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Sensititre broth microdilution plates variation in colistin quality control minimum inhibitory concentration results

To the Editor,

Multi-drug-resistant (MDR) Gram-negative organisms represent a significant healthcare problem. Many are associated with hospital-acquired infections, with *Pseudomonas aeruginosa* being one of the most commonly identified

pathogens.¹ Carbapenem resistance significantly limits available treatment options with polymyxins typically being some of the last-line drugs available.² The current recommended method for susceptibility testing of colistin is broth microdilution.^{3,4} It is also recommended that concurrent quality control testing should be performed with a drug-susceptible strain (*Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853), and a drug-resistant strain (*Escherichia coli* NCTC 13846).⁵ According to current European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, the target minimum inhibitory concentration (MIC) (and range) are as follows: *E. coli* ATCC 25922, 0.5–1 mg/L (0.25–2 mg/L); *P. aeruginosa* ATCC 27853, 1–2 mg/L (0.5–4 mg/L); *E. coli* NCTC 13846, 4 mg/L (2–8 mg/L).⁵ Clinical and Laboratory Standards Institute (CLSI) expected MIC ranges for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 are the same as for EUCAST but do not specify target MICs.^{5,6}

We performed susceptibility testing for colistin and polymyxin B using a commercial broth microdilution method (Sensititre; Thermo Fisher, USA) following an internal evaluation in 2017.⁴ Although polymyxin B was included in the plates, testing with colistin is generally recommended, and there are currently no breakpoints available for polymyxin B. The colistin MIC results were interpreted based on EUCAST breakpoints.³ Testing was usually performed for carbapenem-resistant organisms or on request by clinicians. Since the start of testing, three different plates were in use (sequentially) for colistin and polymyxin B testing: GNX3F, SGP1MBD and SGPMDRO1F. These plates are subsequently referred to as Plates A, B, and C, respectively. Plate A was a research use only plate, and Plates B and C were customised *in vitro* diagnostic plates. All three plates contained colistin and polymyxin B, with wells ranging from 0.25 mg/L to 4 mg/L.

Quality control data and colistin/polymyxin B testing results between September 2017 and May 2021 were collated. Enterobacterales and *P. aeruginosa* (as identified by MALDI Biotyper; Bruker, USA) were included. These data are summarised in Fig. 1. The quality control results demonstrated a rightward shift (increased MIC) in the quality control results of *E. coli* ATCC 25922 during the period when Plate B was in use compared to Plates A and C. Quality control testing with *P. aeruginosa* ATCC 27853 was not performed when Plate A was in use, but a leftward shift was demonstrated between using Plates B and C. Conversely, the majority of the mcr-1 positive *E. coli* NCTC 13846 had MIC >4 mg/L, which is outside of the target MIC of 4 mg/L.

Although within the expected range, many of the quality control (QC) results were outside of the target MICs for *P. aeruginosa* ATCC 27853 when Plate B was in use. A similar rightward shift was also seen in the MIC distribution of clinical isolates during this period. Colistin resistance rates for the tested Enterobacterales stratified by testing plates were: Plate A, 11.8% (4/34); Plate B, 6.5% (5/77); and Plate C, 15.0% (3/20). Colistin resistance rates for *P. aeruginosa* were: Plate A, 5.5% (3/55); Plate B, 46.2% (54/117); and Plate C, 2.6% (1/38). There was a clear increase in the number of *P. aeruginosa* with resistant MICs when Plate B was in use, which was reversed when we switched to Plate C. The most commonly tested Enterobacterales were *Escherichia coli* and *Klebsiella pneumoniae*. The MIC distributions

Sensititre plate	Colistin (MIC in mg/L)						Polymyxin B (MIC in mg/L)					
	0.25	0.5	1	2	4	>4	0.25	0.5	1	2	4	>4
<i>Escherichia coli</i> ATCC 25922												
A	37	2	0	0	0	0	37	2	0	0	0	0
B	0	8	47	4	0	0	1	0	39	19	0	0
C	22	0	0	0	0	0	21	1	0	0	0	0
<i>Escherichia coli</i> NCTC 13846												
A	0	0	0	0	0	25	0	0	0	0	0	25
B	0	0	0	0	17	12	0	0	0	3	22	4
C	0	0	0	0	0	3	0	0	0	0	0	3
<i>Pseudomonas aeruginosa</i> ATCC 27853												
A	N/A											
B	0	0	0	17	12	0	0	0	0	22	7	0
C	0	0	20	0	0	0	0	0	20	0	0	0
Clinical <i>Pseudomonas aeruginosa</i> isolates												
A	2	2	37	11	1	2	0	3	48	5	1	1
B	0	0	12	51	51	3	0	0	10	64	47	1
C	0	1	20	16	0	1	0	0	30	7	0	1
Clinical <i>Escherichia coli</i> isolates												
A	8	0	0	0	0	0	7	1	0	0	0	0
B	1	2	18	0	0	2	1	0	9	11	0	1
C	5	0	0	0	0	0	4	1	0	0	0	0
Clinical <i>Klebsiella pneumoniae</i> isolates												
A	19	0	1	0	0	3	17	2	1	0	0	3
B	3	0	29	7	1	2	1	3	2	34	1	1
C	7	1	1	0	0	2	7	2	0	0	0	2

Fig. 1 Colistin and polymyxin B minimum inhibitory concentration distributions of quality control strains and clinical isolates stratified by Sensititre plates. Green shading indicates target MIC for quality control strain, and orange shading indicates the accepted range.

are shown in Fig. 1, and demonstrated a similar rightward shift among susceptible isolates when Plate B was in use, which was also reversed when we switched to Plate C.

Pseudomonas aeruginosa naturally have higher MICs compared to Enterobacteriales. Wild-type isolates (including *P. aeruginosa* 27853) may be expected to have MICs of up to 4 mg/L, which would be interpreted as resistant, based on current breakpoints (susceptible ≤ 2 , resistant >4), although within the area of technical uncertainty. Due to typical MICs being close to the breakpoint, minor shifts in MIC results in a categorical change of interpretation from susceptible to resistant.

Although broth microdilution is recommended as the test method for colistin, there are potential limitations. It is not practical for diagnostic laboratories to routinely perform broth microdilution and there is still reliance on commercial testing methods such as Sensititre that has been previously validated by EUCAST. Despite previous validation data, variations may occur between different plates, which emphasise the need for adequate quality control testing to monitor the performance of these tests. Shifting MIC ranges of QC strains can result in major errors, particularly for organisms such as *P. aeruginosa* with typical MICs close to the breakpoint.

While the QC results were largely within range, many occurred outside of the target MICs for both colistin-susceptible and colistin-resistant QC isolates. Inclusion of colistin-susceptible and colistin-resistant QC strains is important to ensure reliable results across the tested MIC range. Some of the expected MIC ranges of quality control strains

occur at the lower and upper limits of range of tested MICs. Manufacturers may consider increasing the tested MIC range as an additional measure to improve robustness of their tests. Diagnostic laboratories should be aware of testing limitations and importance of closely monitoring quality control results, particularly if quality control results are skewed towards one end of the expected range outside of target range.

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First human case of infection with *Arsenophonus nasoniae*, the male killer insect pathogen

To the Editor,

Many arthropod-borne human infections have been recognised in recent years, but this is the first reported case of honeybee-associated *Arsenophonus nasoniae* bacteraemia.

We present the case of a 37-year-old male who attended the hospital emergency department with sudden onset of fever, rigors, and myalgia 5 hours after sustaining a bee sting to his left thumb that occurred in the evening during April 2018 behind his home garage in Wollongong, New South Wales, Australia. This reaction was unusual for him as he had sustained several bee stings in the past without any severe effects. His other medical problems included eczema, well-controlled with topical corticosteroid cream, and chronic neck pain.

On presentation, he was well-perfused with blood pressure of 137/81 mmHg but was tachypnoeic with a respiratory rate of 30 breaths per minute and tachycardic with a heart rate of 145 beats per minute. He was febrile with a temperature of 39.0°C. Oxygen saturation was 98% on room air. His left thumb was inflamed but there were no signs of any embedded bee body parts after removal by his wife. The remainder of his physical examination was unremarkable.

Investigations revealed a normal white cell count $10.5 \times 10^9/L$ [reference range (RR) 3.50–11.00] with a mild neutrophilia of $8.29 \times 10^9/L$ (RR 1.7–7.0). Arterial whole blood showed a normal lactate 1.4 mmol/L (RR 0.0–2.2). He had normal renal and liver function test results and his urinalysis, electrocardiogram and chest X-ray were all unremarkable. One set of blood cultures was collected into BD BACTEC Plus Aerobic/F and Plus Anaerobic/F culture vials (Becton Dickinson, USA). They were incubated in the BD BACTEC 9000 automated blood culture system. After 4 hours observation and conservative management in the emergency department (ED), he was discharged home. His aerobic blood culture vial signalled positive 48 hours later with Gram-negative bacilli and he was recalled to the ED for further assessment. Clinically, he was doing well with no fever, thus he was not started on any antibiotic and was discharged home again.

The contents of the aerobic vial were subcultured onto horse blood, chocolate and MacConkey agars and these media were incubated at 35°C. Growth was evident only on horse blood agar and chocolate agar incubated in 5% CO₂ after 48 hours incubation. There was no growth on horse blood agar incubated anaerobically and no growth on

MacConkey agar incubated in ambient air. Colonies were mucoid, grey-white, round, and convex with entire edges. Cells were non-motile, non-spore forming, long rods, occasionally filamentous in young cultures.

The blood culture isolate was further identified by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) using a Bruker MALDI Biotyper with Bruker Biotyper 3.4 software and library version V4.0.0.1 (Bruker Daltonik, Germany), according to the manufacturer's instructions, with a spectral score of 1.78 as *Arsenophonus nasoniae* with good confidence to the genus level.

Confirmation of the species identification was achieved by 16S rRNA PCR and sequencing.¹ DNA extraction was performed from cultures by crude lysate preparation (100°C for 10 minutes in molecular grade water). The sequence obtained from the sample was 98.9% identical to the *Arsenophonus nasoniae* (ATCC 49151), which was deposited in GenBank (NCBI) under accession NR_042811.1, with eight mismatches in 796 bases sequenced.

Antimicrobial susceptibility testing of the *A. nasoniae* blood culture isolate was performed using the Vitek 2 semi-automated system (version 08.01; bioMérieux, USA) with the commercially available Vitek 2 AST-N246 card. Antimicrobial susceptibility results are shown in Table 1.

The patient decided to keep the dead bee in a resealable plastic bag and its remnants were sent to Hawkesbury Institute for the Environment, Western Sydney University for further identification and possible detection of the causative bacterial DNA, 6 weeks after the contact.

The bee was morphologically confirmed as *Apis mellifera*. Culture was attempted on the bee remnant, but we were not able to identify *A. nasoniae* from the mixed growth. The remnant was homogenised in a TissueLyser (Qiagen, Germany) at 30 Hz for 4 min with a 2 mm sterile steel ball. Total DNA was then extracted using TRIsure (Bioline) following the manufacturer's recommendations. To detect the presence of *Arsenophonus* sp. DNA in the bee sample, PCR was performed using the Bioline MyTaq Red PCR kit and primer set Ars23S1 (CGTTTGATGAATTCATAGTCAAA)/

Ars23S2 (GGTCCTCCAGTTAGTGTACCCAAC).² 200 ng DNA was used in a 10 µL reaction (2 µL DNA, 2 µL MyTaq

Table 1 Antimicrobial susceptibility of the *Arsenophonus nasoniae* blood culture isolate using VITEK 2 Systems Version: 08.01 and AST-N246 card

Antimicrobial agent	MIC (mg/L)
Ampicillin	4
Amoxicillin/clavulanic acid	≤2
Cefazolin	≤4
Ceftriaxone	≤1
Ceftazidime	≤1
Cefepime	≤1
Meropenem	≤0.25
Amikacin	4
Gentamicin	≤1
Tobramycin	≤1
Ciprofloxacin	≤0.25
Norfloxacin	≤0.5
Nitrofurantoin	≤16
Trimethoprim	≤0.5
Trimethoprim/sulfamethoxazole	≤20

MIC, minimal inhibitory concentration.