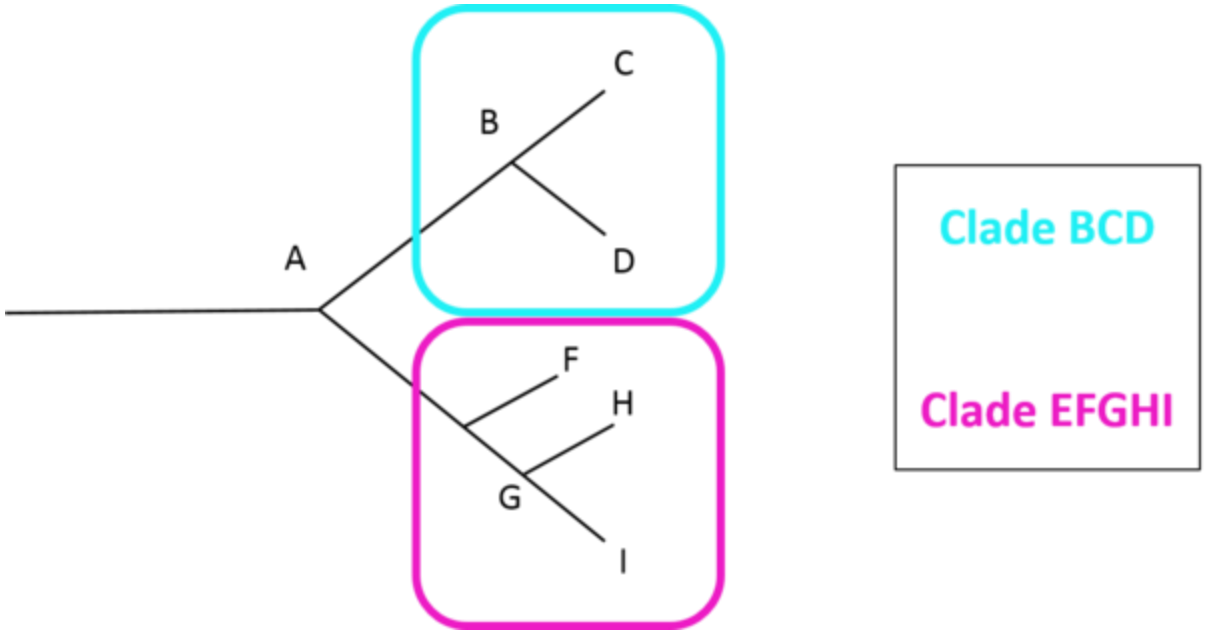
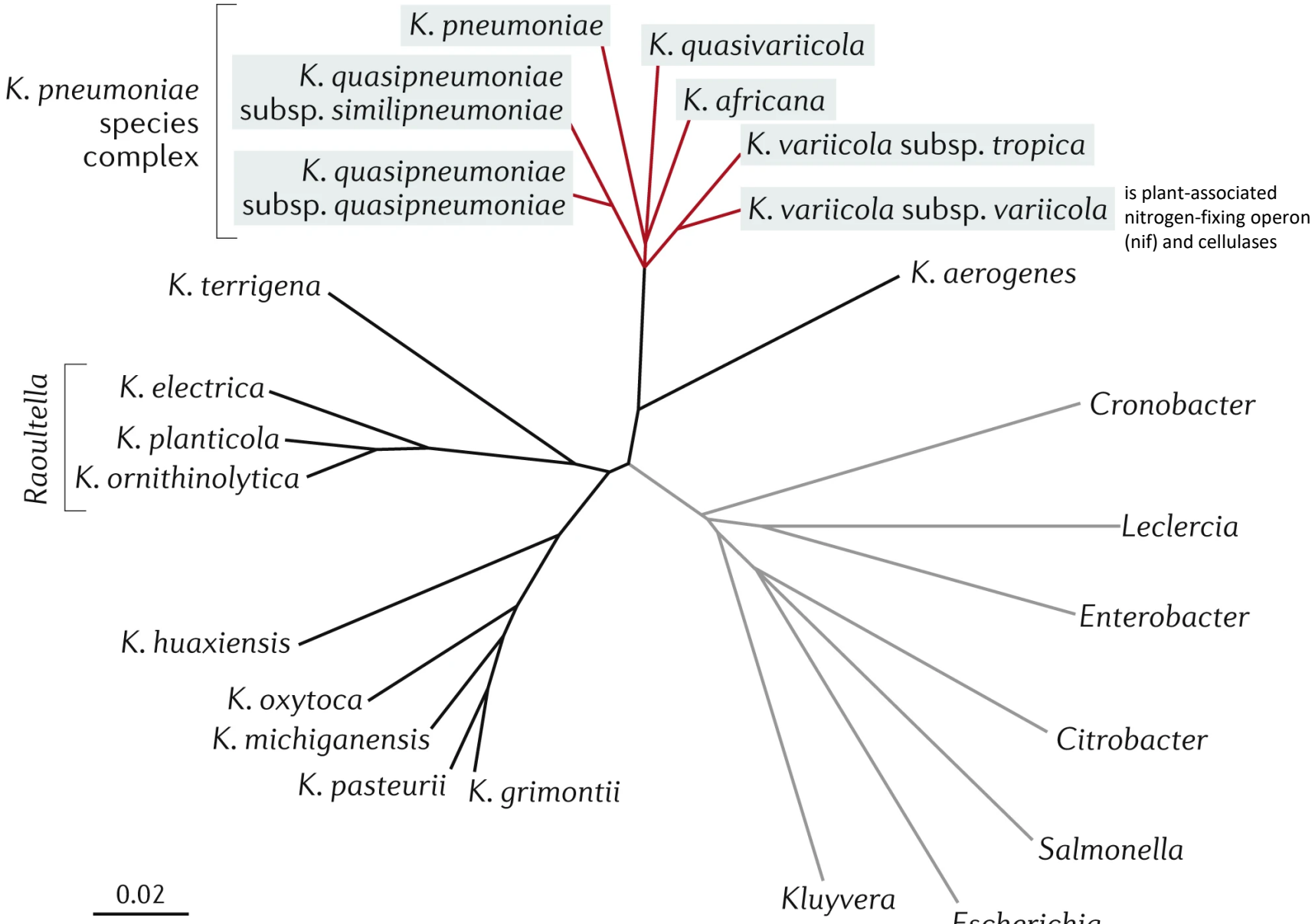
# Molecular phylogenesis of bacteria






**Wyres, K.L., Lam, M.M.C. & Holt, K.E. Population genomics of *Klebsiella pneumoniae*. *Nat Rev Microbiol* 18, 344–359 (2020)**

- *Enterobacteriaceae*

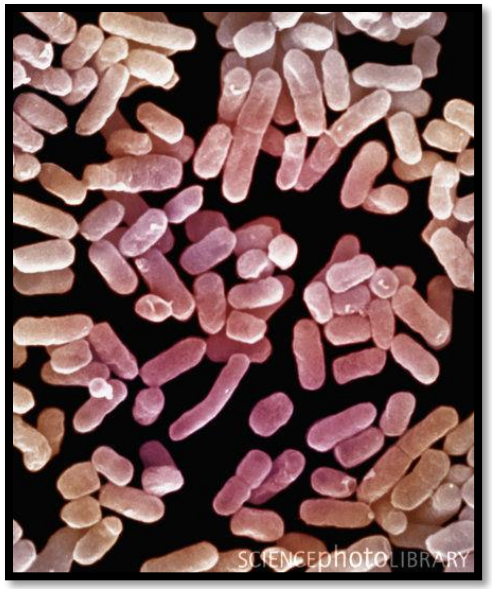
# *Klebsiella pneumoniae*

**2016 ASM Agar Art Contest**

Cherry tree and flamingo:

Klebsiella pneumoniae (pink), Citrobacter freundii (magenta to black), Salmonella Typhimurium (black), Morganella morganii (brown)
Salmonella-Shigella agar

# *Klebsiella pneumoniae*




- Described in 1882 by Carl Friedländer
- *Enterobacterales*
- Gram-negative
- Facoltative aerobic
- capsula

# *Klebsiella pneumoniae*

SURVEILLANCE REPORT

## Antimicrobial resistance in the EU/EEA (EARS-Net)

Annual Epidemiological Report for 2019

### Key facts

- Thirty European Union (EU) or European Economic Area (EEA) countries reported data for 2019 to the European Antimicrobial Resistance Surveillance Network (EARS-Net). Twenty-nine countries reported data for all eight bacterial species under surveillance by EARS-Net (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* species, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*), while one country reported data for all bacterial species except *S. pneumoniae*.
- EARS-Net data for 2019 displayed wide variations in the occurrence of antimicrobial resistance (AMR) across the EU/EEA depending on the bacterial species, antimicrobial group and geographical region.
- The most commonly reported bacterial species was *E. coli* (44.2%), followed by *S. aureus* (20.6%), *K. pneumoniae* (11.3%), *E. faecalis* (6.8%), *P. aeruginosa* (5.6%), *S. pneumoniae* (5.3%), *E. faecium* (4.5%) and *Acinetobacter* species (1.7%).
- In 2019, more than half of the *E. coli* isolates reported to EARS-Net and more than a third of the *K. pneumoniae* isolates were resistant to at least one antimicrobial group under surveillance, and combined resistance to several antimicrobial groups was frequent. Resistance percentages were generally higher in *K. pneumoniae* than in *E. coli*. While carbapenem resistance remained rare in *E. coli*, several countries reported carbapenem resistance percentages above 10% in *K. pneumoniae*. Carbapenem resistance was also common in *P. aeruginosa* and *Acinetobacter* species, and at higher percentages than in *K. pneumoniae*. For most gram-negative bacterial species–antimicrobial group combinations, changes in resistance percentages between 2015 and 2019 were moderate, and resistance remained at previously reported high levels.
- For *S. aureus*, the decline in the percentage of meticillin-resistant (i.e. MRSA) isolates reported in previous years continued in 2019. Nevertheless, MRSA remains an important pathogen in the EU/EEA, with levels still high in several countries, and combined resistance to another antimicrobial group was common. Decreases during the same period were also noted for penicillin non-wild type and macrolide resistance in *S. pneumoniae*.
- One development of particular concern was the increase in the percentage of vancomycin-resistant isolates of *E. faecium* in the EU/EEA, from 10.5% in 2015 to 18.3% in 2019 (EU/EEA population-weighted mean percentage).
- For several bacterial species–antimicrobial group combinations, a north-to-south and west-to-east gradient was evident in the EU/EEA. In general, lower percentages of resistance were reported by countries in the north of Europe and higher percentages were reported by countries in the south and east of Europe. However, for vancomycin-resistant *E. faecium*, no distinct geographical pattern was evident.

Suggested citation: European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2019. Stockholm: ECDC; 2020.

Stockholm, November 2020

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In Europe

>90,000 infections

>7,000 deaths annually

25% of the total disability-adjusted life years lost to multidrug-resistant (MDR) bacterial infections.

11.3% bloodstream infections

In 2019, more than a third of the *K. pneumoniae* isolates were resistant to at least one antimicrobial group under surveillance, and combined resistance to several antimicrobial groups was frequent.

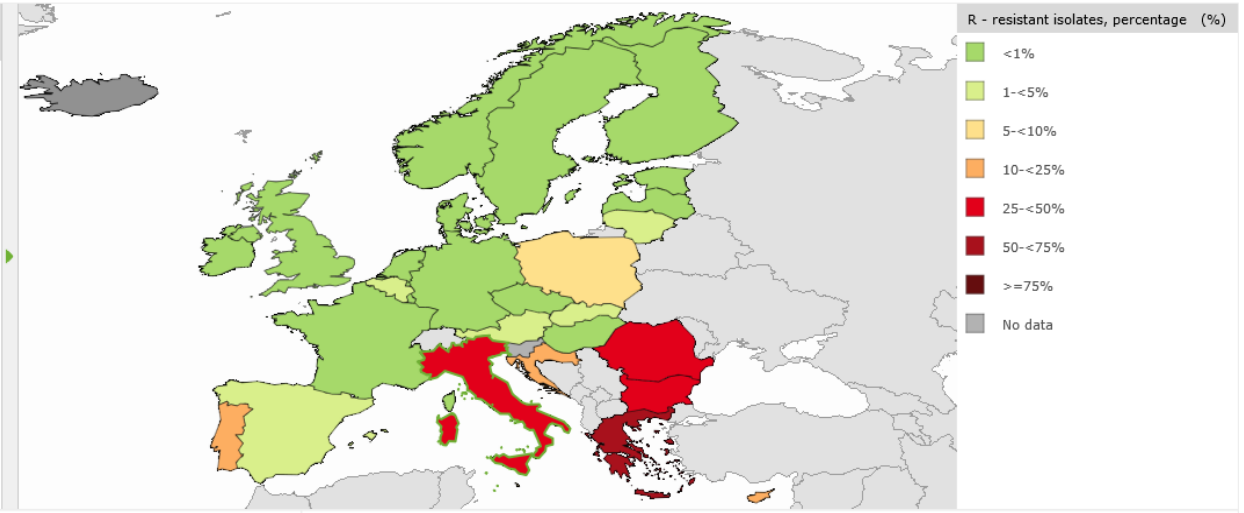
Antimicrobial resistance in the EU/EEA –AER 2019

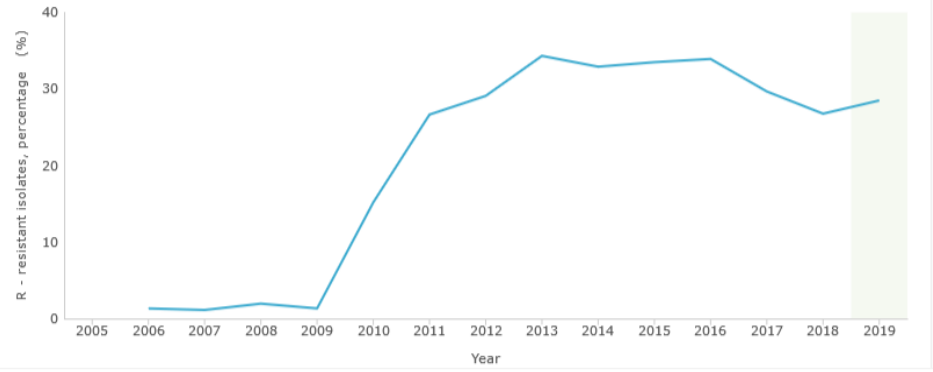
# 28.5% isolated from invasive carbapenem-resistant infections

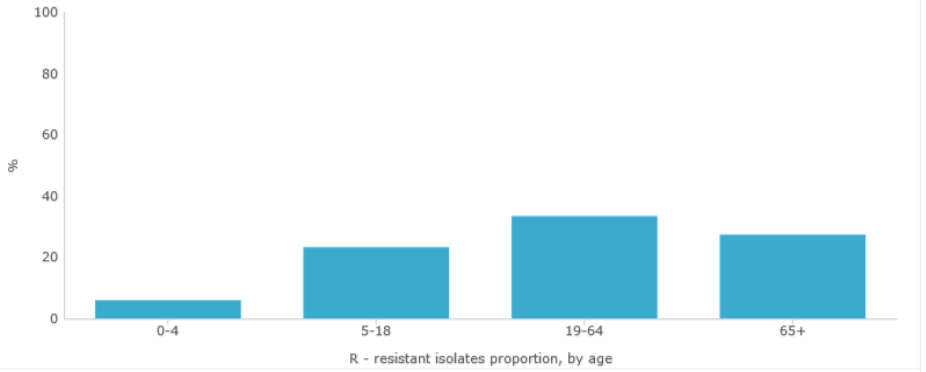
## Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Klebsiella pneumoniae | Carbapenems | R - resistant isolates, percentage | 2019

| Region | R - resistant isolates, percentage (%) |
|---|---|
| Austria | 1.2 |
| Belgium | 1.1 |
| Bulgaria | 27.0 |
| Croatia | 12.0 |
| Cyprus | 13.3 |
| Czechia | 0.6 |
| Denmark | 0.3 |
| Estonia | 0.0 |
| Finland | 0.4 |
| France | 1.0 |
| Germany | 0.9 |
| Greece | 58.3 |
| Hungary | 0.9 |
| Iceland | - |
| Ireland | 0.9 |
| Italy | 28.5 |



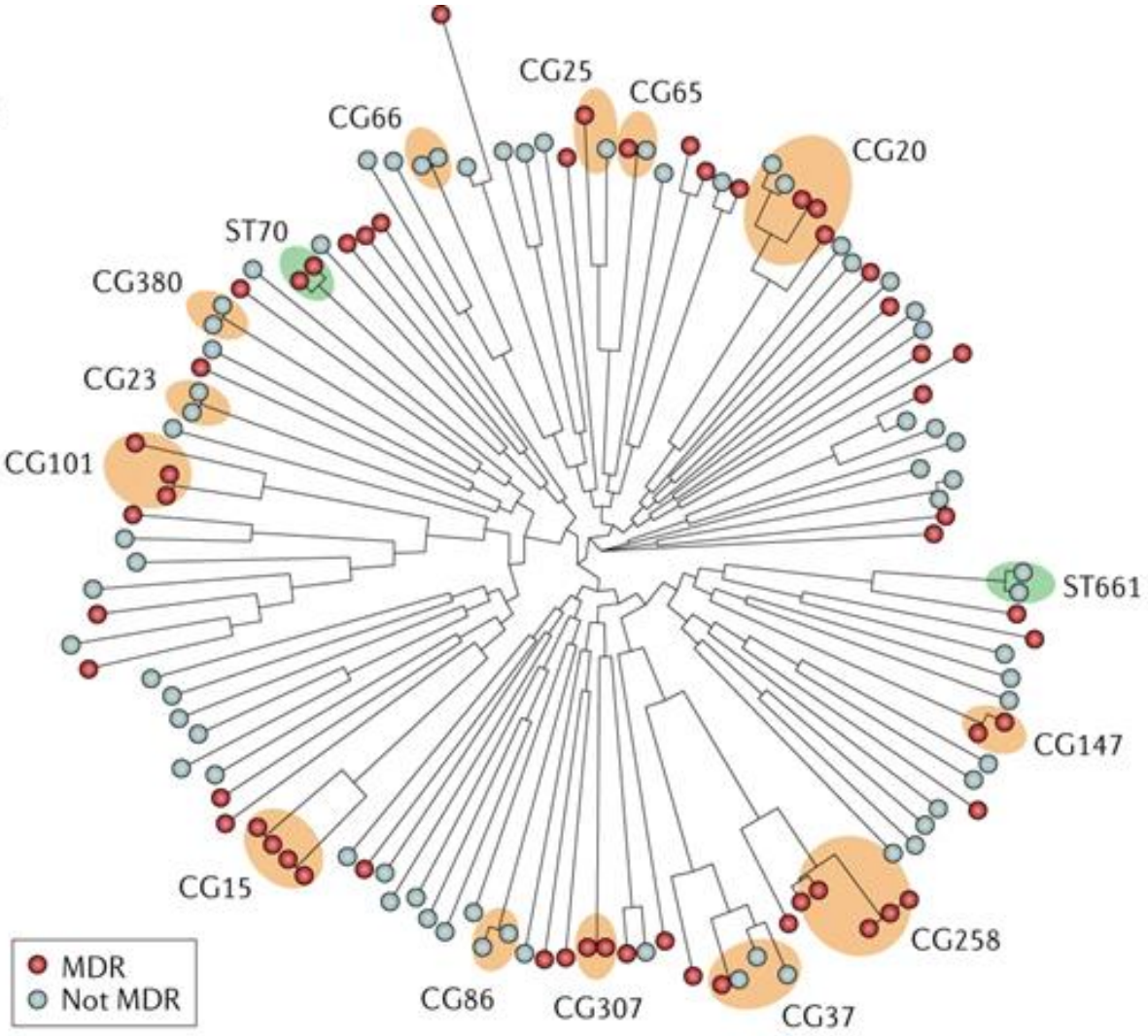

Italy

# *Klebsiella pneumoniae* genome

- Typical *K. pneumoniae* genomes are ~5-6 Mbp in size, encoding ~5,000-6,000 genes. About 1,700 genes are conserved in all members of the species (core genes), while the rest are variously present (accessory genes). The total pan-genome (the sum of all core and accessory genes) is extremely diverse

Phylogenetic analyses based on 1,000-2,000 genes show lineages that differ from each other by a nucleotide divergence of less than 0.5%.

These lineages are referred to as clonal groups (CG), and typically referred to as clones.




**Wyres, K.L., Lam, M.M.C. & Holt, K.E. Population genomics of *Klebsiella pneumoniae*. *Nat Rev Microbiol* 18, 344–359 (2020)**

# 1- Classic *K. pneumoniae (cKp)*

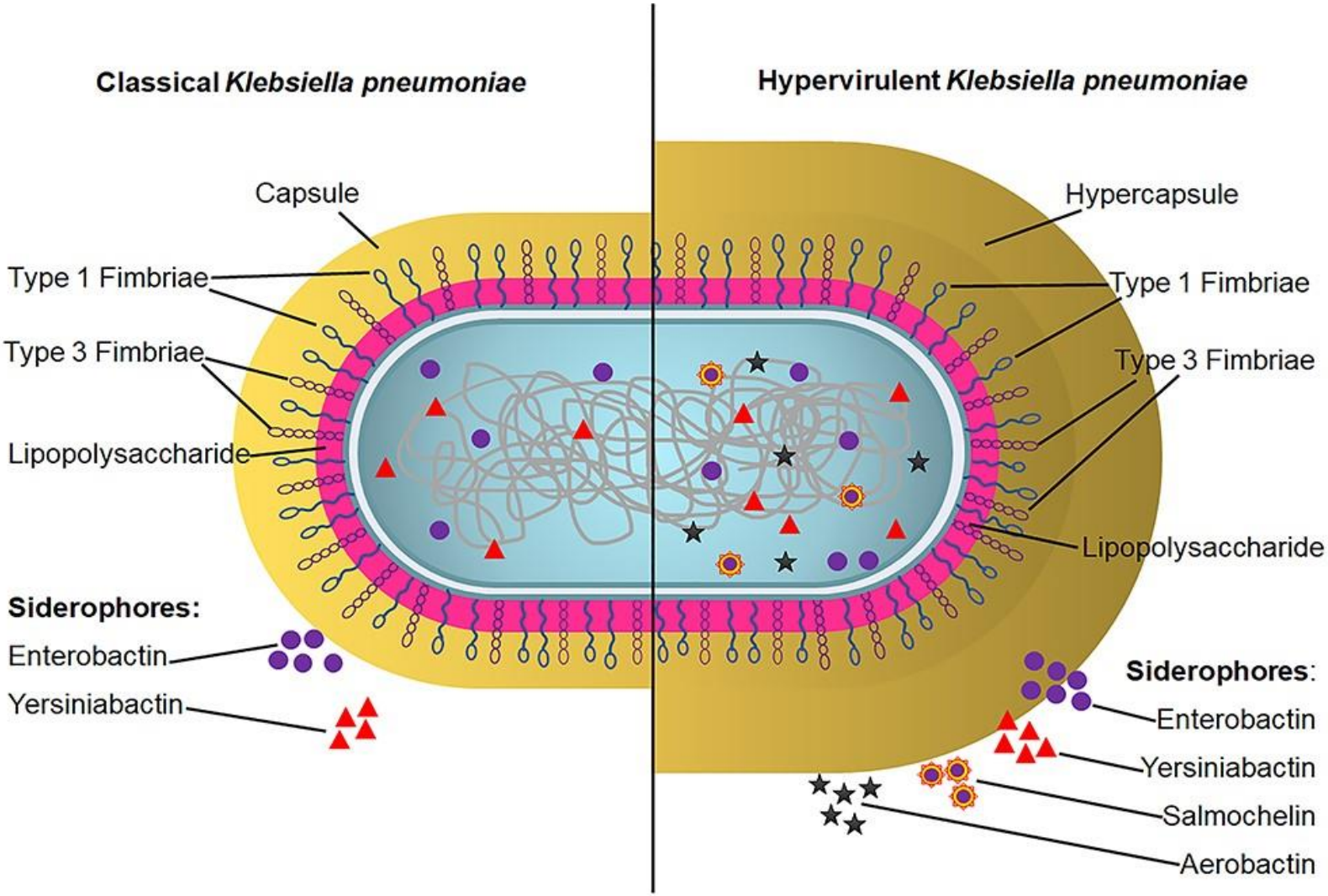
- cKp strains are typically associated with nosocomial infections or urinary and lung infections in patients who are subject to long periods of hospitalization, suggesting that some degree of immune deficiency is necessary to cause a disease
- Resistance to cKp carbapenems (CR-Kp) is considered a serious virulence factor as there are few treatment options for treatment

# 2- Hypervirulent *K. pneumoniae* (hvKp)

- Initially recognized in Asia, hvKp has emerged as the leading cause of pyogenic abscesses to the liver
- hvKp is distinguished from cKp by its ability to metastasize to distant sites, including the eyes, lungs, and central nervous system
- hvKp is also implicated in extra-hepatic infections, including septicemic bacteremia, pneumonia and soft tissue infections
- HvKp strains demonstrate hypermucoviscosity. A phenotypic feature that has become a rapid diagnostic test for the recognition of hypervirulent strains

# String test

Important virulence determinants in classical and hypervirulent K. pneumoniae strains responsible for establishing infection and successful survival inside the host.




*Brief Funct Genomics*, Volume 21, Issue 2, March 2022, Pages 63–77, https://doi.org/10.1093/bfgp/elab038

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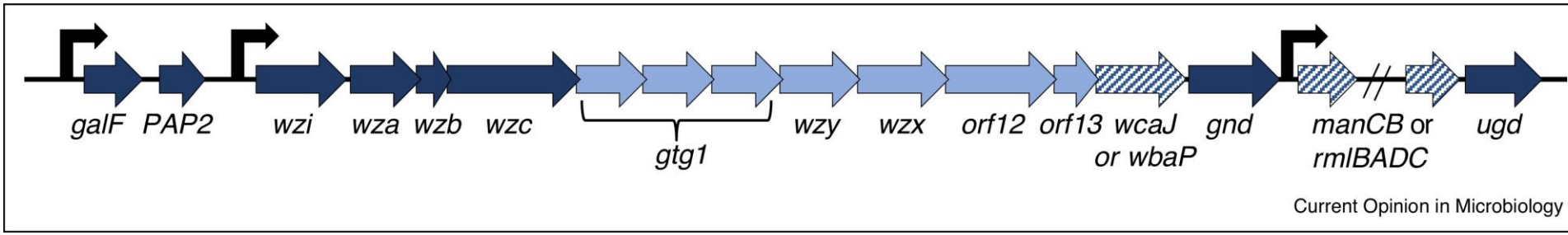
The variation in the oligosaccharide repeats underlies the LPS diversification structurally and functionally.
Based on the composition of the sugar molecules, as many as nine O antigens of *K. pneumoniae* have been identified.
In an example of two of the nine reported O antigens (O1 and O2), each comprises galactans homopolymer, which is D-galactan I polysaccharides. But the O1 antigens differ from the O2 antigens in that they have a D-galactan II cap structure and the capping of O antigens affects the pathogenicity of the pathogen [43]. *Klebsiella pneumoniae* ST258 strains were reported to predominantly express a diverse form of D-galactan I, known as D-galactan III, which confers the bacteria's enhanced survival in host serum

# Capsular biosynthesis cluster




All strains of K. pneumoniae produce an extracellular polysaccharide capsule that represents a virulence factor and more than 130 different types of capsules have been identified in Klebsiella.

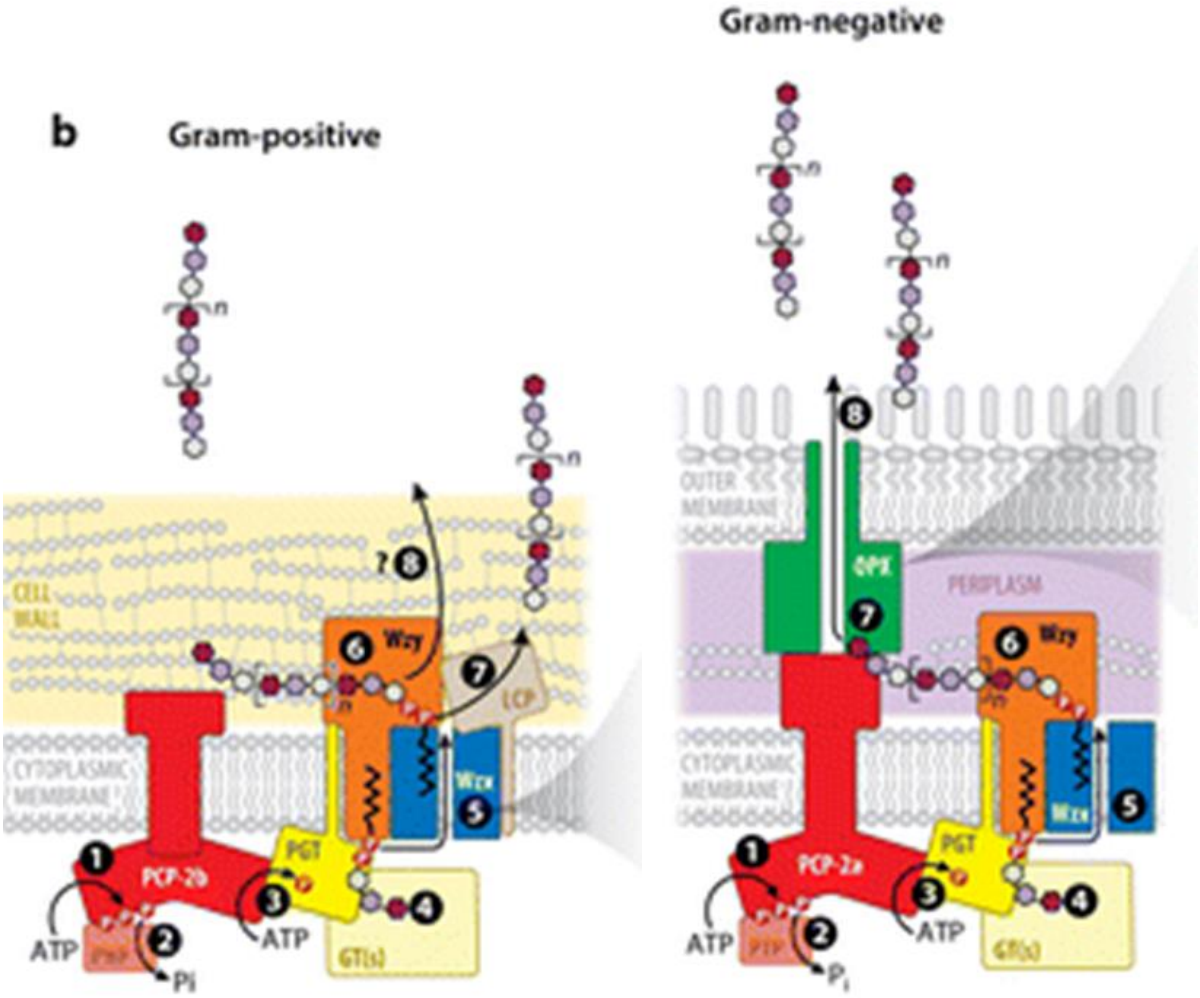
Conserved genes: *galF, PAP2, wzi, wza, wzb and wzc*, encode proteins involved in capsule translocation
GND: glucose-6-phosphate dehydrogenase
*ugd*: UDP-glucose dehydrogenase

Variable region: encode proteins responsible for the polymerization and assembly of specific subunits of the capsule determining the K-type.
nucleotide sugar-dependent glycosyltransferases

**Wzy-dependent pathway.**
Steps ❶–❻ are conserved in both Gram- and Gram+.
❶❷Autophosphorylation/dephosphorylation of the **polysaccharide copolymerase PCP-2a/**b (WbaP) proteins is required for **extracellular polysaccharides (EPSs)** production.
❸ PGT **phosphoglycosyltransferase** may be activated by transphosphorylation
❹ The **undecaprenyl pyrophosphate** Und-PP-linked repeat unit is synthesized by PGT and other assembly enzymes.
❺ Export via Wzx
❻ Blockwise polymerization of Und-PP-repeat units by Wzy.

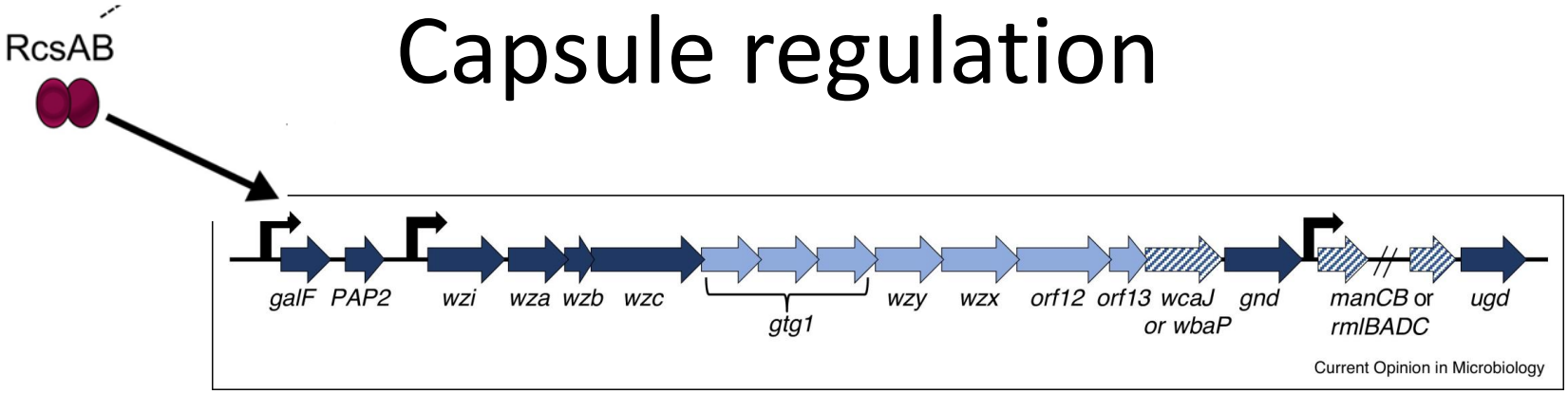
In gram-positive bacteria, nascent EPS is transferred to peptidoglycan assembly intermediates and linked to the cell wall by LCP activity (❼) or potentially released by an unknown mechanism (❽).

In gram-negative bacteria, the nascent polysaccharide is released by an unknown mechanism into a translocation pathway provided by the outer membrane polysaccharide (OPX) and polysaccharide copolymerase PCP-2a proteins (❼). Posttranslocation, the gram-negative EPS remains associated with the cell surface or is secreted into the environment.




**Whitfield, Samantha S. Wear, and Caitlin Sande** Assembly of Bacterial Capsular Polysaccharides and Exopolysaccharides
**Annual Review of Microbiology**
Vol. 74:521-543

# Capsule regulation




- Rcs complex signal transduction system includes RcsC (sensor kinase), RcsD (histidine phosphotransferase), RcsB (response regulator), RcsA (auxiliary protein), and RcsF (outer membrane lipoprotein).
- RcsB dimerizes with RcsA and binds the DNA sequence called RcsAB box that activates capsule cluster expression
- RcsAB binds to promoter ahead of galF/ORF1

# Hypervirulent *K. pneumoniae* (hvKp)

Genes linked to hypervirulence (hv-associated genes)

Three siderophores

*iroBCDN, iutA, iucABCD* e *ybt*

salmochelin (*iroBCDN*),

aerobactin (*iutA, iucABCD*)

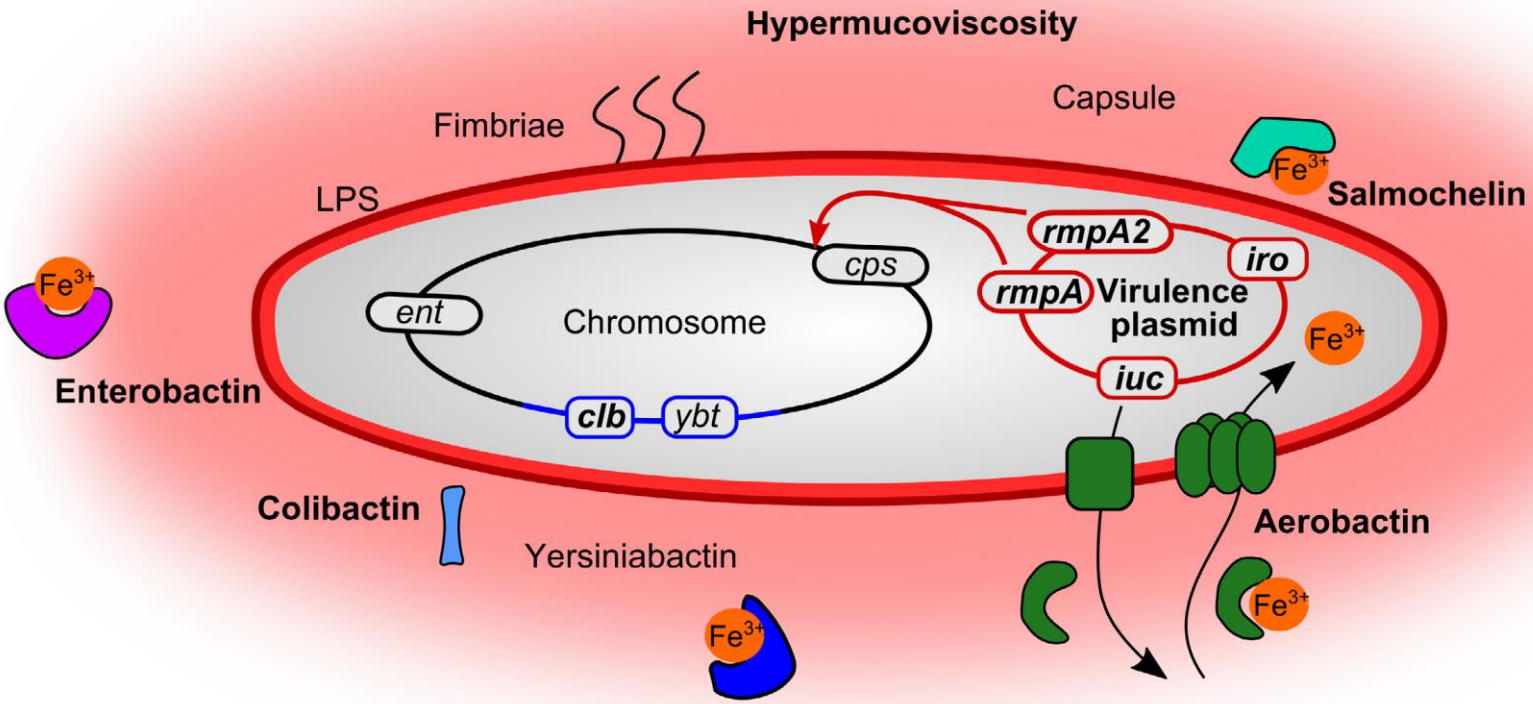
yersiniabactin (*ybt* genes)

Two transcriptional regulators

*rmpA, rmpA2*

Localized on the virulence plasmid pLVPK, but in some strains are on the chromosome as part of an ICE element

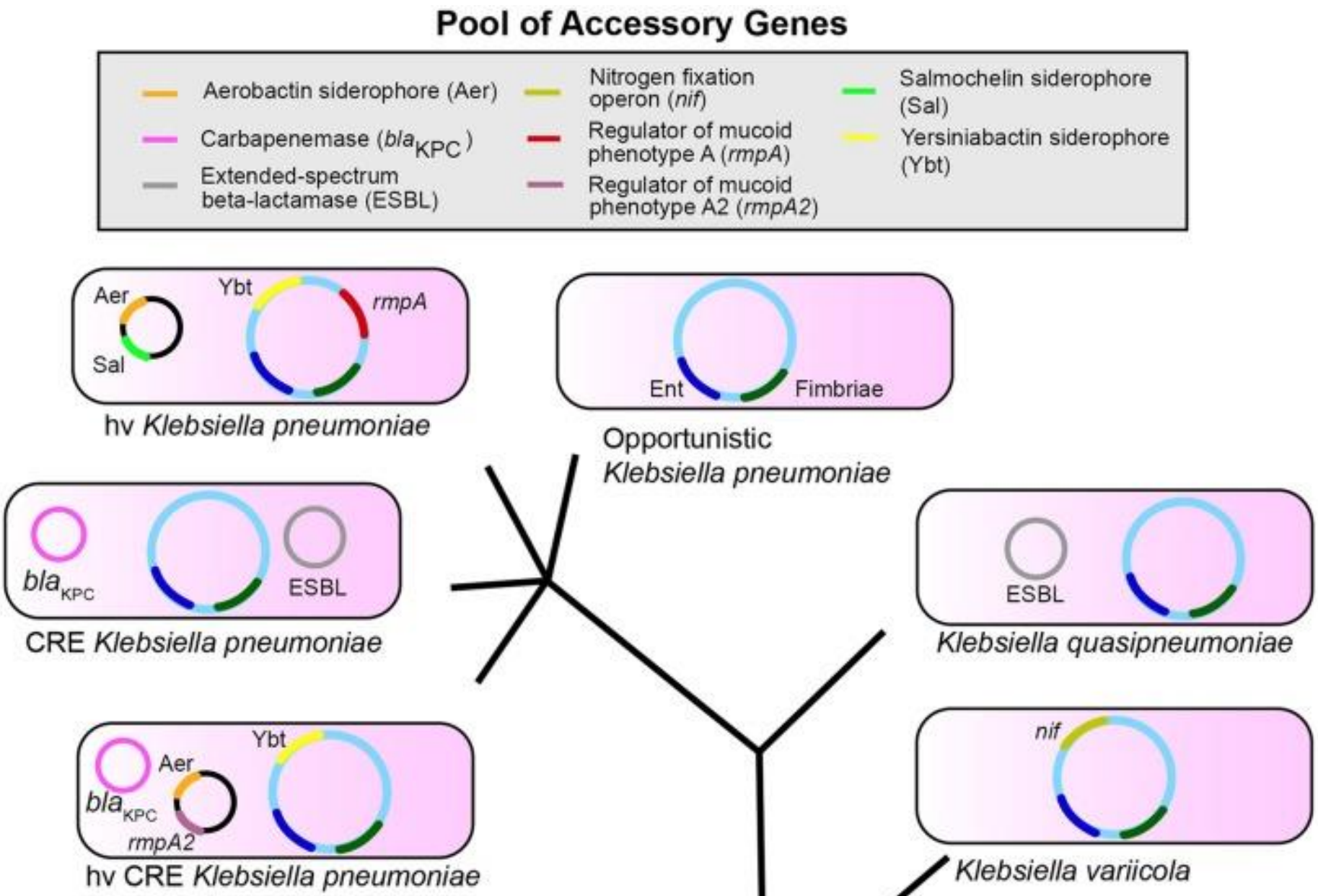
# Hypervirulent *K. pneumoniae* (hvKp)



# The Rmp regulators

**RmpA and RmpA2**

- RmpA and RmpA2 activate *cps* genes and activate the RmpA promoter
- RpmA interacts with RcsB, substituting RcsA in the dimer
- Fur which is a transcriptional regulator that responds to iron binds upstream of the promoter of the *rmpA* gene and represses its expression
- MarR-family regulators: KvrA
- KvgAS and KvhAS two-component systems

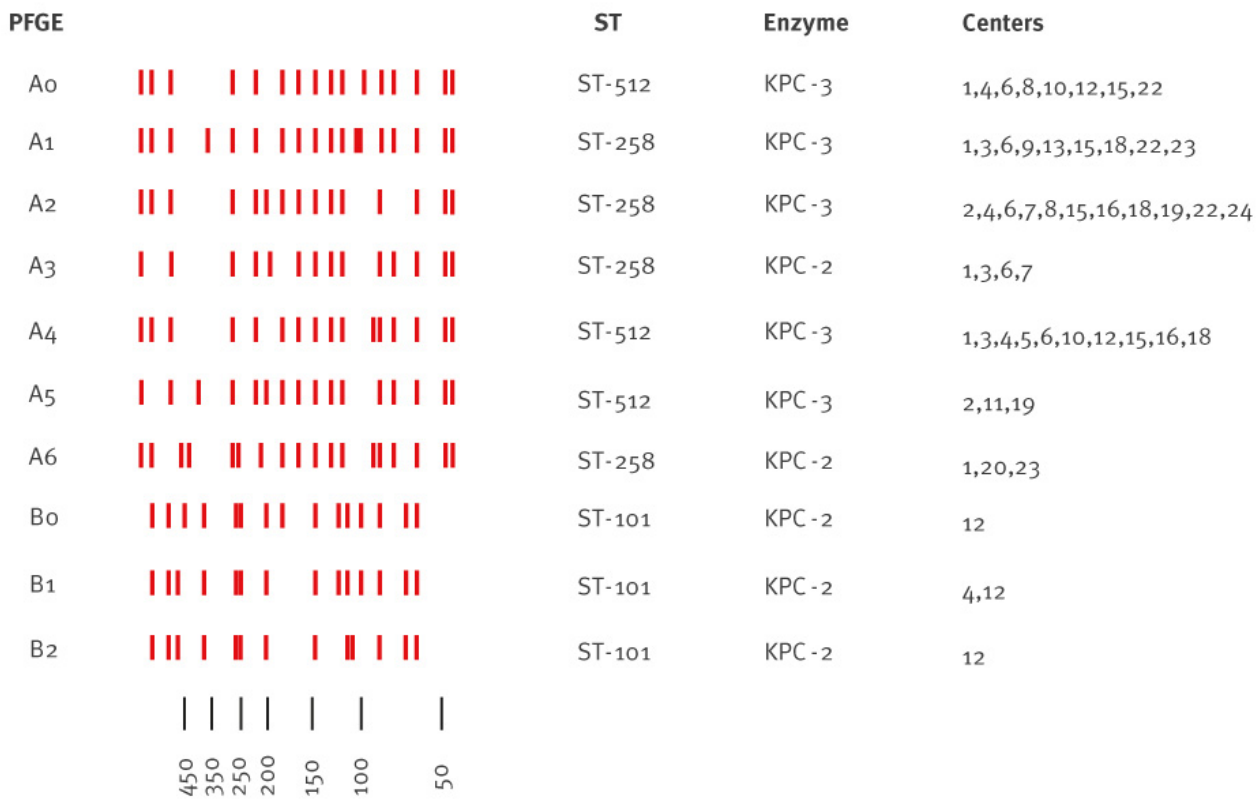



Martin RM, Bachman MA. Colonization, Infection, and the Accessory Genome of *Klebsiella pneumoniae*. Front Cell Infect Microbiol. 2018 Jan 22;8:4. doi: 10.3389/fcimb.2018.00004.

# Studying one *Klebsiella pneumoniae* clone: ST258 and its clonal complex

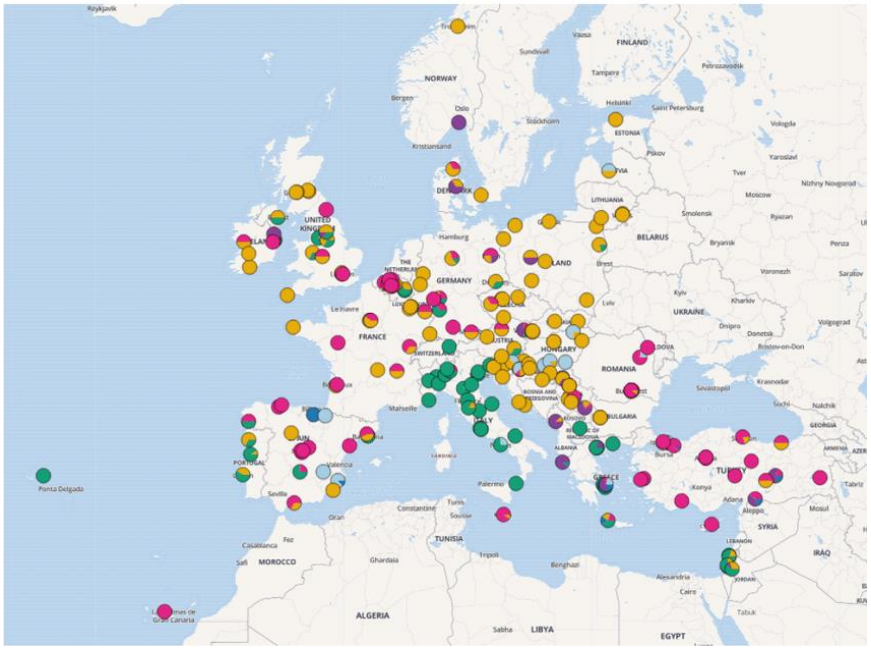
**FIGURE 2**

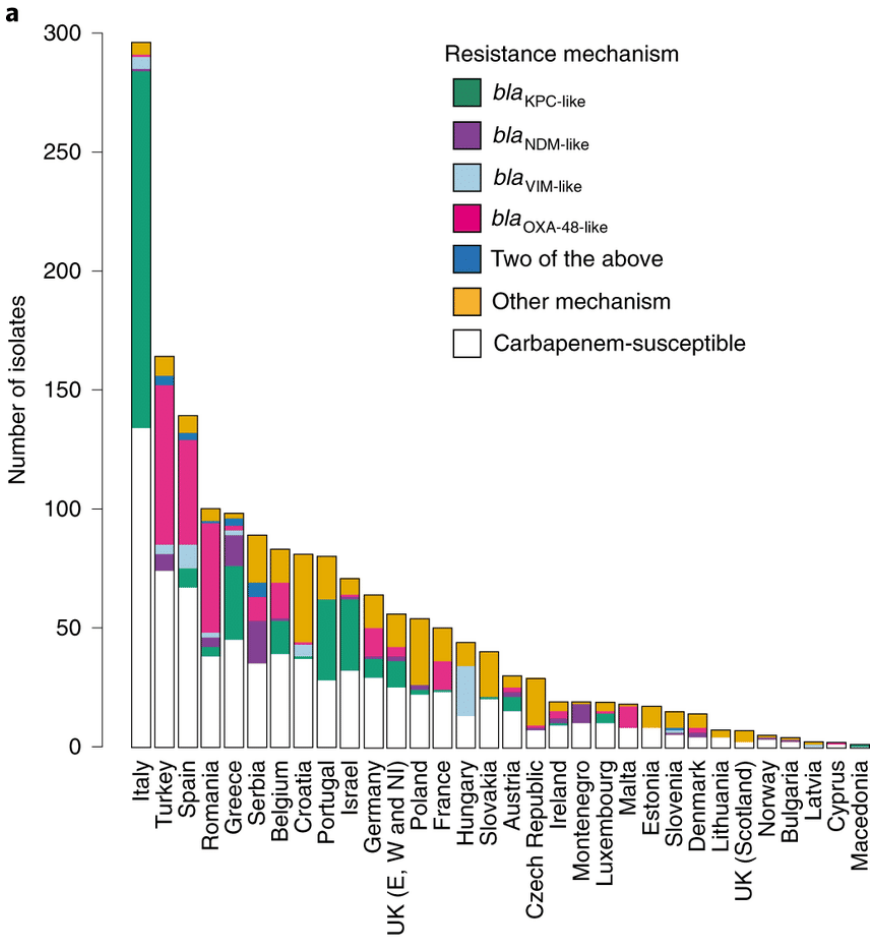
*XbaI* PFGE profiles of KPC-KP in combination with MLST results and KPC-type alleles, Italy, 15 May–30 June 2011 (n=204 )



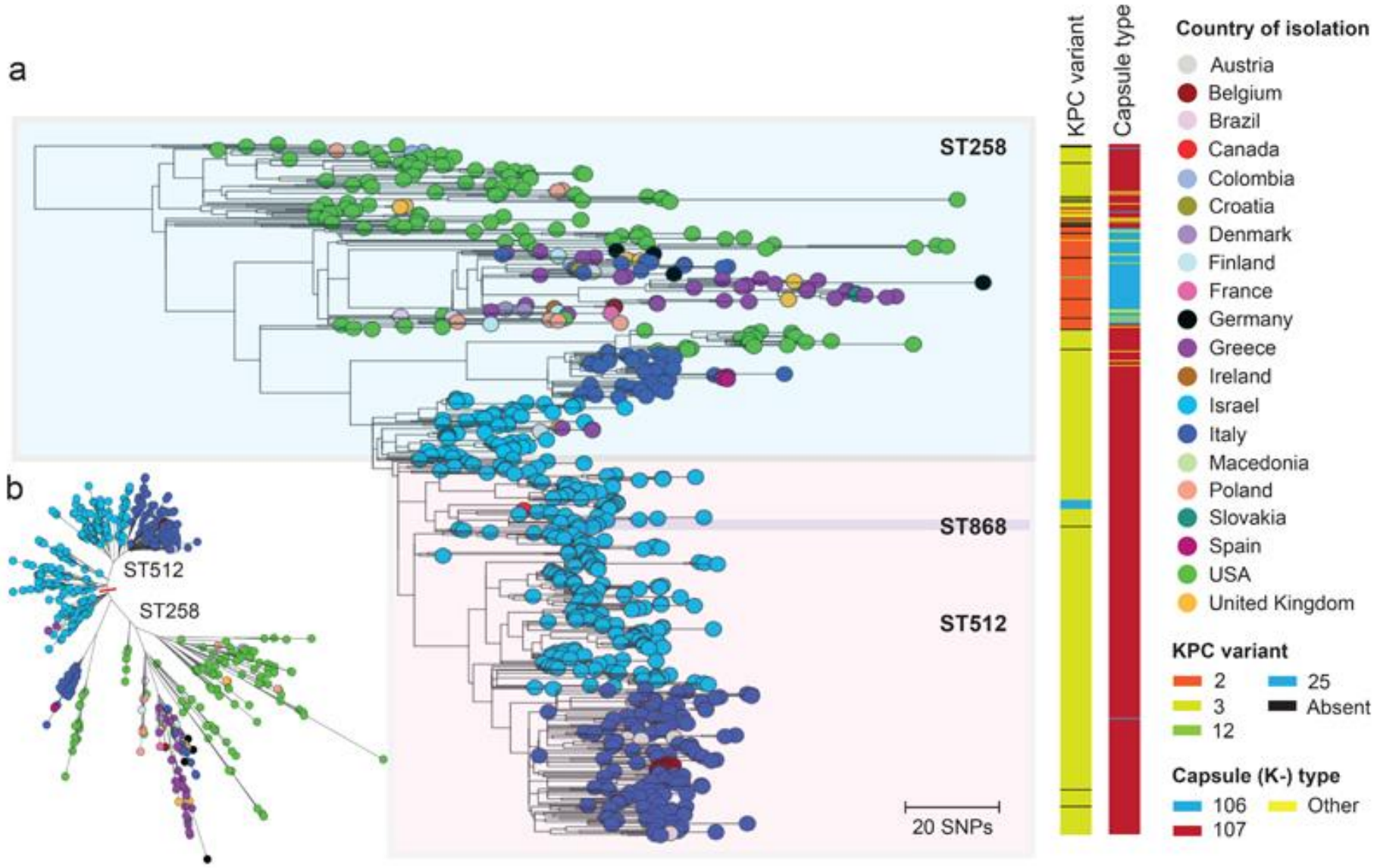

PFGE: pulsed-field gel electrophoresis; KPC-KP: KPC-type carbapenemase-producing *Klebsiella pneumoniae*; MLST: multi-locus sequence typing.

DNA size standards for PFGE profiles are indicated at the bottom. Distribution by centres of different PFGE-types is also indicated: 1: Milan; 2: Varese; 3: Lecco; 4: Turin; 5: Novara; 6: Genoa; 7: Sanremo; 8: Verona; 9: Bolzano; 10-11: Modena; 12: Florence; 13: Siena; 14: Perugia; 15: Ancona; 16: Rome; 17: Pescara; 18: San Giovanni Rotondo; 19: Lecce; 20: Naples; 21: Avellino; 22: Cosenza; 23: Palermo; 24-25: Catania.



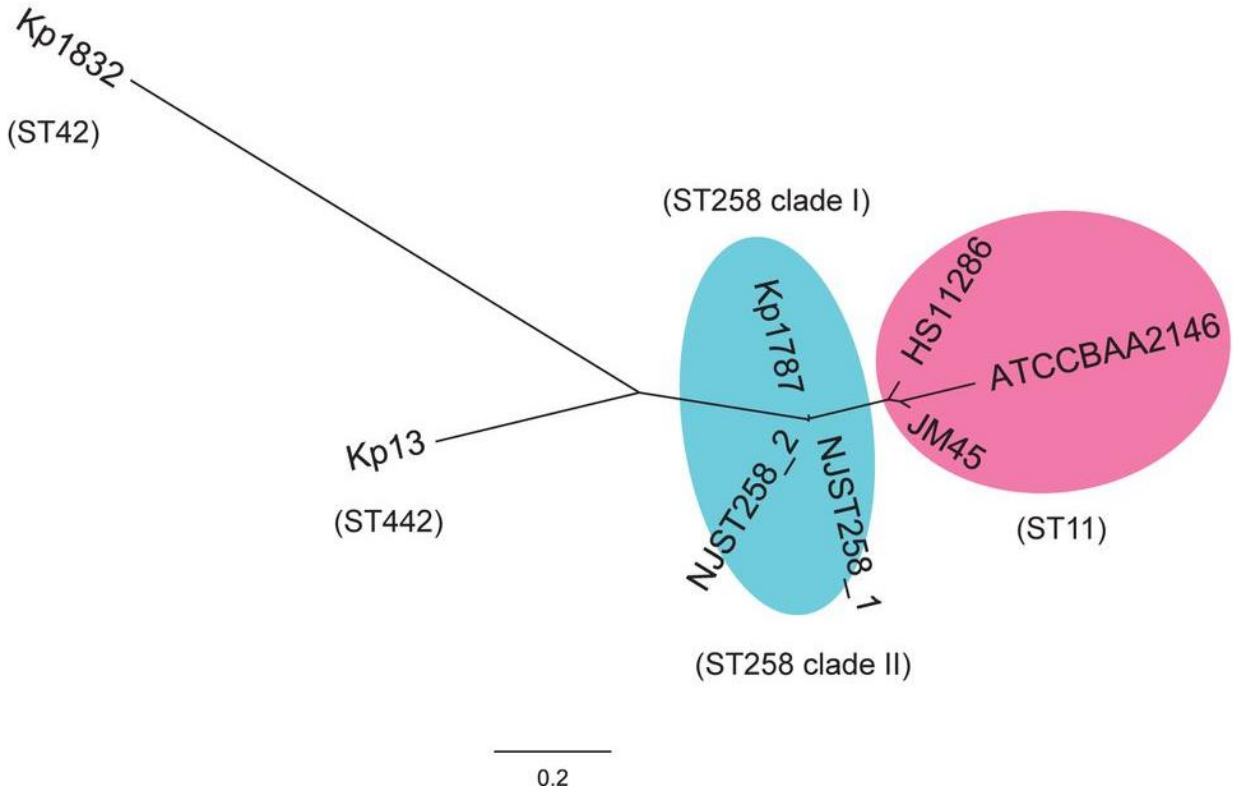

**David et al., 2019. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread**
Nat Microbiol. 4:1919-1929




**David et al., 2019. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread**
Nat Microbiol. 4:1919-1929

nature microbiology

two sequenced reference strains (NJST258_1 and NJST258_2) were genotyped as ST258 clade II strains,
the prototypic ST258 clade I strain (Kp1787) to use as a clade I reference genome.



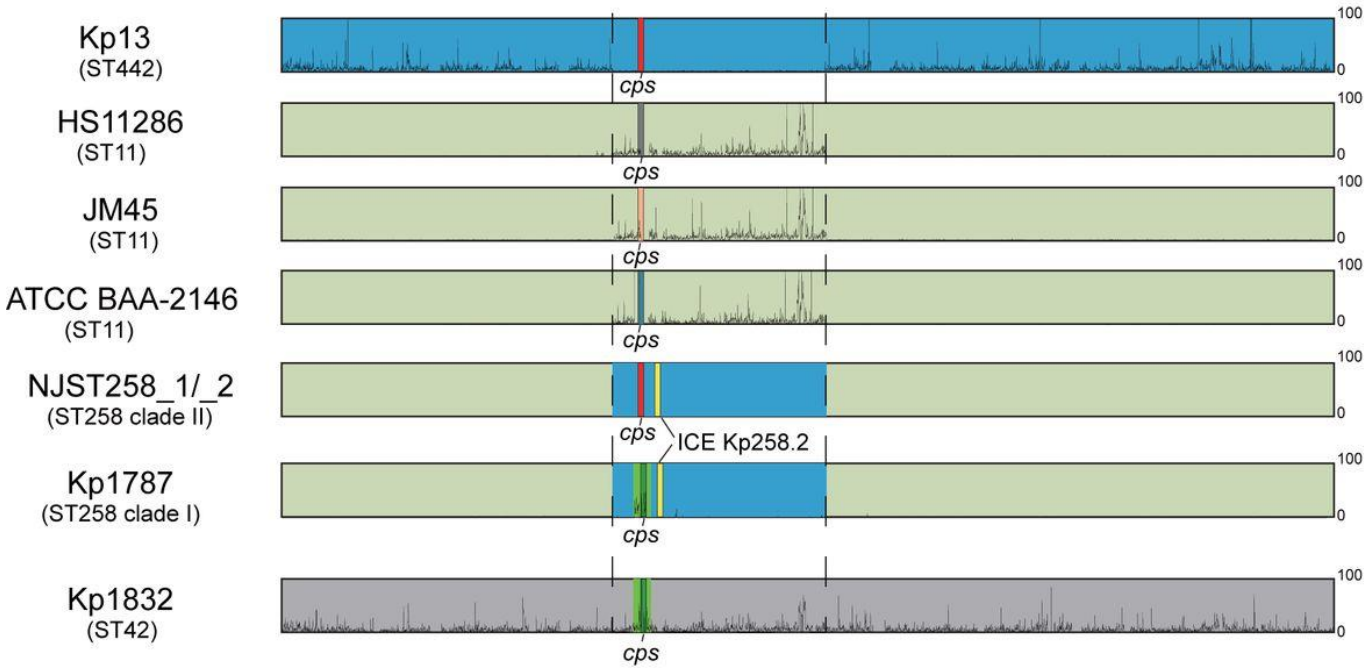
## Large ~1.1-Mbp recombination region in ST258

To elucidate the phylogenetic relationship among ST258, ST11, and ST442 strains, a comparison of the genome sequences of six closed *Klebsiella pneumoniae* strains has been performed

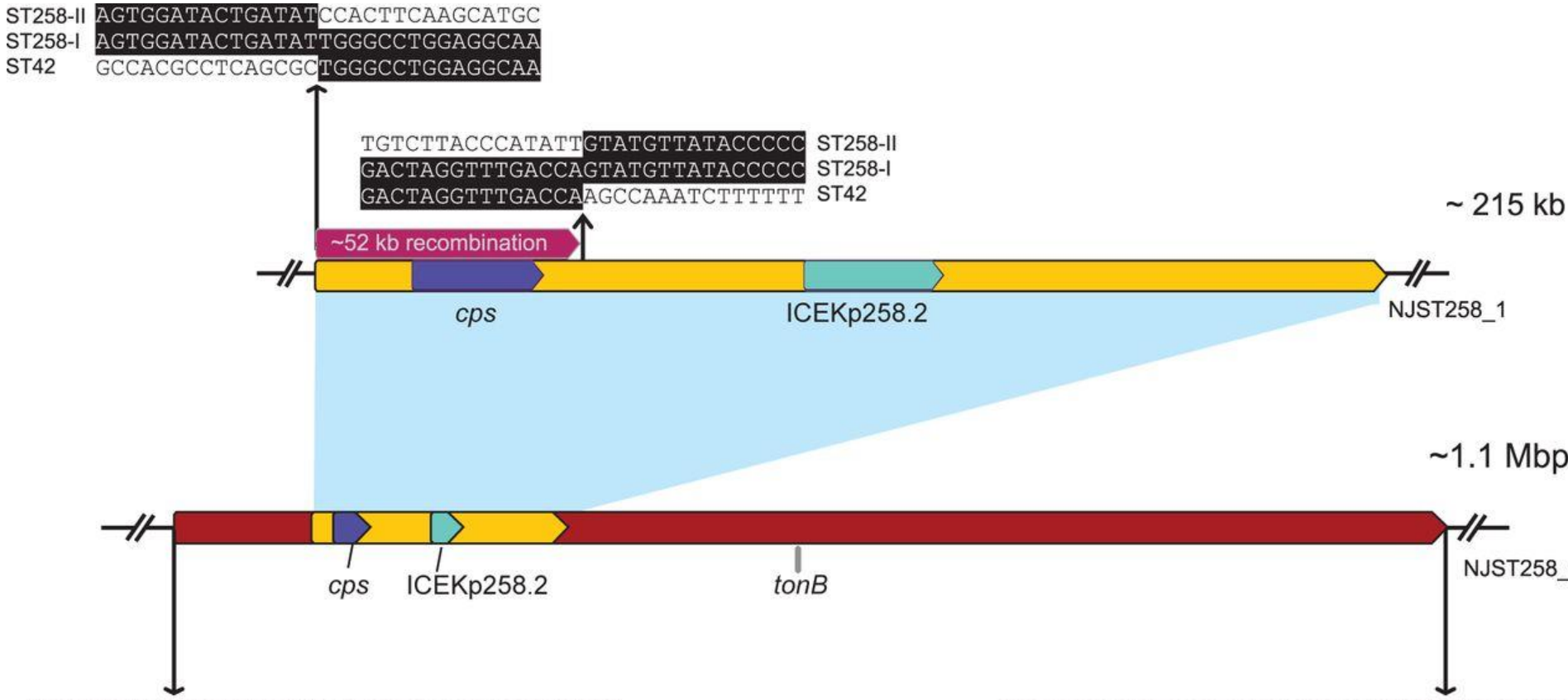
A


| 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 |

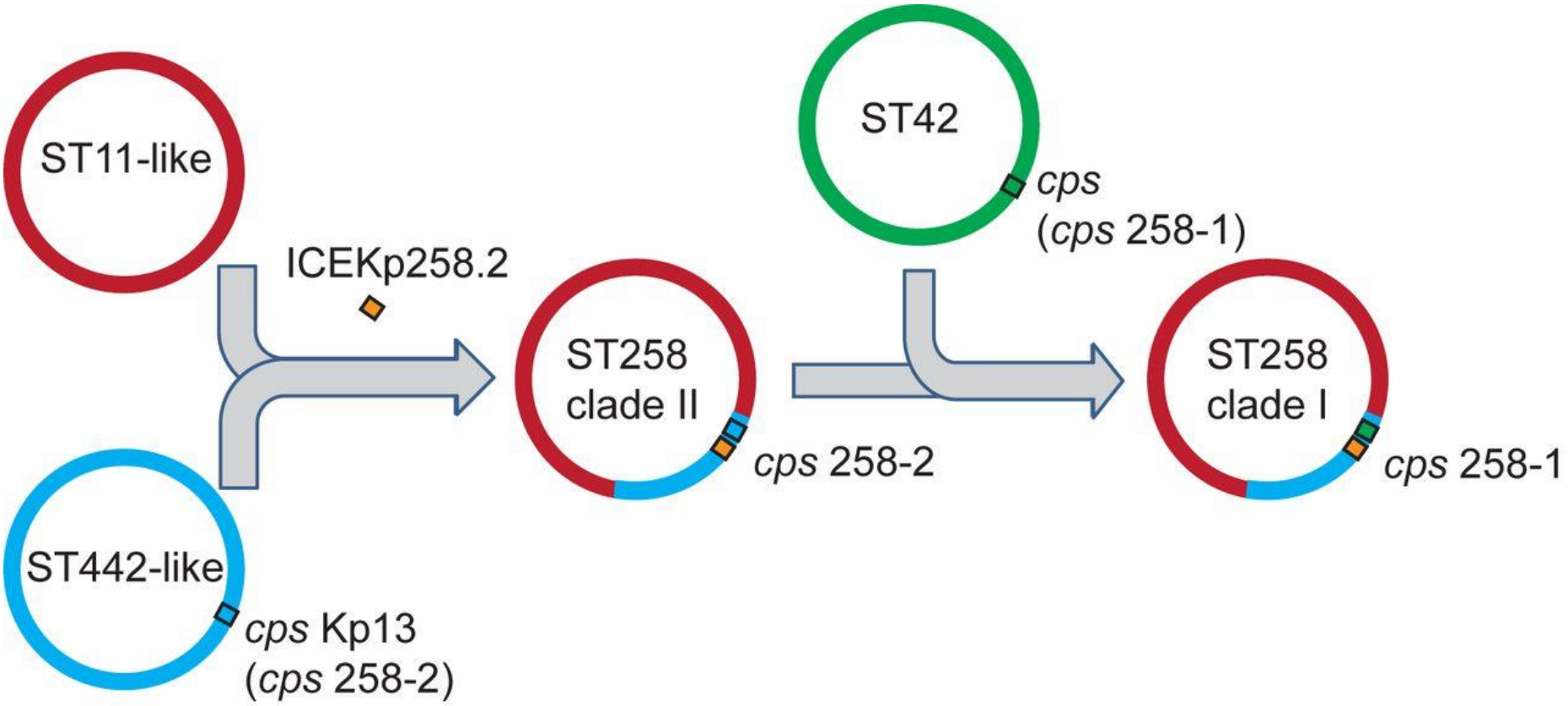
B




L. Chen, B. Mathema, J.D. Pitout, F.R. DeLeo, B.N. Kreiswirth. Epidemic Klebsiella pneumoniae ST258 is a hybrid strain. mBio, 5 (2014)e01355-14




Upstream and downstream junction SNPs for the ~1.1-Mbp recombination fragment and *cps* region in ST258, ST442, and ST42 strains. The start site of the replacement of the ~52-kb *cps*-harboring region is the same as that of the ~215-kb RD in ST258 II clades




Hypothesized evolutionary history in *K. pneumoniae* ST258 strains.

nature medicine

LETTERS

https://doi.org/10.1038/s41591-020-0825-4

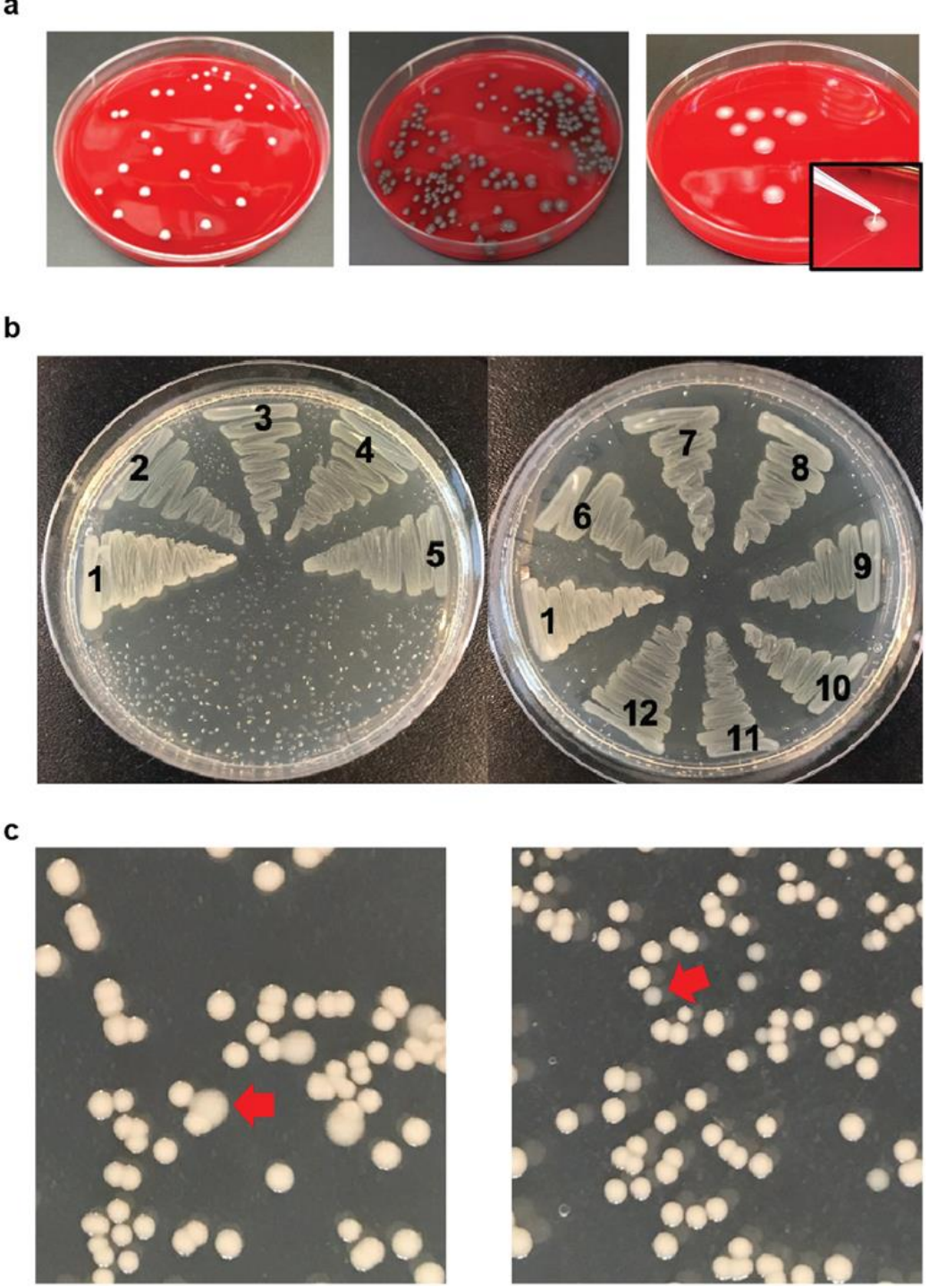
Check for updates

# Adaptive evolution of virulence and persistence in carbapenem-resistant *Klebsiella pneumoniae*

Christoph M. Ernst[1,2,3], Julian R. Braxton[1,2,3,6], Carlos A. Rodriguez-Osorio[1,2,3,6], Anna P. Zagieboylo[1,2,3,6], Li Li[1,2,3], Alejandro Pironti[1], Abigail L. Manson[1], Anil V. Nair[4], Maura Benson[5], Kaelyn Cummins[5], Anne E. Clatworthy[1,2,3], Ashlee M. Earl[1], Lisa A. Cosimi[5] and Deborah T. Hung[1,2,3] ✉

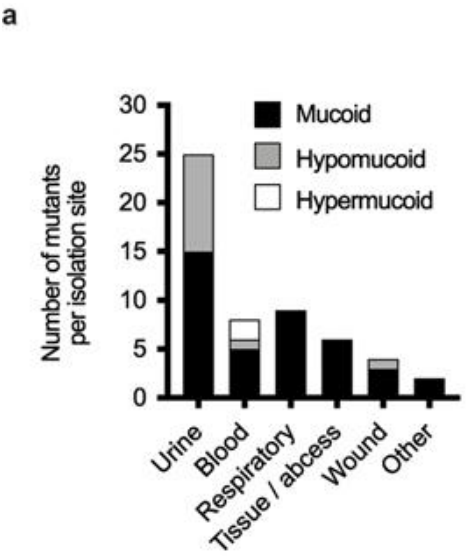
To date, the particular virulence plasmid (pLVPK) has not been detected in carbapenem-resistant *K. pneumoniae* strains of the **ST258** sequence type, which are endemic in the United States and Europe , and only a single ST258 isolate has been reported to carry hypervirulence-associated siderophores. To explore whether the convergence of carbapenem resistance with virulence is more widespread, and could potentially involve alternative virulence mechanisms, we screened a previously reported collection of 54 *K. pneumoniae* ST258 strains collected from US patients for hypercapsule production

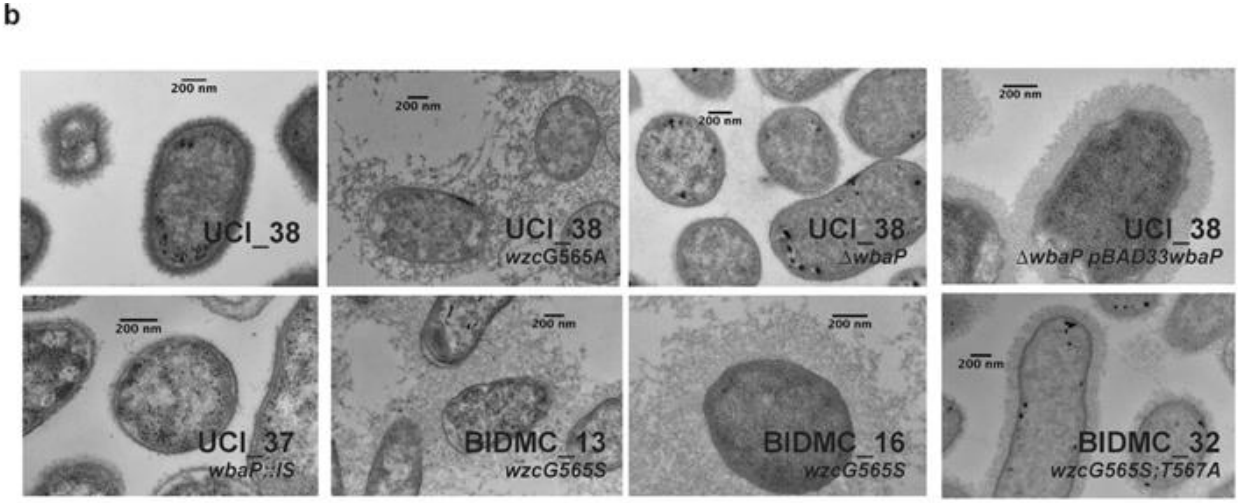
22% (12/54) were hypomucoid and 4% (2/54) were hypermucoid
the two hypermucoid isolates were isolated from bloodstream infections whereas hypomucoidity was significantly associated with isolation from urinary tract infections

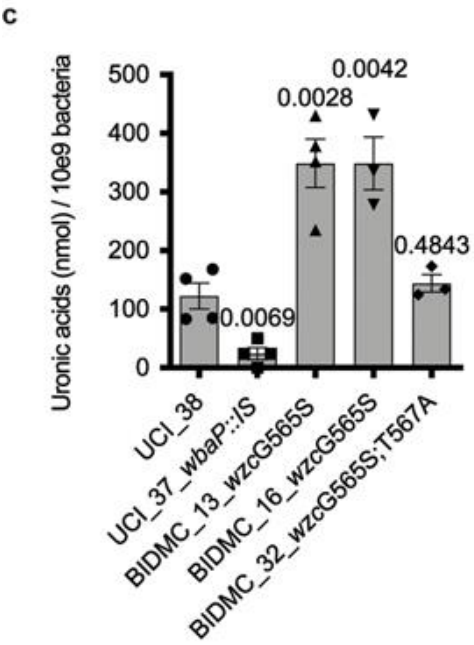


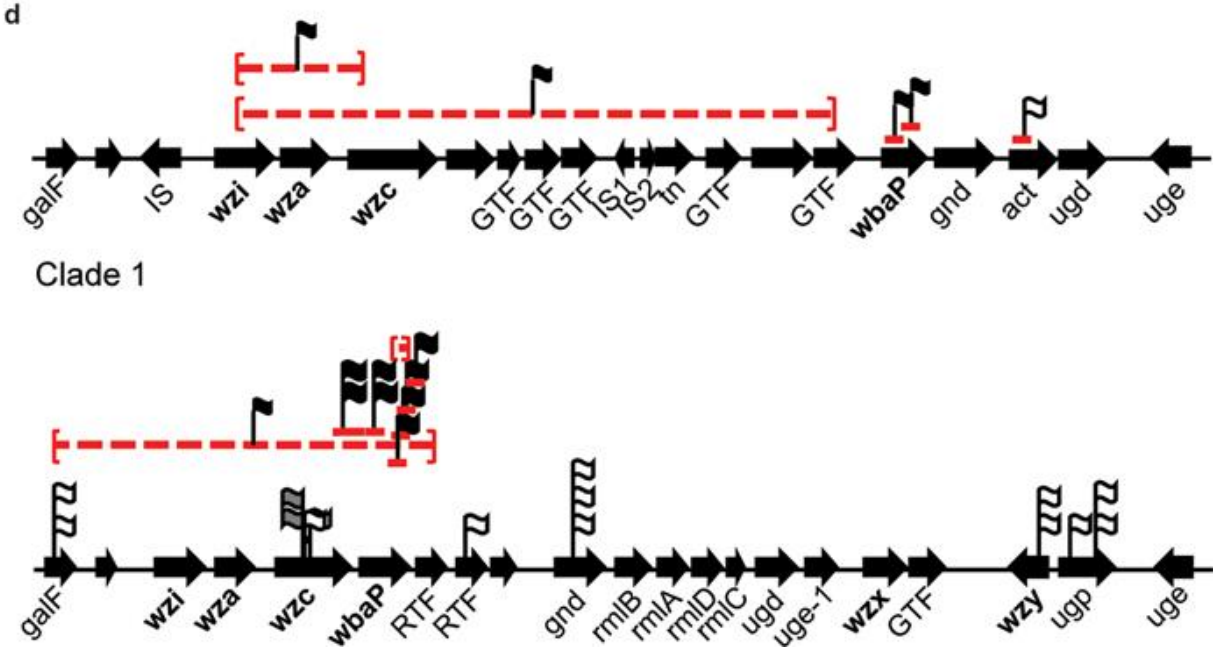
We first considered the capsule-deficient strains. As the capsule is thought to be essential for infection, we were surprised to identify capsule-deficient ST258 strains among the clinical urine isolates, because such strains had been assumed to be avirulent.

The 12 capsule-deficient ST258 isolates had 11 different mutations disrupting capsule-biosynthesis genes. The mutations included **large deletions of several core capsule biosynthesis genes (wzi, wza, wzc)** and, most commonly, insertion sequences (ISs) in ***wbaP***

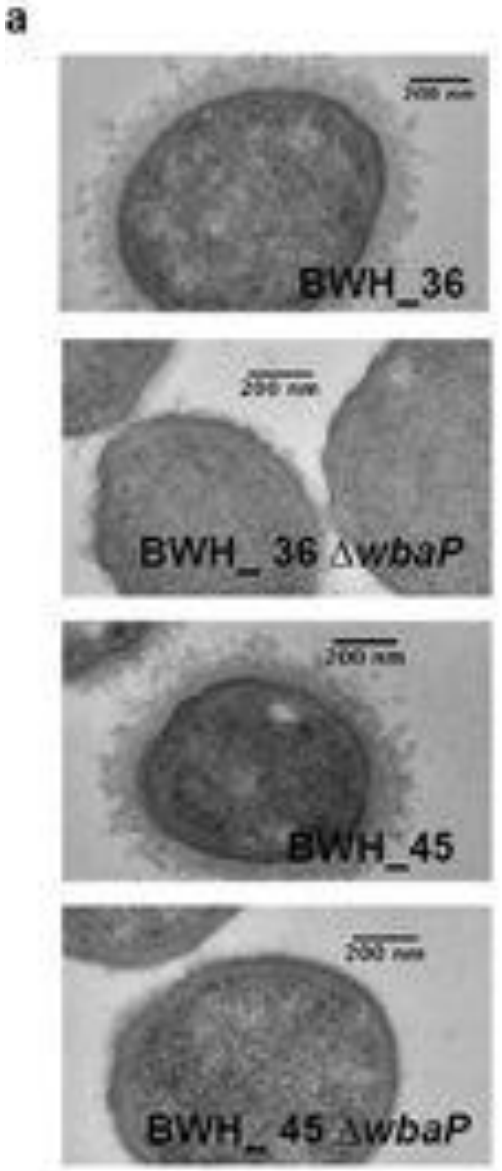


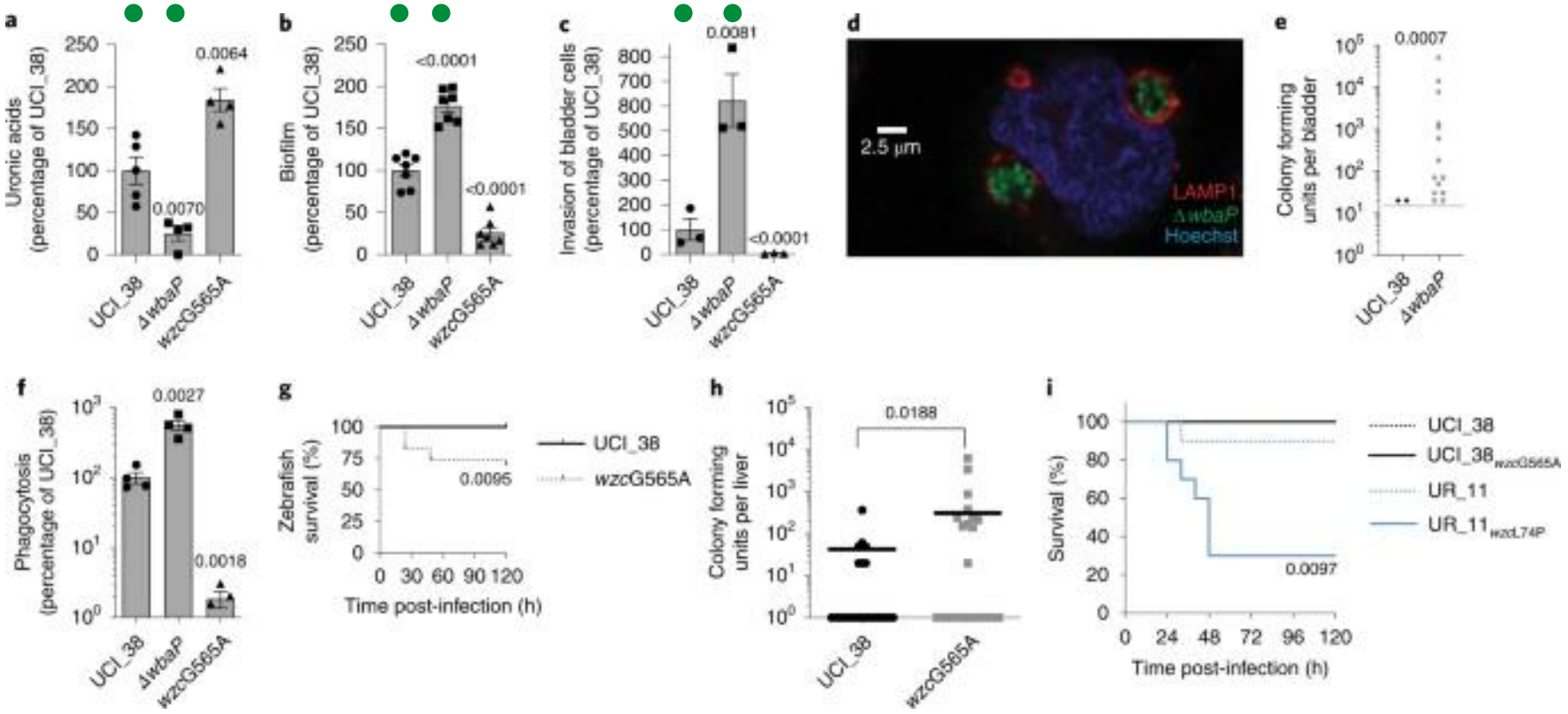



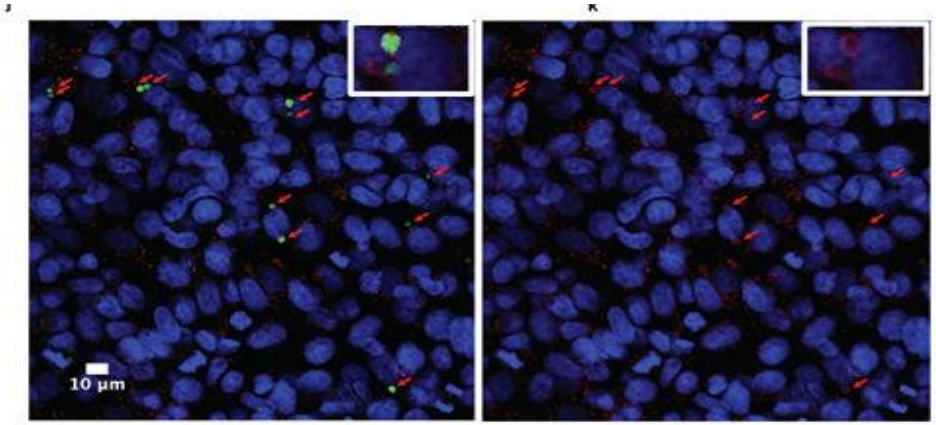
We engineered a mutant strain to confirm that deletion of *wbaP* in the normal capsule-producing clinical strain UCI_38 (UCI_38ΔwbaP) did indeed abolish capsule production, which could be restored by complementation with *wbaP* (Figs. 1b and 2a). Deletion of *wbaP* in two other clinical ST258 isolates (**BWH_36 and BWH_45**) from different ST258 (clade 2) subclades also abolished capsule production (Fig. 2a), demonstrating that *wbaP* plays a similar role in *K. pneumoniae* to that in *E. coli.*

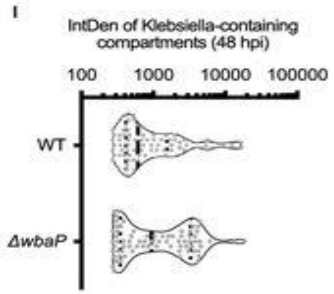


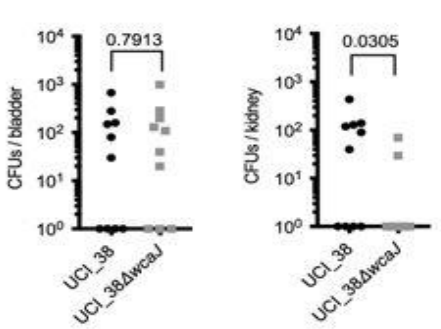
We thus investigated the impact of capsule inactivation on phenotypes associated with UTIs, including biofilm formation and infection of bladder epithelial cells, features crucial for uropathogenic E. coli to establish and persist in UTIs. The engineered deletion strain UCI_38ΔwbaP and the closely related hypocapsule clinical isolate UCI_37wbaP::IS both formed more robust biofilms and invaded bladder epithelial cells more efficiently compared with wild-type UCI_38, a normal capsule strain and the complemented; these same results were recapitulated in *wbaP* deletion strains in two other ST258 strain backgrounds (BWH_36 and BWH_45). Deletion of *wbaP* increased invasion and intracellular replication in LAMP1-positive vacuoles and the capsule-deficient mutant forms more robust biofilms

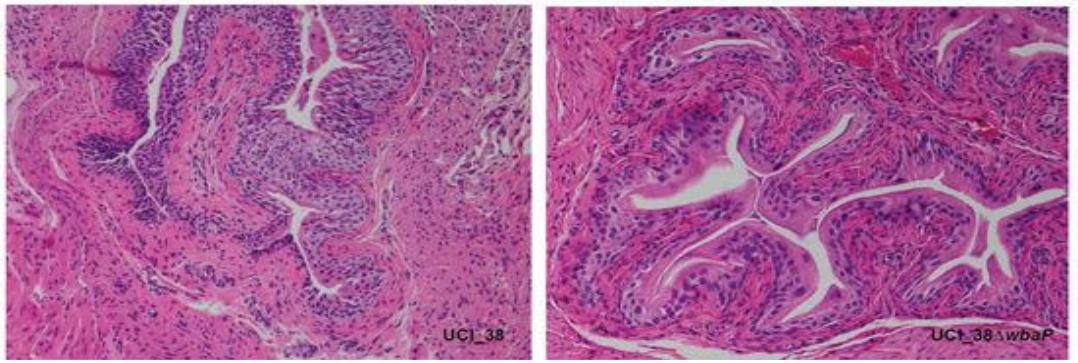


Deletion of wbaP increased invasion and intracellular replication in LAMP1-positive vacuoles, and resulted in a tenfold larger intracellular bacterial reservoir for UCI_38ΔwbaP mutants compared with the isogenic parent UCI_38, which persisted over the course of 48 h.

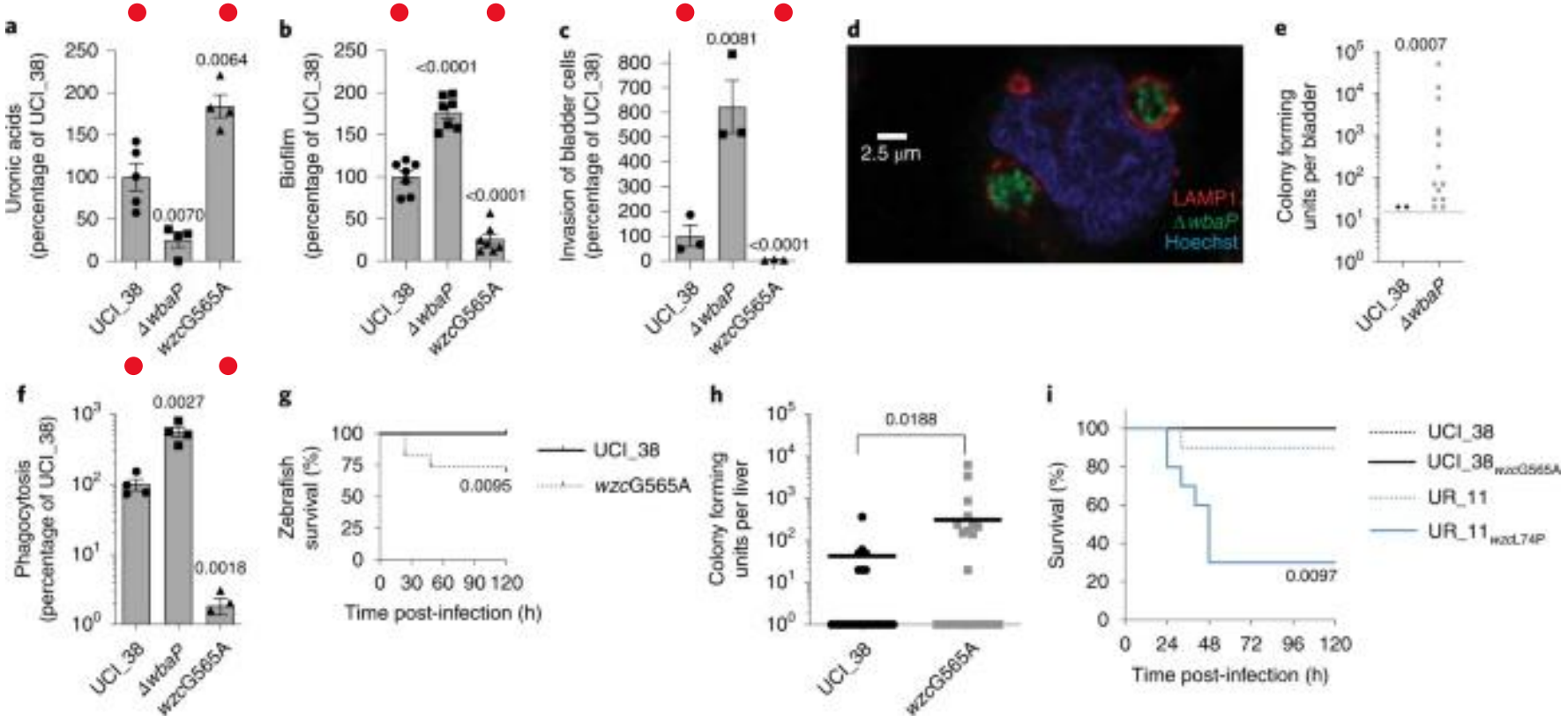


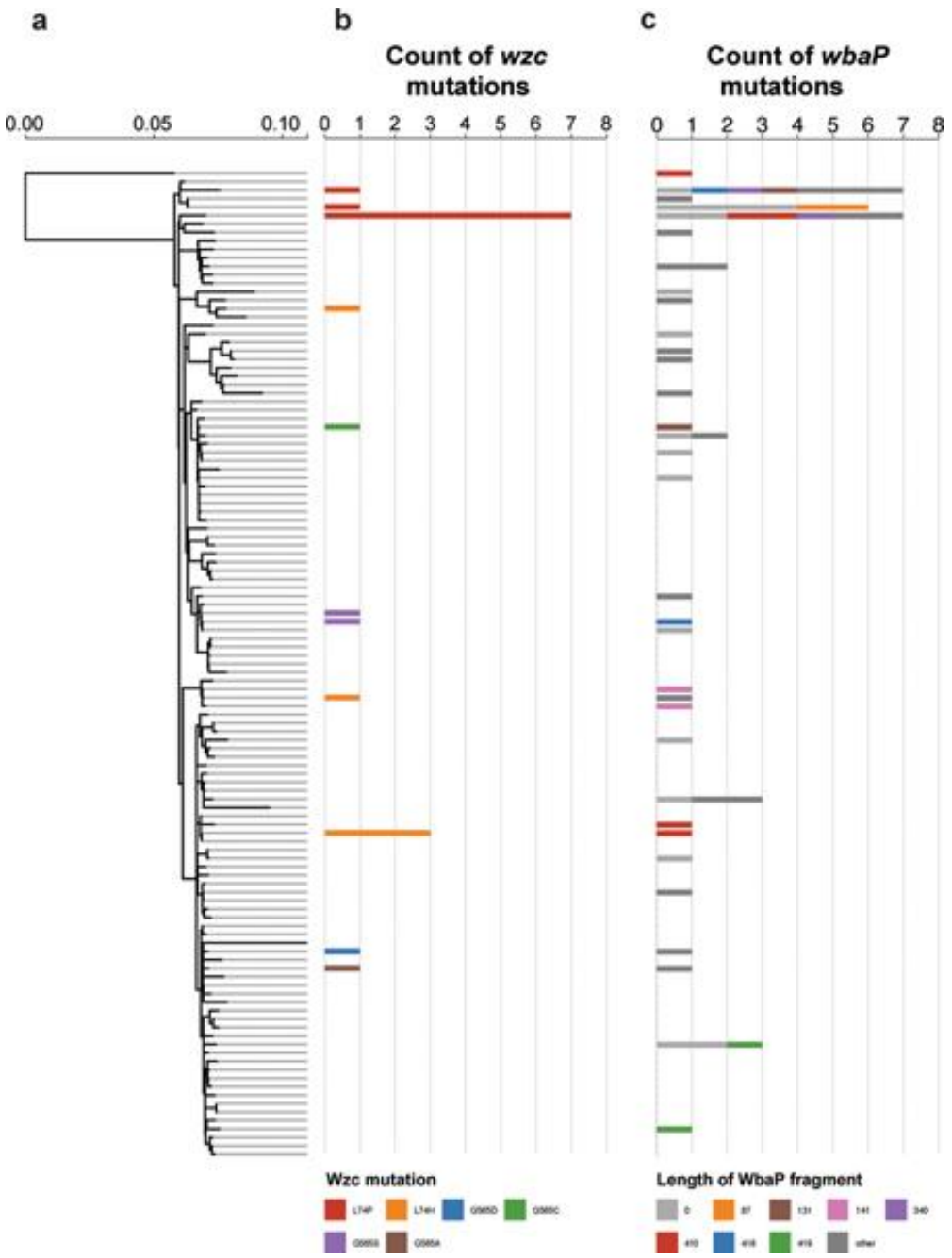



**two hypercapsule blood isolates revealed that they do not carry the capsule transcription factors *rmpA/A2* and the siderophore aerobactin,**
they carry a single missense mutation in the ***wzc* gene, resulting in a glycine-to-serine substitution (Gly-565Ser)**. *Wzc* is required for high-level capsule polymerization in *E. coli.* Episomal expression of the mutated *wzc* allele in UCI_38 confirmed that the Gly-565Ser substitution conferred a hypercapsule, and that an additional Thr-567Ala mutation found in BIDMC_32 suppressed hypercapsule formation.



We thus searched all available ST258 clade 2 genomes in the National Center for Biotechnology Information (NCBI) RefSeq database for the presence of non-synonymous *wzc* mutations and *wbaP* disruptions. Of 966 genomes, 95 strains (10%) harbored mutations in the same *wzc* and *wbaP* capsule genes that we had observed in the original ST258 collection.

The excess of non-synonymous mutations versus synonymous mutations in *wzc* (86.5% versus 13.5%), as well as the absence of synonymous mutations in functional motifs of *wzc*, pointed to the repeated selection of *wzc* mutants. Indeed, we found 20 *wzc* mutations (2.1%; Fig. 3) that altered the same amino-acid positions we had previously identified as conferring hypercapsule production (positions 565 and 74)



Proposed model for the impact of capsule remodeling in catheter-associated UTIs. Classic *K. pneumoniae* strains are relatively resistant to phagocytosis during colonization of the bladder, by establishing biofilms on catheters and bladder epithelium and by invading bladder epithelial cells; the capsule, however, limits biofilm formation and invasion of bladder epithelial cells, thereby restricting infection to a relative degree. Increasingly virulent *K. pneumoniae*, which contain *wzc* mutations resulting in a phagocytosis resistance-conferring hypercapsule, can disseminate more effectively, but are unable to form a biofilm or invade bladder epithelial cells effectively.