COMITATO DI STUDIO PER GLI ANTIMICROBICI
CoSA

Corso Precongressuale B:
*La rilevazione dei meccanismi di resistenza agli antibiotici*

# I test fenotipici

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**S.C. Microbiologia e Virologia**
**Azienda Socio Sanitaria Territoriale di Lecco**

XLVI Congresso Nazionale AMCLI, 11 - 14 Novembre 2017
Palacongressi di Rimini

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# EUCAST guidelines for detection of resistan mechanisms and specific resistances of clinical a epidemiological importance

**Version 2.0[1]**

**July 2017**

[1] Based on version 1.0 from December 2013 by the EUCAST subcommittee for detection of resista

# Importanza clinica ed epidemiologica (controllo delle infezioni, salute pubblica)

## Impostazione terapia basata sull'enzima prodotto

| Antibiotico | MIC mg/L (S/I/R) |
|---|---|
| Ampicillina | > 16 (R) |
| Amoxi-Clav | > 16 (R) |
| Piperacillina | > 16 (R) |
| Pip-Tazo | > 64 (R) |
| Cefotaxime | > 32 (R) |
| Ceftazidime | > 32 (R) |
| Cefepime | > 32 (R) |
| Imipenem | 1 (S) |
| Meropenem | 2 (S) |
| Ertapenem | > 4 (R) |

| Antibiotico | MIC mg/L (S/I/R) |
|---|---|
| Ampicillina | > 16 (R) |
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| Cefepime | > 32 (R) |
| Imipenem | 1 (S) |
| Meropenem | 1 (S) |
| Ertapenem | 2 (R) |

**Italia endemica per molte resistenze agli antibiotici**

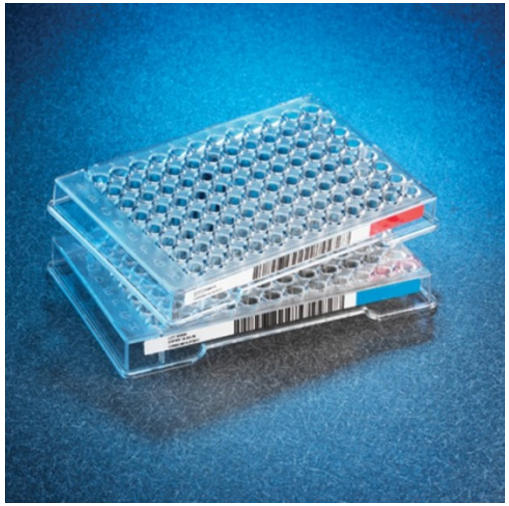
# Conferma dei valori di MIC

**Antibiotici «problematici» se saggiati con metodiche alternative a quella di riferimento:**

- Colistina
- Piperacillina/Tazobactam
- Carbapenemi
- Tigeciclina
- Gentamicina
- Fosfomicina
- Ceftolozano/Tazobactam

L'Etest nelle *Enterobacteriaceae* spesso risulta difficile da interpretare!!

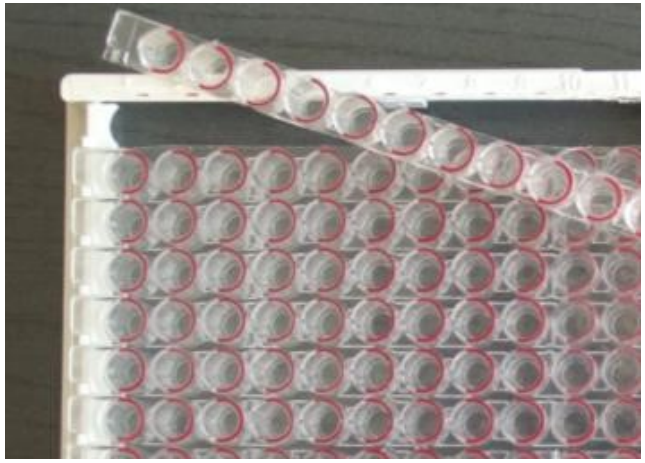
# Microdiluizione in brodo



## Pannello Gram-negativi MDR

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | MERO 0.12 | MERO 0.25 | MERO 0.5 | MERO 1 | MERO 2 | MERO 4 | MERO 8 | MERO 16 | AMI 4 | AM 8 |
| B | GEN 0.5 | GEN 1 | GEN 2 | GEN 4 | GEN 8 | AZT 0.5 | AZT 1 | AZT 2 | AZT 4 | AZ 8 |
| C | CIP 0.06 | CIP 0.12 | CIP 0.25 | CIP 0.5 | CIP 1 | CIP 2 | P/T4 1/4 | P/T4 2/4 | P/T4 4/4 | P/ 8/ |
| D | AUGC 4/2 | AUGC 8/2 | AUGC 16/2 | AUGC 32/2 | AUGC 64/2 | C/T 0.5/4 | C/T 1/4 | C/T 2/4 | C/T 4/4 | C/ 8/4 |
| E | COL 0.25 | COL 0.5 | COL 1 | COL 2 | COL 4 | COL 8 | FOT 0.5 | FOT 1 | FOT 2 | FO 4 |
| F | TGC 0.25 | TGC 0.5 | TGC 1 | TGC 2 | TGC 4 | SXT 1/19 | SXT 2/38 | SXT 4/76 | SXT 8/152 | TO 2 |
| G | TAZ | TAZ | TAZ | TAZ | TAZ | TAZ | CZA | CZA | CZA | CZ |

# Sistemi di microdiluizione in brodo per singolo antibiotico



| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 1 |
|---|---|---|---|---|---|---|---|---|---|
| GC | COL | COL | COL | COL | COL | COL | COL | COL | C |

REVIEW

# EUCAST expert rules in antimicrobial susceptibility testing

## *Staphylococcus aureus*

TABLE 8. Interpretive rules for β-lactam agents and Gram-positive cocci

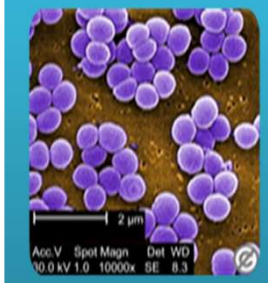
| | | | | | |
|---|---|---|---|---|---|
| 8.2 | *Staphylococcus* spp. | Benzylpenicillin (and β-lactamase detection) | Penicillins apart from isoxazolyl-penicillins and combinations with β-lactamase inhibitors | IF resistant to benzylpenicillin or IF β-lactamase is detected, THEN report as resistant to all penicillins, regardless of MIC, except the isoxazolyl-penicillins and combinations with β-lactamase inhibitors | Testi disc pre >90 pro case |

E' sconsigliato eseguire il test per la produzione di β-lattamasi; in molti Paesi la prevalenza di ceppi produttori di β-lattamasi è >90%, inoltre il test per la produzione di β-lattamasi presenta problemi tecnici inerenti la metodica. In questo contesto, potrebbe essere appropriato riportare tutti gli isolati come resistenti alla benzilpenicillina, ampicillina ed amoxicillina.

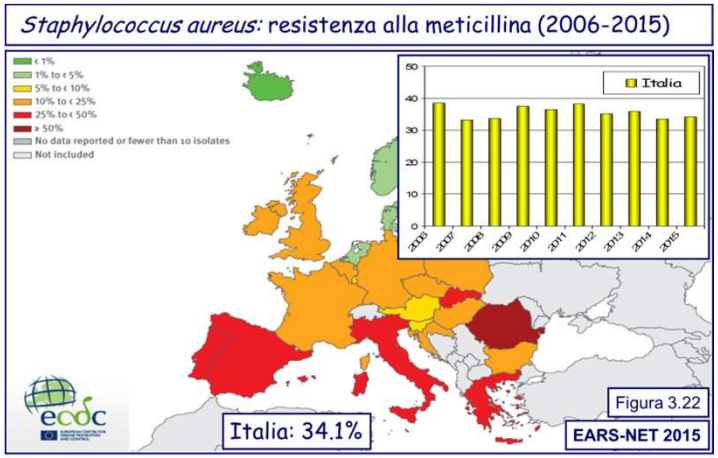
- Un test di induzione della β-lattamasi deve essere eseguito sugli stafilococchi se la penicillina ha…
  - MIC ≤ 0.12 mg/L
  - Alone ≥ 29 (26) mm
  - …..prima di refertare l'isolato come penicillina sensibile

- Inoculare una piastra di Agar Sangue
- Disco di Oxacillina
- Incubare
- Test dell
  (con Nitr
  prelevate
  dell'alone

# Principali meccanismi di resistenza in *Staphylococcus aureus*




**MecA – MecC**



**hVISA/VISA/VRSA**

# La rilevazione degli MRSA deve essere effettuata saggiando l'oxacillina, mediante disco-diffusione?

- *mecA* viene espresso a livelli più alti in presenza di Cefoxitina rispetto a Oxacillina
- L'alone di Cefoxitina è più netto (migliore leggibilità)
- Leggere a luce riflessa

**La cefoxitina è preferita anche in caso di saggio in microdiluizione in brodo**. A causa dell'espressione eterogenea della resistenza, **l'Oxacillina potrebbe risultare sensibile in ceppi meticillino-resistenti.**

Esistono dei ceppi ***mecA* positivi che mostrano sensibilità a Cefoxitina e all'Oxacillina, nei quali *mecA* è presente ma non è espresso** (**OS-MRSA**). La loro frequenza è di circa il **3%**. La rilevazione è basata esclusivamente su metodi molecolari. La reversione in ceppi resistenti all'oxacillina potrebbe avvenire in corso di terapie prolungate.

# Test per la Resistenza *mec*A-mediata

| | | Alone (mm) | | | MIC (mg/L) | |
|---|---|---|---|---|---|---|
| | | S | I | R | S | R |
| *S. aureus*<br>*S. lugdunensis* | Oxacillina | - | - | - | ≤ 2 | > 2 |
| *Stafilococchi CN* | | - | - | - | ≤ 0.25 | >0.25 |
| *S. aureus*<br>*S. lugdunensis* | Cefoxitin<br>30 µg | ≥ 22** | - | < 22 * | ≤ 4 | > 4 |
| *Stafilococchi CN* | | ≥ 25** | - | < 24 * | - | - |

** Refertare Oxacillina-S * Refertare Oxacillina-R

**EUCAST = CLSI**

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

**CLSI M100, 27th Ed., p.59-60 (2017)**

JCM
Journals.ASM.org

# Use of Vitek 2 Antimicrobial Susceptibility Profile To Ide
# in Methicillin-Resistant *Staphylococcus aureus*

TABLE 1 Results of Vitek 2 antimicrobial susceptibility testing of *Staphylococcus aureus* isolates

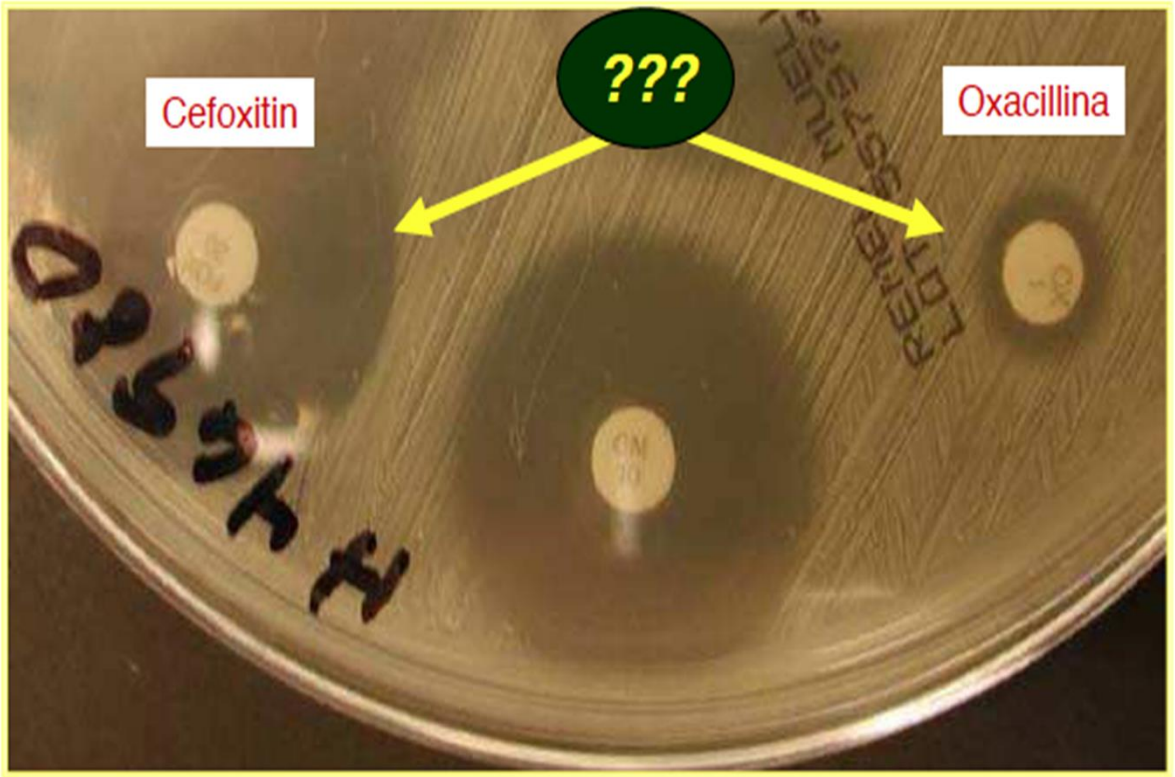
| Identity of *S. aureus* isolate[a] | Total no. of isolates | No. of susceptible and/or resistant isolates/total no. of isolates (% | | |
|---|---|---|---|---|
| | | Oxacillin S and cefoxitin R (S/R) | Oxacillin R and cefoxitin R (R/R) | Oxacillin R cefoxitin S ( |
| MRSA *mecC* positive | 62 | 55/62 (88.7) | 7/62 (11.3) | 0/62 (0) |
| MRSA *mecA* positive | 455 | 4/455 (0.9) | 446/455 (98.0) | 5/455 (1.1) |

August 2013 Volume 51 Number 8

Difficoltà nell'interpretazione dei risultati: discordanza tra cefoxitina ed oxacillina
Alcuni test molecolari rilevano *mecC*

*Staphylococcus aureus*

# Risultati discrepanti?



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nore, some isolates express low-level resistance
are *mecA* and *mecC* negative and do not produce alternative P
susceptible *S. aureus* (BORSA)]. These strains are relatively rare and
of resistance is poorly characterize but may include hyperpı

**Meccanismi coinvolti:**

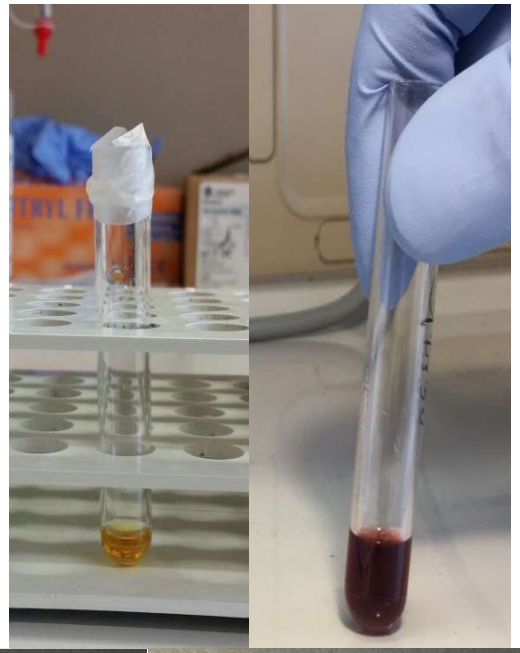
- iperproduzione di β-lattamasi (Sierra-Madero et al., 1988 AAC)
- produzione di meticillinasi (Massida et al., 1992 MDR)
- mutazioni specifiche nelle PBP in prossimità del sito catalitico (Nadarajah, 2006 JMM)

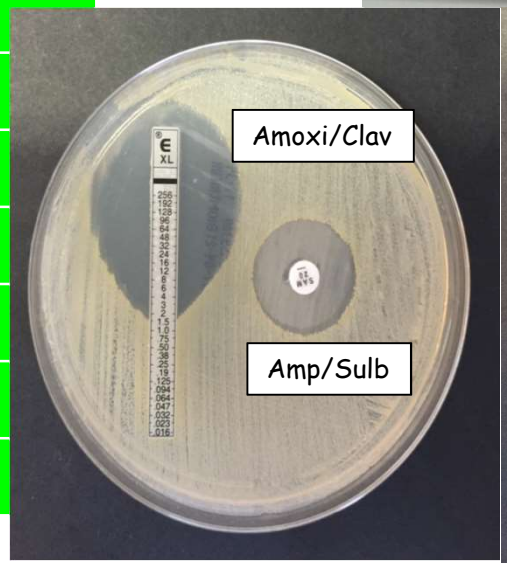
## *Staphylococcus aureus*

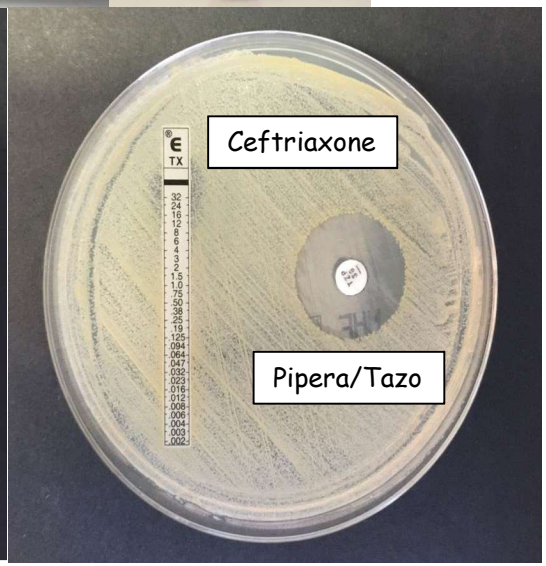
| Antibiotic | MIC mg/L(S/I/R) |
|---|---|
| Cefoxitin screening | Neg |
| Benzilpenicillina | >0.5 (R) |
| Oxacillina | >2 (R) |
| Gentamicina | <=0.5 (S) |
| Levofloxacina | 0.5 (S) |
| Res. Ind. Clinda | Neg |
| Eritromicina | 1 (S) |
| Clindamicina | 0.25 (S) |
| Linezolid | 2 (S) |
| Daptomicina | 0.5 (S) |
| Teicoplanina | <=0.5 (S) |
| Vancomicina | 1 (S) |
| Tigeciclina | <=0.12 (S) |
| Rifampicina | <=0.03 (S) |
| Trimet/Sulfam. | <=10 (S) |

## Test di conferma

| | |
|---|---|
| Disco Cefoxitina | >30 mm (S) |
| MIC Oxacillina BMD | 2 (S) |





**Specimen 3955**

This specimen contained a meticillin sensitive *Staphylococcus aureus* res
and fusidic acid.

The isolate was sensitive to cefoxitin and negative for mecA and me
cefoxitin MIC (4mg/L) was very close to the breakpoint; EUCAST and
breakpoint, ≤4 mg/L. Overall performance was acceptable, with 91.1
reporting a correct sensitive result. However, for both EUCAST a
Although both EUCAST and CLSI provide MIC breakpoints for oxacillin (E
CLSI S≤2, R≥4), they do not provide a disk diffusion method, and both rec
testing for the identification of meticillin resistance. Performance overall wa
of laboratories reporting an incorrect resistant result. As with cefoxitin, per

**Isolato con sensibilità borderline per l'oxacillina**

Cefoxitina

| score | 2 | 1 | 0 | % co |
|---|---|---|---|---|
| BSAC | 29 | 0 | 1 | 96.7 |
| EUCAST | 364 | 3 | 32 | 91.2 |
| CLSI | 60 | 0 | 9 | 87.0 |

**Specimen : 3955** *Staphylococcus aureus*

ISO MIC mg/l

Oxacillina

| Guideline | Method | n (%*) of participants | |
|---|---|---|---|
| | | S (%) | I (%) |
| EUCAST/EUCAST-related | Automated | 84 (36.5) | 0 (0) |
| | Disk diffusion | 24 (64.9) | 0 (0) |
| | MIC | 32 (65.3) | 0 (0) |
| | Multi/other | 21 (63.6) | 0 (0) |

Table 1. Interpretation when oxacillin and cefoxitin results are discrepant.

| | | Cefoxitin result by MIC or disk diffusion | |
|---|---|---|---|
| | | S | R |
| Oxacillin result by MIC | S | Report as oxacillin S | Report as oxacillin R |
| | R | Report as oxacillin R | Report as oxacillin R |

A. Broth microdilution:
Standard methodology (ISO 20776-1) is used and strains with cefoxitin MICs >4 mg/L should be reported as methicillin resistant.

B. Disk diffusion: The EUCAST disk diffusion method is used. Strains with a cefoxitin (30 µg disk) zone diameter <22 mm should be reported as methicillin resistant.

Necessità di utilizzare entrambe le molecole per comprendere i vari fenotipi coinvolti

Saggiare la MIC per l'oxacillina

# Worldwide Epidemiology and An
## Resistance of *Staphylococcus aure*

Monica Monaco, Fernanda Pimentel de Araujo, Melania Cruciani, Eliana M. Coccia and Annalisa Pantosti

**VRSA**

**Table 1** VRSA isolated from 2002 to 2013 reported in indexed journals

| Country or state or city | Date | Source | Vancomycin MIC (μg/ml) | SCC*mec* | *spa* type | MLST | CC | References |
|---|---|---|---|---|---|---|---|---|
| USA/MI | 2002 | Plantar ulcers | 1024 | II | t062 | ST371 | CC5 | Weigel et al. 2003, Limbago et al. 2014 |
| USA/PA | 2002 | Plantar ulcers | 32 | II | t002 | ST5 | CC5 | Tenover et al. 2004, Sievert et al. 2008 |
| USA/NY | 2004 | Urine | 64 | IV | t002 | ST5 | CC5 | Sievert et al. 2008 |
| USA/MI | 2005 | Toe wound | 256 | II | t002 | ST5 | CC5 | Zhu et al. 2008; Sievert et al. 2008 |
| USA/MI | 2005 | Surgical site wound | 512 | II | t002 | ST231 | CC5 | Zhu et al. 2008; Sievert et al. 2008 |
| USA/MI | 2005 | Plantar ulcers | 1024 | NT | t002 | ST85 | CC5 | Zhu et al. 2008, Sievert et al. 2008 |
| USA/MI | 2006 | Triceps wound | 512 | II | t062 | ST231 | CC5 | Zhu et al. 2008, Sievert et al. 2008 |
| USA/MI | 2007 | Toe wound | 1024 | ND | t002 | ST5 | CC5 | Finks et al. 2009 |
| USA/MI | 2007 | Surgical wound | 1024 | ND | t002 | ST5 | CC5 | Finks et al. 2009 |

GISA: glycopeptide intermediate *S. aureus*

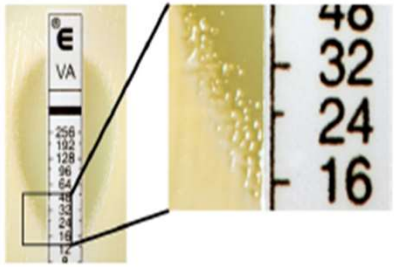
*S. aureus* isolates with low-level resistance to vancomycin (MIC 4 - 8 m

Valori di MIC tra 1 e 2 mg/L

Current Topics in Microbiology and Immunology © Springer International Publishing Switzerland 2016

A. Macro gradient test:

This test gives an indication of reduced vancomycin susceptibility but note that the readings are not MICs. Furthermore, the test does not differentiate between hGISA, GISA and GRSA. The test is set up according to the manufacturer's instructions. Note also that the inoculum is higher (2,0 McFarland) than with standard gradient tests. A positive result is indicated by readings >8mg/L for both vancomycin and teicoplanin.




Introduzione di un commento per gli isolati con MIC confermata a 2 mg/L per la vancomicina
«Isolato con sensibilità borderline per la vancomicina»

As both criteria include teicoplanin, testing of vancomycin could be dependent on the result of the teicoplanin test. The algorithm would then be:

- Teicoplanin reading ≥12 mg/L: GRSA, GISA or hGISA
- Teicoplanin reading 8 mg/L: Test vancomycin. If vancomycin reading is ≥8 mg/L then GRSA, GISA or hGISA
- Teicoplanin reading <8 mg/L: Not GRSA, GISA or hGISA

B. Glycopeptide resistance detection (GRD) gradient test:

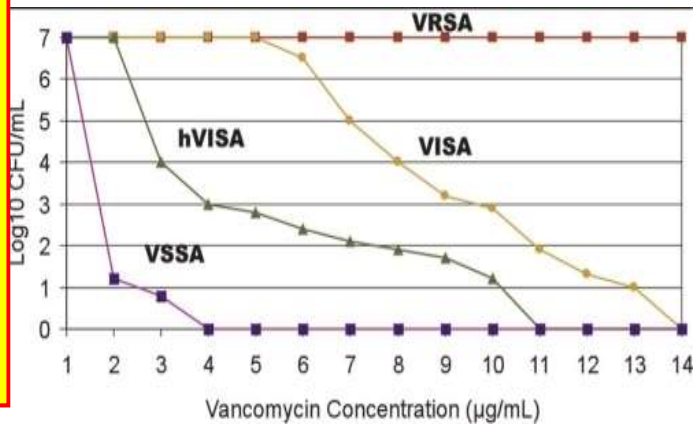
Test according to the manufacturer's instructions. The test is considered positive if the GRD strip result is ≥8 mg/L for either vancomycin or teicoplanin.

D. Confirmatory testing for hGISA/GISA:

Any isolate screening positive for reduced s
GISA by MIC determination may be hGIS
analysis profile-area under curve (PAP-AU
laboratory.

Confronto di AUC del ceppo da saggiare con AUC di un ceppo di riferimento (Mu3, hVISA)

Valori < 0,9: VSSA
Valori tra 0,9 e 1,3: hVISA
Valori > 1,3: VISA



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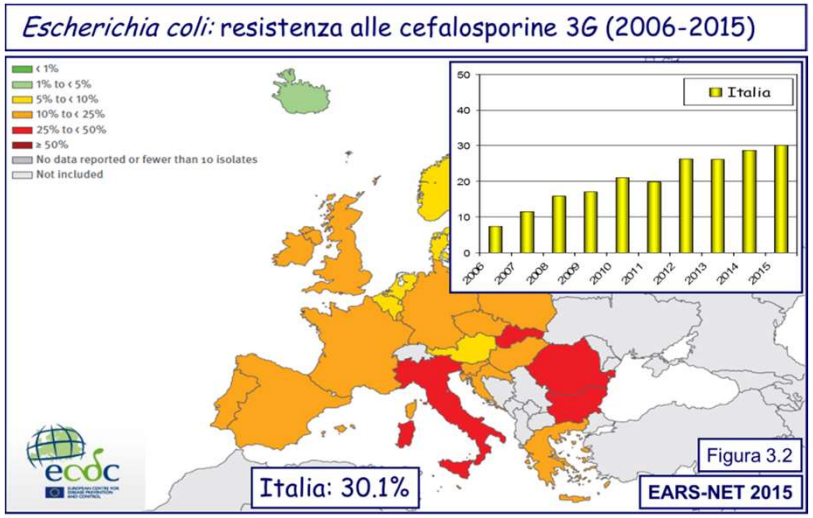


# 3. Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae

## 3.1 Definition

ESBLs are enzymes that hydrolyze most penicillins and cephalosporins, including oxyimino-β-lactam compounds (cefuroxime, third- and fourth-generation cephalosporins and aztreonam) but neither cephamycins nor carbapenems. Most ESBLs belong to the Ambler class A of β-lactamases and are inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) and by diazabicyclooctanones (avibactam) (1).

# 4. Acquired AmpC β-lactamase-producing Enterobacteriaceae

## 4.1 Definition

AmpC-type cephalosporinases are Ambler class C β-lactamases. They hydrolyze penicillins, cephalosporins (including the third-generation but generally not the fourth-generation compounds) and monobactams. In general, AmpC-type enzymes are poorly inhibited by the classical ESBL inhibitors, especially clavulanic acid (1).

TABLE 1. Classification schemes for bacterial β-lactamases, expanded from Bush et al. (1

| Bush-Jacoby group (2009) | Bush-Jacoby-Medeiros group (1995) | Molecular class (subclass) | Distinctive substrate(s) | Inhibited by | | Defining characterist |
|---|---|---|---|---|---|---|
| | | | | CA or TZB[a] | EDTA | |
| 1 | 1 | C | Cephalosporins | No | No | Greater hydrolysis of ceph than benzylpenicillin; hy cephamycins |
| 1e | NI[b] | C | Cephalosporins | No | No | Increased hydrolysis of cef and often other oxyimin lactams |
| 2a | 2a | A | Penicillins | Yes | No | Greater hydrolysis of benzylpenicillin than cephalosporins |
| 2b | 2b | A | Penicillins, early cephalosporins | Yes | No | Similar hydrolysis of benzy and cephalosporins |
| 2be | 2be | A | Extended-spectrum cephalosporins, monobactams | Yes | No | Increased hydrolysis of oxy lactams (cefotaxime, cef ceftriaxone, cefepime, az |
| 2br | 2br | A | Penicillins | No | No | Resistance to clavulanic ac sulbactam, and tazobact |
| 2ber | NI | A | Extended-spectrum cephalosporins, monobactams | No | No | Increased hydrolysis of oxy lactams combined with r to clavulanic acid, sulbac tazobactam |
| 2c | 2c | A | Carbenicillin | Yes | No | Increased hydrolysis of car |
| 2ce | NI | A | Carbenicillin, cefepime | Yes | No | Increased hydrolysis of car cefepime, and cefpirome |
| 2d | 2d | D | Cloxacillin | Variable | No | Increased hydrolysis of clo oxacillin |
| 2de | NI | D | Extended-spectrum cephalosporins | Variable | No | Hydrolyzes cloxacillin or o and oxyimino-β-lactams |
| 2df | NI | D | Carbapenems | Variable | No | Hydrolyzes cloxacillin or o and carbapenems |
| 2e | 2e | A | Extended-spectrum cephalosporins | Yes | No | Hydrolyzes cephalosporins. Inhibited by clavulanic a not aztreonam |
| 2f | 2f | A | Carbapenems | Variable | No | Increased hydrolysis of carbapenems, oxyimino- cephamycins |
| 3a | 3 | B (B1) | Carbapenems | No | Yes | Broad-spectrum hydrolysis |

AmpC

ESBL

# Screening

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A screening breakpoint of >1mg/L is recommended for cefotaxime, ceftriaxone cefpodoxime, in accordance with the guidelines issued by EUCAST and CLSI (Tal EUCAST clinical breakpoint for Enterobacteriaceae is also Susceptible ≤1 mg/L (1 the most sensitive individual indicator cephalosporin for detection of ESBL prod used for screening. However, it is less specific than the combination of cefotaxir and ceftazidime (14, 15) and only the latter compounds are used in the co Corresponding zone diameters for the indicator cephalosporins are shown in Tal

Table 1. ESBL screening methods for Enterobacteriaceae (13-19).

| Method | Antibiotic | Conduct ESBL-testing |
|---|---|---|
| Broth or agar dilution[1] | Cefotaxime/ceftriaxone AND Ceftazidime | MIC >1 mg/L for eithe |
| | Cefpodoxime | MIC >1 mg/L |
| Disk diffusion[1] | Cefotaxime (5 µg) or | Inhibition zone <21 m |
| | Ceftriaxone (30 µg) | Inhibition zone <23 m |
| | AND Ceftazidime (10 µg) | Inhibition zone <22 m |

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

| Method | Antimicrobial agent (disk content) | ESBL confirmation is |
|---|---|---|
| ESBL gradient test | Cefotaxime +/- clavulanic acid | MIC ratio ≥8 or deforme present |
| | Ceftazidime +/- clavulanic acid | MIC ratio ≥8 or deforme present |
| Combination disk diffusion test (CDT) | Cefotaxime (30 µg) +/- clavulanic acid (10 µg) | ≥5 mm increase in inhibi |
| | Ceftazidime (30 µg) +/- clavulanic acid (10 µg) | ≥5 mm increase in inhibi |
| 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 |

Table 3. ESBL confirmation methods for Enterobacteriaceae that are positive in (see Table 1). Group 2 Enterobacteriaceae (see Figure 1).

| Method | Antibiotic | Confirmation is pos |
|---|---|---|
| ESBL gradient test Etest®® ESBL | Cefepime +/- clavulanic acid | MIC ratio ≥8 or defo ellipse present |
| Combination disk diffusion test | Cefepime (30 µg) +/- clavulanic acid (10 µg) | ≥5 mm increase in ir zone |
| Broth microdilution | Cefepime +/- clavulanic acid (fixed concentration 4 mg/L) | MIC ratio ≥8 |
| Double disk synergy test | Cefotaxime, ceftazidime, Cefepime | Expansion of indicat cephalosporin inhibi |

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# Test fenotipici di conferma per ESBL

## Controlli di qualità

| Strain | Mechanism |
|---|---|
| *K. pneumoniae* ATCC 700603 | SHV-18 ESBL |
| *E. coli* CCUG62975 | CTX-M-1 group ESBL and acquired CMY AmpC |
| *E. coli* ATCC 25922 | ESBL-negative |

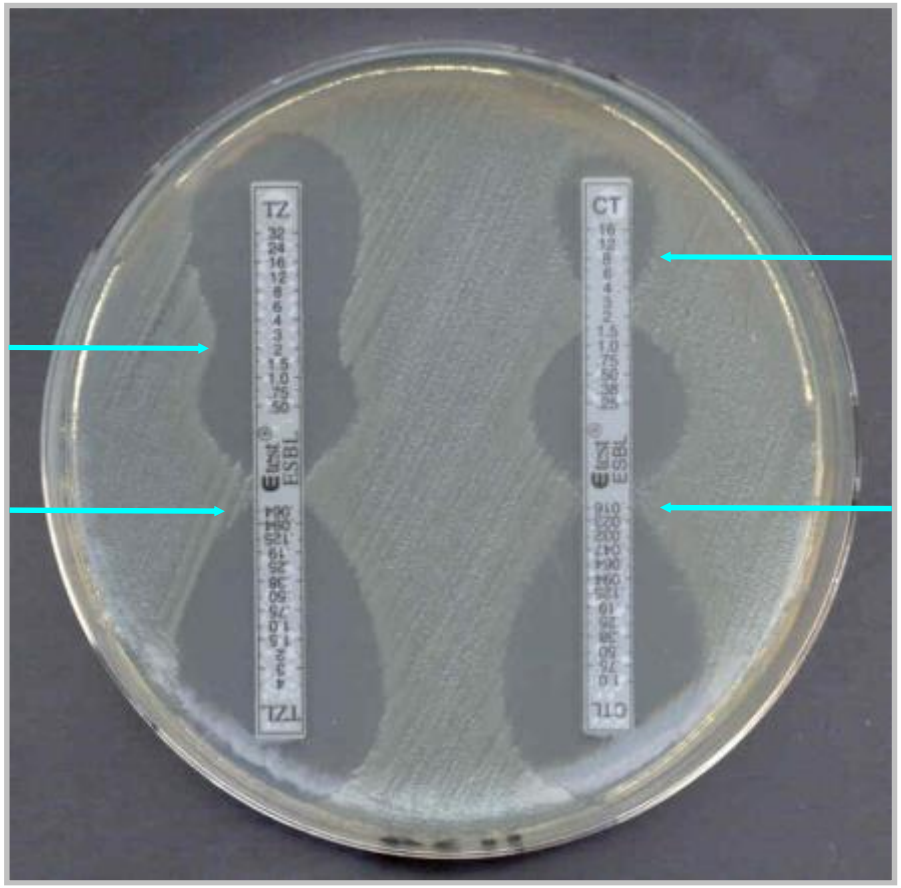
# ESBL gradient test

Ceftazidime

MIC = 2

MIC = 0,064

Ceftazidime
+
Clavulanato

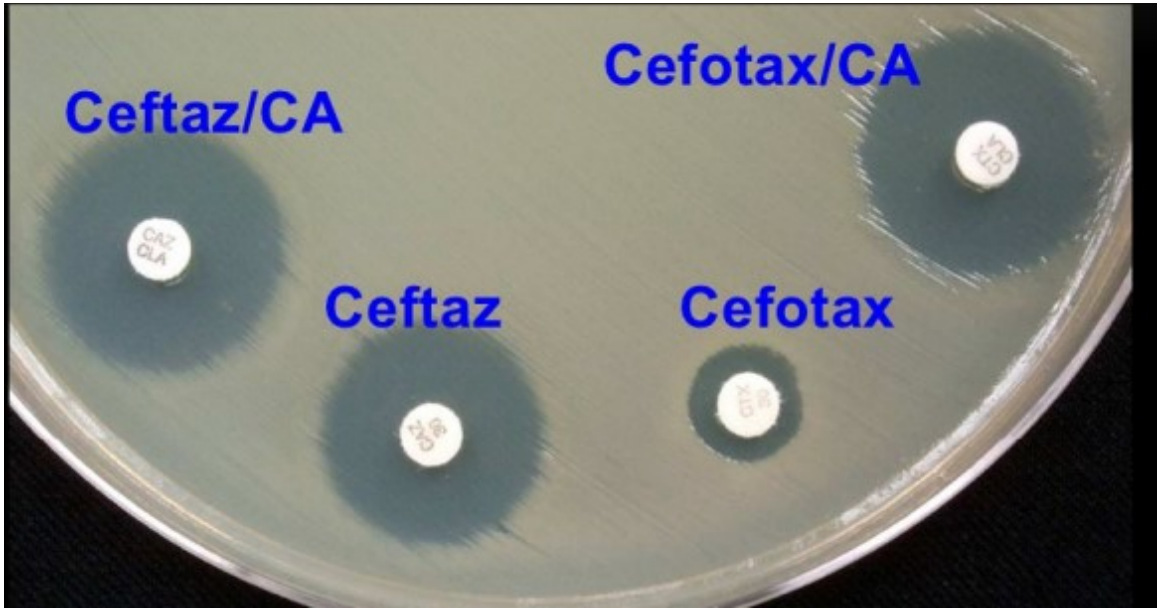



Cefotaxime

MIC = 8

MIC = 0,016

Cefotaxime
+
Clavulanato

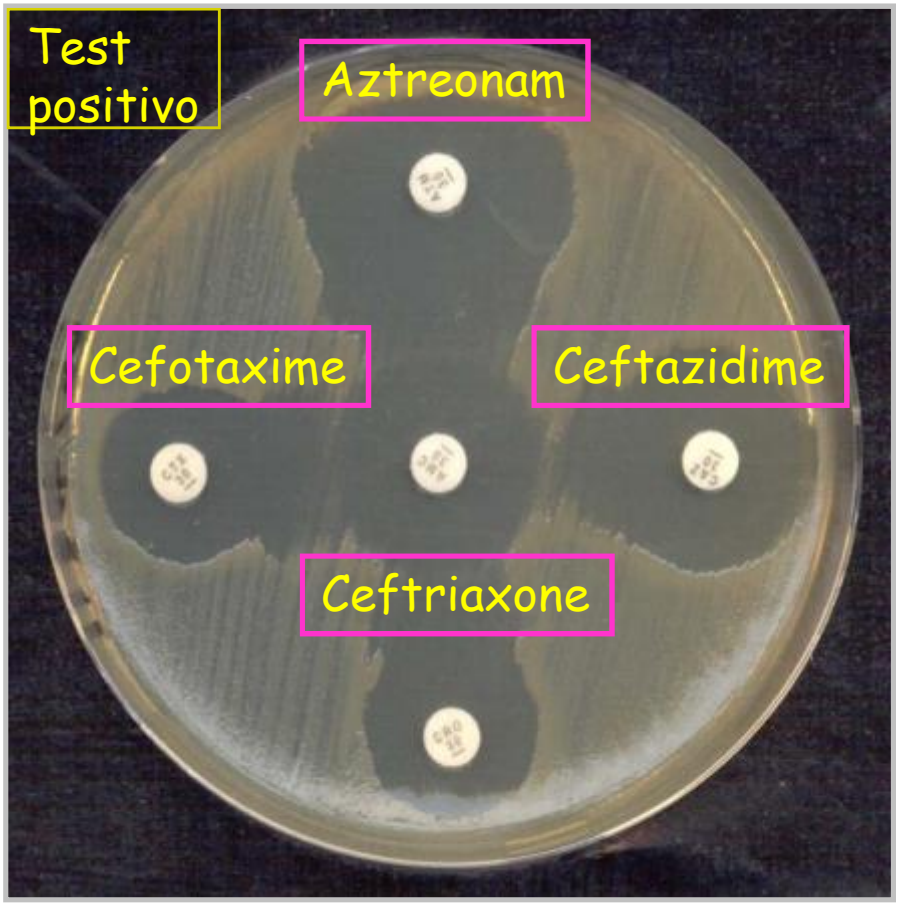


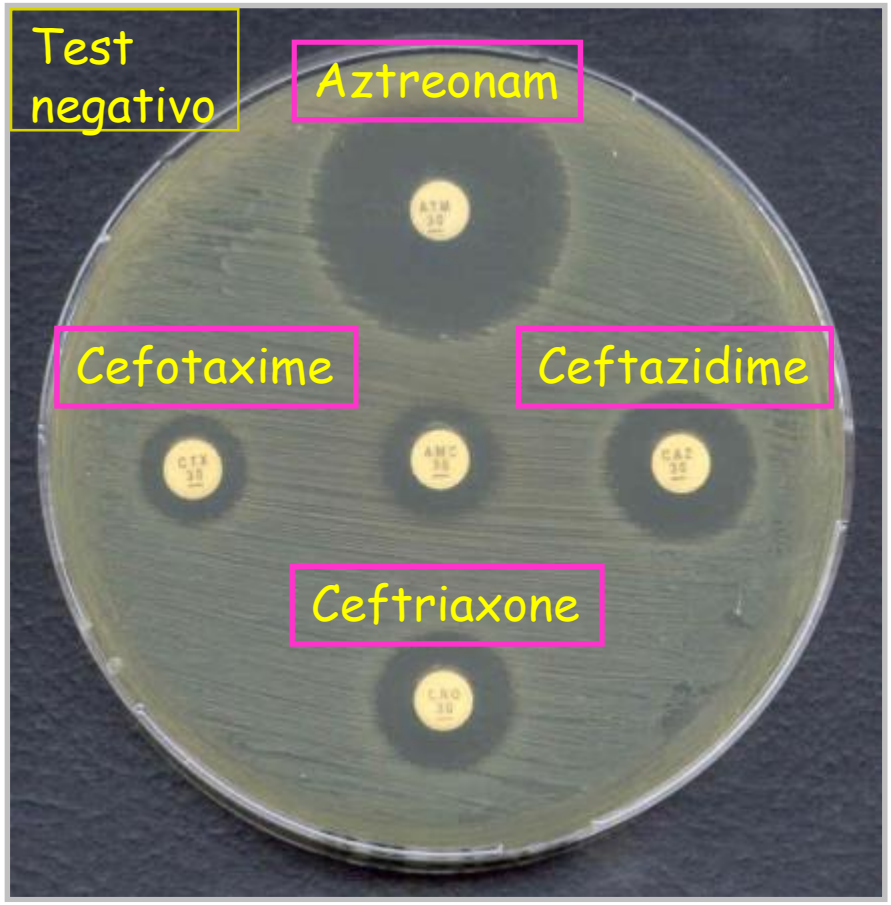

*E. coli*
produttore di ESBL:
dischetti con combinazioni
cefalosporina + clavulanato

Test biochimici
(colorimetrici):
cefalosporina –
cefalosporina + inibitore

Test di sinergia con acido clavulanico

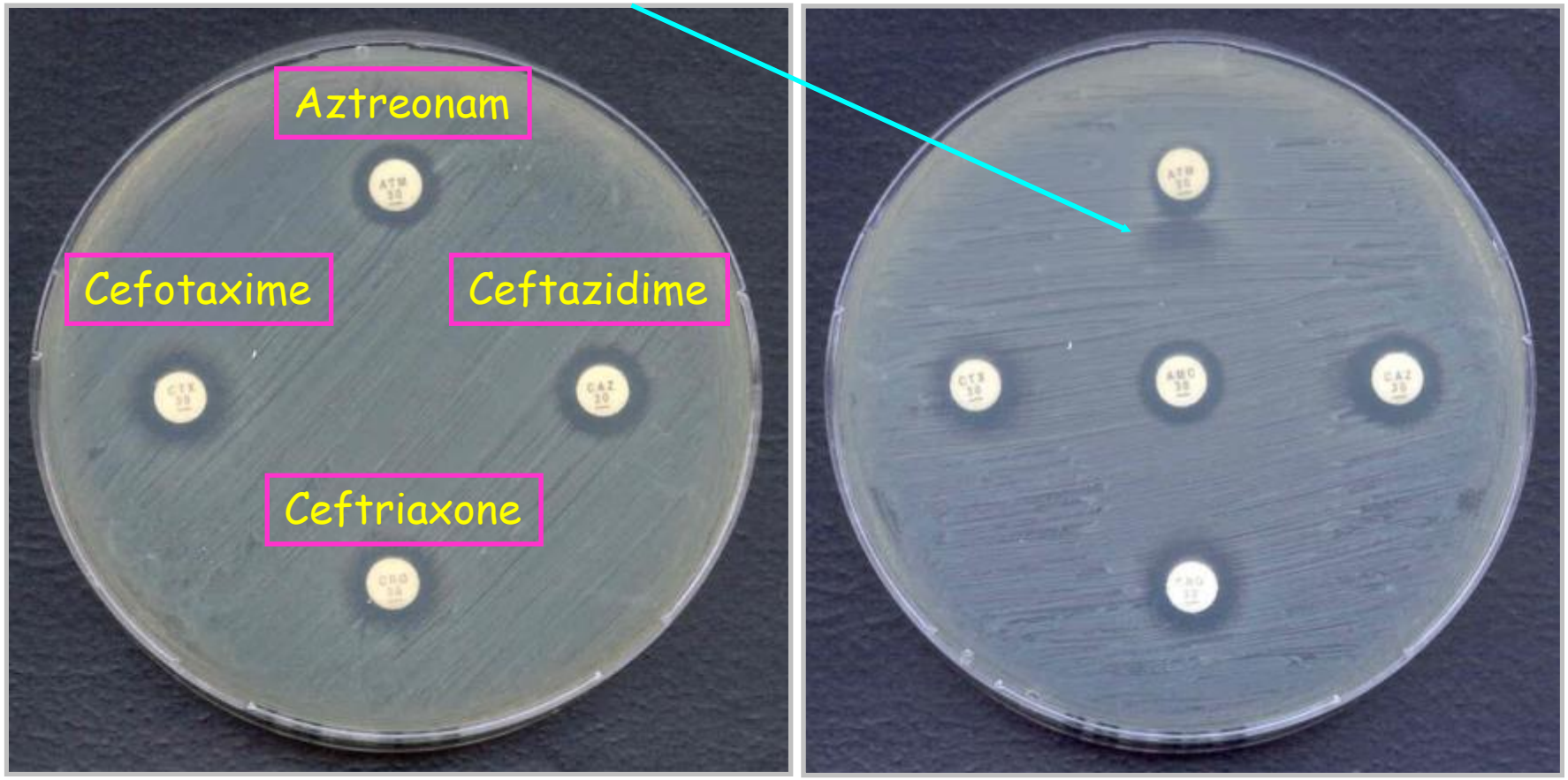
*P. mirabilis* produttore di TEM-92 *P. mirabilis* produttore di CMY-16



# *E. cloacae* iperproduttore di AmpC + ESBL

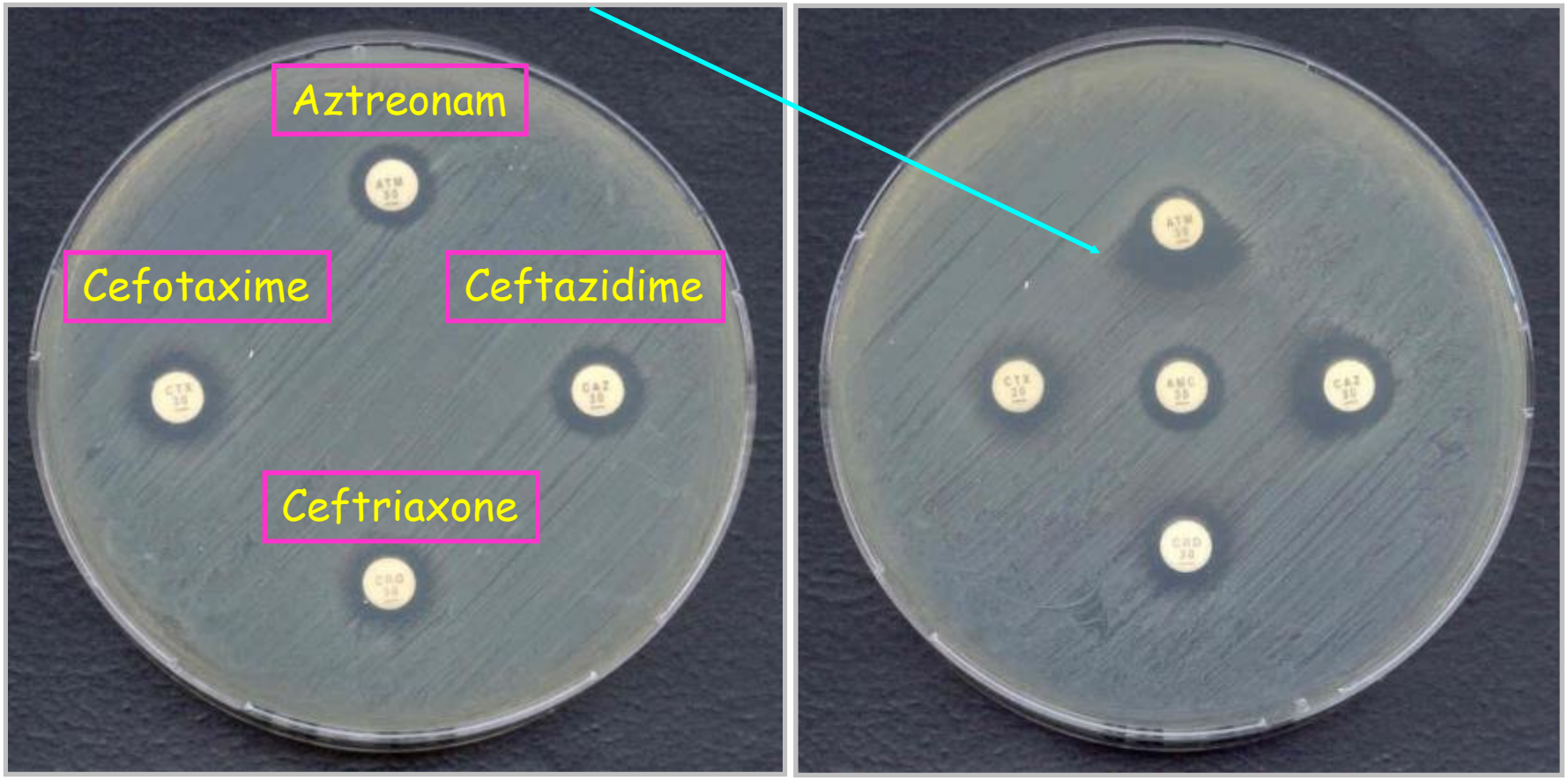
In alcuni enterobatteri l'iperproduzione di β-lattamasi inducibili di classe C può mascherare la produzione di enzimi plasmidici




Diametro aloni:
ceftazidime, 10 mm; cefotaxime, 10 mm; ceftriaxone, 10 mm; aztreonam, 10 mm

# *E. cloacae* iperproduttore di AmpC + ESBL

Diffusione in agar di Mueller-Hinton:
test di sinergia con acido clavulanico (distanza centro-centro 20 mm)

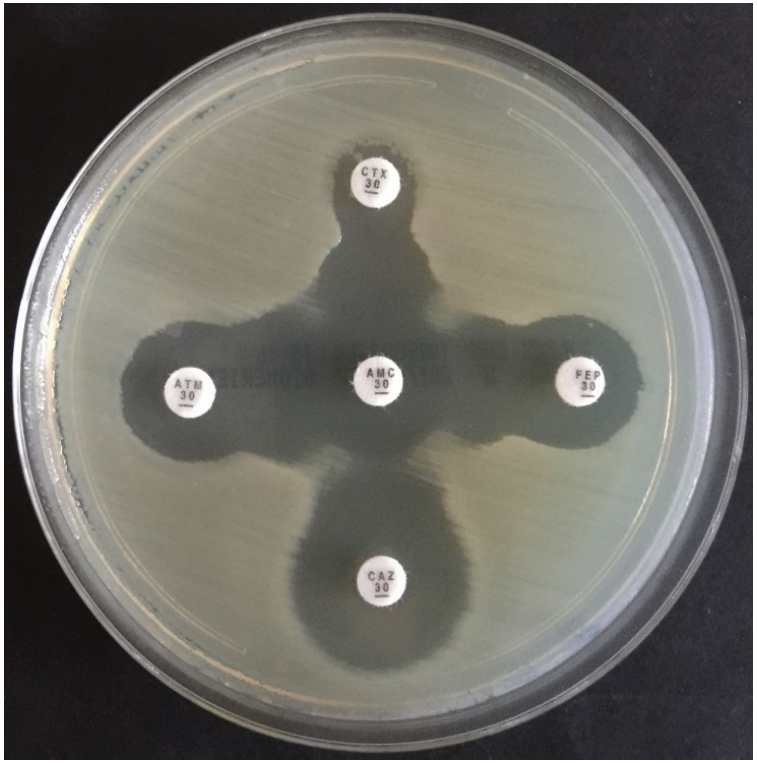



Diametro aloni:
ceftazidime, 10 mm; cefotaxime, 10 mm; ceftriaxone, 10 mm; aztreonam, 10 mm

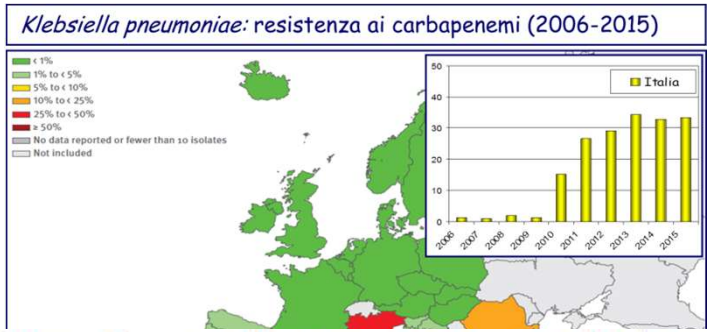
# *Salmonella enterica* ser. Napoli produttore di CTX-M-15

| ***Salmonella enterica* ser. Napoli** | |
|---|---|
| **Antibiotico** | **MIC mg/L(S/I/R)** |
| Amoxi/Clav | 4 (S) |
| Pipera/Tazo | 4 (S) |
| Cefotaxime | >64 (R) |
| Ceftazidime | 4 (I) |
| Cefepime | 4 (I) |
| Ertapenem | <=0.5 (S) |
| Imipenem | <=0.25 (S) |
| Meropenem | <=0.25 (S) |
| Ciprofloxacina | 0.016 (S) |
| Trimet/Sulfam | <=20 (S) |
| Tigeciclina | 1 (S) |

Isolato da coprocoltura
Pz: bambina di 1 anno con sintomi gastroenterici



# Enterobatteri e carbapenemi




**EUCAST – July, 2017**

Table 1. 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

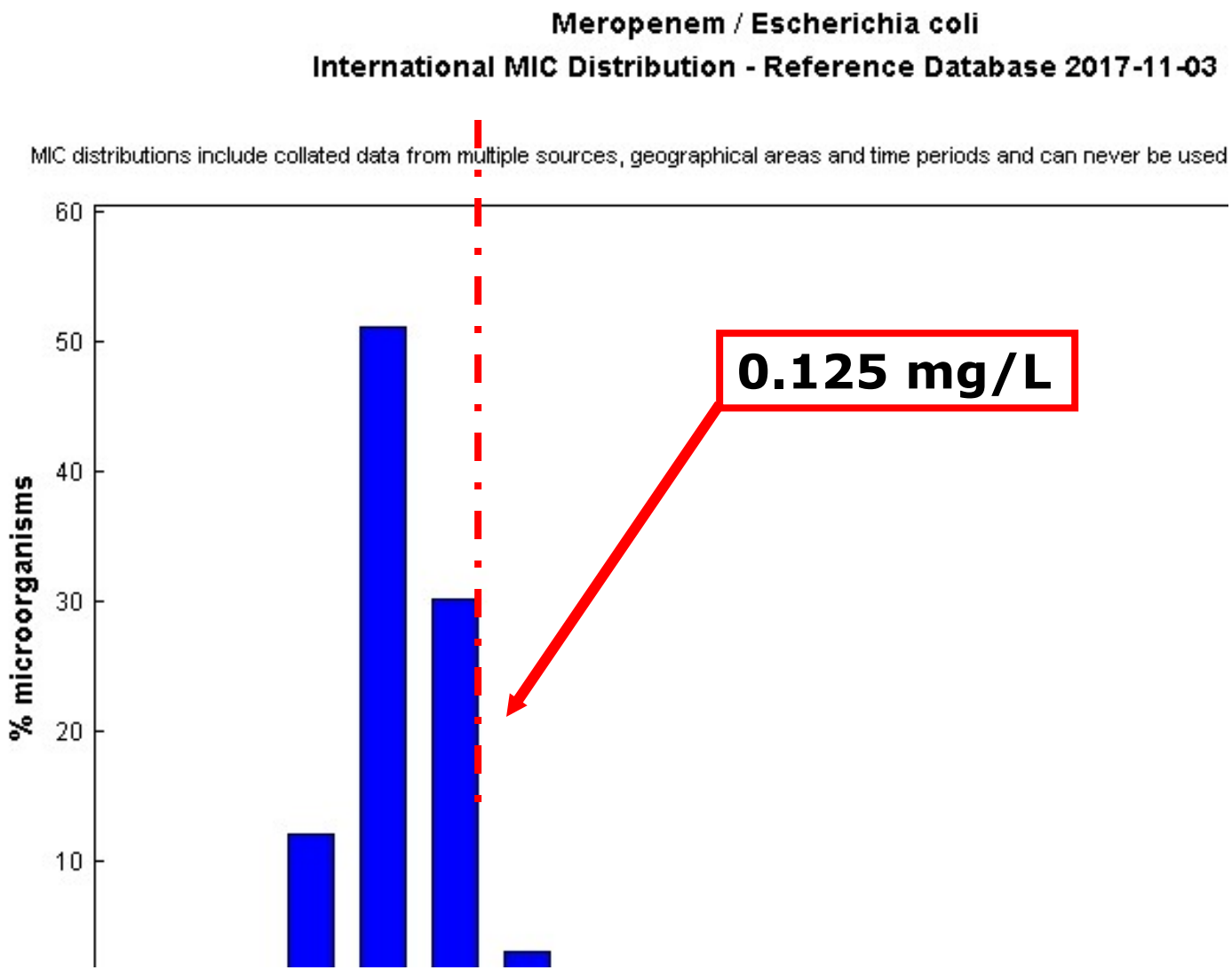
[2]Isolates with 25-27 mm only need to be investigated for carbapenemase-production if they are resistant to piperacillin-tazobactam and/or temocillin (temocillin contributes more to the specificity). Investigation for carbapenemases is always warranted if zone diameter of meropenem is <25 mm.

[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.

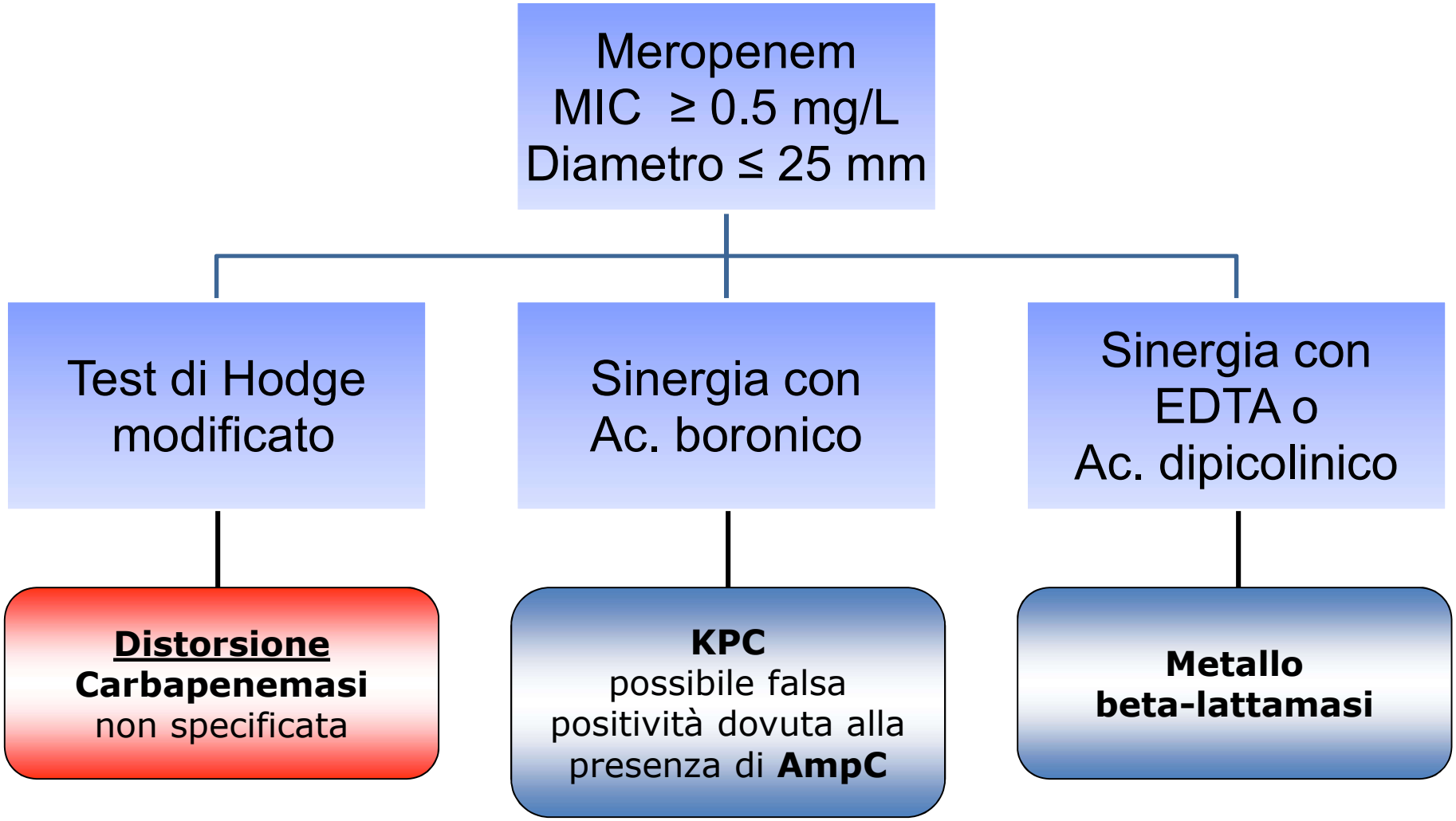
**Test di Screening**

**Breakpoints S ≤2 R >8**

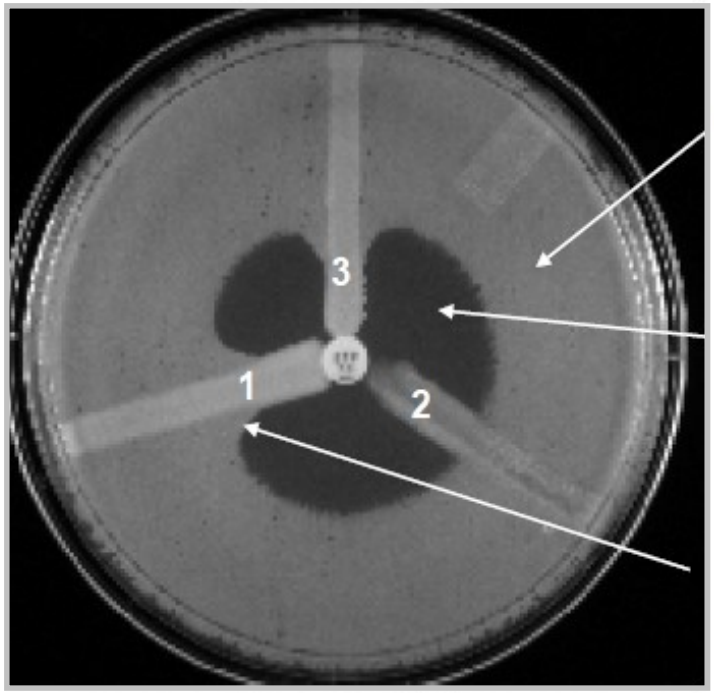
**Cut-off epidemiologico WT ≤0.125**



# Conferma fenotipica della produzione di carbapenemasi negli enterobatteri



# Test di Hodge modificato




*E. coli* ATCC® 25922

Inhibition of *E. coli* ATCC® 25922 by ertapenem

Enhanced growth of *E. coli* ATCC® 25922. Carbapenemase produced by *K. pneumoniae* ATCC ® BAA-1705 inactivated ertapenem that diffused into the media. Thus, there is no longer sufficient ertapenem here to inhibit *E. coli* ATCC® 25922 and an indentation of the zone is noted.

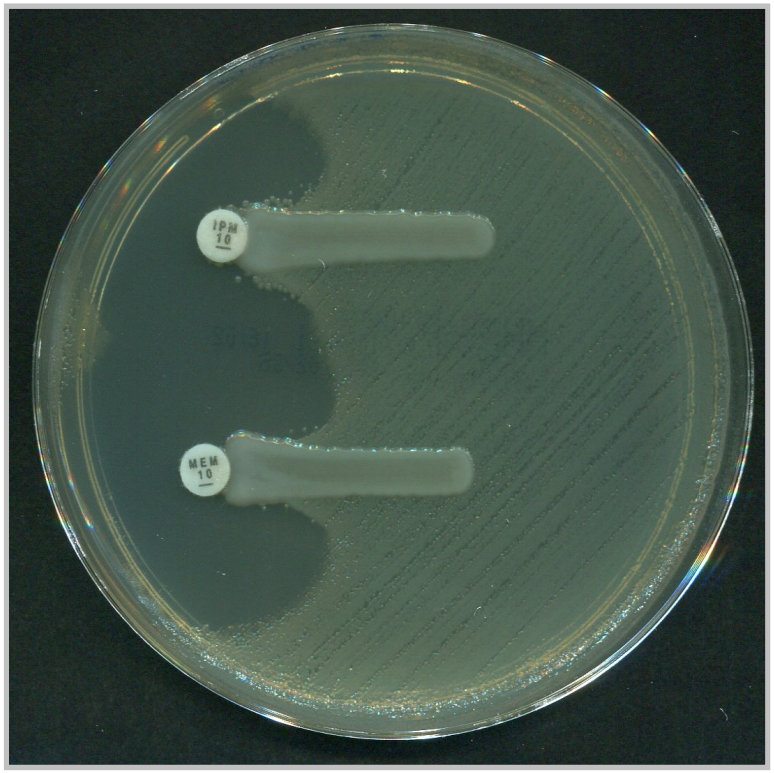
**Figure 1. The MHT Performed on a Small MHA Plate.**
(1) *K. pneumoniae* ATCC®BAA-1705, positive result;
(2) *K. pneumoniae* ATCC®BAA-1706, negative result;
and (3) a clinical isolate, positive result.

# Test di Hodge modificato

Interpretazione talvolta complessa: falsi positivi dovuti a ceppi ahe esprimono ESBL o AmpC associati a perdita di porine

***Klebsiella pneumoniae* OXA-48 ***

**Test di Hodge positivo**




***Klebsiella pneumoniae* con perdita di proteine***

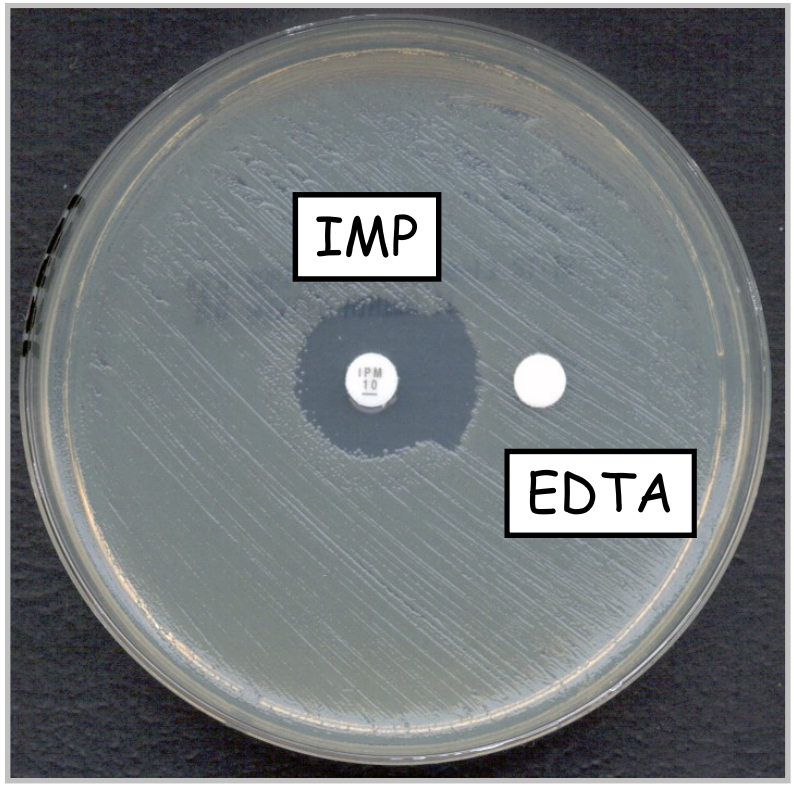
**Test di Hodge negativo**

*associata produzione di ESBL

# Carbapenemasi: test fenotipici di conferma

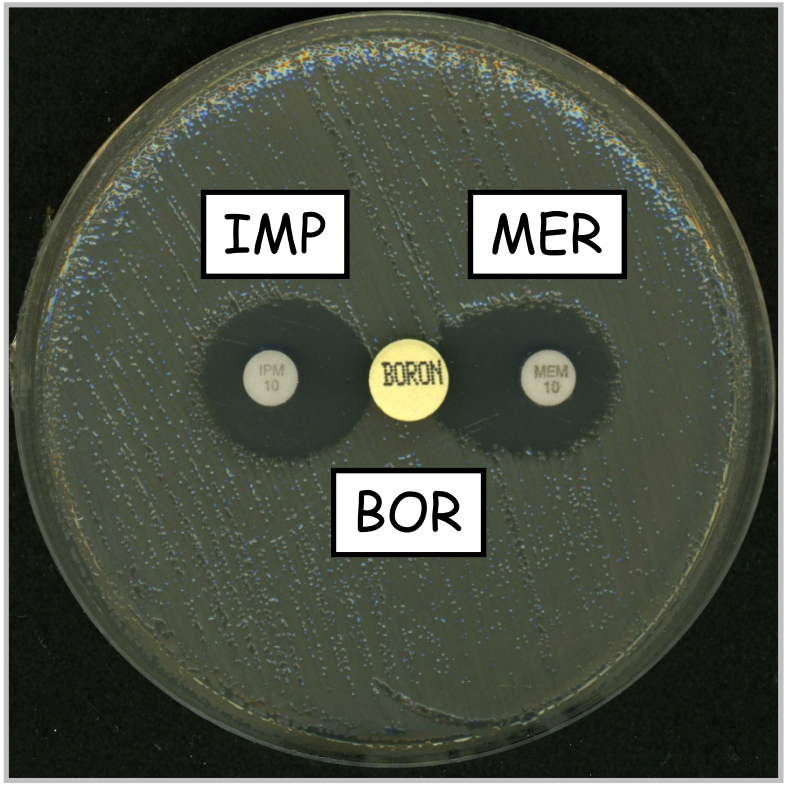
## Test di sinergia mediante approssimazione

Isolato produttore di MBL



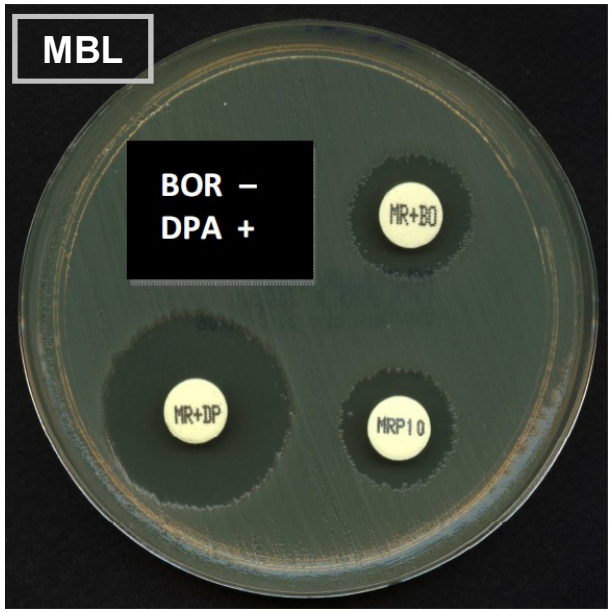

Test con EDTA = positivo

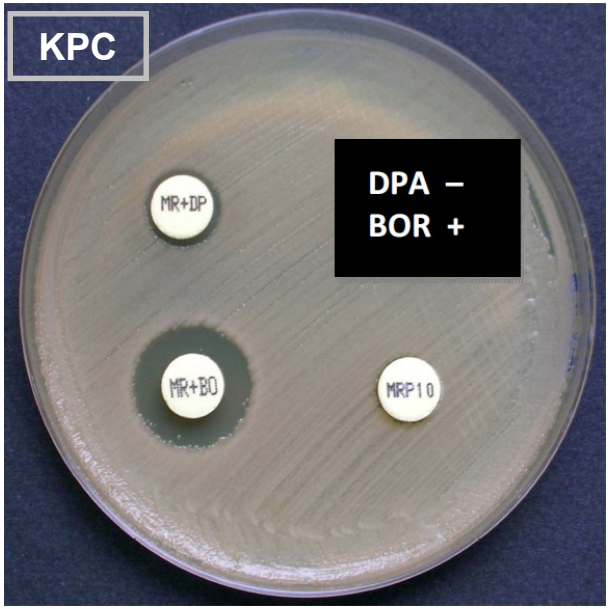
Isolato produttore di KPC




Test con acido boronico: positivo

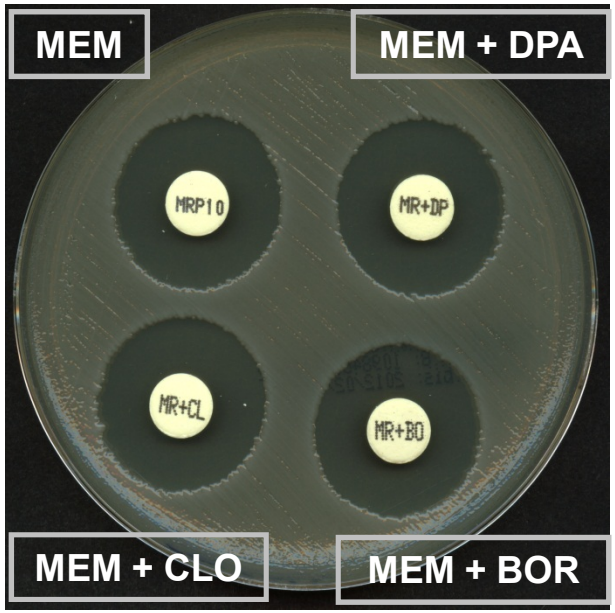
# Carbapenemasi: test fenotipici di conferma




Gli enzimi di tipo OXA non hanno inibitori specifici.
Gli isolati produttori di **OXA-48** hanno elevati livelli di resistenza per la **Temocillina (MIC≥128 mg/L, diametro di inibizione <11 mm)**

**Avibactam** come possibile inibitore




## Test di sinergia mediante dischi di combinazione

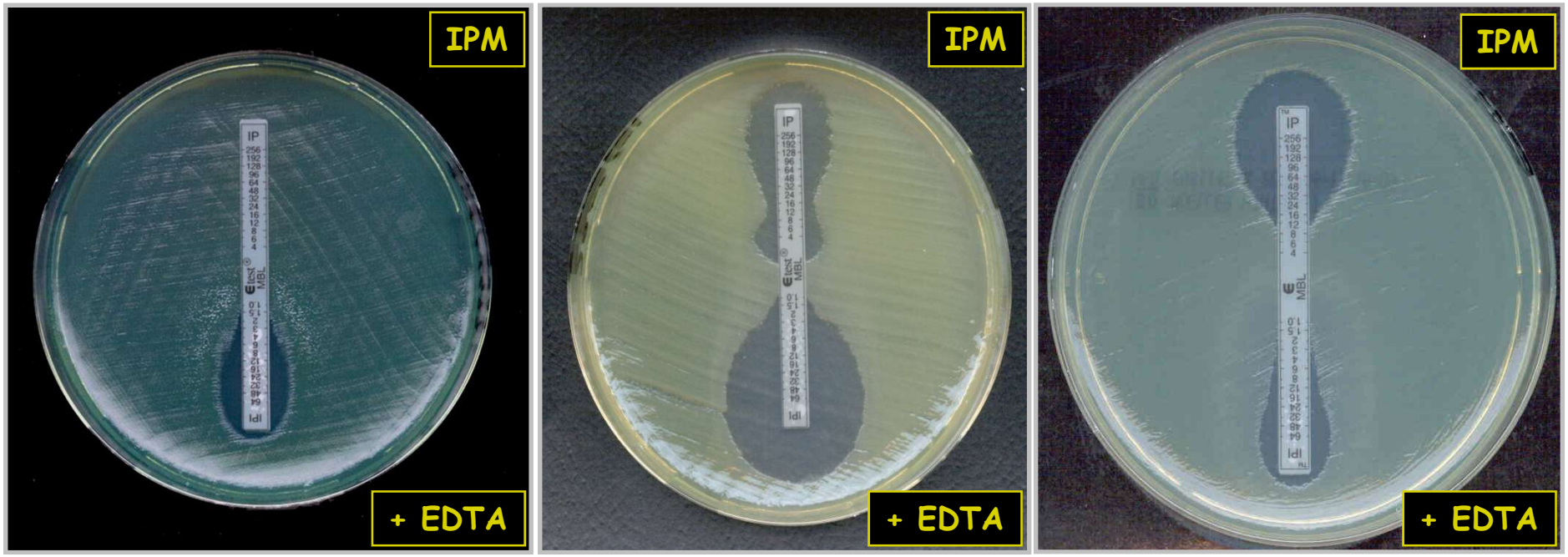
- MBL: BOR -, DPA +
- KPC: DPA -, BOR +
- OXA-48: DPA -, BOR – CLO -

Per la positività del test si valuta la differenza dei diametri di inibizione (**≥5 mm**) dei dischi col meropenem in combinazione rispetto a quello col meropenem da solo

# Etest MBL: sinergia tra imipenem e EDTA

Nel caso di *Pseudomonas* spp. resistente all'imipenem, una riduzione della MIC dell'imipenem ≥ 3 diluizioni in presenza di EDTA è valutata come un risultato positivo

MBL IMP-1 | Phantom zone | MBL IMP-13



**Biolife** Italiana S.r.l.

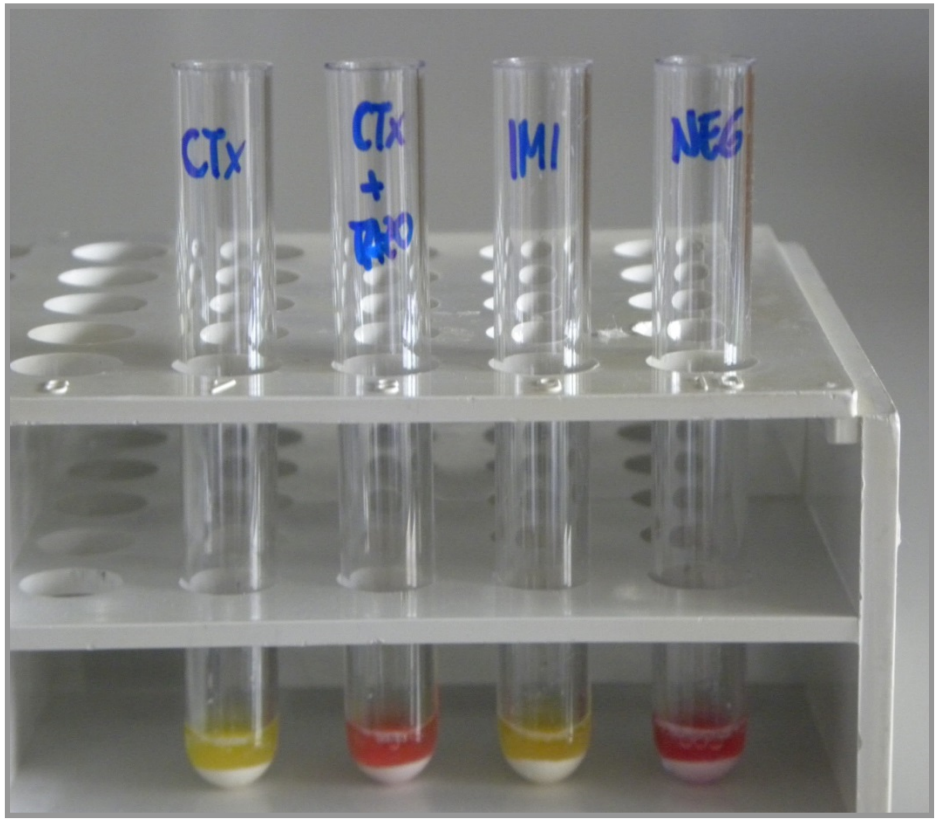
**Scheda Tecnica**

N° 2398024 B I-0 08/2014 Pagina 1 di 2

# Neo-Rapid CARB Screen Kit

**Kit per la rilevazione di meccanismi di resistenza**

Impiego previsto: screening in vitro dei batteri produttori di carbapenemasi (validato per enterobatteri e *Pseudomonas aeruginosa*)




Non validato per *Acinetobacter*

Test basato su idrolisi di:
- imipenem

Il viraggio dal rosso al giallo indica la positività del test

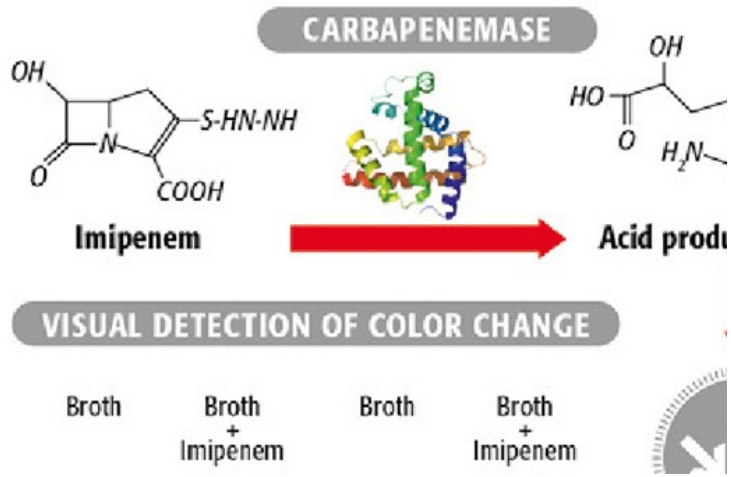
Applicabile su:
- Urine
- Emocolture
- Colonie da terreno agarizzato

# RAPIDEC ® Carba-NP test

The quick and easy way to control carbapenem

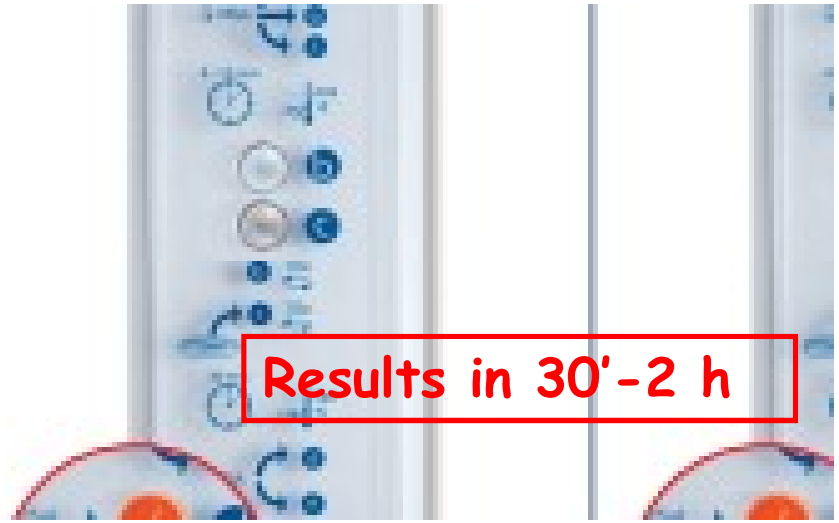
1. **RAPID**:
- Direct detection performed on colonies
- Evaluate the color change
- Approved for *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter*

2. **ACCURATE** and HIGH VALUES of SENSIBILITY AND SPECIFICITY (97.8%)




Carba-NP **POSITIVE carbapenemase-** producing isolate

Carba-NP NEGATIVE



**2017**

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Bacteriology

# Multicenter evaluation of the RAPIDEC® CARBA NP test for rapid screening of carbapenemase-producing *Enterobacteriaceae* and Gram-negative nonfermenters from clinical specimens

**Table 4**
Performance of RAPIDEC® CARBA NP test with clinical isolates.

| Sources | Species/Family | Sensitivity % | Specificity % |
|---|---|---|---|
| **All** | **All (N = 247)** | **70** | **94** |
| | Excluding *A. baumannii* complex (N = 208) | 100 | 94 |
| | *Enterobacteriaceae* (N = 142) | 100 | 98 |
| **Blood cultures** | **All (N = 122)** | **75** | **95** |
| | Excluding *A. baumannii* complex (N = 107) | 100 | 95 |
| | *Enterobacteriaceae* (N = 81) | 100 | 98 |
| **Other materials** | **All (N = 125)** | **65** | **94** |
| | Excluding *A. baumannii* | | |

**Table 1**
Gram-negative strains selected for the evaluation of RAPIDEC® CARBA NP proficiency with the test results obtained in each site.

| Strain | Species | Principal Acquired β-lactamase | Reference | MIC Meropenem (μg/mL) | RAPIDEC® resul (center) | |
|---|---|---|---|---|---|---|
| | | | | | Expected | 1 |
| 6–419 | *Escherichia coli* | No-one | – | 0.5 | – | – |
| 23–1786 | *Enterobacter ludwigii* | NMC-A | Antonelli et al. (2015c) | 32 | + | + |
| 7–556 | *K. pneumoniae* | NDM-1 | – | 32 | + | + |
| 22–1706 | *E. coli* | NDM-5 | – | >32 | + | + |
| 7728 | *P. aeruginosa* | IMP-13 | – | 4 | + | + |
| ATCC 25922 | *E. coli* | no-one | – | 0.5 | – | – |
| 47–3752 | *E. cloacae* complex | IMI-2 | – | >32 | + | + |
| CVB-1 | *E. coli* | NDM-1 | D'Andrea et al. (2011) | 32 | + | + |
| ECBZ-1 | *E. coli* | OXA-48 | Giani et al. (2012) | 1 | + | + |
| FIPP-1 | *K. pneumoniae* | KPC-3 | Giani et al. (2009) | >32 | + | + |
| VA-417/02 | *E. cloacae* complex | VIM-4 | Luzzaro et al. (2004) | 32 | + | + |
| FI-14/157 | *P. aeruginosa* | FIM-1 | Pollini et al. (2013) | >32 | + | + |
| Cfr-FI-07 | *C. freundii* | OXA-372 | Antonelli et al. (2015b) | 16 | + | + |
| 45A02 | *K. pneumoniae* | FOX-7 + porin deficiency | Arena et al. (2013) | 4 | – | – |
| NV132 | *A. baumannii* complex | OXA-58 | – | 8 | + | + |
| 8–27 | *K. pneumoniae* | CTX-M-1-like + OMP deficient | – | 2 | – | – |

# VALUTAZIONE DEL KIT RESIST-3 O.K.N K-SET (CORIS BIOCONCEPT) PER LA RILEVAZIONE DELLE CARBAPENEMASI NEGLI ENTEROBATTERI

Poster 070

| Microrganismo (n) | KPC | OXA-48 | NDM | Altri meccanismi |
|---|---|---|---|---|
| *K. pneumoniae* (48) | 30 | 5 | 2 | 11 |
| *E. coli* (32) | 9 | - | 2 | 21 |
| *E. cloacae* (6) | 1 | - | - | 5 |
| *Citrobacter spp.* (3) | 1 | - | - | 2 |
| *Proteae* (5) | - | - | - | 5 |
| *S. marcescens* (4) | - | - | - | 4 |
| *Salmonella spp.* (2) | - | - | - | 2 |

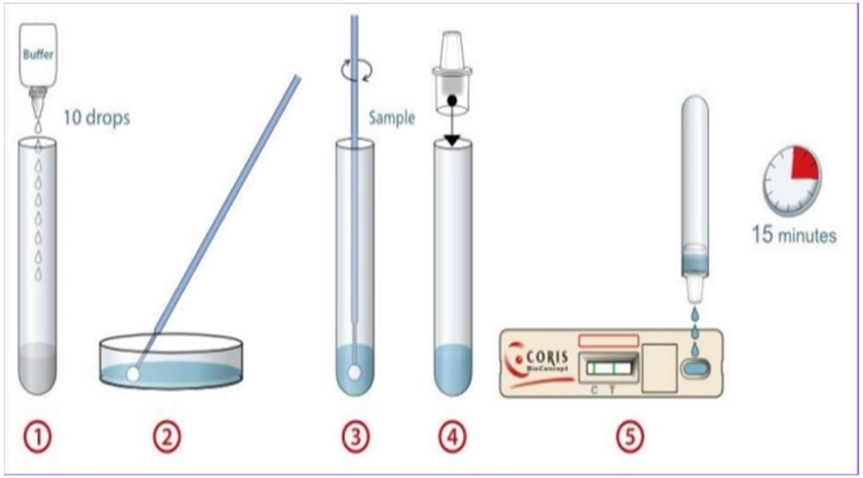



**Figura 1.** Procedura per l'esecuzione del test diagnostico RESIST-3 O.K.N. sulla colonia batterica di interesse.

Non rileva le VIM

Identificazione del determinante di resistenza

Rilevazione di **KPC**, **NDM** ed **OXA-48** da isolati su coltura in agar

Risultato in **15 minuti**

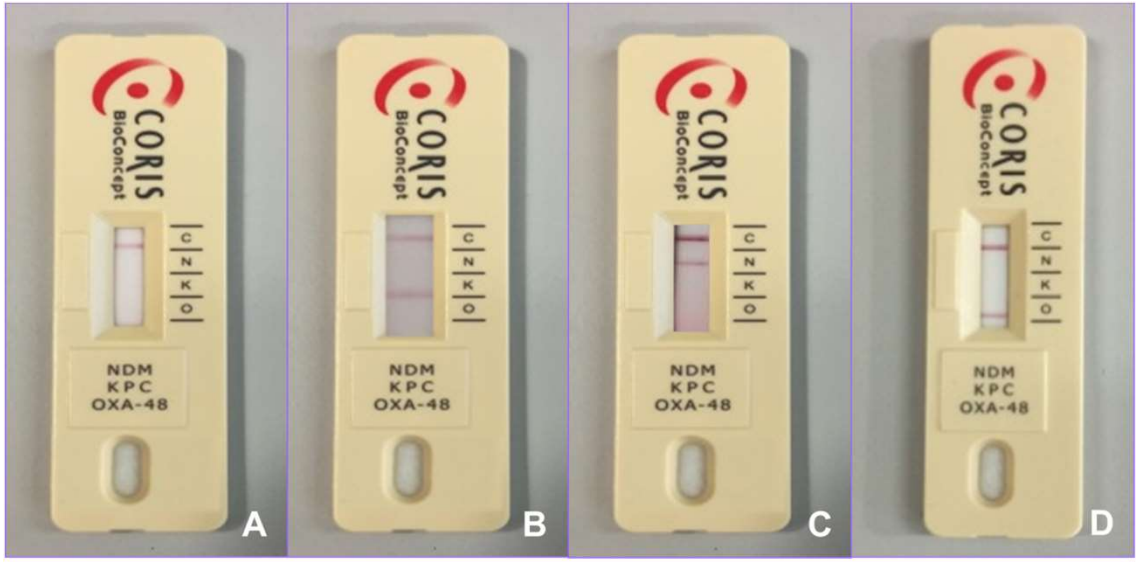
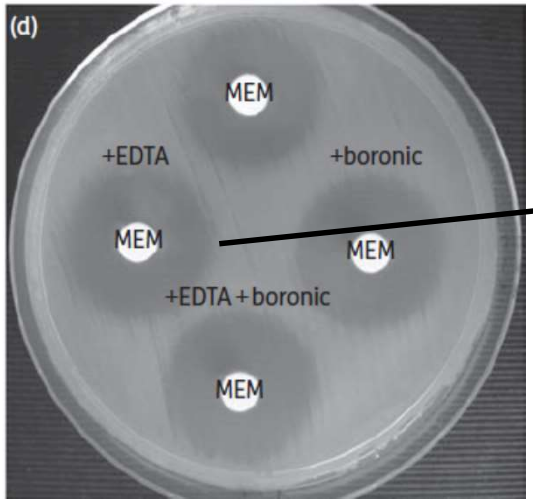
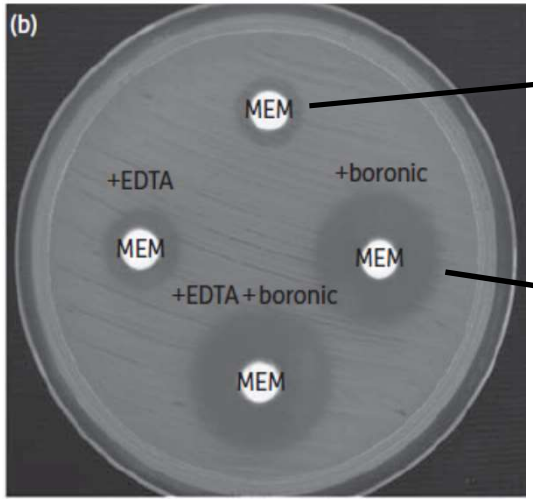
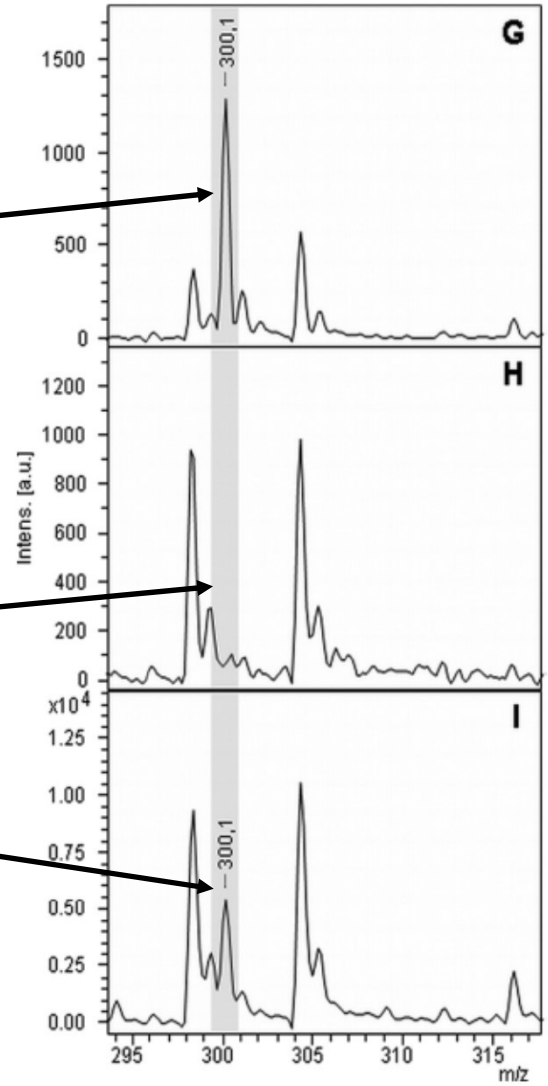



**Figura 2.** 2A: isolato negativo per la produzione di carbapenemasi. 2B: isolato produttore di KPC. 2C: isolato produttore di NDM. 2D: isolato produttore di OXA-48.

# Isolato produttore di carbapenemasi (KPC): spettrometria dopo aggiunta di acido boronico



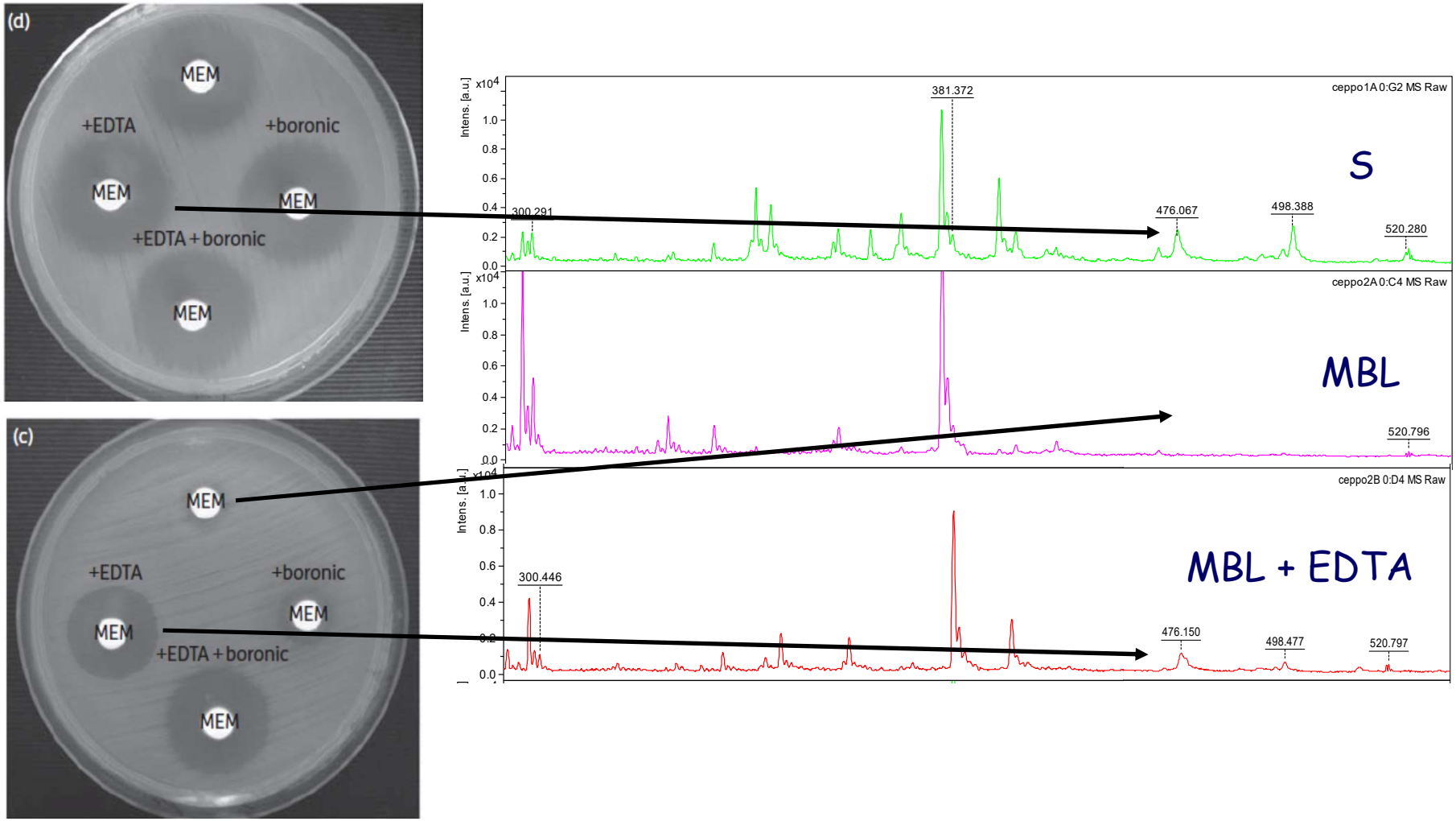




S

KPC

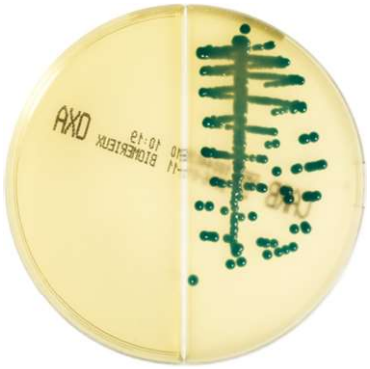
KPC + AB

# Isolato produttore di carbapenemasi (MBL): spettrometria dopo aggiunta di EDTA



# Trasmissione materno-infantile di *K. pneumoniae* produttore di KPC

| *Klebsiella pneumoniae* | |
|---|---|
| **Antibiotico** | **MIC mg/L(S/I/R)** |
| ESBL | Pos |
| Amoxi/Clav | >32 (R) |
| Pipera/Tazo | >64 (R) |
| Cefotaxime | >4 (R) |
| Ceftazidime | >16 (R) |
| Cefepime | >8 (R) |
| Ertapenem | >4 (R) |
| Imipenem | 8 (I) |
| Meropenem | >8 (R) |
| Amikacina | <=1 (S) |
| Gentamicina | <=0.5 (S) |
| Ciprofloxacina | 4 (R) |
| Trimet/Sulfam | <=10 (S) |
| Tigeciclina | 0.5 (S) |
| Colistina | 0.5 (S) |



**Madre** colonizzata da *K. pneumoniae* KPC e trasmissione ai **due gemelli** durante il parto.

L' **urina** delle madre era positiva a bassa carica (**≈$10^3$ CFU/ml**) per *K. pneumoniae* KPC.

Lo stesso ceppo è stato riscontrato a livello **placentare** e dai **tamponi rettali** di screening dei gemelli.
I ceppi presentavano lo **stesso profilo genetico** in seguito a sequenziamento completo del genoma.

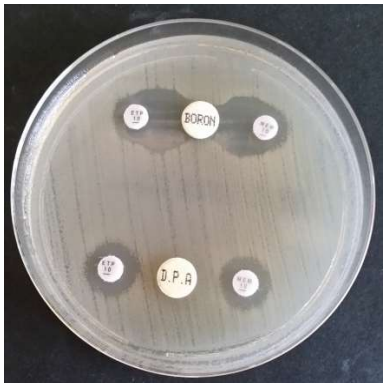
Il **rapido riconoscimento** di *K. pneumoniae* KPC, **anche se a bassa carica da urine**, ha permesso una **pronta comunicazione** al reparto in modo da evitare una diffusione del microrganismo in **ostetricia**

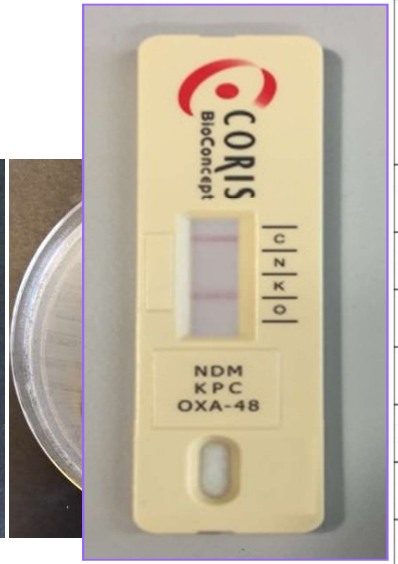
Poster P071

# PRIMO ISOLAMENTO DI *CITROBACTER BRAAKII* PRODUTTORE DI KPC

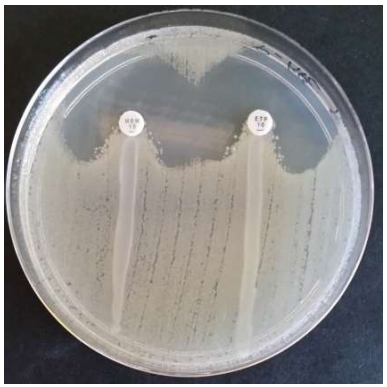
Paziente ricoverato in Terapia intensiva, nessun ricovero precedente (proveniente dal domicilio), tamponi da ulcera e rettali positivi per *C. braakii* (prima) e *K. pneumoniae* (dopo)

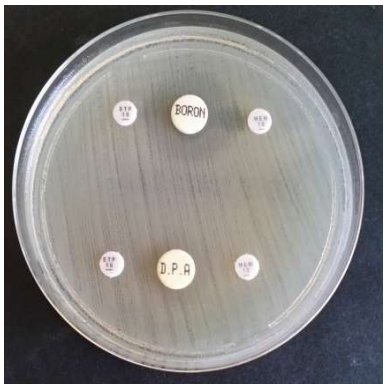
## *C. braakii*

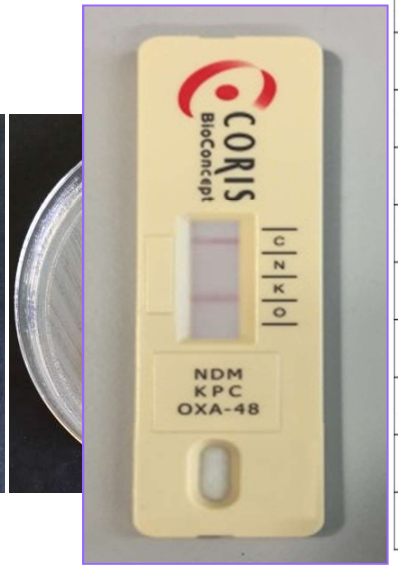



## *K. pneumoniae*





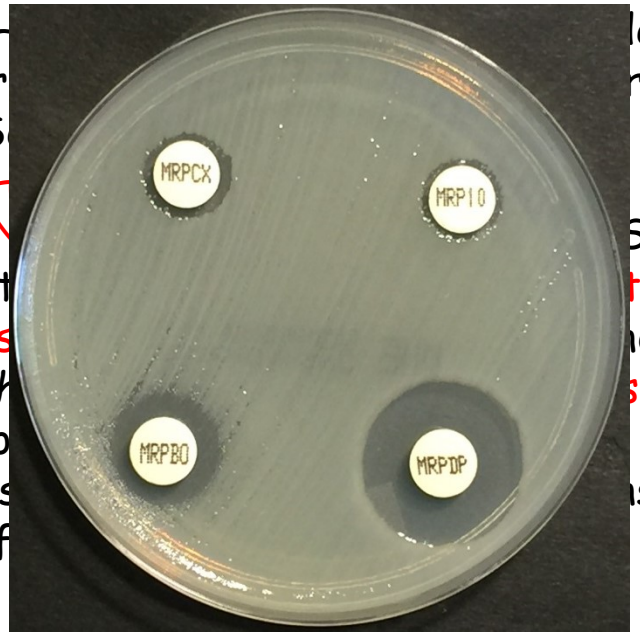

| Antibiotici | MICs mg/L (S, I, R) |
|---|---|
| | *C. braakii* |
| Amoxicillina/acido clavulanico | >64/2 (R) |
| Piperacillina/tazobactam | >32/4 (R) |
| Cefotaxime | 8 (R) |
| Ceftazidime | 16 (R) |
| Ertapenem | >2 (R) |
| Imipenem | 8 (I) |
| Meropenem | 4 (I) |
| Amikacina | 16 (I) |
| Gentamicina | 2 (S) |
| Tobramicina | >8 (R) |
| Ciprofloxacina | >2 (R) |
| Tigeciclina | 1 (S) |
| Cotrimossazolo | 4/76 (I) |
| Colistina | <0.25 (S) |
| Aztreonam | >32 (R) |
| Ceftolozano/tazobactam | 16/4 (R) |
| Ceftazidime/avibactam | <0.5/4 (S) |

# NDM in our experience

Two indipe

**Case #1** - frican
origin,invo
hospitalize .
In Italy, N ST15)
wasisolate age fluid
linked to c
He was dis er 25
days of ho

**Case #2** - leveloped
an acute r ruise in
the Red S the
Red Sea H
In Italy, N ST11)
Was isolat tory
secretions d
*S. maltoph* s caused
by NDM-p
He was dis s after
61 days of

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Short Communication

First report of NDM-1-producing *Klebsiella pneumoniae* imported
Africa to Italy: Evidence of the need for continuous surveillance

***Klebsiella pneumoniae-NDM-1***

| Antibiotico | MIC mg/L(S/I/R) |
|---|---|
| Pip/Tazo | >128 (R) |
| Ceftriaxone | >64 (R) |
| Ceftazidime | >64 (R) |
| Cefepime | >64 (R) |
| Ertapenem | >8 (R) |
| Imipenem | >16 (R) |
| Meropenem | >16 (R) |
| Amikacina | >64 (R) |
| Gentamicina | >16 (R) |
| Ciprofloxacina | >4 (R) |
| Tigeciclina | ≤0.5 (S) |
| Colistina | ≤0.5 (S) |

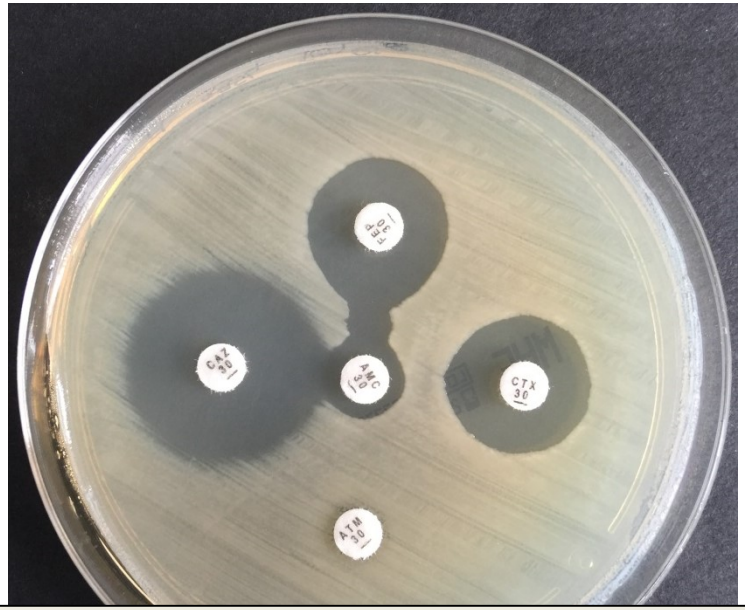
Principe et al., JGAR 2017

UK NEQAS

International Quality Expe

| *Klebsiella oxytoca* | |
|---|---|
| Antibiotico | MIC mg/L(S/I/R) |
| ESBL | Pos |
| Amoxi/Clav | >16 (R) |
| Pipera/Tazo | >64 (R) |
| Cefotaxime | 4 (R) |
| Ceftazidime | <=1 (S) |
| Cefepime | 2 (I) |
| Ertapenem | <=0.5 (S) |
| Imipenem | <=0.25 (S) |
| Meropenem | <=0.25 (S) |
| Amikacina | <=2 (S) |
| Gentamicina | <=1 (S) |
| Ciprofloxacina | <=0.25 (S) |
| Trimet/Sulfam | <=20 (S) |
| Tigeciclina | ≤0.5 (S) |
| Colistina | ≤0.5 (S) |

| Test di conferma | |
|---|---|
| Ceftazidime (E-Test) | 0.75 (S) |
| Cefepime (E-Test) | 3 (I) |
| Cefoxitina (Disco) | 6 mm (R) |




Meccanismo di resistenza presunto:
**Iperespressione di K1**

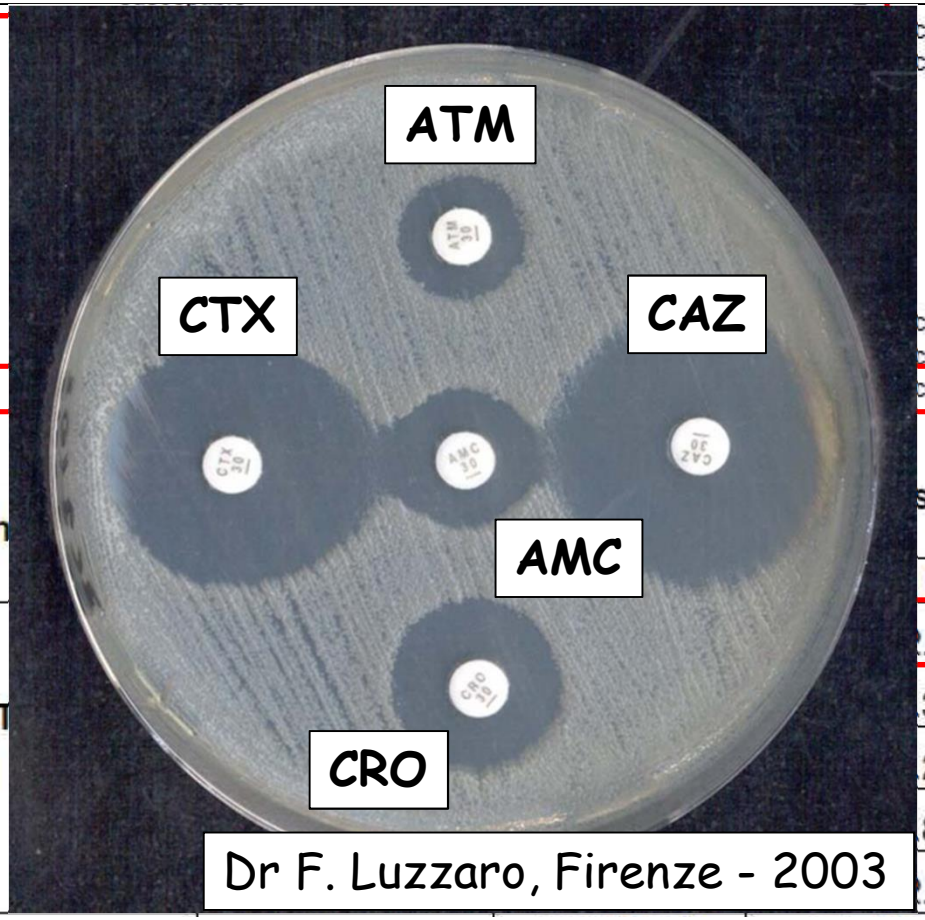
**Specimen 3954**

This specimen contained *Klebsiella oxytoca* hyper-producing the K1 beta-lactamase.

The isolate displayed a phenotype consistent with hyper-production of the K1 beta-lactamase found in *K.oxytoca*; high level resistance to cefuroxime (MIC ≥128 mg/L), piperacillin/tazobactam (MIC ≥128 mg/L), and ceftriaxone (MIC ≥128 mg/L), with relative sparing of cefotaxime (reference MICs 4/8 mg/L), and to a greater extent ceftazidime (MIC 0.5mg/L). Indeed the isolate was sensitive to ceftazidime according to both EUCAST (breakpoints S≤1, R>4) and CLSI (breakpoints S≤4, R≥16) criteria.

***Klebsiella oxytoca* K1+++**

| Antibiotico | MIC mg/L(S/I/R) |
|---|---|
| Ceftriaxone | 16 (R) |
| Cefotaxime | 2 (I) |
| Ceftazidime | 0.5 (S) |
| Cefepime | 4 (I) |
| Aztreonam | 64 (R) |
| Imipenem | <=0.25 (S) |
| Meropenem | <=0.25 (S) |
| Amikacina | 1 (S) |
| Gentamicina | 0.5 (S) |
| Ciprofloxacina | 32 (R) |



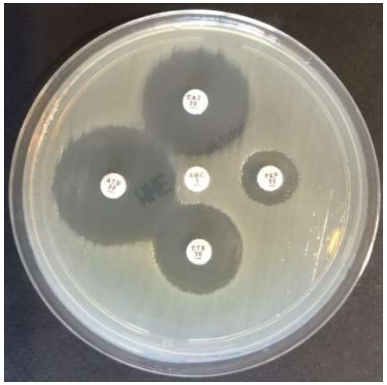

Dr F. Luzzaro, Firenze - 2003

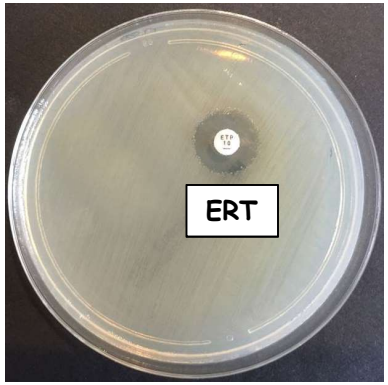
ESBL

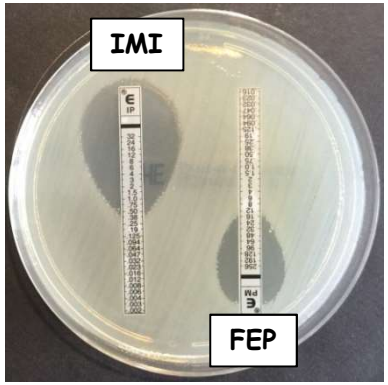
UK NEQAS Microbiology

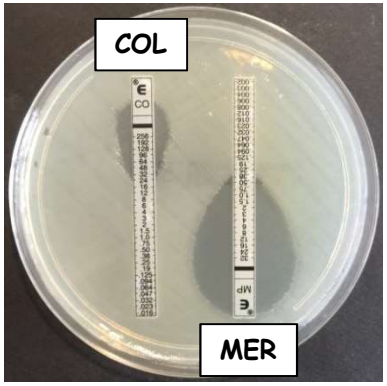
Special Surveys

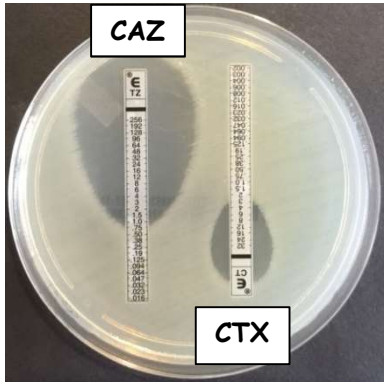
Distribution : **4060**

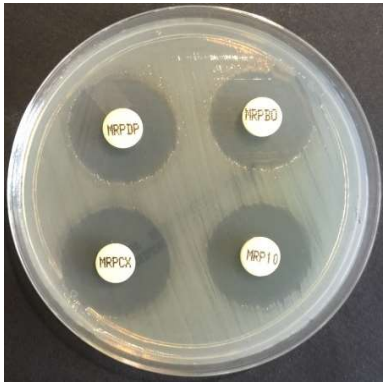


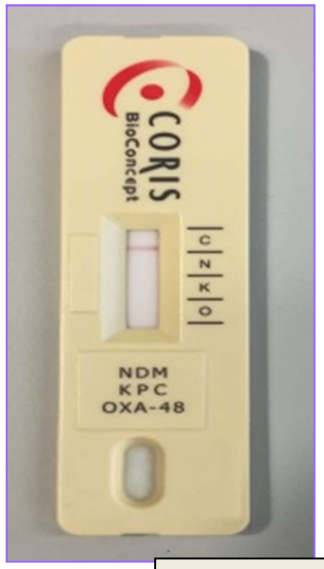





| *Klebsiella pneumoniae* | |
|---|---|
| **Antibiotico** | **MIC mg/L(S/I/R)** |
| ESBL | Neg |
| Pip/Tazo | >64 (R) |
| Amoxi/Clav | >16 (R) |
| Cefotaxime | 2 (I) |
| Ceftazidime | <=1 (S) |
| Cefepime | 16 (R) |
| Ertapenem | 4 (R) |
| Imipenem | 0.5 (S) |
| Meropenem | 1 (S) |
| Amikacina | >32 (R) |
| Gentamicina | >8 (R) |
| Ciprofloxacina | >2 (R) |
| Tigeciclina | >4 (R) |

Meccanismo/i di resistenza presunto/i non rilevabile/i con metodi fenotipici

UK NEQAS Microbiology

Special Surveys

Distribution : **4060**

| | | | |
|---|---|---|---|
| Specimen 3677 | *Klebsiella pneumoniae* | | *Kle* |
| | Amikacin | intermediate | resi |
| | Amoxicillin | resistant | not |
| | Amoxicillin-clavulanic acid | resistant | resi |
| | Ampicillin | resistant | resi |
| | Cefotaxime | intermediate/resistant | resi |
| | Ceftazidime | susceptible | sus |
| | Ceftriaxone | susceptible | not |
| | Ciprofloxacin | resistant | resi |
| | Colistin | resistant | resi |
| | Ertapenem | resistant | resi |
| | Gentamicin | resistant | resi |
| | Imipenem | susceptible | sus |
| | Levofloxacin | resistant | resi |

**Specimen 3677**

This specimen contained a strain of *Klebsiella pneumoniae* with both O
enzymes, expressing resistance to many beta-lactam agents, including inhib
colistin (by EUCAST methods) and quinolones. The isolate was suscepti
meropenem, ceftriaxone and ceftazidime. Cefotaxime was
intermediate/resistant based on an MIC of 2-4 mg/L. The isolate expressed r
gentamicin and tobramycin and intermediate susceptibility to amikacin. A goo
results was seen for quinolones, amoxicillin, third generation cephalospo
dissociated susceptibility among the different agents), inhibitor combinat
tobramycin and ertapenem.

The organism expressed intermediate resistance to amikacin (MIC 16 m
breakpoints. The 787 participants reporting amikacin susceptibility reporte

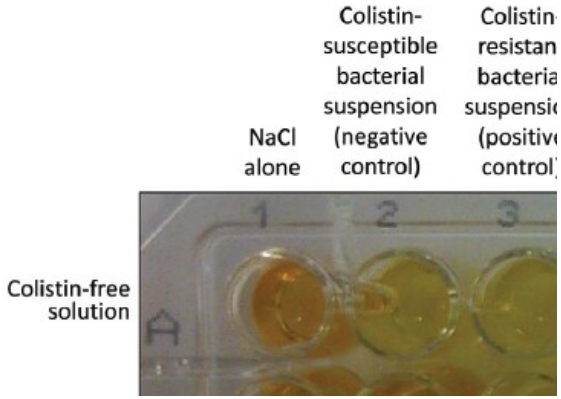
# Resistenza alla colistina

«Recently, it was discovered that **MCR-enzymes are dependent on zinc for their hydrolysis**, and that **zinc chelation can therefore inhibit their activity**. Inhibition tests based on **EDTA** or **dipicolinic acid** are therefore expected»

**(EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance - July, 2017)**

**Rapid Detection of Polym**
**Resistance in *Enterobacter***

**Em. Infect. Dis. 2016**




Format: Abstract

Clin Microbiol Infect. 2017 Aug 19. pii: S1198-743X(17)30457-3. doi: 10.1016/j.cmi.2017.08.011. [Epub ahead of p

Format: Abstract

J Clin Microbiol. 2017 Oct 4. pii: JCM.00835-17. doi: 10.1128/JCM.00835-17. [Epub ahead of print]

**Detection of Colistin-Resistant MCR-1-Positive Escherichia coli Using Inh**

# Conclusioni

| Antibiotico | ESBL | CRE | MDR Pseudomonas | MDR Acinetobacter |
|---|---|---|---|---|
| **Cefiderocol** | SI | KPC e NDM | SI | SI |
| **Ceftolozane/Tazobactam** | SI | NO | SI | NO |
| **Ceftazidime/Avibactam** | SI | KPC e OXA-48 (NO MBL) | SI | NO |
| **Ceftaroline/Avibactam** | SI | KPC e OXA-48 (NO MBL) | NO | NO |
| **Aztreonam/Avibactam** | SI | MBL | SI | NO |
| **Meropenem/Vaborbactam** | SI | KPC | NO | NO |
| **Imipenem/Relebactam** | SI | KPC e OXA-48 (NO MBL) | NO | NO |
| **Plazomicin** | SI | KPC (NO NDM) | NO | NO |
| **Everacycline** | SI | KPC | NO | SI |

- In futuro, necessità di caratterizzare la tipologia enzimatica

AMCLI associazione microbiologi clinici italiani

COMITATO DI STUDIO PER GLI ANTIMICROBICI
CoSA

# Grazie per l'attenzione

Luigi Principe

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Azienda Socio Sanitaria Territoriale di Lecco