OLGU SUNUMU
ANTİBİYOTİK DİRENCİNDE YENİ FENOTİPLER

(ENTEROBACTERIACAEA'DE KARBAPENEMAZLAR)

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İSTANBUL ÜNİVERSİTESİ
Istanbul Tıp Fakültesi
Klinik Mikrobiyoloji AD

OLGU

- 60 yaş, kadın hasta
- Ateş, üşüme titreme , yan ağrısı ve idrar yaparken yanma, sık idrara çıkma şikayetleri ile hastanemizin ilk yardım servisine başvuruyor.
- 6 hafta önce geçirilmiş üriner enfeksiyon nedeniyle başka bir hastanede yattığı ve Seftriakson (2x1 gr) IV tedavisi gördüğü biliniyor.
- Ancak hangi etkenin idrar kültüründen izole edildiği bilinmiyor
- Hastanın bu tedavinin ardından düzelerek taburcu edildiği ve aradan geçen 6 hafta boyunca herhangi bir yakınmasının olmadığı ifade ediliyor
- Hasta tekrar aynı şikayetler ile hastanemize bavuruda bulunuyor

- Hastanın fiziki muayenesi sonucuna göre
  (39.1 °C ateş, Nabız 120 dak/ritmik, AKB:100/60 mm/Hg, , Beyaz küre:17.500/ mm^3, CRP:82, Sedimentasyon78/saat,
  TİT: mikroskopik alanda silme lökosit, küme lökositler)

- Ürosepsis tanısı ile Üroloji servisine yatırılıyor.

- Kan ve İdrar kültürleri alınıyor.
- Rasyonel tedavisi, kültür sonuçlarına göre düzenlenecek şekilde ampirik olarak 4x500 mg/gün imipenem/silastatin IV dozda başlanıyor
- Tedaviden 48 saat geçmesine rağmen kontrol altına alınamayan ateş yüksekliği ve 22.000/ mm^3 e yükselen beyaz küre sayısı nedeniyle tedavisine siprofloksasin 2x400 mg IV ekleniyor.
- Kan kültüründe üreme yok
- İdrar kültüründen *Enterobacter cloacae* izole ediliyor

E.cloacae

Antibiyotik	Duyarlılık
Sefotaksim/Seftazidim	**R**
Sefepim	**R**
Piperasilin/Tazobaktam	**R**
Sefoperazon/Sulbaktam	**R**
Gentamisin	**R**
Amikasin	**R**
Ertapenem/İmipenem/Meropenem	**R**
Siprofloksasin	**R**
Tigesiklin	**S**
Kolistin	**S**

Direnç mekanizması?

- AmpC-plazmidik veya kromozomal?
- GSBL ve AmpC'nin kombinasyonu?
- KPC?
- MBL (VIM veya IMP)?
- NDM-1?
- OXA-48?
- Belirsiz?

MYSTIC ÇALIŞMASI (2004)
İMİPENEM DUYARLILIĞINA KÜRESEL BAKIŞ

The Global View: Imipenem % Susceptibility

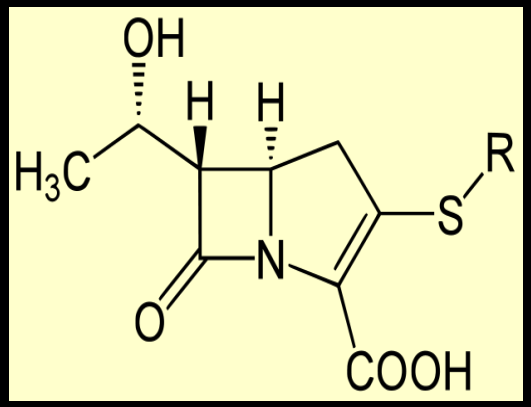
	Enterobacteriacae	*Pseudomonas*	*Acinetobacter*
USA	≥99	85	92
Europe	≥97	79	83
Japan	≥98	52	95
South America	≥98	60	73

MYSTIC (Meropenem Yearly Susceptibility Test Information Collection)

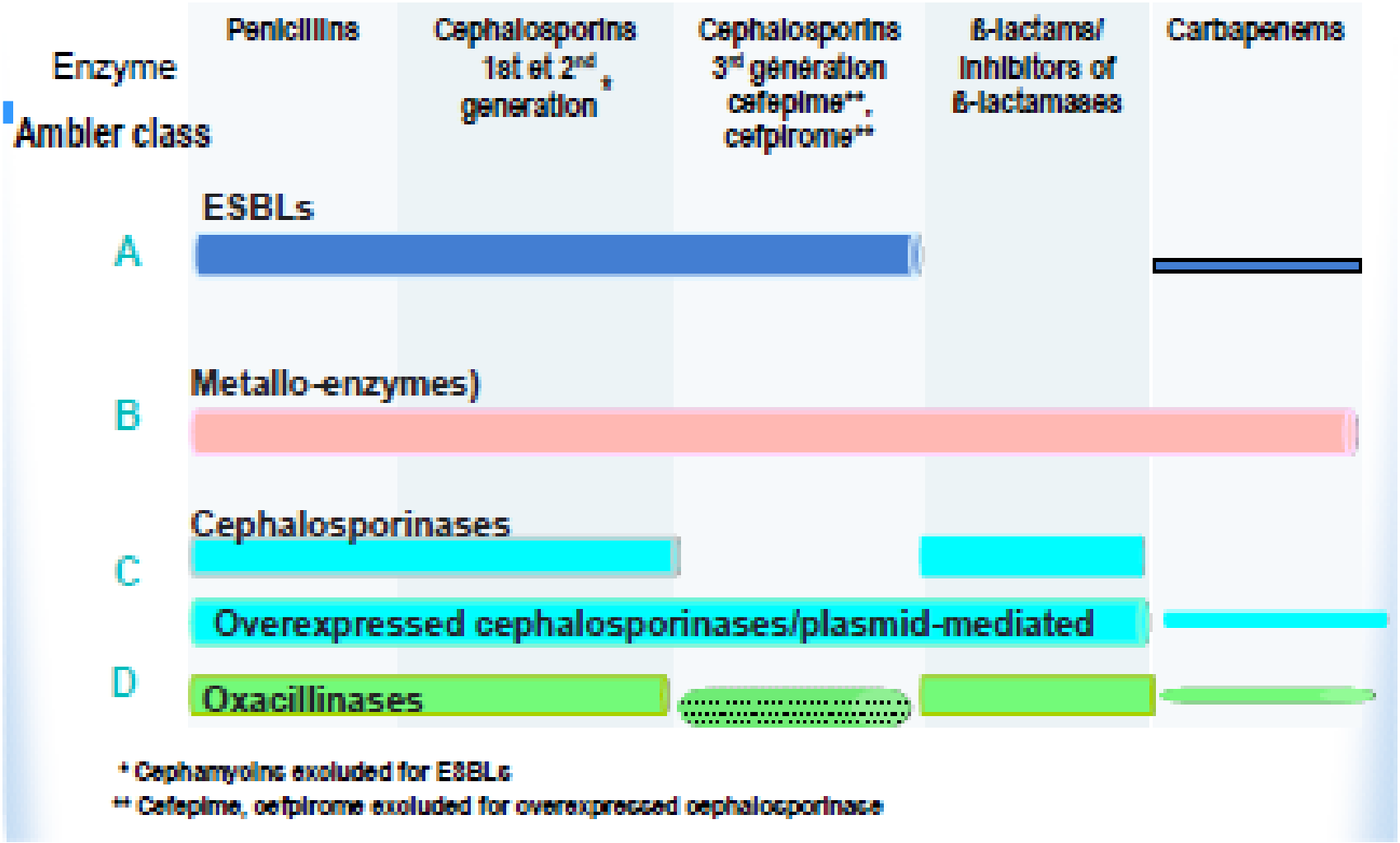
Diag. Microb. Infect. Dis. 2004. Turner. 50:291, Rhomberg et al. 49:273, Jones et al. 49:211.
Int. J. Antimicrob. Agents. 2002. Pfaller et al 19:383

KARBAPENEMLER

- Ertapenem
- Doripenem
- İmipenem
- Meropenem
- Razupenem

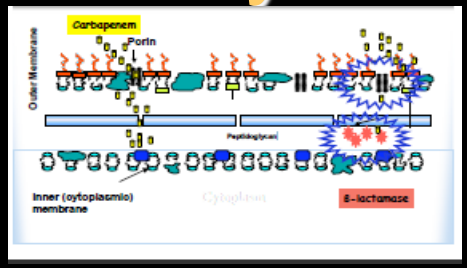


Geniş spektrumlu β-laktamazların etki spektrumu

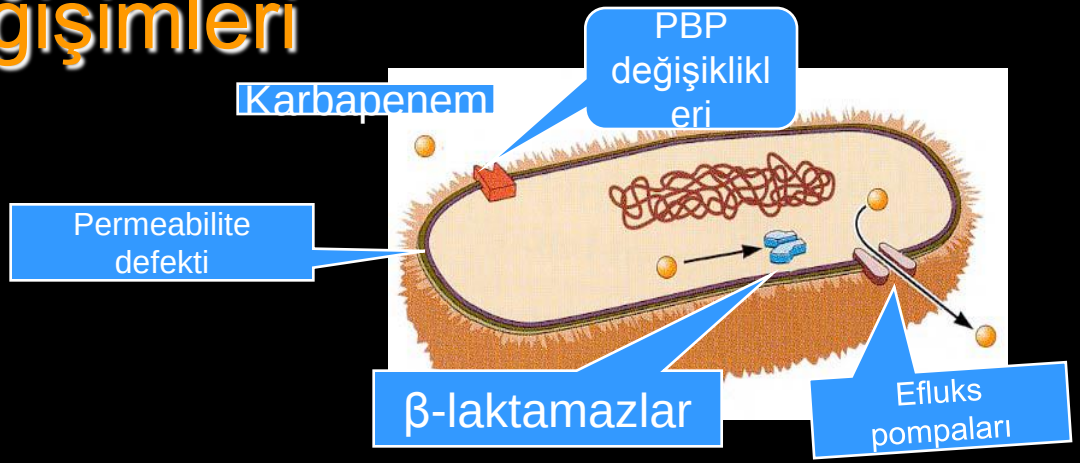


Karbapenemlere Direnç Mekanizmaları

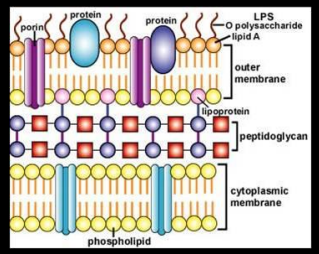
1. İlacın hücre içinde etkin konsantrasyona ulaşamaması

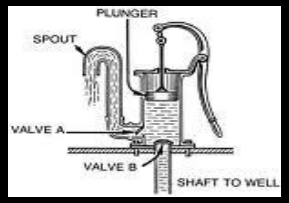


2. Karbapenemleri hidroliz eden enzimlerin varlığı (karbapenemazlar)
3. Hedef PBP değişimleri



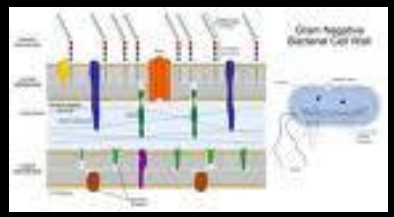
1. İlacın hücre içinde etkin konsantrasyona ulaşamaması



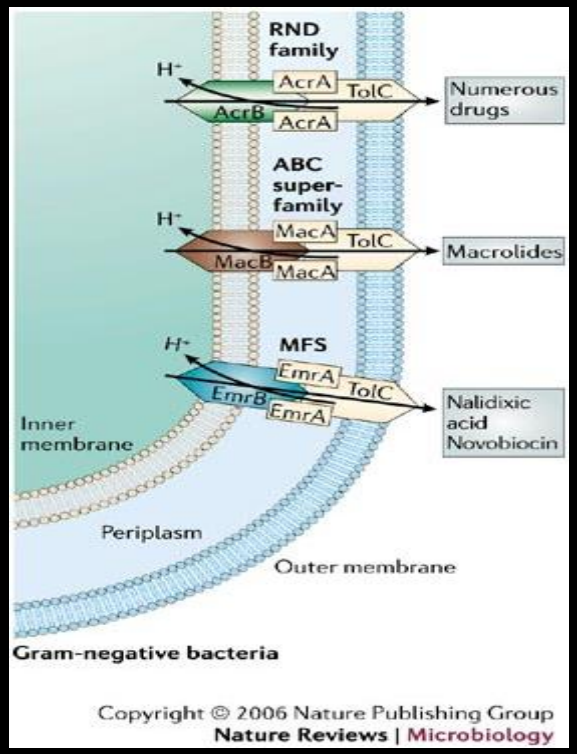


a. Porin değişimleri:

- Porin proteinlerindeki değişiklikler
  - OmpF, OmpC
  - OmpK-35
  - OmpK-36
  - OmpK-37
- Porin kayıbı karbapenemlerin periplazmik aralığa girişini sınırlandırır



b.Aktif pompa sistemlerinin indüklenmesi

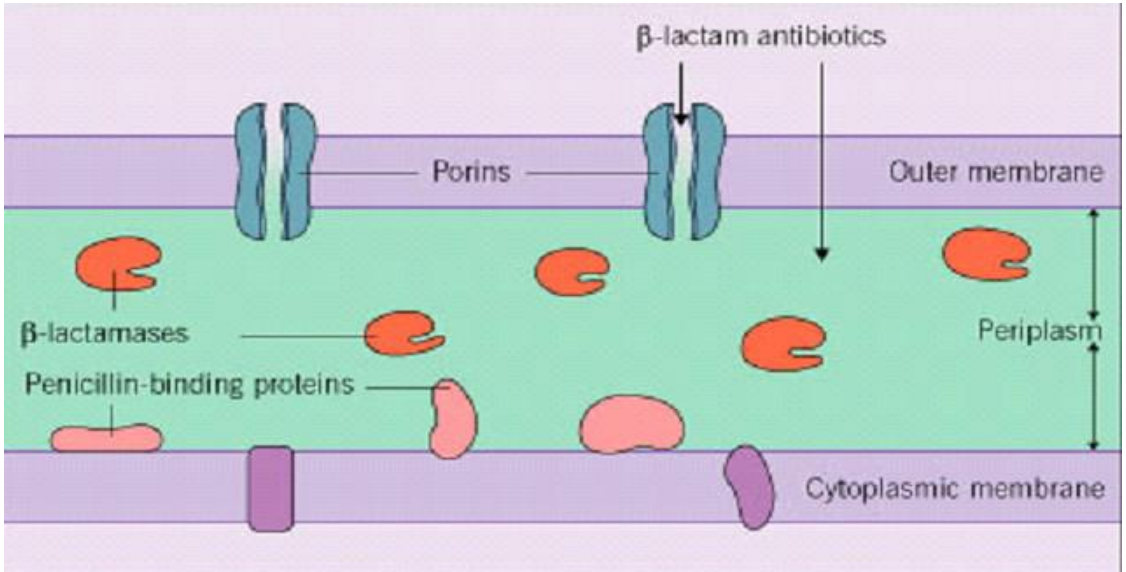


Enterobacteriaceae'de karbapenem direnci

Dış membran porin proteinlerinin kayıbı ile birlikte sefalosporinaz yapımı

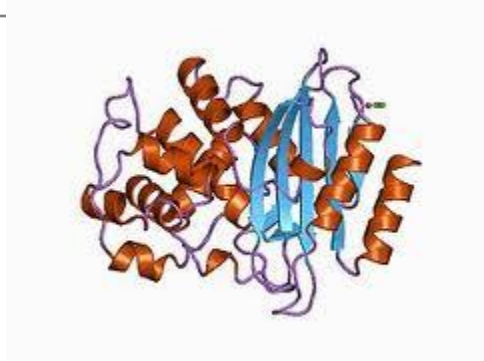
- AmpC-tipi β- laktamazlar veya
- CTX-M tipi GSBL aktivitesi

Düşük düzey karbapenemaz aktivitesi



Bush-Jacoby-Medeiros group[1]	Ambler class[2]	Representative enzymes (examples)	Preferred substrates	Inhibited by		Organisms	Location
				CA[3]	EDTA[4]		
1	C	AmpC	Cephalosporins	-	-	Gramnegative rods	Chromosome (Plasmid)
2d	D	OXA-1-10 PSE-2	Penicillins cloxacillin	±	-	*Enterobacteriaceae* *P.aeruginosa*	Variable
2e	A	CepA FPM-1 L2	Cephalosporins	+	-	*Bacteroides sp* *Proteus sp* *S.maltophilia* (inducible)	Variable
2f	A	NMC-A Sme-1-3 Imi-1-3 KPC-1,2 GES-2 D	Penicillins cephalosporins carbapenems OXA-24-26,40, 51,58,72	+ ±		*E.cloacae* *S.marcescens* *K.pneumoniae* *P.aeruginosa* *A.baumanii*	Chromosome Plasmid Plasmid Chromosome
3	B	VIM, IMP, SPM, GIM L1	Penicillins cephalosporins carbapenems	-	+	*P.aeruginosa* *A.species* *Enterobacteriacae*	Variable

2-Karbapenemazlar



- **Sınıf A penisilinazlar**
  Kromozomal: SME,NMC ,IMI
  Plazmidik: **KPC,GES , IMI-2**
- **Sınıf B: Metalloenzimler:**
- IMP1-27, VIM 1-24 , SPM, GIM,SIM,NDM

- **Sınıf C: Sefalosporinazlar veya Amp C**

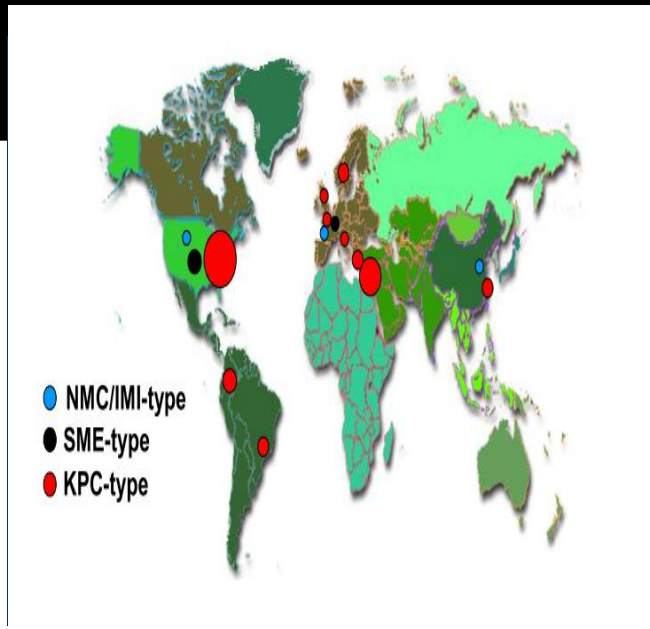
- **Sınıf D: Oksasilinazlar**

Dünyada Artış Gösteren β-Laktamazlar

- Plazmid kontrolündeki Grup-1 (Amp-C Enzimler)
- Grup 2be'de bulunan GSBL enzimleri
- Grup 3 metallo beta laktamazlar

  (IMP, VIM, NDM…)

Sınıf A karbapenemaz aktivitesi olan GSBL'ler



1-Kromozomal karbapenemazlar

- **NMCA** *E. cloacae*
- **IMI-1** *E. cloacae*
- **Sme-1, -2** *S. marcescens*
- **SFC-1** *Serratia fonticola*
- **SHV-38** *K. pneumoniae*
- **2-Plazmidik karbapenemazlar**
- **GES-3,-4,-5** *K. pneumoniae, E. coli, E. cloacae*
- **IMI-2,-3** *Enterobacter asburiae, E. cloacae*
- **KPC-1-11** *K. pneumoniae, E. cloacae, Salmonella, E. coli*

SHV-38 β-Laktamaz
(K.pneumoniae)

- Kromozomal kaynaklı
- 3. kuşak sefalosporinler ve imipeneme azalmış duyarlılık

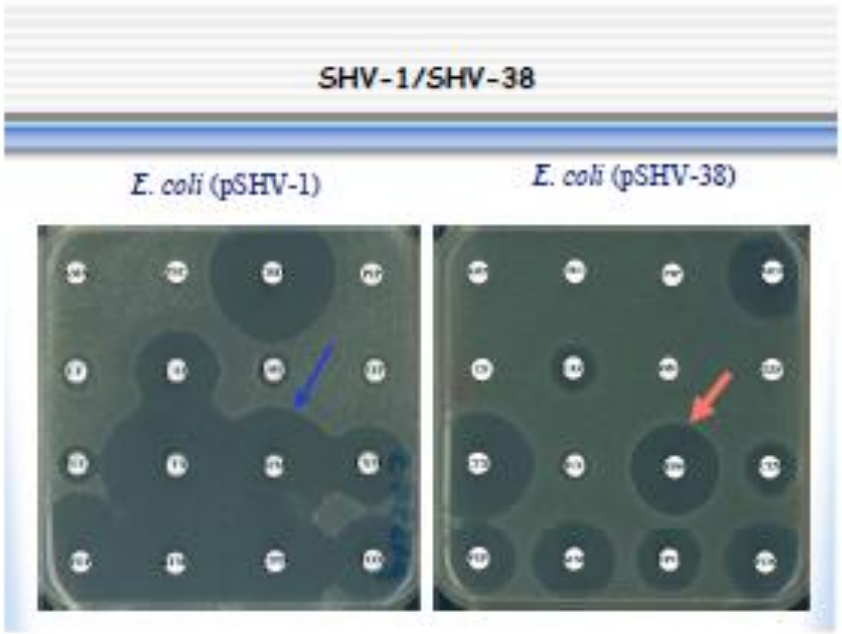


TABLE 1. MICs of β-lactams for *K. pneumoniae* Lor-1, *E. coli* DH10B(pLP-1), *E. coli* DH10B(pLP-2), and *E. coli* DH10B (reference strain)

β-Lactam(s)[a]	MIC (μg/ml) for:			
	K. pneumoniae Lor-1 (SHV-38)	*E. coli* DH10B (pLP-1) (SHV-38)	*E. coli* DH10B (pLP-2) (SHV-1)	*E. coli* DH10B
SHV-38	SHV-1			
Amoxicillin	>512	>512	>512	4
Amoxicillin + CLA	>512	>512	64	4
Ticarcillin	>512	>512	>512	4
Ticarcillin + CLA	>512	>512	128	4
Piperacillin	>512	>512	>512	2
Piperacillin + TZB	>512	>512	256	2
Cephalothin	128	512	32	4
Cefuroxime	4	16	8	4
Cefoxitin	4	8	8	8
Ceftazidime	16	64	2	0.25
Ceftazidime + CLA	2	8	1	0.25
Cefotaxime	0.25	0.5	0.12	0.06
Ceftriaxone	0.12	0.25	0.06	0.06
Cefepime	2	4	0.25	0.03
Cefpirome	2	4	0.5	0.03
Cefpirome + CLA	0.12	0.5	0.06	0.03
Aztreonam	2	4	0.25	0.12
Moxalactam	1	2	0.25	0.06
Moxalactam + CLA	0.25	0.5	0.12	0.12
Imipenem	0.25	0.5	0.06	0.06
Meropenem	0.06	0.12	0.06	0.06

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.

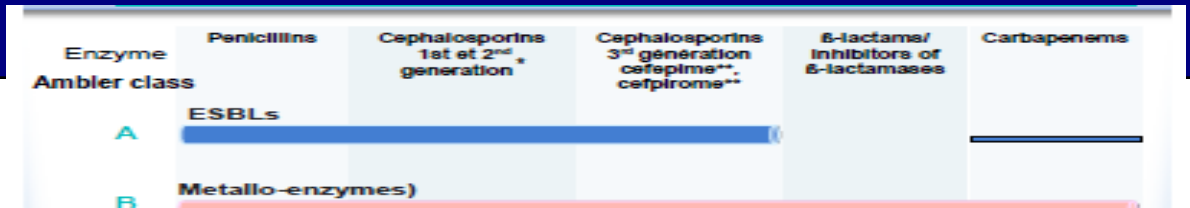
Poirel & Nordmann AAC, 2003

Sınıf A Karbapenemazlar

K pneumoniae carbapenemase (KPC)

Bush-Jacoby-Medeiros group[1]	Ambler class[2]	Representative enzymes (examples)	Preferred substrates	Inhibited by		Organisms	Location
				CA[3]	EDTA[4]		
1	C	AmpC	Cephalosporins	-	-	Gramnegative rods	Chromosome (Plasmid)
2d	D	OXA-1-10 PSE-2	Penicillins cloxacillin	±	-	*Enterobacteriaceae* *P.aeruginosa*	Variable
2e	A	CepA FPM-1 L2	Cephalosporins	+	-	*Bacteroides sp* *Proteus sp* *S.maltophilia* (inducible)	Variable
2f	A	NMC-A Sme-1-3 Imi-1-3	Penicillins cephalosporins carbapenems	+		*E.cloacae* *S.marcescens*	Chromosome
		KPC-1,2				*K.pneumoniae*	Plasmid
		GES-2				*P.aeruginosa*	Plasmid

- Enterobacteriaceae'de nadir
- Penisilinleri, sefalosporinleri, aztreonam ve karbapenemler
- İmipeneme duyarlı olabilir
- Klavulanik asit ve tazobaktam tarafından inhibisyon



Karbapenemaz-Producing *Klebsiella pneumoniae* (KPC)

- KPC-1, 2001

  KPC-2 -11

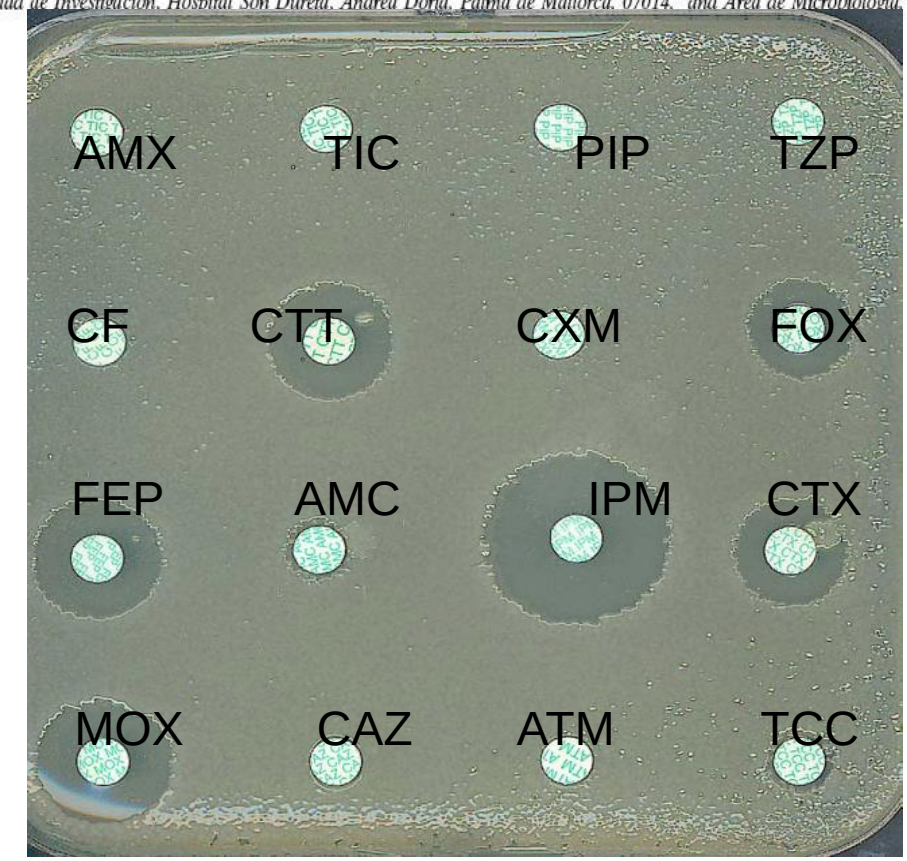
- *K. pneumoniae,*
- *K. oxytoca*
- *Salmonella enterica*
- *S. marcescens*
- *C. freundii*
- *E. coli*
- *E. cloacae*
- *P. aeruginosa*
- *A. baumannii*

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2001, p. 1151–1161 Vol. 45, No. 4
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.4.1151–1161.2001

Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of *Klebsiella pneumoniae*

HESNA YIGIT,[1] ANNE MARIE QUEENAN,[2] GREGORY J. ANDERSON,[1] ANTONIO DOMENECH-SANCHEZ,[3] JAMES W. BIDDLE,[1] CHRISTINE D. STEWARD,[1] SEBASTIAN ALBERTI,[4] KAREN BUSH,[2] AND FRED C. TENOVER[1]*

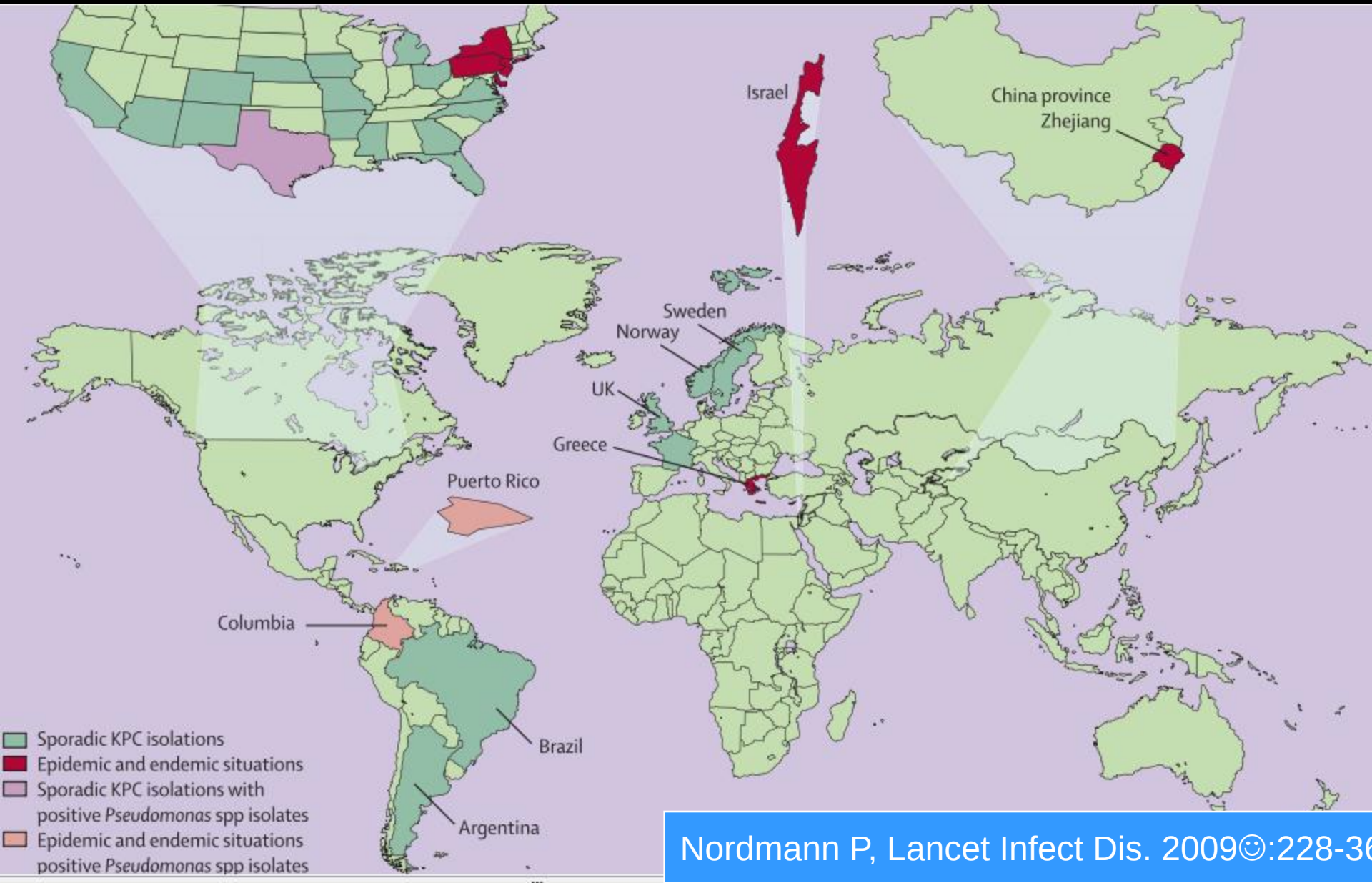
Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[1]; The R. W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey 08869[2]; and Unidad de Investigacion, Hospital Son Dureta, Andrea Doria, Palma de Mallorca, 07014,[4] and Área de Microbiología



KPC Enzimleri

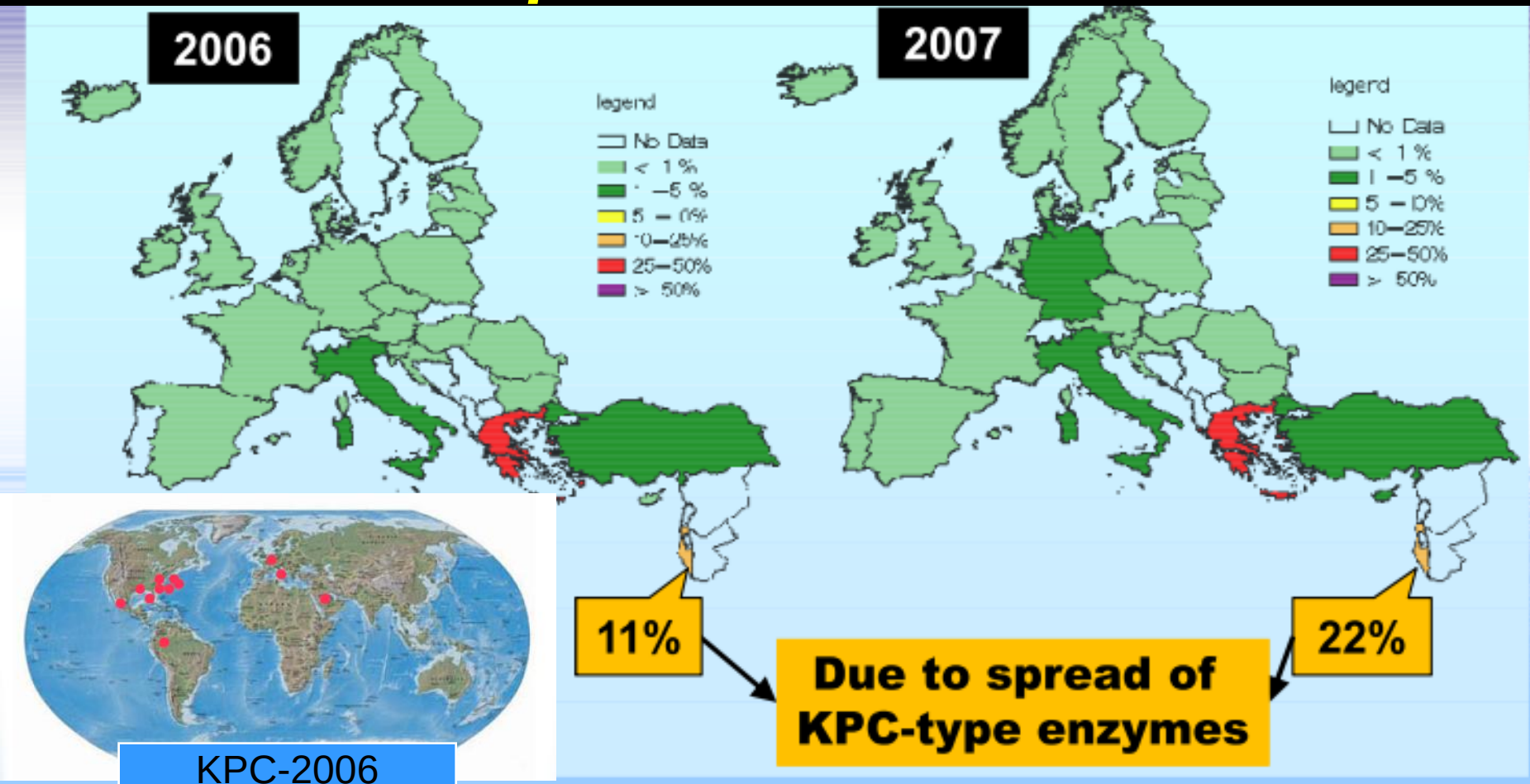
- %45 Sme-1, %44 NMCA ve IMI-1
- *bla*KPC plazmitler üzerinde (50 kb)
  - Dar spektrumlu β-laktamazlar
  - Genişlemiş spektrumlu β-laktamazlar
  - Aminoglikozit direnç geni [AAC(6')-Ib]
  - Plazmit kaynaklı florokinolon direnç genleri

KPC+ suşlarının dünyadaki yayılımı



Nordmann P, Lancet Infect Dis. 2009☺:228-36

KPC ve karbapenem dirençli *K.pneumoniae* İsrail



Both clonal and plasmid spread

EARSS database

Navon- Venezia *et al.* - AAC 2006

Leavitt *et al.* - AAC 2007

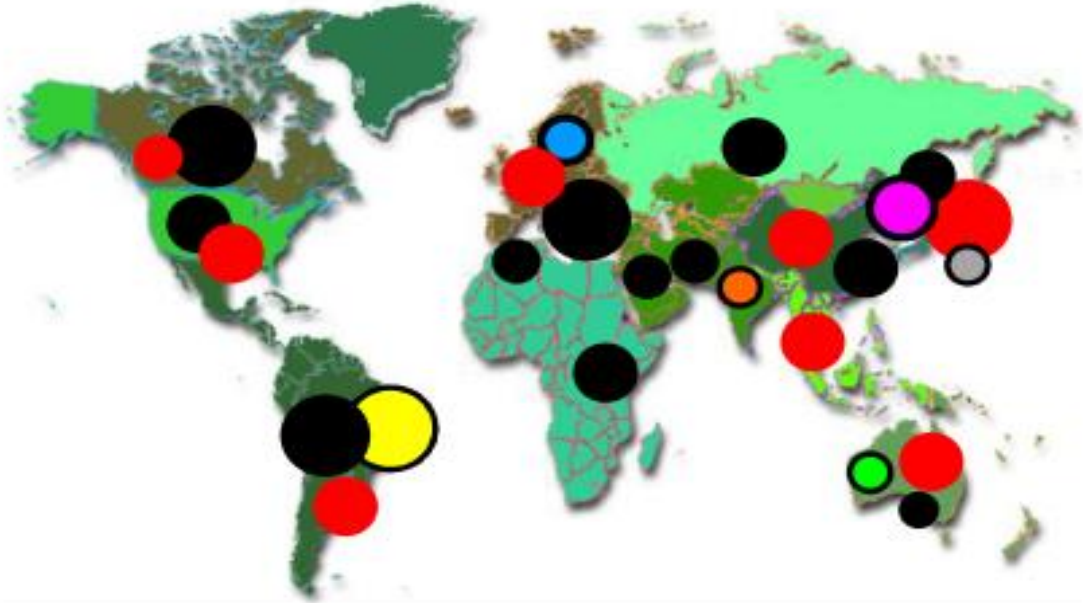
Villegas & Quinn AAC 2006;
Navon-Venezia & Carmeli AAC 2006

Sınıf B β-Laktamazlar (Metallo β-laktamazlar)

Bush-Jacoby-Medeiros group[1]	Ambler class[2]	Representative enzymes (examples)	Preferred substrates	Inhibited by		Organisms	Location
				CA[3]	EDTA[4]		
1							
2d							
2e							
2f							
3	B	VIM, IMP, SPM, GIM	Penicillins cephalosporins carbapenems	-	+	*P.aeruginosa* *A.species* *Enterobacteriacae*	Variable
		L1					

- Aktif bölgelerinde **serin** yerine Zn^{+2} iyonu
- **Monobaktamlar** dışında karbapenemler dahil tüm beta laktamları hidroliz edebilirler.
- Klavulanik asit, tazobaktam, sulbaktam etkilenmez
- EDTA gibi bir metal şelatörü ile inhibe olurlar
  - 10-phenanthroline
  - Dipikolinik asit
- Kromozom, plazmit

MBL enzimlerinin dünyadaki yayılımı



IMP	*P. aeruginosa* *Acinetobacter* oth. GNNFs *Enterics* *Aeromonas*
VIM	*P. aeruginosa* *Acinetobacter* oth. GNNFs *Enterics* *Aeromonas*
SPM-1	*P. aeruginosa*
GIM-1	*P. aeruginosa*
SIM-1	*Acinetobacter*
AIM-1	*P. aeruginosa*
KHM-1	
NDM-1	

Klebsiella, Enterobacter, E.coli, P.mirabilis, Citrobacter, Serratia, Morganella, Providencia

Enterobacteriaceae'de kazanılmış MBL'ler

- 1990, sporadik bildirimler

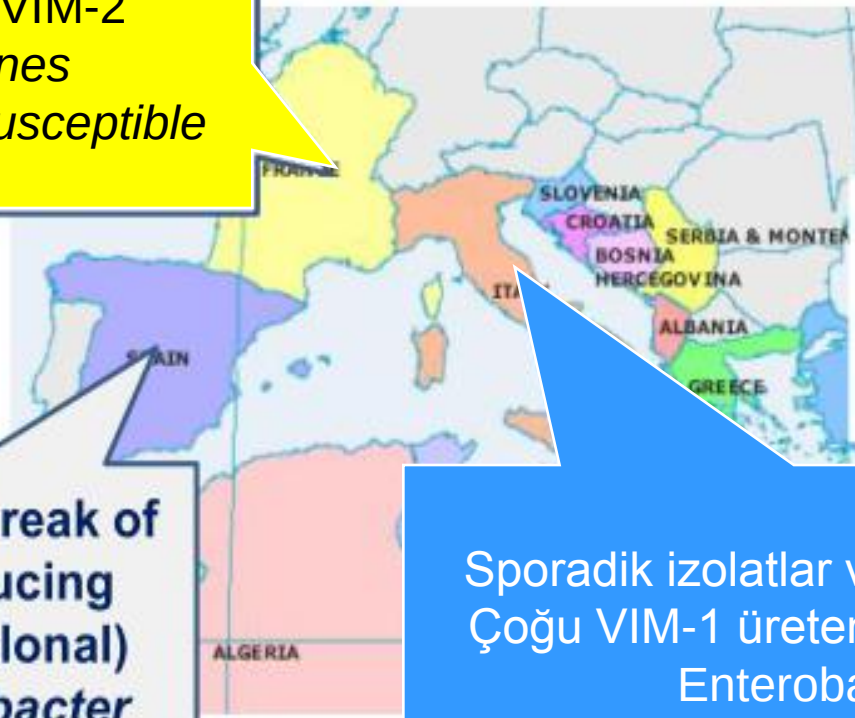
  (Güney Doğu Asya, Avrupa, Avustralya)
- Düşük karbapenem MİK'lerinden dolayı saptanmalarındaki zorluklar
- Saptanan türler

  Klebsiella, Enterobacter, E.coli, P.mirabilis, Citrobacter, Serratia, Morganella, Providencia

Enterobacteriaceae'deki MBL'ler

Biendo *et al.* – JCM 2008

2003-05
IMP-1 veya VIM-2
E.aerogenes
İmipenem *nonsusceptible*



Luzzaro *et al.* – AAC 2004
Rossolini *et al.* AAC 2008
Cagnacci *et al.* – JAC 2008
Aschbacher *et al.* – JAC 2008
Perilli *et al.* – MDR 2008

2005-06: outbreak of VIM-1-producing *Klebsiella* (clonal) and *Enterobacter* (multiclonal)

Sporadik izolatlar veya küçük salgınlar
Çoğu VIM-1 üreten *Klebsiella* ve diğer Enterobacteriacea

Tato *et al.* – CID 2007

- VIM-5
  K.pneumoniae
  [3]Enterobacter cloacae
- VIM-1
  [4]P.aeruginosa, K.pneumoniae, [4]E.cloacae
- [4]IMP-1
  [4]Enterobacter cloacae, [5]K.pneumoniae, E.coli

[1]Poirel L. AAC: 2004;48:15-22.
[2]Nazic H. AAAC: 2005;49:2146-2147
[3]Yıldırm. ECCMID Congress, 2007
[4]SENTRY, ECCMID Congress 2007
Aktaş Z. CMI 2006:12(7):695-96.

New Delhi Metallo-β-laktamaz (NDM-1)

Hindistan, Pakistan, Bangladeş, İngiltere, Fransa,İsveç, Amerika, Avustralya, Japonya, Singapur, Kenya

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2009, p. 5046
0066-4804/09/$12.00 doi:10.1128/AAC.00774-09

Characterization of a New Metallo-β-Lactamase Gene, *bla*$_{NDM-1}$, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in *Klebsiella pneumoniae* Sequence Type 14 from India[▽]

Dongeun Yong,[1,2] Mark A. Toleman,[2] Christian G. Giske,[3] Hyun S. Cho,[4] Kristina Sundman,[5] Kyungwon Lee [1] and Ti… R. Walsh[2]*

Yonsei University College of M… Resistance, Seoul, Republic of Korea[1]; Department of …nical Microbiology, MTC—Karolinska Institutet, …ty College of Life Science and Biotechnology, …biology, Örebro University Hospital,

ESCMID

CMI CLINICAL MICROBIOLOGY AND INFECTION

New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia?

Jean Marc Rolain[1,*], Philippe Parola[1,2], Giuseppe Cornaglia[3]

DOI: 10.1111/j.1469-0691.2010.03385.x

Issue … nd

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

ELSEVIER

Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae

Karen Bush

…ms and other antibiotics in the …associated with plasmidic …ferred among …ingly

J Assoc Physicians India. 2010 Mar;58:147-9.

New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems …compromised.

Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R.

disease com… Dept of Research, P.D.Hinduja National Hospital & Medical Research Centre, Mumbai, India.

gens that ha…

[3], two are …

tive Gram-n… **Abstract**

BACKGROUND: Carbapenems are among the few useful antibiotics against multidrug resistant gram negative bacteria particularly those …

…ciadtamase. However resistance to carbapenems occurs and is mediated by mechanisms like loss of outer membrane proteins and pro…

…olving carbapenems. An alert issued in the UK in 2009 warned of an increasing number of carbapenem resistant Enter…

…recently hospitalized in India and Pakistan and had new type of metallo beta lactamase designat…

…lactamase enzyme in Enterobacteriaceae at a tertiary care centre …

K.pneumoniae
E.coli
C.freundii
E.cloacae
M.morganii

Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study

Karthikeyan K Kumarasamy, Mark A Toleman, Timothy R Walsh, Jay Bagaria, Fafhana Butt, Ravikumar Balakrishnan, Uma Chaudhary, Michel Doumith, Christian G Giske, Seema Irfan, Padma Krishnan, Anil V Kumar, Sunil Maharjan, Shazad Mushtaq, Tabassum Noorie, David L Paterson, Andrew Pearson, Claire Perry, Rachel Pike, Bhargavi Rao, Ujjwayini Ray, Jayanta B Sarma, Madhu Sharma, Elizabeth Sheridan, Mandayam A Thirunarayan, Jane Turton, Supriya Upadhyay, Marina Warner, William Welfare, David M Livermore, Neil Woodford

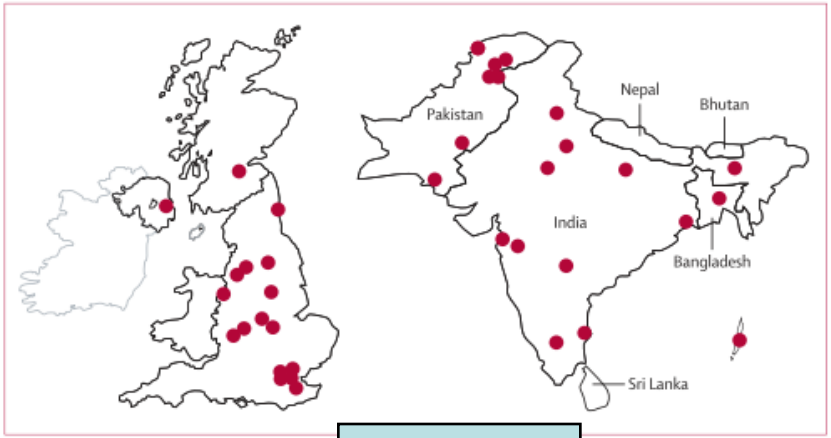
NDM-1

Findings We identified 44 isolates with NDM-1 in Chennai, 26 in Haryana, 37 in the UK, and 73 in other sites in India and Pakistan. NDM-1 was mostly found among *Escherichia coli* (36) and *Klebsiella pneumoniae* (111), which were highly resistant to all antibiotics except to tigecycline and colistin. *K pneumoniae* isolates from Haryana were clonal but NDM-1 producers from the UK and Chennai were clonally diverse. Most isolates carried the NDM-1 gene on plasmids: those from UK and Chennai were readily transferable whereas those from Haryana were not conjugative. Many of the UK NDM-1 positive patients had travelled to India or Pakistan within the past year, or had links with these countries.

Interpretation The potential of NDM-1 to be a worldwide public health problem is great, and co-ordinated international surveillance is needed.

Funding European Union, Wellcome Trust, and Wyeth.

K.pneumoniae, E.coli
C.freundii, E.cloacae, M.morganii



2008-09

Sınıf D OXA β-laktamazlar

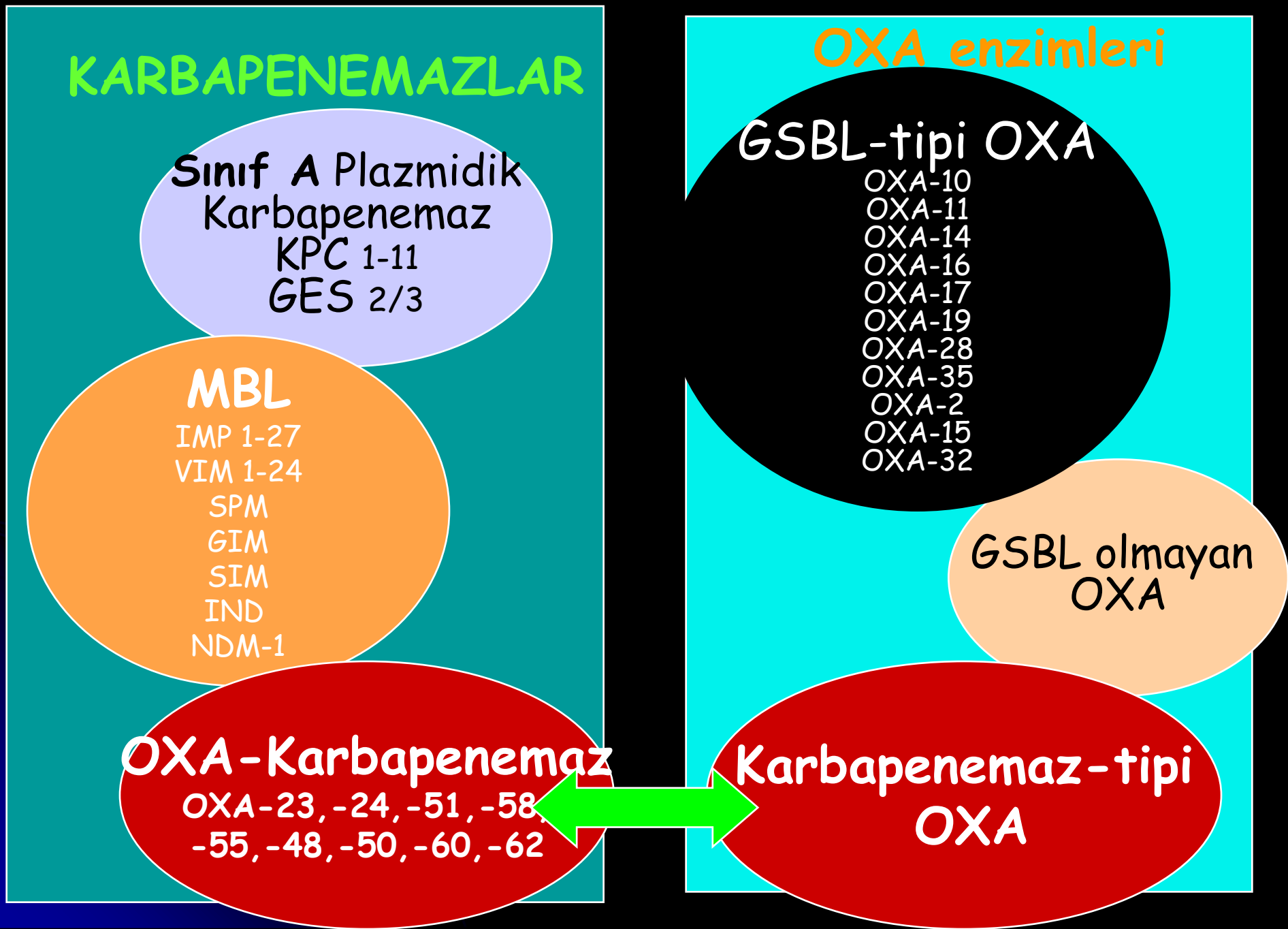
Bush-Jacoby-Medeiros group[1]	Ambler class[2]	Representative enzymes (examples)	Preferred substrates	Inhibited by		Organisms	Location
				CA[3]	EDTA[4]		
1	C	AmpC	Cephalosporins	-	-	Gramnegative rods	Chromosome (Plasmid)
2d	D	OXA-1-10 PSE-2	Penicillins cloxacillin	±	-	*Enterobacteriaceae* *P.aeruginosa*	Variable
2e	A	CepA FPM-1	Cephalosporins	+	-	*Bacteroides sp* *Proteus sp*	Variable
	D	OXA-24-26,40, 51,58,72		±		*A.baumanii*	Chromosome
3	B	VIM, IMP, SPM, GIM L1	Penicillins cephalosporins carbapenems	-	+	*P.aeruginosa* *A.species* *Enterobacteriacae*	Variable

- İki grubu önem taşımaktadır. (GSBL, KARBAPENEMAZ)
- Plazmit veya integron kökenli
- 3.Kuşak sefalosporinlere ve aztreonama etkinlikleri yoktur
- Klavulanik asit, tazobaktama ve sulbaktam ile inhibe olmaz, $NaCl_2$ ile inhibisyon
- EDTA ile inhibe olmamaları ile metallo beta laktamaz enzimlerinden ayrılabilirler

OXA-48

panel C

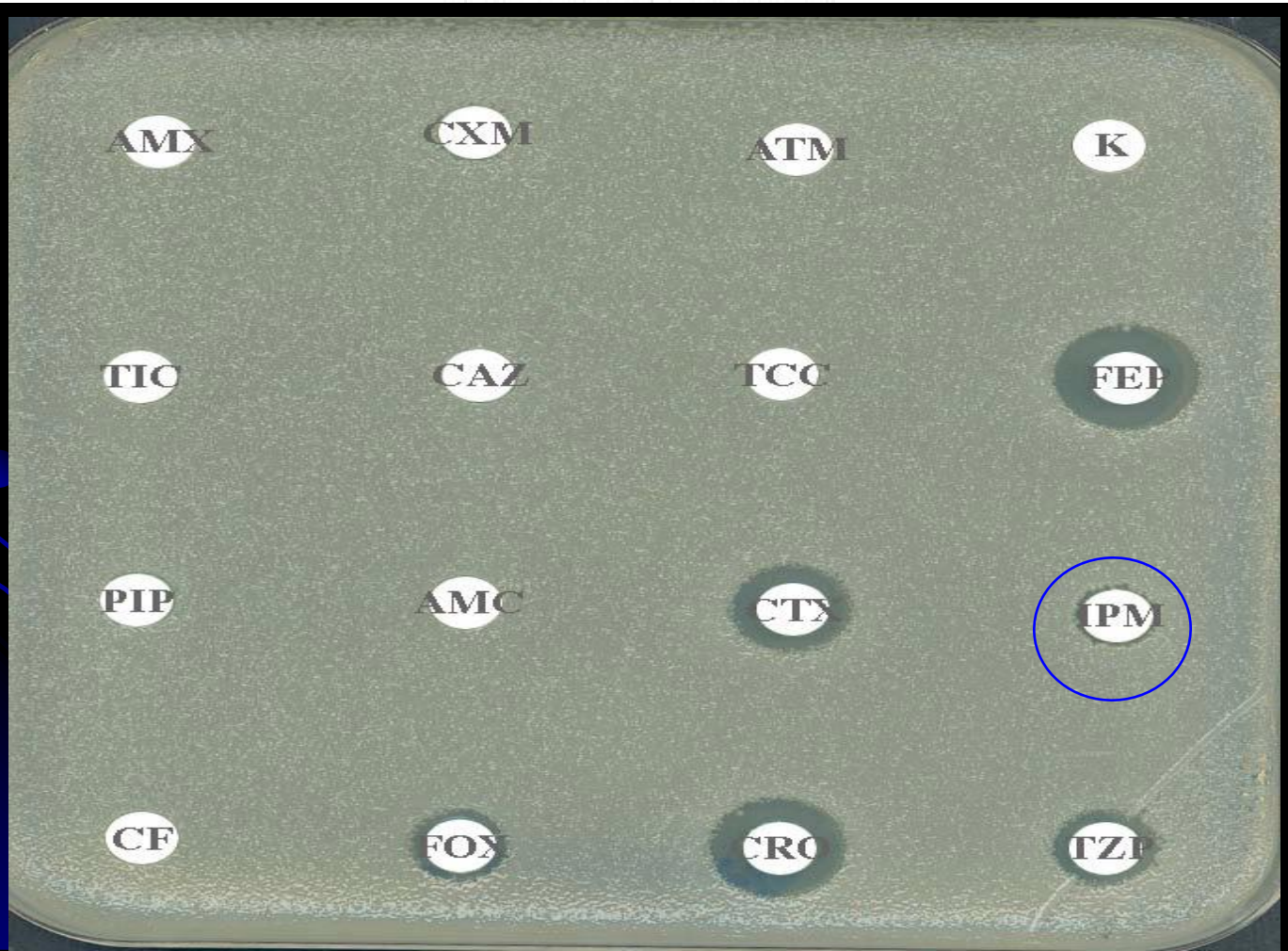
Nordmann P, Carrer A. **Carbapenemases in enterobacteriaceae.** Arch Pediatr. 2010 Sep;17 Suppl 4:S154-62


KARBAPENEMAZLAR
Sınıf A Plazmidik Karbapenemaz
KPC 1-11
GES 2/3
MBL
IMP 1-27
VIM 1-24
SPM
GIM
SIM
IND
NDM-1
OXA-Karbapenemaz
OXA-23,-24,-51,-58,
-55,-48,-50,-60,-62
OXA enzimleri
GSBL-tipi OXA
OXA-10
OXA-11
OXA-14
OXA-16
OXA-17
OXA-19
OXA-28
OXA-35
OXA-2
OXA-15
OXA-32
GSBL olmayan OXA
Karbapenemaz-tipi OXA

0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.6.2004–2006.2002

Chromosome-Encoded Class D β-Lactamase OXA-23 in *Proteus mirabilis*

R. Bonnet,[1]* H. Marchandin,[2] C. Chanal,[1] D. Sirot,[1] R. Labia,[3] C. De Champs,[1] E. Jumas-Bilak,[4] and J. Sirot[1]

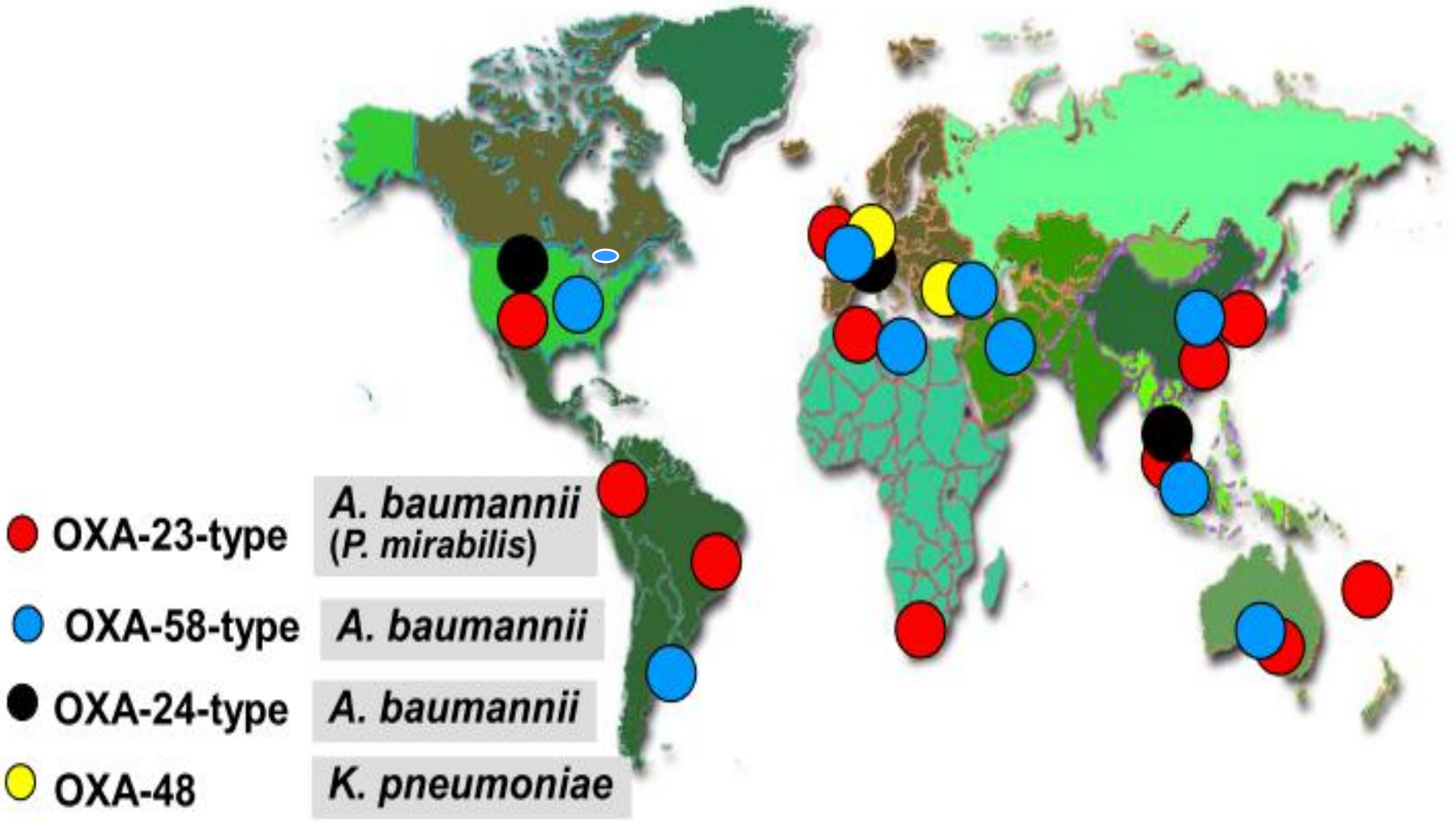


- **Karbapenemaz-tipi OXA (n=45)**
- ***Pseudomonas, Acinetobacter, Klebsiella***

TABLE 6. Carbapenemase subgroups of the OXA family of β-lactamases

Cluster	Enzyme subfamily	Additional OXA member(s)	Referen
1	OXA-23 (ARI-1)	OXA-27, OXA-49	225
2	OXA-24	OXA-25, OXA-26, OXA-40, OXA-72	225
3	OXA-51	OXA-64 to OXA-71, OXA-75 to OXA-78, OXA-83, OXA-84, OXA-86 to OXA-89, OXA-91, OXA-92, OXA-94, OXA-95	213, 22
4	OXA-58	None	225
5	OXA-55	OXA-SHE	225
6	OXA-48	OXA-54, OXA-SAR2	225
7	OXA-50	OXA-50a to OXA-50d, PoxB	225
8	OXA-60	OXA-60a to OXA-60d	225
9	OXA-62	None	192

Kazanılmış Sınıf D serin karbapenemazlar



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2004, p. 15–22 Vol. 48, No. 1
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.1.15–22.2004

Emergence of Oxacillinase-Mediated Resistance to Imipenem in *Klebsiella pneumoniae*

Laurent Poirel,[1] Claire Héritier,[1] Venus Tolün,[2] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, Université Paris XI, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre, France,[1] and Department of Microbiology, Istanbul Medical Faculty, Capa, Istanbul, Turkey[2]

Received 20 March 2003/Returned for modification 7 July 2003/Accepted 22 September 2003

Klebsiella pneumoniae **strain 11978 was isolated in Turkey in 2001 and was found to be resistant to all β-lactams, including carbapenems. Cloning and expression in *Escherichia coli* identified five β-lactamases, including two novel oxacillinases. The β-lactamase OXA-48 hydrolyzed imipenem at a high level and was remotely related (less than 46% amino acid identity) to the other oxacillinases. It hydrolyzed penicillins and imipenem but not expanded-spectrum cephalosporins. The bla_{OXA-48} gene was plasmid encoded and not associated with an integron, in contrast to most of the oxacillinase genes. An insertion sequence, IS*1999*, was**

2001: Klebsiella pneumoniae'de OXA-48

- İTF'deki ilk OXA-48
- Dünyadaki ilk OXA-48

Microbiology

Chemotherapy

Chemotherapy 088
DOI: 10.1159/000XXXXXX

Received: May 2, 2006
Accepted after revision: June 3, 2007
Published online: ■■■

Carbapenem-Hydrolyzing Oxacillinase, OXA-48, Persists in *Klebsiella pneumoniae* in Istanbul, Turkey

Zerrin Aktaş[a] Çiğdem Bal[a] Ines Schneider[c] Barış Can[a] Kenan Midilli[b]
Adolf Bauernfeind[c]

Kasım 2004 + Mart 2005:

2 K.pneumoniae izolatında OXA-48

OXA-48 beta-laktamaz pozitif *K.pneumoniae* ile hastanemizde 2 Salgın

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2008, p. 2950–2954 Vol. 52, No. 8
0066-4804/08/$08.00+0 doi:10.1128/AAC.01672-07

Spread of OXA-48-Positive Carbapenem-Resistant *Klebsiel* *onia* Isolates in Istanbul, Turkey▽

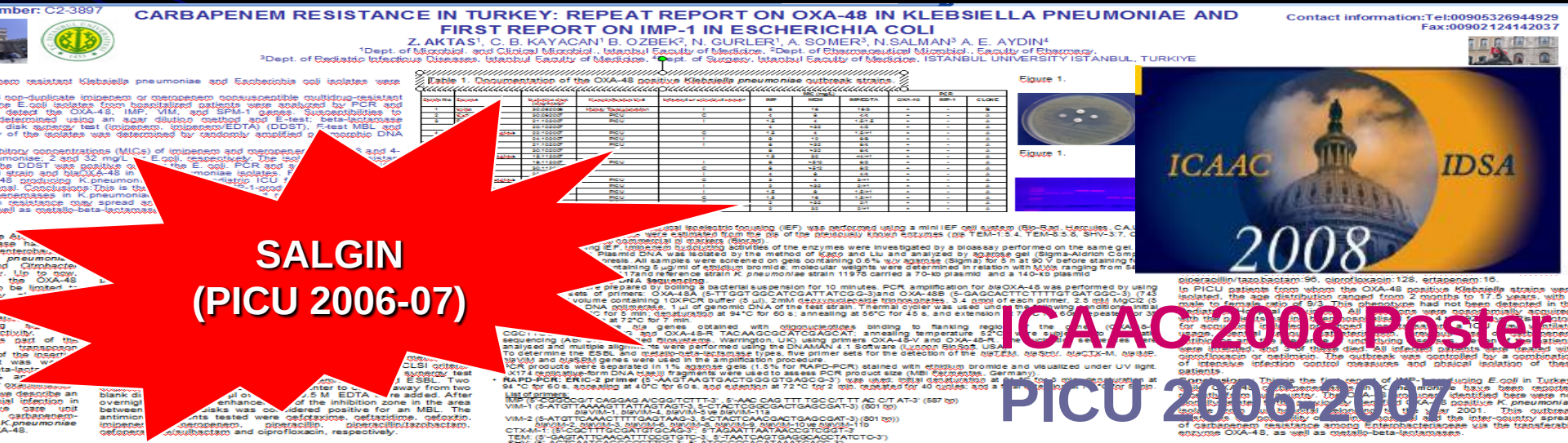
Amélie Carrër,[1] Laurent Poirel,[1] Haluk Eraksoy,[2] Selim Badur,[3] and Patrice Nordma[1]

Service de Bactériologie-Virologie, INSERM U914, Emerging Resistance to Antibiotics, Hôpital de Paris, Faculté de Médecine Paris Sud, Le Kremlin-Bicêtre, France,[1] and Clinical Microbiology[2] and Department of Microbiology,[3] Istanbul Capa, Istanbul, Turkey

Received 27 December 2007/Returned for modification 10 Febru 200

The first outbreak of carbapenem-resistant *Klebsiella pneumoniae* isolates pr ucing the plasmid-encoded carbapenem-hydrolyzing oxacillinase OXA-48 is reported. The 39 isolates belonged to two different clones and were collected at the University Hospital of Istanbul, Turkey, from May 2006 to February 2007, and they coproduced various β-lactamases (SHV-12, OXA-9, and TEM-1 for clone A and CTX-M-15, TEM-1, and OXA-1 for clone B).

SALGIN (ICU 2006)



SALGIN (PICU 2006-07)

ICAAC 2008 Poster

PICU 2006-2007

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2010, p. 1369–1373 Vol. 54, No. 3
0066-4804/10/$12.00 doi:10.1128/AAC.01312-09

Spread of OXA-48-Encoding Plasmid in Turkey and Beyond▽

Amélie Carrër,[1] Laurent Poirel,[1] Mesut Yilmaz,[2] Özay Arikan Akan,[3] Cilli Feriha,[4] Gaëlle Cuzon,[1] Ghassan Matar,[5] Patrick Honderlick,[6] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, INSERM U914 Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris Sud, K-Bicêtre, France[1]; Infectious Diseases and Clinical Microbiology Department, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey[2]; Central Laboratories, Medical School, Ankara University, Ibni Sina Hospital, Ankara, Turkey[3]; Ege University Medical Faculty, Department of Microbiology and Clinical Microbiology, Izmir, Turkey[4]; American University of Beirut, Beirut, Lebanon[5]; and Service de Microbiologie, Hôpital Foch, Suresnes, France[6]

Received 17 September 2009/Returned for modification 24 October 2009/Accepted 4 January 2010

Eighteen carbapenem-resistant, OXA-48-positive enterobacterial isolates recovered from Turkey, Lebanon, Egypt, France, and Belgium were analyzed. In most isolates, similar 70-kb plasmids carrying the carbapenemase gene bla_{OXA-48} were identified. That gene was located within either transposon Tn*1999* or transposon Tn*1999.2*, which was always inserted within the same gene. This work highlights the current plasmid-mediated dissemination of the OXA-48 carbapenemase worldwide.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 20
0066-4804/08/$08.00+0 doi:10.1128/AAC.01672-07
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Spread of OXA-48-Positive Carbapenem-Resistant *Klebsiella pneumoniae* Isolates in Istanbul, Turkey▽

Amélie Carrër,[1] Laurent Poirel,[1] Haluk Eraksoy,[2] A. Arahan Cagatay,[2] Selim Badur,[3] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, INSERM U914, Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris Sud, Le Kremlin-Bicêtre, France,[1] and Departments of Infectious Diseases and Clinical Microbiology[2] and Department of Microbiology,[3] Istanbul Faculty of Medicine, Capa, Istanbul, Turkey

Received 27 December 2007/Returned for modification 10 February 2008/Accepted 24 May 2008

The first outbreak of carbapenem-resistant *Klebsiella pneumoniae* isolates producing the plasmid-encoded carbapenem-hydrolyzing oxacillinase OXA-48 is reported. The 39 isolates belonged to two different clones and were collected at the University Hospital of Istanbul, Turkey, from May 2006 to February 2007, and they coproduced various β-lactamases (SHV-12, OXA-9, and TEM-1 for clone A and CTX-M-15, TEM-1, and OXA-1 for clone B).

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2008, p. 3463–3464 Vol. 52, No. 9
0066-4804/08/$08.00+0 doi:10.1128/AAC.00543-08
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Plasmid-Encoded Carbapenem-Hydrolyzing β-Lactamase OXA-48 in an Imipenem-Susceptible *Klebsiella pneumoniae* Strain from Belgium▽

Int J Antimicrob Agents. 2010 Jul;36(1):91-3. Epub 2010 Mar 30.

Plasmid-mediated carbapenem-hydrolysing OXA-48 beta-lactamase in Klebsiella pneumoniae from Tunisia.

Cuzon G, Naas T, Lesenne A, Benhamou M, Nordmann P.

Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, Assistance Publique-Hôpitaux de Paris, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre, France.

PMID: 20356714 [PubMed - in process]

Int J Antimicrob Agents. 2008 Jun;31(6):523-6. Epub 2008 Mar 12.

Carbapenem-resistant Escherichia coli and Klebsiella pneumoniae isolates from Turkey with OXA-48-like carbapenemases and outer membrane protein loss.

Gülmez D, Woodford N, Palepou MF, Mushtaq S, Metan G, Yakupogullari Y, Kocagoz S, Uzun O, Hascelik G, Livermore DM.

Hacettepe University Faculty of Medicine, Department of Microbiology and Clinical Microbiology, 06100 Sihhiye, Ankara, Turkey. dolunayglm@yahoo.com

Hindistan, İngiltere, Lübnan, Arjantin, Fransa, Tunus, Fas

Abstract C2-650, 49th ICAAC , C2-648, presented at the 49th ICAAC, Abstract C2-647, 49th ICAAC

Sınıf C β-laktamazlar (AmpC)

TABLE 1. Classification schemes for bacterial β-lactamases

Bush-Jacoby-Medeiros group	1989 Bush group (44)	Richmond-Sykes class (253)	Mitsuhashi-Inoue type (194)[a]	Molecular class (2, 121, 132)	Preferred substrates	Inhibited by: CA[b]	Inhibited by: EDTA	Representative enzymes
Group 1 1	1	Ia, Ib, Id	CSase	C	Cephalosporins	−	−	AmpC enzymes from gram-negative bacteria; MIR-1

- **Sefalosporinler ve penisilinleri hidrolize eder**
  - Sefepim ve sefpirom hariç
- **Sefoksitine direnç**
  - Providencia, Serratia ve Morganella için güvenilir değil
  - İndüklenebilir veya dereprese mutantlar S/I olabilir
- **EDTA ile inhibe olmaz**
- **Klavulanat inhibe olmaz**
- **Kloksasilin ve boronik asit ile inhibe olur**

3	3	Not included	Not included	B	Most β-lactams, including carbapenems	−	+	L1 from *Xanthomonas maltophilia*, CcrA from *Bacteroides fragilis*
4	4	Not included	Not included	ND[c]	Penicillins	−	?	Penicillinase from *Pseudomonas cepacia*

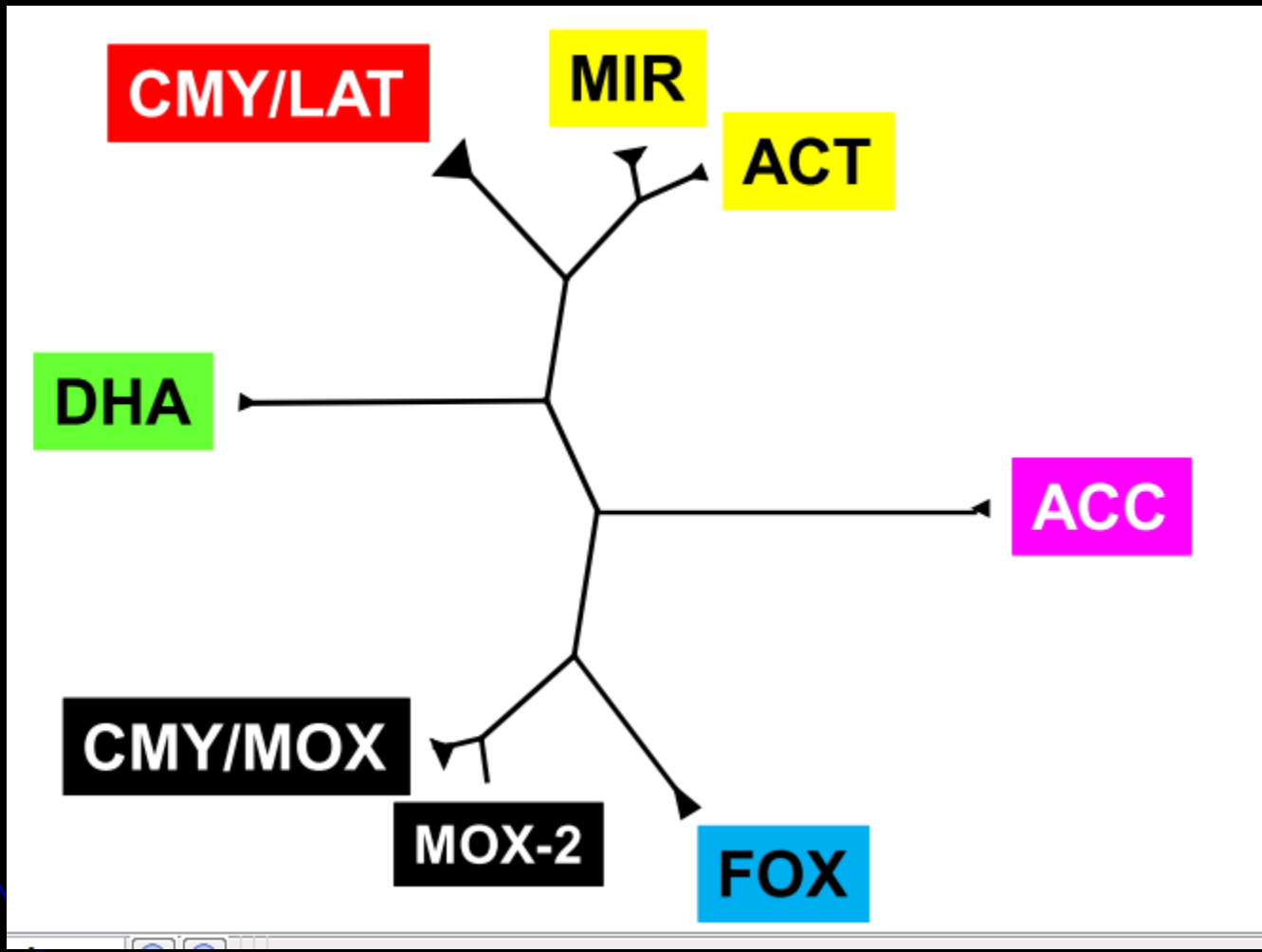
Plazmid Kaynaklı Amp C

- Çoğunlukla *K.pneumoniae, Proteus mirabilis*
  E.coli, Salmonella ve Enterobacter aerogenes…
- Biyokimyasal özellikler kromozomal AmpC ile aynı
- Epidemiyolojik olarak yayılma potansiyeli daha fazla
- GSBL ile mozaik direnç yapı oluşumu
- OMP geçirgenlik azalması ile karbapenem direnci

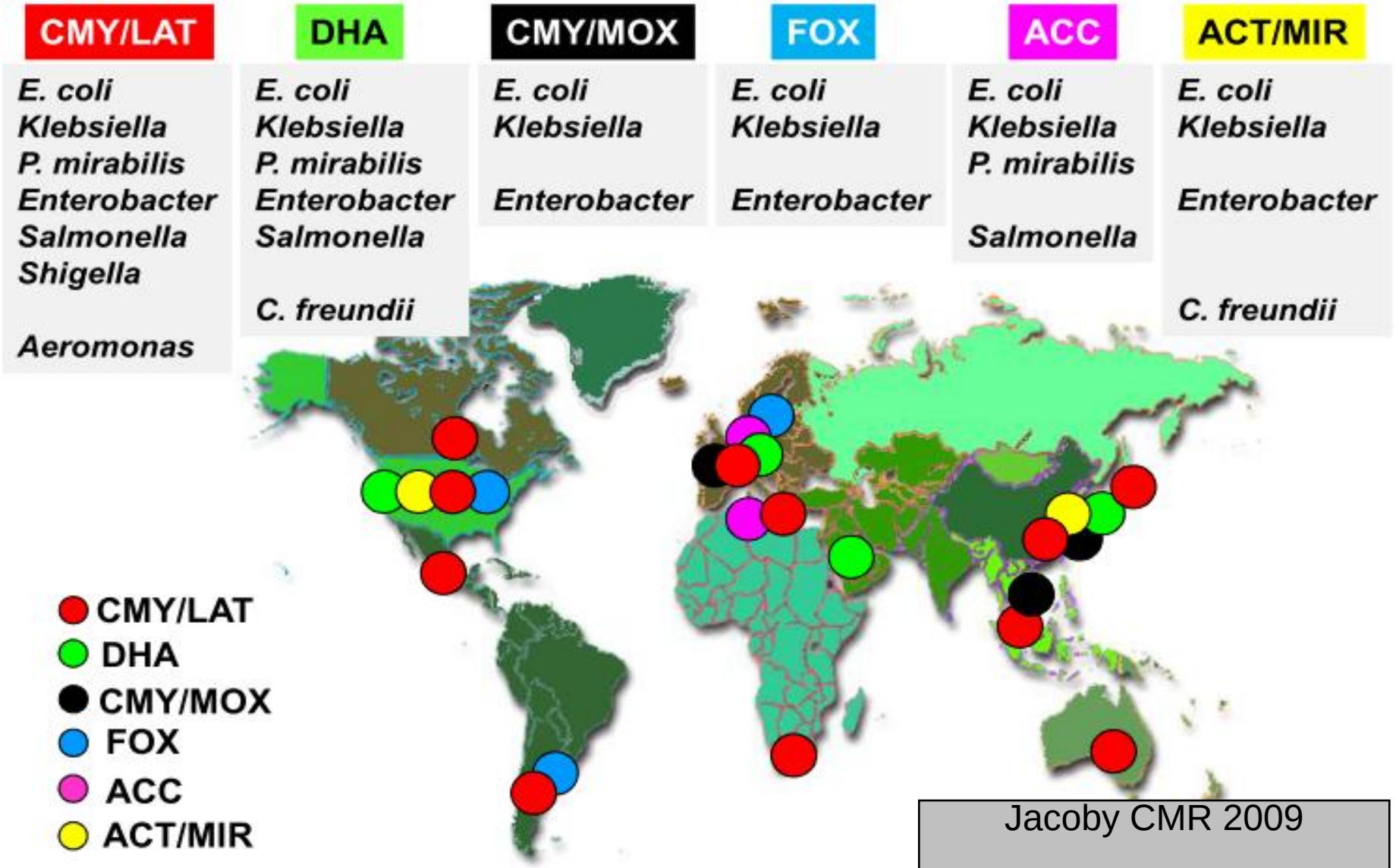
Rodriguez Bano, JCM 2004

Plazmid Kaynaklı AmpC Tipleri

- CMY (53)
- LAT (4)
- MIR (5)
- ACT (8)
- ACC (4)
- FOX (7)
- MOX (8)
- DHA (4)
- CFE (1)
- LAT (1)…



Plazmidik AmpC'nin dünyadaki yayılımı



Jacoby CMR 2009

Neden β-laktamazları belirlemeliyiz?

- Uygun antibiyotik tedavisi
- Enfeksiyon kontrolü
- Epidemiyolojik araştırmalar

Niçin karbapenem direncine neden olan mekanizmaları belirlemeliyiz?

- Porin kayıbı ile birlikte Sınıf A GSBL (CTX-M) + azalmış permeabilite
- Sınıf C aşırı AmpC üretimi + azalmış permeabilite

  Bu özelliğe sahip suşlar ertapenemi meropenem veya imipenemden daha fazla hidrolize eder
- Bu durumda bütün karbapenemlerin dirençli bildirilmesi gerekmez
- Duyarlı karbapenemler duyarlı olarak bildirilmelidir.
- Bazı izolatlar rutin duyarlılık testlerinde duyarlı görülseler de direnç genlerini taşıyor olabilir

Direnç neden yayılıyor?

- Suboptimal Saptama
- Moleküler faktörler
- Antibiyotiklerin seçici baskısı

Otomatize sistemler karbapenem direncini saptamada yetersiz

Table 2. Summary of antimicrobial susceptibility testing results for 15 test isolates*

Method (software)	Card/panel	Imipenem results (n = 15)			Meropenem results (n = 15)		
		Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
Broth microdilution	In-house frozen panel	13	2	0	14	1	0
Disk diffusion	BDDS disks	3	11	1	10	5	0
MicroScan (LabPro1.51, Alert 1.50)	Neg combo 32	7	7	1	13	1	1
Phoenix (4.05W/3.81A)	NMIC/ ID-104	5	8	2	12	1	2
Sensititre AutoReader (3.0.8 SP2)	GN2F	0	2	13	0	3	12
VITEK (R10.01)	Superflex GNS 122 and 127	5	0	10	2	3	10
VITEK 2* (R04.01)	GN07	4	6	5	4	4	5

*No meropenem interpretations were given by the Advanced Expert System for 2 organisms.

For DNA sequence determination, a 989-bp PCR prod- (data not shown). The 15 isolates, which were collected

Comparison of BD Phoenix, Vitek 2, and MicroScan Automated Systems for Detection and Inference of Mechanisms Responsible for Carbapenem Resistance in *Enterobacteriaceae*▽

Neil Woodford,[1]* Anne T. Eastaway,[2] Michael Ford,[3] Alistair Leanord,[4] Chloe Keane,[4] Reinhard M. Quayle,[5] Jane A. Steer,[5] Jiancher

Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infe
HAI & Infection Control Group, Health Protection Scotland, G

OXA-48 zayıf saptanıyor

TABLE 2. Ability of commercial systems to infer carbapenema
defined carbapenem resistan mechanisms[a]

Mechanism	No. of isolates							
	Phoenix		MicroScan NM36		MicroScan NBC39		Vitek 2	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
KPC (n = 8)	8	0	8	0	8	0	8	0
MBL (IMP, VIM, or NDM; n = 20)	20	0	19	1[b]	20	0	16	4[b]
OXA-48 (n = 11)	11	0	6	5	4	7	5	6
E. coli/*Klebsiella* spp., ESBL, porin loss (n = 10)	10	0	10	0	8	2	8	2
Enterobacter spp., AmpC/ESBL, porin loss (n = 6)	6	0	5	1	5	1	2	4

[a] There were 55 test isolates. Phoenix flagged likely carbapenemase producers as potentially having an MBL, whereas the MicroScan flagged them as potentially having KPC enzymes. These inferences were taken to mean carbapenemase positive.

[b] All IMP types.

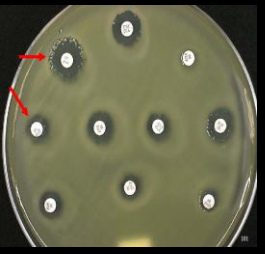
ENTEROBACTERIACEAE'DE HETEROJEN KARBAPENEM DİRENCİ

	AMX	AMC	PIP/TZB	CTX	CAZ	IMP	ERT	AZT
KPC	R	I	R	R	R	S/I/R	I/R	R
IMP/VIM/NDM	R	R	S/I	R	I/R	S/I/R	I/R	S
OXA-48	R	R	R	S	I/S	I/S	I/S	S

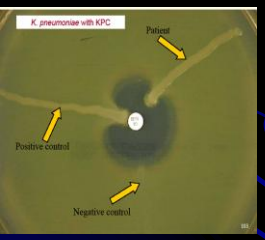
	mg/L		
	İmipenem	Meropenem	Ertapenem
Enterobacteriaceae KPC	0,5 ; >64	1 ; 64	0,5 ; >64
Enterobacteriaceae MBL	0,5 ; >64	0,25 ; >64	0,5 ; >32
Enterobacteriacae OXA-48	1 ; >64	0,5 ; 64	4 ; >64

Nordmann P, Carrer A. **Carbapenemases in enterobacteriaceae.** Arch Pediatr. 2010 Sep;17 Suppl 4:S154-62

Enterobacteriacea'de karbapenemaz üreten suşların saptanmasında kullanılan fenotipik tarama ve doğrulama testleri



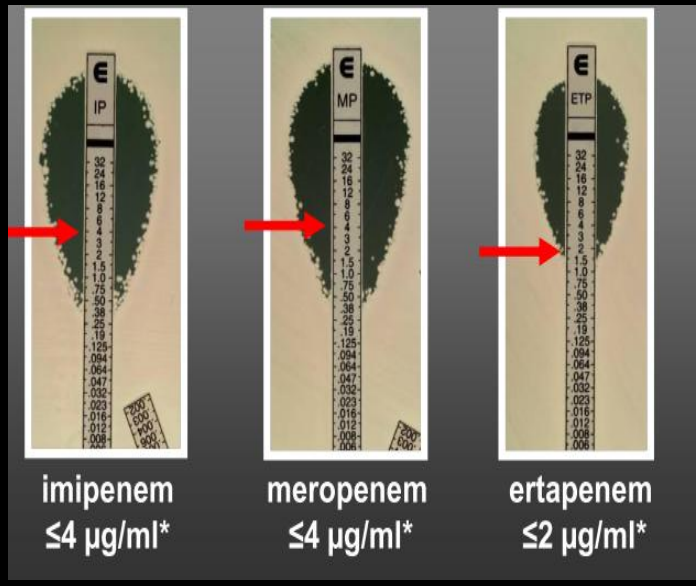
- Disk zon çapı <22 mm ertapenem/ meropenem
- MİK >1 µg/ml imipenem, ertapenem veya meropenem

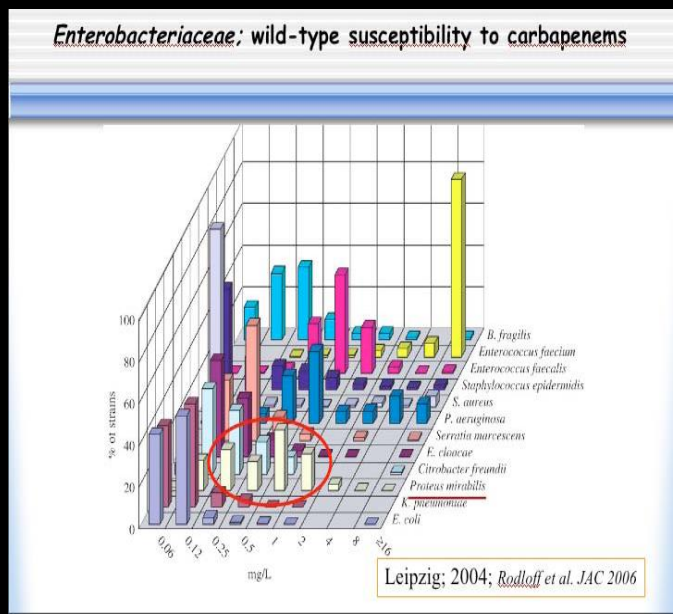


- CLSI, Doğrulama Testi
  (Modifiye Hodge Testi)

- Imipenem disk tarama için uygun değil
- Imipenem MİK'i hafif yükselmiş değerler *Proteus/Providencia/Morganella*

CLSI-M100-S19, Jan 2009)



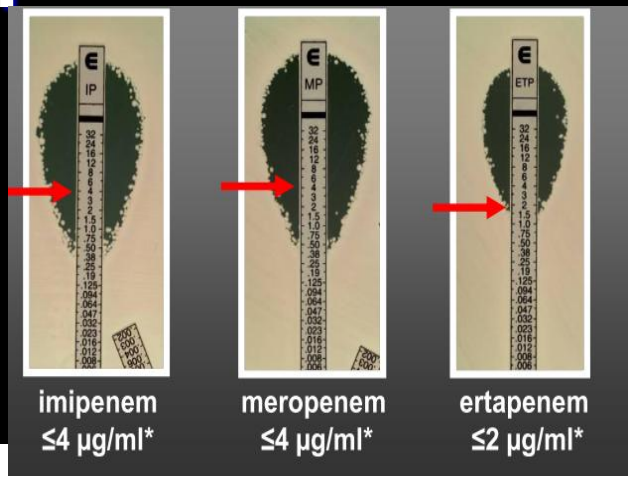


CLSI ve EUCAST karbapenem breakpoints

TABLE 2. CLSI and EUCAST carbapenem clinical breakpoints and epidemiological cut-off values (ECOFFs) (MIC values, mg/L)

Organisms	CLSI S (≤)	CLSI R (≥)	EUCAST S (≤)	EUCAST R (>)	EUCAST ECOFF (>)
Enterobacteriaceae					
Imipenem	4	8	2	8	1–4
Meropenem	4	8	2	8	0.125–0.25
Ertapenem	2	4	0.5	1	0.064
Doripenem	ND	ND	1	4	0.064
Pseudomonas aeruginosa					
Imipenem	4	16	4	8	4
Meropenem	4	16	2	8	2
Doripenem	ND	ND	1	4	1
Acinetobacter spp.					
Imipenem	4	16	2	8	1
Meropenem	4	16	2	8	2
Doripenem	ND	ND	1	4	1

ND, not defined.



	FDA	CLSI (2010)		EUCAST (EMEA) (2010)		
	S	S	R	S	R	ECOFF
Imipenem	≤4	≤1	≥4	≤2	>8	≤1
Meropenem	≤4	≤1	≥4	≤2	>8	≤0.125
Ertapenem	≤2	≤0.25	≥1	≤0.5	>1	≤0.06
Doripenem	≤0.5	≤1	≥4	≤1	>4	≤0.12

- Fenotipik testler bu enzimlerin spesifik inhibitörleri kullanılarak belirlenebilmektedir

- Klinik laboratuvarlarda bu testlerin rutin olarak hızlı ve doğru uygulanması

- **Klinik ve epidemiyolojik açıdan önemlidir**

Plazmidik AmpC enzimlerinin tanınması neden önemli?

- Hastane içinde diğer mikroorganizmalara yayılma riski

- AmpC'yi yüksek düzeyde üreten suşlar
- *in vitro* bazı sefalosporinlere ve aztreonama duyarlı Tedavide başarısızlıklar

Clin Microbiol Rev (2009) 22:161-182.

AmpC'nin fenotipik Saptanması

- AmpC'nin saptanması için CLSI veya diğer onaylanmış bir kriter yok
- Sefoksitine direnç

  E.coli, K.pneumoniae, P.mirabilis,

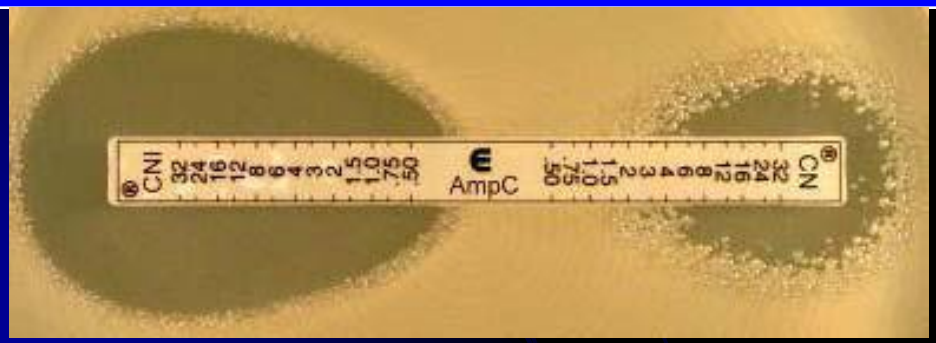
  Salmonella ve Shigella
- Kromozomal ve Kazanılmış AmpC enzimlerinin ayırımı yapılamıyor *E.coli ve Shigella*

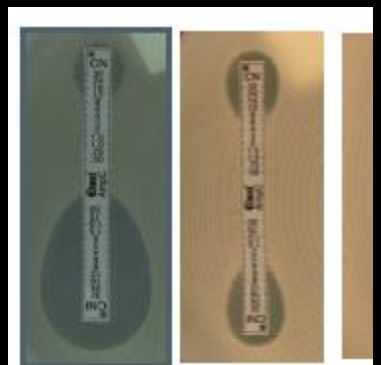
- β-laktam inhibitörleri (Kloksasilin)
- Non β-laktam inhibitörleri (Boronik asit)
- Modifiye Hodge Testi (Sefoksitin)

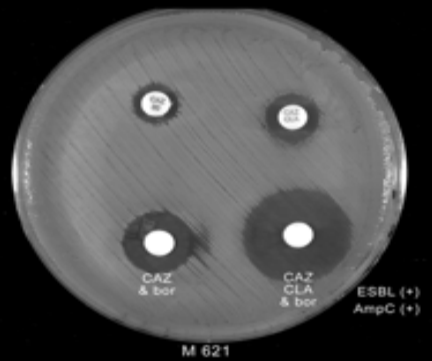
AmpC'nin saptanması- Fenotipik

Sefoksitin diski+ 500 µg kloksasilin
≥4 mm zon çapında artış
Duyarlılığı ve özgüllüğü %95
E-Test
Kazanılmış AmpC için
Duyarlılığı ve özgüllüğü %97

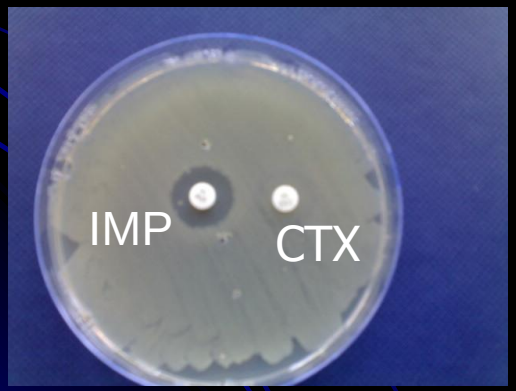
AAC (2009:53:146



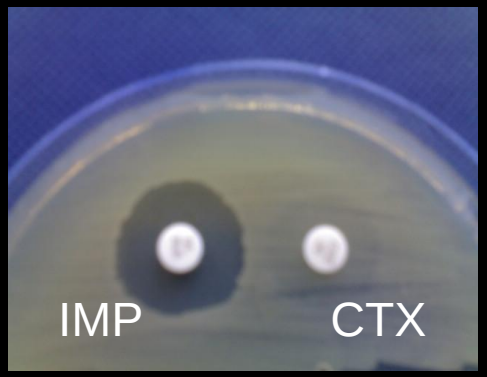




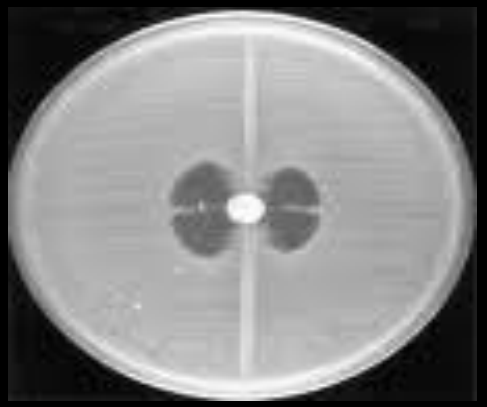
- Kloksasilinli besiyeri (250 µg/ml)
- Modifiye Hodge Testi



Mueller Hinton agar



Kloksasinli besiyeri



Modifiye Hodge testi

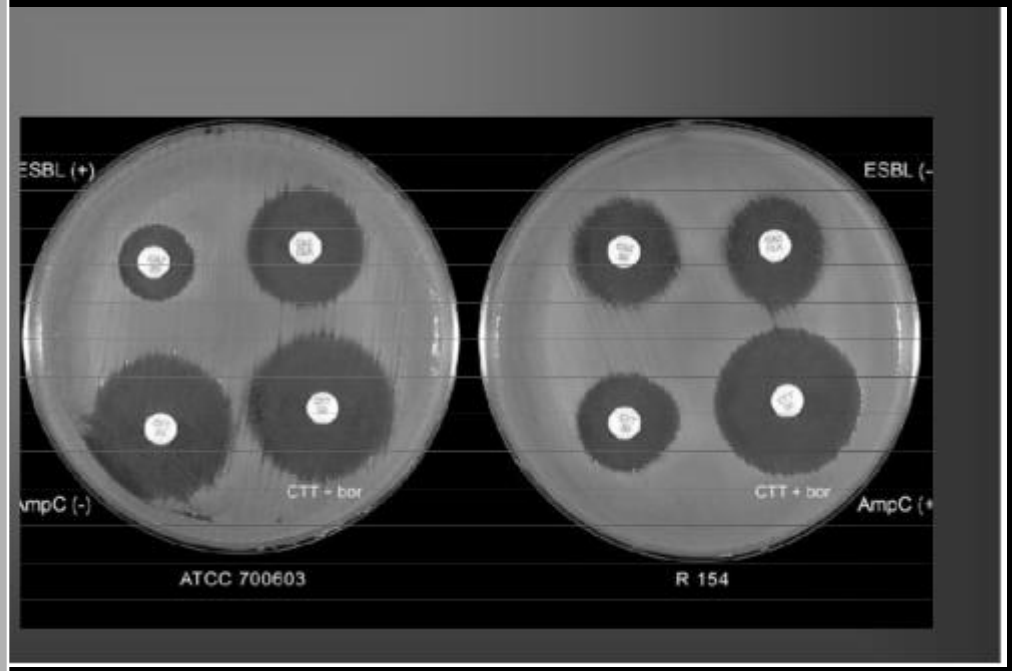
AmpC'nin saptanması-Fenotipik

Boronik asit

120 µg fenilboronik asit 3 ml DMSO

20 µl sefotetan (30 µg) diskine ilave

Zon çapı ≥5 mm



JCM 2005:43:4163-67

TABLE 1: Performance of Etest AmpC strips vs. Genotype/Phenotype Result

Organism	AmpC +[1]	AmpC -	% Sensitivity [2]		% Specificity [3]	
			CN/CNI	FX/FXI	CN/CNI	FX/FXI
C. freundii	2	0	100	0	100	0
E. coli	84	18	87 (97)	64 (83)	89 (91)	69 (84)
K. pneumoniae	87	18	91 (94)	60 (92)	92 (95)	67 (93)
Enterobacter spp	12	0	83	58	83	58
P. mirabilis	0	1	NA	NA	100	100
P. stuartii	1	0	0	0	0	0
S. marcescens	3	0	100	33	100	33
S. typhimurium	1	0	100	0	100	0
Total	**190**	**37**	**88 (91)**	**60 (83)**	**90 (93)**	**67 (87)**

[1] AmpC genotype or phenotype positive

[2] Sensitivity = AmpC phenotype by Etest/reference genotype.

[3] Specificity = Correct Etest phenotype results (+ or -) /reference results

46. ICAAC 2006 D0451

Plazmidik AmpC üreten suşlar

Strains (n)	AmpC Gold status (n)	CN/CN-Clox			CN/CN-Bor			FX/FX-Clo			FX/FX-Bor		
		+	-	ND	+	-	ND	+	-	ND	+	-	ND
E. coli (103)	+ (61)	59	0	2	56	3	2	50	5	6	40	9	12
	- (42)	0	42	0	0	42	0	0	41	1	0	41	1
K. pneumoniae (23)	+ (2)	2	0	0	2	0	0	1	0	1	1	0	1
	- (21)	0	18	3	0	18	3	0	18	3	0	18	3
K. oxytoca (7)	- (7)	0	7	0	0	7	0	0	7	0	0	7	0

Kromozomal AmpC üreten suşlar

Strains (n)	AmpC Gold status (n)	CN/CN-Clox			CN/CN-Bor			FX/FX-Clo			FX/FX-Bor		
		+	-	ND	+	-	ND	+	-	ND	+	-	ND
E. aerogenes (46)	+ (44)	35	2	7	37	5	2	20	3	21	2	4	38
	- (2)	0	2	0	0	2	0	0	2	0	0	2	0
C. freundii (13)	+ (13)	9	4	0	9	4	0	6	2	5	4	2	7
E. cloacae (28)	+ (28)	16	2	10	13	2	13	4	1	23	0	4	24
Morganella spp. (6)	+ (6)	3	3	0	2	4	0	0	6	0	0	6	0
S. marcescens (16)	+ (16)	6	10	0	7	9	0	0	13	3	0	13	3

Plazmidik AmpC üreten suşlar

Strains (n)	AmpC Gold status (n)	CN/CN-Clox		CN/CN-Bor		FX/FX-Clo		FX/FX-Bor	
		Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec
E. coli (103)	+ (61)	97	100	92	100	82	98	68	98
	- (42)								
K. pneumoniae (23)	+ (2)	100	86	100	86	50	86	50	86
	- (21)								
K. oxytoca (7)	- (7)	NA	100	NA	100	NA	100	NA	100
Total	**+ (63)**	**97**	**96**	**92**	**96**	**81**	**94**	**65**	**94**
	- (70)								

Kromozomal AmpC üreten suşlar

Total	**+ (107)**	**64**	**100**	**64**	**100**	**28**	**100**	**6**	**100**
	- (2)								

AmpC Saptanması-Fenotipik

Test suşu

E.cloacae, AmpC derepresse

Duyarlı suş

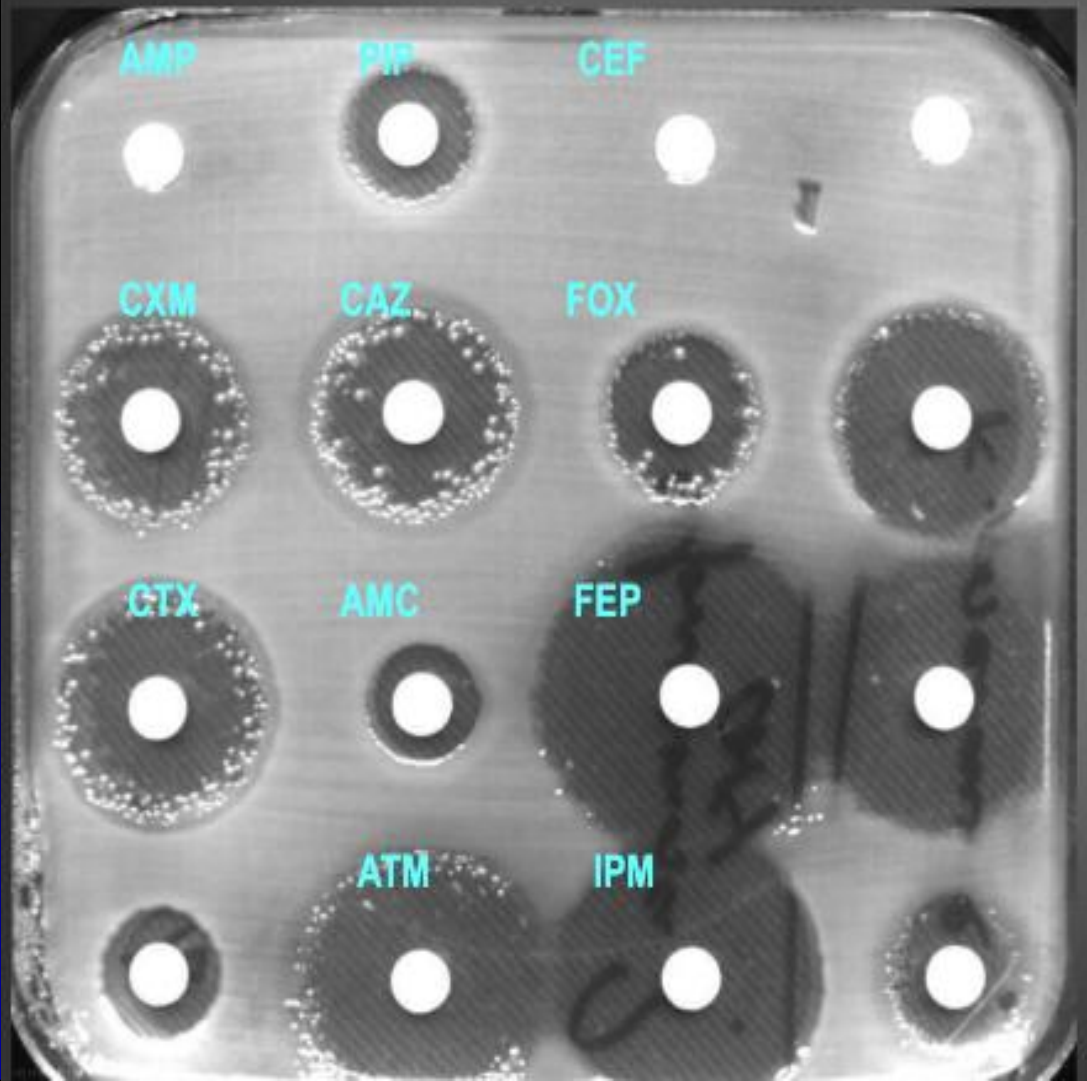
E.Coli ATCC 25922

Disk

Sefoksitin

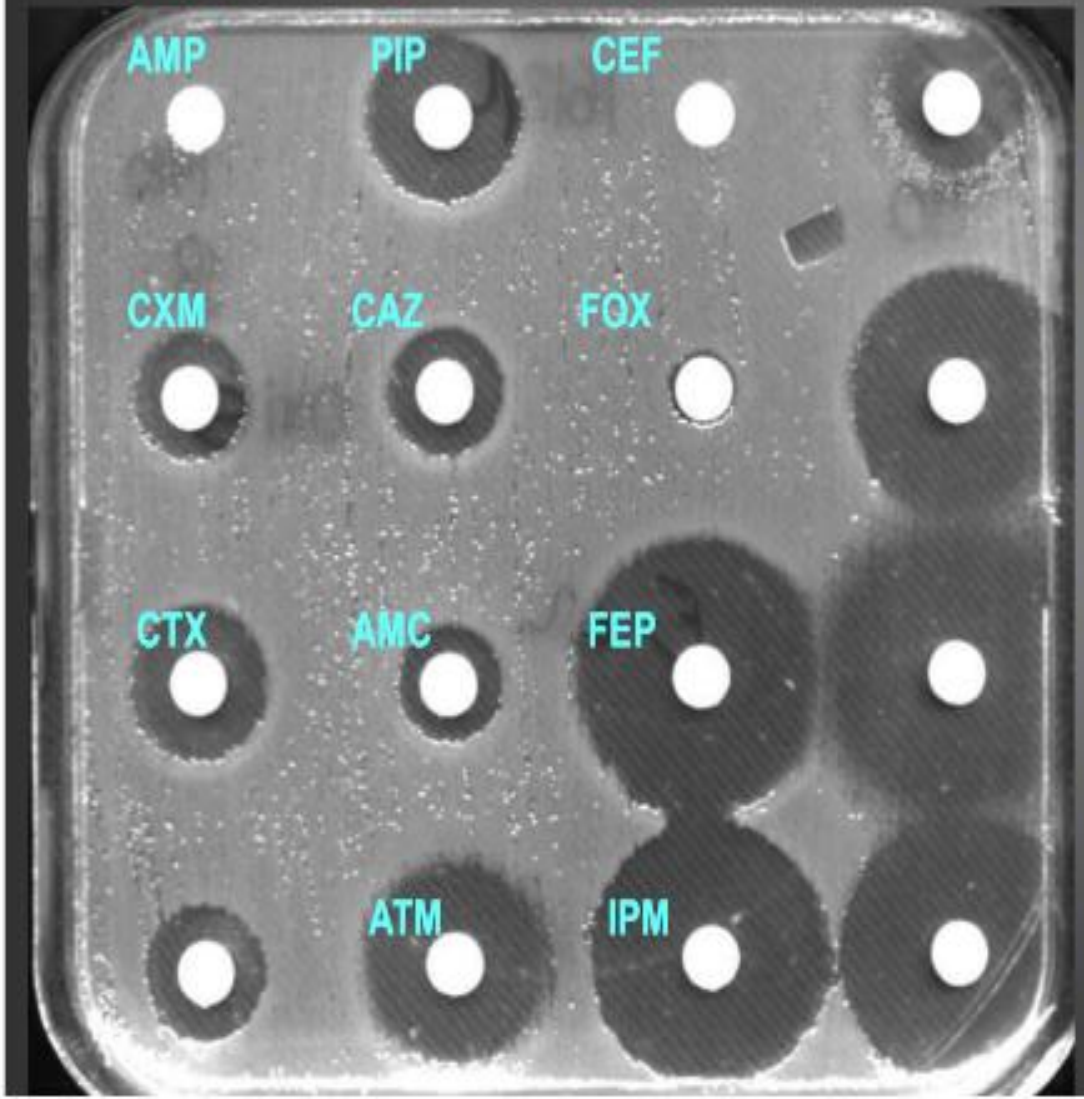
Kazanılmış AmpC'nin saptanması

(*K.pneumoniae* CMY-2)



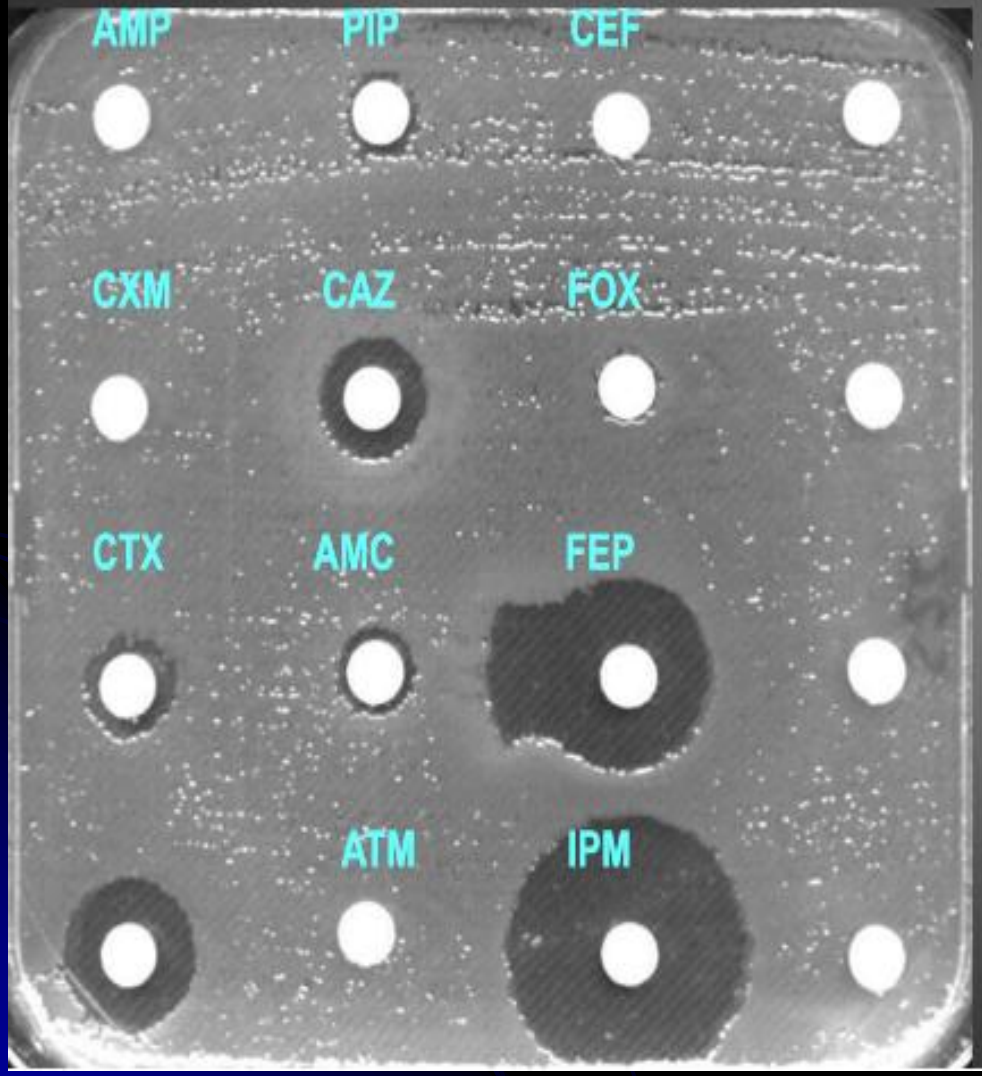
	MIC	Real	Interpr
Ampicillin	>256	R	R
Ticarcillin	64->256	R	R
Piperacillin	32->256	R	R
Piper/Tazo	32- >256	I/R	R
Amox / clav	>256	R	R
Cephalotin	>256	R	R
Cefoxitin	32->256	R	R
Cefuroxime	32->256	R	R
Cefotaxime	2->256	r/R	R
Ceftazidime	2->256	r/R	R
Cefepime	0,12-1	S	S
Aztreonam	2->256	r/R	R
Imipenem	0,06-0.12	S	S

AmpC enziminin aşırı üretimi *E.coli*



	CIM	Real	Interpr
Ampicillin	32->256	R	R
Ticarcillin	8-32	r	I/R
Piperacillin	8-32	r	I/R
Piper/Tazo	8-32	r	I/R
Amox/clav	32->256	R	R
Cephalotin	32->256	R	R
Cefoxitine	16->256	R	R
Cefuroxime	16->256	R	R
Cefotaxine	0,25-2	r	I/R
Ceftazidime	1-4	r	I/R
Cefepime	0,06-0.12	S	S
Aztreonam	0,25-2	r	I/R
Imipenem	0,06-0.12	S	S

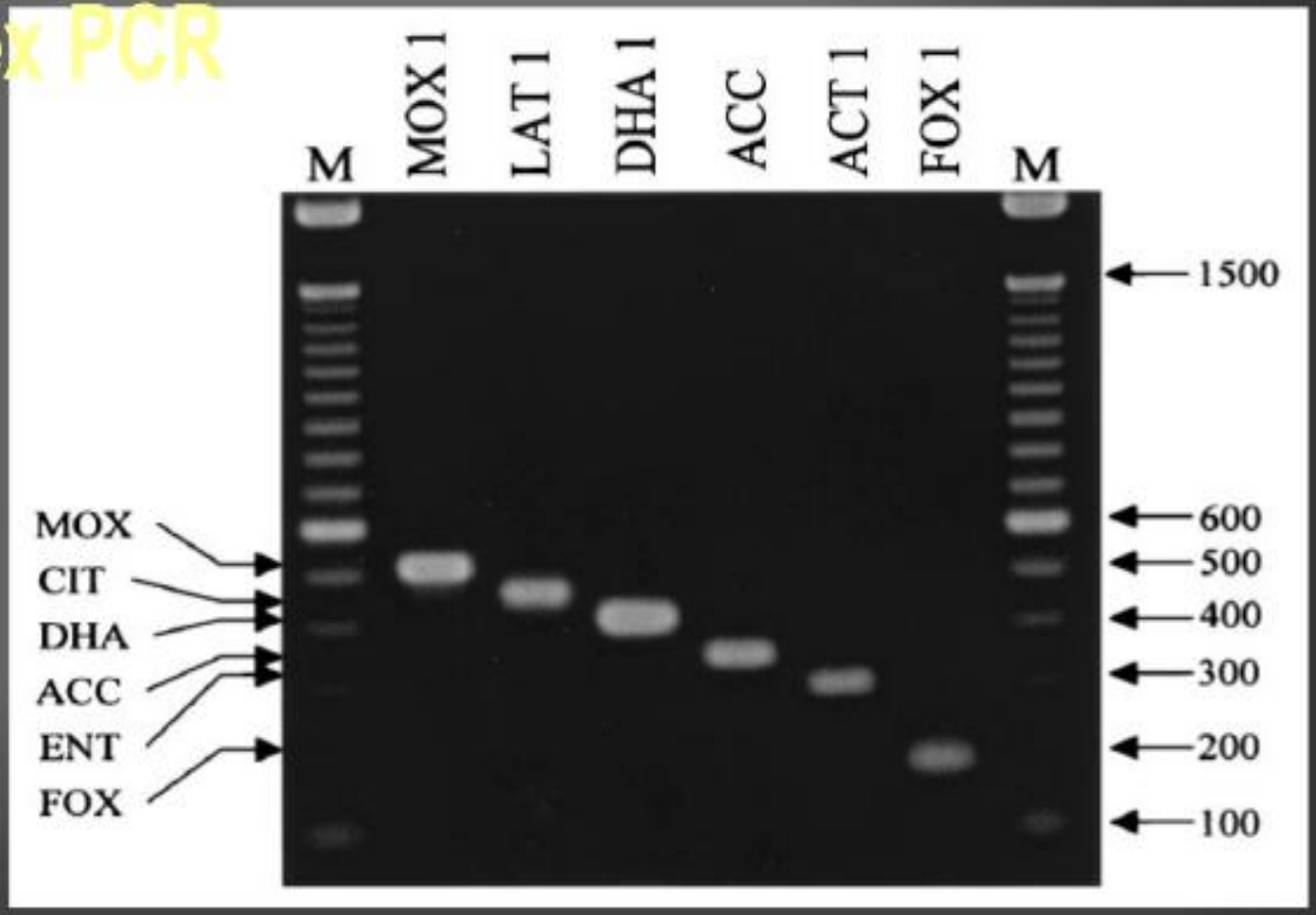
AmpC+GSBL *E.cloacae* SHV-2



	MIC	Real	Interpr
Ampicillin	>256	R	R
Ticarcillin	>256	R	R
Piperacillin	>256	R	R
Piper/Tazo	>256	R	R
Amox/clav	>256	R	R
Cephalotin	>256	R	R
Cefoxitin	>256	R	R
Cefuroxime	>256	R	R
Cefotaxine	16->256	r/R	R
Ceftazidime	8->256	r/R	R
Cefepime	1->256	r/R	R
Aztreonam	8->256	r/R	R
Imipenem	0,12-0,25	S	S

Kazanılmış AmpC'nin Genotipik saptanması (Multipleks PCR)

- Multiplex PCR



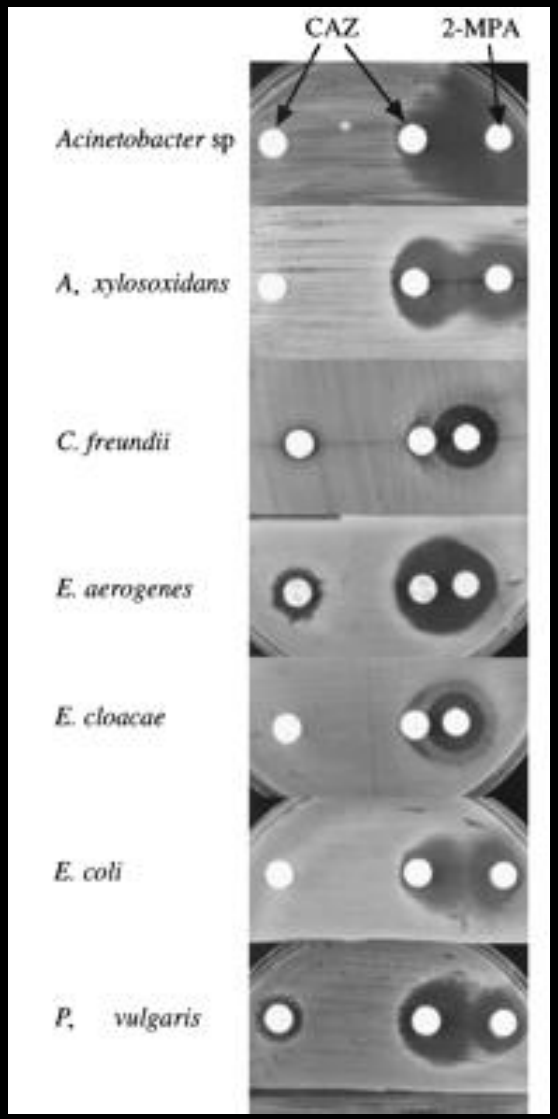
PEREZ-PEREZ&HANSON, JCM 2002:40:2153

MBL'nin Fenotipik Saptanması

- Çift disk sinerji
- Kombine disk
- MBL E-test
- İmipenem/EDTA mikrodilüsyon yöntemi

EDTA, 2-MPA

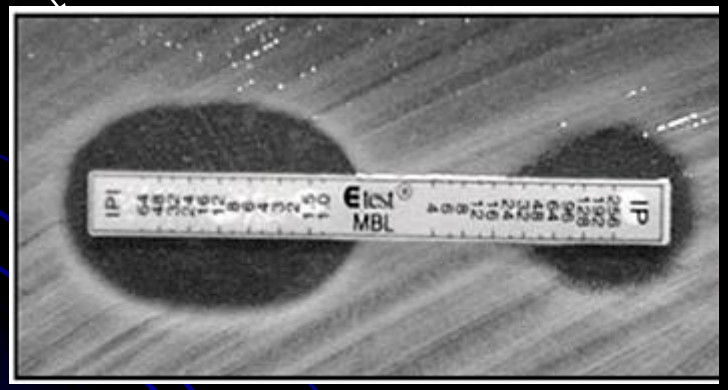
Seftazidim, imipenem

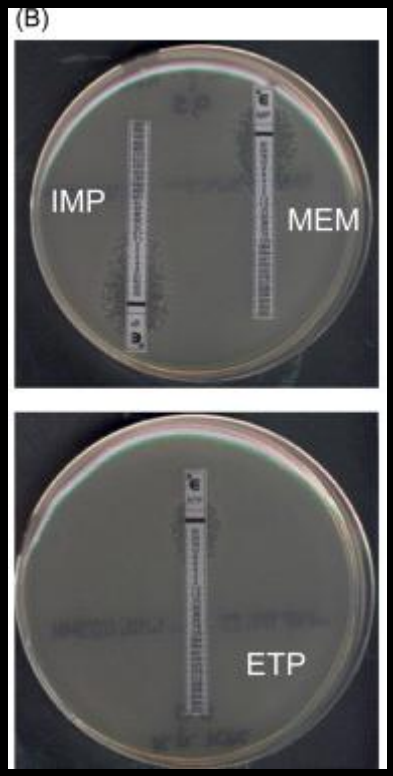


Walsh *et al*. JCM, 2002, 2755-9

MBL E test

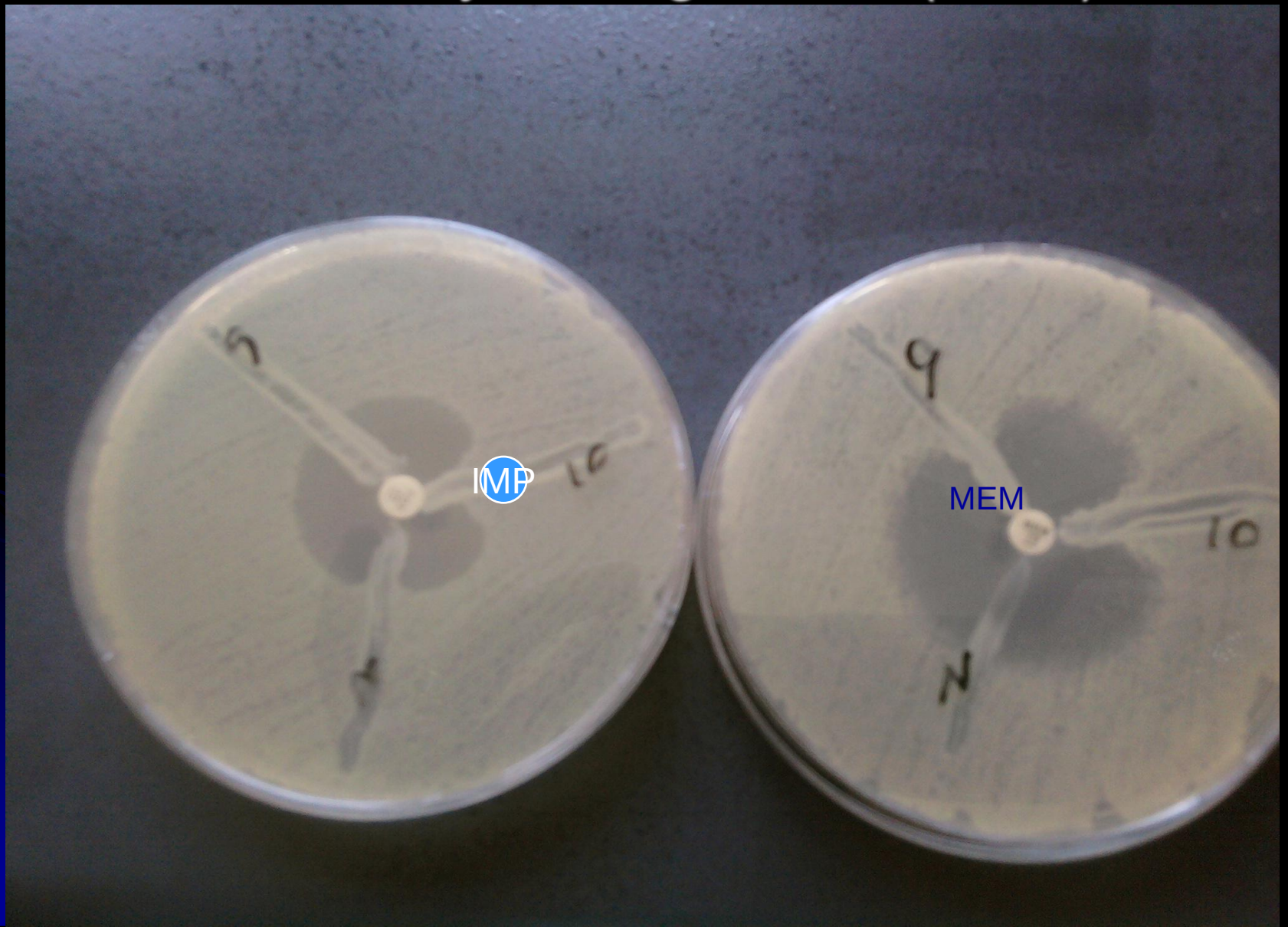
- **MBL E Test Stripleri** (AB BIODISK, Sweden)
- 4–256 µg/ml imipenem,
- 1–64 µg/ml imipenem+EDTA
- ≥8 kat azalma





-Franklin C. *J Clin. Microbiol.* 2006; 44: 139-3144.
-Lee K. *Clin Microbiol Infect* 2001: 7: 88-91.
-Walsh *et al*. JCM, 2002, 2755-9

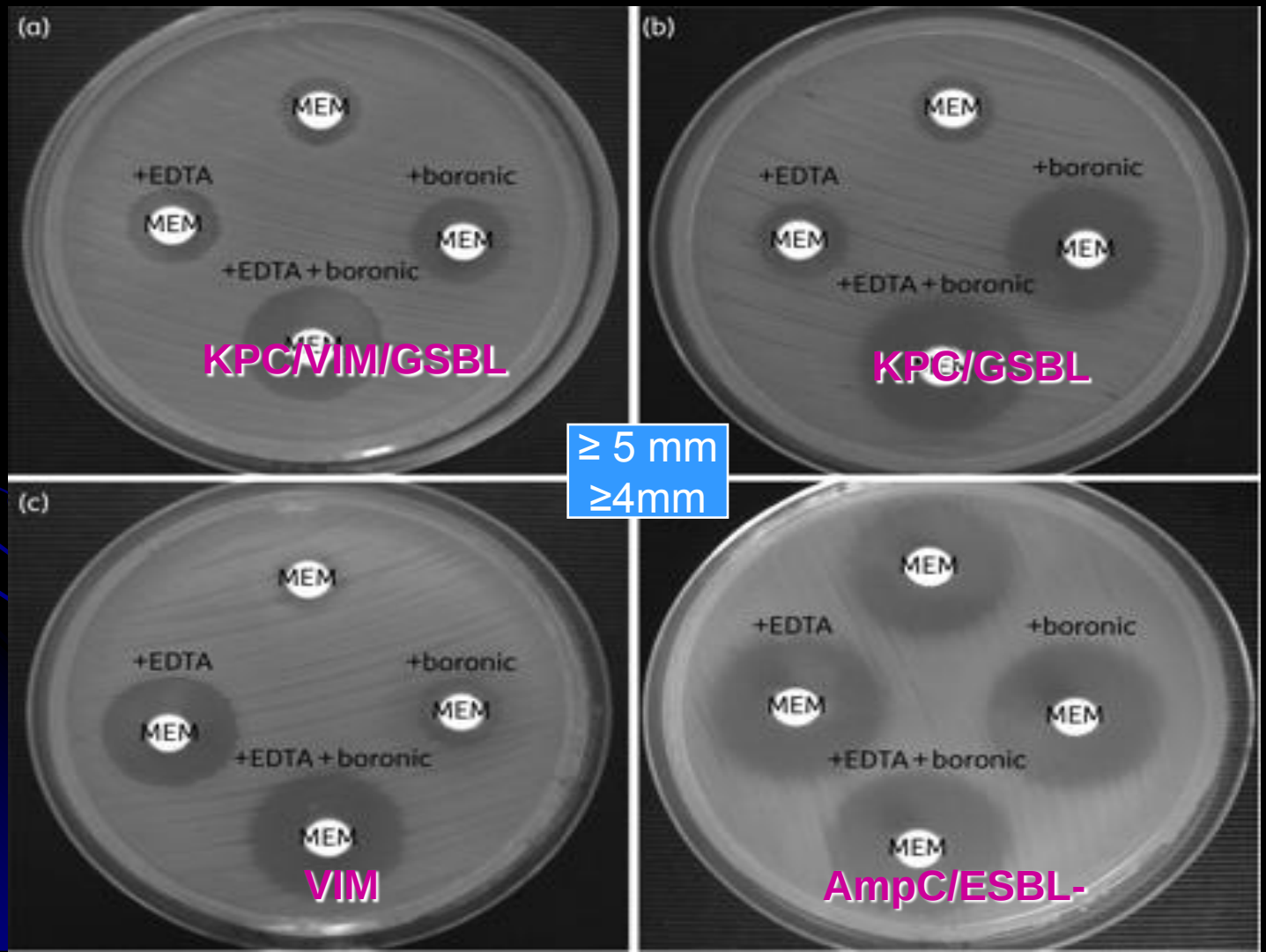
Modifiye Hodge Testi (MHT)



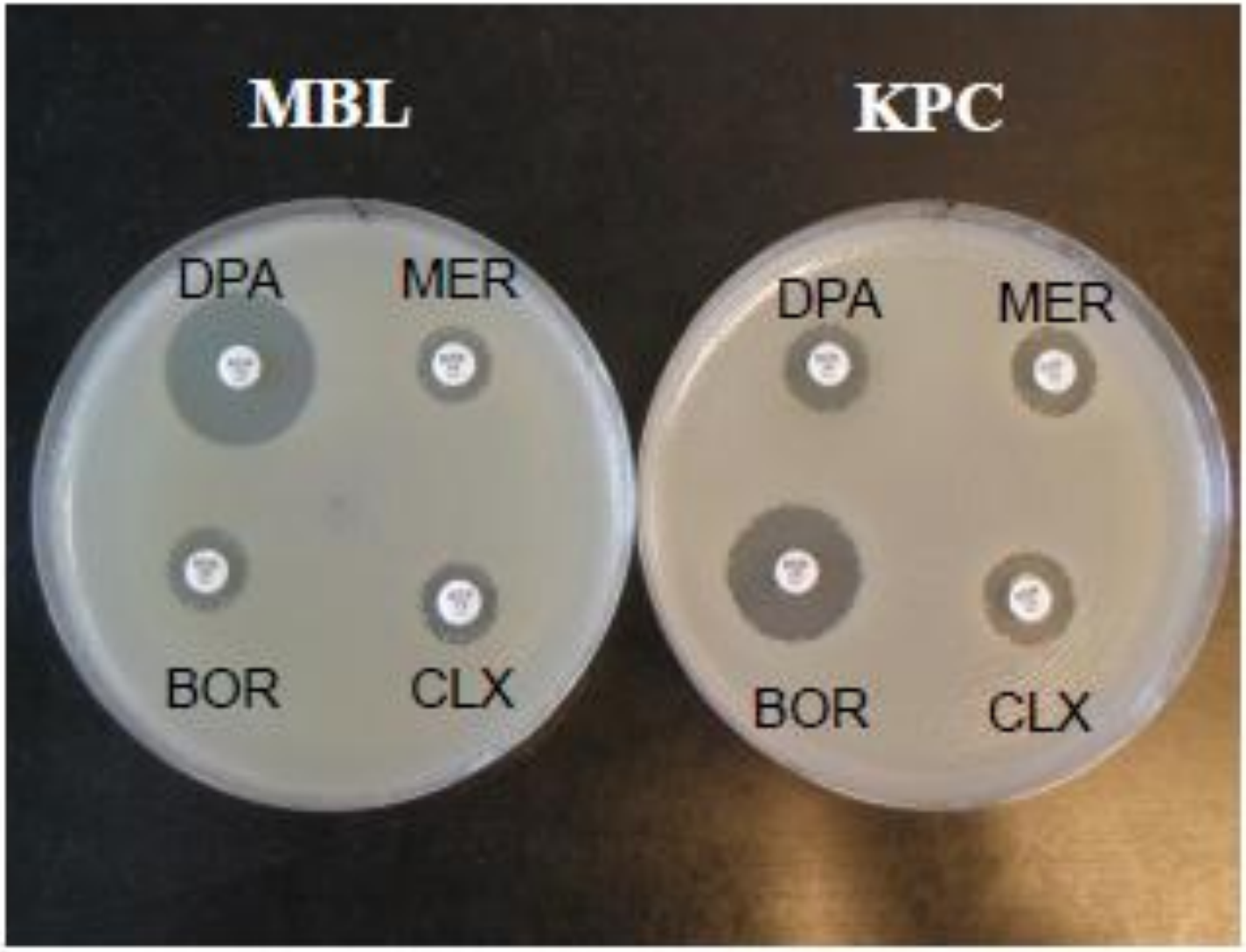
Kombine-disk testleri

Meropenem (MEM) ,

MEM+EDTA, MEM+fenilboronik asit , MEM+ EDTA+fenilboronik asit



Pozitif fenotipik testler



Karbapenemazların fenotipik olarak saptanması

B-laktamaz	İnhibisyon		
	EDTA/DPA	**Boronik asit**	**Kloksasilin**
MBL	Evet	Hayır	Hayır
KPC/Sınıf A karbapenemazlar	Hayır	Evet	Hayır
OXA-48	Hayır	Hayır	Hayır
GSBL	Hayır	Hayır	Hayır
AmpC	Hayır	Evet	Evet

Zon çaplarında artış

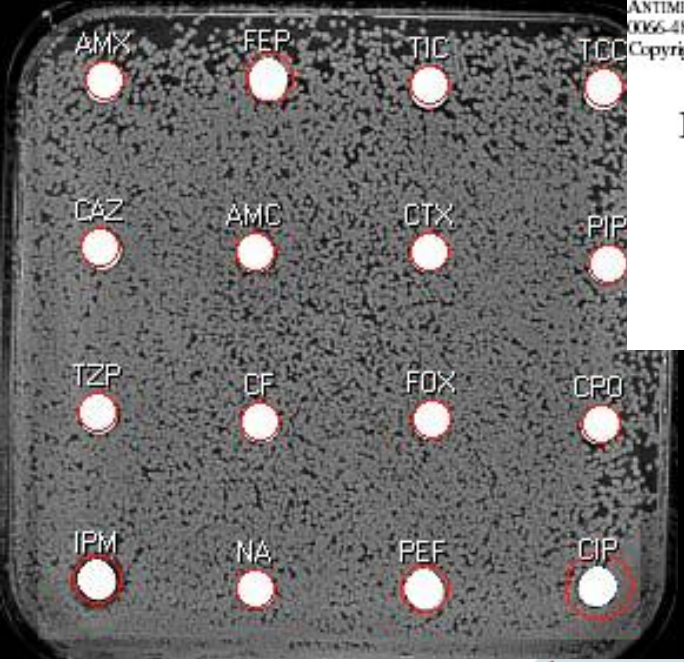
β-lactamase	Zon çapında artış		
	DPA	Boronic acid	Cloxacillin
MBL (n=25)	5-15	-1-2	0-2
KPC (n=34)	-1-3	4-16	0-2
OXA-48 (n=9)	-4-4	-1-4	0-2
ESBL (n=9)	-3-2	-2-3	-1-0
AmpC (n=9)	-3-1	1-7	0-7

Giske et al. CMI 2010. In press

Fenotipik Testlerin Duyarlılık ve Özgüllüğü

TEST	β-laktamaz	Duyarlılık	Özgüllük
APBA	KPC	100%	98%
APBA+CLX	AmpC	80%	100%
DPA	MBL	100%	100%
EDTA	MBL	100%	88%
Modified cloverleaf (Hodge) test	Carbapene-mases	100%	77%

Giske et al. CMI 2010. In press



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2006, p. 4198–4201 Vol. 50, No. 12
0066-4804/06/$08.00+0 doi:10.1128/AAC.00663-06

Emergence of Multidrug-Resistant *Klebsiella pneumoniae* Isolates Producing VIM-4 Metallo-β-Lactamase, CTX-M-15 Extended-Spectrum β-Lactamase, and CMY-4 AmpC β-Lactamase in a Tunisian University Hospital▽

Sonia Ktari,[1] Guillaume Arlet,[2]* Basma Mnif,[1] Valérie Gautier,[2] Fouzia Mahjoubi,[1] Mounir Ben Jmeaa,[3] Mounir Bouaziz,[4] and Adnane Hammami[1]

ORIGINAL ARTICLE ◀ Previous Article | ToC | Next Article ▶

Year : 2009 | Volume : 52 | Issue : 3 | Page : 339-342

Ventilator-associated pneumonia caused by carbapenem-resistant Enterobacteriaceae carrying multiple metallo-beta-lactamase genes

Mayank Dwivedi[1], Anshuman Mishra[2], Afzal Azim[3], RK Singh[3], AK Baronia[3], KN Prasad[1], TN Dhole[1], UN Dwivedi[2]

[1] Department of Microbiology, SGPGIMS, Lucknow, India
[2] Department of Biochemistry, Lucknow University, India
[3] Department of Critical Care Medicine, SGPGIMS, Lucknow, India

K.pn:IMP, SIM-1

Journal of Antimicrobial Chemotherapy (2006) 57, 142–145
doi:10.1093/jac/dki389
Advance Access publication 10 November 2005

JAC

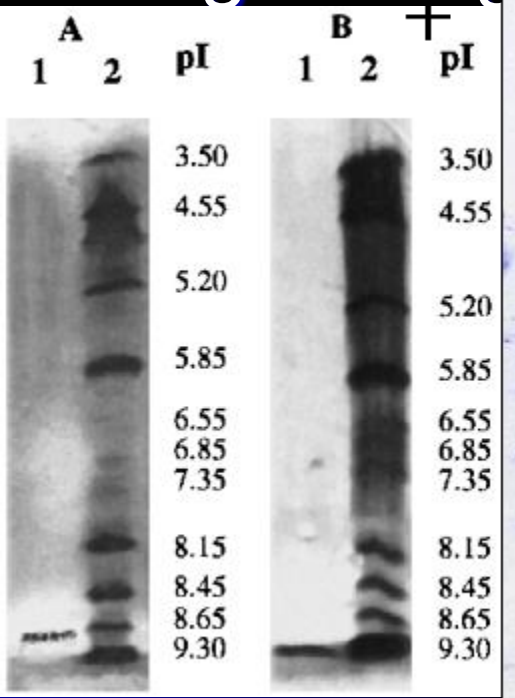
First outbreak of multidrug-resistant *Klebsiella pneumoniae* carrying bla_{VIM-1} and bla_{SHV-5} in a French university hospital

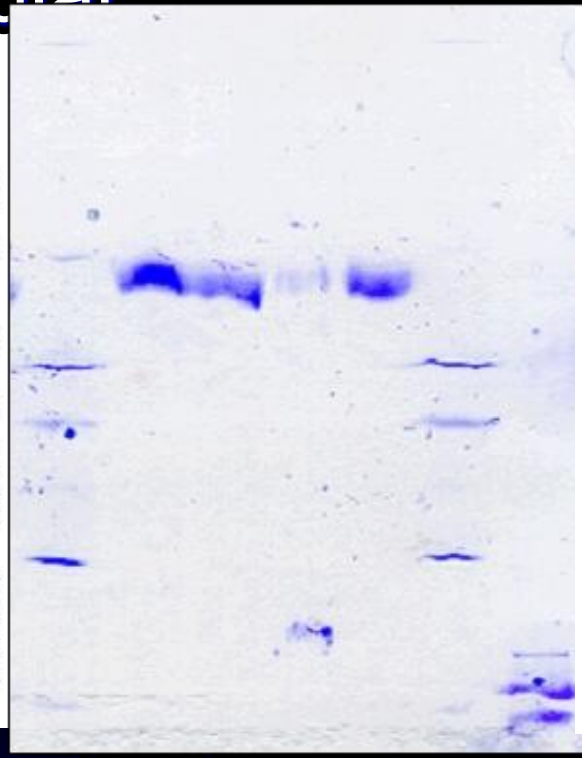
Najiby Kassis-Chikhani[1,2], Dominique Decré[3]*, Valérie Gautier[3], Béatrice Burghoffer[3], Faouzi Saliba[4], Daniele Mathieu[1], Didier Samuel[4], Denis Castaing[4], Jean-Claude Petit[3], Elisabeth Dussaix[1] and Guillaume Arlet[3]

Poirel, Pham & Nordmann, Pathology, 2004, 36; 266-7.

İzoelektrik Odaklama Yöntemi (IEF)

- Biribirinden anlamlı şekilde farklı olan enzimleri pI değerleri hakkında bilgiler sağlar





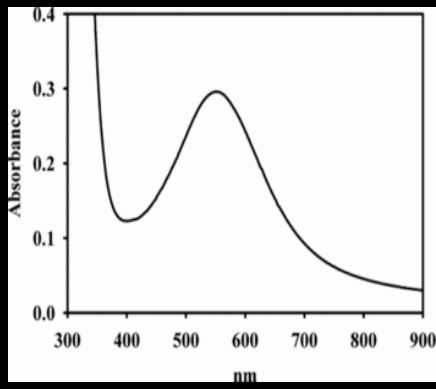
5.2	TEM-55 TEM-57 TEM-58 TEM-70 TEM-122 TEM-125
5.3	TEM-12 TEM-25 PER-1
5.4	TEM-7 TEM-19 TEM-20 TEM-29 TEM-112 TEM-126
5.5	TEM-9 TEM-17 OXA-18
5.6	TEM-5 TEM-10 TEM-11 TEM-13 TEM-26 TEM-50 TEM-59 TEM-63
5.7	TEM-68
5.8	TEM-42 GES-1 GES-2 IBC-2
5.9	TEM-4 TEM-6 TEM-8 TEM-27 TEM-69 TEM-72 TEM-114
6	TEM-15 TEM-47 TEM-48 TEM-49 TEM-52 TEM-66 TEM-109
6.1	TEM-28 TEM-43 OXA-17
6.2	OXA-14 OXA-16
6.3	TEM-3 TEM-16 TEM-18 TEM-22 TEM-113
6.4	TEM-21 TEM-56 TEM-60 OXA-11
6.5	TEM-24 TEM-46 TEM-61
6.6	
6.7	

Enzimatik tarama testi

- İmipenem diski (10 µg)
- İmipenem+10 µl enzim
- İmipenem+10 µl d.su
- Diskler oda ısısında 24 saat inkübe edilir
- 0.5 Mc Farland bulanıklık *E.coli* ATCC 25922 suşu MHA'a inokülasyon ve diskler yerleştirilir
- 35 ºC'de bir gece inkübasyon
- Zon çapı farlılığı ≥2 mm ise pozitif sonuç

Endmiani, JCM, 2010:

Spektrofotometrik Yöntem



- Altın standart
- Bakterilerden ekde edilen enzimlerin
- Spektrofotometre kullanılarak
- Ertapenem, meropenem ve imipenemin hidrolizi
- EDTA gibi bir inhibitör varlığında enzim aktivitesinin inhbisyonu

Kromojenik besiyerleri

- CHROM agar

  Karbapenem MİK> 16 µg/ml olan suşları tespit edebilir

- ChromID GSBL ⟶ IMP/VIM ve KPC tipi karbapenemazlar

  ESBL üretmeyen OXA-48 suşlarını tespit etmede yetersiz

Karbapenemazların Saptanmasında Moleküler Yöntemler

- Klasik PCR
- Real-time PCR
- Multipleks PCR
- Mikroarray teknolojileri
- Dizi analizi
- Klonlama Yöntemleri…

GSBL (TEM, SHV ve CTX-M)

Microarray 8 saat (36 izolat)

3 saat DNA izolasyonu

5 saat ligasyon, amplifikasyon ve saptama

		Sequencing		
		+	-	Total
ESBL	+	104	0	101
	-	4*	111	115
	Total	105	111	216

Specificity: 100 %
Sensitivity: 96 %
PPV: 100 %
NPV: 96 %

Endimiani et al, JCM 2010
Naas et al, AAC 2010
Cohen Stuart et al, JAC 2010

KPC, TEM, SHV ve CTX-M genleri

		Sequencing		
		+	-	Total
ESBL	+	104	0	101
	-	4*	111	115
	Total	105	111	216

Specificity: 100 %
Sensitivity: 96 %
PPV: 100 %
NPV: 96 %

		Sequencing		
		+	-	Total
KPC	+	57	0	57
	-	0	49	49
	Total	57	49	106

Specificity: 100 %
Sensitivity: 100 %
PPV: 100 %
NPV: 100 %

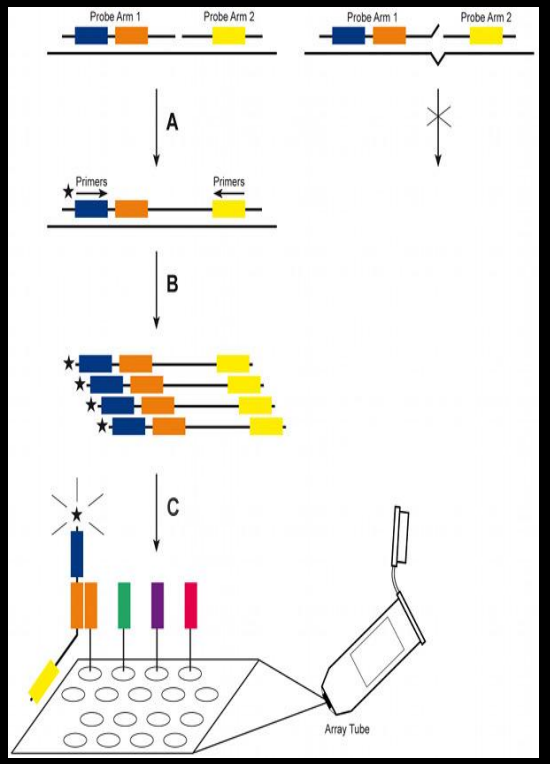
The ESBL Array (Check-Points B.V., Wageningen, The Netherlands)

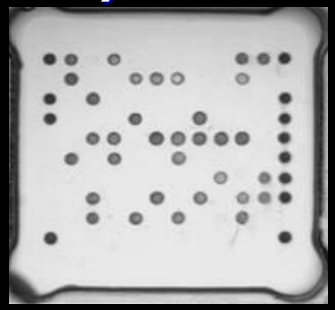
Check-MDR CT101

KPC, CMY, DHA, FOX, MOX, ACC, MIR, ACT and NDM-1

Check-MDR CT102

- Karbapenemazlar
- **KPC, NDM-1**
- GSBL
- **CTX-M, SHV, TEM**
- AmpC
- **CMY, DHA, FOX, MOX, ACC, MIR ACT**





Direnç mekanizması?

- AmpC-plazmidik veya kromozomal?
- GSBL ve AmpC'nin kombinasyonu?
- MBL (VIM veya IMP)?
- NDM-1?
- OXA-48?
- Belirsiz?

E.cloacae
Fenotipik Testlerin Sonuçları

	Sinerji Testi		IEF	BIOASSAY	
IP/klox	IP/IPI (MBL) E-Test	GSBL (ÇDS)	pI	Modifiye Hodge Test MEM	Modifiye Hodge Test FOX
+	+	-	7.2 5.3	+	+

E.cloacae
Moleküler Yöntemlerin Sonuçları

Plazmid (kb)	PCR							
	QnrA	AmpC	PER KPC GES	IMP VIM SPM NDM	OXA - 48	TEM	SHV	CTX-M (Grup 1)
140 70 5	+	-	-	IMP-1 +	+	-	-	-

- GSBL hala major epidemiyolojik önemli
- AmpC ve karbapenemazlar da önemli direnç determinantlarına (salgın, hızlı yayılma)
- Karbapemaz üreten suşlar aynı zamanda çoğul dirençli

- Çok az tedavi seçenekleri olması

SONUÇ-2

- Hastaları klinik servislere, özellikle **yoğun bakım ünitelerine** ve **onkoloji** ünitelerine kabul ederken karbapenemaz üreten bakterilerle kolonizasyonu tespit etmek önlem alınması açısından yararlı olacaktır.

- Bir hastanede MBL, OXA, GSBL pozitif bir izolatın identifiye edilmesi sadece terapötik bir problem değil aynı zamanda infeksiyon kontrol komitesini de ciddi şekilde ilgilendiren bir durumdur.
- Erken tespit çoklu ilaç direncine sahip bu izolatların yayılımını önleyebilir

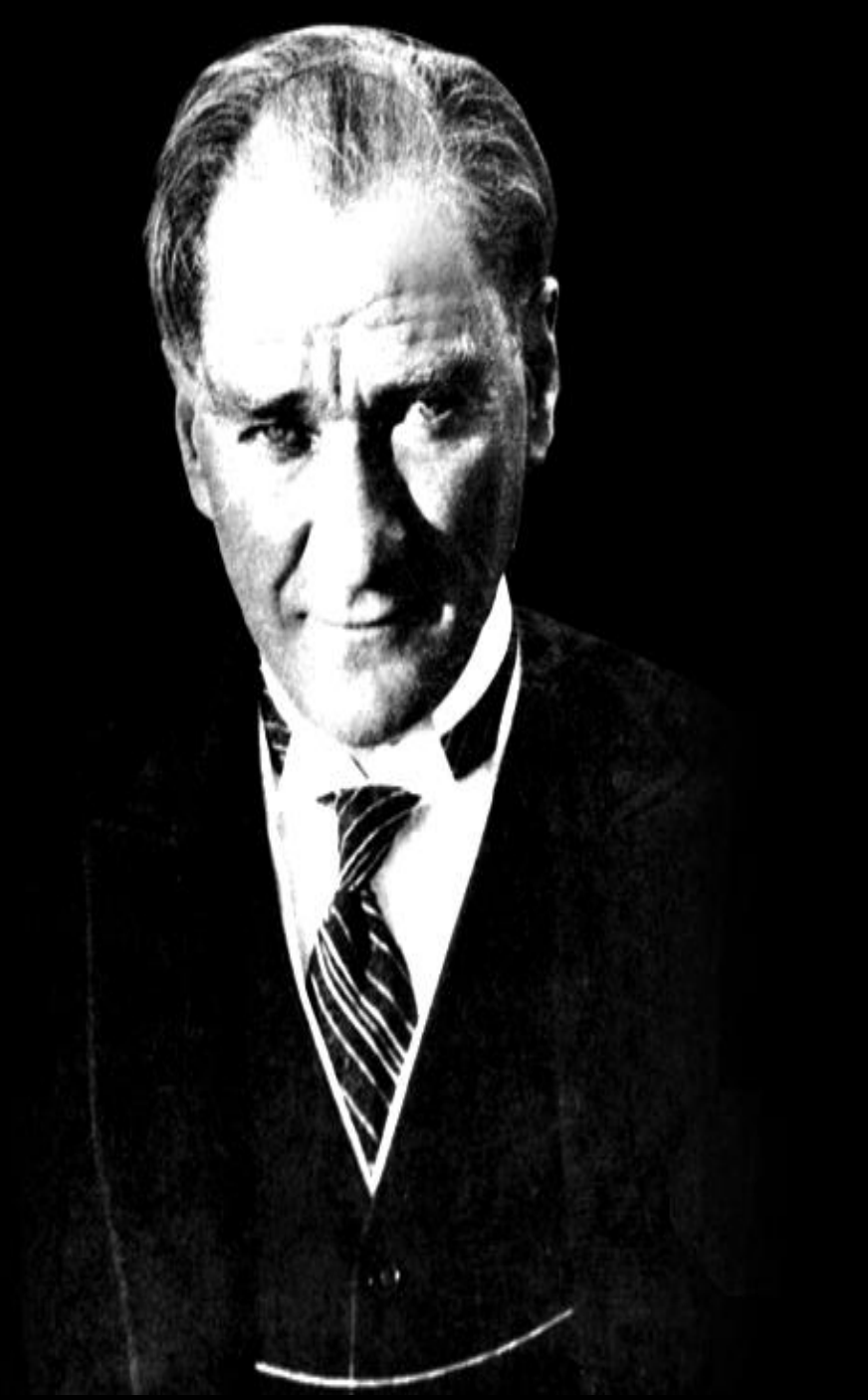
- Mikrobiyoloji laboratuvarı infeksiyon kontrol komitesini acilen bilgilendirmeli,

- Hasta yüksek risk grubunda kabul edilmeli ve uygun izolasyon ölçütleri uygulanmalıdır.

- Eğer gerekliyse, hastanın tıbbi formları, hasta ile temasa geçen klinisyenleri ve diğer tıbbi bakım çalışanlarını bilgilendirecek şekilde infeksiyonun yüksek risk grubundan olduğunu belirtir şekilde düzenlenmelidir.

1881-193∞

ŞÜKRAN GURUR VE ÖZLEMLE ANIYORUZ



J Antimicrob Chemother. 2010 Aug;65(8):1664-71. Epub 2010 Jun 11.

A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates.

Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, Petropoulou D, Sofianou D.

- **Combined-disc tests for the differentiation of MBL and KPC carbapenemases**
- Detailed results of the combined-disc tests are shown in Table 3. All 63 KPC-possessing isolates showed a ≥5 mm increase in the zone diameters of the combined discs with PBA or both PBA and EDTA compared with meropenem alone, whereas the combined-disc test that uses meropenem with and without EDTA was clearly negative in all of them (sensitivity 100%; Table 4). The activity of meropenem was enhanced remarkably by PBA (mean increase 9 mm) or PBA plus EDTA (mean increase 10 mm) in all KPC producers and irrespective of the carbapenem MICs or the co-production of an ESBL. All 47 VIM-possessing isolates showed a ≥5 mm increase in the zone diameters of the combined discs with EDTA or both PBA and EDTA compared with meropenem alone, whereas the combined-disc test that uses meropenem with and without PBA was clearly negative in all VIM producers (sensitivity 100%; Table 4). The activity of meropenem was enhanced remarkably by EDTA (mean increase 10 mm) or PBA plus EDTA (mean increase 11 mm) in all 47 VIM producers and irrespective of the carbapenem MICs or the co-production of an ESBL. All but one of the 31 KPC- and VIM-possessing isolates showed a ≥5 mm increase in the zone diameter of the combined-disc test using meropenem with and without both PBA and EDTA, whereas the combined-disc tests using meropenem with and without PBA or EDTA were negative in all KPC and VIM producers (sensitivity 96.8%; Table 4). The activity of meropenem was enhanced remarkably by the combination of PBA and EDTA (mean increase 10 mm) in all 30 positive isolates and irrespective of the carbapenem MICs or the co-production of an ESBL, clearly indicating the combined inhibitory activity of the two inhibitors against the production of both carbapenemases. Characteristically, the increase in the zone diameters of the combined-disc test using meropenem with and without both PBA and EDTA was similar among KPC-possessing, VIM-possessing, as well as KPC- and VIM-possessing clinical isolates (Figure 1).

- **Check-Points' diagnostic tests are based on our innovative, patented Check-Made system**
- The Check-Made system combines a highly efficient multiplex amplification method with a simple, user-friendly diagnostic microarray detection system. All reagents and disposables for our diagnostic tests are supplied in a kit for 72 samples with a clear manual. The user only needs to add sample DNA: most commercially available DNA preparation methods will yield a fine result. **1. The multiplex amplification method employs two steps, a DNA Recognition step and a PCR**
- The Recognition step enables target DNA recognition and discrimination up to the single nucleotide level if needed (step A). The first step (DNA recognition) creates the template for the PCR step in a way that all templates are equipped with the same two PCR primer sites. This allows for very high multiplex ratio's enabling the robust and efficient detection of many DNA target sequences in parallel. The PCR step (step B) provides for the amplification and labeling required for microarray detection. A one tube protocol is used for the DNA recognition and PCR step with minimum hands-on time. **2. After amplification the PCR products are detected on a microarray**
- Each individual amplification product will be detected on its own unique array position (step C).So called Check-points Tubes are used incorporating a universal diagnostic microarray at the bottom of a standard 1.5 ml reaction tube. The Check-Points Tube format enables easy handling of all microarray detection steps: hybridization, washing and staining.
- **3. Microarray images are captured using a small Array Tube reader connected to a PC through a USB hub**
-
- Data analysis of the microarray results is fully automated using Check-Points' advanced detection software package: E-Ads. A clear and objective test result will be given by the software, and all data will be automatically stored in an accessible manner for future reference if needed.
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