Application analysis of MALDI-TOF MS in rapid identification of anaerobic bacteria

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Abstract

BACKGROUND: Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been rapidly developed and widely used as an analytical technique in the clinical laboratories with high accuracy in the identification of microorganisms. METHOD: This study was designed to evaluate MALDI-TOF MS for identification of clinical pathogenic anaerobes. RESULT: Twenty-eight studies covering 6685 strains of anaerobic bacteria were included in this meta-analysis. Fixed-effects models based on the P-value and the I-squared were used for meta-analysis to consider the possibility of heterogeneity between studies. Statistical analyses were performed by using STATA 12.0. Results shown that the identification accuracy of MALDI-TOF MS at species was 84% (I² = 98.0%, P < 0.1), genus was 92% (I² = 96.6%, P < 0.1). Thereinto, the identification accuracy of Bacteroides was the highest at 96% with a 95% CI of 95% to 97%. Next were Lactobacillus spp., Parabacteroides spp., Clostridium spp., Propionibacterium spp., Prevotella spp., Veillonella spp. and Peptostreptococcus spp., and their correct identification rates were all above 90%, while the accuracy of rare anaerobic bacteria was lower. Meanwhile, the overall capabilities of two MALDI-TOF MS systems were different. The identification accuracy rate of VITEK MS was 90%, compared to 86% by the MALDI biotyper system. CONCLUSON: In summary, our research showed that MALDI-TOF-MS was satisfactory in the identification of genus in clinical pathogenic anaerobic bacteria. However, this method still suffered from different drawbacks in the identification of the rare anaerobes and species levels of common anaerobic bacteria.

Introduction

Anaerobic bacteria exist as part of the normal flora in human intestinal tract, oral cavity and urogenital tract [1], and they could cause infection diseases as a result of microenvironment or immune system impaired. Anaerobic infection could also be induced by deep wounds accompanied by facultative anaerobes and aerobic bacteria invading. The invasive anaerobic infection is life threatening, and the mortality rate of anaerobic bacteremia is high to 40% [2]. Thus, the accurate and fast identification of anaerobic bacteria is pivotal to prompt antimicrobial treatments. Conventional anaerobe identification methods are cumbersome, time-consuming, and costly. It requires a long-term cultivation (not less than 24 hours) to obtain enough inocula, in addition, the identification work is complicated, including colony traits, colony morphology, and staining results, etc. Meanwhile, conventional phenotypic methods and commercial kits are difficulty to identify the rarely or newly identified species [3], and the lab results are often inconclusive or incorrect [4]. Real-time, fast, high-throughput, high-sensitivity, high-selectivity, and low loss have been the goals pursued by analysts in modern analytical science.

The modern mass spectrometry technology enhances the understanding of the whole biological system through direct analysis of biological molecules such as proteins, lipids, carbohydrates and amino acids [5,6], which has been applied to the field of life science [7]. As an emerging technology, Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been widely used in clinical microbial diagnosis in the past few years, and is gradually replacing the traditional identification methods [8,9]. MALDI-TOF MS is a rapid developed mass spectrometry technology in the late 1980s, with
high sensitivity to detect various states samples. It is a useful, fast and accurate tool for routine laboratory analysis and has been used to identify mycobacteria [10,11], nocardia [12], yeasts [13] and anaerobes [14-16] isolated from solid media of clinical specimens. At present, there are few researches to evaluate the identification of anaerobic bacteria by MALDI-TOF MS. Herein, we searched the literature of related publications, and conducted a meta-analysis to determine the reliability of mass spectrometry as a routine diagnostic method for anaerobic bacteria.

Methods

Search strategy

The scientific literature was extensively searched using the MeSH terms "maldi-ms" and "anaerobic bacteria" to query the electronic database of Medline and Web of science (up to 1 April 2018). Selected articles contained studies involving the identification of anaerobes by MALDI-TOF MS. The references cited in these articles were examined to determine other articles. The meta-analysis was performed by referring to (when appropriate) the PRISMA guidelines [17]. EndNote X8 (Thomson Reuters) was used for literature management. We read the titles and abstracts of each searched publications and selected only those relevant articles for full-text reading. There are no restrictions on the language, publication status and geographical distribution of publications.

Inclusion and exclusion criteria

We set up the criteria for the inclusion and exclusion of the literature. The inclusion criteria were as follows: (1) the study objective: the clinical specimens were identified as anaerobic bacteria by reference methods; (2) the study method: the identification of anaerobes by MALDI-TOF MS; (3) the research objective: the accuracy of MALDI-TOF MS identification of anaerobes.

The exclusion criteria included the following aspects: review articles, reviews, case reports, scientific abstracts and lectures; common anaerobes with fewer than 10 strains of anaerobes and less than 5 uncommon anaerobes; direct identification of bacteria in the positive blood culture bottle; The target bacteria could not be extracted, and pathogenic microorganisms or industrial environmental microbes of plants or animals were identified.

Quality assessment

What is important in meta-analysis is whether heterogeneity exists in the included studies and the possible reasons for the existence of heterogeneity, because heterogeneity may lead to deviations in meta-analysis results - known as "mixed apples and oranges"[18]. Subgroup analysis can be used to analyze heterogeneity in meta-analysis [19]. The sources of heterogeneity can be divided into: different research designs, different experimental conditions, different definitions of exposure and/or outcomes, different measurement methods, and the existence of other interference factors, i.e. covariates. In addition, low-quality literature will bring significant heterogeneity [20]. The following modified criteria,
referring to the quality assessment for studies of diagnostic accuracy (QUADAS) [21], were used to assess the quality of original studies: study design, category and geographical distribution of strains, blinded status, reference methods, threshold, strain source, and system database.

Assessment of publication bias and influence analysis

According to statistics, the studies of positive results are more likely to publish than those of negative results, but it can not really represent the overall study population [22]. In fact, the samples may be less representative. This result is called "publication bias" in statistics [20]. Funnel diagrams are commonly used graphical tests to assess publication bias in meta-analysis [23]. Egger's linear regression test of funnel plot asymmetry at the genus level and Begg rank correlation (with continuity correction) showed that little publication bias was detected in this review (t = -1.54 and P = 0.123 for Egger's Test; z = -0.35 and P = 0.727 for Begg's Test).

Results

Results of the systematic literature search

A total of 234 articles were retrieved from the electronic database. Another four articles were identified through manual search, bibliographic search, and commentator suggestions. Finally, a total of 28 studies were included according to the defined inclusion and exclusion criteria (Figure 1).

Bacterial isolates

After comprehensive and detailed data compilation, we collected 6685 strains of anaerobic bacteria. The most 4 common analysis strains in this article were Bacteroides spp. (1952), Clostridium spp. (1599), Propionibacterium spp. (611) and Prevotella spp. (509). A total of 5125 anaerobic bacteria were analyzed by MALDI biotyper which VITEK MS analyzed a total of 1,609 anaerobic bacteria. In addition, 49 anaerobic bacteria were analyzed by both MALDI-TOF MS systems.

Performance of the MS system

The overall statistical results of the meta-analysis at the genera and species level identification were summarized by forest plots of random-effects model (Figure 2 and Figure 3) [24-49]. Of these, 6008 (92%; 95% CI of 90% to 93%) were correctly identified to the genus level, while 5656 (84%; 95% CI of 81% to 87%) were correctly identified to the species level by MALDI-TOF MS with random-effects model.

The pooled identification results of MALDI-TOF MS by random-effects for all anaerobic genera were shown in Table 1. The overall correct identification ratio of MALDI-TOF MS to anaerobic bacteria ranged from 60% to 100% at the genus level and ranged from 51% to 100% at the species level. Significant heterogeneity was found both at the genus level (P < 0.001; I² = 96.6%) and the species level (P < 0.001; I²=98.0%). Identification accuracy of Bacteroides spp. was the highest at 96% with a 95% CI of 95% to 97%. The higher proportion of anaerobic bacteria was Lactobacillus spp., Parabacteroides spp.,
Clostridium spp., Propionibacterium spp., Prevotella spp., Veillonella spp. and Peptostreptococcus spp.. The correct identification rate was higher than 90%. Identification accuracy of Bifidobacterium spp., Solobacterium spp., Finegoldia spp., Capnocytophaga spp., Parvimonas spp., Peptoniphilus spp., Slackia spp., Actinomyces spp., Ruminococcus spp. and Tissierella spp. were similar with an overall correct identification ratio at 80%, followed by Fusobacterium spp., Eggerthella spp. with an identification proportion above 70%. Identification accuracy of Actinobaculum spp., Atopobium spp., Anaerococcus spp. and Flavonifracter spp. were similar with an overall correct identification ratio at 60%. The lowest performance of MALDI-TOF MS was in Eubacterium spp., Bilophila spp., Butyrivibrio spp. and Porphyromonas spp., at 50%. There were many factors contributing to this result, such as the category of strain, the proportion of common and unusual species, or the reference library version.

Subgroup meta-analyses

We selected the genera (sample number not less than 5) which were identified by MALDI biotyper and VITEK MS, to compare the identification accuracy for the same genus of the two systems (Table 2). Identification accuracy rate by MALDI biotyper for Parabacteroides spp., Eggerthella spp., Peptostreptococcus spp., Parvimonas spp., Bacteroides spp., Clostridium spp. and Peptoniphilus spp. was higher than VITEK MS. For Prevotella spp. and Actinomyces spp., the efficacy of the two systems were similar, however, the heterogeneity of MALDI biotyper was more significant. In addition, the correct rate of MALDI biotyper for some strains (such as Finegoldia spp. and Fusobacterium spp.) was lower than VITEK MS, and the heterogeneity of MALDI biotype was higher than the latter. To sum up, the results of Table 2 showed that the correct rate of MALDI biotyper identification of anaerobic bacteria was higher than VITEK MS, while the heterogeneity of the MALDI biotyper was more significant.

It was worth noting that VITEK MS incorrectly identified Actinomyces georgiae as Capnocytophaga gingivalis, MALDI biotyper incorrectly identified Clostridium spp. as Enterococcus spp. (Table 3), and MALDI biotyper also incorrectly identified some rare anaerobic bacteria Mogibacterium timidum and Parvimonas micra as other bacteria. That might be due to the lack of corresponding standard spectrum in the database.

Discussion

MALDI-TOF MS, based on the microbial identification of characteristic protein fingerprints of bacteria, usually takes only a few minutes to rapidly identify the species of different microorganisms, greatly shortening the detection time and improving the diagnostic efficiency of infectious diseases. Anaerobic bacteria are hard to isolate and culture by conventional approach, and MALDI-TOF MS provides a useful technology for its identification. In this study, we conducted a meta-analysis to analyze the differences of independent research results by addressing heterogeneity between studies, potentially providing new insights into the identification of anaerobic bacteria by MALDI-TOF MS [50,51].

According to inclusion and exclusion criteria, 28 anaerobic genera were included, which assessed critically by two available MALDI-TOF MS systems. It is all known that anaerobes are more difficulty to
identify than aerobes in clinical laboratory [52]. However, using MALDI-TOF MS, the overall identification accuracy of anaerobic bacteria at genus level was 92% (95% CI of 0.90 to 0.93) in 28 included articles with 6685 various anaerobes isolates. These results indicated that MALDI-TOF MS was a qualified method for the accurate and rapid identification of pathogenic anaerobes. At the same time, we noticed that the identification property of MALDI-TOF MS against common anaerobe isolate species was variable. Among them, the correct rates of 18 anaerobic genera (Bacteroides spp., Lactobacillus spp., Parabacteroides spp., Clostridium spp., etc.) were more than 80%, the correct rates of identification of 6 anaerobic genera (Fusobacterium spp., Eggerthella spp., Actinobaculum spp., Atopobium spp., Anaerococcus spp., Flavonifractor spp., etc.) were between 60% and 80%, while the other 4 anaerobic genera (Eubacterium spp., Bilophila spp., Butyricimonas spp. and Porphyromonas spp.) identification rates were relative lower (less than 60%). The different identification correct rate might be due to the difficulty of obtaining satisfactory spectra from some species, such as Mogibacterium timidum or Actinomyces georgiae, and the limit of uncommon anaerobes species spectra in commercial reference libraries is also part of reasons. Therefore, it is increasing important to update the library of various anaerobic species, especially, those lacking or poorly represented in the current version. Fortunately, commercial databases are constantly being improved and updated at intervals of about three to six months [53].

In this study, we analyzed two commonly used identification systems, MALDI biotyper and VITEK MS. In order to compare the same anaerobic genus between two systems, we focused analyzing 12 out of 28 anaerobic bacteria genera included in both systems. Among them, Bacteroides spp., Clostridium spp., Propionibacterium spp. and Prevotella spp. were the predominant anaobes. Figure 2 and Figure 3 showed the overall identification rates of the specimens with two identification systems, MALDI biotyper and VITEK MS, respectively. The identification capacities of the two systems in Table 2 and forest plot (Figure 2 and Figure 3) was different. The summary identification rate of MALDI biotyper in Table 2 was higher than the VITEK MS, while the data in forest plot was opposite. It is supposed that low equipment cost leads to a wider range of MALDI biotyper applications. The rare anaerobic specimens identified by MALDI biotyper accounted for a large proportion, most of them were not included in the relevant database as previously described, which led to the decrease of overall identification rate. This is consistent with the data presented in the forest plot.

In addition to instrument itself, the identification correct ratio of anaerobic bacteria is also related to the system paired database. As shown in Table 3, one third of the 28 studies displayed identification errors, most of them were correct genus and wrong species, and some of them were wrong genera. These results might attribute to the similarity protein composition of species, which makes the differentiation of the quality peak difficult, and makes it difficult for MALDI-TOF MS to correctly identify the strain. The similarity of the protein structure leaded to incorrect identification results, which were not only found in anaerobic bacteria, but also in other genera. Prod’ hom et al. [8] indicated that the lower identification scores for MALDI-TOF MS of Streptococcus spp. and Staphylococcus spp. might be related to interspecies correlation and bacterial cell wall composition. Tomoyuki Yunoki et al. also believed that the identification accuracy of MALDI-TOF MS was relatively low among the less common isolated species [28]. Therefore, updating the existing information and completing the database of difficultly identified
organisms (such as *Fusobacterium* spp. and *Porphyromonas micra*) were useful to improve the identification accuracy of MALDI-TOF MS.

In conclusion, MALDI-TOF MS has shown a high degree of accuracy in anaerobic identification in current meta-analysis, although it still lacks in the identification of rare anaerobic species. As a brand new technology, MALDI-TOF MS is not only widely used in the clinical diagnosis of pathogenic diseases, but also developed for other applications, such as strain typing [54], detection of virulence factors [55] and evaluation of drug resistance [56]. In addition, the direct identification of pathogenic bacteria in blood culture [57] and urine [58] is also one of the research hotspots. Therefore, it is necessary to analyze the comprehensive ability of this technique in clinical microbiology diagnosis in the future.

**Abbreviations**

MALDI-TOF MS  Matrix-assisted laser desorption ionization-time of flight mass spectrometry

**Declarations**

**Ethics approval and consent to participate**

This systematic analysis does not need ethical approval or consent to participate because it was a secondary analysis of human subject data published in the public domain.

**Consent for publication**

Manuscript is approved by all authors for publication.

**Availability of data and material**

This study has no additional data and material.

**Competing interests**

The authors declare that they have no conflict of interest.

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**Authors' contributions**
Conceived and designed the study: Bing Gu, Hongchun Li. Searched and screened the literature: Mingzhu Shan, Xuhua Mao, Mingju Yan. Analyzed the data: Mingzhu Shan, Xuhua Mao. Wrote the paper: Ying Li, Mingzhu Shan, Zuobin Zhu, Ying Chen.

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