Proposition de stage M2 à l'UMR GenPhySE (INRA Toulouse)

Estimation of the rate of transfer of resistance genes from complex communities into *E.coli*

Keywords : antibiotic resistance, gut bacterial communities

Context

Antibiotics have substantially improved the means to fight against pathogenic bacteria, which represents an obvious benefit in public health and in livestock farming. The livestock industry, where antibiotics have been used at subtherapeutic concentrations as growth promoters for more than 50 years, is particularly prone to the emergence of antibiotic resistance genes (ARGs).

The (ab)use of antibiotics in livestock led to widespread resistance and multi-resistance phenomenon. Indeed resistant genetic variants have a competitive advantage in environments harboring antibiotics, leading to their diffusion in the population. For example, carbapenem-resistant *Enterobacteriaceae* pathogens that are resistant to most known antibiotics have been detected in livestock. To make the matter worse, the genetic variations leading to antibiotic resistance are not necessarily a major disadvantage in antibiotic-free environments(Perron et al. 2010) so that their decay may be very slow. For example, humans may still carry ARGs in their gut two years after a single antibiotic treatment (Jernberg et al. 2007). The lack of major fitness costs of ARGs is a crucial issue because resistant microbes may accumulate additional ARGs to evolve into multiresistant bacteria.

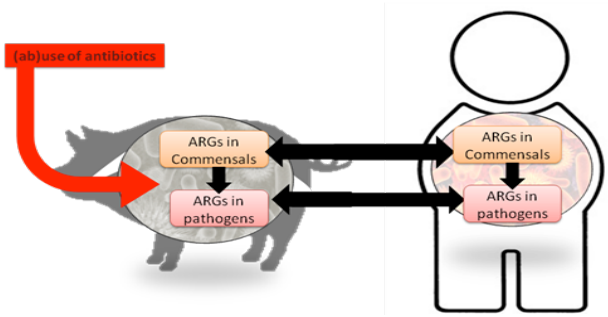

Fig 1 : Direct and indirect diffusion of antibiotic resistance genes (ARGs) to microbes that are pathogenic for humans. While many studies focus on the direct diffusion of pathogenic microbes from animal to human intestinal tract, such as Methicillin-resistant staphylococcus aureus which is now prevalent in US livestock, commensal microbes are probably key players because they represent the vast majority of the gut microbiota and they also develop resistance mechanisms against antibiotics.

Multi-resistance poses a major public health issue because the genes involved may be transferred to the microbes that are pathogenic for humans. MRSA is a typical example of a pathogen that might have acquired resistance genes while residing in livestock (Vandendriessche et al. 2013). The European Union banned the use of antibiotics as growth promoters in 2006 to limit this phenomenon. However, antibiotics may still be added to animal feed or water for veterinary purposes so that gut bacteria of livestock may still experience subtherapeutic concentrations at the end of the therapy. Furthermore, antibiotics are diluted in manure where they meet an enormous microbial biomass, so that appearance and transfer of resistance is very likely to occur also there.

Importantly, multi-resistance is especially an issue when it can be transferred easily to human pathogens. However some rare ARGs remain elusive despite the sequencing depth that can be achieved via next-generation sequencing.

In this context, our goal is to identify ARGs that may be transferred to *E.coli*. We will reproduce the protocol described in Rolland et al. (Rolland et al. 1985). Briefly a nalidixic acid-resistant recipient *E.coli* strain is incubated overnight with the donor strains harboring resistance against tetracyclins. The following day, the solution is plated on a tet+nal plate. Hence, the transfer rate of the plasmids carrying the tet resistance to the nalidixic-resistant strain can be estimated. Here, we aim to adapt this protocol to complex bacterial communities.

Aims of the master student

With the help of other INRA partners, the planning for the master student is the following :

1. *Collect 12 samples of microbial communities in 8 pig farms using tetracyclins (96 samples)*
2. *Culture the recipient E.coli strain with a resistance to nalidixic acid (available in the lab)*
3. *Incubate the recipient strain with the donors (ie the collected complex bacterial communities in Task 1)*

4. Spread 100 ul of the solution on a Macconkey agar plate containing tetracycline and nalidixic acid.

Once the proof of concept achieved (estimated time=3 months), the student will continue the work by one or more of the following :
-automatisation with a pipetting robot for high throughput
-isolate 5 to 10 plasmids carrying antibiotic resistance genes and sequence them.
-check for multiple resistance
-assess the relevance of spontaneous transformation (ie using the purified plasmids)
-increase the rate of spontaneous transformation by adding calcium chloride
-Evaluate the transfer rate in an anaerobic setup instead of the aerobic setup

Abilities

The student should understand english and be highly motivated. Previous knowledge about handling microbial communities would be appreciated but is not necessary.

Contact

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Practical details

- 6 months from Jan to Jun 2016
- INRA Toulouse – UMR GenPhySE à Auzeville (31)
- paid about 550 €/mois – (lunch available on site for ~ 2.50 eur)

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Vandendriessche, S., Vanderhaeghen, W., Soares, F.V., et al. (2013) Prevalence, risk factors and genetic diversity of methicillin-resistant Staphylococcus aureus carried by humans and animals across livestock production sectors. *J Antimicrob Chemother* **68**, 1510-1516.