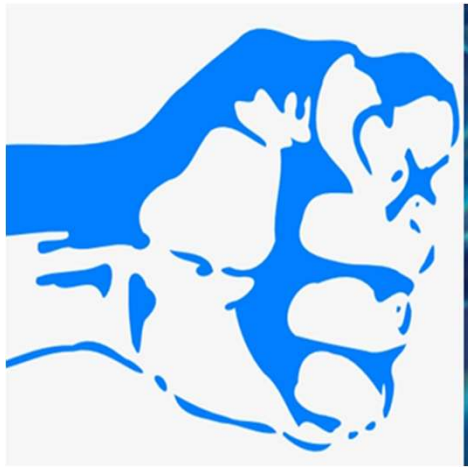
ΔΙΑΤΜΗΜΑΤΙΚΟ ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

«ΕΦΑΡΜΟΓΕΣ ΤΗΣ ΒΙΟΛΟΓΙΑΣ ΣΤΗΝ ΙΑΤΡΙΚΗ»

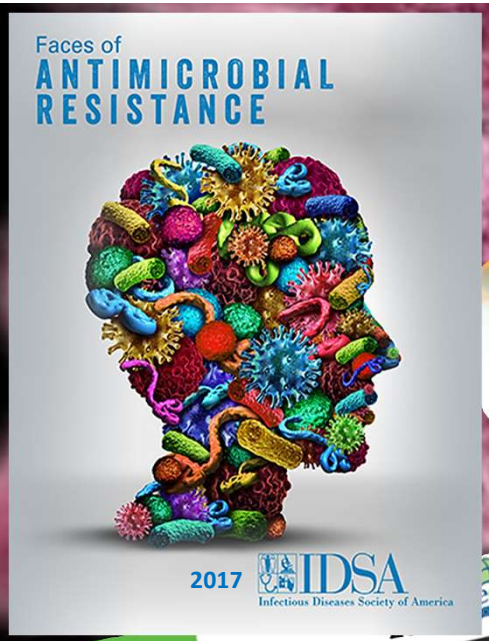
Μάθημα: Μικροβιολογία και Δημόσια Υγεία

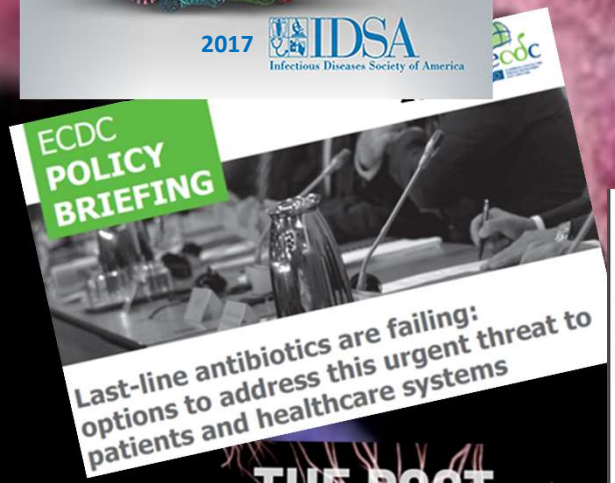


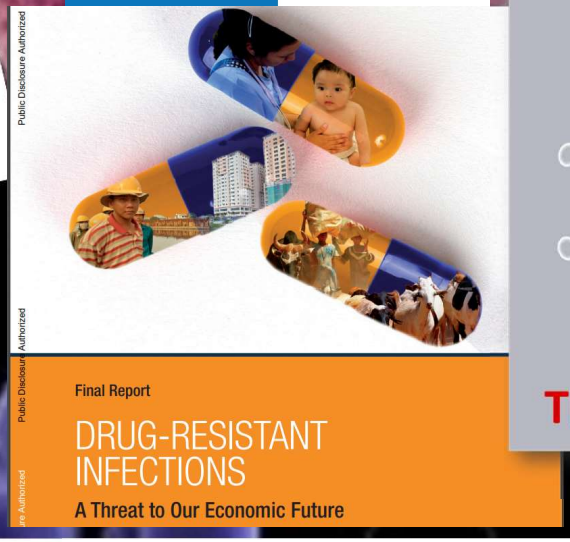
Μέθοδοι διάγνωσης της μικροβιακής αντοχής

Ολυμπία Ζαρκωτού,

Διευθύντρια Μικροβιολογικού Τμήματος, Γ.Ν. Πειραιά «Τζάνειο»







Αν το πρόβλημα της αντοχής δεν αντιμετωπιστεί

>10 εκατ. θάνατοι ετησίως

το 2050 θα πεθαίνει
ένας άνθρωπος κάθε 3 δευτερόλεπτα
εξαιτίας ανθεκτικών λοιμώξεων


AMR
a "slow-moving
tsunami"

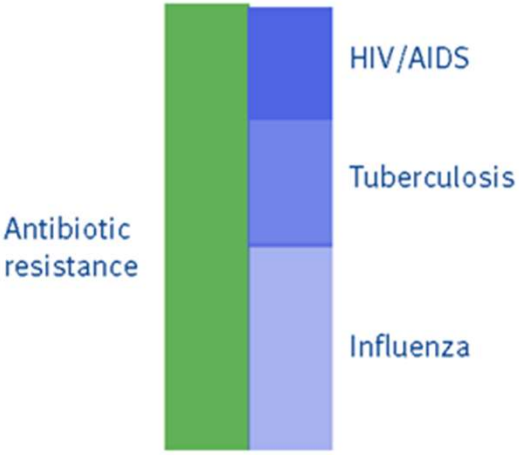
EUROPEAN ANTIBIOTIC AWARENESS DAY
A EUROPEAN HEALTH INITIATIVE

ecdc

Antibiotic resistance: a growing threat to human health

Antibiotic resistance is the ability of bacteria to combat the action of one or more antibiotics. Bacteria, not humans or animals, become antibiotic-resistant.

In Europe, the health impact of antibiotic resistant infections is comparable to that of influenza, tuberculosis and HIV/AIDS combined.



More than 35000 deaths

Each year, more than 35 000 people die from antibiotic-resistant infections in the European Union, Iceland and Norway. This is **equivalent to the number of passengers on 13 cruise ships.**

Antibiotic resistance is a silent pandemic and a growing threat to human health.

13 cruise ships

Over 70 %

healthcare-associated infections

Over 70% of the health impact of antibiotic-resistant infections is directly linked to healthcare-associated infections. This could be minimized through adequate infection prevention and control measures, as well as antibiotic stewardship in healthcare settings.

Increasing burden

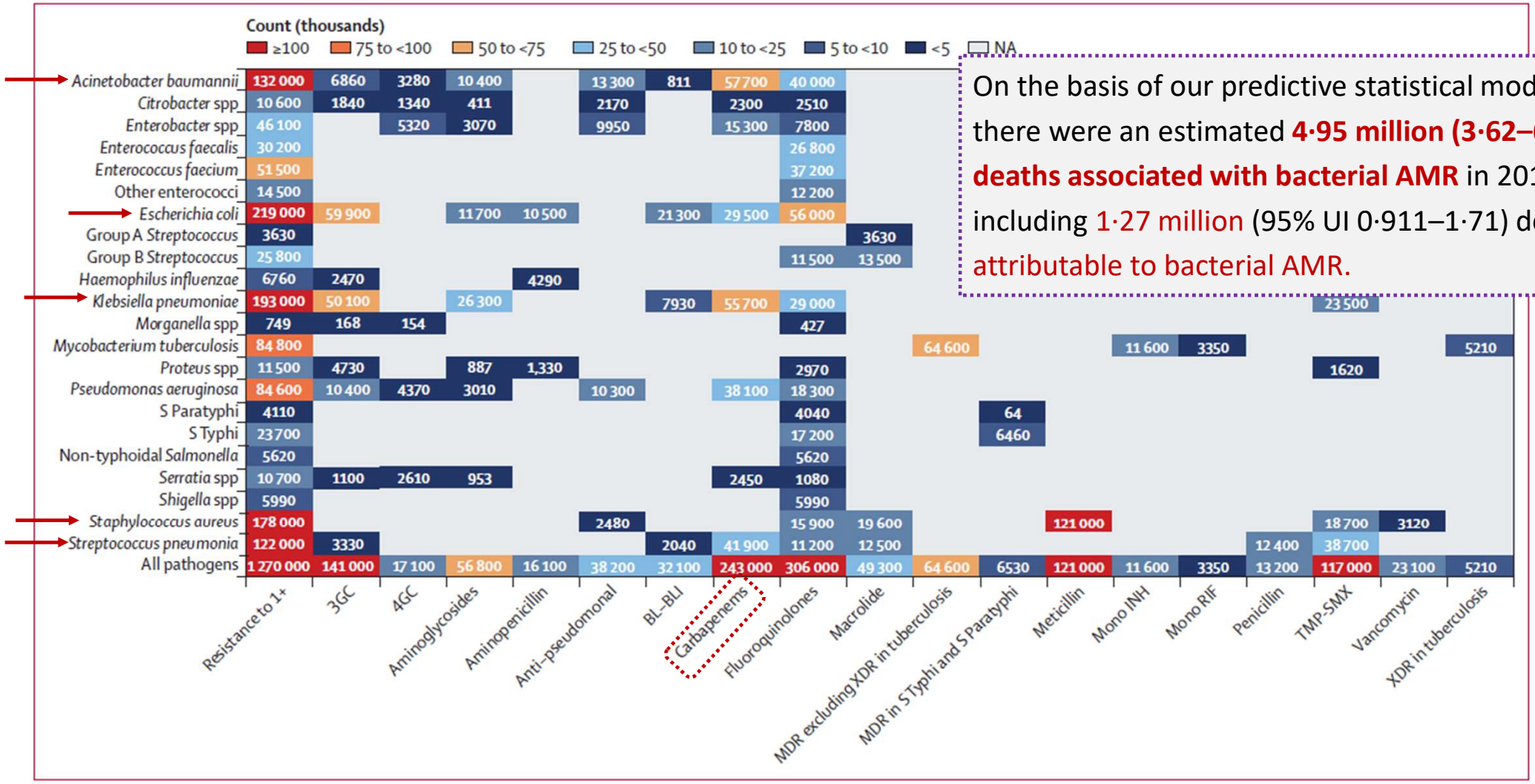
Resistance to antibiotics that are used as last line for treatment of infections, such as the carbapenems, has the highest health impact.

Between 2016 and 2020, the overall number of deaths caused by antibiotic-resistant bacteria under study has increased.

For carbapenem-resistant *Klebsiella pneumoniae* and *Acinetobacter* spp, commonly causing healthcare-associated infections, the number of attributable deaths increased by approximately 50%.

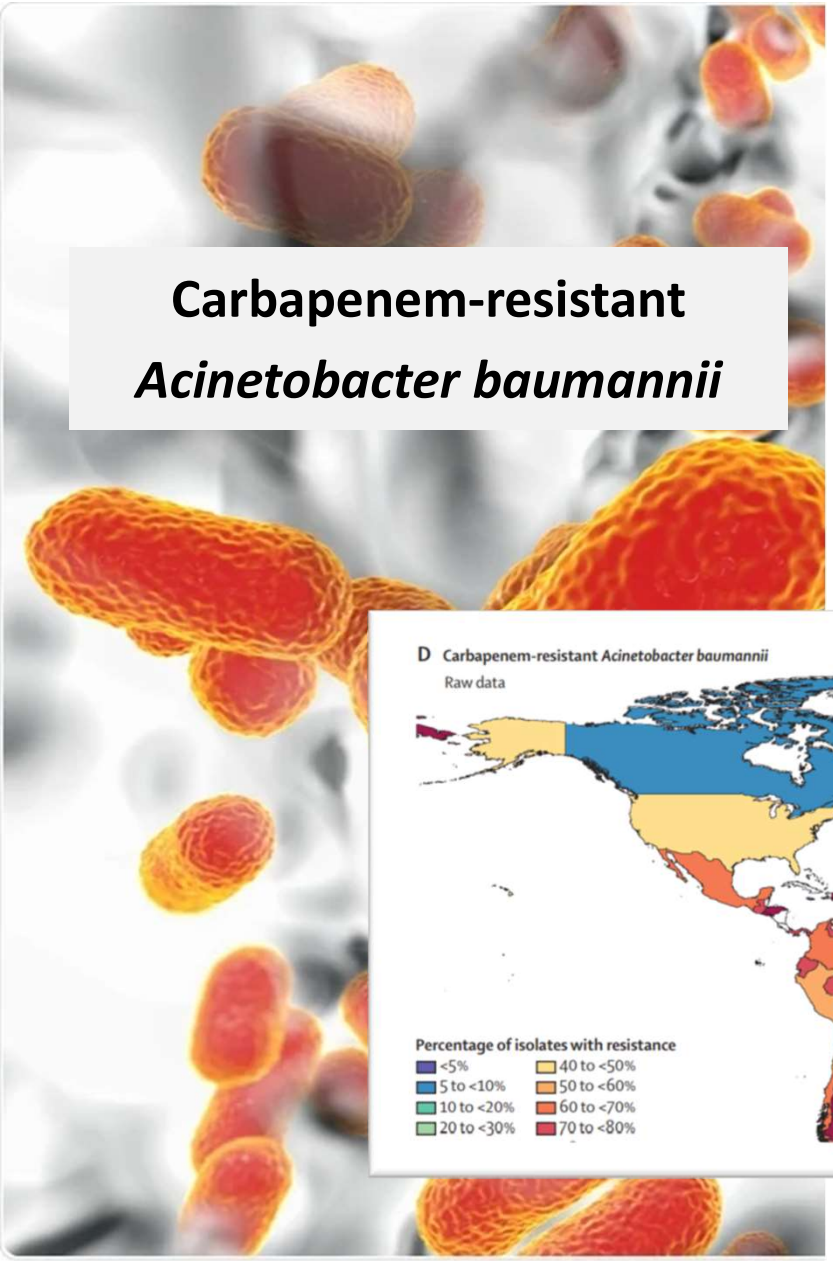
https://antibiotic.ecdc.europa.eu/en/publications-data/antibiotic-resistance-growing-threat-human-health

Global deaths (counts) attributable to bacterial AMR by pathogen–drug combination, 2019

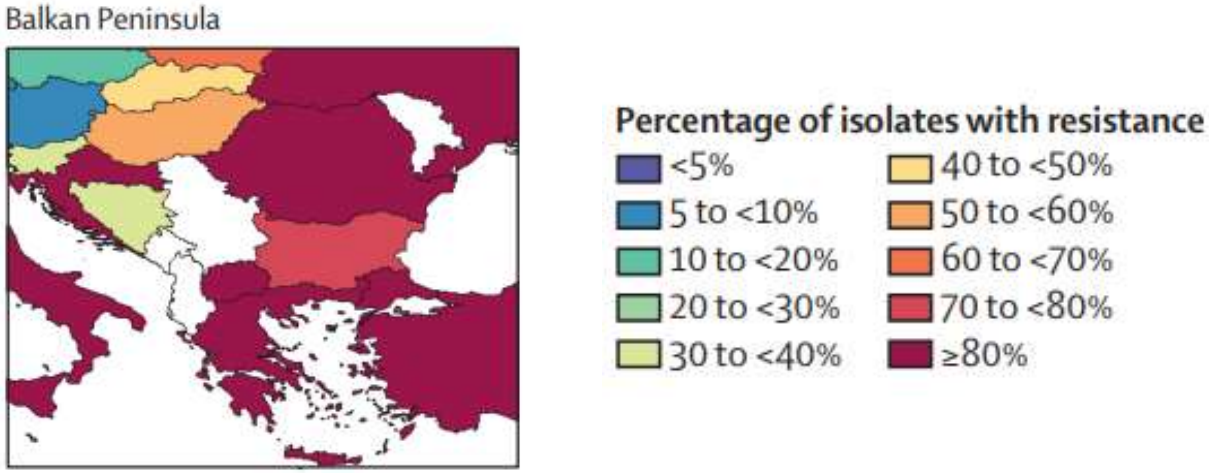


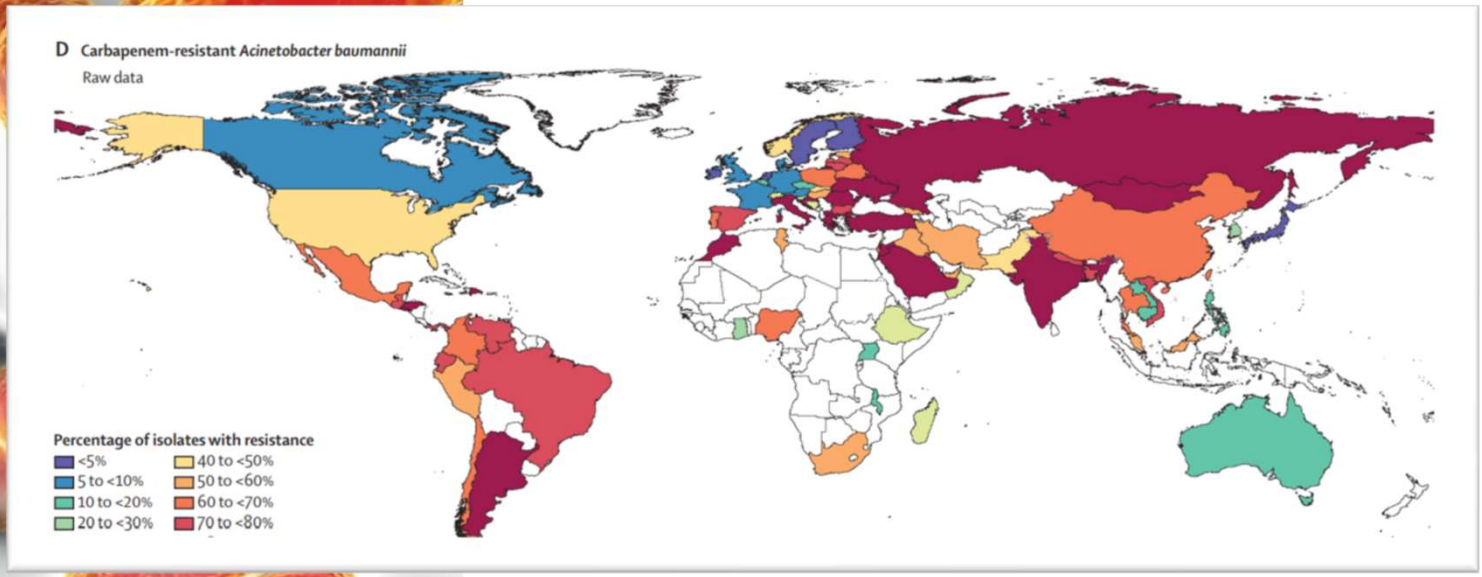
On the basis of our predictive statistical models, there were an estimated **4·95 million (3·62–6·57) deaths associated with bacterial AMR** in 2019, including 1·27 million (95% UI 0·911–1·71) deaths attributable to bacterial AMR.

Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-655.

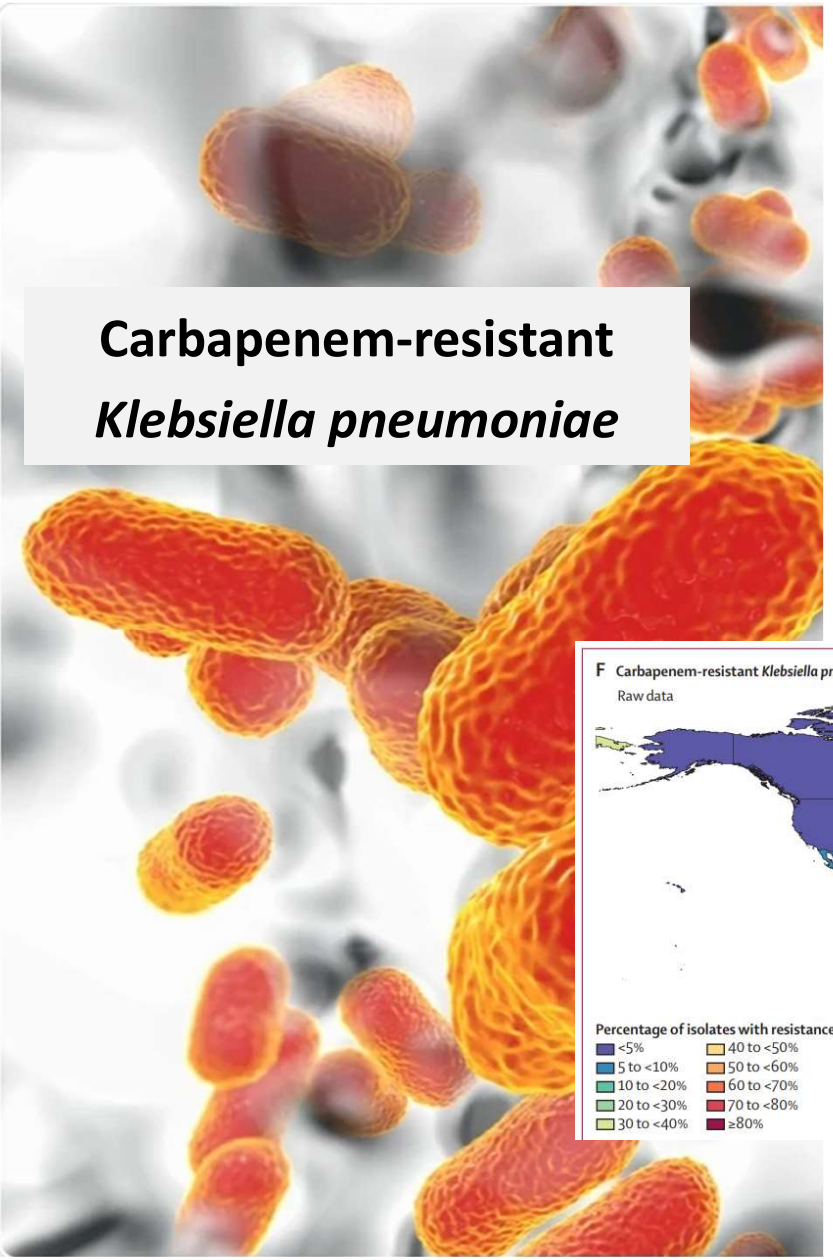


Carbapenem-resistant *Acinetobacter baumannii*

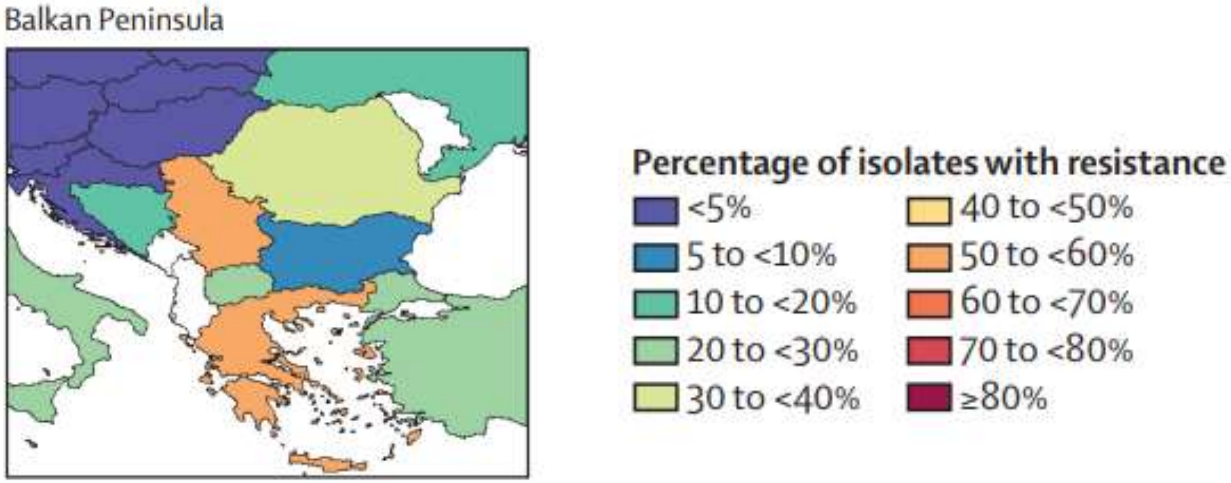


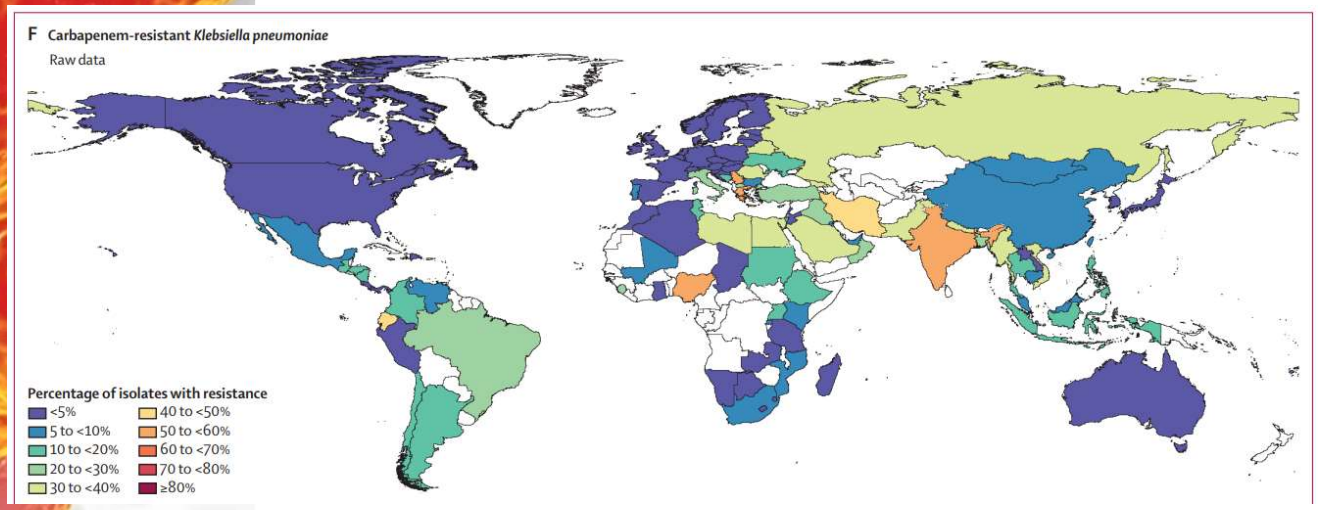


Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet, 2022, 399: 629-55



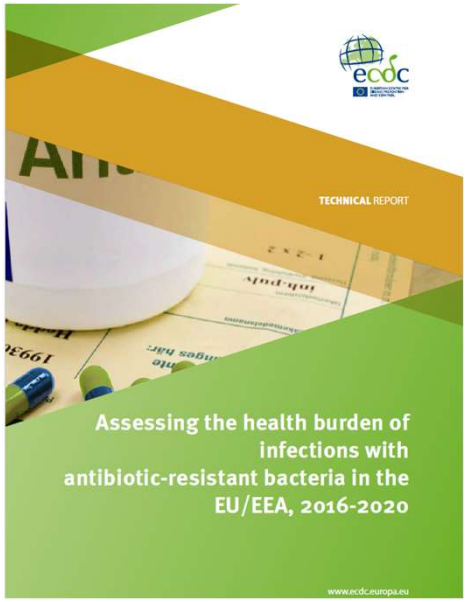
Carbapenem-resistant *Klebsiella pneumoniae*

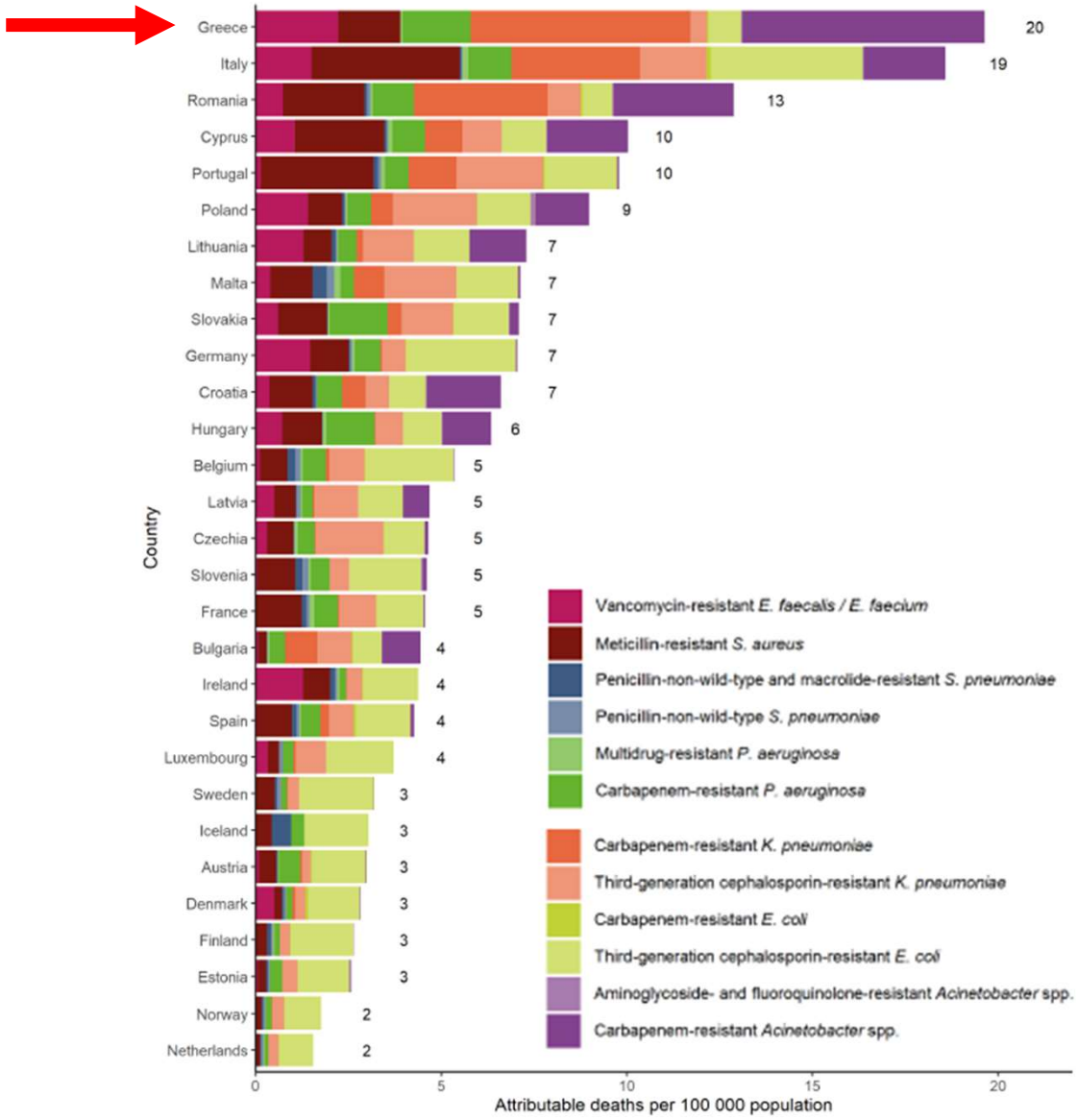




Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet, 2022, 399: 629-55

Estimations of the burden of infections with antibiotic-resistant bacteria presented as attributable deaths per 100 000 population by country*, EU/EEA, 2020





Assessing the health burden of infections with antibiotic-resistant bacteria in the EU/EEA, 2016-2020. Stockholm: ECDC; 2022.

Στόχοι μικροβιακής αντοχής: Ελλάδα

https://antibiotic.ecdc.europa.eu/en/publications-data/country-sheet-2024-greece

EUROPEAN ANTIBIOTIC AWARENESS DAY
A EUROPEAN HEALTH INITIATIVE

Antimicrobial resistance targets[i] -2024 update[ii]-

(2023 data)

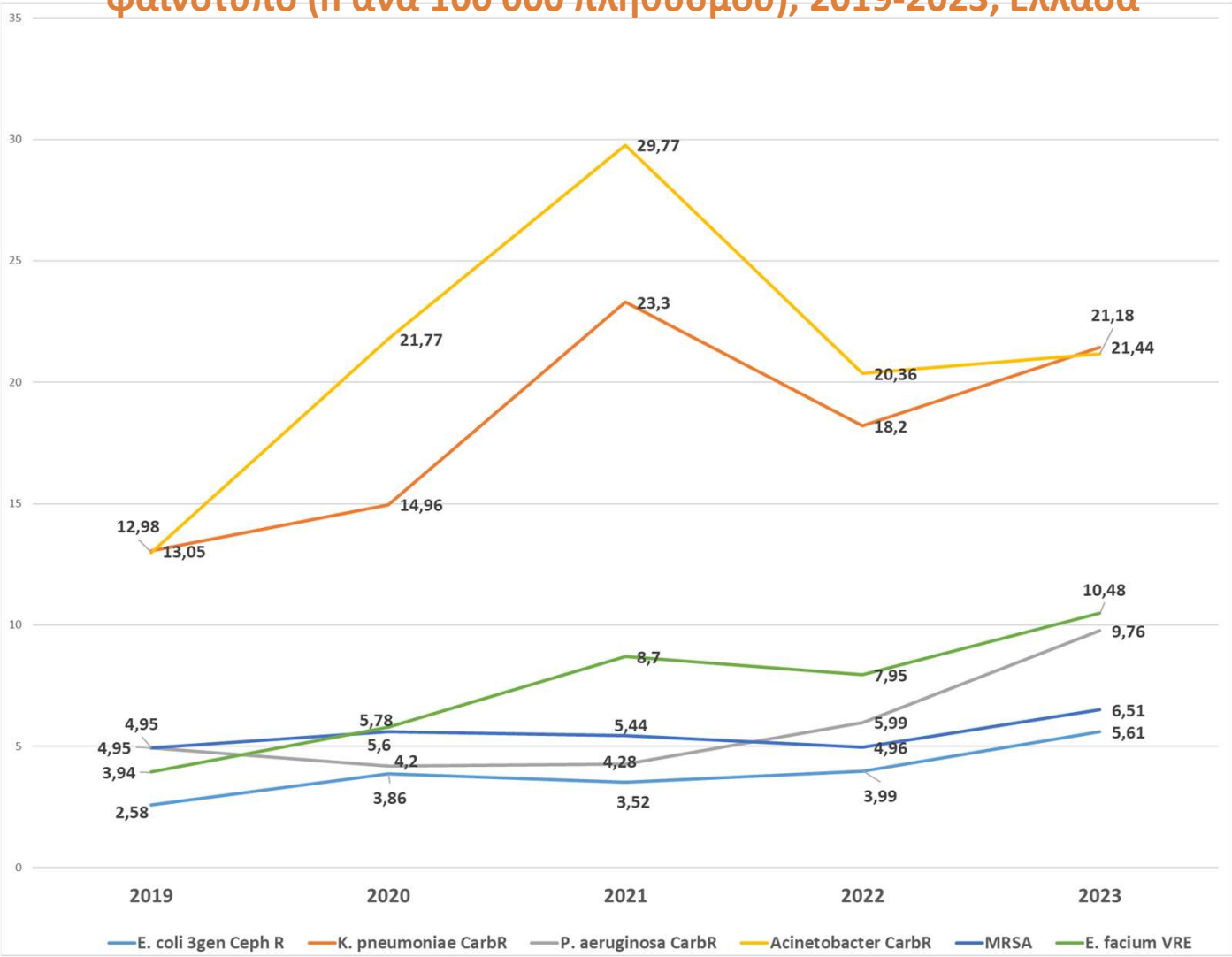
ecdc

Greece

Target achieved | Progress | Regress

Reduce by 27% the total consumption of antibiotics in humans Defined daily doses (DDDs) per 1 000 inhabitants per day	2019 baseline	34.1	-
	2023	28.5	-16.3%
	2030 TARGET	24.9	-27%
At least 65% of the total consumption of antibiotics in humans belongs to the 'Access' group of antibiotics As defined in the AWaRe classification of the WHO *Percentage point difference from 2019.	2019 baseline	46.8%	-
	2023	42.0%	-4.8%*
	2030 TARGET	65%	+18.2%*
Reduce by 10% the total incidence of bloodstream infections with meticillin-resistant *Staphylococcus aureus* (MRSA) Number per 100 000 population	2019 baseline	4.6	-
	2023	6.5	+41.8%
	2030 TARGET	4.1	-10%
Maintain at baseline level the total incidence of bloodstream infections with third-generation cephalosporin-resistant *Escherichia coli* Number per 100 000 population	2019 baseline	2.6	-
	2023	5.6	+117%
	2030 TARGET	2.6	-
Reduce by 5% the total incidence of bloodstream infections with carbapenem-resistant *Klebsiella pneumoniae* Number per 100 000 population	2019 baseline	13.1	-
	2023	21.4	+64.3%
	2030 TARGET	12.4	-5%

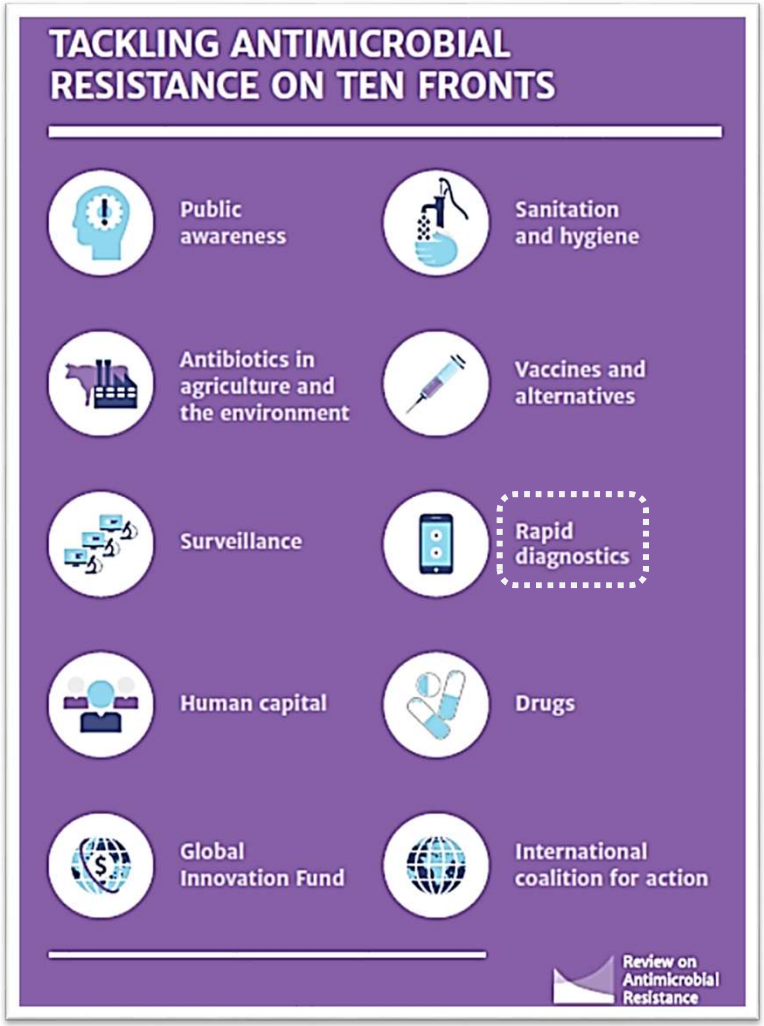
Εκτιμώμενη συνολική επίπτωση βακτηριαιμιών με ανθεκτικό φαινότυπο (n ανά 100 000 πληθυσμού), 2019-2023, Ελλάδα



Μεταβολή %, 2019-2023, ανά παθογόνο

- Στελέχη *Escherichia coli* με αντοχή στις κεφαλοσπορίνες τρίτης γενιάς: αύξηση 117,4%
- Ανθεκτικά στις καρβαπενέμες στελέχη *K. pneumoniae*: αύξηση 64,3%
- Ανθεκτικά στις καρβαπενέμες στελέχη *P. aeruginosa*: αύξηση 97,2%
- Ανθεκτικά στις καρβαπενέμες στελέχη *A. baumanni*: αύξηση 63,2%
- MRSA: αύξηση 41,8%
- *E. faecium* VRE: αύξηση 166%

European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net)- Annual Epidemiological Report 2023. Stockholm: ECDC; 2024.



'Tackling drug-resistant infections globally: Final report and recommendations'. The review on antimicrobial resistance; May 2016

https://health.ec.europa.eu/system/files/2020-01/amr_2017_action-plan_0.pdf

Σύσταση του Συμβουλίου σχετικά με την ενίσχυση των δράσεων της ΕΕ για την καταπολέμηση της μικροβιακής αντοχής στο πλαίσιο της προσέγγισης «Μία υγεία» 2023/C 220/01

https://eur-lex.europa.eu/legal content/EL/TXT/?uri= CELEX:32023H0622(01)

The European Partnership on One Health AMR (EUP OHAMR) brings together 53 partners from 30 countries to strengthen AMR R&I with a One Health approach.
By coordinating research, boosting innovation, improving surveillance and **diagnostics**, and supporting better prevention strategies, the partnership aims to close critical knowledge gaps and translate science into policy

https://cordis.europa.eu/project/id/101217154

‘Best buy’ interventions to tackle AMR

- improving hygiene in healthcare facilities, including promotion of handwashing
- stewardship programmes promoting more prudent use of antibiotics to end decades of over-prescription

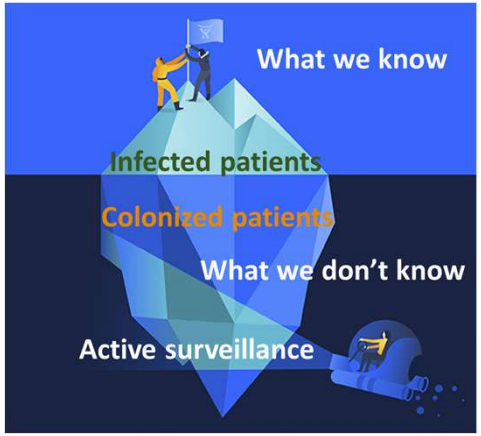
These will decrease the health burden of AMR – measured in DALYs – by about 40%

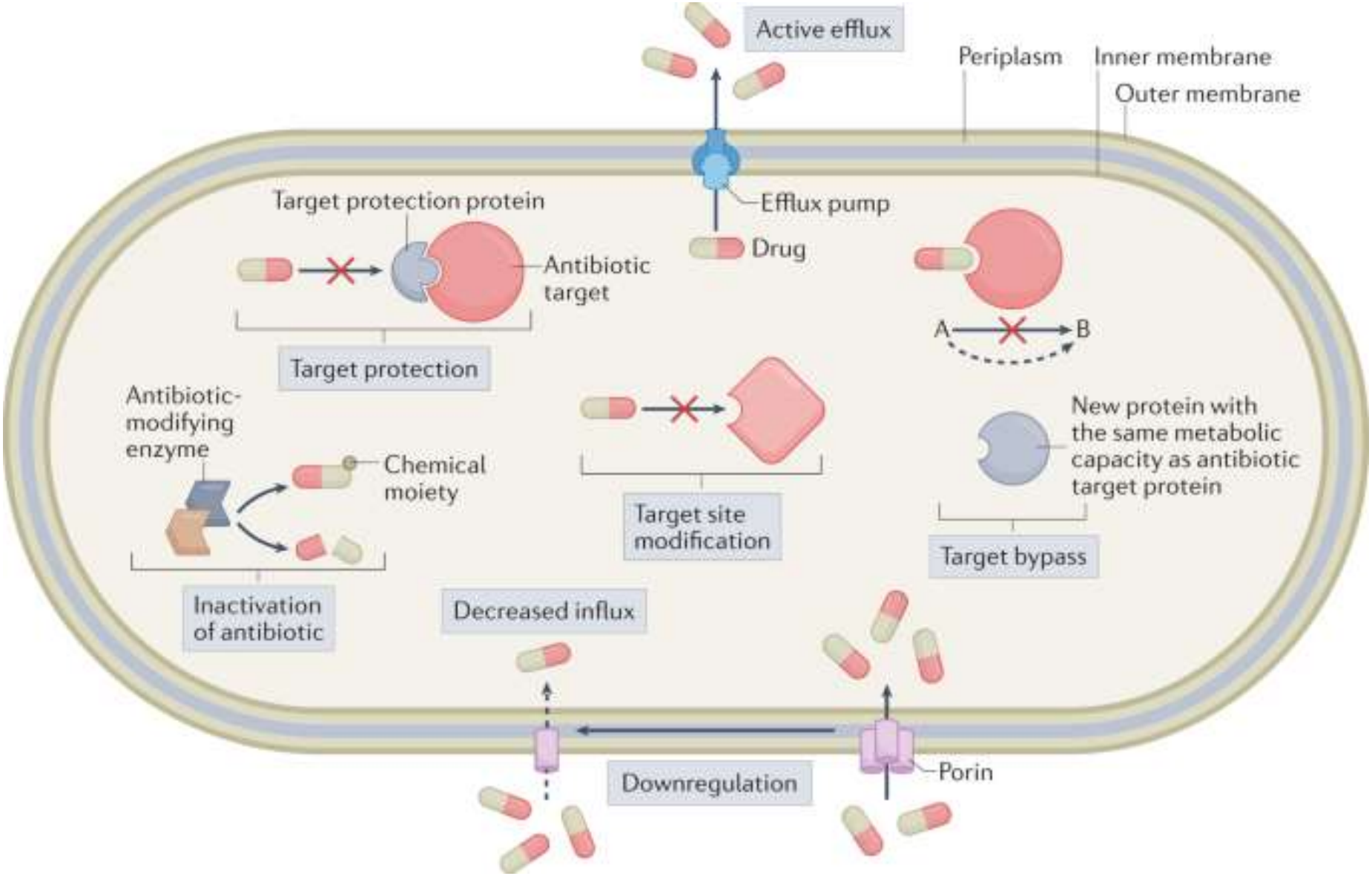
- **use of rapid diagnostic tests**
- delayed prescription
- public awareness campaigns

Source: OECD (2018), Stemming the Superbug Tide: Just a Few Dollars More. Available at: oe.cd/amr-2018.

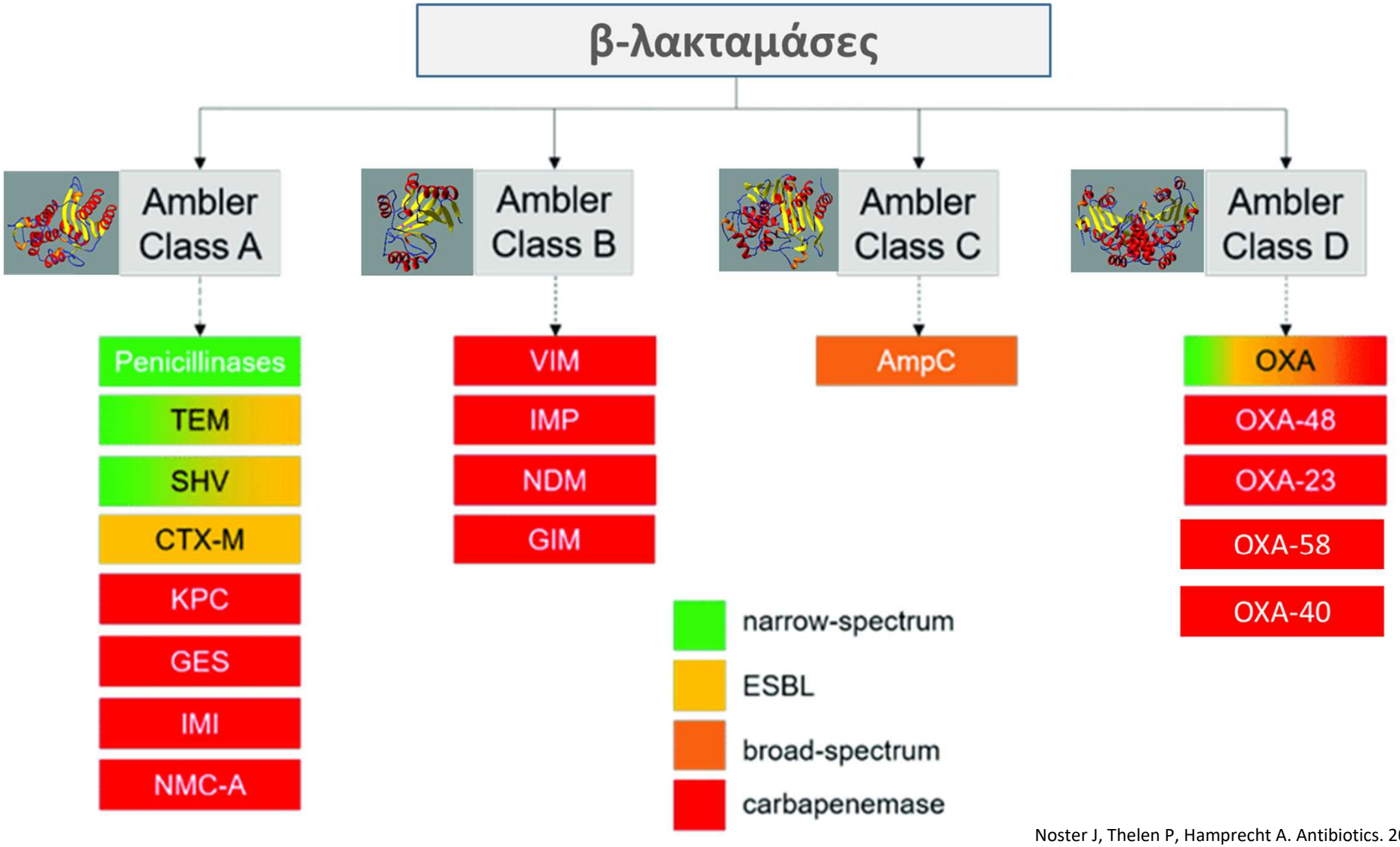
Μικροβιολογικό εργαστήριο και ανίχνευση αντοχής

Πληροφορία



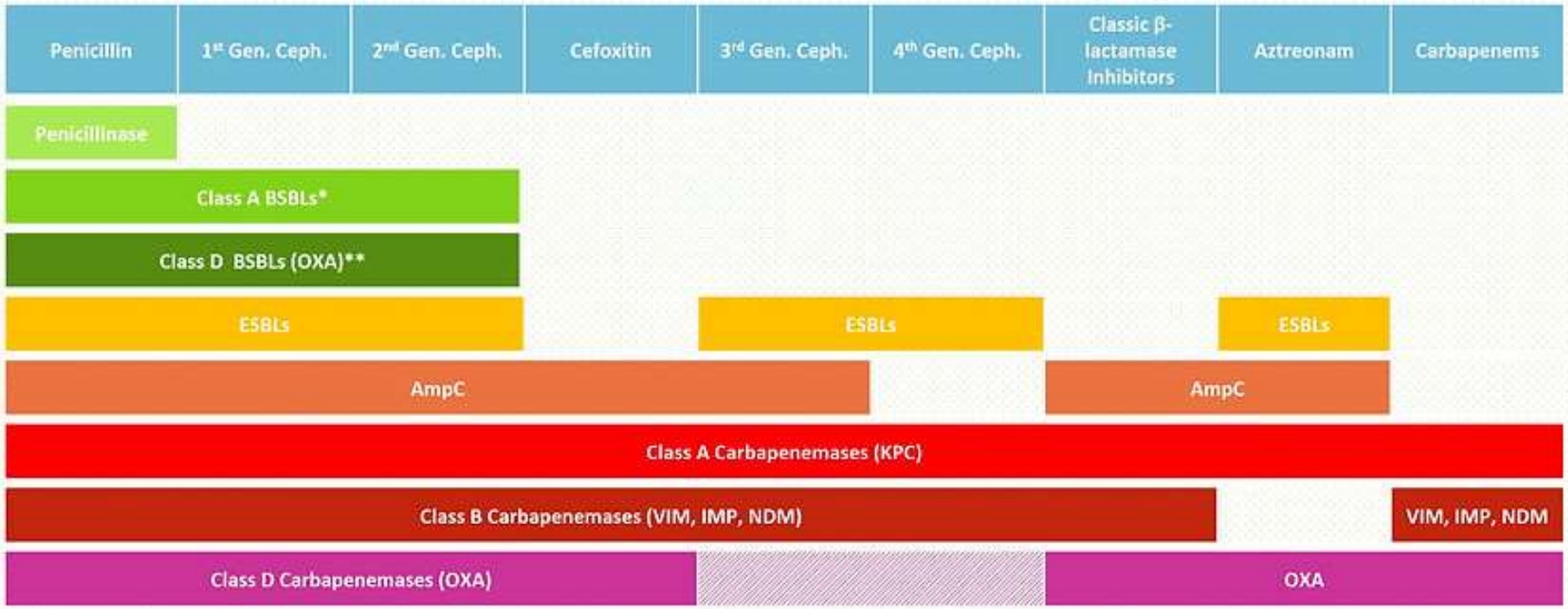


Darby EM et al. Nat Rev Microbiol. 2023;21(5):280-295.



Noster J, Thelen P, Hamprecht A. Antibiotics. 2021; 10(9):1140.
Με τροποποίηση

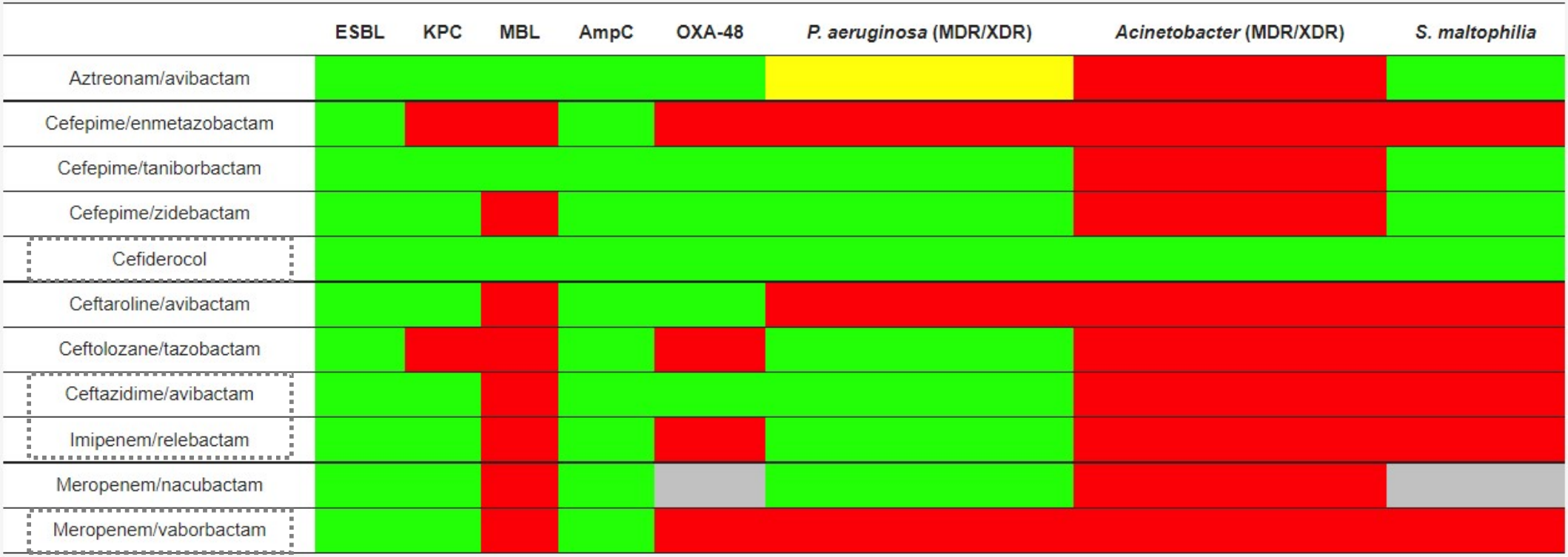
β- λακταμάσες: υδρολυτικό φάσμα



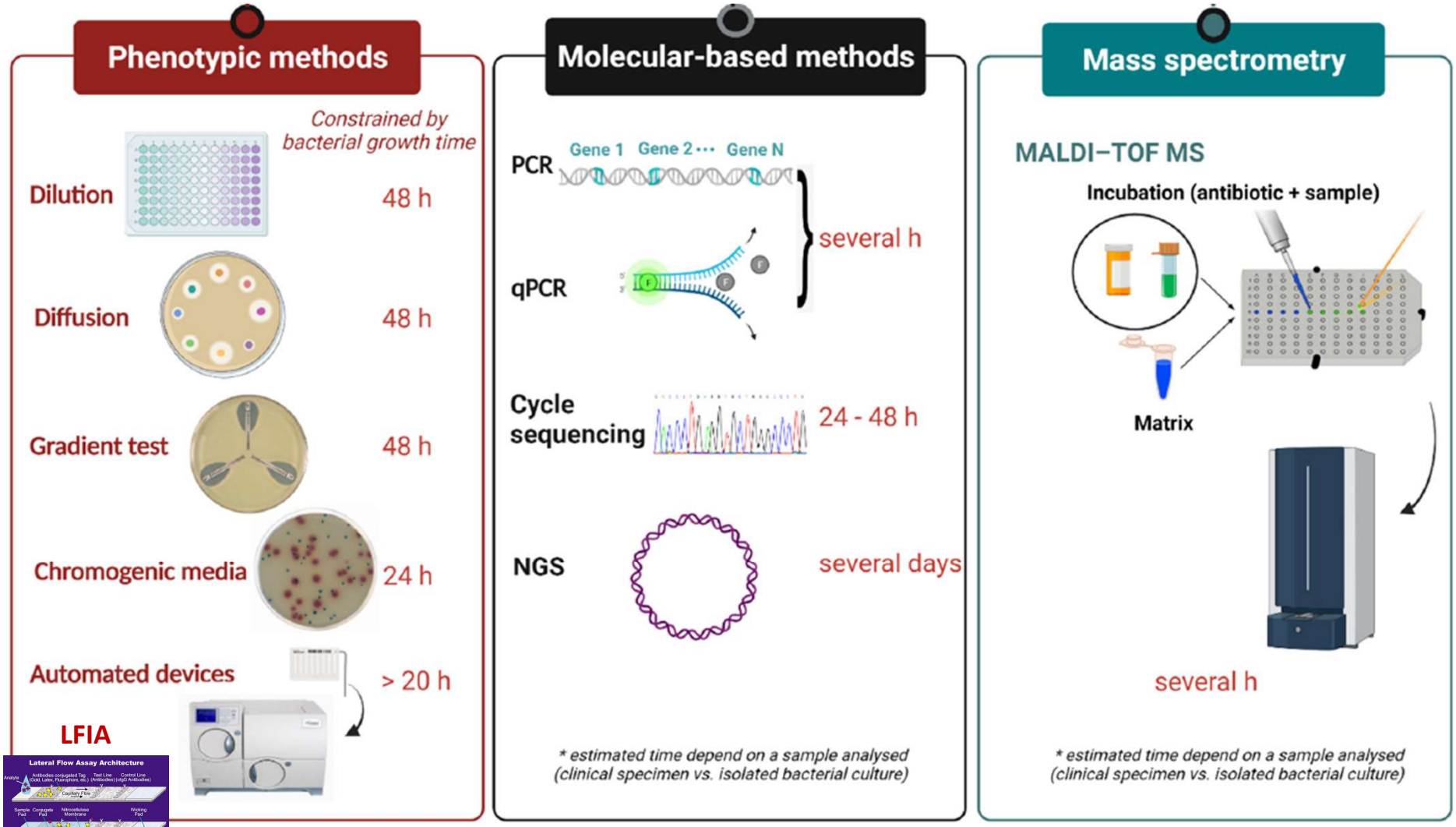
*including benzylpenicillins, aminopenicillins, carboxypenicillins, ureidopenicillin, narrow spectrum cephalosporins (cefazolin and cefuroxime and others)

**BSBL substrates plus oxacillin, nafcillin, and dicloxacillin

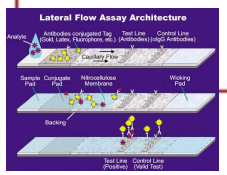
β- λακταμάσες και νεότερα αντιβιοτικά



Μέθοδοι ανίχνευσης της αντοχής



LFIA



Gajic I et al. Antibiotics (Basel). 2022;11(4):427.

Table 1. Differences between phenotypic and genotypic resistance detection methods

Characteristic	Phenotypic methods	Genotypic methods
Question to be answered	Does the antibiotic inhibit bacterial growth at clinically relevant concentrations?	Is a gene or mutation associated with antibiotic resistance present?
Turn-around time	Slow	Fast
Inoculum needed	High	Low
Provides information about resistance mechanism	No	Yes
Predicts antibiotic susceptibility and resistance	Yes	Sometimes. Only detects a gene or mutation associated with resistance; this may not correlate with phenotypic resistance in all isolates (e.g. if a gene is not expressed). If a singular genotypic resistance type is associated with resistance to a particular antibiotic in a particular bacterial species, its absence infers susceptibility. However, when there is more than one genotypic resistance type associated with resistance, such an inference may not always be correct.
Provides MIC	Yes	No
Cost	Moderate	High

Banerjee R, Patel R. JAC Antimicrob Resist. 2023;5(1):dlad018.

Κλινικό δείγμα

ID /AST

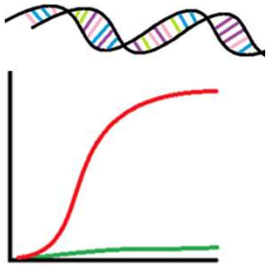
Ζώνες αναστολής

Τιμές MIC

Pheno R Μηχανισμός;

TAT= 48-72 h

CIDT

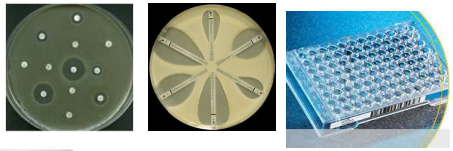


ID / Ανίχνευση γονιδίων αντοχής

TAT= 1-4 h

Διάγνωση Κατάλληλη αγωγή

AST



Ταχύτητα

αξιοπιστία

επαναληψιμότητα

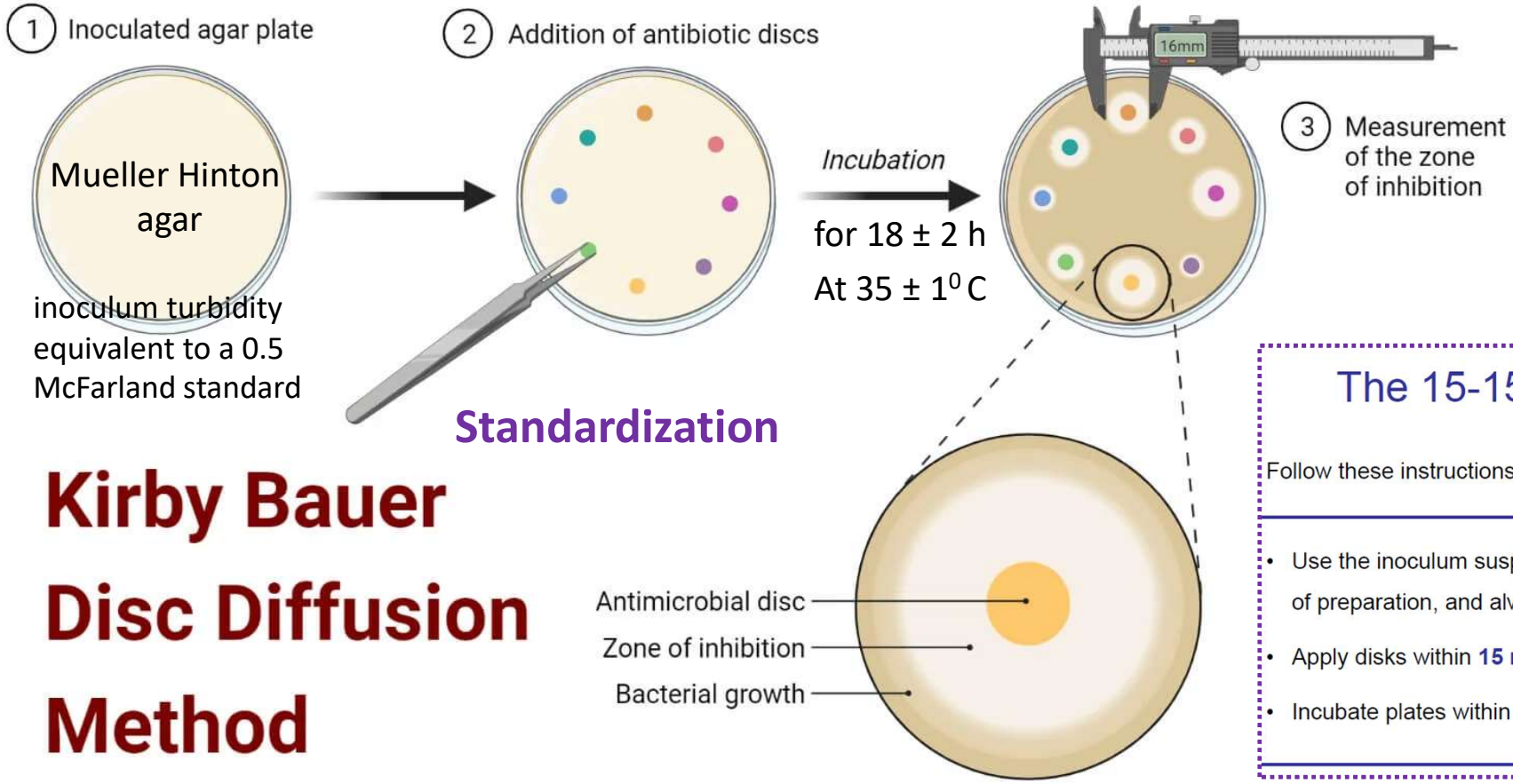
Προαγωγή της φροντίδας του ασθενούς

Διασφάλιση συνταγαγρόφησης του κατάλληλου ΑΒ

Προαγωγή της ορθολογικής χρήσης ΑΒ

Ανίχνευση μηχανισμών αντοχής
Εφαρμογή μέτρων για περιορισμό της διασποράς

Φαινοτυπικές μέθοδοι: Μέθοδος διάχυσης των δίσκων (Kirby-Bauer)



The 15-15-15 minute rule

Follow these instructions for disk diffusion:

- Use the inoculum suspension optimally within **15 minutes** of preparation, and always within 60 minutes.
- Apply disks within **15 minutes** of inoculation.
- Incubate plates within **15 minutes** of disk application.

EUCAST disk diffusion method for AST. Version 13.0, January 2025.

Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M. 1966.
Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493-496

Έλεγχος ευαισθησίας και ερμηνεία των αποτελεσμάτων

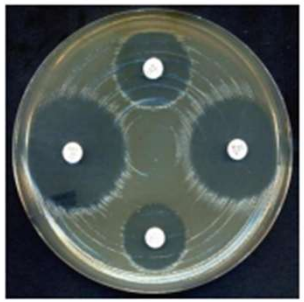
Επιδημιολογικά cut-offs
Ενδογενής αντοχή
Ανίχνευση μηχανισμών αντοχής
Δοσολογικά σχήματα για SDD όρια

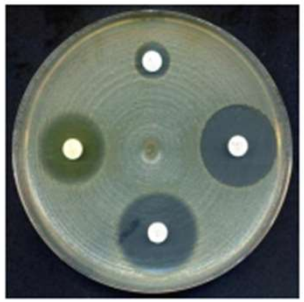
Μη μέλη: $200.00

Κλινικά όρια ευαισθησίας
Δοσολογικά σχήματα
Επιδημιολογικά cut-offs (ECOFFs)
Expert rules / Ενδογενής αντοχή
Ανίχνευση μηχανισμών αντοχής
Rational documents (breakpoint background)

Μέθοδος διάχυσης των δίσκων Kirby-Bauer: ερμηνεία

S, I, R





Enterobacterales *

Expert Rules and Expected Phenotypes

Guidance documents

EUCAST Clinical Breakpoint Tables v. 15.0, valid from 2025-01-01

For abbreviations and explanations of breakpoints, see the Notes sheet

Carbapenems[1]	MIC breakpoints (mg/L)			Disk content	Zone diameter breakpoints (mm)			Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >	ATU	(µg)	S ≥	R <	ATU	
Doripenem	1	2		10	24	21		1. Some isolates that produce carbapenemase are categorised as susceptible with the current breakpoints and should be reported as tested, i.e. the presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility. Carbapenemase detection and characterisation are recommended for public health and infection control purposes. For carbapenemase screening, a meropenem screening cut-off of >0.125 mg/L (zone diameter <28 mm) is recommended. 2. The intrinsically low activity of imipenem against *Morganella morganii*, *Proteus* spp. and *Providencia* spp. requires the high exposure of imipenem. 3. For susceptibility testing purposes, the concentration of relebactam is fixed at 4 mg/L. 4. For susceptibility testing purposes, the concentration of vaborbactam is fixed at 8 mg/L. A. For isolates in the ATU, if resistant to meropenem report resistant to meropenem-vaborbactam. If not resistant to meropenem, investigate further.
Ertapenem	0.5	0.5		10	23	23		
Imipenem, *Enterobacterales* except *Morganellaceae*	2	4		10	22	19		
Imipenem[2], *Morganellaceae*	0.001	4		10	50	19		
Imipenem-relebactam, *Enterobacterales* except *Morganellaceae*	2[3]	2[3]		10-25	22	22	20-22	
Meropenem (indications other than meningitis)	2	8		10	22	16		
Meropenem (meningitis)	2	2		10	22	22		
Meropenem-vaborbactam	8[4]	8[4]		20-10	20	20	15-19[A]	

Monobactams	MIC breakpoints (mg/L)			Disk content	Zone diameter breakpoints (mm)			Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >	ATU	(µg)	S ≥	R <	ATU	
Aztreonam[1]	1	4		30	26	21		1. The aztreonam breakpoints for *Enterobacterales* will detect clinically important resistance mechanisms (including ESBL). Some isolates that produce beta-lactamases are susceptible to aztreonam with these breakpoints and should be reported as tested, i.e. the presence or absence of an ESBL does not in itself influence the categorisation of susceptibility. ESBL detection and characterisation are recommended for public health and infection control purposes. 2. For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L.
Aztreonam-avibactam	4[2]	4[2]		30-20	25	25	22-24	

Fluoroquinolones	MIC breakpoints (mg/L)			Disk content	Zone diameter breakpoints (mm)			Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >	ATU	(µg)	S ≥	R <	ATU	
Ciprofloxacin, *Salmonella* spp.[1]	0.06	0.06			Note[A]	Note[A]		1. There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with any detectable fluoroquinolone resistance mechanisms. The available data relate mainly to *Salmonella* Typhi but there are also case reports of poor response with other *Salmonella* species. 2/B. In meningitis, where all fluoroquinolone resistance mechanisms must be excluded, either perform an MIC test, or infer susceptibility from the pefloxacin 5 µg screening test. 3. Fluoroquinolone breakpoints are available for other agents. A. Tests with a ciprofloxacin 5 µg disk will not reliably exclude all fluoroquinolone resistance mechanisms in *Salmonella* spp. Perform an MIC test, or infer susceptibility from the pefloxacin 5 µg screening test. C. The pefloxacin screening test can also be used to detect fluoroquinolone resistance mechanisms in other *Enterobacterales* such as *E. coli*, *K. pneumoniae* and *Shigella* spp. D. A disk diffusion test awaits action from the responsible pharmaceutical company.
Ciprofloxacin (indications other than meningitis)	0.25	0.5	0.5	5	25	22	22-24	
Ciprofloxacin (meningitis)[2]	0.125	0.125			Note[B]	Note[B]		
Pefloxacin (screen only)	NA	NA		5	24[A,B,C]	24[A,B,C]		
Delafloxacin, *E. coli*	0.125	0.125			Note[D]	Note[D]		
Levofloxacin	0.5	1		5	23	19		
Moxifloxacin, *Enterobacterales* except *Morganella morganii*, *Proteus* spp. and *Serratia* spp.[3]	0.25	0.25		5	22	22		
Nalidixic acid (screen only)	NA	NA			NA	NA		
Norfloxacin (uncomplicated UTI only)	0.5	0.5		10	24	24		
Ofloxacin	0.25	0.5		5	24	22		

The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 15.0, 2025. http://www.eucast.org.

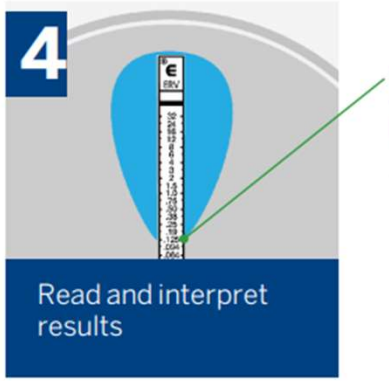
EUCAST, Rapid AST

- Χρήση με φιάλες BACTEC (BD), BacT/ALERT (bioMérieux) και VersaTREK (Thermo Fisher)
- 0 – 18 h μετά τη θετικοποίηση της φιάλης
- Απευθείας ενοφθαλμισμός (MH, MH-F) με 125±25 μl από τη θετική φιάλη
- Το μικροβιακό είδος **πρέπει να είναι γνωστό** πριν την ερμηνεία των αποτελεσμάτων

Table 1. Incubation conditions for antimicrobial susceptibility test plates.

Organism	Incubation time	Medium	Incubation
Escherichia coli *Klebsiella pneumoniae* *Salmonella enterica* *Acinetobacter baumannii* *Staphylococcus aureus* *Enterococcus faecalis* *Enterococcus faecium*	4, 6 and 8 hours 16-20 hours	MH	35±1°C in air
Pseudomonas aeruginosa	6 and 8 hours 16-20 hours	MH	35±1°C in air

Ταινίες διαβαθμισμένης συγκέντρωσης αντιβιοτικών

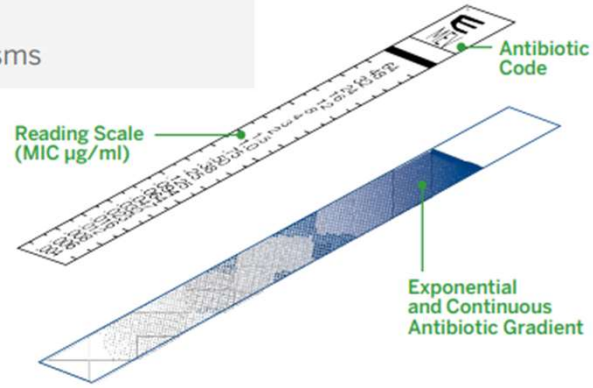


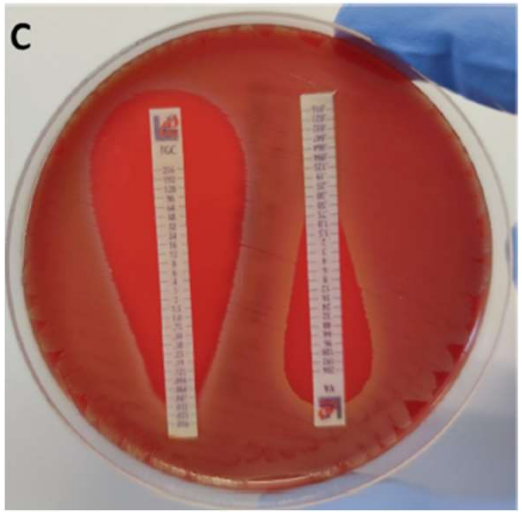
The MIC value is read from the scale in terms of µg/mL at complete inhibition of bacterial growth, where the pointed end of the ellipse intersects the strip.

SET UP TIME:
< 5 Minutes

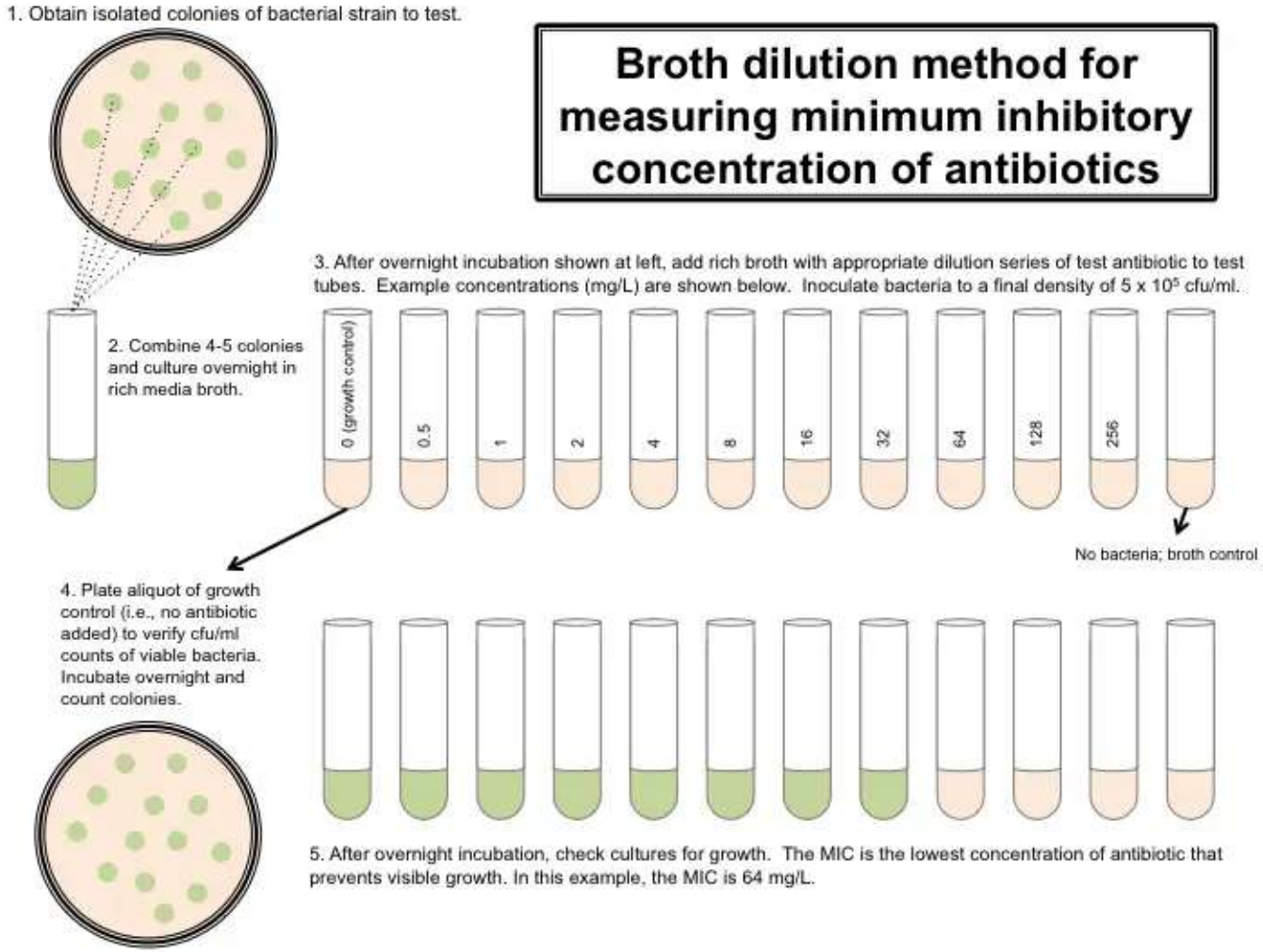
TIME TO RESULT:
16 – 20 hours for most organisms

Τιμή MIC



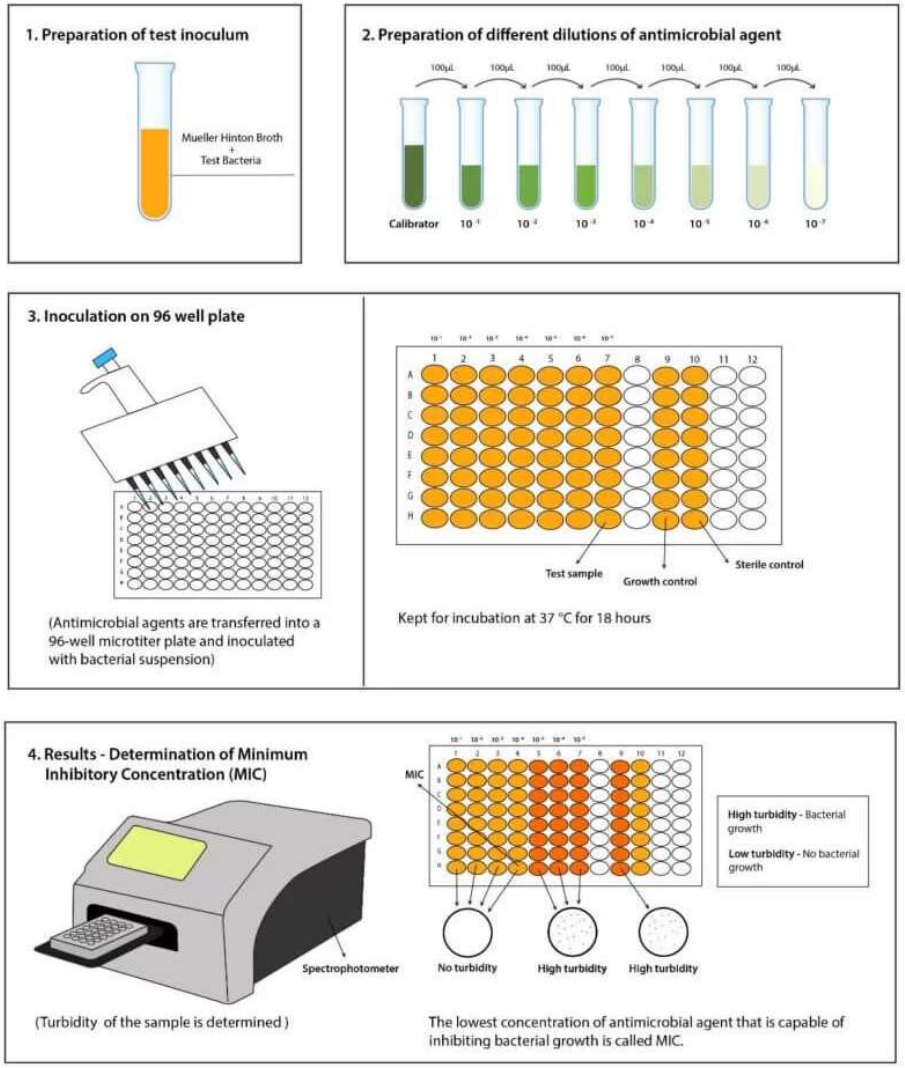


Μέθοδος μακροαραιώσεων σε ζωμό



Μέθοδος μικροαραίωσης σε ζωμό (BMD)

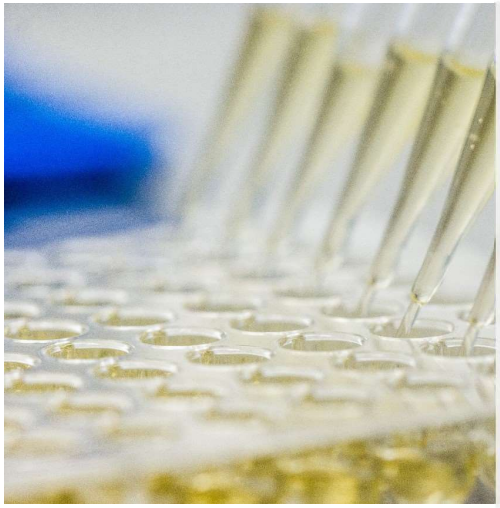
Correct inoculum
5 x 10^5 CFU/ml



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

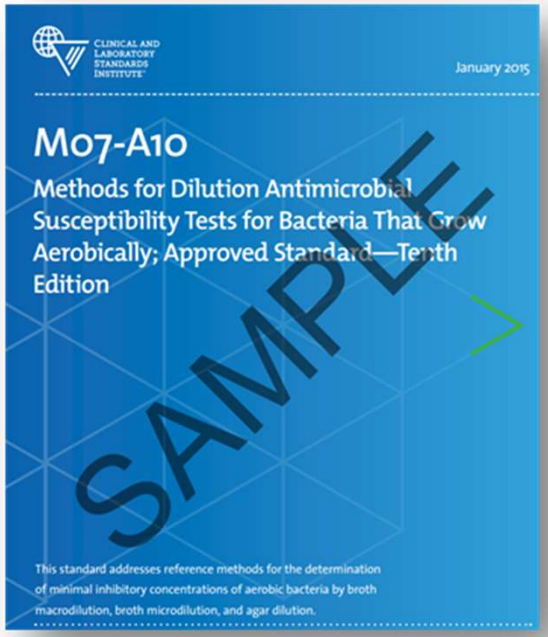
Version 5.0, January 2024

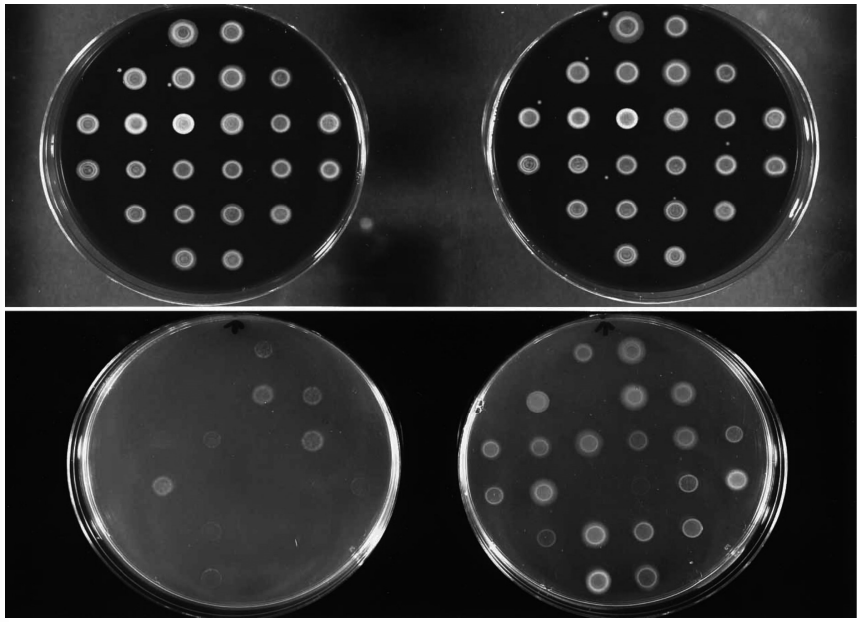
EUCAST reading guide for broth microdilution



- BMD is the reference method for AST of rapidly growing aerobic bacteria, except for mecillinam and fosfomycin, where agar dilution is the reference method.
- EUCAST recommends testing according to the International Standard ISO 20776-1 (with the use of MH-F broth for fastidious organisms).

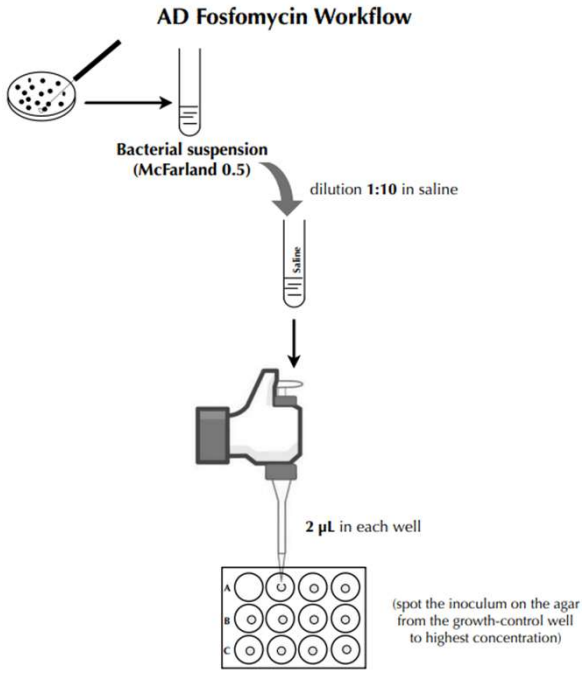
Μέθοδος αραίωσης σε άγαρ (agar dilution)

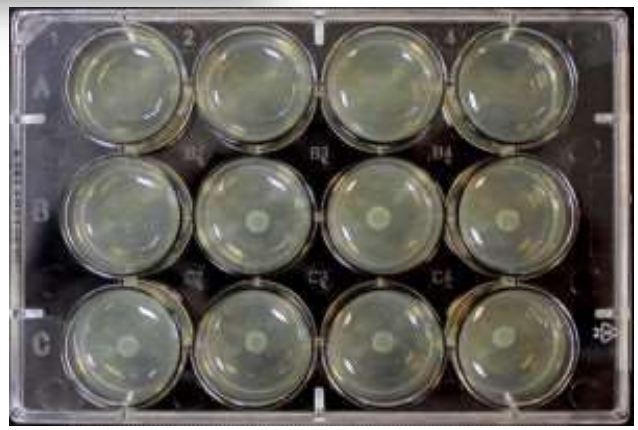




mecillinam and fosfomycin: agar dilution is **the reference method**

Fosfomycin Agar Dilution Panel, Liofilchem





Fosfomycin MIC range: 0.25 - 256 µg/mL

A	256	128	64	32
B	16	8	4	2
C	1	0.5	0.25	Control

Growth-control: No antimicrobial agent in the well.

Αυτοματοποιημένα συστήματα

Detection of AMR facilitated by Advanced Expert System (AES).

The instruments read the kinetics of the growth using an optical system with photometer/ multichannel fluorimeter readings to assess fluorescence / turbidity /colorimetric signals

- Each of the systems has inherent advantages and limitations
- Results can vary widely by antimicrobial drugs, software versions, and cards
- Some of the systems are not reliable for correct categorization of susceptibility for certain drugs, leading to wrong classifications.
- Software updates and synchronization of breakpoints according to the current standards are mandatory.
- Panels usually contain only several concentrations of each antimicrobial agent, and the resulting MIC is not always given as an exact value.

Gajic I et al. Antibiotics (Basel). 2022;11(4):427.

Advantages and disadvantages of the common methods of antimicrobial susceptibility testing

Method	Advantage	Disadvantage	Comments
Broth dilution 	Well-standardised Harmonised Commercially available tests are easy to perform	Time-consuming Individual mistakes	Quantitative **
Agar Dilution	Well-standardised Suitable for testing a large number of isolates	Time-consuming Limited concentration of antimicrobial agents	Quantitative Possible automation in part
Disk diffusion 	Simple to perform Low cost Simple and fast interpretation The high number of test antibiotics per test High flexibility in antibiotic selection Detection of resistance patterns Mass use and the possibility of automatisation A number of a different use (AST, identification, screening, etc.) Detection of heteroresistant population or contamination	Time-consuming No MIC value The inability for some antibiotics to be tested	Qualitative * **Όχι τιμές MIC**
Gradient test	Convenient and flexible Simple to perform Does not require expertise Detection of resistance patterns	Relatively expensive Relatively long incubation	Quantitative
Automated systems	Simple to perform	Relatively expensive	Semi-quantitative ***

Gajic I et al. Antibiotics (Basel). 2022;11(4):427.

Antibiotic resistance threats

Carbapenem-resistant
Enterobacterales (CRE)

Extended-spectrum cephalosporin resistance in Enterobacterales suggestive of extended- spectrum β-lactamase **(ESBL)** production

Multidrug-resistant (MDR)
Pseudomonas aeruginosa

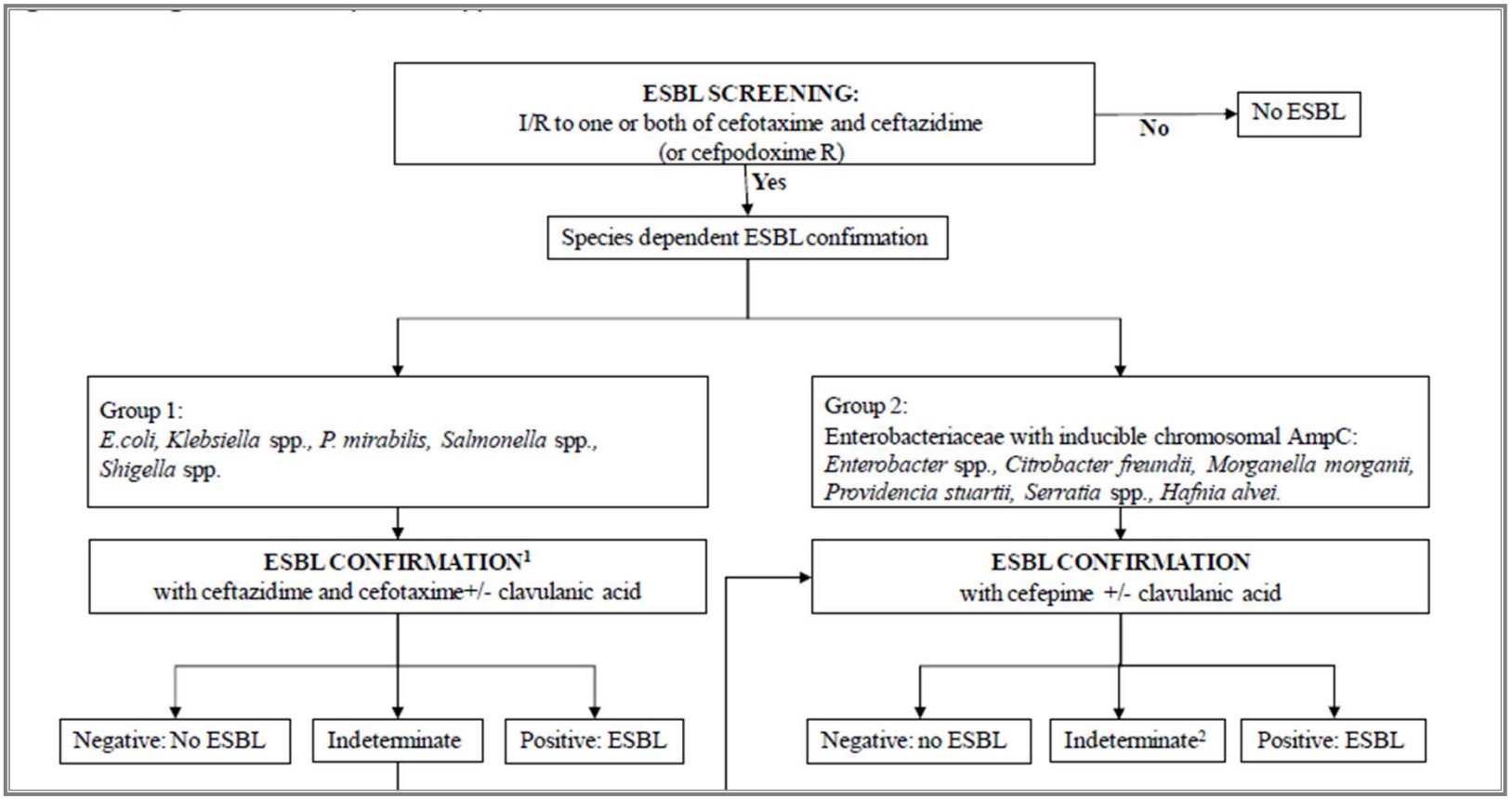
Carbapenem-resistant
Acinetobacter species

Methicillin-resistant
Staphylococcus aureus (MRSA)

Vancomycin-resistant
Enterococcus (VRE)

CDC, Antibiotic Resistance Threats in the United States

Αλγόριθμος για φαινοτυπική ανίχνευση ESBLs σε *Enterobacterales*



[1]If cefoxitin has an MIC >8 mg/L, perform cefepime+/- clavulanic acid confirmation test

If confirmation with cefepime +/- clavulanic acid is still indeterminate, genotypic testing is required.

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.01. July 2017

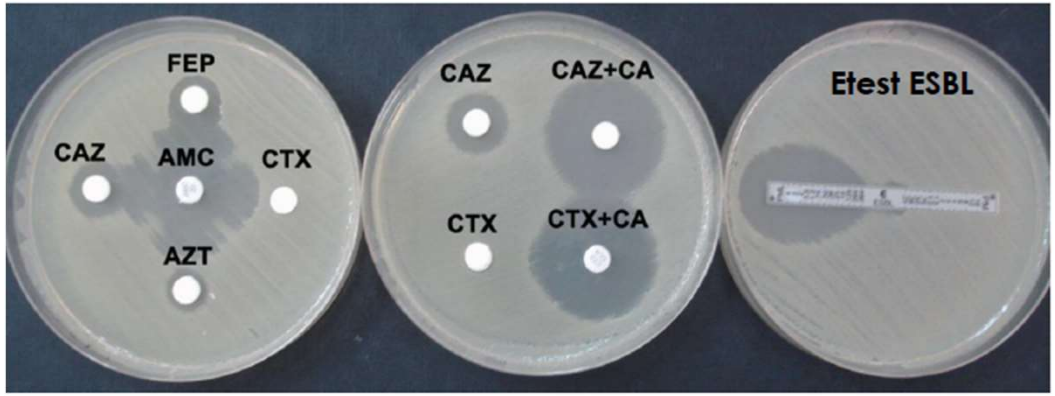
Φαινοτυπική ανίχνευση ESBL

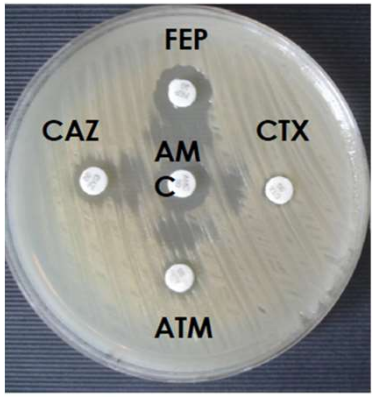
Bionumber: 0405610450106601
Organism Quantity:

Selected Organism: Escherichia coli

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Ertapenem	<= 0.12	S
Temocillin			Imipenem	<= 0.25	S
Urine	<= 4	I	Meropenem		
Other	<= 4		Meningitis	<= 0.25	S
Ampicillin	>= 32	R	Other	<= 0.25	S
Amoxicillin/Clavulanic Acid	8	S	Amikacin	2	S
Piperacillin/Tazobactam	<= 4	S	Gentamicin	<= 1	S
Cefuroxime	>= 64	R	Tobramycin	<= 1	S
Cefuroxime Axetil	>= 64	R	Ciprofloxacin		
Cefoxitin	<= 4	IE	Meningitis	0.5	
Cefditoren			Other	0.5	I
Cefixime	>= 4	R	Levofloxacin	1	I
Cefotaxime			Moxifloxacin	0.5	R
Meningitis	>= 64	R	Minocycline	(-)	(-)
Other	>= 64	R	Tetracycline	(-)	(-)
Ceftazidime	8	R	Tigecycline	<= 0.5	S
Ceftriaxone			Fosfomycin		
Meningitis	>= 64	R	Oral	<= 16	
Other	>= 64	R	Other	<= 16	S
Ceftazidime/Avibactam	<= 0.12	S	Nitrofurantoin	<= 16	S
Ceftolozane/Tazobactam	<= 0.25	S	Chloramphenicol	4	IE
Cefepime	2	I	Colistin	<= 0.5	S
Aztreonam	16	R	Trimethoprim/ Sulfamethoxazole	<= 20	S

DDST **CDT** **Etest ESBL**



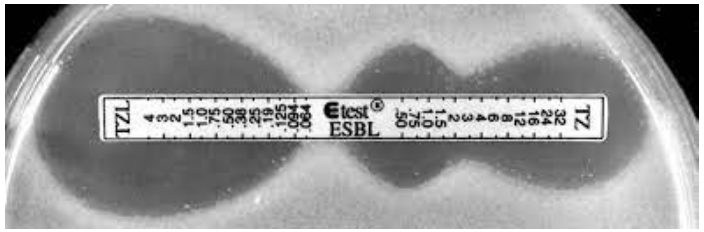


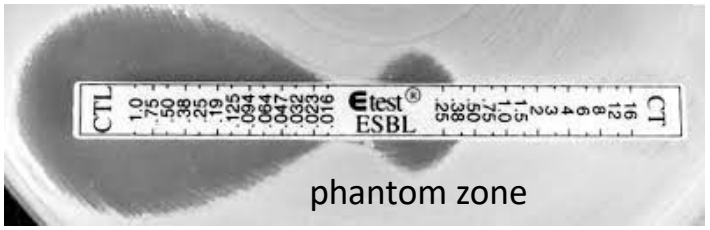
≥5 mm increase in inhibition zone

MIC ratio ≥8 or if a phantom zone or deformed ellipse is present

Φαινοτυπική ανίχνευση ESBL

Etest ESBL

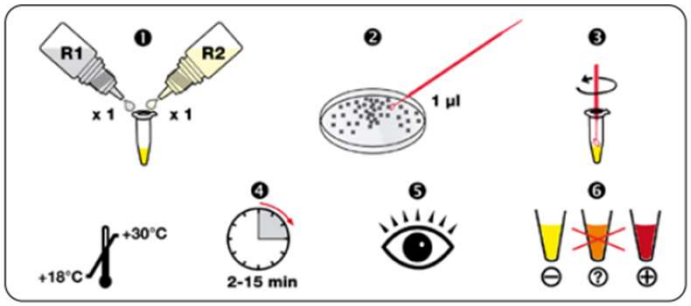




EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.01. July 2017

Χρωματομετρικές μέθοδοι

β LACTA™ test



directly with isolated colonies or with bacterial pellets from positive blood cultures or urines

Se: 98%
Sp: 100%

Χρωμογόνα υλικά

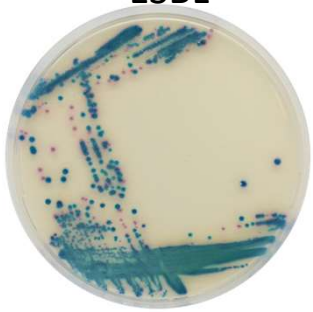
Liofilchem® Chromatic ESBL

Escherichia coli DSM 22311

Brilliance™ ESBL Agar

CHROMID® ESBL

CHROMagar™ ESBL



Del Corpo O et al. Clin Microbiol Infect. 2023:S1198-743X(23)00425-1.

CRE-Vitek reports

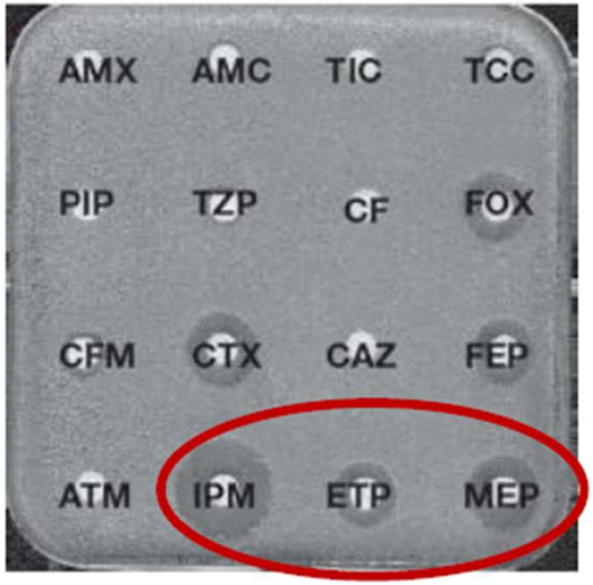
Organism Quantity: **Selected Organism: Klebsiella pneumoniae**

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Ertapenem	>= 8	R
Temocillin			Imipenem	>= 16	R
Urine	>= 32	R	Meropenem		
Other	>= 32		Meningitis	>= 16	R
Ampicillin	>= 32	R	Other	>= 16	R
Amoxicillin/Clavulanic Acid	>= 32	R	Amikacin	16	R
Piperacillin/Tazobactam	>= 128	R	Gentamicin	>= 16	R
Cefuroxime	>= 64	R	Tobramycin	>= 16	R
Cefuroxime Axetil	>= 64	R	Ciprofloxacin		
Cefoxitin	>= 64	IE	Meningitis	>= 4	
Cefditoren			Other	>= 4	R
Cefixime	>= 4	R	Levofloxacin	>= 8	R
Cefotaxime			Moxifloxacin	>= 8	R
Meningitis	>= 64	R	Minocycline	(-)	(-)
Other	>= 64	R	Tetracycline	(-)	(-)
Ceftazidime	>= 64	R	Tigecycline	2	--
Ceftriaxone			Fosfomycin		
Meningitis	>= 64	R	Oral	128	R
Other	>= 64	R	Other	128	R
Ceftazidime/Avibactam	4	S	Nitrofurantoin		
Ceftolozane/Tazobactam	>= 32	R	Chloramphenicol	>= 64	IE
Cefepime	>= 32	R	Colistin	>= 16	R
Aztreonam	>= 64	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

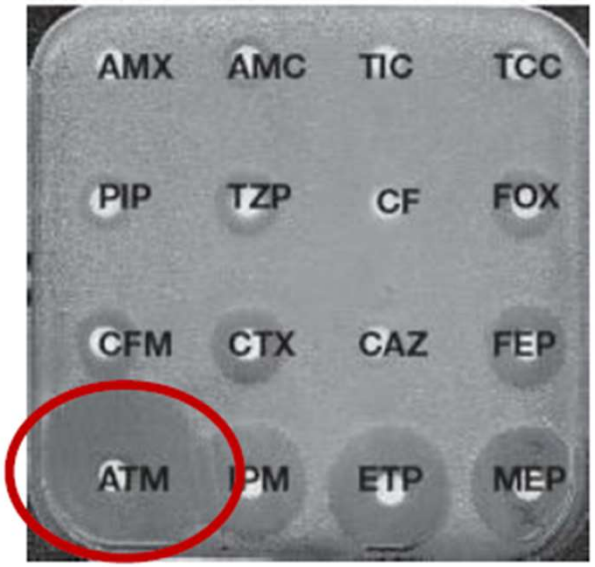
Organism Quantity: **Selected Organism: Klebsiella pneumoniae**

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Ertapenem	>= 8	R
Temocillin			Imipenem	>= 16	R
Urine	>= 32	R	Meropenem		
Other	>= 32		Meningitis	>= 16	R
Ampicillin	>= 32	R	Other	>= 16	R
Amoxicillin/Clavulanic Acid	>= 32	R	Amikacin	4	S
Piperacillin/Tazobactam	>= 128	R	Gentamicin	>= 16	R
Cefuroxime	>= 64	R	Tobramycin	8	R
Cefuroxime Axetil	>= 64	R	Ciprofloxacin		
Cefoxitin	>= 64	IE	Meningitis	>= 4	
Cefditoren			Other	>= 4	R
Cefixime	>= 4	R	Levofloxacin	>= 8	R
Cefotaxime			Moxifloxacin	>= 8	R
Meningitis	>= 64	R	Minocycline	(-)	(-)
Other	>= 64	R	Tetracycline	(-)	(-)
Ceftazidime	>= 64	R	Tigecycline	1	--
Ceftriaxone			Fosfomycin		
Meningitis	>= 64	R	Oral	<= 16	
Other	>= 64	R	Other	<= 16	S
Ceftazidime/Avibactam	>= 16	R	Nitrofurantoin		
Ceftolozane/Tazobactam	>= 32	R	Chloramphenicol	16	IE
Cefepime	>= 32	R	Colistin	<= 0.5	S
Aztreonam	>= 64	R	Trimethoprim/ Sulfamethoxazole	<= 20	S

Ανίχνευση παραγωγής καρβαπενεμασών στα Εντεροβακτηριακά: πληροφορίες από το αντιβιόγραμμα



KPC / ESBL +/- [Caz-avi S]
MBL / ESBL + [Caz-avi R]
OXA-48 / ESBL +



MBL
ESBL negative

OXA-48
ESBL negative

Ανίχνευση μηχανισμών αντοχής: CRE

Clinical breakpoints and **screening cut-off values** for carbapenemase-producing Enterobacteriaceae (according to EUCAST methodology)

Carbapenem	MIC (mg/L)		Disk diffusion zone diameter (mm) with 10 μg disks	
	S/I breakpoint	Screening cut-off	S/I breakpoint	Screening cut-off
Meropenem[1]	≤2	>0.125	≥22	<28[2]
Ertapenem[3]	≤0.5	>0.125	≥25	<25

[1]Best balance of sensitivity and specificity

[2]Isolates with 25-27 mm only need to be investigated for carbapenemase-production if they are resistant to piperacillin-tazobactam and/or temocillin (temocillin contributes more to the specificity).

Investigation for carbapenemases is always warranted if zone diameter of meropenem is <25 mm.

[3]High sensitivity but low specificity. Can be used as an alternative screening agent, but isolates with ESBL and AmpC may be resistant without having carbapenemases.

Phenotypic methods

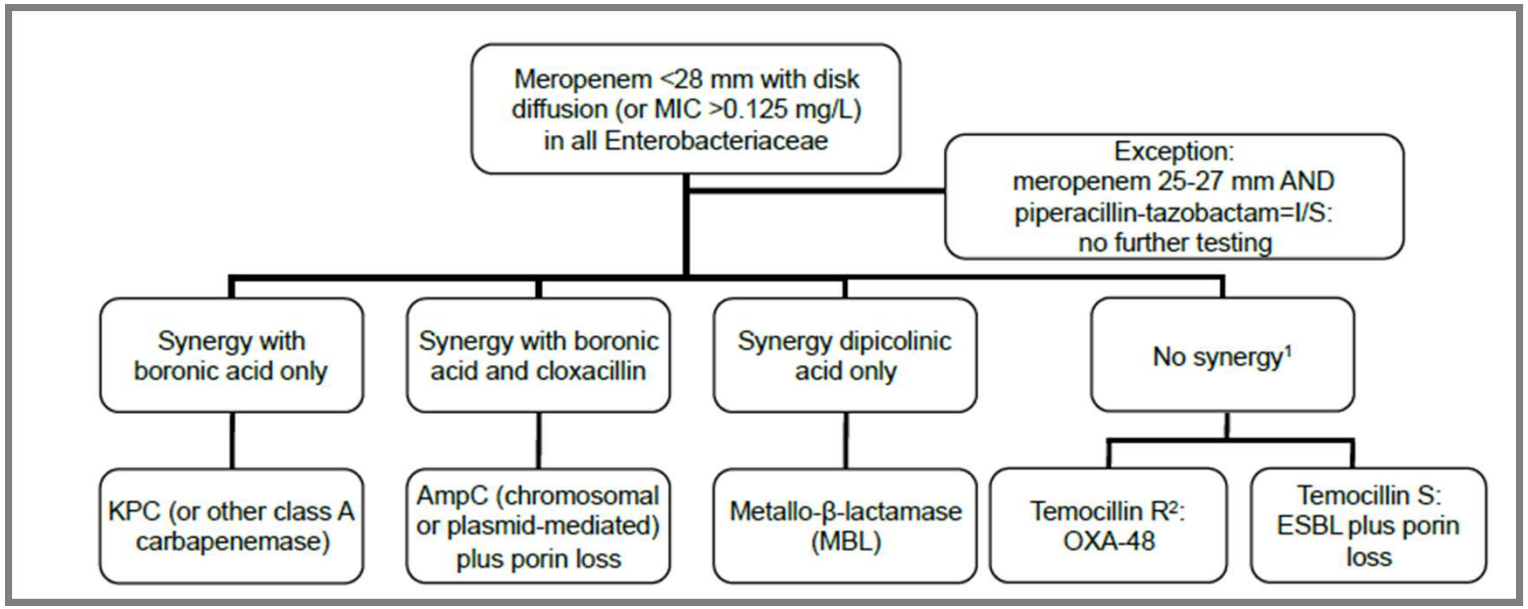
- combination disk test methods
- colorimetric assays based on hydrolysis of carbapenems

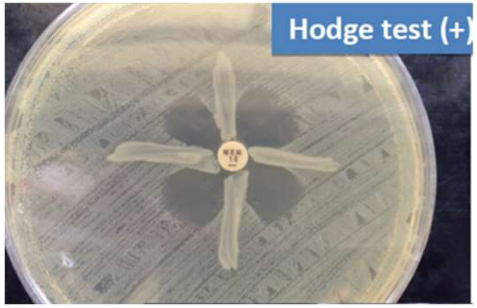
- other methods detecting carbapenem hydrolysis
- lateral flow assays

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.01. July 2017

Ανίχνευση μηχανισμών αντοχής: CRE

Φαινοτυπικός αλγόριθμος για ανίχνευση παραγωγής καρβαπενεμασών στα Εντεροβακτηριακά



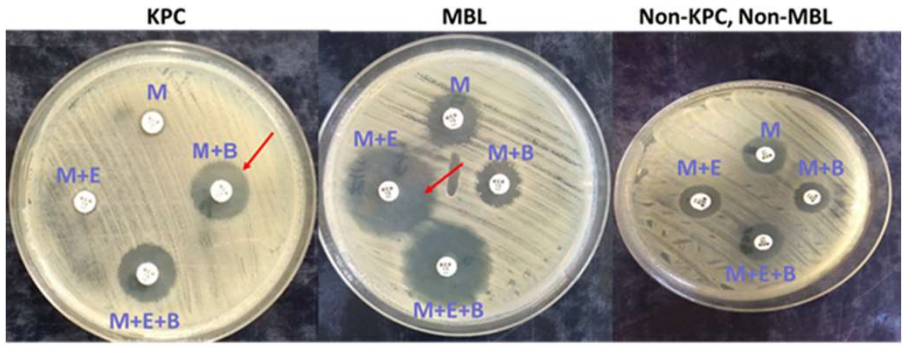


Use of the modified cloverleaf (Hodge) test is **not currently recommended** as results are difficult to interpret, the specificity is poor, and in some cases the sensitivity is also suboptimal

[1]Combination of several carbapenemases can also contribute to no synergy – e.g. MBL and KPC in combination. Molecular testing is usually necessary in such cases

[2]High-level temocillin resistance (>128 mg/L, zone diameter <11 mm) is a phenotypic marker of OXA-48

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.01. July 2017



Journal of Antimicrobial Chemotherapy (2008) **62**, 1257–1260
doi:10.1093/jac/dkn364
Advance Access publication 4 September 2008

JAC

First occurrence of KPC-2-possessing *Klebsiella pneumoniae* in a Greek hospital and recommendation for detection with boronic acid disc tests

Athanassios Tsakris[1]*, Ioulia Kristo[2], Aggeliki Poulou[3], Fani Markou[3], Alexandros Ikonomidis[2] and Spyros Pournaras[2]

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2009, p. 362–367 Vol. 47, No. 2
0095-1137/09/$08.00+0 doi:10.1128/JCM.01922-08

Evaluation of Boronic Acid Disk Tests for Differentiating KPC-Possessing *Klebsiella pneumoniae* Isolates in the Clinical Laboratory[▽]

Athanassios Tsakris,[1]* Ioulia Kristo,[2] Aggeliki Poulou,[3] Katerina Themeli-Digalaki,[4] Alexandros Ikonomidis,[2] Dimitra Petropoulou,[5] Spyros Pournaras,[2] and Danai Sofianou[6]

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3420–3426 Vol. 47, No. 11
0095-1137/09/$12.00 doi:10.1128/JCM.01314-09

Use of Boronic Acid Disk Tests To Detect Extended-Spectrum β-Lactamases in Clinical Isolates of KPC Carbapenemase-Possessing *Enterobacteriaceae*[▽]

Athanassios Tsakris,[1]* Aggeliki Poulou,[2] Katerina Themeli-Digalaki,[3] Evangelia Voulgari,[1] Theodore Pittaras,[1] Danai Sofianou,[4] Spyros Pournaras,[5] and Dimitra Petropoulou[6]

J Antimicrob Chemother 2010; **65**: 1319–1321
doi:10.1093/jac/dkq124 Advance Access publication 15 April 2010

Journal of Antimicrobial Chemotherapy

Inhibitor-based methods for the detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice by using boronic acid compounds

Spyros Pournaras[1], Aggeliki Poulou[2] and Athanassios Tsakris[3]*

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2011, p. 2804–2809 2011;49(8):2804-9. Vol. 49, No. 8
0095-1137/11/$12.00 doi:10.1128/JCM.00666-11

Comparative Evaluation of Combined-Disk Tests Using Different Boronic Acid Compounds for Detection of *Klebsiella pneumoniae* Carbapenemase-Producing *Enterobacteriaceae* Clinical Isolates[▽]

Athanassios Tsakris,[1]* Katerina Themeli-Digalaki,[2] Aggeliki Poulou,[1,3] Georgia Vrioni,[1] Evangelia Voulgari,[1] Vasiliki Koumaki,[1] Antonella Agodi,[4] Spyros Pournaras,[5] and Danai Sofianou[6]

JCM JournalsASM.org 2013;51(9):2986-90.

A Combined Disk Test for Direct Differentiation of Carbapenemase-Producing *Enterobacteriaceae* in Surveillance Rectal Swabs

Spyros Pournaras,[a] Olympia Zarkotou,[a,b] Aggeliki Poulou,[a,c] Ioulia Kristo,[d] Georgia Vrioni,[a] Katerina Themeli-Digalaki,[b] Athanassios Tsakris[a]

Department of Microbiology, Medical School, University of Athens, Athens, Greece[a]; Department of Microbiology, Tzaneio General Hospital, Piraeus, Greece[b]; Department of Microbiology, Serres General Hospital, Serres, Greece[c]; Department of Microbiology, Faculty of Medicine, University of Thessaly, Larissa, Greece[d]

JCM JournalsASM.org 2014;52(5):1483-9.

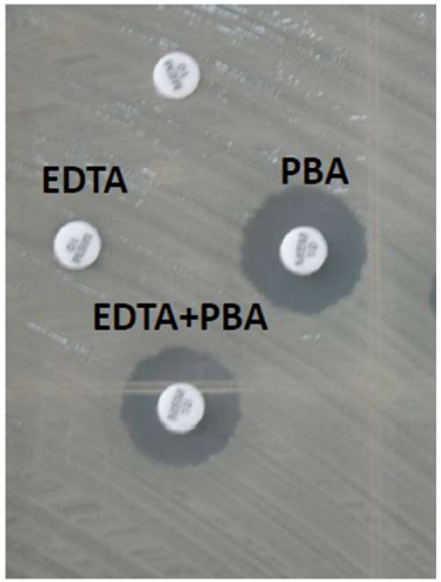
Modified CLSI Extended-Spectrum β-Lactamase (ESBL) Confirmatory Test for Phenotypic Detection of ESBLs among *Enterobacteriaceae* Producing Various β-Lactamases

Aggeliki Poulou,[a,b] Evgenia Grivakou,[a] Georgia Vrioni,[a] Vassiliki Koumaki,[a] Theodoros Pittaras,[a] Spyros Pournaras,[a] Athanassios Tsakris[a]

Department of Microbiology, Medical School, University of Athens, Athens, Greece[a]; Department of Microbiology, General Hospital of Serres, Serres, Greece[b]

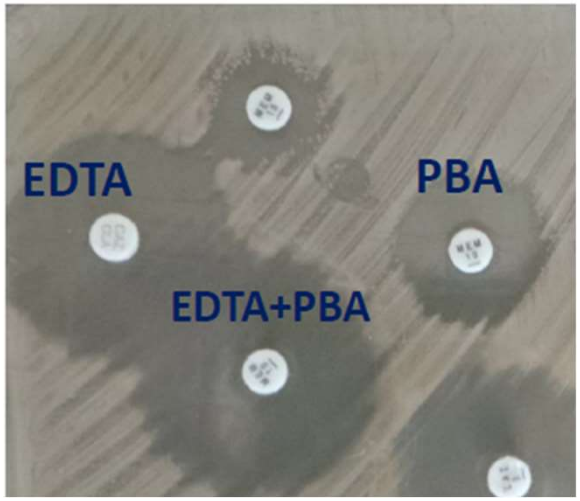
Φαινοτυπική ανίχνευση καρβαπενεμασών στα Εντεροβακτηριακά: χρήση αναστολέων καρβαπενεμασών

CDT



KPC

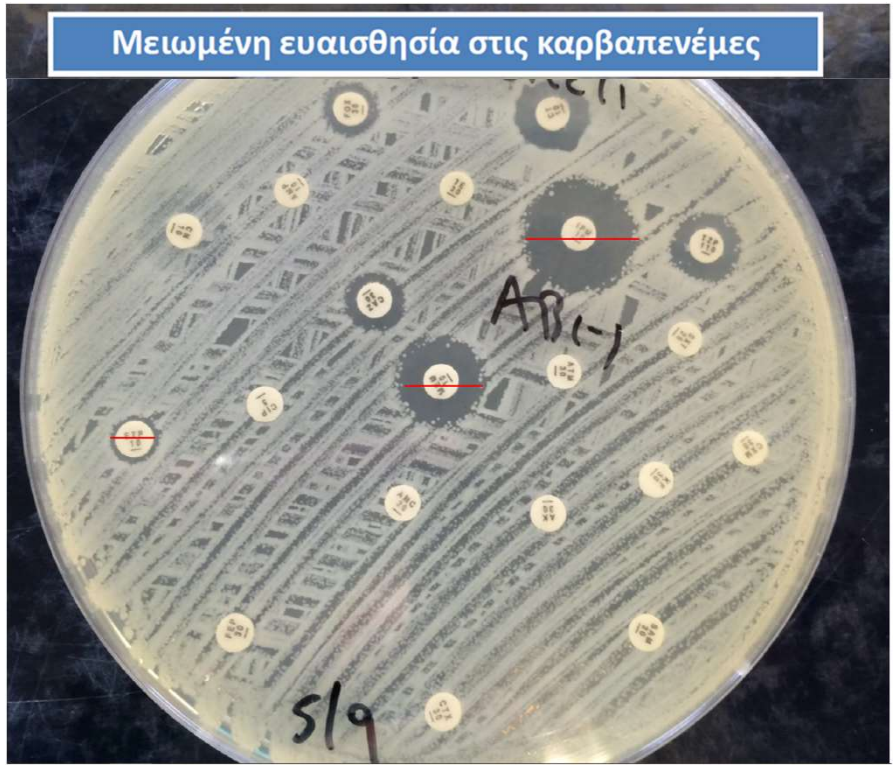
CDT

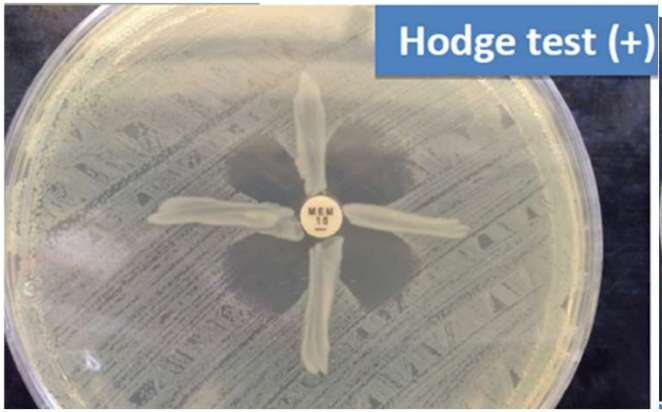


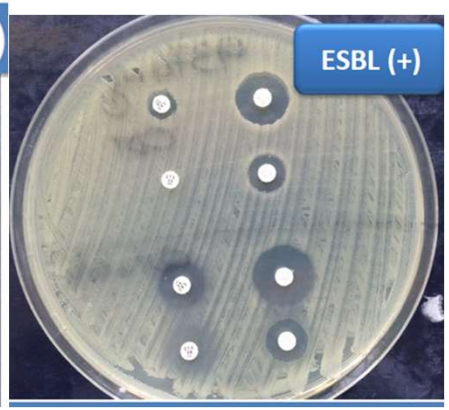
MBL

KPC+MBL

Φαινοτυπική ανίχνευση καρβαπενεμασών στα Εντεροβακτηριακά







VIM (-)	KPC (-)	**OXA-48 (+)**	**CTX-M (+)**	TEM (+)	NDM (-)	**SHV (+)**

E. coli

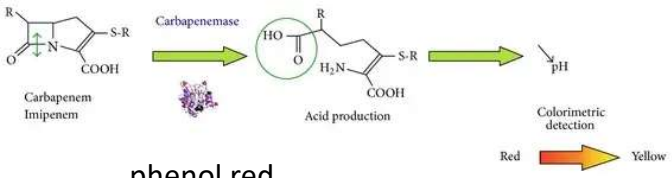
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Ertapenem	0.25	S
Temocillin			Imipenem	<= 0.25	S
Urine	>= 32	R	Meropenem		
Other	>= 32		Meningitis	<= 0.25	S
Ampicillin	>= 32	R	Other	<= 0.25	S
Amoxicillin/Clavulanic Acid	>= 32	R	Amikacin	2	S
Piperacillin/Tazobactam	>= 128	R	Gentamicin	<= 1	S
Cefuroxime	>= 64	R	Tobramycin	<= 1	S
Cefuroxime Axetil	>= 64	R	Ciprofloxacin		
Cefoxitin	<= 4	IE	Meningitis	0.5	
Cefditoren			Other	0.5	I
Cefixime	>= 4	R	Levofloxacin	1	I
Cefotaxime			Moxifloxacin	0.5	R
Meningitis	>= 64	R	Minocycline	(-)	(-)
Other	>= 64	R	Tetracycline	(-)	(-)
Ceftazidime	32	R	Tigecycline	<= 0.5	S
Ceftriaxone			Fosfomycin		
Meningitis	>= 64	R	Oral	<= 16	
Other	>= 64	R	Other	<= 16	S
Ceftazidime/Avibactam	<= 0.12	S	Nitrofurantoin		
Ceftolozane/Tazobactam	8	R	Chloramphenicol	16	IE
Cefepime	>= 32	R	Colistin	<= 0.5	S
Aztreonam	>= 64	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

(-)= Susceptibility testing not recommended; species is a poor target for therapy IE= Insufficient Evidence that species is good target for therapy; MIC may be reported without interpretation

Φαινοτυπική ανίχνευση παραγωγής καρβαπενεμασών στα Εντεροβακτηριακά

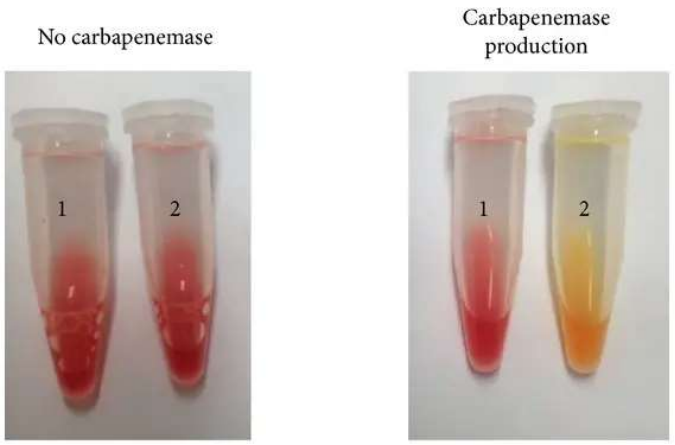
Χρωματομετρικές μέθοδοι (colorimetric tests)

Carba NP Test



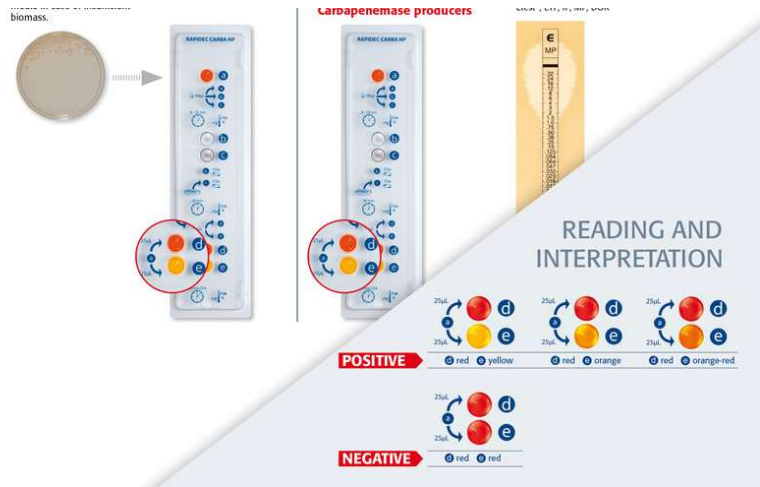
phenol red

(a)



(1) Revealing solution (internal negative control)
(2) Revealing solution + imipenem

(b)



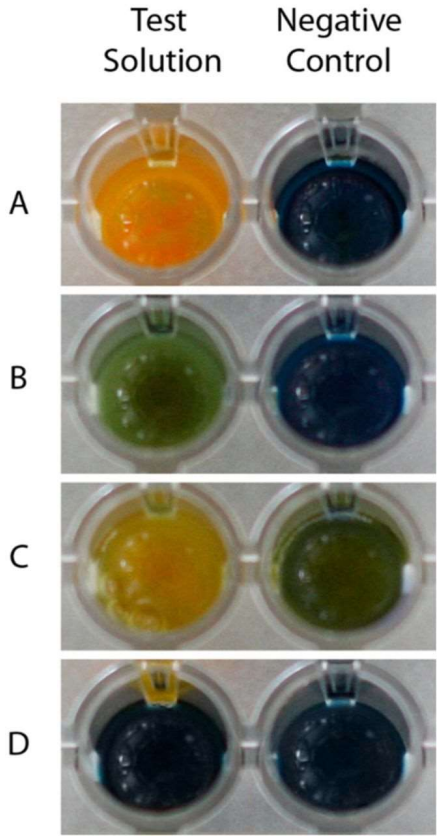
Sensitivity: 73–100% for most carbapenemases

Performed poorly for OXA-48 enzyme

Nordmann P, Poirel L, Dortet L. Emerg Infect Dis. 2012;18:1503-7.
Dortet L, Poirel L, Nordmann P. Antimicrob Agents Chemother. 2012;56:6437-40.
Dortet L et al. J Antimicrob Chemother. 2015 ; 70):3014-22.
Kabir MH et al. J Antimicrob Chemother. 2016 ;71:1213-6.
Noël A et al. J Clin Microbiol. 2017;55(2):510-518
Pasteran F et al. J Clin Microbiol. 2015 ;53(6):1996-8.

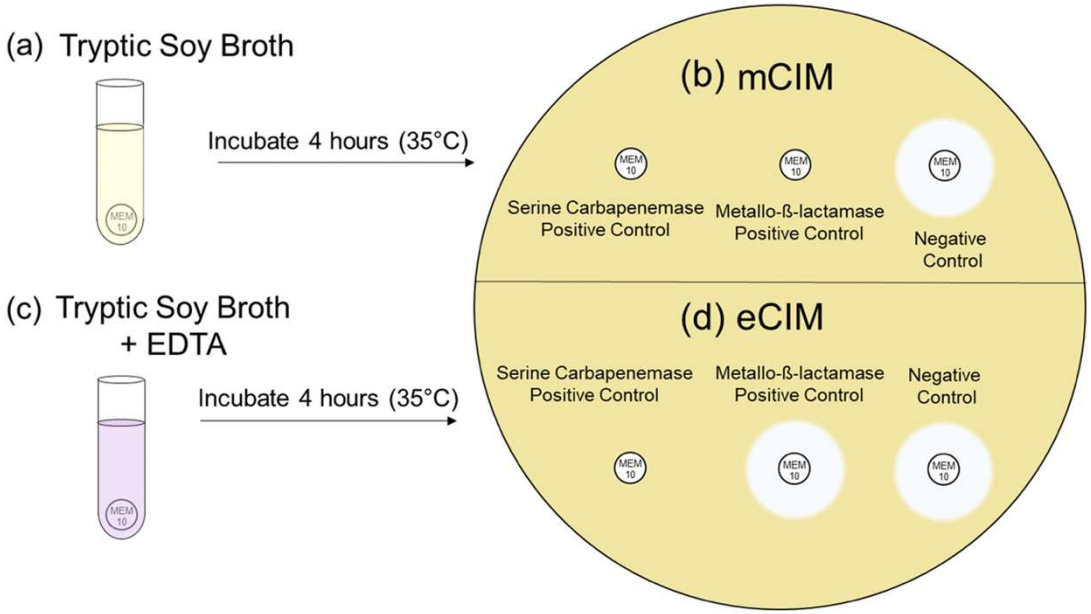
Blue-Carba Test

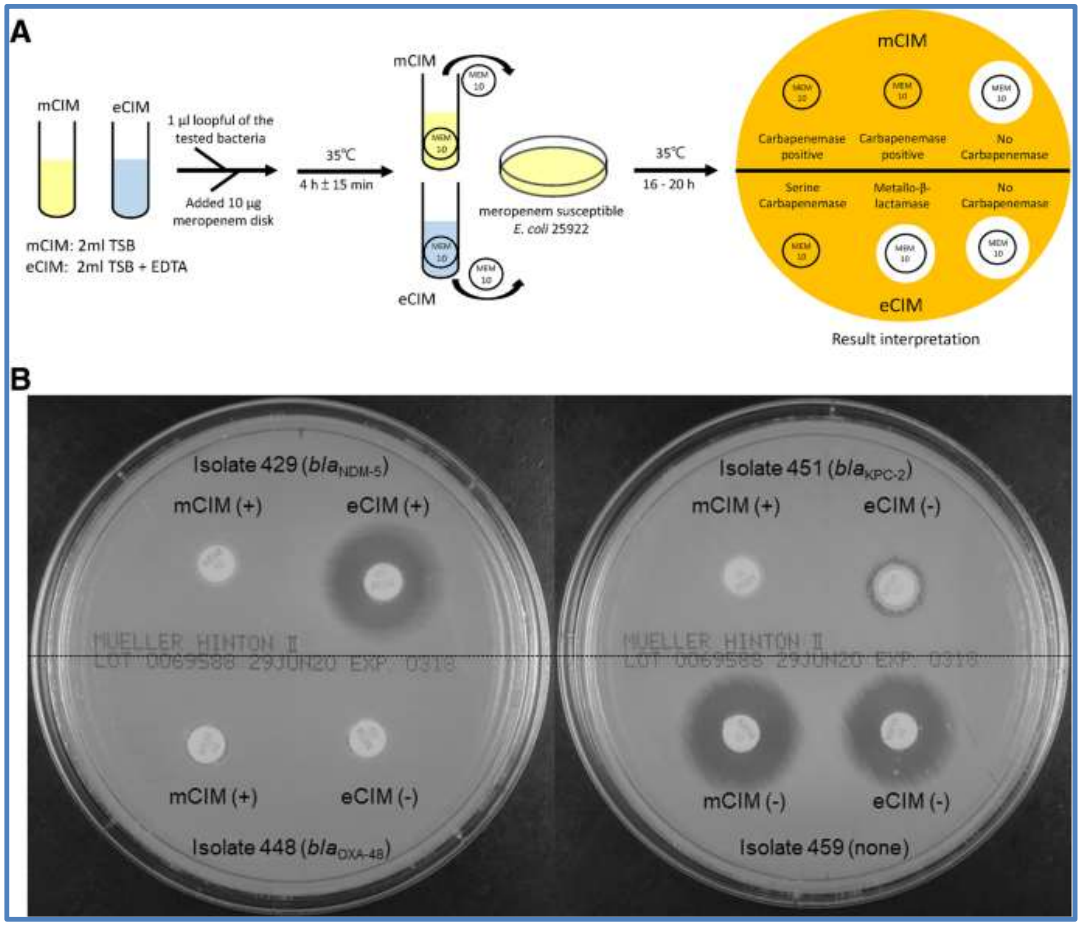
indicator bromothymol blue



Screening test: Carbapenemase Inactivation Methods (mCIM/eCIM)

The Carbapenemase Inactivation Methods (mCIM/eCIM) are Currently Recommended by CLSI





Tamma PD, Simner PJ. J Clin Microbiol. 2018;56(11):e01140–18.
https://asm.org/Articles/2019/May/Opening-the-Black-Box-Phenotypic-Carbapenemase-Det

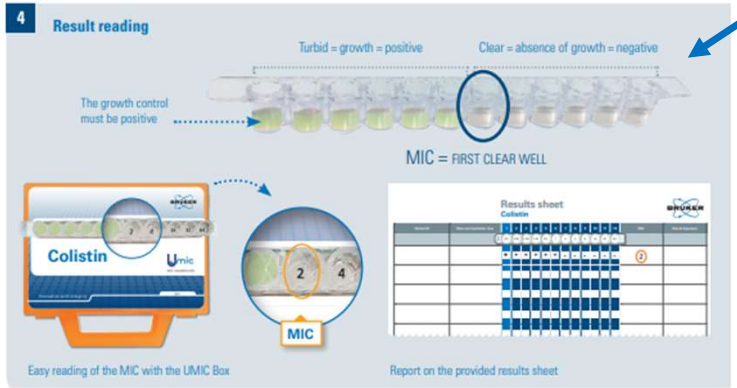
van der Zwaluw K et al. PLoS One. 2015;10(3):e0123690
Yamada K et al. J Microbiol Methods. 2016;128:48-51.
Tijet N, Patel SN, Melano RG. J Antimicrob Chemother. 2016 ;71(1):274-6.

Vitek reports

Bionumber: 0201010103500210
Organism Quantity:

Selected Organism: Acinetobacter baumannii complex

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL			Imipenem	>= 16	R
Temocillin			Meropenem		
Ampicillin	(-)	(-)	Meningitis	>= 16	R
Amoxicillin/Clavulanic Acid			Other	>= 16	R
Piperacillin/Tazobactam	>= 128	IE	Amikacin	>= 64	R
Cefuroxime	(-)	(-)	Gentamicin	>= 16	R
Cefuroxime Axetil	(-)	(-)	Tobramycin	>= 16	R
Cefoxitin	(-)	(-)	Ciprofloxacin	>= 4	R
Cefditoren			Levofloxacin	4	R
Cefixime	(-)	(-)	Moxifloxacin	(-)	(-)
Cefotaxime	(-)	(-)	Minocycline	2	IE
Ceftazidime	(-)	(-)	Tetracycline	(-)	(-)
Ceftriaxone	(-)	(-)	Tigecycline	4	IE
Ceftazidime/Avibactam			Fosfomycin	(-)	(-)
Ceftolozane/Tazobactam			Nitrofurantoin	(-)	(-)
Cefepime	(-)	(-)	Chloramphenicol	(-)	(-)
Aztreonam	(-)	(-)	Colistin	<= 0.5	S
Ertapenem			Trimethoprim/ Sulfamethoxazole	>= 320	R



Organism Quantity:

Selected Organism: Pseudomonas aeruginosa

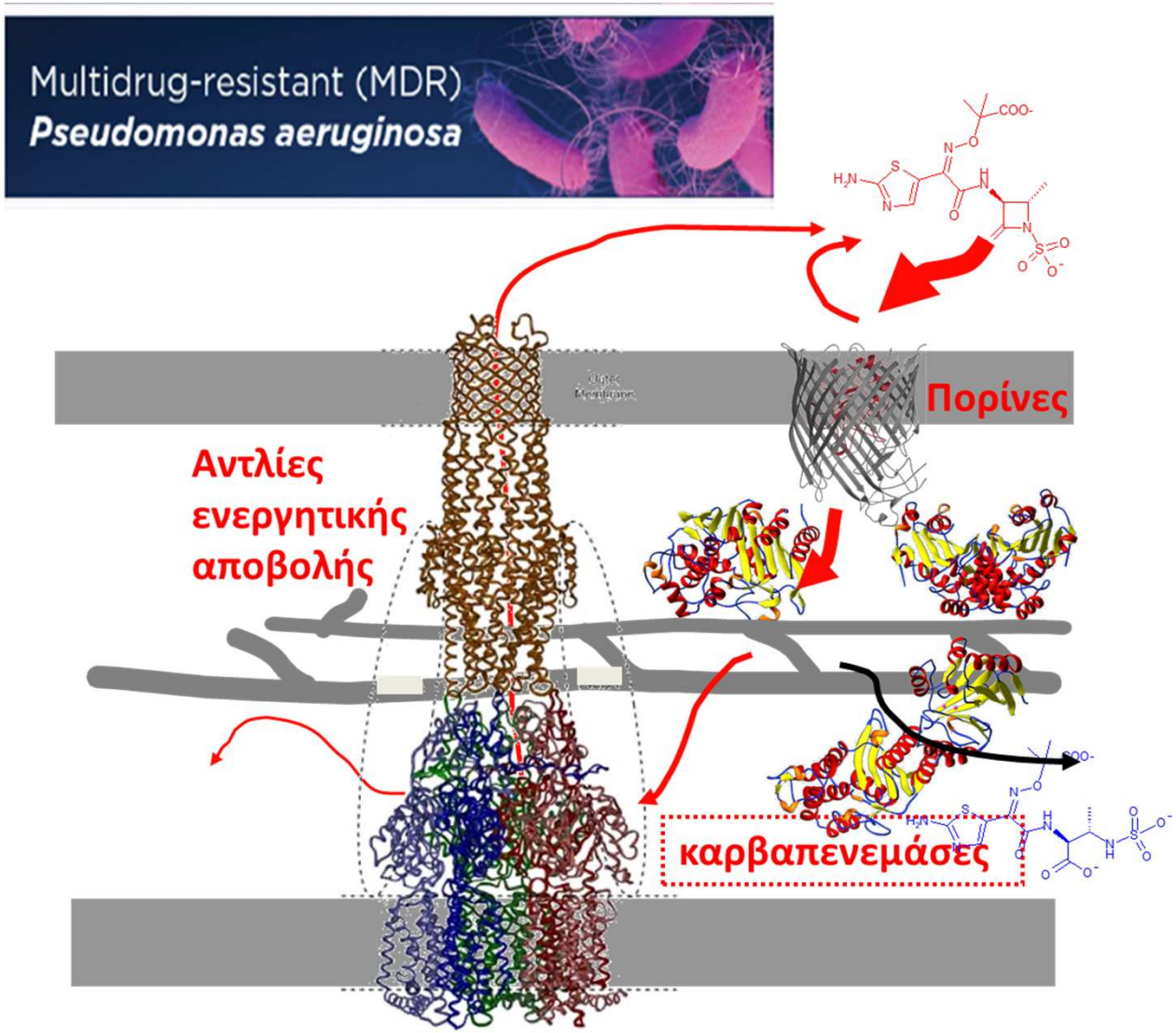
Comments:	Colistin S results / Gram negative bacilli: perform an alternative method of testing prior to reporting results.

Identification Information			
Organism Origin	Technologist		
Selected Organism	Pseudomonas aeruginosa		
	Entered: Sep 15, 2023 13:42 EEST	**By:**	LabTech
Analysis Messages:			

Susceptibility Information	**Card:**	AST-N318	**Lot Number:**	7582293203	**Expires:**	Mar 7, 2024 12:00 EET
	Status:	Final	**Analysis Time:**	9.27 hours	**Completed:**	Sep 15, 2023 22:42 EEST

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
+Ampicillin		(-)	Meropenem		
Ampicillin/Sulbactam			Meningitis	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Other	>= 16	R
Cefoxitin			Amikacin	>= 64	R
+Cefotaxime		(-)	Gentamicin	>= 16	IE
Ceftazidime	>= 64	R	Ciprofloxacin	>= 4	R
Ceftriaxone	(-)	(-)	Levofloxacin	>= 8	R
+Ceftolozane/Tazobactam			Tigecycline	(-)	(-)
Cefepime	>= 64	R	Fosfomycin	(-)	(-)
Aztreonam	>= 64	R	Colistin	2	S
+Ertapenem		(-)	Trimethoprim/ Sulfamethoxazole	(-)	(-)
Imipenem	>= 16	R			

P. aeruginosa και αντοχή στις καρβαπενέμες



Τάξη	Ένζυμα
A	KPC GES
D	OXA (OXA-40, -50 -198)
B	VIM IMP NDM SPM GIM, AIM, FIM

P. aeruginosa και φαινοτυπική ανίχνευση MBL

Δεν υπάρχουν προτυποποιημένες δοκιμασίες

Οι δοκιμασίες στηρίζονται στη χρήση χηλικών παραγόντων EDTA, MPA, DPA

Η απόδοση εξαρτάται από

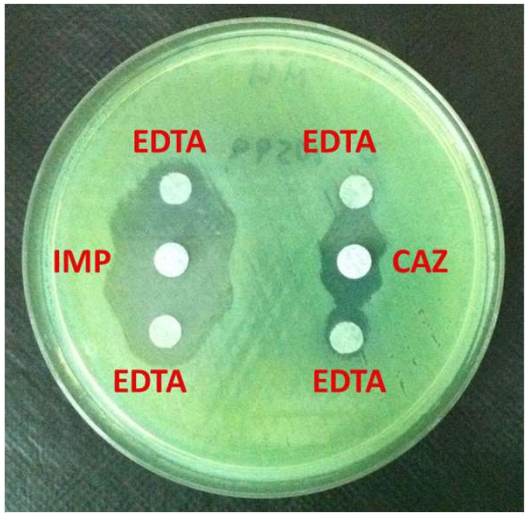
- μεθοδολογία (DDST, CDT, Etest MBL)
- β-λακταμικό υπόστρωμα
- είδος του αναστολέα
- ποσότητα του αναστολέα
- χαρακτηριστικά των στελεχών

All phenotypic combined disk tests lacked either sensitivity or specificity for the detection of MBL in *P. aeruginosa*

CDT

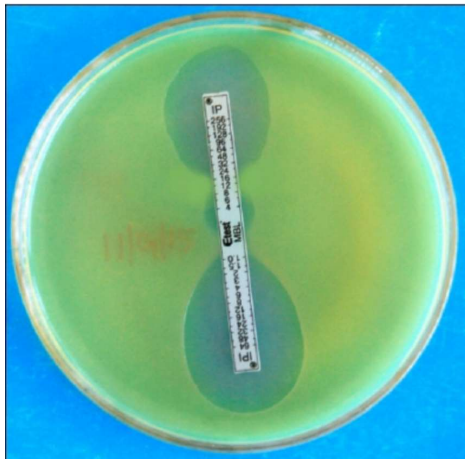
EDTA 292 μg (10 μl EDTA 0.1 M)
Δd > 4 mm
EDTA 750 μg, Δd ≥ 6 mm
100% SN και SP
DPA 835 μg, Δd ≥ 5 mm
SN=93,2% SP= 97,1%

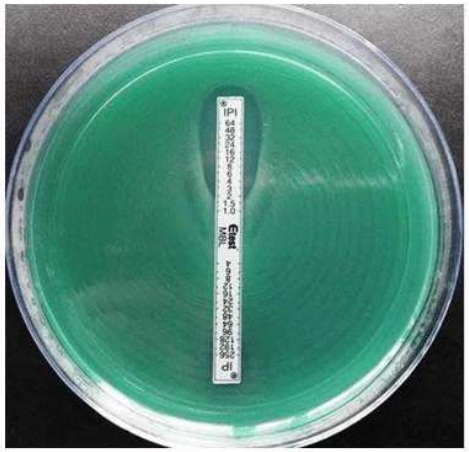
DDST



EDTA 292 μg
5 μl MPA 1.4 Mm
DPA 250 μg

Etest MBL, bioMerieux





IP/ IPI ≥ 8
παρουσία ζώνης-φάντασμα
παραμόρφωση των ελλείψεων IP ή IPI

For *P. aeruginosa* the MBL Etest® as well as disk-based assays have been used for several decades, but are hampered **by poor specificity**.

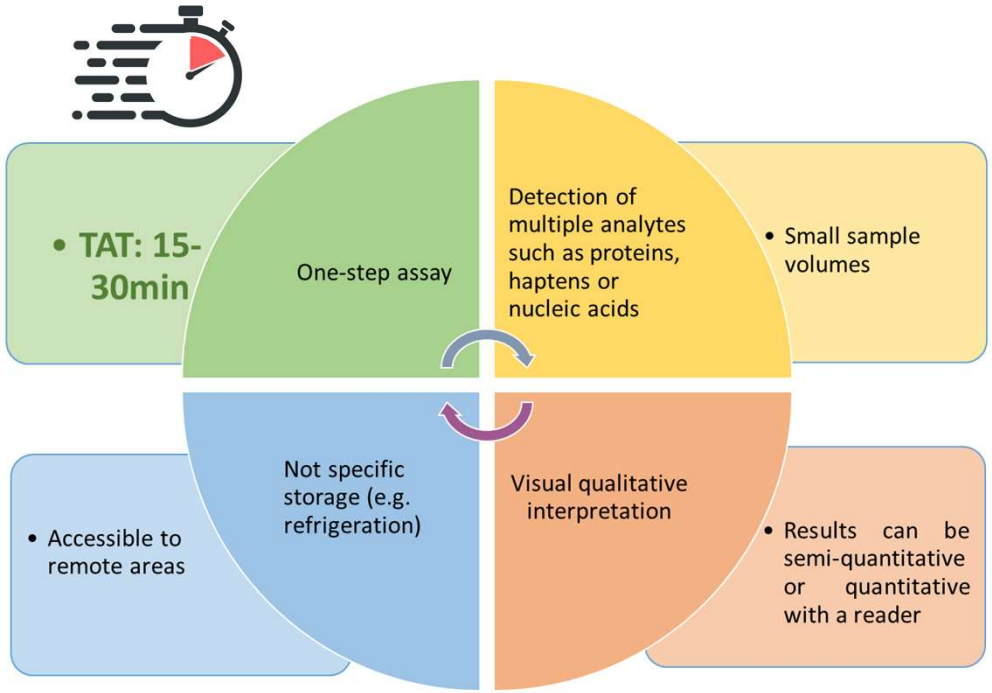
EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance

diagnostics 2022; 12(7):1744. MDPI

Review

The Revolution of Lateral Flow Assay in the Field of AMR Detection

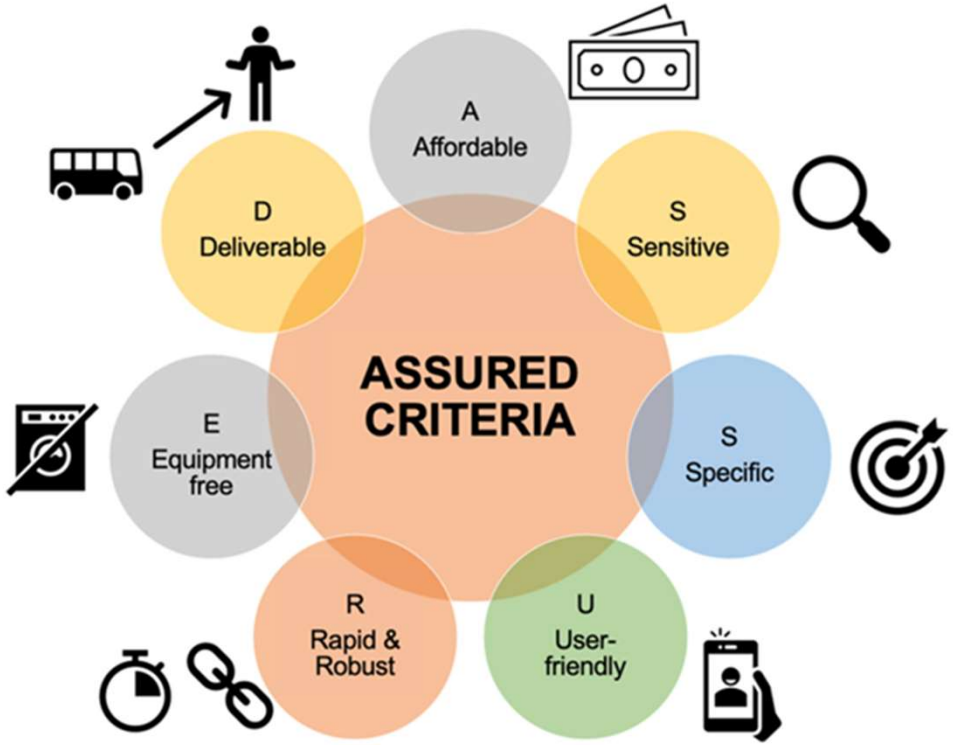
Hervé Boutal [1], Christian Moguet [1], Lilas Pommiès [1], Stéphanie Simon [1], Thierry Naas [2,3,4] and Hervé Volland [1,*]



LFIA performance relies mostly on antibody affinity and specificity

World Health Organization

an ideal PoC test

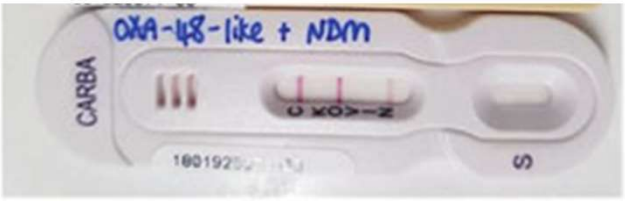


PBC
Se: 98%
Sp: 100%

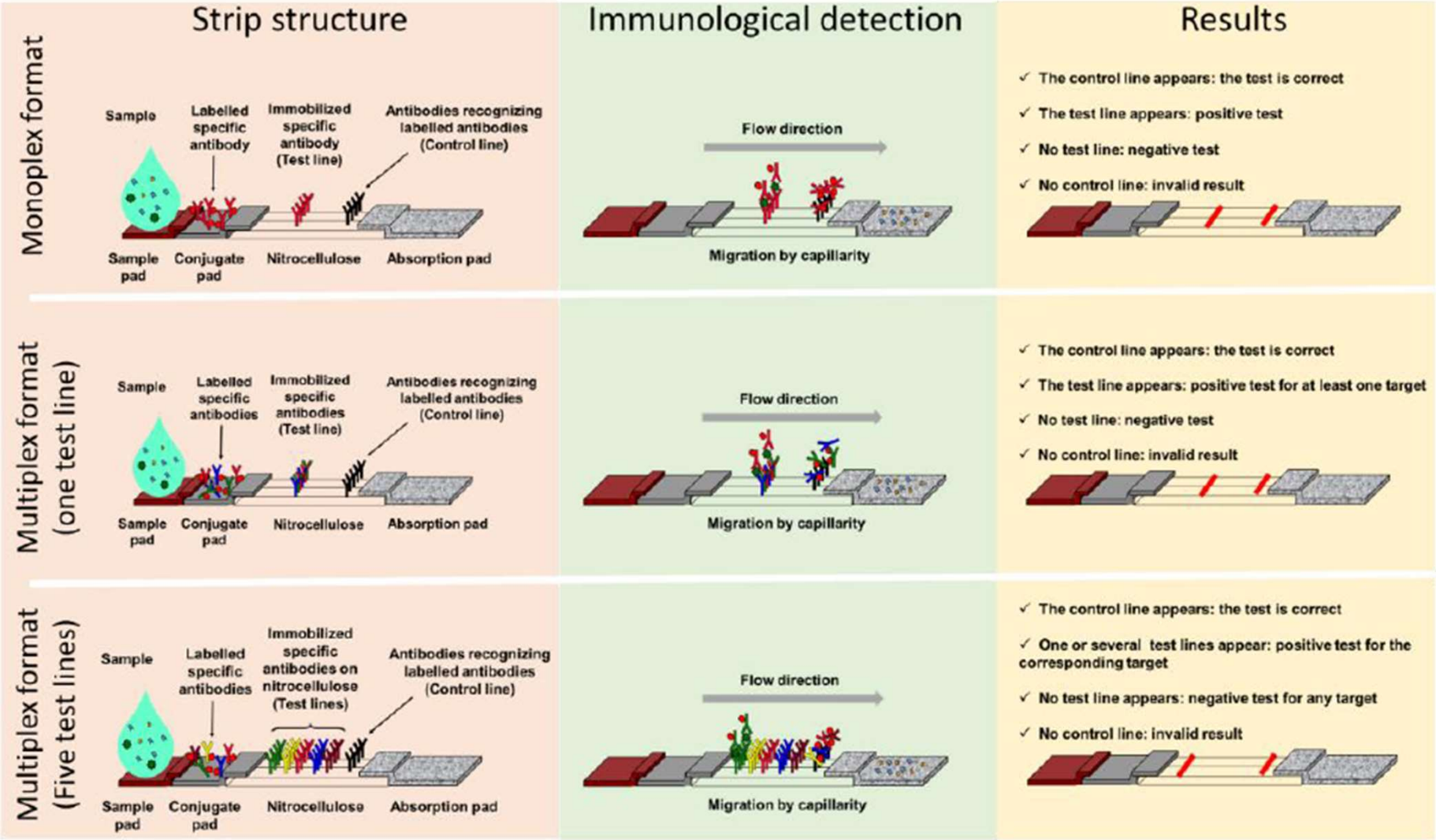
Del Corpo O et al. Clin Microbiol Infect. 2023 Sep 16:S1198-743X(23)00425-1.

Εντεροβακτηριακά με μειωμένη ευαισθησία στις καρβαπενέμες

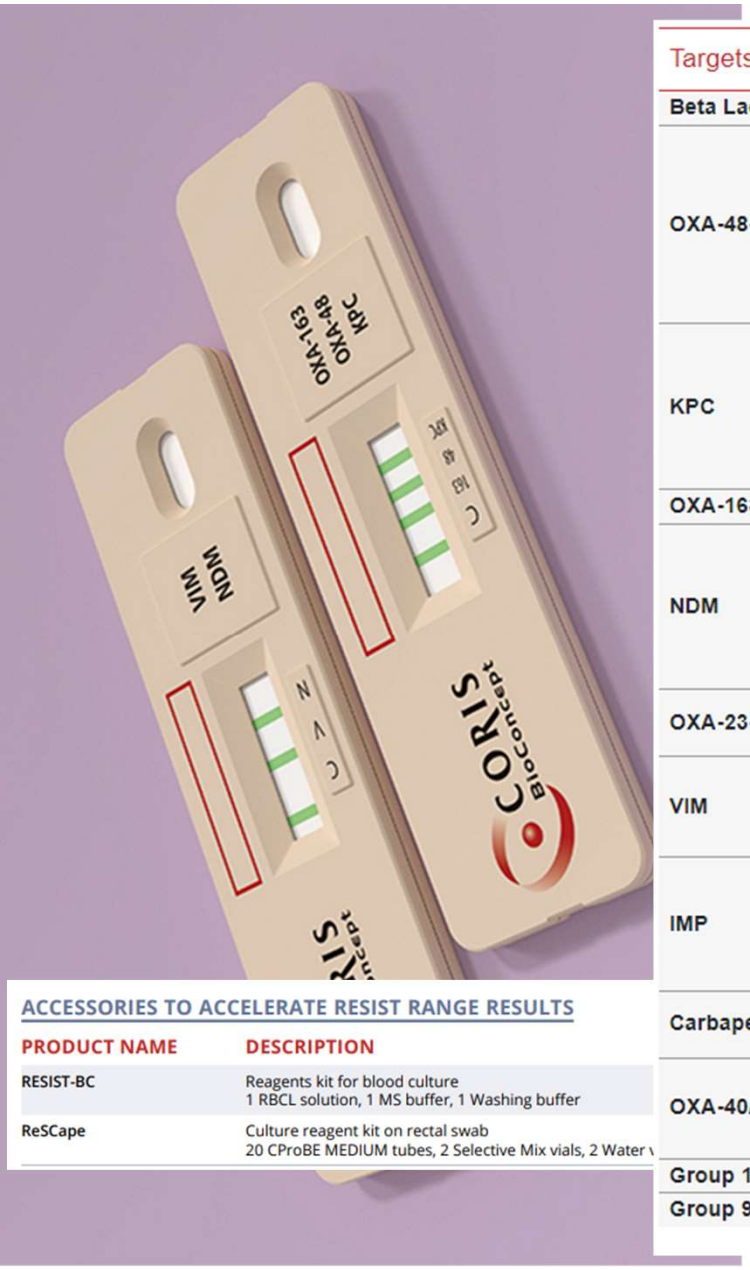
- **LFIA**
  - **Αρνητικό**
    - **CIM eCIM**
      - **Αρνητικό**
        - Πιθανή απώλεια πορινών
          Αποστολή στο κέντρο αναφοράς
      - **Θετικό**
        - Άλλη καρβαπενεμάση;
          Αποστολή στο κέντρο αναφοράς
  - **Θετικό**
    - Αναφορά στην κλινική
      Ενημέρωση ΝΕΛ



Lateral flow assay formats



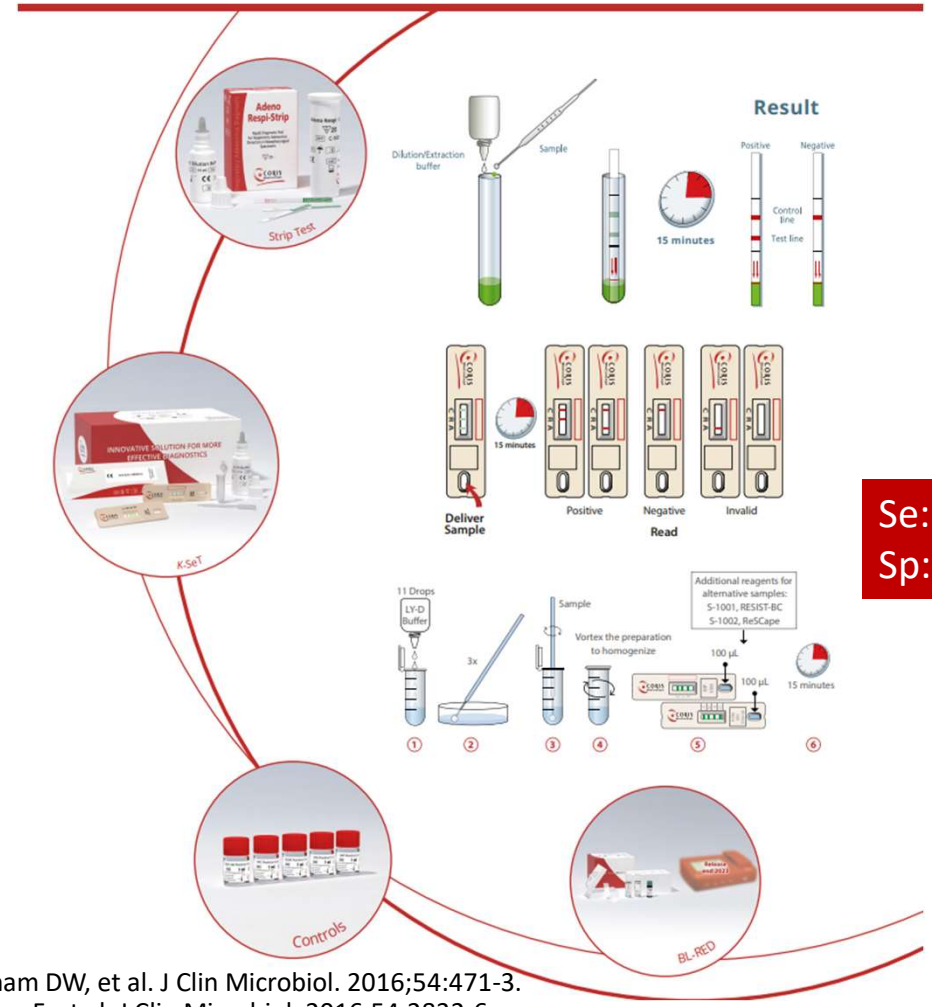
Boutal H et al. Diagnostics. 2022; 12(7):1744.



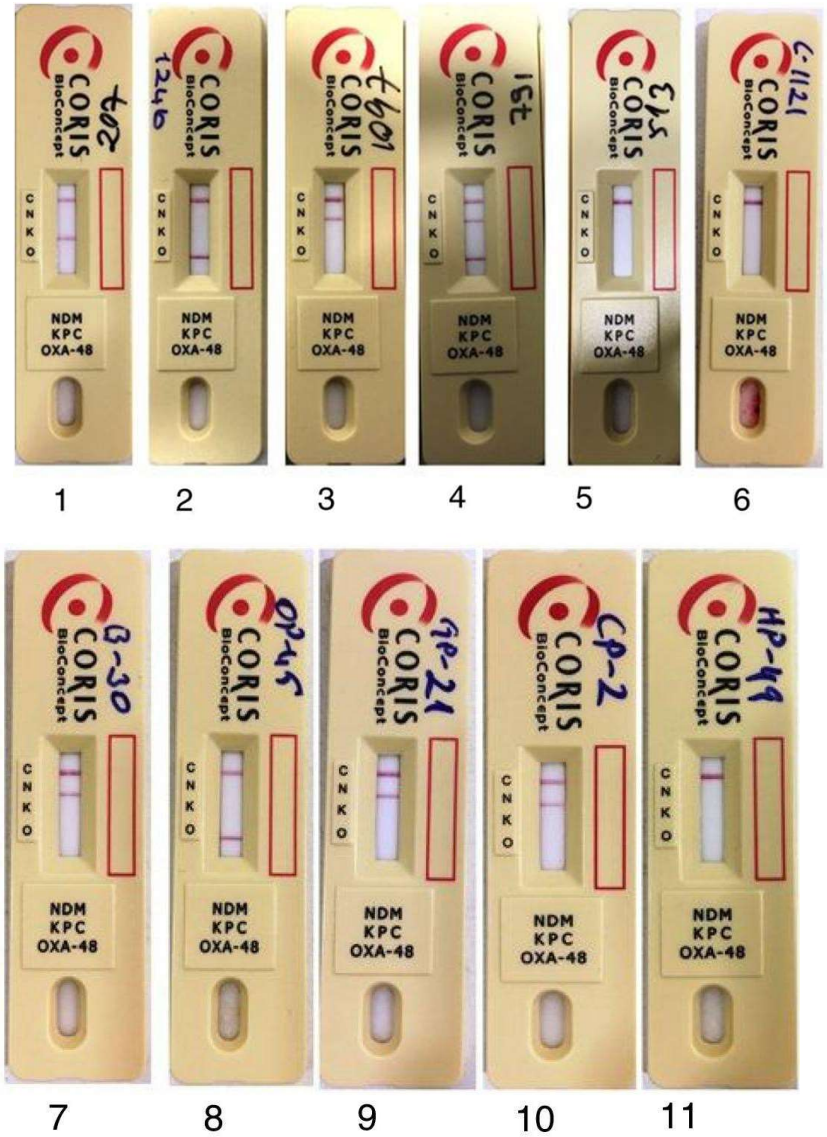
ACCESSORIES TO ACCELERATE RESIST RANGE RESULTS

PRODUCT NAME	DESCRIPTION
RESIST-BC	Reagents kit for blood culture 1 RBCL solution, 1 MS buffer, 1 Washing buffer
ReSCape	Culture reagent kit on rectal swab 20 CProBE MEDIUM tubes, 2 Selective Mix vials, 2 Water v

Targets	Technology	Product	Other identifications
Beta Lactamases	Electrochemistry	BL-RED 25	
OXA-48-like	Lateral flow	OXA-48 *K*-SeT	
	Lateral flow	O.K.N.V.I. RESIST-5	KPC, NDM, VIM, IMP
	Lateral flow	RESIST-3 O.O.K. *K*-SeT	OXA-163-like, KPC
	Lateral flow	RESIST-3 O.K.N. *K*-SeT	KPC, NDM
	Reagents	RESIST-BC	Carbapenemases: KPC, NDM, VIM, IMP, OXA-40/58
	Reagents	ReSCape	Carbapenemases: KPC, NDM, VIM, IMP, OXA-40/58
KPC	Lateral flow	O.K.N.V.I. RESIST-5	OXA-48-like, NDM, VIM, IMP
	Lateral flow	RESIST-3 O.O.K. *K*-SeT	OXA-48-like, OXA-163-like
	Lateral flow	RESIST-3 O.K.N. *K*-SeT	OXA-48-like, NDM
	Reagents	RESIST-BC	Carbapenemases: OXA-48-like, NDM, VIM, IMP, OXA-40/58
	Reagents	ReSCape	Carbapenemases: OXA-48-like, NDM, VIM, IMP, OXA-40/58
OXA-163-like	Lateral flow	RESIST-3 O.O.K. *K*-SeT	OXA-48-like, KPC
NDM	Lateral flow	O.K.N.V.I. RESIST-5	OXA-48-like, KPC, VIM, IMP
	Lateral flow	RESIST-3 O.K.N. *K*-SeT	OXA-48-like, KPC
	Reagents	RESIST-BC	Carbapenemases: OXA-48-like, KPC, VIM, IMP, OXA-40/58
	Reagents	ReSCape	Carbapenemases: OXA-48-like, KPC, VIM, IMP, OXA-40/58
	Lateral flow	RESIST ACINETO	OXA-23-like, OXA-40/58
OXA-23-like	Lateral flow	OXA-23 *K*-SeT	
	Lateral flow	RESIST ACINETO	OXA-40/58, NDM
VIM	Lateral flow	O.K.N.V.I. RESIST-5	OXA-48-like, KPC, NDM, IMP
	Reagents	RESIST-BC	Carbapenemases: OXA-48-like, KPC, NDM, IMP, OXA-40/58
	Reagents	ReSCape	Carbapenemases: OXA-48-like, KPC, NDM, IMP, OXA-40/58
IMP	Lateral flow	O.K.N.V.I. RESIST-5	OXA-48-like, KPC, NDM, VIM
	Lateral flow	IMP *K*-SeT	
	Reagents	RESIST-BC	Carbapenemases: OXA-48-like, KPC, NDM, VIM, OXA-40/58
	Reagents	ReSCape	Carbapenemases: OXA-48-like, KPC, NDM, VIM, OXA-40/58
Carbapenemases	Reagents	RESIST-BC	Carbapenemases: OXA-48-like, KPC, NDM, VIM, IMP, OXA-40/58
	Reagents	ReSCape	Carbapenemases: OXA-48-like, KPC, NDM, VIM, IMP, OXA-40/58
OXA-40/58	Reagents	RESIST-BC	Carbapenemases: OXA-48-like, KPC, NDM, VIM, IMP
	Reagents	ReSCape	Carbapenemases: OXA-48-like, KPC, NDM, VIM, IMP
	Lateral flow	RESIST ACINETO	OXA-23-like, NDM
Group 1 (CTX-M-15,...)	Lateral flow	RESIST CTX-M	Group 9 (CTX-M-14,...)
Group 9 (CTX-M-14,...)	Lateral flow	RESIST CTX-M	Group 1 (CTX-M-15,...)



Se: 98 -100%
Sp: 100%



Wareham DW, et al. J Clin Microbiol. 2016;54:471-3.
Pasteran F, et al. J Clin Microbiol. 2016;54:2832-6.
Glupczynski Y et al. J Antimicrob Chemother. 2017;72(7):1955-1960.
Greissl C et al. Eur J Clin Microbiol Infect Dis. 2019;38(2):331-335.
Song W et al. Ann Lab Med. 2020;40(3):259-263.
Sadek M et al. Diagn Microbiol Infect Dis. 2022;104(4):115761.
Bouvier M et al. Diagn Microbiol Infect Dis. 2023;107(3):116043.

Ανίχνευση καρβαπενεμασών σε στελέχη *Acinetobacter*

N = 174 well-characterized carbapenemase and non-carbapenemase producing isolates belonging to various *Acinetobacter* species

149 *A. baumannii*, 11 *A. pittii*, four *A. radioresistens*, three *A. ursingii*, two *A. calcoaceticus*, two *A. nosocomialis*, one *A. bereziniae*, one *A. lwoffii* and one *A. junii*.

Negative test
A. baumannii

OXA-40/58 +
A. baumannii

OXA-23 +
A. baumannii

NDM +
A. baumannii

OXA-40/58 +
OXA-23 +
A. baumannii

OXA-40/58 +
NDM +
A. baumannii

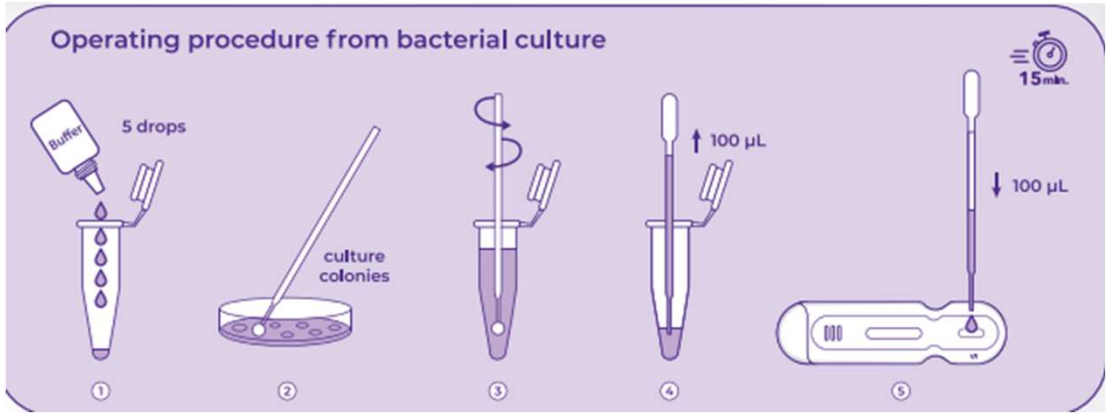
OXA-40/58 +
A. pittii

OXA-23 +
A. radioresistens

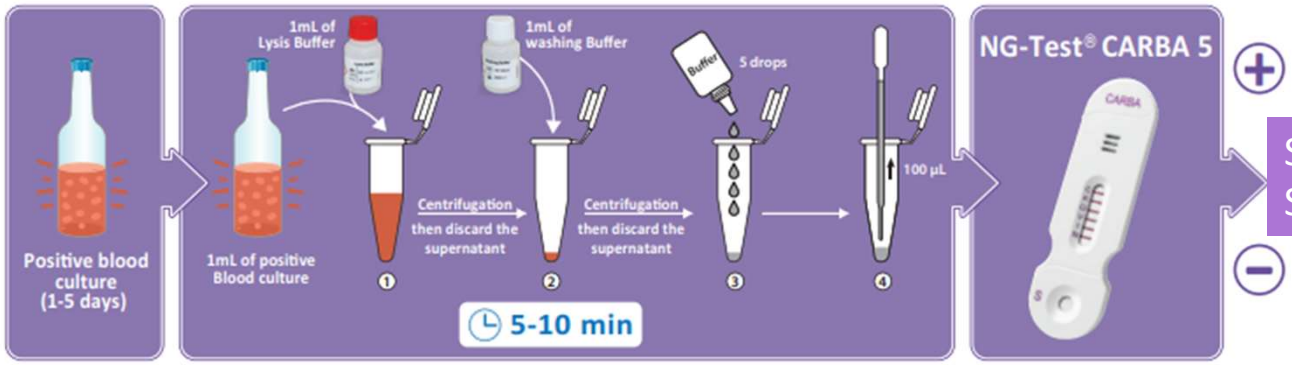
Overall sensitivity and specificity:100% and 96%
PPA and NPA 91% and 100%.

After exclusion of *A. pittii* and *A. radioresistens* results, sensitivity, specificity, PPA and NPA were 100%.

Bouvier M et al. Diagn Microbiol Infect Dis. 2023;107(3):116043.



IDENTIFICATION PROCESS FROM DIRECT BLOOD CULTURE: 20–40 min protocol



Se: 100%
Sp: 100%

Bodendoerfer E et al. J Antimicrob Chemother. 2019;74(6):1749-1751.

NG•TEST®/CARBA-5

Se: 97 -100%
Sp: 95-100%

Boutal H et al. J Antimicrob Chemother. 2018;73:909-15.
Han R et al. Front Microbiol. 2021;11:609856.
Volland H, et al. Antimicrob Agents Chemother. 2019;64(1):e01940-19.

NG•TEST®/CTX-M Multi

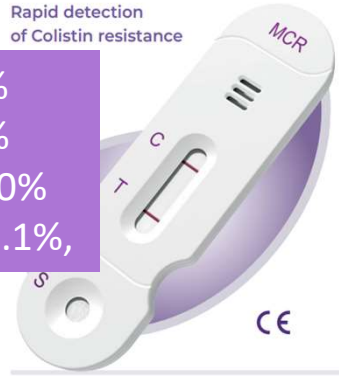
Rapid detection of Extended Spectrum Beta-Lactamase (ESBL)

Se: 100%
Sp: 100%

Bernabeu S et al. Diagnostics (Basel). 2020;10(10):764.
Bianco G, et al. J Hosp Infect. 2020;105(2):341-343.
Boattini M et al. Eur J Clin Microbiol Infect Dis. 2021;40(7):1495-1501.

NG•TEST®/MCR-1

Rapid detection of Colistin resistance



Se: 100%
Sp: 100%
[2]PPA: 100%
[2]NPA: 96.1%,

Volland H et al. J Clin Microbiol. 2019;57:e01454-18.
Fenwick AJ et al. J Clin Microbiol. 2020;58(4):e01823-19.

LFIA και ανίχνευση φορέων απευθείας από το δείγμα

J Antimicrob Chemother. 2019 Feb 1;74(2):357-359. doi: 10.1093/jac/dky429.

Lateral flow immunochromatographic assay for rapid screening of faecal carriage of carbapenemase-producing Enterobacteriaceae

Charlotte Fauconnier [1], Magali Dodemont [2], Angélique Depouhon [1], Ahalieyah Anantharajah [1], Alexia Verroken [1], Hector Rodriguez-Villalobos [1]

Residual rectal swabs (*n*=149), routinely screened for CPE through selective culture and confirmed by PCR

Protocol: 2.5h incubation of the swab in an enrichment medium containing meropenem followed by **O.K.N. *K*-SeT** testing after centrifugation.

Strains: 82 OXA-48, 22 NDM, 5 KPC, 1 IMP, 7 VIM, 3 OXA-23

Detected: 76 OXA-48, 19 NDM, 5 KPC

Sensitivity: 96%

Specificity: 100%

LoD: 10^4-10^5 cfu/ml

Microorganisms. 2021 Apr 27;9(5):942. doi: 10.3390/microorganisms9050942.

Detection of KPC, NDM and VIM-Producing Organisms Directly from Rectal Swabs by a Multiplex Lateral Flow Immunoassay

Alexandra Vasilakopoulou [1], Polyxeni Karakosta [1], Sophia Vourli [1], Eleni Kalogeropoulou [1], Spyros Pournaras [1]

Fecal swabs (*n*=30)

- Culture
- PCR
- **NG-Test® CARBA-5**

Each sample was tested by CARBSA-5 five times:

- Direct testing
- Incubation in TSB for 1h, 2h, 3h, 4h

TSB enriched with 0.25mg/l meropenem

Sensitivity:

80% (direct testing)

88% (1h incubation)

92% (2h incubation)

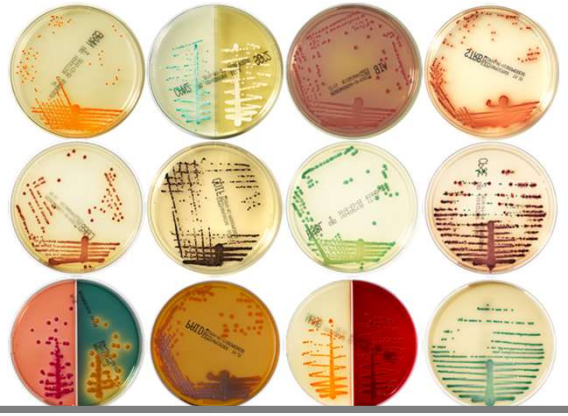
100% (3h incubation)

Specificity: 100%

Concordant results from LFIA and PCR: 11 KPC, 8 VIM, 6 NDM. [In five cases, co-carriage of KPC and VIM]

Χρωμογόνα υλικά

- Purpose: more rapid detection and identification of MDRO
- The target organisms are characterized by specific enzyme systems that metabolize the substrates to release the chromogen.
- The chromogen can then be visually detected by direct observation of a distinct colour change in the medium (coloured colonies).
- Compared with the use of conventional culture media, the use of chromogenic agar often reduces the costs and labor time.
- Their primary use is for screening of patients colonized with various pathogens.
- The sensitivity and specificity depend on the manufacturer and the type of microorganism detected
- Additional confirmation of the resistant bacteria is sometimes needed.

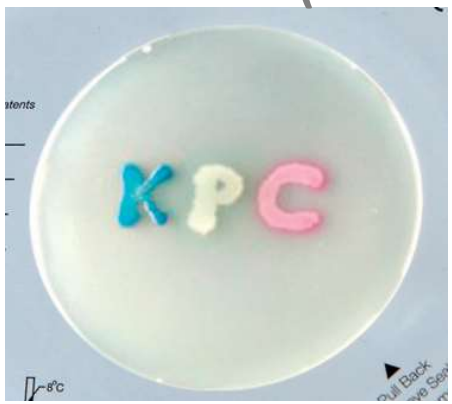


Advantage	
Mass use and the possibility of automatisation	
Simple to perform	
Simple and fast interpretation	
Disadvantage	**Comments**
Not completely susceptible and specific	Qualitative with no interpretation criteria (S, I, R)
Time-consuming	
Limited spectra or single antibiotic	
Relatively expensive	
Screening only or required confirmatory identification	
No MIC value	

Gajic I et al. Antibiotics (Basel). 2022;11(4):427.

Χρωμογόνα εκλεκτικά υλικά για CRE

CHROMagar KPC, Colorex KPC (CHROMagar)

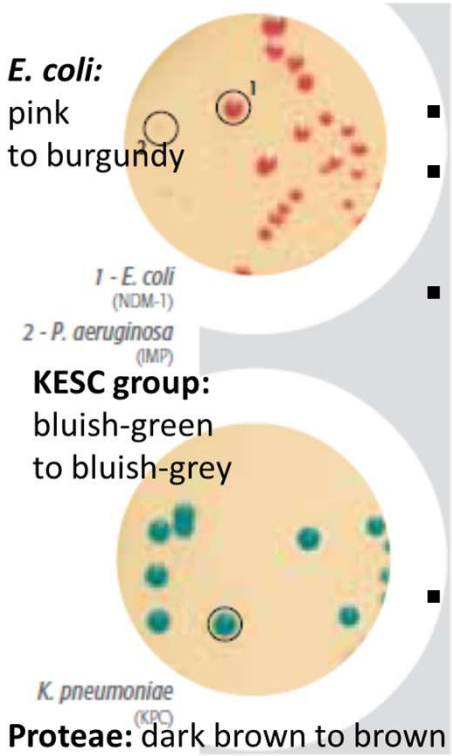


Klebsiella, Enterobacter, Citrobacter CR → Metallic blue
Pseudomonas CR → Cream, translucent
E.coli CR → dark pink to reddish

- Μειονέκτημα: έλλειψη ευαισθησίας
- Δυσκολία ανάπτυξης στελεχών με MIC≤4 μg/ml
- Προβληματική η ανίχνευση στελεχών MBL και OXA-48

Nordmann P et al. J Antimicrob Chemother. 2013; 68(3):487-9.

ChromID Carba (bioMérieux)



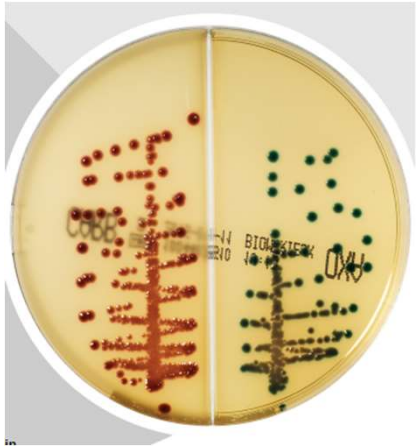
- SN: 92,4%, SP: 96, 9%
- Ικανότητα ανίχνευσης στελεχών με χαμηλές MIC[1]
- Υψηλότερη ικανότητα ανίχνευσης για KPC-στελέχη vs Brilliance CRE /Colorex KPC (σε υψηλό και σε χαμηλό inoculum)[2]
- Υψηλότερη ικανότητα ανίχνευσης NDM-στελεχών[3]

1. Vrioni G, et al. J Clin Microbiol. 2012; 50(6):1841-6.
2. Wilkinson KM, et al. J Clin Microbiol. 2012; 50(9):3102-4.
3. Perry JD, et al. J Antimicrob Chemother. 2011; 66(10):2288-94.

ChromID OXA-48 (bioMérieux)

Incubation : 18 hours
K. pneumoniae OXA-48

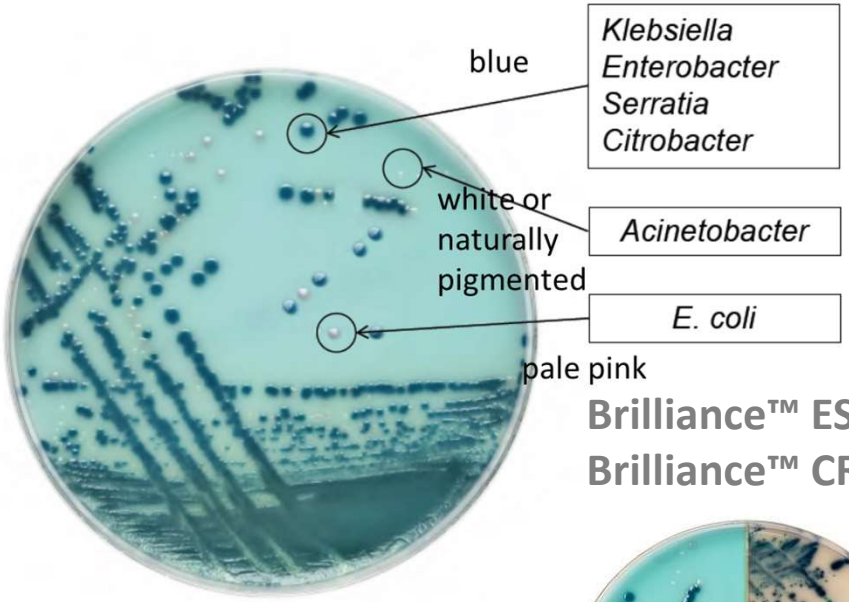
CHROMID® CARBA SMART Agar



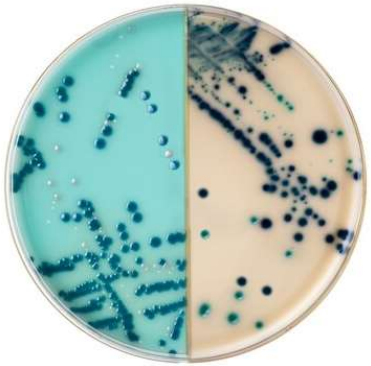
Χρωμογόνα εκλεκτικά υλικά για CRE

Brilliance™ CRE (Oxoid)

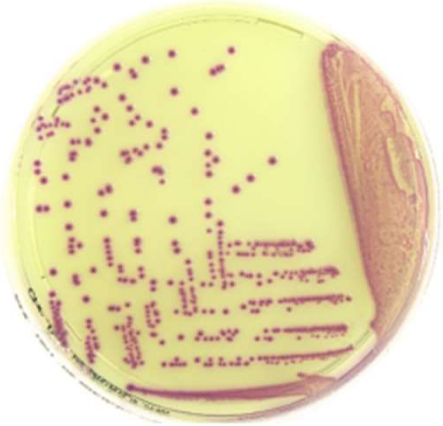
- Χαμηλότερη SN έναντι του ID Carba για την ανίχνευση NDM-στελεχών
- Υπεροχή έναντι του CHROMagar KPC (43%)
- Παρόμοια SP



Brilliance™ ESBL Agar/ Brilliance™ CRE Agar



Liofilchem® Chromatic CRE



Liofilchem® Chromatic OXA-48

OXA-48 positive *Enterobacter cloacae*

Day KM, et al. Diagn Microbiol Infect Dis. 2013; 75(2):187-91.
Girlich D, et al. Diagn Microbiol Infect Dis. 2013;75(2):214-7.

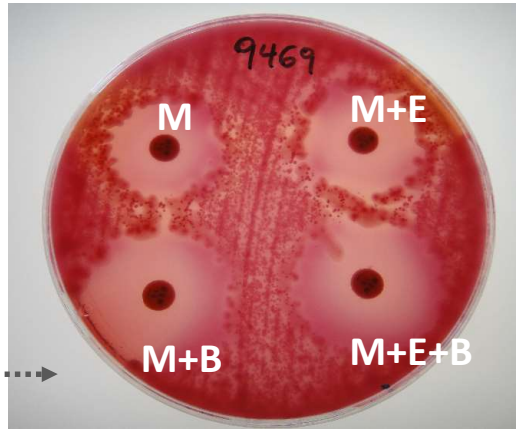
Έλεγχος φορείας για CRE: καλλιεργητικές μέθοδοι

Home-made τρυβλία MacConkey agar:

- Με ενσωματωμένη καρβαπενέμη
  - ικανοποιητική ευαισθησία
- Με δίσκους καρβαπενεμών:
  - SN/SP εξαρτώνται από το όριο
- Με δίσκους + αναστολείς:
  - ανίχνευση + διαφοροποίηση

Χρωμογόνα υλικά

- **chromID CARBA / chromID OXA-48, bioMérieux**
  - Υψηλή ευαισθησία και ειδικότητα
- **Brilliance CRE, Oxoid**
  - Ικανοποιητική ευαισθησία και ειδικότητα
- **CHROMagar KPC/ Colorex KPC, CHROMagar**
  - Ανιχνεύει στελέχη με υψηλού επιπέδου αντοχή



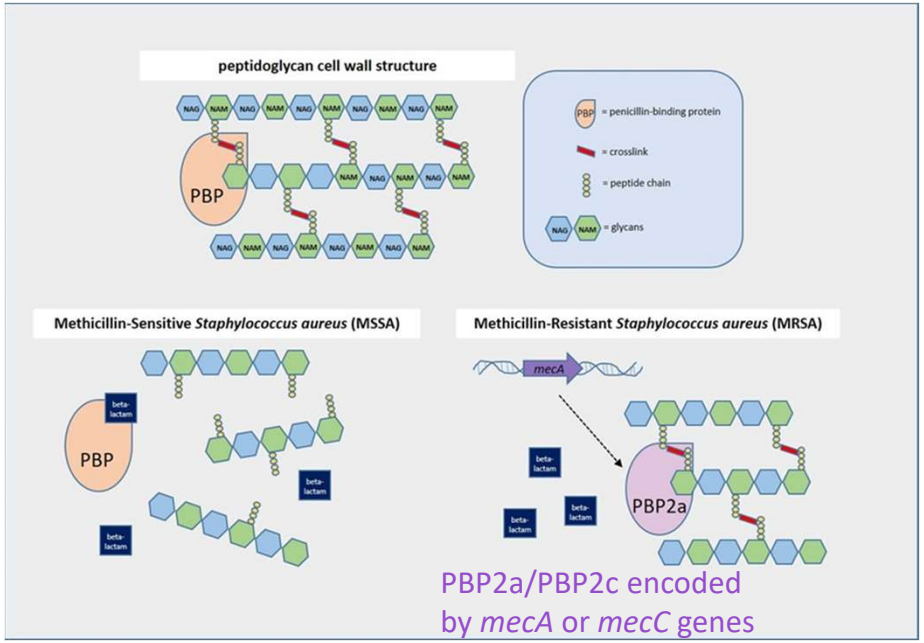
Pournaras S, et al. J Clin Microbiol. 2013; 51(9):2986-90.

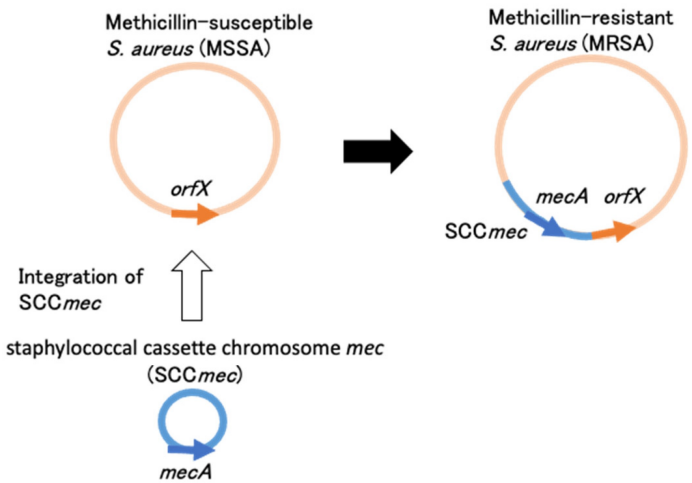
Η επίδοση της κάθε μεθόδου εξαρτάται:

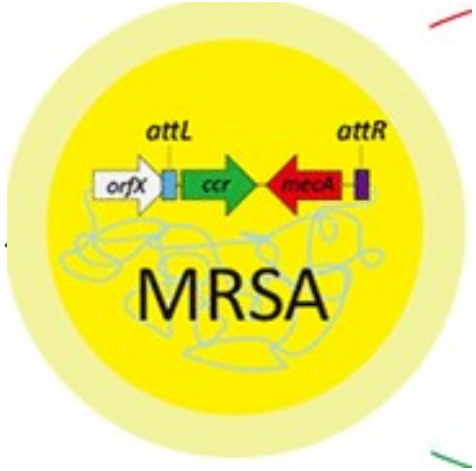
- επίπεδο αντοχής στις καρβαπενέμες
- υδρόλυση άλλων β-λακταμών (φάσμα)
- παρουσία άλλων μηχανισμών αντοχής
- γεωγραφική περιοχή, βακτηριακό είδος, δείγμα, χρόνο επώασης, μέθοδο αναφοράς, ορισμό αληθώς θετικών, …

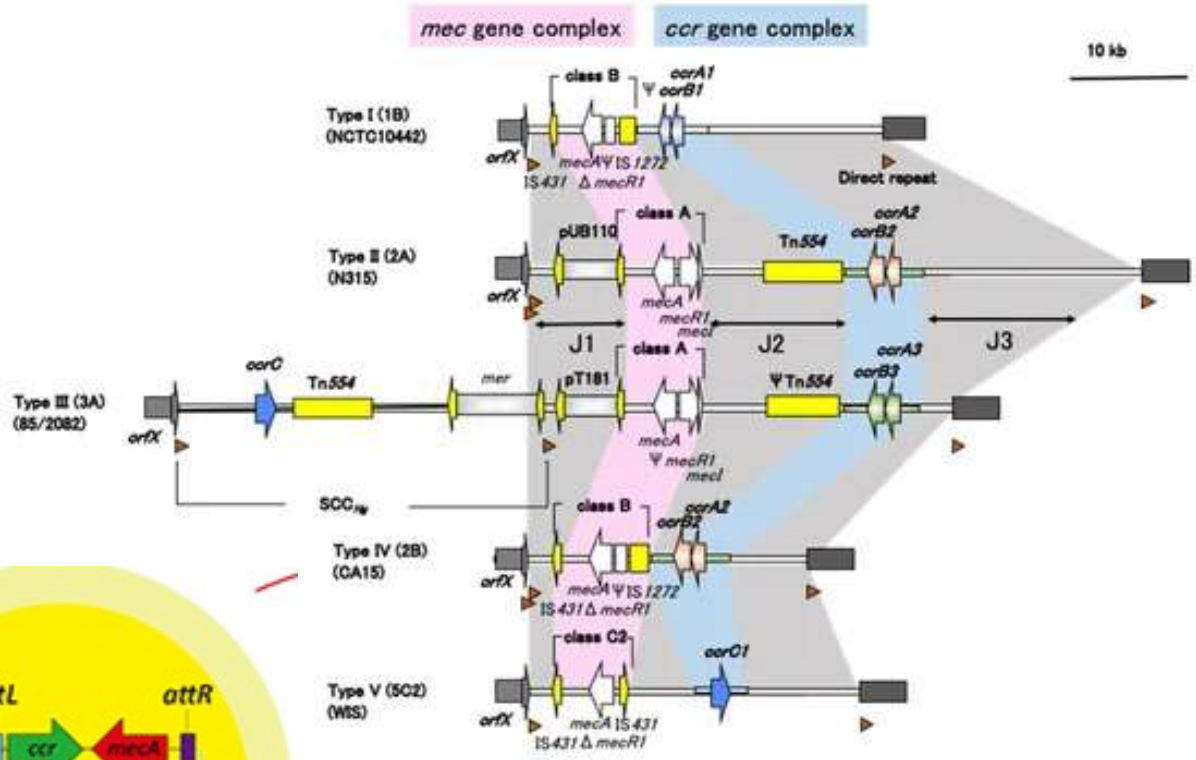
Σύγκριση μελετών-μεθόδων δύσκολη

MRSA





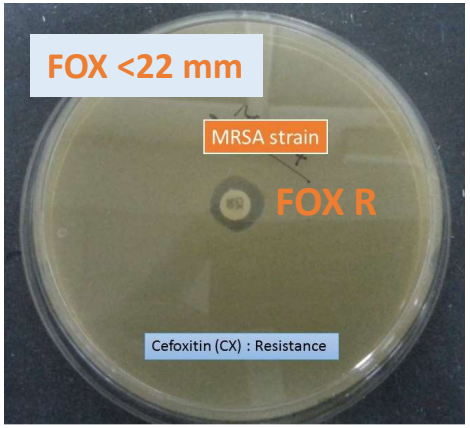




Uehara Y. Current Status of Staphylococcal Cassette Chromosome mec (SCCmec). Antibiotics. 2022; 11(1):86.

Φαινοτυπικές μέθοδοι

Disk diffusion



CEFOXITIN: a very **sensitive and specific marker** of mecA/mecC-mediated methicillin resistance including in heterogeneous expressing strains and **is the agent of choice.**

Broth microdilution

Organism Quantity: **Selected Organism: Staphylococcus aureus**

Comments:	S.aureus - Mupirocin: ECOFF

Identification Information			
Organism Origin	Technologist		
Selected Organism	Staphylococcus aureus		
	Entered: Oct 7, 2023 17:56 EEST	**By:**	LabTech
Analysis Messages:			

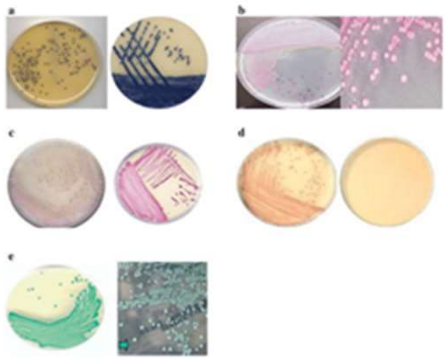
Susceptibility Information	**Card:**	AST-P659	**Lot Number:**	8092252103	**Expires:**	Jan 26, 2024 12:00 EET
	Status:	Final	**Analysis Time:**	8.48 hours	**Completed:**	Oct 8, 2023 02:22 EEST

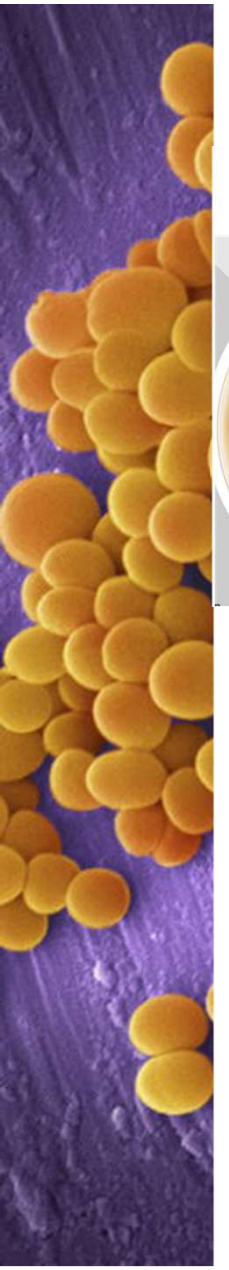
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Linezolid	2	S
Benzylpenicillin	>= 0.5	R	Daptomycin	0.25	S
Oxacillin	>= 4	R	Teicoplanin	<= 0.5	S
Ceftaroline			Vancomycin	<= 0.5	S
Pneumonia	0.25	S	Tetracycline	<= 1	S
Other	0.25	S	Tigecycline	<= 0.12	S
Gentamicin	<= 0.5	S	Fusidic Acid	8	R
Levofloxacin	>= 8	R	Mupirocin	<= 1	S
Inducible Clindamycin Resistance	NEG	-	Rifampicin	<= 0.03	S
Erythromycin	0.5	S	Trimethoprim/ Sulfamethoxazole	<= 10	S
Clindamycin	0.25	S			

Detection of PBP2a with latex agglutination kits

*PBP2c is not detected by the majority of commercial assays

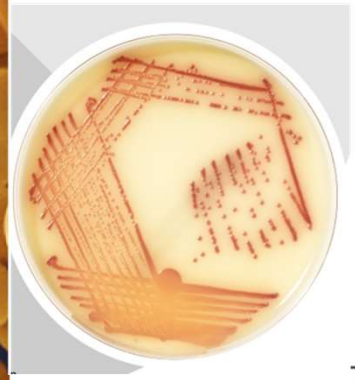
Chromogenic media





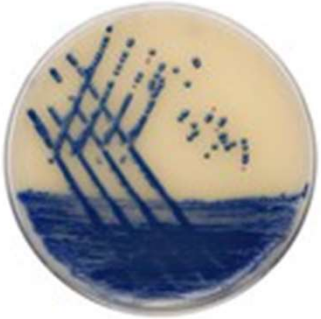
Χρωμογόνα εκλεκτικά υλικά για MRSA

CHROMID® MRSA SMART, bioMerieux

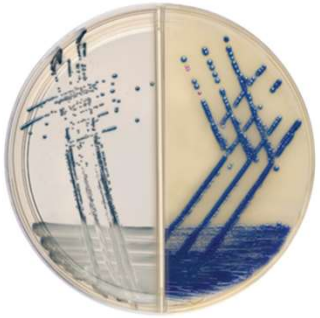


ChromID *S. aureus*/ ChromID MRSA, bioMerieux

Brilliance™ MRSA 2 Agar, Oxoid



Brilliance™ MRSA 2 Agar/ Brilliance™ Staph 24 Agar



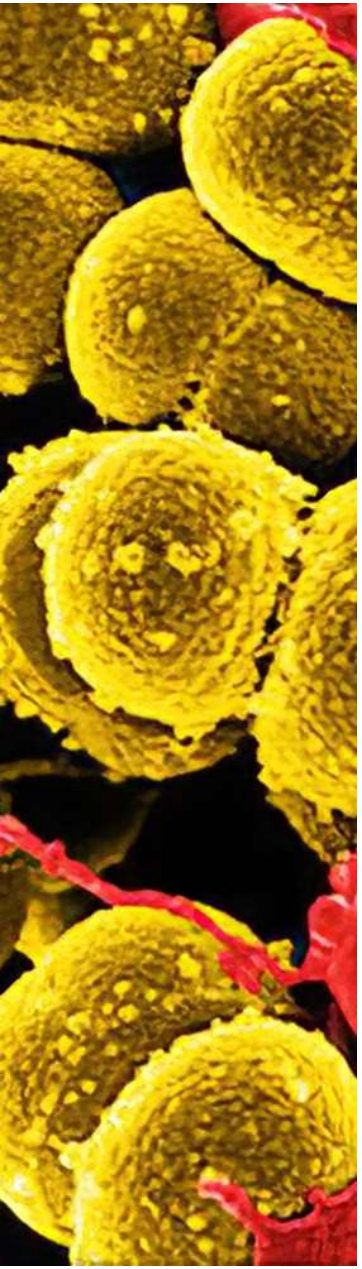
MRSA Select, Bio-Rad

ChromAgar MRSA

Variable	CHROMagar [43]	BBL-CHROMagar [43]	CHROMagar MRSA [35]
Sensitivity (%)	82–93	83–94	96–100
Specificity (%)	97–99	98–99	95–97
Turnaround time (h)	24–48	24–48	24–48
Storage for incubation	2–8 °C; dark	2–8 °C; dark	2–8 °C; dark

Variable	MRSASelect [35]	Brilliance MRSA agar [43]	ChromID [43]
Sensitivity (%)	81–93	90–96	83–94
Specificity (%)	92–97	69–87	90–96
Turnaround time (h)	18–24	18–24	
Storage for incubation	Room temperature	2–10 °C; dark	Room temperature

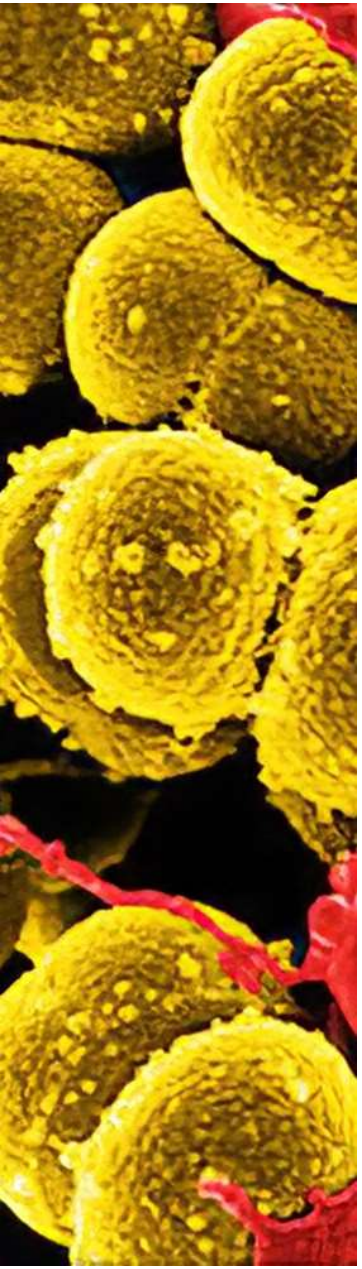
Harbarth S et al. Int J Antimicrob Agents. 2011;37(2):110-7.
Nonhoff C et al. Eur J Clin Microbiol Infect Dis. 2009 Apr;28(4):363-9.
Malhotra-Kumar S et al. J Clin Microbiol. 2010 Apr;48(4):1040-6.



Φαινοτυπικές μέθοδοι για ανίχνευση του MRSA

Phenotypic Method Used	Principle of the Method	On Culture/on Clinical Sample	TAT [1]	Brief Advantages/Disadvantages +/−	No. and Type of *S. aureus* Anlyzed [2]	Major Diagnostic Performance [3]	Reference
Chromogenic media	Selective media, colorimetric colony detection	Nasal swabs, positive blood culture	24 h	+ Easy to perform and interpret, low-cost − No MSSA identification	72 MRSA 28 MSSA	Sensitivity 100% Specificity 78.6%	[28]
					19 MRSA 29 MSSA	Sensitivity 78.9% Specificity 41.3%	[29]
Rapid BMD	Colorimetric assay, MTT dye as oxidation-reactions indicator	Culture	7 h	+ Easy to perform, rapid, low-cost − Larger validations needed	21 MRSA 19 MSSA	Sensitivity 100% Specificity 94.7%	[30]
Disk diffusion on Mueller-Hinton rapid agar MHR-SIR	Disk diffusion with shorter incubation	Positive blood culture	6–8 h	+ Easy to perform, rapid, low-cost − Larger validations needed	23 *S. aureus* tested for several antibiotics	CA 97.8% mEs 1.9% VME 0.3%	[31]
					29 MRSA 50 MSSA	TAT 17h shorter, better therapy de-escalation	[32]
Rapid AST	Disk diffusion with shorter incubation	Positive blood culture	4–6 h	+ Easy to perform, rapid, low-cost − Low growth, diameters margins less demarcated	24 MRSA 313 MSSA	Sensitivity 100% Specificity 100%	[33]
					14 MRSA 183 MSSA	Sensitivity 100% Specificity 99.4% ATU 0.5%	[34]
					9 MRSA 212 MSSA	Sensitivity 100% Specificity 99.1% ATU 0.4% ME 0.5%	[35]

Sanchini A. Diagnostics (Basel). 2022;12(1):208.



Φαινοτυπικές μέθοδοι για ανίχνευση του MRSA

Phenotypic Method Used	Principle of the Method	On Culture/on Clinical Sample	TAT [1]	Brief Advantages/Disadvantages +/−	No. and Type of *S. aureus* Anlyzed [2]	Major Diagnostic Performance [3]	Reference
VITEK 2	Turbidimetric monitoring of bacterial growth	Culture	4–11 h	+ Automated, rapid, standardized interpretation − Fixed range of testable antibiotics, less accurate for linezolid/vancomycin	72 MRSA 28 MSSA	Sensitivity 97.2% Specificity 100%	[28]
					27 MRSA resistant to linezolid	MEs 85.2%	[36]
					28 MRSA with a vancomycin MIC ≥ 2	MEs 46.4%	[37]
BD Phoenix	Turbidimetric and colorimetric growth detection	Culture	6–16 h	+ Automated, rapid, standardized interpretation − Less accurate for SXT	642 *S. aureus* analyzed for SXT resistance	CA 91.9% MEs 7.9% mEs 0.3%	[38]
Copan WASP Colibri	Automated disk diffusion	Culture	16 h	+ Automated, flexible, detect heteroresistance and mixed cultures − Should be compared with standard disk diffusion	107 *S. aureus* tested for several antibiotics	CA 99.9%	[39]
Accelerate PhenoTest™ BC	Single-cell analysis, fluorescent in situ hybridization	Positive blood culture	7 h	+ Automated, monomicrobial call, rapid, identification and AST in the same platform	98 MRSA 86 MSSA	Sensitivity 100% Specificity 98.8% CA 99.5%	[17]
					22 MRSA 2 MSSA	Sensitivity 100% Specificity 100%	[40]
PBP2a latex agglutination assay	Particles with monoclonal antibodies, agglutination reaction	Culture	5 m	+ Rapid, easy to perform and interpret − no PBP2c detection	95 MRSA 10 MSSA	Sensitivity 98.95% Specificity 77.8%	[41]

Sanchini A. Diagnostics (Basel). 2022;12(1):208.

Μοριακές μέθοδοι για ανίχνευση του MRSA απευθείας από το δείγμα: παραδείγματα

Molecular Method Used	Principle of the Method	On Culture/ on Clinical Sample	TAT [1]
Xpert ® SA Nasal Complete (Cepheid)	Real-time PCR for *mecA/C, spa* and SCC*mec-orfX*	Nasal samples	1 h
Xpert ® MRSA/SA BC Assay (Cepheid)	Real-time PCR for *mecA/C, spa* and SCC*mec-orfX*	Positive blood cultures	1.7 h
Xpert ® MRSA/SA SSTI (Cepheid)	Real-time PCR for *mecA/C, spa* and SCC*mec-orfX*	BAL samples	68 m

Se 86-98%
Sp 90-95%

Molecular Method Used	Principle of the Method	On Culture/ on Clinical Sample	TAT [1]	Brief Advantages/Disadvantages +/−
Hologic Panther Fusion ® MRSA	PCR and Invader chemistries for *mecA/C, gap* and SCC*mec-orfX*	Nasal samples	<3 h	+ Can analyze 350 samples in 8 h − Need comparison with a similar method
MRSA/SA ELITe MGB assay (ELITech-Group)	Real time PCR for *mecA/C* and a *S. aureus* specific sequence	Sputum, tracheal aspirate, BAL	<3 h	+ Accurate − Do not target SCC*mec-orfX* junction
Eazyplex ® MRSA	LAMP targeting *S. aureus, S. epidermidis, mecA/C*	Positive blood cultures	1 h	+ Portable; faster than similar methods − Need optimization for CONS

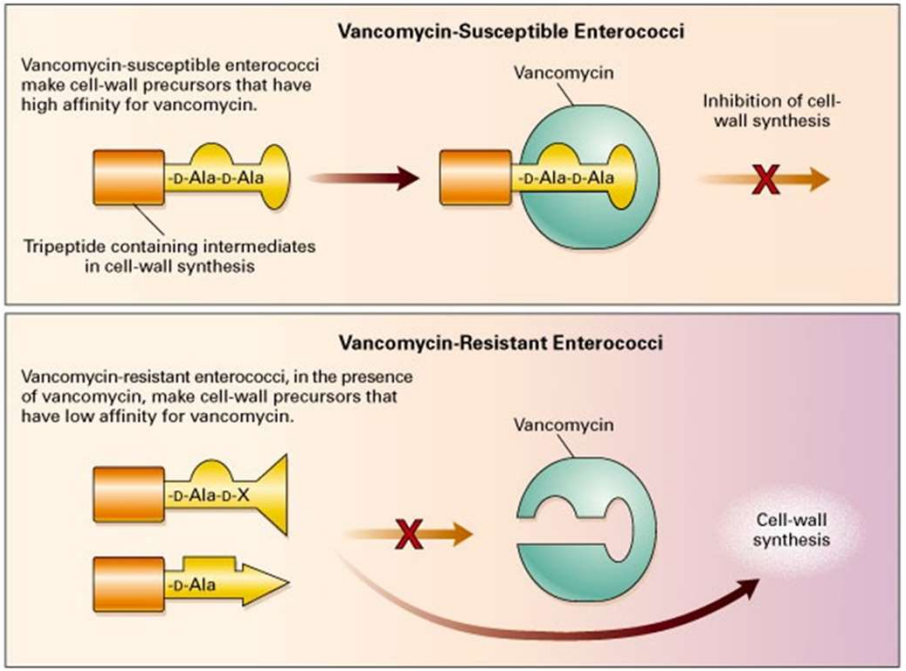
Sanchini A. Diagnostics (Basel). 2022;12(1):208.

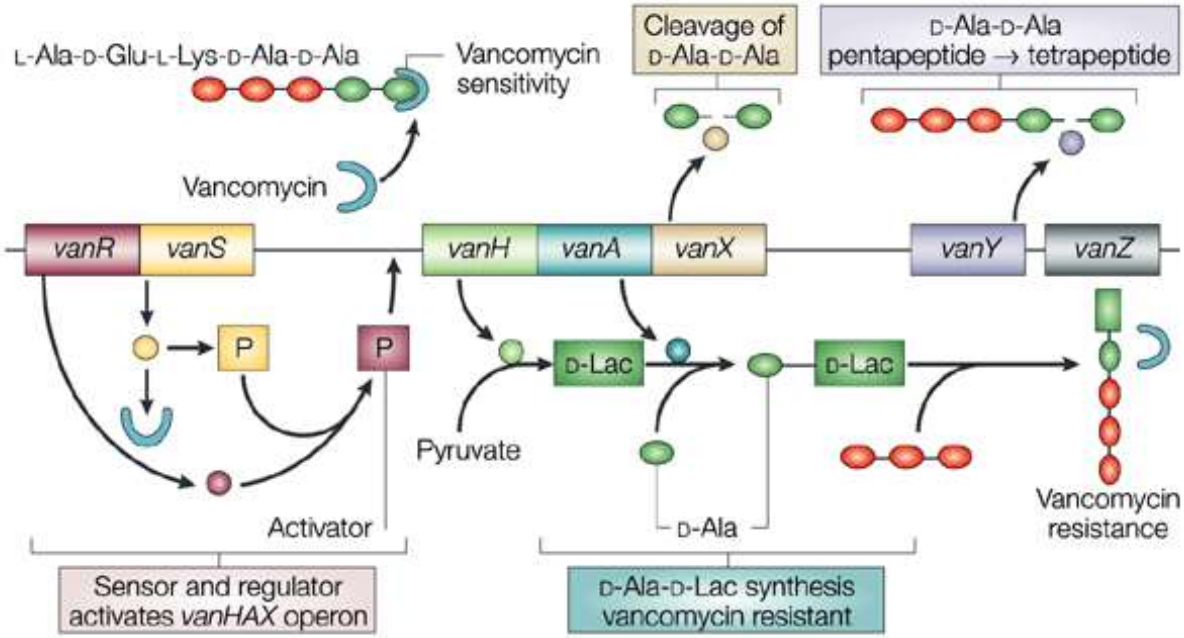
VRE, μηχανισμός αντοχής: τροποποίηση στόχου δράσης

Σύνθεση πεπτιδογλυκάνης που φέρει πλευρικές πενταπεπτιδικές αλυσίδες που καταλήγουν σε

D-ala-D-lactate (VanA, VanB, VanD)

D-ala-D-serine (VanC, VanE, VanG, VanL , VanN)





Nature Reviews | Genetics

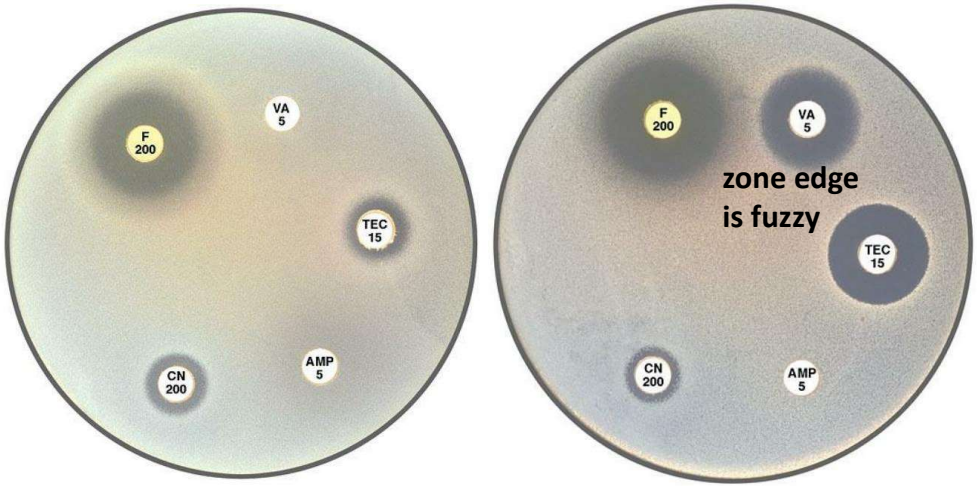
Glycopeptide resistance due to *van*-type gene clusters

	Aquired							Intrinsic
Resistance level	**High**	**Variable**	**Moderate**	**Low**				**Low**
Type	**VanA**	**VanB**	**VanD**	**VanE**	**VanG**	**VanL**	**VanN**	**VanC**
MIC in mg/L:								
Vancomycin	≥16	≥4	≥64	6–32	12–16	8	8	2–32
Teicoplanin	>8	0,5-1	4–64	0,5	0,5			0,5-1
Expression	Inducible	Inducible	Constitutive/ Inducible (*vanD2*)	Inducible/ (Constitutive)	Inducible			Constitutive/ Inducible
van ligase gene	*vanA*	*vanB1-B3*	*vanD1-5*	*vanE*	*vanG1-2*	*vanL*	*vanN*	*vanC1-C3*
Modified target	D-alanine-D-lactate	D-alanine-D-lactate	D-alanine-D-lactate	D-alanine-D-serine	D-alanine-D-serine	D-alanine-D-serine	D-alanine-D-serine?	D-alanine-D-serine
Conjugative transfer	Yes	Yes	No	No	Yes	No	Yes	No
Location	Plasmid/chromosome on transposon(s)	Plasmid/chromosome ± transposon/ICE[a]	Chromosome	Chromosome	Chromosome on possible ICE	Chromosome?	Plasmid	Chromosome
Distribution	*Enterococcus faecium* *Enterococcus faecalis* *Enterococcus avium* *Enterococcus casseliflavus* *Enterococcus durans* *Enterococcus gallinarum* *Enterococcus hirae* *Enterococcus mundtii* *Enterococcus raffinosus* *Staphylococcus aureus* *Bacillus circulans* *Oerskovia turbata* *Arcanobacterium haemolyticum* *Paenibacillus* *Rhodococcus*	*Enterococcus faecium* *Enterococcus faecalis* *Enterococcus casseliflavus* *Enterococcus durans* *Enterococcus gallinarum* *Enterococcus hirae* *Staphylococcus epidermidis* *Streptococcus* *Clostridium* *Ruminococcus* *Eggerthella*	*Enterococcus faecium* *Enterococcus faecalis* *Enterococcus avium* *Enterococcus gallinarum* *Enterococcus raffinosus* Non-enterococcal faecal flora	*Enterococcus faecalis*	*Enterococcus faecalis* Non-enterococcal faecal flora	*Enterococcus faecalis*	*Enterococcus faecium*	*Enterococcus gallinarum* – *vanC1* *Enterococcus casseliflavus* – *vanC2/3*

Hegstad K et al. Clin Microbiol Infect. 2010;16(6):541-54.

Ανίχνευση VRE: παραδείγματα

Μέθοδος διάχυσης των δίσκων

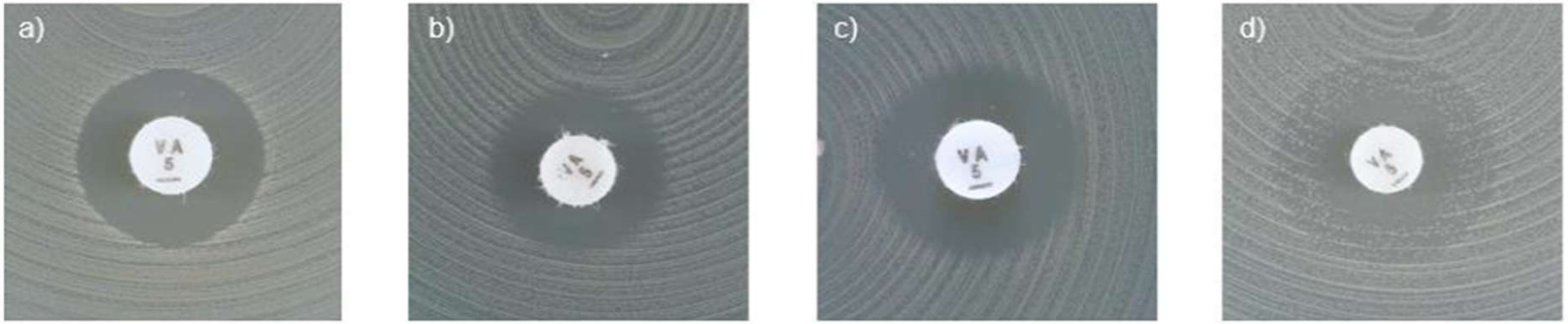


Isolates must not be reported susceptible before 24 h incubation

Ταινίες διαβαθμισμένης συγκέντρωσης αντιβιοτικών

sharp zone edges

Confirmatory testing with PCR or report resistant even if the zone diameter is ≥ 12 mm



The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. http://www.eucast.org.

Ανίχνευση VRE: παραδείγματα

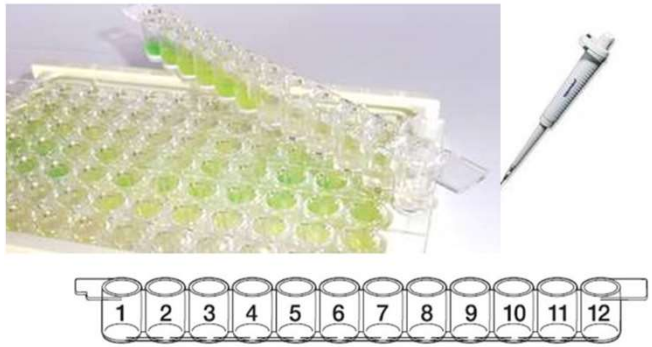
Αυτοματοποιημένα συστήματα

Bionumber: 532047265773771
Organism Quantity:

Selected Organism: Enterococcus faecium

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen			Moxifloxacin	(-)	(-)
Benzylpenicillin	(-)	(-)	Inducible Clindamycin Resistance		
Ampicillin	>= 32	R	Erythromycin	(-)	(-)
Oxacillin			Clindamycin	(-)	(-)
Imipenem	>= 16	R	Quinupristin/Dalfopristin	4	R
Gentamicin High Level (synergy)	SYN-S	S	Linezolid	1	S
Streptomycin High Level (synergy)	SYN-R	R	Daptomycin		
Gentamicin			Teicoplanin	>= 32	R
Ciprofloxacin			Vancomycin	>= 32	R
Urine	>= 8	R	Tigecycline	<= 0.12	S
Other	>= 8		Trimethoprim/ Sulfamethoxazole		

Broth microdilution



Μοριακές μέθοδοι απευθείας από το δείγμα

*Van*A or *Van*B resistance in 20 min, from rectal swabs, blood culture
or as culture confirmation

VRE Cepheid Xpert® vanA/vanB

VanA SN= 87.1%, SP=99.7%, PPV=98.0%, NPV=97.7%

VanB SN=77.6%

The saving of time to results corresponded to 141 saved isolation days and 292 saved transmission risk days.

Holzknecht BJ et al. New Microbes New Infect. 2017;16:54-59.

J Antimicrob Chemother 2021; **76**: 146–151
doi:10.1093/jac/dkaa413 Advance Access publication 9 October 2020

Journal of Antimicrobial Chemotherapy

Development and validation of a lateral flow immunoassay for rapid detection of VanA-producing enterococci

Saoussen Oueslati[1,2]†, Hervé Volland[3]†, Vincent Cattoir[4], Sandrine Bernabeu[1,2], Delphine Girlich[1], Ducan Dulac[3], Marc Plaisance[3], Maxime Laroche[5], Laurent Dortet[1,2,6], Stéphanie Simon[3] and Thierry Naas[1,2,6]*

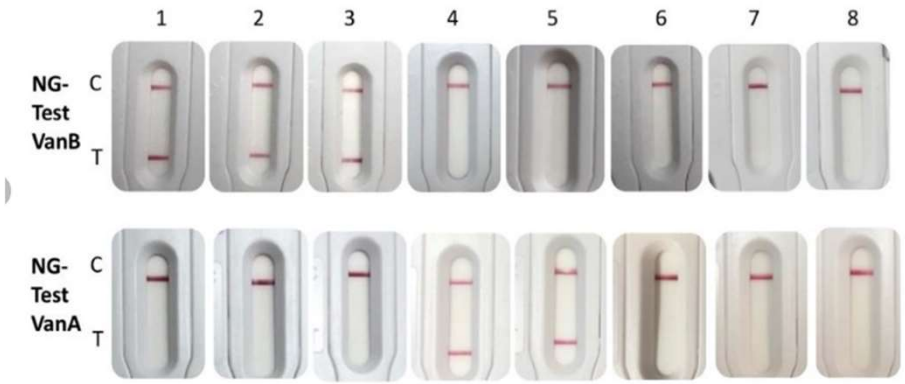
diagnostics

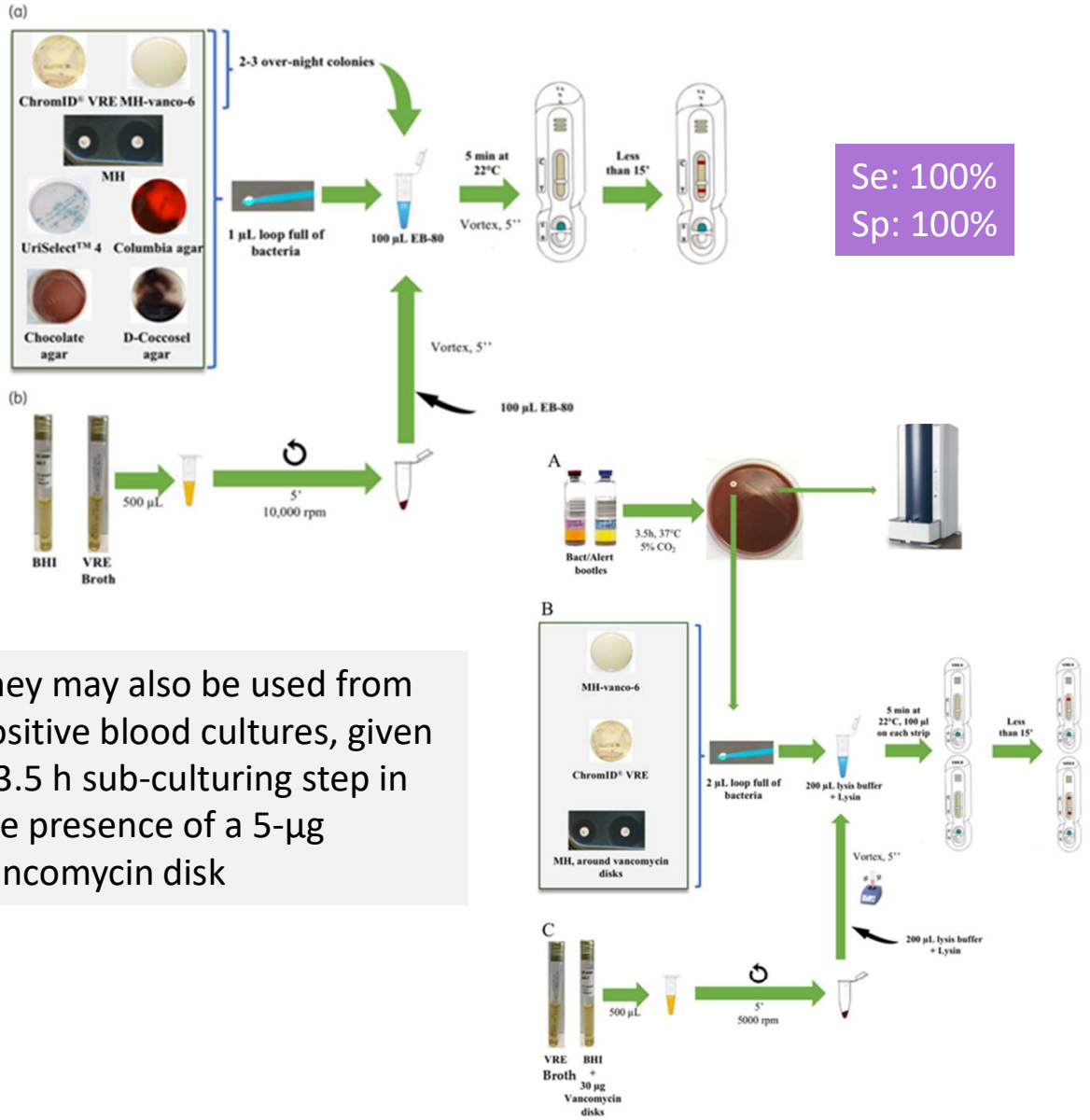
MDPI

Article

Rapid Detection of VanA/B-Producing Vancomycin-Resistant Enterococci Using Lateral Flow Immunoassay

Saoussen Oueslati [1,2,†], Camille Gonzalez [1,2,†], Hervé Volland [3,*,†], Vincent Cattoir [4], Sandrine Bernabeu [1,2], Delphine Girlich [1,2], Duncan Dulac [3], Marc Plaisance [3], Laure Boutigny [5], Laurent Dortet [1,2], Stéphanie Simon [3] and Thierry Naas [1,*]





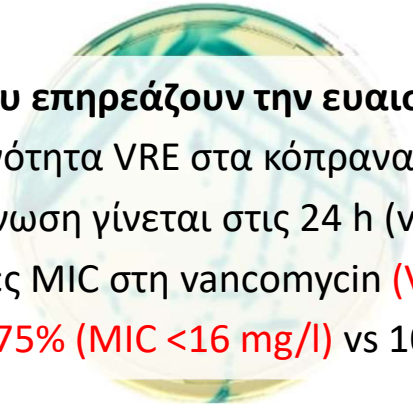
Se: 100%
Sp: 100%

They may also be used from positive blood cultures, given a 3.5 h sub-culturing step in the presence of a 5-µg vancomycin disk

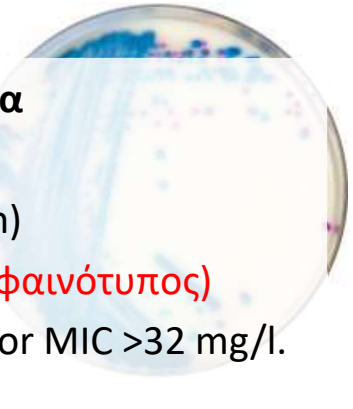
Χρωμογόνα εκλεκτικά υλικά για VRE

Brilliance VRE®, Oxoid

Chromatic VRE, Liofilchem



ChromAgar® VRE



Παράγοντες που επηρεάζουν την ευαισθησία

- χαμηλή πυκνότητα VRE στα κόπρανα
- όταν η ανάγνωση γίνεται στις 24 h (vs 48 h)
- χαμηλές τιμές MIC στη vancomycin (VanB φαινότυπος)
  - SN 25-75% (MIC <16 mg/l) vs 100% for MIC >32 mg/l.

chromID® VRE, bioMerieux

Bile-esculin-azide agar + 6 mg/L vancomycin

TABLE 2 Comparison 396 specimens (combined data)

Medium	Performance (% [95% CI])[b]			
	Sensitivity	Specificity	PPV	NPV
BEAV broth	98.0 (94.7–98.0)	100 (98.9–100)	100 (96.7–100)	99.3 (98.3–99.3)
Enterococcosel (BEAV)	84.8 (80.9–84.8)	100 (98.7–100)	100 (95.3–100)	95.2 (93.9–95.2)
InTray Colorex VRE	91.9 (86.9–94.8)	98.3 (96.6–99.3)	94.8 (89.6–97.9)	97.3 (95.7–98.3)
chromID	94.9 (91.0–95.9)	99.7 (98.3–100)	98.9 (94.8–99.9)	98.3 (97.0–98.7)
VRE*Select*	91.9 (87.8–92.9)	99.7 (98.3–100)	98.9 (94.4–99.9)	97.4 (96.0–97.7)
HardyCHROM VRE	89.9 (85.6–90.9)	99.7 (98.2–100)	98.9 (94.2–99.9)	96.7 (95.4–97.0)
Spectra VRE	93.9 (89.9–94.9)	99.7 (98.3–100)	98.9 (94.7–99.9)	98.0 (96.7–98.3)

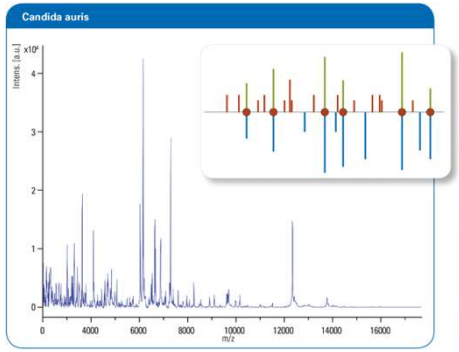
Τα χρωμογόνα υπερέχουν έναντι του BEAV: SN 89.9-93.9% vs 84.8%
Αποτελέσματα διαθέσιμα 24-48 h νωρίτερα

Επιλογή σύμφωνα με:
ευκολία διάκρισης αποικιών, αξιοπιστία, κόστος, ανάγκη επιβεβαιωτικών δοκιμασιών

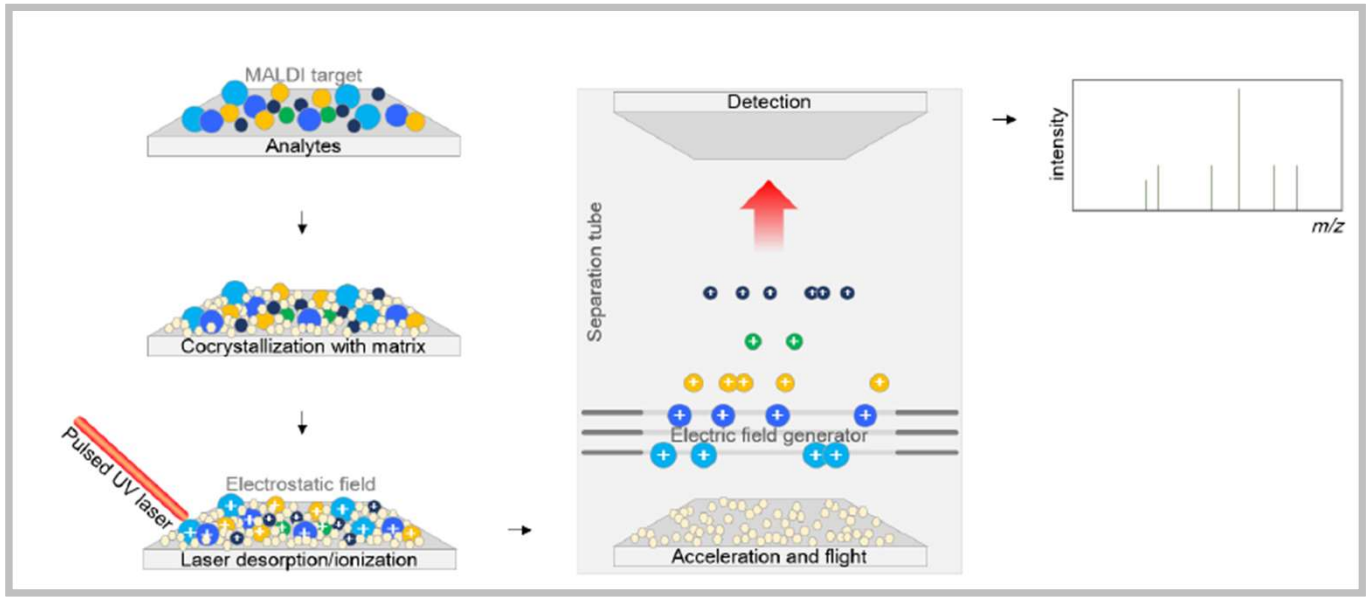
Suwantarat N et al. J Clin Microbiol. 2014;52(11):4039-42.
Wijesuriya TM et al. J Clin Microbiol. 2014 Aug;52(8):2829-33.

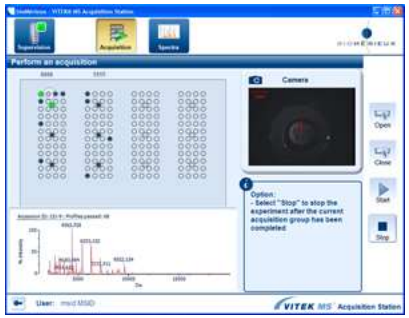
MALDI-TOF Mass Spectrometry και ανίχνευση αντοχής

Matrix-Assisted Laser Desorption Ionization (MALDI) Time-Of-Flight (TOF) Mass Spectrometry

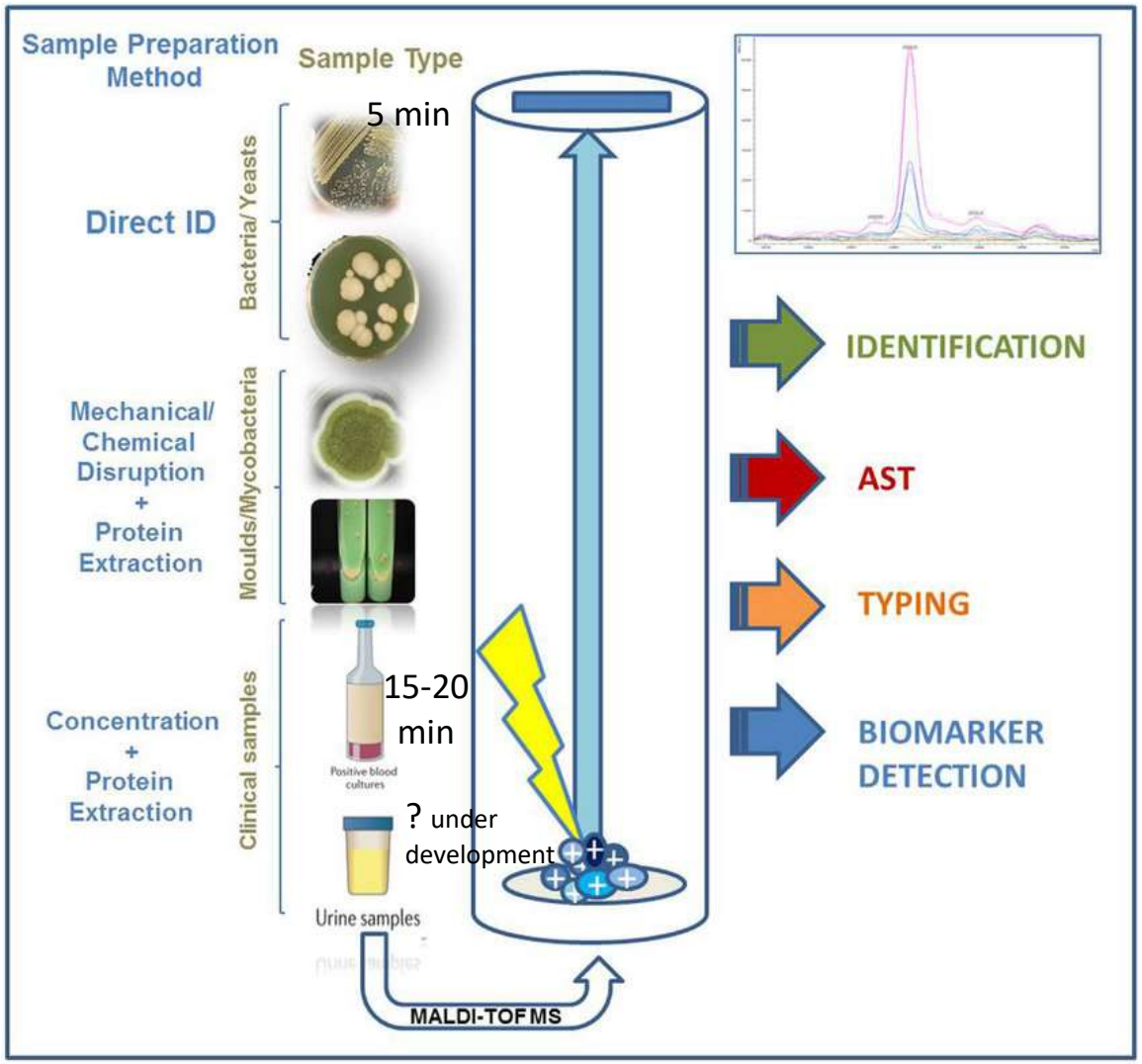


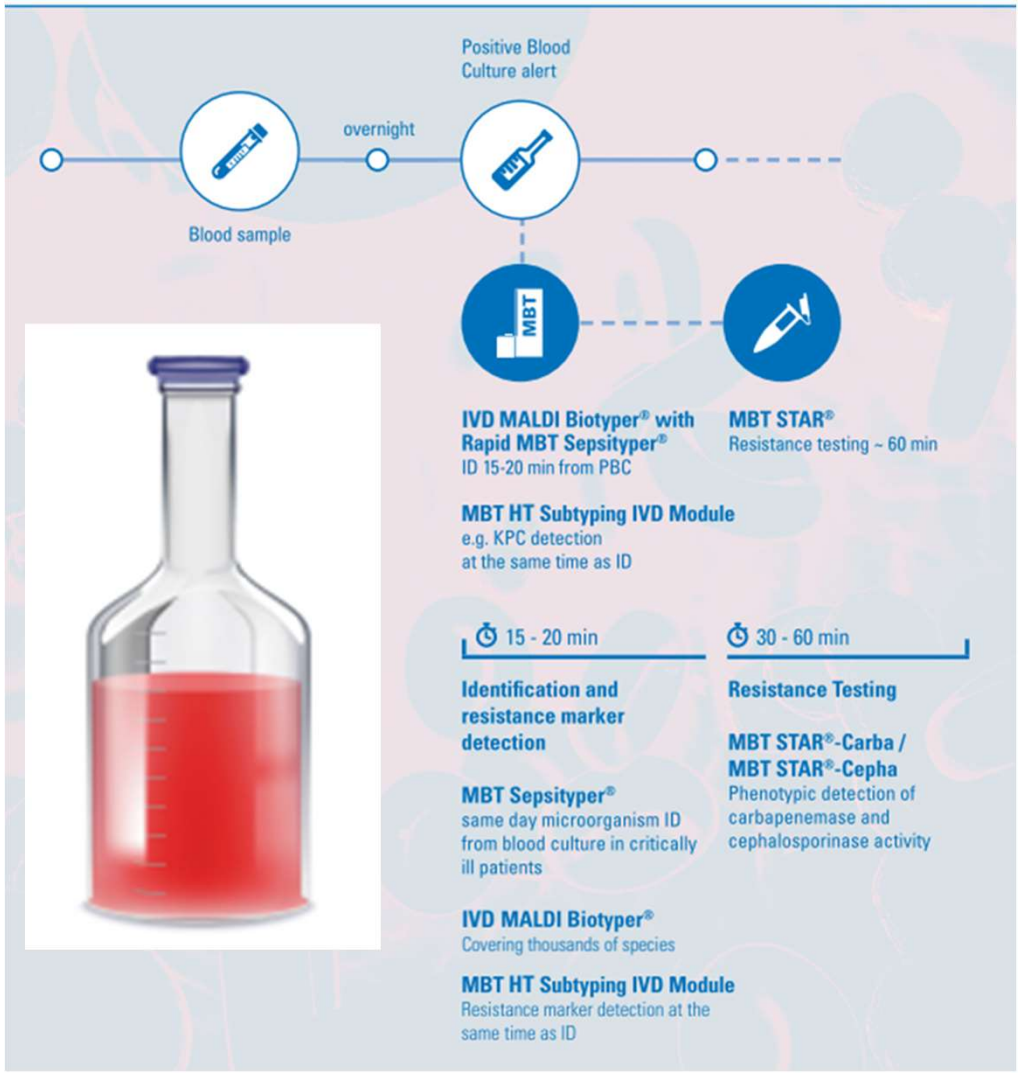
System analysis time to ID result:
95 isolates + 1 QC sample ~5 min





Yoon EJ, Jeong SH. MALDI-TOF Mass Spectrometry Technology as a Tool for the Rapid Diagnosis of Antimicrobial Resistance in Bacteria. Antibiotics (Basel). 2021;10(8):982.


Sample Preparation Method
Sample Type
5 min
Direct ID
Bacteria/ Yeasts
Mechanical/ Chemical Disruption + Protein Extraction
Moulds/Mycobacteria
Concentration + Protein Extraction
Clinical samples
15-20 min
Positive blood cultures
? under development
Urine samples
MALDI-TOF MS
IDENTIFICATION
AST
TYPING
BIOMARKER DETECTION


Positive Blood Culture alert
overnight
Blood sample
MBT
IVD MALDI Biotyper® with Rapid MBT Sepsityper®
ID 15-20 min from PBC
MBT STAR®
Resistance testing ~ 60 min
MBT HT Subtyping IVD Module
e.g. KPC detection
at the same time as ID
15 - 20 min
30 - 60 min
Identification and resistance marker detection
Resistance Testing
MBT STAR®-Carba / MBT STAR®-Cepha
Phenotypic detection of carbapenemase and cephalosporinase activity
MBT Sepsityper®
same day microorganism ID from blood culture in critically ill patients
IVD MALDI Biotyper®
Covering thousands of species
MBT HT Subtyping IVD Module
Resistance marker detection at the same time as ID

MALDI-TOF MS-based rapid diagnostic methods for AMR

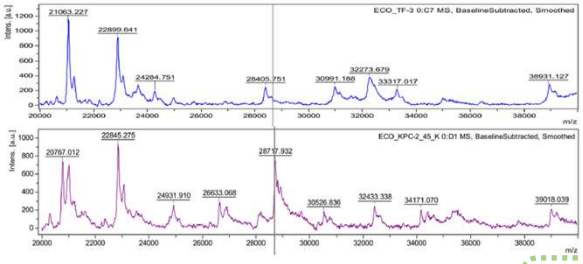
Strategy	Target	Target Resistance	Commercialized (Company) and/or Developing Products	Ref.
Antimicrobial susceptibility testing	AUC [1] shift spectra (isotope)	All antimicrobials	MBT-ASTRA (Bruker) MBT-Resist (Bruker) DoT-MGA [2] (inv. [3])	[22,24,25,70,71]
ID of the AMR clonal group	Clone-specific spectra	Methicillin (MRSA)	ClinProtTools (Bruker)	[72]
ID of the modified antimicrobial drug	Shift peak of the modified drug	Beta-lactams Cephalosporins Carbapenems	MBT STAR®-BL (Bruker) MBT STAR®-Cepha (Bruker) MBT STAR®-Carba (Bruker)	[40,73,74]
ID of the modified antimicrobial target	Shift peak of the modified drug target	Colistin	MALDIxin test (inv.)	[49,75]
Direct detection of the AMR determinant	Protein-specific peak	Carbapenems	Aprodete™ KPC-CPE (Aprilis)	[55]
Biomarker detection	Protein-specific peak	Carbapenems	ClinProtTools (Bruker)	[64,76]

[1] AUC, area under curve. [2] DoT-MGA, Direct-on-Target Microdroplet Growth Assay. [3] inv., under investigation.

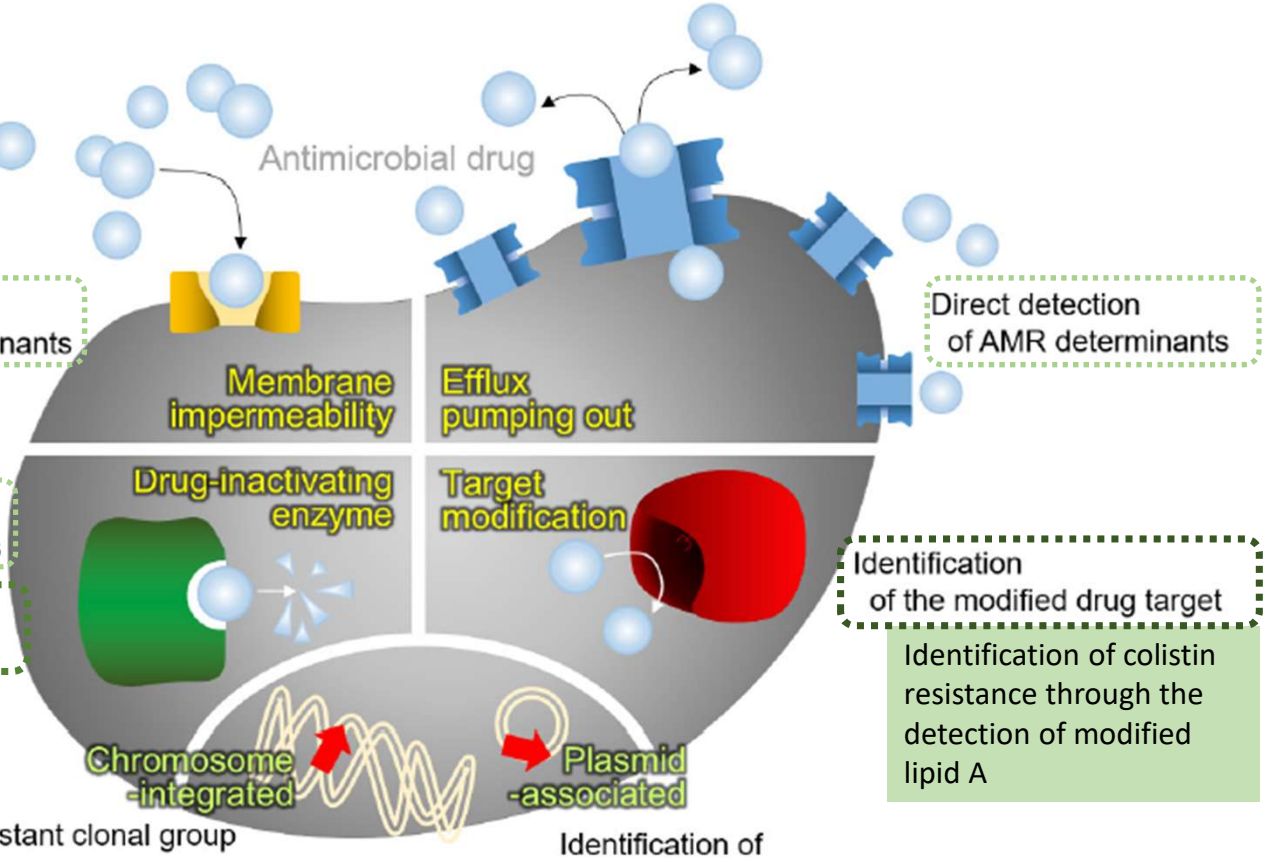
Yoon EJ, Jeong SH. Antibiotics (Basel). 2021;10(8):982.

MALDI-TOF Mass Spectrometry και ανίχνευση αντοχής

Identification of the KPC producer



beta-lactamases
Porin defects



The modified antibiotics change their molecular weights.
AMR is identified by detecting the peak shift using the MALDI-TOF MS methodology

Identification of colistin resistance through the detection of modified lipid A

MALDI-TOF MS protein fingerprints of well-known AMR clonal groups

Yoon EJ, Jeong SH. Antibiotics (Basel). 2021;10(8):982.

MALDI-TOF Mass Spectrometry και ανίχνευση αντοχής

MBT STAR®-Carba IVD Assay

Sensitivity 93.9%
specificity, 100%

Bacteria with active carbapenemase will inactivate the carbapenem antibiotic by hydrolysation of the ß-lactam ring, **which is associated with a detectable mass shift.**

In assays with bacteria without active carbapenemase, only peaks corresponding to the intact antibiotic will be present in the mass spectrum.

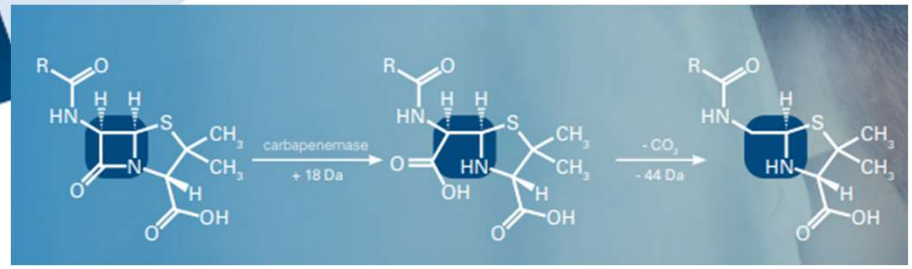
In assays with bacteria with active carbapenemase, peaks of the intact antibiotic will decrease.

Sample	Species	Control ID	Carba	
Sample 1	Klebsiella pneumoniae	confirmed	1.09	H
Sample 2	Klebsiella pneumoniae	confirmed	0.98	H
Sample 3	Escherichia coli	confirmed	0.02	NH
neg.control		not performed	-0.03	NH
pos.control		not performed	1.09	H
Δ controls			1.47	

H	Hydrolyzed[1]
NH	Non-hydrolyzed[2]

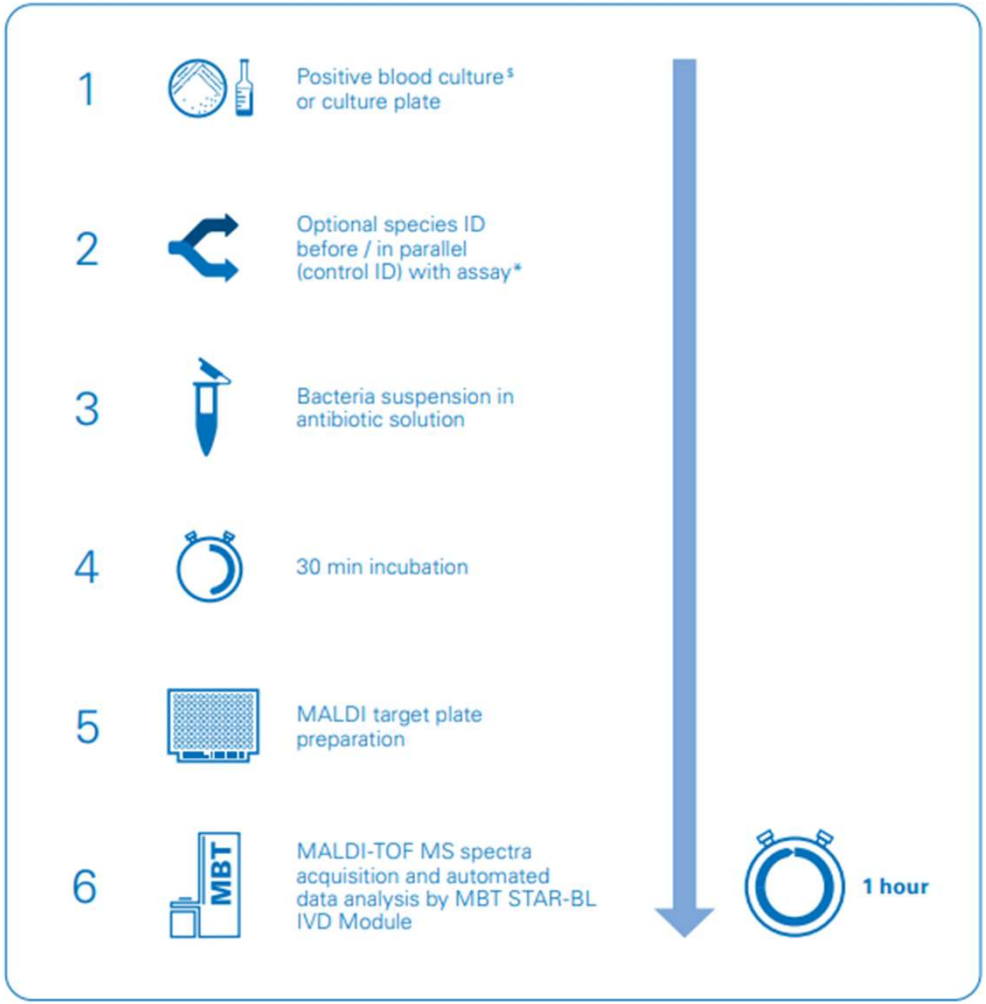
Se: 100%, Sp: 100%

Del Corpo O et al. Clin Microbiol Infect. 2023 Sep 16:S1198-743X(23)00425-1.



MALDI-TOF Mass Spectrometry και ανίχνευση αντοχής

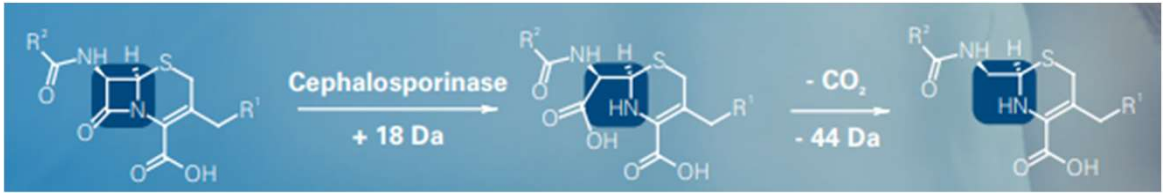
MBT STAR®-Cepha IVD Assay



Se: 94%
Sp: 97%

Del Corpo O et al. Clin Microbiol Infect. 2023 Sep 16:S1198-743X(23)00425-1.

Hydrolysis of β-lactam ring leads to mass shifts that can easily be detected by MALDI-TOF mass spectrometry



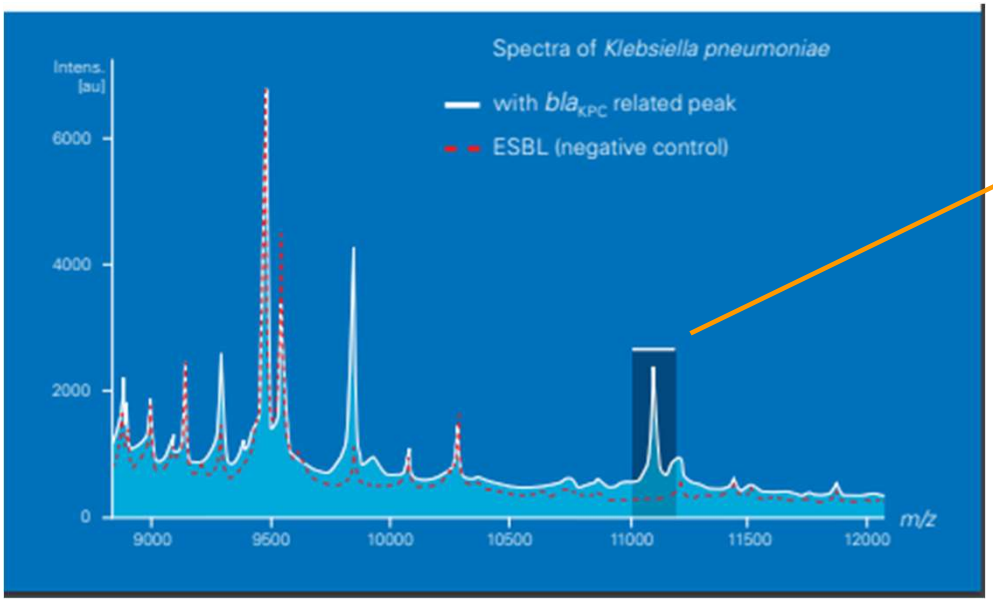
Sample	Species	Control ID	Cepha	
Sample 1	Escherichia coli	confirmed	1.28	H
Sample 2	Escherichia coli	confirmed	1.14	H
Sample 3	Klebsiella variicola	confirmed	-0.55	NH
neg.control		not performed	-0.10	NH
pos.control		not performed	1.04	H
Δcontrols			1.12	

H	Hydrolyzed[1]
NH	Non-hydrolyzed[2]

MALDI-TOF Mass Spectrometry και ανίχνευση αντοχής

MBT Subtyping IVD Module

Quick detection of *bla*$_{KPC}$ expressing *K. pneumoniae* and *E. coli*



*A peak in MALDI-TOF mass spectra of KPC-producing *K. pneumoniae*, related to the plasmid carrying *bla*$_{KPC}$ (pKpQIL). This peak at 11,109 m/z is clearly detectable in MALDI-TOF mass spectra.

Prerequisite for the automated detection process is the successful ID.

The MBT HT Subtyping IVD Module then looks for the *bla*$_{KPC}$ related peak in the sample spectrum and, if present, the software will report this sample as a KPC positive one.

KPC detection of *K. pneumoniae* and *E. coli* **includes only strains** with a *bla*$_{KPC}$ pKpQIL plasmid.

If the gene expression rate is low, there will be no characteristic peak and no KPC subtyping alert.

Cultivation media and conditions might suppress or induce a signal at m/z 11,109 which is not related to a KPC resistance. Therefore, Columbia Agar with 5% sheep blood agar must be used for cultivation of *K. pneumoniae* and *E. coli*.

Lau AF et al. J Clin Microbiol. 2014;52(8):2804-12.
Youn JH et al. J Clin Microbiol. 2016;54(1):35-42.

MALDI-TOF Mass Spectrometry και ανίχνευση αντοχής

MBT Subtyping IVD Module

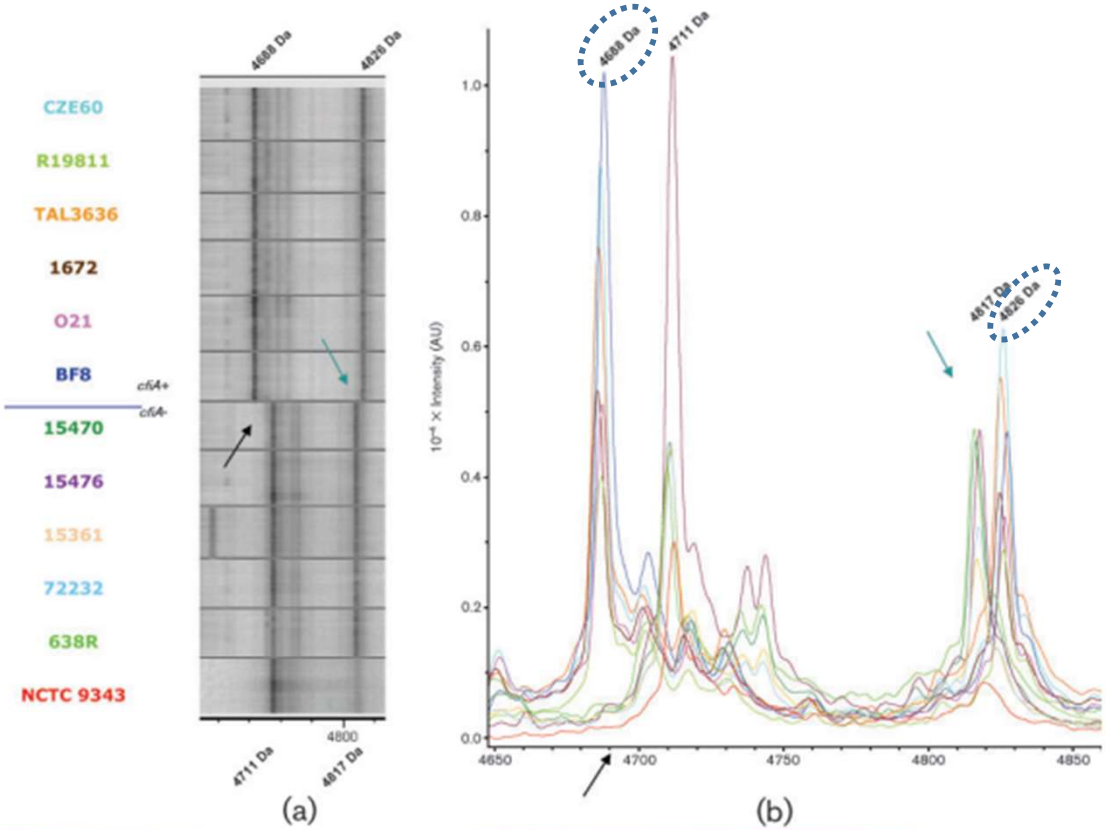
Bacteroides fragilis cfiA subtyping

A cluster of *B. fragilis*, (division II), carries the *cfiA* gene encoding a MBL.

The intrinsic gene is overexpressed when an insertion sequence is integrated upstream of the gene, providing a strong promoter. Then, *cfiA* MBL confers resistance against nearly all beta-lactam drugs to the bacterial host.

Through an extensive screening of *B. fragilis*, the *cfiA*-positive division II strains were distinguished from the *cfiA*-negative strains by spectral shifts between 4000–5500 m/z, and the results were satisfactory.

After successful identification, the MBT HT Subtyping IVD Module looks for specific peaks associated with the protein expressed by *B. fragilis cfiA* positive strains.



Nagy E et al. J Med Microbiol. 2011;60(Pt 11):1584-1590.

Applications of MALDI-TOF MS for specific AMR detection

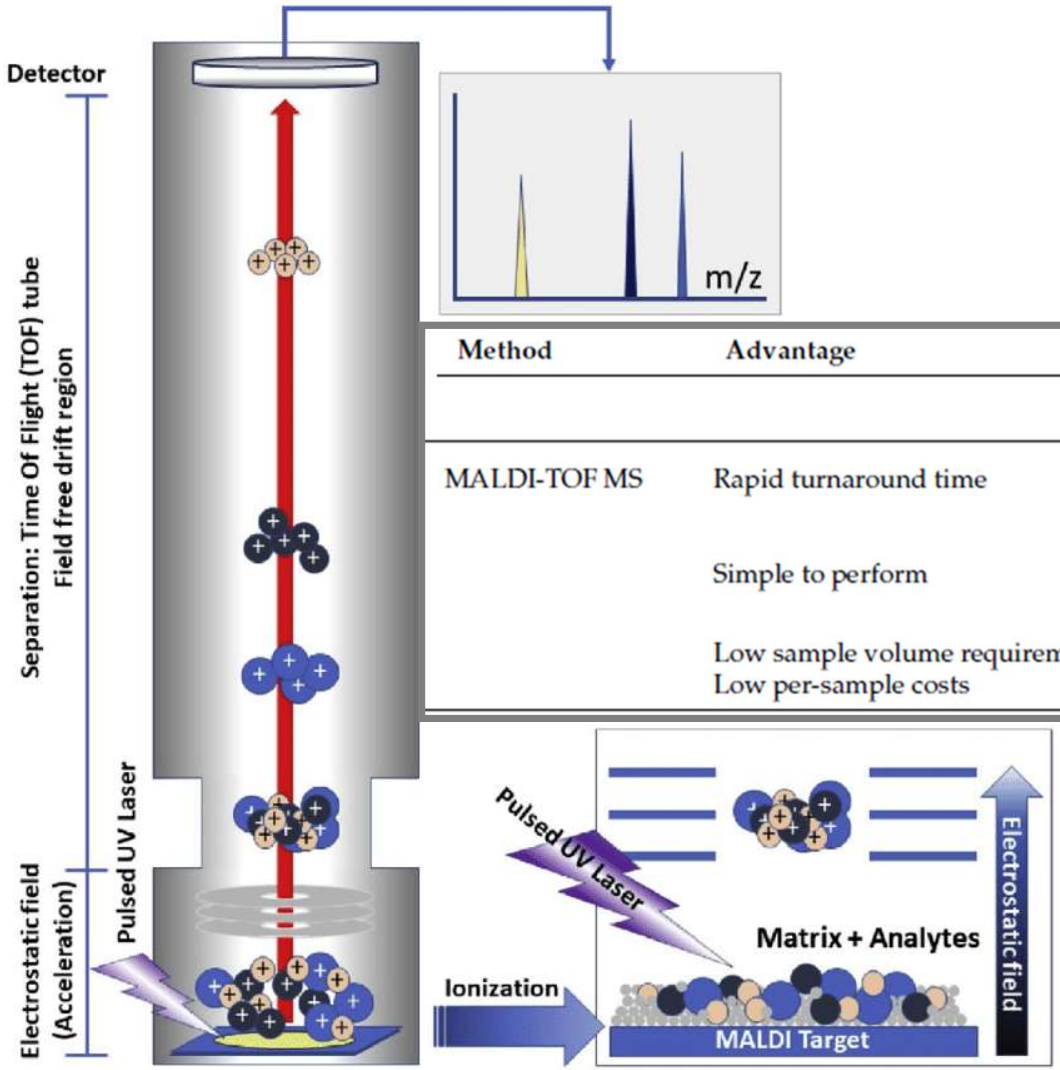
Organism	Antibiotic	Year (Reference)
E. coli	Polymyxins	2018 [122]
E. coli	Colistin	2019 [123]
E. coli *Klebsiella pneumoniae*	Beta-lactams (ESBL-producing isolates)	2019 [124]
Staphylococcus aureus *S. intermedius* *S. pseudintermedius*	Novobiocin Polymyxin B Acriflavine	2019 [125]
S. aureus	Methicillin	2019 [126]
Candida auris	Echinocandins	2019 [127]
K. pneumoniae *Bacteroides fragilis* *S. aureus*	Carbapenems (carbapenemase-producing isolates) Methicillin	2019 [128]
Enterobacteriaceae	Carbapenems (carbapenemase-producing isolates)	2019 [129]
Enterobacteriaceae	Carbapenem	2019 [130]
Pseudomonas aeruginosa	Beta-lactams (MBL)	2019 [131]
Enterococcus faecium	Vancomycin	2019 [132]
K. pneumoniae	Carbapenems (carbapenemase-producing isolates)	2019 [133]
Acinetobacter baumannii	Colistin	2020 [134]
E. coli *K. pneumoniae*	Cefotaxime Meropenem, Ciprofloxacin	2020 [135]
S. aureus *Enterococcus* species *E. coli* *K. pneumoniae*	Oxacillin (methicillin) Vancomycin Ceftriaxone Meropenem	2020 [136]
Enterobacterales	Imipenem/Relebactam	2020 [137]
S. aureus	Methicillin	2020 [138]

Kaprou GD et al. Antibiotics (Basel). 2021;10(2):209.

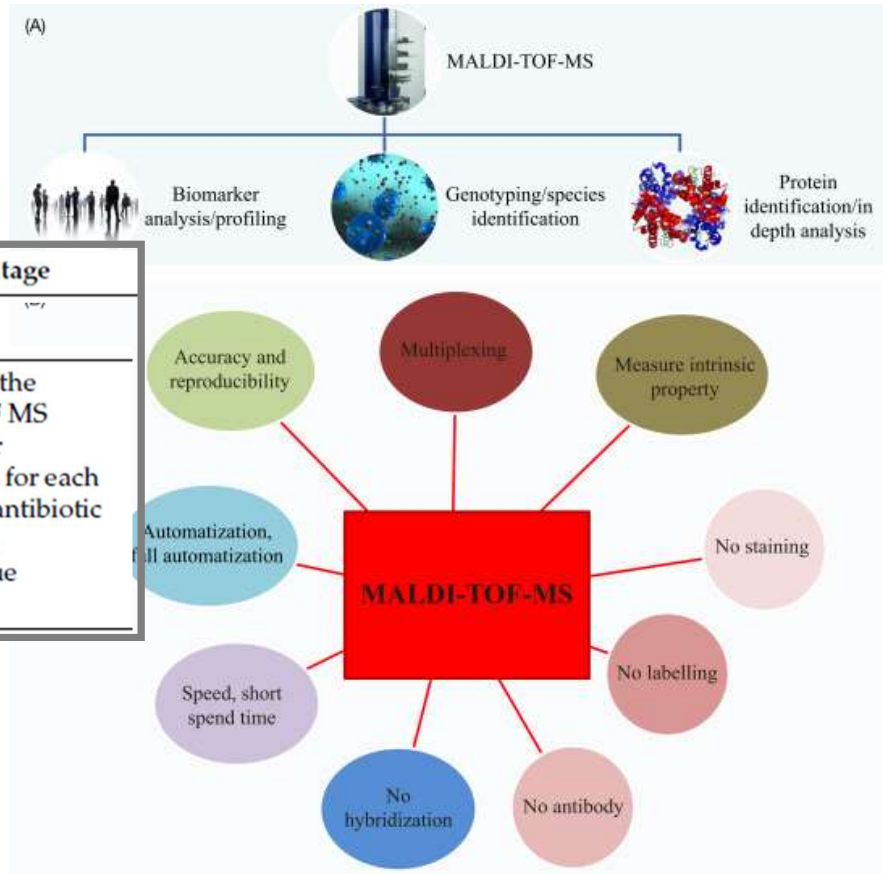
MALDI-TOF MS approaches for AMR detection in *S. aureus*

MALDI-TOF System Used	Principle of the Method	On Culture/ on Clinical Sample	TAT [1]	Brief Advantages/ Disadvantages +/−	No. and Type of *S. aureus* Analyzed [2]	Major Diagnostic Performance [3]	Reference
MALDI Biotyper ® CA System (Bruker Daltonics)	21 peaks to discriminate MRSA/MSSA	Culture	Few m	+ Use a combination of peaks − Low specificity	181 *S. aureus*	Sensitivity 87.6% Specificity 71.4%	[64]
MALDI Biotyper ® CA System (Bruker Daltonics)	Peptide PSM-*mec* and δ-toxin peaks for MRSA identification	Culture	Few m	+ Sensitive for SCC*mec* type II, III and VIII − Not adapted for other SCC*mec* types	35 MRSA	Sensitivity 40%	[65]
MALDI Biotyper ® CA System (Bruker Daltonics)	Peptide PSM-*mec* peak for MRSA identification	Culture	Few m	+ Good specificity − SCC*mec* types of isolates unknown	241 MRSA 106 MSSA	Sensitivity 60.2% Specificity 100%	[66]
MALDI Biotyper ® CA System (Bruker Daltonics)	m/z 4594 peak for MRSA identification	Culture	Few m	+ Potential MRSA biomarker peak − To be tested in an independent set of data	36 MRSA 31 MSSA	Sensitivity 83.3% Specificity 96.8%	[67]
VITEK MS (BioMérieux)	support vector machine algorithm to identify 38 peaks to discriminate MRSA/MSSA	Culture	Few m	+ Use a combination of peaks − MRSA/MSSA classification model needs optimization	194 MRSA 258 MSSA	Sensitivity 84.0% Specificity 88.0%	[68]
MALDI Biotyper ® CA System (Bruker Daltonics)	DOT-MGA	Positive blood cultures	6 h	+ Rapid; tested with cefoxitin − Could be optimized for additional antibiotics	30 MRSA 14 MSSA	Sensitivity 100% Specificity 100%	[69]
VITEK MS (BioMérieux)	DOT-MGA	Culture	5 h	+ Tested with oxacillin − Could be optimized for additional antibiotics	20 MRSA 20 MSSA	Sensitivity 100% Specificity 100%	[70]

Sanchini A. Diagnostics (Basel). 2022;12(1):208.



Method	Advantage	Disadvantage
MALDI-TOF MS	Rapid turnaround time Simple to perform Low sample volume requirements Low per-sample costs	High cost of the MALDI-TOF MS Need further optimisation for each species and antibiotic combination No MIC value

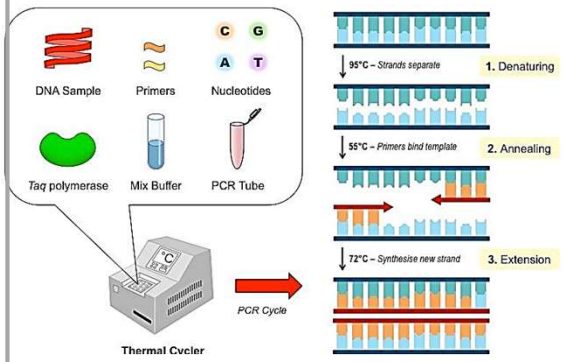


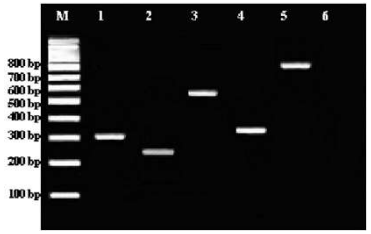
Gajic I et al. Antibiotics (Basel). 2022;11(4):427.

Μοριακές μέθοδοι: από την PCR στο mNGS

Gene(s) amplification + amplicons detection

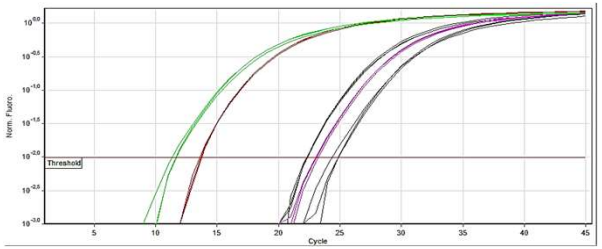
Single / Multiplex Endpoint PCR





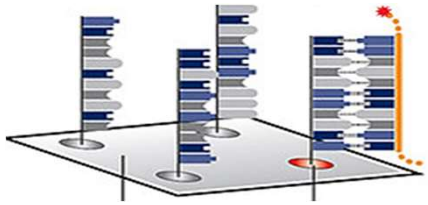
Διαδικασία εργώδης
Κίνδυνος επιμολύνσεων

Single / Multiplex Real time PCR

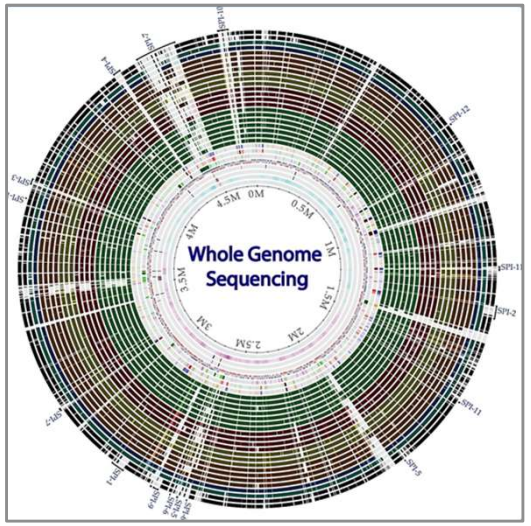


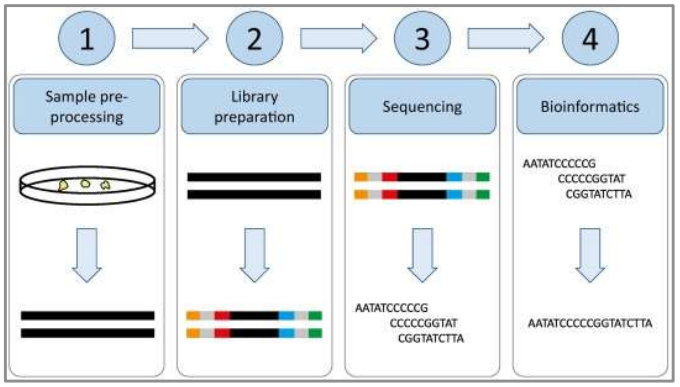
Multiplexing and miniaturization
of the hybridization step

PCR / hybridization σε microarray



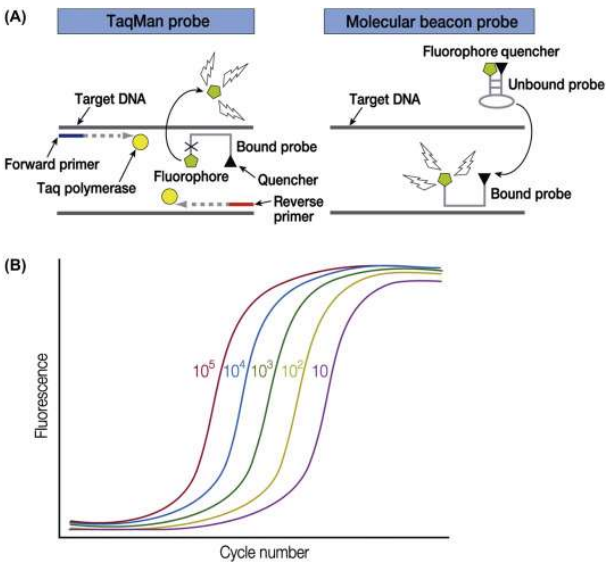
Εμπορικά διαθέσιμες μέθοδοι με:
αυτοματοποίηση
πολυπλεκτικότητα
στανταρισμένα πρωτόκολλα
quality control



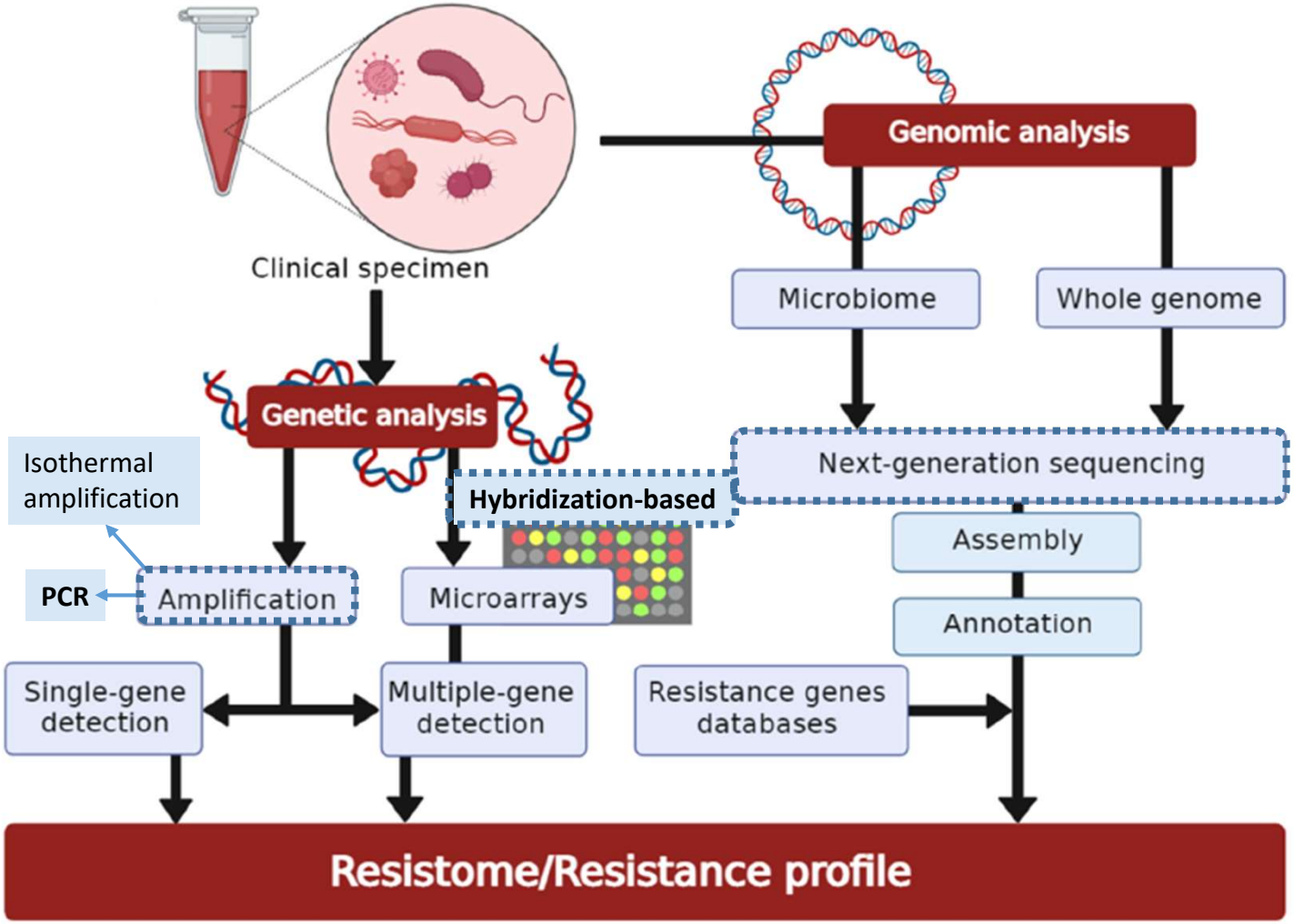


The basic workflow of molecular-based techniques for AMR detection

Real- time PCR (qPCR)



- measurement of data in real-time
- greater sensitivity
- reduced risk of contamination
- greater amenity to multiplexing
- many systems are partially or even completely automated



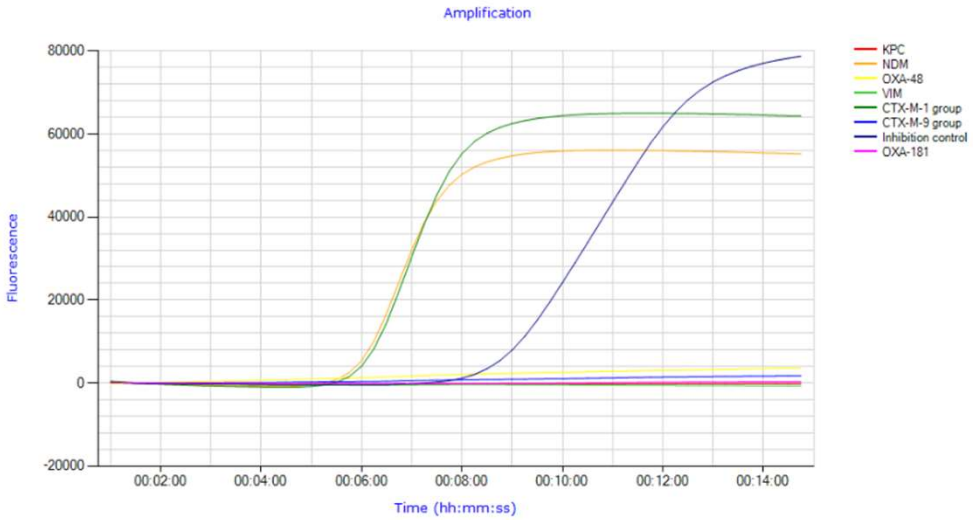
Gajic I et al. Antibiotics (Basel). 2022;11(4):427.

Ισοθερμικές μοριακές μέθοδοι και ανίχνευση αντοχής: παράδειγμα

Loop-mediated isothermal amplification technology and a real-time PCR platform

No DNA/RNA extraction necessary

eazyplex®, Amplex



TAT= 20-30 min

Sample: swab, culture

	SuperBug complete A	SuperBug complete B	SuperBug complete C
NDM	X	X	X
VIM	X	X	X
KPC	X	X	X
OXA-48	X	X	X
OXA-23	X	X	
OXA-40	X	X	
OXA-58	X		
OXA-181		X	X
IMP			X

eazyplex® SuperBug CRE

KPC, NDM, OXA-48, OXA-181 and VIM

ESBL genes: CTX-M-1 and CTX-M-9 group

TAT= 15-20 min

Direct screening from rectal swab and urine

Confirmation from positive BC and agar plate

eazyplex SuperBug Acineto

OXA-51, OXA-23-,-40- and -58-like and NDM

in 15 minutes

Μοριακές μέθοδοι και ανίχνευση αντοχής: παραδείγματα

Xpert® Carba-R, Cepheid

TAT: 48-minutes

Targeted genes	Δείγμα
KPC, NDM, VIM, IMP, OXA-48 (covering OXA-181 & OXA-232)	Ορθικός στυλεός Αποικίες

[1]Overall percentage agreement with WGS: 92.8%

[2]Metanalysis of 17 studies involving 15,972 samples

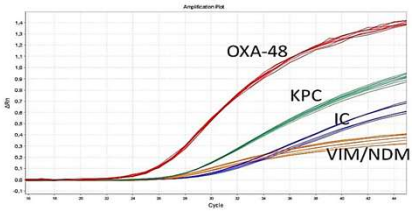
Pooled Se= 95%, Sp= 99%

In high-risk populations: Se= 99%, Sp= 98%

1. Khoo BY et al.J Clin Microbiol. 2023;61(9):e0031623.
2. Bai Y et al. J Mol Diagn. 2021;23(11):1534-1544.

Check-Direct CPE® assay, (Checks points)

TAT: 2 h
(+DNA extraction)



Targeted genes	Specimen	Technology/ instrumentation
KPC, VIM, NDM, OXA-48	Rectal swab Strains	Two-step ligation-mediated multiplex real-time PCR amplification – micro array hybridization / non-dedicated platform

867 samples from 627 patients (Reference method: culture + PCR)

CPO assay Se= 95.7%, SP= 96.5%, PPV= 60.8% and NPV= 99.8%

Xpert assay

Se= 97.9%, Sp= 99.8%, PPV= 95.8%, and NPV= 99.9%

The CPO assay: useful when handling many specimens, as tests are conducted in batches.

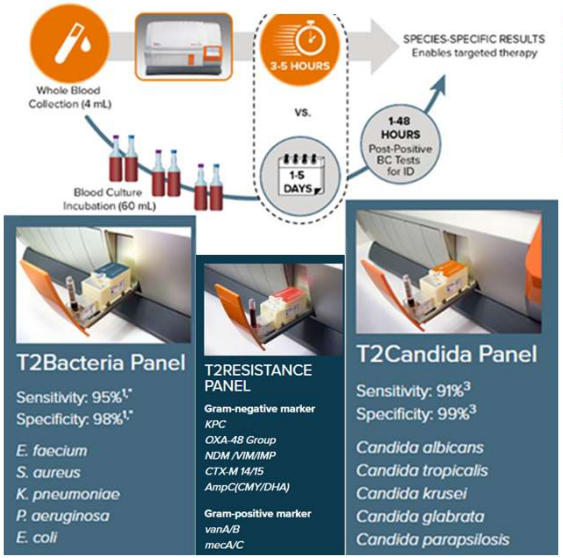
Positive cases showing high Ct values should be confirmed by another assay to rule out false positivity.

Yoo IY et al. Diagn Microbiol Infect Dis. 2022;103(3):115716.

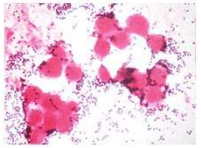
Συμβατική vs. ταχεία διάγνωση

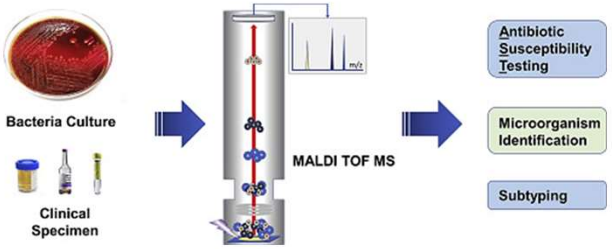
Εμπειρική αγωγή

Ολικό αίμα

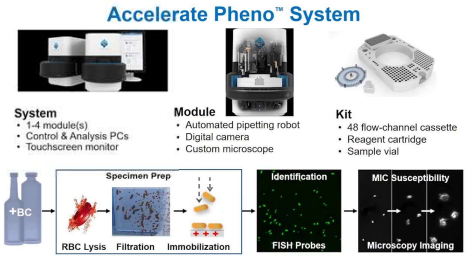


Χρώση Gram





MALDI Sepsityper
VITEK MS blood culture kit



Ροή εργασίας συμβατικών μεθόδων (~48-168 h)

Short subculture → ID /AST

Ροή εργασίας μοριακών μεθόδων

Διάγνωση Στοχευμένη αγωγή

Direct MALDI-TOF MS identification from positive blood cultures		MALDI-TOF MS identification from positive blood cultures using short sub-culture on solid medium		Molecular methods for identification from positive blood cultures	
ADVANTAGES	LIMITATIONS	ADVANTAGES	LIMITATIONS	ADVANTAGES	LIMITATIONS
Very rapid identification Easy Low additional cost	Identification difficulties with some species Confirmation might be needed Mixed cultures	Rapid identification Very easy Easy to integrate in lab workflow Confirmation usually not needed	Slow-growing organisms Mixed cultures	Rapid identification Easy	High cost Confirmation needed Limited pathogen panels

Reduced diagnostic accuracy for polymicrobial cultures

Sensitivity is influenced by:

- the concentration of pathogen-derived nucleic acids
- the presence of PCR inhibitors
- the sample volume

90% των αιτίων μικροβιαιμίας

DNA-based identification from whole blood

Ολικό αίμα

ADVANTAGES	LIMITATIONS
Time advantage compared to culture Advantage in pathogen detection under antibiotics	Limited number of pathogens in panel assays Relatively low sensitivity (only 1-5 ml blood sampled) Resistance detection ≠ susceptibility testing Clinical relevance of DNAemia ? High workload Highly experienced staff required High cost Analytical cut-offs set by some manufacturers Does not substitute culture-based testing

Lamy B et al. Clin Microbiol Infect. 2020;26(2):142-150.

JAMA Network Open
American Medical Association

Prevalence of Antibiotic-Resistant Pathogens in Culture-Proven Sepsis and Outcomes Associated With Inadequate and Broad-Spectrum Empiric Antibiotic Use

N= 17 430 adults with culture-positive sepsis
admitted to 104 US hospitals
67% received empiric broad-spectrum antibiotics

Is there risk associated with the receipt of empiric broad-spectrum antibiotics?

Resistant GP isolated in 13.6% of patients
Resistant GN in 13.2%
Both undertreatment (failure to cover organisms)
and overtreatment (resistant organisms targeted but not isolated)
were associated with higher mortality after detailed risk adjustment.

Table 2.

Outcomes Associated With Inadequate and Unnecessarily Broad Empiric Antibiotic Therapy[a]

Outcome	Inadequate vs adequate empiric therapy						Unnecessarily broad vs not unnecessarily broad empiric therapy[b]					
	No./total No. (%)		Unadjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value	No./total No. (%)		Unadjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
	Inadequate	Adequate empiric therapy					Unnecessarily broad	Not unnecessarily broad				
In-hospital death	488/2785 (17.5)	2011/12 388 (16.3)	1.10 (0.98-1.22)	.09	1.19 (1.03-1.37)	.02	1575/8405 (18.7)	436/3993 (10.9)	1.88 (1.68-2.11)	<.001	1.22 (1.06-1.40)	.007
Hospital-onset acute kidney injury	486/2785 (17.5)	2196/12 398 (17.7)	0.98 (0.88-1.09)	.74	1.02 (0.90-1.16)	.72	1641/8405 (19.5)	555/3993 (13.9)	1.50 (1.35-1.67)	<.001	1.12 (1.00-1.26)	.05
Clostridioides difficile	207/2785 (7.4)	498/12 398 (4.0)	1.92 (1.63-2.27)	<.001	1.19 (0.98-1.45)	.09	367/8405 (4.4)	131/3993 (3.3)	1.34 (1.10-1.65)	.004	1.26 (1.01-1.57)	.04

These findings underscore the need for better tests to rapidly identify patients with resistant pathogens

Rhee C et al. JAMA Netw Open. 2020;3(4):e202899.

FilmArray BCID2: 43 Targets, TAT 1 h

GRAM-NEGATIVE BACTERIA
Acinetobacter baumannii complex
Bacteroides fragilis
Enterobacterales
- *Enterobacter cloacae* complex
- *Escherichia coli*
- *Klebsiella aerogenes*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae* group
- *Proteus* spp.
- *Salmonella* spp.
- *Serratia marcescens*

Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Stenotrophomonas maltophilia

GRAM-POSITIVE BACTERIA
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Listeria monocytogenes*
- *Staphylococcus* spp.

Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
- *Streptococcus* spp.

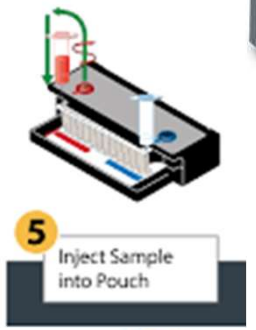
Streptococcus agalactiae
Streptococcus pneumonia
Streptococcus pyogenes

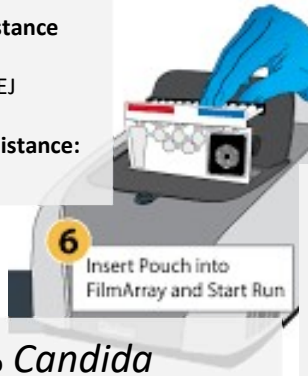
YEAST:
- *Candida albicans*
- *Candida auris*
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Cryptococcus*

(C. neoformans/C. gattii)

AMR GENES
Carbapenemases
- IMP
- KPC
- OXA-48-like
- NDM
- VIM

Colistin Resistance: *mcr-1*
ESBL: CTX-M
Methicillin Resistance
mecA/C
mecA/C and MREJ (MRSA)
Vancomycin Resistance:
vanA/B





Nested-PCR and melting curve analysis dedicated platform

Se: 98.1% GN, 97.3% GP, 99.2% *Candida*
Sp: 99.9% GN, 99.8% GP, 99.9% *Candida*
Se/Sp για AMR γονίδια: 98.4-100/98.3-100%

Salimnia H et al. J Clin Microbiol 2016.

ePlex® BCID Panels

***Se 97-99%**
Sp 96-99%

*Huang TD et al.
J Clin Microbiol. 2019;57(2).

Gram-positive Organisms

20 παθογόνα
4 γονίδια αντοχής

21 παθογόνα
6 γονίδια αντοχής
Gram-Negative Organisms

15 παθογόνα
Fungal Organisms

VERIGENE® Bloodstream Infection Testing Panels

Gram-Positive Blood Culture Test (BC-GP)

Species
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes
Enterococcus faecalis
Enterococcus faecium

Group
Streptococcus anginosus

Genus
Staphylococcus spp.
Streptococcus spp.
Micrococcus spp.+
Listeria spp.

Resistance
mecA (methicillin)
vanA (vancomycin)
vanB (vancomycin)

Gram-Negative Blood Culture Test (BC-GN)

Species
*Escherichia coli**
Klebsiella pneumoniae
Klebsiella oxytoca
Pseudomonas aeruginosa
Serratia marcescens++

Genus
Acinetobacter spp.
Citrobacter spp.
Enterobacter spp.
Proteus spp.

Resistance
CTX-M (ESBL)
IMP (carbapenemase)
KPC (carbapenemase)
NDM (carbapenemase)
OXA (carbapenemase)
VIM (carbapenemase)

Micro-array hybridization dedicated platform

TAT 2-2.5 h

Συνδρομική διάγνωση και ανίχνευση γονιδίων αντοχής: Blood Culture Panels (positive BC bottle)

Parameter	FilmArray BCID	Verigene Gram-positive blood culture	Verigene Gram-negative blood culture
Total no. of targets	27	15	14
Ability to detect presence of resistance gene			
mecA	✓	✓	
vanA	✓	✓	
vanB	✓	✓	
bla_{KPC}	✓		✓
bla_{NDM}	✓		✓
bla_{OXA}	✓		✓
bla_{VIM}	✓		✓
bla_{IMP}	✓		✓
bla_{CTX-M}	✓		✓
mcr-1	✓		
Time to result (h)	~1	~2.5	~2

Se 93.9%

Se: 90.9

NPA >99.9%

Sp> 98 %

Se: 94.9%

PPA 100%

PPA 96.2%

PPA 94.3%

PPA 100%

PPA 100%

PPA 98.9%

ePlex BCID, GenMark

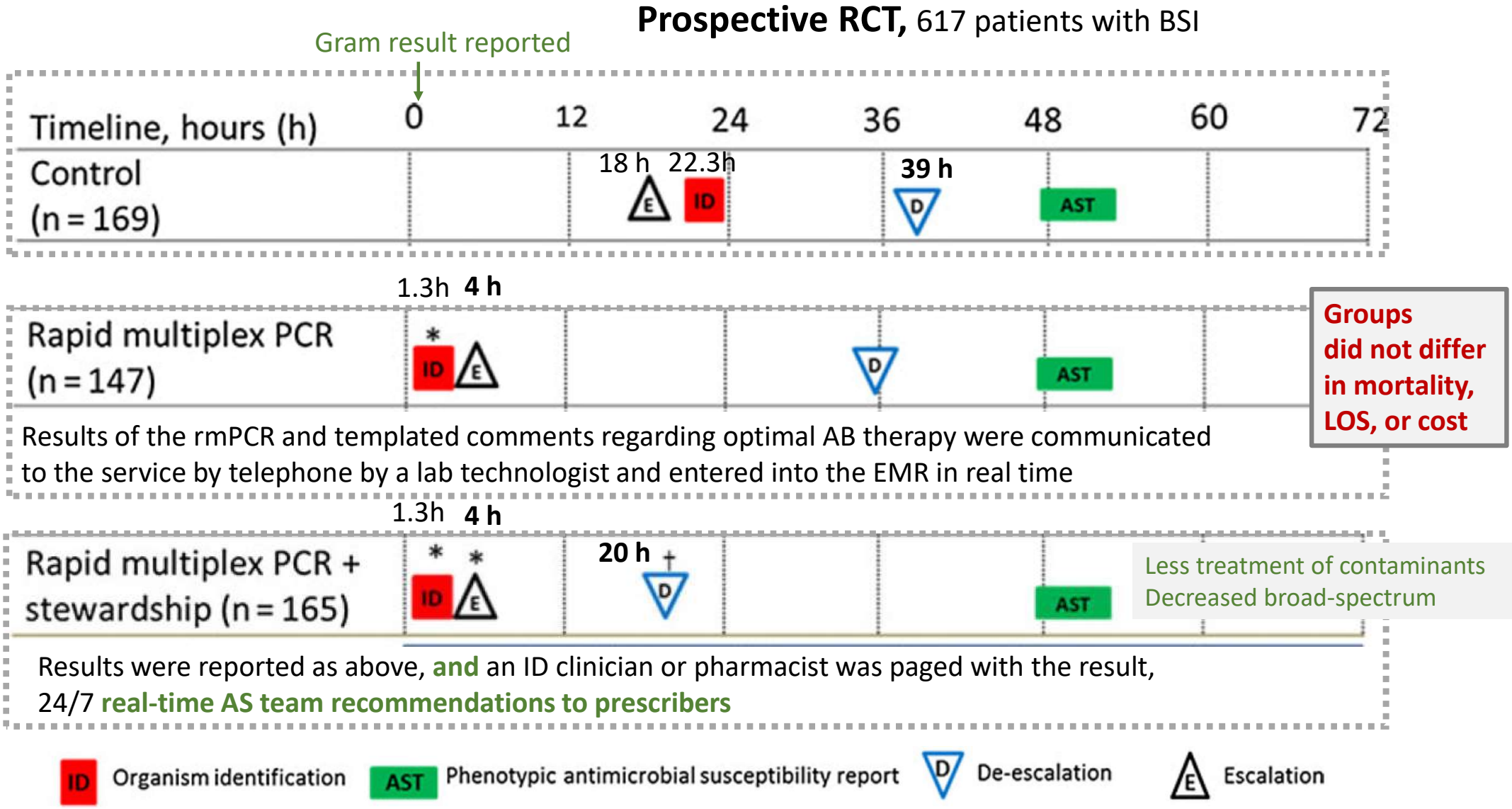
Resistance Genes
CTX-M
KPC
NDM
VIM
IMP
OXA

Resistance Genes
mecA
mecC
vanA
vanB

Multiplexed NAAT and a electrochemical detection technology with a dedicated platform

Peri AM et al. BMC Infect Dis. 2022;22(1):794.
Ramanan P et al. Clin Microbiol Rev. 2017;31(1) (με επικαιροποίηση).
Salimnia H et al. J Clin Microbiol. 2016;54(3):687-98.
Ledeboer NA et al. J Clin Microbiol. 2015;53(8):2460-72.

Prospective RCT, 617 patients with BSI



Banerjee R et al. Clin Infect Dis. 2015;61(7):1071-80.

Clinical Infectious Diseases
MAJOR ARTICLE
IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD
2022;75(12):2066-2075.

Impact of a Rapid Molecular Test for *Klebsiella pneumoniae* Carbapenemase and Ceftazidime-Avibactam Use on Outcomes After Bacteremia Caused by Carbapenem-Resistant Enterobacterales

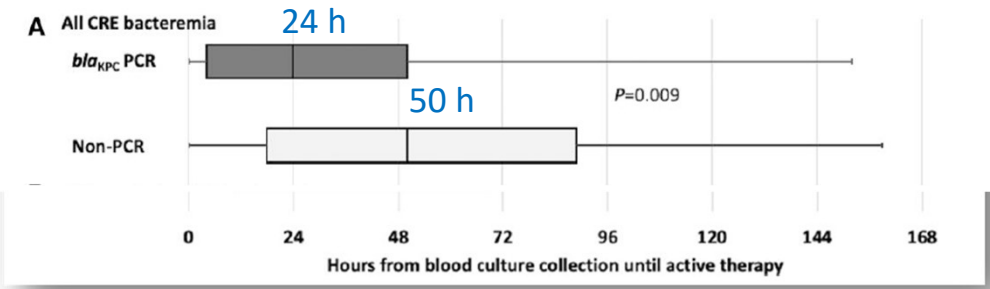
Michael J. Satlin,[1,2] Liang Chen,[3,4] Angela Gomez-Simmonds,[5] Jamie Marino,[2] Gregory Weston,[6] Tanaya Bhowmick,[7] Susan K. Seo,[8] Steven J. Sperber,[9,10] Angela C. Kim,[11] Brandon Eilertson,[12] Sierra Derti,[1] Stephen G. Jenkins,[1,2] Michael H. Levi,[13] Melvin P. Weinstein,[7,14] Yi-Wei Tang,[15] Tao Hong,[16] Stefan Juretschko,[17] Katherine L. Hoffman,[18] Thomas J. Walsh,[1] Lars F. Westblade,[1,2] Anne-Catrin Uhlemann,[5] and Barry N. Kreiswirth[3,4]

N= 137 patients with CRE bacteremia

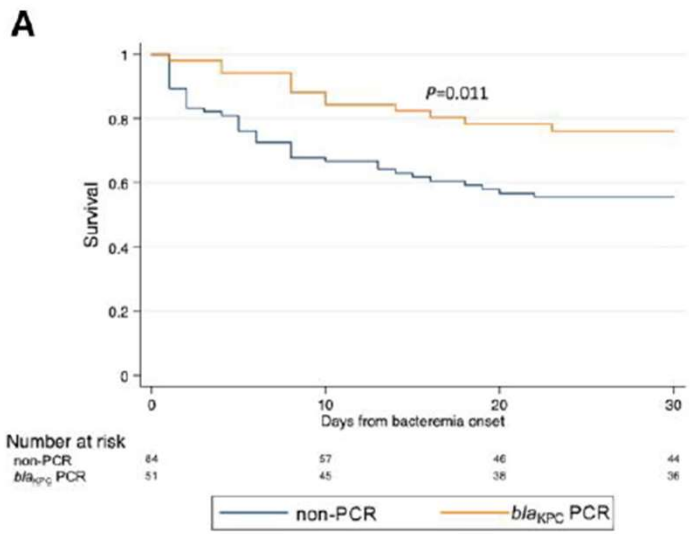
[77% CP-CRE (65% KPC)]

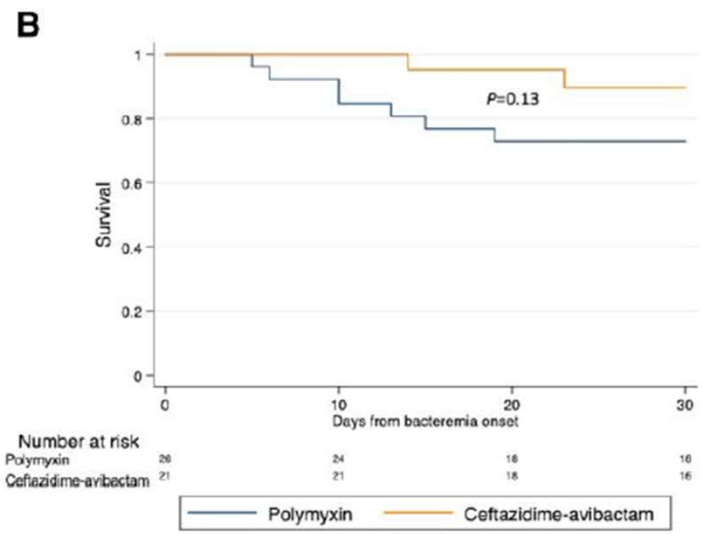
2016-2018, 8 New York and New Jersey medical centers

3/8 study hospitals used the FilmArray BCID



30-day mortality 24% vs 47%; P=.007





30-day mortality 10% with caz-avi monotherapy vs. 31% with polymyxin monotherapy (P=.08)

antibiotics MDPI

Article

Impact of an Antimicrobial Stewardship Program Intervention Associated with the Rapid Identification of Microorganisms by MALDI-TOF and Detection of Resistance Genes in ICU Patients with Gram-Negative Bacteremia

216 episodes of GN bacteremia, ICU

MALDI-TOF και ανίχνευση των γονιδίων AMR απευθείας από τη φιάλη της θετικής αιμοκ/ας

Table 4. Antimicrobial consumption in DOT per 1000 days present and collection (21 days).

Antimicrobial Consumption and Cost	Pre-Intervention	Intervention	*p*-Value
Consumption (DOT/1000 days present, median, IQR)			
All antimicrobials	N = 114 1.381 (1.103–2.251)	N = 102 1.262 (1.063–1.662)	0.032 [a]
Antimicrobial for gram-negative bacteria	N = 114 1.281 (1.004–1.775)	N = 102 1.172 (1.006–1.427)	0.067 [a]
Antimicrobial for gram-positive bacteria	N = 52 475 (238–761)	N = 43 270 (139–467)	0.004 [a]
Carbapenems	N = 76 836 (504–1056)	N = 72 543 (301–991)	0.040 [a]
Colistin/Polymyxin B	N = 45 722 (390–932)	N = 40 881 (462–1001)	0.299 [a]
Ceftazidime/avibactam	N = 3 931 (725–1022)	N = 8 911 (823–940)	0.865 [a]

Καμιά επίδραση στη θνητότητα 30-ημερών
Σημαντικά μικρότερη η συνολική διάρκεια νοσηλείας (44 vs. 39 ημέρες, p = 0.005) και ο χρόνος νοσηλείας στη ΜΕΘ (17 vs. 13 ημέρες, p = 0.033)

Campos AF et al. Antibiotics (Basel). 2022;11(9):1226.

EXPERT REVIEW OF MEDICAL DEVICES
2021, VOL. 18, NO. 5, 473–482
https://doi.org/10.1080/17434440.2021.1919508

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META-ANALYSIS

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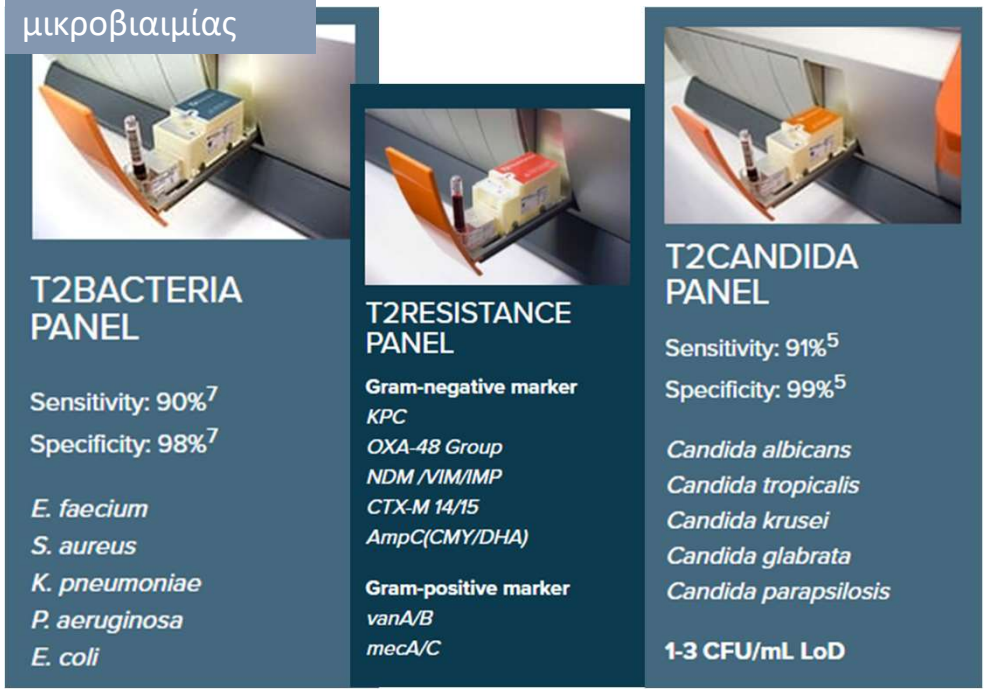
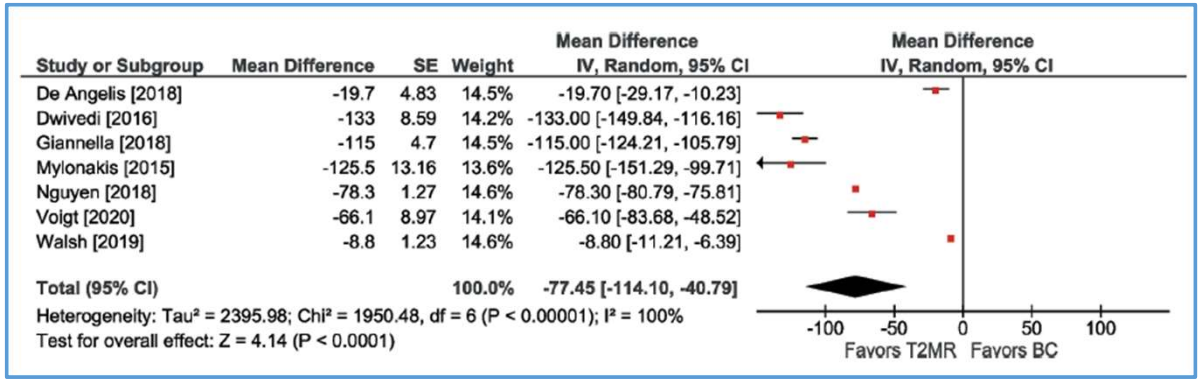
Antimicrobial and resource utilization with T2 magnetic resonance for rapid diagnosis of bloodstream infections: systematic review with meta-analysis of controlled studies

61.4% των αιτίων μικροβιαιμίας

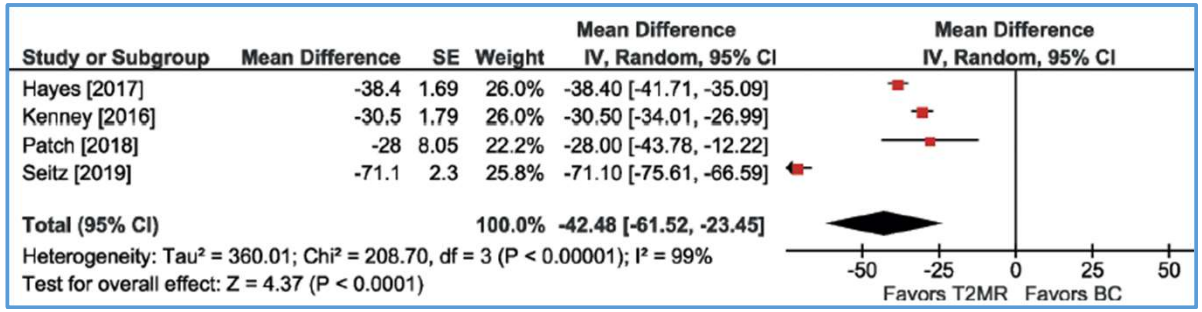


Hours to species identification with T2MR vs. BC



Pooled mean difference = −77 hours (p <0.001)

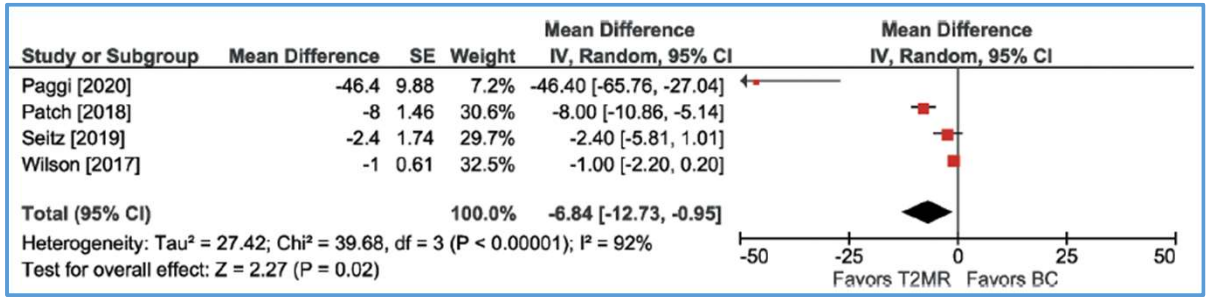
Hours to targeted therapy among T2MR positive cases vs. BC



Pooled mean difference = −42 hours (p <0.001)

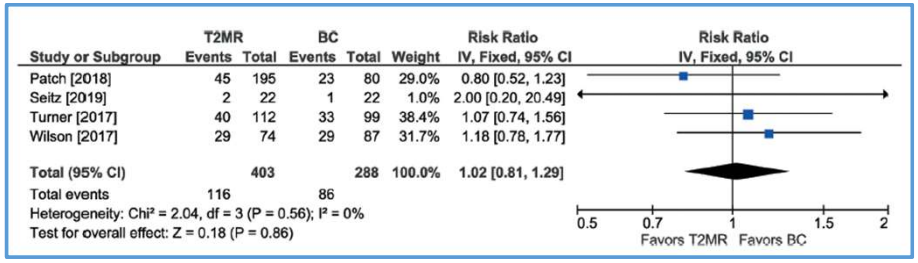
Giannella M et al. Expert Rev Med Devices. 2021;18(5):473-482.

Hours to empirical therapy de-escalation among T2MR negative cases vs. BC



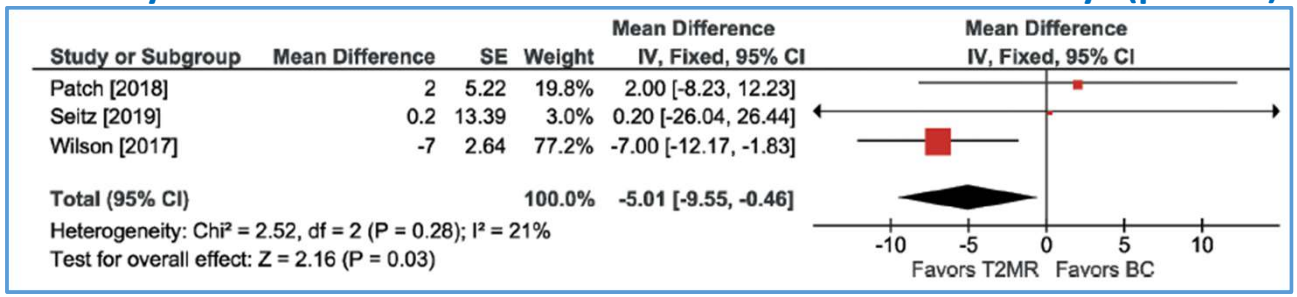
Pooled mean difference = −7 hours (p = 0.02).

Mortality with T2MR vs. BC

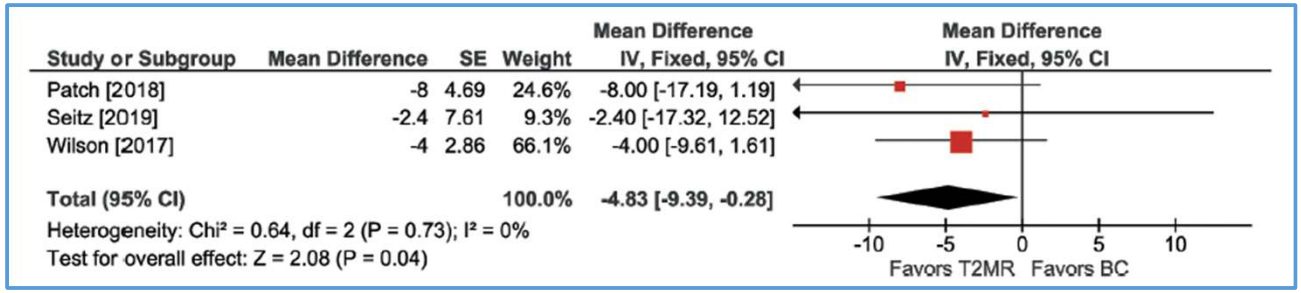


28.9% vs. 29.9%, RR = 1.02, p = 0.86

ICU stay with T2MR vs. BC. Pooled mean difference = −5.0 days (p =0.03)



Hospital stay with T2MR vs. BC. Pooled mean difference = −4.8 days (p =0.04)



Considering hospital costs for sepsis patients range from 2,000 USD to 5,000 USD per day depending on sepsis severity, implementation of T2MR could theoretically reduce hospital costs by 10,000 USD to 25,000 USD per patient tested

Giannella M et al. Expert Rev Med Devices. 2021;18(5):473-482.

Μοριακή ανίχνευση αντοχής: προβληματισμοί

Ασυμφωνία αποτελεσμάτων μοριακών / συμβατικών μεθόδων

Ποια είναι η μέθοδος αναφοράς / gold standard;

Υψηλά ποσοστά **culture-negative / molecular testing-positive** (5% - 55%)

— Αυξανόμενη ευαισθησία σύγχρονων μοριακών μεθόδων
  Πιθανή έλλειψη ευαισθησίας καλλιεργητικών μεθόδων (low inoculum)

— Παρουσία νεκρών ή μη καλλιεργήσιμων βακτηρίων

— Μη έκφραση του γονιδίου αντοχής ή έκφραση σε χαμηλά επίπεδα
  (χαμηλού επιπέδου αντοχή μη ανιχνεύσιμη με τις συμβατικές μεθόδους)

— Παρουσία άλλων βακτηρίων στο δείγμα που δεν ανιχνεύονται
  και φέρουν τα γονίδια αντοχής

Ποιες είναι οι κλινικές επιπτώσεις

FP μοριακές, ME (μη απαραίτητη ευρεία κάλυψη ασθενούς)

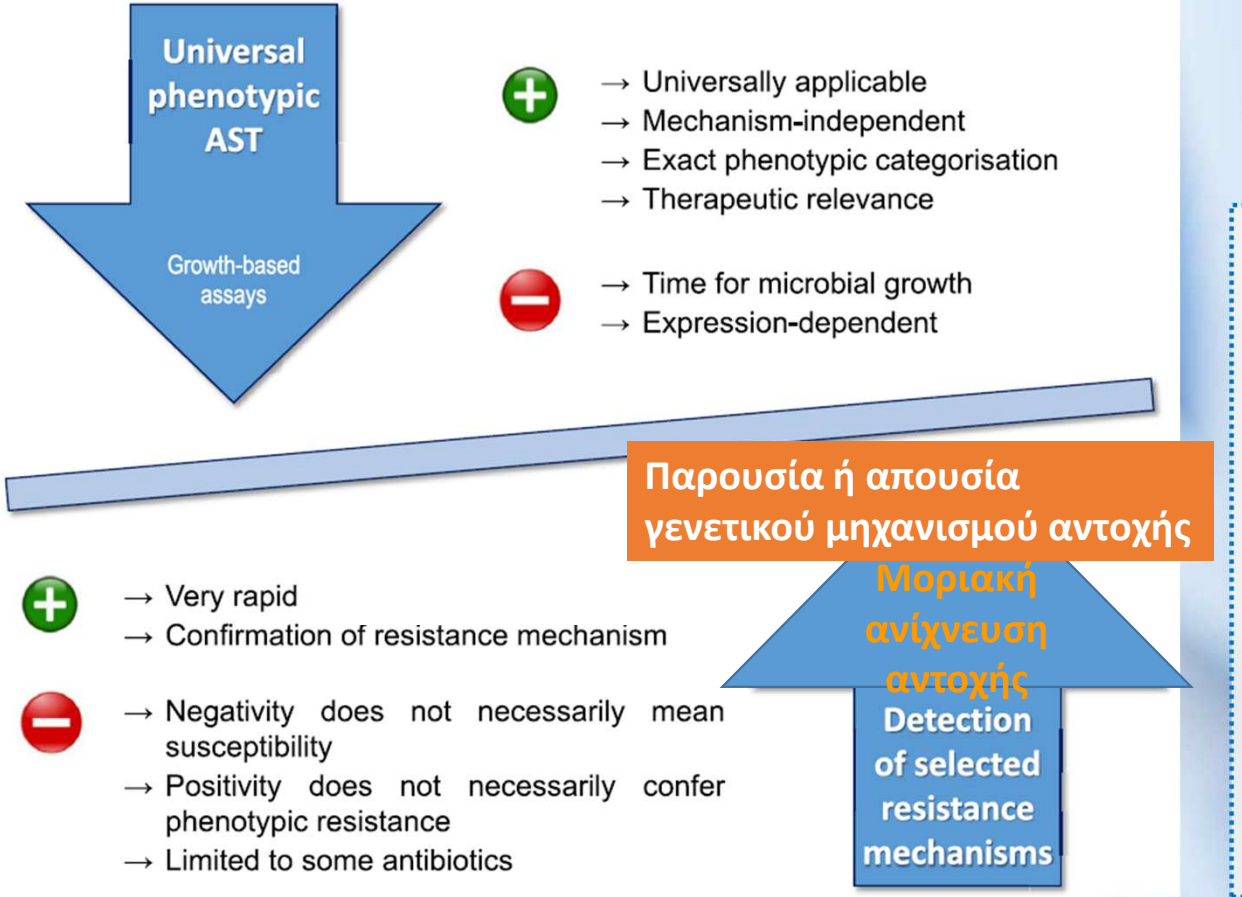
FN καλλιεργητικές, VME ακατάλληλη αγωγή

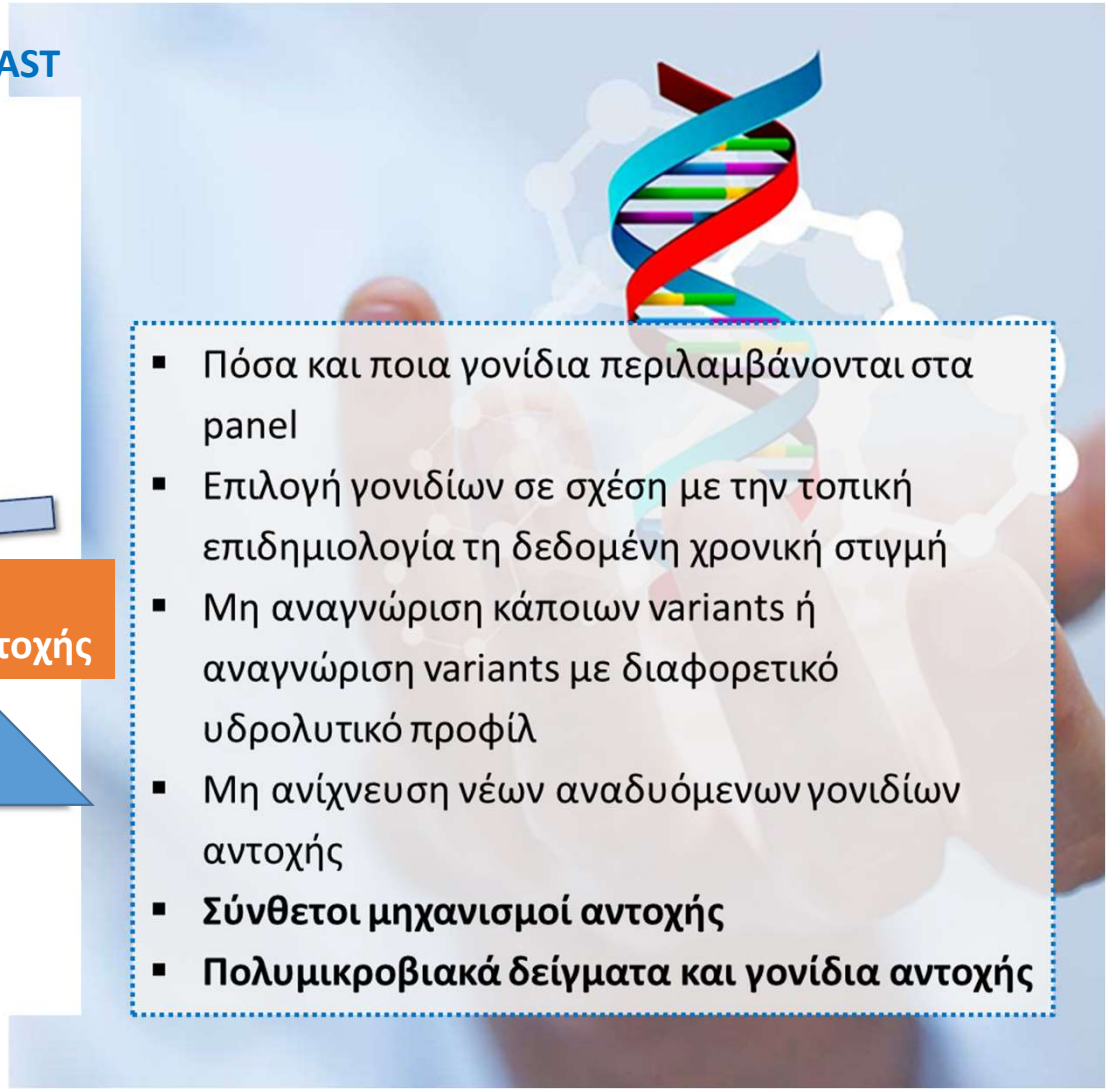
Περιορισμένες επιπτώσεις στην περίπτωση μη έκφρασης του γονιδίου

Decousser JW et al. Expert Rev Mol Diagn. 2017;17(4):327-350.

Μοριακή ανίχνευση αντοχής: προβληματισμοί

Detection of particular resistance mechanisms vs. phenotypic AST





- Πόσα και ποια γονίδια περιλαμβάνονται στα panel
- Επιλογή γονιδίων σε σχέση με την τοπική επιδημιολογία τη δεδομένη χρονική στιγμή
- Μη αναγνώριση κάποιων variants ή αναγνώριση variants με διαφορετικό υδρολυτικό προφίλ
- Μη ανίχνευση νέων αναδυόμενων γονιδίων αντοχής
- **Σύνθετοι μηχανισμοί αντοχής**
- **Πολυμικροβιακά δείγματα και γονίδια αντοχής**

Idelevich EA, Becker K. Clin Microbiol Infect. 2019;25(11):1347-1355. Με τροποποίηση

Είναι ασφαλής η αποκλιμάκωση;

AMERICAN SOCIETY FOR MICROBIOLOGY Antimicrobial Agents and Chemotherapy®

CLINICAL THERAPEUTICS

An Antibiotic Stewardship Program Blueprint for Optimizing Verigene BC-GN within an Institution: a Tale of Two Cities

Δεν υπάρχει σαφής κατεύθυνση για τις περιπτώσεις που δεν ανιχνεύονται καθοριστές αντοχής

1046 GN bloodstream isolates

TABLE 2 Verigene BC-GN analytic results by bug-drug combination[a]

Institution and target organism	n	No. (%) of	No. of isolates	P (%)	PPV (%)	NPV (%)
Detroit Medical Center						
E. coli	3			9	97	98
K. pneumoniae	1			9	97	94
	1			00	100	99
K. oxytoca	2			00	100	95
Proteus spp.	5			00	100	94
Enterobacter spp.	6			00	100	97
Citrobacter spp.	1					100
Acinetobacter spp.	3			3	80	93
Pseudomonas spp.	5					88

AMERICAN SOCIETY FOR MICROBIOLOGY Journal of Clinical Microbiology®

LETTER TO THE EDITOR

2020;58(4):e02098-19.

The Clinical Impact of a Negative Molecular β-Lactamase Gene Test for *Enterobacteriaceae*: Let's Not Let Perfect Be the Enemy of Really Good

Jason M. Pogue,[a] Emily L. Heil[b]

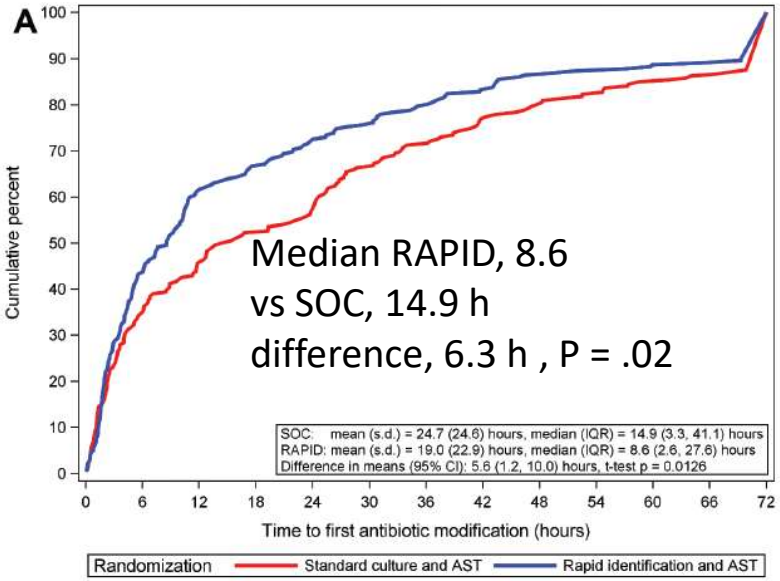
Με εξαίρεση την *P. aeruginosa*, η απουσία καθοριστών αντοχής **μπορούσε να προβλέψει την ευαισθησία στα αντιβιοτικά στόχους**

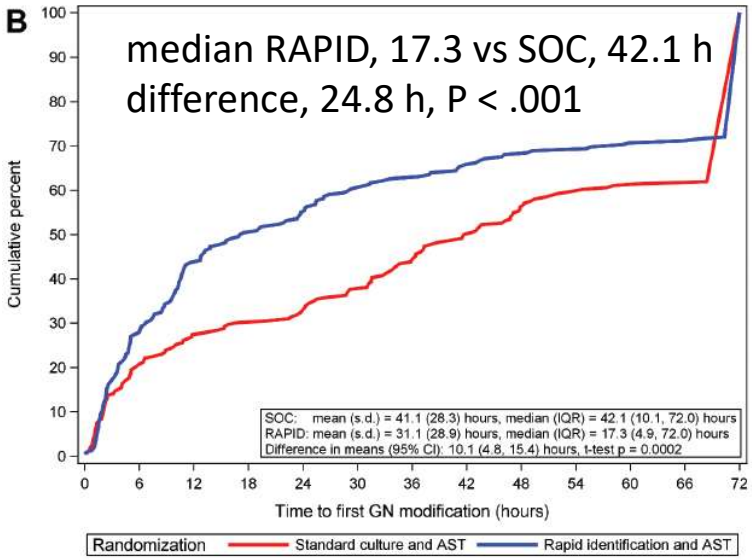
Pogue JM et al. Antimicrob Agents Chemother. 2018;62(5):e02538-17.

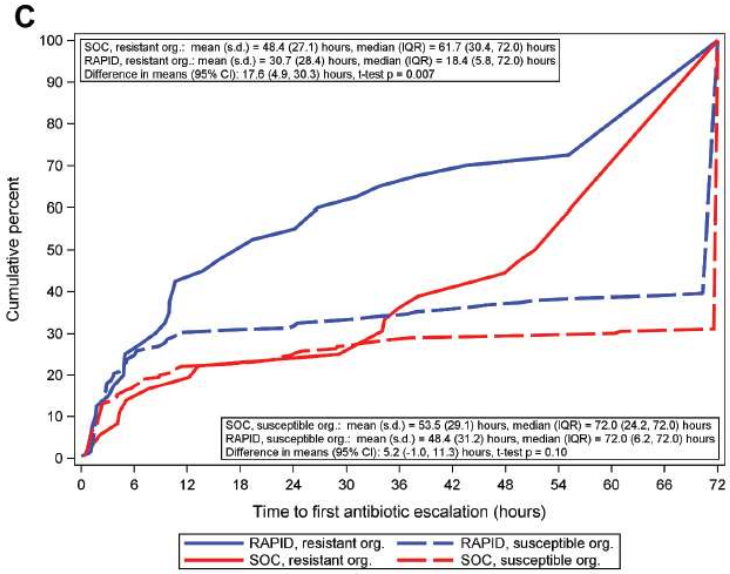
ID and phenotypic AST using the Accelerate Pheno System (RAPID)

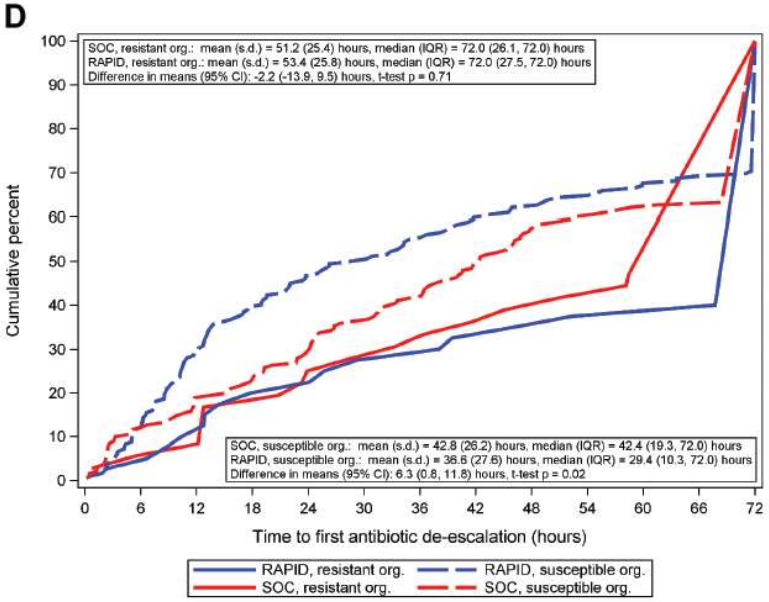
RCT
Patients (N= 448)
with positive BC with GNB
RANDOMIZED
SOC testing with AS review
or RAPID with AS

Patients with MDRO, escalation
Median RAPID, 18.4 vs SOC, 61.7 h
(difference, 43.3 h; P = .01)
Patients with S organisms, de-escalation
median RAPID, 29.4 vs SOC, 42.4 h
(difference, 13 h, P = .02)









Banerjee R et al. Clin Infect Dis. 2021;73(1):e39-e46.

Clinical Infectious Diseases
MAJOR ARTICLE
IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD
2022;75(2):269-277

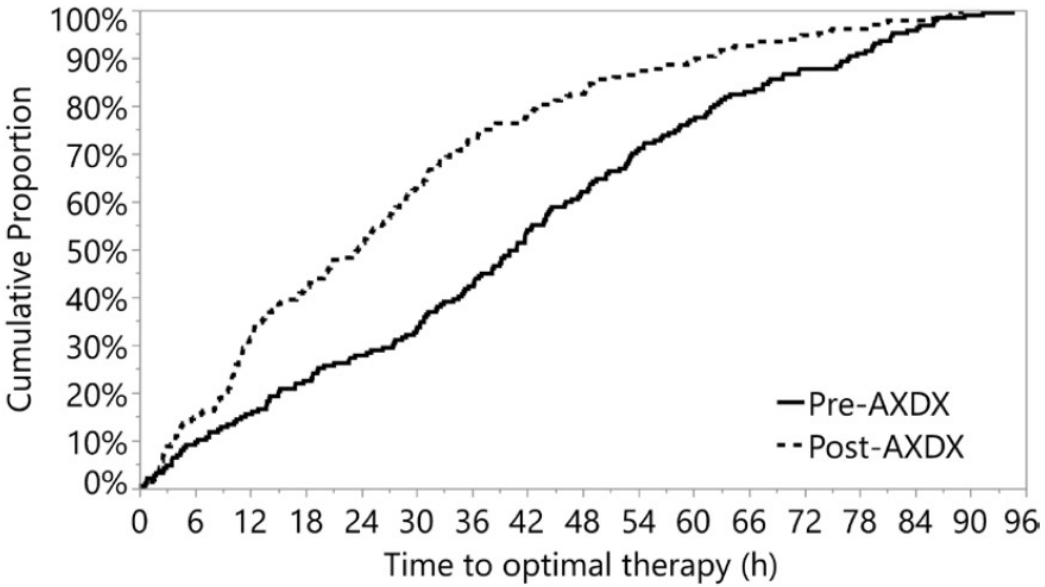
Real-World Impact of the Accelerate PhenoTest BC Kit on Patients With Bloodstream Infections in the Improving Outcomes and Antimicrobial Stewardship Study: A Quasiexperimental Multicenter Study

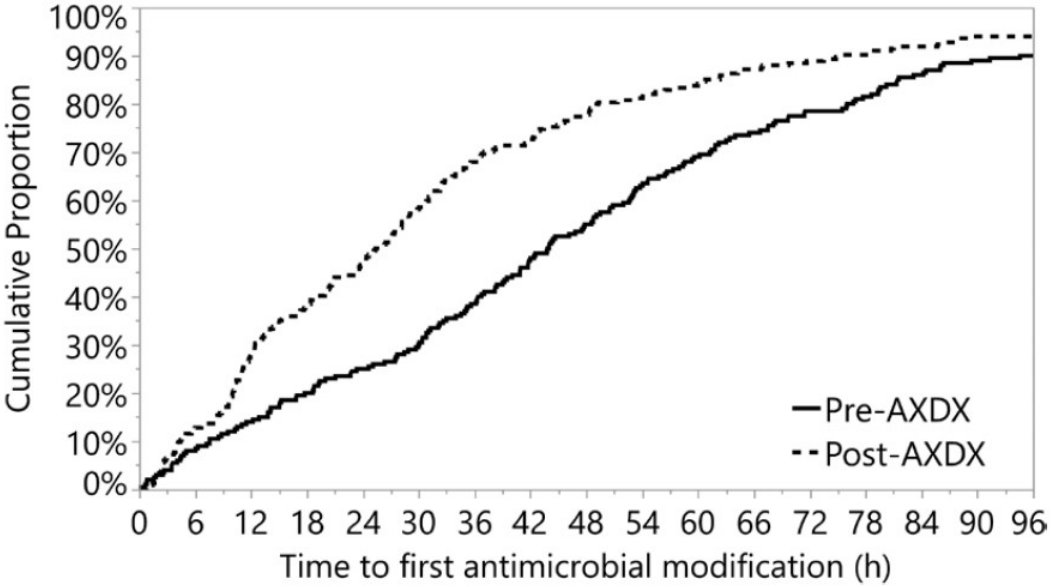
Amira A. Bhalodi,[1,a] Shawn H. MacVane,[1,a] Bradley Ford,[2] Dilek Ince,[3] Patrick M. Kinn,[4] Kelly M. Percival,[4] Derek N. Bremmer,[5] Dustin R. Carr,[5] Thomas L. Walsh,[6] Micah M. Bhatti,[7] Samuel A. Shelburne,[8] Romney M. Humphries,[1,b] Kaleb Wolfe,[9] Eric R. Rosenbaum,[10] Ryan K. Dare,[9] Johann Kolev,[11] Meghan Madhusudhan,[12] Michael A. Ben-Aderet,[11] and Margie A. Morgan[13]

N= 854 ασθενείς με BSIs
5 κέντρα στις ΗΠΑ
Απρίλιος 2017 - Νοέμβριος 2019

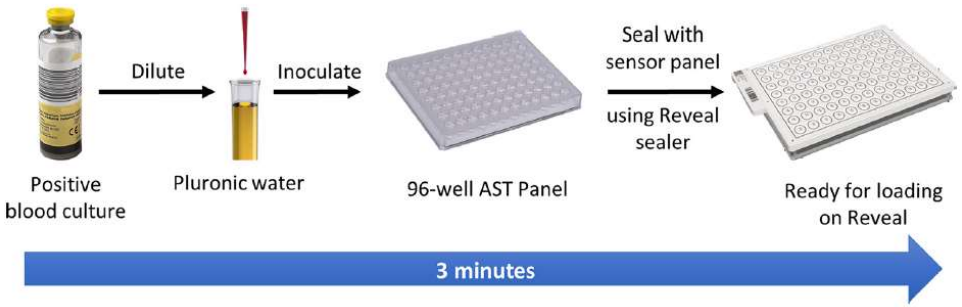
Median TTOT was 17.2 hours shorter in the post-AXDX arm (23.7 h) vs. pre-AXDX arm (40.9 h; P < .0001).

Διάμεσος χρόνος
Για την πρώτη τροποποίηση: 24.2 vs 13.9 h; P < .0001
Για την πρώτη αποκλιμάκωση: 36.0 vs 27.2 h; P = .0004





Rapid AST



RAPID AST SYSTEM

5.5 hours

TABLE 1 Overall performance of the Reveal AST system

Parameter or detail	Performance of Reveal AST against: Sensititre	Vitek 2
Parameter, % (no. positive/total no.)		
EA[c]	98.0 (2,129/2,173)	97.0 (1,482/1,528)
CA[c]	96.3 (2,174/2,258)	96.2 (1,554/1,615)
mE	3.5 (78/2,258)	3.3 (54/1,615)
ME	0.3 (5/1,889)	0.3 (4/1,342)
VME	1.3 (4/313)	1.3 (3/232)

Wide antimicrobial coverage for Gram-negative bloodstream infections, with 176 bug drug combinations.

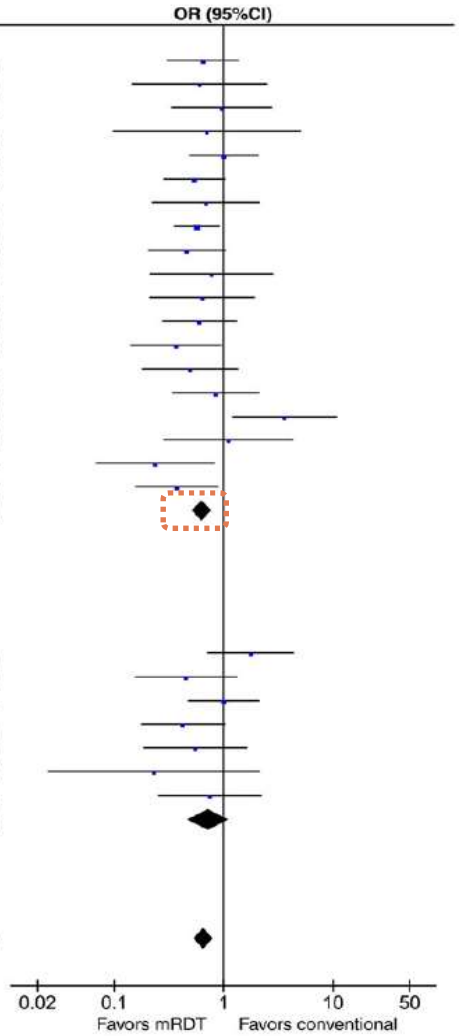
ANTIMICROBIAL DRUG	MIC CALLING RANGE (µg/ml)	A. baumannii	C. freundii	C. koseri	E. cloacae	E. coli	K. aerogenes	K. oxytoca	K. pneumoniae	P. mirabilis	P. aeruginosa	
Amikacin	4 - 16	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Amoxicillin / Clavulanate	4/2 - 16/2			✓		✓		✓	✓	✓		5
Ampicillin	4 - 8					✓				✓		2
Aztreonam	1 - 16		✓	✓	✓	✓	✓	✓	✓		✓	8
Cefepime	0.125 - 64		✓	✓	✓	✓		✓	✓	✓	✓	8
Cefotaxime	0.125 - 4		✓	✓	✓	✓	✓	✓	✓	✓		8
Cefoxitin	8 - 16			✓		✓		✓	✓	✓		5
Ceftazidime	0.125 - 64		✓	✓	✓	✓	✓	✓	✓	✓	✓	9
Ceftazidime / Avibactam	0.25/4, 1/4 - 16/4		✓	✓	✓	✓	✓	✓	✓	✓	✓	9
Ceftazidime / Clavulanate	0.25/4 - 8/4					✓		✓	✓			3
Ceftolozane / Tazobactam	1/4 - 4/4				✓	✓	✓	✓	✓		✓	6
Ciprofloxacin	0.06, 0.25 - 1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Ertapenem	0.125 - 1		✓	✓	✓	✓	✓	✓	✓	✓		8
Gentamicin	2 - 4	✓	✓	✓	✓	✓	✓	✓	✓	✓		9
Imipenem	1 - 8	✓	✓	✓	✓	✓	✓	✓	✓		✓	9
Levofloxacin	0.25 - 1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Meropenem	0.125 - 8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Meropenem / Vaborbactam	2/8 - 8/8		✓	✓	✓	✓	✓	✓	✓	✓	✓	9
Piperacillin	8 - 16		✓		✓	✓	✓	✓	✓	✓	✓	8
Piperacillin / Tazobactam	4/4 - 16/4		✓	✓	✓	✓	✓	✓	✓	✓	✓	9
Tigecycline	0.5 - 1			✓		✓						2
Tobramycin	2 - 4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Trimethoprim / Sulfamethoxazole	2/38 - 4/76	✓	✓	✓	✓	✓	✓	✓	✓	✓		9
TOTAL		8	17	19	18	23	17	21	21	18	14	176

System reports results with S/I/R interpretation according to CA-SFM 2019 and EUCAST 2021 Breakpoints

Tibbetts R et al. J Clin Microbiol. 2022;60(6):e0009822.

Mortality outcomes with molecular rapid diagnostic testing (mRDT) versus conventional testing in bloodstream infection

Study or Subgroup	mRDT Events	mRDT Total	Conventional Testing Events	Conventional Testing Total	Weight, %	OR (95%CI)
1.1.1 mRDT with ASP						
Bauer et al [17] (2010)	15	82	19	74	5.6	0.65 (.30–1.39)
Bias et al [19] (2015)	3	37	7	55	1.8	0.61 (.15–2.51)
Box et al [20] (2015)	6	64	10	103	3.0	0.96 (.33–2.79)
Forrest et al [24] (2006)	2	119	2	84	0.9	0.70 (.10–5.08)
Forrest et al [23] (2006)	19	72	20	76	6.0	1.00 (.48–2.09)
Forrest et al [25] (2008)	17	95	37	129	7.4	0.54 (.28–1.04)
Heil et al [27] (2012)	5	21	19	61	2.7	0.69 (.22–2.16)
Huang et al [29] (2013)	31	245	52	256	11.8	0.57 (.35–.92)
Lockwood et al [30] (2016)	11	241	14	149	4.9	0.46 (.20–.1.04)
Macvane et al [32] (2015)	5	63	5	50	2.1	0.78 (.21–.2.84)
Macvane et al [33] (2016)	6	23	16	45	2.8	0.64 (.21–.1.95)
Nagel et al [36] (2014)	11	117	19	129	5.3	0.60 (.27–.1.32)
Pardo et al [39] (2016)	5	84	37	252	3.6	0.37 (.14–.97)
Perez et al [15] (2013)	6	107	12	112	3.3	0.50 (.18–1.37)
Revolinksi et al [40] (2015)	8	95	13	133	4.0	0.85 (.34–2.14)
Sango et al [42] (2013)	11	28	7	46	2.8	3.61 (1.19–10.89)
Sothoron et al [43] (2015)	5	67	4	59	1.9	1.11 (.28–4.34)
Suzuki et al [44] (2015)	3	88	19	147	2.3	0.24 (.07– .83)
Walker et al [45] (2016)	8	97	19	98	4.3	0.37 (.16– .90)
Subtotal		**1745**		**2058**	**76.5**	**0.64 (.51– .79)**
Total events	177		331			
Heterogeneity: $\tau^2 = 0.01$ $\chi^2 = 19.00$ ($df = 18$; $P = .39$); $I^2 = 5\%$						
Test for overall effect: $z = 4.14$ ($P < .001$)						
1.1.2 mRDT without ASP						
Beuving et al [18] (2015)	14	114	8	109	4.1	1.77 (.71–4.40)
Felsenstein et al [22] (2016)	5	189	11	194	3.0	0.45 (.15–1.33)
Frye et al [26] (2012)	14	110	17	134	5.7	1.00 (.47–2.14)
Ly et al [31] (2008)	8	101	17	101	4.2	0.43 (.17–1.04)
Maslonka et al [34] (2014)	6	55	10	55	2.9	0.55 (.19–1.64)
Neuberger et al [37] (2008)	1	42	4	42	0.7	0.23 (.02–2.17)
Wang et al [46] (2013)	8	48	8	38	2.9	0.75 (.25–2.23)
Subtotal		**659**		**673**	**23.5**	**0.72 (.46–1.12)**
Total events	56		75			
Heterogeneity: $\tau^2 = 0.08$ $\chi^2 = 7.74$ ($df = 6$; $P = .26$); $I^2 = 23\%$						
Test for overall effect: $z = 1.46$ ($P = .15$)						
Total (95% CI)		**2404**		**2731**	**100.0**	**0.66 (.54– .80)**
Total events	233		406			
Heterogeneity: $\tau^2 = 0.02$ $\chi^2 = 27.22$ ($df = 25$; $P = .34$); $I^2 = 8\%$						
Test for overall effect: $z = 4.27$ ($P < .001$)						
Test for subgoup differences: $\chi^2 = 0.25$ ($df = 1$; $P = .62$); $I^2 = 0\%$						



mRDT και ASP

31 μελέτες, 5920 BSI pts

Mortality risk decreased significantly with mRDT in the presence of ASP

Number needed to treat: 20 patients with BSI to prevent 1 death within 30 days

Time to effective therapy decreased by a weighted mean difference of −5.03 hours

Length of stay decreased by −2.48 days

Timbrook TT et al. Clin Infect Dis. 2017;64(1):15-23

AMERICAN SOCIETY FOR MICROBIOLOGY Clinical Microbiology Reviews®

2018;31(3):e00095-17.

REVIEW

The Cost-Effectiveness of Rapid Diagnostic Testing for the Diagnosis of Bloodstream Infections with or without Antimicrobial Stewardship

Elina Eleftheria Pliakos,[a] Nikolaos Andreatos,[a] Fadi Shehadeh,[a] Panayiotis D. Ziakas,[a] Eleftherios Mylonakis[a]

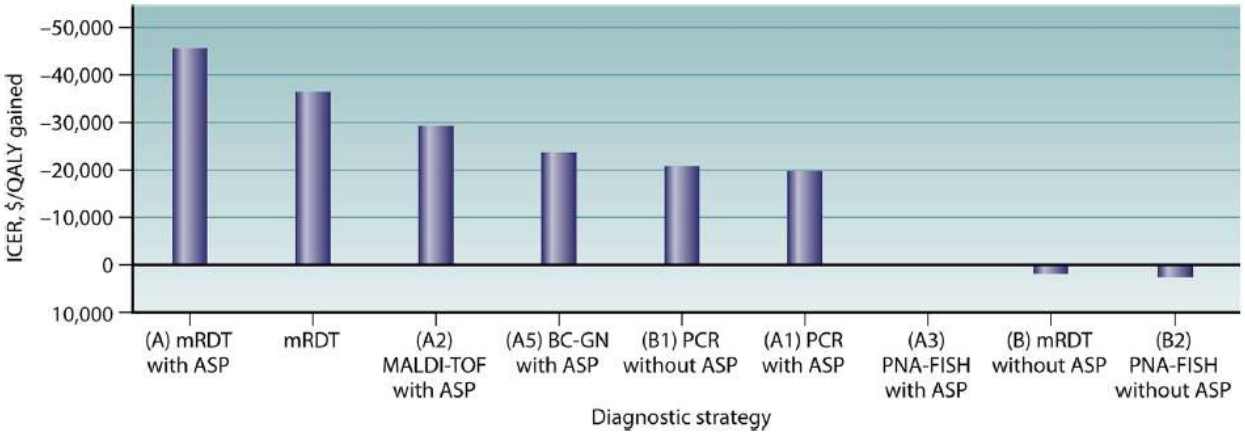


TABLE 3 Base-case analysis results for competing strategies

	Base-case estimate				
		Effect		ICER	
Strategy	Cost ($)	Probability of survival	QALY value	$/death averted	$/QALY gained
mRDT (with and without ASP)	36,301.50	0.89	11.9883	−490,763	−36,434
mRDT with ASP (A)	31,274.24	0.89	11.9883	−616,445	−45,764
mRDT without ASP (B)	57,220.14	0.90	12.1230	25,762	1,913
PCR with ASP (A1)	47,917.58	0.88	11.8536	−267,148	−19,833
MALDI-TOF analysis with ASP (A2)	28,394.21	0.92	12.3924	−393,397	−29,205
PNA-FISH with ASP (A3)	53,226.09	0.85	11.4495	0	0
BC-GP with ASP (A4)	24,904.91	0.84	11.3148	Dominated	Dominated
BC-GN with ASP (A5)	33,691.47	0.92	12.3924	−317,722	−23,587
PCR without ASP (B1)	47,430.21	0.88	11.8536	−283,394	−21,039
PNA-FISH without ASP (B2)	58,284.16	0.92	12.3924	33,602	2,495
Conventional laboratory methods with ASP (C)	41,723.98	0.84	11.3148	Dominated	Dominated
Conventional laboratory methods without ASP (D)	55,932.02	0.85	11.4495	Baseline	Baseline

Ταχεία μοριακή διάγνωση και diagnostic stewardship

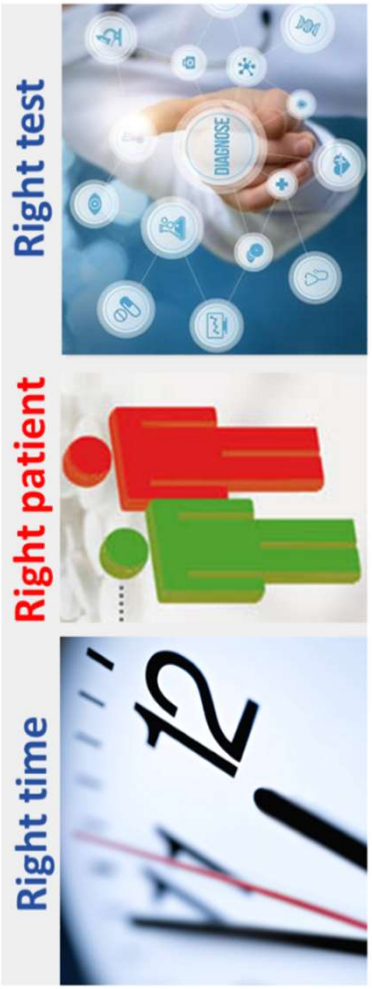


TABLE 1 Key diagnostic stewardship considerations for implementation of rapid infectious disease diagnostics

Goal	Key question	Key considerations and potential strategies
Right test	Is the test appropriate for the clinical setting?	Sensitivity and specificity Predictive values Testing volumes Diagnostic yield Laboratory feasibility Cost Clinical impact
Right patient	Will the clinical care of the patient be affected by the test result?	Laboratory test utilization committee Automatic laboratory reflex CPOE decision support Appropriate use criteria Indication selection Prior authorization Benchmarking Specimen rejection
Right time	Will the result be available in time to optimally affect care? **Λειτουργεία 24/7?**	Time to specimen receipt Centralized vs point-of-care testing On-demand vs batched testing Specimen preparation time Run time Result reporting time

Τρόπος παρουσίασης αποτελεσμάτων

Messacar K et al. J Clin Microbiol, 2017.

Μοριακή ανίχνευση: αναφορά αποτελέσματος

Reporting

"detected" or "not detected" for a specific molecular target

+

interpretative comments with therapeutic guidance

Simner PJ et al. Clin Infect Dis. 2023;76(9):1550-1558.

Organisms Detected: *Enterobacterales* *Klebsiella pneumoniae* group

Controls: Passed

Applicable Antimicrobial Resistance Genes Detected: CTX-M, NDM

Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

		Gram Negative Bacteria
	Not Detected	*Acinetobacter calcoaceticus-baumannii* complex
	Not Detected	*Bacteroides fragilis*
✓	Detected	*Enterobacterales*
	Not Detected	*Enterobacter cloacae* complex
	Not Detected	*Escherichia coli*
	Not Detected	*Klebsiella aerogenes*
	Not Detected	*Klebsiella oxytoca*
✓	Detected	*Klebsiella pneumoniae* group
	Not Detected	*Proteus* spp.
	Not Detected	*Salmonella* spp.
	Not Detected	*Serratia marcescens*
	Not Detected	*Haemophilus influenzae*
	Not Detected	*Neisseria meningitidis*
	Not Detected	*Pseudomonas aeruginosa*
	Not Detected	*Stenotrophomonas maltophilia*
		Yeast
	Not Detected	*Candida albicans*
	Not Detected	*Candida auris*
	Not Detected	*Candida glabrata*
	Not Detected	*Candida krusei*
	Not Detected	*Candida parapsilosis*
	Not Detected	*Candida tropicalis*
	Not Detected	*Cryptococcus neoformans/gattii*

Organisms Detected: *Enterobacterales* *Klebsiella pneumoniae* group

Controls: Passed

Applicable Antimicrobial Resistance Genes Detected: CTX-M, NDM

Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

		Antimicrobial Resistance Genes
✓	Detected	CTX-M
	Not Detected	IMP
	Not Detected	KPC
	Not Detected	*mcr-1*
⊘	N/A	*mecA/C*
⊘	N/A	*mecA/C* and MREJ (MRSA)
✓	Detected	NDM
	Not Detected	OXA-48-like
	N/A	*vanA/B*
	Not Detected	VIM
		Gram Positive Bacteria
	Not Detected	*Enterococcus faecalis*
	Not Detected	*Enterococcus faecium*
	Not Detected	*Listeria monocytogenes*
	Not Detected	*Staphylococcus* spp.
	Not Detected	*Staphylococcus aureus*
	Not Detected	*Staphylococcus epidermidis*
	Not Detected	*Staphylococcus lugdunensis*
	Not Detected	*Streptococcus* spp.
	Not Detected	*Streptococcus agalactiae* (Group B)
	Not Detected	*Streptococcus pneumoniae*
	Not Detected	*Streptococcus pyogenes* (Group A)

Μοριακή ανίχνευση αντοχής: σχόλια για την αναφορά των αποτελεσμάτων

Table 3. Reporting comments for molecular blood culture diagnostics. Adapted from Banerjee *et al.*[29]

Staphylococcus aureus, *mecA* detected	Probable methicillin-resistant *Staphylococcus aureus* (MRSA); further testing in progress. MRSA is predictably resistant to beta-lactam antibiotics (except ceftaroline). Patient requires contact precautions if hospitalized.
Staphylococcus aureus, *mecA* not detected	Methicillin (oxacillin)-susceptible *Staphylococcus aureus*. Preferred therapy is an anti-staphylococcal β-lactam antibiotic, unless clinically contraindicated.
Staphylococcus, coagulase-negative, *mecA* detected	Methicillin (oxacillin)-resistant coagulase-negative *Staphylococcus*. Possible blood culture contaminant (unless isolated from more than one blood culture draw or clinical case suggests pathogenicity). No antibiotic treatment is indicated for blood culture contaminants.
Enterobacter cloacae complex	This organism may contain an inducible β-lactamase. Penicillin or second- or third-generation cephalosporin monotherapy may result in emergence of high-level resistance.
Escherichia coli, $bla_{CTX\text{-}M}$ detected	Extended-spectrum β-lactamase-producer. Carbapenems are drugs of choice for ESBL-producers.
E. coli, bla_{KPC} detected	Carbapenemase producer. Patient requires contact precautions if hospitalized. This organism is resistant to carbapenems and other β-lactam antibiotics. Consult infectious diseases.

Banerjee R, Patel R. JAC Antimicrob Resist. 2023;5(1):dlad018.

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MINIREVIEW

2021;59(6):e00138-20.

The Genotype-to-Phenotype Dilemma: How Should Laboratories Approach Discordant Susceptibility Results?

Rebecca Yee,[a] Jennifer Dien Bard,[a,b] Patricia J. Simner[c]

difficult to **associate the AMR marker with a particular organism** when multiple Gram-negative organisms are identified

Potential steps and reporting structure for specific discrepant examples found in GN

GENOTYPES

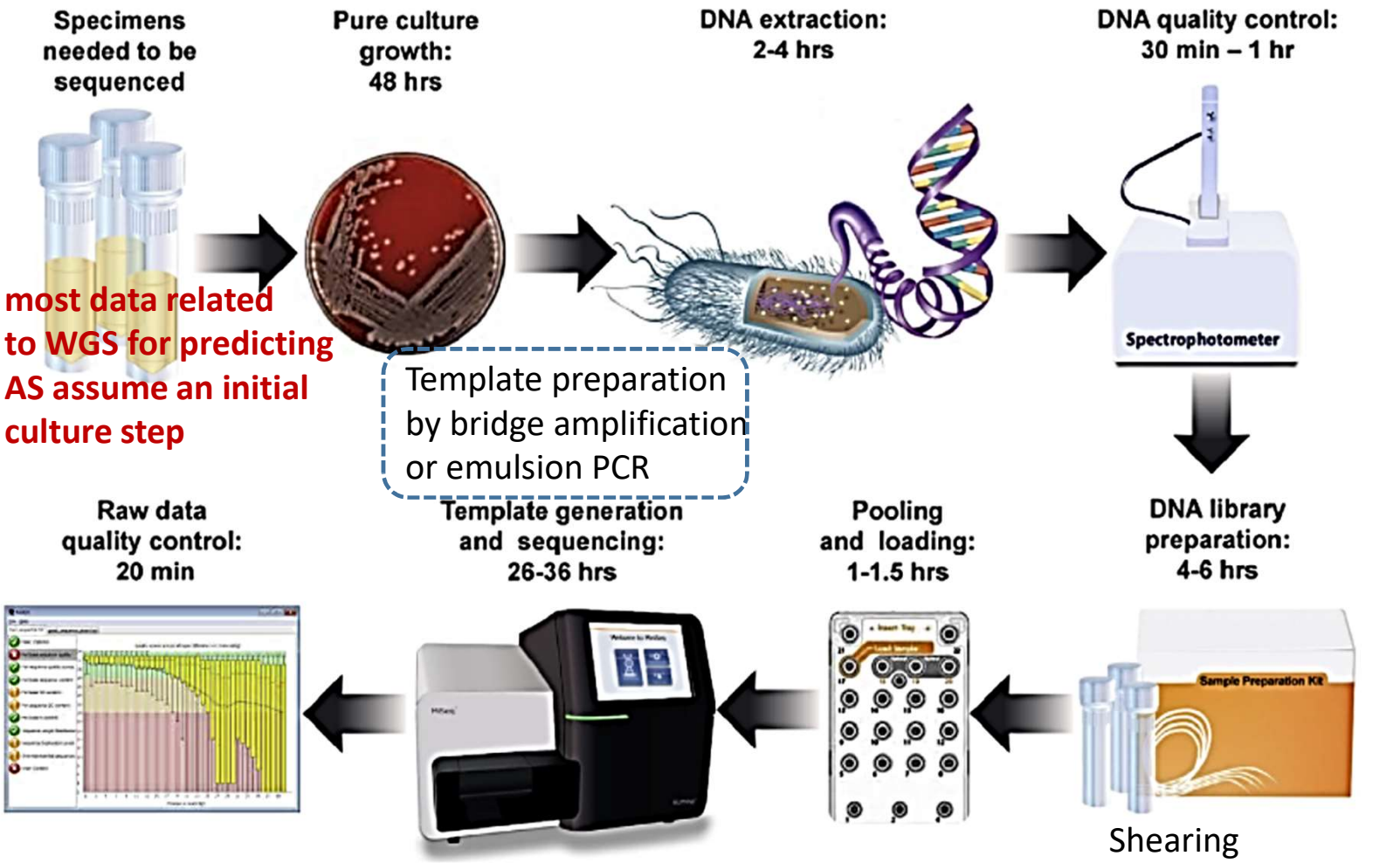
Gram-negative organism detected by NAAT

1) No ESBL or Carbapenemase Markers Detected	2) ESBL marker detected (CTX-M)	3) Carbapenemase marker detected (KPC, NDM, OXA, VIM, IMP)

POTENTIAL DISCORDANT PHENOTYPES

1) 3GC & 4GC variable or resistant +/- carbapenem variable or resistant	2) Susceptible to all 3GC & 4GC*	3) 3GC & 4GC variable or susceptible to all carbapenems*
⬇	⬇	⬇
Most likely scenarios: Off-panel AMR mechanisms (e.g., AmpC, other ESBLs, porins, carbapenemases +/- cell wall permeability defects)	Most likely scenarios: Lack or poor expression of ESBL, mixed population, heteroresistance, loss of plasmid	Most likely scenarios: Lack or poor expression of carbapenemase, mixed population, heteroresistance, loss of plasmid
⬇	⬇	⬇
	Consider the following to resolve: a. Sub the blood culture broth with a 3GC disk to identify resistant population & pursue additional testing b. Repeat discordant 3GC & 4GC AST by a reference method	Consider the following to resolve: a. Sub the blood culture broth with a carbapenem disk to identify resistant population & pursue additional testing b. Repeat discordant 3GC, 4GC and cefepime AST results; preferably by a different method and ideally a reference method
⬇	⬇	⬇
No further testing required in most instances.	Report all 3GC & 4GC as R if unresolved.	Report all 3GC, 4GC and carbapenems as R if unresolved.
⬇	*Variable resistance to 3GC & 4GC may occur depending on the hydrolysis profile of the ESBL detected	*Variable resistance to 3GC, 4GC, & carbapenems may occur depending on the hydrolysis profile of the carbapenemase detected
Report phenotypic results as tested.		

WGS: τα βήματα της διαδικασίας



most data related to WGS for predicting AS assume an initial culture step

Template preparation by bridge amplification or emulsion PCR

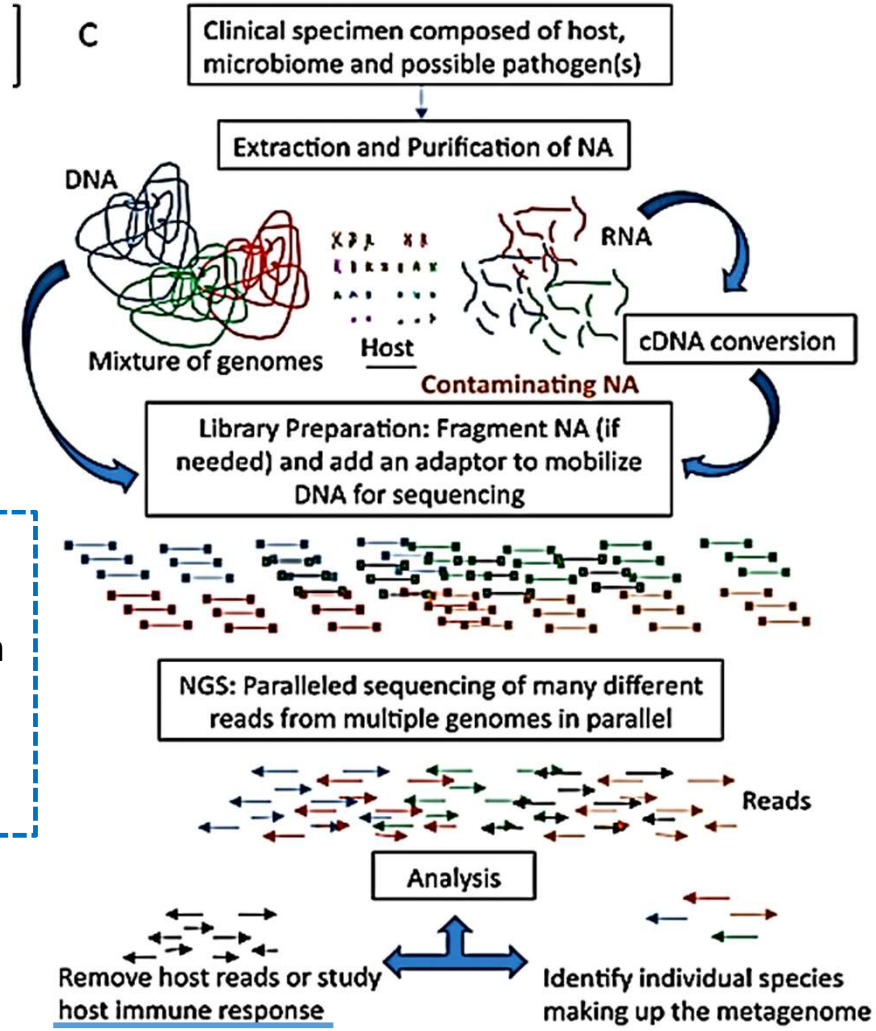
Shearing
Adaptors/Barcodes
Amplification

Besser J et al. Clin Microbiol Infect. 2018;24(4):335-341.

Metagenomic NGS (agnostic NGS): το επόμενο βήμα

hypothesis-free,
culture-independent,
pathogen detection

evaluate the immunologic
response associated
with the presence and type of infection
e.g. biomarkers
provide insight into the pathogenesis
of the microorganisms detected



Ερμηνεία αποτελεσμάτων

Simner PJ et al. Clin Infect Dis. 2018;66(5):778-788.

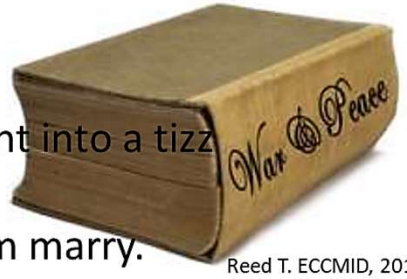
WGS: "one test fits all" approach

NAAT versus Genomic data

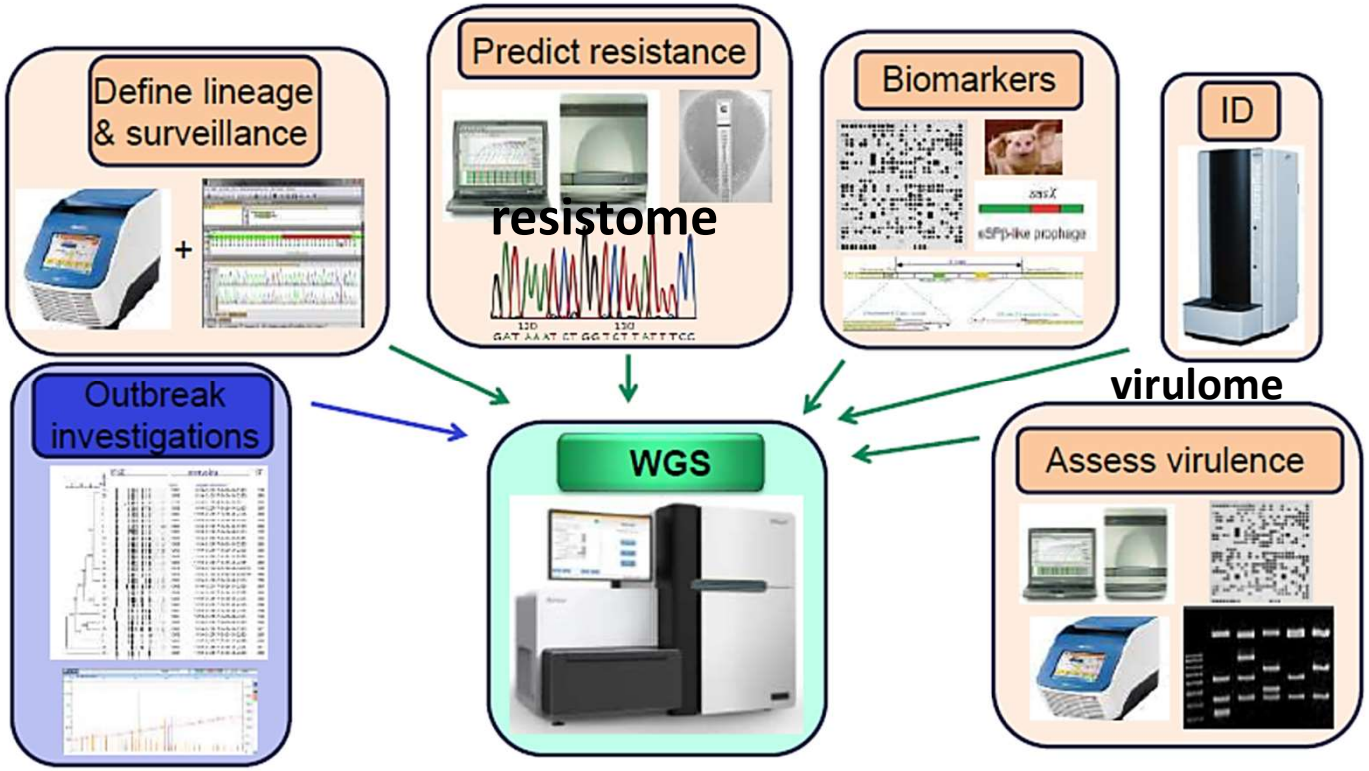
Tweet versus… **Tome**

Leo Tolstoy, War and Peace

Napoleon invades Russia.
Russian aristocratic families sent into a tizz
War ensues. French retreat.
Russians celebrate.Lots of them marry.



Reed T. ECCMID, 2016.



Woodford N. ECCMID, 2016.

frontiers | Frontiers in Microbiology
https://www.frontiersin.org/journals/microbiology
ORIGINAL RESEARCH
published: 10 June 2022
doi: 10.3389/fmicb.2022.804627

Rebelo AR et al. 2022;13:804627.

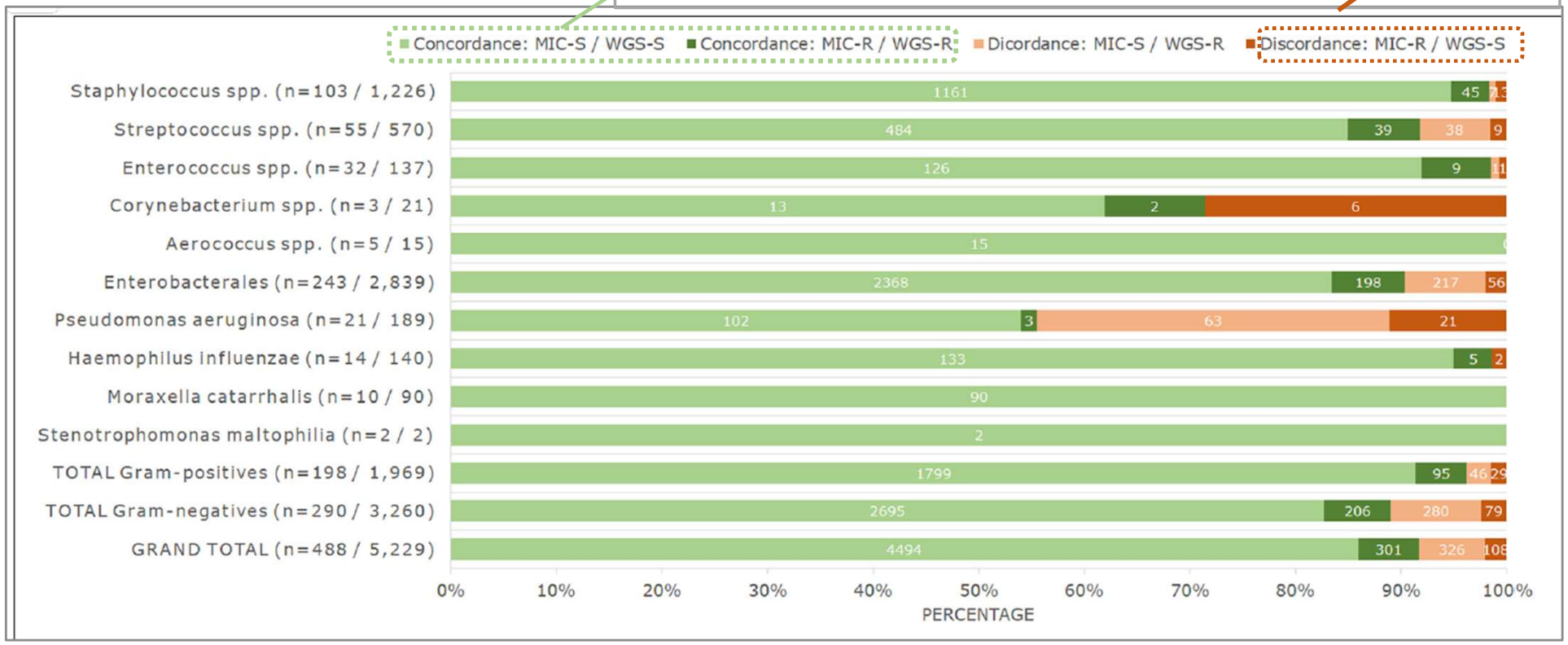
One Day in Denmark: Comparison of Phenotypic and Genotypic Antimicrobial Susceptibility Testing in Bacterial Isolates From Clinical Settings

N= 500 isolates randomly selected

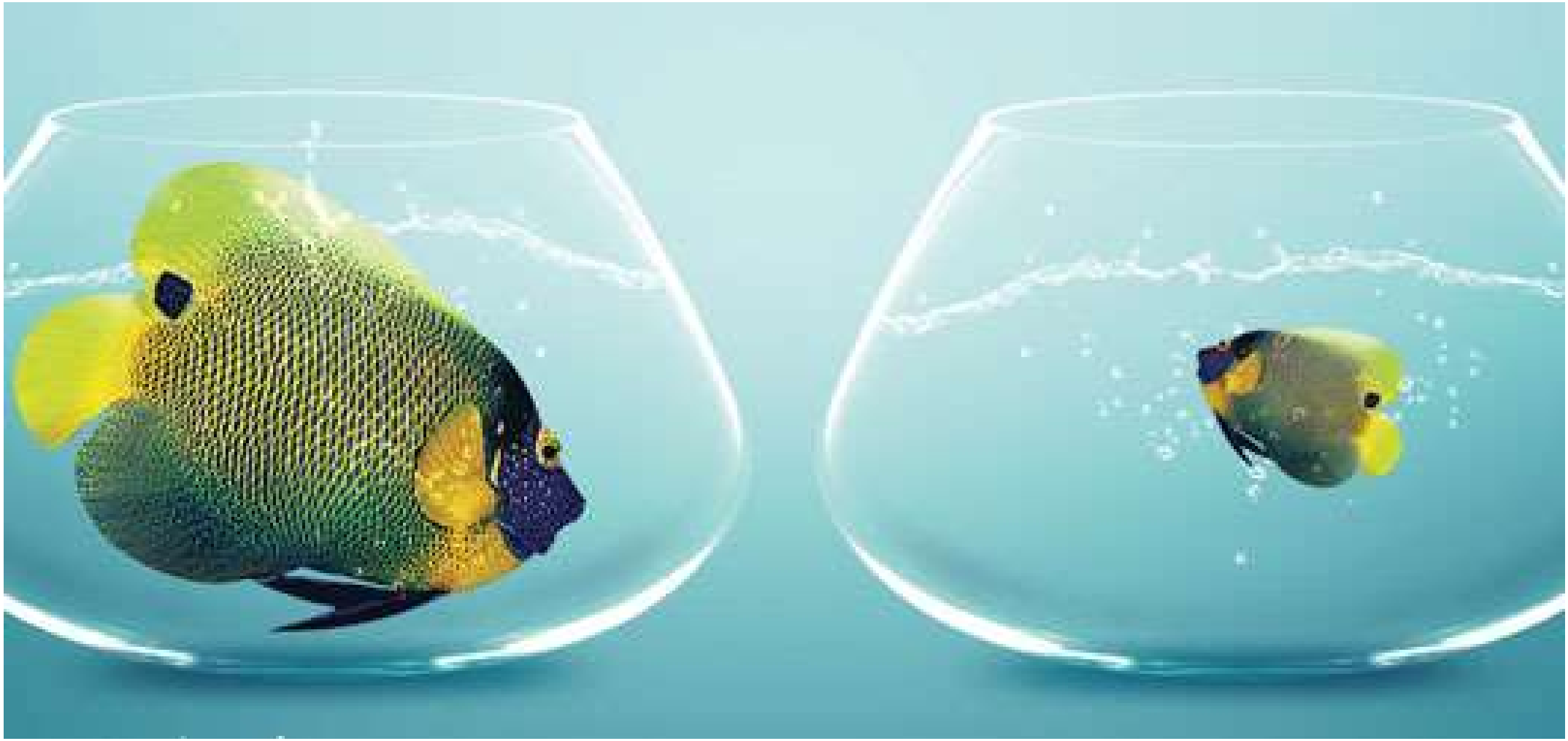
concordance of 91.7%

cases of phenotypically resistant isolates without any known genetic resistance mechanism: 2.1% of all combinations analyzed

Percentage and number of genotype–phenotype concordances and discordances observed for all bacteria analyzed in this study, when comparing MIC and WGS results.



One size does not fit all




stewardship
Noun: the activity or job of
protecting
and being responsible
for something


Our time with antibiotics
is running out.
CHANGE CAN'T WAIT
HANDLE
ANTIBIOTICS
WITH CARE


THE FUTURE OF
ANTIBIOTICS
DEPENDS ON ALL OF US

World AMR
Awareness
Week 2025
18-24 Nov

WAAW 2025 theme

Act Now: Protect Our Present, Secure Our Future

Food and Agriculture Organization of the United Nations
UN environment programme
World Health Organization
World Organisation for Animal Health