

Whole genome sequencing (WGS) in antimicrobial susceptibility testing (AST)

NordicAST Workshop 27th of May 2025
Hyllie, Malmö, Sweden

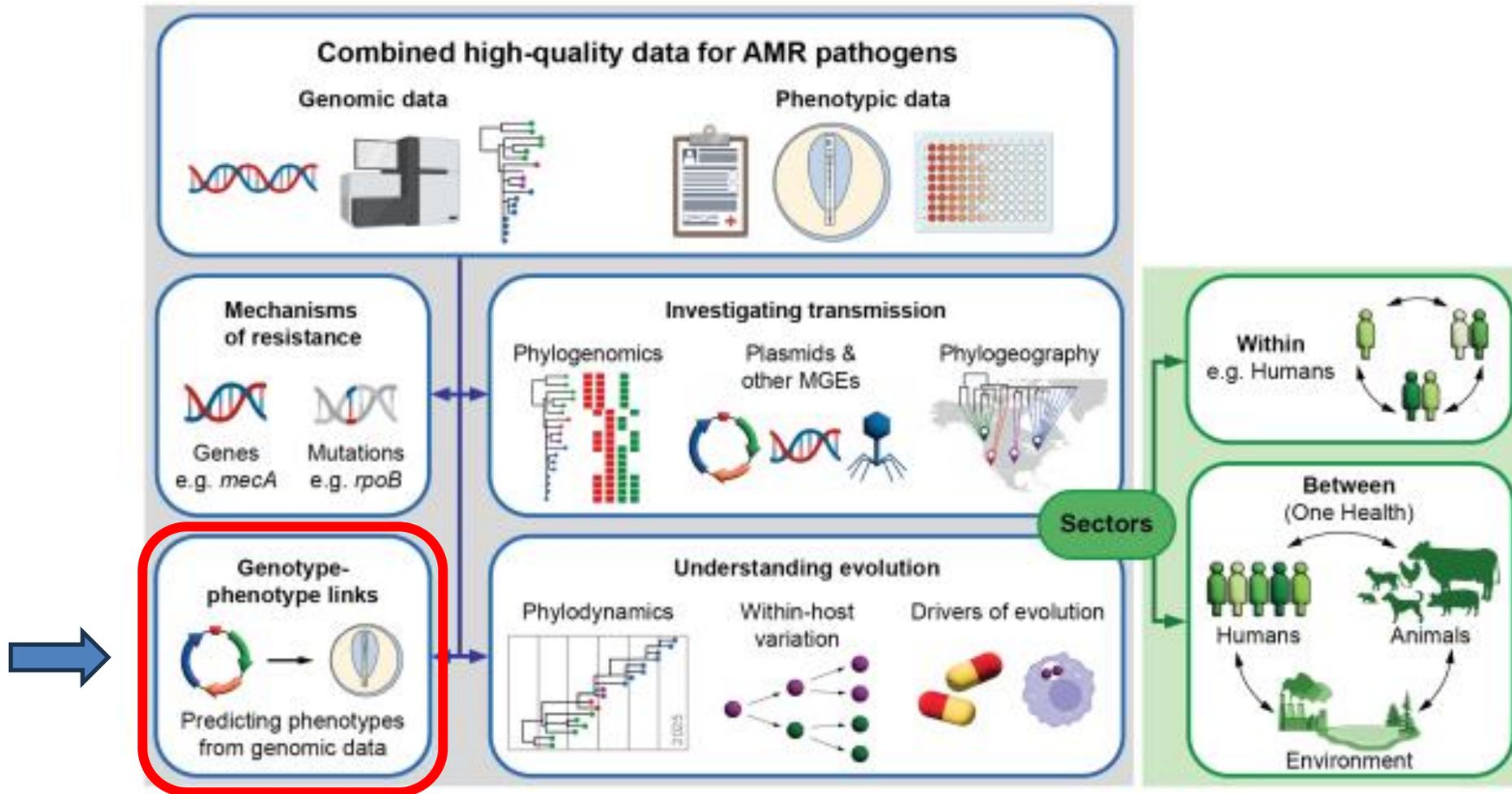
Ørjan Samuelsen, Prof./PhD

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University Hospital of North Norway
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Agenda/outline

- General concepts and considerations
 - Genotype-phenotype relationship
 - Antimicrobial resistance gene/mutation databases
 - Bioinformatical tools
 - Interpretation
 - Implementation
- EUCAST subcommittee 2017 -> 2025
 - Examples of advances key pathogens
- Artificial intelligence in antimicrobial susceptibility testing -> Adrian Egli (tomorrow @ 10:40 😊)

Genomics for antimicrobial resistance

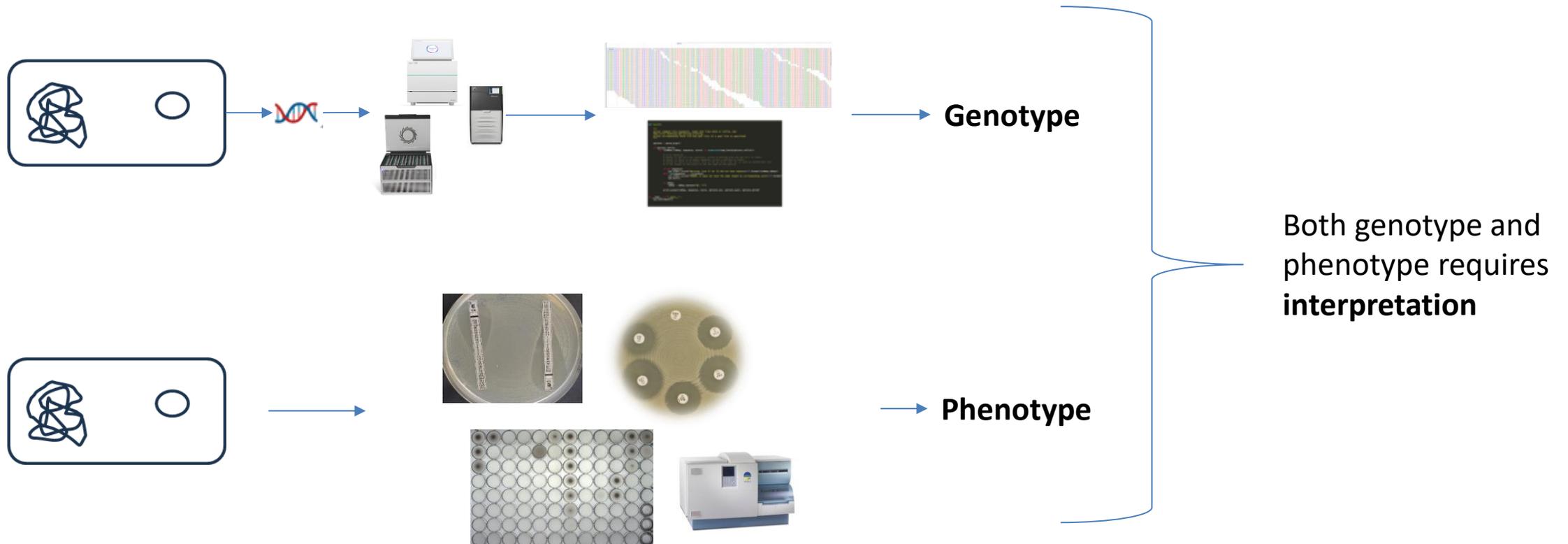


Aim: genotype X \rightarrow phenotype Y (i.e. *bla*_{NDM-1} \rightarrow carbapenem resistance?;)

Genotype and phenotype

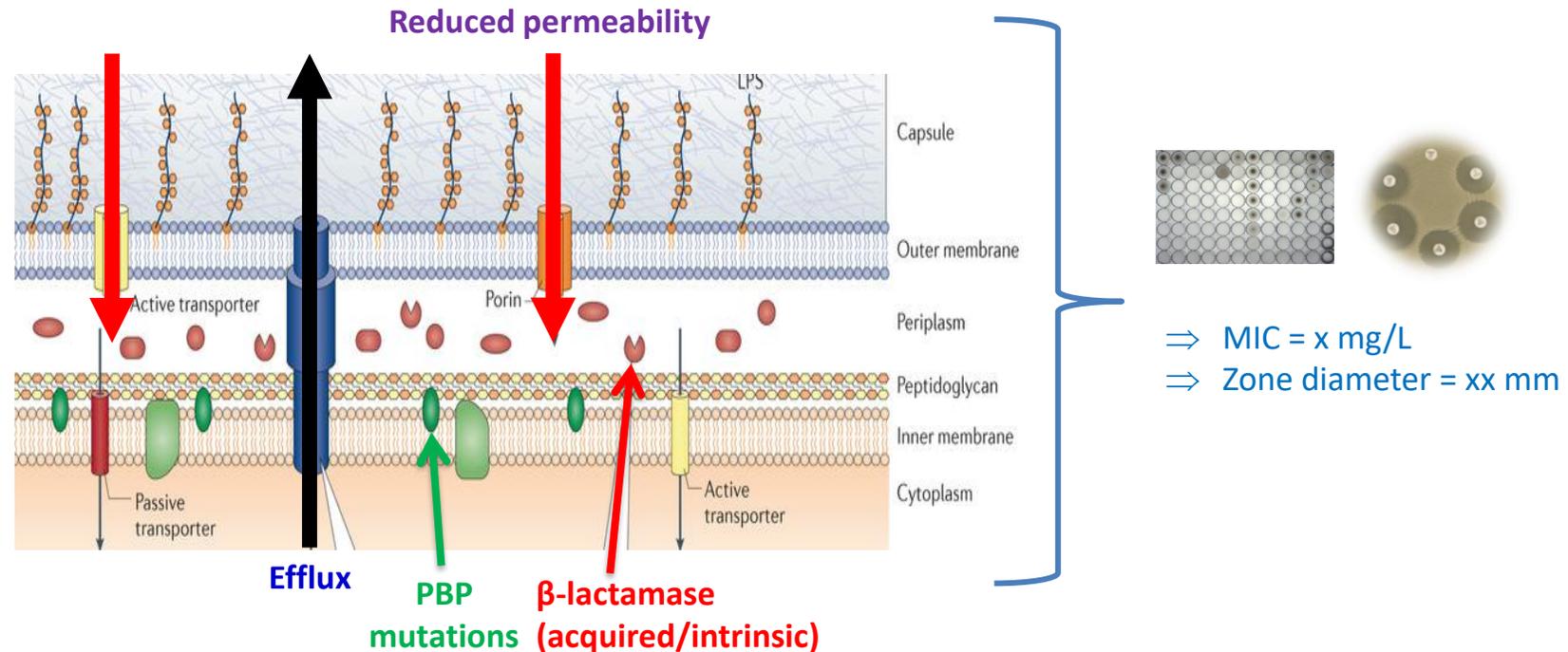
- **Genotype:** organism's genetic profile determined by a **genotypic assay**
- **Phenotype:** organism's observable physical and biochemical characteristic determined by a **biological assay**

→ Both are assay results and influenced by experimental conditions



Genotype-phenotype relationship challenge

Genotype-phenotype relationships are often complex and multifactorial



- Variable gene expression, transcription regulation, gene copy number, IS-provided promoters, promoter mutations.....

Multiple reasons for genotype-phenotype discrepancies

TABLE 1 Examples of genetic alterations resulting in genotypic and phenotypic susceptibility discrepancies^a

Organism	Genotype	Phenotype	Description	Discrepancy reason	Reference(s)
<i>Staphylococcus aureus</i>	<i>mecA</i> detected	Methicillin susceptible	Met-to-Ile change at amino acid residue 483	Elimination of PBP2a transpeptidase activity	38
	PBP2a detected, <i>mecA</i> not detected	Methicillin susceptible	Divergent <i>SCCmec</i> element	Remnant not functional	39
	<i>SCCmec-orfX</i> detected	Methicillin susceptible	<i>SCCmec-orfX</i> junction variants	Low specificity of proprietary target	40
	<i>SCCmec</i> detected	Methicillin susceptible	<i>SCCmec</i> remnant with <i>ccr</i> and partial <i>mecA</i> genes	Remnant not functional	41
	<i>SCCmec</i> detected	Methicillin susceptible	<i>mecA</i> deletions within <i>SCCmec</i>	Detectable by molecular methods, but remnant not functional	43
	<i>SCCmec</i> detected	Methicillin susceptible	Amplification of segments on right-extremity junction sequence of <i>SCCmec</i>	Retained segments do not contain the actual <i>mecA</i> gene	42
	<i>mecA</i> detected	Methicillin susceptible	Heterogeneously resistant large inoculum led to resistant phenotype; oxacillin induction increased MIC	Heterogeneously resistant large inoculum led to resistant phenotype	38
	<i>mecA</i> not detected	Methicillin resistant	Presence of <i>mecA</i> homologue (LGA251)	Not detectable by molecular target	43
<i>Enterococcus</i> species	<i>vanA</i> detected	Vancomycin susceptible	Nucleotide deletions in Tn546	Nonfunctional <i>vanA</i>	44
	<i>vanA</i> detected	Vancomycin variable ^b	Silenced <i>vanA</i> (<i>vanHAX</i>) gene	Unexpressed <i>vanA</i>	45
	<i>vanAB</i> not detected	Vancomycin resistant	Presence of <i>vanC</i>	Not detectable by molecular target	46
	<i>vanAB</i> not detected	Vancomycin resistant	Presence of <i>vanM</i>	Not detectable by molecular target	24
Enterobacterales	<i>bla_{KPC}</i> detected	Carbapenem susceptible	Truncated <i>bla_{KPC}</i>	Nonfunctional carbapenemase	26
	<i>bla_{CTX-M}</i> not detected	Cefotaxime or ceftriaxone resistant	<i>bla_{TEM/SHV}</i> -derived ESBLs	Not detectable by molecular target	16, 17
	<i>bla_{OXA-48}</i> detected	Carbapenem variable ^b	<i>bla_{OXA-48}</i> and other mechanisms (e.g., other β -lactamases)	Alternative resistance mechanisms	17
	Carbapenemase not detected	Carbapenem resistant	Alternative carbapenem resistance mechanism (e.g., AmpC production combined with porin mutations and/or efflux)	Alternative carbapenem resistance mechanism	47
	Carbapenemase not detected	Carbapenem resistant	Test (e.g., Carba-R) cannot detect <i>bla_{OXA-48}</i> -like and some other carbapenemases	Not detectable by molecular target	48
	Carbapenemase not detected	Imipenem resistance	Intrinsic low-level resistance (<i>Morganella</i> , <i>Proteus</i> spp., and <i>Providencia</i> spp.)	Intrinsic low-level resistance	49
	NA	Cefepime variable ^b	ESBL-carrying strains	Alternative resistance mechanisms	50
	NA	β -Lactam/inhibitor combination variable ^b	ESBL-carrying strains	Alternative resistance mechanisms	51
NA	Cephalosporin variable ^b	Presence of multiple β -lactamases	Alternative resistance mechanisms	17	

^aNA, not applicable.

^bVariable describes potentially resistant, intermediate resistant, and susceptible interpretations.

- Truncation of genes – non functional gene
- Mutations in genes that alter properties of the product – i.e. altered substrate profile
- Mutations or inactivation of promoter regions influencing the expression
- Novel gene(s)/mutation(s) – unknown how they influence the phenotype
- Etc, etc.

Requirements for accurate genotype-phenotype relationship

High-quality genomic data

- Depth of genome coverage (e.g. >30x¹)
- Low number of contigs (e.g. <500¹)
- Genome size (within 10% of expected¹)
- Read quality – low error rates
- N50 (>15,000¹)
- ..

High-quality phenotypic data

- AST method - Reference method
- Actual measurements – not only S-I-R
 - Changes in breakpoints
- Included antimicrobials
- QC data

High-quality strain collections

- Clonally diverse
- Diversity of resistance mechanisms
- Diversity of antimicrobial susceptibility – not only selected resistant strains
- Single center/multi center
- Geography and temporal distribution
- Epidemiological metadata

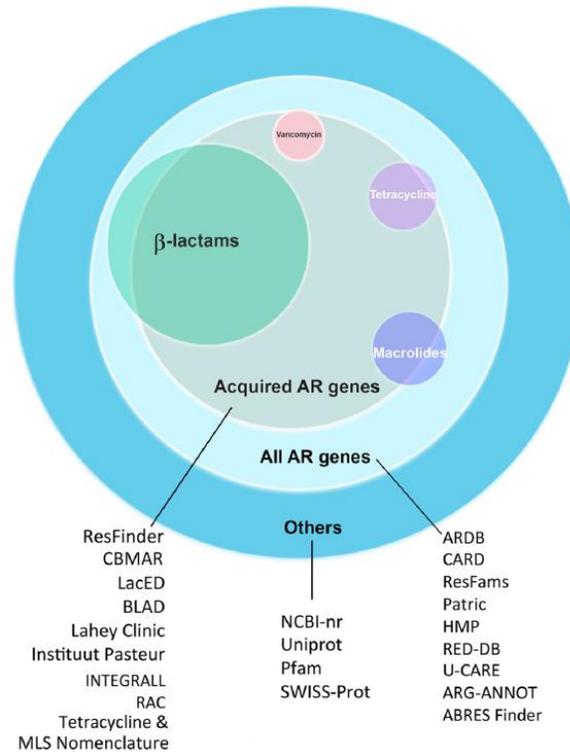
¹From EURGen-RefLabCap protocol on CCRE (<https://www.eurgen-reflabcap.eu/>)

Determination of the AMR genotype - resistance databases

Multiple databases developed over the years for different purposes

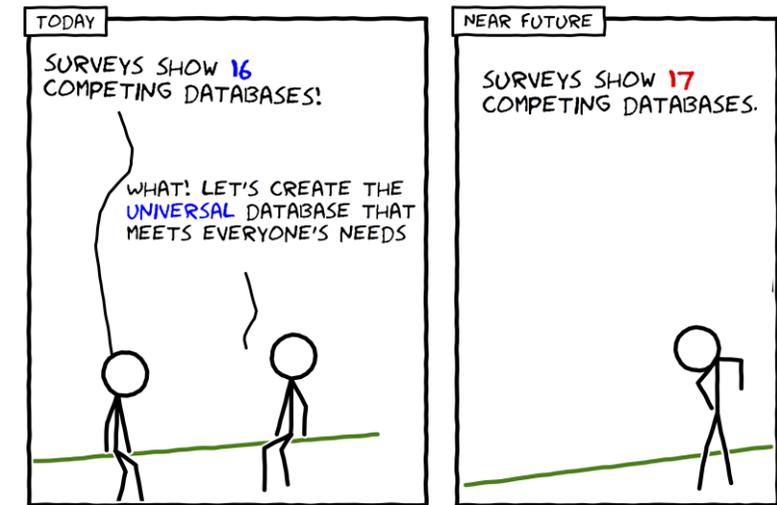
Database	Description	Link	Status
General databases			
CARD ²⁷	• Ontology-based database that provides comprehensive information of AR genes and their resistance mechanisms • Currently contains >2,200 protein homologues and includes a curated set of resistance-conferring chromosomal mutations in protein-coding genes	https://card.mcmaster.ca/	Active; launched in 2013; updated monthly
Resfinder ²²	Collation of AR genes involved in HGT events	https://cge.cbs.dtu.dk/services/ResFinder/	Active; started in 2012; update regularly; last update in February 2019
ResfinderFG ²⁴	Collection of resistance gene variants identified in multiple functional metagenomics studies	https://cge.cbs.dtu.dk/services/ResFinderFG/	Active; last update in November 2016
Resfams ²⁸	A profile HMM-based curated database confirmed for AR function	http://www.dantaslab.org/resfams/	Active; last update in January 2015
ARDB ²⁵	• First centralized resource of AR gene information • Manually curated; contains >4,500 AR sequences	https://ardb.cbc.umed.edu/	Archived; last updated in 2009
MEGARes ¹⁸	• Collation of multiple databases (CARD, ARG-ANNOT and ResFinder) to avoid redundancy between entries • For high-throughput screening and statistical analysis	https://megares.meglab.org/	Active; last update in December 2016
NDARO	• Collated and curated data from multiple databases (CARD, Lahey, Pasteur Institute β -Lactamases and ResFinder) • Contains 4,500 AR sequences	https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047	Active; started in 2016
ARG-ANNOT ⁶	• Repository of >1,800 AR sequences collated from scientific literature and online resources • Also includes point mutation data for select AR-associated chromosomal genes	Not available	Archived; last update in May 2018
Mustard ¹⁵	Resource containing 6,095 AR determinants from 20 families, including curated sets of AR genes identified in functional metagenomics studies	http://mgps.eu/Mustard/	Active; last update in November 2018
FARME database ³¹	Curated set of microbial sequences functionally screened to confer resistance in various functional metagenomics studies of different habitats	http://staff.washington.edu/wallace/farme/	Active; last update in 2017
SARG (v2) ³⁴	• Hierarchical structured database derived from ARDB, CARD and NCBI-NR database • Contains >12,000 AR genes; also includes profile HMMs for 189 AR genes subtypes	http://smile.hku.hk/SARGs	Active
Lahey list of β -lactamases ³⁰	First initiative to compile known β -lactamases and assign nomenclature to novel ones	http://www.lahey.org/Studies/	Archived; last update in 2015
BLDB ³⁷	Manually curated database for AR enzymes classified by class, family and subfamily	http://blbd.eu/	Active; last update in November 2018
LacED ^{38,39}	Curated database of TEM and SHV β -lactamases, including a curated set of known TEM and SHV variants	http://www.laced.uni-stuttgart.de/	TEMLacED active; last update in 2017; SHVED archived; last update in April 2010
CBMAR ²¹	Database that identifies and characterizes novel β -lactamases on the basis of Ambler classification	http://proteininformatics.org/mkumar/lactamasedb/	Last update in September 2014
Species-specific databases			
MUBII-TB-DB ³⁶	Database of mutations associated with AR in Mycobacterium tuberculosis	https://umr5558-bibiserv.univ-lyon1.fr/mubii/mubii-select.cgi	Last update in December 2013
U-CARE ¹⁰	User-friendly, comprehensive AR repository for Escherichia coli	http://www.e-bioinformatics.net/ucare	Last update in 2016

Boolchandani M, et al. *Nat. Rev. Genet.* 2019



Xavier BB et al. *J Clin Microbiol* 2016

HOW BIOLOGICAL DATABASES PROLIFERATE



Helmy M, et al. *PLoS Comput. Biol.* 2016

Determination of the AMR genotype - resistance databases



<https://genepi.dk/>

The Comprehensive Antibiotic Resistance Database

A bioinformatic database of resistance genes, their products and associated phenotypes.

8526 Ontology Terms, 6442 Reference Sequences, 4542 SNPs, 3328 Publications, 6490 AMR Detection Models

<https://card.mcmaster.ca/>



Health > Pathogen Detection > Reference Gene Catalog

<https://www.ncbi.nlm.nih.gov/pathogens/refgene/#>

- Curated databases

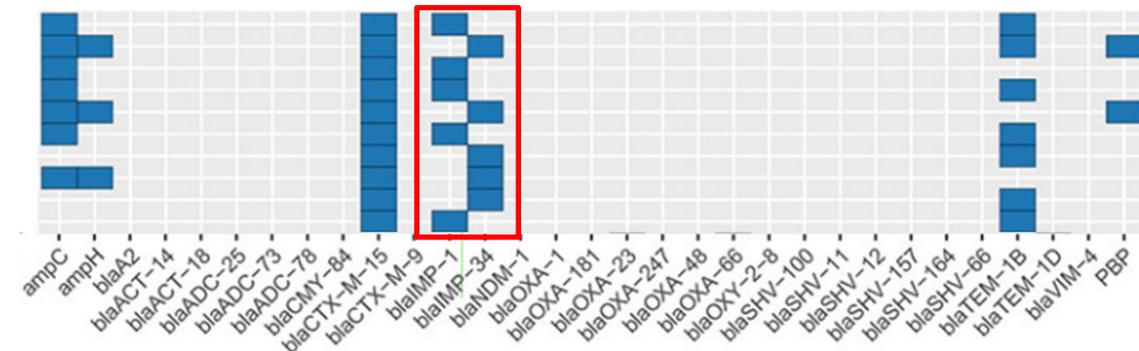
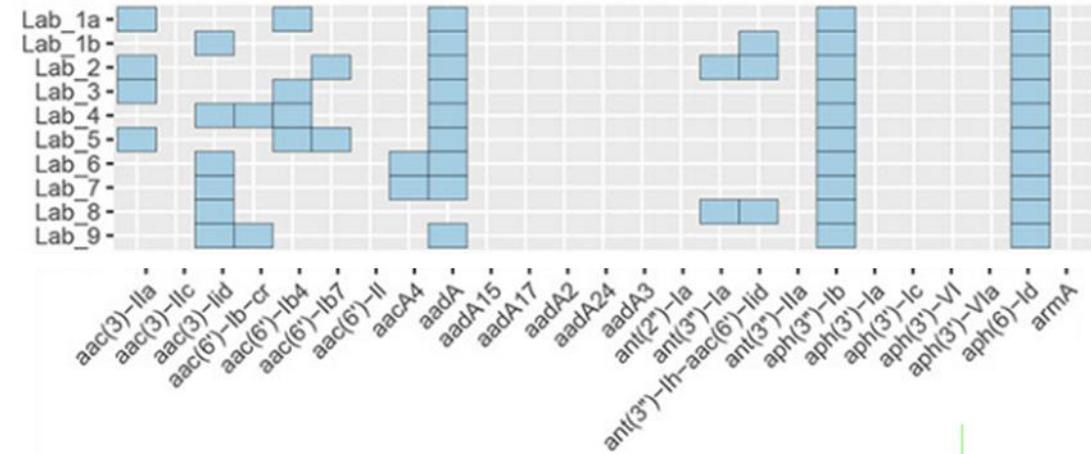
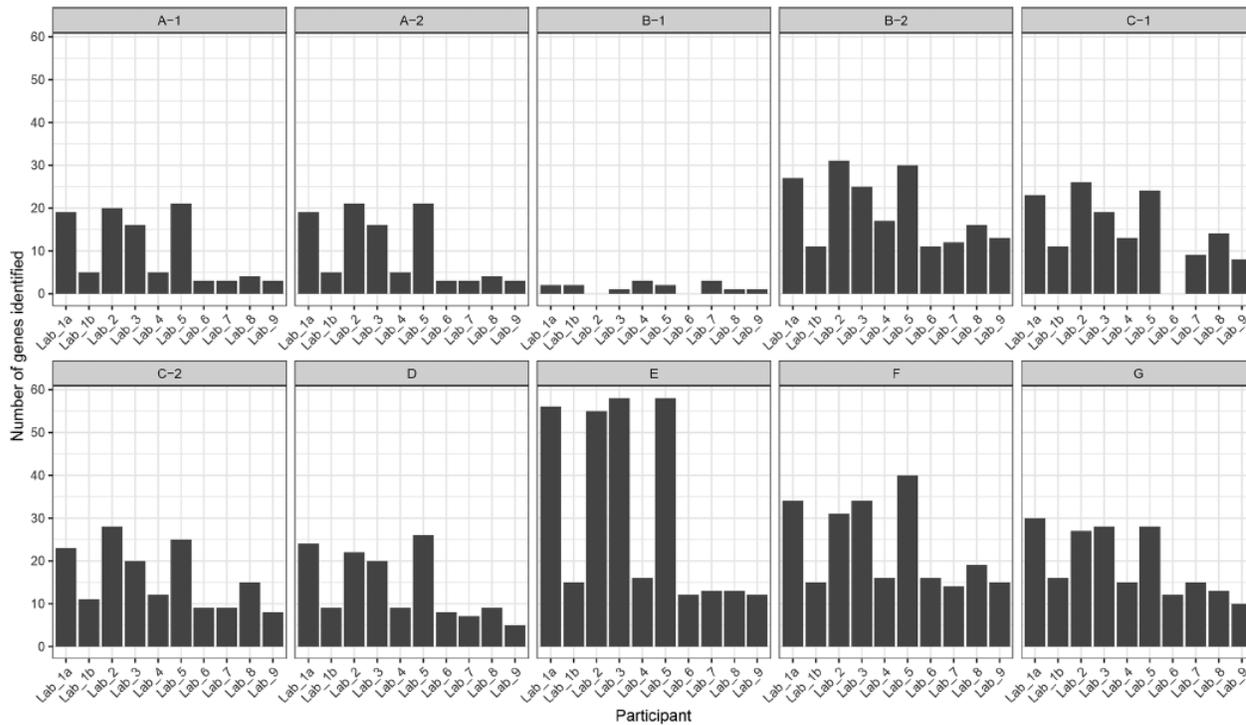
CARD*Shark: automated prioritization of literature curation for the Comprehensive Antibiotic Resistance Database

Arman Edalatmand^{1,2,3} and Andrew G. McArthur^{id 1,2,3,*}

- 'Frequently' updated
- Resistance genes
- Point mutations (species specific)

Database selection matters

Inter-laboratory study (n=9)
(CARD, ResFinder, ARG-ANNOT)



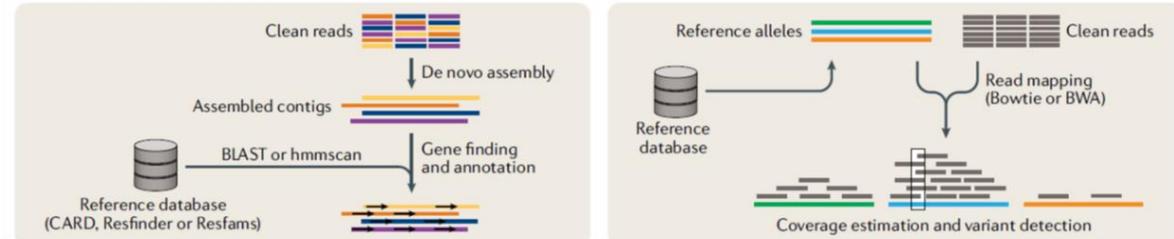
➤ No. of AMR genes detected affected by the choice of database

➤ Different variants of same gene detected

Bioinformatical approach

Assembly-based or read-based approach

b Assembly-based versus read-based approach



Assembly-based approach:

- Computationally expensive and time consuming, particularly in resistome profiling of large complex communities
- Identification of both known and novel resistance genes that share low similarity with reference database; however, requires high genome coverage
- Genomic context such as regulatory and mobile element sequences can be captured

Read-based approach:

- Fast and less computationally demanding, enabling resistome analysis of large data sets
- Identification of resistance genes is dependent on completeness of the reference database of organisms under analysis
- Nearby genes and genomic context are missing; may lead to false positives due to spurious mapping

Boolchandani M, et al. *Nat. Rev. Genet.* 2019

ResFinder

Version

4.7.2

Select input type

FASTA (Assembled Genome/Contigs) ✓

FASTA (Assembled Genome/Contigs)

FASTQ (Non-nanopore Reads)

FASTQ (Nanopore Reads)



RGI main

Use the online Resistance Gene Identifier to predict resistome(s) from **protein, genome, or genome assembly data** based on homology and SNP models. Sequence data must be in FASTA format.

Full documentation and an alternate command line version of RGI are available at <https://github.com/arpCARD/rgi>.

RGI bwt

Use the command line version of the Resistance Gene Identifier to predict resistome(s) from **short sequencing reads** based on reference sequences in CARD and read mapping algorithms (default: *KMA*). Sequence data must be in FASTQ format.

Full documentation and the command line version of RGI are available at <https://github.com/arpCARD/rgi>.

NCBI Antimicrobial Resistance Gene Finder Plus (AMRFinderPlus)

assembled nucleotide sequences

Interpretation – rules based approach

Genotype X -> Phenotype Y

ResFinder

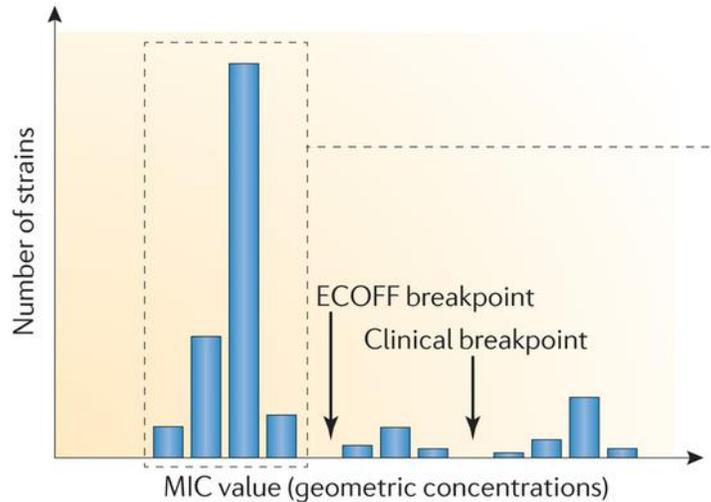
Phenotypes Hide

escherichia coli All

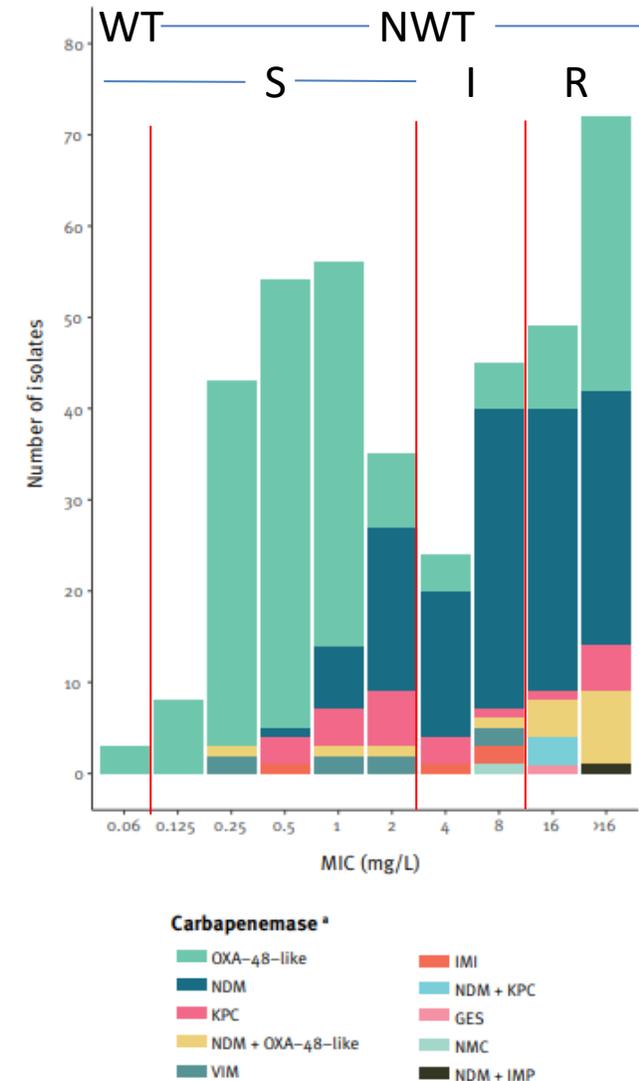
Antimicrobial	Class	WGS-predicted phenotype	Genetic background
gentamicin	aminoglycoside	Resistant	aac(3)-IIId;1;EU022314
tobramycin	aminoglycoside	Resistant	aac(3)-IIId;1;EU022314
amikacin	aminoglycoside	No resistance	
ciprofloxacin	quinolone	Resistant	
nalidixic acid	quinolone	Resistant	
ampicillin	beta-lactam	Resistant	blaTEM-1B;1;AY458016
ampicillin+clavulanic acid	beta-lactam	Resistant	blaOXA-48;1;AY236073
cefepime	beta-lactam	No resistance	
cefotaxime	beta-lactam	No resistance	
cefoxitin	beta-lactam	No resistance	
ceftazidime	beta-lactam	No resistance	
ertapenem	beta-lactam	No resistance	
imipenem	beta-lactam	Resistant	blaOXA-48;1;AY236073
meropenem	beta-lactam	Resistant	blaOXA-48;1;AY236073
piperacillin+tazobactam	beta-lactam	Resistant	blaOXA-48;1;AY236073
temocillin	beta-lactam	No resistance	
sulfamethoxazole	folate pathway antagonist	Resistant	sul1;2;U12338
trimethoprim	folate pathway antagonist	Resistant	dfrA17;1;FJ460238
fosfomicin	fosfomicin	No resistance	
azithromycin	macrolide	Resistant	mph(A);1;D16251
tetracycline	tetracycline	Resistant	tet(D);1;AF467077
tigecycline	tetracycline	No resistance	
chloramphenicol	amphenicol	Resistant	catA1;1;V00622
colistin	polymyxin	No resistance	

- AMRFinderPlus/CARD – assign resistance genes/mutations to specific classes/antibiotics

Interpretation of genotype - phenotypic comparator?



- **Clinical breakpoints – S-I-R categories?**
 - Not static
 - Presence of resistance mechanisms do not always confer clinical resistance
- **Wild type (WT) vs. non-wild type (NWT)?**
 - Genes associated with resistance are not always expressed
 - Intrinsic/'silent' resistance genes



Develop interpretative standards for AMR genotypes

Standardized AMR genotype report

Gene symbol	Class	Subclass
blaSHV-11	BETA-LACTAM	BETA-LACTAM
fosA	FOSFOMYCIN	FOSFOMYCIN
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN

(example NCBI AMRfinderplus output)

Interpreted genotype report

Context	Drug	Interpretation
core	penicillins	wt R
core	fosfomycin	wt S
core	ciprofloxacin	wt S
core	ciprofloxacin	wt S
acquired	ceftriaxone	nwt R



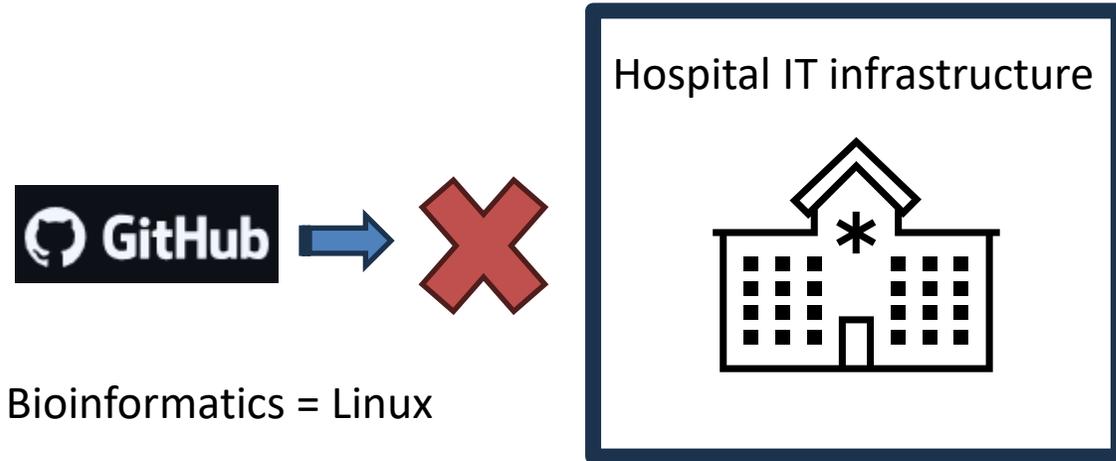
- Expert curation
- Matched genome/phenotype data
- Link to phenotype rules

organism	gene	refseq	ARO	mut.	context	drug	drug class	category	PMID	rule curation note	breakpoint	breakpoint_standard
s_Klebsiella pneumoniae	blaSHV	NF000285.3	ARO:3000.015	-	core	-	penicillins	wt R	32284385	Specific alleles can be ESBL, these are mostly mobile	not applicable	Expected resistant phenotypes v1.2 (13 January, 2023)
s_Klebsiella pneumoniae	oqxA	NF000272.1	ARO:3003.922	-	core	--	fluoroquinolones	wt S	30834112	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promoter	MIC <=0.25 mg/L	EUCAST v14.0 (2024)
s_Klebsiella pneumoniae	oqxB	NF000037.1	ARO:3003.923	-	core	-	fluoroquinolones	wt S	30834112	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promoter	MIC <=0.25 mg/L	EUCAST v14.0 (2024)
s_Klebsiella pneumoniae	fosA5_fam	NF040540.1	-	-	core	fosfomycin	-	wt S	33128341	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promoter (fosA5_family is the parent node for K. pneumoniae chromosomal fosA)	MIC >128 mg/L	ECOFF (January 2024)
s_Klebsiella pneumoniae	fosA5	WP_012579083.1	ARO:3003.209	-	core	fosfomycin	-	wt S	25441705	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promoter	MIC >128 mg/L	ECOFF (January 2024)
s_Klebsiella pneumoniae	fosA6	WP_089174570.1	ARO:3004.111	-	core	fosfomycin	-	wt S	27261267	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promoter	MIC >128 mg/L	ECOFF (January 2024)
s_Klebsiella pneumoniae	fosA10	WP_004214174.1	-	-	core	fosfomycin	-	wt S	32431524	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promoter	MIC >128 mg/L	ECOFF (January 2024)

Example - *Klebsiella pneumoniae*, covering core genes and wildtype phenotypes

Implementation of WGS – bioinformatical challenges

- Bioinformatical competence and infrastructure



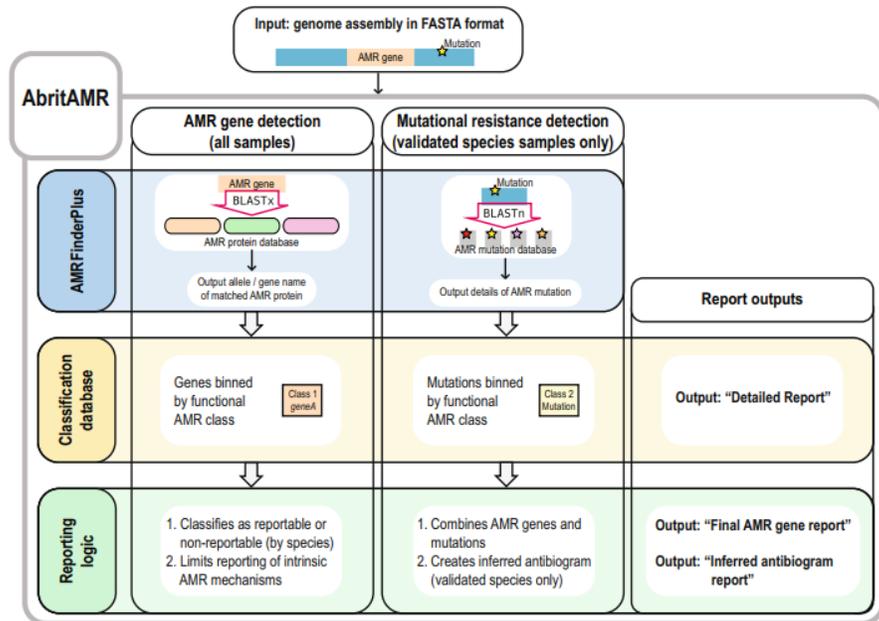
- Bioinformatics = Linux

Alternative – commercial solutions?

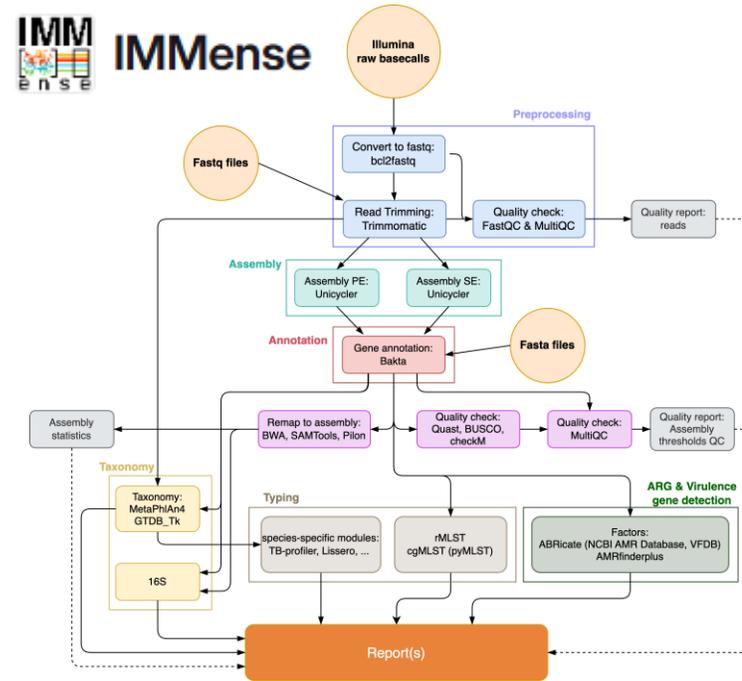
- Bioinformatical approach?
- AMR database? Updated?
- Interpretive rules?

Implementation of WGS – ISO/IVDR

- ISO accredited workflows



Sherry NL et al. Nat. Commun. 2023



IVDR???

<https://gitlab.uzh.ch/appliedmicrobiologyresearch/immense>



Swiss Pathogen Surveillance Platform (SPSP)

Implementation of WGS – Ring trials?

- Lack of external quality assessment providers
- Examples of proficiency tests



Microbial Genetics | Research Article

Whole-genome sequencing for antimicrobial surveillance: species-specific quality thresholds and data evaluation from the network of the European Union Reference Laboratory for Antimicrobial Resistance genomic proficiency tests of 2021 and 2022

Lauge Holm Sørensen,¹ Susanne Karlsmose Pedersen,¹ Jacob Dyring Jensen,¹ Niamh Lacy-Roberts,¹ Athina Andrea,¹ Michael S. M. Brouwer,² Kees T. Veldman,² Yan Lou,² Maria Hoffmann,² Rene S. Hendriksen¹



ORIGINAL RESEARCH
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NGS-Based *S. aureus* Typing and Outbreak Analysis in Clinical Microbiology Laboratories: Lessons Learned From a Swiss-Wide Proficiency Test

OPEN ACCESS

Edited by:

Paoting Lan,
University of New South Wales,
Australia

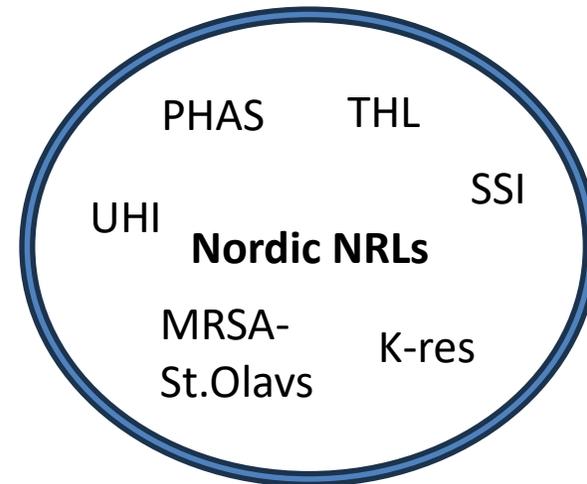
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Where are we now?

2017

Clinical Microbiology and Infection 23 (2017) 2–22



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Contents lists available at ScienceDirect

Clinical Microbiology and Infection

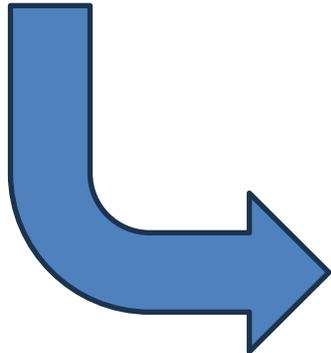
journal homepage: www.clinicalmicrobiologyandinfection.com



Review

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

M.J. Ellington^{1,†}, O. Ekelund^{2,†}, F.M. Aarestrup³, R. Canton⁴, M. Doumith¹, C. Giske⁵, H. Grundman⁶, H. Hasman⁷, M.T.G. Holden⁸, K.L. Hopkins¹, J. Iredell⁹, G. Kahlmeter², C.U. Köser¹⁰, A. MacGowan¹¹, D. Mevius^{12,13}, M. Mulvey¹⁴, T. Naas¹⁵, T. Peto¹⁶, J.-M. Rolain¹⁷, Ø. Samuelsen¹⁸, N. Woodford^{1,*}



2024-2025



The EUCAST Subcommittee on WGS and Phenotypic AST

Review the literature and progress since 2017

Work ongoing

Antonio Oliver (Spain), Chair	Katy Jeannot (France).
Ørjan Samuelsen (Norway), Co-chair	Gunnar Kahlmeter (Sweden).
Carla Lopez-Causape (Spain), Scientific secretary	Claudio Köser (United Kingdom).
Valeria Bortolaia (Denmark).	Amy Mathers (United States).
Mike Brouwer (Netherlands).	Frank Møller Aarestrup (Denmark).
Rafael Cantón (Spain).	Thierry Naas (France).
Adrian Egli (Switzerland).	Spyros Pournaras (Greece).
Christian Giske (Sweden).	Etienne Ruppé (France).
Yonatan Grad (United States).	Thomas Schön (Sweden).
Axel Hamprecht (Germany).	Nicole Stoesser (United Kingdom).
Susanne Haussler (Germany).	John Turnidge (Australia).
Kat Holt (United Kingdom).	Guido Werner (Germany).
Katie Hopkins (United Kingdom).	Gerry Wright (Canada).
Ben Howden (Australia)	

Changes from 2017

- Evidence reports:
 - *Enterobacterales* } *Enterobacterales*
 - *Salmonella* spp. }
 - *Acinetobacter baumannii* and *Pseudomonas aeruginosa* -> evaluated separately
 - Additional organisms added: enterococci, *Haemophilus influenzae*, *Bacteroides fragilis*
- Real world experience – ISO accreditation, IVDR, ring trials, reporting etc.
- Metagenomic developments
- The role of AI and ML -> Adrian Egli (tomorrow @ 10:40 😊)
- Role of other OMICs

Change in terminology:



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→ WGS for antimicrobial susceptibility **testing** (AST)

- WGS is not a antimicrobial susceptibility testing method – it is a genotyping method

→ WGS is a tool that can be used for antimicrobial susceptibility **prediction**

Recent advances - *Enterobacterales*

- Our understanding of genotype-phenotype associations greatly increased:
 - More sequencing
 - Advancement of long-read sequencing
 - Expansion of reference AMR gene databases
 - Species specific tools developed



K. pneumoniae species complex

<https://kleborate.readthedocs.io/en/latest/index.html#>

Lam MMC et al. Nat. Commun. 2021

Genotyping Salmonella Typhi

This repository houses the GenoTyphi genotyping scheme for Salmonella Typhi.

<https://github.com/typhoidgenomics/genotyphi>

Ingle DJ et al. bioRxiv. 2024

Tools not only for AMR – typing, MLST, virulence, plasmid marker, etc.

Expands knowledge and species specific interpretation

Example – The SHV conundrum

- *K. pneumoniae*:
 - Chromosomal copy of *bla*_{SHV} -> ampicillin resistance
 - Mutations in chromosomal *bla*_{SHV} -> ESBL or BLI
 - Plasmid-borne *bla*_{SHV} -> WT, ESBL, BLI

Interpretation of SHV-variants?

MICROBIAL GENOMICS

RESEARCH ARTICLE
Tsang et al., *Microbial Genomics* 2024;10:001294
DOI 10.1099/mgen.0.001294



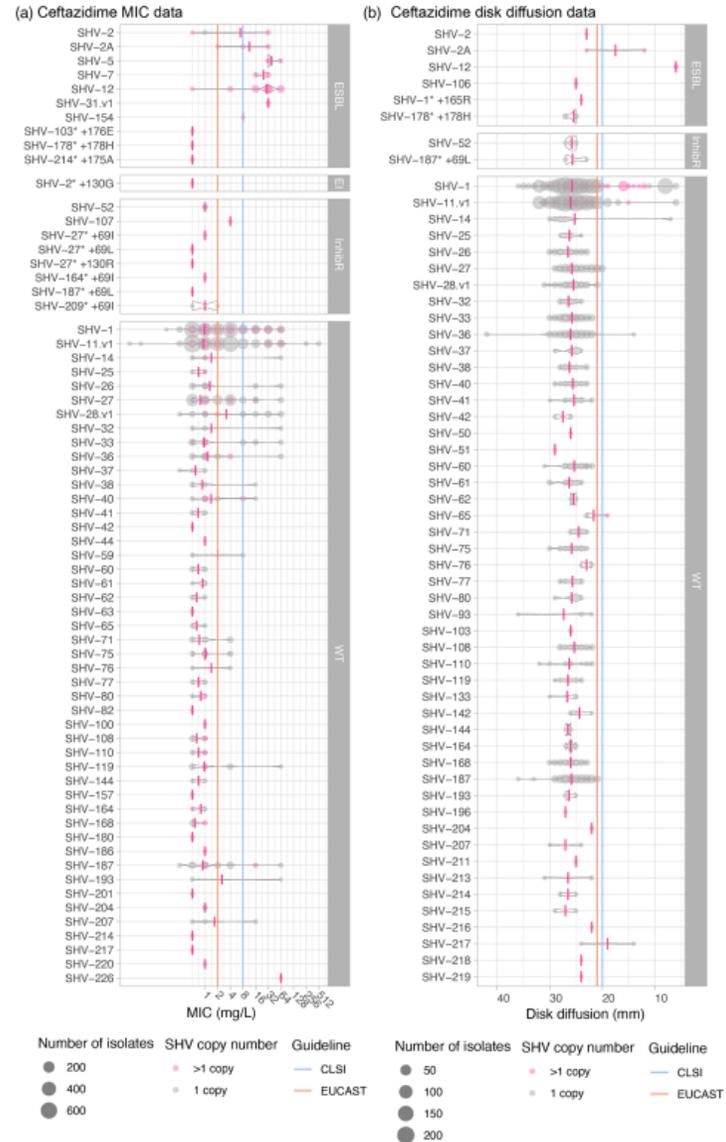
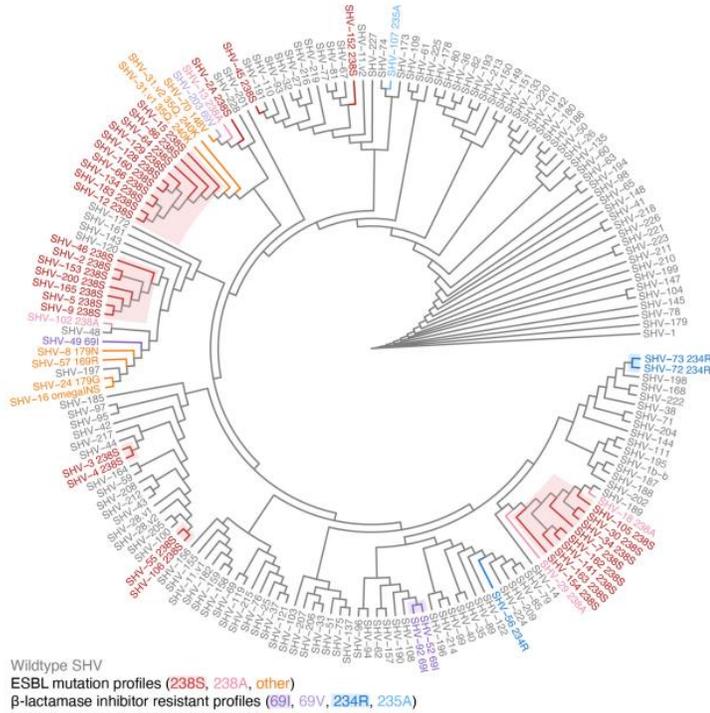
Diversity, functional classification and genotyping of SHV β -lactamases in *Klebsiella pneumoniae*

Kara K. Tsang¹, Margaret M. C. Lam², Ryan R. Wick^{2,3}, Kelly L. Wyres², Michael Bachman⁴, Stephen Baker⁵, Katherine Barry⁶, Sylvain Brisse⁷, Susana Campino¹, Alexandra Chiaverini⁸, Daniela Maria Cirillo⁹, Taane Clark¹, Jukka Corander¹⁰, Marta Corbella¹¹, Alessandra Cornacchia⁸, Aline Cuénod¹², Nicola D'Alterio⁸, Federico Di Marco⁹, Pilar Donado-Godoy¹³, Adrian Egli¹², Refath Farzana¹⁴, Edward J. Feil¹⁵, Aasmund Fostervold¹⁴, Claire L. Gorrie³, Brekhna Hassan¹⁷, Marit Andrea Klokhammer Hetland¹⁶, Le Nguyen Minh Hoa¹⁸, Le Thi Hoi¹⁹, Benjamin Howden³, Odion O. Ikimiukor²⁰, Adam W. J. Jenney³, Håkon Kaspersen²¹, Fahad Khokhar⁵, Thongpan Leangapichart²¹, Małgorzata Ligowska-Marzeta²², Iren Høyland Løhr¹⁶, Scott W. Long²³, Amy J. Mathers⁴, Andrew G. McArthur²⁴, Geetha Nagaraj²⁵, Anderson O. Oaikhena²⁰, Iruka N. Okeke²⁰, João Perdigão²⁶, Hardik Parikh⁶, My H. Pham²⁷, Francesco Pomilio⁸, Niclas Raffelsberger²⁸, Andrianiaina Rakotondrasoa²⁹, K. L. Ravi Kumar²⁵, Leah W. Roberts³⁰, Carla Rodrigues⁷, Ørjan Samuelsen^{31,32}, Kirsty Sands¹⁴, Davide Sasseria^{11,33}, Helena Seth-Smith¹², Varun Shamanna²⁵, Norelle L. Sherry³, Sonia Sia³⁴, Anton Spadar¹, Nicole Stoesser³⁵, Marianne Sundt²¹, Arnfinn Sundsfjord^{31,36}, Pham Ngoc Thach¹⁸, Nicholas R. Thomson²⁷, Harry A. Thorpe¹⁰, M. Estée Torok⁵, Van Dinh Trang¹⁸, Nguyen Vu Trung¹⁹, Jay Vornhagen²⁷, Timothy Walsh¹⁴, Ben Warne⁵, Hayley Wilson³⁸, Gerard D. Wright²⁴, Kathryn E. Holt^{1,2,*} and KlebNET-GSP AMR Genotype-Phenotype Group

- 3999 isolates with ≥ 1 SHV allele but **no other β -lactamase**
- Geographically diverse - 24 countries, 2001-2021
- Human, animal, environmental isolates
- Broth microdilution, Disc diffusion, Agar dilution, Vitek 2, Phoenix

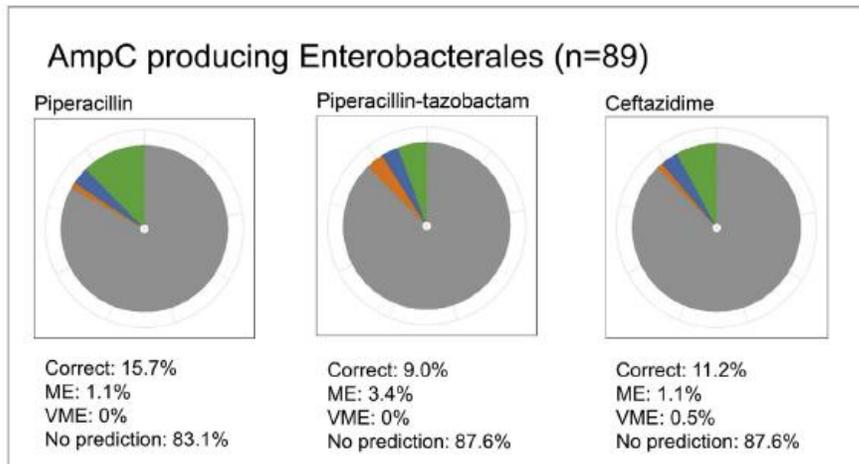
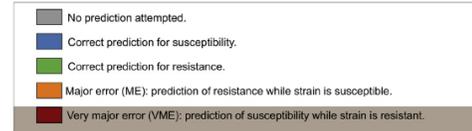
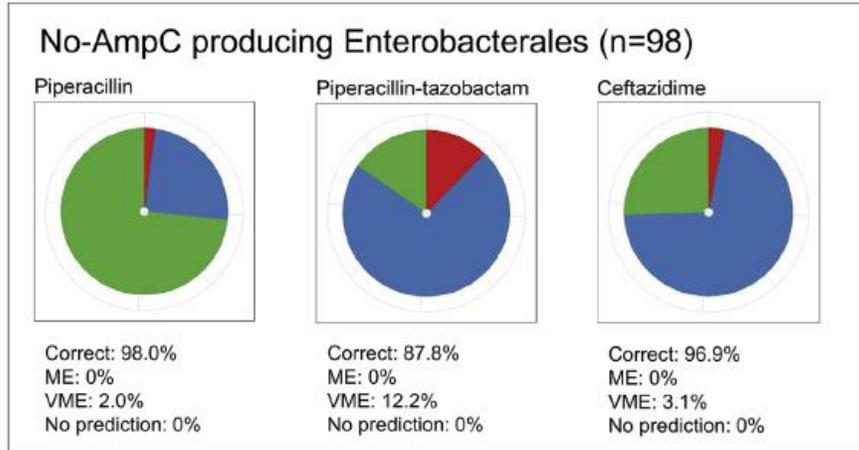
SHV – *K. pneumoniae*

181 distinct *bla*_{SHV} alleles



➤ Re-classification of multiple SHV-alleles

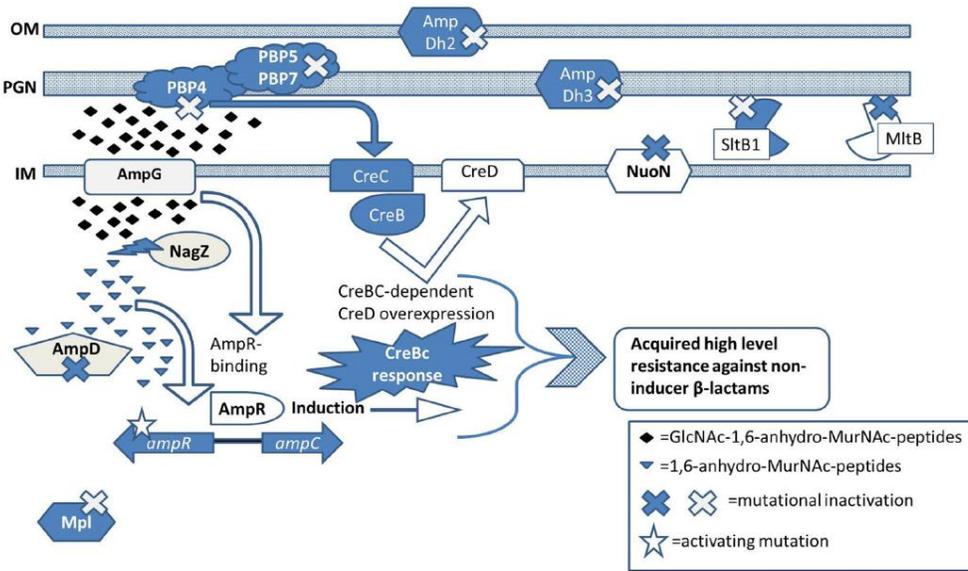
Impact of the intrinsic resistome: AmpC+ vs. AmpC- *Enterobacterales*



- AmpC-negative:
 - 2.0% VME piperacillin
 - 12.2% VME piperacillin-tazobactam
 - 3.1% VME ceftazidime
- AmpC-producing:
 - Prediction not attempted in 83.1-87.6% of AmpC-positive isolates!
- «Prediction of the overexpression of AmpC..require more data»
- Only a minority of AmpC-producing strains had *ampD* and *ampR* genes highly similar to those of the corresponding reference NCBI genome

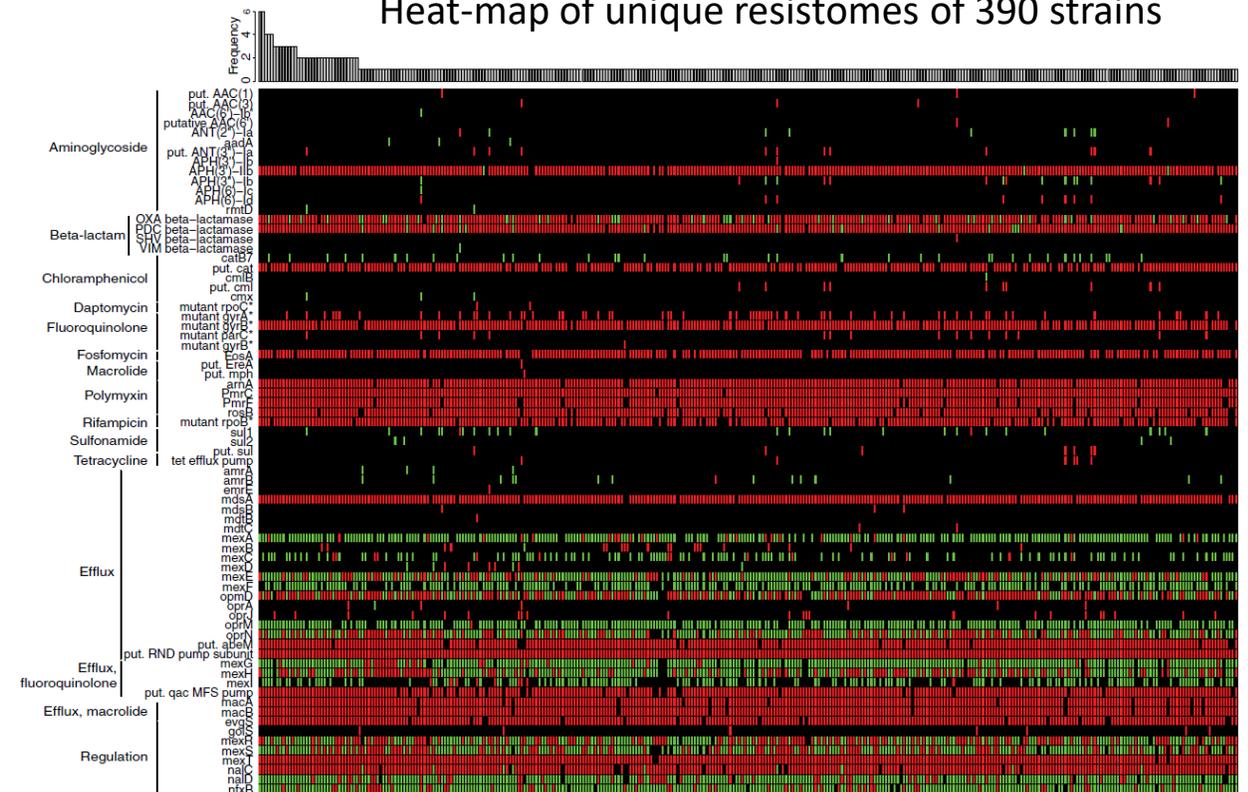
Recent advances – *P. aeruginosa*

- Challenge: large and complex repertoire of mutation-driven mechanisms



Mutational mechanisms driving to AmpC hyperproduction

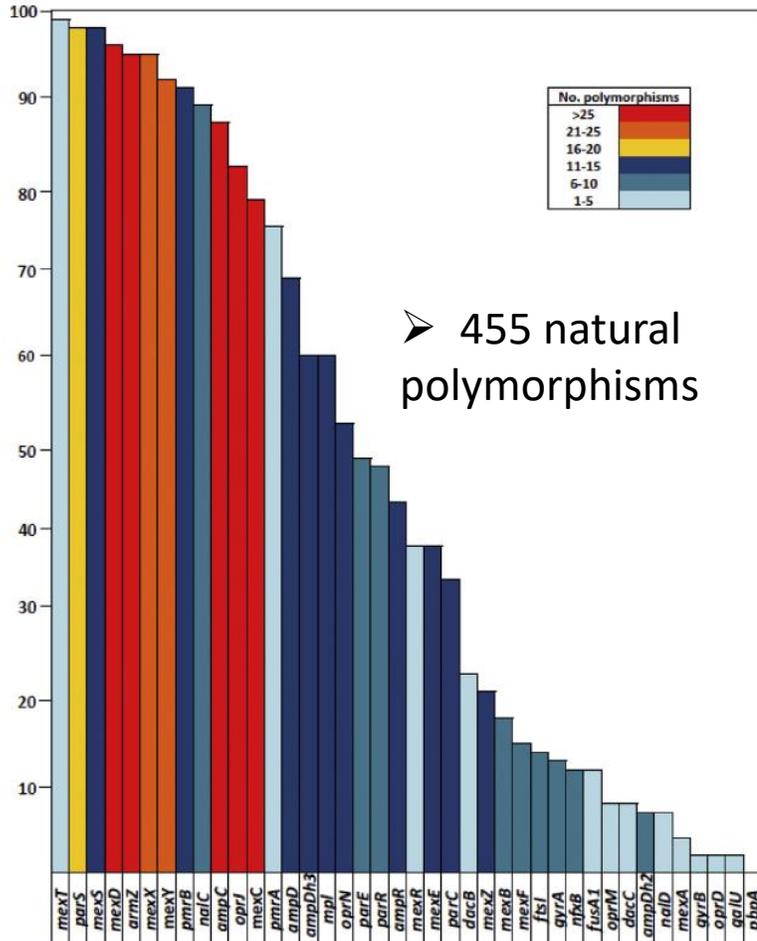
Heat-map of unique resistomes of 390 strains



Resistance mutations vs. natural polymorphisms?

Example - *P. aeruginosa*

1. Natural polymorphism in 100 WT isolates & 40 genes



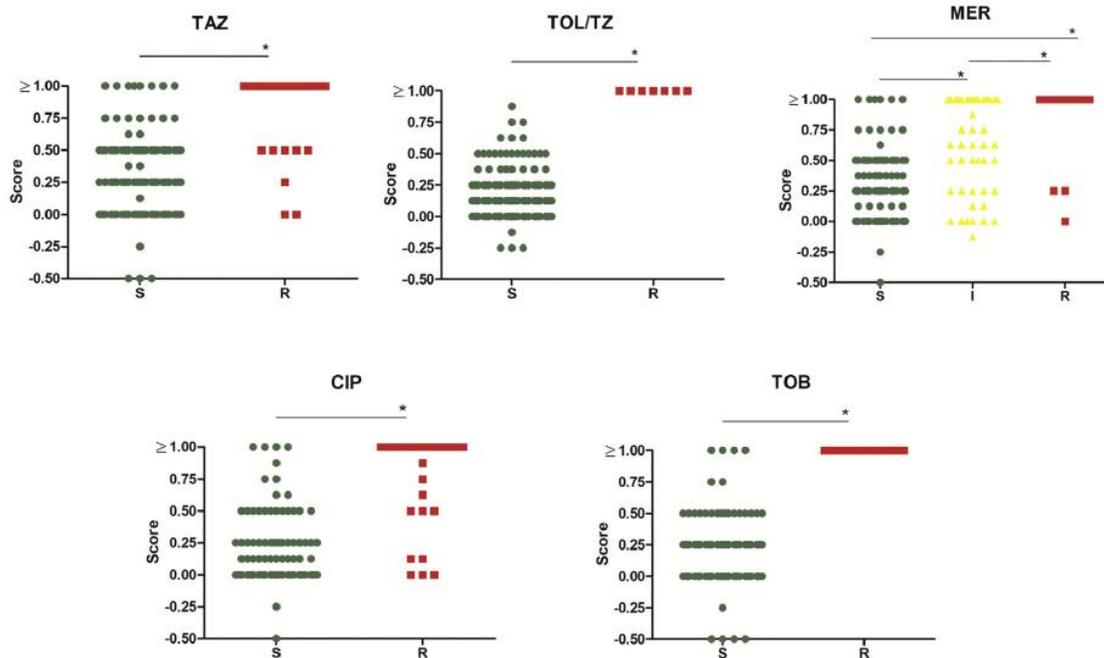
2. Genotypic 'score'

0 (no effect) -> 1 (clinical resistance)

Score <0.5 – predict phenotypic susceptibility

LOCUS GENE	Resistance mechanism/altered target	Antibiotics	Type of mutation	Score values	Notes
PA0807 ampDh3	AmpC overexpression	CAZ	Loss of function	0.25-0.125	Frameshift, stop-codon mutations and gene absence score the maximum value
PA0958 oprD	OprD inactivation	MER	Loss of function	0.5-0.25	Frameshift, stop-codon mutations and gene absence score the maximum value
PA1798 parS	MexXY overexpression	TOB	Gain of function	0.5-0.25	Mutations L14Q, V101M, L137P, A138T, A168V, Q232E and G361R score the max value
PA1798 parS	OprD downregulation and MexXY overexpression	MER	Gain of function	0.5-0.25	Mutations L14Q, V101M, L137P, A138T, A168V, Q232E and G361R score the max value
PA1798 parS	MexXY and MexEF-OprN overexpression	CIP	Gain of function	0.25-0.125	Mutations L14Q, V101M, L137P, A138T, A168V, Q232E and G361R score the max value
PA1799 parR	MexXY overexpression	TOB	Gain of function	0.5-0.25	Mutations M59I and E156K score the max value
PA1799 parR	OprD downregulation and MexXY overexpression	MER	Gain of function	0.5-0.25	Mutations M59I and E156K score the max value
PA1799 parR	MexXY and MexEF-OprN overexpression	CIP	Gain of function	0.25-0.125	Mutations M59I and E156K score the max value

Example - *P. aeruginosa*



Meropenem:

- Genotypic S <0.5: 2.4% phenotypic R
- Genotypic R ≥ 1 : 15.4% phenotypic S
- Not possible to predict 'I' category

Tobramycin:

- Genotypic S <0.5: all phenotypic S
- Genotypic R ≥ 1 : 12.9% phenotypic S

Table 2
Sensitivity of the resistance genotype score to predict phenotypic susceptibility and resistance in the 204 clinical isolates studied

Antibiotic	% (n) S/I/R score <0.5	% (n) S/I/R score 0.5 - <1	% (n) S/I/R score ≥ 1
TAZ	97.7 (129/132)/2.3 (3/132)	88.1 (37/42)/11.9 (5/42)	27.6 (8/29)/72.4 (21/29)
TOL/TZ	100 (177/177)/0 (0/177)	100 (19/19)/0 (0/19)	0 (0/7)/100 (7/7)
MER	86.5 (108/125)/11.1 (14/125)/2.4 (3/125)	60.5 (23/38)/39.5 (15/38)/0 (0/38)	15.4 (6/39)/38.5 (15/39)/46.1 (18/39)
CIP	95.5 (107/112)/4.5 (5/112)	73.9 (17/23)/26.1 (6/23)	5.9 (4/68)/94.1 (64/68)
TOB	100 (153/153)/0 (0/153)	100 (19/19)/0 (0/19)	12.9 (4/31)/87.1 (27/31)

TAZ, ceftazidime; TOL/TZ, ceftolozane/tazobactam; MER, meropenem; CIP, ciprofloxacin; TOB, tobramycin.

Recent advances - Enterococci

Antibiotic resistance determination using *Enterococcus faecium* whole-genome sequences: a diagnostic accuracy study using genotypic and phenotypic data

Francesc Coll, Theodore Goulouris, Beth Blane, Corin A Yeats, Kathy E Raven, Catherine Ludden, Fahad A Khokhar, Hayley J Wilson, Leah W Roberts, Ewan M Harrison, Carolyne S Horner, Le Thi Hoi, Nguyen Thi Hoa, Nguyen Vu Trung, Nicholas M Brown, Mark A Holmes, Julian Parkhill, Mili Estee Török, Sharon J Peacock



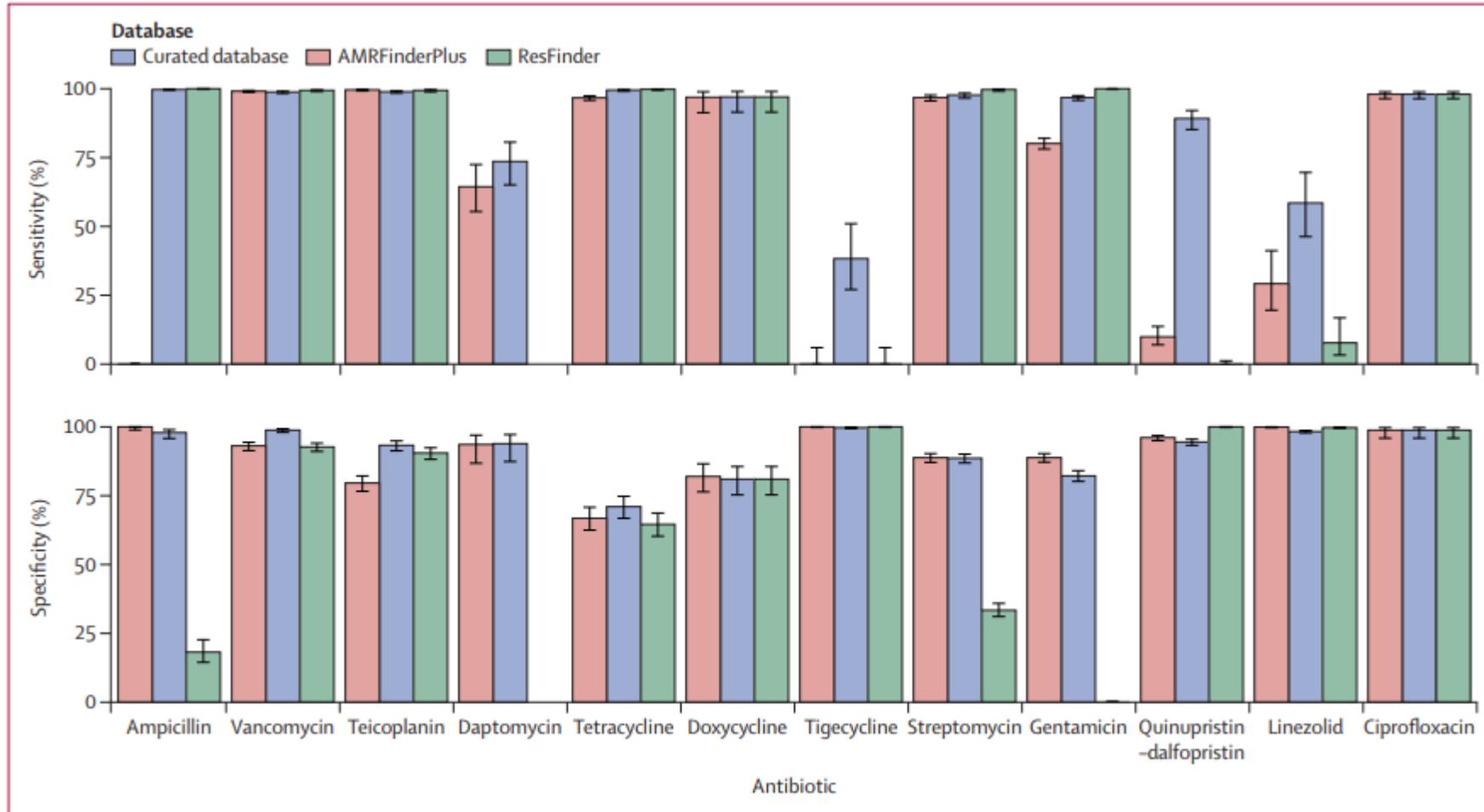
- 4382 *E. faecium* isolates
- 11 countries 2000-2018
- 12 antibiotics – broth microdilution, Vitek 2, disc diffusion
- Curated database – 228 genetic markers

	Resistant isolates	Susceptible isolates	True positive	False negative	True negative	False positive	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Ampicillin (n=3132)	2792 (89.1%)	340 (10.9%)	2785	7	333	7	99.7% (99.5–99.9)	97.9% (95.8–99.0)	99.7% (99.5–99.9)	97.9% (95.8–99.0)
Vancomycin (n=3398)	2309 (68.0%)	1089 (32.0%)	2281	28	1076	13	98.8% (98.3–99.2)	98.8% (98.0–99.3)	99.4% (99.0–99.7)	97.5% (96.4–98.2)
Teicoplanin (n=2760)	1982 (71.8%)	778 (28.2%)	1961	21	726	52	98.9% (98.4–99.3)	93.3% (91.3–94.9)	97.4% (96.6–98.0)	97.2% (95.7–98.2)
Daptomycin (n=220)	121 (55.0%)	99 (45.0%)	89	32	93	6	73.6% (65.1–80.6)	93.9% (87.4–97.2)	93.7% (86.9–97.1)	74.4% (66.1–81.2)
Ciprofloxacin (n=673)	501 (74.4%)	172 (25.6%)	491	10	170	2	98.0% (96.4–98.9)	98.8% (95.9–99.7)	99.6% (98.5–99.9)	94.4% (90.1–97.0)
Tetracycline (n=2358)	1869 (79.3%)	489 (20.7%)	1860	9	347	142	99.5% (99.1–99.7)	71.0% (66.8–74.8)	92.9% (91.7–94.0)	97.5% (95.3–98.7)
Doxycycline (n=320)	99 (30.9%)	221 (69.1%)	96	3	179	42	97.0% (91.5–99.0)	81.0% (75.3–85.6)	69.6% (61.4–76.6)	98.4% (95.3–99.4)
Tigecycline (n=2412)	60 (2.5%)	2352 (97.5%)	23	37	2346	6	38.3% (27.1–51.0)	99.7% (99.4–99.9)	79.3% (61.6–90.2)	98.4% (97.9–98.9)
Streptomycin* (n=2635)	1152 (43.7%)	1483 (56.3%)	1125	27	1314	169	97.7% (96.6–98.4)	88.6% (86.9–90.1)	86.9% (85.0–88.7)	98.0% (97.1–98.6)
Gentamicin* (n=3125)	1610 (51.5%)	1515 (48.5%)	1558	52	1246	269	96.8% (95.8–97.5)	82.2% (80.2–84.1)	85.3% (83.6–86.8)	96.0% (94.8–96.9)
Quinupristin–dalfopristin (n=1864)	314 (16.8%)	1550 (83.2%)	280	34	1465	85	89.2% (85.2–92.1)	94.5% (93.3–95.5)	76.7% (72.1–80.8)	97.7% (96.8–98.4)
Linezolid (n=2957)	65 (2.2%)	2892 (97.8%)	38	27	2843	49	58.5% (46.3–69.6)	98.3% (97.8–98.7)	43.7% (33.7–54.1)	99.1% (98.6–99.4)
Re-test†	38 (1.3%)	2919 (98.7%)	38	0	2870	49	100.0% (90.8–100.0)	98.3% (97.8–98.7)	43.7% (33.7–54.1)	100.0% (99.9–100.0)

Data are n (%) or n, unless otherwise specified. Metrics of diagnostic performance when using the curated database to predict phenotypic antibiotic resistance. Here the intermediate susceptibility category is ignored—ie, isolates with intermediate susceptibility are treated as not tested. See appendix 2 (p 19) for the diagnostic performance achieved when predicting phenotypic non-susceptibility—ie, when treating the intermediate and resistance categories as non-susceptible. See appendix 2 (pp 17–18) for minimum inhibitory concentration and zone diameter breakpoints used in this study. A true positive was defined as a phenotypically resistant isolate carrying an antimicrobial resistance genetic determinant; a false negative was defined as a phenotypically resistant isolate not carrying any antimicrobial resistance genetic determinant; a true negative was defined as a phenotypically susceptible isolate not carrying any antimicrobial resistance genetic determinant; and a false positive was defined as a phenotypically susceptible isolate carrying an antimicrobial resistance genetic determinant. PPV=positive predictive value. NPV=negative predictive value. *High-level resistance to aminoglycoside. †Accuracy of genotypic predictions for linezolid after re-testing 27 false negatives that were susceptible according to E-test (bioMérieux, Marcy l’Étoile, France).

Table 3: Accuracy of genotypic predictions of phenotypic resistance of *Enterococcus faecium* to 12 antibiotics when using the curated database

Recent advances - Enterococci



Example species specific tool – LRE-Finder

- Linezolid resistance enterococci -> mutations in 23S rRNA
- Challenge: multiple copies of 23S rRNA:
 - *E. faecalis*: 4 copies
 - *E. faecium*: 6 copies
- Susceptibility depends on no. of mutated copies

J Antimicrob Chemother 2019; **74**: 1473–1476
doi:10.1093/jac/dkz092 Advance Access publication 12 March 2019

Journal of
Antimicrobial
Chemotherapy

LRE-Finder, a Web tool for detection of the 23S rRNA mutations and the *optrA*, *cfr*, *cfr(B)* and *poxA* genes encoding linezolid resistance in enterococci from whole-genome sequences

Henrik Hasman¹, Philip T. L. C. Clausen², Hülya Kaya¹, Frank Hansen¹, Jenny Dahl Knudsen³, Mikala Wang⁴, Barbara Juliane Holzkecht⁵, Jurgita Samulionienė⁶, Bent L. Røder⁷, Niels Frimodt-Møller³, Ole Lund² and Anette M. Hammerum^{1*}

Hasman H et al. *J. Antimicrob. Chemother.* 2019

Identifiserte mutasjoner i 23S:

Reference | Posisjon i WT ratio [%] | Mutasjon ratio [%] | Predikert fenotype

G2505A	99.6	0.0	S
G2576T	24.3	75.6	R

MIC: >256 mg/L = R

Identifiserte mutasjoner i 23S:

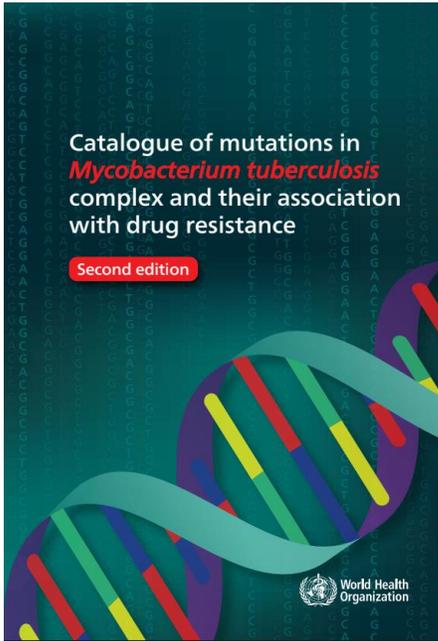
Reference | Posisjon i WT ratio [%] | Mutasjon ratio [%] | Predikert fenotype

G2505A	100.0	0.0	S
G2576T	85.2	14.8	R

MIC: 4 mg/L = S

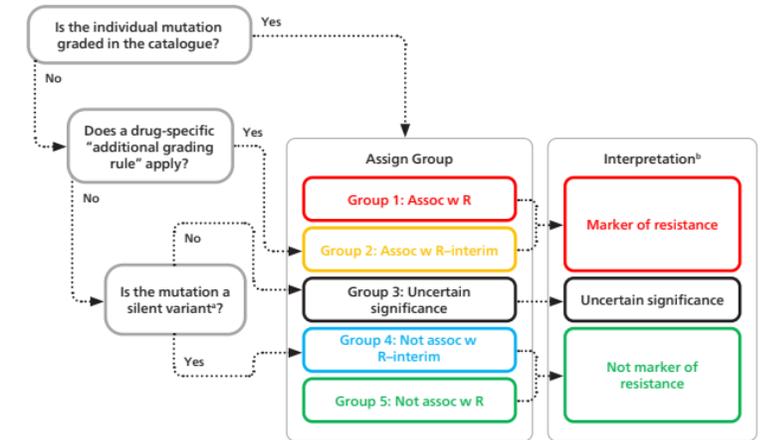
Recent advances – *M. tuberculosis*

2017: *M. tuberculosis* likely the first pathogen where WGS-generated results offers advantage over traditional AST methods



- Global standard for interpreting genotypic results
- ~52000 isolates
- High quality phenotypic data
- 48152 mutations

Fig. 1. Instructions for using the catalogue



Mutation named as described in Section 5.6

Final confidence grading of a mutation

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPVSOLO_ib	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes
RIF	rpoB_p.His445Leu	20	30512	217	16593	1.3%	99.9%	91.6%	91.4%	94.8%	85.8%	19.6	1) AwR	ALL+WHO		1) Assoc w R	
RIF	rpoB_p.Leu430Pro	185	30347	215	16595	1.3%	99.4%	53.8%	25.5%	32.0%	17.1%	0.6	3) Uncertain	ALL+WHO	Borderline	1) Assoc w R	
RIF	rpoB_p.Ser450Phe	1	30531	206	16604	1.2%	100.0%	99.5%	100.0%	100.0%	97.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R	

Drug in focus

Additional grading criteria applied when relevant to reach the Final confidence grading

Also template for targeted NGS

Directly from clinical samples? Metagenomics



Sensitivity: 65.7-85.0% for predicting staphylococcal resistance

Evaluation of the CosmosID Bioinformatics Platform for Prosthetic Joint-Associated Sonicate Fluid Shotgun Metagenomic Data Analysis

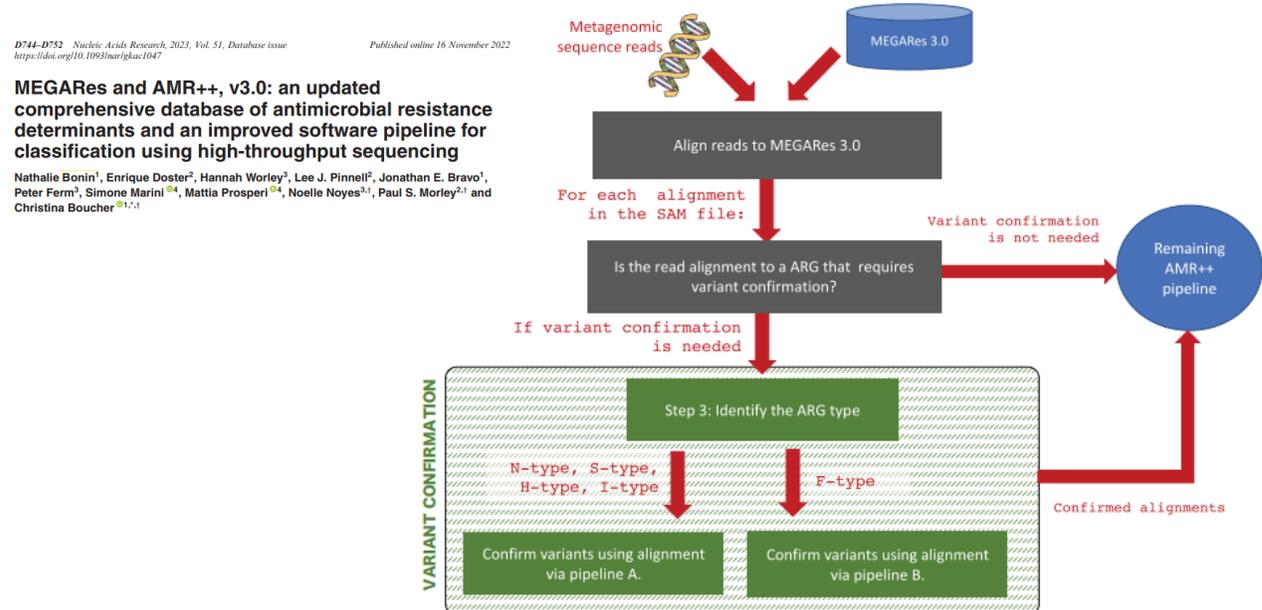
Qun Yan,^{a,b} Yu Mi Wi,^{a,c} Matthew J. Thoendel,^d Yash S. Raval,^a Kerryl E. Greenwood-Quaintance,^a Matthew P. Abdel,^e Patricio R. Jeraldo,^f Nicholas Chia,^f Robin Patel^{a,d}

Yan C et al. J. Clin. Microbiol. 2019

Challenges:

- Human reads vs. bacterial reads
- More bioinformatically challenging
- Connection to bacterial host
- Polymicrobial infections
- Commensal resistome

Customized bioinformatical approaches?



Summary



Review

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

M.J. Ellington^{1,1}, O. Ekelund^{2,1}, F.M. Aarestrup³, R. Canton⁴, M. Doumith¹, C. Giske⁵, H. Grundman⁶, H. Hasman⁷, M.T.G. Holden⁸, K.L. Hopkins¹, J. Iredell⁹, G. Kahlmeter², C.U. Köser¹⁰, A. MacGowan¹¹, D. Mevius^{12,13}, M. Mulvey¹⁴, T. Naas¹⁵, T. Peto¹⁶, J.-M. Rolain¹⁷, Ø. Samuelsen¹⁸, N. Woodford^{1,4}

2017 conclusion:

«*The published evidence for using WGS as a tool to infer antimicrobial susceptibility accurately is significantly improved, but **the evidence/knowledge base requires further expansion for WGS-AST to be used in clinical decision making***»

My thoughts (not official EUCAST subcommittee conclusions):

- The knowledge base has significantly expanded, but also the complexity of genotype-phenotype associations
- Still not there yet for clinical decision making except *M. tuberculosis* -> HIC
- Alternative to AST in surveillance for some species – *S. aureus*, *E. coli*, *K. pneumoniae*, +
- More large-scale studies required with high quality reference AST data
- Several remaining challenges:
 - **Turn-around-time and cost!**
 - Interpretive criteria – WGS ‘breakpoint’ tables? – (ESGEM-AMR, WHO catalogue)
 - Implementation – bioinformatic infrastructure, regulatory aspects (accreditation, IVDR, ++)