Fully Automated Phenotypic Resistance Reporting in 3 Hours, Directly from a Positive Blood Culture

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Background

Bloodstream infections (BSI) require prompt treatment with antibiotics, but empiric therapy can be inadequate in at least 14% of severe infection cases[1]. Especially for high resistance hospital settings, such as the ICU[2–4], the availability of rapid antimicrobial susceptibility testing (AST) methods can significantly reduce length of stay (LoS) per patient and has the potential to save lives[5,6]. In Covid-19 patients in ICU, BSI can result from secondary bacterial infections or superinfections and impact negatively on clinical outcome[7,8]. In this regard, reducing LoS is especially valuable in a time of hospital bed scarcity such as during pandemics. In addition, rapid AST can reduce the risk of developing further antimicrobial resistance, by enabling an earlier start of adequate therapy and faster de-escalation[6].

The ASTar System

The fully-automated ASTar® System delivers rapid phenotypic AST and true Minimum Inhibitory Concentrations (MIC) directly from positive blood cultures and against an extensive AST panel, in ~6 hours. The AST Disc has over 330 chambers available for antimicrobials, covering both fastidious and non-fastidious pathogens. ASTar combines a user-friendly interface with high throughput and random-access load-and-go.

Rationale

When treating BSI or sepsis, it is extremely important to rapidly predict if and what type of resistance may be present for the specific causative bacteria to make relevant antimicrobial choices at an individual patient level. We analyzed how early ESBL-phenotype of Enterobacterales could be flagged, as well as if we could identify I or R phenotypes, calling for adjustment of antimicrobial regimen or dosing, for a larger set of antimicrobials. Both tests were performed directly from positive blood cultures using the ASTar System in combination with a non-CE marked development software.

Results

85 unique isolates from 11 different species of Enterobacterales were screened for ESBL-phenotype as defined by EUCAST (ver2.0 July 2017). After 3-hours AST, 11 of the isolates were identified as ESBL-phenotype. After 18 hours, all of these were also classified as ESBL phenotype by the reference method (BMD) (Table 1). Based on excellent performance of the ESBL phenotype analysis, we continued to evaluate a set of 109 unique isolates (including the 85 in the ESBL screen) against a panel of 21 antimicrobials (Table 2), for identification of I or R classification ("non-susceptible") read out at 3-hours.

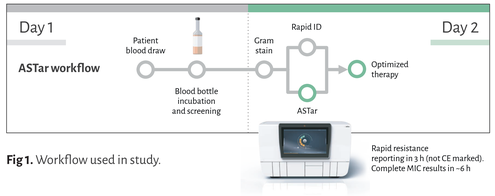

Fig 1. Workflow used in study.

Table 1. ESBL screening of Enterobacterales

Q-linea rapid R	Reference BMD, 18 hours	
	No ESBL phenotype	ESBL phenotype
No ESBL phenotype	74	0
ESBL phenotype	0	11

PPV for ESBL identification = 100%

Table 2. Antibiotic panel and bacteria included in study

Antimicrobial class	Antimicrobial agent	*C. freundii*	*C. koseri*	*E. cloacae complex*	*E. coli*	*K. aerogenes*	*K. oxytoca*	*K. pneumoniae*	*M. morganii*	*P. mirabilis*	*P. vulgaris*	*S. marcescens*	Number of resistant isolates
Penicillin	Amoxicillin-clavulanic acid[1]		•		•		•	•		•	•		11
Penicillin	Ampicillin				•					•			19
Penicillin	Piperacillin-tazobactam[2]	•	•	•	•	•	•	•	•	•	•	•	13
Cephalosporin	Cefepime	•	•	•	•	•	•	•	•	•	•	•	11
Cephalosporin	Cefotaxime	•	•	•	•	•	•	•	•	•	•	•	15
Cephalosporin	Cefoxitin				•		•	•		•			5
Cephalosporin	Ceftazidime	•	•	•	•	•	•	•			•	•	13
Cephalosporin	Ceftazidime-avibactam[3]	•	•	•	•	•	•	•	•	•	•	•	0
Cephalosporin	Ceftolozane-tazobactam[2]	•	•	•	•	•	•	•	•		•	•	10
Cephalosporin	Ceftriaxone	•	•	•	•	•	•	•		•	•	•	15
Carbapenem	Ertapenem	•	•		•		•	•	•				3
Carbapenem	Meropenem	•	•	•	•	•	•	•	•	•	•	•	0
Monobactam	Aztreonam	•	•	•	•	•	•	•	•	•	•	•	15
Fluoroquinolone	Ciprofloxacin	•	•	•	•	•	•	•	•	•	•	•	18
Fluoroquinolone	Levofloxacin	•	•	•	•	•	•	•	•	•	•	•	13
Aminoglycoside	Amikacin	•	•	•	•	•	•	•	•			•	7
Aminoglycoside	Gentamicin	•	•	•	•	•	•	•	•	•	•	•	14
Aminoglycoside	Tobramycin	•	•	•	•	•	•	•	•	•	•	•	12
Tetracycline	Tigecycline		•		•								0
Miscellaneous	Colistin				•			•					0
Miscellaneous	Trimethoprim-sulfamethoxazole[4]	•	•	•	•	•	•	•	•		•	•	0

[1] For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L
[2] For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L
[3] For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L
[4] Trimethoprim:sulfamethoxazole in the ratio 1:19

The selected antimicrobials corresponded to those on the CE/IVD panel for ASTar BC G- panel, except for cefuroxime and cefazolin, as these were not included in the non-CE marked development software used. This generated a total of 1883 results; 193 non-susceptible results were called at 3 hours, of which 189 were confirmed as non-susceptible by the reference method (see Table 3). Three out of the four failed tests were for the antimicrobial TRS. The remaining single failed result was in essential agreement with the reference method but not categorical agreement, warranting further testing to confirm the reference MIC.

Choose AST disc and load | Scan and load cartridge | Tap START RUN

Fig 2. Workflow to load and run a sample in the fully automated system used in the study.

Table 3. Identification of I or R phenotype from positive blood cultures in 3 hours

Q-linea rapid R	Reference BMD, 18 hours	
	Susceptible[1]	Non susceptible[2]
Susceptible[1]	1664	26
Non susceptible[2]	4[3]	189

[1] Susceptible classification according to EUCAST v10.
[2] Non susceptible. Included MIC results that would be classified as I or R according to EUCAST v10.
[3] 3 out of 4 are TRS

PPV for non-susceptible classification = 97.9%

Methods

Blood culture flasks (bcf) were inoculated with blood from healthy donors and clinically derived bacterial isolates and cultured until signaled positive. A 1 ml aliquot from the positive bcf was subjected to fully-automatic sample preparation and inoculation into discrete concentrations of dried antimicrobials in multiple two-fold dilutions. High-speed optical microscopy in combination with proprietary algorithms were used to determine non-susceptibility. Read-out was available after 3-hours total assay time. Sensititre™ broth microdilution (BMD) of the corresponding isolate was used as reference.

References

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Conclusions

Within 3 hours Q-linea's AST technology identified ESBL-phenotypes in Enterobacterales with a positive predictive value of 100%. A positive predictive value of 97.9% for I or R classification was achieved for 21 antibiotics within the test set of 109 isolates. The result indicates that Q-linea AST technology may be used to signal that antimicrobial treatment should be escalated, by selecting another antimicrobial for treatment, or dose-adjusted after final MIC determination. Additionally, in ~6 hours the system provides the final MIC report that can drive de-escalation decisions as well as provide guidance on eventual dose-adjustment.

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