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REVIEW ARTICLE

Rapid Identification Protocols in bacteria by MALDI-TOF from positive blood cultures. A literature review

Protocolos de Identificación Rápida en bacterias por MADI-TOF a partir de Hemocultivos Positivos. Una revisión narrativa

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Abstract

Objective. To identify protocols for rapid identification of bacteria by MALDI-TOF from positive blood cultures that are as accurate as standardized ones. **Methods**. A bibliographic review was conducted on experimental articles, systematic reviews, experimental studies, and diagnostic test studies on the rapid identification of bacteria by MALDI-TOF from positive blood cultures. The QUADAS-2 tool was applied to evaluate the risk of bias in the articles. **Results**. There are various methodologies for the rapid identification of bacteria from positive blood cultures, all with a processing time of no more than one hour and with materials accessible in laboratories. These methodologies, such as Sepsityper and artisanal methods, achieve good accuracy in identifying Gram-negative bacteria, but there are still limitations in identifying Gram-positive bacteria depending on the genus and species of the bacterium. It is important to note that the quality of evidence in most studies is moderate, so the results should be taken with caution. **Conclusion**. There are several methodologies for rapid identification of bacteria in positive blood cultures, with short processing times and accessible materials in laboratories, that achieve good accuracy in identifying Gram-negative bacteria but have limitations in identifying Gram-positive bacteria. The quality of evidence is moderate, indicating that further studies with better design and higher quality and certainty results are needed to draw inferences obtained.

Keywords: rapid identification, protocols, positive blood cultures, literature review, research work analysis (Source: MeSH BIREME).

Resumen

Objetivo. Identificar protocolos de identificación rápida de bacterias por MALDI-TOF a partir de hemocultivos positivos que sean precisos como los estandarizados. **Métodos**. Se hizo una revisión bibliográfica de artículos experimentales, revisiones sistemáticas, estudios experimentales y estudios de pruebas diagnósticas de estudios sobre la identificación rápida de bacterias mediante MALDI-TOF a partir de hemocultivos positivos; donde se aplicó el instrumento del QUADAS-2 para la evaluación de riesgo de sesgo de los artículos. **Resultados**. Se tiene una gran variedad de metodologías en identificación rápida de las bacterias a partir de los hemocultivos positivos, todas ellas tienen un tiempo de procesamiento que no supera una hora y con materiales accesibles en los laboratorios. Estas metodologías como Sepsityper y los métodos artesanales logran una buena precisión en la identificación de las bacterias Gram negativas, aún se tienen limitaciones en la identificación de bacterias Gram positivas dependiendo del género y especie de la bacteria. Se destaca, que la calidad de la evidencia de la mayoría de los estudios es moderada, por lo tanto, tomar con cautela la información. **Conclusión**. Hay varias metodologías de identificación rápida de bacterias en hemocultivos positivos, con tiempo de procesamiento corto y materiales accesibles en los laboratorios, que logran una buena precisión en identificación de bacterias Gram negativas, pero tienen limitaciones en las Gram positivas. La calidad de la evidencia es moderada, por lo que se requieren más estudios con mejor diseño y que puedan generar resultados de mayor calidad y certeza a fin de tomar las inferencias que se hayan obtenido.

Palabras clave: identificación rápida, protocolos, hemocultivos positivos, revisión de la literatura, análisis de trabajos de investigación (Fuente: DeCS BIREME).

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Introduction

Septic shock and severe sepsis are the most important causes of emergency care in the world and the most frequent of admissions to the intensive care unit service, since they are strongly related by their high mortality, morbidity, and high cost of medical care during the hospital stay. Therefore, an adequate identification of the etiological agent is required in the shortest possible time in order to provide correct and timely treatment, thus reducing a potential risk of mortality and / or serious sequelae[1,2].

Given the possibility of not having the identification of the etiological agent in less time, microbiology laboratories have been improving response times and this is how, the mass spectrometry methodology with time-of-flight analyzer and desorption by matrix-assisted laser (MALDI-TOF) has become the standard bacterial identification which has been gradually implemented[1,2]. However, there are some laboratories that do not have this mass spectrometry analyzer, so their sample processing is conventional or artisanal, thus delaying the identification of the etiological agent, even with the high possibility of not being able to identify the correct pathogen[2].

This methodology is of structural determination that allows to study the distribution of the molecules of a substance according to its mass. The mass range for the identification of microorganisms is between 2000 Da and 20000 Da. Most of the mass peaks obtained in this range are ribosomal proteins. The set of these mass peaks are the spectra of the microorganism. This protein profile is compared with the protein profiles stored in the database of the equipment being used, from which it will issue an identification report in a few seconds[3].

The MALDI TOF methodology is standardized from the growth of bacterial colonies obtained from a culture medium which can take from 6 to 24 hours. Different studies have satisfactorily reported bacterial identification from positive blood cultures performing various procedures using MALDI-TOF, but the results are variable.

One of the procedures described is the method of Sepsityper (Bruker Daltonic) which is a commercial method that through a series of processes allows identification in a short time through obtaining the bacterial pellet, being the limitation the cost[2].

Therefore, there are in-house or artisanal methods that many laboratories have been developing to remove human cells and obtain the bacterial pellet from positive blood cultures. Saponin and ammonium chloride are used to lyse blood cells. The separation of microorganisms from blood cells is obtained by differential centrifugation and gel separator tube, as well as the method of staggered sedimentation that have as advantages the lowest cost and easy implementation[2].

Therefore, the rapid identification of the bacteria that cause bloodstream infections would improve patient care, providing an effective and empirical antibiotic treatment for the isolated germ, reducing hospital stay and subsequently mortality due to early therapeutic administration. Likewise, to be able to establish a rapid identification protocol from positive blood cultures that adapt to the resources of each microbiology laboratory under the mass spectrometry methodology with matrix-assisted laser time-of-flight and desorption analyzer (MALDI-TOF).

Methods

A quick review of the available literature was conducted.

A. Eligibility Criteria

1) Inclusion Criteria

- Related to the topic to be treated: rapid identification in MALDI-TOF from blood cultures.
- Types of study: Systematic reviews, experimental studies, diagnostic test studies.
- Language: Those published in English, Spanish.

2) Exclusion Criteria

- Type of study: letters to the editor, editorials, comments, fact sheets, case reports.

PICO QUESTION: Are there protocols for rapid identification of bacteria by MALDI-TOF from positive blood cultures that are accurate as standardized?

- Population: Positive blood cultures.
- Intervention: Rapid identification by MALDI TOF.
- Comparator: Standardized identification.
- Results: Identify bacteria accurately as the standardized method.
- Make it in less time than the standardized method.
- Make it easy to perform.

B. Sources of Information

We reviewed the PUBMED/Medline and EMBASE database, from which articles that were not freely accessible through PUBMED were obtained. Additionally, we manually reviewed the publications that cited the articles of interest, as well as publications cited from the same studies.

C. Search Strategy

The search strategy was based on the terms MeSH (Medical Subject Headings) as it shows in Table 1. Literature in English and Spanish was reviewed. The search date was 8 September 2022 to 24 September 2022 and was updated on 10 October 2022.

The search results were imported to the Zotero reference manager version 6.0.15 for identification and removal of duplicate articles. This stage was performed by a single author.

Table 1. Search strategy

N°	Search Terms	Results
#1	Search: ("blood culture"[tiab] OR "bacteremia"[tiab]) OR "bloodstream infection" [tiab] Filters: in the last 10 years, Humans, English, Spanish ("blood culture"[Title/Abstract] OR "bacteremia"[Title/Abstract] OR "bloodstream infection"[Title/Abstract]) AND ((y_10[Filter]) AND (humans [Filter]) AND (english[Filter] OR spanish[Filter]))	14234
#2	Search: ((maldi ms [tiab]) OR (mass spectrometry [tiab])) OR (matrix-assisted laser desorption/ionization [tiab]) Filters: in the last 10 years, Humans, English, Spanish ("maldi ms"[Title/Abstract] OR "mass spectrometry"[Title/Abstract] OR "matrix assisted laser desorption ionization"[Title/Abstract]) AND ((y_10[Filter]) AND (humans [Filter]) AND (english[Filter] OR spanish[Filter]))	62102
#3	Search: (accuracy [tiab]) OR (rapid identification [tiab]) Filters: in the last 10 years, Humans, English, Spanish ("accuracy"[Title/Abstract] OR "rapid identification"[Title/Abstract]) AND ((y_10[Filter]) AND (humans [Filter]) AND (english[Filter] OR spanish[Filter]))	172566
#1 , #2 AND #3	Search: ("blood culture"[Title/Abstract] OR "bacteremia"[Title/Abstract] OR "bloodstream infection"[tiab]) AND ("maldi ms"[Title/Abstract] OR "mass spectrometry"[Title/Abstract] OR "matrix assisted laser desorption ionization"[Title/Abstract]) AND ("accuracy"[Title/Abstract] OR "rapid identification"[Title/Abstract]) Filters: in the last 10 years, Humans, English, Spanish (("blood culture"[Title/Abstract] OR "bacteremia"[Title/Abstract] OR "bloodstream infection"[Title/Abstract]) AND ("maldi ms"[Title/Abstract] OR "mass spectrometry"[Title/Abstract] OR "matrix assisted laser desorption ionization"[Title/Abstract])) AND ("accuracy"[Title/Abstract] OR "rapid identification"[Title/Abstract]) AND ((y_10[Filter]) AND (humans[Filter]) AND (english[Filter] OR spanish[Filter]))	116

Source: Own Elaboration

D. Risk of BIAS Assessment

Study quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool[29] which was performed using the Review Manager (RevMan) and the meta-analysis was evaluated with the A MeaSurement Tool to Assess systematic Reviews (AMSTAR 2). For this evaluation we performed between an independent reviewer and the author.

Results

The 116 citations were identified and from them 68 studies were discarded in a first review whose causes of exclusion were: title, study design (n = 22), objectives (n = 35) that corresponded to studies where they evaluated the clinical impact from the point of view of costs or treatment, other studies evaluated the MALDI-TOF methodology without having any rapid identification procedure, Identification from subcultures in less time, sample type (n = 3) which were two by vitreous humor and one of amniotic fluid and by intervention (n = 8). Of the 48 potentially appropriate studies that were reviewed in detail, 23 were excluded for the following reasons: objective (n=9), intervention (n=13), and design (n=1) from which a letter to the editor corresponded. Of the group corresponding to the exclusion of intervention, the majority corresponded to blood cultures that came from simulations, that is, positive blood cultures were prepared with ram blood and placing ATCC strains of Gram-

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positive and Gram-negative bacteria. Therefore, the number of studies that were part of this review was 25, as it shows in Table 2.

Table 2. Summary of reviewed article

Author Name	Title	Journal	Year of Publication	Design Type
Dai[4]	Evaluation of a Rapid and Simplified Protocol for Direct Identification of Microorganisms From Positive Blood Cultures by Using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)	Frontiers in Cellular and Infection Microbiology	2021	Experimental
Ponderand [15]	Evaluation of Rapid Sepsityper® protocol and specific MBT-Sepsityper module (Bruker Daltonics) for the rapid diagnosis of bacteremia and fungemia by MALDI-TOF-MS	Annals of Clinical Microbiology and Antimicrobials	2020	Experimental
Cordovana [19]	Rapid Sepsityper in clinical routine: 2 years' successful experience	Journal of Medical Microbiology	2020	Experimental
Samaranayake [16]	Rapid direct identification of positive paediatric blood cultures by MALDI-TOF MS technology and its clinical impact in the paediatric hospital setting	BMC research notes	2020	Experimental
Carretero [10]	Rapid identification of bacteria directly from positive blood cultures by a modified method using a serum separator tube and matrix-assisted laser desorption ionization - time of flight MS	Journal of Medical Microbiology	2020	Experimental
Yuan [6]	Evaluation of an optimized method to directly identify bacteria from positive blood cultures using MALDI-TOF mass spectrometry	Journal of Clinical Laboratory Analysis	2020	Experimental
Simon [14]	Direct Identification of 80 Percent of Bacteria from Blood Culture Bottles by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Using a 10-Minute Extraction Protocol	Journal of Clinical Microbiology	2020	Experimental
Fang [7]	Rapid identification of microorganisms from positive blood cultures in pediatric patients by MALDI-TOF MS: Sepsityper kit versus short-term subculture	Journal of Microbiological Methods	2020	Experimental
Hu [8]	Applicability of an in-house saponin-based extraction method in Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for identifying bacterial and fungal species in positively flagged pediatric VersaTREK blood cultures	Journal of Microbiology, Immunology, and Infection	2020	Experimental
Huang [5]	Evaluation of an in-house MALDI-TOF MS rapid diagnostic method for direct identification of micro-organisms from blood cultures	Journal of Medical Microbiology	2019	Experimental

Martín-Pujol [11]	Comparison of three procedures for the rapid identification of bacteraemia-causing microorganisms. Evaluation of their effectiveness and applicability to microbiology laboratories	Enfermedades infecciosas y microbiologia clinica (English ed.)	2019	Observacional comparativo
Wu [28]	Direct antimicrobial susceptibility tests of bacteria and yeasts from positive blood cultures by using serum separator gel tubes and MALDI–TOF MS	Journal of Microbiological Methods	2019	Experimental
Ruiz-Aragón [2]	Direct bacterial identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry: A systematic review and meta-analysis	Enfermedades Infecciosas Y Microbiologia Clinica (English Ed.)	2018	Revisión sistemática y metaanálisis
Lin [26]	A simple method for rapid microbial identification from positive monomicrobial blood culture bottles through matrix-assisted laser desorption ionization time-of-flight mass spectrometry	Journal of Microbiology, Immunology and Infection	2018	Experimental
Barberino [18]	Direct identification from positive blood broth culture by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS)	The Brazilian Journal of Infectious Diseases: An Official Publication of the Brazilian Society of Infectious Diseases	2017	Experimental
Jo [25]	Direct Identification and Antimicrobial Susceptibility Testing of Bacteria From Positive Blood Culture Bottles by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and the Vitek 2 System	Annals of Laboratory Medicine	2016	Experimental
Yonetani [20]	Direct identification of microorganisms from positive blood cultures by MALDI-TOF MS using an in-house saponin method	International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases	2016	Experimental
Jakovljev [22]	Development of a rapid and simplified protocol for direct bacterial identification from positive blood cultures by using matrix assisted laser desorption ionization time-of- flight mass spectrometry	BMC microbiology	2015	Experimental
Cattani [21]	Rapid identification of microorganisms by mass spectrometry in a blood culture system. Comparison of two procedures	Revista Argentina De Microbiologia	2015	Experimental
Egli [24]	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) directly from positive blood culture flasks allows rapid identification of bloodstream infections in immunosuppressed hosts	Transplant infectious disease: an official journal of the Transplantation Society,	2015	Experimental de evaluación de prueba diagnóstica

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Mestas [13]	Direct identification of bacteria from positive BacT/ALERT blood culture bottles using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry	Diagnostic Microbiology and Infectious Disease	2014	Experimental
Jamal [27]	Rapid identification of pathogens directly from blood culture bottles by Bruker matrix-assisted laser desorption laser ionization-time of flight mass spectrometry versus routine methods	Diagnostic microbiology and infectious disease	2013	Comparativo
Fothergill [12]	Rapid identification of bacteria and yeasts from positive-blood-culture bottles by using a lysis-filtration method and matrix-assisted laser desorption ionization-time of flight mass spectrum analysis with the SARAMIS database	Journal of Clinical Microbiology	2013	Experimental
Foster [17]	Rapid Identification of microbes in positive blood cultures by use of the vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system	Journal of clinical microbiology	2013	Experimental
Lagacé-Weins [23]	Identification of blood culture isolates directly from positive blood cultures by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry and a commercial extraction system: analysis of performance, cost, and turnaround time	Journal of Clinical Microbiology	2012	Experimental

Source: Own Elaboration

The studies selection flowchart is shown in Figure 1 and the reason for exclusion of unselected citations is available as a supplementary table.ísticamente significativa con sexo masculino (OR 3.62 p<0.05) e hipertensión arterial (OR 3.45 p<0.05) (tabla 2).

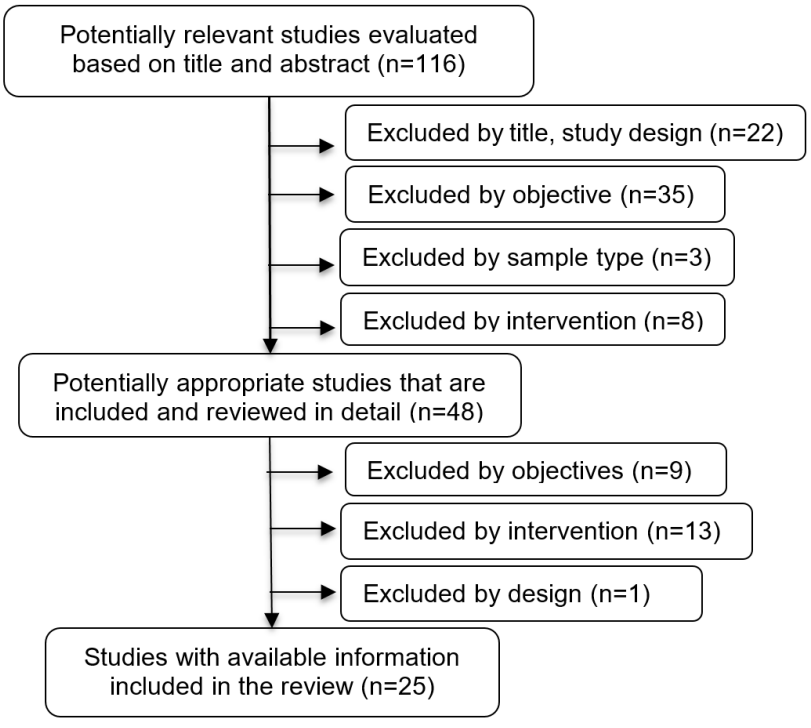

Fig. 1. Flowchart of the reviewed studies

E. Characteristics of the included studies

We identified 24 experimental studies and one study that was a systematic review and meta-analysis. The objective of the 24 studies was to evaluate the performance of the internal method for direct identification of bacteria compared with standardized identification from positive blood cultures and the systematic review study was to assess the accuracy of MALDI-TOF MS for direct identification from positive blood culture vials[5].

The studies reviewed were conducted in China, with the largest number with six studies[4–9], Spain has three studies[2,10,11], the countries where two studies have been published are: United States of America[12,13], French[14,15], Australia[16,17], and other countries such as Brazil[18], Italy[19], Japan[20], Argentina[21], Norway[22], Canada[23], Switzerland[24], South Korea[25], Taiwan[26] and Kuwait[27] have a published study.

Blood cultures that were positive in either the adult or pediatric population were considered as population, being mostly the blood cultures taken in the adult population, only three articles were had where the population was purely pediatric[7,8,16] and a study in immunosuppressed patients[24].

Study samples were variable with the lowest being 62[24] and the one with the highest number of samples was 6918[13].

The positivity of the blood cultures was preferably monomicrobial, that is, a bacterium grew, it was not considered polymicrobial isolates. They were not excluded if the bacteria were isolated from different types of aerobic and anaerobic blood culture bottles. Positive blood cultures performed by simulation were not considered.

The methodologies that were evaluated were filtration[12], lysis-centrifugation[4,17,20], saponina[5,8,11,18,20,22], Filtration-centrifugation[25], centrifugation[20,26], centrifugation with use of gel separator tube[6,9,10] Triton-centrifugation[14,16] and Sepsityper[7,11,15,18–21,23,24,27,28], this last methodology is a commercial kit part of the MALDI TOF team of Bruker Daltonic. The most evaluated methodology was Sepsityper, followed by saponin. All these methodologies were compared with the standard methodology which is to perform the identification of MALDI TOF from the isolated colonies in the cultures according to their routine protocols for bacterial identification. There is a study that describes that they made two comparison methodologies with their standardized method, one was with Sepsityper and another with artisanal methodology but they do not describe what it is called or what procedure they did[21].

These experimental studies took into account the concordance in the identification of Gram-positive and Gram-negative bacteria with standardized identification.

For the identification of bacteria in general through rapid identification methods was achieved in more than 80% correct identification. For the group of Gram-positive bacteria the correct identification was between 50 to 99%, being the lowest with the tube in gel separator[10] and the in-house methodology that achieved the greatest identification was with centrifugation newt[14]. When the evaluation by taxonomy was conducted, it was evident that Staphylococcus aureus has the best agreement rate in identification when compared with standardized methods, instead the lowest concordance rates were with staphylococcus bacteria other than Staphylococcus aureus and streptococci where all methodologies had difficulties in their identification.

Regarding the identification in Gram-negative bacteria, the correct identification was greater than 90% when compared with the standardized methodology. From the taxonomic point of view, the bacteria that presented a better identification were Enterobacteriaceae such as Escherichia coli and Klebsiella pneumoniae, being similar with all methodologies, except for a study where they do not describe the artisanal methodology where it was the lowest concordance rate (78.44%), as well as with the Sepsityper methodology (78.9%)[21]. In non-fermenting bacilli such as Pseudomonas and Acinetobacter they had an identification rate greater than 80% up to 100%[5,6,19].

Some studies identified anaerobic bacteria that were isolated from positive blood cultures but the amount was very low so their evaluation is not significant(4–6).

F. Study characteristics by frequency methodology

1) Sepsityper® [7,11,15,18–21,23,24,27,28]

It was the most studied methodology, achieving a very variable percentage of identification of bacteria, being the highest percentage of identification in Gram-negative bacteria compared to the standard method. In this group, in most studies, Enterobacteriaceae had the identification percentage above 95%, but there are some studies where they reported lower identifications, for example the study by

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Cattani et al.[21], reported in 78.9% joining Enterobacteriaceae with non-fermenting bacteria, and the study of Fang et al.[7] reported that identification was lower in Gram-negative bacteria compared to Gram-positive bacteria (84.8% vs. 86.1% respectively).

The lowest identification was with Gram-positive bacteria reaching up to 35.9%[20]. *S.aureus* and *Enterococcus spp* bacteria achieved the highest identifications (greater than 90%) and most studies reported difficulties with coagulase-negative staphylococcal bacteria and streptococci with this methodology.

Almost all studies performed the Sepsityper methodology based on what was described in their inserts since the procedure took a duration of approximately 30 min. In the study of Ponderand et al.[15] made comparisons of fast Sepsityper (RS) where the protocol took 10 minutes to process, the standard Sepsityper protocol (SS) that had a duration of 30 minutes and the fast Sepsityper protocol where formic acid was added (RS + FA), as well as the comparison of the MBT-Sepsityper RUO and MBT-Compass-IVD software, being the first software that provided a significantly greater identification with the protocols: SR (62.2% vs 43.9, p=0.001), SR+FA (73.2% vs. 64.6%, p=0.023), while in the SS protocol the identification trend was greater (78% vs. 72%) Finally, when comparing by Gram-positive bacteria, the one with the highest percentage of identification was RS+AF and in Gram-negative bacteria there was no significant difference in the three protocols.

2) Saponina[5,8,11,18,20,22]

Of the 6 studies, it is observed that the sample size is variable from 152 to 666 positive blood cultures. Most studies evaluated saponin as a cell lysis reagent and in the study by Jakovljev et al.[22] They evaluated two methods with saponin where A: cell sediment was superimposed with formic acid for protein extraction and B was exposed with formic acid followed by acetonitrile. The best results were obtained with method A, where they achieved the direct identification of 81.9% and 65.8% of organisms with method A and method B, respectively.

The identification of Gram-negative bacteria was higher compared to Gram-positive bacteria. All studies reported more than 90% identification in Gram-negative bacteria. As for Gram-positive bacteria, the percentage of identification was between 76 and 78.5% based on identification in genus, since at the species level the percentages were lower (69.6%). The studies did not report which bacteria had difficulty identifying with[20].

3) Centrifugation with gel separator tube[6,10,28]

From the three studies, the sample size ranges from 158 to 822 positive blood cultures. For the identification of Gram-negative bacteria, the highest percentages were obtained (83.5% to 93.4%) [10,28].

Regarding the identification of Gram-positive bacteria had the lowest percentage (21.8% to 81.3%)[6,10].

In the study of Yuan et al.[6], The highest rate of identification consistency was that of Staphylococcus cephalococcus, Staphylococcus haemolyticus, Staphylococcus intermediate and Staphylococcus goat, which was 100%, followed by that of Staphylococcus *hominis*, Staphylococcus epidermidis, and *Staphylococcus aureus*, which was above 90%. Therefore, there was a significant difference in consistency rates between different species of gram-positive bacteria ($p< 0.01$)

Also in the study of Wu et al.[28] describes that they had inconclusive results in Staphylococcus aureus, coagulase-negative staphylococci and *Enterococcus spp*.

On the other hand, Carretero et al.[10], identified 100% of Streptococcus agalactiae and Streptococcus gallolyticu s and 75% of *Streptococcus pneumoniae* with a score >1.7, being one of the few methodologies that achieves the identification of the streptococci group.

4) Centrifugation lysis[4,13,17]

The studies reviewed had a variable sample size of 168 to 2032[4,13].

For the identification of Gram-positive bacteria, the percentages range from 78.4% to 95.7%, being Foster's study[17] who obtained the highest percentage where *S. aureus* and coagulase-negative staphylococci had no difficulties in their identification. For Gram-negative bacteria, the range goes from 84.7% to 94.06%.

In the study of Mestas et al.[13] evaluated the method of lysis centrifugation (LTD) direct and extracted (formic acid is added after extraction), which for both groups of bacteria the method of LTD extracted obtained better percentage of identification. The processing time by this methodology is 35 to 40 minutes[17].

5) Centrifugation method[20,26]

In the study of Yonetani et al.[20] obtained a correct identification of Gram-positive bacteria in gender of 58.7% and when evaluated in species it was lower (26%). However, the study of Lin et al.[26] showed an identification in 78.2%, being higher than that reported by Yonetani et al.[20]. By taxonomy, the Gram-positive bacteria that had difficulty identifying were with S. epidermidis and coagulase-negative staphylococci.

On the correct identification in Gram negative bacteria was in 75.8% and 85%, showing a marked difference with Gram-positive bacteria[20,26]. The processing time of this methodology is 10 min[26].

6) Triton-centrifugation method[14,16]

The correct identification of Gram-positive bacteria was reported in 75.6% and 84%, while for Gram-negative bacteria the percentage of correct identification was higher (90.5% and 99%), being higher when performed by taxonomy for Enterobacteriaceae 96.5% and for non-fermenting bacilli was 72.7%[14]. The bacterium that had the lowest percentage in identification was S. pneumoniae(70,6%)[14].

G. Other studies

With the filtration method carried out by Fothergill et al.[12] the percentage of correct identification of Gram-positive bacteria was 74.5% and for Gram-negative bacteria it was 84%. The processing time is 15 to 20 min.

In relation to the filtration-centrifugation method of Jo et al.[25] reported that the percentage of correct identification of Gram-positive and Gram-negative bacteria was 73.9% and 92.6% respectively. They had difficulty identifying streptococci and staphylococci other than Staphylococcus aureus.

H. Study quality assessment

The evaluation was made by two reviewers CCE and BMC whose evaluations were coincident. As evidenced in Table 3 and Figure 2, we describe the 24 studies evaluated where the patient selection item presents low risk in most studies since they evaluate the evidence from positive blood cultures of hospitalized patients, as well as a low concern in the applicability of the results.

It is observed that in the item of the reference method the probability of risk is not clear, because the methodologies to be compared with the reference obtained results in less time and in all studies both methodologies were carried out in parallel, but the concern about the applicability of the results was low in most cases. Therefore, most studies are of moderate quality.

Regarding the meta-analysis study, the AMSTAR 2 tool was applied, whose result was of low quality.

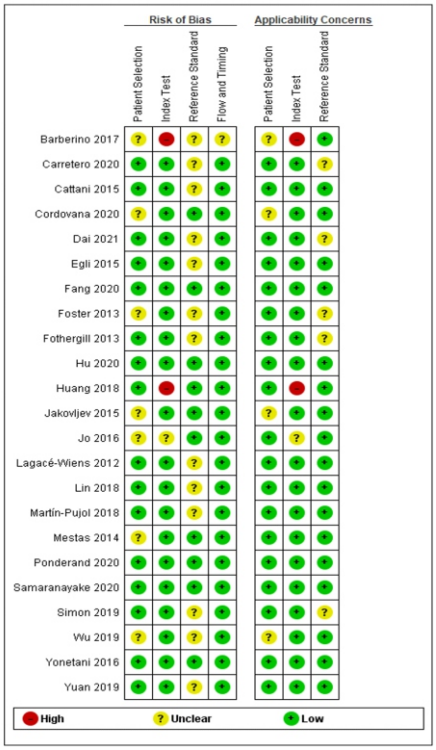

Fig. 2. QUADAS-2: Probability of Risk and Concern about Applicability of Results

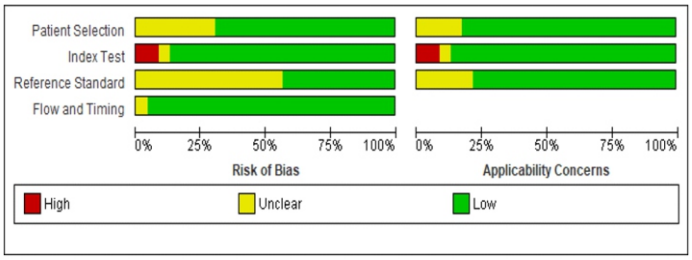

Fig. 2. QUADAS-2: Probability of Risk and Concern about Applicability of Results

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Discussion

For the identification of the different types of germs from blood cultures, it is necessary to execute subcultures in solid media that are incubated for 24 hours and possibly longer, according to the methodology available in the laboratory.

The methodology introduced in microbiology laboratories that has been shown to reduce bacterial identification time is mass spectrometry with matrix-assisted laser-assisted time-of-flight and desorption analyzer (MALDI-TOF). This method is based on producing spectral fingerprints of unique characteristic masses for each germ, proving to be a more direct and accurate bacterial identification method than traditional methods[30].

One of the advantages of MALDI-TOF is the ease of use and the ability to analyze many insulations simultaneously, which increases productivity. Similarly, once the strain is loaded into the instrument, identifications can occur in about 1 to 2 minutes[31].

Despite the great reduction in the identification time, it is still difficult to achieve even less from positive blood cultures to obtain the bacterial sediment, so they have begun to develop various protocols[32].

The identification of bacteria from positive blood cultures is methodologically challenging because the blood contains large amounts of nonbacterial proteins from leukocytes and erythrocytes, which can generate significant residues in the protein expression profile[24].

Another challenge because MALDI-TOF requires approximately 105 colony forming units to obtain a specific and reliable spectrum, a high microbial density is needed to obtain a good protein spectrum[31].

Therefore, it is necessary to concentrate the microorganisms by differential washing and centrifugation or perform another process such as protein extraction, to obtain the microbial sediment[32].

There are several protocols that comply with these procedures for the rapid identification of bacteria by the MALDI-TOF methodology, as demonstrated in the studies described. The most common methods are Sepsityper, saponin®, gel separator tube centrifugation and centrifugation lysis.

As for the Sepsityper® kit, it is an in vitro diagnostic method developed by Bruker, in which it is performed in his equipment, which has shown in numerous studies that it is able to have a good identification of Gram-negative bacteria, as well as in studies where they are compared with artisanal methods; with Gram-positive bacteria according to taxonomic group type, Staphylococcus aureus and Enterococcus spp were successfully discriminated against and showed difficulties with coagulase-negative staphylococcus bacteria and streptococci. A limitation of this method is how expensive compared to the artisanal methods described, as described by Yonetani et al.[20] Compared to saponin, similar identities and processing times were achieved in about 30 min. The reviewed studies have few applicability issues, but with a moderate risk of bias, as more than half of the studies using this method have the uncleared baseline test item.

The saponin method stands out among the procedures evaluated in several reviewed studies and is considered a cell lysis reagent to improve protein profiles[24].

The quality of evidence from these studies is moderate to low. This is because the index test item is at high risk of bias in two studies, with major concerns about the applicability of this item.

The methodology that presents few steps, less effort and minimal materials for its procedure compared to artisanal methods, is centrifugation with gel separation tubes[10]. The quality of the evidence in the studies is moderate due to the unclarified risk of bias in the reference test item and concerns about unclear applicability in the baseline test items and patient selection in the Carretero et al. study. and in the study of Wu et al. respectively It has been shown to have a good agreement with the identification of Gram-negative bacteria when performed with the gel separator tube, but in the identification of Gram-positive bacteria the percentage was lower (21.8% to 81.3%)[6,10].

In the study of Carretero et al.[10] a good identification of streptococci such as S. agalactiae, S. gallolyticus and S. pneumoniae was achieved, of which the rest of the methodologies have had difficulties.

The centrifugation lysis methodology has achieved better bacterial identification when formic acid is added after extraction, as demonstrated by the Mestas et al. study[13].

Consequently, review studies of artisanal methods showed a good concordance rate in identifying especially Gram-negative bacteria compared to Gram-positive bacteria. It should be noted that the largest number of

bacteria isolated from positive blood cultures were Gram-positive bacteria of the genus Staphylococcus, dominated by coagulase-negative staphylococci, and in this group the bacterium Staphylococcus epidermidis stands out, which is generally considered a contaminant[10,24].

One explanation for why all methodologies have some difficulty identifying Gram-positive bacteria with MALDI-TOF is that their cell walls made of peptidoglycan, which are thicker, generate greater cleavage resistance than Gram-negative bacteria[33].

Some studies, such as Carretero et al.[10] They have evaluated whether the type of blood culture bottle (e.g., aerobic adult, anaerobic adult and pediatric) would influence the use of these rapid methodologies (centrifugation in gel separation tubes), demonstrating that they do not differ significantly in bacterial identification.

Regarding the quality of the studies reviewed, they were rated as moderate quality, in the selection of positive blood cultures, most were consecutive over a period of time and some were unclear in the selection; For the index test of artisanal methodologies if the procedure and interpretation are described according to the score issued by the team dividing it by categories, this score was variable in the studies, its interpretation was given without the knowledge of the reference method because these artisanal methods take less procedural time than standardized methods. At the same time, the standardized method and the artisanal method of rapid identification were developed. The potential risks are unclear in the section of the standardized reference method, as it is unclear from the studies whether the reference method was interpreted without knowing the method of comparison.

For the development of these evaluated methodologies, one of the limitations is that before its application it is important to know the Gram stain report of positive blood cultures to find a single type of bacteria, because there is difficulty in polymicrobial growth developing an imprecision in its identification as described by Yonetani et al.[20]. In these cases, molecular tests such as multiplex polymerase chain reaction (PCR), where the samples are blood from positive blood cultures, have allowed better identification and detection of more common resistance genes.

Another limitation of the studies is that we did not have many germs, such as anaerobic bacteria, yeasts, and bacteria rarely isolated from blood cultures and therefore were not considered in this review. In-house methodologies have the advantage of requiring short processing times, so they don't generate a lot of workloads for microbiology staff. They are also applicable because of their low cost and ease of implementation in the routine of microbiology laboratories.

Finally, rapid identification of bacteria isolated from positive blood cultures could improve patient care, shorten hospital stays, and reduce costs, especially when antibiotic therapy is initiated when time is of the essence.

Conclusion

There is a variety of methodologies in rapid identification of bacteria from positive blood cultures, all of them have a processing time that does not exceed one hour and with materials accessible in laboratories, these methodologies such as Sepsityper and artisanal methods achieve good accuracy in the identification of Gram-negative bacteria, there are still limitations in the identification of Gram-positive bacteria depending on the genus and species of the bacterium. Importantly, the quality of evidence from most studies is moderate, therefore the results obtained in the various studies need to be taken with caution. More studies with better design and that can generate results of higher quality and certainty are required to take the inferences that have been obtained. If you want to implement any of the artisanal methods, it is necessary that the laboratories perform a validation since now they are procedures under investigation, while the Sepsityper method is the one that is validated for in vitro diagnosis.

Describe contribution de authors

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2. **Metodología**: Jean Meneses-Claudio, Margaret Condori-Zevallos
3. **Recolección de datos**: Carolina Cucho-Espinoza, Jean Meneses-Claudio, Margaret Condori-Zevallos
4. **Realizó los análisis del estudio**: Carolina Cucho-Espinoza
5. **Escribió el primer borrador del artículo**: Carolina Cucho-Espinoza, Jean Meneses-Claudio, Brian Meneses-Claudio
6. **Realizó la edición crítica del artículo**: Brian Meneses-Claudio
7. **Acepto el contenido final del articulo**: Carolina Cucho-Espinoza, Jean Meneses-Claudio, Margaret Condori-Zevallos, Brian Meneses-Claudio
8. **Aprobaron versión para publicación**: Carolina Cucho-Espinoza, Jean Meneses-Claudio, Margaret

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