Session CPHM-02
22-06-2025

Controlled inoculum functionality of an automated rapid AST system leads to consistent MIC determination

Natalie Brown, Jenny Göransson, Harer Osman, Jan Grawé, Anna Karman, Stina Vincentsson, Lotta Levén, Ylva Molin. Q-linea, Uppsala, Sweden.

Q-linea
+46 18 444 36 10
contact@qlinea.com

Background

Accurate Minimum Inhibitory Concentration (MIC) determination is essential for optimising antimicrobial therapy for critically ill patients with bloodstream infections. Antimicrobial Susceptibility Testing (AST) underpins MIC measurement, but results can be influenced by the initial bacterial load, causing MIC variability and affecting accuracy (1, 2). This is especially relevant for carbapenems, which are vital drugs for managing severe infections. ISO, CLSI and EUCAST all recommend the same specific concentration range for performing AST (3–5), however bacterial concentration in positive blood cultures (PBCs) varies and direct AST from PBCs will inherit this variation if not adjusted for. According to data on bacterial concentration in PBCs at 0 h versus 8 h from positivity, this factor alone can cause a 20–50-fold concentration increase for several *Enterobacterales*, including *E. coli* (6).

ASTar® addresses this by automatically preparing a controlled inoculum. In this study, we assessed how MIC values from ASTar testing of PBCs, adjusted to reflect a realistic range of variable bacterial concentrations expected at positivity, compared to broth microdilution (BMD) MICs where the inoculum densities were adjusted to be approximately what could be expected at a typical 1:1000 dilution of variable PBC samples.

Methods

Four *Enterobacterales* strains carrying beta-lactamase genes were tested against carbapenems using both the ASTar System and BMD (Table 1).

Table 1. The four tested strains.

Strain	Species	Resistance mechanism
SQ001	*K. pneumoniae*	SHV-30, VIM-1
SQ002	*E. cloacae*	ACT-7, TEM-1B, VIM-1
SQ003	*E. coli*	KPC-3, OXA-1
SQ004	*E. coli*	KPC-3, TEM-1B

ASTar using blood cultures with varying bacterial content

To simulate expected variations seen in Gram-negative PBCs and assess MIC determination, each strain was tested at three different bacterial concentrations spanning two orders of magnitude. Strains were inoculated into BacT/ALERT FA Plus Aerobic bottles containing human blood and cultured in a BacT/Alert Virtuo cabinet. Viable counts were performed 8 hours post-positivity to quantify bacterial content. The PBC content was subsequently diluted 10× and 100× into negative bottles to simulate bacterial densities commonly observed in PBCs.

BMD using varying bacterial content

BMD was carried out on the same strains, using inocula spanning two orders of magnitude, centered on the recommended bacterial content for BMD (3, 4). This design mimicked concentrations seen in fixed-dilution PBCs. For example, a 1:1000 dilution from a source that varies between about 5×10^7 and 5×10^9, which reflects the common concentration range expected in clinical PBCs.

Results

Presented results are based on modal MIC values from triplicate runs. For BMD, data is presented as deviation from the modal MIC value obtained at the recommended inoculum from EUCAST/CLSI. In contrast, ASTar data is presented as deviation from the modal MIC value obtained on samples from undiluted 8 h positive blood cultures (Figure 1A).

Across a 100-fold variation in bacterial load, ASTar showed minimal variability, deviating by no more than one MIC step in either direction for a specific strain. In comparison, for BMD, the MIC values deviated more widely, with differences of approximately 2–6 steps across the same 100-fold inoculum range, and up to 3 steps over 10× variation around the target inoculum range specified by ISO 20776-1 and CLSI M07 (3, 4).

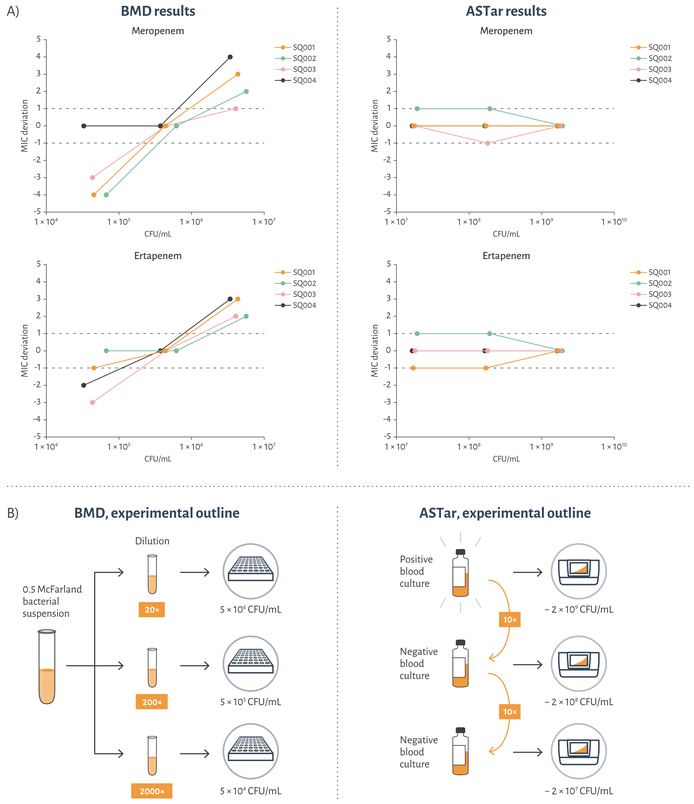

Fig 1. Results and experimental outline. A) Meropenem and Ertapenem MICs were determined for the tested strains and inocula. B) Different inocula of blood culture and bacterial suspensions were prepared and tested with ASTar and BMD, respectively.

The results presented here are in line with published data on the effect of inoculum on MIC determination and categorical interpretation. See, for example, the publications below:

- **Smith and Kirby**, 2018; doi: 10.1128/aac.00433-18
  Tested strains: ~30 *Enterobacterales* strains, CRE, ESBL, susceptible.
  Findings (excerpt): "Our results suggest that IE is sufficiently pronounced for meropenem and cefepime in multidrug-resistant Gram-negative pathogens to affect categorical interpretations during standard laboratory testing."
- **Cartagena *et al.***, 2023 (preprint): doi: 10.1101/2023.05.23.541813
  Tested: 110 clinical CRE isolates across 12 species of *Enterobacterales*
  Findings (excerpt): "Our results confirmed prior reports that minor differences in inoculum can lead to categorical changes in susceptibility interpretation (45), particularly impacting the detection of CP-CRE, the isolates that carry the greatest implications for infection control."

Conclusion

ASTar overcomes the inoculum effect

- For carbapenem-resistant isolates, BMD MIC values for Meropenem and Ertapenem by were strongly affected by the tested inoculum.
- In contrast, ASTar yielded consistent MIC values for both carbapenems, even when testing PBCs with varying bacterial loads over a range of different inoculi.

ASTar delivers reliable MIC values directly from positive blood cultures for antimicrobials and strains where a fixed dilution may give rise to variable MIC results

References

1. Cartagena, A.J. *et al*, The carbapenem inoculum effect provides insight into the molecular mechanisms underlying carbapenem resistance in *Enterobacterales*, 2023, bioRxiv
2. Smith, K., Kirby, J.E. The Inoculum Effect in the Era of Multidrug resistance: Minor Differences in Inoculum Have Dramatic Effect on MIC Determination, 2018, Antimib Agents Chemother 62e00433-18
3. CLSI M07 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 12th Edition
4. ISO 20776-1:2019 Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – part 1: Broth microdilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases
5. Broth microdilution - EUCAST reading guide v 5.0 (1 January 2024)
6. K130914 – 510(k) decision summary for FilmArray® Blood Culture Identification (BCID) panel

www.qlinea.com

D92965 2025-06-09