Spectrum of activity and in vitro susceptibility patterns

(focus on CPE)

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Activity spectra

Substrate profiles in general

TABLE 4. Substrate and inhibition profiles of the carbapenemases

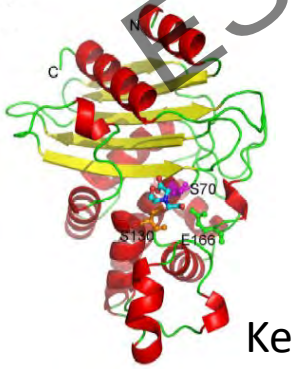
Molecular class	Functional group	Enzyme	Hydrolysis profile[a]					Inhibition profile[b]	
			Penicillins	Early cephalosporins	Extended-spectrum cephalosporins	Aztreonam	Carbapenems	EDTA	Clavulanic acid
A	2f	NMC	+	+	+	+	+	−	+
		IMI	+	+	+	+	+	−	+
		SME	+	+	±	+	+	−	+
		KPC	+	+	+	+	+	−	+
		GES	+	+	+	−	±	−	+
B1	3	IMP	+	+	+	−	+	+	−
		VIM	+	+	+	−	+	+	−
		GIM	+	+	+	−	+	+	−
		SPM	+	+	+	−	+	+	−
D	2d	OXA	+	+	±	−	±	−	±

Queenan & Bush 2007

Class A: KPCs

- **In general KPCs hydrolyse all clinically relevant β-lactams of broader use:**
  - penicillins (penicillinases)
  - 1st & 2nd generation cephalosporins
  - 3rd & 4th generation cephalosporins (ESCs)
  - monobactams
  - carbapenems (carbapenemases)
- **Are inhibited by:**
  - β-lactam inhibitors
  - avibactam
- **Details: weak hydrolysis of FOX & CAZ**

Tzouvelekis et al. 2012; Nordmann & Poirel 2014



Ke et al. 2007

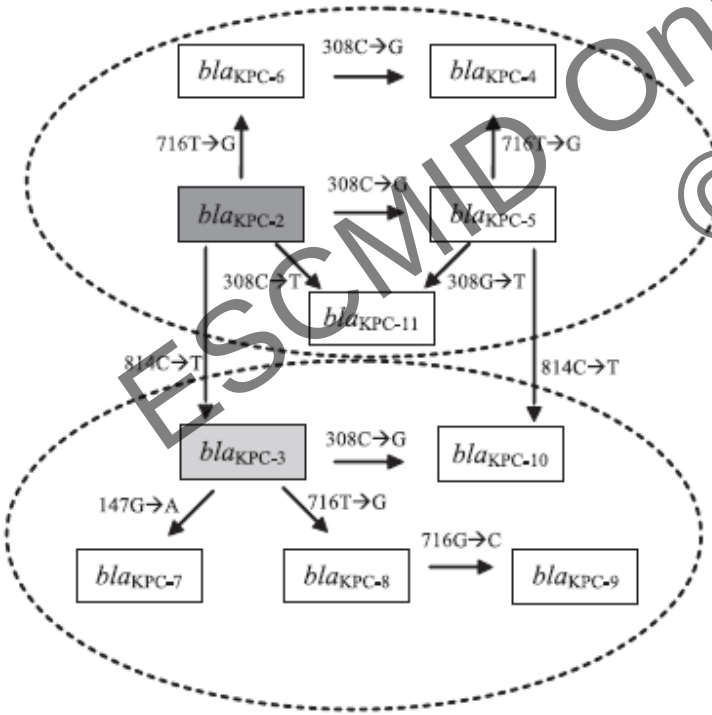
Yigit et al. 2001; Yigit et al. 2003

KPC-2: k_{cat}/K_m [$\mu M^{-1} s^{-1}$]	
Benzylpenicillin	1.4
Ampicillin	0.9
Piperacillin	1.3
Cephalothin	1.1
Cephaloridine	0.9
Cefoxitin	0.002
Cefotaxime	0.1
Ceftazidime	0.001
Aztreonam	0.06
Imipenem	0.3
Meropenem	0.3

KPCs

- **KPCs:**
- 23 variants (www.Lahey.org/studies)
- mainly **KPC-2** i **KPC-3**
- few (?) polymorphic positions
- unclear evolution

Chen et al. 2011



KPC variant (mutations)	k_{cat}/K_m [$\mu M^{-1} s^{-1}$]		
	AMP	IPM	CAZ
KPC-2	0.23	0.19	0.0008
KPC-5 (P104R)	0.22	0.12	0.009
KPC-11 (P104L)	0.1	0.22	0.002
KPC-6 (V240G)	0.46	0.16	0.004
KPC-3 (H274Y)	0.52	0.32	0.007
KPC-4 (P104R+V240G)	0.12	0.14	0.04
KPC-10 (P104R+H274Y)	0.3	0.15	0.06
KPC-9 (V240A+H274Y)	0.33	0.24	0.02
KPC-8 (V240G+H274Y)	0.18	0.22	0.03
KPC-7 (M49I+H274Y)	0.56	0.26	0.006

- **Substitutions P104R, V240G, H274Y:**
  - increase CAZ hydrolysis
  - especially in double combinations
- decrease thermal stability; still well-folded enzymes

Mehta et al. 2015

Class A: GESes

- **ESBL family** with 27 variants known to date (www.Lahey.org/studies)
- **Several carbapenemases: GES-2, -4, -5, -6, -11, -14, -18…** (CPE)
- **Position G170 in the Ω loop:**
  - G170N: GES-1 → GES-2
  - G170S: GES-3 → GES-4; also in GES-5 & -6
- Rather weak carbapenemase activity upon mutations
- Changes in activity against ESCs & cephamycins

Stewart et al. 2015

Catalytic efficiency, k_{cat}/K_m [$\mu M^{-1}\ s^{-1}$]

substrate	GES-1	GES-2	GES-3	GES-4
CTX	0.015	0.003	0.11	0.024
CAZ	0.188	NM	0.023	0.002
FOX	0.033	NH	NH	0.11
IPM	0.00007	0.009	NH	0.081

Poirel et al. 2001; Wachino et al. 2004; Vourli et al. 2004; Kotsakis et al. 2010

Class A: others

- **Like KPCs, 3 types with likely 'intrinsic' carbapenemase activity:**
  - **NMC-A / IMI:**
    - 12 variants
  - **SME:**
    - 5 variants
  - **SFC-1**

k_{cat}/K_m [$\mu M^{-1} s^{-1}$]				
	KPC-2	IMI-1	SME-3	SFC-1
Benzylpenicillin	1.4	0.6	2.9	nd
Ampicillin	0.9	0.2	1.3	0.9
Cephalothin	1.1	0.9	nd	2.2
Cephaloridine	0.9	1.9	3.41	nd
Cefoxitin	0.002	0.007	0.0005	0.05
Cefotaxime	0.1	0.02	0.01	0.09
Ceftazidime	0.001	0.00002	0.0008	0.04
Aztreonam	0.06	0.6	0.2	0.3
Imipenem	0.3	0.5	2.1	0.7
Meropenem	0.3	0.4	0.6	0.3

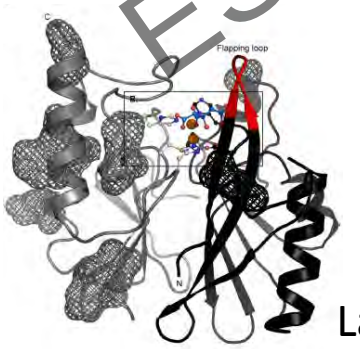
Sougakoff et al. 2002

Naas et al. 1994; Mariotte-Boyer et al. 1996; Rasmussen et al. 1996; Majiduddin & Palzkill 2003; Queenan et al. 2006; Fonseca et al. 2007; www.Lahey.org/studies

Class B: MBLs

- **MBLs hydrolyse most of clinically relevant β-lactams:**
  - penicillins
  - 1st & 2nd generation cephalosporins
  - ESCs
  - carbapenems (carbapenemases)
- **Spare monobactams**
- **Are inhibited by chelators**
  - **EDTA**
  - thiols (MPA, SMA)
  - rhodanine-derived thioenolates
- **Are not inhibited by β-lactam inhibitors**

- **MBL types in CPE:**
- **IMPs**
- VIMs, mainly **VIM-1 types**
- **NDMs**
- rare: GIMs, KHM



Lassaux et al. 2011

Walsh et al. 2005; Tzouvelekis et al. 2012; Nordmann & Poirel 2014; Brem et al. 2014; Wendel & MacKenzie 2015

MBLs

• VIM-1 types evolve modifying activity against carbapenems and other β-lactams (penicillins, cephalosporins)

• Few positions:
- T33A
- N215K
- H224L
- S228R

	k_{cat}/K_m [$\mu M^{-1} s^{-1}$]					
β-Lactam	IMP-1	VIM-2	VIM-1	VIM-4	VIM-19	VIM-26
Benzylpenicillin	0.6	4.0	0.04	3.1	5.0	40.0
Ampicillin	nd	1.4	0.04	0.07	nd	21.0
Cephalothin	2.4	11.8	5.1	36.0	nd	nd
Cefoxitin	2.0	1.2	0.2	nd	0.5	0.01
Cefotaxime	0.4	5.8	0.7	nd	30.0	nd
Ceftazidime	0.2	0.05	0.08	nd	0.02	0.03
Aztreonam	-	-	-	-	-	-
Imipenem	1.2	3.8	1.3	23.0	6.0	0.3
Meropenem	0.1	2.5	0.3	0.9	2.0	0.2

Laraki et al. 1999; Docquier et al. 2003; Franceschini et al. 2000; Lassaux et al. 2011; Rodriguez-Fernandez et al. 2010; Leiros et al. 2015

MBLs

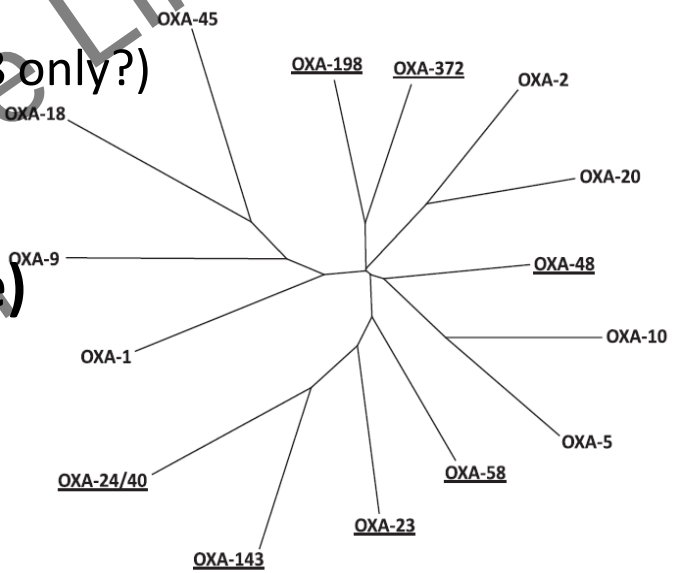
- **The so far NDM evolution does not seem to cause major differences in carbapenem-hydrolysing activity**
  - differences in thermal stability
  - more stable variants with higher MICs; biological relevance unclear

k_{cat}/K_m [$\mu M^{-1} s^{-1}$]							
β-Lactam	**NDM-1**	**NDM-3** D95N	**NDM-4** M154L	**NDM-5** M154L V88L	**NDM-6** A233V	**NDM-7** M154L D130N	**NDM-8** M154L D130G
Ampicillin	**4.1**	**2.8**	**2.9**	**2.6**	**3.0**	**2.3**	**1.2**
Cephalothin	**2.6**	**4.6**	**4.1**	**1.6**	**6.1**	**1.5**	**1.9**
Cefoxitin	**0.2**	**0.1**	**0.2**	**0.1**	**0.2**	**0.2**	**0.2**
Ceftazidime	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.3**
Imipenem	**7.6**	**9.2**	**4.1**	**2.2**	**5.8**	**2.3**	**2.9**
Meropenem	**5.2**	**4.2**	**4.9**	**4.1**	**7.4**	**4.3**	**2.6**
Doripenem	**1.9**	**2.0**	**6.0**	**4.5**	**8.6**	**5.4**	**1.7**

Makena et al. 2015

Class D: CHDLs

- **CHDLs hydrolyse:**
  - penicillins (oxacillinases), incl. temocillin (OXA-48 only?)
  - 1st generation cephalosporins
  - carbapenems (carbapenemases)
- **Low carbapenemase activity (imipenemase)**
- **ESCs: poor (CTX) or no activity (CAZ, FEP)**
- Are inhibited by NaCl
- **Poorly inhibited by β-lactam inhibitors**
  - avibactam?



Antonelli et al. 2015

Docquier et al. 2009

- **CHDL types in CPE:**
  - **OXA-48 types**
  - OXA-372
  - very rare if at all: OXA-23, -40, -51, -58

Poirel et al. 2010; Poirel et al. 2012; Łęski et al. 2013; Antonelli et al. 2015; Docquier & Mangani 2015; Lahiri et al. 2015

OXA-48 types & -372

- **A number of enzymes differing by 1-5 amino-acids from OXA-48**
  - similar activity profiles
- Some others (-163, -247, -405):
  - ESC & ATM-hydrolysing activity; in practice no carbapenemase activity
  - partial inhibition by CLA & TZB; lower temocillin-hydrolysing activity

	k_{cat}/K_m [$\mu M^{-1} s^{-1}$]						
β-Lactam	**OXA-48**	**OXA-162**	**OXA-163**	**OXA-181**	**OXA-204**	**OXA-232**	**OXA-372**
Ampicillin	2.4	0.8	0.07	1.3	0.9	0.6	1.0
Oxacillin	1.4	0.04	0.4	1.1	0.5	1.2	1.2
Temocillin	0.006	0.004	nd	0.005	0.007	0.0005	0.09
Cephalothin	0.2	0.07	0.3	0.05	0.05	0.1	0.003
Cefotaxime	0.01	0.01	0.2	0.01	0.01	0.06	nh
Ceftazidime	nh	nh	0.003	nh	nh	0.0001	nh
Imipenem	0.4	0.4	0.00006	0.6	0.4	0.02	0.2
Meropenem	0.006	0.001	0.00003	0.002	0.0008	0.0003	0.5

Oueslati et al. 2015; Docquier et al. 2009; Gomez et al. 2013; Dortet et al. 2015; Antonelli et al. 2015

In vitro susceptibility patterns = resistance phenotypes

CPE resistance phenotypes

- **Two major issues:**
  - **resistance profiles:**
    - should reflect activity spectra but if no interfering mechanisms
  - **resistance levels:**
    - mainly to carbapenems
- **Early works on CPE:**
  - **carbapenem resistance levels vary largely**
  - **often low or very low level (S)**
  - **interfering mechanisms (*e. g.* ESBLs)**
    - Taiwan 1999-2000: *K. pneumoniae* IMP-8

***K. pneumoniae* IMP-8 (n=17)**	
IPM MIC	**0.25 – >256** (0.5 – 1)
IPM MIC_{50}	**1**
IPM MIC_{90}	**4**
MEM MIC	**0.25 – 16** (0.25 – 0.5)
MEM MIC_{50}	**0.5**
MEM MIC_{90}	**1**
ATM MIC	**0.25 – 2**
+SHV-12 ATM MIC	**64 – >256**

Yan et al. 2001

CPE resistance phenotypes

TABLE I. Resistance phenotypes resulting from the expression of the main carbapenemases reported in *Enterobacteriaceae* without or with extended-spectrum β-lactamases (ESBLs)

	AMX	AMC	TZP	CTX	CAZ	IMP	ETP	MER	ATM
KPC	R	S/I	R	R	R	S/I/R	I/R	S/I/R	R
KPC + ESBL	R	I/R	R	R	R	I/R	I/R	I/R	R
IMP/VIM/NDM	R	R	I/R	R	I/R	S/I/R	I/R	S/I/R	S
IMP/VIM/NDM + ESBL	R	R	I/R	R	R	I/R	R	S/I/R	R
OXA-48/OXA-181	R	R	S/I/R	S/I	S	S/I	S/I	S/I	S
OXA-48/OXA-181 + ESBL	R	R	I/R	R	R	I/R	I/R	I/R	R

AMX, amoxycillin; AMC, amoxycillin–clavulanic acid; TZP, piperacillin–tazobactam; CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; ETP, ertapenem; MER, meropenem; ATM, aztreonam.

Nordmann et al. 2012

TABLE 2. Breakpoint values for carbapenems according to the US (CLSI) and European (EUCAST) guidelines, as updated June 2010 (MIC values, mg/L)

	CLSI		EUCAST	
	S (≤)	R (≥)	S (≤)	R (>)
Imipenem	1	4	2	8
Meropenem	1	4	2	8
Ertapenem	0.5	2	0.5	1
Doripenem	1	4	2	8

TABLE 3. Range of MICs of carbapenems for clinical *Enterobacteriaceae* expressing the main carbapenemases

	MIC (mg/L)		
	Imipenem	Meropenem	Ertapenem
KPC	0.5 to >32	0.5 to >32	0.5 to >32
IMP/VIM/NDM	0.5 to >32	0.5 to >64	0.38 to >32
OXA-48/OXA-181	0.25 to 64	0.38 to 64	0.38 to >32

Presence and expression of a carbapenemase gene alone is not sufficient for conferring 'pharmacological' resistance:

- even with the 'new' breakpoints
- 'biological' resistance
- clinical isolates
- transconjugants & transformants

KPCs

Recombinant strains demonstrate β-lactamase-associated phenotypes:

- natural plasmids vs. expression vectors
  - plasmid copy number
  - promoter strength
  - additional AMR genes in natural plasmids

TABLE 1. Susceptibilities of the *K. pneumoniae* isolates and of the *E. coli* transconjugants obtained in the study

Isolate(s)	MIC[a] (μg/ml) of:														
	AMP	AMC	PIP	TZP	CAZ	CTX	FEP	IPM	MEM	AMK	GEN	CIP	TET	TGC	CST
Clinical isolates	>256	>256	>256	>256	>256	128	>32	2–3	3–4	12–16	1–1.5	>32	32–48	3	0.38–0.75
KPC-2/TEM-1-producing transconjugant 2641/08	>256	24	96	48	3	2	1.5	3	0.75	1.5	0.25	0.023	2	0.38	0.25
SHV-12-producing transconjugants	>256	4–6	32–48	1–1.5	16	1.5–2	0.5	0.25	0.047	1–1.5	0.19–0.25	0.016	1.5–2	0.38–0.5	0.19–0.25
E. coli A15	3	4	1.5	1	0.25	0.047	0.047	0.25	0.032	1	0.19	0.023	2	0.38	0.25

- natural plasmid IncFII$_K$ pKpQIL-like
- Tn*4401*a variant with *bla*$_{KPC-2}$
- *bla*$_{TEM-1}$

Baraniak et al. 2009

KPCs

Does resistance level depend on a KPC variant?

E. coli RB791 transformants with bla_{NDM} genes in the pTP123 vector:

Table 2. Minimum inhibitory concentrations (MIC's) of antibiotics for KPC variants.

		MIC (µg / mL)			
		AMP	CAZ	IMI	MERO
pTP123-*empty*		16	0.125	0.38	0.064
pTP123-bla_{KPC-2}	(KPC-2)	128	0.38	1	0.38
P104R	(KPC-5)	64	2.0	0.75	0.25
P104L	(KPC-11)	64	0.5	0.75	0.25
V240G	(KPC-6)	128	1.5	1.0	0.25
H274Y	(KPC-3)	64	1.5	1	0.25
P104R:V240G	(KPC-4)	64	12	1	0.25
P104R:H274Y	(KPC-10)	32	16	1	0.125
V240A:H274Y	(KPC-9)	128	4	1	0.19
V240G:H274Y	(KPC-8)	128	32	2	0.25
M49I:H274Y	(KPC-7)	64	1.5	1	0.25

Consistent with the kinetic data:

- natural KPC variants confer significant differences in CAZ-R levels
- but not in carba-R levels

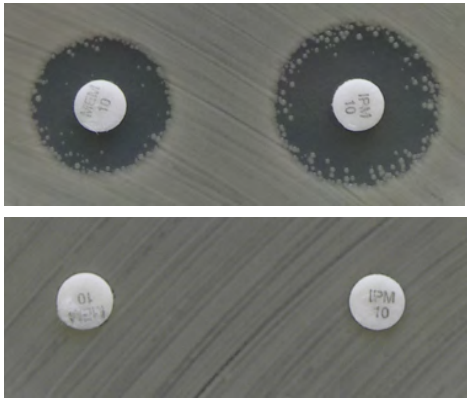
Mehta et al. 2015

KPCs

β-lactam	*Kpn* ST258 KPC-2 TEM-1	*Kpn* ST258 KPC-2 TEM-1	*Kpn* ST258 KPC-2 SHV-12 TEM-1	*Kpn* ST258 KPC-2 CTX-M-3 TEM-1	*Kpn* ST23 KPC-2 TEM-1
AMX / AMC	>256	>256	>256	>256	>256
PIP / TZP	>256	>256	>256	>256	>256
CAZ	24	32	>256	64	8
ATM	>256	>256	>256	>256	64
IPM	1*	>32	1	>32	1*
MEM	2*	>32	2	>32	2*
ETP	6*	>32	4	>32	2*
AMK	8	12	48	>256	2
GEN	2	1	2	>256	0.25
CIP	>32	>32	>32	>32	0.03
TGC	1.5	1	1	0.5	1
CST	0.38	0.38	0.25	0.19	0.38

Issues:

- all β-lactams
- no inhibitor effect
- variability:
  - carba-R level
  - CAZ
- * heteroresistance
- other compounds:
  - MDR / XDR
- 'ST258 phenotype'



Baraniak et al. 2011

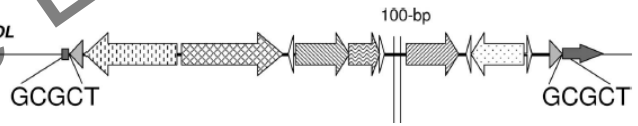
KPCs

Factors increasing the carba-R level:

- transcription efficiency:
  - Tn*4401* microheterogeneity – 'promoter-up deletions'
- gene dosage
- genetic background
  - porin deficiencies (OmpK36)
  - other β-lactamases (incl. carbapenemases)
  - clone-related factors etc. ?

Kitchel et al. 2010

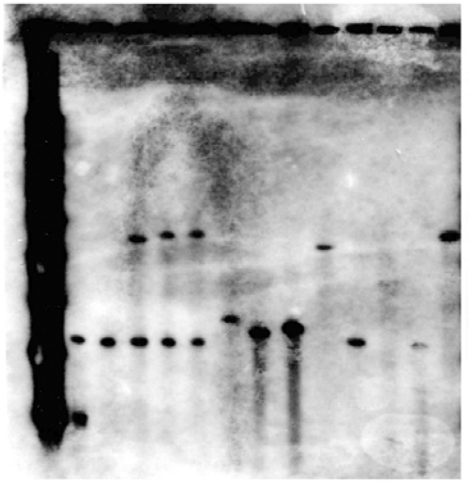
Naas et al. 2008



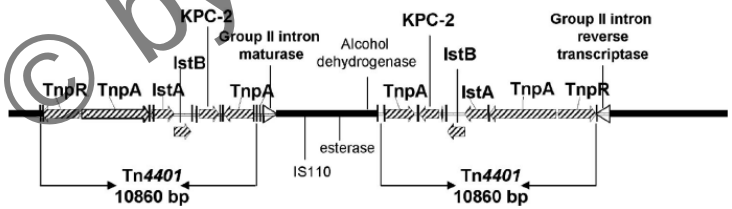
Tn*4401* variants:
- Tn*4401*a-g

Chmelnitsky et al. 2014

Baraniak et al. 2011



Gootz et al. 2009



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Genetic Factors Associated with Elevated Carbapenem Resistance in KPC-Producing *Klebsiella pneumoniae*[▽]

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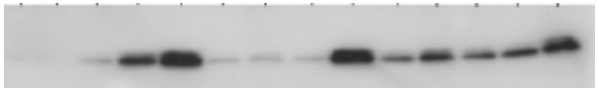


Double Tn*4401* copies
- in-plasmid
- different plasmids

GESes

MICs [mg/L]

β-lactam	*Eco* rec (GES-1)	***Eco* rec (GES-2)**	***Pae* R⁺ (GES-2)**	*Eco* rec (GES-3)	***Eco* rec (GES-4)**	*Kpn* cl.is. (GES-3)	***Kpn* cl.is. (GES-4)**
amoxicillin	>512	**>512**	**>512**	>128	**>128**	>128	**>128**
amoxi-clav	>128	**16**	**>512**	32	**>128**	>128	**>128**
cephalothin	256	**32**	**>512**				
cefoxitin	8	**4**	**>512**	8	**>128**	128	**>128**
cefotaxime	4	**1**	**128**	2	**1**	64	**16**
ceftazidime	128	**8**	**16**	128	**64**	>1,024	**1,024**
ceftazidime-clav	8	**0.5**	**16**	0.25	**8**	256	**512**
aztreonam	1	**0.5**	**8**	4	**2**	64	**32**
imipenem	0.06	**0.25**	**16**	0.13	**0.25**	0.25	**8**
meropenem	0.06	**0.06**	**2**	0.015	**0.25**	0.5	**8**

Low-level carbapenem resistance, consistent with the kinetic data

Poirel et al. 2001; Wachino et al. 2004

MBLs

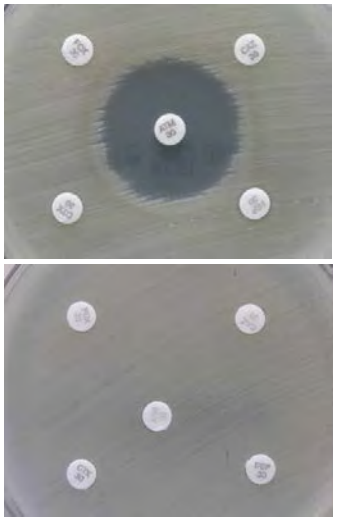
TABLE 1. Characteristics of 17 *bla*$_{VIM-1}$-containing *K. pneumoniae* clinical isolates and 6 *E. coli* transconjugants

Isolate[a]	Hospital	Isolation date (day-mo-yr)	PFGE type	Etest MICs (μg/ml) of β-lactams[b]								Other resistance markers[c]
				PIP	TZP	CTX	CAZ	ATM	IPM	IPM-E	MEM	
Kp1	I	17-09-02	A	>256	256	128	256	256	32	<1	32	Gm, Net, Tb, An, Sxt, C
Ec(trc-1)				>256	128	128	>256	2	1	<1	0.25	Gm, Net, Tb, An, Sxt, C
Kp2	I	18-10-02	A	>256	256	>256	>256	>256	8	<1	4	Gm, Net, Tb, An, Sxt, C
Ec(trc-2)				128	128	128	32	2	0.5	<1	0.12	Gm, Net, Tb, An, Sxt, C
Kp3	I	06-11-02	A	128	128	128	256	128	16	<1	16	Gm, Net, Tb, An, Sxt, C
Kp4	I	10-11-02	A	>256	256	64	128	64	32	<1	32	Gm, Net, Tb, An, Sxt, C
Kp5	I	17-11-02	B	>256	256	64	>256	>256	4	<1	1	Gm, Net, Tb, An, Sxt
Kp6	I	12-12-02	A	256	128	128	256	128	8	<1	4	Gm, Net, Tb, An, Sxt, C
Kp7	II	10-10-02	C	>256	>256	256	256	0.12	32	<1	16	Net, Tb, An, Sxt
Kp8	II	11-10-02	C	>256	>256	128	256	0.25	32	<1	>32	Net, Tb, An, Sxt
Ec(trc-8)				>256	256	64	>256	0.12	4	<1	0.5	Net, Tb, An, Sxt
Kp9	II	22-10-02	C	>256	256	64	>256	0.5	16	<1	8	Net, Tb, An, Sxt
Kp10	II	16-11-02	C	>256	256	64	256	0.5	32	<1	8	Net, Tb, An, Sxt
Kp11	II	27-11-02	C1	>256	>256	256	>256	0.12	4	<1	1	Net, Tb, An, Sxt
Ec(trc-11)				>256	256	32	>256	0.06	2	<1	0.25	Net, Tb, An, Sxt

Giakkoupi et al. 2003

Issues (1):

- resistance to all β-lactams and inhibitor combinations except for ATM
- qualitative differences: ATM-R due to ESBLs, AmpCs, KPCs …
- quantitative differences: large variations in carba-R levels
    - recombinants usually with low-level resistance



Walsh et al. 2005; Tzouvelekis et al. 2012; Nordmann & Poirel 2014

MBLs

TABLE 1. Characteristics of 17 *bla*$_{VIM-1}$-containing *K. pneumoniae* clinical isolates and 6 *E. coli* transconjugants

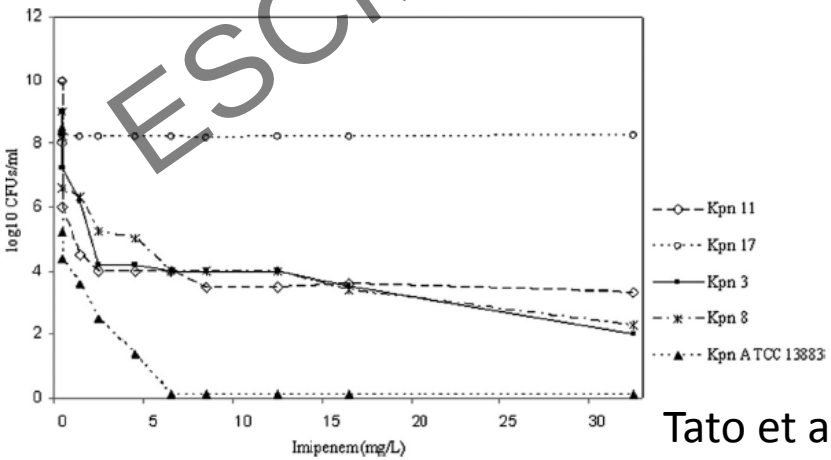
Isolate[a]	Hospital	Isolation date (day-mo-yr)	PFGE type	Etest MICs (μg/ml) of β-lactams[b]								Other resistance markers[c]
				PIP	TZP	CTX	CAZ	ATM	IPM	IPM-E	MEM	
Kp1	I	17-09-02	A	>256	256	128	256	256	32	<1	32	Gm, Net, Tb, An, Sxt, C
Ec(trc-1)				>256	128	128	>256	2	1	<1	0.25	Gm, Net, Tb, An, Sxt, C

Giakkoupi et al. 2003

TABLE 1 Susceptibilities of the *E. coli* 5428/11 isolate producing NDM-1, CTX-M-15, TEM-1, and OXA-1 and its *E. coli* A15 Rif^r transconjugant producing NDM-1 and TEM-1

Isolate	MIC (μg/ml)[a]															
	AMX	AMC	PIP	TZP	CAZ	CTX	FEP	ATM	ERT	IPM	MEM	AMK	GEN	CIP	TET	TGC	CST
E. coli 5428/11	>256	>256	>256	>256	>256	>256	32	32	>32[b]	>32[b]	>32[b]	>256	>256	>32	>256	0.19	0.38
R$^+$ [5428/11][c]	>256	>256	>256	24	>256	32	6	0.064	2	2	4	>256	>256	0.016	2	0.19	0.38

Fiett et al. 2014



Tato et al. 2010

Issues (2):

- no specific differences between MBL types
- heteroresistance:
- scattered colonies / population analysis profiles
- possibly due to expression variations
- almost always MDR / XDR (like ST258 KPC):
- IMPs & VIMs encoded by integrons

MBLs

VIM variants confer varying carba-R levels, consistent with kinetics

E. coli TOP10 transformants with *bla*$_{VIM}$ genes cloned into the pCRBluntII TOPO vector

	MIC [mg/L]				
β-Lactam	VIM-1	VIM-4	VIM-19	VIM-1	VIM-26
AMP / AMX	>512	>512	>512	>256	>256
AMC	>512	>512	>512		
PIP	256	256	256	32	16
TZP	256	256	256	128	32
FOX	256	256	256	32	8
CTX	32	64	64	32	32
CAZ	512	32	32	>256	64
ATM	0.12	0.12	0.12		
IPM	1	4	8	2	4
MEM	0.25	0.5	1	0.25	0.125

Rodriguez-Fernandez et al. 2010; Leiros et al. 2015

MBLs

NDM variants confer varying carba-R levels:

- despite no significant differences in kinetics
- variants with more thermal stability (NDM-5, -7) produce higher MICs

MICs [mg/L]										
	NDM-1	NDM-2	NDM-4	NDM-5	NDM-7	NDM-1 IS*Aba125*	NDM-2 IS*Aba125*	NDM-4 IS*Aba125*	NDM-5 IS*Aba125*	NDM-7 IS*Aba125*
AMP	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
LOT	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
FOX	24	>256	>256	>256	>256	>256	>256	>256	>256	>256
CAZ	>256	>256	>256	>256	32	>256	>256	>256	>256	>256
IPM	0.38	0.38	0.25	0.25	2	8	8	>32	>32	>32
MEM	0.38	0.38	0.38	0.38	1	4	4	8	>32	>32
ETP	0.38	2	2	2	2	8	16	16	>32	>32

E. coli TOP10 transformants with bla_{NDM} genes in the pCRBluntII TOPO vector:
- only CDSes and entire genes with IS*Aba125* promoters

Makena et al. 2015

MBLs

Factors increasing the carba-R level:

Yan et al. 2001; Giakkoupi et al. 2003; Loli et al. 2006

- structural mutations in MBL genes
- transcription efficiency:
  - bla_{IMP} and bla_{VIM} genes within integronic arrays; IS*Aba125* promoter for bla_{NDM}s
  - DNA rearrangements at the 5' end of bla_{VIM-1}-like genes (???)
- gene dosage:
  - duplications of bla_{VIM-1}-like genes
- genetic background
  - porin deficiencies (OmpK36)
  - other β-lactamases
  - clone-related factors etc. ?

Most of MBL producers are high-level resistant:
- VIM producers in Greece
- NDM producers in the UK

Jain et al. 2014

Table 2. MIC distributions for Enterobacteriaceae[a] (n=306) and non-fermenters[b] (n=20) producing NDM carbapenemase

Antibiotic (range tested, mg/L)	Isolates	BSAC breakpoint(s)[13] ≤S/>R	Number of isolates with MIC (mg/L) ≤0.125	0.25	0.5	1	2	4	8	16	32	64	≥128	NA	% S
Ertapenem (0.125–16)	Enterobacteriaceae	≤0.5/>1					1	1	2	10	290[c]			2	0
	non-fermenters	NT													NT
Imipenem (0.06–128)	Enterobacteriaceae	≤2/>8					3	5	22	64	118	66	27	1	1
	non-fermenters	Ac≤2/>8 Ps≤4/>8									1	4	14	1	0
Meropenem (0.06–32)	Enterobacteriaceae	≤2/>8					1	5	9	35	92	164[c]			0.3
	non-fermenters	≤2/>8								1		18[c]		1	0
Amikacin (0.5–64)	Enterobacteriaceae	≤8/>16			1	19	17	19	10	15	6	1	218		22
	non-fermenters	≤8/>16				1	1	1			1	3	12	1	15
Gentamicin (0.125–32)	Enterobacteriaceae	≤2/>4		1	15	14	2		2	1	9	262[c]			10
	non-fermenters	≤4/>4	1		1				1	1	1	14[c]		1	10
Tobramycin (0.125–32)	Enterobacteriaceae	≤2/>4			10	8	3	2	9	19	17	238[c]			7
	non-fermenters	Ac≤4/>4[d] Ps≤4/>4		1				1	2	4	1	10[c]		1	10
Ciprofloxacin (0.125–8)	Enterobacteriaceae	≤0.5/>1	18	14	5	4	8	7	7	243[c]					12
	non-fermenters	Ac≤1/>1 Ps≤0.5/>1	1	1	1				1	15[c]				1	15
Colistin (0.5–32)	Enterobacteriaceae	≤2/>2			171[e]	98	7	1	7	4	3	7[c]		8	90
	non-fermenters	Ac≤2/>2 Ps≤4/>4			11[e]	7	1							1	95
Tigecycline (0.25–16)	Enterobacteriaceae	≤1/>2		49[e]	74	61	68	34	10	3				7	60
	non-fermenters	Ac≤0.25/>0.5[d,f]		3[e]	2	2	3	3	1					6	15

OXA-48 types

Some OXA-48 clinical isolates and often their *E. coli* recombinants show the enzyme-associated β-lactam resistance phenotype:

- resistance to penicillins & penicillin-inhibitor combinations
- increased carbapenem MICs but:
  - low-level or no resistance

	MICs [mg/L]		
	E. co J53	*K. pn* BEL OXA-48	*E. co* J53 OXA-48
AMX	4	>256	>256
AMC	4	>256	>256
PIP	1	>256	128
TZP	1	>256	128
CAZ	0.75	1	0.75
CTX	0.12	0.12	0.12
FEP	0.12	0.12	0.12
ATM	0.06	0.06	0.06
IPM	0.75	0.75	0.75
MEM	0.19	0.5	0.5
ETP	0.25	4	2

Carrër et al. 2010

OXA-48 types

Consistent with the activity data:

OXA-48-like CHDLs confer similar resistance phenotypes

OXA-163 behaves like an ES-oxacillinase

Queslati et al. 2015; Antonelli et al. 2015

MICs [mg/L]									
	E.co TOP10	OXA-48	OXA-162	OXA-163	OXA-181	OXA-204	OXA-232	*E.co* DH5α	OXA-372
AMP	8	>256	>256	>256	>256	>256	>256	4	128
TEM	8	>256	>256	32	>256	256	32	8	128
LOT	8	16	32	64	32	16	64	8	8
CTX	0.06	0.25	0.5	16	1	0.25	0.12	0.06	0.06
CAZ	0.12	0.25	0.5	64	0.5	0.5	1	0.125	0.25
IPM	0.25	2	2	0.5	4	2	1	0.125	2
MEM	0.06	0.25	0.5	0.06	0.5	0.25	0.25	≤0.015	0.06
ETP	0.01	0.5	0.5	0.06	1	0.5	0.5	≤0.015	0.03

E. coli TOP10 or DH5α transformants with bla_{OXA} genes in pCRBluntII TOPO or pLBII

OXA-48 types

OXA-48 clinical isolates much vary in resistance phenotypes:

- even having the same Tn*1999*-like element and plasmid type (IncL pOXA-48-like)
- **ESC-R owing to frequent presence of ESBLs** (and/or other β-lactamases)
- **high-level carba-R**
- **MDR / XDR**

ESBL-negative; low carba-R | ESBL-positive; low carba-R | ESBL-positive; high carba-R

Poirel et al. 2012

OXA-48 types

	MICs [mg/L]							
	K. pn BEL OXA-48	*K. pn* LEB OXA-48	*K. pn* EGY OXA-48 CTX-M-15	*K. pn* TUR OXA-48 CTX-M-15 SHV-5	*P. mi* TUR OXA-48 TEM-101	*E. cl* TUR OXA-48 SHV-5	*C. fr* TUR OXA-48 VEB-1	*E. co* TUR OXA-48 TEM-150
AMX	>256	>256	>512	>512	>256	>512	>512	>256
AMC	>256	>256	>512	>512	>256	>512	>512	>256
PIP	>256	96	>512	>512	>256	>512	>512	>256
TZP	>256	96	>512	>512	>256	>512	>512	>256
CAZ	1	0.75	512	512	512	>512	512	512
CTX	0.12	1.5	64	64	64	>512	64	64
ATM	0.06	0.06	512	512	512	>512	512	512
IPM	0.75	>16	2	>32	>32	0.5	>32	24
MEM	0.5	>16	2	>32	>32	0.5	>32	24
ETP	4	>16	3	>32	>32	0.5	>32	>32

Carrër et al. 2010

OXA-48 types

The role of porins:

- in CHDLs large effect on carba MICs
- LOT & CTX

MICs [mg/L]								
	E.co TOP10	*E.co* HB4	OXA-48	OXA-162	OXA-163	OXA-181	OXA-204	OXA-232
AMP	8	8	>256	>256	>256	>256	>256	>256
TEM	8	32	>256	>256	64 (32)	>256	>256 (256)	>256 (32)
LOT	8	64	>256 (16)	>256 (32)	>256 (64)	>256 (32)	256 (16)	>256 (64)
CTX	0.06	0.5	8 (0.25)	8 (0.5)	128 (16)	8 (1)	4 (0.25)	2 (0.12)
CAZ	0.12	0.5	1 (0.25)	1 (0.5)	256 (64)	0.5 (0.5)	0.5 (0.5)	0.5 (1)
IPM	0.25	0.25	64 (2)	128 (2)	0.5 (0.5)	128 (4)	64 (2)	16 (1)
MEM	0.06	0.12	64 (0.25)	128 (0.5)	4 (0.06)	128 (0.5)	64 (0.25)	32 (0.25)
ETP	0.01	1	256 (0.5)	>256 (0.5)	32 (0.06)	>256 (1)	256 (0.5)	256 (0.5)

E. coli HB4 transformants with bla_{OXA} genes in pCRBluntII TOPO

Oueslati et al. 2015

CPE resistance phenotypes

- **Complex interplay of multiple factors:**
  - carbapenemase type general activity
  - carbapenemase variant specific activity (in some cases)
  - carbapenemase expression level
    - transcription efficiency
    - gene dosage
  - genetic background
    - other β-lactamases
    - cell permeability
- **Large quantitative and qualitative variations**
- **Continuous evolution:**
  - high-level resistance (more & more)
  - MDR / XDR phenotypes