Optimized identification of microorganisms directly from positive blood cultures by MALDI-TOF to improve antimicrobial treatment

ABSTRACT

Introduction. Bacteriemia is a major cause of morbidity and mortality among hospitalized patients worldwide. Early identification of microorganisms from blood culture can lead to improvement of treatment and outcomes.

Methods. The study was divided into two phases. The first phase when a comparison of the methods was made to check the concordance between them, using as a reference the standard method implemented in the laboratory. In a second phase, both methods are combined. We used the rapid identification method and when it could not identify we used the standard method. The microorganisms that were not identified by either of the two methods were identified from colony at 24 hours.

Results. A total of 589 microbial positive blood cultures have been included in the present study. With the rapid method we obtained 96% and 88% identification results for Gram-negative bacilli (GNB) and Gram-positive cocci (GPC) respectively. In this study we observed that the combination of the rapid and standard method achieved identifications of 98% and 97% for GNB and GPC respectively.

Conclusions. The data analysed shows that both methods combined perform better than individually. We achieved an optimization of the identification of microorganisms directly from positive blood cultures by MALDI-TOF. This combination identified 98% of the microorganisms in between ten minutes to one hour and a half since the blood culture flagged positive.

Keywords: Maldi biotyper; Antimicrobial treatment; Direct identification; Combination of methods; Blood cultures.

Optimización en el proceso de identificación directamente de hemocultivos positivos por MALDI-TOF para mejorar el tratamiento antimicrobiano

RESUMEN

Introducción. La bacteriemia es una de las principales causas de morbilidad y mortalidad entre los pacientes hospitalizados de todo el mundo. La identificación temprana de los microorganismos que están en la sangre, permite optimizar los tratamientos y conseguir mejores resultados.

Material y métodos. El estudio se dividió en dos fases. En la primera fase se realizó una comparación entre los métodos para comprobar la concordancia entre ellos, tomando como referencia el método estándar implementado en el laboratorio. La segunda fase combinó ambos métodos para la identificación de hemocultivos positivos. Se utilizó el método de identificación rápida como primera opción y el método estándar solo cuando no se consiguió identificar por la primera opción. Los microorganismos que no fueron identificados por ninguno de los dos métodos, se identificaron directamente de la colonia crecida a las 24 horas.

Resultados. Se analizaron un total de 589 hemocultivos positivos en este estudio. Con el método rápido obtuvimos un 96% y 88% de identificación para bacilos gramnegativos y cocos grampositivos respectivamente. En este estudio observamos que la combinación de los dos métodos, se identificaron directamente de la colonia crecida a las 24 horas.

Conclusiones. Los datos analizados muestran que ambos métodos combinados consiguen mejores resultados que utilizados de forma individual. Logramos una optimización de la identificación de microorganismos directamente a partir de hemocultivos positivos por MALDI-TOF. Con esta combinación se identificó el 98% de los microorganismos entre los primeros 10 minutos y hora y media de hemocultivo positivo.

Palabras clave: tipo Maldi-torf; Tratamiento antimicrobiano; Identificación directa; Combinación de métodos; Hemocultivos.
INTRODUCTION

Bloodstream infections are a major cause of morbidity and mortality. The possibility of speeding up the identification and results of antimicrobial efficacy on bacteria grown in blood cultures and, the consequent adjustment of the appropriate antibiotic therapy is of paramount importance in patients with sepsis to improve their outcome [1,2].

Presence of microorganisms in bloodstream is a life-threatening situation that requires quick identification and treatment. Pathogen identification is of great importance, enabling adjustment of antibiotic care [3]. In most clinical microbiology laboratories, the traditional method for microbial identification includes sampling of an aliquot from the positive blood culture, subculturing it into solid agars for 18–24 h, and bacterial identification according to biochemical features and antimicrobial efficacy testing. The primary disadvantage of this method is that causative pathogen identification is performed only after colony growth and isolation, which leads to a higher turnaround time [4].

In recent years, direct identification from positive blood cultures has demonstrated reliability and a quicker comparison than MS identification from plate colonies. These methodologies have shown over the eighty percent of success in colony’s identification in gram negative bacilli and over the sixty five percent in gram positive cocci [5].

These methods also provide quicker turnarounds and combined with molecular and blood culture direct sensibility methods, they can provide a correct identification and measure sensibility to some antimicrobial agents within hours [6-8].

Our objective is to evaluate if the consecutive performance of two direct blood cultures identification methods is more efficient (percentage of direct positive blood cultures identifications) than the use of both methods individually.

MATERIAL AND METHODS

This study was conducted in University Hospital of La Paz in Madrid (Spain), a 1300-bed tertiary academic center that is a key part of the Spanish National Health Service, which supports a mixed urban and peri urban population of approximately 600,000 people nearby Madrid, Spain, with approximately 48,000 hospital admissions/year. Five hundred and eighty-two (582) consecutive positive blood cultures have been included in the present study.

All aerobic, anaerobic and pediatric/aerobic blood culture bottles have been incubated in a BD BACTEC™ FX automated device (Beckton Dickinson, Madrid, Spain) for up to 5 days at 37 °C until they were identified as positive. Samples were evaluated with the Bruker MALDI-biotyper system of Bruker Daltonik (Bruker Daltonik GmbH, Bremen, Germany). Mass spectra was obtained using a Microflex LT Mass spectrometer (Bruker Daltonik GmbH) and Flex Control software. Bacterial identification was obtained using the MALDI eBiotyper 2.0 software (Bruker Daltonik GmbH). The spectra was calibrated by using Escherichia coli ribosomal proteins (Bruker Daltonik GmbH).

We carried out the study over a period of five months (from March 2019 to July 2019) and we divided it into two phases. On the first phase (March 2019 to May 2019), we used for identification a direct blood culture a standard method which published in the literature [9]. In this first phase we assessed reliability and accuracy in our laboratory of an in-house 10-minute protocol for direct identification method previously validated [10]. We evaluated in parallel this faster and more economic method against our direct blood identification method [9]. On the second stage, we performed consecutively this quick method followed, in the cases in which the identification was not reliable, by the direct blood identification method implemented in our laboratory. Direct identifications have been performed during the routine schedule, so we included in our study every blood culture that tested positive during the morning shift (from 8:00 am to 15:00 pm) and we included one or more blood culture per patient in the study.

The protocols for sample processing used in both methods were followed step by step as they are published [9,10]. A brief description of these methods is following:

Rapid method added 200 µl of blood culture broth to a 1-ml solution of Triton X-100 (Sigma-Aldrich, Lyon, France) at a concentration of 0.1%. The mix was vortexed for 5 second and then centrifuged at 13,000 rpm for 2 min. The supernatant was discarded, and then a further 1 ml of 0.1% Triton X-100 was added before a second cycle of vortexing and centrifugation. The supernatant was removed again and added 20 µl of formic acid to the pellet. We changed this step respect to the original protocol because we found out that the identification was better incorporating 20 µl of acid formic than incorporating only 1,2 µl of acid formic. This mix was centrifuged at 13,000 rpm for 1 min. The supernatant was ready for identification using MALDI-TOF MS.

Standard method centrifuged 4 ml of blood culture at 800 rpm for 5 min. Then the supernatant was centrifuged at 10,000 rpm for 10 min. The pellet was washed once with 1 ml of deionized water. Then, an ethanol/formic acid extraction procedure was applied: the pellet was resuspended in 300 ml of water. 900 ml of absolute ethanol was added and the mixture was centrifuged at 13000 rpm for 2 min. The supernatant was discarded, 20 ml of formic acid (70% v/v) was added to the pellet and mixed thoroughly, 20 ml acetonitrile was added and mixed again. The mixture was centrifuged again at 13000 rpm for 1 min. One microliter of the supernatant was placed onto a spot of the steel target plate (Bruker Daltonik GmbH, Bremen, Germany) and gently mixed with 1 ml of a-cyano-4-hydroxy-cinnamic acid matrix solution in organic solvent (50% acetonitrile and 2.5% trifluoroacetic acid) and air dried at room temperature.

Identification between both methods has been considered reliable and concordant when bacterial identification was the same and the direct bacterial log (score) cut-offs ranged from 1.5 to 2.5. This range was evaluated by Simon et al [10]. They found the lower confidence score that provided the higher percentage of direct identifications without loss off accuracy.
Table 1  Distribution of identifications during phase one

| Microorganisms             | Total number of isolates | Correct identification by both methods | Only identification by rapid method (Simon et al) [10] | Only identification by standard method (Romero-Gómez et al) [9] | Identification by grown colony |
|----------------------------|--------------------------|---------------------------------------|------------------------------------------------------|------------------------------------------------------|--------------------------------|
| Abiotrophia defectiva      | 3                        | 3                                     |                                                      |                                                      |                                |
| Bacillus cereus            | 1                        | 1                                     |                                                      |                                                      |                                |
| Bacillus licheniformis     | 1                        | 1                                     |                                                      |                                                      |                                |
| Bacteroides fragilis       | 2                        | 2                                     |                                                      |                                                      |                                |
| Brevibacillus parabrevis   | 1                        | 1                                     |                                                      |                                                      |                                |
| Candida albicans           | 4                        | 2                                     |                                                      |                                                      | 2                              |
| Candida lusitaniae         | 3                        | 2                                     |                                                      |                                                      | 1                              |
| Candida parapsilosis       | 1                        | 1                                     |                                                      |                                                      |                                |
| Candida tropicalis         | 3                        | 2                                     |                                                      |                                                      | 1                              |
| Capnocytophaga sputigena   | 1                        | 1                                     |                                                      |                                                      |                                |
| Citrobacter sp             | 1                        | 1                                     |                                                      |                                                      |                                |
| Corynebacterium alvei      | 1                        | 1                                     |                                                      |                                                      |                                |
| Curtobacterium acnes       | 1                        | 1                                     |                                                      |                                                      |                                |
| Enterobacter cloacae       | 2                        | 2                                     |                                                      |                                                      |                                |
| Enterobacter kobei         | 1                        | 1                                     |                                                      |                                                      |                                |
| Enterococcus casseliflavus | 1                        | 0                                     |                                                      |                                                      | 1                              |
| Enterococcus faecalis      | 17                       | 16                                    |                                                      |                                                      |                                |
| Enterococcus faecium       | 3                        | 3                                     |                                                      |                                                      |                                |
| Escherichia coli           | 74                       | 72                                    |                                                      |                                                      | 2                              |
| Gemella haemolysans        | 1                        | 1                                     |                                                      |                                                      |                                |
| Hafnia alvei               | 1                        | 1                                     |                                                      |                                                      |                                |
| Klebsiella aerogenes       | 1                        | 1                                     |                                                      |                                                      |                                |
| Klebsiella oxytoca         | 7                        | 6                                     |                                                      |                                                      | 1                              |
| Klebsiella pneumoniae      | 14                       | 14                                    |                                                      |                                                      |                                |
| Kodamaea ohmeri            | 1                        | 1                                     |                                                      |                                                      |                                |
| Listeria innocua           | 2                        | 2                                     |                                                      |                                                      |                                |
| Listeria sp                | 1                        | 1                                     |                                                      |                                                      |                                |
| Micrococcus luteus         | 2                        | 2                                     |                                                      |                                                      |                                |
| Moraxella catarrhalis      | 1                        | 1                                     |                                                      |                                                      |                                |
| Moraxella nonliquefaciens  | 2                        | 0                                     |                                                      |                                                      | 2                              |
| Morganella morganil        | 1                        | 1                                     |                                                      |                                                      |                                |
| Proteus mirabilis          | 1                        | 1                                     |                                                      |                                                      |                                |
| Pseudomonas aeruginosa     | 12                       | 12                                    |                                                      |                                                      |                                |
| Pseudomonas putida         | 1                        | 1                                     |                                                      |                                                      |                                |
| Rothia dentocariosa        | 1                        | 1                                     |                                                      |                                                      |                                |
| Rothia mucilaginosa        | 1                        | 1                                     |                                                      |                                                      |                                |
| Salmonella sp              | 4                        | 3                                     |                                                      |                                                      | 1                              |
| Serratia liquefaciens      | 3                        | 3                                     |                                                      |                                                      |                                |
| Serratia marcescens        | 6                        | 6                                     |                                                      |                                                      |                                |
| Staphylococcus aureus      | 25                       | 18                                    | 6                                                    |                                                      | 1                              |
| Staphylococcus capitis     | 7                        | 5                                     | 2                                                    |                                                      |                                |
| Staphylococcus caprae      | 2                        | 2                                     |                                                      |                                                      |                                |
| Staphylococcus epidermidis | 84                       | 66                                    | 7                                                    |                                                      | 4                              |
| Staphylococcus haemolyticus| 12                       | 9                                     | 1                                                    |                                                      | 2                              |
| Staphylococcus hominis     | 23                       | 19                                    |                                                      |                                                      | 2                              |
| Staphylococcus pettenkoferi| 2                        | 2                                     |                                                      |                                                      |                                |
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Table 1
Distribution of identifications during phase one (cont.)

| Microorganisms                          | Total number of isolates | Correct identification by both methods | Only identification by rapid method (Simon et al) [10] | Only identification by standard method (Romero-Gómez et al) [9] | Identification by grown colony |
|-----------------------------------------|--------------------------|----------------------------------------|--------------------------------------------------------|---------------------------------------------------------------|-------------------------------|
| Staphylococcus pseudointermedius        | 1                        | 0                                      | 1                                                      |                                                               |                                |
| Staphylococcus schleiferi              | 1                        | 1                                      |                                                        |                                                               |                                |
| Staphylococcus warneri                  | 1                        | 1                                      |                                                        |                                                               |                                |
| Stenotrophomonas maltophilia            | 2                        | 2                                      |                                                        |                                                               |                                |
| Streptococcus alactolyticus             | 1                        | 0                                      | 1                                                      |                                                               |                                |
| Streptococcus anginosus                 | 4                        | 2                                      | 2                                                      |                                                               |                                |
| Streptococcus dysgalactiae              | 5                        | 3                                      | 2                                                      |                                                               |                                |
| Streptococcus gallolyticus              | 1                        | 0                                      | 1                                                      |                                                               |                                |
| Streptococcus gordonii                  | 2                        | 0                                      | 2                                                      |                                                               |                                |
| Streptococcus equi                      | 1                        | 0                                      | 1                                                      |                                                               |                                |
| Streptococcus oralis/mitis/pneumoniae   | 14                       | 11                                     | 1                                                      | 2                                                             |                                |
| Streptococcus parasanginis              | 2                        | 2                                      |                                                        |                                                               |                                |
| Streptococcus salivarius                | 2                        | 2                                      |                                                        |                                                               |                                |
| Trichosporon asahii                     | 2                        | 2                                      |                                                        |                                                               |                                |
|                                         | 378                      | 319                                    | 24                                                     | 23                                                            | 12                             |

Table 2
Microorganisms with discordance between both methods

| Rapid method (Simon et al) [10] | Standard method (Romero-Gómez et al) [9] | Identification by grown colony |
|----------------------------------|------------------------------------------|--------------------------------|
| Staphylococcus capitis           | Staphylococcus haemolyticus              | Staphylococcus haemolyticus    |
| Streptococcus alactolyticus      | Streptococcus galolyticus                | Candida albicans              |
| Staphylococcus pseudointermedius |                                          | Candida albicans              |

RESULTS

A total of 582 samples from 499 patients were applied over a five-month period. The period was divided into two phases. The first phase included 382 samples. 378 were monomicrobial, of which 225 (58%) contained Gram-positive organisms and 141 (37%) contained Gram-negative organisms. Four samples from mixed and sterile cultures (false positive blood cultures) were excluded from the study. The second phase included 200 samples. 193 were monomicrobial, of which 115 (59%) contained Gram-positive and 78 (40%) contained Gram-negative organisms. Seven samples from mixed and sterile cultures (false positive blood cultures) were excluded from the study too. During the first phase, both methods were compared, obtaining the results presented in Table 1. Identification percentages observed for rapid method and standard method were very similar, 90.74% and 90.47% respectively. Combining the results from both methods, we achieved an identification of the 96.82% (366). Only 12 microorganisms remained unidentified and they had to be identified from the grown colony. We only observed three discrepancies between both methods (Table 2). Final identification was performed from the grown colony. During the second phase we checked the identification percentage by performing consecutively both methods. We performed in the first place rapid method (the Simon et al. method) to reduce processing time. We observed a 98% of correct microorganisms identifications in less than one hour and a half since blood culture was identified as positive (Table 3).

DISCUSSION

Bloodstream infections are major cause of morbidity and mortality among hospitalized patients worldwide. Early identification of microorganisms from blood culture can facilitate earlier optimization of treatment [11]. The goal of integrating quicker diagnostic microbiology laboratory techniques (ie, pathogen identification and sensibility testing) with antimicrobial stewardship practices is to improve outcomes among
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| Microorganisms            | Total | Rapid method (Simon et al) [10] | Standard method (Romero-Gomez et al) [9] | Id from colony |
|---------------------------|-------|---------------------------------|-------------------------------------------|---------------|
| Achromobacter xylosidans  | 1     | 1                               |                                           |               |
| Acinetobacter baumannii   | 1     | 1                               |                                           |               |
| Acinetobacter pittii      | 1     | 1                               |                                           |               |
| Candida tropicalis        | 1     | 1                               |                                           |               |
| Citrobacter freundii      | 1     | 1                               |                                           |               |
| Clostridium ramosus       | 1     | 1                               |                                           |               |
| Corynebacterium striatum  | 1     | 1                               |                                           |               |
| Cutibacterium acnes       | 1     | 1                               |                                           |               |
| Enterobacter cancerogenus | 1     | 1                               |                                           |               |
| Enterobacter cloacae      | 4     | 2                               |                                           | 1             |
| Enterobacter hormaechei   | 1     | 1                               |                                           |               |
| Enterobacter kobei        | 4     | 4                               |                                           |               |
| Enterococcus faecalis     | 6     | 6                               |                                           |               |
| Enterococcus faecium      | 1     | 1                               |                                           |               |
| Escherichia coli          | 29    |                                 |                                           |               |
| Gardnerella vaginalis     | 1     | 1                               |                                           |               |
| Haemophilus influenzae    | 2     | 2                               |                                           |               |
| Haemophilus parainfluenza | 4     | 3                               |                                           | 1             |
| Klebsiella oxytoca        | 1     | 1                               |                                           |               |
| Klebsiella pneumoniae     | 13    |                                 |                                           |               |
| Proteus mirabilis         | 5     | 5                               |                                           |               |
| Pseudomonas aeruginosa    | 6     | 6                               |                                           |               |
| Rothia dentocariosa       | 1     | 1                               |                                           |               |
| Serratia marcescens       | 2     | 2                               |                                           |               |
| Staphylococcus aureus     | 21    |                                 |                                           |               |
| Staphylococcus epidermidis| 34    | 29                              |                                           | 4             |
| Staphylococcus haemolyticus| 10   | 7                               |                                           | 2             |
| Staphylococcus hominis    | 23    | 22                              |                                           |               |
| Staphylococcus lugdunensis| 1     | 1                               |                                           |               |
| Staphylococcus simulans   | 1     | 1                               |                                           |               |
| Streptococcus constellatus| 3     | 3                               |                                           |               |
| Streptococcus gordonii    | 1     | 1                               |                                           |               |
| Streptococcus oralis/mitis/pneumoniae| 2 | 2 | | |
| Streptococcus pyogenes    | 5     | 5                               |                                           |               |
| Streptococcus salivarius  | 1     | 1                               |                                           |               |
| Streptococcus sanguinis   | 1     | 1                               |                                           |               |
| Veillonella parvula       | 1     | 1                               |                                           |               |

| Total | Rapid method | Standard method | Id from colony |
|-------|--------------|-----------------|---------------|
| 193   | 181          | 8               | 4             |
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Table 4

| Table 4 | Comparison of the percentages of identification between the different protocols described in the bibliography. |
|---------|--------------------------------------------------------------------------------------------------------|
|         | GNB | GPC | Staphylococcus aureus | Coagulase-negative staphylococci | Processing time minutes |
| Combination of methods | 98.68 | 97.25 | 100 | 100 | <60 |
| Simon et al. 2019 [10] | 90.5 | 75.6 | 94.9 | 75.5 | 10 |
| Romero-Gomez et al. 2012 [9] | 97.7 | 97.84 | 75.8 | 63.3 | 60 |
| Yuan Y. et al. 2020 [22] | 91.5 | 88.3 | 95.7 | N | 30 |
| Azrad et al. 2019 [4] | 95 | 92 | 100 | 93 | 15 |
| Melver et al. 2018 [15] | 91.1 | 82 | N | 93 | 10 |
| Lin Jung-Fu et al. 2018 [16] | 85 | 78.2 | 88.2 | N | 10 |
| Zhou et al. 2017 [17] | 92.8 | 82.4 | 100 | 95.7 | 60 |
| Barninis et al. 2016 [18] | 97.5 | 96.1 | 94.11 | 98 | 60 |
| Jakovljev et al. 2015 [19] | 91 | 74.4 | 100 | 57.14 | 25 |
| Monteiro et al. 2015 [20] | 99 | 86.3 | 100 | 80 | N |
| Ferreira et al. 2011 [21] | 98.3 | 93.9 | 30.6 | 71.9 | 50 |

GNB: Gram-negative bacilli, GPC: Gram-positive cocci. N: No data

hospitalized patients. Earlier initiation of active, targeted antimicrobial therapy, informed by quicker identification and susceptibility results, has demonstrated improved patient care outcomes (decreased LOS, decreased mortality) and reduced health care expenses in bloodstream infections [12]. For septic patients, delaying the initiation of antimicrobial therapy or choosing an inappropriate antibiotic can considerably worsen their prognosis [13].

With the combination of quicker diagnostic methods, we achieved the identification of 98% of the microorganisms in less than one hour and a half. We have observed an increase in the percentages of identification compared to others published in the literature (Table 4). The combination of two methods increased the percentages of microorganisms’ identification in a global manner. In our study, the biggest increase was observed with the coagulase negative staphylococci with an identification of 100% compared to 75.5% for Simon et al. [10] and 63.3% for Romero-Gomez et al. [9]. For S. aureus we obtained a percentage of identification of 100%. This allowed us to optimize the molecular diagnosis of methicillin-resistant Staphylococcus aureus (MRSA). We are currently performing molecular MRSA test only in confirmed S. aureus. Before the implementation of this combination of methods, in suspicion of S. aureus infections with no direct blood culture identification, we performed molecular test to anticipate methicillin resistance. This procedure, sometimes reported false methicillin resistance results due to the identification the next day of coagulase negative staphylococci in agar plates.

Another advantage of our combining both methods is the identification of contaminating organisms (coagulase negative staphylococci mainly) from positive blood cultures. This can be beneficial for patient outcomes, drug interactions and adverse events, avoiding unnecessary anti-Gram-positive antibiotic therapy. Early confirmation of contaminated blood cultures is an advantage and can lead to potential de-escalation of antibiotics along with complimentary diagnostic testing and shortening of hospital stay. The correct identification of coagulase negative staphylococci is also an improvement on the neonatal diagnosis of related catheter sepsis and the clinical significance of these isolates [14].

Finally, we would also like to acknowledge the study has some limitations. The first limitation is the direct yeast identifications. We only performed 14 yeasts direct identifications, obtaining an 84% of correct identifications. Due to the low number of isolates, we cannot conclude that this combination of methods is as good as it seems in the case of direct identification of yeasts. The second limitation is the number of isolates identified correctly in the second phase by the Romero et al. methodology [9]. We only need to perform this methodology in 8 isolates. This could be due to the technical staff acquired experience performing the Simon et al. method [10]. On the other hand, combining results by both methods with the phase one isolates, the 96.82% of the isolates were correctly identified. Therefore, we demonstrated in the whole period of the study, an improvement in direct identification from positive blood cultures combining both methods. Another of the limitations that we observed was the variability of the results depending on the experience of the worker. An example of this was the difficulty in identifying species such Streptococcus spp. (S. anginosus, S. dysgalactiae and S. gallolyticus) during phase one which improved significantly with experience in the technique during phase two. During the study period we did not find any anaerobic microorganisms, which was a limitation when checking the identification of this type of microorganisms.
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Our combination of methods has the advantage of being a quick and easy-to-perform procedure. This combination could provide an alternative approach to improve blood culture management in microbiology laboratories without added labor to the workflow. This provides additional time for the technical staff to devote to other areas within the microbiology laboratory such as quality control, equipment maintenance or research. In conclusion, both methods combined are better than individually. We achieved an optimization of the identification of microorganisms directly from positive blood cultures by MALDI-TOF. This combination identified 98% of the microorganisms in an interval of ten minutes to one hour and a half since the blood culture flagged positive.

This practice allows a reliable and fast identification to make a clinical decision for antimicrobial treatment in bacteremia / sepsis / septic shock, improving the effectiveness of the methods performed individually.

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None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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