

# Antibiogramma fenotipico (rapido e non): criticità

Corso Precongressuale D  
ANTIBIOGRAMMA 2024: “QUO VADIS?”

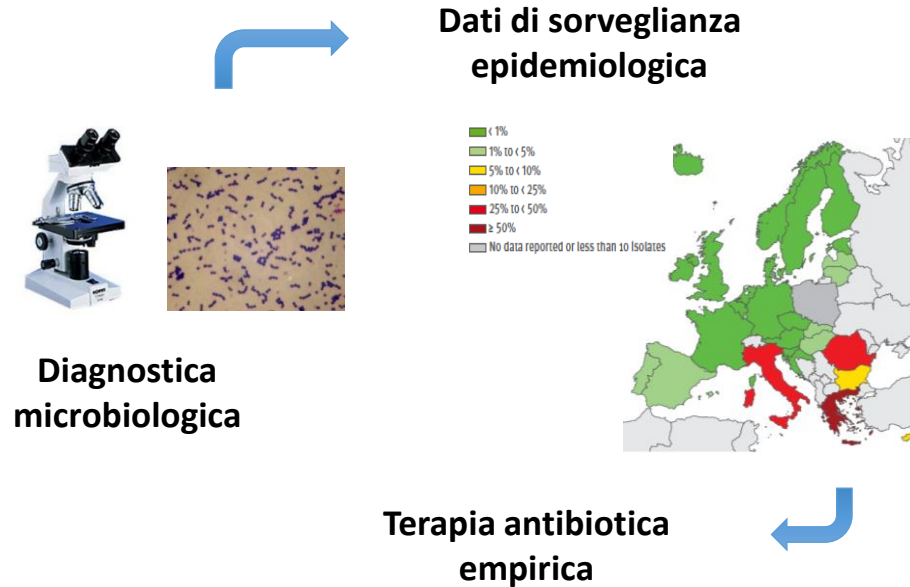
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Milano**

# Saggi di sensibilità in Microbiologia clinica

## Quale ruolo?

- Scelta della terapia antibiotica mirata
- Epidemiologia delle resistenze (locale/nazionale) e loro evoluzione
- Sorveglianza mirata ad interventi di infection control (ceppi MDR/XDR ad alto rischio epidemico)



### Quale antibiotico?

Staphylococcus aureus

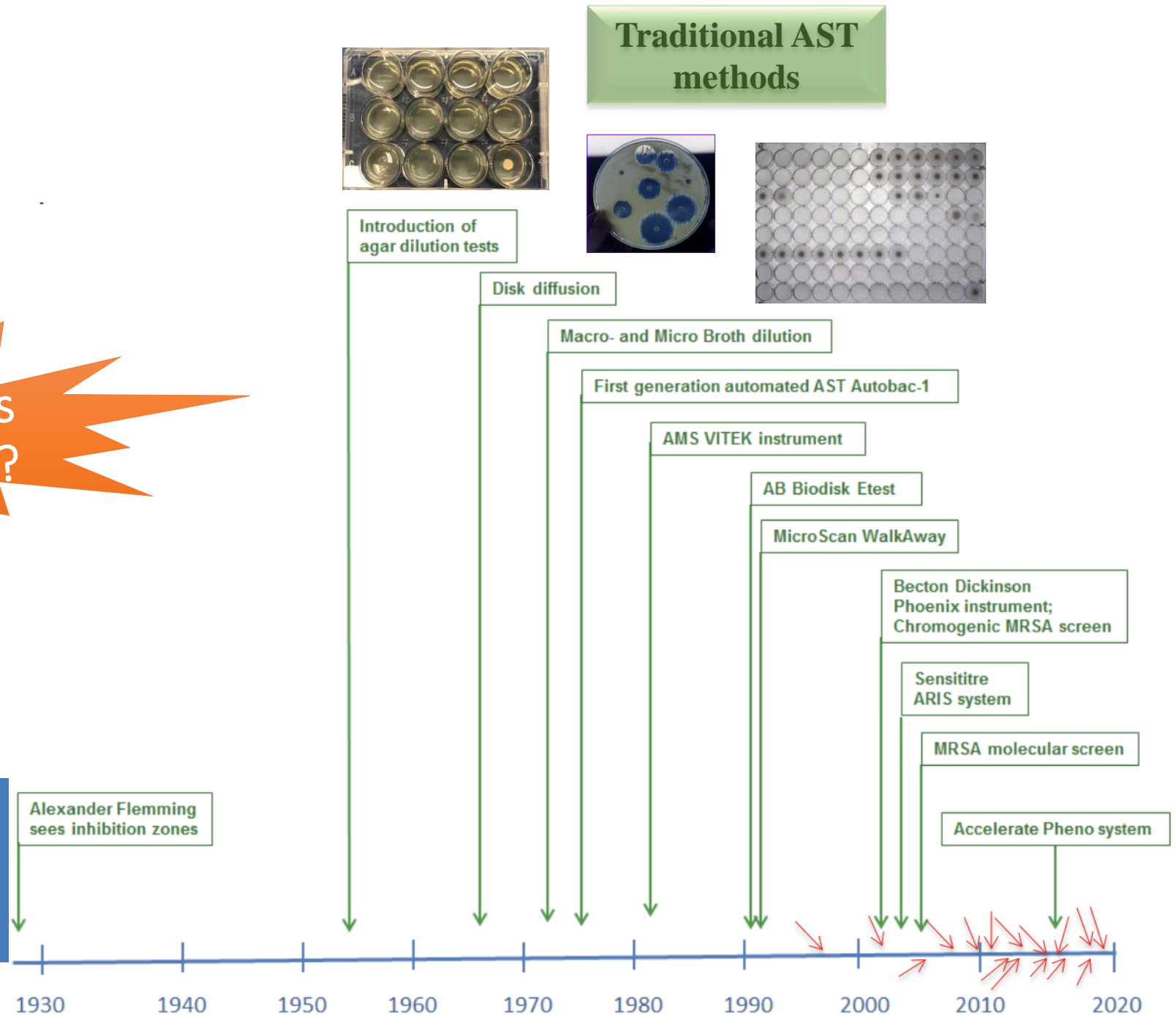
Antibiotico	MIC mg/L (S/I/R)
Cefoxitin screen	POS
Oxacillina	>4 R
Eritromicina	>2 R
Clindamicina	>0.5 R
Daptomicina	0.5 S
Linezolid	2 S
Vancomicina	1 S
Teicoplanina	1 S
Amikacina	>1 R
Gentamicina	>1 R
Levofloxacina	>2 R
Tigeciclina	≤0.12 S

Terapia antibiotica mirata

Routinely used  
traditional and  
automated  
phenotypic  
methods

Which antibiotic is  
most appropriate?

Culture-based strategies remain  
the basis of diagnostic  
microbiology



# ANTIBIOGRAMMA FENOTIPICO

**Standardizzazione**

Test standardizzati

**Inoculo sensibilità**

Test eseguito su colonia

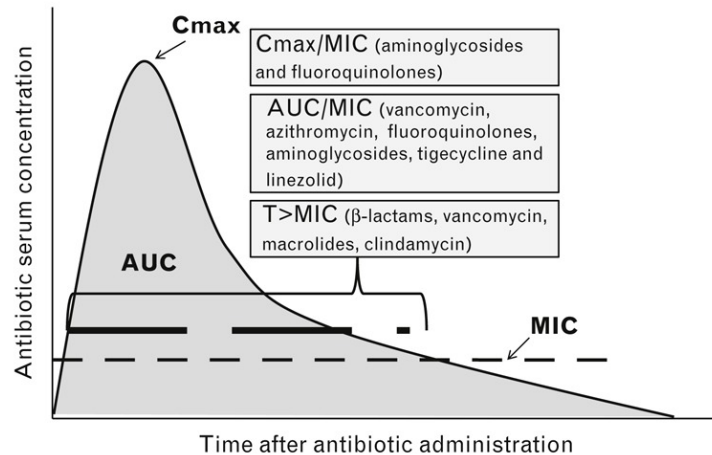
**Tipo di Risultato**

Quantitativo

Breakpoint clinici definiti



**MIC: Minimum Inhibitory Concentration**



Attività e dosaggi di farmaci tarati su MIC

Classe A  
(serina)

TEM  
SHV  
CTX-M

Classe C

AmpC

ESBL/AmpC + perdita di porine

Resistenza a bassi livelli

# ANTIBIOGRAMMA FENOTIPICO

Standardizzazione	Test standardizzati
Inoculo sensibilità	Test eseguito su colonia
Tipo di Risultato	Quantitativo
Resistenze multifattoriali	Si

Antimicrobial <sup>a</sup>	PIP-TZ	CAZ	FEP	TOL-TZ	ATM	IMP	MER	FQ	AMG	COL	FOS	MIC (mg/L)	MPC (mg/L)	Primary R MEC	Secondary R MEC
PIP/TZ	+++	+++	++	-	++	-	+	-/+	-	-	-	2	>32	↑ AmpC	↑ MexAB
CAZ	+++	+++	++	-/+	++	-	+	-/+	-	-	-	1	>32	↑ AmpC	↑ MexAB
FEP	++	++	+++	-/+	+++	-	++	+	+	-	-	1	>32	↑ MexAB/XY	↑ AmpC
TOL/TZ	-/+	+	+	+	-/+	-	-/+	-	-	-	-	0.5	2	AmpC+mut AmpC	PBP3
ATM	++	++	+++	-/+	+++	-	++	+	-	-	-	4	>32	↑ MexAB/XY	↑ AmpC
IMP	-/+	-/+	-/+	-	-/+	+++	++	-/+	-	-	-	1	>32	OprD	MexST (↑ MexEF ↓ OprD)
MER	+	+	+	-	+	++	++	+	-	-	-	0.5	8	OprD	↑ MexAB, PBP3
FQ <sup>b</sup>	+	+	++	-	++	-/+	+	+++	+	-	-	0.12	2	QRDR	↑ MexAB/XY/CD/EF
AMG <sup>c</sup>	-	-	+	-	-	-	-	+	++	-	-	1	8	↑ MexXY	FusA
COL	-	-	-/+	-	-	-/+	-/+	-/+		+	-	0.5	2	pmrAB/phoPQ	parRS
FOS	-	-	-	-	-	-	-	-	-	-	++++	64	>1,024	GlpT	

## *P.aeruginosa*

Hyperproduction of inducible AmpC chromosomal cephalosporinase  
 Repression or inactivation of the OprD porine  
 Hyperexpression of multiple efflux pumps  
 Modification of lipopolysaccharide mediated by mutations

# ANTIBIOGRAMMA FENOTIPICO

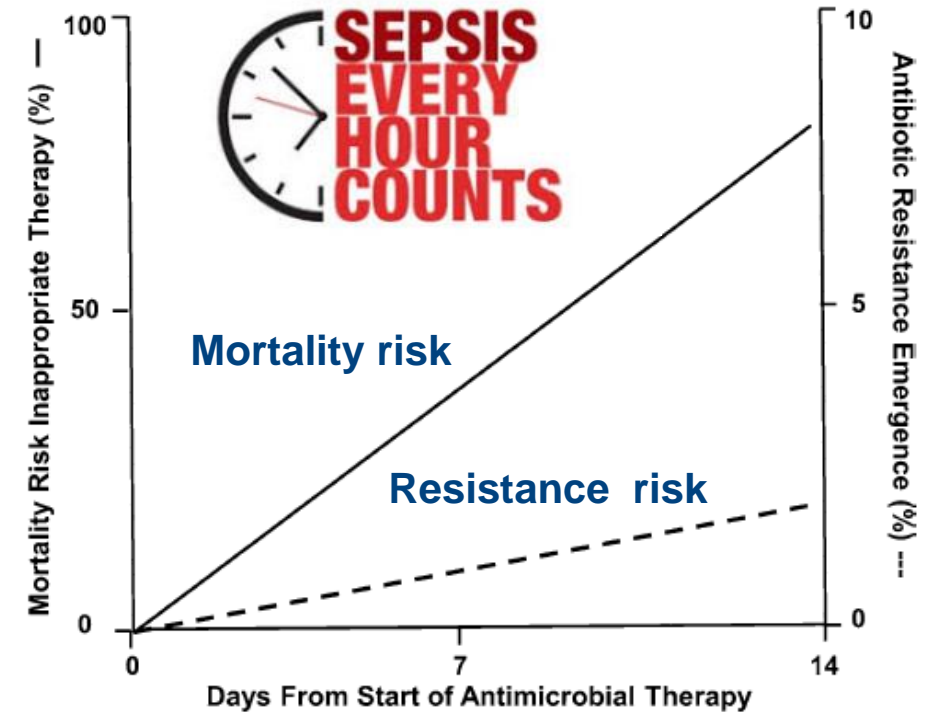
<b>Standardizzazione</b>	Test standardizzati
<b>Inoculo sensibilità</b>	Test eseguito su colonia
<b>Tipo di Risultato</b>	Quantitativo
<b>Resistenze multifattoriali</b>	Si
<b>Target</b>	Pannello ampio
<b>Nuovi Meccanismi</b>	Si

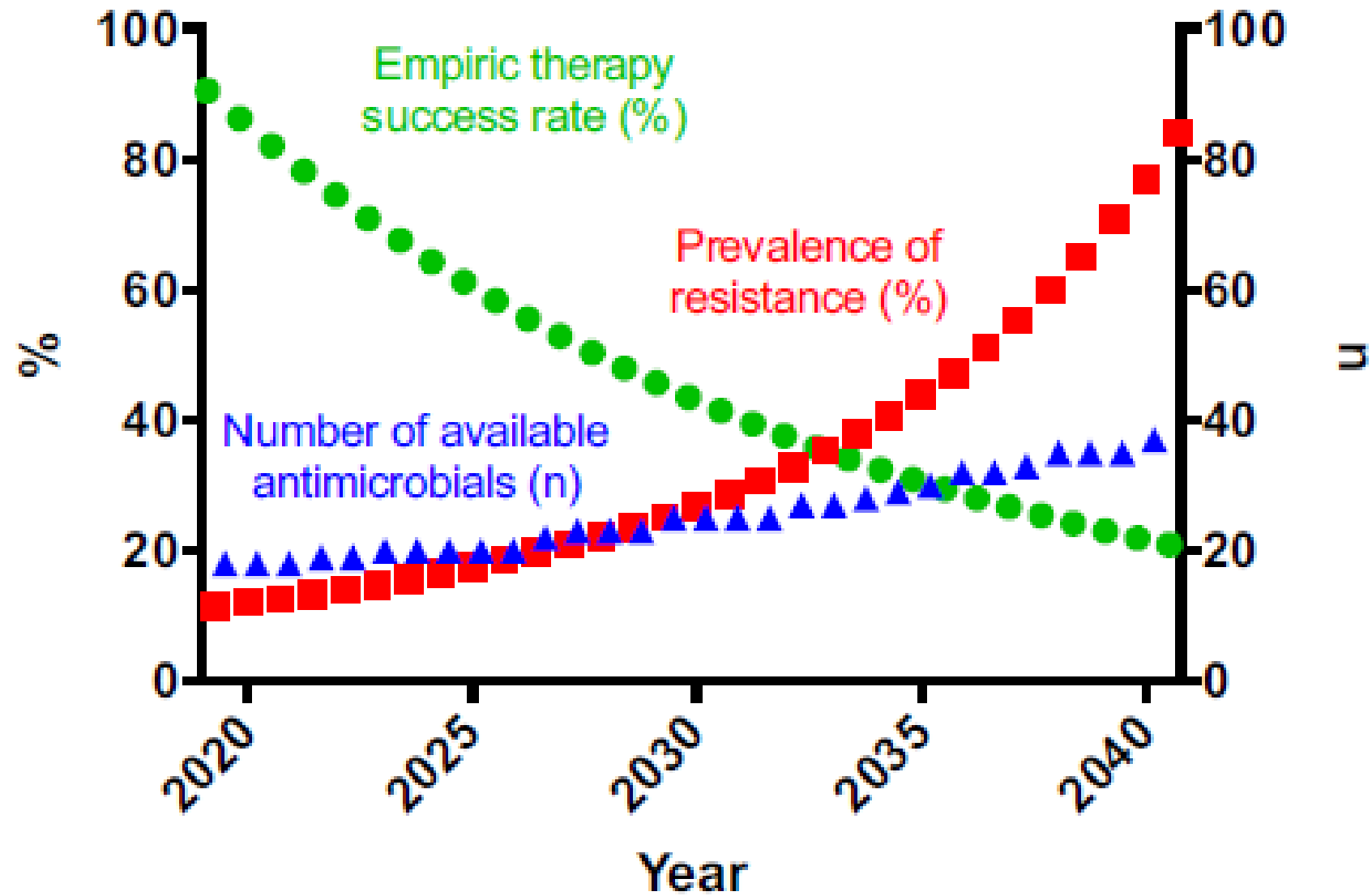


# Appropriate therapy: impact of broad spectrum antimicrobials and delayed administration

Delaying appropriate antibiotic therapy increases the risk of death

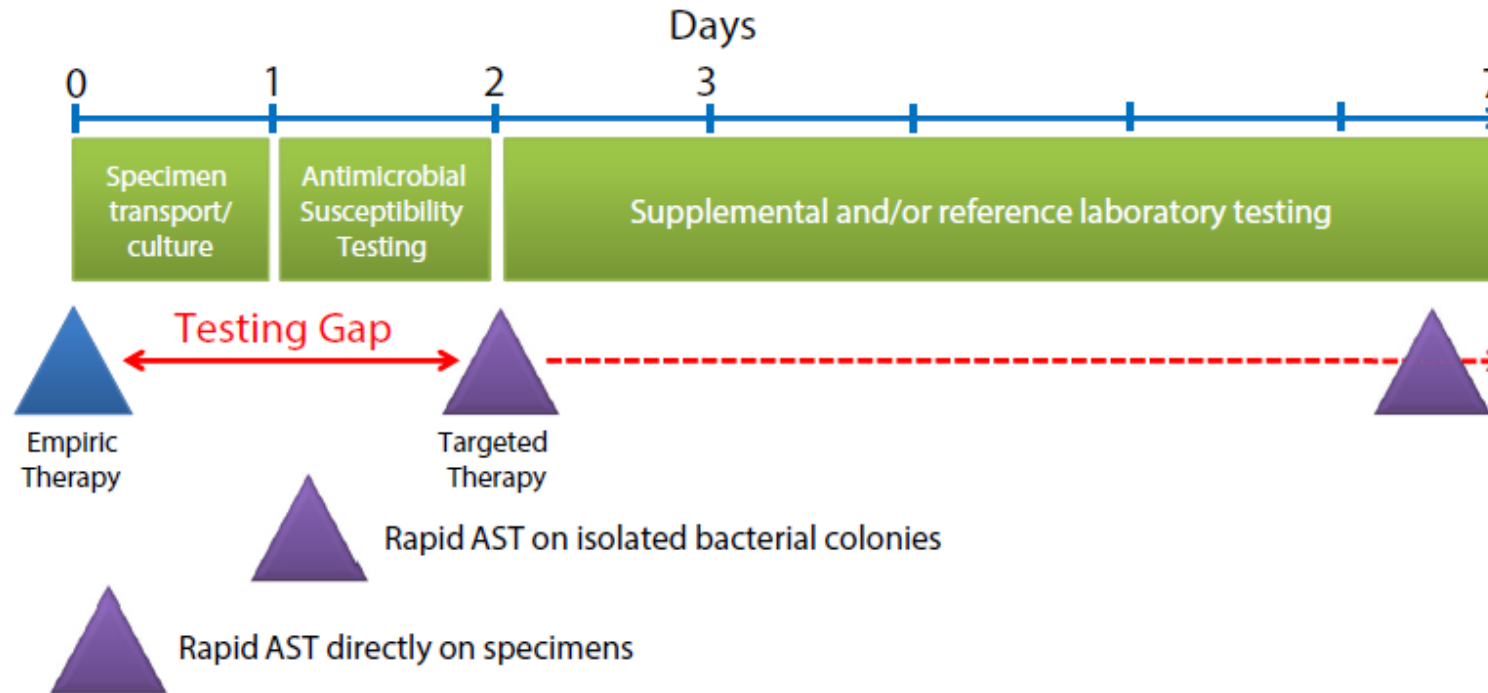
The risk of antibiotic resistance increases as the duration of antibiotic therapy is prolonged







# ANTIBIOGRAMMA RAPIDO



- ✓ Diminuire l'intervallo di refertazione dell'antibiogramma, ovvero il tempo che intercorre dalla raccolta dei campioni microbiologici all'identificazione e il tempo necessario per l'antibiogramma, è fondamentale per supportare la gestione antimicrobica e per fornire un trattamento efficace e mirato.

# ANTIBIOGRAMMA RAPIDO

```
graph TD; A[ANTIBIOGRAMMA RAPIDO] --> B[FENOTIPICO<br/>indicazione della<br/>sensibilità/resistenza con<br/>determinazione accurata<br/>della MIC]; A --> C[GENOTIPICO<br/>rilevamento del<br/>meccanismo AMR]; B --> D[RAST tradizionali]; B --> E[RAST automatizzati];
```

The diagram is a flowchart titled 'ANTIBIOGRAMMA RAPIDO'. It branches into two main categories: 'FENOTIPICO' and 'GENOTIPICO'. 'FENOTIPICO' further branches into 'RAST tradizionali' and 'RAST automatizzati'. 'GENOTIPICO' leads to 'rilevamento del meccanismo AMR'. The flow is indicated by blue arrows. The background features abstract geometric shapes in light blue and yellow.

## FENOTIPICO

indicazione della  
sensibilità/resistenza con  
determinazione accurata  
della MIC

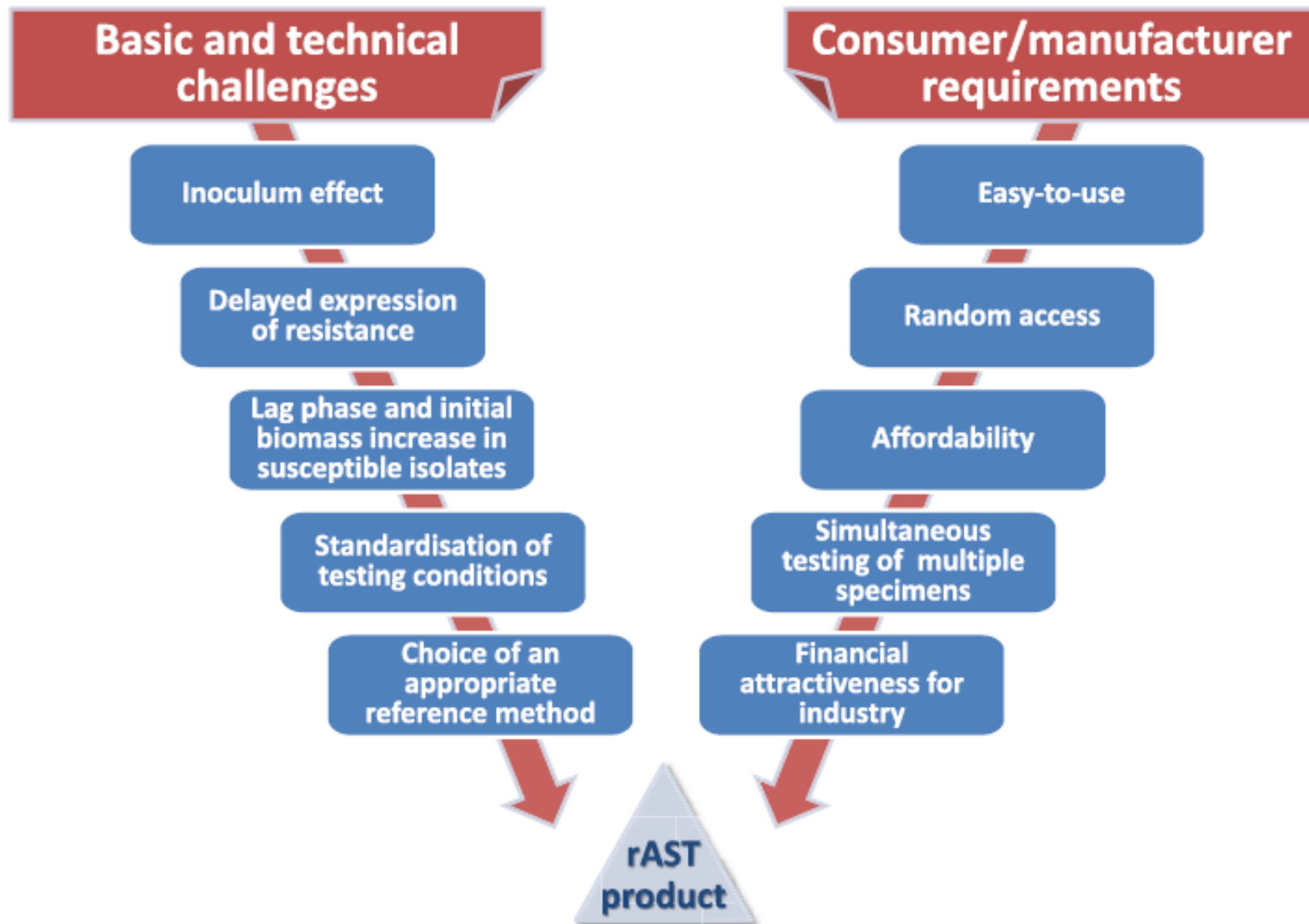
## GENOTIPICO

rilevamento del  
meccanismo AMR

**RAST tradizionali**

**RAST automatizzati**

# Antibiogramma fenotipico rapido e non: sfide e criticità

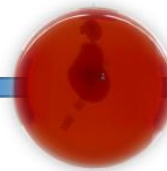


# Antibiogramma fenotipico rapido

Accettazione campione	Coltura positiva	Colonia	
Incubazione/Semina	Lettura	ID /AST	AST
0 h	6-120 h	3-6 h	24-48-72 h



6-120 h



Terapia empirica

Aggiustamento ter. empirica

Referto preliminare da colonia  
(anche patina 3 h per emocolture)

- ID Maldi-Tof
- AST rapido fenotipico




Referto definitivo da colonia  
Antibiogramma fenotipico tradizionale

Terapia mirata

# NUOVE TECNOLOGIE: impatto sul workflow delle emocolture







EMOCOLTURA  
POSITIVA

TEST	INFORMAZIONE	TEMPISTICA
	Gram	Morfologia
	ID (Maldi-Tof) da flacone	ID
	ID (Maldi-Tof) Test rapidi per R AST diretto da colonia	ID AST
		10 minuti
		30 minuti
		3 ore 3 ore 12 ore

# NUOVE TECNOLOGIE: impatto sul workflow delle emocolture



EMOCOLTURA  
POSITIVA

TEST	INFORMAZIONE	TEMPISTICA
	Gram	Morfologia
	ID (Maldi-Tof) da flacone	ID
	ID (Maldi-Tof) Test rapidi per R AST diretto da colonia	ID AST
	Rapid AST da flacone	AST

# RAST mediante disco-diffusione da emocoltura positiva (RAST EUCAST)

## Rapid AST in bloodcultures

Organization

EUCAST News

New definitions of S, I and R

Clinical breakpoints and dosing

Rapid AST in blood cultures

Methods

Breakpoints for short incubation

Expert rules and intrinsic resistance

Resistance mechanisms

Guidance documents

Consultations - New!

MIC and zone distributions and ECOFFs

AST of bacteria

AST of mycobacteria



## Rapid AST directly from blood culture bottles

EUCAST has published recommendations for short incubation (4, 6 and 8 hours) AST directly from positive blood culture bottles:

- direct inoculation of disk diffusion plates (MH, MH-F) using 100 - 150 µL directly from a positive blood culture bottle (BD, bioMérieux and Thermo Fisher).
- no centrifugation or dilution of the inoculum - streak plates as for standard EUCAST disk diffusion.
- shortened incubation - 4, 6 and 8 hours with breakpoints adapted to each incubation time.
- zone diameters are read from the front of the plate after removal of the lid.
- breakpoints for each species and each reading time.
- identity of species must be known prior to interpretation of AST results.

## Rapid AST directly from positive blood culture bottles

- ✓ EUCAST has validated a method for **direct** plating of disk diffusion MH and MHF agar plates for reading after **4, 6 and 8 hours of incubation**
- ✓ Incubation can not be prolonged - the method is only validated for the **short incubation time**.
- ✓ Currently the method is **validated** for:
  - E. coli*
  - K.pneumoniae*
  - Ps. aeruginosa*
  - S. aureus*
  - E. faecalis and faecium*
  - S.pneumoniae*and for a limited panel of antimicrobials.



### **Preparation of blood culture bottles**

The EUCAST RAST method has been validated using blood culture bottles for BACTEC (Becton Dickinson), BacT/ALERT (bioMérieux) and VersaTREK (Thermo Fisher). The RAST method can be performed 0 – 18 hours after blood culture bottles have signalled positive. Do not remove positive bottles from the blood culture instrument until you are ready to proceed with the RAST. However, to allow for transport of positive bottles from one site to another, we have evaluated the impact of keeping bottles at room temperature after having removed them from the instrument. RAST results were not affected by a “delay” of up to 3 hours.

- Metodo da eseguire **entro 18 ore dalla positivizzazione del flacone**
- **Entro 3 ore dallo scarico del flacone dall'incubatore**

### **Inoculation of agar plates directly from blood culture bottles**

Take 125±25 µl of undiluted blood culture broth from the positive blood culture bottle to each 90-mm circular MH/MH-F agar plate. Spread the broth gently over the agar surface by swabbing in three directions or using an automatic plate rotator and apply disks as for standard AST.

### **Incubation and reading of plates**

Incubate plates as described in Table 1. Read inhibition zones at ± 5 minutes of the stated reading time (4, 6 and/or 8 hours). If needed, re-incubate the plates within 10 minutes to enable reading at a later time (6 and/or 8 hours). Do not incubate or read plates beyond 8 hours.

- Prelevare **125 +/- 25 microlitri di brodo di emocoltura positiva non diluita**
- Seminare su **piastra di MH o MH-F da 90 mm** coprendo tutta la superficie (semina manuale o automatica)
- Applicare i dischetti di antibiotico
- **Incubare e leggere la crescita a 4-6-8 ore (più o meno 5 minuti dall'orario previsto)**
- **Reincubare entro 10 minuti per la lettura successiva**



# 2023: EUCAST RAST IN BLOOD CULTURES

EUCAST RAST Breakpoint Tables version 6.1 (2023-06-07)

European Committee on Antimicrobial Susceptibility Testing

## Zone diameter breakpoint tables for rapid antimicrobial susceptibility testing (RAST) directly from blood culture bottles

Version 6.1, valid from 2023-06-07

This document should be cited as "The European Committee on Antimicrobial Susceptibility Testing. Zone diameter Breakpoint Tables for rapid antimicrobial susceptibility testing (RAST) directly from blood culture bottles. Version 6.1, 2023. <http://www.eucast.org>."

**Table 1.** Incubation conditions for antimicrobial susceptibility test plates.

Organism	Incubation time	Medium	Incubation
<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Staphylococcus aureus</i>	4, 6 and 8 hours 16-20 hours	MH	35±1°C in air
<i>Pseudomonas aeruginosa</i>	6 and 8 hours 16-20 hours	MH	35±1°C in air
<i>Acinetobacter baumannii</i> <i>Enterococcus faecalis</i> <i>Enterococcus faecium</i>	4, 6 and 8 hours	MH	35±1°C in air
<i>Streptococcus pneumoniae</i>	4, 6 and 8 hours 16-20 hours	MH-F	35±1°C in 4-6% CO <sub>2</sub> in air

Content	Page	Additional information
Changes	1	
Notes	3	
Guidance on reading EUCAST RAST Breakpoint Tables	5	
Information on technical uncertainty	6	
<i>Escherichia coli</i>	7	Breakpoints for 4, 6, 8 and 16-20 h
<i>Klebsiella pneumoniae</i>	8	Breakpoints for 4, 6, 8 and 16-20 h
<i>Pseudomonas aeruginosa</i>	9	Breakpoints for 6, 8 and 16-20 h
<i>Acinetobacter baumannii</i>	10	Breakpoints for 4, 6, 8 and 16-20 h
<i>Staphylococcus aureus</i>	11	Breakpoints for 4, 6, 8 and 16-20 h
<i>Enterococcus faecalis</i>	12	Breakpoints for 4, 6, 8 and 16-20 h
<i>Enterococcus faecium</i>	13	Breakpoints for 4, 6, 8 and 16-20 h
<i>Streptococcus pneumoniae</i>	14	Breakpoints for 4, 6, 8 and 16-20 h

- The EUCAST RAST method is based on the EUCAST standard disk diffusion methodology, but with modified inoculum, incubation time, modified reading instructions and specific RAST breakpoints. The purpose of the EUCAST RAST method is to allow rapid susceptibility test results directly from positive blood cultures.
- The RAST method provides specific breakpoints for readings **at 4, 6 and/or 8 hours** incubation.
- In addition, RAST breakpoints for 16-20 hours incubation have been developed for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*. Results should only be read after 16-20 hours when it is not possible to read results after 4, 6 and/or 8 hours incubation. Isolates for which results are inside the ATU at all reading times must be tested with the standard methodology and breakpoints**

## *Klebsiella pneumoniae*

Zone diameter breakpoints for RAST directly from blood culture bottles

EUCAST RAST Breakpoint Tables v. 6.1, valid from 2023-06-07

# 2023: EUCAST RAST IN BLOOD CULTURES

EUCAST rapid disk diffusion method directly from positive blood culture bottles

Medium: Mueller-Hinton (MH) agar

Inoculum: 125±25 µL directly from a positive blood culture bottle

Incubation: Air, 35±1°C

Incubation time: 4, 6, 8 and 16-20 hours

General reading instructions: Inhibition zones should only be read when the growth is confluent and zone edges are clearly visible.

Reading 4, 6 and 8 hours: Remove the lid and read zone diameters from the front against a dark background illuminated with reflected light.

Reading 16-20 hours: Read zone diameters from the back of the plate against a dark background illuminated with reflected light.

[QC for implementation of RAST](#)

Breakpoints are valid for *K. pneumoniae*, *K. variicola* and *K. quasipneumoniae*.

Antimicrobial agent	Disk content (µg)	4 hours			6 hours			8 hours			16-20 hours		
		S ≥	ATU	R <	S ≥	ATU	R <	S ≥	ATU	R <	S ≥	ATU	R <
Amoxicillin-clavulanic acid	20-10	15	13-14	13	16	14-15	14	16	14-15	14	18	16-17	16
Piperacillin-tazobactam	30-6	15	13-14	13	16	14-15	14	16	14-15	14	17	15-16	15
Temocillin	30	50	14	14	50	15	15	50	16	16	50	16	16
Cefotaxime <sup>1</sup>	5	15	12-14	12	18	15-17	15	18	15-17	15	16	14-15	14
Ceftazidime <sup>1</sup>	10	15	13-14	13	16	14-15	14	16	14-15	14	18	15-17	15
Ceftazidime-avibactam	10-4	12	10-11	10	13	11-12	11	13	11-12	11	14	12-13	12
Ceftolozane-tazobactam	30-10	16	14-15	14	16	14-15	14	17	15-16	15	20	17-19	17
Imipenem <sup>2</sup>	10	16	14-15	14	17	15-16	15	17	15-16	15	15	12-14	12
Imipenem-relebactam	10-25	15	13-14	13	15	14	14	15	14	14	17	15-16	15
Meropenem <sup>2</sup>	10	15	13-14	13	17	15-16	15	17	15-16	15	15	13-14	13
Meropenem-vaborbactam	20-10	16	14-15	14	17	16	16	17	16	16	15	13-14	13
Ciprofloxacin	5	17	15-16	15	18	16-17	16	18	16-17	16	19	17-18	17
Levofloxacin	5	17	14-16	14	18	15-17	15	18	15-17	15	18	15-17	15
Amikacin <sup>3</sup>	30	(15)	(13-14)	(13)	(14)	(12-13)	(12)	(14)	(12-13)	(12)	(15)	(13-14)	(13)
Gentamicin <sup>3</sup>	10	(14)	(12-13)	(12)	(14)	(12-13)	(12)	(13)	(11-12)	(11)	(14)	(13)	(13)
Tobramycin <sup>3</sup>	10	(14)	(12-13)	(12)	(13)	(11-12)	(11)	(13)	(11-12)	(11)	(14)	(13)	(13)
Trimethoprim-sulfamethoxazole	1.25-23.75	11	9-10	9	11	9-10	9	11	9-10	9	10	8-9	8

### Notes

1. Cephalosporin breakpoints for *K. pneumoniae* will detect all clinically important resistance mechanisms. The presence or absence of an ESBL does not in itself influence the categorisation of susceptibility. However, ESBL detection and characterisation are recommended for public health and infection control purposes.

See document [EUCAST RAST screening for resistance mechanisms \(link to document\)](#) for screening cut-offs.

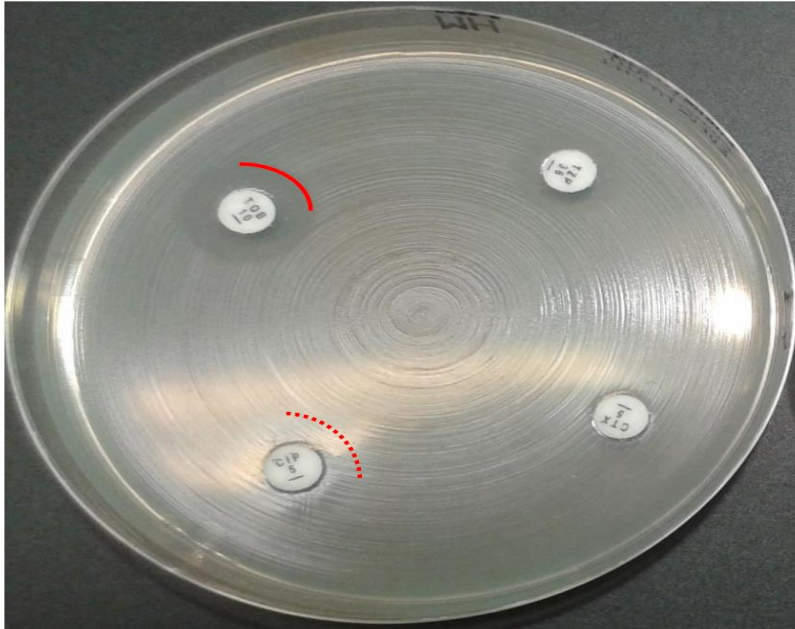
2. Carbapenem breakpoints for *K. pneumoniae* will detect all clinically important resistance mechanisms. The presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility. However, carbapenemase detection and characterisation are recommended for public health and infection control purposes.

See document [EUCAST RAST screening for resistance mechanisms \(link to document\)](#) for screening cut-offs.

3. Aminoglycoside breakpoints distinguish between isolates without and with resistance mechanisms. For blood stream infections, EUCAST recommends that aminoglycosides are used in combination with other active therapy.

### Examination of plates after incubation

With an incubation time of 4-8 hours, the growth on the Muller-Hinton agar plate will often appear less distinct than with standard incubation time (16-20 hours). **Inhibition zones should only be read when the growth is confluent and zone edges are clearly visible, see example in Figure 1.**



**Figure 1.** *E. coli* after 4 hours incubation. Zones with a clearly visible zone edge should be read (solid line) and zones with no clear zone should not be read (dotted line).

### Measurement of zone diameters and interpretation of susceptibility

Read both MH and MH-F plates **manually from the front of the plate with the lid removed** and with reflected light. Read MH plates against a dark background and MH-F plates against a light background. Hold the plate about 30 cm from the eye at a 45 degree angle. Angle the plate to identify sharp zone edges. Measure inhibition zone diameters to the nearest millimetre. Thin growth within an inhibition zone with a clear zone edge should be ignored. This occasionally occurs at early reading for *E. coli* and *K. pneumoniae* and most often for  $\beta$ -lactam antibiotics. Sometimes there is no evident inhibition zone after 4 hours but a zone diameter can easily be measured after 6 hours (Table 2). It is not always possible to read inhibition zones for all tested antibiotic agents.

- A 4-8 ore crescita può apparire meno chiara rispetto al tempo di incubazione standard
- **Le zone di inibizione devono essere lette quando la crescita è confluenta e i bordi degli aloni chiaramente visibili**
- La lettura dei diametri deve essere eseguita manualmente dopo aver rimosso il coperchio, tenendo la piastra a 30 cm dagli occhi con una angolazione di 45°.
- **La crescita fine all'interno di una zona di inibizione evidente deve essere ignorata** (si verifica occasionalmente con i beta-lattamici per *E.coli* e *K.pneumoniae*)



## Screening for ESBL and carbapenemases in *E. coli* and *K. pneumoniae* for epidemiological purposes as part of the RAST procedure.

EUCAST Guidelines for detection of resistance mechanisms and specific resistance of clinical and/or epidemiological importance using EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles.

Version 2.0  
April 2022

### 2. Extended-spectrum $\beta$ -lactamase (ESBL)-producing *E. coli* and *K. pneumoniae*

- With the RAST method, ESBL production in *E. coli* and *K. pneumoniae* can be detected by using cefotaxime and ceftazidime screening cut-off values at 4, 6, 8 and 16-20 hours.
- Test both cefotaxime and ceftazidime.
- Screen-positive organisms (for cefotaxime and/or ceftazidime) should be subjected to ordinary confirmatory and typing procedures.

**Table 1. Screening cut-off values (mm) for ESBL-producing *E. coli* and *K. pneumoniae*.**

Species	Antimicrobial agent	Conduct ESBL testing if			
		4 hours	6 hours	8 hours	16-20 hours
<i>E. coli</i>	Cefotaxime 5 $\mu$ g	<15	<16	<17	<16
	Ceftazidime 10 $\mu$ g	<15	<16	<17	<17
<i>K. pneumoniae</i>	Cefotaxime 5 $\mu$ g	<15	<18	<18	<16
	Ceftazidime 10 $\mu$ g	<15	<16	<16	<18

### 3. Carbapenemase-producing *E. coli* and *K. pneumoniae*

- With the EUCAST RAST method carbapenemase-producing *E. coli* and *K. pneumoniae* can be detected by using meropenem screening cut-off values at 6, 8 and 16-20 hours. There is no screening cut-off value for 4 hours incubation.
- Screen-positive organisms should be subjected to ordinary confirmatory and typing procedures.

**Table 2. Screening cut-off values (mm) for carbapenemase-producing *E. coli* and *K. pneumoniae*.**

Species	Antimicrobial agent	Screening cut-off			
		4 hours	6 hours	8 hours	16-20 hours
<i>E. coli</i>	Meropenem 10 $\mu$ g	-	<20	<20	<20
<i>K. pneumoniae</i>	Meropenem 10 $\mu$ g	-	<18	<18	<20

## Impact of EUCAST rapid antimicrobial susceptibility testing (RAST) on management of Gram-negative bloodstream infection



Emilie Cardot Martin<sup>a,\*</sup>, Marie Alice Colombier<sup>b</sup>, Lucie Limousin<sup>a</sup>, Orianne Daude<sup>a</sup>, Oscar Izarn<sup>a</sup>, Pierre Cahen<sup>a</sup>, Eric Farfour<sup>a</sup>, Philippe Lesprit<sup>c</sup>, Marc Vasse<sup>a</sup>

	RAST	Control group	p
N episodes	61	49	
N Effective antibiotic therapy on blood bottle sampling day (day 0) (%)	39 (64)	34 (69)	0,69
N antibiotic regimen modification after positive blood culture and gram staining result (day 1) (%)	22 (36)	13 (27)	0,31
N Bacterial identification and RAST on blood culture positive day (day 1) (%)	61 (100)	0	<b>&lt;0,001</b>
N antibiotic regimen modification after identification/RAST (day 1) or within 24 hours the positivity for CG (%)	25 (41)	8 (16)	<b>0,01</b>
N Effective antibiotic on blood culture positive day (day 1) (%)	61 (100)	43 (88)	<b>0,007</b>
Time to start effective antibiotic therapy from positive blood culture (hours)*	8 (+/-5)	19 (+/-24)	0,86
Length of hospital stay (days) *	20 (+/-23)	17 (+/-20)	0,24
N transfer to intensive care (%)	3 (4,9)	3 (6,1)	1,00
N Mortality at day 30 (%)	10 (16)	5 (10)	0,41

### Bacterial species

<i>E. coli</i>	34 (69,4)	41 (67,2)	0,84
3rd generation Cephalosporin resistant <i>E coli</i>	6 (12,2)	6 (9,8)	0,76
<i>K. pneumoniae</i>	6 (12,2)	11 (18,0)	0,44
3rd generation Cephalosporin resistant <i>K. pneumoniae</i>	2 (4,1)	2 (3,2)	
<i>P. aeruginosa</i>	9 (18,4)	9 (14,7)	0,62

## RAST mediante disco-diffusione da emocoltura positiva (RAST EUCAST)



### VANTAGGI

- Costi limitati
- Flessibilità
- Applicabilità a tutti i pazienti
- Possibile allestimento subito dopo scarico flacone positivo
- BP per i principali patogeni e antibiotici



### LIMITI

- Lettura a 4-6-8 ore (gestione refertazione?)
- Lettura operatore dipendente
- Risultato fenotipico quantitativo che non fornisce la MIC
  - % ATU (soprattutto per piperacillina-tazobactam)

# **EUCAST rapid antimicrobial susceptibility testing of blood cultures positive for *Escherichia coli* or *Klebsiella pneumoniae*: experience of three laboratories in Italy**

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Tiziana D'Inzeo<sup>1,2</sup>, Barbara Fiori<sup>2,3</sup>, Gioconda Brigante<sup>4</sup>,  
Francesco Luzzaro<sup>5</sup>, Flora Marzia Liotti<sup>1,2</sup>,  
Giulia Menchinelli<sup>1,2</sup>, Maurizio Sanguinetti <sup>1,2\*</sup>,  
Teresa Spanu<sup>1,2‡</sup> and Brunella Posteraro<sup>1,6‡</sup>

Antibiotic	Total	BMD			reading time (h)	RAST <sup>a</sup>			Categorization error (%) <sup>b</sup>			
		S (%)	I (%)	R (%)		ATU (%)	S (%)	R (%)	CA (%)	ME	VME	mE
Piperacillin/tazobactam	200	154 (77.0)		46 (23.0)	4	49 (24.5)	107 (53.5)	44 (22.0)	151 (100)			
	200				6	28 (14.0)	127 (63.5)	45 (22.5)	172 (100)			
	200				8	10 (5.0)	145 (72.5)	45 (22.5)	190 (100)			
Cefotaxime	200	125 (62.5)		75 (37.5)	4	0 (0.0)	125 (62.5)	75 (37.5)	200 (100)			
	200				6	0 (0.0)	125 (62.5)	75 (37.5)	200 (100)			
	200				8	0 (0.0)	125 (62.5)	75 (37.5)	200 (100)			
Ceftazidime	200	123 (61.5)	3 (1.5)	74 (37.0)	4	3 (1.5)	124 (62.0)	73 (36.5)	196 (99.5)			1 <sup>c</sup>
	200				6	2 (1.0)	124 (62.0)	74 (37.0)	197 (100)			1
	200				8	2 (1.0)	124 (62.0)	74 (37.0)	197 (100)			1
Meropenem	200	171 (85.5)	2 (1.0)	27 (13.5)	4	2 (1.0)	170 (85.0)	28 (14.0)	197 (99.5)			1 <sup>c</sup>
	200				6	2 (1.0)	170 (85.0)	28 (14.0)	197 (99.5)			1
	200				8	2 (1.0)	170 (85.0)	28 (14.0)	197 (99.5)			1
Ciprofloxacin	200	108 (54.0)		92 (46.0)	4	23 (11.5)	96 (48.0)	81 (40.5)	176 (99.4)		1 <sup>c</sup>	
	200				6	15 (7.5)	105 (52.5)	80 (40.0)	184 (99.5)		1	
	200				8	11 (5.5)	109 (54.5)	80 (40.0)	188 (99.5)		1	
Amikacin	200	188 (94.0)		12 (6.0)	4	33 (16.5)	158 (79.0)	9 (4.5)	167 (100)			
	200				6	9 (4.5)	182 (91.0)	9 (4.5)	191 (100)			
	200				8	2 (1.0)	188 (94.0)	10 (5.0)	198 (100)			
Gentamicin	200	164 (82.0)		36 (18.0)	4	29 (14.5)	134 (67.0)	37 (18.5)	170 (99.4)	1 <sup>c</sup>		
	200				6	10 (5.0)	154 (77.0)	36 (18.0)	190 (100)			
	200				8	5 (2.5)	159 (79.5)	36 (18.0)	195 (100)			
Tobramycin	200	148 (74.0)		52 (26.0)	4	0 (0.0)	148 (74.0)	52 (26.0)	200 (100)			
	200				6	0 (0.0)	148 (74.0)	52 (26.0)	200 (100)			
	200				8	0 (0.0)	148 (74.0)	52 (26.0)	200 (100)			
Total	4800				All	237 (5.0)	3365 (70.1)	1198 (24.9)	4553 (99.8)	1 (0.02)	3 (0.08)	6 (0.1)



## Esempi di pannelli per l'antibiogramma fenotipico rapido

# MANUALE



EUCAST

EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Rapid AST in bloodcultures

Organization

EUCAST News

New definitions of S, I and R

Clinical breakpoints and dosing

Rapid AST in blood cultures

Methods

Breakpoints for short incubation

Expert rules and intrinsic resistance

## Rapid AST directly from blood culture bottles

EUCAST has published recommendations for short incubation (4, 6 and 8 hours) AST directly from positive blood culture bottles:



[illegible]

**TERAPIA MIRATA  
3-6 ORE**



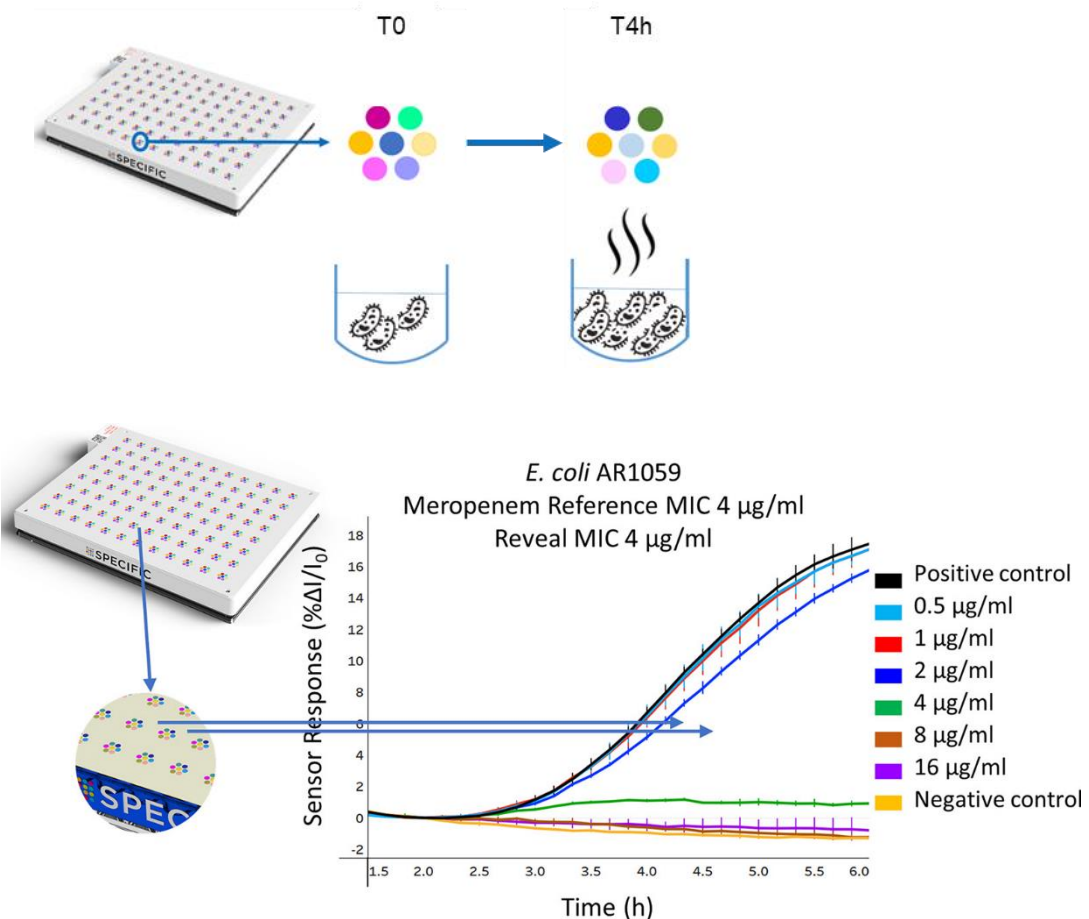
# Rapid phenotypic AST methods for testing positive blood cultures

Fornire dati quantitativi (MIC)  
consente un aggiustamento precoce e  
preciso del trattamento

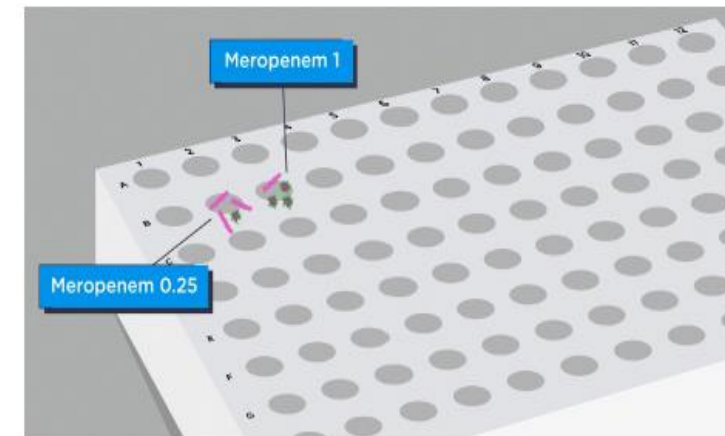
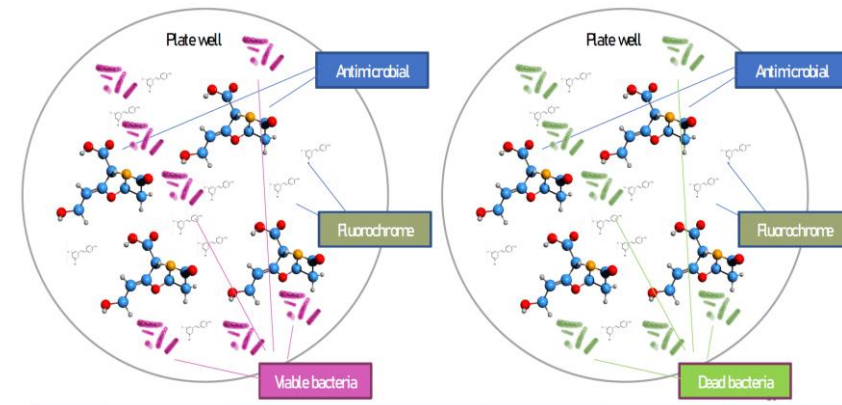
La microscopia automatizzata time-lapse per l'analisi morfo-cinetica cellulare, consente di monitorare le caratteristiche fenotipiche, come dimensione, forma, tasso di divisione delle singole cellule vive, mentre vengono esposte agli antibiotici, valutando le curve di crescita a livello di ogni singola colonia per estrapolare i valori MIC.

		TTR
	Morphological	7 h
Alfred (AliFAX)	Light scattering to detect bacterial growth in liquid culture broth.	3–5 h
dRAST (QuantaMatrix)	Time-lapse imaging of bacterial cells on micropatterned plastic microchips.	6 h
Reveal AST (Specific Diagnostics)	Sensor array for volatile organic compounds emitted during microorganism growth.	4.5 h
ASTar (Q-linea)	Time-lapse imaging of bacterial growth in broth.	3–6 h
Fastinov	Flow cytometry applying fluorescent dyes that reveal cell damage during treatment.	80 min
LifeScale (Affinity Biosensors)	Mass measurement using a microcantilever.	4 h

## Dal colore alla MIC...


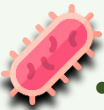









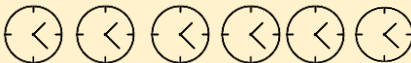





## Dalla citofluorimetria alla MIC...



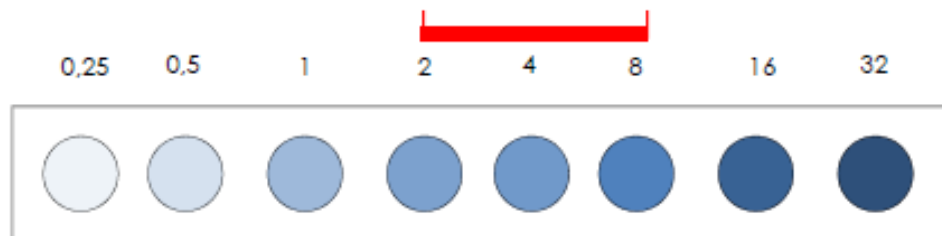
nuova tecnologia di sensori per rilevare la crescita delle popolazioni batteriche attraverso l'emissione di composti organici volatili durante la crescita, utilizzando piastre a 96 pozzetti contenenti 7 sensori posizionati su ciascun pozzetto. Quando le sostanze volatili vengono emesse dai batteri, i sensori rispondono con un cambiamento di colore. Ciascuno dei 7 sensori in ciascuno dei 96 pozzetti viene sottoposto a imaging ogni 10 minuti per monitorare la variazione di intensità nel tempo.



MODULI	TAT (ore)	PANNELLI	ANTIBIOTICI
Moduli da 1	2-4 	 <ul style="list-style-type: none"> <li>Gram -</li> <li>Gram + (in sviluppo)</li> </ul> 	12 
Moduli da 4 (ogni «cassetto» può contenere fino a 2 ATB da far partire insieme)	6 	 <ul style="list-style-type: none"> <li>Gram -</li> </ul>	23 
Non modulare: 12 postazioni indipendenti	6 	 <ul style="list-style-type: none"> <li>Gram -</li> <li>Fastidious</li> </ul> 	23 
Non modulare: 15 postazioni indipendenti	6 	 <ul style="list-style-type: none"> <li>Gram -</li> <li>Gram +</li> </ul> 	17 

Antibiotico					
Amikacina	X	X	X	X	X
Amocillina/Clavulanato	X		X	X	X
Ampicillina			X	X	X
Aztreonam	X			X	
Ceftazolina				X	
Cefepime	X	X	X	X	X
Cefotaxime	X	X	X	X	X

#### MICRO BRODO DILUIZIONE



- Valuta la crescita con concentrazioni di antibiotico al raddoppio.
- Alta variabilità intra e inter laboratorio richiede un attento controllo e una standardizzazione.
- E' generalmente accettato che i test MIC in brodo siano riproducibili entro  $\pm 1$  diluzione.

**GRADIENTE**  
Valuta la crescita batterica con concentrazione continua a gradiente.

Variazione 5%\*



Il gradiente di concentrazione di antibiotico si traduce in un'alta risoluzione e precisione.

Gram negativi:

Enterobacterales, principali specie

*P.aeruginosa*

*A.baumannii*

La disponibilità di avere un valore di **MIC accurato**, consente un aggiustamento preciso del trattamento antimicrobico

Antibiogramma fenotipico vs. antibiogramma fenotipico rapido

E.coli, n.17

Antibiotico	CA (%)	VME	ME	mE
AMIKACIN	88%		2	
AMOX/CLAV	71%	5		
AMPICILLINA	88%	2		
AZTREONAM	87%			
CEFEPIME	88%			
CEFTAZIDIME	82%			1
CEFTAZIDIME/AVI	94%			
CEFTOLOZANE/TAZO	94%			
CIPROFLOXACIN	88%			
ERTAPENEM	100%			
GENTAMICIN	88%			
IMIPENEM	100%			
MEROPENEM	100%			
PIPERACILLIN	100%			
PIPERACILLIN/TAZO	100%			
TRIMETHOPRIM/SUL	100%			

K.pneumoniae,n.13

Antibiotico	CA(%)	VME	ME	me
AMIKACIN	100%			
AMOX/CLAV	93%		1	
AZTREONAM	100%			
CEFEPIME	100%			
CEFTAZIDIME	100%			
CEFTAZIDIME/AVI	100%			
CEFTOLOZANE/TAZO	100%			
CIPROFLOXACIN	92%			1
ERTAPENEM	92%		1	
GENTAMICIN	100%			
IMIPENEM	92%			1
LEVOFLOXACIN	85%	2		
MEROPENEM	100%			
PIPERACILLIN	100%			
PIPERACILLIN/TAZO	92%		1	
TRIMETHOPRIM/SUL	92.9%			1

P.aeruginosa,n.5

Antibiotico	CA(100%)	VME	ME	me
AMIKACIN	60%	1	1	
AZTREONAM	100%			
CEFEPIME	80%			1
CEFTAZIDIME	80%			1
CIPROFLOXACIN	80%			1
IMIPENEM	80%			1
MEROPENEM	100%			
PIPERACILLIN	80%			1
PIPERACILLIN/TAZO	80%			1



Legenda:

CA: categorical Agreement  
VME: very major error - falsely susceptible,  
ME: major error -falsely resistant  
Me: minor error (mE, sensibile/resistente *versus* intermedio),

Includere intervalli di MIC più ampi per una *de-escalation* più efficace nella scelta dell’antibiotico e migliorare lo standard di cura.

# Antibiogramma rapido fenotipico automatizzato

## VANTAGGI

- Elevata automazione
- Informazioni fenotipiche completa (SIR + MIC)
- Riduzione del TAT significativo rispetto all'AST fenotipico tradizionale

## LIMITI

- Costi elevati
- Non funziona su campioni polimicrobrici
- Necessità di attendere colorazione di gram (identificazione?)
- Mancanza di alcuni tra gli antibiotici «nuovi» e di quelli «difficult to test»



# Antibiogramma rapido fenotipico automatizzato



RESEARCH ARTICLE



BACTERIOLOGY



ORIGINAL RESEARCH  
published: 23 March 2022  
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## Systematic Evaluation of the Accelerate Pheno System for Susceptibility Testing of Gram-Negative Bacteria Isolated from Blood Cultures

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## Performance of a System for Rapid Phenotypic Antimicrobial Susceptibility Testing of Gram-Negative Bacteria Directly from Positive Blood Culture Bottles

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Rapid AST of Gram-negative Bacteria from Blood Cultures

**TABLE 1** Diagnostic accuracy of Accelerate Pheno blood culture detection system for Enterobacteriales ( $n = 263$ )<sup>a</sup>

Antibiotics	Categorical agreement		VME		ME		mE	
	N	%	N	%	N	%	N	%
Amikacin	261	97	3	60	1	0.4	4	2
Ampicillin-sulbactam	235	83	2	3	2	2	37	16
Aztreonam	262	93	2	7	3	1	13	5
Cefepime					3	1	20	8
Ceftazidime					10	4	21	8
Ceftazidime-avibactam <sup>b</sup>					3	1	11	4
Ceftriaxone-tazobactam <sup>c</sup>					0	0	26	10
Ciprofloxacin					2	1	2	1
Ertapenem					1	0.4	2	1
Gentamicin					1	0.4	4	2
Meropenem					6	3	25	10
Piperacillin-tazobactam					2	1	16	6
Tobramycin								

<sup>a</sup>Enterobacteriales

<sup>b</sup>Enterobacteriales, 17 Enterobacter, and 10 S.

**TABLE 2** Accuracy study results for each antimicrobial, including contrived and clinical samples, after discrepancy resolution<sup>a</sup>

Antimicrobial agent	EA (%)	CA (%)	VMEs (%)	MDs (%)
Ampicillin	232/241 (96.7)	237/241 (98.3)	1/79 (1.0)	3/142 (2.1)
Amoxicillin-clavulanic acid <sup>b</sup>	341/357 (95.5)	332/357 (93.0)	4/79 (5.1)	21/278 (7.6)
Piperacillin-tazobactam <sup>c</sup>	416/436 (95.4)	426/436 (97.7)	5/69 (7.2)	4/354 (1.1)
Cefazolin	276/286 (96.5)	262/286 (91.6)	0/121 (0)	NA <sup>d</sup>
Cefepime	440/452 (97.3)	435/441 (98.6)	0/65 (0)	0/267 (0)
Ceftazidime	422/443 (95.3)	438/443 (98.9)	0/61 (0)	4/380 (1.1)
Ceftazidime	389/399 (97.5)	387/399 (97.0)	0/68 (0)	3/310 (1.0)
Ceftazidime-avibactam <sup>e</sup>	393/429 (91.6)	422/429 (98.4)	6/419 (1.4)	6/419 (1.4)
Ceftriaxone-tazobactam <sup>c</sup>	416/426 (97.7)	418/426 (98.1)	3/38 (7.9)	5/388 (1.3)
Ceftriaxone	429/444 (96.6)	440/444 (99.1)	0/62 (0)	1/382 (0.3)
Cefuroxime	282/294 (95.9)	285/294 (96.9)	0/44 (0)	NA <sup>d</sup>
Ertapenem			0/374 (0)	0/374 (0)
Imipenem			0/432 (0)	0/432 (0)
Aztreonam			0/345 (0)	0/345 (0)
Ciprofloxacin			0/333 (0)	0/333 (0)
Ciprofloxacin			2/7	1/375 (0.3)
Levofloxacin			5/67 (8.2)	0/383 (0)
Amikacin			2/980 (0.5)	2/980 (0.5)
Gentamicin			1/195 (0.5)	1/195 (0.5)
Tigecycline			0/238 (0)	0/238 (0)
Colistin			1/1	8/332 (2.4)
Trimethoprim-sulfamethoxazole <sup>f</sup>				
Total			39 (2.4)	62/6,845 (0.9)

<sup>a</sup>The concentration of clavulanic acid is fixed

<sup>b</sup>The concentration of tazobactam is fixed

<sup>c</sup>The concentration of avibactam is fixed

<sup>d</sup>Trimethoprim-sulfamethoxazole at a ratio

<sup>e</sup>There is no clinical breakpoint for A. ba

<sup>f</sup>VME and MD calculations were based on

an arbitrary 5 breakpoints [16].

Alternative evaluation criteria could be

used if all discrepancies in such cases

are instead classified as either MD or V

and 5/121 (4.1%) VMEs for ceftazidime, 0/

(0.1%) VMEs for cefuroxime, 0/357 (0%)

(0.3%) VMEs for levofloxacin.

Article

## Performance Evaluation of the Quantamatrix QMAC-dRAST System for Rapid Antibiotic Susceptibility Testing Directly from Blood Cultures

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TABLE 2. Performance characteristics of the QMAC-dRAST by antibiotic and bacterial group.

	N° of Antibiotics Tested	CA	CA%	me	me%	ME	ME%	VME	VME%	S	S%	R	R%
<b>Enterobacteriales</b>													
Amikacin	130	120	100									0	0.0
Amoxicillin-Clavulanate	130	125	96.2			3	5.9	2	2.5			51	39.2
Ampicillin	130	120	100									16	12.3
Ceftazidime	130	114	87.7	11	8.5	5	5.1					98	75.4
Ceftriaxone-Avibactam	129	129	100									129	100
Ciprofloxacin	129	123	95.3	5	3.9	1	1					104	80.6
Cefepime	130	116	89.2	9	6.9	5	4.6	1	5.6			109	83.1
Gentamicin	130	125	96.2	4	3.1							112	86.2
Imipenem	118	101	85.6	15	12.7							97.5	3
Levofloxacin	130	120	92.3	10	7.7							114	87.7
Meropenem	130	129	99.2	1	0.8							129	99.2
Piperacillin-Tazobactam	130	122	93.8	6	4.6	1	1	1	3.4			101	77.7
Trimethoprim-Sulfamethoxazole	130	128	98.5	1	0.8	1	1.1					89	68.5
<b>Non-fermentative GNB</b>													
Amikacin	19	18	94.7	1	5.3							16	84.2
Ceftazidime	13	13	100									6	46.2
Ceftriaxone-Avibactam	12	12	100									9	75
Ciprofloxacin	19	17	89.5	2	10.5							14	73.7
Cefepime	13	11	84.6									9	69.2
Gentamicin												12	66.7
Imipenem												12	63.2
Meropenem												11	57.7
Piperacillin-Tazobactam												14	73.7
Trimethoprim-Sulfamethoxazole												10	76.9
Staphylococcus spp.												6	85.7
Chlamydia												58	84.1
Daptomycin												49	100
Clindamycin												51	73.9
Linezolid												49	100
Levofloxacin												48	69.6
Tetracycline												32	46.4
Chacillin												1	4.1
Penicillin G												65	94.2
Ticoplanin												49	100
Vancomycin												49	100
<b>Endomycetes spp.</b>													
Amphotericin												27	80.6
Caspofungin												27	80.6
Linezolid												100	100
Levofloxacin												18	49.2
Ticoplanin												24	77.4
Vancomycin												21	67.7
Total												2.6	2025

ME: major error; ME%: ME rate (percentage of very major errors); VME%: VME rate (percentage of VMEs); S: susceptible; R: resistant; S%: percentage of percentage of antibiotics that exhibit cm values

## Evaluation of the Speed, Accuracy and Precision of the QuickMIC Rapid Antibiotic Susceptibility Testing Assay With Gram-Negative Bacteria in a Clinical Setting

Christer Malmberg<sup>1,2</sup>, Jessie Torpner<sup>2†</sup>, Jenny Fernberg<sup>2†</sup>, Håkan Öhm<sup>2</sup>, Jonas Ångström<sup>2</sup>, Cecilia Johansson<sup>2</sup>, Thomas Tängdén<sup>2</sup> and Johan Kreuger<sup>1\*</sup>

TABLE 2 | Antibiotic concentrations used for BMD and QuickMIC AST testing.

Antibiotic (μ)	Supplier	Article number	Batch number	Concentration range mg/L (QuickMIC)	Concentration range mg/L (BMD)
Amikacin (AMK)	Sigma	PHR1860	LRAB1258	1 – 20	0.5–30
Cefepime (CEP)	Sigma	PHR1763	LRAB8603	0.5–10	0.25–16
Ciprofloxacin (CIP)	Sigma	PHR1044	LRAB8271	0.125–2.5	0.125–4
Colistin (COL)	Sigma	CA461	080M4881V	0.25–5	0.125–8
Ceftazidime (CTA)	Sigma	Y000420	4.0	0.25–5	0.0156–8
Ceftazidime/CTA	Sigma	C0900500	3	0.5–10	0.25–16
Avicarb				4*	4*
Gentamicin				0.5–10	0.25–16
Meropenem				0.5–10	0.25–16
Piperacillin				2–40*	0.5–64*
Tazobactam				4*	4*
Tigecycline				0.06–1.25	0.0156–2
Tobramycin				0.5–10	0.125–16

\*The concentration of 4 mg/L, as per EUCAST guidelines.

TABLE 7 | QuickMIC and BMD AST of bacteria in clinical blood cultures, by tested antibiotic.

Antibiotic	CTZ (38)	GEN (39)	MER (36)	PIT (37)	TIG (33)	TOB (32)	Total (407)
CA (%)	94.4	91.4	97.1	93.5	45.8	96.3	91.0
EA (%)	96.8	96.3	96.7	100.0	78.6	100.0	96.7
MD (%)	3.2	0.0	0.0	0.0	0.0	0.0	1.0
MD (%)	0.0	0.0	3.3	0.0	21.4	0.0	1.3
MD (%)	0.0	3.7	0.0	0.0	0.0	0.0	1.0

**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?	Time to specimen receipt Centralized vs point-of-care testing On-demand vs batched testing Specimen preparation time Run time Result reporting time

**Probabilità di impatto sulla terapia?  
(patogeno, tipologia resistenza)**

**Possibilità di utilizzo tempestivo del dato?  
(orario, percorso dedicato)**



# Conclusioni

- ✓ **Metodi potenzialmente utili per ridurre il tempo di impostazione di una terapia antimicrobica appropriata**
- ✓ **Diversi metodi attualmente disponibili (EUCAST RAST, RAST automatizzati), necessario valutare sulla base di:**
  - *risorse economiche, strutturali, di personale*
  - *organizzazione ed orari di apertura del laboratorio (flusso di lavoro 24/24 su 7gg)*
  - *percorsi dedicati, tipologia pazienti ed epidemiologia locale*

**FONDAMENTALE INSERIRE RAST ALL'INTERNO DI UN PERCORSO DEDICATO BEN CODIFICATO CHE NE OTTIMIZZI IL VALORE CLINICO**