Minutes of the MedVetKlebs kick-off meeting

(11-12 January 2018, Institut Pasteur, Paris)

Overview of the project – S Brisse: please see attached .ppt

&

5-slides presentations by partners (see attached .ppt files)

Institut Pasteur/Hôpital Bicêtre (P. Glaser/T. Naas – Paris, France)

- APHP – 22000 beds
- CARBA-R collection – 40% Kp (from which 15% are from patients from abroad)

ANSES (J-Y Madec; M. Haenni – Lyon, France)

- Active collaboration with S. Brisse (IP)
- **2498** fecal samples from animals – **230 Kp (9.2%)**
- AST and WGS of the 230 Kp

INRA (P. Piveteau; E. Barbier – Dijon, France)

- Expertise in *Listeria* spp*., E. coli* ESBL; *Mycobacterium*
- qPCR - quantification and detection of Kp in complex matrices (e.g. soil)
- sampling of soils (very complex microbiota – optimized protocol using 100 mg to 2g of sample; double DNA purification)

AGES (F. Allerberger, K. Hauser – Vienna and Graz - Austria)

- Few reports of Kp in the clinical setting
- Outbreak of Kp (susceptible) in a ICU neonatal ward – 22 neonates – 2 of them died
- Kathrin Hauser (supervisor: Claudia Schlagenhaufen) will be active on the project in Graz

NUIG (D. Morris - Galway, Ireland)

- Ongoing projects – AREST, VITOCONEHEALTH, DETER
- 68 Kp already recovered from SCAI medium; 2 clones circulating R to 3GC, AMINO, FQs in clinical samples

UCD (S. Fanning – Dublin, Ireland)

- 26 Kp with AST and WGS performed – CG147, CG258 and CG15 (**high risk clones**)
- Food Kp are susceptible
- Reversing resistance was presented

IZSAM (F. Pomilio – Teramo, Italy)

- Distributed network of agencies throughout Italy
- Core sequencing facility
- WP1 involvement, validation of methods

SSI (E. Möller Nielsen – Copenhagen, Denmark)

- Samples from pigs with ABR and no-ABR
- **Old strains** from (ex-WHO) reference collection – provide an excel table with more information regarding these strains (name, number, year of isolation, …) – interest to perform WGS
- Gosia hired on the MeVetKlebs project

NCOH (R. Willems, W. van Schaik – Utrecht, The Netherlands)

- Working in Kp from different ICU in Europe
- Non clinical samples – 395 fecal samples from pets and ~2000 from volunteers (householders of pets)
- 144 clinical Kp in animals from Vet Faculty incl. 90 dogs
- Plasmid assembly software developments

Presentation by Bich-Tram Huynh (IP): BIRDY project – Kp healthy carriage

- BIRDY – carriage of Kp in pregnant women's from low income countries
- Senegal
- Madagascar (n=408) – 64% Kp complex (32% ESBL)
- Cambodia (n=149)- 67% Kp complex (12% ESBL)
- SCAI medium for isolation, AST, WGS (Illumina – 2x150 paired-ended)
- Evaluation of risk factors (meat consumption, …)

Presentation by Soizic Sergeant (IP): financial and reporting aspects

Each year, partners of MedVetKlebs will need to obtain from their financial/administrative person, the budget spend on MedVetKlebs project (which needs to be declared together with other possible EJP One Health projects run in the same Institute), and send it to Pasteur (M Caillet/S Brisse) for information.

Important point is that the budget can be moved within a partners among different lines (e.g., publications to consumables) and even can be moved between MedVetKlebs partners. This is authorization of the EJP One Health board.

Presentation by Sylvain Brisse (IP): Klebsiella diversity, taxonomy and ecology

See attached .ppt; the point was made to harmonize our vocabulary on Kp lineages, and that very little is known on Kp ecology.

Discussion of work packages

WP1. Methods

1. **Culture media to be used for Kp isolation**

   Presentation by S Brisse of the SCAI (Citrate Simmons agar with 1% inositol) method and alternative strategies (see attached .ppt)

   IP protocol (Van Kregten JCM1984; Passet and Brisse, JCM2014):

   Citrate Simmons agar from Bio-Rad (Ref 64834)
   myo-inositol from Sigma Aldrich (Ref I5125) – filter-sterilized and added to cooled medium (50°C).

   Protocol for faecal samples

- Enrichment in LB broth with amoxicillin 10mg/L - 18h at 37°C
- 100 uL directly plated on SCAI medium 48h/37°C– selection of yellow, moist colonies

please see full protocols in GoogleDrive

Note: Depending on the type of the sample (e.g. plants; soil) a dilution step might be needed.

<u>Further discussion on alternative enrichment/culture strategies</u>:

With or without enrichment? With or without antibiotic?

Use another medium for enrichment (e.g. buffered peptone water)?

Make an enrichment directly in SC broth with inositol and antibiotic; and then plate onto SCAI agar. Evaluate possible bias introduced by ampicillin (amoxicillin) “enrichment” step.

From the enrichment perform a PCR to detect Kp; and plate in SCAI only the positive samples?

Test other media performances (accordingly to ISO protocols – F. Pomilio from Italy)

Need to test for recovery rate of SCAI (in fact this was compared for SCAI and blood agar in Paris using serial dilutions: SCAI provides identical numbers of CFUs/colonies as blood agar.

Decisions: The SCAI procedure will be distributed to all partners (S Brisse – via GoogleDrive) and tested locally by each partner within three months at most.

Other culture strategies will be explored – still open. Partners are welcome to explore. S Brisse will define with D. Morris and K. Hauser (task leaders) and some partners, specific tests.

SCAI medium should be used to test isolation from a minimal set of common samples across partners such as fecal samples, salad (lettuce, incl. roots); parsley; and spinach. To be defined more precisely in the coming weeks (S Brisse and F Pomilio)

2. **Detection of different Kp groups by qPCR**

Presentation by P Piveteau on qPCR strategy – please see attached ppt**.**

Decisions:

We will target the four main phylogroups of the Kp complex. Ideally a multiplex qPCR with one target for each group (Kp1, Kp2, Kp3, Kp4), one target for the entire complex (incl. Kp5, Kp6) plus one universal target (16S) for quantification of overall microbiome/checking for inhibition.

IP team will search for phylogroup specific genes; Dijon will design the specific primers and test the **specificity and sensitivity** of the primers/probes

Verify the equipment availability in the different partners (universal). All partners will send information about qPCR instruments they could use for testing the method.

Define the controls to use (*E. coli* detection?; 16S)

WP2. Sampling

Presentation by S Brisse on the possible strategies, sources, reservoirs (see attached .ppt)

Lots of brainstorming went on about the best environments in which to search for Kp. It was decided to postpone decisions to after the modelling presentations.

WP3. Genomics & modelling

1. Genomics. This task comes later in the project and it was decided not to spend time on it right now.

It will be important to know if all partners have a sequencer in house or if they need to send the isolates/samples to the other partners.

We will discuss these questions at the intermediate meeting; priority is to define sampling.

2. Modelling

Presentation by **S. Pires – Denmark and L. Opatowski –Paris;** please see attached ppt.

So far, literature on Kp transmission models only exists for the case of hospital transmission. We are more interested by the broader Kp ecology/transmission picture. Two approaches were presented: source attribution and dynamic modelling.

Source attribution via the subtyping approach will need identifying subtypes specific for particular sources. The comparative exposure approach is data-hungry and might be difficult to apply to Klebsiella in the near future.

Dynamic modelling will need temporal/longitudinal sampling of all possible components within specific settings, which we might be able to define. Dynamic models need longitudinal data but can work for a low number of individuals.

Difficulties with Klebsiella include the fact that disease does not follow directly the colonization step, so that 'cases' are difficult to identify except perhaps for specific subtypes (e.g. ESBL resistant); and that defining 'subtypes' is a clearly identified problem for Klebsiella pneumoniae sublineages (either too broad at the level of phylogroups, or too narrow at the level of sublineages). Might possibly be applicable for particular genes, gene clusters, mobile genetic elements or plasmids.

Presentation by Jean-Yves Madec – Update on Klebsiella in animals, see attached ppt

Presentation by Sebastian Lopez (SpARK project, Brisse group) – Kp in food and the environment, see attached ppt

Discussions on sampling

Distinction was made between two steps: *broad* sampling (multiple sources, blind survey) and *deep* sampling (targeted sources defined upon broad sampling to frequently contain Kp).

Taking into account the time of collection (e.g – seasonal variability) has been discussed as important. All partners should collect samples within the same conditions – e.g., summer, winter.

Discussion on the type of samples that each partner is able to sample; e.g., Italy, Ireland, Austria and Denmark have access to large, diverse collections of food samples

How many colonies should we take from the plate? Multiple colonies should certainly be considered to access variability within samples; It will depend of the number of colonies that look like Kp; if we have less than 10, perhaps recover all for (Maldi tof) identification; if many colonies like Kp select up to 10, taking into account different morphotypes.

Communication

- It seemed to be consensual that we do not need a dedicated web site.
- Sharing of documents will happen through a Google Drive folder

- A group photo was taken on Day 2, early afternoon – it is available on the GoogleDrive.

Hired personnel

Pasteur (genomics, administrative support), SSI, INRA have already identified the persons hired specifically on the project.

- Genomics: Carla Rodrigues
- Administrative support: Marina Caillet (who will take over from Soizic Sergeant)
- SSI: Gosia Ligowska-Marzeta
- INRA: Elodie Barbier

Pasteur (modelling), ANSES and IZSAM still have to identify/hire their dedicated persons.

External partners and other consortia

Several other groups (SpARK project, EuSKAPE, NorMedVet, …) are interested in Kp ecology and transmission. We may invite some of them at our next plenary meeting in January 2019. Within the One Health EJP, other projects might also be interested in collaborating with MedVetKlebs.

Next steps:

- Need to define 'common denominator' sampling for early SCAI tests
- Need to define alternative culture strategies
- Organize a skype meeting within three or four months to share experience with the SCAI medium procedure.
- Define broad sampling strategy

Date of next plenary meeting:

10th and 11th of January 2019 (Thursday and Friday) – at Institut Pasteur, Paris. Please mark your agendas!