


Rapid Pathogen Identification With Direct Application of MALDI-TOF Mass Spectrometry on an Endophthalmitis Vitreous Sample Without Prior Culture

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Abstract

Purpose: We report a case of a 72-year-old man with bleb-related endophthalmitis (BRE) whose vitreous samples were directly analyzed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to rapidly identify the causative organism, whereas the results from conventional microbiological techniques were negative. **Methods:** We analyzed BRE vitreous samples with MALDI-TOF MS (Vitek MS, bioMérieux) for rapid pathogen identification without prior culture. Samples were also analyzed with standard microbiological methods. **Results:** Within 1 hour of sample acquisition, MALDI-TOF MS identified *Gemella sanguinis* from the undiluted vitreous sample from vitrectomy without prior culture with a confidence value of 99.7%. Gram stain and cultures from aqueous and vitreous samples were negative for 28 days after acquisition. The patient's right-eye vision improved from hand motion to 20/50 2 months later. **Conclusions:** Our findings suggest that the direct analysis of intraocular samples with MALDI-TOF MS could be a novel, promising adjuvant method of rapid endophthalmitis diagnosis.

Keywords

bleb-related endophthalmitis, culture, endophthalmitis, inflammatory and infectious diseases, MALDI-TOF MS, mass spectrometry, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, microbiology, retina, vitreoretinal surgery

Introduction

Endophthalmitis is a serious intraocular infection that can quickly result in irreversible blindness following intraocular surgery, intraocular injections, trauma, or endogenous spread.¹⁻³ Conventional microbiological analysis of endophthalmitis samples often fails to identify pathogens and takes days to provide results.³ Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an analytical tool that can expediently identify pathogens that grow from cultures. This is important for the initiation of appropriate therapies, especially in the face of increasing antibiotic resistance.¹

MALDI-TOF MS (Vitek MS, bioMérieux) uses a laser source to ionize whole-cell extracts to produce peptide fingerprint spectra unique to each microorganism, and compares the peptide profile against databases to enable distinction between strains of the same species.⁴⁻⁷ However, MALDI-TOF MS is currently used only in cases in which there is organism growth from cultures, and limited data exist for the direct analysis of patient samples.^{1,4-6,8} In this report, we investigate the direct analysis of vitreous endophthalmitis samples with

MALDI-TOF MS without prior culture for rapid pathogen identification.

Methods

A 72-year-old man with a history of trabeculectomy 25 years prior presented with vision loss, pain, and redness in his right eye and vision of counting fingers. He was diagnosed with bleb-related endophthalmitis (BRE) with a purulent bleb,

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hypopyon, and dense fibrin reaction in the anterior chamber as well as dense vitritis (Figure 1A). He underwent an immediate anterior chamber tap (vitreous tap was dry), and intravitreal injections of ceftazidime (2.25 mg/0.1 ml) and vancomycin (1 mg/0.1 ml) were administered. Aqueous humor (0.1 ml) from the tap was sent for standard Gram stains and cultures.

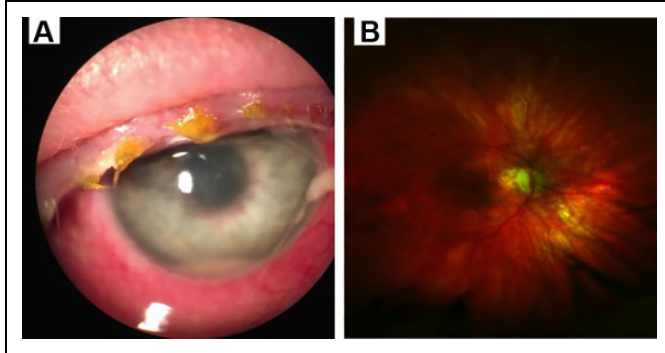


Figure 1. Images of patient with right-eye, bleb-related endophthalmitis. (A) Photo of patient's right eye on day of presentation with hypopyon visible in the anterior chamber. (B) Right eye fundus photo on postoperative month 2.

The patient was started on antibiotic drops in his right eye and oral moxifloxacin.

The following day, the patient's vision deteriorated to hand motion, and the decision was made to proceed with pars plana vitrectomy. During the vitrectomy, ~1.2 ml of undiluted vitreous humor were collected. After the infusion was started, 2 additional samples of ~3 ml each of diluted vitreous humor were collected, for a total of 3 samples. After core vitrectomy and bleb revision, ceftazidime (2.25 mg/0.1 ml) and vancomycin (1 mg/0.1 ml) were injected intravitreally.

Vitreous samples were sent for identification with Gram stain and culture. The remaining samples were prepared for MALDI-TOF MS analysis by centrifuging them at 6000 g for 10 minutes at 4°C to produce 3 separate bacterial pellets. The bacterial pellets were washed with sterile, double-distilled H₂O. The pellets appeared opaque and white, with mucoid consistency (Table 1). Sterile plastic loops were used to apply the bacterial pellets on the spots of the target plate for MALDI-TOF MS (Figure 2, A-C). A colony of *Escherichia coli* (*E coli*) was used as a positive control. Each spot was overlaid with a matrix of α -cyano-4-hydroxycinnamic acid per protocol

Table 1. Vitreous Samples Descriptions and Results From MALDI-TOF MS Analysis.

Sample	Amount Used for MALDI Processing	Description of Fluid	Description of Pellet	Spot on MALDI Target Plate	Result From MALDI-TOF MS (Vitek MS Biotyper)	Confidence Value (%)
Undiluted vitreous sample	1 ml	Cloudy, no obvious blood	White, opaque, mucous consistency	A1	<i>Gemella sanguinis</i>	99.7 ^a
				A2	<i>Gemella sanguinis</i>	99.3 ^a
				A3	No identification	
					<i>Prevotella denticola</i>	15.1
					<i>Gardnerella vaginalis</i>	15.1
Diluted vitreous sample #1	3 ml	Cloudy, no obvious blood	White, opaque, mucous consistency		<i>Mycobacterium flavescens</i>	15
					<i>Lactobacillus delbrueckii</i>	
				A4	No identification	
				B1	No identification	
				B2	No identification	
Diluted vitreous sample #2	2.8 ml	Clear, with some blood	Opaque, bloody, mucous consistency		<i>Listeria grayi</i>	48.3
					<i>Chryseobacterium indologenes</i>	51.6
				B3	No identification	
				B4	No identification	
					<i>Vibrio fluvialis</i>	32.4
					<i>Gemella sanguinis</i>	33.7
					<i>Listeria grayi</i>	33.8
				C1	No identification	
					<i>Gardnerella vaginalis</i>	13.7
					<i>Vibrio fluvialis</i>	13.8
Positive control, <i>Escherichia coli</i>				Positive control spot	<i>Escherichia coli</i>	99.9 ^a

Abbreviation: MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Gemella sanguinis was identified from the undiluted vitreous humor obtained from vitrectomy with >99% confidence value. According to the manufacturer's recommendations, a confidence value between 60% and 99.9% with a single species proposed is considered to be an accurate result. If the resultant peptide fingerprint does not match an organism in the database, the strain is determined to be outside of the scope of the database and the results are classified as "no identification." In some of these cases, a list of low-probability organisms is provided with low confidence values (<60%), which are considered nondiagnostic and not accurate as seen in spots A3, B2, B4, and C1.

^aConfidence values >99%.

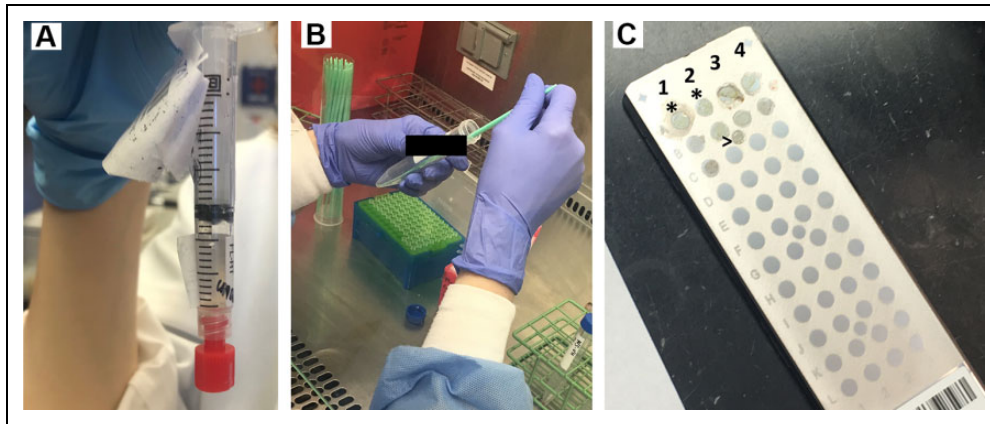


Figure 2. Processing of infected vitreous humor for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. (A) Syringe containing 1 ml of undiluted vitreous sample obtained from vitrectomy that was centrifuged to concentrate bacterial load into a bacterial pellet. (B) A sterile inoculating loop was used to apply a portion of the bacterial pellet formed from centrifugation onto the target plate for MALDI-TOF MS analysis. (C) Target plate shown with vitreous humor pellets applied onto spots (small, round circles). Spots where pathogen identification was achieved with high ($\geq 60\%$) confidence value (asterisks). Spot containing *Escherichia coli*-positive control (arrowhead). Table 1 shows the corresponding spots for each sample and the results.

(Vitek MS, bioMérieux), and the target plate was inserted into the machine.

Results

The pellet from undiluted vitreous was applied onto 4 spots; the first 2 spots had sufficient material for analysis (Table 1). *Gemella sanguinis* was identified on the first 2 spots, with confidence values of 99.7% and 99.3% within 1 hour of sample acquisition. A score of $\geq 60\%$ indicates species-level identification.⁴ The latter 2 spots from undiluted vitreous pellet and the spots from diluted vitreous did not result in an accurate identification (“no identification”). Lists of possible organisms with low confidence values ($< 60\%$), which are considered nondiagnostic, were provided in some cases. The positive control of *E coli* was identified accurately with a confidence value of 99.9% (Table 1).

Gram stains of the tap and vitrectomy samples showed no organisms. There was no bacterial or fungal growth of any of these samples. At the postoperative month 2 visit, the patient’s visual acuity improved to 20/50, intraocular pressure was normal, and the retina was attached on exam (Figure 1B).

Conclusions

MALDI-TOF MS is a promising analytical tool for the characterization of different types of microorganisms, with a gain of time of days compared with traditional methods.^{4,6,8} Although MALDI-TOF MS can identify bacteria, mycobacteria, and fungi within minutes, currently it is used only to analyze organisms from colonies grown in cultures.

Here we describe, for the first time, the successful identification of a pathogen from direct MALDI-TOF MS analysis of a vitreous endophthalmitis sample without prior culture in

a case in which conventional microbiology analysis was negative. MALDI-TOF MS identified *Gemella sanguinis* with high confidence values of 99.7% and 99.3% within 1 hour of sample acquisition. It is very unlikely that the *Gemella sanguinis* from our patient was a contaminant, because MALDI-TOF MS requires at least 10^4 microorganisms for a positive result.⁷ By centrifuging the vitreous samples, we were able to adequately concentrate the bacterial load into a pellet, which was used for analysis, and bypass the need for culture. Results of “no identification” from the diluted vitreous samples were likely due to inadequate bacterial load following intraoperative infusion.

Gemella sanguinis is a gram-positive anaerobe first characterized in 1998.⁹ It has shown sensitivity to vancomycin and cephalosporins, which were used empirically in our case.^{9,10} Though rare, cases of endophthalmitis by *Gemella* species have been reported, and *Gemella* systemic infections are typically associated with immunocompromised status.¹¹ Cases of BRE in the United States are more commonly caused by streptococci, enterococci, and *Haemophilus influenzae*, however, and our case would be the first reported account of BRE by *Gemella sanguinis*.^{2,12}

Conventional cultures take days to provide results.¹¹ Sensitivity rates vary from 22% to 30% for aqueous and 40% to 69% for vitreous samples.¹³ In our case, conventional techniques grew no organisms, likely because of small sample volume, low bacterial load, and the prior administration of antibiotics. *Gemella sanguinis* is an anaerobic species that was relatively recently discovered, possibly indicating it may be a fastidious organism to grow via conventional methods. Although PCR has been shown to improve the diagnostic yield in endophthalmitis, it is costly and relatively time-consuming, and its yield is limited by primer selection for specific microorganisms.¹³

Compared with traditional methods, MALDI-TOF MS can recognize a wider variety of organisms including bacteria, fungi, and mycobacteria within minutes, and the reagents cost

significantly less.^{4,5} Importantly, MALDI-TOF MS was able to identify the pathogen in our case even after the administration of intravitreal and systemic broad-spectrum antibiotics. The laser ionization of samples used to produce the peptide fingerprint is lethal to microorganisms, indicating that MALDI-TOF MS does not require live organisms for identification.

Successful pathogen identification has previously been demonstrated with the direct analysis of cerebrospinal fluid and urine with MALDI-TOF MS without prior culture in cases of meningitis and urinary tract infections, respectively.^{7,14} MALDI-TOF MS has also been used to identify causative pathogens in endophthalmitis samples after they were grown in blood culture bottles.^{4,8} Newer-generation technology like Vitek 2 provides antimicrobial susceptibility test (AST) results faster than conventional methods, and could potentially be used along with MALDI-TOF MS to provide susceptibility information on the identified pathogen.¹⁴ Further preclinical studies should investigate the use of MALDI-TOF MS for in vitro and in vivo models to investigate the effects of inflammatory responses and the minimum sample volume and load of bacteria needed for successful identification without prior culture.

Our report has several limitations. First, the identification of *Gemella sanguinis* was not confirmed with other microbiological methods, and thus the results of this case must be taken with caution. However, it is unlikely that the result of *Gemella sanguinis* was due to contamination because at least 10⁴ microorganisms are needed for positive identification with MALDI-TOF MS, and there was likely no growth of potential bacterial contaminants because our samples were not incubated prior to analysis.⁷

It is important to note that confirmation of the causative organism with existing gold-standard tests such as cultures or PCR with a universal bacterial primer should be mandatory before advising and employing MALDI-TOF MS independently for regular use in clinical practice. Other emerging sequencing techniques may also aid in determining the validity of MALDI-TOF MS. More cases must be studied, and controlled in vitro experiments must also be implemented to establish the value of directly analyzing samples with MALDI-TOF MS.

Although MALDI-TOF MS is unable to determine AST profiles, unpublished preliminary data from our lab suggest that an automated AST system (Vitek 2) may be implemented in parallel to directly analyze intraocular samples and determine both pathogen and AST profiles accurately without culture. Because MALDI-TOF MS requires at least 10⁴ microorganisms for a positive result, the utility of this technique is limited by the yield of intraocular sample obtained and the extent of infection. The centrifugation applied in our case likely concentrated the infectious material, and further studies should determine the optimal processing protocol for accurate results with MALDI-TOF MS.

Rapid and accurate diagnostic approaches for endophthalmitis are crucial, but current diagnostic methods have numerous limitations. Direct analysis of bacterial pellets from vitreous samples with MALDI-TOF MS without prior culture could represent a novel shift in clinical microbiology as a rapid and inexpensive adjuvant technique in combination with standard procedures, at a time when the value of targeted molecular

therapies prevails in the face of increasing antibiotic resistance and its consequent morbidity and costs to health care.^{1,15} As stated, further studies are needed to validate the clinical value and ability of MALDI-TOF MS to provide the diagnostic accuracy needed for effective diagnosis and treatment strategy in ocular and other systemic infections.

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Ethical Approval

The study was conducted according to the tenets of the Declaration of Helsinki and all sensitive data were managed according to HIPAA (Health Insurance Portability and Accountability Act) rules.

Statement of Informed Consent

Written informed consent was obtained from the patient prior to this report.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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