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Antimicrobial susceptibility testing determined by Alfred 60/AST (Alifax®) in a multi-sites lab: performance's evaluation and optimization of workflow

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ABSTRACT

Purpose: New techniques are needed to speed-up the identification and antimicrobial susceptibility testing (AST) of bacteria associated with bloodstream infections. Alfred 60/AST (Alifax®, Polverara, Italy) performs AST by light scattering directly from positive blood cultures.

Methods: We evaluated Alfred 60/AST performances for 4 months. Each new episode of bacteraemia was included and AST were compared to either our rapid automated AST (Vitek® 2) or disk diffusion method. The discrepancies were investigated using Etest®. The time-to-result (TTR) was evaluated by comparing the blood volume inserted into Alfred 60/AST, i.e. 2 versus 7 blood drops. Taking into account the TTR, the workflow of positive blood cultures and the availability of AST results was studied in order to optimize the implementation of Alfred 60/AST.

Results: A total of 249 samples and 1108 antibiotics for AST were tested. After exclusion of unavailable results, 1008 antibiotics were analysed. 94.9% (n = 957/1008) of the antibiotics showed categorical agreement. There were 14 very major errors (VME), 24 major errors (ME) and 13 minor errors (mE). The VME were mostly related to clindamycin (64.3%) whereas meropenem and piperacillin-tazobactam constituted the major part (37.5% and 61.5%) of ME and mE respectively. Results were highly reliable for *Enterobacterales* and enterococci. The mean TTR ranged between 4.3 and 6.3 h and was statistically 20 min faster when applying the 7 blood drops protocol. We showed that Alfred 60/AST could give relievable results within working hours for positive blood culture which are flagged the same day between 12:00 am and 12:00 pm.

Conclusion: Our study confirmed that Alfred 60/AST gives reliable AST results in a short period of time, especially for *Enterobacterales* and enterococci. AST could thus be easily obtained the same day of a positive blood culture. Clinical impact studies are mandatory to validate a 24/24 working.

1. Introduction

Sepsis is a major cause of morbidity and mortality in hospitalized patients (Shankar-Hari et al., 2016; Fleischmann et al., 2016). Delaying the appropriate antimicrobial treatment makes the vital prognosis of septic patients worse (Kumar et al., 2009; Garnacho-Montero et al., 2015; Ferrer et al., 2014). Performing blood cultures (BC) is the standard procedure to assess blood infection and to obtain information about the causal agent and its antimicrobial susceptibility. For many years, the conventional identification (ID) and antimicrobial susceptibility testing (AST) techniques for the analysis of positive BC required at least 48 additional hours (24 h incubation to obtain a bacterial isolate followed by 24 h incubation to get ID and/or AST results). Since the last new century, many achievements of rapid diagnostic assays took place in

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clinical laboratories. This ranges from the implementation of the MALDI-TOF MS (Tan et al., 2012) for the ID aspects to the development of lateral flow immunoassays (Rao et al., 2016; Hamprecht et al., 2018; Riccobono et al., 2018; Takissian et al., 2019), chromogenic (Renvoisé et al., 2013) and molecular assays (Liesenfeld et al., 2014; Sinha et al., 2018) for the AST aspects. Rapid automated AST is also feasible (Höring et al., 2019; Prod'hom et al., 2013). Nowadays, clinical microbiologists and scientists are always trying to optimize the management of BC in their lab. To this end, we evaluated the CE-marked Alifax® Alfred 60/AST that is based on the detection of bacterial growth by turbidimetry through light scattering and may significantly reduce the AST reporting time. Our first objective was to evaluate the performances of this new technology in comparison with our conventional AST techniques. For the pre-analytical step, the use of a needle (BD BACTEC™ Subculture aerobic venting unit) that fits easily on the BC bottle allows the user to pour blood drops instead of pipetting blood from the BC. Our second objective was to evaluate the time to results (TTR) of two protocols which differ by the amount of blood poured. Indeed, the initial bacterial load may be different according to the number of blood drops inoculated. We hypothesized that a greater bacteria load could reduce the time required to reach the 0.5 McFar. Our third and last objective was to optimize Alfred 60/AST implementation in our clinical multi-sites' laboratory in order to maximise its benefit for the clinical management of the patient. Indeed, several key elements are necessary to make a new technique efficient, like the improvement of the sample and result's workflows. This is particularly true for merged laboratories that are located in the same area, and where the place of each procedure or instrument should be continuously rethought according to both the evolution of the lab's infrastructure and the evolution of the techniques. Indeed, not only new techniques are mandatory to reduce TAT and the real question is how we could improve the processing of positive BC at the microbiology lab to maximise the benefit of each techniques at our disposal, new technologies like Alfred 60/AST included. In this context, TAT of the 3 analytical phases must be studied thoroughly. Post-analytical aspects may also be considered because an effective result is the one that is forwarded as soon as possible to an available infectious disease who may adjust the antimicrobial therapy of the patient.

2. Materials and methods

A prospective study was performed between December 16, 2019 to April 17, 2020 at the Laboratoire Hospitalier Universitaire de Bruxelles – Universitair Laboratorium Brussel (LHUB-ULB). Our laboratory works hand-in-hand with 5 hospital partners located in the Brussel Capital Region: Erasme Hospital (academic hospital of the Université Libre de Bruxelles (ULB)), CHU Saint-Pierre, Jules Bordet Institute, CHU Brugmann and the Hôpital Universitaire des Enfants Reine Fabiola (children's hospital). Estimated catchment population is 2680. The laboratory currently had four activity centers (Anderlecht, Porte de Hal, Horta, Brien).

2.1. Samples selection

Blood specimens from patients admitted to the 5 hospital partners of the LHUB-ULB were inoculated into BC bottles (BD BACTEC™ Lytic Anaerobic medium and BD BACTEC™ Plus Aerobic medium). All working days positive BC (from Monday 00:00 am to Friday 17:00 pm) collected from patients with significant bacteraemia caused by *Enterobacterales*, *S. aureus*, enterococci, non-fermenting Gram-negative bacilli ($\geq$ 1 positive BC or coagulase negative staphylococci - coNS ($\geq$2 positive BC) were included. One sample per patient and per new episode ($\geq$ 7 days after a previous one) was included. AST from positive BC were processed with both Alfred 60/AST and our routine AST procedure.

2.2. Routine workflow

Aerobic and anaerobic BC bottles of patients with a suspected bloodstream infection are inserted 24/24 into a BD BACTECTM FX device (Becton Dickinson Diagnostic Systems). For each positive bottle, a Gram stain and culture are performed. For each new episode of bacteraemia, a "rapid" in-house identification method (MALDI-TOF MS directly from positive BC; Bruker Daltonics, Bremen, Germany) (Martiny et al., 2012) is performed during working hours if young subculture is not yet available. Briefly, 8 mL blood of a positive BC is transferred into a 10 mL serum separator tube which is then centrifuged during 10 min at 2000*g*. The supernatant is then discarded, and the pellet is washed with 1 mL of deionised water before being centrifuged a second time at 16,600*g* for 2 min. The bacterial pellet is then ready for MALDI-TOF MS identification. This procedure, a twofold centrifugation protocol, also yields a sufficient bacterial pellet for a rapid Vitek® 2 AST or for performing rapid assays to detect cefoxitin or third generation cephalosporin resistances (AlereTM PBP2A Culture Colony Test and ß LACTA™ test). We routinely perform AST using a rapid Vitek® 2 (rapid automated method from bacterial pellet of centrifuged blood) or the disk diffusion method. In this study, all strains were processed by a rapid Vitek® 2, except for non-fermenting Gram-negative bacilli for which the disk diffusion method was used as soon as the growth of colonies was sufficient (standard EUCAST microbiological procedure). To detect or confirm resistance mechanisms, double disk diffusion (ESBL strains), D-test (inducible clindamycin resistance), lateral flow immunoassays (OXA-48 K-Set CORIS BioConcept) and/or molecular methods are performed.

2.3. AST by Alfred 60/AST

AST by Alfred 60/AST were performed during working hours at the LHUB-ULB Anderlecht site. BC from other LHUB-ULB sites were carried to this site by taxi. Briefly, Alfred 60/AST works in 3 steps. The first step is the inoculation of 10 µL of blood by the user into a 3 mL enrichment broth vial which is then inserted in a 37 °C incubator. According to manufacturer's instructions provided during the training, an alternative procedure was implemented involving the use of needles (BD BACTEC™ Subculture aerobic venting unit) and blood drops instead of pipetting. As recommended, we inoculated the enrichment broth vial pouring 2 or 7 blood drops from the aerobic and anaerobic positive BC respectively. The subsequent times to reach the 0.5 McFarland were analysed. If both BC from a same new episode were positive, we collected drops randomly either from the aerobic or the anaerobic bottle. After this first step, Alfred 60/AST performs AST automatically. The instrument is equipped with a light scattering technology that monitors the turbidity of the broths. As soon as 0.5 McFarland is reached, the suspension is buffered (step 2) into an empty vial located in the fridge in order to stop bacterial growing. The third step involves the inoculation of the 0.5 McFarland suspension into an enrichment vial containing a fixed concentration of the antibiotic (AB) to be tested. Depending on the AB, the analysis is then completed after 3 or 5 h.

AB panels for AST were selected with the antimicrobial stewardships from each hospital partner after a multidisciplinary discussion. Specific panels of EUCAST lyophilized AB were selected for (1) *Enterobacterales* (amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, meropenem and ciprofloxacin); (2) staphylococci (cefoxitin, clindamycin, trimethoprim-sulfamethoxazole and vancomycin); (3) enterococci (ampicillin and vancomycin) and (4) non-fermenting Gram-negative bacilli (piperacillin-tazobactam, meropenem, ceftazidime, amikacin and ciprofloxacin).

2.4. Data analysis

EUCAST version 9.0 breakpoints were used for AST interpretation of Vitek® 2, Etest® and disk diffusion methods results. The interpretation of the Vitek Advanced Expert System™ was taken into account for comparison. Discrepant results between Alfred 60/AST and our routine methods were subjected to Etest® except for coNS. Disk diffusion method in triplicate was performed for discrepancies concerning coNS in order to exclude unsuspected mixed cultures.

After the discrepancies analysis, AST results provided by Alfred 60/AST were classified as concordant, very major errors (VME), major errors (ME) or minor errors (mE) according to Cumitech's recommendations (Sharp and Clark, 2009). A Mann-Withney-Wilcoxon test was applied to compare the time obtained to reach 0.5 McFarland by the 2 protocols (2 versus 7 blood drops). Statistical analyses were performed using GraphPad version 9.1.2 (GraphPad Software San Diego, CA).

3. Results

3.1. Sample population

From December 16, 2019 to April 17, 2020, 249 positive BC (137 aerobic, 112 anaerobic) were included in the evaluation (Fig. 1).

The study was intermittently disrupted by Alfred 60/AST technical issues and by the COVID-19 pandemic. These events stopped us from hiring all positive BC that meet our criteria. A total of 1108 AB were performed for AST with Alfred 60/AST. Among these 100 AB were excluded for comparison because of technical issues ($n = 65$) or irrelevance ($n = 35$) (Fig. 2).

3 coNS ($n = 12$) were excluded of the analysis because of considered as contaminants, AmpC-producing *Enterobacterales* ($n = 23$) were excluded for amoxicillin-clavulanate analysis as the reagent should not be used for AST in these species. Various analytical failures occurred in the automate: technical issues of Alfred 60/AST (35 AB from 8 AST), results reported as "not valid" (13 AB from 10 AST) which could come from pipetting errors, and insufficient growth of the bacteria- reported as "insufficient inoculation" (17 AB from 5 AST). The later occurred in *Pseudomonas oryzihabitans* ($n = 1$), *A. baumanii* (n = 2), *Staphylococcus hominis* (n = 1) and *S. aureus* (n = 1) strains. Regarding the number of samples, 13 had no results because of insufficient bacterial growth ($n = 5$) or automate issues ($n = 8$).

Finally, 233 samples and 1008 AB were considered for the comparison. 138 *Enterobacterales*, 13 *P. aeruginosa*, 61 staphylococci, 21 enterococci were included. Results are summarized in the Table 1 according to the Cumitech's recommendations.

For all the antimicrobials tested, the categorical agreement (CA) was 94.9%. For Gram-positive cocci, total CA was 94.3% and for enterococci, no discrepant results were highlighted between Alfred 60/AST AST and Vitek® 2. Non-fermenting Gram-negative bacilli had a CA of 89.2% and the CA of *Enterobacterales* reached 95.8%.

In total, 5.1% ($n = 51$) AB were wrongly assessed by Alfred 60/AST (Table 1). There were 7.4% (14/189) VME, 3% (24/808) ME and 1.8% (13/715) mE. Clindamycin discrepancies concerned the major part (64.3%) of the VME. Whereas the in vitro AST of clindamycin was defined as sensitive by the Vitek® 2, 9 staphylococci out of the 22 resistant strains were finally expertise resistant to clindamycin by the Vitek Advanced Expert System™. These discrepancies were linked to inducible clindamycin resistance strains which were confirmed by a D-test on Mueller-Hinton agar. ME were principally observed with meropenem ($n = 9$) and with ciprofloxacin for *E. coli* ($n = 7$). Piperacillin-tazobactam AST constituted the major part (61.5%) of the mE, 50% of them were reported as susceptible instead of intermediate result.

Acquired resistance mechanisms were identified in 27 samples (20 ESBL producers, 2 OXA-48 strains, one ESBL + OXA-48 strain, 3 MRSA and one VRE). One MRSA out of 3 was reported as sensitive by Alfred 60/AST.

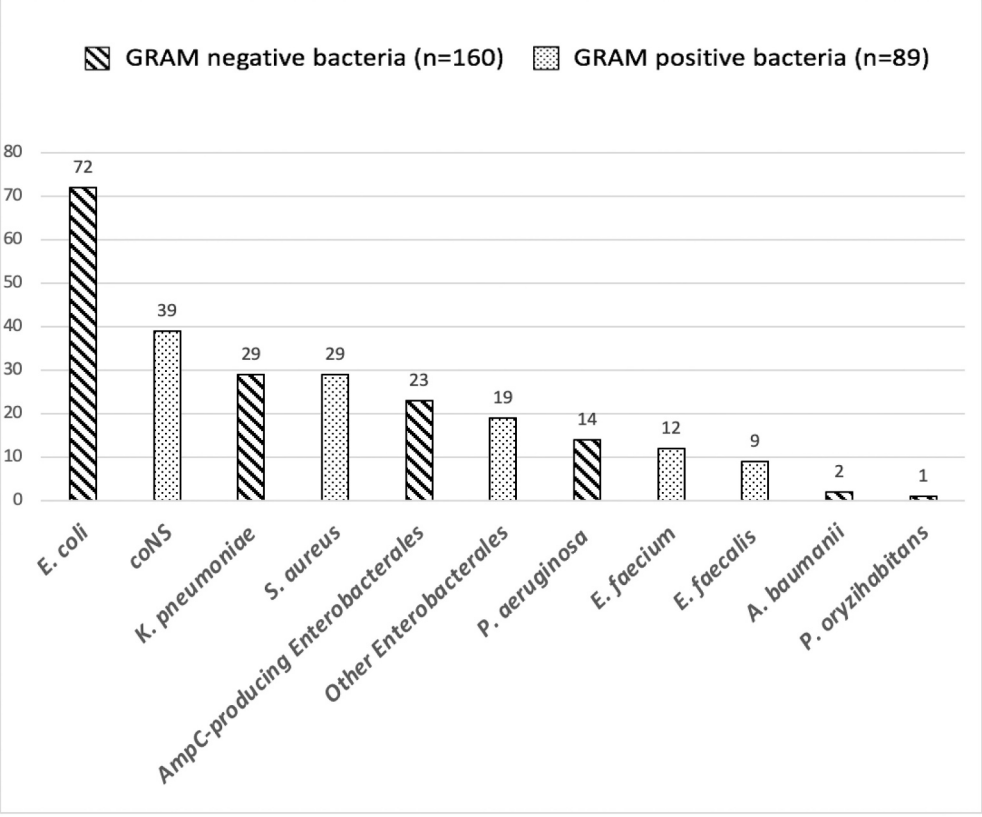

Fig. 1. Overview of the 249 strains performed with Alfred 60/AST. coNS, coagulase negative staphylococci.

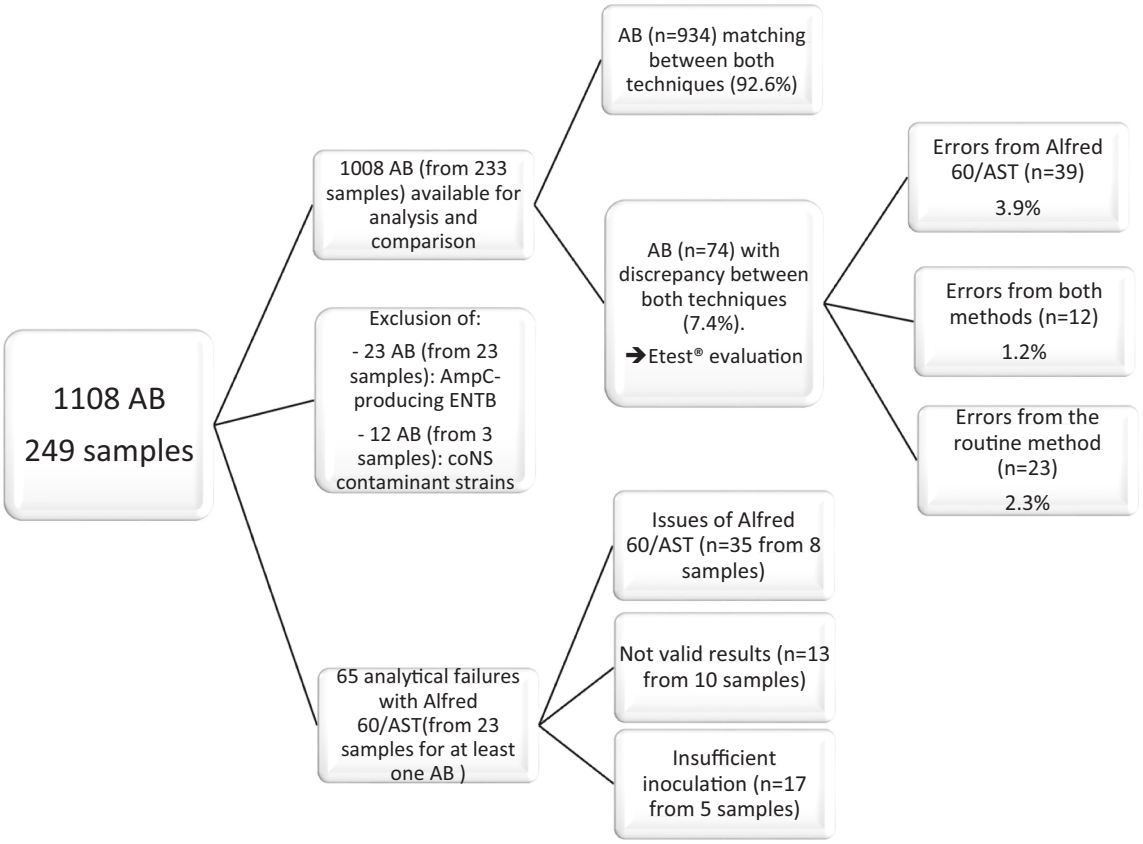

Fig. 2. Diagram which details the number of antibiotics for antimicrobial susceptibility testing and samples, the number of excluded antibiotics for analysis and the percentage of errors from Alfred 60/AST or from our routine method (rapid Vitek® 2 or disk diffusion methods). AB, antibiotic; AMC: amoxicillin-clavulanate; coNS: coagulase negative staphylococci; ENTB, *Enterobacterales.*

3.2. Time to results

Regardless of the amount of blood poured into the enrichment broth vial, the mean time for AST results with Alfred 60/AST was around 4.3 or 6.3 h. *Enterobacterales* (54.5 min) and enterococci (53.5 min) reached the 0.5 McFarland on average faster than staphylococci (82.2 min) and non-fermenting Gram-negative bacilli (126.8 min). Excluding non-fermenting Gram-negative bacilli, a significant difference ($p < 0.0001$) has been observed with other species which needed 53 min and 71 min to reach the 0.5 McFarland with 7 drops and 2 drops respectively (Fig. 3).

We needed to rerun some samples (7.1%) when 7 drops were transferred using the anaerobic bottle since the high turbidity of the vial blinded the photodetectors.

4. Discussion

Alfred 60/AST has been developed by Alifax® in order to speed-up positive BC testing. In this prospective study, we evaluated not only the analytical performances of Alfred 60/AST but also the TTR according to the blood volume poured. Performances evaluation was assessed by comparison to our routine method (rapid Vitek® 2 or disk diffusion method). Eventually, a theoretical TTR diagram has been designed depending on the time of BC positivity.

4.1. First objective: assessment of the analytical performances of Alfred 60/AST

Our study shows a total CA of 94.9% without significant differences between Gram-positive bacteria (95.2%) and Gram-negative bacteria (94.3%). Even the inclusion of newly positive BC was intermittently interrupted by Alfred 60/AST issues or COVID-19 outbreak, the variety of strains assessed is representative of a usual microbiology routine.

The analytical performances of enterococci were very good and showed a CA of 100% for both ampicillin and vancomycin. For staphylococci, Alfred 60/AST cannot detect inducible clindamycin resistance as we obtained a high rate of VME (40.9%) such as those of other studies (12% to 29.4%) (Anton-Vazquez et al., 2019; Van den Poel et al., 2020). For *Enterobacterales*, we highlighted very good results for amoxicillin-clavulanate and better results for cefotaxime (CA at 97.8% versus between 89.7 and 97.8%) and piperacillin-tazobactam (CA at 93.3% versus between 77.3 and 85.7%) AST than in other studies (Barnini et al., 2016; Giordano et al., 2018; Van den Poel et al., 2020). Indeed, piperacillin-tazobactam antibiotic reagent has been reviewed by Alifax® and marketed in October 2018 because of previous high rates of VME and ME. Results of piperacillin-tazobactam AST were improved after the reagent was revised (Boland et al., 2019; Mantzana et al., 2021) as well as in our evaluation where we also found a major decrease in VME and ME. We should note that Mantzana et al. (2021) evaluated Alfred 60/AST during 2 periods (May 2018 to July 2018 and March 2019 to February 2020) but they didn't describe a higher piperacillin-tazobactam discrepancy rate in the first period compared to the second period, as it was discussed by Boland et al. (2019). An unexpected high rate of false resistant results was observed for ciprofloxacin (6.3% of the ME) in comparison with other studies (0% of the ME) (Boland et al., 2019) which could be explained by the fact that our strains showed borderline minimum inhibitory concentration (MIC = 0.25 mg/L).

For meropenem, 9 ME were observed with a rate of 6.2% which was close to that described by Giordano & all. It should be pointed out that meropenem ME rate (1.2% to 14.3%) is variable between studies (Barnini et al., 2016; Giordano et al., 2018; Van den Poel et al., 2020). We hypothesize that this variation could be due to an unstable antibiotic

Table 1
AST results from Alfred 60/AST compared to the rapid Vitek® 2 or to the disk diffusion method (non-fermenting bacilli). Results are expressed after discrepancies analysis.

Group species (n)	Antibiotics	CA (%)	VME (%)	ME (%)	mE (%)
Enterobacterales ($n = 138$)	**Amoxicillin-clavulanate**	113/115 (98.2)	1/35 (2.8)	1/80 (1.2)	NA
	Piperacillin-tazobactam	126/135 (93.3)	0/18 (0)	1/109 (0.9)	8/135 (5.9)
	Cefotaxime	135/138 (97.8)	0/27 (0)	2/110 (1.8)	1/138 (0.7)
	Meropenem	128/135 (94.8)	0/1 (0)	6/133 (4.5)	1/135 (0.7)
	Ciprofloxacin	131/138 (94.9)	0/27 (0)	7/111 (6.3)	0/138 (0)
Non-fermenting Gram-negative bacilli (n = 13)	**Piperacillin-tazobactam**	11/13 (84.6)	1/1 (100)	1/12 (8.3)	NA
	Meropenem	10/13 (76.9)	NA (*)	3/13 (23)	0/13 (0)
	Ceftazidime	12/13 (92.3)	NA (*)	1/13 (7.6)	NA
	Amikacin	12/13 (92.3)	1/1 (100)	0/12 (0)	0/13 (0)
	Ciprofloxacin	13/13 (100)	0/1 (0)	0/12 (0)	NA
Staphylococci ($n = 61$)	**Cefoxitin**	56/59 (94.9)	2/28 (7.1)§	1/31 (3.2)	NA
	Clindamycin	51/61 (83.6)	9/22 (40.9)	0/38 (0)	1/61 (1.6)
	Trimethoprim-sulfamethoxazole	58/61 (95)	0/16 (0)	1/45 (2.2)	2/61 (3.2)
	Vancomycin	59/59 (100)	NA (*)	0/59 (0)	NA
Enterococci (n = 21)	**Ampicillin**	21/21 (100)	0/11 (0)	0/10 (0)	0/21 (0)
	Vancomycin	21/21 (100)	0/1 (0)	0/20 (0)	NA
Total ($n = 233$)	**Total $n = 1008$**	957/1008 (94.9)	14/189 (7.4)	24/808 (3.0)	13/715 (1.8)

(*) no resistant strain; § one MRSA and one coagulase negative staphylococci oxacillin resistant.

reagent. Unlike other regenerated antibiotics that have a 7 days expiration date, meropenem antibiotic reagent is stable for 3 days at 3–5 °C. Alifax® has already taken into account this observation and a more stable version of meropenem with a modified matrix has now been marketed. However, of the 6 meropenem ME cases concerning *Enterobacterales*, 3 were sensitive to amoxicillin-clavulanate/piperacillin-tazobactam and cefotaxime. This kind of general results should draw the attention of microbiologists to suspect a false meropenem resistant result.

One of the interesting points of this study is that there was a high proportion of resistant strains. This allowed Alfred 60/AST assessment on less common strains and the possible impact it could have on hospital hygiene. Alfred 60/AST cannot detect specifically an ESBL producer bacteria. However, *Enterobacterales* with a resistant cefotaxime result should draw the attention of the microbiologist to suspect an ESBL producer bacteria. In this study, all ESBL producers bacteria ($n = 21$) were correctly reported as cefotaxime resistant strains by Alfred 60/AST. In the same way, three OXA-48 strains were correctly reported as meropenem resistant (n = 2) or sensitive ($n = 1$). CA for vancomycin was 100% for Gram-positive cocci, one *Enterococcus faecium* was vancomycin resistant and correctly reported. However, one out of 3 MRSA strain was not detected by Alfred 60/AST (VME rate of 33.3%). A recent study (Mantzana et al., 2021) also highlighted a high VME rate of 15.3% for the cefoxitin evaluation in *S. aureus* strains. This is maybe due to a short incubation method (3 h), Alifax® is working on changing the analysis length to 5 h. Anyway, other rapid diagnostic assays could represent an alternative for quick MRSA detection. The benefit of Alfred 60/AST in this particular case is, to our opinion, rather limited.

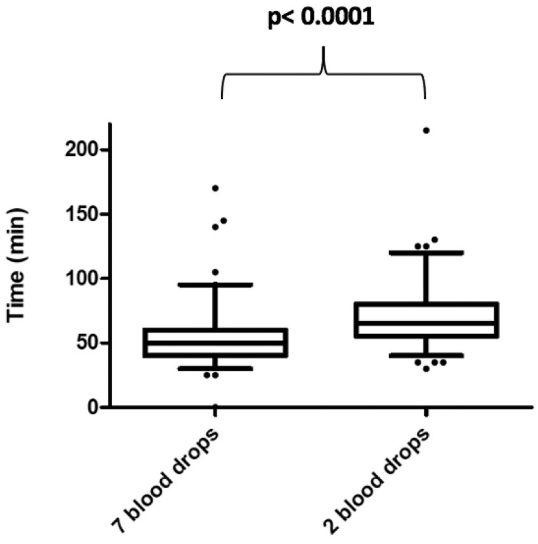

Fig. 3. Time to reach 0.5 McFarland turbidity on Alfred 60/AST pouring 7 ($n = 99$) or 2 ($n = 111$) blood drops into the enrichment broth vial expressed as a boxplot. Non-fermenting Gram-negative bacilli were excluded of the analysis.

We observed that improvements can be made particularly regarding the non-fermenting bacilli AST. As previously reported (Van den Poel et al., 2020), we found that many non-fermenting bacilli have troubles to grow into Alfred 60/AST and the delay to obtain AST is substantially lengthened. Moreover, either results are not as concordant as for other species or remain unavailable. We should however mention that one limitation of our study concerns the low number of evaluated non-fermenting species ($n = 17$). Several major technical issues occurred with Alfred 60/AST- which was a display product- especially at the beginning of our study. That mainly concerned the hardware and the need to replace parts of the system. This led to unavailable results for comparison (3.2% of overall AST). However, Alifax® quickly intervened for troubleshooting and no other issues were observed after their interventions.

An important point to discuss is that all bacteria were accurately identified before performing AST on Alfred 60/AST. Besides the fact that the bacterial identification already helps the infectious diseases' specialist to adjust the empirical antibiotic therapy, it also leads to reduced AST costs in comparison with performing a large antibiotic panel on the basis of the Gram staining alone. Indeed, for a same antibiotic, several conditionings are available that are to be specifically used for a particular strain. As an example, the cefoxitin antibiotic reagent used for *S. aureus* on Alfred 60/AST is different from that used for coNS. Accurate identification therefore avoids unnecessary tests by allowing the selection of the most suitable antibiotic panel. Our study showed that the combination of Alfred 60/AST and the microbial identification by MALDI-TOF MS from pellet of centrifuged blood is an optimal combination. Indeed, not only TTR of the identification and the AST results are shortened but it also allows a decrease in reagents cost. The combination of a rapid identification method by MALDI-TOF MS (Martiny et al., 2012) and a RAST method is now well described (Prod'hom et al., 2013). We think that a rapid identification method should be performed

in addition to the Gram staining even new techniques allow to significantly reduce the TTR of AST.

4.2. Second objective: does blood volume amount have an impact on the time to results?

As the mean time for AST with Alfred 60/AST was around 4.3 or 6.3 h (1.3 h for the time to reach 0.5 McFarland and 3 or 5 h for AST), we can easily obtain an AST the same day of a positive BC. We demonstrated here that the time to reach 0.5 McFarland may be significantly reduced based on the amount of blood drops transferred. Compared to 2 drops, the time needed to reach the 0.5 McFarland is meanly 20 min faster when pouring 7 drops. However, we note here that this TTR decrease is low, and we think that the clinical impacts would not be significant. We suggest nevertheless that the difference in terms of TTR may be due not to the BC bottle (aerobic or anaerobic) but to the bacterial load which is higher when 7 drops are poured. In this case, 0.5 McFarland is achieved faster than the protocol which uses 2 blood drops. We should note that we cannot apply the 7 blood drops protocol using the aerobic bottle because the enrichment vial's turbidity would be too high, and the photodetectors will be systematically blinded. However, we do not specially recommend to apply the 7 drops protocol using the anaerobic bottle because we needed to rerun some samples (7.1% of all samples with the 7 blood drops protocol) and the TTR for these samples were finally worse than the 2 drops protocol. To further decrease TTR, it seems that other/new protocols could be evaluated. Barnini et al. (2016) skipped the step 1 of the procedure (incubation of the enrichment broth vial to reach 0.5 McFarland) and demonstrated that this first step could be performed manually from bacteria's pellet of centrifuged blood without any major impact on the AST results.

4.3. Third objective: TAT and theoretical diagram in a multi-sites' laboratory

The TAT of BC AST has markedly been reduced these last years thanks to the implementation of rapid AST assays (Renvoisé et al., 2013; Rao et al., 2016; Liesenfeld et al., 2014). It would be very challenging to further decrease the TTR of our microbiology methods. Nevertheless, we believe that a workflow which considers the TTR is a critical point. Indeed, to allow clinicians adjusting antimicrobial therapy as soon as possible, an efficient AST result should be reported during working hours. From the time that positive BC is detected, a TTR of around 5 h to obtain a complete AST is a real improvement compared to standard methods. Giving a short TTR and relievable AST results, Alfred 60/AST can perform AST to made possible a same day panel AST of bacteria from positive BC bottles. Indeed, all processed positive BC in the morning (from 8:00 am to 12:00 pm) will have an available AST for the end of the afternoon. At this time, clinicians are still present at the hospital and could adjust antibiotic treatment if they would to. Fig. 4 shows the different day's hours of AST availability according to the positivity hour of the BC and to the instrument used (Alfred 60/AST versus rapid automated Vitek® 2). This diagram has been designed for our main laboratory site (Porte de Hal) and we pointed out here that time of reporting AST is delayed when BC come from other laboratory sites. We should note that Alfred 60/AST TTR depends on the antibiotic tested and some results are already available after 4.3 h. In comparison, the mean TTR to obtain a complete AST using the rapid Vitek® 2 was 11 h in our study. Fig. 4 shows other time frames depending on the time of the flagged positive BC. As an example, AST of a BC which is flagged positive by the BD BACTEC™ FX at 8:00 am will be performed at 9.00 am (day 0). The AST from Alfred 60/AST will be available the same day in the afternoon between 12:30 pm and 02:30 pm whereas the AST from the rapid Vitek® 2 method will be available during the night shift and discussed with the infectious diseases' specialists the next day in the morning. Beyond office hours, the processing of BC by Alfred 60/AST is feasible because the hands-on time is very short, provided that all necessary reagents are loaded into the refrigerated compartment part of Alfred 60/AST and that the daily maintenance was performed earlier. In this diagram, the greatest benefit seems to concern BC that become positive after midnight. Indeed, with a TTR around 4.3 to 6.3 h, AST results can be reported to the clinicians early in the morning joined with the species identification (rapid ID or ID on young subcultured with MALDI-TOF MS). Instead of processing continuously the positive BC during the night shift, a batch process around 4:00 am would be more comfortable for the night technologist. Positive BC (until 3:00 am) from other lab's sites will then have been carried to the main lab's site (Porte

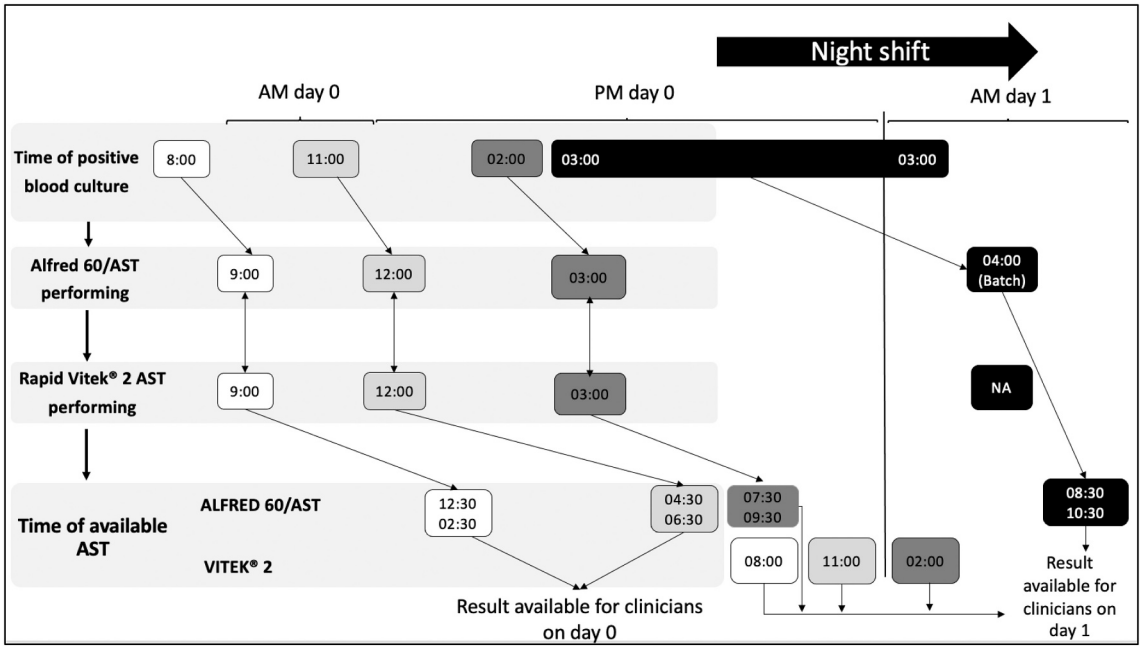

Fig. 4. Expected time-to-result between Alfred 60/AST and our routine method (rapid Vitek® 2 AST), according to the time of flagged positive blood culture. Night shifts are included. This diagram is designed for our main laboratory site (Porte de Hal).

de Hal). During night shift, AST by Alfred 60/AST would be performed only based on the Gram staining because the rapid method identification requires a significant hands-on-time. This workflow proposal has been designed for our multi-sites' laboratory. We showed that Alfred 60/AST could give relievable results within working hours for positive blood culture which are flagged the same day between 12:00 am and 12:00 pm. It seems that the availability of both microbiologist and infectious disease over the night shift could be challenging for the adjustment of antibiotic therapy. However, both microbiologist and infectious disease will be able to discuss and analyse the available morning AST results from last night shift positive BC. A microbiologist who would implement Alfred 60/AST should adjust his workflow depending on the samples volume and the kind of hospital departments for which patients will benefit from this new technology. Moreover, we think that working a night shift in the microbiology lab would be more profitable for a multi-sites' laboratory performing a large number of samples. The study implementation of this new technology during night shift must be evaluated, including the required costs for the equipment and the staff. Our last point of view is related to multidisciplinary work. We indeed must be focused on the patient management. To this end, as soon as the microbiologists report AST results, the escalation or de-escalation of the antibiotic treatment should be evaluated closely with the infectious diseases and the pharmacy department.

5. Conclusion

Our study confirmed that Alfred 60/AST gives reliable AST results for *Enterobacterales*, enterococci and staphylococci (excluding the clindamycin). TTR is between 4.3 and 6.3 h, and 20 min faster when applying the 7 blood drops protocol. Microbiologists may be able to improve the TAT by rethinking the whole lab process and considering the post-analytical aspects as the results report. An additional study will determine the real impact of such technology on both the clinical management of septic patients in our hospitals and the antimicrobial and hospitalisation costs. It is also mandatory to evaluate the interest of performing AST on Alfred 60/AST during night shift, especially in a multi-sites' lab processing a large number of samples.

Funding

Reagents were provided by Alifax. Alfred 60/AST was a display product.

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics approval

No personal information was stored in the study database. All samples taken from patients were used after routine assays were performed. Patient data were anonymized.

Consent for publication

Not applicable.

Declaration of Competing Interest

Alifax had no role in study design, data collection, data analysis.

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