Original Research Article

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) for the proteomic based identification of *Aggregatibacter actinomycetemcomitans* isolated from orodental infections

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Abstract

Aim: *Aggregatibacter actinomycetemcomitans* is a fastidious gram negative cocco bacilli responsible for aggressive and chronic periodontitis as well as other systemic infections. In this study the usefulness of MALDITOF MS a proteomic based study was evaluated for the identification of *A. actinomycetemcomitans* isolates from orodental infections.
Materials and methods: Fifty clinical isolates of *A. actinomycetemcomitans* obtained from orodental infections were subjected for identification with conventional as well as MALDITOF MS analysis.
Results: All the isolates tested were accurately identified by the MALDITOF MS analysis. Among the 50 isolates 35 were identified at secure and probable species level and another 15 were identified at highly probable species level.
Conclusion: MALDITOF MS is reliable, cost effective and rapid test for the identification of slow growing fastidious bacteria like *A. actinomycetemcomitans* compared with conventional methods.

Key words

A.actinomycetemcomitans, Orodental infections, MALDITOF MS.

Introduction

Aggregatibacter actinomycetemcomitans is a gram negative capnoic, fastidious bacteria implicated in the etiology of aggressive form of periodontitis and other systemic infections [1-5]. It is a member of HACEK (*Haemophilus aphrophilus,Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae*) group of bacteria that are responsible for infections of the endocardium [3].

Early and accurate identification of pathogen is essential for adequate antibiotic therapy and management. The diagnostic microbiology laboratory are supposed to identify infectious agents in rapid accurate and cost effective manner. This process may be delayed when it deals with fastidious as well as microorganisms which are difficult to identify. MALDI TOF MS analysis is used as an alternate method for the identification of various aerobic, non fastidious organisms as reported in the literature [6]. This technique is based upon the detection of highly abundant proteins in a mass range between 2 and 20 kilo Dalton (kDa) by computing their mass (m) to charge (z), m/z values. The organism were identified by comparing the typical score value generated for each microorganism with the stored standard reference spectra in the data base [7]. In this study clinical strains of *A.actinomycetemcomitans* obtained from orodental infections were subjected to MALDITOF MS analysis. Reports are lacking from India in this particular aspect.

Materials and methods

Isolation of the strains from cases

The study was conducted at a tertiary care hospital at Mangaluru, coastal Karnataka, South India. The study was approved by the Institutional Ethics Committee (Ref. No FMMC /IEC /395 /2010) and the study was registered at CTRI (clinical trial Registry India) by National Institute of Medical Statistics hosted at the Indian Council of Medical research. (ICMR) (Registration No CTRI/2014/09/005031).

A. actinomycetemcomitans was isolated from patients with orodental infections attending the dental colleges in and around Mangaluru. The paper points specimens obtained from the sub gingival sites as well as infected root canal of the patients were transported in reduced transport fluid and sub cultured on Dentaid -1 media. The plates were incubated at 37^0C in a candle jar for 48-72 hours. Pin point (1mm diameter), glistening colonies with central 4-6 pointed star like configuration were presumptively identified as *A. actinomycetemcomitans* and were confirmed by conventional methods like Grams stain, positive catalase test, spot indole test and biochemical reactions [8].

MALDITOF MS analysis using Microflex LT™

Fifty strains of *A. actinomycetemcomitans* which were identified by morphological characteristics and biochemical reactions were subjected to MALDITOF MS analysis by tube extraction method. This analysis was performed at Microbiological Laboratory, Coimbatore, Tamil Nadu.

MALDI-TOF MS, analysis was done using a Microflex LT™, bench top mass spectrometer (Bruker Daltonics, Bremen, Germany). The software for the control of the instrument was Flex Control 3.3 and Maldi Biotyper 3.0 (Bruker Daltonics) for the analysis of the spectra and comparison with the database. A bacterial test standard provided by the manufacturer was included in every run for calibration purposes. Default settings (acquisition of mass spectra in the linear positive mode within the 2–20 kDa range, Ion Source 1 [IS1] 20 kV, IS2 18.05 kV, lens 6.0 kV, linear detector 2560 V) were applied according to manufacturer's instructions.

Tube based extraction method

The procedure was followed according to manufacturer's instructions. Briefly, 2-3 pure colonies of bacteria, resuspended in a mixture containing 900-μL absolute ethanol and 300 μL of sterile water in a sterile vial. This solution was centrifuged at 13,000 rpm for 2 minutes. The supernatant was discarded and the pellet was resuspended again in 25–100 μL of 70% formic acid in water and the same amount of 100% acetonitrile. This solution was centrifuged at 13,000 rpm for 2 minute and 1 μL of the supernatant was spotted onto a polished steel MALDI target plate. The spots were overlaid with 1 μL of 70% formic acid after drying at room temperature. This was kept for air drying, once dried, samples were overlaid again with 1 μL of matrix (a-cyano-4-hydroxycinnamic acid solution in 50% acetonitrile and 2.5% trifluoroacetic acid).When the matrix was air dried, spectra were acquired by the mass spectrometer and compared with the database.

Criteria for assigning positivity

The score values obtained for each strain was compared with the values given below by the manufacturer (**Table - 1**).

Table - 1: Meaning of score value.

Range	Description
2.300-3.000	Highly probable species identification
2.000-2.299	Secure genus identification, probable species identification
1.700-1.999	Probable genus identification
0.000-1.699	Not reliable identification

Results

The 50 clinical strains of *A. actinomycetemcomitans* identified by conventional methods were correctly identified by MALDITOF MS. Among the 50 strains 35(70%) isolates showed score value between 2.000 and 2.299 and another 15 (30%) isolates were showed score value between 2.300 and 3.000 (**Table - 2**).

Fifty clinical strains isolated in this study were best matched with 3 reference *A. actinomycetemcomitans* strains from the data base (**Table - 3**).

In the mass spectra obtained, two maximum peaks were observed at 4681 and 9364 m/z and two minor peaks at 6409 and 7180 m/z, which represents for *A. actinomycetemcomitans* (**Figure – 1**).

Discussion

In recent years the implementation of MALDITOF MS in the microbiology laboratory allows identification of a wide variety of bacterial and fungal pathogens. Review of the literature shows that MALDITOF MS was used for the identification of commonly encountered aerobic bacterial and fungal pathogens along with few gram negative anaerobes with reliable results [6, 9-13]. No documented reports were available from India regarding the usefulness of MALDITOF MS for the identification of *A. actinomycetemcomitans*.

In the present study, all the 50 isolates tested were accurately identified by conventional as well as MALDITOF MS analysis (100%). Among the 50 isolates tested with MALDITOF MS, 35 strains showed score values between 2.000 and 2.299 with secure genus and probable species identification (70%) and remaining 15 strains were between 2.299 and 3.000 with highly probable species identification (30%). Similar type of results were reported by Couturier MR, et al. [13] when used MALDI TOF for identification of HACEK group. In that study all the 5 *A. actinomycetemcomitans* tested were identified up to species level (100%).

In MALDITOF analysis, majority of the spectral peaks lies between 5000 to 20000 kDa. In this region, peaks would typically represent singly protonated protein molecule while most

lipoologosaccharides and peptidoglycans would appear at m/z less than 3500 kDa [14]. In the present study 2 major peaks of 4681 and 9364m/z and two minor peaks at 6409 and 7180 m/z were obtained, which represents the *A. actinomycetemcomitans* and emphasized the fact that these proteins were mainly ribosomal in nature [16].
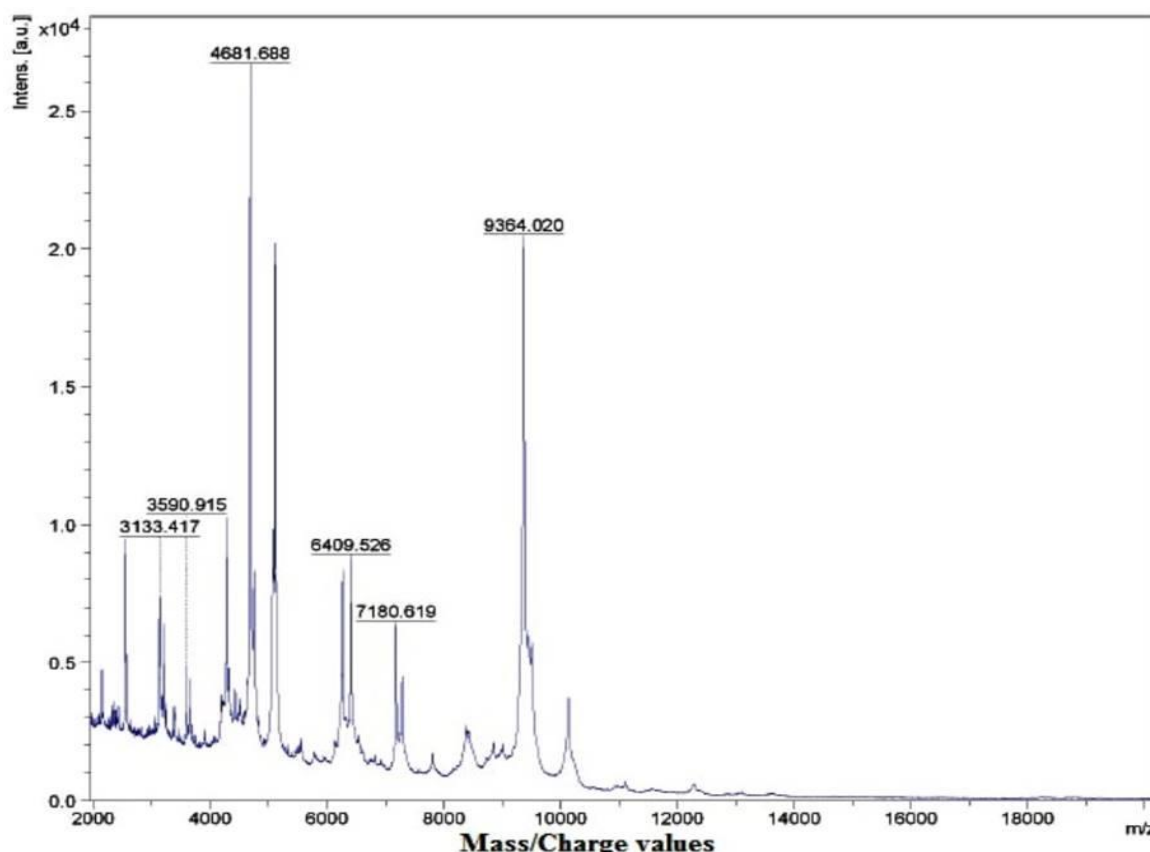
Table - 2: Results of the MALDITOF MS analysis done for 50 clinical strains of *A.actinomycetemcomitans.*

Total number of isolates tested	Score value distribution among tested isolates and percentage.			
	(0.000-1.699) Test Not reliable	(1.700-1.999) Probable genus identification	(2.000-2.299) Secure genus and probable species identification	(2.300-3.000) Highly probable species
50	0	0	35 (70%)	15 (30%)

Table - 3: Clinical strains best matched with reference strains in the data base.

Reference organism in data base	Number of clinical strains matched with reference strain
A.actinomycetemcomitans CIP 52_106T CTL	20
A.actinomycetemcomitans CCM 4688[T]	18
A.actinomycetemcomitans CCM 6053	12

Figure - 1: MALDITOF MS - Mass spectra of *A. actinomycetemcomitans.*

Two different methods were performed on the bacterial isolates prior to MALDITOF MS identification. In the tube based extraction method, proteins in the bacterial cells were extracted using ethanol. This method was found superior to the simple direct colony method where colonies were subjected directly to MALDITOF MS analysis [15]. In the present study, we employed the tube based extraction for sample preparations and the results were conclusive.

The usual conventional phenotypic method for the identification of bacteria include culture and growth pattern on various media, gram stain and biochemical characteristics. Complete identification is usually time consuming and require specialized staffs for the correct interpretation. Recent molecular techniques like polymerase chain reaction, sequencing and microarray analysis have found some applications in microbiology laboratory; however these methods are also time consuming, costly and require trained staff [7]. The turnaround time for the MALDITOF analysis was less than 30 minutes, provided a fresh pure culture of organism is available with tube based extraction method whereas for conventional identification it was more than 48 hours. In a study by Panda A et al the turnaround time for MALDITOF MS analysis was less than 23minutes [16]. Hence, MALDITOF MS analysis has proved to be a rapid technique for the identification of *A. actinomycetemcomitans*.

Conclusion

MALDITOF MS, a proteomic based identification method is rapid, accurate and reproducible procedure for the identification of clinical isolates of *A. actinomycetemcomitans* than the conventional biochemical identification methods. The rapid nature of the test saves considerable amount of time for earlier therapeutic interventions and better patient care.

Acknowledgement

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