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NanoVengers:

Oxford Nanopore Technologies sequencing takes on antimicrobial resistant pathogens and vaccine development

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Department of Molecular Biology and Biochemistry
Simon Fraser University

Lee Lab

SFU

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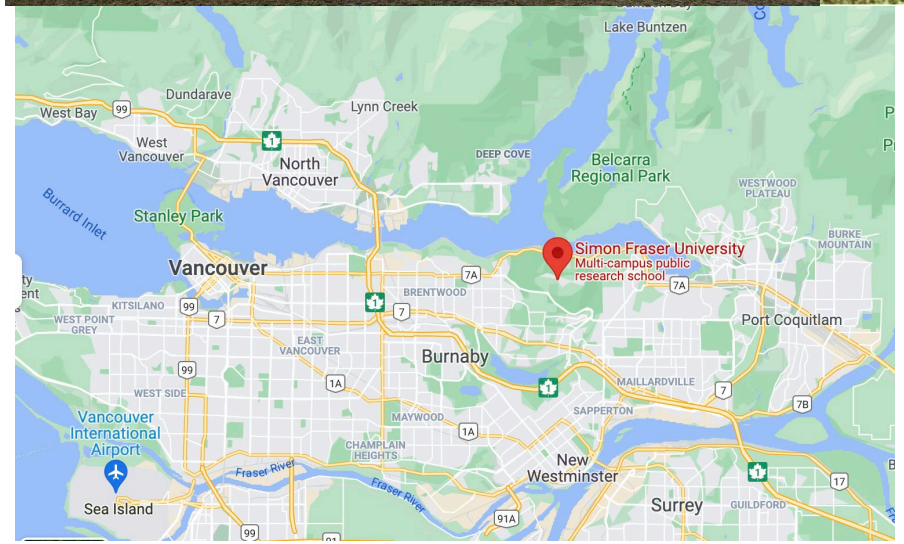


Simon Fraser University acknowledges the unceded Traditional Coast Salish Lands including the Tsleil-Waututh (səl̓ilw̓ətaʔɬ), Kwikwetlem (kʷikʷəƛ̓əm), Squamish (Sḵwx̱wú7mesh Úxwumixw) and Musqueam (xʷməθkʷəy̓əm) Nations upon which our campus resides.

Lee Lab

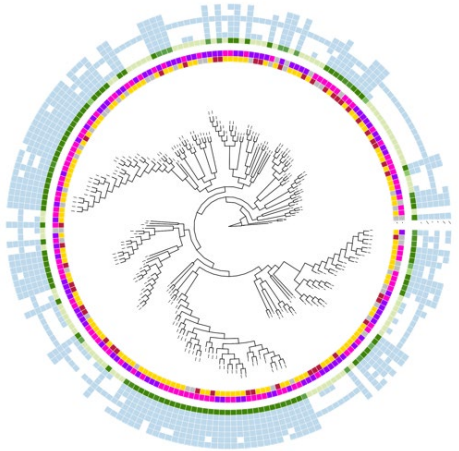
Systems biology approaches provide novel diagnostic, preventative and therapeutic strategies

Host Immune Responses

PMID: 39472236
PMID: 38769383
PMID: 30862783
PMID: 32479514

Microbiome

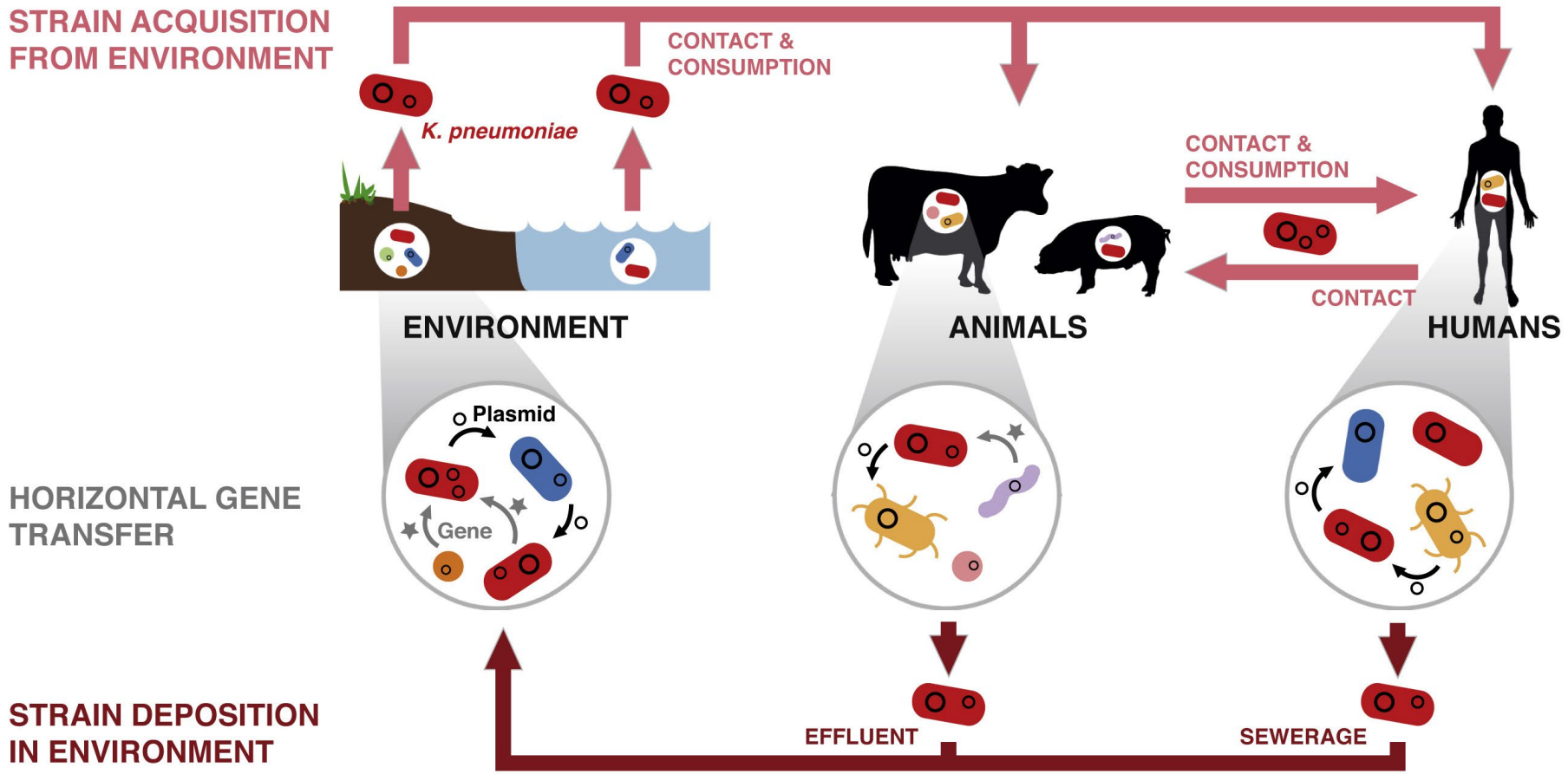
Pathogen



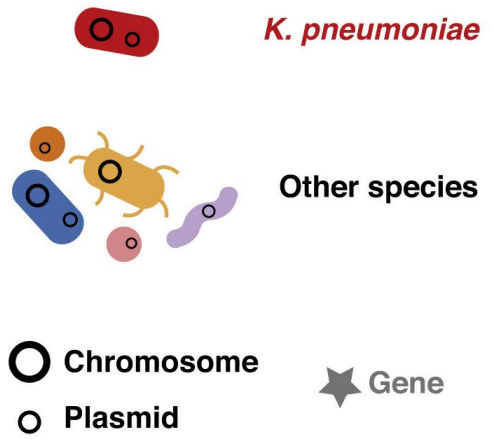
PMID: 34424159

PMID: 33329547

K. pneumoniae is an AMR One Health pathogen



K. pneumoniae **strains acquire AMR genes and plasmids in all niches, which they can carry between niches and donate to other human pathogens.**

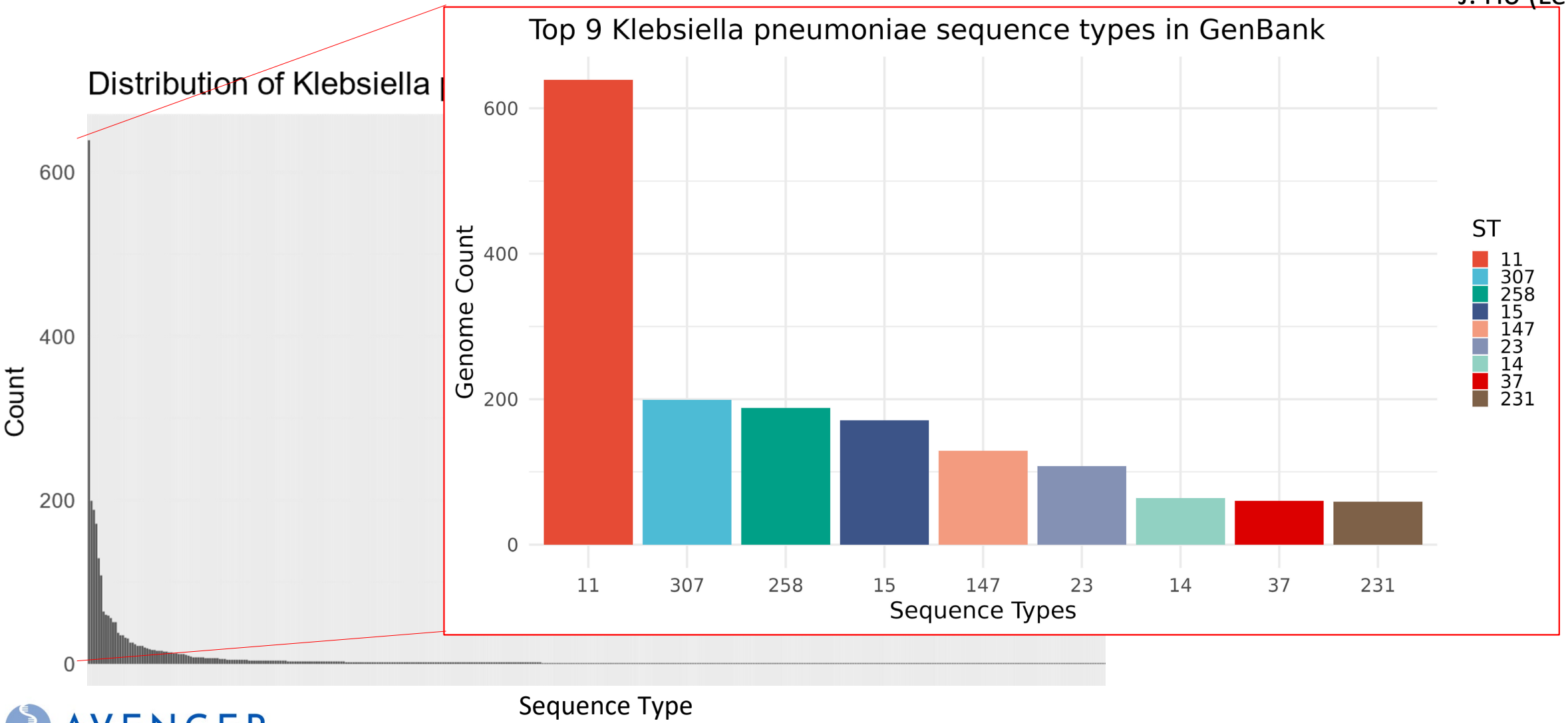


Current Opinion in Microbiology

Wyres & Holt, 2019

NCBI reference genomes do not capture sufficient genetic diversity

J. Ho (Lee Lab)



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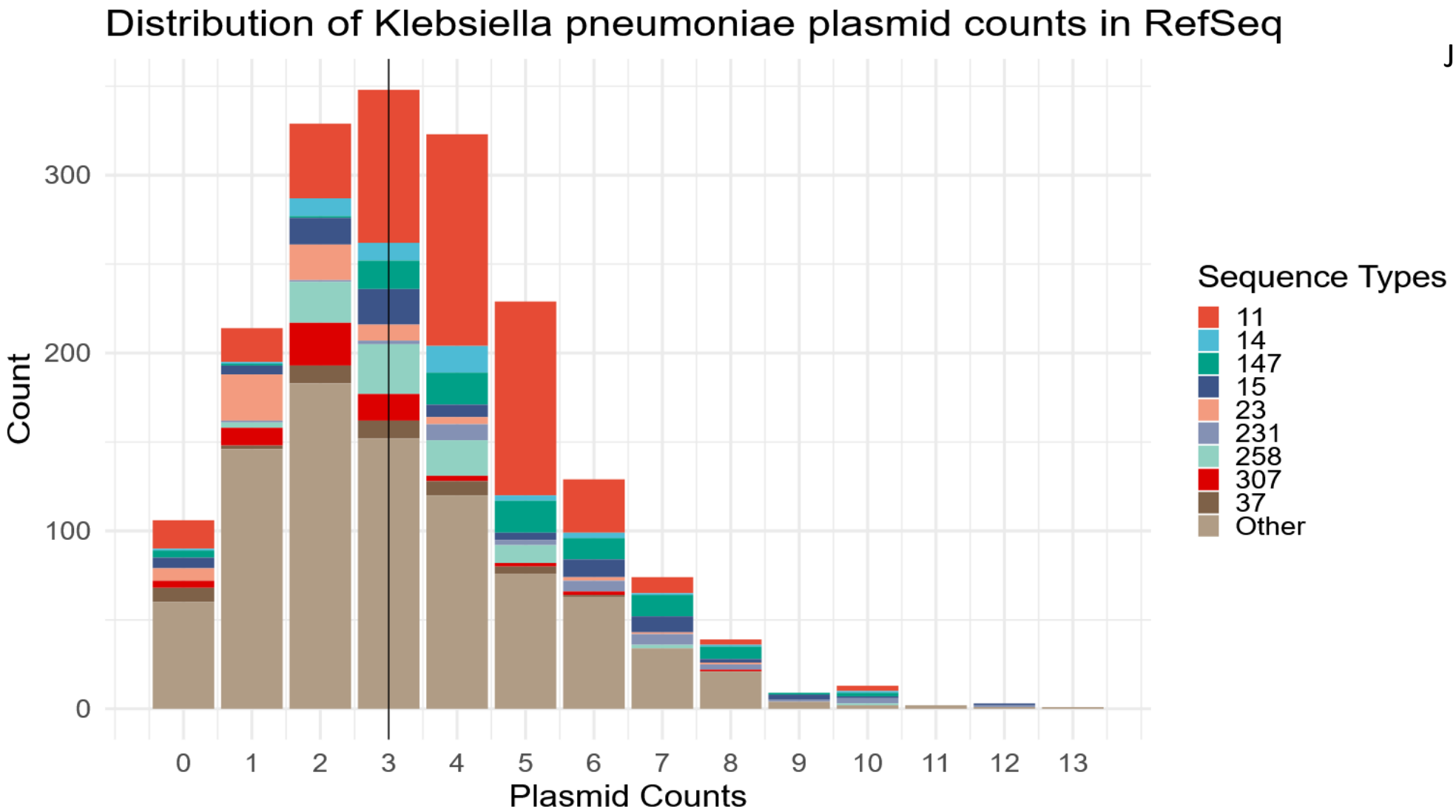
K. pneumoniae: AMR trafficker with lots of plasmids

J. Ho (Lee Lab)



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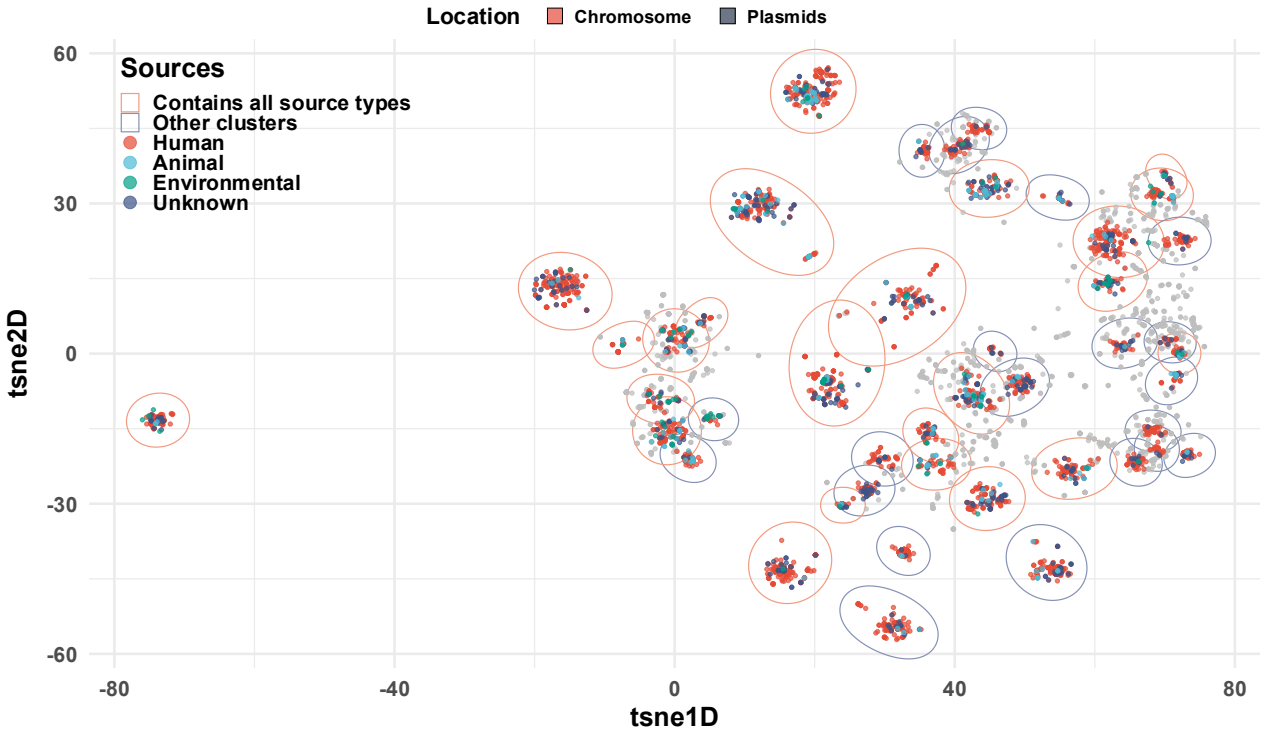
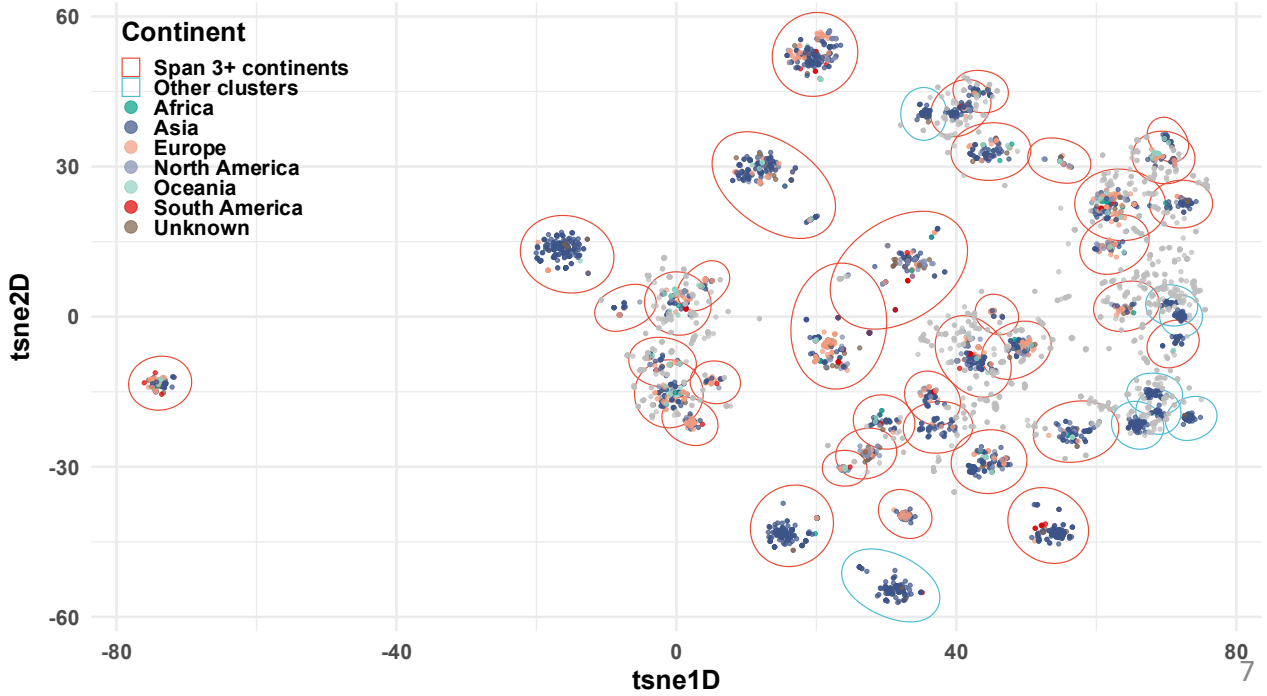
AMR and plasmids in *Klebsiella pneumoniae* are widespread

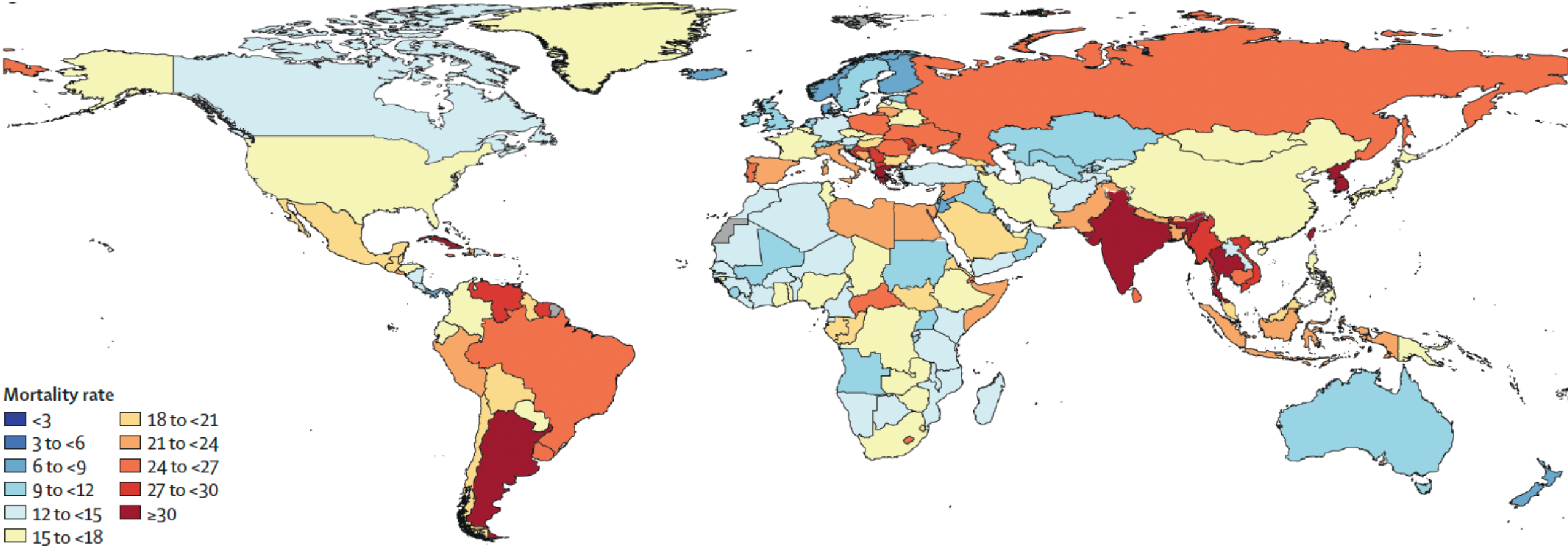
J. Ho (Lee Lab)

- Resistance is primarily carried on plasmids regardless of isolation source
- Similar plasmids are found across all isolation sources and around the globe





The Hidden Pandemic: Antimicrobial Resistance (AMR)



- ~ 1.2 million people died in 2019 from antibiotic-resistant bacterial infections
- More than 39 million deaths from AMR infections estimated between now and 2050

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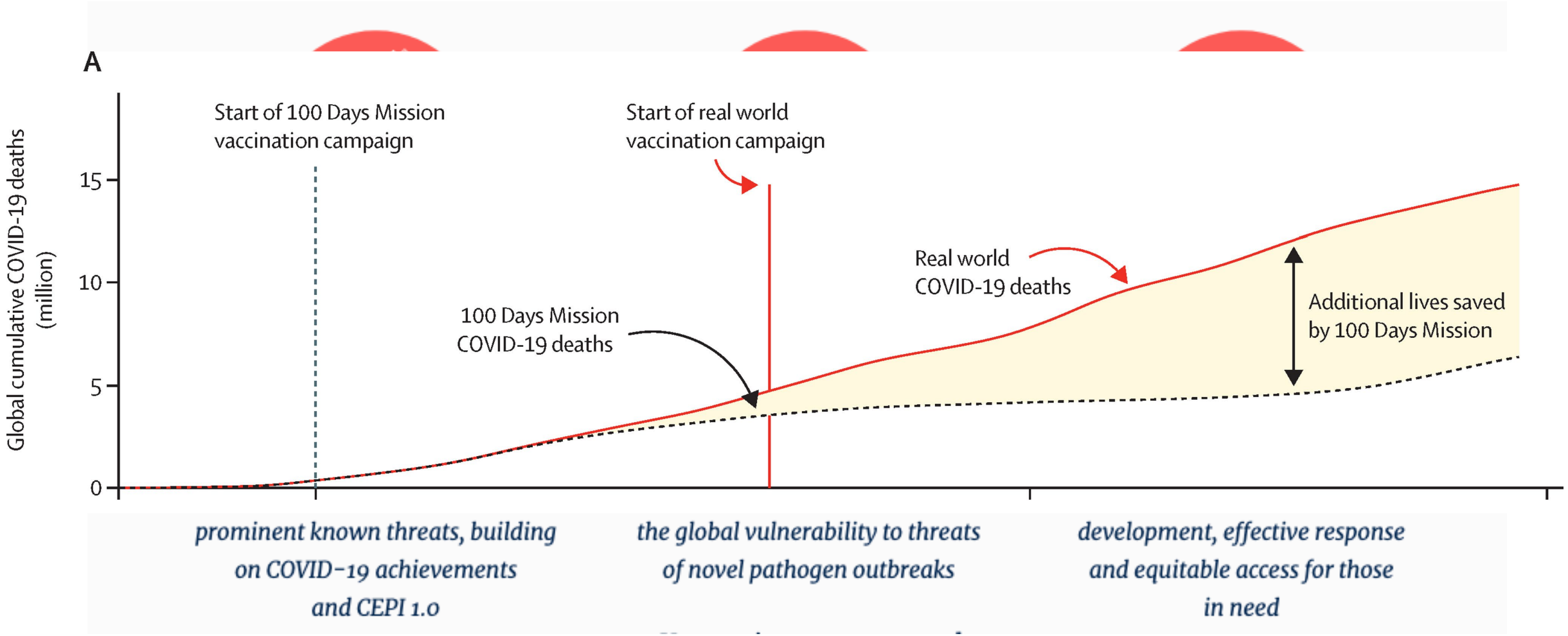
GBD 2021 Antimicrobial Resistance Collaborators, Lancet, 404(10459): 1199-1226

CEPI
Coalition for Epidemic Preparedness Innovations

The 100 Days Mission – Vaccine Development



https://cepi.net/cepi-20-and-100-days-mission

Barnsley, Gregory et al. 2024. The Lancet Global Health, V12, I11

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Advanced RNA LNP **V**accines **E**ngineered with **N**ext-**G**eneration designs to **E**nhance pandemic **R**eadiness

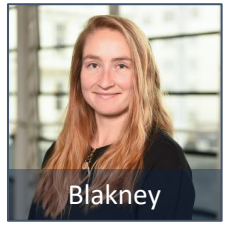
Program Directors: Dr. Pieter Cullis and Dr. Anna Blakney

RNA LNP Technology

Immunology

Antigen Design

THE UNIVERSITY OF BRITISH COLUMBIA

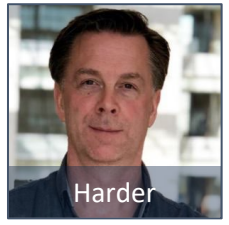

Blakney


Cullis


Jan


Kizhakkedathu

DALHOUSIE UNIVERSITY


Harder


McNagny


Rossi


Gujar


Howlett


Lee

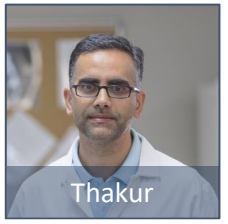
SFU


Russell


Sadarangani


Strynadka

Translation


Thakur

UNIVERSITY OF SASKATCHEWAN

VIDO

Biomanufacturing

EVONIK Leading Beyond Chemistry

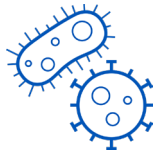
NANOVATION therapeutics

cytiva

NORTHERN RNA MANUFACTURING A BETTER FUTURE

AVENGER will build domestic capacity for a 100-day response using next-generation RNA LNP technology

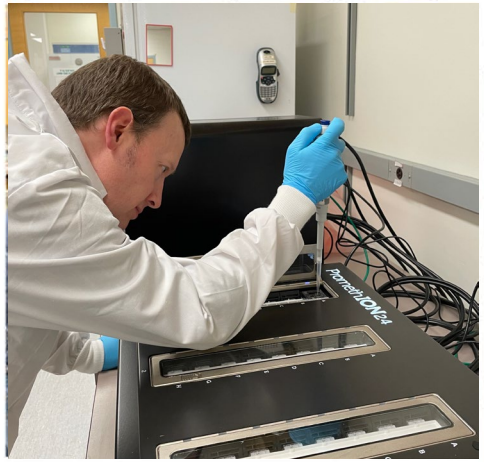
- **Aim 1: Develop high-performing, RNA LNP vaccines with improved efficacy**
  - **Pillar – Research and innovation, Infection prevention and control**
    - LNP with improved potency, stability, and safety
    - saRNA and mRNA optimized for low-dose vaccines
    - Formulations that induce long-lived adaptive responses
    - Formulations for aged and frail populations



- **Aim 2: Develop vaccine candidates against antimicrobial-resistant (AMR) bacteria (*K. pneumoniae*, *S. pneumoniae*, *S. aureus*)**
  - **Pillar – Surveillance; R&I, Infection prevention and control**
    - Build a strategy and extensive database for bacterial vaccine antigen selection and optimization
    - Obtain and sequence clinical isolates – long read sequencing (Nanopore)
    - At least one RNA LNP vaccine for an AMR bacterial pathogen

- **Aim 3: Bio-manufacture a candidate vaccine and test it in an NHP infection model**
  - **Pillar – R&I, Infection prevention and control**
    - Logistics and implementation of a biomanufacturing pipeline
    - Adaptable platform technology

Sequencing of clinical isolates:



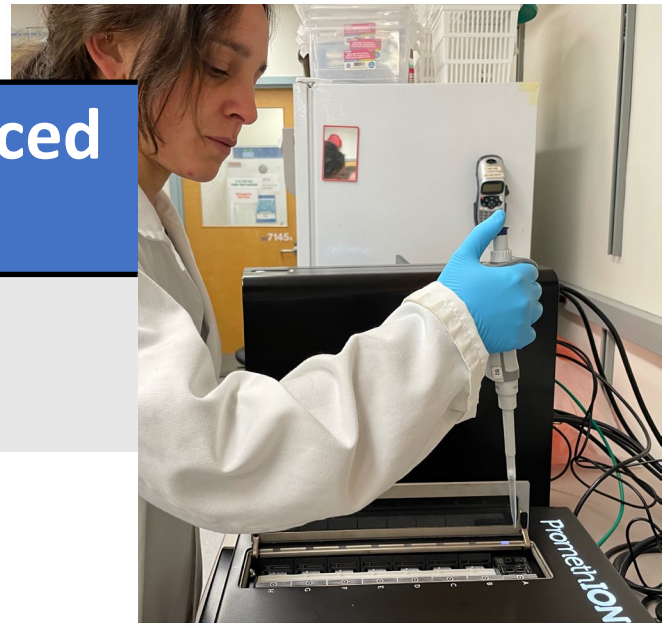
Dr. M. Trimble (Lee Lab)

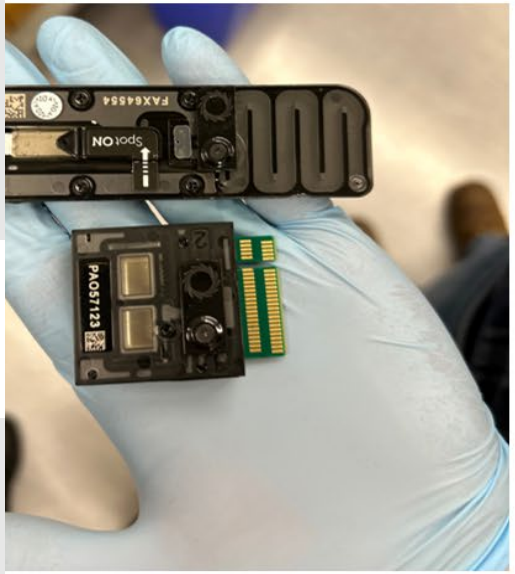
Dr. B. Raymond; A. Redey (Lee

- Thanks to Carmen Li an

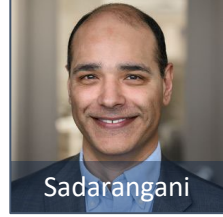
Species	To be sequenced	Sequenced
K. pneumoniae	101	> 400
S. aureus	356	> 60
S. pneumoniae	114	-
MRSA	17	-
Total	**588**	

Dr. O. Pacios (Russell/Lee Labs)





Reverse Vaccinology to the Rescue!



Klebsiella pneumoniae
NCBI

fasta

Annotate genomes
Bakta

Pangenome analysis
Panaroo

Entropy & divergence
BMGE

Human homology
BLASTp

Extracellular targets
PSORTb

Core genome SNPs
snp-sites

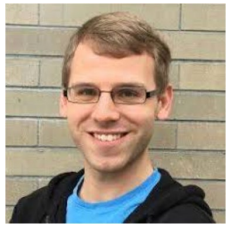
Expression analysis
DESeq2/Limma

Construct phylogeny
FastTree

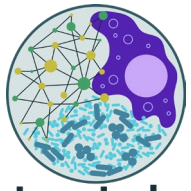
Strain metadata
NCBI

Structural prediction
AlphaFold

Visualize phylogeny
ggtree



T. Blimkie; J. Ho (Lee Lab)



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AVENGER Next Steps

RNA LNP technology development

- Identified PEG substitutes
- Designed new alphaviral vectors for saRNA backbones
- Establishing immunological assessments of benchmark vaccines
- Establishing immune alterations in variably aged and frail mice
- Standardizing immunoassays for evaluating new formulations

Bacterial antigen discovery and engineering

- Candidate antigens for *K. pneumoniae* and *S. aureus* have been engineered from existing data sets
- Acquiring clinical isolates of AMR bacteria (~900)
- Building a whole-genome assembly and reverse vaccinology pipeline
- Establishing murine infection models

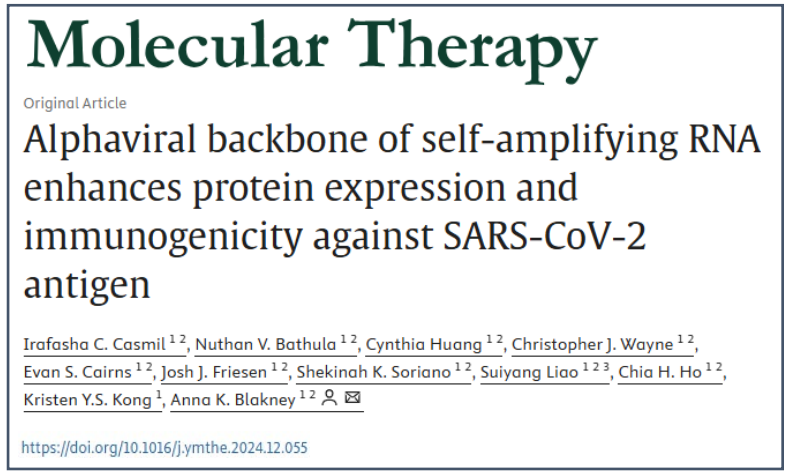

ADVANCED FUNCTIONAL MATERIALS

Research Article | Open Access

An Ultrahydrating Polymer that Protects Protein Therapeutics and RNA-Lipid Nanoparticles Against Freezing, Heat and Lyophilization Stress

Anna Herrmann, Srinivas Abbina, Nuthan Vikas Bathula, Peyman Malek Mohammadi Nouri, Irina Chafeeva, Iren Constantinescu, Emaan Abbasi, Usama Abbasi, Matthew Drayton, Haiming Daniel Luo, Haisle Moon, Arshdeep Gill, Yu Xi, Allan K. Bertram, Caigan Du, Rainer Haag, Anna K. Blakney, Jayachandran N. Kizhakkedathu

First published: 01 August 2024 | https://doi.org/10.1002/adfm.202406878


Molecular Therapy

Original Article

Alphaviral backbone of self-amplifying RNA enhances protein expression and immunogenicity against SARS-CoV-2 antigen

Irafasha C. Casmil [1,2], Nuthan V. Bathula [1,2], Cynthia Huang [1,2], Christopher J. Wayne [1,2], Evan S. Cairns [1,2], Josh J. Friesen [1,2], Shekinah K. Soriano [1,2], Suiyang Liao [1,2,3], Chia H. Ho [1,2], Kristen Y.S. Kong [1], Anna K. Blakney [1,2]

https://doi.org/10.1016/j.ymthe.2024.12.055

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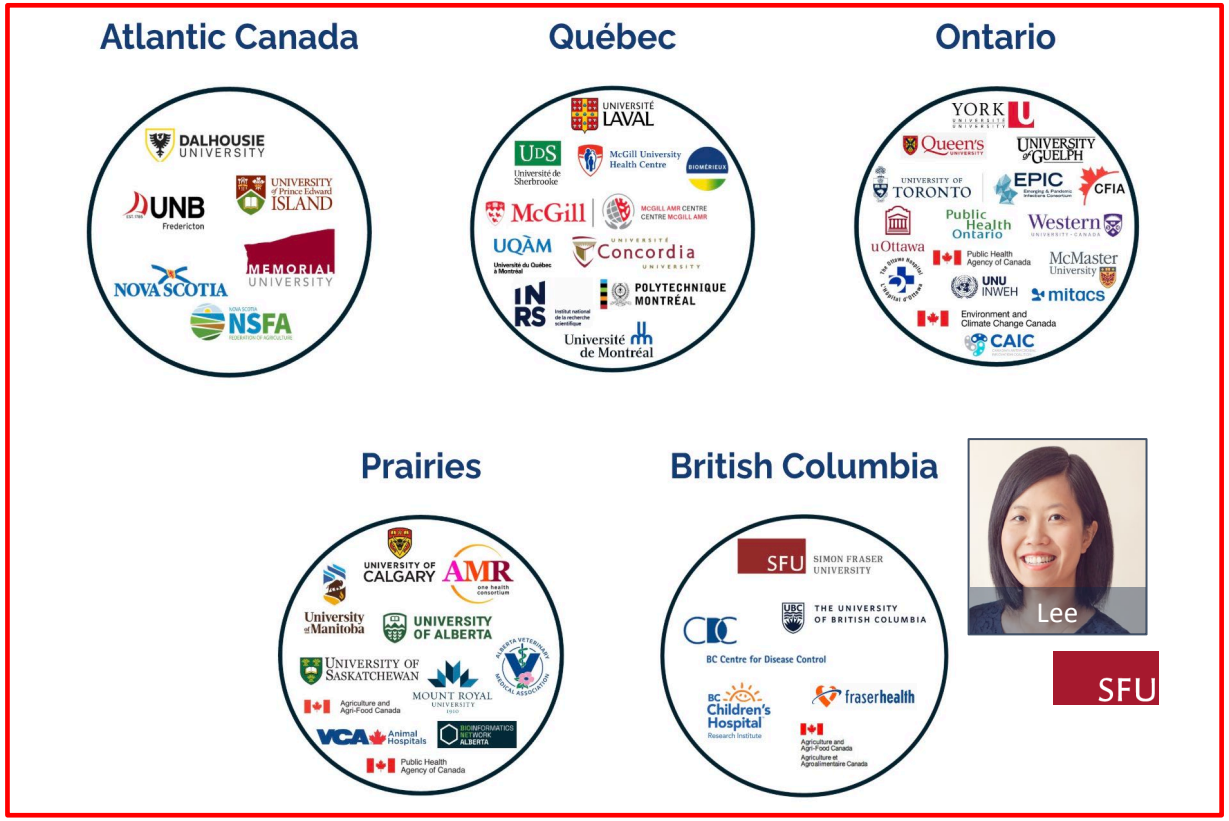
CIHR Funded One Health AMR Training Platform: CAN-AMR-NET

CAN-AMR-Net

https://canamrnet.org/

- Central Coordination through the University of Calgary

- Encompassing **5 Regional** hubs under 4 themes
  1. One Health/AMR Translational Competencies
  2. Transdisciplinary Training & Career Readiness
  3. Excellence in Knowledge Translation & Communication
  4. Integration of Indigenous Knowledge and Community-Led Research



Acknowledgements

Lee Lab

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Next-generation vaccines for Canadians

SFU Collaborators:

BCCHRI (Malawi & 16S Diagnostics):

Dalhousie (Neonatal Mouse Sepsis):

Funding Support:

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

NSERC CRSNG

BANTING RESEARCH FOUNDATION

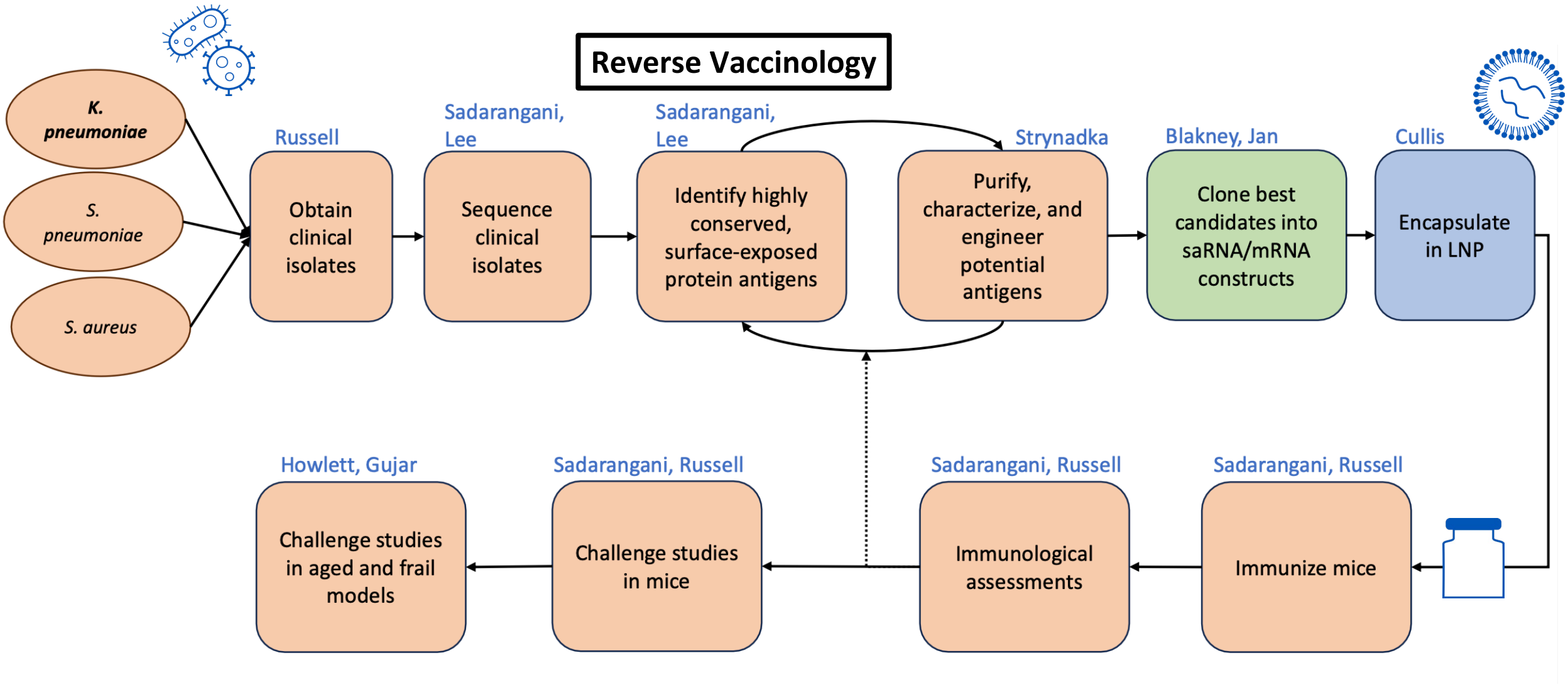
Michael Smith Health Research BC

Australian Government National Health and Medical Research Council

NHMRC

INNOVATION Canada Foundation for Innovation / Fondation canadienne pour l'innovation

AVENGER takes on 3 Key Priority AMR Pathogens



Klebsiella pneumoniae: a Global AMR Threat

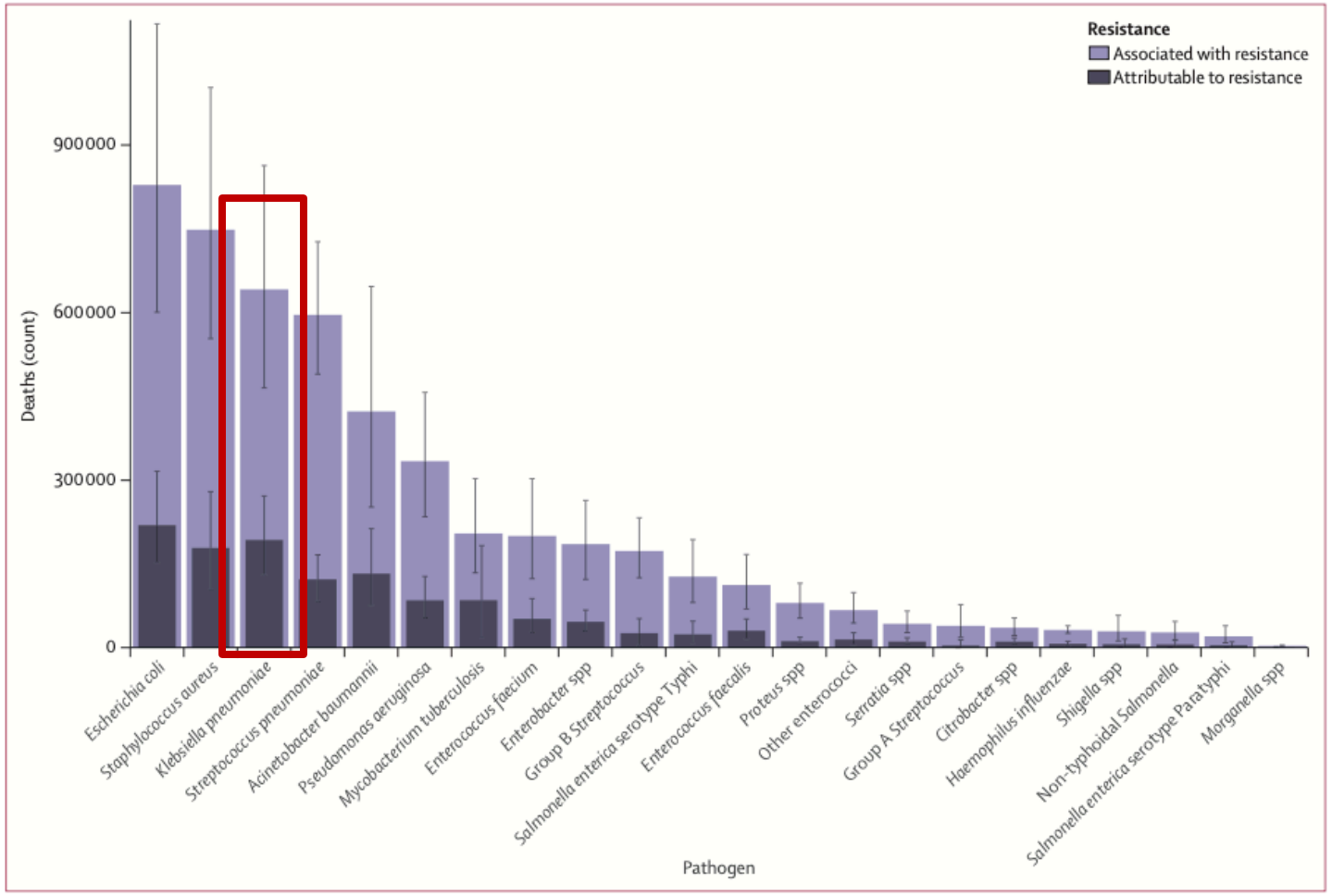
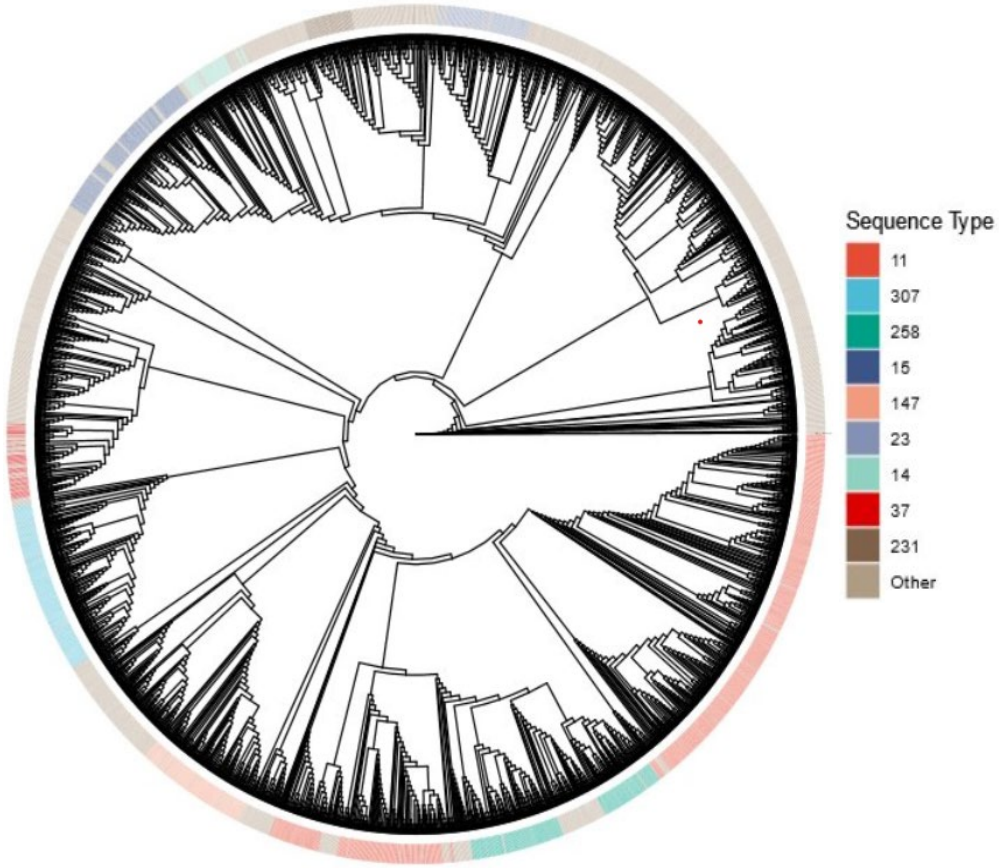


Figure 4: **Global deaths (counts) attributable to and associated with bacterial antimicrobial resistance by pathogen, 2019**
Estimates were aggregated across drugs, accounting for the co-occurrence of resistance to multiple drugs. Error bars show 95% uncertainty intervals.



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GBD 2019 Antimicrobial Resistance Collaborators, Lancet, 399(10325): 629-655

K. pneumoniae: Vaccine Evaluation Profile

KLEBSIELLA PNEUMONIAE

Health impact:

Direct health impact

1.0	0.0
Mortality	Morbidity

Impact on AMR reduction

1.0	2.0
Antibiotic use	Urgency of AMR threat

Secondary health impact

None identified

Sub-population benefits

Individuals with recurrent urinary tract infections, HIV, chronic lung conditions or diabetes

Alternative interventions

Probability of R&D success:

0.0	0.5	0.5
Pipeline robustness	Pathogen biology	Pre-clinical and clinical R&D

Combination potential

None identified

Acceleration potential

None identified

Major barriers to development

Lack of understanding of disease epidemiology

Probability of uptake:

1.0	0.0	1.0	1.0
Commercial attractiveness	Expected policy stance	Payer, government or Gavi support	Barriers to uptake