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RESEARCH ARTICLE

Performance of MALDI-TOF Mass Spectrometry for the Identification of the HACEK Group and Other Fastidious Gram-Negative Rods

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Abstract:

Objective:

The aim of this study was to determine the capacity of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) to identify 155 HACEK clinical isolates and other fastidious or infrequently isolated Gram-negative rods (*e.g.*, *Actinobacillus*, *Capnocytophaga, Pasteurella, Neisseria, Moraxella, Dysgonomonas*, among others).

Methods:

All the isolates were identified by standard biochemical tests and MALDI-TOF MS. Two different extraction methods (direct transfer formic acid method on spot and ethanol formic acid extraction method) and different cut-offs for genus/specie level identification were used. MALDI-TOF MS identification was considered correct when the result obtained from the MS database agreed with the phenotypic identification result. When both the methods gave discordant results, the 16S rDNA gene sequencing was considered as the gold standard identification method.

Results:

Employing the score cut-offs suggested by the manufacturer, 93.55% and 69.03% isolates were correctly identified at the genus and species level, respectively. On the contrary , employing lower cut-off scores for identification, 98.06% and 92.09% isolates were properly identified at the genus and species level respectively and no significant differences between the results obtained with two extraction methods were observed .

Conclusion:

The accurate identification of 14 genera showed the reliability of MALDI-TOF MS as an optional methodology to the routine identification methods currently used in laboratories.

Keywords: MALDI-TOF MS, HACEK group, Fastidious Gram-negative rods, Extraction methods, Gene sequencing, Mass spectrometry.

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1. INTRODUCTION

The identification of the HACEK group (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella*) and other fastidious Gram-negative rods (*e.g.*, *Actinobacillus*, *Capnocytophaga, Pasteurella, Neisseria, Moraxella, Dysgonomonas*, among others) by conventional phenotypic methodology is difficult, mainly because of their slow growth and low reactivity in biochemical tests. Their specific growth requirements also make the biochemical identification of these isolates difficult.

It is well known that conventional identification methods for the HACEK group and other fastidious Gram-negative rods are time-consuming, which contribute to the problems in their routine identification in the microbiology laboratory. In addition, identification using molecular methods requires trained staff. Because of these factors, we thought it would be valuable to assess the capacity of matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) Mass Spectrometry (MS) for the identification of HACEK group members and other fastidious or infrequently isolated Gram-negative rods (*e.g.*, *Actinobacillus*, *Capnocytophaga, Pasteurella, Neisseria,*

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Moraxella, Dysgonomonas, among others). In the past few years, this technology has been demonstrated to be very useful in clinical microbiology because it allows for the definitive identification of different bacterial species within a few minutes [1 - 6].

2. OBJECTIVE

The study aimed to evaluate the capacity of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) to identify 155 HACEK clinical isolates and other fastidious or infrequently isolated Gram-negative rods (*e.g.*, *Actinobacillus*, *Capnocytophaga, Pasteurella, Neisseria, Moraxella, Dysgonomonas*, among others).

3. MATERIALS AND METHODS

3.1. Bacterial Isolates

A total of 155 clinical strains from a culture collection assembled from 2010-2015 at the university teaching hospital, Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, Argentina, were used in this study. All of the isolates were recovered from significant clinical samples. Bacterial strains previously conserved at -70°C in stock medium (Brain Heart Infusion Broth(Difco) supplemented with 20% glycerol) were subcultured twice prior to testing on 5% sheep blood agar and were incubated for 24-48 h in a 5% CO_2 atmosphere at 35°C.

3.2. Phenotypic and Molecular Identification

All of the isolates were previously identified by conventional biochemical tests [7, 8]. 16S rRNA gene sequencing was applied to solve any discrepancies between the identification obtained *via* conventional methodology and the identification obtained by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS). 16S rRNA gene sequencing was also applied for definitive species identification in cases in which phenotypic tests were not reliable. This represented a total of 70 isolates, (approximately 45% of the isolates), (*e.g.*, for *Capnocytophaga* spp., *Cardiobacterium hominis, Dysgonomonas* spp., *Moraxella* spp., *Neisseria* spp., *Sutonella indologenes*, *Kingella denitrificans*, and *Leptotrichia trevisani*). PCR products of the 16S rRNA gene were obtained with Taq DNA polymerase based on the manufacturer's specifications (Promega) using the primers described by Weisburg *et al.* [9]. Both DNA strands of the PCR products were sequenced using an ABIPrism 3100 Bioanalyzer at the Macrogen Inc. sequencing facility in South Korea. The sequences were analyzed by BLAST and compared using the GenBank database.

3.3. MALDI-TOF Mass Spectrometry

The clinical strains were subcultured on Columbia agar with 5% sheep blood (Laboratorios Britania, Argentina) at 37°C with 5% CO_2 for 24 to 48 h for MALDI-TOF MS evaluation.

The study was performed using a Bruker Daltonics Microflex LT instrument with Biotyper software 3.1 (Bruker Daltonics, Bremen, Germany).

3.4. Sample Preparation for MALDI-TOF MS

Two extraction methods were used to prepare the bacteria for identification by MALDI-TOF MS: direct transfer formic acid method on spot and ethanol formic acid extraction method) as described by Schulthess B *et al*. [10]. MALDI-TOF MS analysis was conducted according to the specifications described in previous reports [10, 11].

3.5. MALDI-TOF MS Data Interpretation

We investigated percent identification using two different cut-offs for the genus-level identification results. The cutoffs proposed by the manufacturer were used to establish species-level identification (≥ 2.000), genus level identification (1.7 to 1.999), and unreliable results (<1.700). Also, based on previous reports [1, 4, 12] and on our own results [11, 13], lower cutoff scores were employed for identification: ≥ 1.5 for the genus level and ≥ 1.7 for the species-level; A score < 1.5 was considered as unreliable. Additionally, a 10% difference bet-ween the first two species having the best coincidences in the database was mandatory for species identification. If this condition was not fulfilled, the identification was considered correct only at the genus level [12]. MALDI-TOF identification was considered correct when the result obtained from the MS database was in agreement with identification results obtained by conventional biochemical tests.

When both the methods gave discordant results, the 16S rDNA sequencing as the gold standard identification method was carried out. The MS results concordant with 16S rDNA sequencing identification were considered correct. MS results discordant with 16S rDNA identification were considered incorrect.

3.6. Data Analysis

The population parameters for both extraction methods were compared using the z test [14].

4. RESULTS AND DISCUSSION

Using the direct extraction formic acid method and lower cutoff scores for identification, 152 isolates (98.06%) were correctly identified to the genus level and 144 isolates (92.90%) were correctly identified to the species level by MALDI-TOF MS (Table **1**). Using the ethanol-formic acid-acetonitrile extraction method, 148 isolates (95.48%) were correctly identified to the genus level and 145 isolates (93.55%) to the species level. A comparison of both the extraction methods showed no significant differences between the results at confidence levels of 95% and 99% (Table **S1**).

As the genus level identification score decreased from ≥ 1.7 to ≥ 1.5, the correct genus-level identification percentage increased from 93.55% to 98.06%. The *Cardiobacterium hominis* and *Neisseria canis* isolates were correctly identified using the lower score. Decreasing the species-level score identification (≥ 2.0 to ≥ 1.7) also improved the identification to the species level from 69.03% to 92.90% for *Aggregatibacter aphrophilus*, *Haemophilus parainfluenzae*, *Kingella denitrificans, Moraxella lacunata*, *Moraxella osloensis* and *Neisseria weaveri*. However, one *Dysgonomonas capnocytophagoides* isolate was incorrectly identified as *D. gadei*

using the lower score (Table **1**).

To our knowledge, this is the first work that evaluates the use of MALDI-TOF MS for the identification of HACEK group members in conjunction with other nutritionally demanding or infrequently isolated Gram-negative rods, such as *Capnocytophaga*, *Dysgonomonas* spp., *Pasteurella* spp., *Leptotrichia trevisanii*, *Moraxella* spp. and saprophytic species of *Neisseria*. Our work is notable because we included 14 genera and 32 species. Powell *et al.* [15], who studied 140 HACEK group clinical isolates (*i.e.*, *Aggregatibacter*, *Haemophilus*, *Eikenella* and *Kingella*), reported the excellent efficiency of MALDI-TOF MS for the identification of these microorganisms. However, the misidentification of *A. aphrophilus* as *Aggregatibacter segnis* or as *H. parainfluenzae* was reported by these authors. Conversely, in our study, *A. aphrophilus* was rightly identified, but it should be noted that different equipment was used in our study; the results reported by Powell *et al.* were obtained using a bioMérieux Vitek MALDI-TOF MS (bioMérieux, Durham, NC), and the spectra were compared against the Vitek MS SARAMIS database for Research Use Only of (RUO) version 4.09 [15].

Table 1. Agreement of MALDI-TOF MS with standard biochemical or molecular identification for HACEK and related genera using different identification cutoff scores.

Conventional or Molecular Identification Method	No. of Isolates	Genus ID[a] (Score ≥ 1.5)	Species ID (Score ≥ 1.7)	No. ID[b] (Score < 1.5)	Erroneous ID	Genus ID (Score ≥ 1.7)	Species ID (Score ≥ 2.0)	No. ID (Score < 1.7)
Actinobacillus ureae	5	5	5	–	–	5	5	–
Aggregatibacter actinomycetemcomitans	4	4	4	–	–	4	4	–
Aggregatibacter aphrophilus	10	10	9	–	–	9	6	1
Aggregatibacter segnis	2	2	2	–	–	2	2	–
Capnocytophaga gingivalis	2	2	2	–	–	2	1	–
Capnocytophaga granulosa	1	1	1	–	–	1	1	–
Capnocytophaga ochracea	4	4	4	–	–	4	4	–
Capnocytophaga sputigena	15	15	15	–	–	15	14	–
Cardiobacterium hominis	2	2	0	–	–	0	0	2
Dysgonomonas capnocytophagoides	1	–	–	–	1	1	0	–
Dysgonomonas gadei	1	–	–	1	–	–	–	1
Eikenella corrodens	16	16	15	–	–	15	14	1
Haemophilus influenzae	11	11	11	–	–	11	10	–
Haemophilus parahaemolyticus	1	1	1	–	–	1	1	–
Haemophilus parainfluenzae	12	12	12	–	–	12	9	–
Kingella denitrificans	13	13	13	–	–	1	–	
Kingella kingae	4	4	4	–	–	4	4	–
Leptotrichia trevisanii	2	2	2	–	–	2	2	–
Mannheimia haemolytica	1	1	1	–	–	1	1	–
Moraxella lacunata	6	6	6	–	–	6	0	–
Moraxella nonliquefaciens	5	5	5	–	–	5	5	–
Moraxella osloensis	11	11	9	–	–	9	3	2
Neisseria bacilliformis	1	1	1	–	–	1	0	–
Neisseria elongata spp. *glycolytica*	1	–	–	1	–	–	–	1
Neisseria canis	2	2	0	–	–	–	–	2
Neisseria weaveri	1	1	1	–	–	1	0	–
Pasteurella canis	6	6	6	–	–	6	6	–
Pasteurella dagmatis	2	2	2	–	–	2	1	–
Pasteurella multocida	10	10	10	–	–	10	10	–
Pasteurella pneumotrophica	1	1	1	–	–	1	1	–
Pasteurella stomatis	1	1	1	–	–	1	1	–
Sutonella indologenes	1	1	1	–	–	1	1	–
Total n (%)	155	152 (98.06)	144 (92.90)	2 (1.29)	1 (0.64)	145(93.55)	107 (69.03)	10 (6.45)

[a]ID: identification; [b]NO ID: not reliable identification

Couturier *et al.* [16] also assessed the identification of 103 HACEK isolates and 20 *H. influenzae* clinical isolates by MALDI TOF MS. According to our results, *H. parainfluenzae*, *A. actinomycetemcomitans*, *E. corrodens*, *K. kingae*, and *H. influenzae* were correctly identified, but various isolates of *C. hominis* gave lower scores. In addition, and contrary to our findings, the misidentification of *A. aphrophilus* as *H. influenzae* was noted by these authors.

For the genus *Capnocytophaga*, MALDI-TOF MS identification reported in the literature has been limited only to the species *C. canimorsus* and *C. cynodegmi* [17, 18]. Our work demonstrated that MALDI- TOF MS accurately identified 100% of *Capnocytophaga* spp. isolates of human origin (*i.e.*, *C. sputigena*, *C. gingivalis*, *C. ochracea* and *C. granulosa*) at the species level.

The utility of MALDI-TOF to identify *Pasteurella* species has been noted by other authors [19, 20]. In particular, the identification of *P. dagmatis* is challenging in the microbiology laboratory because this species is wrongly identified as *P. pneumotropica* by the automated VITEK 2 system. In addition, 16S rRNA gene sequencing does not discriminate between *P. dagmatis* and *P. stomatis,* as the two species share more than 99% similarity. Zangenah *et al.* [19] evaluated four methods for the identification of species of *Pasteurella*: VITEK 2, VITEK MS (BioMerieux), and Bruker Biotyper MS (Bruker), along with the traditional biochemical tests. Their results indicated that while Vitek 2 system identified only 48.5% of the *Pasteurella* isolates, traditional biochemical tests accurately identified 94% of the isolates. Additionally, although both Bruker and Vitek MALDI-TOF MS identified 89% of isolates, the average time required for identification with conventional phenotypic tests was 20 hours, in contrast to 10 minutes required by MALDI-TOF MS. These findings clearly showed that MALDI-TOF MS is a valuable diagnostic tool in the clinical laboratory [19]. All of the different species of *Pasteurella* included in our study were accurately identified (Table **1**).

The identification of asaccharolytic and microscopic bacillary species of *Neisseria* and the differentiation of *Moraxella* spp. are very difficult. For *Neisseria* species of animal origin, while *N. weaveri* was accurately identified at the species level, *N. canis* was correctly identified only at the genus level by MALDI-TOF MS. However, it is known that the identification of these organisms should be polyphasic – more than one method should be required for reliable identification. In fact, the two *N. canis* isolates included in our study were previously identified using two methodologies: conventional phenotypic tests and 16S rRNA gene sequencing. This methodology did not discriminate between *N. canis* and *N. animaloris* since these species share more than 99% identity in 16S rRNA [21, 22], which indicated that more than one method is required for the correct identification. While *Neisseria* species of human source that show bacillary morphology on Gram stains, such as *N. bacilliformis* and *N. elongata* subsp. *glycolytica*, the former was accurately iden-tified, an unreliable identification was obtained for *N. elongata* subsp. *glycolytica*. This discrepancy could be explained because the spectrum for this species was not included in MALDI Biotyper library version 3.0. As noted by other authors, *N. bacilliformis* and *N. elongata* can be reliably distinguished by their 16S rRNA gene sequences or cellular fatty acid profiles, but their discrimination may be complex using conventional biochemical tests [23]. Likewise, the particular characteristics of *N. bacilliformis* (bacillary morphology and inability to metabolize carbohydrates) may also lead to an erroneous identification as *Moraxella* spp, [23]. However, MALDI-TOF MS correctly identified both *Moraxella lacunata*, *M. nonliquefaciens* and 9 of 11 *M. osloensis* isolates to the species level, along with *N. bacilliformis* (Table **1**).

There are no publications in the literature that describe the capacity of MALDI-TOF MS to identify *Dysgonomonas* spp. We included *D. gadei* and *D. capnocytophagoides* in this work. Using the manufacturer's proposed cutoff scores, only *D. capnocytophagoides* was correctly identified at the genus level, but it was incorrectly identified as *D. gadei* when the cutoff score decreased because *D. capnocytophagoides* was not included in the MALDI Biotyper library. However, an unreliable identification score (1.393) was obtained for *D. gadei*, regardless of the cutoff used.

The only two *Leptotrichia trevisanii* isolates included in our study were properly identified at the species level with a score of more than 2.0, and their identification was confirmed by 16S rRNA gene sequencing. The correct identification of *Leptotrichia* spp. has been reported by other authors [24].

CONCLUSION

The results obtained in the present study showed that MALDI-TOF MS is a reliable tool for the identification of members of the HACEK group and other nutritionally demanding or infrequently isolated Gram-negative rods. Our results allowed us to conclude that the identification rate was not improved using the ethanol-formic acid extraction method instead of the direct transferformic acid technique. Moreover, we observed that decreasing the cutoff scores for species and genus-level identification enhanced the performance of MALDI-TOF MS. The percentage of unreliable or erroneous identifications could be lowered by expanding the MALDI-TOF MS database by incorporating spectra for new species and increasing the number of spectra for uncommon species.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data used for the analysis of the current study will be provided by the corresponding author on request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared None.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers website along with the published article.

REFERENCES

[1] Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J Clin Microbiol 2010; 48(5): 1549-54. [http://dx.doi.org/10.1128/JCM.01794-09]. [PMID: 20220166].
[http://dx.doi.org/10.1128/JCM.01794-09] [PMID: 20220166]

[2] van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 2010; 48(3): 900-7. [http://dx.doi.org /10.1128/JCM.02071-09]. [PMID: 20053859].
[http://dx.doi.org/10.1128/JCM.02071-09] [PMID: 20053859]

[3] Bizzini A, Jaton K, Romo D, Bille J, Prod'hom G, Greub G. Matrix-assisted laser desorption ionization-time of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficult-to-identify bacterial strains. J Clin Microbiol 2011; 49(2): 693-6. [http://dx.doi.org/10.1128/JCM.01463-10]. [PMID: 21106794].
[http://dx.doi.org/10.1128/JCM.01463-10] [PMID: 21106794]

[4] Cherkaoui A, Hibbs J, Emonet S, *et al.* Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J Clin Microbiol 2010; 48(4): 1169-75. [http://dx.doi.org/10.1128/JCM.01881-09]. [PMID: 20164271].
[http://dx.doi.org/10.1128/JCM.01881-09] [PMID: 20164271]

[5] Neville SA, Lecordier A, Ziochos H, *et al.* Utility of matrix-assisted laser desorption ionization-time of flight mass spectrometry following introduction for routine laboratory bacterial identification. J Clin Microbiol 2011; 49(8): 2980-4. [http://dx.doi.org/ 10.1128/JCM.00431-11]. [PMID: 21632894].
[http://dx.doi.org/10.1128/JCM.00431-11] [PMID: 21632894]

[6] Sogawa K, Watanabe M, Sato K, *et al.* Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms. Anal Bioanal Chem 2011; 400(7): 1905-11. [http://dx.doi.org/ 10.1007/s00216-011-4877-7]. [PMID: 21442367].
[http://dx.doi.org/10.1007/s00216-011-4877-7] [PMID: 21442367]

[7] Zbinden R. *Aggregatibacter, Capnocytophaga, Eikenella, Kingella, Pasteurella*, and Other Fastidious or Rarely Encountered Gram-Negative Rods.Manual of Clinical Microbiology. 11th ed. Washington, DC: ASM Press 2015; pp. 652-66.
[http://dx.doi.org/10.1128/9781555817381.ch35]

[8] Ledeboer NA, Doern GV. Haemophilus.Manual of Clinical Microbiology. 11th ed. Washington, DC: ASM Press 2015; pp. 667-84.
[http://dx.doi.org/10.1128/9781555817381.ch36]

[9] Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 1991; 173(2): 697-703. [http://dx.doi.org/10.1128/jb.173.2.697-703.1991]. [PMID: 1987160].
[http://dx.doi.org/10.1128/jb.173.2.697-703.1991] [PMID: 1987160]

[10] Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Böttger EC, Hombach M. Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. J Clin Microbiol 2013; 51(6): 1834-40. [http://dx.doi.org/ 10.1128/JCM.02654-12]. [PMID: 23554198].
[http://dx.doi.org/10.1128/JCM.02654-12] [PMID: 23554198]

[11] Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) as a reliable tool to identify species of catalase-negative gram-positive Cocci not belonging to the *streptococcus* Genus. Open Microbiol J 2016; 10: 202-8. [http://dx.doi.org/10.2174/1874285801610010202]. [PMID: 28217192].
[http://dx.doi.org/10.2174/1874285801610010202] [PMID: 28217192]

[12] Alatoom AA, Cazanave CJ, Cunningham SA, Ihde SM, Patel R. Identification of non-*diphtheriae corynebacterium* by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012; 50(1): 160-3. [http://dx.doi.org/ 10.1128/JCM.05889-11]. [PMID: 22075579].
[http://dx.doi.org/10.1128/JCM.05889-11] [PMID: 22075579]

[13] Barberis C, Almuzara M, Join-Lambert O, Ramírez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014; 9(9)e106303 [http://dx.doi.org/ 10.1371/journal.pone.0106303]. [PMID: 25184254].
[http://dx.doi.org/10.1371/journal.pone.0106303] [PMID: 25184254]

[14] Sprinthall RC, Ed. Basic statistical analysis. 9th ed. Upper Saddle River, NJ: Pearson Education 2011.

[15] Powell EA, Blecker-Shelly D, Montgomery S, Mortensen JE. Application of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of the fastidious pediatric pathogens *Aggregatibacter, Eikenella, Haemophilus*, and *Kingella.* J Clin Microbiol 2013; 51(11): 3862-4. [http://dx.doi.org/ 10.1128/JCM.02233-13]. [PMID: 23966506].
[http://dx.doi.org/10.1128/JCM.02233-13] [PMID: 23966506]

[16] Couturier MR, Mehinovic E, Croft AC, Fisher MA. Identification of HACEK clinical isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2011; 49(3): 1104-6. [http://dx.doi.org/ 10.1128/JCM.01777-10]. [PMID: 21227988].
[http://dx.doi.org/10.1128/JCM.01777-10] [PMID: 21227988]

[17] Zangenah S, Özenci V, Boräng S, Bergman P. Identification of blood and wound isolates of *C. canimorsus* and *C. cynodegmi* using VITEK2 and MALDI-TOF. Eur J Clin Microbiol Infect Dis 2012; 31(10): 2631-7.
[http://dx.doi.org/10.1007/s10096-012-1606-x] [PMID: 22454088]

[18] Sotiriou A, Sventzouri S, Nepka M, Magira EE. Acute generalized livedo racemosa caused by *Capnocytophaga canimorsus* identified by MALDI-TOF MS. Int J Infect Dis 2015; 33(4): 196-8.
[http://dx.doi.org/10.1016/j.ijid.2015.02.008] [PMID: 25677725]

[19] Zangenah S, Güleryüz G, Boräng S, Ullberg M, Bergman P, Özenci V. Identification of clinical *Pasteurella* isolates by MALDI-TOF - a comparison with VITEK 2 and conventional microbiological methods. Diagn Microbiol Infect Dis 2013; 77(2): 96-8.
[http://dx.doi.org/10.1016/j.diagmicrobio.2013.06.024] [PMID: 23886788]

[20] Xiong J, Krajden S, Kus JV, *et al.* Bacteremia due to *Pasteurella dagmatis* acquired from a dog bite, with a review of systemic infections and challenges in laboratory identification. Can J Infect Dis Med Microbiol 2015; 26(5): 273-6.
[http://dx.doi.org/10.1155/2015/946812] [PMID: 26600817]

[21] Wolfgang WJ, Carpenter AN, Cole JA, *et al. Neisseria wadsworthii* sp. nov. and *Neisseria shayeganii* sp. nov., isolated from clinical specimens. Int J Syst Evol Microbiol 2011; 61(Pt 1): 91-8.
[http://dx.doi.org/10.1099/ijs.0.022426-0] [PMID: 20173010]

[22] Vandamme P, Holmes B, Bercovier H, Coenye T. Classification of centers for disease control group eugonic fermenter (EF)-4a and EF-4b as *neisseria animaloris* sp. nov. and *neisseria zoodegmatis* sp. nov., respectively. Int J Syst Evol Microbiol 2006; 56(Pt 8): 1801-5. [http://dx.doi.org/10.1099/ijs.0.64142-0] [PMID: 16902011]

[23] Han XY, Hong T, Falsen E. *Neisseria bacilliformis* sp. nov. isolated from human infections. J Clin Microbiol 2006; 44(2): 474-9. [http://dx.doi.org/10.1128/JCM.44.2.474-479.2006] [PMID: 16455901]

[24] Smid MC, Dotters-Katz SK, Plongla R, Boggess KA. *Leptotrichia buccalis*: a novel cause of Chorioamnionitis. Infect Dis Rep 2015; 7(2): 5801. [http://dx.doi.org/10.4081/idr.2015.5801] [PMID: 26294950]