Mass spectrometry pathogen identification and phenotypic antimicrobial susceptibility testing delivering MICs in hours, directly from clinical positive blood cultures

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Introduction

Current workflows and methodologies used in clinical microbiology laboratories for sepsis diagnosis are based on a sequential chain of events that delivers identification (ID) and antimicrobial susceptibility testing (AST) at the earliest 48 hours after blood draw, although workflows may differ between laboratories. As a result of this delay, clinicians have resorted to empiric therapy during the first days of treatment.

We have evaluated a workflow combining a novel prototype AST system, ASTar™ (Q-linea), with the rapid identification of pathogens. Using this workflow, both ID and AST can be delivered within six hours from positive blood culture, which translates into bringing forward targeted treatment by more than 29 hours compared to current practice.

Fig 1. The ASTar system performs rapid automated AST directly from clinical samples using the sample preparation cartridge in combination with the AST panel disc.

Materials and methods

Clinical samples from patients with suspected bacteremia were collected in aerobic, anaerobic or pediatric BacT/ALERT Plus® (bioMérieux) blood culture bottles and cultured in a BacT/ALERT VIRTUO® system (bioMérieux). Samples from positive cultures were directly plated on agar plates. After a short incubation of 4–6 hours, colonies were identified with Microflex™ LT MALDI-TOF System (Bruker) according to Uppsala University Hospital (UUH) routine procedures. Aliquots were also taken and transported 400 meters to Q-linea for AST. A 500 µl aliquot was subjected to automated sample preparation followed by inoculation into 5 to 11 concentrations of different antimicrobials in two-fold dilutions. Time-lapse images were acquired, and proprietary algorithms translated growth results into minimum inhibitory concentration (MIC) values within six hours. For reference, the clinical isolates were also run with broth microdilution (BMD) from colonies after overnight incubation.

Experimental set-up

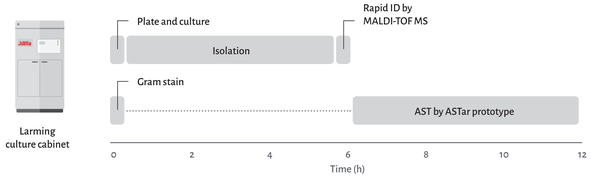

Fig 2. The experimental set-up. Samples were cultured for 4–6 hours at the clinical microbiology laboratory, UUH, and thereafter identified with MALDI-TOF MS. An aliquot from the positive blood culture was then transported to Q-linea for AST analysis using an ASTar prototype system. In this experiment, ID was run prior to AST to avoid running AST for samples containing microbes not in the pathogen panel.

ASTar workflow

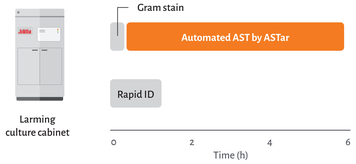

Fig 3. Illustration of a workflow with ASTar in parallel with rapid ID. ASTar can be started independently of ID, which is entered before, during or after the AST run to create the final MIC report.

Results

Short-time incubation and subsequent mass spectrometry (MS) for identification gave results for both gram-positive and gram-negative colonies with scores above 1.9 for all samples and above 2.0 for 23 of 26 samples (Table 1). One sample was suspected polymicrobial after MS of short-term cultured samples and was excluded from AST since the first version of ASTar does not handle polymicrobial samples. Three samples were excluded due to technical failures.

Rapid AST with ASTar was run from clinical positive blood cultures. The different blood cultures contained *H. influenzae*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. cloacae* complex, *P. mirabilis*, *S. aureus*, or *S. epidermidis*, as reported by MS. In total, 199 data points were included, and we reached an overall Essential Agreement (EA) of 91.8% and a Categorical Agreement (CA) of 96.5% compared to reference BMD MIC (Table 2). In total, 2 major errors and 5 minor errors were observed. No very major errors were identified. Two samples that were reported to contain only a single pathogen by MS, with scores > 2.0, showed multiple pathogens after overnight incubation on plates. However, ASTar MIC results matched reference BMD for the species reported as monomicrobial by short culture MS.

Table 1. Identification of pathogens by MALDI-TOF MS after short-term culture.

	Species	Score		Species	Score
1	*S. epidermidis*	1.98	14	*H. influenzae*	2.19
2	*S. aureus*	2.39	15	*P. mirabilis*	2.31
3	*E. coli*	2.20	16	*S. epidermidis*	1.97
4	*K. pneumoniae*	2.49	17	*S. epidermidis*	1.91
5	*P. aeruginosa*	2.21	18	*S. aureus*	2.38
6	*E. coli*	2.41	19	*E. cloacae* complex	2.15
7	*E. cloacae* complex	2.20	20	*E. coli*	2.23
8	*S. epidermidis*	2.15	21	*S. aureus*	2.49
9	*S. epidermidis*	2.05	22	*S. aureus*	2.43
10	*E. coli*	2.42	23	*S. aureus*†	2.16
11	*S. aureus*	2.36	24	*P. aeruginosa*	2.32
12	*S. aureus*	2.05	25	*C. koseri*	2.47
13	*S. epidermidis**	2.03	26	*P. mirabilis**	2.42

* Multiple species identified in the sample after overnight cultivation.
† This sample contained cultured synovial fluid instead of blood.

Table 2. ASTar susceptibility data from clinically positive blood cultures, and one synovial fluid sample.

				No. of tests					
Antimicrobial agent	Total no. of tests	EA (%)	CA (%)	S	I	R	mE	ME	VME
Amoxicillin-clavulanic acid	11	11 (100%)	11 (100%)	9		2			
Benzylpenicillin	4	3 (75%)	4 (100%)			4			
Piperacillin-tazobactam	12	12 (100%)	12 (100%)	10		2			
Cefotaxime	11	10 (91%)	11 (100%)	10		1			
Cefoxitin screen	4	-	4 (100%)	4					
Ceftazidime	12	10 (83%)	10 (83%)	11		1	1	1	
Ceftolozane-tazobactam	12	12 (100%)	12 (100%)	11		1			
Meropenem	13	11 (85%)	13 (100%)	12		1			
Ciprofloxacin	13	13 (100%)	12 (92%)	12		1	1		
Levofloxacin	9	8 (89%)	9 (100%)	6		3			
Gentamicin	12	11 (92%)	12 (100%)	11		1			
Tobramycin	12	10 (83%)	12 (100%)	12					
Vancomycin	9	9 (100%)	9 (100%)	9					
Erythromycin	9	9 (100%)	9 (100%)	4		5			
Clindamycin	9	8 (89%)	8 (89%)	5		4	1		
Tetracycline	9	8 (89%)	7 (78%)	6	2	1	1	1	
Tigecycline	9	7 (78%)	9 (100%)	9					
Colistin	11	11 (100%)	11 (100%)	9		2			
Daptomycin	9	9 (100%)	9 (100%)	9					
Trimethoprim-sulfamethoxazole	9	7 (78%)	8 (89%)	7	2		1		
Total	**199**	**91.8%**	**96.5%**						

mE = minor error, ME = major error, VME = very major error

Conclusions

- AST direct from positive blood culture using a prototype ASTar was in good concordance with reference BMD, showing an EA and CA of 91.8% and 96.5% respectively.
- While the two samples that were subsequently identified as polymicrobial have ASTar results in concordance with BMD data for the species initially identified by MS, performance of ASTar on unidentified polymicrobial samples will be further investigated.
- ASTar along with rapid ID offers a parallelized workflow delivering actionable results within six hours from positive blood culture.

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