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A new assay for rapid antimicrobial susceptibility testing of bloodstream infection-causing gram-negative bacteria

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

Giulia De Angelis[1,2], Tiziana Di Martino[3], Giorgia Sanna[1], Giulia Menchinelli[2], Silvia Zelli[1], Venere Cortazzo[1], Tiziana D'Inzeo[1], Barbara Fiori[2], Teresa Spanu[1], Brunella Posteraro[1,2], Maurizio Sanguinetti[1,2]

1. Università Cattolica del Sacro Cuore, Rome, Italy; 2. Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; 3. Q-linea, Uppsala, Sweden.

Background

Antimicrobial susceptibility testing (AST) of bacterial organisms causing bloodstream infection (BSI) is required for optimal antimicrobial therapy management. While conventional AST technologies usually take 2–3 days to provide results, rapid AST methods allow clinicians to determine which antibiotics will treat bloodstream infections in hours instead of days. This allows for a timely administration of optimal antimicrobial therapy, thereby reducing mortality and the length of hospital stay. These observations highlight the value of timely reporting MIC values by rapid AST technologies.

The Q-linea ASTar System assay (hereafter referred to as ASTar) performs AST based on the high-speed time-lapse microscopy imaging of Gram-negative bacteria in broth microdilution to determine MIC values. ASTar provides results approximately 6 hours after direct testing of positive blood culture (BC) broths.

We sought to assess the performance of ASTar by testing BCs from Gram-negative BSI patients in a large Italian hospital, considered an endemic area for multidrug-resistant Gram-negative bacteria.

Materials and methods

We included ten BC broths that were positive for Gram-negative bacteria, as documented by Gram-stain microscopy followed by MALDI-TOF mass spectrometry identification. Broths were tested using the ASTar BC G– Kit and performed according to the manufacturer's instructions. ASTar results were compared with those obtained by singlicate EUCAST broth microdilution (BMD) testing on the organisms isolated from BC subcultures. The isolates were characterized by CTX-M-type extended spectrum b-lactamases and KPC-, VIM-, NDM-, IMP- or OXA-48-type carbapenemases. BMD and ASTar MICs were interpreted according to 2022 EUCAST breakpoints. ASTar performance was assessed regarding essential and categorical agreements with BMD results.

ASTar Instrument

The ASTar Instrument is an *in vitro* diagnostic medical device for automated quantitative determination of antimicrobial susceptibility. The ASTar Instrument is a fully automated instrument for rapid AST, that provides robust and consistent inoculum preparation for AST, with high-speed time-lapse microscope imaging of organisms in broth microdilution (BMD) to determine minimum inhibitory concentrations (MIC).

The instrument (Figure 1) is designed to process up to 12 samples in parallel. New samples may be loaded randomly when available slots are available. The ASTar BC G– Consumable kit is intended for in vitro quantitative determination of antimicrobial susceptibility of on-panel Gram-negative, fastidious, and non-fastidious bacteria sampled from positive blood culture bottles. Positive blood culture samples can be run up until 16 h from positivity. The disc contains culturing chambers with prefilled antimicrobials in various concentrations used for AST, chambers without antimicrobials used as controls, and chambers used to determine the concentration in the added sample. The cartridge contains all reagents and disposable articles needed for sample preparation, concentration determination, dilution, and growth medium adaptation.

Fig 1. ASTar Instrument.

Results

We studied ten positive BC broths for *Escherichia coli* (n=4), *Klebsiella pneumoniae* (n=2), *Pseudomonas aeruginosa* (n=2), *Proteus mirabilis* (n=1) and *Proteus vulgaris* (n=1). Regarding B-lactamase associated resistance mechanisms, we found that one *E. coli* isolate was VIM producer, one *P. aeruginosa* isolate was NDM producer, and two *K. pneumoniae* were, respectively, CTX-M producer and KPC/CTX-M producer. Compared to BMD, ASTar showed an overall essential agreement of 95.0% (113 of 119 organisms/AST results) and an overall categorical agreement of 98.3% (117 of 119 organisms/AST results) (Table 1). There were only two (1.7%) minor errors, which regarded cefepime in one of two *P. aeruginosa* isolates (susceptible, increased exposure [8 mg/L] by BMD and misclassified as resistant [16 mg/L] by ASTar) and meropenem in the VIM-producing *E. coli* isolate (susceptible, increased exposure [8 mg/L] by BMD and misclassified as susceptible [2 mg/L] by ASTar).

Table 1. The essential and categorical agreement results of ASTar compared to BMD. The highlighted cells represent disagreements between ASTar and BMD. However, considering the low numbers of results for each antimicrobial-microorganism combination, it is difficult to infer anything from these disagreements.

Antibiotic	Microorganism	Number of isolates			Essential agreement	Categorical agreement	VME	ME	mE
		S	I	R					
Amikacin	*E. coli*	4	0	0	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	P. aeruginosa	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	9	0	1	10/10 (100%)	10/10 (100%)	0	0	0
Amoxicillin-clavulanate	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	0	0	2	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	4	0	4	8/8 (100%)	8/8 (100%)	0	0	0
Aztreonam	*P. aeruginosa*	0	1	1	2/2 (100%)	2/2 (100%)	0	0	0
Cefepime	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	0	0	2	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	1	0	1	1/2 (50%)	2/2 (100%)	0	0	0
	P. aeruginosa	1	1	0	2/2 (100%)	1/2 (50%)	0	0	1
	All	5	1	4	9/10 (90%)	9/10 (90%)	0	0	1
Cefotaxime	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	0	0	2	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	4	0	4	8/8 (100%)	8/8 (100%)	0	0	0
Ceftazidime	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	0	0	2	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	P. aeruginosa	0	1	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	4	1	5	10/10 (100%)	10/10 (100%)	0	0	0
Ceftazidime-avibactam	*E. coli*	3	0	1	3/4 (75%)	4/4 (100%)	0	0	0
	K. pneumoniae	2	0	0	1/2 (50%)	2/2 (100%)	0	0	0
	Proteus spp.	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	P. aeruginosa	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	8	0	2	8/10 (80%)	10/10 (100%)	0	0	0
Ceftolozane-tazobactam	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	1	0	1	1/2 (50%)	2/2 (100%)	0	0	0
	Proteus spp.	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	P. aeruginosa	1	0	1	1/2 (50%)	2/2 (100%)	0	0	0
	All	7	0	3	8/10 (80%)	10/10 (100%)	0	0	0
Ciprofloxacin	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	0	0	2	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	P. aeruginosa	0	1	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	4	1	5	10/10 (100%)	10/10 (100%)	0	0	0
Colistin	*E. coli*	4	0	0	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	All	6	0	0	6/6 (100%)	6/6 (100%)	0	0	0
Gentamicin	*E. coli*	4	0	0	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	0	0	2	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	5	0	3	8/8 (100%)	8/8 (100%)	0	0	0
Meropenem	*E. coli*	3	1	0	3/4 (75%)	3/4 (75%)	0	0	1
	K. pneumoniae	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	P. aeruginosa	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	7	1	2	9/10 (90%)	9/10 (90%)	0	0	1
Piperacillin-tazobactam	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	0	0	2	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	P. aeruginosa	0	1	1	2/2 (100%)	2/2 (100%)	0	0	0
	All	5	1	4	10/10 (100%)	10/10 (100%)	0	0	0
Thrimetoprim-sulphametoxazole	*E. coli*	3	0	1	4/4 (100%)	4/4 (100%)	0	0	0
	K. pneumoniae	1	0	1	2/2 (100%)	2/2 (100%)	0	0	0
	Proteus spp.	2	0	0	2/2 (100%)	2/2 (100%)	0	0	0
	All	6	0	2	8/8 (100%)	8/8 (100%)	0	0	0
All	**All**	**74**	**6**	**39**	**113/119 (95.0%)**	**117/119 (98.3%)**	**0**	**0**	**2**

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Conclusion

Based on these findings, ASTar may be a valid laboratory tool for rapid AST of BSI-causing Gram-negative bacteria.

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