Performance and potential clinical impact of Alfred60AST (Alifax®) for direct antimicrobial susceptibility testing on positive blood culture bottles

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Abstract

Rapid pathogen identification (ID) and antimicrobial susceptibility testing (AST) of bacteria-causing bloodstream infections can improve patients' outcome. In this study, we evaluated the performance of Alfred60^{AST} (Alifax) which provides AST directly on positive blood culture (BC) bottles by light scattering. In a selected group of patients with a clinical suspicion of severe sepsis or at risk for infections with multiresistant organisms, we compared Alfred60^{AST} AST results with traditional AST results (Vitek2 (bioMérieux) or disk diffusion). Discrepancy analysis was performed by Etest (bioMérieux) or broth microdilution. In total, 222 samples were evaluated. On 595 susceptibility determinations, 93.4% showed categorical agreement (CA) with the standard method. Eighty-one percent of isolates showed a 100% categorical agreement (CA) which increased to 84.3% after discrepancy analysis. There were 8 very major discrepancies (VMD), 18 major discrepancies (MD), and 13 minor discrepancies (MiD). Most discrepant results were observed for piperacillin-tazobactam (15.6%) and clindamycin (18.9%). Analysis time was 6–6.5 h for a complete Alfred60^{AST} AST result. In addition, we evaluated the behavior of clinicians in adjusting antibiotic therapy according to the routine AST results. In 37% of all patients, antibiotic therapy was altered after reporting of AST result and adjustment was more frequent for Gram-negative than for Gram-positive isolates. With some improvements, Alfred60^{AST} provides accurate and rapid preliminary AST results for organisms causing bloodstream infections and may have at least a potential clinical benefit in about one-third of patients with severe sepsis, by delivering faster results compared with conventional methods.

Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [1, 2]. The early administration of appropriate antibiotics in patients with severe sepsis and septic shock improves mortality, reduces the length of hospital stay, and limits the development of resistance [1, 3,4,5,6]. However, defining the "appropriate" antibiotic is challenging since it is based on the ID and the antimicrobial susceptibility of the causative microorganism. In about one-third of septic cases, blood cultures (BCs) can identify the causative organism and are still the gold standard in diagnosis of bloodstream infections. Therefore, as a clinical laboratory, the processing of BCs and the identification (ID) and the antimicrobial susceptibility testing (AST) of the causative microorganism(s) should be performed as soon as possible [1].

The current standard in processing BCs is to perform ID and AST of the organism when there is visible growth on subcultured agar plates. Since this workflow depends on the growth characteristics of the organism, time to ID and time to AST results can reach up to 24–48 h after positivity detection of the BC bottle. These long time-to-results may lead to a delay in the optimization of patients' antibiotic therapy, potentially increasing mortality. Speeding up the process of ID and AST of the causative microorganism has a clinical impact only if reporting is followed by antimicrobial stewardship intervention [7,8,9]. Therefore, it is important to evaluate changes in the analytical process of positive BCs in terms of potential clinical benefit for the patients.

In order to speed up the processing of BCs, different methods have been developed to perform direct AST on the positive BC bottles [10, 11]. Alifax® SpA (Italy) developed Alfred60^{AST}, an automated system for enrichment and AST by light scattering. Without any preparation steps, aliquots of positive BCs can be loaded directly on Alfred60^{AST}. The system performs enrichment of the aliquot which can be used for either MALDI-TOF-based ID (off-board analysis), AST (on-board analysis), or both. Antibiotic panels can be composed by the customer from a total of 35 antibiotics used against Gram-positive (staphylococci and enterococci) or Gram-negative (Enterobacterales and *Pseudomonas*) species. Both broad-spectrum and narrow-spectrum antibiotics are available.

In UZ Leuven, a tertiary hospital with a positivity rate of around 900 positive BC bottles per month, the current median time to obtain AST result of the microorganism is approximately 40 h after positivity detection by the BacT/alert system (bioMérieux) [2]. In order to reduce the turnaround time, we evaluated the impact of introducing Alfred60^{AST} for direct susceptibility testing on positive BC bottles from a selected patient group. Its performance will be assessed by comparing AST results obtained by Alfred60^{AST} with those obtained by traditional methods: Vitek 2 system or disk diffusion method on isolated colonies. In addition to the performance of the system, we also assessed the potential clinical impact of having more rapid AST results by gaining insight in the behavior of clinicians in adjusting antibiotic therapy after the availability of BC AST results.

Materials and methods

The study was conducted from October 2017 until February 2018 at the University Hospitals of Leuven, Belgium, a tertiary hospital with about 2000 beds and almost 60,000 hospital admissions per year.

Patient selection

During the study period, all adult patients (≥ 18 years) with significant bacteremia (at least 1/4 positive BC bottle with Gram-negative rods or 2/4 with staphylococci or streptococci from the same collection moment) and admitted to the emergency room, intensive care unit, or hospitalized ≥ 10 days were included.

Samples

Per patient meeting the above criteria, one aerobic (BacT/alert FA Plus, bioMérieux, Marcy L'Etoile, France) or anaerobic (BacT/alert FN Plus, bioMérieux) BC bottle from a new episode (≥10 days after a previous one) that was flagged positive by the BACT/ALERT® system (bioMérieux) during weekdays between 2 a.m. and 17:30 p.m. and of which Gram stain result showed pure growth of staphylococci, streptococci, or Gram-negative rods was included.

Traditional workflow of blood cultures

After being sent to the laboratory, aerobic (BacT/alert FA Plus, bioMérieux, Marcy L'Etoile, France) and anaerobic (BacT/alert FN Plus, bioMérieux) BC bottles are inserted 24/24 h into the BACT/ALERT® system (bioMérieux). After positivity detection, a Gram stain is made from each sample during working hours, and according to the Gram stain result, samples are subcultured on appropriate agar plates. After an incubation time of at least 6 h, grown colonies are identified using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and AST is performed by the Vitek 2 system (bioMérieux, Marcy L'Etoile, France) for all Gram-negative aerobic rods (except *H. influenzae*) and staphylococci. The following Vitek 2 cards are used: AST-N353 for Gram-negative rods and Vitek 2 AST-P650 for staphylococci. For enterococci, the disk diffusion method with ROSCO Neo-Sensitabs™ disks (Rosco Diagnostica A/S, Taastrup, Denmark) is used on the Mueller-Hinton agar according to EUCAST guidelines 6.0 (2016). All Gram stain results are discussed immediately with the prescribers. ID and AST results requiring antibiotic therapy modification are actively communicated to the clinician during working hours (8 a.m.–6 p.m.).

AST by Alfred60^{AST} is performed according to the instructions of the manufacturer with the use of 1 drop blood volume instead of 500 µL blood volume diluted 1:10 in the saline solution proposed by the official protocol. This method was approved by the manufacturer in order to facilitate the process and keep extra steps at risk for contamination as low as possible. In brief, from each positive BC bottle meeting inclusion criteria, one drop is transferred to an "Enrichment" or "HB&L culture" vial (Alifax®) for analysis with Alfred60^{AST} (Alifax®). After inoculation, culture broth vials are incubated on board for enrichment. The system contains its own turbidity monitor, and at the moment a turbidity level of 0.5 McFarland is detected, the sample is buffered automatically. If a turbidity of 0.5 cannot be reached after a maximum time of 5 h, the sample is not further processed. According to our settings, the system automatically starts AST after buffering the sample. The sample is inoculated into 6 vials containing a fixed antibiotic concentration and an additional antibiotic-free vial to serve as a growth reference, called the "reference vial." "G-NF AST Broth" and "Automation kit" vials (Alifax®) are used for respectively Gram-negative and Gram-positive AST.

In order to start AST analysis with Alfred60^{AST}, lyophilized antibiotics need to be regenerated off-board with 2 mL regenerating solution before they can be loaded to the system. The following antibiotics were selected and tested: ampicillin, clindamycin, cefoxitin, and vancomycin for Gram-positive cocci; and levofloxacin, cefotaxime, piperacillin-tazobactam, and meropenem for Gram-negative rods.

Lyophilized antibiotics are stored at 4 °C on board. The shelf life of regenerated antibiotics is 7 days, with the exception of 3 days for meropenem.

Real-time bacterial growth curve information is available on the display of the system. Results are reported as the percentage of resistance by comparing bacterial growth in the reference vial with growth in antibiotic-containing vials. Resistance percentages are categorized automatically by the system into “sensitive,” “intermediate,” or “resistant” if the resistance percentage is between respectively 0–35%, 35–50%, and > 50%. Time to AST result is 180 or 300 min, according to the type of antibiotic and organism.

For every sample analyzed with Alfred60^{AST}, the time to reach 0.5 McFarland and the McFarland level at which buffering occurred were collected.

Discrepancy analysis

Discrepancy testing was performed for isolates that do not show categorical agreement (CA) between Alfred60^{AST} AST and Vitek 2 or disk diffusion. For the interpretation of Vitek 2 results, EUCAST breakpoints (version 6.0) were used, without the interpretation by the Vitek Advanced Expert System™. Discrepant results regarding clindamycin, vancomycin, ampicillin, meropenem, cefotaxime, levofloxacin, and oxacillin were subjected to Etest® (bioMérieux, Marcy L’Etoile, France) and a MIC value was read after overnight incubation on 35° Celsius. In case of discordance regarding piperacillin-tazobactam, broth microdilution was performed by Sensititre™ DKMGN (Thermo Scientific, USA) in addition to disk diffusion with ROSCO Neo-Sensitabs™ disks containing 30 + 6 µg piperacillin-tazobactam. Susceptibility results obtained by Etest® or Sensititre™ were used as the golden standard.

The percentage of categorical agreement (CA), the number of very major discrepancies (VMD), major discrepancies (MD), and minor discrepancies (MiDs) were determined. CA is the percentage of the total test results with the same categorical interpretation result as the reference result.

A discrepant antibiotic result is defined as “very major” if it is reported sensitive instead of resistant, “major” if it is reported resistant instead of sensitive, and “minor” if it is reported “intermediate” instead of sensitive or resistant compared with the reference method.

All polymicrobial cultures were excluded from the analysis.

Clinical breakpoints

During the study period, the Alifax® software version 0.2.12.01 was used. All MIC values and zone diameters obtained by Vitek 2, Etest®, Sensititre™, and disk diffusion were interpreted according to EUCAST version 6.0 breakpoints.

Patient clinical information

In order to evaluate the clinical impact of a direct AST result on a specific group of patients, we retrospectively investigate the impact of traditional AST results of the BC on the antibiotic therapy of the patient. For each sample included in the study, the medical record of the patient was investigated and the source of bacteremia, empiric antibiotic therapy (i.e., antibiotic therapy before Gram stain result), and antibiotic therapy after Gram stain, ID, and AST results were retrospectively assessed. Date and time of every modification in antibiotic therapy were collected and compared with date and time of reporting Gram stain, ID, and AST results. The effect of AST results on antibiotic therapy of the patient and defining the absence or presence of therapy modifications as "adequate" or "inadequate," were retrospectively discussed with a panel of microbiologists and clinicians.

Statistical analysis

A Fisher's exact test by the GraphPad Software (GraphPad Software, San Diego, CA) was used to calculate the difference in the performance of Alfred60^{AST} AST and therapeutic adjustments between Gram-positive and Gram-negative samples.

Ethics statement

The study protocol was approved by the Ethical Committee of UZ Leuven.

Results

From October 2017 until February 2018, 3642 BC samples from 971 patients became positive. Of those positive BC samples, 222 samples (6%) from 220 patients were analyzed with the Alifax® AST software by Alfred60^{AST}. Forty-seven samples (15 Gram-negative and 32 Gram-positive) needed to be excluded from further analysis due to analytical failures (N = 22), polymicrobial culture (N = 9), not fulfilling inclusion criteria (e.g., only 1 BC bottle positive for staphylococci) (N = 3), or antibiotic panel not validated for the species (e.g., streptococci) (N = 13) (Table 1).

Of the 22 samples with analytical failure, 16 (6 Gram-negative and 10 Gram-positive) failed since growth in the reference vial did not exceed ≥ 700,000 CFU/mL. All antibiotics tested for these samples were reported as having "insufficient inoculation" and no result was reported. Table 1 shows the species with insufficient growth in the reference vial. Remarkably, this occurred in all tested *Staphylococcus pettenkoferi* species and in 3 out of 9 (33%) tested *P. aeruginosa*. For 6 other samples, no turbidity of 0.5 McFarland was reached and no AST was started. This was due to the presence of anaerobic (*Bacteroides* ssp., N = 5) or microaerophilic (*Campylobacter fetus*, N = 1) species that were not able to grow since incubation on Alfred60^{AST} occurs in an aerobic atmosphere.

At the moment, no validated antibiotic panels for streptococci are available on Alfred60^{AST}. In our study, 6 *S. pneumoniae*, 3 beta-hemolytic streptococci, and 3 viridans streptococci were therefore excluded from further analysis (Table 1).

In total, 175 samples (103 aerobic and 72 anaerobic BC bottles) from 168 patients were included for analysis. Eighty-five Enterobacterales, 6 *Pseudomonas* species, 54 coagulase-negative staphylococci, 19 *S. aureus*, and 11 *Enterococcus* species (Table 1) were analyzed.

Twenty-seven samples (19 Gram-negative and 8 Gram-positive) had discordant results compared with the gold standard AST (Table 2). Twenty-two samples had only one discordant antibiotic result but 4 samples had 2 discrepancies: one *M. morganii* for cefotaxime and piperacillin-tazobactam and three *P. aeruginosa* of which two were discordant for levofloxacin and meropenem and one for meropenem and piperacillin-tazobactam (Table 3). There was one *P. mirabilis* that had 3 discrepancies (for cefotaxime, meropenem, and piperacillin-tazobactam). However, after reanalysis on Alfred60^{AST}, all antibiotic results for this species were concordant. For *S. aureus* ($N = 19$) and enterococci ($N = 11$), no discrepant antibiotic results were detected between Alifax® AST and Vitek 2/disk diffusion.

In total, 595 antibiotics were tested with Alfred60^{AST} of which 556 (93.4%) were concordant with the Vitek 2/disk diffusion result (Table 4). Of the 39 discrepancies (12 Gram-positive and 27 Gram-negative panels), 18 MD, and 13 MiDs were detected (Tables 3 and 4). In total, 8 VMD results were detected, including piperacillin-tazobactam (Enterobacterales) for *E. coli* ($N = 1$), *E. cloacae* ($N = 1$), and *E. aerogenes* ($N = 1$); levofloxacin for *K. pneumoniae* ($N = 1$) and *P. aeruginosa* ($N = 1$); and clindamycin for *S. epidermidis* ($N = 2$) and *S. hominis* ($N = 1$). One VMD (piperacillin-tazobactam for *E. coli*) was resolved after discrepancy analysis and 2 others (piperacillin-tazobactam for *E. cloacae* and levofloxacin for *P. aeruginosa*) were reclassified as MiD.

On the other hand, 2 MiD results between Alfred60^{AST} and Vitek 2 (1 piperacillin-tazobactam for *S. marcescens* and 1 clindamycin for *S. hominis*) were reclassified as VMD after discrepancy analysis (Table 3).

Major discrepancies were more frequently seen in Gram-negative samples than in Gram-positive samples and included the following antibiotics: piperacillin-tazobactam ($N = 9$), meropenem ($N = 3$), cefotaxime ($N = 3$), cefoxitin ($N = 2$), and clindamycin ($N = 1$). All MDs with Vitek 2 were confirmed with broth microdilution or Etest®.

In total, 14 MiDs were seen of which 6 involved clindamycin for coagulase-negative staphylococci. The Alifax® software reported all 6 as “sensitive” while the Vitek 2 analysis measured a MIC value of 0.5 mg/L which is categorized into “intermediate” according to the EUCAST 6.0 breakpoint table. However, 4 of these samples with MiD resolved after analysis by Etest®. In 17 samples containing staphylococci (4 *S. aureus* and 13 coagulase-negative staphylococci), inducible clindamycin resistance was detected by Vitek 2 or by a D test on on Mueller-Hinton agar. Except for 1 sample, the Alifax® software reported all these samples as clindamycin susceptible. Five of these 17 samples with inducible clindamycin resistance showed discrepant clindamycin susceptibility results between Alfred60^{AST} and Vitek 2 as shown in Table 3.

In our study population, the presence of bacteria with resistant phenotypes was limited. One methicillin-resistant *S. aureus* (MRSA) was correctly reported as cefoxitin resistant by the Alifax® software. Unfortunately, none of the included samples contained carbapenem-resistant Enterobacterales. There was 1 vancomycin-resistant *E. faecium* (VRE) in our population, but due to polymicrobial culture (the sample also contained a *S. maltophilia*), the sample was excluded from further analysis.

Time to results

Time to Alfred60^{AST} AST result is dependent on time to reach 0.5 McFarland turbidity and susceptibility analysis time per antibiotic. The latter is fixed: 180 min for ampicillin, cefotaxime, cefoxitin, clindamycin, and levofloxacin, and 300 min for piperacillin-tazobactam, meropenem, and vancomycin. Variation of analysis time is only influenced by the time to reach 0.5 McFarland turbidity. The mean time to reach 0.5 McFarland was 1 h and 30 min for Gram-positive cocci and 1 h 6 min for Gram-negative rods (Fig. 1). For Gram-positive cocci, results were more dispersed than for Gram-negative rods. The mean McFarland level before buffering was 0.51 for Gram-positive cocci and 0.48 for Gram-negative rods.

Clinical data

Of the 168 patients included in the study, 90 were male and 78 female. The majority of BCs were taken in the emergency department (82/175 BCs, 47%), but at the time of Gram stain reporting, most patients were hospitalized in the intensive care unit (57/175 BCs, 32.6%) (Fig. 2).

For Gram-negative isolates ($N = 91$), the source of bacteremia was urinary ($N = 36$), abdominal ($N = 28$), respiratory ($N = 5$), catheter-related ($N = 4$), wound infection ($N = 1$), or unknown/unclear ($N = 17$). For Gram-positive isolates, the source of infection was catheter-related ($N = 34$), circulatory (endocarditis or septic thrombophlebitis) ($N = 8$), respiratory ($N = 2$), abdominal ($N = 3$), joint infection ($N = 1$), skin and soft tissue infection ($N = 1$), unknown ($N = 17$), or considered contamination ($N = 15$).

Clinical data regarding antibiotic therapy in the 175 episodes of bacteriemia in 168 different patients are summarized in Table 5. Eleven patients died or were on palliative care during the study. Fifteen patients with Gram-positive bacteremia did not receive any antibiotic therapy since the catheter was removed in case of catheter-related infection or the sample was considered to be contaminated. Of the 149 patients who did receive antibiotics, the antimicrobial therapy was initiated or adjusted according to the Gram stain or ID result in 75 of them (50%). The adjustment was more frequent for Gram-positive isolates (51%) than for Gram-negative isolates (35%) ($p < 0.01$).

In 64/175 (37%) samples, the clinician was influenced by the AST result to adjust antibiotic therapy within 4 days. In 49 of them (77%), the adjustment was executed within 24 h after the AST result reporting. Of all patients that have been switched according to the AST result, the antibiotic therapy was de-escalated in 75% (48/64) (results not shown). Gram-negative AST results had more impact than Gram-positive

AST results on the antibiotic regimen of the patient (48% versus 24%, $p = 0.01$) while the impact of Gram stain and/or ID result was higher for Gram-positive samples than for Gram-negatives (51% versus 35%, $p < 0.01$). In 15 episodes of bacteremia, adjustment of antibiotic therapy was done more than 24 h after reporting of the AST result.

In 46% of all patients, the patients received adequate therapy and the AST result of the BC did not have an impact on the antibiotic therapy. The most frequent reason for not adjusting antibiotic therapy was based on the resistance profile of the microorganism (e.g., ampicillin-resistant *E. faecium*) or due to patient characteristics (e.g., penicillin allergy) ($N = 67$). Other reasons were the earlier adjustment of the antibiotic therapy based on the AST of another sample (i.e., urine in case of urosepsis) ($N = 3$) or the lack of clinical improvement or the presence of neutropenia ($N = 2$). For 8 samples (5 Gram-negative and 3 Gram-positive), the reason for not adjusting antibiotic therapy was unknown.

Discussion

We evaluated the performance of Alfred60^{AST} (Alifax®) for direct AST on positive BC bottles and estimated its potential clinical impact on the antibiotic therapy of selected patients. Alfred60^{AST} performs enrichment of the positive BC and automatically starts AST of a selected antibiotic panel by light scattering.

The Alifax® system is able to deliver faster (6–6.5 h) AST results in comparison with conventional AST based on overnight growth and accurate AST results similar to conventional AST, but some improvements are needed, in particular for piperacillin-tazobactam, meropenem for *Pseudomonas* species, and clindamycin. Results are still preliminary as polymicrobial cultures need to be excluded and only a limited number of antibiotics are tested. By analyzing the impact of reporting AST results on the antibiotic therapy of the patient, our study indicates that (rapid) AST results can have an impact on 37% of the patients. This impact seems to be higher for Gram-negative bacteremia compared with Gram-positive bacteremia. For Gram-positive bacteremia, focus on rapid ID may be more important to guide antibiotic therapy in clinical practice.

Alfred60^{AST} AST results were in complete CA with Vitek2/disk diffusion results (i.e., AST agreement for all tested antibiotics) in 81.3% of samples (85.4% Gram-positive and 77.5% Gram-negative isolates). The categorical agreement increased to 84.2% (90.2% for Gram-positive species and 78.7% for Gram-negative species) if Etest®/broth microdilution was performed on discordant results. The overall CA of Alifax® AST was higher for Gram-positive organisms than for Gram-negative pathogens. In particular, for *S. aureus* and enterococci, no discrepant results were observed.

Fontana et al. and Barnini et al. performed similar evaluations of Alfred60^{AST} and showed complete AST agreement in respectively 88.8% and 62.1% of samples with also less discrepancies for Gram-positive than for Gram-negative isolates [11, 12]. Results are not completely comparable with our data since different antibiotic panels and different comparators were used. Moreover, different patient populations were studied in different hospitals. In our study, due to the limited capacity, Alfred60^{AST}

was only used on a selected group of patients for which the potential clinical impact was expected to be the greatest: patients with severe sepsis (i.e., admitted to the emergency department or intensive care unit) or patients with a high risk for multiresistant species (i.e., hospitalized > 10 days).

In our study, 39 discrepant results were seen on 595 measurements (6.6%) with 8 VMDs, 18 MDs, and 13 MiDs. Other studies reported categorical agreements of 87%, 96.3%, and 97% between Alfred60^{AST} and routine AST with respectively Phoenix™ (BD), Vitek 2 (bioMérieux), and broth microdilution [13,14,15]. Giordana et al. evaluated Alfred60^{AST} and compared it with another technique for direct AST testing: Pheno™ (Accelerate). The Pheno™ system is able to perform AST within 7 h by analyzing the bacterial growth rate with an automated digital microscopy. Giordana et al. used microdilution (Sensititre, Thermo Fisher Scientific) assay as the gold standard. In their study, AST with Alfred60^{AST} showed higher agreement for Gram-negative organisms (91.1% versus 90.6%) but lower agreement for Gram-positive species (95.7% versus 100%) compared with AST with Pheno™ [16].

Besides Alfred60^{AST}, Alifax® also provides HB&L™, another system with the same light scattering technology for direct susceptibility testing for which literature reports agreement of 83–85% [12, 17, 18].

In our study, most discrepant results were seen for piperacillin-tazobactam, meropenem for *Pseudomonas* species, and clindamycin. For piperacillin-tazobactam, 9 out of 14 discrepancies (including both Enterobacterales and Pseudomonaceae) were major and none resolved after broth microdilution. Remarkably, none of the discrepancies occurred in *Klebsiella* species, which was the second most abundant Enterobacterium after *E. coli*. The method-dependent variation in susceptibility testing for piperacillin-tazobactam is a known problem [19]. After modification of the drug formulation by the manufacturer, 5 discrepant results considering Enterobacterales were corrected (results not shown).

We also showed that Alfred60^{AST} was unable to detect inducible clindamycin resistance. From a clinical perspective, the need for rapid clindamycin susceptibility result is debatable since, in the case of bacteremia, monotherapy with clindamycin is not the first-line regimen in our hospital. We suggest confirming these rapid AST results of clindamycin with traditional methods.

Although the EUCAST version 8.0 guideline was already implemented in our laboratory at the time of the study, all results were interpreted according to the EUCAST version 6.0 breakpoints as the new version of Alifax® software was not available at that time. If EUCAST version 8.0 breakpoints would have been used, four additional discrepancies would have been detected for levofloxacin.

Except for levofloxacin, clindamycin, piperacillin-tazobactam, and meropenem for Pseudomonaceae, the agreement with Vitek 2 or microdilution for all other antibiotics was ≥ 90% which is acceptable according to the criteria proposed by CLSI or Jorgensen [20, 21].

Twenty-one percent of the samples analyzed on Alfred60^{AST} were excluded. Reasons for exclusion were diverse: no growth of the organism (e.g., anaerobic or microaerophilic organisms), insufficient growth in the reference vial (e.g., encapsulated *P. aeruginosa*), polymicrobial culture, or no validated antibiotic panel for the species (i.e., streptococci). Moreover, none of the four *S. pettenkoferi*–positive samples could be analyzed by Alfred60^{AST} due to insufficient inoculation. The use of a slightly adapted protocol of inoculation of the vial can be an explanation of the high number of analytical failures of insufficient growth. We used one drop of the BC bottle, instead of 10 μL. This high percentage of exclusions needs to be considered when performing cost-effectiveness studies of Alfred60^{AST}.

The mean time to reach complete AST result was 6–6.5 h (1–1.5 h for enrichment plus 5 h for antibiotic analysis). This is significantly lower than 40 h with our traditional methods. With Alfred60^{AST}, AST results can be available on the same day as Gram stain reporting, i.e., if analysis starts at 10–11 a.m., results will be available around 5–6 p.m. when the majority of clinicians are still present in the hospital to adjust antimicrobial therapy if necessary. As already has been shown in literature, MALDI-TOF MS–based ID can correctly identify > 80% of species on a short-incubated culture (e.g., 5 h). At the time Alfred60^{AST} AST result can be reported, the ID of the species will be known for the majority of samples [22,23,24,25]. Result of ID is important because for some species (e.g., streptococci or *S. maltophilia*), AST analysis with Alfred60^{AST} is not (yet) validated and AST result may not be reported.

A major limitation of this prospective study is the limited number of some species. For example, only 6 *P. aeruginosa* were included and no other *Pseudomonas* species or *Acinetobacter* species were isolated in this period. There was 1 positive BC for *S. maltophilia* that needed to be excluded since none of the tested antibiotics were validated for this species (no EUCAST breakpoint available). However, intrinsic resistance for meropenem was correctly reported by Alfred60^{AST}. Besides the limited number of nonfermenting species, the number of resistant phenotypes was also limited: only 1 MRSA and 13 Enterobacterales resistant to third-generation cephalosporins were included. No carbapenem-resistant Enterobacterales were observed during the study. A vancomycin-resistant enterococcus from a clinical sample was unfortunately excluded because of no pure growth (the sample also contained *S. maltophilia*). However, an in-house VRE strain was tested with Alfred60^{AST} (results not shown), and ampicillin and vancomycin were correctly reported as resistant.

Other limitations of the study are that only BCs from weekdays were included, clinical data were retrospectively assessed, and we performed the analysis only on adult patients.

In order to assess the potential clinical impact of introducing Alfred60^{AST}, we evaluated the behavior of clinicians in adjusting antibiotic therapy based on the AST result of the pathogen. We showed that in 37.8% of bacteremia cases, antibiotic therapy was changed after reporting the AST result, which is in agreement with the study of Hayakawa et al. [26]. The influence of AST from Gram-negative organisms was significantly higher than for Gram-positive organisms while Gram stain and/or ID results were more important for Gram-positive samples than for Gram-negative samples. This was also shown by Meda et al. [27]. In general, tests that improve time

to ID for Gram-positive pathogens and time to AST for Gram-negative organisms have the greatest potential impact in optimizing antibiotic therapy [27, 28].

However, Alfred60^{AST} provides only partial AST results. We did not study the impact of partial AST results on the behavior of the clinicians. Verroken et al. implemented rapid ID and partial susceptibility testing by the detection of cephalosporin-resistant Enterobacterales and MRSA. With these partial susceptibility results, they showed a significant reduction in time to optimal antimicrobial therapy indicating that clinicians can be influenced by partial but important AST results [7].

To conclude, Alfred60^{AST} provides rapid AST results directly on positive BC samples but accuracy needs to be improved. Also, more prospective studies are needed to assess the clinical impact of rapid but only partial AST results and to evaluate cost-efficiency of the system including hands-on-time.

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This study protocol was approved by the Ethical Committee of the University Hospital of Leuven.

Informed consent

No informed consent was needed for this study since no personal data were involved.

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Table 1 Overview of blood cultures and identified microorganisms that were analyzed with Alfred60AST. The reason for the exclusion of samples for analysis is indicated between brackets

Results				
			Microorganism	**Number**
Gram-negative	Total	106		
	Excluded	15	*P. aeruginosa* (insufficient inoculation)	3
			Acinetobacter species (insufficient inoculation)	1
			H. influenzae (insufficient inoculation)	1
			E. coli (insufficient inoculation)	1
			B. fragilis (no growth)	3
			B. vulgatus (no growth)	2
			C. fetus (no growth)	1
			C. sedlakii + *K. pneumoniae* (mixed sample)	1
			E. coli + *Neisseria species* (mixed sample)	1
			S. maltophilia (not validated)	1
	Included	91	*E. coli*	50
			K. pneumoniae	11
			P. aeruginosa	6
			K. oxytoca	5
			P. mirabilis	4
			E. cloacae	4
			S. marcescens	3
			P. rettgeri	2
			C. braakii	1
			E. aerogenens	1
			M. morganii	1
			P. stuartii	1
			P. shigelloides	1
			Proteus species	1

Gram-positive	Total	116		
	Excluded	32	*S. pneumoniae* (not validated)	6
			S. agalactiae (not validated)	2
			S. anginosus (not validated)	1
			S. mitis (not validated)	1
			S. sanguinis (not validated)	1
			Streptococcus group G (not validated)	1
			S. pettenkoferi (insufficient inoculation)	4
			S. epidermidis (insufficient inoculation)	3
			S. hominis (insufficient inoculation)	2
			S. capitis (insufficient inoculation)	1
			S. epidermidis + *S. hominis* (mixed sample)	2
			S. epidermidis + *S. capitis* + *S. hominis* (mixed sample)	1
			S. aureus + *S. epidermidis* (mixed sample)	1
			S. lugdunensis + *S. hominis* (mixed sample)	1
			E. faecalis + *C. albicans* (mixed sample)	1
			E. faecium + *S. maltophilia* (mixed sample))	1
			S. epidermidis (not meeting inclusion criteria)	1
			S. capitis (not meeting inclusion criteria)	1
			S. haemolyticus (not meeting inclusion criteria)	1
	Included	84	*S. epidermidis*	36
			S. aureus	19
			S. hominis	13
			E. faecalis	5
			E. faecium	5
			S. haemolyticus	3
			S. capitis	2
			E. avium	1

Table 2 Categorical agreement and discrepancies of Alfred60^{AST}, compared with Vitek 2/disk diffusion and the gold standard (Vitek 2/disk diffusion and Etest or Sensititre for discrepant result). Four samples with no complete AST result were excluded

Alfred60 ^{AST} versus Vitek 2/disk diffusion				
	100% categorical agreement	1 discrepancy	2 discrepancies	3 discrepancies
Total (*N* = 171)	139 (81.3%)	26 (15.2%)	5 (2.9%)	1 (0.6%)
Gram-positive (*N* = 82)	70 (85.4%)	12 (14.6%)	0	0
Gram-negative (*N* = 89)	69 (77.5%)*	14 (15.7%)	5 (5.6%)	1 (1.1%)
Alfred60 ^{AST} versus gold standard				
	100% categorical agreement	1 discrepancy	2 discrepancies	3 discrepancies
Total (*N* = 171)	144 (84.2%)	22 (12.9%)	4 (2.3%)	1 (0.6%)
Gram-positive (*N* = 82)	74 (90.2%)	8 (9.8%)	0	0
Gram-negative (*N* = 89)	70 (78.7%)*	14 (15.7%)	4 (4.5%)	1 (1.1%)

*No significant difference between Gram-positive and Gram-negative samples (p = 0.06)

Table 3 Summary of discrepant results among 39 samples

Antibiotic	Species	Alfred60^{AST}	Vitek 2 (MIC mg/L)	Etest (MIC mg/L)/disk diffusion (diameter mm) (EUCAST v. 6.0)	Sensititre (MIC mg/L)	Discrepancy (compared with Vitek)	Discrepa (compar with gol standarc
Piperacillin-tazobactam (EB)	*E. coli*	S	R (≥ 128)	S (21 mm)	S (8)	Very major	None
		R	S (8)	S (20 mm)	S (4)	Major	Major
		R	S (≤ 4)	S (20 mm)	S (4)	Major	Major
		R	S (≤ 4)	I (19 mm)	S (8)	Major	Major
		R	S (≤ 4)	I (19 mm)	S (4)	Major	Major
		R	S (8)	I (19 mm)	S (8)	Major	Major
		I	S (8)	S (20 mm)	S (4)	Minor	Minor
		I	S (8)	I (17 mm)	S (8)	Minor	Minor
	E. cloacae	S	R (≥ 128)	R (16 mm)	I (16)	Very major	Minor
	E. aerogenes	S	R (≥ 128)	I (17 mm)	R (32)	Very major	Very maj
	M. morganii	R	S (≤ 4)	S (30 mm)	S (≤ 1)	Major	Major
	P. mirabilis	R	S (≤ 4)	S (25 mm)	S (≤ 1)	Major	Major
Piperacillin-tazobactam (PS)	*P. aeruginosa*	R	S (≤ 4)	S (22 mm)	S (8)	Major	Major
		R	S (8)	S (23 mm)	S (4)	Major	Major

Levofloxacin	*K. pneumoniae*	S	R (4)	R (3)		Very major	Very maj
	P. aeruginosa	S	R (4)	I (2)		Very major	Minor
	S. marcescens	S	I (2)	R (4)		Minor	Very maj
	P. aeruginosa	R	I (2)	I (2)		Minor	Minor
	K. pneumoniae	S	I (2)	S (1)		Minor	None
Cefotaxime	*K. pneumoniae*	R	S (≤ 0.25)	S (0.125)		Major	Major
	P. mirabilis	R	S (≤ 0.25)	S (0.023)		Major	Major
	M. morganii	R	S (≤ 0.25)	S (0.016)		Major	Major
Meropenem (EB)	*P. mirabilis*	R	S (0.5)	S (0.023)		Major	Major
Meropenem (PS)	*P. aeruginosa*	R	S (1)	S (2)		Major	Major
		R	S (1)	S (1)		Major	Major
		I	S (1)	S (0.25)		Minor	Minor
		I	S (0.5)	S (0.38)		Minor	Minor
Cefoxitin	*S. epidermidis*	R	S (≤ 0.25)	S (0.125)		Major	Major
		R	S (≤ 0.25)	S (0.19)		Major	Major

Clindamycin	*S. epidermidis*	S	R (≥ 4)	R (> 256)		Very major	Very maj
		S	R (≥ 4)	R (1.5)		Very major	Very maj
		S	I (0.5)[£]	S (0.125)		Minor	None
		S	I (0.5)	S (0.064)		Minor	None
		S	I (0.5)	S (0.38)		Minor	Minor
	S. hominis	S	R (≥ 4)	R (> 256)		Very major	Very maj
		R	S (0.25)[£]	S (0.064)		Major	Major
		S	I (0.5)[£]	R (> 256)		Minor	Very maj
		S	I (0.5)[£]	S (0.25)		Minor	None
		S	I (0.5)[£]	S (0.19)		Minor	None

*Gold standard includes broth microdilution (for piperacillin-tazobactam) and Etest® (other)

[£]Inducible clindamycin resistance detected by Vitek 2 or positive D test

EB Enterobacterales, *PS* Pseudomonas species

Table 4 Correlation results of Alfred60^{AST} with results from Vitek 2/disk diffusion (DD). Number of very major discrepancies (VMD), major discrepancies (MD), and minor discrepancies (MiDs) per antibiotic and microorganism are indicated. Susceptibility categorization results by traditional methods are indicated

Alfred60^{AST} versus Vitek 2/DD							
		No discrepancy/total measurements	**% agreement**	**% disagreement**	**VMD**	**MD**	**Mi**
Enterobacterales (*N* = 85)	Cefotaxime	82/85	96.5	3.5	0	3	0
	Levofloxacin	82/85	96.5	3.5	1	0	2
	Meropenem	83/84	98.8	1.2	0	1	0
	Piperacillin-tazobactam	72/84	85.7	14.3	3	7	2
Pseudomonaceae (*N* = 6)	Levofloxacin	4/6	66.6	33.3	1	0	1
	Meropenem	2/6	33.3	66.7	0	2	2
	Piperacillin-tazobactam	4/6	66.7	33.3	0	2	0
Coagulase-negative Staphylococci (*N* = 54)	Cefoxitin	51/53	96.2	3.8	0	2	0
	Clindamycin	43/53	81.1	18.9	3	1	6
	Vancomycin	54/54	100	0.0	0	0	0
S. aureus (*N* = 19)	Cefoxitin	19/19	100	0.0	0	0	0
	Clindamycin	19/19	100	0.0	0	0	0
	Vancomycin	19/19	100	0.0	0	0	0
Enterococci (*N* = 11)	Ampicillin	11/11	100	0.0	0	0	0
	Vancomycin	11/11	100	0.0	0	0	0
Total (*N* = 175)		556/595	93.4	6.6	8	18	13

S sensitive, *I* intermediate, *R* resistant, *DD* disk diffusion, *Sta* staphylococci, *CNS* coagulase-negative staphylococci

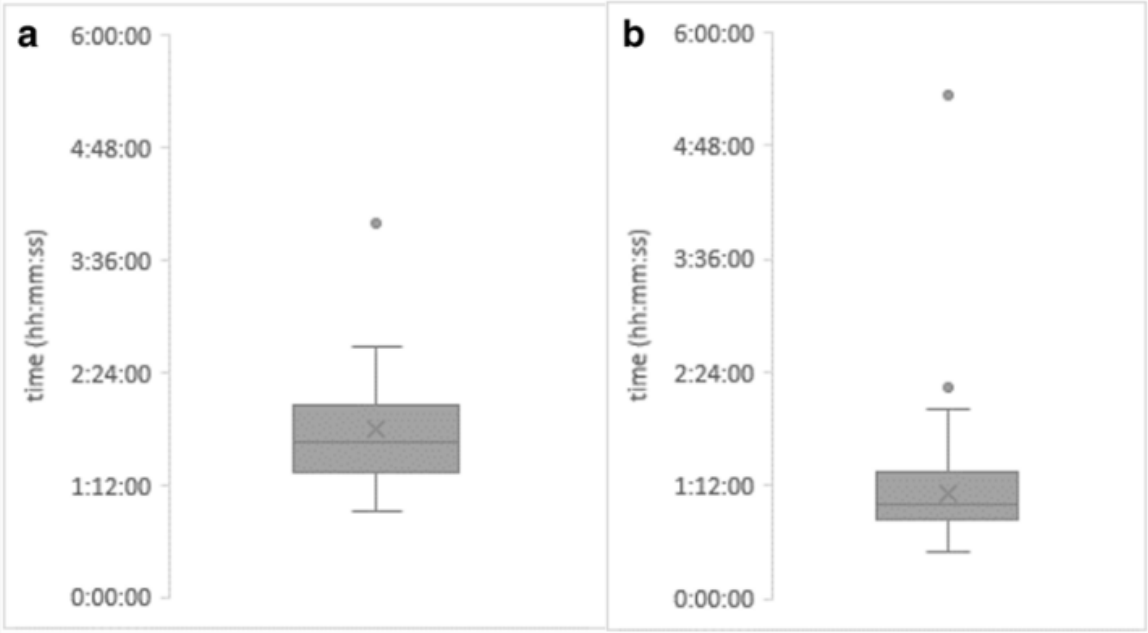

Fig. 1
Time to reach 0.5 McFarland turbidity on Alfred60^{AST} for Gram-positive cocci (**a**) and Gram-negative rods (**b**) expressed as a box-and-whisker plot

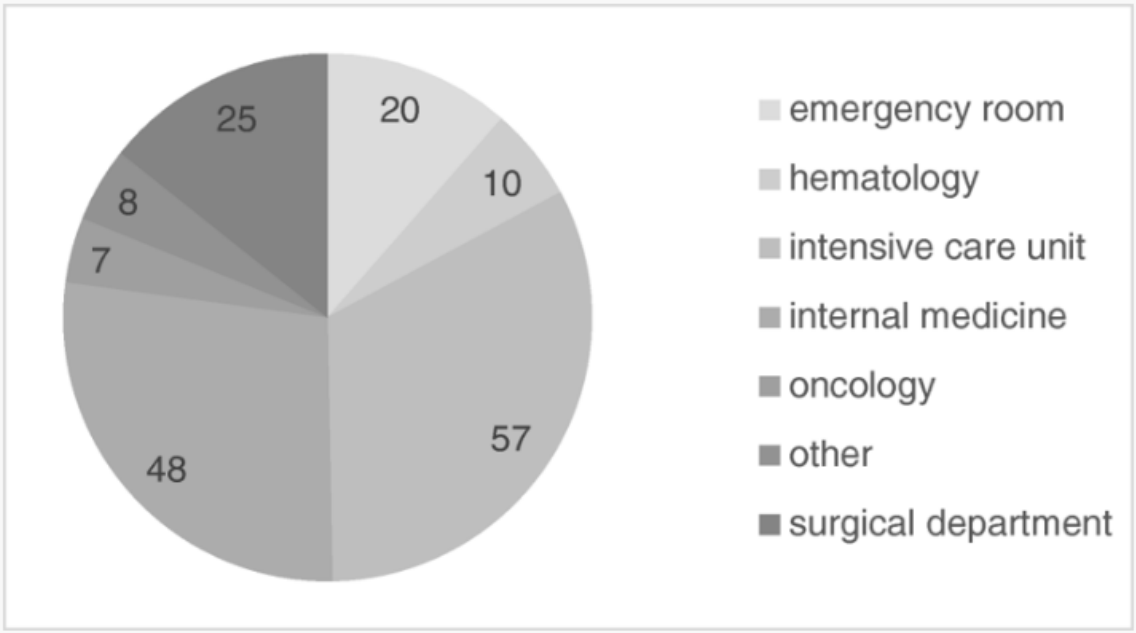

Fig. 2
Hospitalization department of patients at the moment of Gram stain reporting. "Other" includes physical medicine and rehabilitation, obstetrics and gynecology, otorhinolaryngology, and neurology

Table 5 Antibiotic therapy and antibiotic adjustments of all bacteremia episodes included

		Gram-negative bacteremia (*N* = 91)		Gram-positive bacteremia (*N* = 84)		Total (*N* = 175)	
		N	% of Gram-negatives	*N*	% of Gram-positives	*N*	% of all patients
No antibiotic therapy (catheter removal or considered contamination)		0	0	15	18	15	9
Antibiotic therapy		84	92	65	77	149	85
AB therapy adjustment after Gram stain/ID result		32	35	43	51	75	43
AB therapy adjustment after AST result (< 4 days)							
Yes		44	48	20	24	64	37
Adjustment in < 24 h		34	37	15	18	49	28
Adjustment in ≥ 24 h		10	11	5	6	15	9
No		40	44	40	48	80	46
Adequate	No adjustment possible	*30*	33	37	44	67	38
	Adjusted to AST from other samples	*3*	3	0	0	3	2
	No adjustment due to clinical status	*2*	2	0	0	2	1
Not adequate/reason unknown		*5*	6	3	4	8	5
AB therapy stopped < 4 days after AST result (considered contamination)		0	0	5	6	5	3
Deceased/palliative setting		7	8	4	5	11	6