MLST is also being utilised as a useful technique for comparing plasmids (Dierikx et al. 2010). Comparing the characteristics of host bacterial strains, ESBL genotypes, plasmids and other genetic elements will be invaluable in tracing the spread and evolution of strains and in detecting potential links between different host animal species and humans as there is little data available that has been collected in a consistent manner.

3.4   Recommendations

Standardised data should be collected and published on the use of antimicrobials in hospital and community settings, as well as in veterinary practice.

Medical and veterinary laboratories should provide prevalence data of ESBLPCs which should include the capacity to identify both epidemic and emergent bacterial clones carrying ESBLs as well as epidemic promiscuous plasmids bearing ESBLs and any increases in prevalence. Data should be provided to

clinical teams and to the local HPU along with details of agreed actions to be taken following a significant rise in cases.

Local data on rates of infection caused by ESBLPCs should be used for risk assessment and should include targeted prevalence surveys in critical areas e.g. intensive care, renal and other specialised units. Data should be collected and disseminated through the CCDC and DIPCs to relevant clinicians with appropriate advice and guidance for action to reduce rates of infection.

4 THERAPY

4.1 Medical

4.1.1 Introduction

In humans, infections caused by ESBL-producing bacteraemia occur predominantly in elderly patients, particularly those who are catheterised or have urinary tract pathology. Most of these patients will have received prior antibiotic therapy particularly quinolones or cephalosporins (Woodford et al. 2004). International travel is also a risk factor (Tham et al 2010). Most ESBL-producing bacteria are resistant to several non-carbapenem β-lactams, including piperacillin/ tazobactam, fluoroquinolones and trimethoprim and many are also resistant to gentamicin. The O25, ST131 clone of CTX-M ESBL-producing *E. coli* often express pathogenicity factors, and a high proportion of infections result in bacteraemia with resultant mortality (Tumbarello et al. 2007). The choice of antibiotic will depend on the severity and site of infection and whether the antimicrobial sensitivity pattern of the organism is known (Table 4.1 lists the agents that have been used). Empirical treatment strategy should be based on the local susceptibility patterns. Delay in adequate therapy will lead to adverse outcomes and increased mortality (Kumar et al. 2006; Schwaber & Carmeli 2007).

4.1.2 Treatment of urinary tract infections

There is consensus that severe infections due to ESBL producers should be treated with a carbapenem, but there is less agreement with respect to uncomplicated urinary tract infections. Oral options are limited and include nitrofurantoin (for lower UTIs only) and fosfomycin. Nitrofurantoin is widely used, but there is a significant failure rate, perhaps associated with undiagnosed ascending infection. Moreover, the duration of nitrofurantoin therapy is longer than normal for a UTI and the drug is poorly tolerated by some patients. Anecdotal reports from Spain favour fosfomycin, which is licensed but not marketed in the UK.

Combinations of agents such as cefixime (or cefpodoxime or pivmecillinam) with agents containing clavulanic acid such as co-amoxiclav (Brenwald et al. 2006; Thomas et al. 2006) have been used to treat UTIs caused by CTX-M ESBL-producing *E. coli*. However, these are unlicensed applications and reports of such use in the literature are rare. These combinations are not effective against Amp C producing Enterobacteriaceae and thus should not be used empirically or to treat infections caused by these organisms. Treatment failures have been reported when pivmecillinam has been used alone (Garau 2008; Livermore et al. 2008). Intravenous options that can be given once a day include gentamicin (also suitable for intramuscular injection) and ertapenem (Garau 2008, Livermore et al. 2003). (Table 4.2)

Mecillinam (licensed and available, but not widely used) also appears active against some producers *in vitro* but there is little clinical data and its MICs are

inoculum dependent. Some centres are co-administering various oral β-lactams – mecillinam, cefpodoxime or cefixime – along with co-amoxiclav (thereby using its clavulanate to protect the mecillinam or cephalosporin). The efficacy of these various treatments needs analysis, or better, prospective clinical trials.

Asymptomatic bacteriuria is common, especially in the elderly. It is important to treat symptomatic infections only, as unnecessary use of antibiotics will select for resistance. Urinary catheters soon become colonised and antibiotics are unlikely to eradicate the bacteria but will select resistant colonising flora (SIGN 2006).

4.1.3 Treatment of bacteraemia and deep sites of infection

Carbapenems, such as meropenem and imipenem are broad-spectrum agents that can be used as empirical therapy for severe sepsis that may be caused by ESBL-producing bacteria. They currently offer the most reliable treatment for severely ill patients, but excessive use will increase resistance levels. Ertapenem and temocillin are reserved mainly for treatment of a bacterium of known sensitivity or a situation where *Pseudomonas* spp. is unlikely (e.g. community acquired), as they are both inactive against *Pseudomonas* spp. but are also stable to AmpC β-lactamase. Temocillin is inactive against Gram-positive bacteria and *Bacteroides* spp*. Carbapenemase-producing *E. coli* and *Klebsiella pneumoniae* are extremely rare in the UK, although ertapenem and temocillin resistance is slightly more common (Livermore et al. 2003; Livermore & Tulkens 2009, Hammerum & Walsh et al 2010). Providing the locally encountered Gram-negative bacilli remain sensitive to gentamicin, gentamicin may be used as empirical therapy in combination with agents other than carbapenems to treat a severe infection. Amikacin has been used as an alternative where gentamicin resistance is common. Once the bacterium has been identified and susceptibilities are known, therapy should be de-escalated if possible to a narrow spectrum agent.

Although tigecycline has activity against ESBL-producing bacteria, urinary levels are low and thus it is not a first line antibiotic for treatment of these infections. It is also inactive against *Proteus* and *Pseudomonas* spp. and resistance in some Enterobacteriaceae has been noted (Anthony et al. 2008; Garau 2008; Hawkey & Finch 2007).

Intravenous therapy with colistin has been used to treat infections due to multi-resistant Gram-negative organisms. Although studies have shown that there has been acceptable efficacy and less nephro- and neuro-toxicity than previously reported, at present its use is mainly reserved for ESBL-producing bacteria that are also resistant to gentamicin and carbapenems (Falagas & Kasiakou 2005).

Antibiotic	Used for	Contraindication	Advantages	Disadvantages/ side effects
Nitrofurantoin 100 mg 6 hourly 7-day course advisable	Oral only Treatment of uncomplicated urinary tract infections	Renal impairment (GFR<60) Not active against *Proteus* spp. and *Pseudomonas* spp.	Widely available. Been in use for > 50 years Resistance rare in *E. coli* more common in other Enterobacteriaceae	Causes nausea and vomiting. Rarely peripheral neuropathy with long-term use Resistance develops on treatment
Fosfomycin 3 g sachet each day for 3 days recommended (IV and oral capsules also available)	Oral Licensed for treatment of uncomplicated urinary tract infections		Resistance rare even in Spain where it is used widely including treatment of severe infections	Not marketed in the UK and thus difficult to obtain Headache or diarrhoea in 10% of patients
Gentamicin Im or IV Refer to guidelines re dosing regimens	Can use for outpatient therapy when given once a day	Contraindicated in severe renal failure	Resistance common in some areas but useful if Strain A is locally predominant	Side effects include nephrotoxicity and vestibular and auditory damage Pre dose levels are required to assess further dosing
Temocillin IV 1 g [2 g in severe infections] IV 12 hourly	Treatment of urinary tract and other infections caused by ESBL and AmpC-producing bacteria sensitive to this agent	Narrow spectrum, inactive against Gram positive bacteria, *Bacteroides* spp. and Pseudomonasb spp. Penicillin allergy	Good in vitro-activity against multi resistant ESBLs including AmpC-producing bacteria	Side effects as for penicillins Low-level serum levels Provenance outside the urinary tract to be established Not used widely in UK
Ertapenem IV 1 g once a day and thus useful for outpatient therapy	Treatment of infections caused by ESBL-producing bacteria sensitive to this agent	Does not cover infections caused by *Pseudomonas* spp. Penicillin anaphylaxis	Effective for treatment of ESBL *E. coli* and convenient ods regime	Side effects as for cephalosporins Seizure rate 0.5% from clinical trials Resistance more likely (but rare) than other carbapenems because of combinations of impermeability with an ESBL or AmpC Not licensed for UTI in EU though licensed for this indication elsewhere
Meropenem IV 1 g 8 hourly	Treatment of severe infections when organism is unknown	Penicillin anaphylaxis	Broad spectrum of activity including *Pseudomonas* spp. and ESBL-producing bacteria	Side effects as per cephalosporins

Imipenem with Cilastin IV 1-2g daily in 3 to 4 divided doses	Treatment of severe infections	Penicillin anaphylaxis Renal Impairment CNS disorders	Broad spectrum of activity including *Pseudomonas* spp and ESBL-producing bacteria	Side effects as for cephalosporins Seizure rate > ertapenem
Doripenem IV 500 mg 8 hourly	Treatment of severe infections when organism is unknown	Penicillin anaphylaxis	Broad spectrum of activity including Pseudomonas spp and ESBL-producing bacteria	Side effects as for cephalosporins Caution in renal impairment
Tigecycline IV 100 mg loading dose followed by 50 mg 12 hourly	Skin and intra abdominal infections	Does not penetrate urinary tract Does not cover infections caused by *Pseudomonas* spp. or *Proteus* spp. Cannot be given to children younger than 12 years of age	Wide range of Gram-negative bacilli including ESBL-producing organisms	Nausea and vomiting in up to one-third of patients
Colistin sulphate IV 1–2 million units 8 hourly (15,000–25,000 units/kg 8 hourly if <60 kg)	Bacteraemia, pneumonia, urinary tract infection caused by sensitive Gram-negative bacteria resistant to other agents	Not active against Gram-positive bacteria, anerobes, *Proteus* spp*., *S*erratia spp., etc. Monitor renal function Discontinue if nephrotoxicity occurs	Wide range of resistant Gram-negative bacteria including ESBL-producing organisms and *Acinetobacter* spp.	Nephrotoxicity (up to 20% in seriously-ill hospitalised patients); neurotoxicity (up to 25% of patients – generally mild)

Table 4.1. Antibiotics commonly used to treat infections caused by ESBL-producing bacteria in humans

Antibiotic	Used for	Contraindication	Advantages	Disadvantages/ side effects
Cefixime 200 mg 12 hourly or **Pivmecillinam** 400 mg 8 hourly or **Cefpodoxime** 100–200 mg 12 hourly all for a total of 7 days with **Clavulanic** acid in the form of **Co-amoxiclav** 375 mg 8 hourly	Oral treatment of uncomplicated UTIs	Penicillin allergy High risk for selecting for super-added infections such as *Clostridium difficile* and *Candida* spp.	Clavulanic acid inhibits ESBLs Cefixime is more stable than amoxicillin to ESBLs	Side effects as for penicillins/ cephalosporins clavulanic acid can induce AmpC enzymes e.g. in Enterobacter spp. possibly negating the effect of inhibiting the ESBL. These Enterobacter spp. are however rare pathogens in community Use as directed therapy

Table 4.2 Combinations of antibiotics that have been used specifically for the treatment of uncomplicated urinary tract infections (UTIs) caused by ESBL-producing bacteria. (These combinations are unlicensed for use in this form and are not effective for the treatment of infections due to AmpC Producing Enterobacteriaceae)

4.1.4 Treatment of infections due to carbapenem producers

Most carbapenemases producers are extremely drug resistant. β –Lactams and Aminoglycosides resistance to the whole class is common. Polymyxins, tigecycline and fosfomycin are the agents with the most frequent *in vitro* activity, but all have limitations (Table 4.3.).

Antibiotic	Advantages and disadvantages
β –Lactams	
No available β-lactamase inhibitor inactivates carbapenemases. β –Lactams resistance to the whole class is common. Carbapenems may still be active vs. producers with low level resistance	
Aztreonam	Is stable to metallo-carbapenemases, including IMP, VIM and NDM, but most producers are resistant owing to co-production of AmpC or ESBL enzymes; It is NOT stable to non-metallo-carbapenemases including OXA-48 and KPC types
Ceftazidime, cefotaxime and aztreonam	These remain active against Enterobacteriaceae with OXA-48 unless these also have AmpC or an ESBL
Temocillin	Is relatively stable to KPC enzymes (not others), but MICs mostly are narrowly out of range at licensed dosage (2g q12h)
Aminoglycosides	
Resistance to the whole class is common	
	Strains with NDM-1 almost always have a 16S rRNA methylase, conferring resistance to all aminoglycosides suitable for human use
	ST258 *K. pneumoniae* with KPC are mostly susceptible to gentamicin (not others)
	Other strains with KPC, VIM, IMP and OXA-48 enzyme are variably resistant to aminoglycosides, reflecting multiple modifying enzymes. Isepamicin is active against some isolates resistant to other analogues, but is not available in the UK

Others	
Polymyxins, tigecycline and fosfomycin	These are the agents with most frequent *in vitro* activity, but all have limitations. Dosage will vary with the patient and infection site, but should be on the principle of 'highest safe' rather than 'minimum potentially effective'; durations should be as standard for the infection type
Colistin sulphate IV 1–2 million units 8 hourly (15,000–25,000 units/kg 8 hourly if <60 kg)	Active vs. >90% of producers. Case reports of successful use in a range of infections due to carbapenemase producers. Significant nephro- and neuro-toxicity and poor lung penetration. Use high dose and possible addition of nebulised colistin in pneumonia.
Tigecycline IV 100 mg loading dose followed by 50 mg 12 hourly	Active in vitro vs. most carbapenem-resistant *E. coli*. Licensed for skin and soft tissue and complicated intra-abdominal infections. Case reports of success in various infections with carbapenemase producers. Low blood concentrations; low urine level; off-label use should be cautious; unsuitable in urinary infections as only 22% excreted in urine. Excess deaths in some trials, esp. ventilator pneumonia (not a licensed indication). Many *Klebsiella* only intermediately susceptible (MIC, 2 mg/L); some resistant.
Fosfomycin 3 g sachet each day for 3 days recommended (IV and oral capsules also available)	Active against most *E. coli* with carbapenemases including NDM-1. Effective in urinary infections. Borderline susceptibility common in *Klebsiella* spp. Risk of mutational resistance. Not licensed for use in the UK, but pharmacists can import.
A few isolates are susceptible to other antibiotics including e.g. chloramphenicol, ciprofloxacin and cotrimoxazole. Most producers, however, are resistant to these drugs.	

Table 4.3 Infections due to carbapenemase producing organisms

4.2 Veterinary

In animals, most isolates of ESBL-producing bacteria identified and investigated by the Veterinary Laboratories Agency do not appear to have caused clinical disease. In contrast to the human situation, many of these isolates come from younger (e.g. calves) rather than older animals. In a number of cases, there has been prior antibiotic treatment of the animal and/or in the herd concerned, but investigations to understand any link between prior use of antibiotics and the carriage of ESBL-producing bacteria are continuing.

Unless ESBL-producing bacteria are causing disease in animals, antimicrobial therapy is not warranted. The identification of ESBL-producing bacteria in specimens should not lead to an automatic instigation of antimicrobial therapy. Should a specimen yield a mixture of bacterial specimens including an ESBL, which is not believed to be the cause of the clinical problem, care should be taken not to choose an antimicrobial that simultaneously selects for ESBL-producing bacteria that are also present. An example would be the treatment of a soft tissue infection caused by *Staphylococcus aureus* with some ESBL *E. coli* also present treated with a 3GC.

Caution should also be observed in choosing an appropriate antimicrobial on the basis of culture and sensitivity results, whether derived from the veterinary practice's laboratory or from a microbiological laboratory. The range of different antimicrobial drugs against which resistance is being assessed does not always

include all the antimicrobials authorised by the VMD for use in a particular species (Table 4.4) and, occasionally, might include (instead and/or as well) drugs authorised only for human use, dependent on local practices in force at the particular laboratory used for testing. Whilst resistant bacteria may be shown to be sensitive to a drug licensed for human use, which may be validly prescribed under the cascade, such a drug may not be appropriate if sensitivity testing has not been carried out for all the available licensed veterinary drugs.

Apparent clinical failure should be assessed by collecting a second specimen for culture to identify resistant strains. Should a specific veterinary clinical infection with a bacterial isolate not respond to antimicrobial therapy when it would normally be anticipated to do so, this should be reported using the Suspected Adverse Reaction Surveillance Scheme (SARSS) to the VMD.

Best practice is in prescribing appropriate antibiotics to treat specific disease on the basis of culture and sensitivity testing results. Other measures are important, such as improving ventilation in cases of respiratory disease, improving (or initiating) cleansing and disinfection. Good bio-security, improving the nutrition plan and /or otherwise reducing stress in the affected animal(s) will help minimise the spread of resistant bacteria.

One of the first cases of a fatal clinical infection in animals caused by an ESBL producing E.coli was investigated by the VLA in 2009. The VLA isolated an ESBL-producing *E. coli* from a dairy cow, which was one of three on the same farm that had died with clinical signs of acute mastitis (infection of the mammary gland). Mastitis is a common problem in dairy cattle frequently caused by *E. coli* as well as other pathogens. Acute fatal mastitis is less common. The udder from one of the three dead cows yielded a profuse growth of ESBL *E. coli*. Histopathological examination of the udder did not reveal marked changes associated with local infection, possibly reflecting the acute nature of the condition. The cow probably died as a result of endotoxic shock which had developed as a result of the mammary infection. The cephalosporin cefquinome had been administered whilst the cows were alive; however, ESBL *E. coli* would in general be expected to be resistant to all veterinary cephalosporins.

Cephalosporin (generation)	Species	Route of Administration	Indications (Summarised from Datasheets)
Cephalexin (first)	Dogs	Oral	*S. intermedius* pyoderma
	Dogs, Cats	Oral	Range of Gram-positive and Gram-negative infections, including skin infections and urinary tract infection (UTI)
		S/C, I/M	
	Dogs, Cats		Range of Gram-positive and Gram-negative infections, including respiratory and skin infections and UTI
		Intra-mammary	Mastitis.
	Cattle	I/M	Metritis, foot infections, wounds, septicaemic mastitis
	Cattle		
Cephalonium (first)	Cattle	Intra-mammary	Dry cow therapy
Cefapirin (first)	Cattle	Intra-uterine	Metritis
		Intra-mammary	Mastitis
Cefoperazone (third)	Cattle	Intra-mammary	Mastitis

Cefovecin (third)	Dogs, Cats	S/C	Range of Gram-positive and Gram-negative infections, including pyoderma, wounds, UTI
Cefquinome (fourth)	Cattle	Intra-mammary	Dry cow therapy
	Cattle	Intra-mammary	Mastitis
	Cattle	I/M	Bacterial respiratory disease, digital dermatitis, foul in the foot, infectious bulbar necrosis, systemic *E. coli* mastitis, colisepticaemia in calves
	Pigs	I/M	Bacterial respiratory disease, MMA, meningitis, arthritis, epidermitis
	Horses	I/M or I/V	Bacterial respiratory disease (*Streptococcus equi, zooepidemicus*), colisepticaemia in foals
Ceftiofur (third)	Cattle	S/C, I/M	Bacterial respiratory disease, metritis, acute interdigital necrobacillosis
	Pigs	I/M	Bacterial respiratory disease, septicaemia, polyarthritis, polyserositis associated with *Streptococcus suis* infection
	Horses	I/M	Bacterial respiratory disease

Table 4.4 Cephalosporins authorised for use in veterinary medicine in the UK, including their summarised datasheet indications

4.3 Recommendations

Appropriate national guidelines on antimicrobial prescribing, and good antibiotic stewardship should be followed and should include education and training of staff. Critically important agents used for treating human infections (e.g. carbapenems) should not be licensed for use in animals. Agents such as carbapenems, glycylcyclines and polymyxins should be used extremely judiciously. Sensitivity tests should be undertaken whenever possible with proper medical assessment before these agents are used.

Professional bodies (e.g. BVA, BSAC) should actively inform practicing clinicians and veterinarians about the problem of ESBLs, via sharing of information in appropriate journals.

Local and national point prevalence surveys on combination therapies or treatment options which have been tried in outbreak and/ or difficult cases, should be published to provide information for others to consider. The efficacy of the various treatment regimes for ESBL-associated urinary tract infections needs analysis, or better, prospective clinical trials.

5 CONTROL OPTIONS

5.1   Introduction

The route(s) of transmission of CTX-M producing strains to animals and the general environment and their cycling between animals, humans and the environment are not well understood, and so effective control measures are, for the most part, currently unproven.

Control options should be based on professional advice, good risk assessment and best practice evidence to decide the level of control required in different settings. Well-established general principles of infection prevention and control still apply. Consideration should be given to an ongoing education programme, which raises awareness and helps maintain vigilance.

Organisations should consider developing guidance to include procedures to be followed for the recognition and management of an outbreak of ESBL-assoicated infection. The importance of co-ordinated working of different organisations cannot be underestimated.
Appropriate national guidelines on antimicrobial prescribing, infection prevention and control, environmental cleaning and decontamination should be followed.

5.2   Medical

5.2.1   Management responsibilities

The Code of Practice (DH, 2009) identifies Chief Executives or equivalent of registered healthcare providers as being ultimately responsible for infection prevention and control in their organisations. Effective prevention and control of ESBLPCs requires joint strategies and a co-ordinated approach between healthcare facilities in hospitals, the community, care homes and general practice. Policies should include specific guidance on alerting all organisations of increased rates of ESBLPC infection and colonisation in any one of them, and informing general practitioners and receiving organisations whenever an affected patient is transferred.

Professional advice and guidance should be sought from the local Infection Control Team (ICT), the Director of Infection Control and Prevention (DIPC) and the Consultant in Communicable Diseases Control (CCDC) via the Health Protection Unit.

Local data on rates of infections caused by ESBLPCs by infection site, age and other risk factors should be used for risk assessment. Data should be collected and disseminated through the CCDC and DIPCs to the relevant clinicians with advice and guidance for action to reduce rates of infection.

All Healthcare providers should have clear guidance on good infection control practices, which include how to deal with multi-drug resistant Enterobacteriaceae, including management of outbreaks. Providers should

produce written information and guidelines for patients and staff on the control of ESBLPCs that are compatible with national guidance and appropriate to their settings.

There should be a regular review of the guidance to ensure compliance with best evidence based practice available.

5.2.2   Clinical responsibilities

Senior medical, nursing, infection control and pharmacy staff in all healthcare settings must understand the significance and importance of ESBLPCs. National and local guidance drawn from the HPA and the local HPU, DIPC and CCDC, should be in place covering appropriate plans for infection prevention and control. Clinical staff should have appropriate training and education in infection prevention and control and antibiotic stewardship.

5.2.3   Laboratory responsibilities

Laboratories should use appropriate methods to identify ESBLPCs. In particular, coliforms should be identified to species level and reliable tests for the detection of ESBLs used. Guidance is provided by the HPA (HPA 2008b). Protocols for appropriate use of reference facilities should be in place. Laboratories should provide results data to the infection control team to carry out local alert organism surveillance for ESBLPCs and this information should be shared with clinical teams and with the local HPU.

5.2.4   Drugs and therapeutics committee

The appropriate local professional group that deals with antibiotics should be kept informed of the level of ESBLPCs in their healthcare environment. They should continuously review the evidence that certain antibiotics favour the emergence and spread of these organisms and modify their prescribing policies, educational programmes and audits to minimize this effect. A local antimicrobial management team should be responsible for monitoring this.

5.2.5   Principles of infection prevention and control of ESBLPCs

Good infection prevention and control standards may be sufficient to prevent and control the spread of ESBLPCs in hospitals, care homes and community. Recent work shows high levels of asymptomatic gut colonisation in nursing home residents which may require screening and cohort isolation if there is an outbreak in a care home.

In normal domestic environments (i.e. patients at home) the risk of transmission to family members is unknown and probably varies with the strain. The risk of true infection occurring in home contacts without indwelling devises or serious underlying disease is low.   Therefore, healthy family and domestic contacts (including children) should be re-assured about their safety but encouraged to maintain sensible high standards of general hygiene. The patient and contacts should wash their hands before eating, after using the toilet and after contact

with urine or faeces. Soiled linen should be laundered at high temperatures. Patients with devices (e.g. urinary and intravenous catheters, tracheostomies) should adhere to strict hygiene and infection control advice from their medical and nursing advisors. Healthcare staff caring for patients carrying ESBL-producing coliforms who require transfer to a hospital, care home or other healthcare facility, should inform the receiving institution.

Hospital outbreaks of ESBLPCs have been recognised for many years. These usually involve *Klebsiella pneumoniae* (predominantly), *Enterobacter* and *Serratia* (KES). These organisms usually colonise the intestine and urinary tract of hospitalised patients and are spread from patient to patient mainly by hands. Prevention and control of spread depends on appropriate infection control precautions by staff, prudent antimicrobial prescribing in hospitals and identification and isolation of cases and carriers.

CTX-M-producing strains of *E. coli* are more virulent and affect predominantly community patients but can spread in hospitals causing a full spectrum of infections. Patients admitted to hospital (or care homes) with ESBL-producing *E. coli* may become the source of nosocomial transmission, and prevention and control strategies for such hospital outbreaks are similar to those required for *Klebsiella*. However, the frequency of ESBL-producing *E. coli* transmission in hospitals is unknown (it is very uncommon for non-ESBL-producing *E. coli* strains) and the routes of transmission in the community are not well understood.

There is general agreement on the principles of infection prevention and control of MDR KES in hospitals and these should continue to be applied to hospitalised patients with ESBL-producing *E. coli* infections while their hospital epidemiology is being elucidated. There is even less of an evidence base for the prevention and control of community infections with ESBL-producing *E. coli* and the guidance given here is pragmatic. The principles of control in both hospitals and the community must be kept under review in the light of emerging new evidence. There must be on-going programmes of education in general infection prevention and control and in prudent antibiotic prescribing. Performance should be monitored by appropriate audits where possible.

5.2.6 General principles

The evidence based for the general principles of control outlined in the table below are drawn from the following publications and reports Friedman et al. 2005; Pratt et al. 2007; Shlaes et al. 1997; Siegel et al. 2007; and STRAMA 2007.

Recommendation Grade	Strength of evidence
[A]	Strongly recommended and supported by systematic review of randomised control trials or individual randomised control trials
[B]	Strongly recommended and supported by non randomised control trials and/or by clinical governance reports and/or the Code of Practice
[C]	Recommended and supported by group consensus and/or strong theoretical rationale

The essential elements of infection prevention and control in humans are to:

- ensure effective general systems of infection prevention and control and prudent antimicrobial prescribing (including education and control) are in place **[B]**
- establish appropriate specific control policies for ESBLPCs based on available evidence **[B]**
- institute appropriate local surveillance to rapidly identify patients with ESBLPCs and any increase in incidence **[A]**
- reduce antimicrobial pressure that favours the emergence and spread of ESBLPCs **[B]**
- minimise interventions such as urinary catheterisation and intubation that encourage colonisation and invasive infection **[B]**
- maintain high standards of hygienic clinical practice and environmental cleaning and decontamination **[B]**
- limit unnecessary patient movement between wards, units and institutions **[C]**
- isolate affected patients if a risk assessment indicates this is necessary **[B]**
- screen high risk patients within high risk units for asymptomatic carriage on admission or during outbreaks and implement appropriate controls for them also **[C]**
- inform receiving wards or institutions of patient status when infected/colonised patients are transferred **[B]**
- during outbreaks, implement an appropriate outbreak control plan. When the outbreak is over, perform root cause analyses to identify actions to prevent repeat incidents **[C]**.

5.2.7 Implementation of enhanced control measures for ESBLPCs

Enhanced control measures should be based on risk assessment and considered when alert organism surveillance identifies that:

- the risk of transmission to others is high (e.g. in hospitals or care homes or where affected patients have diarrhoea, urinary incontinence or are in contact with others with indwelling devices)

- the colonising organism is known, or suspected to be highly multi-resistant, highly transmissible and/or highly virulent

- other individuals likely to be in contact are highly vulnerable to infection and/or at risk of poor outcomes should they become infected.

- the first case of extended-spectrum beta (β)-lactamase producing coliform (ESBLPC) is identified within a unit that has never had any patients with this type of infection before

- the first case of ESBLPC is identified within a unit that has previously been free of ESBLPCs and cares for patients at high risk of poor outcomes if they become infected (e.g. a special care baby unit, renal dialysis unit)

- the incidence or prevalence of ESBLPCs increases beyond the usual institutional level, despite implementation of routine infection control measures

- there is evidence of continued patient-to-patient transmission despite implementation of routine control measures.

5.2.8 Enhanced control measures including incident/outbreak control measures

The Director of Infection Prevention and Control (DIPC) or equivalent lead for infection control should quickly establish the extent and significance of the incident/ outbreak and inform senior management of the organisation.

A risk assessment, to decide whether this is a serious incident that requires convening a multidisciplinary Outbreak/Incident Control Group (OCG) to manage the incident/outbreak should be carried out. This may require rapid action to screen and identify previously unrecognised colonised patients and to analyse ward stays and movements of all affected patients to check if there is evidence of transmission.

If an outbreak is suspected, an OCG should be convened. A risk assessment should be undertaken and dependent on findings, a meeting should be held to review the situation and make a decision on how to manage the incident.

- The OCG should be chaired by the DIPC or nominee and should include the infection prevention and control team, HPU, clinical and managerial representatives, appropriate Senior Management representatives and the local CCDC.

- Other members should be co-opted as required.

- The OCG should obtain expert professional advice from those with experience in infection prevention and control of ESBLPCs either in-house or by outside consultation via the local HPU.

- A case definition must be created and the extent of the outbreak measured by retrospective and prospective case finding. This may require extensive and repeated patient screening, analysis of patient transfers and enhanced surveillance of clinical isolates.

- At an early stage criteria for closing (and then re-opening) the ward or unit to admissions should be agreed based on a risk assessment.

5.2.8.1 *Isolation of hospitalised patients*

In hospitals, patients colonised or infected with ESBLPCs must be isolated in single rooms if possible or cohorted depending on the risk assessment. Risks include the number and severity of infections, number of antimicrobial agents

still effective against the strain(s), case-mix issues such as incontinence, confusion in the elderly and staff skill mix required and available.

Since bowel colonisation is prolonged and untreatable and represents the largest source, control of faecal contamination of the environment and fomites is most important. Consider the use of hydrogen peroxide disinfection or using steam cleaning as part of terminal cleaning.

Affected patients should remain in isolation for the duration of their stay or until a risk assessment and the success of interventions indicates that the risk to other patients is minimal.

5.2.8.2 *Isolation of affected residents in care home*

Isolate affected residents in single rooms or cohorts when indicated by a risk assessment. Note that the risk of transmission is increased if the resident is incontinent of faeces or has a colonised catheterised urinary tract. Discontinue isolation if there is no evidence of on-going transmission, active infection or diarrhoea.

5.2.8.3 *Strict control of antimicrobial agents and antibiotic stewardship*

A local multi-disciplinary group should ensure stewardship measures are in place to promote optimal and safe usage of antimicrobials in order to minimise the acquisition and spread of resistance. There should also be awareness that changes in antimicrobial policy may adversely affect patient outcome. Where practicable, lengths of stay and mortality should be monitored.

A group should review antimicrobial use and consider limiting the use of agents associated with increased prevalence of ESBLPCs, e.g., third generation cephalosporins, aminoglycosides and quinolones.

Agree policies for treating patients with serious infections due to the outbreak organism. Colonised patients should not be treated with antibiotics.

There is no evidence that attempting to decolonise faecal carriers with antimicrobials is effective; protocols have not been established and evidence from SDD studies shows that 3GCs select from ESBLPC faecal carriage.

5.2.9 Organism typing, enhanced surveillance and screening

- The outbreak organism may have identifying characteristics such as a specific antibiogram. If necessary, send isolates to Regional or Central HPA laboratories for typing.

- Be aware that outbreaks may involve more than one strain or species (simultaneous or sequential outbreaks).

- Do not delay control interventions by waiting for typing results.

- Consider extending screening to:

    - All patients on admission to affected wards and high-risk units, such as intensive care units.

    - All patients admitted or transferred from units or institutions known or suspected to have a high incidence of ESBLPCs. Pre-emptively isolate such patients until the results of screening are known.

    - Pre-emptively isolate re-admitted patients known to have had previous colonisation or infection with ESBLPCs until the results of screening are known.

- Screen all potentially affected patients at intervals (e.g. weekly) to assess the effectiveness of control interventions and to determine if transmission has ended.

- Do not screen staff for carriage of ESBLPCs unless other routes of transmission have been eliminated, or there is very strong evidence that a staff member is the source of ongoing transmission.

5.2.10 Enhanced infection control precautions and environmental cleaning

- Assign dedicated nursing staff (and other staff where possible) to the care of ESBLPC patients in cohort areas.

- Implement single patient use of non-critical equipment (e.g. blood pressure cuffs, stethoscopes).

- Ensure that environmental cleaning and disinfection is supervised by staff trained in the role of the environment in transmission of ESBLPCs. Implement appropriate and consistent cleaning and disinfection of surfaces likely to be touched by the patients and staff (e.g. bedrails, carts, bedside commodes, doorknobs and taps).

- Do not culture the environment to identify a possible source of transmission unless the ICT decides this is necessary.

- If transmission continues, despite enhanced control procedures, the ward may need to be closed to admissions (see above). When affected patients are discharged, perform a terminal clean and disinfection before re-opening the ward to further admissions.

- Consider the use of steam cleaning or use of hydrogen peroxide disinfection as part of terminal cleaning.

5.2.11 Education and training of staff

- Implement refresher training and education on clinical risks for acquisition and transmission of ESBLPCs for staff involved in the outbreak.

- Consider the use of appraisals and organisational objective setting in ensuring continuous compliance with best practice.

5.2.12 Communications

- The DIPC or nominee should keep the Management Board informed of events and actions; and the CCDC should inform the HPA and the Director of Public Health.

- Involve the communications team to help prepare and distribute patient, staff and visitor information leaflets on ESBLPCs and keep staff and visitors fully updated on the progress and control of the outbreak.

- Systems should be in place to identify and review all deaths in patients affected by an ESBLPC outbreak. The OCG should agree criteria for deciding whether ESBLPC infection probably or definitely contributed to death, which must be recorded on the death certificate. The OCG should maintain a daily updated list of the numbers of affected patients and the number of deaths probably and definitely associated with the infection.

- The OCG meetings must be recorded and a report produced when the outbreak/incident has ended. A final report should include a root cause analysis of the outbreak, lessons learned and proposals to prevent future incidents. This report must be shared with the Management Board and the OCG and if appropriate with national organisations.

5.3   Carbapenem- resistant Enterobacteriaceae

Carbapenem-resistant Enterobacteriaceae remain an uncommon cause of human infections in England. They are extensively antibiotic-resistant and there are limited therapeutic options available for serious infections.

Carbapenem resistance can usually be detected by routine tests in clinical laboratories. However, confirmation of the resistance mechanism requires molecular analysis by a reference laboratory.

Enhanced control measures as outlined above are applicable to prevent and control the spread of these organisms. The following additional control measures should be considered if a patient is identified as being colonised or infected with carbapenem-resistant Enterobacteriaceae.

5.3.1 Endoscopy and related procedures

It should be noted that

- several endoscope-related transmissions of carbapenem resistant organisms have been reported in the UK and France. Similar risks are likely e.g. with colonoscopy.
- care should be taken to disinfect or protect equipment used with endoscopes, e.g. cameras that do not undergo the same routine steralisation.

Trusts should ensure that relevant staff understand the risks and take aequate precautions.

> www.mhra.gov.uk/Publications/Postersandleaflets/CON2022584 and www.mhra.gov.uk/Publications/Safetywarnings/MedicalDeviceAlerts/CON 087958

5.3.2 Additional control options - key actions

- Any Enterobacteriaceae isolate suspected of being carbapenem-resistant must be sent to the HPA reference laboratory for confirmation and typing.

- All patients colonised or infected with carbapenemase-producing Enterobacteriaceae must be placed in single room isolation.

- A six month retrospective review of microbiology records should be carried out to check that previously unrecognised cases have not occurred.

- If previously unrecognised cases are identified consideration should be given to a point prevalence survey of high risk facilities (e.g. intensive care units) and screening of other patients with epidemiological links to the index case to identify any additional patients colonised with carbapenemase producing Enterobacteriaceae.

- If other cases are identified amongst patients with epidemiologic links (i.e. suggesting patient-to-patient transmission) strict infection control and prevention measures should be reinforced, supported by weekly surveillance cultures until no new cases of infection or colonisation are identified.

- If an uncontrolled outbreak is identified and/or is affecting very high risk patients (such as neonates) consideration should be given to closing the ward to further admissions while the outbreak is brought under control (see above).

- When the point prevalence survey fails to identify other colonised patients suggesting that infection control measures are adequate active surveillance can be discontinued.

- Consideration should be given to carrying out regular point prevalence surveys to monitor the prevalence of these organisms.

- Routine microbiological surveillance is not recommended at present.

- Screening should consist of rectal or perirectal swabs; limited data indicate that surveillance screening of stool specimens, rectal swabs or perirectal swabs might produce higher yields than testing of other body sites (e.g., nares or skin).

- When affected patients are discharged, perform a terminal clean and disinfection before re-opening the room or ward to further admissions. Consider using either steam cleaning or use of hydrogen peroxide disinfection as part of terminal cleaning. Cosider the use of hydrogen peroxide disinfection or using steam cleaning as part of terminal cleaning.

5.4   Veterinary

Some similar strategies to those described above have been pursued on occasions where the VLA has identified CTX-M producing strains in animals. Further investigations on farms where ESBL isolates have been detected are being carried out where possible by the VLA with the following objectives to:

- assist with control of endemic disease problems to minimise the need for use of antimicrobials on the farms;

- advise, in conjunction with the private veterinary surgeon, on appropriate antimicrobial usage to treat the endemic disease problems currently affecting the herds;

- assist with control of the ESBL *bacteria* that have been identified and advise on practical measures to attempt to limit their spread and hasten their decline or elimination;

- monitor how the situation is developing by collecting and testing further samples during follow up visits;

- investigate possible sources of the ESBLs (though it should be noted that it is notoriously difficult to determine the source of a bacterium once secondary spread and multiplication have occurred after its introduction to these complex ecosystems).

5.4.1 Approach to control when ESBLPCs are identified in animals

In general, for animals, the risks of acquiring resistant bacterial infections also tend to be highest in those individuals that are ill and/or under antimicrobial treatment, followed by those kept in higher stocking density situations and/or mixed with other animals on a regular basis and lowest for animals kept singly or extensively. However, individually kept companion or pet animals may represent (and be at risk of) higher potential levels of human transmission to direct contacts than transmission to or from other animals. Thus, specific policies developed for these different facilities must be based on an initial risk assessment. Decisions on how to manage affected individual(s) should be

based on an assessment of all pertinent issues when each new case is identified.

To date, when VLA have identified ESBLPCs in animals they have responded by means of advice, such as described above. On previous occasions where ESBLPCs have been identified, the possibility of imposing movement restrictions on the animals involved and/or their products, or even culling them, to prevent the spread of the ESBLPC has been considered in discussions with stakeholders, including public health officials. Up until now it has not been thought appropriate to implement these types of control measures on any animals on the farms involved for the following reasons.

- The majority of strains of ESBL *E. coli* that VLA have isolated are not known to be a significant cause of disease in animals – *E. coli* is present in the intestinal tract of most healthy animals as a normal commensal organism (an organism that occurs naturally in the animal gut).

- The vast majority of ESBLPCs identified in animals by the VLA have been in commensal rather than pathogenic organisms. To date there was a possible link between the resistant bacteria and clinical disease only on a handful of farms where the VLA has identified an ESBLPC.

- The *E. coli* strains identified to date are not known to be those commonly associated with human infections.

There is currently ongoing surveillance of clinical veterinary diagnostic material submitted to the VLA for ESBLs. However, organisms containing these resistance genes may be present on livestock premises which have not yet been detected, because not all affected premises will necessarily submit diagnostic material to VLA. As not all affected premises are being identified, the imposition of movement restrictions on known positive premises is unlikely to be effective at containing spread.

- Current surveillance for ESBLPCs in the UK is dependent on the submission of clinical veterinary diagnostic material to the VLA, SAC and AFBI. Automatic imposition of control measures, such as movement controls, because of the isolation of ESBLPCs could have significant economic and/or other animal management consequences, including compromising welfare in certain circumstances. Therefore, the automatic imposition of specific controls could lead to a significant decrease in the submission of bacteriology samples from farm animals to these government laboratories. Such a change would impede future surveillance and the understanding of trends for many different issues, not just ESBLs.

- ESBLPCs have the ability to exist in the environment and colonise a number of different animal species, including wildlife vectors such as birds. This aspect of the ecology of the bacteria means that controlling its spread is extremely difficult because of the many and varied potential routes of dissemination and the diverse environmental niches that it can occupy.

In future, as further ESBLPCs are isolated by the VLA, a risk assessment of the circumstances relating both to the farm and to the type of bacteria and its likely impact on both animal and human health will be considered in deciding the most appropriate response in terms of either monitoring, controlling and/or attempting to eradicate the resistant bacteria. Options such as imposing movement restrictions on the animals involved and/or their products, or possibly culling affected animals in extreme situations (in addition to the advisory approach and monitoring that VLA are currently undertaking) will be selected as appropriate to the situation.

The VLA can be contacted for suitable advice via the nearest Regional Laboratory if private veterinary laboratories isolate ESBLPCs, or if individual veterinary surgeons require specific advice on managing a case involving ESBLPCs.

In addition, should a specific veterinary bacterial isolate not respond to antimicrobial therapy when it would normally be anticipated to do so, such as in the case of an ESBLPC, as ESBL-mediated resistance is currently uncommon in veterinary isolates (and the same approach would also apply to other rare or novel forms of antimicrobial resistance) this should be reported using the Suspected Adverse Reaction Surveillance Scheme (SARSS) to the VMD. SARSS is a national surveillance scheme run by the VMD, and reports may be made on-line or through the yellow report form bound into the NOAH Compendium or available directly from the VMD. If failure of antibiotics to be effective because of ESBL-mediated resistance (or other novel mechanisms) is routinely reported to SARSS this would provide a valuable extra means to monitor occurrence of such resistance.

5.4.2 Control measures

A range of control measures to promote local containment in ESBL cases identified by VLA on farms in England and Wales have been considered. These include:

- thorough terminal hygiene and adoption of 'all-in, all-out' management systems;

- review of antimicrobial prescribing measures to minimise selection for ESBL resistance;

- the use of disinfection to promote local containment.

5.5   Recommendations

All organisations should consider developing clear guidance on good infection control practices to include recognition and management of an outbreak of multi-resistant Gram-negative bacterial infections (including ESBLs infections). The guidance should be based on national guidance and be appropriate for their

settings and should include education and training of staff and appropriate written information for patients and the public.

A review of the practicalities of changing the amount, type and timing of administration of antimicrobials to animals to reduce the prevalence of ESBLPCs should be considered. The use of cephalosporin (and quinolone if appropriate) medication should be stopped or greatly reduced, with other medicines being used in their place on any farm known or suspected to have ESBLs.

The practice of co-administration of a 3GCs with Marek's disease vaccine to young chicks should stop.

6   OUTCOME MEASURES

6.1   Introduction

Outcome measures relevant to both medical and veterinary medicine include process, patient and public health outcomes.

6.2   Medical outcomes

6.2.1   Process outcomes

All healthcare providers should have appropriate infection prevention and control procedures in place for the prevention and control of ESBLPCs as outlined in the Code of Practice (DH 2009).

6.2.2   Patient outcomes

Mandatory surveillance of MRSA bacteraemia rates by Primary Care Trust, with public reporting has been effective in promoting improvements in the control of MRSA. A specific quarterly report, should be published on episodes of ESBLPC bacteraemia rates to assess the overall impact of control activity. Consideration should be given to use the mandatory *E. coli* bacteraemia surveillance to monitor prevalence.

A second assessment could be the mandatory incidence/prevalence of ESBL-producing *E. coli* in UTIs, again measured by episode (perhaps the first isolate in each patient). This could be done by repeated surveillance of laboratory reports. The aim would not be to have league tables but for Trusts to show local reductions in rates year on year. Selective surveillance of UTIs in general practice, outpatients, general medical (including elderly patients) and surgical wards and specialities such as intensive care could also be done. Specialities with chronic or recurrent urosepsis, such as renal medicine and urology, would be inappropriate sites for monitoring.

It is vital to remember that certain areas of the country will have higher ESBLPC rates than others. This is due to variation within different communities of multiple gene pools, bacterial strains and importation in the gut from other areas of the world. As control does not rest with an individual institution, the construction of league tables to measure improvement of organisations should be avoided.

6.3   Veterinary outcomes

Animal welfare is of concern to society as a whole. Antimicrobials are not the only means of sustaining animal health and welfare but, in specific situations, may be the only means to safeguard animal health and avoid animal suffering. Therefore, banning even some aspects of currently permitted veterinary antimicrobial use (such as a possible ban of certain currently authorised veterinary medicines as previously proposed by the Chief Medical Officer) may well cause increased animal suffering. Similarly culling animals as a means of controlling antimicrobial resistance will be perceived with mixed opinions by

society as a whole. Probably the most contentious aspect would be if UK or EU inspired control measures meant that a pet could no longer be successfully treated in the UK. An unintended consequence could be an increase in such animals being taken abroad for treatment with the products banned from veterinary use in the UK and the consequent risk of their acting as a vector for bringing back other resistant organisms on their return.

Another veterinary specific outcome measure is that the majority of veterinary antimicrobials are prescribed for use in livestock. The vast majority of these animals are kept for commercial reasons. The implementation of UK (or EU)-only antimicrobial resistance control measures could put the economic viability of those keeping livestock at a disadvantage.

This could be both due to an increase in costs per unit of produce (either as fewer animals survive (whether due to death because of illness or due to culling to control the spread of antimicrobial resistance) to be productive (e.g. produce milk or eggs or to be slaughtered in premium condition), or because overheads per unit of production must be increased as a consequence (examples may be that livestock have to be kept more extensively or in better buildings to minimise risks of becoming infected, such as avoiding pneumonia by building better designed, well-ventilated buildings).

Such measures would have a direct affect on the economics of livestock farming in the UK and could also lead to an increase in imported foodstuffs not produced under such stringent requirements. As a consequence, measures suggested to help control antimicrobial resistance that may be proposed at a UK-only or EU-only level could place the UK population at a greater risk in antimicrobial resistance terms. Similarly protecting the UK population (human and animal) by trade measures on imported foods and livestock would be seen internationally as a trade barrier to protect British farming because of the global economic significance of livestock farming and so may be impossible to implement.

Unless consumers are prepared to pay a premium for food produced by means designed to lower the risk of transmitting antimicrobial resistance (while not compromising animal welfare) the potential for unintended consequences of certain measures that may be used to control antimicrobial resistance is high. In fact some measures could ultimately put consumers at higher risk, for example by exporting livestock keeping from the UK and then importing animal derived produce produced to poorer standards (in terms of control measures applied to minimise antimicrobial resistance risks) than would have been met in the UK.

6.3.1 Process outcomes

Veterinary treatment to animals, with the exception of some statutory disease control measures, provided on a private, fee-paying basis. Practice and professional standards are governed by the Royal College of Veterinary Surgeons and the British Veterinary Association and the Responsible Use of Medicines in Agriculture Alliance provide advice to veterinary surgeons on the responsible use of antimicrobials. There are practical divisions between

companion animal and farm animal practice. The VMD oversees the authorisation of veterinary medicines; Defra and the devolved administrations in Scotland, Wales and Northern Ireland have overall responsibility for animal health. The VLA provides large animal diagnostic laboratory facilities in England and Wales; AFBI and SAC provide a similar function in Northern Ireland and Scotland, where SAC also provide diagnostic facilities for companion animals. A number of private veterinary laboratories and some human clinical laboratories provide laboratory testing facilities for companion and other animals. All of the above have a role in some or all of the following:

- Raise awareness of ESBL among the veterinary profession.
- Ensure adequate laboratory support to provide an accurate diagnosis.
- Provide treatment options for veterinary ESBL infections and antimicrobial prescribing guidelines/policies.
- Undertake infection control measures in the veterinary setting (veterinary practice and farm environment).

6.3.2 Animal patient outcomes

The benefits to animal health of 3GC treatment with possible negative effects on animal health (treatment failure in the individual animal or widespread development and spread of ESBL resistance) should be balanced. The potential of possible negative public health outcomes should also be considered. To support this there should be:

- adequate provision of accurate laboratory diagnosis
- awareness of the issues and treatment options amongst the veterinary profession
- antimicrobial prescribing policies and their influence on the development of ESBL infections in the veterinary setting
- appropriate treatment of ESBL infections
- appropriate surveillance and reaction to surveillance results
- development of guidance relating to usage of drugs of last resort in companion animal patients.

6.3.3 Veterinary and medical public health outcomes

A major public health goal should be the preservation of the efficacy of carbapenems as treatment options for human infections. There are no carbapenems currently authorised specifically for veterinary use and that appears to be the situation globally. However, carbapenems are being used in companion animal practice in the UK under the prescribing cascade to treat some resistant infections in pets. The extent of such usage is unknown but is probably limited.

Factors allowing the environmental dissemination of resistance genes should be considered because these may provide a mechanism for the introduction of resistance genes into the animal population. The presence of plasmid addiction systems on some successful epidemic plasmids mean that slow dissemination and ongoing persistence of resistance plasmids in bacteria in animals may be

able to occur independently of any antimicrobial usage. The widespread environmental release of carbapenemase resistance genes might, therefore, provide a source for entry of those genes into the animal population and consequent passage along the food chain.

Surveillance for types, spread and prevalence of ESBL resistance should be considered:

- in clinical diagnostic veterinary material, which would provide an early indication of the diversity of ESBLs present in animals.
- through abattoir surveillance of animals to provide an indication of ESBL resistance entering the food chain.
- through comparison of medical and veterinary data to look for common types of ESBL plasmids or *E. coli* and other bacterial isolates carrying ESBLs occurring in both humans and animals.
- through source attribution – the number of human ESBL infections which may result from contact with animals or food derived from animals.
- via the degree of spread of ESBL resistance into zoonotic organisms especially *Salmonella*.

6.4   Recommendations

The *Health and Social Care Act 2008 Code of Practice on the prevention and control of infections and related guidance* (DH 2009) should be adhered to.

Quarterly reports on ESBLPC bacteraemia rates and selective surveillance of urinary tract infections (UTIs) caused by ESBLPCs in primary and secondary care should be published by the Health Protection Agency (HPA).

The HPA Microbiology Services Division network of automated antimicrobial resistance surveillance systems should be used to support surveillance. Surveillance for types, spread and prevalence of ESBL resistance should be continued in clinical diagnostic veterinary material.

Antimicrobial prescribing policies should be developed. Adherence to such policies and their consequential influence on the development and spread of ESBLPC infections in the veterinary setting should be monitored.

7   RESEARCH GAPS

7.1   Introduction

Whilst much has been discovered since ESBL-producing *E. coli* began to proliferate in the UK and elsewhere around 2003, much remains uncertain. Some major resolvable issues are highlighted below, but others will only be answered by good surveillance and the passage of time. The most important question is: will ESBL *E. coli* remain a problem of hospital and 'complicated community' infections or will they become prevalent in simple cystitis, as in South and South East Asia or will they reduce in their occurrence, perhaps as clinicians move away from cephalosporins and quinolones, largely predicated on concern that these also select for *Clostridium difficile*? The second question for consideration is whether ESBLPCs will extend their clinical impact beyond that currently exerted by ESBL *E. coli.*

7.2   Surveillance

7.2.1   Prevalence in urinary infections

Although complicated urinary tract infections (UTIs) are the main site for human infections with ESBL *E. coli* current surveillance is weak.   This is being addressed by installation and networking of Vitek machines across the HPA's Microbiology Services Division network, but there are still limitations with this strategy: (i) that many UTIs are treated empirically with no specimen taken; (ii) that UTIs where a specimen is taken disproportionately represent difficult/recalcitrant cases; and (iii) that review of patient notes will be required to categorize UTIs as complicated or not. Networking of the automated systems should therefore only be seen as the first step towards better surveillance.

7.2.2   Prevalence of gut carriage of ESBL *E. coli* in different population groups

This is critical, since most *E. coli* urinary infection is by strains that were already resident in the human gut, but has only been studied to a limited extent in the UK and elsewhere. A survey of faecal specimens passing through York Hospital in 2003 found ESBL-positive isolates in 2%, but the investigation was potentially distorted because the source patients were being investigated (in the most part) for diarrhoeal diseases and may have had an abnormal gut flora. Recent studies in 18 Belfast care homes found 40% of residents carried ESBL *E. coli*, a similar rate to that found in Argentina but the representativeness of this finding for the wider UK is unknown. A small study in Spain found 4% carriage in the general public, whilst another study among job applicants at a Saudi hospital found a carriage rate of 13%. In Bolzano, Italy, 70% of residents had ESBL producers and 5% had strains with metallo-carbapenamases.

We would suggest that a wider surveillance of gut carriage of ESBLs in humans should be incorporated into the Infectious Intestinal Disease (IID) programme or into the pilot studies for colonic cancer screening.   Alternatively, sewage sampling could be undertaken in rural and urban areas.

7.2.3 Carriage in domestic and imported food

Farm sampling in the UK indicates moderate carriage rates of ESBL-positive *E. coli* among food-animal species. Limited previous work has found a higher prevalence of ESBLs in certain imported foodstuffs than in domestically reared produce (Warren et al., 2008). It is unclear whether imported food is a major reservoir of ESBL-positive *E. coli* and, if so, whether the strains and enzymes present have any relationship to those found in human infections. In Warren et al's study, which examined the flora of supermarket chickens bought in the West Midlands found frequent carriage; the main ESBLs identified were CTX-M-2 and -9- group enzymes, not CTX-M-15. Since CTX-M-2 is extremely rare in *E. coli* from human infections in the UK, the clinical significance is uncertain. Nevertheless, this sampling represents just one food type from one region therefore this is an area which warrants wider and systematic investigation.

7.3 Laboratory management

The effects of using rapid detection methods (ideally at 'point of care') in the management and outcome of patients and appropriate antibiotic usage needs to be investigated.

7.3.1 Molecular investigation of strain/enzyme traits

7.3.1.1 *Reasons for the success of ST131 and its CTX-M-15 plasmids*

There are limited data to show that ST131 occurred as a quinolone- (but not cephalosporin-) resistant lineage before it acquired plasmids encoding for the CTX-M-15 enzyme, and to show that it has acquired different CTX-M-ESBL encoding plasmids at different times and places, some of which encode, for example aminoglycoside resistance and some of which do not. This implies that the lineage has survival traits independent of its cephalosporin resistance. Early work failed to identify pathogenicity factors specific to the A–E strains in the UK, and showed variation in pathogenicity factor carriage among them. However, these studies pre-dated the realisation that all these five were ST131 variants; these aspects therefore need re-review and further investigation.

7.4 Novel management strategies

7.4.1 Selective gut decontamination

Selective gut decontamination has the potential to reduce the carriage, and the incidence of nosocomial infection by multi-resistant Gram-negative bacteria. It has not been widely adopted outside the Netherlands, due to fears about long-term resistance consequences. Nevertheless, it may be worthwhile to review its utility in patients carrying ESBL producers and about to undergo immunosuppressive therapy. Many regimens have used a quinolone or a 3GC given systemically (to which most ESBL producers would be resistant) along with non-absorbed colistin. Therefore, the effectiveness of selective gut decontamination needs to be reviewed.

7.4.2 Gamma-radiation

If food, rather than human to human oro-faecal transmission, is shown to be a major vector of ESBL-positive (or quinolone resistant) *E. coli* in the UK, then the utility of gamma-radiation (cold pasteurisation) in reducing or eradicating food contamination may warrant re-consideration.

7.4.3 Vaccine

In 2004, the Veterinary Laboratories Agency (VLA) isolated the first ESBL *E. coli* from a UK cattle farm. Despite changes in antimicrobial usage and other aspects of farm management, the prevalence of ESBL *E. coli* continued to rise. Following administration of an autogenous *E. coli* vaccine, a significant reduction in the prevalence of ESBL *E. coli* was observed. To what extent this reduction in prevalence was due to vaccination or to other factors is unknown, but this observation does raise the question as to whether vaccination could represent a possible means of controlling ESBLs in people and/or animals in the future. Defra have recently commissioned the VLA to replicate the vaccination of ESBL-colonised calves to determine whether vaccination was a significant factor in the observed reduction in ESBL prevalence. Should vaccination be found to have a significant effect on ESBL prevalence, further research to optimise the vaccine approach would be necessary before this approach could be used for management of clinical disease and/or individuals carrying ESBL *E. coli.*

7.4.4 Environmental contamination

Environmental contamination with ESBL-carrying bacteria, and any consequent human–animal cycling/recycling is not well understood. Some farms feed waste milk to calves whilst others dispose of it in slurry and/or use it to treat land. Such uses present a risk of environmental contamination, and the potential significance of these practices needs to be assessed.

Work to determine the importance of this route of infection and persistence of ESBL-positive organisms is required. Should human–animal cycling be found to be a significant route of transmission, further research into appropriate methodologies to minimise such transmission may be appropriate.

7.4.5 Antibiotic treatment

It has become apparent during a number of recent VLA investigations on farms where ESBLs have been identified, that ceftiofur has been in use (generally to treat clinical disease or metaphylactically). However, we do not currently have information on antibiotic usage on the majority of the farms where VLA have so far isolated ESBLs. Therefore, in future where VLA carry out such investigations, specific information on antimicrobial usage should be recorded, and the results analysed against similar paired control farms where no ESBLs have been identified. This will enable possible risk factors associated with, for example antimicrobial selection and manner of use to be assessed and ensure

that current best practice guidance for the prescribing and usage of antimicrobials in animals is appropriate.

Defra/VMD have commissioned VLA to carry out research in this area. Appendix 3 provides details of currently funded Defra research on ESBLs.

7.5 Recommendations

A wider surveillance of gut carriage of ESBLPCs in humans should be incorporated into Infectious Intestinal Disease (IID) programmes.

Carriage of ESBLPCs in domestic and imported foods warrants further investigation.

Point of care' testing or other rapid detection methods for ESBLPCs should be explored.

The effectiveness of selective gut decontamination needs to be reviewed.

Further research into novel therapies (e.g. gamma-radiation of food and the use of vaccines) should be undertaken.

Further research into routes of transmission and human–animal cycling should be carried out and if appropriate, further research into methodologies to minimise such transmission. The possibility of reducing ESBLPCs in human and animal waste prior to release into the environment should be evaluated as a control option.

Research on the carriage patterns of resistant bacteria in animals compared to humans and the influence of the usage of various antimicrobials would be useful.

8 SUMMARY OF RECOMMENDATIONS

Characterisation

1. All clinical laboratories should identify coliforms to species level and use reliable tests for detection of ESBLPCs adhering to EUCAST breakpoints whilst testing all presumptive ESBL- producing isolates for the presence of ESBLs.

2. All clinical laboratories should report overall prevalence of ESBL phenotypes on coliforms and they should increase the usage of rapid ESBL tests to ensure patients are given optimal treatment.

3. Protocols for appropriate use of reference laboratories should be in place. These should include any Enterobacteriaceae isolate suspected of being carbapenem-resistant must be sent to the HPA reference laboratory for confirmation and typing. All reference laboratories should be capable of providing genotyping of both ESBL genes (e.g. CTX-M) and producer strains to elucidate epidemiology and support surveillance for new successful clones.

Transfer pathways

1. Further work should be carried out to understand what happens to bacteria with antimicrobial resistance genes in human and animal waste during storage and associated processes before it is applied to land.

2. The carriage rate of ESBLPCs in the healthy human population and in travellers to high prevalence areas should be determined as denominator data from which to compare rates of ESBLPCs in patients with exposure to healthcare facilities.

3. The prevalence of ESBL-carrying organisms or resistance determinants in retail food samples, environmental samples and all categories of food handlers should be determined to elucidate the resistance gene cycle.

Surveillance

1. Standardised data should be collected and published on the use of antimicrobials in hospital and community settings, as well as in veterinary practice.

2. Medical and veterinary laboratories should provide prevalence data of ESBLPCs which should include the capacity to identify both epidemic and emergent bacterial clones carrying ESBLs as well as epidemic promiscuous plasmids bearing ESBLs and any increases in prevalence. Data should be

provided to clinical teams and to the local HPU along with details of agreed actions to be taken following a significant rise in cases.

3. Local data on rates of infection caused by ESBLPCs should be used for risk assessment and should include targeted prevalence surveys in critical areas e.g. intensive care, renal and other specialised units. Data should be collected and disseminated through the CCDC and DIPCs to relevant clinicians with appropriate advice and guidance for action to reduce rates of infection.

Therapy

1. Appropriate national guidelines on antimicrobial prescribing, and good antibiotic stewardship should be followed and should include education and training of staff. Critically important agents used for treating human infections (e.g. carbapenems) should not be licensed for use in animals. Agents such as carbapenems, glycylcyclines and polymyxins should be used extremely judiciously. Sensitivity tests should be undertaken whenever possible with proper medical assessment before these agents are used.

2. Professional bodies (e.g. BVA, BSAC) should actively inform practicing clinicians and veterinarians about the problem of ESBLs, via sharing of information in appropriate journals.

3. Local and national point prevalence surveys on combination therapies or treatment options which have been tried in outbreak and/ or difficult cases, should be published to provide information for others to consider. The efficacy of the various treatment regimes for ESBL-associated urinary tract infections needs analysis, or better, prospective clinical trials.

Control options

1. All organisations should consider developing clear guidance on good infection control practices to include recognition and management of an outbreak of multi-resistant Gram-negative bacterial infections (including ESBLs infections). The guidance should be based on national guidance and be appropriate for their settings and should include education and training of staff and appropriate written information for patients and the public.

2. A review of the practicalities of changing the amount, type and timing of administration of antimicrobials to animals to reduce the prevalence of ESBLPCs should be considered. The use of cephalosporin (and quinolone if appropriate) medication should be stopped or greatly reduced, with other medicines being used in their place on any farm known or suspected to have ESBLs.

3. The practice of co-administration of a 3GCs with Marek's disease vaccine to young chicks should stop.

Outcome measures

1. The *Health and Social Care Act 2008 Code of Practice on the prevention and control of infections and related guidance* (DH 2009) should be adhered to.

2. Quarterly reports on ESBLPC bacteraemia rates and selective surveillance of urinary tract infections (UTIs) caused by ESBLPCs in primary and secondary care should be published by the Health Protection Agency (HPA).

3. The HPA Microbiology Services Division network of automated antimicrobial resistance surveillance systems should be used to support surveillance. Surveillance for types, spread and prevalence of ESBL resistance should be continued in clinical diagnostic veterinary material.

4. Antimicrobial prescribing policies should be developed. Adherence to such policies and their consequential influence on the development and spread of ESBLPC infections in the veterinary setting should be monitored.

Research gaps

1. A wider surveillance of gut carriage of ESBLPCs in humans should be incorporated into Infectious Intestinal Disease (IID) programmes.

2. Carriage of ESBLPCs in domestic and imported foods warrants further investigation.

3. 'Point of care' testing or other rapid detection methods for ESBLPCs should be explored.

4. The effectiveness of selective gut decontamination needs to be reviewed.

5. Further research into novel therapies (e.g. gamma-radiation of food and the use of vaccines) should be undertaken.

6. Further research into routes of transmission and human–animal cycling should be carried out and if appropriate, further research into methodologies to minimise such transmission. The possibility of reducing ESBLPCs in human and animal waste prior to release into the environment should be evaluated as a control option.

7. Research on the carriage patterns of resistant bacteria in animals compared to humans and the influence of the usage of various antimicrobials would be useful.

APPENDIX 1
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APPENDIX 2
GLOSSARY OF TERMS

Aerobic bacteria

An aerobic bacteria is an organism that can survive and grow in an oxygenated environment

AFBI

Agri-Food and Biosciences Institute (of Northern Ireland)

AmpC

A type of beta (β) lactamase

Antibiotic

A substance produced by or derived from a micro-organism, which selectively destroys or inhibits the growth of other micro-organisms

Antifungal

Products that are destructive to or suppress the reproduction or growth of fungi

Antimicrobial

A compound which at low concentrations exerts an action against micro-organisms and exhibits selective toxicity towards them. The term includes any substance of natural, synthetic or semi-synthetic origin that is used to kill, or inhibit the growth of micro-organisms. Antimicrobials include antibiotics, disinfectants, preservatives and other substances

Antimicrobial resistance

The ability of a micro-organism to grow or survive in the presence of an antimicrobial that is usually sufficient to inhibit or kill micro-organisms of the same species

Antiprotozoal

A drug primarily used in the treatment and/or prevention of protozoal infections

ARHAI

Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infections (ARHAI). Established in April 2007 to provide practical and scientific advice to the Government on strategies to minimise the incidence of healthcare associated infections (HCAI) and to maintain the effectiveness of antimicrobial agents in the treatment and prevention of microbial infections in humans and animals

ARMRL[*]	Antibiotic Resistance Monitoring and Reference Laboratory is the national reference laboratory responsible for the detection and investigation of antibiotic resistance in medical isolates
Asymptomatic bacteriuria	Asymptomatic bacteriuria is the presence of bacteria in the urine without the accompanying symptoms of a urinary tract infection
ATCC	Amercian Type Culture Collection
Bacteria	A large group of single-celled, prokaryote (cells that lack membrane-bound nuclei) micro-organisms
β-Lactam	Semi-synthetic antibiotics derived from penicillin G or cephalosporin C, natural antibiotics produced by the mould *Cephalosporium acremonium.*
β-lactamase enzymes	Enzymes produced by some bacteria and that are responsible for their resistance to beta (β) - lactam antibiotics. Whilst a broader definition would include all β-lactamases capable of hydrolysing 3GCs (Giske et al. 2009), this joint group has retained the more widely used and accepted definition outlined below.

ESBLs are β-lactamase enzymes, generally acquired rather than inherent to a bacterial species, that confers resistance to oxyimino-cephalosporins (but not carbapenems). Some are mutant derivatives of well-established plasmid-mediated β-lactamases (e.g. TEM/SHV) which, in their un-mutated form, are not able to degrade oxyimino-cephalosporins. Others (e.g. CTX-M types) have been mobilised on plasmids from chromosomal genes in environmental/opportunistic bacteria.

Plasmid-mediated AmpC β-lactamase types were also considered in this report. These too have been mobilised from the chromosomes of environmental/ opportunistic bacteria and can confer resistance to oxyimino-cephalosporins. However, they belong to a different molecular class to the generality of ESBLs and have |

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[*] Suspect isolates of carbapenemases producers should be sent for confirmation to ARMRL,HPA Microbiology Services Colindale, 61 Colindale Ave., London, NW9 5EQ for the attention of Dr David Livermore

	significant differences in spectrum and susceptibility to inhibitors.
Breakpoints	An organism is defined as susceptible or resistant at a level of antimicrobial agent activity (breakpoint) in a defined phenotypic test system associated with a high likelihood of treatment success or failure
BSAC	British Society of Antimicrobial Chemotherapy
Carbapenemases	These are β-lactamase enzymes (e.g. KPC, NDM etc) that are capable of destroying carbapenem antibiotics (e.g. imipenem, meropenem etc) and 3GCs
Cephalosporin	A large class of antibiotics similar both chemically and in mode of action to penicillins. Cephalosporins are grouped into 'generations' by their antimicrobial properties. The first cephalosporins were designated first-generation, whereas, later, more extended-spectrum cephalosporins were classified as second-, third- or fourth-generation. Each newer generation of cephalosporins has significantly greater Gram-negative antimicrobial properties than the preceding generation, in most cases with decreased activity against Gram-positive organisms
Chromosomes	A threadlike strand of DNA in the cell nucleus that carries the genes in a linear order
CLED	Cystine-lactose-electrolyte-deficient agar
CLSI	Clinical and Laboratory Standards Institute
Coliform	Rod-shaped gram-negative non-spore forming organisms belonging to the Enterobacteriaceae family
Commensal	Living together of one population (or individual) with another where neither is benefited nor harmed
CVMP	Committee for Medicinal Products for Veterinary Use

DARC	Department for Environment, Food and Rural Affairs (Defra) Antimicrobial Resistance Coordination Group
DARD	Department of Agriculture and Rural Development
DARDNI	Department of Agriculture and Rural Development Northern Ireland
Defra	Department for Environment, Food and Rural Affairs
DH	Department of Health
DIPC	Director of Infection Prevention and Control
Disinfectant	An agent that destroys, neutralizes or inhibits the growth of disease-carrying micro-organisms
EARSS	EARSS is an international network of national surveillance systems and monitors resistance in a number of bacterial isolates. More than 750 laboratories from 28 countries participate in the EARSS programme
E. coli	*Escherichia coli* (commonly abbreviated *E. coli*; named after Theodor Escherich) is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. *E.coli* is the major aerobic bacterial species present in the normal gut flora of humans and animals and is the most common cause of urinary tract and blood stream infections in humans. Certain strains of *E. coli*, and other bacteria, produce enzymes called extended-spectrum beta (β) lactamases (ESBLs), which make them resistant to a wide range of cephalosporin and penicillin antibiotics (the β-lactams). Many ESBL producers are also resistant to other, unrelated, antimicrobials (e.g. fluoroquinolones and aminoglycosides) owing to the presence of additional resistance mechanisms.
EDTA	Ethylenediaminetetraacetic acid is a polyamino carboxylic acid and a colourless, water-soluble solid. Its usefulness arises because of its role

	as a hexadentate ("six-toothed") ligand and chelating agent
EFSA	European Food Safety Authority
EMA (was EMEA)	European Medicines Agency. The central EU regulatory agency for the evaluation of medicines developed by pharmaceutical companies for use in the EU
Enterobacteriaceae	Enterobacteriaceae are a large family of gram-negative rod-shaped bacteria which are facultative anaerobes, fermenting sugars to produce lactic acid and various other end products
ESBL	Extended-Spectrum Beta (β) -Lactamases (ESBLs) are enzymes that can be produced by bacteria making them resistant to penicillins and cephalosporins
ESBLPCs	Extended Spectrum Beta (β) -Lactamase-Producing coliforms (see above)
Etest	The Epsilometer test (usually abbreviated Etest) is a laboratory test used by microbiologists to determine whether or not a specific strain of bacterium or fungus is susceptible to the action of a specific antibiotic
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
Exogenous isolation	A special technique to isolate plasmids from bacteria
Facultative anaerobes	An organism, such as a bacterium, that can live in the absence as well as in the presence of atmospheric oxygen
Food animals	Animals reared for food including cattle, sheep, pigs, poultry, salmon and trout. Bees are also included as a food animal because they produce honey
Fungi	Small, often microscopic, plant-like organisms that absorb food directly through cell walls and lack chlorophyll and cellulose in their cell walls

Gene cassettes	A modular DNA sequence encoding one or more genes for a single biochemical function. It can exist by itself in circular form or can be integrated into an integron
Genotype	The genotype is the genetic constitution of a cell, an organism or an individual, usually with reference to a specific character under consideration
Gram-negative bacteria	Gram-negative bacteria are those bacteria that do not retain crystal violet dye in the Gram staining procedure
HPA	Health Protection Agency
ICT	Infection Control Team
IID	Infectious Intestinal Disease
Integron	A two component gene capture and dissemination system, initially discovered in relation to antibiotic resistance, which is found in plasmids, chromosomes and transposons
IMP	IMP- a type of carbapenemase (one of the metallo-β-lactamases)
Isolates	Terminology used to refer to cultured bacteria of one type
KES	*Klebsiella pneumoniae*, *Enterobacter* and *Serratia*
Klebsiella spp	Type of enterobacteria
KPC	A type of carbapenemase (*K. pneumoniae* carbapenemase)
LHCAI	Laboratory of Healthcare Associated Infection. Reference laboratory producing specialist reference functions as well as comprehensive infection prevention and control advice
MARAN	Monitoring of Antimicrobial Resistance and antibiotic usage in Animals in the Netherlands
Metaphylaxis	A method of secondary prevention using antibiotics to treat sub clinical infections in animals

MBL	Mannose binding lectin
MDR	Multi-drug resistant - resistant to three or more families of antimicrobial
MIC	Is the lowest concentration of an antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation
MLST	Multi-locus sequence typing
MYSTIC	Meropenem Yearly Susceptibility Test Information Collection
NCTC	National Collection of Type Cultures, a Health Protection Agency Culture Collection
NDM	New Delhi Metallo-beta-lactamase - an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics
NEQAS	National External Quality Assessment Service
Nephrotoxic	Toxic to the kidney
NOAH	National Office of Animal Health
Non food animals	Animals not reared for food. These are mainly companion animals including, dogs, cats, horses, birds and small mammals (e.g. rabbits)
Nosocomial	Hospital acquired infection
OCG	Outbreak control group
Oro-faecal transmission	Organisms from human faeces that enter a person's mouth and are swallowed
OXA	Oxacillinase - A group of β-lactamases
PCR	The polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence
PFGE	Pulsed-field gel electrophoresis (PFGE) is a standard gel electrophoresis technique used for separation of high molecular weight DNA fragments that are greater than 50 kilobases in size

Phenotype	A phenotype is any observable characteristic or trait of an organism e.g. its shape, colour or chemical properties.
Plasmid	A DNA molecule that is separate from, and can replicate independently of, the chromosomal DNA.
Protozoa	A group of one-celled organisms
Quaternary ammonium compounds	Powerful disinfectants with additional surfactant (detergent) action
SAC	Scottish Agricultural College
SAGAM	Scientific Advisory Group on Antimicrobials
SARSS	Suspected Adverse Reaction Surveillance Scheme
SDD	Selective decontamination of the digestive tract
ST131	A clone of *E.coli* bacteria
Therapeutic product	A product which treats or prevents disease
Transposons	Mobile elements of DNA that can insert into new locations in the chromosomal DNA and that can affect the function of genes at or near the insertion site. (Transposons should not be confused with plasmids, since they are incapable of autonomous replication)
UKNEQAS	United Kingdom National External Quality Assessment Service
UTI	Urinary Tract Infection
UWWTD	Urban Waste Water Treatment Directive
Virus	A virus (from the Latin virus meaning toxin or poison) is a small infectious agent that can replicate only inside the cells of other organisms
VIM	Verona integron-encoded metallo-β-lactamase

VLA	Veterinary Laboratories Agency - an executive agency of Defra (in April 2011 merged with Animal Health to form AHVLA)
VMD	Veterinary Medicines Directorate - an executive agency of Defra
Waste milk	Milk that cannot be submitted to a dairy for human consumption as it contains antimicrobial residues or is not fit for human consumption for other reasons
Xenobiotics	A chemical compound that is not produced by a living organism and not normally present in a biological system, e.g. a chemical disinfectant

APPENDIX 3
CURRENT FUNDED DEFRA RESEARCH ON ESBLS

OD2023: *Potential risk to human and animal health from the emergence and spread of beta-lactamase resistance in animals in GB.* This large project covers a number of areas including: (i) optimisation of sampling strategies for research and surveillance; (ii) investigations into incidence rates and risk factors for infection of calves and the dynamics of CTX-M transmission within a positive population; (iii) a pilot study to establish whether ESBL *E. coli* are present in a high-risk population in close geographical proximity to the index farm; (iv) retrospective screening of samples from semi-structured surveys; and (v) analysis of samples from the 2008 abattoir survey of broilers in the UK; and studies of human populations in contact with the index farm. (This project has been completed and is awaiting assessment before publication).

OD2025: *Antimicrobial use and carriage of antimicrobial-resistant E. coli and staphylococci in dogs and horses in the community.* This project includes: (i) a community-based study on the prevalence of antimicrobial resistant *E. coli*, including ESBLs, in dogs; (ii) a nationwide study on the prevalence of AMR *E. coli* in dogs and horses; (iii) a longitudinal study on the duration of shedding of AMR *E. coli* in dogs and horses; and (iv) construction of risk pathways, identification of data requirements, evaluation of approaches, and assessment of the risk of antimicrobial resistance being transferred from companion animals to humans.

OD2026: *The clinical treatment of pet dogs and antibiotic resistance in commensal and potentially pathogenic bacteria.* This project is looking at the development of bacterial resistance due to clinical treatment of dogs with antibiotics. *E. coli* are tested for resistance to ceftazidime and cefotaxime.

OD2028: *A generic toolbox for the molecular epidemiology of CTX-M bearing verotoxigenic E. coli and vaccine trial.* This project aims to provide: (i) Understanding of the survival and persistence on-farm of CTX-M *E. coli*, and plasmids carrying CTX-M genes in different bacteria; (ii) A trial on the effect of vaccination on the prevalence of CTX-M14-bearing *E. coli* in cattle; (iii) A generic toolbox for the molecular characterisation of plasmids.

OD2031: *Assessment of the risk of selection of Extended Spectrum Beta Lactamase (ESBL) resistance in calves fed waste milk containing antibiotic residues.* This project aims to investigate whether the practice of feeding of waste milk containing antibiotic residues to calves, particularly third and fourth generation cephalosporins, is associated with the selection of antimicrobial resistance, particularly ESBL resistance in gut commensal bacterial such as *E. coli.*

OZ0328: *A monitoring, control and education package to assist the turkey industry with reduction of* Salmonella *and antimicrobial resistance, and achieving EU targets.* This project includes assessment of 3GC resistance in *E. coli* isolates from the EU baseline survey for Salmonella in turkeys. (This project has been completed and is awaiting assessment before publication).

VM02207: *Measures to control the dissemination of plasmid borne antibiotic resistance in food producing animals.* This project will: analyse a panel of CTX-M producing *E. coli* for other antibiotic resistance to provide advice on which antibiotics may be used to minimise co-selection of CTX-M genes; and analyse plasmid curing ability of a range of natural food additives for possible treatment of animals on outbreak farms.

VM02210: S*tudy to collect antimicrobial usage, antimicrobial resistance and other relevant data from dairy farms with and without ESBL E. coli.* Gathering on-farm information on antimicrobial usage and farm management practices in order to investigate the relationship between antimicrobial usage and ESBL prevalence. ESBL levels in calves will also be assessed.

Summaries of on-going projects and final reports for completed projects may be found by going to http://randd.defra.gov.uk and searching using the project code or a keyword.

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