All you should Know About

Klebsiella pneumoniae

Sylvain Brisse

Biodiversity and Epidemiology of Bacterial Pathogens
Institut Pasteur, Paris, France

All you should Know About

Klebsiella pneumoniae

especially if you are embarking

in 'One Health' Kp studies

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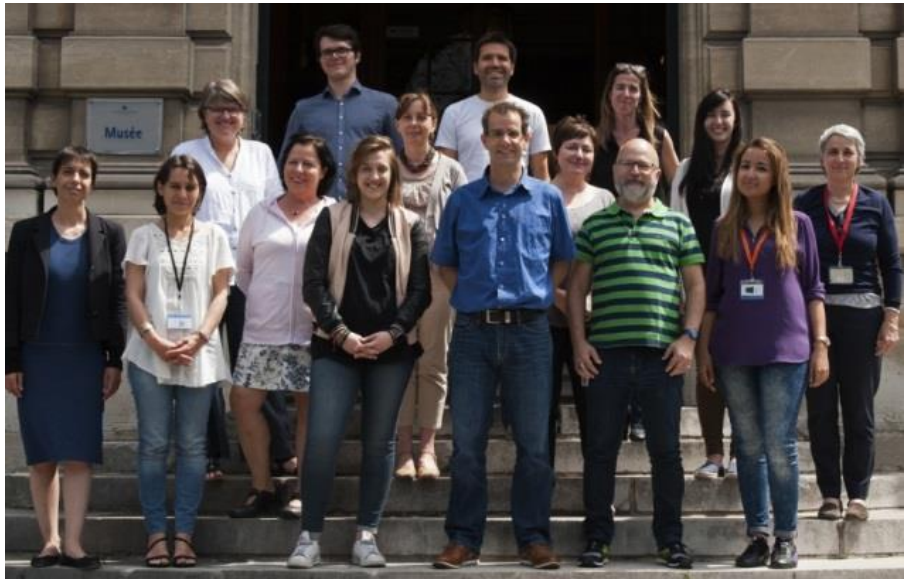
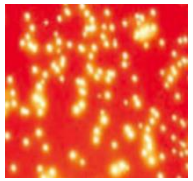
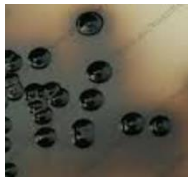
Biodiversity and Epidemiology of Bacterial Pathogens



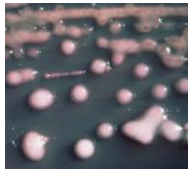
Institut Pasteur



Bordetella pertussis (French National Ref. Center)



Corynebacterium diphtheriae (French Natl. Ref. Center)



Klebsiella pneumoniae

Klebsiella pneumoniae antimicrobial resistance

ESKAPE

- 5 - 10% nosocomial infections

- ESBLs
- Carbapenems
- Colistin, tygecycline

- Death rate > 50% for CR-Kp bacteremia

‘Urgent threat’ (CDC 2013)
WHO priority list
ECDC priority list


A Woman Was Killed by a Superbug Resistant to All 26 American Antibiotics

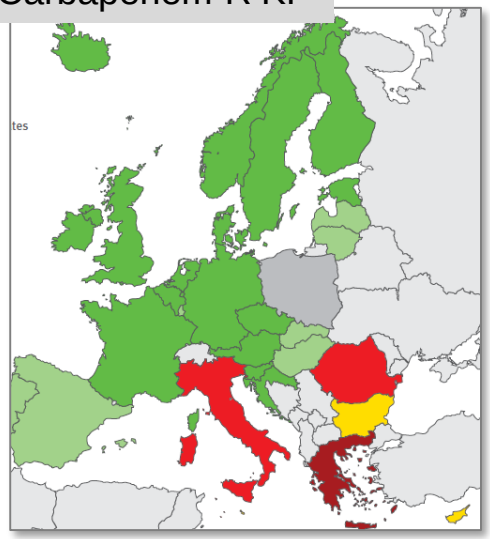
She won't be the last.

SARAH ZHANG | JAN 13, 2017 | HEALTH

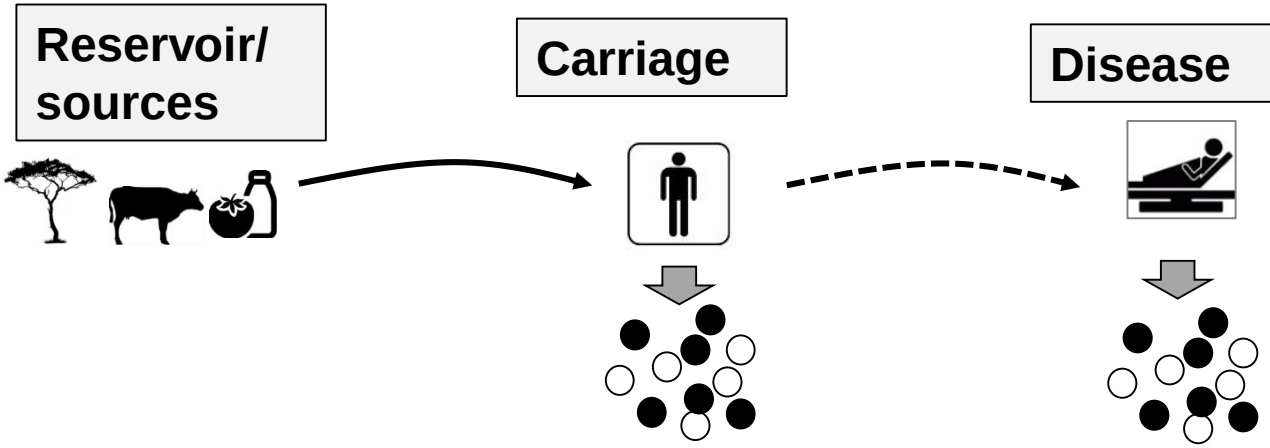
Share Tweet ...

TEXT SIZE – +

Carbapenem-R KP



Klebsiella pneumoniae: carriage precedes disease



Montgomerie 1979
Martin *et al*. 2016
Gorrie *et al*. 2017

- Infection by endogenous strain in most (>50%) cases
- Nosocomial transmission in minority (~10%) of cases

Naive hypothesis:
Knowledge of Kp sources (*e.g.*food) will help control transmission

Naive approach:
Let's compare strains from different ecological compartments and identify the sources

Challenges to ‘One Health *Klebsiella*’ projects

Technological

Kp detection, isolation, identification, genotyping

Genomic data handling & analysis, metagenomics

Organisational

There is no dedicated surveillance for Kp

Biological

Lack of rapid onset infection after ingestion: silent transmission

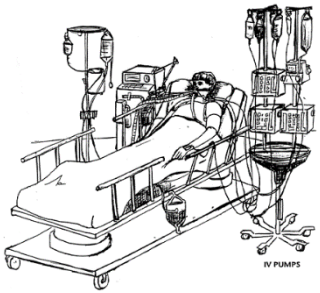
Klebsiella diversity

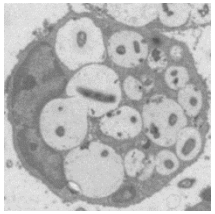
Klebsiella ubiquity

Klebsiella?

Genus *Klebsiella: pre-genomics* taxonomy

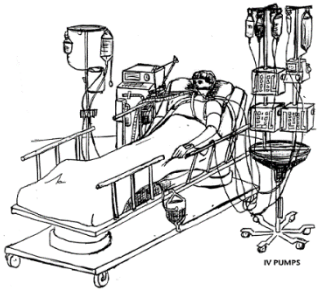
K. pneumoniae
- *subsp. pneumoniae*
- *subsp. rhinoscleromatis*
- *subsp. ozaenae*





- *K. granulomatis (← Calymmatobacterium granulomatis)*
  granuloma inguinale

- *K. oxytoca*

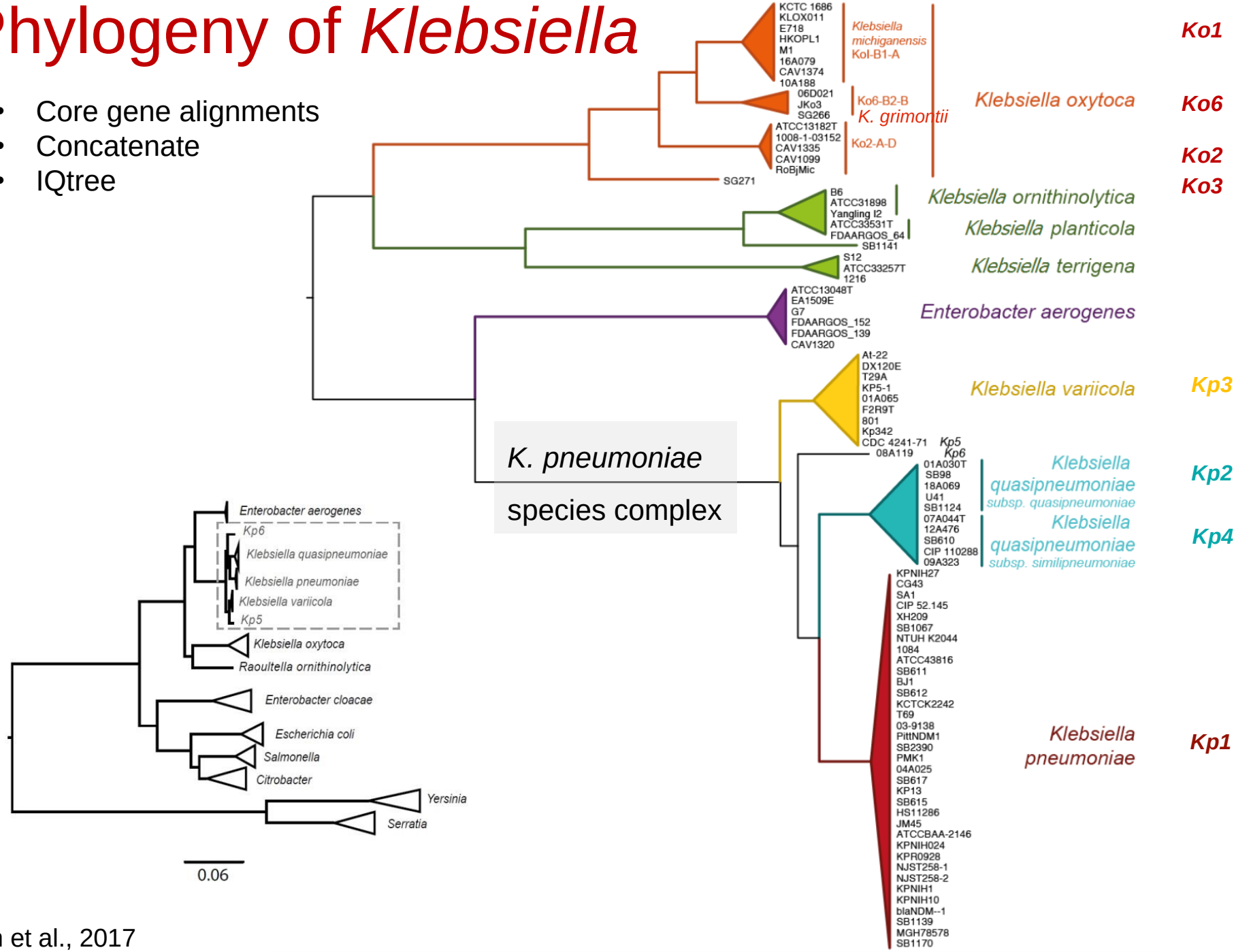


- *Enterobacter aerogenes (K. mobilis)*

- *K. planticola (→ Raoultella)*
- *K. ornithinolytica (→ Raoultella)*
- *K. terrigena (→ Raoultella)*

Phylogeny of *Klebsiella*

- Core gene alignments
- Concatenate
- IQtree

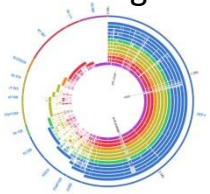


Blin et al., 2017

Average Nucleotide Identity (ANI)

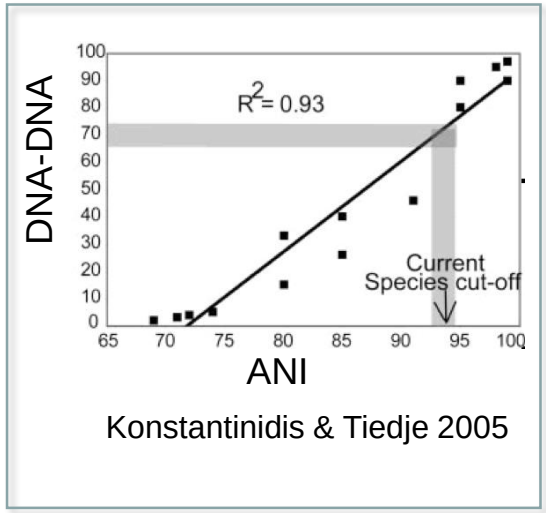
Average Nucleotide Identity (ANI):

1. Search homologous genes between two genomes

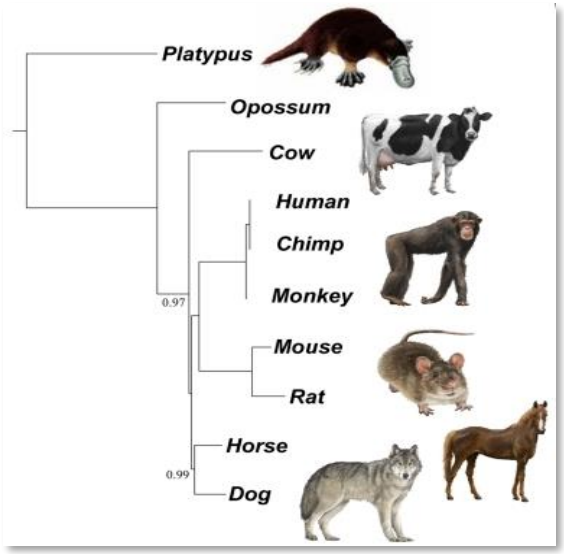


2. Align & calculate % identity

3. Average over all genes



Konstantinidis & Tiedje 2005



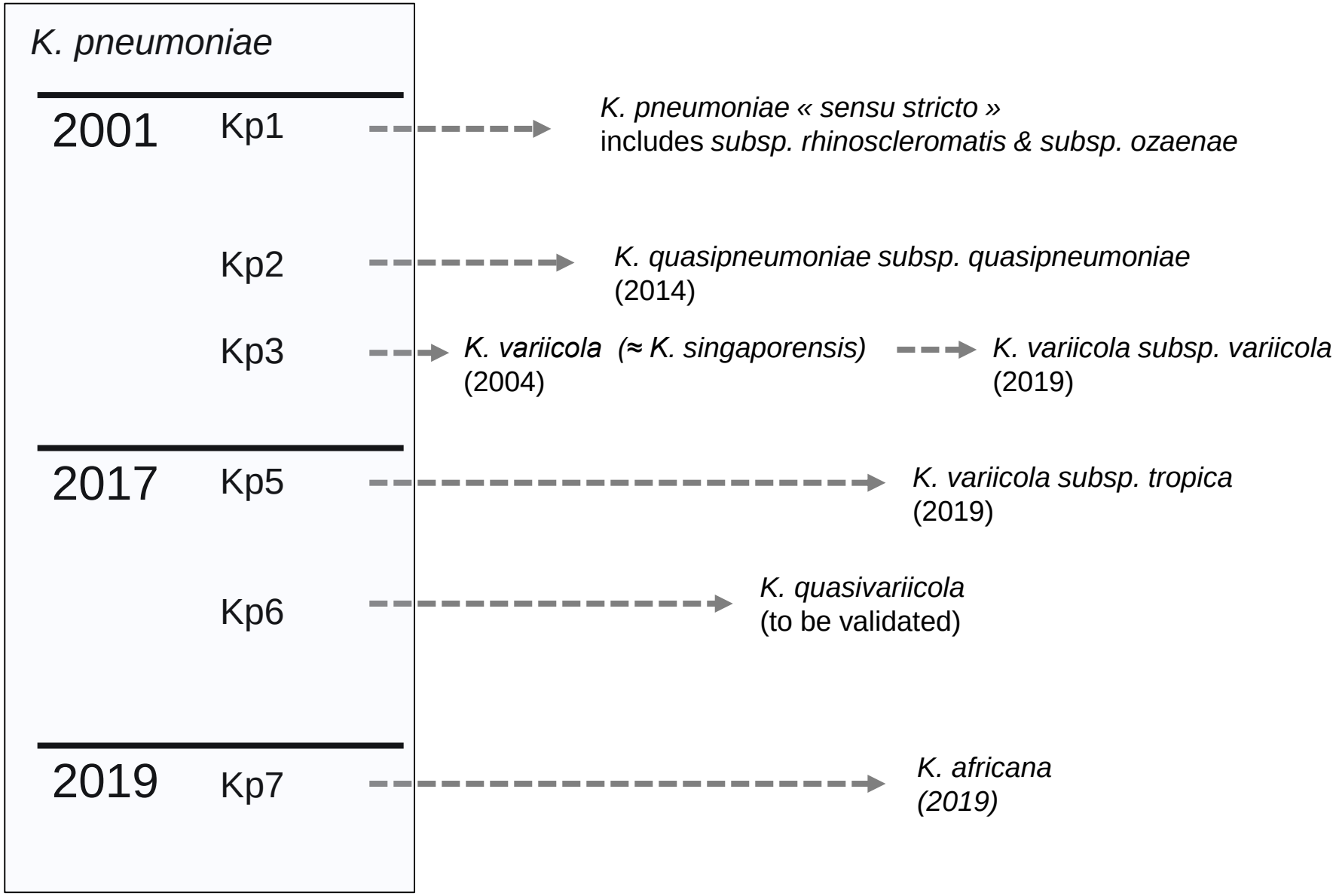
Human- chimp.: ~1.2% nt divergence

5% ANI
70% reassociation

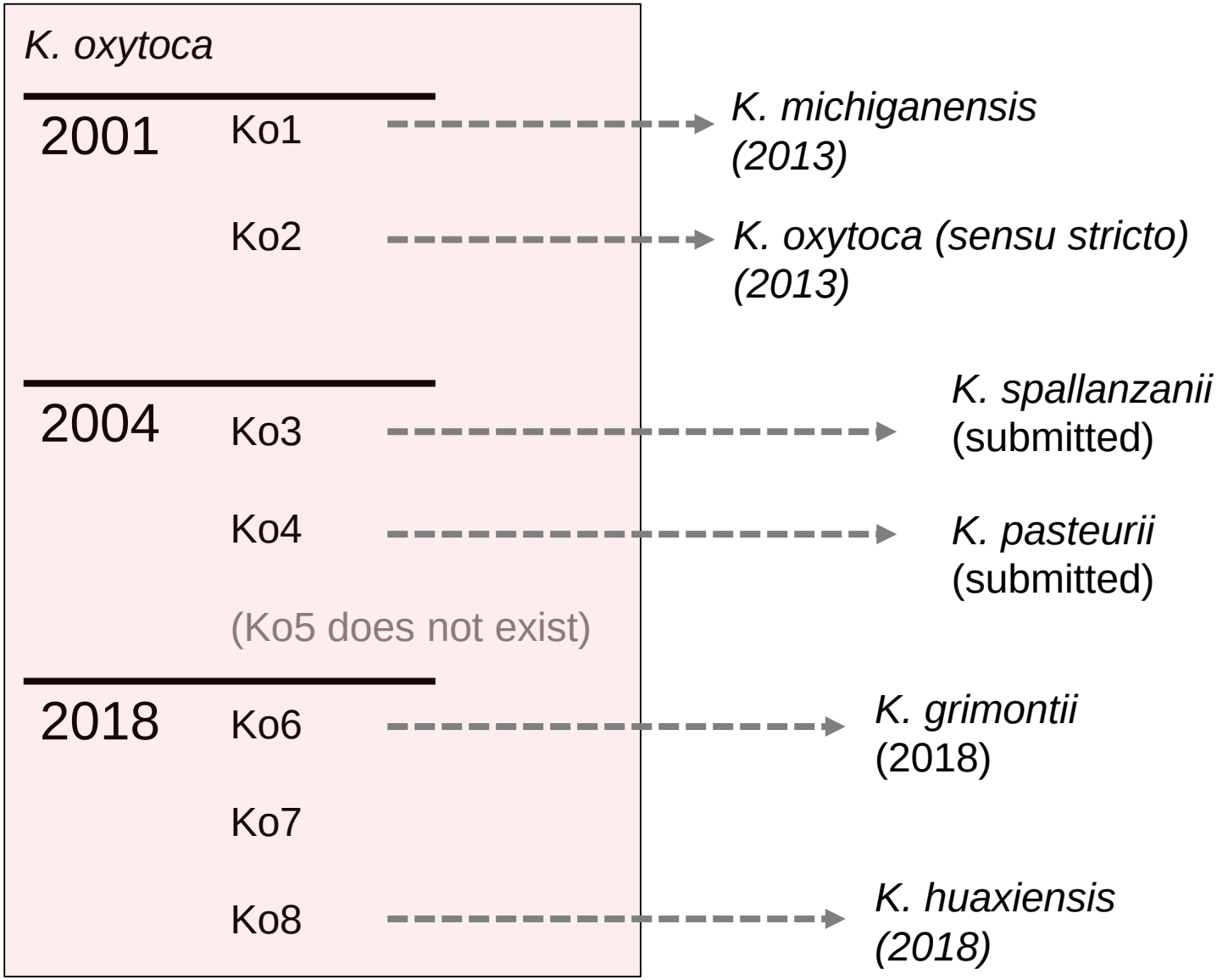
Microbial species borders

Strains within a microbial (bacterial) species can differ by up to 5%

Taxonomic history of the *K. pneumoniae* complex



Taxonomic history of the *K. oxytoca* complex



Klebsiella taxonomy (2019)

- *K. pneumoniae*
  - *subsp. pneumoniae*
  - *subsp. rhinoscleromatis*
  - *subsp. ozaenae*
- *K. granulomatis* (← *Calymmatobacterium granulomatis*)
- *K. variicola* ~ *K. singaporensis*
  - *subsp. variicola*
  - *subsp. tropica*
- *K. quasipneumoniae*
  - *subsp. quasipneumoniae*
  - *subsp. similipneumoniae*
- *K. quasivariicola*
- *K. africana*

K. pneumoniae complex

- *K. planticola* (→ *Raoultella*)
- *K. ornithinolytica* (→ *Raoultella*)
- *K. terrigena* (→ *Raoultella*)

- *K. aerogenes* (← *K. mobilis*, *E. aerogenes*)

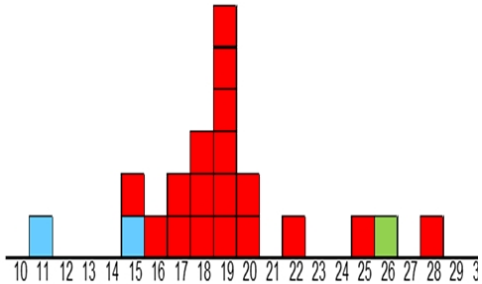
- *K. oxytoca*
- *K. michiganensis*
- *K. grimontii*
- *K. huaxiensis*
- *K. spallanzanii*
- *K. pasteurii*

K. oxytoca complex

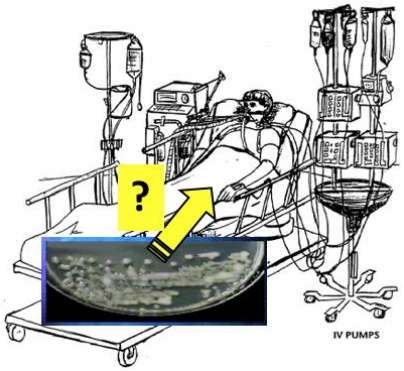
Taxonomy of *strains*

Detecting and tracking the ennemy

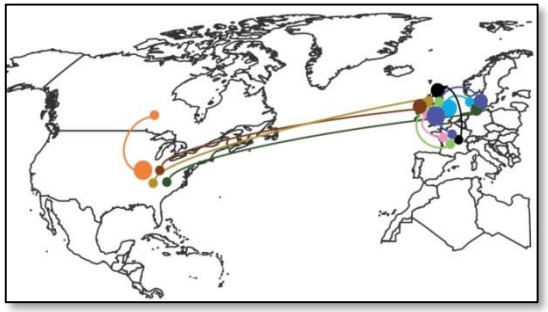
Outbreak detection



Source identification



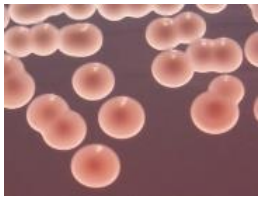
Global spread routes



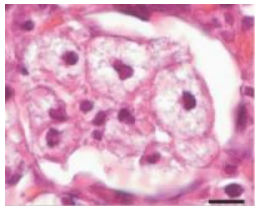
➡ **Public health, Global Health & 'One Health'**

Knowing the ennemy

Biology



Pathophysiology



➡ **Cumulative knowledge on (important) strains**

Strain taxonomies

PFGE type

spoligotype

serotype

Sequence Type

biotype

pathovar

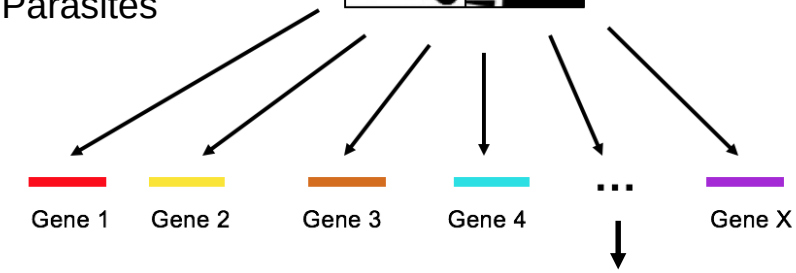
clonal group

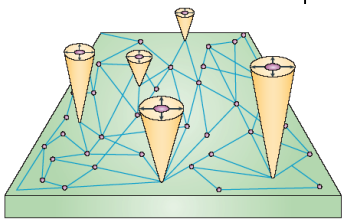
sublineage

The Multilocus Sequence Typing standard

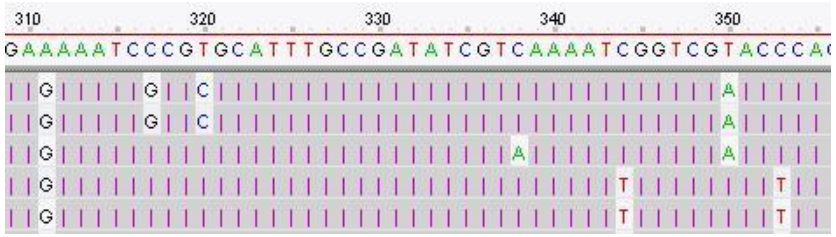
Bacteria
Fungi
Parasites

Maiden, Achtman, Spratt *et al*.





• Sequence of one gene (*rpoB*, 501 bp)

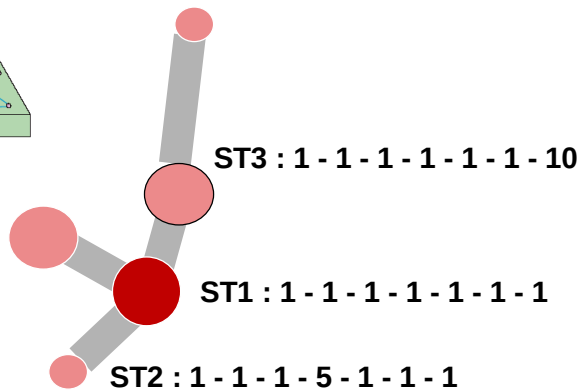


•Allele

1
1
2
3
3

gapA	*infB*	*mdh*	*pgi*	*phoE*	*rpoB*	*tonB*
3	3	1	1	1	1	79

Sequence type (ST)	Alleles
1	4 4 1 1 7 4 10
11	3 3 1 1 1 1 4
258	3 3 1 1 1 1 79

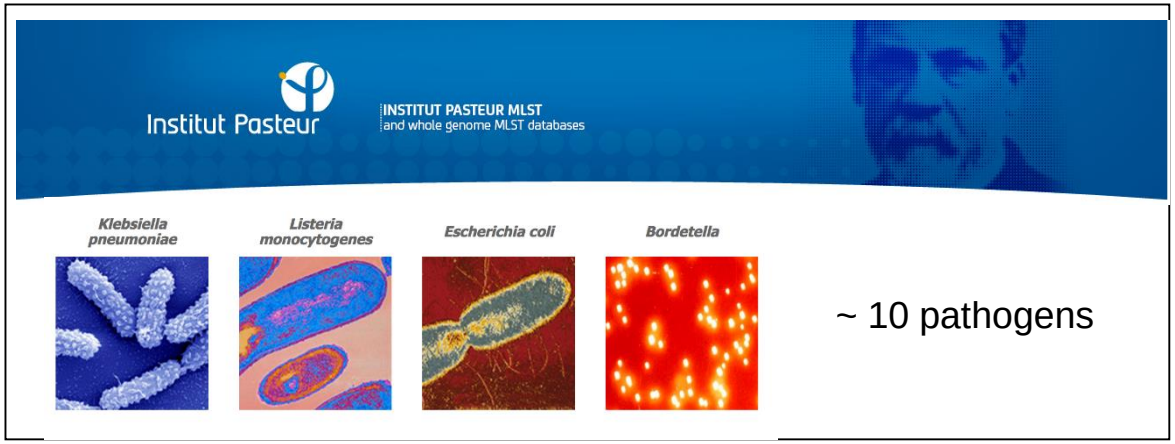


Single-linkage clustering

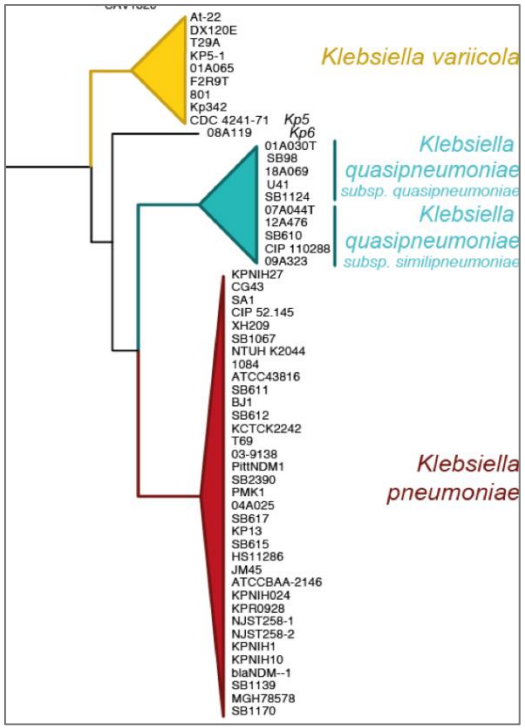
Classification:
Clonal complexes,
Clonal groups,…

Klebsiella pneumoniae MLST database

Hosted at http://bigsdb.pasteur.fr



~ 10 pathogens



Comprises:

K pneumoniae
K variicola
K quasipneumoniae
'K africana'
'K variicola subsp. tropica'

Common language
'Lingua franca'
'Strain esperanto'

Contents:

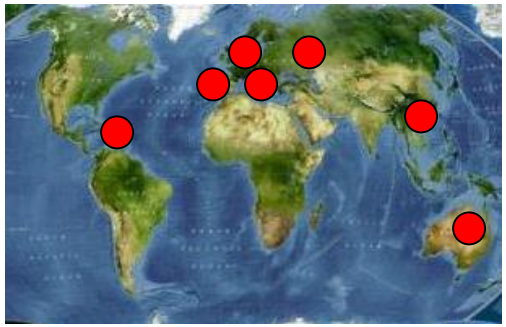
http://bigsdb.pasteur.fr/klebsiella

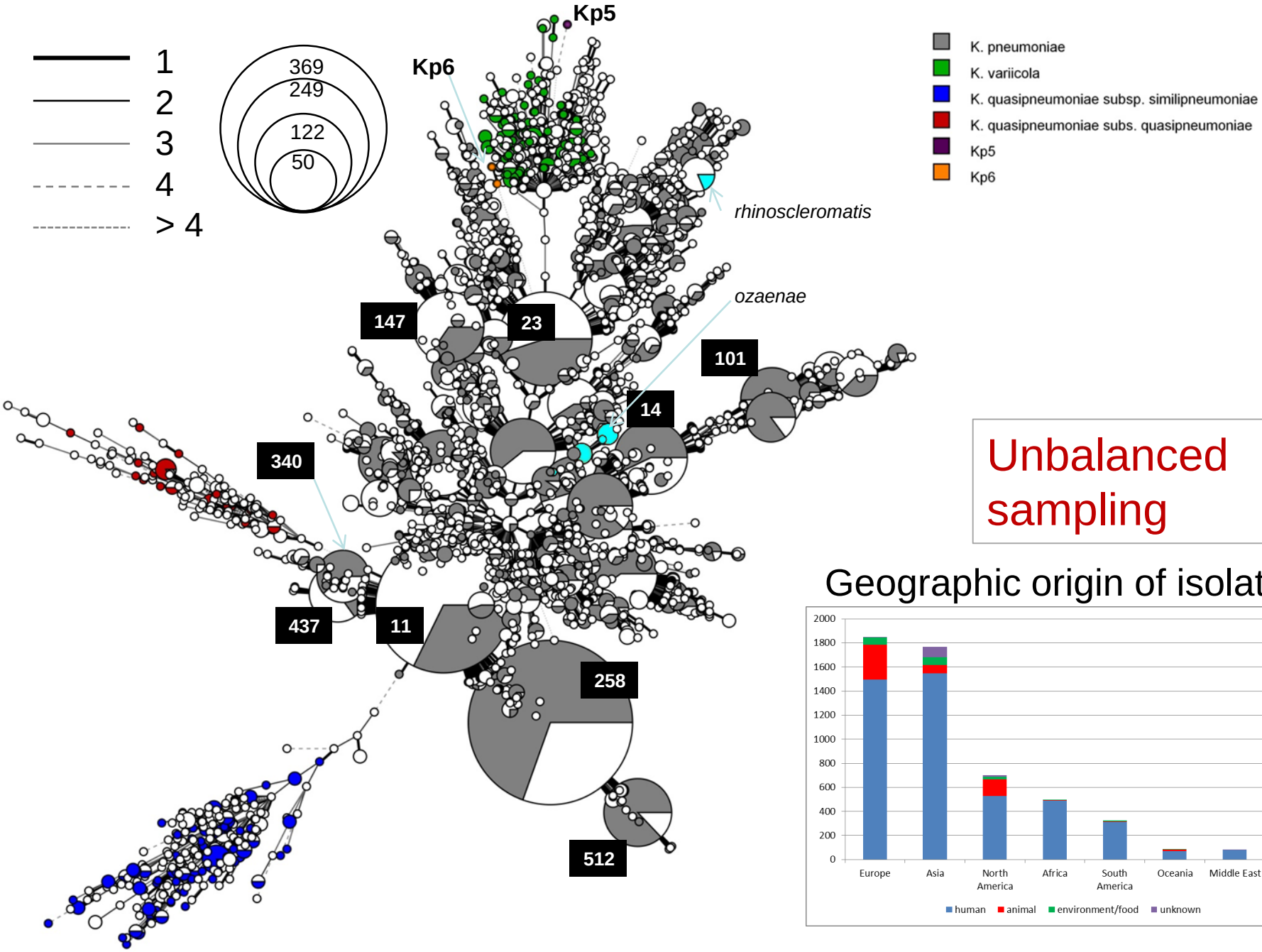
Sequences and profiles database

Isolates database

- 3539 STs
- blaOKP, LEN, OXY
- AbSTs, CbSTs,…

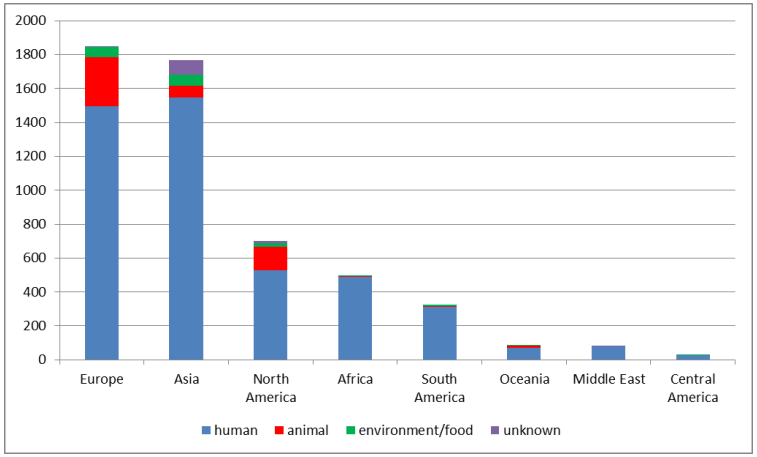
- 5000 public isolates
- 3000 private isolates
- 250 submitters



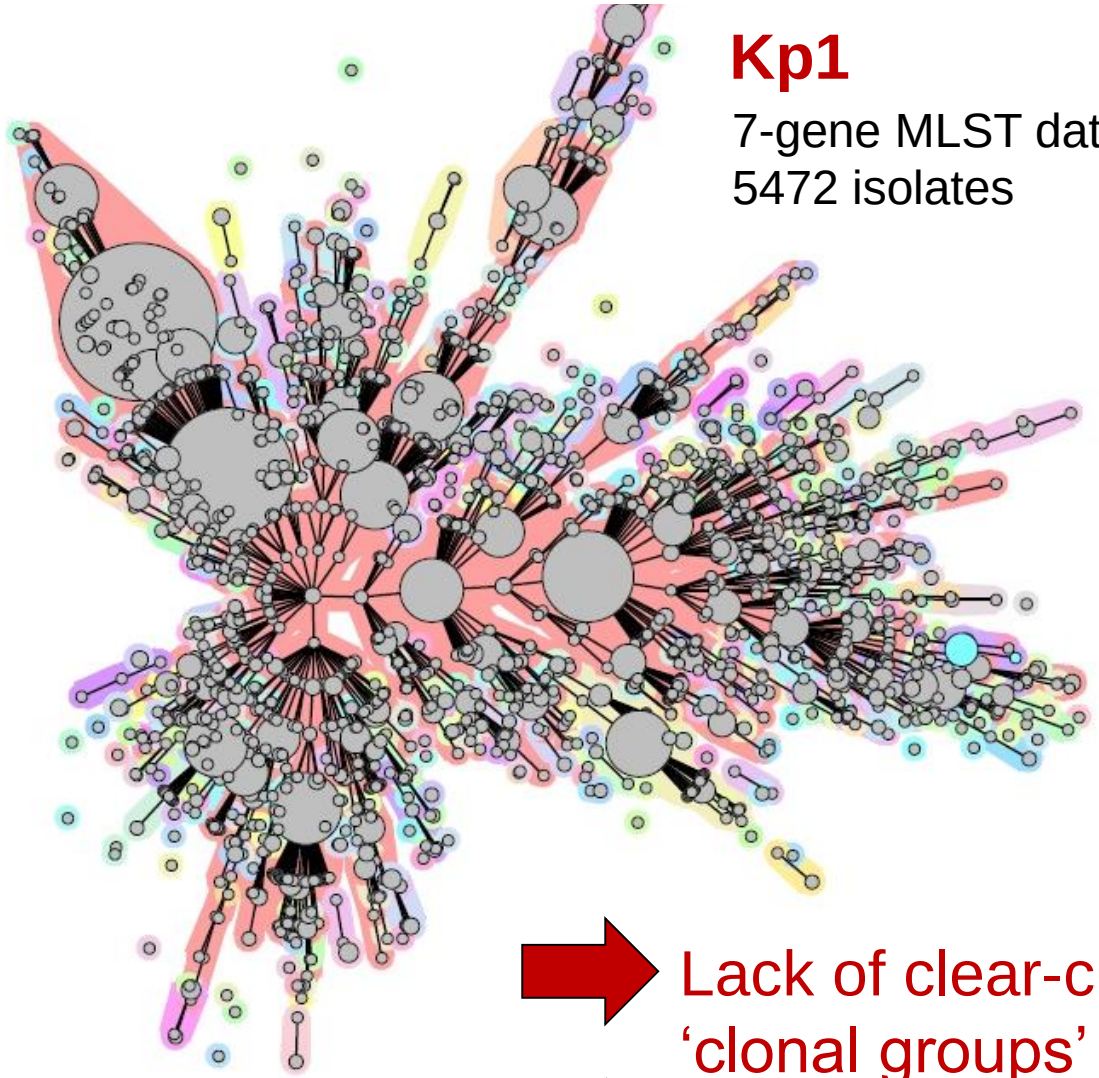


Unbalanced sampling

Geographic origin of isolates

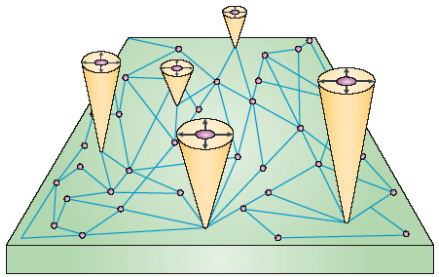


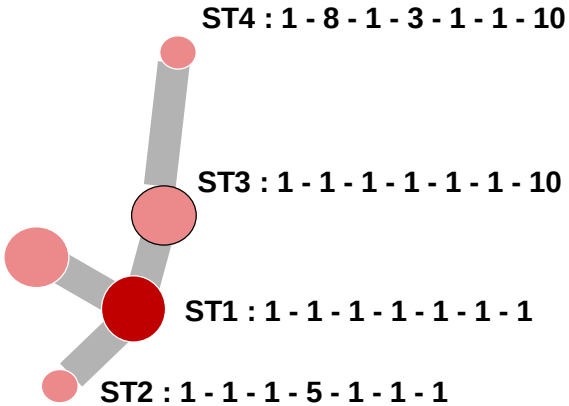
7-gene MLST limitations



Kp1

7-gene MLST database
5472 isolates

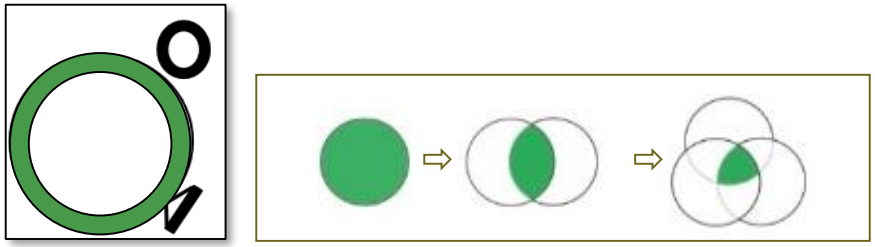




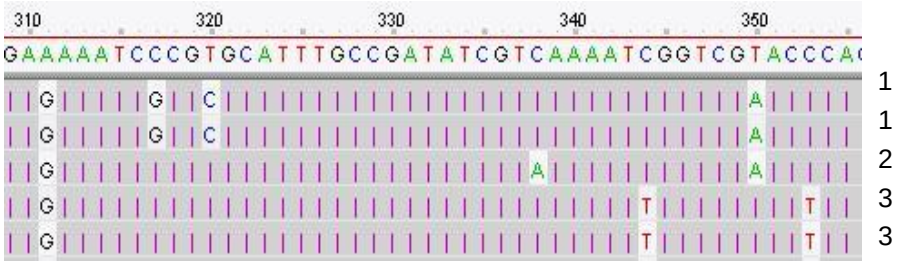
- Lack of clear-cut delimitations of ‘clonal groups’
- Lack of discrimination for small-scale epidemiology

Core genome MLST

Maiden *et al*. 2010



Step 1. Define core genome



Step 2. Define variation at core genes

1	15	12	37	3	16	22	11
1	5	4	24	4	4	1	1
27	28	9	31	1	25	10	15

Step 3. Define allelic profiles of genomes

Kp: 634 highly conserved syntenic gene loci (Bialek, EID 2014)

Sharing genomic nomenclatures

Klebsiella pneumoniae Genome Database

http://bigsdb.pasteur.fr

Please select locus/scheme

cgMLST v1.0

Order results by

locus

Enter query sequence (single or multiple contigs up to whole genome in size)

```
>NC_003112.2                                MC58 chromosome, complete genome
TTCGGCTTAAACCTTATCCACATCCAAACGCATAACCGTAACCCATTCACCGTTATGGAAATGTCGCCCG
ACAACCACCC
AGCCGAATGATTCATAAAATATTTGCACATCAGGCGTATAAAGATACAAGAACTTTATCCCCAGCGAACG
CGCTGCGCCT
ATGCAGTGGGCGACCAGCCTCCTGCCAATGCCTTTTCCGCGATATTCAGGTAAAACAAAGACATCCCCCA
```

Alternatively upload FASTA file

Select FASTA file:

Parcourir... Aucun fichier sélectionné.

or enter Genbank accession

Action

Reset Submit

Matching profiles

Closest profile: cgST-13

Mismatches: 2

Powered by BIGSdb
(Jolley *et al*. 2010, 2018)

Genomic nomenclatures: conclusions

- 7-gene MLST is the main language and will be continuously extended… curator volunteers are welcome!
- Genomic nomenclature: work in progress
- BIGSdb-Kp database will soon be open for submitters, and structured into private datasets, shareable with chosen users

Isolation of *Klebsiella*

Isolation of *Klebsiella:* principles

- *Klebsiella* members harbor a constitutive beta-lactamase chromosomal gene
- *Klebsiella* can utilize inositol and citrate as sole carbon sources

(Fecal) Sample → LB+Ampicillin 18 h, 37˚ → Spread on SCAi agar, 37- 44˚ , 48 h

SCAi: Simmon's citrate with inositol

SCAi: unbiased selective enrichment procedure

Van Kregten *et al*., 1984

Most Kp STs grow on SCAi

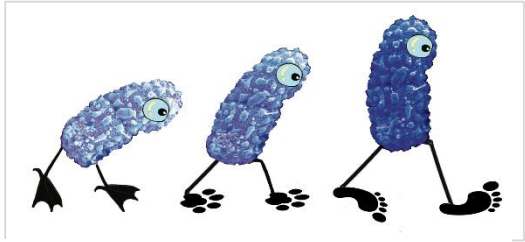
Passet & Brisse, 2015

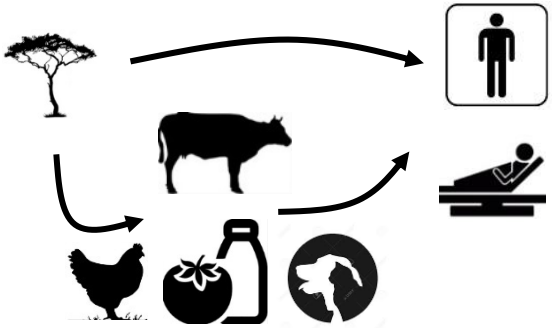
Fecal Sample → LB+Ampicillin 18h, 37° C → Spread on SCAi Agar, 37° C, 48h

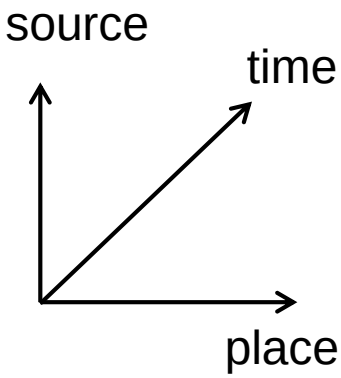
Confirm identification (MALDI-TOF)

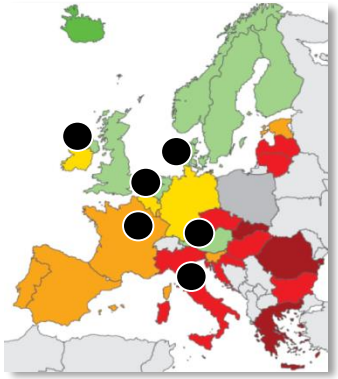
- Antibiotic susceptibility
- Extract DNA
- Genomic sequencing

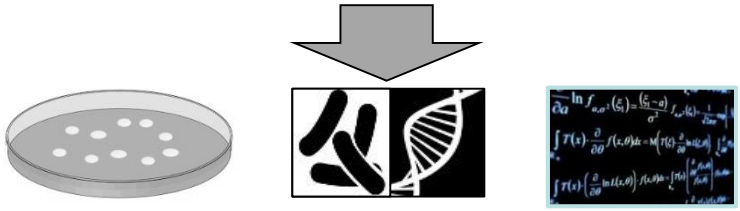
MedVetKlebs











WP1:
E. Barbier (INRA)
D. Morris (NUIG)
K. Hauser (AGES)
F. Pomilio (ISZAM)
E. Moller (SSI)
S. Brisse (IP)

WP1. Detection & isolation methods

WP2. Broad sampling of potential reservoirs & sources

WP3. Population genomics, gene flux & modeling

SCAi allows growth of all phylogroups, diverse STs

52 strains (Kp1 – Kp6; Ko1, Ko2, Ko6; K. planticola, K. ornithinolytica, K. aerogenes)

approx 100 CFU expected; growth at 37C; recovery counts

As ‘productive’ as non-selective, Sigma and LioFilChem

F. Pomilio, IZSAM, Italy (unpublished)

One SCAi, sample-specific protocols

Water (30 L) filtered	Buffered peptone water 24 h, 42 C	Spread on SCAi 37 C, 48 h
Fecal (10 ul)	LB (TCS) + Amp 24 h, 37 C	Spread on SCAi 37 C, 48 h
Meat (25 g, 225 ml BPW)	BPW 24 h, 37 C	Spread on SCAi 44 C, 48 h
Soil	90 ml LB+Amp 24 h, 30 C	Spread on SCAi 37 C, 48 h

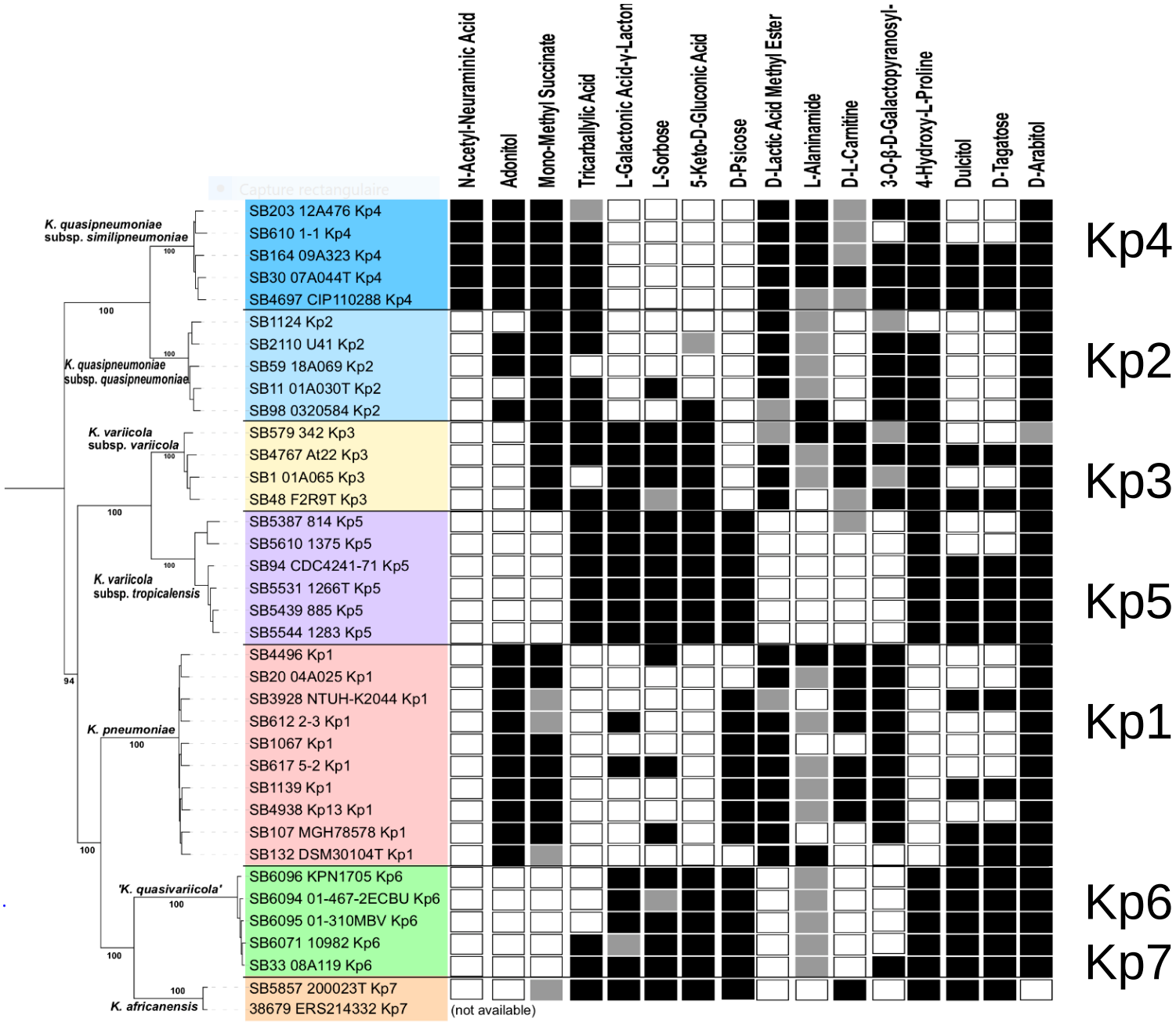
Protocols available upon request

SCAi medium : conclusions

- SCAi recovers all/most *Klebsiella*, with similar productivity
- SCAI agar incubation at 44C can remove contaminants (e.g., *Proteus*)
- Adapt pre-treatment to sample type
- Protocols available upon request

Identification of *Klebsiella*

Catabolic diversity within the Kp complex

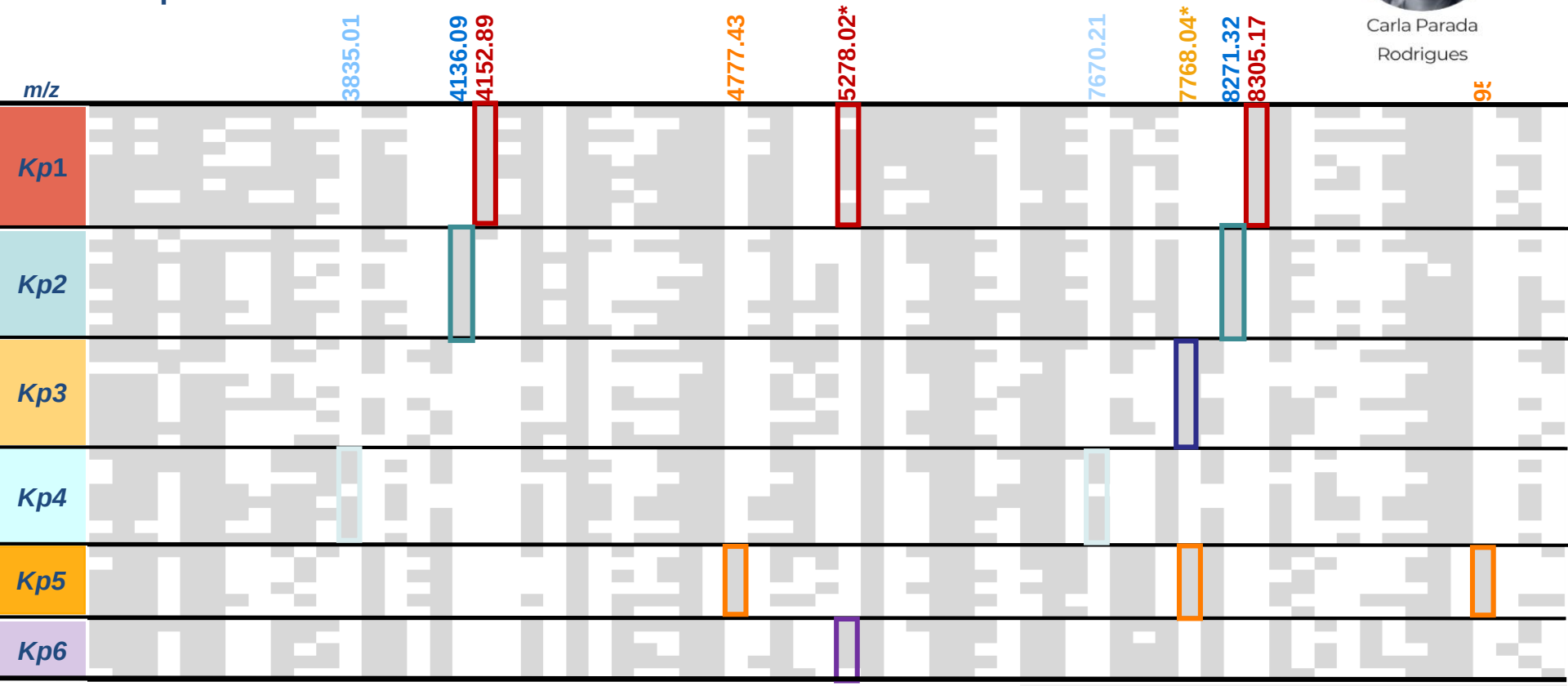


MALDI-ToF MS discriminates *Klebsiella* taxa

- **Microflex LT mass spectrometer**
  (Bruker Daltonics, Germany)
- **24 spectra for each isolate**

Rodrigues, Passet, Rakotondrasoa & Brisse, 2018

Carla Parada Rodrigues



Sensitivity – **80-100%**; Specificity - **97-100%**

- **Currently in microbiol labs MS: only Kp1 / Kp3 reference spectra**
- **Need for integration / evaluation in clinical microbiology settings**
- **Open database identification system?**

Kp identification in practice

- MALDI-ToF works, but needs implementation
- Biochemical tests possible but not practical
- blaSHV, blaOXY or other genes (*gyrA, rpoB*)
- Genomics & ANI
- **Need for simple methods; culture-free a plus**

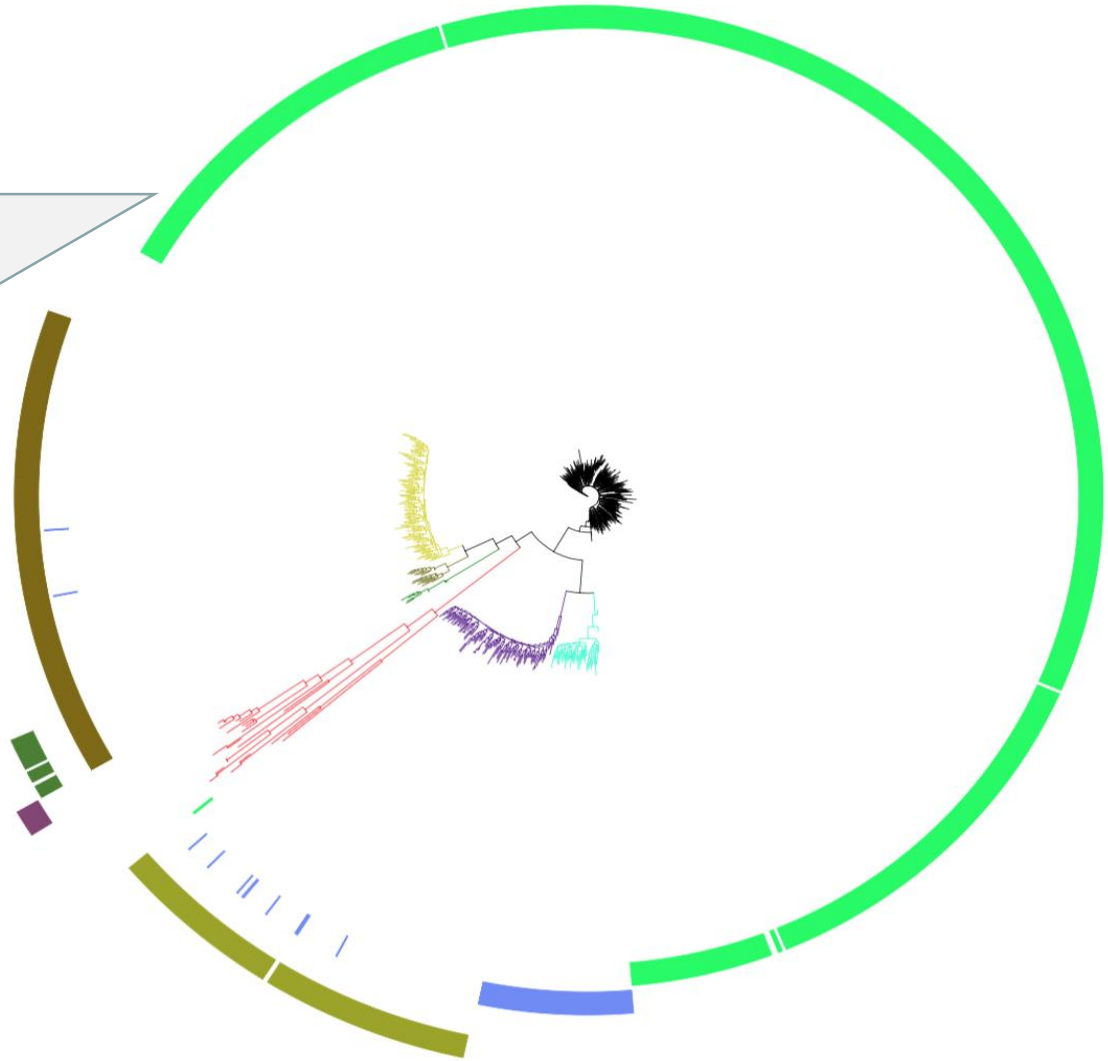
Phylogroup-specific qPCR assays

1001 ST-deduplicated genomes

Pangenome distribution, Specificity, G+C% filtering

6 specific target genes

- **Kp1**
- **Kp2**
- **Kp4**
- **Kp3 & 5**
- **Kp5**
- **Kp6**



Taqman qPCR screens

E. Barbier

5 systems

Kp_1, Kp_2, Kp_4, Kp_6* and *$Kp_{3\text{-}5}$

- **Specificity tested on reference strains**

***Kp* Complex**	
Kp_1	10
Kp_2	9
Kp_3	9
Kp_4	9
Kp_5	5
Kp_6	5

Other *bacteria*	
K. oxytoca	8
Raoultella	5
Enterobacter	3

☺ **100% *specificity for Kp_1, Kp_2, Kp_4 and Kp_6***

😐 **Lack of discrimination between *Kp_3* and *Kp_5***

- **Specificity tested on environmental isolates**

$Kp_{1\text{-}2\text{-}4}$	114
$Kp_{3\text{-}5}$	58

R. ornithino	18
K. oxytoca	5
R. planticola	2
E. aerogenes	2

☺ **100 % agreement real time PCR – Maldi Tof**

- *K. pneumoniae* ⇨ ***Kp_1* or *Kp_2* or *Kp_4***
- *K. variicola* ⇨ ***$Kp_{3\text{-}5}$***

Culture-free detection of *K. pneumoniae*

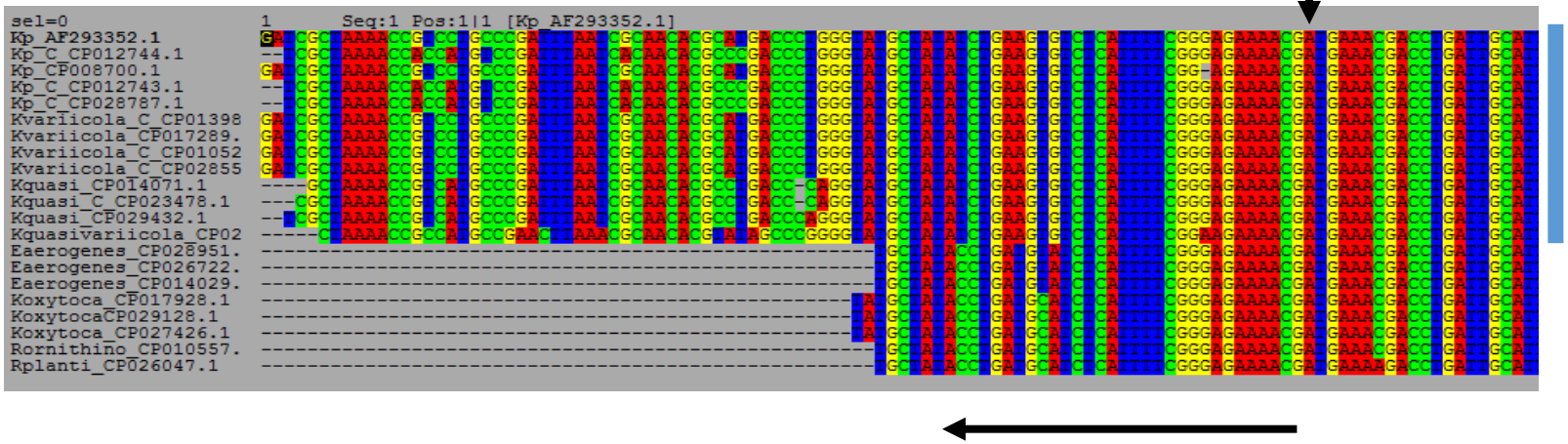
Available PCR assays

Target	Target phylogroup stated in the publication	Presence in Kp Phylogroups
KPK_2677_1_KV770	Kp3	Not specific
mtnC	-	*Klebsiella* spp., *Enterobacter* and *Raoutella*
KpI50233a_KP1	Kp1	Kp1
KPK_2449_1_KV1000	Kp3	Not specific
KPK_1825_1_KV1615	Kp3	Not specific
KP1_4125_1_yphG	Kp complex	Not specific
KP1_2389_1_KP888	Kp complex	Kp1+Kp6
16S-23S rRNA IS	Kp complex	Not specific
16S-23S rRNA IS	Kp complex	Not specific
16S-23S rRNA IS	Kp complex	Not specific
16S-23S rRNA IS	Kp complex	Not specific
16S-23S rRNA IS	Kp complex	Not specific
pehX	*K. oxytoca*	only *K. oxytoca* complex
khe	*Klebsiella* spp.	*Klebsiella* spp.
tyrB	Kp complex	Kp complex

Most/all : Before taxonomic updates

zur – *khe* intergenic region (ZKIR)

khe start



KpC

zkir_F CTAAAACCGCCATGTCCGATTTAA

zkir_R TTCCGAAAATGAGACACTTCAGA

- Uniquely present in *K. pneumoniae* complex
- Expected detection in Kp1 to Kp7

Assay development based on SYBR green chemistry

ZKIR qPCR is highly specific

E. Barbier

- **Specificity tests on reference strains**

***Kp* Complex**		**Other *Klebsiella* and *Raoultella***		**Other *bacteria***	
				Enterobacter aerogenes	3
Kp_1	10 strains	*K. oxytoca*	*8*	*Escherichia coli*	1
Kp_2	9	*R. planticola*	*7*	*Salmonella enteritidis*	1
Kp_3	9	*R. terrigena*	*5*	*A. tumefasciens*	1
Kp_4	9	*R. ornithinolytica*	*2*	*Enterococcus sp.*	6
Kp_5	5			*S. aureus/capitis*	2
Kp_6	5			*Bacillus cereus*	1
Kp_7	1				

☺ **All the *Kp* phylogroup strains: positive**
☺ **All other tested bacteria: negative**

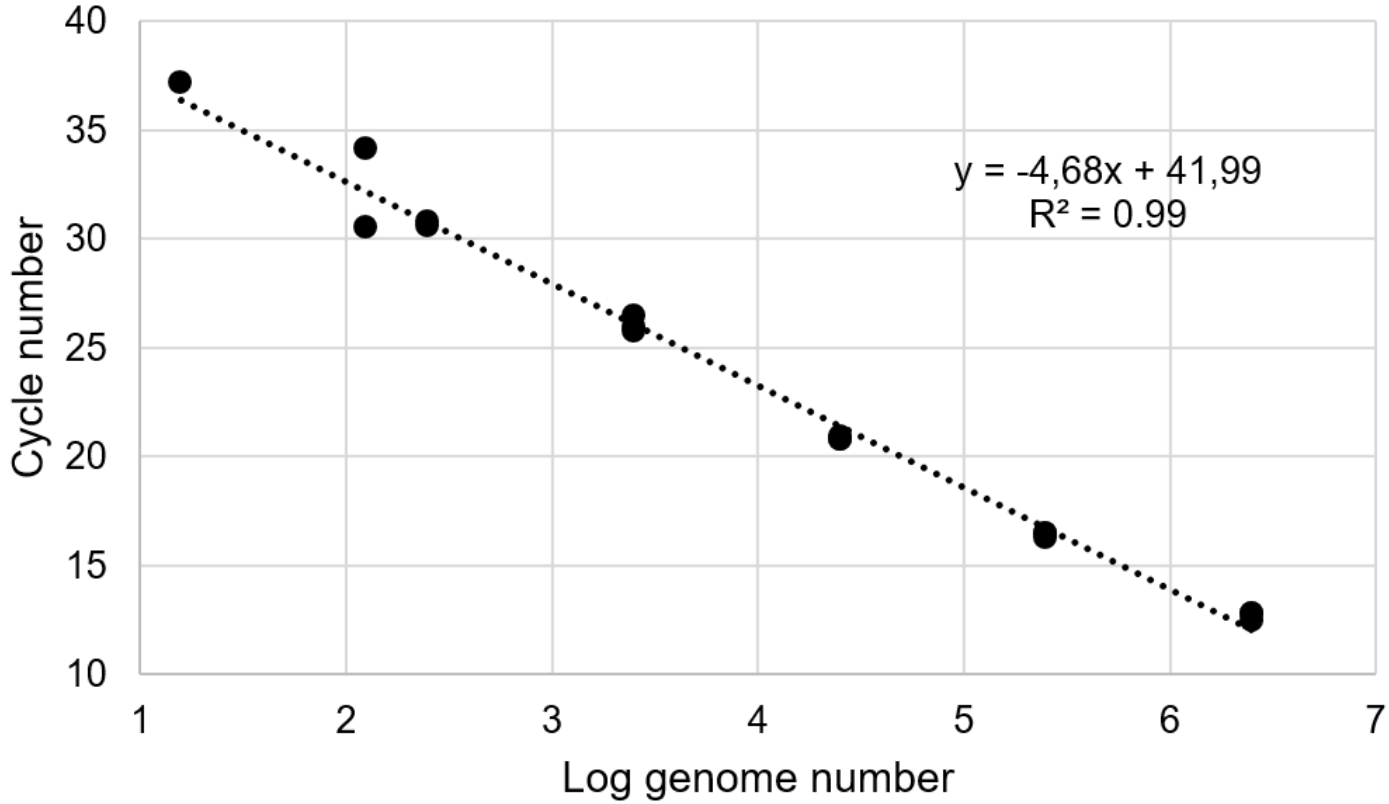
- **Specificity tests on environmental isolates**

172 zkir + isolates
75 zkir - isolates

☺ **100 % agreement realtime PCR – Maldi Tof**

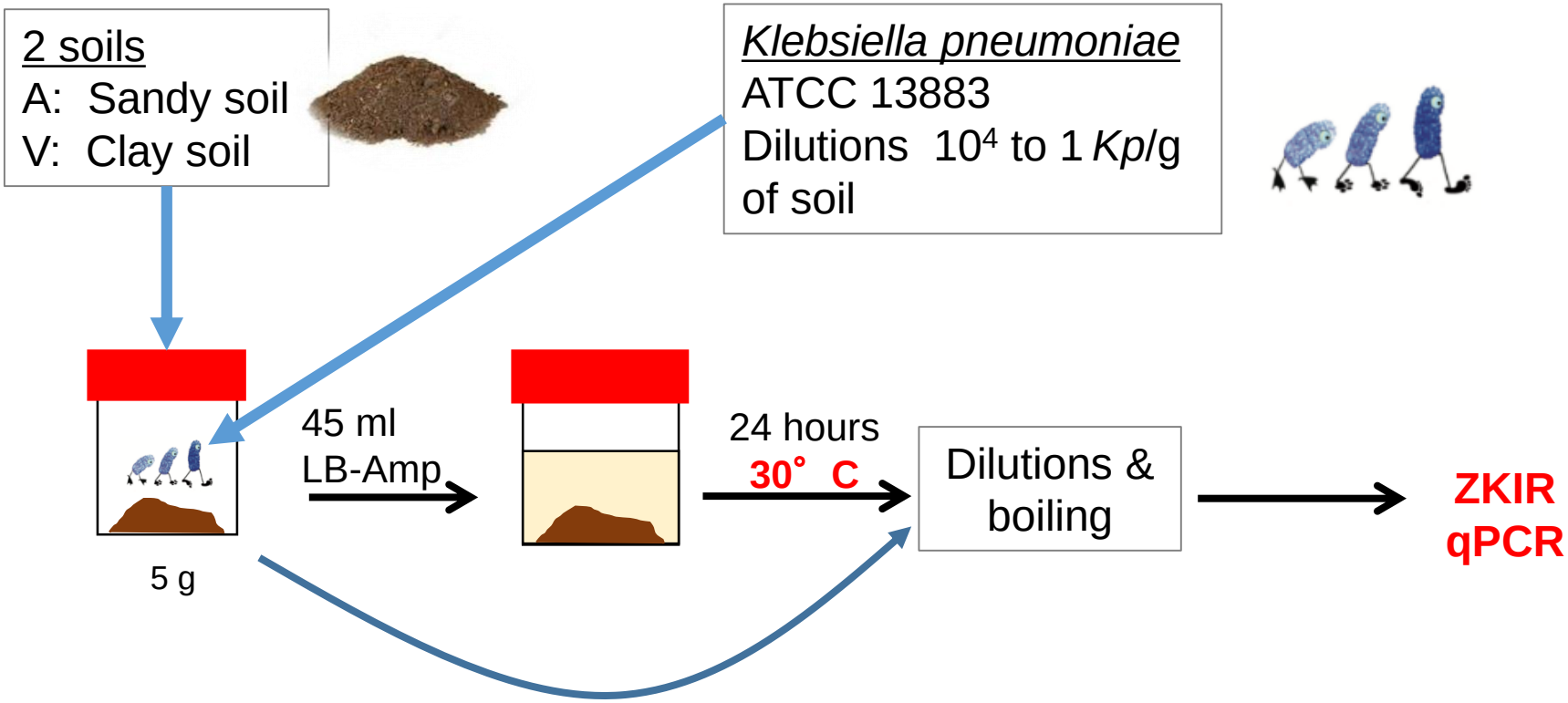
- zkir + ⇨ *Kp* complex (*pneumoniae* or *variicola*)
- zkir - ⇨ Non *Kp* Complex (*K. oxytoca*, *Raoultella*, …)

Analytical sensitivity of ZKIR qPCR



- Good linearity
- Limit of detection: 15 genomes (45 fg) per reaction

Sensitivity in spiked soil

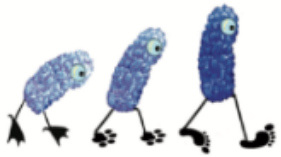


Limit of detection:

1.5 CFU g^{-1} after enrichment

1.5×10^3 to 1.5×10^4 CFU g^{-1} directly from soil extraction

ZKIR qPCR assay and culture-based method



Environmental samples

650 samples tested with ZKIR qPCR

- 98 zikr +
- 93 (95%) recovery of *Kp* isolates on SCAI

Food samples

240 samples tested with ZKIR qPCR

- Higher sensitivity than SCAI
- Good concordance

ZKIR assay: (Barbier *et al*., in prep)

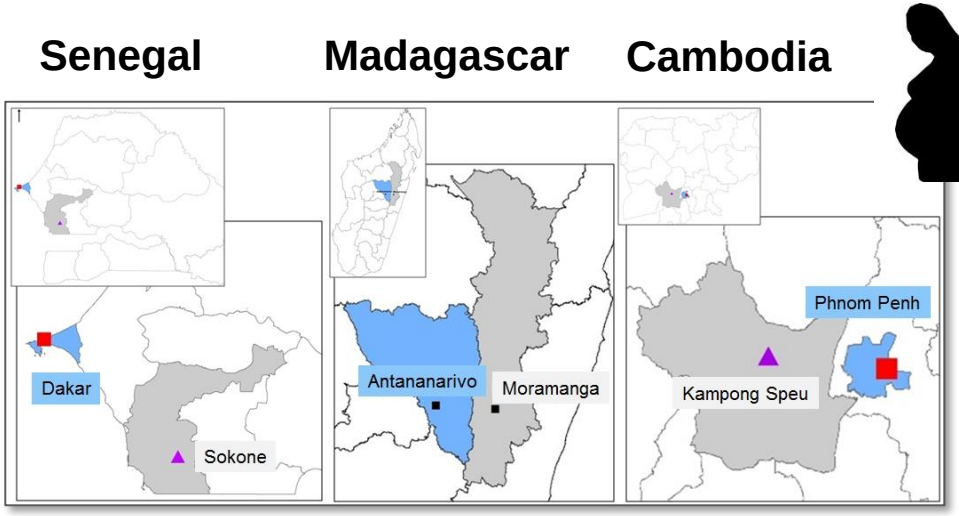
Sensitive, specific, fast & cheap screening method for Kp presence in environmental samples

Available on protocols.io

Institut Pasteur

Klebsiella pneumoniae carriage in three low-income countries

Multicentric cohort of pregnant women



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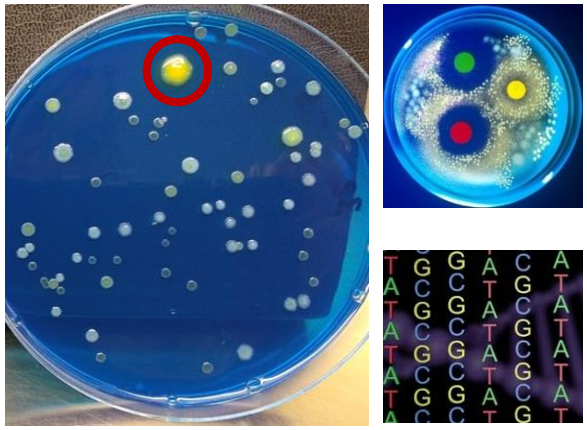


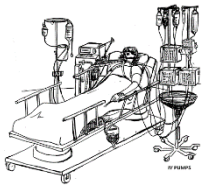
« Single isolate »
LB+Amp, SCAi

Urban Rural

Cross-sectional study : enrollment during one year (2015-2016)

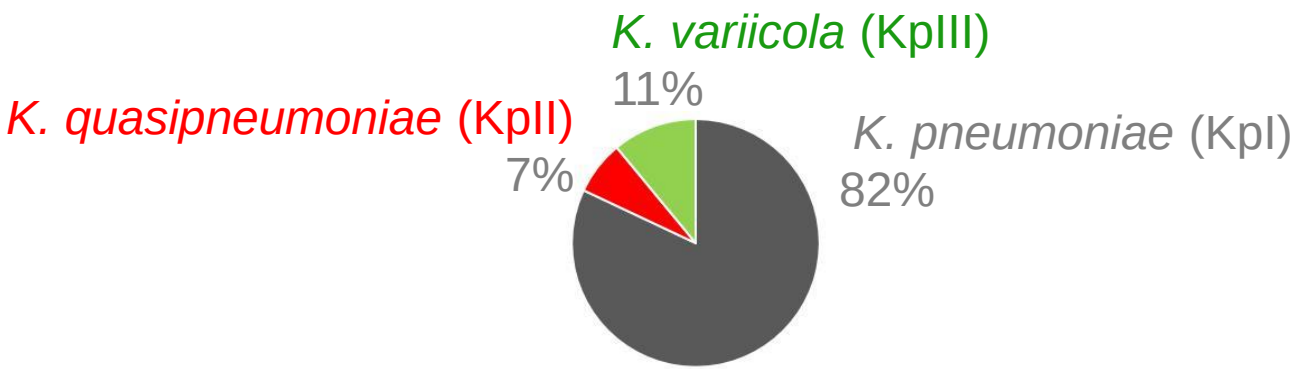
- ✓ Socio-demographics, medical history, environmental exposure, dietary habits, antibiotic intake
- ✓ Stool sample or rectal swab collected at delivery
- ✓ *Kp* isolation, antimicrobial susceptibility, genomic sequencing

Clinical prevalence of *Kp* phylogroups



420 nosocomial isolates:
- Blood (n = 305)
- RTI (n = 56)
- UTI (n = 35)
- Wound (n = 24)

Brisse et al., CMI 2004

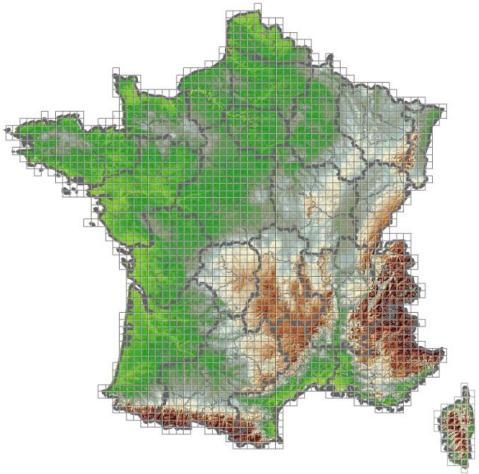


Hypothesis

Carriage rate, rather than attack rate, determines phylogroup frequency in <u>*opportunistic*</u> infections

Detection of Kp in the environment

E. Barbier

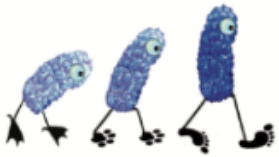


- **RMQS soils**: French soil quality monitoring network (Infosol, INRA Orleans)
  16 km^2 grid pattern- 2200 sites - sampling 2016-2027

  ↳ **130 soils analysed (April to November)**

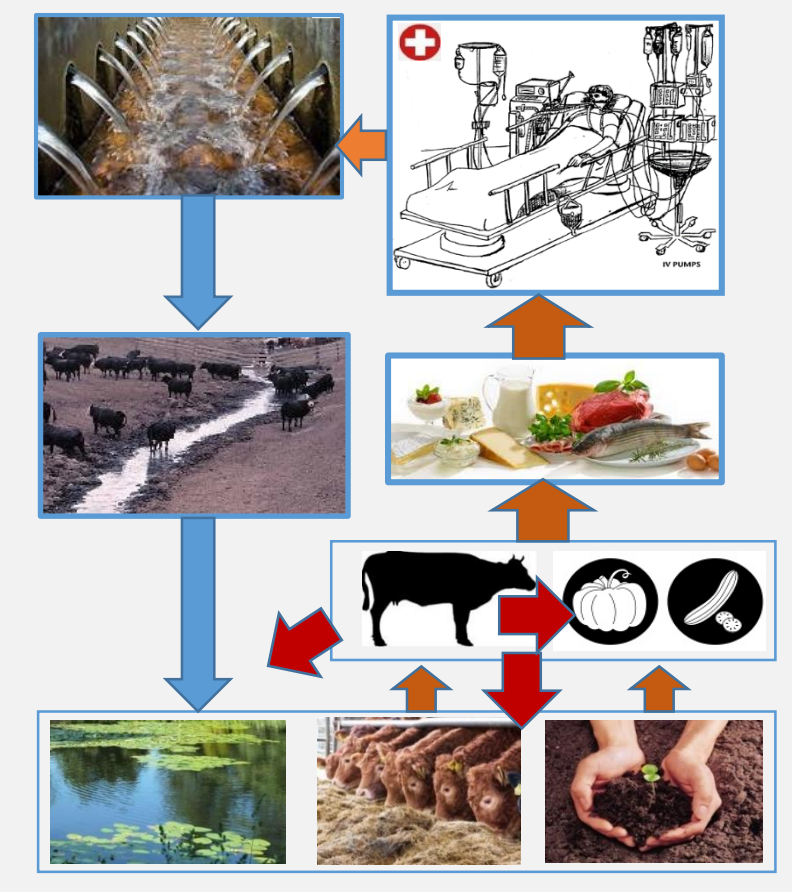
- **210 various samples** from different regions of France and Europe (**soil, mud, feces, water, sewage**…) (One Health EJP - ListAdapt)

- **Wastewater treatment plants from Côte d'Or: treated water** (7 samples) **and sewage sludges** (30 samples)



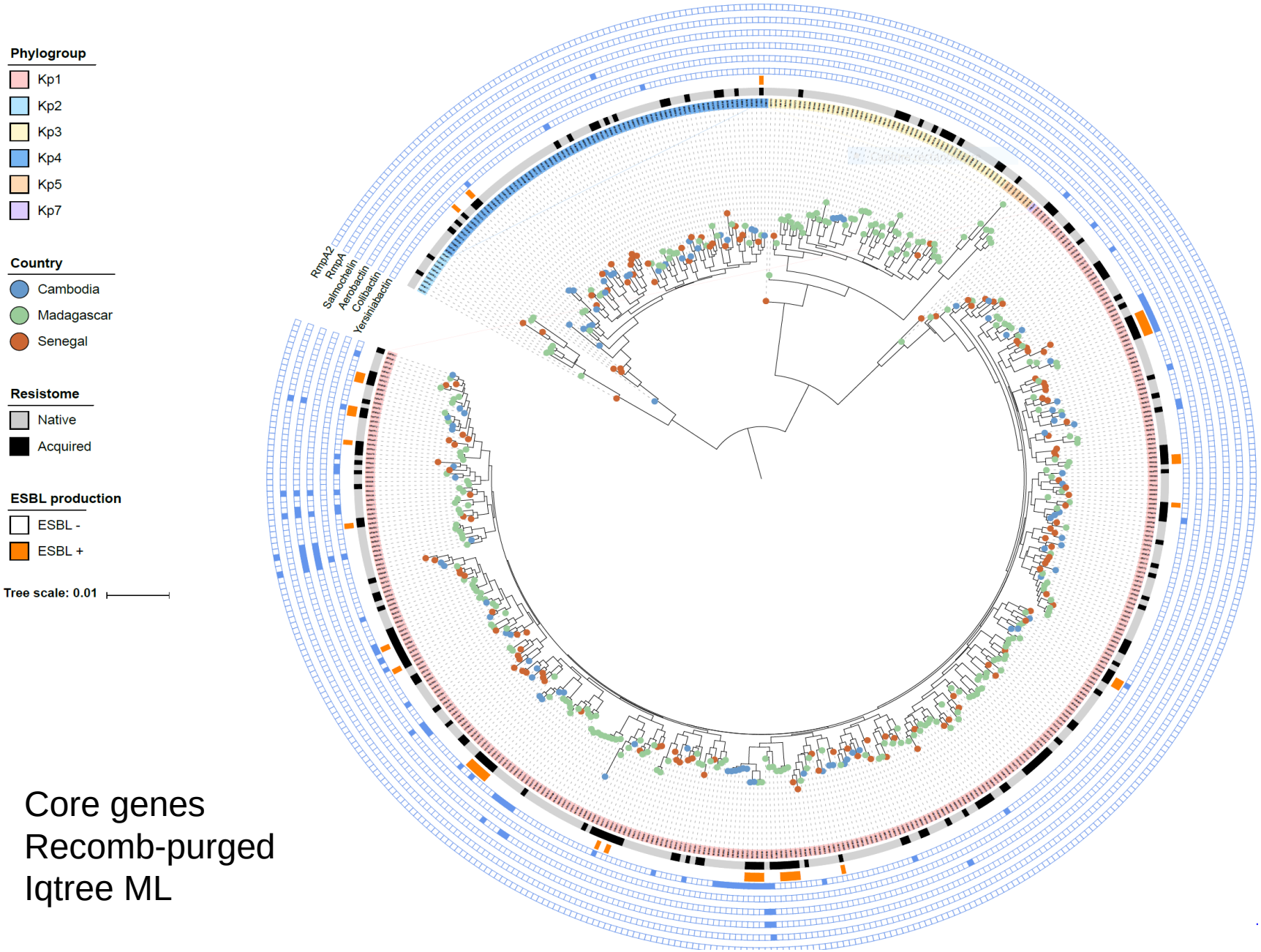
Kp sampling: conclusions

- High rate of *Kp* carriage (approx. 60%) in LMIC community
- Heterogeneous situation among countries; Kp1 predominant
- Kp present in multiple environmental sources; Kp1 predominant



Transmission?

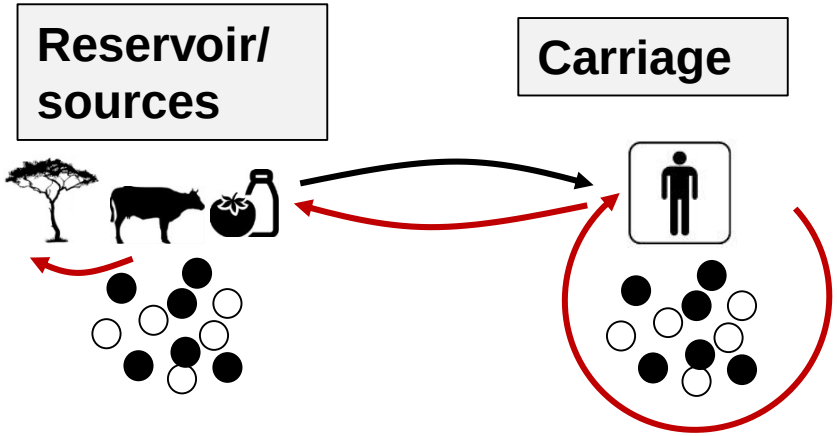
Phylogenetics of carriage Kp



Core genes
Recomb-purged
Iqtree ML

Kp transmission studies challenges

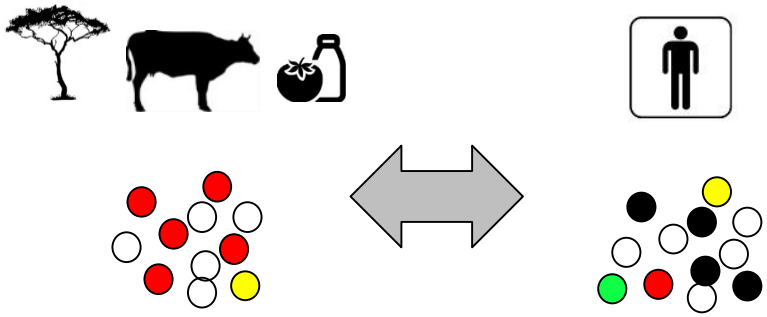
- Kp diversity (and ubiquity) makes transmission studies highly challenging



- Need for local, controled, longitudinal transmission studies
- Metagenomics & gene flux

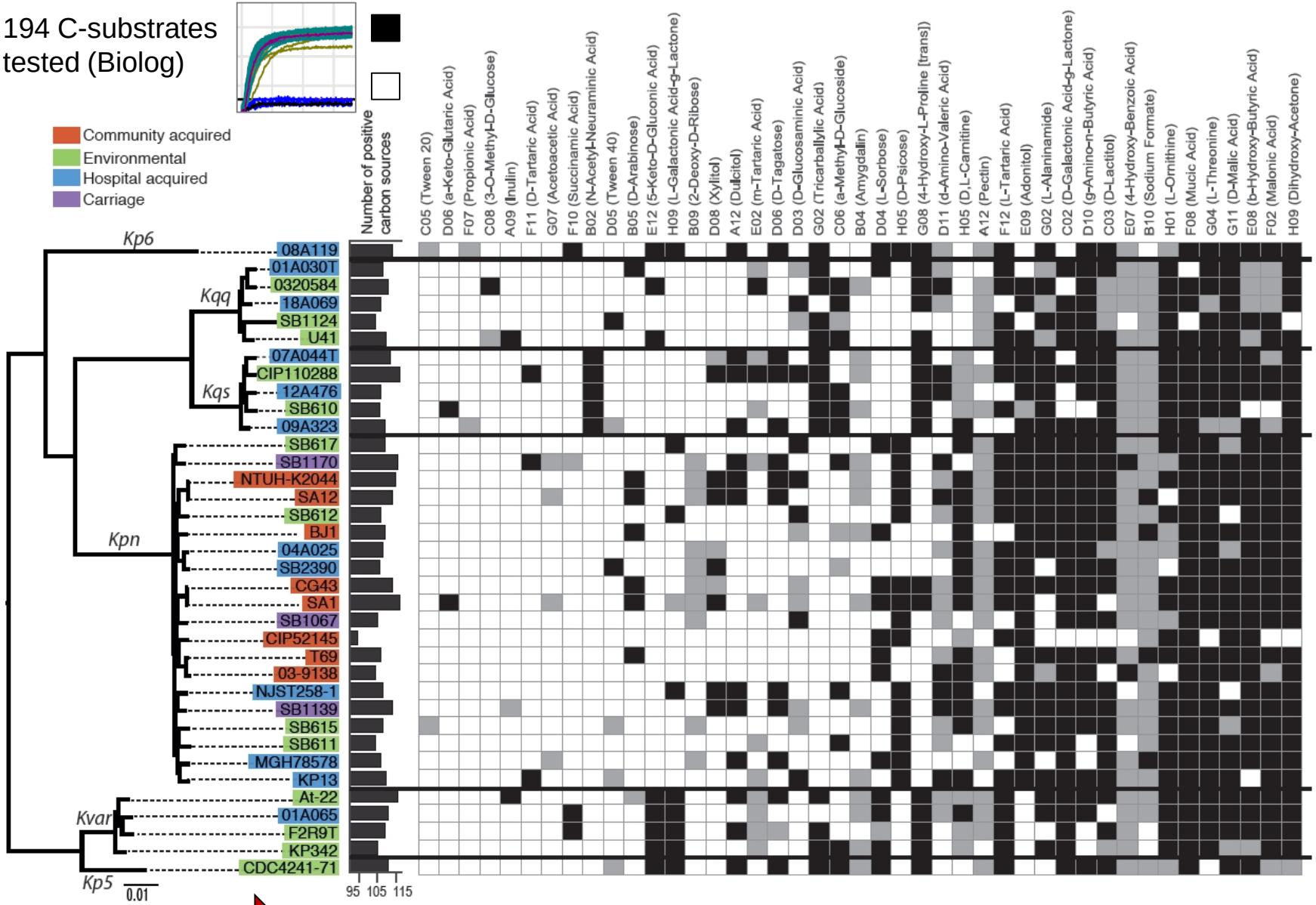
Ecology, evolution and biology of Kp

- Kp phylogroups, sublineages have unique genes
- Genomes from multiple niches should lead to identify eco-adaptive (enriched) genes
- Analyzing these genes functionally will contribute to understand the biology of Kp



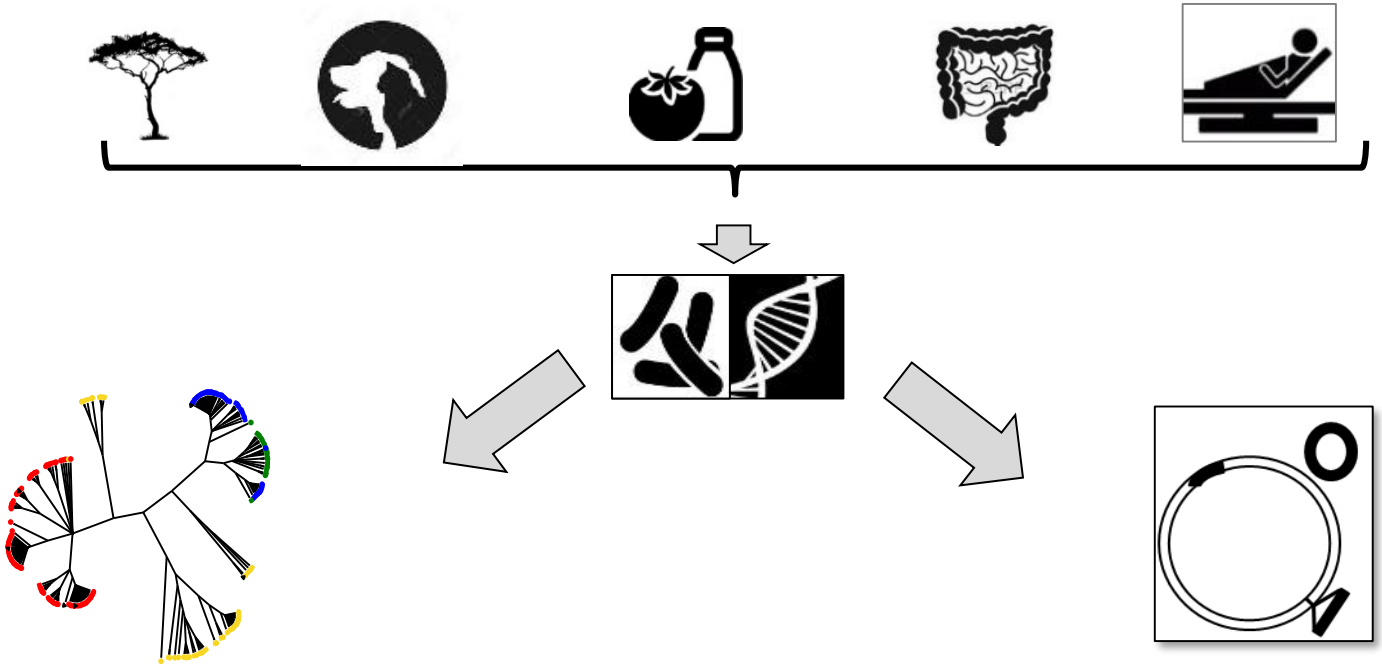
Extensive metabolic diversity of *Kp*

194 C-substrates tested (Biolog)



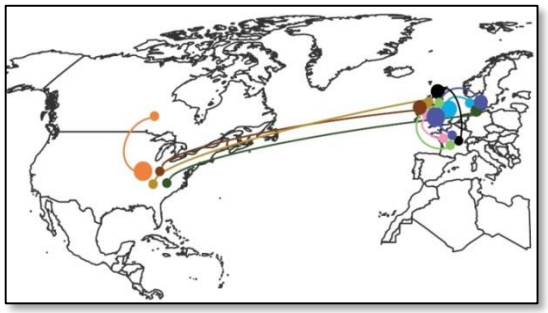
Mean: 109 substrates used; average distance: 14 substrates

Integrated epidemiology and population biology



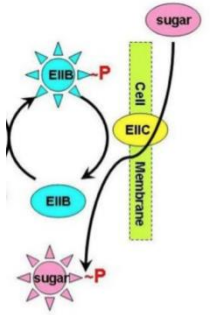
Population structure & Epidemiology

Genotype – phenotype associations



Novel understanding

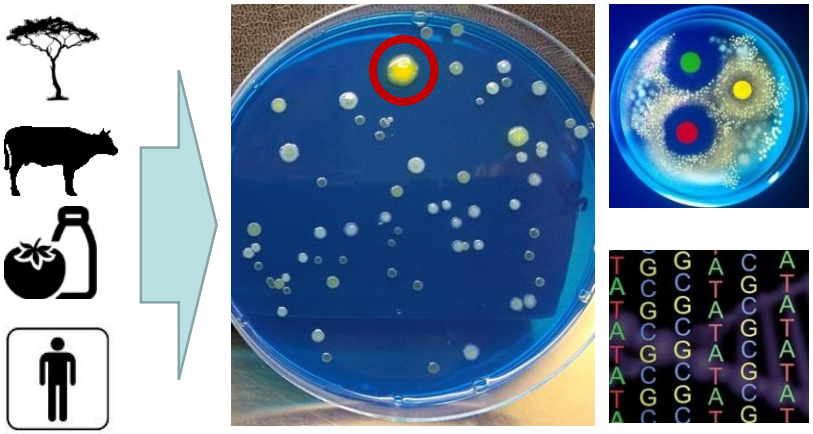
Improved sampling



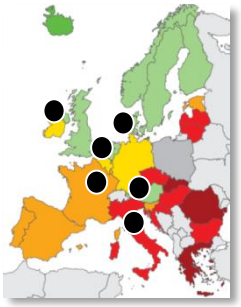
Transmission studies

Biology, pathophysiology

Funded Kp ecology/transmission projects



« Single isolate »
« Mixed-colony seq »



- ✓ MedVetKlebs (S. Brisse)
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- ✓ Kleb-GAP (A. Sundsfjord)
- ✓ KlebNET, ‘network of networks’ (S. Brisse)

One HEALTH EJP

jpiamr

SpARK

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