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

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Background

Antimicrobial treatment optimisation relies on accurate Minimum Inhibitory Concentration (MIC) results determined by Antimicrobial Susceptibility Testing (AST). Carbapenems are key antimicrobials for treating critically ill patients with bloodstream infections, but variation in inoculum at AST can cause MIC variability, affecting accuracy (1, 2). 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. This study compared MIC values for ASTar on PBCs contrived to have differing bacterial concentration that can be expected at positivity, to broth microdilution (BMD) MICs where the inoculum densities were adjusted to be approximately what could be expected if a fixed 1:1000 dilution would be performed e.g. on PBCs with an inherent variability in bacterial concentration.

Conclusion

ASTar overcomes the inoculum effect

- For isolates with carbapenem resistance, MIC values for Meropenem and Ertapenem by BMD are strongly affected by the tested inoculum.
- When the same isolates are analyzed by ASTar as positive blood cultures with differing bacterial content, MIC values for both carbapenems are virtually unchanged over the tested range of 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.

Materials and methods

Four *Enterobacterales* strains carrying beta-lactamase genes were tested against carbapenems using ASTar 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 in Gram-negative PBCs and assess MIC determination, three bacterial concentrations spanning two orders of magnitude were tested. Each tested strain was inoculated to BacT/ALERT FA Plus Aerobic bottles with human blood and cultured in a BacT/Alert Virtuo cabinet. Bacterial content was determined for positive blood cultures using viable count and performed 8 h after positivity. The PBC content was then diluted 10× and 100× into negative bottles to simulate commonly encountered Gram-negative PBC bacterial contents.

BMD using varying bacterial content

BMD was run on the same strains using inoculum spanning two orders of magnitude, centered on recommended bacterial content for BMD (3, 4). This experiment then mimic concentrations that could be expected if using a fixed dilution scheme from PBCs. The dilution points reflect an example 1:1000 dilution from a source that varies between about 5×10^7 and 5×10^9, close to the span expected in PBCs.

Results

Presented results are based on modal MIC values of triplicate runs. For BMD, data is presented as deviation from the modal MIC value obtained at recommended EUCAST/CLSI inoculum, while for ASTar results, data is presented as deviation from the modal MIC value obtained on samples from undiluted 8 h positive blood cultures, see figure 1A. Over a 100-fold variation, ASTar data deviated by a maximum of one MIC step up or down for a specific strain. For BMD, the MIC deviated by 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).

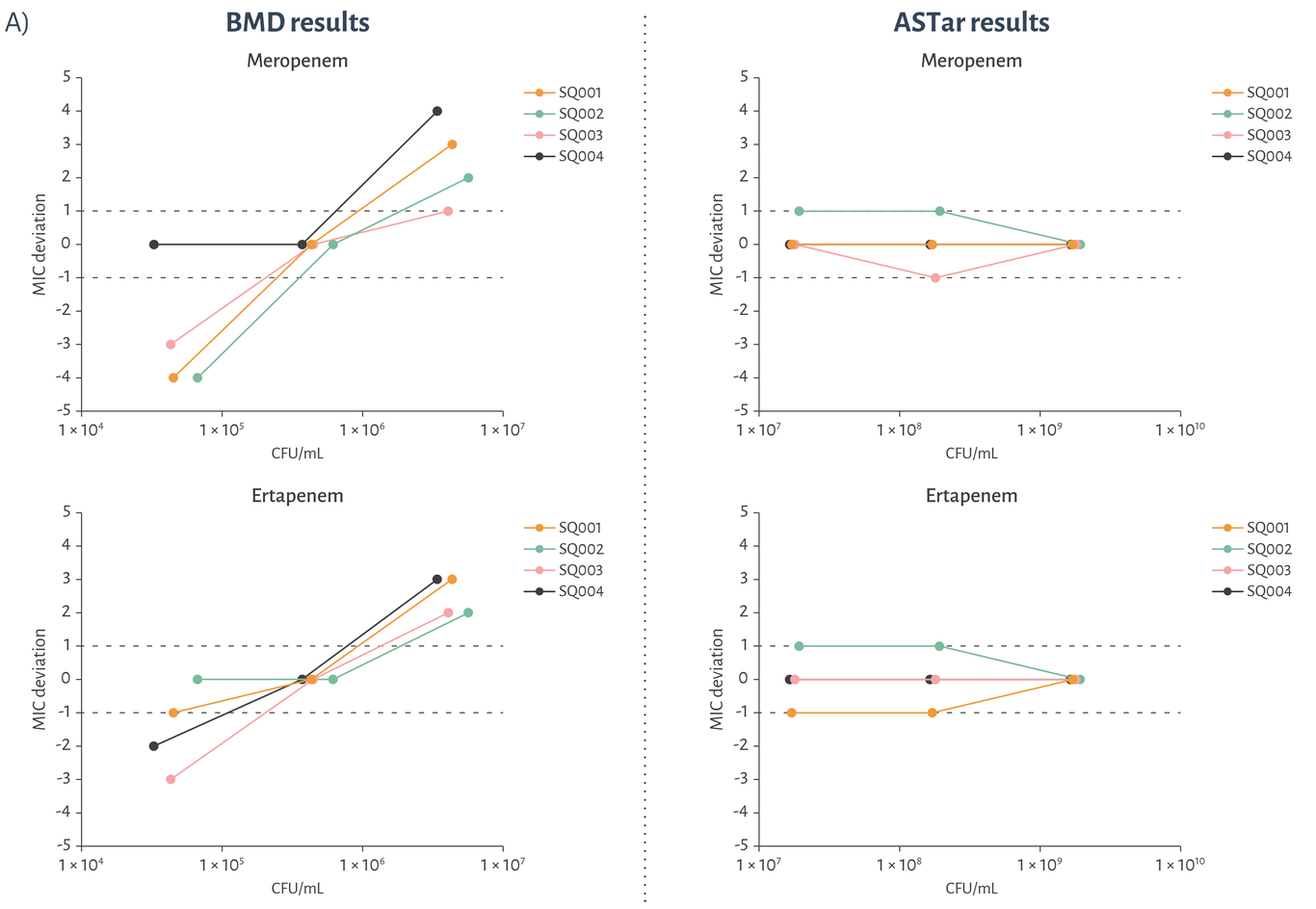

Fig 1. Results and experimental outline. A) Meropenem and Ertapenem MICs were determined for the tested strains and inocula. B) Different inocula of blood culture and bacterial suspensions were prepared and tested with ASTar and BMD, respectively.

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 micro-dilution 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

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