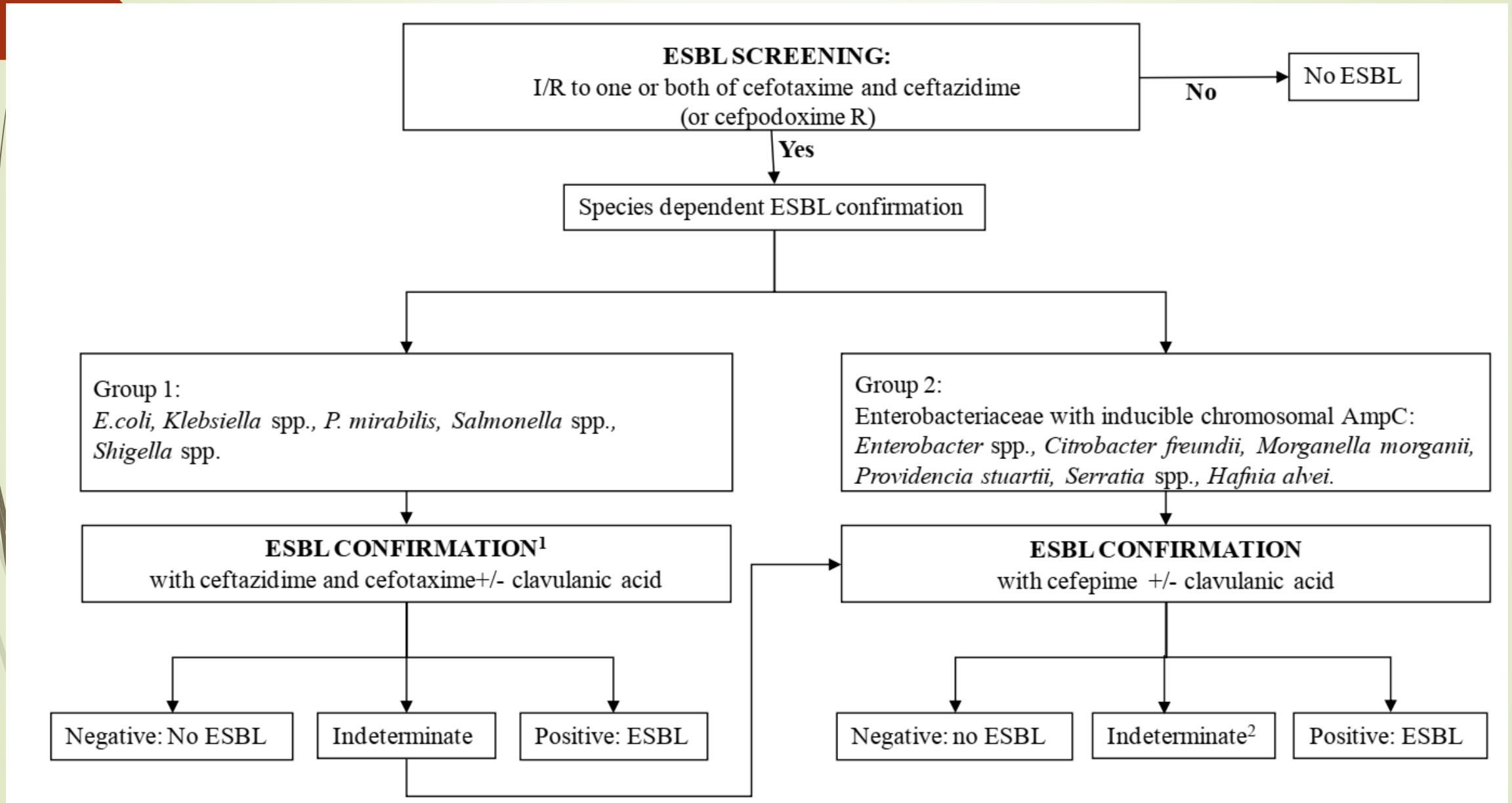
Detection methods of B-lactamases in Enterobacterales

Dr. MAIRI A.

1. Extended-Spectrum β-lactamase (ESBL)

- 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 avibactam.

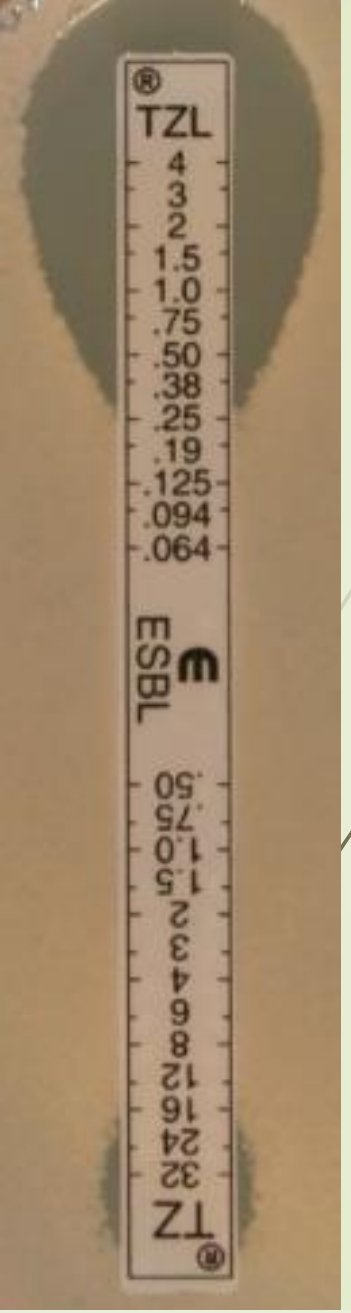
1.1. ESBL-screening in Enterobacterales



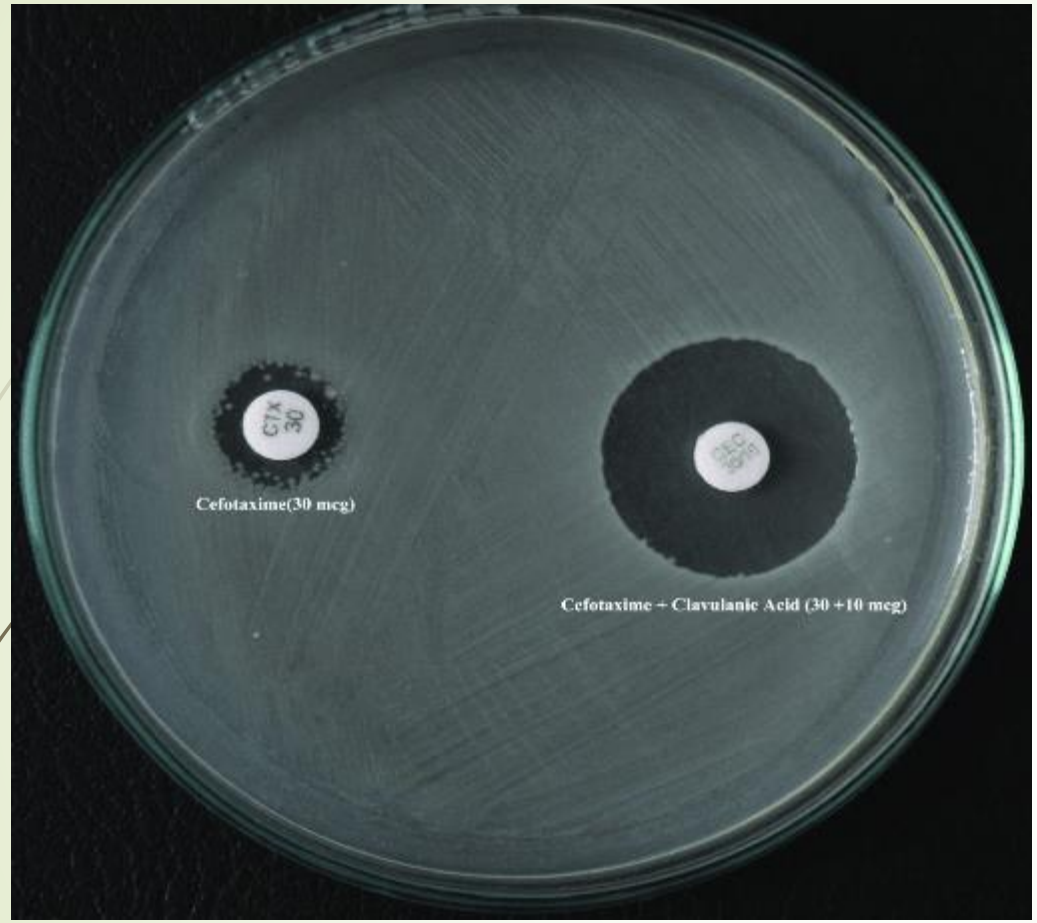
1.2. Phenotypic confirmation methods

- Four of the several phenotypic methods based on the *in vitro* inhibition of ESBL activity by clavulanic acid are recommended for ESBL confirmation:
    - the combination disk test (CDT)
    - the double-disk synergy test (DDST)
    - the ESBL gradient test
    - the broth microdilution test

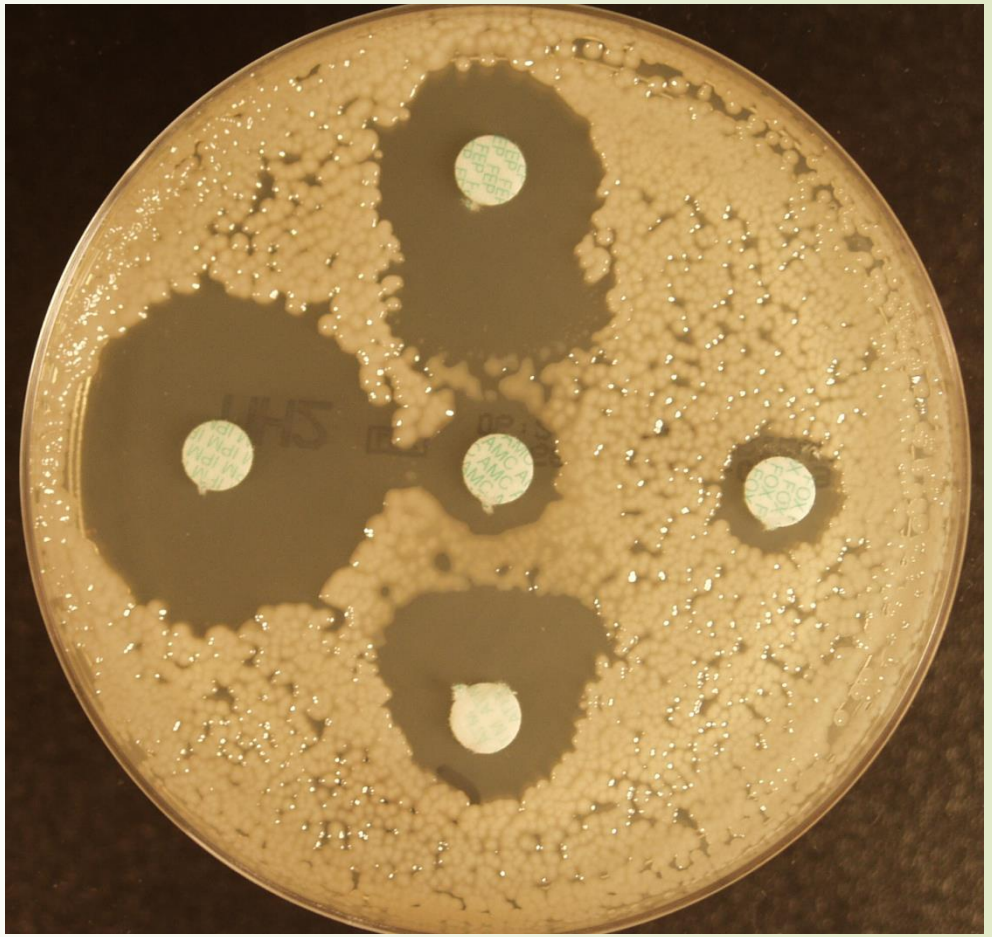
Method	Antimicrobial agent (disk content)	ESBL confirmation is positive if
ESBL gradient test	Cefotaxime +/- clavulanic acid	MIC ratio ≥8 or deformed ellipse present
	Ceftazidime +/- clavulanic acid	MIC ratio ≥8 or deformed ellipse present
Combination disk diffusion test (CDT)	Cefotaxime (30 µg) +/- 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 gradient test

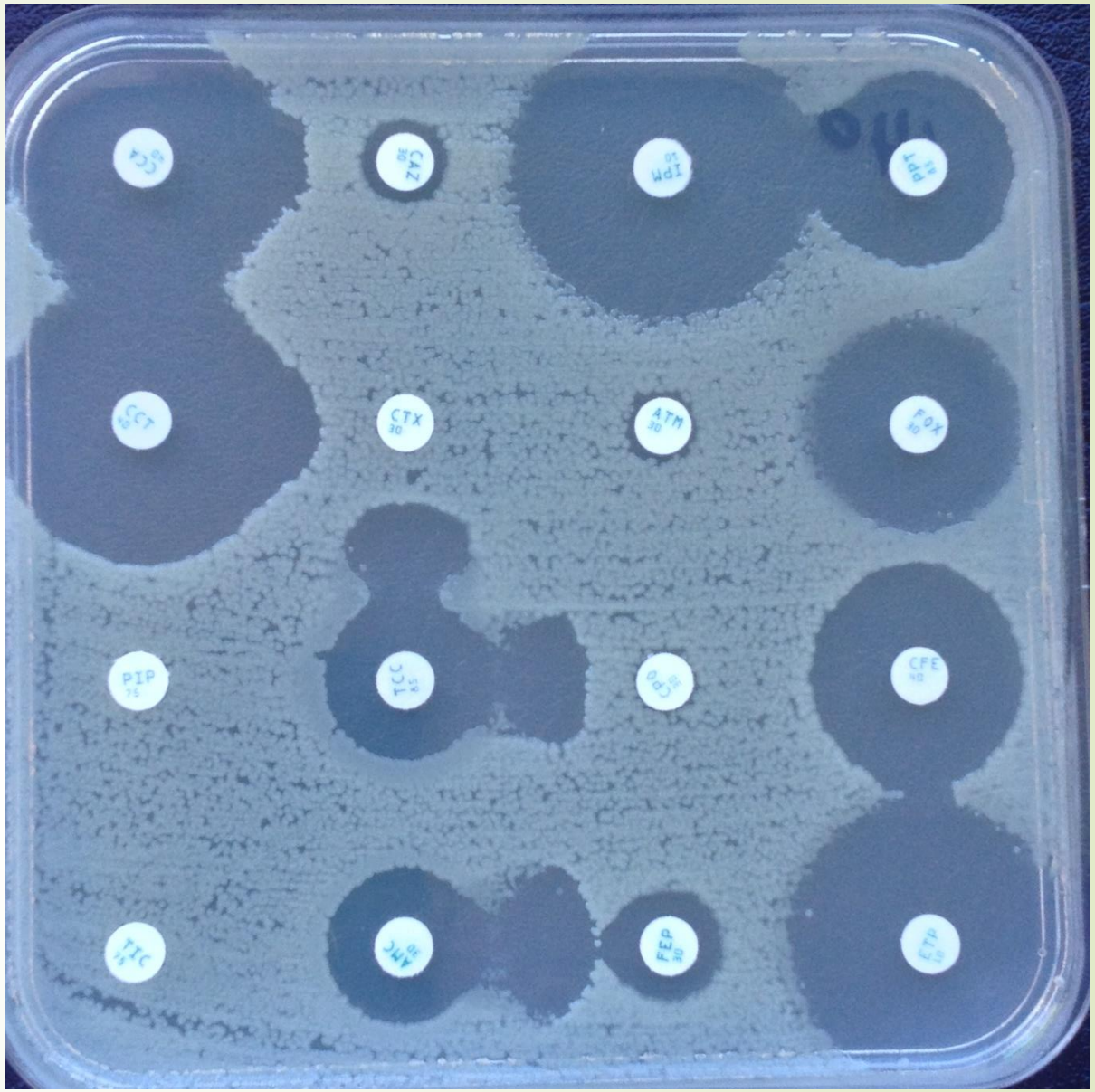


Combination disk diffusion test (CDT)



Double disk synergy test (DDST)


PPT 85
FOX 30
CFE 30
ETP 10
IPM 10
ATM 30
FEP 30
AMC 30
CAZ 30
CTX 30
TCC 85
AMC 30
CCA 10
CCT 30
PIP 75
TIC 75


CCA 10
CAZ 30
IPM 10
PPT 85
CCT 30
CTX 30
ATM 30
FOX 30
PIP 75
TCC 85
CFO 30
CFE 30
TIC 75
AMC 30
FEP 30
ETP 10

1.3. Phenotypic detection of ESBL in the presence of other β-lactamases that mask synergy

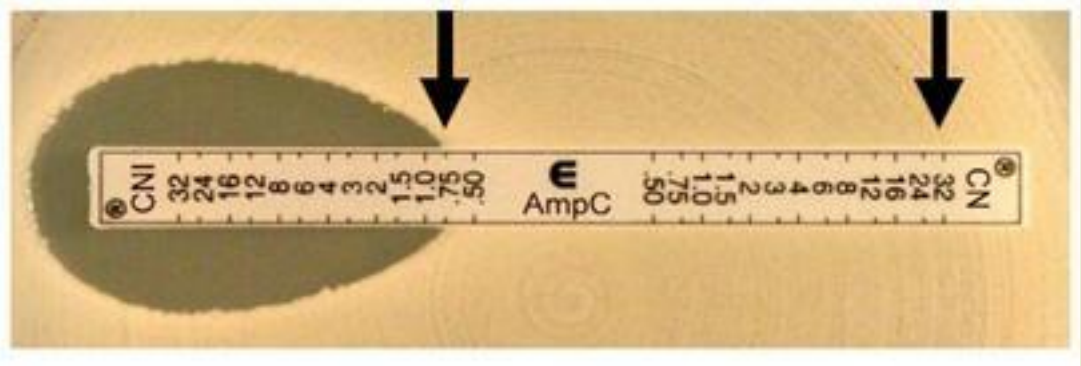
- To confirm the presence of ESBLs in isolates with high-level expression of AmpC β-lactamases it is recommended that an additional ESBL confirmation test be performed with cefepime.
- Alternative approaches include use of cloxacillin. Test formats include CDT with disks containing the two cephalosporin indicators with both clavulanic acid and cloxacillin together, and standard CDT or DDST on agar plates supplemented with 200-250 mg/L cloxacillin.

2. Acquired AmpC β-lactamase

- 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

Cefotaxime R or ceftazidime R **AND** cefoxitin R[1] in *E. coli, K. pneumoniae*, *P. mirabilis*, *Salmonella* spp, *Shigella* spp

- Cloxacillin synergy detected
  - *E. coli* and *Shigella* spp: PCR is required to discriminate between plasmid-acquired and chromosomal AmpC
  - *K.pneumoniae*, *P. mirabilis*, *Salmonella* (lack chromosomal AmpC) plasmid-mediated AmpC detected
- Cloxacillin synergy not detected
  - Other mechanisms (e.g. porin loss)



MH

MH+cloxacilline

3. Carbapenemases

- Carbapenemases are β-lactamases that hydrolyze penicillins, in most cases cephalosporins, and to various degrees carbapenems and monobactams (the latter are not hydrolyzed by metallo-β-lactamases).
- Class A, B and D

3.1. The modified Hodge test

- An overnight culture of indicator organism *E. coli* ATCC 25922 was adjusted to a turbidity of McFarland No. 0.5. These suspension was used to swab inoculate the surface of the agar plates.
- After drying the surface, test organisms were heavily streaked from the center to the periphery of the plate using an inoculating loop and a 10-µg imipenem or ertapenem disk was placed at the center, and incubated overnight.
- The Hodge test is interpreted as positive by the presence of distortion of the inhibition zone.

Improved performance of the modified Hodge test with MacConkey agar for screening carbapenemase-producing Gram-negative bacilli

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ABSTRACT

The detection of carbapenemases in Gram-negative bacilli is important for optimal patient treatment and to control spread of the resistance. The modified Hodge test can detect carbapenemase-producing Gram-negative bacilli. In this study, we compared the performance of MacConkey agar and Mueller-Hinton agar for metallo-β-lactamase (MBL) and OXA carbapenemase screening. Overall, the performance of Hodge test was better with MacConkey agar due to enhanced release of β-lactamases from the cells in the presence of bile compounds. Concomitant use of the modified Hodge test could resolve most of the problems with uncertain double-disk synergy tests in MBL detection.

1. Introduction

The detection of metallo-β-lactamases (MBL) and OXA-type carbapenemases in clinical isolates of Gram-negative bacilli (GNB) is important, because most of them are resistant to virtually all β-lactams and because the resistance can be transferred horizontally (Walsh et al., 2005; Walther-Rasmussen and Høiby, 2006). However, the CLSI publication (2010) does not contain any guideline for phenotypic detection of these carbapenemases. For detection of MBL-producing GNB, inhibitor based tests, i.e., the E-test MBL (Lee et al., 2005), double-disk synergy (DDS) tests (Arakawa et al., 2000; Lee et al., 2003), and a combined-disk test (Yong et al., 2002), were reported, but all of these tests showed either low sensitivity or specificity (Lee et al., 2003; Scoulica et al., 2004; Yan et al., 2004; Franklin et al., 2006). A recent evaluation confirmed that the performance of the DDS test depends on β-lactam substrate, MBL inhibitor, and bacterial species tested (Picao et al., 2008). Also a DDS test with ceftazidime disk may fail to detect MBLs when the isolates are AmpC hyperproducers (Lee et al., 2003). Franklin et al. (2006) used a DDS test together with a combined-disk test, to improve performance, and reported a sensitivity of 100% in detecting 84 MBL-producing isolates, but a specificity of 98% with 52 MBL-negative isolates. These studies indicate that development of novel phenotypic tests not dependent on MBL inhibitors is needed.

Hodge et al. (1978) made a simple modification of the cloverleaf test (Østravik and Ødegaard, 1971) to detect penicillinase-producing gonococci by inoculating a plate with indicator organism, *Escherichia coli*, in a manner similar to the current disk diffusion susceptibility testing. We modified the method of Hodge et al. to screen MBL-producing GNB (Lee et al., 2001). Anderson et al. (2007) evaluated the test and reported a 100% sensitivity and specificity in detecting a class A carbapenemases, KPC, in Enterobacteriaceae isolates. Our original modified Hodge test using Mueller-Hinton agar (MHA) occasionally gave negative or equivocal results in detecting MBL-producing *Pseudomonas* spp. and *Acinetobacter* spp. Galani et al. (2008) reported that 4.2% of MBL-producing Enterobacteriaceae isolates gave false-negative Hodge test. Although our preliminary study showed that the use of MacConkey agar could improve the Hodge test, the effect on screening MBL-producing GNB and on OXA-type carbapenemase-producing *Acinetobacter* has not been investigated.

The aims of this study were to identify the component of MacConkey agar and other factors that improve the Hodge test performance in the screening of MBL and OXA-type carbapenemases, and to determine whether the improvement is due to increased production of β-lactamases or enhanced release of them.

2. Materials and methods

2.1. Bacterial strains

Clinical isolates of GNB producing MBLs, OXA carbapenemases, and other β-lactamases were used (Table 1). One strain each of *Serratia marcescens* with SME-1, and *Pseudomonas aeruginosa* with VIM-1 were

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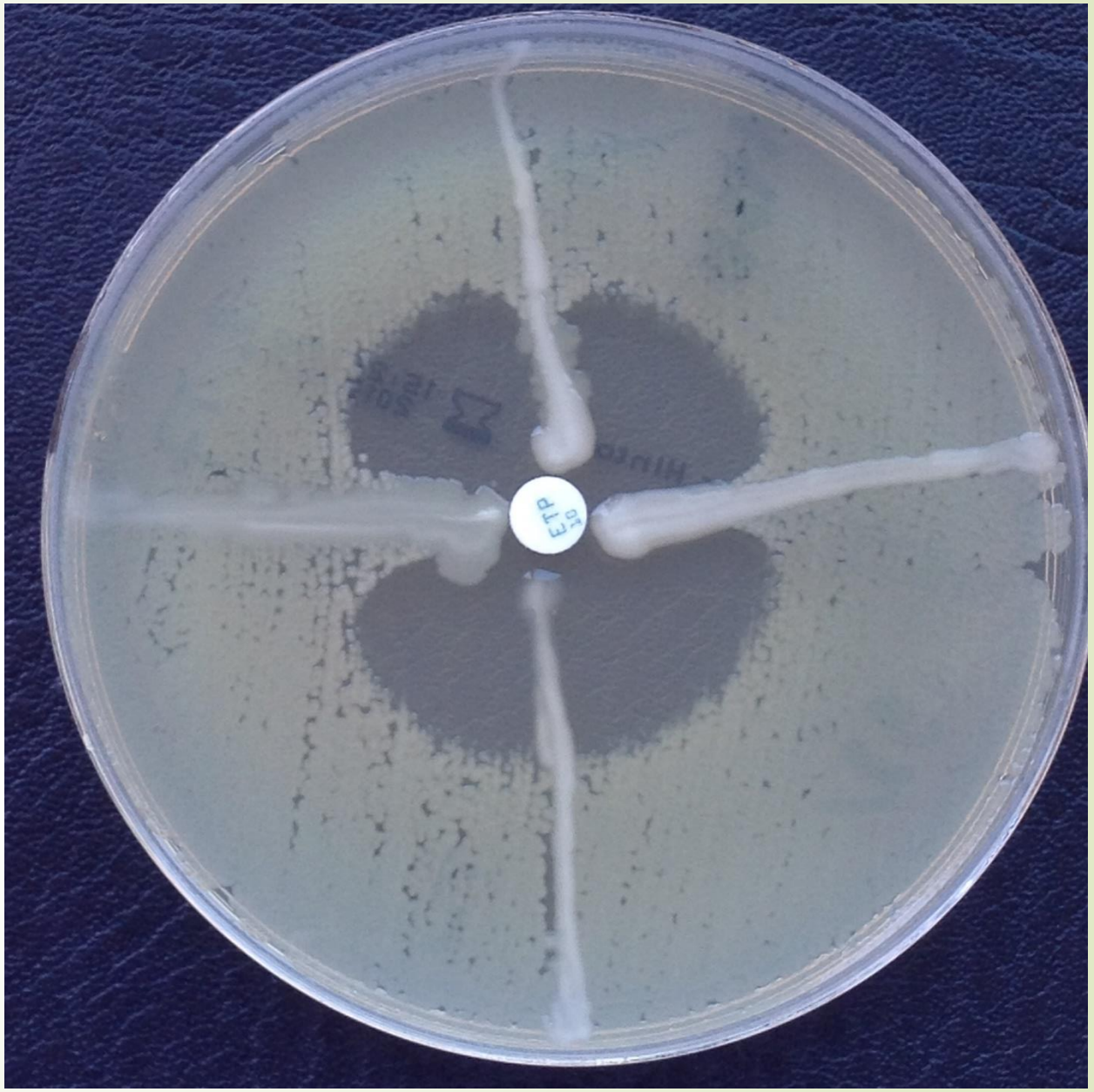


3.2. Screening for carbapenemase-production

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

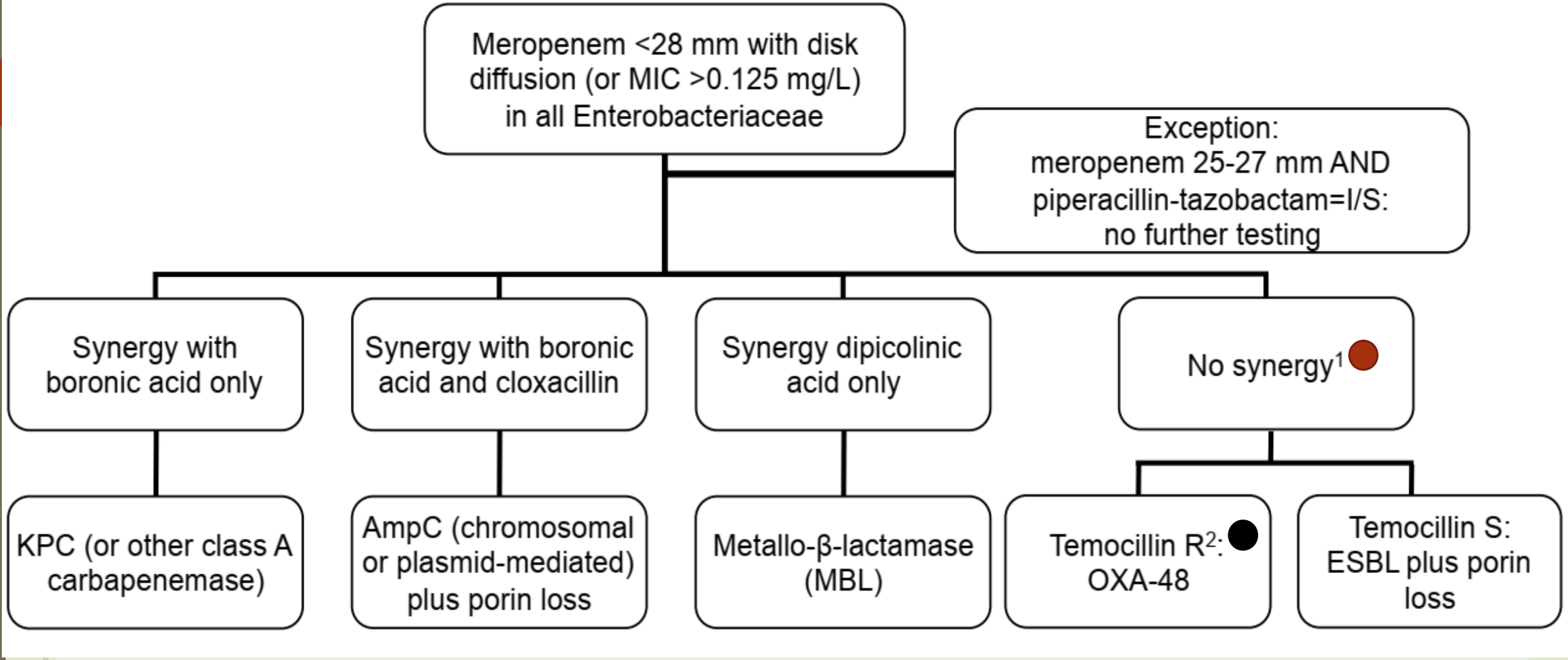
1: Meropenem offers the best balance of sensitivity and specificity

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).

3: High sensitivity but low specificity (isolates with ESBL and AmpC may be resistant without having carbapenemases)

3.3. Combination disk testing

- The disks contain **meropenem** ± various inhibitors.
- **Boronic acid** inhibits class A carbapenemases
- **Dipicolinic acid** and **EDTA** inhibit class B carbapenemases.
- **Avibactam** inhibits OXA-48
- **Cloxacillin** (inhibits AmpC β-lactamases), has been added to the tests to differentiate between [AmpC hyperproduction + porin loss] and [carbapenemase-production]

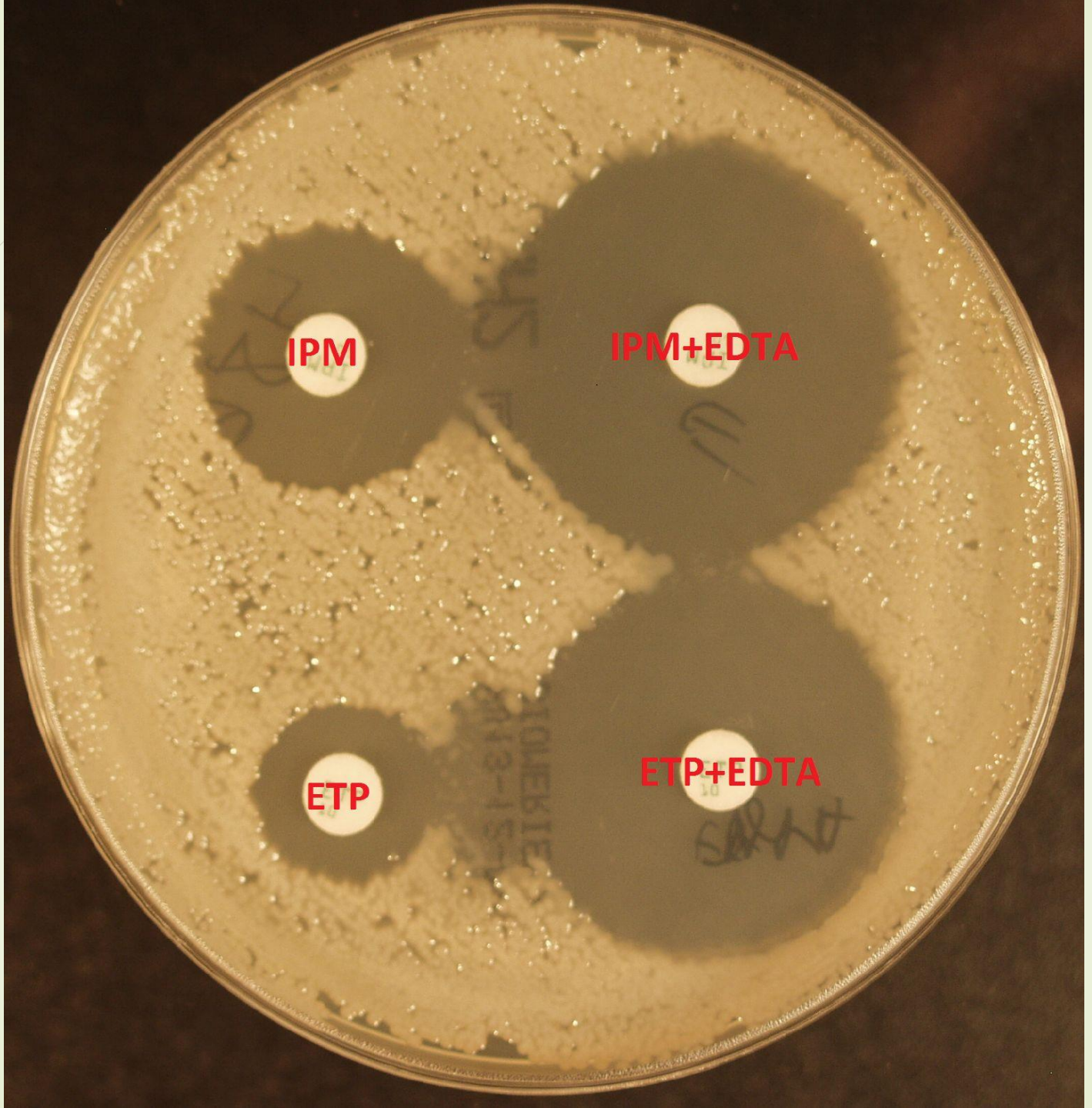


Algorithm for carbapenemase detection

- 1: Combination of several carbapenemases can also contribute to no synergy
- 2: High-level temocillin resistance (>128 mg/L, zone diameter <11 mm) is a phenotypic marker of OXA-48

B-lactamase	Synergy observed as increase in zone diameter (mm) with 10 µg meropenem disk/tablet				Temocillin MIC >128 mg/L or zone diameter <11 mm
	DPA/EDTA	APBA/PBA	DPA+APBA	CLX	
MBL	+	-	-	-	Variable[1]
KPC	-	+	-	-	Variable[1]
MBL + KPC[2]	Variable	Variable	+	-	Variable[1]
OXA-48-like	-	-	-	-	Yes
AmpC + porin loss	-	+	-	+	Variable[1]
ESBL + porin loss	-	-	-	-	No

Abbreviations: DPA=dipicolinic acid, APBA= aminophenyl boronic acid, PBA= phenyl boronic acid, CLX=cloxacillin


IPM
IPM+EDTA
ETP
ETP+EDTA

3.4. Biochemical (colorimetric) tests (Carba NP-test)

- The CarbaNP test is a rapid (<2 h) test for detection of carbapenem hydrolysis, which will give rise to a pH-change resulting in a colour shift from red to yellow with phenol red solution.
- The Carba NP test has been validated with bacterial colonies grown on Mueller-Hinton agar plates, blood agar plates, trypticase soy agar plates, and most selective media used in screening for carbapenemase producers.
- The Carba NP test should not be performed with bacterial colonies grown on Drigalski or McConkey agar plates.

Rapid Detection of Carbapenemase-producing *Enterobacteriaceae*

Patrice Nordmann, Laurent Poirel, and Laurent Dortet

To rapidly identify carbapenemase producers in *Enterobacteriaceae*, we developed the Carba NP test. The test uses isolated bacterial colonies and is based on in vitro hydrolysis of a carbapenem, imipenem. It was 100% sensitive and specific compared with molecular-based techniques. This rapid (<2 hours), inexpensive technique may be implemented in any laboratory.

Multidrug resistance is emerging worldwide at an alarming rate among a variety of bacterial species, causing both community-acquired and nosocomial infections (*1*). Carbapenems, the last line of therapy, are now frequently needed to treat nosocomial infections, and increasing resistance to this class of β-lactams leaves the health care system with almost no effective drugs (*1*). However, reports of carbapenem-resistant *Enterobacteriaceae* have increased (*2,3*). Resistance may be related to association of a decrease in bacterial outer-membrane permeability, with overexpression of β-lactamases with no carbapenemase activity or to expression of carbapenemases (*2,4,5*). Spread of carbapenemase producers is a relevant clinical issue because carbapenemases confer resistance to most β-lactams (*2*). Various carbapenemases have been reported in *Enterobacteriaceae,* such as the following types: *Klebsiella pneumoniae* carbapenemase (KPC; Ambler class A); Verona integron–encoded metallo-β-lactamase (VIM), imipenemase (IMP), New Delhi metallo-β-lactamase (NDM) (all Ambler class B); and oxacillinase-48 (OXA-48; Ambler class D) (*2,4–6*). In addition, carbapenemase producers are usually associated with many other non–β-lactam resistance determinants, which give rise to multidrug- and pandrug-resistant isolates (*2,3,7*).

Potential carbapenemase producers are currently screened first by susceptibility testing, using breakpoint values for carbapenems (*2,8*). However, this technique is time-consuming, and many carbapenemase producers do not confer obvious resistance levels to carbapenems. There is a need for laboratories to search for carbapenemase producers (*9*). Phenotype-based techniques for identifying in vitro production of carbapenemase, such as the modified Hodge test, are not highly sensitive and specific (*2,8,10*). Detection of metallo-β-lactamase producers (IMP, VIM, NDM) and of KPC producers may be based on the inhibitory properties of several molecules but requires additional expertise and time (usually an extra 24–48 hours) (*2,8,11,12*). Furthermore, no inhibitors are available for detecting OXA-48–type producers that are spreading rapidly, at least in northern Africa, the Middle East, and Europe (*2*). Molecular detection of carbapenemase genes remains costly and requires substantial expertise. Both the phenotype-based techniques and molecular tests are time-consuming (at least 12–24 hours) and are poorly adapted to the clinical need for isolating patients rapidly to prevent nosocomial outbreaks.

We developed a novel test, described here, based on a technique designed to identify the hydrolysis of the b-lactam ring of a carbapenem. This test is rapid, sensitive and specific, and adaptable to any laboratory in the world.

The Study

We included in the study 162 carbapenemase-producing strains of various enterobacterial species isolated from clinical samples (e.g., blood cultures, urine, sputum) and of global origin (Table 1). This collection of strains also included 46 strains that were fully susceptible to carbapenems or showed a decreased susceptibility to carbapenems as a consequence of non–carbapenemase-based mechanisms (Table 2). Antibiograms were carried out for all strains on Mueller-Hinton agar (Becton Dickinson, Le Point de Chaix, France) according to guidelines of the Clinical and Laboratory Standards Institute (*13*). The Carba NP (Carbapenemase Nordmann-Poirel) test was performed as follows. One calibrated dose (10 mL) of the tested strain directly recovered from the antibiogram was resuspended in a Tris-HCl 20 mmol/L lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific Pierce, Rockford, IL, USA), vortexed for 1 minute and further incubated at room temperature for 30 minutes. This bacterial suspension was centrifuged at 10,000 × *g* at room temperature for 5 minutes. Thirty μL of the supernatant, corresponding to the enzymatic bacterial suspension, was mixed in a 96-well tray with 100 μL of a 1-mL solution made of 3 mg of imipenem monohydrate (Sigma, Saint-Quentin Fallavier, France), pH 7.8, phenol red solution, and 0.1 mmol/L $ZnSO_4$ (Merck Millipore, Guyancourt, France). The phenol red solution was prepared by mixing 2 mL of a phenol red (Merck Millipore) solution 0.5% (wt/vol) with 16.6 mL of distilled water. The pH value was then adjusted to 7.8 by adding drops of 1 N NaOH. A mixture of the phe-

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Rapid Detection of Carbapenemase-Producing *Pseudomonas* spp.

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A novel biochemical technique, the Carba NP test, has been evaluated to detect carbapenemase production in *Pseudomonas* spp. This test was specific (100%), sensitive (94.4%), and rapid (<2 h). This cost-effective test, which could be implemented in any microbiology laboratory, offers a reliable technique for identification of carbapenemase-producing *Pseudomonas* spp.

Pseudomonas aeruginosa is intrinsically resistant to a number of β-lactams due to the low permeability of its outer membrane, the constitutive expression of various efflux pumps, and the production of β-lactamases (5). Acquired resistance to broad-spectrum β-lactams is increasingly observed in *P. aeruginosa*. Currently, PER-, VEB-, and GES-type enzymes are the most frequently observed extended-spectrum β-lactamases (ESBLs) identified in *Pseudomonas* spp. (5, 7). Therefore, carbapenems are considered crucial for treating many *P. aeruginosa*-associated infections.

In *Pseudomonas* spp., carbapenem resistance may be related either to a decreased bacterial outer membrane permeability (e.g., loss or modification of the OprD2 porin or overexpression of efflux pumps), often associated with overexpression of β-lactamases possessing no significant carbapenemase activity (AmpCs), or to expression of true carbapenemases (5, 14). In *Pseudomonas* spp., those carbapenemases are mostly metallo-β-lactamases (MBLs) of the VIM, IMP, SPM, GIM, AIM, DIM, and NDM types and, to a lesser extent, Ambler class A carbapenemases of the KPC and GES types (GES-2, -4, -5, -6, and -11) (2, 3, 12).

Screening of carbapenemase producers among carbapenem-resistant *P. aeruginosa* isolates is important since many carbapenemase genes are plasmid carried and easily transferable. Phenotypic techniques for *in vitro* identification of carbapenemase

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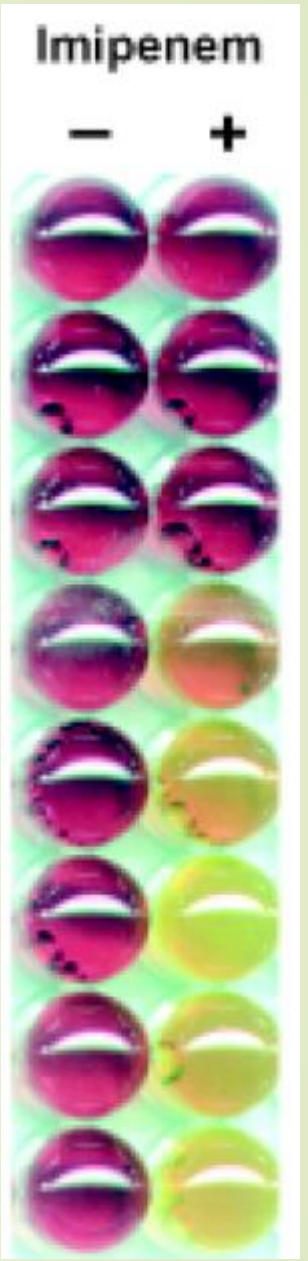
TABLE 1 Detection of carbapenemase activity in carbapenemase producers by using the Carba NP test

Ambler class	Carbapenemase type	Organism	β-Lactamase	MIC (mg/liter) IMP	MIC (mg/liter) MER	Carba NP test result
A	KPC	*P. aeruginosa* COL	KPC-2	>32	>32	+
		P. aeruginosa P13	KPC-2	>32	>32	+
		P. aeruginosa PA-2	KPC-2	>32	>32	+
		P. aeruginosa PA-3	KPC-2	>32	>32	+
	GES	*P. aeruginosa* GW-1	GES-2	3	1	−
		P. aeruginosa P35	GES-5	>32	>32	−
B	VIM	*P. aeruginosa* P0510	VIM-1	>32	>32	+
		Pseudomonas fluorescens COU	VIM-2	>32	>32	+
		P. aeruginosa REZ	VIM-2	>32	>32	+
		P. putida 9335	VIM-2	>32	>32	+
		P. stutzeri P511503100	VIM-2	>32	>32	+
		P. aeruginosa BY25753	VIM-2	>32	>32	+
		P. aeruginosa V919005	VIM-2	>32	>32	+
		P. aeruginosa AK5493	VIM-2	>32	>32	+
		P. aeruginosa KA-209	VIM-2	>32	>32	+
		P. putida NTU 91/99	VIM-2	>32	>32	+
		P. aeruginosa CAS	VIM-4	>32	>32	+
		P. aeruginosa JAC	VIM-4	>32	>32	+
	IMP	*P. aeruginosa* 12870	IMP-1	12	>32	+
		P. stutzeri PB207	IMP-1	2	4	+
		P. putida NTU 92/99	IMP-1	1	0.19	+
		P. aeruginosa	IMP-1	>32	>32	+
		P. aeruginosa 0607097	IMP-2	>32	>32	+
		P. aeruginosa ITA	IMP-13	>32	>32	+
	NDM	*P. aeruginosa* 453	NDM-1	>32	>32	+
		P. aeruginosa 353	NDM-1	>32	>32	+
	GIM	*P. aeruginosa* 73-12198	GIM-1	3	0.19	+
		P. aeruginosa 73-15574	GIM-1	>32	>32	+
		P. aeruginosa 73-15553A	GIM-1	>32	>32	+
		P. aeruginosa 73-5674	GIM-1	>32	>32	+
	AIM	*P. aeruginosa* WCH2677	AIM-1	>32	>32	+
		P. aeruginosa WCH2813	AIM-1	>32	>32	+
		P. aeruginosa WCH2837	AIM-1	>32	>32	+
	SPM	*P. aeruginosa* 16	SPM-1	>32	>32	+
	DIM	*P. stutzeri* 13	DIM-1	>32	>32	+
	BIC	*P. fluorescens*	BIC-1	>32	4	+



Rapid identification of carbapenemase-producing *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* using a modified Carba NP test

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Abstract

Biochemical tests have been previously developed to identify carbapenemase-producing *Enterobacteriaceae, Pseudomonas* spp. (Carba NP test) and *Acinetobacter* spp. (CarbAcineto NP test). We evaluated a modified Carba NP test to detect carbapenemase production in *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species using a single protocol with rapid results and found good reliability and speed.

Keywords: Carba NP test, carbapenemase, carbapenems, Gram negative, multidrug-resistant bacteria

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Multidrug-resistant Gram-negative bacteria (GNB) are increasingly being reported worldwide. The spread of carbapenemase-producing *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species have become a global threat. The emergence of resistance to carbapenems makes the treatment for infections caused by these carbapenem-resistant strains very limited [1–3]. Different types of carbapenemases have been reported, such as Ambler class A *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana extended spectrum (GES) β-lactamase, Ambler class B metallo-β-lactamases (MBL) and Ambler class D oxacillinase type [1].

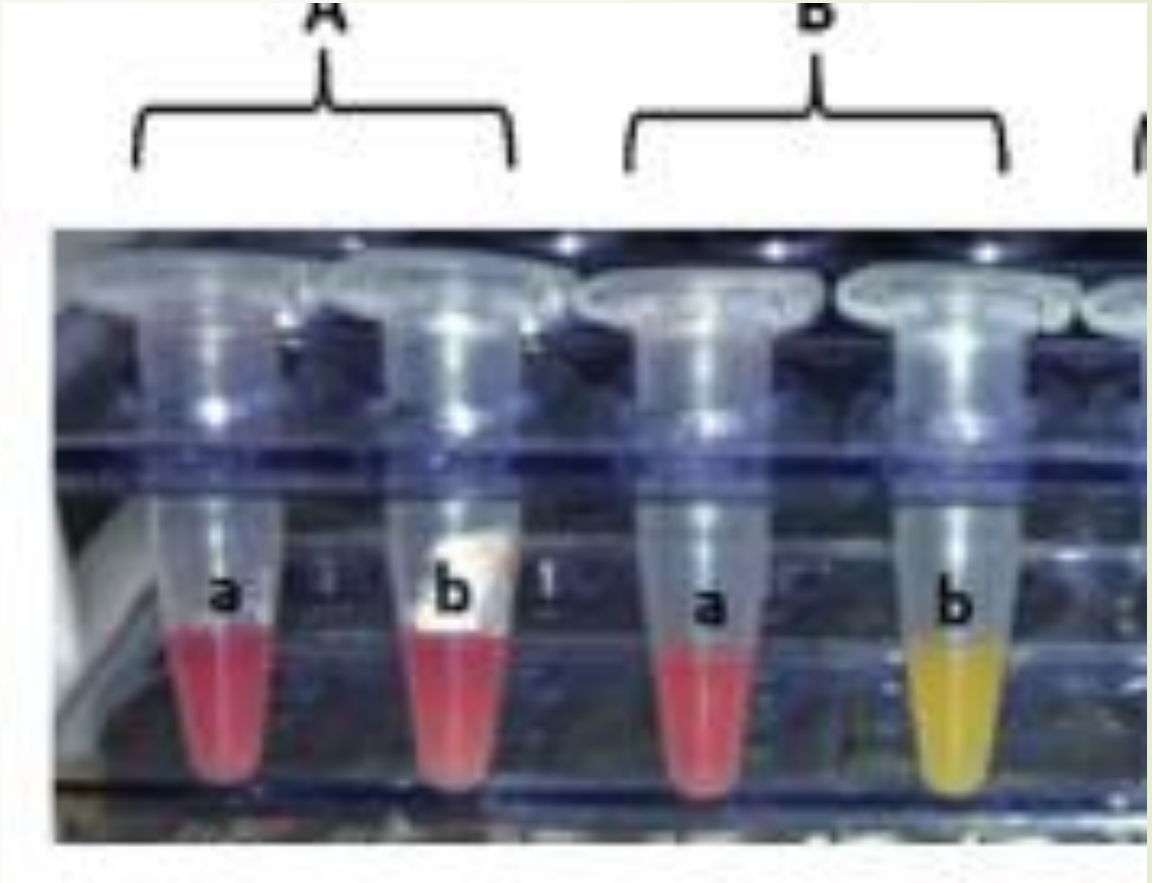
Rapid methods for detecting carbapenemase producers have been described, such as the MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) carbapenemase assay [4]. Previous studies have described a rapid biochemical carbapenemase detection method based on imipenem hydrolysis, the Carba NP test, for *Enterobacteriaceae* [5] and *Pseudomonas* species [6], as well as the CarbAcineto NP test for *Acinetobacter* species [7]. Recently, however, several authors have published evaluations of the Carba NP and the CarbAcineto NP tests; their criticisms focussed essentially on the absence of detection of oxacillinase (OXA) type carbapenemases [8–10].

Here we describe a modified Carba NP (MCNP) test which enables the rapid detection of different carbapenemases (KPC, MBL and OXA types) from *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species using a single protocol.

One hundred ten previously characterized GNB, including 69 carbapenemase-producing GNB (*Enterobacteriaceae* n = 14, *Pseudomonas aeruginosa* n = 11 and *Acinetobacter baumannii* n = 44), and 41 non-carbapenemase-producing GNB, including *Enterobacteriaceae* (n = 24), *P. aeruginosa* (n = 5) and *A. baumannii* (n = 12), were tested in two laboratories including Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE), Aix-Marseille University, Marseille, France, and Microbial Ecology laboratory, Béjaia University, Béjaia, Algeria (Table 1). Carbapenemase activity was assessed using phenotypic and genotypic tests, including the modified Hodge test, MALDI-TOF MS assay, PCR amplification and sequencing [4,11].

The Carba NP and the CarbAcineto NP tests are straightforward biochemical tests which identify carbapenemase production in GNB by detecting imipenem hydrolysis using phenol red solution as a colour indicator and a bacterial lysis buffer (B-PER II, Bacterial Protein Extraction Reagent) for *Enterobacteriaceae* and *Pseudomonas* species (Carba NP test) [5,6] and 5 M NaCl for *Acinetobacter* species (CarbAcineto NP test) [7].

In order to use a single protocol to detect the production of carbapenemases in the three types of bacteria



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3.5. Carbapenem Inactivation Method (CIM-test)

- The CIM-test utilizes antibiotic susceptibility-testing disks as substrate aliquots.
- Following 2H of incubation of a full loop of bacteria with a meropenem disk, the disk is placed on an MH agar inoculated with *E. coli* ATCC 25922.
- Enzymatic inactivation will produce no zone, whereas no carbapenemase activity will imply there will be a zone.
- One main disadvantage of this technique is that it requires usually at least 18 hours to obtain the results.

RESEARCH ARTICLE

The Carbapenem Inactivation Method (CIM), a Simple and Low-Cost Alternative for the Carba NP Test to Assess Phenotypic Carbapenemase Activity in Gram-Negative Rods

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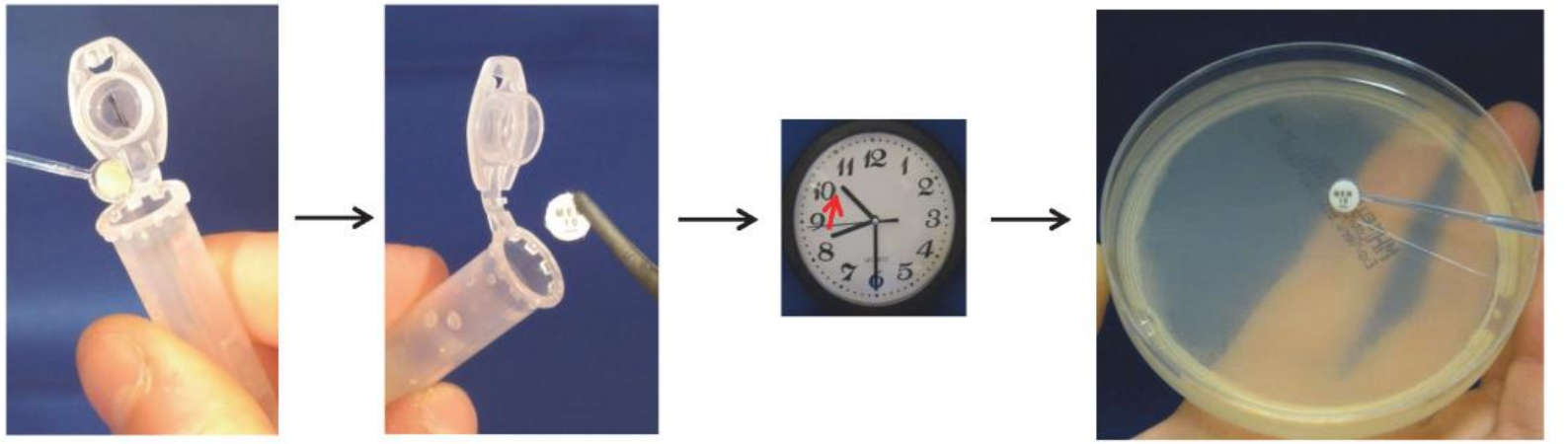
Abstract

A new phenotypic test, called the Carbapenem Inactivation Method (CIM), was developed to detect carbapenemase activity in Gram-negative rods within eight hours. This method showed high concordance with results obtained by PCR to detect genes coding for the carbapenemases KPC, NDM, OXA-48, VIM, IMP and OXA-23. It allows reliable detection of carbapenemase activity encoded by various genes in species of *Enterobacteriaceae* (e.g., *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*), but also in non-fermenters *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The CIM was shown to be a cost-effective and highly robust phenotypic screening method that can reliably detect carbapenemase activity.

Introduction

The emergence and spread of carbapenemase-producing Gram-negative rods is a worldwide emerging public health threat [1–3]. Particularly in health care centers, this may pose a major problem as carbapenems are becoming more frequently needed to treat infections caused by Gram-negative bacteria that produce extended spectrum beta-lactamases (ESBL) [4,5]. To prevent spread of carbapenemase producers, rapid detection of these bacteria has become imperative [6]. Resistance to carbapenems is assessed in phenotypic susceptibility assays either on agar plates or in automated microbiology systems. However, high or low minimal inhibitory concentrations (MICs) do not necessarily reflect the production of carbapenemases, as other mechanisms such as porin loss or increased efflux pump activity, due to alterations in chromosomally located genes, can also cause resistance [7,8]. Since carbapenemase-encoding genes are often located on plasmids, this type of resistance is much more likely to spread [9].



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Original Article

Performance of a new in-house medium Carba MTL-broth for the rapid detection of carbapenemase-producing *Enterobacteriaceae*

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Abstract

Introduction: The spread of carbapenemase-producing *Enterobacteriaceae* (CPE) represents a major public health issue. Methods allowing rapid detection of carbapenemases in developing countries are therefore urgently needed. In the current study, we developed a new in-house medium for the rapid detection of CPE isolates, especially OXA-48 producers.

Methodology: A panel of 144 clinical strains previously characterized was tested on in-house Carba MTL-broth medium using four different concentrations of ertapenem (0.5 to 2 mg/L), and compared to chromID® OXA-48 and chromID® CARBA (BioMérieux) media.

Results: Comparative evaluation of the Carba MTL-broth with chromID® OXA-48 and chromID® CARBA showed that chromID® OXA-48 and Carba MTL-broth had the highest sensitivity for detection of OXA-48 producers (93.9% and 100%, respectively) comparatively to chromID® CARBA (21.2%). The chromID® OXA-48 had the highest specificity (100%), as compared to the Carba MTL-broth (65.5%) and chromID® CARBA (84.4%) for the detection of OXA-48 producers.

Conclusions: The in-house Carba MTL-broth developed in this study is sensitive, inexpensive, an easy-to-use phenotypic method for the detection of OXA-48-producing enterobacteria. Given the burden of pan-drug resistance, its implementation in the microbiology laboratory of developing countries could be a useful tool for rapid detection of these bacteria.

Key words: Carbapenemase-producing *Enterobacteriaceae*; carba MTL-broth; developing countries; OXA-48-like; screening medium.

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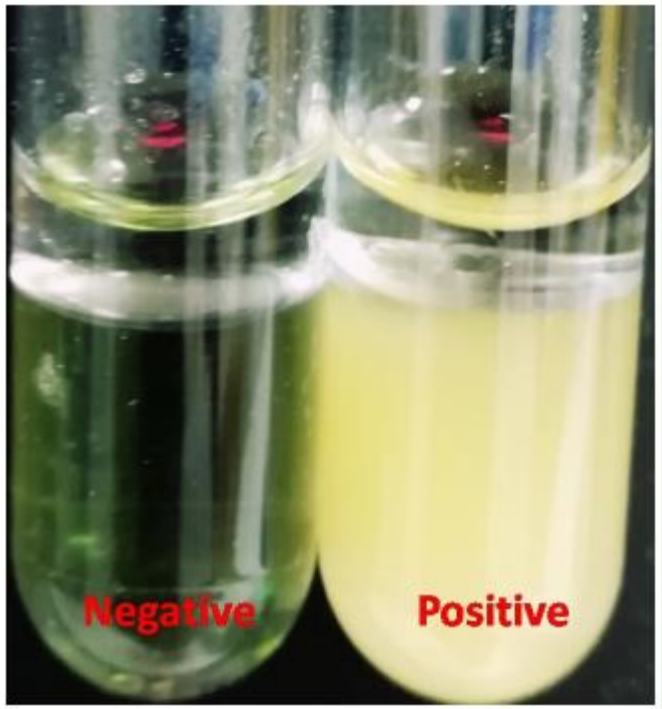
Introduction

Multidrug resistance is an important Public Health problem and has been increasingly reported throughout the world especially concerning the *Enterobacteriaceae* isolates. One of the most important emerging resistance traits corresponds to the production of carbapenemases hydrolyzing carbapenems, which confer resistance to almost all β-lactams [1,2]. The main groups of carbapenemases identified in *Enterobacteriaceae* are Ambler class A (KPC-type) that are able to hydrolyze all β-lactams except cephamycins, the zinc-dependent metallo-β-lactamases (MBL) Ambler class B (NDM, VIM, and IMP) of hydrolyzing all β-lactams except aztreonam, and the Ambler class D (OXA-48-like) carbapenemases, hydrolyzing carbapenems and broad-spectrum cephalosporins only weakly [3-5]. The level of resistance to carbapenems conferred by those carbapenemase producers may vary significantly, making their detection difficult [6].

Vigilant surveillance, rapid and reliable identification of these strains by the personnel in the clinical microbiology laboratory are essential to effective infection control [7]. In this aim, it is important to define robust standardized screening methods for the detection of carbapenemase-producing *Enterobacteriaceae* (CPEs) which can be used in all laboratories, particularly in developing countries where the carbapenemases are diffused [3,4,8,9]. Currently, different methods are used to detect enzymatic resistance to carbapenems including phenotypic methods (*e.g.* chromogenic media), mass spectrometry, biochemical-based methods (*e.g.* Carba NP test and

Figure 1. Interpretation colors of Carba MTL-broth.



Note

Comparison of the Modified-Hodge test, Carba NP test, and carbapenem inactivation method as screening methods for carbapenemase-producing *Enterobacteriaceae*

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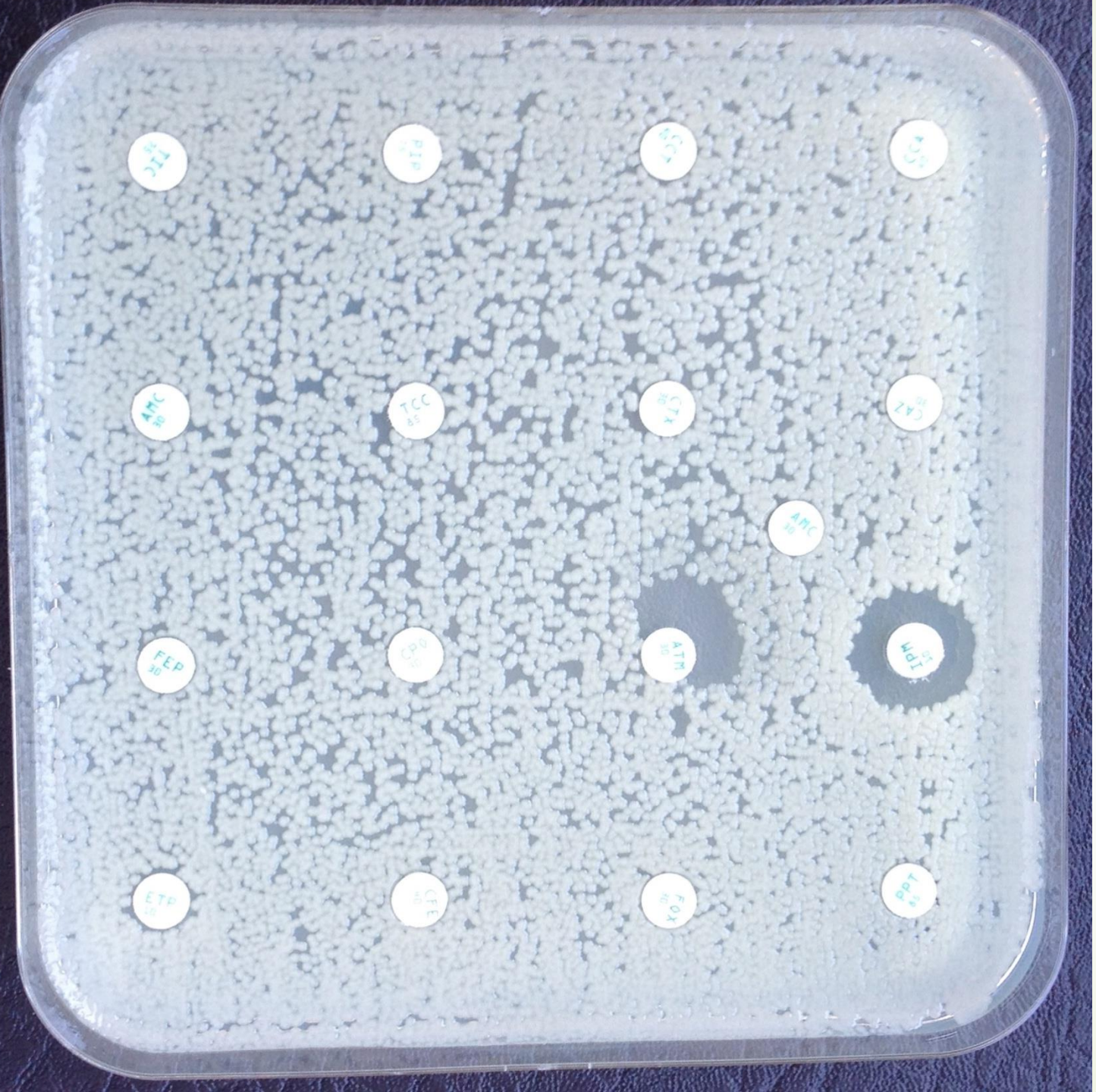
ABSTRACT

We compared three screening methods for carbapenemase-producing *Enterobacteriaceae*. While the Modified-Hodge test and Carba NP test produced false-negative results for OXA-48-like and mucoid NDM producers, the carbapenem inactivation method (CIM) showed positive results for these isolates. Although the CIM required cultivation time, it is well suited for general clinical laboratories.

Table 3
Sensitivity and specificity of each screening method.

	CIM			MHT		CNPt
	Imipenem	Meropenem	Ertapenem	Meropenem	Ertapenem	
Sensitivity (%)[a]	100	97.0	93.9	81.8	90.9	87.9
Specificity (%)[a]	56.5	95.7	100	82.6	65.0	100


TIC 75
PIP 100
CCV 30
CTX 30
AMC 20
TCC 65
CTX 30
CAZ 30
AMC 30
FEP 30
CPO 30
ATM 30
IPM 10
ETP 10
CTX 30
FOX 30
TOB 10