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Review

Value of matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry in clinical microbiology and infectious diseases in Africa and tropical areas

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Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MALDI-TOF MS) is a revolutionary technique with multiple applications. Its use in clinical microbiology is now becoming widespread as the method is an easy, rapid, effective, accurate, and cheap way to identify cultured bacteria and fungi. It is, therefore, an ideal tool to replace conventional methods still used in Africa and tropical areas for routine microbiological diagnosis. The recent installation of a MALDI-TOF MS for diagnostic purposes in a hospital in Senegal has confirmed that this tool is not only valuable but also robust in tropical Africa, providing further evidence that this technique should be widely distributed there. However, despite its value for clinical microbiology in Africa, the acquisition and installation of MALDI-TOF MS is subject to several constraints. This review provides general information on aspects of MALDI-TOF MS. The specific aspects and constraints observed in Africa and tropical countries are also addressed with suggestions for appropriate solutions.

Key words: Microorganism, infectious diseases, quick identification, matrix-assisted laser desorption-ionization time-of-flight, matrix assisted laser desorption ionization-time of flight (MALDI-TOF).

INTRODUCTION

Cardiovascular diseases are the leading cause of death in developed countries, while in Africa and low-income countries, thousands of deaths linked to infectious diseases are recorded every year (Prost, 2000; Lopez et al., 2000; Bryce et al., 2005; Williams et al., 2002).

Against this backdrop of the high incidence of infectious diseases, including emerging and reemerging pathogens (Desenclos and De Valk, 2005), improving tools for the

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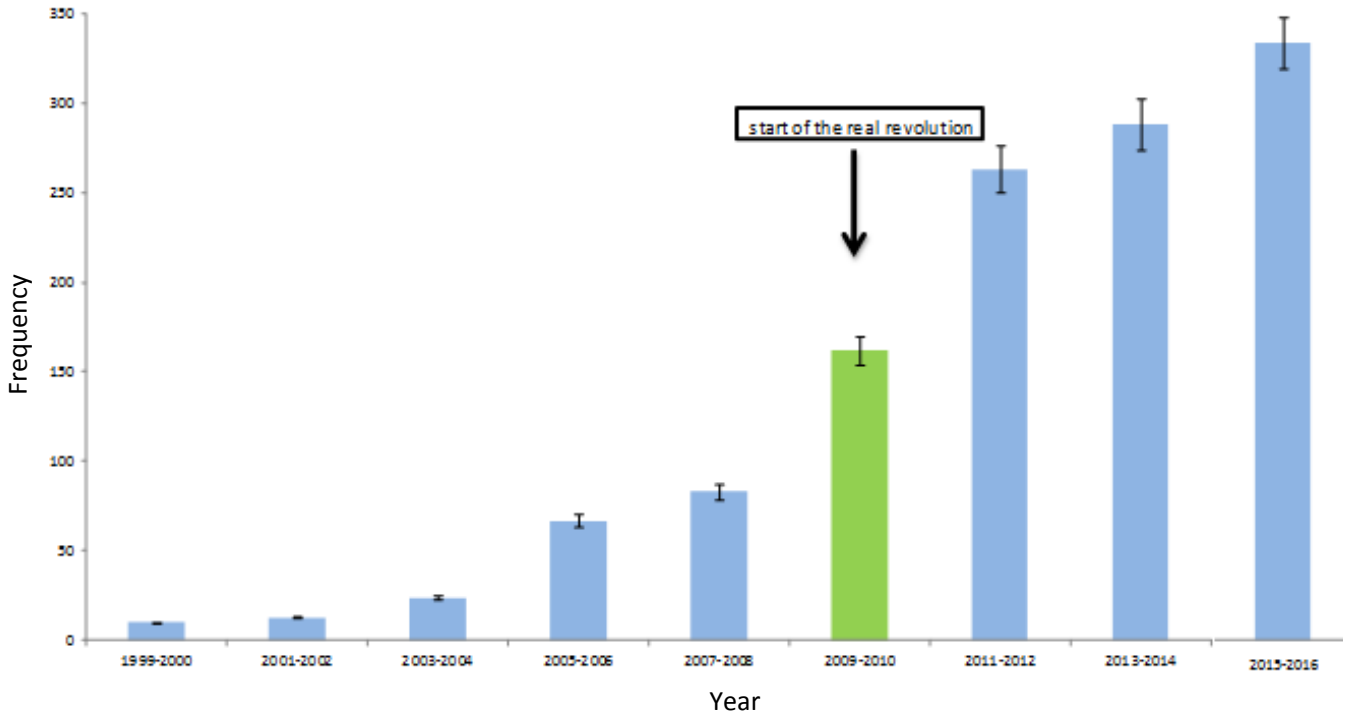

Figure 1. Increasing number of publications related to MALDI-TOF MS applications in medical microbiology from 1999 to 2014. It shows also that 2009 marks the massive use of MALDI-TOF MS in clinical microbiology laboratories. The following Mesh terms through bibliographic NCBI database were used to built this graph: ("spectrometry, mass, matrix-assisted laser desorption-ionization"(MeSH Terms) OR ("spectrometry""(All Fields) AND "mass"(All Fields) AND "matrix-assisted"(All Fields) AND "laser" (All Fields) AND "desorption-ionization"(All Fields]) OR "matrix-assisted laser desorption-ionization mass spectrometry" (All Fields) OR "maldi" (All Fields) AND "tof" (All Fields) AND ("microbiology" (Subheading) OR "microbiology" (All Fields) OR "microbiology" (MeSH Terms) AND ("1999/01/01" (PDAT): "2014/12/31" (PDAT).

identification of microorganisms in clinical microbiology laboratories is urgently required.

In African countries, routine diagnostic methods are generally based on culture media, followed by growth characteristics and biochemical patterns. These steps are fastidious, requiring large quantities of expensive reagents and *a priori* knowledge of the isolated microorganism; identification may take place after several hours or days, depending on the microorganism, and even then is sometimes inaccurate (Seng et al., 2009).

Recently, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has enabled a revolution in the routine work of clinical microbiology laboratories with the quick, inexpensive, and accurate identification of bacteria and fungi. Without any *a priori* knowledge, it is possible to quickly adapt first line anti-infective treatment as the best possible treatment (Courcol, 2009). MALDI-TOF MS is having a real impact on global health and its implementation will be of great value in Africa and other tropical areas, as recently shown in Senegal, where its broad applicability and robustness have been recently proven (Fall et al., 2015; Lo et al., 2015).

Here, we will review the general aspects of MALDI-TOF MS but will also focus on the specific aspects and constraints observed in Africa and tropical countries. We will also propose appropriate solutions.

GENERAL ASPECTS OF MALDI-TOF MS TECHNOLOGY

In 1975, the scientific literature began to combine MS with pyrolysis for the detection of bacterial proteins (Intelicato-Young and Fox, 2013). In 2009, a new revolution in clinical microbiology began when the efficiency of MALDI-TOF MS for the routine identification of bacteria was demonstrated with a correct identification of 95 and 84% of the genus and species levels, respectively, for 1,660 bacteria (Seng et al., 2009; Seng et al., 2010).

Since then, an explosion in scientific publications on the use of MALDI-TOF MS in clinical microbiology has been observed (Figure 1), supporting the fact that the method is a fast and reliable means of identifying microorganisms and is clearly more efficient than conventional methods (Eigner et al., 2009; Blondiaux et al., 2010). It has been estimated that ten bacterial strains can be identified in parallel in less than 15 min with MS, while it takes more than 360 min to do so using

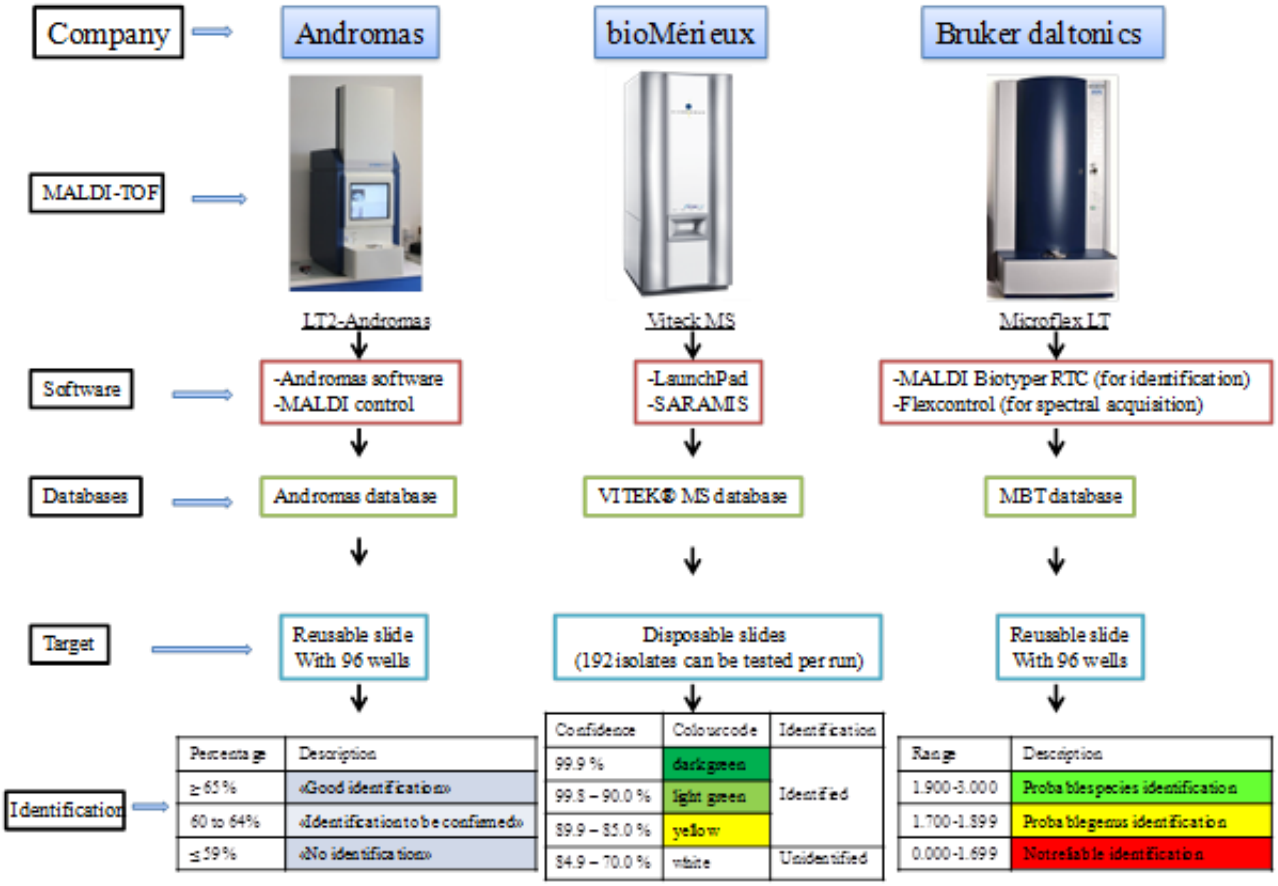

Figure 2. The various MALDI-TOF MS instruments are currently commercialized for the identification of microorganisms in clinical laboratories. The LT2-Andromas, Vitek MS, and MALDI Biotyper have been accredited for identification purposes in clinical microbiology laboratories under EU directive EC/98/79 in several European countries. The VITEK® MS and the MALDI Biotyper were cleared by the US Food and Drug Administration (FDA) for the identification of cultured bacteria and, in the case of the former system, yeast in 2013.

conventional automated systems (Biswas and Rolain, 2013; Cherkaoui et al., 2010).

Currently, three MALDI-TOF mass spectrometers are on the market (Figure 2); the Andromas system (Paris, France) (Bille et al., 2012), the Microflex LT (Bruker Daltonics, Heidelberg, Germany, in collaboration with Becton Dickinson, Franklin Lakes, NJ, USA) (Lee et al., 2015; Saffert et al., 2011), and the VITEK® MS (bioMérieux, Marcy l'Etoile, France) (Patel, 2013).

For bacterial and fungal identification, one isolated colony is picked and directly deposited on a well of a MALDI-TOF plate, preferentially in duplicate, as the deposit is crucial for accurate identification (Figure 3) (Fenselau and Demirev, 2001). This preparation must then be overlaid with a matrix solution (solution with alpha-cyano-4-hydroxycinnamic acid, acetonitrile, trifluoroacetic acid, etc.), and air dried at room temperature (about five minutes) to permit co-crystallization (Shunsuke et al., 2014) before placing the plate in the MALDI-TOF instrument for analysis. Identification is achieved by comparing the spectra of analyzed species against the reference spectra present in the MALDI-TOF database (Coltella et al., 2013; Martiny et al., 2012).

Identification robustness depends on the richness of the databases, which have been regularly and substantially updated since 2009 (Seng et al., 2010). The database provided with the Vitek MS installed in Dakar, is annually updated by bioMérieux. Indeed, commercial database are regularly updated and released (approximately one time per year). Besides, depending on the MALDI-TOF mass spectrometer, database can be incremented directly with spectra from local bacteria paving way for data with local epidemiology. Specific database can also be created for entomology (Sambou et al., 2015).

Recently, Tran et al. (2015) performed a huge study to evaluate the cost savings of implementing routine microbiological identification by MALDI-TOF MS (bioMérieux Vitek, Durham, NC, USA) in their laboratory.

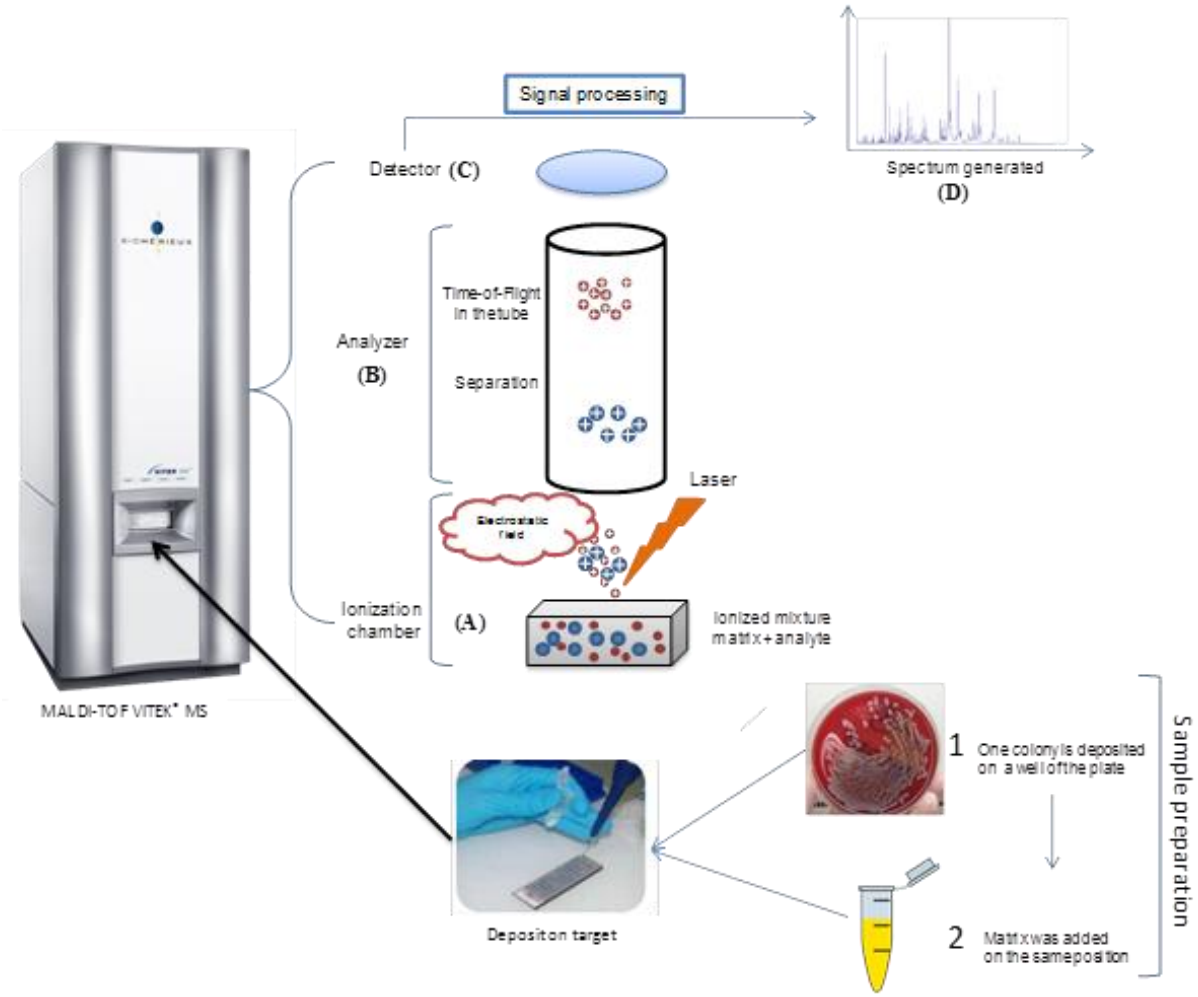

Figure 3. MALDI-TOF MS's operating principle and the sample preparation step for identification. The principle of this measurement is based on the ability of an electric and/or magnetic field to deflect a flow of ions, each with a mass and a charge proportional to their trajectories. Overall, mass spectrometry can be divided into three steps: the ionization chamber that produces ions in the gas phase (A), the analyzer which selects ions by mass-to-charge ratio (m/z) (B), and the detector that converts the ionic current into electric current (C). Bombing with a laser beam generates ions in the ionization chamber. These ions are accelerated into an electric field which directs them to the analyzer that separates them according to their time-of-free flight (TOF: Time-Of-Flight). The smaller molecules grasp the detector first, followed by the biggest, according to the m/z ratio. Those which have the same m/z ratio are then separated by an electrostatic mirror. The detector converts the received ions into electrical current which is amplified and digitized (D).

Overall, reagent costs for the conventional methods averaged $3.59 per isolate, while those for MS were $0.43. The use of MS was equated to a net saving of $69,108.61 (87.8%) in reagent costs annually. When technologists' time and maintenance costs were included, conventional identification cost would be $142,532.69 versus $68,886.51 with MS, resulting in a laboratory saving of $73,646.18 (51.7%) annually. They also estimated that the initial cost of the instrument at their usage level would be offset in about three years (Tran et al., 2015). Comparing MALDI-TOF MS to other identification methods which is usually used in microbiology laboratories shows that it is very cost-effective in terms of reagent cost and working time (Table 1) (Musser, 2014).

The direct identification of microorganisms in specimens such as blood cultures, urine, or cerebrospinal fluid has been proposed using home-made (Segawa et al., 2014; Ferreira et al., 2010; Yonetani et al., 2016; Foster, 2013; Ferroni et al., 2010; Ferreira et al., 2011) or commercial kits for blood cultures (Jamal et al., 2013; Haigh et al., 2013; Nonnemann et al., 2013).

Currently, direct identification is mainly available for blood cultures after a pre-incubation step but it allows the quick identification of the involved microorganism and the opportunity of considerably earlier treatment adaptation,

Table 1. Cost comparisons between MALDI-TOF MS and tests used in clinical microbiological laboratories for bacterial identification.

Tests	Reagents cost only by test	Estimated staff time needed to perform test (h)	Estimated cost in staff time per test	Total cost per test (staff and reagents)
16S rDNA sequence analysis	$11.97	03	$218.87	$230.84
Real-Time PCR	$4.99	0.5	$36.47	$41.47
Individual biochemical test	$0.99	0.15	$10.94	$11.94[1]
MALDI-TOF MS	$0.50	0.5	$36.47	$36.98

[1]This represents only one biochemical test not a panel of tests required for bacterial identification.

with a direct clinical impact (Kohlmann et al., 2015).

In addition, MALDI-TOF MS is a promising, easy, inexpensive, and rapid tool for investigating an outbreak (Croxatto et al., 2012; Gaia et al., 2011; Fujinami et al., 2011; Williamson et al., 2008). For example, the epidemiological investigation of a nosocomial outbreak of multidrug resistant *Corynebacterium striatum* showed that all outbreak-related strains are clustered in a single clone with a MALDI-TOF MS dendrogram (Verroken et al., 2013). It has also enabled the accurate and reproducible discrimination of major methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complexes observed in outbreaks, belonging to strains prepared with the same extraction protocol (Wolters et al., 2011; Josten et al., 2013).

MALDI-TOF MS has also made it possible to differentiate the five most frequently-isolated *Salmonella enterica* serovars (Enteritidis, Typhimurium, Virchow, Infantis, and Hadar) (Dieckmann and Malorny, 2011) as well as to identify *Escherichia coli* pathotypes (Clark et al., 2013; Barbuddhe et al., 2008). The validity of MALDI-TOF MS for typing extended-spectrum β-lactamase-producing *E. coli* in a previously published nosocomial outbreak was recently assessed (Egli et al., 2015). Thus, all these data clearly show that MALDI-TOF MS has a promising future in the epidemiological surveillance of infectious diseases (Doern and Butler-Wu, 2016).

Africa is prey to endemic diseases such as malaria. This is why the use of rapid and effective control methods could permit the prevention and control of vector-borne diseases. Several studies have shown that MALDI-TOF MS has also enabled the rapid detection of arthropod vectors, such as ticks, mosquitoes, fleas (Yssouf et al., 2014), phlebotomine sand flies (Mathis et al., 2015), and *Culicoides* without any expertise or skills in entomology (Sambou et al., 2015; Yssouf et al., 2013a).

Recently, the utility of MALDI-TOF MS for a dual identification of tick species and bacteria has been demonstrated. Intracellular *Rickettsia* spp. has been detected using MALDI-TOF MS in ticks (Yssouf et al., 2015), as well as *Borrelia crocidurae* in *Ornithodoros sonrai* ticks (Fotso et al., 2014). This concept offers new perspectives for monitoring other vector borne diseases that present public health concerns.

Finally, MALDI-TOF MS has also facilitated the identification of meat origin in raw and processed meats, and fish in culinary preparations (Mazzeo et al., 2008; Flaudrops et al., 2015). Key stages in the use of MALDI-TOF MS for identification purposes other than microbial purposes are summarized in Table 2 (Yssouf et al., 2014; Yssouf et al., 2013a; Mazzeo et al., 2008; Kaufmann et al., 2012; Yssouf et al., 2013b; Steinmann et al., 2013; Flaudrops et al., 2015).

VALUE OF MALDI-TOF MS IN AFRICA

Bacterial and fungal identification in Africa

Conventional biochemical identification methods (Figure 4) are in standard use in Africa, although performance limitations sometimes exist (Patel, 2013; Samb-Ba et al., 2014). Storage of the various reagents requires strict conditions and compliance with expiration dates. Difficulties with cold storage are also observed, which can have a real impact on reagents. Reagent supply issues have also been experienced. Finally, identification is often based on interpretation of the few biochemical tests available, sometimes leading to inaccurate identification which can have a clinical impact on patient treatment. Thus, use of new-generation technologies such as MALDI-TOF MS may resolve many of these difficulties. The low cost, speed, and accuracy of identification without prior knowledge supports the claim that the use of

Table 2. Key stages in the use of MALDI-TOF MS for identification purposes other than microbial.

References	First use of MALDI-TOF MS for identification purpose	Year
Mazzaeo et al.	Fish	2008
Kaufmann et al.	Culicoides	2012
Yssouf et al.	Ticks	2013[a]
Steinmann et al.	Ceratopogonid and culicid larvae	2013
Yssouf et al.	Mosquitoes	2013[b]
Yssouf et al.	Fleas	2014
Flaudrops et al.	Meat from raw and processed meat in culinary preparations	2015

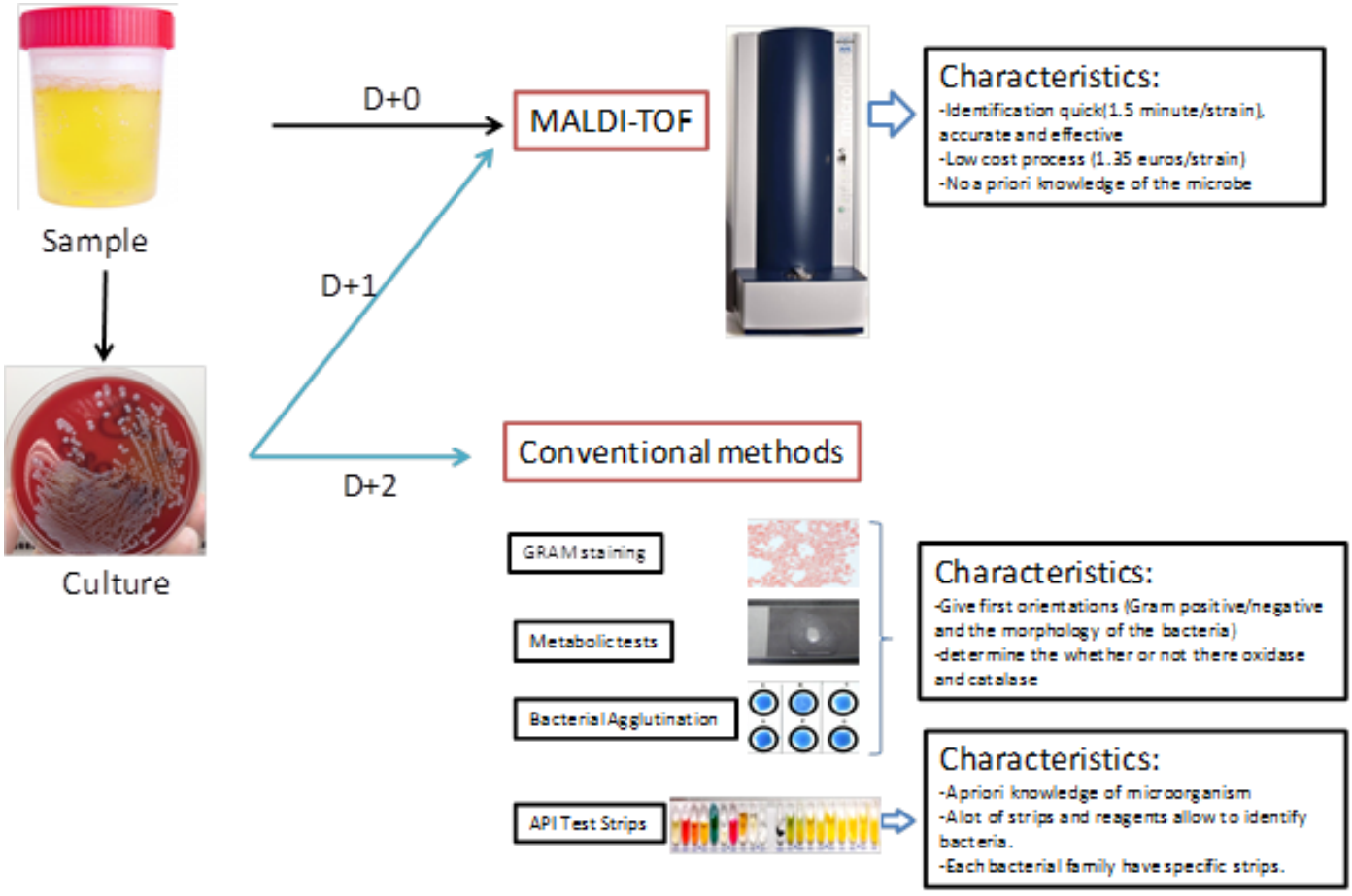

Figure 4. MALDI-TOF MS performance compared to conventional methods is routinely used in some clinical laboratories in Africa.

MALDI-TOF MS will help in microbiology laboratories in Africa (Cherkaoui et al., 2010; Bizzini and Greub, 2010). When we implemented a VITEK® mass spectrometer RUO (bioMérieux, Marcy l'Etoile, France) in Senegal (Hôpital Principal de Dakar) in 2012, conventional methods such as API strips were immediately stopped. In just ten months, the instrument correctly identified 2,082 bacteria and fungi at the species level (85.7%) (Fall et al., 2015).

Specific aspects and constraints for MALDI-TOF MS in Africa

Constraints for acquisition and installation

The primary obstacle in performing microbial identification using MALDI-TOF MS is the cost of the equipment, which is estimated at between $120,000 and $270,000 (Tran et al., 2015). Electricity is another constraint,

as it must be supplied continuously for MS. Thus, the presence of an electric generator is required to prevent power failure. Moreover, the instrument, as well as all the connected computers, must be equipped with an inverter in case of micro-power cuts. The room in which the equipment is housed must be protected from insects and dust, and must be thermo-isolated; air conditioning is mandatory.

Constraints for routine microbial identification

The main reagent required to perform MALDI-TOF MS is the chemical matrix, which is not expensive, particularly when it is home-made (Seng et al., 2009; Martiny et al., 2014). Home-made solutions can also be freshly prepared each day in not more than 10 min and stored at room temperature for the day. None of the reagents (acetonitrile solution, water, trifluoroacetic acid solution, and α-Cyano-4-hydroxycinnamic acid) need to be frozen; α -Cyano-4-hydroxycinnamic acid is the only reagent that must be stored away from light. The chemical matrix must be stored at +4°C only when purchased matrices or home-made matrices prepared a few days before are used. Commercialized standards also need to be frozen at -20°C, but fresh *E. coli* cultures can also be used as standard. Thus, reagents are not a limitation to the process of microbial identification when home-made matrices are prepared on a daily basis and *E. coli* is freshly cultivated. Each system includes spot target plates, but the plates are reusable steel targets for the Microflex LT, while the VITEK® MS uses disposable plastic targets (Deak et al., 2015). Humans may be a constraint as staff must be previously and specifically trained in the use of MALDI-TOF MS. However, it is an easy system which does not require specific prior expertise. Moreover, the required skills are quickly acquired. For example, in Senegal, after a four-day course including theoretical and practical training, the four people who completed the training course provided by two engineers from bioMérieux were autonomous in the use of MALDI-TOF MS (Fall et al., 2015).

Constraints for maintenance

The second main obstacle to the use of MALDI-TOF MS in Africa is maintenance. Annual maintenance is recommended by the manufacturers, which raises two problems: its cost, including the cost of spare parts, labor and maintenance contracts; and the lack of trained personnel in Africa to perform it. The spare parts that need to be changed most frequently are the laser and detector (depending on frequency of use) and the primary pump (a lifespan of three to four years). Overall, for the MALDI-TOF mass spectrometer that was implanted in Senegal, when moderate problems are observed (two or three times per year), a web connection is established between the local instrument in Dakar and the company in France. This kind of maintenance concerns the fine tuning and the diagnostic of eventual issues. In parallel, maintenance is done once per year by moving an engineer from France.

Solutions

Funding for the acquisition and maintenance of MALDI-TOF MS in Africa is the main constraint for implementing the technology. Routine identification does not actually raise problems or limitations.

For this study, the cost of acquiring the apparatus in Dakar was covered and shared between several organizations, including the Institute of Research for Development, a public French organization involved in research with and for southern countries, the Mediterranean Infection Hospital-University Institute, which promotes the fight against infectious diseases on a global scale, and the French Ministry of Foreign Affairs (Fall et al., 2015; Lo et al., 2015). For others, research organizations, non-governmental organizations, or charity foundations, such as the Mérieux Foundation or the Melinda and Bill Gates Foundation, which are both already involved in the use of new tools to prevent and treat deadly diseases in Africa, could help fund this equipment.

Currently, the strategy applied in several countries to lower management costs involves grouping clinical microbiology laboratories into large core laboratories. Thus, the development of a common MS platform between several clinical microbiology laboratories in nearby areas would appear to be the best option to share the costs. The experience of MALDI-TOF MS networking in university hospitals in Belgium has recently been reported for identifying microorganisms in Brussels (Martiny et al., 2014).

Over a one-month period, 1,055 isolates were identified using conventional techniques from the first hospital and analyzed by MALDI-TOF MS in another hospital situated at 7.5 km away; target plates and identification projects were sent. Identification by the MALDI-TOF networking system was more accurate and faster than that carried out in parallel with conventional methods which led to a substantial annual cost savings (Martiny et al., 2014).

Twelve months ago, the study clinical microbiology laboratory (University Hospital, Marseille, France) also opened up access to MALDI-TOF MS platform for use by other hospitals: the public health hospital from Salon de Provence, a remote town 52 km away with 400 beds, and the military teaching hospital of Marseille (Laveran), a general hospital with open access for both military personnel and civilians with 303 beds (personal data). Every week, hundreds of bacteria were correctly identified at a low cost without moving patients. Thus, the

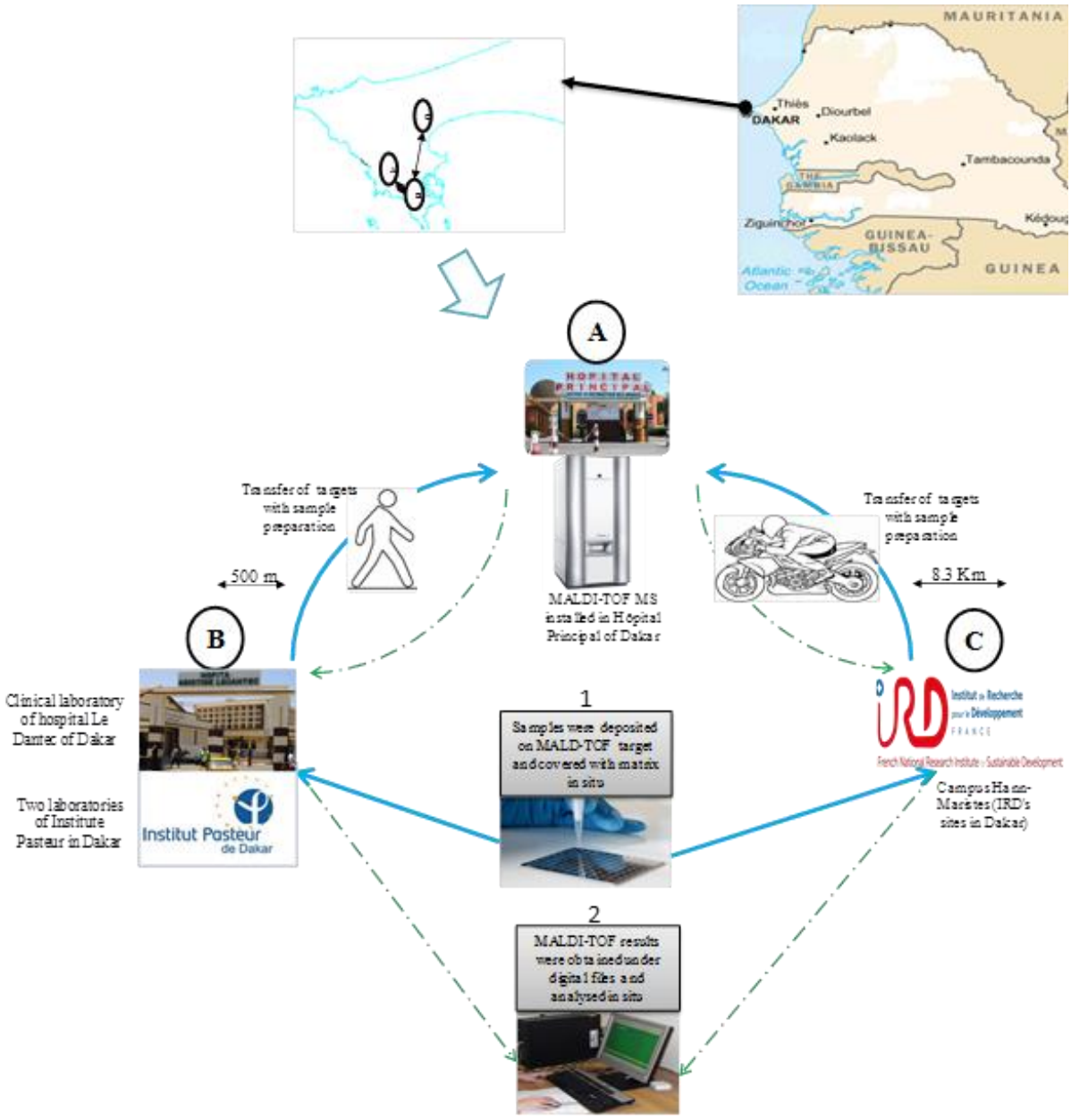

Figure 5. Schematization of the circuit for the use of a MALDI-TOF platform in Dakar (Senegal). MALDI-TOF MS is located in the clinical microbiology laboratory of Hôpital Principal de Dakar (A), but it is also used for diagnostic and research purposes by Hospital Le Dantec, the Institute Pasteur of Dakar (B), and by the Institut de Recherche pour le Développement (IRD) situated at the east of Dakar (C).

use of the same MALDI-TOF MS platform enables skills to be shared and reduces the cost of acquiring and maintaining the instrument (Martiny et al., 2014).

The MALDI-TOF mass spectrometer, that we managed, is installed at the Hôpital Principal de Dakar (Senegal) since 2012 (Fall et al., 2015). This platform is open to other health structures as well as research centers located in Dakar and its periphery as indicated on Figure 5. The samples shipment to the platform is frequent for centers like the Institute of Research for Development (IRD) and the Pasteur Institute but it is rarer for structures such as the Le Dantec Hospital and the public center of biologic and medical analysis of the Hôpital Abass Ndao.

Indeed, the platform becomes a real support for the identification of microorganisms isolated from patients in these structures. IRD prepares its own target plates; all the other structures send the strains they have been unable to correctly identify using conventional methods directly to the hospital. The time for target plate transfer

to the platform varies depending on the road traffic but it never exceeds an hour and a half. A low quality of deposit linked to transfer between sites and temperature has never been observed. When the target plates arrived at the platform, only the qualified personnel of the platform perform the plate's analysis. Interpretation of the data is also performed by the personnel of the platform, except for the plates from the IRD.

Indeed, qualified people and Saramis software (bioMérieux) are both available at the IRD. Thus, raw data can be retrieved and interpreted there. For other structures, interpreted data can be recuperated directly or send by email. If a MALDI-TOF MS platform is established, a cooperation agreement and a convention should be established between the various teams in order to specify not only the organization of workflows but also the tasks and responsibilities of everyone involved. Finally, local maintenance staff should be specifically trained.

CONCLUSION

The rapidity, efficiency, and low cost have led many laboratories to adopt the MALDI-TOF as a tool for routine diagnosis, resulting to an improvement in patient care. The first successful use of a MALDI-TOF mass spectrometer in Senegal supports the fact that it is a robust and potentially valuable tool in tropical Africa which should be widely distributed there. The development of shared MALDI-TOF MS platforms in nearby geographic areas will allow equipment, skills, and costs to be shared.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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