Emayella augustorita, New Member of Pasteurellaceae, Isolated from Blood Cultures of Septic Patient

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We report discovery of a new bacterial genus and species of the family Pasteurellaceae by using phylogenetic and metabolic analysis. The bacterium, *Emayella augustorita*, was isolated from blood cultures of a patient in France diagnosed with an adenocarcinoma of the intestines and who was treated with a biliary prosthesis placement.

Pasteurellaceae bacteria have been identified in many vertebrates as members of the microbiota but can occasionally cause human infections (*1*). Currently, the family Pasteurellaceae contains 34 genera and 105 species (https://pasteurellaceae.eu). Few acquired antibiotic resistances are reported (occasionally β-lactamases, macrolides, tetracyclines, or fluoroquinolones), but recently some strains were reported to have multidrug resistance profiles (*2*–*4*). We describe a new bacterium belonging to the Pasteurellaceae family isolated from positive blood cultures of a septic patient.

A 74-year-old woman was admitted January 2022 to the emergency department at the Limoges teaching hospital in France. She reported complaints of an occlusive syndrome with nausea, vomiting, and fever. She was previously diagnosed in 2015 with an adenocarcinoma of the small intestine with metastases in the lungs and liver with biliary compression. After multiple chemotherapies and surgeries, she was diagnosed with severe bacteremia of digestive origin (Figure). A metallic biliary prosthesis through a biliary drain was placed in December 2021. No documentation of any contact with farm animals or pets was reported.

We conducted an abdominal computed tomography scan that detected a hepatic lesion with a heterogeneous hypodense area of the tip of the VI segment of the liver on the path of the biliary drain, suggesting a biloma. Laboratory results showed evidence of possible infection with a total leukocyte count of 24.4 × 10^9 leukocytes/L (reference range 3.78–9.42 × 10^9 leukocytes/L) and a C-reactive protein result of 150 mg/L (reference range <5 mg/L). Two blood cultures were collected, and we initiated empiric antibiotic therapy with ceftriaxone-metronidazole (Figure). Four blood culture bottles, aerobic and anaerobic incubation detected growth after 16 hours of incubation. A Gram stain of the positive bottles showed a short gram-negative rod (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/30/8/23-1651-App1.pdf). Small, bright colonies grew in a 5% CO_2 atmosphere on blood agar and PolyVitex plates (bioMérieux, https://www.biomerieux.com) after 24 hours of incubation at 35°C (Appendix Figure 1). We attempted bacterial identification by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (bioMérieux) and did not match any known bacterial species. We determined MICs by using E-tests (bioMérieux) on Mueller Hinton agar with 5% horse blood (bioMérieux) for amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, levofloxacin, and trimethoprim/sulfamethoxazole, according to pharmacokinetic-pharmacodynamic EUCAST breakpoints

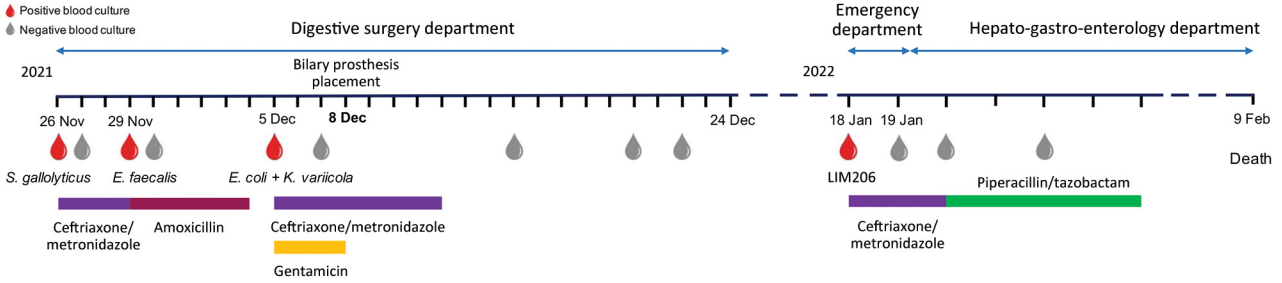

Figure. Timeline of main events for case report on the isolation of *Emayella augustorita*, a novel bacterium of the *Pasteurellaceae* family recovered from a patient with sepsis, France. *E. coli*, *Escherichia coli*; *E. faecalis*, *Enterococcus faecalis*; *K. variicola*, *Klebsiella variicola*; *S. gallolyticus*, *Streptococcus gallolyticus*.

Table. MIC values observed for LIM206, *Emayella augustorita*, a novel bacteria of the Pasteurellaceae family recovered from a patient with sepsis, France*

Antibiotic	PK/PD breakpoints, mg/L†	MIC, mg/L
Amoxicillin	2–8	0.75
Amoxicillin + clavulanic acid	2–8	0.75
Piperacillin + tazobactam	8–16	1.0
Cefotaxime	1–2	0.032
Levofloxacin	0.5–1	0.064
Trimethoprim/sulfamethoxazole	0.5	0.004

*PK/PD, pharmacokinetics/pharmacodynamics.
†All breakpoints were from EUCAST except for trimethoprim/sulfamethoxazole, which was epidemiologic cutoff value.

(https://www.eucast.org). No phenotypic resistance was detected (Table). The patient's successive blood cultures became negative, but her general status worsened, leading to death in February 2022 (Figure).

Sanger sequencing of the whole 16S rRNA gene (Genbank accession no. OR046993) did not show sufficient identification (94.78% similarity with *Pasteurella oralis*). A 16S phylogenetic tree confirmed the result but also emphasized the wrong affiliation of genera in this family (Appendix Figure 2). Because of the low identity percentage (<97%), we conducted whole-genome sequencing by using the Ion GeneStudio S5 Plus platform (ThermoFisher Scientific, https://www.fishersci.com), as previously described (*5*). The genome size was 2.68 Mbp, and the total DNA guanine and cytosine mol% content was 45.3 mol%. Annotation identified 2462 coding sequence, 45 tRNA, and 6 rRNA. No resistance genes were detected (Appendix). We identified the type strain as LIM206 (Genbank accession no. JAWHQP010000000).

A phylogenetic tree based on single nucleotide polymorphisms comparison between whole genomes of different Pasteurellaceae species showed LIM206 was placed on a separate branch from all other genera. The average nucleotide identity between LIM206 and the closest members that shared a phylogenetic branch was 73.48% with *Actinobacillus succinogenes*, 74.09% with *Basfia succiniciproducens*, 72.13% with *Lonepinella koalarum*, 73.20% with *Mesocricetibacter intestinalis*, and 72.13% with *Pasteurella bettyae* (Appendix Figure 3). Results of those combined analyses suggested LIM206 belonged to a new species and a new genus of Pasteurellaceae. We conducted multilocus sequence analysis on 16S rRNA, *infB*, *recN*, *rpoA*, and *rpoB* genes according to previous recommendations (*6*,*7*) (Appendix). LIM206 was separated from existing genera of Pasteurellaceae and closely linked to *A. succinogenes* and *B. succiniciproducens* (Appendix Figure 4). To confirm the new genus, we conducted amino acid identity analysis and found a maximum amino acid identity value of 77.67% with *Basfia succiniciproducens* (Appendix Figure 5). This identity value was considered below the genus identity threshold of ≈83% compared to other Pasteurellaceae genera.

We compared the biochemical characteristics of LIM206 to those of different species of the Pasteurellaceae family (Appendix Table 1). We detected the presence of urease activity, the acidification of L-arabinose and D-xylose, and the absence of acidification of D-mannitol and D-trehalose, which are not frequently observed in Pasteurellaceae. Those characteristics are absent in the genetically closest species (*8*,*9*).

In conclusion, we report the description of a new genus and species of the Pasteurellaceae family found in blood cultures of a septic patient in France followed for metastatic adenocarcinoma of the intestines. We derived the genus name *Emayella* from the word enamel; the species name *augustorita* is in reference to the Roman name of Limoges. The bacterium is a short gram-negative coccoid to rod shape. It is catalase negative, oxidase positive, nonmotile, fermentative, capnophilic, and nonhemolytic. The bacterium does not require β-nicotinamide adenine dinucleotide or hemin-factors for growth.

About the Author

Dr. Meyer is a clinical microbiologist at the Limoges University Hospital Center, France. His research focuses on molecular bacteriology, clinical metagenomics, and antibiotic resistance.

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Persistence of Influenza H5N1 and H1N1 Viruses in Unpasteurized Milk on Milking Unit Surfaces

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Examining the persistence of highly pathogenic avian influenza A(H5N1) from cattle and human influenza A(H1N1)pdm09 pandemic viruses in unpasteurized milk revealed that both remain infectious on milking equipment materials for several hours. Those findings highlight the risk for H5N1 virus transmission to humans from contaminated surfaces during the milking process.

Highly pathogenic avian influenza A(H5N1) virus was detected in US domestic dairy cattle in late March 2024, after which it spread to herds across multiple states and resulted in at least 3 confirmed human infections (*1*). Assessment of milk from infected dairy cows indicated that unpasteurized milk contained high levels of infectious influenza virus (*2*; L.C. Caserta et al., unpub. data, https://doi.org/10.1101/2024.05.22.595317). Exposure of dairy farm workers to contaminated unpasteurized milk during the milking process could lead to increased human H5 virus infections. Such infections could enable H5 viruses to adapt through viral evolution within humans and gain the capability for human-to-human transmission.

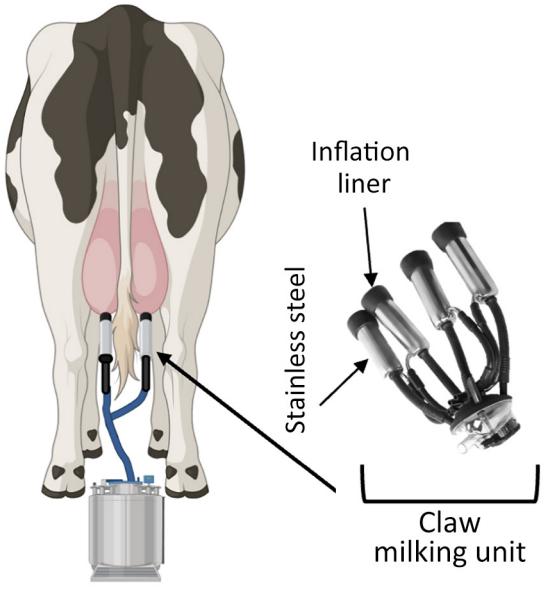

Figure 1. Illustration of milking unit surfaces tested in a study of persistence of influenza H5N1 and H1N1 viruses in unpasteurized milk. Before attaching the milking unit (claw), a dairy worker disinfects the teat ends, performs forestripping of each teat to detect abnormal milk, and then wipes each teat with a clean dry towel. Workers then attach the milking unit to the cow teats. A pulsation system opens and closes the rubber inflation liner (at left) around the teat to massage it, mimicking a human stripping action. A vacuum pump is controlled by a variable speed drive and adjusts the suction to allow milk to flow down a pipeline away from the cow into a bulk tank or directly onto a truck. Additional sources of exposure to humans include handling of raw unpasteurized milk collected separately from sick cows or during the pasteurization process. Schematic created in BioRender (https://www.biorender.com).

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Appendix

Sanger sequencing of the whole 16S rRNA gene was performed using previously published primers (*1*) and sequences were compared on the EzBioCloud database 2021.07.07 (*2*). Construction of the Neighbor-joining 16S phylogenetic tree was performed on Geneious® Prime 2020.0 software using Tamura-Nei genetic distance model with 1000 bootstraps.

After WGS, a total number of 4,197,739 reads of 219 bp mean length were generated. Reads were assembled with SPADES v3.15.4. yielding 88 contigs above 500 bp. Annotation of the genome was performed with Prokka v1.14.6. Resistance genes were detected from whole genome sequences on CARD v3.2.5 database with standard parameters (*3*).

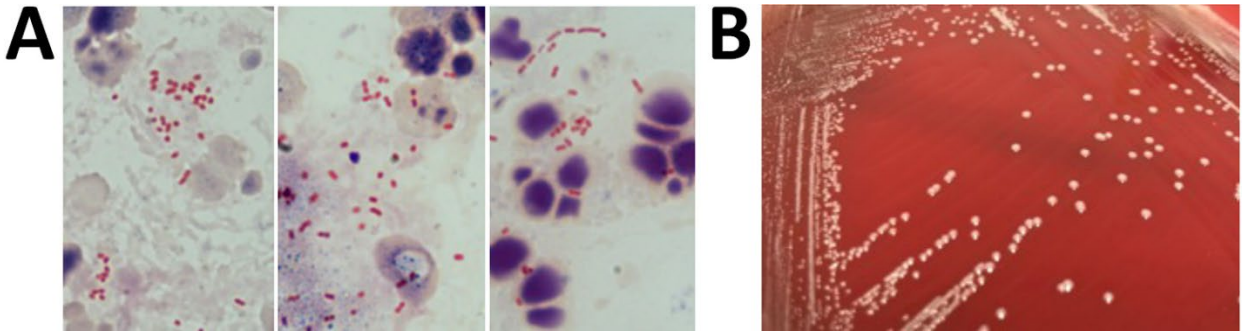
Single nucleotide polymorphisms (SNP) comparison between whole genomes of different *Pasteurellaceae* species was constructed with snippy v4.4.3 (https://github.com/tseemann/snippy) and Fasttree v2.1.10 programs. Average Nucleotide Identity (ANI) scores were obtained on EZBiocloud platform. Considering Multi Locus Sequence Analysis (MLSA), 16S rRNA, *infB, recN, rpoA* and *rpoB* genes of each genome were merged, aligned using MUSCLE on Geneious® Prime 2020.0 and a Neighbor-joining tree was constructed after 1000 bootstrap replications on MEGA 11 software. Amino-acid identity (AAI) analysis was performed using the EzAAI v1.2.3 pipeline (https://github.com/endixk/ezaai) with the parameters of 40% amino acid identity and 50% coverage length, as recommended by Nicholson et al. (*4*)

Biochemical characteristics were obtained using API20E gallery, Vitek2® NH and Vitek2® ANC cards (bioMérieux).

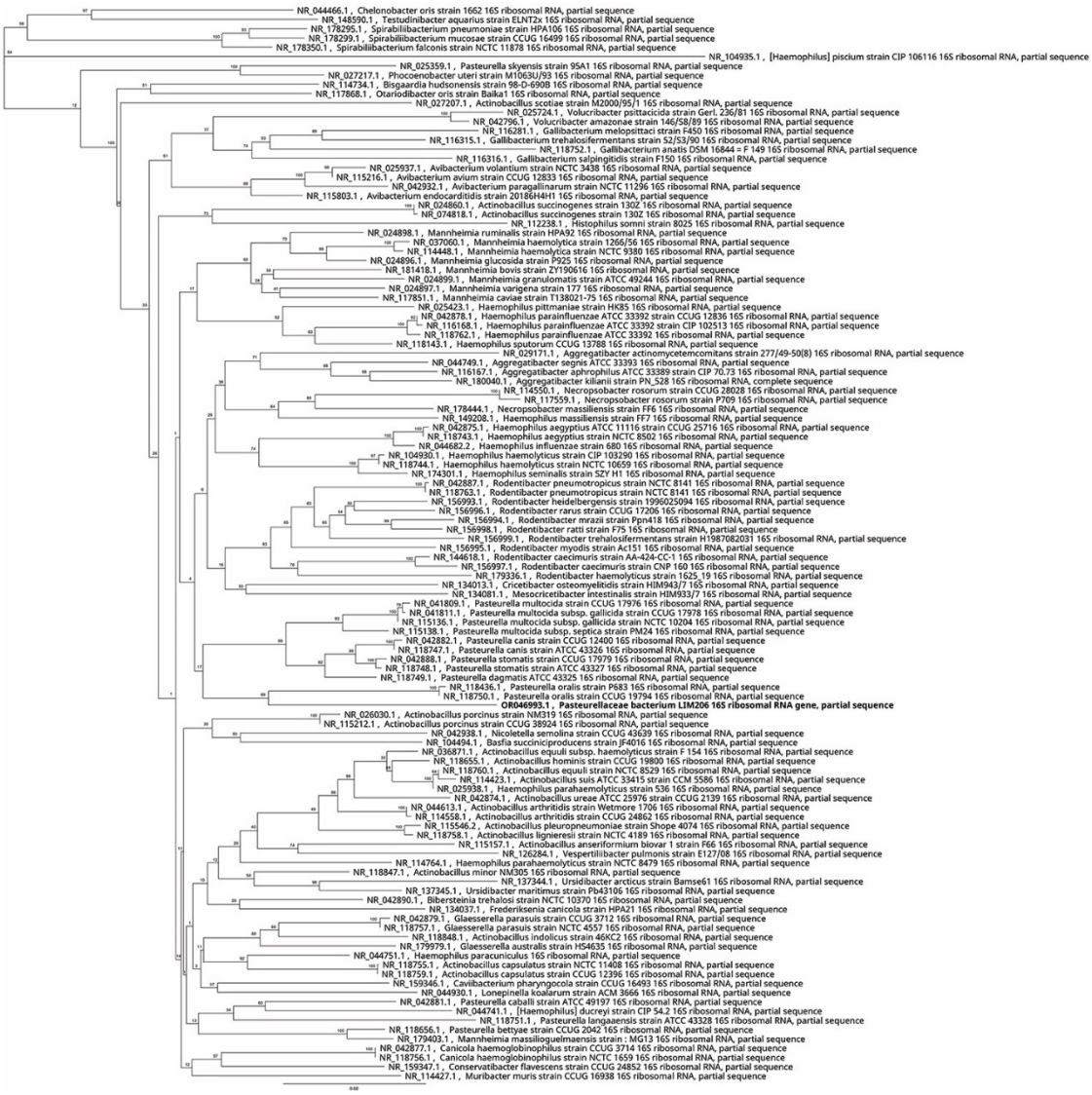
Table. Phenotypic and biochemical characteristics of LIM206 *Emayella* gen. nov. from other genera of the family Pasteurellaceae.

Genera	1	2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28	29	31	32	33	34
X and/or V dependency	−/−	-/D	-/D	-/D	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	-/+	+/+	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	-/+	−/−
β-haemolysis	−	−	−	−	−	D	−	−	+	−	−	−	+	−	D	D	−	D	−	−	−	−	−	−	−	−	+	−	−	+	D
Oxydase	+	−	D	+	+	D	+	+	+	+	+	+	D	+	+	+	−	D	+	+	+	+	+	D	+	+	+	+	+	+	D
Catalase	−	D	D	D	−	D	+	−	+	−	−	+	D	+	D	−	−	+	−	+	+	+	+	+	−	+	+	+	+	+	D
Urease	+	−	+	−	−	−	−	−	−	−	−	−	D	−	D	−	−	-	+	D	−	+	D	D	−	+	−	−	−	−	−
Indole	−	−	−	−	−	−	−	−	−	D	+	+	D	−	D	+	+	-	+	−	−	−	+	+	−	D	−	+	ND	−	−
Alkaline phosphatase	+	+	+	D	+	+	+	+	+	−	+	+	+	+	+	+	-	+	+	−	+	D	+	+	+	+	+	−	−	+	+
PNPG (α-glucosidase)	−	ND	D	D	−	D	−	−	−	+	−	+	D	ND	−	ND	ND	ND	−	+	+	ND	ND	D	ND	+	ND	−	+	D	−
Ornithine decarboxylase (ODC)	−	−	−	D	−	−	−	−	−	+	−	−	−	ND	D	−	−	D	−	−	−	−	−	D	−	D	ND	+	−	−	−
Acid produced from:																															
L-Arabinose	+	−	D	D	−	−	−	+	+	+	+	ND	D	−	ND	ND	+	D	−	−	−	−	+	D	ND	D	+	+	+	+	D
D-Galactose	+	D	D	D	+	−	+	+	ND	+	ND	ND	+	+	+	−	ND	+	ND	+	+	−	ND	+	ND	+	−	ND	ND	ND	+
D-Mannitol	−	D	+	D	+	+	D	−	+	+	+	−	+	−	−	ND	−	+	+	D	−	−	−	D	−	−	−	+	+	D	−
D-Mannose	+	D	D	+	+	+	+	+	+	+	+	+	+	+	D	ND	+	-	+	+	+	−	−	+	−	+	+	ND	+	−	+
L-Rhamnose	−	ND	−	−	−	−	D	−	ND	ND	+	ND	−	−	−	ND	ND	D	D	ND	+	−	−	−	−	−	−	+	+	+	−
Sucrose	+	D	+	+	+	+	+	+	+	+	+	+	+	+	D	−	D	+	+	+	+	−	−	+	−	+	−	+	−	−	+
D-Tréhalose	−	D	D	D	+	+	+	-	+	+	−	D	D	−	−	−	ND	−	−	+	+	−	+	D	−	+	−	+	+	D	−
D-Xylose	+	D	+	−	+	−	−	−	+	−	ND	ND	+	−	D	−	ND	+	ND	D	+	−	−	−	−	+	−	ND	ND	+	−

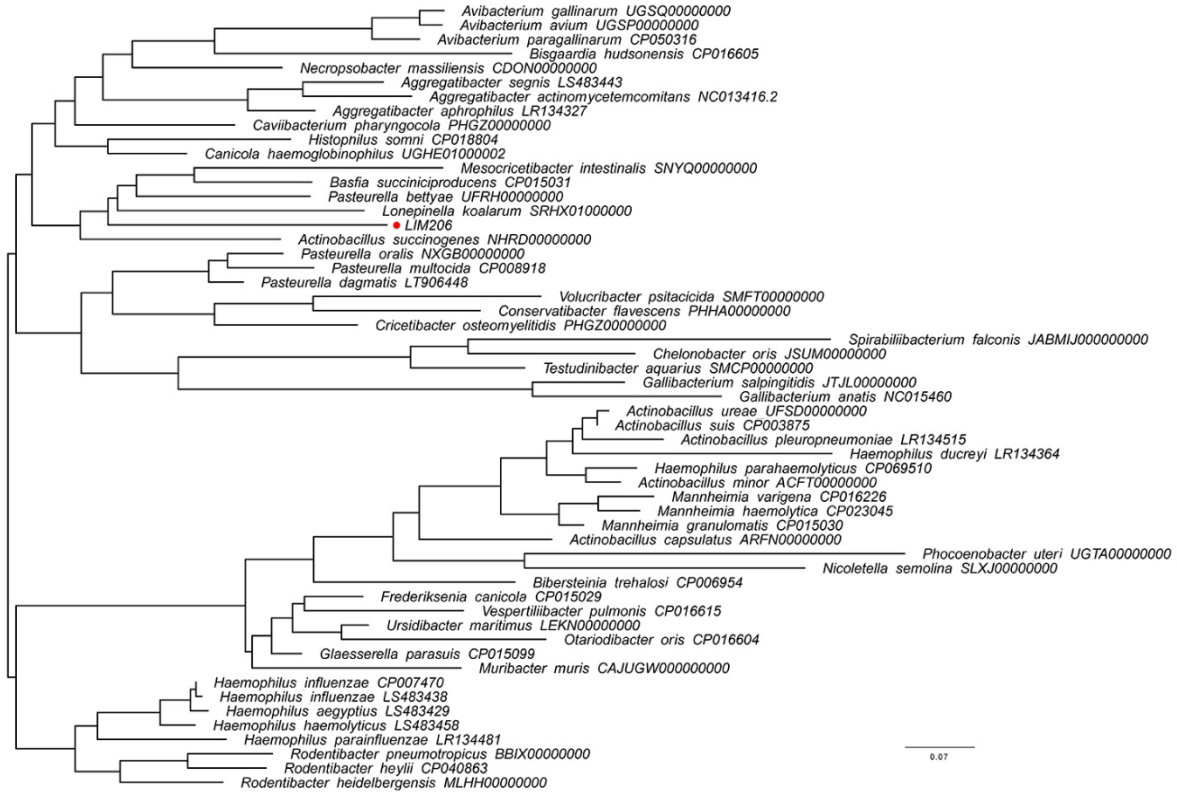
Genera: 1. LIM206 (data from this study); 2. *Aggregatibacter*; 3. *Actinobacillus sensu stricto*; 4. *Avibacterium*; 5. *Basfia*; 6. *Bibersteinia*; 7. *Bisgaardia;* 8. *Caviibacterium*; 9. *Chelonobacter*; 10. *Conservatibacter*; 11. *Cricetibacter*; 12. *Frederiksenia*; 13. *Gallibacterium*; 14. *Glaesserella*; 15. *Haemophilus sensu stricto*; 16. *Histophilus*; 17. *Lonepinella*; 18. *Mannheimia*; 19. *Mesocricetibacter*; 20. *Muribacter* 21. *Necropsobacter*; 22. *Nicoletella*; 23. *Otariodibacter*; 24. *Pasteurella sensu stricto*; 25. *Phocoenobacter*; 26. *Rodentibacter*; 27. *Seminibacterium*; 28. *Testudinibacter*; 29. *Ursidibacter*; 30. *Vespertiliibacter*; 31. *Volucribacter*; +, positive reaction; -, negative reaction; D, variable reaction; ND, not defined



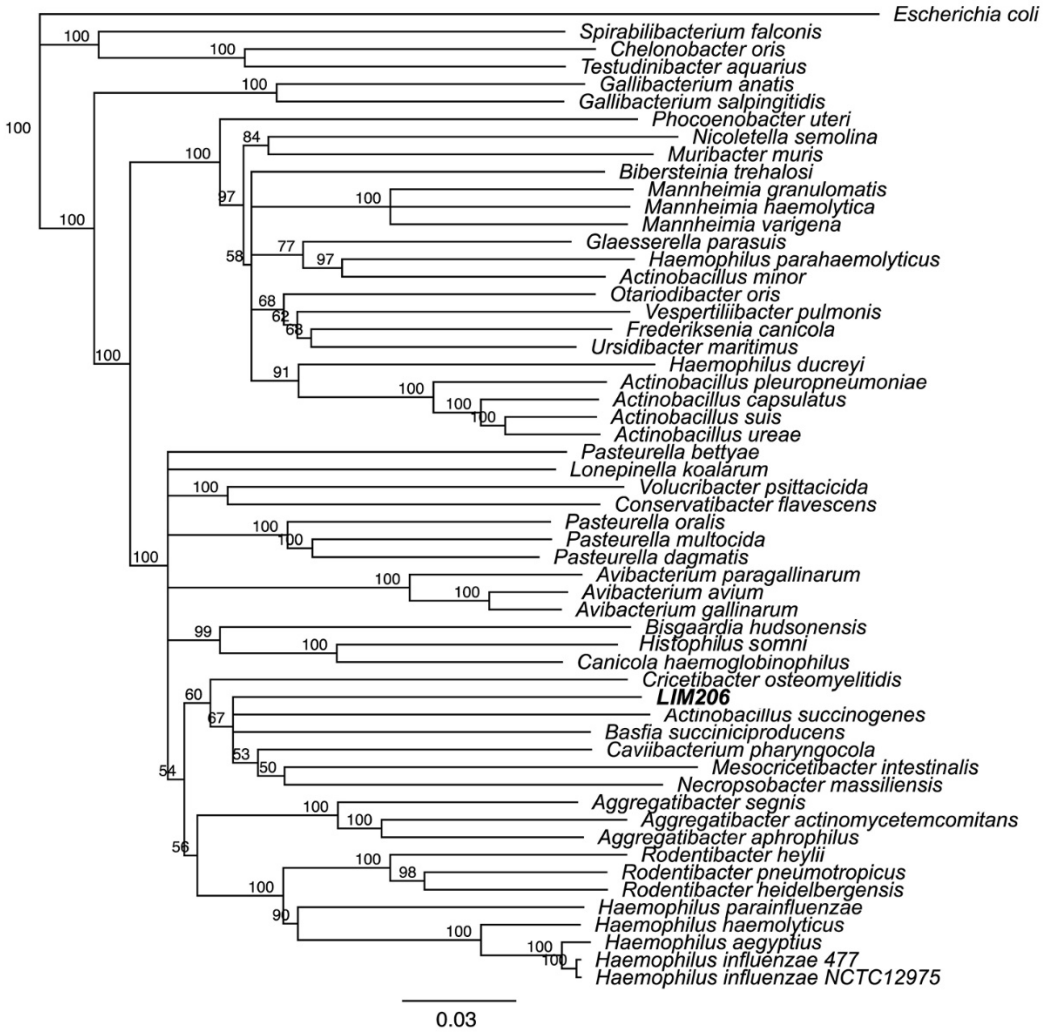
Appendix Figure 1. Gram staining and culture on blood agar of LIM206.



Appendix Figure 2. Neighbor-joining phylogenetic tree based on full 16SrRNA sequences of LIM206 compared to other *Pasteurellaceae* species. This phylogenetic tree has been generated using Geneious® Prime 2020.0 software with Tamura-Nei genetic distance model with 1000 bootstraps. In bold is represented the position of LIM206. Scale bar represents the phylogenetic distances based on 16S sequence differences.



Appendix Figure 3. Maximum likelihood phylogenetic tree based on whole genome sequencing SNP comparison of different *Pasteurellaceae* genomes. SNP differences were generated using snippy v4.4.3. Fasttree v2.1.10 was then used to generate phylogenetic tree with standard parameters. Visualization of the tree was executed on RStudio v2023.9.1. Scale bar represents the distance of nucleotide substitutions per site. Redpoint shows the position of LIM206.



Appendix Figure 4. Neighbor-joining tree based on MLSA of *16SrRNA*, *infB, recN, rpoA* and *rpoB* genes alignment of 54 *Pasteurellaceae* genomes after 1000 boostraps replications**.** Sequences of strain *E. coli* MG1655 (NC_000913.3) were added to root the tree. In bold is represented the position of LIM206.

Appendix Figure 5. Matrix of amino-acid identity scores (in %) obtained after comparing main species of the *Pasteurellaceae* family. Analysis was performed on EzAAI v1.2.3 pipeline with standard parameters (40% amino acid identity and 50% coverage length).

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