

Escherichia coli

- ONE DAY NATIONAL MEETING

Thursday 5 June 2008
Royal College of Physicians of London

PROGRAMME

& available abstracts



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Welcome

The emergence of resistance in Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella* spp., is of increasing and continuing concern, not least because its' prevalence has been steadily increasing in both healthcare-associated and community infections.

Through plenary lectures this conference will examine a range of issues relating to *E. coli*, including antimicrobial resistance, the role of animal carriage in the environment, the evolution of multiple resistances and the clinical impact of resistance in *E. coli*.

Delegates will have the opportunity to participate in interactive clinical case presentation discussions that will relate to the diagnosis and treatment of clinical manifestations of *E. coli*.

The conference, which is supported by a grant from Merck Sharp & Dohme Limited, will enable delegates to update their knowledge in this important area, in addition to sharing local experiences with colleagues from across the UK.

We hope you find this important event both informative and enjoyable.

Nicholas Brown,
BSAC Meetings Secretary

Escherichia coli

- ONE DAY NATIONAL MEETING

programme

0945-1030 REGISTRATION & COFFEE

1030 Welcome address
Nicholas Brown, Cambridge

1040 – 1245 SESSION ONE

Chair: Nicholas Brown, Cambridge

1040 Antimicrobial resistance in E. coli – the national picture
Neil Woodford, London

1105 The role of animal carriage and the environment
Peter Hawkey, Birmingham

1130 Carriage and transmission in care homes
Anne Loughrey, Belfast

1155 Evolution of multiple resistances in E. coli
John Cheesbrough, Preston

1220 Clinical impact of antimicrobial resistance in E. coli
Jesus Rodriguez-Bano, Seville

1245 – 1330 LUNCH & POSTER VIEWING

1330-1600 SESSION TWO: CLINICAL CASE PRESENTATIONS

Chair: Peter Hawkey, Birmingham

This session comprises five clinical case presentations relating to the diagnosis and treatment of clinical manifestations of E. coli. Each presentation will comprise 15 minute presentation and 15 minute Q&A/discussion time.

1330 CASE 1
Laboratory detection problems
Hugo Donaldson, Belfast

1400 CASE 2
A simple UTI without an oral solution
Ann Pallett, Southampton

1430 CASE 3
Complicated hospital acquired infection 1
Graham Harvey, Shrewsbury

1500 CASE 4
Complicated hospital acquired infection 2
Nicholas Brown, Cambridge

1530 CASE 5
Complicated hospital acquired infection 3
David Enoch, Cambridge

1600 CLOSING REMARKS

Complete nucleotide sequences of plasmids pEK204 and pEK516, encoding CTX-M enzymes in two major UK *Escherichia coli* strains

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Objectives: *E. coli* strains with the CTX-M-15 ESBL have become prevalent in the UK since 2003. They include 5 ancestrally-related strains, A-E: A is the UK's most prevalent ESBL-producing *E. coli* strain and has the plasmid pEK499 (117,536-bp), sequenced previously; D is local to one site; B, C and E are scattered. Some strain C isolates produce CTX-M-3, rather than CTX-M-15 enzyme. We sequenced the $bla_{CTX-M-3}$ and $bla_{CTX-M-15}$ -harbouring plasmids pEK204 and pEK516 from strain C and D representatives, respectively, and compared them with pEK499 of strain A.

Methods: Plasmids pEK204 and pEK516 were transferred by conjugation into *E. coli* J-53. Randomly sheared plasmid fragments were cloned into pGEM-Teasy vector and transformed into *E. coli* DH10b. Inserts were sequenced by dye terminator chemistry. Sequences were assembled using the Staden Package. Combinatorial PCRs, directed PCRs, and walking reads were used to assemble the sequences and to fill-in gaps.

Results: Plasmid pEK516 (64,471-bp, strain D) harboured 103 predicted open reading frames, with 7 antibiotic resistance genes —*aac6'-Ib-cr*, *aac3-IIa*, $bla_{CTX-M-15}$, bla_{OXA-1} , bla_{TEM-1} , *catB4* and *tet(A)*— clustered in a 22-kb region. Plasmid pEK204 (93,732-bp, strain C) carried only $bla_{CTX-M-3}$ and bla_{TEM-1} . Both were conjugative IncFII plasmids, but variations in their *oriV* sequences suggested that they belonged to different sub-types. bla_{CTX-M} genes were linked to an *ISEcp1* element in both cases, with a 128-bp link for $bla_{CTX-M-3}$ on pEK204 and a 48-bp link for $bla_{CTX-M-15}$ on pEK516. Plasmid pEK516 shared 75% of its DNA sequence with pEK499, albeit with considerable rearrangements. Plasmid pEK204 showed only c. 10% homology with these plasmids.

Conclusion: Plasmid pEK516 (strain D) was highly related to plasmid pEK499, which encodes CTX-M-15 enzyme in epidemic strain A, but was 53-kb (45%) smaller, encoded gentamicin resistance, and was conjugative. Plasmid pEK204 (strain C) was very different and had a longer *ISEcp1*- bla_{CTX-M} link, as in the Polish plasmid, pCTX-M-3. Ancestrally-related *E. coli* strains have acquired different plasmids encoding CTX-M ESBLs or separate occasions.

Locusta migratoria brain lysates exhibit potent broad spectrum antibacterial activity

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Due to the changing and worsening trends of antimicrobial resistance among bacteria that are commonly responsible for serious human infections, there is a need for newer and more powerful antibiotic agents. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections. The search for new antibiotic compounds originating from natural resources is a promising research area. Insects are the largest (80% of all fauna) and the most widespread group within the animal Kingdom. In this study, lysates from the brain, muscle and fat body from the locust, *Locusta migratoria* were investigated for their antimicrobial activity against Gram positive and Gram negative bacteria. The muscle and fat body did not show any antimicrobial activity. Brain lysates, however showed remarkable bactericidal properties killing almost 100% of different bacteria including *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *S. epidermidis*, and a neuropathogenic *Escherichia coli* K1. The bactericidal activity was heat-resistant, but SDS-labile indicating its proteinaceous nature. Brain lysates had no and/or minimal cytotoxic effects on human brain microvascular endothelial cell cytotoxicity. Active brain material(s) are being subjected to ¹H and ¹³C NMR spectroscopy and full structure elucidation is being carried out using heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC) experiments.

The Rate Of Horizontal Transmission Of ABR Plasmids Is Increased In Stressed *Escherichia coli*

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Aim: The aim of this study was to investigate the possibility that sub-lethal stresses (high/low temperature, osmotic and pH stress) can alter the rate of horizontal transmission of antibiotic resistance (ABR) plasmids between *E. coli* strains and between *E. coli* and *Salmonella* Typhimurium.

Methods and Results: *E. coli* donor cultures, carrying F1 plasmid R386 and Inc I1 plasmid TP307 and *E. coli* and *Salmonella* Typhimurium recipient cultures were pre-stressed under a range of sub-lethal environmental conditions (high/low temperature, osmotic and pH stress). The pre-stressed donor and recipient cultures were then mated and the transmission rate calculated. The study found that the horizontal transmission rate of plasmids R386 and TP307 was significantly increased ($p < 0.05$) when pre-stressed donor and recipient cells are mated under conditions of environmental stress.

Conclusions: The results from this study indicate that the sub-lethal stresses *E. coli* strains encounter e.g in the human gut / wound, the environment or in food preservation systems increases the inter- and intra-specific horizontal transmission of selected ABR plasmids and may be contributing to the dissemination of ABR among important food borne pathogens.

Complete nucleotide sequence of pEK499, a multi-drug resistance plasmid from the UK's most prevalent *Escherichia coli* strain with CTX-M-15 β -lactamase

N Woodford, E Karisik, A Underwood, MJ Ellington, DM Livermore
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Objectives: Multi-resistant *E. coli* with CTX-M (mostly CTX-M-15) β -lactamases are prevalent in the UK, with several epidemic strains and many unrelated producers. Strain A is the most widespread producer clone, recorded from >45 centres. We report the complete nucleotide sequence of pEK499, a multi-resistance plasmid encoding CTX-M-15 enzyme in epidemic strain A.

Method: pEK499 was randomly sheared and the 2-3 kb fraction was cloned into the pGEM-Teasy vector, prior to transformation into *E. coli* DH10b. Inserts were sequenced by dye terminator chemistry. Sequences were assembled using the Staden Package. Combinatorial PCRs, directed PCRs, and walking reads on selected clones were used to assemble the sequences and to fill-in gaps.

Results: pEK499 was found to be a circular molecule of 117,536 bp belonging to incompatibility group FII. It harboured up to 185 predicted genes and encoded multi-resistance and virulence factors. With the exception of bla_{TEM-1} , all antibiotic resistance genes were clustered in a 25-kb region. They included $bla_{CTX-M-15}$ and bla_{OXA-1} as well as genes conferring resistance to aminoglycosides and ciprofloxacin ($aac6'-Ib-cr$), macrolides [$mph(A)$], chloramphenicol ($catB3$) and tetracycline [$tet(A)$]. A 1.8-kb class I integron was present within the multi-resistance region; this carried $dfxA17$ and $aadA5$, encoding trimethoprim and streptomycin resistance respectively, also $sulI$ encoding sulphonamide resistance. Virulence-associated genes present on pEK499 included the serum survival gene $traT$, as well as $vagC/D$. pEK499 also encoded the F-plasmid-derived CcdA/B toxin/antitoxin addiction system.

Conclusion. Plasmid pEK499 harboured 10 antibiotic resistance genes affecting 8 antibiotic classes, along with different virulence determinants. It may contribute towards the epidemiological success of *E. coli* strain A in the UK. The toxin/antitoxin addiction system will ensure its maintenance in the absence of antibiotic pressure.

Developing a novel model for the study of *Escherichia coli* K1 meningitis

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A whole-organism approach to the study of disease is recognised as essential to gaining a full understanding of the interrelationships between infectious agents and their hosts. While vertebrate model systems are seen as immediately more relevant, it is proposed here that the use of an invertebrate model at an early stage can offer several advantages in terms of speed, cost, technical convenience, and ethical acceptance. Here, we showed that locusts can be used as a model to study *Escherichia coli* K1 pathogenesis. *E. coli* K12 strain HB101 has very low pathogenicity to locusts and does not invade the locust brain, whereas injection of 2×10^6 *E. coli* K1 strain RS218 (O18:K1:H7) kills almost 100% of locusts within 72 h, and invades the brain within 24 h of injection. Both mortality and invasion of the brain in locusts after injection of *E. coli* K1 require at least two of the known virulence determinants shown for mammals. Thus, deletion mutants that lack OmpA (Outer membrane protein A) or CNF1 (cytotoxic necrotizing factor 1) have reduced abilities both to kill locusts and invade the locust brain compared with the parent *E. coli* K1. Interestingly, deletion mutants lacking FimH or the NeuDB gene cluster are still able to cause high mortality and invade the locust brain *in vivo*. It is argued that the likely existence of additional virulence determinants can be investigated *in vivo* using this insect system. Overall, these results suggest that locusts can be used as a model to study *E. coli* pathogenesis *in vivo*. Because insects rely for their protection against infection on an entirely innate immune system, the use of an insect model is particularly relevant in the study of neonatal *E. coli* K1 meningitis, the control of which has significant dependency on the innate immune system.

Genetic Diversity and Population Genetic Structure of Uropathogenic *Escherichia coli* Isolated in Manchester

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Despite the frequent reports of emerging uropathogenic *E. coli* (UPEC) lineages with unusual characterization that cause sporadic outbreaks, the population structure of UPEC is still not well defined. This study examines the population structure and demonstrates the genetic diversity of 100 UPEC isolates collected in Manchester between (16/July/2007 – 25/August / 2007). Using multilocus sequence typing, the tested isolates were classified into 38 sequence type with no predominant sequence type. Sequence types ST73, ST69, ST95, and ST131 were the most frequently detected and accounted for 20%, 11%, 10% and 10%, respectively.

Multilocus sequence analysis further confirms that recombination has played a significant role in the evolution of UPEC population, but is not sufficiently frequent to prevent the emergence of clonal lineage. In addition, there was some evidence of association of certain antibiotic resistance patterns and certain sequence types, which could be used to predict the presence of different clonal groups such as clonal group A. Although further investigations are needed to confirm the existence of such genetic lineages, MLST could provide an accurate monitoring tool for antibiotic resistance uropathogenic *E. coli* lineages.

Molecular fingerprinting of extended spectrum beta lactamase *Escherichia coli* using the DiversiLab repetitive sequence based PCR system

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Several studies using multi-locus sequence typing (MLST) have identified a globally disseminated, drug resistant, uropathogenic *E. coli* strain (O25:H4 ST-131) producing a CTX-M-15 extended spectrum beta lactamase (ESBL) enzyme. It is important to have rapid typing systems that can identify this strain in order to track its emergence and spread and assist in implementation of appropriate prevention strategies to control possible spread within the community and hospital environments.

The aim of the present study was to evaluate the DiversiLab repetitive sequence based PCR (rep-PCR) system as a rapid method (processing time of <4 hours) for subtyping ESBL uropathogenic *E. coli* (UPEC).

A total of 36 UPEC isolates (comprising 26 cefpodoxime resistant isolates and 10 cefpodoxime susceptible isolates) had been previously sequence typed were included in the study. A selection of these isolates had been previously analyzed by pulse field gel electrophoresis (PFGE). DNA was extracted and typed by rep-PCR. Sample relationships were designated as follows: different – three or more band differences (similarity < 95%); similar – one to two band differences (similarity >95%); and indistinguishable – no band differences (similarity >97%).

Among 36 *E. coli* isolates, 16 rep-PCR profiles were identified. Cefpodoxime resistant and cefpodoxime susceptible *E. coli* isolates were assigned to different rep-PCR profiles. The method was able to cluster *E. coli* isolates in lineages similar to those identified by MLST. It clustered all of ST-131 *E. coli* isolates together and clustered different sequence type *E. coli* isolates together. However, there was no obvious correlation between rep-PCR profiles and PFGE types, where isolates with the same rep-PCR profile had different PFGE types.

The DiversiLab system has the potential to be an alternative technique to sequence typing for strain characterisation and rapid identification of the emergence of new strains. More importantly, it can provide data in real time for investigation of ESBL *E. coli* infection. The relationship between rep-PCR profiles and PFGE requires further investigation.

Impact of agriculture on the prevalence of antimicrobial resistant *E.coli* in rivers in Ireland

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Objectives: The role that the agricultural industry can have on the spread of antimicrobial resistance has rarely been investigated. This study examines the impact various farming types can have on the prevalence of antimicrobial resistance patterns in *E.coli* isolated from river water in particular catchment areas.

Methods: 1 litre water samples were collected between May and June 2007 from rivers both upstream (U) and downstream (D) of particular farming types, namely; pig, tillage, organic beef, sheep and dairy farms. Slurry (SI) samples were also collected from each farm, with soil (So) in the case of tillage. Samples were screened for the presence of *E.coli* resistant to ampicillin (A), streptomycin (S), tetracycline (T), sulphonamides (Su), ciprofloxacin (C), cefotaxime (F) and ceftiofuran (X) using a quantitative culture method. Representative resistant isolates were screened for additional resistance to sixteen antimicrobial agents by Clinical Laboratory Standards Institute (CLSI) disk diffusion methods.

Results: Overall samples taken upstream and downstream from each farm and the slurry / soil samples showed levels of resistance to Su, T, A, S. The highest level of resistance from each farm was to A (0-100% of isolates resistant). T resistance was highest near the organic beef farm (U=54% SI=3%, D=54%). The overall highest resistance towards Su, T, A, S was in the tillage samples followed by; organic beef, pig, dairy and sheep. Ciprofloxacin resistance was observed in the following samples; Tillage U=0.34%, D=5%, pig D =1% and organic beef U=5%, D=5%.

Conclusion: Antimicrobial resistance is frequently present in *E. coli* in rivers in rural areas of Ireland. Broadly similar resistance patterns were detected in the vicinity of all farming practices. Given that samples were collected on a single occasion and that factors other than the identified agricultural practice may impact on levels of resistant *E. coli* detected the results must be interpreted with caution.

Comparison of gentamicin-resistant (gent-R), CTX-M-producing and non-producing *Escherichia coli* isolates from community-onset urinary tract infections

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Background: Multi-resistant *E. coli* strains from community-onset infections are an increasing public health concern, especially those with CTX-M ESBLs. We studied the clonality of ESBL-producing *E. coli* from urines of patients attending general practitioners in Cornwall (south-west England), and compared them with gent-R, ESBL-negative isolates.

Methods: Isolates were identified by the API system. Susceptibilities were determined and interpreted using BSAC methodology. *bla*_{CTX-M} genes were sought by PCR. Isolates were compared by PFGE of XbaI-digested genomic DNA, and representatives were subjected to multi-locus sequence typing.

Results: In 2004-05, 69 *E. coli* were received from Truro (Cornwall) laboratory, mainly for confirmation of ESBL production: these included 45 CTX-M group 1 producers; 7 CTX-M group 9 producers; 3 producers of non-CTX-M ESBLs; and 14 gent-R, ESBL-negative isolates. By PFGE, 9 gent-R, ESBL-negative *E. coli* were distinct (<85% similarity) from the ESBL producers. However, 3 isolates were related to CTX-M group 1 producers, and 2 isolates were related to a non-CTX-M ESBL producer. One outbreak strain, represented by 12 CTX-M group 1 producers and 2 gent-R, ESBL-negative isolates was identified, and was distinct by PFGE from nationally-distributed CTX-M-producing strains; 3 representatives belonged to sequence type (ST) 131.

Conclusions: Community-onset, ESBL-producing *E. coli* were diverse. Two ESBL-negative isolates were closely related to a local CTX-M-producing outbreak strain, suggesting gain or loss of a *bla*_{CTX-M}-carrying plasmid by a clone circulating in the community. This outbreak strain had the same ST as several epidemic CTX-M-producing strains from the UK, which is consistent with repeated selection of a successful clonal lineage.

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