Next-Generation Sequencing in Companion Animal Practice for Infectious Disease Diagnostics and Characterizing Normal Microbiomes

Nora Jean Nealon, DVM, PhD

KEYWORDS

• Microbiome • 16S rRNA • Whole genome sequencing • Metagenome sequencing • Quantitative PCR (qPCR)

KEY POINTS

- Next-generation sequencing diagnostics are noteworthy for their high-througput capacity and rapid processing times.
- Uses for next-generation sequencing in veterinary medicine include infectious disease monitoring and microbiome profiling diagnostics.
- Due in part to the surge in medical research and consumer interest in the microbiota, there is increased interest in companion animal microbiome profiling diagnostics.
- The relative cost, ease of interpretation, and turnaround time are important factors to consider when selecting the ideal next generation sequencing test.
- Practitioners need to consider the limitations of each next-generation sequencing method when recommending one as a diagnostic tool and when interpreting results.

INTRODUCTION

Small animal practice is becoming increasingly specialized, including in the techniques and expertise that clinicians need for performing and interpreting diagnostics.[1] The past decade has seen rapid advancements in the technology by which veterinarians detect and diagnose microbial and infectious diseases with methodologies called next-generation sequencing (NGS), which allow for the rapid analysis of large numbers of DNA and RNA sequences.[2] At the same time, NGS-driven research examining the microbiome, the collection of microorganisms living on or within canine and feline

Department of Veterinary Biomedical Sciences, Shreiber School of Veterinary Medicine, Rowan University, South Harrison, NJ, USA
E-mail address: nealon@rowan.edu

Vet Clin Small Anim 56 (2026) 1323–1336
https://doi.org/10.1016/j.cvsm.2026.06.005 **vetsmall.theclinics.com**
0195-5616/26/© 2026 Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Abbreviations	
BLAST	basic local alignment search tool
cDNA	complementary DNA
NGS	next-generation sequencing
rRNA	ribosomal RNA

patients, has illustrated the critical roles that the microbiome plays in health and disease.[3]

It is important for clinicians to develop a basic understanding of NGS methodologies, especially as these tools become integrated into patient assessments. To address this need, this review will provide a comparative overview of the most common NGS methodologies in small animal medicine, discuss what each identifies, and describe how each works, with a focus on the detection and evaluation of bacterial diseases, which are where these methodologies are the most widely used and best developed. To put this information into clinical context, this review will additionally discuss the strengths and limitations of each methodology relevant to veterinary practice. Collectively, these considerations will help to guide clinicians with test selection and results interpretation for NGS methodologies.

WHAT IS NEXT-GENERATION SEQUENCING?

Diagnostic development has been guided by the clinical need for rapid, sensitive, specific, and cost-effective tests. Simultaneously, consumer, clinician, and scientific interests have pushed for the integration of microbiome diagnostics into veterinary.[4] A growing area of research addressing these needs uses NGS methodologies. NGS includes methodologies that allow for the sequencing of prokaryotic and/or eukaryotic nucleotides. Genetic sequencing refers to the methodologies that determine the order of nucleotides in DNA and RNA. These sequencing methodologies include Sanger sequencing and Maxam-Gilbert sequencing (first-generation), Roche-454 sequencing, Illumina sequencing, and solid sequencing (second-generation), and long-read sequencing (third-generation). While first-generation sequencing processes a single fragment of nucleotides with each experimental run, second and third generation sequencing processes millions of nucleotide fragments simultaneously.[2,5] Because of this high-throughput, high-output capacity, these newer second and third generation sequencing methodologies are collectively referred to as NGS.

TYPES OF NEXT-GENERATION SEQUENCING AND THEIR APPLICATIONS TO SMALL ANIMAL PRACTICE

Since its creation, NGS has been increasingly applied to clinical cases. In veterinary medicine, NGS assists with infectious disease diagnosis, identifies new and emerging pathogens, and helps to monitor responses to treatment especially those that modulate the gut microbiome.[6–9] Veterinary medicine is seeing a rise in NGS methodologies that allow clinicians and owners to profile a pet's fecal microbiome.[3,4] To establish these endpoints, the common and emergent NGS methods used in small animal veterinary medicine include whole genome sequencing, 16S ribosomal RNA (rRNA) sequencing, shotgun metagenomic sequencing, and transcriptomic sequencing.[3,6,7,9] Each of these methods is discussed below. Additionally, **Fig. 1** provides a graphical overview of each NGS technique and **Fig. 2** highlights clinical applications of NGS techniques in small animal medicine. **Table 1** summarizes the clinical care points

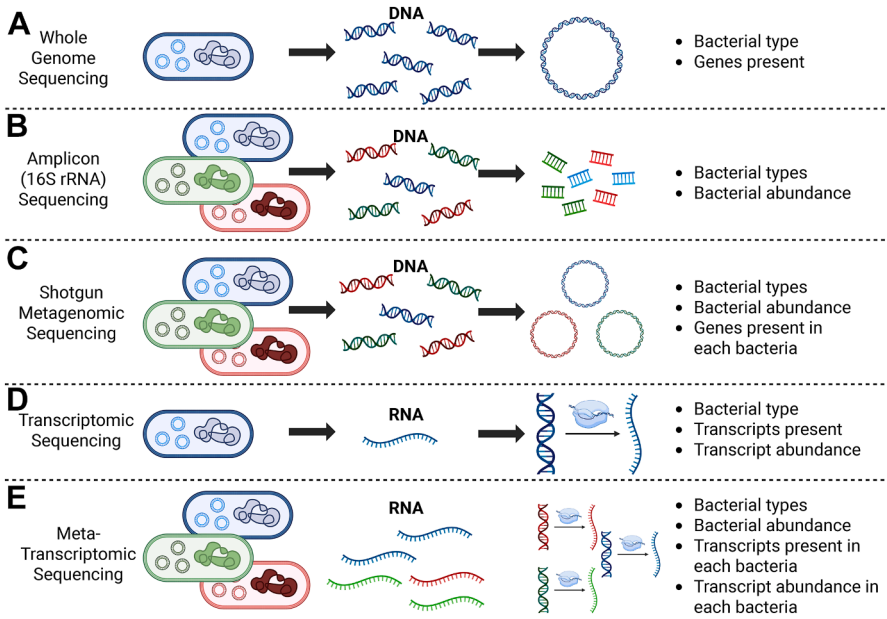


Fig. 1. Methodologies underlying the routine and emergent bacterial next generation. Sequencing methodologies used in small animal medicine. (*A*) Whole genome sequencing evaluates all DNA in a single bacterial species or strain. This methodology provides information on the bacteria's taxonomic identity as well as the genes it carries. (*B*) Amplicon (16S rRNA) sequencies compares the 16S rRNA gene of DNA for all bacteria in a sample. This methodology provides information on taxonomy and abundance. (*C*) Shotgun metagenomics compares all the DNA for all bacteria in a sample. This methodology provides taxonomy, the abundance of each taxon, and the genes each bacterium carries. (*D*) Transcriptomics measures the RNA in a single bacterial species or strain. This methodology provides information on gene expression. (*E*) Metatranscriptomics compares all the RNA for all bacteria in a sample. This methodology provides taxonomy and abundance for each taxon and indicates the genes each taxon is expressing. (*Created in* BioRender. Nealon NJ. (2026) https://BioRender.com/dghl4k0.)

and includes an overview of each methodology, clinical applications, advantages, and limitations of each technique.

Whole Genome Sequencing

Whole genome sequencing characterizes the entire DNA in a single organism. In small animal patients, this often includes bacteria isolated from a urine sample, fecal sample, or skin swab.[10–12] Following extraction, DNA is prepared for genome sequencing. To turn DNA fragments into an assembled genome, computational and statistical algorithms are applied to the sequencing files, often using supercomputing resources designed to rapidly process large file sizes.[13] While some workflows assemble sequences based on known species sequences, de novo approaches use mathematical modeling and do not rely on reference sequences.[14] Bacterial genomes often contain extrachromosomal DNA called plasmids, which have clinical implications in the spread of antimicrobial resistance genes between bacteria.[15] Consequently, NGS workflows developed tools allowing for the assembly and identification of plasmids that bacteria may be harboring.[16]

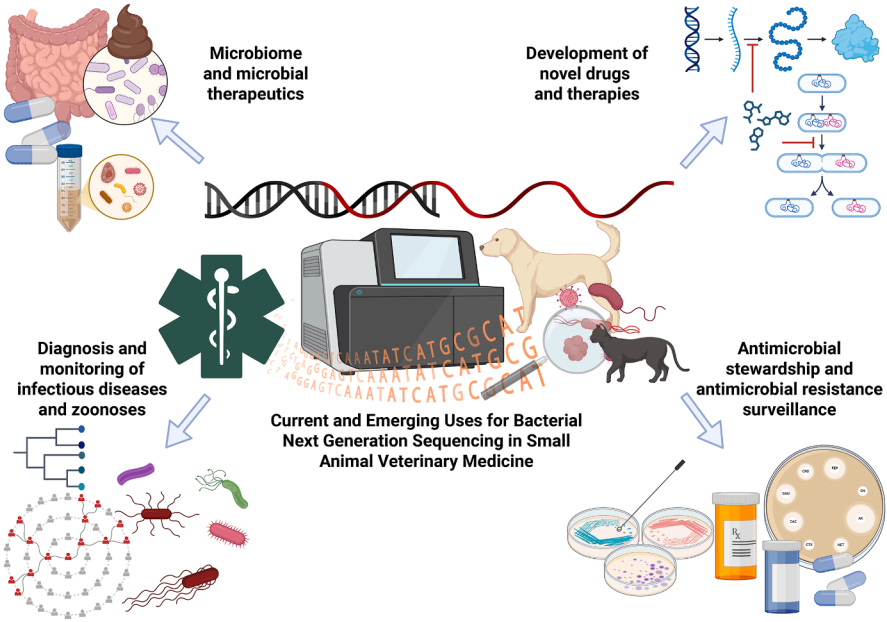


Fig. 2. Existing and emergent uses for bacterial NGS methodologies in small animal veterinary medicine. Bacterial NGS methodologies have multiple existing and emerging uses in small animal medicine. Clinically, these methodologies help to develop and interpret the microbiome diagnostics used in patient disease management and/or health monitoring, including with consumer microbiome test kits. During disease outbreaks, NGS can inform contract tracing and identify etiologic agents, including for polymicrobial or atypical bacterial diseases. NGS is routinely used in hospital disease surveillance and antimicrobial stewardship initiatives to improve antimicrobial use and patient safety. Ongoing research additional incorporates NGS methodologies to identify and test novel therapeutics for microbiome manipulation and/or for bacterial disease treatments. (*Created in* BioRender. Nealon NJ. (2026) https://BioRender.com/obst3f7.)

Two important and common clinical questions with whole genome sequencing data include identifying what organism is present and/or what genes it carries. To address these, assembled genomes can be compared to libraries of reference genomes and gene sequences. The process of aligning DNA sequences from a sample with those in a reference library is referred to as *basic local alignment search tool* (BLAST).[17] This approach is often used in outbreak investigations, especially those with the potential to infect animals and people.[18–20] During these scenarios, whole genome guided taxonomic identifications are often sensitive enough to determine the species, strain, and strain divergence across cases, letting clinicians implement appropriate preventive and treatment measures.[21–23] In veterinary medicine, BLAST searches on whole genome sequencing samples have been used in hospital surveillance programs that monitor antimicrobial resistant pathogens.[24–26] Following environmental sampling, bacterial culture, and genome sequencing, genes associated with resistance to high-priority antimicrobials (eg, carbapenem resistance) are BLAST searched in the genomes of clinically concerning isolates.[24] The presence of these genes then informs hospital-wide disinfection and patient management protocols.[24,27,28]

When compared to other methodologies, whole genome sequencing is relatively inexpensive, with an average sample cost of ~$200.00.[29] However, practitioners

Table 1
Advantages and limitastions of microbial next generation sequencing techniques in small animal practice

Technique	Description	Examples of Current and Future Uses in Small Animal Practice	Advantages	Limitations	References
Whole genome sequencing	Analysis of the entire genome and/or the plasmids of a microorganism	• Infectious disease outbreak investigations, including when diseases represent high morbidity, mortality and/or zoonotic public health risks • Hospital pathogen surveillance plans (eg, screening environments for pathogens that carry antimicrobial resistance genes of clinical importance)	• Identify the microbe down to species or strain level • Identify presence vs absence of clinically important genes (eg, those that confer antimicrobial resistance or pathogenicity)	• Moderately expensive • Cannot directly infer microbial function (eg, pathogenicity) • Time and resource intensive (longer turnaround times, access to supercomputing resources)	13,16,19,21,23,29,30,54
Amplicon (16S rRNA) Sequencing	Analysis of one or more regions of the 16S rRNA genes in a microbiome	• Commercial canine and feline gastrointestinal and oral health test kits (eg, AnimalBiomeBasePaws, owned by Zoetis, Purina Petivity) • Assessing fecal microbiota transplant engraftment • Establishing the links between microbiome composition and health vs disease for the development of novel diagnostics and treatments	• Identify types of microbes and their relative abundances in a sample. • Serial monitoring of patient response to treatment or disease progression • Relatively inexpensive	• Does not provide absolute quantities or concentrations of microbes • Cannot directly infer microbial function (eg, pathogenicity) • Limited resolution for genus and species-level taxonomic identifications • Significant variability in sample processing and analysis methods may impact data interpretation and clinical translation	4,9,46–48,50–52

(*continued on next page*)

Table 1
(*continued*)

Technique	Description	Examples of Current and Future Uses in Small Animal Practice	Advantages	Limitations	References
Shotgun metagenomic sequencing	Analysis of whole genomes in a microbiome	• Expand interpretation of amplicon sequencing datasets, including those used to interpret patient clinical health, such as gut microbiota test kits • Adjunctive screening test for infectious disease outbreak investigations, especially in cases where potential polymicrobial, atypical, and/or unknown etiologic agents may be driving the outbreak	• Identify types of microbes in a sample along with their relative abundance and the genes they carry • Drive mechanistic research into gene and disease association, especially for health conditions associated with dysbiosis	• Expensive • Time and resource intensive (longer turnaround times, access to supercomputing resources) • Cannot directly infer microbial function (eg, pathogenicity)	36,53,55,56,63
Transcriptome and metatranscriptome sequencing	Analysis of the RNA sequences produced by one microorganism during transcription (transcriptomics) or those produced by a microbiome (metatranscriptomics)	• Further assessment of pathogens and opportunistic pathogens (examine samples for expression of virulence factors and/or antimicrobial resistance genes) • Identify novel targets for antimicrobial therapeutics (eg, inhibit expression of key pathogen genes)	• Identify the genes microbes are expressing and their relative levels of expression • Identify types of microbes in a sample, their abundance and the genes they express, and the relative levels of gene expression (metatranscriptomics) • Provide information on gene expression	• Specialized sample collection methods may be needed to prevent RNA degradation • Expensive • Time and resource intensive (longer turnaround times)	58–62

should consider the limitations of this methodology. Importantly, whole genome sequencing can identify bacterial genes, but it does not indicate gene expression.[30] For example, whole genome sequencing may detect antimicrobial resistance genes to carbapenems in an *Escherichia coli* isolate identified during a hospital surveillance program. However, further testing is needed to determine if this translates into functional resistance against carbapenems rendering this ineffective as a treatment option. When considering these limitations, it is recommended that clinicians interpret whole genome sequencing data alongside diagnostics that infer phenotype. In clinical practice, this can include antimicrobial susceptibility profiles, which examine how bacteria grow in the presence of different antimicrobials[31]; here, whole genome sequencing data can provide information on which genes impart this resistant phenotype.

Metagenome Sequencing: Amplicon (16S Ribosomal RNA) Sequencing

Metagenome sequencing refers to the simultaneous sequencing of an entire ecosystem of DNA. In bacteria, metagenome sequencing is often used to characterize the microbiome, the collection of microorganisms and genes inhabiting an ecological niche, including the fecal microbiome, oral microbiome, and skin microbiome.[9,32,33] Common metagenome NGS methodologies with applications to small animal medicine include 16S rRNA (amplicon) sequencing and shotgun metagenomic sequencing.

Amplicon sequencing involves the amplification and sequencing of a single gene region for all organisms in a sample. Following DNA extraction, gene amplification occurs via polymerase chain reaction, an assay that produces large concentrations of specified DNA regions or genes.[6,7] With amplicon sequencing, the gene being amplified must vary enough between bacteria so that analysis can discriminate between taxa. In bacteria, this gene is typically a region of the 16S rRNA gene, which is widely used to determine phylogenetic relationships between diverse bacterial taxa.[34] In bacteria, there are 9 distinct regions of the 16S rRNA gene used for amplicon sequencing, providing a variety of methodology options.[35] Following sequencing, similar DNA sequences are grouped together into an amplicon consensus sequence; amplicons are then matched to reference amplicon sequences to determine taxonomy.[36]

The growing use of amplicon sequencing has contributed to its decreased costs, helping to make it more accessible as an NGS diagnostic tool for small animal practice.[37,38] The genetic sequences produced by 16S rRNA sequencing allow clinicians to describe what taxa are present and how abundant each is. An increasing body of research has demonstrated that within bacterial microbiomes, taxa presence and abundance change with health and disease.[3,4,9,32,33] In small animal, this has been extensively investigated within the fecal microbiome, where perturbations have been observed with obesity, antimicrobial use, chronic enteropathies, parasitic infections, epilepsy, lymphoma and chemotherapy, and many other conditions.[9,39–43] This emergent microbiome research has significantly impacted antimicrobial stewardship practices, supported the increased use of probiotic therapeutics for patient management, and advanced foundational veterinary knowledge of physiology and pathology, through an expanded understanding of host–microbial interactions.[4,9,41] Amplicon sequencing technologies targeted toward pet owners have seen increased use in small animal veterinary practice, with the purpose of encouraging people to explore these results with their veterinarian to identify diagnostic and/or treatment needs for their pet. This includes gastrointestinal health test kits, such as those developed by AnimalBiome and Nestle Purina PetCare.[44,45] These kits have owners mail in a stool sample and they provide a microbiome profile analysis alongside product recommendations for diet, probiotic, and/or fecal microbiota transplant products.[4,46–49] Other NGS-based microbiome test kits include oral health test kits for cats sold by

BasePaws (Zoetis Inc.) and AnimalBiome. These tests associate feline oral microbiomes with clinical measures of oral health including halitosis and tooth resorption and encourage owners to use these results to pursue additional oral health assessments by a veterinarian.[50,51]

Although amplicon sequencing is readily used in multiple small animal veterinary applications, it has several limitations. First, amplicon sequencing rarely possesses the resolution to adequately identify past the genus level.[38] This lower resolution consequently reduces the usefulness of amplicon sequencing as a tool for determining bacterial species or strains for diagnosis and disease outbreak management. The various analysis strategies used to process amplicon sequencing data can lead to large differences in the types and abundance of DNA sequences that are used in downstream statistical comparisons. In other words, the same amplicon sequencing dataset, when analyzed with 2 different methods, can produce noticeably different results.[38,52] This complicates data interpretation and weakens the clinical reliability of the data, especially if serial testing on one patient uses several analysis strategies.

Metagenome Sequencing: Shotgun Metagenomic Sequencing

Shotgun metagenomic sequencing is a second emerging type of metagenome sequencing being explored for its applications in small animal veterinary medicine. While amplicon sequencing involves the targeting and amplification of the 16S rRNA gene, shotgun metagenome sequencing examines the entire genome of all bacteria in the sample.[6,7] As with whole genome sequencing and amplicon sequencing, shotgun metagenomics begins with DNA extraction. However, following extraction, all DNA in the sample is processed, which distinguishes shotgun metagenomics from amplicon sequencing. The sequenced genomes are processed using various bioinformatic tools that filter out unwanted sequences (eg, removal of host DNA from clinical specimens when the goal is bacterial assessment) and align sequences to their taxonomic identity using libraries of reference genomes.[53] By sequencing the entire genome of all bacteria in the sample, shotgun metagenomics provides information on identity, abundance, and the genes each bacterium contains.[6,7]

Shotgun metagenomics is being explored for its ability to expand on the information generated for veterinary patients with amplicon sequencing and whole genome sequencing methodologies. For example, amplicon sequencing studies have established associations between small animal fecal microbiomes and health.[9,45] To further understand the contributions of the microbiome, shotgun metagenomics can reveal the microbial genes underlying each condition. In turn, this approach allows for targeted analysis of these genes and the microbial species harboring them. For example, 16S studies have identified alterations to the canine fecal microbiome occurring during glucose intolerance, and shotgun metagenomics has revealed that this is associated with altered patterns of branched chain amino acid genes, which are important regulators of glucose homeostasis.[54] Following this observation, these branched chain amino acid genes can now be explored mechanistically as a novel management strategy for diabetes mellitus. During bacterial disease outbreaks, shotgun metagenomic sequencing can expand the diagnostic targets being evaluated as part of a screening test used to determine the cause of the outbreak, especially when differential diagnoses point to several potential microorganisms or the etiologic agent is unknown or atypical.[55] In this case, shotgun metagenomics offers an advantage over whole genome sequencing, where the latter may not capture unexpected bacterial contributors or characterize the polymicrobial infections.

Shotgun metagenomics provides robust and high-resolution information about the bacteria contained in veterinary patients. However, this methodology comes with

processing time and cost tradeoffs that should be considered when using it as part of patient or population diagnostic assessments. When compared to whole genome and amplicon sequencing, shotgun metagenomics often takes longer to process and carries a significantly higher cost per sample.[56] Given the importance of intervention time during infectious disease management, this long processing and analysis time can limit the routine use of shotgun metagenomics in small animal practice. With the rising cost of veterinary care,[57] small animal clinicians must consider the overall utility and impact of these results on patient care decisions. Additionally, as with amplicon and whole genome sequencing, shotgun metagenomics examines DNA. Consequently, it does not provide direct information about gene expression, which is important to consider when linking genes with health and disease phenotypes.

Developments in RNA Sequencing: Transcriptomics and Metatranscriptomics

The majority of NGS methodologies used in small animal veterinary medicine focus on DNA analysis. Although NGS-guided DNA analysis provides important clinical insights into patient health, it does not provide assess gene expression. To address this limitation, emergent NGS technologies are exploring RNA sequencing. Collectively, these RNA sequencing methodologies are called transcriptomics.[58,59] A common way of evaluating gene expression is by measuring how cells produce RNA during transcription. Following transcription, RNA is used to produce proteins or impact cellular function via other mechanisms. By providing an indication of DNA expression, RNA transcripts are a valuable proxy for predicting the function of DNA sequences and offer a link between genotype and phenotype.[58,59]

To isolate and sequence RNA contained in bacterial samples, they are prepared using specialized reagents that purify RNA and prevent its degradation.[59] In many workflows, the resultant RNA fragments are converted into their complementary DNA strands (cDNA) using a purified viral enzyme called reverse transcriptase before analysis.[58] In newer workflows, RNA can be subjected to sequencing without conversion into cDNA.[59] Sequencing data are compared to reference gene libraries to determine what genes are being expressed.[59] Like metagenomics, transcriptomic and metatranscriptomic analyses provide information on the levels of bacterial gene expression in a sample and/or in the presence versus absence of different experimental or clinical variables.[58,59] Compared to DNA-based analyses, the sequences matches identified during transcriptomics represent the portions of genetic material in a cell that are being converted into RNA (eg, presumably expressed genes).[60]

As with DNA-based methodologies, transcriptomics can be performed on single bacterial species (transcriptomics) or be applied to populations such as a microbiome (metatranscriptomics). Outside of this difference transcriptomics and metatranscriptomics are methodologically similar.[60] Clinical applications for transcriptomics and metatranscriptomics are emerging within veterinary medicine. One area of research involves assessing zoonotic disease transmission risks between companion animals and people. When the microbiome of oral and rectal swabs from companion animals were examined, they unexpectedly identified presumed commensal microbes expressing pathogenicity-related genes,[61] a finding which DNA-based NGS methodologies would not have captured. Other investigations using canine *E coli* are exploring mechanisms of antimicrobial resistance gene expression,[62] which could impact how antimicrobials are used in practice. While transcriptomics and metatranscriptomics methodologies have potential for advancing veterinary medicine, they are still expensive, have long turnaround times, and may require specialized sample collection techniques to limit RNA degradation.[58,59] Consequently, their use in small animal clinical

practice is still limited, and ongoing research will be needed before they are more accessible to practitioners.

SUMMARY

NGS methodologies are emerging diagnostic tools in small animal medicine, especially in the management of bacterial diseases and microbiome assessments. Their continued incorporation into veterinary medical settings is anticipated given the growing consumer demand for and use of NGS-based diagnostic test kits and their increasing use in clinical research studies. While whole genome and amplicon sequencing are more widely used, shotgun metagenomics and transcriptomics are becoming increasingly explored for their utility in clinical practice. As small animal medicine becomes increasingly specialized and clients demand personalized medical approaches for their pets, the ability of clinicians to incorporate NGS methodologies into clinical assessments will become increasingly important. Therefore, practitioners will need to understand the benefits and limitations of each test and communicate these with owners. Ultimately, reflecting on the methodologies underlying each of these NGS methodologies will help to elevate the care that veterinarians provide to patients, especially when managing bacterial and microbiome-associated conditions.

CLINICS CARE POINTS

- The relative cost, ease of interpretation, and turnaround time are important factors to consider when selecting the ideal next generation sequencing test.
- Practitioners need to consider the limitations of each next-generation sequencing method when recommendiong one as a diagnostic tool and when interpreting results.

ACKNOWLEDGMENTS

The author would like to acknowledge their mentors and colleagues, past and present, for helping them build expertise in next-generation sequencing and understanding its applications to veterinary clinical medicine.

DISCLOSURE

The author has nothing to disclose. Publication of this article was supported using institutional startup funding provided to Dr N.J. Nealon by the Shreiber School of Veterinary Medicine of Rowan University.

REFERENCES

1. Drzemalla L, Kleinsorgen C, Tipold A. Clinical training in veterinary medicine: perspectives on quality and the role of specialists. Front Vet Sci 2025;12. https://doi.org/10.3389/fvets.2025.1601783.
2. Eren K, Taktakoğlu N, Pirim I. DNA sequencing methods: from past to present. Eurasian J Med 2022;54(Suppl 1):S47–56.
3. Shah H, Trivedi M, Gurjar T, et al. Decoding the gut microbiome in companion animals: impacts and innovations. Microorganisms 2024;12(9):1831.
4. Smith B. The heroes within: utilizing the gut microbiome, microbiome therapeutics, and fecal microbiota transplant in managing companion animal health and disease. JAVMA 2025;263(2). https://doi.org/10.2460/javma.25.03.0218.

5. Chaves M, Hashish A, Goraichuk IV, et al. Nanopore sequencing in veterinary medicine: from concepts to clinical applications. Front Cell Infect Microbiol 2025;15:1701570. https://doi.org/10.3389/fcimb.2025.1701570.
6. Domrazek K, Jurka P. Application of next-generation sequencing (NGS) techniques for selected companion animals. Animals (Basel) 2024;14(11):1578.
7. Van Borm S, Belák S, Freimanis G, et al. Next-generation sequencing in veterinary medicine: how can the massive amount of information arising from high-throughput technologies improve diagnosis, control, and management of infectious diseases? Methods Mol Biol 2015;1247:415–36.
8. Luo J, Lu W, Liu R, et al. From panels to pathogen networks: the expanding role of targeted sequencing in veterinary medicine. Biology 2025;14(8):1075.
9. Suchodolski JS. Analysis of the gut microbiome in dogs and cats. Vet Clin Pathol 2022;50(Suppl 1):6–17.
10. Saengsawang P, Tanonkaew R, Kimseng R, et al. Whole genome sequence analysis of multidrug-resistant Staphylococcus aureus and Staphylococcus pseudintermedius isolated from superficial pyoderma in dogs and cats. Antibiotics (Basel) 2025;14(7):643.
11. Jousserand N, Auvray F, Chagneau C, et al. Zoonotic potential of uropathogenic Escherichia coli lineages from companion animals. Vet Res 2025;56(1):69.
12. Boehmer T, Vogler AJ, Thomas A, et al. Phenotypic characterization and whole genome analysis of extended-spectrum beta-lactamase-producing bacteria isolated from dogs in Germany. PLoS One 2018. https://doi.org/10.1371/journal.pone.0206252.
13. Bagger FO, Borgwardt L, Jespersen AS, et al. Whole genome sequencing in clinical practice. BMC Med Genomics 2024;17:39.
14. Li IC, Yu GY, Huang JF, et al. Comparison of Reference-Based Assembly and De Novo Assembly for Bacterial Plasmid Reconstruction and AMR Gene Localization in Salmonella enterica Serovar Schwarzengrund Isolates. Microorganisms 2022; 10(2):227.
15. Hallal Ferreira Raro O, Poirel L, Tocco M, et al. Impact of veterinary antibiotics on plasmid-encoded antibiotic resistance transfer. J Antimicrob Chemother 2023; 78(9). https://doi.org/10.1093/jac/dkad226.
16. Orlek A, Stoesser N, Anjum MF, et al. Plasmid classification in an era of whole-genome sequencing: application in studies of antibiotic resistance epidemiology. Front Microbiol 2017;8:182.
17. Price TK, Realegeno S, Mirasol R, et al. Validation, implementation, and clinical utility of whole genome sequence-based bacterial identification in the clinical microbiology laboratory. J Mol Diagn 2021;23(11):1468–77.
18. Puangseree J, Hein ST, Prathan R, et al. Genomic insights into multidrug - resistant Salmonella enterica isolates from pet dogs and cats. Sci Rep 2025;15(1): 22104. https://doi.org/10.1038/s41598-025-06301-5.
19. Gilchrist CA, Turner SD, Riley MF, et al. Whole-genome sequencing in outbreak analysis. Clin Microbiol Rev 2015;28(3):541–63.
20. Holmes A, Allison L, Ward M, et al. Utility of whole-genome sequencing of Escherichia coli o157 for outbreak detection and epidemiological surveillance. J Clin Microbiol 2015;53(11):3565–73.
21. Jones JL, Wang L, Ceric O, et al. Whole genome sequencing confirms source of pathogens associated with bacterial foodborne illness in pets fed raw pet food. J Vet Diagn Invest 2019;31(2):235–40.

22. Jørgensen HJ, Valheim M, Sekse C, et al. An official outbreak investigation of acute haemorrhagic diarrhoea in dogs in Norway points to Providencia alcalifaciens as a likely cause. Animals 2021;11(11):3201.
23. Robb AR, Ure R, Chaput DL, et al. Emergence of novel methicillin resistant Staphylococcus pseudintermedius lineages revealed by whole genome sequencing of isolates from companion animals and humans in Scotland. PLoS One 2024;19(7): e0305211. https://doi.org/10.1371/journal.pone.0305211.
24. King C, Diaz-Campos D, Mollenkopf D, et al. Management of carbapenem-resistant Enterobacterales in a veterinary hospital environment utilizing surveillance with infection control and prevention measures. J Vet Intern Med 2026; 40(1):aalaf023. https://doi.org/10.1093/jvimsj/aalaf023.
25. Hoet AE, Johnson A, Nava-Hoet RC, et al. Environmental methicillin-resistant Staphylococcus aureus in a veterinary teaching Hospital during a nonoutbreak period. Vector Borne Zoonotic Dis 2011;11(6):609–15.
26. Mathys DA, Mollenkopf DF, Van Balen JC, et al. β-Lactam and fluoroquinolone-resistant enterobacteriaceae recovered from the environment of human and veterinary tertiary care hospitals. Vector Borne Zoonotic Dis 2018;18(11):620–3.
27. Maddock KJ, Bowden R, Cole SD, et al. Current state and future directions for veterinary antimicrobial resistance research. Am J Vet Res 2024. https://doi.org/10.2460/ajvr.23.12.0294.
28. DeStefano I, Fellman CL, Bergeron AC, et al. Effective mitigation of an outbreak of New Delhi metallo-β-lactamase–producing Escherichia coli infections in a small animal veterinary teaching hospital. J Am Vet Med Assoc 2025. https://doi.org/10.2460/javma.24.09.0572.
29. Price V, Ngwira LG, Lewis JM, et al. A systematic review of economic evaluations of whole-genome sequencing for the surveillance of bacterial pathogens. Microb Genom 2023;9(2):mgen000947. https://doi.org/10.1099/mgen.0.000947.
30. Quainoo S, Coolen JPM, van Hijum SAFT, et al. Whole-genome sequencing of bacterial pathogens: the future of nosocomial outbreak analysis. Clin Microbiol Rev 2017;31(1). Available at: doi/full/10.1128/cmr.00082-17.
31. Maddock KJ, Burbick CR, Cole SD, et al. A one health perspective on the use of genotypic methods for antimicrobial resistance prediction. J Am Vet Med Assoc 2024. https://doi.org/10.2460/javma.23.12.0687.
32. Davis EM, Weese JS. Oral microbiome in dogs and cats: dysbiosis and the utility of antimicrobial therapy in the treatment of periodontal disease. Vet Clin North Am Small Anim Pract 2022;52(1):107–19.
33. Weese JS. The canine and feline skin microbiome in health and disease. Vet Dermatol 2013;24(1):137–45.e31.
34. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol 2007; 45(9):2761–4.
35. Weinroth MD, Belk AD, Dean C, et al. Considerations and best practices in animal science 16S ribosomal RNA gene sequencing microbiome studies. J Anim Sci 2022;100(2):skab346. https://doi.org/10.1093/jas/skab346.
36. Liu YX, Qin Y, Chen T, et al. A practical guide to amplicon and metagenomic analysis of microbiome data. Protein Cell 2021;12(5):315–30.
37. Minich JJ, Humphrey G, Benitez RAS, et al. High-throughput miniaturized 16S rRNA amplicon library preparation reduces costs while preserving microbiome integrity. mSystems 2018;3(6). https://doi.org/10.1128/msystems.00166-18.
38. Boers SA, Jansen R, Hays JP. Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine

clinical microbiological diagnostic laboratory. Eur J Clin Microbiol Infect Dis 2019; 38(6):1059–70.
39. Kim H, Seo J, Park T, et al. Obese dogs exhibit different fecal microbiome and specific microbial networks compared with normal weight dogs. Sci Rep 2023; 13(1):723.
40. Berry ASF, Johnson K, Martins R, et al. Natural infection with giardia is associated with altered community structure of the human and canine gut microbiome. mSphere 2020;5(4). https://doi.org/10.1128/mSphere.00670-20. e00670-20.
41. Pilla R, Gaschen FP, Barr JW, et al. Effects of metronidazole on the fecal microbiome and metabolome in healthy dogs. J Vet Intern Med 2020;34(5):1853–66.
42. Verdoodt F, Hesta M, Goossens E, et al. The fecal metabolome and microbiome are altered in dogs with idiopathic epilepsy compared to healthy dogs. Sci Rep 2025;15(1):27024. https://doi.org/10.1038/s41598-025-09919-7.
43. Aragon J, Weber AM, Tipton M, et al. Impacts of vincristine and prednisolone chemotherapy on the canine gut microbiota in dogs undergoing treatment for lymphoma. Vet Comp Oncol 2025;23(3):388–400.
44. Rojas CA, Park B, Scarsella E, et al. Species-level characterization of the core microbiome in healthy dogs using full-length 16S rRNA gene sequencing. Front Vet Sci 2024;11. https://doi.org/10.3389/fvets.2024.1405470.
45. Ganz HH, Jospin G, Rojas CA, et al. The kitty microbiome project: defining the healthy fecal "Core Microbiome" in pet domestic cats. Vet Sci 2022;9(11):635.
46. Basic Dog & Cat Microbiome Analysis Kit | petivity. Available at: https://www.petivity.com/products/microbiome-analysis-kit. Accessed May 7, 2026.
47. Advanced dog & cat microbiome analysis kit. Petivity. Available at: https://www.petivity.com/products/advanced-microbiome-analysis-kit. Accessed May 7, 2026.
48. Comprehensive dog & cat microbiome analysis kit | petivity. Available at: https://www.petivity.com/products/microbiome-analysis-kit-comprehensive. Accessed May 7, 2026.
49. Carapeto S, Cunha E, Serrano I, et al. Effect of the administration of a lyophilised faecal capsules on the intestinal microbiome of dogs: a pilot study. Genes 2023; 14(9):1676.
50. Kao D, Yang J, Nisperos S, et al. Development of an oral swab based microbiome test for the detection of feline dental disease. bioRxiv 2021. https://doi.org/10.1101/2021.04.23.441192. 04.23.441192.
51. Oral microbiome test for cats. AnimalBiome. Available at: https://www.animalbiome.com/products/kittybiome-oral-health-test. Accessed May 7, 2026.
52. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, et al. Microbiome datasets are compositional: and this is not optional. Front Microbiol 2017;8. https://doi.org/10.3389/fmicb.2017.02224.
53. Setubal JC. Metagenome-assembled genomes: concepts, analogies, and challenges. Biophys Rev 2021;13(6):905–9.
54. Lewis S, Nash A, Li Q, et al. Comparison of 16S and whole genome dog microbiomes using machine learning. BioData Min 2021;14:41.
55. Chen H, Li J, Yan S, et al. Identification of pathogen(s) in infectious diseases using shotgun metagenomic sequencing and conventional culture: a comparative study. PeerJ 2021;9:e11699. https://doi.org/10.7717/peerj.11699.
56. Dulanto Chiang A, Dekker JP. From the pipeline to the bedside: advances and challenges in clinical metagenomics. J Infect Dis 2020;221(Suppl 3):S331–40.
57. Benson J, Tincher EM. Cost of care, access to care, and payment options in veterinary practice. Vet Clin North Am Small Anim Pract 2024;54(2):235–50.

58. Khodadadian A, Darzi S, Haghi-Daredeh S, et al. Genomics and transcriptomics: the powerful technologies in precision medicine. Int J Gen Med 2020;13:627–40.
59. Lowe R, Shirley N, Bleackley M, et al. Transcriptomics technologies. PLoS Comput Biol 2017;13(5):e1005457. https://doi.org/10.1371/journal.pcbi.1005457.
60. Ojala T, Häkkinen AE, Kankuri E, et al. Current concepts, advances, and challenges in deciphering the human microbiota with metatranscriptomics. Trends Genet 2023;39(9):686–702.
61. Wu WC, Pan YF, Zhou WD, et al. Meta-transcriptomic analysis of companion animal infectomes reveals their diversity and potential roles in animal and human disease. mSphere 2024;9(8). https://doi.org/10.1128/msphere.00439-24. e00439-24.
62. Ceres K, Zehr JD, Murrell C, et al. Evolutionary genomic analyses of canine E. coli infections identify a relic capsular locus associated with resistance to multiple classes of antimicrobials. Appl Environ Microbiol 2024;90(8). https://doi.org/10.1128/aem.00354-24. e00354-24.
63. Shah N, Tang H, Doak TG, et al. Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics. Pac Symp Biocomput 2011;165–76. https://doi.org/10.1142/9789814335058_0018.