Closed Genome Sequences and Antibiograms of 16 *Pasteurella multocida* Isolates from Bovine Respiratory Disease Complex Cases and Apparently Healthy Controls

Gregory P. Harhay,[a] Dayna M. Harhay,[a] James L. Bono,[a] Timothy P. L. Smith,[a] Sarah F. Capik,[b,c] Keith D. DeDonder,[d] Michael D. Apley,[e] Brian V. Lubbers,[e] Bradley J. White,[e] Robert L. Larson[e]

[a]USDA, Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, Nebraska, USA
[b]Texas A&M AgriLife Research, Texas A&M University System, College Station, Texas, USA
[c]Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA
[d]Veterinary and Biomedical Research Center, Inc., Manhattan, Kansas, USA
[e]College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA

ABSTRACT *Pasteurella multocida* is an animal-associated Gram-negative member of the *Pasteurellaceae* family. It is an opportunistic pathogen and is one of the principal bacterial species contributing to bovine respiratory disease complex (BRDC) in feedlot cattle. We present 16 closed genome sequences and antibiograms of isolates cultured from calves exhibiting clinical signs of BRDC and from control calves not showing signs of BRDC.

Bovine respiratory disease complex (BRDC) outbreaks in feedlot cattle are the primary drivers of disease-related antibiotic treatments and have a significant economic impact (1). *Pasteurella multocida* is associated with not only BRDC but also diseases of swine, rabbits, birds, cats, and dogs and zoonotic infections of humans (2).

Nasopharyngeal and bronchoalveolar lavage isolates were cultured from calves exhibiting clinical signs of BRDC and control calves not showing signs of BRDC at a Kansas feedlot in 2013 during a 28-day retrospective study of 180 calves originating from 3 different southeastern U.S. states (3–7). The genome sequences presented here are from isolates collected from case animals in the Kansas feedlot (morbidity samples) and control animals at the source sale barn (day 0, not showing signs of BRDC). Isolates from control animals were selected based on being matched with cases, as closely as possible, for origin and position in the cattle transport trailer. If *P. multocida* was isolated from a BRDC morbidity sample, or from a sample collected on day 0 from an animal that later became a case, the isolate was included in this data set. Closed genome sequences and methylomes of these isolates were obtained using Pacific Biosciences (PB) single-molecule real-time sequencing. Antibiograms were generated from overnight 37°C chocolate agar cultures using a Sensititre BOPO6F plate (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's and Clinical and Laboratory Standards Institute guidelines (8) with *Escherichia coli* ATCC 25992 as the reference control strain. Each isolate's published genome sequence is associated with an antibiogram and is accessible on their respective BioSample pages. All 16 genomes exhibited N6-adenine methylation at the GATC motif, while no other base modifications were detected. Antibiograms demonstrated variation in antimicrobial resistances between the sequenced isolates.

The *P. multocida* isolates were grown overnight at 37°C in brain heart infusion broth shaken at 190 rpm. Cells were pelleted at 6,000 × *g* for 10 min at 4°C, and genomic DNA

TABLE 1 Chromosome and BioSample accession numbers with metadata

GenBank accession no.	SRA run no.	Size (bp)	No. of genes	BioSample accession no.	Animal no.	Animal class	Sample day	Morbidity sample
CP015559	SRR7721909	2,344,126	2,206	SAMN04622935	243	Case	0	
CP015572	SRR7721913	2,344,125	2,207	SAMN04622939	217	Control	0	
CP015562	SRR7721905	2,345,801	2,210	SAMN04622931	245	Case	0	
CP015569	SRR7721914	2,530,586	2,453	SAMN04622940	230	Control	0	
CP015571	SRR7721908	2,333,691	2,257	SAMN04622934	262	Case	0	
CP015558	SRR7721917	2,342,910	2,181	SAMN04622941	204	Case	19	✔
CP015573	SRR7721915	2,342,911	2,180	SAMN04622945	204	Case	19	✔
CP015568	SRR7721911	2,258,592	2,089	SAMN04622937	235	Control	0	
CP015567	SRR7721918	2,462,567	2,365	SAMN04622942	262	Case	14	✔
CP015564	SRR7721920	2,334,467	2,175	SAMN04622944	175	Case	20	✔
CP015566	SRR7721919	2,334,516	2,174	SAMN04622943	175	Case	20	✔
CP015570	SRR7721906	2,337,902	2,180	SAMN04622932	204	Case	0	
CP015565	SRR7721910	2,337,801	2,178	SAMN04622936	175	Case	0	
CP015560	SRR7721916	2,327,616	2,168	SAMN04622946	243	Case	5	✔
CP015561	SRR7721907	2,327,607	2,168	SAMN04622933	229	Case	0	
CP015563	SRR7721912	2,334,845	2,176	SAMN04622938	220	Control	0	

was isolated using Qiagen Genomic-tip 100/G columns according to the manufacturer's directions. From the genomic DNA, PB 20-kb insert libraries were created and sequenced on a PB RS II system using C4/P6 (chemistry/polymerase) to at least 100-fold coverage. The genomes were assembled using PB HGAP3 V.3 with default parameters to generate a single contig with overlapping 3′ and 5′ ends of at least 1 kb with greater than 99% identity. For each isolate's contig, the overlapping region was deleted from the 3′ end, the two ends were joined to circularize the chromosome, and GenSkew V.1 (http://genskew.csb.univie.ac.at) was used with default parameters to localize the approximate origin of replication so that the base pair numbering could be reindexed to reflect the origin of replication at base pair position 1. The PB RS_Resequencing pipeline V.2.3.0.139497 (SMRTPIPE) with default parameters was used to map the reads back to the references to generate final consensus concordance assemblies that were manually inspected to ensure that the assemblies were free from low-quality read-mapping regions and inadequate read coverage (9). The genomes were annotated with the NCBI Prokaryotic Genome Annotation Pipeline. The use of cattle in this study was approved by the Kansas State University Institutional Animal Care and Use Committee. Base modification data are available for each assembly by accessing the hyperlink in the "comment" area on that assembly's GenBank accession number page.

Data availability. GenBank genome sequence and BioSample accession numbers and links to raw sequence read data are given in Table 1. Raw HDF5 sequence data for this study are available at the Sequence Read Archive under the number SRP158403.

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