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ORIGINAL ARTICLE

Direct antimicrobial susceptibility testing from the blood culture pellet obtained for MALDI-TOF identification of Enterobacterales and *Pseudomonas aeruginosa*

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Abstract

To standardize the methodology for conducting direct antimicrobial susceptibility testing (AST) of Enterobacterales and *Pseudomonas aeruginosa* causing bacteremia from positive blood culture pellets. Two methods for processing positive blood cultures with Enterobacterales and *P. aeruginosa* were compared: a conventional method for identification and AST versus a direct method obtaining a pellet for both matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) identification and direct AST. A total of 157 (145 Enterobacterales, 12 *P. aeruginosa*) positive blood cultures were included. Microorganism identification showed 100% concordance between both methods at species and genus level. Definitive AST results were obtained 24 h earlier with the rapid method than the conventional one ($p < 0.001$). Of the 2814 MICs generated, there were discrepancies with respect to the conventional method in 47 (1.7%), 0.3% being very major (VME) and 1.3% major (ME) errors. Better results for AST were obtained when colony counts with the pellet were $\geq 10^5$ cfu/ml. The essential agreement (EA) for antibiotics tested in Enterobacterales was at least 97%, except for ampicillin (95%). Regardless of colony count, the greatest discrepancies were observed for first/s-generation cephalosporins and aminoglycosides. In *P. aeruginosa*, EA was at least 92%, except for piperacillin-tazobactam (84%) and cefepime (76%). No VME occurred except for ceftazidime (8%). ME occurred in piperacillin/tazobactam (16%), ticarcillin, ceftazidime, tobramycin, amikacin, and colistin (8% each). Direct use of the blood culture pellet permits fast AST in bacteremia of Enterobacterales, enabling the clinicians to perform an early treatment adjustment. However, for *Pseudomonas aeruginosa*, the data needs expanding to improve the reliability of this technique.

Keywords Bacteraemia · Gram-negative bacilli · Blood culture pellet · Direct antimicrobial susceptibility testing · MALDI-TOF

Introduction

A key function of Clinical Microbiology laboratories is to obtain accurate and timely antimicrobial susceptibility testing (AST) data, especially in sepsis or critically ill patients. Bloodstream infections (BSI) are the main cause of morbidity and mortality in hospitalized patients and timely start of appropriate antibiotics has an important impact on patient outcome. A reduction of this timeline to less than one working day is very useful, reducing morbidity and mortality as well as the overall costs for healthcare systems [1]. Moreover, accelerating the AST has a great impact on patient therapy and is also critical for the overall success of health care institutions' antimicrobial stewardship programs, particularly in critically ill patients [2–4]. In the hospital setting, microorganisms are subjected to a high antibiotic pressure, resulting in multidrug-resistant pathogens which imply great difficulties for their correct treatment [5].

Different methods to obtain earlier AST results than with current standard methods are under development. Both EUCAST and CLSI are developing new clinical breakpoints

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for early reading of disk-diffusion antibiograms performed directly from positive blood cultures [6, 7]. Other methods, such as Accelerate Pheno™ System that uses PNA-FISH technology for bacterial identification and morphokinetic analysis using time-lapse imaging for AST provides results in 7–8 h [8]. Molecular techniques are rapid and sensitive, but nowadays they are not affordable for all laboratories due to their costs [9]. There are also some groups working on the use of flow-cytometry to obtain AST results in less than 2 h [10, 11]. To also achieve earlier AST results, we propose a new and simple method to rapidly obtain bacterial antibiotic susceptibility. This is based on the use of the blood culture pellet for both MALDI-TOF identification and for the phenotypic AST using a commercial automated broth microdilution method.

Material and methods

Study design

The study was performed at Ramón y Cajal University Hospital (Madrid, Spain), a tertiary hospital with 1161 beds and more than 30,000 admissions per year, in which all medical and surgical specialties are represented. The Microbiology Department provides routine blood culture microbiological results from 8:00 am to 18:00 pm (Monday through Friday) and from 8:00 am to 15:00 pm (Saturday and Sunday). From September 2016 to January 2017 (20 weeks), identification from pellets using MALDI-TOF and both conventional and rapid AST methods were performed sequentially on all positive blood cultures with Gram-negative bacilli. Isolates other than Enterobacterales and *Pseudomonas aeruginosa* were excluded.

Aerobic, anaerobic, and pediatric blood culture bottles (BD BACTEC™ Plus Aerobic/F, BD BACTEC™ Lytic/10 Anaerobic/F and BD BACTEC™ Peds Plus™/F, respectively) from patients with suspected BSI were incubated in a BACTEC™ FX instrument (Becton & Dickinson, Erembodegem, Belgium), according to the manufacturer's instructions. If both the aerobic and the anaerobic bottles were positive, the aerobic was prioritized for processing.

The study was approved by the local ethics committee.

Conventional method

Positive blood culture bottles were immediately processed including Gram stain and plating onto both blood and chocolate agar. All plates were incubated at 37 °C. Simultaneously, identification was performed using a bacterial pellet obtained using a protocol slightly different from that described by Prod'hom et al. [12] (Fig. 1) and MALDI-TOF MS Microflex LT platform (Bruker Daltonics, Bremen, Germany).

To obtain the pellet, a sterile microcentrifuge tube is filled with 1 ml of the blood culture and mixed with 200 μL of 5% sodium dodecyl sulfate (SDS) solution using a vortex. After that, the tube is centrifuged 1 min at 13,000 rounds per minute (RPM). The supernatant in the tube is discarded, and the pellet is resuspended adding 1 ml of sterile distilled water and mixed with vortex. Intensive mixing of the sample to a homogenous suspension is a crucial step in this procedure. The wash-centrifugation-remove cycle using sterile water is repeated two more times. After the last discarding of the supernatant, a pellet of approximately 0.1 ml remains at the bottom of the microcentrifuge tube.

Two spots were prepared on a MALDI-TOF target plate using a wooden toothpick. After the spots were dried, α-cyano-4-hydroxycinnamic acid (HCCA) matrix was added. The target plate was placed in the MALDI Biotyper and the analysis was initiated. If the score was higher than 1.7 in one spot, or higher than 1.5 in both of them, with the same result in genus and species, the isolate identification was accepted. The result of the Gram stain and the MALDI-TOF identification were immediately communicated to a physician on the respective ward by phone. After overnight incubation, isolated colonies were submitted to identification and AST with the automated system MicroScan (Beckman, West Sacramento, CA, US), using the Negative Combo Panel NP53 for Enterobacterales and the Negative Combo Panel NP58 for *P. aeruginosa*, according to the manufacturer's guidelines.

Rapid method

Positive blood culture bottles were processed in order to rapidly obtain a bacterial pellet; as previously described (see Fig. 1) in conventional method. The same process for MALDI-TOF identification from the pellet was performed. If the score was higher than 1.7 in one spot, or higher than 1.5 in both of them with the same result in genus and species, the isolate identification was accepted as correct, and the pellet was submitted to identification and AST directly. Isolates that did not meet these conditions were disregarded for further analysis.

In order to inoculate the MicroScan panels, a suspension was prepared directly from the pellet using the Prompt™ Inoculation System Wands, removing the standardizer before punching the pellet. The density of the inoculum was verified by sub-cultivating 100 μL of this suspension onto a blood agar plate, which was incubated 24 h at 37 °C. According to the inoculum density, two groups were established: group 1 ($\geq 10^5$ cfu/ml) and group 2 ($< 10^5$ cfu/ml). Direct AST from the pellet was further processed as with the conventional method. MIC data interpretation was performed in both methods using EUCAST breakpoint criteria, version 7.0 (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf).

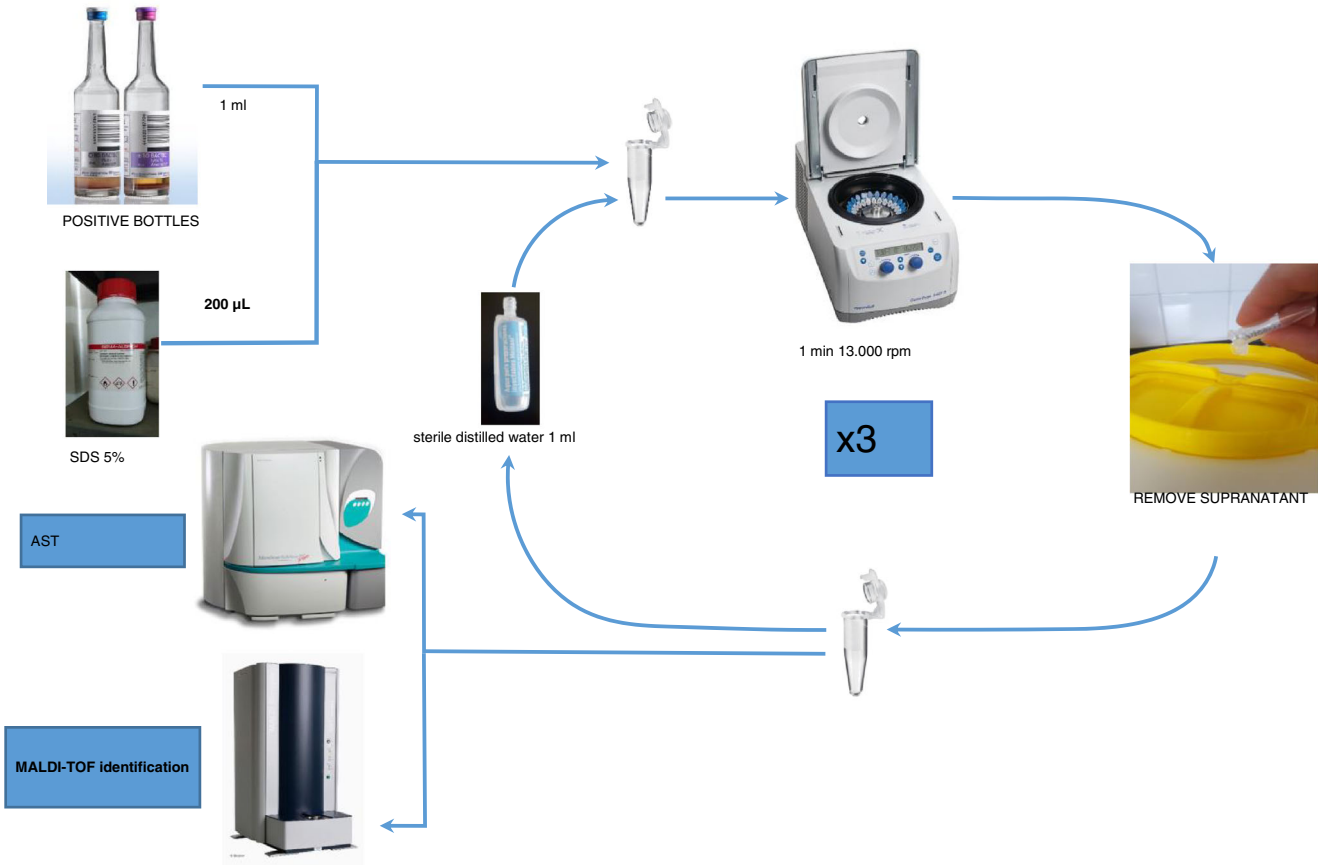


Fig. 1 Pellet obtention method: a microcentrifuge tube is filled with 1 ml of the blood culture and mixed using vortex with 200 µL of 5% sodium dodecyl sulfate (SDS) solution. After 1 min of centrifugation at 13,000 rounds per minute (RPM), the supernatant is discarded, and the pellet is resuspended in 1 ml of sterile distilled water and mixed with vortex. Intensive mixing of the sample to a homogenous suspension is a crucial step in this procedure. The wash-centrifugation-remove cycle is repeated, two more times. A pellet of approximately 0.1 ml remains in the microcentrifuge tube

Data analysis

The identifications obtained through the biochemical tests of the MicroScan (Beckman, West Sacramento, CA, USA) panels were compared with those obtained through MALDI-TOF. A comparison was made of the time from when a blood culture was positive until the result was obtained with its corresponding AST for each of the methods (conventional and rapid).

The AST results for both procedures were compared using the following parameters: categorical agreement (CA), essential agreement (EA), minor (mE), major (ME) and very major (VME) errors [13], and kappa coefficient. AST errors were calculated, considering the conventional procedure as the reference method. Error levels were expressed as percentages. Values for the kappa coefficient were interpreted according to the Landis and Koch classification [14].

Due to the antibiotic concentrations included in the panels, it was not possible to determine the exact differences in MICs between the conventional and the rapid method when MIC values were in the lower of upper limit of the studied concentration range and, consequently, were included in the Essential Agreement (% of determinations with MIC of ± 1 dilution with respect to the standard method).

Results

A total of 157 blood cultures were included in the study: 145 with Enterobacterales and 12 with *P. aeruginosa* isolates. Blood cultures in which these microorganisms were isolated were obtained from patients admitted at medical (66%) and surgical (14%) wards and intensive care units (7%) or attended at the emergency room department (13%).

None of the isolates had to be excluded as they were all identified from the pellet with scores higher than 1.7 in one spot or higher than 1.5 in two spots with the same result in genus and species on MALDI-TOF target plate. Almost 89% of Enterobacterales (129/145) and 83.3% of *P. aeruginosa* (10/12) isolates were identified from the pellet with a score higher than 1.7. We identified 107 *E. coli*, 28 *Klebsiella* spp. (24 *Klebsiella pneumoniae* and 4 *Klebsiella oxytoca*), 4

Proteus mirabilis, 3 *Serratia marcescens*, 2 *Enterobacter cloacae*, 1 *Salmonella* spp., and 12 *P. aeruginosa* isolates. No discrepancy was found between the bacterial identification obtained through MALDI-TOF and the biochemical identification obtained in the MicroScan panels.

In all cases, definitive AST results were obtained 24 h earlier with the rapid method compared with the conventional one ($p < 0.001$).

Starting from 157 isolates, a total of 2814 MIC results were generated, observing 47 cases (1.7%) of discrepancies with respect to the conventional method, 0.3% being VME and 1.3% ME.

Overall, and considering the density of the inoculum used with the rapid method, discrepancies compared with the conventional method were: (i) Group 1 (count $\geq 10^5$ cfu/ml) (96 isolates, 90 Enterobacterales and 6 *P. aeruginosa*): 1.9% (32/1722 MICs) with 0.2% VME and 1.4% ME; (ii) Group 2 (count $< 10^5$ cfu/ml) (61 isolates, 55 Enterobacterales and 6 *P. aeruginosa*), 1.5% (15/1092 MICs) with 0.7% VME and 1% ME.

Considering 145 Enterobacterales alone, discrepancies were observed in 35 out of 2610 MIC results (1.3%), 0.3% being VME and 1.1% ME. For all *P. aeruginosa*, discrepancies were observed in 12 out of the 204 MIC results (5.9%), 0.6% being VME and 4.3% ME.

Overall, essential agreement on Enterobacterales ($n = 145$) was 100% for amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, ertapenem, tobramycin, amikacin, and tigecycline. For cefoxitin, ceftazidime, cefotaxime, cefepime, and ciprofloxacin, EA was 99%. EA was 98% for aztreonam and 97% for cefazoline cefuroxime and gentamicin. The lowest EA was 95% for ampicillin. CA was 100% for amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, ertapenem, and tigecycline. For cefepime and amikacin, CA was 99%. Ceftazidime, cefotaxime, aztreonam, and ciprofloxacin had a CA of 98%. For cefuroxime and gentamicin, CA was 96%. Tobramycin CA was 94% and ampicillin CA was 93%. VME was 1% for ampicillin, cefuroxime, cefepime, gentamicin, and tobramycin. ME was 6% for ampicillin, 3% for gentamicin, 2% for aztreonam, and 1% for cefuroxime, ceftazidime, cefotaxime, tobramycin, and ciprofloxacin. Minor error was 4% for tobramycin, 2% for cefuroxime, and 1% for ceftazidime, cefotaxime, gentamicin, amikacin, and ciprofloxacin. Kappa index had values between 0.8 and 1 for ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefuroxime, ceftazidime, cefotaxime, cefepime, aztreonam, amikacin, and ciprofloxacin. The only antibiotics with lower kappa values were tobramycin and gentamicin (see Supplementary Table 1).

Tables 1 and 2 summarize the data of Enterobacterales according to the antibiotic and broken down by the colony count. Among Enterobacterales, there was complete agreement in 18 *E. coli* (12 with an inoculum $\geq 10^5$ cfu/ml and 6 with $< 10^5$ cfu/ml), 8 *Klebsiella* spp. (6 with $\geq 10^5$ cfu/ml and 2 with lower counts), 1 *Enterobacter* ($\geq 10^5$ cfu/ml), 1 *S. enterica* ($\geq 10^5$ cfu/ml), and 1 *S. marcescens* ($< 10^5$ cfu/ml). As to resistance mechanisms, no carbapenemases were detected but of the 107 *E. coli*, 10 were extended-spectrum beta-lactamase (ESBL)-producers. Specific data referring to the most frequent microorganisms (*E. coli* and *Klebsiella* spp.) are shown in the Supplementary Table 2.

In *P. aeruginosa*, EA was 100% for imipenem, gentamicin, ciprofloxacin, levofloxacin, colistin, minocycline, and fosfomycin. Ticarcillin, ceftazidime, aztreonam, meropenem, tobramycin, and amikacin had an EA of 92%. For piperacillin-tazobactam EA was 84%, and 76% for cefepime. CA was 100% for imipenem and ciprofloxacin and 92% for ticarcillin, cefepime, gentamicin, and levofloxacin. Piperacillin-tazobactam, meropenem, tobramycin, amikacin, and colistin had a CA of 84% whereas ceftazidime and aztreonam had a CA of 76%. Only ceftazidime reported VME (one out of 12, 8.3%). Piperacillin-tazobactam had an ME of 16.6% (2/12), and ticarcillin, ceftazidime, tobramycin, amikacin, and colistin had ME of 8.3% (1/12). Minor error was 25% (3/12) for aztreonam; 16.6% (2/12) for meropenem; and 8.3% (1/12) each for ceftazidime, cefepime, gentamicin, tobramycin, amikacin, levofloxacin, and colistin. Ticarcillin, imipenem, and ciprofloxacin had kappa values between 0.8 and 1. Other kappa results for *P. aeruginosa* are included in Supplementary Table 3.

Tables 3 and 4 summarize the data of *P. aeruginosa* according to the antibiotic and broken down by the colony count.

Discussion

The main goal of this study was to develop an accurate and fast technique by using the pellet of positive blood cultures, to allow the direct identification and AST of Enterobacterales and *P. aeruginosa* isolates. This easy and inexpensive methodology could be implemented in any microbiology laboratory using MALDI-TOF and a semiautomatic method for AST, such as MicroScan. This method confers a wide advantage over other techniques which use specific equipment that is not always available in all laboratories [8, 10, 11].

Results obtained in this study show that the identification of Enterobacterales and *P. aeruginosa* isolates using the pellet is highly reliable, and no identification errors were found when compared with the conventional method from bacterial colonies, similar to that described in prior studies [15].

On the other hand, the main reason to accelerate AST results of BSI is to offer the clinicians the possibility of an early treatment adjustment. Our method decreased this time in 24 h as is shown in Fig. 2. This method, included within antimicrobial stewardship programs, can contribute to diminish morbidity and mortality in patients with bacteremia, especially in

Table 1 Results from the conventional method and variations produced by the rapid method for Enterobacterales according to count colony and kappa coefficient

UFC/ml	Antibiotic	Eucast interpretation with conventional method (no. of isolates)			Changes in Eucast interpretation with rapid method (no. of isolates)									
		S	I	R	Stay S	Stay R	R ➔ S (VME)	S ➔ R (ME)	R ➔ I	I ➔ S	I ➔ R	S ➔ I	Kappa	Interpretation
$\geq 10^5$ ($n = 90$)	Ampicillin	63		27	56	26	1	7	0	0	0	0	0.801	Very good
	Amoxicillin-clavulanic ac.	76		14	76	14	0	0	0	0	0	0	1	Very good
	Piperacillin-tazobactam	82	1	7	82	7	0	0	0	0	0	0	1	Very good
	Cefuroxime	76		14	75	11	1	1	2	0	0	0	0.822	Very good
	Ceftazidime	83		7	82	7	0	0	0	0	0	1	0.932	Very good
	Cefotaxime	82	1	7	82	7	0	0	0	0	0	0	1	Very good
	Cefepime	82	1	7	82	7	0	0	0	0	0	0	1	Very good
	Aztreonam	73	3	14	73	14	0	0	0	0	0	0	1	Very good
	Imipenem	90			90	0	0	0	0	0	0	0	1	Very good
	Ertapenem	90			90	0	0	0	0	0	0	0	1	Very good
	Gentamicin	79		11	75	8	1	4	2	0	0	0	0.733	Good
	Tobramycin	80	3	7	78	6	0	1	1	0	3	1	0.698	Good
	Amikacin	86	2	2	86	2	0	0	0	1	0	0	0.828	Very good
	Tigecycline	89		1	89	1	0	0	0	0	0	0	1	Very good
	Ciprofloxacin	66	4	20	66	19	0	1	0	0	0	0	0.967	Very good
$< 10^5$ ($n = 55$)	Ampicillin	36		19	35	18	1	1	0	0	0	0	0.92	Very good
	Amoxicillin-clavulanic ac.	39		16	39	16	0	0	0	0	0	0	1	Very good
	Piperacillin-tazobactam	51		4	51	4	0	0	0	0	0	0	1	Very good
	Cefuroxime	48		7	47	6	1	1	0	0	0	0	0.836	Very good
	Ceftazidime	50	1	4	49	4	0	1	0	0	0	0	0.879	Very good
	Cefotaxime	49	2	4	46	4	0	2	0	0	0	1	0.722	Good
	Cefepime	51	1	3	51	2	1	0	0	0	0	0	0.791	Good
	Aztreonam	48		7	46	7	0	2	0	0	0	0	0.854	Very good
	Imipenem	55			55	0	0	0	0	0	0	0	1	Very good
	Ertapenem	55			55	0	0	0	0	0	0	0	1	Very good
	Gentamicin	47	3	5	47	4	1	0	0	0	0	0	0.879	Very good
	Tobramycin	49	2	4	47	4	1	0	1	0	0	0	0.782	Good
	Amikacin	51	2	2	51	2	0	0	0	0	1	0	0.825	Very good
	Tigecycline	53		2	53	2	0	0	0	0	0	0	1	Very good
	Ciprofloxacin	44	5	6	44	6	0	0	0	2	0	0	0.855	Very good

Table 2 Differences between direct AST method and the reference one for Enterobacterales ($n = 145$) according to colony count and percentage of minor errors (mE), major errors (ME), and very major errors (VME) for different antibiotics

Antibiotics	Count $\geq 10^5$ cfu/ml ($N = 90$)					Count $< 10^5$ cfu/ml ($N = 55$)				
	EA (%)	CA (%)	VME (%)	ME (%)	mE (%)	EA (%)	CA (%)	VME (%)	ME (%)	mE (%)
Ampicillin	93	91	1	8	0	96	96	2	2	0
Amoxicillin-clavulanic ac.	100	100	0	0	0	100	100	0	0	0
Piperacillin-tazobactam	100	100	0	0	0	100	100	0	0	0
Cefuroxime	96	96	1	1	2	96	96	2	2	0
Ceftazidime	99	99	0	0	1	98	98	0	2	0
Cefotaxime	100	100	0	0	0	96	94	0	4	2
Cefepime	99	100	0	0	0	98	98	2	0	0
Aztreonam	99	100	0	0	0	96	96	0	4	0
Imipenem	100	100	0	0	0	100	100	0	0	0
Ertapenem	100	100	0	0	0	100	100	0	0	0
Gentamicin	97	93	1	4	2	98	98	2	0	0
Tobramycin	100	94	0	1	5	100	96	2	0	2
Amikacin	100	99	0	0	1	100	98	0	0	2
Tigecycline	100	100	0	0	0	100	100	0	0	0
Ciprofloxacin	99	99	0	1	0	100	96	0	0	4

Total: 0.33% VME y 1.1% ME

EA essential agreement, *CA* categorical agreement, *ME* major errors, *VME* very major errors, *mE* minor errors

those who are critically ill or infected with multidrug-resistant pathogens [16–20].

As to MIC results, differences were only found in 1.7% of the MIC determinations, 0.3% being VME and 1.3% ME. Therefore, this AST technique is in agreement with standard evaluation criteria (< 10% of total errors, including < 1.5% VME, and < 3% ME [21]). For validation of the procedure, at least 90% agreement in AST results is mandatory [13].

Although Enterobacterales fulfilled this validation standard, one of the limitations of the study was that the inclusion of Enterobacterales consisted of 107 *E. coli* and 24 *K. pneumoniae*, which is 90.3% (131/145 Enterobacterales) of the included isolates, so this study does not allow analyzing the differences in the performance of these tests in relation to the species.

In *P. aeruginosa*, the validation criterion for ME was not reached, which translated into false resistance implying a reduction of the therapeutic options available.

The reason for these discrepancies might be due to the poor homogeneity of the bacterial load in the pellet [12, 22–24]. A greater number of congruent data were obtained with an inoculum $\geq 10^5$ cfu/ml (group 1), which is similar to the standard recommended for AST. This means that one of the critical points of the procedure is to obtain an adequate inoculum. For this purpose, a homogeneous pellet suitable for inoculation in each panel is required.

In the case of Enterobacterales, the biggest problems were found with aminoglycosides and first-/second-generation cephalosporins, and cefazolin was the antibiotic exhibiting greater discrepancies. Despite these discrepancies, there was only 0.3% VME, so this rapid AST method appears to be reliable to be used routinely. ME occurs mostly with ampicillin and gentamicin. These discrepancies might, in general, have a limited impact. In the actual context of multidrug-resistant pathogens, gentamicin is usually prescribed in combination with a β-lactam antibiotic. According to genus and species, it should be noted that discrepancies with aminoglycosides were only observed with *E. coli*. It is of note that *K. pneumoniae* and *K. oxytoca* did not exhibit false resistance (ME) for any antibiotic thus not limiting the available therapeutic options.

In the case of *P. aeruginosa*, as the number of isolates included in the study was small, congruent results were lower than for Enterobacterales. Nevertheless, the incidence of bacteremia due to *P. aeruginosa* is also lower than for Enterobacteriaceae [25]. The EA for antibiotics tested in *P. aeruginosa* was at least 92%, except for piperacillin/tazobactam (84%) and cefepime (76%). The CA was at least 84%, except for ceftazidime and aztreonam, both with 76%. No VME occurred, except for ceftazidime (one out of 12 isolates, 8.3%). ME were due to piperacillin-tazobactam (two out of 12 isolates, 16.6%), ticarcillin, ceftazidime, tobramycin, amikacin, and colistin (one out of 12 isolates, 8.3%). Correlation (kappa index) was poor for aminoglycosides and weak for ceftazidime and colistin, the most common therapeutic options. The

Table 3 Results from the conventional method and variations produced by the rapid method for *Pseudomonas aeruginosa* according to colony count and kappa coefficient

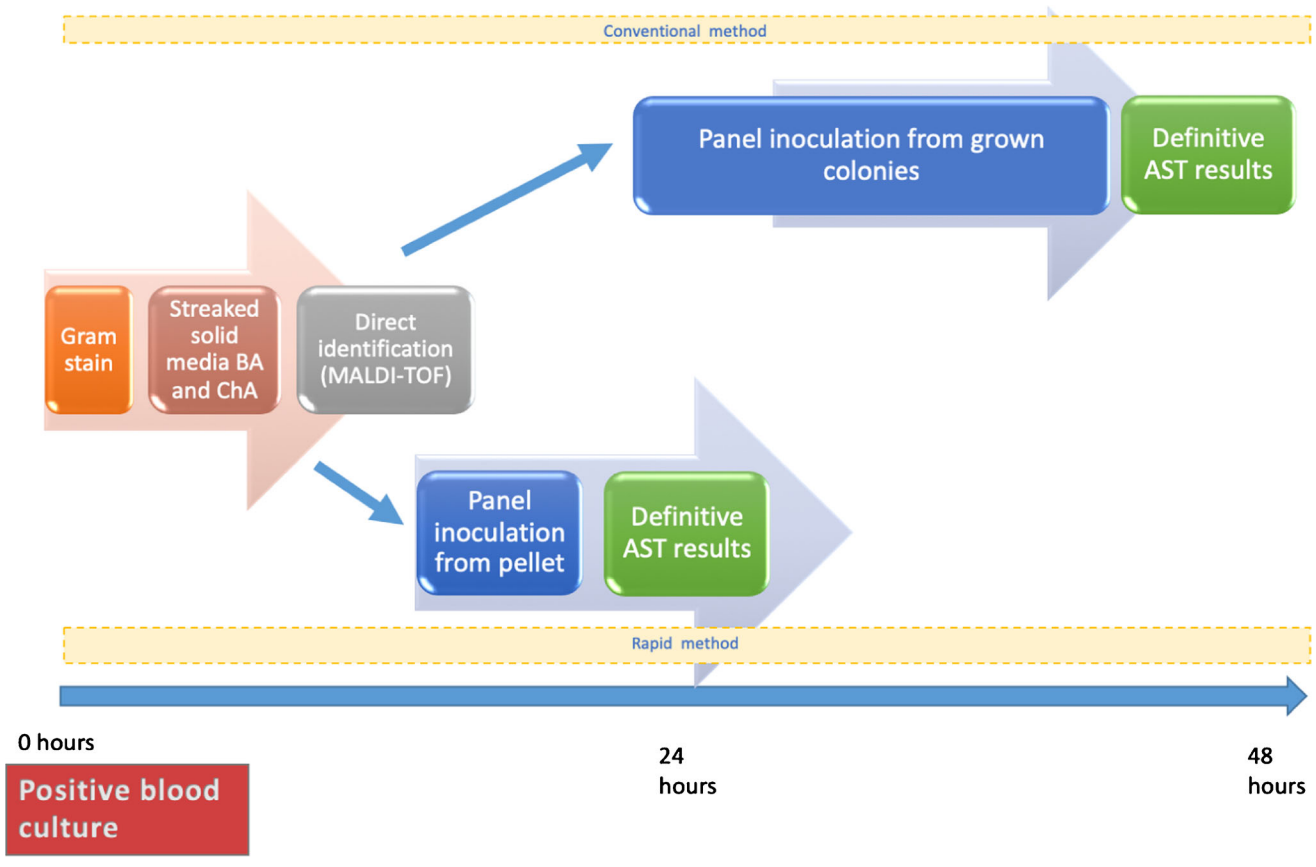
UFC/ml	Antibiotic	Eucast interpretation with conventional method (no. of isolates)			Changes in Eucast interpretation with rapid method (no. of isolates)									
		S	I	R	Stay S	Stay R	R ➔ S (VME)	S ➔ R (ME)	R ➔ I	I ➔ S	I ➔ R	S ➔ I	Kappa	Interpretation
$\geq 10^5$ ($n = 6$)	Ticarcillin	3		3	2	3	0	1	0	0	0	0	0.667	Good
	Piperacillin/tazobactam	5		1	3	1	0	2	0	0	0	0	0.333	Weak
	Ceftazidime	6		0	5	0	0	1	0	0	0	0	0	Poor
	Cefepime	5		1	5	1	0	0	0	0	0	0	1	Very good
	Aztreonam	1	4	1	1	1	0	0	0	3	0	0	0.118	Poor
	Imipenem	6	0	0	6	0	0	0	0	0	0	0		
	Meropenem	5	1	0	4	0	0	0	0	1	0	1	0.333	Weak
	Gentamicin	6		0	6	0	0	0	0	0	0	0		
	Tobramycin	6		0	5	0	0	1	0	0	0	0	0	Poor
	Amikacin	6		0	4	0	0	1	0	0	0	1	0.143	Poor
	Ciprofloxacin	4		2	4	2	0	0	0	0	0	0	1	Very good
	Levofloxacin	5		1	5	1	0	0	0	0	0	0	1	Very good
	Colistin	6		0	5	0	0	0	0	0	0	1	0.4	Weak
$< 10^5$ ($n = 6$)	Ticarcillin	1		5	1	5	0	0	0	0	0	0	1	Very good
	Piperacillin/tazobactam	4		2	4	2	0	0	0	0	0	0	1	Very good
	Ceftazidime	4		2	3	1	1	0	1	0	0	0	0.386	Weak
	Cefepime	5		1	5	0	0	0	1	0	0	0	0	Poor
	Aztreonam	0	6	0	0	0	0	0	0	0	0	0	1	Very good
	Imipenem	5	1	0	5	0	0	0	0	0	0	0	1	Very good
	Meropenem	6	0	0	6	0	0	0	0	0	0	0	1	Very good
	Gentamicin	5		1	5	0	0	0	1	0	0	0	0	Poor
	Tobramycin	5		1	5	0	0	0	1	0	0	0	0	Poor
	Amikacin	5	1	0	5	0	0	0	0	0	0	0	1	Very good
	Ciprofloxacin	5		1	5	1	0	0	0	0	0	0	1	Very good
	Levofloxacin	5		1	4	1	0	0	0	0	0	1	0.667	Good
	Colistin	6		0	5	0	0	1	0	0	0	0	0	Poor

Table 4 Differences between direct AST method and the reference one for *Pseudomonas aeruginosa* ($n = 12$) according to colony count and percentage of minor errors (mE), major errors (ME), and very major errors (VME) for different antibiotics

Antibiotics	Count $\geq 10^5$ cfu/ml ($N = 6$)					Count $< 10^5$ cfu/ml ($N = 6$)				
	EA (%)	CA (%)	VME (%)	ME (%)	mE (%)	EA (%)	CA (%)	VME (%)	ME (%)	mE (%)
Ticarcillin	83	83	0	17	0	100	100	0	0	0
Piperacillin/tazobactam	66	66	0	33	0	100	100	0	0	0
Ceftazidime	83	83	0	17	0	100	66	17	0	17
Cefepime	83	100	0	0	0	66	83	0	0	17
Aztreonam	83	50	0	0	50	100	100	0	0	0
Imipenem	100	100	0	0	0	100	100	0	0	0
Meropenem	83	66	0	0	33	100	100	0	0	0
Gentamicin	100	100	0	0	0	100	83	0	0	17
Tobramycin	83	83	0	17	0	100	83	0	0	17
Amikacin	83	66	0	17	17	100	100	0	0	0
Ciprofloxacin	100	100	0	0	0	100	100	0	0	0
Levofloxacin	100	100	0	0	0	100	83	0	0	17
Colistin	100	83	0	0	17	100	83	0	17	0

Total: 0.6% VME y 4.3% ME

EA essential agreement, *CA* categorical agreement, *ME* major errors, *VME* very major errors, *mE* minor errors



BA → Blood Agar ChA → Chocolate agar AST → Antimicrobial susceptibility testing

Fig. 2 Temporal comparison of conventional and rapid method workflows. BA, blood agar; ChA, chocolate agar; AST, antimicrobial susceptibility testing

ME was important on piperacillin-tazobactam, limited patient's therapeutic options, while ceftazidime ME and VME hindered results' accuracy. Analyzing data by colony count, in *P. aeruginosa* with $\geq 10^5$ cfu/ml counts, the antibiotic that produced most discrepancies was piperacillin-tazobactam (CA = 66% and ME = 33%), whereas ceftazidime discrepancies were found in those with reported counts $< 10^5$ cfu/ml (CA = 66% and VME = 17%).

Another limitation of this study is that only 10% of *E. coli* were ESBL-producers but good concordance results were observed (data not shown). It would be desirable to increase the number of isolates with this phenotype and to test carbapenemase-producing strains. Also, a limitation is that the pellet method is not applicable in polymicrobial bacteremia, as results of AST might be unreliable. Moreover, we have not included other Gram-negative bacilli different from Enterobacterales or *P. aeruginosa*, which might have relevance in the nosocomial setting such as *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, or *Achromobacter xylosoxidans*.

In the case of anaerobes as well as yeasts and Gram-positive cocci, direct MALDI-TOF identification is reliable with some limitations or modifications in the extraction process, whereas direct AST still needs to improve its reliability [12, 22, 24, 26].

In summary, using the pellet from positive blood cultures for the identification and direct AST of Enterobacterales (mostly *E. coli* and *Klebsiella* sp.) isolates is as reliable as colony-based automated microdilution methods but with the advantage of providing results 24 h earlier. For *P. aeruginosa* and Enterobacterales different than *E. coli* or *Klebsiella* sp. more studies are needed in order to be able to conclude that direct method is also reliable. This processing method appears to be a valuable tool for improving the turnaround time in blood culture diagnostics, which could make a difference in therapeutic management, especially in high-risk patients.

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Compliance with ethical standards

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

Ethical approval The study was approved by the local ethics committee as it stated in the record number 332 of this committee.

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