Le carbapenemasi: cosa sono ed epidemiologia nazionale e locale

Trento, 26/02/2014

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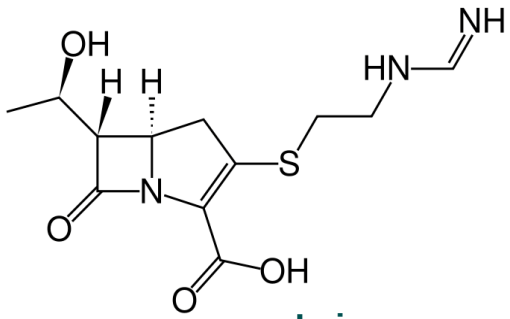
Programma

- ✓ Le carbapenemasi. Cosa sono?
- ✓ Epidemiologia negli enterobatteri
- ✓ Enterobatteri carbapenemasi+ in Italia e Trentino/Alto Adige
- ✓ Acinetobacter baumannii
- ✓ Pseudomonas aeruginosa

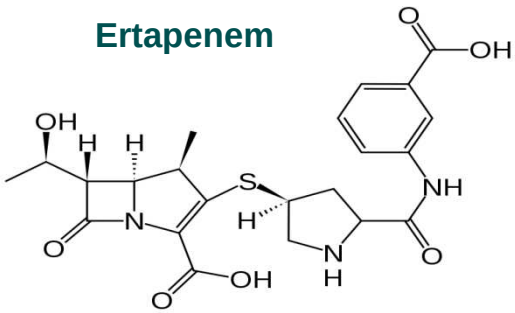
Antibiotici carbapenemici

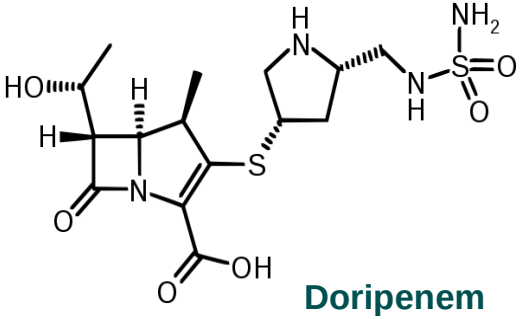
- Ertapenem
- Imipenem
- Meropenem
- Doripenem



Imipenem

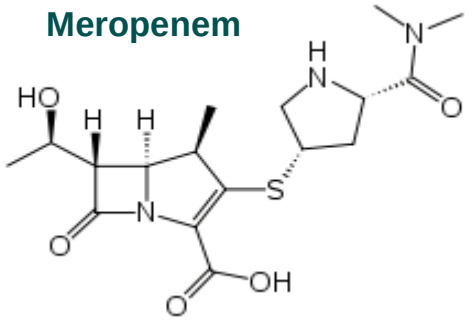
Ertapenem





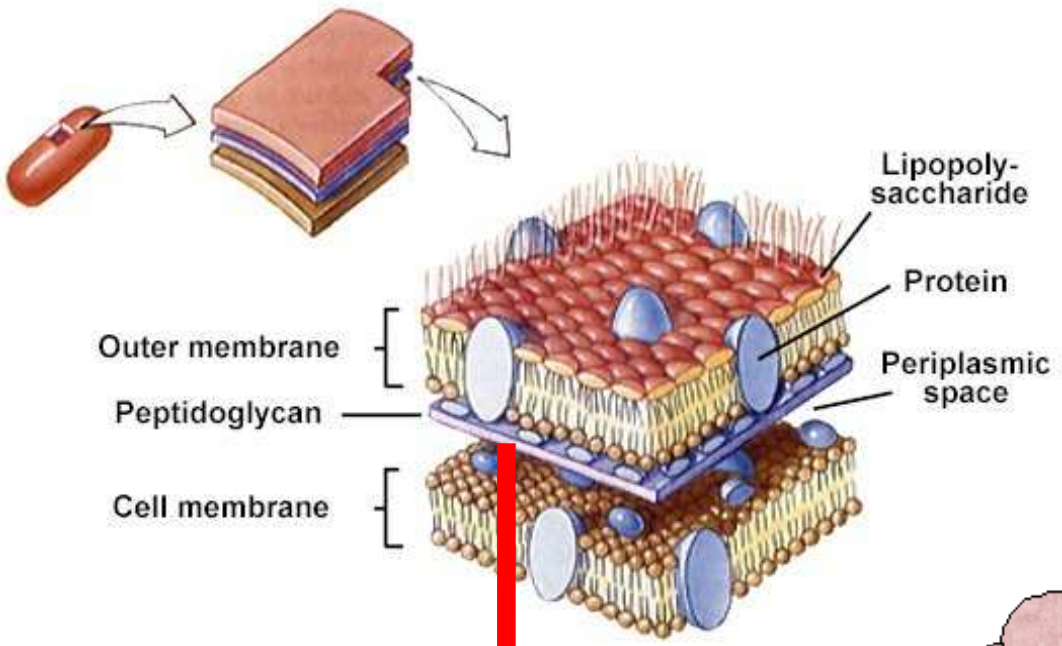
Doripenem

Meropenem



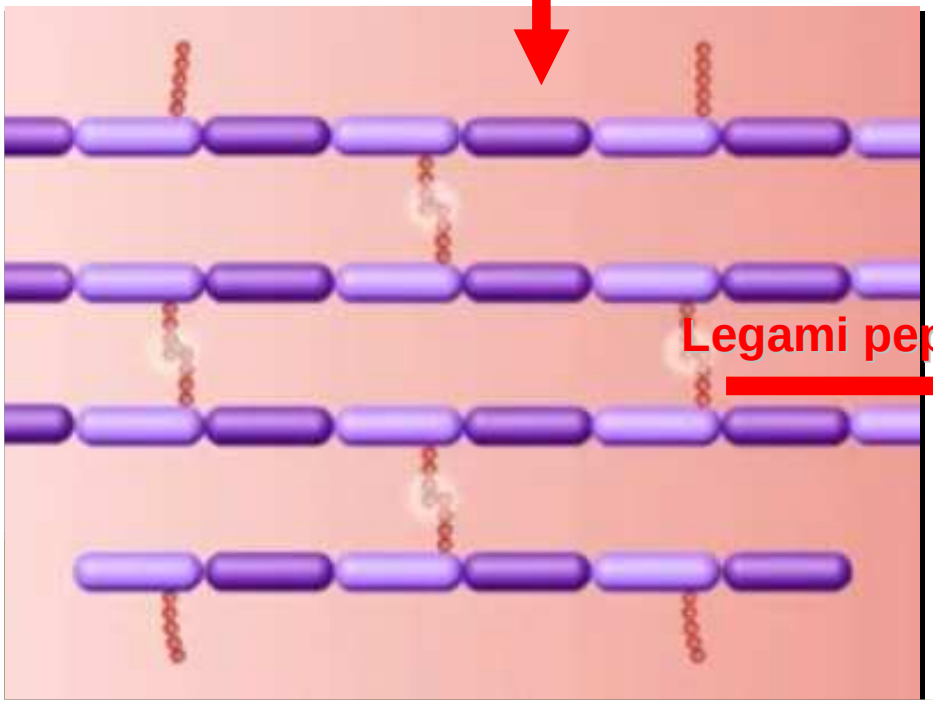
Antibiotici carbapenemici: sottoclasse di antibiotici ß-lattamici

Parete cellulare di enterobatteri (*E.coli, Klebsiella*,…)

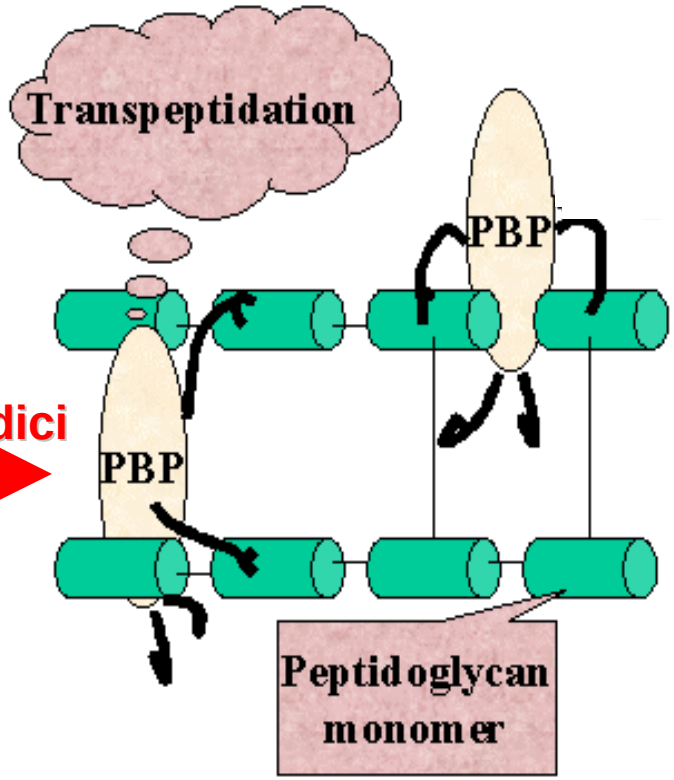


Meccanismo d‘azione di antibiotici carbapenemici:

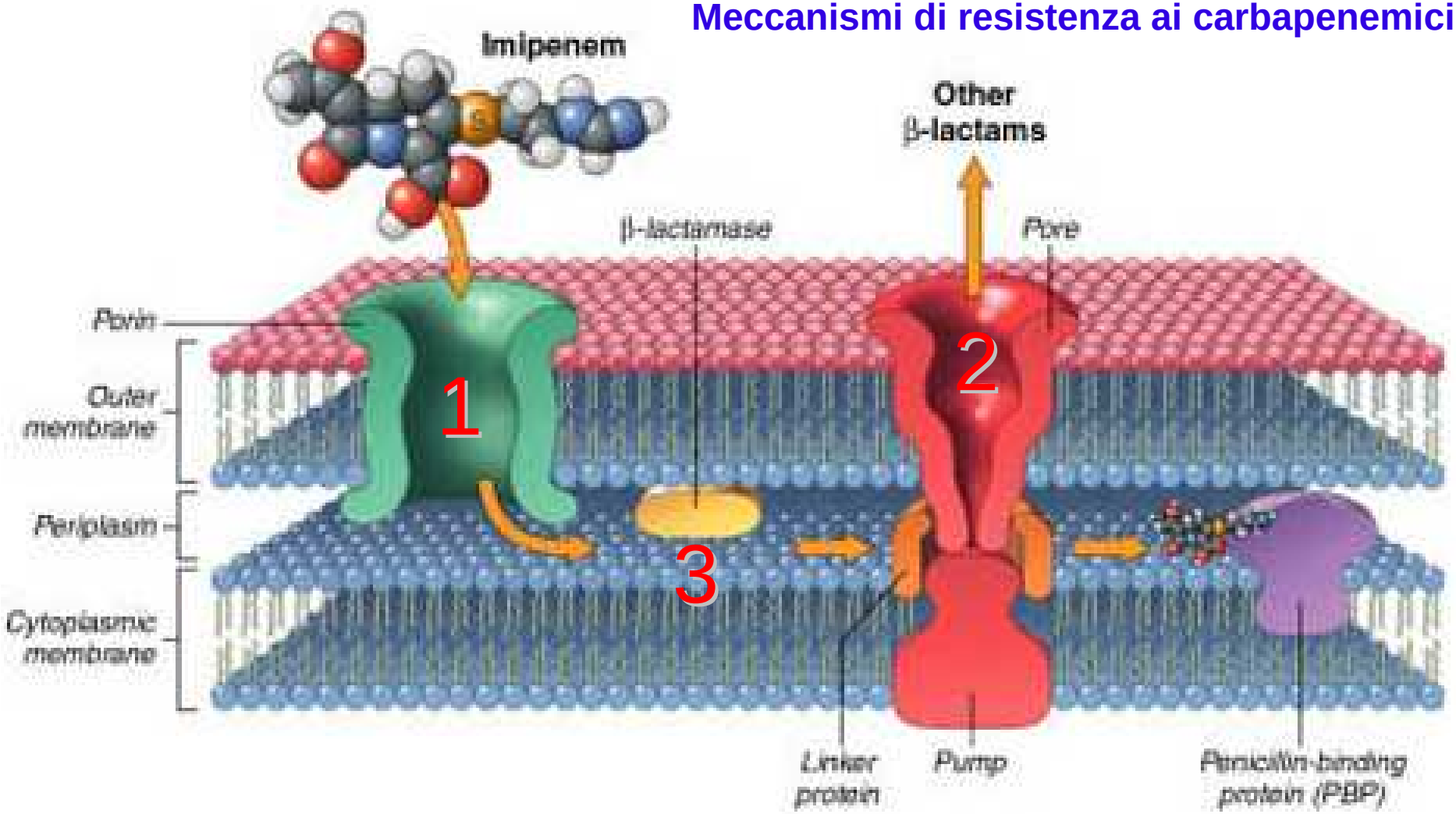
Le PBP (Penicillin-Binding-Proteins), coinvolte nella formazione di legami peptidici nel peptidoglicano, fungono come sito d‘attacco dei carbapenemici



Legami peptidici



Meccanismi di resistenza ai carbapenemici



1) Impossibilità di raggiungere il recettore (perdita di porine)

2) Sistemi di efflusso (pompe d'efflusso)

3) Distruzione enzimatica del farmaco (β-lattamasi)

Classificazione delle carbapenemasi

	Serina carbapenemasi		Metallo ß-lattamasi
Classe molecolare di Ambler *	**A**	**D**	**B**
Gruppo funzionale di Bush **	**2f**	**2df**	**3**
Esempi	**KPC** (1-17) SME (1-5) GES (1-24) IMI (1-4) NMC-A SFC-1 SHV-38	**OXA-48** OXA-163 OXA-181 OXA-23 OXA-24 OXA-51 OXA-58 OXA-143	**VIM** (1-40) **NDM** (1-10) **IMP** (1-47) SPM GIM SIM DIM AIM KHM FIM

*** Ambler RP. The structure of β-lactamases. *B Biol Sciences* 1980. 289:321-331. ** Bush et al. Updated functional classification of ß-lactamases. *AAC* 2010; 54: 969-76. Website di Karen Bush della Lahey Clinic: http://www.lahey.org/Studies/.**

Antibiogrammi di enterobatteri produttori di carbapenemasi da Bolzano: VIM (Verona integron-encoded metallo-ß-lactamase), KPC (*Klebsiella pneumoniae* carbapenemasi), OXA-48 (*Escherichia coli*)

	Klebsiella oxytoca MBL di tipo **VIM (+ ESBL)** (Bolzano)		*Klebsiella pneumonia carbapenemasi* **KPC** (Bolzano)		*Escherichia coli carbapenemasi* **OXA-48** (Bolzano)	
	MIC	Interpret.	MIC	Interpret.	MIC	Interpret.
Ampicillina	≥ 32	R	≥ 32	R	≥ 32	R
Amox./clavulanato	≥ 32	R	≥ 32	R	≥ 32	R
Piperacillina/tazobact.	≥ 128	R	≥ 128	R	≥ 128	R
Cefotaxime	16	R	16	R	≤ 1	S
Ceftazidime	≥ 64	R	≥ 64	R	≤ 1	S
Cefepime	4	R	8	R	≤ 1	S
Meropenem	1	R?	≥ 16	R	0,25	R?
Imipenem	≥ 16	R	8	R	4	R
Ertapenem	≤ 0,5	R?	≥ 8	R	4	R
Gentamicina	≤ 1	S	≥ 16	R	≤ 1	S
Amikacina	≤ 2	S	8	I	≤ 2	S
Tobramicina	8	R	≥ 16	R	≤ 2	S
Ciprofloxacina	≥ 4	R	≥ 4	R	≤ 0,25	S
Levofloxacina	≥ 8	R	≥ 8	R	≤ 0,12	S
Trimetoprim/sulfa.	≥ 320	R	≥ 320	R	≥ 320	R
Tigeciclina	2	I	2	I	≤ 0,5	S
Colistina	≤ 0,5	S	≤ 0,5	S	≤ 0,5	S
Nitrofurantoina	≤ 16	S	≥ 512	R	≤ 16	S
Fosfomicina	≤ 16	S	32	S	≤ 16	S

Geni per carbapenemasi circondati da elementi mobili e localizzati sul cromosoma o frequentemente su plasmidi

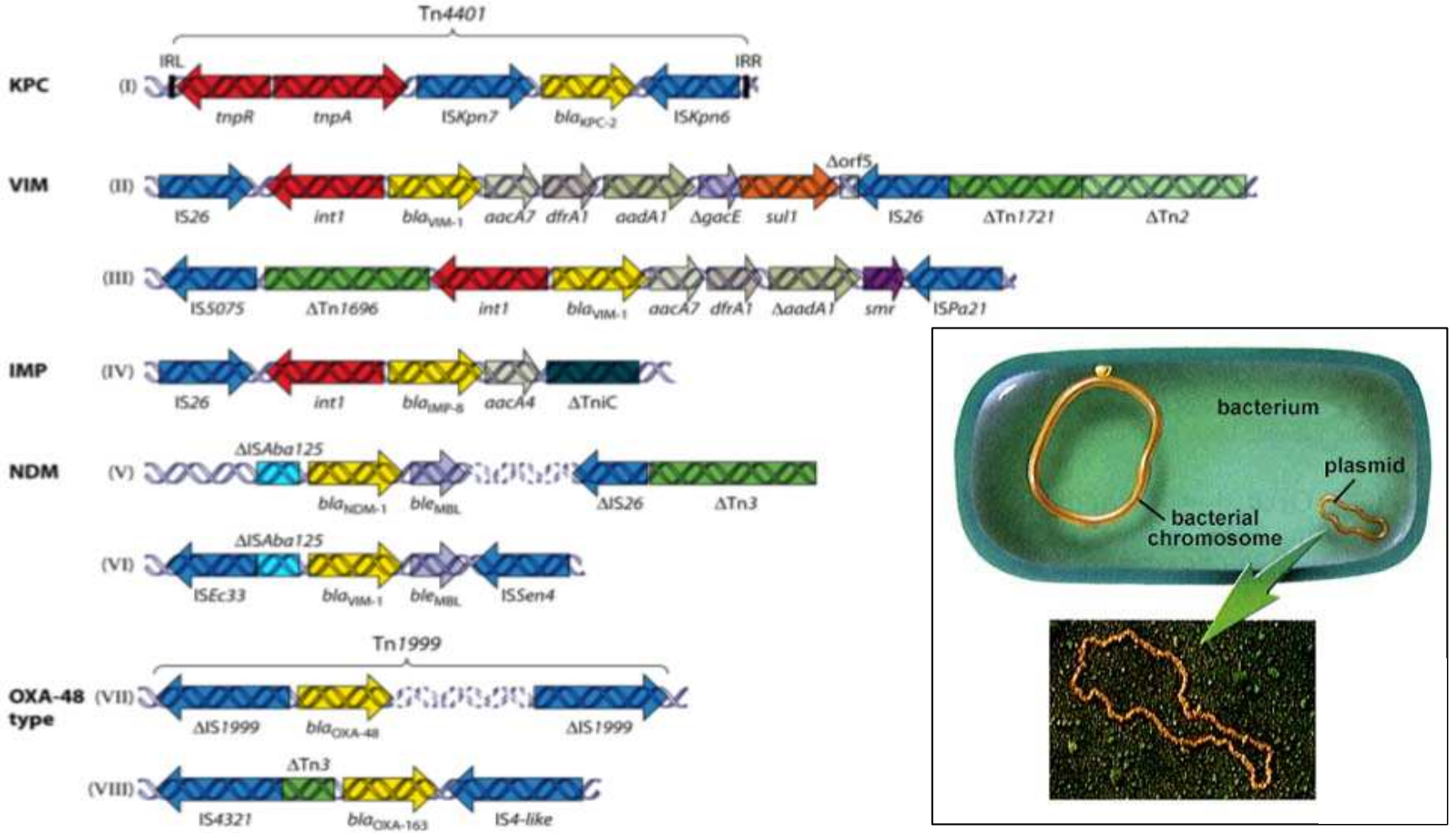
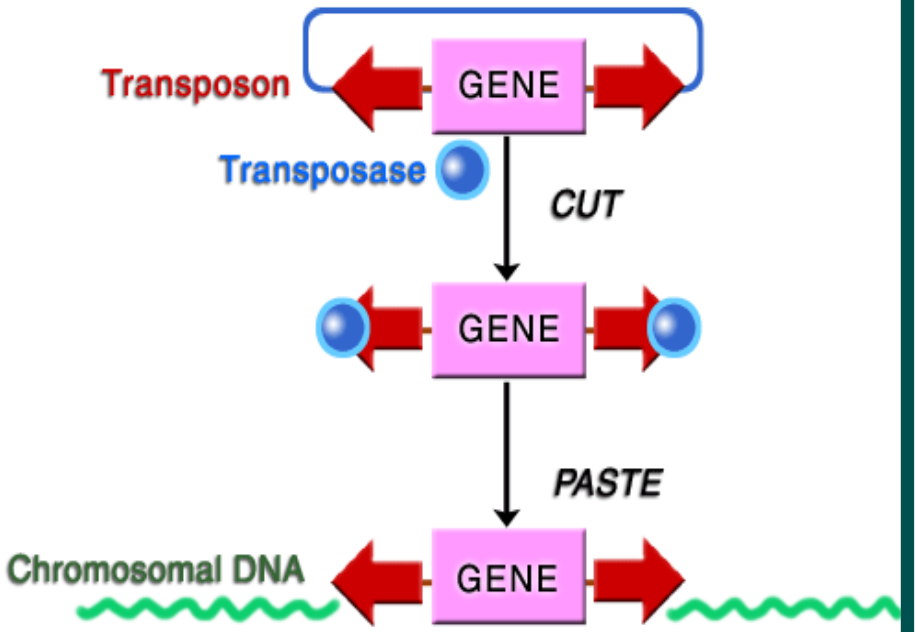


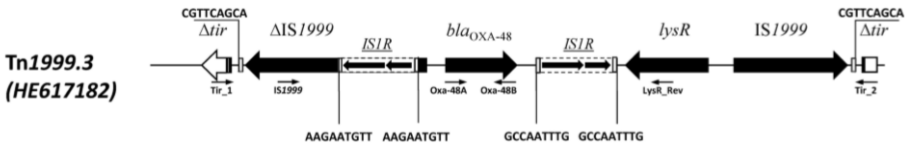
FIG 1 Schematic depiction of representative sequences from enterobacterial plasmids, showing the association of carbapenemase-encoding genes with various mobile elements. (I) The bla_{KPC-2}-containing Tn*4401* transposon from plasmid pNYC (GenBank accession no. EU176011) (180). (II and III) Representative VIM-encoding sequences from plasmids pNL194 (GenBank accession no. GU585907) (167) and pCC416 (GenBank accession no. AJ704863) (59), respectively. (IV) A bla_{IMP}-carrying sequence from plasmid pFP10-2 (GenBank accession no. HQ651093) (146). (V and VI) Sequences containing bla_{NDM-1} carried by a plasmid from *K. pneumoniae* 05-506 (GenBank accession no. FN396876) (273) and by plasmid p271A (GenBank accession no. HQ162469) (218), respectively. (VII and VIII) The OXA-48-encoding transposon Tn*1999* from plasmid pA-1 (GenBank accession no. AY236073) (217) and the $bla_{OXA-163}$-containing segment from plasmid p6299 (GenBank accession no. HQ700343) (216), respectively.

Tzouvelekis LS et al. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clinical Microbiology Reviews 2012; 25: 682-707.

Trasposoni e elementi IS

- Elementi genetici **mobili**
- **Si spostano** tra diversi siti nel genoma
- **Trasposizione** catalizzata da trasposasi



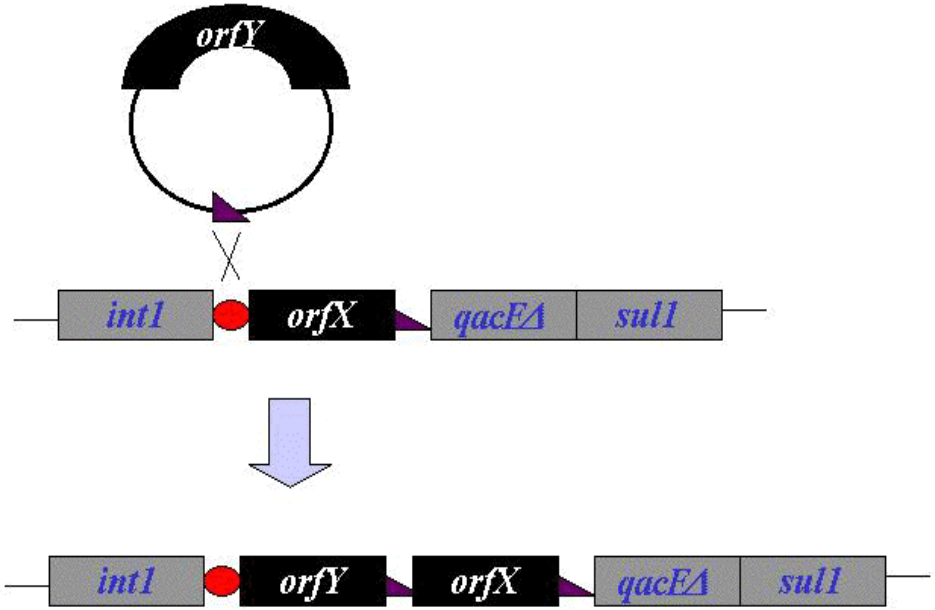


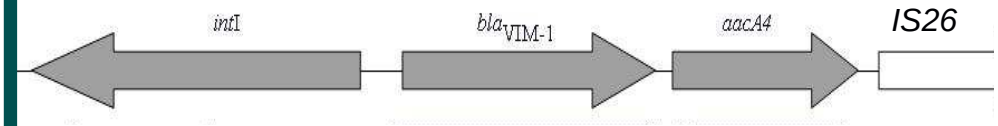
Elementi IS associati con gene OXA-48 in *E.coli* da Bolzano

Giani T et al. Escherichia coli from Italy producing OXA-48 carbapenemase encoded by a novel Tn1999 transposon derivative. AAC 2012; 56: 2211-2213.

Integron

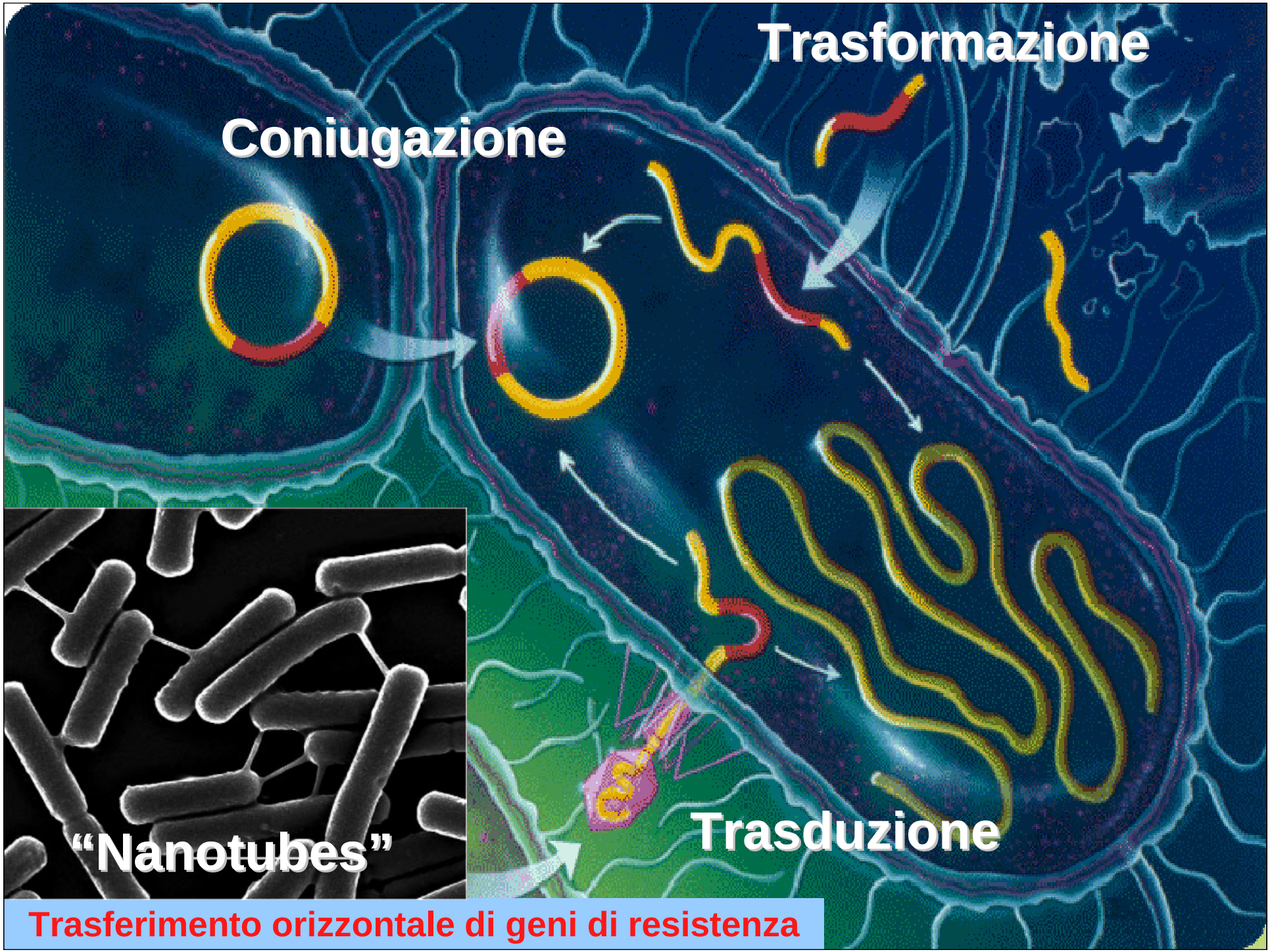
- Elementi per la **cattura di cassette di geni**
- 3 regioni: integrasi, regione variabile, regione conservata (*qacEΔ-sul1*)
- **Integrazione di nuove cassette di geni**





Integron e elemento IS in enterobatteri VIM-1 produttori da Bolzano

Aschbacher R et al. Linkage of acquired quinolone resistance (qnrS1) and metallo-ß-lactamase (bla_{VIM1}) genes in multiple species of Enterobact. from Bolzano, Italy. JAC 2008; 61: 515-23



Trasferimento orizzontale di geni di resistenza

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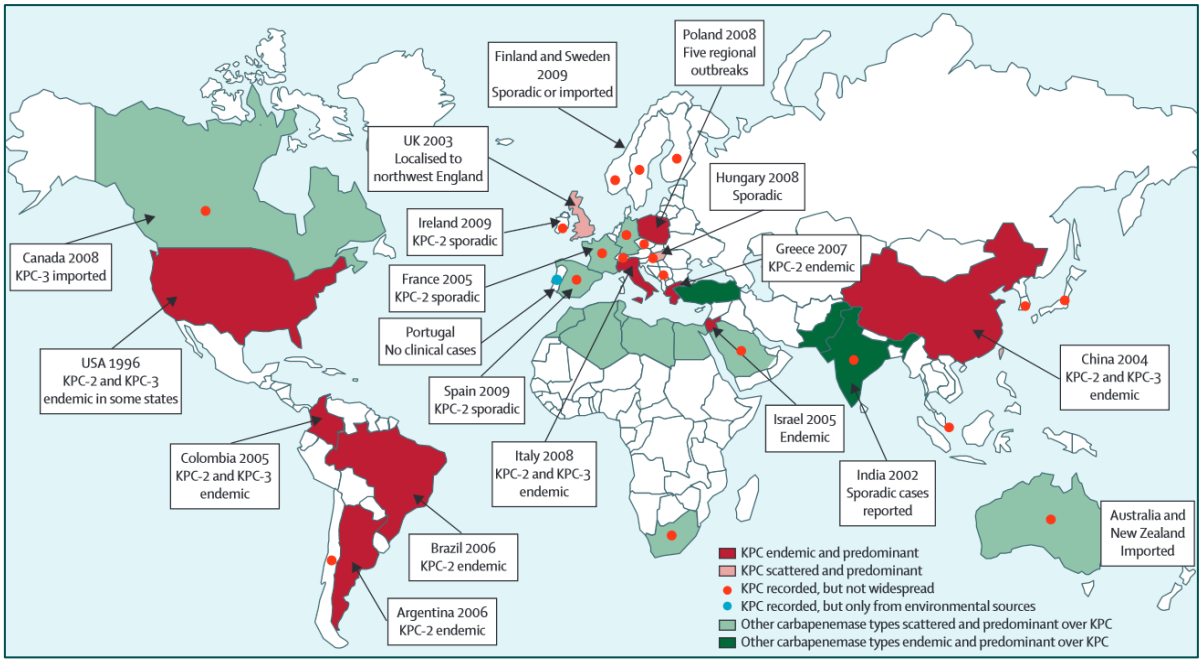
- ✓ Pseudomonas aeruginosa

KPC (*K.pneumoniae* carbapenemasi)

- ✓ 1˚ isol. 1996, USA
- ✓ Soprattutto in *K.pneumoniae*
- ✓ Cloni epidemici, soprattutto **ST258**
- ✓ Diffuso in USA, America del Sud, Cina, Israele, Grecia,…

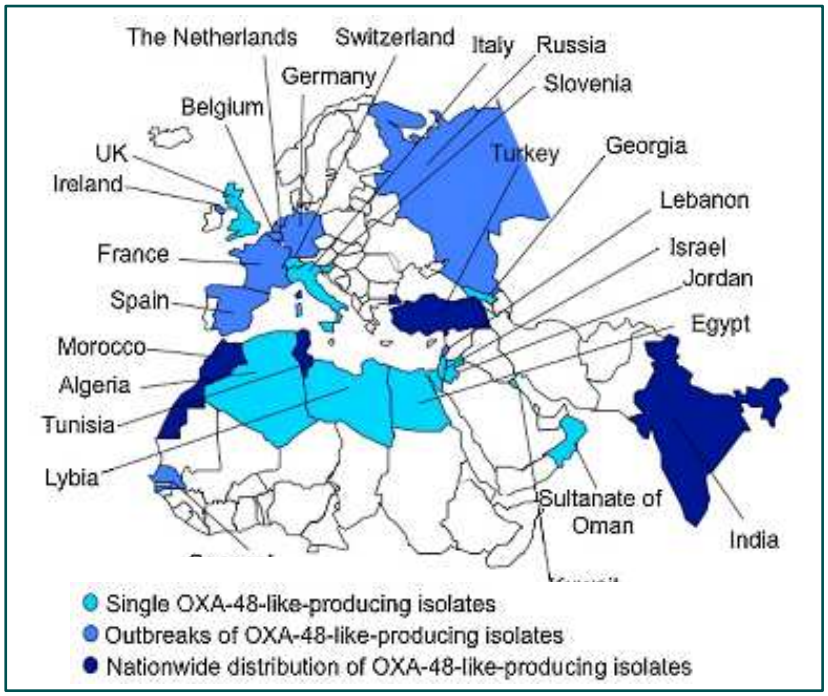


- ✓ **Italia: endemia di alto livello**
- ✓ **Provincia di Trento: finora 9 isolati (dati: Dott. Lanzafame)**
- ✓ **Provincia di Bolzano: finora 8 isolati, generalmente da pazienti rivoverati precedentemente in ospedali italiani**

Munoz-Price et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis 2013;13:785-796.

OXA-48 carbapenemasi

- ✓1° isolato 2001 in **Turchia**
- ✓Prevalente in India, Turchia, Nordafrica, Francia, Germania
- ✓Italia: **sporadici**

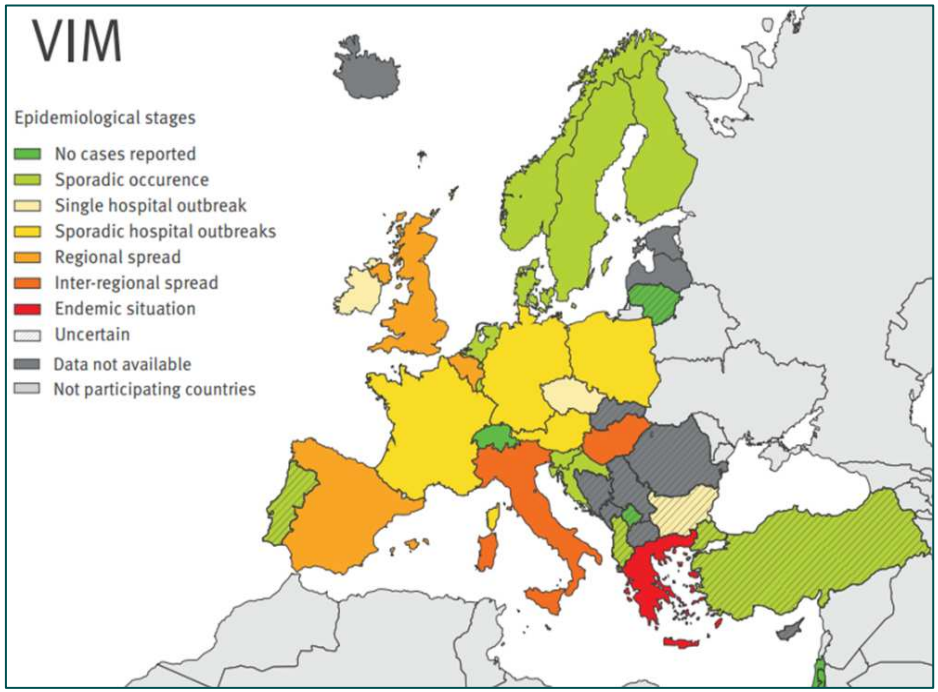


- ✓Provincia di Trento: **nessuno** (dati: Dott. Lanzafame)
- ✓Provincia di Bolzano: **1 isolato** da urocoltura (Paziente in vacanze in Egitto)

Nordmann P. Carbapenemase-producing Enterobacteriaceae: Overview of a major public health challenge. Med Mal Infect 2013; 18.

VIM (Verona imipenemasi)

- ✓ 1˚ VIM isolato nel 1997 a Verona in *P.aeruginosa*
- ✓ Enterobatteri: diffusione mondiale, in Europa soprattutto in Grecia e Cipro Italia: epidemie locali, vari isolati sporadici

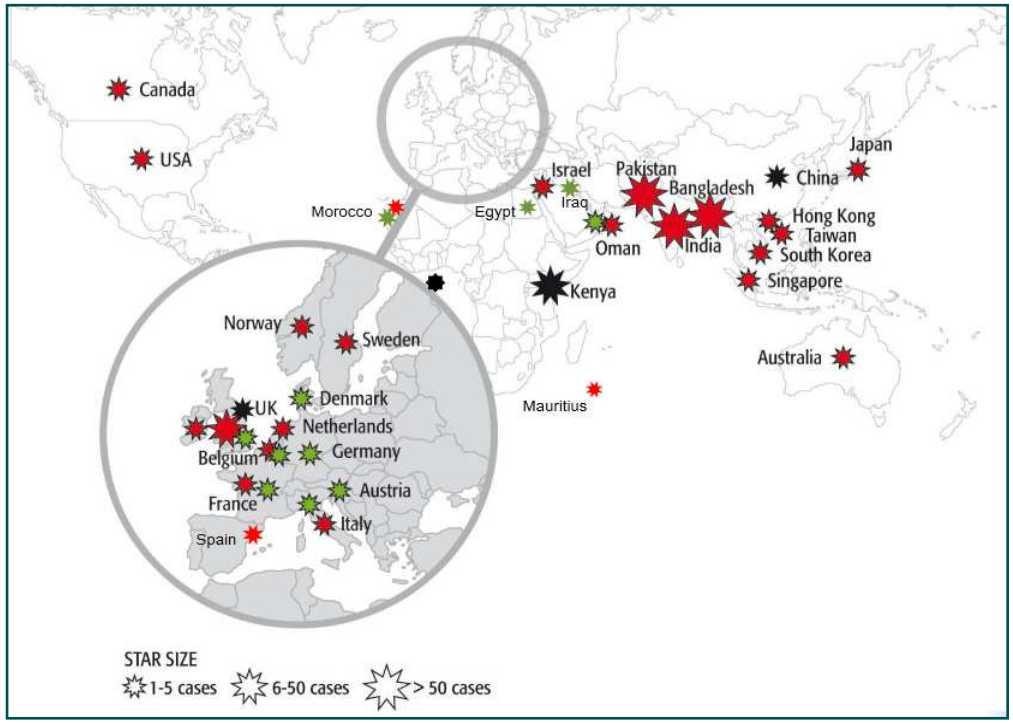


- ✓ Provincia di Trento: 11 nel 2013; 6 specie (dati: Dott. Lanzafame)
- ✓ Provincia di Bolzano: evidenziati dal 2005, endemia di basso livello (20-30 isolati/anno); 11 specie, associazione con "*high risk epidemic clone*" *E. coli* ST131

Glasner C et al. Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries. Euro Surveill 2013;18.

NDM (New Delhi metallo-ß-lactamase)

- ✓ 1° NDM nel 2009 in *Klebsiella pneumoniae* in paziente Swedese ricoverato precedente-mente a New Delhi
- ✓ Epidemiologia: India, Pakistan, Bangladesh, Balkani, Gran Bretagna



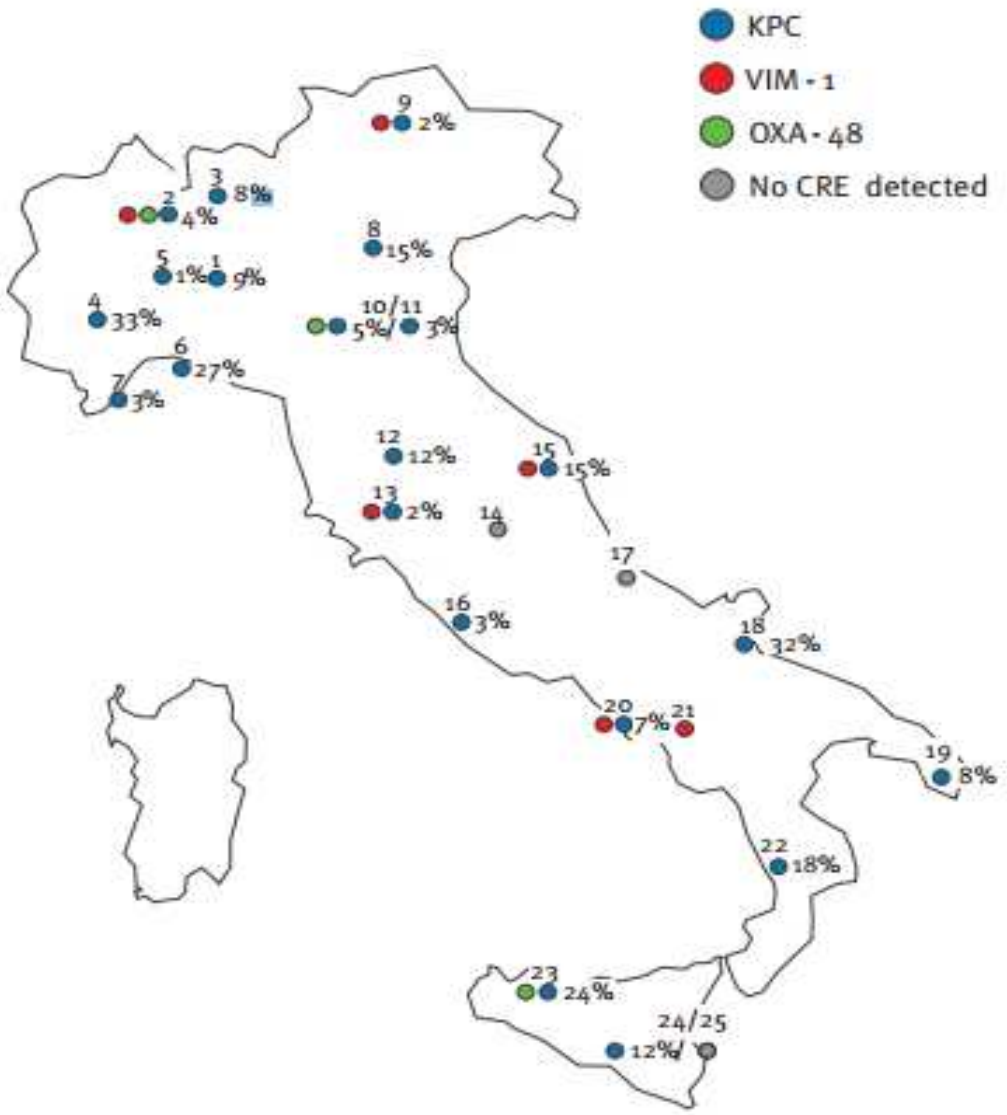
- ✓ **Italia:** singole epidemie locali, isolati sporadici
- ✓ **Provincia di Trento: 1 isolato** (dati: Dott. Lanzafame)
- ✓ **Provincia di Bolzano: nessuno**

Nordmann P et al. Global Spread of Carbapenemase-producing Enterobacteriaceae. Emerging Inf Dis 2011. 17;10: 1791-1798

Epidemiologia enterobatteri carbapenemasi produttori in Italia

Italia: alta prevalenza di enterobatteri carba-penemasi+ (nel 2011)

- ✓Trovati in 23/25 ospedali (compreso Bolzano)
- ✓1,6% degli isolati di enterobatteri
- ✓Materiali: urina (42%), sangue (18%), tratto respira-torio (18%), altri (22%)
- ✓Prev. *K.pneumoniae* (97%)
- ✓*K.pneumoniae* con KPC (90%), generalmente ST258
- ✓VIM e OXA-48 sporadici

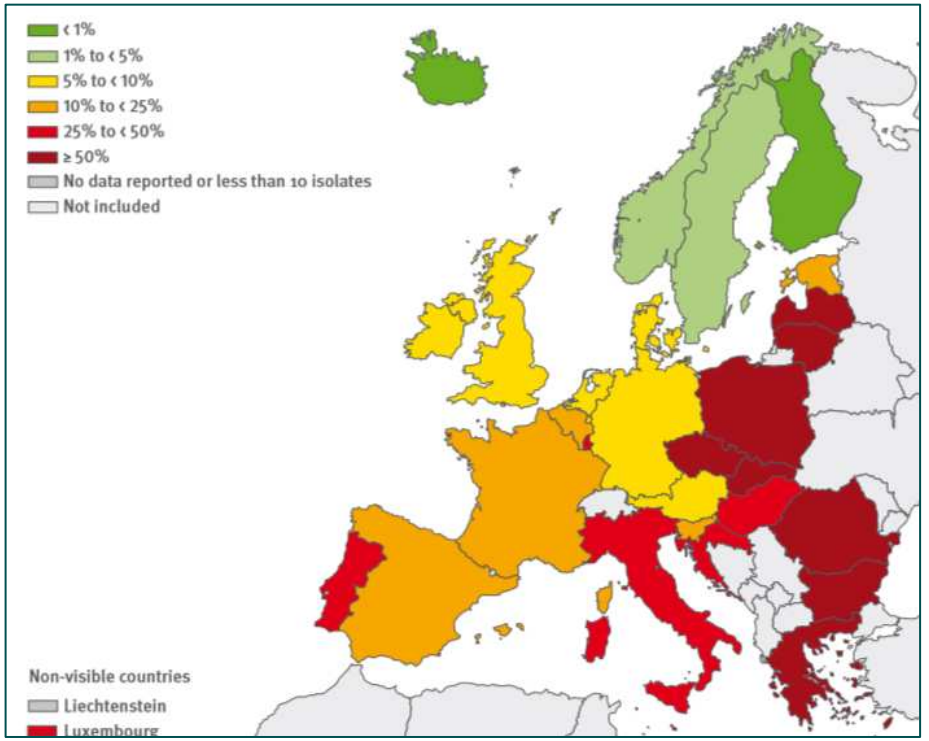


1: Milan; 2: Varese; 3: Lecco; 4: Turin; 5: Novara; 6: Genoa; 7: Sanremo; 8: Verona; 9: Bolzano; 10-11: Modena; 12: Florence; 13: Siena; 14: Perugia; 15: Ancona; 16: Rome; 17: Pescara; 18: San Giovanni Rotondo; 19: Lecce; 20: Naples; 21: Avellino; 22: Cosenza; 23: Palermo; 24-25: Catania. The types of carbapenemases detected in different laboratories, and the proportion of KPC-producing *Klebsiella pneumoniae* versus the total number of *K. pneumoniae* isolates are indicated.

Giani T et al. Epidemic diffusion of KPC carbapene-mase-producing Klebsiella pneumoniae in Italy: results of the first countrywide survey. Euro Surveill 2013; 18.

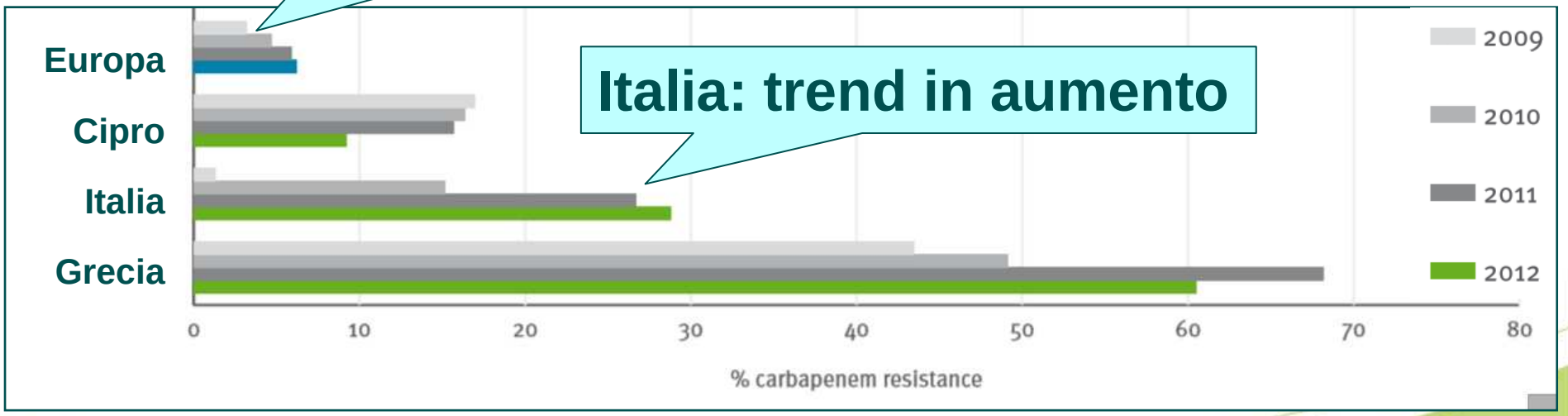
Klebsiella pneumoniae da sangue

resistenza a carbapenemici Italia 2012: 29%



Media europea

Italia: trend in aumento



EARS-Net (European Antimicrobial Resistance Surveillance Network) 2012: http://ecdc.europa.eu/

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- ✓ Pseudomonas aeruginosa

Epidemiologia enterobatteri carbapenemasi produttori in Provincia di Trento

Dati forniti da:

Dott. Paolo Lanzafame

Direttore U.O. Microbiologia e Virologia

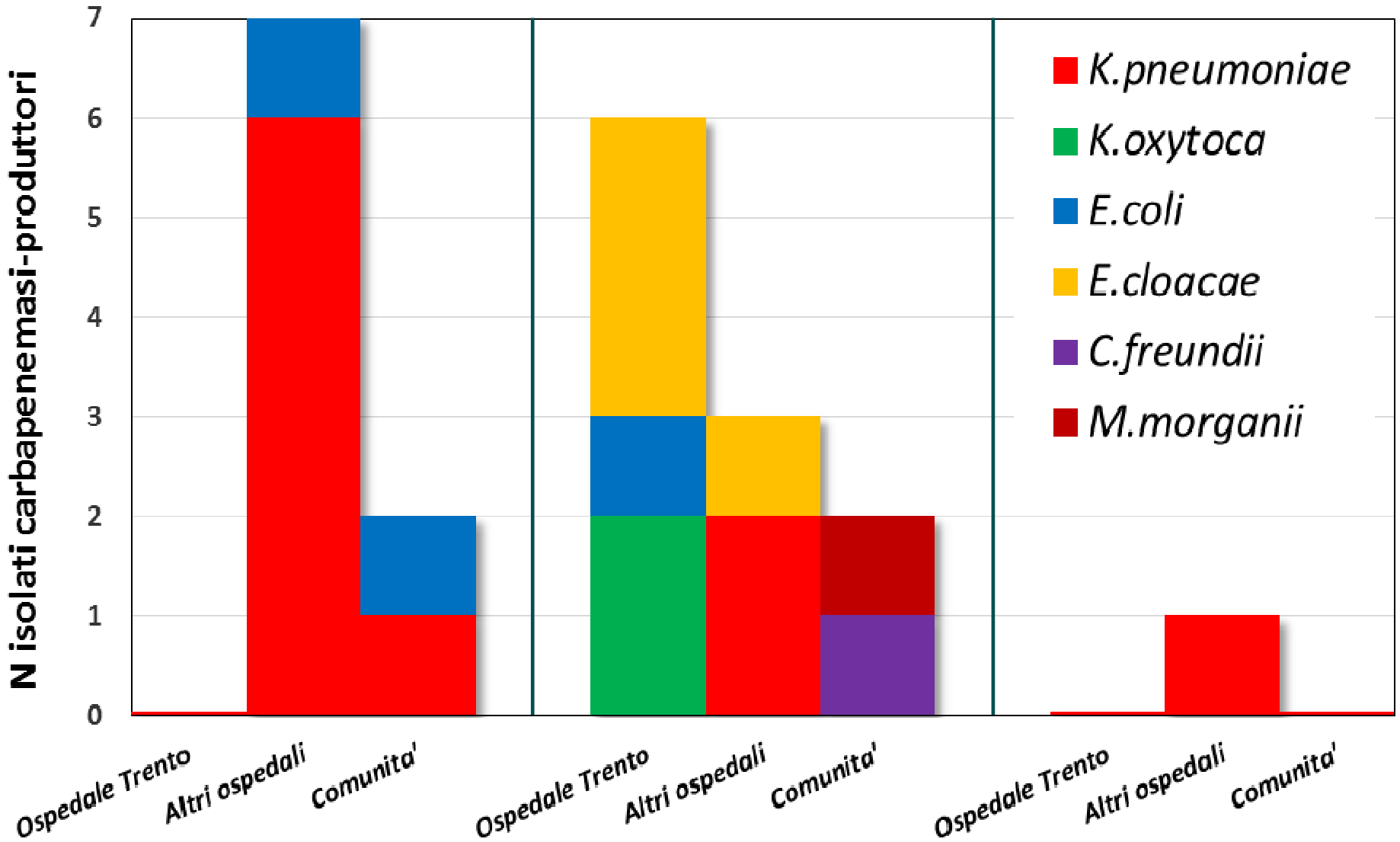
Azienda Provinciale per i Servizi Sanitari

Provincia Autonoma di Trento

Ospedale S.Chiara

Provincia di Trento: enterobatteri carbapenemasi+, 2013, 21 isolati

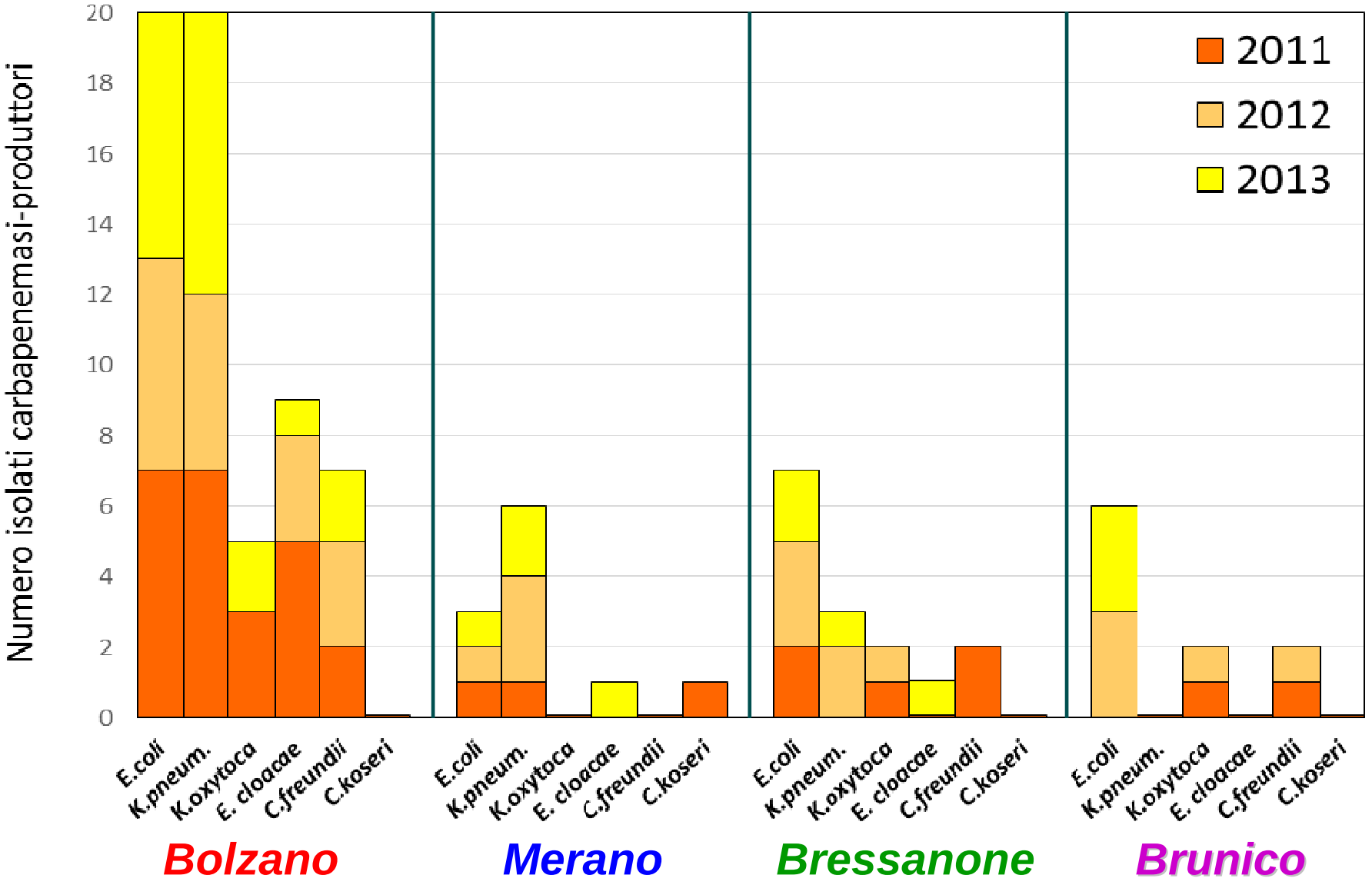
Materiali: urina (15), t.auricolare (2), sangue (1), drenaggio (1), BAL (1)



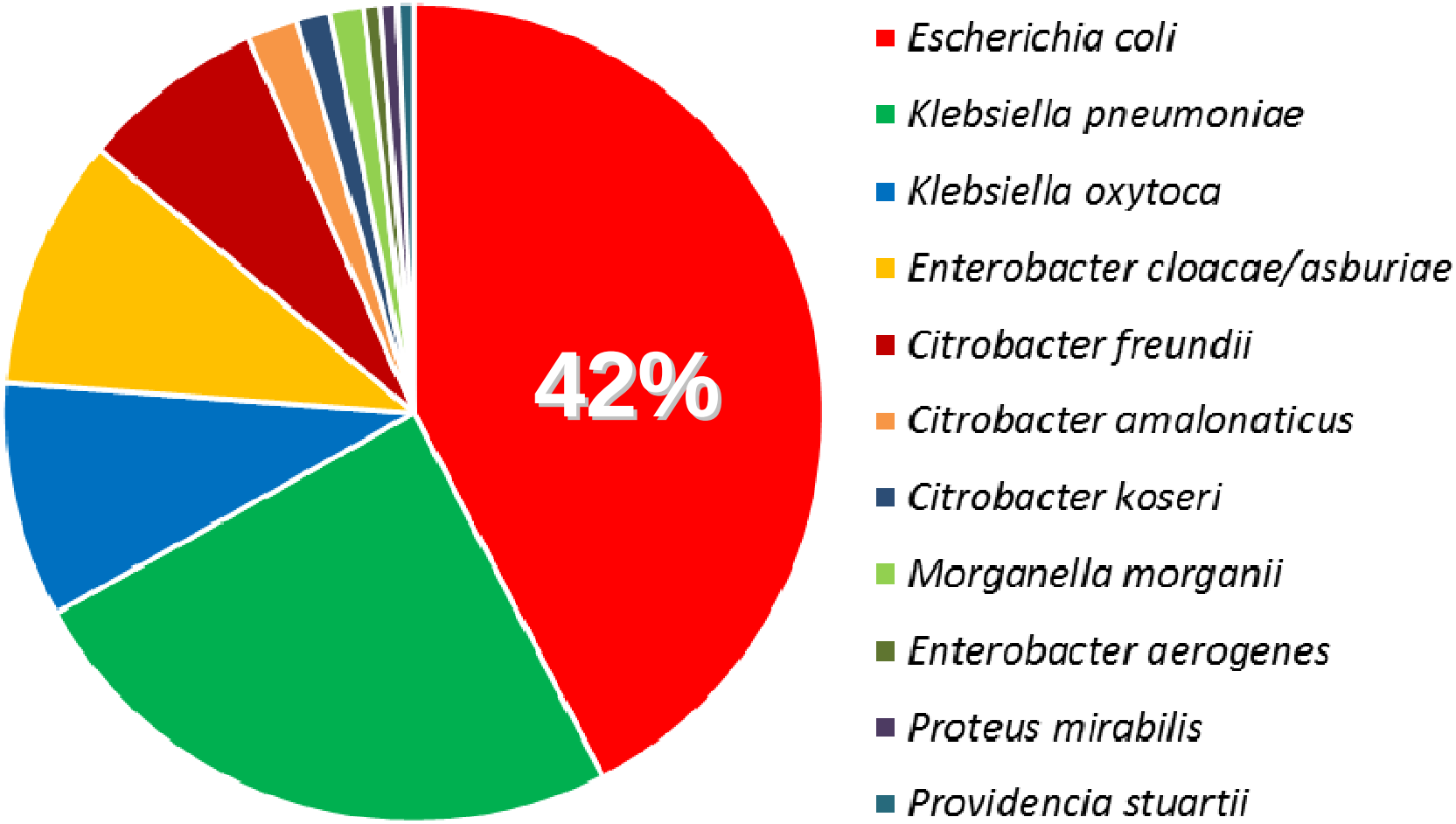
Dati Provincia di Trento: Dott. Paolo Lanzafame, Direttore U.O. Microbiologia e Virologia, Trento

Epidemiologia enterobatteri carbapenemasi produttori in Provincia di Bolzano

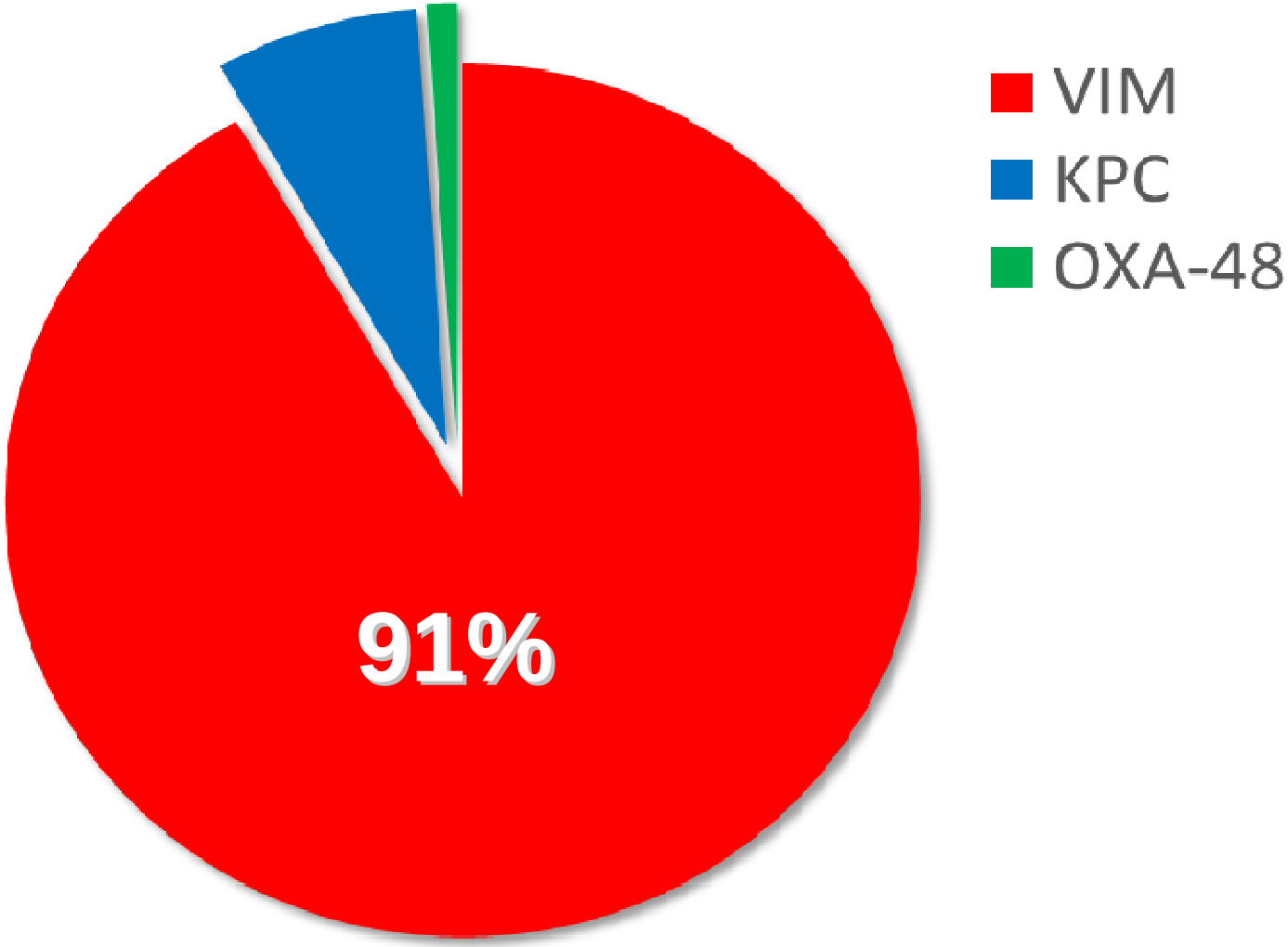
Provincia di Bolzano: enterobatteri carbapenemasi+, tutti i tipi di campioni



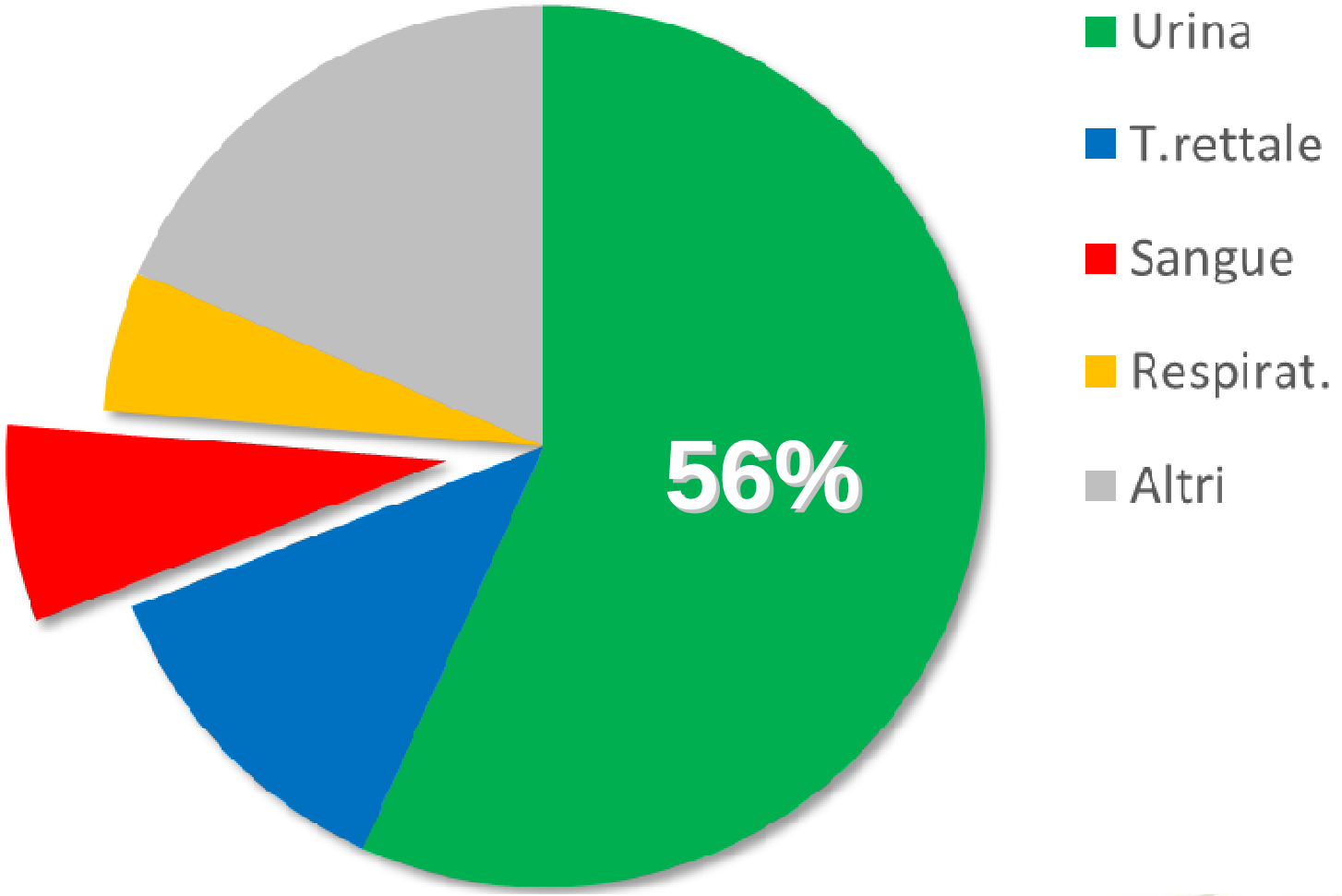
Specie enterobatteriche carbapenemasi-produttori, 150 isolati, 2008-2013, Bolzano



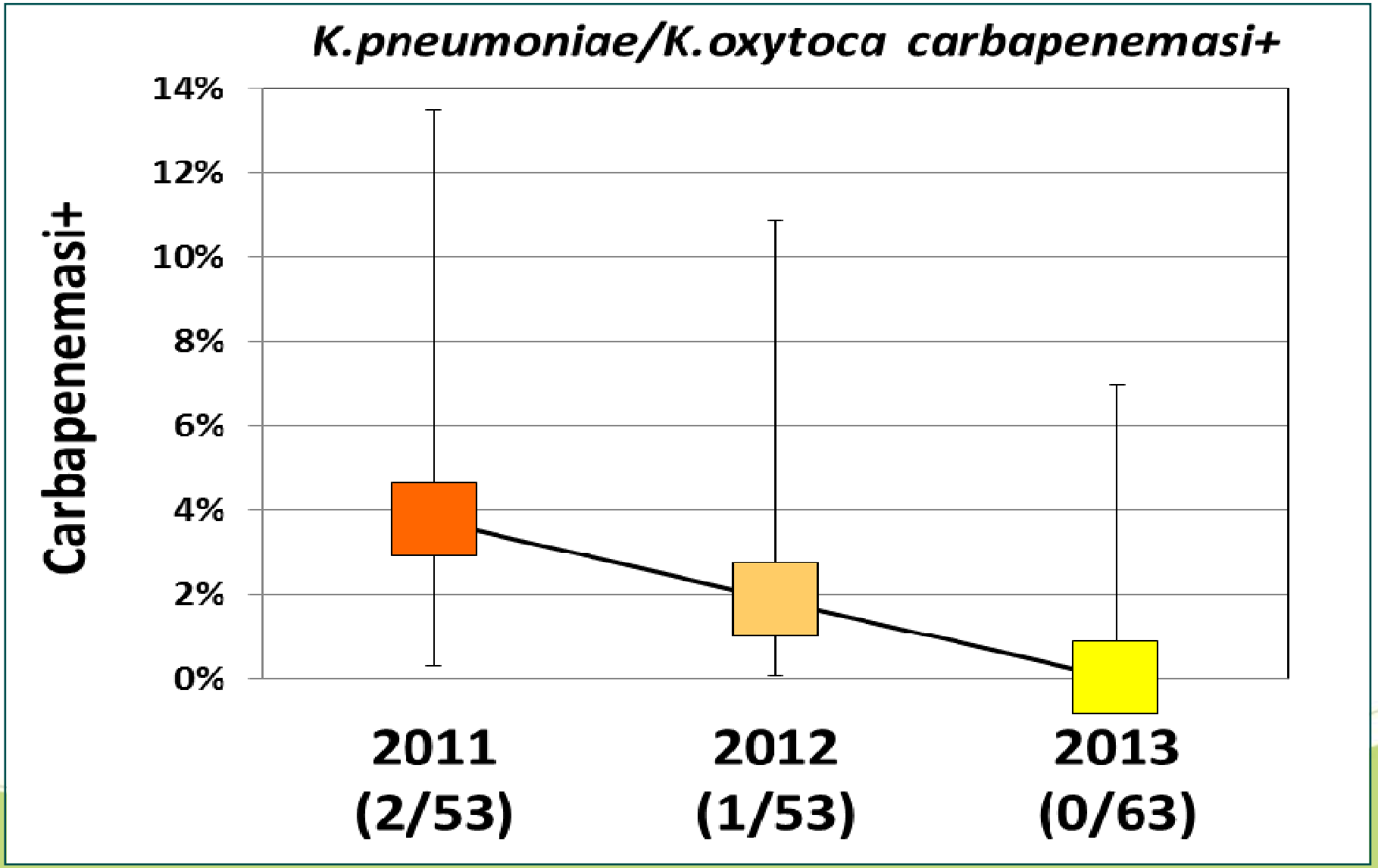
Provincia di Bolzano: tipi di carbapenemasi in entero-batteri (97 isolati), 2011-2013, tutti i tipi di materiali



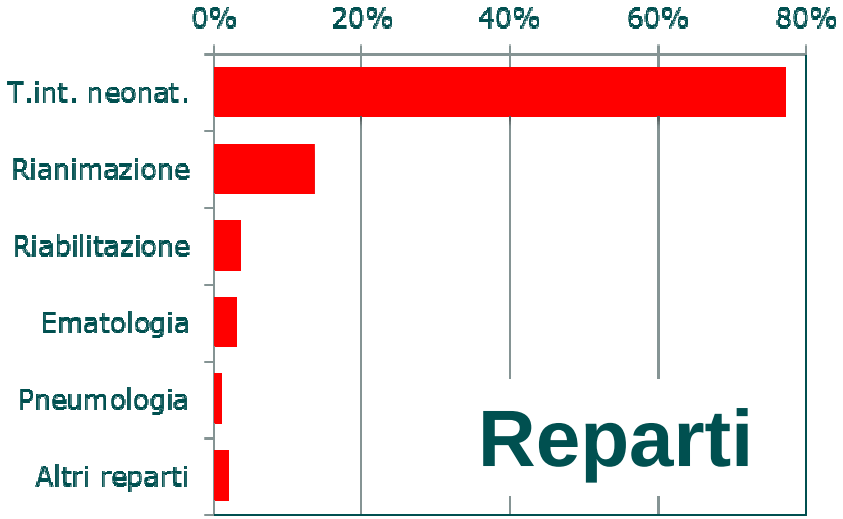
Tipi di campione, enterobatteri carbapenemasi+ (97 isolati), 2011-2013, Provincia di Bolzano

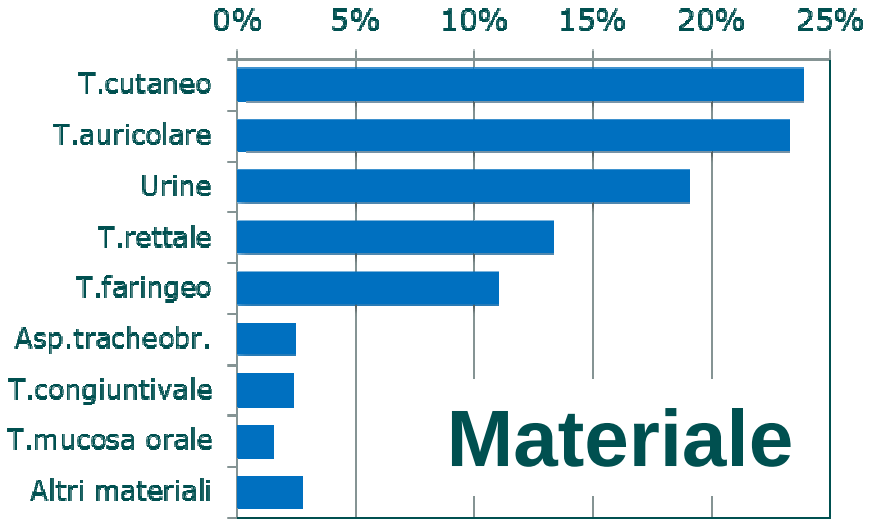


Klebsiella pneumoniae, Klebsiella oxytoca, emocolture, 2011-2013, Provincia di Bolzano



*Screening carbapenemasi in enterobatteri **

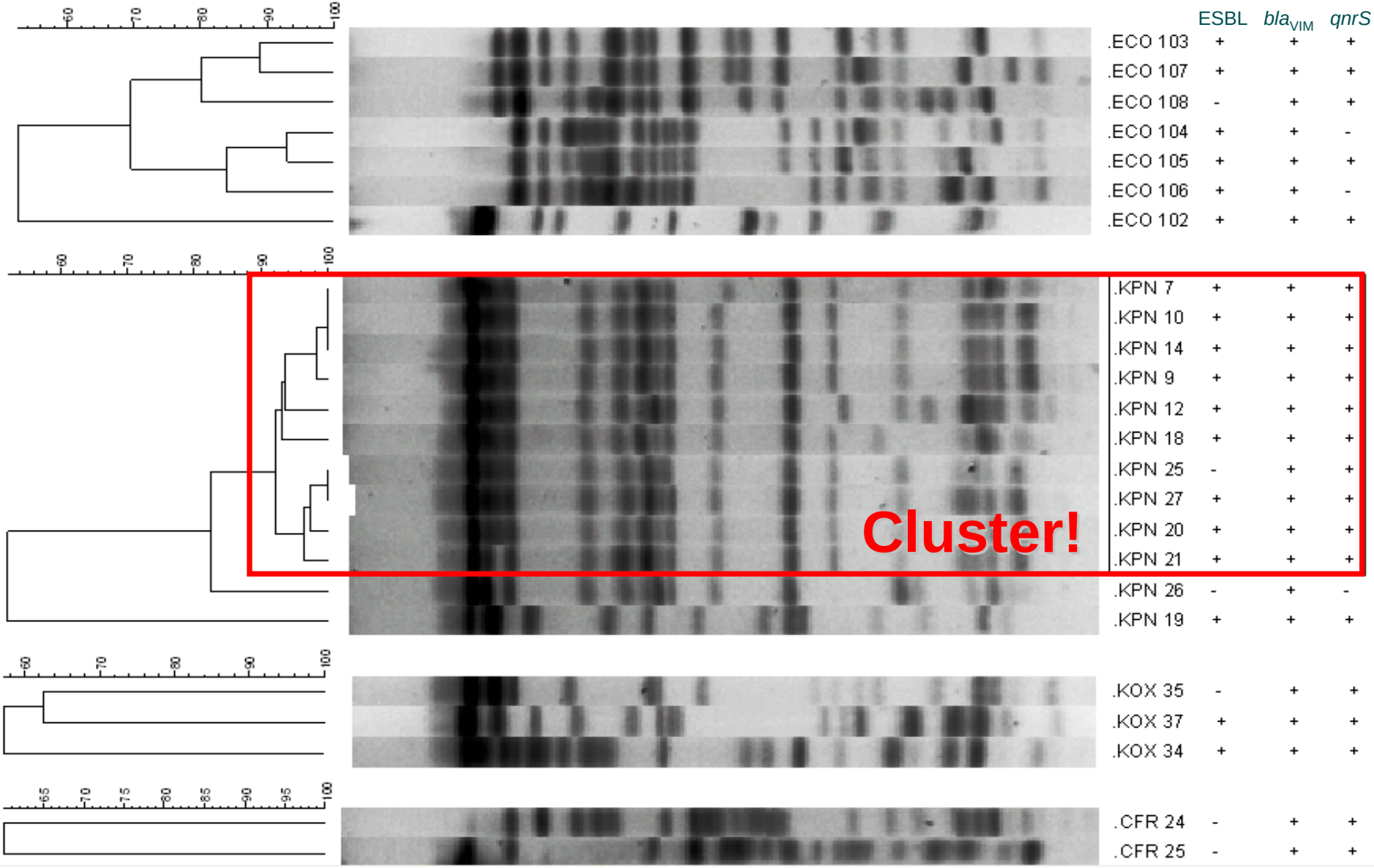




Pazienti positivi

** Screening eseguito nel periodo 2010-2014 con terreno Chromagar ESBL su 3900 campioni da 1450 pazienti*

PFGE: dendrogrammi per illustrare la somiglianza di 7 *E.coli* (ECO),12 *K.pneumoniae* (KPN), 3 *K.oxytoca* (KOX) e 2 *C.freundii* (CFR) contenenti i geni *bla*VIM e *qnr*S. La gradazione della scala rappresenta il grado di somiglianza; membri di un cluster con >85% di somiglianza in rettangolo.



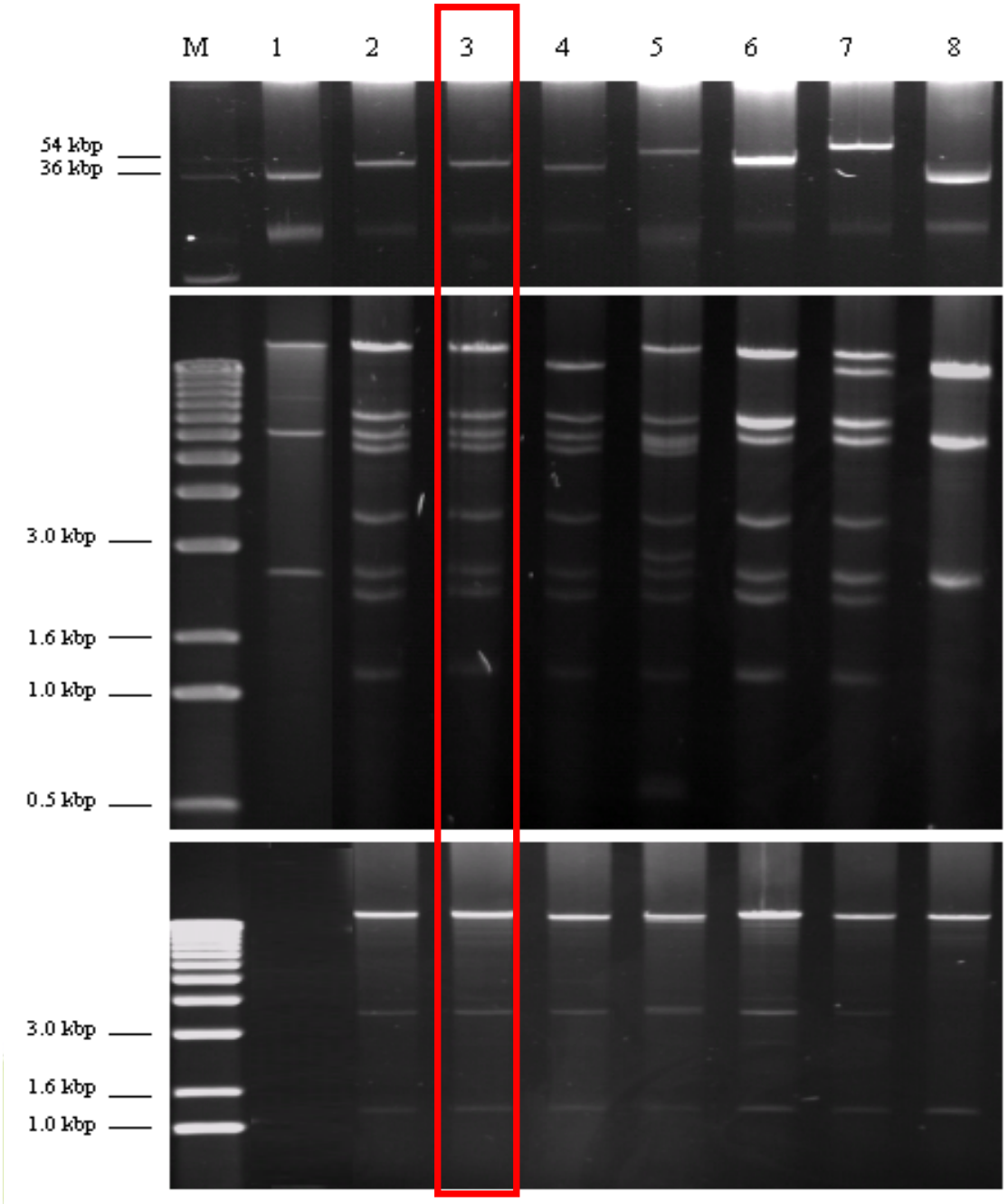
Aschbacher R et al. Linkage of acquired quinolone resistance (qnrS1) and metallo-ß-lactamase (bla_{VIM1}) genes in multiple species of Enterobacteriaceae from Bolzano, Italy. Journal of Antimicrobial Chemotherapy 2008; 61: 515-523.

Table 1: Carbapenemase-producing *Enterobacteriaceae* from the Bolzano area, 2011-2012.

Isolate (Patient)	Date of isol.	Age	Specimen	Department	Species	Gene	MLST[a]	PFGE[b]
988 (1)	May 2011	54	Urine	Outpatient	*E. coli*	bla_{OXA-48}	ST2076	ND[h]
1008 (2)	Apr 2011	59	Urine, perineal swab	Rehabilitation	*K. pneumoniae*	bla_{KPC-3}	ST258	KP-A
1061 (3)	Jul 2011	65	Bronchial aspirate	Intensive care unit	*K. pneumoniae*	bla_{KPC-3}	ST258	KP-A
1240 (4)	Jan 2012	78	Urine	Nephrology	*C. freundii*	bla_{VIM-1}	ND	ND
1056 (5)	Jul 2011	14	Blood	Paediatrics	*E. asburiae*[i]	bla_{VIM-1}	ND	ND
1076 (6)	Jul 2011	63	Sputum	Haematology	*E. asburiae*[i]	bla_{VIM-1}	ND	ND
972 (7)	Apr 2011	62	Urine	Rehabilitation	*E. cloacae*[i]	bla_{VIM-1}	ND	ND
1027 (8)	Jun 2011	78	Urine	Nephrology	*E. coli*	bla_{VIM-1}	ST131	EC-A
1063 (9)	Jul 2011	91	Urine	Geriatrics	*E. coli*	bla_{VIM-1}	ST131	EC-A1
1160 (10)	Oct 2011	83	Urine	LTCF[j]	*E. coli*	bla_{VIM-1}	ST34	EC-D
1185 (11)[k]	Nov 2011	74	Blood	LTCF	*E. coli*	bla_{VIM-1}	ST131	EC-B
1193 (12)[k]	Nov 2011	81	Urine	Geriatrics	*E. coli*	bla_{VIM-1}	ST131	EC-A
1321 (13)[k]	Mar 2012	92	Urine	Outpatient	*E. coli*	bla_{VIM-1}	ST131	EC-A
1448 (14)	Jun 2012	61	Perineal swab	Haematology	*E. coli*	bla_{VIM-1}	ST23	EC-C
1042 (7)	Jul 2011	62	Urine	LTCF	*K. oxytoca*	bla_{VIM-1}	ND	ND
1195 (15)	Nov 2011	70	Perineal swab	Intensive care unit	*K. oxytoca*	bla_{VIM-1}	ND	ND
931 (16)	Feb 2011	69	Urine	Internal Medicine	*K. pneumoniae*	bla_{VIM-1}	ST54	KP-B
1025 (17)	Jun 2011	56	Urine	Orthopedics	*K. pneumoniae*	bla_{VIM-1}	ST475	KP-C
1043 (7)	Jul 2011	62	Urine	LTCF	*K. pneumoniae*	bla_{VIM-1}	ST730	KP-D
1159 (18)[k]	Jul 2011	80	Pleural fluid	Gastroenterology	*K. pneumoniae*	bla_{VIM-1}	ST721	KP-E
1182 (19)	Nov 2011	83	Ulcer	Outpatient	*K. pneumoniae*	bla_{VIM-1}	ST54	KP-B1
1415 (20)	May 2012	64	Urine	Internal Medicine	*K. pneumoniae*	bla_{VIM-1}	ST623	KP-F
1444 (14)	Jun 2012	61	Pharyngeal swab	Haematology	*K. pneumoniae*	bla_{VIM-1}	ST25	KP-G

Aschbacher R et al. Carbapenemase-producing Enterobacteriaceae during 2011-12 in the Bolzano area (Northern Italy): increasing diversity in a low-endemicity setting. Diagn Microbiol Infect Dis 2013; 77:354-6

Plasmidi da enterobatteri VIM-1 produttori, Bolznano



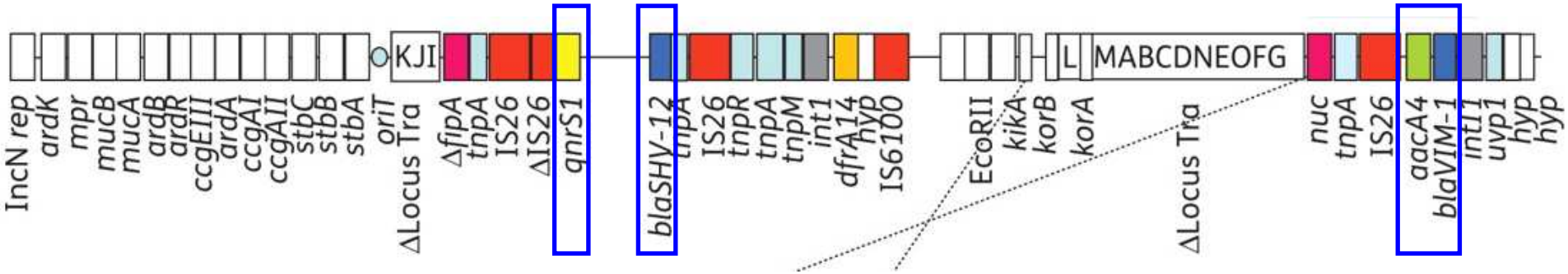
Sopra: elettroforesi su un gel di agarosio (0.7%) di plasmidi estratti da vari trasformanti. Lane M: control strain *E.coli* 39R861, lanes 1-8: vari plasmidi da enterobatteri VIM-produttori.

Centro: plasmidi estratti dai trasformanti come sopra, digeriti con enzima di restrizione *Hpa*I, separati su gel di agarosio 0,7%. In lane M MW marker.

Sotto: plasmidi come nell'immagine di centro digeriti con *BamHI*/*Sac*I. M MW marker, lane 1: nessun campione.

Quadro rosso: plasmide sequenziato

Sequenziamento di un plasmide da *Klebsiella oxytoca VIM+ da Bolzano*



Sul plasmide geni per:

- Carbapenemasi (*bla*$_{VIM-1}$)
- ESBL (*bla*$_{SHV-12}$)
- Resistenza a fluorochinoloni (*qnrS1*)
- Resistenza a aminoglicosidi (*aacA4*)

Carattoli A et al. Complete nucleotide sequence of the IncN plasmid pKOX105 encoding VIM-1, QnrS1 and SHV-12 proteins in Enterobacteriaceae from Bolzano, Italy compared with IncN plasmids encoding KPC enzymes in the USA. Journal of Antimicrobial Chemotherapy 2010; 65:2070-5.

Antibiotici	*E.coli 108 (Isolato clinico)*	*E.coli DH10B*	*E.coli DH10B (pECO108)*
	MIC	MIC	MIC
Ampicillina	> 64	8	> 64
Amoxicillina/clav.	64	8	64
Piperacillina	> 64	4	> 64
Piperacillina/tazo.	> 64	4	> 64
Aztreonam	0.25	≤ 0.125	0.25
Cefotaxime	256	≤ 0.125	128
Ceftazidime	> 256	0.5	> 256
Cefpirome	> 64	≤ 0.125	> 64
Cefoxitina	> 64	8	> 64
Imipenem	8	0.5	8
Meropenem	8	≤ 0.06	2
Ertapenem	8	≤ 0.06	1
Tobramicina	> 32	0.5	16
Gentamicina	> 32	0.5	2
Netilmicina	16	0.25	2
Amikacina	4	1	2
Acido nalidixico	> 256	1	4
Ciprofloxacina	> 32	0.002	0.25
Levofloxacina	> 32	0.008	0.25
Moxifloxacina	> 32	0.002	0.125
Ofloxacina	> 32	0.032	0.25

Presenza di ulteriori meccanismi di resistenza:
E.coli 108: isolato clinico di Bolzano.
E.coli DH10B: ceppo di laboratorio usato come accettore per trasformazione.
E.coli DH10B (pECO108): *E.coli* DH10B contenente il plasmide con i geni per VIM-1 (ed i geni *aacA4* e *qnrS1*, aumentando la MIC per aminoglicosidi e fluorochinolonici, rispettivamente).

Aschbacher R et al. Linkage of acquired quinolone resistance (qnrS1) and metallo-ß-lactamase (bla_{VIM1}) genes in multiple species of Enterobacteriaceae from Bolzano, Italy. Journal of Antimicrobial Chemotherapy 2008; 61: 515-523.

Bolzano: riassunto plasmidi con *bla*$_{VIM}$

- ✓ Plasmidi di grandezza variabile: ca. 30-150 kb
- ✓ Frequente rimaneggiamento dei plasmidi
- ✓ Tutti appartenenti al gruppo d'incompatibilitá IncN
- ✓ Facilmente trasferibili:
  - ✓ Per trasformazione: successo 10/10
  - ✓ Per coniugazione: successo 3/5 (frequenza: 5 x 10^{-5} – 5 x 10^{-4})
- ✓ Associazione di geni *bla*$_{VIM}$, *bla*$_{SHV}$, *qnrS* e *aacA4* sullo stesso plasmide + altri geni di resistenza cromosomali → fenotipo MDR

Aschbacher R et al. Linkage of acquired quinolone resistance (qnrS1) and metallo-ß-lactamase (bla$_{VIM1}$) genes in multiple species of Enterobacteriaceae from Bolzano, Italy. Journal of Antimicrobial Chemotherapy 2008; 61: 515-523. Aschbacher R et al. Metallo-β-lactamases among Enterobacteriaceae from routine samples in an Italian tertiary-care hospital and long-term care facilities during 2008. Clin Microbiol Infect 2011; 17:181-189. Carattoli A et al. Complete nucleotide sequence of the IncN plasmid pKOX105 encoding VIM-1, QnrS1 and SHV-12 proteins in Enterobacteriaceae from Bolzano, Italy compared with IncN plasmids encoding KPC enzymes in the USA. Journal of Antimicrobial Chemotherapy 2010; 65:2070-2075.

Bolzano: 24 ceppi di enterobatteri VIM +

Enterobacterial isolate	AMC	TZP	ATM	ATM-DDS	CTX	CTXC	CAZ	CAZC	CPR	CPRC	FOX	IPME	IPM	MEM	ETP	CIP	GEN	TOB	AMK	TIG	COL
E. coli 102	64	32	>64	+	64	16	128	32	32	16	64	0.25	2	0.25	0.25	0.5	2	8	2	0.25	≤0.5
E. coli 103	64	>64	64	+	128	>32	256	>32	64	32	>64	0.125	4	1	0.5	>8.0	32	>32	2	0.125	≤0.5
E. coli 104	64	32	>64	+	>256	16	256	>32	>64	8	32	0.25	2	0.25	0.25	>8.0	>32	32	2	1	≤0.5
E. coli 105	64	64	>64	+	>256	16	>256	32	>64	8	32	0.25	2	0.5	0.25	>8.0	>32	>32	8	0.5	≤0.5
E. coli 106	64	>64	>64	+	>256	>32	>256	>32	>64	>32	>64	0.25	8	8	4	>8.0	>32	>32	16	1	≤0.5
E. coli 107	64	>64	>64	+	256	>32	>256	>32	64	>32	64	0.25	8	2	2	>8.0	4	16	4	0.25	≤0.5
E. coli 108	64	>64	0.5	−	256	>32	>256	>32	>64	>32	>64	0.25	8	8	8	>8.0	>32	>32	4	0.125	1
K. pneumoniae 7	64	32	32	+	32	8	64	32	16	4	16	0.25	2	0.25	≤0.125	0.5	2	8	1	0.5	≤0.5
K. pneumoniae 9	32	>64	>64	+	128	>32	>256	>32	64	>32	>64	0.25	8	4	8	8	1	4	1	4	1
K. pneumoniae 10	64	>64	>64	+	64	>32	>256	>32	32	>32	>64	0.25	8	4	2	1	2	8	1	0.5	≤0.5
K. pneumoniae 12	32	>64	>64	+	64	32	>256	>32	32	32	>64	1	4	2	2	8	1	4	1	1	1
K. pneumoniae 14	32	>64	>64	+	64	>32	>256	>32	64	>32	>64	0.25	8	2	4	8	1	4	≤0.5	4	2
K. pneumoniae 18	32	64	>64	+	64	32	>256	>32	32	32	64	0.5	4	1	1	>8.0	1	4	1	0.5	1
K. pneumoniae 19	64	>64	>64	+	128	>32	>256	>32	64	>32	64	0.25	8	2	1	2	2	8	2	0.5	≤0.5
K. pneumoniae 20	64	>64	>64	+	256	>32	>256	>32	64	>32	>64	0.25	16	4	4	8	2	8	2	1	1
K. pneumoniae 21	64	>64	>64	+	128	>32	>256	>32	32	32	64	0.25	8	2	2	1	2	8	2	0.5	≤0.5
K. pneumoniae 25	64	>64	0.25	−	64	>32	256	>32	64	>32	>64	0.5	8	2	1	0.5	1	8	1	0.5	≤0.5
K. pneumoniae 26	32	>64	≤0.125	−	32	32	256	>32	32	32	64	0.25	4	0.5	0.5	0.25	2	8	4	0.25	≤0.5
K. pneumoniae 27	32	>64	>64	+	128	>32	>256	>32	32	>32	>64	0.25	8	4	4	>8.0	2	8	1	1	≤0.5
K. oxytoca 34	64	>64	>64	+	128	>32	>256	>32	64	>32	>64	0.25	8	4	4	>8.0	2	8	64	1	≤0.5
K. oxytoca 35	64	>64	0.25	−	64	>32	256	>32	>64	>32	>64	0.25	16	2	1	0.5	2	16	2	≤0.25	1
K. oxytoca 37	>64	>64	>64	+	>256	>32	>256	>32	>64	>32	>64	1	>32	>32	>16	4	32	>32	64	1	≤0.5
C. freundii 24	64	>64	≤0.125	−	64	>32	>256	>32	64	32	>64	0.5	8	2	0.5	>8.0	2	16	2	0.5	≤0.5
C. freundii 25	64	>64	0.25	−	64	>32	>256	>32	64	32	>64	0.5	8	1	0.5	0.5	2	16	2	≤0.25	≤0.5

AMC, amoxicillin/clavulanic acid 2:1; ATM, aztreonam; ATM-DDS, ESBL double disc synergy test between aztreonam and clavulanic acid; CTX, cefotaxime; CTXC, cefotaxime + 4 mg/L clavulanic acid; CAZ, ceftazidime; CAZC, ceftazidime + 4 mg/L clavulanic acid; CPR, cefpirome; CPRC, cefpirome + 4 mg/L clavulanic acid; FOX, cefoxitin; TZP, piperacillin + 4 mg/L tazobactam; IPME, imipenem + 400 mg/L EDTA; IPM, imipenem; MEM, meropenem; ETP, ertapenem; CIP, ciprofloxacin; TOB, tobramycin; AMK, amikacin; GEN, gentamicin; TIG, tigecycline; COL, colistin.

Aschbacher R et al. Linkage of acquired quinolone resistance (qnrS1) and metallo-ß-lactamase (bla_{VIM1}) genes in multiple species of Enterobacteriaceae from Bolzano, Italy. Journal of Antimicrobial Chemotherapy 2008; 61: 515-523.

Carbapenem MICs (mg/L) for carbapenemase-producing isolates determined by different methods, and susceptibilities to other antimicrobial agents.

Isolate	Gene	Imipenem				Meropenem				Ertapenem			Other agents[c]								
		Etest[a]	Wider	Vitek 2	BMD[b]	Etest[a]	Wider	Vitek 2	BMD	Etest[a]	Vitek 2	BMD	Cip	Amk	Gen	Tig	Col	Sxt	Fep	Caz	Ctx
988	bla_{OXA-48}	0.5	2	≤1	1	0.25	0.5	≤0.25	1	2	4	2	S	S	S	S	S	R	S	S	S
1008	bla_{KPC-3}	>16	>8	>8	16	16	>8	2	32	>16	>4	64	R	S	R	I	S	R	R	R	R
1061	bla_{KPC-3}	>16	>8	4	16	8	>8	>8	32	>16	>4	128	R	R	S	I	S	R	R	R	R
1240	bla_{VIM-1}	16	4	8	4	0.25	2	4	1	0.25	1	0.25	R	S	S	S	S	S	S	R	R
1056	bla_{VIM-1}	>16	>8	8	16	4	>8	1	8	1	≤0.5	2	S	S	I	S	S	R	I	R	R
1076	bla_{VIM-1}	>16	>8	8	64	8	>8	2	64	32	2	4	R	S	S	S	R	S	R	R	R
972	bla_{VIM-1}	2	4	4	8	0.5	1	≤0.25	8	2	≤0.5	4	R	S	I	R	S	S	I	R	R
1027	bla_{VIM-1}	2	4	>8	4	0.25	4	≤0.25	4	0.12	≤0.5	1	R	S	I	S	S	S	I	R	R
1063	bla_{VIM-1}	2	4	4	8	0.25	1	≤0.25	8	0.5	≤0.5	2	R	S	I	S	S	S	R	R	R
1160	bla_{VIM-1}	2	8	4	4	0.12	0.5	≤0.25	2	0.06	≤0.5	0.25	S	S	I	S	S	S	S	R	R
1185	bla_{VIM-1}	1	8	≤1	4	0.5	4	≤0.25	1	0.5	≤0.5	0.25	R	S	I	S	S	S	R	R	R
1193	bla_{VIM-1}	2	2	≤1	8	0.25	0.5	≤0.25	2	0.25	≤0.5	1	R	S	I	S	S	I	I	R	R
1321	bla_{VIM-1}	4	4	≤1	4	0.25	0.5	≤0.25	1	0.25	≤0.5	0.25	R	S	I	S	S	R	I	R	R
1448	bla_{VIM-1}	1	0.5	2	2	0.06	≤0.12	≤0.25	0.25	0.25	≤0.5	<0.25	R	S	S	S	S	R	I	R	R
1042	bla_{VIM-1}	1	4	8	8	0.25	8	1	4	0.5	≤0.5	1	S	S	I	S	S	R	S	R	R
1195	bla_{VIM-1}	16	4	>8	16	2	>8	2	32	2	4	16	R	S	S	S	S	R	R	R	R
931	bla_{VIM-1}	4	4	8	8	0.5	2	≤0.25	4	2	≤0.5	0.5	I	S	S	S	S	S	I	R	R
1025	bla_{VIM-1}	1	4	8	8	1	2	1	2	1	≤0.5	0.5	I	S	S	S	S	S	S	R	R
1043	bla_{VIM-1}	1	2	2	4	0.25	0.5	≤0.25	1	0.25	≤0.5	0.25	S	S	S	S	S	S	R	R	R
1159	bla_{VIM-1}	2	0.5	≤1	4	1	1	≤0.25	4	1	≤0.5	4	R	S	S	R	S	R	I	R	R
1182	bla_{VIM-1}	8	>8	>8	32	1	>8	1	64	1	4	8	I	S	I	S	S	S	R	R	R
1415	bla_{VIM-1}	4	4	2	4	0.25	0.5	≤0.25	2	0.5	≤0.5	0.5	I	S	S	S	S	S	S	R	R
1444	bla_{VIM-1}	1	1	2	4	0.25	0.5	≤0.25	4	0.5	≤0.5	1	R	S	S	R	S	S	I	R	R
Cumulative susceptibilities and agreement with BMD																					
%S		57	26	39	9	83	61	92	43	52	70	39	22	96	52	78	96	57	26	4	4
%I		17	52	44	65	13	13	4	35	17	4	17	17	0	44	9	0	4	39	0	0
%R		26	22	17	26	4	26	4	22	31	26	44	61	4	4	13	4	39	35	96	96
EA (BMD)		56	78	69	NA	17	65	13	NA	61	74	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
CA (BMD)		43	48	48	NA	56	69	43	NA	56	61	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

S = susceptible; I = intermediate; R = resistant; EA (BMD) = essential agreement relative to BMD; CA (BMD) = category agreement relative to BMD; NA = not applicable.

[a] Etest MICs for carbapenems are rounded up to the next doubling dilution for comparison.

[b] Reference broth microdilution.

[c] Susceptibility categories based on data from Vitek 2, interpreted according to EUCAST breakpoints. Cip = ciprofloxacin; Amk = amikacin; Gen = gentamicin; Tig = tigecycline; Col = colistin; Sxt = trimethoprim-sulphamethoxazole; Fep = cefepime; Caz = ceftazidime; Ctx = cefotaxime.

Aschbacher R et al. Carbapenemase-producing Enterobacteriaceae during 2011-12 in the Bolzano area (Northern Italy): increasing diversity in a low-endemicity setting. Diag Microbiol Infect Dis 2013; 77:354-6

Refertazione carbapenemici secondo le MIC in enterobatteri carbapenemasi +

- ✓ **MIC determinate in laboratorio: concordanza fra metodi e ripetibilitá scarse.**
- ✓ **MIC per cefalosporine o carbapenemici non correlano sempre con la risposta clinica.**
- ✓ **Importanza epidemiologica e di sorveglianza di fenotipo ("microrganismi sentinella").**

Provincia di Bolzano: R a carbapenemici!

(Decisione di UHAC: Unit for Hospital Antimicrobial Chemotherapy). Aggiunta di nota al referto, vedi sotto!

Attenzione: isolato microbiologico con importanti implicazioni di prevenzione e controllo della trasmissione.
Si raccomanda di applicare i protocolli elaborati dal Servizio di Igiene ospedaliera.
La terapia richiede una valutazione da parte di uno specialista del reparto Malattie Infettive.

Livermore DM et al. Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly? JAC 2012; 67: 1569-1577

TABLE I. Epidemiological and clinical characteristics of patients infected or colonized with *bla*$_{VIM}$-positive isolates

Isolate	Sex/age (years)	Comorbities	Department	Hospitalization in last 6 months	In hospital at diagnosis	MBL-positive Specime	Clinical relevance of MBL-producer	rior arbapenem herapy within 6 nonths)	Ongoing antimicrobial therapy	Species	Targeted antimicrobial therapy	Treatment outcome (on day 7)	Overall outcome (within 6 months)
370	M/81	DM, senile dementia	LTCF	Yes	No	Urine	Colonization	AV	NAV	*Proteus mirabilis*	NAP	NAP	Dead
402	F/63	Multiple sclerosis, UTI	LTCF	No	No	Urine	UTI	o	Yes	*Escherichia coli*	NAV	NAV	Alive
406, 424	M/50	Demyelinizing encephalopathy, autoimmune thyroiditis, multifocal intestinal infarction, HCV+	Intensive care unit, rehabilitation unit	Yes	Yes	Umbilical drainage bronchia aspirate	Surgical site infection, pneu monia	es	Aerosolized COL + TZP	*Enterobacter cloacae*, *Klebsiella oxytoca*	TGC + AMK + LVX + MEM	Success	Dead
420	F/81	AF,CHF, Parkinson's disease	LTCF	No	No	Urine	Colonization	AV	NAV	*Escherichia coli*	NAP	NAP	Dead
425	F/95	Gastric cancer, hypertension	LTCF	No	No	Urine	Colonization	o	None	*Escherichia coli*	None	NAP	Dead
426	M/61	AMI, necrotizing enterocolitis	Intensive care unit	No	Yes	Bronchial aspirate	Colonization	o	TZP + LVX + MTZ	*Klebsiella pneumoniae*	None	NAP	Dead
428	M/84	Stroke	LTCF	Yes	No	Urine	Colonization	AV	NAV	*Escherichia coli*	NAP	NAP	Dead
429	F/49	Perinatal hypoxia, congenital heart disease, hypothyroidism, Hepatitis C	Internal medicine	No	Yes	Urine	Colonization	o	TIG + LVX	*Citrobacter freundii*	TGC + LVX	NAP	Alive
450, 451, 520	M/5	Prune-belly syndrome	Paediatrics	Yes	No	Gland swa urine	Colonization	o	None	*Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*	None	NAP	Alive
430	F/69	Kidney transplantation type 2 DM, Hypertension	Nephrology	Yes	No	Urine	Colonization	o	None	*Citrobacter freundii*	None	NAP	Alive
445, 446	M/94	COPD, hemiplegia	LTCF	Yes	No	Urine	Colonization	AV	NAV	*Morganella morganii*, *Providencia stuartii*	NAP	NAP	Dead
457, 462, 474	F/63	AML, ABMT, intracranial haemorrhages	Haematology	Yes	Yes	Blood, wound swab, vaginal swab	CRBSI, wound infection, vaginitis	es	TZP + MEM + LZD	*Klebsiella pneumoniae*, *Citrobacter amalonaticus*, *Citrobacter freundii*	TGC	Success	Alive
458	F/88	Senile dementia, UTI	LTCF	No	No	Urine	Colonization	AV	NAV	*Escherichia coli*	NAP	NAP	Alive
464	F/78	CHF, aortic bioprosthesis, hypertension, autoimmune thyroiditis	Internal medicine	Yes	No	Urine	UTI	o	LVX	*Escherichia coli*	SXT	Success	Alive
472	F/80	AMI, CHF, hypertension	LTCF	No	No	Urine	Colonization	o	None	*Klebsiella oxytoca*	NAP	NAP	Dead
476	F/78	Obesity, hip prosthesis, colonic diverticolosis	Orthopaedics	No	No	Wound	Colonization	o	LVX + TEC	*Escherichia coli*	None	NAP	Alive
480	M/76	Biliary ducts cancer, hypertension, stroke	LTCF	Yes	No	Urine	UTI	o	No	*Escherichia coli*	NIT	NAV	Alive
481	M/65	AML, AMI	Haematology	No	No	Blood	CRBSI	es	VAN + MEM	*Enterobacter aerogenes*	TZP + LVX	Success	Alive
488	F/5 months	Premature infant, necrotizing enterocolitis	NICU	Yes	Yes	Urine	Colonization	es	CAZ + TEC	*Enterobacter cloacae*	None	NAP	Alive
490	F/87	Hypertension, COPD, senile dementia	LTCF	Yes	No	Urine	Colonization	AV	NAV	*Escherichia coli*	NAP	NAP	Alive
491	F/69	CRF, Hypertension, UTI, CMV-infection, vasculitis, *Clostridium difficile* enterocolitis	Nephrology	Yes	Yes	Urine	Colonization	o	None	*Klebsiella pneumoniae*	None	NAP	Alive
497	F/87	AML, CHF, intestinal B-NHL	Orthopaedics	No	Yes	Urine	Colonization	o	None	*Klebsiella pneumoniae*	None	NAP	NAV
505	F/79	Stroke, type 2 DM, UTI, AMI, breast cancer, hypertension	Internal medicine	Yes	Yes	Urine	Colonization	o	None	*Escherichia coli*	None	NAP	NAV
508	F/76	Alzheimer's disease	LTCF	Yes	No	Urine	Colonization	AV	NAV	*Klebsiella pneumoniae*	NAP	NAP	Alive
512, 513	M/59	HCV+, hypertension, intracranial haemorrhages	Neurosurgery	No	Yes	Bronchial secretio	Colonization	o	None	*Klebsiella oxytoca*, *Citrobacter freundii*	None	NAP	Alive
515	F/84	CRF, type 2 DM, anaemia, cerebral vasculopathy	Internal medicine	Yes	Yes	Urine	Colonization	o	None	*Escherichia coli*	None	NAP	Alive
518	M/78	AF, CHF, type 2 DM, brain infarction, urinary incontinence	Internal medicine	Yes	Yes	Urine	UTI	o	None	*Escherichia coli*	SXT	Success	Alive

Aschbacher R et al. Metallo-β-lactamases among Enterobacteria-ceae from routine samples in an Italian tertiary-care hospital and long-term care facilities during 2008. Clin Microbiol Infect 2011; 17:181-189.

Programma

- ✓ Le carbapenemasi. Cosa sono?
- ✓ Epidemiologia negli enterobatteri
- ✓ Enterobatteri carbapenemasi+ in Italia e Trentino/Alto Adige
- ✓ Acinetobacter baumannii
- ✓ Pseudomonas aeruginosa

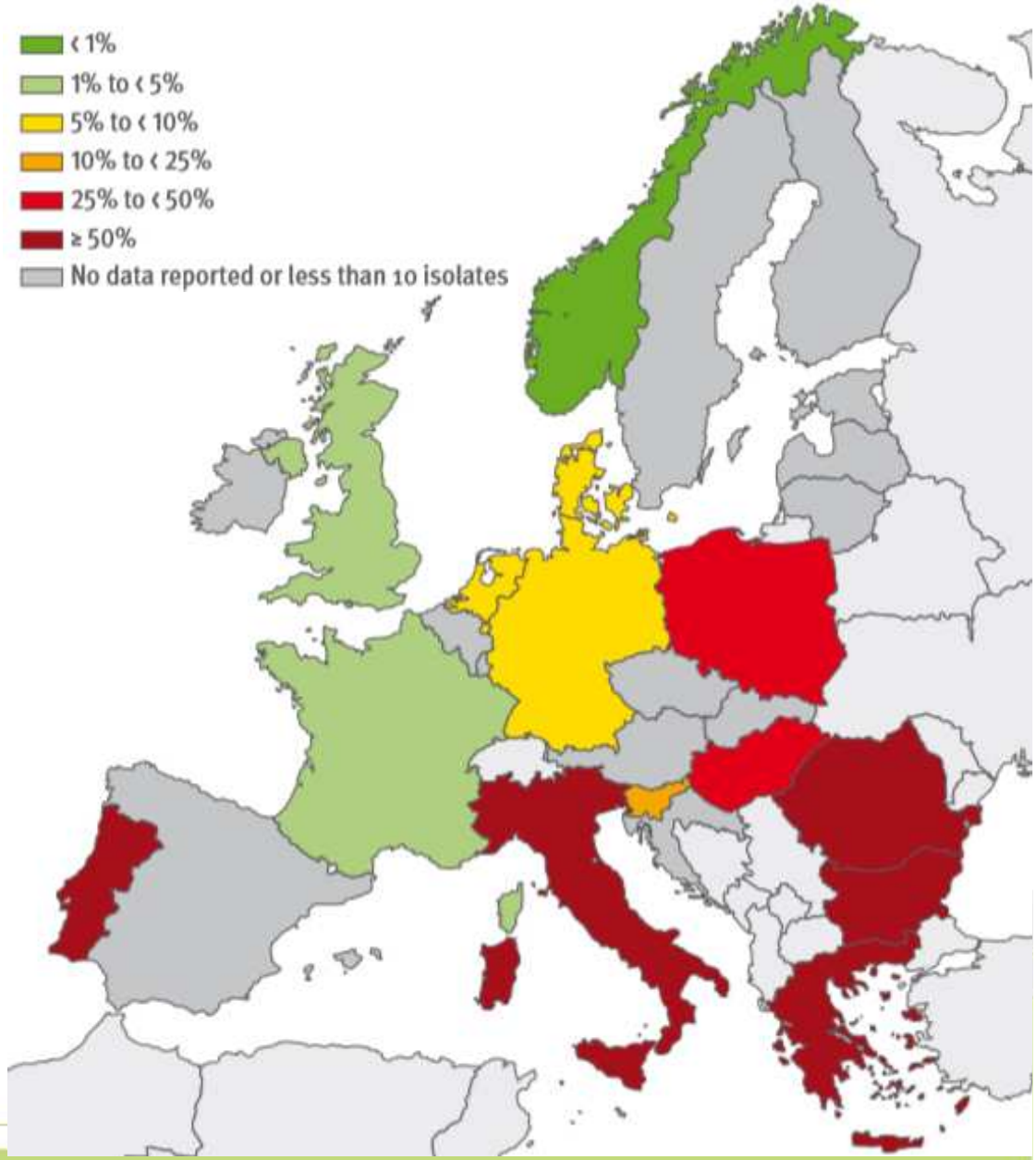
Acinetobacter baumannii carbapenemasi+

- ✓ R a carba-penemici gen. da carbapenemasi di tipo **OXA**
- ✓ Sottogruppi: OXA-51, -58,-23,-24/40,-143 (Italia: soprat-tutto **OXA-58, OXA-23**)
- ✓ Raramente: IMP, VIM, SIM, NDM
- ✓ Alto grado di multiresistenza

Acinetobacter baumannii, carbapenemasi di tipo OXA Isolato a Bolzano da tampone ferita		
	MIC	**Interpret.**
Ceftazidime	≥ 64	R
Meropenem	≥ 16	R
Imipenem	≥ 16	R
Gentamicina	≥ 16	R
Amikacina	≥ 64	R
Tobramicina	≥ 16	R
Ciprofloxacina	≥ 4	R
Levofloxacina	≥ 8	R
Trimet./ sulfamet.	≥ 320	R
Tigeciclina	2	I
Colistina	≤ 0.5	S

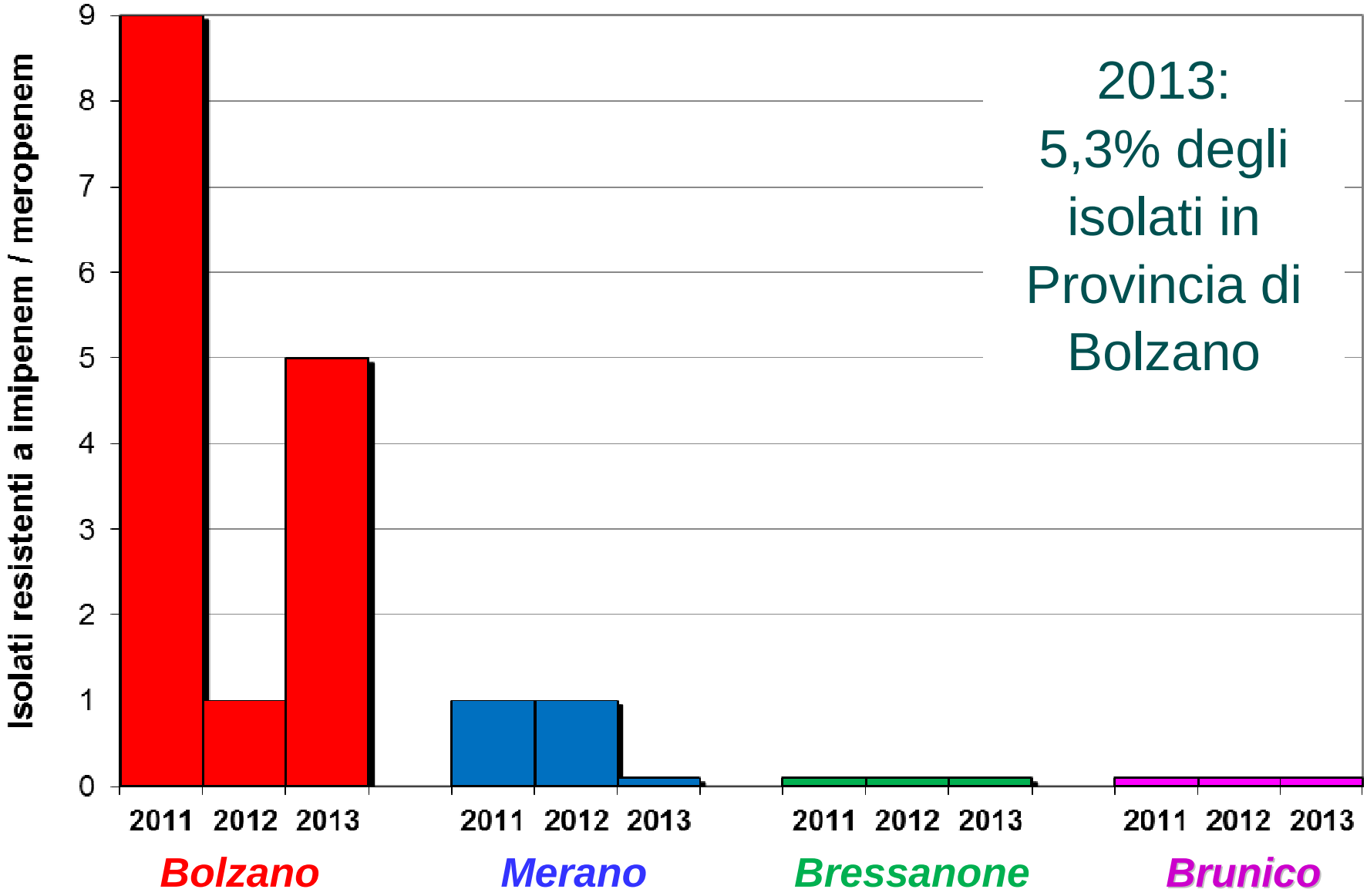
Acinetobacter baumannii carbapenemasi+

- ✓ **Italia: *A.baumannii* carbapenemasi produttori (tipo OXA) sono frequenti (ad es. isolati da sangue >50%, vedi figura!)**
- ✓ **Provincia di Trento: 2 isolati nel 2013**
  (Dati: Dott.Lanzafame)
- ✓ **Provincia di Bolzano: 5 nel 2013; 18 isolati in tutto**



EARS-Net (European Antimicrobial Resistance Surveillance Network) 2012: http://ecdc.europa.eu/

Provincia di Bolzano: *Acinetobacter baumannii* R a imipenem / meropenem, tutti i tipi di campioni



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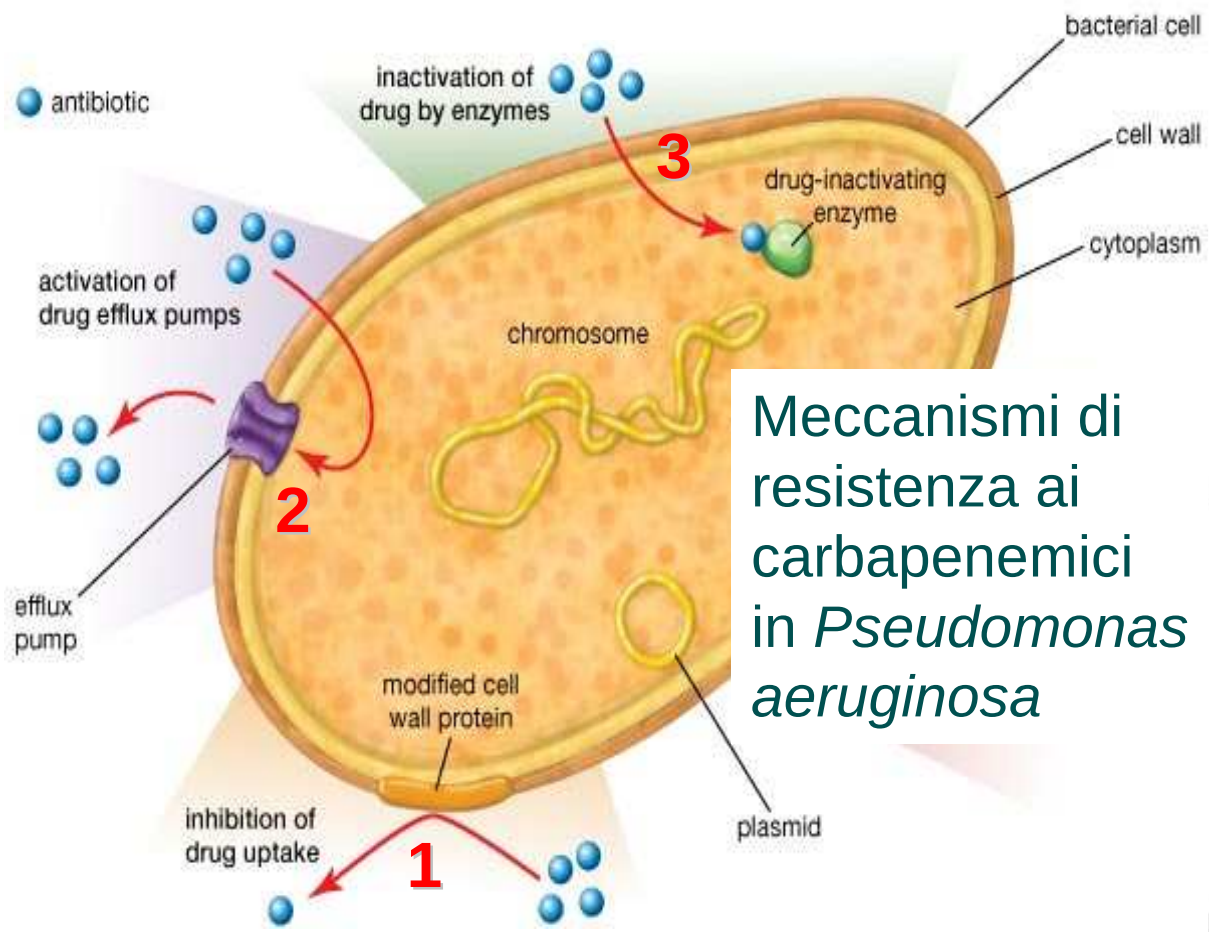
Resistenza acquisita ai carbapenemici in *P. aeruginosa*

1) **Ridotta permeabilità della parete (Porina OprD)**
2) **Sovraespressione pompe d'efflusso (MexAB-OprM)**
3) **ß-lattamasi:**
   - ✓ **Metallo-ß-lattamasi (carbapenemasi):**
     - ✓ **VIM**
     - ✓ **IMP**
     - ✓ SPM, GIM, FIM, NDM
   - ✓ **Serina-carbapenemasi:**
     - ✓ KPC, GES-2, GES-5
     - ✓ OXA-40, OXA-50
   - ✓ **AmpC a spettro esteso (ESAmpC)**

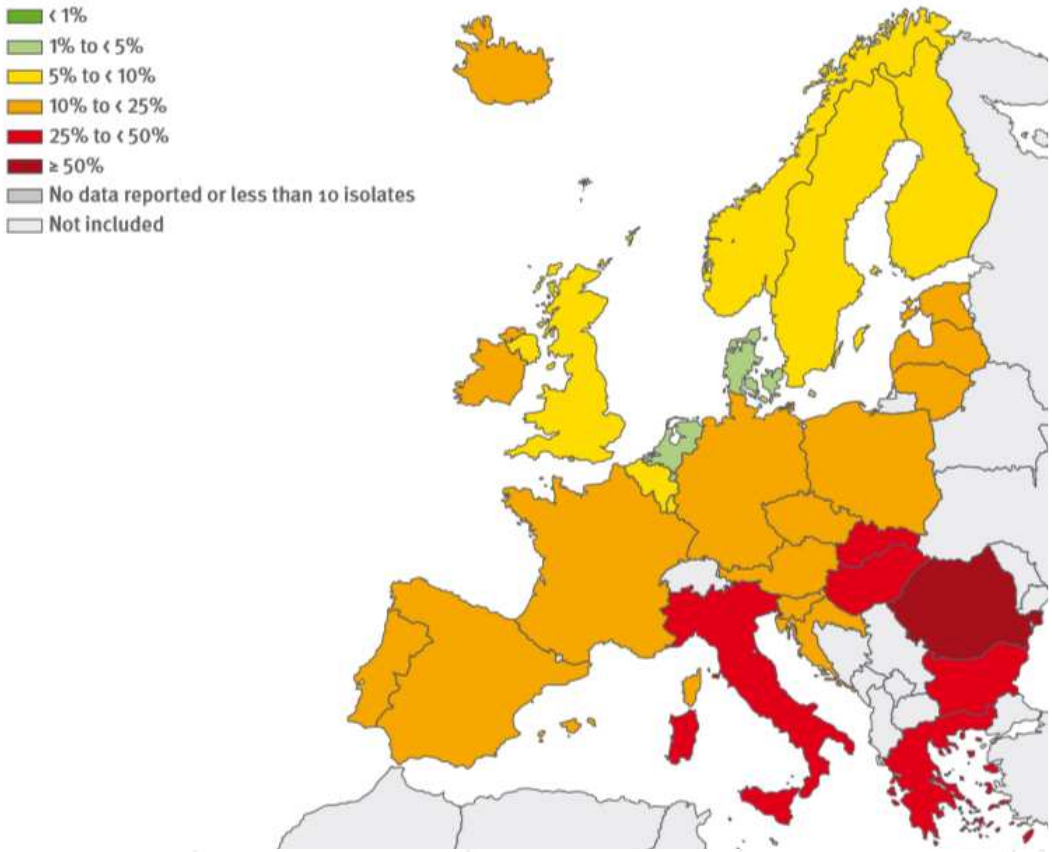


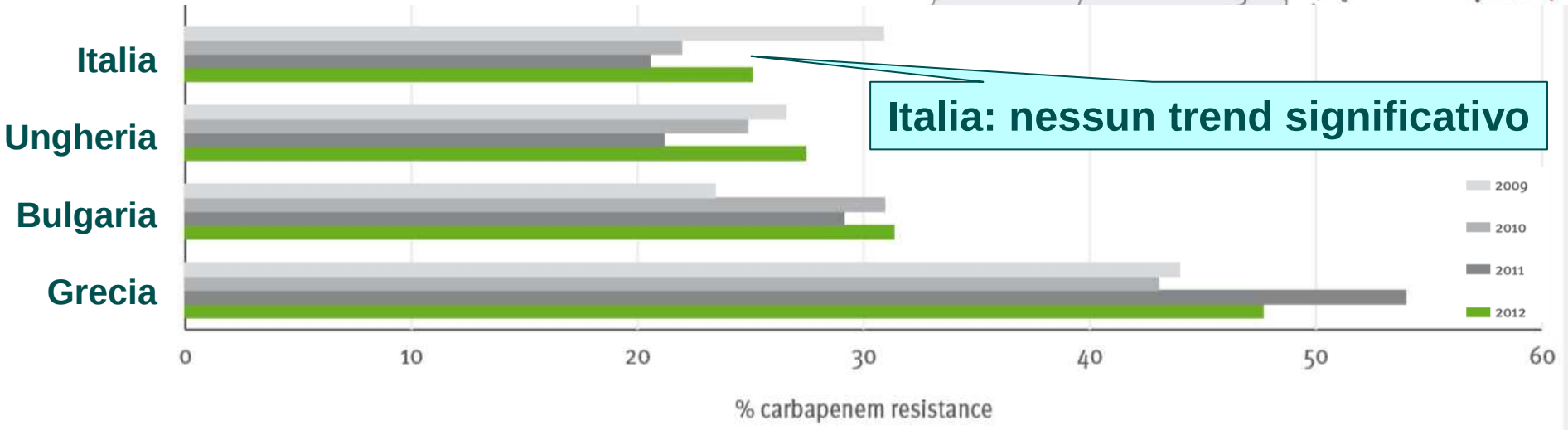
Meccanismi di resistenza ai carbapenemici in *Pseudomonas aeruginosa*

La resistenza ai carbapenemici è generalmente causata da una combinazione di meccanismi di resistenza

Wolter DJ et al. Mechanisms of ß-lactam resistance among Pseudomonas aeruginosa. Curr Pharm Design 2013; 19: 209-222

Pseudomonas aeruginosa da sangue
resistenza a carbapenemici
Italia 2012: 25%

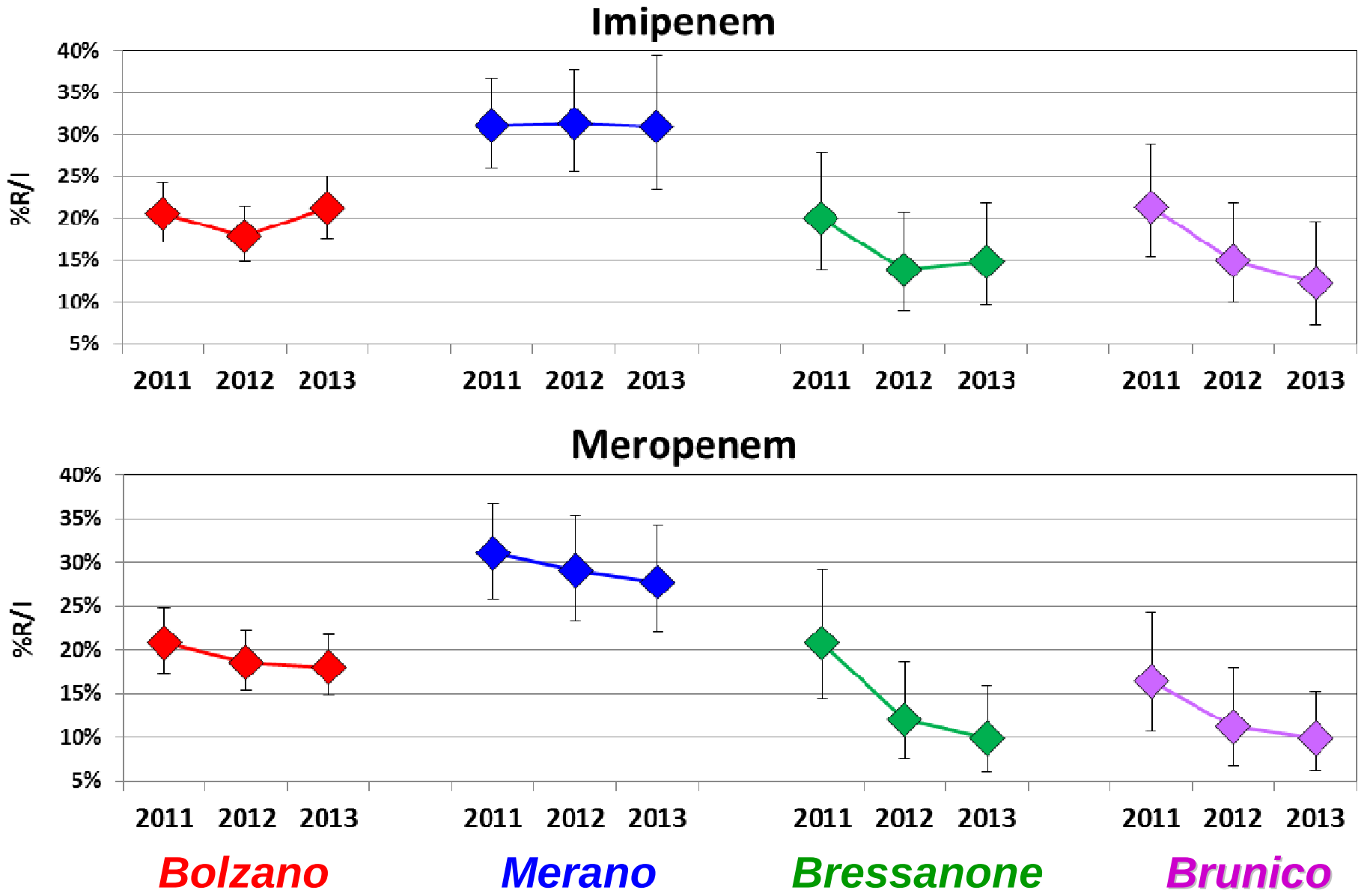




Italia: nessun trend significativo

EARS-Net (European Antimicrobial Resistance Surveillance Network) 2012: http://ecdc.europa.eu/

Provincia di Bolzano: R/I ai carbapenemici in *Pseudomonas aeruginosa, tutti i materiali*



Antibiogramma di un isolato di *Pseudomonas aeruginosa* con metallo-β-lattamasi (MBL)

	MIC (mg/L)	Interpretazione
Piperacillina/tazobactam	≥ 128	R
Aztreonam	8	I
Ceftazidime	≥ 64	R
Cefepime	≥ 64	R
Imipenem	≥ 256	R
Meropenem	≥ 64	R
Tobramicina	≥ 64	R
Gentamicina	≥ 64	R
Amikacina	≥ 128	R
Ciprofloxacina	≥ 16	R
Colistina	1	S

- ✓ Gen. resistenza a tutti i β-lattamici ecc. aztreonam
- ✓ Gen. MIC per i carbapenemici fortemente aumentate
- ✓ Multiresistenza
- ✓ **Provincia di Trento: nel 2013 32 isolati (13,1%); tutti VIM** (Dati: Dott. Lanzafame)
- ✓ **Provincia di Bolzano: 15 MBL dal 2009**, tipi VIM, IMP-13 (Tipizzazione: Prof. Cornaglia, Verona)

Conclusioni carbapenemasi

- ✓ ***Enterobatteri:*** **alta prevalenza di KPC in *K.pneumoniae* in Italia. In Trentino/Alto Adige endemia di basso livello, principalmente da VIM-1.**
- ✓ ***Acinetobacter baumannii*: in Italia alta prevalenza, in Trentino/Alto Adige isolati sporadici, con bassa prevalenza.**
- ✓ ***Pseudomonas aeruginosa*: alta prevalenza di isolati VIM+ in Provincia di Trento. Isolati sporadici in Provincia di Bolzano.**