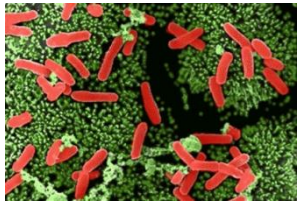
BAŞKENT ÜNİVERSİTESİ

Gram-Negatif Bakterilerde Direncin Laboratuvar Tanısı ve Yorumlanması

Dr. Özlem Kurt-Azap
Başkent Üniversitesi Tıp Fakültesi
Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji AD



Sunum planı

- Antimikrobiyal duyarlılığı belirleme yöntemleri
- Rehberler
- Tanımlar
- Olgular
- Sonuç

Yöntemler

- **Fenotipik**

- MİK belirleme
- Disk diffüzyon
- Otomatize sistemler

- **Genotipik**

- Direnç geni (mec A..) veya genin ürünü (PB2..)

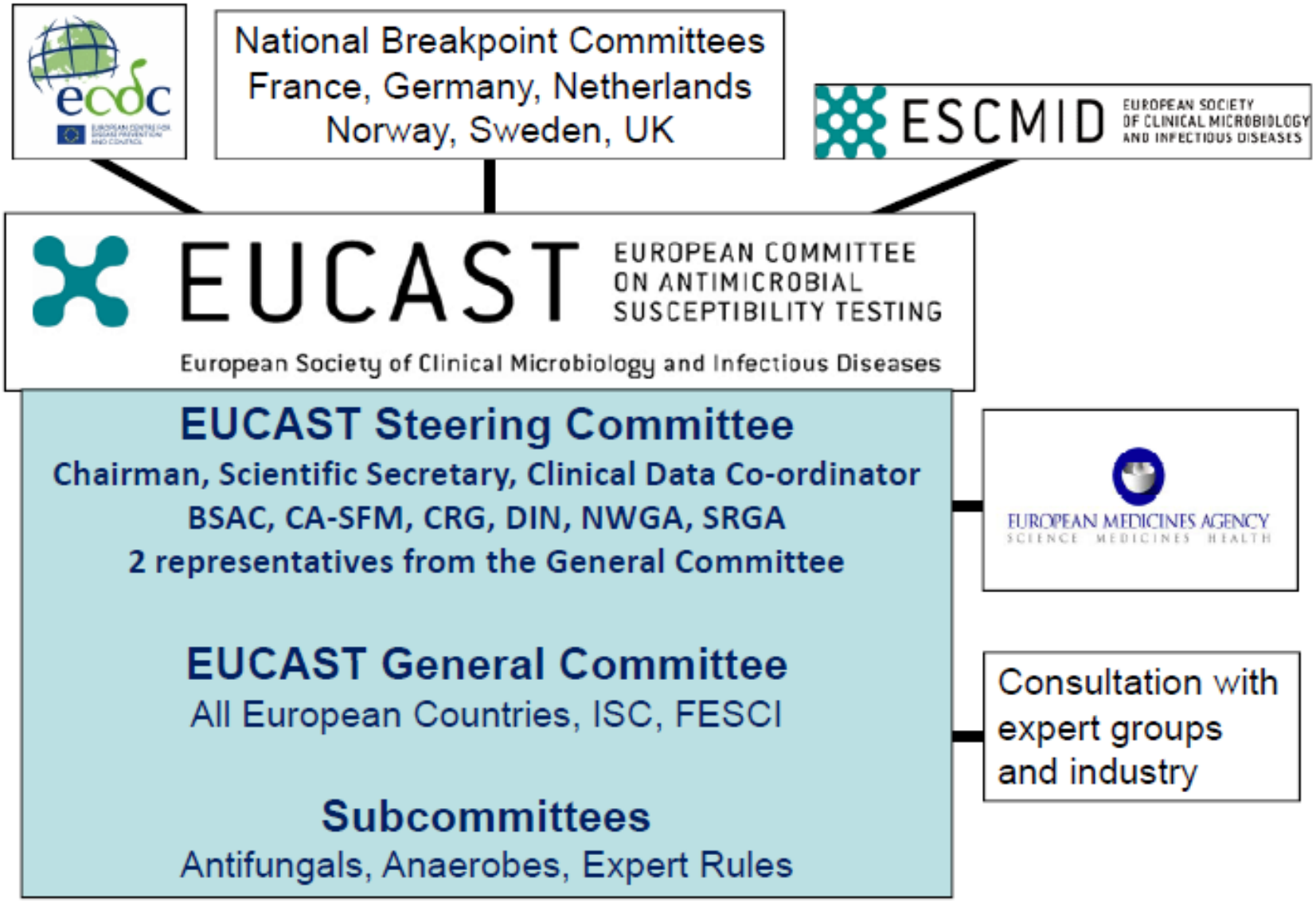
- **Uzman kuralları ("Expert rules")**

- Eritromisine dirençli ise azitromisine ve roksitromisine de dirençli

Rehberler

- CLSI: ABD
- EUCAST: Avrupa
- BSAC: İngiltere
- SRGA: İsveç
- NWGA: Norveç
- CRG: Hollanda
- DIN: Almanya

EUCAST


ecdc
National Breakpoint Committees
France, Germany, Netherlands
Norway, Sweden, UK
ESCMID
EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases
EUCAST Steering Committee
Chairman, Scientific Secretary, Clinical Data Co-ordinator
BSAC, CA-SFM, CRG, DIN, NWGA, SRGA
2 representatives from the General Committee
EUCAST General Committee
All European Countries, ISC, FESCI
Subcommittees
Antifungals, Anaerobes, Expert Rules
EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH
Consultation with expert groups and industry

REHBERLER- “NEQAS” Dış Kalite Kontrol Sistemine Katılan Laboratuvarlar

Results by guideline

	S	I	R	% concordance
score	2	1	0	
BSAC	123	0	0	100
CRG	1	0	0	100
EUCAST	322	2	2	98.8
CLSI	162	1	0	99.4
NWGA	1	0	0	100
SRGA	7	0	0	100
All	616	3	2	99.2
TR	2	0	0	100.0

Tanımlar- I

Duyarlı

Orta duyarlı: MİK değerleri, kan ve doku düzeylerine yakın değerlerde olan izolatlardır

- İlacın normalden yüksek dozda kullanılabileceği durumlarda veya ilacın enfeksiyon bölgesinde yüksek konsantrasyona ulaştığı durumlarda kullanılabilir

Dirençli

Duyarlı değil: Sadece "duyarlı" yorumlama kategorisi olup orta duyarlı veya dirençli kategorisi olmayan gruptur

İntrensek dirençli

Heterorezistans: Dirençli subpopülasyonun bulunması

Tanımlar- II

- **ÇİD (MDR)**
- **XDR:** Kolistin ve tigesiklin dışındaki antibiyotiklere dirençli suşlar
- **PDR:** Tüm antibiyotiklere dirençli suşlar

Clin Microbiol Infect 2012; **18**: 268–281

Tanımlar- III

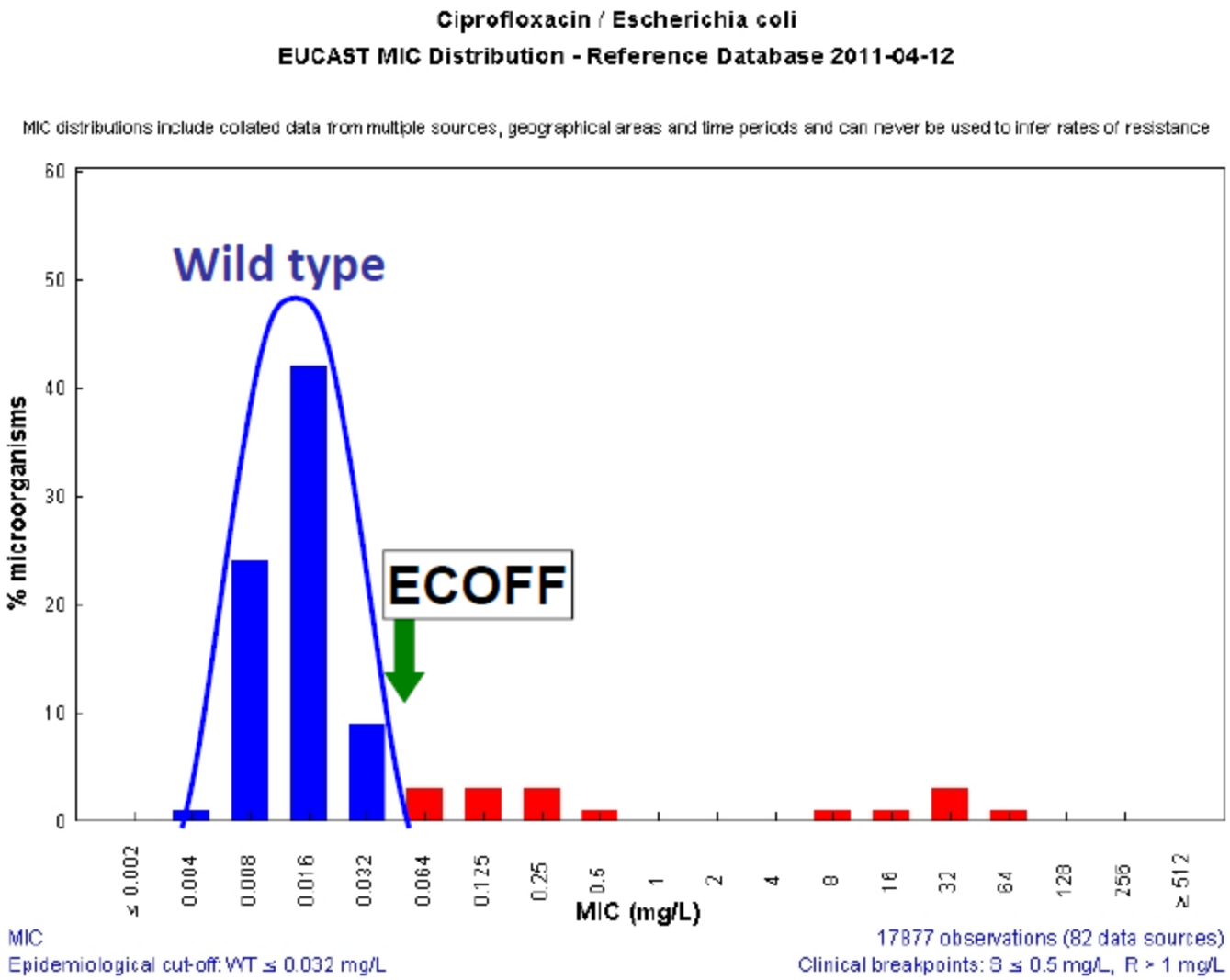
Epidemiological cut-off value (ECOFF)

- MIC value identifying the upper limit of the wild type population

- A microorganism is defined as wild type (WT) for a species by the absence of acquired and mutational mechanisms of resistance to the agent.

EUCAST

EUCAST wild type and ECOFF



EUCAST

REHBERLER VE SINIR DEĞERLER

Breakpoints prior to harmonisation (mg/L) S≤ R>							
	BSAC	CA-SFM	CRG	DIN	NWGA	SRGA	CLSI
General breakpoints		1/2	1/2	1/2	0.125/2	1/2	
Species related breakpoints							
Enterobacteriaceae	1/1				0.12/2	0.12/1	1/2
Pseudomonas spp.	1/4					1/1	1/2
Acinetobacter spp.						1/1	1/2
Staphylococci	1/1				0.12/2	0.06/2	1/2
Streptococci	1/1				0.12/2	0.12/2	
S. pneumoniae	2/2 (I)*				0.12/2 (I)*	0.12/2 (I)*	
Enterococci					0.12/2	0.12/2	1/2
Haemophilus/Moraxella spp.	1/1				0.12/0.5	0.12/0.25	1/-
Corynebacteria							
N. meningitidis	1/1				0.06/0.12	0.03/0.25	
N. gonorrhoeae	0.06/-		0.06/1		0.06/0.12	0.06/0.25	0.06/0.5
P. multocida						0.12/0.25	
Anaerobes							
Campylobacter spp.	1/1						
Helicobacter pylori	2/2						

CLSI and FDA and EUCAST breakpoints may differ because …..

- Different criteria used to set breakpoints
- Sometimes different dosing regimens; different clinical indications
- Some breakpoints set many years ago and not yet reassessed by respective agency

Examples (Enterobacteriaceae):

Agent	CLSI	FDA	EUCAST
Ceftriaxone	≤1*	≤8	≤1
Gentamicin	≤4	≤4	≤2
Meropenem	≤1*	≤4	≤2

*new 2010

Janet Hindler, 2010

Sefalosporin sınır değerleri (CLSI)
2009 vs **2010**

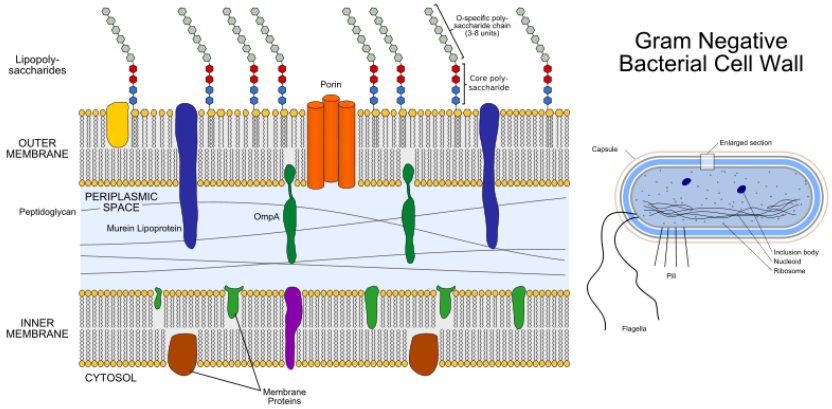
Agent	CLSI 2009 (Old)			CLSI 2010 (New)		
	Susc	Int	Res	Susc	Int	Res
Cefazolin	≤8	16	≥32	≤1	2	≥4
Cefotaxime	≤8	16-32	≥64	≤1	2	≥4
Ceftriaxone	≤8	16-32	≥64	≤1	2	≥4
Ceftazidime	≤8	16	≥32	≤4	8	≥16
Aztreonam	≤8	16	≥32	≤4	8	≥16
*Cefepime***	*≤8*	*16*	*≥32*	*No change*		

Karbapenem sınır değerlerindeki değişiklikler (CLSI-**2010**)

Agent	CLSI 2010 (Old)			CLSI June 2010 (New)		
	Susc	Int	Res	Susc	Int	Res
Doripenem	-	-	-	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.25	0.5	≥1
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

“Sorunlu” gram-negatif bakteriler

- *Acinetobacter baumannii*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*
- Diğer enterik bakteriler
- Diğer non-fermentatif bakteriler



Gram negatif bakterilerde bulunan beta laktamazlar

Beta-Lactamase	1st Gen. Cephs	2nd Gen. Cephs	Cephamycins	3rd Gen. Cephs	4th Gen. Cephs (Cefepime)	Carbapenems	Monobactams	Beta-Lactamase Inhibitor Effective?
KPC	Resistant	Resistant	Variable	Resistant	Resistant	Resistant	Resistant	Weakly
NDM-1	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Susceptible	No
Low Level AmpC	Variable	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	No
Hyper-produced AmpC	Resistant	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	No
ESBL	Resistant	Resistant	Susceptible	Resistant	Variable	Susceptible	Resistant	Yes

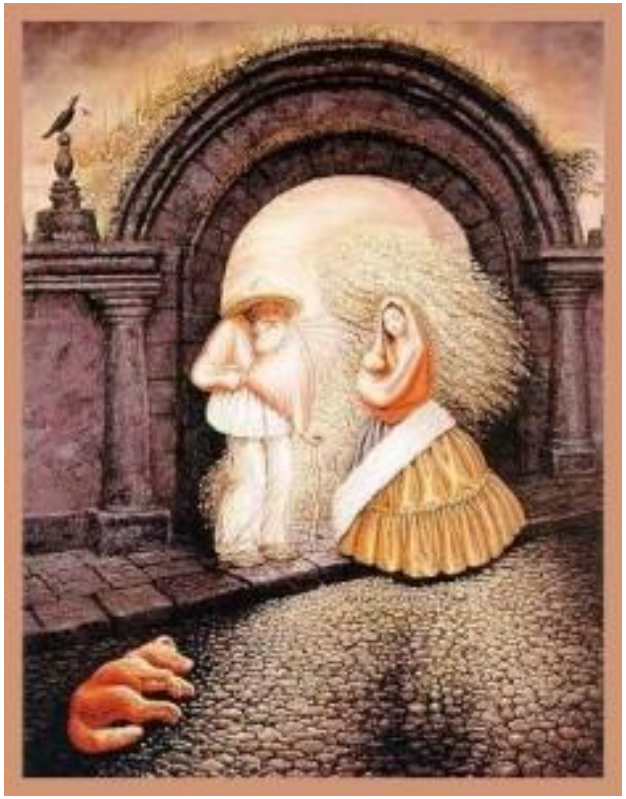
KARBAPENEMAZLAR

Moleküler sınıf	Bush-Jacoby	Özellikler	Örnek enzimler
A	2f	Karbapenem, sefalosporin ve penisilin hidrolizi. KLA ve TAZ ile düşük inhibisyon.	KPC-2, KPC-3, GES-2, IMI-2
B	3a	Monobaktamlar dışında tüm karbapenemlerin hidrolizi. KLA ve TAZ etkisiz. EDTA ve diğer şelatörler ile inhibisyon.	IMP-1, VIM-1, NDM-1
D	2df	Karbapenem, kloksasilin ve oksasilin hidrolizi. KLA bazılarında etkili.	OXA-23, OXA-48

İdentifikasyon

DOĞRU TANIMLAMA ÇOK ÖNEMLİ!

- Konvansiyonel yöntemler
- Tanımlama kitleri
- Otomatize sistemler
- Kromojenik besiyerleri



Evaluation of CHROMagar KPC for Rapid Detection of Carbapenem-Resistant *Enterobacteriaceae*[▽]

Zmira Samra,[1] Judi Bahar,[2] Liora Madar-Shapiro,[2] Nazi Aziz,[1] Sara Israel,[2] and Jihad Bishara[3]*

Laboratory of Clinical Microbiology[1] and Unit of Infectious Diseases,[3] Rabin Medical Center, Beilinson Hospital, Sackler School of Medicine, Tel-Aviv University, Petah-Tiqwa 49100, Israel, and Hylab, Rehovot, Israel[2]

Received 6 February 2008/Returned for modification 18 March 2008/Accepted 3 July 2008

A new CHROMagar KPC medium was compared to MacConkey agar with carbapenem discs and PCR for the *bla*$_{KPC}$ gene for rapid detection of carbapenem-resistant *Klebsiella pneumoniae*. The sensitivity and specificity relative to PCR were 100% and 98.4%, respectively, for CHROMagar KPC and 92.7% and 95.9%, respectively, for MacConkey agar.

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2008, p. 3110–3111

CHROMagar™ KPC

Test yöntemi

- 1966 Kirby-Bauer
  Disk diffüzyon

Am J Clin Pathol. 1966 Apr;45(4):493-6.
Antibiotic susceptibility testing by a standardized single disk method.
Bauer AW, Kirby WM, Sherris JC, Turck M.

- 1977- National Reference System for the Clinical Laboratory (NRSCL), Şimdi **CLSI**

- Her bakteri ve antibiyotik için bir disk diffüzyon sınır değeri yok!

- Şu anda kullanmakta olduğumuz bazı antibiyotikler için uyguladığımız rehberlerde sınır değer yok!

TİGESİKLİN- I

Acinetobacter spp. için:

- **CLSI**'da, **EUCAST'**de, **FDA**'da sınır değer yok
- **BSAC:** ≤1 mg/L

  disk diffüzyon öneriliyor
- Genelde çalışmalarda ≤2 mg/L duyarlı kabul edilmiş

Enterik bakteriler için:

- **CLSI**'da sınır değer yok
- **EUCAST**: ≤1 mg/L

  sadece *E.coli* için disk diffüzyon sınır değerleri var
- **FDA:** ≤2 mg/L

TİGESİKLİN- II

İntrensek dirençli olan mo'lar

- *Pseudomonas aeruginosa*
- *Morganella spp.*
- *Providencia spp.*
- *Proteus spp.*

KOLİSTİN- I

***Acinetobacter* spp. için: Disk diffüzyon önerilmez**

- **CLSI:** ≤ 2 mg/L duyarlı, ≥4 mg/L dirençli
- **EUCAST:** ≤ 2 mg/L duyarlı, >2 mg/L dirençli

***Pseudomonas spp.* için: Disk diffüzyon ÖNERİLİR**

- **CLSI:** ≤2mg/L duyarlı, 4mg/L orta duyarlı, ≥8 mg/L dirençli
- **EUCAST:** ≤ 4 mg/L duyarlı, >4 mg/L dirençli

Enterik bakteriler için:

- **CLSI:** Sınır değer yok
- **EUCAST: Disk diffüzyon önerilmez**

  ≤ 2 mg/L duyarlı, >2 mg/L dirençli

KOLİSTİN- II

İntrensek dirençli olan mikroorganizmalar

- *Serratia marcescens*
- *Providencia stuartii*
- *Proteus mirabilis*
- *Morganella morganii*
- *Burkholderia cepacia*

Fosfomisin

- ***Acinetobacter*** spp. için sınır değer yok
- ***Pseudomonas*** spp. için sınır değer yok
- **Enterik bakteriler** için EUCAST'te MİK için sınır değer var

Olgu 1

İdrar kültürü: 100000 kob/ml *E.coli*

	MIC (µg/ml)
amikacin	>32 R
ampicillin	>32 R
cefazolin	>32 R
cefepime	8 S
ceftazidime	1 S
ceftriaxone	>32 R
ciprofloxacin	≤0.25 S
gentamicin	≤0.5 S
piper-tazobactam	>128 R
tobramycin	1 S
trimeth-sulfa	>4/76 R

GSBL pozitif *E.coli*

CLSI- Yeni öneriler

- Sınır değerleri düşürüldü

- GSBL tanımlaması sadece enfeksiyon kontrolü amacıyla öneriliyor

Antibiyotik	**Eski M100-S19 (2009)** MİK değerleri mg/L			**Yeni M100-S20 (2010)** MİK değerleri mg/L		
	Duyarlı (S)	I	Dirençli (R)	Duyarlı (S)	I	Dirençli (R)
Sefazolin	**≤8**	16	≥32	**≤1**	2	≥4
Sefotaksim	**≤8**	16-32	≥64	**≤1**	2	≥4
Seftizoksim	**≤8**	16-32	≥64	**≤1**	2	≥4
Seftriakson	**≤8**	16-32	≥64	**≤1**	2	≥4
Seftazidim	**≤8**	16	≥32	**≤4**	8	≥16
Aztreonam	**≤8**	16	≥32	**≤4**	8	≥16

GSBL- Sorular/Sorunlar

- Sadece enfeksiyon kontrolü amacıyla kullanıldığında labotatuvarlar test edecek mi?
- Daha önceki yıllarda konuşulan tedavi başarısızlıkları?
- Test tekrarında çıkabilecek farklı sonuçlarda ne olacak?
- Sonuç gecikiyor!

GSBL saptanması

Yöntem	Duyarlılık	Özgüllük	PPV	NPV
Microscan	83.4	72.9	81.6	75.5
Phoenix	98.8	52.2	75.0	96.6
Vitek 2	85.9	78.0	84.9	79.3
ÇDS	94.1	81.4	87.9	90.6
Kombine Disk	92.9	96.6	97.5	90.5
ETest	94.1	84.7	89.9	90.9

Wiengand JCM 2007;45:1167

Table. Antimicrobial susceptibilities of cephalosporins againt ESBL positive strains according to CLSI 2011 criteria

	Ceftriaxone	Cefotaxime	Ceftazidime	Cefepime
E.coli (n=100)	0	3 (3%)	13 (13%)	16 (16%)
Klebsiella spp. (n=92)	1 (1%)	0	5 (5.4%)	21 (22.8%)

Antibiyotik	Eski M100-S19 (2009) MİK değerleri mg/L			Yeni M100-S20 (2010) MİK değerleri mg/L		
	Duyarlı (S)	I	Dirençli (R)	Duyarlı (S)	I	Dirençli (R)
Sefazolin	≤8	16	≥32	≤1	2	≥4
Sefotaksim	≤8	16-32	≥64	≤1	2	≥4
Seftizoksim	≤8	16-32	≥64	≤1	2	≥4
Seftriakson	≤8	16-32	≥64	≤1	2	≥4
Seftazidim	≤8	16	≥32	≤4	8	≥16
Aztreonam	≤8	16	≥32	≤4	8	≥16

ECCMID 2011, Poster

Olgu 2

Kan kültürü: *Klebsiella pneumoniae* üredi

Gentamicin	1	S
Ampicillin	>128	R
Pip/Tazo	<=2	S
Cefotaxime	1	S
Cefepime	4	S
Cefazolin	>32	R
Ceftazidime	4	R
Aztreonam	8	R
Meropenem	<0.5	S

Antibiyotik	**Eski M100-S19 (2009)** MİK değerleri mg/L			**Yeni M100-S20 (2010)** MİK değerleri mg/L		
	Duyarlı (S)	I	Dirençli (R)	Duyarlı (S)	I	Dirençli (R)
Sefazolin	**≤8**	16	≥32	**≤1**	2	≥4
Sefotaksim	**≤8**	16-32	≥64	**≤1**	2	≥4
Seftizoksim	**≤8**	16-32	≥64	**≤1**	2	≥4
Seftriakson	**≤8**	16-32	≥64	**≤1**	2	≥4
Seftazidim	**≤8**	16	≥32	**≤4**	8	≥16
Aztreonam	**≤8**	16	≥32	**≤4**	8	≥16

EUCAST Ceftazidime	1	4

Olgu 3

Yara kültürü: *Klebsiella pneumoniae* üredi

- Sefoksitin: Dirençli
- Seftriakson: Dirençli
- Seftazidim: Dirençli

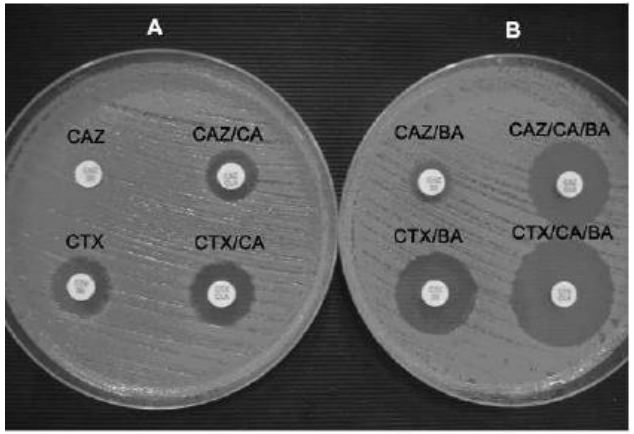
Amp C ve GSBL birlikteliği

- Plazmid geçişli Amp C direnci artıyor

Ayıredebilmek için:

- Sefepim ile çift disk sinerji

- Amp C inhibitörleri: Kloksasilin
  Boronik asit



Olgu 4

Kan kültürü: *Enterobacter aerogenes* üredi

Ertapenem: Dirençli

İmipenem: Duyarlı

Meropenem: Duyarlı

Karbapenem direnç mekanizmaları

- Karbapenemazlar
- Sınıf A GSBL (CTX-M) **+** geçirgenlikte azalma
- Sınıf C Amp C tipi direnç **+** geçirgenlikte azalma

Olgu 5

Kan kültürü: *Klebsiella pneumoniae* üredi

Ertapenem: Dirençli

İmipenem: Dirençli

Meropenem: Dirençli

Klebsiella pneumoniae

- Karbapenem direnci çıkmakta/artmakta
- ABD ve Avrupa'da KPC, NDM mevcut
- Türkiye'de OXA-48 mevcut

  bir olguda NDM (2012)

Karbapenemazlar

Sınıf	Enzim	En sık görülen tür
Sınıf A	KPC, SME, IMI, NMC, GES	Enterobacteriaceae (*P. aeruginosa*'da nadir)
Sınıf B (metallo-β-laktamaz)	IMP, VIM, GIM, SPM, IND, NDM-1	*P. aeruginosa* Enterobacteriacea *Acinetobacter* spp.
Sınıf D	OXA	*Acinetobacter* spp. Enterobacteriaceae (OXA-48)

Review

Guideline for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae

James Cohen Stuart[a,*], Maurine A. Leverstein-Van Hall[a,b], on behalf of members of the Dutch Working Party on the Detection of Highly Resistant Microorganisms[1]

Species	Meropenem		Imipenem			Ertapenem
	Escherichia coli, *Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp.	*Citrobacter* spp., *Serratia* spp., *Proteus mirabilis*, *Morganella morganii*, *Providencia* spp.	*E. coli*	*Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp., *Citrobacter* spp.	*Serratia* spp., *P. mirabilis*, *M. morganii*, *Providencia* spp.	*E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp., *Serratia* spp., *P. mirabilis*, *M. morganii*, *Providencia* spp.
Carbapenemase screening breakpoint	≥0.5[a]	≥0.5[a]	≥2[b]	≥2[b]	N/A	≥0.5[c]
Epidemiological cut-off wild-type	S ≤0.125	S ≤0.25	S ≤0.5	S ≤1	S ≤4 (*Serratia* S ≤2)	S ≤0.064
EUCAST breakpoint	S ≤2	S ≤2	S ≤2	S ≤2	S ≤2	S ≤0.5
CLSI breakpoint	S ≤1	S ≤1	S ≤1	S ≤1	S ≤1	S ≤1

International Journal of Antimicrobial Agents 36 (2010) 205–210

Karbapenemazların saptanması

Table 1. MIC range of carbapenems for *Enterobacteriaceae* that produce several types of carbapenemases*

	MIC, mg/L		
Carbapenemase	Imipenem	Meropenem	Ertapenem
KPC	0.5–>64	1–>64	0.5–>64
Metallo β-lactamases†	0.5–>64	0.25–>64	0.5–>64
OXA-48 type	1–>64	0.5–>64	0.25–>64

*KPC, *Klebsiella pneumoniae* carbapenemase; OXA-48, oxacillinase-48.
†Including New Delhi metallo-β-lactamase-1.

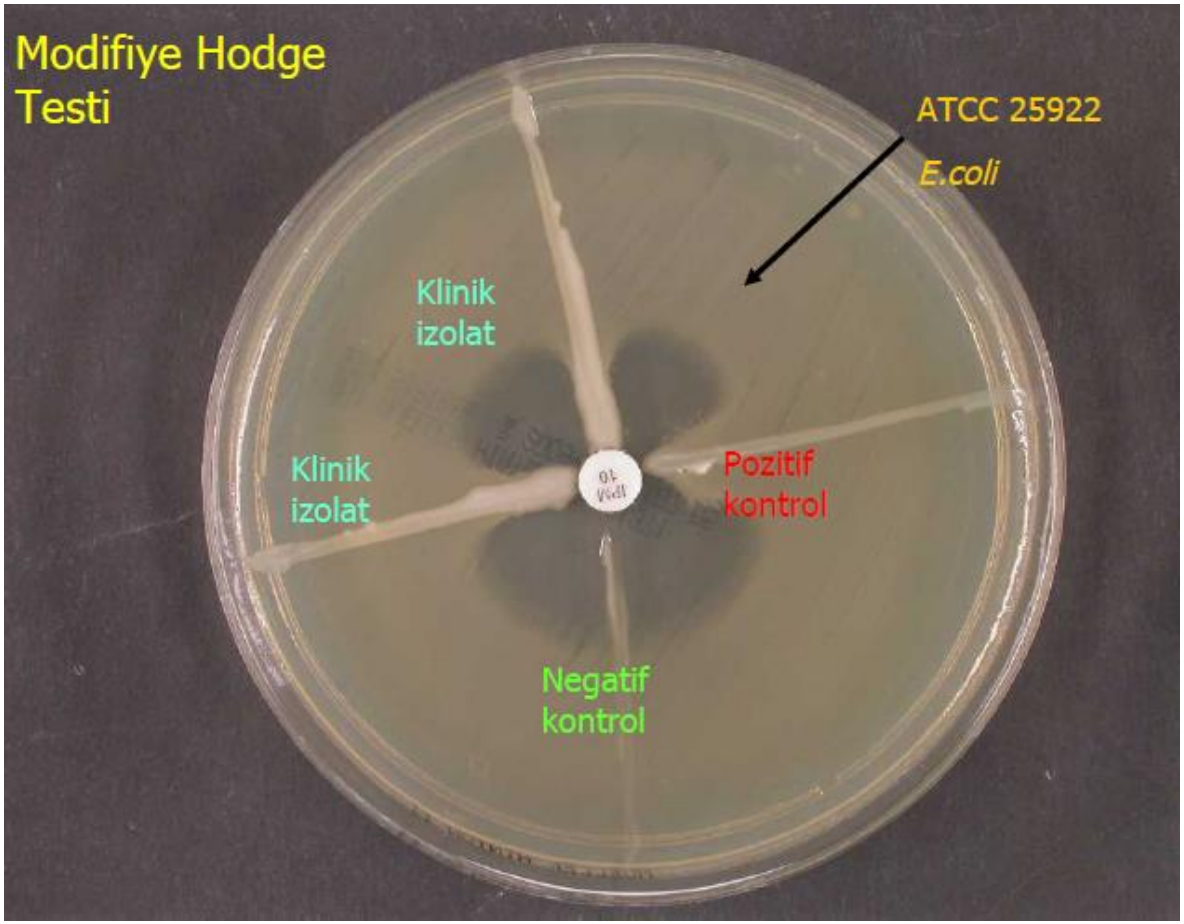
Carbapenem Resistance in *Klebsiella pneumoniae* Not Detected by Automated Susceptibility Testing

Fred C. Tenover,* Rajinder K. Kalsi,† Portia P. Williams,‡ Roberta B. Carey,* Sheila Stocker,* David Lonsway,* J. Kamile Rasheed,* James W. Biddle,* John E. McGowan Jr.,‡ and Bruce Hanna†

Detecting β-lactamase–mediated carbapenem resistance among *Klebsiella pneumoniae* isolates and other *Enterobacteriaceae* is an emerging problem. In this study, 15 bla_{KPC}-positive *Klebsiella pneumoniae* that showed discrepant results for imipenem and meropenem from 4 New York City hospitals were characterized by isoelectric focusing; broth microdilution (BMD); disk diffusion (DD); and MicroScan, Phoenix, Sensititre, VITEK, and VITEK 2 automated systems. All 15 isolates were either intermediate or resistant to imipenem and meropenem by BMD; 1 was susceptible to imipenem by DD. MicroScan and Phoenix reported 1 (6.7%) and 2 (13.3%) isolates, respectively, as imipenem susceptible. VITEK and VITEK 2 reported 10 (67%) and 5 (33%) isolates, respectively, as imipenem susceptible. By Sensititre, 13 (87%) isolates were susceptible to imipenem, and 12 (80%) were susceptible to meropenem. The VITEK 2 Advanced Expert System changed 2 imipenem MIC results from ≥16 μg/mL to ≤2 μg/mL but kept the interpretation as resistant. The recognition of carbapenem-resistant *K. pneumoniae* continues to challenge automated susceptibility systems.

KPC pozitif suşların %6.7-87'si atlanıyor

2010 EYLÜL ÖNCESİ CLSI



Modifiye Hodge Testi pozitif ise " bakteri karbapenemaz üretmektedir. Bazı karbapenemlere duyarlı gözükse de klinik tedavi sırasında direnç gelişebilir" notu ile raporlama

KPC

Sınıf A beta-laktamazlar (KPC 2-11)

- Karbapenemler ve 3. Kuşak sefalosporinler dahil olmak üzere **TÜM** beta-laktamlara direnc yol açar

Özellikle ABD'de Enterobacteriaceae üyelerinde bildirilmiş---- Tüm dünya

Saptanması güç: imipenem ve meropenem (S) olabilir; ertapenem daha iyi bir göstergedir.

Boronik asit ile inhibe olur (Amp C tipi enzimlere DİKKAT!: kloksasilin/oksasilin inhibisyonu*

KPC ENZİMLERİNİN YAYILIMI

Table. Bacterial species that have been found to produce KPC resistance enzymes[a]

Enterobacteriaceae	Non-*Enterobacteriaceae*
Citrobacter freundii	*Pseudomonas aeruginosa*
Escherichia coli	*Pseudomonas putida*
Enterobacter aerogenes	*Acinetobacter* spp.
Enterobacter cloacae	
Enterobacter gergoviae	
Klebsiella pneumoniae	
Klebsiella oxytoca	
Proteus mirabilis	
Salmonella enterica	
Serratia marcescens	

Klebsiella pneumoniae *remains the most common organism to harbor KPCs; however, relative frequency in other organisms has not been reported.*
[a]*KPC,* Klebsiella pneumoniae *carbapenemase.*

Southern Medical Journal • Volume 104, Number 1, January 2011

OXA-tipi karbapenemazlar

- İlk olarak Türkiye'den bildirildi

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
Copyright © 2004, American Society for Microbiology. All Rights Reserved.

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 found immediately upstream of bla_{OXA-48}. Another plasmid that encoded a second oxacillinase gene, bla_{OXA-47}, located inside a class 1 integron was identified in *K. pneumoniae* 11978. OXA-47 had a narrow spectrum of hydrolysis activity and did not hydrolyze ceftazidime or imipenem, as is found for the β-lactamase (OXA-1) to which it is related. In addition, β-lactamases TEM-1 and SHV-2a were expressed from the same *K. pneumoniae* isolate. Analysis of the outer membrane proteins of this isolate revealed that it lacked a porin of ca. 36 kDa. Thus, the high-level resistance to β-lactams of this clinical isolate resulted from peculiar β-lactamases and modification of outer membrane proteins.**

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
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Spread of OXA-48-Positive Carbapenem-Resistant *Klebsiella pneumoniae* Isolates in Istanbul, Turkey[▽]

Amélie Carrër,[1] Laurent Poirel,[1] Haluk Eraksoy,[2] A. Atahan 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 Department 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, Mar. 2010, p. 1369–1373 Vol. 54, No. 3
0066-4804/10/$12.00 doi:10.1128/AAC.01312-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

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]*

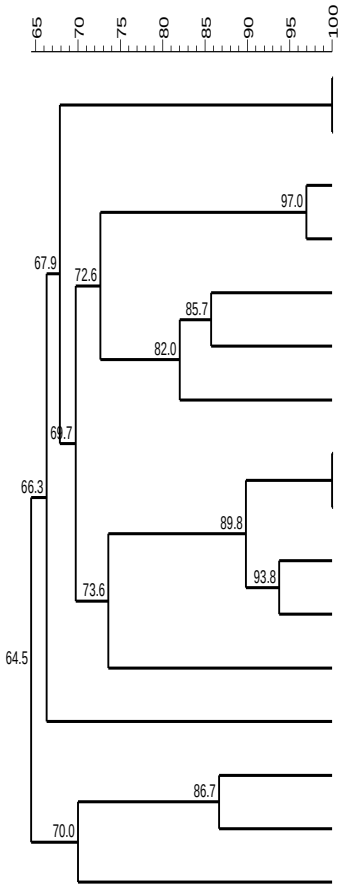
Service de Bactériologie-Virologie, INSERM U914 Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris Sud, K.-Bicêtre, France[1]; Infectious Diseases and Clinical Microbiology Department, Cerrahpasa Medical Faculty, University of Istanbul, 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

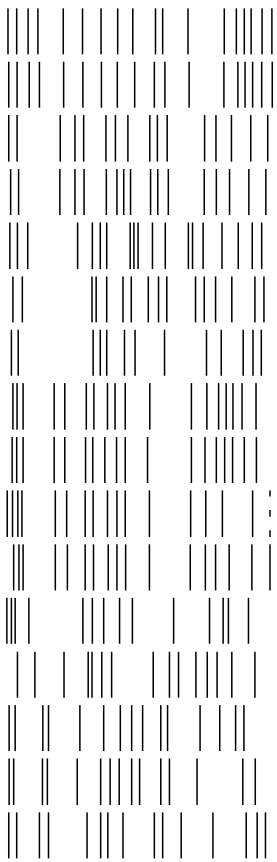
Eighteen carbapenem-resistant, OXA-48-positive enterobacterial isolates recovered from Turkey, Lebanon, Egypt, France, and Belgium were analyzed. In most isolates, similar 70-kb plasmids carrying the carbapenemase gene bla_{OXA-48} were identified. That gene was located within either transposon Tn*1999* 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.

Başkent Deneyimi: 16 OXA-48 pozitif suş

K. pneumoniae Xba-1



K. pneumoniae Xba-1



No	Genotype	Susceptible Antibiotics*	Associated ESBL Genes
8		CN	TEM, CTX-M
11		CN	TEM, CTX-M
12		ATM, FEP, CTX, CAZ	None
14		None	CTX-M
1		CN	TEM
9		ATM, FEP, CTX, CAZ, CN, SXT	None
7		ATM, FEP, CTX, CAZ, CIP, CN, LEV, SXT	None
6		CN	TEM, CTX-M
4		None	TEM
15		None	CTX-M
16		Cn	CTX-M
3		ATM, FEP, CAZ, CIP, CN, LEV	None
2		CIP, LEV, SXT	CTX-M
5		ATM, FEP, CAZ, CN, SXT	None
13		None	CTX-M
10		CIP, LEV	CTX-M

Metallo-beta-laktamazlar

- Karbapenemleri ve aztreonam hariç tüm beta laktamları parçalayabilen enzimler

- *Stenotrophomonas maltophilia'*da kromozomal metallo-beta-laktamaz mevcut

- NDM: Enterik bakterilerde saptanmış olan plazmid geçişli metallo-beta-laktamaz

İLK NDM

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2009, p. 5046–5054 Vol. 53, No. 12
0066-4804/09/$12.00 doi:10.1128/AAC.00774-09
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

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 Timothy R. Walsh[2]*

Yonsei University College of Medicine, Research Institute of Antimicrobial Resistance, Seoul, Republic of Korea[1]; Department of Medical Microbiology, Cardiff University, Cardiff, United Kingdom[2]; Clinical Microbiology, MTC—Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden[3]; Yonsei University College of Life Science and Biotechnology, Seoul, Republic of Korea[4]; and Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden[5]

Received 10 June 2009/Returned for modification 7 August 2009/Accepted 10 September 2009

A Swedish patient of Indian origin traveled to New Delhi, India, and acquired a urinary tract infection caused by a carbapenem-resistant *Klebsiella pneumoniae* strain that typed to the sequence type 14 complex. The isolate, *Klebsiella pneumoniae* 05-506, was shown to possess a metallo-β-lactamase (MBL) but was negative for previously known MBL genes. Gene libraries and amplification of class 1 integrons revealed three resistance-conferring regions; the first contained bla_{CMY-4} flanked by IS*EcP1* and *blc*. The second region of 4.8 kb contained a complex class 1 integron with the gene cassettes *arr-2*, a new erythromycin esterase gene; *ereC*; *aadA1*; and *cmlA7*. An intact IS*CR1* element was shown to be downstream from the *qac/sul* genes. The third region consisted of a new MBL gene, designated bla_{NDM-1}, flanked on one side by *K. pneumoniae* DNA and a truncated IS*26* element on its other side. The last two regions lie adjacent to one another, and all three regions are found on a 180-kb region that is easily transferable to recipient strains and that confers resistance to all antibiotics except fluoroquinolones and colistin. NDM-1 shares very little identity with other MBLs, with the most similar MBLs being VIM-1/VIM-2, with which it has only 32.4% identity. As well as possessing unique residues near the active site, NDM-1 also has an additional insert between positions 162 and 166 not present in other MBLs. NDM-1 has a molecular mass of 28 kDa, is monomeric, and can hydrolyze all β-lactams except aztreonam. Compared to VIM-2, NDM-1 displays tighter binding to most cephalosporins, in particular, cefuroxime, cefotaxime, and cephalothin (cefalotin), and also to the penicillins. NDM-1 does not bind to the carbapenems as tightly as IMP-1 or VIM-2 and turns over the carbapenems at a rate similar to that of VIM-2. In addition to *K. pneumoniae* 05-506, bla_{NDM-1} was found on a 140-kb plasmid in an *Escherichia coli* strain isolated from the patient's feces, inferring the possibility of in vivo conjugation. The broad resistance carried on these plasmids is a further worrying development for India, which already has high levels of antibiotic resistance.

J Antimicrob Chemother 2011; **66**: 689–692
doi:10.1093/jac/dkq520 Advance Access publication 28 January 2011

Journal of Antimicrobial Chemotherapy

Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria?

Patrice Nordmann[1]*, Laurent Poirel[1], Mark A. Toleman[2] and Timothy R. Walsh[2]

[1]*Service de Bactériologie-Virologie, INSERM U914 'Emerging Resistance to Antibiotics', Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K.-Bicêtre, France;* [2]*Department of Medical Microbiology, School of Medicine, Cardiff University, Cardiff, UK*

*Corresponding author. Tel: +33-1-45-21-36-32; Fax: +33-1-45-21-63-40; E-mail: nordmann.patrice@bct.aphp.fr

The NDM-1 gene, first identified in Sweden in 2008 in *Klebsiella pneumoniae* from a patient hospitalized in New Delhi, encodes a metallo-β-lactamase that inactivates all β-lactams except aztreonam. This bla_{NDM-1} gene has been identified in hospital-acquired bacterial species, such as *K. pneumoniae*, but also in the typical community-acquired species, *Escherichia coli*. This gene has been identified in strains that possess other resistance mechanisms contributing to their multidrug resistance patterns. It has been recently extensively reported from the UK, India and Pakistan and, albeit to a lesser extent, from a number of other countries worldwide. In most of the cases a link with the Indian subcontinent has also been established. To stem the onslaught of NDM producers, early identification of cases of NDM-related infections and prevention of their spread by implementing screening, hygiene measures and the isolation of carriers is needed.

TÜRKİYE'DEN NDM

NDM-1-Producing *Klebsiella pneumoniae* Now in Turkey

- Irak'tan gelen KİT hastası
- Kan kültüründe üremiş
- Eksitus olmuş

Antimicrob Agents Chemother. 2012 May; 56(5): 2784–2785.

YENİ CLSI KRİTERLERİ (2010 EYLÜL) KULLANILDIĞINDA NİÇİN KARABAPENEMAZLARI RUTİNDE ARAMAYA GEREK YOK?

Çünkü duyarlı sınırlarda olduğu sürece tedavi başarısında fark yok

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 1868–1873 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.00782-08
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

Prospective Observational Study of the Impact of VIM-1 Metallo-β-Lactamase on the Outcome of Patients with *Klebsiella pneumoniae* Bloodstream Infections[▽]

George L. Daikos,[1*] Panayiotis Petrikkos,[1] Mina Psichogiou,[1] Chris Kosmidis,[1] Evangelos Vryonis,[2] Athanasios Skoutelis,[2] Kleoniki Georgousi,[3] Leonidas S. Tzouvelekis,[4] Panayotis T. Tassios,[4] Christina Bamia,[5] and George Petrikkos[1]

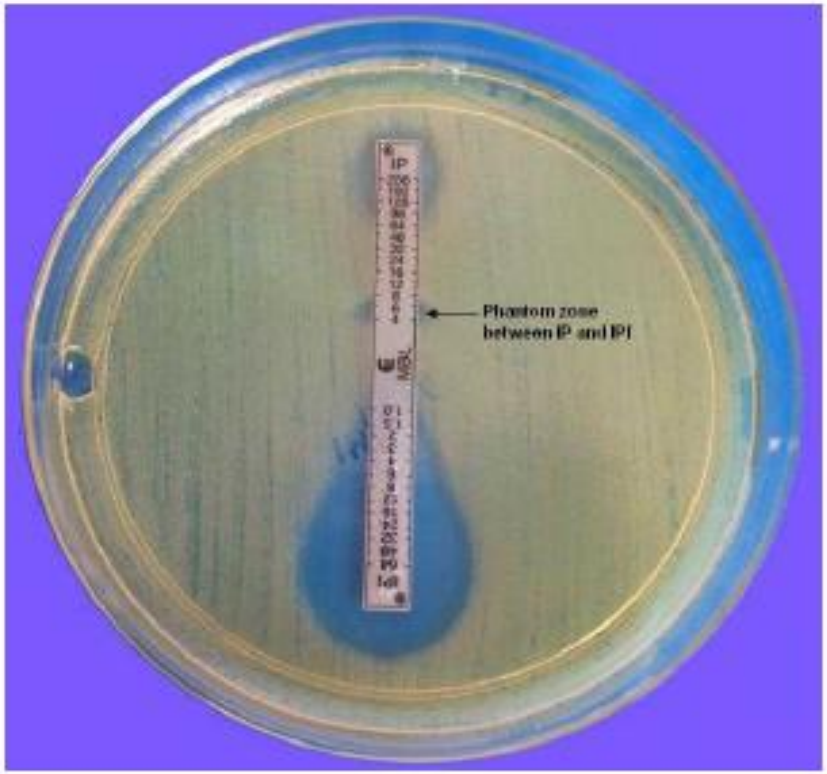
Metallo-beta-laktamazların tanımlanması

Tanımlanmalarında aktif bölgedeki Zn iyonuna bağlanarak enzimi inaktive eden bileşikler kullanılır: EDTA, dipikolinik asit, tiyol bileşikleri gibi

İmipenem / EDTA ile MİK'de

>8 kat veya >3 log2 dilüsyon

azalma



NDM-1 + CTX-15

TZP
PIP
TIC
AMX
ETP
TCC
CAZ
CF
FOX
IPM
AMC
CTX
CXM
MEM
ATM
FEP
CIP
CS
NET
RA
OFX
TE
C
TM
NOR
TGC
SXT
AN
FT
FOS
SSS
GM

Olgu 6

Yara kültürü: *Serratia marcescens* üredi

İmipenem: Duyarlı

Meropenem: Duyarlı

Ertapenem: Duyarlı

Siprofloksasin: Dirençli

Kolistin: Duyarlı

Kolistin

İntrensek dirençli olan mikroorganizmalar

- *Serratia marcescens*
- *Providencia stuartii*
- *Proteus mirabilis*
- *Morganella morganii*
- *Burkholderia cepacia*

Olgu 7

Kan kültürü: *Acinetobacter baumannii* üredi

İmipenem: Dirençli

Meropenem: Dirençli

Ertapenem: Dirençli

Siprofloksasin: Dirençli

Kolistin: Dirençli

Tigesiklin: Dirençli

Olgu 7

Kan kültürü: *Acinetobacter baumannii* üredi

İmipenem: Dirençli

Meropenem: Dirençli

Ertapenem: Dirençli

Siprofloksasin: Dirençli

Kolistin: Dirençli

Tigesiklin: Dirençli ??

TİGESİKLİN

Acinetobacter spp. için:

CLSI'da, **EUCAST'**de, **FDA**'da sınır değer yok

BSAC: ≤1 mg/L

disk diffüzyon öneriliyor

Genelde çalışmalarda ≤2 mg/L duyarlı kabul edilmiş

Olgu 8

Kan kültürü: Non-fermentatif gram negatif basil üredi

Antibiyogram sonucu (Disk difüzyon):

Siprofloksasin: Dirençli

Seftazidim: Duyarlı

İmipenem: Duyarlı

Meropenem: Duyarlı

CLSI Önerisi

- *Psedomonas aeruginosa*
- *Acinetobacter spp.*
- *Burkholderia cepacia.*
- *Stenotrophomonas maltophilia*

dışındaki non-fermentatif gram negatif basiller için duyarlılık sonucu MİK çalışılarak verilmelidir

MEVCUT DURUM: BİR YIĞIN SORUN

- Antibiyotik duyarlılık sınır değerleri değişmekte
- Değişiklikleri rutine sokmak çok zor olmakta
- Çok ilaca dirençli suşlar gün geçtikçe artmakta
- Otomatize sistemler dirençli bazı dirençli suşları atlayabilmekte
- İdentifikasyon aşamasında sorunlar yaşanmakta

ÇÖZÜM

- “İyi” çalışan bir laborauvar
- Sıkı bir “laboratuvar-klinik iletişimi”
- Hastalarını ve sonuçlarını çok iyi takip eden klinisyenler ve laboratuvar çalışanları

BAŞKENT ÜNİVERSİTESİ

HEPİMİZE KOLAY GELSİN!