II Jornada del Comité Español del Antibiograma (CoEsAnt)

Madrid, 12 de febrero de 2026

Análisis del resistoma por WGS en la predicción de la sensibilidad antibiótica

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II Jornada del Comité Español del Antibiograma (CoEsAnt)

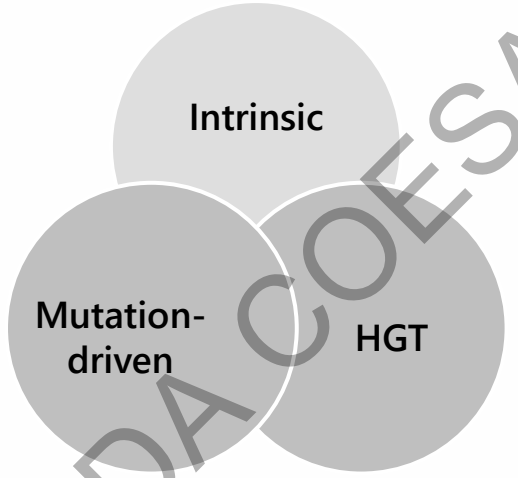
WGS Resistome analysis

Martínez JL *et al* Nat Rev Microbiol 2015

Intrinsic resistome. All intrinsic genes, common to all taxonomically related bacteria, that contribute to phenotypic resistance

Mutational resistome. all acquired point mutations within the bacteria intrinsic genes that lead to phenotypic resistance.

Antibiotic resistome



Horizontally acquired resistome (mobilome): all new resistance determinants acquired in a bacteria by horizontal gene transfer".

To study AR mechanisms

- To expand AR mechanisms knowledge
- To understand genotype-phenotype correlation

For epidemiological surveillance

- To prevent the spread of resistant pathogens

To predict SIR (WGS-ASP)

- To guide antibiotic therapies

Time constrains

WGS and susceptibility testing

2017

Clinical Microbiology and Infection 23 (2017) 2–22

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. Aarestrup [3], R. Canton [4], M. Doumith [1], C. Giske [5], H. Grundman [6], H. Hasman [7], M.T.G. Holden [8], K.L. Hopkins [1], J. Iredell [9], G. Kahlmeter [2], C.U. Köser [10], A. MacGowan [11], D. Mevius [12, 13], M. Mulvey [14], T. Naas [15], T. Peto [16], J.-M. Rolain [17], Ø. Samuelsen [18], N. Woodford [1, *]

- This EUCAST Subcomittee report **reviewed the state-of-the-art of WGS-based AST**: 200 published works, late 2015-early 2016.
- **Focus on WHO priority**: *Enterobacteriaceae*, *Salmonella spp.*, *S. aureus*, *S. pneumoniae*, *N. gonorrhoeae*, *M. tuberculosis*, *C. difficile*, *A. baumannii* and *P. aeruginosa*.

Conclusions and recommendations:

- Insufficient data to present a definitive document on the topic.
- The quality of data needs to improve and to be assured via more rigorous and 'standardized' approaches to data analysis.
- ECOFF should be the primary comparator for WGS-based prediction.
- Performance of different tools should be calibrated against a single global reference database.
- WGS-based approaches may be used to better understand and improve some areas of phenotypic AST.

S. aureus, M. tuberculosis

***Enterobacteriaceae* (including *Salmonella*)**

S. pneumoniae, N. gonorrhoeae, P. aeruginosa, A. baumannii, C. difficile

Currently under revision

EUCAST WGS-ASP SubC 2024

Antonio Oliver (Spain), Chair

Ørjan Samuelsen (Norway), Co-chair

Carla Lopez-Causape (Spain), Scientific secretary

DNA sequencing technologies

Plataforma	Secuenciador	Amplificación de la librería	Tecnología de secuenciación	Máximo de lecturas por carrera (/día*)	Longitud máxima de las lecturas	Tiempo carrera	Output máx. por carrera (/día*)	Precisión por base	Tipos de errores más frecuentes	Coste mín. Gb (/muestra)
ABI Sanger	SeqStudio	No aplica	Sanger	67K*	800 pb	30 min	192 muestras	<0,01%	-	$
	3500 series	No aplica	Sanger	3500: 138K * 3500xL: 403K*	> 850 pb	30 min	3500: 384 muestras 3500xL: 1152 muestras	<0,01%	-	$
	Refreshed 3730 series	No aplica	Sanger	3730: 1.38 M * 3730xL: 2.76 M*	900 pb	20 min	3730: 3456 muestras 3730xL: 6912 muestras	<0,01%	-	$
Illumina	MiSeq	Puente (*bridge*-PCR)	Secuenciación por síntesis	50 M	300x2 pb	4-56 h	15 Gb	0,1-1%	Sustituciones de nucleótidos	$$
	NextSeq	Puente (*bridge*-PCR)	Secuenciación por síntesis	800 M	150x2pb	11-29 h	120 Gb	0,1-1%	Sustituciones de nucleótidos	$$
	HiSeq 4000	Puente (*bridge*-PCR)	Secuenciación por síntesis	2,5 B	150x2pb	1-3,5 días	750 Gb	0,1-1%	Sustituciones de nucleótidos	$$
Ion Torrent	PGM	PCR en emulsión (emPCR)	Secuenciación por ligación	5,5 M	400 pb	2-7 h	1 Gb	1%	InDels	$$
	S5 series	PCR en emulsión (emPCR)	Secuenciación por ligación	S5: 80M S5Plus: 130M S5Prime: 130M	600 pb	3-21,5 h	25 Gb	1%	InDels	$$
PacBio	RS	No aplica	SMRT	50 K	10-15 Kb	4 h (máx.)	1 Gb	10-15%	InDels	$$$
	Sequel	No aplica	SMRT	500 K	10-15 Kb	4 h (máx.)	10 Gb	10-15%	InDels	$$$
Oxford nanopore	MiniON	No aplica	SMRT	1M (máx)	10-20 Kb	1-48 h	5 Gb	5-15%	Sustituciones e Indels	$$$

Short vs long reads for antibiotic resistance

Table 1
Advantages and disadvantages of short-read sequencing versus long-read sequencing for detection of antimicrobial resistance

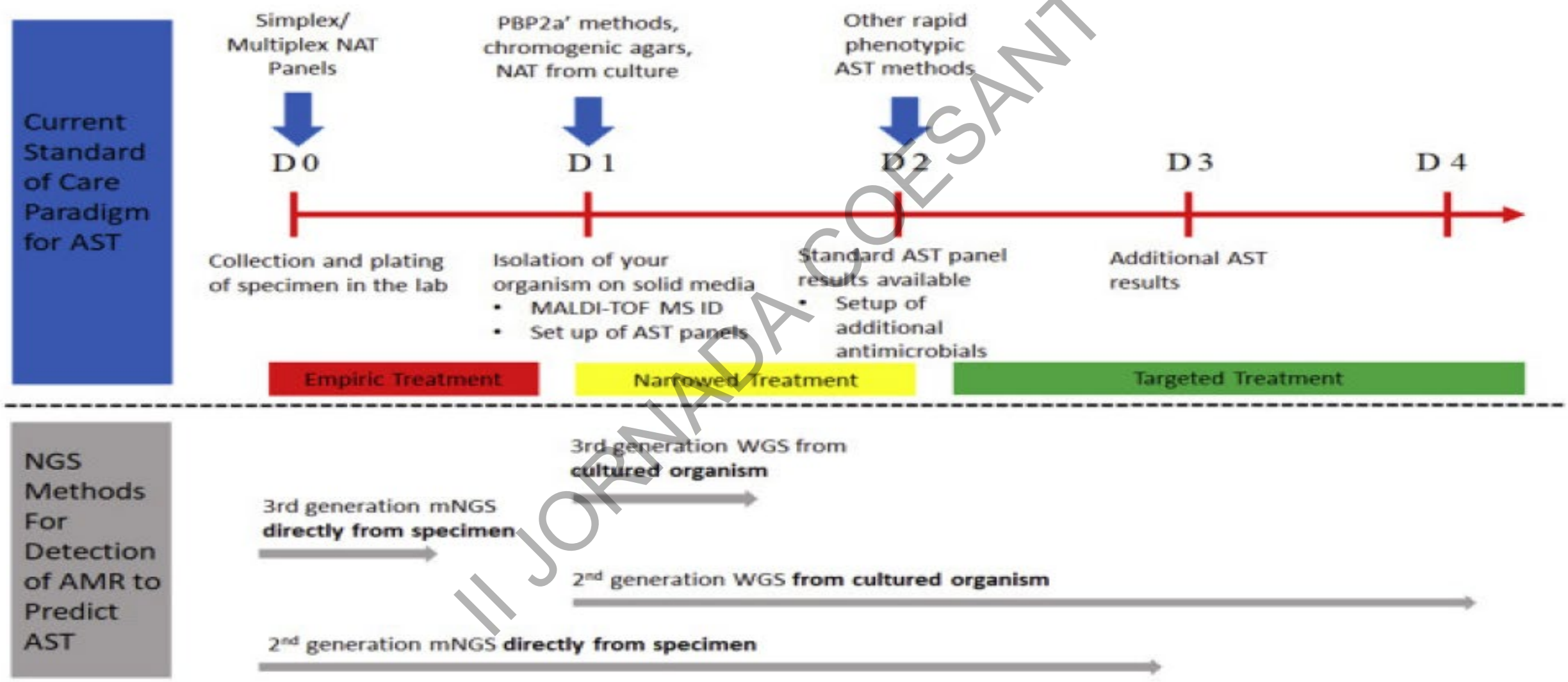
	Definition	Advantages	Disadvantages

Second-generation short-read sequencing 	NGS technologies where nucleic acid is fragmented and amplified followed by sequencing of many different clusters of short reads (50–500 bp) taking place in parallel where base detection is monitored by different methods, including pyrophosphate release (454), hydrogen release (Ion Torrent), release of fluorescent reversible-terminator nucleotides (Illumina), or fluorescent ligated probes (SOLiD)	• High accuracy (~0.1% error rate)
		  ○ Strain typing capability
		  ○ SNP level analysis
		  ○ Detection of chromosomal mutations leading to AMR, entire AMR genes, and distinction of allelic variants
		• Multiplexing capability driving lower costs
			• Long TAT
			• Whole genomes are often difficult to assemble around repetitive regions leading to fragmented assemblies
			• Unable to link AMR genes to genetic context by mNGS methods

Third-generation long-read sequencing 	NGS technologies that allow for long-read (1–100kb) single-molecular sequencing by monitoring incorporation of fluorescently labeled nucleotides (Pacific Biosciences, PacBio) or monitoring an electrical signal as nucleic acid is fed through a nano-sized pore (Oxford Nanopore Technology; ONT)	• Real-time analysis
		• Shorter TAT
		• Live streaming capability (ONT)
		• Easier to assemble WGS due to long reads
		• Plasmids are easily assembled and typed
		• AMR genes are easily detected
		• Provides genetic context directly from clinical specimens
			• Lower accuracy (3%–15% error rate)
			  ○ SNP level analysis is not possible
			  ○ Chromosomal mutations leading to AMR or allelic variants are not reliably detected
			• High concentration of input nucleic acid is required

Clin Lab Med 42 (2022) 557–572
https://doi.org/10.1016/j.cll.2022.09.011

Phenotypic AST vs WGS & TAT: impact on treatment

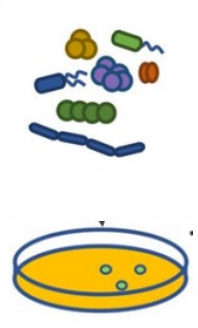


Clin Lab Med 42 (2022) 557–572
https://doi.org/10.1016/j.cll.2022.09.011

WGS process overview

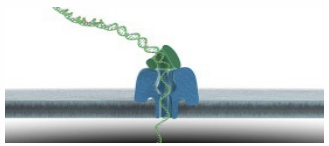
WET-LAB

1



2

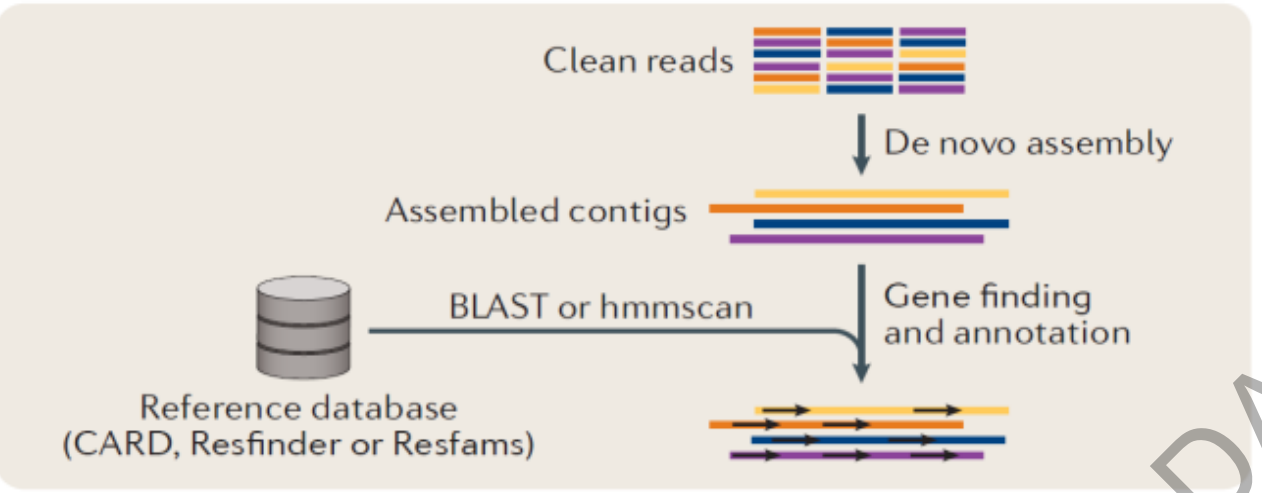
3

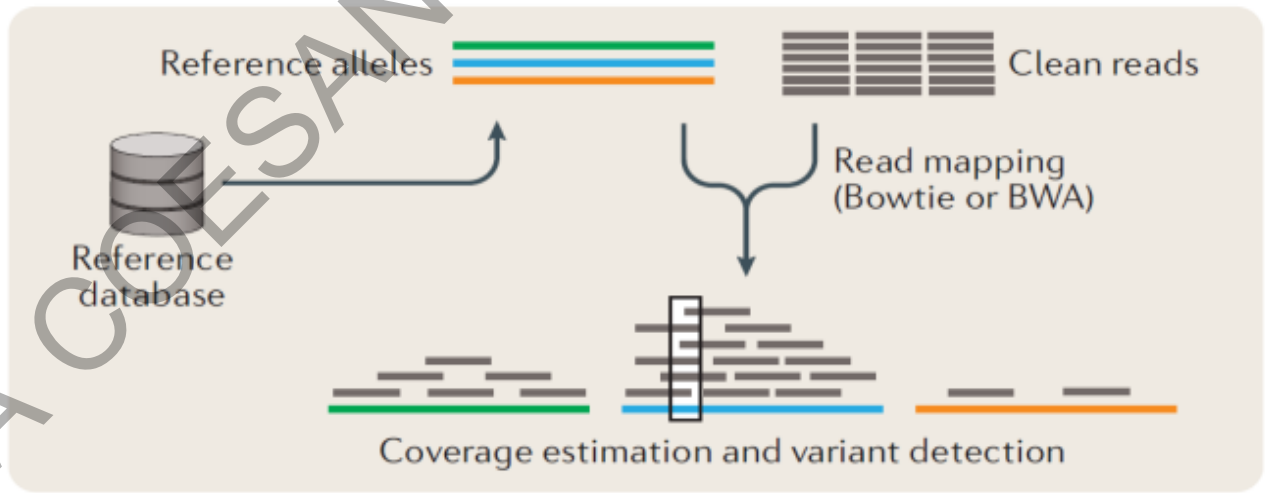


DRY-LAB

4

WGS-ASP: Assembly-based or read-based methods?





- X Computationally expensive
- X Time consuming
- X Information loss
- ✓ Identification of novel AR genes
- ✓ Up/Downstream regulatory elements

Whole Genome Sequencing

- X Inflate false-positive predictions
- X Completely dependent on databases
- X No information of up/downstream genes
- ✓ Fast and less computationally demanding
- ✓ AR genes from low abundance microorganisms

Whole Metagenome Sequencing

López-Causapé *et al* Procedimiento SEIMC 71. 2021

Relevance of data quality, analysis and interpretation

MICROBIAL GENOMICS

RESEARCH ARTICLE

Doyle *et al.*, *Microbial Genomics* 2020;6

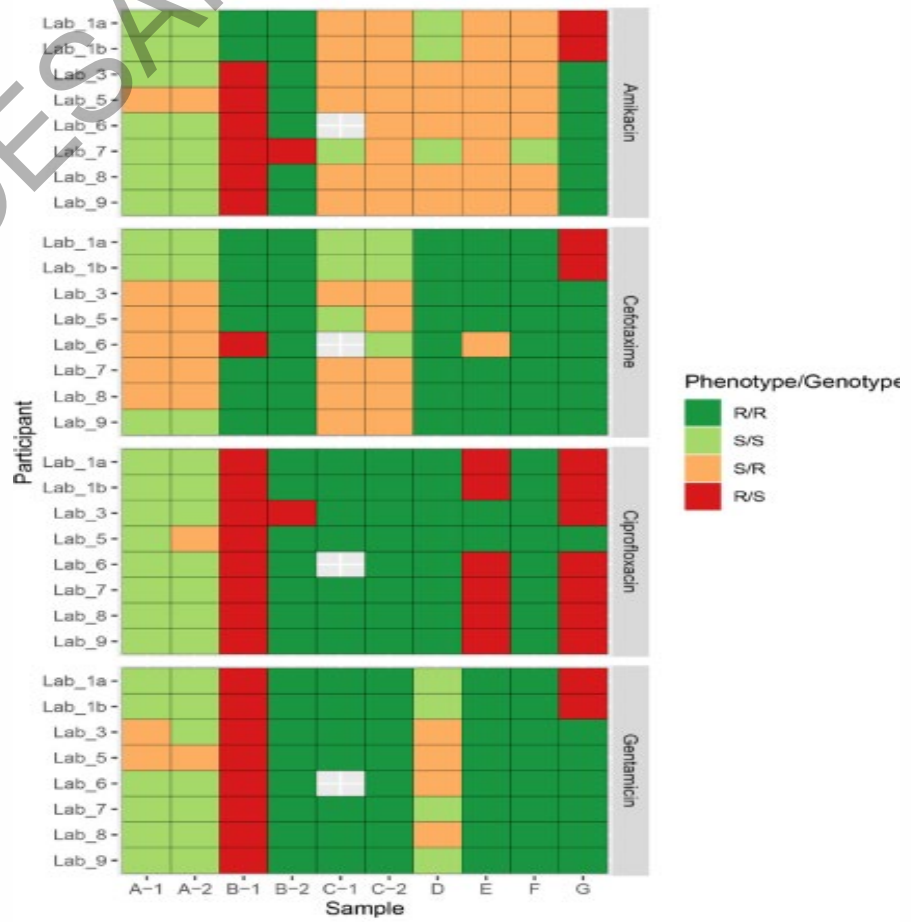
DOI 10.1099/mgen.0.000335

Discordant bioinformatic predictions of antimicrobial resistance from whole-genome sequencing data of bacterial isolates: an inter-laboratory study

Ronan M. Doyle[1,2,*], Denise M. O'Sullivan[3], Sean D. Aller[4], Sebastian Bruchmann[5], Taane Clark[6], Andreu Coello Pelegrin[7,8], Martin Cormican[9], Ernest Diez Benavente[6], Matthew J. Ellington[10], Elaine McGrath[11], Yair Motro[12], Thi Phuong Thuy Nguyen[13], Jody Phelan[6], Liam P. Shaw[14], Richard A. Stabler[15], Alex van Belkum[7], Lucy van Dorp[16], Neil Woodford[10], Jacob Moran-Gilad[12], Jim F. Huggett[3,17] and Kathryn A. Harris[2]

Table 1. Inter-laboratory study sample characteristics

Study ID	Isolate species	Sequencing method	Carbapenemase gene	Median depth of coverage	Comment
A-1	*Klebsiella pneumoniae*	NEBNext Ultra II+NextSeq 150 bp PE	OXA-48-like	190.2×	Exact duplicate of A-2
A-2	*Klebsiella pneumoniae*	NEBNext Ultra II+NextSeq 150 bp PE	OXA-48-like	190.2×	Exact duplicate of A-1
B-1	*Enterobacter cloacae* complex	NEBNext Ultra II+NextSeq 150 bp PE	OXA-48-like	1.4×	Very low coverage duplicate of B-2
B-2	*Enterobacter cloacae* complex	NEBNext Ultra II+NextSeq 150 bp PE	OXA-48-like	142.9×	High coverage duplicate of B-1
C-1	*Klebsiella oxytoca*	Nextera DNA +HiSeq 100 bp PE	OXA-48-like	37.4×	Same original isolate as C-2
C-2	*Klebsiella oxytoca*	NEBNext Ultra II+NextSeq 150 bp PE	OXA-48-like	156.4×	Same original isolate as C-1
D	*Klebsiella pneumoniae*	NEBNext Ultra II+NextSeq 150 bp PE	NDM	83.5×	
E	*Escherichia coli*	Nextera DNA +HiSeq 100 bp PE	IMP	20.6×	
F	*Citrobacter freundii*	NEBNext Ultra II+NextSeq 150 bp PE	VIM	32.5×	
G	*Acinetobacter baumannii*	NEBNext Ultra II+NextSeq 150 bp PE	OXA-23-like and OXA-51-like	22.2×	



Rules-based and Model-based WGS-ASP

Rules-based WGS-ASP	Uses AR databases
	Identify AR determinants Horizontally-acquired genes Cromosomal mutations
	Correlation to phenotype
Model-based WGS-ASP	Uses machine-learning or statistical approaches
	Entire genome/gene markers associated phenotype

Performance migth be equivalent, but model-based approaches do not provide information about antibiotic resistance mechanisms

Yee R *et al* Clin Lab Med 2022

Tools and databases for AR detection

Tabla 2. Recursos bioinformáticos para la detección de mecanismos de resistencia a los antibióticos.

Recurso	Microorganismo diana	Aplicación	Herramienta de análisis	Base de datos	Detección de mutaciones cromosómicas	Input	Link
ResFinder	General	Genomas Metagenomas	BLAST	Propia	Sí	FASTA (nt) FASTQ (nt)	https://cge.cbs.dtu.dk/services/ResFinder/
CARD	General	Genomas Metagenomas	BLAST, RGI	Propia	Sí	FASTA (nt) FASTA (aa)	https://card.mcmaster.ca/home
NCBI AMRFinderPlus	General	Genomas	BLAST, HMMER	Propia	Sí	FASTA (nt) FASTA (aa) GFF	https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/
ABRICATE	General	Genomas Metagenomas	BLAST	ResFinder CARD ARG-ANNOT NCBI AMRFinder EcOH PlasmidFinder Ecoli_VF VFDB	No	FASTA (nt)	https://github.com/tseemann/abricate
ARIBA	General	Genomas	Minimap, Bowtie2	Derivado de: ARG-ANNOT CARD PlasmidFinder ResFinder VFDB	Sí	FASTQ (nt)	https://github.com/sanger-pathogens/ariba
MEGARes	General	Genomas Metagenomas	BWA	Derivado de: ARG-ANNOT CARD NCBI Lahey Clinic beta-lactamase archive ResFinder	No	FASTQ (nt)	https://megares.meglab.org/
Kmer resistance	General	Genomas Metagenomas	KMA	ResFinder	Sí	FASTQ (nt) FASTA (nt)	https://cge.cbs.dtu.dk/services/KmerResistance-2.2/
LREfinder	Resistencia a linezolid en *Enterococcus* spp.	Genomas	KMA	Propia	Si	FASTA (nt) FASTQ (nt)	https://cge.cbs.dtu.dk/services/LRE-finder/
SCCmec Finder	Búsqueda de elementos SCCmec en *S. aureus*	Genomas	BLAST, KMA	Propia	No	FASTA (nt) FASTQ (nt)	https://cge.cbs.dtu.dk/services/SCCmecFinder/
Mykrobe	Resistencia en *M. tuberculosis* y *S. aureus*	Genomas	Propio (basado en gráficos de De Bruijn)	Propia	Sí	FASTQ (nt)	https://www.mykrobe.com/
DRAGdb	Resistencia mutacional en microorganismos SKAPE y *M. tuberculosis*	Genomas	BLAST	Propia	Sí	FASTA (nt)	http://bicresources.jcbose.ac.in/ssaha4/drag/index.php

CARD, ResFinder and AMRFinder databases

Nucleic Acids Research

D690–D699 Nucleic Acids Research, 2023, Vol. 51, Database issue *Published online 20 October 2022*
https://doi.org/10.1093/nar/gkac920

CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database

Brian P. Alcock[1,2,3], William Huynh[1,2,3], Romeo Chalil[1,2,3], Keaton W. Smith[1,2,3], Amogelang R. Raphenya[1,2,3], Mateusz A. Wlodarski[1,2,3], Arman Edalatmand[1,2,3], Aaron Petkau[4,5], Sohaib A. Syed[1,2,3], Kara K. Tsang[1,2,3], Sheridan J.C. Baker[1,2,3], Mugdha Dave[1,2,3], Madeline C. McCarthy[1,2,3], Karyn M. Mukiri[1,2,3], Jalees A. Nasir[1,2,3], Bahar Golbon[1,2,3], Hamna Imtiaz[1,2,3], Xingjian Jiang[1,2,3], Komal Kaur[1,2,3], Megan Kwong[1,2,3], Zi Cheng Liang[1,2,3], Keyu C. Niu[1,2,3], Prabakar Shan[1,2,3], Jasmine Y.J. Yang[1,2,3], Kristen L. Gray[6], Gemma R. Hoad[7], Baofeng Jia[6], Timsy Bhando[1,2,3], Lindsey A. Carfrae[1,2,3], Maya A. Farha[1,2,3], Shawn French[1,2,3], Rodion Gordzevich[1,2,3], Kenneth Rachwalski[1,2,3], Megan M. Tu[1,2,3], Emily Bordeleau[1,2,3], Damion Dooley[8], Emma Griffiths[8], Haley L. Zubyk[1,2,3], Eric D. Brown[1,2,3], Finlay Maguire[9,10,11], Robert G. Beiko[9,10], William W.L. Hsiao[6,8], Fiona S.L. Brinkman[6], Gary Van Domselaar[5,12] and Andrew G. McArthur[1,2,3,*]

scientific reports

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]

MICROBIAL GENOMICS

BIORESOURCE

Florensa *et al.*, *Microbial Genomics* 2022;8:000748
DOI 10.1099/mgen.0.000748

MICROBIOLOGY SOCIETY

OPEN DATA OPEN ACCESS

ResFinder – an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes

Alfred Ferrer Florensa, Rolf Sommer Kaas, Philip Thomas Lanken Conradsen Clausen, Derya Aytan-Aktug and Frank M. Aarestrup*

- ✓ Focus on mobile AR determinants
- ✓ Limited allele variants
- ✓ Limited chromosomal mutations (e.g. FQ)
- ✓ Presence/absence
- ✓ Interpretation challenges

ResFinder in real life

frontiers | Frontiers in Microbiology

ORIGINAL RESEARCH
published: 10 June 2022
doi: 10.3389/fmicb.2022.804627

One Day in Denmark: Comparison of Phenotypic and Genotypic Antimicrobial Susceptibility Testing in Bacterial Isolates From Clinical Settings

Ana Rita Rebelo[1], Valeria Bortolaia[1,2], Pimlapas Leekitcharoenphon[1], Dennis Schrøder Hansen[3], Hans Linde Nielsen[4,5], Svend Ellermann-Eriksen[6], Michael Kemp[7], Bent Løwe Røder[8], Niels Frimodt-Møller[9], Turid Snekloth Søndergaard[10], John Eugenio Cola[11], Claus Østergaard[12], Henrik Westh[13,14] and Frank M. Aarestrup[1]*

OPEN ACCESS

MALDI-TOF
SensititreTM panels (except for 7 anaerobic bacteria)
EUCAST clinical breakpoints version 12.0.

NextSeq 500 sequencing platform
De novo assembled (max 500 contigs, 0.5 Mbp deviation)
KmerFinder2 and rMLST3
ResFinder 4.04 (min alignment 60%, min identity 90%)
Absence/presence of ARGs/chromosomal point mutations

Random selection of clinical isolates obtained during a single day from all clinical microbiological laboratories in Denmark.

TABLE 1 | Distribution by genera of the 488 bacterial isolates analyzed in this study.

Genera	Number of isolates	Percentage
Escherichia	171	35.0
Staphylococcus	103	21.1
Streptococcus	55	11.3
Klebsiella	34	7.0
Enterococcus	32	6.6
Pseudomonas	21	4.3
Haemophilus	14	2.9
Moraxella	10	2.0
Proteus	10	2.0
Enterobacter	10	2.0
Citrobacter	10	2.0
Aerococcus	5	1.0
Corynebacterium	3	0.6
Stenotrophomonas	2	0.4
Serratia	2	0.4
Erwinia	1	0.2
Morganella	1	0.2
Providencia	1	0.2
Raoultella	1	0.2
Salmonella	1	0.2
Yersinia	1	0.2
Grand total	**488**	**100**

ResFinder in real life

5,229 isolate-ATB combinations

434 discordances (8.3%)

- 75 in GP (75/1969, 3.8%)
- 359 in GN (359/3260, 11%)
- 326 major error (6.2%)
- 108 very major error (2.1%)

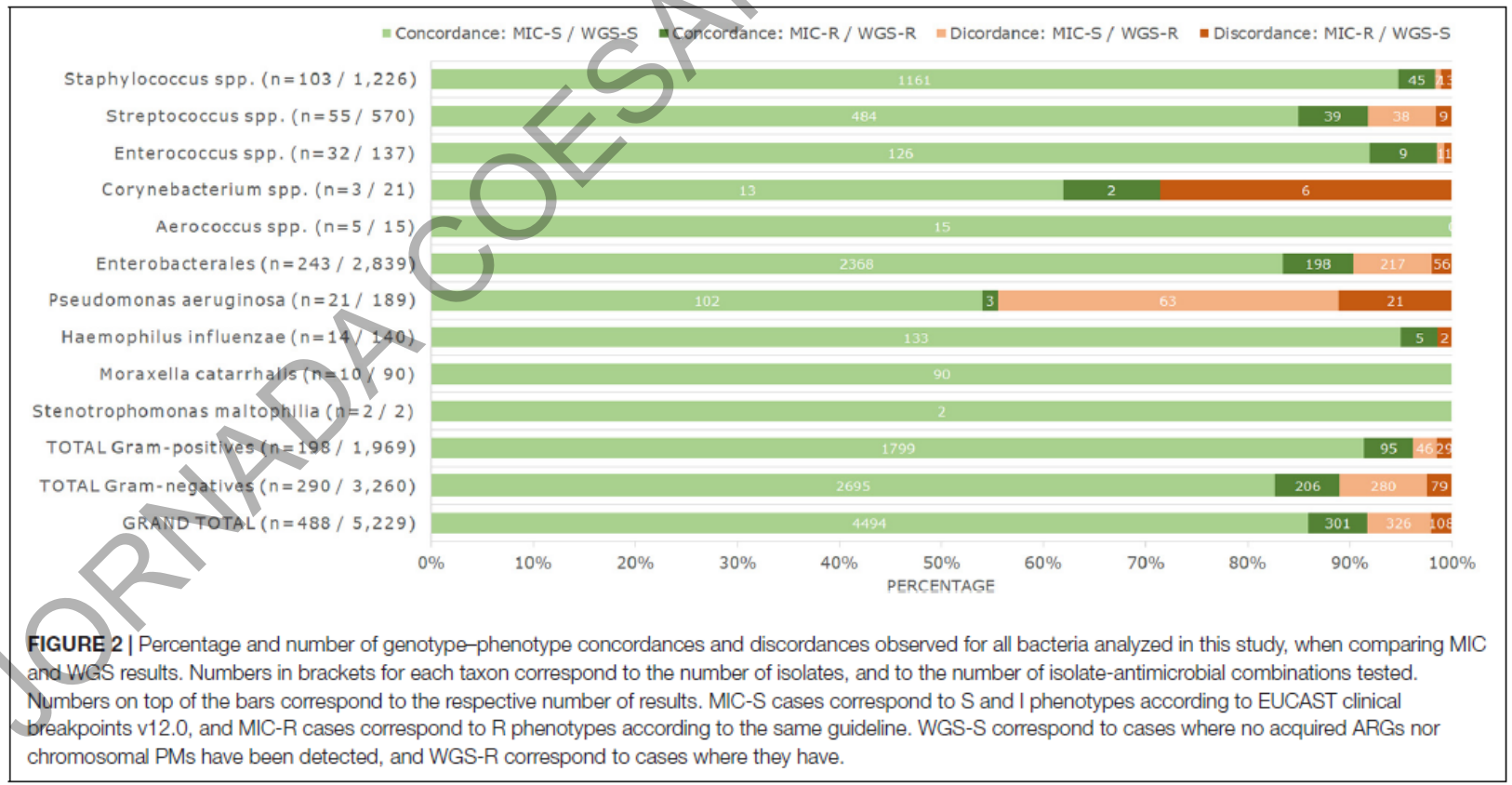


FIGURE 2 | Percentage and number of genotype–phenotype concordances and discordances observed for all bacteria analyzed in this study, when comparing MIC and WGS results. Numbers in brackets for each taxon correspond to the number of isolates, and to the number of isolate-antimicrobial combinations tested. Numbers on top of the bars correspond to the respective number of results. MIC-S cases correspond to S and I phenotypes according to EUCAST clinical breakpoints v12.0, and MIC-R cases correspond to R phenotypes according to the same guideline. WGS-S correspond to cases where no acquired ARGs nor chromosomal PMs have been detected, and WGS-R correspond to cases where they have.

Rebelo AR *et al* Front Microbiol 2022

Rapid WGS-ASP

Antimicrobial Agents and Chemotherapy

Applying Rapid Whole-Genome Sequencing To Predict Phenotypic Antimicrobial Susceptibility Testing Results among Carbapenem-Resistant *Klebsiella pneumoniae* Clinical Isolates

Pranita D. Tamma,[a] Yunfan Fan,[b] Yehudit Bergman,[c] Geo Pertea,[d] Abida Q. Kazmi,[c] Shawna Lewis,[c] Karen C. Carroll,[e] Michael C. Schatz,[d,f] Winston Timp,[b] Patricia J. Simner[e]

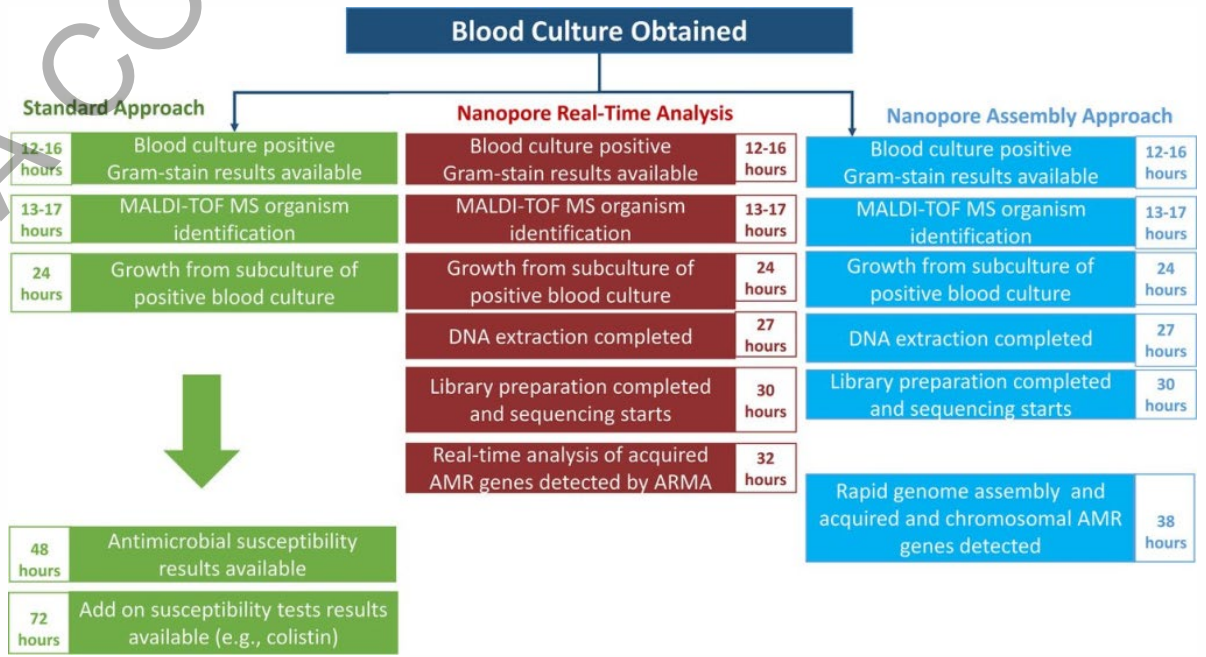
TABLE 1 Percent agreement between three different sequencing and analysis approaches compared to phenotypic antimicrobial susceptibility testing results for 40 *Klebsiella pneumoniae* clinical isolates (Table view)

Antibiotic	Phenotypic antimicrobial susceptibility testing results (%)		% agreement with antimicrobial susceptibility testing results		
	Susceptible	Not susceptible	Real-time approach	Assembly approach	Hybrid Nanopore-Illumina assembly
Piperacillin-tazobactam	25	75	80	85	85
Ceftriaxone	25	75	93	95	95
Cefepime	28	72	95	98	98
Ceftazidime-avibactam	93	7	100	100	100
Ertapenem	78	22	83	85	85
Meropenem	40	60	93	95	95
Amikacin	78	22	78	85	85
Gentamicin	60	40	45	93	95
Ciprofloxacin	33	67	30	98	98
Colistin	93	7	93	98	98
Doxycycline	50	50	63	80	80
Trimethoprim-sulfamethoxazole	35	65	68	93	93
Overall agreement			77	92	92

The real-time approach was unable to identify allelic variants

Detected blaSHV were assumed to be non-ESBL blaSHV (β-lactams)
Unable to quantify the number of AMEs
Unable to identify chromosomal mutations (FQ and COL)

Oxford Nanopore sequencing (real time, 3rd generation)
(1) Real time approach
(2) Assembly-based approach
(3) Hybrid approach (Illumina+ nanopore)



Both the real-time and assembly approaches were significantly faster than the standard approach

Rapid WGS-ASP

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| Bacteriology | Research Article

Rapid nanopore sequencing and predictive susceptibility testing of positive blood cultures from intensive care patients with sepsis

Patrick N. A. Harris,[1,2,3] Michelle J. Bauer,[1] Lukas Lüftinger,[4] Stephan Beisken,[4] Brian M. Forde,[1] Ross Balch,[1] Menino Cotta,[1] Luregn Schlapbach,[5,6] Sainath Raman,[6,7] Kiran Shekar,[8,9] Peter Kruger,[10,11] Jeff Lipman,[1,12,13,14] Seweryn Bialasiewicz,[15] Lachlan Coin,[16] Jason A. Roberts,[1,3,13,17] David L. Paterson,[1,18] Adam D. Irwin[1,19]

TABLE 3 Performance of predictive AST by species compared to Vitek 2, for samples with high quality predictions[a]

Species	*N*	Categorical agreement (%)	Major error (%)	Very major error (%)
Staphylococcus aureus	9	97.2	3.2	–
Escherichia coli	6	87.8	9.2	23.5
Klebsiella pneumoniae	3	93.8	6.5	0
Pseudomonas aeruginosa	3	52.4	47.6	–
Enterococcus faecalis	1	100	0	–
Klebsiella aerogenes	1	83.3	20	0
Streptococcus pneumoniae	1	66.7	33.3	–
Overall	24	89.3	10.5	12.1

[a]Note: blank cells reflect no data for calculations (e.g., no resistance seen in that species by reference method).

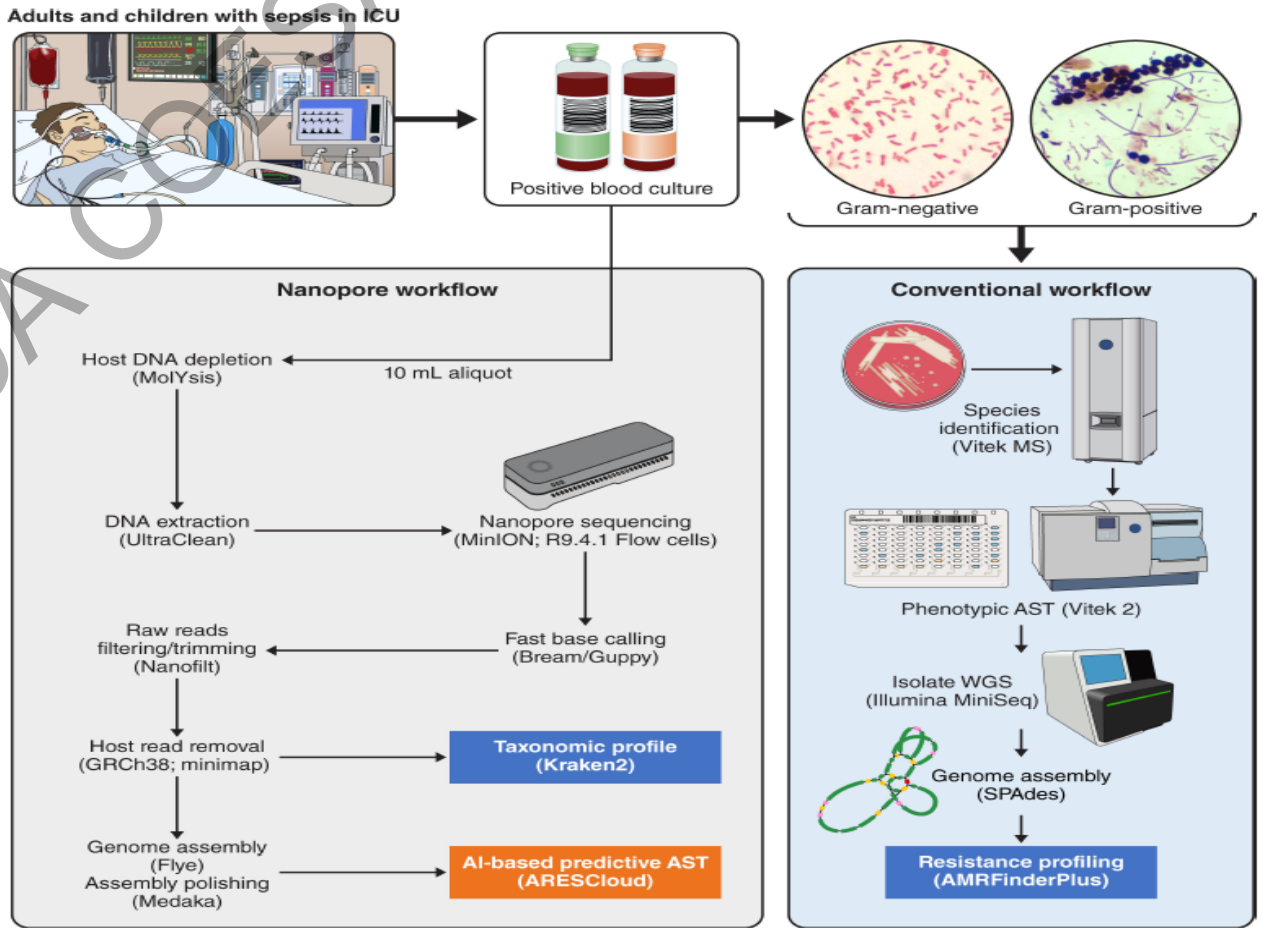


FIG 1 Workflow for Nanopore sequencing and conventional analysis of positive blood cultures.

Difficulties for assessing phenotypes from WGS

- Insufficient knowledge on involved resistance mechanisms
- Impact of genetic background
- Crossresistances and collateral susceptibilities
- Combinations of resistance mechanisms
- Expression related mechanisms (efflux pumps, β-lactamases…)
- Mutation-driven resistance mechanisms
- Emerging resistance mechanisms to novel and classical antibiotics

ESGEM AMRrules

AMRrules

ESCMID

ESGEM-ESGARS-EUCAST

1

ESKAPEE pathogens

- *Enterococcus faecium*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae* species complex
- *Acinetobacter baumannii*
- Pseudomonas aeruginosa
- Enterobacter cloacae complex
- *E. coli*

2

Other organisms on the WHO Priority Pathogens list

- *Salmonellae*, *Shigella* spp., other *Enterobacteriaceae*
- *Neisseria gonorrhoeae*
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Helicobacter pylori*
- *Campylobacter* spp.

3

Other organisms of clinical relevance where sufficient expertise and data is available, prioritising those with EUCAST Expected Resistant phenotypes

Enterobacter

Teresa Coque
Rafael Canton
Paul Higgins
Fernando Lazaro Perona
Po-Yu Liu
Elena Martinez
Rietie Venter
Ana Budimir
Angela Novais
Patrick Harris
Valeria Bortolaia

P. aeruginosa

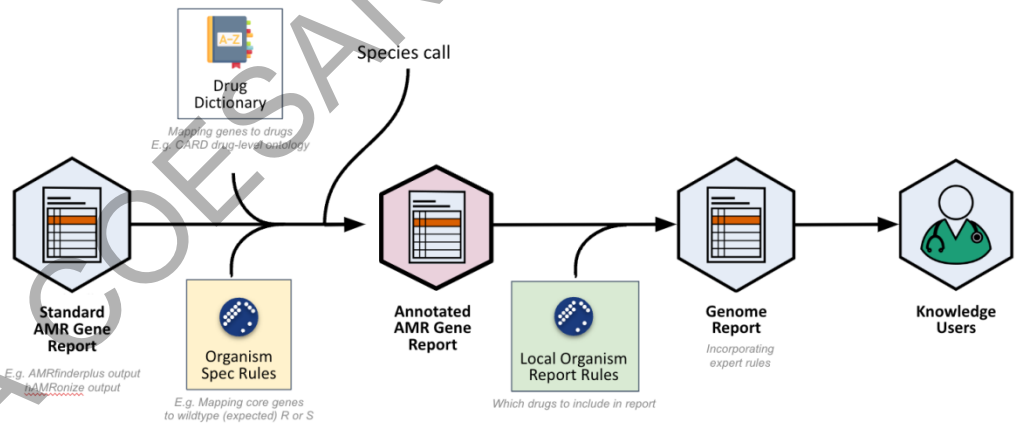
Antonio Oliver
Adriana Cabal Rosel
Alasdair Hubbard
Bogdan Lorga
Xena Li
Carla Lopez Causape
Juliette Severin
David Wareham
Adam Witney
Ørjan Samuelsen
Bela Kocsis

ESGEM AMRrules

AMRrules

ESCMID

ESGEM-ESGARS-EUCAST



1

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- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Helicobacter pylori*
- *Campylobacter* spp.

3

Other organisms of clinical relevance where sufficient expertise and data is available, prioritising those with EUCAST Expected Resistant phenotypes

ESGEM-AMR: Initial (Phase 1) Priorities

Create AMRrules that clearly delineate **core genes** associated with **'wildtype'** phenotypes for each species (**wt S** or **wt R**)

- Use expert knowledge, literature, and matched genome/AST data where available
- Focus on EUCAST breakpoints/ECOFFs as the target for definition
- Aim to explain all Expected Resistances with core genes

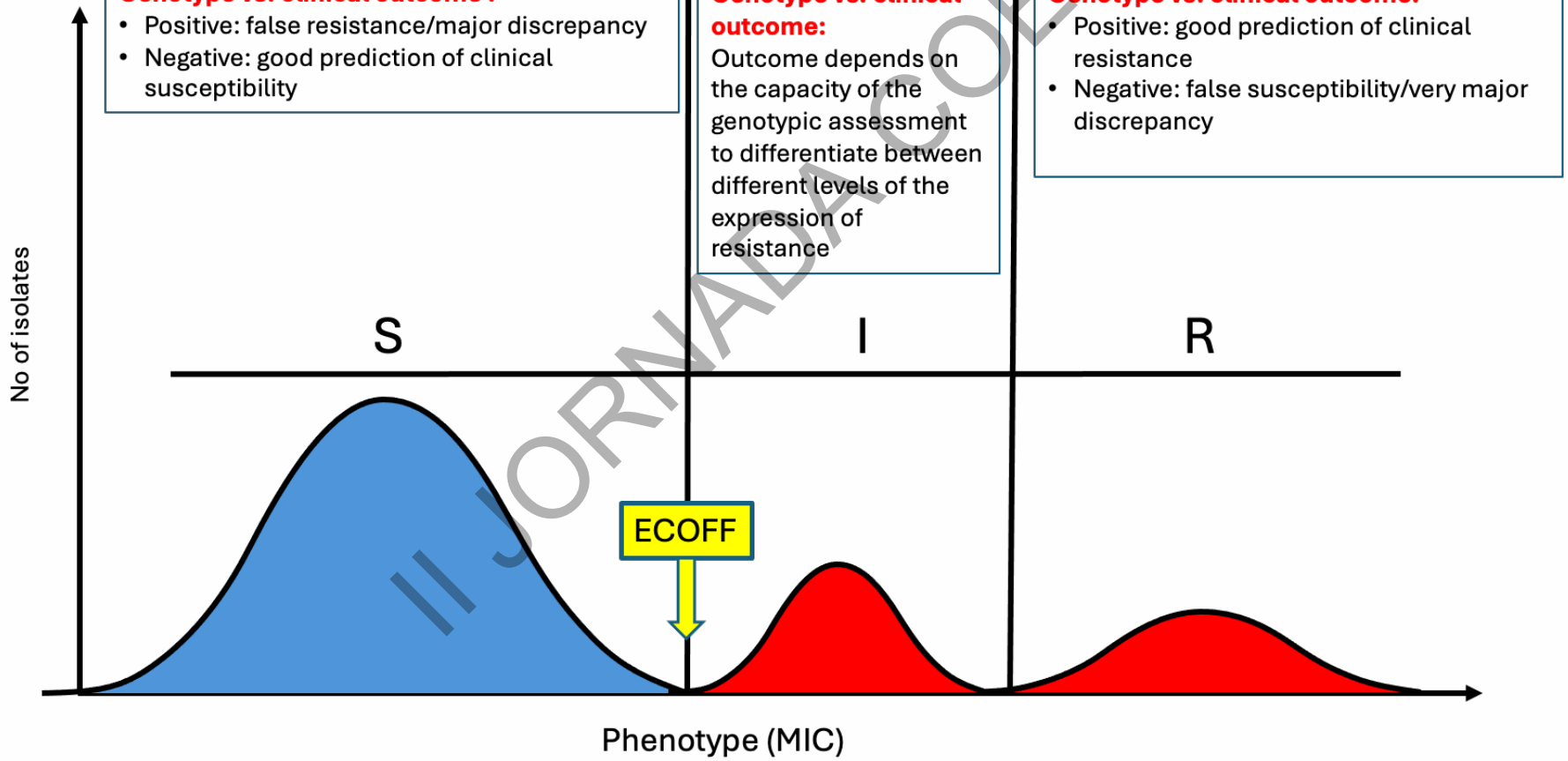
Resistance genotypes vs ECOFF and SIR breakpoints

Genotype vs. ECOFF:
- Positive: good phenotype-genotype correlation
- Negative: poor phenotype-genotype correlation

Genotype vs. clinical outcome :
- Positive: false resistance/major discrepancy
- Negative: good prediction of clinical susceptibility

Genotype vs. clinical outcome:
Outcome depends on the capacity of the genotypic assessment to differentiate between different levels of the expression of resistance

Genotype vs. clinical outcome:
- Positive: good prediction of clinical resistance
- Negative: false susceptibility/very major discrepancy



The challenge of P. aeruginosa antimicrobial resistance

- Intrinsic resistance
- Development of (multi)drug resistance during antimicrobial therapy
- Horizontally-acquired resistance (ESBLs and carbapenemases)
- Global dissemination of MDR/XDR/DTR strains: high-risk clones
- Emerging and complex resistance mechanisms to newer β-lactams

Antibiotic	AmpC ↑	MexAB↑	OprD-	AmpC Ω-loop*	OXA ESBL	ESBL	CarbA	CarbA Mut**	CarbB	Iron transp.
Piperacillin/tazobactam	R	r	S	S/r	R	R	R	R	R	S
Ceftazidime	R	r	S	R	R	R	R	R	R	S
Cefepime	r/R	r/R	S	R	R	R	R	R	R	S
Aztreonam	r/R	R	S	R	r/R	R	R	R	S	S
Imipenem	S	S	r/R	S	S	S	R	S	R	S
Meropenem	S	r	r	S	S	S	R	S	R	S
Ceftolozane/tazobactam	S	S	S	R	R	r/R	R	R	R	S
Ceftazidime/avibactam	S/r	r	S	r/R	r/R	S/r	S	R	R	S
Meropenem/vaborbactam	S	r	r	S	S	S	r/R	S	R	S
Imipenem/relebactam	S	r	r	S	S	S	r/R	S	R	S
Cefiderocol	S	S	S	S/r	S/r	S/r	S/r	S/r	S/r	r
Aztreonam/avibactam	S	R	S	r/R	r/R	S/r	S/r	r/R	S	S
Cefepime/zidebactam	S	r/R	S	S/r	S/r	S/r	S/r	S/r	r/R	S
Cefepime/taniborbactam	S	r/R	S	S/r	S/r	S/r	S/r	S/r	r/R	S

Oliver A *et al* CMI 2024

Article

2020

TRANSPARENT PROCESS OPEN ACCESS

EMBO Molecular Medicine

Predicting antimicrobial resistance in *Pseudomonas aeruginosa* with machine learning-enabled molecular diagnostics

Ariane Khaledi[1,2,†], Aaron Weimann[2,3,4,†], Monika Schniederjans[1,2,‡], Ehsaneddin Asgari[3,5,‡], Tzu-Hao Kuo[3], Antonio Oliver[6], Gabriel Cabot[6], Axel Kola[7], Petra Gastmeier[7], Michael Hogardt[8], Daniel Jonas[9], Mohammad RK Mofrad[5,10], Andreas Bremges[3,4], Alice C McHardy[3,4,§,*] & Susanne Häussler[1,2,§,**]

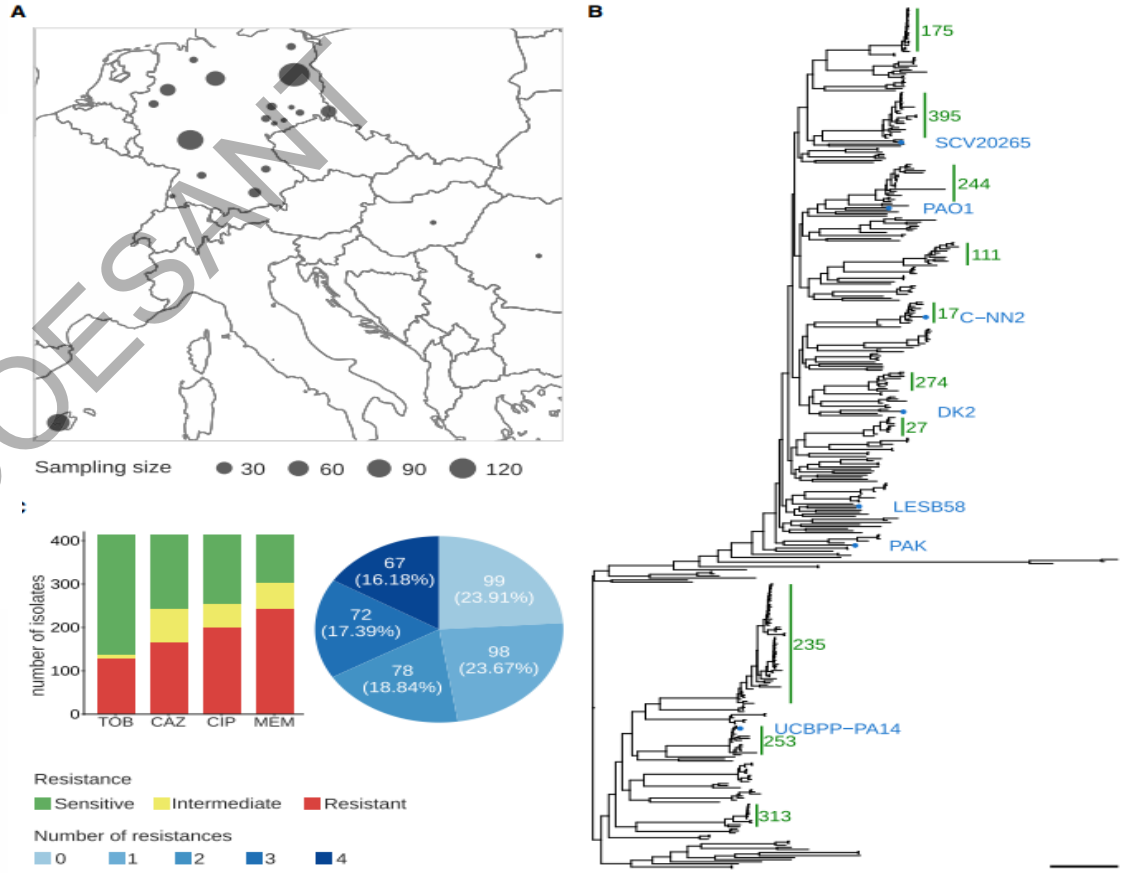


Table 1. Performance of support vector machine (SVM) classifier to predict sensitivity or resistance to four different antibiotics.

Antibiotic	Markers used	Sensitivity (resistance)	Sensitivity (susceptibility)	Predictive value (resistance)	Predictive value (susceptibility)	F1-score	Number of markers*
CAZ	GPA+EXPR	0.83 ± 0.02	0.81 ± 0.02	0.81 ± 0.02	0.83 ± 0.01	0.82 ± 0.01	37
TOB	GPA+EXPR	0.89 ± 0.01	0.94 ± 0.01	0.88 ± 0.01	0.95 ± 0.01	0.92 ± 0.01	59
MEM	GPA+EXPR	0.91 ± 0.02	0.86 ± 0.01	0.93 ± 0.01	0.81 ± 0.03	0.87 ± 0.01	93
CIP	SNPs	0.92 ± 0.01	0.87 ± 0.01	0.91 ± 0.01	0.90 ± 0.01	0.90 ± 0.01	50

*The number of markers indicates the number of (combined) features that resulted in the least complex SVM model within one standard deviation of the peak performance, i.e., with the best macro F1-score and as few as possible features for each drug.

ORIGINAL ARTICLE | VOLUME 27, ISSUE 11, P1631-1637, NOVEMBER 2021 Download Full Issue

Predicting *Pseudomonas aeruginosa* susceptibility phenotypes from whole genome sequence resistome analysis

Sara Cortes-Lara • Ester del Barrio-Tofiño • Carla López-Causapé • Antonio Oliver •
on behalf of the GEMARA-SEIMC/REIPI Pseudomonas study Group † • Show footnotes

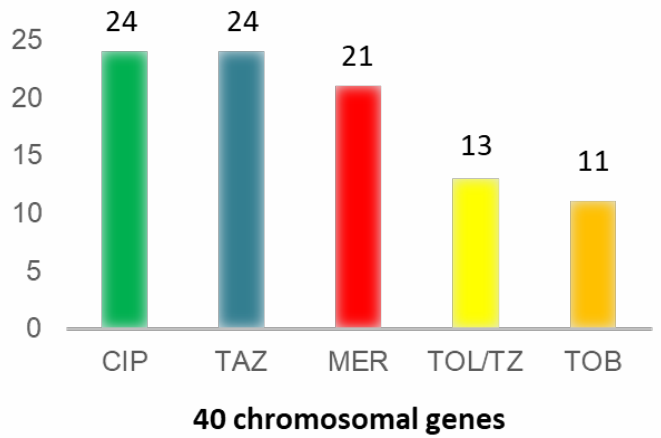
Open Archive • Published: May 17, 2021 • DOI: https://doi.org/10.1016/j.cmi.2021.05.011

Objective: to develop a genotypic score based in WGS to predict clinical (EUCAST) SIR profiles for ceftazidime, ceftolozane/tazobactam, meropenem, tobramycin and ciprofloxacin

- Define a set of genes for each antibiotic
- Ponderate the impact on resistance level
- Stablish a resistance genotypic score
- Develop a catalog of natural polymorphisms (to differentiate from resistance mutations)
- Validate the genotypic score in a multicentre collection of clinical isolates

Defining the *P. aeruginosa* genotypic resistance scores

- A total of 40 chromosomal genes were selected after detailed review of existing literature.
- Genes/mutations were scored according to their expected effect on resistance: no effect (0); low-level resistance (when two mutations are required for clinical resistance) (0.5); and clinical resistance (1).
- Well-characterized gain-of-function mutations and inactivating loss-of-function mutations were given the whole score values, whereas amino acid changes of unknown effect only 1/2.
- Mutations known to increase susceptibility were also considered and scored (negative values).
- Horizontally-acquired resistance determinants (Resfinder) were individually scored based on the published literature.



Score	Phenotype
<0.5	Susceptible
≥0.5-<1	Low-level R, uncertain clinical relevance
≥1	Resistant

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Defining the *P. aeruginosa* genotypic resistance scores: ceftazidime

LOCUS | GENE	Resistance mechanism/altered target	Type of mutation	Effect	Score values
PA0424|mexR	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA0425|mexA	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0426|mexB	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0427|oprM	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0807|ampDh3	AmpC overexpression	Loss of function	+	0.25-0.125
PA2023|galU	Lipopolysacharide sinthesis	Loss of function	+	0.5-0.25
PA3047|PBP4	AmpC overexpression	Loss of function	+	1-0.5
PA3574|nalD	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA3721|nalC	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA3999|dacC	Penicillin-binding protein PBP5	Gain of function	+	0.125
PA4020|mpl	AmpC overexpression	Loss of function	+	0.5-0.25
PA4109|ampR	AmpC overexpression	Gain of function	+	1
PA4110|ampC	AmpC structural modification	Gain of function	+	0.5-0.25
PA4418|ftsI	Penicillin-binding protein PBP3	Gain of function	+	0.5-0.25
PA4522|ampD	AmpC overexpression	Loss of function	+	1-0.5
PA5485|ampDh2	AmpC overexpression	Loss of function	+	0.25-0.125

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Defining the *P. aeruginosa* genotypic resistance scores: ceftolozane/tazobactam

LOCUS | GENE	Resistance mechanism/altered target	Type of mutation	Effect	Score values
PA0424|mexR	MexAB-OprM overexpression	Loss of function	+	0.25-0.125
PA0425|mexA	Intrinsic antibiotic resistance	Loss of function	-	-0.25
PA0426|mexB	Intrinsic antibiotic resistance	Loss of function	-	-0.25
PA0427|oprM	Intrinsic antibiotic resistance	Loss of function	-	-0.25
PA2023|galU	Lipopolysacharide sinthesis	Loss of function	+	0.5-0.25
PA3047|PBP4	AmpC overexpression	Loss of function	+	0.25-0.125
PA3574|nalD	MexAB-OprM overexpression	Loss of function	+	0.25-0.125
PA3721|nalC	MexAB-OprM overexpression	Loss of function	+	0.25-0.125
PA4020|mpl	AmpC overexpression	Loss of function	+	0.25-0.125
PA4109|ampR	AmpC overexpression	Gain of function	+	0.25
PA4110|ampC	AmpC structural modification	Gain of function	+	0.75-0.375
PA4418|ftsI	Penicillin-binding protein PBP3	Gain of function	+	0.5-0.25
PA4522|ampD	AmpC overexpression	Loss of function	+	0.25-0.125

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Defining the *P. aeruginosa* genotypic resistance scores: Meropenem

LOCUS | GENE	Resistance mechanism/altered target	Type of mutation	Effect	Score values
PA0424|mexR	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA0425|mexA	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0426|mexB	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0427|oprM	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0958|oprD	OprD inactivation	Loss of function	+	0.5-0.25
PA1798|parS	OprD downregulation and MexXY overexpression	Gain of function	+	0.5-0.25
PA1799|parR	OprD downregulation and MexXY overexpression	Gain of function	+	0.5-0.25
PA2018|mexY	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA2019|mexX	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA2020|mexZ	MexXY overexpression	Loss of function	+	0.5-0.25
PA2023|galU	Lipopolysacharide sinthesis	Loss of function	+	0.5-0.25
PA2491|mexS	OprD downregulation	Loss of function	+	0.5-0.25
PA2492|mexT	OprD downregulation	Gain of function	+	0.5-0.25
PA3047|PBP4	AmpC overexpression	Loss of function	+	0.25-0.125
PA3574|nalD	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA3721|nalC	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA4003|pbpA	Penicillin-binding protein PBP2	Gain of function	+	0.25
PA4020|mpl	AmpC overexpression	Loss of function	+	0.25-0.125
PA4109|ampR	AmpC overexpression	Gain of function	+	0.25
PA4418|ftsI	Penicillin-binding protein PBP3	Gain of function	+	0.5-0.25
PA4522|ampD	AmpC overexpression	Loss of function	+	0.25-0.125

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Defining the *P. aeruginosa* genotypic resistance scores: ciprofloxacin

LOCUS | GENE	Resistance mechanism/altered target	Type of mutation	Effect	Score values
PA0004|gyrB	DNA gyrase-QRDR	Gain of function	+	1
PA0424|mexR	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA0425|mexA	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0426|mexB	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA0427|oprM	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA1798|parS	MexXY and MexEF-OprN overexpression	Gain of function	+	0.25-0.125
PA1799|parR	MexXY and MexEF-OprN overexpression	Gain of function	+	0.25-0.125
PA2018|mexY	Intrinsic antibiotic resistance	Loss of function	-	-0.25
PA2019|mexX	Intrinsic antibiotic resistance	Loss of function	-	-0.25
PA2020|mexZ	MexXY overexpression	Loss of function	+	0.25-0.125
PA2491|mexS	MexEF-OprN overexpression	Loss of function	+	1-0.5
PA2492|mexT	MexEF-OprN overexpression	Gain of function	+	1-0.5
PA2493|mexE	MexEF-OprN overexpression	Loss of function	-	
PA2494|mexF	MexEF-OprN overexpression	Loss of function	-	
PA2495|oprN	MexEF-OprN overexpression	Loss of function	-	
PA3168|gyrA	DNA gyrase-QRDR	Gain of function	+	1
PA3574|nalD	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA3721|nalC	MexAB-OprM overexpression	Loss of function	+	0.5-0.25
PA4597|oprJ	MexCD-OprJ overexpression	Loss of function	-	
PA4598|mexD	MexCD-OprJ overexpression	Loss of function	-	
PA4599|mexC	MexCD-OprJ overexpression	Loss of function	-	
PA4600|nfxB	MexCD-OprJ overexpression	Loss of function	+	1-0.5
PA4964|parC	DNA topoisomerase IV-QRDR	Gain of function	+	0.5
PA4967|parE	DNA topoisomerase IV-QRDR	Gain of function	+	0.5

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Defining the *P. aeruginosa* genotypic resistance scores: tobramycin

LOCUS | GENE	Resistance mechanism/altered target	Type of mutation	Effect	Score values
PA0427|oprM	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA1798|parS	MexXY overexpression	Gain of function	+	0.5-0.25
PA1799|parR	MexXY overexpression	Gain of function	+	0.5-0.25
PA2018|mexY	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA2019|mexX	Intrinsic antibiotic resistance	Loss of function	-	-0.5
PA2020|mexZ	MexXY overexpression	Loss of function	+	0.5-0.25
PA4266|fusA1	Elongation factor G	Gain of function	+	0.5-0.25
PA4775|pmrA	Lipopolysacharide sinthesis	Gain of function	+	0.5-0.25
PA4776|pmrB	Lipopolysacharide sinthesis	Gain of function	+	0.5-0.25
PA5471.1|armZ	MexXY overexpression	Loss of function	+	0.5-0.25

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Genomic analysis of wild-type *P. aeruginosa* and development of a catalog of natural polymorphisms in genes involved in resistance

100 fully wild-type *P. aeruginosa* isolates (2-3 isolates from each of 51 hospitals participating in a Spanish nation-wide study, del Barrio-Tofiño *et al* JAC 2019)

Isolates with a wild-type susceptibility profile were defined, according to EUCAST epidemiological cut-off (ECOFF) values and/or MIC within 2-fold dilutions of the reference strain PAO1

Antibiotic	EUCAST 2021 ≤S - >R	EUCAST ECOFF	PAO1 MIC	Break point applied
PIP/tz	0.001-16	16	2	≤4
ATM	0.001-16	16	4	≤8
CAZ	0.001-8	8	1	≤4
FEP	0.001-8	8	1	≤4
IMP	0.001-4	4	2	≤2
MER	2-8	2	0.5	≤1
TOL/tz	4-4	4	0.5	≤1
CIP	0.001-0.5	0.5	0.12	≤0.25
TOB	2-2	2	1	≤2
AMK	16-16	16	2	≤4
COL	2-2	4	2	≤2

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Genomic analysis of wild-type *P. aeruginosa* and development of a catalog of natural polymorphisms in genes involved in resistance

High clonal diversity among the100 wild-type isoles:

- 81 different STs
- 68 unique STs
- 20 non-previously described STs

However, isolates belonging to “top 10” world-wide high-risk clones (Del barrio-Tofiño *et al* IJAA 2020) were also detected: ST235 (n=3), ST244 (n=5), ST308 (n=2), ST654 (n=2)

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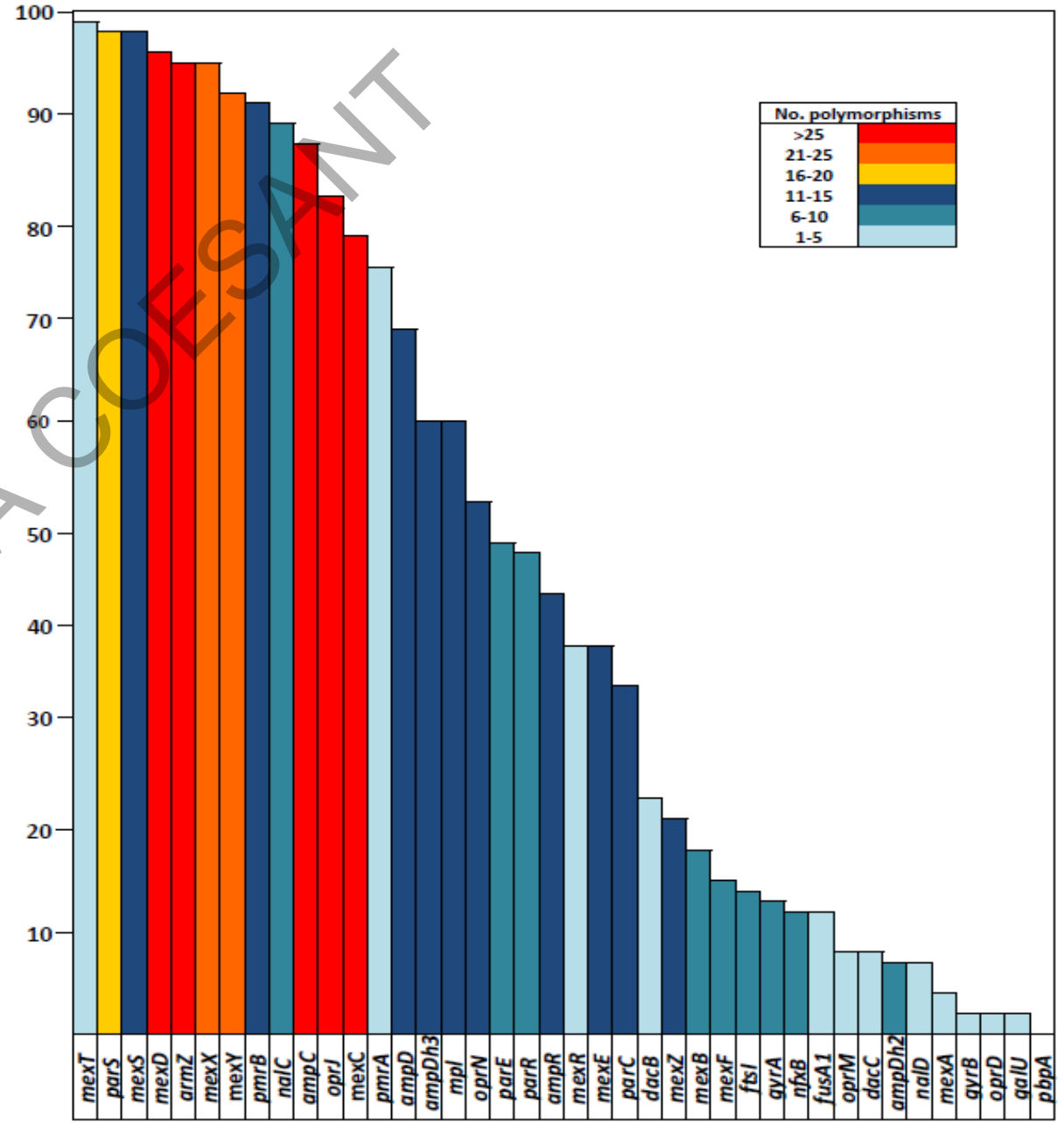
Genomic analysis of wild-type *P. aeruginosa* and development of a catalog of natural polymorphisms in genes involved in resistance

Deffinition of natural polymorphisms: aa change present in at least 2 different STs of wild-type isolates (or 1 if supported by available Genbank data)

- A catalog of 455 natural polymorphisms was stablished for the 40 chromosomal genes
- All genes except that of PBP2
- Highest for AmpC and MexXY and mexCD-OprJ efflux pumps



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Genomic analysis of wild-type *P. aeruginosa* and development of a catalog of natural polymorphisms in genes involved in resistance

List of mutations in resistance genes detected for the 100 wild-type isolates

LOCUS	GENE	MUTATIONS
PA0004	*gyrB*	A623T, A720V
PA0424	*mexR*	
PA0425	*mexA*	C24G, Q69X, A111T, D342E
PA0426	*mexB*	S183L, L672Q, R817P, I963V, aa36Δ5, aa287Δ4, nt1345Δ8, nt1435Δ1
PA0427	*oprM*	nt1451InsC, nt426Δ1
PA0807	*ampDh3*	Y207X
PA0958	*oprD*	F77L, F89L, L96I, Q250K, nt88IS
PA1798	*parS*	E90X, R165H, D294A, S386N, nt1086Δ1
PA1799	*parR*	
PA2018	*mexY*	V24A, N98S, D149Y, V240A, A400T, G423C, V431I, E505G, A527V, I531T, E731G, V1021L, T1034S
PA2019	*mexX*	Q396X
PA2020	*mexZ*	G195S, nt9InsC, nt350Δ19
PA2023	*galU*	
PA2491	*mexS*	P94S, T236P, nt390Δ11, nt769Δ1, Δ803nt
PA2492	*mexT*	S19W, P28L, R40H, R45X, D59A, nt844Δ1, aa92Δ4
PA2493	*mexE*	S5F, P337A, Q372L, Δ*mexE*
PA2494	*mexF*	A258T, G501S, D648A, A850E, E860G, L893V, V957I, Δ2646nt
PA2495	*oprN*	T37A, A277V , G364S
PA3047	*dacB* (PBP4)	A35T, N44K
PA3168	*gyrA*	N200S, aa914InsPK
PA3574	*nalD*	A19T
PA3721	*nalC*	G213D, Δ239nt, nt444Δ1
PA3999	*dacC* (PBP5)	
PA4003	*pbpA* (PBP2)	V29I, E107D, P503L, P621L
PA4020	*mpl*	H98Y, D382N
PA4109	*ampR*	M241I
PA4110	*ampC*	L133I, G194D, D263V, S306T
PA4266	*fusA1*	S695T
PA4418	*ftsI* (PBP3)	G8S, V373I, D515N
PA4522	*ampD*	R14H
PA4597	*oprJ*	L66P, G278D, G322A
PA4598	*mexD*	M118T, G199D, T659M, G686R, N775K
PA4599	*mexC*	A106T, A227V, nt205Δ2
PA4600	*nfxB*	L88F, nt295Δ14
PA4776	*pmrA*	H139Y
PA4777	*pmrB*	E269K, T343N, G439D, G441D, V451L, T458I
PA4964	*parC*	D62N, A233P, E498D
PA4967	*parE*	
PA5471	*armZ*	T32I, G51C, A58V, R320P, R322S, S348T
PA5485	*ampDh2*	V40I

- Frequent mutations leading to the inactivation of intrinsic efflux pumps (particularly MexAB-OprM)
- Frequent mutations leading efflux pump overexpression (such as *nalC*, *mexZ* or *mexS*)
- At least 1 OprD inactivating mutation (IS)
- Several PBP2, PBP3, and AmpC mutations of uncertain effect

Distribution of the genotypic resistance score values for the 100 wildt-ype *P. aeruginosa*.

Antibiotic	No. Isolates		
	Score <0.5	Score 0.5 - <1	Score ≥1
TAZ	96	4	0
TOL/TZ	100	0	0
MER	96	4	0
CIP	93	5	2
TOB	97	3	0

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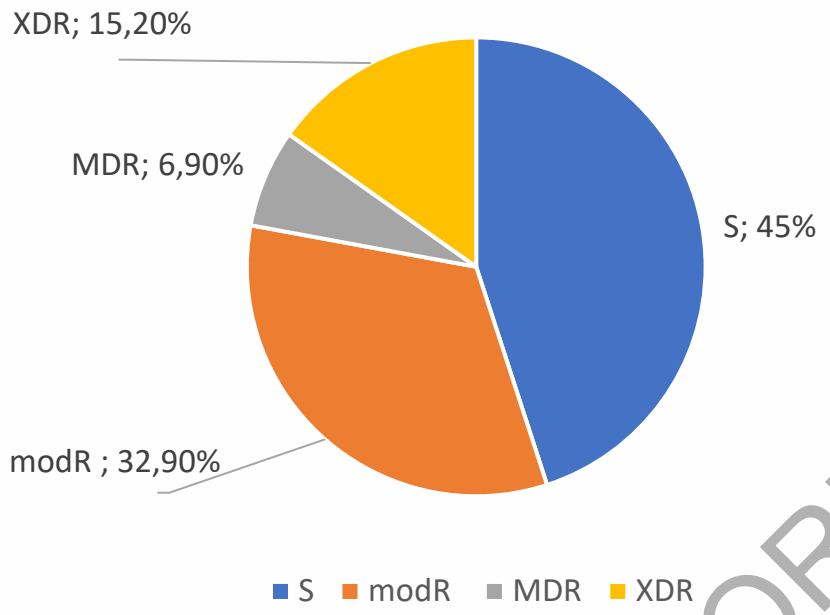
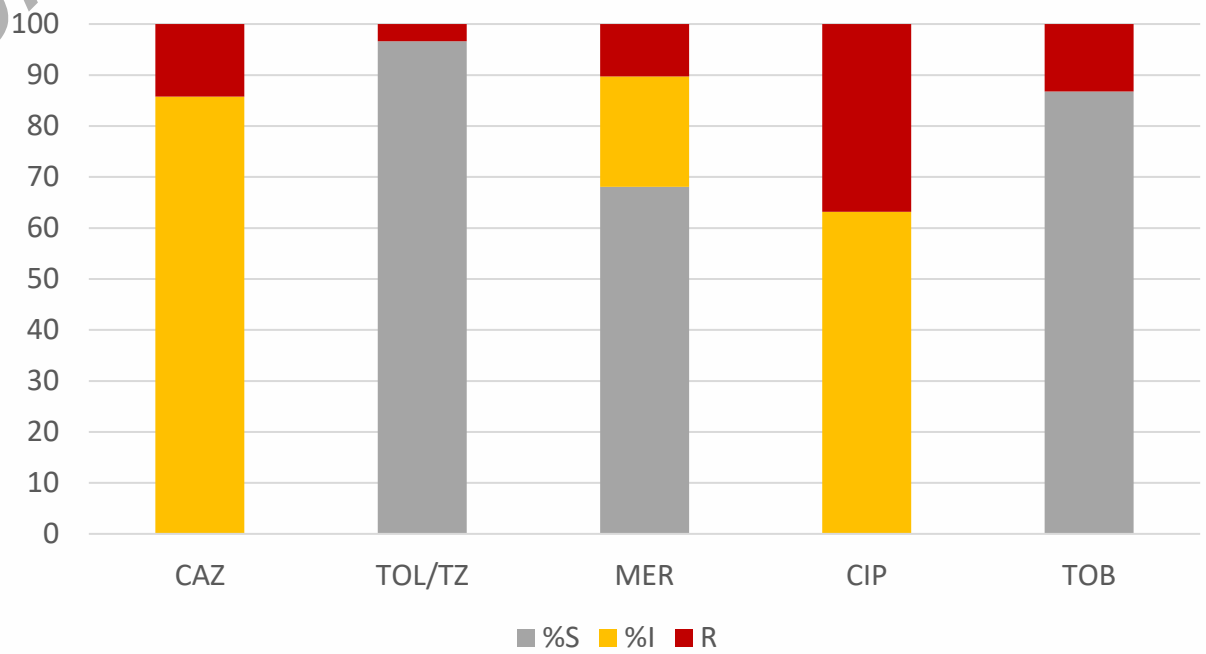
Validation of the *P. aeruginosa* genotypic resistance score in a multicenter collection of clinical isolates

The score was validated in 204 isolates from the 51 hospitals (4/hospital) participating in the Spanish multicentre survey (del Barrio-tofiño *et al* JAC 2019)





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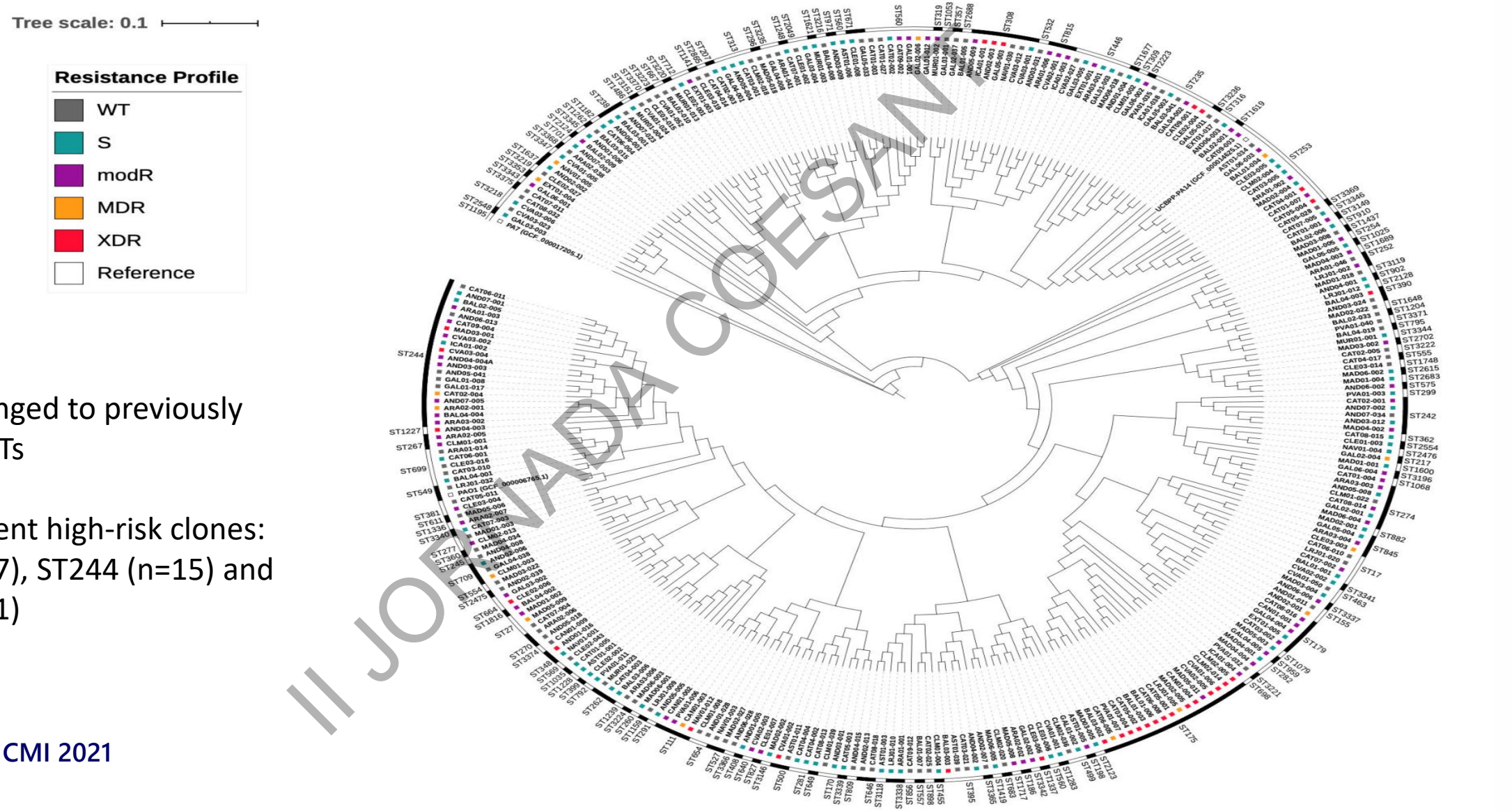
Validation of the *P. aeruginosa* genotypic resistance score in a multicenter collection of clinical isolates



- 92.3% belonged to previously described STs
- Most frequent high-risk clones: ST175 (n=17), ST244 (n=15) and ST253 (n=11)

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Figure . Core-genome phylogenetic reconstruction of the 304 (100 WT+ 204 validation set) *P. aeruginosa* sequenced isolates and PAO1, PA14 and PA7 reference strains.

Validation of the *P. aeruginosa* genotypic resistance score in a multicenter collection of clinical isolates

Accuracy of the resistance genotypic score to predict phenotypic susceptibility and resistance in the 204 clinical isolates studied

Antibiotic	% S/I/R score <0.5	% S/I/R score 0.5 - <1	% S/I/R score ≥1
TAZ	97.7 / 2.3	88.1 / 11.9	27.6 / 72.4
TOL/TZ	100 / 0	100 / 0	0 / 100
MER	86.5 / 11.1 / 2.4	60.5 / 39.5 / 0	15.4 / 38.5 / 46.1
CIP	95.5 / 4.5	73.9 / 26.1	5.9 / 94.1
TOB	100 / 0	100 / 0	12.9 / 87.1

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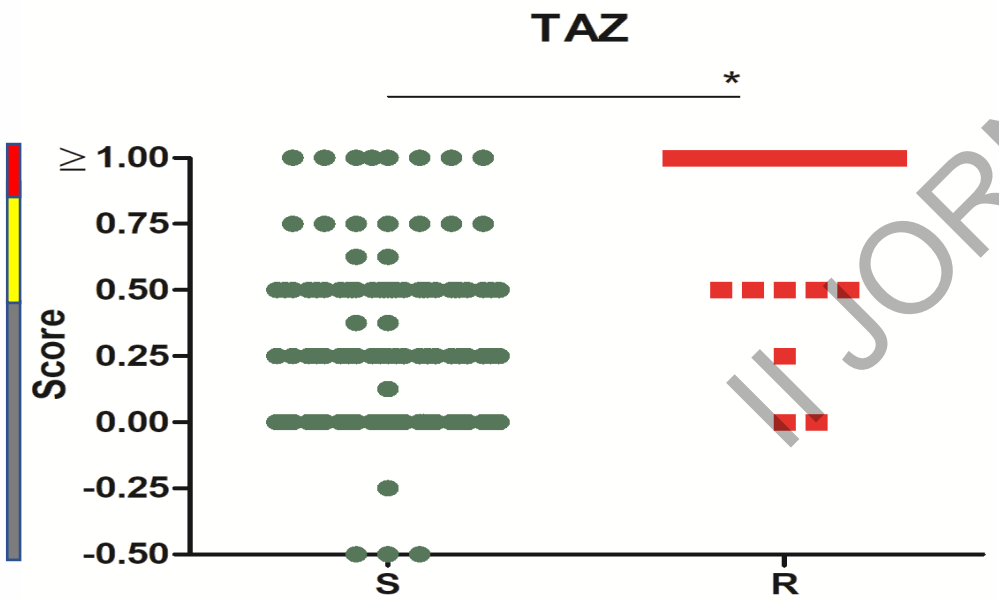

- 97.7% of the isolates with scores <0.5 were CAZ susceptible. Only 3 isolates (2.3%) with scores <0.5 were phenotypically resistant (CAZ MICs ≥ 32 mg/L). Unkwon R mechanisms?

- 72.4% of the isolates showing scores ≥1 were resistant. Thus, 8 isolates (27.6%) of isolates showing scores ≥1 were phenotypically susceptible, despite showing mutations in *ampC* regulators. Moreover, *ampC* overexpression was confirmed at transcriptional level.

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Validation of the *P. aeruginosa* genotypic resistance score in a multicenter collection of clinical isolates

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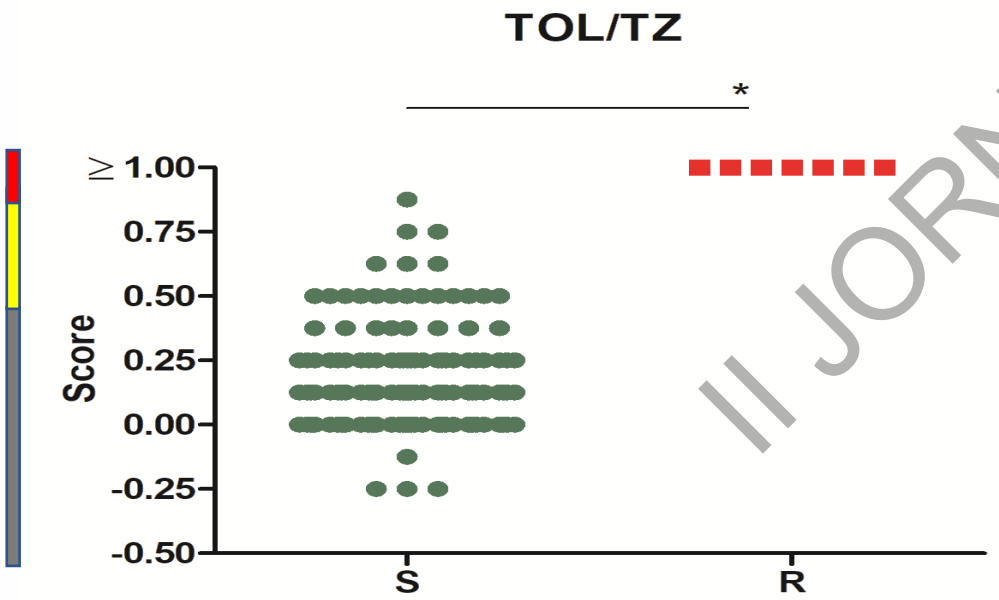

- 100% of the isolates with scores <0.5 were TOL/TZ susceptible. Moreover all isolates with scores ≥0.5-<1(low level R mechanisms) were also susceptible.
- 100% of the isolates showing scores ≥1 were TOL/tz resistant. Included isolates producing horizontally-acquired carbapenemases or AmpC overexpression + Ω-loop mutations.

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Validation of the *P. aeruginosa* genotypic resistance score in a multicenter collection of clinical isolates

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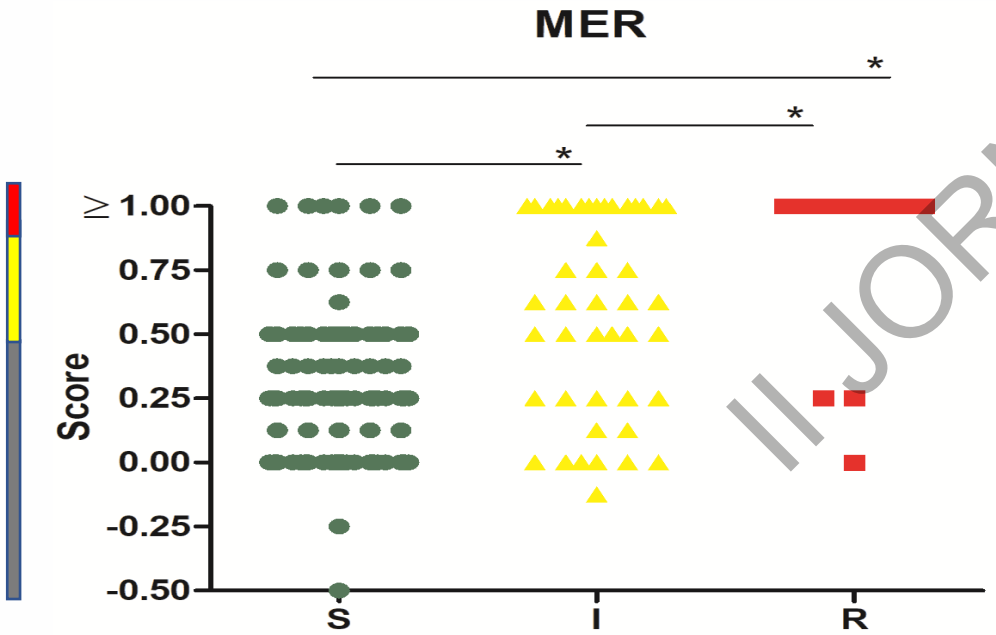

Assement was complicated with the I category (MICs 4-8 mg/L) that microbiologically correlates with nonwild-type population (low level resistance).

- 97.6% of the isolates showing scores <0.5 were MER susceptible (S+I). Only 3 isolates (2.4%) showing scores <0.5 were phenotypically R (unknown R mechanisms?)
- R prediction was low since only 46.1% of isolates showing scores ≥1 were phenotypically R (despite all showing mutations known to be involved in MER R such as OprD inactivation).

Cortes-Lara *et al* CMI 2021

Validation of the *P. aeruginosa* genotypic resistance score in a multicenter collection of clinical isolates

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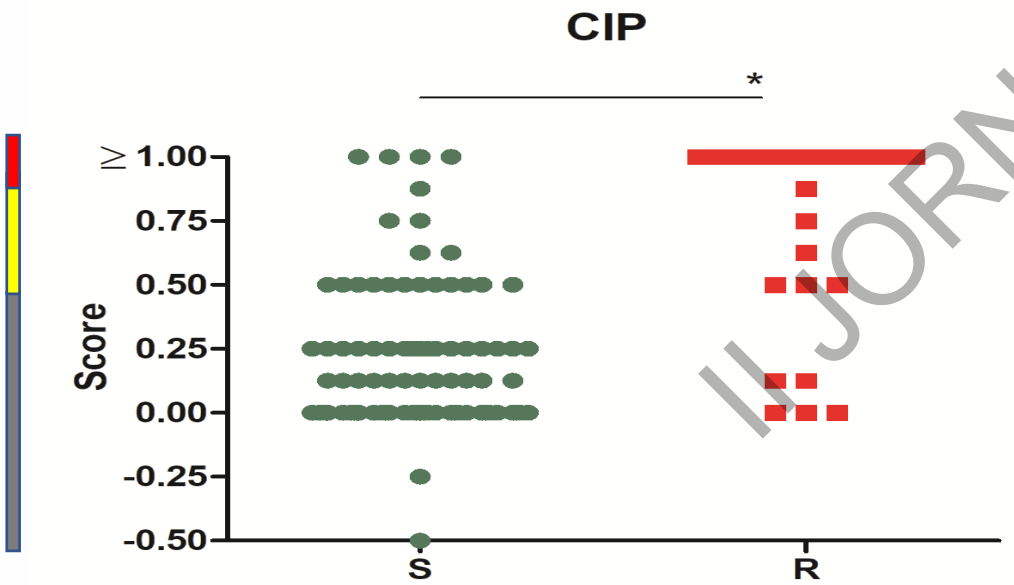

95.5% of the isolates with scores <0.5 were CIP susceptible.

94.1% of the isolates showing scores ≥1 were CIP resistant.

Cortes-Lara *et al* CMI 2021

Validation of the *P. aeruginosa* genotypic resistance score in a multicenter collection of clinical isolates

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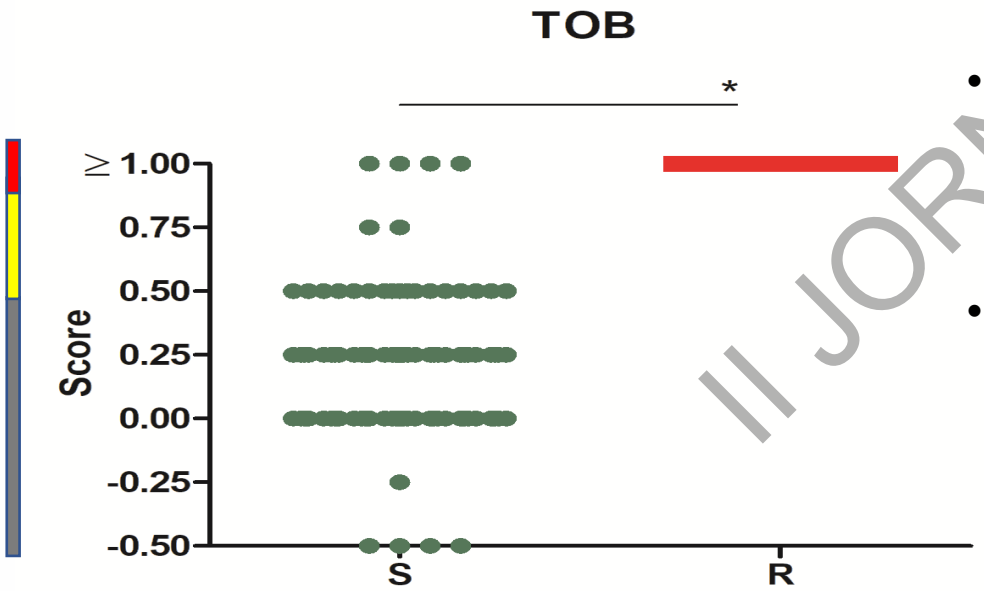

- 100% of the isolates with scores <0.5 were TOB susceptible. Moreover, all isolates with scores ≥0.5-<1(low level R mechanisms) were also susceptible.

- 87.2% of the isolates showing scores ≥1 were TOB resistant. Thus, 4 isolates (12.9%) showing a score ≥1were phenotypically susceptible. Nevertheless, all showed TOB R resistance mechanisms, including an horizontally-acquired *aacA4* gene in 2 of them.

Cortes-Lara *et al* CMI 2021

J Antimicrob Chemother 2022; **77**: 1862–1872
https://doi.org/10.1093/jac/dkac122 Advance Access publication 22 April 2022

Journal of Antimicrobial Chemotherapy

Susceptibility profiles and resistance genomics of *Pseudomonas aeruginosa* isolates from European ICUs participating in the ASPIRE-ICU trial

Gabriel Torrens[1], Thomas Ewout van der Schalk [2], Sara Cortes-Lara[1], Leen Timbermont[2], Ester del Barrio-Tofiño[1], Basil Britto Xavier[2], Laura Zamorano[1], Christine Lammens[2], Omar Ali[3], Alexey Ruzin[3], Herman Goossens[2], Samir Kumar-Singh[2], Jan Kluytmans[4], Fleur Paling[4], R. Craig MacLean[5], Thilo Köhler[6], Carla López-Causapé[1], Surbhi Malhotra-Kumar [2], and Antonio Oliver[1]* on behalf of the ASPIRE-ICU study team

Table 2. Distribution of the resistance genotypic score values among 102 *P. aeruginosa* respiratory isolates

	Number (%) of isolates S/I/R[b]		
Antibiotic[a]	Score <0.5 (susceptible genotype)	Score 0.5–<1 (undetermined genotype)	Score ≥1 (resistant genotype)
CAZ	44 (100)/0 (0)	7 (58.3)/5 (41.7)	2 (4.3)/44 (95.7)
C/T	58 (100)/0 (0)	1 (33.3)/2 (66.6)	0(0)/41 (100)
MEM	30 (90.9)/2 (6.1)/1 (3)	12 (60)/7 (35)/1 (5)	0 (0)/3 (6.1)/46 (93.9)
CIP	33 (100)/0 (0)	9 (81.8)/2 (18.2)	1 (1.7)/57 (98.3)
TOB	51 (100)/0 (0)	9 (100)/0 (0)	0 (0)/42 (100)

[a]CAZ, ceftazidime; C/T, ceftolozane/tazobactam; MEM, meropenem; CIP, ciprofloxacin; TOB, tobramycin.
[b]I/R for CAZ and CIP, S/R for C/T and TOB, S/I/R for MEM according to EUCAST breakpoints.

EUCAST *P. aeruginosa* panel, Phenotype vs WGS

Reference MIC values (mg/L) for *Pseudomonas aeruginosa* in a EUCAST/CCUG collection of defined strains with varying levels of susceptibility to antimicrobial agents from different classes.

Listed MICs represent consensus MICs from repeated tests (≥9) using broth microdilution according to ISO 20776-1 on freeze-dried Sensititre panels.

The 9 strains were selected, tested and quality controlled before and after freeze drying by the EUCAST Development Laboratory. The freeze dried material is prepared by and available from CCUG (Culture Collection at University of Gothenburg, Sweden).

Whole Genome Sequencing (WGS) amnd analysis was performed independently by Antonio Oliver, Hospital Son Espases, Palma de Mallorca, as president of the Spanish National AST committee (COESANT) and Ørjan Samuelsen, Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, University Hospital of North Norway.

This collection can be used together with *Pseudomonas aeruginosa* ATCC 27853 to evaluate MIC methods for *P. aeruginosa*.

When tested with MIC products or AST devices, MICs should comply with the consensus MICs, either being identical or with a value which is within ± 1 dilution of the consensus MIC. Some degree of random variation must be accepted whereas systematic deviation (i.e. MICs for a specific agent or several agents being systematically lower or higher than consensus MICs), should be further investigated, even if MICs are within ± 1 dilution of consensus MICs.

The EUCAST/CCUG collection is procured under the same rules as for other type culture collection strains.

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

NASJONAL KOMPETANSETJENESTE
for påvisning av antibiotikaresistens

CCUG
Culture Collection
University of Gothenburg

https://www.eucast.org/ast_of_bacteria/panels_of_defined_strains/

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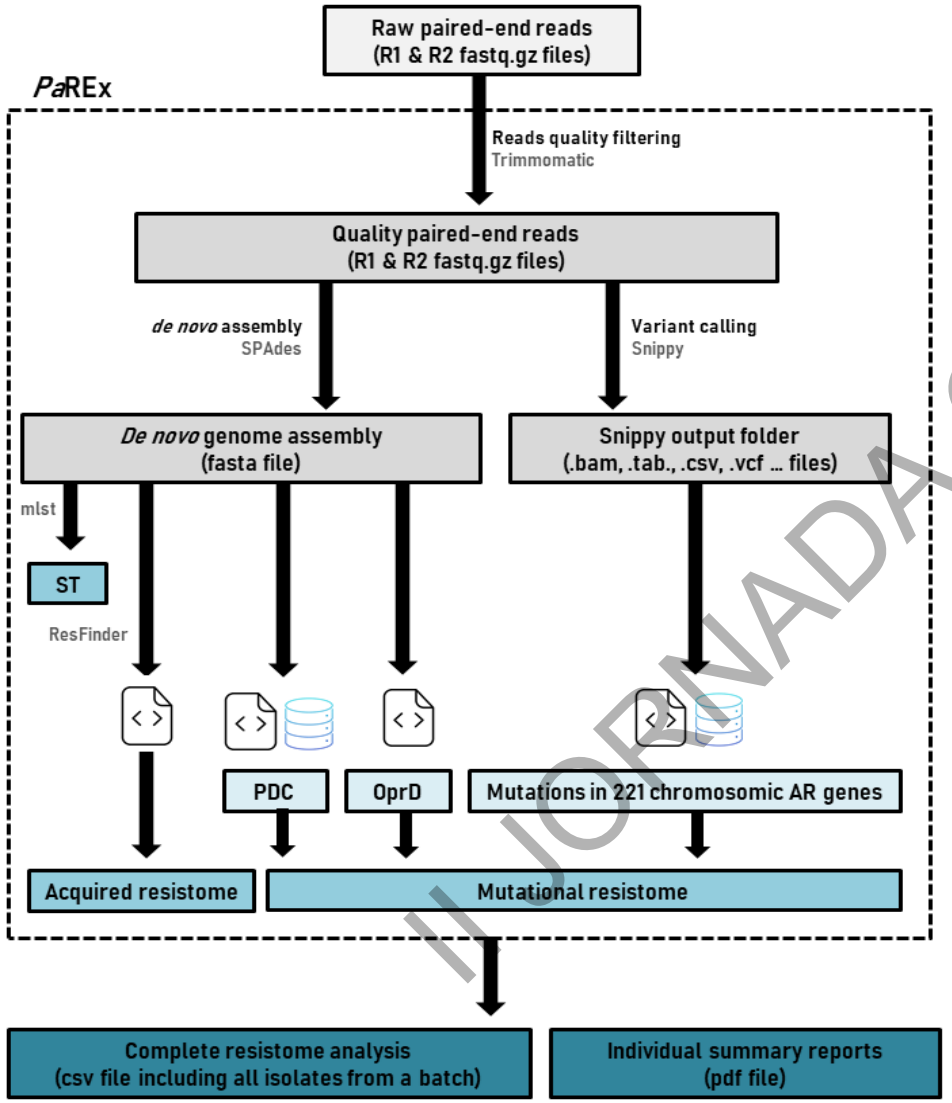
CCUG 75455
***Pseudomonas aeruginosa* (ST244)**

Animicrobial agent	MIC (mg/L)	Main resistance mechanisms[a,d]	Strength of resistance genotype[b,d]	Correlation with phenotype[c,d]
Piperacillin-tazobactam[1]	>32	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Cefepime	32	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Ceftazidime	>16	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Ceftazidime-avibactam[2]	8-16	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	Moderate	Full correlation
Ceftolozane-tazobactam[1]	4	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	Moderate	Full correlation
Imipenem	32	Inactivation of OprD (K407X), AmpC overexpression (AmpD aa162InsPERIQGHCDIA)	High	Full correlation
Meropenem	32	Inactivation of OprD (K407X), AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Aztreonam	64	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Ciprofloxacin	1	MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted)	Moderate	Full correlation
Levofloxacin	4	MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted)	Moderate	Full correlation
Amikacin	16	MexXY overpexpression (mexZnt343Δ1)	Moderate	Full correlation
Tobramycin	1	MexXY overpexpression (mexZnt343Δ1)	Weak	Full correlation
Colistin	Note[3]	LPS colistin resistance mutation (ParR E214K)	High	Full correlation

[1] Fixed 4 mg/L tazobactam
[2] Fixed 4 mg/L avibactam
[3] A reference MIC value could not be established due to poor reproducibility in repeated tests.
[a] List of resistance mechanisms/mutations
[b] Expected effect of the summatory of mutations (weak, moderate, high)
[c] Correlation between genotype and phenotype (full correlation, partial correlation, no correlation)
[d] Adapted from S. Cortes-lara et al Clin Microbiol Infect 2021; 27: 1631-1637

https://www.eucast.org/ast_of_bacteria/panels_of_defined_strains/

P. aeruginosa resistome explorer



Sequence Type	
ST	235
MLST allelic profile	acsA(38) aroE(11) guaA(3) mutL(13) nuoD(1) ppsA(2) trpE(4)
PDC	
Aminoacid substitutions (vs PDC-1)	G27D, A97V, T105A, V205L, G391A
PDC variant (RefSeq protein ID)	PDC-35 (WP_003093423.1)
Horizontally acquired resistome	
Beta-lactamases	blaOXA-2 (100.00%), blaVIM-2 (100.00%)
AMEs	aac(6')-31 (94.03%), aac(6')-Il (100.00%), aac(6')-Ib-Hangzhou (99.42%)
Quinolones resistance genes	
Other resistance genes	aadA6 (100.00%), sul1 (100.00%)
Mutational resistome	
cpxS (E259Q), *gyrA* (T83I), *mexR* (K44M), *mexT* (nt240_247del), *mexZ* (V48A), *mpl* (V124G), *parC* (S87L), *pmrB* (V344M)	

López-Causapé et al., AAC (in press)

eBioMedicine
Part of THE LANCET Discovery Science

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Monitoring of *Pseudomonas aeruginosa* mutational resistome dynamics using an enrichment panel for direct sequencing of clinical samples

Sara Cortes-Lara,[a,g] Paola Medina-Reatiga,[a,g] Ester del Barrio-Tofiño,[a] María A. Gomis-Font,[a] Gabriel Cabot,[a] Fernando Gómez-Romano,[a] Ignacio Ayestarán,[b] Asunción Colomar,[b] Alexandre Palou-Rotger,[c] Jesús Oteo-Iglesias,[d] Rosa del Campo,[e] Rafael Cantón,[e] Juan P. Horcajada,[f] Carla López-Causapé,[a,*] and Antonio Oliver[a,**]

Ca. 200 genes (resistome, MLST, hypermutation, virulence)

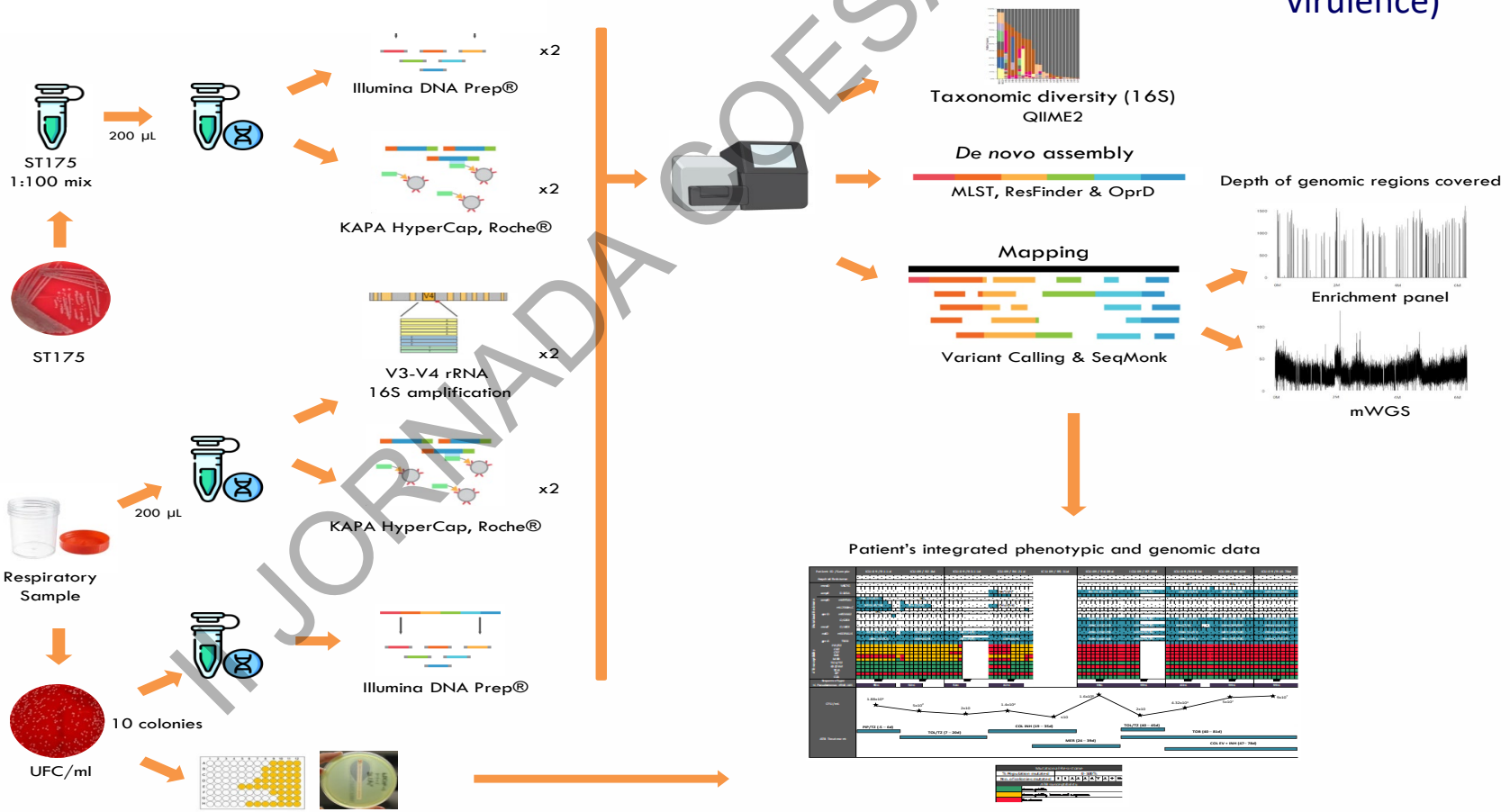


Figure 1. Wet-lab and dry-lab workflow overview of the *in vitro* and *in vivo* validation of the *P. aeruginosa* resistome capture panel

EUCAST WGS-ASP subcommittee 2025 update

EUCAST European Committee on Antimicrobial Susceptibility Testing

Public Consultation

The role of whole genome sequencing in antimicrobial susceptibility prediction of bacteria: 2025 update from the EUCAST Subcommittee

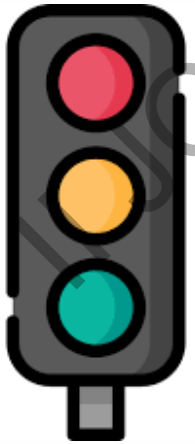
Samuelsen Ø, López-Causapé C, Aarestrup FM, Bortolaia V, Brouwer MSM, Cantón R, Egli A, Grad YH, Hamprecht A, Haussler S, Holt KE, Hopkins KL, Howden BP, Jeannot K, Kahlmeter G, Köser CU, Mathers AJ, Naas T, Pournaras S, Ruppé E, Schön T, Stoesser N, Turnidge J, Werner G, Wright G, Giske CG, Oliver A

- The phenotype, genotype and definitions of resistance
- Advancements in technologies for WGS-ASP.
- Quality metrics for WGS-ASP.
- Standardization and availability of Antimicrobial Resistance Gene databases.
- Recent advances in WGS-ASP for Gram-negative bacteria.
- Recent advances in WGS-ASP for Gram-positive bacteria
- Recent advances in WGS-ASP for *M. tuberculosis*.
- Real World experience of the application of WGS-AST in the clinical microbiology lab routine
- ASP in metagenomes
- Role of artificial intelligence and machine learning in WGS-ASP
- Role of other OMICs in WGS-ASP

S. pneumoniae, N. gonorrhoeae, P. aeruginosa, A. baumannii, C. difficile

Enterobacteriaceae (including Salmonella)

S. aureus, M. tuberculosis



C. difficile, B. fragilis

S. pneumoniae, N. gonorrhoeae, P. aeruginosa, A. baumannii, Enterococci, H. influenzae

S. aureus, M. tuberculosis, Enterobacterales

2017 **2025**

Table 6. State of the art of WGS-ASP for the targeted microoorganisms.

Microorganism	Current WGS-ASP evidence (Low, Medium, High)	Major advances since 2017	Remaining challenges
Enterobacterales	High	Development of species-specific tools. Large scale studies elucidating the phenotype-genotype relationship of specific species-drug combinations.	Quantitative impact and combinatorial effects of resistance mechanisms. Novel agents. Tools to identify gene-copy number effects. Species and lineage effects.

.....

EUCAST WGS-ASP subcommittee 2025 update

Textbox: Summary of conclusion and recomendations

1. Expansion of the evidence base: Although our understanding of genotype–phenotype associations has grown, further expansion of the evidence base is essential—particularly for complex resistance mechanisms such as mutational overexpression, gene copy number variations, porin expression/modification and efflux pumps. Additionally, newer antimicrobial agents (e.g., β-lactam–β-lactamase inhibitor combinations, cefiderocol, gepotidacin, zoliflodacin) require more comprehensive data. Ongoing, integrated phenotypic and genotypic AMR surveillance remains critical.

2. To advance the applicability of WGS-ASP comparative assessment should use both ECOFF and, if different, clinical breakpoints.

3. Generation of high-quality phenotype-genotype data: Phenotypic data should be obtained using reference antimicrobial susceptibility testing (AST) methods (ISO-standard broth microdilution, or with some microorganisms /antibiotics agar dilution). Surrogate AST methods should be avoided. MIC values are preferred over categorical S-I-R data to allow for reinterpretation if breakpoints are revised. Genomic data must meet stringent quality thresholds, tailored to species and sequencing platforms, to ensure reliability.

4. Representative strain collections and metadata: Datasets used to evaluate phenotype–genotype relationships should reflect the target population and encompass the full susceptibility spectrum, including wild-type (WT), non-wild-type (NWT), and borderline strains with MICs near clinical breakpoints. Rich contextual metadata is essential for accurate interpretation and to minimize bias.

5. Standardisation and advancement of organism specific interpretive criteria: Further development of unified, organism-specific genotype interpretation frameworks is needed—similar to the WHO mutation catalogue for *M. tuberculosis*. Harmonization efforts should be supported by tools that standardize outputs from bioinformatics pipelines to ensure consistency across platforms.

6. Compliance with international quality standards: establishment of robust external quality assessments, proficiency testing, and ring trials are vital for validating WGS-ASP in clinical settings. These measures are also necessary to meet regulatory requirements such as IVDR (EMA) and FDA standards – particularly if the results are aimed to be used in patient management decisions.

7. Leverage AI/ML approaches: The potential of AI and ML models should be further explored for WGS-ASP. For this field to advance, it is essential to train the models on reference data, and to compose strain collections according to the principles described above.

8. Further advancements in technology are required in terms of cost and turn-around-time for implementation of WGS as a primary tool for antimicrobial susceptibility prediction; setting-specific cost-effectiveness of WGS-ASP should be considered.

Take home messages

- Technological advances reducing turn around time, long read sequencing and direct sample sequencing.
- Useful for understanding resistance mechanisms, genotype-phenotype correlation and surveillance, but not to replace phenotypic AST.
- Correlation with SIR clinical breakpoints still challenging when different from ECOFFs.
- Standardization of data analysis and QC still needs to be improved.
- Several useful web-based platforms, but global reference databases still lacking.
- Phenotypic predictions from WGS still challenging for some pathogens and R mechanisms (such as *P. aeruginosa* and most mutation driven resistance mechanisms).
- Growing role of machine learning and AI.
- Increasing complexity of both genotypes and phenotypes with the introduction of novel β-lactams.
- ESCMID-ESGEM-AMR working group on Interpretive Standards for AMR Genotypes.
- EUCAST WGS-ASP subcommittee reestablished, Updated document under final revision.

Acknowledgments

Microbioloy Department and Research Unit, H. Son Espases

EUCAST

European Committee on Antimicrobial Susceptibility Testing

Subcommittee on WGS and Phenotypic AST

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Chair
Spain

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Co-Chair
Norway

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