

# Identification of Pasteurellaceae by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

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## Objectives

The taxonomic systematic of Pasteurellaceae is a difficult topic. Some species has been reclassified from the genus *Haemophilus* to *Aggregatibacter* (e.g. *Aggregatibacter aphrophilus* and *paraaphrophilus*). Other species left the genus *Pasteurella* and new genus were defined (e.g. *Avibacterium* or *Gallibacterium*). In daily routine in the microbiology lab many of them are difficult to identify in commercially available systems using biochemical parameters. Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry is a rapid and accurate tool for the identification of many microorganisms. We assessed this technology for more than 30 Pasteurellaceae species from the Medical Culture Collection Marburg (MCCM). In addition we determined the presence of  $\beta$ -lactamases in the strains, an important marker for effective treatment. recommendations.

## Materials and Methods

Pasteurellaceae strains were grown on blood or chocolate agar under aerobic or microaerophilic conditions between 24 to 72 hours. Identification with mass spectrometry was done by smear preparations (direct inoculation of the target from the plate) and after a protein extraction protocol. The results were compared to the MCCM database and all identifications were confirmed by 16S-rDNA sequencing. In addition the presence of  $\beta$ -lactamases was determined by MALDI-TOF using a modification of our recently described protocol for carbapenemase detection (Burckhardt et al., JCM 2011).

## Results

No	Strain	MCCM	Sec	MS result (db-2010)	DNA Sequencing	MS result 2 <sup>1</sup> (db-2012)
2	<i>Pasteurella stomatis</i>	0104	17	<i>Pasteurella multocida</i> (Genus)	ndb	<i>Pasteurella stomatis</i>
3	<i>Pasteurella pneumotropica</i>	3303	18	<i>Haemophilus influenzae</i> (Genus)	ndb	<i>Pasteurella pneumotropica</i>
4	<i>Pasteurella pneumotropica</i>	3409	18	<i>Haemophilus influenzae</i>	ndb	<i>Pasteurella pneumotropica</i>
5	<i>Pasteurella multocida septica</i>	0378	23	<i>Pasteurella multocida</i>	ndb	<i>Pasteurella multocida</i>
6	<i>Pasteurella multocida</i>	3024	22	<i>Pasteurella multocida</i>	ndb	<i>Pasteurella multocida</i>
7	<i>Pasteurella multocida gallicida</i>	3280	24	<i>Pasteurella multocida</i>	ndb	<i>Pasteurella multocida</i>
9	<i>Avibacterium gallinarum</i>	0033	18	<i>Avibacterium gallinarum</i>	ndb	<i>Avibacterium gallinarum</i>
10	<i>Avibacterium gallinarum</i>	0003 <sup>1</sup>	15	<i>Avibacterium gallinarum</i>	ndb	<i>Avibacterium gallinarum</i>
11	<i>Pasteurella dagmatis</i>	3139	20	<i>Pasteurella multocida</i> (Genus)	ndb	<i>Pasteurella pneumotropica</i>
12	<i>Pasteurella dagmatis</i>	3191	16	<i>Pasteurella multocida</i>	ndb	<i>Pasteurella dagmatis</i>
13	<i>Pasteurella canis</i>	0045 <sup>1</sup>	15	<i>Pasteurella multocida</i>	ndb	<i>Pasteurella canis</i>
14	<i>Pasteurella canis</i>	3430	13	<i>Pasteurella multocida</i>	ndb	<i>Pasteurella canis</i>
15	<i>Pasteurella bettyae</i>	0450 <sup>1</sup>	16	<i>Pasteurella multocida</i> (Genus)	ndb	<i>Pasteurella bettyae</i>
17	<i>Pasteurella avium</i>	0272 <sup>1</sup>	23	<i>Pasteurella avium</i>	ndb	<i>Pasteurella avium</i>
18	<i>Pasteurella anatis</i>	0140 <sup>1</sup>	21	<i>Pasteurella anatis</i>	ndb	<i>Pasteurella anatis</i>
19	<i>Pasteurella anatis</i>	0141	22	<i>Pasteurella anatis</i>	ndb	<i>Pasteurella anatis</i>
22	<i>Mannheimia varigena</i>	2528	20	<i>Mannheimia haemolytica</i>	ndb	<i>M. glucosida/M. haemolytica</i>
23	<i>Mannheimia glucosida</i>	0675	21	<i>Mannheimia haemolytica</i>	ndb	<i>Mannheimia glucosida</i>
24	<i>Mannheimia glucosida</i>	0388	18	<i>Mannheimia haemolytica</i>	ndb	<i>Mannheimia glucosida</i>
25	<i>Histophilus ovis</i>	0338	20	<i>Histophilus somni</i>	ndb	<i>Histophilus somni</i>
26	<i>Histophilus ovis</i>	1381	15	<i>Mannheimia glucosida</i>	ndb	<i>Mannheimia glucosida</i>
27	<i>Haemophilus parainfluenzae</i>	3215	23	<i>Haemophilus parainfluenzae</i>	ndb	<i>Haemophilus parainfluenzae</i>
28	<i>Haemophilus parainfluenzae</i>	2641	24	<i>Haemophilus parainfluenzae</i>	ndb	<i>Haemophilus parainfluenzae</i>
29	<i>Haemophilus parahaeolyticus</i>	3212	21	<i>Haemophilus parainfluenzae</i>	ndb	<i>Haemophilus parainfluenzae</i>
30	<i>Haemophilus influenzae</i>	0617	24	<i>Haemophilus influenzae</i>	ndb	<i>Haemophilus influenzae</i>
31	<i>Haemophilus haemolyticus</i>	0352	19	<i>Haemophilus influenzae</i>	ndb	<i>Haemophilus sp.</i>
32	<i>Haemophilus haemolyticus</i>	2169	20	<i>Haemophilus influenzae</i>	ndb	<i>Haemophilus haemolyticus</i>
33	<i>Haemophilus haemoglobinophilus</i>	0723 <sup>1</sup>	15	<i>Haemophilus influenzae</i>	ndb	<i>Haemophilus haemoglobinophilus</i>
34	<i>Haemophilus felis</i>	2738	15	<i>Pasteurella multocida</i>	ndb	<i>Haemophilus felis</i>
35	<i>Haemophilus felis</i>	3143	18	<i>Pasteurella multocida</i>	ndb	<i>Haemophilus felis</i>
40	<i>Actinobacillus muris</i>	821	13	<i>Haemophilus influenzae</i>	ndb	<i>Actinobacillus muris</i>
41	<i>Actinobacillus lignieresii</i>	2712	21	<i>Aggregatibacter aphrophilus</i>	ndb	<i>Aggregatibacter aphrophilus</i>
43	<i>Aggregatibacter actinomycetemcomitans</i>	0135	23	<i>Aggregatibacter actinomycetemcomitans</i>	ndb	<i>Aggregatibacter actinomycetemcomitans</i>

Table1: Identification of different Pasteurellaceae strains by MALDI-TOF MS  
27 of 33 strains were identified on species level (4 on genus level, 2 unsolved).  
[MCCM: Medical Culture Collection Marburg; ndb: not in database]

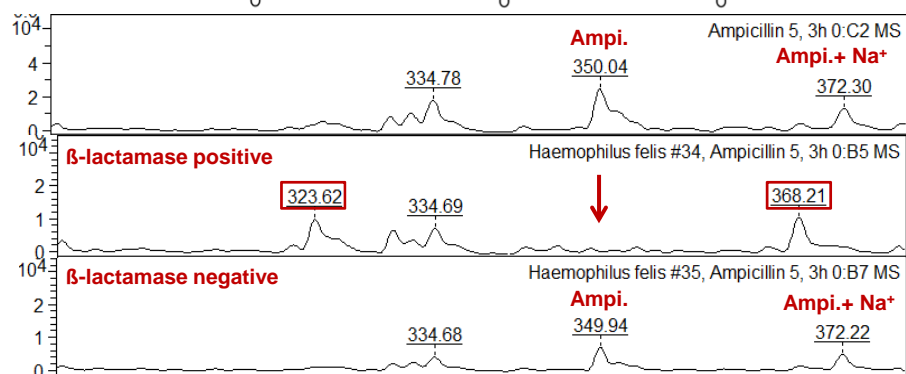
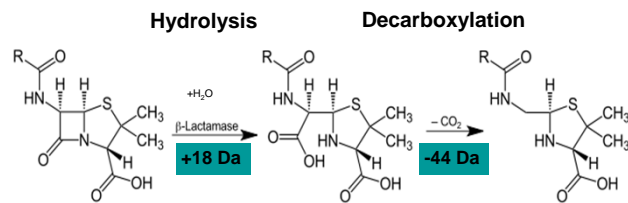


Figure1:  $\beta$ -lactamase detection by MALDI-TOF MS

Ampicillin (350 Da) is hydrolyzed (368 Da) and partially decarboxylated (324 Da) by the enzyme.

## Conclusions

1. MALDI-TOF is a fast and reliable method to identify Pasteurellaceae in daily lab routine.
2. The presence of  $\beta$ -lactamases can be investigated on the mass spectrometer.