Genomic Surveillance for Antimicrobial Resistance in *Mannheimia haemolytica* Using Nanopore Single Molecule Sequencing Technology

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Abstract

Disruptive innovations in long-range, cost-effective direct template nucleic acid sequencing are transforming clinical and diagnostic medicine. A multidrug resistant strain and a pan-susceptible strain of *Mannheimia haemolytica*, isolated from pneumonic bovine lung samples, were respectively sequenced at 146x and 111x coverage with Oxford Nanopore Technologies MinION. *De novo* assembly produced a complete genome for the non-resistant strain and a nearly complete assembly for the drug resistant strain. Functional annotation using RAST (Rapid Annotations using Subsystems Technology), CARD (Comprehensive Antibiotic Resistance Database) and ResFinder databases identified genes conferring resistance to different classes of antibiotics including beta lactams, tetracyclines, lincosamides, phenicols, aminoglycosides, sulfonamides and macrolides. Antibiotic resistance phenotypes of the *M. haemolytica* strains were confirmed with minimum inhibitory concentration (MIC) assays. The sequencing capacity of highly portable MinION devices was verified by sub-sampling sequencing reads; potential for antimicrobial resistance determined by identification of resistance genes in the draft assemblies with as little as 5,437 MinION reads corresponded to all classes of MIC assays. The resulting quality assemblies and AMR gene annotation highlight efficiency of ultra long-read, whole-genome sequencing (WGS) as a valuable tool in diagnostic veterinary medicine.

Introduction

Emergence of antimicrobial resistance (AMR) among the most important bacterial pathogens is recognized as a major public health concern. Not only has AMR emerged in hospital environments, it is often identified in community settings, in livestock feedlots and in aquaculture and crop production, suggesting an ever-increasing range of reservoirs of antibiotic-resistant bacteria (1-4). Bacterial response to the antibiotic “attack” is the prime example of genetic adaptation through the interplay of immense genetic plasticity, ranging from mutational adaptations and acquisition of genetic material to alteration of gene expression with fitness consequence of the pathogen (5). As a result, understanding the genetic basis of resistance is of paramount importance to design strategies to curtail the emergence and spread of AMR, as well as to devise innovative therapeutic approaches against multidrug-resistant organisms (6).

With an increased population of pet and farm animals there is a significant risk of humans acquiring drug resistant bacteria from animal sources. One of the major deficiencies in veterinary medicine is the lack of validated data to determine minimum inhibitory concentration (MIC) breakpoints for drug-microbe-host combinations, based on which scientifically sound interpretations can be made regarding whether a pathogen is susceptible or resistant to a specific drug. This is especially true for anaerobic bacteria. The large diversity of domestic and exotic animal species and their numerous microbial pathogens pose significant challenges to developing reliable interpretation criteria for all antimicrobial drugs.

Recently, whole-genome sequencing (WGS) has become an invaluable tool to combat antibiotic resistance (7-12). In addition to the bench-top sequencing platforms like Illumina machines, in 2014 Oxford Nanopore Technologies (ONT) released a portable MinION device through an early access program (the MinION Access Program). MinION devices can be

powered by a standard USB3 port and the base-calling step that turns electrical signals into nucleotides is enabled by a cloud-computing platform (13), providing the portability needed for various clinical microbiology settings, especially for political or logistical reasons (14-16). The landmark case for diagnostic investigation of pathogen genomes was the Ebola outbreak in Guinea (17). Using three MinION devices and four laptop computers, 148 sequencing runs were conducted to cover 142 samples, and a rapid turnaround was demonstrated that sequencing process could be performed within an hour with the whole work-flow from amplification, library preparation and sequencing runs brought to completion in 24 hours (18). Currently, sequencing capacity of MinION has reached about 450 bases per second; as the throughput and speed increases, ONT platform might become suitable for real-time pathogen surveillance and clinical diagnostic applications (19).

In veterinary medicine, approaches such as mass spectrometry and multiplexed PCR are commonly used for diagnostic applications. WGS, however, has yet to be adopted as a part of routine diagnostic procedures. In this report we describe the use of WGS for evaluating AMR of *Mannheimia haemolytica*, an important bacterial pathogen of cattle causing bovine respiratory disease (BRD). The annual loss to the cattle industry from BRD is estimated to be around one billion USD in North America (20). Increase in antimicrobial resistant *M. haemolytica* over the years has been recognized (21, 22); and, high prevalence of multidrug resistance of *M. haemolytica* that underscores critical challenges regarding treatments and management practices was also strongly reckoned in a recent study (23). In this manuscript WGS-based detection of AMR of two *Mannheimia* strains was determined using ONT's MinION devices; with the assemblies constructed with as little as 5,347 ONT ultra-long reads, potential bacterial AMR was supported by all genome annotation databases and the genetic AMR of these strains was further

102 confirmed with MIC assays. Using this comparative study as an example, the feasibility and
103 effectiveness of integrating ONT's MinION technology in clinical investigations was explored.

Materials and Methods

DNA extraction for bacterial isolates

M. haemolytica OADDL-1 was isolated from the lung of a 3-year-old bull and *M. haemolytica* OADDL-2 was isolated from the lung of a calf of unknown age. Both animals from which the bacterial isolates were obtained had a history of sudden death and a histologic diagnosis of severe acute fibrinous pleuropneumonia. The calf from which the resistant *M. haemolytica* isolate was obtained also had a history of antimicrobial treatment with Tilmicosin and Enrofloxacin. Bacterial isolates were cultured on 5% sheep blood agar (Hardy Diagnostics); five to 10 colonies of bacteria were carefully selected to avoid agar contamination and then suspended in Tris EDTA buffer. Genomic DNA was extracted using the OMEGA Bio-tek EZNA® bacterial DNA kit D3350-01 protocol (OMEGA Bio-tek, Norcross, GA).

MIC (minimum inhibitory concentration)

Antimicrobial susceptibility of *M. haemolytica* isolates was determined by MIC method on the Sensititre automated system (Thermo Scientific, Waltham, MA, USA), using the Bovine/Porcine panel (BOPO 6F, Thermo Scientific, Waltham, MA, USA) following manufacturer recommendations. Interpretations regarding susceptibility or resistance to antimicrobial were made based on CLSI VET01S guidelines (24).

MinION Library Preparation and Barcoding

Library preparation was performed following the procedures outlined for SQK-LSK208 sequencing kit and barcoded for multiplexing using the complimentary EXP-NDB002 barcoding kit (Oxford Nanopore Technologies, United Kingdom) with the following protocol adjustments. A total of 1.5 µg of gDNA from each bacterial isolate was sheared in g-tubes (Covaris) at 4200 RPM for a targeted fragment size of 20 Kb. End-repair was performed following the

recommended protocol of the manufacturer for Ultra II End-prep enzyme mix (NEB). Adapter ligation reaction incubations were increased to 15 minutes. Bead clean-ups used 0.4x AMPureXP beads (Beckman Coulter, Brea, CA) for additional size selection and elutions were performed at 37°C for 20 minutes. DNA concentration of the library was quantified using Quant-IT PicoGreen[®] dsDNA Assay Kit (ThermoFisher Scientific), measured on Synergy H1, hybrid multi-mode microplate reader (BioTek), confirming final library yields above the recommended 200 ng. In total, two MinION barcoded libraries were prepared separately for sequencing on two flow cells (listed as Library A and B in Table 2).

MinION Sequencing

Two MinION R9.4 SpotON flow cells, labeled FAB 42646 and FAB 42472, were used for sequencing. The MinKNOW GUI application (version 1.4.2) was used to perform a platform quality check to ensure flow cell quality. Flow cells were primed as instructed prior to sequencing. MinKNOW version 1.4.2 was executed to sequence using the protocol script NC_48Hr_Sequencing_Run_FLO-MIN105_SQK-LSK208.py. Both libraries were sequenced for approximately 48 hours, with no live base-calling performed.

Albacore Base-calling

Due to discontinued support of 2-D reads by ONT and Nanopolish (0.6-dev), sequenced reads were subsequently base-called using the newest version of Albacore v 2.1.0. for 1-D base-calling, where both template and complement strands were considered as individual reads.

Albacore performs base-calling on the raw reads using event detection to categorize the signal-level data, after which the event detection is compared to existing models and associated with a nitrogenous base. All raw reads were evaluated and filtered for a cut-off quality score of seven. Base-called reads were further split into their respective barcode folders of *M*.

haemolytica OADDL-1 and OADDL-2 for downstream analyses. All reads were trimmed using Porechop (v0.2.1, github.com/rrwick/Porechop) to remove barcoded adapter sequences found in the read files.

Genome Assembly using Canu

Genome assembly was performed using the software package Canu (version 1.6). Canu implements the overlap-layout-consensus method and is partitioned into three primary stages: correction, trimming and assembly (25). Default Canu command and parameters for assembly were used with a suggested genome size of 2.7 million bp. QUAST was used to evaluate assembly statistics (26).

Genome Polishing with Nanopolish

Typically, Nanopore sequencing reads are prone to deletion errors, particularly in homopolymer regions (27). In this study, Nanopolish (0.6-dev), a software program that utilizes a trained hidden Markov model to improve the final consensus assembly was used for error correction. Reads were aligned back to the Canu assembly using bwa (28), with the `-x ont` parameters for noisy ONT reads; samtools was used to sort and index the reads (29). Nanopolish was used to compute a polished consensus genome by correcting substitution, insertion and deletion errors that may have occurred during sequencing or base-calling. Following the github protocol for Nanopolish (<https://github.com/jts/nanopolish>), the default commands and parameters for computing a consensus sequence were used, with thread count set to four and parallel count set to eight.

RAST, CARD and ResFinder Annotation

Predicted genes were called from the assembled contigs using the prokaryote gene calling software Prodigal (30). All amino acid sequences were annotated using a combination of

homology search with NCBI blast+ (31), and domain identification using hmmscan (32) with PFAM v28.0 database (33). AMR gene determination was performed using NCBI blast+ with three different tools and databases: RAST (Rapid Annotation using Subsystem Technology, (34)), CARD (the Comprehensive Antibiotic Resistance Database, (35, 36)) and ResFinder (37). RAST is a web-based service that offers rapid annotation of prokaryotic genomes (<http://rast.nmpdr.org/rast.cgi>), using an automated system that requires a genome submission. Similarly, ResFinder is also a web-based service (<https://cge.cbs.dtu.dk/services/ResFinder>), where AMR genes are identified with the threshold and minimum length set to 90% and 60%, respectively. The CARD database (<http://arpcard.mcmaster.ca>), which integrates disparate molecular and sequence data, also provides Antibiotic Resistance Ontology (ARO) (36).

Genome Sub-sampling for AMR Gene Identification

To evaluate the degree to which multiplexity would affect assembly quality for AMR detection, sub-sampling of reads was conducted. Six sub-divisions of randomly sampled reads were created from the total sequencing reads; each subdivision represented 1/10th, 1/6th, 2/6th, 3/6th, 4/6th and 5/6th of the total reads. Five replicates were generated for each sub-division of reads; the replications were created to examine the variability derived from random sampling of raw sequencing reads. AMR predictability was then assessed by the quality of the genome assemblies and annotation methods described earlier. For all sub-divided group of reads and replicates, assemblies were generated using Canu v1.6; assembly qualities were evaluated using QUAST and polished with Nanopolish. And, *in silico* determination for AMR genes was conducted by CARD and ResFinder.

Results

AMR Phenotyping by MIC

Details of the MIC results are listed in Table 1. Overall, *M. haemolytica* OADDL-1 was found to be susceptible and *M. haemolytica* OADDL-2 was resistant to most of the antimicrobials used in routine therapeutics, with the only exception of OADDL-2 susceptible to Ceftiofur. In most cases for antimicrobials with no CLSI interpretation guidelines (NI), the MIC values for OADDL-2 were found higher than the susceptible OADDL-1; however, both OADDL-1 and OADDL-2 showed similar MIC levels for Sulfadimethoxine and Sulfamethoxazole Trimethoprim (Table 1).

Sequencing and Base-calling

Initial MinION sequencing yielded 138,689 and 116,073 raw reads for flow cell FAB 42646 and FAB 42472, respectively, for a total of 254,762 reads to be assembled. As shown in Table 2, Albacore 1-D base-calling resulted in 53,478 reads that passed the quality filter for OADDL-1, and 28,162 total reads for OADDL-2. The mean read length for OADDL-1 was 7,562 bp for FAB 42646 and 6,969 bp for FAB 42472 with an N50 value at 6,807 for flow cell FAB 42646 and 8,796 for flow cell FAB 42472. The mean read length for *M. haemolytica* OADDL-2 was 11,611 for FAB 42646 and 10,629 for FAB 42472 and an N50 of 17,018 bp for FAB 42646 and 15,722 bp for FAB 42472 (Table 2).

Genome Assembly and Genome Polishing

Genome assembly with Canu v1.6 of quality sequencing reads for OADDL-1 resulted in a single contig of 2,644,744 bp with a mean GC content of 41.19%. The draft genome assembly of OADDL-2 was composed of five contigs totaling 2,804,188 bp with an average GC content of

41.24% (Table 3). Polishing (Nanopolish) resulted in increased genome length for both OADDL-1 (2,656,520 bp) and OADDL-2 (2,815,485 bp) (Table 3).

The assembly of OADDL-2 also revealed the presence of a 5,265 bp plasmid; a BLAST search returned a 99% identity and 80% coverage of a known plasmid *Pasturella multocida* pCCK411, strain U-B411 (FIG 1); the GC content, and over- and under- representation of GC regions of the plasmid is depicted in FIG 1. A native plasmid found in *M. haemolytica*, plasmid *P. haemolytica*, which encodes for the Bla gene that confers beta-lactam resistance (38) was also found with a BLAST search and returned with a 99% identity and 80% coverage.

Genome Annotation and AMR Gene Identification

Annotation by RAST for OADDL-1 revealed 3,593 coding sequences, which are further categorized under subsystems such as carbohydrates, DNA metabolism, virulence, disease and defense, to name a few. Detailed information of RAST annotations is provided in Table 1S. The RAST annotation for OADDL-2 detected 4,030 coding sequences, of which 45 were related to virulence, disease and defense (Table 2S). Amongst these 4,030 coding sequences, the RAST annotation for OADDL-2 revealed multiple genes that may confer resistance; these include beta-lactamase (bl), Aminoglycoside N6'-acetyltransferase (N6'-ac), and Streptomycin 3"-O-adenylyltransferase (AadA1), for instance (Table 4). The locations of these AMR related genes are showed in FIG. 2.

ResFinder, which by default searches resistant genes that share 98% identity to the genes within the databases, was used to identify 18 AMR genes in OADDL-2 strain (Table 5); examples of these AMR genes are blaOXA-2_1 and blaROB-1_1 Beta-lactam. AMR genes commonly found in *M. haemolytic* resistant strains (21, 39), such as tet(H)_3 (Tetracycline) and

aph(3')-Ia_1 (Aminoglycoside), were also identified in OADDL-2, whereas the susceptible OADDL-1 returned no results for AMR genes on ResFinder.

Alternatively, the AMR gene homology search using CARD identified 16 AMR genes in the OADDL-2 resistant strain; common AMR genes like ROB-1 Beta-lactam, tet(H) for Tetracycline and APH for Aminoglycoside were present in the resistant *M. haemolytica* strain, and two other published strains 89010807N (GenBank accession no. CP011098) (40) and M42548 (GenBank accession no. CP005383) (41) (Table 5). Interestingly, *macB* that confers resistance to macrolide antibiotics was identified for both OADDL-1 and OADDL-2 (Table 5), however not in homologous regions (dark red triangles in FIG 2). This *macB* annotation, though present in both by CARD, showed sequence coverage as low as 10.65% (OADDL-1).

A global alignment to identify homologous genomic regions between OADDL-1 and OADDL-2 was performed using Mauve (42); the aligned regions by Mauve were visualized using Circos (43), with the annotations for AMR genes by both CARD and ResFinder. In FIG 2, regions with shared colors indicate sequence homology. Though the overall sequence similarity was estimated at 95.2%; significant structural variation is also identified, including four reverse regions in OADDL-2 (FIG 2). Among these reverse regions, two genome clusters in the orange colored region of OADDL-2, homologous to the 760,000 to 950,000 bp region of the susceptible OADDL-1, accommodate most of the annotated AMR related genes (details in the A and B boxes of FIG 2).

Genome Assembly Quality Assessment and AMR Gene Identification

To assess adequate amounts of long reads for microbial genome assembly and subsequent AMR gene detection, subsampling was conducted at 1/10th, and from 1/6th to 5/6th of the total reads used for original assembly. In OADDL-1, the base pair length was consistent across all replicates

and partitions (mean=2,649,246 bp), with standard deviations ranging from 9,575 bp ($1/6^{\text{th}}$) to 36,605 bp ($1/10^{\text{th}}$). Mean GC content was also stable across all samples with a range of 41.16% to 41.23% (Table 6). Assembly quality began to decline at $2/6^{\text{th}}$ of the original total reads (8,913 reads); average N50 reduced to 1,864,819 bp, and averaged number of mismatches with original assembly increased to 17,233 bp. At $1/10^{\text{th}}$ of the total reads, the number of contigs increased 10 fold (19 to 24 contigs) and the N50 of the assemblies dropped to 351,563 bp, showing a significantly reduced assembly quality compared to the other subsamples.

For OADDL-2, subsampling of the reads generated comparable results. The average genome size of all subsampled assemblies was 2,777,982 bp, with a standard deviation of 24,724 bp; mean GC content remained consistent amongst these assemblies with a range of 41.22% to 41.33% (Table 7). The plasmid was found in all assemblies. Partitioning of sequencing reads at $1/10^{\text{th}}$ showed a rapid degradation in assembly quality: the number of contigs increased to at least 29 (maximum at 40), and the N50 value dropped to an average of 133,657 bp (Table 7).

For all assemblies using subsets of reads, annotation was done by ResFinder and CARD databases. Overall, there were no observable changes compared to the original annotation for AMR genes found in OADDL-2. CARD AMR annotation varied slightly due to the low sequence identity of the *macB* gene. With the exception of the *macB* gene present at low coverage, there were no resistance genes found on OADDL-1.

Discussion

Massive parallelization of sequencing reaction, such as the popular Illumina platforms (44), has remarkably increased the read numbers per run to a few billion of short read fragments, transforming DNA molecular readers into diagnostic tools in virtually every field of biomedical research (45-47). Among all available technologies, growing interest was recognized in the field of long-read sequencing, owing to the apparent genome complexity in lengthy repetitive elements, copy number and structural variations that lead to difficulty in assembling data from short-read platforms (44). Currently, the dominant platforms for direct typing long read fragments are Pacific Biosciences (PacBio) and Oxford Nanopore, both employing a single-molecule real-time approach. In addition to the improved assembly when kilobase sized reads are available (48), single molecular technologies are amplification free and thereby not hampered by PCR-based artifacts and GC-bias, giving a much more uniform coverage and span across GC-rich regions (49). The feasibility of ONT has been studied in clinical plasmid isolates of *Escherichia coli* (14), *Salmonella typhimurium* (15), *Vibrio parahaemolyticus* (15) and *Klebsiella pneumoniae* (14, 15). Nanopore-only assembly yielded > 99% (0.2% mismatches and 0.5-0.6% gaps) consensus accuracy compared to Illumina MiSeq, and with polishing using MiSeq paired-end reads, assembly accuracy increased to ~ 99.9% (14). However, when multiplexing 12 plasmids by Oxford's Rapid Barcoding Sequencing kit, the average accuracy of two plasmids compared to the known reference assembly turned out to be considerably lower (87% in (15)). In Wick et al. (2017), 12 isolates of *K. pneumoniae* were barcoded, multiplexed and sequenced with 1-D technology on a single flow cell; the most accurate ONT-only assembly had an estimated 0.349% error rate after polishing (50). These results have recommended an alternative approach of using both Illumina and ONT reads for hybrid assemblies (51).

To study AMR detection capacity of ONT, we sampled reads from MinION R9.4 flow-cells. When using the full capacity, one *M. haemolytica* strain was fully assembled while the other produced 5 contigs with N50 that covers over 85% of the polished genome. The averaged genome coverage was at ~146x and ~111x for OADDL-1 and OADDL-2, respectively; both assemblies showed a GC content (Table 3) similar to other published strains (~ 41% in (40, 41, 52, 53)). Results from our barcoded, multiplexed *M. haemolytica* strains suggest that consistent taxonomic status and genome evolvability and transduction capacity could be correctly determined (54-56). Capacity of ONT's single molecule long-read sequencing platform for strain identification is thus confirmed.

In this study, AMR profiles of both *M. haemolytica* strains were determined by standard MIC method (results see Table 1). In CLSI guidelines (24), drugs with host specific MIC interpretation are categorized as Group A drugs. Several of the Group A drugs listed in Table 1 are commonly used to treat bacterial respiratory disease in cattle. However for cases like Danofloxacin, though it is classified as Group A, bovine specific MIC breakpoints for resistance has yet not been defined, making accurate interpretations of resistant phenotypes ambiguous. For drugs not classified under Group A, interpretations of MIC values impose greater challenges, since they must be predicted based on guidelines for human or other host species. From a public health perspective it is significant to understand the resistance mechanisms for all groups of antimicrobials whether they are used in animals or not. Direct identification of the genomic basis for AMR, such as our study case in *M. haemolytica*, could be a more reliable approach.

We also examined different aspects that could impact the cost-effectiveness for broader applicability of ONT to for AMR detection. Aside from the issues associated with the biased input of barcoded DNA samples (50), at a greater degree of multiplexity assembly quality could

be concerning, which might subsequently lead to mis-identification of AMR genes. Current ONT R9.4.1 flow cell technology with SQK-LSK 109 sequencing kits averages over 10 billion bp per flow cell. When genome coverage and assembly quality are not the major concerns, our results indicate that rapid AMR diagnosis using ONT long-read sequencing technology could provide sufficient capacity for genotyping over 12 microbial genomes simultaneously in one ONT flow cell in 48 hours. The limitation, in principle, is due to the use of ONT barcoding kit (EXP-NBD103), which works in conjunction with the current genomic DNA sequencing kit SQK-LSK109. Here we also should note that, unlike Wick et al. (2017) (50), our simulated genome assemblies listed in Table 6 and Table 7 were assembled using ONT-only reads, but polished on the constructed genome of OADDL-1 with all reads (17,714 reads; 244 million bp). There are other proven cases that demonstrate how superior assembly quality can be obtained through hybrid assembly by ONT-Illumina (57, 58) and PacBio-Illumina (59, 60). Based on our ONT-only read assemblies, AMR genes identified in *M. haemolytica* strains were congruous to the MIC results (Table 1). Amongst the tested antibiotics, Ceftiofur, a third generation of cephalosporin antibiotics as well as a beta-lactam, was found to be susceptible in both strains of *M. haemolytica* (Table 1). It has been suggested that resistance to broad spectrum cephalosporins such as Ceftiofur is conferred by AmpC type beta-lactamases (61, 62), which were not detected in either of the *M. haemolytica* isolates used in this study. Studies focusing on comparisons of phenotypic susceptibility testing with sequence-based AMR prediction have already been performed for a variety of bacterial pathogens (8, 63-65), now to include this report on *Mannheimia* strains. Amongst these, promising results of 97% sensitivity and 99% specificity for AMR detection was reported from a large study conducted in U.K. for *Staphylococcus aureus*

strains (8), clearly demonstrating that WGS is transforming the practice of outbreak investigation with remarkable accuracy.

Equipped with such sequencing capacity, we believe direct genome sequencing approaches for AMR detection has started to shed light on genomic surveillance for disease control. At present, routine drug susceptibility testing is undertaken using phenotype-based methods, including disc diffusion, gradient diffusion and broth dilution methods that have been automated in a number of commercial platforms (64, 66). Despite the problems with specificity for certain organism-antimicrobial combinations (67, 68) and common operation errors in practices and result interpretation (69), drug susceptibility testing requires laboratory procedures that could be completed within 48 ~ 96 hours. Further, standard susceptibility testing protocols can only be done for culturable bacteria, creating significant challenges for risk assessment when the entire AMR reservoir needs to be investigated for both culturable and unculturable pathogens (70). Bacterial unculturability is therefore the most problematic for complete AMR detection, as it is possible that only about 1% of what is present in a sample can be grown with current cultivation techniques (71, 72). Determination of AMR of anaerobic and other difficult to grow fastidious bacteria is also challenging. Genomic approaches (73, 74), on the other hand, can provide sufficient information to re-assemble genomics of AMR for ‘unculturable’ samples (12, 73). To fulfill the need for timely response in disease control, WGS approaches that rely on growing, isolating and purifying bacterial isolates might still fall short. To effectively reduce the response time and expand genomic surveillance for “unculturable” pathogens, direct sequencing approaches should be considered. This culture-independent approach is encouraged by the recent successes in meta-genomics (sequencing all genomes in a sample) (74-76), though much still

remains to be studied, like contaminants and low biomass specimens, prior to the broad adoption of meta-genomics in diagnostic laboratories (77).

Accession number(s)

The MinION data obtained in this study have been deposited in the NCBI Sequence Read Archive under BioProject PRJNAxxxxxxx. (please contact charles.chen@okstate.edu for early access).

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Figure Legends

FIG 1. BLAST genome alignment between the plasmid found in *M. haemolytica* OADDL-2 and the highest scored plasmid found in a BLAST alignment plasmid pCCK411. GC-content and GC-skew are also included. GC Skew is shown to visualize regions where there is an over- (+) or under- (-) abundance of guanine and cytosine compared to adenine and thymine.

FIG 2. Global genome alignment between bacterial species *M. haemolytica* OADDL-1 and *M. haemolytica* OADDL-2. Homologous genome regions are marked by colors using *M. haemolytica* OADDL-1 as basal frame; the 5' to 3' reverse regions in *M. haemolytica* OADDL-2 are indicated by the arrows. The *macB* genes, annotated only by CARD, are found in non-homologous regions of *M. haemolytica* (marked by the red triangles). Two genome clusters, one from 35,000 to 45,000 bp (A) and another from 156,000 to 168,000 bp (B) in a reverse region of *M. haemolytica* OADDL-2, accommodate most of the genetics of AMR.

References:

1. Thanner S, Drissner D, Walsh F. 2016. Antimicrobial Resistance in Agriculture. *mBio* 7.
2. FAO. 2017. Antimicrobial resistance in food and agriculture.
3. FAO. 2016. The FAO action plan on antimicrobial resistance 2016-2020. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Rome.
4. WHO. 2018. Antimicrobial resistance.
5. Hughes D, Andersson DI. 2015. Evolutionary consequences of drug resistance: shared principles across diverse targets and organisms. *Nature Reviews Genetics* 16:459.
6. Munita JM, Arias CA. 2016. Mechanisms of Antibiotic Resistance. *Microbiol Spectr* 4.
7. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52:1501-10.
8. Gordon NC, Price JR, Cole K, Everitt R, Morgan M, Finney J, Kearns AM, Pichon B, Young B, Wilson DJ, Llewelyn MJ, Paul J, Peto TEA, Crook DW, Walker AS, Golubchik T. 2014. Prediction of *Staphylococcus aureus* Antimicrobial Resistance by Whole-Genome Sequencing. *Journal of Clinical Microbiology* 52:1182-1191.
9. Didelot X, Bowden R, Wilson DJ, Peto TEA, Crook DW. 2012. Transforming clinical microbiology with bacterial genome sequencing. *Nat Rev Genet* 13:601-612.
10. Tagini F, Greub G. 2017. Bacterial genome sequencing in clinical microbiology: a pathogen-oriented review. *Eur J Clin Microbiol Infect Dis* 36:2007-2020.
11. Fricke WF, Rasko DA. 2014. Bacterial genome sequencing in the clinic: bioinformatic challenges and solutions. *Nat Rev Genet* 15:49-55.
12. Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Møller N, Aarestrup FM. 2014. Rapid Whole-Genome Sequencing for Detection and Characterization of Microorganisms Directly from Clinical Samples. *Journal of Clinical Microbiology* 52:139-146.
13. Jain M, Olsen HE, Paten B, Akeson M. 2016. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome Biology* 17:239.
14. Lemon JK, Khil PP, Frank KM, Dekker JP. 2017. Rapid Nanopore Sequencing of Plasmids and Resistance Gene Detection in Clinical Isolates. *J Clin Microbiol* 55:3530-3543.
15. Li R, Xie M, Dong N, Lin D, Yang X, Wong MHY, Chan EW, Chen S. 2018. Efficient generation of complete sequences of MDR-encoding plasmids by rapid assembly of MinION barcoding sequencing data. *Gigascience* doi:10.1093/gigascience/gix132.
16. Quick J, Ashton P, Calus S, Chatt C, Gossain S, Hawker J, Nair S, Neal K, Nye K, Peters T, De Pinna E, Robinson E, Struthers K, Webber M, Catto A, Dallman TJ, Hawkey P, Loman NJ. 2015. Rapid draft sequencing and real-time nanopore sequencing in a hospital outbreak of *Salmonella*. *Genome Biol* 16:114.
17. Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L, Bore JA, Koundouno R, Dudas G, Mikhail A, Ouedraogo N, Afrough B, Bah A, Baum JH, Becker-Ziaja B, Boettcher JP, Cabeza-Cabrerizo M, Camino-Sanchez A, Carter LL, Doerrbecker J, Enkirch T, Dorival IGG, Hetzelt N, Hinzmann J, Holm T, Kafetzopoulou LE, Koropogui M, Kosgey A, Kuisma E, Logue CH, Mazzarelli A, Meisel S, Mertens M, Michel J, Ngabo D, Nitzsche K, Pallash E, Patrono LV, Portmann J, Repits JG, Rickett NY, Sachse A, Singethan K, Vitoriano I, Yemanaberhan RL, Zekeng EG, Trina R, Bello A, Sall AA, Faye O, et al. 2016. Real-time, portable genome sequencing for Ebola surveillance. *Nature* 530:228-232.
18. Lu H, Giordano F, Ning Z. 2016. Oxford Nanopore MinION Sequencing and Genome Assembly. *Genomics, Proteomics & Bioinformatics* 14:265-279.

19. Jain M, Koren S, Miga KH, Quick J, Rand AC, Sasani TA, Tyson JR, Beggs AD, Dilthey AT, Fiddes IT, Malla S, Marriott H, Nieto T, O'Grady J, Olsen HE, Pedersen BS, Rhie A, Richardson H, Quinlan AR, Snutch TP, Tee L, Paten B, Phillippy AM, Simpson JT, Loman NJ, Loose M. 2018. Nanopore sequencing and assembly of a human genome with ultra-long reads. *Nature Biotechnology* 36:338.
20. Noyes NR, Benedict KM, Gow SP, Booker CW, Hannon SJ, McAllister TA, Morley PS. 2015. *Mannheimia haemolytica* in feedlot cattle: prevalence of recovery and associations with antimicrobial use, resistance, and health outcomes. *J Vet Intern Med* 29:705-13.
21. Clawson ML, Murray RW, Sweeney MT, Apley MD, DeDonder KD, Capik SF, Larson RL, Lubbers BV, White BJ, Kalbfleisch TS, Schuller G, Dickey AM, Harhay GP, Heaton MP, Chitko-McKown CG, Brichta-Harhay DM, Bono JL, Smith TPL. 2016. Genomic signatures of *Mannheimia haemolytica* that associate with the lungs of cattle with respiratory disease, an integrative conjugative element, and antibiotic resistance genes. *BMC Genomics* 17:982.
22. Lubbers BV, Hanzlicek GA. 2013. Antimicrobial multidrug resistance and coresistance patterns of *Mannheimia haemolytica* isolated from bovine respiratory disease cases--a three-year (2009-2011) retrospective analysis. *J Vet Diagn Invest* 25:413-7.
23. Woolums AR, Karisch BB, Frye JG, Epperson W, Smith DR, Blanton J, Austin F, Kaplan R, Hiott L, Woodley T, Gupta SK, Jackson CR, McClelland M. 2018. Multidrug resistant *Mannheimia haemolytica* isolated from high-risk beef stocker cattle after antimicrobial metaphylaxis and treatment for bovine respiratory disease. *Veterinary Microbiology* 221:143-152.
24. CLSI. 2015. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 3rd ed, Wayne, PA.
25. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res* 27:722-736.
26. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072-5.
27. Sárközy P, Molnár V, Fogl D, Szalai C, Antal P. 2017. Beyond Homopolymer Errors: a Systematic Investigation of Nanopore-based DNA Sequencing Characteristics Using HLA-DQA2. *Periodica Polytechnica Electrical Engineering and Computer Science* 61:231-237.
28. Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv:13033997 [q-bioGN]*.
29. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078-9.
30. Hyatt D, LoCascio PF, Hauser LJ, Uberbacher EC. 2012. Gene and translation initiation site prediction in metagenomic sequences. *Bioinformatics* 28:2223-30.
31. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10:421.
32. Finn RD, Clements J, Arndt W, Miller BL, Wheeler TJ, Schreiber F, Bateman A, Eddy SR. 2015. HMMER web server: 2015 update. *Nucleic Acids Research* 43:W30-W38.
33. Finn RD, Coghill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A. 2016. The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Res* 44:D279-85.
34. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206-14.
35. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL,

- Pawlowski AC, Johnson TA, Brinkman FS, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 45:D566-d573.
36. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJV, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD. 2013. The Comprehensive Antibiotic Resistance Database. *Antimicrobial Agents and Chemotherapy* 57:3348-3357.
37. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy* 67:2640-2644.
38. Wood AR, Lainson FA, Wright F, Baird GD, Donachie W. 1995. A native plasmid of *Pasteurella haemolytica* serotype A1: DNA sequence analysis and investigation of its potential as a vector. *Res Vet Sci* 58:163-8.
39. Kehrenberg C, Catry B, Haesebrouck F, de Kruif A, Schwarz S. 2005. tet(L)-mediated tetracycline resistance in bovine Mannheimia and Pasteurella isolates. *J Antimicrob Chemother* 56:403-6.
40. Heaton MP, Harhay GP, Smith TPL, Bono JL, Chitko-McKown CG. 2015. Complete Closed Genome Sequences of a Mannheimia haemolytica Serotype A1 Leukotoxin Deletion Mutant and Its Wild-Type Parent Strain. *Genome Announcements* 3.
41. Eidam C, Poehlein A, Brenner Michael G, Kadlec K, Liesegang H, Brzuszkiewicz E, Daniel R, Sweeney MT, Murray RW, Watts JL, Schwarz S. 2013. Complete Genome Sequence of Mannheimia haemolytica Strain 42548 from a Case of Bovine Respiratory Disease. *Genome Announc* 1.
42. Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res* 14:1394-403.
43. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. 2009. Circos: an information aesthetic for comparative genomics. *Genome Res* 19:1639-45.
44. Goodwin S, McPherson JD, McCombie WR. 2016. Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews Genetics* 17:333.
45. Buermans HPJ, den Dunnen JT. 2014. Next generation sequencing technology: Advances and applications. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1842:1932-1941.
46. Katsanis SH, Katsanis N. 2013. Molecular genetic testing and the future of clinical genomics. *Nature Reviews Genetics* 14:415.
47. Vrijenhoek T, Kraaijeveld K, Elferink M, de Ligt J, Kranendonk E, Santen G, Nijman IJ, Butler D, Claes G, Costessi A, Dorlijn W, van Eyndhoven W, Halley DJJ, van den Hout MCGN, van Hove S, Johansson LF, Jongbloed JDH, Kamps R, Kockx CEM, de Koning B, Kriek M, Lekanne dit Deprez R, Lunstroo H, Mannens M, Mook OR, Nelen M, Ploem C, Rijnen M, Saris JJ, Sinke R, Sistermans E, van Slegtenhorst M, Sleutels F, van der Stoep N, van Tienhoven M, Vermaat M, Vogel M, Waisfisz Q, Marjan Weiss J, van den Wijngaard A, van Workum W, Ijntema H, van der Zwaag B, van Ijcken WFJ, den Dunnen J, Veltman JA, Hennekam R, Cuppen E. 2015. Next-generation sequencing-based genome diagnostics across clinical genetics centers: implementation choices and their effects. *European Journal Of Human Genetics* 23:1142.
48. English AC, Richards S, Han Y, Wang M, Vee V, Qu J, Qin X, Muzny DM, Reid JG, Worley KC, Gibbs RA. 2012. Mind the Gap: Upgrading Genomes with Pacific Biosciences RS Long-Read Sequencing Technology. *PLOS ONE* 7:e47768.
49. Loomis EW, Eid JS, Peluso P, Yin J, Hickey L, Rank D, McCalmon S, Hagerman RJ, Tassone F, Hagerman PJ. 2013. Sequencing the unsequenceable: Expanded CGG-repeat alleles of the fragile X gene. *Genome Research* 23:121-128.

50. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microb Genom* 3:e000132.
51. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595.
52. Harhay GP, Koren S, Phillippy AM, McVey DS, Kuszak J, Clawson ML, Harhay DM, Heaton MP, Chitko-McKown CG, Smith TP. 2013. Complete Closed Genome Sequences of *Mannheimia haemolytica* Serotypes A1 and A6, Isolated from Cattle. *Genome Announc* 1.
53. Kidanemariam Gelaw A, Bihon W, Faranani R, Mafofo J, Rees J, Madoroba E. 2015. Complete Genome Sequence of *Mannheimia haemolytica* Strain Mh10517, Isolated from Sheep in South Africa. *Genome Announcements* 3.
54. Mann S, Chen Y-PP. 2010. Bacterial genomic G+C composition-eliciting environmental adaptation. *Genomics* 95:7-15.
55. Canchaya C, Fournous G, Brüssow H. 2004. The impact of prophages on bacterial chromosomes. *Molecular Microbiology* 53:9-18.
56. Lassalle F, Périan S, Bataillon T, Nesme X, Duret L, Daubin V. 2015. GC-Content Evolution in Bacterial Genomes: The Biased Gene Conversion Hypothesis Expands. *PLOS Genetics* 11:e1004941.
57. Ashton PM, Nair S, Dallman T, Rubino S, Rabsch W, Mwaigwisya S, Wain J, O'Grady J. 2015. MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. *Nat Biotechnol* 33:296-300.
58. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963.
59. Utturkar SM, Klingeman DM, Hurt RA, Brown SD. 2017. A Case Study into Microbial Genome Assembly Gap Sequences and Finishing Strategies. *Frontiers in Microbiology* 8:1272.
60. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563-9.
61. Folster JP, Tolar B, Pecic G, Sheehan D, Rickert R, Hise K, Zhao S, Fedorka-Cray PJ, McDermott P, Whichard JM. 2014. Characterization of blaCMY plasmids and their possible role in source attribution of *Salmonella enterica* serotype Typhimurium infections. *Foodborne Pathog Dis* 11:301-6.
62. Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. 2006. Molecular Epidemiology of Ceftiofur-Resistant *Escherichia coli* Isolates from Dairy Calves. *Applied and Environmental Microbiology* 72:3940-3948.
63. Köser CU, Ellington MJ, Peacock SJ. 2014. Whole-genome sequencing to control antimicrobial resistance. *Trends in Genetics* 30:401-407.
64. Stoesser N, Batty EM, Eyre DW, Morgan M, Wyllie DH, Del Ojo Elias C, Johnson JR, Walker AS, Peto TEA, Crook DW. 2013. Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data. *Journal of Antimicrobial Chemotherapy* 68:2234-2244.
65. Zankari E, Hasman H, Kaas RS, Seyfarth AM, Agersø Y, Lund O, Larsen MV, Aarestrup FM. 2013. Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. *Journal of Antimicrobial Chemotherapy* 68:771-777.
66. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. 2009. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clinical Infectious Diseases* 49:1749-1755.

67. Andrews JM. 2001. The development of the BSAC standardized method of disc diffusion testing. *J Antimicrob Chemother* 48 Suppl 1:29-42.
68. Bulik CC, Fauntleroy KA, Jenkins SG, Abuali M, LaBombardi VJ, Nicolau DP, Kuti JL. 2010. Comparison of meropenem MICs and susceptibilities for carbapenemase-producing *Klebsiella pneumoniae* isolates by various testing methods. *J Clin Microbiol* 48:2402-6.
69. Jorgensen JH, Ferraro MJ. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis* 49:1749-55.
70. Wang L, Yu Z. 2012. Antimicrobial Resistance Arising from Food-Animal Productions and Its Mitigation. InTech, Rijeka, Croatia.
71. Staley JT, Konopka A. 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu Rev Microbiol* 39:321-46.
72. Adam D, Maciejewska M, Naômé A, Martinet L, Coppieters W, Karim L, Baurain D, Rigali S. 2018. Isolation, Characterization, and Antibacterial Activity of Hard-to-Culture Actinobacteria from Cave Moonmilk Deposits. *Antibiotics* 7:28.
73. Chitsaz H, Yee-Greenbaum JL, Tesler G, Lombardo MJ, Dupont CL, Badger JH, Novotny M, Rusch DB, Fraser LJ, Gormley NA, Schulz-Trieglaff O, Smith GP, Evers DJ, Pevzner PA, Lasken RS. 2011. Efficient de novo assembly of single-cell bacterial genomes from short-read data sets. *Nat Biotechnol* 29:915-21.
74. Rasko DA, Webster DR, Sahl JW, Bashir A, Boisen N, Scheutz F, Paxinos EE, Sebra R, Chin CS, Iliopoulos D, Klammer A, Peluso P, Lee L, Kislyuk AO, Bullard J, Kasarskis A, Wang S, Eid J, Rank D, Redman JC, Steyert SR, Frimodt-Moller J, Struve C, Petersen AM, Krogfelt KA, Nataro JP, Schadt EE, Waldor MK. 2011. Origins of the *E. coli* strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med* 365:709-17.
75. Fang G, Munera D, Friedman DI, Mandlik A, Chao MC, Banerjee O, Feng Z, Losic B, Mahajan MC, Jabado OJ, Deikus G, Clark TA, Luong K, Murray IA, Davis BM, Keren-Paz A, Chess A, Roberts RJ, Korlach J, Turner SW, Kumar V, Waldor MK, Schadt EE. 2012. Genome-wide mapping of methylated adenine residues in pathogenic *Escherichia coli* using single-molecule real-time sequencing. *Nat Biotechnol* 30:1232-9.
76. Afshinnkoo E, Chou C, Alexander N, Ahsanuddin S, Schuetz AN, Mason CE. 2017. Precision Metagenomics: Rapid Metagenomic Analyses for Infectious Disease Diagnostics and Public Health Surveillance. *Journal of Biomolecular Techniques : JBT* 28:40-45.
77. Forbes JD, Knox NC, Ronholm J, Pagotto F, Reimer A. 2017. Metagenomics: The Next Culture-Independent Game Changer. *Frontiers in Microbiology* 8.

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624 **Tables**

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626 Table 1. Phenotypic susceptibility testing results for bacterial strains *M. haemolytica* OADDL-1 and *M. haemolytica* OADDL-2.

CLSI Group	Antibacterial Name	Antibacterial Class	<i>M. haemolytica</i> OADDL-2	<i>M. haemolytica</i> OADDL-1
A	Spectinomycin	Aminocyclitol	R (> 64)	S (= 16)
A	Ceftiofur	Cephalosporin	S (≤ 0.25)	S (≤ 0.25)
A	Tilmicosin	Macrolide	R (> 64)	S (≤ 4)
A	Tulathromycin	Macrolide	R (> 64)	S (= 4)
A	Penicillin	Penicillin	R (> 8)	S (= 0.25)
A	Florfenicol	Phenicol	R (> 8)	S (= 0.5)
A	Danofloxacin	Fluoroquinolone	NI (> 1)	S (≤ 0.12)
A	Enrofloxacin	Fluoroquinolone	R (> 2)	S (≤ 0.12)
A	Oxytetracycline	Tetracycline	R (> 8)	S (≤ 0.5)
B	Sulfadimethoxine	Sulfonamide	NI (> 256)	NI (> 256)
B	Ampicillin	Penicillin	NI (> 16)	NI (≤ 0.25)
C	Tylosin	Macrolide	NI (> 32)	NI (= 32)
D	Gentamicin	Aminoglycoside	NI (> 16)	NI (= 2)
D	Sulfamethoxazole Trimethoprim	Potentiased Sulfa	NI (≤ 2)	NI (≤ 2)
Ungrouped	Clindamycin	Lincosamide	NI (> 16)	NI (= 8)

627 **R** = Resistant, **S** = Susceptible, **NI** = No interpretation in CLSI group

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630 Table 2. 1D base-called read statistics from Albacore for both *Mannheimia haemolytica* strains.

	Total Reads	Mean (bp)	Median (bp)	Min (bp)	Max (bp)	N25	N50	N75
<i>M. haemolytica</i> OADDL-1								
FAB 42646	27,304	7,562	5,267	210	77,585	19,083	11,974	6,444
FAB 42472	26,174	6,969	4,818	69	65,212	17,903	10,837	5,831
<i>M. haemolytica</i> OADDL-2								
FAB 42646	14,132	11,611	9,132	241	75,460	23,851	17,018	9,985
FAB 42472	14,030	10,629	8,665	249	68,470	22,657	15,722	8,982

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638 Table 3. Assembly statistics generated by Canu and improved by Nanopolish.

	Number of contigs	Total length	Total length polished	N50	GC content (%)
<i>M. haemolytica</i> OADDL-1	1	2,644,744 bp	2,656,520 bp	-	41.19
<i>M. haemolytica</i> OADDL-2	5	2,804,188 bp	2,815,485 bp	2,381,899 bp	41.24

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Table 4. Unique AMR genes found in *M. haemolytica* OADDL-2 using the RAST annotation web server.

Gene Abbreviations	Functional Role
BL	Beta-lactamase (EC 3.5.2.6)
BLc	Beta-lactamase class C and other penicillin binding proteins
BLI	Metal-dependent hydrolases of the beta-lactamase superfamily I
BLR	Negative regulator of beta-lactamase expression
bl	Beta-lactamase
BLII	Metal-dependent hydrolases of the beta-lactamase superfamily II
BLIII	Metal-dependent hydrolases of the beta-lactamase superfamily III
BLA	Beta-lactamase class A
CTX	Beta-lactamase CTX-M-16
blaR	Regulatory protein BlaR1
blaI	Beta-lactamase repressor BlaI
BLD	Beta-lactamase class D
OXA	Beta-lactamase OXA-18 precursor (EC 3.5.2.6)
Top	DNA Topoisomerase III (EC 5.99.1.2)
AmpS	Beta-lactamase AmpS
gyrase	DNA gyrase subunit A and B (EC 5.99.1.3)
PSE2	Beta-lactamase PSE-2 precursor (EC 3.5.2.6)
ybxI	Probable beta-lactamase ybxI precursor (EC 3.5.2.6)
CS	Beta-lactamase (Cephalosporinase) (EC 3.5.2.6)
AadA1	Streptomycin 3"-O-adenylyltransferase (EC 2.7.7.47)
AadA2	Spectinomycin 9-O-adenylyltransferase
N6'-ac	Aminoglycoside N6'-acetyltransferase (EC 2.3.1.82)

Table 5. Identification of AMR genes with ResFinder and CARD databases.

AMR Genes	Database	Antibiotic Class	<i>M. haemolytica</i> OADDL-2	<i>M. haemolytica</i> OADDL-1	<i>M. haemolytica</i> 89010807N	<i>M. haemolytica</i> M42548
ant(2'')-Ia_18	ResFinder	Aminoglycoside	Present	-	-	-
aadA8b_1	ResFinder	Aminoglycoside	Present	-	-	-
blaOXA-2_1	ResFinder	Beta-lactam	Present	-	-	-
msr(E)_4	ResFinder	MLSB	Present	-	-	-
mph(E)_3	ResFinder	Macrolide	Present	-	-	-
catB4_1	ResFinder	Phenicol	Present	-	-	-
tet(H)_3	ResFinder	Tetracycline	Present	-	Present	Present
blaROB-1_1	ResFinder	Beta-lactam	Present	-	-	-
blaROB-1_1	ResFinder	Beta-lactam	Present	-	-	-
aph(3')-Ia_1	ResFinder	Aminoglycoside	Present	-	-	Present
aph(6)-Id_1	ResFinder	Aminoglycoside	Present	-	-	Present
aph(3'')-Ib_3	ResFinder	Aminoglycoside	Present	-	-	-
sul2_11	ResFinder	Sulfonamide	Present	-	-	-
floR_2	ResFinder	Phenicol	Present	-	-	-
erm(42)_2	ResFinder	MLSB	Present	-	-	-
strA_1	ResFinder	Aminoglycoside	-	-	-	Present
sul2_2	ResFinder	Aminoglycoside	-	-	-	Present
ANT(2'')-Ia	CARD	Aminoglycoside	Present	-	-	-
aadA	CARD	Aminoglycoside	Present	-	-	-
aadA25	CARD	Streptomycin/Spectinomycin	Present	-	-	-
OXA-15	CARD	Beta-lactam	Present	-	-	-
msrE	CARD	Erythromycin/Streptogramin	Present	-	-	-
mphD	CARD	Macrolide	Present	-	-	-
tet(H)	CARD	Tetracycline	Present	-	Present	Present
ROB-1	CARD	Beta-lactam	Present	-	-	-
macB	CARD	Macrolide	Present	Present	-	-
APH(3')-Ia	CARD	Aminoglycoside	Present	-	-	Present
APH(6)-Id	CARD	Aminoglycoside	Present	-	-	Present
APH(3'')-Ib	CARD	Aminoglycoside	Present	-	-	Present
sul2	CARD	Sulfonamide	Present	-	-	Present
floR	CARD	Chloramphenicol	Present	-	-	-
Erm(42)	CARD	MLSB	Present	-	-	-

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653 Table 6. Assembly quality assessment of sequencing read subsets; results of *M. haemolytica* OADDL-1.

Subsample	Average base-pair length (std. dev.)	Contig count	Average N50 (std. dev.)	GC Content (%)	Ave. Tot. Mismatches (std. dev.)
1/10 th of reads	2,628,537 (36,605)	19 - 24	351,563 (224,531)	41.2 – 41.3	30,993.6 (531.6)
1/6 th of reads	2,624,083 (9,575)	4 - 10	1,292,922 (518,613)	41.2 – 41.2	20,988.0 (131.9)
2/6 th of reads	2,668,633 (13,312)	2 - 5	1,864,819 (652,239)	41.2 – 41.2	17,233.4 (231.7)
3/6 th of reads	2,662,841 (13,994)	1 - 4	2,610,430 (41,359)	41.2 – 41.2	15,802.6 (365.1)
4/6 th of reads	2,660,579 (14,754)	1 - 4	2,576,912 (71,524)	41.2 – 41.2	14,632.6 (185.2)
5/6 th of reads	2,650,805 (16,078)	1 - 3	2,589,962 (111,922)	41.2 – 41.2	13,939.0 (181.4)
Mean	2,649,246 (25,931)	-	-	-	18,931.5 (271.2)

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660 Table 7. Assembly quality assessment of sequencing read subsets and AMR gene identification; results of *M. haemolytica* OADDL-2.

Subsamples	Average base-pair length (std. dev.)	Contig count	Average N50 (std. dev.)	GC Content (%)	Plasmid (Y/N)	AMR genes ResFinder (mean)	AMR genes CARD (mean)	Ave. Tot. Mismatches (std. dev.)
1/10 th of reads	2,619,204 (43,898)	29 - 40	133,657 (9,800)	41.2 - 41.3	Y	15-17 (15.6)	14-16 (15)	37,875.2 (599.8)
1/6 th of reads	2,800,552 (16,129)	6 - 20	606,852 (471,233)	41.3 - 41.3	Y	15-17 (15.6)	14-16 (15)	25,032.6 (559.5)
2/6 th of reads	2,806,768 (16,792)	4 - 6	2,178,862 (407,453)	41.2 - 41.3	Y	15-16 (15.4)	14-15 (14.6)	17,772.2 (292.8)
3/6 th of reads	2,792,790 (14,509)	3 - 6	2,197,805 (532,021)	41.2 - 41.3	Y	15-17 (15.6)	14-15 (14.6)	15,962.6 (145.8)
4/6 th of reads	2,844,871 (40,342)	3 - 8	2,254,699 (412,158)	41.2 - 41.3	Y	14-16 (15)	14-15 (14.6)	14,807.2 (221.7)
5/6 th of reads	2,803,710 (16,678)	3 - 4	2,602,409 (349,320)	41.3 - 41.3	Y	15-16 (15.2)	14-15 (14.4)	14,064.8 (173.2)
Mean	2,777,982 (24,724)	-	-	-	-	15.4	14.7	20,919.1 (332.1)

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FIG 1.

FIG 2.