

Rapid reshuffling of Tn4401 transposon variants and plasmids carrying *bla*_{KPC} in *Klebsiella pneumoniae* ST196 within a single hospital outbreak

Anna E Sheppard, Nicole Stoesser, David Eyre, Robert Sebra, Kasi Vegesana, Ian Mesner, John Ainsworth, Tim E Peto, A. Sarah Walker, Derrick W Crook, Amy J Mathers

23rd April 2017

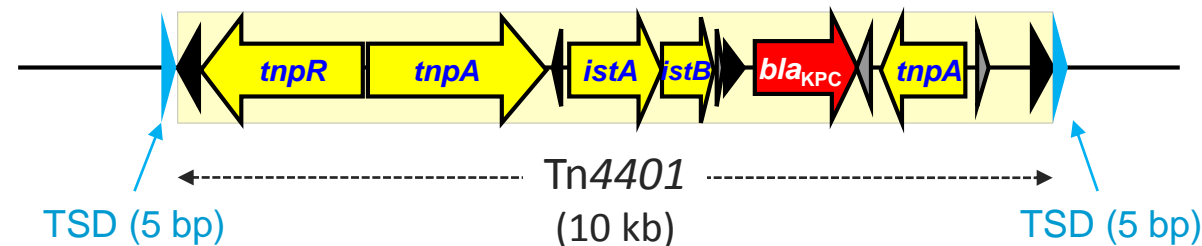


Conflicts of interest

- None

Klebsiella pneumoniae carbapenemase (KPC)

- Ambler Class A serine β -lactamase
- Hydrolyzes all β -lactams; penicillins, extended spectrum cephalosporins, aztreonam and carbapenems
- Found across various *Enterobacteriaceae* species
- bla_{KPC} is usually found within Tn4401, which undergoes replicative transposition with 5 bp target site duplication (TSD)



Long-term multi-species KPC outbreak



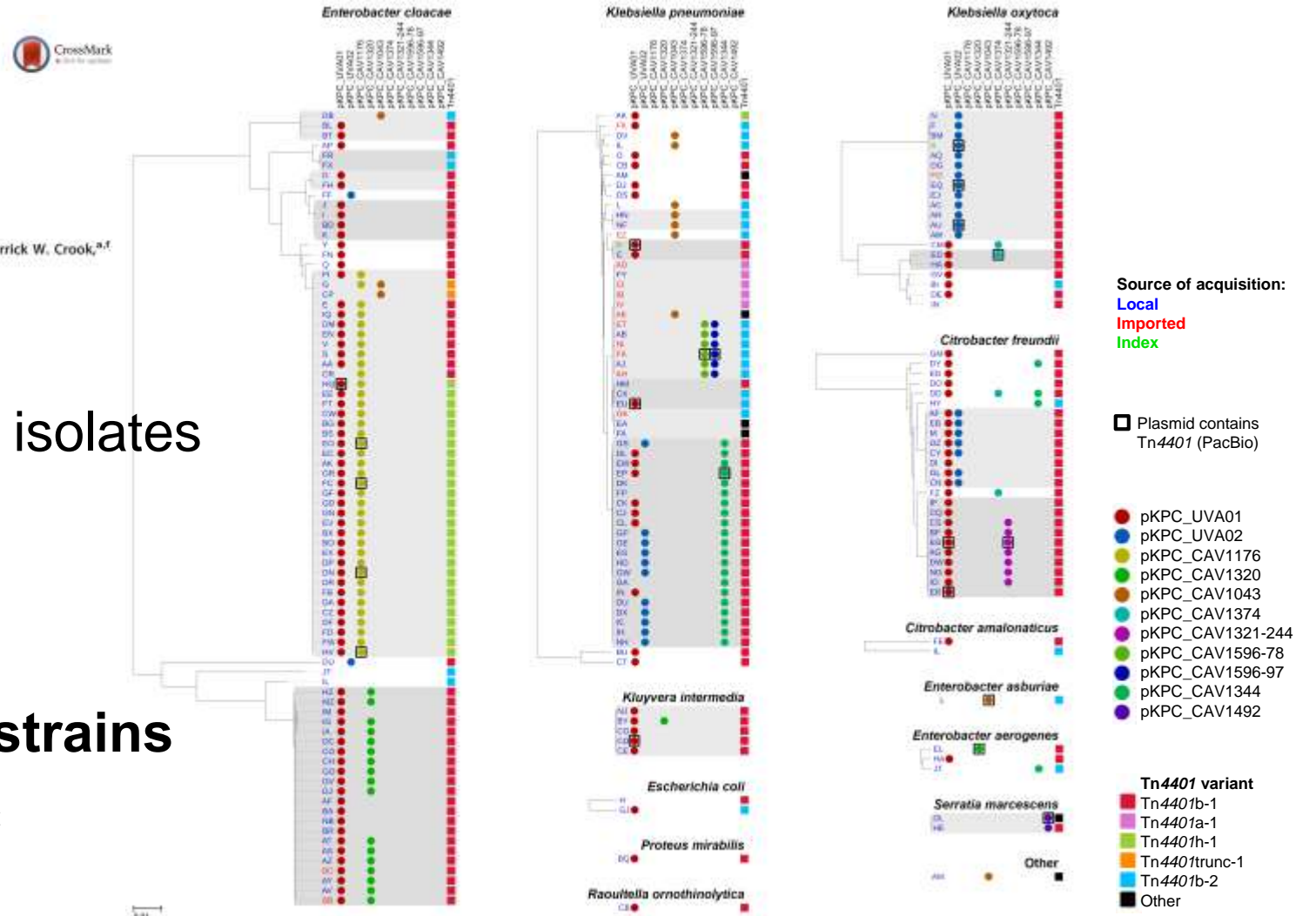
Nested Russian Doll-Like Genetic Mobility Drives Rapid Dissemination of the Carbapenem Resistance Gene *bla*_{KPC}

Anna E. Sheppard,^a Nicole Stoesser,^a Daniel J. Wilson,^a Robert Sebra,^b Andrew Kasarskis,^b Luke W. Anson,^a Adam Giess,^{a*} Louise J. Pankhurst,^a Alison Vaughan,^a Christopher J. Grim,^c Heather L. Cox,^d Anthony J. Yeh,^{da} the Modernising Medical Microbiology (MMM) Informatics Group, Costi D. Sifri,^{da*} A. Sarah Walker,^a Tim E. Peto,^a Derrick W. Crook,^{a,f} Amy J. Mathers^{da}

281 *bla*_{KPC}-Enterobacteriaceae isolates
(182 patients, 2007-2012)

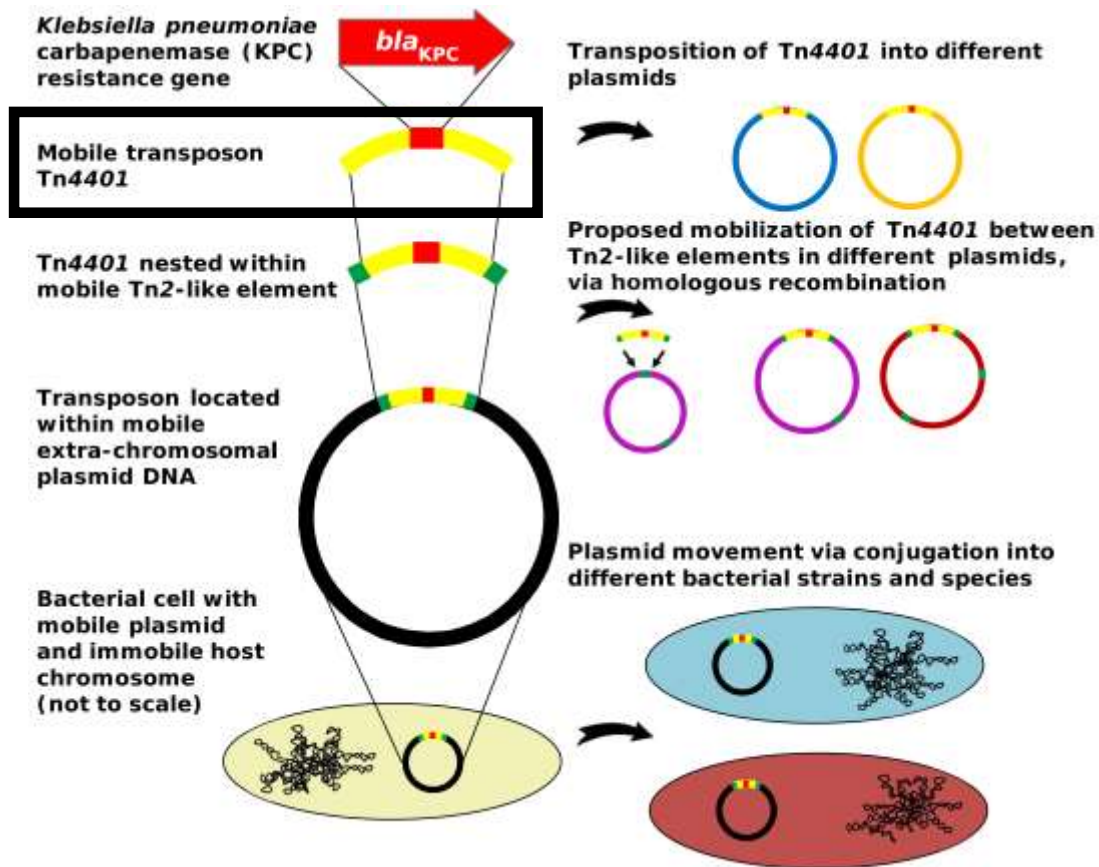
- Illumina: all isolates
- PacBio: select isolates

→ High diversity in species, strains and plasmids carrying *bla*_{KPC}



Long-term multi-species KPC outbreak

Multiple levels of *bla*_{KPC} mobility:



Mobilisation of *bla*_{KPC} via:

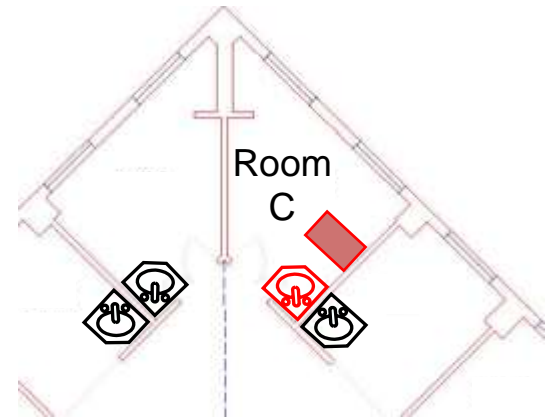
- Tn4401 transposition
- Recombination involving Tn2-like elements
- Plasmid conjugation

Transmission routes remain largely elusive

Can we find ways to track *bla*_{KPC} through multiple patients, strains and plasmids?

→ Variation within Tn4401?

K. pneumoniae ST196

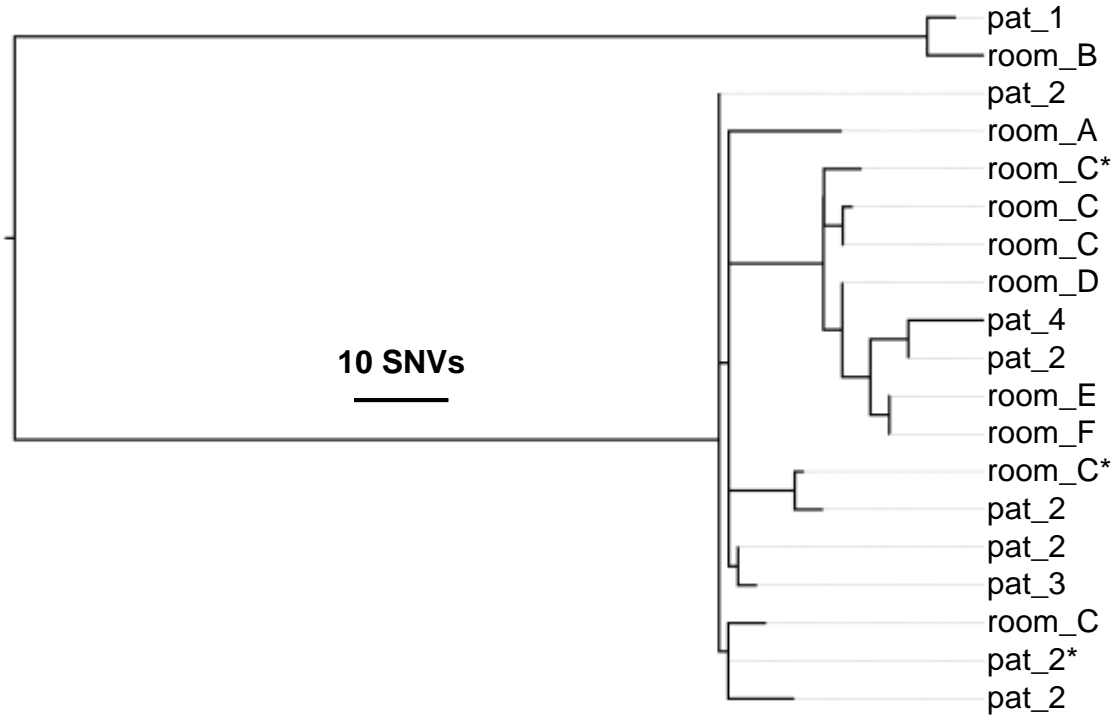


- 19 KPC isolates belonging to this lineage (from our collection of ~950 Illumina-sequenced KPC isolates)
 - 4 patients involved (patients 1-4)
 - Patient 2 had six isolates (Nov 2013 – Sep 2014)
 - 6 environmental locations (sink/shower drains, rooms A-F)
 - Room C had five isolates (Feb 2014 – May 2014)
- In December 2013, SICU (including rooms C, D, E) was closed for 9 weeks and all sink pipes replaced
- Immediately following this, room C tested positive for KPC 10 days AFTER patient 2 went into this room

Bioinformatic approaches

- 19 *K. pneumoniae* ST196 isolates Illumina sequenced

Results: Illumina

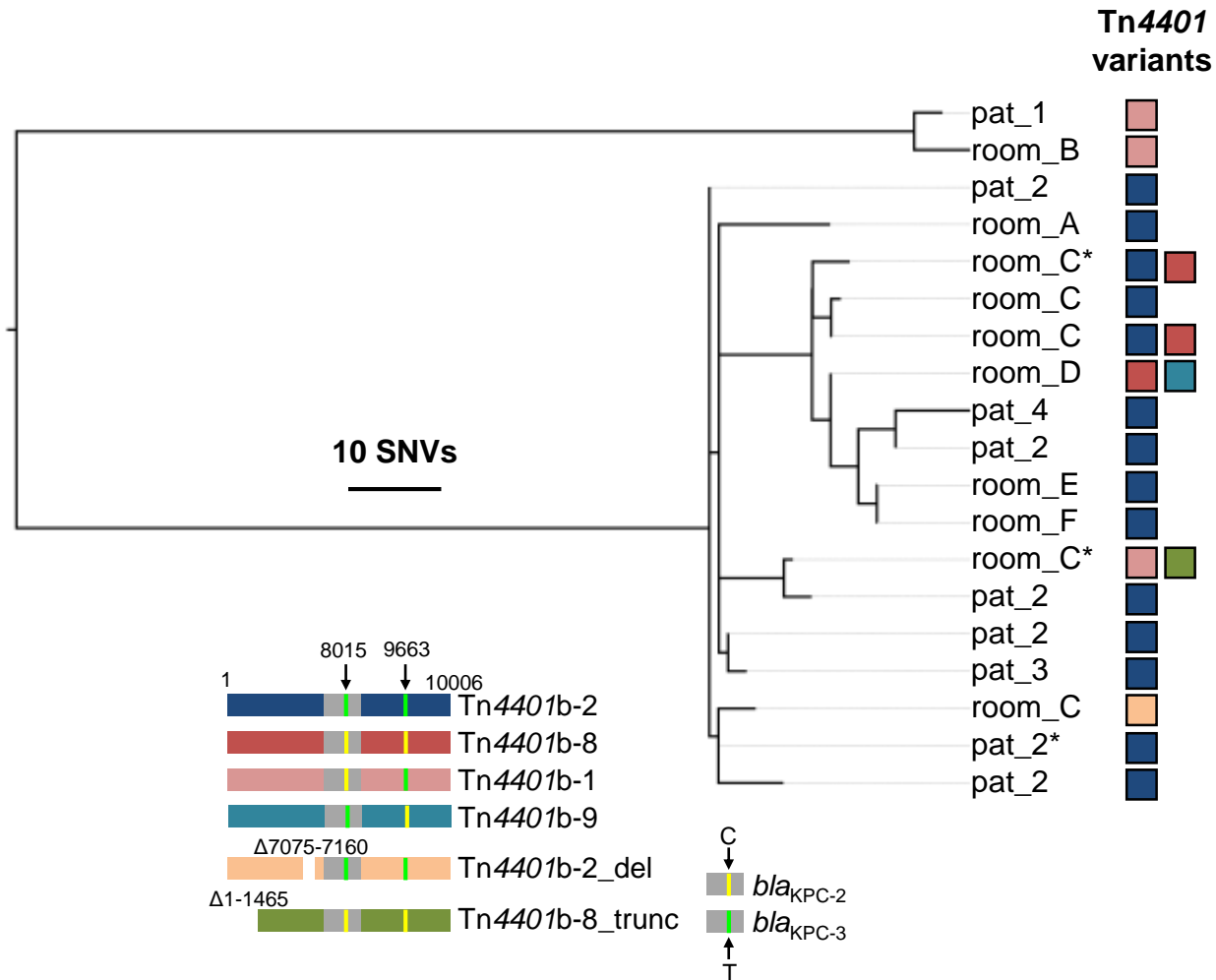


Low variation in core genome
(<40 SNVs excluding divergent isolates)

Bioinformatic approaches

- 19 *K. pneumoniae* ST196 isolates Illumina sequenced
- Tn4401 variation
 - Structural variation assessed by *de novo* assembly and BLASTn
 - SNV variation assessed by mapping to Tn4401b-1 reference

Results: Illumina



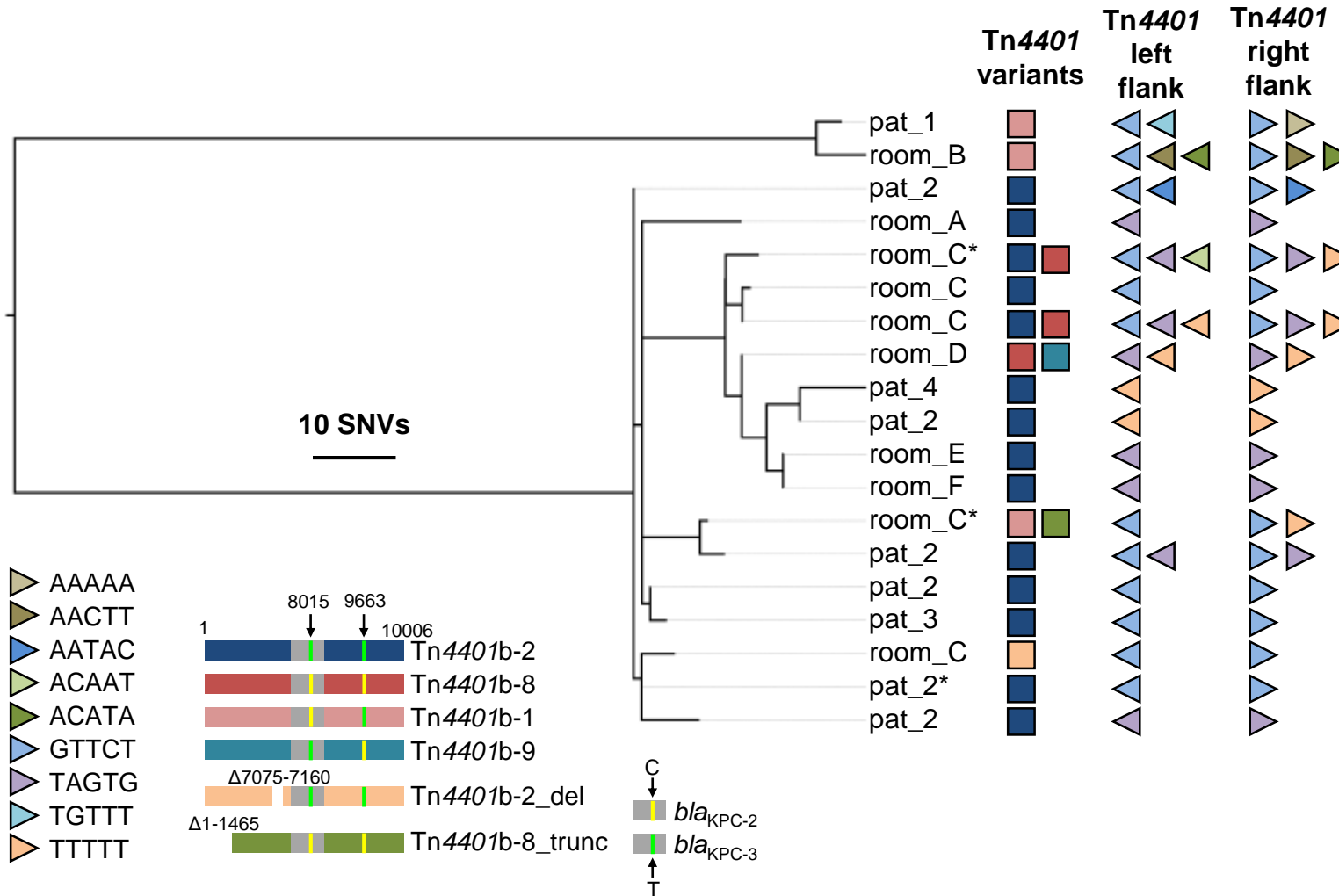
Low variation in core genome
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6 different Tn4401 variants
→ 4 isolates (21%) with multiple Tn4401 variants

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- Plasmid variation assessed by mapping to Tn4401b-1 reference and extracting 5 bp flanking sequences
 - Enables detection of multiple Tn4401 copies

Results: Illumina



Low variation in core genome
 (<40 SNVs excluding divergent isolates)

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 → 4 isolates (21%) with multiple Tn4401 variants

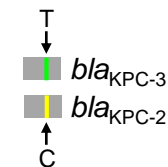
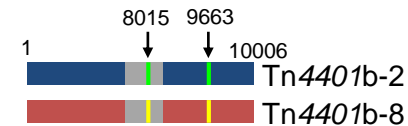
Likely ≥7 different plasmid contexts
 → 8 isolates (42%) with multiple flanking sequences

Bioinformatic approaches

- 19 *K. pneumoniae* ST196 isolates Illumina sequenced
- Tn4401 variation
 - Structural variation assessed by *de novo* assembly and BLASTn
 - SNV variation assessed by mapping to Tn4401b-1 reference
- Plasmid variation assessed by mapping to Tn4401b-1 reference and extracting 5 bp flanking sequences
 - Enables detection of multiple Tn4401 copies
- 3 isolates PacBio sequenced to fully resolve plasmid structures (one from patient 2, two from room C)
 - Plus one *Serratia marcescens* isolate from patient 2

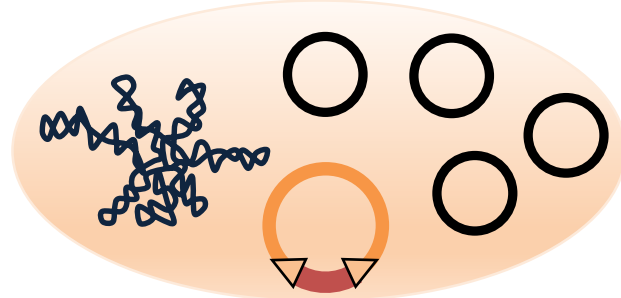
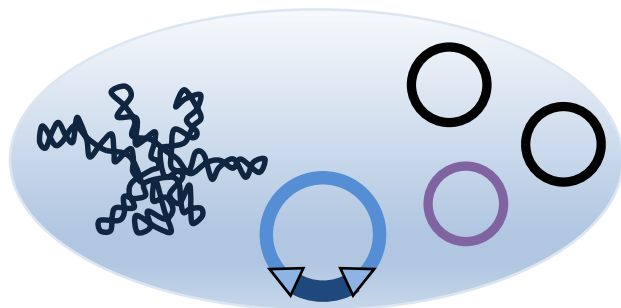
Results: PacBio

- ▶ GTTCT
- ▶ TTTT
- ▶ TAGTG
- ▶ ACAAT

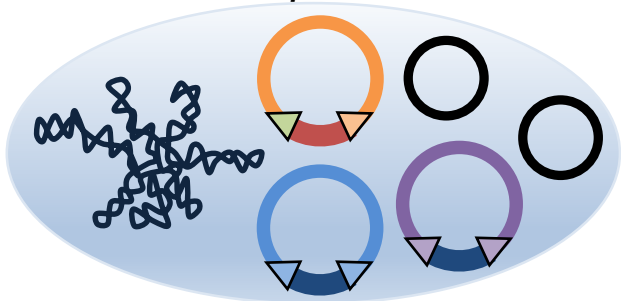


Patient 2: *K. pneumoniae*

Patient 2: *S. marcescens*

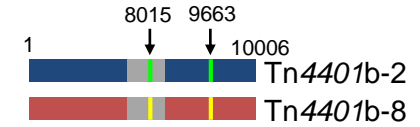


Room C: *K. pneumoniae*



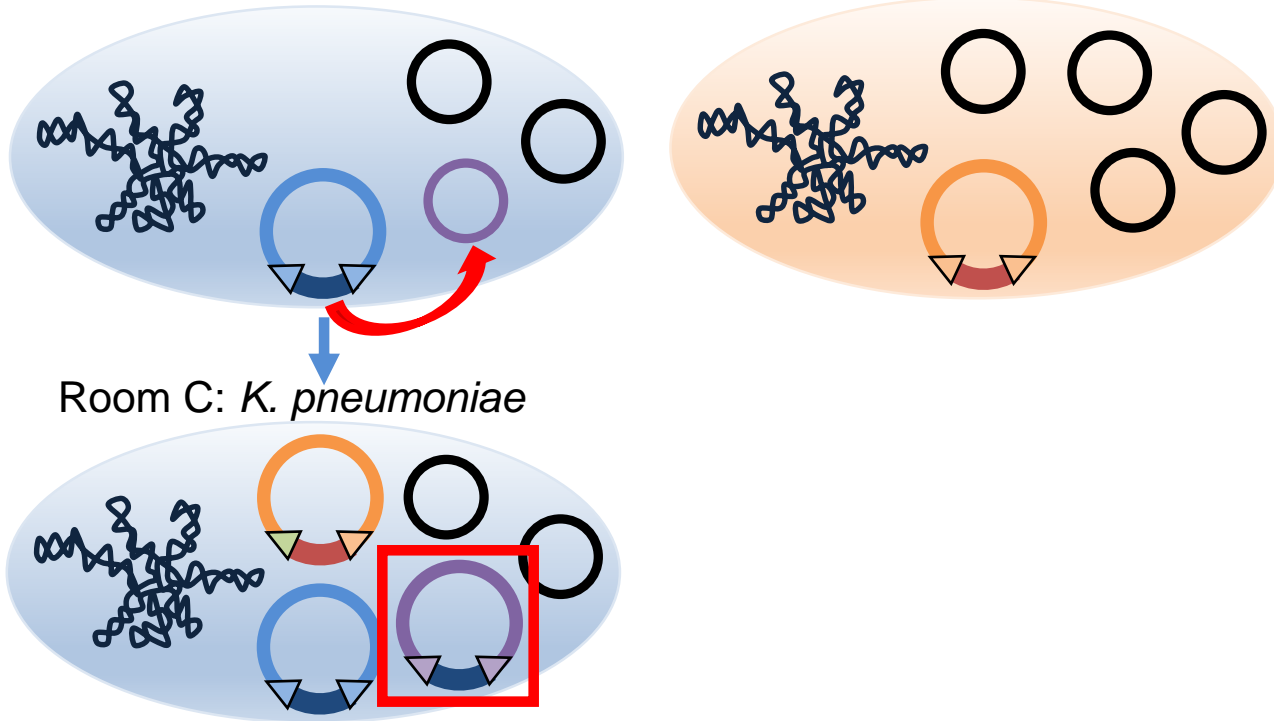
Results: PacBio

- ▶ GTTCT
- ▶ TTTT
- ▶ TAGTG
- ▶ ACAAT



Patient 2: *K. pneumoniae*

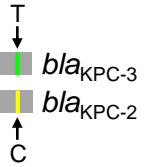
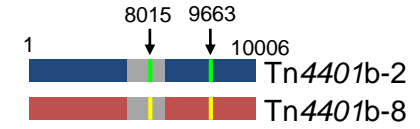
Patient 2: *S. marcescens*



→ **Tn4401** transposition to a new plasmid within *K. pneumoniae*

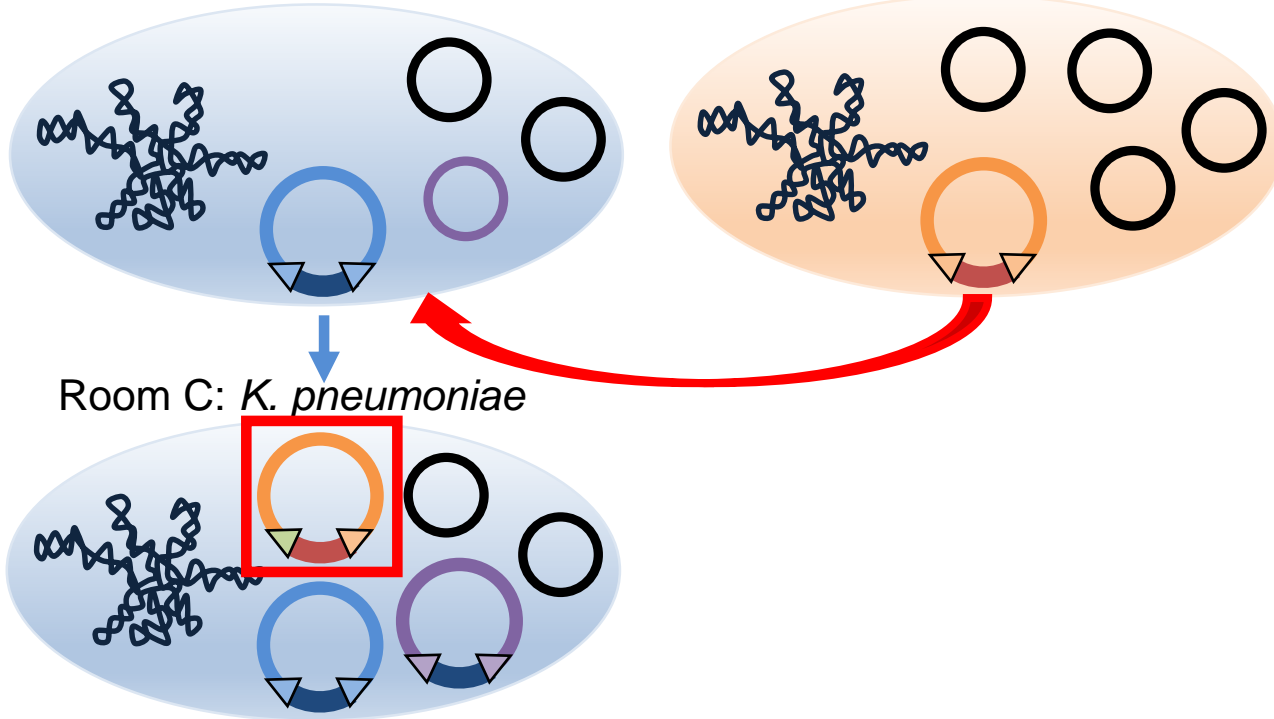
Results: PacBio

- ▶ GTTCT
- ▶ TTTT
- ▶ TAGTG
- ▶ ACAAT



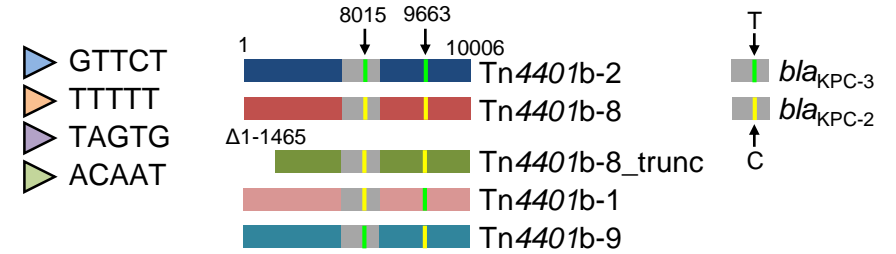
Patient 2: *K. pneumoniae*

Patient 2: *S. marcescens*



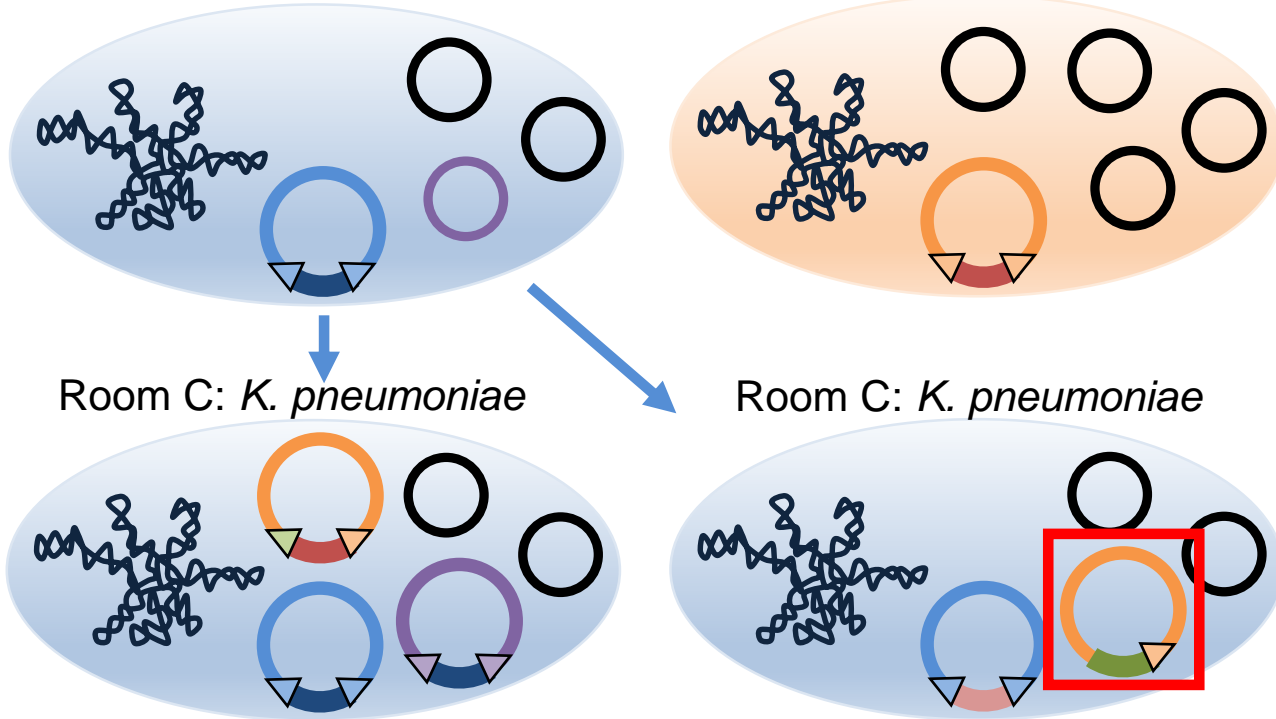
→ **Plasmid transfer from KPC-Sm to KPC-Kp**

Results: PacBio



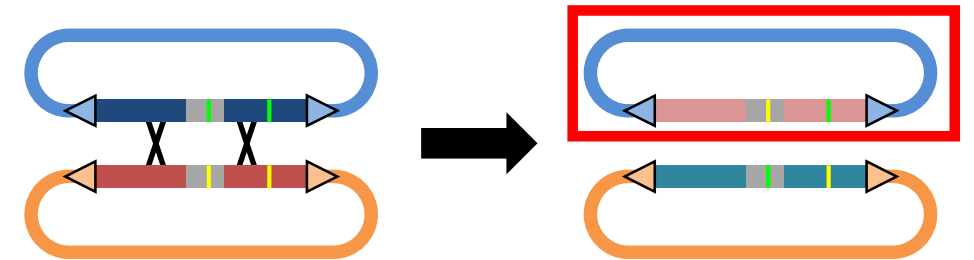
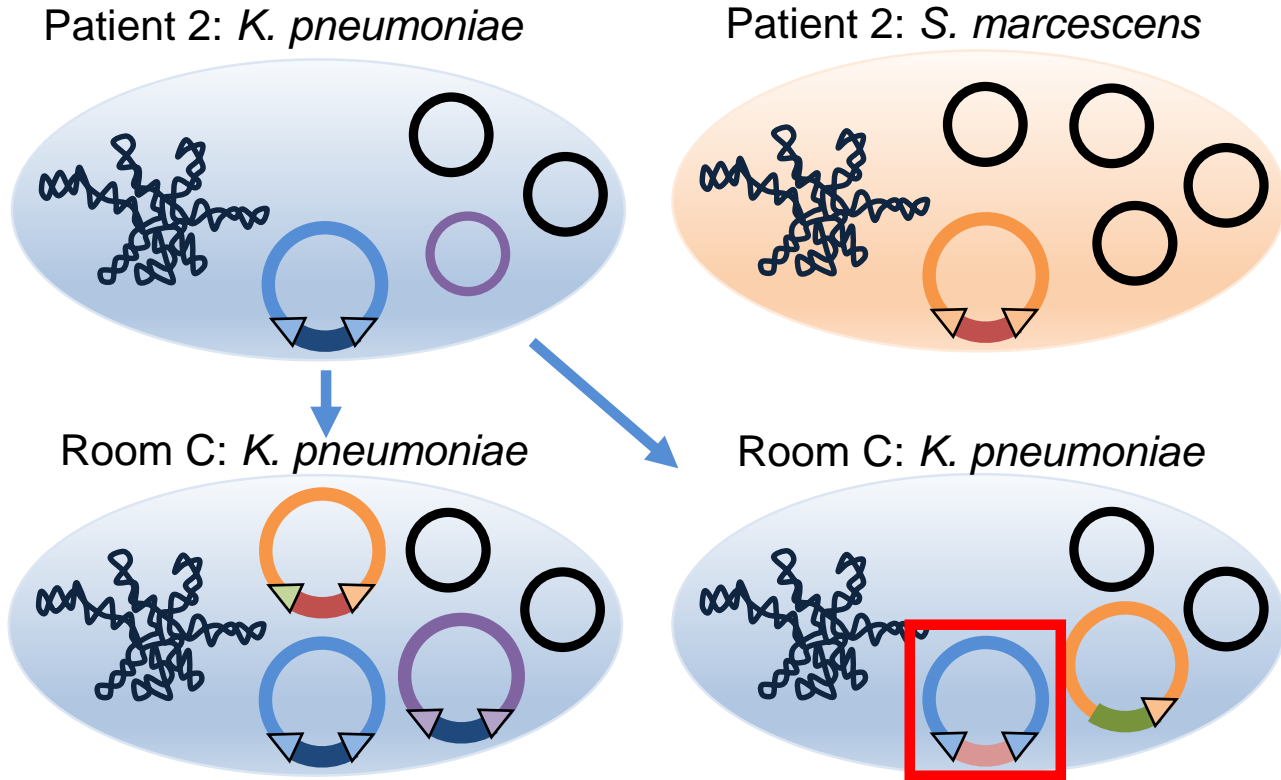
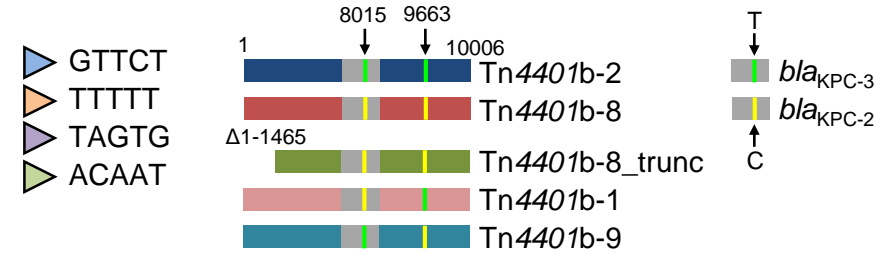
Patient 2: *K. pneumoniae*

Patient 2: *S. marcescens*



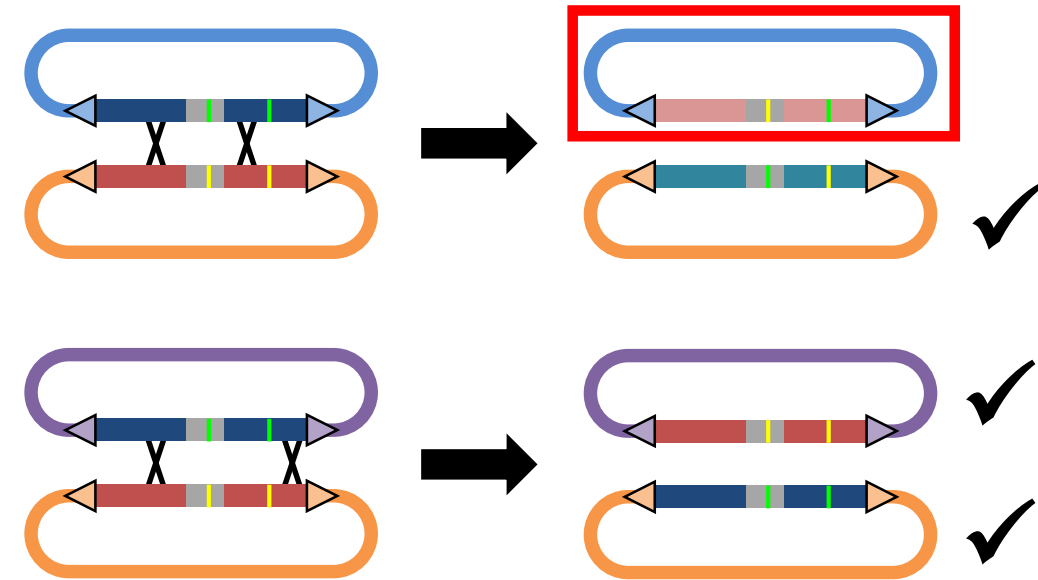
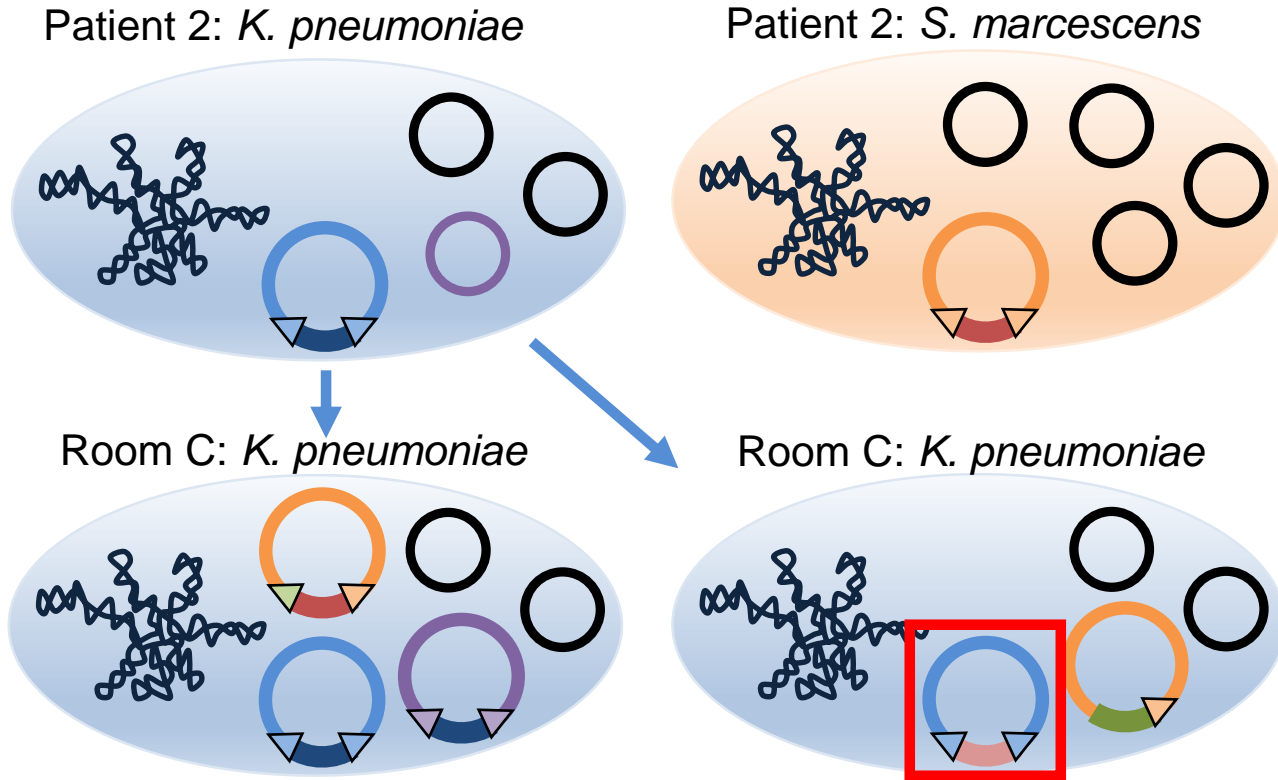
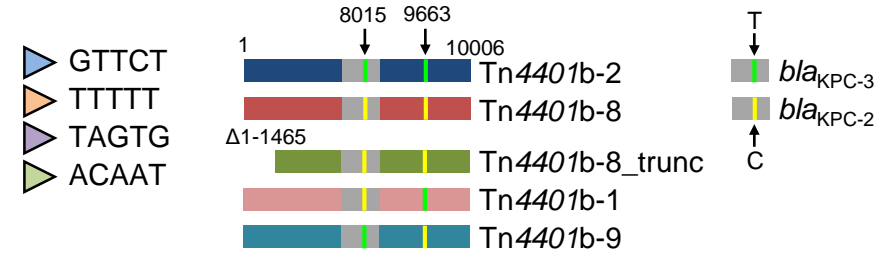
→ **Generation of new Tn4401 variants via deletion**

Results: PacBio



→ **Generation of new Tn4401 variants via homologous recombination**

Results: PacBio



→ **Exchange of Tn4401 variants between plasmids via homologous recombination**

Summary

- From 19 isolates of a single KPC-*K. pneumoniae* lineage (ST196):
 - Multiple *bla*_{KPC} plasmids (putatively 7 total, 3 confirmed by PacBio)
 - Tn4401 transposition
 - At least two independent *bla*_{KPC} plasmid acquisitions
 - Multiple Tn4401 variants (6 total; 2 variable sites and 2 deletions)
 - Homologous recombination
 - Tn4401 partial deletions
 - Many different plasmid/Tn4401 variant combinations (putatively 13 total)
- Applications to transmission tracking
 - Tn4401 variants are not stable within specific host strains / plasmids

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