Welcome to the Summer AMRHAI Newsletter. Since our last issue, the two new Health Protection Research Units for HCAI and AMR (at Imperial College London and Oxford) have had to submit two-year business plans to NIHR and they have now begun recruiting. We’re looking forward to some great collaborative research to come over the next five years.

In this issue, we highlight: AST categorization of R/S versus whole genome sequencing, new anti-Gram-negative antibiotics (yes, really!), molecular tests for carbapenemases, a non-metallo-carbapenemase in a handful of Pseudomonas aeruginosa isolates, intrinsic carbapenemases in a Gram-positive species, overseas training ventures, and resistant organisms that we don’t need for reference investigation (but thanks anyway). Lastly, you may have noticed that antibiotic resistance recently won the Longitude Prize 2014, meaning that it was considered to be the most pressing of six ‘greatest issues of our time’. Given that voting was open to anyone, not just professionals with a vested interest, this suggests that perhaps public engagement activities and the ‘critical concern’ message that we’ve been sowing for so long are taking root, but will they bear fruit? Let’s hope so.

Neil Woodford

Can whole genome sequencing replace susceptibility testing?

We all rely on phenotypic susceptibility testing methods (disc, strip, broth and agar dilution, or automated methods) to categorise isolates as sensitive, intermediate or resistant to antibiotics. These assess resistance directly, and provide our benchmark for evaluating novel methods. However, key genes responsible for resistance may be detected more rapidly using molecular methods. Genetic tests for resistance are currently applied most widely for particular organisms (eg Mycobacterium tuberculosis) or for key resistance determinants (eg mec genes in staphylococci, van glycopeptide resistance genes in enterococci). There is a growing market for rapid tests to detect genes encoding ESBLs and carbapenemases in Gram-negative bacteria. While walking round the ECCMID tradeshow in May, it occurred to me that the acronym could easily stand for ‘Emerging Carbapenemases Create Markets for Innovative Diagnostics’!

Presence of a resistance gene or mutation may be used to infer resistance either ahead of or instead of a phenotypic result, but phenotypic/genotypic concordance is never perfect. Some resistance genes are expressed poorly or not at all leading to strains that exhibit susceptibility or levels of diminished susceptibility still below clinical breakpoints. Some resistant strains have genuinely novel resistance mechanisms that won’t be detected by molecular methods. In other strains, resistance may rely on the complex interplay of multiple mechanisms with no single predictive genetic marker.

Absence of known resistance genes does not always mean susceptibility and so, given this latter caveat, will molecular tests ever replace phenotypic methods? Some would argue that the answer is an emphatic ‘No!’ but advances in whole genome sequencing (WGS) are making me reconsider. I am increasingly convinced that WGS might be able to
replace much phenotypic testing in some laboratories, but this isn’t likely to happen for several years.

AMRHAI is now using Colindale’s WGS facility for selected isolates and is screening the data for resistance genes in Staphylococcus aureus, Escherichia coli, Klebsiella species and carbapenemase-producing bacteria. Our hypothesis is that these WGS-derived genotypic antibiograms will be good enough to eliminate the need to determine phenotypic susceptibility, or to restrict phenotypic testing to agents with poorest genotypic/phenotypic concordance. Determining whether AMRHAI could make this switch is just one of the research activities for the PHE/Oxford Health Protection Research Unit. Our challenge is to generate robust evidence!

**NEIL WOODFORD**

### New anti-Gram-negatives: compassionate use and clinical trials

It has been a truism for years that ‘there are no new anti-Gram-negative agents’. Yet it is ceasing to be true. Ceftolozane-tazobactam, ceftazidime-avibactam, plazomicin and eravacycline are all advancing through Phase III, and meropenem-Rpx7009 (‘Carbavance’) is about to enter these trials. Consequently we no longer have to answer an inevitable ‘No’ when we are asked about new alternatives, although we are still in the uncertain period before these agents are fully trialled and licensed.

AMRHAI will consider susceptibility testing of late stage investigational agents when no existing treatment option can be identified or when existing options are contraindicated for the particular patient. We will then help to liaise with the company’s medical department about supplying drugs to the patient’s hospital, though the decision of whether or not to make the antibiotic available is entirely at the company’s discretion. It also has to be remembered that there are unlikely to be any proven breakpoint guidelines. Both Cubist (ceftolozane-tazobactam) and AstraZeneca (ceftazidime-avibactam) have compassionate use programmes in place and are able to supply correctly labelled material in the UK. Different companies’ criteria for compassionate supply vary, but the general principles are that there must be no alternative licensed therapy, that the agent must appear active in vitro, and that it must be undergoing trials for similar infections in patients of the same age group.

Instances where new agents may have a unique microbiological activity include P. aeruginosa with mutation-mediated pan-resistance (not carbapenemase-associated pan-resistance) in the case of ceftolozane-tazobactam, and production of KPC or OXA-48 (not NDM/VIM metallo-) carbapenemases in the case of ceftazidime-avibactam. Both these β-lactamase inhibitor combinations are in clinical trials for adult patients with intra-abdominal infections, complicated urinary infections and hospital-acquired or ventilator-associated pneumonias.

AMRHAI also receives occasional requests from companies to identify hospitals with this or that resistance with a view to enrolling them in clinical trials. We have had recent approaches with respect to plazomicin, an aminoglycoside that evades modifying enzymes, but not ribosome-modifying methyltransferases, and meropenem-Rpx7009, which should extend the activity of meropenem to cover strains with KPC, but not other carbapenemase types. Confidentiality considerations prevent us from disclosing affected hospitals to companies (or to anyone else for that matter). What we do instead is to tell relevant sites that a company is interested in recruiting sites with ‘their’ resistance types and leave it to them to make contact. Please let us know if you’d like to be involved in such trials.

**DAVID LIVERMORE**
Are there multi-resistant *Staphylococcus capitis* strains on your NNU?

We have noted a particular clone of *S. capitis* in a handful of NNUs in the UK, most often causing line infections or sepsis in very low birth weight infants. The isolates are closely related and markedly distinct from *S. capitis* from adult patients, suggesting they represent an epidemic lineage. The isolates are multiply resistant, including oxacillin (*mecA*), gentamicin and fusidic acid; and they have reduced susceptibility to teicoplanin and vancomycin. Intriguingly, a similar strain has been identified in NNUs in other countries including France, Belgium and Australia. If you note any such isolates from your NNU, we would welcome them being sent to us for characterisation.

ANGELA KEARNS

PVL revisited

As many of you know, national guidance on the diagnosis and management of PVL-positive *S. aureus* infections was published in 2008. You may be interested to know that a group has re-convened to revise the document. We plan to expand the guidance to include various forms of staphylococcal toxin-mediated disease (except enteric disease) such as toxic shock, septic shock, scalded skin syndrome, and serious skin and soft tissue infections (SSTIs). We’re aiming for NICE accreditation so this is likely to be a lengthy process, but the good news is that we’re seeking to provide a more evidence-based guidance (where data are available). We hope to have a draft document available for consultation in Autumn 2015. Watch this space for further updates!

ANGELA KEARNS

Teaching in South Africa…

In May, Peter Hoffman flew from British summer to South African winter to teach building design and infection control for a week on the Postgraduate Diploma in Infection Control run by Stellenbosch University at Tygerberg Hospital in Cape Town. Students on the course were from five countries in southern Africa and from a range of health economies. The essence of the week was to establish how building design can affect or facilitate infection control and how, rather than follow guidelines written for wealthy health economies, general cost effective principles can be employed. Highlights of the course were a trip to the hospital roof to see water tanks and cooling towers (with a distant view of Table Mountain), and a visit to a clinic for MDR and XDR TB in the township of Khayelitsha – itself a fine example of how to be highly effective within local constraints.

PETER HOFFMAN

…and Georgia

Nan Shetty represented AMRHAI’s WHO Collaborating Centre for Reference & Research on Antimicrobial Resistance and Healthcare Associated Infections at a multi-country seminar on antimicrobial resistance held in Tbilisi, Georgia on 30th June to 1st July 2014. The seminar sought to raise awareness of infection control principles and antimicrobial resistance among healthcare professionals from non-EU member states. Participants came from all spheres of
healthcare delivery including hospital directors/managers, surgeons, medical microbiologists, infectious disease specialists, epidemiologists and professionals in infection prevention and control. National microbiology focal points from the surrounding countries were also present. Participants came from Georgia, Armenia, Azerbaijan, Kazakhstan, Moldova, Russia, Turkey and Uzbekistan.

Nan spoke on the practical aspects of translating theory into practice and promoting a viable infection prevention and control programme in hospitals. She also presented a real-life case of a complicated surgical site infection. The importance of having an MRSA policy, an isolation policy, a hand hygiene policy, and a central and peripheral venous catheter care bundle was emphasised. Nan was asked to provide a take-home message in a single sentence, which was: “Follow the principles of standard precautions and break the chain of infection”.

NAN SHETTY

Bacillus species

Other than its association with food poisoning, the isolation of Bacillus species, notably but not exclusively Bacillus cereus, from clinical specimens was thought to be due to contamination. But not so! We receive some 30 specimens per year isolated from blood, wounds, bone and joint, ocular, endocarditis and, more recently, total parenteral nutrition (TPN) fluid. The type of patients include the immunosuppressed, neonates, the elderly and postsurgical patients, especially when prosthetic implants are involved. Taking B. cereus as the major species, a common MIC profile is resistance to penicillin, ampicillin and cefotaxime, with MICs ≥8 mg/L. This is beta-lactamase driven – no surprise there – but did you realise that the beta-lactamase in B. cereus is a metallo-beta-lactamase (MBL)? This was the first MBL ever discovered, not recently, but in 1967 by Kuwabara and Abraham. Little did they realise the impact that MBLs in general would have today. B. cereus should therefore be resistant to all beta-lactams. Because of the increased numbers of submissions from neonates we have started to insert the following standard report comment to make this fact clear to laboratories and clinicians alike: ‘Bacillus cereus naturally possesses a chromosomally located MBL, known as BC-II. This MBL is not transmissible, but should result in resistance to all beta-lactams regardless of any inhibitor combination. In vitro results indicating sensitivity to any beta-lactam, including carbapenems, should be treated with caution, as clinical efficacy could be impaired.’

BC-II is not a plasmid-mediated carbapenemase, but we have come across other Bacillus species that have unexpected \( \beta \)-lactamase activity: B. clausii possesses a class A enzyme, BCL-1, and some isolates of this and other species, including Paenibacillus species have MIC profiles very similar to those of B. cereus. This is unexpected and we are examining genome sequences for verification. The true carbapenemase status of these species and whether tests similar to those used for Gram-negatives can be used needs to be confirmed. The only readily available MIC guidelines for bacillus species are from CLSI; disc diffusion advice does not seem to exist. The most reliably active agent is ciprofloxacin, as MICs are commonly 0.125-0.5 mg/L (bpt = 1 mg/L) although we recognise that this agent may not be licensed for use in neonates or suitable for the elderly patient (due to the risk of C. difficile-associated disease). Infections of joint prosthesis or osteomyelitis caused by B. cereus may be a problem in the elderly as not all isolates are susceptible to vancomycin and teicoplanin (MIC ranges are 0.125-8 mg/L; breakpoint = 4 mg/L). Co-trimoxazole and gentamicin are usefully active, with MICs of 0.25-1 mg/L (bpt = 2 mg/L) and 0.25-2 mg/L (bpt = 4 and 8 mg/L), respectively. MICs of linezolid are 2-4 mg/L (bpt = 4 mg/L). We hope to
provide some advice on disc testing in the future but before we are in a good position to
provide clinical guidance.

ROBERT HILL, GAURI GODBOLE AND NANDINI SHETTY

Faster testing for carbapenemase-producers: help us to help you

In our last Newsletter we announced that we had started fast-tracking molecular testing of
Enterobacteriaceae isolates submitted for investigation of carbapenem resistance as they
arrive daily. Using our knowledge of the national epidemiology of carbapenemase producers
and/or information provided on the submission form, we undertake PCR for three key
carbapenemases, namely KPC, OXA-48 and NDM. However, it’s clear from telephone calls
from requesting laboratories and clinicians that key information that would guide our
selection of urgent molecular testing is not making it on to referral forms. Frequently we also
receive repeat isolates from patients that we have previously identified as carrying a
carbapenemase-producing organism. As a reminder:

- **DO** tick the appropriate boxes on our submission form: ‘MIC evaluation’ (and include
reason for request on the form) and ‘carbapenem resistance’
- **DO** indicate clearly on the form if you require only molecular investigation of carbapenem
resistance; if known, please state the resistance gene(s) you would like us to screen for
- **DO** indicate details of: (a) travel history (particularly if there was healthcare contact
overseas); (b) transfer from a UK hospital known to have had problems with
carbapenemase-producing Enterobacteriaceae; (c) a patient previously colonised or
infected by carbapenemase-producing Enterobacteriaceae (of a different species); and
(d) results of your own susceptibility testing or methods for confirmation of
carbapenemase production
- **DON’T** just tick the ‘unusual resistance’ box or just write ‘? resistance mechanism’
without any further details; this may lead to unnecessary work, or may result in your
isolate not being selected for fast-tracking
- **DON’T** just write ‘previous CPE’; including previous AMRHAI test results on the referral
form together with the PHE reference number for the isolate could help us narrow down
our initial selection of PCR(s) to perform

KATIE HOPKINS AND DANIELE MEUNIER

Focussing on key resistance mechanisms: a service change

Since we implemented fast-track PCR testing for carbapenemase detection, the resistance
mechanisms section has seen a 55% increase in its workload in the first six months of 2014
compared to the same time period last year. In an effort to keep this manageable for
available staff we will no longer offer molecular detection of acquired AmpC genes in *E. coli*
and *Klebsiella* species. Our MIC reports will continue to infer AmpC production based on
antibiograms. This change to our molecular services will be reviewed regularly. Eventually
we hope that whole genome sequencing will provide the means to define a full resistance
genotype for referred isolates and, as Neil writes, this is something that we’re working on
with HPRU colleagues.

KATIE HOPKINS
Bacterial identification section: work in progress

We are currently in the process of merging work streams for the identification of unknown bacteria in MISU and AMRHAI to create a new bacterial identification section (BIDS) within AMRHAI. This will eventually offer a consolidated approach for processing and handling unknowns and atypical bacterial pathogens within microbiology services at Colindale for both isolates and normally sterile site clinical samples. Benefits to the user will include the creation of a single portal for customers, ready availability of clinical advice via BRD’s Consultant Medical Microbiologists and enhanced testing algorithms to provide an accurate and timely bacterial identification service. Look out for further details of BIDS in our Winter issue.

Julie Logan

Confirmation of glycopeptide resistance in enterococci

We constantly seek to focus our susceptibility testing reference service on the investigation of clinical isolates with unusual resistance, detection of emerging resistances and evaluation of susceptibility for therapeutic guidance. Our resources are limited and it is critical they are deployed effectively against emerging resistances of public health concern.

In this context, we do not seek routine submission of suspected VRE for confirmation of glycopeptide resistance. The great majority of enterococci with the common VanA form of glycopeptide resistance are clearly resistant to vancomycin and teicoplanin, whilst those with VanB are typically resistant to vancomycin but not teicoplanin. UK NEQAS distributions show that these can be detected by disc or automated methods and do not need reference confirmation by determination of MICs.

We will no longer test VRE sent in simply for confirmation, but we remain happy to adjudicate on any enterococci giving doubtful results with vancomycin, and we do want any S. aureus suspected to have glycopeptide resistance.

Robert Hill

Deluged with carbapenem-resistant P. aeruginosa

In the last issue we outlined which carbapenem-resistant Pseudomonas spp. genuinely merit submission to AMRHAI for investigation of their underlying resistance mechanism(s). Despite our plea for local triage, we are being inundated with isolates of P. aeruginosa that are resistant to carbapenems while remaining susceptible to other β-lactams – these almost always have mutational resistance associated with combinations of porin loss, up-regulated efflux, or derepressed AmpC – and we are even asked to investigate resistance to ertapenem (which is intrinsic to the genus!) at least once or twice every week. These submissions needlessly overburden our extremely hard-working susceptibility testing team.

To reiterate then, we seek only those Pseudomonas isolates that are suspected validly to produce a carbapenemase (that is a transferable carbapenem resistance mechanism). Such isolates should be resistant to all relevant carbapenems (that is imipenem, meropenem and doripenem) and piperacillin-tazobactam and usually ceftazidime; susceptibility or resistance to aztreonam is variable because most carbapenemase-producing Pseudomonas have metallo-enzymes, usually VIM-types (but see Jane and Laura’s discovery below). By considering more carefully the isolates you submit, you will not only reduce our workloads (which will please the team), but also contribute to faster testing and reporting (which will please you).

Neil Woodford
GES-5 carbapenemase in *P. aeruginosa* ST235

There is an association between particular *P. aeruginosa* sequence types (STs) and metallo-carbapenemases (MBLs), especially VIM enzymes. ST235 is one of several multi-resistant lineages found internationally, and corresponds to a VNTR profile of 13,3,6,4,5,1,x,2,x (where x is variable). As part of Laura Wright’s PhD studies, we noticed a few highly carbapenem-resistant isolates of this lineage (with imipenem and meropenem MICs >32 mg/L) that lacked an MBL. We sent a representative for whole genome sequencing …and found the gene for GES-5 *non-metallo*-carbapenemase as part of an integron (a Gram-negative gene capture and expression system); we’ve not detected this particular enzyme previously in the UK. So far we’ve found eight other GES-5 producers (so a very small proportion of the hundreds of *P. aeruginosa* you send us) from five different hospitals and all belong to ST235. We’re currently analysing their antibiograms for any tell-tale patterns to aid recognition in the future!

**JANE TURTON AND LAURA WRIGHT**

**Surveillance studies update**

Thanks to all who participated in our surveys of *P. aeruginosa* and *K. pneumoniae* from bacteraemia and the EuSCAPE study, which are now finished (except, that is, for the data analysis and paper writing). Participants have received the overall report on the *P. aeruginosa* survey, and the reports for your own laboratories on the *Klebsiella* survey. In both cases we found a large number of types associated with blood infections, with common types that we had seen among screening isolates also represented. We look forward to working with you to get these published – apologies for the delay in progressing that on our part, but we are determined to get there, and many thanks to those of you who have already provided comments. As you may know, Kim Mallard, who carried out these surveys, has sadly moved on to pastures new (we miss you, Kim!) However, she has a very worthy successor in the form of Amy Coward, who began her new post on 4th August. Amy is already well known to us as she has been doing a sterling job for us in AMRHAI in another role for the last two years, and we are thrilled that she is taking on the surveillance. Her first survey is going to be on types of enterococci from clinically significant infections. As you may be aware, we have seen a large increase in submissions of enterococci for typing, but many of them are from screening of patients and we need to get a handle on which types are associated with infection. In addition, thanks to lots of hard work from Kate Martin, we have seen marked geographical clustering of types over large regions, and we hope this survey may also shed some light on this, perhaps by including some centres from which we would not normally receive isolates. If you are interested in participating, we would love to hear from you! Your isolates will be typed and you will receive a report on each. At the end of the study period, you will receive an overall report for isolates submitted from your laboratory and a report that summarises the results for all the participating laboratories. Then the plan is to submit the findings for publication with everyone included as the ‘AMRHAI Surveillance group’. We would love to welcome you on board! If you are interested, please drop Amy or myself a line.

**AMY COWARD & JANE TURTON**
WGS of an international clone of *P. aeruginosa*

In the last *Newsletter*, we mentioned that we were having next generation sequencing (NGS) done on representatives of the most prevalent type of *P. aeruginosa* associated with metallo-carbapenemases (MBLs) that we see in the UK — this has a VNTR profile of 11,3,4,3,2,2,x,4,x (where x is variable) and corresponds to sequence type (ST) 111, and is most often associated with a VIM carbapenemase gene. It is an international lineage found in geographically disparate locations, and we need the extra resolution provided by NGS to try to work out to what extent, and by what pathways, this type is spreading in the UK, a particular concern being the increasing number of hospitals in the London area that are affected. The isolates were collected between 2005 and 2014. This work is now almost complete. Isolates were sequenced on the HiSeq by Cath Arnold’s team at Colindale and analysed by the Bioinformatics team, led by Anthony Underwood. SNP analysis indicated that isolates fell into a number of clearly separated clusters, which did fit with the epidemiological information we have on these isolates. Most isolates carried *bla*<sub>VIM</sub>, but some carried *bla*<sub>IMP</sub>; one carried *bla*<sub>NDM-1</sub>, and some had no MBL — those with different or no carbapenemase genes clearly clustered separately from one another. There was one very tight cluster of VIM-positive isolates involving two London hospitals with close links between them and documented patient transfers, which was very well separated from the other clusters. But there was a broad cluster involving a number of London hospitals, which is concerning. Welsh isolates fell into a separate cluster. BEAST (Bayesian Evolutionary Analysis Sampling Trees) analysis, which produces time-measured phylogenies where each sample on the tree and putative common ancestor can be assigned a time, gave essentially the same results. We conclude from all this that:

- SNP or BEAST analysis of whole genome sequences can provide discrimination among closely related isolates belonging to an international clonal lineage, which does appear to reflect the known epidemiology
- information from WGS can be used to provide comparative studies of genes found in particular subsets of isolates, to provide information on them and to identify targets for rapidly seeking them

We have begun to use SNP analysis of NGS sequences of representatives of ST111 and also of ST235 using a workflow that Anthony wrote for us on Galaxy (https://usegalaxy.org/ Data intensive biology for everyone). We therefore can provide retrospective information on how closely particular isolates within these lineages may be linked, although we regret we can’t currently do that in real time.

JANE TURTON

Staffing and visitors

Since our last issue, AMRHAI has sadly said farewell to Ineka Gow, who has returned to Australia, and Kim Mallard, who left to join industry. We wish them both well.

We extend warm welcomes to our new starters and congratulations to several members who’ve moved internally to new positions: Natasha Bundock, Christine Carr, Daniel Godoy, Maimuna Kimuli, James Rogers and Catherine Wiggins will all support service development across the Bacteriology Reference Department; Christiana Moigboi joined our Staphylococcus Section; and Noor Patwari moved to the Antibiotic Sensitivity Section.

Welcome also to Pascale Trépanier from Canada, who is visiting for two months to help analyse EuSCAPE data as part of her MSc project, and to Valia Dimou from Greece, who returns for five months to undertake a study of VIM-producing Enterobacteriaceae funded by an award from the HIS.
Publications: January – June 2014

## AMRhai Senior Staff… for when you need advice

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