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ORIGINAL ARTICLE

Evaluation of the Alfred AST® system for rapid antimicrobial susceptibility testing directly from positive blood cultures

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Abstract

To assess the concordance of antimicrobial susceptibility testing results obtained by the Alfred AST® system performed directly from positive blood cultures in comparison with the standard susceptibility test results performed from isolated colonies by an automated broth microdilution method and to determine the applicability of Alfred AST® system in the routine of our blood culture laboratory. This system is based on the detection of growth by turbidimetry through a technology based on light scattering. Antimicrobial susceptibility testing was performed directly from positive bottles by the Alfred AST® system (Alifax, Padova, Italy). The broth microdilution method (MicroScan, Beckman Coulter, CA, USA) performed to the isolates was considered the standard for comparison. We evaluated 115 significant episodes of bacteremia produced by 51 Gram-negative Enterobacterales, 8 *Pseudomonas* spp., 2 non-fermenting Gram-negative rods, 7 *Staphylococcus aureus*, 23 coagulase-negative *Staphylococcus*, 12 *Enterococcus* spp., and 12 *Streptococcus* spp. We performed 828 susceptibility determinations with a categorical agreement with the standard method of 97.1%. Only 24 errors (2.9%) were detected. It should be pointed out that for staphylococci and glycopeptides the correlation was only 87% and for non-fermenting Gram-negative rods and piperacillin/tazobactam was only 88.9%. Time to get antibiogram results by Alfred AST® system was 5 versus 48 h for the standard microdilution method from the isolated colonies. The Alfred AST® system is a useful and rapid method to obtain antimicrobial susceptibility results within the same work shift after blood culture positivity.

Keywords Blood cultures · Rapid antimicrobial testing · Sepsis · Bactec FX · Bacteremia · Alfred 60AST

Introduction

The presence of living microorganisms in the blood of a patient is an event of major diagnostic and prognostic importance. Rapid detection of pathogens (bacteria and fungi) in bloodstream infections is one of the major challenges in clinical microbiology laboratories. Since blood cultures are still considered the gold standard diagnostic procedure for sepsis, processing of blood cultures is a priority [1, 2].

When blood cultures yield a clinically important pathogen, not only an infectious cause is established for the patient's illness but also the etiologic agent becomes available for antimicrobial susceptibility testing (AST) and optimization of therapy.

The microbiology laboratory plays a key role in the diagnosis of bloodstream infections (BSIs) and the reduction of the time to identification and AST is a major goal [3]. The rapid availability of results should allow early administration of targeted antimicrobial treatment, hereby potentially improving the clinical outcome and also reducing the length of hospital stay and associated costs. A more rapid and optimal therapy furthermore limits antibiotic usage and the development of resistance [4–6].

Nowadays, after a blood culture is positive, information regarding AST can be delayed for 24 h for preliminary results (if an antibiogram is performed directly from the blood culture bottle, which is not standardized) and up to 48–72 h for definite standard AST results and minimum inhibitory concentration (MIC) determination. New molecular methods applied directly in the blood culture can provide bacterial identification and detection of resistance genes in less than 2 h, but this information is limited to certain microorganisms and genes

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(i.e., methicillin resistance, carbapenemase production, extended-spectrum ß-lactamase production, and glycopeptide resistance) and do not provide data on AST to different antimicrobials. The Alfred AST® (Alifax, Padova, Italy) is an automated system for the rapid determination of AST directly from blood cultures. The system is based on the detection of growth by turbidimetry through a technology based on light scattering. The Alfred AST® system is able to provide susceptibility results of different microorganisms and different antimicrobial agents at the same time in 5–7 h from positive blood culture bottles. Few data are available related to the concordance of this method with standard or commercialized broth microdilution methods and/or the usefulness of the Alfred AST® system in the rapid determination of AST from blood culture isolates.

The objectives of this study were to assess the concordance of AST results obtained by the Alfred AST® system performed directly from positive blood cultures in comparison with the standard susceptibility test results performed from isolated colonies by an automated broth microdilution method and to determine the applicability of Alfred AST® system in the routine of our blood culture laboratory.

Table 1 Organism distribution

Organism	No. significant episodes of bacteremia
Enterobacterales	51
Escherichia coli	35
Klebsiella pneumoniae	6
Proteus mirabilis	4
Klebsiella oxytoca	3
Serratia marcescens	1
Pantoea agglomerans	1
Salmonella spp.	1
Non-fermenting GNR	8
Pseudomonas aeruginosa	5
Pseudomonas stutzeri	1
Stenotrophomonas maltophilia	1
Acinetobacter spp.	1
Coagulase-negative Staphylococci	23
Staphylococcus aureus	7
Enterococcus spp.	12
Enterococcus faecalis	10
Enterococcus faecium	2
Streptococcus spp.	12
Streptococcus pneumoniae	7
Group *viridans* streptococci	3
Streptococcus pyogenes	1
Streptococcus agalactiae	1
Total no. of organisms	115

GNR Gram-negative rod

Methods

Hospital setting

The study was conducted at the Hospital General Universitario Gregorio Marañón, a 1200-bed tertiary hospital in Madrid, Spain, attending a population of 715,000 inhabitants. Our laboratory processes approximately 35,000 blood cultures per year and detects about 1500 episodes of bacteremia per year.

Study definitions

An episode of bacteremia was considered significant according to the American Society for Microbiology (ASM) criteria [7].

The types of errors were defined according also to the ASM criteria [8]: a *very major error* was considered when Alfred AST® categorized the isolate as susceptible and the reference method as resistant, a *major error* was considered when Alfred AST® categorized the isolate as resistant and the reference method as susceptible, and a *minor error* was considered when Alfred AST® or the standard method categorized the isolate as intermediate and the other method as susceptible or resistant.

Microbiology workflow

The study was performed from November 2015 to January 2016. We included all positive significant episodes of bacteremia detected at the beginning of our morning shift (8 am to 3 pm) in order to be able to perform all the procedures required for the Alfred AST® system to provide the susceptibility results within the same morning shift. The system allows the selection of the antimicrobials to be tested, as determined by the microbiologist, according to the microorganism. Episodes of bacteremia with more than one bacterial species were excluded. Episodes of bacteremia by anaerobes were also excluded.

Blood cultures were processed using the Bactec FX® automated system (Becton Dickinson, Franklin Lakes, NJ, USA) and incubated for 5 days. Gram stain was performed to all positive bottles. Microorganism identification was performed directly from the positive bottles by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) using the Bruker Biotyper System (Bruker Daltonik GmbH, Bremen, Germany) only for significant episodes of bacteremia.

AST was performed directly from positive bottles by the Alfred AST® system (Alifax, Padova, Italy) assigning one of the following antimicrobial panels according to the direct identification obtained by MALDI-TOF:

Table 2 Correlation (%) between the susceptibility of Alfred® AST system and the reference method (broth microdilution by MicroScan) using Enterobacterales

Enterobacterales ($N = 51$)	Ampicillin	Ampicillin/ sulbactam	Piperacillin/ tazobactam	Cefotaxime	Aztreonam	Meropenem	Gentamicin	Amikacin	Ciprofloxacin	Cotrimoxazole
No. of comparisons	51	48	51	51	49	48	31	51	51	46
Correlation (%)	98.0	91.6	100	96.1	100	100	100	100	98.0	97.8

1. Enterobacterales: ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefotaxime, aztreonam, meropenem, gentamicin, amikacin, ciprofloxacin, and cotrimoxazole
2. Non-fermenting Gram-negative rods: piperacillin/tazobactam, meropenem, gentamicin, amikacin, and ciprofloxacin
3. Gram-positive cocci in clusters: cefoxitin, gentamicin, amikacin, clindamycin, levofloxacin, cotrimoxazole, rifampin, linezolid, vancomycin, and teicoplanin
4. Gram-positive cocci in pairs or chains: ampicillin, linezolid, vancomycin, and high-level gentamicin

In addition, we cultured all positive bottles onto Columbia blood agar (incubation in air), chocolate agar (incubation in 5% CO_2), and Brucella agar (incubation in anaerobic atmosphere) at 35–37 °C for 48 h.

For comparison, AST was performed from colonies grown onto Columbia blood agar by the automated broth microdilution method (MicroScan, Beckman Coulter, CA, USA) that was considered the standard for comparison. Interpretation of clinical categories of susceptible and resistant was determined following the EUCAST recommendations. The categorical agreement between the two susceptibility methods was determined. Identification of the isolates was performed by MALDI-TOF and/or MicroScan.

Results

From November 2015 to January 2016, we studied 115 episodes of bacteremia. The most commonly identified microorganisms were *Escherichia coli*, coagulase-negative staphylococci, and *Enterococcus faecalis* (Table 1). Six Enterobacterales produced extended-spectrum beta-lactamases (ESBL) and one also produced an OXA-48-like carbapenemase.

We were able to compare 828 susceptibility determinations (Alfred AST® system from positive bottles vs MicroScan from isolates). The categorical agreement was 97.1% and 24 errors were detected (2.9%): 1 minor error, 22 corresponding to major errors, and only 1 very major error (one *Staphylococcus epidermidis* isolate resistant to levofloxacin according to the reference method and susceptible according to Alfred AST® system). We performed e-testing from the isolate and disc diffusion directly from the positive bottle to verify the very major error.

The results of the correlation between the two susceptibility methods are shown in Tables 2, 3, 4, and 5 for the different groups of microorganisms. For Enterobacterales, the categorical agreement was 97.9% with one minor error and nine major errors. With the exception of ampicillin/sulbactam (91.6% agreement), all the antimicrobials showed correlations greater than 95%. The production of ESBL by six isolates of Enterobacterales (four *E. coli* and two *Klebsiella pneumoniae*)

Table 3 Correlation (%) between the susceptibility of the Alfred® AST system and the reference method (broth microdilution by MicroScan) using non-fermenting Gram-negative rods

Pseudomonas spp./other NFGNR ($N = 8$)					
	Piperacillin/ tazobactam	Meropenem	Gentamicin	Amikacin	Ciprofloxacin
No. of comparisons	8	8	5	8	5
Correlation (%)	88.9	90	85.7	100	100

was detected by the interpretation of the antibiogram obtained by Alfred AST®. In the case of the OXA-48-like-ESBL-producing *K. pneumoniae*, we only detected the production of the ESBL since the carbapenem that we included in the Alfred AST panel was meropenem and this carbapenemase showed only resistance to ertapenem and very low hydrolytic activity against meropenem. In fact, by using the MicroScan system, the isolate was only resistant to ertapenem.

The categorical agreement for staphylococci was 96.4%. For all the antimicrobials tested, the correlation was very high (greater than 96%) with the exception of the glycopeptides for which the correlation was 87%. We detected eight errors in the susceptibility determinations against staphylococci, and one of them was a very major error. All these errors were detected for coagulase-negative staphylococci. In the case of *S. aureus* (seven isolates), the categorical agreement for all antimicrobials tested was 100.0% (total agreement).

The categorical agreement for enterococci (12 isolates) was 97.6%. There was only one mayor error for the detection of high-level gentamicin resistance in one *Enterococcus faecium* isolate. The categorical agreement for *Streptococcus* spp. was 97.7% with only one mayor error in the detection of vancomycin resistance in an *S. pneumoniae* isolate.

For non-fermenting Gram-negative rods, the categorical agreement was 90.9% with four major errors. We included one major error in the detection of trimethoprim/sulfamethoxazole susceptibility of an isolate of *Stenotrophomonas maltophilia*.

The turnaround time for the detection of the direct antibiogram by Alfred AST® system was 5 versus 48 h for the reference method. This time to results allows Alfred AST® to be applied and informed in the routine of our blood culture laboratory in the same work shift.

Discussion

In this study including 115 BSIs, Alfred AST® system proved to be an adequate method to test antimicrobial susceptibilities of the bacterial isolates from positive blood cultures with an excellent categorical correlation (97.1%) with the reference method. Performing of the technique directly from the positive blood culture bottles reduced the time of the determination of antimicrobial susceptibility. Results were available 5 h after the processing of the positive blood cultures is started.

We only detected one very major error, 22 major errors, and one minor error. These make the system more reliable as the great majority (95.8%) of the errors of Alfred AST® system consisted in categorizing the bacteria as resistant, while with the reference method, the isolates were reported as susceptible. Categorical agreement rates for glycopeptides in coagulase-staphylococci were 86%. For us, this was a matter of concern. Some studies have described the lack of concordance in the detection of glycopeptide susceptibility for staphylococci isolates using different standardized susceptibility methods [9–11]. Anyway, we consider that Alfred AST® system should improve the detection of glycopeptide susceptibility.

MALDI-TOF MS has demonstrated to be useful in the rapid identification of microorganisms directly from positive blood cultures [12]. In a study, the authors evaluate the combination of MALDI-TOF and Vitek-2 Compact System to achieve rapid identification and susceptibility testing by direct inoculation from positive blood cultures [13]. The average times required to obtain AST testing results were 6.45 ± 4.52 and 9.55 ± 2.97 h for Gram-negative rods and Gram-positive cocci, respectively. In our setting, time to susceptibility results

Table 4 Correlation (%) between the susceptibility of the Alfred® AST system and the reference method (broth microdilution by MicroScan) using *Staphylococcus* spp.

Staphylococcus spp. ($N = 30$)										
	Cefoxitin	Amikacin	Gentamicin	Clindamycin	Levofloxacin	Cotrimoxazole	Rifampin	Linezolid	Vancomycin	Teicoplanin
No. of comparisons	29	28	27	27	26	24	24	22	23	15
Correlation (%)	96.5	96.4	100	100	96.1	100	100	100	86.9	86.7

Table 5 Correlation (%) between the susceptibility of the Alfred® AST system and the reference method (broth microdilution by MicroScan) using Gram-positive cocci arranged in pairs or chains

	Enterococcus spp. (*N* = 12)				*Streptococcus* spp. (*N* = 12)		
	Ampicillin	Linezolid	Vancomycin	High-level gentamicin	Ampicillin	Linezolid	Vancomycin
No. of comparisons	12	10	12	8	11	9	10
Correlation (%)	100	100	100	87.5	100	100	90

should be available in less than 5–6 h in order to communicate the information to the responsible physician or to the infectious diseases consultants who are physically present at the hospital only in the morning shift. Alfred AST® system provides real-time growth curves for the bacteria with or without antimicrobials. Although final time to results are 3 or 5 h depending on the antimicrobial, the manual reading of the curves may anticipate the definitive results as most of the curves show a clear resistance or susceptible pattern prior to the final readings.

Other molecular methods, like Verigene® and Filmarray®, allow rapid identification for a limited number of bacterial or yeast identification and detection of some resistance markers directly from blood culture bottles is available [14–16]. The time to result of these two former systems is shorter than the time to result of the method we evaluated; however, the number of antimicrobials available by Alfred AST® system is much higher and the system is flexible to design the more adequate panel of antimicrobials for every setting. However, using the Alfred AST® system involves prior identification method like MALDI-TOF in order to assign the proper antimicrobial panel.

Our study has limitations. First, the study was performed in a single center with a small number of clinical samples. Besides, as we could only include seven cases of bacteremia caused by non-fermenting Gram-negative rods, the conclusion for this group of microorganism was limited. Besides, the growth of these microorganisms in the system was not always adequate. Twenty-seven percent of the cases of bacteremia caused by *Pseudomonas aeruginosa* could not be evaluated due to insufficient growth. The exclusion of the polymicrobial bacteremia is another limitation of this study. In our setting, 10% of all cases of bacteremia are polymicrobial.

In conclusion, the evaluation of the Alfred AST® system for the determination of antimicrobial susceptibility directly from positive blood culture bottles showed an excellent correlation of this rapid method with the standard microdilution method at least 1 day earlier than the preliminary results were obtained. This allows clinical decisions in the same work shift that the episode of bacteremia is detected. Studies are needed to assess the clinical impact of the application of the Alfred AST® system on antimicrobial treatment, length of hospitalization, medical costs, and mortality.

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Compliance with ethical standards

Conflict of interest The authors declare that they do not have conflicts of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Baron EJ, Weinstein MP, Dunne WM, Yagupsky P, Welch DF, Wilson DM (2005) In: Coordinating ed., Baron EJ (eds) Cumitech 1C: blood cultures IV. American Society for Microbiology, Washington, DC
2. Kim TJ, Weinstein MP (2013) Update on blood cultures: how to obtain, process, report, and interpret. Clin Microbiol Infect 19:513–520
3. Munson EL, Diekema DJ, Beekmann SE, Chapin KC, Doern GV (2003) Detection and treatment of bloodstream infection: laboratory reporting and antimicrobial management. J Clin Microbiol 41:495–497
4. Angela MH, Newton D, Kunapuli A, Gandhi TN, Washer LL, Isip J et al (2013) Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. Clin Infect Dis 57:1237–1245
5. Bauer KA, West JE, Balada-Llasat JM, Pancholi P, Stevenson KB, Goff DA (2010) An antimicrobial stewardship program's impact with rapid polymerase chain reaction methicillin-resistant *Staphylococcus aureus*/*S. aureus* blood culture test in patients with *S. aureus* bacteremia. Clin Infect Dis 51:1076–1080
6. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Land GA et al (2013) Integrating rapid pathogen identification and

antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med 137:1247–1254
7. York MK, Henry M, Gilligan P (2007) Blood cultures. Clinical detection and interpretation. In: García LS (ed) Clinical microbiology procedures handbook, Third edn. ASM Press, Washington, DC, pp 3.4.1.1–3.4.1.20
8. Clark RB, Lewinski MA, Loeffelholz MJ, Tibbetts RJ (2009) In: Coordinating ed., Sharp SE (eds) Cumitech 31a, verification and validation procedures in the clinical microbiology laboratory. American Society for Microbiology, Washington, DC
9. Swenson JM, Anderson KF, Lonsway DR, Thompson A, McAllister SK, Limbago BM et al (2009) Accuracy of commercial and reference susceptibility testing methods for detecting vancomycin-intermediate Staphylococcus aureus. J Clin Microbiol 47:2013–2017
10. Kruzel MC, Lewis CT, Welsh KJ, Lewis EM, Dundas NE, Mohr JF et al (2011) Determination of vancomycin and daptomycin MICs by different testing methods for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 49:2272–2273
11. Rybak MJ, Vidaillac C, Sader HS, Rhomberg PR, Salimnia H, Briski LE et al (2013) Evaluation of vancomycin susceptibility testing for methicillin-resistant *Staphylococcus aureus*: comparison of Etest and three automated testing methods. J Clin Microbiol 51:2077–2081
12. Rodríguez-Sánchez B, Sánchez-Carrillo C, Ruiz A, Marín M, Cercenado E, Rodríguez-Créixems M et al (2014) Direct identification of pathogens from positive blood cultures using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. Clin Microbiolol Infect 20:421–427
13. Romero-Gómez MP, Gómez-Gil R, Paño-Pardo JR, Mignorance J (2012) Identification and susceptibility testing of microorganism by direct inoculation from positive blood culture bottles by combining MALDI-TOF and Vitek-2 compact is rapid and effective. J Inf Secur 65:513–520
14. Beal SG, Ciurca J, Smith G, John J, Lee F, Doern CD et al (2013) Evaluation of the Nanosphere Verigene gram-positive blood culture assay with the VersaTREK blood culture system and assessment of possible impact on selected patients. J Clin Microbiol 51:3988–3992
15. Bork JT, Leekha S, Heil EL, Zhao L, Badamas R, Johnson JK (2015) Rapid testing using the Verigene gram-negative blood culture nucleic acid test in combination with antimicrobial stewardship intervention against gram-negative bacteremia. Antimicrob Agents Chemother 59:1588–1595
16. Ward C, Stocker K, Begum J, Wade P, Ebrahimsa U, Goldenberg SD (2015) Performance evaluation of the Verigene® (Nanosphere) and FilmArray® (BioFire®) molecular assays for identification of causative organisms in bacterial bloodstream infections. Eur J Clin Microbiol Infect Dis 34:487–496