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ORIGINAL ARTICLE

Evaluation of the Alfred™ turbidity monitoring system (Alifax®) following sonication in the diagnosis of central venous catheter colonization

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Abstract

The conventional diagnostic techniques for catheter colonization (CC) take at least 48 h to yield results. Therefore, new diagnostic procedures that speed up the time necessary for results are needed. Our main objective was to assess the efficacy of the combination of sonication, turbidity monitoring, and MALDI-TOF to detect CC and catheter-related bloodstream infection (C-RBSI). For 1 year, we assessed central venous catheter (CVC) tips that arrived at the microbiology laboratory from adult patients admitted to our institution. CVC tips were cut, inoculated into 2.5 ml of BHI, and sonicated for 1 min. The suspension was then processed using Gram stain, quantitative culture (gold standard), and preincubation on the Alfred™ system. We analyzed the validity values of our new diagnostic approach for prediction of CC and C-RBSI and compared them with those of the gold standard. We collected a total of 167 catheters, 33 (19.8%) of which were colonized. We confirmed 21 episodes of C-RBSI. The distribution of microorganisms in colonized CVCs was as follows: Gram-positive, 68.4%; Gram-negative, 5.3%; and yeasts, 26.3%. The validity values for CC and C-RBSI using the new procedure were as follows: S, 39.4%/61.9%; Sp, 100%/100%; PPV, 100%/100%; and NPV, 87.0%/94.8%. The combination of sonication with a pre-incubation period based on turbidity monitoring using the Alfred™ system followed by MALDI-TOF proved to be a useful tool that was faster than conventional culture for ruling out C-RBSI. Future studies are needed to assess the clinical and economic impact of this diagnostic approach.

Keywords Catheter-related bloodstream infection · Turbidity · Sonication · Diagnostic optimization

Introduction

Catheter-related bloodstream infections (C-RBSIs) are the most frequent nosocomial infections (1), and between 15% and 30% of cases of nosocomial bacteremia are catheter-related (1). C-RBSIs have significant associated morbidity and increase both hospital costs (estimated at approximately €18,000 per episode) and length of stay (2). Attributable mortality ranges between 12 and 25% (3). The complexity of this type of infection makes it necessary to start antibiotic therapy early to improve prognosis (4). C-RBSIs occur after catheter tip colonization, which is used to identify at-risk populations (5).

It is essential to find systems for the diagnosis of catheter colonization that are faster than conventional culture-based methods. International guidelines currently recommend the use of the Maki or sonication techniques as the gold standard

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diagnostic methods. However, both techniques require at least 24–48 h before results are available, and this delay is excessive when early therapy is essential.

Measurement of turbidimetry (HB&L™ and Alfred60^{AST}, Alifax®) is an attractive alternative that could be implemented in microbiology laboratories for early detection of colonized catheters. In their prospective study, Fontana et al. (6) demonstrated that this approach was more successful for diagnosing C-RBSI than Maki's technique. However, despite the greater diagnostic yield, they did not use matrix-associated laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) as an additional method for the detection of early colonization and did not compare their efficacy with regard to sonication.

Therefore, our main objective was to assess the validity of the Alfred60^{AST} system from Alifax® combined with MALDI-TOF for the early detection of colonized catheters and C-RBSI and compare this approach with sonication.

Material and methods

The study sample comprised central venous catheter (CVC) tips that arrived at the microbiology laboratory over a 1-year period from adult patients who had been admitted to our institution. Catheter tips were processed by cutting the 5-cm distal part into 1–2-mm fragments, which were inoculated in 2.5 ml of BHI and sonicated for 1 min followed by vigorous vortexing. The suspension was then processed for Gram stain, 100 μl was plated onto blood agar for quantitative culture (gold standard), and 500 μl was placed into HB&L medium (Alifax®) and incubated in the Alfred60^{AST} automated system for 8 h or until turbidity was achieved (0.5 McFarland). Next, 4 aliquots were obtained for Gram stain, MALDI-TOF, and qualitative conventional culture on blood agar plates (24–48 h incubation). A Microflex LT benchtop mass spectrometer (Bruker Daltonics, Bremen, Germany) and the BDAL V6 library (Bruker Daltonics) containing 6903 MSPs were used in this study. FlexControl 3.3 and Maldi Biotyper 3.1 (Bruker Daltonics) were applied for the mass spectrometer control and comparison with the database, respectively. Procedure for microorganism identification by MALDI-TOF is described elsewhere (7).

In the case of negative results, the incubation period in the Alfred60^{AST} system was extended to 24 h. Figure 1 shows the experimental procedure.

In patients receiving antibiotic therapy, the sonicate was diluted 1:10, 1:100, and 1:50 in HB&L to reduce the antibiotic concentration. We analyzed the validity values for Gram stain of the sonicate and for our new diagnostic combination approach for predicting colonization and C-RBSI using incubation periods of ≤ 8 h and from 8 to 24 h.

All patients provided their written informed consent for their data to be used.

Definitions

Catheter-related bloodstream infection (C-RBSI): Isolation of the same microorganism in at least 1 peripheral blood culture and catheter tip culture.

Catheter colonization by conventional culture "Gold standard": Isolation of ≥ 100 cfu in the culture of the catheter tip sonicate.

Catheter colonization by Alfred: a turbidity value of 0.5 McFarland obtained by the Alfred60^{AST} automated system.

Statistical analysis

Qualitative variables are expressed as frequencies. Quantitative variables are expressed as median (IQR). Normally distributed continuous variables were compared using the *t* test, and non-normally distributed variables were compared using the Kruskal-Wallis test. Differences between groups were compared using the Mann-Whitney test with a Bonferroni correction. All statistical tests were 2-tailed.

Statistical significance was set at $p < 0.05$. The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Armonk, New York, USA).

The validity values of Gram-stain and turbidity combined with MALDI-TOF were calculated for the CVC tip to sonicate according to the gold standard (culture of sonicate) and for both incubation times (≤ 8 h and > 8 h).

Results

A total of 167 catheters were included in the study as follows: 53.3% were CVC, 20.5% peripheral venous catheters, 14.4% peripherally inserted CVCs, and 10% introducers. Table 1 shows catheter and patient characteristics.

Using conventional culture, 33 (19.8%) colonized CVC were detected, and 21 episodes of C-RBSI were confirmed. Of the 33 positive CVCs and 21 C-RBSI episodes, 2 and 1, respectively, were polymicrobial. The etiology of colonized catheters and C-RBSI are shown in Table 2. Details of which microorganisms were detected by conventional culture, Alfred, or both are shown as Supplemental data file 1. In colonized catheters, Gram-positive cocci (68.4%) accounted for most episodes, followed by yeasts (26.3%) and Gram-negative bacilli (5.3%).

Using Alfred60^{AST}, we detected 13 colonized catheters for incubation periods of ≤ 8 h. We recovered a further 8 colonized catheters when incubation was extended for 24 h, the longest time to positivity being 20 h. As for C-RBSI, the new device was able to detect 13 and 2 episodes for incubations of ≤ 8 h and > 8 h, respectively. Table 3 details the validity values for all the diagnostic methods (Gram stain of the sonicate and

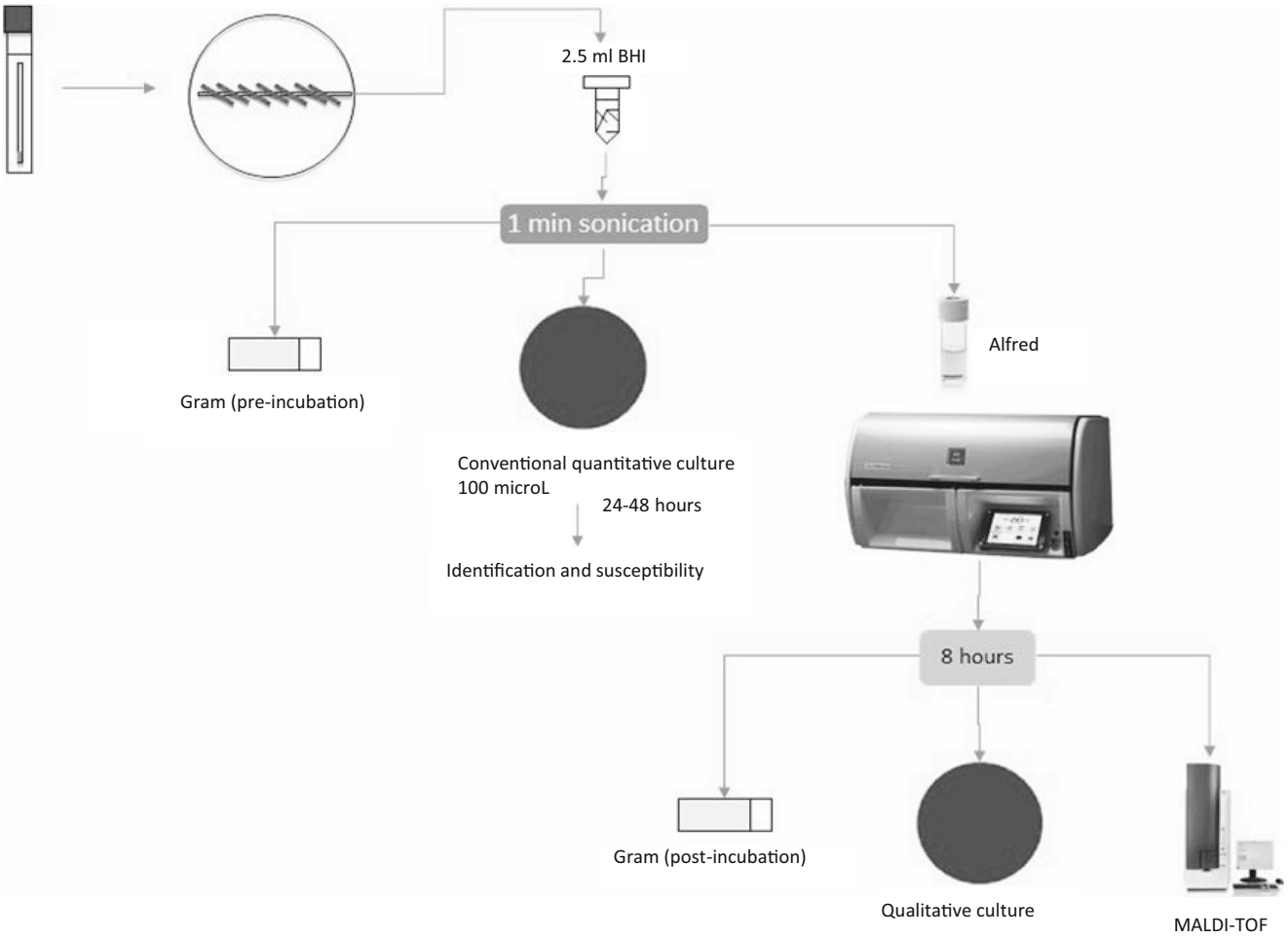

Fig. 1 Study procedure

the new combination diagnostic approach) compared with the gold standard (quantitative culture). Although sensitivity was not sufficiently high for catheter colonization and C-RSBI with respect to Gram stain or the new procedure (52.4% and 39.4–61.9%, respectively), the NPV was 93.5% for C-RBSI using Gram stain, and 87.0% and 94.8% for catheter colonization and C-RSBI using the new procedure.

Worse results were obtained with yeasts and CoNS, as only 1 of the 4 catheters colonized by *C. glabrata* was detected by Alfred60^{AST}, with an incubation time of > 8 h (9 h and 20 min). In the case of *S. epidermidis*, only 3 out of the 14 colonized catheters were detected at ≤ 8 h of incubation. The remaining 11 were detected after > 8 h of incubation or proved to be negative after 24 h.

Discussion

We demonstrated that the combination of MALDI-TOF with Alfred60^{AST} (the first automated system for bacterial culture and susceptibility testing) had a PPV of 100% for detecting both colonization and C-RBSI in ≤ 8 h. However, it had a sensitivity of 63.6% for detecting catheter colonization in 24 h. Although PPV was independent of incubation time, sensitivity decreased to 39.4% for incubations of ≤ 8 h.

Maki's technique only detects colonization on the external surface of the catheter, in contrast to sonication, which can detect both extra- and intraluminal colonization (8). Culture after sonication has not proven to be superior to the reference technique, although a few studies have evaluated the effectiveness of sonication combined with other innovative techniques to speed up diagnosis (9, 10). Given that quantitative methods are time-consuming, the simplicity of semiquantitative techniques has contributed to their widespread use in clinical microbiology laboratories (11, 12). Several prospective studies have compared Maki's semiquantitative technique with quantitative methods (sonication and vortexing) for detection of C-RBSI and concluded that the 3 methods exhibited similar reliability; however, Maki's semiquantitative technique was simpler to use (10, 13). Hence, diagnosis of catheter colonization is based on conventional culture, which takes at least 24–48 h before yielding a result and, although simple, it can be time-consuming and laborious (14). Moreover, developments in technology have led to the introduction of automated

Table 1 Patient and catheter characteristics

Characteristic	N (%)
Patients, *N* = 130	
Male sex	91 (70.0)
Median (IQR) age, years	62.00 (50.00–73.25)
Ward	
ICU	69 (53.1)
Surgery	16 (12.3)
Post-cardiac ICU	14 (10.8)
Cardiology	8 (6.2)
Gastroenterology	8 (6.2)
Internal medicine	7 (5.4)
Onco-hematology	5 (3.8)
Nephrology	3 (2.3)
Catheters, *N* = 167	
Type of catheter	
Central venous catheter	99 (53.3)
Peripheral venous catheter	34 (20.4)
Peripherally inserted central venous catheter	24 (14.4)
Introducer	10 (6.0)

IQR interquartile range, *ICU* intensive care unit

diagnostic systems in microbiology laboratories (15, 16). Automation enables a high degree of standardization, which may be beneficial not only in terms of its cost-effectiveness but also with respect to diagnostic quality, thus leading to early preliminary results (15). Therefore, before using any automated system, it is imperative to validate its diagnostic capacity with respect to reference tests.

We showed that although Alfred60^{AST}, the first automated system for bacterial culture and susceptibility testing, had good NPV (97.4%) for predicting C-RBSI and a 100% PPV for both catheter colonization and C-RBSI, it was still not sufficiently sensitive to detect colonization compared with sonication as the standard culture. Certain microorganisms were difficult to detect, and in some cases, incubation time had to be extended for > 8 h. However, these preliminary results suggested that combination of Alfred60^{AST} with MALDI-TOF detected catheter colonization and C-RBSI faster than conventional culture. Despite Gram stain showed better S and NPV values than Alfred for 8-h incubations, it cannot provide information about the species of the microorganisms in order to apply an appropriate and targeted treatment for a specific microorganism, which may have important consequences when CoNS and *S. aureus* or *C. albicans* and *C. tropicalis* infections have to be differentiated. Gram can be useful as a preliminary approach to detect catheter colonization.

The Alfred60^{AST} system measures the turbidity of specific liquid samples by generating real-time growth curves, as specified in the manufacturer's instructions. Turbidity is assessed based on the Beer-Lambert law, which reflects the direct proportionality between turbidity and cell concentration (17). Since we hypothesized that Alfred60^{AST} was better for detecting samples with high initial microbial loads and faster growth rates, sensitivity was higher for C-RBSI than for catheter colonization. HB&L medium is composed of an animal tissue infusion, and although it is an enriched medium, it was unable to enhance the growth of certain microorganisms, such as *C. glabrata* and *S. epidermidis*. The components of the medium should be re-evaluated in order to improve and facilitate growth of *C. glabrata* and *S. epidermidis*, for example, by increasing the glucose concentration (18, 19).

To the best of our knowledge, the present study is the first to evaluate Alfred60^{AST} system in combination with MALDI-TOF for diagnosis of colonization in CVC sonicates. Fontana et al. (6) used the Alfred60-like HB&L UROQUATTRO™ system to improve diagnosis of C-RBSI and found that it was

Table 2 Etiology of the colonized catheters and C-RBSI episodes

Microorganism	Colonized catheters, *N* (%)	C-RBSI episodes, *N* (%)
Gram-positive	26 (68.4)	12 (57.1)
Staphylococcus epidermidis	14 (36.8)	5 (23.8)
Other CoNS	6 (15.8)	4 (19.0)
Staphylococcus aureus	2 (5.3)	1 (4.8)
Enterococcus faecalis	2 (5.3)	2 (9.5)
Enterococcus faecium	2 (5.3)	–
Gram-negative	2 (5.3)	2 (9.5)
Enterobacter cloacae	2 (5.3)	2 (9.5)
Yeasts	10 (26.3)	7 (33.3)
Candida glabrata	4 (10.5)	3 (14.3)
Candida albicans	3 (7.9)	3 (14.3)
Candida parapsilosis	2 (5.3)	1 (4.8)
Candida tropicalis	1 (2.6)	–

CoNS coagulase-negative staphylococci

Table 3 Validity values of Gram and of the turbidity and MALDI-TOF combination on the CVC tip sonicate

	Prevalence	S	SP	PPV	NPV	LR+	LR–
Catheter colonization							
Gram	12.6	52.4	98.0	78.6	93.5	25.5	0.5
Alfred-MALDI (≤ 8 h)	19.8	39.4	100.0	100.0	87.0	NA	0.6
Alfred-MALDI (> 8 h)	19.8	63.6	100.0	100.0	91.8	NA	0.4
C-RBSI							
Alfred-MALDI (≤ 8 h)	12.6	61.9	100.0	100.0	94.8	NA	0.4
Alfred-MALDI (> 8 h)	12.6	71.4	100.0	100.0	96.1	NA	0.3

Incubations > 8 h included waits of between 8 h and 24 h, with the longest time of positivization of 20 h

S sensitivity, *SP* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR–* negative likelihood ratio, *NA* not applicable

more sensitive than Maki's technique (571 vs. 374 colonized catheters, respectively). However, they did not use MALDI-TOF and needed to culture the positive samples to identify the microorganism, thus extending the time to diagnosis (6). In contrast, we found that Alfred60^{AST} was not able to detect all positive CVC tips when compared with sonication as a conventional culture, although we did not perform Maki's technique. By using the same segment of the catheter for culture and measurement of turbidity, we ensured that the initial concentration of microorganism(s) was equal for both tests, thus explaining the differences between our results and those of Fontana et al. Moreover, although sonication did not improve the diagnosis of C-RBSI compared with Maki's technique, it can detach cells grown in biofilms in the inner surface of the catheter, thus facilitating the detection of colonization (9).

A key aspect of our methodology was that we introduced MALDI-TOF after detecting a positive sample using the Alfred60^{AST} system. This approach made it possible to identify the microorganism in a 0.5 McFarland solution of the sonicate, thus significantly shortening the time to diagnosis.

In conclusion, the combination of Alfred60^{AST} with MALDI-TOF could be a suitable alternative to conventional culture for the diagnosis of catheter colonization and C-RBSI by reducing the time to detection to a maximum of 20 h in positive samples and a minimum of 8 h in negative samples. However, improvements must be made to increase microbial growth in turbidimetry systems before using them in clinical microbiology laboratories. It is also important to assess the clinical impact of this approach.

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Compliance with ethical standards

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

Ethics statement The study was approved by the Ethics Committee of Hospital Gregorio Marañón. The patients gave their written informed consent for their data to be used in the study.

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