

CARBAPENEMASAS EN ENTEROBACTERIAS

A decorative graphic consisting of a white circle with a teal outline, positioned centrally on a horizontal dotted line that separates the title area from the author information area.

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Carbapenemasas: definición



- Las carbapenemasas son enzimas que inactivan todos los antibióticos betalactámicos (penicilinas, cefalosporinas y carbapanemes)
- Presentan un importante problema clínico: **Livermore DM. Has the era of untreatable infections arrived? J Antimicrob Chemother. 2009;64 Suppl 1:i29-36.**
- Pueden estar presentes algunas de las especies bacterianas con más importancia clínica, sobre todo asociadas a infección nosocomial:
 - *Escherichia coli*
 - *Klebsiella pneumoniae*

Carbapenemas: panorama actual



Carbapenemases in *Klebsiella pneumoniae* and Other *Enterobacteriaceae*: an Evolving Crisis of Global Dimensions

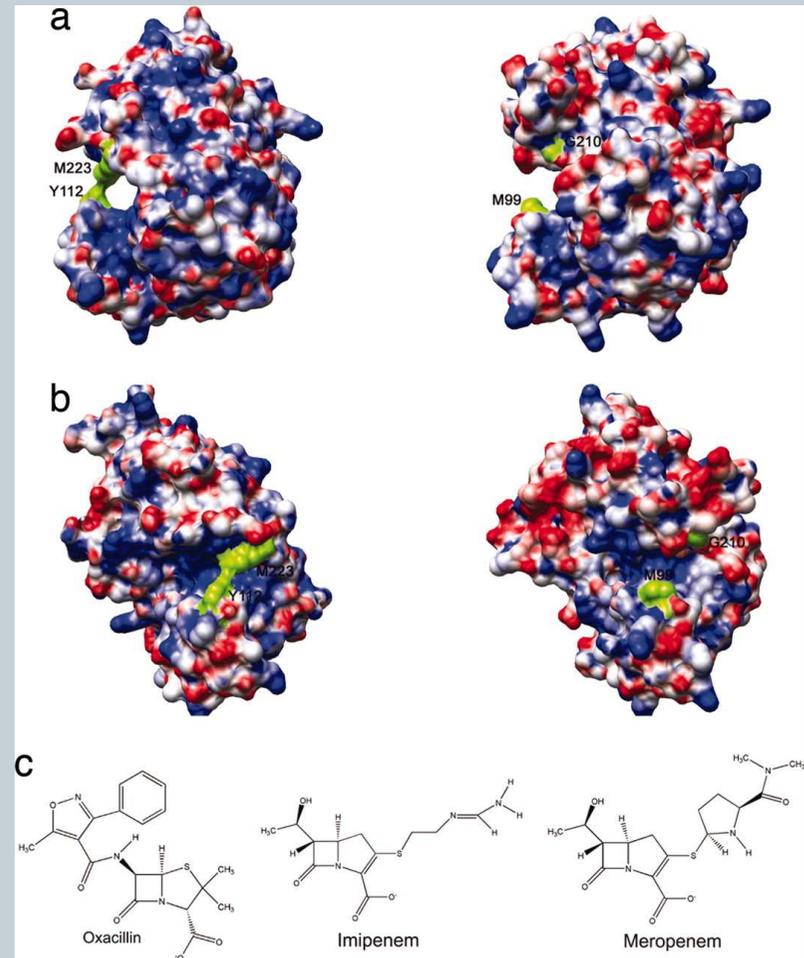
L. S. Tzouveleki^a, A. Markogiannaki^b, M. Psychogiou^c, P. T. Tassios^a and G. L. Daikos^c

Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe

R. Cantón^{1,2}, M. Akóva³, Y. Carmeli⁴, C. G. Giske⁵, Y. Glupczynski⁶, M. Gniadkowski⁷, D. M. Livermore^{8,9}, V. Miriagou¹⁰, T. Naas¹¹, G. M. Rossolini¹², Ø. Samuelsen¹³, H. Seifert¹⁴, N. Woodford⁹ and P. Nordmann¹¹; the European Network on Carbapenemases*

Carbapenemasas: características generales

- Los estudios fenotípicos clásicos (antibiograma) presentan muchas limitaciones en su detección, ya que estas bacterias **pueden tener valores de concentración mínima inhibitoria sólo ligeramente elevados**
- Como estos enzimas pertenecen a muchos grupos, su detección genotípica también es difícil



Carbapenemasas: perfiles de resistencia



TABLE 4. Expected phenotypes of carbapenemase producers for selected β -lactams

Organism	Enzyme	AMP	TIC	TZP	CAZ	ATM	IMP	ETP
<i>Pseudomonas aeruginosa</i>	MBL	–	R	R	R	S	R	–
	KPC	–	R	R	R	R	R	–
<i>Acinetobacter baumannii</i>	MBL	R	R	R	R	S	I/R	–
	OXA	R	R	I	S	S	I/R	–
<i>Enterobacteriaceae</i> AmpC(–)	MBL	R	R	S/I	R	S	S/I/R	I/R
<i>Enterobacteriaceae</i> AmpC(+)	MBL	–	R	S/I	R	S	S/I/R	I/R
<i>Enterobacteriaceae</i> AmpC(–)	KPC/GES	R	R	R	I/R	R	S/I/R	I/R
<i>Enterobacteriaceae</i> AmpC(+)	KPC/GES	–	R	R	R	R	I/R	I/R
<i>Enterobacteriaceae</i> AmpC(–)	OXA-48	R	R	R	I/S	S	S/I	S/I

MBL, metallo- β -lactamase; AMP, ampicillin; TIC, ticarcillin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; ATM, aztreonam; IMP, imipenem; ETP, ertapenem. Phenotypes may vary for many organism–compound combinations, depending on enzyme variants, expression level or additional mechanisms.

Carbapenemasas: características generales



Grupos de carbapenemasas

Clase A: Familias SME, IMI, NMC, GES y KPC

Metalobetalactamasas: Familias IMP, VIM, SPM, GIM y SIM

Clase D: Tipo OXA-beta-lactamasas (50 tipos)

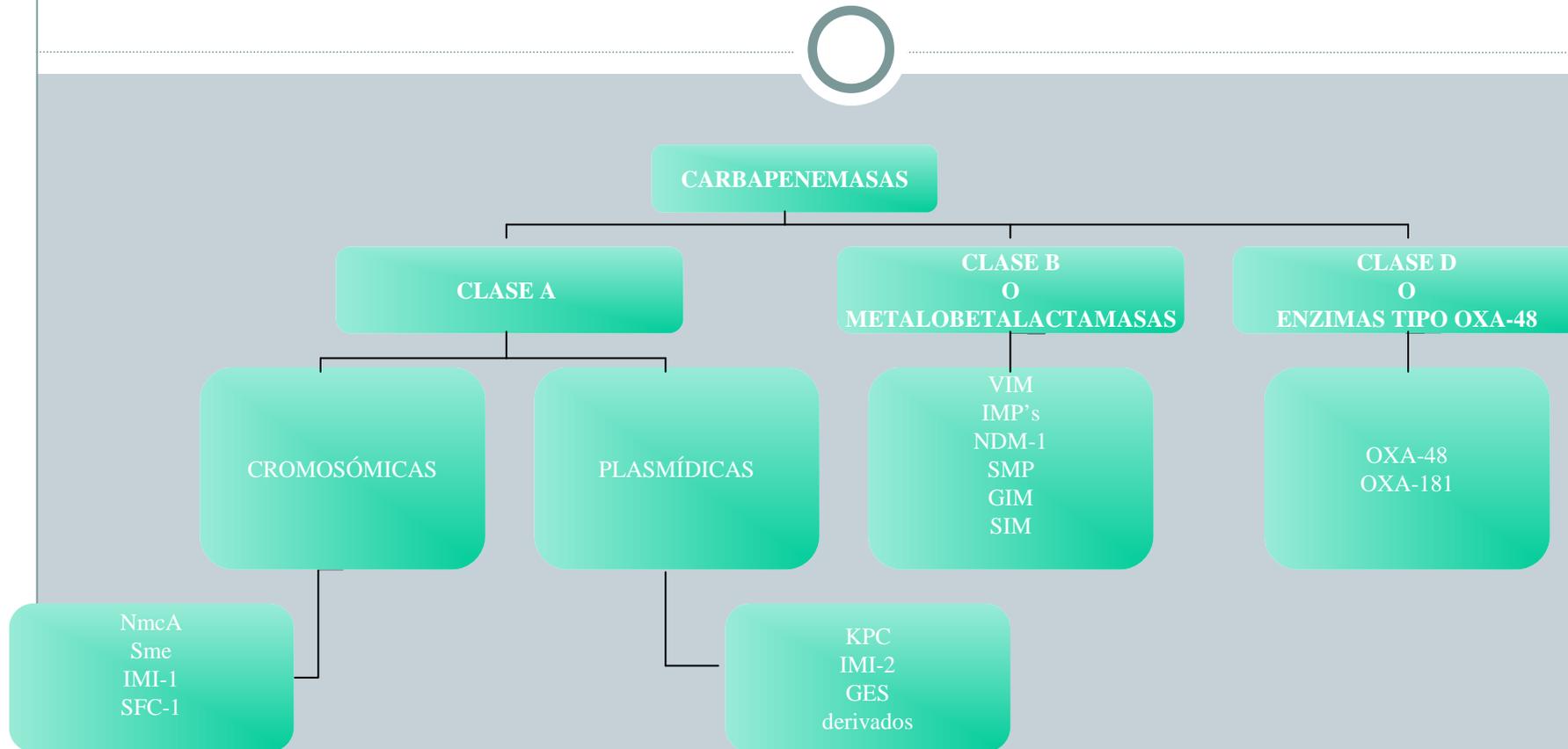
TABLE 4. Substrate and inhibition profiles of the carbapenemases

Molecular class	Functional group	Enzyme	Hydrolysis profile ^a					Inhibition profile ^b		Reference(s)
			Penicillins	Early cephalosporins	Extended-spectrum cephalosporins	Aztreonam	Carbapenems	EDTA	Clavulanic acid	
A	2f	NMC	+	+	+	+	+	-	+	124
		IMI	+	+	+	+	+	-	+	183
		SME	+	+	±	+	+	-	+	179
		KPC	+	+	+	+	+	-	+	4
		GES	+	+	+	-	±	-	+	174, 219
B1	3	IMP	+	+	+	-	+	+	-	224
		VIM	+	+	+	-	+	+	-	224
		GIM	+	+	+	-	+	+	-	224
		SPM	+	+	+	-	+	+	-	224
D	2d	OXA	+	+	±	-	±	-	±	225

^a Symbols: +, strong hydrolysis (generally, k_{cat} of $>2 \text{ s}^{-1}$); ±, weak hydrolysis (generally, k_{cat} of 0.5 to 2 s^{-1}); -, no measurable hydrolysis reported (generally, k_{cat} of $<0.5 \text{ s}^{-1}$).

^b Symbols: +, reported inhibition; ±, variable inhibition among β -lactamase family members; -, no inhibition reported.

Carbapenemasas: Clasificación



Carattoli A. Plasmids and the spread of resistance. *Int J Med Microbiol.* 2013; S1438-4221

Carbapenemasas: características generales

TABLE I. Species distribution of clinically relevant acquired carbapenemases

Organism	MBLs (class B)	Class A KPC (GES)	OXA (class D)
Pseudomonads			
<i>Pseudomonas aeruginosa</i>	++	+	+
<i>Pseudomonas putida</i>	+	+	
<i>Acinetobacter baumannii</i>	+ ^a		++
<i>Acinetobacter</i> spp.	+		+
Enterobacteria			
<i>Klebsiella pneumoniae</i>	+ ^a	++	+
<i>Escherichia coli</i>	+	+	+
<i>Proteus mirabilis</i>	+		+
<i>Providencia</i> spp.	+		
<i>Klebsiella oxytoca</i>	+	+	
<i>Serratia marcescens</i>	+ ^a	+	
<i>Enterobacter</i> spp.	+ ^a	+	
<i>Citrobacter freundii</i>	+	+	
<i>Morganella morganii</i>	+		
<i>Salmonella enterica</i>		+	
<i>Raoultella</i> spp.		+	

MBL, metallo- β -lactamase.

++, prevalent species–enzyme type combinations; +, occasionally reported species–enzyme type combinations.

^aEndemic in certain regions.

Crosses in bold denote higher prevalence in the respective species.

¿Qué concentraciones utilizar como cribado?



Willems E, et al. Towards a phenotypic screening strategy for emerging β -lactamases in Gram-negative bacilli. *Int J Antimicrob Agents*. 2013;41:99-109.

Cribado en Enterobacteriaceae: 0.5 μ g/mL meropenem

Confirmatory Test for Suspected Carbapenemase Production in *Enterobacteriaceae* for Use With Table 2A

When to do this test:

Institutional infection control procedures or epidemiological investigations may require identification of carbapenemase-producing *Enterobacteriaceae*. Carbapenemase-producing isolates usually test intermediate or resistant to one or more carbapenems using the **current** interpretive criteria as listed in Table 2A (Note: **Ertapenem nonsusceptibility is the most sensitive indicator of carbapenemase production**), and test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). Therefore, for infection control or epidemiological investigations, testing could be limited to isolates with these characteristics.

Clinical and Laboratory Standards Institute 2012

Sistemas automatizados comercializados



Sistema	Panel	Rango ($\mu\text{g/mL}$) Meropenem	Rango ($\mu\text{g/mL}$) Ertapenem
MicroScan [®]	NM40	0.5 - 8	0.5 - 1
	NM37	1 - 8	0.5 - 4
	NC53	-	0.5 - 4
	NU52	-	1
VITEK 2 [™]	AST-N245	0.5 - 12 *	-
	AST-N244	-	0.5 - 6
BD Phoenix [™]	NMIC-64	1 - 8	-
	NMIC-70	1 - 8	0.5 - 4
	UNMIC-53	1 - 8	-

* Realiza una interpretación cinética que amplía el rango de CMI a 0.25 – 16 $\mu\text{g/mL}$

Puntos de corte actuales



Carbapenems	CLSI 2012						EUCAST 2013			
	CMI (µg/mL)			Diámetro (mm)			CMI (µg/mL)		Diámetro (mm)	
	≤S	I	R≥	≥S	I	R≤	≤S	R>	≥S	R>
Doripenem	1	2	4	23	20-22	19	1	4	24	18
Ertapenem	0.5	1	2	22	19-21	18	0.5	1	25	22
Imipenem	1	2	4	23	20-22	19	2	8	22	16
Meropenem	1	2	4	23	20-22	19	2	8	22	16

CMI a carbapenems



Organism/enzyme type	MICs (mg/L)		
	Imipenem	Meropenem	Ertapenem
<i>Enterobacteriaceae</i> AmpC(-)/MBL	0.5 to >64	0.25 to >64	0.5–4(?) Not enough data ^a
<i>Enterobacteriaceae</i> AmpC(+)/MBL	1 to >32	1 to >32	Not enough data ^a
<i>Enterobacteriaceae</i> AmpC(-)/KPC	0.5 to >64	1 to >32	0.5 to >64
<i>Enterobacteriaceae</i> AmpC(+)/KPC	8 to >64	4–64	8 to >64
<i>Enterobacteriaceae</i> AmpC(-)/OXA (OXA-48)	1 to >64	0.5–64	4 to >64

MBL, metallo- β -lactamase.

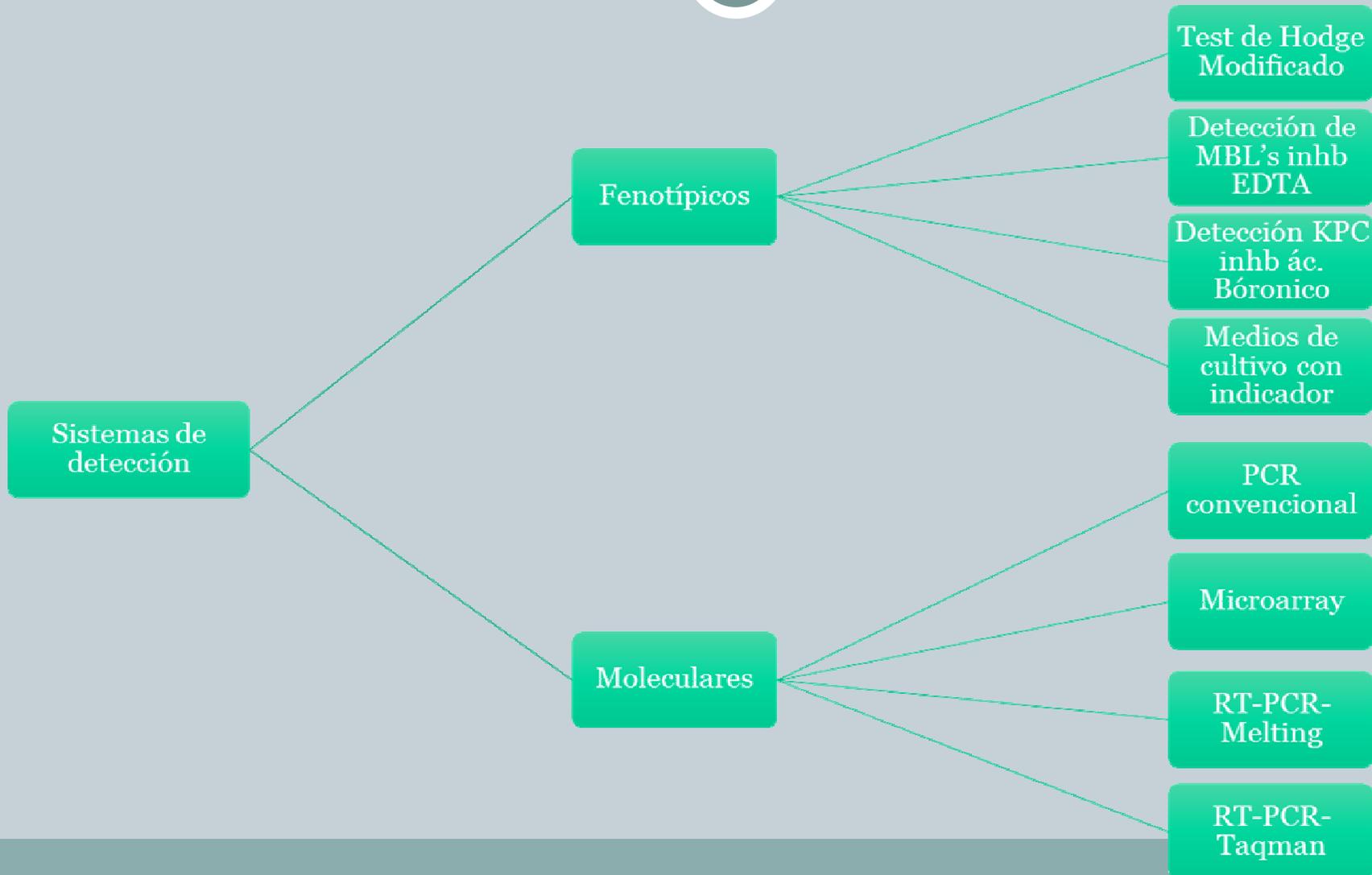
^aMost of the papers do not include ertapenem MIC values.

(?)Upper limit uncertain.

Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues.

Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, Malamou-Lada E, Martinez-Martinez L, Navarro F, Nordmann P, Peixe L, Pournaras S, Rossolini GM, Tsakris A, Vatopoulos A, Cantón R.

Carbapenemasas: Sistemas de detección



Carbapenemasas: Detección



Métodos fenotípicos

Métodos fenotípicos



Test de Hodge



**Etest con carbapenems y EDTA
(Clase B)**



Borónico (Clase A)

¿Qué placas cromogénicas podemos usar?



Comparison of four laboratory methods in the detection of carbapenemase-producing Enterobacteriaceae.

Frawley J, Mangan L, Boo TW.

J Med Microbiol. 2013 Mar 28.

Comparación de:

- Brilliance CRE agar (Oxoid)
- ChromID ESBL agar (bioMerieux)
- CHROMagar KPC

Conclusiones:

- Para KPC y NDM las tres placas tienen el mismo límite de detección
- ChromID ESBL y CHROMagar KPC fallan en la detección de OXA-48
- ChromID ESBL agar fue el único medio para detectar VIM

¿Qué placas cromogénicas podemos usar?



Comparison of the SUPERCARBA, CHROMagar KPC, and Brilliance CRE screening media for detection of Enterobacteriaceae with reduced susceptibility to carbapenems.

Girlich D, Poirel L, Nordmann P.

Diagn Microbiol Infect Dis. 2013 Feb; 75(2):214-7.

SUPERCARBA medium: (96.5%)
Brilliance CRE (76.3%)
CHROMagar KPC (43%)
Especificidad: Entre 57% y 68%.



CHROMagar™ KPC
Typical Appearance of Microorganisms

E.coli CarbapenemR



Dark pink
to reddish

Klebsiella, Enterobacter, Citrobacter CarbapenemR



Metallic blue

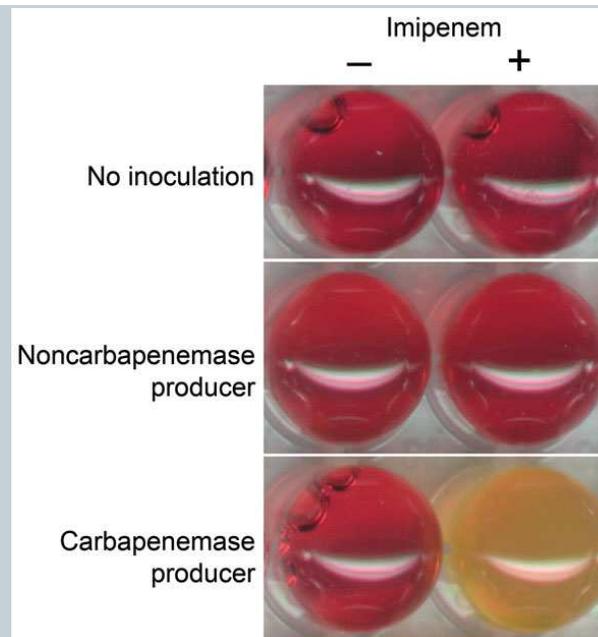


Nueva prueba bioquímica de cribado



Test bioquímico: (**Carba NP test II**)

- Permite identificar y discriminar entre las clases del carbapenemasas: (A, B, and D)
- Se basa de la detección de la acidificación resultante de la hidrólisis del imipenem
- Sensibilidad y especificidad del 100%



Rapid identification of carbapenemase types in Enterobacteriaceae and Pseudomonas spp. by using a biochemical test.

Dortet L, Poirel L, Nordmann P.

Antimicrob Agents Chemother. 2012 Dec;56(12):6437-40

Strategies for identification of carbapenemase-producing Enterobacteriaceae.

Nordmann P, Poirel L.

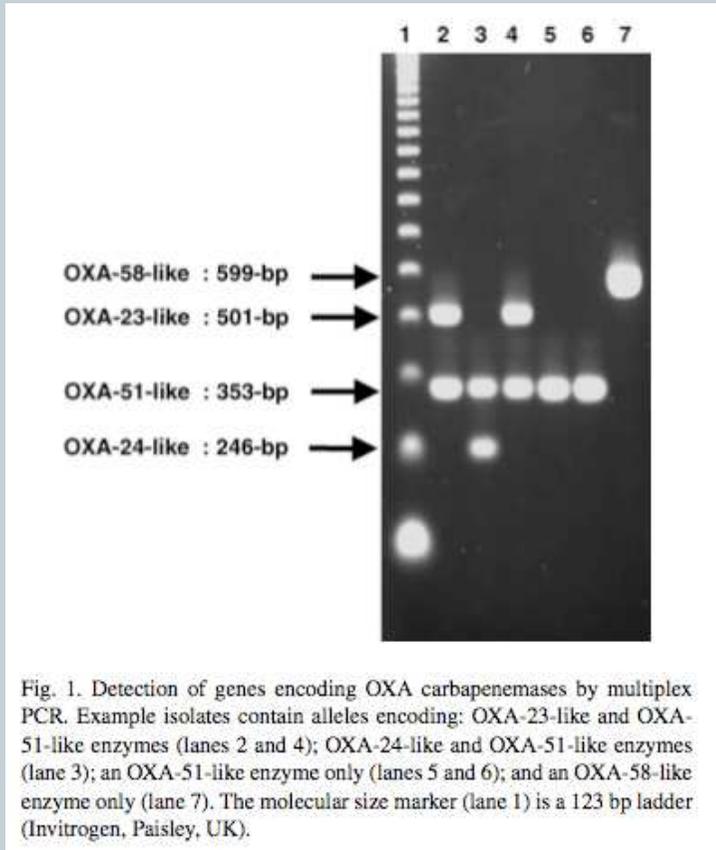
J Antimicrob Chemother. 2013 Mar;68(3):487-9.

Carbapenemasas: Detección

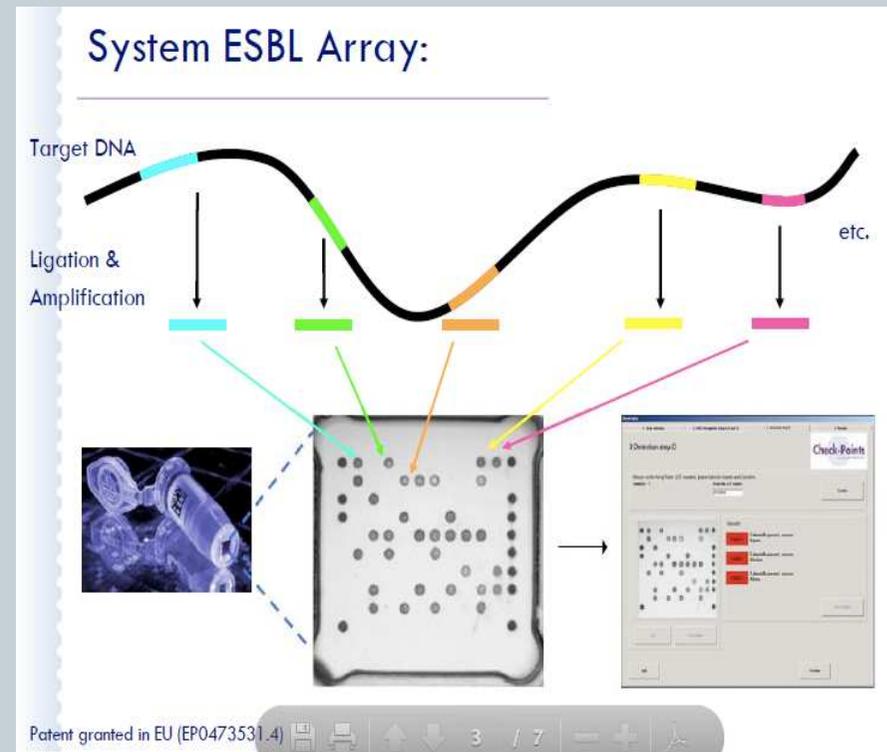


Test no
fenotípicos

Métodos moleculares

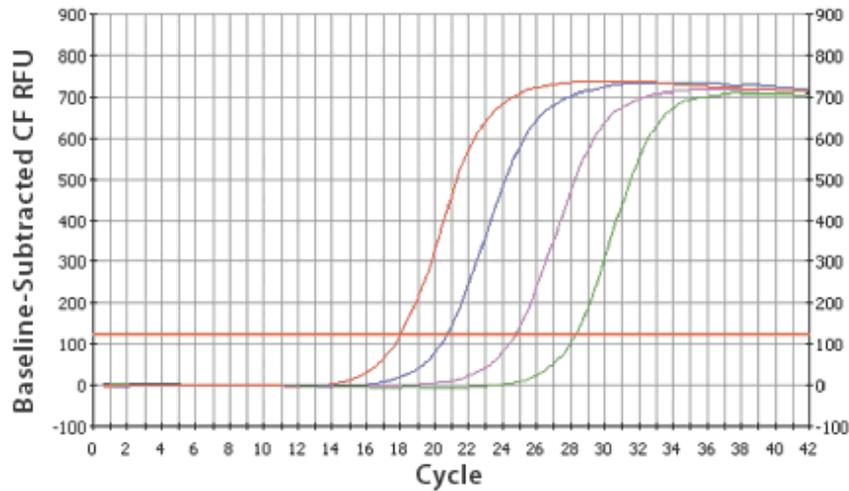


PCR convencional

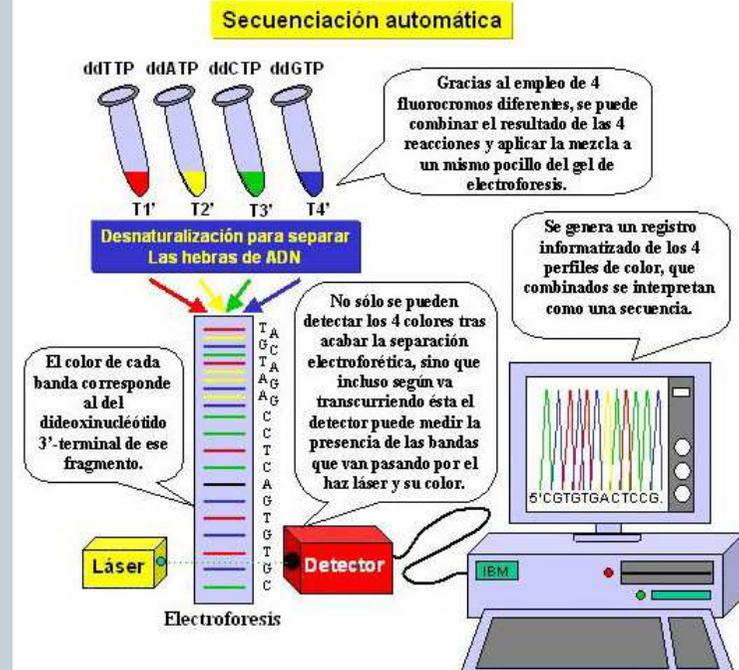


Detección por check-points

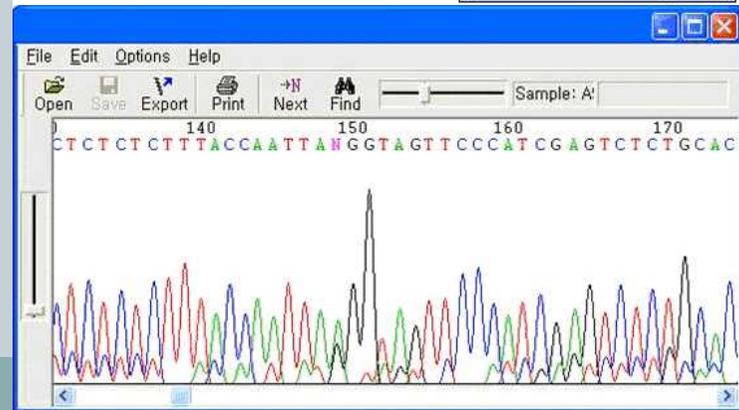
Métodos moleculares



Real Time PCR



Secuenciación

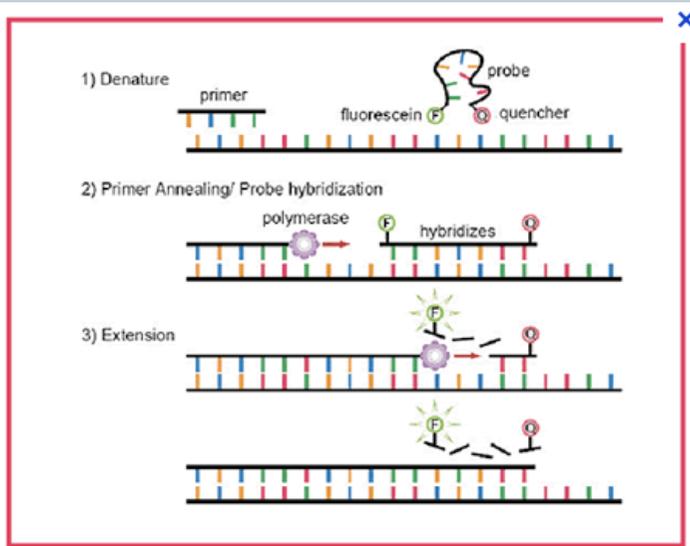


Real time PCR: en proceso de patente

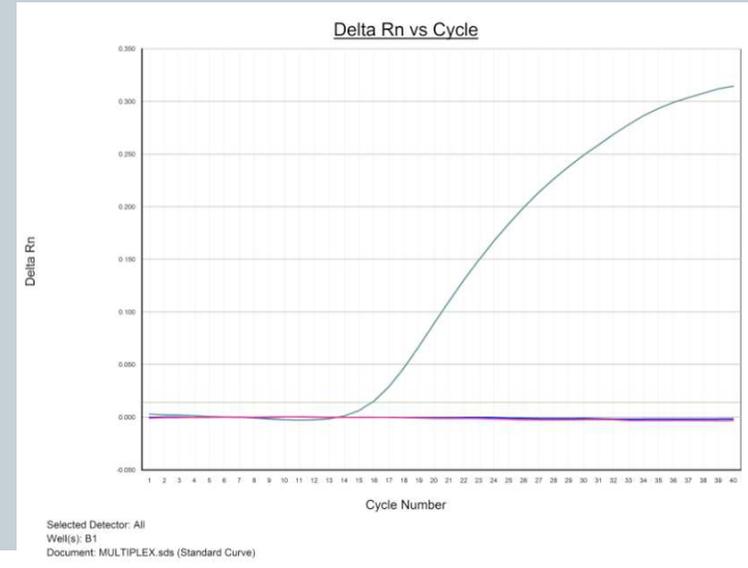


Reacción MPX	Dianas que se detectan	Fluoróforos empleados
1	Oxa-23	CY5
	Oxa-51	TAMRA
	Oxa-58	JOE
2	Oxa-133	TAMRA
	Oxa-182	JOE
	Oxa-48	CY5
3	Oxa-24	JOE
	Oxa-54	TAMRA
	Oxa-62	CY5
4	KPC	CY5
	NMC/IMI	JOE
	SME	TAMRA
5	GES-CARB	CY5
6	IMP-15	TAMRA
	IMP-19	JOE
	IMP-3	CY5
7	GIM	TAMRA
	SPM	JOE
	VIM	CY5
8	NDM	CY5
	SIM	TAMRA

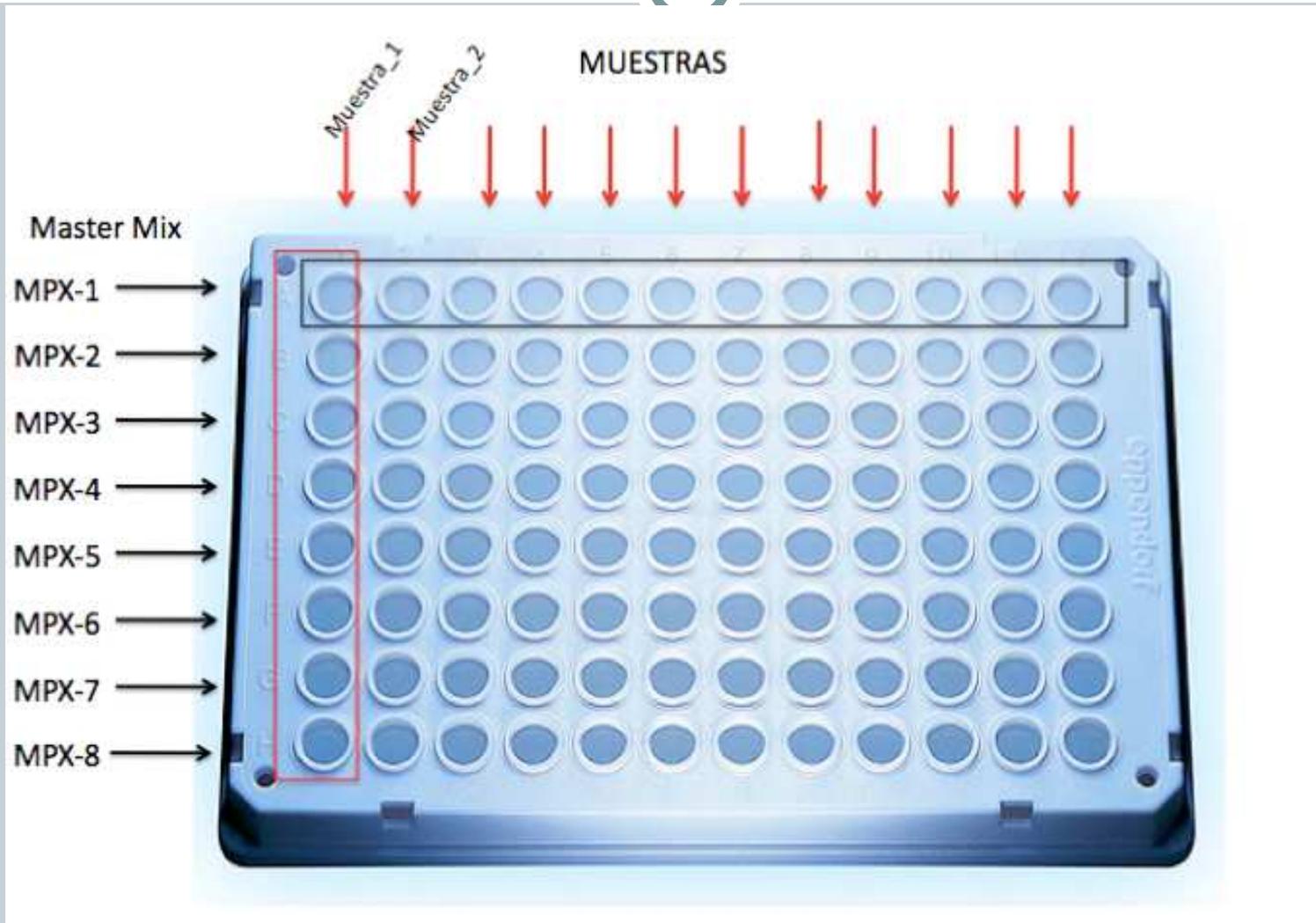
Real time PCR: en proceso de patente



TaqMan® Probe M



Real time PCR: en proceso de patente



Carbapenemasas: nuevos métodos de detección

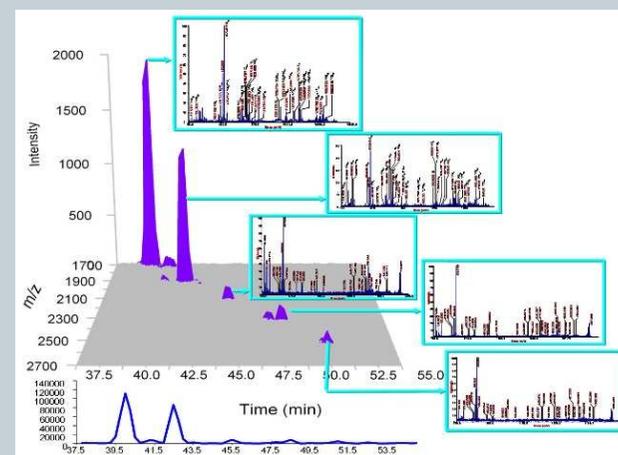
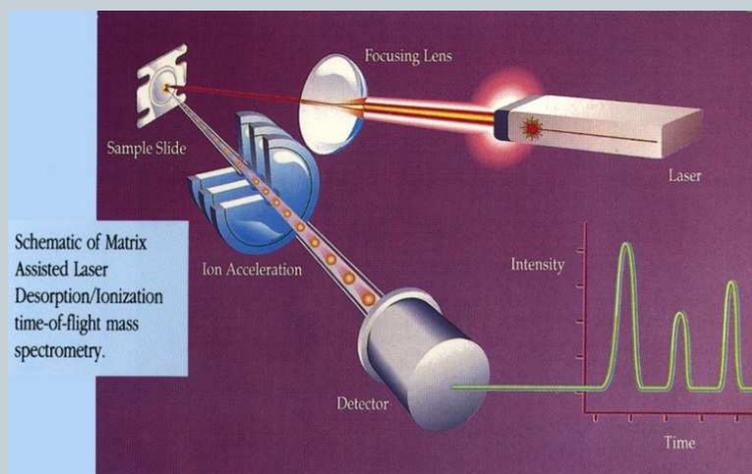


Detección de los metabolitos del carbapenem tras incubación con la bacteria

MALDI-TOF MS applied to indirect carbapenemase detection: a validated procedure to clearly distinguish between carbapenemase-positive and carbapenemase-negative bacterial strains.

Wang L. Han C. Sui W. Wang M. Lu X.

Anal Bioanal Chem. 2013 Jun;405(15):5259-66.



Carbapenemas: Comunidad Valenciana

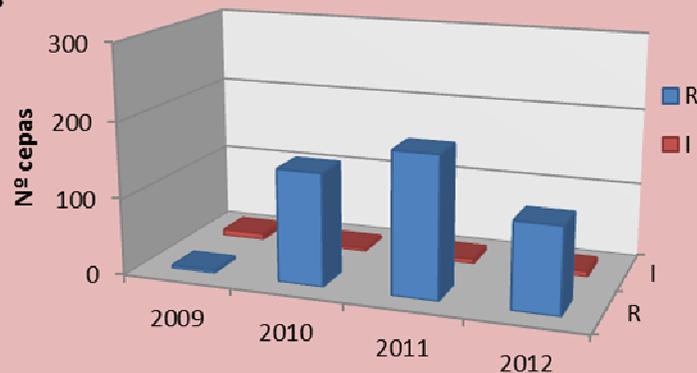


Situación en nuestro medio

Escherichia coli 2009-2012 en la Comunidad Valenciana

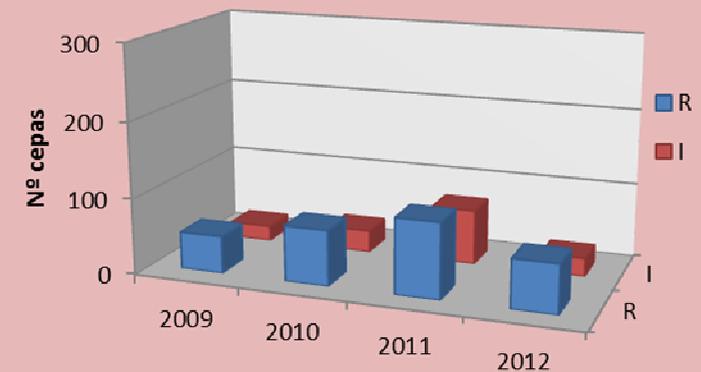
Resistencia a Ertapenem

Año	Nº aislados
2009	6438
2010	10949
2011	17225
2012	21948



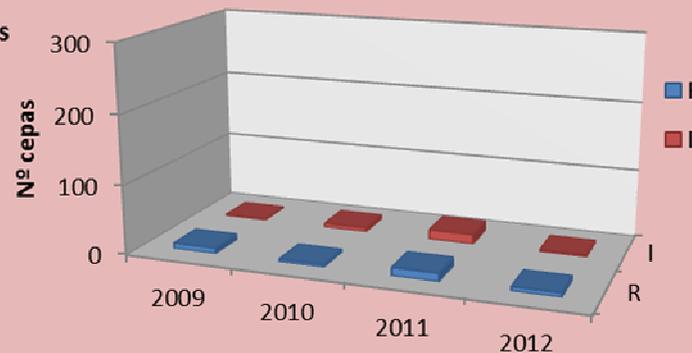
Resistencia a Imipenem

Año	Nº aislados
2009	31063
2010	38298
2011	44904
2012	46560



Resistencia a Meropenem

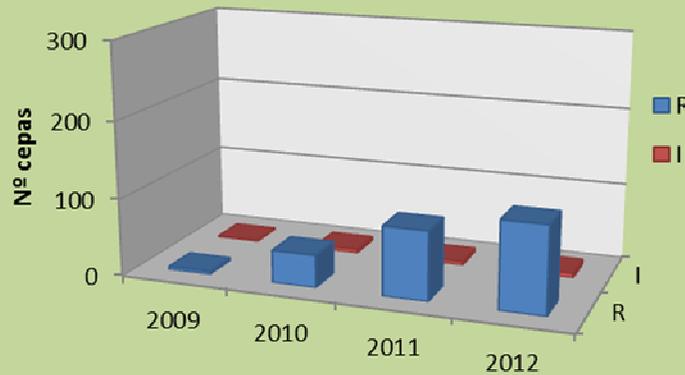
Año	Nº aislados
2009	11012
2010	9285
2011	8535
2012	5925



Klebsiella spp. 2009-2012 en la Comunidad Valenciana

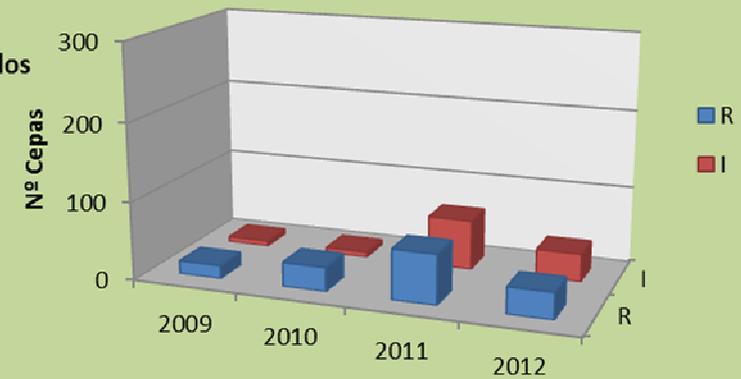
RESISTENCIA A ERTAPENEM

Año	Nº aislados
2009	2022
2010	3082
2011	5285
2012	6527



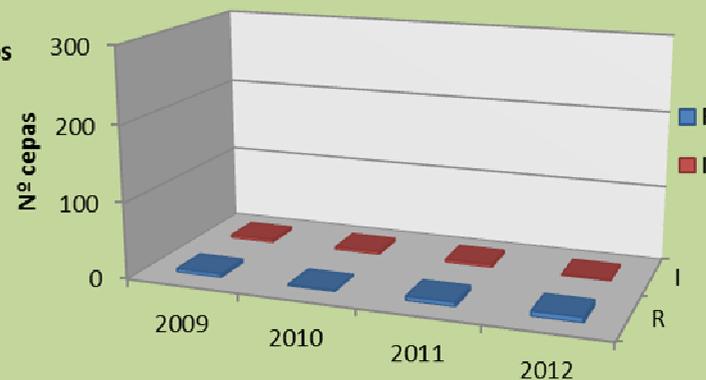
RESISTENCIA A IMIPENEM

Año	Nº aislados
2009	7602
2010	9345
2011	11671
2012	12379



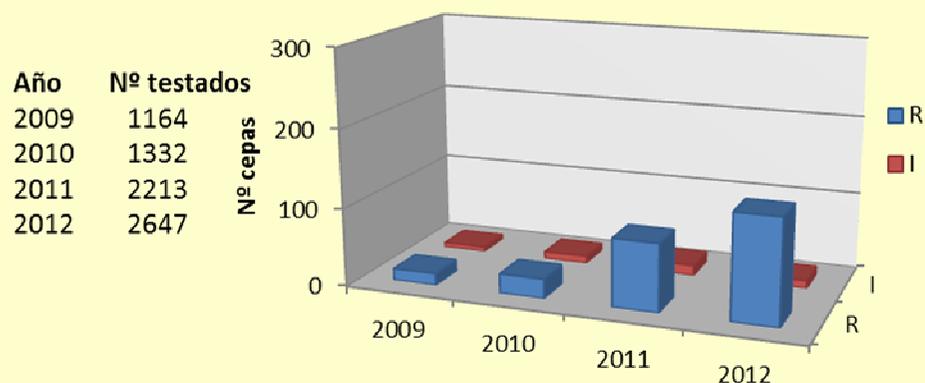
RESISTENCIA A MEROPENEM

Año	Nº aislados
2009	3204
2010	2558
2011	2235
2012	1731

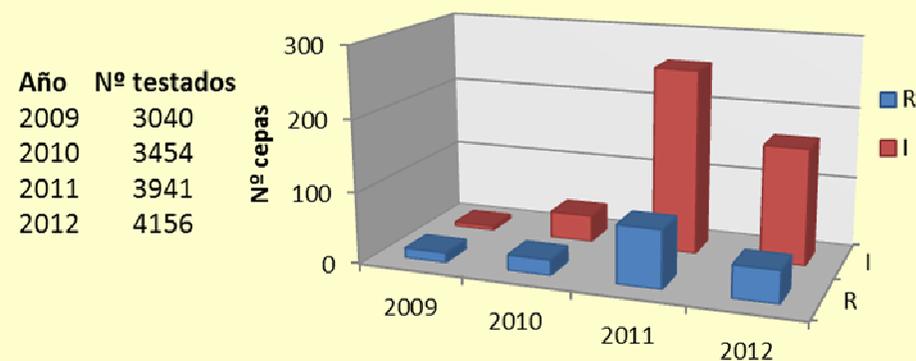


Enterobacter spp. 2009-2012 en la Comunidad Valenciana

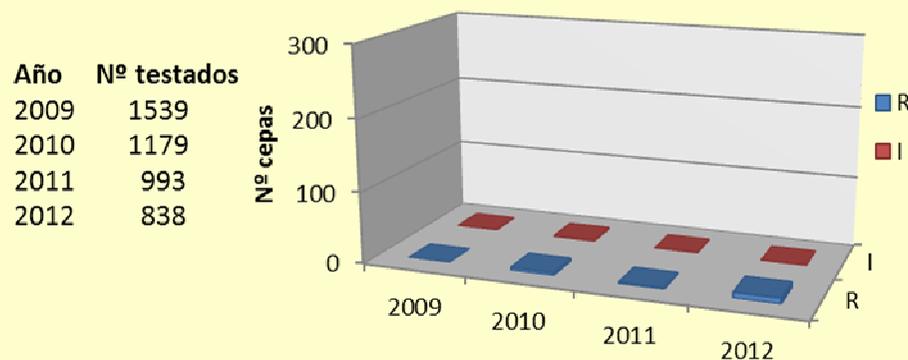
Resistencia a Ertapenem



Resistencia a Imipenen

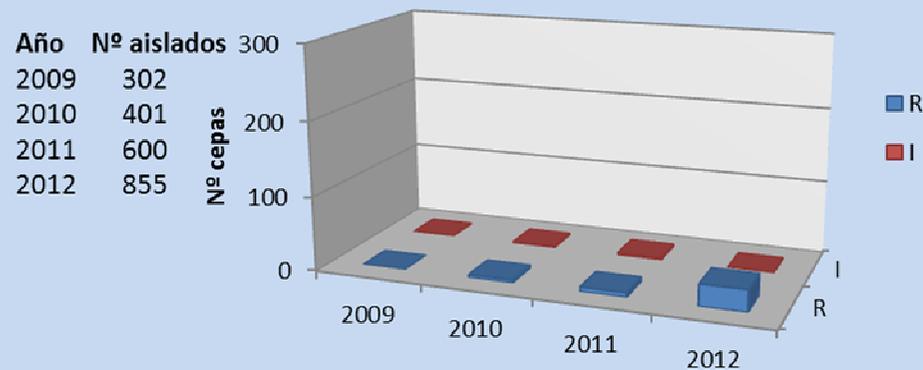


Resistencia a Meropenem

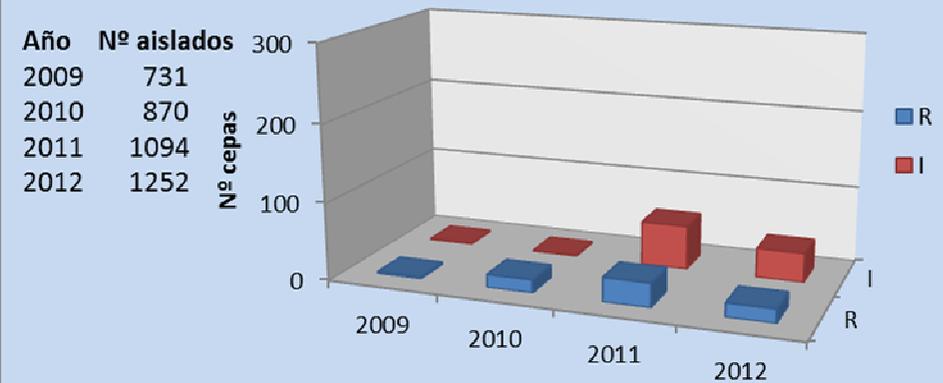


Serratia marcescens 2009-2012 en la Comunidad Valenciana

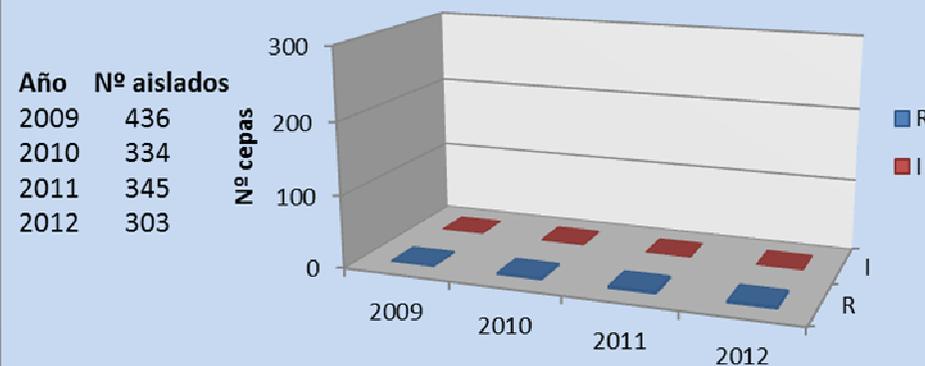
Resistencia a Ertapenem



Resistencia a Imipenem



Resistencia a Meropenem



Carbapenemasas: conclusiones



Jones RN, et al. Educational antimicrobial susceptibility testing as a **critical component** of microbiology laboratory proficiency programs: American Proficiency Institute results for 2007-2011. *Diagn Microbiol Infect Dis.* 2013;75:357-60.



Carbapenemasas



Control de brotes y prevención

¿Hay que controlar a los portadores asintomáticos?



Depende de la prevalencia de estos enzimas

El control de portadores fecales en una herramienta básica de control

Active surveillance for asymptomatic carriers of carbapenemase-producing *Klebsiella pneumoniae* in a hospital setting.

Gagliotti C, Ciccarese V, Sarti M, Giordani S, Barozzi A, Braglia C, Gallerani C, Gargiulo R, Lenzotti G, Manzi O, Martella D, Moro ML.
J Hosp Infect. 2013 Apr;83(4):330-2. c

El riesgo de colonización se asocia a tratamientos previos con imipenem

Emergence of imipenem-resistant gram-negative bacilli in intestinal flora of intensive care patients.

Armand-Lefèvre L, Angebault C, Barbier F, Hamelet E, Defrance G, Ruppé E, Bronchard R, Lepeule R, Lucet JC, El Mniai A, Wolff M, Montravers P, Plésiat P, Andremont A.
Antimicrob Agents Chemother. 2013 Mar;57(3):1488-95.

¿Hay que controlar a los portadores asintomáticos?



¿Donde buscar?

- Exudados rectales o heces: sensibilidad: 88%
- Más muestra de piel a nivel inguinal: Sensibilidad: 100%
- Muestras ambientales: 0,5% de muestras positivas

Anatomic sites of patient colonization and environmental contamination with *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae at long-term acute care hospitals.

Thurlow CJ, Prabaker K, Lin MY, Lolans K, Weinstein RA, Hayden MK; Centers for Disease Control and Prevention Epicenters Program.

Infect Control Hosp Epidemiol. 2013 Jan;34(1):56-61.

Carbapenemasas



¿Cómo
interpretar?

¿Cómo interpretar?

Reporting

The following applies ONLY when using interpretive criteria for carbapenems described in M100-S20 (January 2010).

For isolates that are **MHT positive** and have an ertapenem MIC of 2–4 µg/mL, imipenem MIC of 2–8 µg/mL, or meropenem MIC of 2–8 µg/mL, **report all carbapenems as resistant.**

If the MHT is negative, interpret the carbapenem MICs using CLSI interpretive criteria as listed in Table 2A in M100-S20 (January 2010).

NOTE: Not all carbapenemase-producing isolates of *Enterobacteriaceae* are MHT positive and MHT-positive results may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.

Clinical and Laboratory Standards Institute 2010

Reporting

Report results of the MHT to infection control or those requesting epidemiological information.

No change in the interpretation of carbapenems susceptibility test results is required for MHT-positive isolates.

Clinical and Laboratory Standards Institute 2012

Notes

Numbers for comments on MIC breakpoints

Letters for comments on disk diffusion

1. The carbapenem breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including the majority of carbapenemases). Some isolates that produce carbapenemase are categorised as susceptible with these breakpoints and should be reported as tested, *i.e.* the **presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility**. In many areas, carbapenemase detection and characterisation is recommended or mandatory for infection control purposes.

EUCAST 2013

¿CMI o mecanismo de resistencia?



Discrepancia entre los estudios que asocian la respuesta clínica en cepas con CMI poco elevada pero con presencia de carbapenemasas.



Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly?

Livermore DM, Andrews JM, Hawkey PM, Ho PL, Keness Y, Doi Y, Paterson D, Woodford N.

J Antimicrob Chemother. 2012 Jul;67(7):1569-77.

