Institut Pasteur

Klebsiella pneumoniae:

Biodiversity, epidemiology,

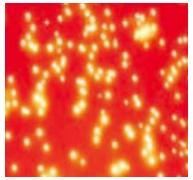
and links with pathogenesis

Sylvain Brisse

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

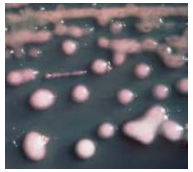
Biodiversity and Epidemiology of Bacterial Pathogens

Institut Pasteur



Bordetella pertussis (French National Ref. Center)

Corynebacterium diphtheriae (French Natl. Ref. Center)



Klebsiella pneumoniae

New Superbug Discovered in the USA

The Atlantic

SUBSCRIBE SEARCH MENU

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

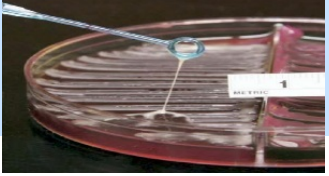
She won't be the last.

SARAH ZHANG | JAN 13, 2017 | HEALTH

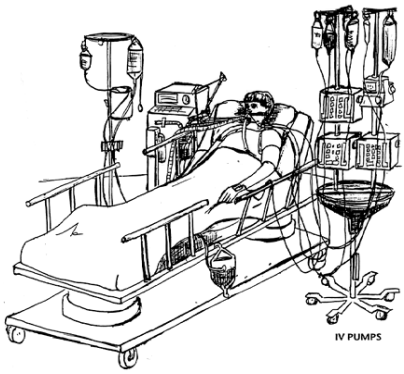
Share Tweet ...

TEXT SIZE − +

A Nevada woman died in September after being infected with type of drug-resistant bacteria called Klebsiella pneumonaiae that was resistant to all antibiotics available in the U.S., the CDC reported on Friday.

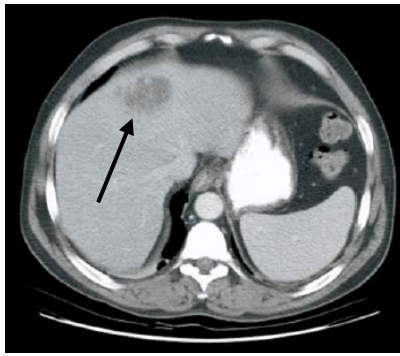


Klebsiella pneumoniae



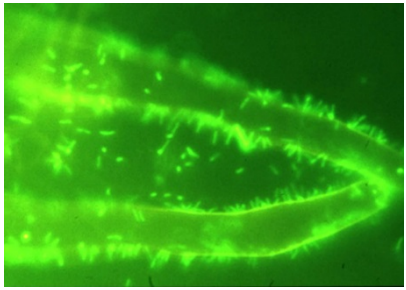
Nosocomial pathogen

- 5 - 8% nosocomial infections Europe / USA
- Urinary, respiratory, bacteremia; outbreaks
- Multidrug resistant (MDR) strains



Community pathogen

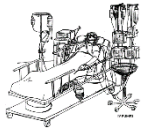
- Severe pneumonia; pyogenic liver abscess; meningitis
- Hypervirulent *K. pneumoniae* (HVKP)
- Mostly capsular types K1 and K2



Ubiquitous

- Human and animal carriage, plants, water, soil,…

Objective:
Understand *Klebsiella* epidemiological patterns at the genetic and functional levels

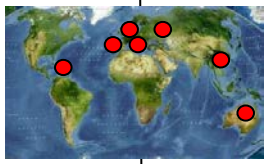


Humans

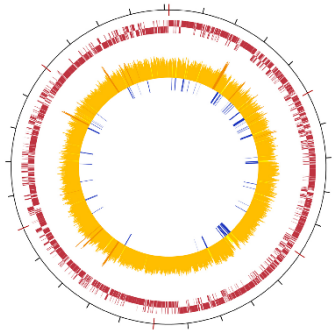
Animal

Environment

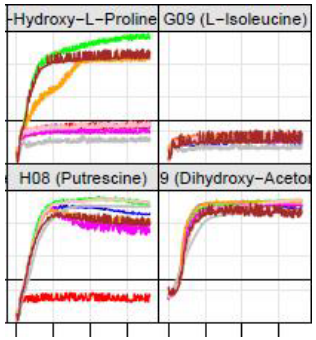
Historical collection



Dynamics



Comparative genomics

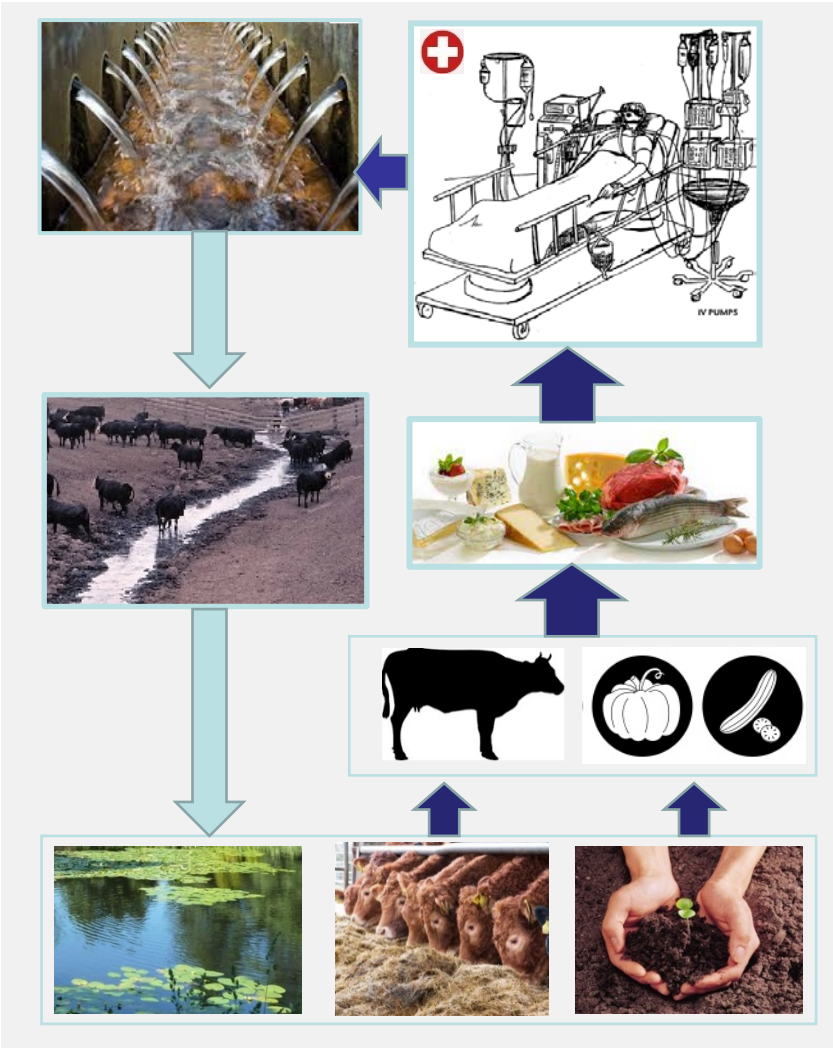


Phenotyping

Genetics
In-vitro
In-vivo

Virulence / adaptive genes

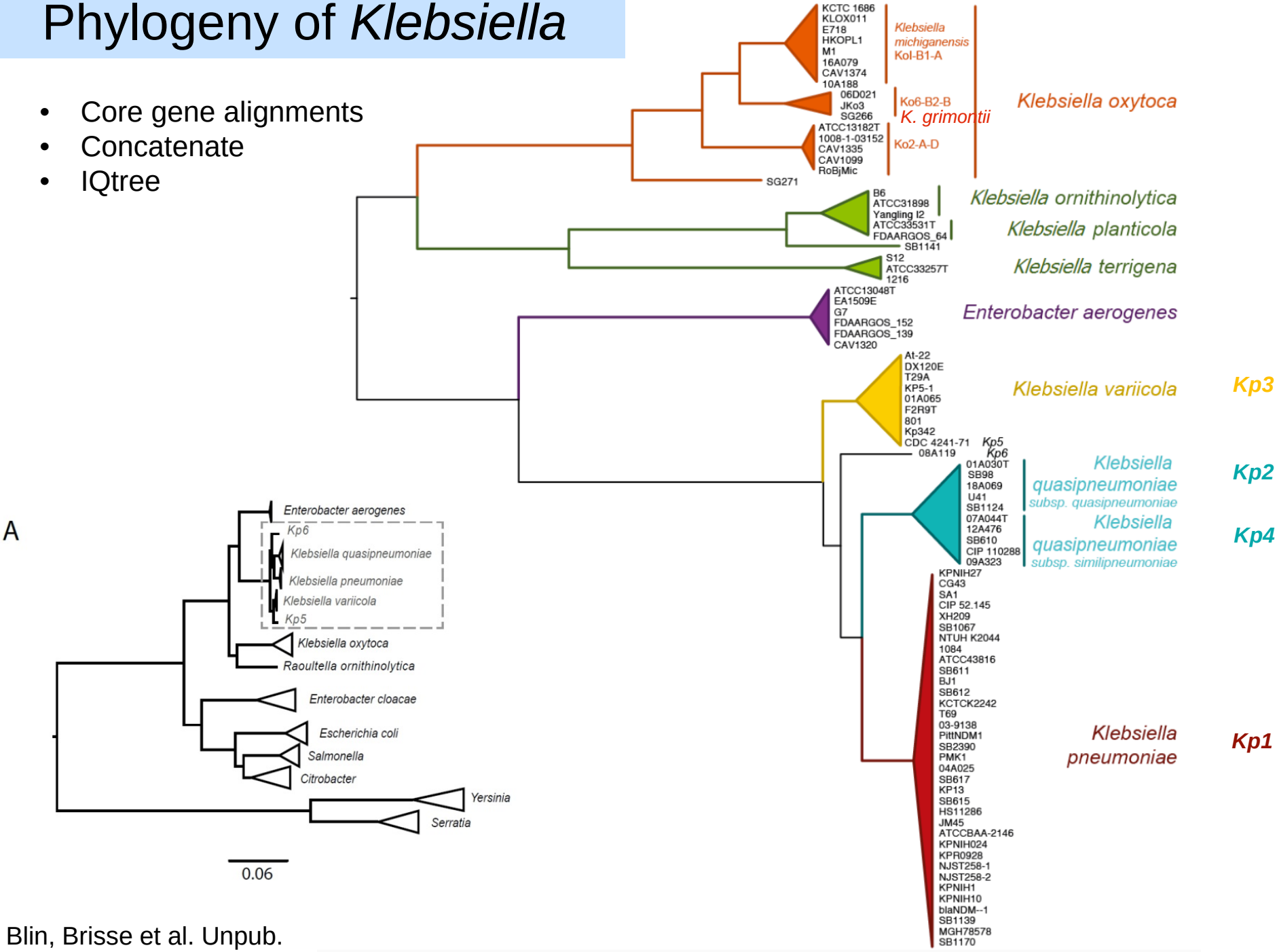
High-risk *K. pneumoniae* strains & genes



- Tools to track Kp strains internationally
- Genomic features of high-risk strains
- What are the reservoirs and transmission routes of high-risk Kp strains?

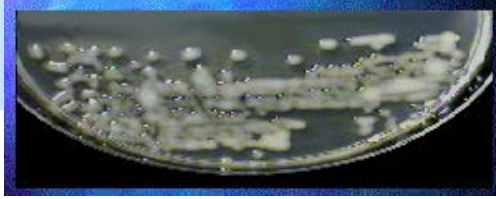
Phylogeny of *Klebsiella*

- Core gene alignments
- Concatenate
- IQtree



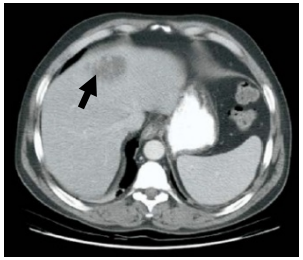
Blin, Brisse et al. Unpub.

Genus *Klebsiella:* taxonomy

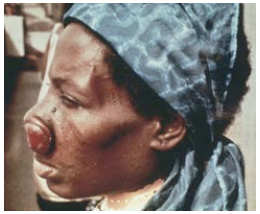


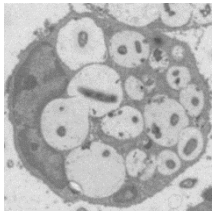
K. pneumoniae

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



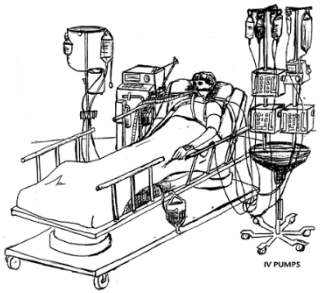
- *K. granulomatis (← Calymmatobacterium granulomatis)*
- *K. variicola ~ K. singaporensis*





- *K. quasipneumoniae*
  - *subsp. quasipneumoniae*
  - *subsp. similipneumoniae*

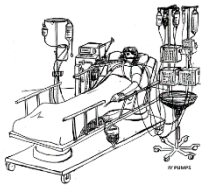
- *K. oxytoca*
- *K. michiganensis*
- *K. grimontii*



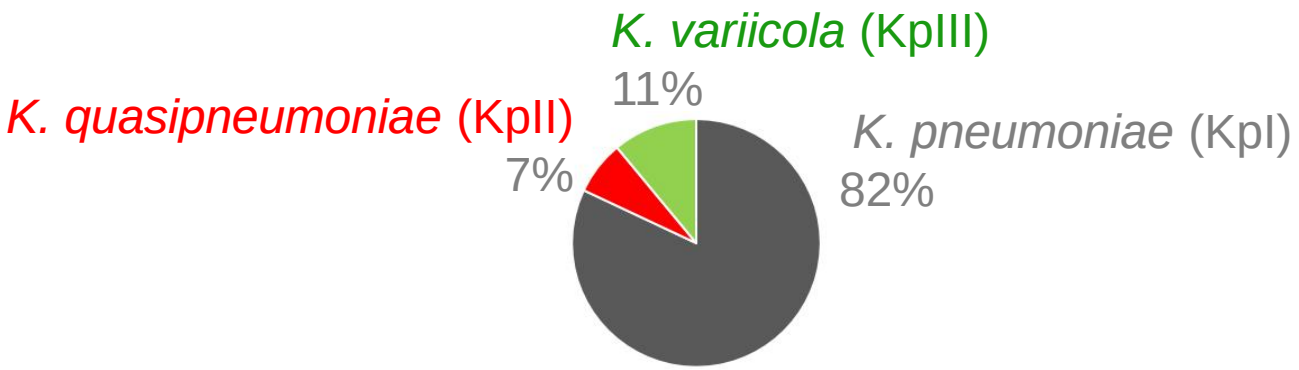
- *K. planticola (→ Raoultella)*
- *K. ornithinolytica (→ Raoultella)*
- *K. terrigena (→ Raoultella)*

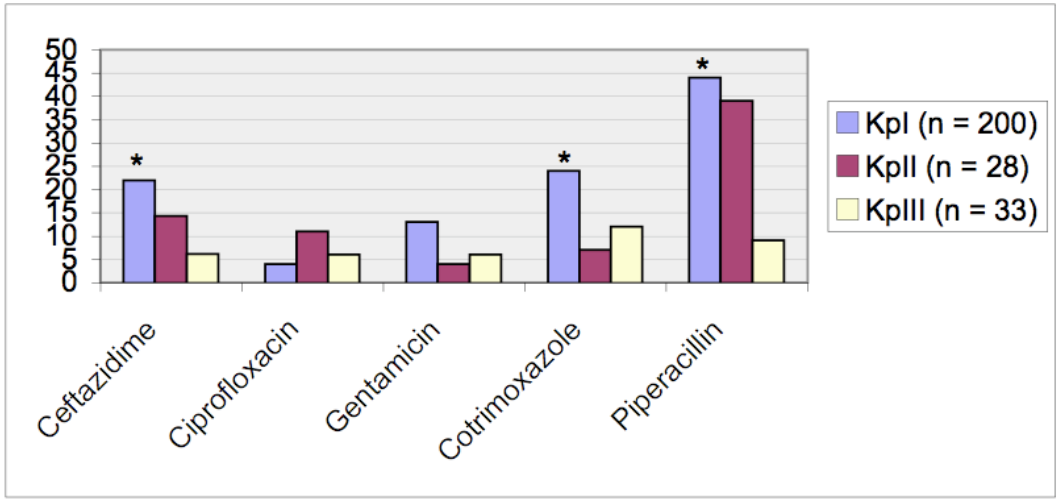
Clinical importance of *Klebsiella pneumoniae* and related species

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



Brisse et al., CMI 2004





Antimicrobial resistance:

Kp1 > Kp3 ~ Kp2/4

Clinical importance of *Klebsiella pneumoniae* and related species

Klebsiella variicola Is a Frequent Cause of Bloodstream Infection in the Stockholm Area, and Associated with Higher Mortality Compared to *K. pneumoniae*

Maatallah et al. 2015 PLoS One

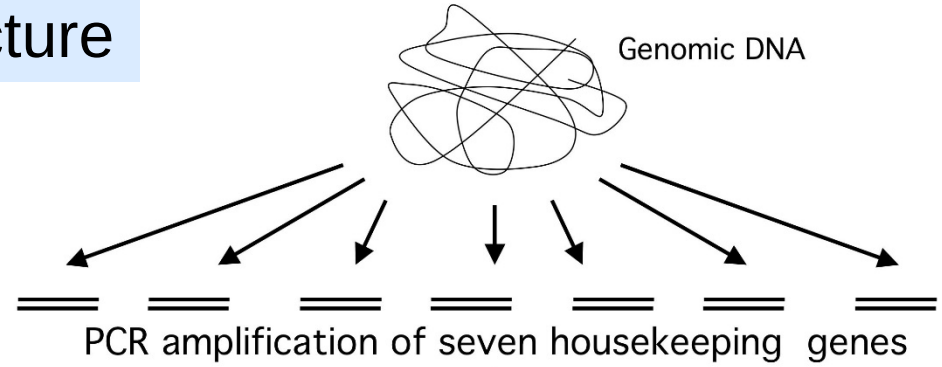
Liver Abscess Caused by Infection with Community-Acquired *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*

Breurec et al. 2016 Emerg Inf Dis

- *K. quasipneumoniae* and *K. variicola* still largely under-recognized in medical microbiology practice

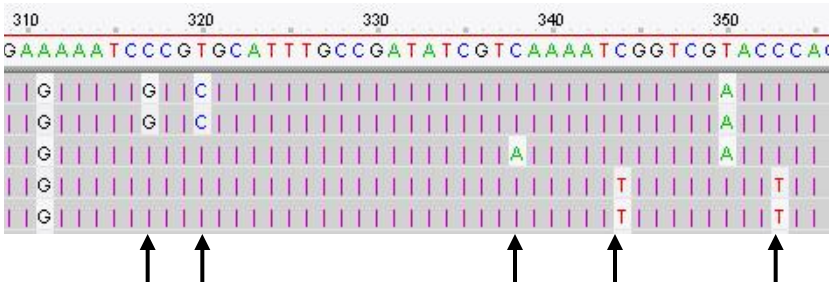
Population structure

MLST: Multilocus Sequence Typing



Sequencing on both strands, approx. 500 bp

• Sequence of one gene



•Allele

1
1
2
3
3

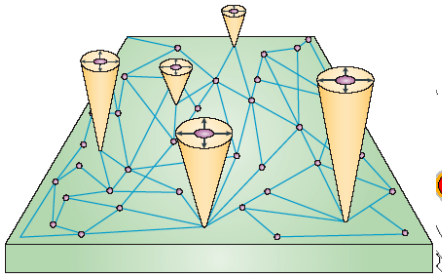
Alleles:

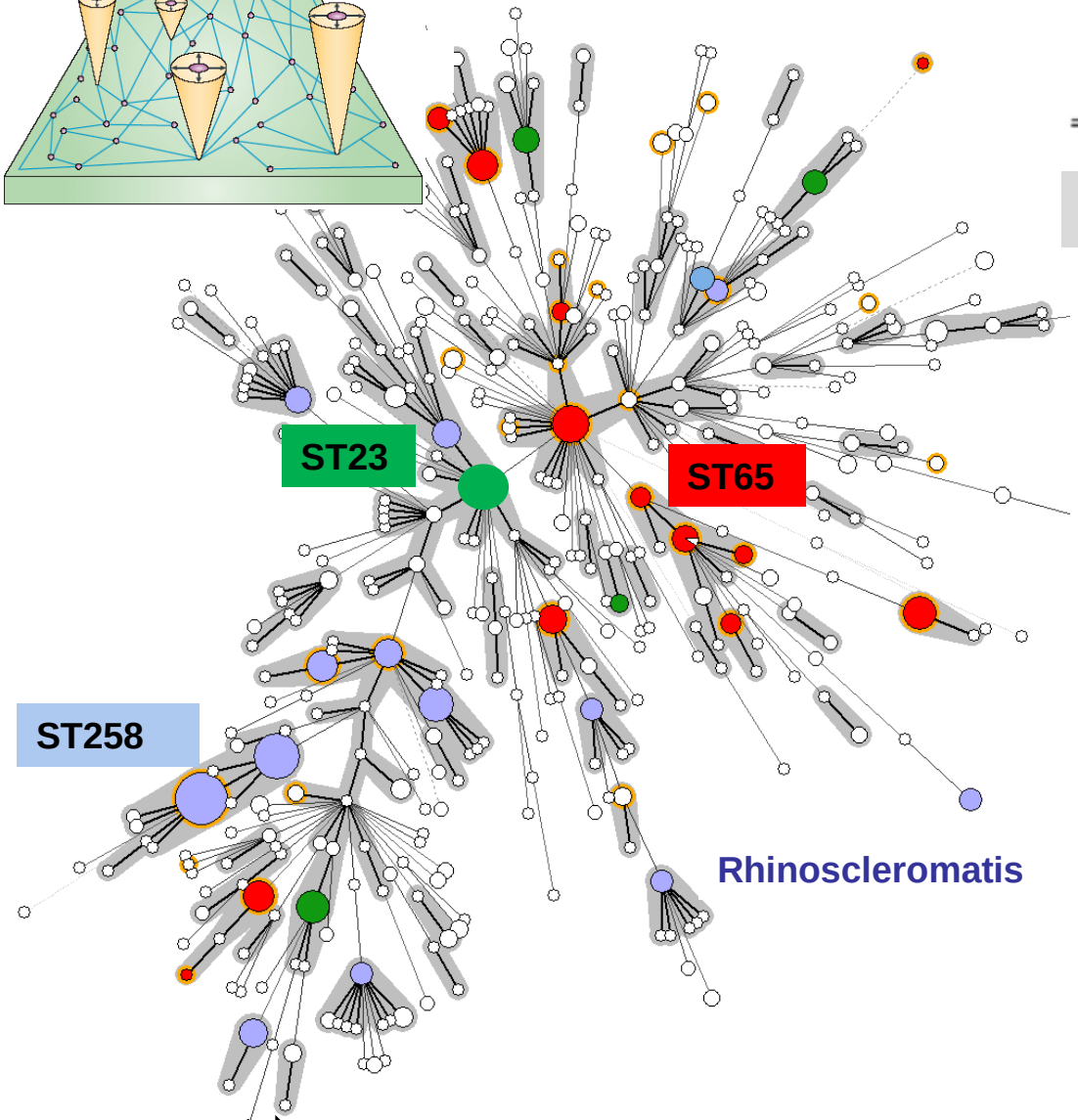
gapA	*infB*	*mdh*	*pgi*	*phoE*	*rpoB*	*tonB*
2	1	1	1	9	4	12

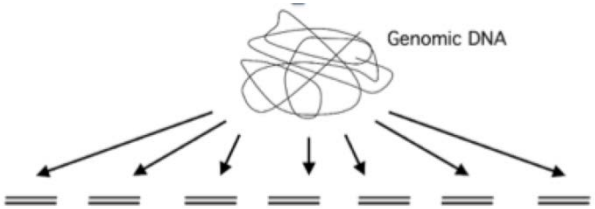
Sequence type (ST)	Alleles
23	2 1 1 1 9 4 12
2	3 4 1 1 9 4 17
57	2 1 1 1 19 4 12

Single Locus Variants (SLV)

MLST-based *K. pneumoniae* population structure

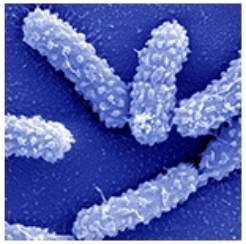






Diancourt *et al.* 2005 JCM

Klebsiella Sequence Typing



Sequences and profiles database

Isolates database

http://www.pasteur.fr/mlst

> 3000 STs
> 6000 isolates
> 400 submitters

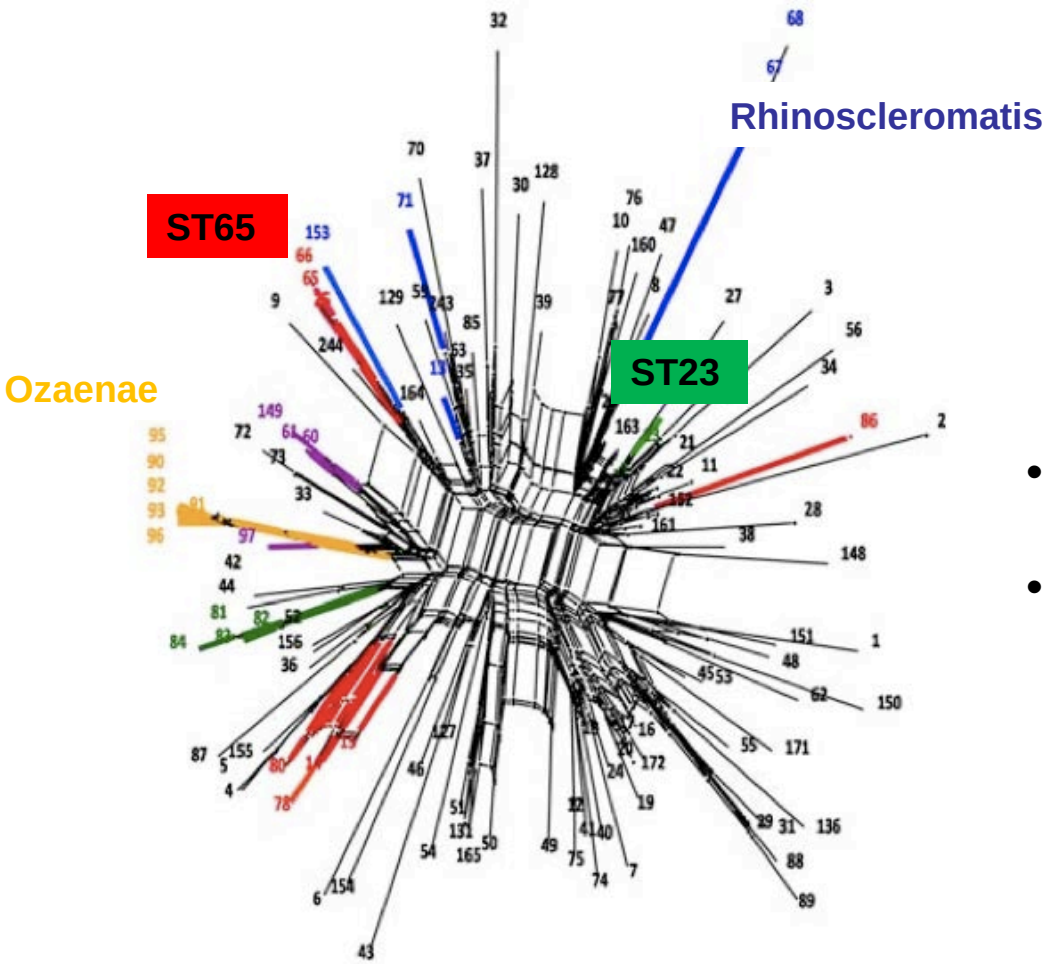
Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*

MLST: Universal language

K. pneumoniae population structure

K. pneumoniae (Kpl)

(splits graph, MLST data)

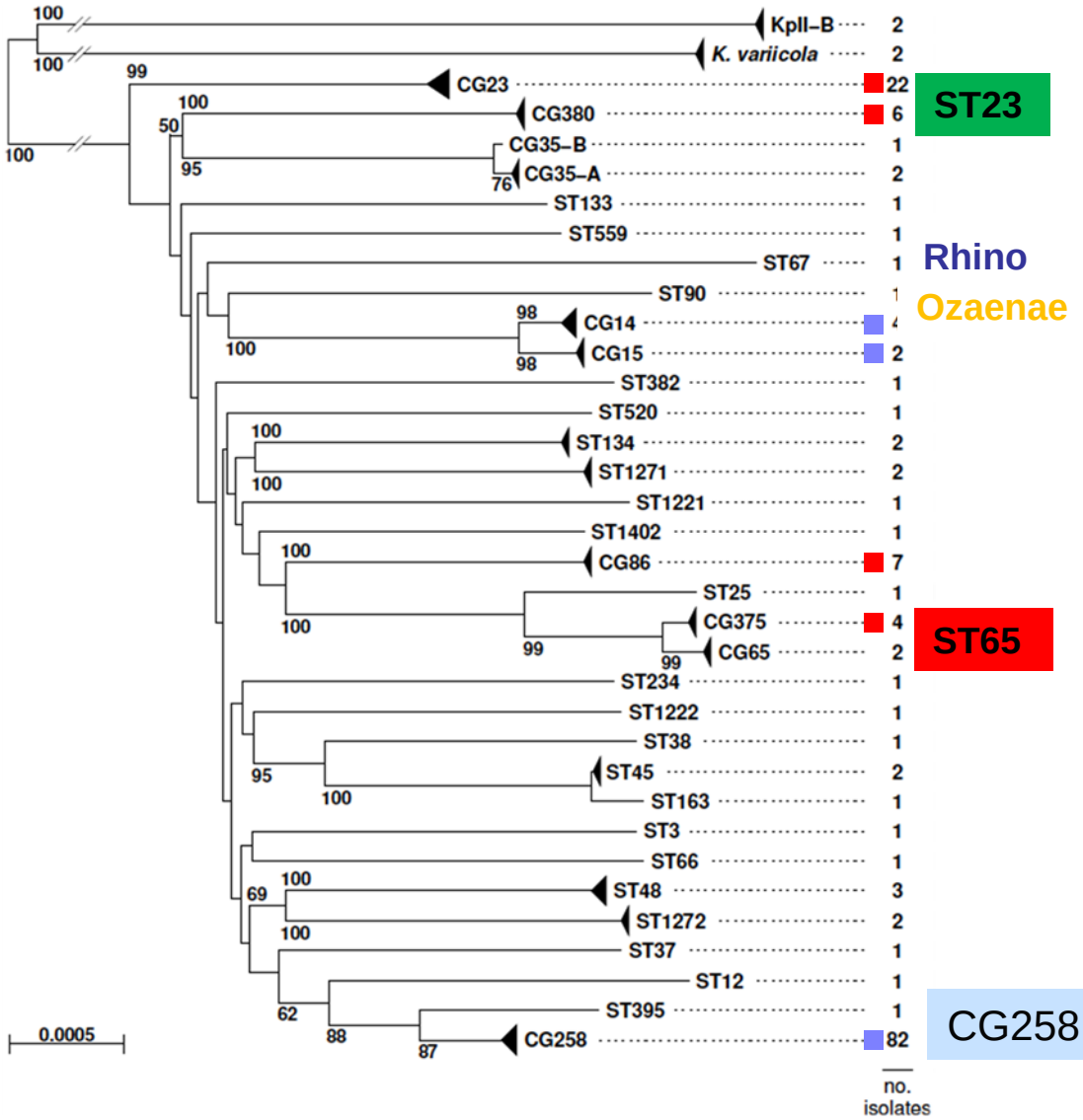


- Lack of phylogenetic structure
- High recombination rate (r/m ~ 20)

Brisse et al., PLOS One, 2009

K. pneumoniae genomic population structure

Bialek, Criscuolo et al., 2014

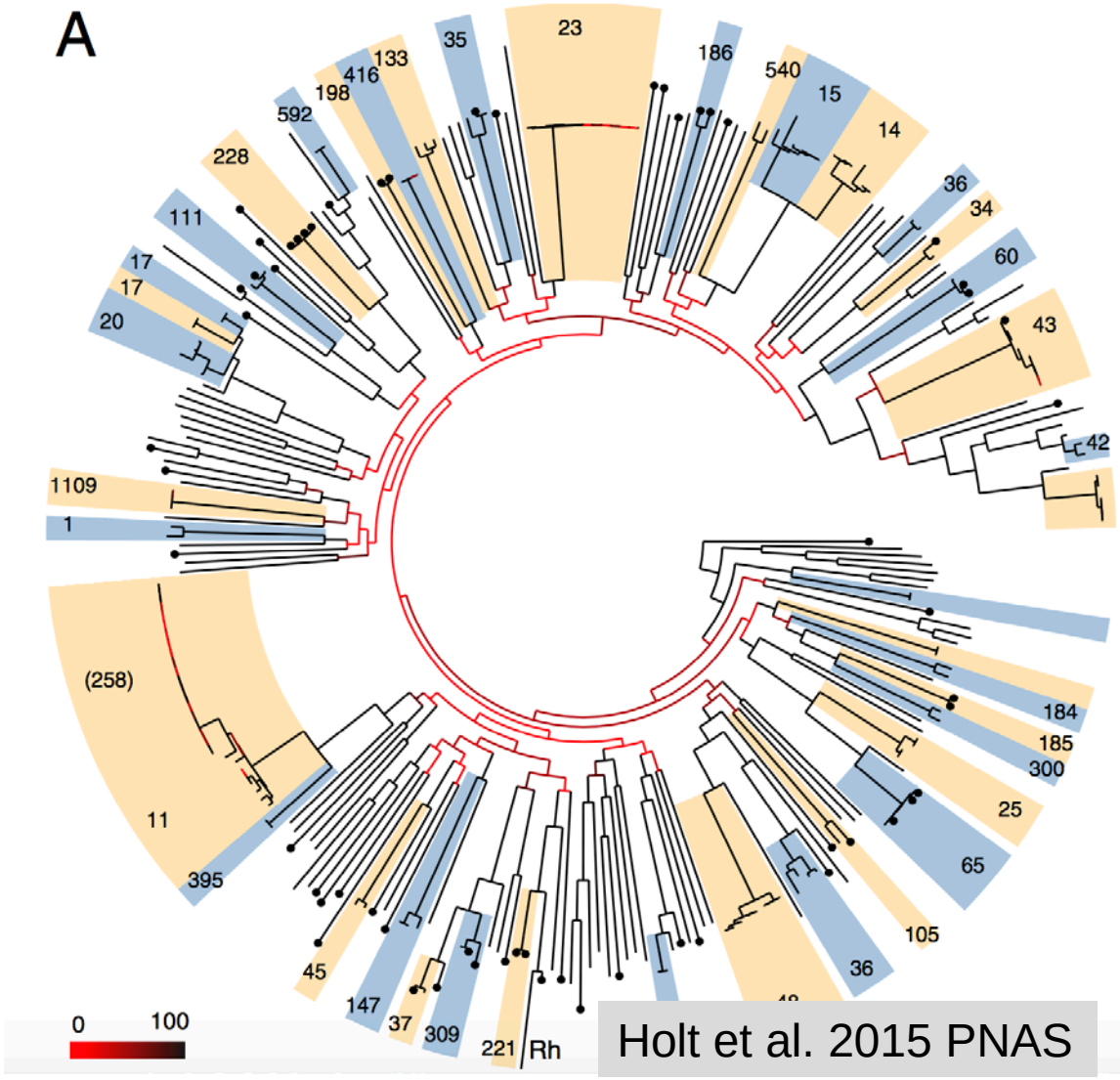


Phylogenetic analysis

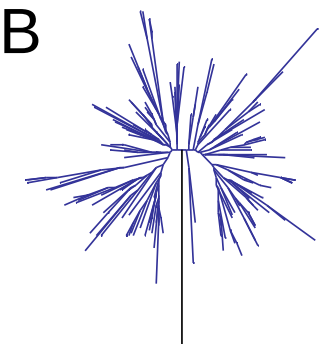
- Pairwise distance estimates
- Elimination of extreme values (~ imports)
- Mean evolutionary distance
- FastME

- Resolution of clonal groups
- Star-like phylogeny

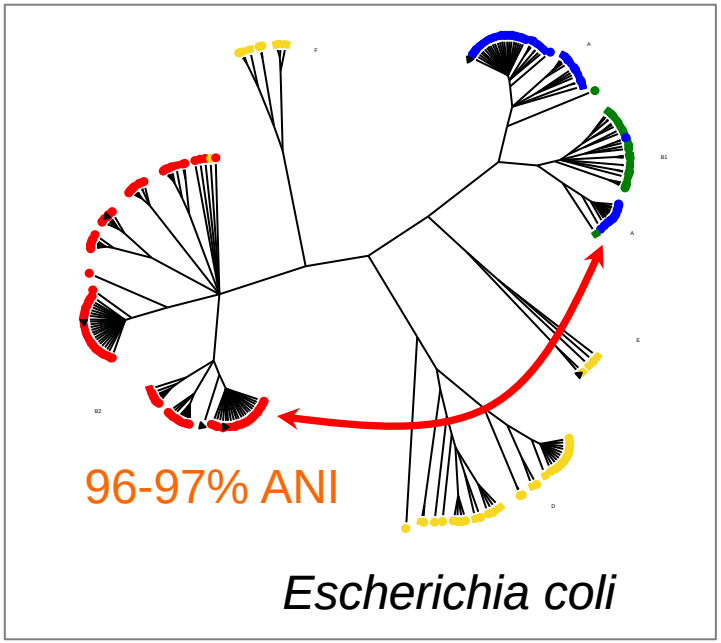
Genome-based *K. pneumoniae* population structure



Holt et al. 2015 PNAS



99.6% ANI



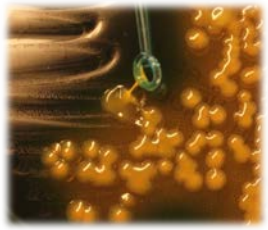
Kp: Multiple unordered closely-related lineages

Evolutionary radiation or pervasive recombination?

Klebsiella pneumoniae pathogenicity

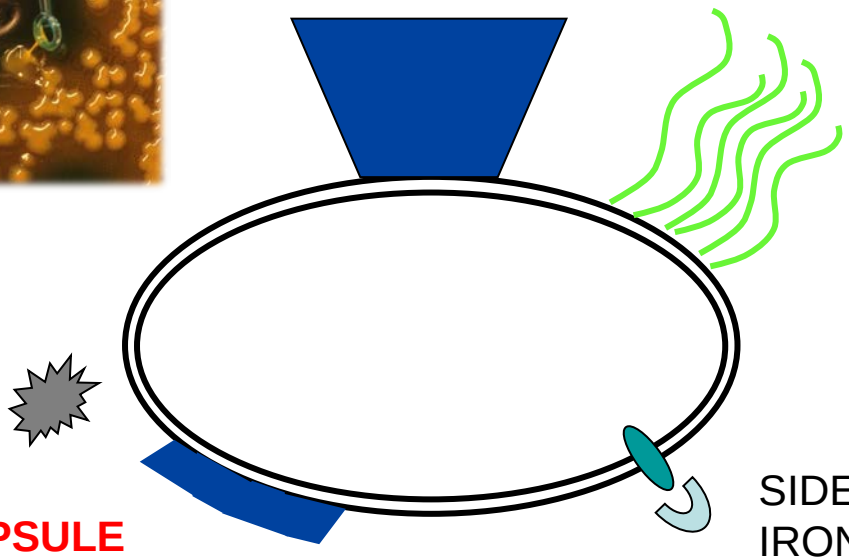
Virulence factors confirmed in mouse model

✧ Virulence factors:



HYPERMUCOVISCOSITY
(***rmpA***, *rmpA2*)

FIMBRIAE
type 3 pili (*mrkABCDF*)
type 1 pili (fim)



TOXINS
Microcin E492
Colibactin

METABOLISM
Allantoinase

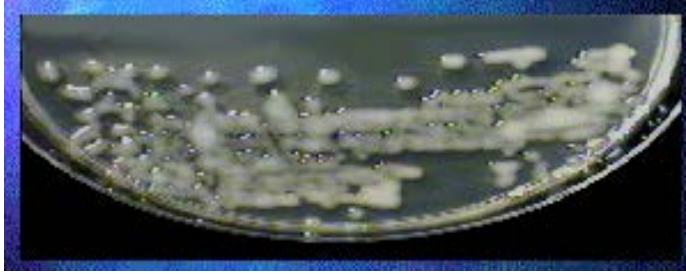
CAPSULE
77 serotypes : *wzi*, *wzc*
(serum & phagocytosis resistance)

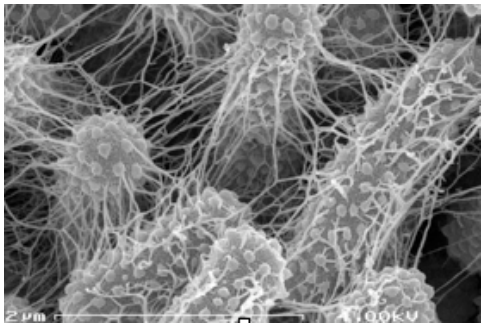
SIDEROPHORES /
IRON TRANSPORT
Enterobactin
Aerobactin
Salmochelin
Yersiniabactin
kvgAS
kfuABC

✧ Heavy metal resistance-associated clusters:

pbrABCR, *pcoABCDERS*, *silCERS*, and *terWXY*

K. pneumoniae capsule



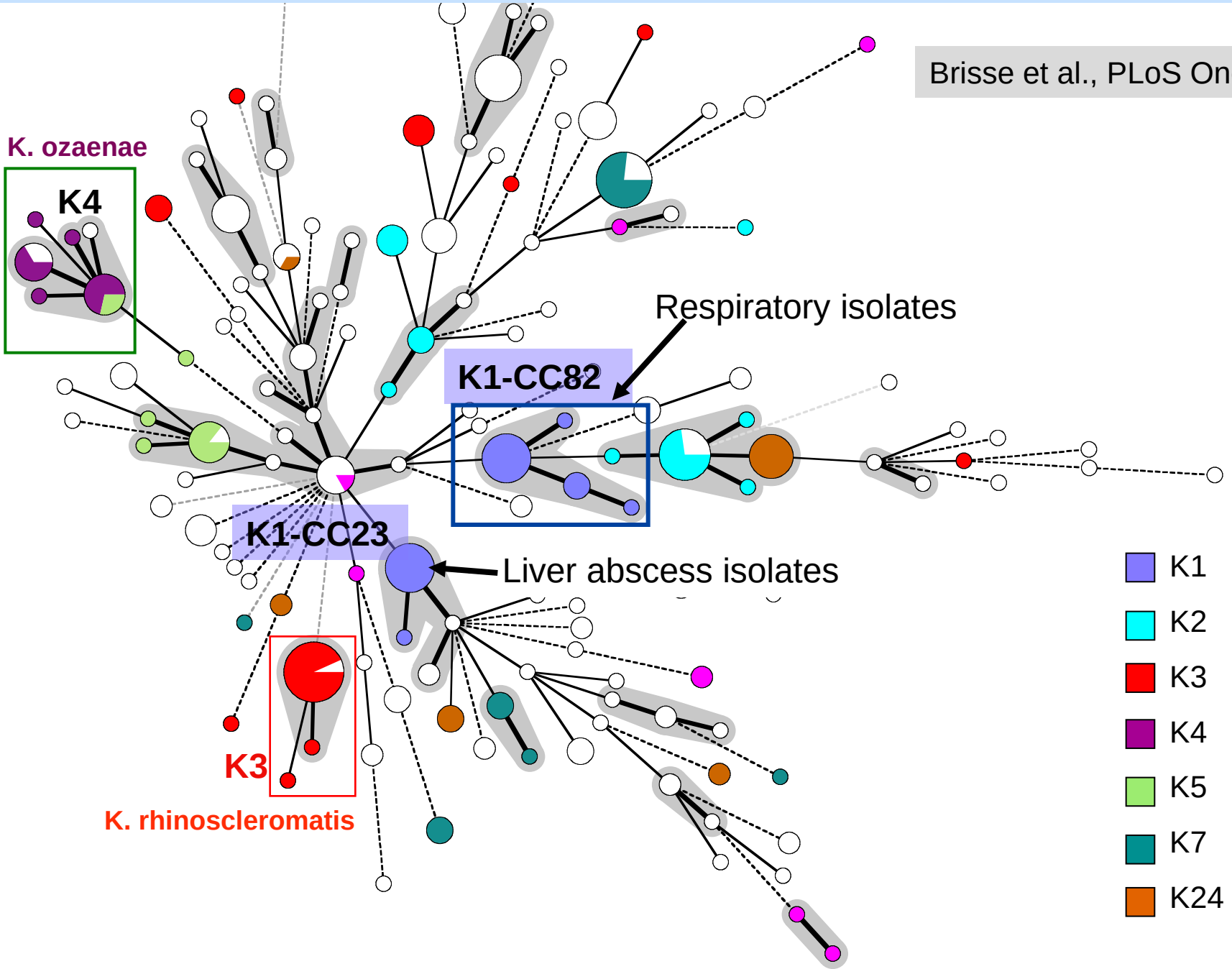


- Polysaccharidic, abundant >> mucoidy
- 77 K types
- K-typing for epidemiology

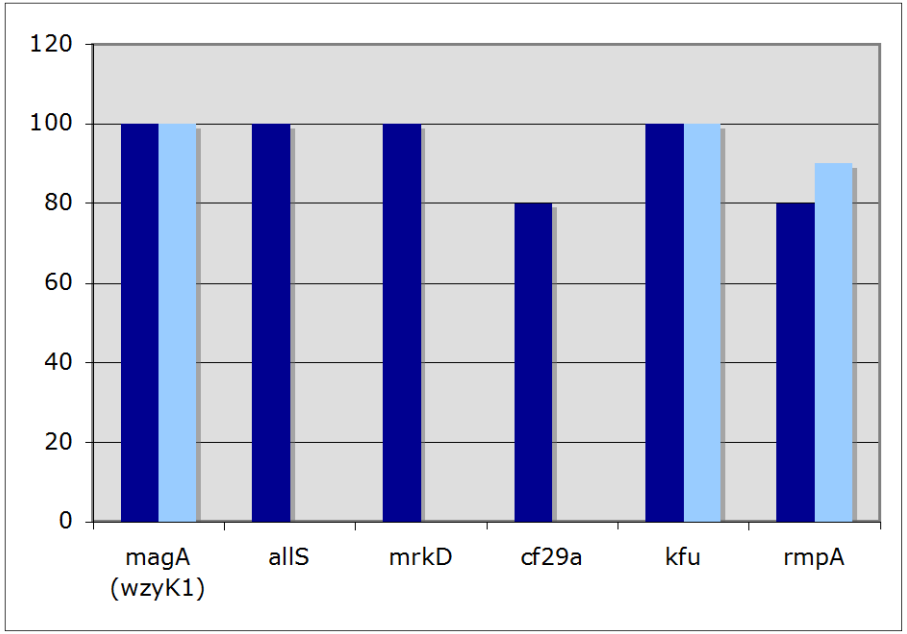
- Confirms identification of
  - K. pneumoniae subsp. ozaenae (K4)
  - K. pneumoniae subsp. rhinoscleromatis (K3)

- Major virulence factor
  - K1, K2 >>> K7, K21 (humans, mice, horses)
  - K1 (& K2): pyogenic liver abscess (PLA)

MLST clones *versus* capsular serotypes



K1 clones: virulence in mouse respiratory model



Proportion virulent strains

CC23-K1 (n = 10) 4/5

CC82-K1 (n = 15) 1/4

Brisse et al., 2009

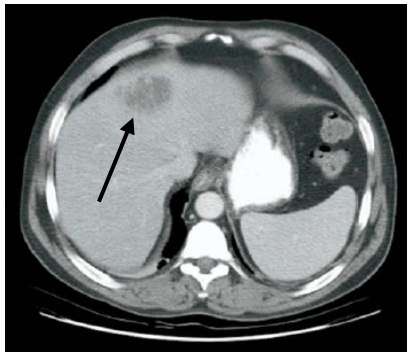
K1 clones differ in source, virulence & gene content

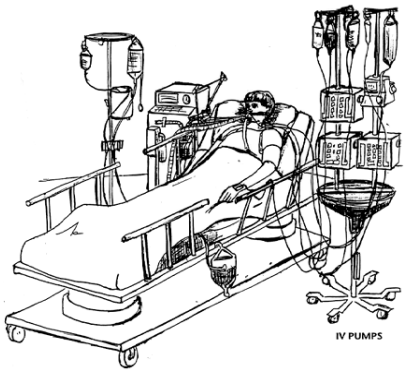
Brisse et al., PLoS One 2009

Population structure of ‘high-risk’ clones

Genomic Definition of Hypervirulent and Multidrug-Resistant *Klebsiella pneumoniae* Clonal Groups

Suzanne Bialek-Davenet,[1] Alexis Criscuolo,[1] Florent Ailloud, Virginie Passet, Louis Jones, Anne-Sophie Delannoy-Vieillard, Benoit Garin, Simon Le Hello, Guillaume Arlet, Marie-Hélène Nicolas-Chanoine, Dominique Decré, and Sylvain Brisse





- 48 K1/K2 strains, incl. 21 HVKP strains (liver abscess, pneumonia, meningitis)

  Illumina sequencing (~240 contigs)

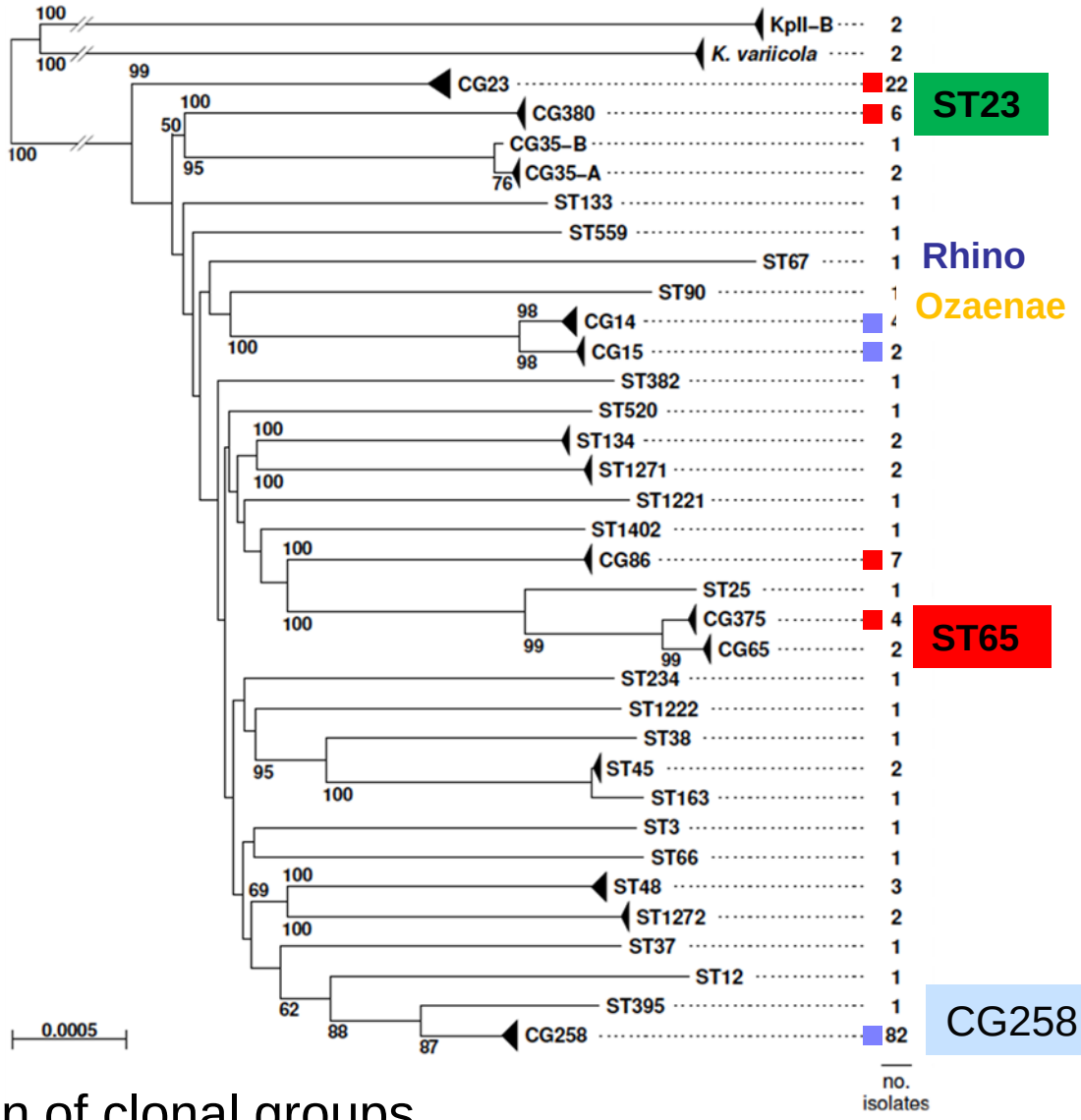
- 119 genomes from NCBI genome repository

  including 20 ST258 isolates from Bethesda 2011 outbreak (Snitkin et al. 2012)

K. pneumoniae population structure (based on genome sequences)

Phylogenetic analysis

- Pairwise distance estimates
- Elimination of extreme values (~ imports)
- Mean evolutionary distance
- FastME

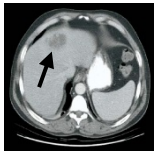


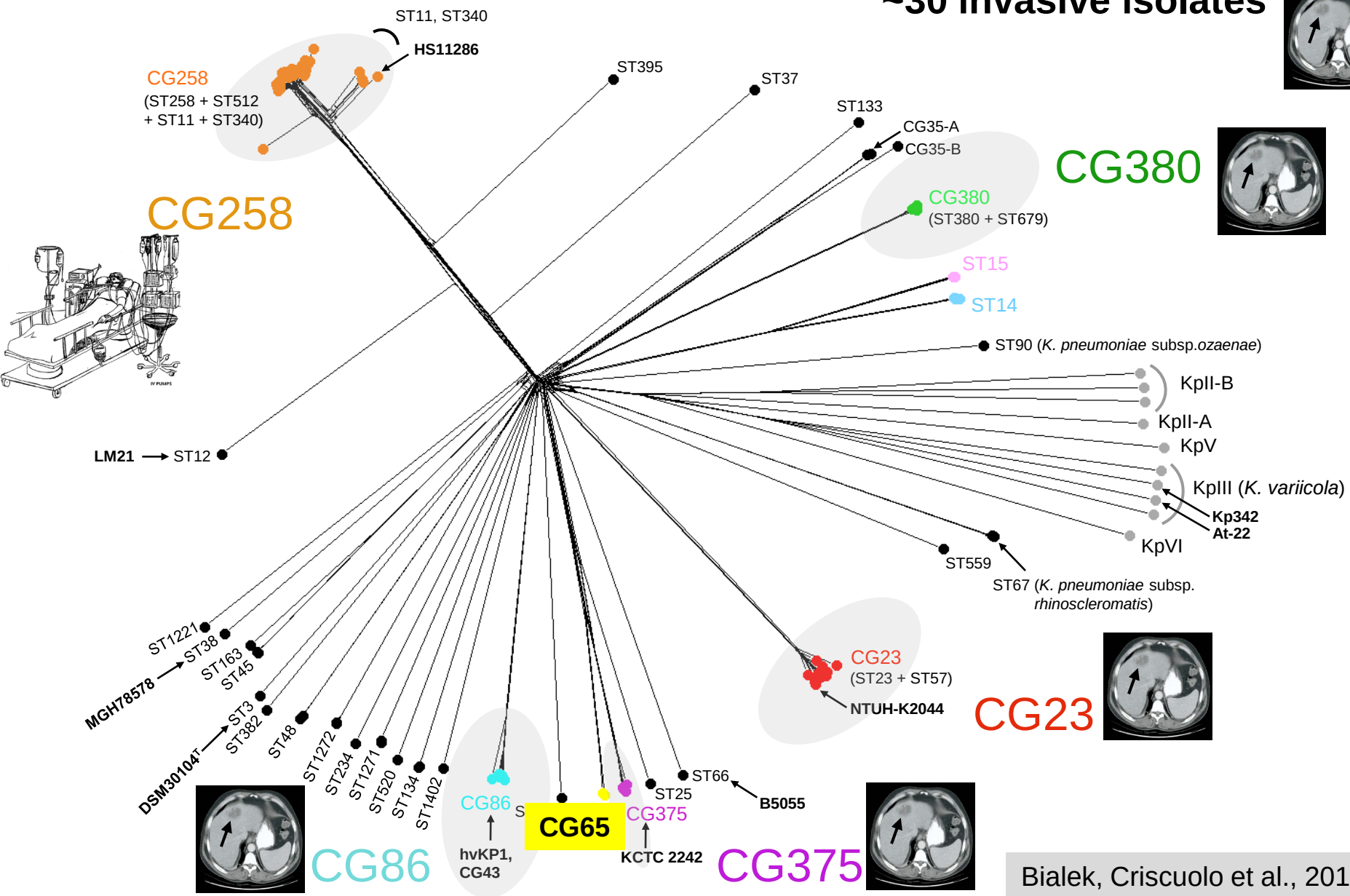
- Resolution of clonal groups
- Star-like phylogeny

Bialek, Criscuolo et al., 2014

Clonal groups *versus* pathogenicity

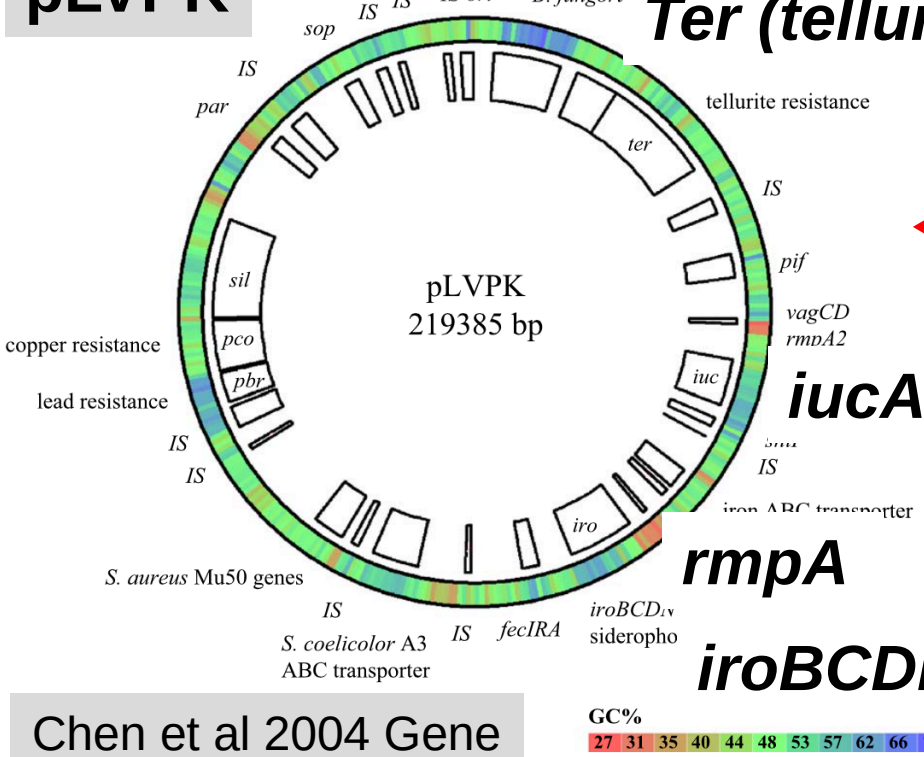
~30 invasive isolates





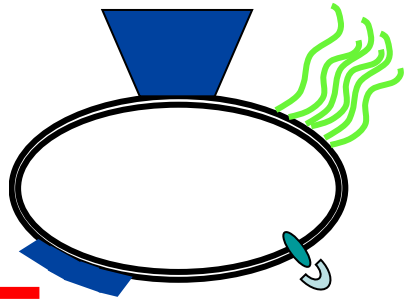
Bialek, Criscuolo et al., 2014

pLVPK



Ter (tellurite)

K1, K2
serotypes



iucABCDiutA (aerobactin)

rmpA

iroBCDN (salmochelin)

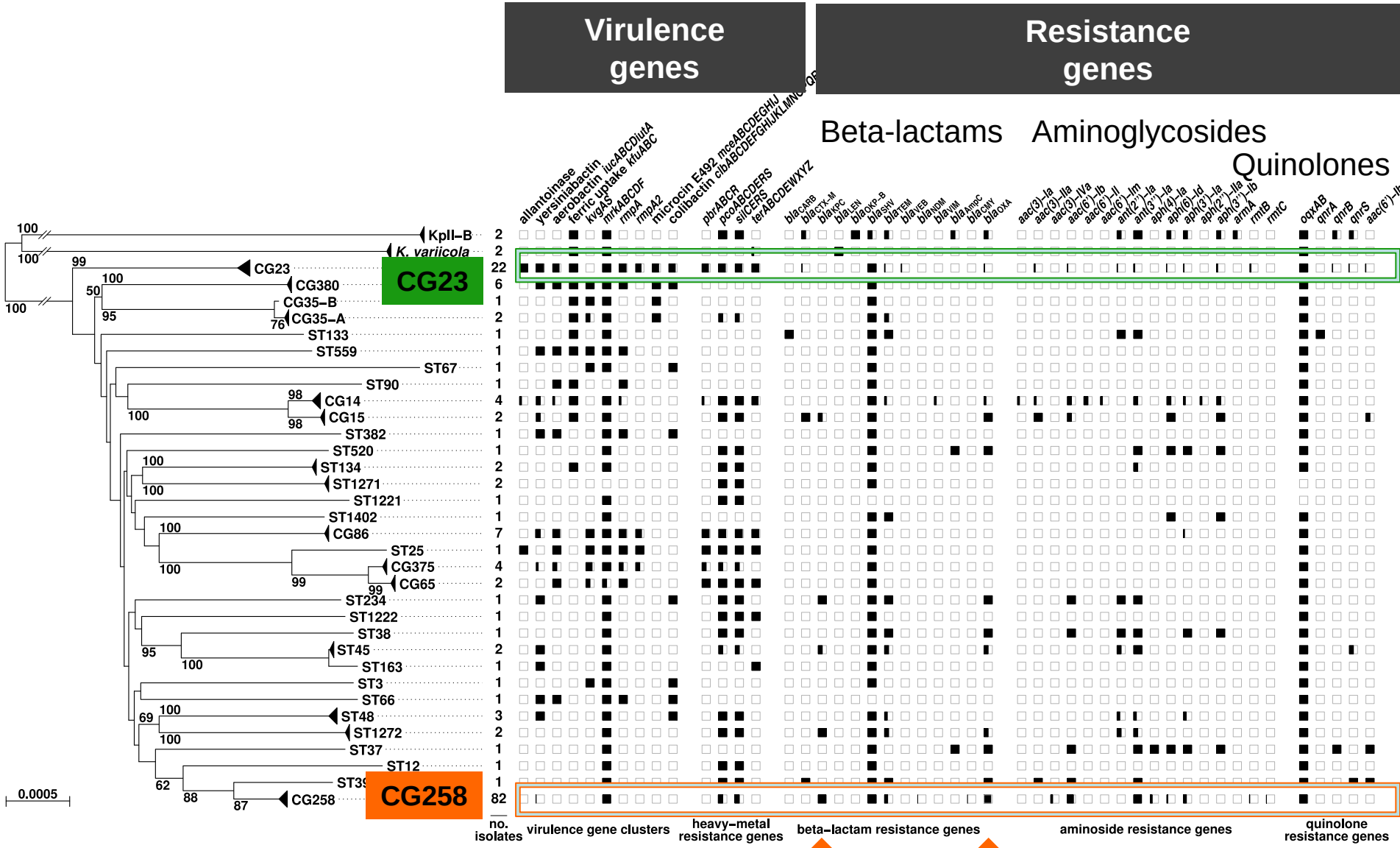
Chen et al 2004 Gene

Integrative and Conjugative element ICE*Kp*1

yersiniabactin **salmochelin + *rmpA*** T4SS vir/mob

Lin et al 2008 J Bact

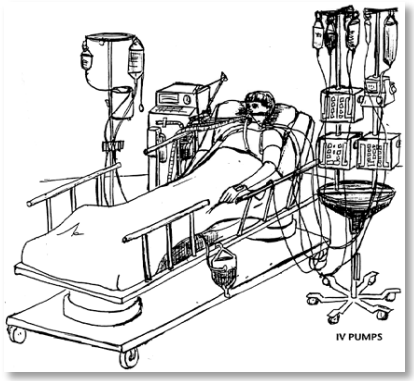
Gene content breakdown by clonal group



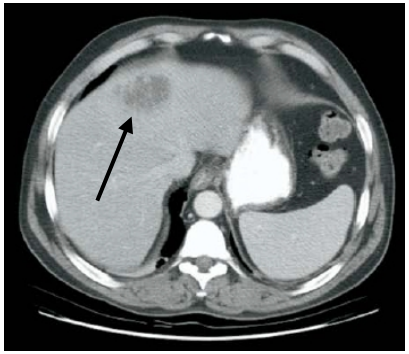
Bialek, Criscuolo et al., 2014

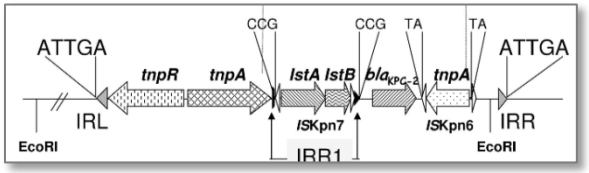
Emergence of ‘dual-risk’ *K. pneumoniae*

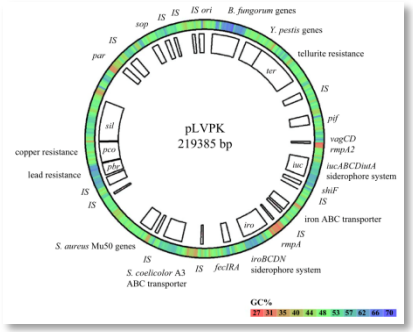
Resistance



Virulence







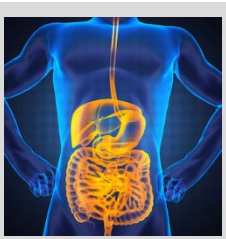
Lancet Inf. Dis. 2017

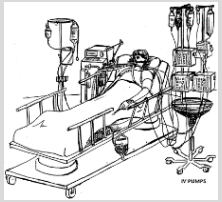
A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study

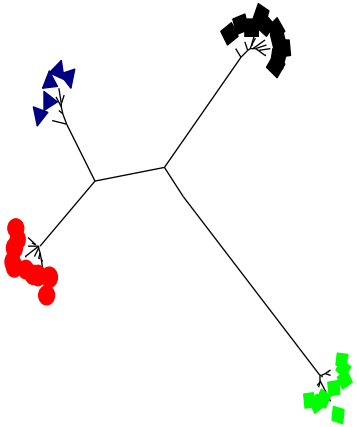
Danxia Gu*, Ning Dong*, Zhiwei Zheng, Di Lin, Man Huang, Lihua Wang, Edward Wai-Chi Chan, Lingbin Shu, Jiang Yu, Rong Zhang, Sheng Chen

Ecology of *K. pneumoniae* & relatives

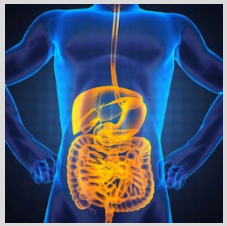
KpII / KpIV
(mainly, *K. quasipneumoniae subsp. similipneumoniae*)

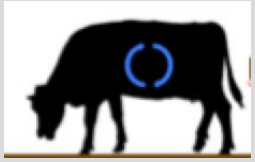


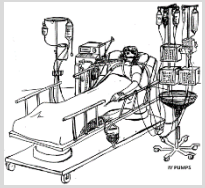


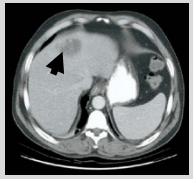


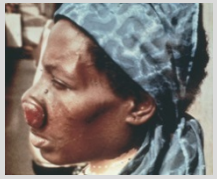
KpI (*K. pneumoniae*)



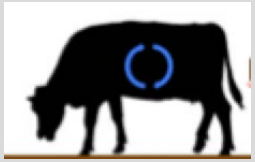


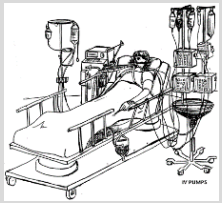






KpIII (*K. variicola*)



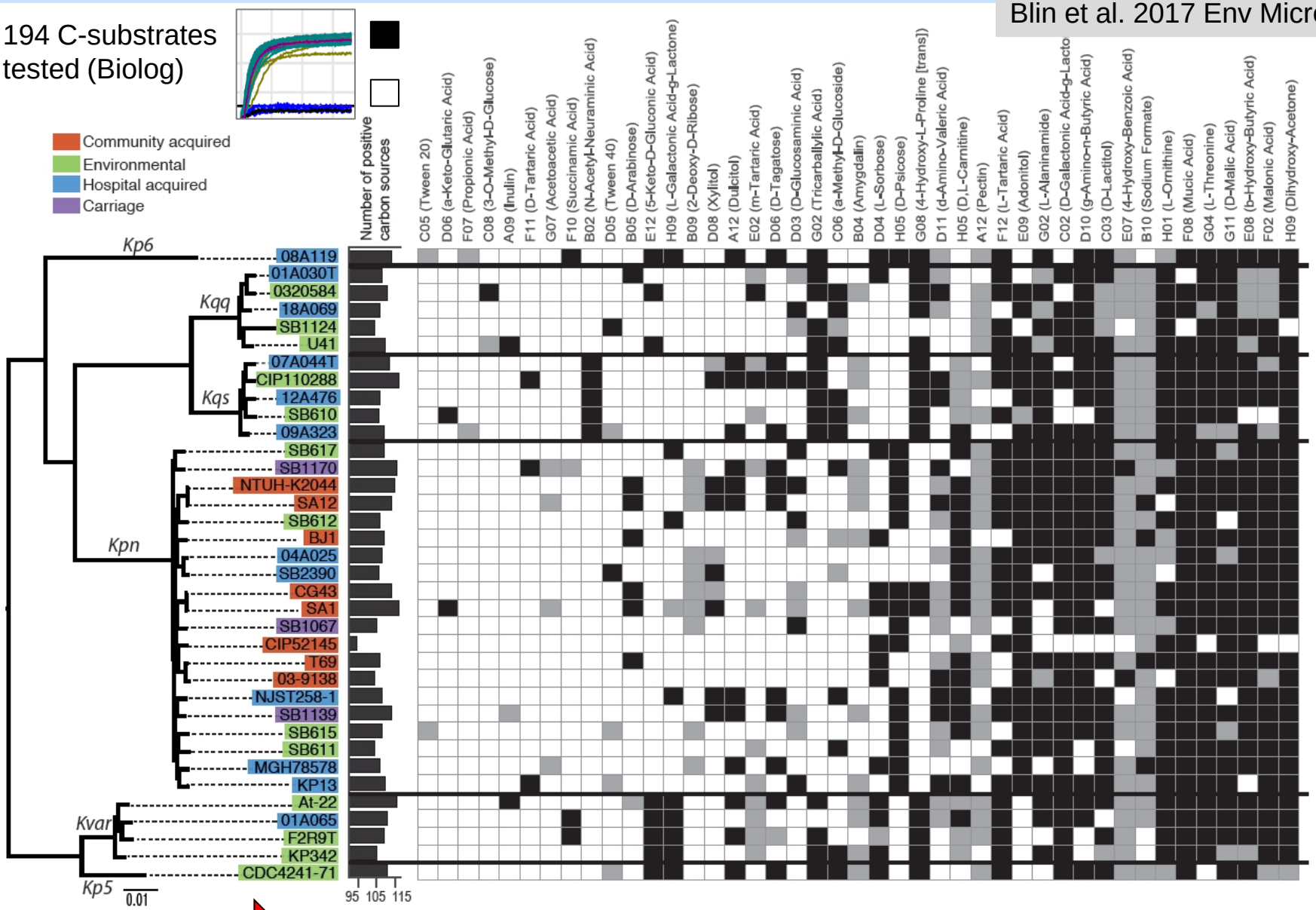


Brisse et al 2004, 2009, 2015
Holt et al. 2015 PNAS

Metabolic diversity of *Kp*

Blin et al. 2017 Env Microbiol

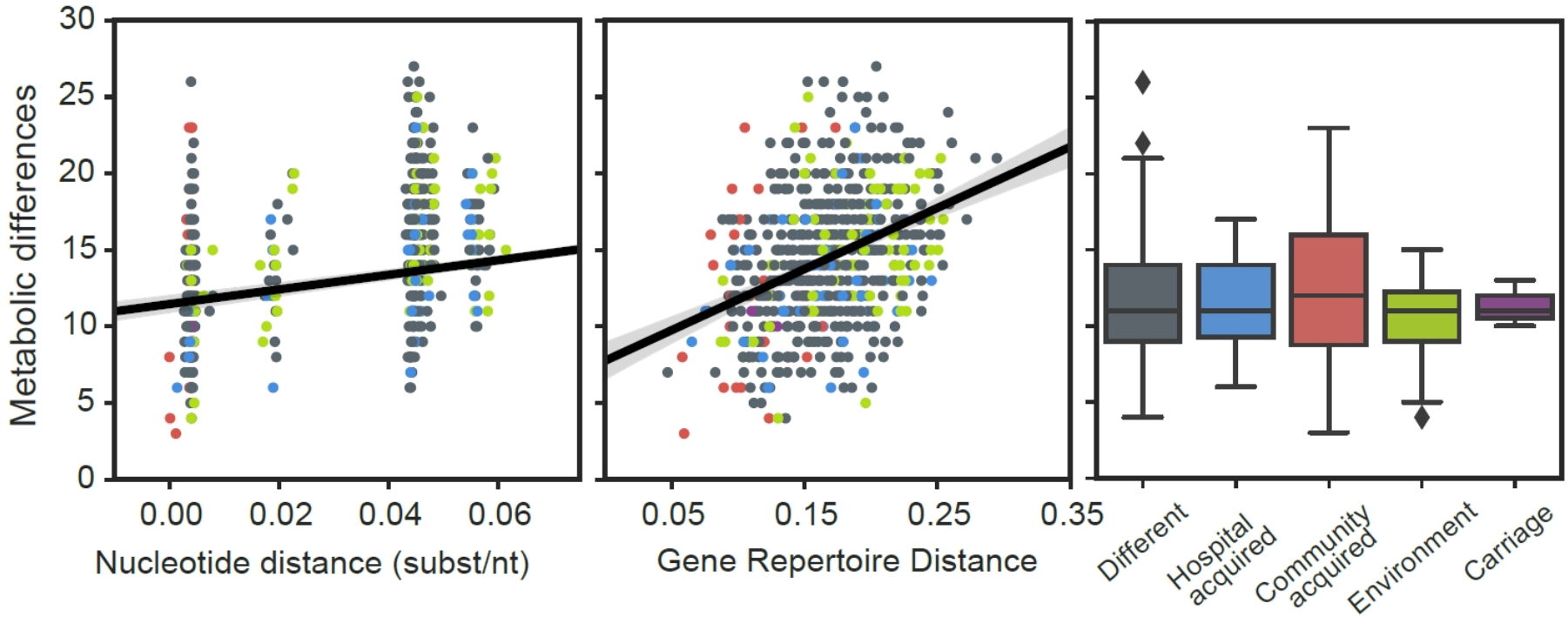
194 C-substrates tested (Biolog)



Mean: 109 substrates used; average distance: 14 substrates

Structure of metabolic diversity of *Kp*

Blin et al. 2017 Env Microbiol

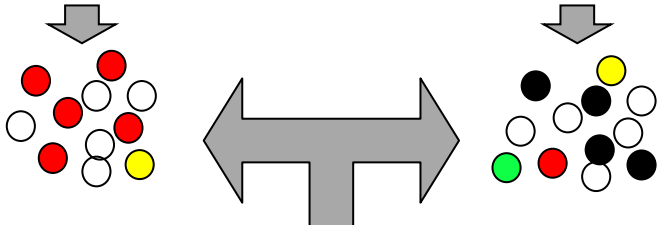


- Catabolic capacities correlate weakly with *Klebsiella* species
- All clonal groups are generalists (except specialized pathogenic clones)
- No metabolic signature of HVKP or MDR-Kp clones found

Potential reservoirs and sources of *K. pneumoniae*

Potential reservoirs/ sources

Carriage



Food habits
Animal contacts
...

Risk factors

- Population genomics (~5000 isolates)
- Targetted metagenomics (~1000 pool-seq)

Abundance profiles

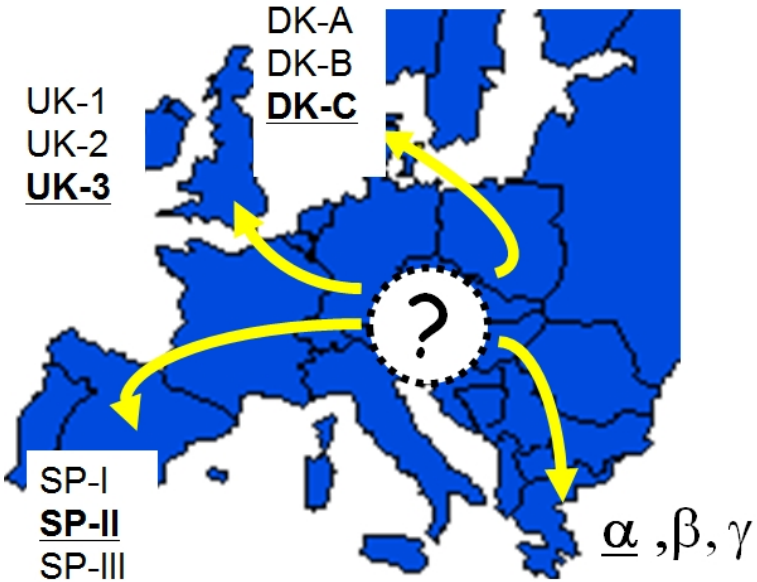
Modelling

Reservoirs & Transmission routes

EJP Co-fund H2020
MedVetKlebs (coord: Brisse)
2018-2020, 9 partners

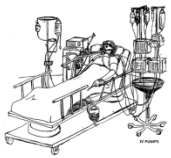
JPIAMR SPARK (E. Feil)
2017-2019
6 partners

Epidemiology & international spread of *Klebsiella*: Need for unified strain nomenclatures

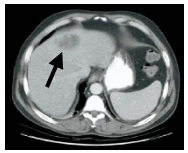


- Detecting multi-county outbreaks
- Spread of emerging pathogens
- Rate of inter-country mixing
- Dependency on vaccination, antimicrobial strategies

Klebsiella pneumoniae core genome MLST



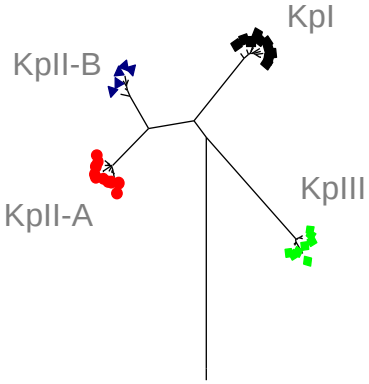
Hospital strains

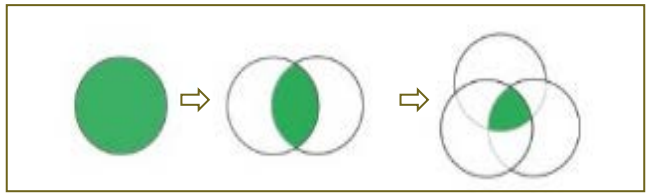


Community-onset

Environment

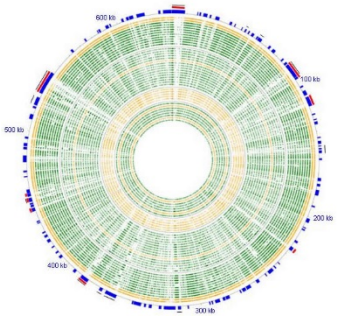
Historical collection





694 core genes (cgMLST)

Core genome MLST of *K. pneumoniae*



Genes in highly conserved, syntenic regions → 634 scgMLST

Ribosomal genes → 53 rMLST (Jolley et al.)

MLST genes → 7 MLST (Diancourt et al.)

694 core genes (cgMLST)

Genomic Definition of Hypervirulent and Multidrug-Resistant *Klebsiella pneumoniae* Clonal Groups

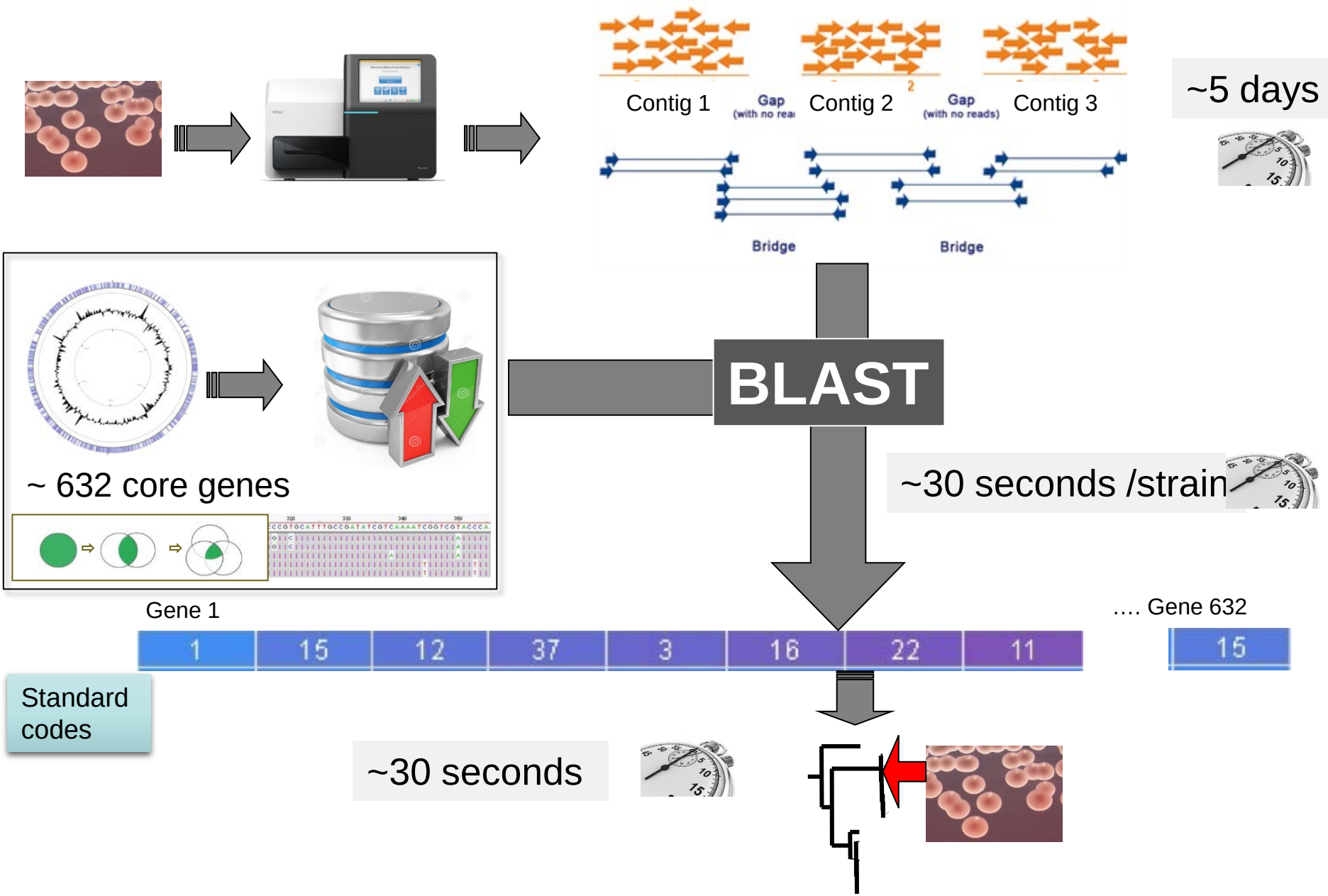
Suzanne Bialek-Davenet,[1] Alexis Criscuolo,[1] Florent Ailloud, Virginie Passet, Louis Jones, Anne-Sophie Delannoy-Vieillard, Benoit Garin, Simon Le Hello, Guillaume Arlet, Marie-Hélène Nicolas-Chanoine, Dominique Decré, and Sylvain Brisse

Emerging Infectious Diseases, 2014

2017 update:

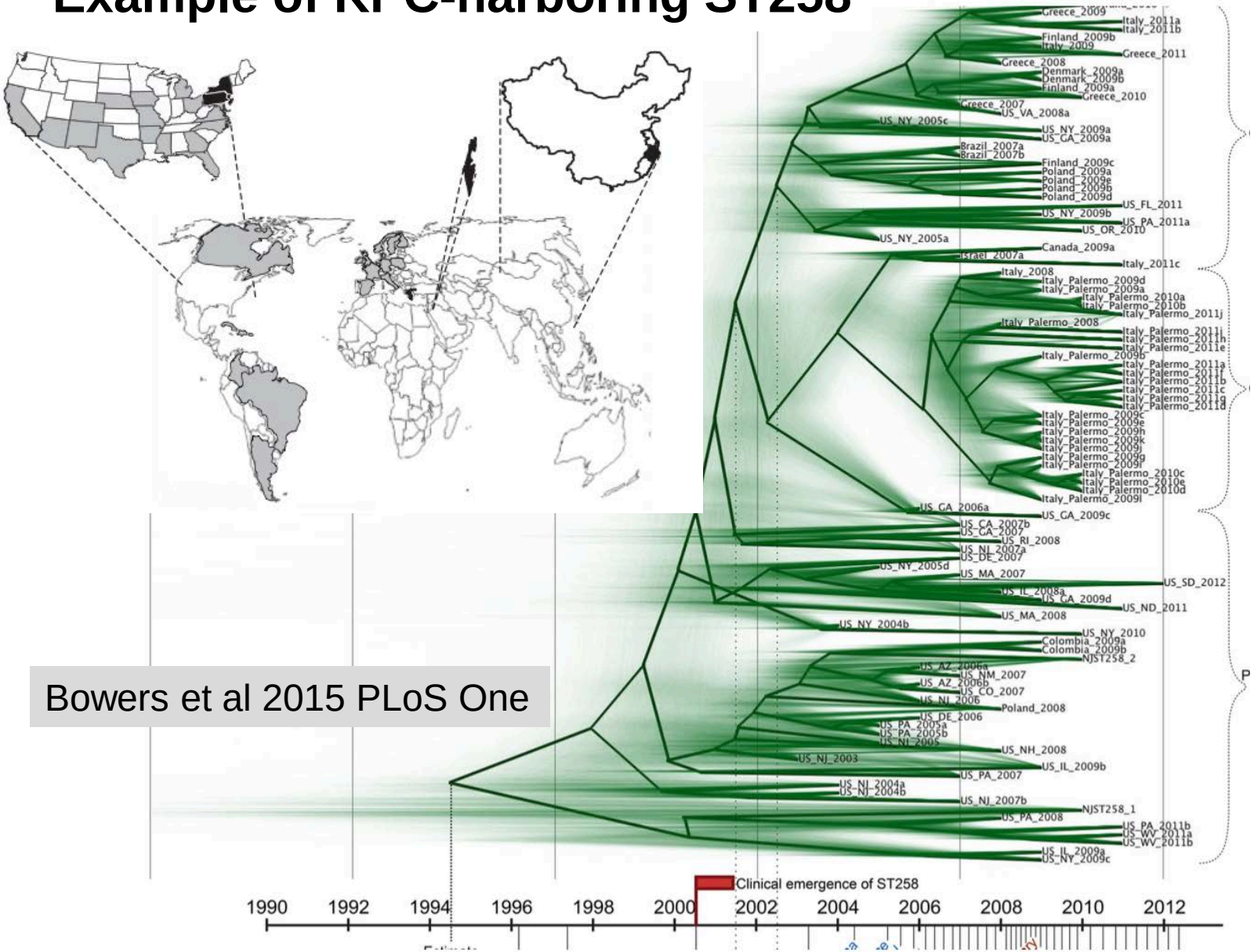
- 632 scgMLST loci were selected
- Complete CDS were used

Core-genome genotyping (cgMLST)



Rapid emergence of MDR *K. pneumoniae*

Example of KPC-harboring ST258



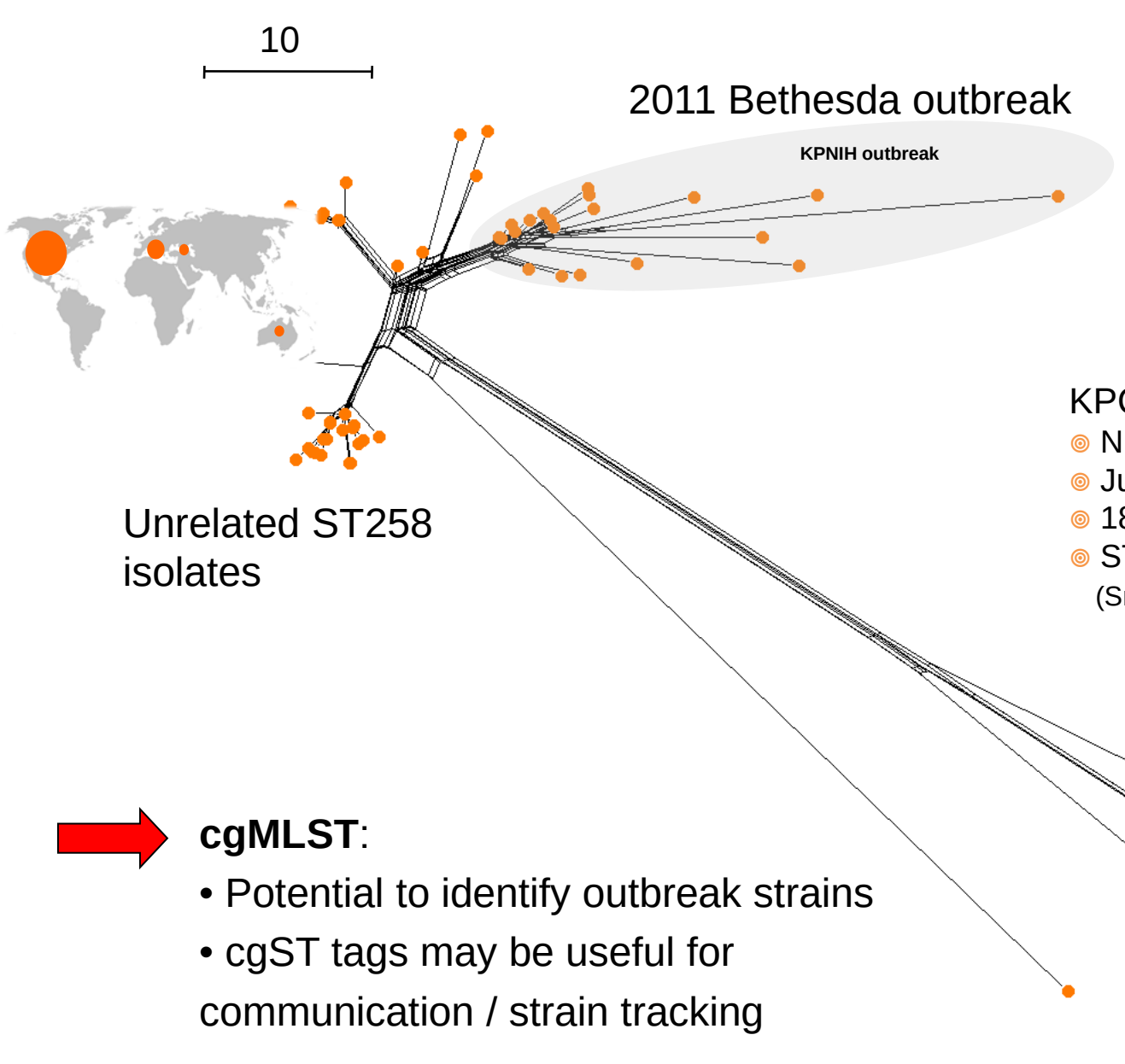
USA
Finland
Poland
Greece
Israel

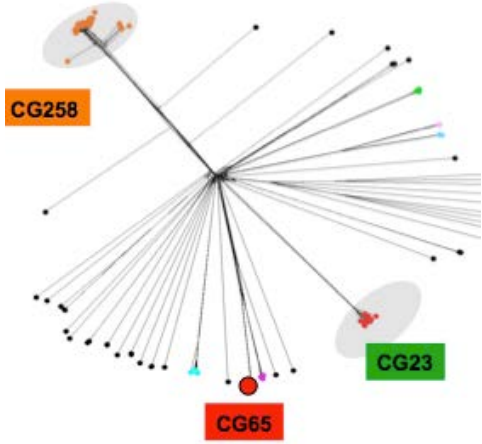
Israel
Italy

USA
mainly

Bowers et al 2015 PLoS One

Characterization of outbreaks





KPC-producing *K. pneumoniae*

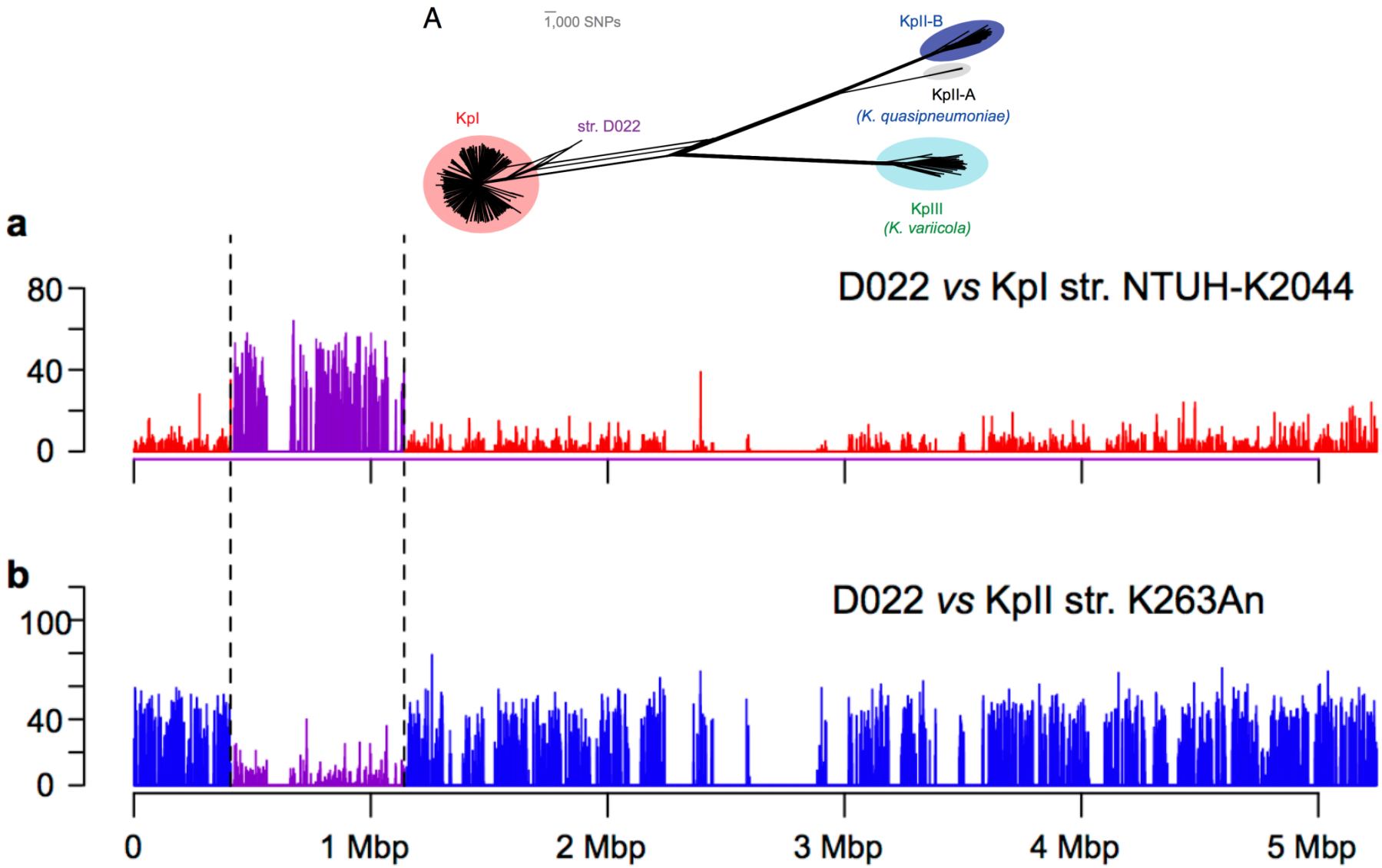
- NIH Clinical Center, Bethesda, USA
- June-Dec. 2011
- 18 patients, 11 deaths
- ST258

(Snitkin et al. 2012)

cgMLST:

- Potential to identify outbreak strains
- cgST tags may be useful for communication / strain tracking

Evolution of *K. pneumoniae* lineages by large recombination

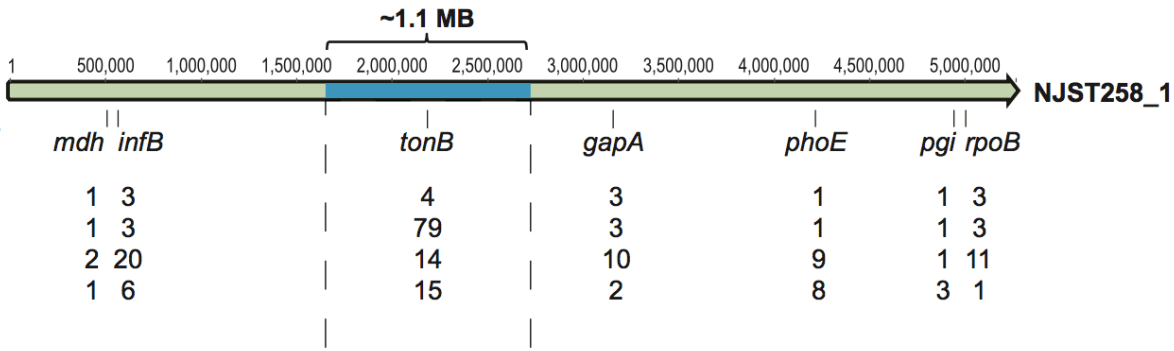


Holt et al 2015 PNAS

Evolution of *K. pneumoniae* clonal groups

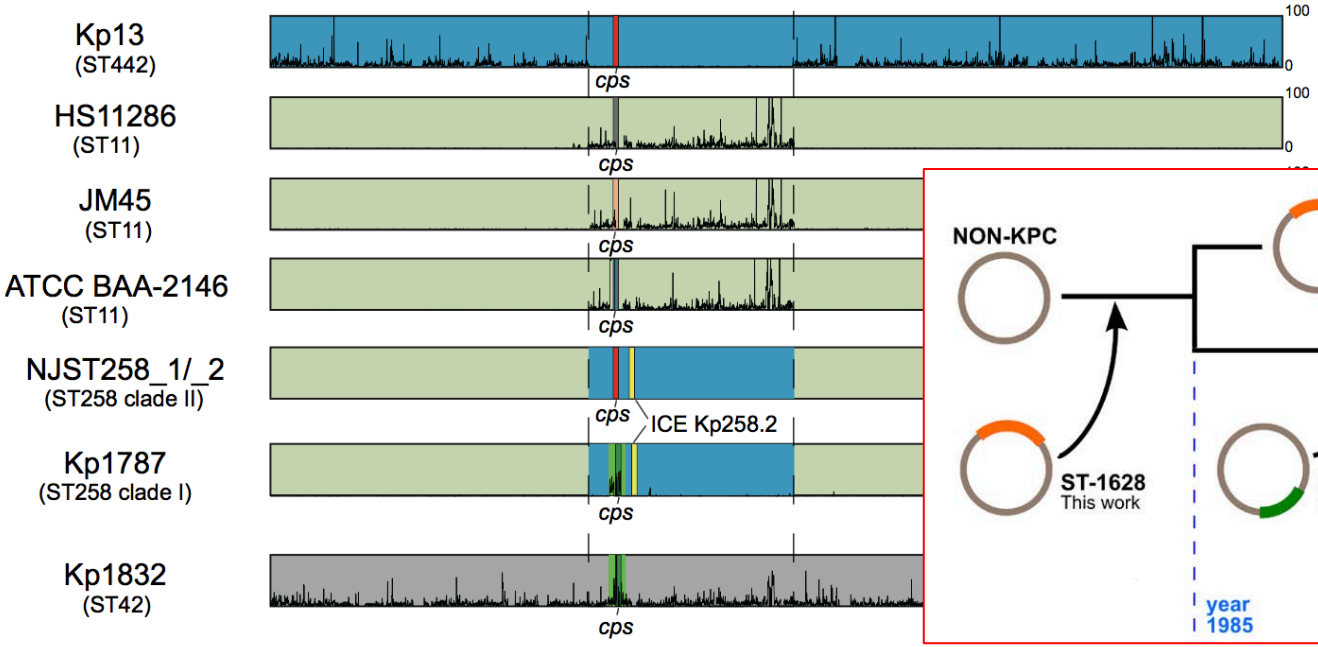
Example of CG258

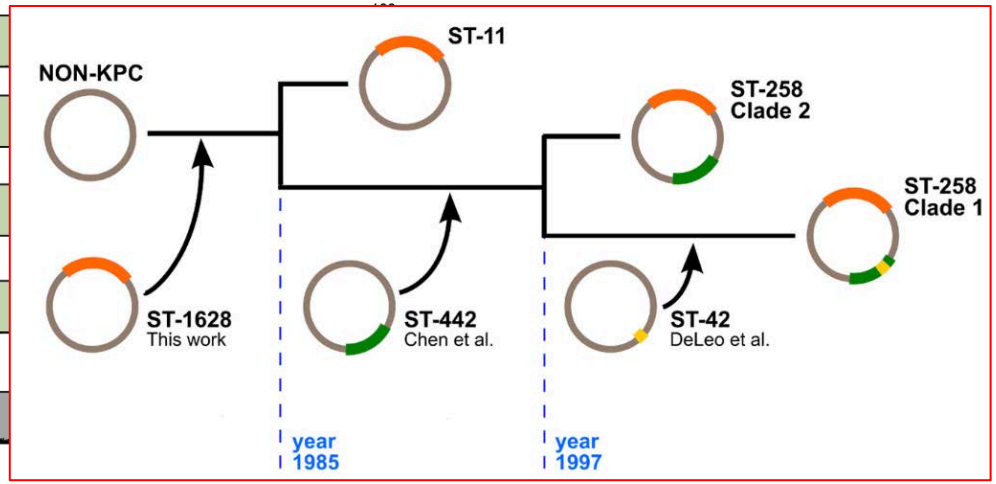
A



MLST	*mdh*	*infB*	*tonB*	*gapA*	*phoE*	*pgi*	*rpoB*
ST11	1	3	4	3	1	1	3
ST258	1	3	79	3	1	1	3
ST442	2	20	14	10	9	1	11
ST42	1	6	15	2	8	3	1

B





Chen et al 2014 mBio

Gaiarsa et al 2015 AAC

Institut Pasteur

INSTITUT PASTEUR MLST
and whole genome MLST databases

BIGSdb
Jolley & Maiden 2010

Klebsiella pneumoniae Genome Database

http://bigsdb.pasteur.fr

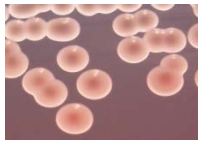
id	isolate	aliases	taxonomic designation	world region	host	clonal group	accession number	gapA	infB	mdh	pgi	phoE	rpoB	tonB	ST
3076	QMP_M1-378		K. pneumoniae	North America	Bovine	76	ERS011957	4	34	1	1	21	1	35	199
3002	DR85_08		K. quasipneumoniae subsp. quasipneumoniae	Asia	Environmental	734	ERS011922	18	15	26	22	94	13	165	734
2949	AJ026		K. variicola	Oceania	Human	695	ERS005747	16	24	21	53	104	17	67	695
2972	AJ182		K. variicola	Oceania	Human	695	ERS005771	16	24	21	53	104	17	67	695
1635	ATCC 13884 T		K. pneumoniae subsp. rhinoscleromatis	North America	Unknown	67	ACZD01	2	1	9	1	15	5	28	67
2937	558m		K. pneumoniae	Asia	Human	660	ERS012030	2	1	2	1	4	1	25	660

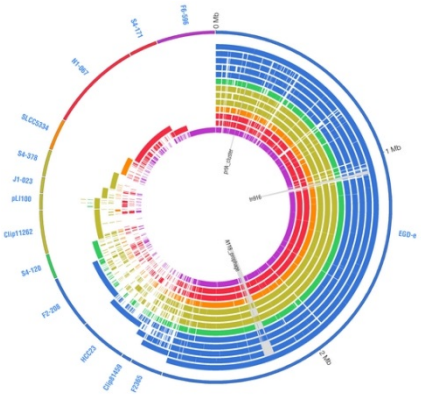
- > 2000 genomes incorporated
- Characterize isolates with species, clonal groups (CG) and ST:

***K. pneumoniae* – CG76 – ST199**

cgMLST type designations

Genome sequencing: ‘all in one technology’

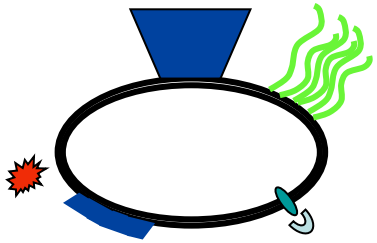




Genotype

Resistance elements

Virulence elements

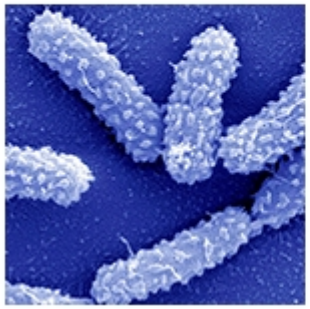


Institut Pasteur

INSTITUT PASTEUR MLST
and whole genome MLST databases

Klebsiella Sequence Typing

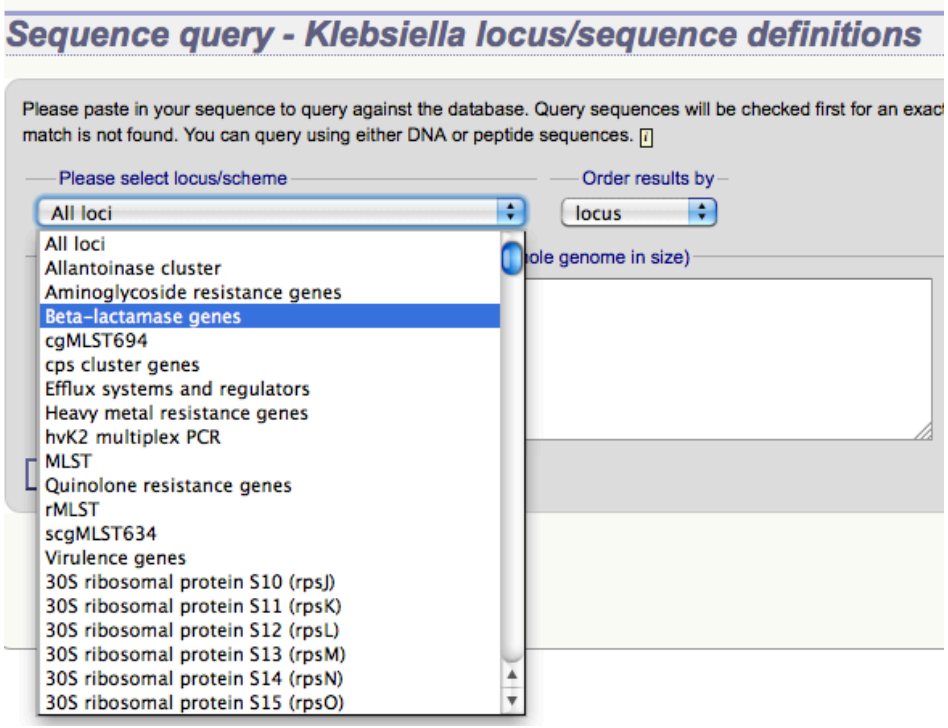
http://bigsdb.pasteur.fr



Sequences and profiles database

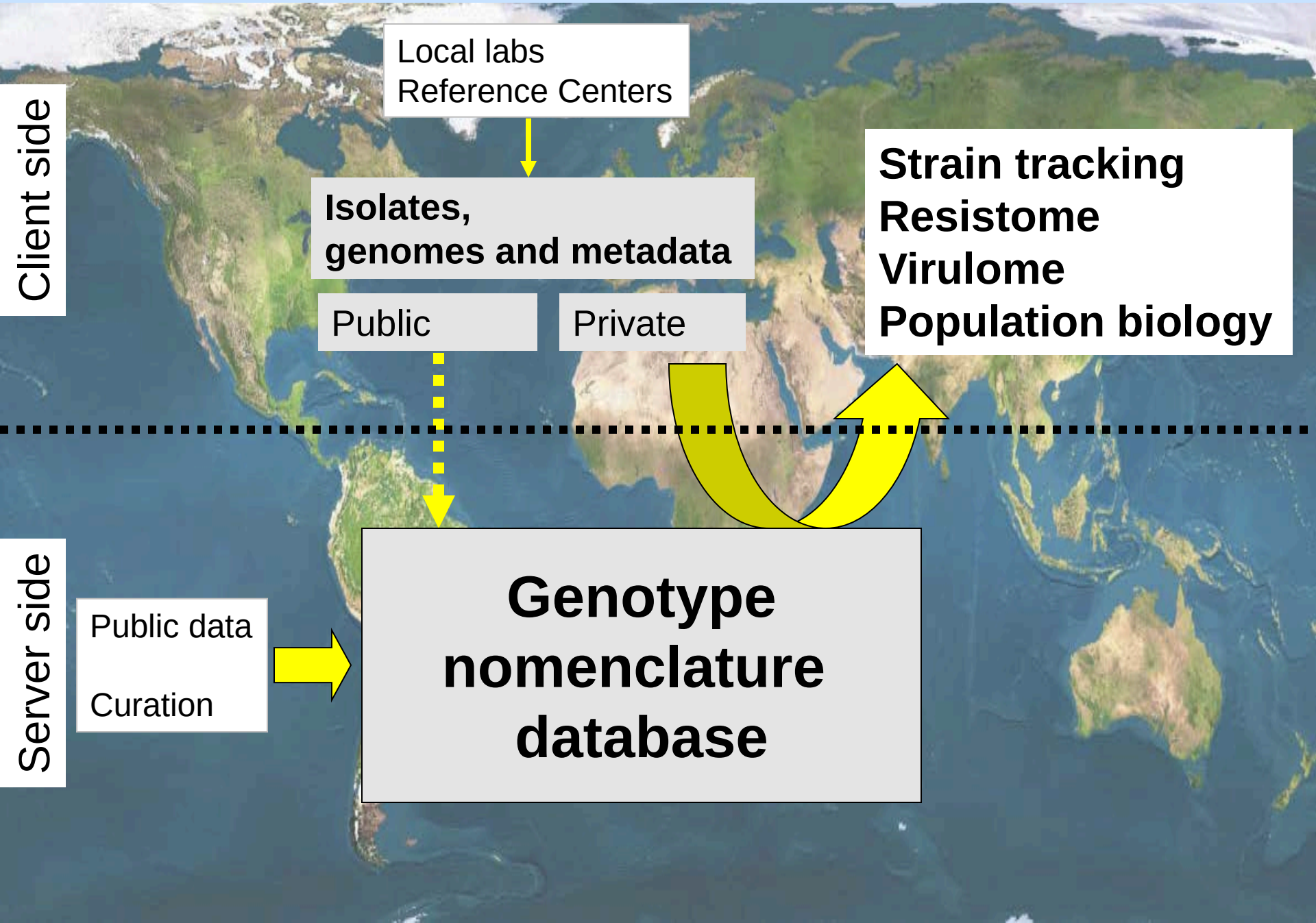
Isolates database

MLST
cgMLST
Virulence genes
Resistance genes
wzi (capsular typing)



Powered by BIGSdb
(Jolley & Maiden, 2010)

Databases for genomic epidemiology & population biology



Acknowledgements

Biodiversity and Epidemiology of Bacterial Pathogens

Virginie PASSET, Carla RODRIGUES, Sebastian LOPEZ-FERNANDES, Radek IZDEBSKI, Melanie HENNART

Microbial Evolutionary Genomics

Suzanne BIALEK, Camille BLIN, Marie TOUCHON, Eduardo ROCHA

Center for Biostatistics, Bioinformatics and Integrative Biology, Institut Pasteur

Alexis CRISCUOLO, Julien GUGLIELMINI, Emmanuel QUEVILLON

Epidemiology Unit

Bich-Tram HUYNH, Didier GUILLEMOT

Institut Pasteur

ANSES, Lyon

Marisa HAENNI, Jean-Yves MADEC

Paris Hospitals (AP-HP)

Dominique DECRE, Marie-Hélène NICOLAS-CHANOINE

Oxford University, UK

Keith JOLLEY, Martin MAIDEN

Univ. Melbourne, Australia

Kat HOLT

INVESTISSEMENTS D'AVENIR

îledeFrance

One HEALTH EJP

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

JPIAMR