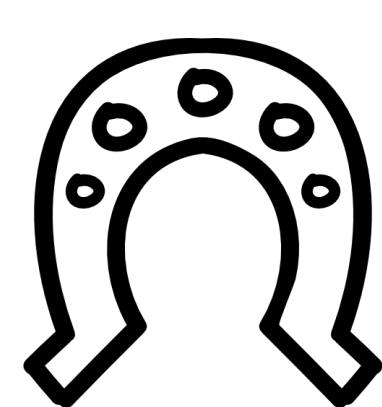




NANOPORE SEQUENCING FOR ONE-HEALTH: genome and plasmid characterization of a methicillin-resistant *Staphylococcus pseudintermedius* strain isolated from canine otitis and metagenomic samples from horse rectum



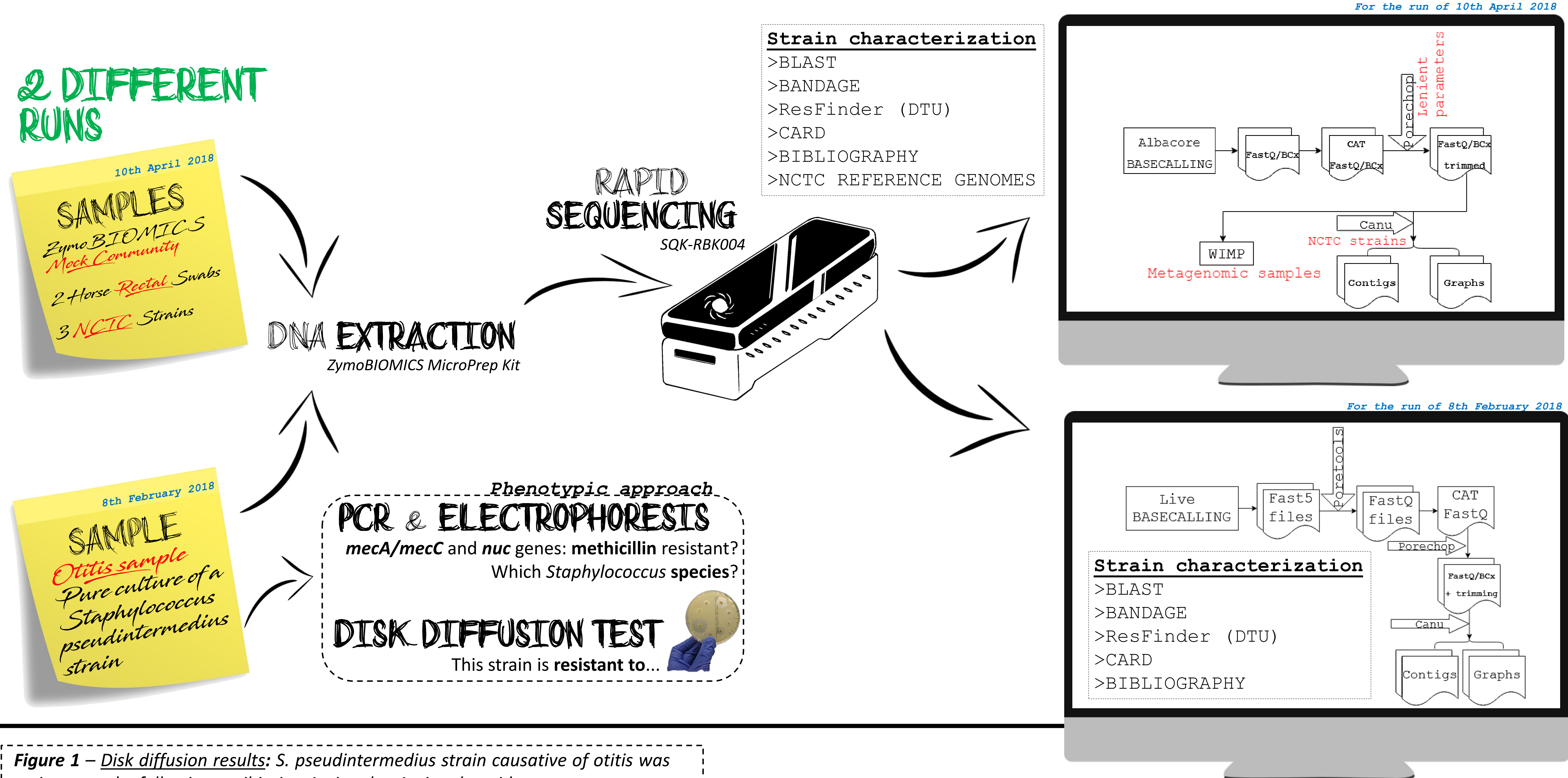
Joaquim Viñes^{a,b}, Olga Francino^a, Anna Cuscó^b
^a SVGM, Servei Veterinari de Genètica Molecular, Universitat Autònoma de Barcelona
^b Vetgenomics, Edifici EUREKA, Parc de Recerca de la UAB



Introduction

- **One-Health** studies rely on the interdisciplinary collaboration in all aspects of health care for humans, animals and the environment.
- *Staphylococcus pseudintermedius* is prone to cause opportunistic infections in skin of dogs and cats, with zoonotic potential.
- Methicillin-Resistant *S. pseudintermedius* (MRSP) is gaining ground, so rapid characterization may improve treatment.
- Clinical metagenomics samples are still challenging. The application of new sequencing approaches may help in rapid diagnostics.

Material and methods



Results

Figure 1 – Disk diffusion results: *S. pseudintermedius* strain causative of otitis was resistant to the following antibiotics. Aminogl. = Aminoglycosides

Aminogl.	Quinolones	Tetracyclines	Macrolides	Methicillin resistance markers	Others
Gentamicin	Ciprofloxacin	Tetracycline	Erythromycin	Oxacillin	Clindamycin
Tobramycin	Marbofloxacin	Doxycycline		Cefoxitin	Co-trimoxazole
	Pradofloxacin	Minocycline			
	Orbifloxacin				

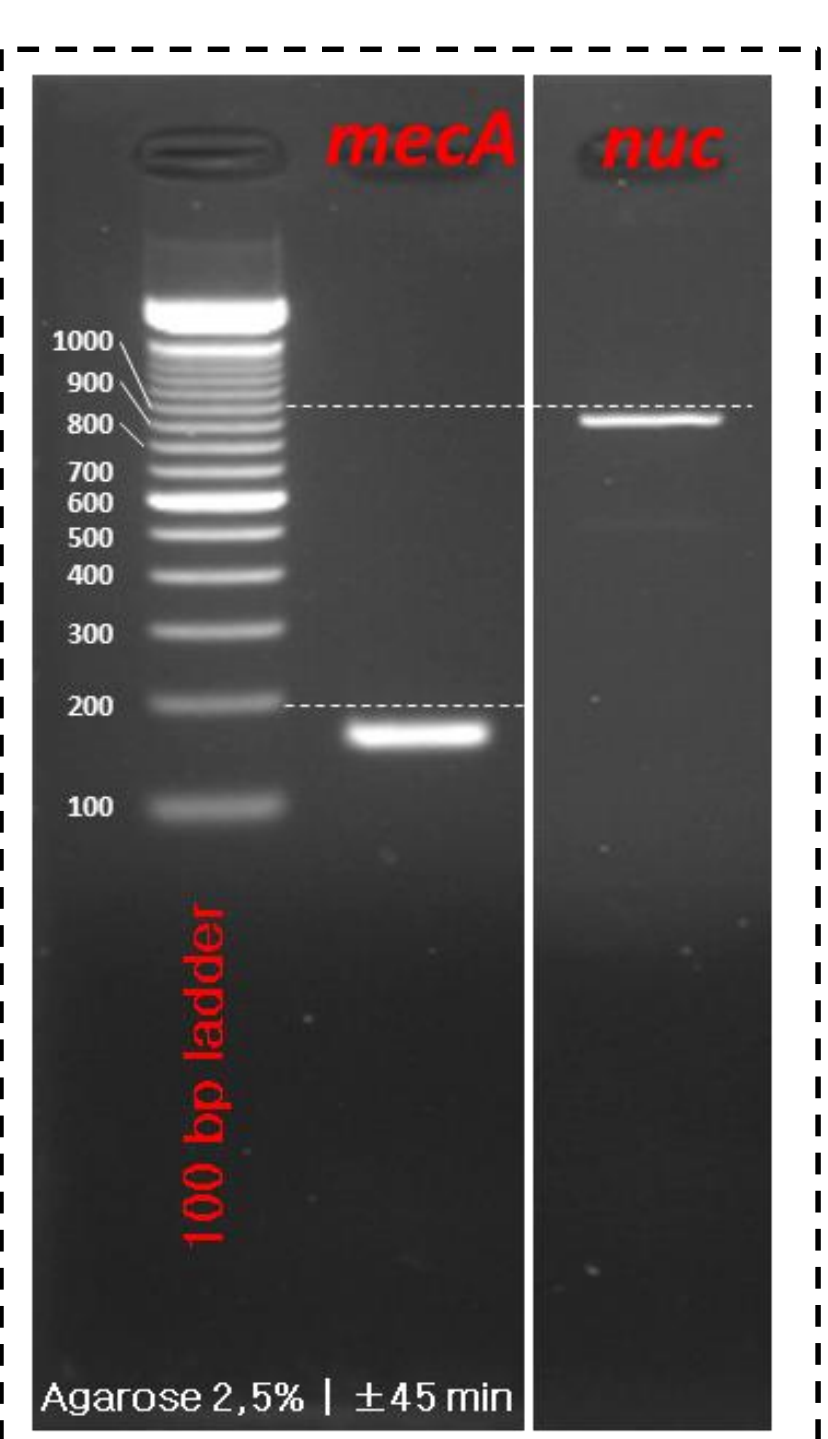
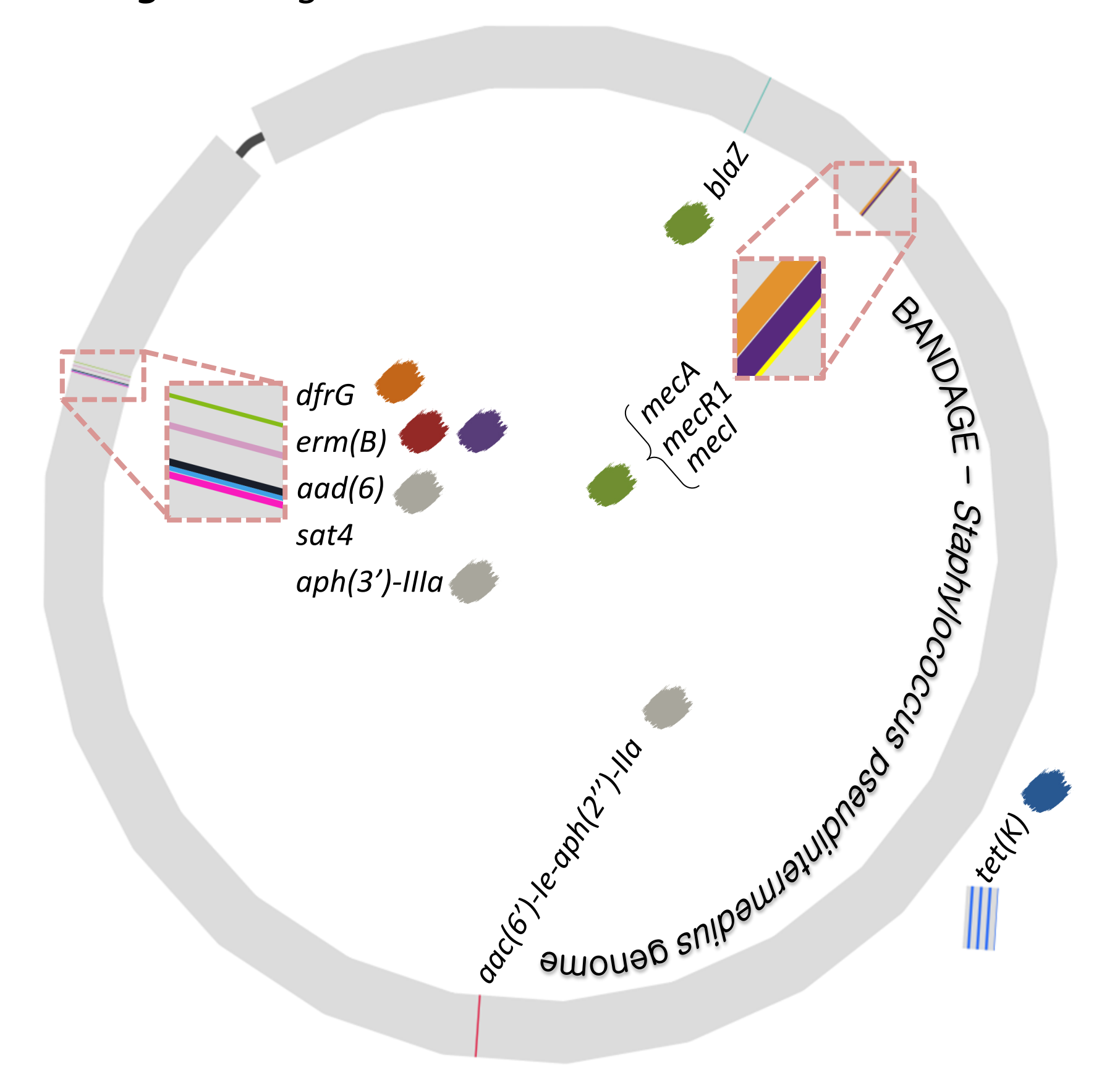


Figure 2 – PCR of mecA and nuc gene. A band of approximately 900 bp determines the presence of *S. pseudintermedius*

Figure 3 – Assembled genome and plasmid (Canu) from *S. pseudintermedius*. Resistance genes can be visualized in Bandage through BLAST search



QUINOLONES
Resistance to quinolones are due to mutations in *gyrA* gene. Using MinION we found **point mutations described** in the bibliography to confer resistance to that kind of antibiotics!

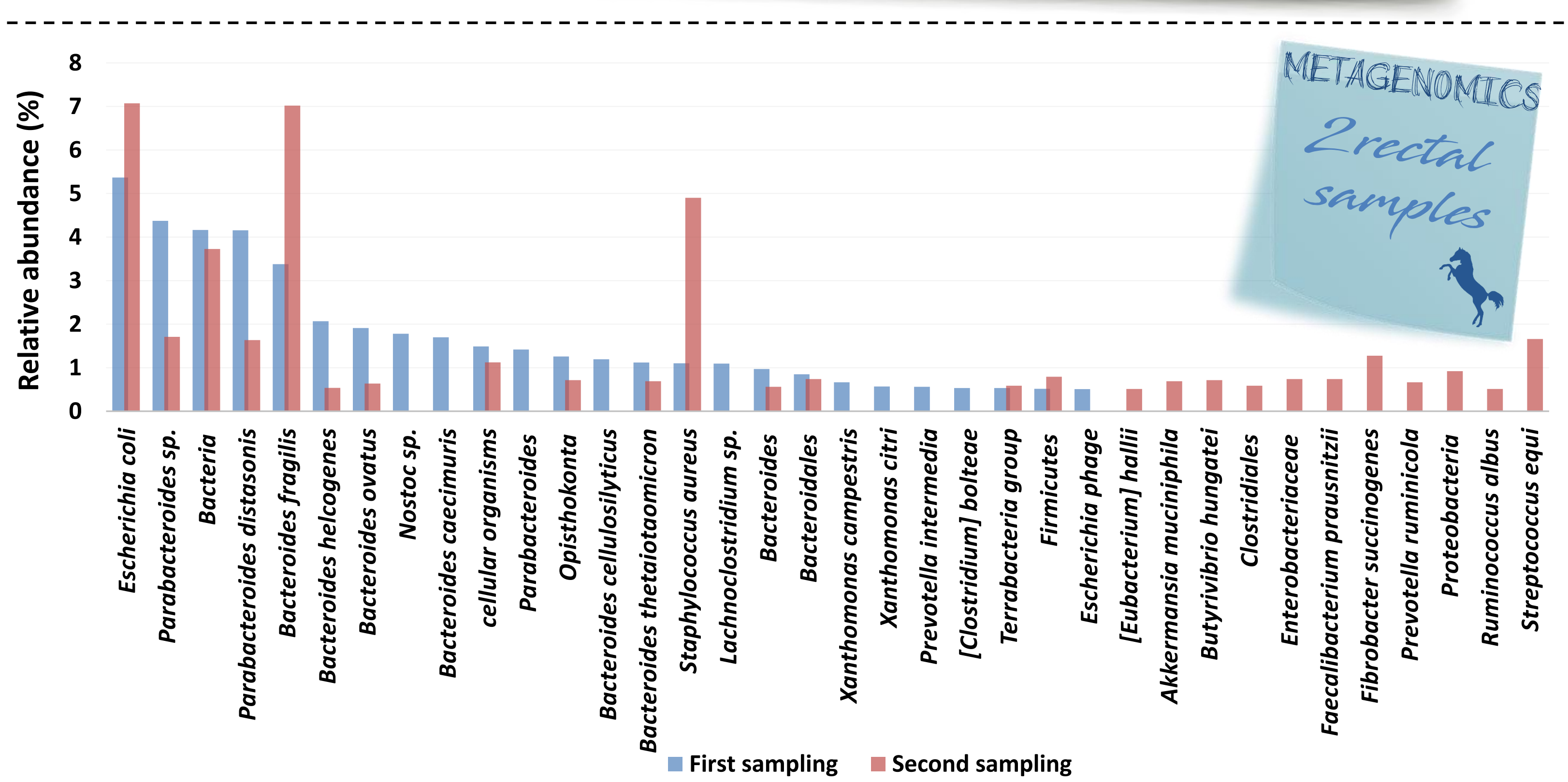


Figure 4 – Relative abundances (%) of the microbial species from the horse rectal swabs. While the first point of sampling corresponds to the admission of a horse with left colon displacement, the second point is after surgery, which incision got infected and followed with endotoxemia

Conclusions

MinION sequencing using Rapid Barcoding Kit (Oxford Nanopore) allowed us to describe all resistances found with **disk diffusion test**, even resistances caused by point mutations
We could found other resistance gene: *sat4*

The **microbiota taxonomy** of horse rectal samples obtained from metagenomics analyses with WIMP is consistent with the bibliography
The **change of microbiota** profile in the second point of sampling may be indicative of the infection (e.g. increase in *S. aureus* or *E. coli*)

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