MEETING MULTIDISCIPLINARE
SUI MICRORGANISMI MULTIDRUG RESISTANT
E LE RELATIVE PROBLEMATICHE MICROBIOLOGICHE,
EPIDEMIOLOGICHE, DIAGNOSTICHE E CLINICHE

CEUB | BERTINORO- 27 NOVEMBRE 2019

Metodi microbiologici per l'identificazione di microrganismi multiresistenti

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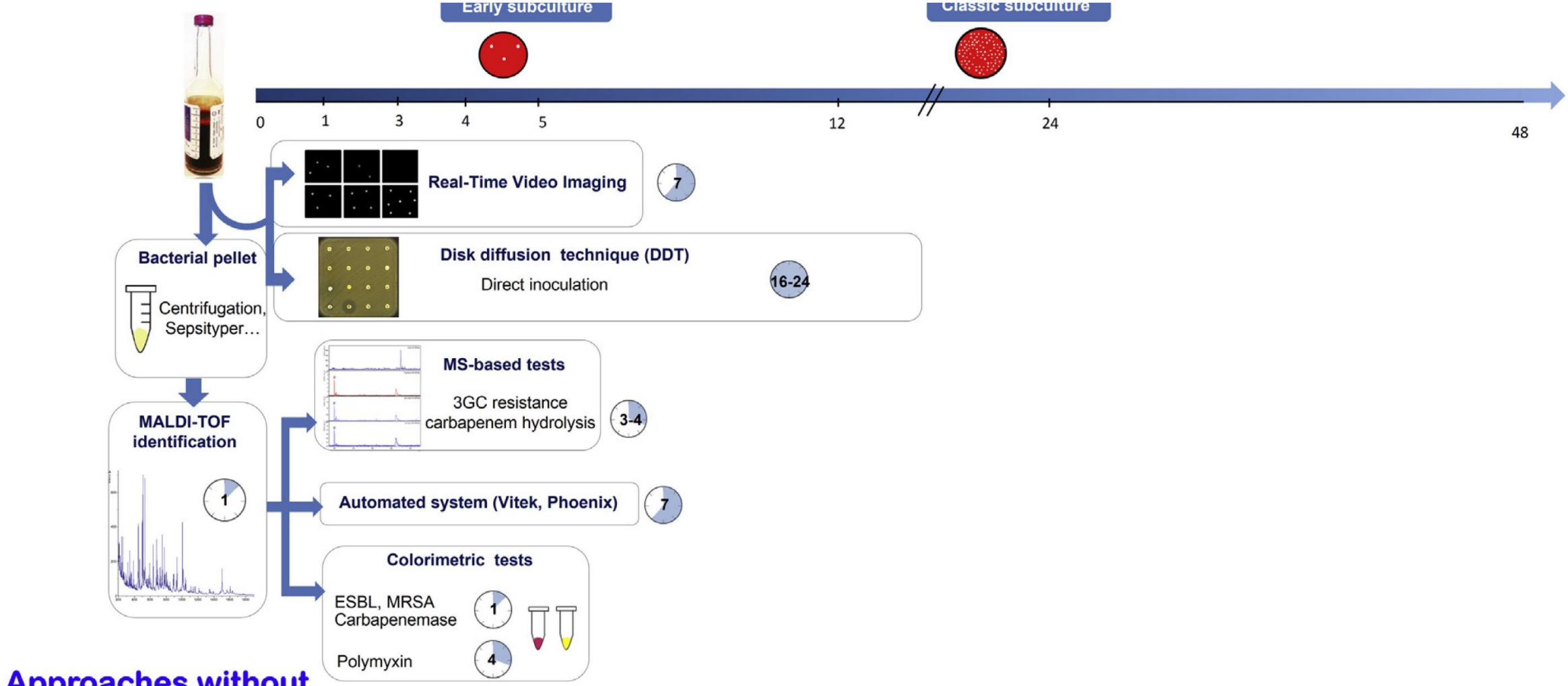
Ha senso parlare di fenotipo nel 2020?

- Possibilità di identificare una vasta gamma di taxa microbici
- Capacità di rilevare la resistenze effettivamente espresse
- Superare la variabilità genetica nel determinismo delle resistenze
- Risposte non così lente come spesso supposto



Approaches without a sub-culture step

All'inizio del processo… i cromogeni selettivi

- Disponibili per una vasta gamma di target (MRSA, VRE, ESBL, CRE, colistina)
- Utilizzo contestualizzato alle esigenze e agli algoritmi interni
- In generale, trovano impiego nello screening e non in algoritmi diagnostici diretti
- Non risentono della variabilità genetica
- Necessario conoscere sensibilità e specificità per meglio indirizzare la scelta in base alle proprie necessità
- Richiedono tempo per la corretta valutazione

AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews

Intestinal Carriage of Carbapenemase-Producing Organisms: Current Status of Surveillance Methods

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TABLE 5 Comparison of model estimates of diagnostic performance for different screening methods using pure cultures[a]

Method (no. of studies)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	DOR (95% CI)	Aggregate no. of isolates (no. of positive isolates)
Brilliance CRE (4)	81.3 (77.1–84.88)	58.89 (39.31–76.02)	6.23 (2.6–14.91)	774 (439)
CDC protocol for ertapenem (1)	79.23 (71.21–85.47)	68.57 (30.95–91.4)	8.32 (1.49–46.57)	200 (130)
CDC protocol for meropenem (1)	46.92 (38.24–55.8)	78.57 (42.41–94.81)	3.24 (0.58–18.19)	200 (130)
CHROMagar KPC (2)	42.11 (35.69–48.81)	78.13 (52.18–92.12)	2.6 (0.73–9.27)	318 (228)
chromID Carba (1)	90.77 (84.36–94.72)	88.57 (59.31–97.63)	76.21 (11.97–485.11)	200 (130)
chromID ESBL (2)	91.01 (86.26–94.23)	21.27 (7.95–45.8)	2.74 (0.75–9.95)	376 (244)
Colorex KPC (2)	52.45 (45.78–59.04)	59.13 (32.06–81.6)	1.6 (0.48–5.35)	377 (230)
Supercarba (3)	96.27 (93.53–97.87)	60.38 (37.33–79.59)	39.29 (12.52–123.33)	176 (114)

[a] CI, confidence interval; DOR, diagnostic odds ratio.

ESBL: come identificarle e perché

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

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

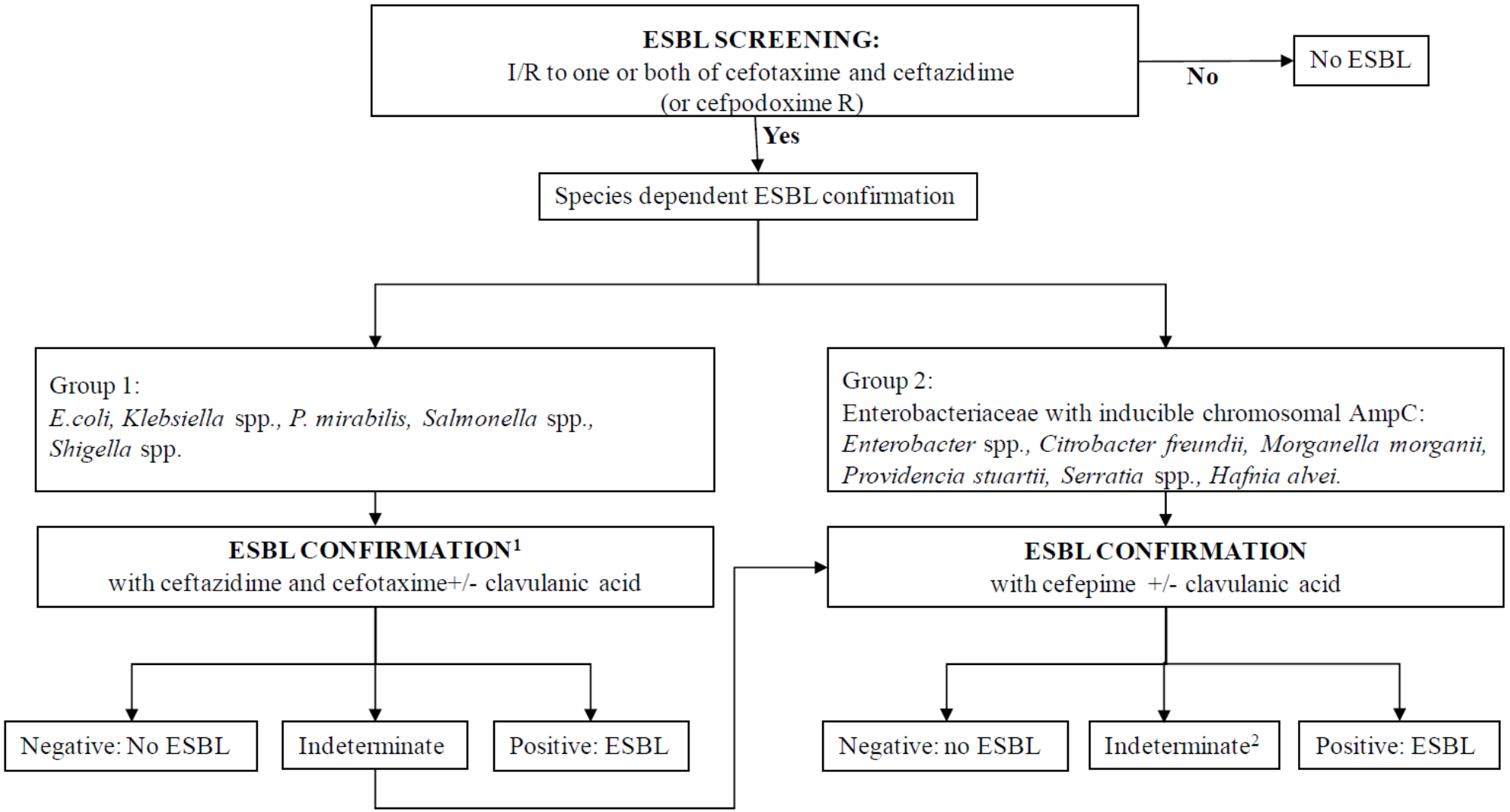
Version 2.0[1]

July 2017

[1] **Based on version 1.0 from December 2013 by the EUCAST subcommittee for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance.** Authors of the original version are acknowledged: Christian G. Giske (Sweden, EUCAST and EARS-Net Coordination Group; chairman), Luis Martinez-Martinez (Spain), Rafael Cantón (Spain, EUCAST), Stefania Stefani (Italy), Robert Skov (Germany), Youri Glupczynski (Belgium), Patrice Nordmann (France), Mandy Wootton (UK), Vivi Miriagou (Greece), Gunnar Skov Simonsen (Norway, EARS-Net Coordination Group), Helena Zemlickova (Czech Republic, EARS-Net Coordination Group), James Cohen-Stuart (The Netherlands), and Marek Gniadkowski (Poland).

- Quando si utilizzano gli attuali criteri interpretativi CLSI o EUCAST, la rilevazione della resistenza tipo ESBL non è necessaria nel report routinario dei risultati (e non è quindi necessario modificare i risultati per cefalosporine, aztreonam o penicilline).
- Tuttavia, la rilevazione della resistenza tipo ESBL può essere utile a fini epidemiologici o per il controllo delle infezioni.

Figure 1. Algorithm for phenotypic detection of ESBLs



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

[2] Cannot be determined as either positive or negative (e.g. if a gradient diffusion strip cannot be read due to growth beyond the MIC range of the strip or there is no clear synergy in combination-disk and double-disk synergy tests). In confirmation with cefepime +/- clavulanic acid is still indeterminate, genotypic testing is required.

Table 2. ESBL confirmation methods for Enterobacteriaceae that are positive in the ESBL screening test (see Table 1). Group 1 Enterobacteriaceae (see Figure 1).

Method	Antimicrobial agent (disk content)	ESBL confirmation is positive if
ESBL gradient test	Cefotaxime +/- clavulanic acid	MIC ratio ≥8 or deformed ellipse present
	Ceftazidime +/- clavulanic acid	MIC ratio ≥8 or deformed ellipse present
Combination disk diffusion test (CDT)	Cefotaxime (30 µg) +/- clavulanic acid (10 µg)	≥5 mm increase in inhibition zone
	Ceftazidime (30 µg) +/- clavulanic acid (10 µg)	≥5 mm increase in inhibition zone
Broth microdilution	Cefotaxime +/- clavulanic acid (4 mg/L)	MIC ratio ≥8
	Ceftazidime +/- clavulanic acid (4 mg/L)	MIC ratio ≥8
	Cefepime +/- clavulanic acid (4 mg/L)	MIC ratio ≥8
Double disk synergy test (DDST)	Cefotaxime, ceftazidime and cefepime	Expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disk

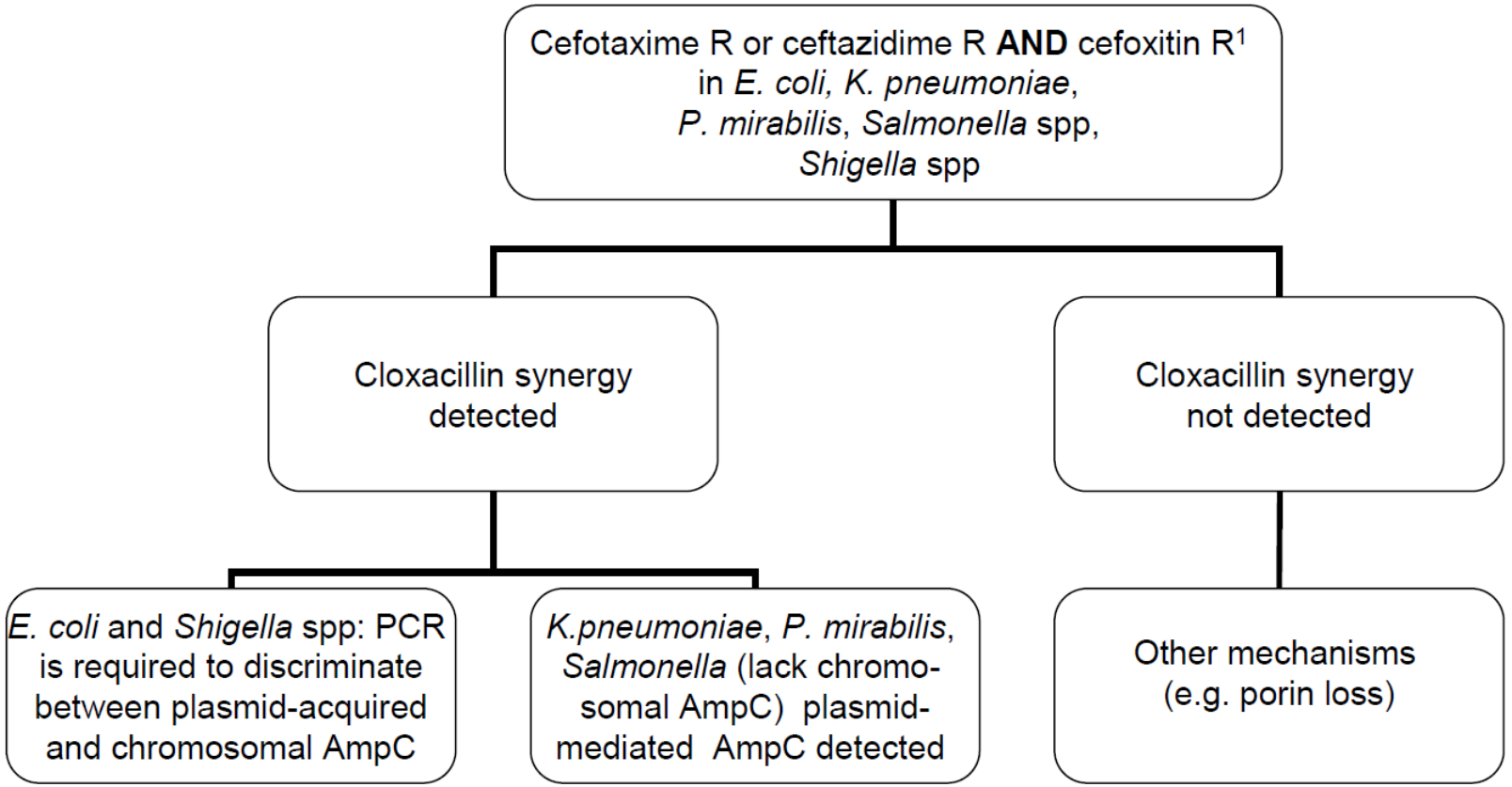
ESBL, come identificarle

- Sinergia delle combinazioni (CDT) - Vengono utilizzati dischetti contenenti cefalosporine (cefotaxime, ceftazidime, cefepime) da sole e in combinazione con acido clavulanico. La zona di inibizione attorno dischetto di cefalosporina combinata con acido clavulanico viene confrontata con quella della sola cefalosporina: il test è positivo se il diametro della zona di inibizione è maggiore di ≥5 mm per la coniugazione cefalosporina - acido clavulanico rispetto alla sola cefalosporina.
- Double-disk synergy test (DDST) - I dischetti contenenti cefalosporine (cefotaxime, ceftazidime, cefepime) vengono posti su piastre accanto a un disco con acido clavulanico (es., amoxicillina-clavulanato). Un risultato positivo si ha quando le zone di inibizione attorno a uno qualsiasi dei dischi di cefalosporine sono deformate nella zona di incontro delle inibizioni con. La distanza tra i dischetti è critica: 20 mm da centro a centro si è rivelata ottimale per i dischetti da 30 µg; tuttavia può essere ridotta (15 mm) o aumentata (30 mm) per evidenziare deformazioni con livelli di resistenza molto alti o bassi, rispettivamente.

ESBL, come identificarle

- Test di diffusione a gradiente – Il test si considera positivo se si osserva un rapporto ≥ 8 volte fra la MIC della cefalosporina associata ad acido clavulanico rispetto a quella della sola cefalosporina, o in caso sia presente una zona fantasma o un'ellisse deformata. Il risultato del test è indeterminato se la striscia non può essere letta a causa della crescita oltre l'intervallo MIC della striscia. In tutti gli altri casi il risultato del test è negativo. Il test a gradiente ESBL deve essere utilizzato solo per confermare la produzione ESBL e non può essere utilizzato per la determinazione della MIC.
- Microdiluizione in brodo – Si esegue con brodo Mueller-Hinton supplementato con cationi e contenente due diluizioni seriali di cefotaxime, ceftazidime e cefepime a concentrazioni comprese tra 0,25 e 512 mcg/ml, con e senza acido clavulanico a concentrazione fissa di 4 mcg/ml. Il test è positivo se si osserva una riduzione ≥ 8 volte nella MIC di una qualsiasi cefalosporina combinata con acido clavulanico rispetto a quella della sola cefalosporina, altrimenti il risultato del test viene interpretato come negativo.

Figure 1. Algorithm for AmpC detection.

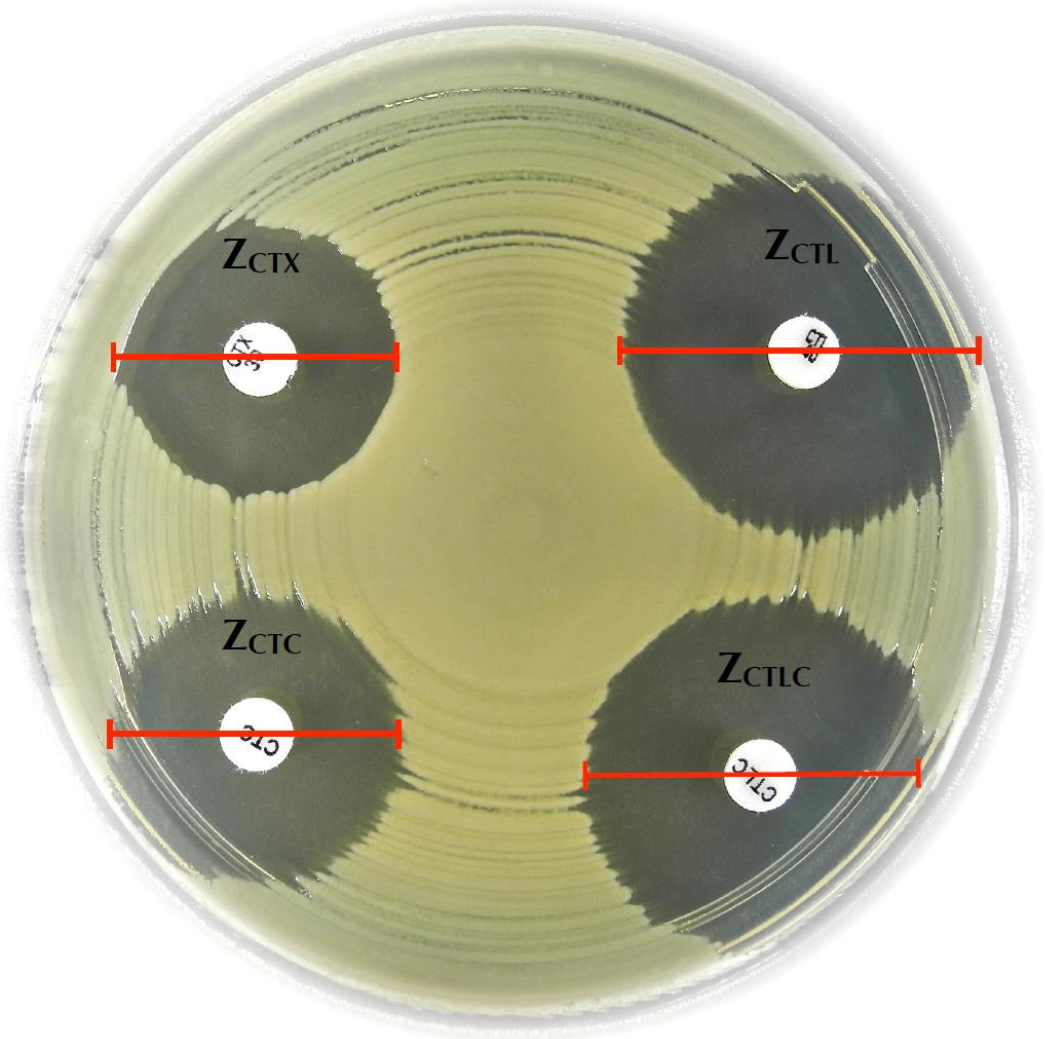


[1] Cefoxitin 'R' is here defined as non-wild type (MIC >8 mg/L or zone diameter <19 mm). For cefotaxime and ceftazidime 'R' is the result obtained using the current EUCAST breakpoints. Investigation of isolates with non-susceptibility to cefotaxime and ceftazidime is an approach with higher sensitivity but lower specificity compared with focusing on cefoxitin resistant isolates (7). AmpC can also be present in isolates with a positive ESBL-test (clavulanic acid synergy). For laboratories not testing cefoxitin, susceptibility to cefepime together with resistance to cefotaxime and/or ceftazidime is another phenotypic indicator of AmpC, although less specific.

AmpC, come rilevarlo

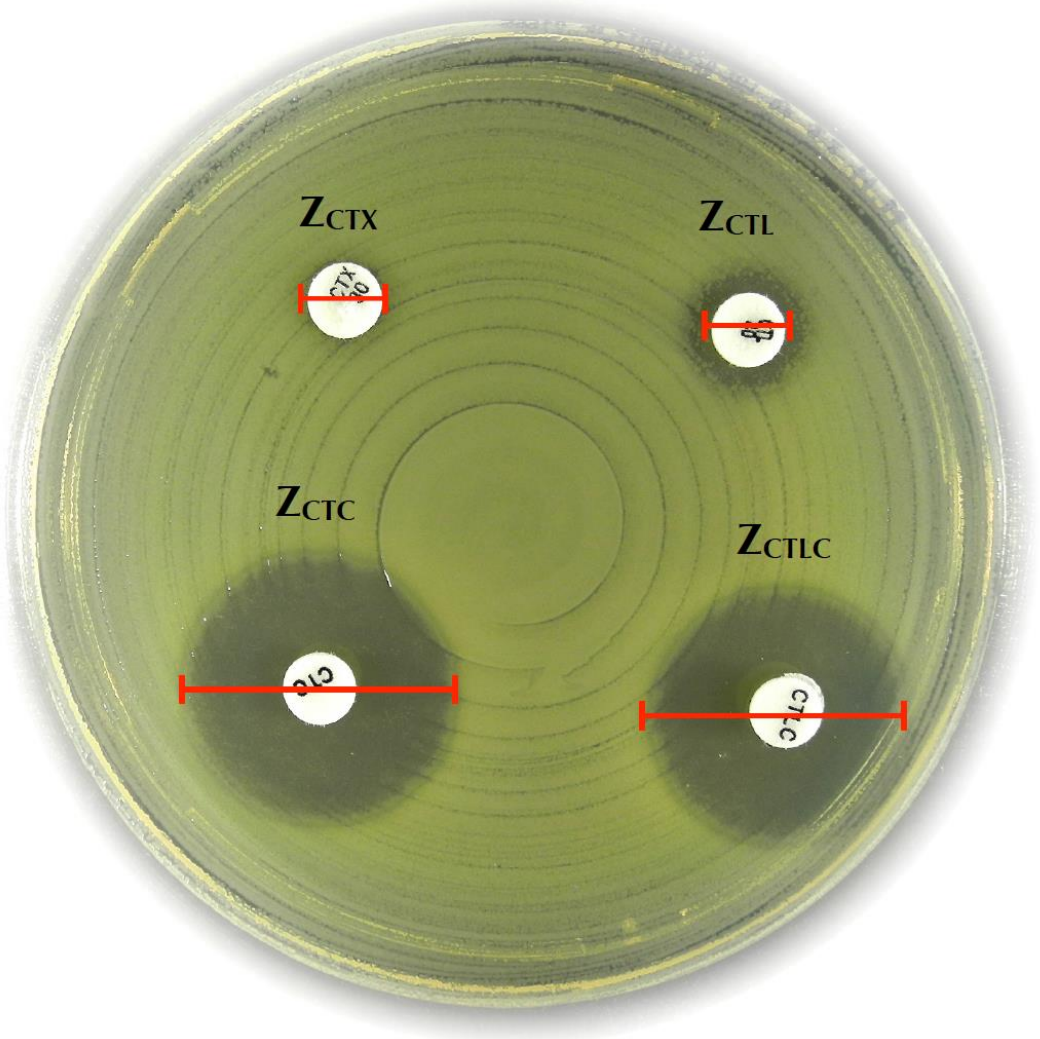
- I test di conferma fenotipica di AmpC si basano generalmente sull'inibizione di AmpC da parte della cloxacillina o dei derivati dell'acido boronico.
- Possono essere utilizzati dischetti contenenti cefotaxima-cloxacillina e ceftazidima-cloxacillina o test a gradiente; tuttavia, per *E. coli*, questi test di conferma possono discriminare tra AmpC plasmidico e iperproduzione costitutiva di un AmpC cromosomico.

ESBL positive strain



$Z_{CTL} - Z_{CTX} \geq 5$ mm
or
$Z_{CTLC} - Z_{CTC} \geq 5$ mm
$(Z_{CTLC} - Z_{CTL} < 5$ mm)

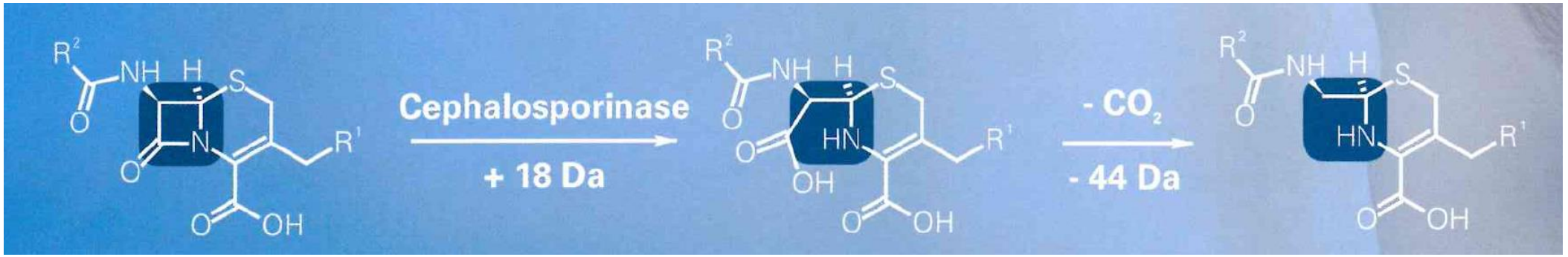
AmpC positive strain



$Z_{CTC} - Z_{CTX} \geq 5$ mm
or
$Z_{CTLC} - Z_{CTL} \geq 5$ mm
$(Z_{CTLC} - Z_{CTC} < 5$ mm)

ESBL e AmpC, rilevazione con MALDI-ToF

- L'incubazione dei batteri produttori di cefalosporinasi con una molecola di cefalosporine provoca l'idrolisi dell'anello β-lattamico, convertendo l'antibiotico in un metabolita inattivo.
- Il tempo di incubazione è di soli 30 minuti per *Enterobacterales*.
- Dopo l'incubazione, la scissione dell'antibiotico di riferimento viene monitorata mediante il rilevamento di uno spostamento di massa specifico nello spettro di massa MALDI-TOF.
- Sono disponibili metodiche home-made e del commercio. Per l'interpretazione, è necessaria un'implementazione del software di rilevazione del MALDI-ToF.



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RESEARCH ARTICLE

A rapid diagnostic workflow for cefotaxime-resistant *Escherichia coli* and *Klebsiella pneumoniae* detection from blood cultures by MALDI-TOF mass spectrometry

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‡ These authors also contributed equally to this work.
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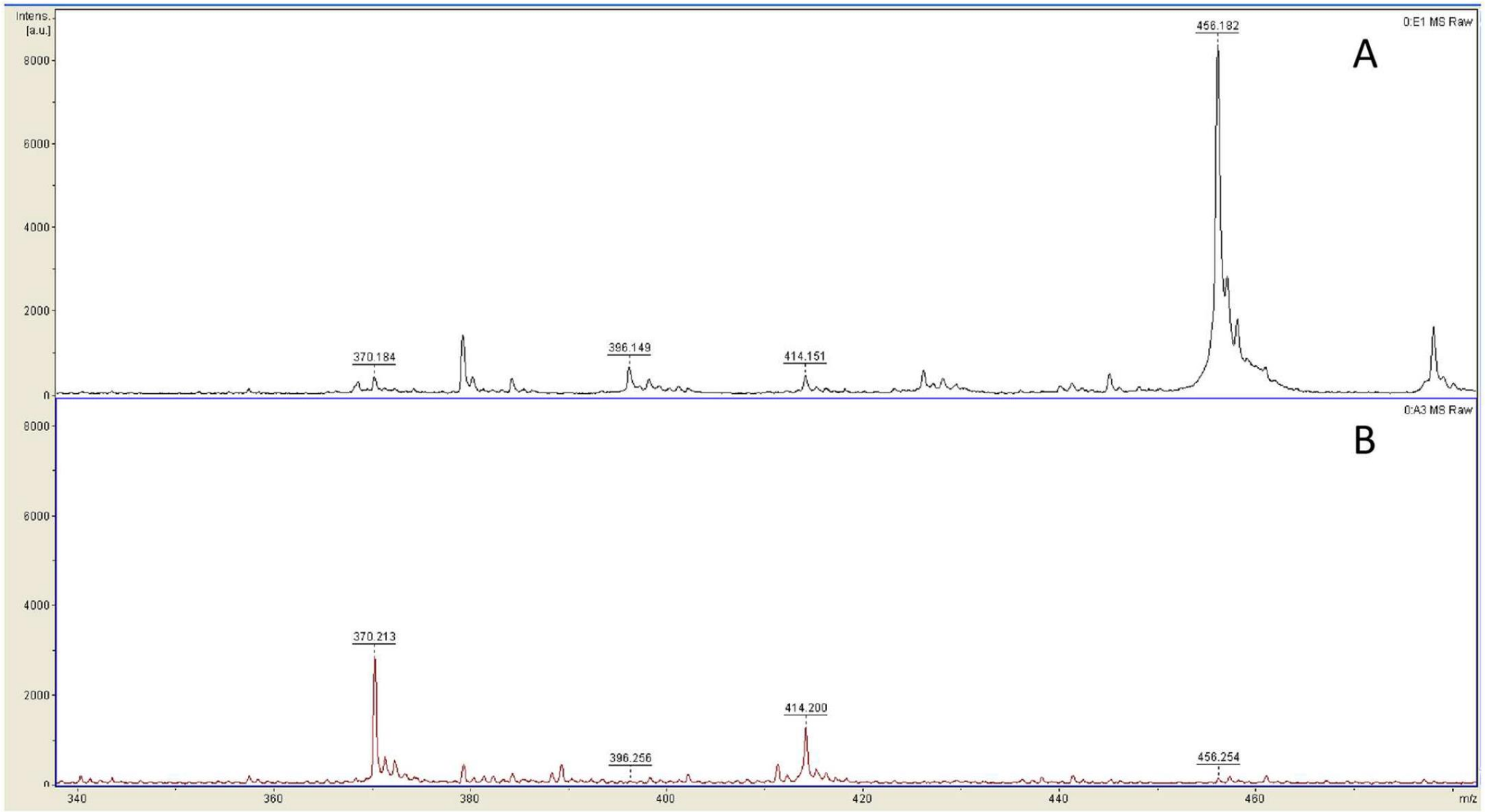
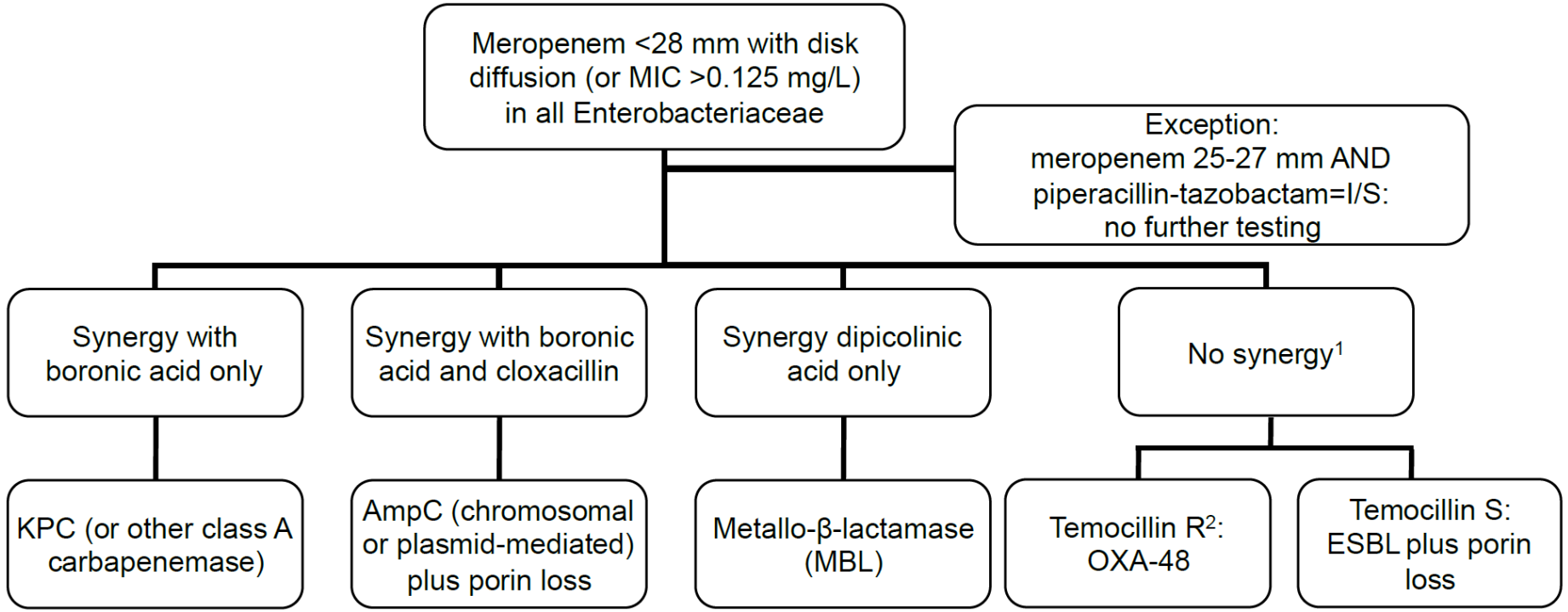


Carbapenemasi, come rilevarle

Figure 1. Algorithm for carbapenemase detection.

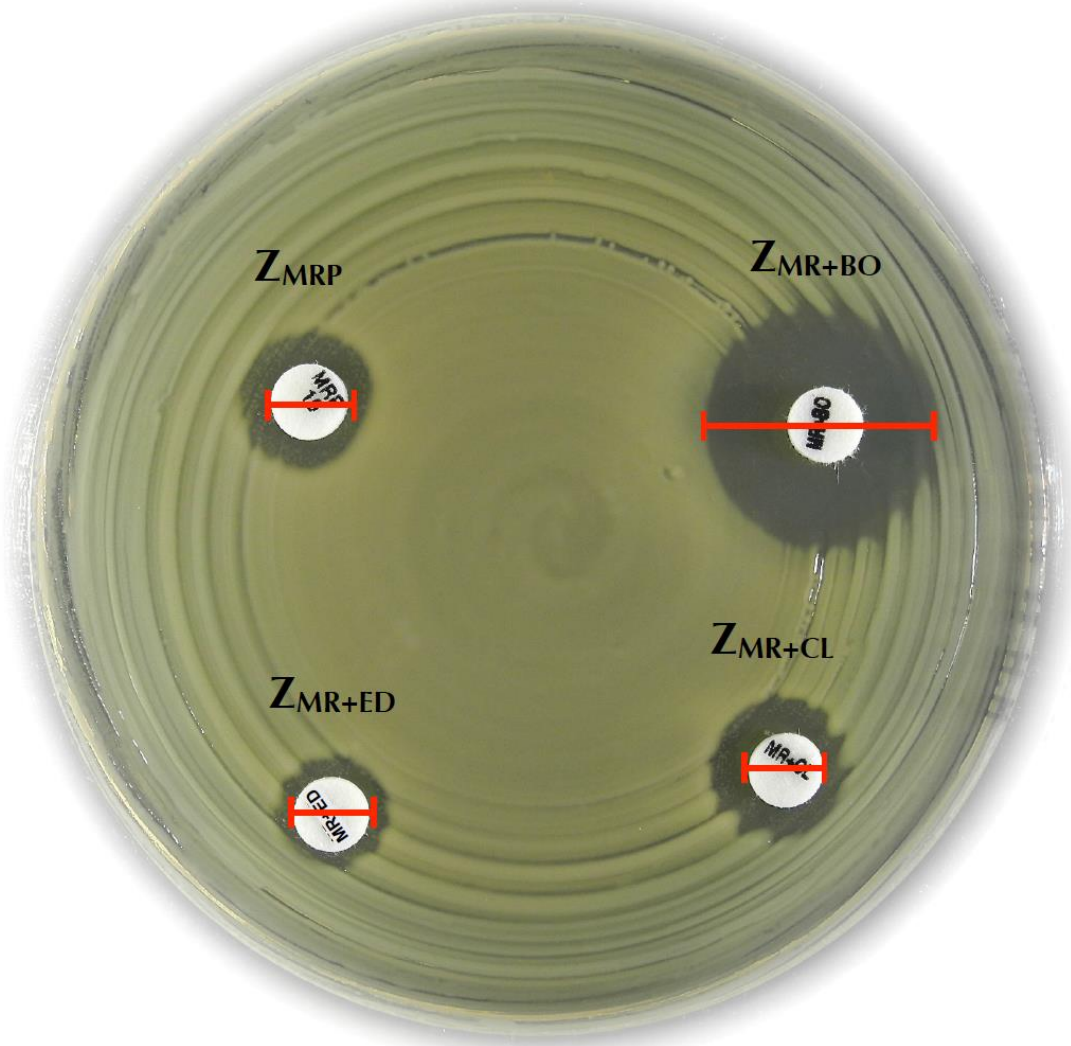


[1]Combination of several carbapenemases can also contribute to no synergy – e.g. MBL and KPC in combination. Molecular testing is usually necessary in such cases

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

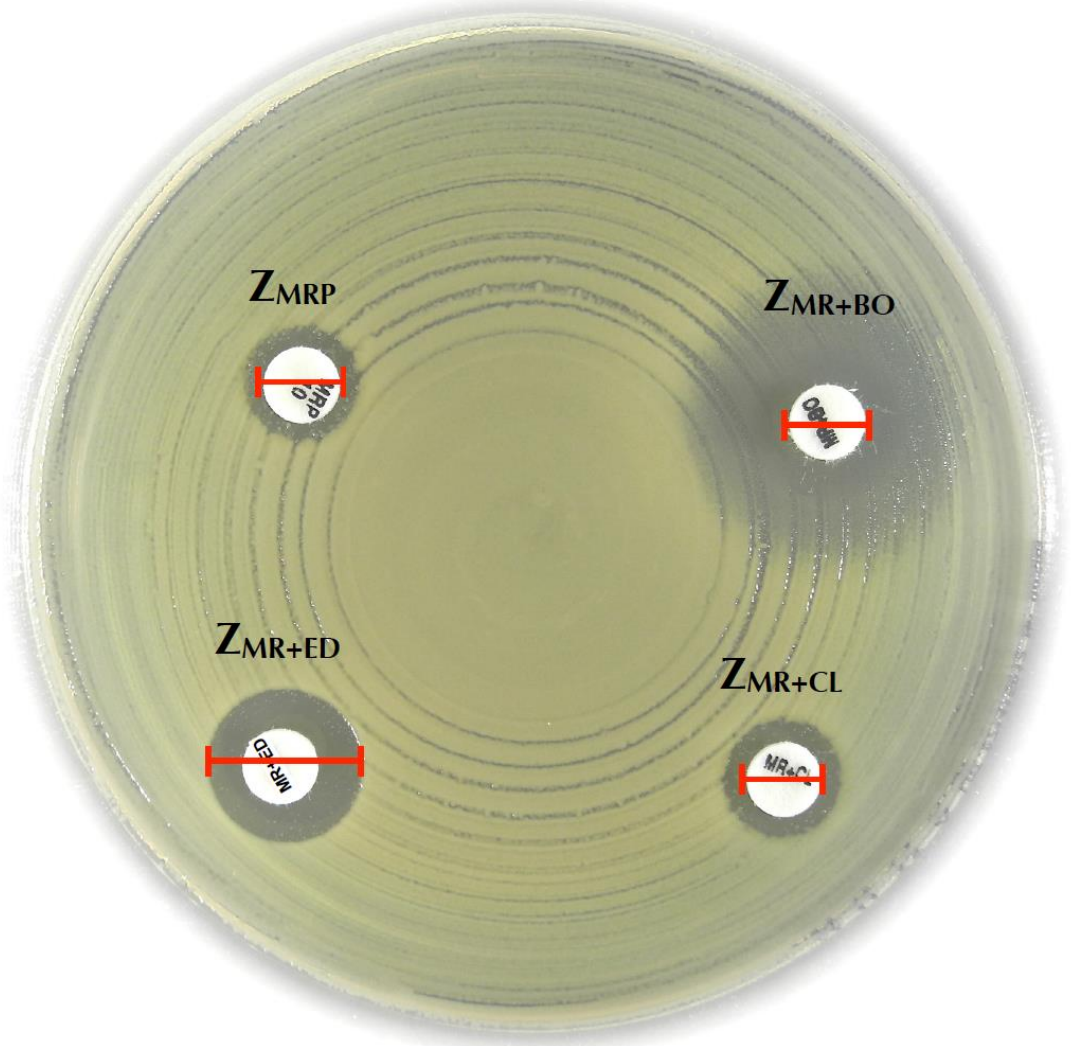
ght, E.C. 2019

KPC positive strain



$Z_{MR+BO} - Z_{MRP} \geq 4$ mm
$Z_{MR+ED} - Z_{MRP} < 5$ mm
$Z_{MR+CL} - Z_{MRP} < 5$ mm

MBL positive strain



$Z_{MR+BO} - Z_{MRP} < 5$ mm
$Z_{MR+ED} - Z_{MRP} \geq 5$ mm
$Z_{MR+CL} - Z_{MRP} < 5$ mm

Carbapenemasi, come rilevarle

- I metodi colorimetrici (CarbaNP test, Blue-Carba test, β CARBA test™) sono test rapidi per la rilevazione dell'idrolisi dei carbapenemi (<2 h), basati su una variazione di pH che determina uno spostamento del colore a seconda del substrato utilizzato.
- Mancano ancora ampi studi di convalida.
- Sono possibili problemi di interpretazione dovuti alla valutazione visiva del cambiamento di colore per risultati dubbi, nonché una certa proporzione (3-5%) di risultati non interpretabili, principalmente relativi agli isolati OXA-48+.
- Questi metodi non sono in grado di distinguere tra i diversi tipi di carbapenemasi.

Carbapenemasi, come rilevarle

- Il Carbapenem Inactivation Method (CIM) rileva l'idrolisi enzimatica incubando un carbapenemico con una sospensione batterica.
- Dopo due ore di incubazione di un'ansata di batteri (loop da 1 mcl) con un dischetto di meropenem, il dischetto viene posizionato su un agar inoculato con *Escherichia coli* ATCC 25922. L'inattivazione enzimatica non produrrà alcuna zona di inibizione, mentre l'assenza di carbapenemasi sarà rivelata dalla presenza di una zona di inibizione.
- Il test CIM ha mostrato prestazioni variabili in diversi studi, richiede tempo (richiede almeno 18 ore) e non è in grado di distinguere tra i diversi tipi di carbapenemasi.

Carbapenemasi, come rilevarle

- Test immunocromatografici sono stati recentemente commercializzati: si basano sulla rilevazione immunologica di epitopi delle diverse carbapenemasi, utilizzando anticorpi monoclonali legati a una membrana di nitrocellulosa.
- Questi saggi hanno prestazioni eccellenti, richiedono pochi minuti e sono stati validati sia da colonia che direttamente da flaconi di emocolture. Mancano ancora validazioni in studi multicentrici o metanalisi da esperienze in centri singoli.

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Original article

Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm

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ABSTRACT

Objectives: The aim of this study was to evaluate the performance of five different carbapenemase tests and to develop an algorithm which will permit the detection of most common and rare carbapenemases in routine microbiology laboratories.

Methods: The immunochromatographic tests CARBA-5 (NG), RESIST-4 O.K.N.V. (Coris), the colorimetric β-CARBA (BioRad), a newly developed carbapenem-inactivation method (CIM) supplemented with zinc (zCIM), and the Xpert Carba-R (Cepheid) were challenged with a collection of 189 molecularly characterized *Enterobacterales* isolates, including 146 carbapenemase producers (CPE): VIM ($n = 48$), OXA-48-like ($n = 40$), NDM ($n = 29$), KPC ($n = 13$), IMI ($n = 9$), IMP ($n = 9$), OXA-58 ($n = 2$), and GES ($n = 2$).

Results: The overall sensitivity/specificity values for the five carbapenemase detection tests were 84.2% (CI 77.6–89.2%)/100% (CI 91.8–100%) for RESIST-4, 88.2% (CI 82.1–92.4%)/100% (CI 91.8–100%) for CARBA-5, 88.2% (CI 82.1–92.4%)/100% (CI 91.8–100%) for Xpert Carba-R, 73.7% (CI 66.2–80.0%)/100% (CI 93.4–99.0%) for β-CARBA, and 97.4% (CI 87.9–99.6%)/97.7% (CI 87.9–99.6%) for zCIM. The four common carbapenemases (KPC, OXA-48-like, NDM, and VIM) were detected with ≥97.6% sensitivity by all tests except for β-CARBA (76.6% (CI 68.4–83.2%)). IMI and GES were only detected by zCIM (sensitivity 90.9% (CI 62.3–98.4%)). Based on these results a new algorithm was developed, consisting of an immunochromatographic assay as the first test followed by zCIM, which allows detection of 99.3% of all carbapenemases assessed.

Conclusions: Except for β-CARBA, all methods showed excellent sensitivity/specificity for the detection of the four most frequent carbapenemases. With the new algorithm, rare variants can also be detected. It is rapid, simple, and inexpensive and can be performed in any microbiology laboratory, as no PCR equipment is required. **L. Lucena Baeza, Clin Microbiol Infect 2019;25:1286.e9–1286.e15**

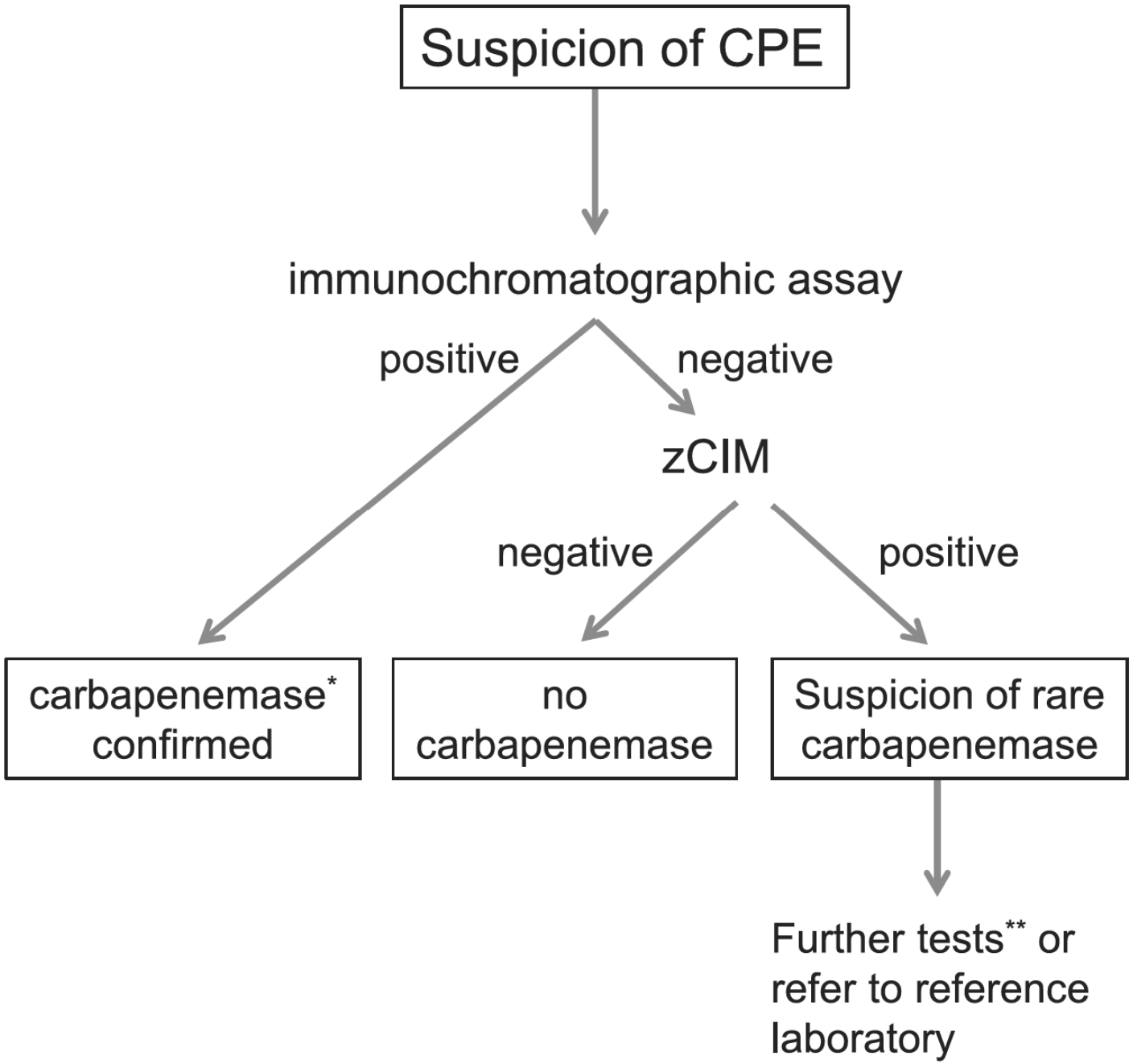
Table 3
Comparison of main features of each carbapenemase detection method

	RESIST-4 O.K.N.V.	CARBA-5	β-CARBA	zCIM
Special equipment necessary	All supplied by manufacturer	All supplied by manufacturer	All supplied by manufacturer	Conventional laboratory equipment
Storing conditions	Room temperature	Room temperature	4°C	4°C
Time for				
Preparation	5 min	5 min	5 min	10 min
Incubation/ reaction	15 min	15 min	30 min	2 h plus overnight culture
Reading/ Interpretation	1 min	1 min	1 min	5 min
Definitive result	21 min	21 min	36 min	20 h 15 min
Price per test*	~15€	~15€	~5€	~1€
Test principle	Immunochromatographic test	Immunochromatographic test	Colorimetric test	Carbapenem hydrolysis assay (activity test)
Result	OXA-48 ,KPC, NDM, VIM	OXA-48, KPC, NDM, VIM, some IMP variants	Carbapenemase positive/negative	Carbapenemase positive/negative
Sample type	Bacterial colonies, some clinical samples (e.g.urine or blood cultures [15])	Bacterial colonies	Bacterial colonies, some clinical samples (blood cultures [28])	Bacterial colonies
Interpretation	Straightforward for most isolates; faint bands can occur for some NDM/VIM isolates	Straightforward for most isolates; faint bands can occur for some NDM/VIM isolates	Subjective (colour change)	Subjective if microcolonies present
Strengths	Fast, simple moderate costs differentiation of the 4 most common carbapenemases	Fast, simple moderate costs differentiation of the 5 most common carbapenemases	Fast, simple differentiation CPE/non-CPE only detection of some rare carbapenemase variants (e.g. OXA-58) possible	Simple, inexpensive differentiation CPE/non-CPE detection of rare/new carbapenemases
Limitations	False-negatives with weak MBLs when harvested from MHA in absence of antibiotic pressure [10,16]	False-negatives with weak or low-level carbapenemase producers, especially withMBLs	False-negatives with weak or low-level carbapenemase producers, especially with MBLs and IMI/GES	False-positives in some isolates with AmpC and porin loss False-negatives with weak carbapenemases Long time-to-result
Reference	[9]	[7]	[3]	This study

Table 2
Performance of the five assays for detection of carbapenemase production

	RESIST-4 O.K.N.V.			CARBA-5			Xpert Carba R			β-CARBA			zCIM		
	Sens % (CI)	Spec % (CI)	Youden index	Sens % (CI)	Spec % (CI)	Youden index	Sens % (CI)	Spec % (CI)	Youden index	Sens % (CI)	Spec % (CI)	Youden index	Sens % (CI)	Spec % (CI)	Youden index
All carbapenemases ($n = 152$)	84.2 (77.6–89.2)	100 (91.8–100)	0.84	88.2 (82.1–92.4)	100 (91.8–100)	0.88	88.2 (82.1–92.4)	100 (91.8–100)	0.88	73.7 (66.2–80.0)	100 (91.8–100)	0.73	97.4 (93.4–99.0)	97.7 (87.9–99.6)	0.95
Ambler class A ($n = 24$)	54.2 (35.1–72.1)			54.2 (35.1–72.1)			54.2 (35.1–72.1)			54.2 (35.1–72.1)			95.8 (79.8-99.3)		
KPC ($n = 13$)	100 (77.2–100)			100 (77.2–100)			100 (77.2–100)			100 (77.2–100)			100 (77.2–100)		
GES ($n = 2$)	0 (0–65.8)			0 (0–65.8)			0 (0–65.8)			0 (0–65.8)			50 (9.5–90.6)		
IMI ($n = 9$)	0 (0–29.9)			0 (0–29.9)			0 (0.0–29.9)			0 (0–29.9)			100 (70.1–100)		
Ambler class B **($n = 86$)**	87.2 (78.5–92.7)			94.2 (87.1–97.5)			94.2 (87.1–97.5)			66.3 (55.8–75.4)			96.5 (90.2–98.8)		
NDM ($n = 29$)	93.1 (78.0–98.1)			96.6 (82.8–99.4)			100 (88.3–100)			41.4 (25.5–59.3)			100 (88.3–100)		
VIM ($n = 48$)	100 (92.6–100)			100 (92.6–100)			100 (92.6–100)			75.0 (61.2–85.1)			93.8 (83.2–97.9)		
IMP ($n = 9$)	0 (0–29.9)			55.6 (26.7–81.1)			44.4 (18.9–73.3)			100 (70.1–100)			100 (70.1–100)		
Ambler class D ($n = 42$)	95.2 (84.2–98.7)			95.2 (84.2–98.7)			95.2 (84.2–98.7)			100 (91.6–100)			100 (91.6–100)		
OXA-48-like ($n = 40$)	100 (91.2–100)			100 (91.2–100)			100 (91.2–100)			100 (91.2–100)			100 (91.2–100)		
OXA-58 ($n = 2$)	0 (0.0–65.8)			0 (0.0–65.8)			0 (0–65.8)			100 (34.2–100)			100 (34.2–100)		

Sens, sensitivity; Spec, specificity; CI, confidence interval.



Carbapenemasi, come rilevarle

- L'incubazione dei batteri produttori di carbapenemasi con una molecola di carbapenemico provoca l'idrolisi dell'anello β-lattamico, convertendo l'antibiotico in un metabolita inattivo.
- Il tempo di incubazione è di soli 30 minuti per *Enterobacterales* e *Pseudomonas* spp. e 60 minuti per *Acinetobacter* spp..
- Dopo l'incubazione, la scissione dell'antibiotico di riferimento viene monitorata mediante il rilevamento di uno spostamento di massa specifico nello spettro di massa MALDI-TOF.
- Sono disponibili metodiche home-made e del commercio. Per l'interpretazione, è necessaria un'implementazione del software di rilevazione del MALDI-ToF.

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Journal of Antimicrobial Chemotherapy

MALDI-TOF for the rapid detection of carbapenemase-producing Enterobacteriaceae: comparison of the commercialized MBT STAR®-Carba IVD Kit with two in-house MALDI-TOF techniques and the RAPIDEC® CARBA NP

Objectives: There is an urgent need for accurate and fast diagnostic tests to identify carbapenemase-producing bacteria. Here, we have evaluated three MALDI-TOF-based techniques to detect carbapenemase-producing Enterobacteriaceae (CPE) from cultured colonies.

Methods: The performance of three MALDI-TOF-based techniques, including the commercialized MBT STAR®-Carba IVD Kit (Bruker Daltonics) and two in-house protocols performed on the Microflex LT Biotyper (Bruker Daltonics) and the VITEK® MS Plus (bioMérieux), were compared with those of the RAPIDEC® CARBA NP (bioMérieux). A collection of 175 isolates including 120 carbapenemase producers and 55 non-carbapenemase producers was tested. Samples were tested blind in the three participating centres. The repeatability of the MBT STAR®-Carba IVD Kit was also evaluated.

Results: The three MALDI-TOF techniques possess sensitivities ranging from 95% to 100% and specificities from 98.2% to 100% compared with 99.2% and 100%, respectively, for the RAPIDEC® CARBA NP. The MBT STAR®-Carba IVD Kit gave highly reproducible results and is the only technique able to provide a concomitant identification of the bacterial isolate. The three MALDI-TOF techniques possess a fast turnaround time (less than 1.5 h).

Conclusions: Overall, MALDI-TOF is a reliable technique for the rapid detection of CPE from cultured colonies. MBT STAR®-Carba IVD Kit, the only commercially available assay, could easily be implemented in a clinical microbiology laboratory if it is already equipped with a Microflex LT Biotyper mass spectrometer.

Resistenza alla colistina: come rilevarla?

- EUCAST ha evidenziato in alcuni warnings che la rilevazione delle CMI per colistina utilizzando la diffusione a gradiente ha un inaccettabile livello di accuratezza.
- Anche per metodi automatici si sono riscontrati livelli di very major errors non accettabili; almeno due produttori raccomandano di non refertare i valori ottenuti senza un controllo con una metodica di riferimento.
- Gold standard «reale»: metodica di agar-diluizione; gold standard «pratico» la microdiluizione in brodo (diverse metodiche commerciali)
- Come tradurre nella pratica clinica queste osservazioni? Refertare la colistina? Quale dato epidemiologico?

Diagnostic Microbiology and Infectious Disease 90 (2018) 7–10

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Rapid Polymyxin NP test for the detection of polymyxin resistance mediated by the *mcr-1/mcr-2* genes

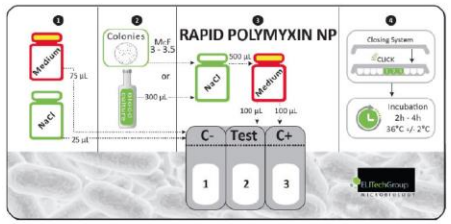
Laurent Poirel [a,b,c], Yu Larpin [a,b], Jan Dobias [a], Roger Stephan [d], Jean-Winoc Decousser [e,f], Jean-Yves Madec [g], Patrice Nordmann [a,b,c,h,*]

PRINCIPLE

The **Rapid Polymyxin NP, Rapid Polymyxin *Pseudomonas* and Rapid Polymyxin *Acinetobacter* tests** have been designed for detecting Polymyxin resistance and susceptibility of *Enterobacteriaceae, P. aeruginosa, A. baumannii,* respectively, from colonies grown on agar plates. For enterobacterial species, the test can even be performed directly from positive blood cultures.This liquid method relies on the rapid and easy visualization of colorimetric reactions.

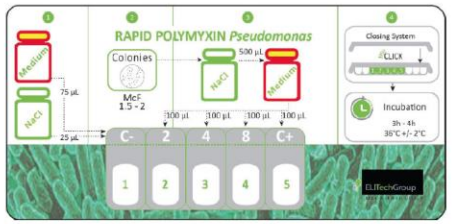
METHODOLOGY

An easy and visual protocol sticked on each test:



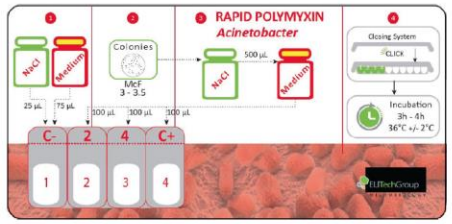
Rapid Polymyxin NP

Resistance at 2mg/L*



Rapid Polymyxin *Pseudomonas*

Resistance at 2-4-8mg/L*



Rapid Polymyxin *Acinetobacter*

Sensitivity at 2-4mg/L*

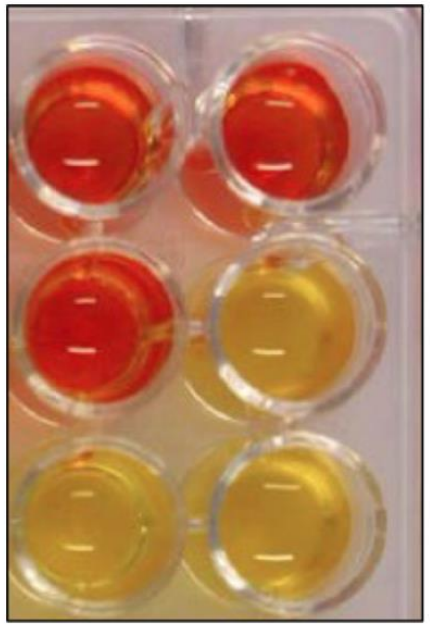
** Pictures illustrating the products are only indicative*

Solution with colistin **Colistin-free solution**

NaCl alone

Colistin-susceptible isolate

Colistin-resistant MCR-1-producing isolate



Clinical Microbiology and Infection 24 (2018) 865–870

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.

E. Matuschek*, J. Åhman, C. Webster, G. Kahlmeter

EUCAST Development Laboratory, Växjö, Sweden

Table 2
Essential and categorical agreements for colistin MIC tests for 75 Gram-negative bacteria with MICs on frozen broth microdilution panels as reference

	Organism	*E. coli* and *K. pneumoniae* (*n*=32)	*P. aeruginosa* (*n*=21)	*Acinetobacter* spp. (*n*=22)	All isolates (*n*=75)
	Colistin reference MIC range (mg/L)	0.25–32	0.25–128	0.5–32	0.25–128
% Essential agreement (EA)[a]	Sensititre custom plate[b]	96	100	91	96
	MICRONAUT-S	97	100	91	96
	MICRONAUT MIC-Strip	97	100	100	99
	SensiTest[c]	96	93	71	88
	UMIC[d]	91	75	77	82
	Etest, Oxoid MH	84	62	59	71
	Etest, BBL MH	63	52	4.5	43
	Etest, MHE	75	43	9.1	47
	MTS, Oxoid MH	59	57	41	53
	MTS, BBL MH	75	57	59	65
% Categorical agreement (CA)[e]	Sensititre custom plate	97	95	91	95
	MICRONAUT-S	94	86	86	89
	MICRONAUT MIC-Strip	94	91	86	91
	SensiTest	94	91	82	89
	UMIC	94	91	91	92
	Etest, Oxoid MH	94	71	73	81
	Etest, BBL MH	94	67	68	79
	Etest, MHE	94	76	82	85
	MTS, Oxoid MH	81	71	82	79
	MTS, BBL MH	84	71	68	76
Number of major errors (ME)[f]	Sensititre custom plate	1	1	2	4
	MICRONAUT-S	2	1	3	6
	MICRONAUT MIC-Strip	2	0	3	5
	SensiTest	2	1	4	7
	UMIC	2	1	0	3
	Etest, Oxoid MH	2	0	0	2
	Etest, BBL MH	1	0	0	1
	Etest, MHE	2	0	0	2
	MTS, Oxoid MH	0	0	0	0
	MTS, BBL MH	0	0	0	0
Number of very major errors (VME)[g]	Sensititre custom plate	0	0	0	0
	MICRONAUT-S	0	2	0	2
	MICRONAUT MIC-Strip	0	2	0	2
	SensiTest	0	1	0	1
	UMIC	0	1	2	3
	Etest, Oxoid MH	0	6	6	12
	Etest, BBL MH	1	7	7	15
	Etest, MHE	0	5	4	9
	MTS, Oxoid MH	6	6	4	16
	MTS, BBL MH	5	6	7	18

[a] MICs being within ± 1 dilution of reference MICs.
[b] Because of truncations in the MIC dilutions, the total number of tests for calculation of EA was 28 for *E. coli/K. pneumoniae* and 19 for *P. aeruginosa*.
[c] Because of truncations in the MIC dilutions, the total number of tests for calculation of EA was 26 for *E. coli/K. pneumoniae*, 15 for *P. aeruginosa* and 17 for *Acinetobacter* spp.
[d] Because of truncations in the MIC dilutions, the total number of tests for calculation of EA was 20 for *P. aeruginosa*.
[e] Test results with correct susceptibility categorization.
[f] Resistant with test method, susceptible with reference method = false resistant.
[g] Susceptible with test method, resistant with reference method = false susceptible.

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BACTERIOLOGY

Clinical Validation of SensiTest Colistin, a Broth Microdilution-Based Method To Evaluate Colistin MICs

Edoardo Carretto,[a] Flavia Brovarone,[a] Giuseppe Russello,[a] Paola Nardini,[a] Maisra M. El-Bouseary,[b] Ali F. Aboklaish,[b] Timothy R. Walsh,[b] Jonathan M. Tyrrell[b]

[a]Clinical Microbiology Laboratory, IRCCS Arcispedale S. Maria Nuova, Reggio Emilia, Italy

[b]Department of Medical Microbiology and Infectious Diseases, Institute of Infection and Immunity, Heath Park Hospital, Cardiff, Wales, United Kingdom

ABSTRACT The global spread of multidrug-resistant Gram-negative bacteria has led to the return of colistin for treating severe infections. Recently, different plasmid-mediated genes conferring resistance to this drug were described and reported worldwide. International committees (EUCAST/CLSI) reevaluated inconsistencies surrounding colistin antimicrobial susceptibility testing (AST), concluding that broth microdilution (BMD) should serve as the reference method for AST. The development of an accurate, reproducible commercial test based on BMD is therefore highly desirable. SensiTest Colistin (STC), a BMD-based compact 4-test panel containing the lyophilized antibiotic in 7 2-fold dilutions (0.25 to 16 μg/ml) was here compared with the EUCAST-CLSI standard reference method (BMD) and, for some isolates, with the automated Phoenix 100 system (PHX). A total of 353 bacterial strains were evaluated by two different laboratories; 137 isolates were resistant to colistin (19 were intrinsically resistant, 83 harbored the *mcr-1* gene). Essential agreement (EA) between STC and BMD was obtained for 339 out of the 353 strains tested (96.0%). Overall categorical agreement was obtained for 349 out of the 353 strains analyzed (98.9%). Two major errors (MEs; 0.93%) and two very major errors (VMEs; 1.46%) were documented. STC appeared to be a simple but highly reliable test with good reproducibility even with panels stored at room temperature or at 35°C. Moreover, STC showed a good performance with strains carrying the *mcr-1* gene, with a 98.8% EA. As the secondary endpoint of our study, VMEs for PHX were documented for 6 isolates (10%).

KEYWORDS multidrug resistance, colistin, MIC, SensiTest Colistin, antimicrobial susceptibility testing, Phoenix 100 system, Liofilchem, Becton Dickinson

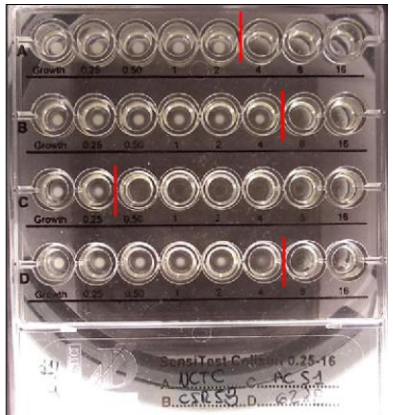
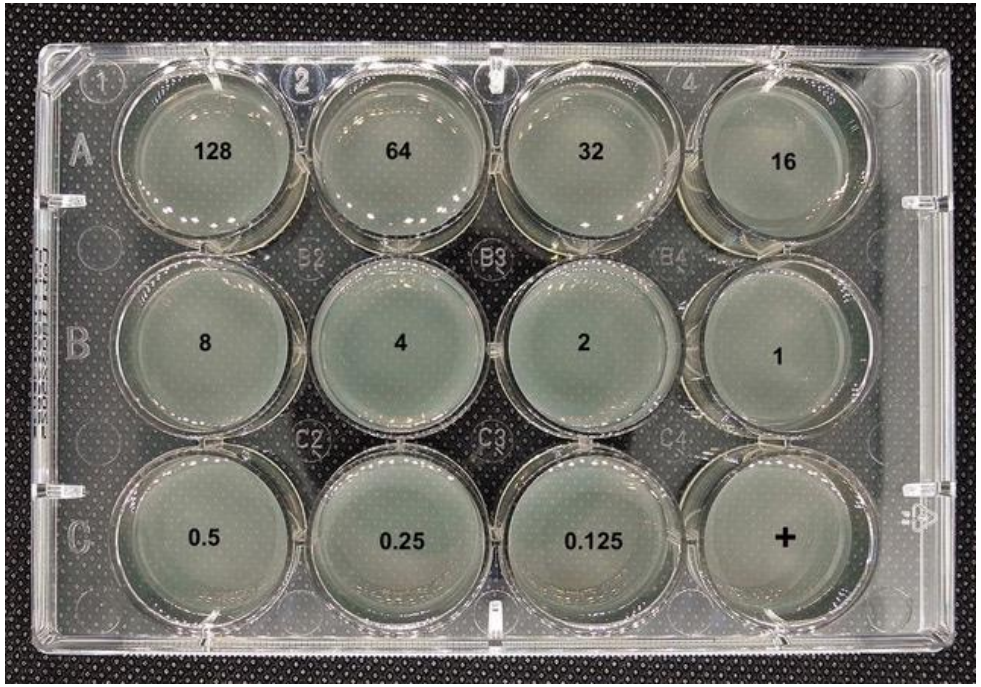


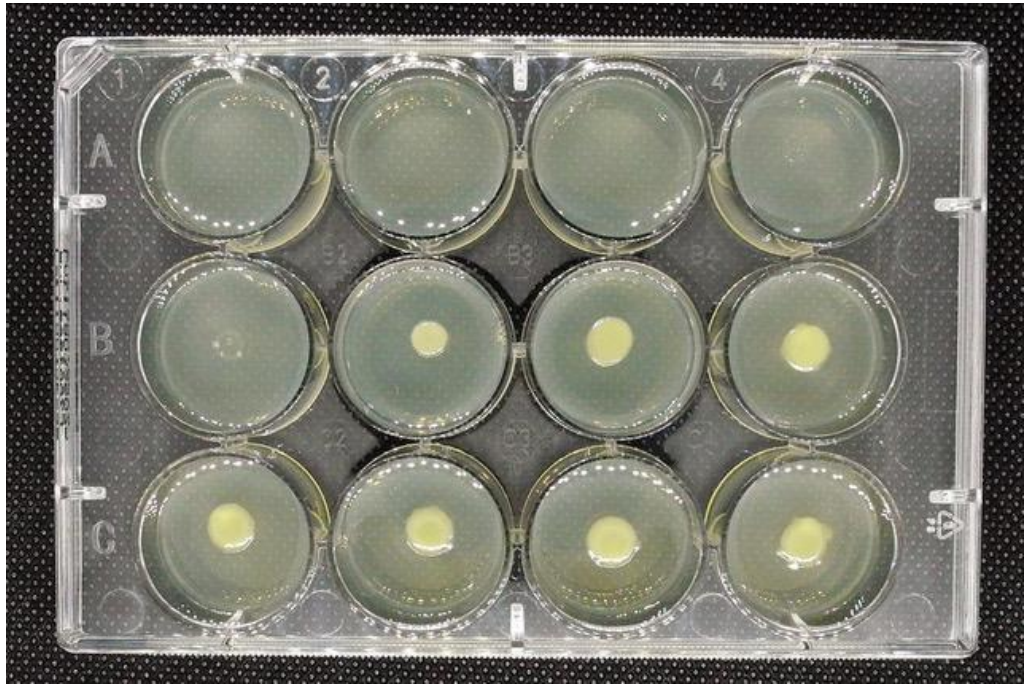
TABLE 1 Study results[a]

Strain	Total no. of isolates (no. colistin resistant[b])	No. of isolates for which MIC was:		% EA	No. (%) of strains for which the following were obtained:		
		Same	±1 dilution		CA	ME	VME
Acinetobacter baumannii	6 (0)	1	5	100.0	6 (100)	0	0
Acinetobacter species[c]	4 (1)	3	1	100.0	4 (100)	0	0
Citrobacter koseri	1 (0)	0	1	100.0	1 (100)	0	0
Enterobacter aerogenes	4 (0)	1	1	50.0	3 (75.0)	1	0
Enterobacter cloacae complex	4 (2)	1	3	100.0	4 (100)	0	0
Escherichia coli	205 (89)	80	119	97.1	204 (99.5)	0	1
Hafnia alvei	6 (6)	3	2	83.3	6 (100)	0	0
Klebsiella oxytoca	6 (0)	2	4	100.0	6 (100)	0	0
Klebsiella pneumoniae	76 (23)	34	38	94.7	75 (98.7)	1	0
Leclercia adecarboxylata	1 (0)	0	1	100.0	1 (100)	0	0
Morganella morganii	2 (2)	2	0	100.0	2 (100)	0	0
Proteus mirabilis	5 (5)	5	0	100.0	5 (100)	0	0
Providencia species	2 (2)	2	0	100.0	2 (100)	0	0
Pseudomonas aeruginosa	19 (0)	9	10	100.0	19 (100)	0	0
Salmonella species	7 (2)	3	3	85.7	6 (85.7)	0	1
Serratia marcescens	4 (4)	4	0	100.0	4 (100)	0	0
Shigella species	1 (1)	1	0	100.0	1 (100	0	0
Total	353	151	188	96.0	349 (98.9)	2	2
Colistin susceptible	216	81	124	94.9	214 (99.1)	2	NA
Colistin resistant (not due to *mcr-1*)	54	43	9	96.3	53 (98.1)	NA	1
Colistin resistant (due to *mcr-1*)	83	27	55	98.8	82 (98.8)	NA	1
Total	353	151	188	96	349 (98.9)	2	2

[a]EA, essential agreement; CA, categorical agreement; ME, major errors; VME, very major errors; NA, not applicable.
[b]Colistin resistance was defined according to EUCAST breakpoints.
[c]These included one strain each of *Acinetobacter ursingii*, *A. lwoffii*, *A. junii*, and *A. nosocomialis*.

Vecchie molecole, vecchi «nuovi» metodi…






ACCELERATE
pheno
ACCELERATE
pheno
ORDERS
DASHBOARD
RESULTS
Dashboard
Doe, Jane
ID: In Progress
AST: In Progress
:22
4:38
Smith, John
ID: E. coli
AST: In Progress
3:46

1. Add positive blood culture

The sample preparation cartridge automatically isolates bacterial cells from the sample matrix and adjusts the concentration for a controlled inoculation to the AST panel.

2. Choose AST panel disc

Q-linea's unique proprietary technology – the AST panel disc – allows automated time-lapse imaging of bacterial population growth in wells containing different concentrations of antimicrobial agents.

3. Scan and load – tap START RUN

Proprietary algorithms translates visual information into MIC values. Based on EUCAST or CLSI breakpoints, MIC values are interpreted as S, I, or R.

EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Accelerate PhenoTest BC Kit

DECISION SUMMARY

A. DEN Number:

DEN160032

Summary	
Summary of the Benefit(s)	• The PhenoTest BC Kit is the first multiplexed in vitro diagnostic platform to use rapid nucleic acid fluorescence in situ hybridization (FISH) identification and quantitative AST to provide identification and susceptibility testing for sixteen pathogenic species of bacteria and yeast to aid in the diagnosis of bacteremia and fungemia. • The PhenoTest BC Kit returns results to patients and healthcare providers within six and a half hours of positive blood culture result, compared to current methods which may take 1-3 days to return results. • The PhenoTest BC Kit demonstrated PPA greater than 95%, NPA greater than 99%, and EA/CA greater than 90% for all microbial and drug targets.
Summary of the Risk(s)	• False positive results, false negative results, misidentifications and incorrect AST results are the primary risks associated with use of the PhenoTest BC Kit. • A false positive result may lead to unnecessary antimicrobial therapy or incorrect antimicrobial therapy with subsequent delay in diagnosis. Patients may receive unnecessary infection control measures, such as contact isolation, if a highly resistant organism is thought to be present. • A false negative result may result in the inappropriate discontinuation or delay of antimicrobial therapy, with subsequent worsening of infection. Patients may also not receive sufficient infection control measures, such as contact isolation. • Misidentifications may occur if the device identifies the wrong microorganism and could result in the ineffective antimicrobial therapy and/or delay to appropriate therapy. Misidentifications could also result in incorrect infection control measures. • Errors in AST result could result in the wrong antibiotic choice with subsequent worsening of infection, or unnecessarily broad antibiotic treatment with the potential for the development of antimicrobial resistance or increased drug side effects.

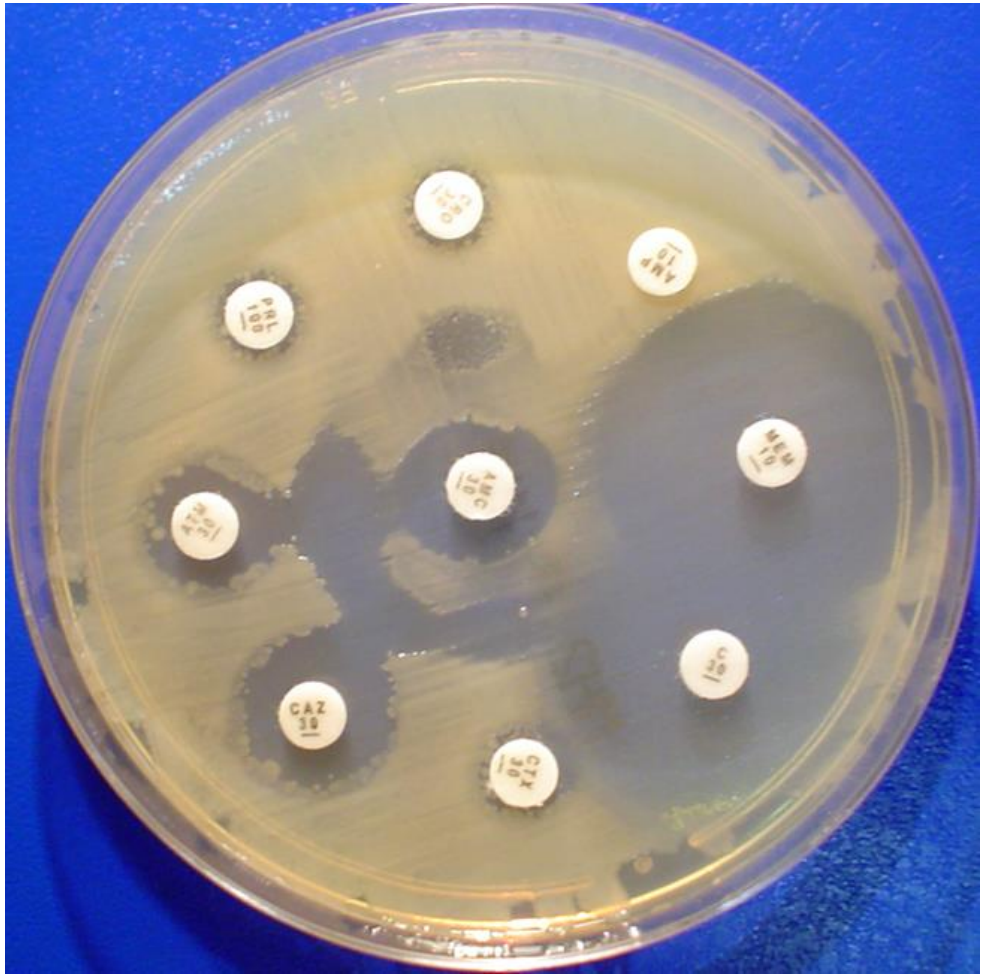
Antibiogramma fenotipico rapido, ad oggi…

- Problemi nella sensibilità analitica?
- Etica nella disponibilità?
- Costi elevati

Secondo tradizione… l'agar diffusione

- Fornisce informazioni relative non solo alla sensibilità – resistenza dei microrganismi, ma anche permette di intuire i meccanismi di resistenza sottostanti

- Richiede tempo per la corretta valutazione

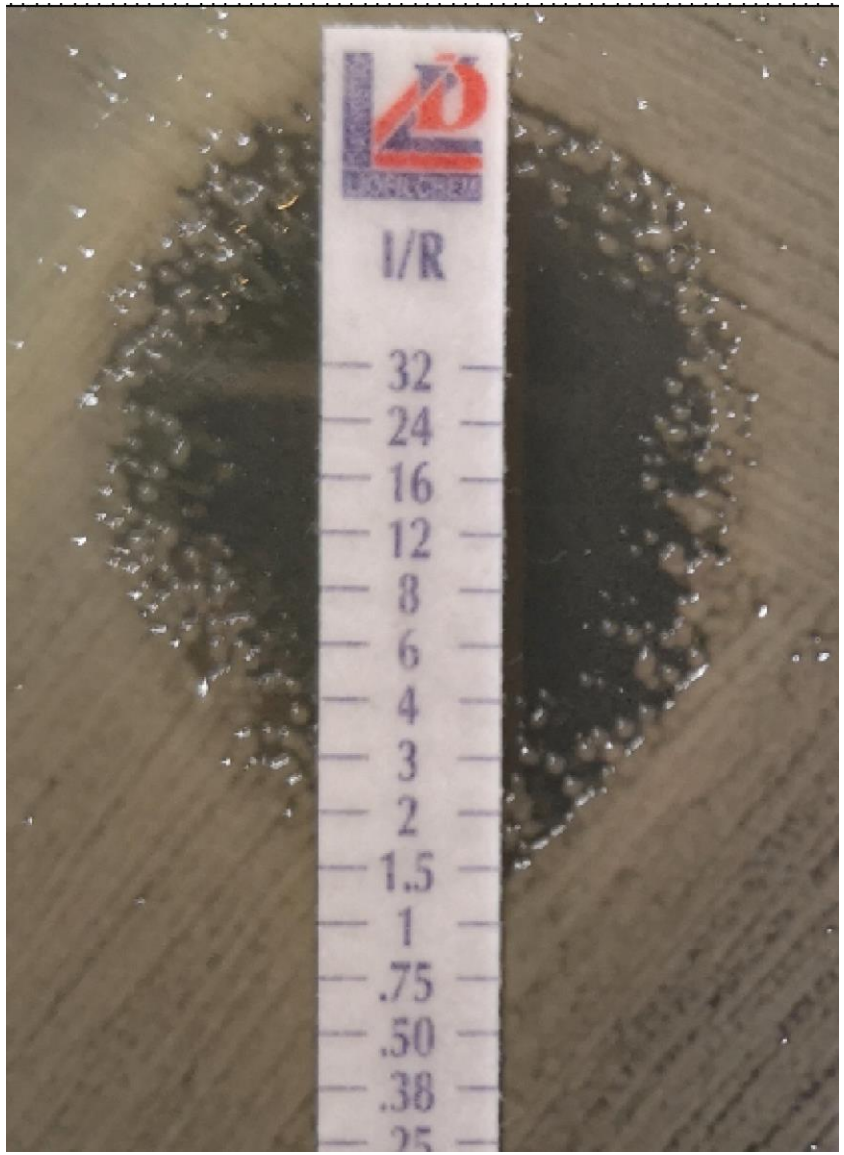


Secondo tradizione… l'agar diffusione

- Nel corso degli anni, abbiamo acquisito nuove competenze e abbiamo imparato a interpretare nuovi meccanismi di resistenza

Secondo tradizione… l'agar diffusione

- Ci aiuta con le nuove molecole, a volte sprofondandoci nei dubbi



ARTICLES
https://doi.org/10.1038/s41564-018-0342-0
nature microbiology

The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification

Hervé Nicoloff[1,3], Karin Hjort[1,3], Bruce R. Levin[2] and Dan I. Andersson[1*]

When choosing antibiotics to treat bacterial infections, it is assumed that the susceptibility of the target bacteria to an antibiotic is reflected by laboratory estimates of the minimum inhibitory concentration (MIC) needed to prevent bacterial growth. A caveat of using MIC data for this purpose is heteroresistance, the presence of a resistant subpopulation in a main population of susceptible cells. We investigated the prevalence and mechanisms of heteroresistance in 41 clinical isolates of the pathogens *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* against 28 different antibiotics. For the 766 bacteria–antibiotic combinations tested, as much as 27.4% of the total was heteroresistant. Genetic analysis demonstrated that a majority of heteroresistance cases were unstable, with an increased resistance of the subpopulations resulting from spontaneous tandem amplifications, typically including known resistance genes. Using mathematical modelling, we show how heteroresistance in the parameter range estimated in this study can result in the failure of antibiotic treatment of infections with bacteria that are classified as antibiotic susceptible. The high prevalence of heteroresistance with the potential for treatment failure highlights the limitations of MIC as the sole criterion for susceptibility determinations. These results call for the development of facile and rapid protocols to identify heteroresistance in pathogens.

Secondo tradizione… l'agar diffusione

Table 5. Substrate profiles of 6'-*N*-aminoglycoside acetyl-transferases [AAC(6')]

Antibiotic	Type of AAC(6')	
	I	II
Gentamicin	–	+
Tobramycin	+	+
Netilmicin	+	+
Amikacin	+	–
Isepamicin	(+)	–
2'-*N*-ethylnetilmicin	+	+
6'-*N*-ethylnetilmicin	–	–
Fortimicin	–	–
Apramycin	–	–
5-episisomicin	+	+

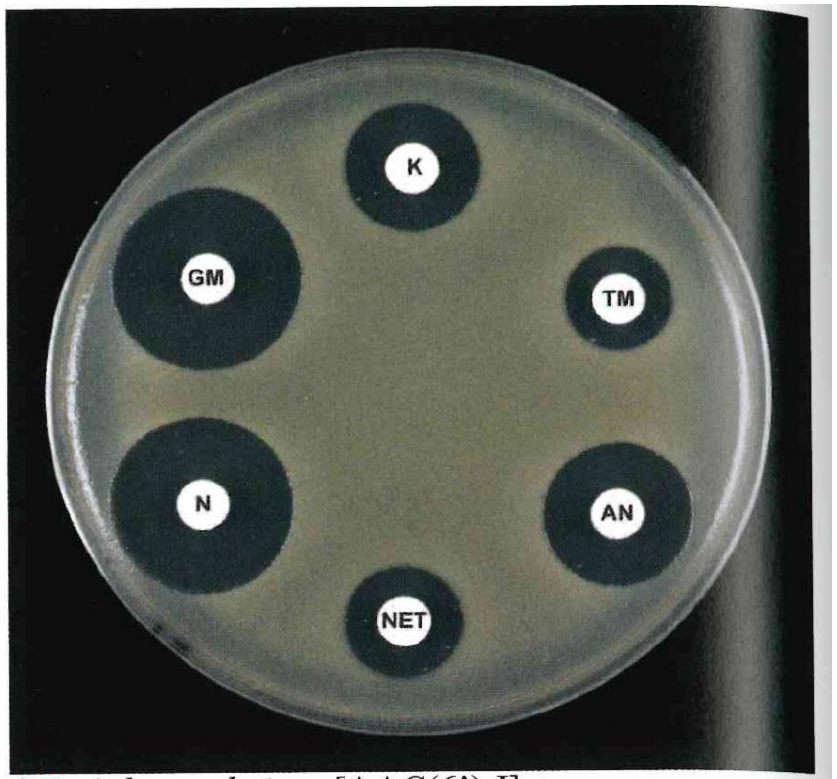
- CI mette di fronte ai nostri limiti di conoscenza, facendoci rendere conto che non abbiamo mai inserito marce oltre la seconda…

Table 4. Genus or species-specific inactivating enzymes in Gram-negative bacilli[a]

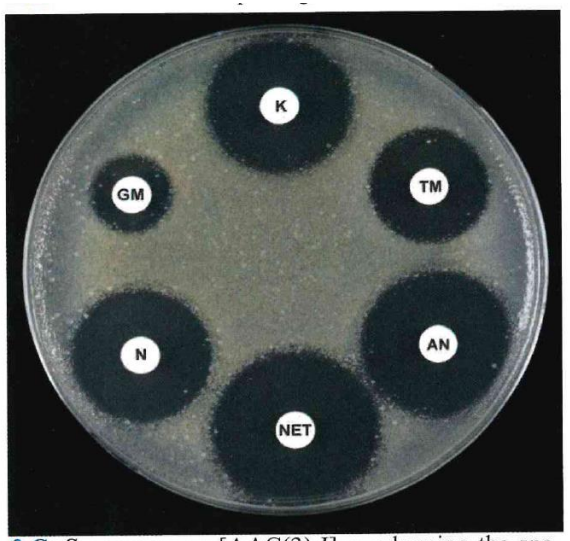
Enzyme	Gene	Organism
AAC(2')	*aac(2')-Ia*	*Providencia* spp.
AAC(3)-III	*aac(3)*	*Pseudomonas* spp.
AAC(6')-Ic	*aac(6')-Ic*	*Serratia marcescens*
AAC(6')-Ig	*aac(6')-Ig*	*A. haemolyticus*
AAC(6')-II	*aac(6')-IIa*	*Pseudomonas* spp.
	aac(6')-IIb	*Pseudomonas* spp.
APH(3')-VI	*aphA-6*	*Acinetobacter* spp.
APH(3')-VII	*aphA-7*	*Campylobacter* spp.

[a] Specificity is absolute for AAC(6')-Ic, AAC(6')-Ig, AAC(3)-III, APH(3')-VII, and AAC(2') and relative in the other cases.

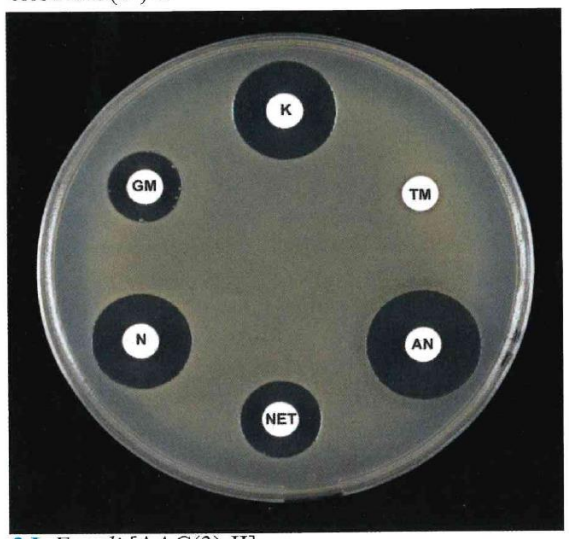
Secondo tradizione… l'agar diffusione



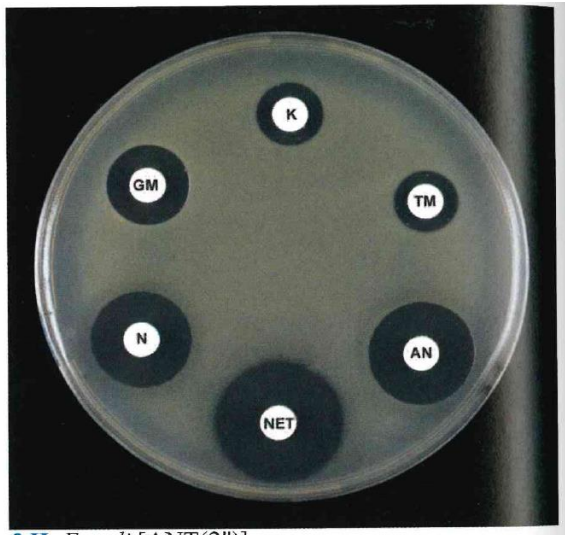
3.F. *A. haemolyticus* [AAC(6')-I].



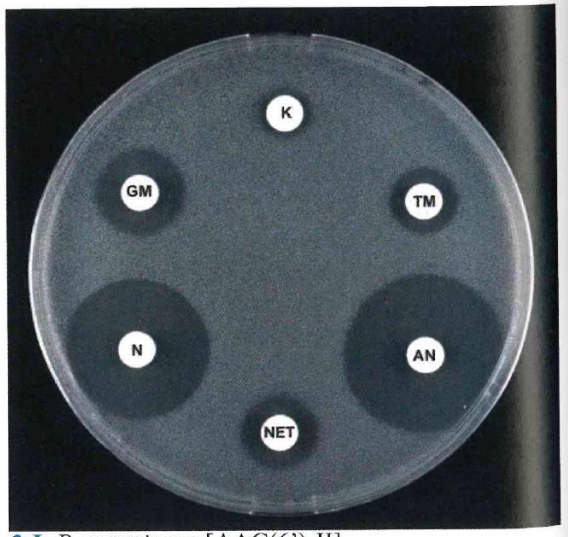
3.G. *S. marcescens* [AAC(3)-I] overlapping the species AAC(6')-I.



3.I. *E. coli* [AAC(3)-II].



3.H. *E. coli* [ANT(2")].



3.J. *P. aeruginosa* [AAC(6')-II].

Secondo tradizione… l'agar diffusione

- Ancora, dopo tanti e tanti anni, non finisce di stupirci…