

NGS to trace the historical spread of antimicrobial resistance

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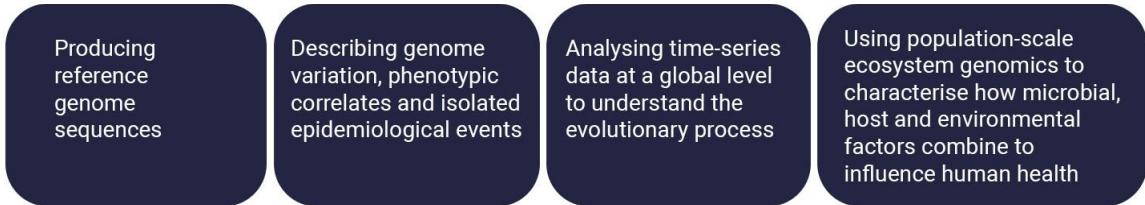
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Parasites and Microbes Programme (PAM)

*PAM's
contribution
to infectious
disease
research*

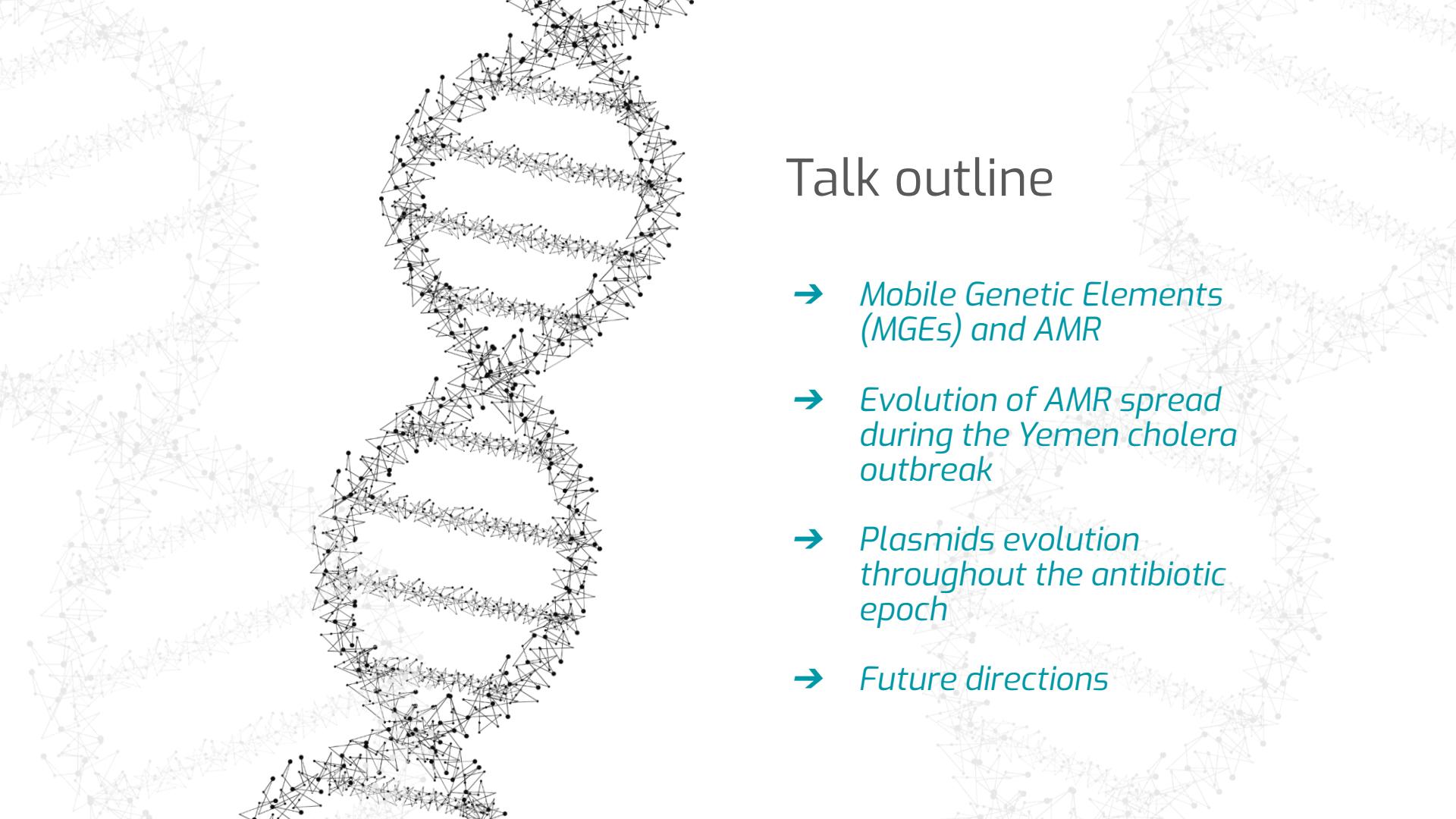


1993 2006 2021 2026 2036

*Thomson
team
Bacterial
Genomics
and
Evolution*



We use genomics approaches to understand the way pathogens have moved, and continue to move, through populations and across continents with a view to providing high-resolution insights aimed at tracking and limiting their spread.

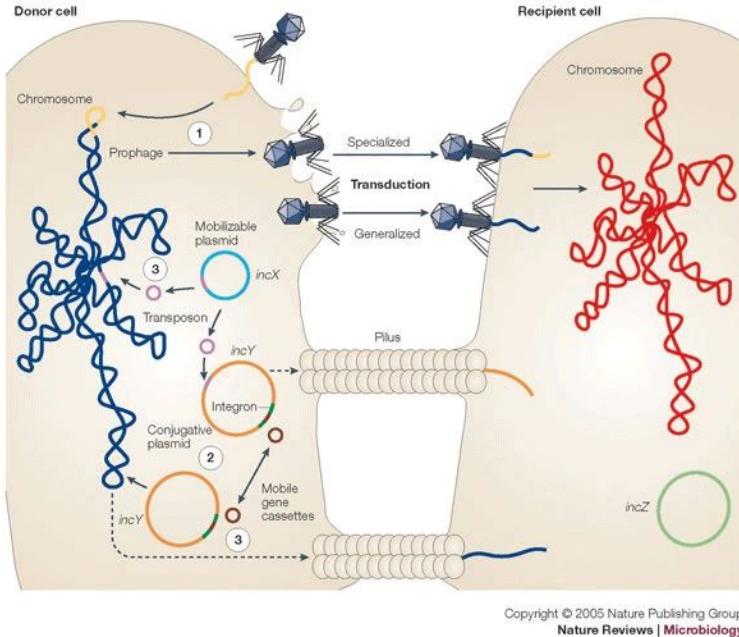


Talk outline

- *Mobile Genetic Elements (MGEs) and AMR*
- *Evolution of AMR spread during the Yemen cholera outbreak*
- *Plasmids evolution throughout the antibiotic epoch*
- *Future directions*

The mobilome's role in AMR Gene Transfer

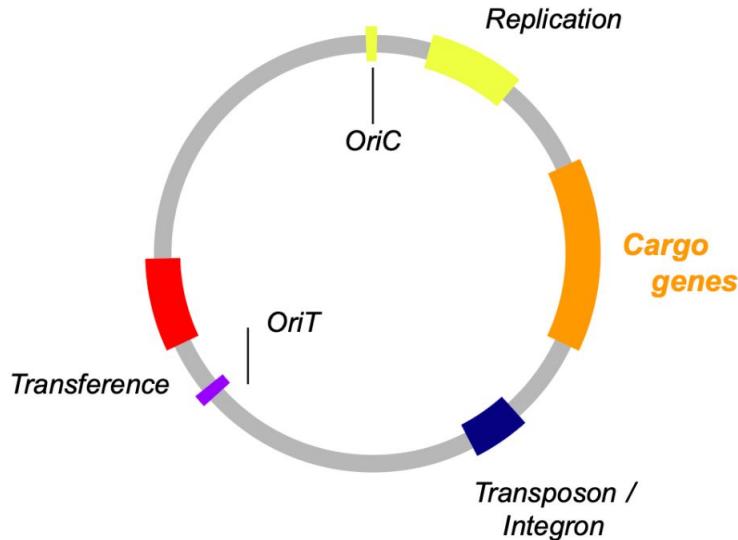
Transfer of DNA between bacterial cells



- MGEs: elements that promote intracellular and intercellular DNA mobility.
- The concerted activity of both types of MGEs results in Horizontal Gene Transfer.
- Bacterial pathogens are able to meet the evolutionary challenge of combating antimicrobials, often by acquiring preexisting resistance determinants from the bacterial gene pool.
- Widespread multidrug resistance is commonly achieved by acquisition of preexisting determinants followed by amplification in response to selection.

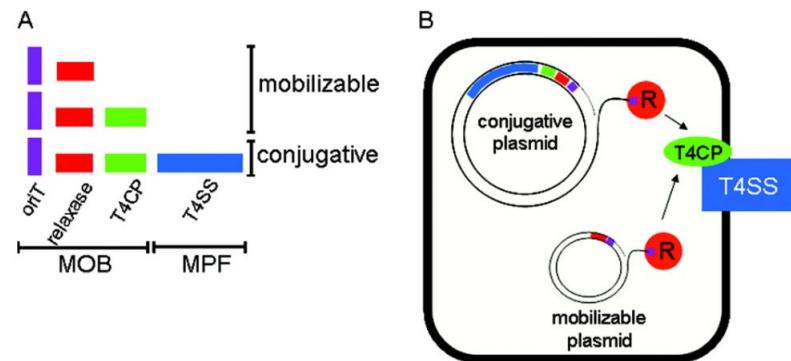
(Figure) Frost et al. 2005; Hulter et al. 2017

Plasmids, the main vehicles of AMR spread



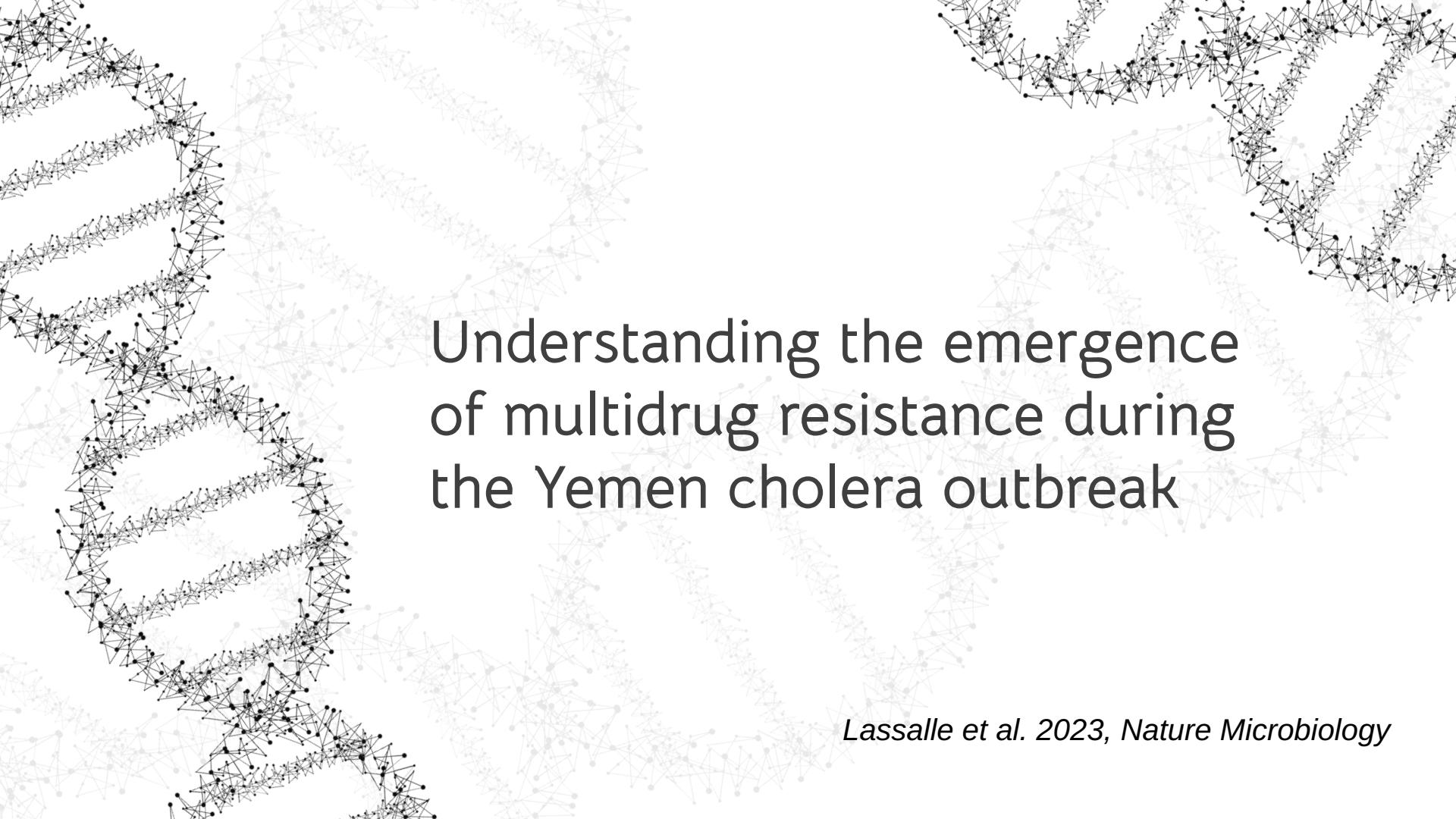
Plasmids are extrachromosomal and (mostly) circular DNA molecules with self-controlled replication and capable of self-transmission between cells.

Source: Rozwandowicz et al. 2018 - J Antimicrob Chemother; Conjugation - Modified from Adenosine (https://en.wikipedia.org/wiki/Bacterial_conjugation#/media/File:Conjugation.svg)



(A) Schematic view of the genetic constitution of transmissible plasmids. (B) Scheme of some essential interactions in the process of conjugation.

Source: Smillie et al. 2010. Mobility of Plasmids.

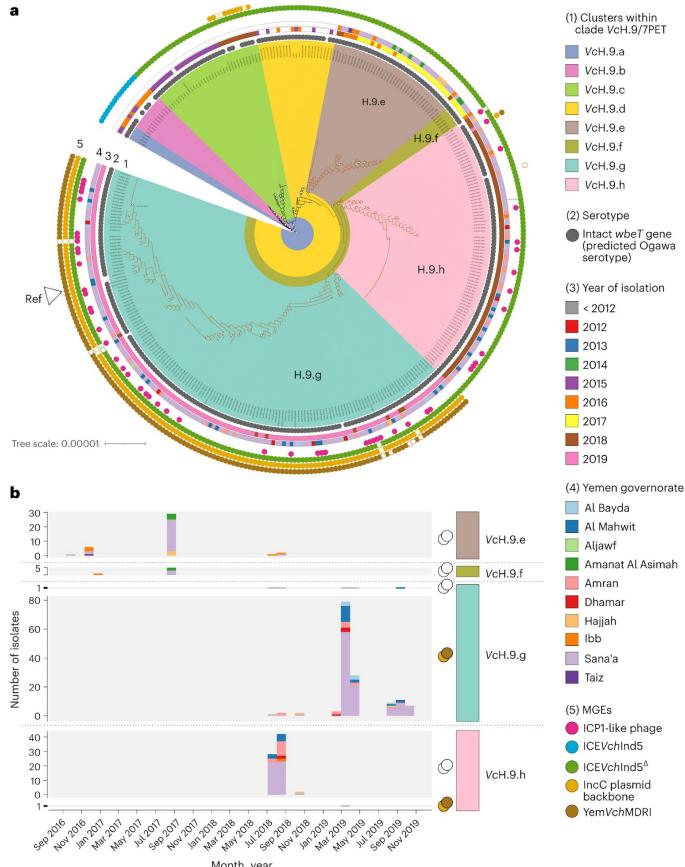


Understanding the emergence of multidrug resistance during the Yemen cholera outbreak

Lassalle et al. 2023, Nature Microbiology

Genomic epidemiology of *Vibrio cholerae* in Yemen

Phylogenetic diversity and spatio-temporal distribution of *V. cholerae* 7PET-T13 isolates (Vch.9) from Yemen.



* Cholera is an acute diarrhoeal disease caused by ingesting food or water contaminated with the bacterium *Vibrio cholerae*.

* Only selected strains have potential to cause epidemics and pandemics, mainly due to their ability to produce cholera toxin. The current pandemic is associated with the emergence of *V. cholerae* biotype known as 7PET.

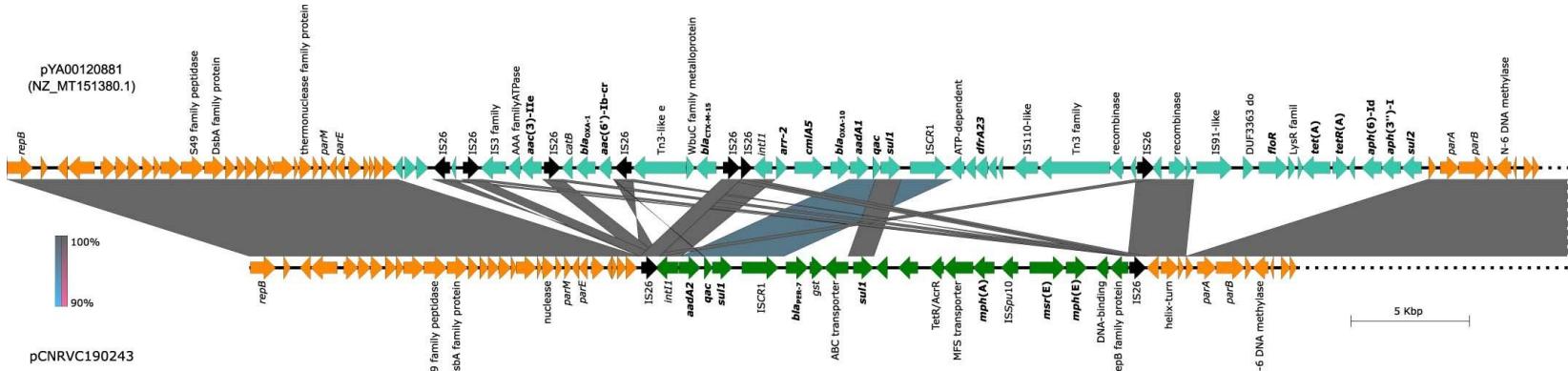
* *V. cholerae* reached Yemen in 2016, causing the largest cholera outbreak in modern history.

* Prior genomic surveillance revealed the introduction of the 7PET T13 in Yemen, which was susceptible to all antibiotics.

* Ongoing surveillance coupled with genomics and microbiology characterisation in the region between 2018 and 2019 documented the emergence of plasmid-mediated multidrug resistance.

The Yemen MRD plasmid is spreading across the species

Comparison of IncC plasmids pCNRVC190243 (*Yemen plasmid*) and pYA00120881 (*plasmid from V. cholerae strains collected in Zimbabwe*).



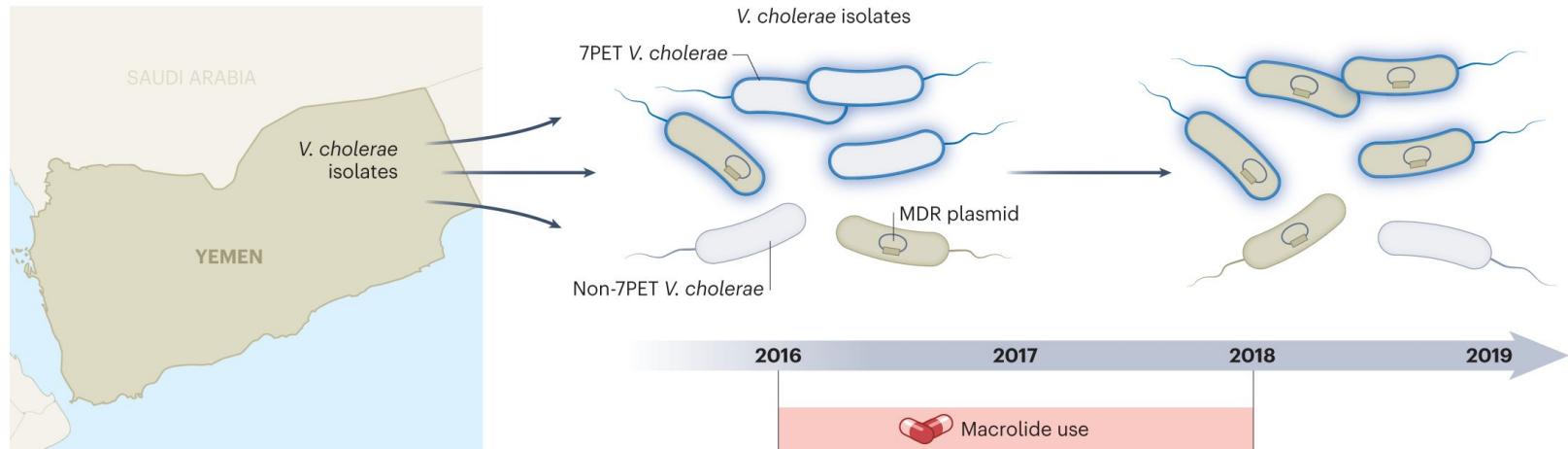
* By looking for the plasmid sequence, we found instances across 7PET and Non-7PET strains, both locally and globally.

* We also found evidence of movement of the MDR region into the chromosome.

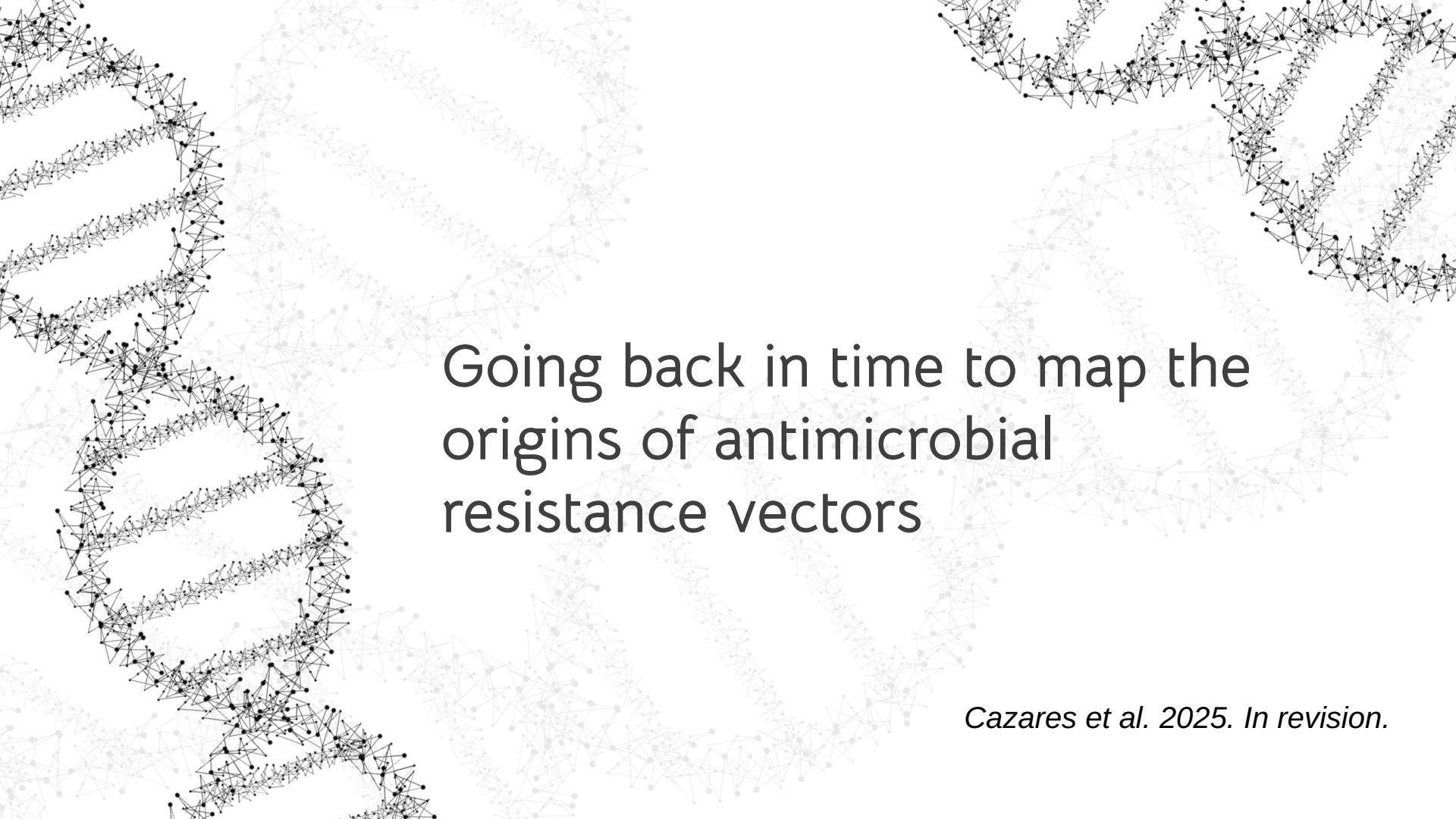
* The MDR region seems to be specific of the Yemen plasmid.

Take home messages

Expansion of multidrug resistance in Yemeni 7PET *V. cholerae* isolates.



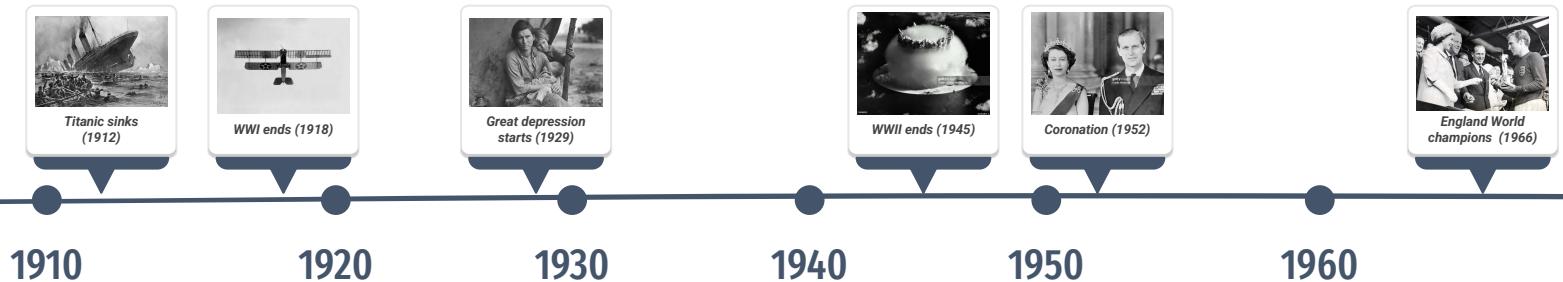
- Genomic analysis of *V. cholerae* isolates recovered in Yemen revealed that clinical macrolide use initiated in 2016 to treat cholera preceded a shift in *V. cholerae* genotype from antibiotic susceptibility to multidrug resistance between 2018 and 2019.
- This was due to the spread of a newly acquired MDR plasmid conferring resistance (beige) in 7PET epidemic *V. cholerae* strains (blue).
- This MDR plasmid was also carried by non-epidemic *V. cholerae* (grey) present, indicating that genetic exchange between epidemic and endemic strains may have contributed to the emergence of multidrug resistance.



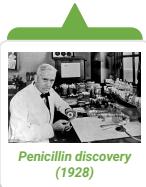
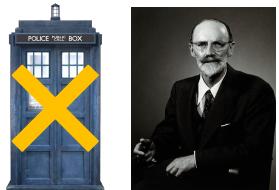
Going back in time to map the origins of antimicrobial resistance vectors

Cazares et al. 2025. In revision.

The historical context of antibiotics discovery



Pre-antibiotic Era (PAE)

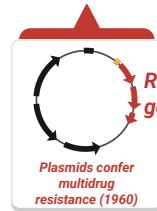


Penicillin discovery
(1928)



Fresh air treatment
for TB (1936)

Antibiotics Industrialisation & Golden Era



Plasmids confer
multidrug
resistance (1960)

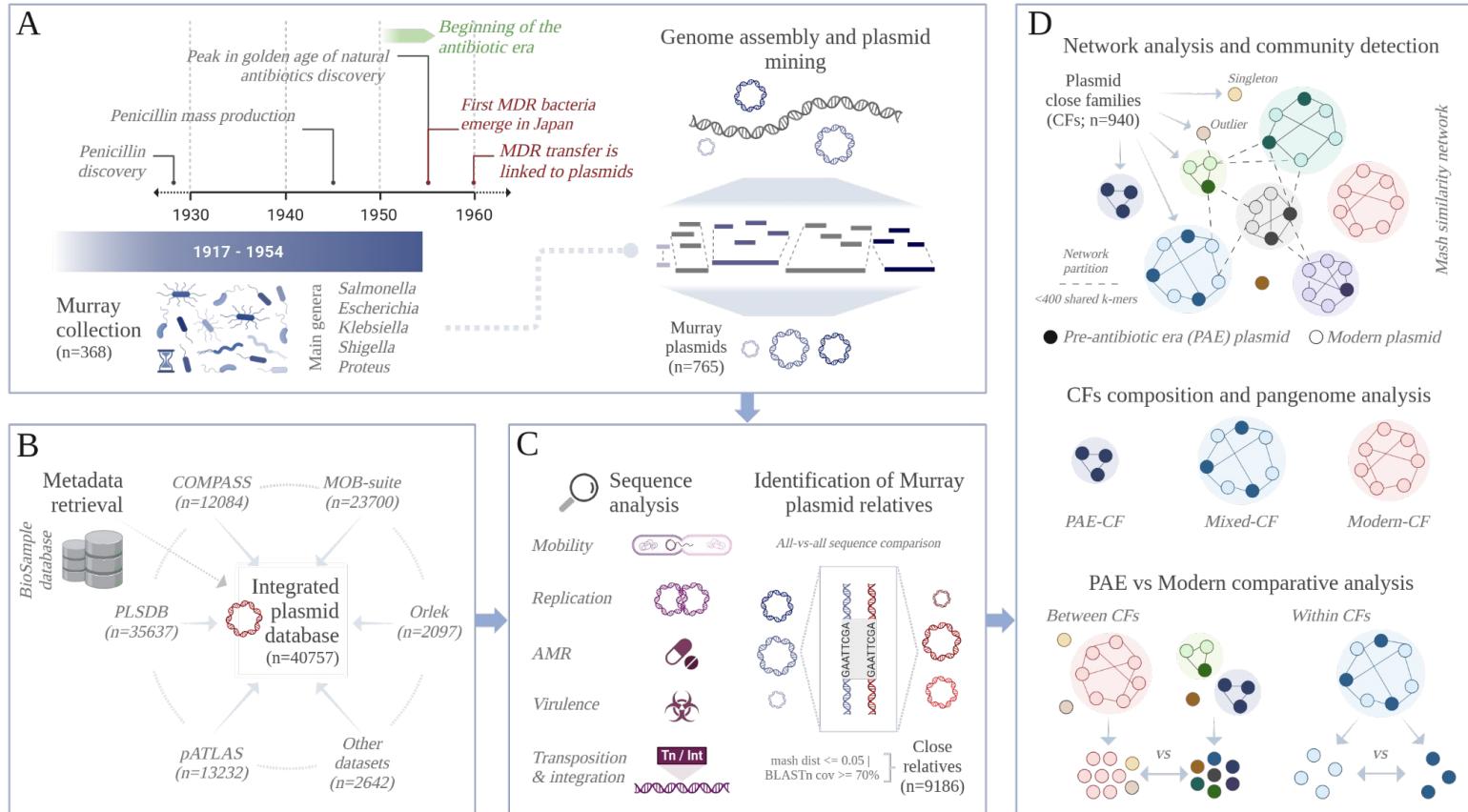
The Murray Collection (1917 - 1954)

Prof. Everitt George
Dunne Murray

Reviving and sequencing pre-antibiotic era pathogens



Study overview



Reconstructed pre-antibiotic era (PAE) plasmids

*PAE plasmids
discovered*

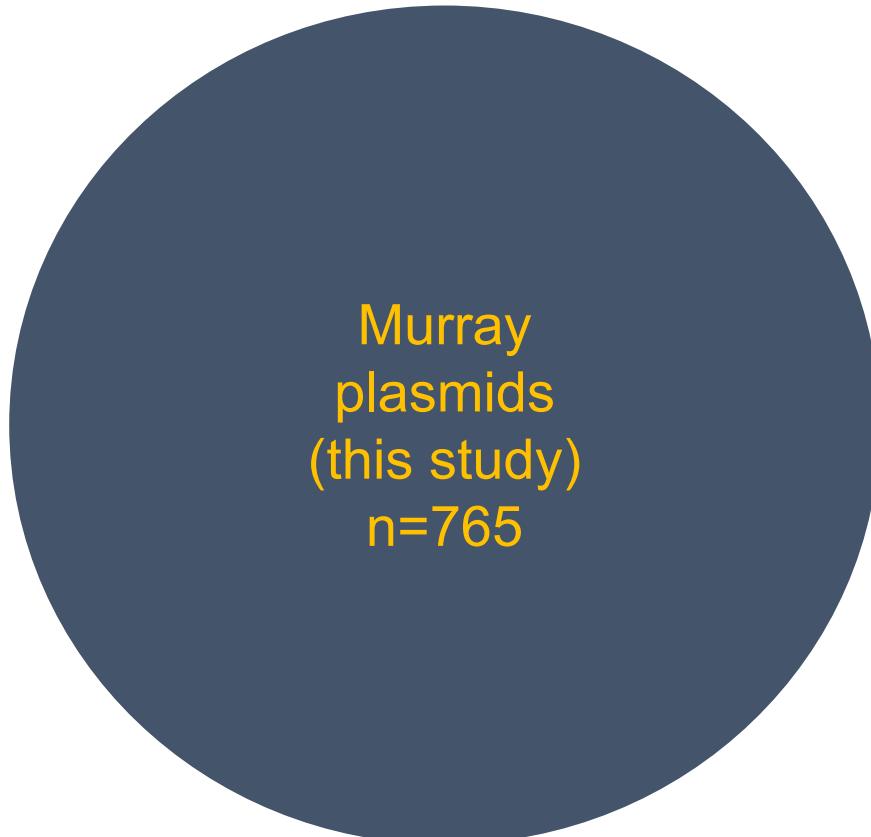
PAE plasmids
in GenBank



n=22

**35-fold
increase**

Murray
plasmids
(this study)
n=765

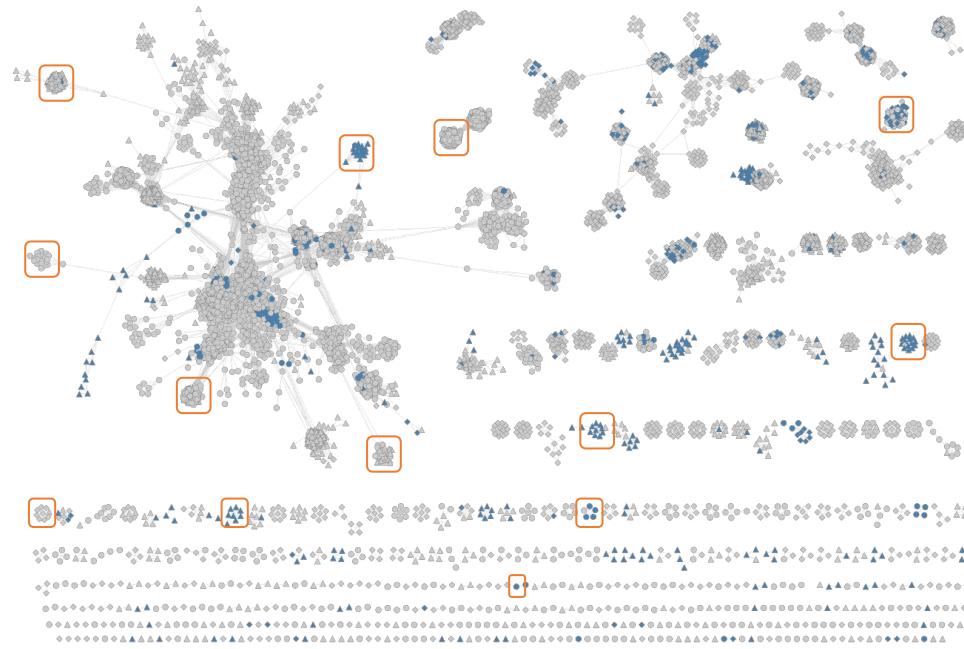


Relationship between PAE plasmids and their modern relatives

**PAE plasmids were
highly diverse**

Sequence similarity network

Examples
of plasmid
species



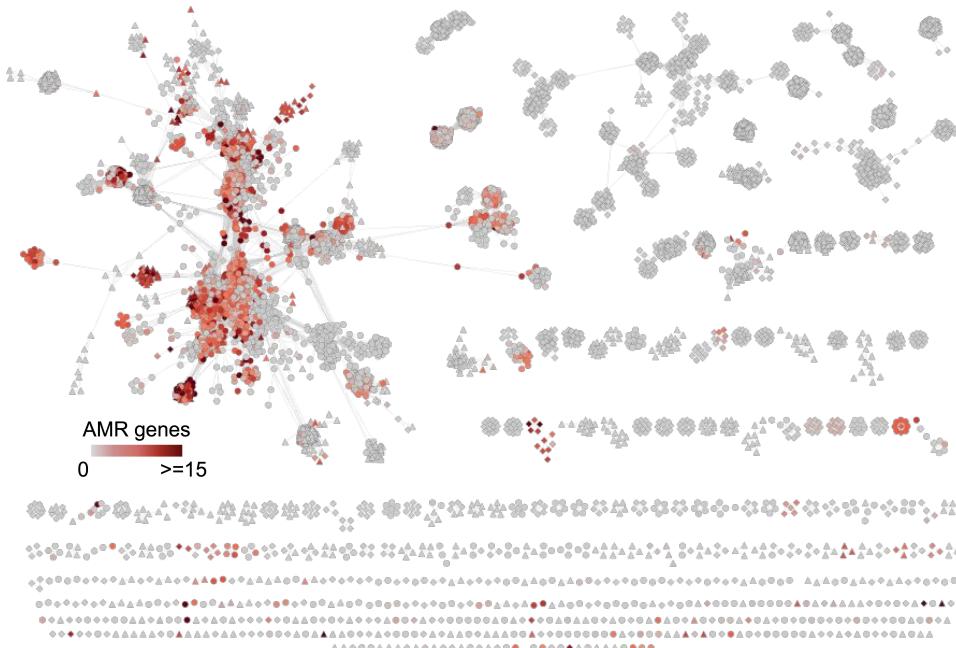
Source: PAE ■ Modern ■

**~10,000 modern
plasmids linked to PAE
relatives**

**More than 900 plasmid
“species” identified**

Plasmids contribution to AMR spread

AMR genes concentrate in a minority of plasmids and plasmid species

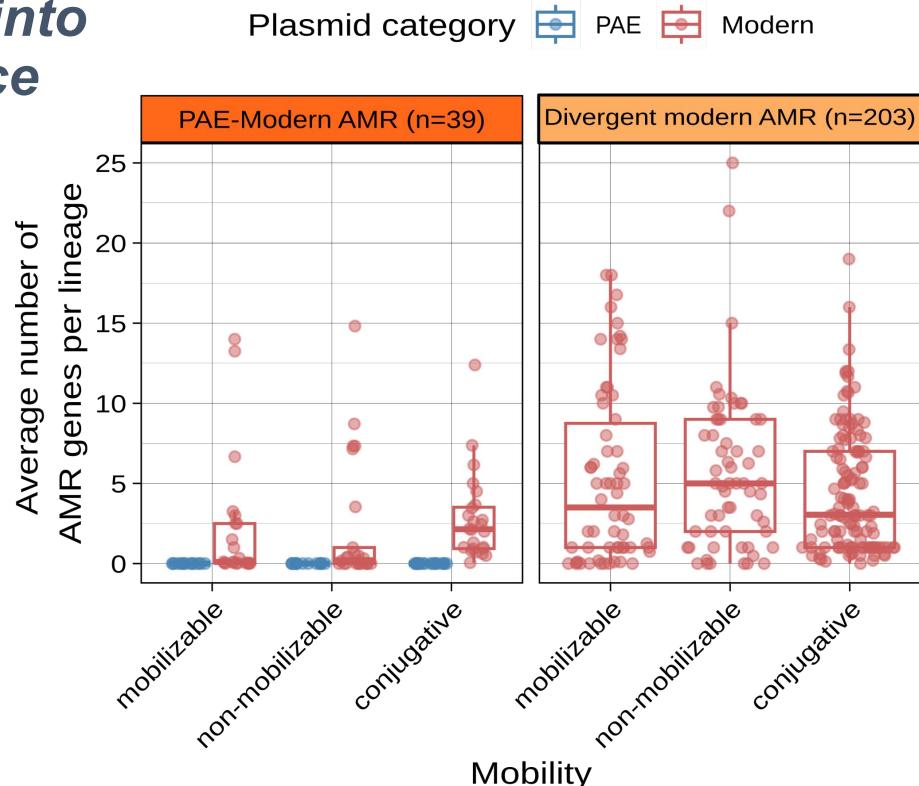


Resistance plasmids are not randomly distributed in the network.

Not all plasmids are the same. AMR gene carriage is confined to 38% of all plasmids.

PAE plasmids contribution to AMR spread

**PAE plasmids
evolved into
resistance
vectors**

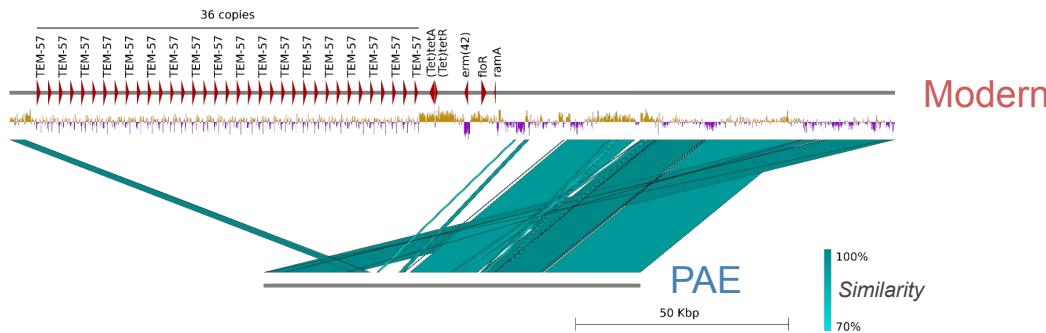
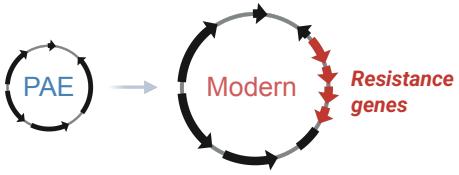


No specific resistance genes in PAE plasmids

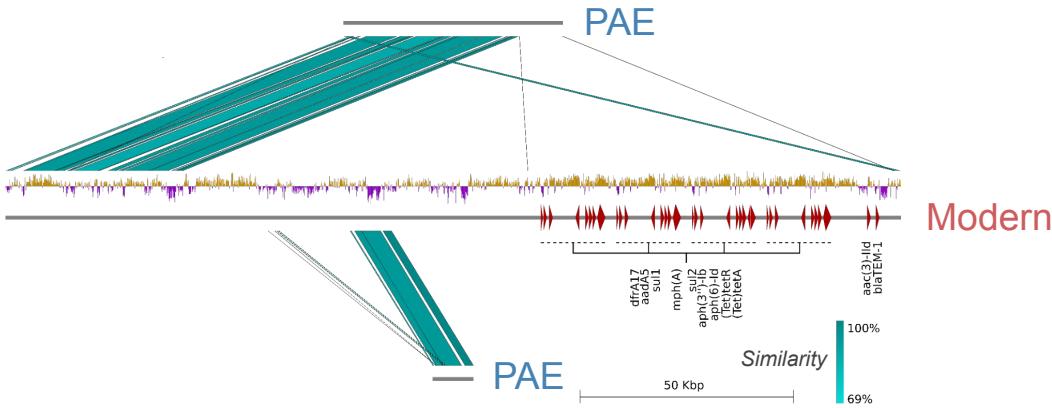
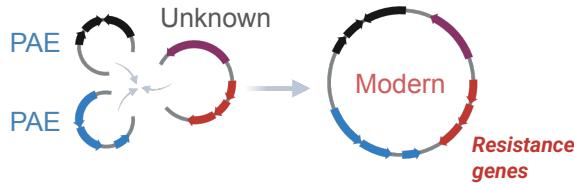
Thousands of resistance genes in modern relatives

Evolutionary strategies followed by 244 resistance plasmid species

Microevolution



Plasmid fusion



Take home messages

- Only a minority of PAE plasmid species evolved to carry resistance genes and they share common characteristics and evolutionary strategies.
- We can focus on these plasmids to enhance genomics surveillance and investigate what makes them good resistance vectors.
- Humans distorted plasmids identity, their role and the way bacteria face antibiotics. We have promoted cooperation for survival.

Thanks!



Wendy Figueroa



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**PAM core,
samples & IT
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