NZ NAC New Zealand National Antimicrobial Susceptibility Testing Committee

Colistin antimicrobial susceptibility testing

Not all mechanisms that contribute to resistance to colistin and other polymyxins have been explained; but those that have are complex. Resistance can be naturally occurring or acquired. Intrinsic resistance, found in *Proteus*, *Morganella*, *Providencia,* and *Serratia*, is constitutively expressed and is linked to the addition of cationic (positively charged) groups to the lipopolysaccharide (LPS), the initial target of polymyxins. These cations increase the charge of the LPS which results in less antibiotic binding. While acquired resistance has been attributed to a number of LPS modifications, it can also be due to specific modifications of outer membrane porins, efflux pumps, capsule hyper-production or release of capsular material that traps the antibiotic, or the loss of LPS thereby removing the antibiotic binding sites (1). The discovery of a readily transferable, plasmid-mediated gene, *mcr*-1, by Lui *et al*, has complicated detection of resistance (2). Additional mcr types have since been reported (3). Furthermore, the interpretation of susceptibility tests has been confounded by reports of heteroresistance described in *Enterobacter*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (1, 4-5).

In July 2016, EUCAST issued a warning regarding the poor performance of disc diffusion for reliably detecting colistin resistance in Gram-negative bacilli. The issues include the poor diffusion of colistin molecules into the agar, drug powder composition and heteroresistance. Further updates and other publications have extended this warning to include MIC gradient strips (6). In addition, automated systems, with a limited number of colistin dilutions, may produce false susceptible results due to the skipped well phenomenon. As such, EUCAST currently recommends broth microdilution (BMD) as the only valid method to determine colistin susceptibility.

The NZ NAC conducted a small study 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. The results were compared against ESR reference BMD and PCR for the *mcr-1* gene. The study isolates consisted of 25 Enterobacteriaceae, including 6 carbapenemase-producing isolates, 4 isolates with intrinsic resistance to colistin, *Escherichia coli* ATCC 25922 (negative control) and *E. coli* NCTC 13846 (*mcr-1* positive control).

21 of the 25 isolates were colistin resistant (MIC >2 mg/L) by ESR BMD and the remaining 4 isolates were susceptible (MIC ≤2 mg/L). The results are summarised below and in the table:

- The MTS produced 4/21 very major errors (VMEs, that is, resistant isolates incorrectly determined to be susceptible), with MICs of ≤2 mg/L for these 4 isolates.
- The Phoenix produced 2/21 VMEs, although one resistant *Enterobacter cloacae* that had an initial MIC of ≤1 mg/L had an MIC of >4 mg/L on repeat; perhaps a consequence of a hetero-resistant population or skipped wells.
- The Rapid Polymyxin NP test was positive for all colistin-resistant isolates (ie, produced no VMEs), but produced 3/4 false positive results. We found the negative/positive colour differentiation was often difficult to interpret.
- The SensiTest Colistin BMD produced 1/21 VME, with one resistant *E. coli* having an MIC of 2 mg/L.
- The Sensititre EURGNCOL BMD was tested in two different laboratories. It produced 2/21 VMEs, including the same *E. coli* that was falsely susceptible in the SensiTest Colistin BMD.

Method	Number isolates tested	% false susceptible (very major errors)	% false resistance (major errors)
Liofilchem MIC Test Strip	25	19.0 (4/21)	25.0 (1/4)
BD Phoenix NMIC-404	25	9.5 (2/21)	25.0 (1/4)
Rapid Polymyxin NP	24	0.0 (0/20)	75.0 (3/4)
Liofilchem SensiTest Colistin	25	4.8 (1/21)	0.0 (0/4)
Trek Sensititre EURGNCOL	25	9.5 (2/21)	0.0 (0/4)

Both the SensiTest Colistin BMD and the Sensititre EURGNCOL BMD were easy to use and to interpret end-points, and the long shelf life is suitable for infrequent use. The SensiTest Colistin consists of a 4-test microtitre tray with a growth well and colistin concentrations ranging from 0.25 to 16 mg/L. The Sensititre EURGNCOL consists of a 3-test microtitre tray with a growth control well and colistin concentrations ranging from 0.25 to 8 mg/L. The Sensititre EURGNCOL plate also includes piperacillin-tazobactam, ceftolozane-tazobactam, ceftazidime-avibactam and meropenem. Furthermore, recently published papers have shown good performances for both of these systems (6-8).

A notable finding of this study was the first identification in New Zealand of a *mcr-1* producing isolate, found in an *E. coli* from a patient with a community-acquired UTI. This isolate tested as colistin resistant in all methods trialled in the study, and had a colistin MIC of 16 mg/L in the reference BMD.

References:

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