Scholars Journal of Dental Sciences (SJDS) **ISSN 2394-4951 (Print)**
Abbreviated Key Title: Sch. J. Dent. Sci. **ISSN 2394-496X (Online)**

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com DOI:10.36347/sjds.2018.v05i03.010

Use of MALDI-TOF Mass Spectrometry in Rapid Identification of Bacteria in Patients with Periodontal Diseases in Dakar (Senegal)

Guirassy Mouhamadou Lamine[1*], Gueye Mamadou Wague[2], Diawara Papa Silman[2,] Fall Bécaye[2], Camara Makhtar[3], Faye Babacar[4], Benoist Henri Michel[1], Diallo Papa Demba[1]
[1]Department of Periodontology, Department of Odontology, University Cheikh Anta Diop of Dakar, Senegal
[2]Department of Microbiology, federation of laboratories Hospital Principal of Dakar, Senegal
[3]Department of Bacteriology-Virology CHNU Aristides the Dantec Dakar, Senegal
[4]Department of Parasitology-Mycology Faculty of Medicine, Pharmacy and Odontology University Cheikh Anta DIOP Dakar, Senegal

Original Research Article

***Corresponding author**
Guirassy Mouhamadou Lamine

Article History
Received: 12.03.2018
Accepted: 25.03.2018
Published: 30.03.2018

DOI:
10.21276/sjds.2018.5.3.10

Abstract: The oral ecosystem consists of polymorphic flora with bacteria, viruses, fungi and parasites. Conventional methods of identification have shown their limitations related to the complexity of the protocols. The mass spectrometry system for identifying microorganisms via their protein is an alternative to these difficulties. The objective of this work was to assess the MALDI-TOF mass spectrometry use in bacteriological identification of the oral microbiota. This was a descriptive cross-sectional study over a period of 3 months and on a sample of 25 patients aged 20 to 76 years. Then one hundred samples of subgingival biofilm and 25 swabs were made at the Clinic of periodontology of the University Cheikh Anta Diop of Dakar and then transported and analyzed at the microbiological laboratory of Hôpital principal de Dakar. The periodontal examination showed a prevalence of 44% for periodontitis and 56% of cases of dental plaque-induced gingivitis. Culture and identification of colonies by MALDI-TOF has isolated a total of 43 bacterial species including 33 optional aero-anaerobic, 06 anaerobic and 04 aerobic. The speed and reliability of MALDI-TOF make it an innovative identification technique in periodontology to optimize patient management. However, other studies with more structured methodological qualities are necessary
Keywords: MALDI-TOF, mass spectrometry, bacteria, identification, periodontology.

INTRODUCTION

The human oral cavity contains an abundance of flora and polymorphic microorganisms, the majority of which is made up of bacteria. It also contains viruses, yeasts and protozoa. This natural cavity constitutes with the colon the most septic part of the body: 1 milligram of plaque carries about 100 million bacteria [1].

Periodontal health is a fragile balance between the aggressiveness of this ecosystem and the response of the host [2]. Any imbalance would be likely to bring about infectious and inflammatory clinical events such as gingivitis or periodontitis [3]. Periodontal diseases are predominantly anaerobic polymicrobial infectious diseases resulting in the destruction of dental support tissue in a permissive host.

The effectiveness of a periodontal treatment results in the disappearance of clinical symptoms and the disappearance of the main pathogens involved in this disease. The complexity and specificity of this oral flora and the fact that it is very difficult to identify have generated more and more attention from clinicians and clinical microbiology laboratories [4]. Bacterial identification allows the confirmation of a diagnosis of periodontitis established by clinical and radiographic examinations. This identification is also a means to evaluate the bacterial load and to analyze the composition of the sub-gingival plaque in oral pathogen [5,6].

The identification and typing techniques used in clinical microbiology are constantly evolving. Conventional methods based mainly on biochemical and / or genotypic tests (molecular biology) have their shortcomings, mainly related to the complexity of the protocols, the availability of the necessary know-how, the costs of the reagents, the use of probes or specific nucleotide sequences for each species and finally the significant time required for identification [7, 8]. These conventional identification methods are time-consuming because large amounts of inocula are needed and the final results may be inconclusive or incorrect. An alternative method for bacterial identification that is

emerging in clinical microbiology is Matrix- Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS). MALDI-TOF MS has been described as a rapid, cost-effective, and reliable method for the identification of bacteria in the clinical laboratory [9-11].

As far as we know, no assessment of the usefulness of MALDI-TOF in bacteriological identification of the oral flora in the field of dentistry has ever been performed in Dakar. However, some techniques based on biochemical identification or PCR have already been the subject of several studies.

The objective of this work was therefore to evaluate the usefulness of MALDI-TOF mass spectrometry in the bacteriological study of the oral microbiota.

MATERIALS AND METHODS

The study was conducted by the Periodontics Clinic of the Department of Odontology of the Faculty of Medicine, Pharmacy and Odontology of the Cheikh Anta Diop University of Dakar and the Microbiology Laboratory of the Hospital Principal of Dakar.

This was a descriptive cross-sectional study .The sample population was derived from the usual patients attending periodontics clinic of the Department of Odontology. It consisted of two groups.

Group1: 14 patients with gingivitis (2 < pocket depth ≤ 3mm).

Group 2: 11 patients with periodontitis (pocket depth ≥ 4)

Periodontitis was defined as 4 or more teeth with a pocket depth ≥ 4mm or a clinical attachment loss > 2mm with Bleeding on Probing> 35% of the sites tested.

Patients with known risk factors such as tobacco, alcohol, diabetes and any other systemic disease that may be a risk factor for periodontitis progression were not included in the study. Patients were required not to have taken any antibiotics or antiseptics over the last 3 months prior to the study and not have undergone Scaling and Root Planning (SRP) during the same period.

The study was conducted in compliance with the legal and ethical rules relevant to the protection of individuals involved in biomedical research. The patients signed a written consent.

A questionnaire allowed the collection of clinical and bacteriological socio-demographic data. Pocket depth and clinical attachment loss data were collected at 6 sites per tooth: vestibular, mesio vestibular, disto vestibular, lingual, mesio-lingual, disto lingual. Periodontitis is characterized as moderate when the average attachment loss is between 3 and 4 mm while the subgroup of severe chronic periodontitis is characterized by a clinical attachment loss ≥ 5mm. The extent of periodontal damage is viewed as generalized when more than 30% of sites are affected.

The level of hygiene is appreciated by calculating the O'Leary plaque index.Gingival inflammation is assessed by calculating the BOP (Bleeding on Probing) gingival index of Ainamo and Bay in 1975 [1].

Four samples were taken at the deepest sites (more than 5mm deep) using endodontic paper tips. Samples of mucosal surfaces (jugal and lingual) were collected using sterile swabs on an approximate area of 1 cm2. These samples were then placed in 9 ml of heart-brain broth as a transport medium and analyzed in the Microbiology laboratory of the Hôspital Principal of Dakar. It must be recalled that two types of samples were collected from each patient: a suspension and a swab.

For the suspension we prepared:

- A thioglycolate broth (BT) to describe the respiratory type of the bacteria and facilitate the growth of the anaerobic ones ;
- A blood and chocolate agar which is an enriched medium for the growth of almost all bacteria.

For the swab we also prepared a cooked blood agar as before. After 24/48 H incubation, the well-isolated colonies were identified using MALDI-TOF-MS.

The principle of MALDI-TOF mass spectrometry lies in the separation during the gas phase of charged molecules obtained by breaking down a sample of the bacterial colony to be studied under the effect of a strong magnetic field. Those molecules are then directed towards a detector. For each of them a height peak proportional to the quantity of the molecule present in the inoculum is recorded. The set of peaks will form a characteristic spectrum of the pathogen to be studied. Precise identification can then be done by comparison with a multitude of perfectly identified reference spectrum. The equipment used for this identification is VITEK MS from Biomerieux. Excel then analyzes the data obtained.

RESULTS

Twenty-five patients were selected and 100 samples of subgingival biofilm were identified. 25 swabs were taken from the jugal, lingual and supra-gingival mucous membranes. The average age was 39 years with a standard deviation of 17 years and extremes were 20 and 76 years. The study population

was made up of 16 women (64%) and 09 men (36%). The sex ratio was therefore 0.56.

Clinical features

Breakdown according to diagnosis

In the study population, the prevalence of severe chronic periodontitis was 20% while that of moderate chronic periodontitis was 24% .with 44% of chronic periodontitis with a greater prevalence in the age group 45-76 years. Gingival disease caused by plaque was found in 56% of patients (Figure 1). Distribution according to periodontal pocket depth. The presence of periodontal pockets (≥ 4mm) was observed in 40% of the sample (Figure 2).

BACTERIOLOGICAL CHARACTERISTICS

Forty-three bacteria were isolated with a different distribution according to the clinical profile of the periodontal disease and the respiratory type of the bacteria in the 25 patients. 14 other colonies of microorganisms not included in the MALDI-TOF database appeared without spectrum (no matches).

An analysis of the distribution of bacteria identified according to the respiratory type revealed the presence of 6 anaerobes out of the 43 versus 33 optional aero-anaerobes. A few aerobes were, however isolated (Figure 3).

The distribution of bacterial species according to the clinical profile shows a predominance of AAF (Aero-Anaerobic Facultative) both in gingivitis and in chronic periodontitis with a common presence of aerobes and anaerobes in gingivitis and periodontitis. In cases of gingivitis, 02 anaerobes were identified (A *odontolyticus* and C *perfringens*) but AAF remained predominant.

In cases of moderate generalized chronic periodontitis (MGCP), gram-positive (S. *aureus*, S. *Gordini* and S. *hominis*) and gram-negative (Y. *enterolitica*) AAFs were found.

In patients with severe generalized chronic periodontitis (SGCP) the analysis revealed a predominance of both Gram positive (*S. arlettae, S. sanguinis, S. pseudopneumoniae S. epidermidis, S. vestibularis*) and gram negative (*A. actinomycetemcomitans, H. influenzae, N. elongata, Neisseria sp*). *Note the presence of 2 anaerobes* (*B. bifidum, Gemellasp*).

ILLUSTRATIONS

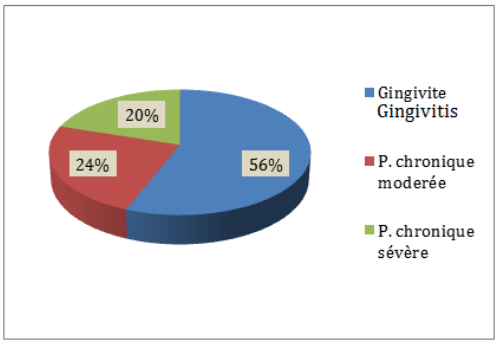

Fig-1: Breakdown of the population sample by diagnosis

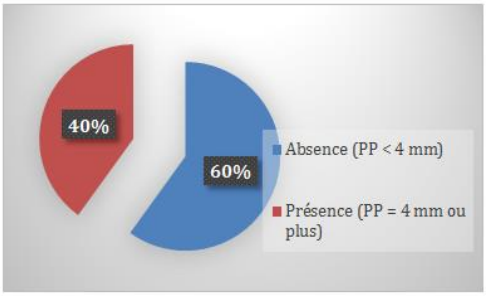

Fig-2: Breakdown by pocket depth

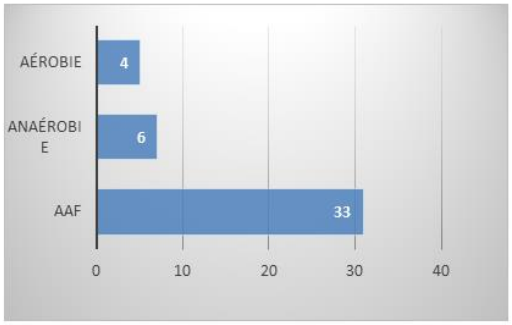

Fig-3: Breakdown by respiratory type

Table-I: Profile of corresponding bacteria

AAF	Gram	Positive	Bacilli (2)	*Bacillus subtilis* *Corynebacteriumamycolatum*
			Cocci (18)	*Staphylococcus cohnii* *Staphylococcus hyicus* *Staphylococcus warneri* *Staphylococcus aureus* *Staphylococcus haemolyticus* *Streptococcus gordonii* *Staphylococcus hominis* *Staphylococcus arlettae* *Staphylococcus epidermidis* *Streptococcus pseudopneumoniae* *Streptococcus anginosus* *Streptococcus mitis* *Streptococcus parasanguinis* *Streptococcus spp* *Streptococcus oralis* *Streptococcus salivarius* *Streptococcus sanguinis* *Streptococcus vestibularis*
		Negative	Bacilli (7)	*Leclerciaadecarboxylata* *Proteusmirabilis* *Providencia stuartii* *Yersiniaenterocolitica* *Aggregatibacter-actinomycetemcomitans* *Haemophilusinfluenzae* *Haemophilusparainfluenzae*
			Cocci (4)	*Actinobacillusliginieresii* *Neisseriaelongata* *Neisseriaspp* *Neisseriasubflava*
			Cocci (1)	*Gemellaspp*
		Negative	Bacilli (1)	*Aeromonaseucrenophila*
			Cocci (2)	*Klebiellapneumonaie* *Veillonellaspp*
Aerobe	Gram	Positive	Bacilli (1)	*Burkholderiacenocepacia*
			Cocci	
		Negative	Bacilli (3)	*Burkholderiastabilis* *Burkholderiaspp* *Pseudomonas aeruginosa*
			Cocci (1)	*Micrococcusluteus*

DISCUSSION

The use of MALDI-TOF mass spectrometry in this descriptive and cross-sectional study allowed the rapid (less than 48 hours) identification of aerobic and facultative aero-anerobic bacteria in the oral microbiota.

Limits and Methodological considerations

This preliminary study based on the identification technique using MALDI TOF had never been performed in the dental services in Dakar in general and even less so in the field of periodontology, in particular at the Department of Odontology of the Faculty of Medicine, Pharmacy and of Odontology of Dakar. Our sample (25 patients) was not very different from Stingu's in 2008 [12] with 33 patients with aggressive periodontitis. In the same vein, Vielkind in 2015 [13] worked on 20 patients with chronic periodontitis and 15 healthy subjects.

In order to maximize the chances of isolating the pathogens involved, 4 samples were taken from each patient, with the endodontic paper tips, i e 100 samples of subgingival biofilm associated with 1 swabbing from the oral and supragingival mucosa. In addition, 25 swab specimens were taken in addition to 100 samples of sub-gingival biofilm.

Social and professional features

The study population had an average age of 39 years with a standard deviation of 17 years which is significantly similar to Stingu *et al*. in 2008 [12] who had an average age of 39.39 with a standard deviation of 10.47 years. The most representative age group in the study was 20 to 30 years old, which characterized a young population. A predominance of female individuals with a sex ratio of 0.56 is noted which is different from the sex ratio found by Sambe in 2013 [14] which was 1.3 with a male predominance therefore.

Clinical features

The distribution and severity of periodontal tissue loss assessed by measuring clinical attachment loss indicates that the prevalence of chronic periodontitis was 44% with a greater prevalence in the 45-76 age group. Bourgeois et al in 2007 [9] found a statistically significant association between the severity of attachment loss and subjects over 50 years of age ($p < 0.01$).

Bacteriological features

In this study, culture and identification by MALDI-TOF allowed the isolation of 43 bacterial species. The quantitative results obtained in this work are inferior to those of Stingu *et al*. in 2008 [12] who isolated 75 bacterial strains in a study of the same nature. This could be explained by the fact that some 14 bacterial colonies that are not in the database of the MALDI-TOF used are not correctly identified and appear as "no matches".

The specific environment of the gingival sulcus seems to be conducive to the development of a more diverse bacterial community. Most bacteria are anaerobes and have a proteolytic metabolism. For periodontal diseases, the infection cannot be blamed on a single bacterium. It will rather be attributed to a consortium of bacteria organized on a biofilm.

Identification according to the respiratory type indicates a predominance of AAFs numbering 33 out of a total of 43 or 76.9%. These quantitative results are inferior to those found by Socransky *et al*. 1982 [15] where the flora consisted of 60% of facultative facultative anaerobic Gram + bacteria or strict anaerobes.

In this study, four anaerobes were found and only six aerobic bacteria have been identified. This stark difference could be due to a lack of control over anaerobiosis throughout the sample handling circuit. Losses in the course of the cultural processes could also partly explain this difference. Indeed, such media as blood agar used can foster the growth of anaerobes. It would therefore be preferable to use Shaedler type media.

Identification based on clinical diagnosis

With respect to gingivitis, most of the identified bacteria were facultative aero-anaerobes. We were also able to isolate a few aerobes and anaerobes. The anaerobes found in gingivitis were *Actinomyces odontolyticus* and *Clostridium perfringens*. Compared with the Socransky *et al*. study in 1982 [15], a small percentage of strict anaerobic Gram-negative bacilli such as *Fusobacterium nucleatum* and *Prevotella intermedia* were also found in this pathological situation.

Some bacteria have been identified repeatedly in gum disease. Thus, Streptococcus sp was the most encountered (10 times) in our study unlike that of Koukos G in 2015 [16] where *Staphylococcus aureus* was the most detected.

In patients with periodontitis, MALDI-TOF was able to identify AAF bacteria and 2 anaerobic bacteria. The most prominent were: *Streptococcus oralis*, *Streptococcus mitis* and *Neisseriae longata*. Choi Nakagawa *et al*. [17], however, found a predominance of *Porphyromonas gingivalis* in chronic periodontitis lesions while it was little or not present in healthy subjects or with gingivitis.

However, for a better evaluation of the potential contribution of MALDI-TOF in periodontics further studies of a greater scope are needed especially in respect of patients with refractory periodontitis or aggressive periodontitis and also in those who would be candidates for complex treatments such as periodontal regeneration or implants.

CONCLUSION

The importance of accurate identification of bacteria to species level is well known in relation to administration of suitable antibiotic treatment. The results of this study suggest that MALDI-TOF-MS might become a useful method for the identification of oral microbiota especially for those that cannot readily be identified by biochemical analysis. This innovative technique will thus optimize the conventional treatment of patients with aggressive or refractory periodontitis, especially when these patients are candidates for complex treatments such as periodontal regeneration and implants.

REFERENCES

1. PASTER BJ et COL. Principe de la spectrometry de masse, 2001; 77: 112-133
2. Plombas M, Gobert B, De March Ak, Sarda Mn, Sixou M, Bene Mc, Miller N, Faure Gc**.** Isotypic antibody response to plaque anaerobes in periodontal disease.J Periodontol, 2002; 73(12): [1507-1511].
3. Vergnes Jn, Sixou M**.** Preterm low birth weight and maternal periodontal status: a meta-analysis. American Journal of Obstetrics Gynecology, 2007; (2): 135-147.
4. LOOMER PM**.** Microbiological diagnostic testing In the treatment of periodontal diseases. Periodontol. 2000,2004; 34: [49-56].
5. Van Winkelhoff AJ**.** Microbiological diagnostic testing in the treatment of periodontal diseases.Int J Dent Hygiene, 2003; 1: 131-137.
6. Mombelli A, Nyman S, Brägger U, Wennström J, Lang NP. Clinical and microbiological changes associated with an altered subgingival environment induced by periodontal pocket reduction. Journal of clinical periodontology. 1995 Oct 1;22(10):780-7.
7. Nagy E, Becker S, Kostrzewa M, Barta N, Urbán E. The value of MALDI-TOF MS for the identification of clinically relevant anaerobic bacteria in routine laboratories. Journal of medical microbiology. 2012 Oct 1;61(10):1393-400.
8. Cariello C, Tissières Lovey L. La spectrométrie de masse MALDI-TOF et le diagnostic microbiologique. Tiré de http://www. essante. ch/wpcontent/uploads/2013/02/La-spectrométrie-de-masse-MALDI-TOF-et-lediagnostic-microbiologique. pdf. 2012.
9. Shah HN, Keys CJ, Schmid O, Gharbia SE. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and proteomics: a new era in anaerobic microbiology. Clinical infectious diseases. 2002 Sep 1;35(Supplement_1):S58-64.
10. Pappin DJ. Peptide mass fingerprinting using MALDI-TOF mass spectrometry. InProtein Sequencing Protocols 1997 (pp. 165-173). Humana Press, Totowa, NJ.
11. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Infectious Diseases. 2009 Aug 15;49(4):543-51.
12. Stingu CS, Rodloff AC, Jentsch H, Schaumann R, Eschrich K. Rapid identification of oral anaerobic bacteria cultivated from subgingival biofilm by MALDI-TOF-MS. Molecular Oral Microbiology. 2008 Oct 1;23(5):372-6.
13. Vielkind P, Jentsch H, Eschrich K, Rodloff AC, Stingu CS. Prevalence of Actinomyces spp. in patients with chronic periodontitis. International Journal of Medical Microbiology. 2015 Oct 1;305(7):682-8.
14. Samb-Ba B, Mazenot C, Gassama-Sow A, Dubourg G, Richet H, Hugon P, Lagier JC, Raoult D, Fenollar F. MALDI-TOF identification of the human gut microbiome in people with and without diarrhea in Senegal. PloS one. 2014 May 1;9(5):e87419.
15. Socransky SS. Present status of studies on the microbial etiology of periodontal disease. Host Parasite Interaction in Periodontal Disease. 1982:1-2.
16. Choil JI, Nakagawa T, Yamada S, Takazoe I, Okuda K. Clinical, microbiological and immunological studies on recurrent periodontal disease. Journal of clinical periodontology. 1990 Aug 1;17(7):426-34.
17. Koukos G, Sakellari D, Arsenakis M, Tsalikis L, Slini T, Konstantinidis A. Prevalence of Staphylococcus aureus and methicillin resistant Staphylococcus aureus (MRSA) in the oral cavity. Archives of oral biology. 2015 Sep 1;60(9):1410-5.
18. Pahumunto N, Ruangsri P, Wongsuwanlert M, Piwat S, Dahlen G, Teanpaisan R. Virulence of Aggregatibacter actinomycetemcomitans serotypes and DGGE subtypes isolated from chronic adult periodontitis in Thailand. Anaerobe. 2015 Dec 1;36:60-4.