Matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of bacteria isolated from pharmaceutical clean rooms

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(Received: September 3, 2017; Revised manuscript received: October 29, 2017; Accepted: November 13, 2017)

Abstract: *Introduction:* During the manufacturing of sterile drugs, it is of the utmost importance to meet the minimum requirements for asepsis recommended by the legislations on good manufacturing practices-based efficient environmental monitoring. *Aims and methods:* The availability of relatively simple to use matrix-assisted laser desorption ionization–time of flight mass spectromtomy (MALDI-TOF MS) devices in the last years has changed the laboratory workflows for the microbial identification, mainly in the clinical area. Thus, the objective of this work was to evaluate the suitability of the MALDI-TOF MS technique for the identification of bacteria isolated from the environment of clean rooms used in some stages of the production of a viral vaccine. Eighteen known bacterial species commonly isolated from clean rooms studied were identified by MALDI-TOF technique and by a biochemical technique (BBL Crystal® System). *Results:* Performance of MALDI-TOF MS was better than biochemical technique for correct species identifications (88.89% and 38.89%, respectively) and produced less unreliable identification (5.55% and 22.22%). *Conclusion:* MALDI-TOF MS can be implemented for routine identification of bacteria in a pharmaceutical quality control laboratory, but as a database-dependent system, maybe some isolated not identified by this technique must be additionally studied and, if appropriate, added to an in-house database.

Keywords: MALDI-TOF MS identification, bacterial identification, biochemical identification, rapid microbiological methods, pharmaceutical clean room, environmental monitoring

Introduction

Biological products are the largest source of innovation in the pharmaceutical industry and have played an important role in the treatment of various diseases that had not been effectively treated with traditional therapies to date [1, 2]. Specifically, vaccines are the group of biological products, which represent the historically most effective ways of preventing diseases [3]. During the vaccine-production process, comprehensive and rigid guidelines must be followed to obtain a safe product. Among the requirements, clean rooms are prerequisite to obtain a safe product [4]. According to the United States Pharmacopeia 38 (USP 38) compendium, in addition to attending different analytical aspects of the product, it is necessary to evaluate a set of particularities of the process for the release of sterile products. When considering the non-applicability of terminal

sterilization, special emphasis is given to environmental monitoring [5].

Clean rooms are controlled environments that require an efficient Environmental Monitoring Program. Thus, this program must be capable of detecting an adverse event in microbiological conditions in time to allow effective corrective actions. The principle aim is controlling the levels of microorganisms and particles within specific limits, in addition to monitoring microbial diversity [6]. Knowledge of the normal microbiota of clean rooms is important to provide useful information in the investigation of sources of contamination, especially when the limits of action are exceeded [4, 6]. Identifying this microbiota is also necessary to establish which microorganism should be used in the validation and evaluation of the growth promoter capacity of culture media for environmental monitoring [7].

Many technologies aimed at microbial identification with different evolutionary levels and applying varied resources have been developed. In the early stages of this evolutionary process, the research was directed and applied to the clinical area, to identify and correct the antibiotic therapy. Following this, great efforts were made in the area of food: the nature and origin of the inputs used in this segment provide the possibility of a wide range of contaminants. Finally, all current targeting is aimed at drugs, complex group as to route of administration, distinct risk groups, and among others. However, the implementation of new technologies in the pharmaceutical field has been delayed due to the highly regulated environment in which the pharmaceutical industry works with a conservative approach and the need for strict validation requirements [4]. Currently, the classical microbial identification methods available to the microbiological pharmaceutical control laboratories and accepted by regulatory authorities are based on culture methods [4, 8]. By adopting the biochemical and physiological comparison of certain characteristics, such as the morphology of the colonies and the potential to ferment certain sugars, a profile of the microorganisms present in the clean rooms can be obtained. However, these conventional methods are often time-consuming and can take up to 6 weeks to obtain the result [9–11]. Thus, it is necessary to constantly search for new technologies to accelerate this response time.

The development of rapid microbiology methods by pharmaceutical companies can be considered as an irreversible tendency in the pharmaceutical field, since they are currently looking for new and more efficient tools to speed up and improve the quality level of the analytical results, including those of microbial identification. In addition to reducing the time of analysis, these methods are more assertive and efficient, require a small amount of material, and reduce the added value of the analysis [4, 12]. Different technologies have originated products for the rapid identification of microorganisms using biochemical methods, such as the API®, VITEK®, and Biolog® galleries; systems based on the use of substrates to obtain the identification of the isolates, such as Crystal® miniaturized identification system and Micro-ID®; gas chromatography of fatty acids; and molecular biology-based systems such as Gene-Trak®, BAX®, MicroSeq®, and Riboprinter® [4].

From the techniques available for rapid and automated methods, mass spectrometry based on the matrix-assisted laser desorption ionization technology (MALDI-TOF) is the one that arouses the highest interest [13]. It is an accurate and cost-effective method of bacterial identification developed in mid-1980s [14, 15]. Among the physicochemical techniques that allow the detection and characterization of microorganisms, it presents a unique combination of rapidity, high specificity, and excellent sensitivity essential for microbial analysis. These attributes combined with their ready adaptability to the direct analysis of fungi and bacteria and the demand for low-cost reagents makes this technique an attractive option for laboratories in clinical, pharmaceutical, environmental, and food microbiology [16].

Parenteral Drug Association technical report no. 33 is intended to fill the gap that prevents the widespread adoption of alternative rapid microbiological methods. The aim was to achieve this objective by establishing how to demonstrate the equivalence of these methods to existing ones in a manner acceptable to regulatory agencies and how to validate equipment associated with the alternative rapid method [12]. Both USP and European Pharmacopeia have specified the steps necessary for the validation of an alternative microbiological method (USP General Information Chapter 1,223 Validation of Alternative Microbiological Methods and Ph. Eur. Informational Chapter 5.1.6 Alternative Methods for Control of Microbiological Quality, respectively) [17, 18]. Validation of Compendial Procedures chapter 1,225 (USP 38) defines some essential characteristics in their application to analytical methods, as detection limit, quantification limit, linearity, and range [17]. The necessary procedure for verification of an alternative microbial identification method can be done in three ways as follows: (1) using an existing system for parallel testing of microbial isolates obtained from routine testing (the number of isolates tested may be as high as 50, and any discrepancies in identification can be arbitrated using a referee method); (2) testing 12–15 known representative stock cultures of different commonly isolated species for a total of 50 tests; or (3) confirming that 20–50 organism identifications, including 15–20 different species, agree with the results of a reference laboratory testing of split sample.

In this study, we selected the most appropriate study conditions (number 2) and followed the steps established to USP 38, the main guide for verification of microbial identification methods. Thus, the objective of this work

was to evaluate the suitability of the MALDI-TOF MS technique for the identification of bacteria isolated from the environment of clean rooms used in some stages of the production of a viral vaccine in comparison with a biochemical identification method.

Materials and Methods

Bacterial isolates and sampling

Thirteen bacterial isolates were recovered from routine identification of samples deriving from the Environmental Monitoring Program of Clean Rooms and selected for the study. Such samples were taken from the air and surfaces of clean rooms used in some stages of the production of a viral vaccine as well as operators of these rooms. Five reference microorganisms from the American Type Culture Collection (ATCC) were also included in the study *(Table I)*.

The sampling of airborne microorganisms was made with impaction sampler M Air T® (Merck Millipore, Billerica, MA, USA) and sterile Petri dishes, adaptable to the apparatus, containing commercially purchased tryptic casein soy agar. About 1 m^3 of air was tested during an exposure time of 15 min. Settling plates containing tryptic casein soy agar commercially purchased were also exposed for sampling airborne microorganisms during 4-h period. The sampling of regular or flat surfaces and personnel were accomplished using contact plates containing tryptic casein soy agar commercially purchased.

Table I Microorganisms selected for the study

Reference microorganisms	ATCC strain
Staphylococcus aureus	6538
Bacillus spizizenii	6633
Salmonella typhimurium	14028
Pseudomonas aeruginosa	9027
Escherichia coli	8739
Microorganisms isolated from clean rooms studied	
Gram-positive	Gram-negative
Micrococcus luteus	*Stenotrophomonas maltophilia*
Staphylococcus epidermidis	*Acinetobacter johnsonii*
Staphylococcus cohnii	*Chryseobacterium indologenes*
Staphylococcus capitis	*Pseudomonas aeruginosa*
Staphylococcus saprophyticus	*Delftia acidorovans*
Staphylococcus warneri	*Ralstonia panila*
Bacillus cereus group	–

Obs.: The stock cultures of the bacterial species selected for the study were identified by MALDI-TOF mass spectrometry method

Culture conditions and stock culture

The isolates were recovered after aerobic incubation at 32.5 ± 2.5 °C for 48 h, and in the sequence at 22.5 ± 2.5 °C for 72 h, totaling 120 h of incubation. Then, the colony forming units were identified by MALDI-TOF MS in duplicate and the stock cultures were maintained on tryptic casein soy agar at 32.5 ± 2.5 °C and subcultured each month until fifth passage. From this stock culture, previously identified by MALDI-TOF MS for reference results were made identifications in triplicate for comparison between biochemical and MALDI-TOF MS methods.

Biochemical identification

After Gram staining and determination of catalase and oxidase activities, bacterial isolates and reference strains were identified by appropriate BBL Crystal® identification systems for Gram-positive and Gram-negative bacteria, both according to the manufacturer's instructions.

Mass spectrometry

A thin smear of bacteria was deposited on a MALDI plate in triplicate. Each smear was overlaid with 1 μL of matrix solution (α-ciano-4-hydroxycinnamic acid). The matrix sample was co-crystallized by air drying at room temperature. Measurements were performed with a Biomerieux mass spectrometer (Vitek® MS) using the software LaunchPad – Shimadzu Biotech MALDI-MS (version 2.8, Shimadzu Scientific Instruments, Kyoto, Japan). Saramis Target Manager program and Saramis Premium system were used for interpretation of spectra obtained. For each spectrum, 100 shots from different positions of the target spot (automatic mode) were collected and analyzed. Spectra were internally calibrated using *Escherichia coli* ATCC 8739 cultivated in MacConkey agar. Results were expressed as proposed by the manufacturer with scores ranging from 0% to 99.9%. Scores below 90% were considered not to have generated a reliable identification; scores above 90% were considered reliable both for genus and species identification.

Criteria for identification

The previously MALDI-TOF identifications were considered final (reference identifications). The results of the tested methods were considered correct only when they had exactly the same identification to the species level of the reference identifications. Scores below 90% were considered not to have generated a reliable identification. Discrepant results on a triplicate test within the same

range (above 90%) (e.g., *Staphylococcus simulans* vs. *Staphylococcus saprophyticus*) were considered not uniform. For comparisons between biochemical and MALDI-TOF MS identification methods, a Fisher's exact test was performed using GraphPad Prism version 5.00, (GraphPad Software, La Jolla, CA, USA), because it is the most appropriate test for the calculation of *p* value when the samples are small.

Results

The results of bacteria identifications by both MALDI-TOF MS and biochemical method are presented in *Tables II* and *III*, respectively. Of the 18 bacterial species selected for evaluation of the MALDI-TOF MS method, 16 (88.9%) were correctly identified by this technique at the genus and species levels: *Staphylococcus cohnii, S. saprophyticus, Micrococcus luteus, Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus warneri, Bacillus cereus* group, *Stenotrophomonas maltophilia, Acinetobacter johnsonii, Pseudomonas aeruginosa, Delftia acidorovans, Staphylococcus aureus* ATCC 6538, *Bacillus spizizenii* ATCC 6633, *Salmonella typhimurium* ATCC 14028, *P. aeruginosa* ATCC 9027, and *E. coli* ATCC 8739. Only one species (*Ralstonia panila*) was not correctly identified (5.5%) and another (*Chryseobacterium indologenes*) (5.5%) resulted in a score below that considered reliable by the manufacturer (90.0%).

The identifications by BBL Crystal® system yielded 12 (66.6%) correct identifications at the genus level: *S. cohnii, S. saprophyticus, M. luteus, S. epidermidis, S. capitis, S. warneri, B. cereus* group, *S. maltophilia, B. spizizenii* ATCC 6633, *S. typhimurium* ATCC 14028,

Table II Identification results of bacterial species selected for the study by MALDI-TOF MS

Bacterial species selected for the study	MALDI-TOF MS analysis (no replicates)[a]					
	Genus correct	Species correct	Misidentification	No uniform identification	No identification	Unreliable identification[b]
Staphylococcus cohnii	2	2	–	–	–	1
Staphylococcus saprophyticus	3	3	–	–	–	–
Micrococcus luteus	3	3	–	–	–	–
Staphylococcus epidermidis	3	3	–	–	–	–
Staphylococcus capitis	3	3	–	–	–	–
Staphylococcus warneri	3	3	–	–	–	–
Bacillus cereus group	3	3	–	–	–	–
Stenotrophomonas maltophilia	1	1	–	–	–	2
Acinetobacter johnsonii	2	2	–	–	–	1
Chryseobacterium indologenes	–	–	–	–	–	3
Pseudomonas aeruginosa	3	3	–	–	–	–
Delftia acidorovans	1	1	–	–	–	2
Ralstonia panila	–	–	3	–	–	–
Staphylococcus aureus ATCC 6538	3	3	–	–	–	–
Bacillus spizizenii ATCC 6633	3	3	–	–	–	–
Salmonella typhimurium ATCC 14028	2	2	–	–	–	1
Pseudomonas aeruginosa ATCC 9027	2	2	–	–	–	1
Escherichia coli ATCC 8739	3	3	–	–	–	–

[a]Bacterial species were tested in triplicate by MALDI-TOF MS and by biochemical identification methods according to manufacturer's descriptions, for a total of 54 tests.
[b]Scores below 90% were considered not to have generated a reliable identification

Table III Identification results of bacterial species selected for the study by BBL Crystal®

Bacterial species selected for the study	BBL Crystal analysis (no replicates)[a]					
	Genus correct	Species correct	Misidentification	No uniform identification	No identification	Unreliable identification[b]
Staphylococcus cohnii	1	1	–	–	–	2
Staphylococcus saprophyticus	3	2	–	1	–	–
Micrococcus luteus	3	1	–	–	–	–
Staphylococcus epidermidis	3	3	–	–	–	–
Staphylococcus capitis	3	3	–	–	–	–
Staphylococcus warneri	3	–	3	–	–	–
Bacillus cereus group	3	–	3	–	–	–
Stenotrophomonas maltophilia	3	3	–	–	–	–
Acinetobacter johnsonii	–	–	–	–	2	1
Chryseobacterium indologenes	–	–	3	–	–	–
Pseudomonas aeruginosa	–	–	–	–	–	3
Delftia acidorovans	–	–	2	–	1	–
Ralstonia panila	–	–	–	–	–	3
Staphylococcus aureus ATCC 6538	–	–	–	–	–	3
Bacillus spizizenii ATCC 6633	3	3	–	–	–	–
Salmonella typhimurium ATCC 14028	3	–	–	–	–	–
Pseudomonas aeruginosa ATCC 9027	1	–	–	–	–	2
Escherichia coli ATCC 8739	3	3	–	–	–	–

[a]Bacterial species were tested in triplicate by MALDI-TOF MS and by biochemical identification methods according to manufacturer's descriptions, for a total of 54 tests.
[b]Scores below 90% were considered not to have generated a reliable identification

P. aeruginosa ATCC 9027, and *E. coli* ATCC 8739. Of these, seven (38.8%) bacterial species were correctly identified at the species level: *S. cohnii, S. epidermidis, S. capitis, S. maltophilia, B. spizizenii, P. aeruginosa* ATCC 9027, and *E. coli*.

Two (11.1%) species were misidentified by BBL Crystal® system (*C. indologenes* and *D. acidorovans*), four (22.2%) did not obtain a sufficient score value to be accepted (90.0%) (*A. johnsonii, P. aeruginosa, R. panila*, and *S. aureus* ATCC 6538) and two (11.1%) resulted in non-uniform identifications (*S. saprophyticus* and *M. luteus*), where acceptable scores were obtained, but with discrepant identifications to the species level or no identification to the species level in some of the three replicates.

Performance of MALDI-TOF MS was better than biochemical technique in all aspects analyzed: correct species identifications (88.89% and 38.89%, respectively), correct identification only at the genus level (0% and 16.67%, respectively), unreliable identifications (scores below 90%) (5.55% and 22.22%), non-uniform identifications between triplicates (0% and 11.11%), and misidentifications (5.55% and 11.11%, respectively) *(Table IV)*.

P values were calculated by comparison of MALDI-TOF MS identification with biochemical identification (Fisher's exact test) *(Table V)*. The measures of accuracy at genus and species level are shown in *Table VI*.

Discussion

Microorganisms are virtually found in all environments. Since the optimal conditions of survival and growth of

Table IV Concordance between MALDI-TOF MS identification and BBL Crystal® system identification

	MALDI-TOF MS					
BBL Crystal®	Species ID (%)	Genus ID (%)	No ID (%)	No uniform ID (%)	Misidentification (%)	Total (%)
Species ID (%)	7 (38.89%)	0	0	0	0	7 (38.89%)
Genus ID (%)	3 (16.67%)	0	0	0	0	3 (16.67%)
No ID (%)	3 (16.67%)	0	0	0	1 (5.55%)	4 (22.22%)
No uniform ID (%)	2 (11.11%)	0	0	0	0	2 (11.11%)
Misidentification (%)	1 (5.55%)	0	1 (5.55%)	0	0	2 (11.11%)
Total (%)	16 (88.89%)	0	1 (5.55%)	0	1 (5.55%)	18 (100%)

The selected bacterial species were tested in triplicate by MALDI-TOF MS and BBL Crystal® system, according to the manufacturer's instructions. For both methods, the acceptable minimum score was 90% for both genus and species identifications. ID species: identification at the species level; Genus ID = correct identification only at genus level; No ID: scores below 90%; No uniform ID: discrepant results on triplicate test within the same range (above 90%); Misidenification: misidentification at the genus or species level

Table V Identifications by MALDI-TOF MS and BBL Crystal® system compared to the final identifications

Identification parameter	MALDI-TOF MS identification [no. of isolates (%)]	BBL Crystal® identification [no. of isolates (%)]	*P* value[a]
Genus correct	16 (88.89%)	12 (66.67%)	NS
Species correct	16 (88.89%)	7 (38.89%)	<0.01
No identification	1 (5.55%)	4 (22.22%)	NS
No uniform identification	0	2 (11.11%)	NS
Misidentification	1 (5.55%)	2 (11.11%)	NS

The selected bacterial species were tested in triplicate by MALDI-TOF MS and BBL Crystal® system. Final identifications were established by prior analysis performed by MALDI-TOF MS with scores above 90%, as recommended by the manufacturer.
[a]NS: not statistically significant

Table VI Measure of accuracy at genus and species level for MALDI-TOF MS and BBL Crystal system

	MALDI-TOF MS		BBL Crystal	
Criteria for identification	Number of correct results	Accuracy (%)	Number of correct results	Accuracy (%)
Species level	16	88.89	7	38.89
Genus level	16	88.89	12	66.67

many of them coincide with which the human populations live, it is inevitable to coexist with large numbers of them. They are on the surface of the human body, as well as in the digestive tract and other natural orifices. This ubiquity and the risk that the microbial contamination of drugs offers to the users evidence the importance of the microbiological control of the productive areas [19].

In an Environmental Monitoring Program, the sampling frequency depends on the criticality of the monitoring sites and the treatment to which the product is subjected after the aseptic process. Environmental monitoring needs considerable attention when the product is potential to personal contact during manufacturing or not submitted to the terminal sterilization. The greater the potential for personal contact with the product, the greater the importance of environmental monitoring, as well as for products not submitted to terminal sterilization [17]. Independently of the degree of sophistication, any Environmental Monitoring Program will not be able to identify and quantify all microbial contaminants present in controlled environments. However, a well-planned and executed routine monitoring provides sufficient information to determine that the controlled environment is operating within a suitable control state [19].

The presence of certain microbial species in clean pharmaceutical rooms may be related to risk factors. It is emphasized that in injectable products, the criticality is independent of the nature of the microorganism. Nevertheless, it should be considered that Gram negative, besides offering microbiological risk, can induce pyrogenic reactions in the patient. On the other hand, those that are characterized by high pathogenicity may be producing different toxins [4].

In this study, the choice of microorganisms was based on high isolation frequency of these species in clean rooms as evident from the literature [20–22]. Utescher et al. [20], in an evaluation study of the Environmental Monitoring Program for clean pharmaceutical rooms in a Brazilian production of biological medicines, observed that the typical microbial population was composed of bacteria of the genera *Staphylococcus* sp., *Micrococcus* sp., and *Bacillus* sp., with predominance of *Staphylococcus spin* all the areas. These three bacterial genera have a ubiquitous distribution. They have been isolated from the environment or on human skin and mucous membranes. Therefore, the study of presence of the species of these genera needs more attention in microbial identification methods in pharmaceutical production. Therefore, the importance of the presence of species of these genera in studies on the adequacy of microbial identification methods is used in the context of pharmaceutical production.

Sandle [21], in his review on the typical microflora of clean pharmaceutical rooms, examined several clean areas located in various establishments in England and Wales used for the manufacture of blood products from 2001 to 2009. Recovered microorganisms were represented by six main genera, among them, *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., and *Pseudomonas* sp. The most frequent species in these environments were *M. luteus, S. epidermidis* and *S. capitis*. Abreu et al. [22] also found a high frequency of the genus *Bacillus* sp. and *Staphylococcus* sp. in their study on environmental monitoring of clean areas.

Fungi and yeast have not been primarily addressed in this study because they were not frequently isolated in clean rooms studied also because, as described in the literature, the analysis of these microorganisms by MALDI-TOF MS is still fragile due to the low reproducibility. In addition, the effects of their different physiological states on the reliability of identification methods are still controversial [23]. MALDI-TOF MS-based identification of yeast also requires a pretreatment of the yeast sample before the acquisition of the spectra, involving a complete extraction of the fungal material [24]. This process needs to be studied more deeply for the identification of fungi isolated from the environment of pharmaceutical clean rooms.

Rapid and accurate identification of microorganisms is an essential part of pharmaceutical analysis and besides having these characteristics, MALDI-TOF technology has been widely used in microbiological laboratories, especially those in the clinical area [25]. However, in the case of laboratories for microbiological quality control of pharmaceutical products, studies are limited, making it difficult to compare the results obtained from this technique with those obtained in the same context of environmental isolates.

The spectra libraries that make up the databases of the mass spectrometers available for microbial identification have their focus on clinical microbiology. This makes it difficult to apply the technique in the clean room area, since it is not possible to know in advance the characteristics of the microorganisms, which will be isolated. This difficulty demands improvement of the libraries and techniques of sample preparation, since the compositional variability of the microorganisms that can be found in the environment is very large and interferes with the accuracy of the technique [25–27].

However, the results of this study revealed an excellent performance of MALDI-TOF MS in comparison with conventional identification technique (biochemical identification) for correct species identification (88.89% and 38.89%, respectively). Other authors also found the same evidence, although it has not been isolated microorganisms from the environmental monitoring of pharmaceutical clean rooms. Veen et al. [28] performed a study with 1,307 clinical isolates of bacteria and yeasts of several genus, such as *Klebsiella* sp., *Pseudomonas* sp., *Salmonella* sp., *Staphylococcus* sp., *Streptococcus* sp., and *Candida* sp. Overall performance of MALDI-TOF MS was significantly better than conventional biochemical systems (Vitek-II, API, and biochemical tests) for correct species identification (92.2% and 83.1%, respectively) [28].

Guo et al. [29], in a comparative study of MALDI-TOF MS and Vitek 2 in bacteria identification, found an accuracy of 96.5% and a lower identification error rate in MALDI-TOF MS performance, making it better at identifying bacteria than Vitek 2. The bacteria were routinely isolated from clinical patients of a General Hospital in Beijing]. Seng et al. [30] also reported that MALDI-TOF MS can replace conventional systems for identification of bacteria in a conventional clinical laboratory. Of 1,660 bacterial isolates analyzed, 95.4% were correctly identified by MALDI-TOF MS; 84.1% were identified at the species level, and 11.3% were identified at the genus level.

In most of the study, Vitek 2 system is used as a biochemical method for comparison with the MALDI-TOF technique. No studies were found using the BBL Crystal system as a conventional method to compare the results.

The microbiological method of BBL Crystal® biochemical identification was chosen for comparison with the MALDI-TOF MS technique, although it is also considered a rapid method. This is due to the fact that this miniaturized system was developed according to the principle of microbiological methods for microbial identification, that is, culture in appropriate culture media to demonstrate the physiological characteristics of each microorganism capable of differentiating them from the others. Thus, some limitations of these methods can still be found in BBL Crystal®, such as the variability of the microorganisms in their response to the culture media, causing subjectivity in the reading of the results, which, in turn, can lead to misidentifications [31].

The greater importance of this study is the implementation of MALDI-TOF MS in a routine setting of pharmaceutical clean rooms and the comparison of MALDI-TOF MS with conventional identification systems on environmental isolates. A limitation of this study is the lack of use of 16S DNA sequencing for analysis of discrepancies, once the traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods [32]. However, good results obtained with MALDI-TOF technology and described in the literature over the years can minimize this limitation [33].

In addition to being a much less labor-intensive technique capable of delivering accurate and reliable results in a few minutes from a single isolated colony, MALDI-TOF MS can also generate significant cost savings for laboratories. Tran et al. [34] reported the advantages of MALDI-TOF MS technique compared with conventional methods. The authors carried out a comparative study to determine the amount that can be saved by the laboratories when replacing the biochemical identification methods with the MALDI-TOF MS technique. A total cost analysis was done, including reagent costs, technologist time, and maintenance service expenses. The use of the MALDI-TOF MS technique resulted in an annual saving of 51.7% and the initial acquisition cost of the equipment, although high can be recovered in approximately 3 years.

Conclusions

In conclusion, MALDI-TOF MS is a simple, rapid, and inexpensive technique for identification of bacteria, including bacteria isolated from pharmaceutical clean room environmental monitoring, and can be implemented in a conventional pharmaceutical quality control laboratory setting. Although the technique has a high accuracy for bacterial identification, the performance can be improved with the improvement of the databases.

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Funding sources: This work was financially supported by National Council for Scientific and Technological Development (CNPq).

Authors' contribution: The work presented was performed in collaboration with all authors. All authors participated in the study's conception and design. LdOA and TdJAP performed laboratory experiments. All authors participated in the interpretation of data, preparation, and editing of manuscript for intellectual content. They also reviewed and approved the final manuscript.

Conflict of interest: All authors confirm no conflict of interest involved with this manuscript.

Statement of human and animal rights: This article does not contain any studies with human or animal subjects performed by any of the authors.

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