Mitigating the Spread of Antimicrobial Resistance in Healthcare Settings through Real-time Genomic Surveillance of Bacterial Plasmids

Nathan J. Raabe, MPH

Epidemiology PhD Candidate, Univ of Pittsburgh School of Public Health, Pittsburgh, PA

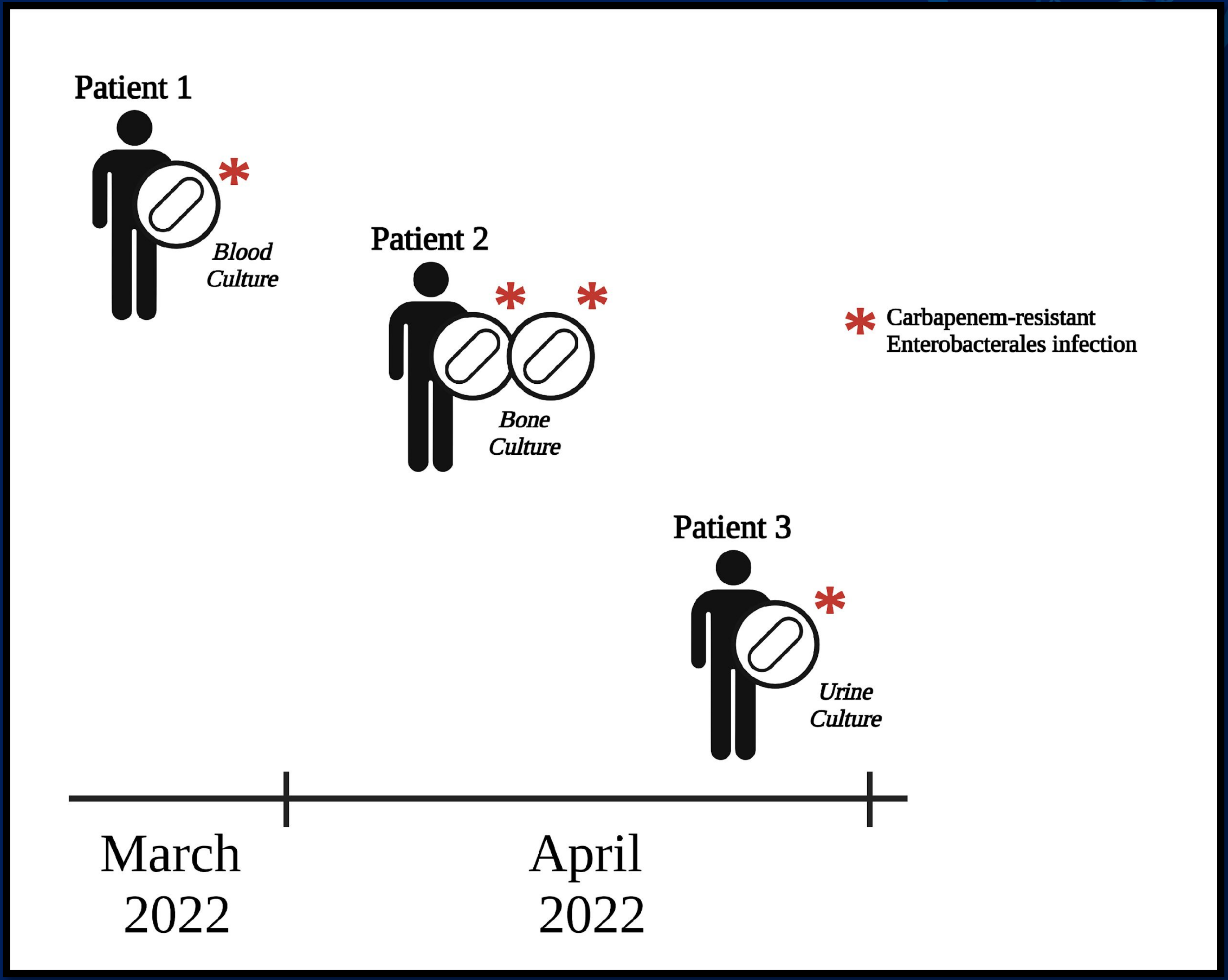
Pitt TPAR Predoctoral Trainee - Microbial Genomics Epidemiology Laboratory (MiGEL)

University of Pittsburgh

Early Signs of an Outbreak

Investigation prompted in April 2022:

- 3 patients (*March-April 2022*) at UPMC Presbyterian Hospital
- Clinical cultures yielded 4 total bacterial isolates.
  - Blood, Bone, Urine
- All 4 isolates were flagged as carbapenem resistant Enterobacterales (CREs).

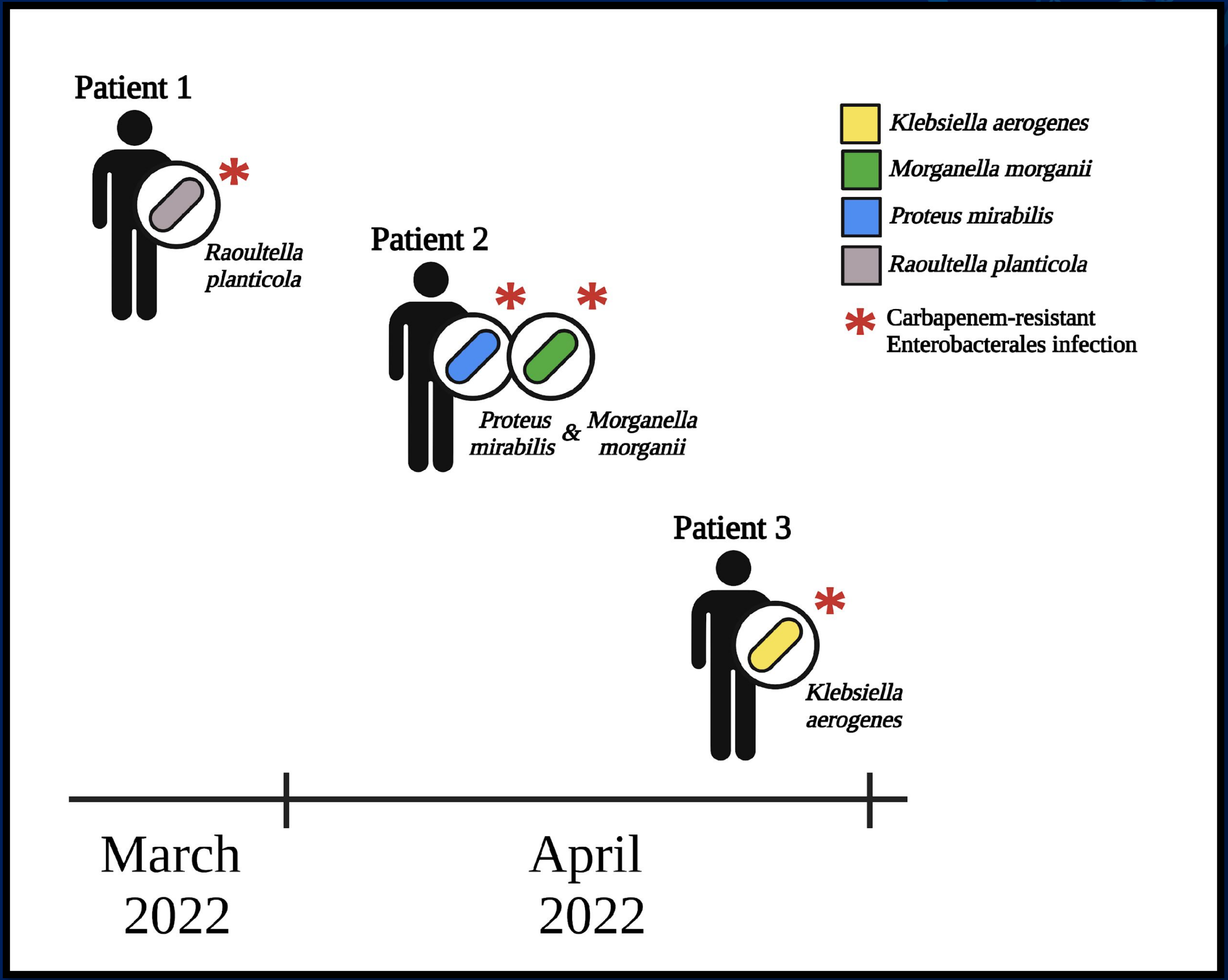


University of Pittsburgh

Early Signs of an Outbreak

Species classification:

- 4 isolates belonged to distinct genera:
  - *Raoultella*
  - *Proteus*
  - *Morganella*
  - *Klebsiella*



University of Pittsburgh

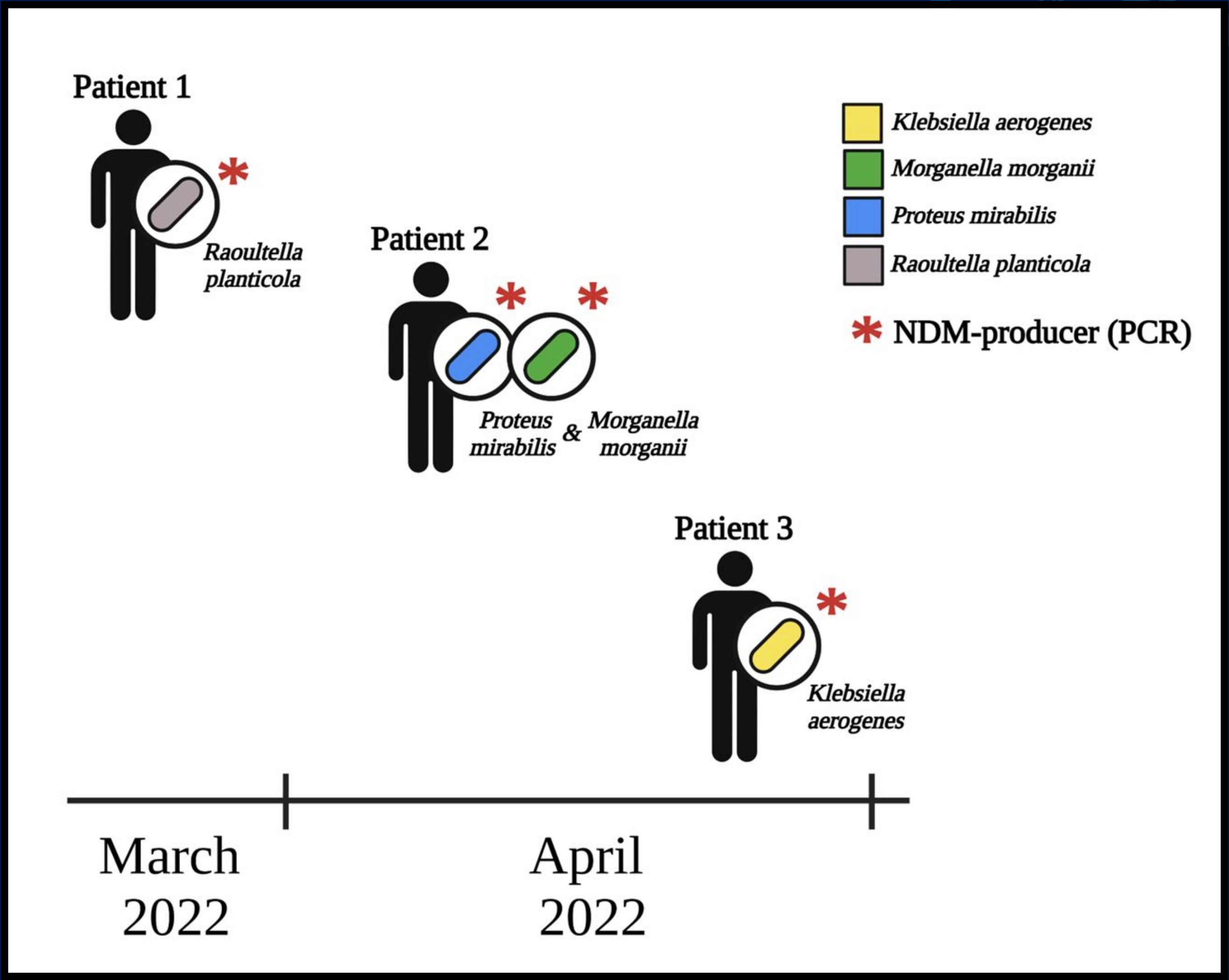
Early Signs of an Outbreak

AMR classification:

- PCR confirmed carbapenemase in all 4 organisms as New Delhi metallo-β-lactamase (NDM).

New Delhi metallo-β-lactamase (NDM):

- Emerging mechanism of carbapenem resistance seen in healthcare-associated infections (HAIs).
- First isolated in 2008, NDM is associated with an increased risk of mortality and limited therapeutic options.[1]
- Encoded by the *bla*$_{\text{NDM}}$ gene.

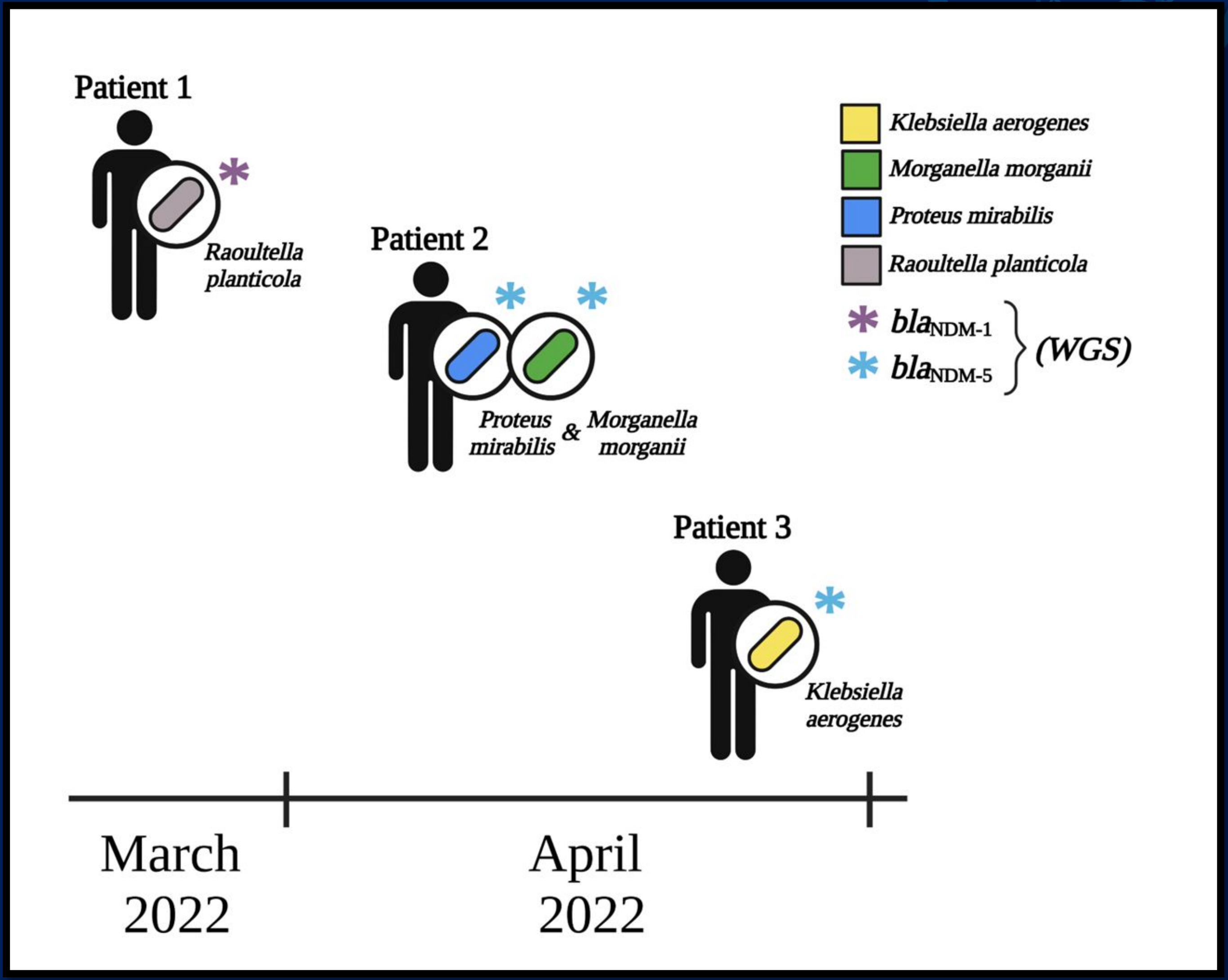


University of Pittsburgh

Early Signs of an Outbreak

Whole Genome Sequencing (WGS):

- Dozens of different NDM variants have been identified in more than 60 host species, spanning multiple bacterial families.[2]
- Illumina short-read WGS data identified 2 NDM variants present amongst the 4 isolates.
    - $bla_{\text{NDM-1}}$
    - $bla_{\text{NDM-5}}$



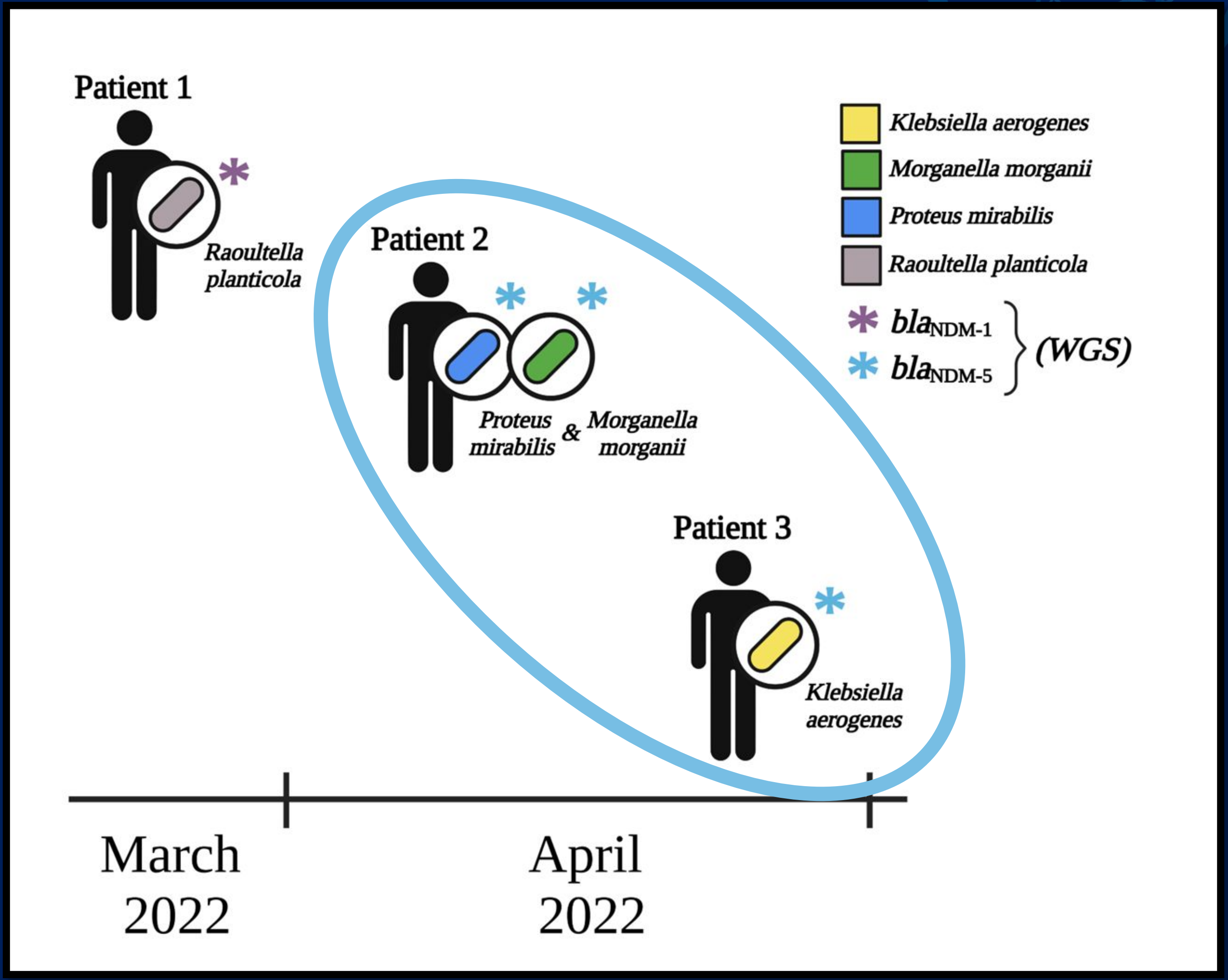
University of Pittsburgh

Early Signs of an Outbreak

Suspicions of mobile AMR:

1. NDM had rarely been observed previously at our center.
2. NDM-5 variant appeared in 3 bacterial isolates of <u>different Enterobacterales species</u>.
3. Patient 2 was <u>co-colonized</u> with two species, <u>each harboring</u> the NDM-5 variant.

Conclusion: the NDM-5 gene may be <u>carried on a mobile genetic element</u>, such as a bacterial plasmid.



University of Pittsburgh

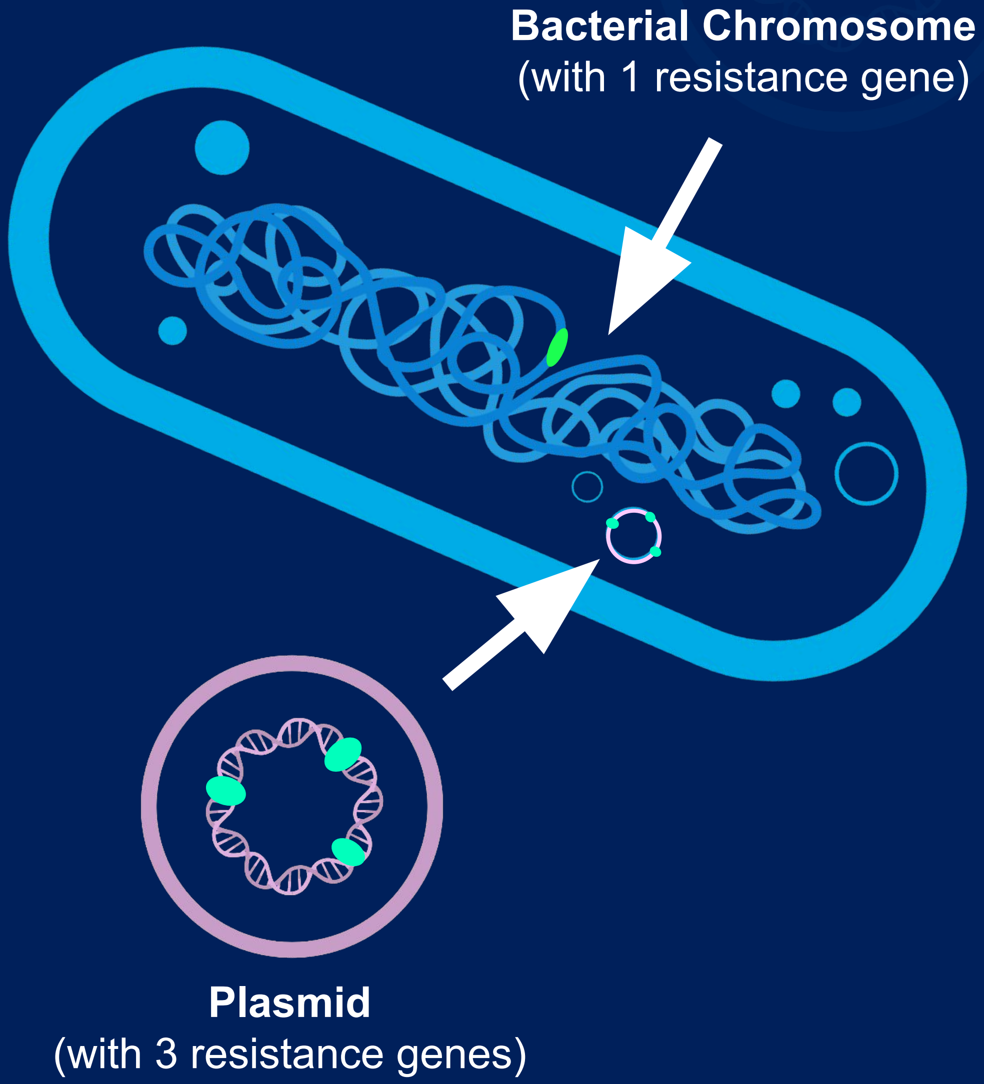
The Bacterial Genome

Bacterial Chromosome (2-8Mbp)

- Single, circular strand of double stranded DNA.
- Chromosome can harbor AMR genes.

Plasmids (5-500kbp)

- Small, circular pieces of DNA outside the bacterial chromosome.
- Bacterial HAIs often carry plasmids encoding AMR, leading to infections with complicated therapy and poor patient outcomes. [3]
- Bacterial plasmids can be acquired, carried, and shared through a mechanism called horizontal plasmid transfer.



Bacterial Chromosome
(with 1 resistance gene)

Plasmid
(with 3 resistance genes)

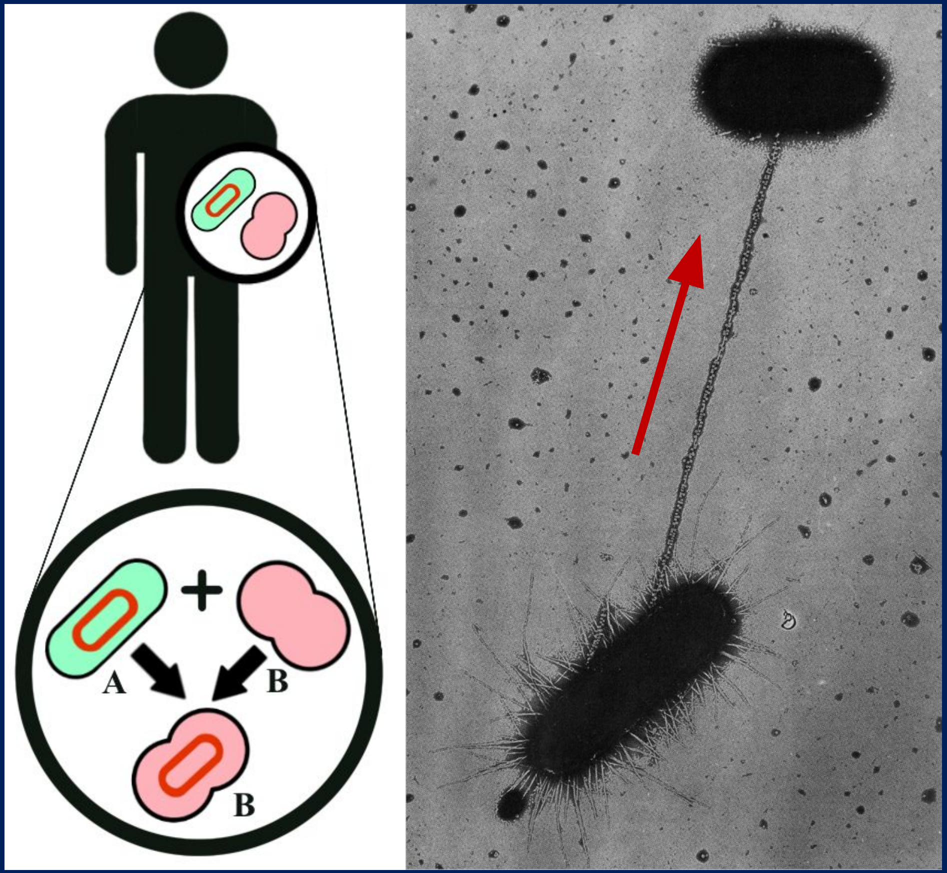
University of Pittsburgh

Horizontal Plasmid Transfer

- Can occur within a co-colonized patient or a hospital environment contaminated with two or more bacterial species.

- These types of settings provide the opportunity for a plasmid to transfer between species.

- May result in transmission of the newly resistant bacterial species harboring the resistance-encoding plasmid to other hospitalized patients.



Horizontal plasmid transfer between bacteria species A and B.

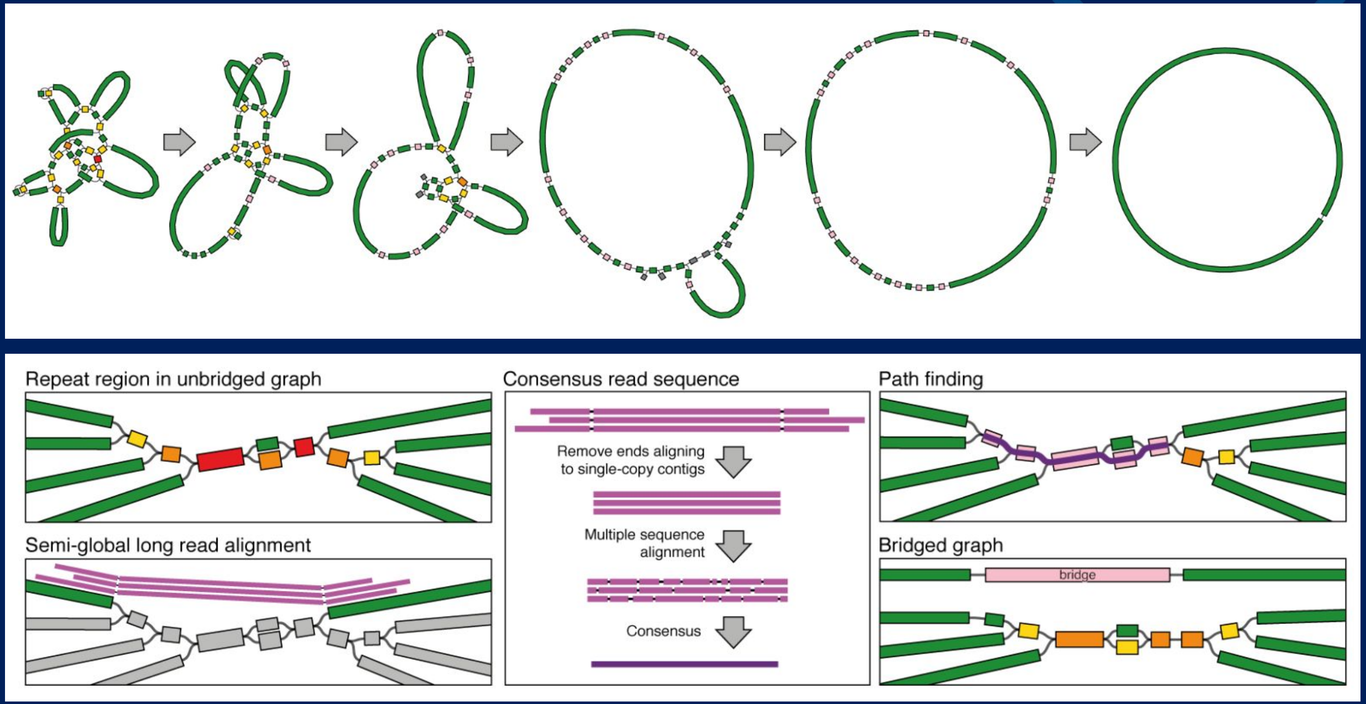
Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol. 2005;3(9):711-721. doi:10.1038/nrmicro1234

Plasmid Investigation

Suspicion of plasmid-mediated AMR:

- We conducted long-read WGS on isolates to achieve greater resolution.
- In conjunction with Illumina data, Nanopore long-read WGS data for the same isolates was used to create hybrid assembled genomes.
- Hybrid assemblies offer several advantages:
  - Maintain the accuracy of SR assemblies
  - More easily resolve repeat regions, resulting in assemblies with fewer, larger contigs.

https://github.com/rrwick/Unicycler

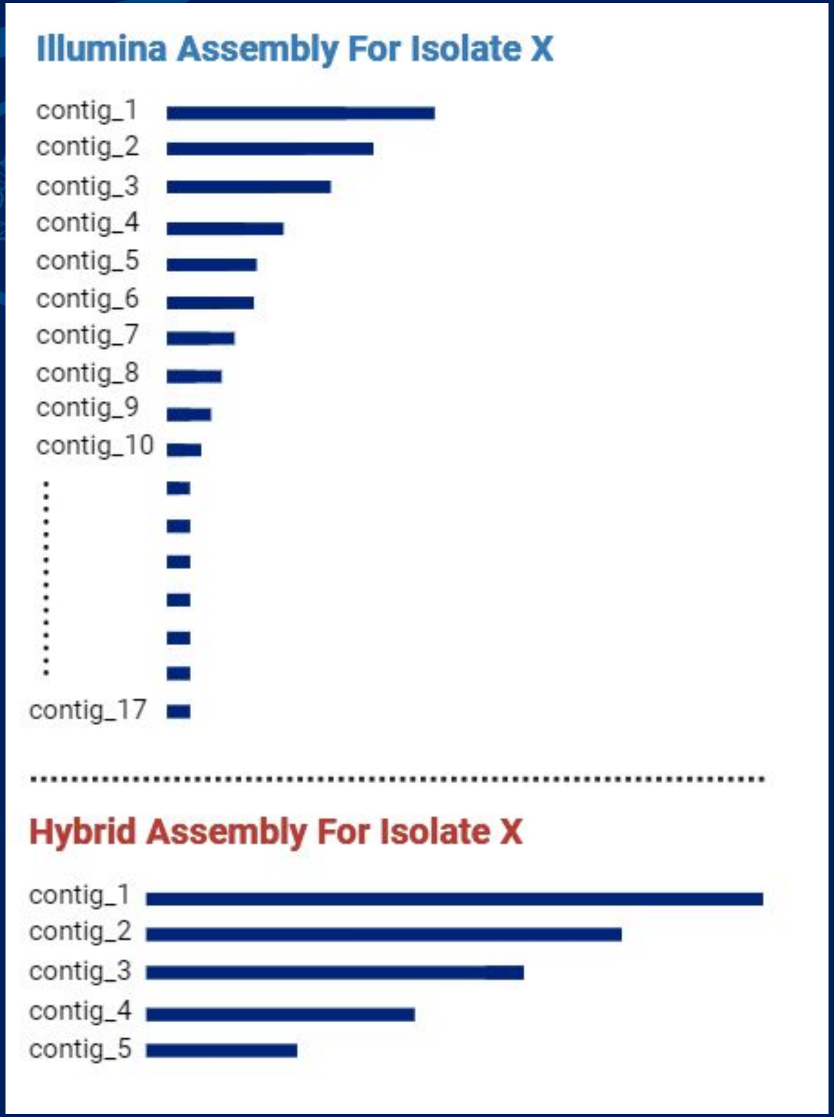


Hybrid Assembly: tangled short-read genome assembly resolved through direct long-read bridging. (Unicycler)

University of Pittsburgh

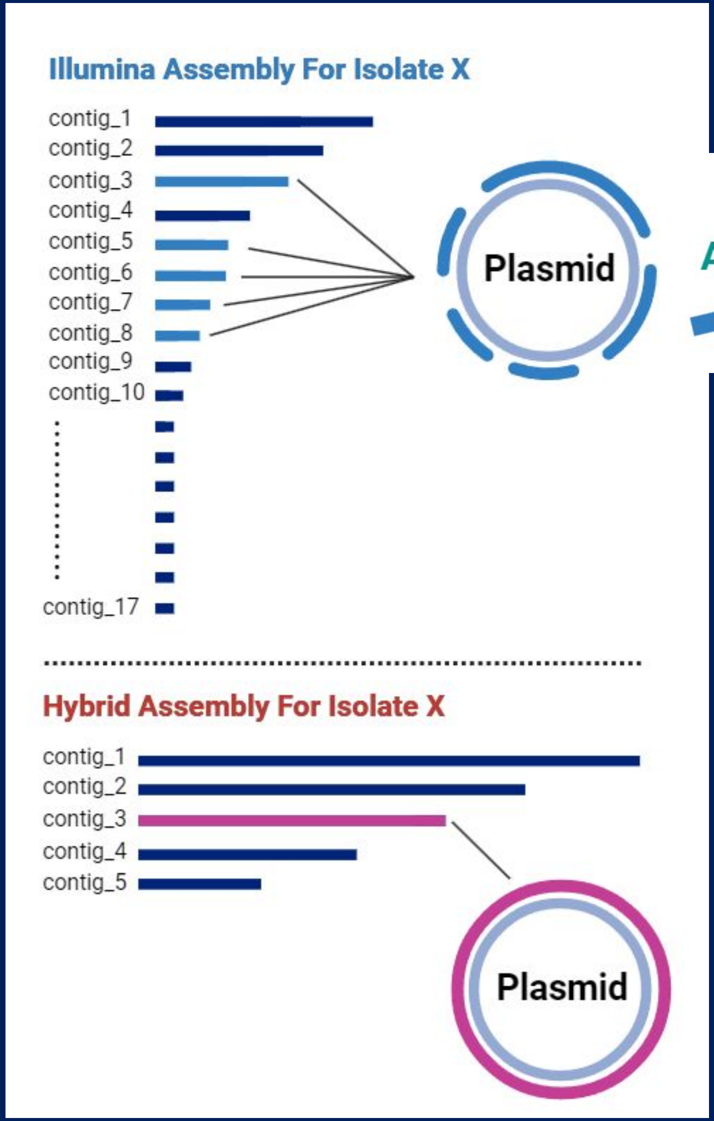
Plasmid Investigation

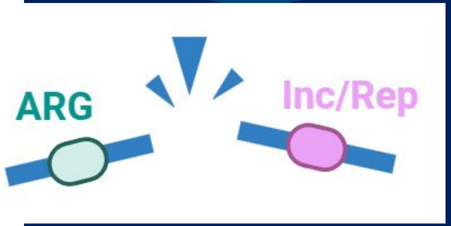
- More easily associate AMR genes with the chromosome or extrachromosomal elements.



Plasmid Reconstruction

from Assembly



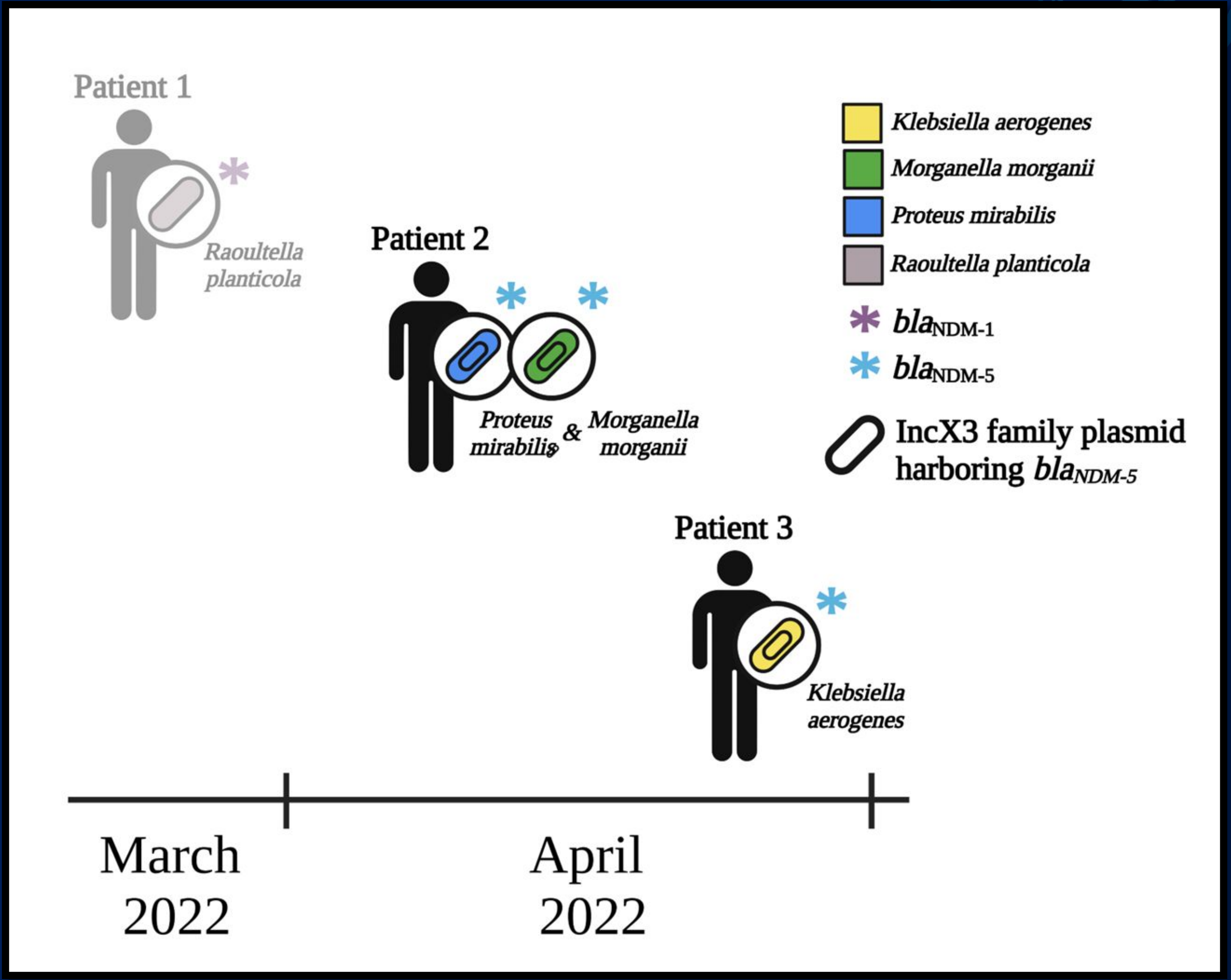


ARG and plasmid typing genes present on different Illumina contigs.

Plasmid Investigation

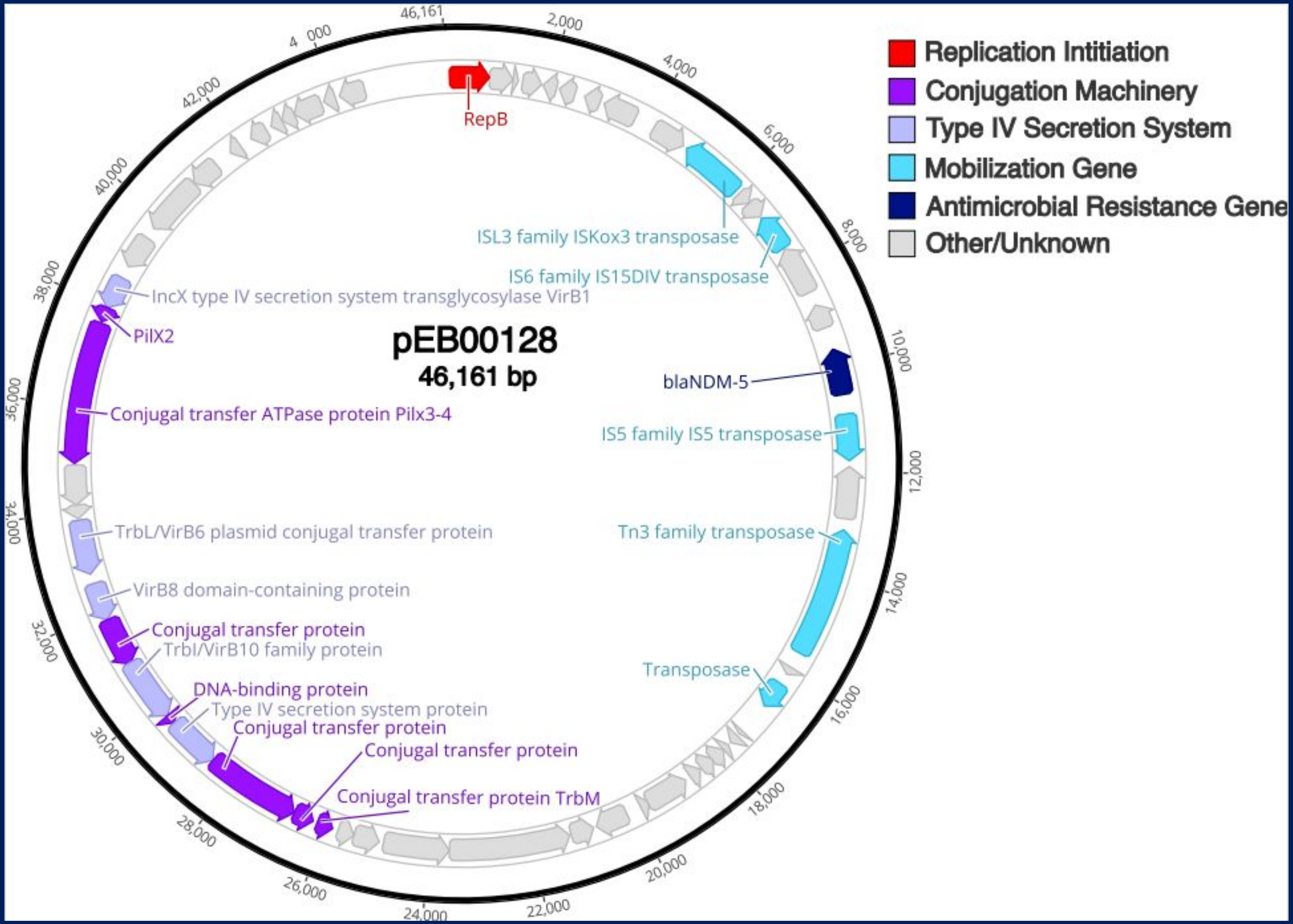
Plasmid Characterization:

- Hybrid assemblies of the isolates from Patients 2 and 3 showed NDM-5 was carried on a near-identical IncX3 family plasmid.
- The plasmid carried additional mobility genes classifying it as conjugative.
- Taken together, this was convincing evidence of a plasmid-associated outbreak of AMR.



University of Pittsburgh

The IncX3 NDM-5 Outbreak Plasmid

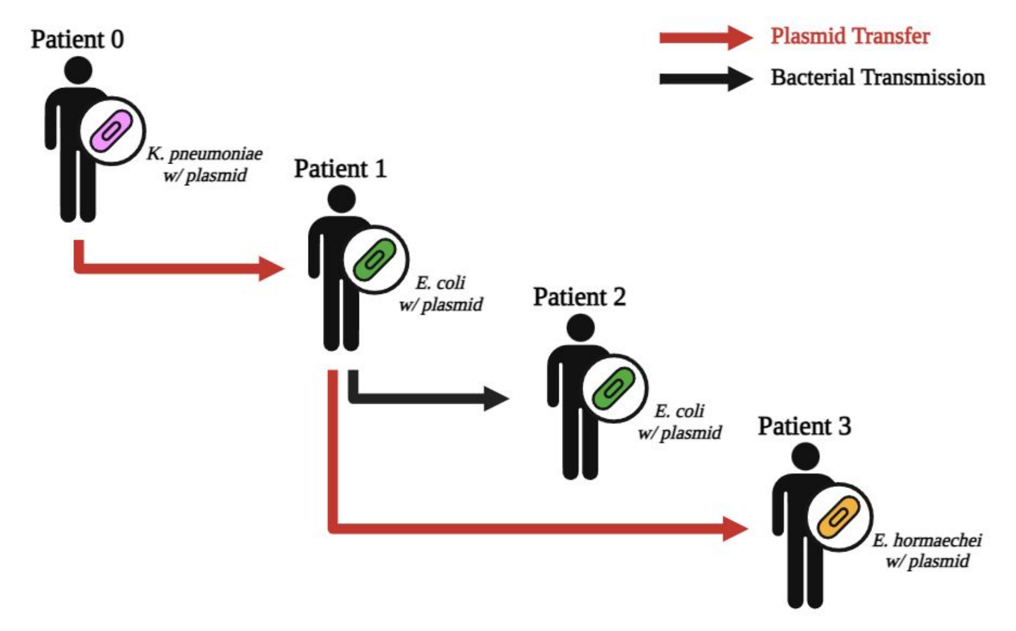


Plasmid Investigation

- WGS as used in outbreak investigations detects same-species transmission, and is largely not designed to identify *multispecies, plasmid-associated outbreaks*, because they involve both:[6]

1) Horizontal plasmid transfer of a common resistance gene-encoding plasmid among *different* bacterial species.

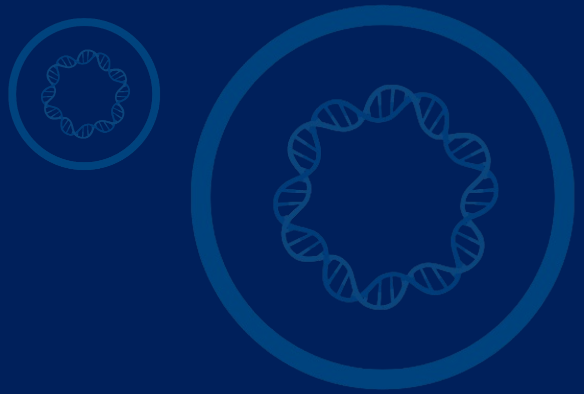
2) Bacterial transmission of plasmid-harboring clonal strains.



Plasmid-associated outbreak of drug-resistant bacteria involving plasmid transfer and bacterial transmission.

Thus, in most cases specialized WGS and bioinformatic approaches must be incorporated to resolve plasmid sequences from isolates.

Building a Response



1. Begin a Traditional Outbreak Investigation

- Obtain swabs from eligible individuals to detect plasmid carriage through:
  - Conducting repeated active surveillance around identified index patients.
  - Continuing routine carbapenem-resistant organism surveillance to identify additional carriers.

Building a Response

2. Leverage the Enhanced Detection System for Healthcare-Associated Transmission (EDS-HAT) to collect and connect isolates using WGS

- **Collection:** High-priority pathogens are collected from patients with prior UPMC admissions or encounters for WGS surveillance as part of EDS-HAT.
- **Connection:** EDS-HAT performs real-time bacterial WGS (Illumina) at UPMC Presbyterian Hospital to detect healthcare-associated bacterial transmission events using genetic relatedness of isolates (<15 SNPs).

3. Supplement EDS-HAT's short-read Illumina sequencing with Nanopore long-read sequencing of CRE's and create hybrid assemblies to resolve plasmids.

Building a Response

4. Conduct a review of the Electronic Health Record to identify the plausible epidemiological routes of plasmid transfer and bacterial transmission events.

Plasmid Transfer

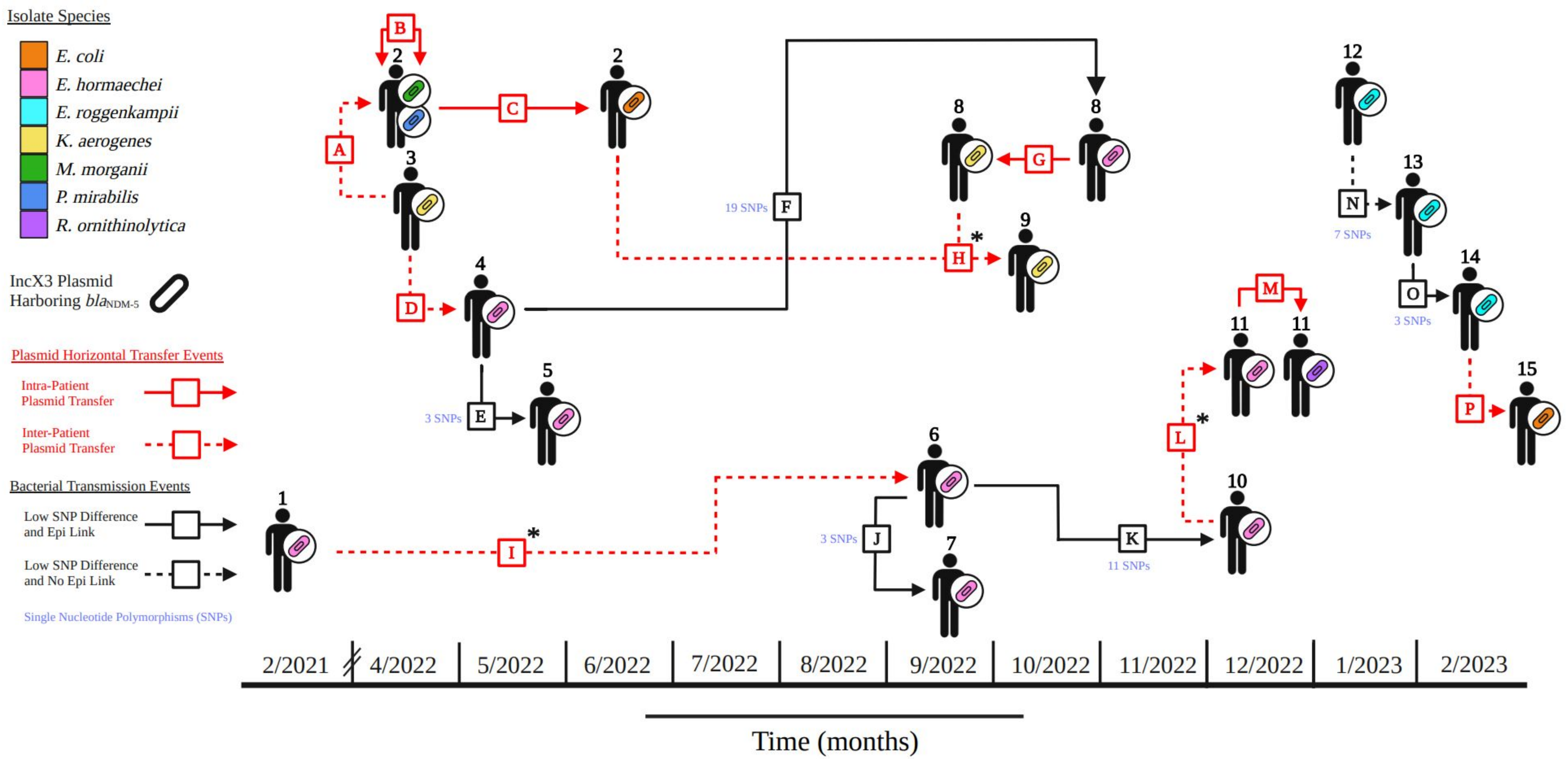
- Intra-patient Plasmid Transfer
  (same plasmid, same patient, different species)
- Putative Inter-Patient Plasmid Transfer
  (same plasmid, different patient, different species, + requires strong epi-route identified between patients)

Bacterial Transmission

- Low SNP difference, with or without epi-route identified

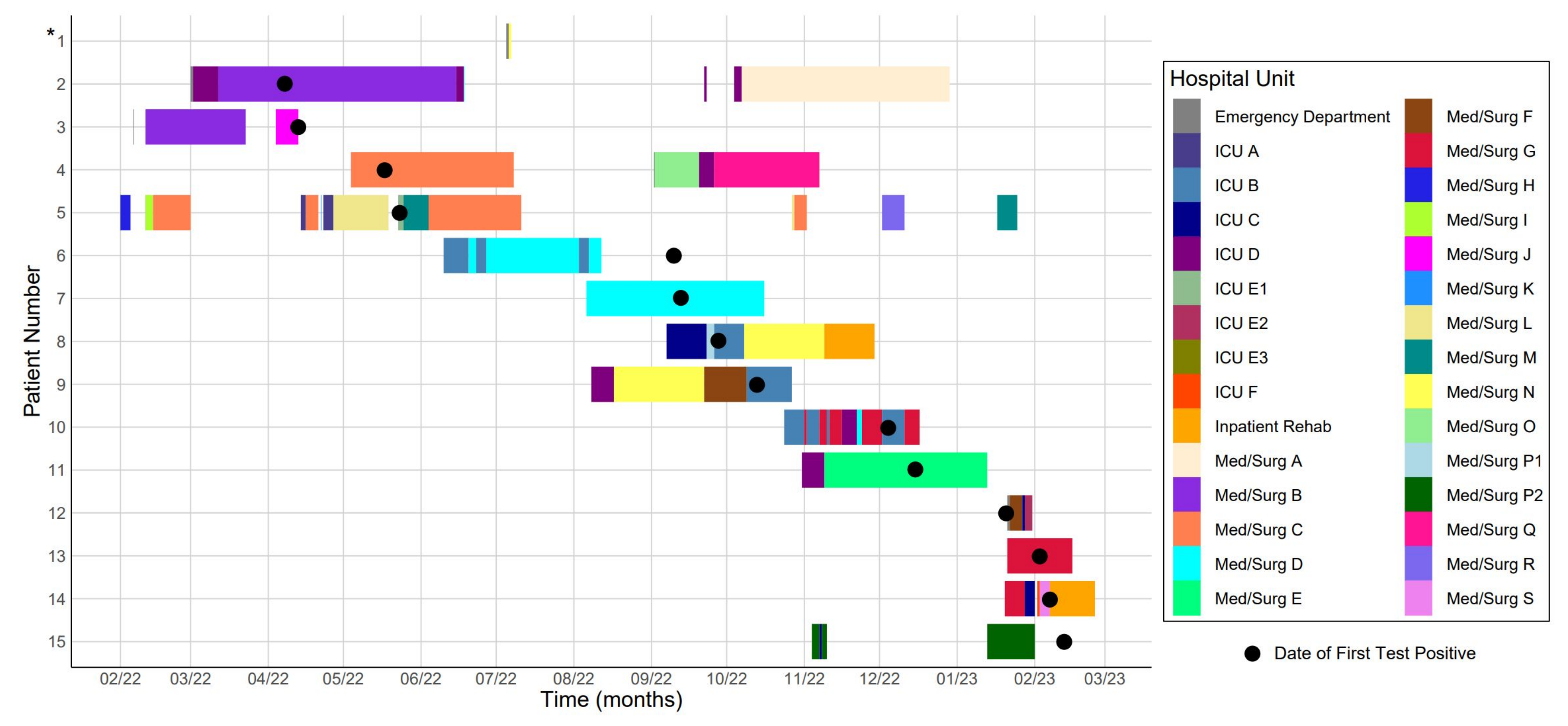
The IncX3 NDM-5 Plasmid Outbreak:

Bacterial Transmission and Plasmid Transfer Events



Raabe et al. Real-time genomic epidemiologic investigation of a multispecies plasmid-associated hospital outbreak of NDM-5-producing Enterobacterales infections, IJID (2024)

The NDM-5 Outbreak by Hospital Unit



Raabe et al. Real-time genomic epidemiologic investigation of a multispecies plasmid-associated hospital outbreak of NDM-5-producing Enterobacterales infections, IJID (2024)

University of Pittsburgh

UPMC
LIFE CHANGING MEDICINE

Building a Response

5. Develop and employ infection prevention interventions which target the identified epidemiological routes of plasmid transfer and transmission.

- **16 total plasmid transfer or bacterial transmission events** across various hospital units.
  - 6 bacterial transmissions
  - 6 inter-patient plasmid transfers
  - 4 intra-patient plasmid transfers
- **We instituted preventions targeting these event routes.**
  - Education and terminal cleans
  - Outbreak appeared to stop in early 2023, where 4 months passed without detecting the outbreak plasmid.

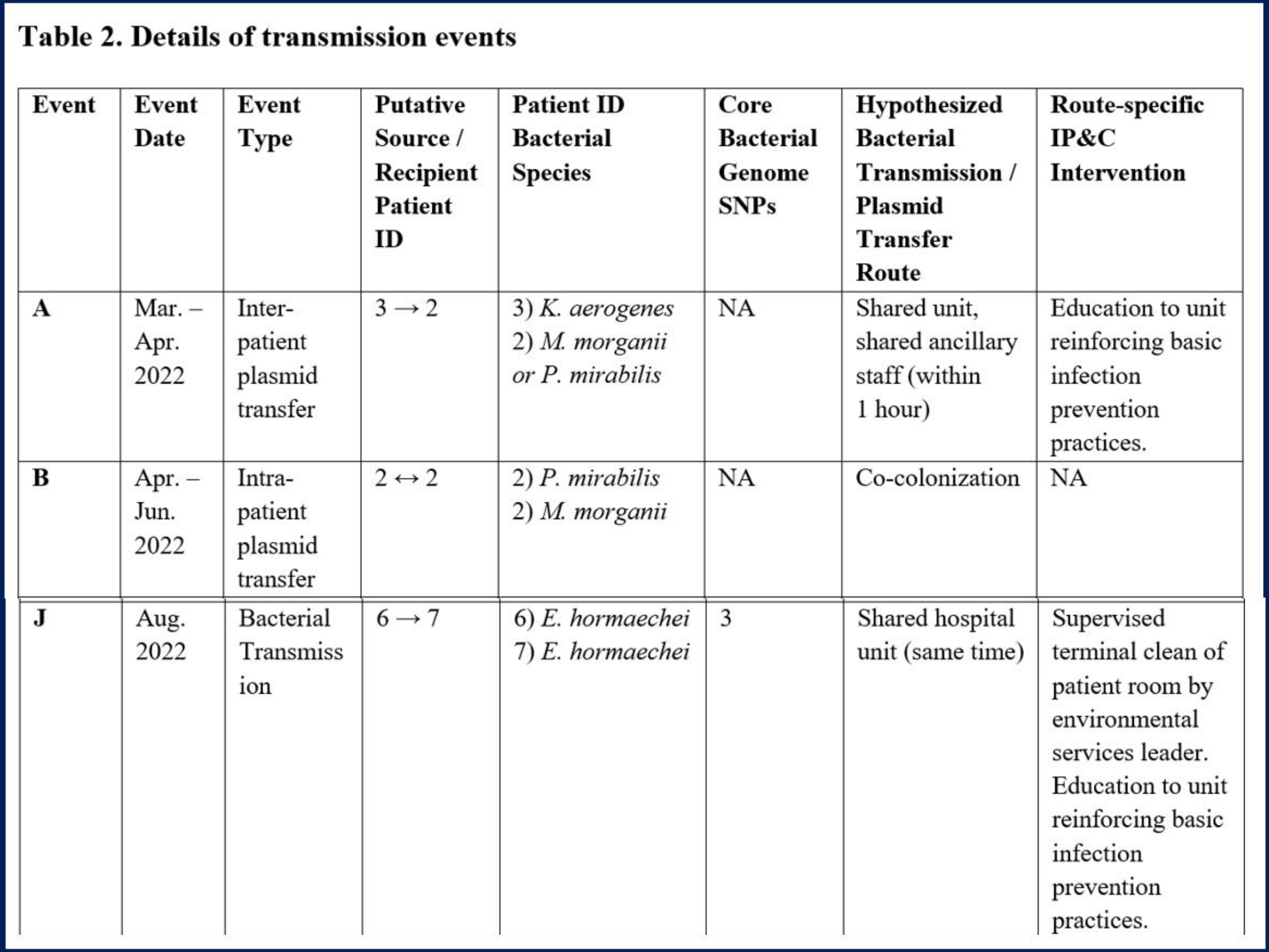


Table 2. Details of transmission events

Event	**Event Date**	**Event Type**	**Putative Source / Recipient Patient ID**	**Patient ID Bacterial Species**	**Core Bacterial Genome SNPs**	**Hypothesized Bacterial Transmission / Plasmid Transfer Route**	**Route-specific IP&C Intervention**
A	Mar. – Apr. 2022	Inter-patient plasmid transfer	3 → 2	3) *K. aerogenes* 2) *M. morganii or P. mirabilis*	NA	Shared unit, shared ancillary staff (within 1 hour)	Education to unit reinforcing basic infection prevention practices.
B	Apr. – Jun. 2022	Intra-patient plasmid transfer	2 ↔ 2	2) *P. mirabilis* 2) *M. morganii*	NA	Co-colonization	NA
J	Aug. 2022	Bacterial Transmission	6 → 7	6) *E. hormaechei* 7) *E. hormaechei*	3	Shared hospital unit (same time)	Supervised terminal clean of patient room by environmental services leader. Education to unit reinforcing basic infection prevention practices.

Raabe et al. Real-time genomic epidemiologic investigation of a multispecies plasmid-associated hospital outbreak of NDM-5-producing Enterobacterales infections, IJID (2024)

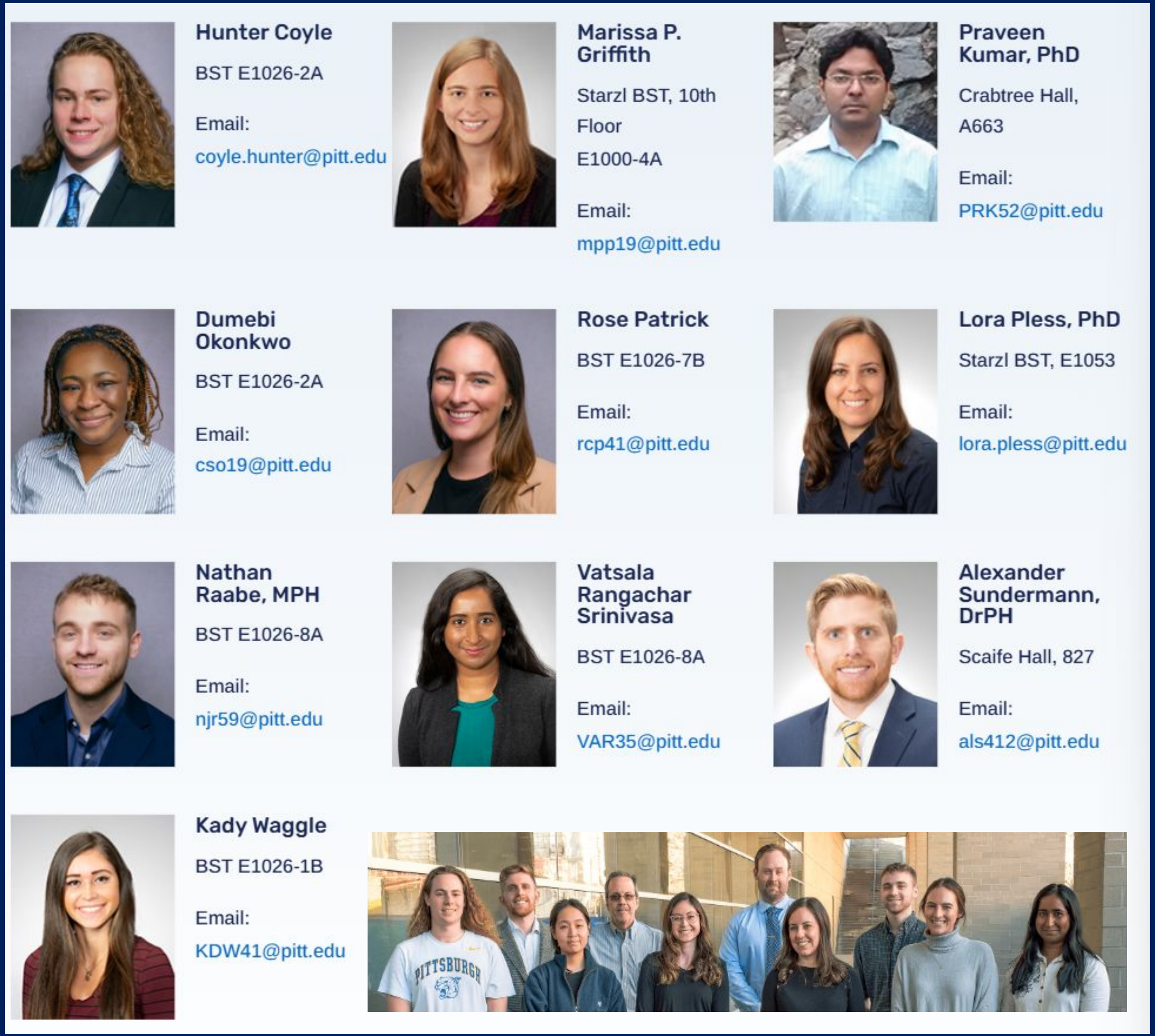
Key Points

- **Identified a multispecies, IncX3 *bla*$_{NDM-5}$ plasmid outbreak** by combining WGS with epidemiological data to track bacterial transmission and plasmid transfer in real time.
- **Sequencing data informed interventions** targeting routes of plasmid transfer and bacterial transmission to mitigate the AMR outbreak.

Real-time plasmid surveillance could detect hidden multi-species outbreaks that current genomic surveillance methods miss, enabling earlier intervention to halt the spread of antimicrobial resistance.

Acknowledgements

Microbial Genomics Epidemiology Laboratory (MiGEL)



Hunter Coyle
BST E1026-2A
Email:
coyle.hunter@pitt.edu

Marissa P. Griffith
Starzl BST, 10th Floor
E1000-4A
Email:
mpp19@pitt.edu

Praveen Kumar, PhD
Crabtree Hall, A663
Email:
PRK52@pitt.edu

Dumebi Okonkwo
BST E1026-2A
Email:
cso19@pitt.edu

Rose Patrick
BST E1026-7B
Email:
rcp41@pitt.edu

Lora Pless, PhD
Starzl BST, E1053
Email:
lora.pless@pitt.edu

Nathan Raabe, MPH
BST E1026-8A
Email:
njr59@pitt.edu

Vatsala Rangachar Srinivasa
BST E1026-8A
Email:
VAR35@pitt.edu

Alexander Sundermann, DrPH
Scaife Hall, 827
Email:
als412@pitt.edu

Kady Waggle
BST E1026-1B
Email:
KDW41@pitt.edu

Lee H. Harrison, MD

Professor of Medicine and Epidemiology
Director, Center for Genomic Epidemiology
University of Pittsburgh

Daria Van Tyne, PhD

Associate Professor of Medicine
Infectious Disease
University of Pittsburgh

Graham M. Snyder, MD, MS

Medical Director,
Infection Prevention and Hospital Epidemiology, UPMC

UPMC
LIFE CHANGING MEDICINE

EDS-HAT Funding

UPMC
LIFE CHANGING MEDICINE

NIH
National Institutes of Health

EDS-HAT and this work is funded in part by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH):

R01AI127472

ENHANCED DETECTION SYSTEM FOR HEALTHCARE-ASSOCIATED TRANSMISSION OF INFECTION

R21AI178369

TRACKING PLASMID SPREAD AND TRANSMISSION IN THE HOSPITAL: A NOVEL TOOL FOR INFECTION PREVENTION AND CONTROL

References:

[1] Snyder BM, Montague BT, Anandan S, Madabhushi AG, Pragasam AK, Verghese VP, et al. Risk factors and epidemiologic predictors of blood stream infections with New Delhi metallo-b-lactamase (NDM-1) producing Enterobacteriaceae. Epidemiol Infect 2019;147:e137. doi: 10.1017/S0950268819000256.
[2] Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM metallo-β-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev 2019;32:e00115–18. doi: 10.1128/CMR.00115-18.
[3] San Millan A Evolution of plasmid-mediated antibiotic resistance in the clinical context. Trends Microbiol 2018;26:978–85. doi: 10.1016/j.tim.2018.06.007.
[4] Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol. 2005;3(9):711-721. doi:10.1038/nrmicro1234
[5] Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13(6): e1005595. https://doi.org/10.1371/journal.pcbi.1005595
[6] Raabe NJ, Valek AL, Griffith MP, Mills E, Waggle K, Srinivasa VR, Ayres AM, Bradford C, Creager HM, Pless LL, Sundermann AJ, Van Tyne D, Snyder GM, Harrison LH. Real-time genomic epidemiologic investigation of a multispecies plasmid-associated hospital outbreak of NDM-5-producing Enterobacterales infections. Int J Infect Dis. 2024 May;142:106971. doi: 10.1016/j.ijid.2024.02.014. Epub 2024 Feb 17. PMID: 38373647; PMCID: PMC11055495.

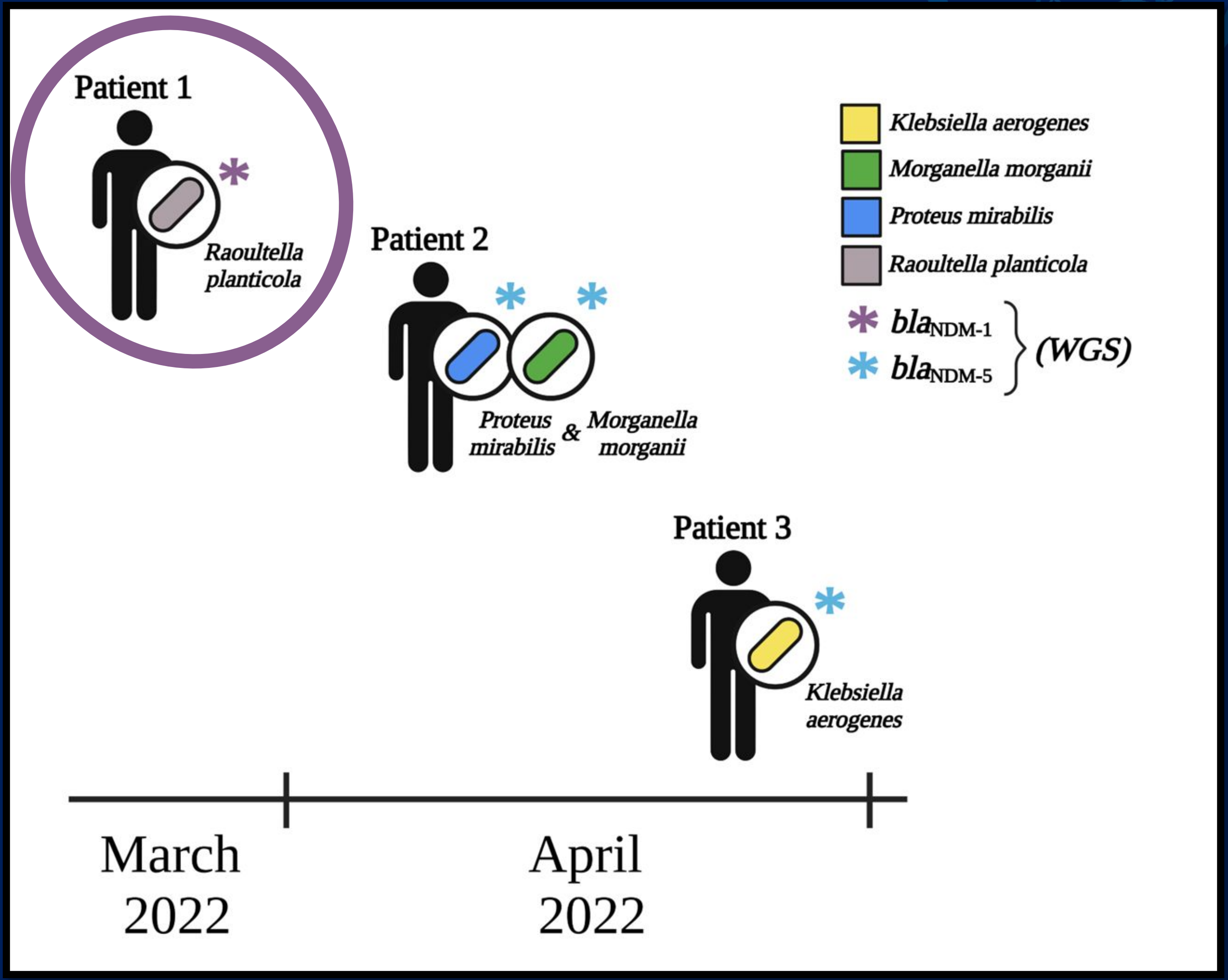
Supplemental Material

Continued plasmid surveillance

Identifying other plasmid-associated outbreaks of NDM at our center:

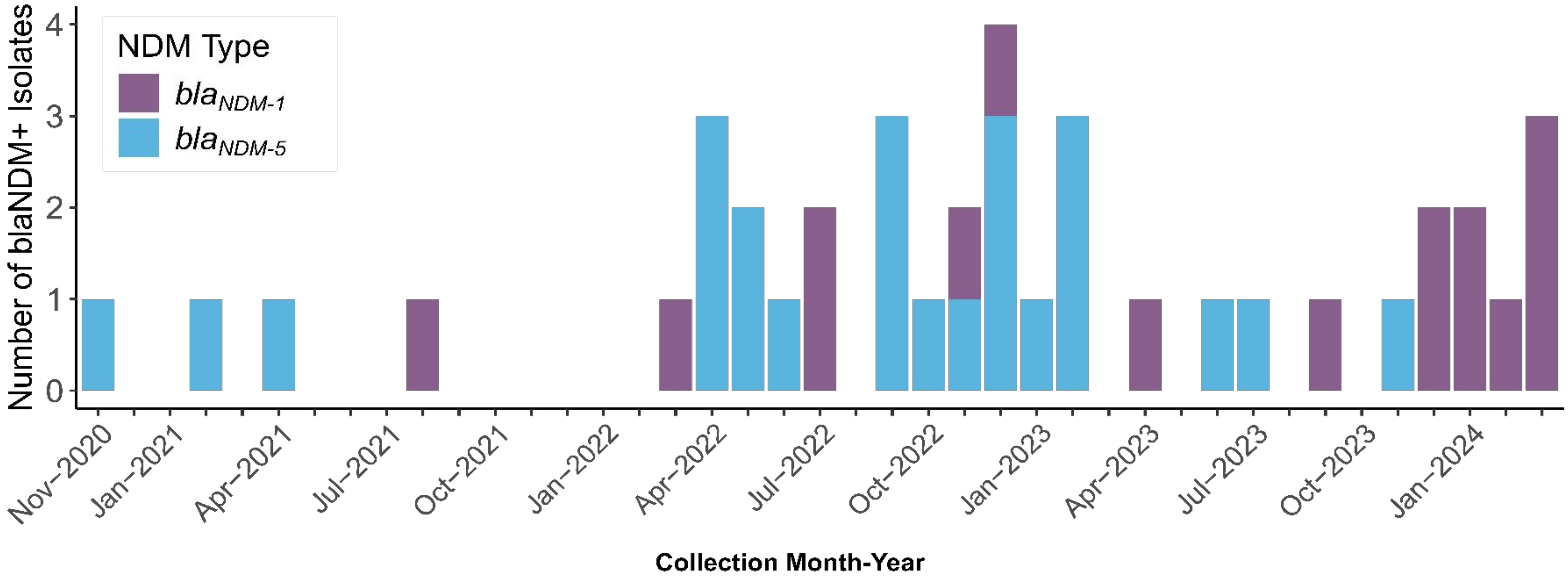
- Continued collection of CREs and retrospective analyses led to the identification of instances of NDM variants which were not part of the IncX3 NDM-5 plasmid outbreak.

- Only *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{NDM-5}}$ were identified in our 40 isolate collection from November 2020 to March 2024.



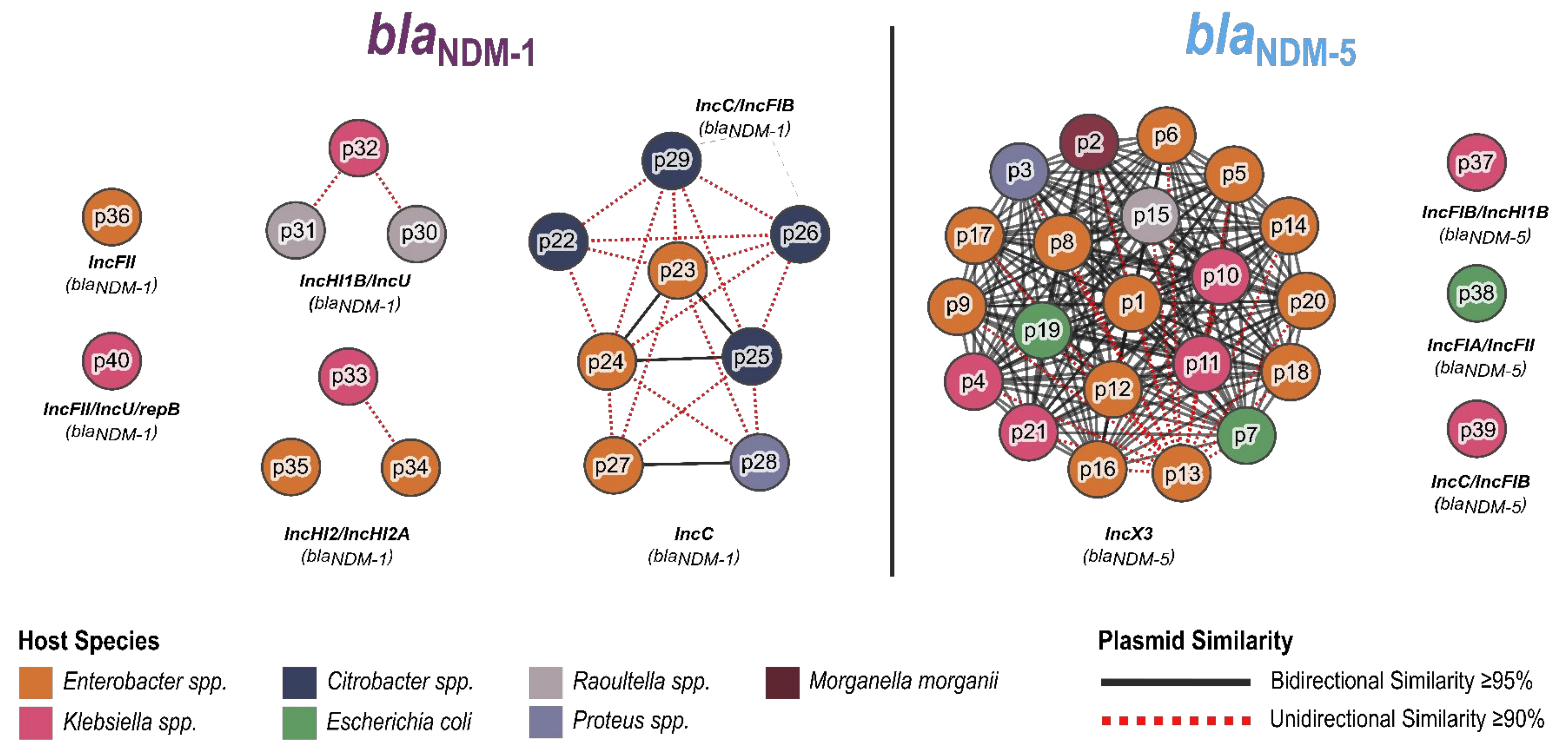
University of Pittsburgh

Incidence of NDM Variants



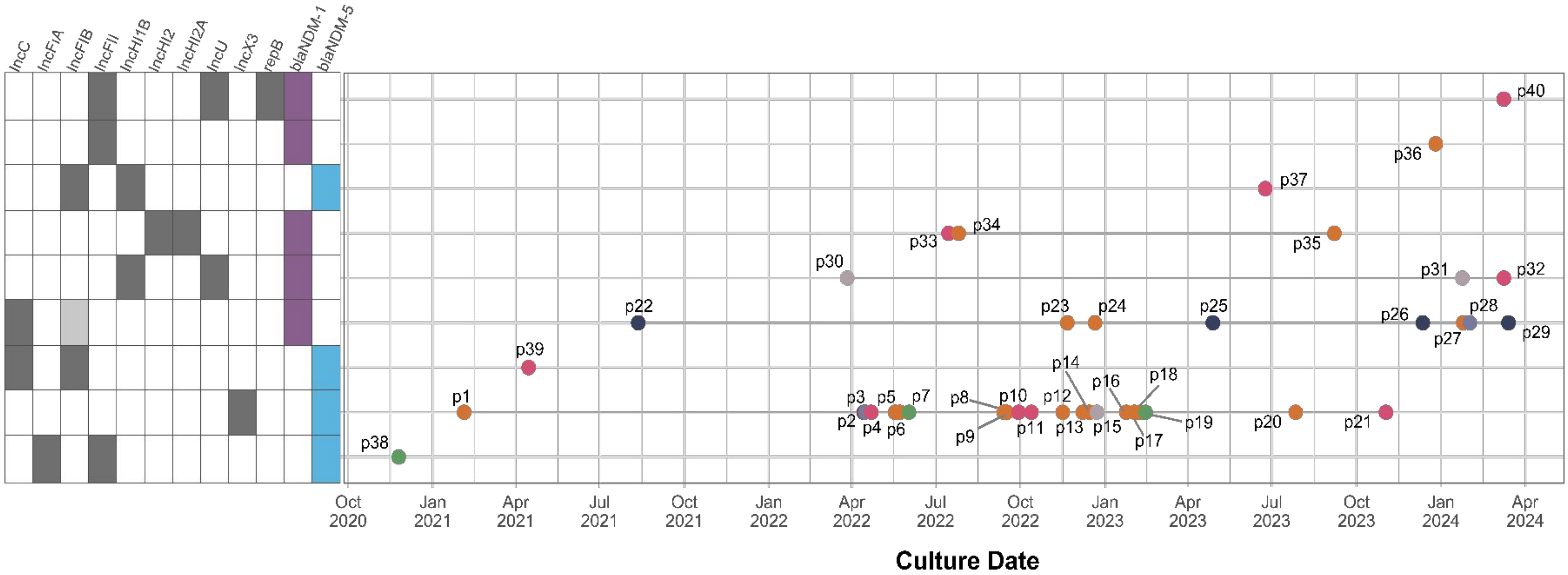
__Distribution of 40 NDM plasmids (NDM-1 & NDM-5) over time, observed in 34 patients from Nov. 2020 to March 2024.__ The most frequent species was *E. hormaechei* (n=15) and *bla*$_{\text{NDM-5}}$ was the dominant variant (n=24).

Plasmid Clusters



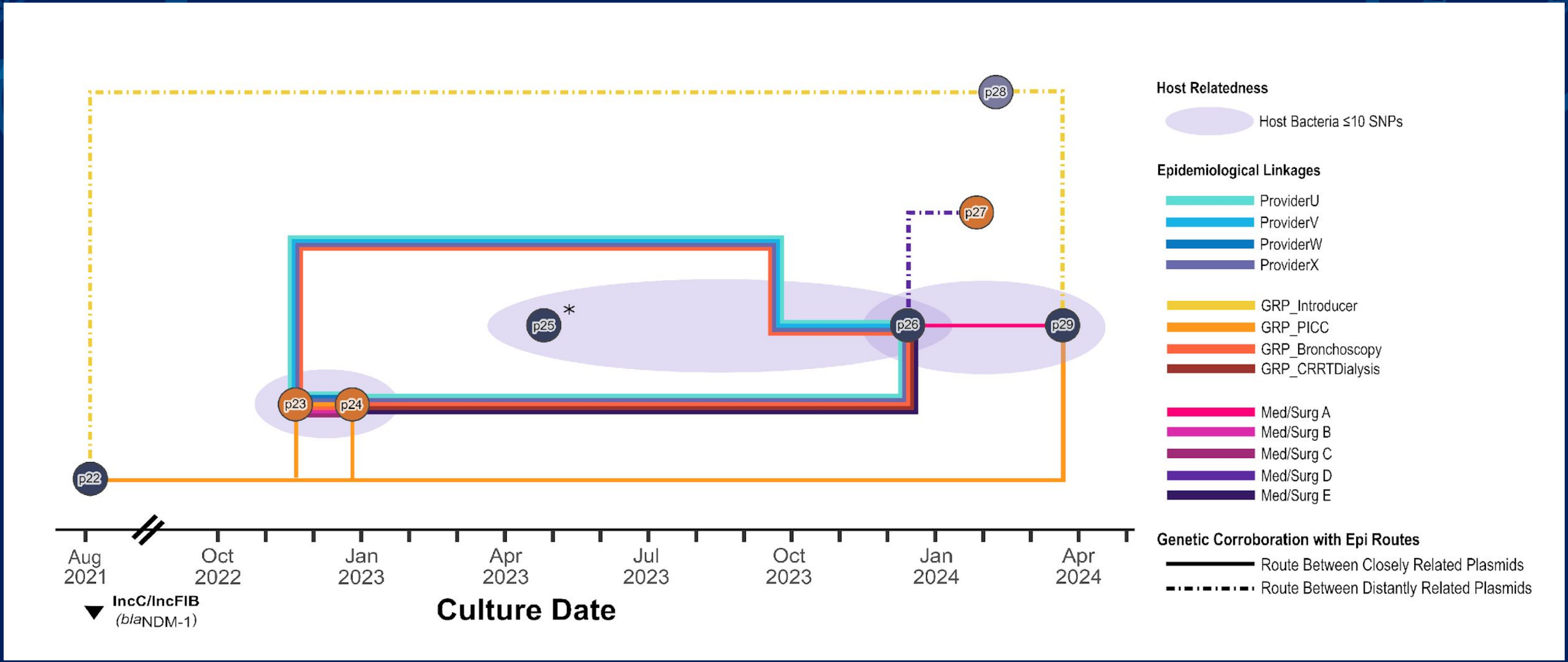
Genetic relatedness of the plasmids based on BLASTn similarity scores. Two major clusters identified: 21 highly conserved IncX3(*bla*$_{\text{NDM-5}}$) plasmids in 8 species and 8 more variable IncC(*bla*$_{\text{NDM-1}}$) plasmids in 4 species.

Plasmid Clusters over Time



__Presence of each NDM plasmid type over extended time periods.__ Grid shows presence (filled) and absence (empty) of replicons and NDM-type.

Epidemiological Investigation



<u>Epidemiological investigation using ML identified potential transmission routes;</u> including 24 shared units, 8 rooms, 20 procedures, and 24 providers with significant odds ratios (ORs: 8.64–390.93, p: <1e-4–0.0308)