



This Newsletter comes at a time of uncertainty for the HPA and the public sector as a whole, budgets are more likely to shrink than to grow...

Against this background, we are doing our utmost to concentrate reference services and investigations where the resistance threats are greatest and where there is most potential for impact. I write below on what we are doing to identify current

and future treatments for infections caused by carbapenemase-producing Enterobacteriaceae. While their numbers are still small, these increasingly-referred organisms cause great concern, exactly because the treatment options are so few. Russell Hope writes overleaf that there is growing evidence that the ESBL problem, long a cause of concern, is finally peaking, reflecting prescribing shifts away from cephalosporins and quinolones. Owing to the importance of these modes of resistance many laboratories write to us seeking ESBL and carbapenemase producers as controls and, to facilitate this, we have

lodged representatives with the National Collection of Type Cultures (NCTC). Neil Woodford details the organisms that are now available on the back page. We will happily lodge other resistance types where demand exists. Last, I'm afraid that we can't do everything free, especially where the public, and individual, health benefit of our work is questionable. Accordingly, with regret, we will start to charge for MIC testing of *Pseudomonas* from cystic fibrosis from 1 January 2011. Sorry, but we hope you appreciate the reasons detailed overleaf.

DAVID LIVERMORE

What's active against carbapenemase producers?

The numbers of carbapenemase-producing Enterobacteriaceae referred to ARMRL from patients in the UK continue to grow: 11 in all the years to 2007, 22 in 2008, 73 in 2009 and 61 in the first half of 2010. The organisms are diverse, though *K. pneumoniae* dominates, followed by *E. coli*. NDM and KPC are increasingly the dominant carbapenemase enzymes followed by VIM. Many of the isolates with NDM metallo-enzymes are epidemiologically linked to the Indian subcontinent, isolated from patients previously hospitalised there. Many with VIM enzymes and some with KPC are similarly linked to Greece, though—disturbingly—other strains with KPC enzymes are spreading locally, especially around Manchester.

Many strains with carbapenemases are extremely multi-resistant and we are regularly asked for treatment advice. Although each isolate must be examined individually, a few general comments can be made. First, colistin and polymyxin B are active against the majority of producers, exceptions being (i) the few

carbapenemase-producing *M. morgani* and *Serratia* spp., which are inherently resistant, (ii) variants of one KPC-positive *K. pneumoniae* clone circulating around Manchester and (iii) occasional 'one-off' *Enterobacter* and *Klebsiella* isolates. Tigecycline too is widely active, especially against carbapenemase-producing *E. coli*, where its MICs are usually <1 mg/L. Many carbapenemase-producing *K. pneumoniae* however fall into the intermediate category, with tigecycline MICs of 2 mg/L, and a few have frank resistance, with MICs of >4 mg/L.

Aminoglycosides show strain-specific activity. Most isolates with NDM carbapenemases also have 16S rRNA methylases which alter their ribosomes to block binding of ALL human-use aminoglycosides, though the veterinary analogue apramycin remains active—an observation of no practical use! Many of the *K. pneumoniae* isolates with KPC carbapenemases collected in the UK belong to the international ST258 clone and are susceptible to gentamicin, though not to amikacin and tobramycin, whereas non-ST258 isolates are more often susceptible to amikacin. Virtually

all carbapenemase producers, except those with NDM enzyme and 16S RNA methylases, are susceptible to isepamicin, an aminoglycoside analogue licensed in Belgium but not the UK. Fosfomicin—available as an i.v. drug in Germany and marketed more widely as an oral agent for urinary infections—is active against most carbapenemase-producing *E. coli*, including those with NDM-1 enzyme, but *K. pneumoniae* and *Enterobacter* spp. are inherently less susceptible, and activity is often borderline at best. Temocillin retains borderline activity against *K. pneumoniae* with KPC enzymes and may be genuinely active if high, ticarcillin-like doses can be safely administered—an aspect that needs full and urgent evaluation.

Beyond this, it's a case of rummaging for long shots, with aztreonam active against the minority of metallo- (NDM, IMP and VIM) carbapenemase-producing strains that don't also have extended-spectrum or AmpC β -lactamases, and with cotrimoxazole and chloramphenicol each active against near random assortments of isolates.

Novel antibiotics, presently in Phase II development (proof of clinical efficacy), may offer future options and ARMRL has tested several of these against a wide battery of producers. Papers are submitted. Combinations of ceftaroline or ceftazidime with the new β -lactamase inhibitor NXL104 proved active against nearly all strains with KPC and OXA-48 carbapenemases, but not those with

metallo-carbapenemases, which are not inhibited by NXL104. Nevertheless, a combination of aztreonam (which is stable to MBLs) plus NXL104 (to inhibit ESBLs and AmpC) was active against all carbapenemase producers, including those with metallo-enzymes. No company is yet developing such a combination, but we would urge them to consider doing so.

ACHN-490, a novel sisomicin-derived aminoglycoside, proved more active than isepamicin against most aminoglycoside-resistant carbapenemase producers, but those with NDM enzyme and 16S rRNA methylases remained resistant. None of these agents, we should add, will be available for least two to three years, but we'll keep you posted on their progress.

DAVID LIVERMORE

Charging for *P. aeruginosa* from cystic fibrosis

As flagged in our last Newsletter, we have been reviewing antibiotic testing of *P. aeruginosa* and other *Pseudomonas* referred from cystic fibrosis.

Two public health points are clear. First, there is very little if any direct transmission of already resistant strains among patients. Even strains with a predilection for CF (e.g. the 'Liverpool' strain) seemingly are acquired from the environment, and acquire different patterns of resistance in each patient whom they affect. Secondly, following from this, virtually all resistance reflects the accumulation of successive random mutations, not gene dissemination.

Our MIC testing therefore does little to track resistant strains or genes usefully.

Moreover the relationship between in-vitro susceptibility and in vivo response is extremely weak for i.v antibiotics in CF and there are no breakpoints for nebulised antibiotics. We therefore also doubt that our MICs provide any better guide for therapy than a hospital's routine disc or automated results and, whilst we are often asked about new or non-conventional antibiotics, the simple fact, for *Pseudomonas*, is that there are none.

In these circumstances we have decided to suspend free reference testing of *P. aeruginosa* from CF as of 1 January 2011. We appreciate that some laboratories disagree with our view of the MICs not being useful for treatment. To accommodate this view,

we will provide a charged service for such testing, also starting from 1st January 2011.

We remain happy to test non-CF *P. aeruginosa* with 'unusual' resistance and will write more fully on what we consider 'unusual' in the next *Newsletter*. The Laboratory of Healthcare Associated Infection remains happy to type CF patients' initial *P. aeruginosa* isolates to identify whether these belong to a major clones and this service remains free to NHS laboratories. We also will continue to test, gratis, *B. cepacia* from CF for the time being, based on greater evidence of clonal spread of already-resistant strains among patients.

DAVID LIVERMORE

Decline of the ESBLs?

During the early 'Noughties' we saw a dramatic rise in cephalosporin-resistant Enterobacteriaceae, especially *Klebsiella* spp. and *E. coli*. This rise mostly reflected the spread of CTX-M ESBLs, which became a major public health concern. The concern was increased as the setting shifted, with producer isolates increasingly seen in community as well as hospital acquired infections.

Encouragingly, the last three years has seen signs of a plateau, then a decline, in the prevalence of cephalosporin-resistant Enterobacteriaceae. Data from both the BSAC Bacteraemia Surveillance (www.bsacsurv.org) and the HPA's LabBase show that cephalosporin non-susceptibility has reduced year on year since 2007, with downtrends also observed for fluoroquinolones and aminoglycosides. The as-yet-unreleased data for the 2009 BSAC bacteraemia programme confirm this trend, with resistance rates around half their 2006-7 peak levels; the HPA data—with a larger sample—show smaller reductions but confirm the reversal of trend. The reasons potentially include:

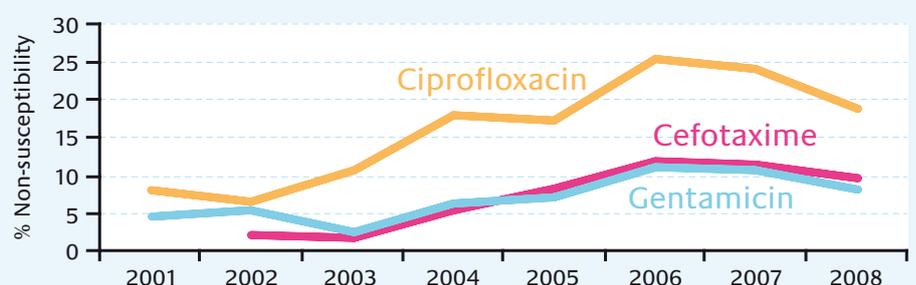
- Better infection control, especially against *Klebsiella* as a hospital-transmitted organism.
- 'Burn out' of hitherto successful clones—ESBL producers submitted to ARMRL seem more diverse and less dominated by ST131 *E. coli*—though this may be submission bias.
- Altered selection pressure with a move away from cephalosporins and fluoroquinolones.

The evidence is strongest for the last of these. Data kindly provided by IMS show sales of cephalosporins and

fluoroquinolones to hospitals falling by around one third from 2006, roughly coinciding with the peaking of ESBLs and quinolone resistance. This fall is balanced by rising use of β -lactamase inhibitor combinations and, less so, carbapenems. We believe it was largely driven by concerns about cephalosporins and quinolones selecting for *C. difficile*, but it may have had beneficial consequences for ESBLs too. These data were presented at the 2010 ECCMID and a paper is in preparation.

RUSSELL HOPE

Trends for *E. coli* from bacteraemia: BSAC data



ARMRL Services

MIC determinations (Antibiotic Resistance & Evaluations Unit)

We determine and interpret antibiograms of referred isolates for four main reasons:

1. Investigation of exceptional resistance (see www.hpa.org.uk/cfi/armrl).
 2. Evaluation of resistance mechanisms.
 3. Therapeutic guidance, particularly in multi-resistant infections.
 4. When the sending lab gets different results by different methods.
- Our standard method is BSAC agar dilution, with weekly runs for gram-negative and -positive organisms. Etests are also used in certain circumstances, e.g. for urgent requests (please phone first). Investigation is not charged for NHS labs.
 - We do not undertake disc testing, but do offer interpretations over the telephone, based on your results and our current experience.
 - The only species we do not accept are Category 3 organisms, *Mycobacterium tuberculosis*, *Neisseria* spp. most anaerobes and fungi, which should be sent to the appropriate HPA laboratories (see www.hpa.org.uk). We do accept non-TB mycobacteria, *Nocardia*, *Actinomycetes*, etc., providing therapeutic guidance.
 - Endocarditis isolates can be referred to ARMRL for sensitivity testing and therapeutic guidance irrespective of resistance, with MICs reported by phone. This is a charged service, but charges are waived where the isolate is confirmed by us to have exceptional resistances.

Molecular Investigation (Resistance Mechanisms Monitoring Unit)

- Genetic tests for resistances are performed (i) to determine whether *S. aureus* with borderline methicillin-resistance have *mecA* (charged); (ii) to test *S. aureus* for *mupA*, determining high-level mupirocin resistance (charged); (iii) to detect quinupristin/dalfopristin resistance genes; (iv)

to detect carbapenemase genes, (v) to identify mutations conferring oxazolidinone resistance and (vi) to detect genes encoding acquired AmpC enzymes. Except for *mecA* and *mupA* detection, tests are done once phenotypes have been confirmed.

New antibiotic evaluations

- ARMRL liaises with pharmaceutical companies on in-vitro evaluation of antibiotics and can undertake multi-centre surveys of the activity of new or established agents. Please contact David Livermore for details.

Sentinel surveillance and multi-centre surveys

- We have a proven track record of running large surveillance/multi-centre surveys of the activity of new or established agents. Please contact Russell Hope or David Livermore for details.

Most of all, ARMRL offers advice...

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Staffing



Farewell to Mike Hornsey, who has left us to join David Wareham's group at Barts and the London School of Medicine, undertaking a project

that extends his PhD work at ARMRL on tigecycline resistance.



Farewell too to Alex—Alejandro Beceiro Casas—who has been working with us for a year on polymyxin resistance in *Acinetobacter* spp. and on Enterobacteriaceae

strains resistant to fourth- but not third-generation cephalosporins. We wish both of you very well for the future.



Welcome to Lenise Teixeira who has joined us for six months from Rio de Janeiro to work on the plasmids and strains of KPC *K. pneumoniae*.... of which we are seeing more and more.

DAVID LIVERMORE

ESBL and carbapenemase controls

We are regularly asked to provide strains with known resistance mechanisms, particularly characterised β -lactamases, as controls for phenotypic tests or PCR assays. To make this process less arduous for ARMRL staff and more efficient for our customers, we have deposited representative strains into the NCTC. Those already lodged are listed in the table below.

- **The ESBL producers** include representatives of the five major CTX-M groups. These can be used to control single or multiplex PCR assays (e.g. Woodford et al. *J Antimicrob Chemother* 2006;**57**:154-5). Also deposited are: laboratory-derived *E. coli* strains with three fully-sequenced

plasmids encoding CTX-M-15 or CTX-M-3 enzymes (Woodford et al. *Antimicrob Agents Chemother.* 2009;**53**: 4472-82) and a representative of “UK strain A”, which is a nationally-prevalent variant of the internationally disseminated O25:H4-ST131 uropathogenic *E. coli* clone (Lau et al. *J Antimicrob Chemother.* 2008;**62**: 1241-4). Producers of TEM-, SHV- and VEB-type ESBLs are also available.

- **Carbapenemase producers** include Enterobacteriaceae and non-fermenters with class A (KPC), B (IMP, VIM and NDM) and D (OXA-type) enzymes. The *Acinetobacter* strains include representatives of the major, regionally-prevalent, UK lineages, OXA-23 clone 1

and SE (south-east) clone (Coelho et al. *J Clin Microbiol.* 2006;**44**: 3623-7).

Laboratories wishing to purchase any of these strains should first search the NCTC on-line catalogue (www.hpacultures.org.uk/). Cultures listed then can be ordered direct from the website. The newest acquisitions, including many below, remain to be catalogued, in which case purchasers should download, complete and submit the order form (www.hpacultures.org.uk/media/5C9/C7/Order_Form_2.doc). Where ARMRL receives regular requests for other resistance types we will deposit these with the NCTC to facilitate supply; suggestions are welcome.

NEIL WOODFORD

Enzyme	NCTC accession number	Species	Notes
ESBL			
TEM-3	13351	<i>E. coli</i>	Representative broad-spectrum ESBL, useful as phenotypic control
TEM-10	13352	<i>E. coli</i>	Ceftazidimase type: useful as phenotypic control
SHV-18	13368	<i>K. pneumoniae</i>	
CTX-M-15 (group 1)	13441	<i>E. coli</i>	Representative of ST131 uropathogenic clone; UK strain A; has multiresistance plasmid pEK499
CTX-M-15 (group 1)	13450	<i>E. coli</i>	Strain DH5 α with plasmid pEK516
CTX-M-15 (group 1)	13451	<i>E. coli</i>	Strain J53 with plasmid pEK499
CTX-M-3 (group 1)	13452	<i>E. coli</i>	Strain J53 with plasmid pEK204
CTX-M group 2 (unsequenced allele)	13462	<i>E. coli</i>	
CTX-M group 8 (unsequenced allele)	13463	<i>E. coli</i>	
CTX-M group 9 (unsequenced allele)	13464	<i>E. cloacae</i>	
CTX-M-26 (group 25)	13465	<i>K. pneumoniae</i>	
VEB-1	13437	<i>P. aeruginosa</i>	Also has VIM-10
Carbapenemase			
KPC-3 (class A)	13438	<i>K. pneumoniae</i>	
VIM-10 (class B; MBL)	13437	<i>P. aeruginosa</i>	Also has VEB-1
VIM-1 (class B; MBL)	13439	<i>K. pneumoniae</i>	Also has QnrS1
NDM-1 (class B; MBL)	13443	<i>K. pneumoniae</i>	
IMP-type (class B; MBL)	13476	<i>E. coli</i>	
OXA-23 (class D)	13424	<i>A. baumannii</i>	Representative of UK ‘OXA-23 clone 1’; also has an intrinsic OXA-51-like enzyme
OXA-40-like (class D)	13302	<i>A. baumannii</i>	Also has an intrinsic OXA-51-like enzyme
OXA-58 (class D)	13305	<i>A. baumannii</i>	Also has an intrinsic OXA-51-like enzyme
OXA-51-like only	13420	<i>A. baumannii</i>	UK ‘SE clone’; has <i>ISaba-1</i> upregulated expression of OXA-51 chromosomal enzyme

Endnote: *Enterococcus faecalis* penicillin^R, ampicillin^S

We get regular queries about these, and referred isolates, because the Vitek 2 flags them as likely β -lactamase producers and advises editing the ampicillin result to R. They almost certainly aren’t, and you shouldn’t.... In reality their (often borderline) penicillin resistance likely involves PBP changes. A few β -lactamase-positive enterococci are recorded, but never (yet) in the UK. If you’re concerned, check your isolate with nitrocefin.

NEIL WOODFORD

Publications

The following papers, published in print during January to June 2010 or available online ahead of print, were written by ARMRL or result from collaborations involving ARMRL staff.

1. Aschbacher *et al.* [Metallo- \$\beta\$ -lactamases among Enterobacteriaceae from routine samples in an Italian tertiary care hospital and long-term care facilities during 2008.](#) *Clin Microbiol Infect*
2. Boakes *et al.* [Molecular diversity within CC22 methicillin-resistant *Staphylococcus aureus* encoding Panton-Valentine Leukocidin in England and Wales.](#) *Clin Microbiol Infect*
3. Ellington *et al.* [Decline of EMRSA-16 amongst methicillin-resistant *Staphylococcus aureus* causing bacteraemias in the UK between 2001 and 2007.](#) *J Antimicrob Chemother.*
4. Ellington *et al.* [Polyclonal multiply antibiotic-resistant methicillin-resistant *Staphylococcus aureus* with Panton-Valentine leucocidin in England.](#) *J Antimicrob Chemother.*
5. Ellington *et al.* [First international spread and dissemination of the virulent Queensland community-associated methicillin-resistant *Staphylococcus aureus* strain.](#) *Clin Microbiol Infect.*
6. Henderson-Begg *et al.* [Mutation frequency in antibiotic-resistant and -susceptible isolates of *Streptococcus pneumoniae*.](#) *Int J Antimicrob Agents.*
7. Hill *et al.* [Linezolid-resistant ST36 methicillin-resistant *Staphylococcus aureus* associated with prolonged linezolid treatment in two paediatric cystic fibrosis patients.](#) *J Antimicrob Chemother.*
8. Hope *et al.* [Proposed disc zone breakpoints for doripenem for use with the BSAC disc susceptibility testing method.](#) *J Antimicrob Chemother*
9. Hornsey *et al.* [Tigecycline resistance in *Serratia marcescens* associated with up-regulation of the SdeXY-HasF efflux system also active against ciprofloxacin and ceftiofime.](#) *J Antimicrob Chemother*
10. Hornsey *et al.* [Emergence of AcrAB-mediated tigecycline resistance in a clinical isolate of *Enterobacter cloacae* during ciprofloxacin treatment.](#) *Int J Antimicrob Agents*
11. Hornsey *et al.* [AdeABC-mediated efflux and tigecycline MICs for epidemic clones of *Acinetobacter baumannii*.](#) *J Antimicrob Chemother*
12. Hunter *et al.* [Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies.](#) *J Antimicrob Chemother.*
13. Livermore *et al.* [Antimicrobial treatment and clinical outcome for infections with carbapenem- and multiply-resistant *Acinetobacter baumannii* around London.](#) *Int J Antimicrob Agents.*
14. March *et al.* [Colonization of residents and staff of a long-term-care facility and adjacent acute-care hospital geriatric unit by multiresistant bacteria.](#) *Clin Microbiol Infect.*
15. Mushtaq & Livermore. [AmpC induction by ceftaroline.](#) *J Antimicrob Chemother.*
16. Mushtaq *et al.* [Activity of checkerboard combinations of ceftaroline and NXL104 versus \$\beta\$ -lactamase-producing Enterobacteriaceae.](#) *J Antimicrob Chemother.*
17. Mushtaq *et al.* [Performance of the Oxoid M.I.C.Evaluator™ Strips compared with the Etest® assay and BSAC agar dilution.](#) *J Antimicrob Chemother.*
18. Schwarz *et al.* [Assessing the antimicrobial susceptibility of bacteria obtained from animals.](#) *Vet Microbiol.*
19. Schwarz *et al.* [Editorial: Assessing the antimicrobial susceptibility of bacteria obtained from animals.](#) *J Antimicrob Chemother.*
20. Tomas *et al.* [Efflux pumps, OprD porin, AmpC \$\beta\$ -lactamase and multiresistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis.](#) *Antimicrob Agents Chemother.*
21. Turton *et al.* [Incidence of *Acinetobacter* species other than *A. baumannii* among clinical isolates of *Acinetobacter*: evidence for emerging species.](#) *J Clin Microbiol.*
22. Wilson *et al.* [Trends among pathogens reported as causing bacteraemia in England, 2004 to 2008.](#) *Clin Microbiol Infect.*
23. Woodford N. [Rapid characterization of *b*-lactamases by multiplex PCR.](#) *Methods Mol Biol.*
24. Woodford *et al.* [Comparison of BD Phoenix™, Vitek® 2 and MicroScan® automated systems for detection and inference of mechanisms responsible for carbapenem resistance in Enterobacteriaceae.](#) *J Clin Microbiol.*