

Real-time Nanopore sequencing for One Health approaches in Argentina

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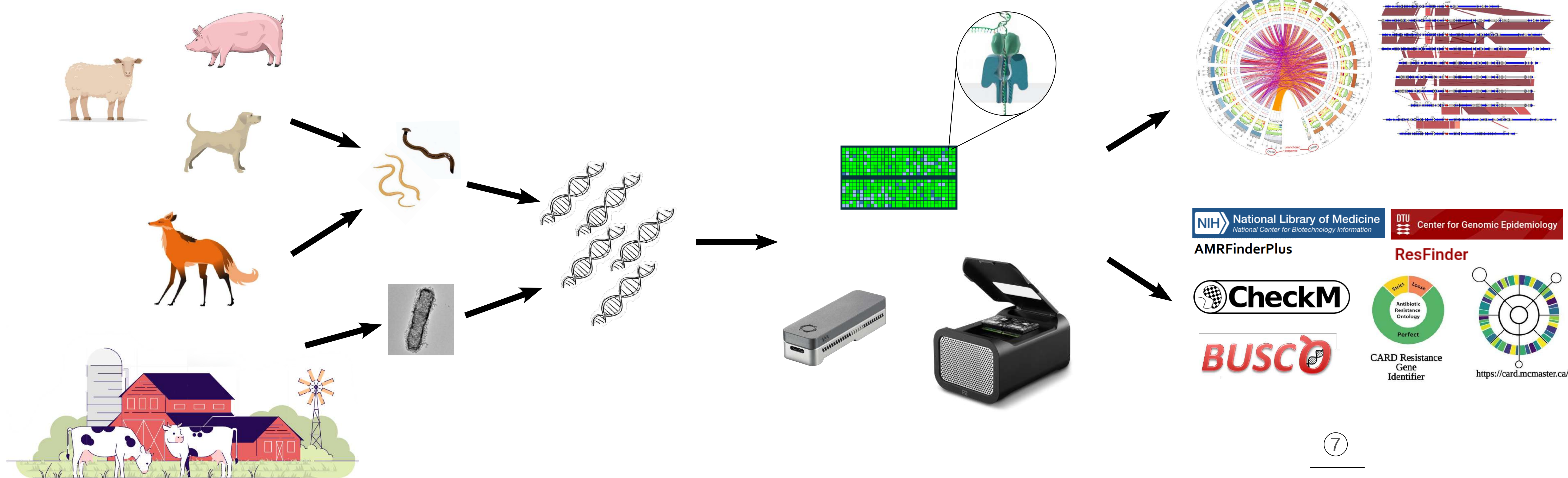
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Introduction and Methods

We applied several strategies within our country to analyze genomes of animal pathogens under a One Health view of the environment. We used Nanopore sequencing to study a group of bacteria and parasites, some of them with the potential to infect humans. In our country there is a health problem with the parasite *Dioctophyme renale*, commonly called "giant kidney worm", which is considered the largest parasitic nematode of terrestrial vertebrates described so far. *D. renale* is found mainly in dogs that live near aquatic environments with a high risk of causing infections in human populations in the Rivera. The adult worm develops in the mammalian kidney, destroying it completely, making it a debilitating and potentially fatal disease for humans and threatened domestic and wild animals.

The only methods available for the diagnosis of the infection are urine analysis, ultrasound, surgery or necropsy. The low sensitivity and specificity of these methods inevitably lead to false negatives, and the absence of nuclear DNA sequences in public databases further complicates this situation. This parasite also affects wild species. We have also analyzed isolated infectious agents (bacteria) related to several bovine diseases, like mastitis (*Staphylococcus sp*), keratoconjunctivitis (*Moraxella sp*), brucellosis (*Brucella sp*), *Campylobacter sp*, and related with human diseases as *E. coli* (O157:H7).

For all samples, high quality DNA was obtained and rapid and ligation libraries were constructed (using previous and Q20 kits). Libraries were sequenced on MinION and PromethION P2 Solo platforms.



Results

All the bacterial genomes analyzed were assembled in complete circular molecules, including chromosomes and plasmids (Table 1). Bacterial genomes were analyzed for its completeness, gene content and the presence of AMR genes. For *D. renale*, 2.1Gb of high quality reads were assembled. The complete mitochondrial genome was reconstructed for the first time, and in less than a week from the sampling. Mitochondrial markers were identified and a PCR designed to analyze genetic variability among domestic and wild animals.

Table 1.

Sample	Species	Sequencing			Assembly		
		Reads (bp)	Bases (Mbp)	Coverage	Contigs	Chromosome	Plasmids
Bm16M_st1024	<i>Brucella melitensis</i>	657,205	1,999	606X	2	2	0
Bm16M_wt	<i>Brucella melitensis</i>	395,425	1,157	351X	2	2	0
Bm_Rev1	<i>Brucella melitensis</i>	570,617	1,563	474X	2	2	0
Cb23	<i>Corynebacterium bovis</i>	26,064	74	28X	1	1	1
Cb24	<i>Corynebacterium bovis</i>	14,928	79	29X	2	1	1
EcNah	<i>Escherichia coli</i>	149,746	899	160X	4	1	3
Mbs21	<i>Moraxella bovis</i>	301,588	668	248X	6	1	5
Mbc19	<i>Moraxella bovoculi</i>	218,955	643	306X	1	1	0
Mbc24	<i>Moraxella bovoculi</i>	175,996	953	454X	2	1	1
Mbc30	<i>Moraxella bovoculi</i>	241,400	1,293	616X	4	1	3
Sa19	<i>Staphylococcus aureus</i>	227,540	1,460	521X	4	1	3
Sa20	<i>Staphylococcus aureus</i>	155,565	1,093	390X	3	1	2
Sa21	<i>Staphylococcus aureus</i>	123,208	1,410	504X	1	1	1
Sa22	<i>Staphylococcus aureus</i>	141,288	1,359	485X	1	1	1

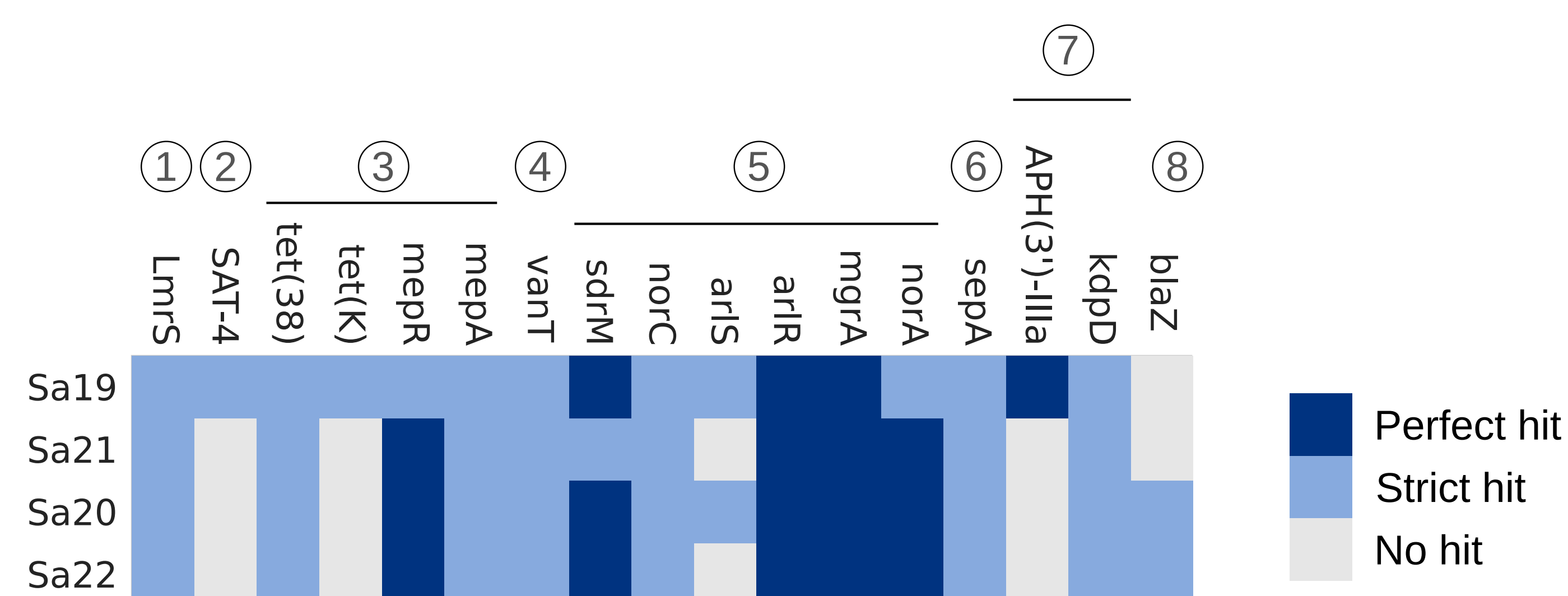


Figure 1. Identification of AMR genes for *Staphylococcus* strains sequenced using CARD RGI. References: (1) macrolide ATB, (2) nucleoside ATB, (3) tetracycline ATB, (4) glycopeptide ATB, (5) fluoroquinolone ATB, (6) disinfecting agents, (7) aminoglycoside ATB, (8) beta-lactamase ATB.

Conclusions

We highlight here only some of the results produced by our groups, as proof of concept of the possibilities of using Nanopore sequencing in the analysis of the genomic content of different types of pathogens. For *D. renale*, a species without molecular information on databases, we developed molecular markers within a week from sampling. For bacteria, the reconstruction of complete genomes by long reads allowed the analysis of structural variations, complete resolution of plasmid content and AMR genes. These results are part of the group's ongoing projects, given the installed sequencing capacity, as there are many more projects underway, including whole genomes, amplicon sequencing, metagenomics, real-time pathogen detection in clinical samples and more.