Diagnostic Microbiology and Infectious Disease 107 (2023) 116032

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Evaluation of VITEK® 2 AST cards (AST-N376 and AST-N397) for susceptibility testing of challenging Gram negatives

E. Riccobono[a,b], N. Aiezza[a], C. Niccolai[a], T. Giani[a,c], G.M. Rossolini[a,c,*]

[a] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[b] Department of Medical Biotechnologies, University of Siena, Siena, Italy
[c] Clinical Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy

ARTICLE INFO

Article history:
Received 22 February 2023
Revised in revised form 11 July 2023
Accepted 15 July 2023
Available online 20 July 2023

Keywords:
antibiotic susceptibility testing
automated system
VITEK
broth microdilution
antibiotic resistance

ABSTRACT

Due to the increasing diffusion of MDR/XDR Gram-negatives it is necessary to offer reliable antibiotic susceptibility testing (AST), which also include new drugs. Here we evaluated the performances of the VITEK®2 AST-N376 and the AST-N397 cards. A collection of 180 clinical Gram-negative bacteria, producing relevant resistance mechanisms, were tested using VITEK 2 and MERLIN, in parallel. Discrepancies between the 2 systems were solved by the reference broth microdilution method. The workflow timing of the VITEK®2 system was also assessed. Overall, the VITEK®2 cards proved to be reliable in determining AST for the molecules evaluated, even if compliance with ISO acceptance criteria for accuracy assessment was not reached for some combinations and showed a short hands-on time for panels preparation. In conclusion, VITEK®2 is a valid system that ensures accurate results for AST of the molecules evaluated in this study and speeds up the workflow in the laboratory of diagnostic microbiology.

1. Introduction

The increase of infections by multi-drug resistant and extensively drug resistant (MDR/XDR) Gram-negatives, together with the increasing complexity of patients, underscores the need for reliable diagnostic tools to support antimicrobial stewardship [1,2].

Several automated *in vitro* diagnostic systems are currently available and broadly used in clinical laboratories for antimicrobial susceptibility testing (AST), including VITEK® 2 (bioMérieux, Marcy l'Etoile, France), Phoenix (BD Diagnostics, Sparks, MD, USA), and MicroScan WalkAway (Beckman Coulter, Sacramento, CA) systems [1]. These systems involve sophisticated instrumentation and software, which provide improved testing standardization (e.g., objective reading of results and closed-system incubation), and are generally associated with an easy workflow, cost-effectiveness, and reduced time to results compared to traditional testing methods [3–5]. Since the introduction of automated *in vitro* diagnostic systems for AST, clinical laboratories have become increasingly reliant on them, and the experience and habit of performing manual AST have gradually dwindled [3].

This global trend must deal with the fact that the process of developing AST devices for new antimicrobial drugs is complex, expensive, and time-consuming, so that, generally, the only AST devices available for recently approved antimicrobial drugs are manual tests that slow down the laboratory workflow, require technical expertise, expose personnel to biological risks and are subject to misinterpretations. Furthermore, low accuracy of AST methods versus reference methodologies has previously been reported for different combinations of pathogens and clinically important molecules, sometimes with negative effects on clinical outcomes [6,7]. For all these reasons, there is an urgent need to keep automated AST devices and related software constantly updated to offer reliable and comprehensive antibiotic panels, which also include new drugs [7,8].

The aim of this study was to evaluate the performances, in terms of accuracy and error rates [3], of the VITEK® 2 AST-N376 card and the AST-N397 card, updated with new formulations and/or extended concentration ranges of old antibiotics (i.e., amikacin, cefepime, ceftazidime, ciprofloxacin, ertapenem, gentamicin, tigecycline, tobramycin) and introduction of new drugs (i.e., ceftolozane/tazobactam, ceftazidime/avibactam) using a collection of previously characterized Gram-negative bacteria including strains with challenging resistance profiles collected from clinical specimens.

2. Material and methods

2.1. Bacterial strains

A total of 180 nonduplicated Gram-negatives representative of the national epidemiology and having well-defined challenging

* Corresponding author. Tel.: +39 055 7949239.
E-mail address: gianmaria.rossolini@unifi.it (G.M. Rossolini).

https://doi.org/10.1016/j.diagmicrobio.2023.116032

phenotypes and/or well-characterized resistance mechanisms, collected at Florence Careggi University Hospital, were selected for testing (Supplementary Table 1). Overall, the collection consisted of *Enterobacterales* [68% (n = 123)], including strains with challenging phenotypes such as MDR/XDR, extended spectrum beta-lactamase (ESBL) producers, class A- and/or class B-carbapenemase producers [(83%, (n = 102)] and fully susceptible strains [17% (n = 21); *Pseudomonas aeruginosa* [25% (n = 45)] and *Acinetobacter baumannii* [7% (n = 12)], including carbapenemase-producing strains [27% (n = 12) and 83% (n = 10), respectively]. Before testing, frozen isolates were subcultured twice to ensure purity onto Columbia Agar with 5% sheep blood and incubated for 18 to 24 h at 35°C ± 2°C. Before susceptibility testing, species identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) (Bruker Daltonics, USA), and the presence of resistance determinants was verified by end-point polymerase chain reaction (PCR) or Real-Time PCR (RT-PCR) (Supplementary Table 2).

2.2. Antibiotic susceptibility testing

The evaluation was performed comparing VITEK® 2 AST-N376 and AST-N397 cards to MICRONAUT-S MDR MRGN−Screening (MERLIN Diagnostika GmbH, Bornheim-Hersel, Germany), which is a commercial broth microdilution method (BMD), based on the rehydration of lyophilized antibiotics by adding a bacterial suspension, previously used as a reference method due to its overall compliance with International Organization for Standardization (ISO) 20776-1:2019 for BMD in terms of volumes, inoculum size, and broth composition [9–11].

VITEK® 2 cards and MERLIN panel were set up following the Manufacturer's instructions. For each isolate included in the study, a suspension was prepared according to ISO criteria 20776-1:2019 and used to inoculate both the AST cards and the MERLIN plates [9].

Since tobramycin was not included in the MERLIN panel, this molecule was directly compared with the broth microdilution reference method (BMD), carried out according to the ISO standard 20776-1: 2019 and using antibiotic powder provided by bioMérieux [9]. All antibiotics tested and their respective ranges are listed in Supplementary Table 3.

VITEK® 2 results were read and interpreted automatically using the "advanced expert system" software provided by bioMérieux (version 9.02), while MERLIN plates were read and interpreted manually using EUCAST interpretative criteria v. 9.0 (www.eucast.org), corresponding to the breakpoints that were validated for VITEK® 2 at the time of the study.

2.3. Data analysis

Susceptibility results were analyzed to assess the performance of the VITEK® 2 cards in relation to the MERLIN panel, in terms of essential agreement (EA), categorical agreement (CA), major discrepancies (MD), very major discrepancies (VMD), minor discrepancies (mD). CA, MD and VMD were evaluated according to ISO criteria 20776-2:2007 [12].

All tests resulted as MDs or VMDs were repeated with both methods. When discrepancies were confirmed, the adjudication of results was determined by comparing results from VITEK®2 and MERLIN to those obtained with the reference BMD following ISO criteria 20776-1:2019, with all techniques performed in parallel using the same bacterial suspension [9].

After adjudication, bias was also evaluated according to ISO 20776-2:2021 criteria [13]. Since the study collection included a limited number of isolates, bias was calculated including all the collection isolates.

2.4. Quality control (QC) testing

For QC purposes, the following ATCC reference strains were tested as quality controls for the entire duration of the study: *E. coli* ATCC 25922 for all drugs; *P. aeruginosa* ATCC 27853 for all drugs except tigecycline and ertapenem; *E. coli* ATCC 35218 for ceftolozane/tazobactam only; *K. pneumoniae* ATCC 700603 for ceftolozane/tazobactam and ceftazidime/avibactam only. If the results for an antimicrobial were not within the expected range, all results for the specific drug obtained during that study day were excluded from the dataset and repeated upon resolution of the issue.

2.5. Workflow analysis

Besides the VITEK® 2 AST cards evaluation, the workflow timing of the VITEK®2 system was assessed compared to the MERLIN system with a selection of strains (n = 73). This analysis was performed per batches of strains, recording the time needed to complete the following steps: preparing suspension (time 1), setting up VITEK 2 (time 2), setting up MERLIN (time 3), and reading MERLIN (time 4). A detailed description of steps and recording times is reported in Supplementary Table 4.

The total time per batch was calculated by adding up time 1 and time 2 for VITEK 2 and time 1, time 3, and time 4 for MERLIN. The mean time per isolate was calculated for each batch (MTI/B), according to the number of isolates included in that batch. The overall mean time per isolate was calculated as the mean of MTI/Bs ± standard deviation. Statistical significance of differences in time measurements was determined by the two-sample independent *t* test using GraphPad Prism version 8.0.1 for Windows (GraphPad Software, Inc., San Diego, California USA; www.graphpad.com).

3. Results

3.1. Evaluation of VITEK®2 AST cards performance

A total of 1571 organism-antimicrobial agent combinations were analyzed. The studied organisms included 123 *Enterobacterales*, 45 *Pseudomonas aeruginosa*, and 12 *Acinetobacter baumannii* (Supplementary Table 1). All QCs were in range in each session.

For each antibiotic, the performances of VITEK®2 compared with MERLIN against *Enterobacterales* are reported in Table 1. Briefly, the EA values reached compliance with ISO acceptance criteria for all antibiotics except for cefepime and ceftolozane/tazobactam with a percentage of 80.5% and 87.8%, respectively. The CA was in agreement with ISO acceptance criteria for all molecules tested, with percentages ≥92.7% (Table 1). Discordances included VMD (n = 1) for ceftolozane/tazobactam (1.4%), MD (n = 1) for ceftazidime/avibactam (1.0%), MDs (n = 6) for ertapenem (8.6%), and MD (n = 1) for gentamicin (1.3%), mDs were also reported, but they were always within the limits allowed by ISO criteria. When discrepancies were repeated for adjudication, BMD assigned to VITEK®2 a VME for ceftolozane/tazobactam (n = 1) and an ME for ceftazidime/avibactam (n = 1), and to MERLIN VMEs for ertapenem (n = 6) and gentamicin (n = 1) (Supplementary Table 5). After the BMD adjudication, all discrepancies were within the limits allowed (Table 1).

The performances of VITEK®2 challenged with MERLIN against *P. aeruginosa* reported an overall compliance with ISO acceptance criteria for all antibiotics except for EA of ceftazidime/avibactam (84.4%) (Table 2). Among discrepancies, a VMD for both cefepime (n = 1) and ceftazidime (n = 1) and an MD for cefepime (n = 1), ceftolozane/tazobactam (n = 1) and gentamicin (n = 1) were reported (Table 2). When discrepancies were repeated for the adjudication, BMD assigned to VITEK®2 a VME for cefepime (n = 1), a VME for ceftazidime (n = 1), and a ME for ceftolozane-tazobactam (n = 1) and to MERLIN a VME for cefepime (n = 1) and gentamicin (n = 1) (Supplementary Table 5).

Table 1
Performance of the VITEK®2 vs MERLIN in *Enterobacterales* isolates (n = 123).

Antimicrobial agents	No. of organism-antimicrobial agent combinations categorized as:			EA[d] (% before BMD adjudication) [%after BMD adjudication]	CA[e] (% before BMD adjudication) [% after BMD adjudication]	Discrepancies (% before BMD adjudication) [% after BMD adjudication]		
	R[a] (%)	S[b] (%)	I[c] (%)			mD	MD	VMD
Amikacin	15 (12.2)	100 (81.3)	8 (6.5)	(100)	(97.6)	(2.4)	(0)	(0)
Cefepime	99 (80.5)	23 (18.7)	1 (0.8)	(80.5)	(92.7)	(7.3)	(0)	(0)
Ceftazidime	101 (82.1)	19 (15.5)	3 (2.4)	(97.6)	(98.4)	(1.6)	(0)	(0)
Ceftolozane-Tazobactam	69 (56.1)	54 (43.9)	-	(87.8)	(99.2)	-	(0)	1.4 [1.4]
Ceftazidime-Avibactam	27 (21.9)	96 (78.1)	-	(91.9)	(99.2)	-	(1.0) [1.0]	(0)
Ciprofloxacin	93 (75.6)	28 (22.8)	2 (1.6)	(99.2)	(94.3)	(5.7)	(0)	(0)
Ertapenem	53 (43.1)	70 (56.9)	0 (0)	(91.1) [94.3]	(95.1) [100]	(0)	(8.6) [0]	(0)
Gentamicin	41 (33.3)	80 (65.1)	2 (1.6)	(97.6) [98.4]	(96.7) [97.6]	(2.4)	(1.3) [0]	(0)
Tigecycline[f]	1 (1.8)	55 (98.2)	0 (0)	(98.2)	(100)	(0)	(0)	(0)
Tobramycin[g]	81 (65.8)	38 (30.9)	4 (3.3)	[83.7]	[95.1]	[4.9]	[0]	[0]

[a] R, Resistant.
[b] S, Susceptible, standard dosing regimen.
[c] I, Susceptible, increased exposure.
[d] EA, Essential Agreement.
[e] CA, Category agreement.
[f] tygecycline was tested for *E. coli* and *C. koseri* only (n=56).
[g] tobramycin was not included in MERLIN panel and was directly compared with BMD method.

After BMD adjudication, the performances of VITEK®2 for *P. aeruginosa* showed an EA in compliance with ISO acceptance criteria for all antibiotics except for ceftazidime/avibactam (84.4%) and an excellent CA for all antibiotics (Table 2). However, ME for ceftolozane/tazobactam and VME for cefepime and ceftazidime were reported with a percentage higher than the limits allowed by ISO criteria (Table 2).

The performances of VITEK®2 challenged with MERLIN against *A. baumannii* isolates showed that EA and CA values were in accordance with ISO acceptance criteria for all antibiotics. A VMD was reported for gentamicin (Table 3). When repeated for adjudication, BMD assigned this discrepancy as VME of VITEK®2 (Supplementary Table 5).

The overall EA and bias of VITEK® 2 were reported in Table 4. The study collection showed EA ≥90% and −30%≤ bias ≤+30% for all antibiotics tested except for EA of cefepime (85.12%) and bias of tobramycin (-33.78%), pointing out a tendency to underestimate MICs for this antibiotic.

3.2. Workflow timing of VITEK®2 system compared to MERLIN system

The times measured during the workflow analysis (Supplementary Table 6) showed that the mean time needed to perform antibiotic susceptibility testing for a single isolate by VITEK®2 was 181.7 seconds (SD ± 43 seconds), while the mean time required by MERLIN was 1,6-fold higher with 291.2 seconds (SD ± 36.5 seconds) ($P < 0.0001$) (Fig. 1).

4. Discussion

The accuracy of antibiotic susceptibility testing (AST) has a crucial importance for the clinical diagnostic laboratory of microbiology since the results of AST are used to predict the clinical efficacy of the tested molecules.

One of the hardest challenges for the clinical diagnostic laboratories of microbiology is to produce accurate AST results using feasible, rapid, reproducible, and cost-effective methods. In this perspective, the implementation of fully- or semiautomated AST systems has a major role since these methods significantly minimize hands-on time and reduce the turnaround time and the variability of results due to highly standardized procedures.

However, one of the most relevant drawbacks of AST systems is the inaccuracy in providing reliable AST results for some molecules compared to reference methodologies [14,15], with possible impact on guiding targeted therapy [7].

In this study, we evaluated the performance of the VITEK® 2 AST-N376 card and AST-N397 card after their renewal (introduction of

Table 2
Performance of the VITEK®2 vs MERLIN in *P. aeruginosa* isolates (n = 45).

Antimicrobial agents	No. of organism-antimicrobial agent combinations categorized as			EA[d] (% before BMD adjudication) [%after BMD adjudication]	CA[e] (% before BMD adjudication) [% after BMD adjudication]	Discrepancies (% before BMD adjudication) [% after BMD adjudication]		
	R[a] (%)	S[b] (%)	I[c] (%)			mD	MD	VMD
Amikacin	20 (44.4)	22 (48.9)	3 (6.7)	(95.6)	(91.1)	(8.9)	(0)	(0)
Cefepime	27 (60.0)	18 (40.0)	-	(97.8) [97.8]	(95.6) [97.8]	-	(5.5) [0]	(3.7) [3.7]
Ceftazidime	30 (66.7)	15 (33.3)	-	(93.3) [93.3]	(97.8) [97.8]	-	(0)	(3.3) [3.3]
Ceftolozane-Tazobactam	20 (44.4)	25 (55.6)	-	(95.6) [97.8]	(97.8) [97.8]	-	(4.0) [4.0]	(0)
Ceftazidime-Avibactam	17 (37.8)	28 (62.2)	-	(84.4)	(100)	-	(0)	(0)
Ciprofloxacin	35 (77.8)	10 (22.2)	0	(100)	(100)	-	(0)	(0)
Gentamicin	31 (68.9)	14 (31.1)	-	(100) [100]	(97.8) [100]	-	(7.1) [0]	(0)
Tobramycin	33 (73.3)	12 (26.7)	-	(97.8)	(100)	-	(0)	(0)

[a] R, Resistant.
[b] S, Susceptible, standard dosing regimen.
[c] I, Susceptible, increased exposure.
[d] EA, Essential Agreement.
[e] CA, Category agreement.

Table 3
Performance of the VITEK®2 vs MERLIN in *Acinetobacter* isolates (n = 12).

Antimicrobial agents	No. of organism-antimicrobial agent combinations categorized as			EA[d] (% before BMD adjudication) [% after BMD adjudication]	CA[e] (% before BMD adjudication) [% after BMD adjudication]	Discrepancies (% before BMD adjudication) [% after BMD adjudication]		
	R[a] (%)	S[b] (%)	I[c] (%)			mD	MD	VMD
Amikacin	10 (83.3)	2 (16.7)	-	(100)	(100)	-	(0)	(0)
Ciprofloxacin	10 (83.3)	0 (0)	2 (16.7)	(100)	(100)	-	(0)	(0)
Gentamicin	11 (91.7)	1 (8.3)	0 (0)	(100) [91.7]	(91.7) [91.7]	-	(0)	9.1 [9.1]
Tobramycin	10 (83.3)	2 (16.7)	0 (0)	(91.7)	(100)	-	(0)	(0)

[a] R, Resistant.
[b] S, Susceptible, standard dosing regimen.
[c] I, Susceptible, increased exposure.
[d] EA, Essential Agreement.
[e] CA, Category agreement.

Table 4
Overall essential agreement (EA) and bias of the VITEK®2.

Antimicrobial agents	EA (%)	Bias (%)
Amikacin	100	−4.18
Cefepime	85.12	−14.52
Ceftazidime	96.43	−13.53
Ceftolozane-Tazobactam	94.05	−18.00
Ceftazidime-Avibactam	91.07	−11.59
Ciprofloxacin	100	+10.73
Ertapenem	98.37	+0.33
Gentamicin	98.33	+12.40
Tigecycline	98.21	+1.79
Tobramycin	96.67	−33.78

novel β-lactams/β-lactamase inhibitors, new drug formulations and/or extended concentration range of antibiotics) using a collection of challenging isolates, including relevant resistance mechanisms such as ESBL and carbapenemase-producing isolates.

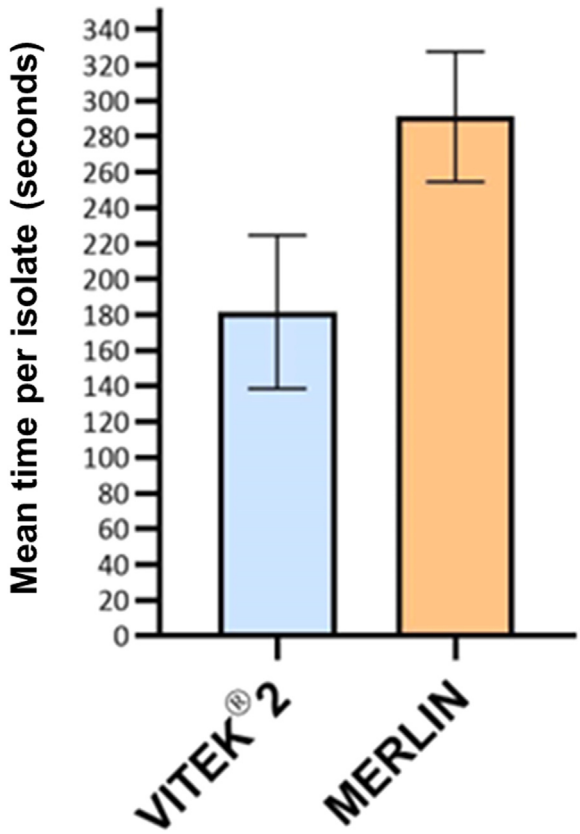

Fig. 1. Overall mean time per isolate of VITEK®2 and MERLIN methods.

Data obtained showed that acceptance criteria for accuracy assessment agreed with those described by the ISO standard for almost all drug-bug combinations. Among the exceptions, the most clinically relevant molecules (cefepime, ceftolozane-tazobactam in *Enterobacterales* and of ceftazidime-avibactam in *P. aeruginosa*) showed CA values and an overall bias within the ISO acceptance criteria [12,13]. However, these data could be influenced by the small number of isolates tested and should be further investigated. A recent evaluation study of VITEK 2 AST-N397 against MERLIN broth microdilution plates showed better results for ceftolozane/tazobactam in *Enterobacterales* (98.7%) [11].

A possible limitation of this study is that the performance of VITEK® 2 has been evaluated in comparison to another commercial system. Indeed, although MICRONAUT-S system can be considered a reliable method for AST and was already used in other similar evaluation studies [10,11,16,17] it is not equivalent to the gold standard based on BMD. For this reason, BMD has been used for discrepancies resolution.

The workflow assessment performed between VITEK® 2 and MERLIN methods for a selection of isolates showed that VITEK® 2 system required a mean time per isolate 1.6 folds shorter than MERLIN system with a statistical significance ($P < 0.0001$). This finding pointed out that the hands-on time to prepare panels with VITEK® 2 system is shorter than hands-on time required by MERLIN system. This data has not a clinical impact but highlights a technical advantage. Indeed, VITEK® 2 almost halves the processing time for each antibiotic susceptibility test, streamlining considerably the workflow in overwhelmed laboratories.

Overall, the new VITEK® 2 cards (AST-N376 and AST-N397) proved to be reliable in determining the antibiotic susceptibility for the combinations of antibiotic-microorganism evaluated in this study and showed a short hands-on time to prepare panels. This can be considered of notable importance since having an automated AST system able to provide a reliable antibiogram is a key factor for the appropriate use of antibiotics and for a better outcome for patients as stated in a recent multicenter retrospective study where the authors demonstrated that MEs and VMEs observed with some AST system were associated with an inappropriate use of antibiotics and poorer outcomes [7].

Declaration of Competing Interest

TG: speakers bureaus for bioMérieux; GMR: research grants, consultancies, speakers bureaus for bioMérieux.

Authors' contributions

ER: conceptualization, methodology, formal analysis, writing original draft; NA, CN: methodology, formal analysis; TG: formal analysis, reviewing and editing; GMR: conceptualization, supervision,

reviewing and editing. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgments

This study was supported by a grant from bioMérieux.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2023.116032.

References

[1] Vasala A, Hytönen VP, Laitinen OH. Modern tools for rapid diagnostics of antimicrobial resistance. Front Cell Infect Microbiol 2020;10:308.
[2] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.
[3] Humphries RM, Kircher S, Ferrell A, Krause KM, Malherbe R, Hsiung A, et al. The continued value of disk diffusion for assessing antimicrobial susceptibility in clinical laboratories: report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. J Clin Microbiol 2018;56 e00437-18.
[4] Khan A, Arias CA, Abbott A, Dien Bard J, Bhatti MM, Humphries RM. Evaluation of the Vitek 2, Phoenix, and MicroScan for antimicrobial susceptibility testing of *Stenotrophomonas maltophilia*. J Clin Microbiol 2021;59:e0065421.
[5] Rampacci E, Trotta M, Fani C, Silvestri S, Stefanetti V, Brachelente C, et al. Comparative performances of Vitek-2, disk diffusion, and broth microdilution for antimicrobial susceptibility testing of canine *Staphylococcus pseudintermedius*. J Clin Microbiol 2021;59:e0034921.
[6] Gaibani P, Giani T, Bovo F, Lombardo D, Amadesi S, Lazzarotto T, et al. Resistance to ceftazidime/avibactam, meropenem/vaborbactam and imipenem/relebactam in Gram-Negative MDR Bacilli: molecular mechanisms and susceptibility testing. *Antibiotics* (Basel) 2022;11:628.
[7] Bartoletti M, Antonelli A, Bussini L, Corcione S, Giacobbe DR, Marconi L, et al. Clinical consequences of very major errors with semi-automated testing systems for antimicrobial susceptibility of carbapenem-resistant Enterobacterales. Clin Microbiol Infect 2022;17:1290.e1-4.
[8] Humphries RM, Hindler JA. Emerging resistance, new antimicrobial agents…but No Tests! The challenge of antimicrobial susceptibility testing in the current US regulatory landscape. Clin Infect Dis 2016;63:83–8.
[9] Clinical laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—Part 1: reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. Geneva, Switzerland: International Organization for Standardization—ISO; 2019.
[10] Mantzana P, Netsika F, Arhonti M, Meletis G, Kandilioti E, Kiriakopoulou M, et al. Performance evaluation of Alfred60AST rapid susceptibility testing directly from positive blood cultures in the routine laboratory workflow. Eur J Clin Microbiol Infect Dis 2021;40:1487–94.
[11] Cortazzo V, Posteraro B, Menchinelli G, Liotti FM, D'Inzeo T, Fiori B, et al. Susceptibility of meropenem-resistant and/or carbapenemase-Producing Clinical Isolates of *Enterobacterales* (*Enterobacteriaceae*) and *Pseudomonas aeruginosa* to ceftazidime-avibactam and ceftolozane-tazobactam as assessed by in vitro testing methods. Antibiotics 2022;11:1023.
[12] Clinical laboratory testing and in vitro diagnostic test systems — susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 2: evaluation of performance of antimicrobial susceptibility test devices. Geneva, Switzerland: International Organization for Standardization—ISO; 2007.
[13] Clinical laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—Part 2: evaluation of performance of antimicrobial susceptibility test devices against reference broth micro-dilution. Geneva, Switzerland: International Organization for Standardization—ISO; 2021.
[14] Arena F, Giani T, Vaggelli G, Terenzi G, Pecile P, Rossolini GM. Accuracy of different methods for susceptibility testing of gentamicin with KPC carbapenemase-producing *Klebsiella pneumoniae*. Diagn Microbiol Infect Dis 2015; 81:132–4.
[15] Idelevich EA, Freeborn DA, Seifert H, Becker K. Comparison of tigecycline susceptibility testing methods for multidrug-resistant *Acinetobacter baumannii*. Diagn Microbiol Infect Dis 2018;91:360–2.
[16] Matuschek E, Åhman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin - evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Acinetobacter* spp. Clin Microbiol Infect 2018;24:865–70.
[17] Balke B, Hoy L, Weissbrodt H, Häussler S. Comparison of the Micronaut Merlin automated broth microtiter system with the standard agar dilution method for antimicrobial susceptibility testing of mucoid and nonmucoid *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. Eur J Clin Microbiol Infect Dis 2004;23:765–71.