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RESEARCH

Impact of reporting rapid susceptibility results in Gram negative bloodstream infections: a real world prospective study

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Abstract

Purpose Mortality and morbidity of patients with bloodstream infection (BSI) remain high despite advances in diagnostic methods and efforts to speed up reporting. This study investigated the impact of reporting rapid Minimum Inhibitory Concentration (MIC)-results in Gram negative BSIs with the ASTar system (Q-linea, Uppsala, Sweden) on the adaptation of empirically started antimicrobial therapy. We performed a real-world study during which antimicrobial susceptibility testing (AST) results were instantly reported to the treating physician in an established multidisciplinary antimicrobial stewardship setting.

Methods Consecutive patients with Gram negative bacteremia were included in the study (monomicrobial Gram stain, life expectancy of at least 48 h and flagging positive before 2 PM). Rapid AST (RAST) reporting with ASTar was added on top of the standard workflow. Technical performance of the system was evaluated as well as the impact on antimicrobial treatment and timelines of achieving effective and optimal antimicrobial therapy.

Results A total of 79 analyses were performed in 77 patients, of which 68 episodes were eligible for analysis. A categorical agreement was observed in 97,5% of 1160 MIC results without false susceptible results. All patients on ineffective empirical therapy (12/68) were switched after a median time of approximately one hour (5 min – 15 h) after communication of the result. Furthermore, 20/55 non-optimal therapies were adapted within a median period of 3 h after communication.

Conclusion The implementation of rapid MICs, measured by the ASTar system, in our low antimicrobial resistance setting with elaborate antimicrobial guidelines, was easy and led to early adaptation of empirical treatment in 32/55 instances (12 ineffective and 20 non-optimal therapy).

Trial Registration Number NCT06218277 (date of registration: 18–12-2023).

Keywords RAST · Gram negative bacteremia · MIC · Antimicrobial stewardship · EUCAST

Introduction

Mortality and morbidity of patients with bloodstream infection (BSI), remain high despite advances in diagnostic methods and efforts to speed up reporting of results [1]. For many

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decades, therapy of BSIs was mainly guided by early Gram stain results as both identification and susceptibility results needed a long time. The introduction of MALDI-TOF MS in the clinical microbiology laboratories has boosted the speed of the identification of the causing pathogen and opened opportunities for rapid antimicrobial susceptibility testing (AST). EUCAST developed and validated the RAST (rapid AST) method for disk diffusion in 2020 [2]. Fast molecular panels, combining identification and susceptibility testing based on the detection of well-known resistance genes are also marketed [3], however these systems have to be implemented in the right setting and results communicated by an experienced microbiologist [4].

The most recent developments in the field are systems that provide growth based AST results and sometimes MIC values, such as VITEK REVEAL (Biomerieux, Marcy-L'étoile, France), QuantaMatrix dRAST (QuantaMatrix, Seoul, Republic of Korea) and ASTar (Q-Linea, Uppsala, Sweden) [5–9]. The ASTar system provides automated rapid phenotypic susceptibility results within 6 h, directly on positive blood cultures.

In this study, we investigated the impact of reporting rapid MIC-results of Gram negative bloodstream infections with the ASTar system on the adaptation of empirically started antimicrobial therapy. Therefore we performed a real world study in which the results were reported immediately to the treating physician, by phone as well as via reporting in the electronic health record. The study was embedded in an established multidisciplinary antimicrobial stewardship setting.

Materials and methods

Study design

We performed a prospective real world clinical study in a + 800 beds tertiary care teaching hospital. We studied consecutive patients with Gram negative bacteremia between 18–12–2023 and 07–06–2024. Patients were included when Gram stain of a positive blood culture bottle revealed Gram negative rods only (no polymicrobial culture) and if they had a life expectancy of at least 48 h, based on the estimation of the treating physician. Every first positive bottle of an episode (defined as a 7 day period) was included. Due to work flow restrictions, only bottles flagging positive before 2 PM could be included (time period of inclusion between 8 AM and 2 PM).

All patients gave written informed consent to participate and the study was approved by the Local Ethics Committee (B6702023000134) and registered on Clinical Trials (NCT06218277).

Workflow

Blood cultures were accepted 24/7 and incubated immediately upon receipt. Bactec bottles Bactec Plus Aerobic/F, anaerobic/F lytic and Peds Plus/F (Becton Dickinson GmbH, Heidelberg, Germany) were used and incubated in the BACTEC FX system (Becton Dickinson GmbH) upon arrival in the laboratory. In case a blood culture bottle flagged for growth, a Gram stain was performed by a specialized microbiology technician and the results were communicated by phone to the attending physician, immediately (between 7 AM and 8 PM) or the next morning. Identification of the organism was performed on a subculture with MALDI-TOF MS (Microflex LT, Bruker Germany). In addition, for bottles flagged positive between 7 AM and 2 PM, identification of the pathogen with MALDI-TOF MS following short incubation (6 h at 35 °C and 5% CO_2) was performed.

All positive blood cultures with a monomicrobial Gram stain (only Gram-negative rods) in patients without a Gram-negative bacteremia in the previous 7 days, simultaneously entered the standard and study workflow.

Standard workflow

Direct AST (directly from positive blood culture bottle without subculture, DAST) was performed with disk diffusion and automated reading with Adagio (Bio-Rad, Hercules, USA) using EUCAST breakpoints (v. 13.1) following 18–24 h of incubation [10]. For Enterobacterales 13 antimicrobials and for *Pseudomonas aeruginosa* 9 antimicrobials were tested. The complete overview is available in the Supplemental data, Table 1S. Only temocillin was not included in the study workflow.

Study workflow

Blood cultures with a monomicrobial Gram stain (only Gram negative rods and no Gram negative bacteremia in the previous 7 days) flagging before 2 PM (to be able to achieve early identification of the pathogen to permit the timely interpretation of the ASTar results and discussion during a multidisciplinary team meeting) were tested with the ASTar system.

The ASTar System consists of the ASTar instrument (Instrument software: ASTar Application computer image, version 1.5.5 and ASTar Instrument computer image, version 1.7.3 and the following Kit software: ASTar BC G- Kit software, (EUCAST), version 1.7.4.) and the ASTar BC G- Kit. The test was performed according to the manufacturer's instructions. Briefly, 1 mL of the positive blood was transferred directly in the ASTar cartridge. After placing the

ASTar BC-G frozen insert in the cartridge, the cartridge was placed in the ASTar system to start the analysis. The system provided MIC-values and EUCAST categorical interpretation after 6 h for monomicrobial Gram-negative blood cultures for 14 Gram negatives and 26 antimicrobials. The overview can be found in the Supplemental data, Table 2S. Results were reported in real-time in the electronic medical record of the patient via a bidirectional connection with the laboratory information system, in combination with active antimicrobial stewardship interventions (including a phone call to the treating physician and discussion of the case at the daily multidisciplinary infection meeting gathering infectious disease specialists (in training), medical microbiologists (in training) and clinical pharmacists (in training) [11].

Measured parameters and outcomes

Patient- and disease characteristics (age, sex, suspected origin of the blood stream infection) were collected from the patient files.

Different time points were noted: time of blood culture collection, time of culture arrival at the laboratory, time of incubation of culture, time of positive flagging of culture bottle, time of result of the MALDI-TOF MS identification and the ASTar, time to result of the disk diffusion AST, time of notification of the different (sub)results to the treating physician, time of administration of the antimicrobials and timing of antimicrobial switches. All time intervals were calculated from the time point the bottle flagged positive. The time between positive flagging of the culture bottle to the result of AST by ASTar and by disk diffusion (DAST) was compared. Also the timing of switch to a more adequate (effective as well as optimal, see 4. Definitions and interpretation of therapy) antibiotic regimen was measured.

For more technical comparison of the DAST with the study AST results, the standard results were considered as the correct result. A very major error was defined as a resistant (EUCAST R) DAST result but a susceptible, standard dose (EUCAST S) study result, a major error was defined as a susceptible, standard dose (EUCAST S) standard result but a resistant (EUCAST R) study result. A minor error was defined as a disagreement between a susceptible standard dose (EUCAST S) against a susceptible, increased dose (EUCAST I) result. The discrepant results were further tested with an E-test (BioMérieux, Marcy-l'étoile, France) which was considered the decisive result.

Definitions and interpretation of therapy

The appropriateness of the empirical therapy was evaluated. The therapy was regarded **effective** if the isolated microorganism tested susceptible (standard dose, EUCAST S) for the prescribed antimicrobial. The antimicrobial therapy was considered **optimal** if it was the least broad-spectrum antimicrobial available according to susceptibility results, if there was no unnecessary anaerobic or *Pseudomonas aeruginosa* coverage in the empirical therapy, or if a suitable oral option was administered. In all cases these factors were balanced against the clinical context of the patient. We considered the severity and source of the infection, the clinical status of the patient, the type of the infection (mono- versus polymicrobial) and the performance of source control.

The interpretation of the effective and optimal character of the antimicrobial therapy was evaluated by a panel consisting of a medical microbiologist and an infectious diseases specialist.

Data handling and statistical analysis

All data were registered in a REDCap Database. Data were analyzed descriptive and using Excel.

Results

Patient characteristics

A total of 79 episodes (in 77 patients) with a Gram stain confirming Gram negative bloodstream infection were enrolled in the study. Patients' characteristics at the time of inclusion are presented in Table 1Sa (Supplemental data). Forty four patients were male, thirty three were female. The median age at presentation was 65 years (range 1–92). For every episode, the presumed source of infection was registered with urinary tract infections as the most prevalent, followed by gastro-intestinal infections and mucosal barrier injury in patients with neutropenic fever.

Microbiological characteristics

The identified bacteria were mainly Enterobacterales, with the majority being *Escherichia coli* (n = 40). The second most prevalent species were *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (both n = 8). Table 1Sb (Supplemental data) gives an overview of the complete spectrum of identifications.

Eleven out of 79 episodes could not be further evaluated in the study because of following reasons: no subculture growth (n = 1), insufficient growth in the ASTar panel (n = 2; 1 *E. coli* and 1 *P. aeruginosa*), and micro-organisms not included in the validated ASTar panel (n = 8; 2 in a mixed culture).

In the further evaluated group of 68 episodes, despite a monomorphic Gram stain, 6 cases revealed mixed Gram negative cultures. Table 1Sc (Supplemental data) shows the

detailed information of these mixed cultures. The underlined species names were used for the ASTar interpretation.

Comparison of AST results between the different methods (ASTar versus DAST)

In the 68 episodes with ASTar results, we observed 23 episodes with a categorical disagreement between ASTar- and DAST results for at least one isolate-antibiotic combination and for a total of 29 results (1160 total number of MICs provided for antibiotics on the hospital formulary, 97.5% categorical agreement). For 18 results we checked the discrepancy with an E-test. Four E-test AST results were congruent with the ASTar result. Figure 1 shows an overview of the discrepancies. Most cases were observed for amoxicillin-clavulanic acid where 10 major errors (ME, resistant with ASTar, susceptible, standard dose with DAST) were observed. Of interest, the most important finding was observed in an OXA-48 carbapenemase-producing *K. pneumoniae* with an ASTar meropenem MIC of 2 µg/mL, which was a trigger for further investigation of the strain and adaptation of the therapy [12]. The disk diffusion result the next day showed a "susceptible, increased exposure" categorization for meropenem.

Of notice, there were no disagreements for the mixed Gram negative cultures when comparing the ASTar results with the DAST results for the separate species.

Timing of results and impact on the empirical antimicrobial therapy

Sixty-eight episodes were eligible for further evaluation concerning therapeutic decisions based on the microbiological results. Figure 2 gives an overview of the results.

The median time to detection of growth in the culture bottle was 11 h (range 6–48 h) in 51 aerobic, 27 anaerobic and 1 pediatric aerobic bottle.

The median time needed for identification by MALDI-TOF MS, from flagging positive, was 10 h (range 4–23 h) which was only slightly shorter than the 11 h time interval between flagging positive and availability of the ASTar result (range 6–24 h) and demonstrates the feasibility of the ASTar-based workflow in a standard blood culture work flow. There was no case where lack of or delay in identification caused delay in interpreting the ASTar results. There was no episode where the identification result as such should have forced an adaptation of the antimicrobial therapy.

The DAST results were available after a median time of 27 h and 45 min after flagging positive (range 21–42 h), a difference of 17 h with the ASTar results.

In 52/68 episodes (76%) the patient was already on empirical antimicrobial therapy at the moment of telephonic notification of the positive Gram stain result, and therapy was changed in 21% of these. This consisted of a change in antibiotic choice or an adaptation of the dose. In all patients

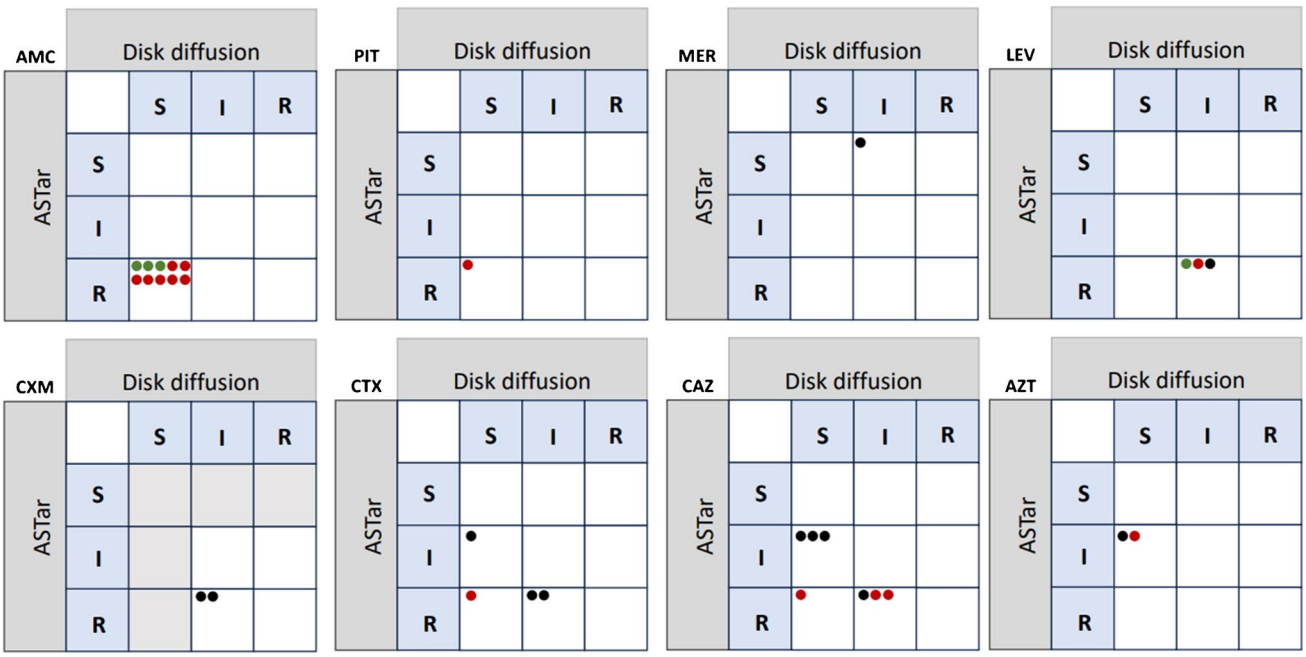

Fig. 1 Categorical discrepancies between ASTar and disk diffusion (DAST). S: susceptible, standard dosing regimen; I: susceptible, increased exposure; R: resistant; AMC: amoxicillin-clavulanic acid; PIT: piperacillin-tazobactam; MER: meropenem; LEV: levofloxacin; CXM: cefuroxime; CTX: cefotaxime; CAZ: ceftazidime; AZT: aztreonam; red dot: ASTar result not confirmed with E-test; green dot: ASTar result confirmed with E-test; black dot: no E-test performed

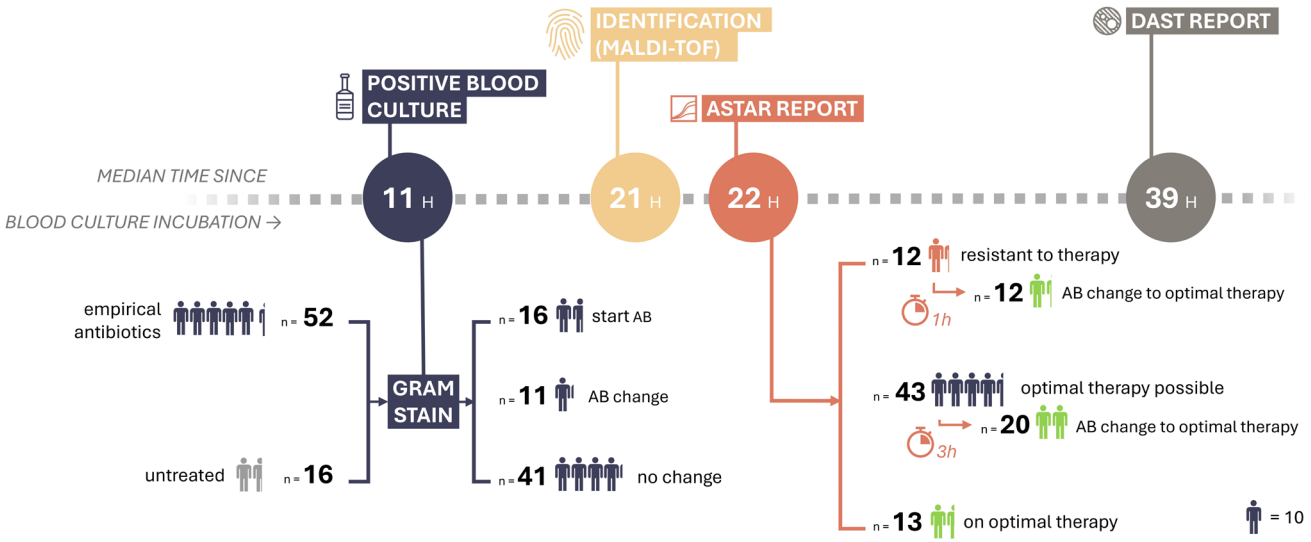

Fig. 2 Timing of results and impact on antimicrobial therapy. n: number; AB: antimicrobial therapy. AB change after gram stain includes change of dose as well as change of antimicrobial

not on antimicrobial therapy at the moment of notification, antimicrobial therapy was started.

In 12/68 episodes the ASTar result showed resistance for the current antibiotic therapy and the treatment was classified as not effective. Notification of the physician led to adaptation of the therapy in all cases, with a median time interval of 1 h (range 5 min-15 h) after notification. In two of these cases the DAST and E-test results showed susceptibility despite MIC values on ASTar for amoxicillin-clavulanic acid of 16 and > 32 µg/mL respectively and both confirmed with E-test.

Briefly, ASTar facilitated switch to effective antimicrobial therapy in 12/68 episodes (17,6%) within a median time of about 1 h after release of the results.

In 43/68 episodes (63%) the results of ASTar showed opportunities for adapting the antimicrobial therapy, allowing for optimal therapy. Half of these opportunities (20/43) was utilized in the hours following the prompt communication with adaptations made after a median time interval of 3 h (n = 20; range 5 min – 18 h). The most important reasons for not optimizing therapy swiftly were cultures at other sites with more resistant bacteria, lack of source control, unknown source of the bacteremia or the severe clinical status of the patient. Although in these cases the panel of the infectious diseases specialist and the medical microbiologist could agree with the decision of the treating physician, we did not counted these cases as "good" to not overestimate the impact of our intervention. Sometimes there was a delay in the start of the more optimal therapy because the treating physician only made a prescription (prescription made immediately after communication of the results) starting the following day. In 17 episodes the switch to optimal therapy was performed later, based on AST results and following improvement of the clinical status of the patient. In this subgroup where optimal therapy was achieved apart from the ASTar results, the median time needed for this therapeutic switch was 38 h from flagging positive, so 35 h later than in the ASTar based subgroup.

There were no episodes where therapy had to be adapted because of resistance with DAST which was not detected with ASTar (no very major errors) There were however 6 therapeutic adaptations partly based on DAST results. In two episodes temocillin (not tested by ASTar) was considered more appropriate for a bacteremia of urinary origin and in four episodes other, newly available, culture results prompted to an antimicrobial therapy change.

Discussion

We studied the impact on therapeutic decisions based on the rapid susceptibility results with the ASTar system for patients suffering from Gram-negative BSI. The study was embedded in a laboratory standard workflow using early identification with MALDI-TOF MS on young cultures and DAST. Comparison of the ASTar with the DAST for categorical agreement (97.5%) was in line with previous data, using different AST systems [9, 13, 14]. Remarkably, in the current study no very major errors were observed.

This is the first study, to our knowledge, evaluating the real-world adaptation of antimicrobial therapy for Gram negative BSI, based on rapid phenotypic ASTar results. One of our concerns at the start, that the treating physicians would not be willing to adapt the empirical therapy at such

an early time point, less than 24 h after drawing the blood culture, was not realized. On the contrary, for the resistant strains antimicrobial escalation generally followed almost immediately after reporting and even for optimization of therapy, in 49% of cases (21/43) this was performed in an acceptable time frame of less than 26 h after the start of incubation of the bottle.

We have to mention explicitly the dedicated communication on several levels that was added as part of the study, embedded in a multidisciplinary stewardship program of more than 10 years in our institution. Every non-ICU patient included in the study was discussed on the daily multidisciplinary patient meeting where infectious disease specialists (in training), medical microbiologists (in training) and clinical pharmacists (in training) give advice (written in the patient record as well as by telephone) concerning antimicrobial therapy. The advices given by this team have a high rate of acceptance [11].

We were surprised by the number of mixed Gram negative cultures in our population and therefore found it interesting to evaluate these results more in detail. The strategy that was (unintentionally) used, interpreting the ASTar results with the micro-organism found from the first identification round on the young colonies (which was the case in 4 mixed cultured) or ignoring the identification of another gram-negative pathogen from the paired bottle, turned out correct in our setting. However, this off-label use of the ASTar results needs caution with critical appraisal of the results when doing so.

We were able to compare the results of the present population with a historical cohort of EUCAST RAST-tested BSIs [15]. In the EUCAST RAST cohort we performed a retrospective analysis of the impact of the AST results on a population of 99 patients with Gram-negative BSI (only *E. coli*, *K. pneumoniae* and *P. aeruginosa* were included as the analysis was embedded in an evaluation of the EUCAST RAST method). The appropriateness of the definitive antimicrobial therapy was reviewed by an experienced microbiologist, based on local antimicrobial prescribing guidelines, AST results and clinical information.

For categorical agreement with the DAST results, ASTar performed better than the EUCAST RAST method, with a categorical disagreement of 2.5% versus 3% and 3% (6 and 8 h of incubation respectively) and absence of very major errors with ASTar (incorrect susceptible result) versus 2/557 results (6 h) and 2/553 (8 h) with EUCAST RAST. Remarkable as well is the absence of ATU-results with ASTar as this was a major issue for piperacillin-tazobactam in the EUCAST RAST methodology.

ASTar AST led to very early antimicrobial adaptations in 48.5% of cases (33/68), both leading to effective as well as to optimized therapy. In the retrospective population, a comparable adaptation of therapy to the AST result was observed (44,5%). However, data are lacking whether the EUCAST RAST results led to a shorter time to effective or optimal therapy.

Overall, the number of adaptation opportunities as well as the implemented adaptations are in line in both studies. This may not be surprising as both studies were performed in the same center and within a time frame of less than 5 years apart. However, this study, using the ASTar BC G- kit, allows for a broader evaluation of both antimicrobials and micro-organisms within the setting of Gram-negative BSIs.

The limitations of our study are the monocentric character and the limited amount of patients included. Furthermore, we did not study the effect of the accelerated switches on microbiological and clinical cure, length of stay, mortality and overall cost. As the ASTar system comes with a cost exceeding that of standard of care, studying the broader impact of implementing the system should be subject of further research.

Although the willingness of performing therapeutic adaptations based on the ASTar results was quite high, our character as a teaching hospital, with a lot of medical specialists in training, may have influenced the timing of the therapeutic switches in a negative way. The less experienced clinicians may have wanted to consult with their supervisors first.

In conclusion, the implementation of the very rapid MICs, measured with the ASTar system, in our setting with low antimicrobial resistance and elaborate antimicrobial guidelines, led to adaptations of the empirical therapy in 33/68 (48.5%) opportunities. Both adaptations leading to effective as well as optimal therapy were performed. In 15% of cases (= 10/68) the ineffective empirical therapy was changed based on ASTar results after a median time of 12 h after flagging of positive culture bottle compared to availability of the DAST results after a median time of 27 h and 45 min. The proportion of adaptations suggests that, when supported by active communication and established multidisciplinary stewardship, even very early antimicrobial switches are achievable and are accepted by the treating physician. More early therapeutic adaptations towards effective and optimal antimicrobial treatment, may lead to better patient outcomes and influence development of resistance, however this warrants further research.

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performed by J.B. The first draft of the manuscript was written by J.B. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors. Q-linea provided the system and kit for the experiments, but the company had no role in study design, data collection and analysis, the decision to publish, or the preparation of the manuscript.

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