NZ NAC New Zealand National Antimicrobial Susceptibility Testing Committee

**December 2019**

# Introduction from the Chair

Kia ora koutou,

Since forming in late 2017, the NZ NAC has worked hard to improve and standardise antimicrobial susceptibility testing among laboratories in NZ that follow EUCAST methods. This newsletter highlights some of the recent work we've been doing and includes some practical suggestions we hope you'll find interesting and useful.

As exiting Chair, I'd like to sincerely thank all NZ NAC members for their valuable contribution over the last year despite having to fit it in over and above already busy workloads. If in the coming year there are questions or issues relating to antimicrobial susceptibility testing that you'd like to put to the NZ NAC, please don't hesitate to get in touch with Sarah (sarah.underwood@esr.cri.nz) who will forward to the new Chair and ensure the question is on the agenda of the next meeting.

Best wishes for the Christmas season,

Josh Freeman
Chair, NZ NAC

## NZ NAC CPE isolate survey, July 2019 and updated CPE minimum standards for detection of CPE document

In August 2018, the NZ NAC introduced guidelines for the detection of CPE from clinical samples and screening specimens. As part of an annual review process to identify any shortfalls in the document, and to assess compliance to the standards, 3 isolates and a questionnaire were sent to each laboratory in June 2019. Overall, laboratories performed very well, with most being able to comply with the minimum standards guidelines, identifying the presence or possibility of a carbapenemase in each isolate. The most challenging isolate was an OXA-48-producing *E. coli*, which coproduced an ESBL, had only low-level resistance to meropenem, and was a weak carbapenem hydrolyser. Temocillin is a sensitive, but not specific, marker for OXA-48, and might be a useful addition to routine screens for resistance mechanism detection, particularly when testing *E. coli*. For more details about the survey results, click here.

The minimum laboratory standard document has now been revised and a couple of paragraphs added about the use of temocillin in resistance mechanism screens as well as the detection of carbapenamase enzymes in *Acinetobacter baumanni* and *Pseudomonas aeruginosa*. Laboratories are encouraged to read the document and implement the recommendations.

## *mcr* genes mediating colistin resistance in NZ

The first NZ NAC newsletter contained an item on a small study that the NAC conducted to evaluate a variety of commercial methods for the detection of colistin resistance: Liofilchem colistin MIC Test Strips (MTSs), BD Phoenix NMIC-404, Rapid Polymyxin NP, Liofilchem SensiTest Colistin BMD, and Trek Sensititre EURGNCOL BMD. A notable finding of this study was the first identification in New Zealand of a *mcr-1* producing isolate, found in an ESBL-producing *E. coli* from a patient with a community-acquired UTI. We subsequently discovered that this isolate co-produced *mcr*-1 and *mcr*-3.[1] Interestingly, co-occurrence of *mcr-1* and *mcr-3* from clinical isolates have rarely been reported globally: one Salmonella isolate (also harbouring $bla_{\text{CTX-M-55}}$) and one *E. coli* (also harbouring

*bla*$_{NDM-5}$).[2]

Since this finding Canterbury Health Laboratories have sent any colistin-resistant ESBL-producing Enterobacterales to ESR for detection of *mcr* genes by PCR. Among approximately 20 isolates, 4 *E. coli* were found positive for *mcr*-1. Three of the four *E. coli* isolates were found in screening samples, with the remaining *E. coli* cultured from midstream urine. The four patients were all in-patients, and only two of them had recent (<12 months) international travel – one to China and one to Thailand (both countries are known to have a high prevalence of *mcr* genes); the other two patients had no recent travel, although both had frequent hospital admissions.

Although colistin wasn't required for treatment in any of these patients, it is important to note that *mcr* genes are present in New Zealand and the true prevalence remains to be determined. If colistin is to be considered as a treatment option, then susceptibility testing should be performed by the EUCAST reference broth microdilution method or a commercial equivalent. Any colistin resistant isolates should be sent to ESR for detection of *mcr* genes by PCR.

**References:**

1. Creighton J, Anderson T, Howard J, Dyet K, Ren X, Freeman J, Co-occurrence of *mcr-1* and *mcr-3* genes in a single *Escherichia coli* in New Zealand. *Journal of Antimicrobial Chemotherapy*, 2019. https://doi.org/10.1093/jac/dkz311.

2. Sun J, Zhang H, Liu YH, Feng Y. Towards understanding MCR-like colistin resistance. *Trends Microbiol*. 2018; **29**: 794-808.

## *Neisseria gonorrhoeae* Azithromycin MIC Testing

Azithromycin is a macrolide antibiotic that can be used to treat a variety of infections including whooping cough, chlamydia, and gonorrhoea. Treatment for gonorrhoea usually consists of a single dose of ceftriaxone by injection and azithromycin by tablet. Bacterial isolates of *Neisseria gonorrhoeae* should always have susceptibility testing performed which should include a ceftriaxone minimum inhibitory concentration (MIC), and if testing is available azithromycin MIC.

During 2018 it was noted that *N. gonorrhoeae* isolates tested using azithromycin gradient MIC strips from different manufacturers were giving different results, with one

higher than the other. Using the 2018 EUCAST interpretive criteria* a number of these isolates fell into the intermediate or resistant categories using the Liofilchem azithromycin MIC gradient strip but were susceptible by the bioMérieux azithromycin Etest MIC gradient strip. It was decided to investigate further to determine if this was consistently the case and whether the strips were close to QC isolate target MIC values. This is all overshadowed by the fact that EUCAST had interpretive criteria for *N. gonorrhoeae* azithromycin but no recommended method or media. CLSI have a method for *N. gonorrhoeae* susceptibility testing but no interpretive criteria for azithromycin. Testing of *N. gonorrhoeae* control and wild strains was performed across three different laboratories to account for any inter-laboratory variation. The method and media used was CLSI, and the interpretive criteria was EUCAST. The following parameters were investigated by one or more of the laboratories.

- Direct comparison of the two different strips using same inoculum and media (44 isolates)
- Accuracy, testing QC isolates with known target azithromycin MIC value
- Storage temperature (fridge versus frozen), over time (6 months)
- Reproducibility, multiple MIC tests on same isolate over time

One variable that could not be tested was different media types as they were not available.

As would be expected there was some inter-laboratory variation but overall the Liofilchem azithromycin MIC strip consistently provided higher MIC results than the bioMérieux azithromycin Etest MIC strip. Both strip types provided some MIC results near or at the target value for the QC isolates. The storage temperature had no impact on the performance of either strip type. The two strip types showed good reproducibility across multiple tests of the same isolate.

The determination of an *N. gonorrhoeae* azithromycin MIC value has technical merit by assisting in the detection of acquired resistance but does not change treatment and clinical management. It is therefore over to each individual laboratory to decide whether they will perform *N. gonorrhoeae* azithromycin MIC's, and if they do which azithromycin gradient strip performs best in their laboratory setting.

* EUCAST 2018 Clinical breakpoints (v8.1) Azithromycin interpretive criteria: Susceptible ≤0.25 mg/L, Resistant >0.5 mg/L.

EUAST 2019 Clinical breakpoints (v9.0) Removed the interpretive criteria and replaced it with the following note, "Azithromycin is always used in conjunction with another

effective agent. For testing purposes with the aim of detecting acquired resistance mechanisms, the ECOFF is 1 mg/L."

A LabPLUS study into variability in azithromycin susceptibility testing for *N. gonorrhoeae* has been published and is available from http://jcm.asm.org/content/early/2019/09/26/JCM.01353-19 The study showed that Liofilchem azithromycin gradient test strip MICs were significantly higher compared with azithromycin Etest and azithromycin agar dilution. The Etest MICs were higher than the agar dilution.

## EUCAST: The 'New I'

In the 2019 EUCAST Breakpoint Tables v9.0, a tighter definition of the 'I' category was introduced. The 'new I' should really be the 'new S' because it is now defined as "Susceptible, **I**ncreased Exposure", whereby "increased exposure" relates to the physiological concentration of the agent and the dosing strategy (dose, frequency and mode of administration).

The EUCAST breakpoint (BP) committee considered that the previous definition of the I category encompassed too many variables and could mean:

1. The therapeutic effect is uncertain at this BP
2. High doses of drugs need to be used
3. The infection is treatable if at a site where an active agent is concentrated e.g. the urinary tract
4. A buffer zone against testing method variables

CLSI has the term Susceptible Dose-Dependent (SDD), which is defined by "a breakpoint that implies that the susceptibility of an isolate depends on the dosing regimen that is used in the patient." However, in EUCAST's opinion, all BPs are dose dependent, therefore SDD is an imprecise meaning. EUCAST wanted to narrow the definition of I, focusing on bug/drug exposure options (hence removing the uncertain aspects of the 'old I'). Therefore, if "increased drug exposure" can be achieved, then the I category can be redefined as Susceptible, **I**ncreased

Exposure.

Increased exposure can be achieved by

1. Increasing the dose
2. Increasing the dosing frequency
3. Changing the mode of administration to continuous infusion (especially applicable for β-lactam antibiotics)
4. Concentrating the antibiotic at the site of infection

**The new definitions are**:

**S - Susceptible, standard dosing regimen:** A microorganism is categorised as "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic succcess using a standard dosing regimen of the agent.

**I - Susceptible, increased exposure***: A microorganism is categorised as "Susceptible, increased exposure*" providing higher exposure to the microorganism can be achieved (dose, frequency, mode of administration).

**R - Resistant:** A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.

*Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

**Note:** The I category should not be converted to R or reported as S. Laboratories should amend their reporting systems to indicate the new meaning for I. For statistical analysis EUCAST recommend that S, I and R are presented separately, but if there is a need to combine then I should be included with S.

**References**:

1. http://www.eucast.org/newsiandr/

2. Kahlmeter G. et al. Point-Counterpoint: Differences between the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute Recommendations for Reporting Antimicrobial Susceptibility Results. *J Clin Microbiol*. 2019: **57**: e01129-19.

## EUCAST: Area of Technical Uncertainty (ATU)

The introduction of the ATU ranges in the 2019 EUCAST Breakpoint Tables v9.0 seemed to create much uncertainty (pardon the pun) with microbiology laboratories! At the Australian Society of Antimicrobials EUCAST workshop in February 2019 there was a very sparse show of hands from delegates indicating that they had already implemented the new tables. Most laboratories seemed unsure of just how to tackle the concept of ATU.

When the EUCAST committee looked at the old I category, its definition contained a buffer zone against testing method variables as well as some bug/drug combinations which are difficult to accurately determine the category result with phenotypic methods – aspects which were definitely not wanted in the new I category definition of susceptible, Increased exposure.

Routine antimicrobial susceptibility testing is *mostly* reliable – providing your methods are robust, your QC is within range, you use good quality consumables, and the process is performed by trained staff. However there is normal variability within systems, with an aim to achieve:

- A target MIC value +/- 1 dilution
- A target zone diameter +/- 2 mm

The ATU is a warning to the laboratory that interpretation at that BP range is difficult. There is currently only a small proportion of bug/drug combinations that are affected by an ATU range. The most common ATUs are with β-lactam agents and this is largely due

to problems with conflicting phenotypic and genotypic results e.g. *Haemophilus influenzae* possessing PBP3 mutations but having MIC values in the susceptible range, and a similar problem with *Staphylococci* and PBP2a.

- ATU is defined by a single MIC value or a short range of zone diameter values
- How the ATU is dealt with depends on the situation i.e.
    - The sample e.g. urine or blood culture
    - The agent e.g. appropriate for site, or last option
    - The infecting organism e.g. presence of intrinsic resistant mechanisms (perform MIC tests or PCR if available)

Laboratories might chose to perform an MIC (automated, gradient strip, broth microdilution) if these tests are readily available. In general, most laboratories will choose to downgrade the result (i.e. from S to I/R – as appropriate), especially if there are other suitable alternative agents. Repeating the test should only be performed if there were method errors.

**Reference**:
http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Area_of_Technical_Uncertainty_-_guidance_2019-1.pdf