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PRO DAIRY Education & Applied Research

ANTIMICROBIAL RESISTANCE AND DAIRY MANURE SYSTEMS

3.B. Antibiotic resistance genes (ARGs) - Measuring ARGs in dairy manure

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Antibiotic resistance genes (ARGs)?

A gene is a unit of heredity - an inheritable segment of DNA that functions as a blueprint, encoding a specific protein or cellular function. ARGs are genes naturally present in bacterial populations that give a bacteria the specific ability to resist the effects of an antibiotic(s).

Detecting

Studies have revealed that specific genes across diverse functional categories of bacteria are associated with resistance to particular antibiotics. These ARGs (see Fact sheet 3.A.) can be used as a measure of a bacteria's ability to resist an antibiotic. There are many molecular approaches to determine the presence of an ARG in a bacteria. Quantitative polymerase chain reaction (qPCR), sometimes called real-time PCR, and genome sequencing are the most common.

Quantitative PCR[1]

Polymerase chain reaction is a technique used to generate thousands or millions of copies of a particular segment of DNA from just a single or few copies. Primers serve as a starting point for DNA replication, and can be used with PCR to targeted replicate particular genes or ARGs. In qPCR, florescent tags are used to label the targeted genes as they are replicated. This florescence can then be very accurately measured enabling the quantification of target genes. Small volume qPCR reactions targeting different ARGs can be prepared commercially in multi-well plates. Sometimes referred to as a microarrays or biochips, this approach allows for the rapid screening of multiple genes from a single bacterium simultaneously.

Genome sequencing[1]

Genome sequencing is an approach where the entire set of DNA in a bacterium or cell is mapped. DNA is made up of four chemical compounds called bases. These are adenine (A), thymine (T), guanine (G), and cytosine (C). The specific four letter language that these bases make up, constitutes the specific genes in a cell. By replicated this four letter language and reading it a genome is sequenced.

There are many different ways to sequence a genome. Most fragment extracted DNA into manageable lengths. Then, DNA fragments are replicated using synthetic bases containing uniquely colored fluorescent tags so they can be read. Sophisticated algorithms are then used to align these fragment reads and recreate the sequence (Figure 1). The recreated genome can then be compared to databases so that genes (such as ARGs) can be identified.

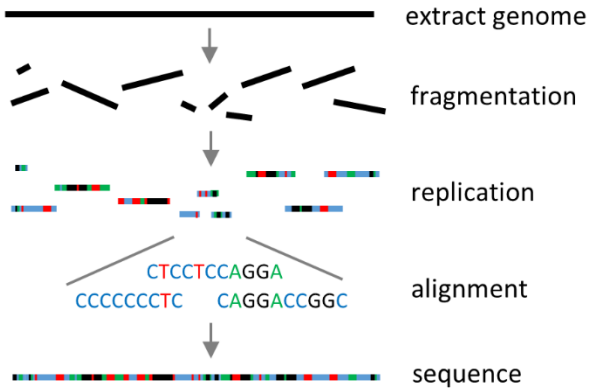

FIGURE 1. GENERALIZED SEQUENCING PROCEDURE.

Limitations of ARG assessments[2,3]

The rapid growth of molecular techniques and genetic data is rapidly outpacing phenotypic studies that are need to test the actual antibiotic resistance associated with a potential ARG. Molecular data depends on an up-to-date and well-curated database. While these are in development, databases and the ways molecular data are organized and interpreted against a database (known as pipelines) are not currently standardized.

Often genes that are very similar in genetic sequence to previously described ARGs are also listed in a database as ARGs without further vetting. While they may likely encode for antibiotic resistance, without phenotypic testing this cannot be verified. Only phenotypic testing can resolve if the gene and its products effectively reduce the susceptibility of a bacteria to an antibiotic. For instance, the presence of a gene does not mean it is functioning, or the regulatory processes to activate it are.

The genetic mechanisms of antibiotic resistance are also diverse and sometimes multiple genes may be involved. Here again phenotypic testing is needed to improve the curation of molecular data. It is also important to note that bacterial genomes are not static and are rapidly evolving. This creates a moving target and further complicates the interpretation of molecular data.

In spite of these limitations, molecular data is revolutionizing the ability to study ARGs and provides an approach to monitor ARGs on farm, in the human medical clinics, and in the environment. Such surveillance will be needed to resolve the influence of management practices on the prevalence of ARGs and to help inform on- and off-farm decision making. As these big data issue of volume, velocity, variety, and veracity are overcome, genomic ARG testing will likely become a routine tool important to the management of herd health and environmental stewardship on modern dairy farms.

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References

[1] Maria-Neto et al. 2015. *Biochimica et Biophysica Acta* 1848:3078–3088 [2] Simjee et al. 2018 *Microbiol Spectrum* 6(4):ARBA-0028-2017. [3] McArthur & Tsang. 2017. *Ann. N.Y. Acad. Sci.* 1388: 78–91

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