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

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**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
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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**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          |                 |
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