Metagenomic Analysis using Real-Time DNA Sequence Analysis as a Diagnostic Tool for Naturally-Occurring Urinary Tract Infections in Dogs

Lauren Achille, BS, MLAS, RLAT[1*]; Annika Linde, DVM, PhD, MPH[2]; Janina Krumbeck, PhD[3]; Gustavo A. Ramirez, PhD[1,4]; J. Santiago Aguilar, PhD[1]; David Forster, DVM, MRCVS, MAICS[1]; Tonatiuh Melgarejo, DVM, MS, PhD[1,2#].

[1]Western University of Health Sciences, CVM, Pomona, CA; [2]CePahtli Research Alliance, Claremont, CA; [3]MiDOG, LLC, Irvine, CA; [4]University of North Carolina, Chapel Hill, NC

*Corresponding Author: lauren.achille@westernu.edu; #Faculty Mentor (Summer 2019)

Background & Rationale

Urinary tract infections (UTIs) are a common type of pathology in human as well as small animal medicine, where it often afflicts canine patients. Recurrent UTIs are not only uncomfortable for the canine patients, but also constitute a therapeutic challenge because of delays in diagnostic microbiology. Consequently, patients with a clinical UTI diagnosis will typically be discharged with empirical antibiotic treatment until laboratory results become available. This situation is currently unavoidable due to the time lag between routine requests for urinalysis (UA), urine culture and sensitivity tests, and availability of laboratory results - which are typically released to the clinician within 3-5 days. This delay constitutes an important problem in the small animal clinic, because UTI cases often are discharged with an ineffective choice of antibiotics while awaiting lab results, which may fuel antimicrobial resistance in pathogens. This pilot study was focused on addressing some of the inadequacies of treating UTIs in dogs by using a portable, highly efficient, feasible, and integrated approach for pathogen detection. The MinION (Oxford Nanopore Technologies (ONT), Oxford, UK) is a new, palm-sized, DNA sequencer that has recently been used for accelerated detection of clinically relevant (difficult to diagnose/culture) human bacterial pathogens in less than two hours post urine collection. Consequently, we expect this technology can be translated for clinical use in a veterinary medicine framework. We also used the Illumina MiSeq (MiDOG LLC) technology to compare microbial analyses data [the poster presents the Illumina MiSeq data only].

Hypothesis & Objectives

Hypothesis: Real-time analysis of metagenomic DNA sequencing, using the Illumina MiSeq from MiDOG LLC (or MinION from ONT), will enable accelerated and accurate identification of UTI pathogens (including antibiotic resistant plasmids) from canine clinical urine samples, and consequently allow for refinement of antibiotic therapy.

Objectives: 1) Amplify and sequence DNA purified from urine samples collected from dogs presented with clinical signs consistent with UTI; 2) Identify and classify bacterial taxa from infected urine samples using the Illumina MiSeq and MinION technologies; 3) Establish the urine microbiome, and its antimicrobial resistant plasmids, in the study subjects; and 4) Compare the urine metagenomic analysis with the culture and sensitivity results obtained from a commercial laboratory.

Design & Methods

Seven dogs presenting with signs of UTI (reports of polyuria, dysuria, hematuria, cloudy/malodorous urine, polydipsia, excessive licking of genital area, breaking housetraining, incontinence) and five controls were included in the study. A total of 10 ml of urine was collected from each dog (5 ml was submitted for culture and sensitivity, 5 ml was used for urinary metagenomic analysis at WesternU). A Quick DNA Urine Kit and an Illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit were used for DNA isolation and amplification. Urinary microbiome was established using the Illumina MiSeq and the MinION DNA sequencing methodologies from MiDOG LLC and ONT, respectively (only Illumina MiSeq data is presented here). Bacterial taxa and antimicrobial resistance plasmids were recorded from each urine sample. Data originated from third generation DNA sequencing was also compared with urine culture and sensitivity test results from a commercial diagnostic laboratory.

1. Sample Collection
2. DNA Isolation
3. DNA Amplification
4. DNA Sequencing & Taxonomic Identification (using Illumina & MinION technologies)

Results

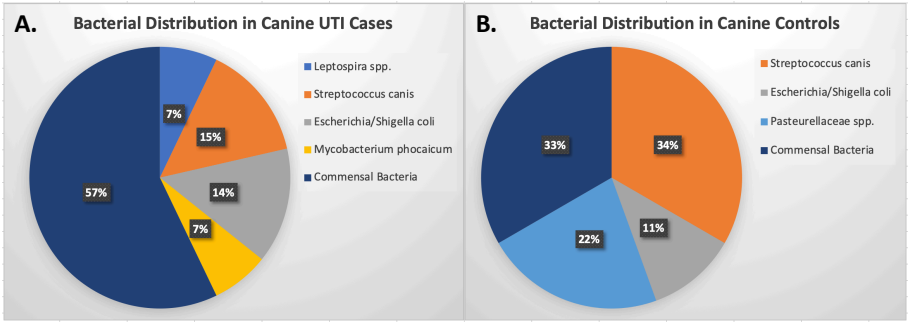

Figure 1 – Pathogenic bacteria (species shown in panel A & B) were identified in urine samples from 86% of dogs with signs of urinary tract infection (UTI), as well as 80% of asymptomatic controls. Bacterial species were detected a total of fourteen and nine times in UTI cases (n = 7) and controls (n = 5), respectively, with varying relative abundance of bacteria across urine samples (not shown). The figure illustrates the percentage of times that pathogenic bacteria (distributed across 3-4 different species) and commensal bacteria were detected in UTI cases (A) vs. controls (B) out of total no. of occurrences.

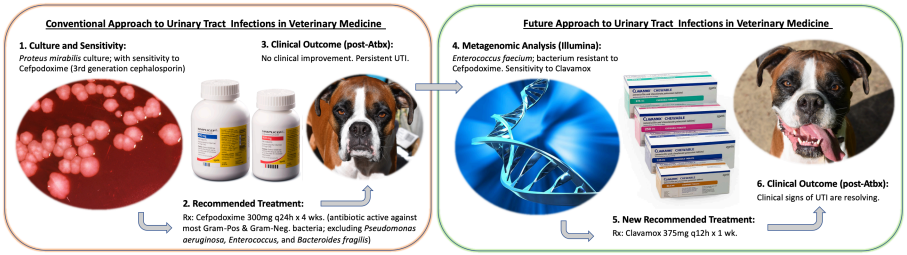

Figure 2 – Example of clinical application of metagenomic analysis in management of a canine urinary tract infection case (K9 UTI-07). An 8-year-old female spayed Boxer presented with a seven days history of UTI signs. Prescribed Cefpodoxime based on culture & sensitivity testing, without clinical improvement. Antibiotic selection revised to Clavamox based on metagenomic data, followed by gradual resolution of UTI signs and clinical improvement.

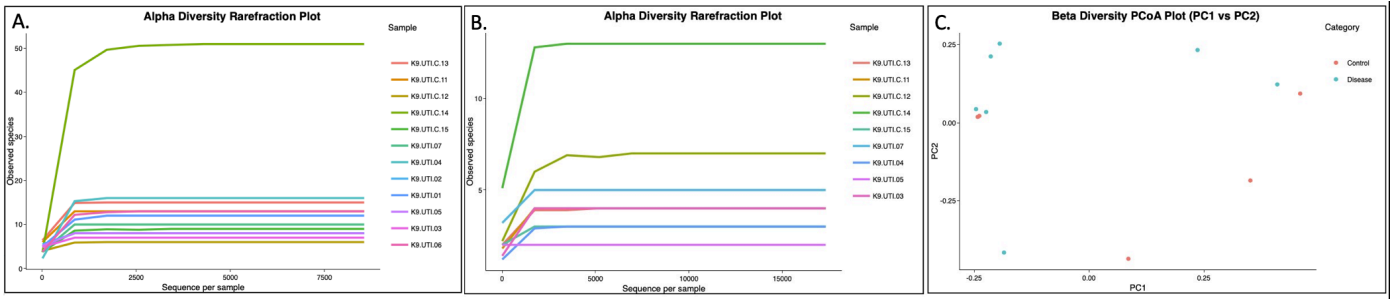

Figure 3 – **Panel A**: represents *bacterial* diversity of individual urine samples, including dogs exhibiting UTI signs (samples 1-7) as well as asymptomatic controls (samples 11-15). **Panel B**: represents *fungal* diversity of individual urine samples from five controls and four dogs with UTI signs. While *alpha-diversity* measures microbial diversity in individual samples (panel A & B), *beta-diversity* (panel C) measures microbial diversity between different samples. **Panel C**: Principal Coordinate Analysis (PCoA), calculated using Bray-Curtis dissimilarity, shows no evidence of any distinct clustering patterns between groups (dogs with UTI vs. asymptomatic controls). The PCoA is used to evaluate microbial structure diversity between different samples. Each dot represents the whole microbial composition profile in a given biological sample (samples with similar profiles would, as such, be closer to each other on the beta diversity plot).

Discussion & Conclusion

Our results indicate that contrary to general belief in veterinary medicine, the canine urinary system is not a sterile environment (Figure 3). Both symptomatic (UTI positive) and clinically healthy dogs host pathogenic bacteria incl. *Streptococcus canis*, *Escherichia/Shigella coli*, and Pasteurella Spp. (Figure 1). Interestingly enough, even though these species were detected in both UTI positive dogs and control samples, clinically healthy dogs did not show signs of infection. Culture results were negative in several urine samples, however, the Illustra DNA amplification kit is sensitive enough to amplify even minute quantities of bacterial DNA in a sample. Our results show bacterial and fungal species ranging in quantities of 5-50, and 2-13 per urine sample, respectively. In clinical practice, when urine samples on dogs suspected of having UTIs are cultured with a negative result, access to the MiDOG (or MinION) technology would provide a more comprehensive analysis.

The need for this technology in veterinary medicine is demonstrated by our clinical case (Figure 2), a patient who was prescribed an antibiotic that was not effective in treating the type of bacteria identified in her urine sample. However, the results from her MiDOG report suggest that one of the species of bacteria present in her urine may contain a plasmid for an intrinsic resistance to the antibiotic she was prescribed. If metagenomic analysis had been applied during her initial sampling, a different antibiotic choice could have been selected prior to discharge.

Acknowledgements & References

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