

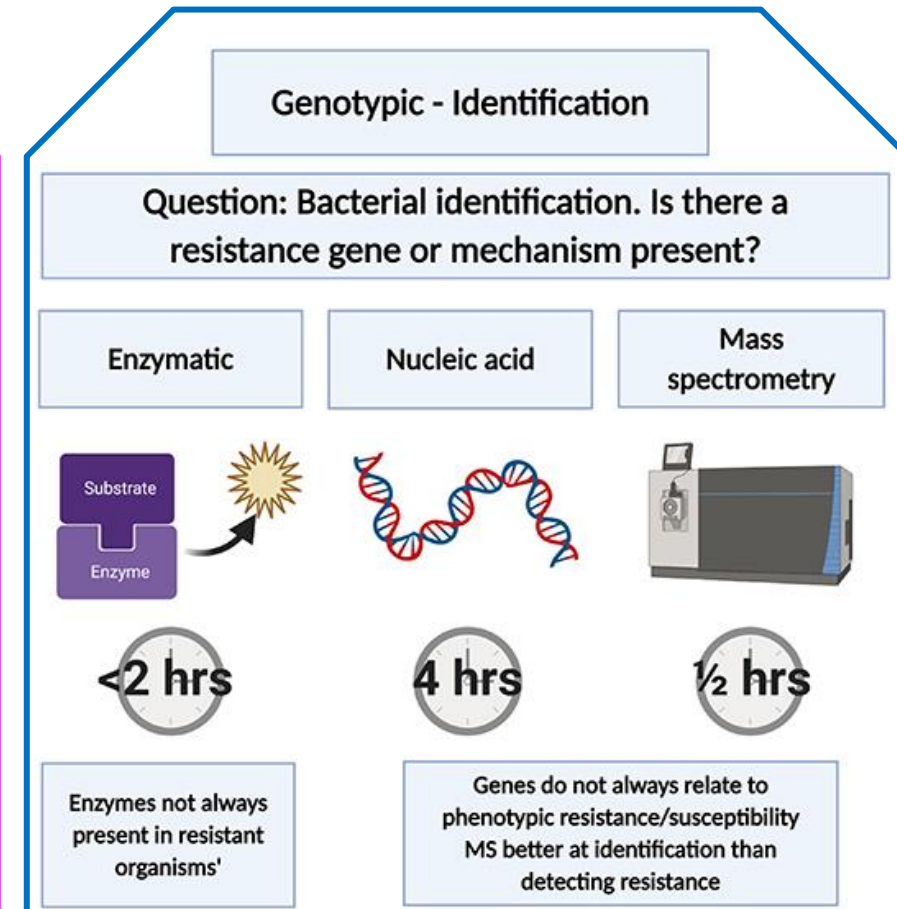
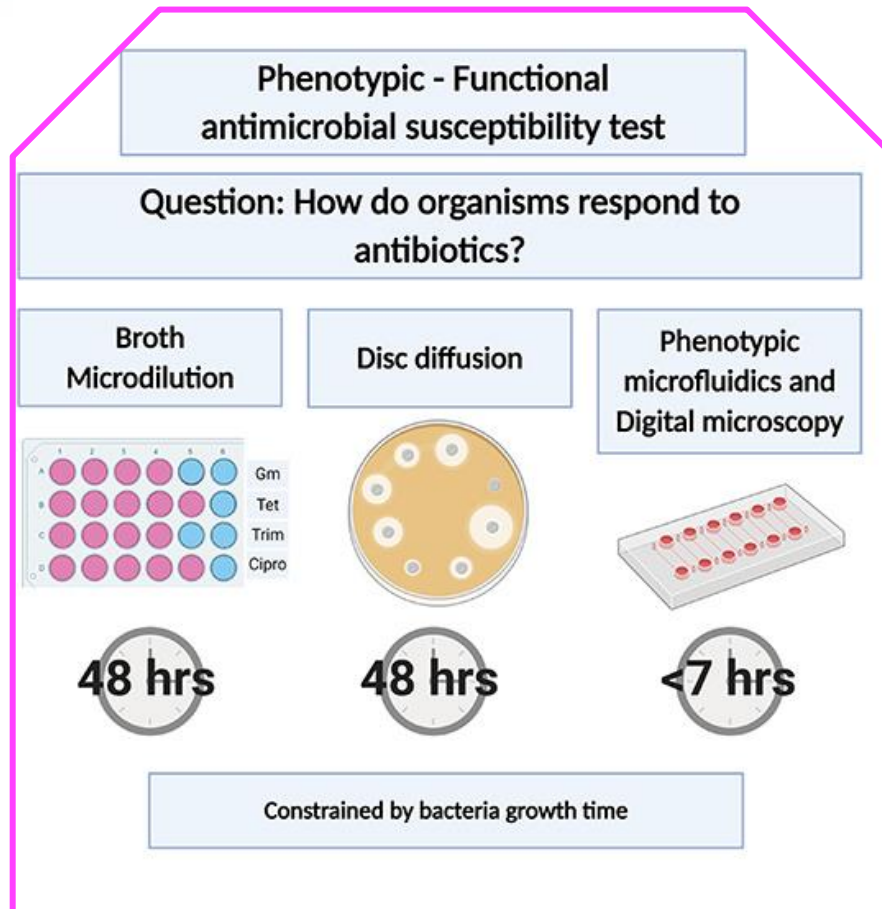
Antibiogramma molecolare per la predizione del fenotipo di resistenza

ANTIBIOGRAMMA 2024: “QUO VADIS?”

Floriana Campanile



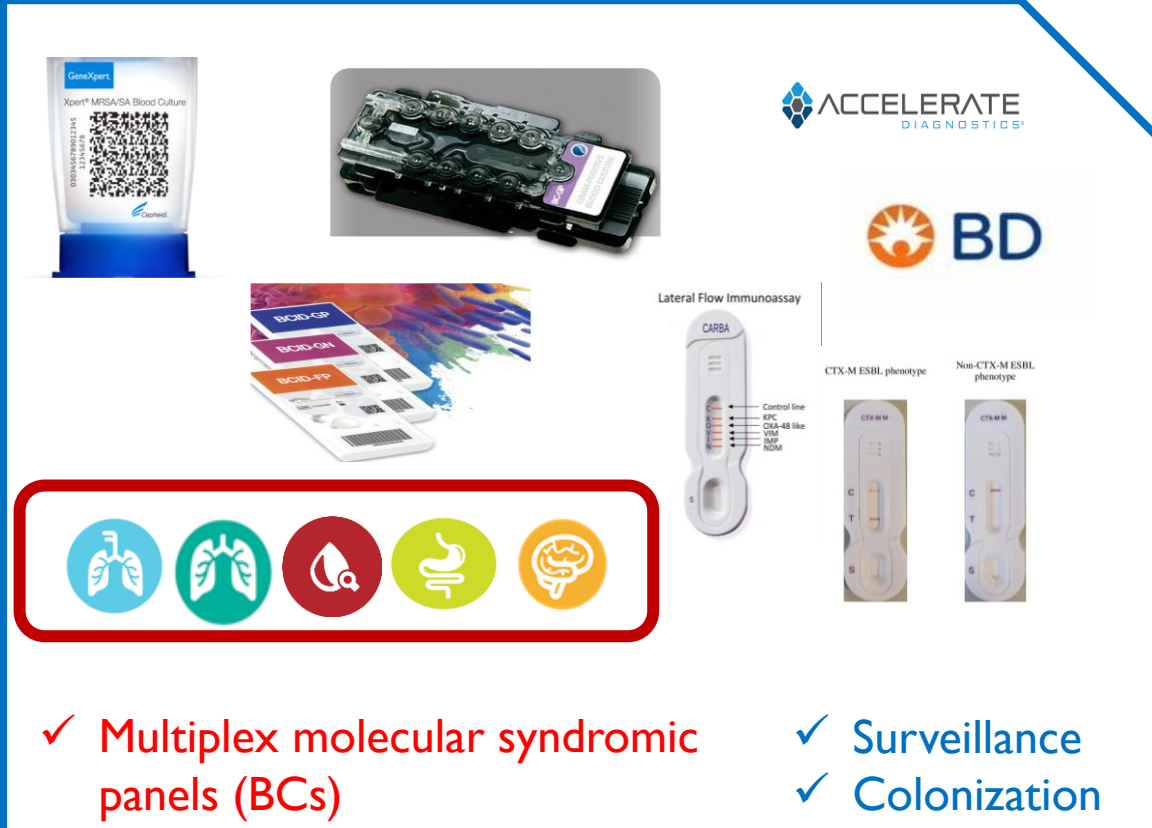
GENOTYPIC RESISTANCE DETECTION ASSAYS



RAPID MOLECULAR (BIOCHEMICAL) DIAGNOSTICS FOR AMR DETECTION → GAME-CHANGER

Although termed as “molecular”, are based on a variety of technologies:

- *RT-PCR*
- *PNA-FISH*
- *Nested-PCR*
- *Microarrays*
- *DNA-hybridization*
- *Immunochromatography (enzyme)*



✓ Multiplex molecular syndromic panels (BCs)

✓ Surveillance
✓ Colonization

FDA-cleared diagnostics identify ID and detect AMR genes (simultaneously or not)





PROS.....

Technical efficacy

- Faster Turn-Around-Times (**TAT**)
- Simultaneously **ID/AMR** gene
- **Multiplex** targeting of AMR

Identification (ID) accuracy

- In case of **difficult or not possible growth**
Independent on live cells
- Previous/under **antibiotic therapy**

Antimicrobial resistance (AMR)

- **Specific** AMR targets
- Insights into **mechanism of R**
- **Prediction** of phenotypic AST

Patient Outcome & lower hospital cost

- **Reduced Time** to effective therapy
- Reduce **antibiotic consumption**
- Reduce **Hospital costs**
- Improve existing **stewardship** programs





CONS.....

Technical efficacy

- CANNOT define **MICs**
- Predict **R NOT S**
- No standardization in clinical practice
- Higher cost

Identification (ID) accuracy

- Less accurate in **polymicrobial** detection

Antimicrobial resistance (AMR)

- **NO Off-panel** - new – AMR variants
- **MAY NOT DETECT non-enzymatic** mechanisms (porin loss; efflux-pump up-regulation).
- May overcall R (AMR genes detected but **NOT expressed**)

Impact on patient outcome

- **Limited** to some antibiotics
- **Cannot rule** AM therapy options
- **Inappropriate** de-escalation or unnecessary broad-spectrum therapy.



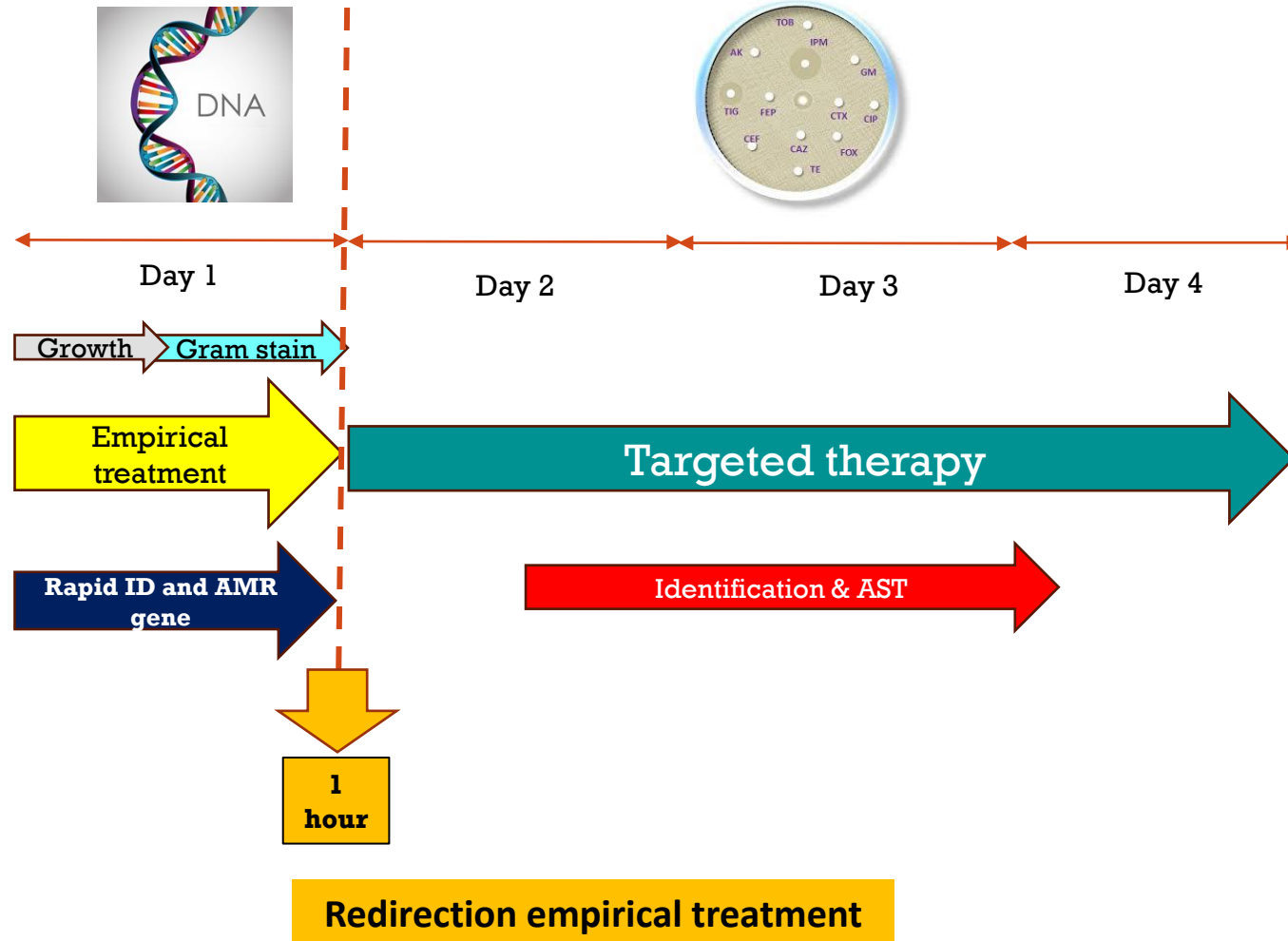
COMMON AMR GENES DETECTED BY COMMERCIALY AVAILABLE GENETIC TESTS

Gene	Organism(s)	Prediction of Resistance	Prediction of Susceptibility
<i>mecA</i> and <i>mecC</i>	Staphylococci	Resistance to all β -lactams except ceftaroline.	Absence of these genes predicts susceptibility to penicillinase stable penicillins with high sensitivity.
<i>vanA</i> and <i>vanB</i>	<i>Enterococcus faecium</i> <i>Enterococcus faecalis</i>	Resistance to vancomycin	Absence of these genes generally indicates vancomycin susceptibility for these species of <i>Enterococcus</i> .
<i>blaCTX-M</i>	Enterobacterales	Resistance to most cephalosporins	Negative result does not confer susceptibility.
<i>blaKPC</i>	Enterobacterales <i>P. aeruginosa</i>	Resistance to β -lactams, except newer agents like cefiderocol, ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam Carried by MDR plasmids \rightarrow cross-R	Negative result does not confer susceptibility. Presence of KPC does not necessarily infer susceptibility to ceftazidime-avibactam.
<i>blaOXA-48</i>	Enterobacterales <i>P. aeruginosa</i>	Resistance to β -lactams, except newer agents like cefiderocol, and novel BLIC ceftazidime-avibactam.	Negative result does not confer susceptibility.
<i>blaIMP</i>	Enterobacterales <i>P. aeruginosa</i>	Resistance to β -lactams except cefiderocol.	Negative result does not confer susceptibility.
<i>blaVIM</i>	Enterobacterales <i>P. aeruginosa</i>	Resistance to β -lactams, except cefiderocol.	Negative result does not confer susceptibility.
<i>blaNDM</i>	Enterobacterales <i>P. aeruginosa</i>	Resistance to β -lactams except aztreonam and cefiderocol. Carried by MDR plasmids \rightarrow cross-R	Negative result does not confer susceptibility.

THE GENOTYPE-TO-PHENOTYPE DILEMMA

Preliminary genotypic AMR (1h)

DEFINITIVE phenotypic AMR (+ 48h)



WHAT THE
POSSIBLE
SCENARIOS?



— COMPARISON BETWEEN PHENOTYPIC AND GENOTYPIC RESISTANCE DETECTION METHODS



THREE DIFFERENT SCENARIOS

1. Genotype correlates with phenotype → **no further** testing required
2. Genotypically R (**AMR gene detected**) and phenotypically S
3. Genotypically S (**AMR gene not detected**) and phenotypically R



HOW CLINICAL LAB SHOULD INTERPRET RAPID MOLECULAR RESULTS IN RELATION TO DISCORDANT PHENOTYPIC AST?



SCENARIO 1: GENOTYPE CORRELATES WITH PHENOTYPE

Staphylococcus aureus

mecA/C gene detected

MRSA (or MRCoNS)



**Decreased duration of
piperacillin/tazobactam →
Anti-MRSA therapy**

**Vancomycin;
Daptomycin**

mecA/C gene not detected

MSSA



**Decreased unnecessary
vancomycin use**

Oxacillin; cefazolin

1 h

Switching therapy



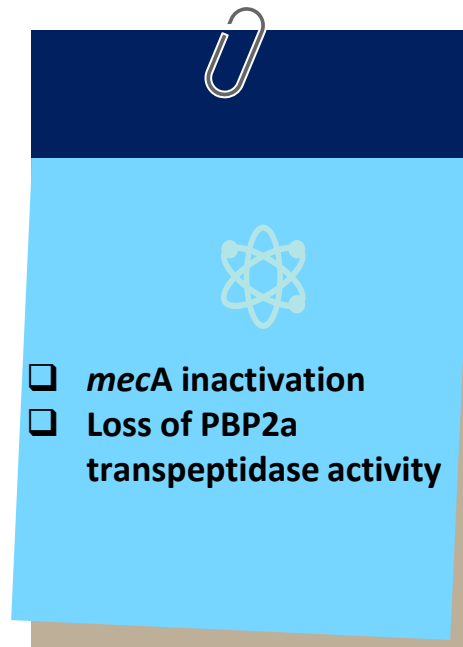
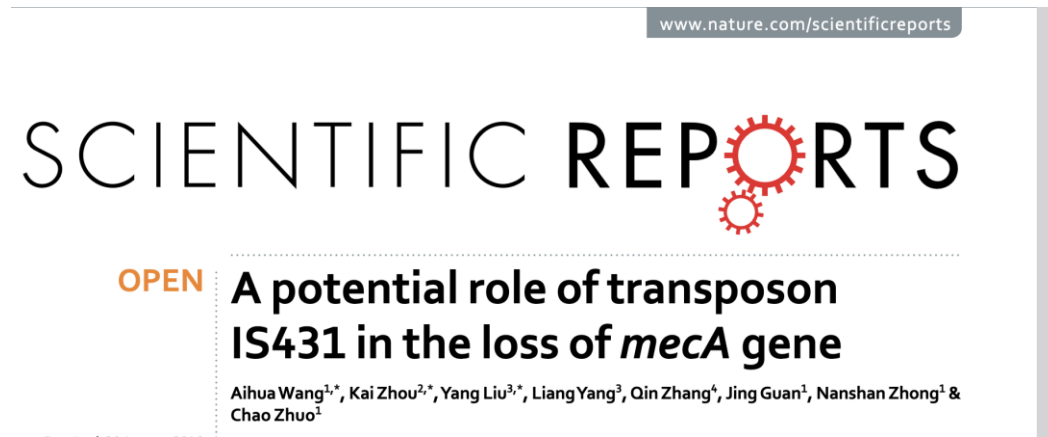
GENETIC ALTERATIONS RESULTING IN GENOTYPIC/PHENOTYPIC DISCREPANCIES

Staphylococcus aureus

Genotype	Phenotype	Description	Discrepancy reason
<i>mecA</i> detected	Methicillin susceptible	<i>mecA</i> inactivation → PBP2a aminoacidic substitution	Loss of PBP2a transpeptidase activity
<i>mecA</i> detected	Methicillin susceptible	Heterogeneously resistant large inoculum led to resistant phenotype	Oxacillin induction increased MIC
<i>mecA</i> not detected	<i>S. aureus</i> with borderline-oxacillin resistance (BORSA)	<ul style="list-style-type: none">• Beta-lactamases over-expression• PBP alterations• Adaptative mutations in cell-wall genes	Oxa R Fox S



SCENARIO 2: AMR GENE DETECTED - PHENOTYPICALLY S

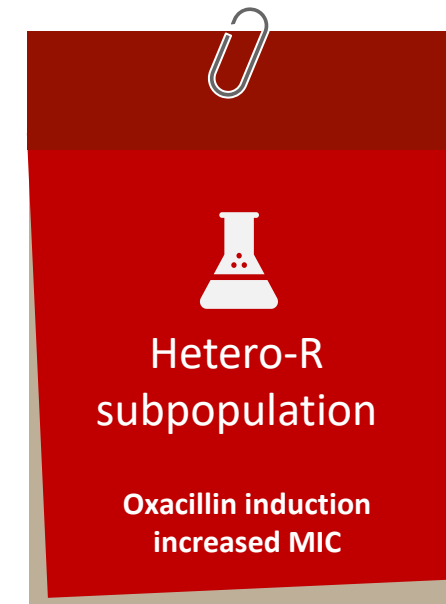


European Journal of Clinical Microbiology & Infectious Diseases (2023) 42:1125–1133
<https://doi.org/10.1007/s10096-023-04646-1>

ORIGINAL ARTICLE

Phenotypic and genomic characteristics of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus*, rapid selection of high-level resistance to beta-lactams

Vladimir Gostev^{1,2} · Ksenia Sabinova¹ · Julia Sopova^{3,4} · Olga Kalinogorskaya¹ · Ofeliia Sulian¹ · Polina Chulkova¹ · Maria Velizhanina^{4,5} · Polina Pavlova^{1,3} · Lavrentii Danilov³ · Lyudmila Kraeva⁶ · Dmitrii Polev⁶ · Elvira Martens^{1,2} · Sergey Sidorenko^{1,2}



SCENARIO 3: AMR GENE NOT DETECTED - PHENOTYPICALLY R

MEC-INDEPENDENT OXACILLIN RESISTANCE (MIOSA)

JOURNAL
OF MEDICAL
MICROBIOLOGY



2017;66:1367–

**β -lactamase
hyperproduction**

Borderline oxacillin resistance in *Staphylococcus aureus* (BORSAs) – a more common phenomenon?

Maria M. Hryniewicz* and Katarzyna M. Hryniewicz

Staphylococcus aureus (BORSAs) – a more common phenomenon?



Inactivation of the Monofunctional Peptidoglycan Glycosyltransferase SgtB Allows *Staphylococcus aureus* to Survive in the Absence of Lipoteichoic Acid

Eleni Karinou,^{a,*} Christopher F. Schuster,^a Manuel Pazos,^b Waldemar Vollmer,^b Angelika Vollmer,^b and Christopher F. Schuster,^a

**Inactivation of
the peptidoglycan
transferase *sgtB*
gene**



RESEARCH ARTICLE
Clinical Science and Epidemiology



Comprehensive Genomic Profiling of Adaptive Mutations Driving the Oxacillin Resistance Phenotype in *Staphylococcus aureus*

Stefano G. Giulieri,^{a,b}
Sarah Baines,^a Noreen M. O'Connell,^a
Timothy P. Stinear,^a

**Adaptive mutations
in core genes
related to cell-wall
metabolism**

Genomic Profiling of Adaptive Mutations Driving the Oxacillin Resistance Phenotype in *Staphylococcus aureus*

Ashleigh S. Hayes,^a Diane Daniel,^a
Xiaoliang Ba,^a Torsten Seemann,^{a,d}



Inactivation of the Monofunctional Peptidoglycan Glycosyltransferase SgtB Allows *Staphylococcus aureus* to Survive in the Absence of Lipoteichoic Acid

Eleni Karinou,^{a,*} Christopher F. Schuster,^a Manuel Pazos,^b Waldemar Vollmer,^b Angelika Vollmer,^b and Christopher F. Schuster,^a

**Inactivation of
the peptidoglycan
transferase *sgtB*
gene**

J Antimicrob Chemother 2019; 74: 1182–1191
doi:10.1093/jac/dkz013 Advance Access publication 12 February 2019

Journal of
Antimicrobial
Chemotherapy

Truncation of GdpP mediates β -lactam resistance in clinical isolates of *Staphylococcus aureus*

Xiaoliang Ba¹, Lajos Kalmar¹, Nazreen F. Hadjirin¹, Heidrun Kerschner², Petra Apfalter², Fiona J. Morgan³, Gavin K. Paterson⁴, Samantha L. Girvan¹, Rui Zhou⁵, Ewan M. Harrison^{6–8,†} and Mark A. Holmes^{1,*†}



GENETIC ALTERATIONS RESULTING IN GENOTYPIC/PHENOTYPIC DISCREPANCIES

Enterococcus faecalis and *E. faecium*

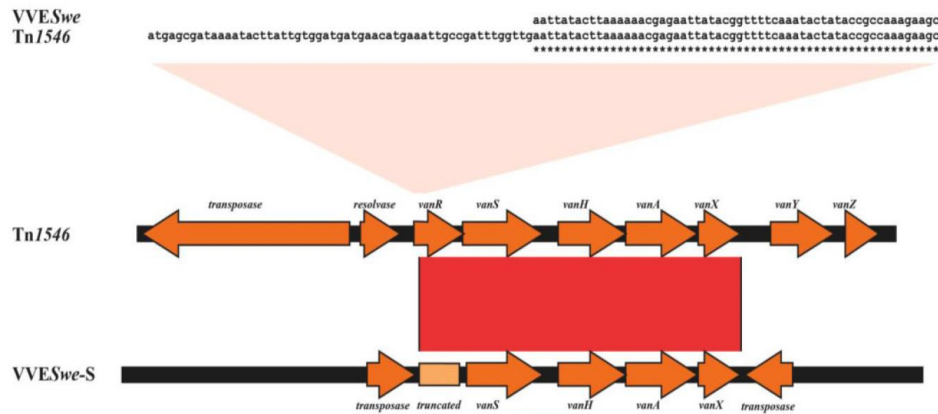
Genotype	Phenotype	Description	Discrepancy reason
<i>vanA</i> detected	Vancomycin susceptible	<ul style="list-style-type: none">• Deletions in Tn1546	<ul style="list-style-type: none">• Nonfunctional <i>vanA</i>
<i>vanA</i> detected	Vancomycin variable (VVE)	<ul style="list-style-type: none">• Rearrangement in the regulator <i>vanRS</i> locus• Silenced <i>vanA</i> (<i>vanHAX</i>) gene	<ul style="list-style-type: none">• Unexpressed <i>vanA</i> Vancomycin induction restores R
<i>vanB</i> detected	Vancomycin susceptible	<ul style="list-style-type: none">• Presence of <i>vanB</i>	<ul style="list-style-type: none">• Lower <i>vanB</i> expression levels (susceptibility range)
<i>vanAB</i> not detected	Vancomycin resistant	<ul style="list-style-type: none">• Presence of <i>vanM</i> or <i>vanD</i>	<ul style="list-style-type: none">• Not detectable by molecular target



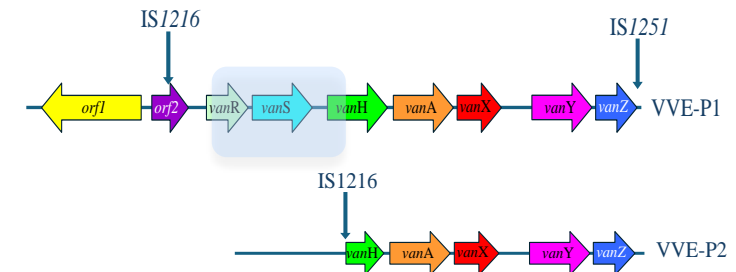
SCENARIO 2: AMR GENE DETECTED - PHENOTYPICALLY S

VANCOMYCIN VARIABLE ENTEROCOCCI (VVE)

- ✓ Vancomycin-susceptible enterococci
- ✓ *vanA* gene detected
- ✓ Rearrangement in the regulator *vanRS* locus
- ✓ Ability to revert to a constitutive VRE phenotype **under vancomycin exposure**



P201



e-POSTER
communication
Sabato 9 Marzo
ore 13:30-14:30
pannello 8



SCENARIO 3: AMR GENE NOT DETECTED - PHENOTYPICALLY R

J Antimicrob Chemother 2020; **75**: 283–291
doi:10.1093/jac/dkz461 Advance Access publication 19 November 2019

Journal of
Antimicrobial
Chemotherapy

Tandem amplification of the *vanM* gene cluster drives vancomycin resistance in vancomycin-variable enterococci

Lingyan Sun^{1,2,†}, Yan Chen^{1,2,†}, Xiaoting Hua^{1,2}, Yiyi Chen^{1,2}, Jinjing Hong³, Xueqing Wu^{1,2}, Yan Jiang^{1,2}, Willem van Schaik⁴, Tingting Qu^{3,‡} and Yunsong Yu^{1,2,‡*}

Received: 26 February 2023 | Revised: 15 March 2023 | Accepted: 16 March 2023
DOI: 10.1111/1440-1681.13772

ORIGINAL ARTICLE

CEPR Clinical and Experimental
Pharmacology and Physiology WILEY

Disk diffusion-based method aids in the detection of *vanM*-positive *Enterococcus faecium* with low vancomycin minimum inhibitory concentrations

Jue Wang^{1,2} | Pei Li^{1,2} | Lin Xin^{1,2} | Xiaogang Xu^{1,2,3} | Minggui Wang^{1,2,3}

vanM & *vanD*

2023 Mar 24;5(2):dlad026.

JAC Antimicrob Resist
<https://doi.org/10.1093/jacamr/dlad026>

JAC-
Antimicrobial
Resistance

Characterization of vancomycin-resistance *vanD* gene clusters in the human intestinal microbiota by metagenomics and culture-enriched metagenomics

Elie Brochu¹, Ann Huletsky¹, Dominique K. Boudreau¹, Frédéric Raymond^{2,3}, Ève Bérubé¹, Amin Ahmed Ouameur¹, Johanne Frenette¹, Maurice Boissinot¹, Jacques Corbeil^{4,5} and Michel G. Bergeron^{1*}



Journal of Microbiological Methods
Volume 204, January 2023, 106646



Check the melting temperature of the FilmArray BCID panel to avoid missed detection of *vanM*-type enterococci

Ying Zhou^{a,1}, Yang Yang^{a,1}, Jing Wu^c, Yan Guo^a, Renru Han^a, Xiaogang Xu^{a,b,d}

2022 Jan 4;4(1):dlab189.

JAC Antimicrob Resist
<https://doi.org/10.1093/jacamr/dlab189>

JAC-
Antimicrobial
Resistance

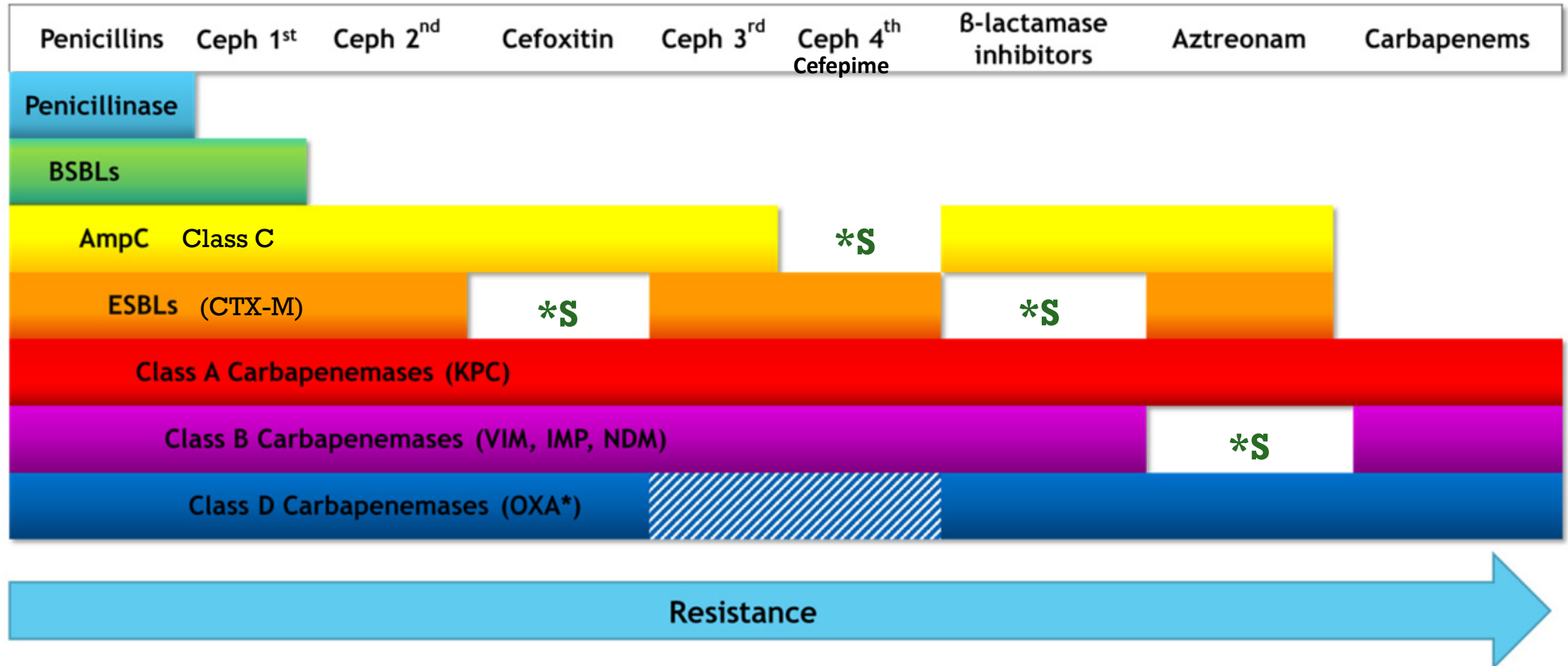
Elucidation of host diversity of the *VanD*-carrying genomic islands in enterococci and anaerobes

Yusuke Hashimoto¹, Junzo Hisatsune^{2,3,4}, Masato Suzuki⁴, Jun Kurushima¹, Takahiro Nomura¹, Hidetada Hirakawa¹, Naoko Kojima⁵, Yuichi Ono⁵, Yutaka Hasegawa⁵, Koichi Tanimoto⁶, Motoyuki Sugai^{2,3,4} and Haruyoshi Tomita^{1,6*}



DIAGNOSTIC AND THERAPEUTIC CHALLENGES IN ENTEROBACTERIALES

IMPORTANT TO DIFFERENTIATE BETWEEN CARBAPENEM RESISTANT AND NON CARBAPENEM RESISTANT



ESBL *versus* AmpC BETA-LACTAMASES

CTX-M **POSITIVO**

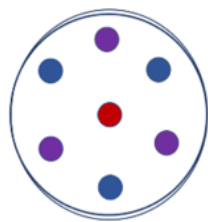
KPC **negativo**

OXA 48 **negativo**

VIM **negativo**

IMP **negativo**

NDM **negativo**



<i>E.coli</i> ESBL	MIC	SIR
Amoxicillina/ac clavulanico	32	R
Ampicillina	>8	R
Cefoxitina	<=8	S
Cefepime	>8	R
Cefotaxime	>32	R
Ceftazidime	32	R
Ceftazime/avibactam	<=2	S
Ceftolozano/tazobactam	<=1	S
Ciprofloxacina	>1	S
Ertapenem	<=0.12	S
Gentamicina	<=2	S
Meropenem	<=0.12	S
Piperacillina/tazobactam	<=8	S
Sulfa/trimethropim	<=2/38	S

CTX-M **negativo**

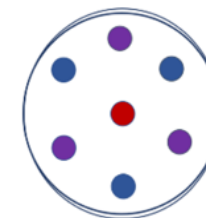
KPC **negativo**

OXA 48 **negativo**

VIM **negativo**

IMP **negativo**

NDM **negativo**



<i>E.cloacae</i> AmpC cromosomica	MIC	SIR
Amoxicillina/ac clavulanico	32	R
Ampicillina	>8	R
Cefoxitina	>16	R
Cefepime	<=8	S
Cefotaxime	16	R
Ceftazidime	16	R
Ceftazime/avibactam	<=2	S
Ceftolozano/tazobactam	<=1	S
Ciprofloxacina	<=0.06	S
Ertapenem	0.5	S
Gentamicina	<=2	S
Meropenem	<=0.12	S
Piperacillina/tazobactam	16	R
Sulfa/trimethropim	<=2/38	S

Clinically relevant bacteria
mediated chromosomally
inducible AmpC β Ls:
SPACE

- *Serratia* spp.
- *Pseudomonas* spp.
- *Acinetobacter* spp.
- *Citrobacter* spp
- *Enterobacter* spp (CTX)

*Cefoxitin hydrolysis → to differentiate AmpC from ESBLs

BLIC IN DIFFERENT COMBINATIONS.....

**BUT NONE OF THESE INHIBITORS WORK AGAINST
METALLO-BETA-LACTAMASES**

Mechanism	CZA	M/V	C/T	I/R	ATM/AVI	FDC
KPC	+	+	-	+	+	+
OXA-48	+	-	-	-	+	+
VIM	-	-	-	-	+	+
IMP	-	-	-	-	+	+
NDM	-	-	-	-	+	+



SCENARIO 1: GENOTYPE CORRELATES WITH PHENOTYPE

Klebsiella pneumoniae – CTX-M

CTX-M **POSITIVO**
KPC **negativo**
OXA 48 **negativo**
VIM **negativo**
IMP **negativo**
NDM **negativo**



ANTIBIOTICO	MIC	
Amoxicillina/ac.clavulanico	>32	R
Piperacillina/tazobactam	>16	R
Cefotaxime	>32	R
Ceftazidime	>32	R
Cefepime	>8	R
Aztreonam	>4	R
Gentamicina	<=2	S
Amikacina	<=8	S
Ciprofloxacina	>1	R
Levofloxacina	>1	R
Colistina	<=2	S
Imipenem	<=1	S
Ertapenem	0.5	S
Meropenem	<=0.12	S
Ceftolozano/Tazobactam	<=1	S
Ceftazidime/Avibactam	<=2	S

Klebsiella pneumoniae - KPC

CTX-M **POSITIVO**
KPC **POSITIVO**
OXA 48 **negativo**
VIM **negativo**
IMP **negativo**
NDM **negativo**



ANTIBIOTICO	MIC	
Amoxicillina/ac.clavulanico	>32	R
Piperacillina/tazobactam	>16	R
Cefotaxime	>32	R
Ceftazidime	>32	R
Cefepime	>8	R
Aztreonam	>4	R
Gentamicina	>4	R
Amikacina	<=8	S
Ciprofloxacina	>1	R
Levofloxacina	>1	R
Colistina	<=2	S
Imipenem	4	R
Ertapenem	>1	R
Meropenem	16	R
Ceftolozano/Tazobactam	>4	R
Ceftazidime/Avibactam	<=2	S

Klebsiella pneumoniae - VIM

CTX-M **POSITIVO**
KPC **POSITIVO**
OXA 48 **negativo**
VIM **POSITIVO**
IMP **negativo**
NDM **negativo**



ANTIBIOTICO	MIC	
Amoxicillina/ac.clavulanico	>32	R
Piperacillina/tazobactam	>16	R
Cefotaxime	>32	R
Ceftazidime	>32	R
Cefepime	>8	R
Aztreonam	>4	R
Gentamicina	>4	R
Amikacina	>16	R
Ciprofloxacina	>1	R
Levofloxacina	>1	R
Colistina	1	S
Imipenem	8	R
Ertapenem	>1	R
Meropenem	>64	R
Ceftolozano/Tazobactam	>4	R
Ceftazidime/Avibactam	>8	R

SCENARIO 2: AMR GENE DETECTED - PHENOTYPICALLY S

ESCHERICHIA COLI DOUBLE POPULATION



DEFINITIVE AMR (+ 48h)

Preliminary genotypic AMR

Phenotypic assays



1h



24h



CTX-M **POSITIVO**
KPC **negativo**
OXA 48 **negativo**
VIM
IMP **negativo**
NDM **negativo**

Double population
- *E.coli* Wild type
- *E.coli* CTX-M

ANTIBIOTICI	<i>E.coli</i> Wild type		<i>E.coli</i> CTX-M	
	MIC	RIS	MIC	RIS
Ampicillina	<=2	S	>8	R
Amoxicillina/Ac. Clav	<=2/1	S	4/2	S
Piperacil/tazobactam	<=4	S	<=4	S
Ceftaxidime	<=0.5	S	>64	R
Cefotaxime	<=0.5	S	8	R
Cefepime	<=0.5	S	>8	R
Aztreonam	<=1	S	>4	R
Meropenem	<=0.12	S	<=0.12	S
Imipenem	<=1	S	<=1	S
Ertapenem	<=0.12	S	<=0.12	S
Amikacina	<=8	S	<=8	S
Ciprofloxacina	<=0.25	S	>1	R
Levofloxacina	<=0.12	S	>1	R
Cotrimossazolo	<=2/38	S	>4/76	R
Claramfenicolo	<=8	S	<=8	S
Colistina	<=2	S	1	S
Tigeciclina	<=0.25	S	<=0.25	S

SCENARIO 2: AMR GENE DETECTED - PHENOTYPICALLY S

Klebsiella pneumoniae – **KPC + VIM**

Preliminary genotypic AMR



1h



CTX-M	negativo
KPC	POSITIVO
OXA 48	negativo
VIM	POSITIVO
IMP	negativo
NDM	negativo

SUSCEPTIBLE TO.... ?

DEFINITIVE AMR (+ 48h)



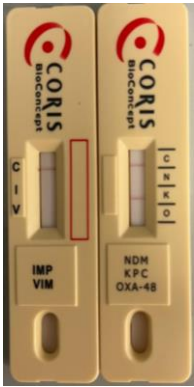
ANTIBIOTICO	MIC	
Amoxicillina/ac.clavulanico	>16	R
Piperacillina/tazobactam	>128	R
Cefotaxime	>4	R
Ceftazidime	>128	R
Cefepime	>8	R
Gentamicina	>4	R
Tigeciclina	0.5	*
Ciprofloxacina	>4	R
Colistina	1	S
Fosfomicina	32	S
Ceftalozano/Tazobactam	>4	R
Meropenem	>32	R
Ceftazidime/Avibactam	4	S
Meropenem/Vaborbactam	1	S
Cefiderocol	0.5	S

Carbapenemase detection

Immunochromatographic assay:

KPC: Positivo

VIM: Negativo



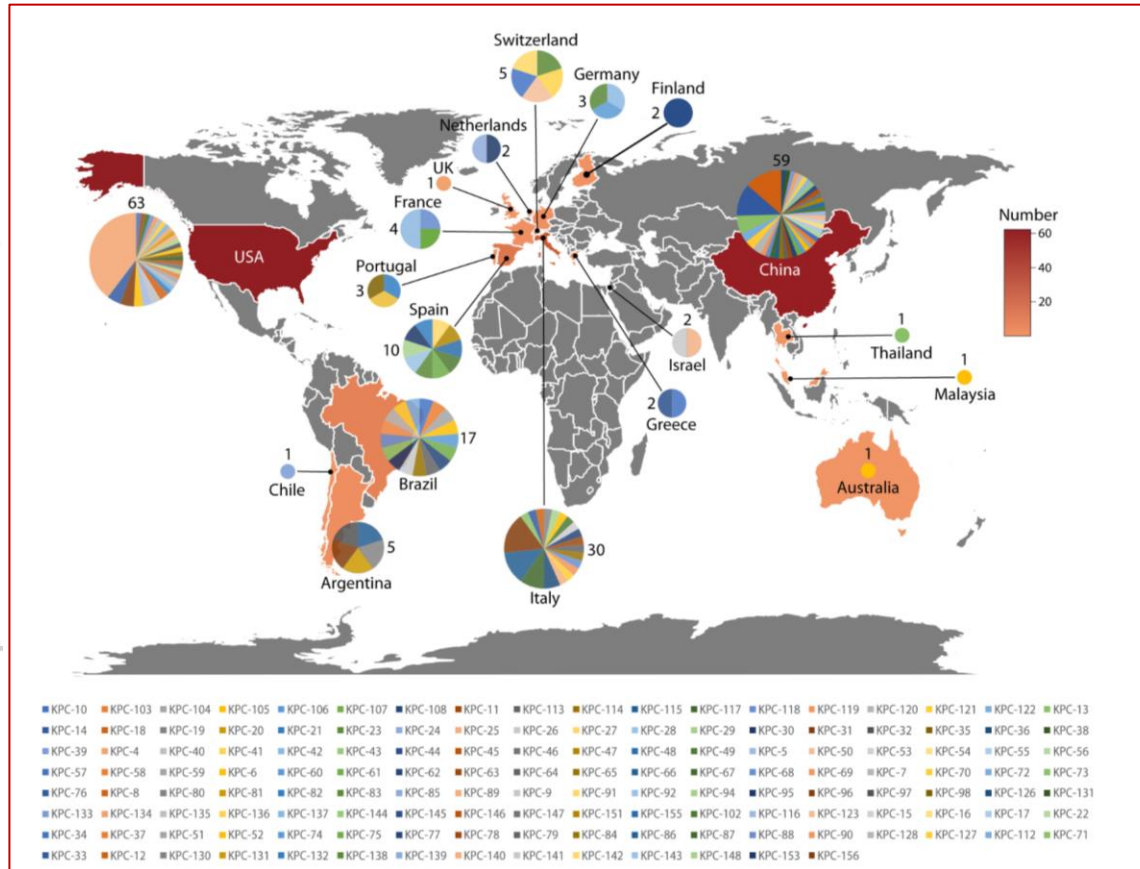
INTERPRETATION

WGS:
*bla*_{KPC-3}
*bla*_{VIM-1::IS26}

EMERGENCE OF KPC VARIANTS

INABILITY TO INFER CAZ/AVI SUSCEPTIBILITY FROM *bla*_{KPC} GENE DETECTION

More than 150 *bla*_{KPC} variants have been reported worldwide



Ding L, et al. Clin Microbiol Rev. 2023.

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 15 November 2023
DOI 10.3389/fmicb.2023.1261261

Check for updates

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Fudan University, China
Shangshang Qin,
Zhengzhou University, China

*CORRESPONDENCE
Jiachang Cai

Mobilization of the *bla*_{KPC-14} gene among heterogenous plasmids in extensively drug-resistant hypervirulent *Klebsiella pneumoniae*

Lin Wang¹, Weiyei Shen¹ and Jiachang Cai^{1*}

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MECHANISMS OF RESISTANCE

Check for updates

In Vitro Selection of Meropenem Resistance among Ceftazidime-Avibactam-Resistant, Meropenem-Susceptible *Klebsiella pneumoniae* Isolates with Variant KPC-3 Carbapenemases

Ryan K. Shields,^{a,b} M. Hong Nguyen,^{a,b} Ellen G. Press,^a Liang Chen,^a Barry N. Kreiswirth,^a Cornelius J. Clancy^{a,b,d}

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MECHANISMS OF RESISTANCE

Check for updates

Mutations in *bla*_{KPC-3} That Confer Ceftazidime-Avibactam Resistance Encode Novel KPC-3 Variants That Function as Extended-Spectrum β -Lactamases

Ghady Haidar,^a Cornelius J. Clancy,^{b,c,d} Ryan K. Shields,^{b,c} Binghua Hao,^a Shaohui Cheng,^b M. Hong Nguyen^{a,b,c}

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

RESEARCH ARTICLE
Month YYYY Volume XX Issue XX e04111-23

Check for updates

KPC-2 allelic variants in *Klebsiella pneumoniae* isolates resistant to ceftazidime-avibactam from Argentina: *bla*_{KPC-80}, *bla*_{KPC-81}, *bla*_{KPC-96} and *bla*_{KPC-97}

María Belén Sanz¹, Fernando Pasteran¹, Juan Manuel de Mendieta¹, Florencia Brunetti^{2,3}, Ezequiel Albornoz¹, Melina Rapoport¹, Celeste Lucero¹, Laura Errecalde⁴, María Rosa Nuñez⁵, Renata Monge⁶, Magdalena Pennini⁷, Pablo Power^{2,3}, Alejandra Corso¹, Sonia A. Gomez^{1,3}

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 21 August 2023
DOI 10.3389/fcimb.2023.1244511

Check for updates

OPEN ACCESS

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Dynamic evolution of ceftazidime-avibactam resistance due to interchanges between *bla*_{KPC-2} and *bla*_{KPC-145} during treatment of *Klebsiella pneumoniae* infection

Yili Chen¹, Runshi Yang², Penghao Guo¹, Pingjuan Liu¹, Jiankai Deng¹, Zhongwen Wu¹, Qingping Wu², Junqi Huang^{1,3,4,5*} and Kang Liao^{6*}

AMERICAN SOCIETY FOR MICROBIOLOGY | mSystems

Open Peer Review | Evolution | Research Article

Genome sequencing unveils *bla*_{KPC-2}-harboring plasmids as drivers of enhanced resistance and virulence in nosocomial *Klebsiella pneumoniae*

Xinhong Han,^{1,2,3} Junxin Zhou,^{1,2,3} Lifei Yu,⁴ Lina Shao,¹ Shiqi Cai,^{1,2,3} Huangdu Hu,^{1,2,3} Qiucheng Shi,^{1,2,3} Zhengnan Wang,^{1,2,3} Xiaoting Hua,^{1,2,3} Yan Jiang,^{1,2,3} Yunsong Yu^{1,2,3}

SCENARIO 2: AMR GENE DETECTED - PHENOTYPICALLY S

Klebsiella pneumoniae – KPC + CTX-M

Preliminary genotypic AMR



1h



CTX-M **POSITIVO**
KPC **POSITIVO**
OXA 48 **negativo**
VIM **negativo**
IMP **negativo**
NDM **negativo**

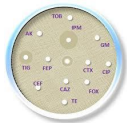
SUSCEPTIBLE TO....

?

RESISTENT TO....

?

DEFINITIVE AMR (+ 48h)



ANTIBIOTICO	MIC	
Amoxicillina/ac.clavulanico	>32	R
Piperacillina/tazobactam	16	I
Cefotaxime	>32	R
Ceftazidime	>32	R
Cefepime	>8	R
Aztreonam	>4	R
Gentamicina	>4	R
Amikacina	<=8	S
Ciprofloxacina	>1	R
Levofloxacina	>1	R
Colistina	<=2	S
Imipenem	<=1	S
Ertapenem	<=0.12	S
Meropenem	<=0.12	S
Ceftolozano/Tazobactam	>4	R
Ceftazidime/Avibactam	>8	R

INTERPRETATION

***bla*_{KPC-166}**

1. Loss of carbapenemasic activity
2. Increased affinity to ceftazidime

CAZ/AVI Resistance



BETA-LACTAM RESISTANCE IN *Pseudomonas aeruginosa* IS COMPLEX AND MULTIFACETED



ELSEVIER

International Journal of Antimicrobial Agents

Volume 45, Issue 5, May 2015, Pages 529-532



Short Communication

Carbapenem resistance in cystic fibrosis strains of *Pseudomonas aeruginosa* as a result of amino acid substitutions in **porin OprD**



Charlotte Richardot^a, Patrick Plésiat^a, Damien Fournier^a, Laura Monlezun^b, Isabelle Broutin^b, Catherine Llanes^a  



antibiotics

Article

Genomic Analysis of Ceftazidime/Avibactam-Resistant GES-Producing Sequence Type 235 *Pseudomonas aeruginosa* Isolates

Raúl Recio^{1,*} , Jennifer Villa¹ , Sara González-Bodí¹, Patricia Brañas¹, María Ángeles Orellana¹, Mikel Mancheño-Losa^{2,3}, Jaime Lora-Tamayo^{2,3}, Fernando Chaves¹ and Esther Viedma¹



AMERICAN SOCIETY FOR MICROBIOLOGY

Antimicrobial Agents and Chemotherapy

AAC 2019

Acquisition of Extended-Spectrum β -Lactamase **GES-6** Leading to Resistance to Ceftolozane-Tazobactam Combination in *Pseudomonas aeruginosa*

Laurent Poiriel^{a,b,c}, José-Manuel Ortiz De La Rosa^{a,b}, Nicolas Kieffer^{a,c}, Véronique Dubois^{d,e}, Aurélie Jayol^{a,b,c,f}, Patrice Nordmann^{a,b,c,g}

Arch Microbiol (2016) 198:565–571
DOI 10.1007/s00203-016-1215-7

ORIGINAL PAPER

Overexpression of **MexAB-OprM efflux pump** in carbapenem-resistant *Pseudomonas aeruginosa*

Ya-ping Pan¹ · Yuan-hong Xu¹ · Zhong-xin Wang¹ · Ya-ping Fang² · Ji-lu Shen¹

- Cell permeability
- Drug uptake and PBP binding
- Reduction of the effective drug concentration



AMERICAN SOCIETY FOR MICROBIOLOGY



RESEARCH ARTICLE

Molecular Biology and Physiology

November/December 2019 Volume 4 Issue 6 10.1128/mSystems.00524-19
<https://doi.org/10.1128/mSystems.00524-19>

Regulation of **AmpC-Driven β -Lactam Resistance** in *Pseudomonas aeruginosa*: Different Pathways, Different Signaling

Gabriel Torrens^a, Sara Belén Hernández^b, Juan Alfonso Ayala^c, Bartolome Moya^{a,d} , Carlos Juan^a , Felipe Cava^b, Antonio Oliver^a 

Antimicrobial Agents and Chemotherapy

AAC 2023

Characterization of *Pseudomonas aeruginosa* resistance to ceftolozane-tazobactam due to **ampC and/or ampD** mutations observed during treatment using semi-mechanistic PKPD modeling

Luc Deroche^{1,2,3}, Vincent Aranzana-Climent¹, Albane Rozenholc¹, Laure Prouvensier^{1,4}, Léa Darnaud¹, Nicolas Grégoire^{1,4}, Sandrine Marchand^{1,4}, Marie-Cécile Ploy^{3,5}, Bruno François^{3,6,7}, William Couet^{1,4}, Olivier Barraud^{3,5,7}, Julien M. Buyck¹

AmpC over-expression, under induction

SCENARIO 3: AMR GENE NOT DETECTED - PHENOTYPICALLY R

Pseudomonas aeruginosa — GES

Preliminary genotypic AMR



1h



CTX-M **negativo**
KPC **negativo**
OXA 48 **negativo**
VIM **negativo**
IMP **negativo**
NDM **negativo**

RESISTANCE TO....

DEFINITIVE AMR (+ 48h)



ANTIBIOTICO	MIC	
Piperacillina/tazobactam	16	R
Ceftazidime	>8	R
Cefepime	>8	R
Aztreonam	16	I
Gentamicina	>16	R
Ciprofloxacina	>16	R
Colistina	≤1	S
Imipenem	>32	R
Meropenem	>32	R
Ceftolozano/Tazobactam	>8	R
Ceftazidime/Avibactam	4	S
Meropenem/Vaborbactam	>16	R

INTERPRETATION

bla_{GES}

Plasmid/Integron carried or
chromosomally encoded (ST235)

1. CAZ/AVI → **good** *in vitro* activity
2. Meropenem/vaborbactam → **poor** activity, similar to meropenem alone



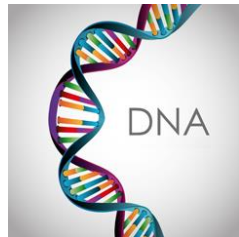
SCENARIO 3: AMR GENE NOT DETECTED - PHENOTYPICALLY R

Pseudomonas aeruginosa — **AMP_C**

Preliminary genotypic AMR



1h



CTX-M negativo
KPC negativo
OXA 48 negativo
VIM negativo
IMP negativo
NDM negativo

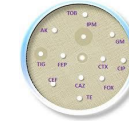
RESISTANCE TO....



SUSCEPTIBLE TO....



DEFINITIVE AMR (+ 48h)



ANTIBIOTICO	MIC	
Piperacillina/tazobactam	32	R
Ceftazidime	>64	R
Cefepime	8	I
Aztreonam	>4	R
Gentamicina	>4	R
Ciprofloxacina	>1	R
Colistina	>=2	S
Imipenem	2	S
Meropenem	4	S
Ceftolozano/Tazobactam	4	S
Ceftazidime/Avibactam	8	S
Meropenem/Vaborbactam	4	S
Cefiderocol	<2	S

INTERPRETATION

Amp_C
over-expression



**THESE DISCREPANCIES COULD IMPACT PATIENT
TREATMENT BY CAUSING INAPPROPRIATE
DE-ESCALATION OF ANTIMICROBIALS OR
UNNECESSARY EXPOSURE TO
BROAD-SPECTRUM
THERAPY**





**Take
home message*

WHAT CAN LABS DO
TO SOLVE
DISCREPANCIES?

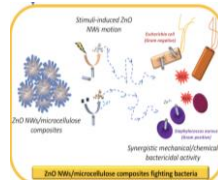
- ✓ **HARMONIZE GENOTYPE-TO-PHENOTYPE RESULTS**
- ✓ **CRITICALLY JUSTIFY DISCREPANCIES**
- ✓ **REPORT AS “**R**” IF A DISCREPANCY IS NOT RESOLVED**
- ✓ **CLEAR RESULT COMMUNICATION**



ACKNOWLEDGEMENT



PRIN 2022 N. 2022WZK874
SOTERIA project



8TH MARCH
happy women's day



“È qui con suo marito?”
“Sono io mio marito”.
Rita Levi Montalcini.

THANK YOU FOR YOUR ATTENTION