Public Health England

AMRHAI *News*

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It was an unexpected honour to win the '*Science in Health Research*' award at the Chief Scientific Officer's Healthcare Science Awards 2017 (see here). Thanks (!) to those who nominated me. Elsewhere, AMRHAI's long-term contribution to global carbapenem resistance research was also highlighted in a recent bibliometric analysis (see here), which showed my team's sustained performance particularly in terms of our multi-country collaborations and the impact of our publications. So much of what we achieve in science relies on solid team efforts and this is seldom fully recognised. My deep appreciation goes to all those who have contributed and collaborated along the way, and to those who still do. Of course, we're always looking for new opportunities and collaborators…

As ever, this issue of our newsletter contains an eclectic mix of topics for your coffee-time delight: from tattooing to colistin resistance; from whole genome sequencing of *S. aureus* to our need to rationalise requests for typing of opportunist pathogens; from the positioning of new β-lactam / β-lactamase inhibitor combinations to AMR-related training and capacity building overseas. The issue ably illustrates AMRHAI's increasingly diverse portfolio. Enjoy!

Neil Woodford

Infection control illustrated

The Infection Control section addresses issues wider than just those that are healthcare associated; one such area is tattooing. Working closely with others in PHE, the Chartered Institute of Environmental Health (representing those who will have to enforce standards) and tattooists, the Tattooing and Body Piercing Guidance Toolkit (available **here**) was produced in 2013. There is now progress on the production of a European Standard on safe tattooing, to be used both by tattoo artists and national regulatory authorities.

Whatever the individual opinions on matters aesthetic, you just have to look around to appreciate that tattooing has become mainstream. Some may be surprised by the apparently low observed level of associated infection but the potential is definite. The tattooing industry is keen to adopt measures both to reassure clients and that can be used to control "scratchers"; the industry term for untrained individuals who practice unsafely (frequently only in their clients' homes, making them difficult to trace), often producing grotesque results.

The production of this standard probably has more complexity than most. Standards typically draw on existing industry standards (very little exists here on an international basis) or on extrapolation from analogous situations. Whilst the obvious analogy is standards of hygiene and sterilization in medical practice, it has to be accepted that tattooing does not pose the same risks as major surgical procedures. A pathway ensuring safety whilst remaining practical for legitimate tattooists is being mapped out, but this is very much a work in progress.

Peter Hoffman

Typing of opportunistic pathogens

In the Opportunistic Pathogens section, we receive far more submissions for typing than we can reasonably process, particularly of the enterococci. Also, the justification for many typing requests is poor or not given. In the last newsletter, we gave notice that from April 2017 we will **cease typing isolates of any genus** submitted without a clear and clinically justifiable reason, which needs to include **details of the comparisons sought** and of the **underlying question posed**. We are now triaging all typing requests and have started to issue reports to explain that we do not type isolate(s) if the request does not fulfil our criteria for processing; these isolates will be archived and could be retrieved for comparative purposes should a need arise. So we hope the following will be helpful:

- for suspected cross-infection episodes, we will be happy to compare up to 10 **potentially related** isolates of the same species (related in time and space) submitted on a multiple isolates form (H1, available **here**); you should outline the epidemiological information to support a possible link
- where cross-infection/an outbreak has been confirmed by our typing, only send further isolates if you need to investigate apparent ineffectiveness of interventions put in place to control it (ie from post-interventional cases)
- we will not be typing duplicates or multiple isolates of the same species from the same patient, unless you can provide a very good reason for us to do so
- we cannot provide comparisons with undefined previous isolates, with no idea of whether any potential match is relevant or not. Single isolates submitted with the query 'same as previous' with no other detail will simply be archived, as will those just saying 'VRE'; we cannot type every isolate on the off-chance that there may be a match. This also applies to isolates submitted on a multiple isolates form with no clear thread joining them
- it is not always clear if typing would be helpful at the time of submission, especially if isolates are submitted primarily for another reason eg carbapenemase detection. Please remember that you can request comparisons of archived specimens retrospectively if you subsequently suspect a link
- consider carefully if there is any value in sending environmental isolates that could represent contamination from the patient – such as from toilet bowls or sink plugholes
- provide as much relevant information as possible, always including the ward if from hospital in-patients

Jane Turton, Nandini Shetty, Neil Woodford

Whole-genome sequencing for *Staphylococcus aureus*

By now, some of you will have received reports from us detailing the results of *S. aureus* reference tests (typing, toxin gene profiling and AMR gene detection) derived from whole-genome sequencing (WGS). Although it's early days in this transition, we hope you can see improvements that benefit you and your patients. We of course welcome your feedback! Examples where we've used WGS for characterising *S. aureus* can be found **here**, **here** and **here**.

Aside from reference testing, we seek to focus on areas where WGS will enhance national public health surveillance. In recent years, as MRSA bacteraemia rates have declined we've **noted** a marked change in the molecular epidemiology. To monitor this change more fully, we aim to perform WGS on all MRSA bacteraemia isolates from England from April 2017 - March 2019. This work will help us better understand changes in molecular epidemiology and how MRSA clones emerge and spread, both locally and nationally. We plan to link WGS data outputs with the demographic, clinical and geographic information from the national Mandatory Bacteraemia Surveillance Programme and will disseminate our findings via an

annual report. It is hoped that increased understanding of the epidemiology of MRSA may inform interventions aimed at further reducing rates of MRSA infection.

Accordingly, with effect from 1st April 2017, we invite you to refer to us a single MRSA isolate from each episode of MRSA bacteraemia in England using our standard **referral form**. As is currently the case, referrals to support national surveillance will be funded by GIA. For each isolate referred, you will receive a strain characterisation report detailing the Multi-Locus Sequence Type (MLST) of the isolate alongside the presence/absence of a panel of AMR and virulence genes; sequence data will subsequently be deposited in an open access database. For further information, please contact Angela Kearns (**angela.kearns@phe.gov.uk**).

Angela Kearns, Bruno Pichon, Michel Doumith, Peter Staves, Russell Hope

Antimicrobial resistance in *Neiserria gonorrhoeae*

The **2015 report** for the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) was published in November 2016. To summarise, the first documented case worldwide of treatment failure to dual therapy with ceftriaxone and azithromycin was reported in England and confirmed at PHE. There were no further cases of ceftriaxone resistance reported. Azithromycin resistance on the other hand is of concern; GRASP reported 10% resistance (MIC >0.5 mg/L) to azithromycin, compared with 1% in 2014. It should be noted, however, that the MICs for the majority (91%) of these isolates were 1 mg/L and that a change of the medium used for susceptibility testing contributed to this increase in resistance. Testing of the 2016 GRASP collection is well underway, as is streamlined processing. For example, whilst full MICs continue to be determined for clinically-relevant antibiotics (ie azithromycin and ceftriaxone), breakpoint plates are now in use for historical antibiotics (eg ciprofloxacin) or where susceptibility distributions are clearly defined (eg spectinomycin). Further changes are proposed for the 2017 GRASP collection; primarily the introduction of electronic data submission for collaborating laboratories. We hope that these changes will help to improve laboratory efficiency and timeliness of reporting whilst still providing the completeness of data that has come to be expected from this world-renowned surveillance programme.

The European Gonococcal Antimicrobial Surveillance Programme is also run in AMRHAI, and the 2015 data show a similar picture to GRASP - very low ceftriaxone resistance (one isolate in 2015) and 'high' azithromycin resistance at 7.1%. The report will be available on ECDC's website soon.

Michelle Cole and Rachel Pitt

Ceftazidime-avibactam and carbapenemase producers

Ceftazidime-avibactam ('CAZ-AVI') is the first commercialised β-lactamase inhibitor combination to inhibit bacteria with KPC or OXA-48 (not metallo-) carbapenemases. It was licensed by the EMA in mid-2016 and is among the antibiotics recently purchased from AstraZeneca by Pfizer, who are now making it available in the UK. Licensed indications are complicated UTI, hospital-acquired pneumonia and –with metronidazole– complicated intra-abdominal infections. Based on the licensed dose of 2+0.5g q8h, EUCAST has breakpoints of S ≤8+4 and R >8+4 mg/L for both Enterobacteriaceae and *Pseudomonas aeruginosa*.

AMRHAI began testing CAZ-AVI in July 2015 and, during the first year, found susceptibility for 200/202 Enterobacteriaceae with KPC carbapenemases and 328/333 with OXA-48-like enzymes. All the KPC isolates were non-susceptible to ceftazidime alone, as were 212 of those with OXA-48 (OXA-48 itself doesn't attack ceftazidime, so producers are only ceftazidime non-susceptible if they also have ESBL or AmpC activity). ESBL- and AmpC-producing Enterobacteriaceae were almost all susceptible to CAZ-AVI, whereas those with metallo-carbapenemases were resistant. For *P. aeruginosa* CAZ-AVI inhibited >90% of ceftazidime-resistant isolates with derepressed AmpC, but resistance remained in those with upregulated efflux, VEB-type ESBLs or MBLs. CAZ-AVI thus contrasts with ceftolozane-tazobactam, launched a little earlier, which is generally active at breakpoint against both AmpC-derepressed and efflux type *P. aeruginosa*, but lacks activity against all carbapenemase-producing Enterobacteriaceae except for the minority of ceftazidime-susceptible OXA-48 isolates. In other words, and from a microbiological perspective, CAZ-AVI and ceftolozane-tazobactam aren't 'competitors' for the formulary committee: rather, they belong to different initial niches, one labelled 'problem *Pseudomonas*' and the other 'KPC and OXA-48 Enterobacteriaceae'.

A concern is that, in **laboratory studies**, we have selected CAZ-AVI-resistant mutants of *Klebsiella* and *Enterobacter* with altered KPC genes. Since other avibactam combinations were less affected we deduced that the enzyme was becoming a 'better' ceftazidimase, not avibactam-resistant, a conclusion supported by **later biochemical work in the US**. Many of these mutations simultaneously degrade the KPC enzyme's ability to confer carbapenem resistance, with MICs reduced. No outcome data for patients with carbapenemase producers were reported from the licensing trials, and it is unclear if any producers were represented. Recently, however, a case-series was published from Pittsburgh, describing 31 severe infections due to KPC producers treated with CAZ-AVI (**here** and **here**). Encouragingly, 59% of patients were clinically cured, but resistance – with the same mutations we saw *in vitro* – arose in 3/31. This is concerning. One answer – off-label and needing research – might be to co-administer meropenem with CAZ-AVI, protecting both β-lactams and aiming to counter-select an evolutionary path that reduces carbapenemase activity.

David Livermore

Detecting transferable colistin resistance mechanisms

Faced with the increasing public health threat posed by multidrug-resistant Gram-negative bacteria, colistin often remains an important 'last-line' option for treatment of serious infections. Most colistin resistance in the Enterobacteriaceae results from mutations in several different chromosomally-encoded genes (for a recent review, see **here**). However, in 2015 the first transferable colistin resistance mechanism encoded by the *mcr-1* gene and located on a plasmid was reported in *Escherichia coli* isolated in **China** and later described worldwide in various enterobacterial species isolated from the environment, vegetable and meat products, animals and humans. Subsequently, *mcr-1.2* and *mcr-1.3* – variants of *mcr-1* exhibiting single amino acid substitutions – were described in **Italy** and **China**, respectively, whilst *mcr-2*, which shares 81% amino acid identity with *mcr-1*, has been identified in ***E. coli* from pigs and cattle** in Belgium. To date, there have been few clinical carbapenemase-producing organisms confirmed as carrying *mcr* genes. However, prior carbapenem usage was found to be a **risk factor for carriage of *mcr-1*-positive *E. coli*** in China, suggesting that the association between *mcr-1* and carbapenem resistance may become stronger in the future.

Chromosomally-encoded forms of colistin resistance are not transferable between strains, with resistance resulting either from *de novo* emergence or clonal expansion of resistant

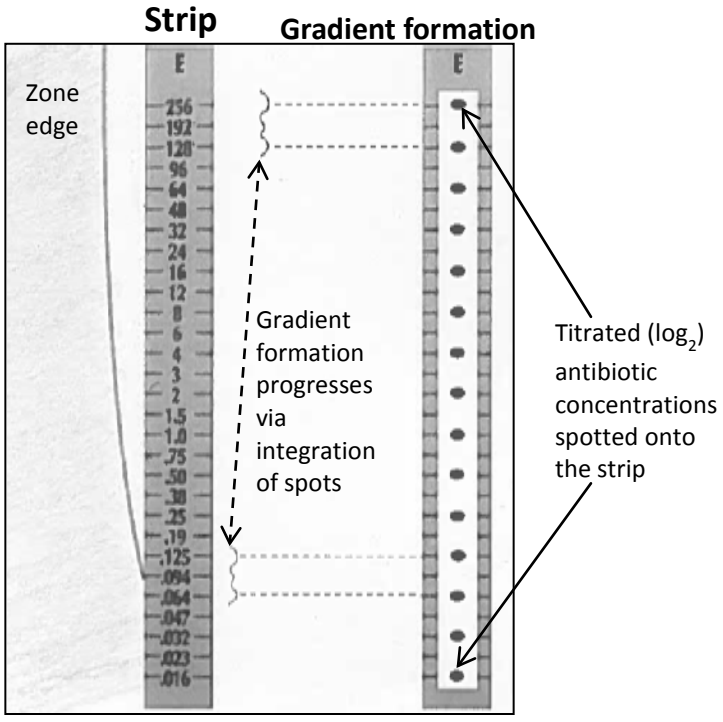
isolates. In contrast, *mcr* genes have greater potential for impact on public health than mutational colistin resistance since infection prevention and control measures may not successfully contain the resistance as the *mcr* gene may "escape", transferring among strains, species and genera. Rapid identification of bacterial strains producing *mcr* genes is therefore important for prompt implementation of infection prevention and control measures in order to prevent further spread of resistance.

A **retrospective analysis** of the PHE archive of whole-genome sequences of ~24,000 bacterial isolates from surveillance collections, submissions to reference services and research projects identified 15 *mcr-1*-positive isolates consisting of *Salmonella enterica* and *Escherichia coli* isolated from humans and poultry meat. In order to address this emerging resistance threat the Resistance Mechanisms Section has implemented a multiplex PCR to seek *mcr-1* (and its variants) and *mcr-2* in isolates of Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* that are confirmed as colistin resistant by AMRHAI. *mcr* genes have yet to be described in *Pseudomonas* or *Acinetobacter* spp. (though a plasmid bearing *mcr-1* was shown to be **stably maintained in *P. aeruginosa***), but if we don't look we won't find! We will also be seeking further information, via a questionnaire, on patients with *mcr*-positive isolates in order to try to understand the risk factors associated with acquisition of a *mcr*-producing organism.

Katie Hopkins

Gradient MIC strips: the diffusion confusion and colistin

The gradient MIC strip (*eg* Etest®, M.I.C.E.™) is a quantitative technique for determining antimicrobial susceptibility (AST) in terms of an MIC in µg/mL (= mg/L). This method uses a stepped antibiotic titration on a strip of material that, when placed on to an agar surface, allows diffusion of the antibiotic concentrations into agar to form a gradient. The figure below shows different concentrations of an antibiotic spotted on a $\log_2$ scale coincident with the scaling on the upper surface of the strip. After incubation, bacteria inhibited by the antibiotic under test form an elliptical zone around the strip (hence the term epsilometry) such that, with appropriate calibration, the elliptical zone reads the MIC at the point of intersection on the calibrated scale.



There are many reports in the literature of the good correlation of results of gradient MIC strip tests with those determined by a dilution method (agar or broth). However, **EUCAST** has found that determining the MICs of some antibiotics, notably and most recently colistin, by gradient MIC strips is unreliable and the variation non-systematic. Indeed, non-dilution testing of colistin is fraught with difficulties; its diffusion characteristics do not produce zones that accurately reflect the dilution-determined MIC, and lead to inaccuracies in categorisation against clinical breakpoints. The zone depends on variable parameters such as the molecular weight of the antibiotic and its charge in the agar

matrix. A further problem most notable with larger molecular weight compounds occurs with the alteration in generation time as the zone edge is approached in the agar. In dilution methods, the antibiotic and any accompanying inhibitor is in a set concentration at each $\log_2$ step; there is no variation.

There are three important messages:

1. False antimicrobial susceptibility testing (AST) interpretations for colistin, arising from non-recommended methodologies, can cause either major or very major errors.
2. Both **EUCAST** and CLSI only recommend microbroth dilution determination of MICs for colistin. We are undertaking further evaluation with a robotic system over the next few months.
3. AST for colistin can be readily undertaken locally with commercial microbroth dilution kits. There is no need to refer isolates to AMRHAI for colistin susceptibility testing unless *acquired* resistance has been demonstrated locally (*ie* please do not refer isolates of intrinsically colistin-resistant species – see **here**).

Robert Hill

Resistance determination and mixed cultures

The Resistance Mechanisms Section and Antibiotic Resistance Evaluation Section receive growing numbers of mixed cultures, usually from a small number of 'persistent offenders'. Whilst molecular detection of resistance genes does not require a pure culture *per se*, pure cultures are essential for further work, particularly MIC determination, and resolution of mixed cultures represents additional work for the reference laboratory.

We will only separate and work on any submissions that grow a maximum of distinct two colony types. Any received that produce more than two colony types on subculture will not be processed. Sending laboratories will receive a report indicating their submission was unsuitable for testing due to a mixed culture along with an invitation to resubmit, and a fixed administration fee will be charged.

Katie Hopkins, Danièle Meunier and Robert Hill

The WHO Collaborating Centre for Reference and Research in AMR and HCAI: the CAESAR experience

As part of its WHO Collaborating Centre activities (see **here**), AMRHAI is actively involved in laboratory capacity building in the Central Asian and Eastern European countries to enable them to provide quality data to the Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) programme and the Global Antimicrobial Surveillance System (GLASS), both organised by the WHO.

Nan Shetty, Daniele Meunier and Neil Woodford play a key role in building laboratory capacity, establishing quality assured and standardised methodology and mentoring laboratories involved in detection of AMR. In 2016, Nan and Daniele were involved in country missions to Albania, Armenia, Georgia, the Russian Federation, Turkmenistan and

Uzbekistan. We also worked with colleagues in UK NEQAS and NCTC to provide the EQA scheme for the CAESAR programme, and panels of control strains for in-country training.

Daniele was one of two trainers responsible for delivering a 4-day 'wet-lab' training course for microbiologists in Ashgabat, Turkmenistan. Theoretical lectures provided the background information needed to perform wet lab activities. The participants were shown techniques to determine antibiotic susceptibility by disc diffusion, gradient strips and automated systems according to EUCAST methodology with an emphasis on detection of ESBLs, vancomycin-resistant enterococci, and inducible resistance to clindamycin in *S. aureus.*

Nan participated in a mission to Yerevan, Armenia and Tashkent, Uzbekistan to provide training and discuss antimicrobial stewardship and infection prevention control (IPC). She also participated in visits to key hospitals and met staff in order to discuss the creation and delivery of a robust IPC programme and stewardship protocols, and provide clarification where relevant.

Danièle Meunier and Nandini Shetty

Training in China

Neil, Nan and Carmel Curtis (from UCLH) recently completed a programme of work, undertaken as part of a Prosperity Fund award to Professor Xiao Yonghong from Zhejiang University, to train Clinical Microbiologists and colleagues from related disciplines in China in aspects of AMR, antibiotic stewardship and infection prevention and control. The overarching objectives were to show Chinese colleagues: (i) how Consultant Microbiologists in the UK play a critical role in the multidisciplinary teams that manage our hospital patients; and (ii) the importance of an expert antibiotic reference laboratory. In contrast, many Clinical Microbiologists in China are almost entirely lab-based, with little or no patient contact, and there is no currently designated national reference laboratory.

The training programme began last September with a pilot course at Colindale, followed by lectures and hospital visits in Hangzhou and Jinan in October. The final deliverable was a 3-day course, run last month in Beijing for almost 60 Chinese colleagues. This comprised lectures, highly interactive breakout groups to discuss complex clinical cases chosen to emphasize issues that surround growing multidrug resistance, and a quiz. A questionnaire on current capability and practice was completed in more than 100 Chinese hospitals and sets out areas for improvement, which may be addressed in future projects.

Neil Woodford and Nandini Shetty

AMRHAI senior staff …for when you need advice.

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Dr Julie Logan **julie.logan@phe.gov.uk**; Tel 020-8327-6059	Bacterial identification (unknown, atypical, fastidious, emerging bacteria); culture negative clinical specimens (16S rDNA sequencing)
Dr. Jane Turton **jane.Turton@phe.gov.uk**; Tel 020-8327-7224	Gram-negative typing and serodiagnosis; enterococci; identification of opportunistic and CF pathogens