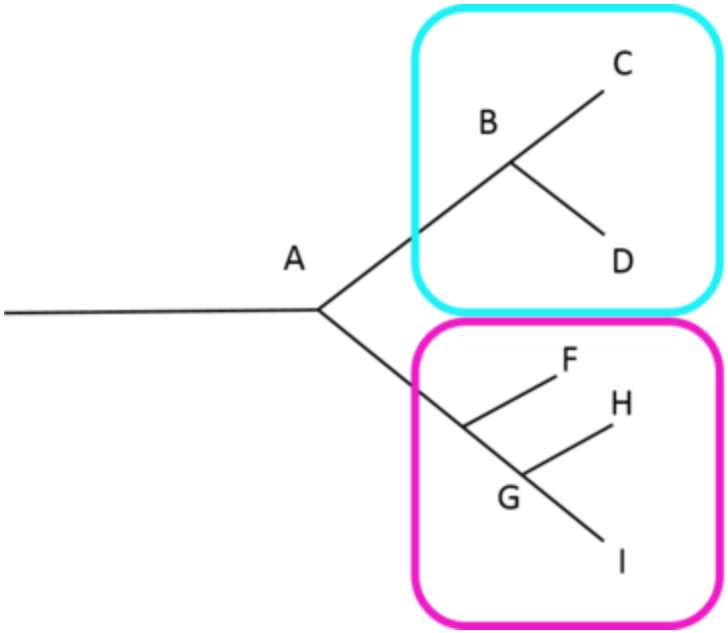
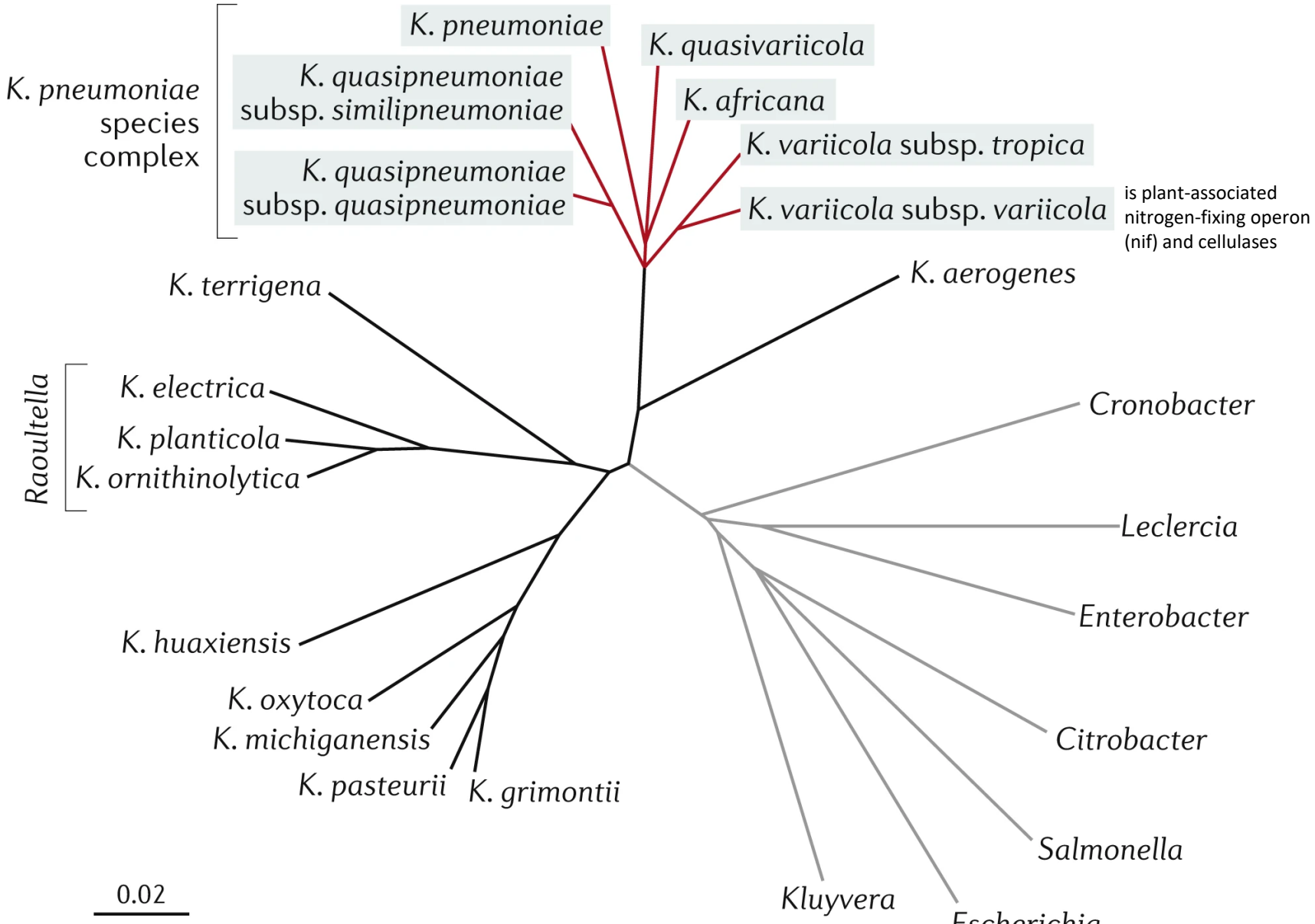
Filogenesi molecolare



Clade BCD

Clade EFGHI



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

- *Enterobacteriaceae*

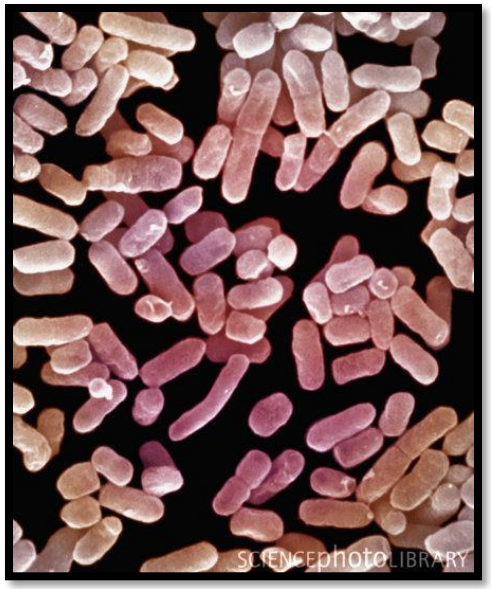
Klebsiella pneumoniae

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

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.

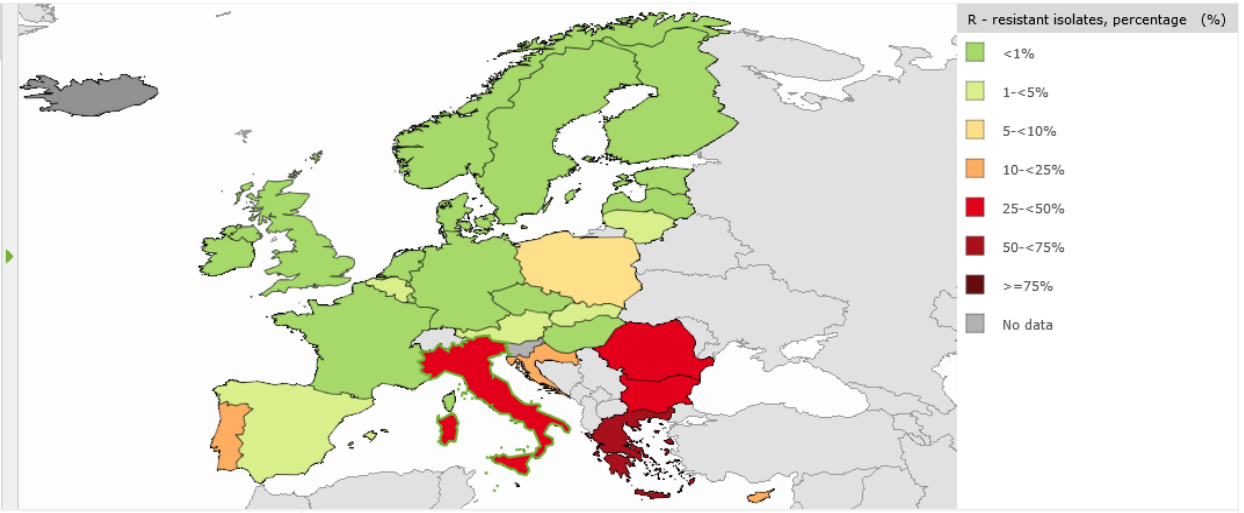
Antimicrobial resistance in the EU/EEA –AER 2019

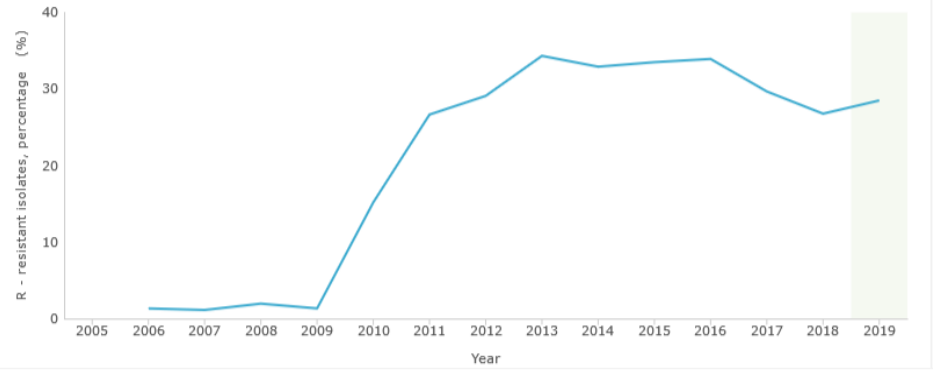
28.5% isolated from invasive carbapenem-resistant infections

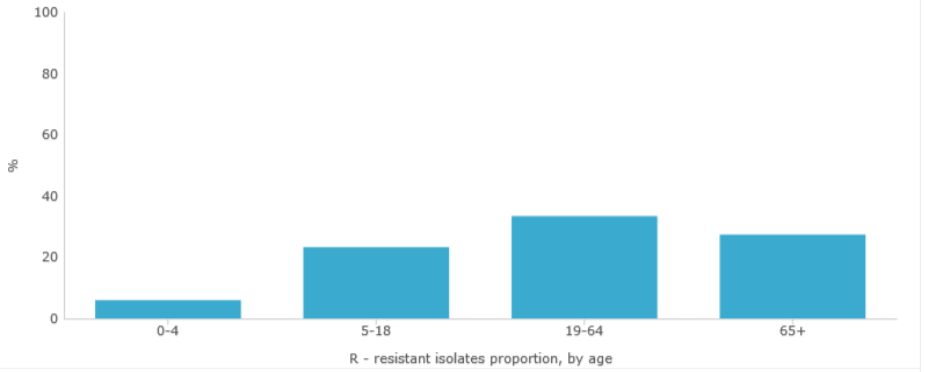
Surveillance Atlas of Infectious Diseases

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







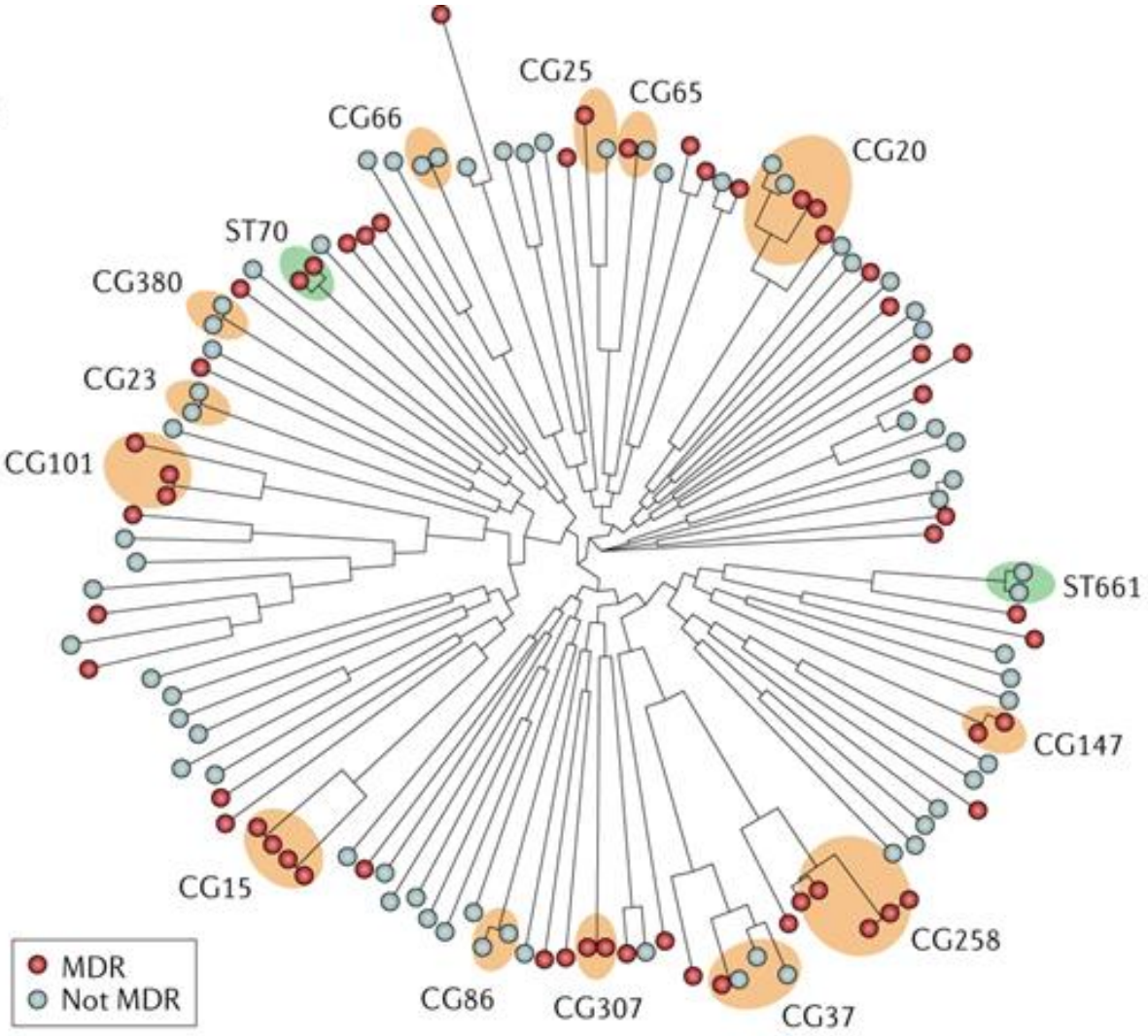
Italy

Klebsiella pneumoniae genome

I genomi di *K. pneumoniae* hanno una dimensione di circa 5-6 Mbp e codificano circa 5.000-6.000 geni. In tutti i membri della specie sono conservati circa 1.700 geni (geni core), mentre i restanti sono variamente presenti (geni accessori). Il pan-genoma totale (la somma di tutti i geni centrali e accessori) è estremamente diversificato

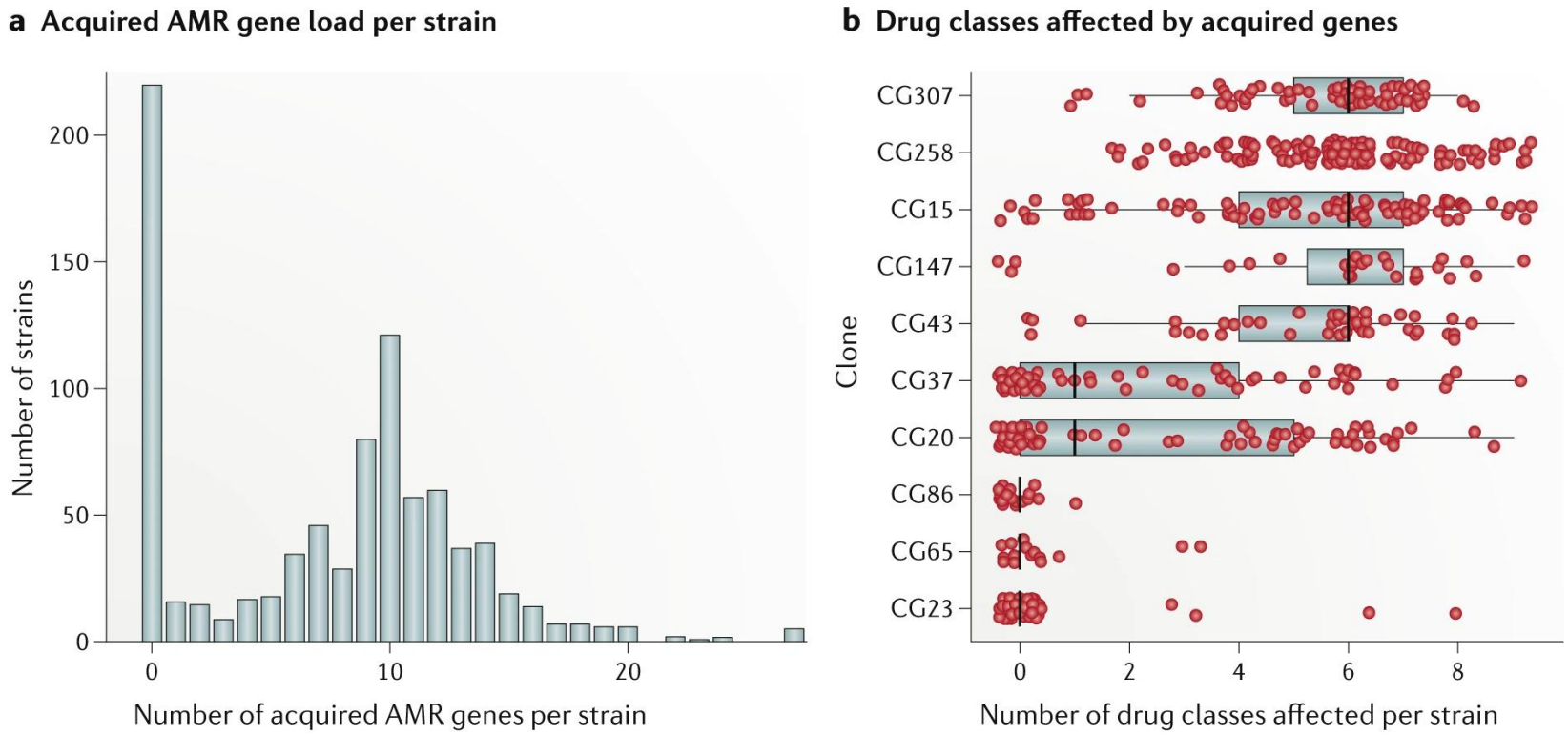
Analisi filogenetiche basate su 1.000-2.000 geni mostrano *lineages* che differiscono tra loro per una divergenza nucleotidica inferiore allo 0,5%.

Questi *lineages* sono indicati come gruppi clonali (CG) e tipicamente indicati come cloni.



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

Acquired antimicrobial resistance in *Klebsiella pneumoniae*



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)*

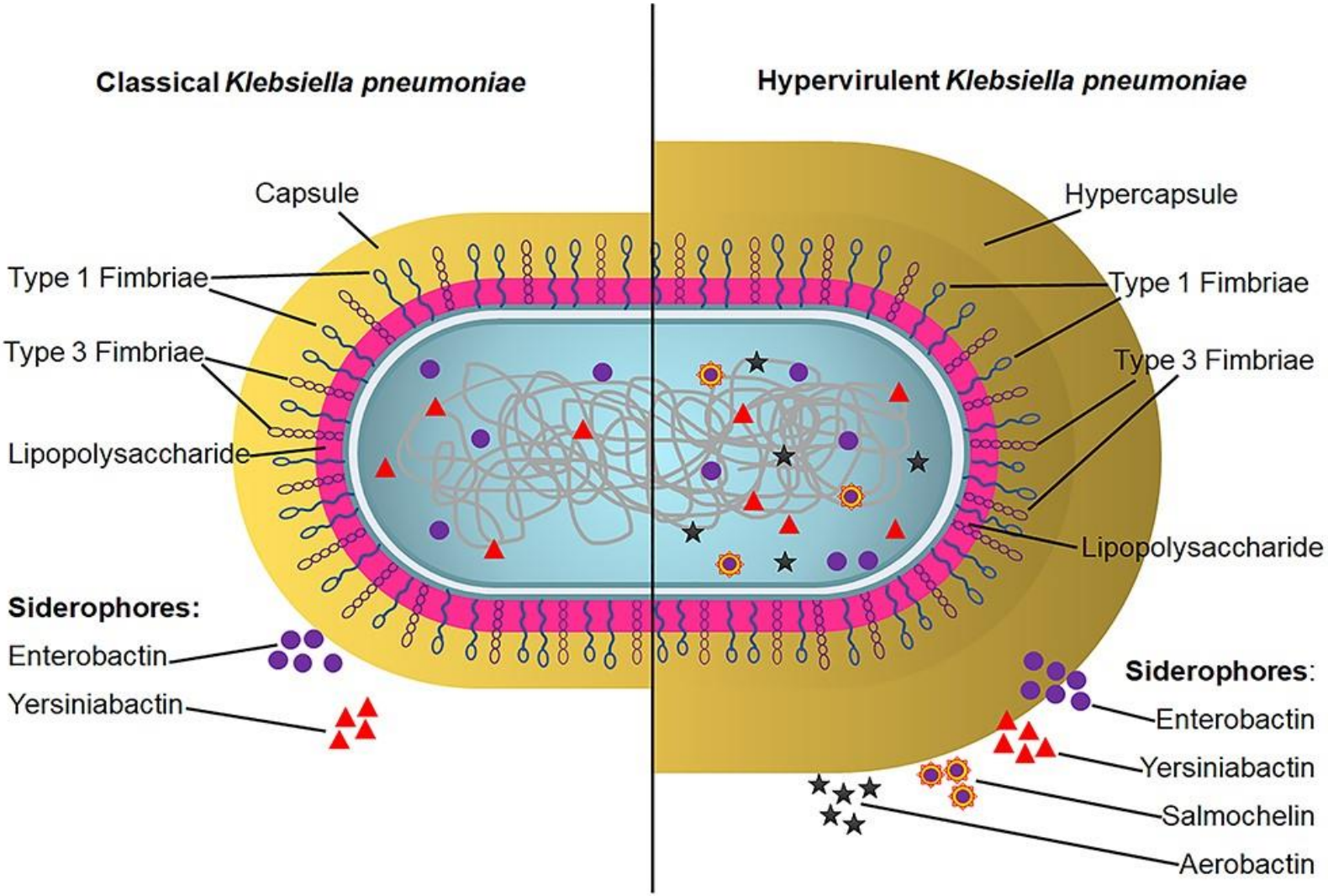
- I ceppi cKp sono tipicamente associati a infezioni nosocomiali o infezioni urinarie e polmonari in pazienti soggetti a lunghi periodi di ospedalizzazione, suggerendo che un certo grado di deficienza immunitaria è necessario per causare una malattia
- La resistenza ai carbapenemi cKp (CR-Kp) è considerata un grave fattore di virulenza poiché esistono poche opzioni terapeutiche per il trattamento

2- Hypervirulent *K. pneumoniae* (hvKp)

- Riconosciuta inizialmente in Asia, l'hvKp è emersa come la principale causa di ascessi piogenici al fegato
- hvKp si distingue da cKp per la sua capacità di metastatizzare in siti distanti, inclusi occhi, polmoni e sistema nervoso centrale
- L'hvKp è implicata anche nelle infezioni extraepatiche, tra cui batteriemia setticemica, polmonite e infezioni dei tessuti molli
- I ceppi HvKp mostrano ipermucoviscosità. Una caratteristica fenotipica che è diventata un test diagnostico rapido per il riconoscimento dei ceppi ipervirulenti

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

ANNUAL REVIEWS

Annual Review of Microbiology

Assembly of Bacterial Capsular Polysaccharides and Exopolysaccharides

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Annu. Rev. Microbiol. 2020. 74:521–43

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Keywords

extracellular polysaccharide, capsular polysaccharide, capsule, exopolysaccharide, glycan biosynthesis, glycan export

Abstract

Polysaccharides are dominant features of most bacterial surfaces and are displayed in different formats. Many bacteria produce abundant long-chain capsular polysaccharides, which can maintain a strong association and form a capsule structure enveloping the cell and/or take the form of exopolysaccharides that are mostly secreted into the immediate environment. These polymers afford the producing bacteria protection from a wide range of physical,

I glicoconiugati superficiali (macromolecole complesse contenenti carboidrati) si trovano in una varietà di forme strutturali con distribuzioni variabili:

I lipopolisaccaridi (LPS) nelle membrane esterne dei gram-negativi
Gli Acidi teicoici della parete (WTA) e polimeri della parete secondaria neigram-positivi

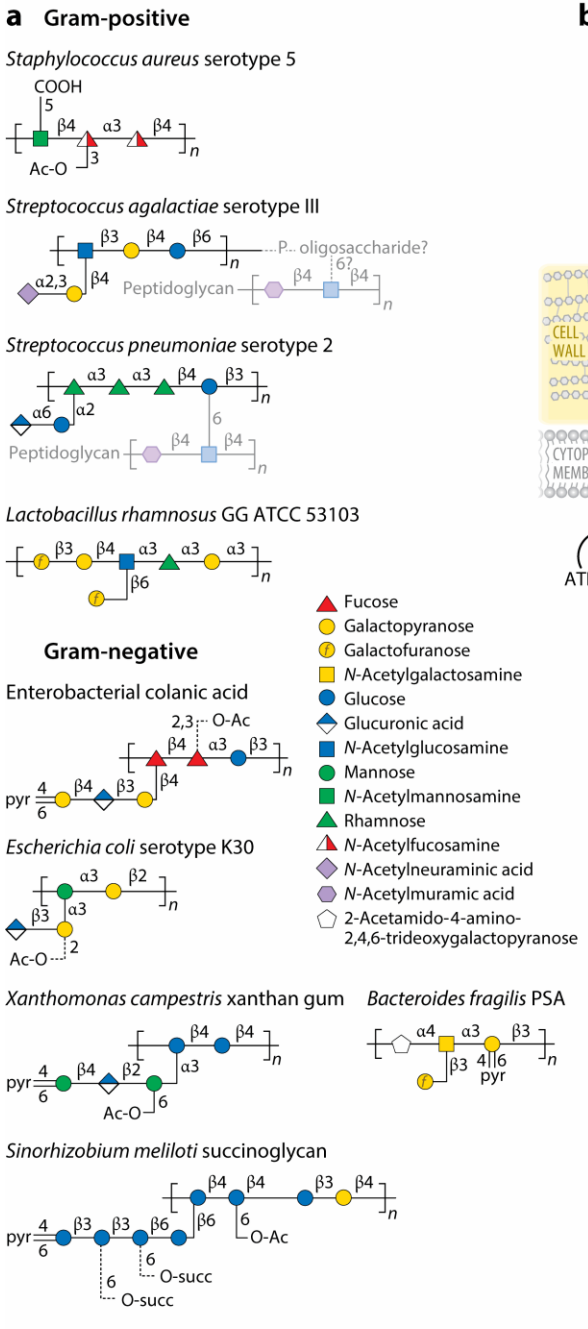
Altri tipi di glicoconiugati:
Proteine glicosilate, nonché polisaccaridi capsulari (CPS) ed esopolisaccaridi secreti

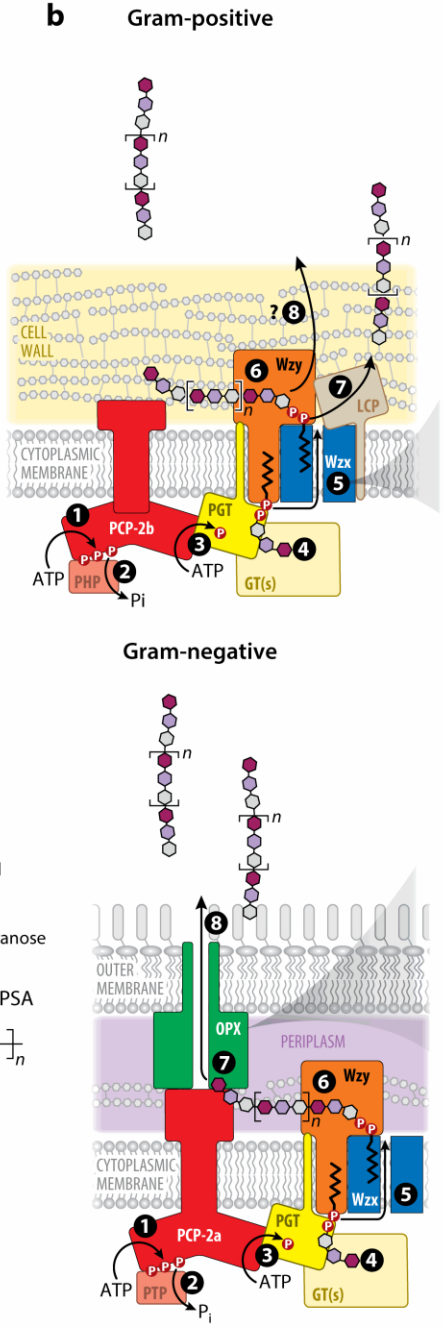
I CPS formano una struttura a capsula che copre la superficie del batterio. Sebbene le capsule siano state segnalate in molte specie, i sistemi di ancoraggio covalente sono attualmente limitati ai glicolipidi capsulari presenti in vari agenti patogeni, indicati come polisaccaridi extracellulari (EPS).

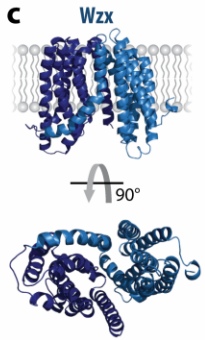
A) Esempi di strutture di unità ripetitive di EPS assemblate

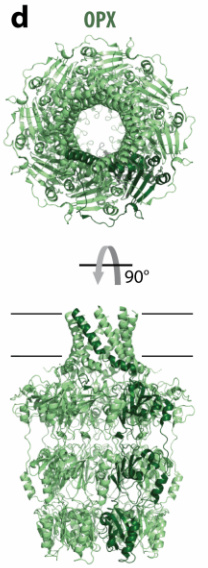
B) Modelli ipotetici per l'assemblaggio e l'esportazione in batteri gram-positivi e gram-negativi.

C) Modello di delle strutture di tipo Wzx (*Escherichia coli* K30) (d) la struttura del Ottamero di E. coli K30 OPX (Wza).







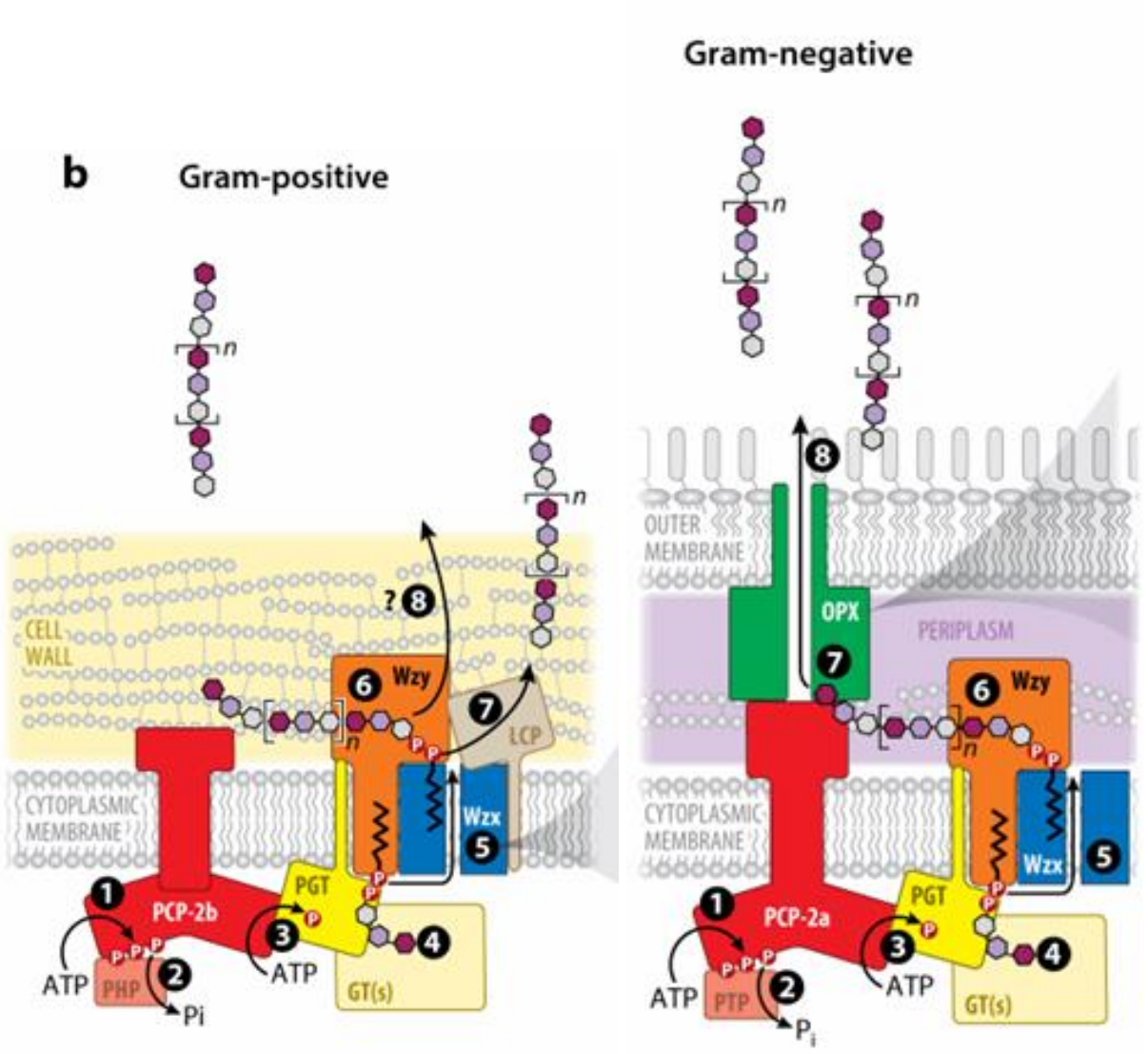


Whitfield C, et al. 2020.
Annu. Rev. Microbiol. 74:521–43

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

1- ASSEMBLAGGIO Wzy-DIPENDENTE DI ETEROPOLISACCARIDI RAMIFICATI COMPLESSI

La strategia più diffusa per la produzione di EPS è nota come percorso Wzy-dipendente e si trova sia nei batteri gram-positivi che in quelli -negativi (Figura 1a).

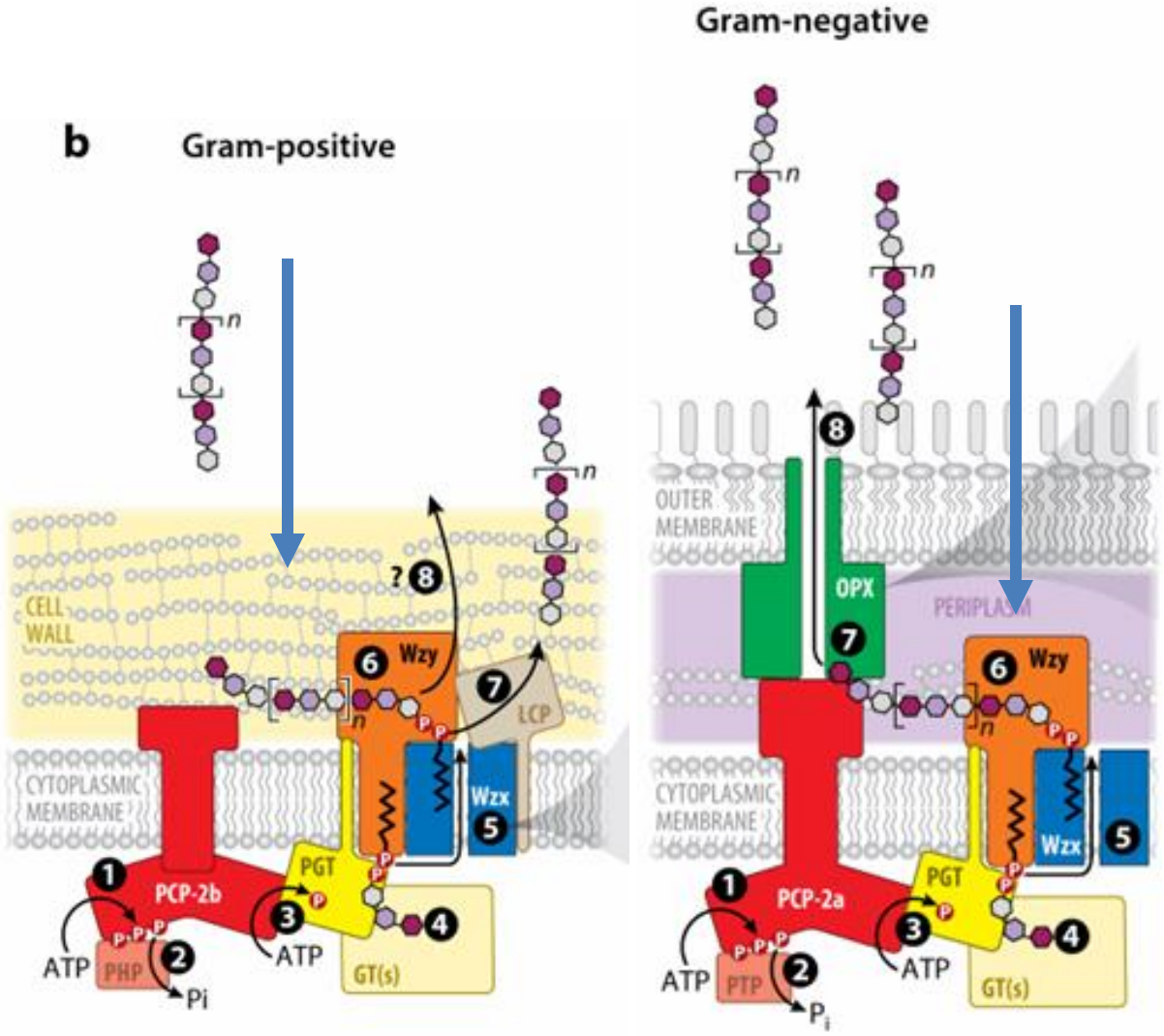
Il basi di questo sistema sono state descritte per la prima volta come pathways della biosintesi dell'antigene O dell' LPS
La polimerasi si chiama Wzy (precedentemente Rfc).

Data la conservazione della topologia di Wzy, la sua sequenza, e la funzione presunta, la designazione WZY è ora ampiamente applicata alla biosintesi dei polisaccaridi in diversi generi e specie batteriche.

Il sistema Wzy-dipendente viene utilizzato per costruire molte capsule, che proteggono i patogeni dalle difese immunitarie innate dell'ospite sopprimendo la risposta infiammatoria e fornendo resistenza ai peptidi antimicrobici, e all'uccisione mediata dal complemento e alla fagocitosi

1-Biosintesi ed esporto di unità ripetute di polisaccaridi extracellulari

PGT **phosphoglycosyltransferase**



2-Polimerizzazione di Unità Ripetute

Wzy polymerase reaction

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

Controllo della polimerizzazione—Polysaccharide Copolymerase Proteins

PCP protein

La dimensione dell'EPS e la distribuzione della lunghezza della catena richiede un processo che promuova l'estensione della catena. Le proteine PCP possiedono un dominio periplasmatico prevalentemente ad α-elica fiancheggiato da due TMH e oligomerizzano in una struttura che si estende dalla membrana. La maggior parte dei sistemi EPS Wzy-dipendenti si distinguono per le proteine PCP-2, che possiedono un'ulteriore chinasi citosolica della tirosina P loop (BY) assente nelle proteine PCP-1 dalla biosintesi dell'antigene O.
La fosforilazione della tirosina del componente PCP-2 e di altre proteine nella via della biosintesi gioca un ruolo essenziale nella capacità di polimerizzare l'EPS. È stato descritto che la fosforilazione della tirosina è necessaria per l'attività dell'enzima PGT (WcaJ) nella *K. pneumoniae*

4- Terminal Step nei Gram-Positivi—Ligation to Peptidoglycan or Release?

Le fasi di postpolimerizzazione divergono nei batteri gram-positivi e -negativi. Nei batteri gram-positivi, l'attacco dell'EPS al peptidoglicano è catalizzato da enzimi appartenenti alla famiglia LytR-CpsA-Psr (LCP).

Wzy-dependent pathway.
Steps ❶–❻ are conserved in both Gram- and Gram+.
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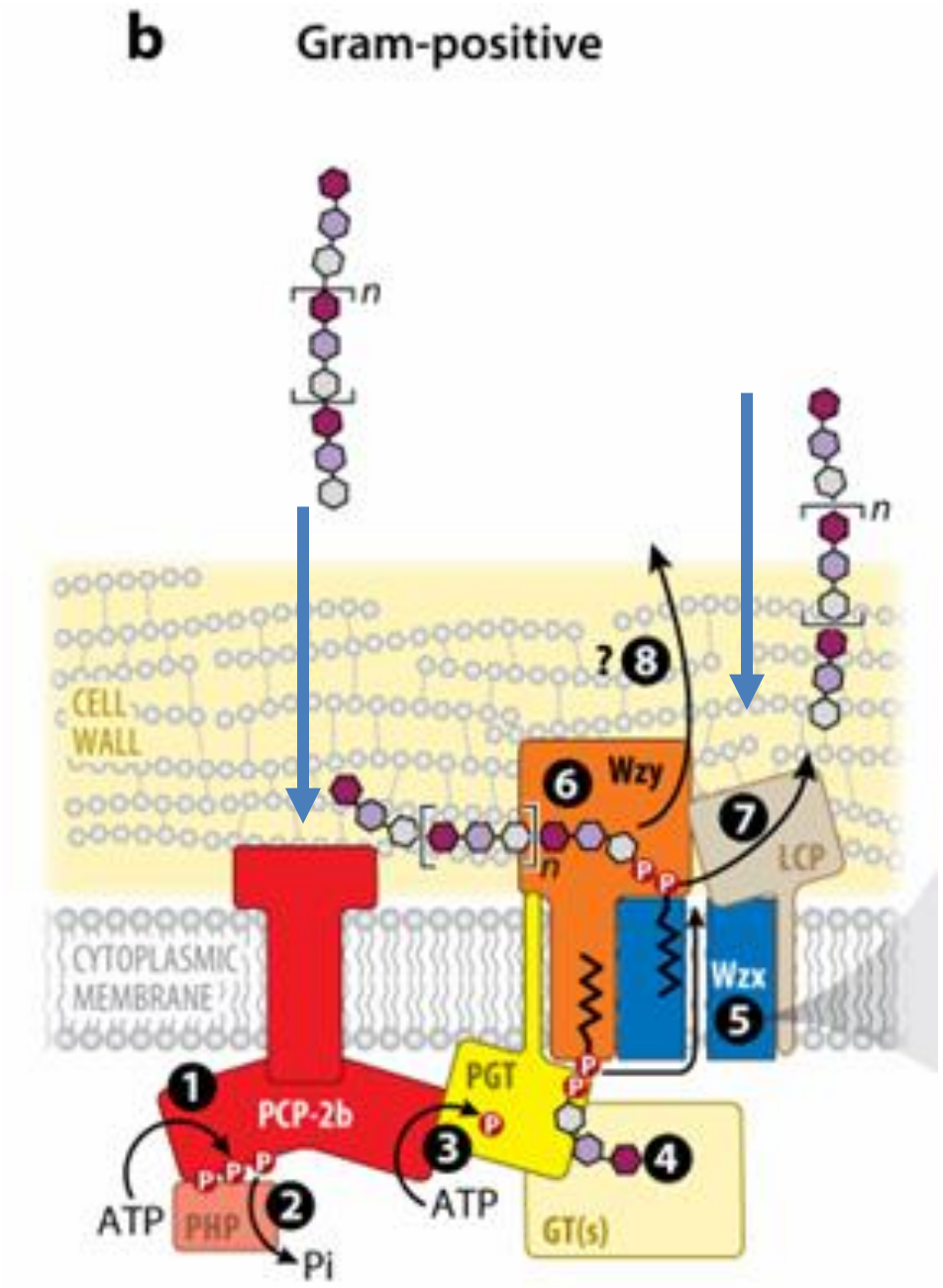
In gram-negative bacteria, the nascent polysaccharide is released by an unknown mechanism into a translocation pathway provided by the outer membrane polysaccharide (OPX) and polysaccharide copolymerase PCP-2a proteins (❼). Posttranslocation, the gram-negative EPS remains associated with the cell surface or is secreted into the environment.



Whitfield,
Polysaccharides and Exopolysaccharides **Annual Review of Microbiology**Vol. 74:521-543

5- Wza, the Outer Membrane Translocon in Gram-Negative Bacteria

La barriera di esclusione costituita dalla membrana esterna deve essere aggirata per la traslocazione dell'EPS nell'ambiente esterno, e ciò è ottenuto dalle proteine polisaccaridiche della membrana esterna (OPX), con E. coli Wza come prototipo.

Wza è una lipoproteina che forma un ottamero stabile (diametro interno 17 Å) composto da eliche α anfipatiche anziché come nelle solite proteine della membrana esterna del β barrels. La maggior parte dell'ottamero racchiude un lume periplasmatico di dimensioni sufficienti ad accogliere una catena EPS.

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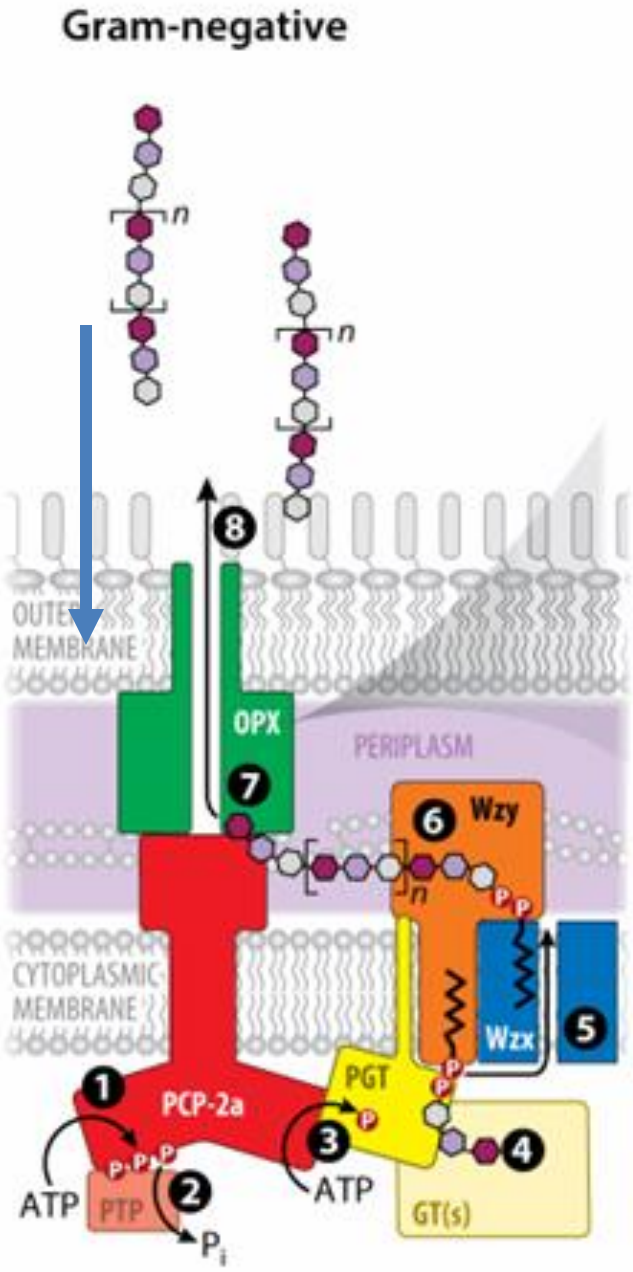
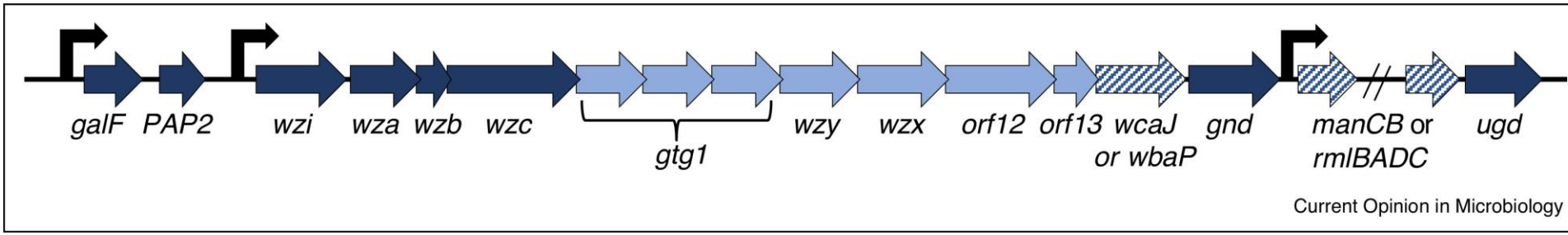


Capsular biosynthesis cluster



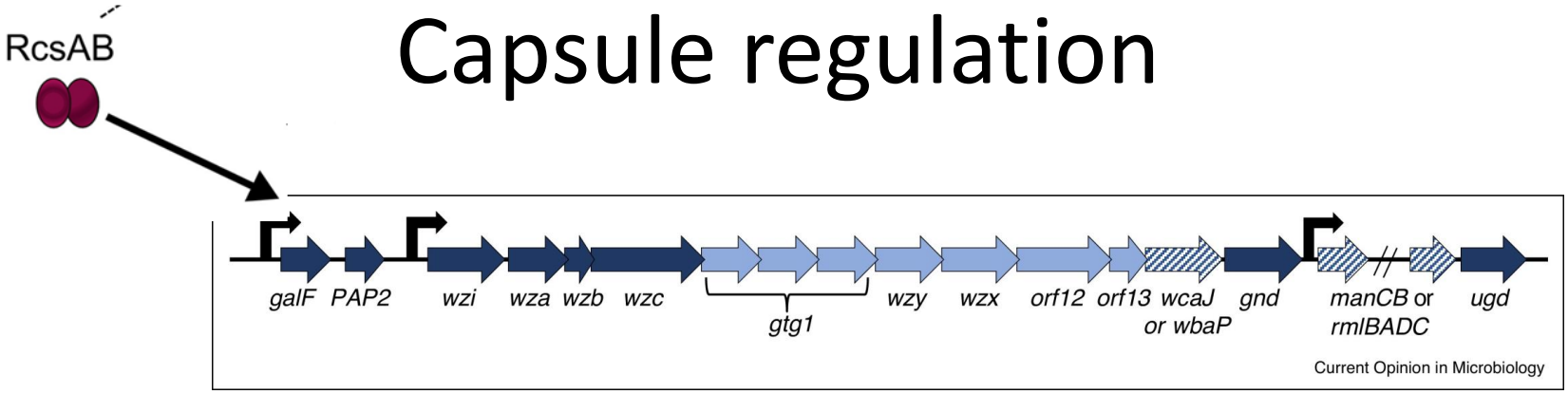
Tutti i ceppi di *K. pneumoniae* producono una capsula polisaccaridica extracellulare che rappresenta un fattore di virulenza e nella *Klebsiella* sono stati identificati più di 130 tipi diversi di capsule.

Geni conservati: *galF, pap2, wzi, wza, wzb and wzc*

GND: glucose-6-phosphate dehydrogenase
ugd: UDP-glucose dehydrogenase

Regione variabile: codificano per le proteine responsabili della polimerizzazione e dell'assemblaggio di specifiche subunità della capsula determinandone il tipo K.
nucleotide sugar-dependent glycosyltransferases

Capsule regulation



- Il complesso sistema di trasduzione del segnale Rcs comprende RcsC (sensore chinasi), RcsD (istidina fosfotransferasi), RcsB (regolatore della risposta), RcsA (proteina ausiliaria) e RcsF (lipoproteina della membrana esterna).
- RcsB dimerizza con RcsA e lega la sequenza di DNA chiamata box RcsAB che attiva l'espressione del cluster di capsule
- RcsAB si lega al promotore prima di galF/ORF1

Hypervirulent *K. pneumoniae* (hvKp)

Genes linked to hypervirulence (hv-associated genes)

3 siderofori

iroBCDN, iutA, iucABCD e *ybt*

salmochelin (*iroBCDN*),

aerobactin (*iutA, iucABCD*)

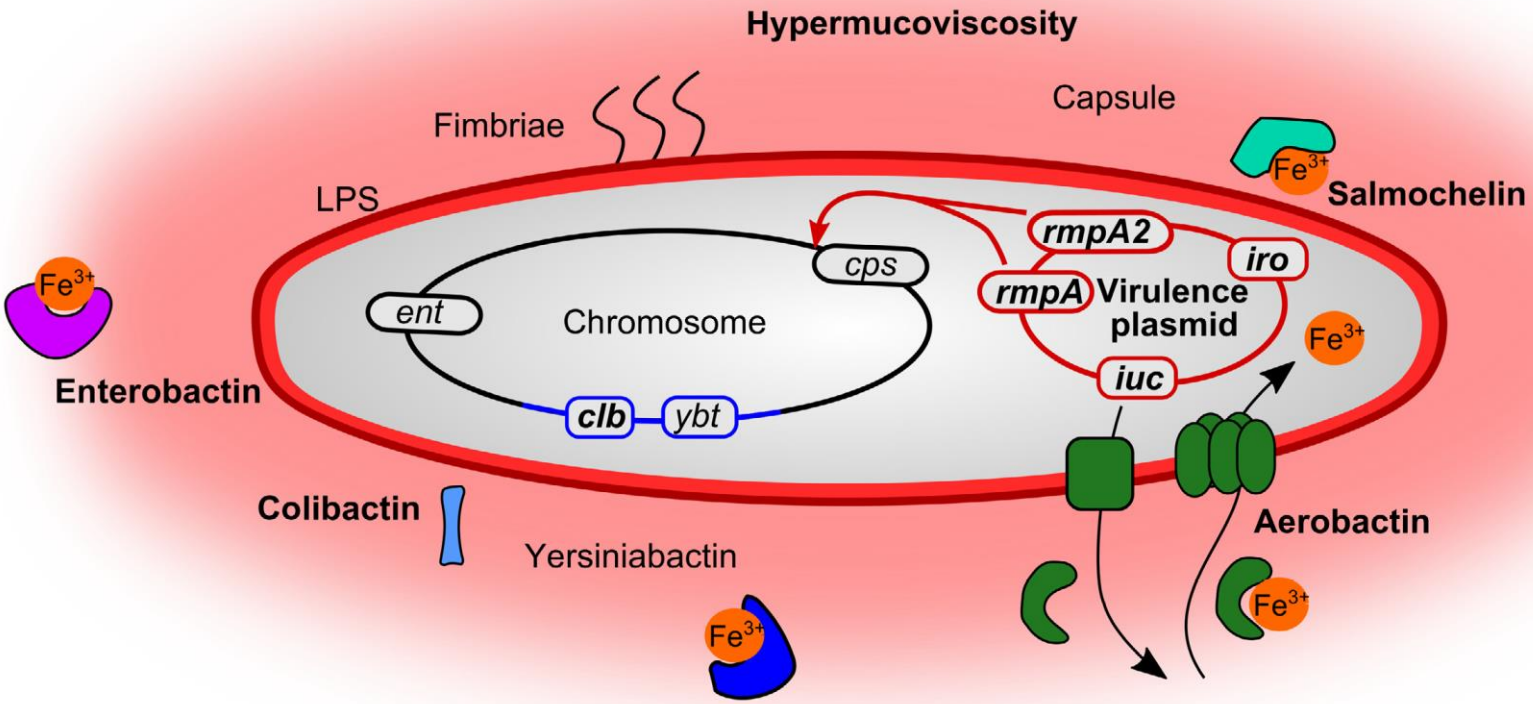
yersiniabactin (*ybt* genes)

Due fattori di regolazione trascrizionale

rmpA, rmpA2

Localizzati sul plasmide di virulenza pLVPK, in alcuni ceppi anche nel cromosoma

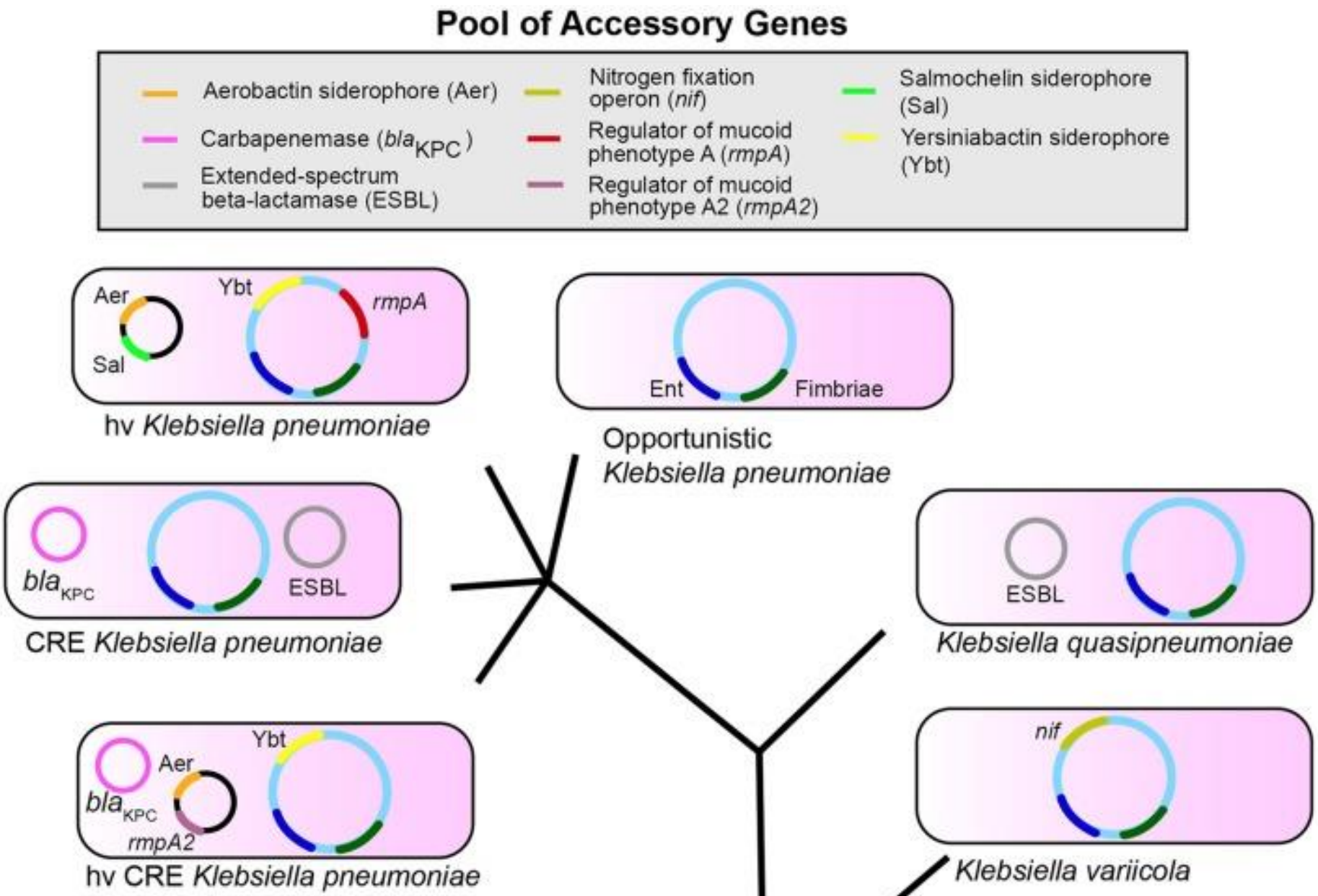
Hypervirulent *K. pneumoniae* (hvKp)



The Rmp regulators

RmpA and RmpA2

- RmpA e RmpA2 attivano i geni cps e attivano il promotore RmpA
- RpmA interagisce con RcsB, sostituendo RcsA nel dimero
- Fur che è un regolatore trascrizionale che risponde al ferro si lega a monte del promotore del gene *rmpA* e ne reprime l'espressione
- Regolatori della famiglia MarR: KvrA
- Sistemi KvgAS e KvhAS bicomponenti

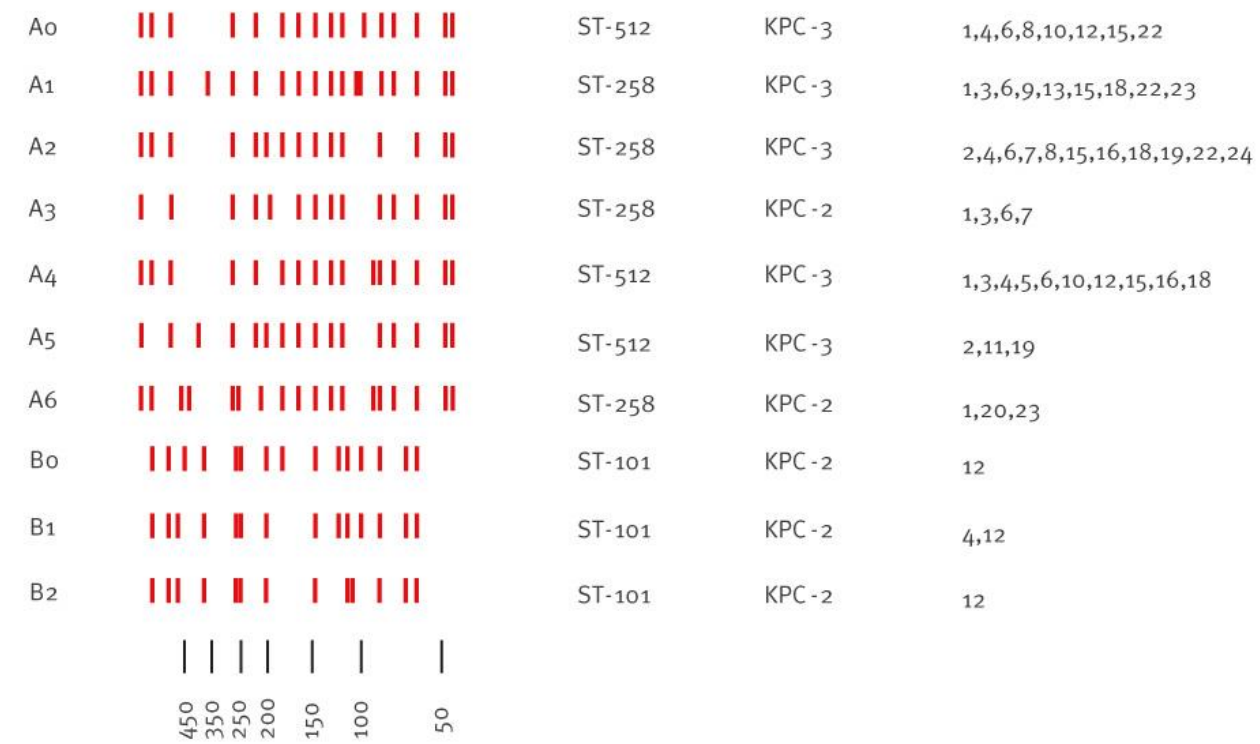


Martin RM, Bachman MA. Colonization, Infection, and the Accessory Genome of *Klebsiella pneumoniae*. Front Cell Infect Microbiol. 2018 Jan 22;8:4. doi: 10.3389/fcimb.2018.00004.

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

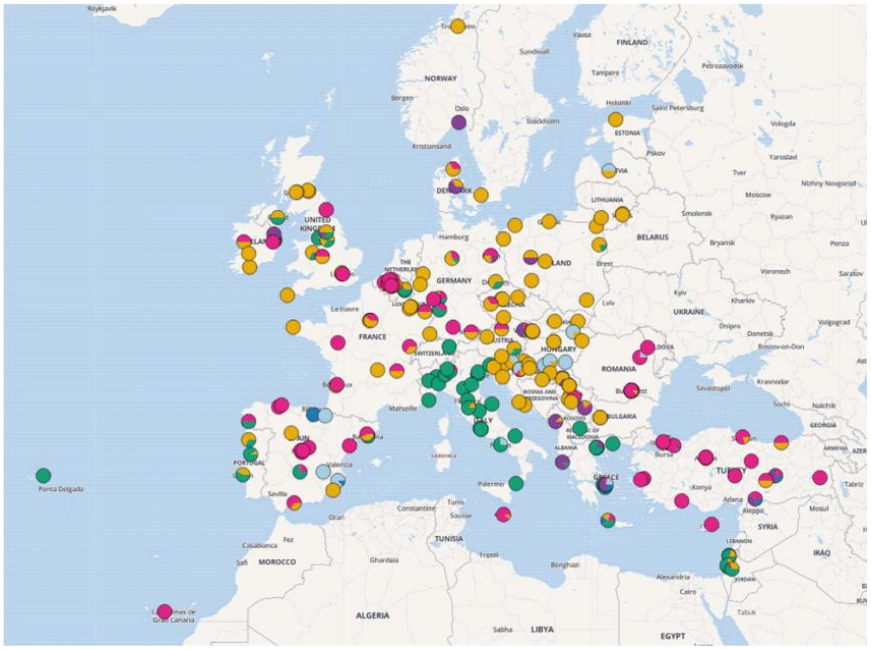
FIGURE 2

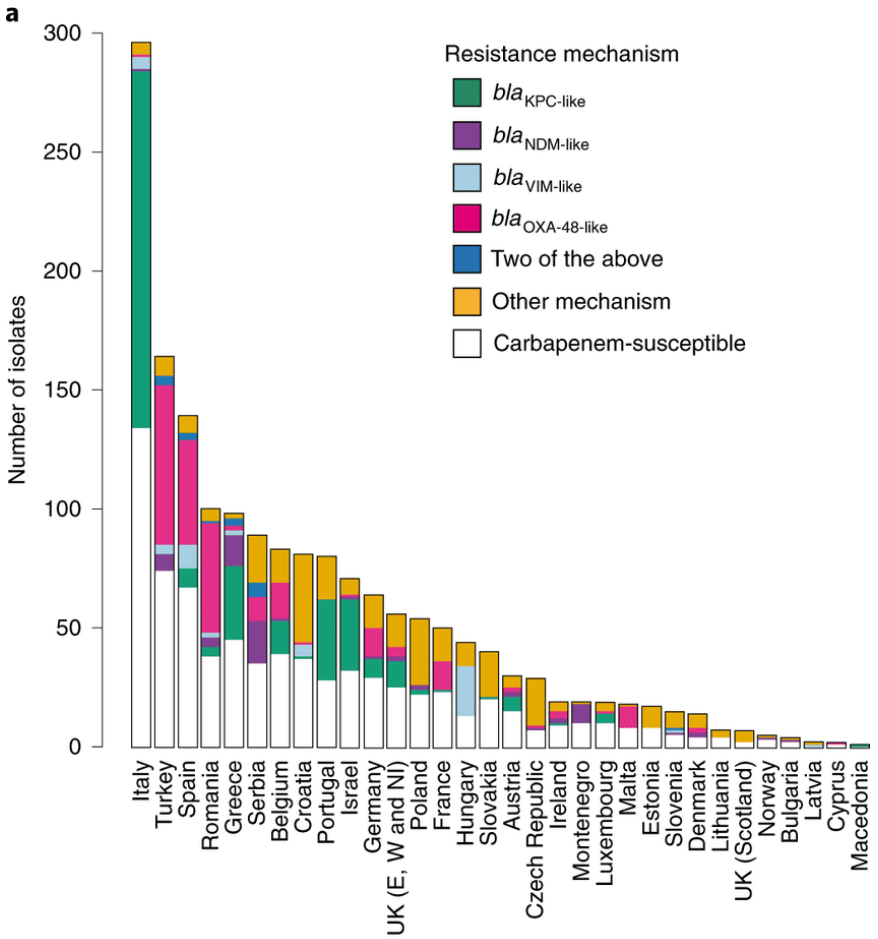
XbaI PFGE profiles of KPC-KP in combination with MLST results and KPC-type alleles, Italy, 15 May–30 June 2011 (n=204)



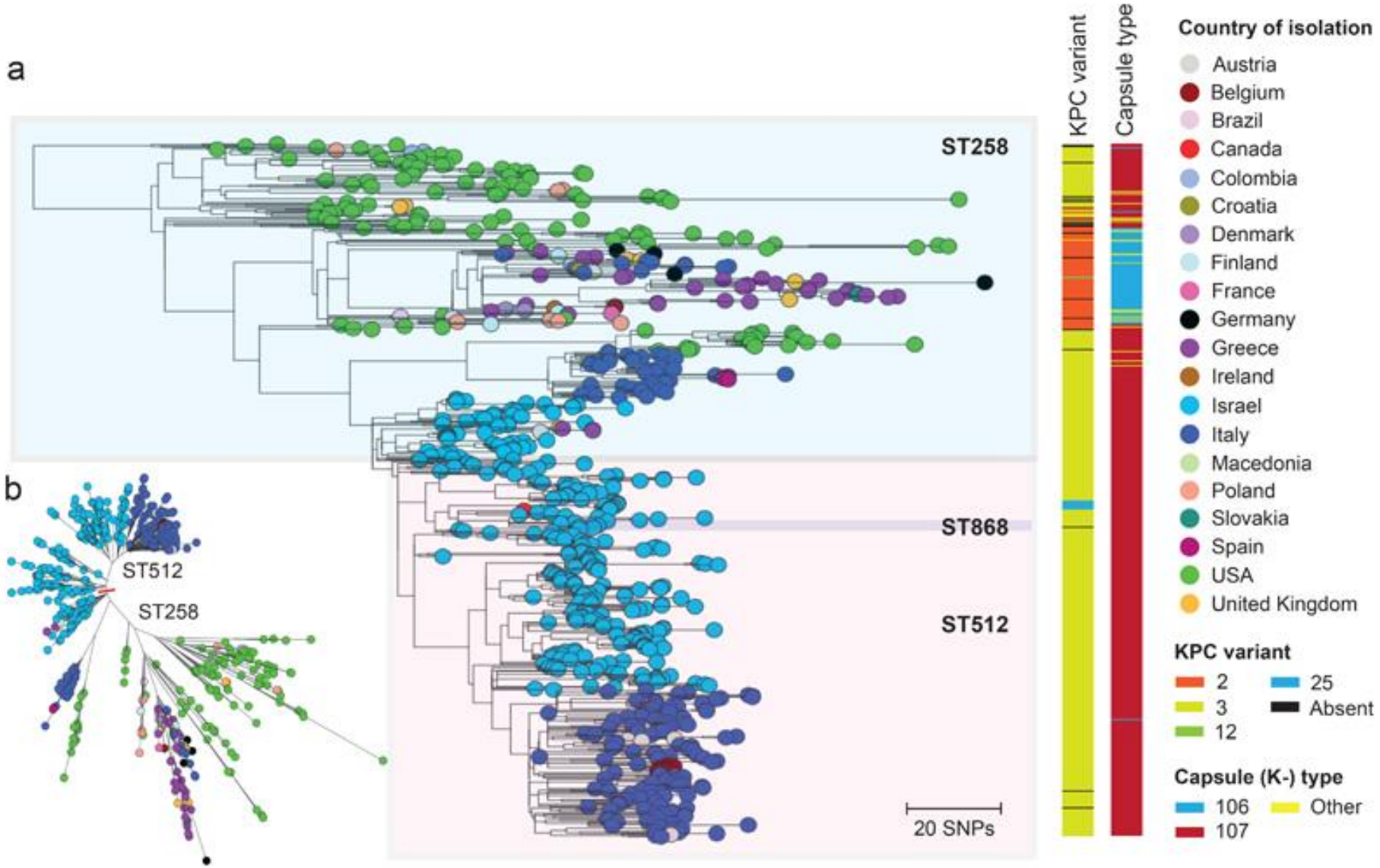
PFGE: pulsed-field gel electrophoresis; KPC-KP: KPC-type carbapenemase-producing *Klebsiella pneumoniae*; MLST: multi-locus sequence typing.

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

› mBio. 2014 Jun 24;5(3):e01355-14. doi: 10.1128/mBio.01355-14.

Epidemic Klebsiella pneumoniae ST258 is a hybrid strain

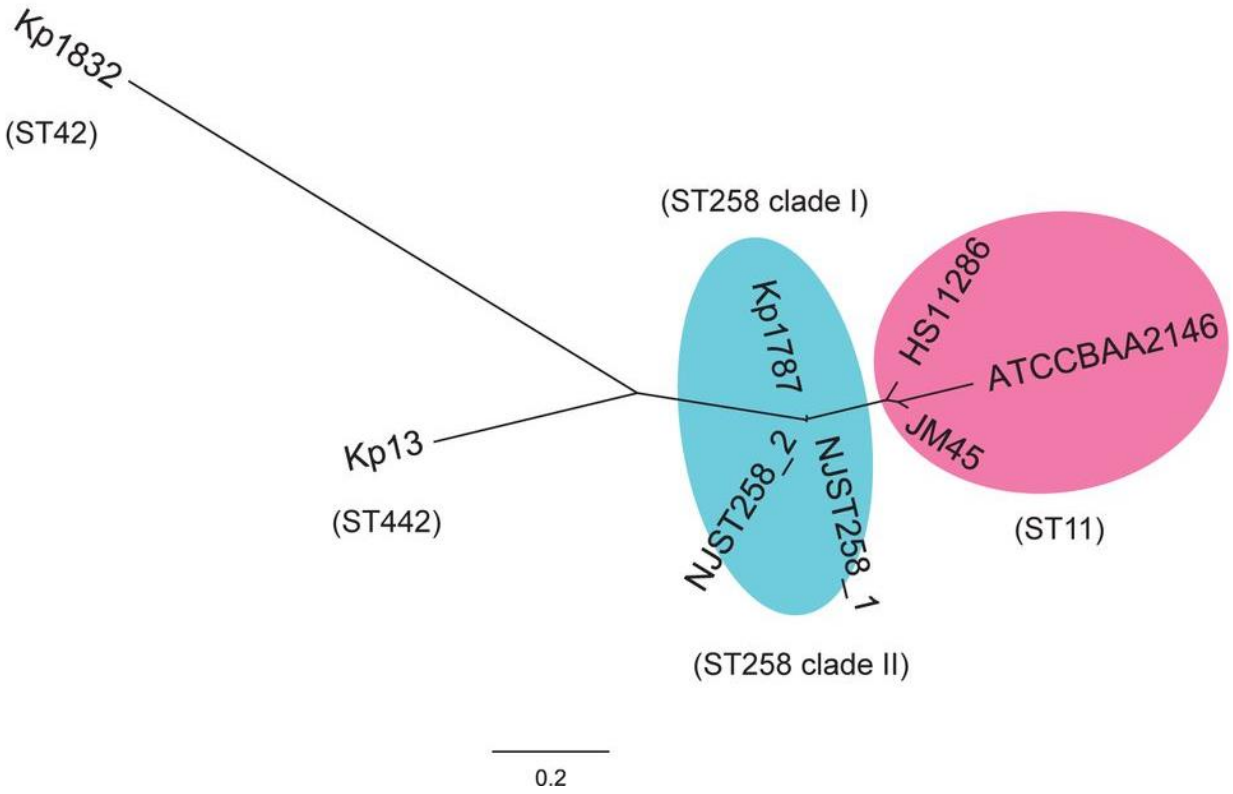
Liang Chen [1], Barun Mathema, Johann D D Pitout, Frank R DeLeo [2], Barry N Kreiswirth [3]

Affiliations + expand

PMID: 24961694 PMCID: PMC4073492 DOI: 10.1128/mBio.01355-14

due ceppi di riferimento (NJST258_1 and NJST258_2) genotipizzati come ST258 clade II, ceppo prototipico ST258 clade I (Kp1787) fa da reference per il clade I

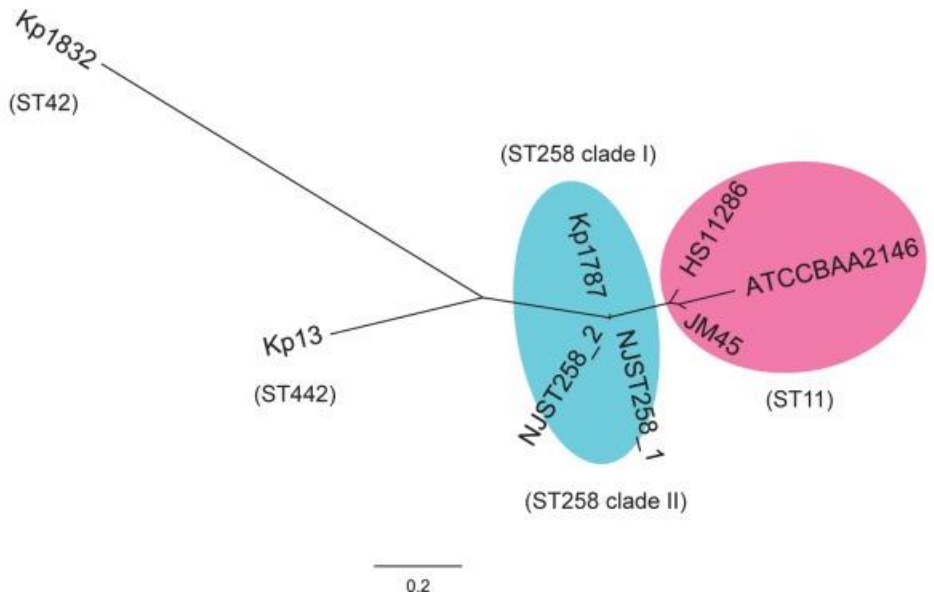
Here we compared the genetic structures and single-nucleotide polymorphism (SNP) distributions in the core genomes of strains from two ST258 clades and other STs (ST11, ST442, and ST42). We identified an ~1.1-Mbp region on ST258 genomes that is homogeneous to that of ST442, while the rest of the ST258 genome resembles that of ST11.



A

	NJST258_2	Kp1787	JM45	HS11286	ATCC BAA-2146	Kp13	Kp1832
NJST258_1	175/43	508/360	8180/8027	8056/7742	12829/12661	21035/217	31304/8566
NJST258_2		445/337	8117/8004	7993/7719	12766/12638	20966/194	31240/8543
Kp1787			8087/7968	8056/7771	12743/12609	21284/503	30927/8222
JM45				3621/3370	6784/6681	28841/8086	30395/7718
HS11286					8183/7915	28522/7761	30080/7414
ATCC BAA-2146						33493/12721	35032/12338
Kp13							31848/8590

B

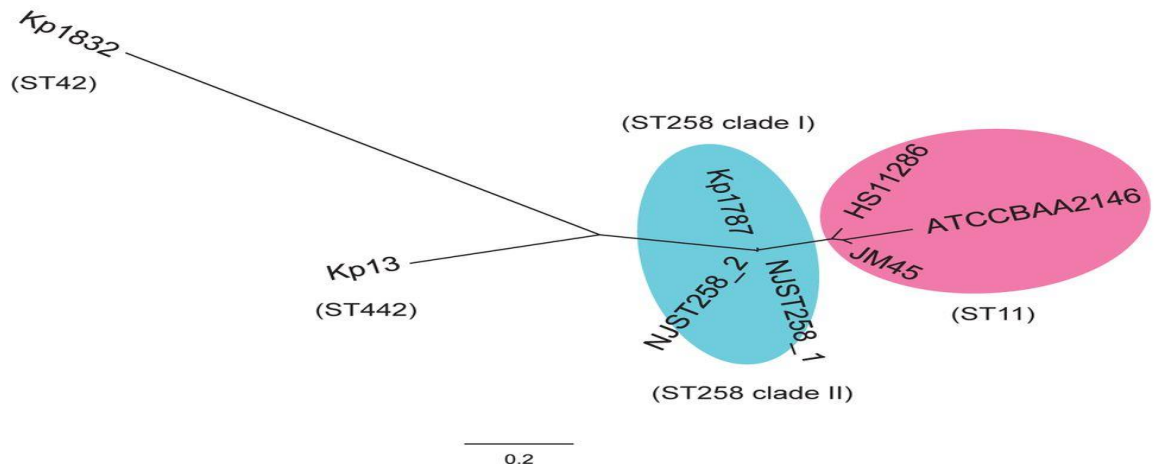


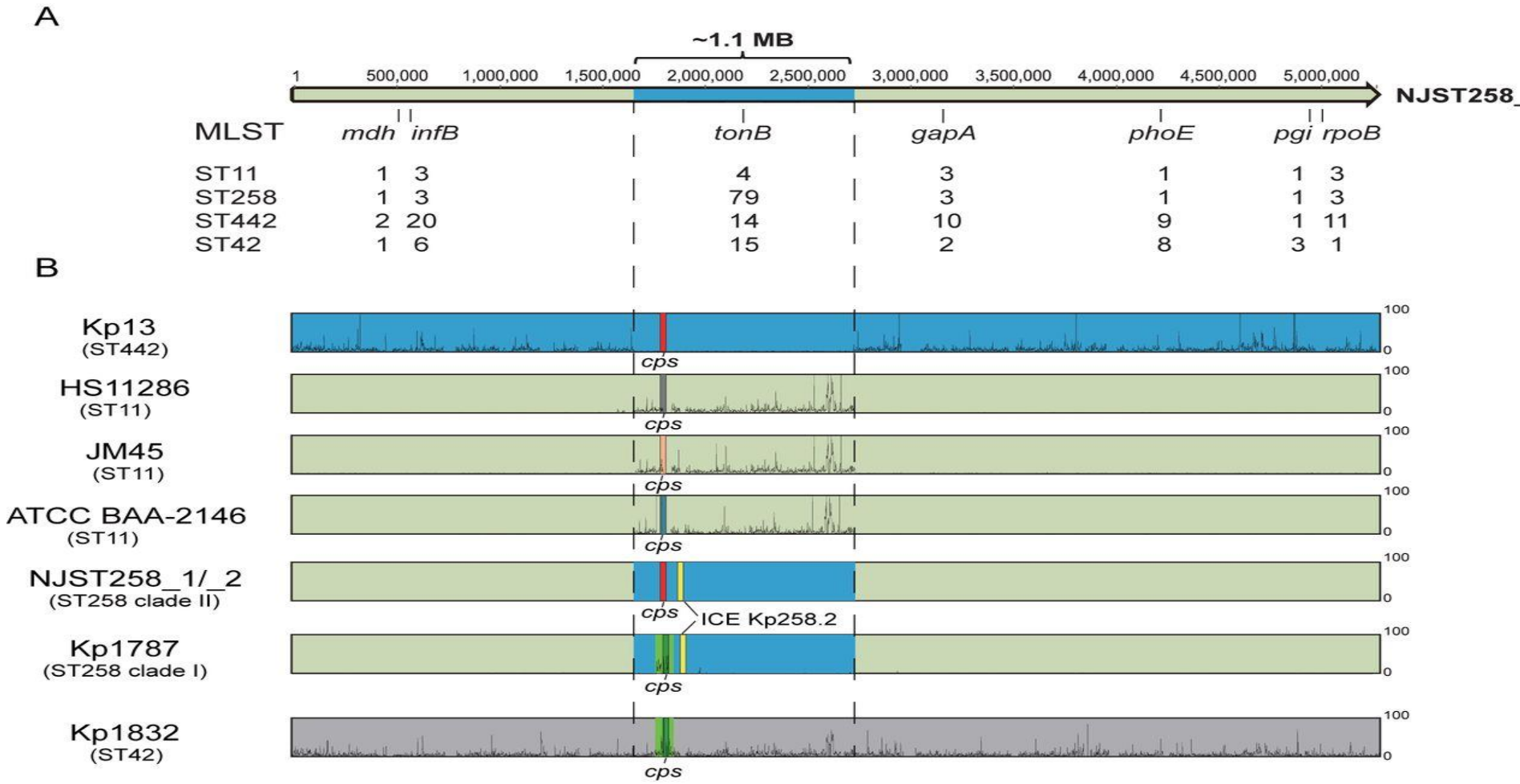
Our results suggest ST258 is a hybrid clone 80% of the genome originated from ST11-like strains and 20% from ST442-like strains. Meanwhile, we sequenced an ST42 strain that carries the same K-antigen-encoding capsule polysaccharide biosynthesis gene (cps) region as ST258 clade I strains. Comparison of the cps-harboring regions between the ST42 and ST258 strains (clades I and II) suggests the ST258 clade I strains evolved from a clade II strain as a result of cps region replacement.

A

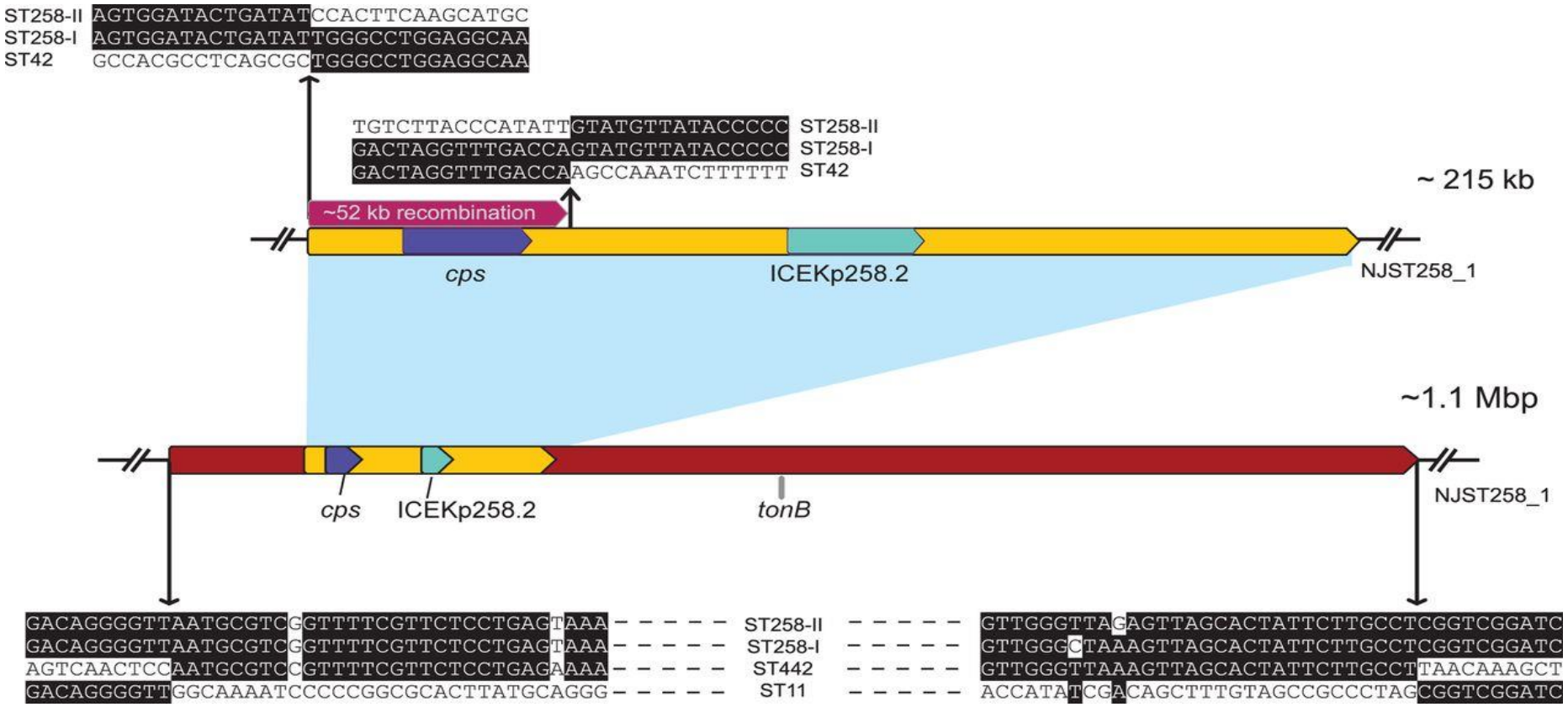
	NJST258_2	Kp1787	JM45	HS11286	ATCC BAA-2146	Kp13	Kp1832
NJST258_1	175/43	508/360	8180/8027	8056/7742	12829/12661	21035/217	31304/8566
NJST258_2		445/337	8117/8004	7993/7719	12766/12638	20966/194	31240/8543
Kp1787			8087/7968	8056/7771	12743/12609	21284/503	30927/8222
JM45				3621/3370	6784/6681	28841/8086	30395/7718
HS11286					8183/7915	28522/7761	30080/7414
ATCC BAA-2146						33493/12721	35032/12338
Kp13							31848/8590

B

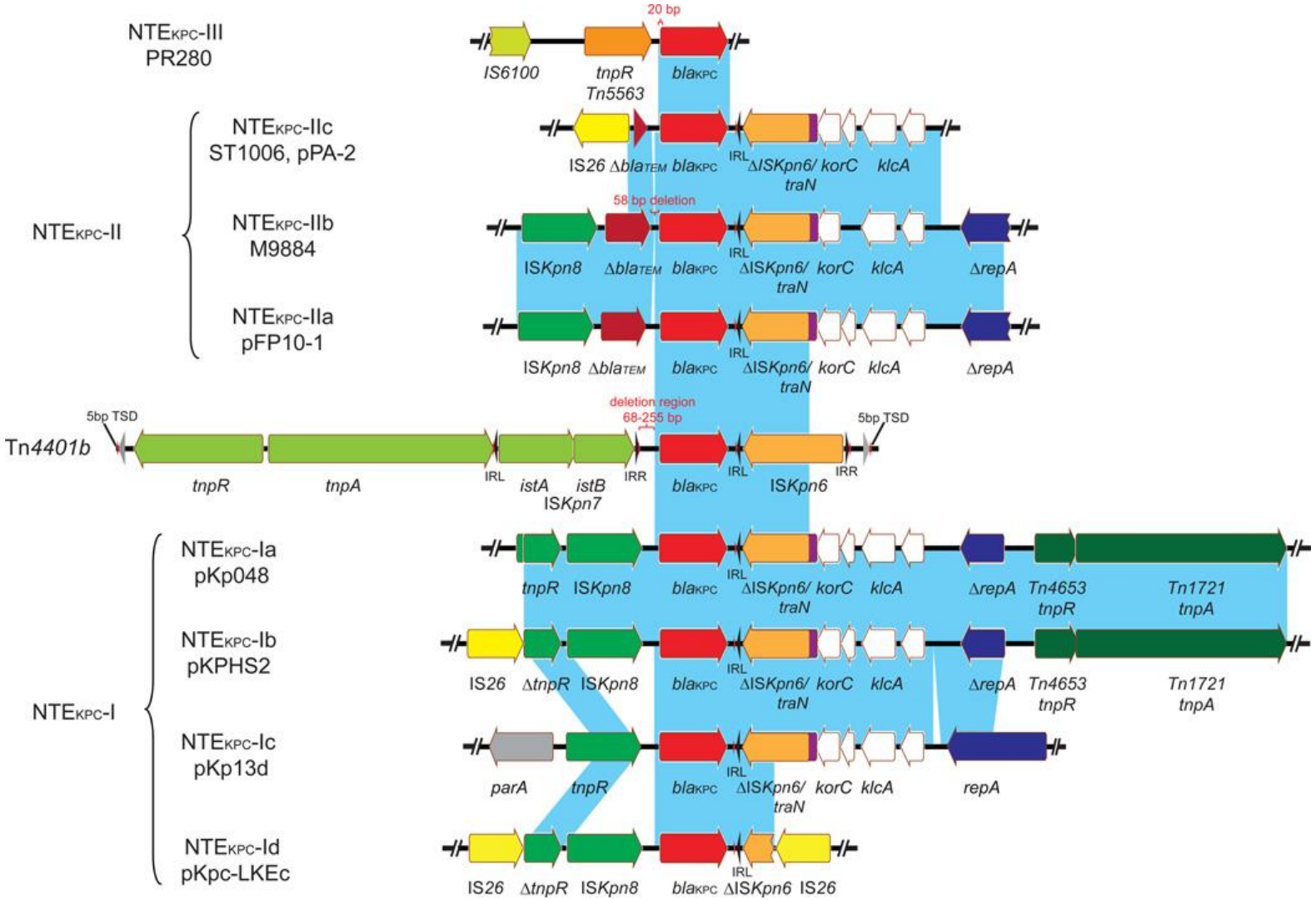




Upstream and downstream junction SNPs for the ~1.1-Mbp recombination fragment and *cps* region in ST258, ST442, and ST42 strains. The start site of the replacement of the ~52-kb *cps*-harboring region is the same as that of the ~215-kb RD in ST258 II clades



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



bla$_{KPC}$-harboring genetic elements (Tn*4401* and NTE$_{KPC}$). Based on the insertion sequence upstream of *bla*$_{KPC}$, NTE$_{KPC}$ can be divided into three groups: NTE$_{KPC}$-I, no insertion [48]; NTE$_{KPC}$-II, insertion of Δ*bla*$_{TEM}$ [48]; and NTE$_{KPC}$-III, insertion of Tn*5563*/IS*6100* [78]. NTE$_{KPC}$-I can be further classified as -Ia (prototype, pKp048) [48], -Ib (pKPHS2) [29], -Ic (pKp13d) [23] and -Id (pKPC-LKEc) [79] based on the insertion sites of upstream and/or downstream of IS*26* and the presence of IS*Kpn8*. NTE$_{KPC}$-II can be subgrouped as -IIa (pFP10-1, and *bla*$_{KPC}$-harboring plasmids from strain M9196 and M11180) [49, 80], -IIb (from strain M9884 and M9988) [49], and -IIc (pPA-2) [50], based on the differences of the length of Δ*bla*$_{TEM}$ and the deletions. Light-blue shading denotes shared regions of homology.

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LETTERS

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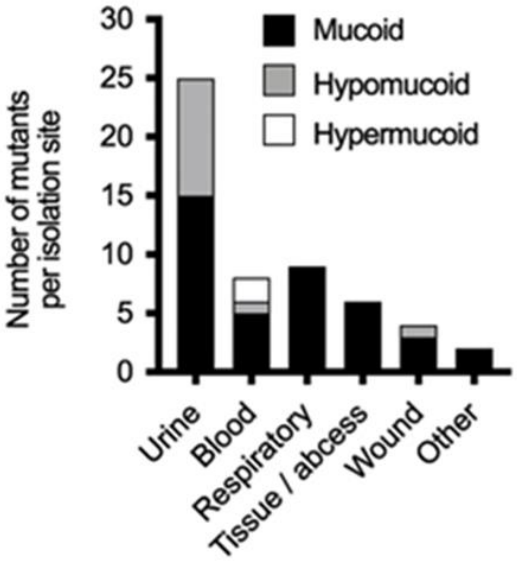
Adaptive evolution of virulence and persistence in carbapenem-resistant *Klebsiella pneumoniae*

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] ✉

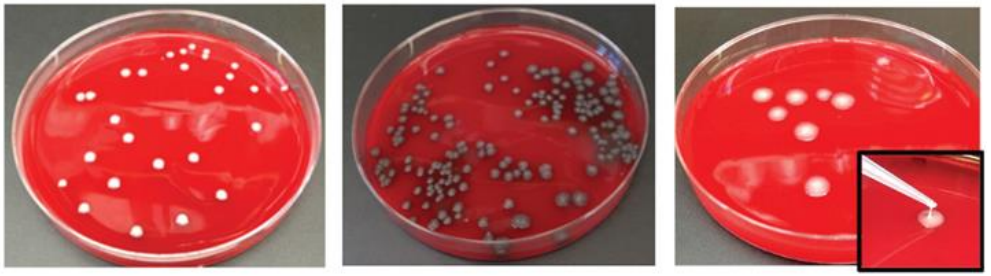
To date, the particular virulence plasmid (pLVPK) has not been detected in carbapenem-resistant *K. pneumoniae* strains of the **ST258** sequence type, which are endemic in the United States and Europe , and only a single ST258 isolate has been reported to carry hypervirulence-associated siderophores. 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

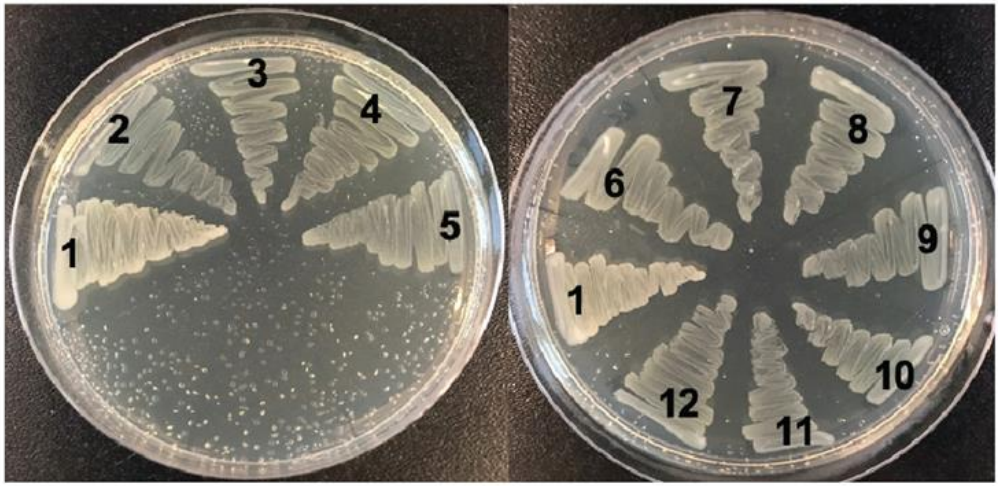
a



a



b



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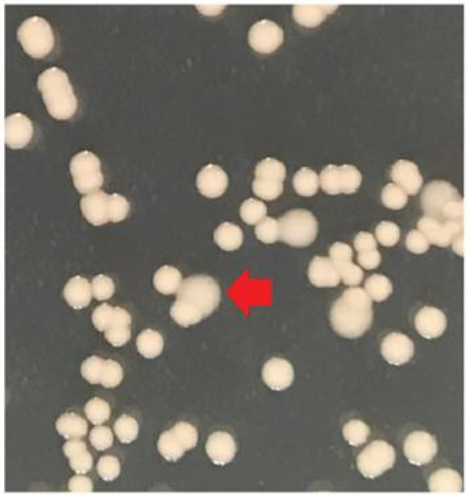
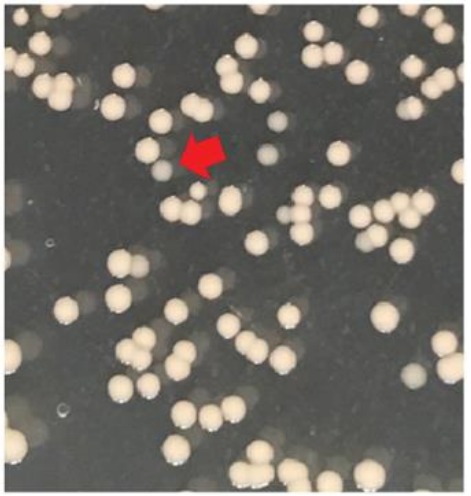
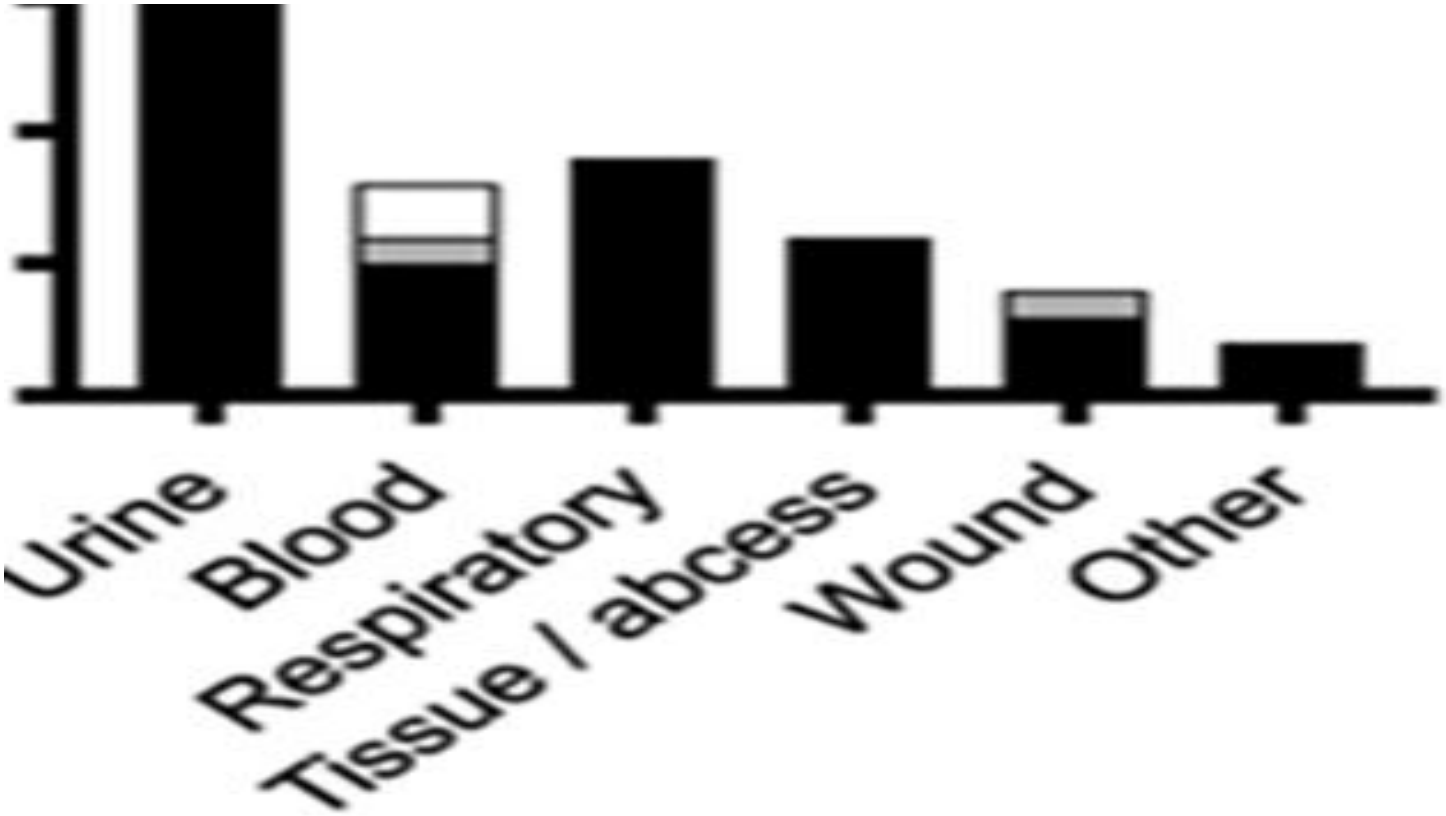
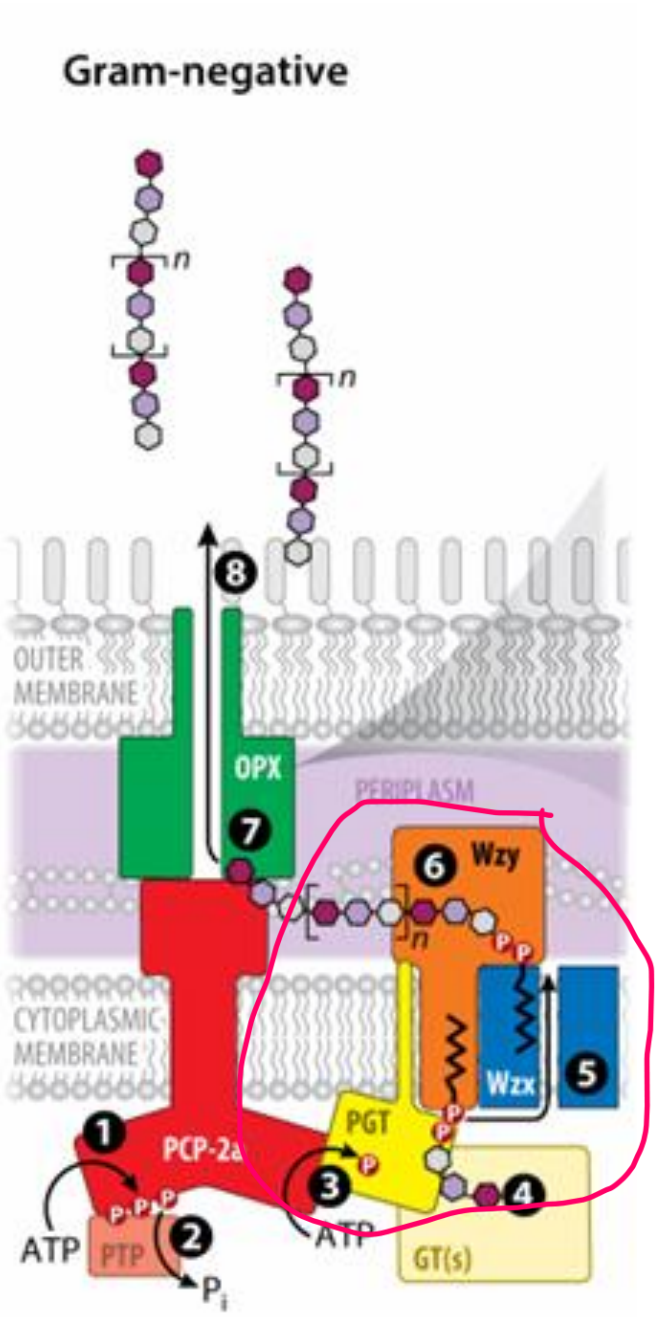




We first considered the capsule-deficient strains. As the capsule is thought to be essential for infection, we were surprised to identify capsule-deficient ST258 strains among the clinical urine isolates, because such strains had been assumed to be avirulent.

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***



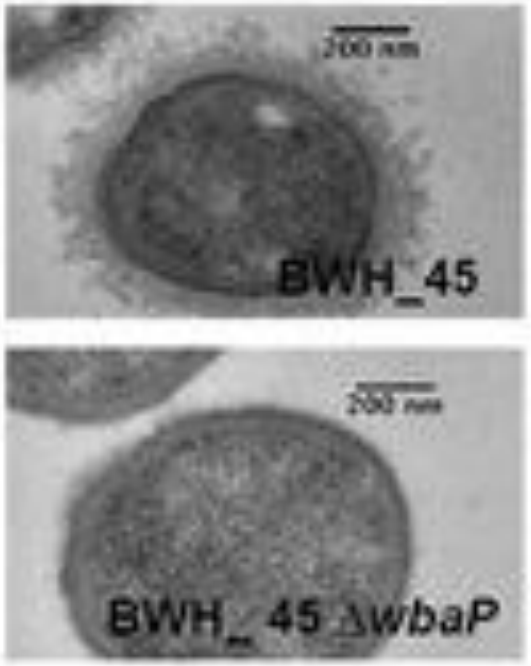


PGTs generate Und-PP-linked hexoses (e.g., CpsE in some *S. pneumoniae* capsule serotypes and **WbaP**

Wzy reaction transfers a growing glycan from its Und-PP carrier to the nonreducing terminus of the incoming Und-PP-repeat unit

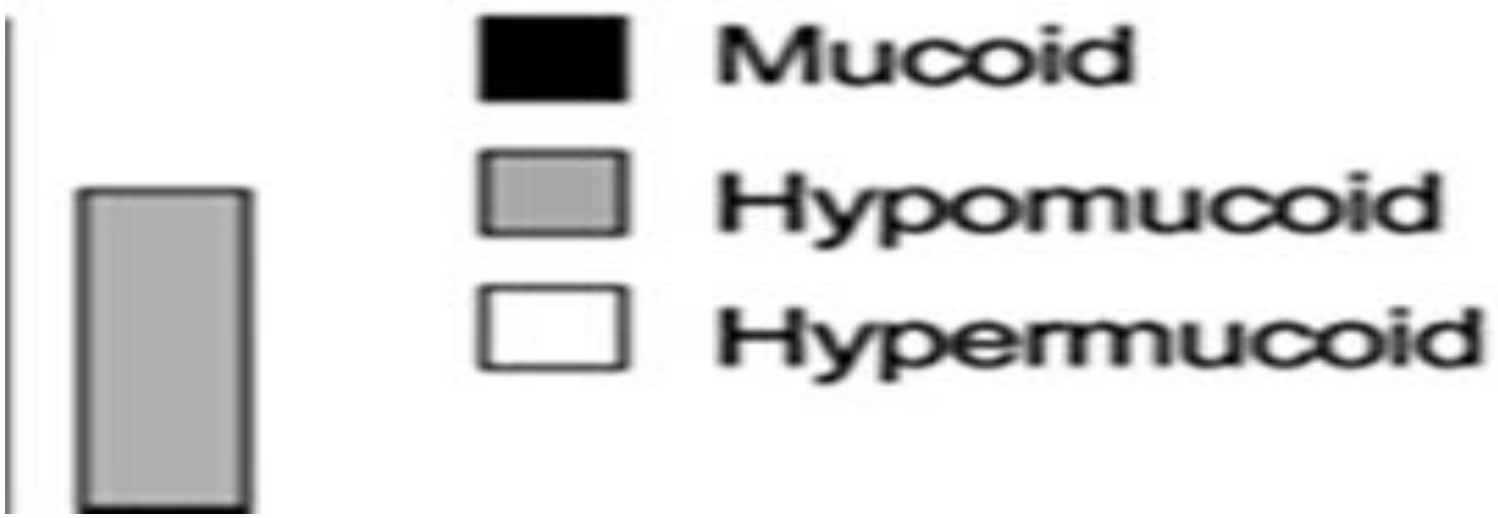
We engineered a mutant strain to confirm that deletion of *wbaP* in the normal capsule-producing clinical strain UCI_38 (UCI_38ΔwbaP) did indeed abolish capsule production, which could be restored by complementation with *wbaP* (Figs. 1b and 2a). Deletion of *wbaP* in two other clinical ST258 isolates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

Reintroducing the *wbaP* gene the capsule production is restored



oid

Analysis of the hypercapsulated strains
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.

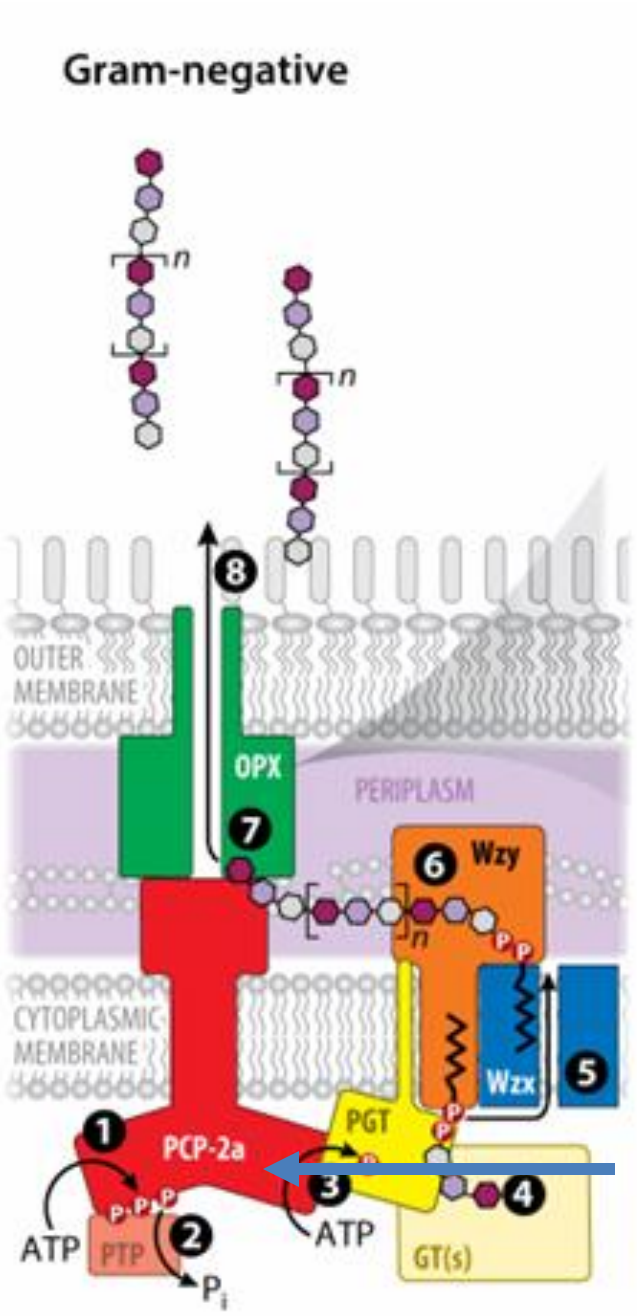


Number per iso

10

5

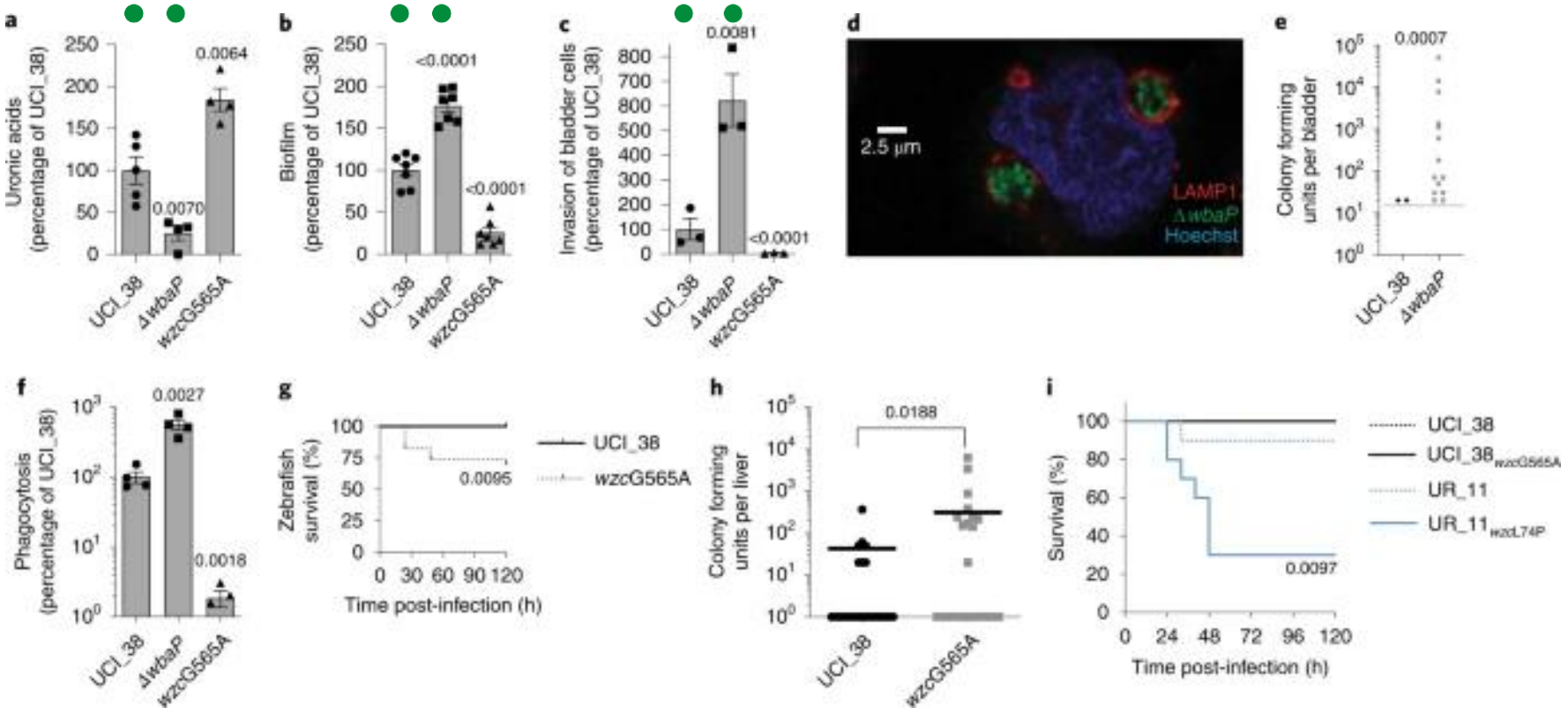
0



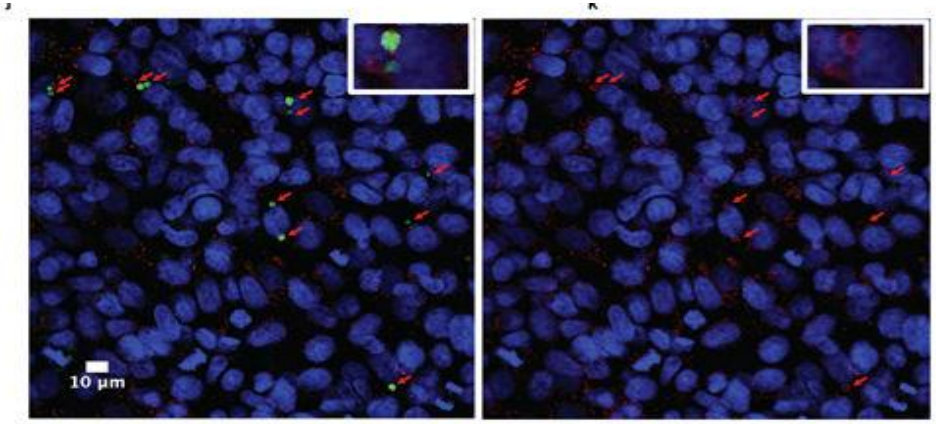
3-Controlling Polymerization—The Polysaccharide Copolymerase Proteins

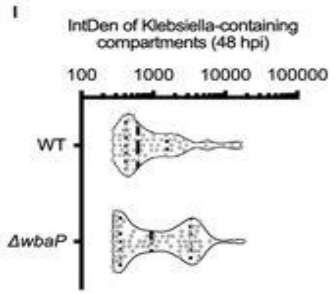
Tyrosine phosphorylation of the PCP-2 component, and other proteins in the biosynthesis pathway, plays an essential role in the ability to polymerize EPS. In most gram-negative examples, the transmembrane and C-terminal kinase domains are fused in a single PCP-2a protein; the prototype (**Wzc**) is from *E. coli* serotype K30 group 1 capsule and colanic acid biosynthesis.

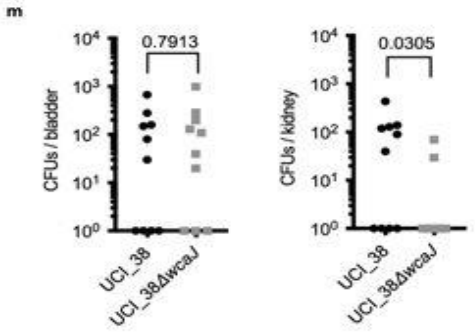
We thus investigated the impact of capsule inactivation on phenotypes associated with UTIs, including biofilm formation and infection of bladder epithelial cells, features crucial for uropathogenic E. coli to establish and persist in UTIs. The engineered deletion strain UCI_38ΔwbaP and the closely related hypocapsule clinical isolate UCI_38wbaP::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

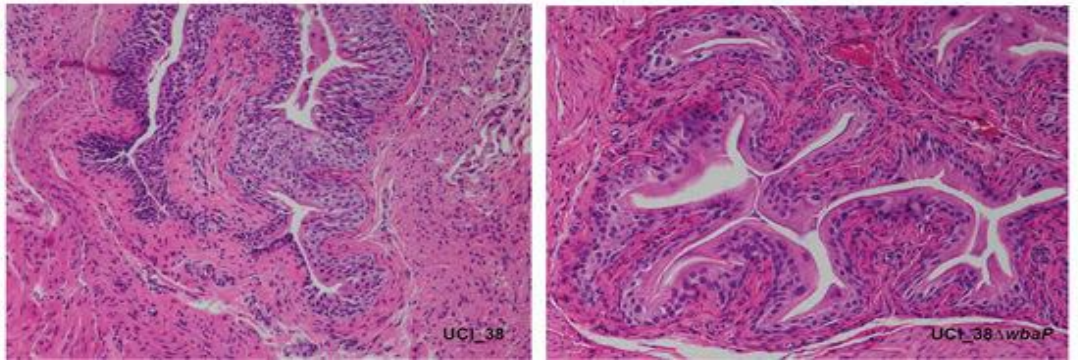


Deletion of *wbaP* increased invasion and intracellular replication in LAMP1-positive vacuoles, and resulted in a tenfold larger intracellular bacterial reservoir for UCI_38ΔwbaP mutants compared with the isogenic parent UCI_38, which persisted over the course of 48 h.

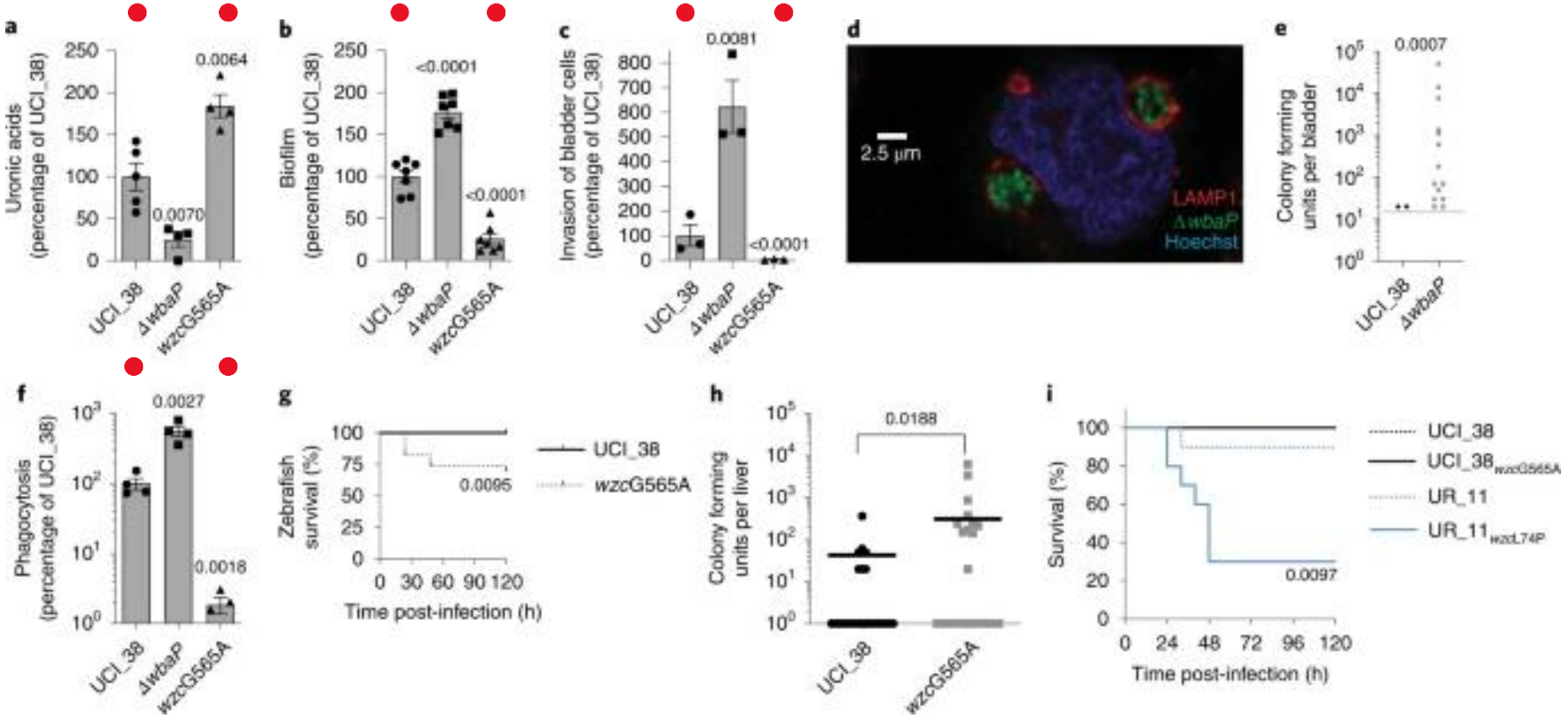






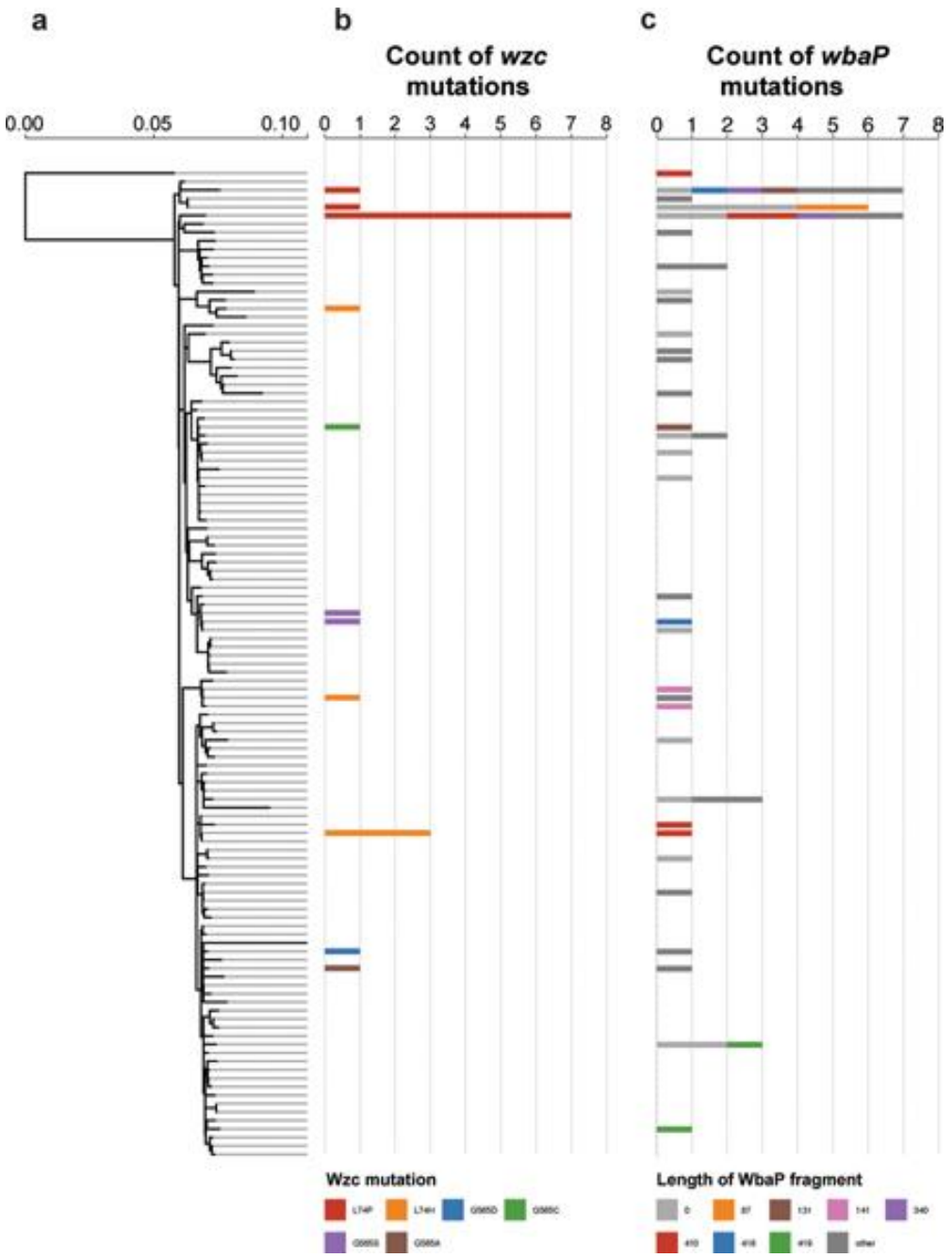


- single mutations in **wzc** (wzcG565S/A/R, wzcA535E) confer **phagocytosis resistance and consistently enhance the virulence of ST258 in multiple animal infection models**. Bloodstream infection of zebrafish larvae with a non-lethal dose of UCI_38 results in lethality after infection with the isogenic hypercapsule wzc mutant UCI_38wzcG565A
- Dissemination of UCI_38 and UCI_38wzcG565A from the bladder to the liver in murine UTIs. The hypercapsule mutant displays greater dissemination to the liver than the isogenic, wild-type parent, as demonstrated by bacterial numbers (c.f.u.) recovered in mouse livers



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.