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AMRHAI *News*

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(contact: amrhai@phe.gov.uk**)**

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Big changes will be happening in PHE's National Infection Service this year, with many appointments being made to its new Senior Management Team. As always, we will aim to minimize the impact of these changes on reference service users, although big changes will be happening in AMRHAI, too. The arrival of robots heralds our intention to make a long-overdue switch to broth microdilution susceptibility testing (after validation, of course!) Another new robot is automating our CPE detection service and allowing us to test for a wider panel of resistance genes more rapidly.

Elsewhere in this issue, you'll find updates on our STI services, on enhanced MRSA bacteraemia surveillance, on *Pseudomonas aeruginosa* and ear piercing, and even an earworm ditty to help you decide what NOT to refer for susceptibility testing.

Finally, I have recently begun work with Fiona Carragher (Deputy Chief Scientific Officer of NHS England) on the Diagnostic Stewardship work stream for the new *UK AMR Diagnostics Collaborative*. As part of a broad brief, our multi-organisational group will consider what AMR tests exist and where they could be most effectively used for patient benefit and safety, which leads us rather nicely into this Newsletter's first article.

Neil Woodford

Where to deploy AMR diagnostics?

In September, Derrick Crook (the Director of PHE's National infection Service) issued a letter to the NHS in which he reiterated the rationale for charging for some services. From April 2018, we will charge for more of AMRHAI's resistance-related services, albeit reluctantly. Regular readers know that my Unit constantly reconsiders its service provision to ensure that we remain focussed on emerging issues of public health concern. Over the last year our limited resources have been stretched beyond what is reasonable by hugely increased workloads, often with over 20,000 MICs determined per month! We are introducing robotics for both molecular and susceptibility testing, and will switch in 2018 to broth microdilution testing to manage rising numbers but, even then, our current workloads are simply not sustainable.

Currently, many referrals ask for colistin MICs or for confirmation of carbapenemase production. Commercial solutions for both exist, meaning that such testing can be deployed in frontline diagnostic laboratories to accelerate impact on individual patient management and infection prevention and control decisions. Several suppliers offer microdilution panels for colistin testing and these allow labs to conform to EUCAST and CLSI recommendations for colistin susceptibility testing. Similarly, multiple commercial tests are available to detect the UK's four major carbapenemase families. These are KPC, NDM, OXA-48-like and VIM and, although they still have low national prevalence, they are widely scattered and regularly found, as many readers will know first-hand. Locally-determined molecular results can be entered into **PHE's Electronic Reporting System** for carbapenemase producers to contribute to national surveillance, understanding of risk factors and national policies to limit spread.

To be clear with regards to charging: (i) extended molecular testing for IMP, GES and other rarer carbapenemase types in carbapenem-resistant isolates that we confirm negative for the 'big 4' families will remain free of charge; (ii) typing of CPE to support outbreak investigations will remain free of charge, irrespective of carbapenemase type (although, as we indicated in the **last Newsletter**, typing will not be done 'just in case' on an isolate-by-isolate prospective basis, and must address a defined hypothesis); and (iii) mechanism studies of isolates confirmed to have acquired colistin resistance and epidemiological typing for any clusters will remain free of charge.

Investigating and seeking to explain unusual or exceptional resistance of public health importance remains a fundamental function of my Unit, as it should be for any reference laboratory. In part, however, we must define 'unusual' by the absence of commercial tests suitable for local use.

Neil Woodford

Some bacteria NOT to refer…. We've got a little list

AMRHAI's core role is to investigate unusual resistance or potential public health importance, and to do so as swiftly as possible. One of the problems we face, with limited staff and resources, is the large number of unexceptional isolates that come in for investigation, with resistances that are commonplace for their species. We appreciate that our commonplace may be rare in your part of the country, but some types are widespread.

And, like Ko-Ko in the *Mikado*…..

We've got a little list; we've got a little list,
Of bugs we all agree, we far too often see…

There's the dratted CNS that comes for teicoplanin MIC
There's the little *Pseudomonas* that has lost its porin D
And the pestilential *E. cloacae* with over-much AmpC
There's every sort of *Enterococcus* with VanA, B, C
And there's the susceptible bacillus, 'Wanting a colistin MIC'

They're on our little list; they're on our little list
They'd none of them be missed; they'd none of them be missed;

With apologies to William S Gilbert

To explain:

Coagulase-negative staphylococci for teicoplanin MICs. Unlike vancomycin, dalbavancin and daptomycin, teicoplanin is unreliable against CNS, with 20-25% of isolates resistant. This has always been the case, though the mechanisms are unclear. Local gradient strip results are adequate to guide use and there is no reason for reference investigation. We would counsel against use of the drug wherever results are borderline or equivocal, especially in bone and joint infections. Please continue to refer CNS or *S. aureus* that are resistant to vancomycin, dalbavancin, ceftaroline or daptomycin. We also remain happy to look at *S. aureus* with suspected teicoplanin resistance.

***Pseudomonas* resistant to carbapenems, but susceptible to cephalosporins and piperacillin/tazobactam.** These have just lost the carbapenem-specific OprD porin; they aren't carbapenemase producers. Don't waste the stamps! We increasingly view ceftolozane-tazobactam resistance as the best marker of an unusual *P. aeruginosa*

deserving reference investigation, but remain happy to look at those with broad resistance to cephalosporins, carbapenems and penicillins.

***Enterobacter* spp. resistant to cephalosporins and (maybe) ertapenem, but fully susceptible to meropenem.** These mostly have derepressed chromosomal AmpC β-lactamase together with reduced permeability, though a few have ESBLs and reduced permeability. Ertapenem has low breakpoints and is less stable than other carbapenems to these mechanisms. Please only refer *Enterobacter* isolates if they meet EUCAST screening criteria for likely carbapenemase production (meropenem MIC >0.125 mg/L, or meropenem disk diffusion zone diameter <28 mm).

Vancomycin-resistant enterococci. Detection of these organisms is well within the capacity of diagnostic laboratories, as shown by numerous NEQAS exercises. Note that *Enterococcus casseliflavus* and *Enterococcus gallinarum* have chromosomal VanC and are inherently resistant at low level to vancomycin, not teicoplanin. We remain happy to examine suspected linezolid or tigecycline resistance in enterococci.

Colistin resistance. EUCAST guidance that gradient strips are unreliable for colistin has led to an upsurge in referrals of colistin-susceptible bacteria 'For colistin MIC determination.' This is not appropriate use of reference services. Convenient broth microdilution tests for colistin are now available commercially and should be used by diagnostic labs. Only isolates found resistant should be referred. We will then be happy to (try to) confirm resistance and, as appropriate, screen for *mcr* genes.

David Livermore

All change in the Resistance Mechanisms Section (again…!)

Since 2015 our carbapenemase molecular detection service has revolved around an initial real-time PCR that detects KPC, OXA-48-like, NDM and VIM carbapenemase genes. Whilst these 'big 4' carbapenemases remain the most common carbapenemases circulating in the UK (and globally), other carbapenemases belonging to the DIM, FRI, GES, IMI/NMC-A, IMP, SME and SPM families have been identified by AMRHAI in isolates referred by UK laboratories. However, screening for these additional carbapenemase genes, and for transferable colistin resistance genes, has relied on a number of conventional PCR assays and Sanger sequencing, which require significant hands-on time. As application of these assays is directed by our knowledge of local epidemiology and/or interpretive reading of antibiograms this can lead to a delay in further testing whilst we await MICs. In addition, the increasing numbers of isolates submitted for investigation of carbapenem resistance has meant that we needed to rethink our approach.

In January 2018, our suite of PCRs was replaced by a single assay run on a commercial platform. The assay has been evaluated by AMRHAI against a panel of 393 isolates with previously defined carbapenem and/or colistin resistance mechanisms (see **here**). The platform performs automated sample preparation and PCR set-up, thereby saving staff time, and will allow the Resistance Mechanisms Section to invest time in further development work to ensure our service remains relevant to emerging resistance issues. An additional benefit will be the ability to screen all potential carbapenemase-producing isolates for transferable colistin resistance (*mcr*) genes, leading to better alert surveillance of these emerging resistance genes that threaten the use of colistin.

Reference service users will still receive reports for Enterobacteriaceae isolates in which a carbapenemase gene has been detected within one to two days after receipt of the isolate in AMRHAI (isolates negative in the assay will still require MIC determination to rule out presence of a rare/novel carbapenemase). Molecular testing of *Pseudomonas* spp. will remain dictated by AMRHAI's MIC profiles, as will metallo-carbapenemase gene detection in *Acinetobacter* spp. (OXA-type carbapenemases are sought in all referred isolates). You will

also notice some minor changes to the layout of the reports you receive from us, which hopefully make our testing process easier to understand.

Katie Hopkins and Danièle Meunier

New MIC robotics

An accurate AST result is crucial in guiding antibiotic treatment. This is particularly relevant in treating drug-resistant infections with few treatment options. Agar dilution (a.k.a. agar incorporation) methodology has been used in AMRHAI (and its predecessors) to determine MICs for decades, basically employing a method developed at the end of the 1970s at the then London Hospital Medical College under Professor JD Williams (which built on earlier methods, including some used by Fleming). The inoculum was realised to be a fundamental variable and a long and tedious photometric method for preparing inocula was described by **Moosdeen *et al.* (1988)**. A simplified procedure was subsequently developed whereby McFarland density standards were used to adjust inocula. This was easier to manage in diagnostic laboratories but inoculum density can still vary widely.

Although guideline-setting bodies such as EUCAST and CLSI have used agar dilution methods for MIC determination, these have mainly been replaced in recommendations by broth microdilution (BMD) testing, largely because the latter can be automated and is more flexible. Automation can reduce inherent variables that exist in systems that rely on manual processing and allow labs to cope with greater workloads. Importantly, the international standard method for MIC determination (ISO 20776) is based on MBD. Nevertheless, agar dilution must be used for some antibiotics, such as fosfomycin and mecillinam, and for testing some species, such as *Neisseria gonorrhoeae*.

We are in the process of transforming our MIC service over to BMD testing. Robots are now available that will pick colonies and produce an inoculum the density of which is set at a specified wavelength using photometry, which basically realises the quality inherent in Moosdeen's method. We have taken delivery of a bespoke system, which is now being set up and validated. It should be operational by the end of March 2018. Unlike our current agar dilution method, which is very rigid and can only be run once per week (because staff have to pour huge numbers of plates for each antibiotic), the robotic system uses microdilution plates containing preformed and bespoke antibiotic ranges. Samples will be processed on a rolling basis (though still within our working hours of Monday to Friday 09.00 -17.30) and should provide additional quality in inoculum setting and plate reading. This will result in improved turnaround times and ability to cope with the ever-increasing numbers of specimens we are receiving. We will also be more readily able to keep up with determining susceptibility to newly licensed antibiotics and combinations.

Robert Hill

Update from the Antimicrobial Resistance in Sexually Transmitted Infections (AMRSTI) Section

Antimicrobial resistance in *Neisseria gonorrhoeae.* The 2016 report for the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) was published in October 2017 (and is available **here**). Briefly, there were no reported cases of ceftriaxone resistance (ie all MICs were ≤0.125 mg/L), and azithromycin resistance (MICs >0.5 mg/L) fell to 4.7% (down from 9.8% in 2015). This reduction in azithromycin resistance is encouraging and we will monitor it closely over the coming year to see if it is maintained.

Testing of the 2017 GRASP collection is underway and, whilst full MICs continue to be determined for clinically-relevant antibiotics (ie azithromycin and ceftriaxone), breakpoint plates are now used for 'historical' antibiotics (eg ciprofloxacin and penicillin) or where susceptibility distributions are clearly defined (eg spectinomycin). Also, there has been

positive feedback from laboratories submitting their data electronically and so electronic data submission will be rolled out to all collaborating laboratories for the 2018 collection.

AMRSTI to co-ordinate Euro-GASP for the next four years. The AMRSTI section has won the tender (again!) from ECDC to co-ordinate the European Gonococcal Surveillance Programme (Euro-GASP) for the next four years. The objectives include: (i) monitoring the susceptibility of *N. gonorrhoeae* isolates in Europe; (ii) improving the quality of epidemiological data reported through Euro-GASP; (iii) assessing the accuracy of *N. gonorrhoeae* antimicrobial susceptibility testing through an EQA scheme; (iv) performing WGS of *N. gonorrhoeae* isolates in order to inform our understanding of the geographic and temporal distribution patterns of public health relevant strains in Europe, including associations between genotype, antimicrobial resistance and patient characteristics; and (v) providing training on STI laboratory diagnostics, *N. gonorrhoeae* susceptibility testing and molecular typing including WGS.

New macrolide AMR assay launched for *Mycoplasma genitalium*. AMRSTI has recently introduced an assay for detecting mutations at loci 2058 and 2059 of the 23S rRNA gene that are most often associated with macrolide resistance in *Mycoplasma genitalium*. This AMR detection will be undertaken automatically on all clinical specimens found positive for *M. genitalium*; there is no opt-out. Turnaround times and specimen requirements for the service remain the same (a minimum of 400 µL NAATs buffer, 3 mL of neat urine, a dry swab collected from an appropriate site or DNA extract). AMRSTI will accept specimens for *M. genitalium* detection from symptomatic patients, known contact cases or for test-of-cure. We believe this service is of great clinical value in these times of high azithromycin resistance in *M. genitalium* and we welcome any feedback from laboratories or clinics.

***N. gonorrhoeae* reference service.** AMRSTI is responsible for monitoring antimicrobial resistance in *N. gonorrhoeae* so we actively encourage all diagnostic laboratories to send us confirmed isolates of *N. gonorrhoeae* suspected to have resistance to ceftriaxone and/or azithromycin (first-line antimicrobial therapy). We particularly encourage all primary diagnostic laboratories that report ceftriaxone-resistant isolates to the second generation surveillance system (SGSS) also to send them to us at AMRSTI so we can (try to) confirm the reported resistance. In addition, please send us isolates that have been related to clinical treatment failure to enable MIC determination and any enhanced surveillance to be initiated.

Rachel Pitt and Michelle Cole

Control of *Pseudomonas aeruginosa* infections following ear piercings

In the autumn of 2016, AMRHAI received isolates of *Pseudomonas aeruginosa* from numerous patients with infections following ‘scaffold’ cartilage upper ear piercings carried out in reputable studios in various locations around the country. Some of these infections were sufficiently serious to require surgical intervention. We showed that the vast majority of the isolates were of the same unusual type. An outbreak investigation team was formed, headed by Richard Puleston, which included PHE epidemiologists, AMRHAI and the Food, Water and Environment (FWE) network, who worked with staff from local authorities and Environmental Health Departments. As well as providing typing, AMRHAI provided sampling guidance based on the probable habitats of *P. aeruginosa* and, with FWE, suitable sampling strategies.

Sampling included a wide variety of equipment used in the piercings, but past experience led AMRHAI to advise a particular focus on liquids, in particular an ‘aftercare’ solution provided by the piercers, obtained from an external company, which seemed common to the infected piercings. Samples of this grew *P. aeruginosa* in high numbers and typing showed that it matched the patient isolates. The trail was then followed back to the manufacturers and the same organism was also recovered from equipment used to make the solution. The ‘aftercare’ solution was swiftly withdrawn from use - and the outbreak stopped! It was a

wonderful example of a multidisciplinary team co-operating together to effect a rapid and decisive end to an outbreak, with each member of the team playing a crucial role. And so gratifying that the sampling guidance and typing we provided were so central to the investigation!

Jane Turton, Dervla Kenna, Zoë Payne, Amy Coward, Noshin Sajedi and Peter Hoffman

MRSA bacteraemia cases: Thanks for your help …please continue!

From 1 April 2017, we invited you to refer a single MRSA isolate from each episode of MRSA bacteraemia in England. Each isolate is subjected to whole-genome sequencing (WGS) and a report issued to you detailing the Multi-Locus Sequence Type (MLST) alongside the presence/absence of a panel of antimicrobial resistance and virulence genes.

In early November we started reviewing the first six months' worth of data, including linking the WGS data outputs with the demographic, clinical and geographic information from the national Mandatory Surveillance Programme for Healthcare Associated Infections. At this stage, **thanks to you**, we estimate to have captured isolates from 306/406 (75%) cases nationally, which far exceeds any previous sentinel-based surveillance initiatives.

We plan to continue this study until the end of March 2019, so please continue to send us your MRSA bacteraemia isolates. For those of you who may have forgotten to send an isolate from cases with a specimen date on or after 1st April 2017, it's not too late – please forward them.

Looking ahead, we plan to disseminate the overall findings via an annual report. It is hoped that an increased understanding of the current epidemiology of MRSA bacteraemia may inform interventions aimed at further reducing rates of MRSA infection. For further information, please contact Angela Kearns (**angela.kearns@phe.gov.uk**).

Angela Kearns, Bruno Pichon, Michel Doumith, Peter Staves, Olisaeloka Nsonwu, John Davies, Russell Hope

Already submitted an isolate to AMRHAI, but require further testing?

More and more often we're finding submissions to AMRHAI that are duplicates of isolates we've already received (same patient details and external laboratory reference number). Commonly these are isolates that were initially submitted for investigation of carbapenem resistance and subsequently a duplicate slope is submitted with a request for MICs or typing (this is a particular issue with isolates referred via the Electronic Reporting System for suspected carbapenemase-producers). Not only does this lead to duplication of testing by AMRHAI, but will lead to duplicated charging for reference services that incur a charge.

Please note that AMRHAI archives all slopes submitted for investigation of antibiotic resistance for a minimum of one year. Therefore, should you require further testing by any of AMRHAI's reference services following submission of an isolate, please contact us by email (**amrhai@phe.gov.uk**) or telephone to discuss your requirements.

Katie Hopkins

Addendum in proof

I regret that Robert Hill will be leaving AMRHAI in March 2018. We wish him the very best for the future and thank him for 13 years of service as head of our susceptibility testing section. He will be missed by colleagues and service users alike.

Neil Woodford

AMRHAI senior staff …for when you need advice.

General enquiries ***amrhai@phe.gov.uk***	Reference Services; placements and visits.
Bacteriology Triage; Tel 020-8327-7887	Specimen / result / report queries
Consultant Microbiologists **colindalemedmicro@phe.gov.uk**; Tel 020-8327-6736	Advice on medical management of cases, incidents or outbreaks. This service is not to access laboratory results.
Prof. Neil Woodford (Head of Unit) **neil.woodford@phe.gov.uk**; Tel 020-8327-6511	Resistance mechanisms; R&D opportunities; commercial opportunities
Ms Hemanti Patel **hemanti.patel@phe.gov.uk**; Tel 020-8327-6986	Technical Manager, AMRHAI; Quality matters arising
Dr Michelle Cole **michelle.cole@phe.gov.uk**; Tel 020-8327-6465	Antimicrobial resistance in sexually transmitted infections
Dr Matthew Ellington **matthew.ellington@phe.gov.uk**; Tel 020-8327-7306	R&D opportunities; genomics and antimicrobial resistance
Dr. Robert Hill **robert.hill@phe.gov.uk**; Tel 020-8327-7237	Susceptibility testing; interpreting antibiograms; treatment
Mr. Peter Hoffman **peter.hoffman@phe.gov.uk**; Tel 020-8327-7274	Infection prevention and control; site visits
Dr. Katie Hopkins **katie.hopkins@phe.gov.uk** ; Tel 020-8327-7061	Resistance mechanisms; inferring mechanisms from antibiograms; commercial opportunities (esp. molecular test evaluations)
Prof. Angela Kearns **angela.kearns@phe.gov.uk**; Tel 020-8327-7227	Staphylococci; ID & typing; PVL/other toxins
Prof. David Livermore **david.livermore@phe.gov.uk**; Tel 020-8327-6511	Commercial opportunities (esp. antibiotic evaluations); surveys
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; enterococci; identification of opportunistic and CF pathogens