GRAM-NEGATIVE THERAPEUTICS IN THE 21ST CENTURY

THE good THE bad AND THE ugly

PROGRAMME

British Society for Antimicrobial Chemotherapy
SPRING MEETING 2006
Thursday 16 March 2006
International Convention Centre, Birmingham

CPD ACCREDITED
Acknowledgements

The Society wishes to thank the following companies for their invaluable support:

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- Merck, Sharpe & Dohme for the provision of an educational grant.
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Delegates are encouraged to visit our sponsors during coffee and lunch breaks.

Poster abstracts

- Development and optimisation of reverse hybridisation method for characterising genotypes of CTX-M phylogenetic Group 1
  VM Ensor, L Xu, J Evans, DM Livermore, PM Hawkey
  University of Birmingham

- Evolution of extended-spectrum beta-lactamases in 4 hypermutable Pseudomonas aeruginosa
  KL Driffield, J Hobbs, JM Bostock, K Miller, AJ O’Neill, I Chopra
  University of Leeds

- An audit of the appropriateness of prescribing of Teicoplanin within Blackpool, Fylde and Wyre Hospital NHS Trust (BFWH)
  A Liu, J Chard
  Blackpool, Fylde and Wyre Hospital NHS Trust

- Evaluation of clinical and economic impact of antibiotic pharmacist input in microbiology ward round to patient care in antimicrobial prescribing
  A Liu, S Reddy
  Blackpool, Fylde and Wyre Hospital NHS Trust

- North West Antibiotic Pharmacist Advisory Group – Education Subgroup
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- An unwelcome gift from the Carribean- treating multidrug resistant Acinetobacter meningitis in the neurosurgical intensive care unit
  R Sutherland, ICJW Bowler
  Oxford Radcliffe Hospitals NHS Trust

- Fitness costs associated with resistance to fluoroquinolone and coumarin antimicrobial agents in Staphylococcus aureus
  University of Leeds

- Evidence for Antibiotic Resistance Gene Silencing in Escherichia coli
  VI Enne, AA Delsol, JM Roe, PM Bennett
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- Antimicrobial Point Prevelance study in a Children’s Hospital
  C Kerrison, FAI Riordan
  Royal Liverpool Children’s NHS Trust

- Failure of mupirocin to eradicate MRSA following in vivo acquisition of high-level mupirocin resistance from S. epidermidis
  JG Hurdle, AJ O’Neill, L Mody, SF Bradley, I Chopra
  University of Leeds

- A novel medium for the isolation and differentiation of multi-drug resistant Gram negative organisms
  S Dimmer
  Oxoid Ltd, Wade Road, Basingstoke

- The rational choice of antibiotics in neutropenic sepsis – the experience of a large teaching hospital
  M Ashcroft, M Karanth, P Mahendra, TSJ Elliott
  University Hospitals Birmingham NHS Trust

- Cefepime-resistant, ceftazidime and cefotaxime-susceptible ESBL phenotype in Salmonella Typhimurium isolates from Africa and Europe. Cefepimases?
  D Morris, V Buckley, J O’Connor, S Kariuki, CA Hart, M Cormican
  National University of Ireland, Galway

- Novel Topoisomerase inhibitors with broad spectrum antibacterial activity
  C Gray, GE Dale, M Cappi, H Schulz, S Piaura
  Morphochem AG, Basel, Switzerland
**British Society for Antimicrobial Chemotherapy**

**SPRING MEETING 2006**

**Programme**

**9.15 - 09.50**
REGISTRATION & COFFEE

**09.50 - 10.00**
Welcome
*Alasdair MacGowan, Bristol*

**10.00 - 11.15**
**A few mechanisms more**

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<td>Extended-spectrum-beta-lactamases</td>
<td>Peter Hawkey, Birmingham</td>
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<tr>
<td>10.15</td>
<td>Metallo-beta-lactamases</td>
<td>Timothy Walsh, Bristol</td>
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<td>10.30</td>
<td>Resistance mechanisms in <em>Acinetobacter</em></td>
<td>Kevin Towner, Nottingham</td>
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<td>10.45</td>
<td>Resistance in Gram-negative rods: recent epidemiology</td>
<td>Alan Johnson, London</td>
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**11.15 - 11.45**
COFFEE

**11.45 - 12.30**
**PARALLEL SESSION A:**

**Where microbiologists dare**

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<td>Common problems and pitfalls in the susceptibility testing of Gram-negative organisms</td>
<td>Jenny Andrews, Birmingham</td>
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<td>Combination testing in the laboratory to support Gram-negative therapy</td>
<td>Ian Gould, Aberdeen</td>
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<td>12.15</td>
<td>Predictive value of MIC/disc testing with <em>Acinetobacter</em></td>
<td>Derek Brown, Cambridge</td>
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**12.30 - 13.00**
**BSAC ANNUAL GENERAL MEETING**

**13.00 - 14.00**
LUNCH

**14.00 - 14.15**
**FREE PAPER**

Development and optimisation of reverse hybridisation method for characterising genotypes of CTX-M phylogenetic group 1

*Vicki Ensor, Birmingham*

**14.15 - 15.30**
**Do you feel lucky? Well, do you? - Topics in clinical therapeutics**

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<td>14.15</td>
<td>The evidence for combination Gram therapy</td>
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<td>Therapy of <em>Burkholderia cepacia</em></td>
<td>Robin Howe, Cardiff</td>
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<td>14.45</td>
<td>Therapy of multi-resistance <em>Acinetobacter</em></td>
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<td>Future Gram-negative agents</td>
<td>Chris Thomson, London</td>
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<td>15.15</td>
<td>Discussion</td>
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**15.30 - 15.45**
CLOSING REMARKS

**Gram-Negative Therapeutics**

**IN THE 21ST CENTURY**
Development and optimisation of reverse hybridisation method for characterising genotypes of CTX-M phylogenetic Group 1

VM Ensor1,2, L Xu3, J Evans2, DM Livermore3, PM Hawkey1,2

Antimicrobial Agents Research Group, University of Birmingham1
West Midlands Health Protection Agency, Heart of England Foundation NHS Trust, Birmingham2
Centre for Infections, Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency, Colindale3

Background
CTX-M extended-spectrum beta-lactamases (ESBLs) have been reported worldwide; prevalence and predominant type varies geographically. CTX-M-15 (group 1) has rapidly become predominant in UK and merits close monitoring. We aimed to develop a novel reverse hybridisation method that may be used in place of sequencing for identifying genotypes of Group 1 CTX-Ms.

Methods
CTX-M encoding regions of the 12 members of Group 1 were aligned using Clustal W. Sequence specific oligonucleotides (SSO’s) were designed around key mutations, producing a unique profile for each CTX-M. Primers were designed to amplify a 666 bp fragment covering all SSO’s. Biotinylated amplicon was obtained using biotin-labelled reverse primer. SSO’s were immobilised onto a membrane and hybridised with amplicons. Following incubation with streptavidin-peroxidase conjugate hybrids were visualised using enhanced chemiluminescense and exposure to a light-sensitive film. Control strains included examples of 4 phylogenetic groups (Group 1 n=5, Group 2 n=3, Group 25/26 n=2, Group 9 n=3) and ESBL-negative E. coli NCTC 10418. A series of experiments to determine optimal SSO concentration, hybridisation and washing conditions was performed.

Results
A 666bp fragment was obtained for all Group 1 CTX-M strains. No amplicon was obtained for CTX-M strains belonging to the other 3 groups. Predicted hybridisation profiles were obtained for all Group 1 control strains tested. No cross activity was obtained for CTX-M strains belonging to other groups or from E. coli NCTC 10418.

Conclusion
This novel reverse hybridisation method has enabled identification of all Group 1 CTX-M genotypes. Only sequence-specific hybrids were obtained, demonstrating high discriminatory power of the method. This method can be used in place of sequencing for genotyping CTX-M’s belonging to Group 1.

Evolution of extended-spectrum beta-lactamases in hypermutable Pseudomonas aeruginosa

KL Driffield, J Hobbs, JM Bostock, K Miller, AJ O’Neill, I Chopra

Antimicrobial Research Centre and Research Institute of Molecular and Cellular Biology, University of Leeds

Objectives
Extended-spectrum beta-lactamases (ESBLs) derived from OXA-10 and TEM-2, exist in clinical isolates of P. aeruginosa. Since hypermutable strains of P. aeruginosa occur in the clinical setting, their superior mutation activities could facilitate ESBL evolution. However, whether P. aeruginosa hypermutators contribute to ESBL evolution is unknown. We therefore examined whether a hypermutable derivative of P. aeruginosa PAO1 permitted the evolution of clinically important TEM-2 and OXA-10 derived ESBLs.

Methods
P. aeruginosa PAO? mutS strains, encoding either TEM-2 or OXA-10 beta-lactamases on naturally occurring plasmids, were used to select mutants conferring ceftazidime resistance. Mutants were characterised by sequencing of the blaTEM-2 and blaOXA-10 genes.

Results
ESBL mutants of TEM-2 and OXA-10 capable of conferring resistance to ceftazidime were selected in hypermutable P. aeruginosa. Three amino acid substitutions were identified in the TEM mutants, which corresponded to TEM-11, TEM-61 and a third novel enzyme. A single amino acid substitution that did not correspond to any naturally occurring beta-lactamase was identified in the OXA mutants.

Conclusions
Hypermutable P. aeruginosa is a host for the evolution of TEM derived ESBLs, although neither of the ESBLs we observed (TEM-11 and TEM-61) have been reported in clinical isolates of ESBL producing P. aeruginosa. An ESBL derived from OXA-10 could also be generated in hypermutable P. aeruginosa. However, this enzyme is not a naturally occurring ESBLs. Consequently, although hypermutable P. aeruginosa have the capacity to generate ESBLs, such strains may not be the source of ESBLs found in clinical isolates of this organism.

This work was supported by BSAC grant to Ian Chopra.
An audit of the appropriateness of prescribing of Teicoplanin within Blackpool, Fylde and Wyre Hospital NHS Trust (BFWH)
A Liu, J Chard
Pharmacy, Blackpool, Fylde and Wyre Hospital NHS Trust

Aim
- To assess the appropriateness of teicoplanin prescribing in BFWH according to the guidance that was issued
- To recommend changes to improve current prescribing

Methodology
- A 3-month retrospective study of prescribing habits of teicoplanin was reviewed in surgical wards against a designed proforma.

Results and Discussion
- Thirteen patients (n=13) were prescribed teicoplanin during the study period, mainly for cellulitis / wound infection (39%) or abdominal sepsis (23%).
- Forty-six percent of the treatment was for MRSA infection.
- The length of teicoplanin treatment was often too long, 14.3 days on average.
- Assays were taken in 4 out of 13 patients. None had reached therapeutic range in the 1st assay.
- Subtherapeutic dosing of teicoplanin could lead to prolonged treatment, treatment failures, addition of unnecessary antibiotic(s) or use of a more expensive antibiotic (e.g. linezolid).
- Increasing antibiotic expenditure and uncertainty of safety profile of high-dosage of teicoplanin from manufacturer warranted more research and audit to review its value on current MRSA treatment.

Conclusion
- Teicoplanin should be reserved for significant sepsis. Clinicians should discuss with Microbiology before initiating teicoplanin treatment to ensure its appropriate use.

Evaluation of clinical and economic impact of antibiotic pharmacist input in microbiology ward round to patient care in antimicrobial prescribing
A Liu*, S Reddy**
* Pharmacy Department, Blackpool, Fylde and Wyre Hospital NHS Trust
** Microbiology Department, Blackpool, Fylde and Wyre Hospital NHS Trust

Introduction
A microbiology ward round was set up in October 2004 to monitor antimicrobial use and promote prudent antimicrobial prescribing within the Trust in supporting the recent Department of Health (DoH) initiatives. It was aimed to increase microbiology input in clinical areas and reduce the unnecessary use of expensive antibiotics. The ward round was attended twice weekly by Microbiology Specialist Registrar and Antibiotic Pharmacist.

Objective
To evaluate the impact of Microbiology ward round over a 4-month period (October 2004 – January 2005) in terms of appropriateness of antibiotic prescribing. To assess the potential for cost-saving in antimicrobial drug budgets during the Microbiology Ward Round.

Method
The Antimicrobial Alert System was set up in Summer 2004. Restricted use antibiotics including Tazocin®, Teicoplanin and Linezolid were reserved for serious infections or the failure of 1st or 2nd line treatments on the recommendation of Consultant Microbiologists. Linezolid could only prescribed with Microbiologist’s approval.

Patients who were on those three antibiotics during the study period were obtained from the Pharmacy Ascribe® System twice a week (Tuesdays and Fridays). Patients from peripheral hospitals were excluded from the study. Interventions by the Microbiology Ward Round team were reported on a designated audit form. The results of all findings were presented in alphanumeric tables and graphic charts.

Results
Sixty-eight patients were seen in the Microbiology Ward Round during study period, 29 (43%) from the Medical Directorate and 22 (32%) from the Surgical Directorate. 58 patients were on restricted use antibiotics: Tazocin (16), Teicoplanin (36) and Linezolid (6). One patient who was on Linezolid was not discussed with Microbiology. Of the thirty-six patients who were on teicoplanin, 83% needed microbiology intervention during ward round. 63% of them were requested to discontinue teicoplanin treatment or change to other antibiotics, which were expected to save £727.7 daily in total. Of the sixteen patients who were on Tazocin®, only one third consulted the Microbiologist before treatment. 62.5% needed microbiology intervention during the ward round. 90% of them were requested to discontinue Tazocin® or changed to other antibiotics, which were expected to save £430.11 daily in total.
North West Antibiotic Pharmacist Advisory Group – Education Subgroup

A Liu
Pharmacy Department, Blackpool, Fylde and Wyre Hospital NHS Trust

Overview
Since its inception in Spring 2005 the Education Subgroup has met quarterly. The aim of the group is to support the North West Antibiotic Pharmacist Network Advisory Group (NWAPAG) to promote clinically effective and prudent use of antimicrobial agents throughout the region by providing education and training resources for antibiotic pharmacists and other health professionals.

So what have we done so far?
A training package was launched in January 2006 to support education of basic microbiology and antibiotic prescribing for healthcare professionals. It has been developed into a series of power point presentations with accompanying ‘homework’ exercises which can be accessed by all NWAPAG members via our antibiotic pharmacists’ website. The programme has been primarily targeted to pharmacists but it can be used to aid training of medical and nursing staff. The training pack consists of two main topics:

- Bugs & Drugs And Antimicrobial Resistance
- Infection and the Role of Antibiotic Pharmacist

Each training session is accompanied with a series of objectives and reflective questions. This is to support participants in maintaining their CPD records. In order to evaluate the contents of the training package with regard to its appropriateness and usefulness, tutors, namely Trust antibiotic pharmacists and participants are requested to complete the accompanying evaluation and feedback forms.

What have we planned for the future?
- Clinical Case Presentations
- Competency Checklist for Band 6/7 and Specialist pharmacists on Microbiology and antibiotic usage
- Links with other groups. Examples include:
  - Other regional pharmacy group to share good practice
  - North West Microbiologist Group

An unwelcome gift from the Carribean- treating multidrug resistant Acinetobacter meningitis in the neurosurgical intensive care unit

R Sutherland, ICJW Bowler
Department Microbiology, Oxford Radcliffe Hospitals NHS Trust, Oxford

A 61 yr old female was transferred to the Oxford Neurosurgical Intensive Care Unit (NITU) from Puerto Rico via the British Virgin Isles Hospital (BVIII). She had sustained a grade 4 subarachnoid haemorrhage whilst holidaying on a cruise ship. The haemorrhage was treated with craniotomy and clipping of an intracerebral artery aneurysm. On arrival at the Oxford NITU her GCS was 6 with stable vital signs.

The neurosurgical plan was to stabilise the patient and insert a ventriculo-peritoneal (V-P) shunt to relieve raised intracranial pressure. Early on, there were signs of intercurrent infection and serial LPs were performed. The first LP was consistent with recent SAH and CSF culture was negative. On Day 4 Gram negative rods and Gram positive cocci in clusters grew from blood cultures taken from a central line. The patient was febrile and meropenem and gentamicin were commenced. The blood culture isolates were identified as multi drug resistant (MDR) Acinetobacter spp (resistant to ceftazidime, gentamicin and meropenem but susceptible to colistin) and coagulase negative Staphylococci. As the central line had been removed and peripheral blood cultures were no growth at day 5, the treatment plan was not changed.

On day 16 a lumbar drain, inserted early in the NITU stay, was dislodged and subsequently sewn back in. The patient’s clinical condition deteriorated. Subsequent LPs had raised white cell counts consistent with neurosurgical meningitis and grew MDR Acinetobacter spp, susceptible only to colistin. CSF cultured from the lumbar drain collecting system grew coagulase negative Staphylococci in addition to the MDR Acinetobacter.

The infection was successfully eradicated with a combination of intravenous and intrathecal colistin therapy along with rifampicin and vancomycin. A V-P shunt was placed on Day 40 following repeated CSFs with no sign of infection. This case highlights the restricted choice available for antimicrobial therapy of MDR Acinetobacter meningitis.
Fitness costs associated with resistance to fluoroquinolone and coumarin antimicrobial agents in Staphylococcus aureus
Antimicrobial Research Centre and Research Institute of Molecular and Cellular Biology, University of Leeds

Background and Objectives
Fluoroquinolones (FQs) and coumarins target bacterial type II topoisomerases and both classes of antimicrobials have been used to treat staphylococcal infections. However, rapid emergence of resistance (particularly to FQs), limits the use of these compounds as single therapeutic agents. This work examines concurrent resistance to FQs and coumarins in Staphylococcus aureus, in order to predict whether there might be issues of resistance in their potential application in combination for anti-staphylococcal chemotherapy. Antibiotic resistance is frequently associated with a biological fitness cost to the bacteria. Information about the fitness of antibiotic-resistant mutants selected in the laboratory can provide a useful indication of the likely maintenance of antibiotic resistance determinants in the clinical setting.

Methods
Selections were made (at 4 x MIC of each drug) for mutants of S. aureus strain SH1000 resistant to FQs and coumarins. Mutants were characterised by sequencing of target genes (grlA and gyrA in FQ-resistant strains, gryB in coumarin-resistant strains). Fitness of mutants was examined by in vitro competition assays.

Results
Sequencing of target genes showed that point mutations conferring resistance were identical to those previously described in clinical isolates (FQ resistance-associated: grlA Ser-80 and gyrA Ser-84 mutations), and in laboratory-selected strains (coumarin resistance-associated gryB: Thr-173, Arg-144 and Gly-85). Mutants resistant to FQs or coumarin agents independently do not carry a significant fitness burden in vitro. Mutants selected for concomitant resistance to both FQ and coumarin agents do show reduced fitness in vitro (up to 35% loss of fitness in competition assays).

Conclusions
The absence of fitness costs associated with FQ-conferring mutations may explain the prevalence of these genotypes among clinical isolates. Mutants resistant to both FQs and coumarins, showing impaired fitness, would be at a selective disadvantage relative to their antibiotic-sensitive counterparts in the antibiotic-free environment and may not persist among in vivo bacterial populations. We therefore suggest that these agents could be of value in combination for the treatment of infections caused by S. aureus.

This work was supported by grant from the British Society for Antimicrobial Chemotherapy.

Evidence for antibiotic resistance gene silencing in Escherichia coli
VI Enne1, AA Delsol1, JM Roe2, PM Bennett1
Bristol Centre for Antimicrobial Research and Evaluation, Department of Cellular and Molecular Medicine, University of Bristol1
Division of Farm Animal Science, Department of Clinical Veterinary Science, University of Bristol2

Objectives
The possibility that unexpressed antibiotic resistance genes are coded by bacterial genomes is rarely studied. We investigated whether plasmid-borne antibiotic resistance genes can be silenced.

Methods
Potential silencing of resistance genes carried on the unrelated plasmids pVE46 (blaTEM-2, aadA1, sul1, tetA) or RP1 (blaOXA-2, aphA, tetA) in Escherichia coli 345-2rifC was investigated following oral inoculation of the strains into organic piglets. Isolates were recovered from faeces by plating onto MacConkey agar containing rifampicin. Those that failed to express the expected resistance profile were characterised further by MIC testing, RAPD typing, plasmid-extraction, PCR, DNA sequencing, RT-PCR and conjugative transfer studies.

Results
52 isolates of E. coli 345-2rifC/pVE46 recovered from pig faeces did not express one or more pVE46-encoded resistance genes, despite retaining the plasmid. Different combinations of unexpressed resistances were observed and twelve representative isolates were selected for further study. Surprisingly, although not expressed, in most cases the resistance genes and their promoters were intact, with fully wild-type sequence. Apart from four isolates exhibiting intermediate level tetracycline resistance, no mRNA for the unexpressed genes was detected. Silencing of resistance genes was reversible at low frequencies between 10-6 and 10-7. Introduction of the plasmid from silenced isolates to another strain restored expression, indicating that gene silencing was a property of the host chromosome rather than the plasmid itself. When E. coli 345-2rifC/RP1 was inoculated into piglets, three isolates were recovered that no longer expressed RP1-encoded resistance genes. As with pVE46, in most cases the coding sequences and promoter regions of these genes were found to be intact, but they were not transcribed.

Conclusions
Our data show that expression of unrelated antibiotic resistance genes can be silenced, whilst gene and promoter sequences necessary for expression of resistance are retained. This phenomenon indicates a previously unrecognised form of transcriptional control that over-rides standard expression signals to shut down gene expression. These findings suggest that unexpressed resistance genes may occur in the wild and may hence have clinical implications.
Antimicrobial Point Prevalence study in a Children's Hospital
C Kerrison, FAI Riordan
Royal Liverpool Children's NHS Trust

Antimicrobial resistance has been linked to injudicious use of antimicrobial agents both in the hospital and community setting. We present a point prevalence study of antimicrobial prescribing practice in a tertiary paediatric hospital.

Objectives
To ascertain current antimicrobial prescribing practice for inpatients within a tertiary paediatric hospital compared with local anti-infective guidelines. To target areas for intervention or clarification during the current revision of trust antimicrobial guidelines.

Methods
Prescription charts/case notes of all inpatients on acute wards within the trust on one day were reviewed by medical or pharmacy staff. Children on any antimicrobial therapy had data recorded on a standardised proforma (ward, age, consultant, diagnosis, antimicrobial; name, dose, route, number of days of use, and indication (suspected/confirmed infection or prophylaxis)). This was compared with microbiological results available on the day of the audit from the hospital computer system. Decision on appropriateness of prescription was made by comparison to current local antimicrobial guidelines.

Results
On the day of the audit 99/203 (49%) patients were on at least one antimicrobial, median agents per child on therapy; 2 (range 2-7). The most frequently prescribed agents were; Selective Decontamination of Digestive tract (n=16), Vancomycin (n=15), Teicoplanin (n=13), Cefaclor (n=2). Only 112/197 (57 %), antimicrobial prescriptions complied with current trust guidelines. The agents most commonly prescribed against guidelines were; Cephalexin (n=6), metronidazole (n=4), azythromicin (n=4), Teicoplanin (n=4). Of patients on antimicrobial therapy, 39 (39%) were colonised or infected with Gram negative organisms. 50 children (50%) were being treated for confirmed or suspected Gram negative infection/colonisation. There were 11 cases of confirmed ESBL carriage. One colonised patient had had an episode of Klebsiella Pneumoniae ESBL bacteraemia in a previous admission.

Conclusions
The rate of antimicrobial prescriptions is comparable to other paediatric studies. Compliance with current hospital guidelines is poor. Further work is required to improve compliance with guidelines and to assess impact on nosocomial bacterial ecology.

Failure of mupirocin to eradicate MRSA following in vivo acquisition of high-level mupirocin resistance from S. epidermidis
JG Hurdle, AJO Neill, L Mody, SF Bradley, I Chopra
Antimicrobial Research Centre, University of Leeds, GRECC, VA Ann Arbor & U Michigan Healthcare Systems, Ann Arbor, MI, USA

Objectives
We sought to examine the molecular basis of the emergence of high-level resistance to mupirocin in a methicillin-resistant Staphylococcus aureus (MRSA) strain that colonized a nursing home resident undergoing mupirocin prophylaxis.

Patients and method
A 73-year old male patient who was a persistent carrier of mupirocin-susceptible MRSA was enrolled in a study to evaluate the efficacy of mupirocin in eradicating nasal carriage of S. aureus among nursing home residents. During prophylaxis a high-level mupirocin-resistant MRSA emerged in the nasal isolates recovered from this patient only. MRSA and coagulase-negative staphylococci were also isolated before, during and after 14 days of prophylaxis with mupirocin. The staphylococcal isolates and their antibiotic resistance plasmids were examined by standard molecular genetic methods.

Results
Genotypic analysis by pulse-field gel electrophoresis and spa typing confirmed that all mupirocin-susceptible and –resistant MRSA possessed the same genotype. The patient was also colonized by a single mupirocin-resistant S. epidermidis strain, which was only detected following the start of mupirocin decolonization treatment. In both mupirocin-resistant MRSA and S. epidermidis strains, identical plasmids of approximately 37 kb were found which carried the mupA determinant and genes for conjugative DNA transfer in staphylococci. These plasmids could be transferred in vitro, by filter mating, from both clinical isolates to the recipient S. aureus RN2677, resulting in transconjugants which expressed mupA-mediated high-level resistance to mupirocin.

Conclusions
The MRSA strain contained a conjugative plasmid expressing mupA that was identical to that found in the S. epidermidis co-colonist. It therefore appears that during nasal decolonization prophylaxis high-level resistance was acquired by the patient’s pre-therapy MRSA through conjugative transfer of a plasmid bearing mupA from S. epidermidis.

This work was presented at the 44th ICAAC Washington through travel support funding from BSAC grant.
A novel medium for the isolation and differentiation of multi-drug resistant Gram negative organisms

S Dimmer
Oxoid Ltd, Wade Road, Basingstoke

The transferable nature of resistance genes has contributed to the spread of Gram negative organisms producing extended-spectrum \( \beta \)-lactamase and metallo-\( \beta \)-lactamase enzymes. In recent years, organisms such as \textit{Acinetobacter} spp, and \textit{Pseudomonas} spp, which are inherently resistant to many antibiotics, have been found with carbapenemase activity. Eradication of these organisms from colonised or infected patients is difficult as treatment options are limited, in some cases, to just one or two antibiotics.

Bacteria deemed to be commensal flora are not usually tested for antibiotic susceptibility and, consequently, recognition of multi-drug resistant (MDR) Gram negatives is usually limited to those which are clinically significant. Few laboratories have implemented screening for MDR Gram negatives and those that do are limited to “traditional” culture media, such as MacConkey or Columbia Agar, with antibiotic discs to indicate resistance. Growth around the disc is identified using tests such as oxidase, catalase and biochemical profile testing. This can often extend turn-around times to 48 hours.

A novel medium, Oxoid Chromogenic Sensitivity Test Agar, has been developed that can be used to isolate and rapidly differentiate MDR Gram negative organisms. The medium has been formulated using specific raw materials to reduce antagonism to antibiotics, which can be added at a level such that only resistant organisms grow. The presence of two chromogens, X-glucopyranoside and red-galactopyranoside, allows rapid identification of the main Gram negative species. The need for further off-plate testing is reduced, and a result can be achieved within 24 hours. This allows infection control teams to act quickly once a MDR Gram negative organism has been isolated and allows for rapid screening of new patient admissions.

The introduction of routine screening for MDR Gram negatives in hospitals will help to eradicate these organisms before they can become established as the “new MRSA.”

The rational choice of antibiotics in neutropenic sepsis – the experience of a large teaching hospital

M Ashcroft, M Karanth*, P Mahendra*, TSJ Elliott
Microbiology Department, University Hospitals Birmingham NHS Trust
*Haematology Department, University Hospitals Birmingham NHS Trust

Introduction
Whilst it is accepted that broad spectrum \( \beta \)-lactams as monotherapy are appropriate as the initial treatment of choice in neutropenic sepsis, which \( \beta \)-lactam to use is not always clear. Treatment must always be tailored to the microbiology of the patient population. We describe the results of a retrospective survey of haematology patients with bacteraemia undertaken to inform the development of new local prescribing guidelines for the treatment of neutropenic sepsis. Existing guidelines recommended tazocin\(^{\circledast}\) as the first line agent with the addition of vancomycin if line sepsis is suspected or gentamicin if the patient is systemically unwell due to sepsis.

Methods
We conducted a computer search for all blood cultures obtained from haematology patients in a 28 month period. The results were then divided into 2 patient groups –
1) patient neutropenic at time of blood culture ( neutrophils d” 1.0)
2) patient non-neutropenic at time of culture ( neutrophils > 1.0)

Summary of results
3025 sets of blood cultures were identified of which 763 (25%) were positive, of these 335 sets were from neutropenic patients. These yielded 275 gram positive organisms ( predominantly CNS 67%, Enterococci 11%, MRSA 4%) 60 gram negative organisms ( predominantly \textit{E.Coli} 42%, other \textit{Enterobacteriaceae} 27%, non-fermentative GNR 13%, \textit{pseudomonas} 7%) and 7 fungi. Of 54 gram negative isolates from which we had sensitivity data, after excluding 3 \textit{S.Maltophilia} , 9 were resistant to tazocin alone ( including 5 \textit{E.Coli} , \textit{E.Cloacae}), 13 resistant to gentamicin alone (these included 8 \textit{E.Coli} , 2 \textit{P.aeruginosa}), 2 resistant to both tazocin and gentamicin ( \textit{E.Coli}) and only 1 resistant to meropenem ( a non-fermentative GNR).

Conclusion
These results suggest that for our patient group either tazocin and gentamicin in combination or meropenem alone are appropriate agents for empirical use in neutropenic sepsis. Monitoring of resistance rates should be ongoing so that guidelines can be updated in response to any changing patterns of resistance.
Cefepime-resistant, ceftazidime and cefotaxime-susceptible ESBL phenotype in *Salmonella* Typhimurium isolates from Africa and Europe. *Cefepimases?*

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**Objectives**

Nontyphoidal salmonella species are a major cause of human gastroenteritis. Extended spectrum beta-lactamase (ESBL) producing salmonella have been reported worldwide. ESBL production confers resistance to the oxyimino cephalosporins. In studies of a large number of salmonella isolates we observed a phenotype of apparent ESBL mediated frank cefepime-resistance with relatively little change in susceptibility to cefotaxime and ceftazidime. This study describes 8 isolates of *S. Typhimurium* with this previously undescribed phenotype.

**Materials and Methods**

Eight isolates of *Salmonella Typhimurium* (7 isolated in Kenya and 1 isolated in Ireland associated with travel to Andorra) were screened for ESBL production in accordance with the Clinical Laboratory Standards Institute (CLSI) disk diffusion methods. ESBL production was confirmed using three ESBL Etest strips (AB Biodisk, Solna, Sweden): ceftazidime/ceftazidime plus clavulanic acid (TZ/TZL); cefotaxime/cefotaxime plus clavulanic acid (CT/CTL); and cefepime/cefeime plus clavulanic acid (PM/PML). Susceptibility to a panel of 20 antimicrobial agents was assessed in accordance with CLSI disk diffusion methods. All isolates were examined for *bla*<sub>TEM</sub>, *bla*<sub>SERV</sub>, *bla*<sub>TIP</sub>, and for the presence of Class I integrons by PCR using specific primers. Isoelectric focusing (IEF) and plasmid analysis was performed on all isolates.

**Results**

All 8 isolates had cefepime MICs of >16mg/ml falling to 0.19 to 0.5 mg/ml in the presence of clavulanic acid, cefotaxime MICs were 1.5 to 6 mg/ml falling to 0.19 to 0.38 mg/ml in the presence of clavulanic acid and ceftazidime MICs were ≤ 0.5 mg/ml. Isolates had varying antibiograms but all were resistant to ampicillin, streptomycin and sulphonamides. The *bla*<sub>TEM</sub> gene was detected in 7 (all isolated in Kenya) of the 8 isolates by PCR. Class I integrons (~ 700bp) were detected in 5 of 8 isolates analysed. A beta-lactamase of pl 7.6, and a plasmid of approximately 126MDa was detected in all isolates.

**Conclusions**

This is the first observation of higher level resistance to cefepime than to other oxyimino cephalosporins among the *Enterobacteriaceae*. The observation of this resistance phenotype in isolates of *Salmonella Typhimurium* from distinct geographical locations suggests a potentially novel beta-lactamase which we tentatively designate a “cefepeimase”.

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**Novel Topoisomerase inhibitors with broad spectrum antibacterial activity**

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We report the discovery and characterization of a novel topoisomerase inhibitor class that exhibits selective and broad-spectrum antibacterial activity. Compounds in this class inhibit growth of many gram-positive and gram-negative bacteria, including the common respiratory pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Pseudomonas Aeruginosa* as well as the newly emerging pathogens, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* and are non-toxic to eukaryotic cell lines. The compounds display a bactericidal mode of action and have a very low tendency to generate resistance. The compounds have been shown to be potent inhibitors of both DNA-gyrase and topoisomerase IV. Furthermore, the compounds appear to inhibit bacterial topoisomerases by a new mechanism, because resistant strains are not cross-resistant to other topoisomerase inhibitors, such as quinolone or coumarin antibiotics. However, mutations conferring resistance map to the QRDR domains of DNA-gyrase. Selected compounds display favorable pharmacokinetic properties in Rat and have been shown to be effective in a mouse pneumonia model.

The compounds are a promising new antibacterial class with activity against all major drug-resistant respiratory pathogens.
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