Klebsiella antimicrobial resistance (AMR) typing

Assistant Professor Zoe A. Dyson

London School of Hygiene and Tropical Medicine

zoe.dyson@lshtm.ac.uk

@msmicrobiocode

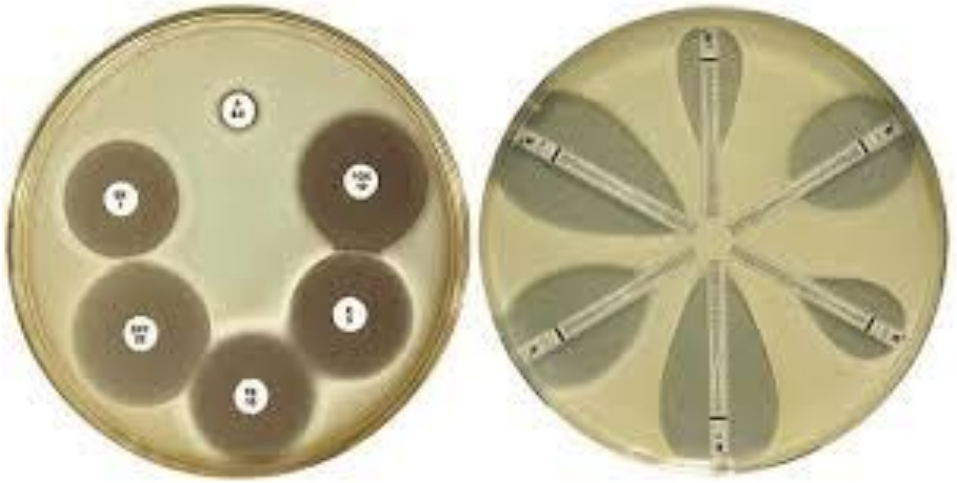
Today’s schedule

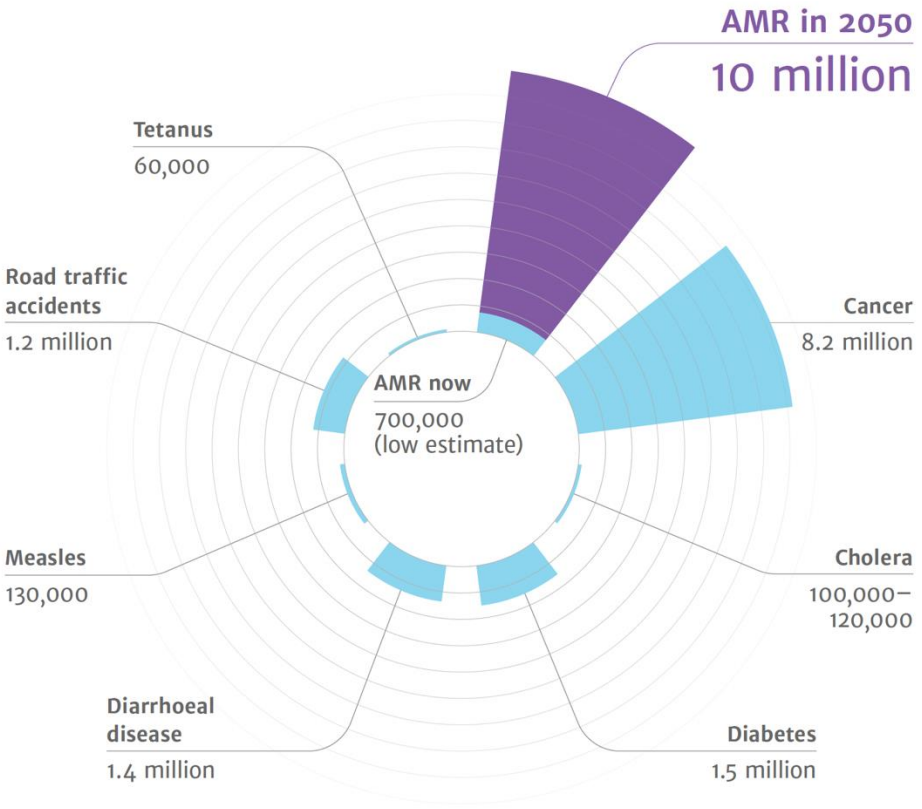
Time	Activity
11:15-12:00 (45 mins)	**Lecture: *Klebsiella* antimicrobial resistance (AMR) typing** • An introduction to AMR determinant detection • AMR in *Klebsiella pnuemoniae* • AMR detection & score analysis with Kleborate
12:00-12:10 (10 mins)	**Class discussion**
12:10-13:00 (50 mins)	**Kleborate hands on practical**
13:00-14:00 (1 hour)	**Lunch**
14:00-15:15 (1 hour 15 mins)	**Kleborate hands on practical**
15:15-15:30 (15 mins)	**Break**
15:30-16:30 (1 hour)	**Kleborate hands on practical (continued)**

An introduction to AMR determinant detection

Antimicrobial resistance

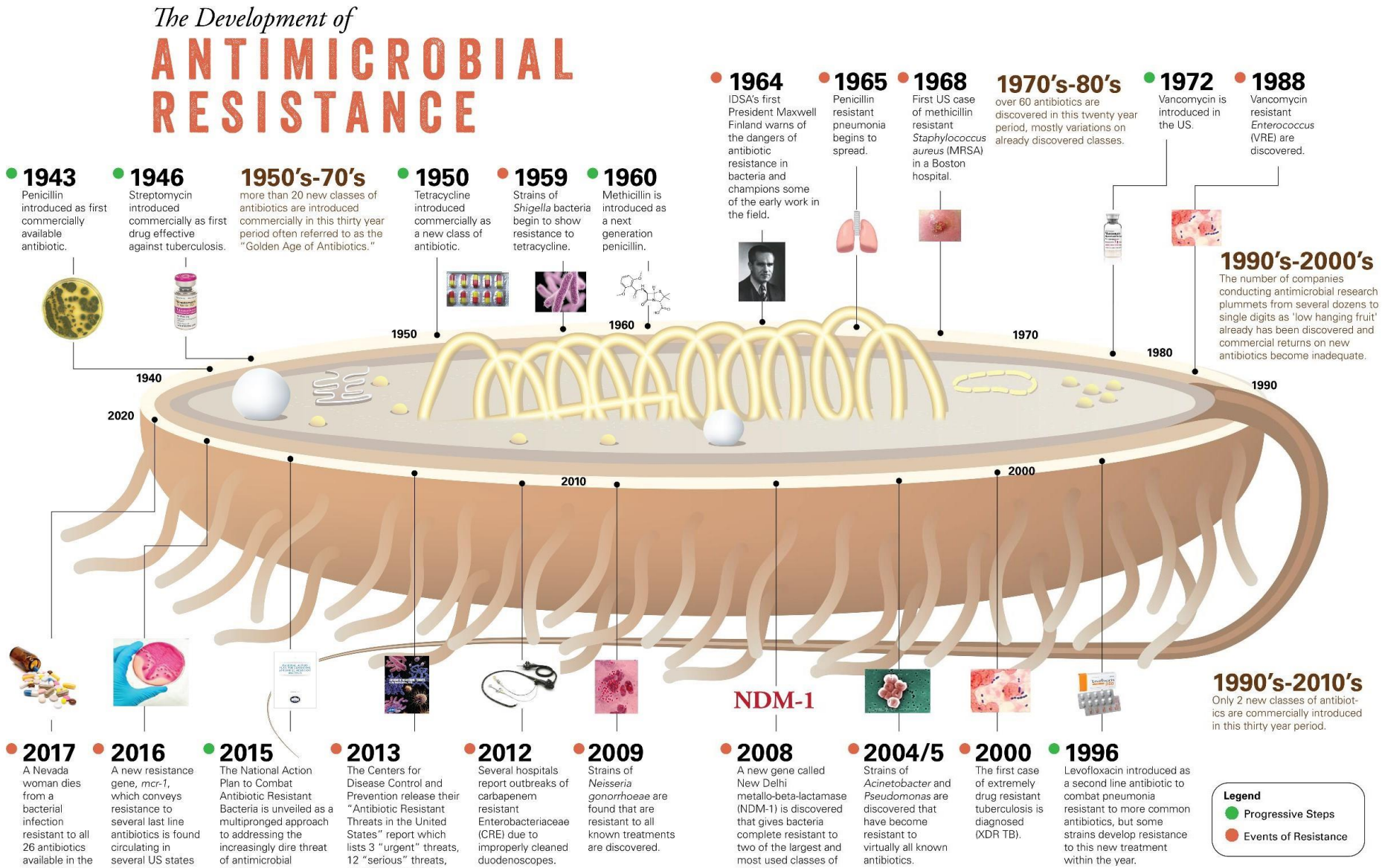
- Antimicrobial resistance (AMR) occurs when pathogens evolve over time and no longer respond to medicines used to treat infections
  - Infections become harder to treat
  - Risk of disease spread, severe illness, and death increase
- O'Neill estimated AMR inaction to lead to:
  - 10 million deaths by 2050
  - Economic cost of $100 trillion USD
  - Failure to meet United Nations Sustainable Development Goals
- AMR national action plans & surveillance
- 2024 United Nations General Assembly





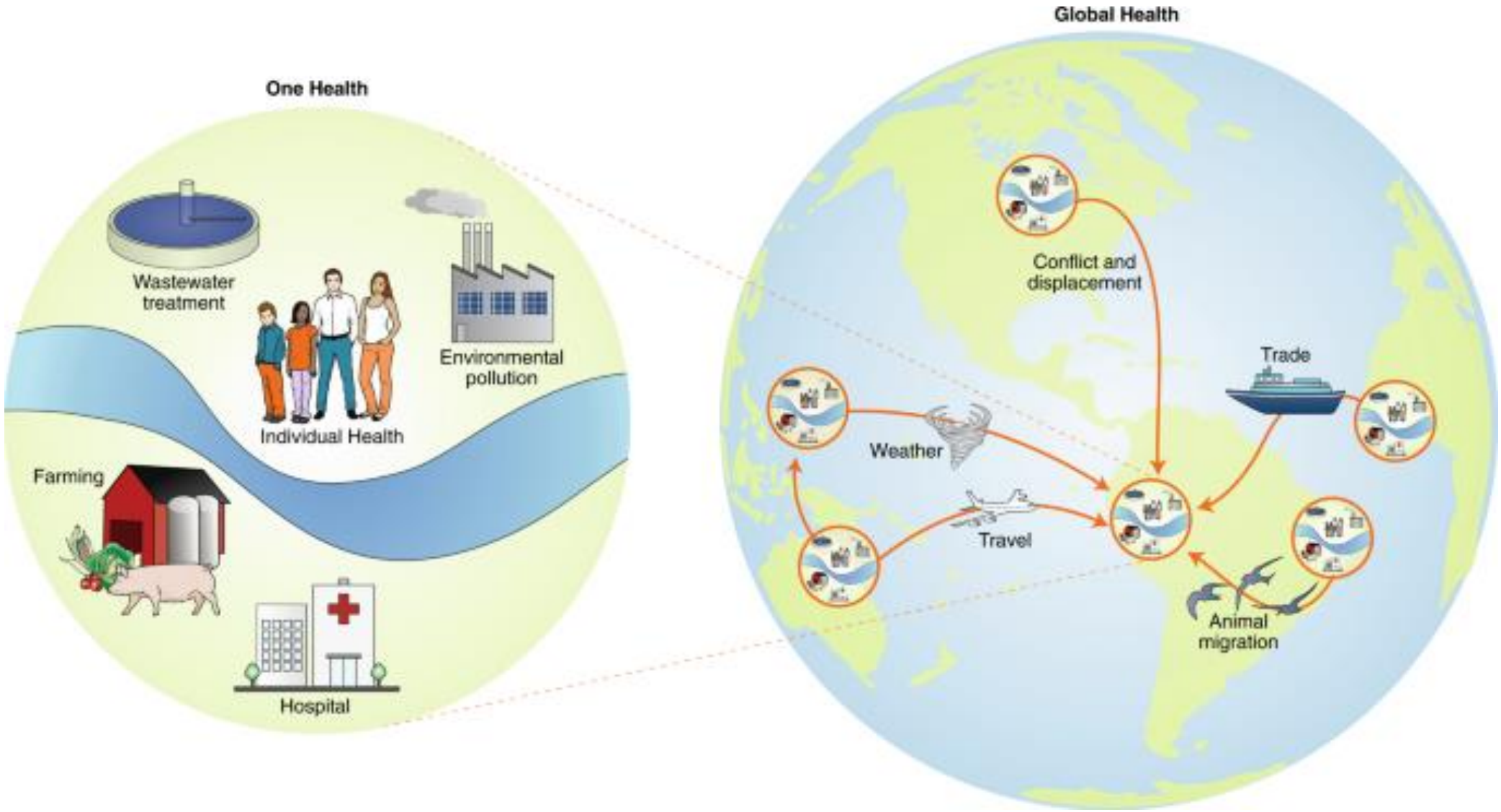
O'Neill 2016

Antimicrobial resistance: a growing problem



FOAR report, ISDA

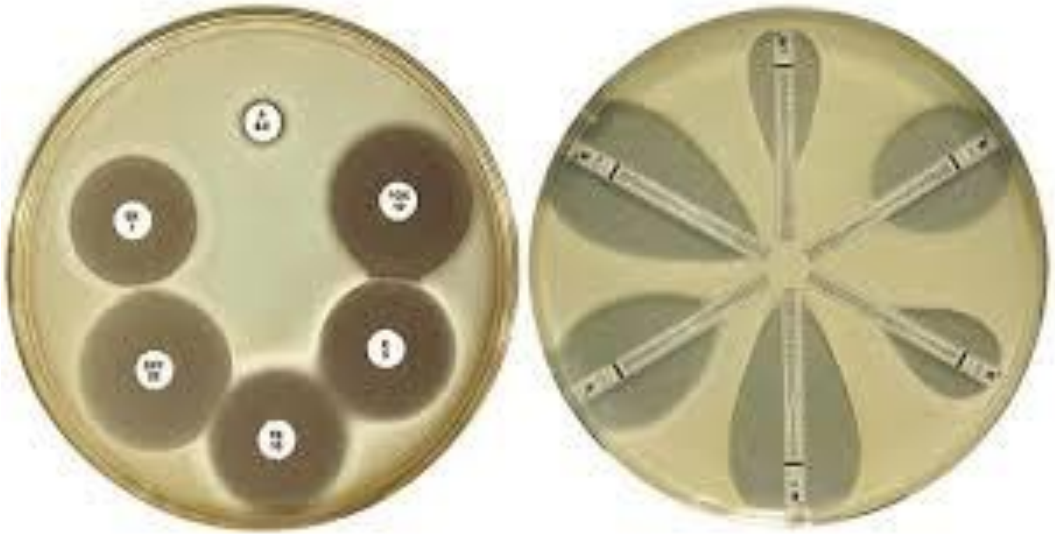
Antimicrobial resistance: many drivers



Hernando-Amado et al. 2019

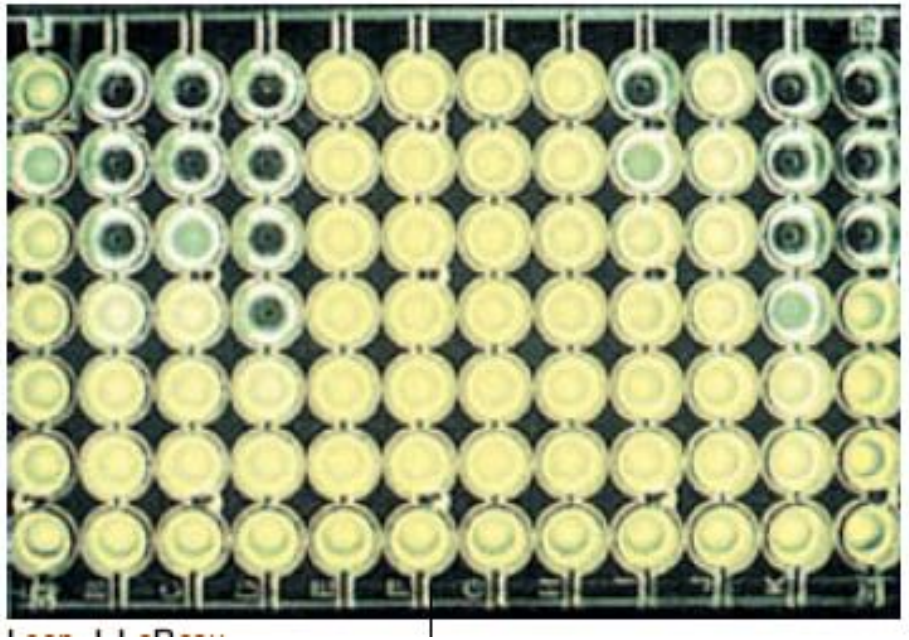
AMR phenotyping methods

- Multiple laboratory methods exist for assessing antimicrobial resistance phenotypes



- Some of the most common ones include:
  - Disk-diffusion
  - Etest
  - Broth dilution

- **Minimal Inhibitory Concentration (MIC)** is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation



Intrinsic and acquired AMR

- AMR can be **intrinsic** or **acquired**

- **Intrinsic resistance** of a bacterial species to a particular antibiotic due to inherent structural or functional characteristics (i.e. the drug never had activity against the pathogen)
  - e.g. daptomycin is active against Gram-positive bacteria but not effective against Gram-negative bacteria due to differences in the composition of the cytoplasmic membrane

- **Acquired resistance** is the result of the evolution of mutations in chromosomal genes or the horizontal transfer of genes that confer resistance to antimicrobials

- Acquired resistance determinants can be readily detected from WGS data

Blair et al. 2015, Nat Rev Microbiol

Acquired AMR determinants detectable via WGS

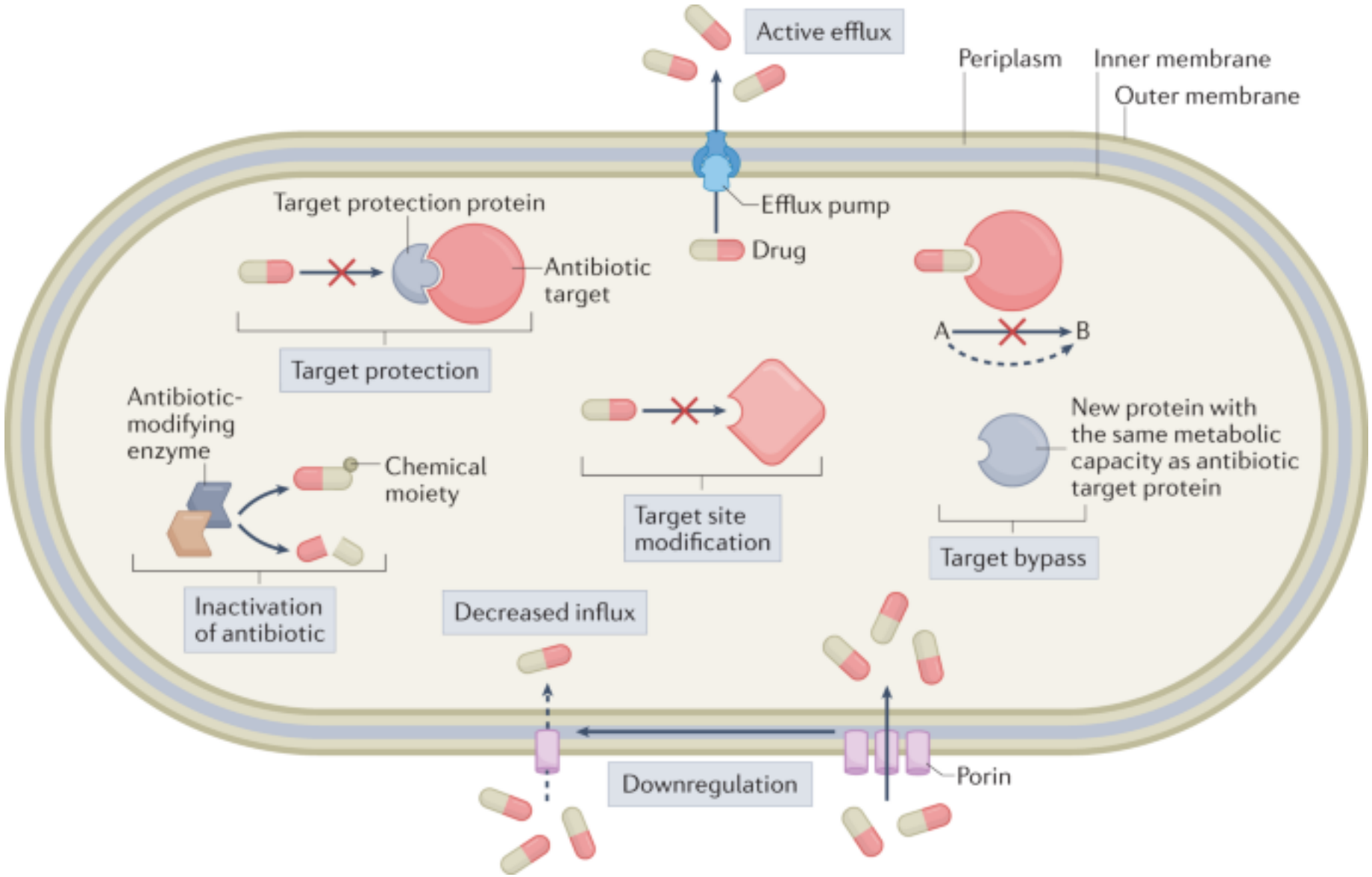
Acquired AMR genes

e.g. Ceftriaxone

Point mutations

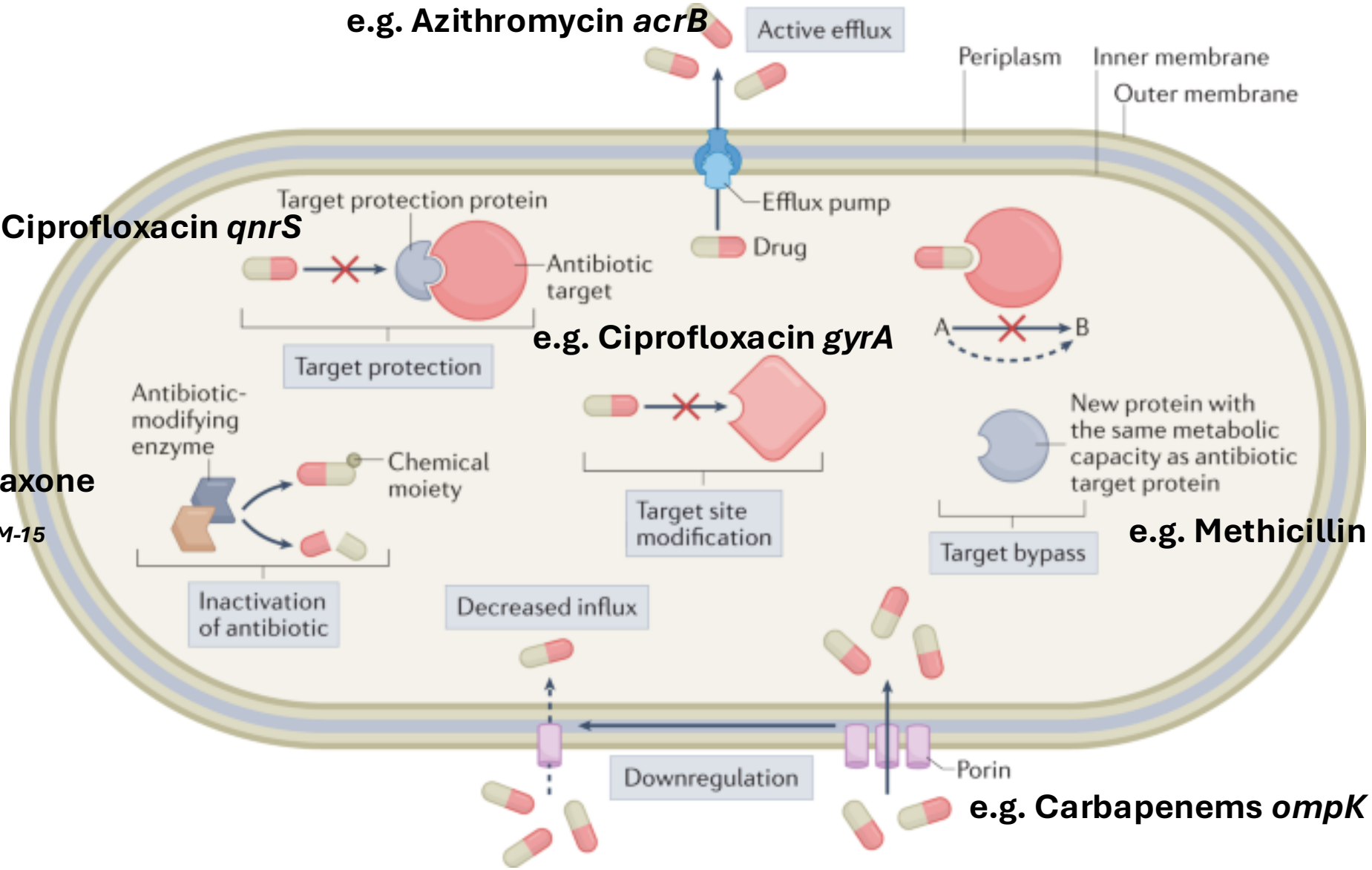
e.g. Fluoroquinolones

Biological mechanisms of AMR



Darby et al. 2023, Nat Rev Genet; Boolchandani et al. 2019, Nat Rev G

Biological & genetic mechanisms of AMR



e.g. Azithromycin *acrB*

e.g. Ciprofloxacin *qnrS*

e.g. Ciprofloxacin *gyrA*

e.g. Ceftriaxone *bla*$_{CTX\text{-}M\text{-}15}$

e.g. Methicillin *mecA*

e.g. Carbapenems *ompK*

Darby et al. 2023, Nat Rev Genet; Boolchandani et al. 2019, Nat Rev G

Antimicrobial resistance determinant databases

scientific reports

Check for updates

OPEN

AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence

Michael Feldgarden[1✉], Vyacheslav Brover[1], Narjol Gonzalez-Escalona[2], Jonathan G. Frye[4], Julie Haendiges[2], Daniel H. Haft[1], Maria Hoffmann[2], James B. Pettengill[2], Arjun B. Prasad[1], Glenn E. Tillman[5], Gregory H. Tyson[3] & William Klimke[1]

Published online 29 October 2019 *Nucleic Acids Research, 2020, Vol. 48, Database issue* **D517–D525**
doi: 10.1093/nar/gkz935

CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database

Brian P. Alcock[1,2,3], Amogelang R. Raphenya[1,2,3], Tammy T.Y. Lau[2,3], Kara K. Tsang[1,2,3], Mégane Bouchard[2,4], Arman Edalatmand[2,3], William Huynh[2,3], Anna-Lisa V. Nguyen[2,4], Annie A. Cheng[2,3], Sihan Liu[2,3], Sally Y. Min[2,3], Anatoly Miroshnichenko[2,3], Hiu-Ki Tran[2,3], Rafik E. Werfalli[2,3], Jalees A. Nasir[2,3], Martins Oloni[2,3], David J. Speicher[2,3], Alexandra Florescu[2,4], Bhavya Singh[5], Mateusz Faltyn[2,6], Anastasia Hernandez-Koutoucheva[7], Arjun N. Sharma[2,3], Emily Bordeleau[1,2,3], Andrew C. Pawlowski[8], Haley L. Zubyk[1,2,3], Damion Dooley[9], Emma Griffiths[10], Finlay Maguire[11], Geoff L. Winsor[10], Robert G. Beiko[11], Fiona S.L. Brinkman[10], William W.L. Hsiao[9,10,12], Gary V. Do

AAC
Journals.ASM.org

ARG-ANNOT, a New Bioinformatic Tool To Discover Antibiotic Resistance Genes in Bacterial Genomes

Sushim Kumar Gupta,[a] Babu Roshan Padmanabhan,[a] Seydina M. Diene,[a] Rafael Lopez-Rojas,[b] Marie Kempf,[c] Luce Landraud,[d] Jean-Marc Rolain[a]

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UMR CNRS 7278—IRD 198 IHU, Méditerranée Infection, Faculté de Médecine et de P
Aix-Marseille Université, Marseille, France[a]; Servicio de Enfermedades Infecciosas, Hospitales Universitarios Virgen del Rocıo, Seville, Spain[b]; Laboratoire de Bacteriologie, Institut de Biologie en santé-PHB, CHU, Angers, France[c]; INSERM U895, Nice, France[d]

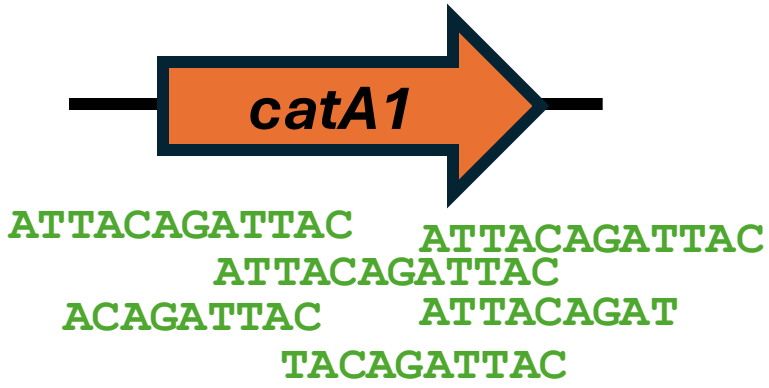
J Antimicrob Chemother 2020; **75**: 3491–3500
doi:10.1093/jac/dkaa345 Advance Access publication 11 August 2020

Journal of Antimicrobial Chemotherapy

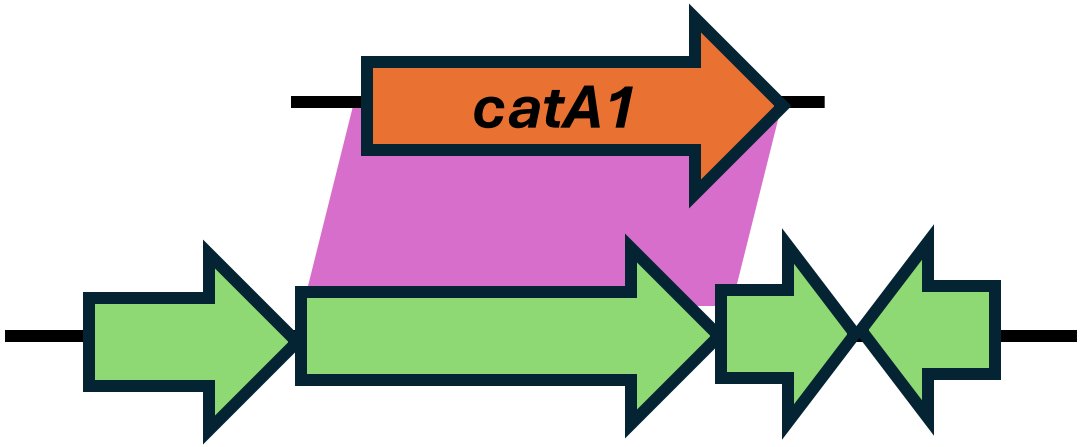
ResFinder 4.0 for predictions of phenotypes from genotypes

Valeria Bortolaia[1]†, Rolf S. Kaas[1]†, Etienne Ruppe[2], Marilyn C. Roberts[3], Stefan Schwarz[4], Vincent Cattoir[5,6,7], Alain Philippon[8], Rosa L. Allesoe[1,9], Ana Rita Rebelo[1], Alfred Ferrer Florensa[1], Linda Fagelhauer[10,11,12], Trinad Chakraborty[10,11], Bernd Neumann[13], Guido Werner[13], Jennifer K. Bender[13], Kerstin Stingl[14], Minh Nguyen[15], Jasmine Coppens[15], Basil Britto Xavier[15], Surbhi Malhotra-Kumar[15], Henrik Westh[16,17], Mette Pinholt[16], Muna F. Anjum[18], Nicholas A. Duggett[18], Isabelle Kempf[19], Suvi Nykäsenoja[20], Satu Olkkola[20], Kinga Wieczorek[21], Ana Amaro[22], Lurdes Clemente[22], Joël Mossong[23], Serge Losch[24], Catherine Ragimbeau[23], Ole Lund[1] and Frank M. Aarestrup[1]*

General software tools & methods



Mapping based



Assembly based

Inouye *et al. Genome Medicine* 2014, **6**:90
http://genomemedicine.com/content/6/11/90

Genome Medicine

SOFTWARE Open Access

SRST2: Rapid genomic surveillance for public health and hospital microbiology labs

Michael Inouye[1,2], Harriet Dashnow[3,4], Lesley-Ann Raven[1], Mark B Schultz[3], Bernard J Pope[4,5], Takehiro Tomita[2,6], Justin Zobel[5] and Kathryn E Holt[3*]

MICROBIAL GENOMICS

RESEARCH ARTICLE

Hunt *et al.*, *Microbial Genomics* 2017;3
DOI 10.1099/mgen.0.000131

MICROBIOLOGY SOCIETY

OPEN DATA OPEN MICROBIOLOGY

ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads

Martin Hunt,[1] Alison E Mather,[1,2] Leonor Sánchez-Busó,[1] Andrew J Page,[1] Julian Parkhill,[1] Jacqueline A Keane[1] and Simon R Harris[1,*]

nature communications

Article

https://doi.org/10.1038/s41467-022-35713-4

An ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance

ed: 1 June 2022
ed: 21 December 2022
d online: 04 January 2023

Norelle L. Sherry[1,2,3], Kristy A. Horan[1], Susan A. Ballard[1], Anders Gonçalves da Silva[1], Claire L. Gorrie[3], Mark B. Schultz[1], Kerrie Stevens[1], Mary Valcanis[1], Michelle L. Sait[1], Timothy P. Stinear[3], Benjamin P. Howden[1,2,3,4] & Torsten Seemann[1,3,4]

Pathogen specific tools: Kleborate & Pathogenwatch

KLEBORATE

Pathogenwatch

https://pathogen.watch/

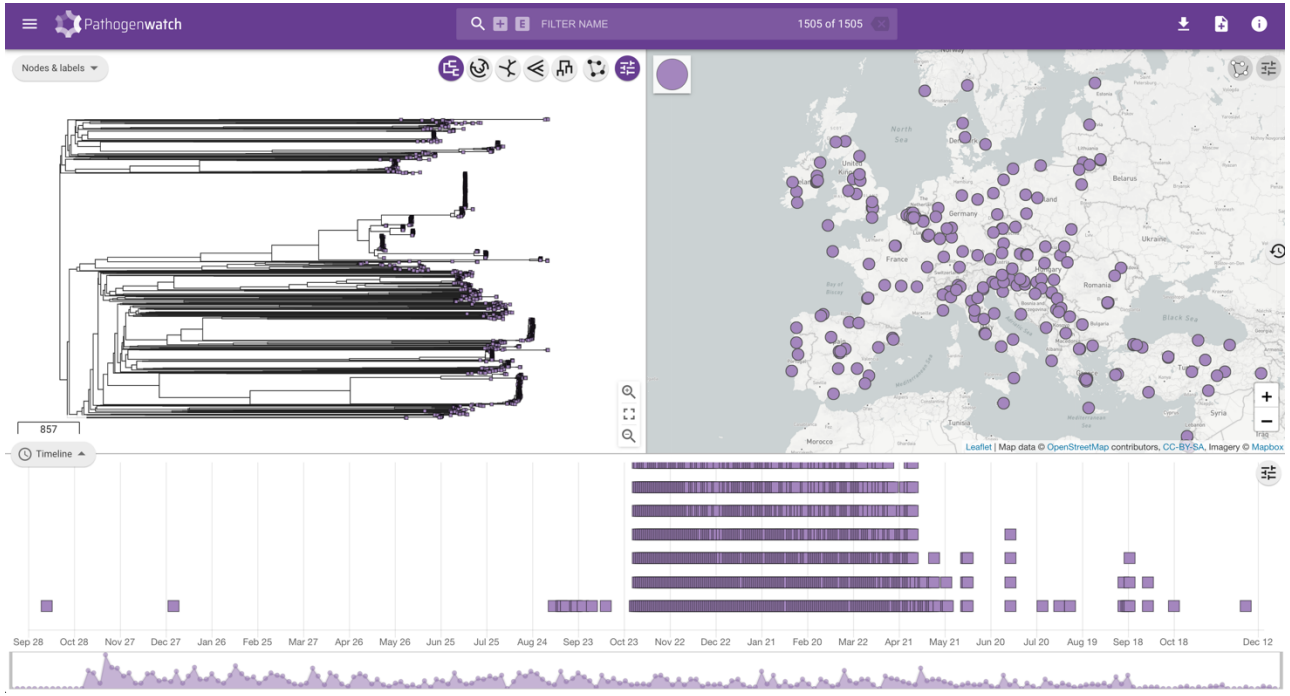
Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Rapid Genomic Characterization and Global Surveillance of *Klebsiella* Using Pathogenwatch

Silvia Argimón,[1,a] Sophia David,[1,a] Anthony Underwood,[1] Monica Abrudan,[1] Nicole E. Wheeler,[1] Mihir Kekre,[1] Khalil Abudahab,[1] Corin A. Yeats,[1,2] Richard Goater,[1] Ben Taylor,[1,2] Harry Harste,[1] Dawn Muddyman,[1] Edward J. Feil,[3] Sylvain Brisse,[4] Kathryn Holt,[5,6] Pilar Donado-Godoy,[7] K. L. Ravikumar,[8] Iruka N. Okeke,[9] Celia Carlos,[10] and David M. Aanensen[1,2]; for the NIHR Global Health Research Unit on Genomic Surveillance of Antimicrobial Resistance[b]

Interactive view of core-genome phylogeny



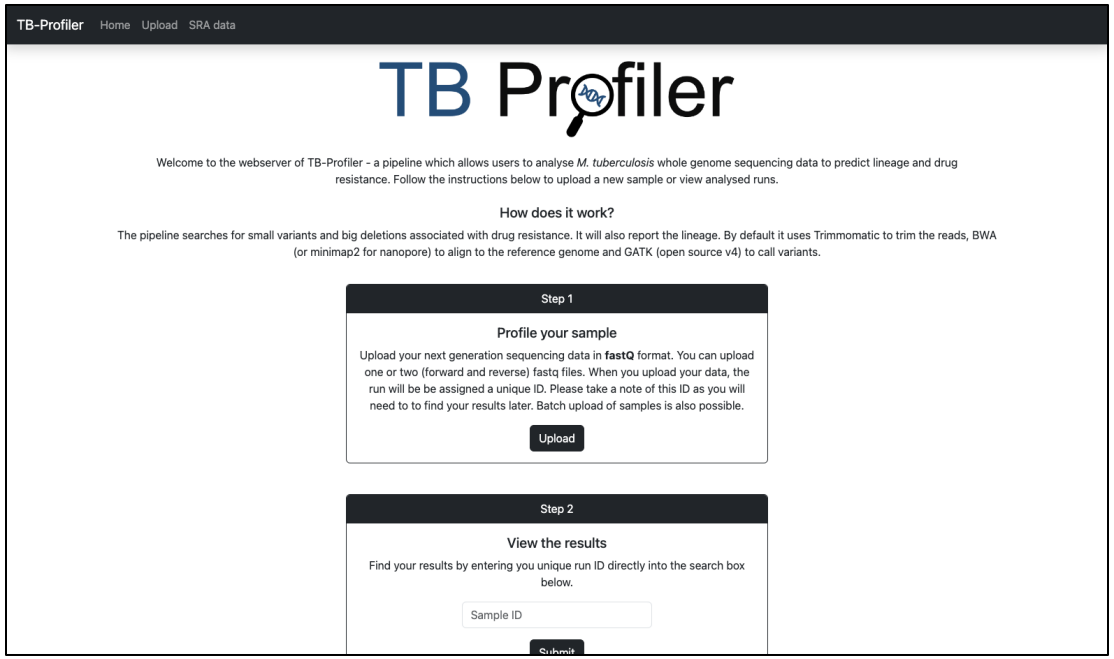
Drug-level genome report in online tool

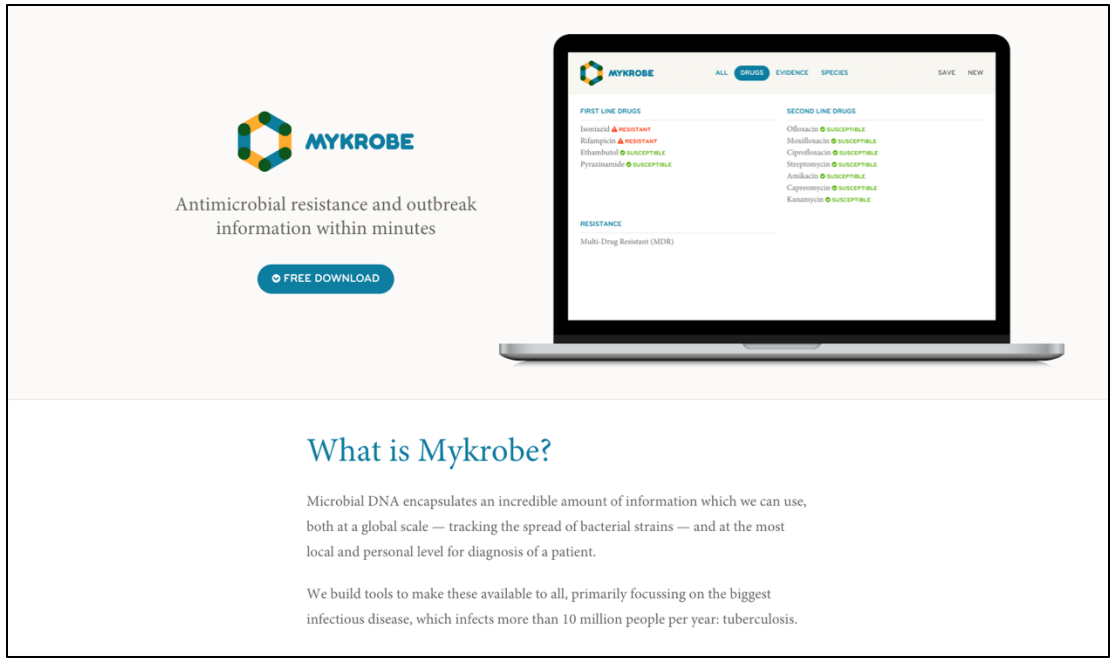
Antimicrobial resistance (AMR)

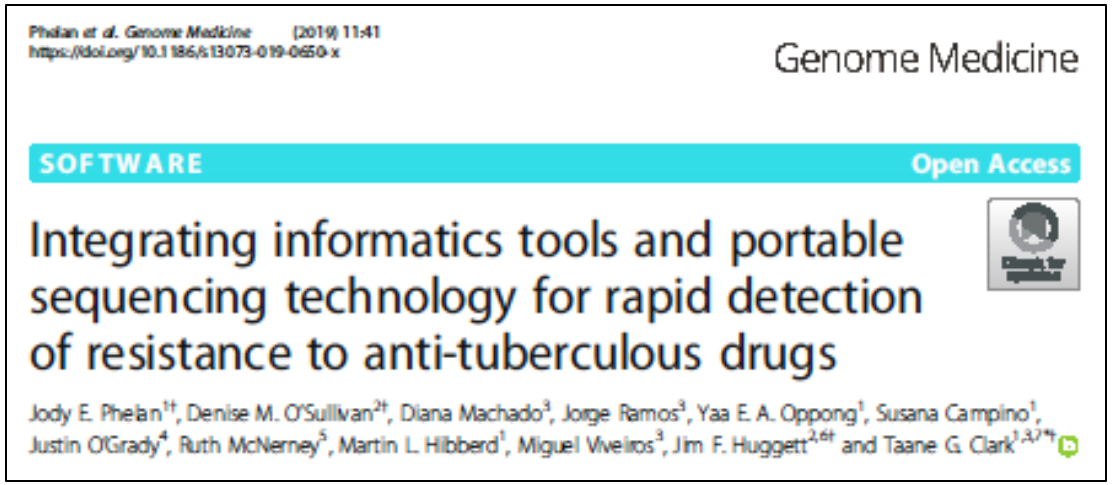
Sourced from Kleborate

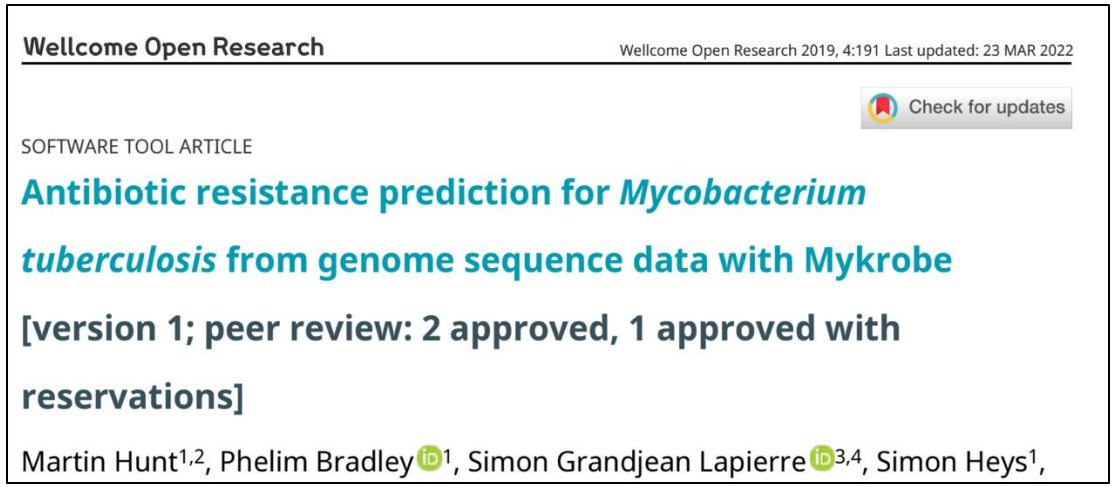
Drug/Class	Resistance Determinants
Aminoglycosides	**aph3-Ia, strA, strB**
Carbapenems	None found
Cephalosporins (3rd gen.)	**CTX-M-15**
Cephalosporins (3rd gen.) + β-lactamase inhibitors	None found
Colistin	None found
Fluoroquinolones	**qnrS1**
Fosfomycin	None found
Penicillins	**TEM-1D, SHV-69 (homolog)**
Penicillins + β-lactamase inhibitors	None found
Phenicols	**catA1**
Sulfonamides	**sul1, sul2 (homolog)**
Tetracycline	**tet(A)**

Other pathogen specific tools



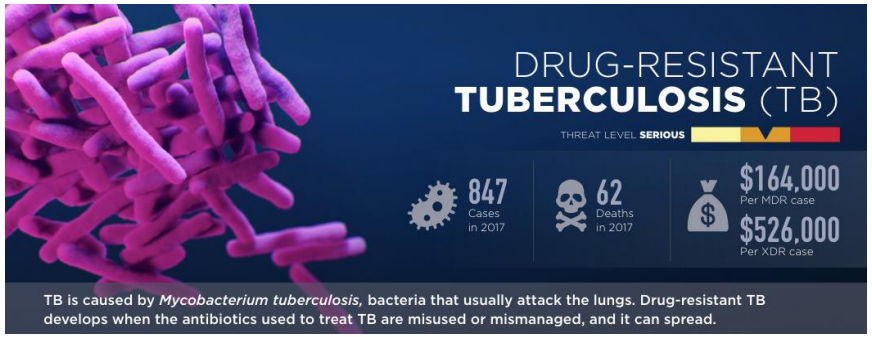




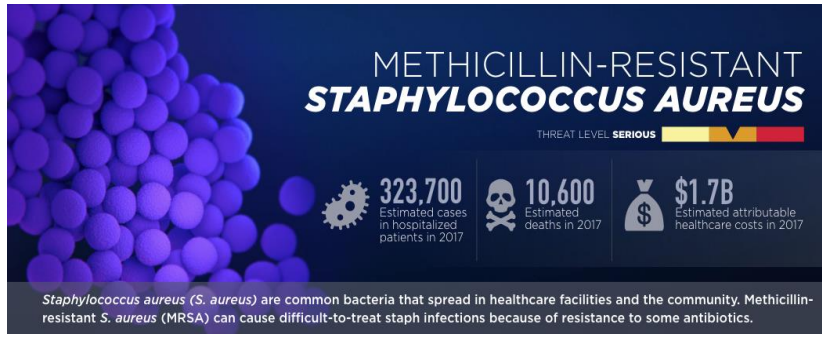


High concordance between AMR genotypes & phenotypes

Salmonella Typhi bacteria cause typhoid fever, a potentially life-threatening disease. Symptoms include high fever, abdominal pain, and headache. Infection can lead to bowel rupture, shock, and death.



TB is caused by *Mycobacterium tuberculosis*, bacteria that usually attack the lungs. Drug-resistant TB develops when the antibiotics used to treat TB are misused or mismanaged, and it can spread.



Staphylococcus aureus (S. aureus) are common bacteria that spread in healthcare facilities and the community. Methicillin-resistant *S. aureus* (MRSA) can cause difficult-to-treat staph infections because of resistance to some antibiotics.

>99.9% concordance

Antibiotic	MIC no.*	Phenotype: susceptible		Phenotype: resistant	
		Genotype: resistant	Genotype: susceptible	Genotype: resistant	Genotype: susceptible
AMX	1034	0	726	308	0
AMX-CL	1034	0	726	308	0
CAZ	1034	0	983	51	0
CRO	1034	0	983	51	0
ETP	1034	0	1034	0	0
GEN	1034	0	1034	0	0
CIP	1034	0	83	950	1
AZM	1034	0	1034	0	0
TMP	1034	1	704	328	1
FOS	1013	0	1012	1	0
TET	1034	0	1006	28	0
SXT	1034	0	698	336	0
CHL	1034	0	711	323	0
COL	1034	0	1034	0	0
Total combinations	14455	–	–	–	–

AMX, Amoxicillin; AMX-CL, amoxicillin/clavulanic acid; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; ETP, ertapenem; GEN, gentamicin; AZM, azithromycin; TMP, trimethoprim; FOS, fosfomycin; TET, tetracycline; SXT, trimethoprim/sulphonamide; CHL, chloramphenicol; COL, colistin. Numbers in bold relate to descrepancies between genotype and phenotype.
*The number of isolates that had phenotypic MIC testing.

>91% sensitivity & specificity

Analysis and Drug	Resistant Phenotype					Susceptible Phenotype					Sensitivity (95% CI)	Specificity (95% CI)
	R	S	U	F	Total	R	S	U	F	Total		
	number of isolates											
WGS, all isolates												
Isoniazid	3067	90	93	44	3294	65	6313	215	117	6710	97.1 (96.5–97.7)	99.0 (98.7–99.2)
Rifampin	2743	69	7	84	2903	85	6763	232	147	7227	97.5 (96.9–98.1)	98.8 (98.5–99.0)
Ethambutol	1410	81	94	55	1640	468	6835	781	70	8154	94.6 (93.3–95.7)	93.6 (93.0–94.1)
Pyrazinamide	863	82	117	77	1139	204	6146	197	108	6655	91.3 (89.3–93.0)	96.8 (96.3–97.2)

>99% accuracy

Drugs	True positives	True negatives	False positives	False negatives	Accuracy (%)
Cefoxitin	778	0	0	0	100
Erythromycin	324	384	1	1	99.72
Tetracycline	101	609	0	0	100
Rifampicin	8	699	0	0	100
Fusidic acid	220	494	1	1	99.72
Gentamicin	53	668	0	0	100
Chloramphenicol	4	684	0	4	99.42
Mupirocin	31	571	6	0	99.01
Linezolid	0	692	0	0	100
Ciprofloxacin	398	289	0	5	99.28
Trimethoprim	0	0	0	3	0
Overall	1917	5090	8	14	99.69

Chattaway et al. 2021, Microb Genom; Walker et al. 1998, NJEM; Kumar et al. 2020, JAC.

High concordance between AMR genotypes & phenotypes in *Klebsiella*

J Antimicrob Chemother 2013; **68**: 2234–2244
doi:10.1093/jac/dkt180 Advance Access publication 30 May 2013

Journal of Antimicrobial Chemotherapy

Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data

N. Stoesser[1,2*], E. M. Batty[3], D. W. Eyre[1], M. Morgan[2], D. H. Wyllie[4], C. Del Ojo Elias[1], J. R. Johnson[5], A. S. Walker[1], T. E. A. Peto[1] and D. W. Crook[1,2]

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Predictability of Phenotype in Relation to Common β-Lactam Resistance Mechanisms in *Escherichia coli* and *Klebsiella pneumoniae*

Alex Agyekum,[a] Alicia Fajardo-Lubián,[a] Xiaoman Ai,[a*] Andrew N. Ginn,[a] Zhiyong Zong,[a*] Xuejun Guo,[a*] John Turnidge,[b,c] Sally R. Partridge,[a] Jonathan R. Iredell[a]

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

ELSEVIER | ISC

Short Communication

Prediction of major antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* in Singapore, USA and China using a limited set of gene targets

Andrew N. Ginn[a,b,c], Agnieszka M. Wiklendt[a], Zhiyong Zong[d], Raymond T.P. Lin[e,f], Jeanette W.P. Teo[e], Paul A. Tambyah[g,h], Lance R. Peterson[i,j,k], Karen Kaul[i,k], Sally R. Partridge[a,b,c], Jonathan R. Iredell[a,b,c,*]

Contents lists available at SciVerse ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

ELSEVIER | ISC

Limited diversity in the gene pool allows prediction of third-generation cephalosporin and aminoglycoside resistance in *Escherichia coli* and *Klebsiella pneumoniae*

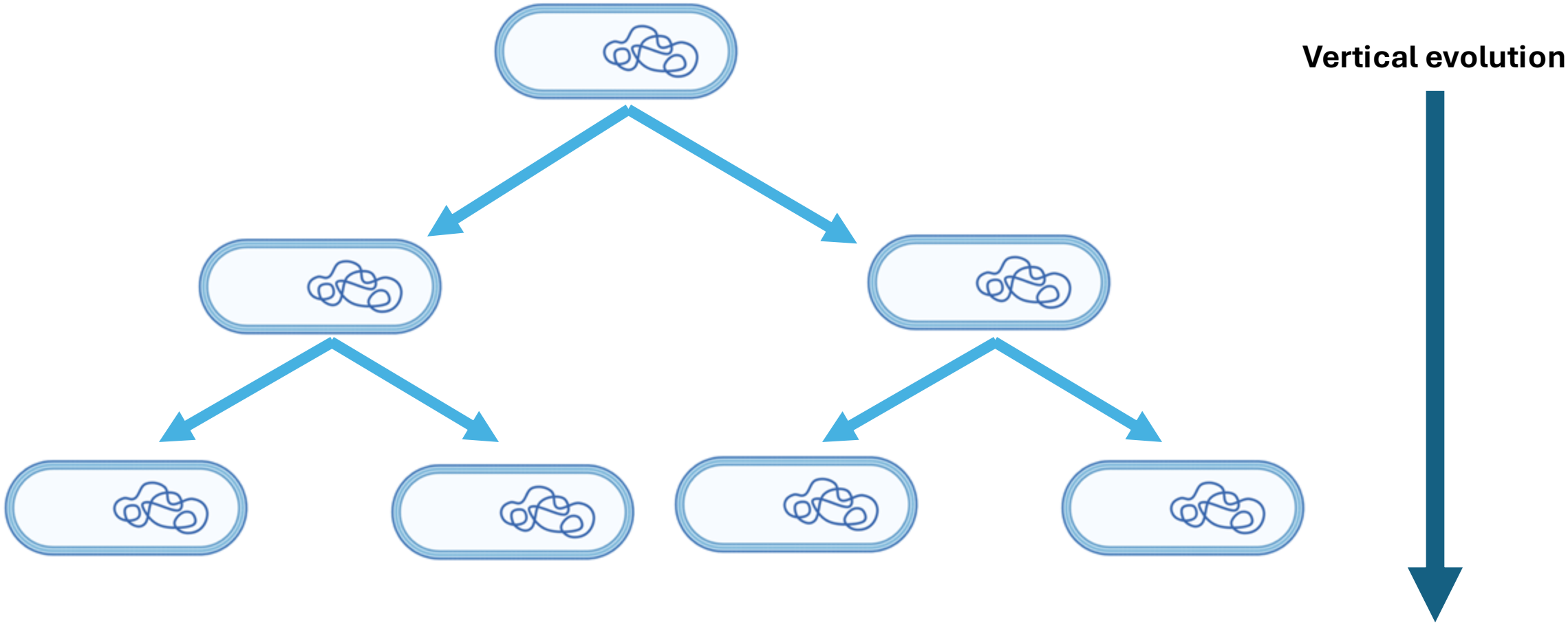
Andrew N. Ginn[a,b,c], Zhiyong Zong[a,d], Agnieszka M. Wiklendt[a], Lee C. Thomas[a], John Merlino[e], Thomas Gottlieb[e], Sebastiaan van Hal[f,1], Jock Harkness[g], Colin Macleod[h], Sydney M. Bell[i], Marcel J. Leroi[j], Sally R. Partridge[a,b,c], Jonathan R. Iredell[a,b,c,*]

Molecular determinants detectable via WGS

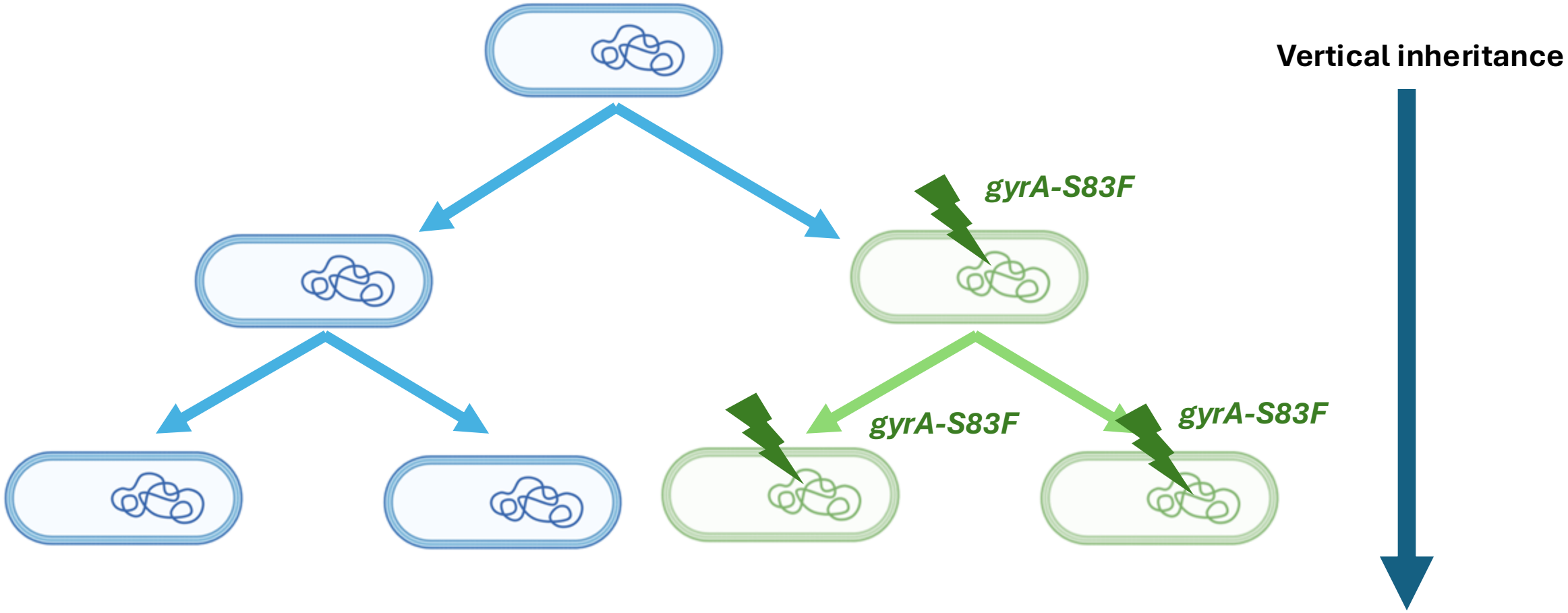
Acquired AMR genes

Point mutations

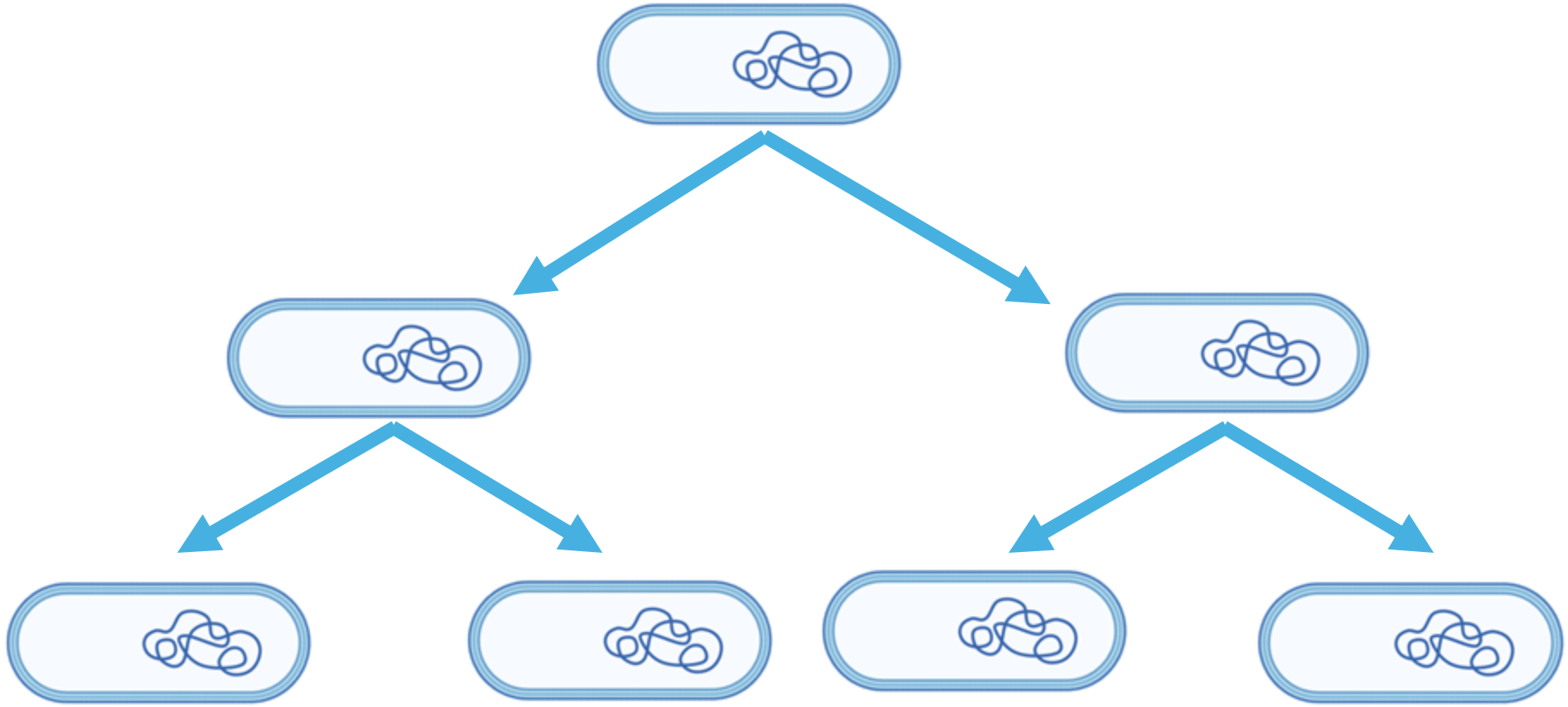
Antimicrobial resistance transmission: mutations



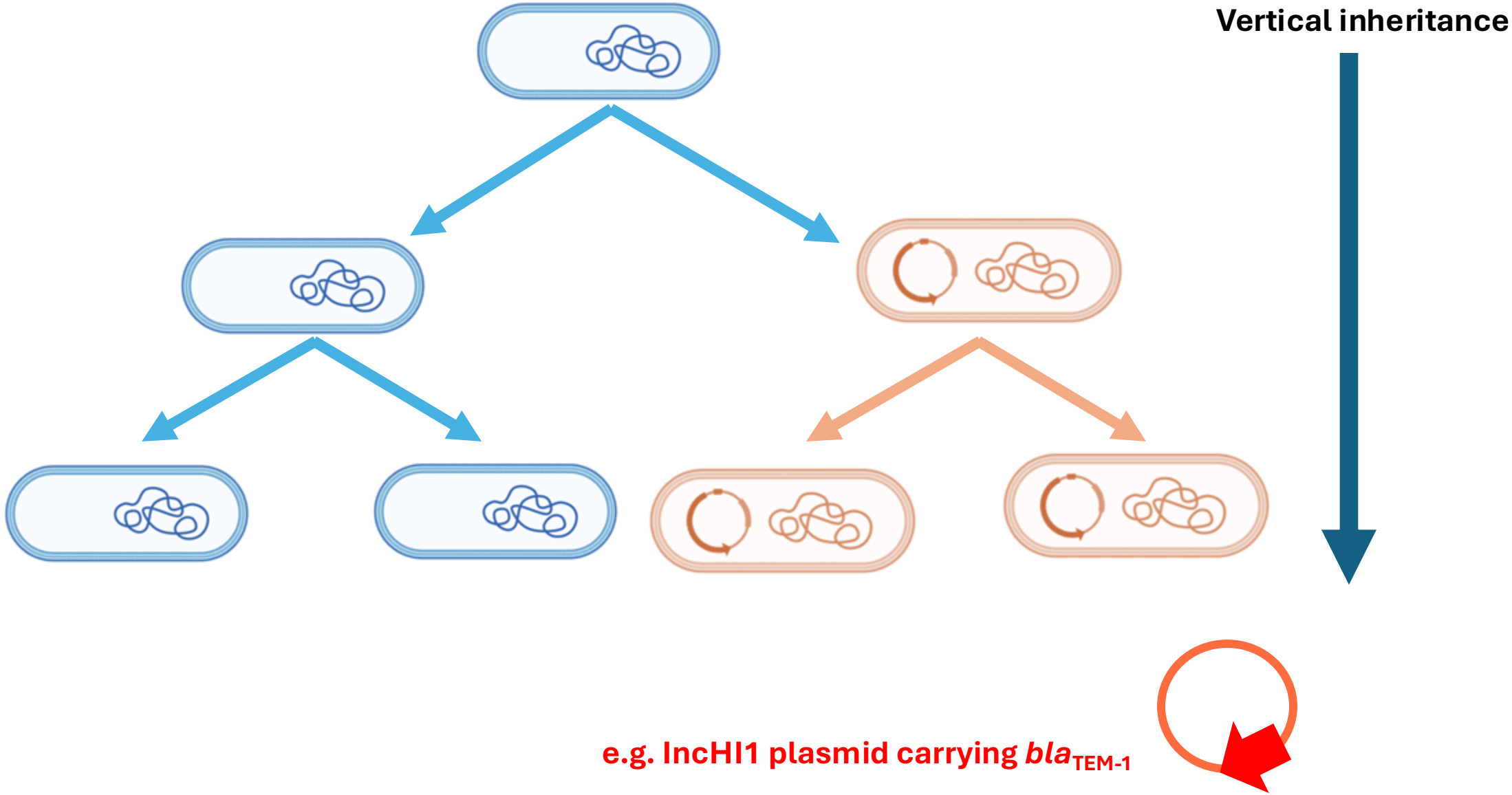
Antimicrobial resistance transmission: mutations



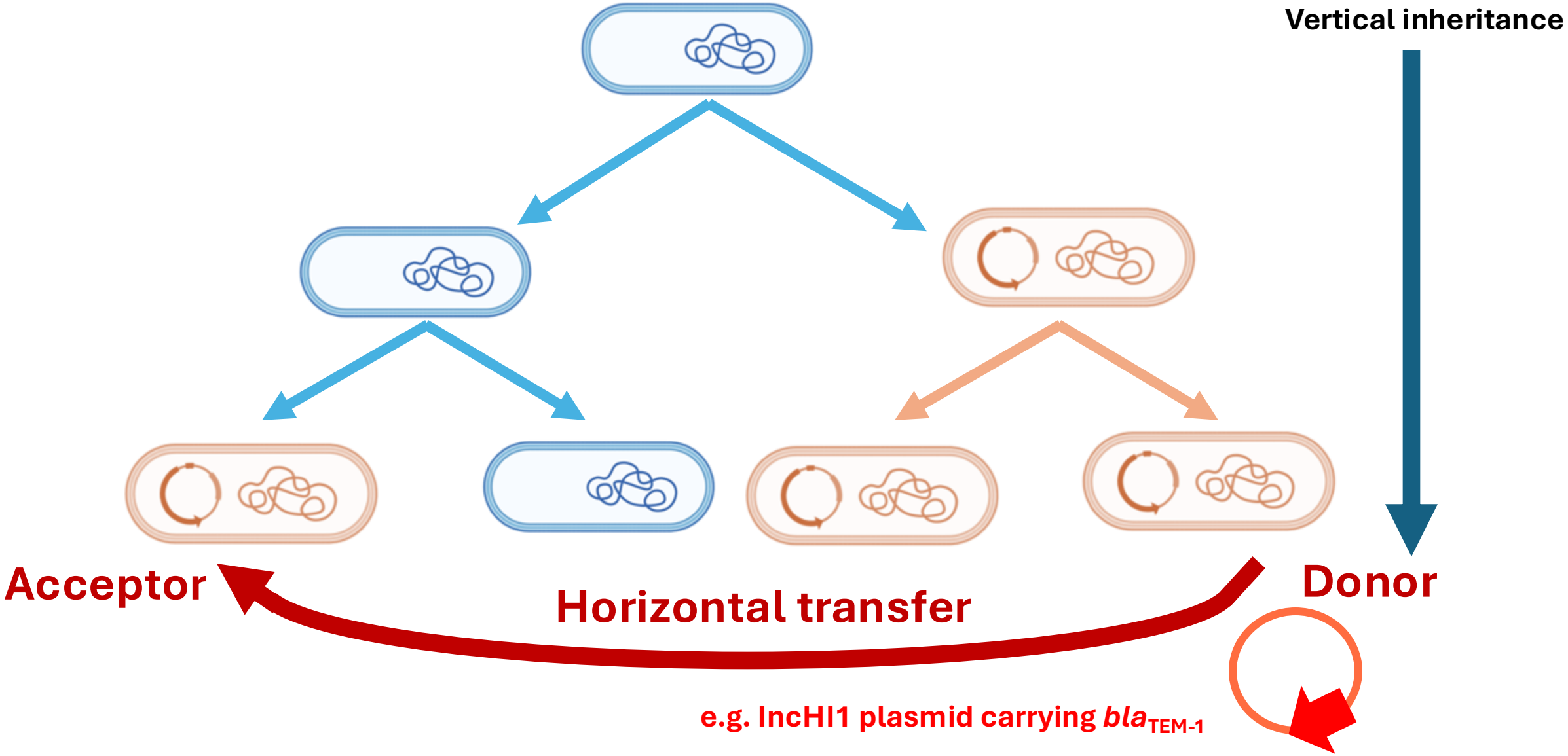
Antimicrobial resistance transmission: genes



Antimicrobial resistance transmission: genes

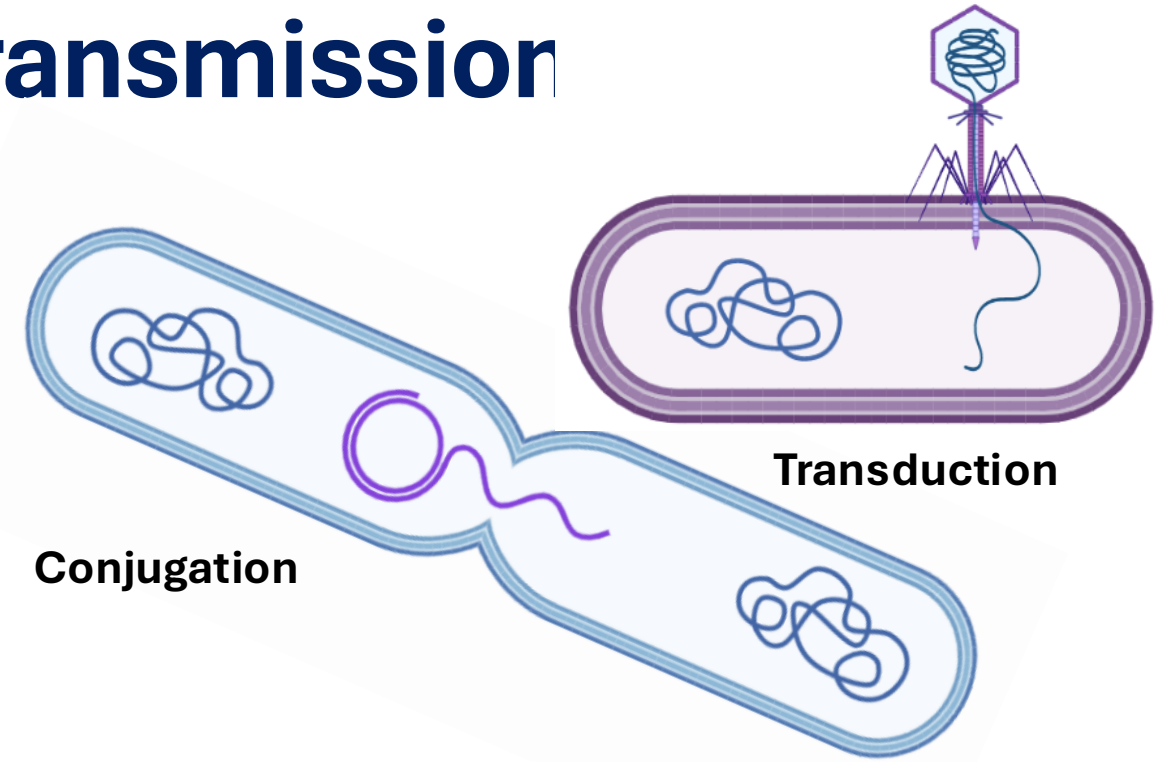


Antimicrobial resistance transmission: genes



Antimicrobial resistance transmission

- Antimicrobial resistance genes can be horizontally acquired and spread via mobile genetic elements
- **Mobile genetic elements** include plasmids, bacteriophages, transposons, integrons, and insertion sequences
- Detection of maker genes associate with plamids and other mobile genetic elements can be carried out using most tools for detecting AMR genes
- Long read sequencing (e.g. Nanopore) can also be used to resolve complex mobile genetic elements



Common mechanisms of horizontal gene transfe

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EPIDEMIOLOGY AND SURVEILLANCE
July 2014 Volume 58 Issue 7
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***In Silico* Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing**

Alessandra Carattoli[a], Ea Zankari[b], Aurora García-Fernández[a], Mette Voldby Larsen[c], Ole Lund[c], Laura Villa[a], Frank Møller Aarestrup[b], Henrik Hasman[b]

Antimicrobial resistance: transmission

- UK nosocomial outbreak of KPC-producing Enterobacteriaceae (KPC-E) outbreak among 20 patients

- Following an *K. pneumoniae* index case harbouring a pKpQIL-D2 plasmid carrying a bla_{KPC} gene 10 months prior, carbapenemase resistance disseminated via multiple routes:

    - Clonal spread of *K. pneumoniae* ST661 carrying a pKpQIL-D2 plasmid (15 cases)

    - Horizontal transfer of pKpQIL-D2 plasmid to other KpSC STs and other bacterial species (5 cases)

    - Transmission of bla_{KPC} via a *Tn4401a* transposabe element from the outbreak pKpQIL-D2 plasmid to other plasmids

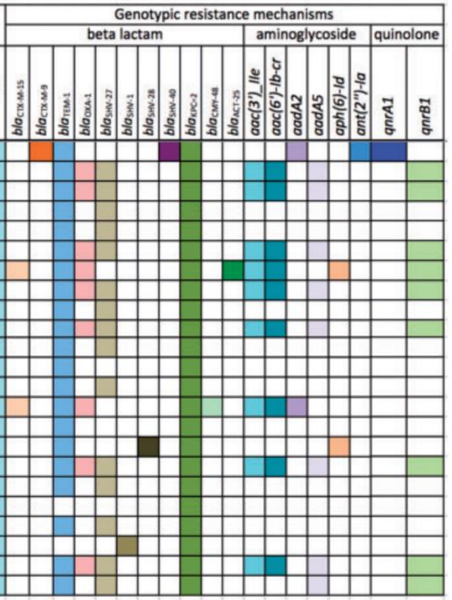
J Antimicrob Chemother 2017; **72**: 3025–3034
doi:10.1093/jac/dkx264 Advance Access publication 7 August 2017

Journal of Antimicrobial Chemotherapy

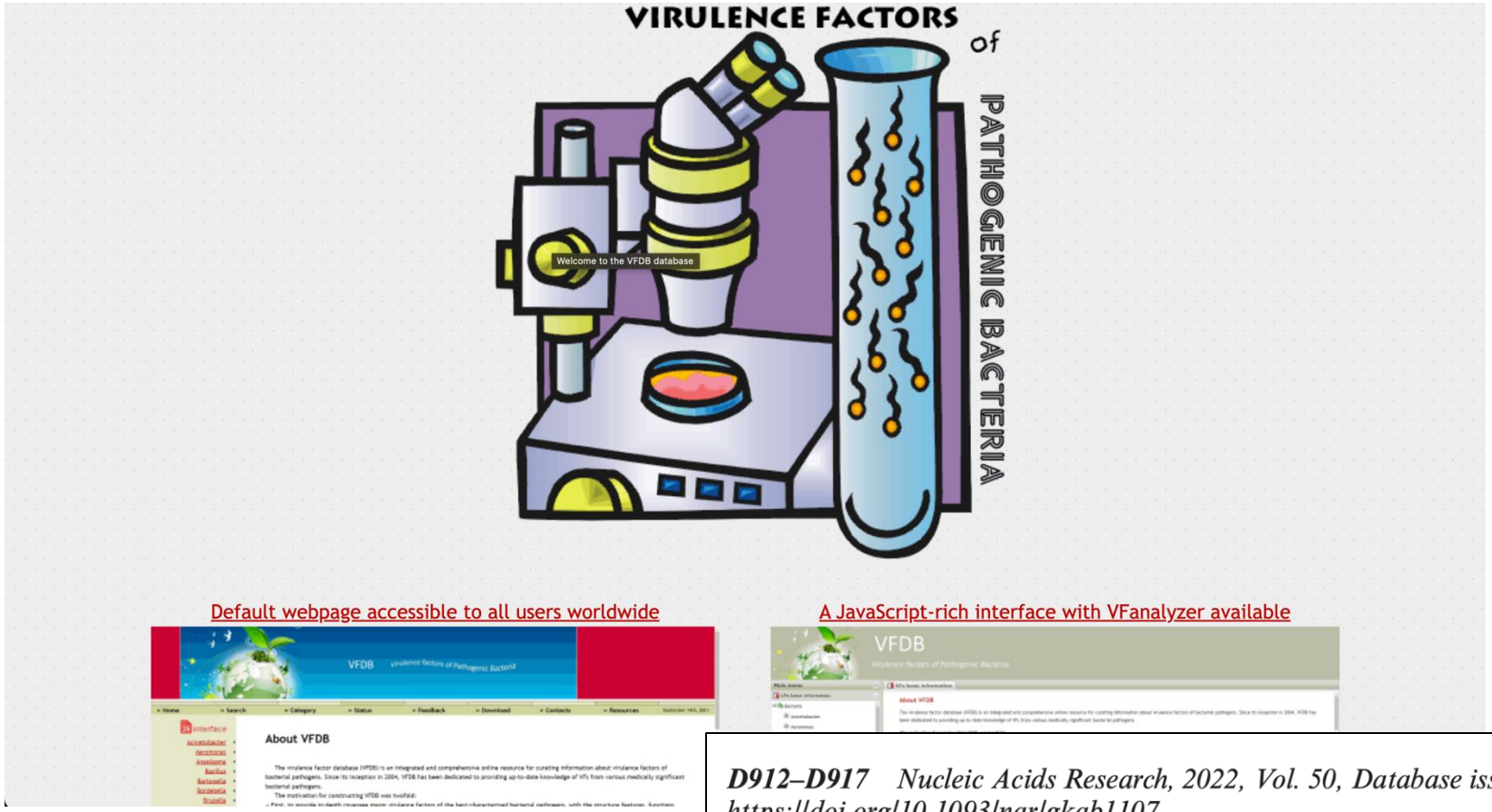
Covert dissemination of carbapenemase-producing *Klebsiella pneumoniae* (KPC) in a successfully controlled outbreak: long- and short-read whole-genome sequencing demonstrate multiple genetic modes of transmission

Jessica Martin[1,2]†, Hang T. T. Phan[3,4]*†, Jacqueline Findlay[5], Nicole Stoesser[3], Louise Pankhurst[3], Indre Navickaite[3], Nicola De Maio[3], David W. Eyre[3], Giles Toogood[6], Nicolas M. Orsi[1,2], Andrew Kirby[1,2], Nicola Young[2], Jane F. Turton[5], Robert L. R. Hill[4], Katie L. Hopkins[4,5], Neil Woodford[4,5], Tim E. A. Peto[3,4], A. Sarah Walker[3,4], Derrick W. Crook[3,4] and Mark H. Wilcox[1,2]

Case Number	Date	Species/ST info	Antibiotic resistance phenotypes													
			AMC	FOX	CIP	TZP	MEM	IPM	ETP	GEN	CAZ	TOB	COL	AMK	TEM	TGC
1	13/12/12	Kpne-ST-491	64	8	≤0.125	>64	32	32	>16	0.5	4	0.5	≤0.5	1	2	1
2	10/07/13	Kpne-ST-661	>64	8	8	>64	16	16	>16	>32	4	>32	≤0.5	8	8	2
2a	10/07/13	Kpne-ST-661	>64	16	8	>64	>32	32	>16	32	8	16	≤0.5	32	>128	>16
3	13/07/13	Kpne-ST-661	64	8	≤0.125	>64	8	16	>16	0.5	8	0.5	1	1	4	0.5
4	03/10/13	Kpne-ST-661	>64	16	≤0.125	>64	32	32	>16	1	32	2	1	4	32	0.5
5	09/10/13	Kpne-ST-661	>64	8	8	>64	16	16	>16	>32	16	>32	1	8	8	2
6	13/10/13	*E.cloacae*	>64	>64	4	>64	8	16	16	>32	16	>32	≤0.5	8	8	2
6	13/10/13	Kpne-ST-661	>64	8	8	>64	32	16	>16	>32	4	>32	≤0.5	8	8	1
7	17/10/13	Kpne-ST-661	>64	8	8	>64	16	16	>16	>32	4	>32	≤0.5	8	2	2
8	20/10/13	Kpne-ST-661	>64	>64	>8.0	>64	>32	128	>16	>32	16	32	≤0.5	8	32	16
9	25/10/13	Kpne-ST-661	>64	8	≤0.125	>64	16	16	>16	1	4	1	≤0.5	4	8	0.5
10	25/10/13	*K.oxytoca*	>64	16	≤0.125	>64	32	32	>16	0.5	32	0.5	≤0.5	1	8	0.5
11	01/11/13	Kpne-ST-661	>64	8	≤0.125	>64	16	16	>16	0.5	8	0.5	1	1	8	0.5
12	04/11/13	*C.freundii*	>64	>64	8	>64	32	32	>16	>32	64	>32	1	8	32	2
12	04/11/13	Kpne-ST-250	>64	16	≤0.125	>64	32	32	>16	1	32	1	≤0.5	2	16	1
13	08/11/13	Kpne-ST-14	>64	32	2	>64	32	16	>16	1	32	0.5	≤0.5	2	16	2
14	19/11/13	Kpne-ST-661	>64	8	4	>64	8	8	16	>32	16	32	≤0.5	4	null	1
15	25/11/13	Kpne-ST-661	>64	32	0.25	>64	16	16	>16	0.5	4	0.5	≤0.5	2	16	0.5
16	19/12/13	Kpne-ST-1820	>64	16	≤0.125	>64	8	16	>16	0.5	32	1	≤0.5	1	16	0.5
17	07/01/14	Kpne-ST-661	>64	8	≤0.125	>64	16	32	>16	0.5	4	0.5	8	1	4	1
18	08/01/14	Kpne-ST-321	>64	16	≤0.125	>64	16	16	>16	1	32	0.5	≤0.5	2	16	1
19	16/01/14	Kpne-ST-661	>64	64	>8.0	>64	32	32	>16	>32	32	>32	≤0.5	4	64	4
20	20/01/14	Kpne-ST-661	>64	32	8	>64	16	16	>16	0.25	16	0.25	≤0.5	≤0.5	16	2



Virulence factor detection



The Virulence Factor Database can be used with most software tools for detecting AMR genes

http://www.mgc.ac.cn/VFs/

D912–D917 Nucleic Acids Research, 2022, Vol. 50, Database issue *Published online 30 November 2021*
https://doi.org/10.1093/nar/gkab1107

VFDB 2022: a general classification scheme for bacterial virulence factors

Bo Liu†, Dandan Zheng†, Siyu Zhou, Lihong Chen* and Jian Yang*

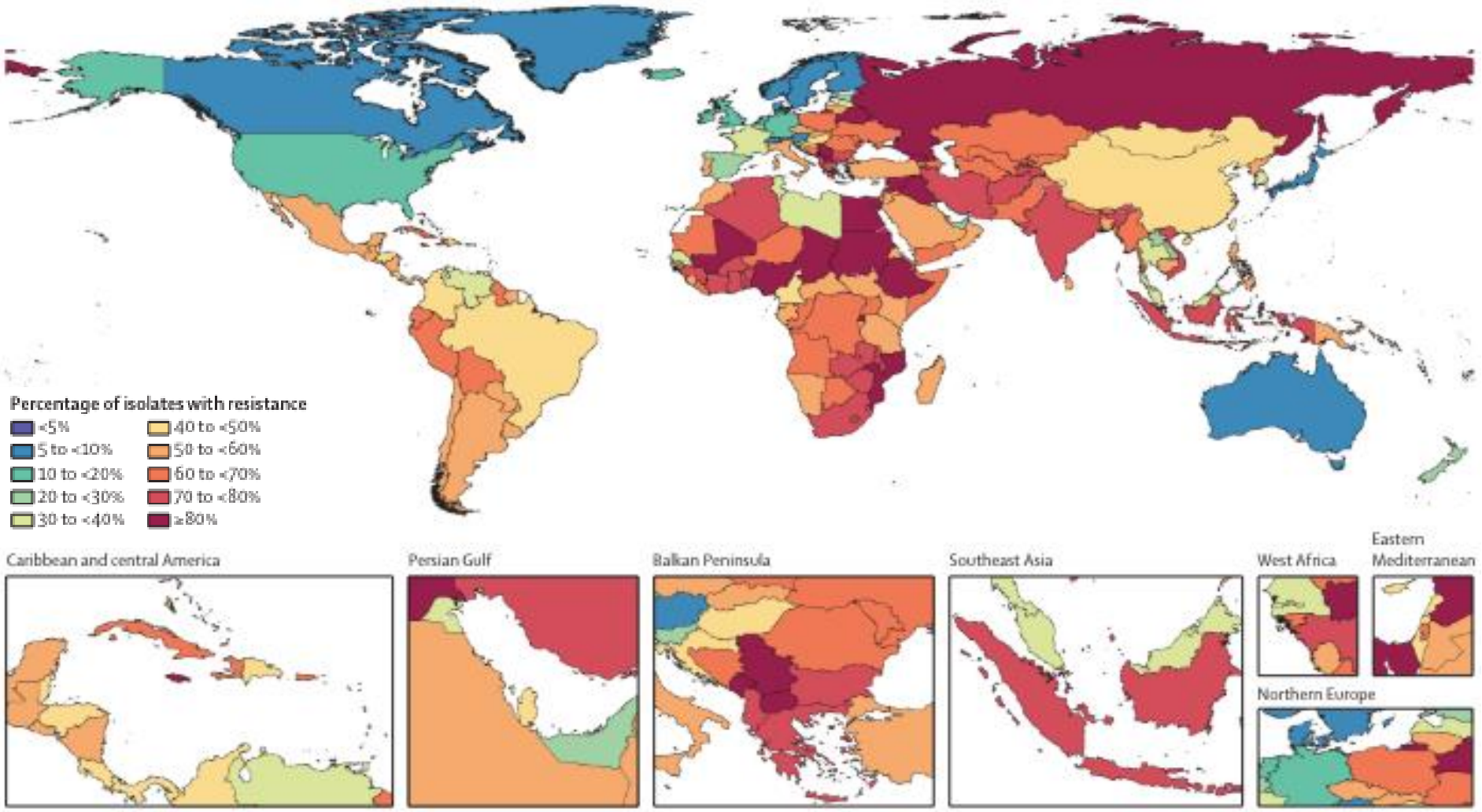
Antimicrobial resistance (AMR) in *Klebsiella pneumoniae*

Antimicrobial resistance in *Klebsiella pneumoniae*

- **Multidrug-resistance (MDR)** is defined as resistance to ≥3 antimicrobial classes, in addition to ampicillin
- MDR is increasing, e.g. >75% of *K. pneumoniae* bloodstream infections in Malawi are MDR
- MDR cases are have mostly evolved from 'classical' strains that are associated with healthcare associated infections (HAI)
- Of particular concern are strains that are resistant to last line antimicrobials; the carbapenems and colistin
- Resistance to all drug classes used to treat *K. pneumoniae* has been observed
- Convergent evolution of hypervirulent and antimicrobial resistant strains has been observed

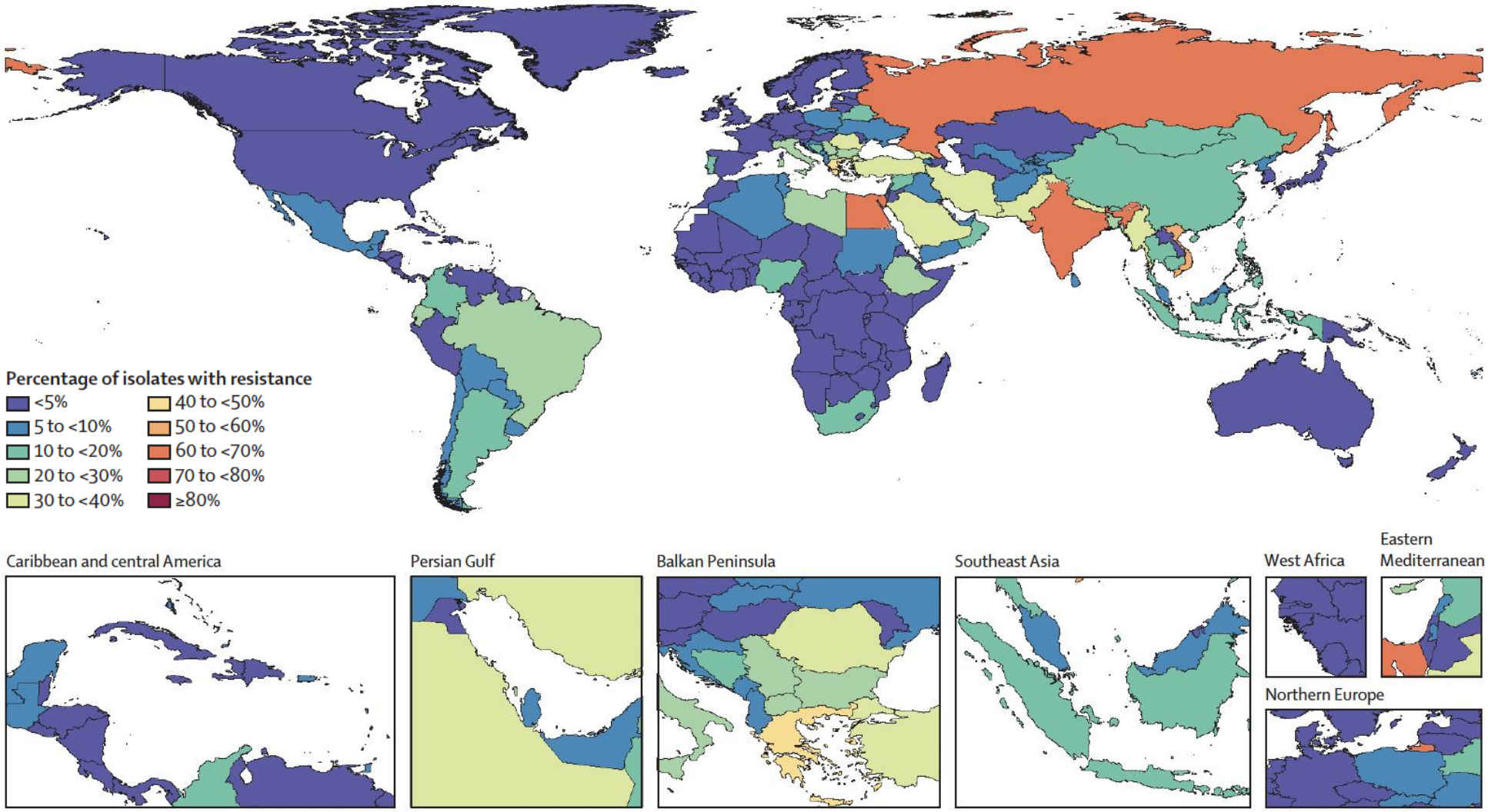
Wyres et al. 2020, Nat Rev Microbiol

Prevalence of *Klebsiella pneumoniae* resistant to third generation cephalosporins



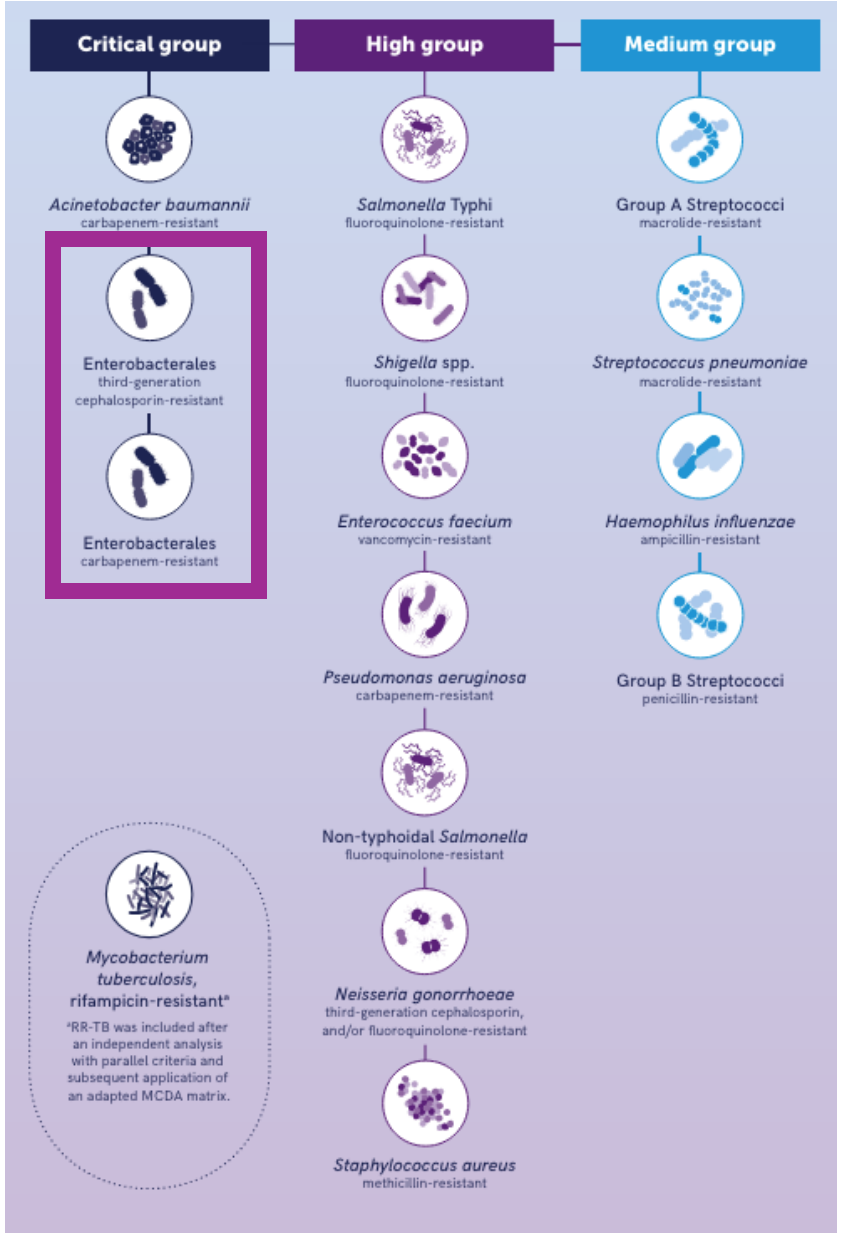
AMR collaborators et al. 2022, Lancet

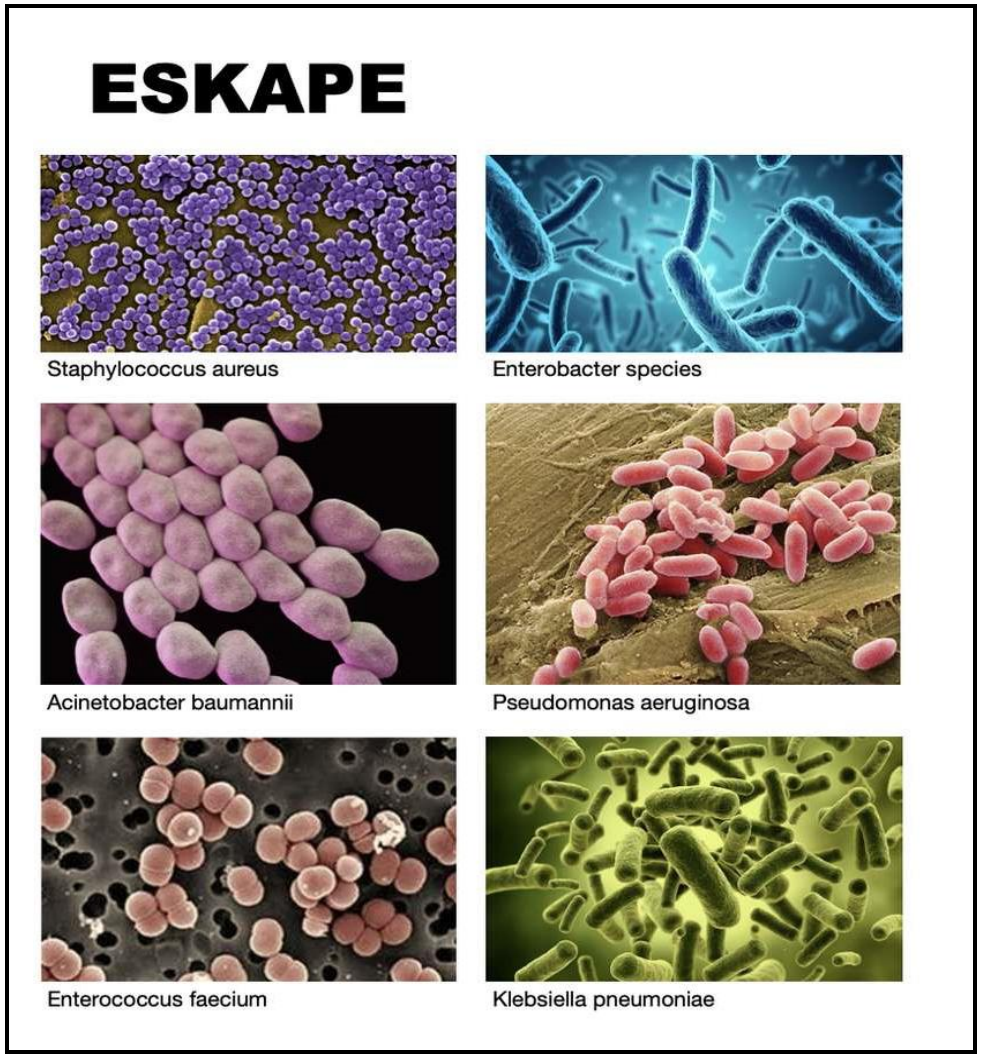
Prevalence of *Klebsiella pneumoniae* resistant to carbapenems (CRKp)



AMR collaborators et al. 2022, Lancet

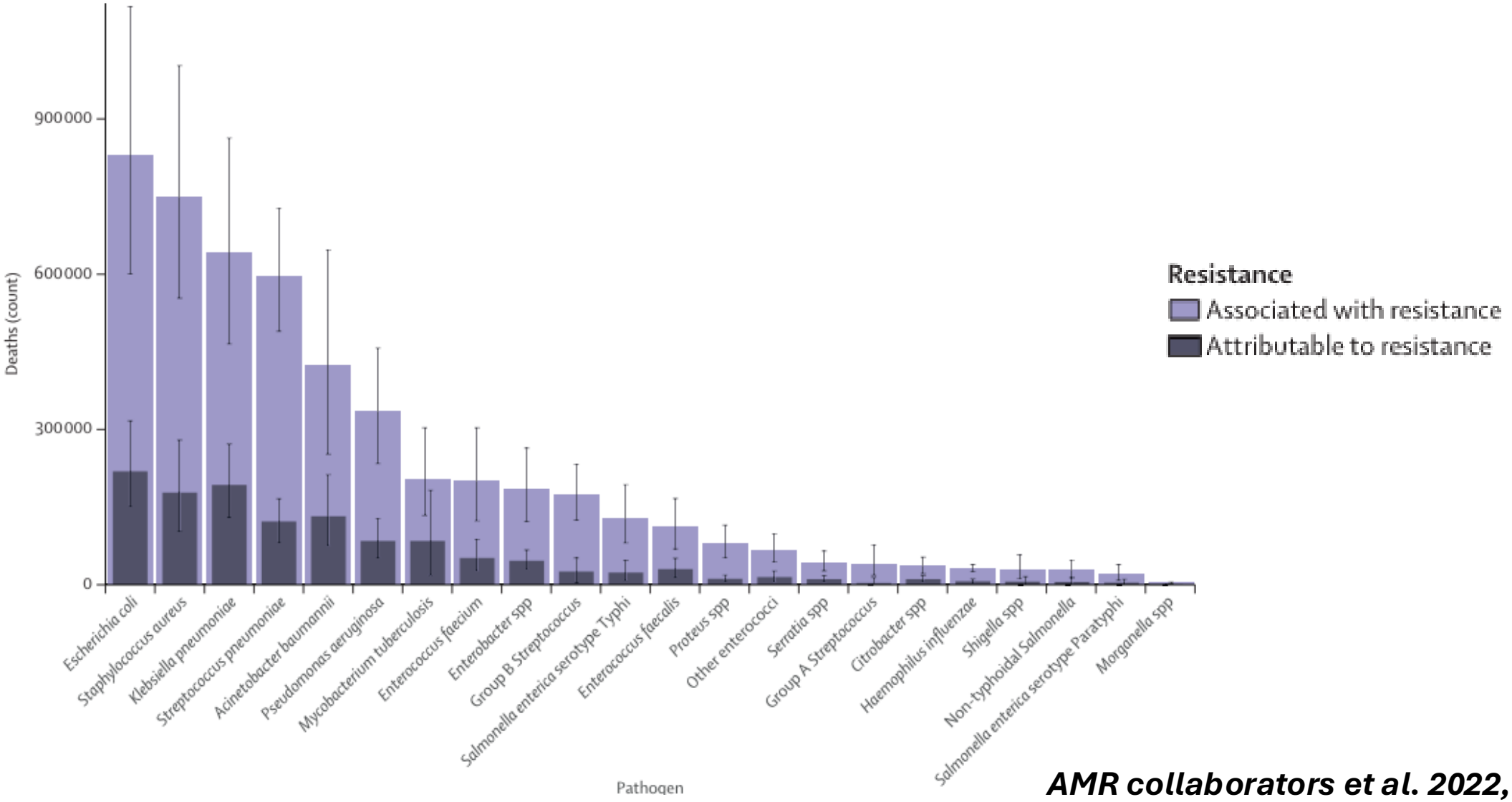
K. pneumoniae is a priority & ESKAPE pathogen





WHO 2024

Antimicrobial resistant (AMR) *K. pneumoniae* is a leading cause of illness and mortality



AMR collaborators et al. 2022, Lancet

Klebsiella is a major driver of neonatal sepsis

- *Klebsiella* is the second largest driver of neonatal sepsis in from sub-Saharan Africa from 1980-2018
- Together with *Staphylococcus aureus* and *Escherichia coli*, *Klebsiella* spp. drove 25% of cases

Articles

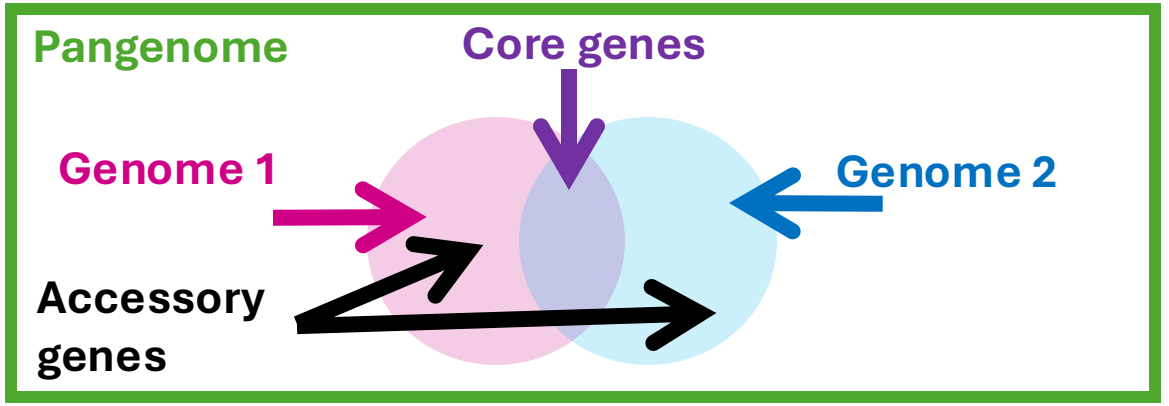
Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines

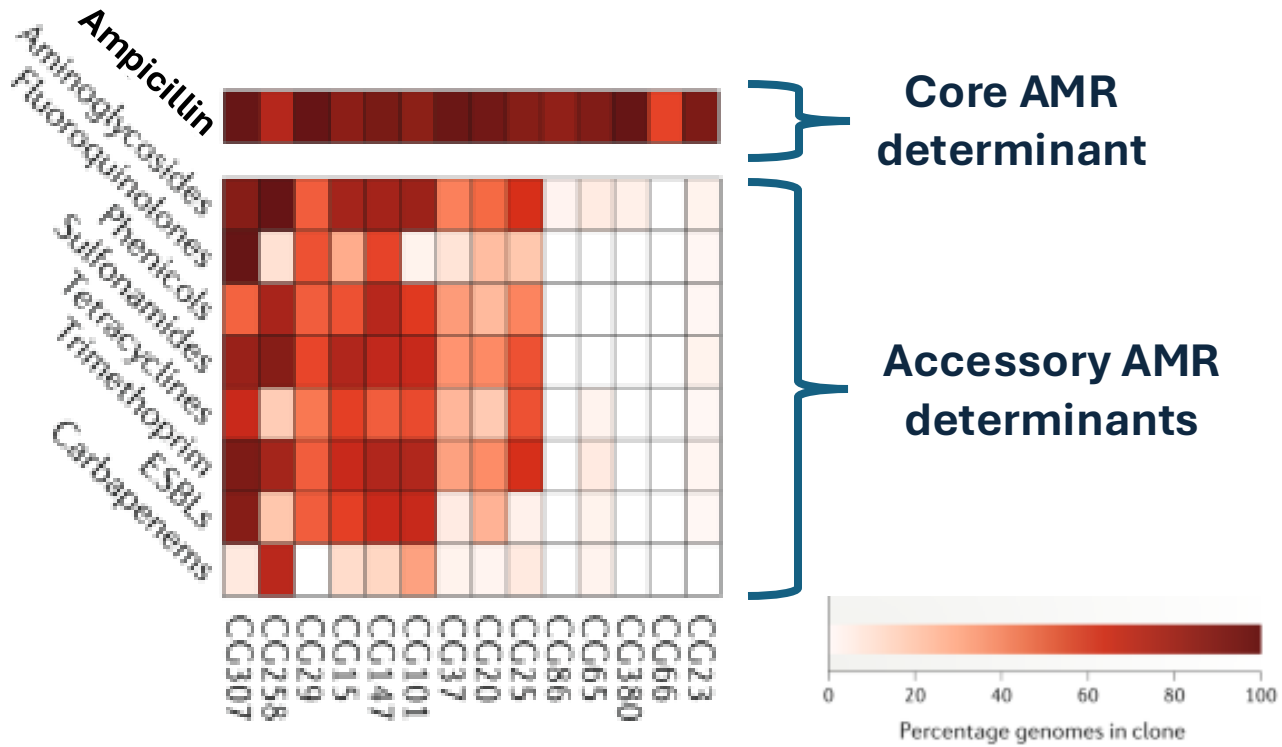
Uduak Okomo, Edem N K Akpalu, Kirsty Le Doare, Anna Roca, Simon Cousens, Alexander Jarde, Mike Sharland, Beate Kampmann, Joy E Lawn**

	1980–2007		2008–18	
	Number of isolates	Proportion (95% CI)	Number of isolates	Proportion (95% CI)
Bacteraemia or sepsis				
Gram-positive				
Staphylococcus aureus	912	0·25 (0·19–0·31)	2080	0·25 (0·21–0·29)
Streptococcus pyogenes	75	0·04 (0·02–0·08)	117	0·04 (0·02–0·07)
Group B streptococci	213	0·07 (0·03–0·12)	342	0·06 (0·03–0·10)
Group D streptococci or enterococcus	139	0·05 (0·03–0·07)	449	0·05 (0·04–0·07)
Streptococcus pneumoniae	72	0·04 (0·02–0·08)	114	0·02 (0·01–0·04)
Viridians streptococci	7	0·01 (0–0·05)	71	0·03 (0·01–0·05)
Other *Streptococcus* species	63	0·03 (0·01–0·05)	209	0·05 (0·03–0·07)
Other or unspecified Gram-positives	86	0·04 (0·01–0·08)	155	0·06 (0·03–0·09)
Gram-negative				
Klebsiella species	644	0·15 (0·11–0·20)	1730	0·21 (0·16–0·27)
Escherichia coli	377	0·10 (0·08–0·13)	856	0·10 (0·08–0·13)
Pseudomonas species	146	0·04 (0·02–0·05)	189	0·03 (0·02–0·04)
Enterobacter species	270	0·08 (0·03–0·13)	263	0·04 (0·03–0·05)
Serratia species	0	..	129	0·03 (0·01–0·07)
Proteus species	54	0·02 (0·01–0·04)	126	0·03 (0·02–0·04)
Salmonella species	162	0·03 (0·02–0·05)	176	0·04 (0·02–0·06)
Citrobacter species	61	0·04 (0·01–0·07)	122	0·02 (0·02–0·04)
Haemophilus influenzae	11	0·01 (0–0·02)	10	0·01 (0–0·03)
Neisseria meningitidis	0	..	17	0·03 (0–0·08)
Acinetobacter species	94	0·05 (0·02–0·07)	299	0·05 (0·03–0·07)
Other or unspecified Gram-negatives	522	0·20 (0·14–0·27)	508	0·10 (0·06–0·14)

Core and accessory AMR determinants

- Resistance can be intrinsic or acquired
- Members of the KpSC carry some chromosomal antimicrobial resistance genes that can be considered core AMR genes
- There are two main interpretations, depending on the core gene:
  1. The pathogen is expected to be resistant to an antimicrobial
  2. That the gene does not confer resistance





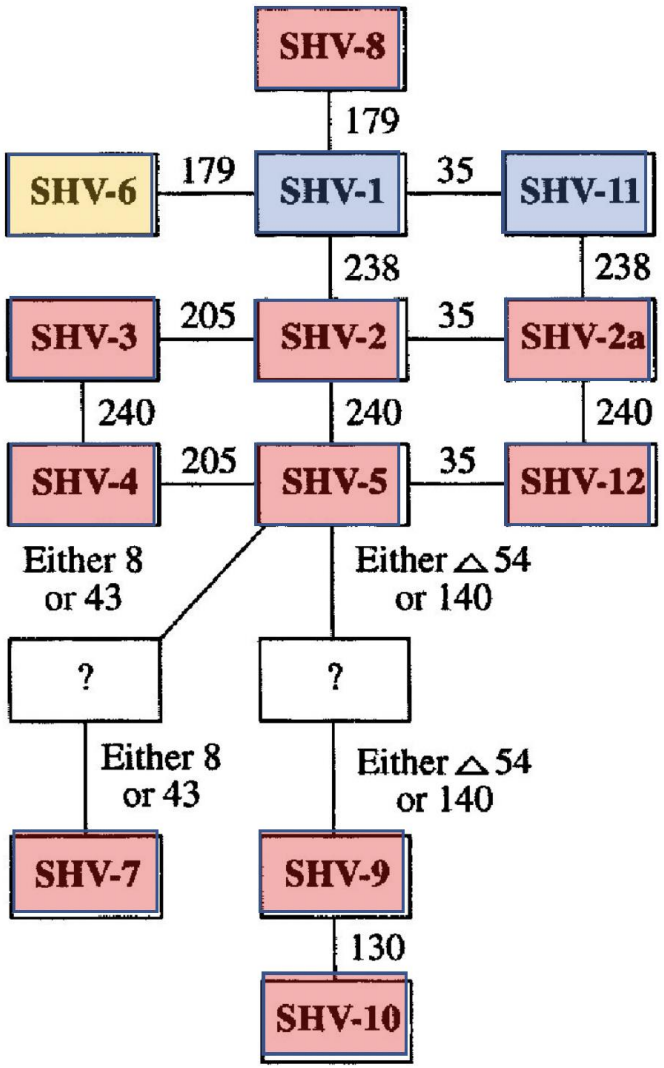
https://www.eucast.org/expert_rules_and_expected_phenotypes/expected_phenotypes

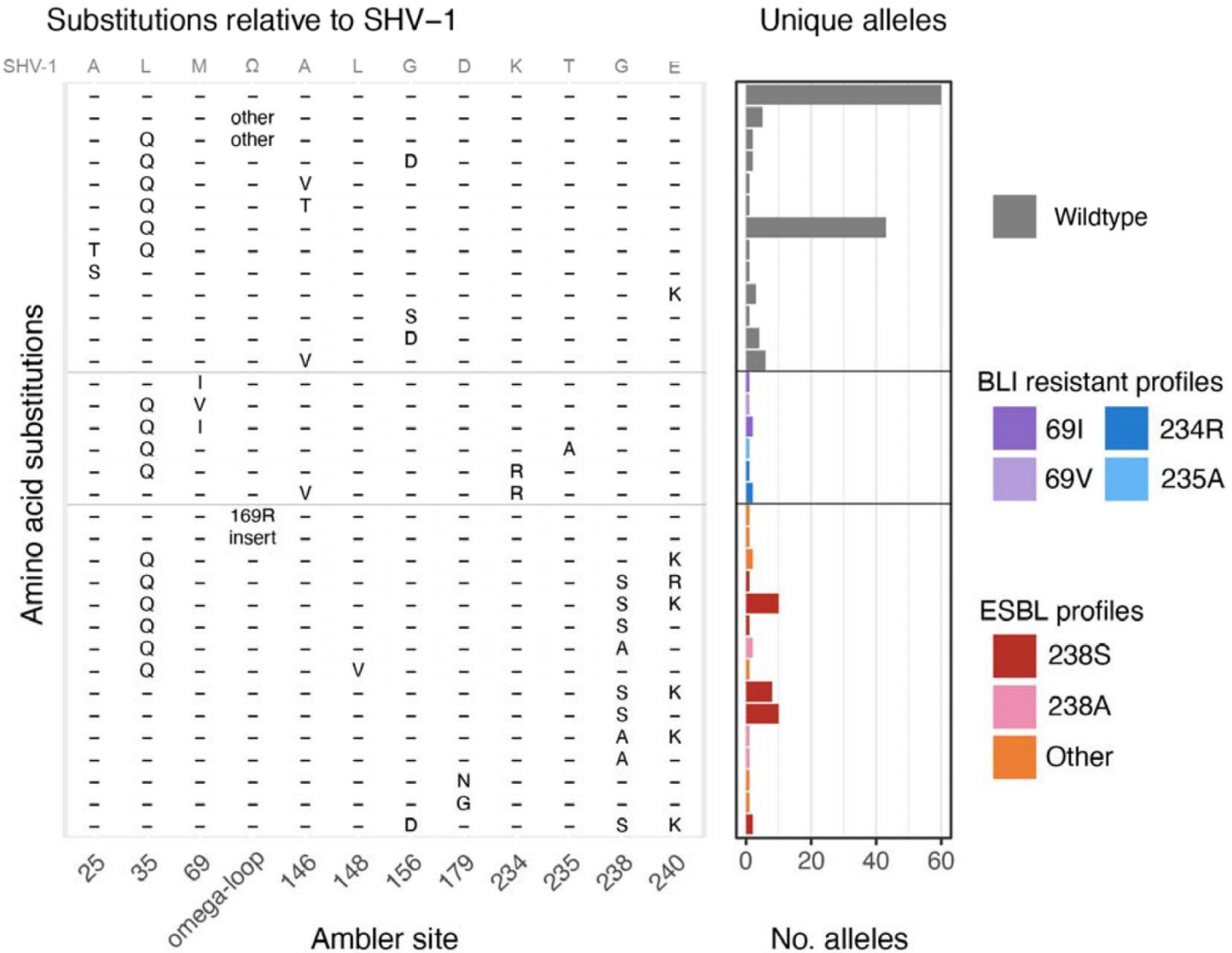
Expected resistance to ampicillin

- All KpSC members are expected to be resistant to ampicillin
- Ampicillin resistance is driven by specific core chromosomal alleles of beta-lactamase genes:
  - *bla*$_{SHV}$ in *K. pneumoniae sensu stricto*
  - *bla*$_{LEN}$ in *K. variicola*
  - *bla*$_{OKP}$ *in K. quasipneumoniae*
- In *K. pneumoniae bla*$_{SHV}$ can become mobilizable by insertion sequences such as IS*26*, forming a mobile genetic element that facilitates dissemination to other bacteria via plasmids
- Mobilised variants of *bla*$_{SHV}$ can acquire mutations that result in extended spectrum beta-lactamase activity (ESBL) conferring resistance to third generation cephalosporins, β-lactamase inhibitor (BLI) resistance, and occasionally carbapenems
- Mobilised forms of *bla*$_{SHV}$ can be hyperexpressed under stronger promotors from IS (insertion sequences)
- A single isolate of *K. pneumoniae* can carry multiple chromosomal and mobilised forms

Tsang et al. 2024, bioRxiv; Wyres et al. 2020, Nat Rev Microbiol

*bla*SHV mutations and spectrum of activity





Heritage et al. 1999, JAC

Tsang et al 2024, bioRxiv

Other core AMR genes

- Both *fosA* (glutathione S-transferase) and the *oqxAB* (efflux pump) genes are considered core AMR genes in *K. pneumoniae*

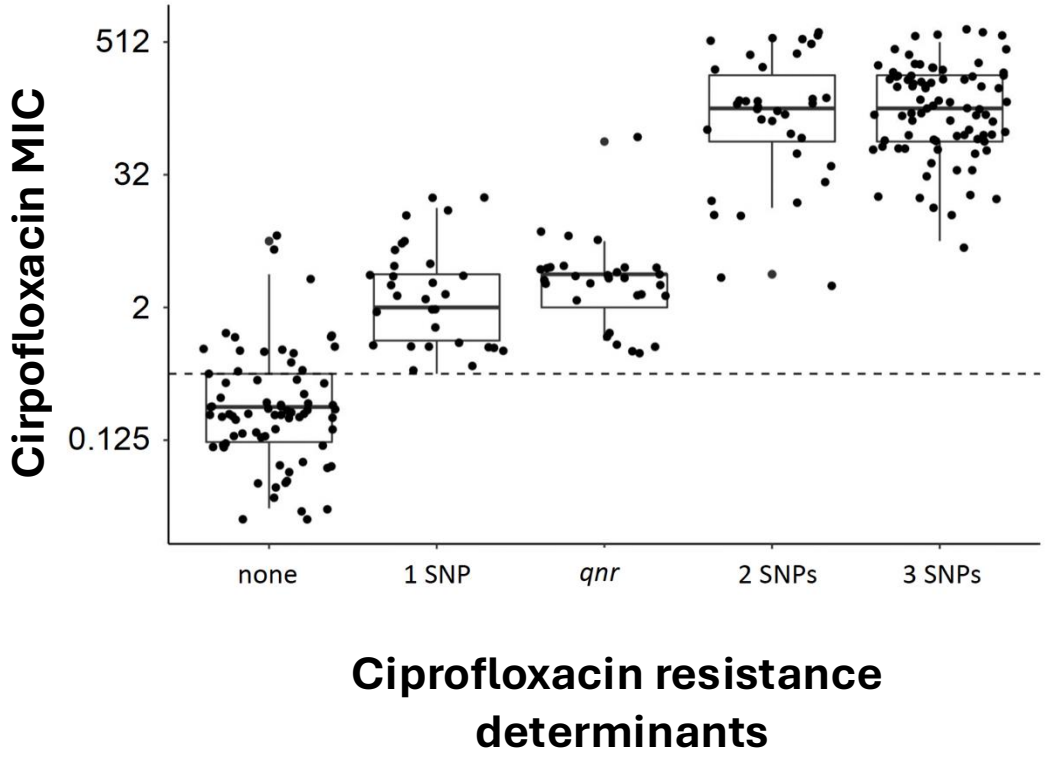
- While *fosA* and *oqxAB* do confer reduced susceptibility at wild type expression levels to both fosfomycin and fluoroquinolones, respectively, this is does not meet recognised break points and is therefore not clinically significant
  - In other bacteria *fosA* and *oqxAB* can be associated with clinical resistance
  - In *K. pneumoniae,* mobililised forms are more highly expressed by strong promotors, e.g. via Insertion Sequences (IS) and can can confer a resistance phenotype – these are reported by Kleborate

Ito, R. et al 2017, mBio; Li et al. 2019, Antimicrob Resist Infect Control; Wyres et al. 2020, Nat Rev Microbiol

Resistance to fluoroquinolones

- Resistance to fluoroquinolones such as ciprofloxacin can be driven either:
  1. Acquired genes (e.g. *qnr*)
  2. Mutations in the Quinolone Resistance Determining Region (QRDR) of core genes *gyrA* and/or *parC*

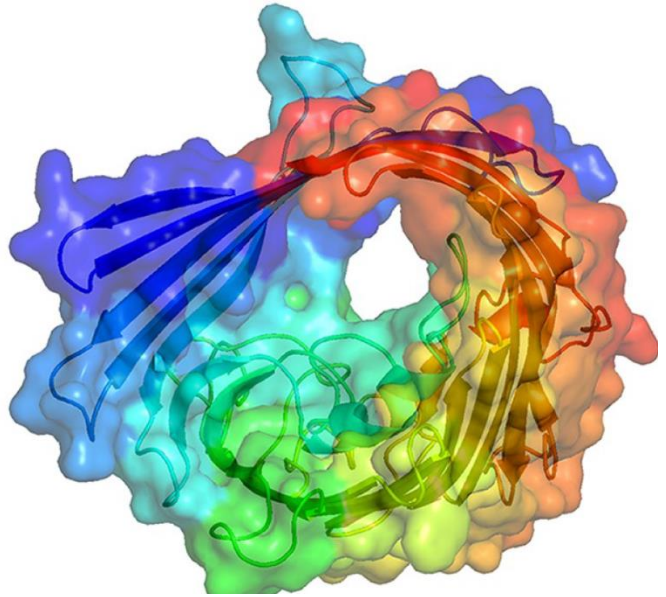
- Fluoroquinolone resistance mutations are synergistic
  - i.e. isolates with 2-3 QRDR mutations elevate the minimal inhibitory concentration (MIC) of the pathogen

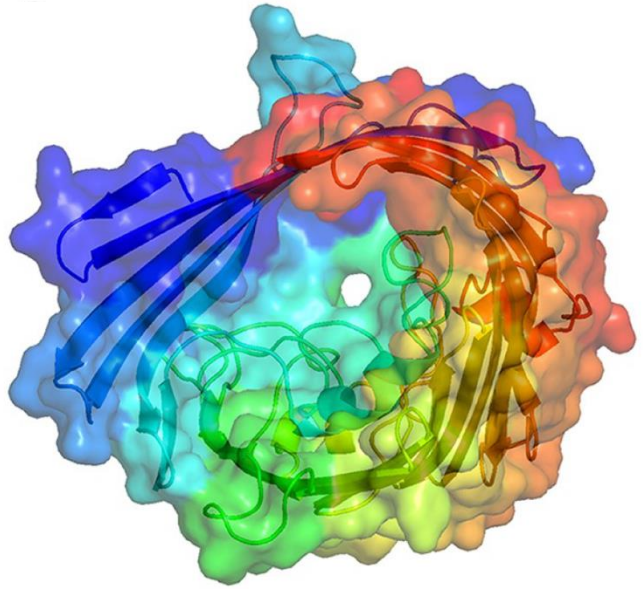


Adapted from Moradigaravand et al, 2017. MBio

Mechanisms of carbapenem resistance

- Resistance to carbapenems can be driven by acquired AMR genes, e.g. *bla*$_{KPC}$, and others
- *K. pneumoniae* encodes 2 major non-specific co-regulated outer membrane porins that allow nutrients and other hydrophilic molecules to diffuse into the cell e.g. beta-lactams
- Expression of these major porins is strongly linked with beta-lactam susceptibility
- Resistance to carbapenems can also arise though mutations in the genes encoding these due to constriction of the inner pore channel/eyelet, i.e.
  - *ompK35* truncation
  - *ompK36* truncation
  - *ompK36* synonymous point mutation *ompK36*-C25T
  - *ompK36* beta-strand loop insertion/duplication - OmpK36GD





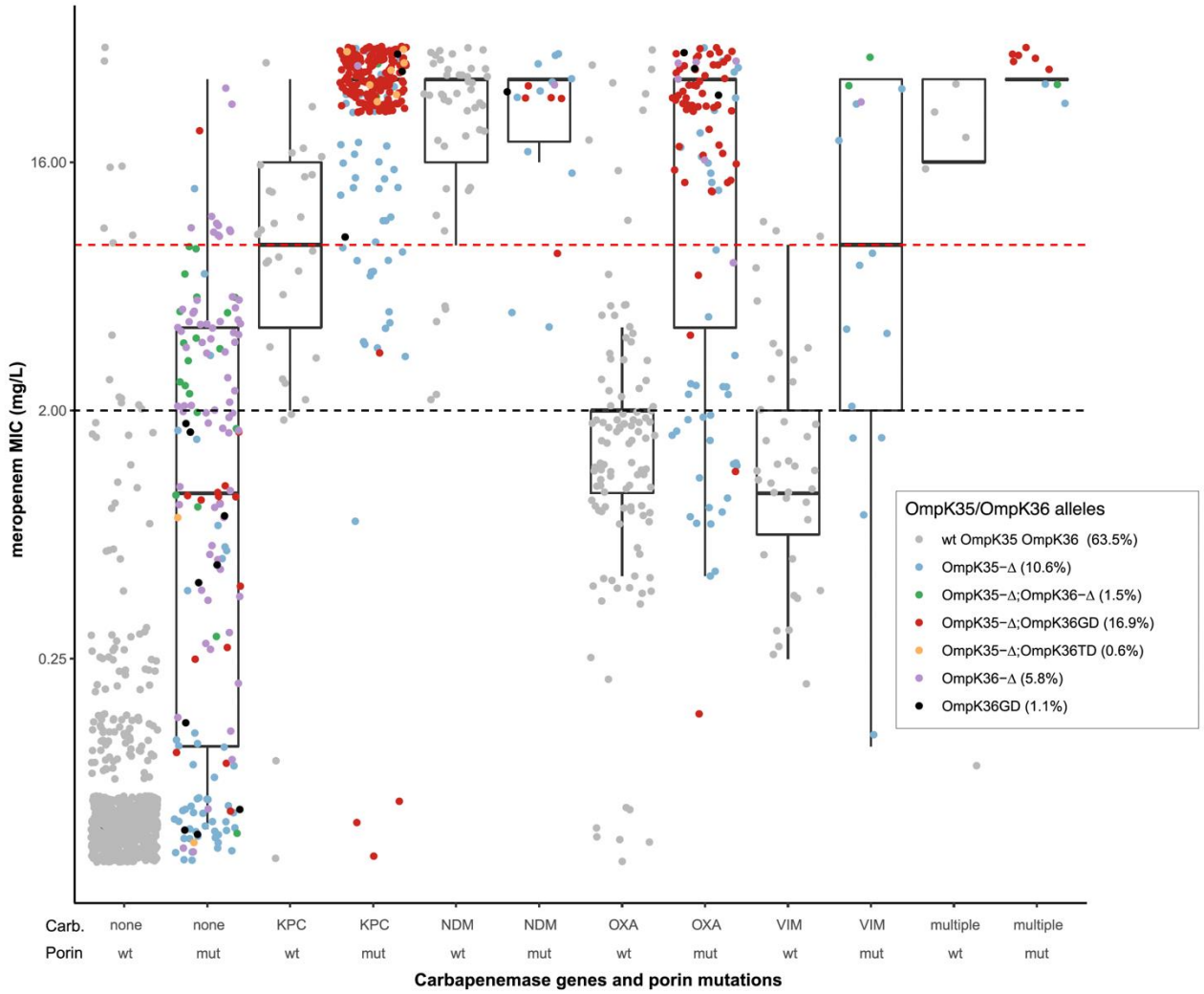
Wong et al. 2022, PNAS; Wong et al. 2019, Nat Commun; Fajardo-Lubian et al. 2019, PLOS Pathogens

Mechanisms of carbapenem resistance

- *bla*$_{OXA}$ and *bla*$_{VIM}$ do not raise the minimal inhibitory concentrations (MIC) above clinical break points if *ompK35* & *ompK36* are wildtype

- *ompK36* mutations increase MIC without acquired carbapenemase genes

- Combinations of acquired carbapenemase genes results in the highest MICs



Lam et al. 2021, Nat Commun

Colistin resistance mechanisms

Carbapenem resistant *K. pneumoniae* (CRKp) infections are often treated with the last-line drug colistin

Resistance to colistin can be driven by:

1. Acquired genes (e.g. *mcr*)

2. Mutations in chromosomal genes
   - Truncation of *mgrB* (encodes a small transmembrane protein that regulates the PhoP/PhoQ system)
   - Truncation of *pmrB* (sensor kinase which also controls lipopolysaccharide modification)

J Antimicrob Chemother 2015; **70**: 75–80
doi:10.1093/jac/dku323 Advance Access publication 3 September 2014

Journal of Antimicrobial Chemotherapy

The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*

Laurent Poirel[1,2], Aurélie Jayol[2], Séverine Bontron[1], Maria-Virginia Villegas[3], Melda Ozdamar[4], Salih Türkoglu[4] and Patrice Nordmann[1,2,5*]

AAC Journals.ASM.org

In Vivo Evolution to Colistin Resistance by PmrB Sensor Kinase Mutation in KPC-Producing *Klebsiella pneumoniae* Is Associated with Low-Dosage Colistin Treatment

Antonio Cannatelli,[a] Vincenzo Di Pilato,[a] Tommaso Giani,[a] Fabio Arena,[a] Simone Ambretti,[b] Paolo Gaibani,[c] Marco Maria D'Andrea,[a] Gian Maria Rossolini[a,d,e]

Antimicrobial resistance (AMR) determinant detection and score analysis with Kleborate

Kleborate: genotyping & surveillance framework

Bioinformatics software for analysing KpSC whole genome sequencing data.

In a single analysis, Kleborate provides data on:

1. Assembly Quality Control Statistics
2. Species typing
3. Multilocus sequence typing (MLST)
4. *In silico* serotyping: K- and O-antigen typing
5. Virulence determinants
6. Antimicrobial Resistance determinants
7. Virulence and AMR scores

Lam et al. 2021, Nat Commun

AMR determinant detection with Kleborate

Kleborate screens for **acquired AMR determinants** (not intrinsic):

1. **Acquired AMR genes**
   - Kleborate uses a version of the generalised CARD AMR database curated for AMR determinants relevant to the KpSC
   - Excludes wildtype *fosA* + *oqxAB* (but includes mobilised forms)
   - *bla*SHV alleles are included

2. **Specific mutations that occur in core chromosomal genes**
   - Quinolone Resistance Determining Region (QRDR) of *gyrA* & *parC* for fluroquinolones
   - OmpK35 and OmpK36 for carbapenem resistance
   - *mgrB & pmrB* for colistin resistance
   - Mutations in bla_{SHV} that mediate ESBL and/or inhibitor resistance

AMR determinant detection with Kleborate

Kleborate AMR reporting:

- Determinants are organised in columns of a delimited text file by drug class
- Mutations are reported in separate columns
- *bla*$_{SHV}$ alleles are reported separately as chromosomal or acquired variants
- Only mobilised forms of *fosA* and *oxqAB* are reported

Kleborate AMR results should not be treated as direct predictions of antimicrobial resistance (AMR) phenotypes

Revision: Kleborate virulence scores

Summary of the relative level of acquired virulence/pathogenicity

Low

High

Virulence score	Virulence determinants*
0	No accessory virulence determinants
1	Yersiniabactin (*ybt*) only
2	Colibactin (clb), either with or without yersiniabactin (*ybt*)**
3	Aerobactin (*iuc*), either with or without yersiniabactin + Colibactin
4	Aerobactin (*iuc*) + yersiniabactin (ybt), without Colibactin (*cbl*)
5	Aerobactin (*iuc*) + yersiniabactin (*ybt*) + Colibactin (*cbl*)

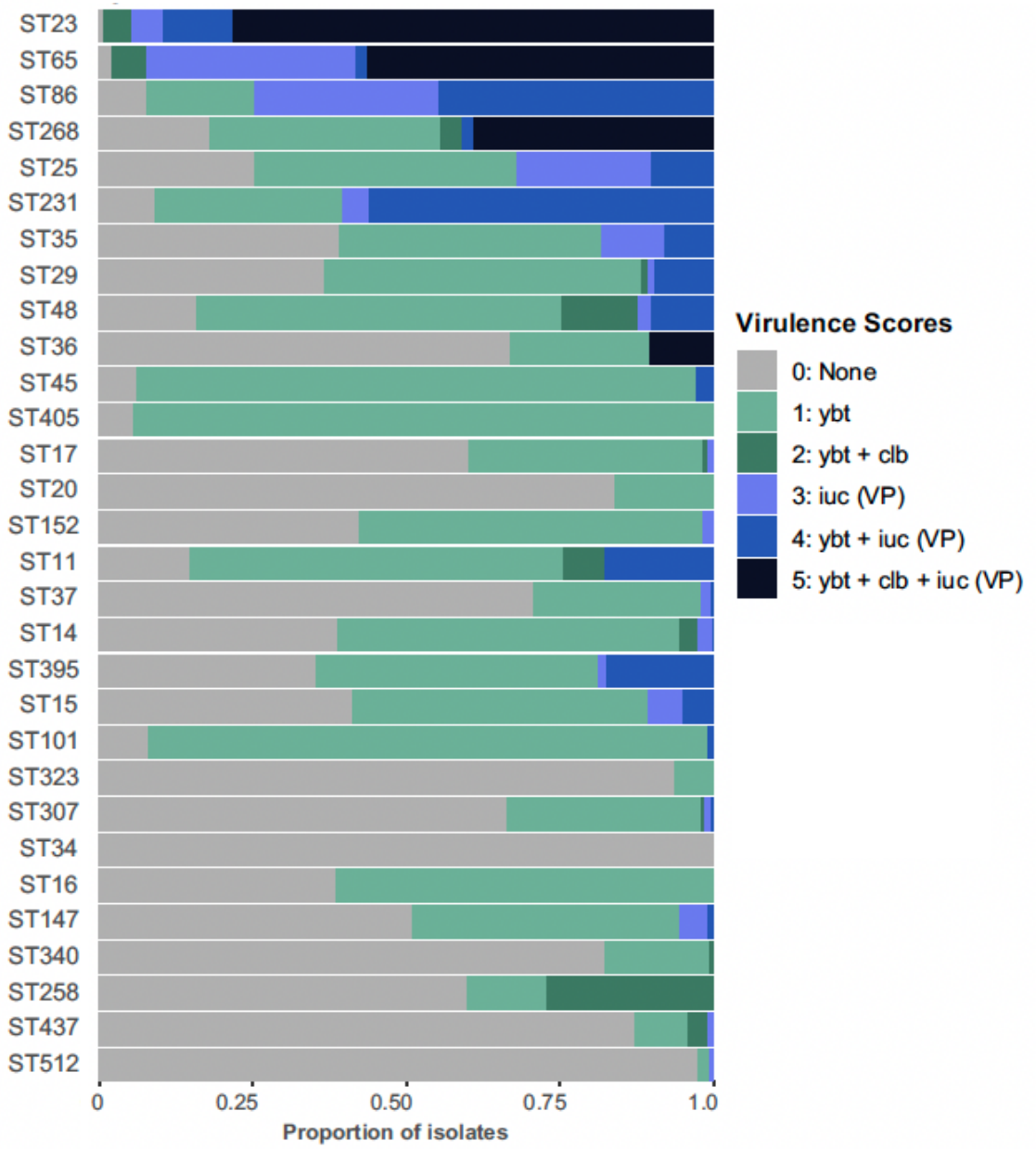
** rmp & Salmochelin (iro) not considered in scoring, but commonly co-carried with aerobactin (iuc) on virulence plasmids (KpVP)*

*** High levels of co-carriage of colibactin and yersiniabactin on ICEKp10*

Lam et al. 2021, Nat Commun

Revision: Kleborate virulence scores

Virulence score	Virulence determinants*
0	No accessory virulence determinants
1	Yersiniabactin (*ybt*) only
2	Colibactin (clb), either with or without yersiniabactin (*ybt*)**
3	Aerobactin (*iuc*), either with or without yersiniabactin + Colibactin
4	Aerobactin (*iuc*) + yersiniabactin (ybt), without Colibactin (*cbl*)
5	Aerobactin (*iuc*) + yersiniabactin (*ybt*) + Colibactin (*cbl*)



Lam et al. 2021, Nat Commun

Kleborate AMR scoring

Summary of the relative level of acquired antimicrobial resistance (AMR) based on the number of resistance classes and determinants. These calculations exclude intrinsic ampicillin resistance.

Low

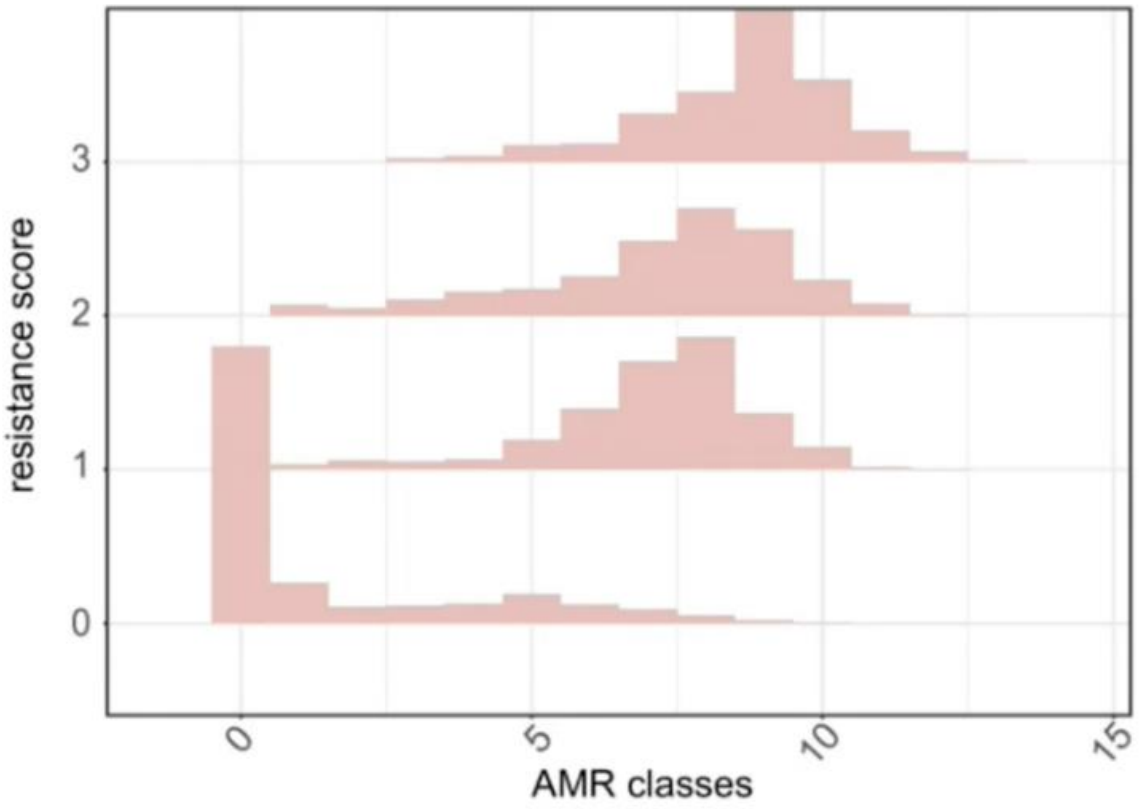
High

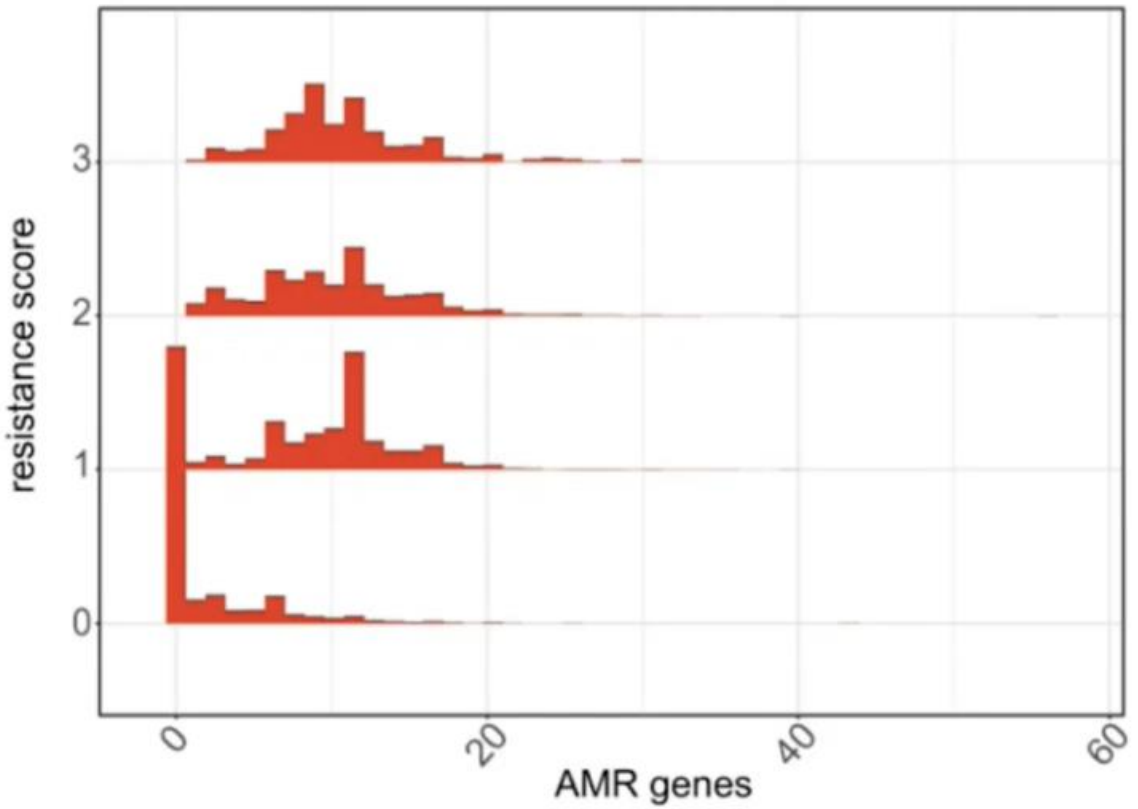
Resistance score	Resistance determinants
0	No ESBL, no carbapenemases (regardless of the presence/absence of colistin resistance determinants)
1	ESBL, no carbapenemases (regardless of the presence/absence of colistin resistance determinants)
2	Carbapenemase without colistin resistance (regardless of ESBL or OmpK)
3	Carbapenemase and colistin resistance (regardless of ESBL or OmpK)

Lam et al. 2021, Nat Comun

Kleborate AMR scoring

A resistance score of 1 or higher is associated with multidrug-resistance (MDR)

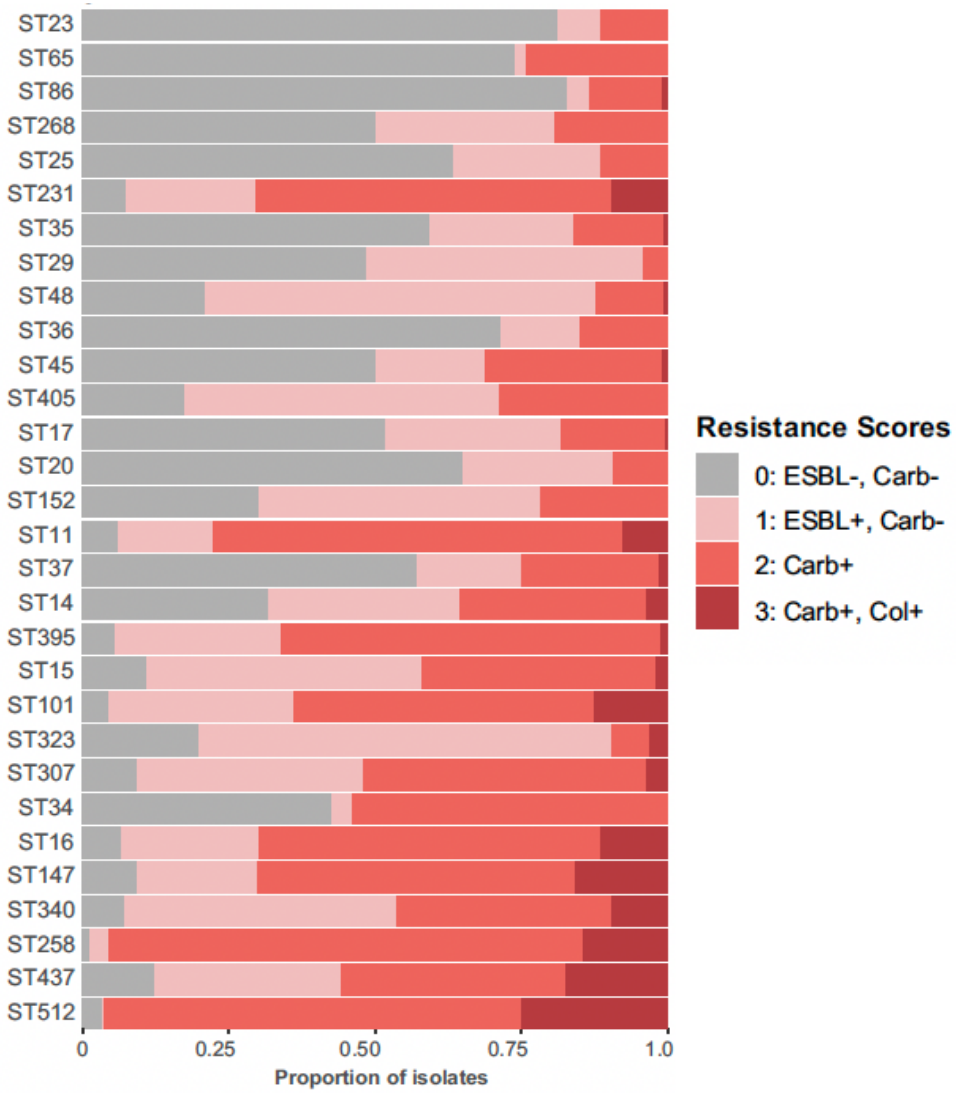




Lam et al. 2021, Nat Comun

Kleborate AMR scoring

Resistance score	Resistance determinants
0	No ESBL, no carbapenemases (regardless of the presence/absence of colistin resistance determinants)
1	ESBL, no carbapenemases (regardless of the presence/absence of colistin resistance determinants)
2	Carbapenemase without colistin resistance (regardless of ESBL or OmpK)
3	Carbapenemase and colistin resistance (regardless of ESBL or OmpK)



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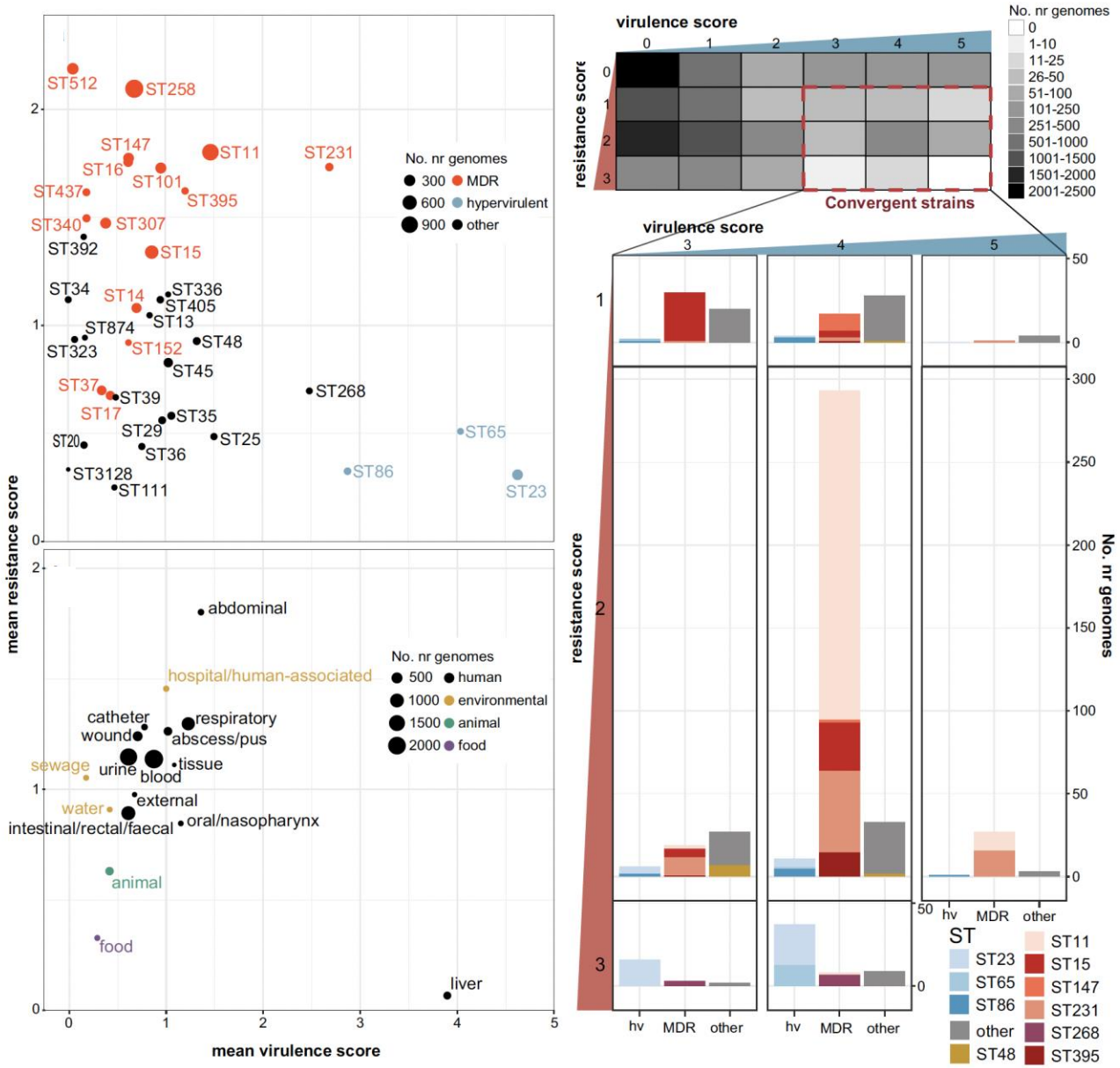
Convergent evolution of MDR + hypervirulence



Lam et al. 2021, Nat Comun

Convergent evolution of MDR + hypervirulence

Articles

A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study

Danxia Gu*, Ning Dong*, Zhiwei Zheng, Di Lin, Man Huang, Lihua Wang, Edward Wai-Chi Chan, Lingbin Shu, Jiang Yu, Rong Zhang, Sheng Chen

Hospital outbreak – 5 patients with severe pneumonia following surgery for severe trauma and subsequent mechanical ventilation. 100% mortality rate.

Carbapenem strain acquired the pLVPK virulence plasmid harbouring salmochelin, aerobactin, and rmp genes.

Any questions or reflections?