

KlebNET-GSP

LECTURE SERIES

Klebsiella pneumoniae Genomic Epidemiology and Antimicrobial Resistance

Introduction to *Klebsiella pneumoniae*

Kat Holt, London School of Hygiene and Tropical Medicine

Intended Learning Objectives

Specific objectives of this session:

1. Understand the basic features of *Klebsiella pneumoniae* genomes
2. Learn about genetic diversity relevant to public health, including:
 1. Population structure and lineages
 2. Polysaccharide loci (capsule and O antigen)
 3. Antimicrobial resistance mechanisms
 4. Virulence and hypervirulence factors
3. Understand the challenges of defining hypervirulence, and convergence of hypervirulence with resistance
4. Learn where to find resources to support genomic surveillance and typing of *Klebsiella pneumoniae*

Outline

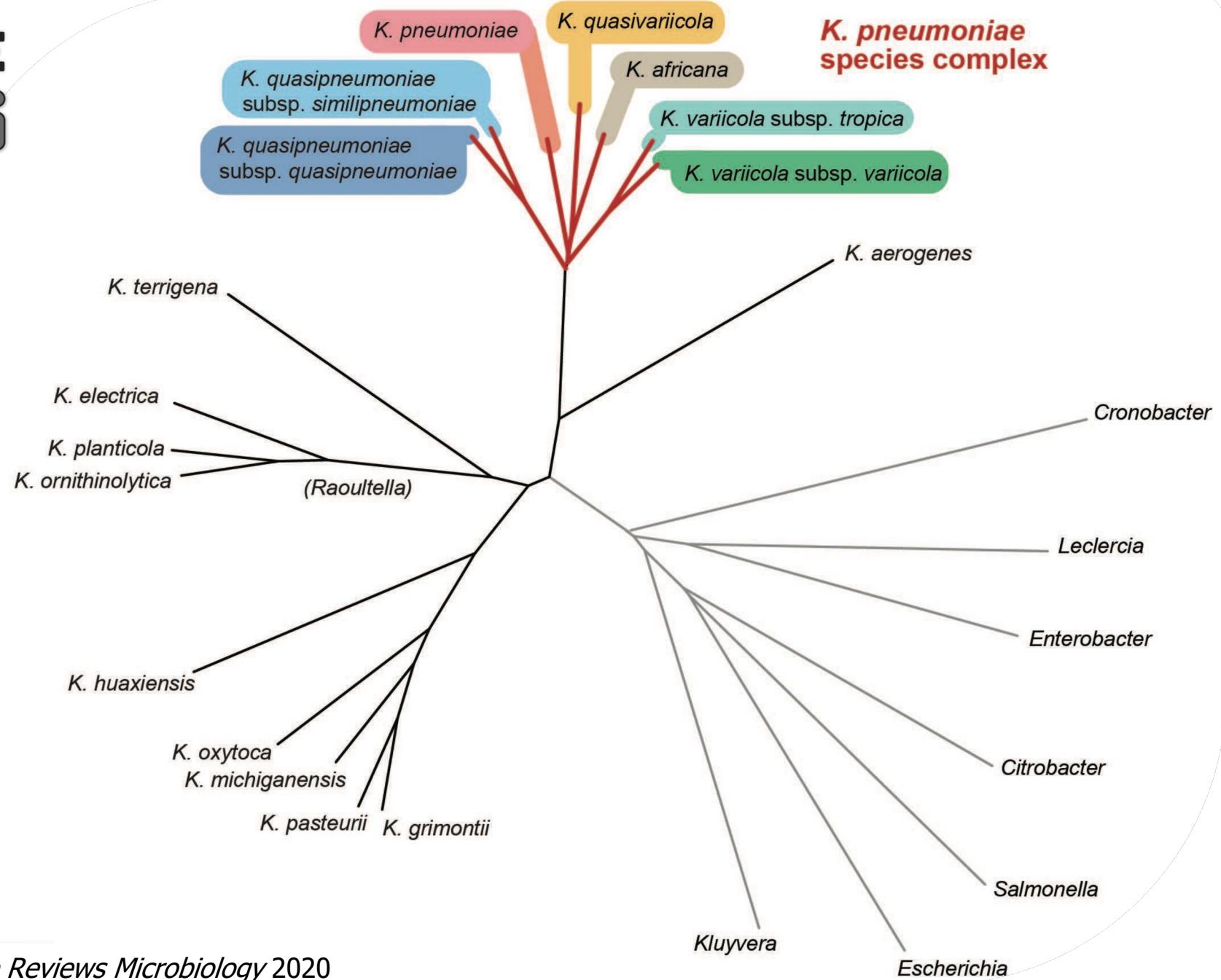
This session consists of the following elements

1. Introduction to *Klebsiella pneumoniae*
2. Overview of genomic features and strain diversity
3. Challenges interpreting virulence markers and hypervirulence
4. KlebNET Genomic Surveillance Platform: resources and collaborative opportunities

Klebsiella pneumoniae (Kp)

- Gram-negative bacterium of the Enterobacterales
- Colonises gut of humans and other animals
- Primarily opportunistic healthcare-associated infections
 - Especially in infants, elderly, immunocompromised
 - Pneumonia, urinary tract infection, wound infection, sepsis
 - Often multidrug resistant, can be challenging to treat
- In Europe: ESBL, carbapenemase-producing or colistin resistant *Kp* causes >90 thousand infections annually¹
 - 25% of total DALYs associated with AMR infections
- Globally: Drug resistant *Kp* causes >735 thousand deaths annually²
 - >100 thousand neonatal sepsis deaths





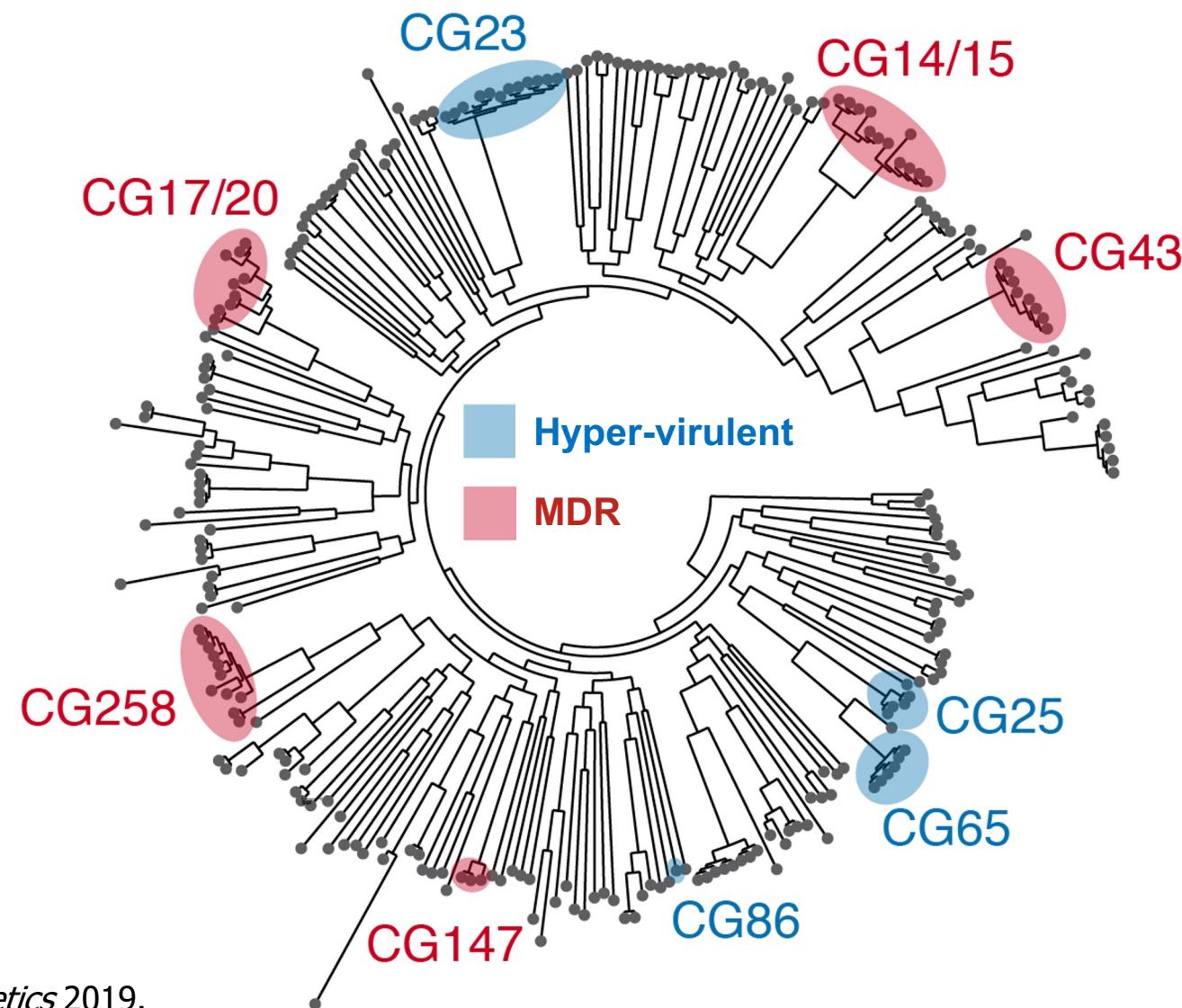
Genomic features and strain diversity

Approx. 5,500,000 base pair chromosome

0-10 plasmids per genome

Thousands of deep branching lineages or 'clonal groups', which differ in gene content

Diverse phylogenetic lineages

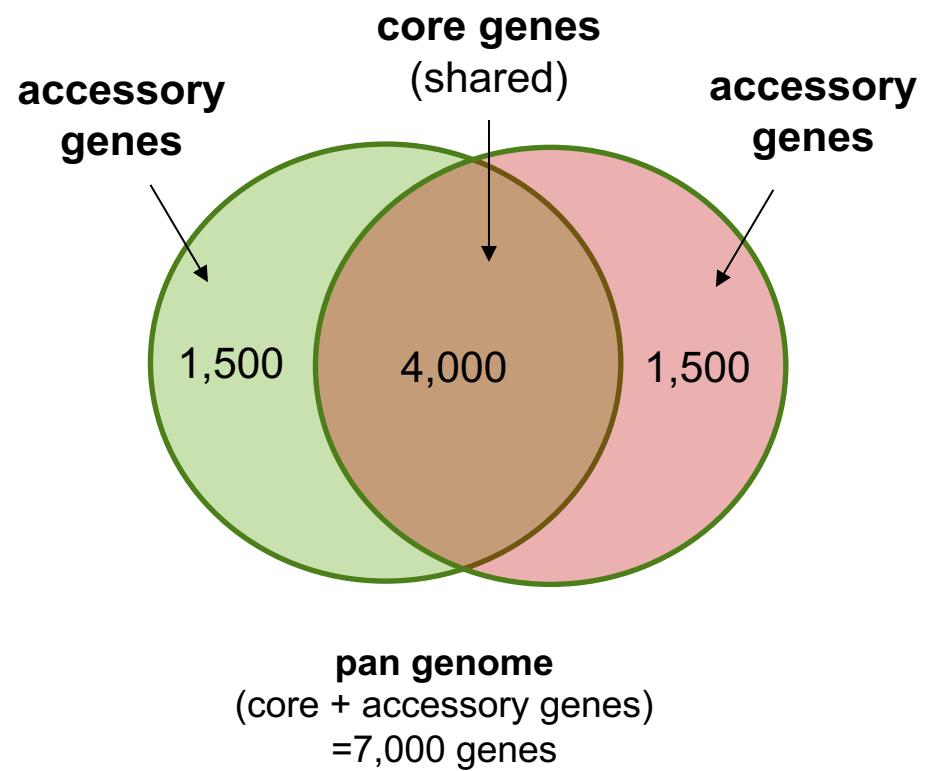


Population structure
Core-genome phylogeny
~0.5% divergence between lineages

Clonal groups (CG)
see next lecture (Sylvain Brisse)

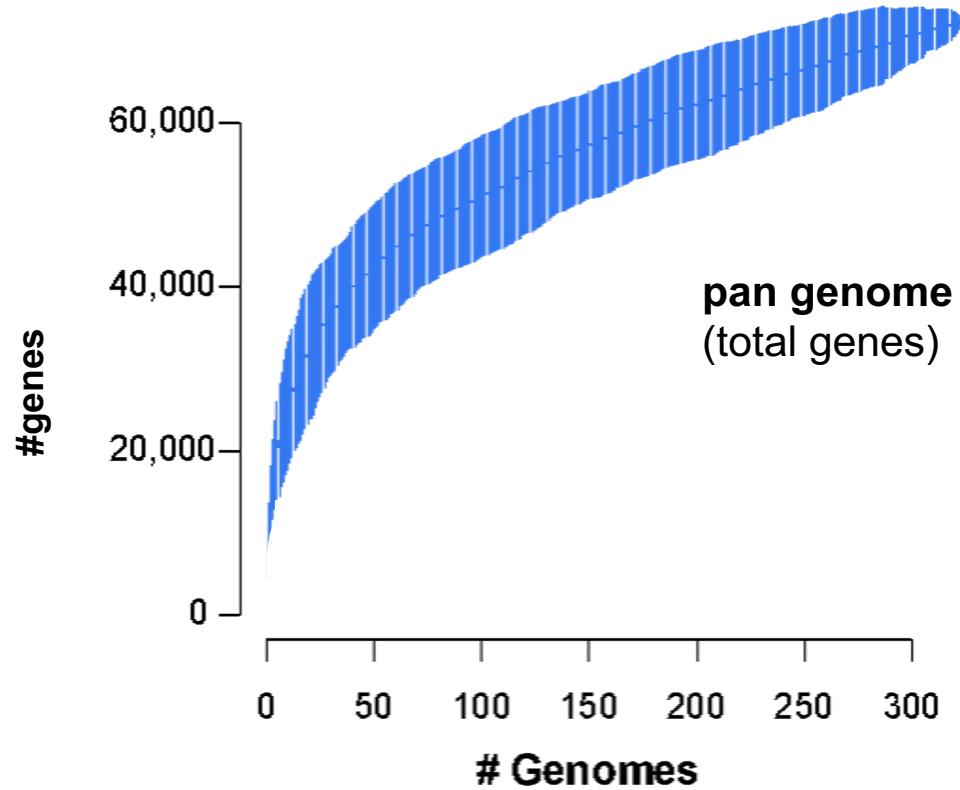
Variable gene content

Comparing 2 *Kp* genomes:



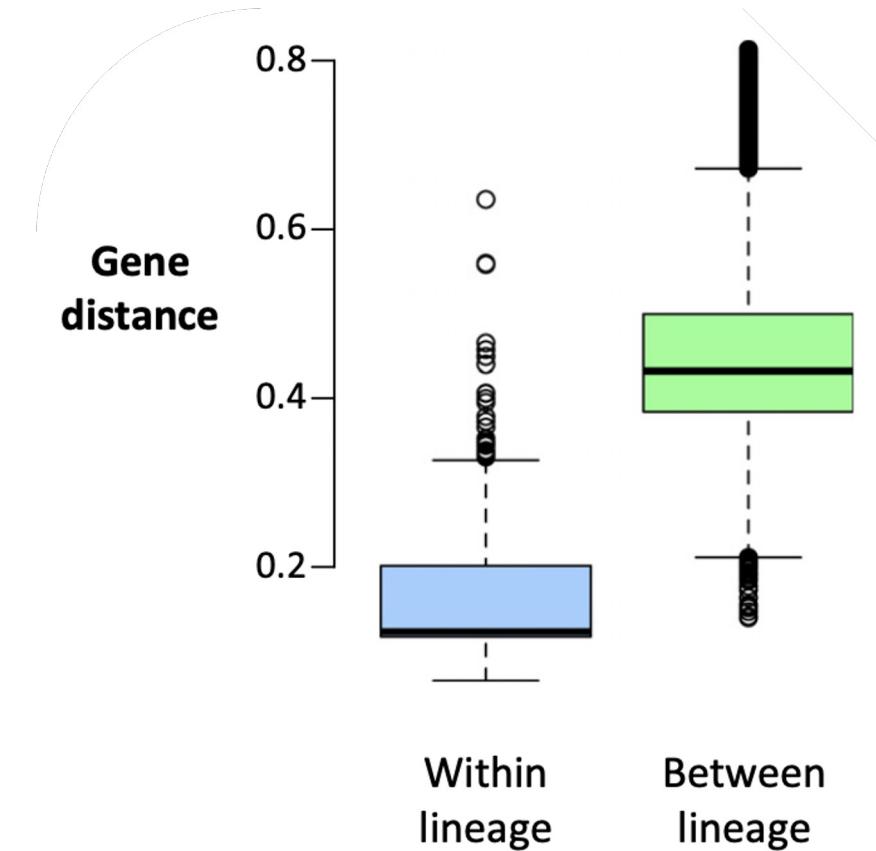
Variable gene content

Comparing 300 *Kp* genomes:

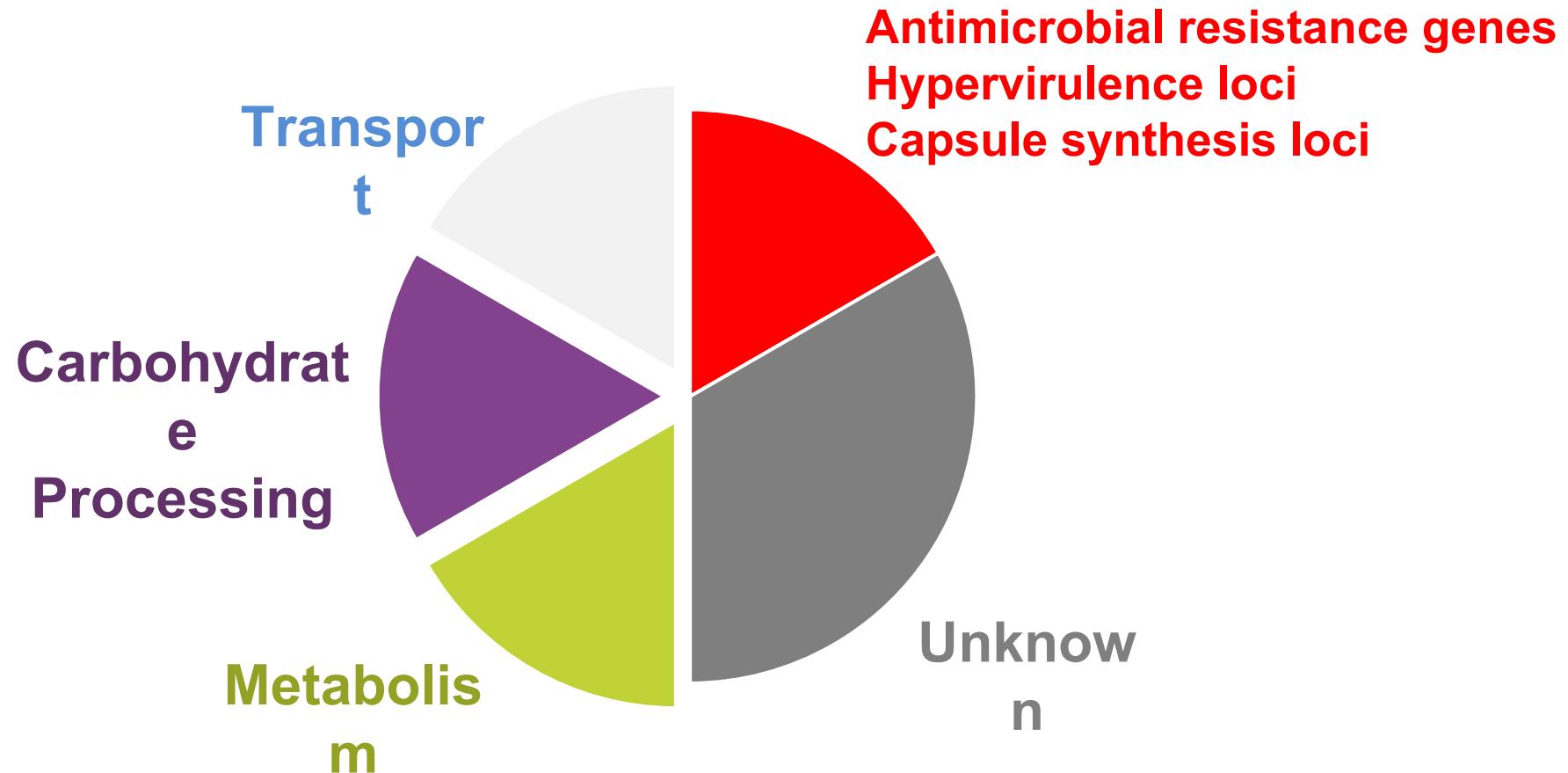


Variable gene content

Comparing between *Kp* lineages:

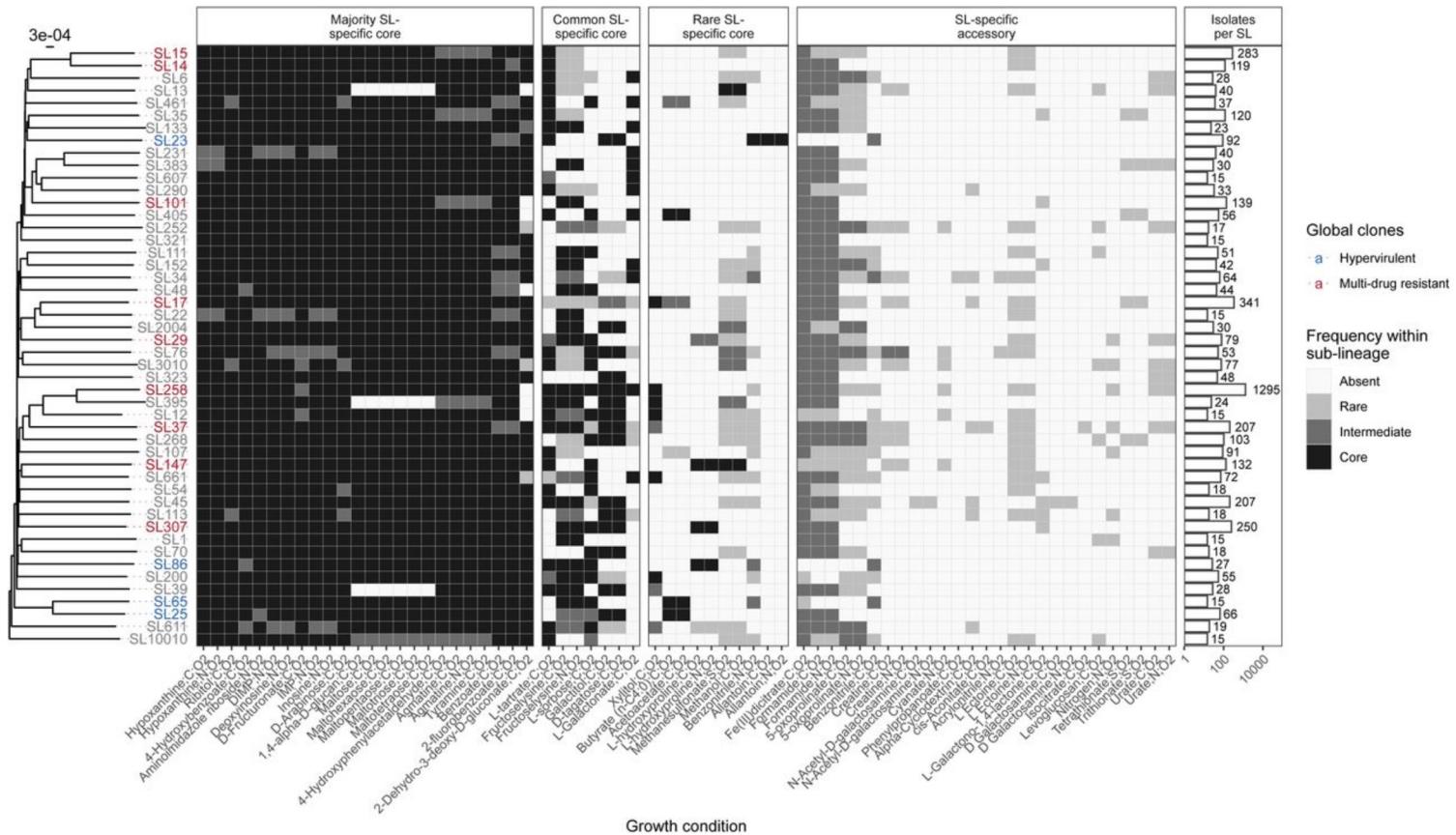


Accessory gene functions



Variation in metabolic genes & predicted phenotypes

A metabolic atlas of the *Klebsiella pneumoniae* species complex reveals lineage-specific metabolism that supports persistent co-existence of diverse lineages

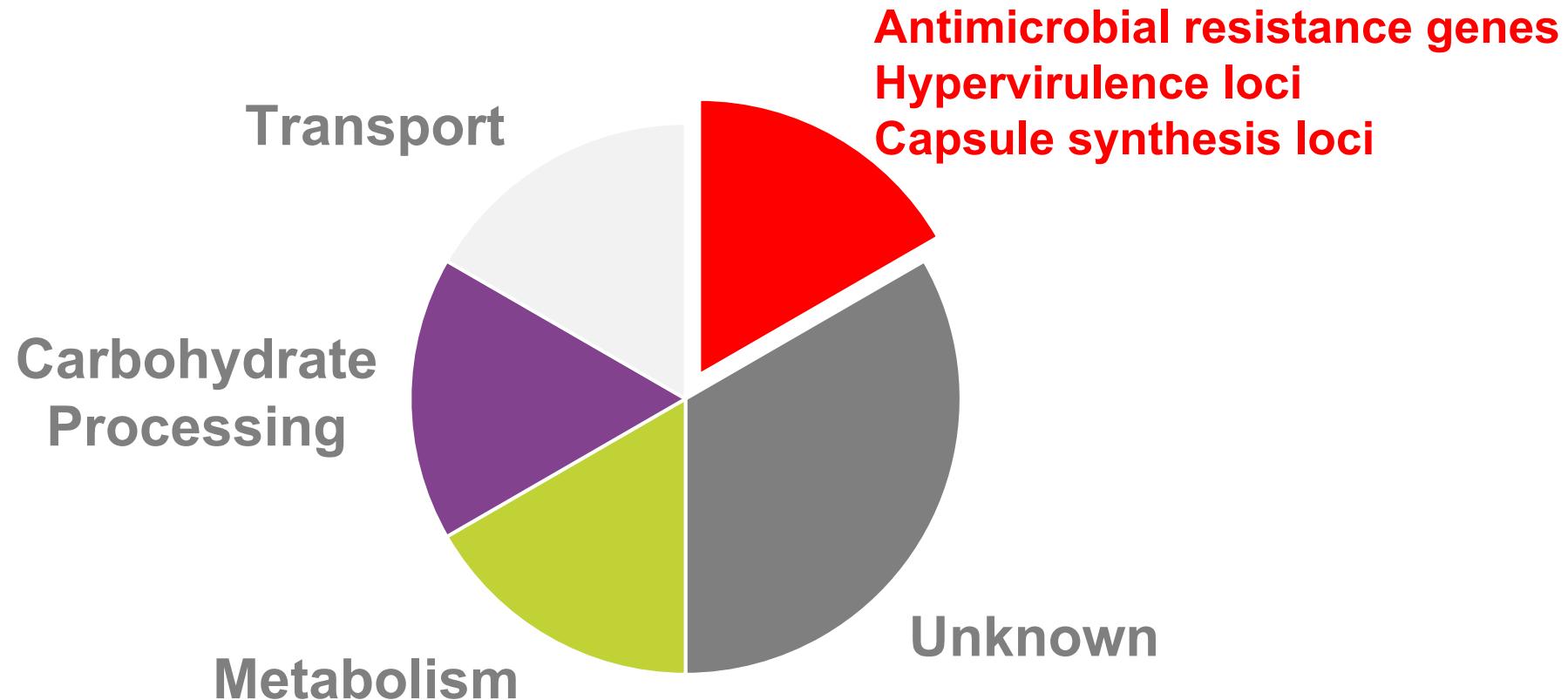


Plot shows variation in substrate utilization per lineage, predicted from genomes using Bactabolize

Bactabolize

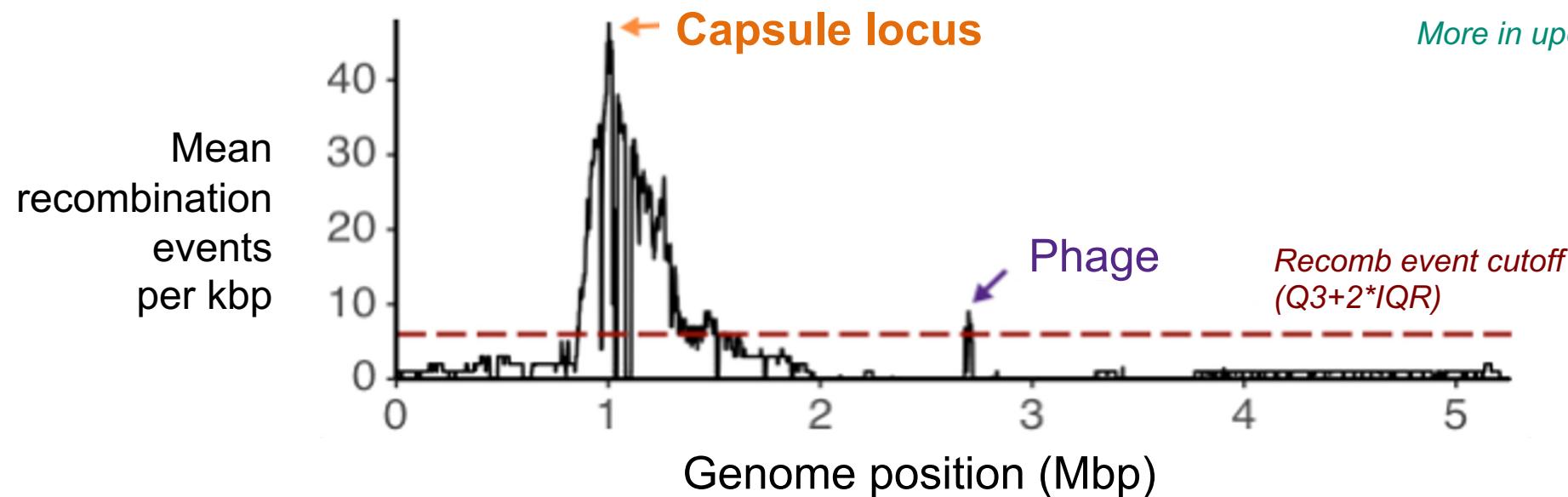
A high-throughput genome-scale metabolic reconstruction and growth simulation pipeline.

Genes of known public health importance



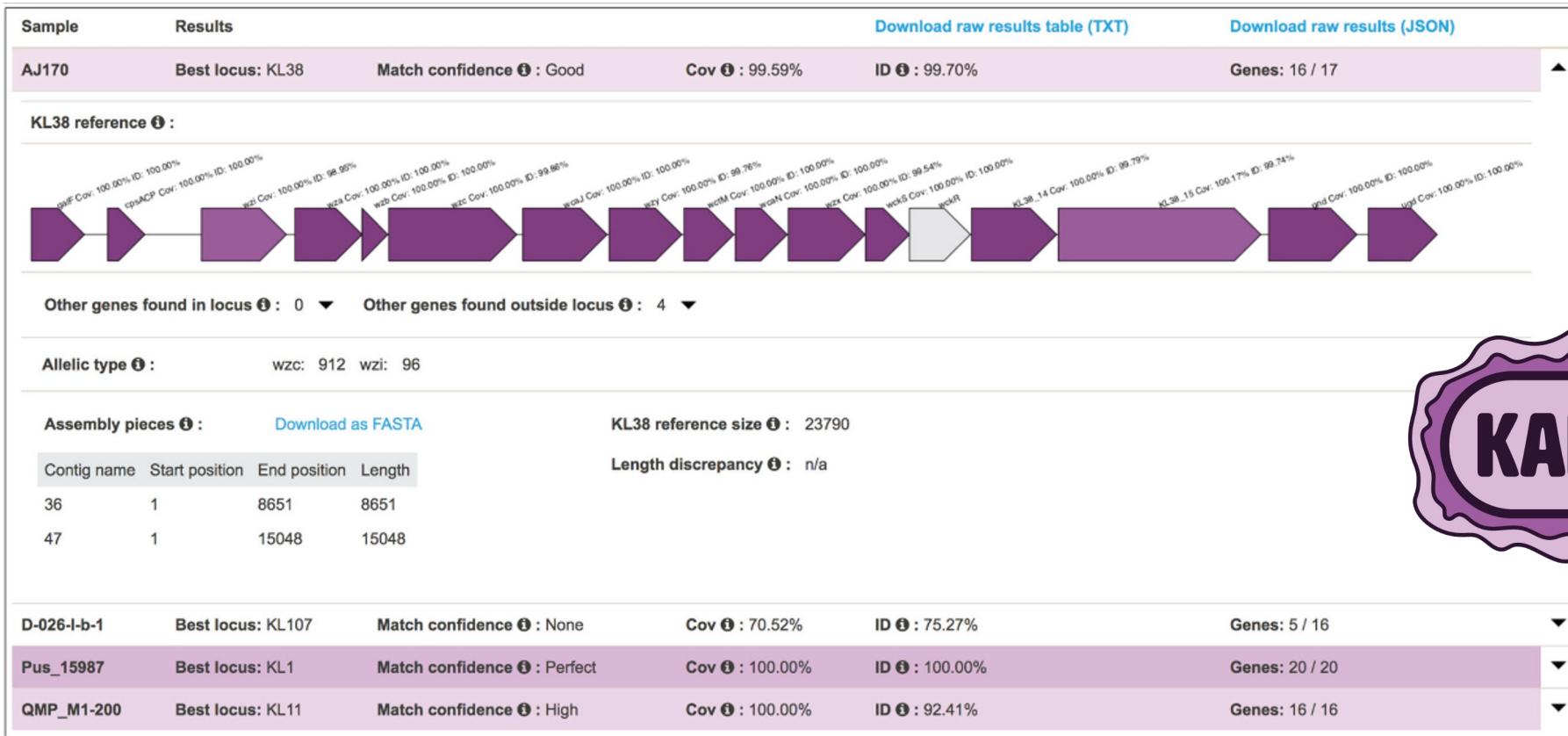
Capsule (K) and O antigen synthesis loci

- >150 capsule (K) loci and 15 O loci defined in *Kp*¹
- Capsule locus is a recombination hotspot in the *Kp* chromosome²



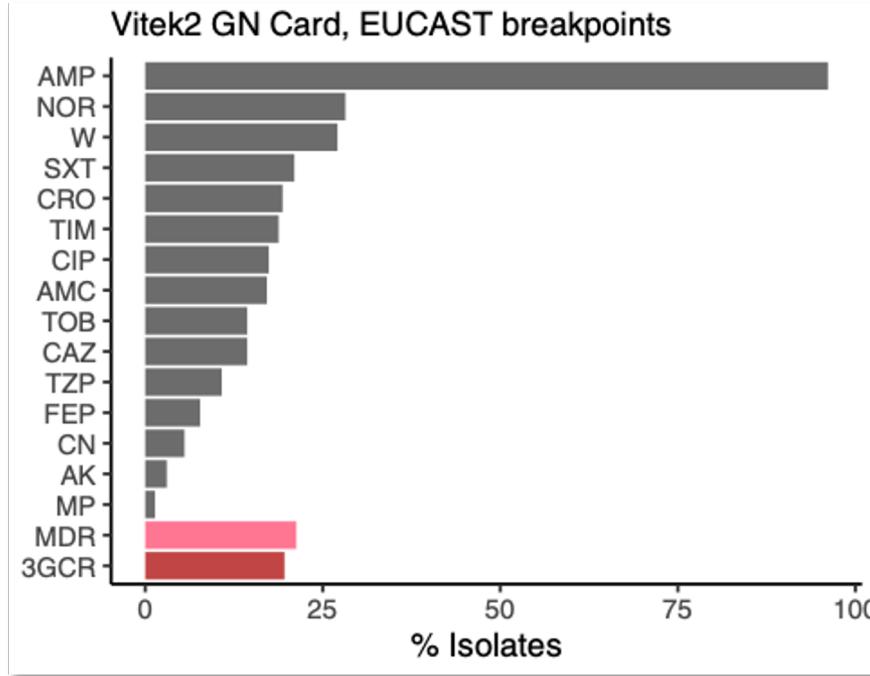
Capsule (K) and O locus typing

More in upcoming lecture from Tom Stanton

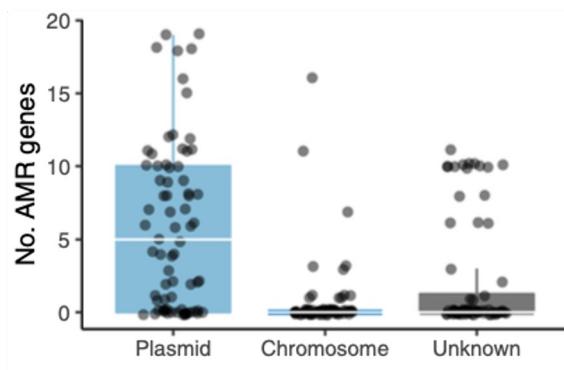


Antimicrobial resistance (AMR)

Snapshot of hospital *Kp* resistance
(all clinical isolates for 1 year)



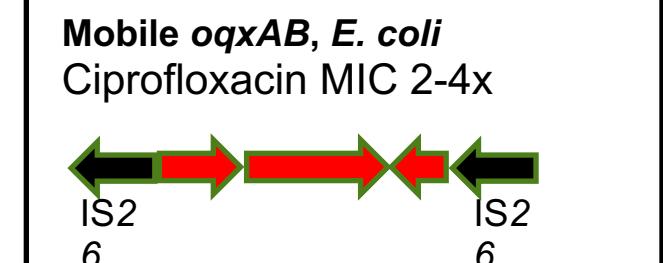
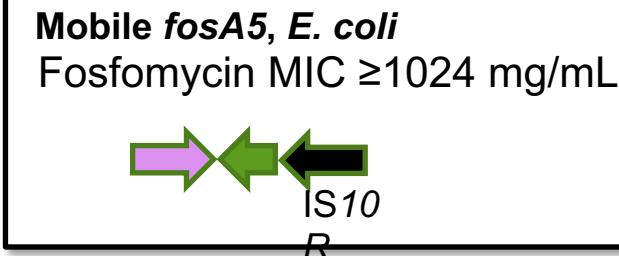
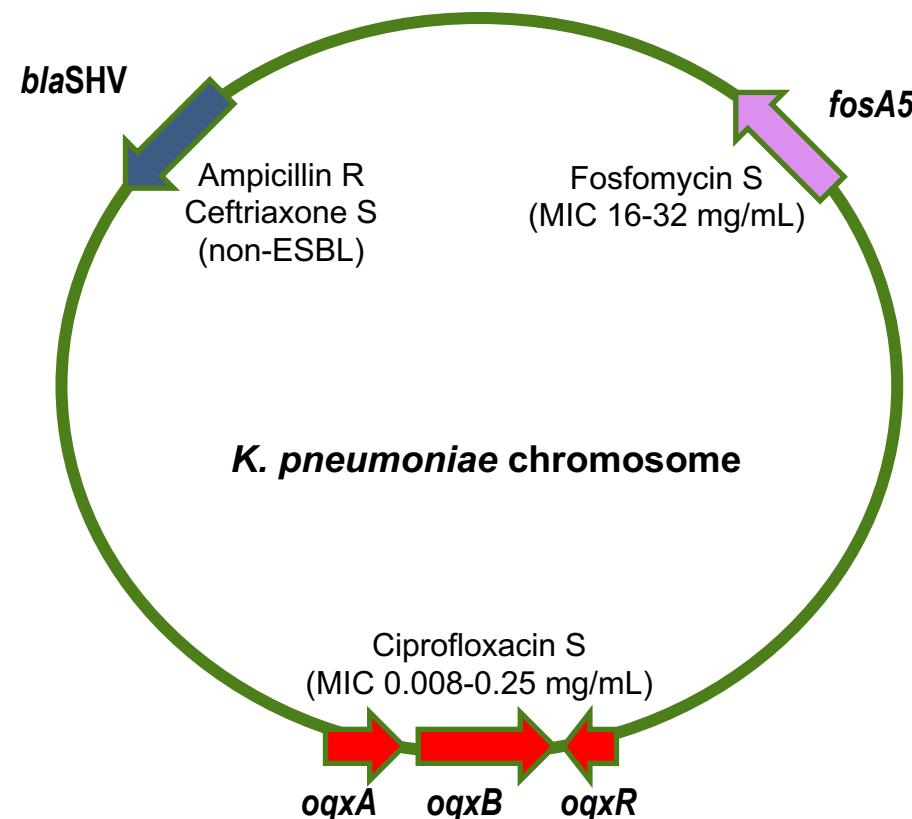
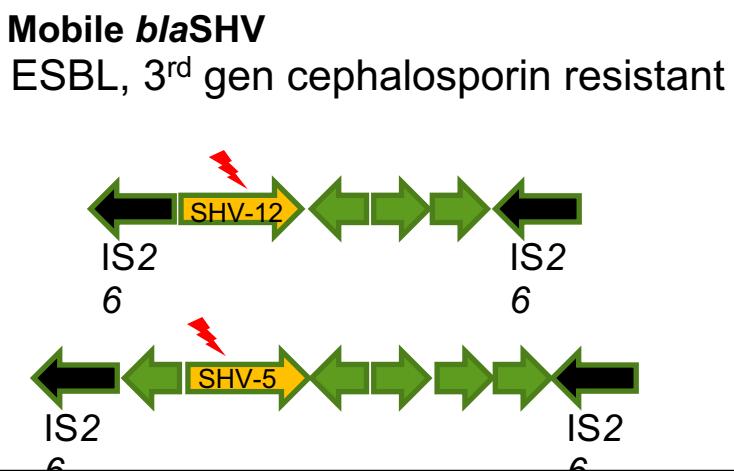
Antimicrobial	Determinants (% of resistance explained)	Major error N (%)	Very major error N (%)
Ceftriaxone	<i>bla</i> _{CTX-M-15} (71%), <i>bla</i> _{CTX-M-14} (13%)	2 (0.8%)	5 (11%)
Meropenem	<i>bla</i> _{IMP-4} (50%), <i>bla</i> _{OXA-48} (50%)	1 (0.3%)	0 (0%)
Ciprofloxacin	<i>qnrB1</i> (61%), <i>qnrS1</i> (7%), GyrA-83 (32%), GyrA-87 (16%), ParC-80 (23%) *	15 (6.0%)	6 (14%)
Gentamicin	<i>aac(6')-lb-cr</i> (79%), <i>rmtB</i> (15%), <i>aac3-IIa</i> (15%), <i>aac3-IId</i> (12%), <i>aadA2</i> (9%), <i>ant(2")-la</i> (9%), <i>aac(6')-lb4</i> (6%)	0 (0%)	1 (9%)
Tobramycin		1 (0.4%)	1 (3%)
Amikacin		§0 (0%)	§2 (29%)
Trimethoprim +Sulfamethoxazole	<i>dfrA14</i> (51%), <i>dfrA12</i> (6%); + <i>sul2</i> (89%), + <i>sul1</i> (29%)	2 (0.9%)	14 (20%)
		4 (1.7%)	4 (7%)



AMR-related core genes

Protein identifier	Gene symbol	Sequence name	Element type	Class
FNMJAFEK_00618	fosA	FosA5 family fosfomycin resistance glutathione transferase	AMR	FOSFOMYCIN
FNMJAFEK_04094	blaSHV	class A broad-spectrum beta-lactamase SHV-1	AMR	BETA-LACTAM
FNMJAFEK_05097	oqxB19	multidrug efflux RND transporter permease subunit OqxB19	AMR	PHENICOL/QUINOLONE
FNMJAFEK_05098	oqxA	multidrug efflux RND transporter periplasmic adaptor subunit OqxA	AMR	PHENICOL/QUINOLONE

AMRFinderPlus output for wildtype *K. pneumoniae* (expected ampicillin resistance only)



Custom AMR dictionary in Kleborate



Commandline result:

Species	Klebsiella pneumoniae
MLST	ST113
K_locus	KL114
O_type	O3/O3a
Ybt	ybt 8; ICEKp3
AGly_acquired	-
Col_acquired	-
Fcyn_acquired	-
Flq_acquired	-
Gly_acquired	-
MLS_acquired	-
Phe_acquired	-
Rif_acquired	-
Sul_acquired	-
Tet_acquired	-
Tgc_acquired	-
Tmt_acquired	-
Bla_acquired	-
Bla_inhR_acquired	-
Bla_ESBL_acquired	-
Bla_ESBL_inhR_acquired	-
Bla_Carb_acquired	-
Bla_chr	SHV-1^
SHV_mutations	-
Omp_mutations	-
Col_mutations	-
Flq_mutations	-

See upcoming lecture from Margaret Lam



Antimicrobial resistance (AMR)

Sourced from Kleborate

Drug/Class	Resistance Determinants
Aminoglycosides	None found
Carbapenems	None found
Cephalosporins (3rd gen.)	None found
Cephalosporins (3rd gen.) + β-lactamase inhibitors	None found
Colistin	None found
Fluoroquinolones	None found
Fosfomycin	None found
Penicillins	SHV-1
Penicillins + β-lactamase inhibitors	None found
Phenicols	None found
Sulfonamides	None found
Tetracycline	None found
Tigecycline	None found
Trimethoprim	None found

Detailed SHV typing in Kleborate



MICROBIAL GENOMICS

RESEARCH ARTICLE

Tsang et al., *Microbial Genomics*
DOI 10.1099/mgen.0.001294



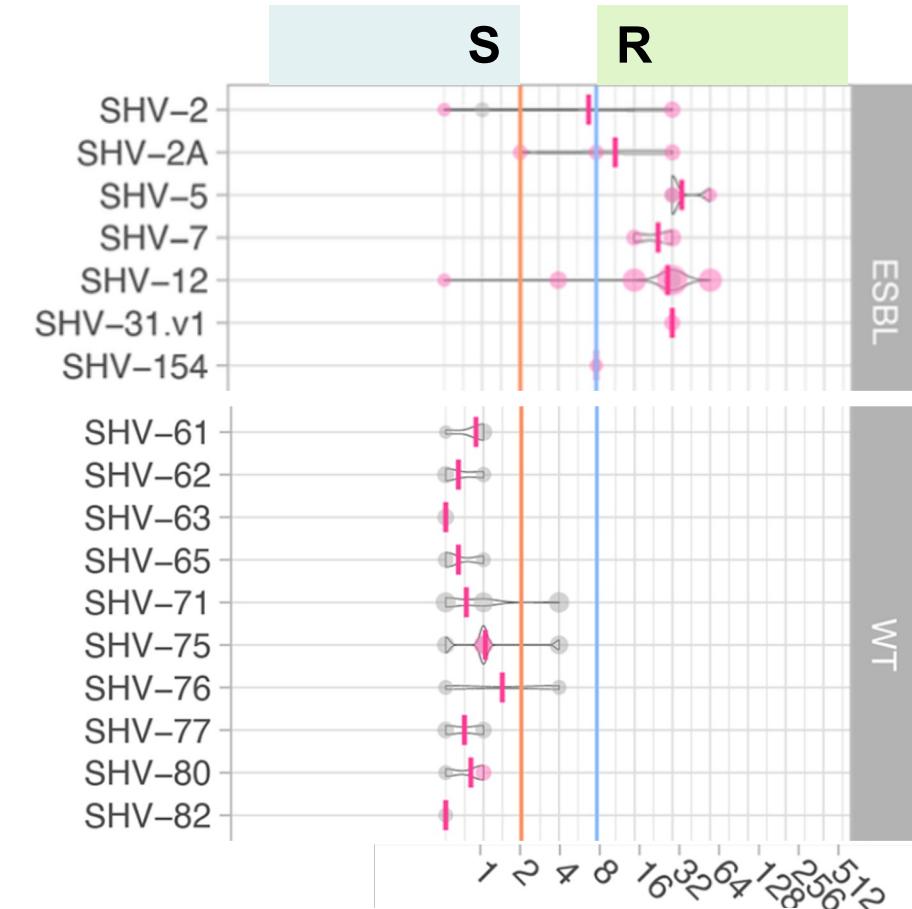
Diversity, functional classification and genotyping of SHV β -lactamases in *Klebsiella pneumoniae*

Kara K. Tsang¹, Margaret M. C. Lam², Ryan R. Wick^{2,3}, Kelly L. Wyres², Michael Bachman⁴, Stephen Baker⁵, Katherine Barry⁶, Sylvain Brisse⁷, Susana Campino¹, Alexandra Chiaverini⁸, Daniela Maria Cirillo⁹, Taane Clark¹, Jukka Corander¹⁰, Marta Corbella¹¹, Alessandra Cornacchia⁸, Aline Cuénod¹², Nicola D'Alterio⁸, Federico Di Marco⁹, Pilar Donado-Godoy¹³, Adrian Egli¹², Refath Farzana¹⁴, Edward J. Feil¹⁵, Aasmund Fostervold¹⁶, Claire L. Gorrie³, Brekhna Hassan¹⁷, Marit Andrea Klokkammer Hetland¹⁶, Le Nguyen Minh Hoa¹⁸, Le Thi Hoi¹⁹, Benjamin Howden³, Odion O. Ikhimiukor²⁰, Adam W. J. Jenney³, Håkon Kaspersen²¹, Fahad Khokhar⁵, Thongpan Leangapichart²¹, Małgorzata Ligowska-Marzeta²², Iren Høyland Löhr¹⁶, Scott W. Long²³, Amy J. Mathers⁶, Andrew G. McArthur²⁴, Geetha Nagaraj²⁵, Anderson O. Oaikhena²⁰, Iruka N. Okeke²⁰, João Perdigão²⁶, Hardik Parikh⁶, My H. Pham²⁷, Francesco Pomilio⁸, Niclas Raffelsberger²⁸, Andriniaina Rakotondrasoa²⁹, K. L. Ravi Kumar²⁵, Leah W. Roberts³⁰, Carla Rodrigues⁷, Ørjan Samuelsen^{31,32}, Kirsty Sands¹⁴, Davide Sassera^{11,33}, Helena Seth-Smith¹², Varun Shamma²⁵, Norelle L. Sherry³, Sonia Sia³⁴, Anton Spadar¹, Nicole Stoesser³⁵, Marianne Sunde²¹, Arnfinn Sundsfjord^{31,36}, Pham Ngoc Thach¹⁸, Nicholas R. Thomson²⁷, Harry A. Thorpe¹⁰, M. Estée Torok⁵, Van Dinh Trang¹⁸, Nguyen Vu Trung¹⁹, Jay Vornhagen³⁷, Timothy Walsh¹⁴, Ben Warne⁵, Hayley Wilson³⁸, Gerard D. Wright²⁴, Kathryn E. Holt^{1,2,*} and KlebNET-GSP AMR Genotype-Phenotype Group

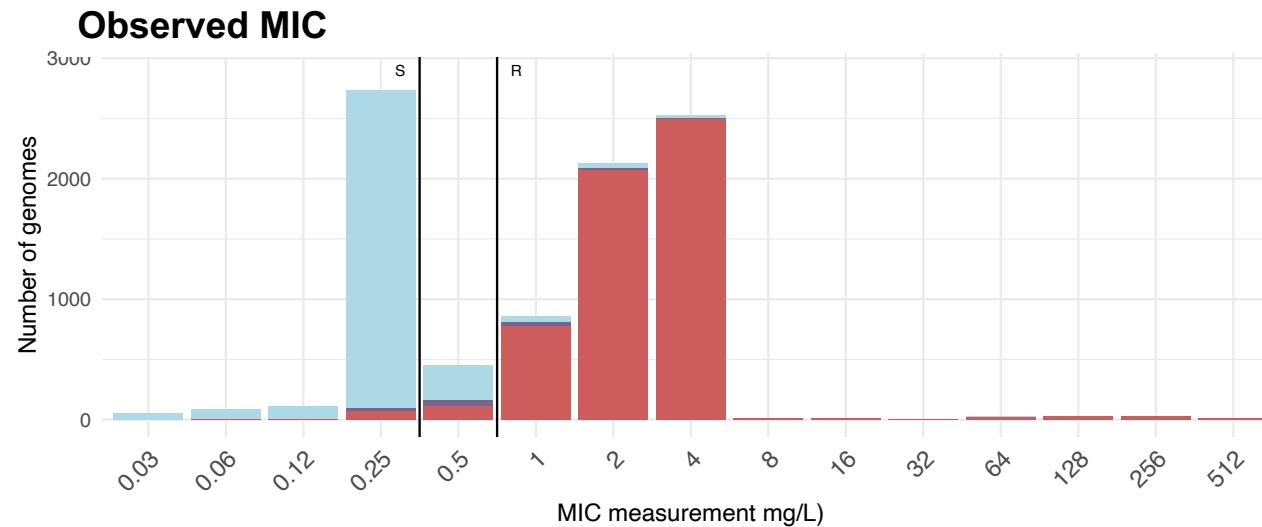


KlebNET-GSP

13,000+ genomes with matched AMR phenotypes from 27 countries



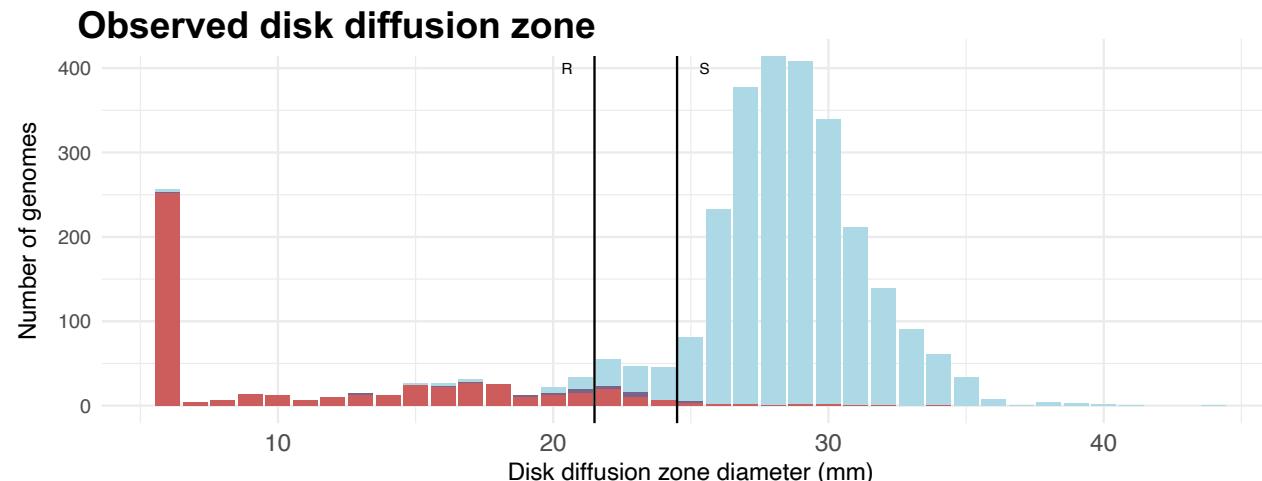
Ciprofloxacin resistance prediction



Predicted phenotype

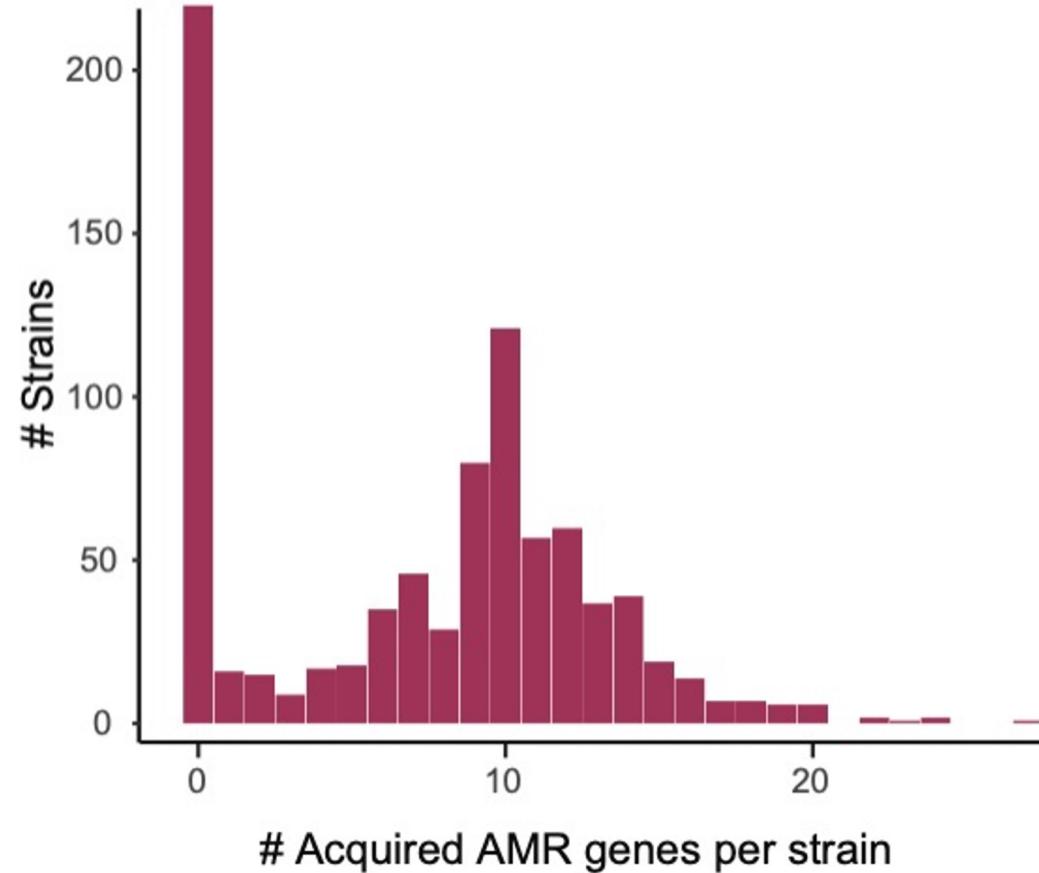
- S
- I
- R

(available in Kleborate v3.2.2+)

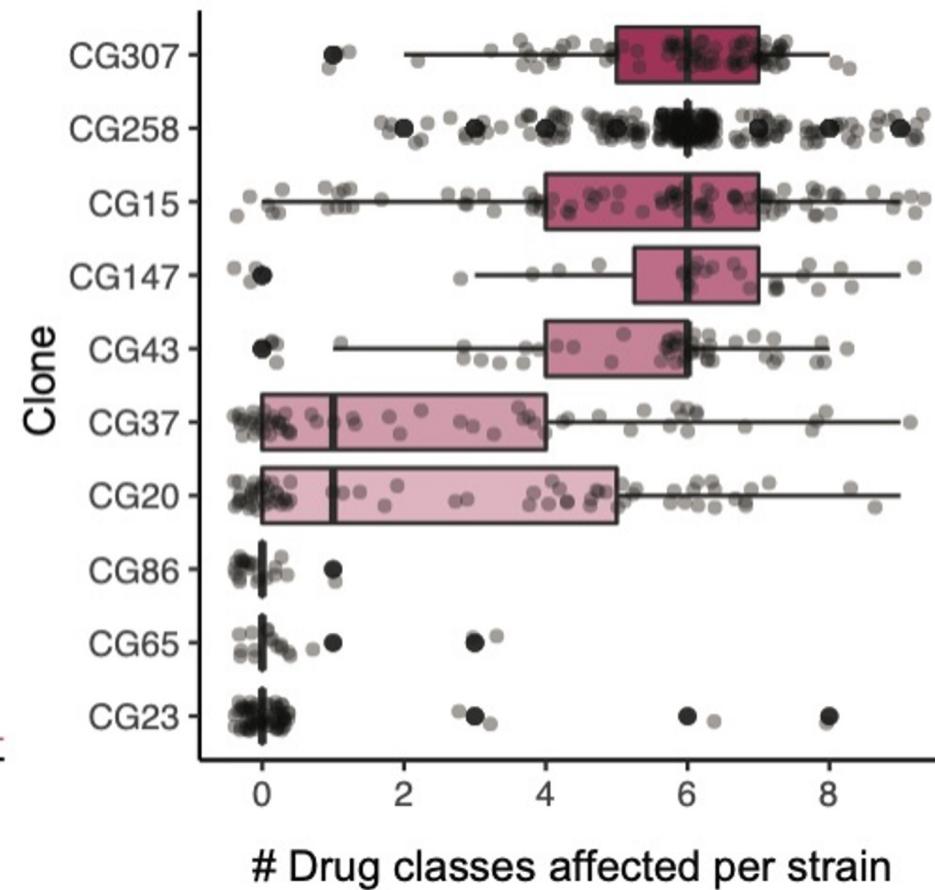


Acquired AMR genes

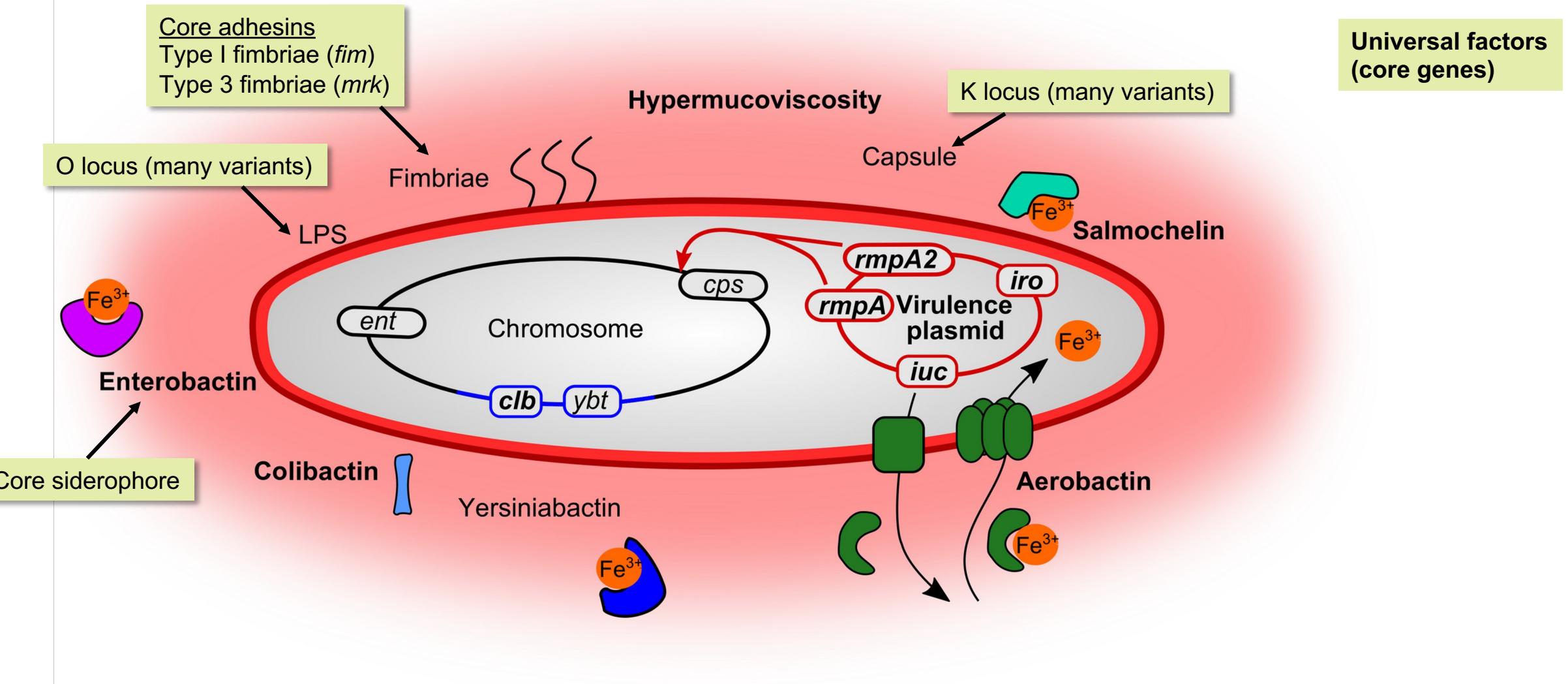
a Acquired AMR gene load per strain



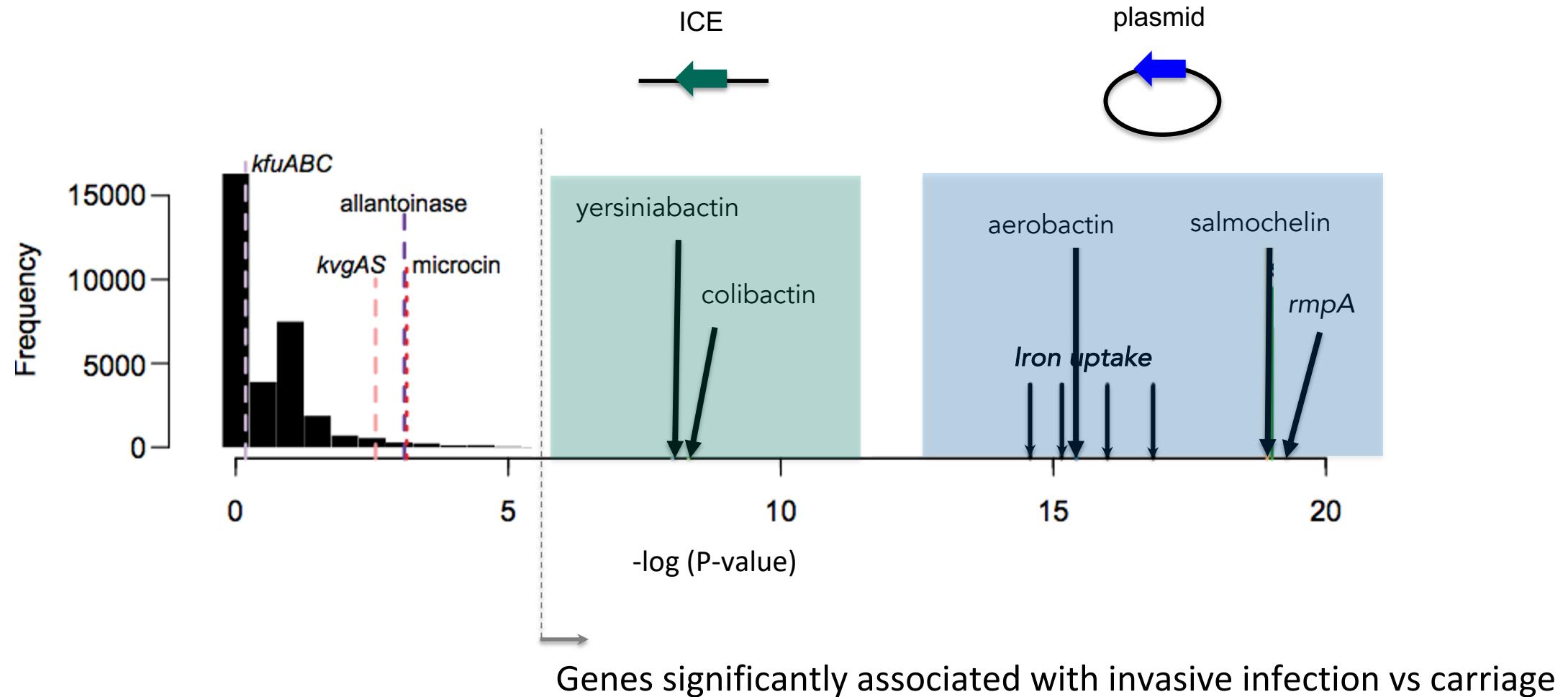
b Drug classes affected by acquired genes



Klebsiella pneumoniae pathogenicity



Accessory genes associated with invasive infections



Virulence factors (ICE *Kp*)

See upcoming lecture from Margaret Lam

Yersiniabactin (ybt)

- siderophore synthesis and receptor
- evades lipocalin-2 signalling, facilitating growth in tissue and immune evasion¹

Colibactin (clb or pks)

- polyketide synthase
- produces genotoxin, which damages host cells (linked to GI/bowel cancer²)
- promotes gut colonisation by killing microbiota³

Virulence factors (virulence plasmid)

Aerobactin (*iuc*) & salmochelin (*iro*) loci

- siderophore
- facilitate growth in host niches¹

See upcoming lecture from Margaret Lam

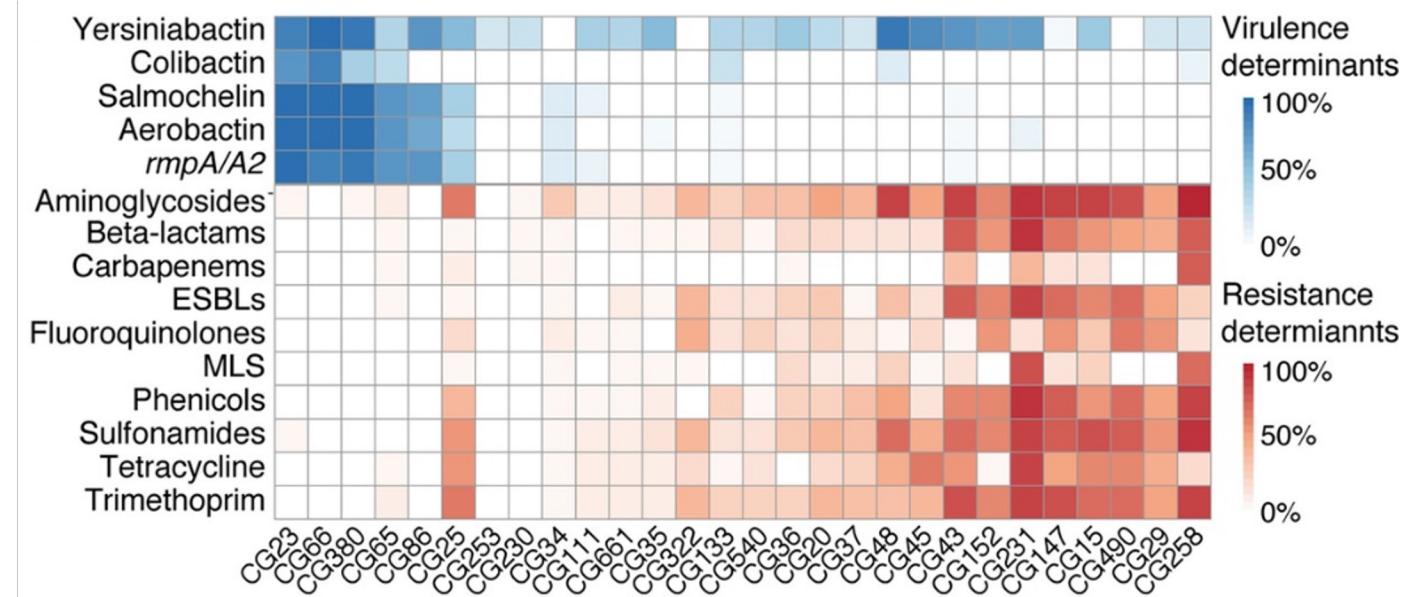
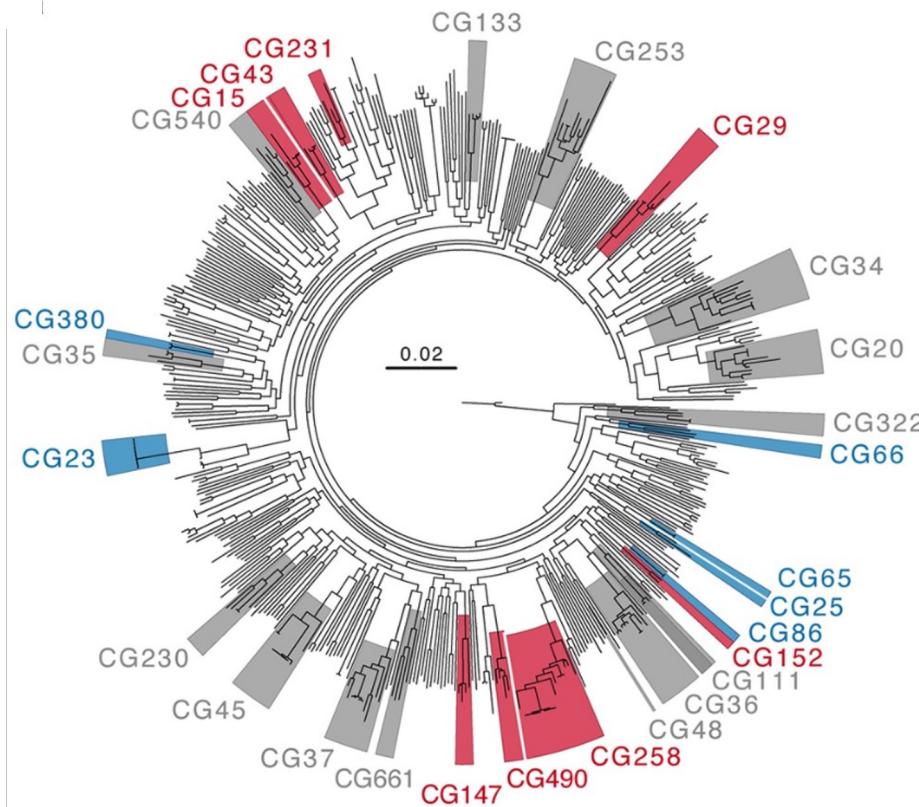
rmp locus (*rmpADC*)

- confers capsule over-production and hypermucoidy²
- increases serum resistance³

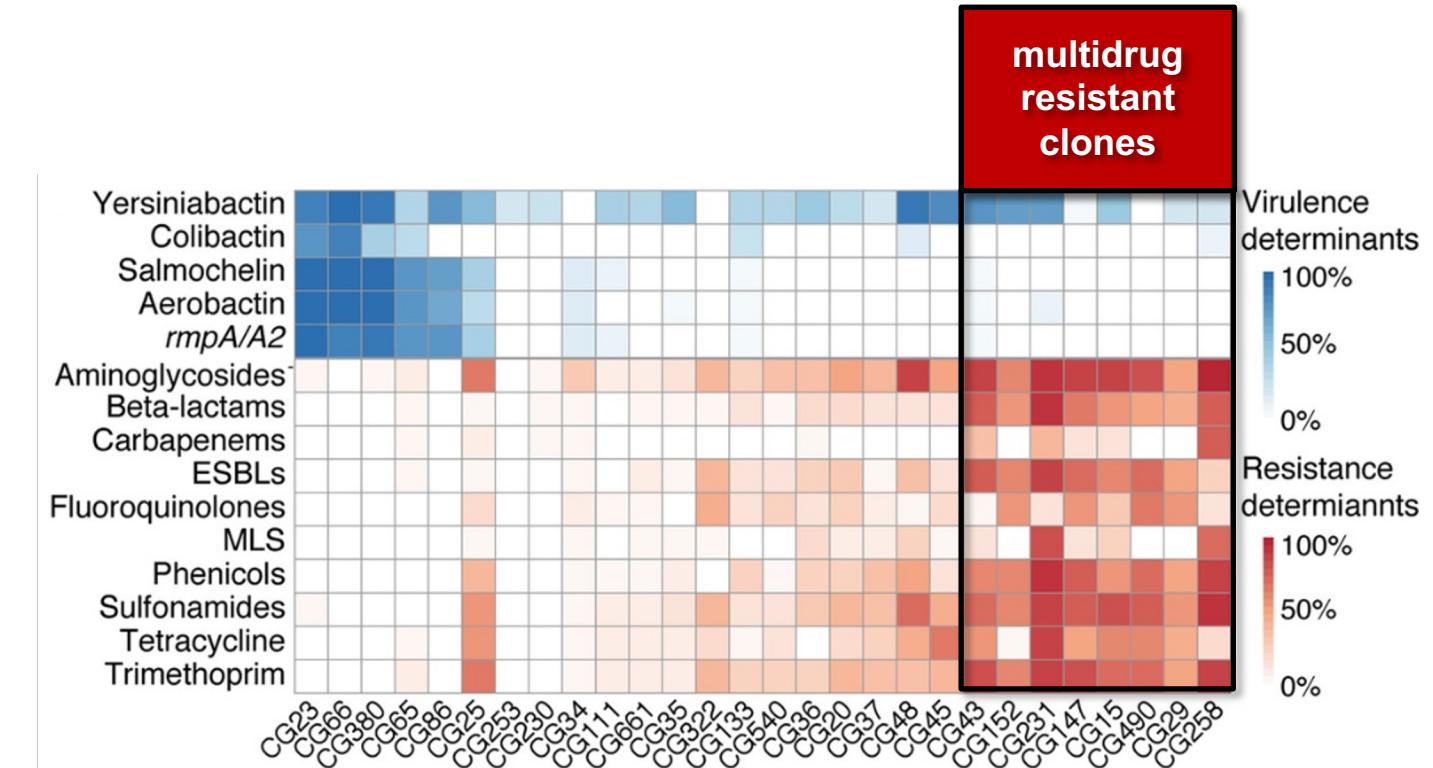
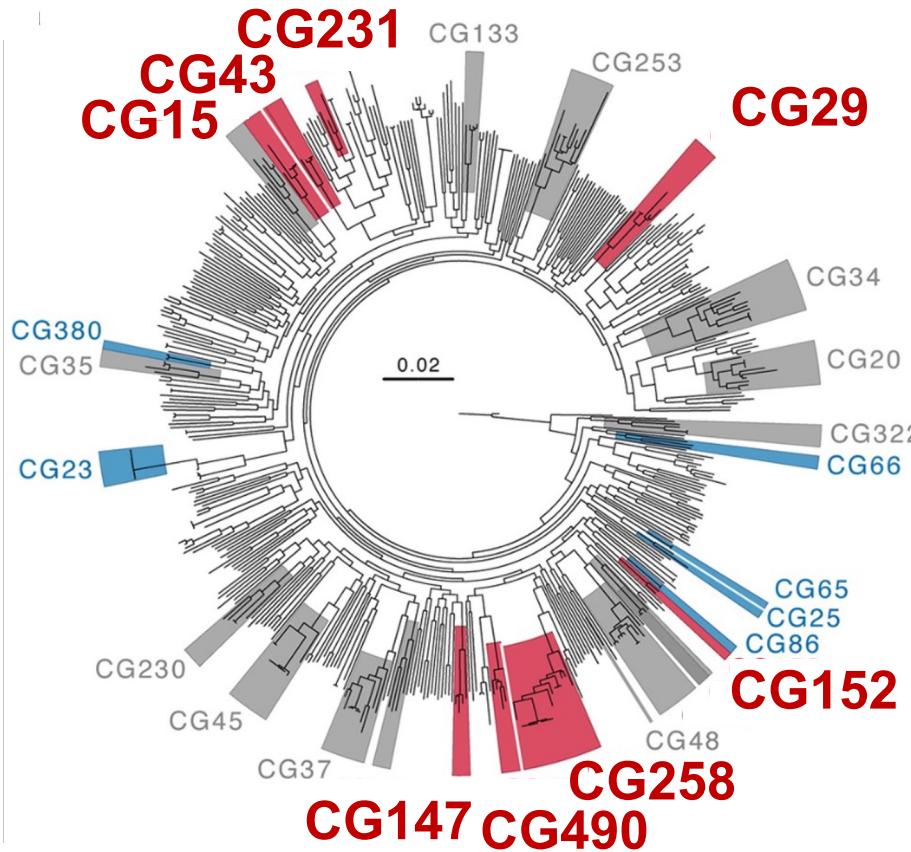
rmpA2 gene

- homolog of *rmpA* in *rmp* locus, but not clear if function is the same
- typically (~90%) disrupted, so not clear if functional

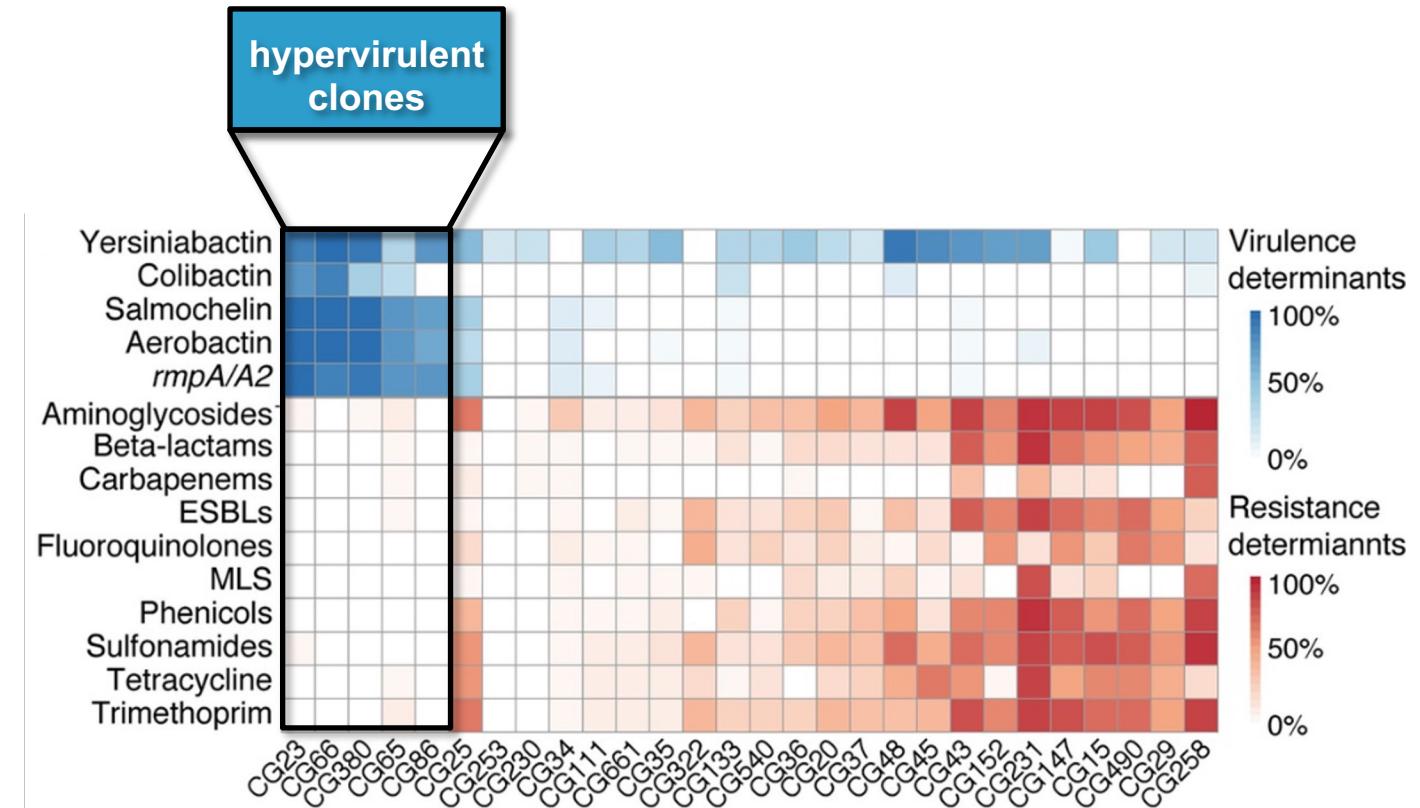
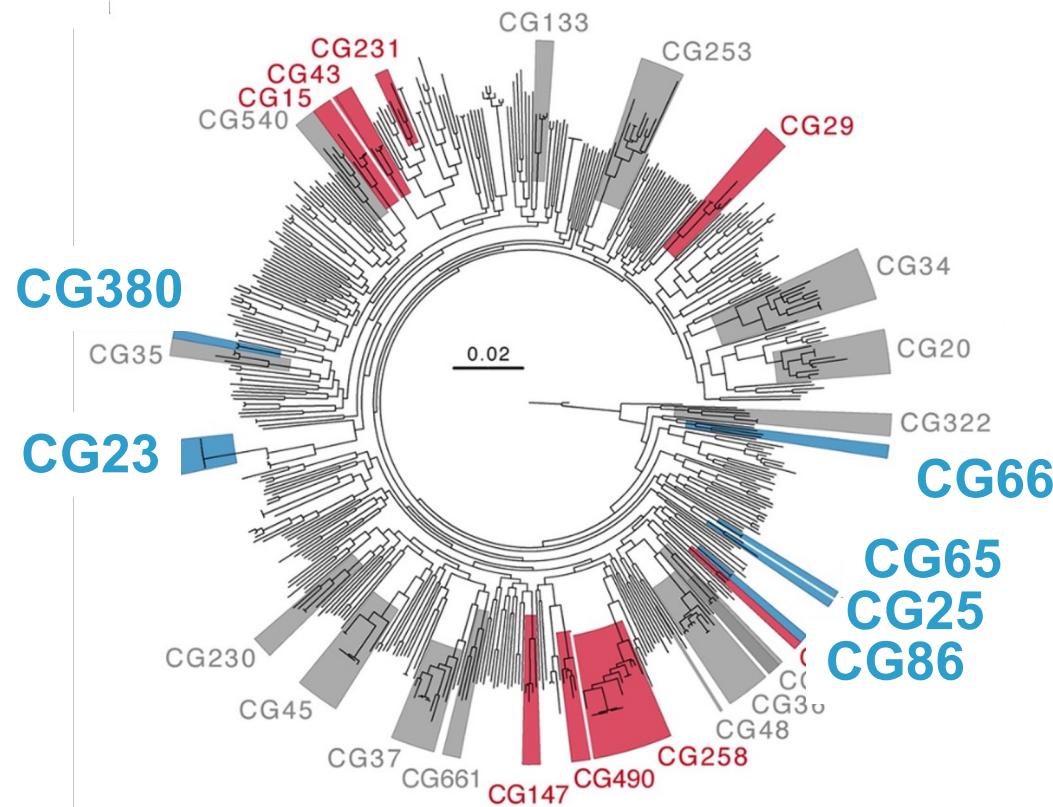
Distribution of AMR and virulence genes



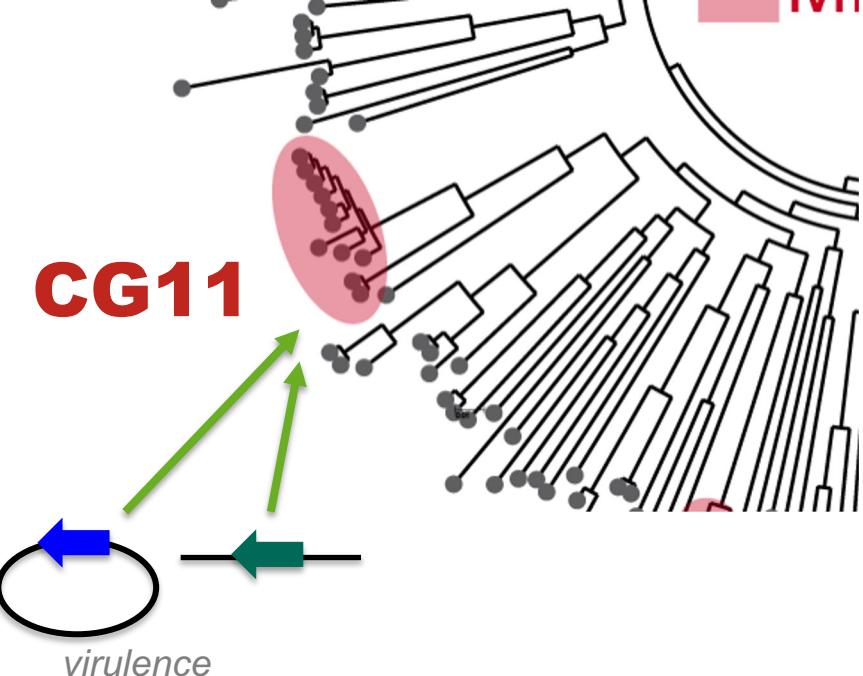
Distribution of AMR and virulence genes



Distribution of AMR and virulence genes



Convergence of AMR and virulence



THE LANCET
Infectious Diseases

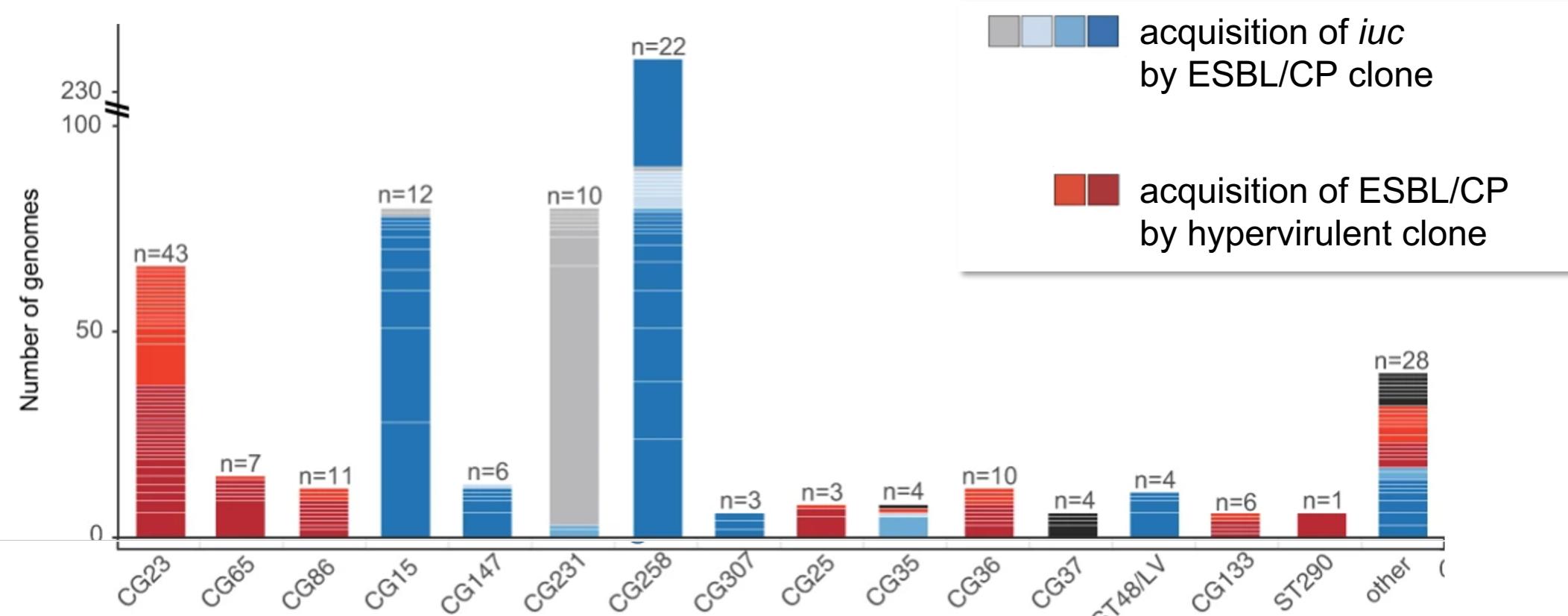
A fatal outbreak of ST11 carbapenem-resistant hypervirulent
Klebsiella pneumoniae in a Chinese hospital: a molecular
epidemiological study

Danxia Gu, MS[†], Ning Dong, MS[†], Zhiwei Zheng, BS, Di Lin, MS, Man Huang, MD, Lihua Wang, MS, Edward Wai-Chi Chan, PhD, Lingbin Shu, MS, Jiang Yu, MS, Dr Rong Zhang, PhD✉✉, Dr Sheng Chen, PhD✉✉

Convergence of AMR and virulence

See upcoming lecture from Margaret Lam

Detected quite frequently now, but clinical significance not clear



Convergence of AMR and hypervirulence?

See upcoming lecture from Margaret Lam



Disease Outbreak News

**Antimicrobial Resistance,
Hypervirulent Klebsiella
pneumoniae - Global
situation**

31 July 2024

THE LANCET
Microbe

**Call for prudent use of the term hypervirulence in
carbapenem-resistant *Klebsiella pneumoniae***

Yang, McNally & Zhong, 2025.

Convergence of AMR and hypervirulence?

Hypervirulent *Kp* infections are defined as

- tissue-invasive infections
- in otherwise healthy individuals from the community
- often involving infections in multiple body sites (metastasis)

Most typical presentation is pyogenic liver abscess, subsequently metastasising to the eye, lung, or central nervous system.

'Hypervirulent *Kp*' are strains associated with such infections, but there is no formal definition.

Molecular markers of hypervirulence?

Hypervirulent *Kp* infections associated with a few clones, which possess ALL of:

- Specific K types (mostly K1, K2)
- Capsule over-production and hypermucoidy due to *rmp*
- Virulence plasmid including *iuc*, *iro*, *rmp*
- ICEKp including *ybt* (sometimes also *c/b*)

Detection of **these clones (CG23, CG86, CG25, CG66, CG65, CG380)** is a strong indicator of hypervirulence, especially if you can confirm all or most of the markers are present.

As these factors are mobile, they appear in various combinations in other lineages...

- ❑ Which marker combinations indicate 'hypervirulent'?
- ❑ What clinical risk is associated with each marker, or combination?

Virulence markers associated with hypervirulence

[

Gene	Marker of	Genetic context	Classifier accuracy, human cohorts	Odds ratio (95% CI), human cohorts	Hazard ratio (95% CI), mouse model
<i>peg-344</i>	putative transporter	VP	0.97	1,428.0 (163.4, 12,483.1)	51.7 (22.7, 118.1)
<i>iroB</i>	salmochelin	VP	0.97	892.3 (159.1, 5002.6)	59.2 (25.8, 135.5)
<i>iucA</i>	aerobactin	VP	0.96	464.7 (107.6, 2,007.2)	31.6 (15.8, 63.6)
<i>rmpA</i>	hypermucoidy (rmp)	VP	0.96	581.0 (114.0, 2,961.8)	41.0 (19.1, 88.2)
<i>rmpA2</i>	?hypermucoidy (rmp2)	VP	0.95	381.8 (92.4, 1,578.1)	31.2 (16.0, 60.7)
<i>terB</i>	tellurite resistance	VP	0.89	69.0 (26.3, 180.7)	14.3 (8.3, 24.8)
<i>irp2</i>	yersiniabactin	ICEKp	0.79	13.9 (6.7, 28.7)	7.8 (4.7, 12.9)
string test	hypermucoidy	-	0.90	86.6 (31.8, 235.8)	15.5 (8.8, 27.2)

Comparing markers in isolates from two patient cohorts:

- 1) Hypervirulent infection: healthy, ambulatory patient with a clinical syndrome of tissue-invasive infection (e.g., hepatic and extrahepatic abscesses, necrotizing fasciitis, or endophthalmitis)
[n=85, from USA and Taiwan]
- 2) Classical infection: randomly chosen, deidentified blood isolates
[n=90, from USA, Canada, UK]

- All these markers were associated with hypervirulent infections
- 5 markers (*peg-344*, *iro*, *iuc*, *rmpA*, *rmpA2*) each with classification accuracy $\geq 95\%$
- ...but these are all in strong genetic linkage on the virulence plasmid

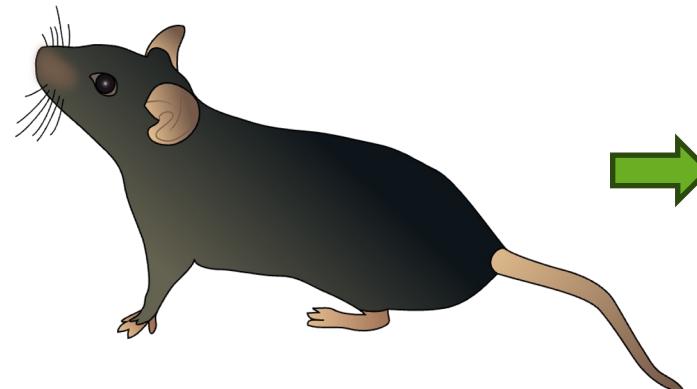
Which markers are essential for hypervirulence?

Isogenic mutants in 4
hypervirulent clinical isolates



ST23 / K1
ST86 / K2
ST1544 / K20
ST29 / K54

Subcutaneous challenge
infection in mice



Hypervirulence lost if we delete:
virulence plasmid
rmpA
iucA (2/4 strains only)

Hypervirulence retained if we delete:
X *irp2* (yersiniabactin)
X *clb* (colibactin)
X *rmpA2* (truncated)

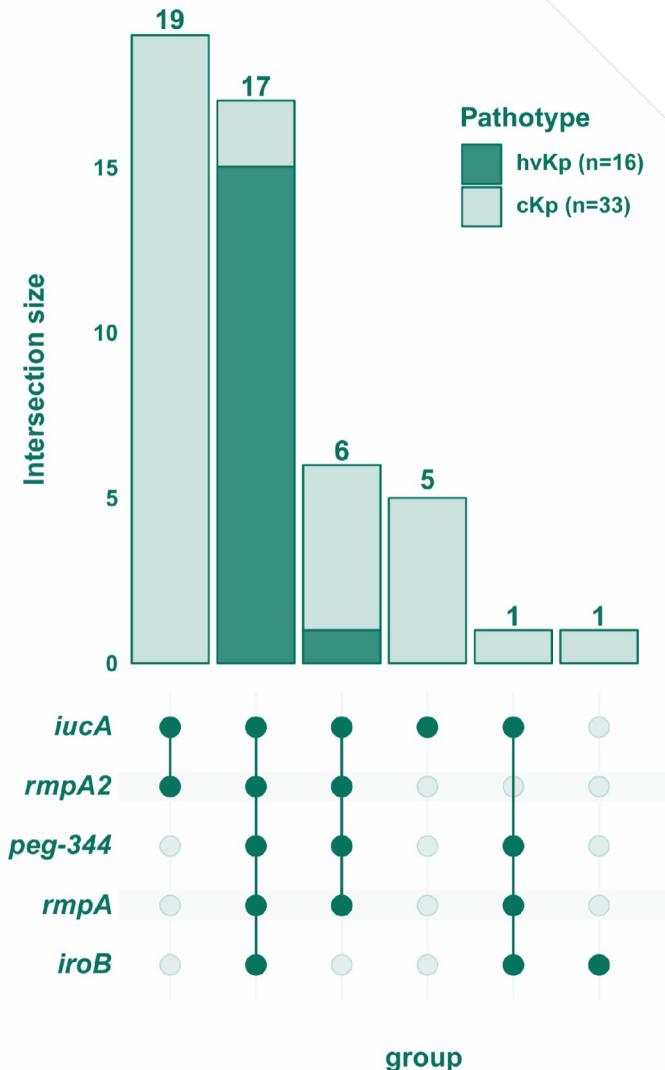
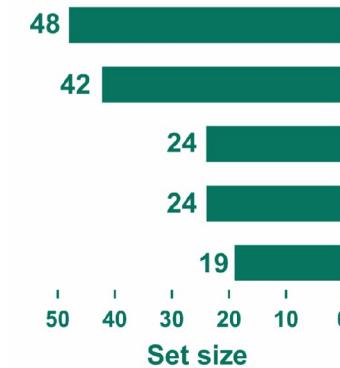
Marker combinations associated with hypervirulence

45 AMR clinical isolates with different combinations of 5 markers:

- *peg-344*
- *iroB*
- *iucA*
- *rmpA*
- *rmpA2*

Subcutaneous infection in mice

? classify as hypervirulent / not



All 5 markers (n=17):
hypervirulent

X *iucA/rmpA2* alone (n=19):
not hypervirulent

? other combinations:
insufficient samples

How to interpret virulence markers?

Best indication of hypervirulence (including community onset and metastasis) is intact copies of loci:

- *rmp* AND *iuc* AND *iro*

***rmp* without *iuc/iro*? – not hypervirulent**

- Probably hypermucoid & hyperencapsulated, leading to enhanced serum resistance
- Not clear the effect on outcome, but unlikely to metastasise without siderophores

***iuc* or *iro* without *rmp*? (including *iuc/rmpA2* without *rmp*) – not hypervirulent**

- Reasonable to expect enhanced colonization potential and increased virulence, but not clear the overall effect on outcome

***ybt* alone? – not hypervirulent**

- Enhanced likelihood of healthcare associated pneumonia (and subsequent sepsis), but not hypervirulent (e.g. do not expect community acquired infection, metastasis, etc)

How to understand convergence?

In the absence of a formal definition for hypervirulence, propose to define convergence of hypervirulence and resistance as:

- Presence of a complete virulence plasmid with intact *iuc*, *rmp*, and *iro* in a drug resistant strain

Presence of partial virulence plasmid with *iuc+rmpA2* in AMR clones is common, but these are likely not hypervirulent.

More clinical research is needed to understand whether such strains are associated with:

- increased clinical risk? (e.g. disease severity, metastasis, mortality)
- increased dissemination risk? (e.g. increased colonization efficiency, or transmission efficiency/ R_0 in different patient groups)

How to understand convergence?



RAPID RISK ASSESSMENT

Emergence of hypervirulent *Klebsiella pneumoniae* ST23 carrying carbapenemase genes in EU/EEA countries

17 March 2021



RAPID COMMUNICATION

Cross-border spread of a mosaic resistance (OXA-48) and virulence (aerobactin) plasmid in *Klebsiella pneumoniae*: a European Antimicrobial Resistance Genes Surveillance Network investigation, Europe, February 2019 to October 2024

Convergence of AMR and hypervirulence

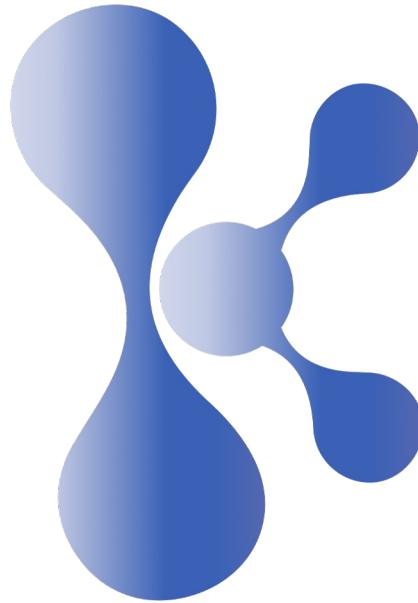
- Known hypervirulent clone, with *iuc* + *iro* + *rmp*
- Clear link to clinical syndrome and confirmed in animal models

RISK: Severe invasive infection with limited treatment options

Convergence of AMR and virulence

- Presence of *iuc* but without *rmp*
- Only 10% blood isolates, majority isolated from UTI or screening

RISK: Clinical risk vs OXA-48+ *iuc*- strains not known, but dissemination of combined plasmid concerning



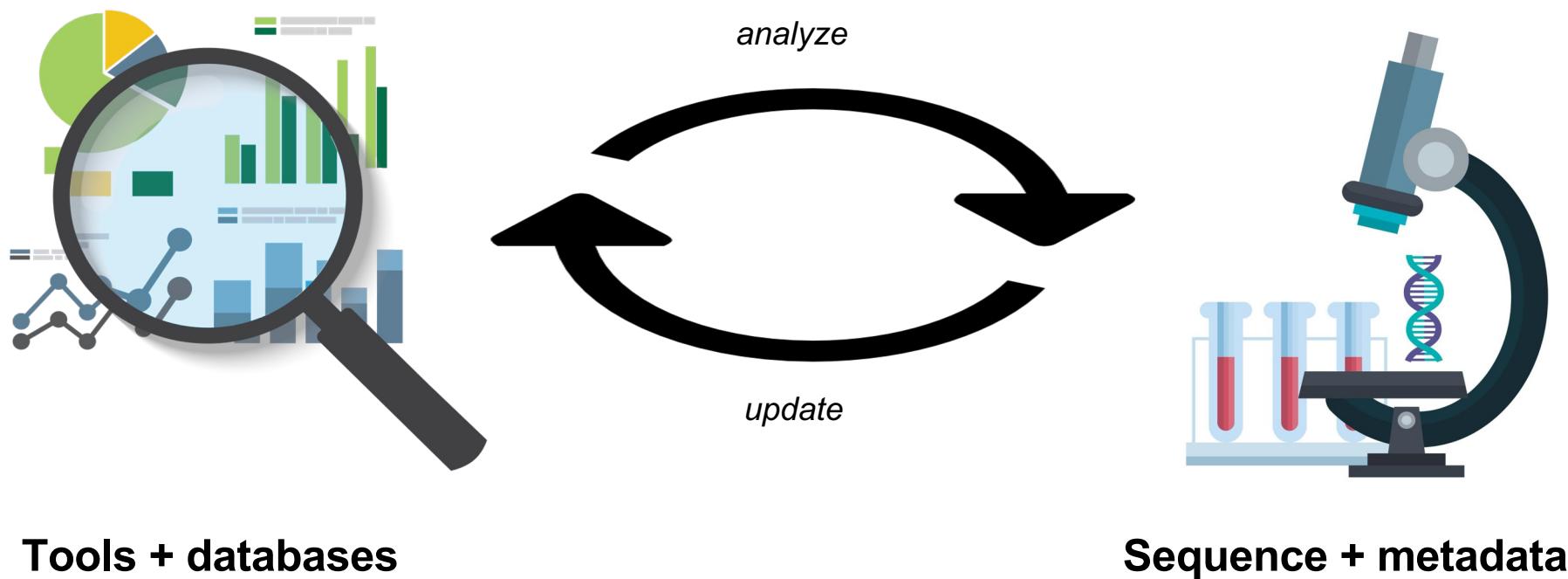
KlebNET

Klebsiella pneumoniae
Genomic Surveillance Platform

klebnet.org



Data sharing is essential to support the digital tools we all need for *Klebsiella* genomics



KlebNET AMR Geno-Pheno Consortium

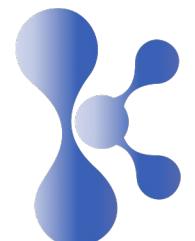
Collating matched genotype and phenotype data from the *Kp* species complex isolated from diverse sources to test and improve tools for prediction of AMR from genomic data, including Kleborate and AMRrules.

- Partners include **ESGEM-AMR**, **CARD**, **NCBI Pathogens**, and **BLDB**.

KlebNET-GSP Epidemiology Consortium

Collating publicly available *Kp* species complex whole genome sequences with matched isolate source and sampling information, to support:

- **KlebNET Clone Reviews** – collaborative genomic epidemiology reviews of globally distributed clones (e.g multi-drug resistant or hypervirulent clones);
- **KlebNET Clone Risk Framework** – a systematic risk framework to support global genomic surveillance of *Kp*;
- **KlebNET Metadata Repository** – a comprehensive open-access repository of enhanced contextual meta-data, facilitating use and reuse of publicly available data by the global research community by enabling robust epidemiology and genomic meta-analyses.



klebnet.org

Other KlebNET resources

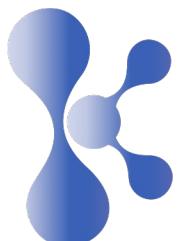
Lab protocols for isolating, identifying and sequencing *Klebsiella*

Curated genome data collections

- Neonatal sepsis isolates
- Bacterial strains available in public reference collections

Data standards

- Metadata template
- QC standards for *K. pneumoniae* genome assemblies



klebnet.org

In summary

List of learning points in this session:

- *Kp* population structured into lineages, characterised by differences in gene content drawn from large pan-genome
- Key genomic features relevant to clinical & public health are K/O loci, AMR variants, virulence markers
- Hypervirulent *Kp* lacks a formal definition, but seems to require both acquired siderophores (especially *iuc*) and hypermucoidy (*rmp*)
- Typing tools for all these loci are available from KlebNET, which also provides resources on QC, metadata, curated genome collections, and collaborative consortia to support further development of public health genomics tools

Further reading

Specific further reading for this session

Population genomics of *Klebsiella pneumoniae*

Wyres, Lam & Holt, *Nature Reviews Microbiology*, 2020

PMID: 32055025

References

- Holt et al, PNAS 2015. PMID: 26100894.
- Wyres et al, PLoS Genetics 2019. PMID: 30986243.
- Wyres & Holt, Trends Micro 2016. PMID: 27742466.
- Vezina et al, eLife 2023. PMID: 37815531.
- Vezina et al, BioRxiv 2024. DOI: 10.1101/2024.07.24.605038.
- Wyres et al, 2016 Microbial Genomics. PMID: 28348840.
- Wick et al, 2018 J Clin Microbiol. PMID: 29618504.
- Gorrie et al, Nature Comms 2022. PMID: 35641522.
- Tsang et al, Microbial Genomics 2024. PMID: 39432416.
- Bachman et al, mBio 2012. PMID: 23169997.
- Kaur et al, Microorganisms 2023. PMID: 36838407.
- Tan et al, ISME Journal 2024. PMID: 38547398.
- Lim et al, eBioMedicine 2025. PMID: 40184910.
- Lam et al, Genome Medicine 2025. PMID: 40205597.
- Xu et al, Virulence 2021. PMID: 34339346.
- Gu et al, Lancet Infect Dis 2018. PMID: 28864030.
- Lam et al, Nature Comms 2021. PMID: 34234121.
- Yang, McNally & Zhong, Lancet Microbe 2025. PMID: 39993405.
- Choby et al, J Intern Med 2019. PMID: 31677303.
- Russo et al, J Clin Microbiol 2018. PMID: 29925642.
- Russo et al, eBioMedicine 2024. PMID: 39178743.
- Russo et al, mBio 2024. PMID: 38231533.
- Linkevicius et al, Eurosurveillance 2025. PMID: 40642770.

Acknowledgements

**Original content created by Professor Kathryn Holt at the London School of Hygiene and Tropical Medicine, United Kingdom.
Formatting by Shaojie Bao, London School of Hygiene and Tropical Medicine, United Kingdom.
Used as training material for KlebNet.org.**