

Title: Whole genome shotgun sequencing from chicken clinical tracheal samples for bacterial and novel bacteriophage identification

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Interpretive Summary: Diagnostic testing of clinical samples is critical to providing client care for food animals. Current submission procedures rely on culturing of samples to determine the causative agent. Newer technologies, such as whole genome sequencing (WGS), offer a faster and more precise mechanism to identify pathogens in a sample. In these studies, we directly identified novel bacteria and viruses from direct DNA sequencing of clinical samples of chickens undergoing respiratory distress. Results were obtained in days following submission compared to weeks that it can take to culture organisms. These studies demonstrate WGS can be a favorable method for pathogen identification in clinical cases.

Technical Abstract: A whole-genome shotgun sequencing (WGS) approach was applied to chicken clinical tracheal swab samples during metagenomics investigations to identify possible microorganisms in poultry with respiratory disease. After applying WGS, *Ornithobacterium rhinotracheale* (ORT) and a putative phage virus candidate were found in one of the swab samples. Multi-locus sequence typing (MLST) scheme of ORT genome involved *adk*, *aroE*, *fumC*, *gdhA*, *pgi*, and *pmi* genes. Antibiotic resistant analysis

demonstrated tetracycline-resistant ribosomal protection protein, tetQ, aminoglycoside-(3)-acetyltransferase IV gene, aminoglycoside antibiotic inactivation and macrolide resistance, ermX gene, in the ORT genome. A putative phage virus candidate was predicted by Prophage Hunter and PFAST, while BLAST analyses were utilized to identify genes encoding bacteriophage proteins. Interestingly, genes encoding endolysins were detected in bacteriophage genomes. The gene products encoded in the prophage sequence were most closely related to bacteriophages in the N4-like family among the Podoviridae in the Caudovirales. This study demonstrates the potential of WGS for rapid detection and characterization of etiologic agents found in clinical samples.