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Rapid phenotypic antimicrobial susceptibility testing of Gram-negative rods directly from positive blood cultures using the novel Q-linea ASTar system

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ABSTRACT Adequate and timely antibiotic therapy is crucial for the treatment of sepsis. Innovative systems, like the Q-linea ASTar, have been developed to perform rapid antimicrobial susceptibility testing (AST) directly from positive blood cultures (BCs). We conducted a prospective study to evaluate ASTar under real-life conditions with a focus on time-to-result and impact on antimicrobial therapy. Over 2 months, all positive BCs that showed Gram-negative rods upon microscopy were tested with the ASTar and our standard procedure (VITEK 2 from short-term culture). Additionally, we included multidrug-resistant Gram-negative bacteria from our archive. Both methods were compared to broth microdilution. In total, 78 bacterial strains (51 prospective and 27 archived) were tested. ASTar covered 94% of the species encountered. The categorical and essential agreement was 95.6% and 90.7%, respectively. ASTar caused 2.4% minor, 2.0% major, and 2.4% very major errors. The categorical agreement was similar to standard procedure. The average time between BC sampling and the availability of the antibiogram for the attending physician was 28 h 49 min for ASTar and 44 h 18 min for standard procedure. ASTar correctly identified all patients who required an escalation of antimicrobial therapy and 75% of those who were eligible for de-escalation. In conclusion, ASTar provided reliable AST results and significantly shortened the time to obtain an antibiogram. However, the percentage of patients that will profit from ASTar in a low-resistance setting is limited, and it is currently unclear if a change of therapy 29 h after BC sampling will have a significant impact on the patient's prognosis.

KEYWORDS AST, RAST, sepsis, blood stream infection, VITEK, scum plate method

Adequate and timely antibiotic therapy is crucial for the successful treatment of bacterial bloodstream infections (BSI) (1). In areas with low resistance rates, empirical therapy is normally appropriate. However, due to rising resistance rates, especially in *Enterobacterales*, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii*, the empirical therapy of a septic patient may fail, causing significant morbidity and mortality (2).

Detection of the pathogen by blood culture (BC) and antimicrobial susceptibility testing (AST) can identify cases of ineffective empirical antibiotic therapy and thereby help to reduce mortality. In classical BC diagnostics, the time span from the collection of the BCs to the availability of the antibiogram consists of the sample transport (approximately 2 h), the time-to-positivity of BCs [approximately 12–18 h for Gram-negative rods (3)], and the duration of AST (approximately 18 h for a non-standardized AST directly from the positive BC bottle). Therefore, at least 32 h pass before an ineffective empirical therapy is revealed by classical BC diagnostics. As many laboratories do not work around the clock, additional time is lost if BC bottles are not processed immediately after positivity or if the AST results are not reported to the physician promptly.

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Disk diffusion-based methods and various systems using innovative technologies have now been developed to accelerate AST and to provide an antibiogram on the day the BC was reported positive (4–8). The ASTar system (Q-linea, Sweden) is one of these systems and performs fully automated microdilution AST directly from positive BCs in about 6 h (9). At present, there is an AST panel for a broad range of Gram-negative microorganisms available (Table S1). Twelve samples can be tested simultaneously with random access and minimal hands-on-time. The identification of the bacterium is not part of the ASTar system, but it is necessary for the interpretation of AST results. Therefore, identification has to be performed within 6 h using another technique.

In this study, we prospectively investigated the performance of the novel Q-linea ASTar system under clinical real-life conditions and compared it with the standard procedure in our laboratory (VITEK 2 AST from short-term culture; Fig. 1). Besides the diagnostic performance [categorical agreement (CA) and essential agreement (EA)], special attention was given to the impact on empirical antimicrobial therapy and the time to the availability of the AST result for the attending physician.

MATERIALS AND METHODS

We performed a diagnostic accuracy study at the University Hospital Erlangen, Germany, a 1,400-bed tertiary care center. Standards for reporting diagnostic accuracy studies were followed. The study was approved by the local ethics committee (application number 21-488-Bm). The need for informed consent was waived.

The study consisted of two parts: in part one, BCs from clinical routine were prospectively tested over a period of 2 months (February until April 2022). For this purpose, the first positive BC of each patient showing Gram-negative rods upon microscopy was included. In part two, BCs were spiked with multidrug-resistant Gram-negative (MDRGN) isolates from our culture collection according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) rapid AST quality control procedure (10). Our culture collection contains strains out of clinical routine. MDRGN strains needed to have resistance against third-generation cephalosporins or carbapenems (e.g., extended-spectrum beta-lactamase or carbapenemase producers). Carbapenemases were confirmed by the detection of the resistance gene. All strains were stored at −70°C before use.

For both parts, the BACTEC PLUS Aerobic/F and Lytic/10 Anaerobic/F BC bottles were used together with the BACTEC FX BC system (Becton Dickinson GmbH, Heidelberg, Germany). The positive BCs were then processed according to our standard procedure and in parallel with the ASTar system. Only BCs reported positive by 11 a.m. were included in the study, as ASTar results would otherwise not be available within laboratory working hours. This procedure would correspond to a later use in diagnostic routine.

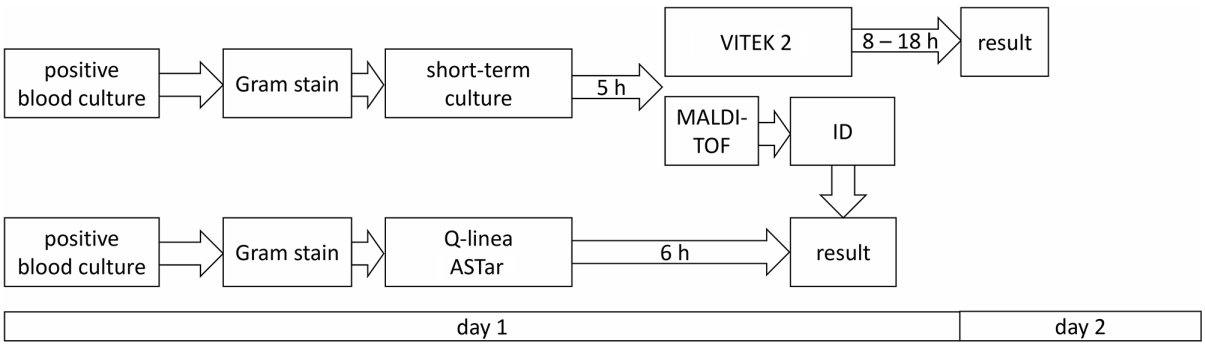

FIG 1 Study workflow.

Standard procedure

Two drops of blood from a positive BC bottle were streaked out on appropriate agar media and immediately incubated at 36°C with 5% CO_2. After a short-term culture for 5 h (scum plate method), an automated AST using the VITEK 2 with the AST-N289 card for *Enterobacterales* and *Acinetobacter species* and the AST-N389 card for *Pseudomonas species* (bioMérieux SA, Marcy-l'Étoile, France) was performed. The scum plate method was internally validated on 100 isolates with results that were comparable to VITEK 2 testing from overnight cultures. Bacteria were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MALDI Biotyper, Bruker Daltonik GmbH, Bremen, Germany) from short-term culture. VITEK 2 AST and identification by MALDI-TOF were carried out according to the manufacturers' recommendations.

ASTar principle

The ASTar system (Q-linea, Uppsala, Sweden) performs fully automated microdilution AST directly from positive BCs in about 6 h. For this purpose, the microorganisms are separated from blood in a sample preparation cartridge and are then transferred automatically to an AST panel. The AST panel has over 330 chambers allowing 6–14 twofold dilutions of each antimicrobial (Table S2). Bacterial population growth in the wells is detected by automated time-lapse imaging, and proprietary algorithms translate visual information into minimum inhibitory concentrations (MICs).

ASTar procedure

After the addition of the ASTar BC G- frozen insert to the ASTar BC G- test cartridge, 1 mL of blood from the positive BC bottle was transferred to the cartridge using a syringe. Both the cartridge and the ASTar AST panel were loaded into the ASTar system and the run was started. The identification of the bacterium was entered into the ASTar system as soon as it was available from the standard procedure. After approximately 6 h, the MIC and the interpretation according to EUCAST breakpoints (V10.0, 2020) were automatically displayed by the ASTar BC G- Kit Software [EU] 1.4.

Reference method

All bacterial strains were frozen at −20°C and were tested in batches by microbroth dilution using the MICRONAUT-S MH Hannover GN3 MIC plate for *Enterobacterales* and *Acinetobacter species* and the MICRONAUT-S *Pseudomonas* MIC plate for *Pseudomonas* species (MERLIN Diagnostika GmbH, Bornheim-Hersel, Germany). For testing amoxicillin/clavulanic acid, the Sensititre EUGNF plate (ThermoFisher Scientific, Wesel, Germany) was used. The layout and the dilution ranges of the MIC plates are given in Tables S3 to S5. Briefly, strains were cultured on Columbia blood agar overnight and a bacterial suspension with a density of McFarland 0.5 was prepared in 0.9% saline. A total of 50 µL of this suspension was transferred to 11.5 mL Müller-Hinton broth. After mixing, 100 µL was added to each well of a MICRONAUT MIC plate. The plate was then sealed with plastic foil and incubated at 36°C for 18 h. Measurement of optical density was performed with a TECAN SUNRISE photometer (TECAN Trading AG, Männedorf, Switzerland) and MICs were calculated by the MCN6 software provided by MERLIN Diagnostika GmbH. The performers of the reference method were not blinded to the results of the index tests.

Comparison of AST results

CA and EA were determined between the reference method and the ASTar or the standard procedure, respectively. The reason for including the standard procedure in this study was to allow a direct comparison of the new method with the laboratory standard, which provides valuable information for deciding whether to change a system or not.

Analysis was performed for routine samples (study part one) and overall samples tested (study parts one and two). CA was achieved if the AST method under evaluation and the reference method yielded the same categorical interpretation (susceptible, susceptible upon increased exposure, and resistant). Very major error (VME), major error (ME), and minor error (mE) were defined as a false susceptible result, a false resistant result, and an error involving the susceptible increased exposure category, respectively (11). EA was evaluated according to the suggestions of Humphries et al. (12), i.e., MIC agreement was defined as "target" (no MIC deviation), "range" (MIC deviation of no more than one $\log^2$ dilution), or "out-of-range" (MIC deviation of more than one $\log^2$ dilution). EA was fulfilled for "target" and "range" MIC agreement (Table S6).

In 2019, EUCAST introduced the "area of technical uncertainty" (ATU). The ATU is used for certain MICs or disk diffusion zone diameters to warn laboratories that the result is in an area where there are interpretative difficulties. ATUs are mostly located between susceptible and resistant MICs or disk diffusion zone diameters. For example, an MIC of 0.5 mg/L for ciprofloxacin in *Enterobacterales* is in the increased (intermediate) category but resistance cannot be completely excluded. Therefore, in routine testing, a MIC within the ATU should be confirmed by a second method or it should be reported as resistant, or the interpretation should not be reported at all. For our study, it was determined that when the reference method for a bacterial isolate and an antibiotic gave a result within the ATU, the antibiotic in question was not evaluated by any AST system. If VITEK 2 AST or ASTar gave a result within the ATU, the antibiotic in question was not evaluated for the respective AST system.

ASTar reported results for cefepime, ceftazidime/avibactam, ceftolozane/tazobactam, and aztreonam only for *Pseudomonas aeruginosa*. Therefore, the number of AST results for these antibiotics is limited.

VITEK 2 AST determined the susceptibility to ampicillin/sulbactam, while ASTar uses amoxicillin/clavulanic acid. Because the MIC distributions of organisms differ for these two antimicrobials, only CA was determined.

Evaluation of clinical impact

In Germany and other European countries, microbiological laboratories are run by physicians with a specialization in medical microbiology. Such physicians often have additional training in antibiotic stewardship and provide advice, which in other countries is provided by infectious disease specialists. In Germany, it is a requirement that all blood culture results are immediately telephoned by such a medical microbiologist to the attending physician. Accordingly, as soon as the result of Gram-staining from the positive BC was available, optimal antibiotic therapy was discussed with the attending physician. The therapy recommended at that time was termed the initial therapy. After the results from ASTar or VITEK 2 AST were available, a potential change in beta-lactam antibiotic therapy was categorized as "escalation" (initial therapy considered ineffective with necessity to use a beta-lactam antibiotic with a broader spectrum), "de-escalation" (initial therapy considered effective with the possibility to use a narrower-spectrum beta-lactam antibiotic), and "no change" (initial therapy considered effective but no possibility of de-escalation). For non-beta-lactam antibiotics, initial treatment was assessed as effective or ineffective only.

Evaluation of time to result

The opening hours of the microbiology laboratory were 8.00 a.m. to 6 p.m. on workdays, 8 a.m. to 4 p.m. on Saturdays, and 9 a.m. to 4 p.m. on Sundays. If AST testing was finished outside the opening hours, then the opening of the laboratory on the next day was considered as the time when the results were available.

Statistical analysis

The statistical analyses were performed as a two-tailed Fisher's exact test using SPSS, V28.0 (IBM, Armonk, USA).

RESULTS

Altogether, 56 patients with positive BCs obtained during routine diagnostics were eligible for part one of the study. Five patients were removed from the analysis due to the presence of an initially unrecognized mixed culture (n = 2) or the growth of pathogens for which the VITEK 2 AST or the reference method were not validated (*Bacteroides fragilis* n = 2, *Haemophilus influenzae* n = 1). Finally, 51 patients were included in part one of the study. For part two, BCs were spiked with 28 MDRGN isolates. One *Pseudomonas aeruginosa* did not grow in the ASTar and, therefore, was removed from analysis. Altogether, 27 additional isolates were included in part two of the study (Fig. S1).

The pathogen spectrum of both study parts is given in Table 1. *Escherichia coli* was the most prevalent pathogen, accounting for 57% of the routine BC isolates. Other *Enterobacterales* were encountered in 31%, *Pseudomonas* spp. in 8%, and *Acinetobacter* spp. in 4%, respectively. Altogether, the validated species of the ASTar covered 94% of the pathogens encountered during routine testing. The MIC distribution of the BC isolates of both study parts, as determined by broth microdilution, is shown in Fig. S2 and S3.

From now on, specific statements on CA and EA will only be made for antibiotics with more than 10 measurements.

ASTar compared to reference method

Overall, 890 determinations of MICs were performed with ASTar in both study parts. Of these, two measurements (0.2%) yielded no result (ampicillin n = 1; piperacillin/tazobactam n = 1). CA could not be determined in another 18 measurements because of lacking EUCAST breakpoints (ceftazidime/avibactam n = 2; ceftolozane/tazobactam n = 2) or because the results were within the ATU of the ASTar (piperacillin/tazobactam n = 3; ciprofloxacin n = 3) or the reference method (piperacillin/tazobactam n = 2; ciprofloxacin n = 6), respectively. Finally, 870 measurements could be evaluated for CA and 831 for EA.

The overall (i.e., study parts one and two) CA of the ASTar was 95.6% (832/870). There were 2.4% (21/870) mE, 2.0% (12/593) ME, and 2.4% (5/212) VME (Table 2; Fig. 2 and

TABLE 1 Species spectrum and frequency of tested pathogens

Species	Study part one (routine)	Study part two (MDRGN)	Study part one + two (overall)
Total (number of strains) (%)	51 (100)	27 (100)	78 (100)
Escherichia coli (number of strains) (%)	29 (57)	9 (33)	38 (49)
Enterobacter cloacae complex (number of strains) (%)	4 (8)	3 (11)	7 (9)
Serratia marcescens (number of strains) (%)	3 (6)	0 (0)	3 (4)
Klebsiella oxytoca (number of strains) (%)	2 (4)	1 (4)	3 (4)
Proteus mirabilis (number of strains) (%)	2 (4)	1 (4)	3 (4)
Pseudomonas aeruginosa (number of strains) (%)	2 (4)	3 (11)	5 (6)
Pseudomonas putida[a] (number of strains) (%)	2 (4)	0 (0)	2 (3)
Citrobacter freundii (number of strains) (%)	1 (2)	0 (0)	1 (1)
Citrobacter koseri (number of strains) (%)	1 (2)	0 (0)	1 (1)
Klebsiella aerogenes (number of strains) (%)	1 (2)	4 (4)	5 (6)
Klebsiella pneumoniae (number of strains) (%)	1 (2)	5 (19)	6 (8)
Klebsiella variicola[a] (number of strains) (%)	1 (2)	0 (0)	1 (1)
Acinetobacter baumannii (number of strains) (%)	1 (2)	1 (4)	2 (3)
Acinetobacter species[a] (number of strains) (%)	1 (2)	0 (0)	1 (1)

[a]Pathogen not validated for ASTar.

TABLE 2 Overall categorical agreement, error, and ATU rates[g]

Antibiotic	Categorical agreement		Minor error		Major error		Very major error		ATU	
	ASTar	VITEK 2	ASTar	VITEK 2	ASTar	VITEK 2	ASTar	VITEK 2	ASTar	VITEK 2
Ampicillin[a] (number/number of tested isolates) (%)	40/40 (100.0)	41/41 (100.0)	0/40 (0.0)	0/41 (0.0)	0/17 (0.0)	0/17 (0.0)	0/23 (0.0)	0/24 (0.0)	n/a	n/a
Amoxicillin/clavulanic acid; ampicillin/sulbactam[b] (number/number of tested isolates) (%)	46/52 (88.5)[c]	50/52 (96.2)	0/52 (0.0)	0/52 (0.0)	6/26 (23.1)[c]	1/26 (3.9)[c]	0/26 (0.0)	1/26 (3.9)[c]	n/a	n/a
Piperacillin/tazobactam[a] (number/number of tested isolates) (%)	67/69 (97.1)	67/71 (94.4)	0/69 (0.0)	1//71 (1.4)	0/46 (0.0)	1/47 (2.1)	2/22 (9.1)[c]	2/22 (9.1)[c]	3/75 (4.0)	3/75 (4.0)
Cefuroxime (number/number of tested isolates) (%)	47/51 (92.2)	48/51 (94.1)	4/51 (7.8)	3/51 (5.9)	n/a	n/a	n/a	n/a	n/a	n/a
Cefotaxime (number/number of tested isolates) (%)	65/68 (95.6)	65/68 (95.6)	2/68 (2.9)	2/68 (2.9)	0/39 (0.0)	0/39 (0.0)	1/28 (3.6)[c]	1/28 (3.6)[c]	n/a	n/a
Ceftazidime (number/number of tested isolates) (%)	66/72 (91.7)	63/72 (87.5)[c]	6/72 (8.3)	8/72 (11.1)[c]	0/39 (0.0)	0/39 (0.0)	0/25 (0.0)	1/25 (4.0)[c]	n/a	n/a
Cefepime[e] (number/number of tested isolates) (%)	7/7 (100.0)	7/7 (100.0)	0/7 (0.0)	0/7 (0.0)	n/a	n/a	n/a	n/a	n/a	n/a
Ceftazidime/avibactam[c,d] (number/number of tested isolates) (%)	4/5 (80.0)[c]	n/a	0/5 (0.0)	n/a	0/2 (0.0)	n/a	1/3 (33.3)[c]	n/a	n/a	n/a
Meropenem (number/number of tested isolates) (%)	68/75 (90.7)	66/75 (88.0)[c]	7/75 (9.3)	8/75 (10.7)[c]	0/67 (0.0)	1/67 (1.5)	0/3 (0.0)	0/3 (0.0)	n/a	n/a
Gentamicin (number/number of tested isolates) (%)	71/71 (100.0)	70/71 (98.6)	0/71 (0.0)	0/71 (0.0)	0/65 (0.0)	1/65 (1.5)	0/6 (0.0)	0/6 (0.0)	n/a	n/a
Tobramycin (number/number of tested isolates) (%)	78/78 (100.0)	78/78 (100.0)	0/78 (0.0)	0/78 (0.0)	0/67 (0.0)	0/67 (0.0)	0/11 (0.0)	0/11 (0.0)	n/a	n/a
Ciprofloxacin (number/number of tested isolates) (%)	69/70 (98.6)	71/71 (100.0)	0/70 (0.0)	0/71 (0.0)	1/46 (2.2)	0/46 (0.0)	0/19 (0.0)	0/19 (0.0)	3/78 (3.8)	4/78 (5.1)
Trimethoprim/sulfamethoxazole (number/number of tested isolates) (%)	62/65 (95.4)	63/65 (96.9)	1/65 (1.5)	1/65 (1.5)	1/44 (2.3)	0/44 (0.0)	1/20 (5.0)[c]	1/20 (5.0)[c]	n/a	n/a
Tigecycline (number/number of tested isolates) (%)	39/39 (100.0)	37/39 (94.9)	0/39 (0.0)	0/39 (0.0)	0/39 (0.0)	2/39 (5.1)[c]	0/0 (0.0)	0/0 (0.0)	n/a	n/a
Colistin[d] (number/number of tested isolates) (%)	42/46 (91.3)	n/a	0/46 (0.0)	n/a	4/46 (8.7)[c]	n/a	0/0 (0.0)	n/a	n/a	n/a
Aztreonam[c] (number/number of tested isolates) (%)	6/7 (85.7)[c]	4/5 (80.0)[c]	1/7 (14.3)[c]	1/5 (20.0)[c]	n/a	n/a	n/a	n/a	n/a	n/a
Ertapenem[d] (number/number of tested isolates) (%)	50/50 (100.0)	n/a	0/50 (0.0)	n/a	0/46 (0.0)	n/a	0/4 (0.0)	n/a	n/a	n/a
Ceftolozane/tazobactam (number/number of tested isolates) (%)	5/5 (100.0)	5/5 (100.0)	0/5 (0.0)	0/5 (0.0)	0/4 (0.0)	0/4 (0.0)	0/1 (0.0)	0/1 (0.0)	n/a	n/a
Total (number of agreements/number of tested isolates) (%)	832/870 (95.6)	735/771 (95.3)	21/870 (2.4)	24/771 (3.1)	12/593 (2.0)	6/500 (1.2)	5/212 (2.4)[f]	6/207 (2.9)[f]	6/153 (3.9)	7/153 (4.6)

[a]One measurement failure in ASTar.
[b]ASTar measured amoxicillin/clavulanic acid and VITEK 2 ampicillin/sulbactam.
[c]Less than 10 strains analyzed.
[d]Not included in the VITEK 2 AST card.
[e]CLSI and FDA requirements not fulfilled.
[f]FDA requirements not fulfilled; n/a, not applicable.
[g]Categorical agreement was present if the AST method and the reference method gave the same categorical result. Very major error, major error, and minor error are defined as a false susceptible result, a false resistant result, and an error involving the susceptible increased exposure category, respectively.

3). These results were within the limits required by the Clinical & Laboratory Standards Institute (CLSI; CA ≥ 90%, mE ≤10%, ME <3%, and VME <3%). However, the US Food and Drug Administration (FDA) limit for VME was exceeded (<1.5%). Minor errors occurred predominantly with meropenem (9.3%; 7/75), ceftazidime (8.3%; 6/72), and cefuroxime (7.8%; 4/51), ME mainly with amoxicillin/clavulanic acid (23.1%; 6/26) and colistin (8.7%; 4/46) and VME with piperacillin/tazobactam (9.1%; 2/22), trimethoprim/sulfamethoxazole (5.0%; 1/20) and cefotaxime (3.6%; 1/28).

Between Enterobacteriaceae and *Pseudomonas* spp., the overall categorical agreement (95.6% and 94.9%, $P = 0.740$) was similar. Interestingly, the analysis of the categorical errors by ASTar together with the species revealed that mE and VME occurred in Enterobacteriaceae and *Pseudomonas* spp. but ME only with Enterobacteriaceae. However, the number of ME was small ($n = 12$) and with a ratio of Enterobacteriaceae to *Pseudomonas* spp. of 9.7 to 1, the absence of ME in *Pseudomonas* spp. is perhaps only a coincidence. It is also striking that amoxicillin/clavulanic acid MEs occurred almost exclusively in *Escherichia coli* and colistin MEs exclusively in *Klebsiella pneumoniae* (Tables S7 and S8).

The overall EA of ASTar and the reference method was 90.7% and, therefore, within the limits of CLSI and FDA (≥90%; Table 3). Especially, the EA for ceftazidime (88.9%), cefuroxime (88.2%), piperacillin/tazobactam (83.8%), cefotaxime (82.4%), and colistin (78.3%) were often out of range (Fig. 2). The MIC deviations of ASTar from the reference method are listed in Table 4.

The sub-analysis of the samples from study part one ($n = 51$) yielded comparable results for CA (95.7%, $P = 1.00$; Table S9) and EA (90.3%, $P = 0.78$; Table S10).

Standard procedure compared to reference method

Overall, 782 measurements of MICs were carried out with VITEK 2 AST in both study parts. Of these, seven measurements were in the ATU of the VITEK 2 (piperacillin/tazobactam $n = 3$; ciprofloxacin $n = 4$) and eight in the ATU of the reference method (piperacillin/tazobactam $n = 2$; ciprofloxacin $n = 6$). In the end, 771 MIC measurements were available for CA and 782 measurements for EA assessment.

The overall CA was 95.3%, with 3.1% mE, 1.2% ME, and 2.9% VME (Table 2). Thus, the overall performance and error rate were within the limits required by the CLSI. However, the FDA limit for VME was exceeded. Minor errors occurred predominately with ceftazidime (11.1%), meropenem (10.7%), and cefuroxime (5.9%), whereas MEs were especially observed with tigecycline (5.1%) and ampicillin/sulbactam (3.9%). VME occurred with piperacillin/tazobactam (9.1%), trimethoprim/sulfamethoxazole (5.0%), ceftazidime (4.0%), ampicillin/sulbactam (3.9%), and cefotaxime (3.6%).

The overall EA was 95.5% so the CLSI and FDA requirements were met. Cefuroxime (EA 88.2%) did not meet the CLSI/FDA requirements (Table 3).

Comparison of ASTar with standard procedure

The percentage of CA and categorical errors was nearly identical between the two methods, and the minimal differences were statistically not significant. In contrast, the difference in EA was significant ($P < 0.001$) (Table 5).

Time-to-result (study part one)

The results of this section are depicted in Fig. 4. The mean (±SD) time between BC sampling and arrival in the laboratory was 2 h 25 min (±1 h 38 min). BCs with Gram-negative rods were reported positive by the BACTEC FX BC system after an additional 11 h 28 min (±3 h 38 min). However, immediate further processing only occurred in 14 out of 50 BCs (28%), as the remaining BCs became positive outside of the laboratory's working hours (weekdays 8 a.m. to 6 p.m.; Saturdays 8 a.m. to 4 p.m.; Sundays 9 a.m. to 4 p.m.). The mean time from BC positivity to laboratory opening was 7 h 3 min (±4 h 16 min, range 2 min to 14 h 49 min) and from laboratory opening to Gram-staining result was 1 h 16 min (±47 min). The mean time from Gram-staining result to the completion of

TABLE 3 Overall essential agreement[g]

Antibiotic	Essential agreement		"target" agreement		"range" agreement		"out-of-range" agreement	
	ASTar	VITEK 2	ASTar	VITEK 2	ASTar	VITEK 2	ASTar	VITEK 2
Ampicillin[e] (number of agreements/number of tested isolates) (%)	37/40 (92.5)	39/41 (95.1)	26/40 (65.0)	32/41 (78.1)	11/40 (27.5)	7/41 (17.1)	3/40 (7.5)	2/41 (4.9)
Piperacillin/tazobactam[b] (number of agreements/number of tested isolates) (%)	62/74 (83.8)[b]	69/75 (92.0)	26/74 (35.1)	63/75 (84.0)	36/74 (48.7)	6/75 (8.0)	12/74 (16.2)	6/75 (8.0)
Cefuroxime (number of agreements/number of tested isolates) (%)	45/51 (88.2)[b]	45/51 (88.2)[a]	30/51 (58.8)	31/51 (60.8)	15/51 (29.4)	14/51 (27.5)	6/51 (11.8)	6/51 (11.8)
Cefotaxime (number of agreements/number of tested isolates) (%)	56/68 (82.4)[b]	66/68 (97.1)	51/68 (75.0)	61/68 (89.7)	5/68 (7.3)	5/68 (7.4)	12/68 (17.7)	2/68 (2.9)
Ceftazidime (number of agreements/number of tested isolates) (%)	64/72 (88.9)	67/72 (93.1)	51/72 (70.8)	57/72 (79.2)	13/72 (18.1)	10/72 (13.9)	8/72 (11.1)	5/72 (6.9)
Cefepime[c] (number of agreements/number of tested isolates) (%)	7/7 (100.0)	7/7 (100.0)	5/7 (71.4)	5/7 (71.4)	2/7 (28.6)	2/7 (28.6)	0/7 (0.0)	0/7 (0.0)
Ceftazidime/avibactam[cd] (number of agreements/number of tested isolates) (%)	4/5 (80.0)[b]	n/a	3/5 (60.0)	n/a	1/5 (20.0)	n/a	1/5 (20.0)	n/a
Meropenem (number of agreements/number of tested isolates) (%)	69/75 (92.0)	69/75 (92.0)	65/75 (86.7)	64/75 (85.3)	4/75 (5.3)	5/75 (6.7)	6/75 (8.0)	6/75 (8.0)
Gentamicin (number of agreements/number of tested isolates) (%)	69/71 (97.2)	70/71 (98.6)	30/71 (42.3)	68/71 (95.8)	39/71 (54.9)	2/71 (2.8)	2/71 (2.8)	1/71 (1.4)
Tobramycin (number of agreements/number of tested isolates) (%)	71/78 (92.3)	77/78 (98.7)	44/78 (56.4)	71/78 (91.0)	27/78 (35.9)	6/78 (7.7)	6/78 (7.7)	1/78 (1.3)
Ciprofloxacin (number of agreements/number of tested isolates) (%)	75/78 (96.2)	77/78 (98.7)	55/78 (70.5)	73/78 (93.6)	20/78 (25.6)	4/78 (5.1)	3/78 (3.9)	1/78 (1.3)
Trimethoprim/sulfamethoxazole (number of agreements/number of tested isolates) (%)	60/65 (92.3)	64/65 (98.5)	58/65 (89.2)	63/65 (96.9)	2/65 (3.1)	1/65 (1.5)	5/65 (7.7)	1/65 (1.5)
Tigecycline (number of agreements/number of tested isolates) (%)	37/39 (97.4)	38/39 (97.4)	36 (92.3)	37/39 (94.9)	2/39 (5.1)	1/39 (2.6)	1/39 (2.6)	1/39 (2.6)
Colistin[e] (number of agreements/number of tested isolates) (%)	36/46 (78.3)[b]	n/a	23/46 (50.0)	n/a	13/46 (28.3)	n/a	10/46 (21.7)	n/a
Aztreonam[c] (number of agreements/number of tested isolates) (%)	6/7 (85.7)[b]	5/5 (100.0)	4/7 (57.1)	2/5 (40.0)	2/7 (28.6)	3/5 (60.0)	1/7 (14.3)	0/7 (0.0)
Ertapenem[d] (number of agreements/number of tested isolates) (%)	49/50 (98.0)	n/a	48/50 (96.0)	n/a	1/50 (2.0)	n/a	1/50 (2.0)	n/a
Ceftolozane/tazobactam[c] (number of agreements/number of tested isolates) (%)	5/5 (100.0)[b]	5/5 (100.0)	3/5 (60.0)	4/5 (80.0)	2/5 (40.0)	1/5 (20.0)	0/5 (0.0)	0/5 (0.0)
Total (number of agreements/number of tested isolates) (%)	754/831 (90.7)	747/782 (95.5)	558/831 (67.2)	671/782 (85.8)	196/831 (23.6)	76/782 (9.7)	77/831 (9.3)	35/782 (4.5)

[a]One measurement error in ASTar.
[b]ASTar measured amoxicillin/clavulanic acid instead of ampicillin/sulbactam.
[c]Less than 10 strains analyzed.
[d]Not included in the VITEK 2 AST card.
[e]CLSI and FDA requirements not fulfilled.
[f]FDA requirements not fulfilled; n/a, not applicable.
[g]Essential agreement was defined as "target" (no MIC deviation), "range" (MIC deviation of no more than one $\log^2$ dilution), or "out-of-range" (MIC deviation of more than one $\log^2$ dilution). Both target and range fulfilled the essential agreement (gray background).

TABLE 4 MIC deviation of ASTar from reference method (broth microdilution)[b]

MIC deviation	≤ −3	2	1	0	+1	+2	≥ +3
Ampicillin	0/40	1/40	0/40	26/40	11/40	1/40	1/40
(number of deviations/number of tested isolates) (%)	(0.0)	(2.5)	(0.0)	(65.0)	(27.5)	(2.5)	(2.5)
Piperacillin/tazobactam	3/74	6/74	9/74	26/74	27/74	2/74	1/74
(number of deviations/number of tested isolates) (%)	(4.1)	(8.1)	(12.2)	(35.1)	(36.5)	(2.7)	(1.4)
Cefuroxime	0/51	0/51	6/51	30/51	9/51	4/51	2/51
(number of deviations/number of tested isolates) (%)	(0.0)	(0.0)	(11.8)	(58.8)	(17.7)	(7.8)	(3.9)
Cefotaxime	9/68	2/68	4/68	51/68	1/68	0/68	1/68
(number of deviations/number of tested isolates) (%)	(13.2)	(2.9)	(5.9)	(75.0)	(1.5)	(0.0)	(1.5)
Ceftazidime	2/72	5/72	5/72	51/72	8/72	1/72	0/72
(number of deviations/number of tested isolates) (%)	(2.8)	(6.9)	(6.9)	(70.8)	(11.1)	(1.4)	(0.0)
Cefepime[a]	0/7	0/7	0/7	5/7	2/7	0/7	0/7
(number of deviations/number of tested isolates) (%)	(0.0)	(0.0)	(0.0)	(71.4)	(28.6)	(0.0)	(0.0)
Ceftazidime/avibactam[a]	0/5	1/5	0/5	3/5	1/5	0/5	0/5
(number of deviations/number of tested isolates) (%)	(0.0)	(20.0)	(0.0)	(60.0)	(20.0)	(0.0)	(0.0)
Meropenem	2/75	2/75	1/75	65/75	3/75	2/75	0/75
(number of deviations/number of tested isolates) (%)	(2.7)	(2.7)	(1.3)	(86.7)	(4.0)	(2.7)	(0.0)
Gentamicin	0/71	0/71	2/71	30/71	37/71	2/71	0/71
(number of deviations/number of tested isolates) (%)	(0.0)	(0.0)	(2.8)	(42.3)	(52.1)	(2.8)	(0.0)
Tobramycin	0/78	4/78	21/78	44/78	7/78	2/78	0/78
(number of deviations/number of tested isolates) (%)	(0.0)	(5.1)	(26.9)	(56.4)	(9.0)	(2.6)	(0.0)
Ciprofloxacin	0/78	1/78	16/78	55/78	4/78	1/78	1/78
(number of deviations/number of tested isolates) (%)	(0.0)	(1.3)	(20.5)	(70.5)	(5.1)	(1.3)	(1.3)
Trimethoprim/sulfamethoxazole	4/65	0/65	1/65	58/65	1/65	0/65	1/65
(number of deviations/number of tested isolates) (%)	(6.2)	(0.0)	(1.5)	(89.2)	(1.5)	(0.0)	(1.5)
Tigecyclin	0/39	1/39	0/39	36/39	2/39	0/39	0/39
(number of deviations/number of tested isolates) (%)	(0.0)	(2.6)	(0.0)	(92.3)	(5.1)	(0.0)	(0.0)
Colistin	0/46	1/46	2/46	23/46	11/46	5/46	4/46
(number of deviations/number of tested isolates) (%)	(0.0)	(2.2)	(4.4)	(50.0)	(23.9)	(10.9)	(8.7)
Aztreonam[a]	0/7	0/7	0/7	4/7	2/7	1/7	0/7
(number of deviations/number of tested isolates) (%)	(0.0)	(0.0)	(0.0)	(57.1)	(28.6)	(14.3)	(0.0)
Ertapenem	1/50	0/50	0/50	48/50	1/50	0/50	0/50
(number of deviations/number of tested isolates) (%)	(2.0)	(0.0)	(0.0)	(96.0)	(2.0)	(0.0)	(0.0)
Ceftolozane/tazobactam[a]	0/5	0/5	1/5	3/5	1/5	0/5	0/5
(number of deviations/number of tested isolates) (%)	(0.0)	(0.0)	(20.0)	(60.0)	(20.0)	(0.0)	(0.0)
Total	21/831	24/831	68/831	558/831	128/831	21/831	11/831
(number of deviations/number of tested isolates) (%)	(2.5)	(2.9)	(8.2)	(67.2)	(15.4)	(2.5)	(1.3)

[a]Less than 10 strains analyzed.
[b]Deviations between -1 and +1 MIC dilution (gray background) are defined as fulfilled essential agreement.

ASTar and the standard procedure was 6 h 47 min (±49 min) and 14 h 13 min (±3 h 29 min), respectively. The difference (7 h 26 min) was statistically significant ($P < 0.001$). Altogether, the mean time from BC sampling to the completion of ASTar and standard procedure was 28 h 59 min and 36 h 25 min, respectively. Even though ASTar and VITEK 2 were performed only with BCs that turned positive until 11 a.m., VITEK 2 measurements, unlike the ASTar determinations, were finished without exception outside the regular working hours and reporting of the results was delayed until the next morning, i.e., after an additional mean 7 h 53 min (±3 h 25 min). Therefore, the mean total timespan from BC sampling to AST report for ASTar and standard procedure was 28 h 59 min and 44 h 18 min, respectively.

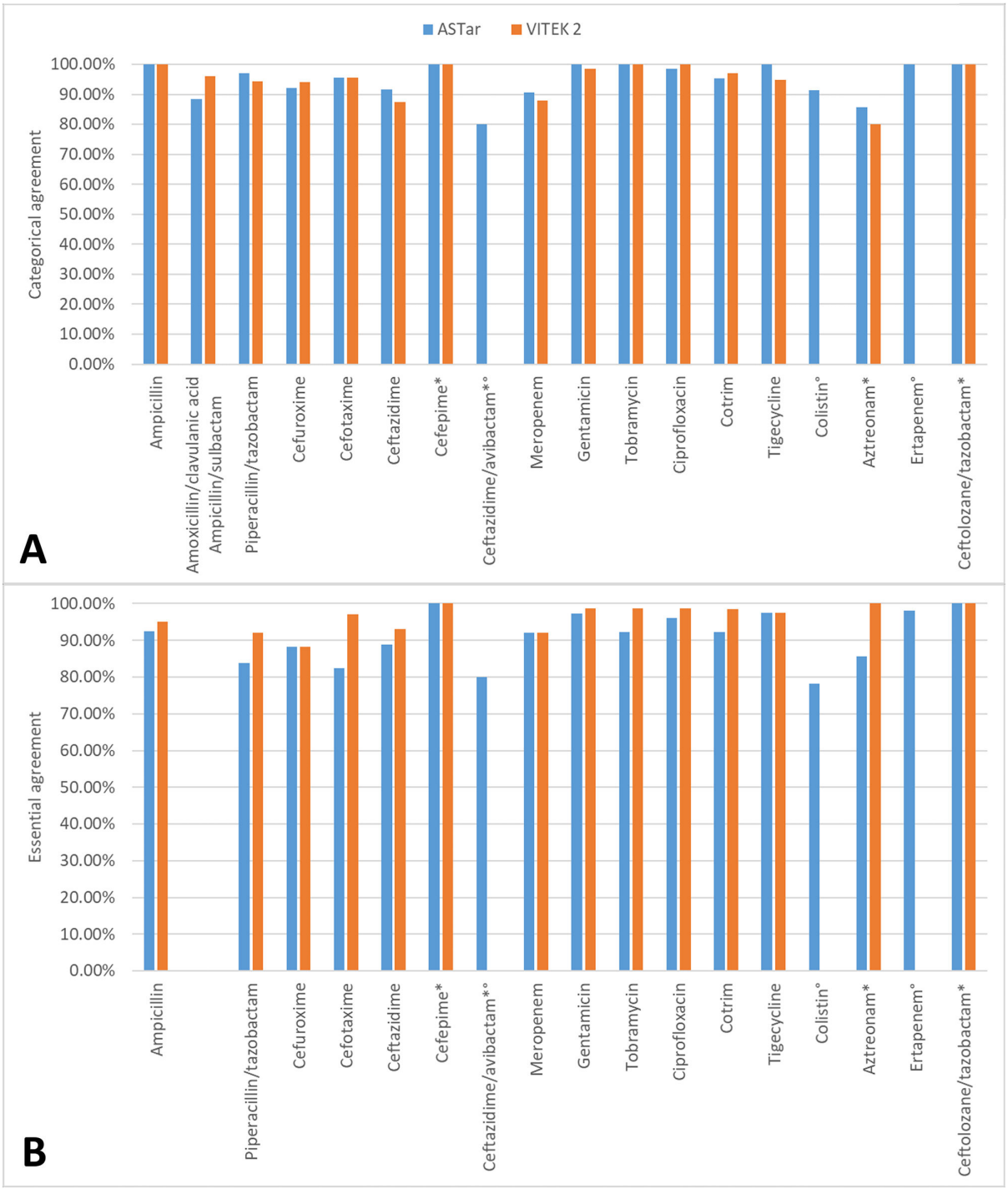

FIG 2 Categorical and essential agreement of ASTar and standard procedure. * indicates antibiotic agents with less than 10 measurements and ^{O} indicates antibiotic agents are not part of the VITEK 2 AST panel. Cotrim, trimethoprim/sulfamethoxazole.

Clinical impact in study part one

Two patients were excluded from this analysis, because one did not receive antibiotic therapy due to a palliative care situation and in the other optimal treatment could not be determined because the reference method yielded results within the ATU. In all other patients, anti-infective therapy after considering the results of Gram staining consisted of piperacillin/tazobactam in 33 cases (68.8%), meropenem in 9 cases (18.8%), ampicillin/

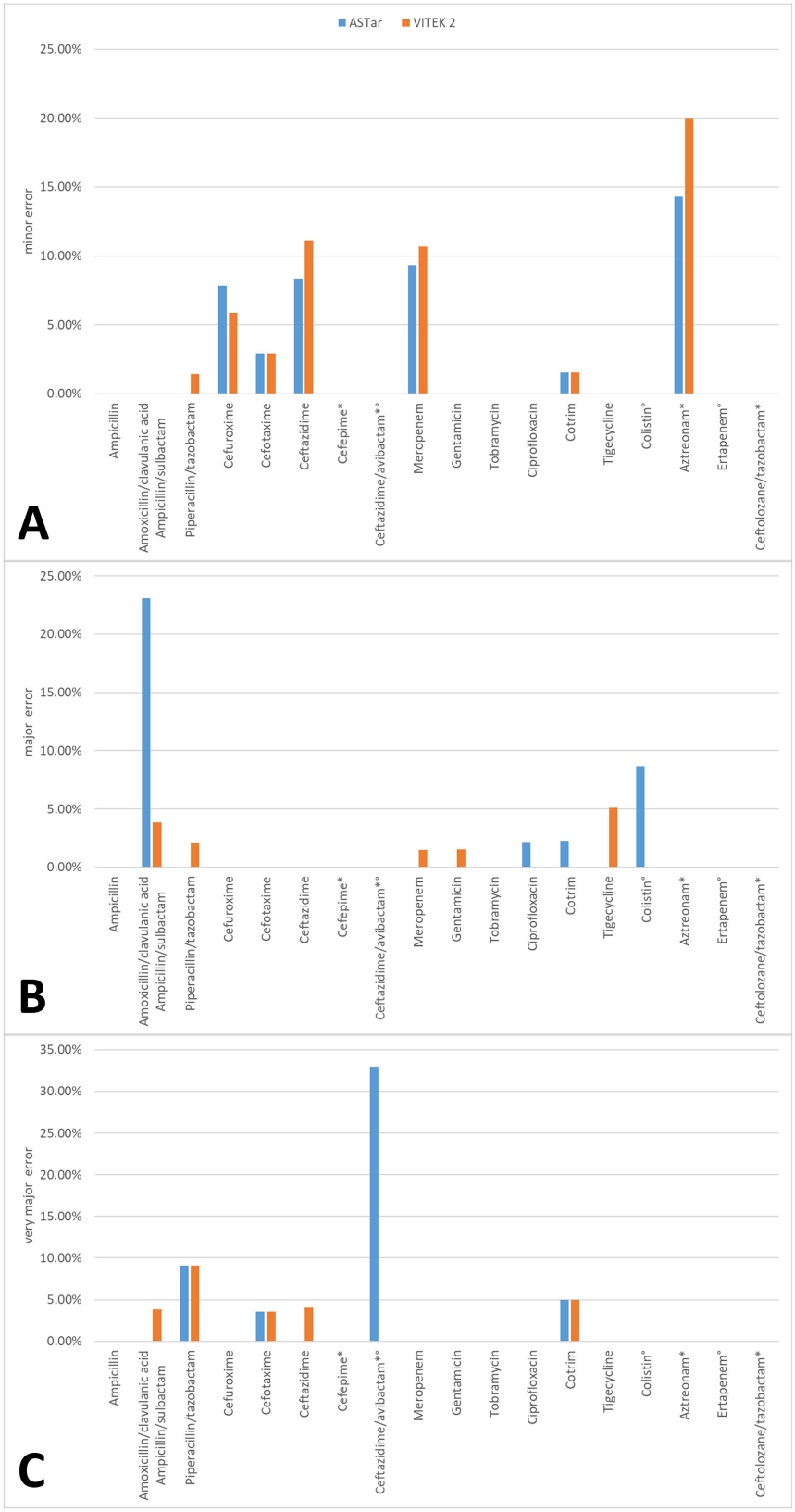

FIG 3 Categorical error rates of ASTar and standard procedure. * indicates antibiotic agents with less than 10 measurements and ^{O} indicates antibiotic agents are not part of the VITEK 2 AST panel. Cotrim, trimethoprim/sulfamethoxazole.

sulbactam and third generation cephalosporins in 2 cases each (4.1%), and ciprofloxacin and cotrimoxazole in 1 case each (2.1%).

The initial antibiotic therapy covered the pathogen detected in the BC in 43 (89.6%) of 48 patients according to the results of broth microdilution. In five patients (10.4%), an escalation of the therapy was necessary [switch from ampicillin/sulbactam to piperacillin/tazobactam (n = 2) and from piperacillin/tazobactam to meropenem (n = 3)]. In 20 patients (41.7%), a de-escalation of the antibiotic therapy would have been possible (piperacillin/tazobactam to ampicillin/sulbactam, n = 14; meropenem to ampicillin/sulbactam, n = 4; meropenem to piperacillin/tazobactam, n = 1; cefotaxime to ampicillin/sulbactam, n = 1). ASTar results for the recommended antibiotic treatment were available from 44 patients. Four patients were excluded, because the ASTar result was in the ATU (n = 2) and the ASTar system does not provide results for meropenem in *Acinetobacter baumannii* with the ASTar BC G- Kit software version used in this study (n = 2). With the help of ASTar, all patients who required an escalation could be correctly identified. However, ASTar failed to identify 5 (25%) of 20 patients for whom de-escalation was possible. In two (4.7%) patients, the ASTar result would have led to unnecessary escalation of therapy. Overall, the results obtained with ASTar led to the correct recommendation of antibiotic therapy in 84.1% of patients. For comparison, standard procedure led to the correct recommendation in 44 (95.7%) of 46 patients. One possible de-escalation from piperacillin/tazobactam to ampicillin/sulbactam was not recognized and one escalation of therapy was recommended unnecessarily. There was a trend towards an overall better prediction of the optimal antibiotic therapy for MIC measurements obtained with VITEK 2 compared to ASTar (P = 0.087).

Heteroresistance

One *Enterobacter cloacae* complex isolate showed delayed heteroresistance, i.e., after 18 h of incubation, a resistant subclone appeared in the inhibition zone of the disk diffusion test with piperacillin/tazobactam and the third generation cephalosporins (Fig. 5). The observed resistance was confirmed by broth microdilution (18 h incubation). However, due to the short incubation time in ASTar and VITEK 2 AST, these systems failed to detect this heteroresistance. A disk diffusion testing was performed on this isolate because it was also included in another study.

DISCUSSION

We conducted a prospective study to evaluate the performance of the ASTar system in routine diagnostics. The ASTar proved to be robust. Only one sample (1.3%) could not be processed due to a system failure. The pathogens included in the ASTar BC G-kit were well-selected by the manufacturer because they covered 94% of the bacterial species encountered during routine testing.

In terms of CA, the ASTar system performed well (95.6%). The rate of MEs was within the CLSI and FDA limits, and the rate of VMEs (2.4%) was within the CLSI limits. A common ME occurred with amoxicillin/clavulanic acid. In five cases, *Escherichia coli* isolates were rated as susceptible to ampicillin and piperacillin/tazobactam but resistant to amoxicillin/clavulanic acid. For *Enterobacterales*, this result is not plausible and prevented a possible de-escalation in 5 of 20 cases. Problems of the ASTar with amoxicillin/clavulanic acid CA were also noted in the only study on the performance of ASTar conducted to date by Göransson et al. together with the manufacturer (9). VME occurred with piperacillin/tazobactam, cephalosporins, and trimethoprim/sulfamethoxazole in Enterobacteriaceae and *Pseudomonas* species. In particular, the VME rate of 9.1% with piperacillin/tazobactam, the most commonly used empirical antibiotic in our setting, is of great concern and could have prevented a switch to an effective therapy. However, we analyzed only 22 piperacillin-/tazobactam-resistant isolates, so the VME rate of 9.1% corresponds to only two VME errors. Larger numbers of resistant isolates need to be assessed in future research to better characterize the VME rate of key antibiotics. Until then, however, we would recommend that ASTar results be verified under standardized

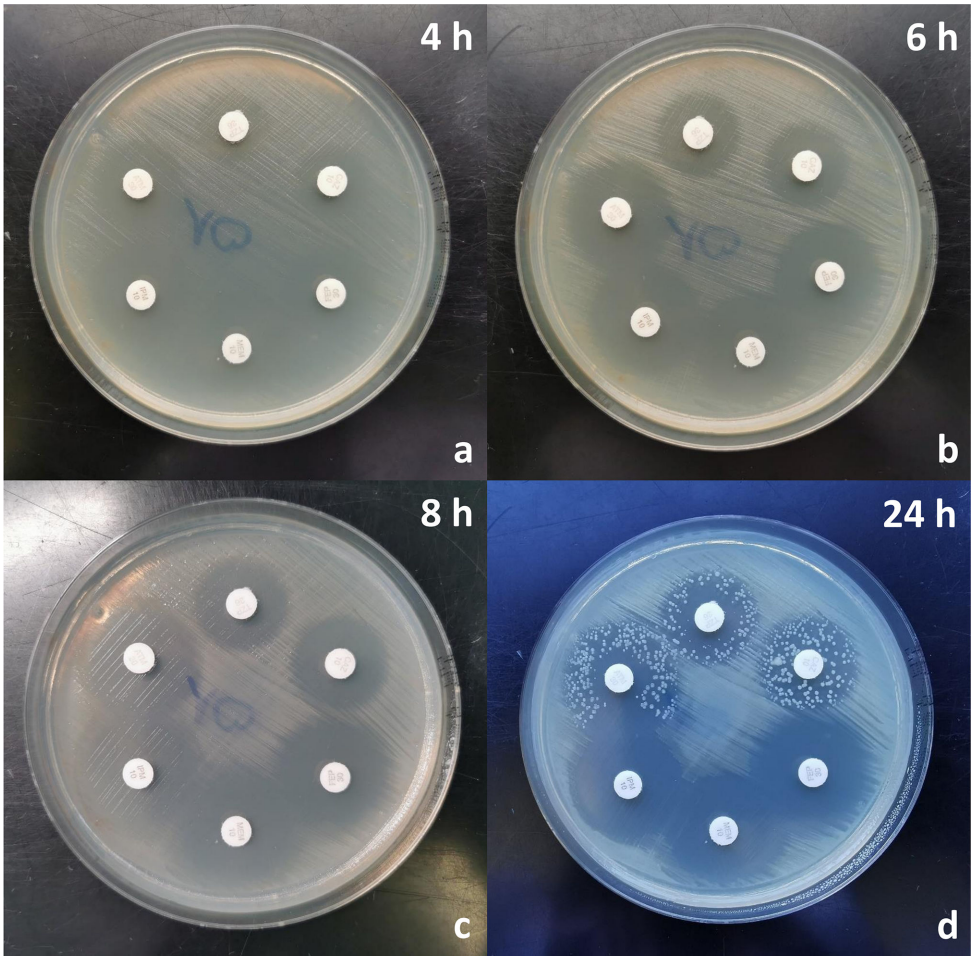

FIG 5 Disk diffusion testing of a heteroresistant isolate of *Enterobacter cloacae* complex at different time points. (a) 4-h incubation, (b) 6-h incubation, (c) 8-h incubation, and (d) 24-h incubation. Antibiotics (clockwise from 12 o'clock): piperacillin/tazobactam, ceftazidime, cefepime, meropenem, imipenem, aztreonam. Piperacillin/tazobactam, ceftazidime, and aztreonam show growth within the inhibition zone only after 24-h incubation.

conditions. Overall, the ASTar results obtained were comparable to the established standard procedure. The addition of isolates from our strain collection, which were mainly MDRGN bacteria, did not significantly change the results compared to the prospectively tested routine samples. Therefore, reliable categorical AST results in regions with higher resistance rates can also be expected.

The EA of the ASTar (90.7%) met the FDA and CLSI requirements. Nevertheless, the standard procedure was superior to ASTar with significantly less MICs out of range (4.5% versus 9.3%, $P < 0.001$). However, ASTar measures a wider range of MICs in comparison to VITEK 2 and this could be due to the disadvantage of ASTar as EA deviations are more likely to occur. Göransson et al. found a similar CA (97.6%) but significantly better EA (95.8%) using an ISO 20776-2:2007-based analytical approach (9). Overall, we believe that CA is for rapid AST systems of much higher importance than EA because a deviation in EA without a categorical error will not result in an ineffective antibiotic therapy.

Loading and operation of ASTar were simple and did not require special skills. The hands-on-time was minimal (approximately 2 min). According to the manufacturer, a test run takes 6 h. However, this only applies to the first sample as simultaneously added further samples are sequentially analyzed at intervals of 10 min each, thus extending their time-to-result (nearly 8 h for the last sample with a full load of 12 samples). In our laboratory, which is responsible for a 1,400-bed hospital and processes around 22,000 BC pairs per year, no more than three samples had to be loaded into the ASTar

TABLE 5 Statistical comparison of categorical and essential agreement between ASTar and standard procedure[a]

	ASTar	VITEK 2	*P*-value
No. of categorical agreements/total no. of measurements (%)	832/870 (95.6)	735/771 (95.3)	1.000
No. of minor errors/total no. of measurements (%)	21/870 (2.4)	24/771 (3.1)	0.449
No. of major errors/total no. of susceptible measurements (%)	12/592 (2.0)	6/500 (1.2)	0.345
No. of very major errors/total no. of resistant measurements (%)	5/210 (2.4)	6/207 (2.9)	1.000
No. of measurements in the ATU/total no. of measurements with ATU (%)	6/153 (3.9)	7/153 (4.6)	1.000
No. of essential agreement/total no. of measurements (%)	754/831 (90.7)	747/782 (95.5)	<0.001
No. of "target" measurements /total no. of measurements (%)	558/831 (67.2)	671/782 (85.8)	<0.001
No. of "range" measurements /total no. of measurements (%)	196/831 (23.6)	76/782 (9.7)	<0.001
No. of "out-of-range" measurements /total no. of measurements (%)	77/831 (9.3)	35/782 (4.5)	<0.001

[a]No., number; ATU, area of technical uncertainty.

simultaneously and the time delay mentioned above was negligible. In real life, the average time from completed Gram staining to the ASTAR results was 7 h 20 min, which is noticeably lower than the average 14 h 30 min with standard procedure. However, three additional time spans have to be added for a correct estimation of the overall time required until a rapid AST result reaches the respective clinical department. First, the time from BC sampling to BC positivity, second, the time from BC positivity until the BC is processed further, and third, the time from the completion of the AST until the results are passed on to the attending physician. For a correct calculation of these time periods, the opening hours of the laboratory must be taken into account. The first two time periods were similar for both diagnostic approaches. However, the third time period was significantly shorter for ASTar because all ASTs from BCs that were positive until 11 a.m. were completed on the same day during regular working hours. In contrast, AST results of the standard procedure were consistently available only after 6 p.m. so that the results were transmitted to the clinician on the next day. Consequently, the mean time span from BC sampling to the availability of the AST results for the attending physician was 28 h 59 min for ASTar and 44 h 18 min for the standard procedure. Although this difference was highly significant, it is currently unclear, whether the adjustment of antibiotic therapy after nearly 29 h compared to 44 h will have a substantial impact on parameters like mortality. A recent retrospective study of patients who developed sepsis in the emergency department or in the general ward showed that there was no difference in the risk of death after 6 h, regardless of whether antibiotic therapy was effective or not. However, after 12 h, the risk of death increased in the group with ineffective therapy compared to the group with effective therapy, and this difference became greater over time (13). Similarly, in the highly cited study by Kumar et al., only 10% of sepsis patients was still alive after 24–36 h if they had received an ineffective empiric antibiotic therapy (1). Accordingly, in terms of mortality, 10% of sepsis patients might benefit from an acceleration of AST by ASTar. However, based on our study data, only 10% of patients did not receive adequate antibiotic therapy. Thus, only 1% of all patients studied would profit from ASTar, assuming patients with BSI to be in septic shock, as the severity of infection was not assessed by us. This number, however, could increase if resistance rates were higher because the rate of effective empiric antibiotic therapy would be lower (14). For this reason, systems like ASTar will be most effective in regions with a high prevalence of MDRGN bacteria.

Even though the reduction in time until an AST is available may have a limited impact on the mortality in areas with low to moderate prevalence of MDRGN bacteria, it is likely to have a positive impact on morbidity and the patients' health in general. In this context, earlier de-escalation can help to prevent the emergence of antibiotic resistance (15) and to limit therapy costs (16). ASTar enabled rapid de-escalation in 15 (35%) patients with 5 of them receiving carbapenem therapy. Compared to standard procedure, however, only two single doses of carbapenem per patient could be saved, provided the attending physicians actually carried out the recommended de-escalation.

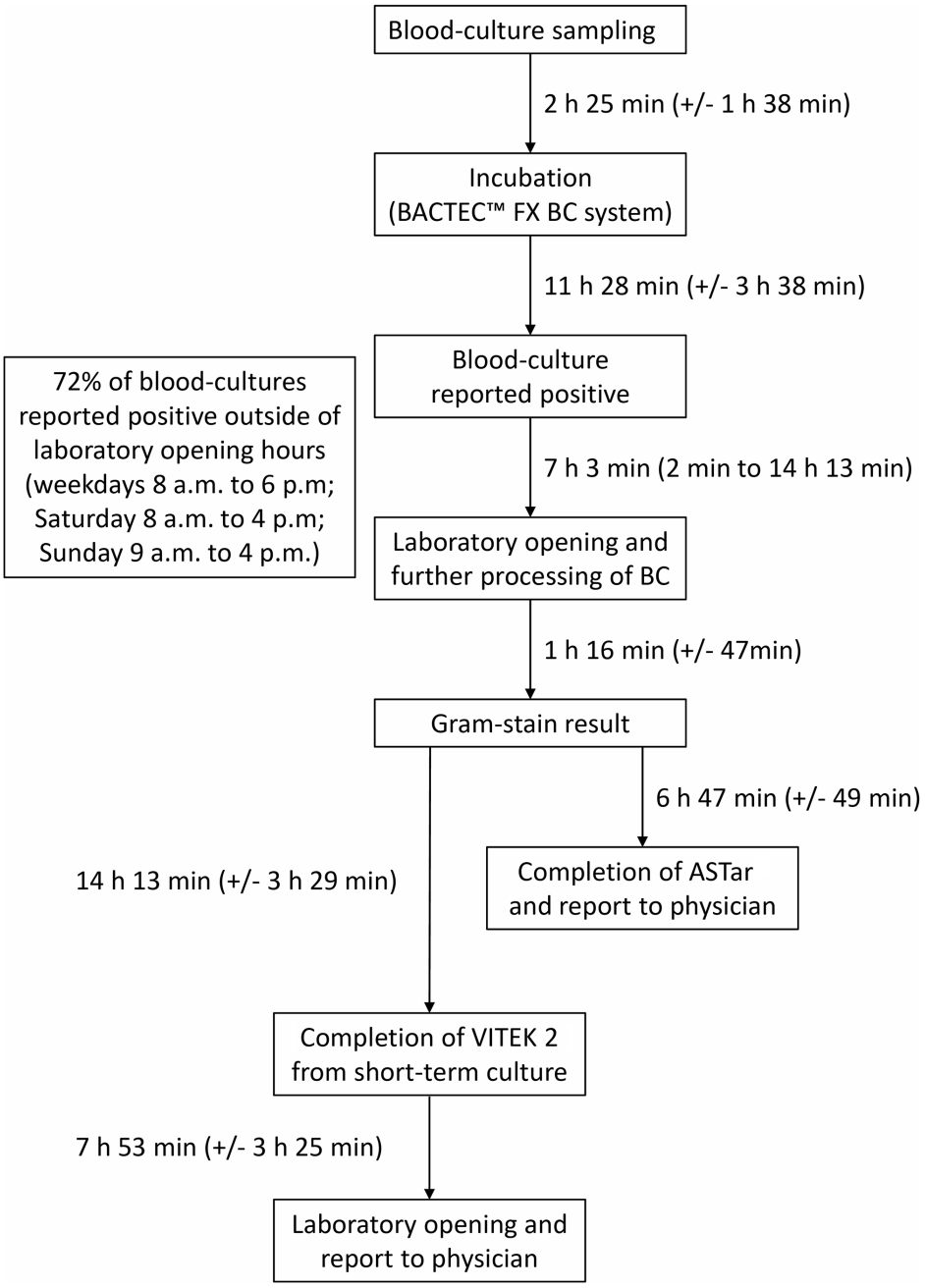

FIG 4 Time periods between BC sampling and transmission of the antibiogram to the physician.

An inherent problem of all rapid AST systems was seen with an isolate of *Enterobacter cloacae* complex. This isolate showed delayed heteroresistance, i.e., the resistance against piperacillin/tazobactam and third-generation cephalosporins was only detectable after 18 h of incubation. All rapid AST systems, ASTar as well as the standard procedure, failed to detect this heteroresistance. One way to solve this problem is to retest all isolates with a standard AST method and incubate for 18 hr.

The novel technique used by ASTar comes at a cost. Laboratories will have to invest over $300,000 for the ASTar instrument and over $100 per test. Quite an investment compared to less than $5 for the standard method used in our laboratory.

The main limitation of our study was the small number of BCs tested. However, our pathogen spectrum was very diverse, and it seems unlikely that ASTar will fail to meet

CLSI requirements if a higher number of isolates were tested. The rather low to moderate number of MDRGN bacteria, reflecting the prevalence in Germany, was compensated by including additional strains from our archive. Therefore, we are convinced that our results are also applicable to countries with a high prevalence of antibiotic resistance.

In conclusion, the ASTar system provided reliable AST results and delivered an antibiogram from the majority of positive BCs on the same day during working hours. The use of ASTar significantly shortened the time from BC sampling to the delivery of the antibiogram to the attending physician when compared to the VITEK 2 system from 5 h short-term cultures. However, the percentage of patients that will benefit from ASTar in a setting with low to moderate antibiotic resistance is limited, raising the question whether ASTar justifies the significant additional costs. The cost-benefit ratio of ASTar will certainly be different in areas with high rates of antimicrobial resistance.

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Thermo-Fisher-Scientific Inc. provided the ASTar system free of charge for the duration of the study and discounted prices for the test reagents.

All authors declare no conflict of interest in connection with the study.

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DATA AVAILABILITY

The original data sets of this study are available from the corresponding author (J.H.) upon reasonable request.

ETHICS APPROVAL

This study was approved by the Ethics Committee of the Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg (21-488-Bm). The need for informed consent was waived.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplementary Tables 1-10 and Supplementary Figures 1-3 (JCM00549-23-S0001.pdf). Supplementary Tables 1-10 and Supplementary Figures 1-3

REFERENCES

1. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M. 2006. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 34:1589–1596. https://doi.org/10.1097/01.CCM.0000217961.75225.E9
2. Kumar A, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE, Dodek P, Wood G, Kumar A, Simon D, Peters C, Ahsan M, Chateau D, Cooperative antimicrobial therapy of Septic shock database research group. 2009. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. Chest 136:1237–1248. https://doi.org/10.1378/chest.09-0087
3. MacBrayne CE, Williams MC, Prinzi A, Pearce K, Lamb D, Parker SK. 2021. Time to blood culture positivity by pathogen and primary service. Hosp Pediatr 11:953–961. https://doi.org/10.1542/hpeds.2021-005873
4. Vasala A, Hytönen VP, Laitinen OH. 2020. Modern tools for rapid diagnostics of antimicrobial resistance. Front Cell Infect Microbiol 10:308. https://doi.org/10.3389/fcimb.2020.00308
5. Åkerlund A, Jonasson E, Matuschek E, Serrander L, Sundqvist M, Kahlmeter G, RAST Study Group. 2020. EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 european laboratories. J Antimicrob Chemother 75:3230–3238. https://doi.org/10.1093/jac/dkaa333
6. Zalas-Więcek P, Bogiel T, Gospodarek-Komkowska E. 2022. The accelerate pheno system-A new tool in microbiological diagnostics of bloodstream infections: a pilot study from Poland. Pathogens 11:1415. https://doi.org/10.3390/pathogens11121415
7. Tibbetts R, George S, Burwell R, Rajeev L, Rhodes PA, Singh P, Samuel L. 2022. Performance of the reveal rapid antibiotic susceptibility testing system on gram-negative blood cultures at a large urban hospital. J Clin Microbiol 60:e0009822. https://doi.org/10.1128/jcm.00098-22
8. Wong AYW, Johnsson ATA, Özenci V. 2022. Performance of dRAST on prospective clinical blood culture samples in a simulated clinical setting and on multidrug-resistant bacteria. Microbiol Spectr 10:e0210721. https://doi.org/10.1128/spectrum.02107-21
9. Göransson J, Sundqvist M, Ghaderi E, Lisby JG, Molin Y, Eriksson E, Carlsson S, Cederlöf A, Ellis L, Melin J. 2023. Performance of a system for rapid phenotypic antimicrobial susceptibility testing of gram-negative bacteria directly from positive blood culture bottles. J Clin Microbiol 61:e0152522. https://doi.org/10.1128/jcm.01525-22
10. The European Committee on antimicrobial susceptibility testing. 2022. Quality control criteria for the implementation of the RAST Method. Version 5.0
11. Jorgensen JH, Ferraro MJ. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis 49:1749–1755. https://doi.org/10.1086/647952
12. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, Koeth L, Sei K, CLSI Methods development and standardization working group of the subcommittee on antimicrobial susceptibility tests. 2018. J Clin Microbiol 56:e01934-17. https://doi.org/10.1128/JCM.01934-17
13. Van Heuverswyn J, Valik JK, Desirée van der Werff S, Hedberg P, Giske C, Nauclér P. 2023. Association between time to appropriate antimicrobial treatment and 30-day mortality in patients with bloodstream infections: a retrospective cohort study. Clin Infect Dis 76:469–478. https://doi.org/10.1093/cid/ciac727
14. Peralta G, Sánchez MB, Garrido JC, De Benito I, Cano ME, Martínez-Martínez L, Roiz MP. 2007. Impact of antibiotic resistance and of adequate empirical antibiotic treatment in the prognosis of patients with *Escherichia coli* bacteraemia. J Antimicrob Chemother 60:855–863. https://doi.org/10.1093/jac/dkm279
15. de With K WK, Kern WK, StraußR, KrammeE, FriedrichsA, HolzmannT, GeissHK, IsnerC, FellhauerM, von Ameln-MayerhoferA, Abele-HornM, HäckerG, WalgerP, DejaM, VehreschildJJ, KatherA, Friese E, PorscheU, JanataO, KrauseR, Wechsler-FördösA. 2018. S3- Leitlinie: Strategien zur Sicherung rationaler Antibiotika-Anwendung im Krankenhaus. AWMF-Registernummer 092/001 – update 2018
16. Tumbarello M, Spanu T, Di Bidino R, Marchetti M, Ruggeri M, Trecarichi EM, De Pascale G, Proli EM, Cauda R, Cicchetti A, Fadda G. 2010. Costs of bloodstream infections caused by *Escherichia coli* and influence of extended-spectrum-β-Lactamase production and inadequate initial antibiotic therapy. Antimicrob Agents Chemother 54:4085–4091. https://doi.org/10.1128/AAC.00143-10