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ORIGINAL ARTICLE

Rapid antibiotic susceptibility testing in blood culture diagnostics performed by direct inoculation using the VITEK®-2 and BD Phoenix™ platforms

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Abstract

Early availability of microbiological results can improve treatment decisions of patients suffering from bloodstream infections. Direct inoculation of automated susceptibility testing (AST) platforms is an approach to shorten time-to-result in blood culture diagnostics. We performed a comparative evaluation of the two commercial AST systems VITEK®-2 and BD Phoenix™ for the direct inoculation with blood culture samples. Furthermore, two different methods of sample preparation were compared in this study. Positive blood cultures were prepared for direct inoculation by use of serum separator tubes and twofold centrifugation. AST was performed with the VITEK®-2 and the BD Phoenix™ system by the standard method according to the manufacturer's recommendations using subcultures on solid media and by direct inoculation of blood culture samples. A hundred clinical samples from blood cultures were included in this study. Rapid AST by direct inoculation showed inter-test agreement rates ranging from 92.45 to 97.7%. Comparing both AST platforms, the VITEK®-2 system demonstrated a higher test accuracy for direct inoculation. No relevant difference was observed for the two different sample preparation methods. Direct inoculation is an easy and inexpensive approach to obtain early full panel phenotypic AST results in blood culture diagnostics. Sample preparation is sufficiently performed by a simple centrifugation method. Both commercial platforms, the VITEK®-2 and the BD Phoenix™, have proven suitable for the use of direct inoculation.

Introduction

Worldwide, sepsis is causing a high disease burden and high mortality rates [1, 2]. Early adequate antimicrobial therapy improves the clinical outcome of patients with sepsis [3–5], but conventional diagnosis based on blood cultures is time-consuming with turnaround times of up to 72 h and more [6]. The introduction of mass spectrometry and molecular methods decreased turnaround times in sepsis diagnostics [7–9]. Today, commercially available molecular platforms yield preliminary results by detecting pathogen identity and genetic resistance markers within only a few hours [7, 8]. Nevertheless, full panel phenotypic susceptibility testing is still indispensable for a complete and reliable resistogram, especially for gram-negative bacteria [7].

In blood culture diagnostics, phenotypic susceptibility testing is usually performed with commercial platforms for automated susceptibility testing (AST). To apply these systems in blood culture diagnostics, a prior overnight subculture step is required according to the manufacturer's instructions. Inoculating the AST platform directly with aliquots from positive blood culture bottles is a method to spare out subcultures and to obtain the test results one day earlier compared to the standard procedure. Besides standard lab equipment, no additional devices are required for this approach, additional material costs are low, and hands-on-time is short [10]. Earlier studies demonstrate high concordance between the rapid direct inoculation method and the standard procedure for antibiotic susceptibility testing [11–13].

In this study, we performed a direct comparison of the VITEK®-2 (bioMérieux, France) and the BD Phoenix™ (BD, USA) platform for rapid and conventional antibiotic susceptibility testing of gram-negative bacteria from positive blood cultures. Two different methods were used to prepare

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the samples for direct inoculation: one using serum separator tubes and one using twofold centrifugation.

Material and methods

Clinical samples

This study was conducted between April and August 2018 at the Institute of Medical Microbiology at the Jena University Hospital. A total of 100 blood culture specimens that had been sent to our laboratory for routine diagnostics were included in this study. Eligible samples were aerobic blood culture flasks (Becton Dickinson, USA) that were flagged positive by the instrumented BACTEC™ blood culture system (Becton Dickinson, USA) and of which gram staining showed gram-negative, rod-shaped bacteria. No blood culture flasks of pediatric patients were included. Only one sample per patient was included in the study.

Standard method for antimicrobial susceptibility testing of positive blood cultures

Positive blood cultures were streaked onto Columbia agar containing 5% sheep blood (Oxoid, Thermo Fisher Scientific, Germany) and incubated overnight at 37 °C in an aerobic atmosphere containing 5% CO_2. The next day, bacterial pellets were picked with a loop from subcultures on solid media and further processed according to the manufacturer's instructions.

For the VITEK®-2 platform, a bacterial suspension using 0.45% sodium chloride solution (CareFusion, USA) was adjusted to an optical density of 0.5–0.63 McFarland units. The VITEK®-2 AST-N233 test card (bioMérieux, France) was inoculated with the bacterial suspension. The cards were loaded into the VITEK®-2 system and results were automatically reported by VITEK®-2 Software release 8.01.

AST with the BD Phoenix™ system was performed by first suspending a bacterial pellet in 4.5 ml of the BD ID broth (Becton Dickinson, USA) and adjusting the solution to 0.5–0.63 McFarland units. A 25 μl aliquot was subsequently pipetted in 8 ml BD AST broth (Becton Dickinson, USA) and in 4.5 ml BD AST broth. One drop of indicator solution was added to each tube. The Phoenix™ NMIC-502 panel was entirely filled with the suspensions (4.5 ml BD AST broth in the left chamber, 8 ml BD AST broth in the right chamber) and loaded into the Phoenix™ platform. Results were automatically reported by BD EpiCenter™ version 9.10.

Sample preparation for direct inoculation

Each positive blood culture sample was processed using a serum separator tube (SST) protocol and by applying a twofold centrifugation protocol (2XC) to yield a bacterial pellet for direct inoculation.

For the 2XC protocol, a 3.5 ml aliquot of the blood culture was transferred into a 15-ml centrifugation tube (Falcon Plastics, USA) and centrifuged with 160*g* for 5 min to sediment blood cells. In the next step, the supernatant was transferred into a 15-ml centrifugation tube (Falcon Plastics, USA) and centrifuged by 650*g* for 10 min. The supernatant was discarded and the bacterial pellet at the bottom was processed for AST as described above for the standard method.

The SST protocol was performed by pipetting a 3.5 ml aliquot into a 4.9-ml serum separator tube (Sarstedt, Germany) and subsequently centrifuging it with 2000*g* for 10 min. After centrifugation, blood cells were sunk into the gel layer and the bacterial layer on the gel surface was cautiously picked with a swap and processed for AST as described above for the standard method.

Confirmatory tests for divergent test results

Discrepant test results between the VITEK®-2 platform and the Phoenix™ platform that were obtained by standard AST were controlled by additional susceptibility testing with an alternative method. E-Test strips (bestbion^{dx}, Germany) were used according to the manufacturer's instructions for controlling test results of ampicillin, cefuroxime, ciprofloxacin, gentamicin, cotrimoxazole, and tigecycline. A commercial MICRONAUT-S microdilution test (MERLIN, Germany) was performed and read out manually to double check minimal inhibitory concentrations (MIC) of ceftazidime, piperacillin, and piperacillin-tazobactam.

Data analysis and statistics

For conventional testing and rapid AST, MICs were documented as issued by the AST platform. MIC values were manually categorized in susceptible, intermediate, and resistant test results in accordance with EUCAST clinical breakpoints version 8.1. Comparison of test results was performed on a categorical level. Deviating test results were classified in error or deviation categories as shown in Table 1.

In a first step, the BD Phoenix™ and VITEK®-2 platforms were checked for deviating results that were derived by the standard testing method. Since the test panels applied (Phoenix™ NMIC-502 and VITEK®-2 AST-N233) had a different selection of antibiotics, we focused our comparison on 12 antibiotics included in both test panels. In case of divergent test results fulfilling "major deviation," "very major deviation," or "minor deviation" criteria, a second confirmatory test was performed to attribute the error to one of the platforms.

For the evaluation of the direct inoculation method, AST results yielded by different sample preparation methods were compared to results derived from standard AST. Divergent

Table 1 Error categorization of divergent test results

			Standard method/confirmatory test	Direct inoculation/divergent result
Very major error/deviation	VME	False susceptible test result	Resistant	Susceptible
Major error/deviation	ME	False resistant test result	Susceptible	Resistant
Minor error/deviation	MI	Imprecise categorical deviation	Intermediate vs resistant/susceptible	

results were interpreted as errors by direct inoculation. Results of all antibiotics included in the test panel were compared in the rapid AST analysis.

To directly compare the performance of both platforms for the direct inoculation method, we performed a subgroup analysis of antibiotics relevant for bloodstream infections (BSI panel) [14, 15] including piperacillin-tazobactam, a third-generation cephalosporin with (ceftazidime) and without activity against *Pseudomonas aeruginosa* (ceftriaxone or cefotaxime), meropenem, ciprofloxacin, and gentamicin. Error rates and agreement rates were compared for each BSI panel antibiotic by performing chi-squared test or Fisher's exact test. A p value < 0.05 was considered significant. The statistical analyses were conducted using IBM SPSS version 25.

Results

Study samples

From a total amount of 100 eligible blood cultures with gram-negative bacteria, 2 polymicrobial blood cultures were excluded from the final data analysis. The remaining 98 specimens represented a gram-negative pathogen spectrum similar to the gram-negative pathogen distribution in our routine blood culture diagnostics. The following species were isolated from blood cultures tested in this study: 66 *Escherichia coli*, 7 *Enterobacter spp.*, 5 *Klebsiella pneumoniae*, 8 *Proteus spp.*, 1 *Serratia marcescens*, 1 *Achromobacter xylosoxidans*, 1 *Leclercia adecarboxylata*, 1 *Citrobacter koseri*, 1 *Aeromonas hydrophila*, and 7 *Pseudomonas aeruginosa*.

Comparison of VITEK®-2 versus BD Phoenix™ for conventional AST

Direct comparison of both commercial platforms, VITEK®-2 and BD Phoenix™, was performed for conventional AST as recommended by the manufacturer. From 98 tests performed for each isolate on each platform, 1 test run was aborted by the VITEK®-2 system, and 3 runs were aborted by the BD Phoenix™ platform. In total, 1117 bacteria/antibiotic agent combinations for each platform were compared. Data of aborted runs were excluded from the final analysis.

In 45 bacteria/antibiotic agent combinations, a divergent test result was found for both AST platforms. Confirmatory susceptibility testing allowed attributing the errors to one of the AST platforms as shown in Table 2.

For both platforms, an error accumulation for certain antibiotic was observed (data not shown). The VITEK® platform generated 1 VME and 4 ME, and the BD Phoenix™ yielded 2 VME and 2 ME when testing piperacillin and piperacillin/tazobactam. Furthermore, 3 VME were observed for VITEK®-2 testing cotrimoxazole and 11 MI occurred for susceptibility testing of tigecycline using the BD Phoenix™ system.

AST by direct inoculation

Each blood culture specimen was prepared with the 2XC and the STT method. Bacteria pellets yielded were used for direct inoculation on both AST platforms. Of 98 specimens tested, 18 rapid AST test runs (8 SST, 10 2XC) were aborted by BD Phoenix™. Direct inoculation AST was aborted 3 times by the VITEK®-2 system (1 SST, 3 2XC). Aborted test runs were excluded from the final analysis. In total, 1455 (SST) and 1438(2XC) bacteria/antibiotic agent combinations tested by direct inoculation on the VITEK®-2 platform were analyzed. On the BD Phoenix™ susceptibility, testing of 2039 (SST) and 2013 (2CX) bacteria/antibiotic agent combinations was performed. Error and agreement rates for each sample preparation method attributed to antibiotics tested are shown in Table 3 for the VITEK®-2 system and in Table 4 for the Phoenix BD™ platform.

Direct comparison of both platforms for AST by direct inoculation was conducted by a subgroup analysis focusing on the antibiotics of the BSI panel. Looking at the BSI panel only, total agreement rates for both platforms converged. For the VITEK®-2 system, total agreement rates of 98.41% (SST) and 98.39% (2XC) were observed, while agreement rates of

Table 2 Error rates for conventional AST compared to confirmatory test

	No. of very major errors (%)	No. of major errors (%)	No. of minor errors (%)
VITEK®-2	5 (0.45)	4 (0.36)	10 (0.9)
BD Phoenix™	6 (0.54)	6 (0.54)	14 (1.3)

Table 3 Direct inoculation AST results for the VITEK®-2 system

Antimicrobial agent	Serum separator tubes (SST)					Twofold centrifugation (2XC)				
	No. of very major errors	No. of major errors	No. of minor errors	No. of agreements	Total	No. of very major errors	No. of major errors	No. of minor errors	No. of agreements	Total
Ampicillin	0	0	0	89	89	0	1	0	87	88
Ampicillin/sulbactam	1	0	3	85	89	1	2	2	83	88
Piperacillin	3	0	1	92	96	3	1	2	89	95
Piperacillin/tazobactam	1	0	2	93	96	1	1	0	93	95
Cefuroxime	1	0	0	34	35	0	0	0	34	34
Cefuroxime axetil	1	0	0	40	41	0	0	0	40	40
Cefpodoxime	0	0	0	88	88	1	1	0	85	87
Cefotaxime	0	0	0	88	88	0	1	0	86	87
Ceftazidime	0	0	5	91	96	0	1	4	90	95
Ertapenem	0	0	0	89	89	0	0	0	88	88
Imipenem	0	0	2	94	96	0	0	2	93	95
Meropenem	0	0	0	96	96	0	0	0	95	95
Gentamicin	0	0	0	95	95	0	0	0	94	94
Ciprofloxacin	0	0	1	95	96	0	1	0	94	95
Moxifloxacin	0	1	0	88	89	0	3	0	85	88
Tigecycline	1	0	3	84	88	0	0	4	83	87
Cotrimoxazole	4	2	1	81	88	4	1	1	81	87
Total (%)	12 (0.82)	3 (0.21)	18 (1.24)	1422 (97.7)	1455 (100.0)	10 (0.70)	13 (0.90)	15 (1.04)	1400 (97.36)	1438 (100.00)

Table 4 Direct inoculation AST results for the BD Phoenix™ system

	Serum separator tubes (SST)					Twofold centrifugation (2XC)				
Antimicrobial agent	No. of very major errors	No. of major errors	No. of minor errors	No. of agreements	Total	No. of very major errors	No. of major errors	No. of minor errors	No. of agreements	Total
Amikacin	0	0	1	79	80	0	0	0	79	79
Amoxicillin-clavulanate	0	7	0	72	79	1	9	0	68	78
Ampicillin	1	0	0	79	80	0	1	0	78	79
Aztreonam	2	13	6	58	79	2	5	2	69	78
Cefepime	0	2	3	74	79	0	1	0	77	78
Cefixime	3	12	0	65	80	3	8	0	68	79
Ceftazidime	0	3	2	75	80	2	3	1	73	79
Ceftazidime-avibactam	1	3	0	74	78	0	4	0	73	77
Ceftriaxone	0	1	1	76	78	0	1	0	76	77
Cefuroxime	1	6	5	68	80	1	4	3	71	79
Cephalexin	1	8	0	71	80	1	3	0	75	79
Ciprofloxacin	0	0	3	76	79	0	0	3	75	78
Colistin	2	0	0	78	80	2	0	0	77	79
Ertapenem	0	0	0	78	78	0	0	0	77	77
Fosfomycin	5	3	0	72	80	4	1	0	74	79
Gentamicin	0	0	1	79	80	1	0	2	76	79
Imipenem	0	0	3	70	73	0	0	2	70	72
Levofloxacin	0	0	4	75	79	0	0	7	71	78
Mecillinam	0	3	0	61	64	0	2	0	61	63
Meropenem	0	0	4	76	80	0	0	1	78	79
Nitrofurantoin	2	6	0	72	80	1	6	0	72	79
Piperacillin	2	2	1	75	80	1	3	2	73	79
Piperacillin-tazobactam	1	6	2	70	79	0	4	2	72	78
Tigecycline	0	2	15	58	75	0	1	15	58	74
Tobramycin	0	0	1	79	80	1	0	2	76	79
Cotrimoxazole	4	0	0	75	79	4	0	0	74	78
Total (%)	25 (1.23)	77 (3.78)	52 (2.55)	1885 (92.45)	2039 (100.00)	24 (1.19)	56 (2.78)	42 (2.09)	1891 (93.94)	2013 (100.00)

Table 5 Direct comparison of both platforms for direct inoculation (BSI core panel)

Method	Platform	Piperacillin-tazobactam	Ceftriaxone/ cefotaxime	Ceftazidime	Meropenem	Ciprofloxacin	Gentamicin
SST	VITEK®-2	96.7% (3/93)	100.0% (0/88)	94.5% (91/5)	100.0% (0/96)	99.0% (1/95)	100.0% (0/95)
	Phoenix™	87.1% (9/70)	97.4% (2/76)	93.3% (75/5)	94.7% (4/76)	96.1% (3/76)	96.0% (3/76)
	p value	0.038*	0.219	0.766	0.041*	0.329	0.092
2XC	VITEK®-2	98.16% (92/2)	100% (0/88)	91.8% (6/73)	100.0% (0/95)	98.9% (1/94)	100.0% (0/94)
	Phoenix™	95.68% (72/6)	98.7% (1/76)	94.5% (5/91)	98.7% (1/78)	96.0% (3/75)	98.7% (1/79)
	p value	0.142	0.467	0.517	0.454	0.328	0.460

*Significant *p* values

94.95% (SST) and 95.74% (2XC) were detected for the BD Phoenix™ platform. Checking for statistically significant differences in test performance was conducted on a single substance level as shown in Table 5. In this analysis, only 2 out of 12 test constellations showed a significant difference in test performance. In both cases, when testing piperacillin-tazobactam and meropenem with the SST method, the VITEK®-2 system outperformed the BD Phoenix™ platform.

Discussion

Both platforms demonstrated a very good performance when applied for standard AST using subcultures on solid media. Divergent results were observed for rapid AST by direct inoculation. While no essential difference in outcome was observed for the two methods of sample preparation, the commercial AST platform applied seems to be more crucial for direct inoculation test performance.

Assessment of error rates in antibiotic susceptibility testing requires defined thresholds. Jorgensen proposed criteria for acceptance of AST methods as follows: A very major error rate of less than 3%, a combined major and minor error rate of less than 7%, and an overall agreement of more than 90% are needed to verify a new test [16]. Applying these criteria to our study, both preparation methods and both platforms have proven suitable for AST by direct inoculation.

Nevertheless, the VITEK®-2 system showed a higher inter-test agreement for direct inoculation AST. This was observed when comparing the AST results for all antibiotics tested as well as for the subgroup analysis of the BSI core panel.

For the VITEK®-2 system, an overall inter-test agreement between direct and conventional AST of 97.7% (SST) and 97.36% (2XC) was measured for full panel testing. The BD Phoenix™ system showed higher error rates with a general inter-test agreement of only 92.45% (SST) and 93.94% (2XC) for rapid AST when regarding all antibiotics tested.

Since both test panels, the Phoenix™ NMIC-502 cartouche and the VITEK®-2 AST-N233 card, differed in their selection of antibiotics, a subgroup analysis of essential antibiotics contained in both tests, the BSI panel, was performed. Here, overall interest agreement increased for both systems, but still, the BD Phoenix™ demonstrated inferior performance compared to the VITEK®-2 system. However, the difference in performance was, except for testing of piperacillin-tazobactam and meropenem with the SST method, not statistically significant.

The results for the VITEK®-2 systems are supported by multiple former studies using the SST or 2XC sample preparation method on this platform for direct inoculation of gram-negative bacteria [11, 17–20]. The inferior performance of the BD Phoenix ™ was not described in studies before, but also fewer studies for direct inoculation were undertaken on this platform so far. Three previous studies reported agreement rates of approximately 99% [20–22] for the BD Phoenix™ system. The study of Gherardi et al. also directly compared the VITEK®-2 and the BD Phoenix™ directly. In this publication, even a slightly better performance of the BD Phoenix platform for rapid AST of gram-negative bacteria was found [20].

Although our results were statistically not significant for the overall performance of the two platforms, our findings still showed a tendency for inferior performance and thereby contradict prior studies. All previous BD Phoenix™ studies used the STT or 2XC sample preparation methods as done in our study and protocols only varied slightly in centrifugation settings. It is unlikely that these minor differences trigger result deviations to such extent. Another, maybe more decisive difference with former studies, is the test panel applied in our study. The Phoenix™ NMIC-502 test panel uses both chambers of the test cartouche which have to be filled separately. Bacterial suspensions for each chamber were partially prepared in separate tubes. Thus, the multiple work steps required for the BD Phoenix™ test runs immanently increase the likelihood of pipetting errors. Nevertheless, since test runs were performed by skilled and trained staff, it is unlikely that pipetting errors caused the majority of inter-test deviations observed in our study.

Another important aspect is the number of aborted runs. While only few rapid AST runs were aborted by the VITEK®-2 system (1 SST, 3 2XC), more direct inoculation

tests were stopped without a final AST result by the BD Phoenix™ platform (8 SST, 10 2XC). Although these errors do not endanger patients as, for example, false-susceptible test results do, they still represent a crucial disadvantage by losing the chance of an early test result. Since these errors occurred in approximately one out of ten cases for the BD Phoenix™, this platform seems not to be as suitable for our method of direct inoculation as the VITEK®-2 system.

Regarding the sample preparation method, no essential differences for the SST and 2XC method were observed when using the same AST platform. Previous studies already demonstrated that a purely mechanical sample preparation method is sufficient for direct inoculation [11, 17, 18, 20–22]. A more elaborate, combined chemical and mechanical sample preparation used in earlier studies did not result in improved agreement rates or even showed lower inter-test agreement and therefore seems not to be beneficial for AST by direct inoculation [23, 24].

Limitations

Compared to earlier studies that examined AST by direct inoculation, the sample size of this study with 98 clinical isolates meets the approximate average. Only few prior studies used more than 250 clinical samples [18, 21, 23, 25], whereas most studies used 100–150 strains [10, 11, 13, 20, 24, 26] or even less than 60 isolates [12, 27]. The samples included in this study yielded a representative pathogen spectrum, similar to the pathogen distribution occurring in routine blood culture diagnostics in our laboratory. Comparable incidence rates in bloodstream infections, with *E. coli* as the leading pathogen and other *Enterobacteriaceae* species or non-fermenting bacteria representing smaller proportions among gram-negative bacteria, have also been reported from other regions or in different clinical settings [28–30]. From these observations, it can be concluded that our sample size is large enough to reflect clinical reality. However, our study size is too small to check for statistically significant differences on a single substance level as performed in the BSI subgroup analyses. Furthermore, comparison of AST results on bacterial species level would not have been feasible.

Second, a risk-corrected error rate could not be determined due to the small numbers of antibiotic resistances occurred in the study cohort. For example, to verify the method for carbapenem susceptibility testing, more carbapenem-resistant strains must be included in the study to rule out false-susceptible results sufficiently [16]. In a low prevalence setting such as Germany, this cannot be achieved with clinical samples only [31]. To tackle this issue in future studies, the deployment of the seeded specimen must be taken into consideration.

Although the clinical benefit of receiving AST results 24 h earlier seems obvious, this study was not designed to analyze the clinical impact of rapid AST. Since the method was not evaluated according to ISO 15189 prior to the study, reporting results derived from direct inoculation would have potentially endangered patients' safety. As a consequence, the study design was merely diagnostic and non-interventional. The influence of rapid AST on clinical outcomes was not analyzed.

Conclusion

In line with prior research, this study demonstrated that the direct inoculation method for AST is a reliable approach to accelerate phenotypic full panel susceptibility testing in blood culture diagnostics. Ideally, the procedure should be implemented in routine diagnostics in combination with a rapid method for pathogen identification, such as direct mass-spectrometry analysis from blood culture pellets. While the different methods of sample preparation had no significant influence on test results, the choice of AST platform seems to be more crucial. In our study, the VITEK™-2 system demonstrated a better, tough statistically not significant, overall performance for testing gram-negative bacteria by direct inoculation from blood cultures. Overall, direct inoculation is an easy and inexpensive approach to obtain full panel phenotypic AST results one day earlier than in conventional blood culture diagnostics. Since this approach requires no additional equipment, it is also feasible in low-resource settings. Future research must focus on the long-term performance of direct inoculation in routine diagnostics and analyze the clinical impact of reporting early results obtained by this method.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study has been approved by the local research ethics committee (5358-11/17).

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