

# **Antibiyotik Direnci Saptamada Yenilikler:**

# **Antibiyotik Direnci Saptamada**

# **Moleküler Tanı Testleri**



**8.** ULUSAL  
MOLEKÜLER VE  
TANISAL MİKROBİYOLOJİ  
KONGRESİ

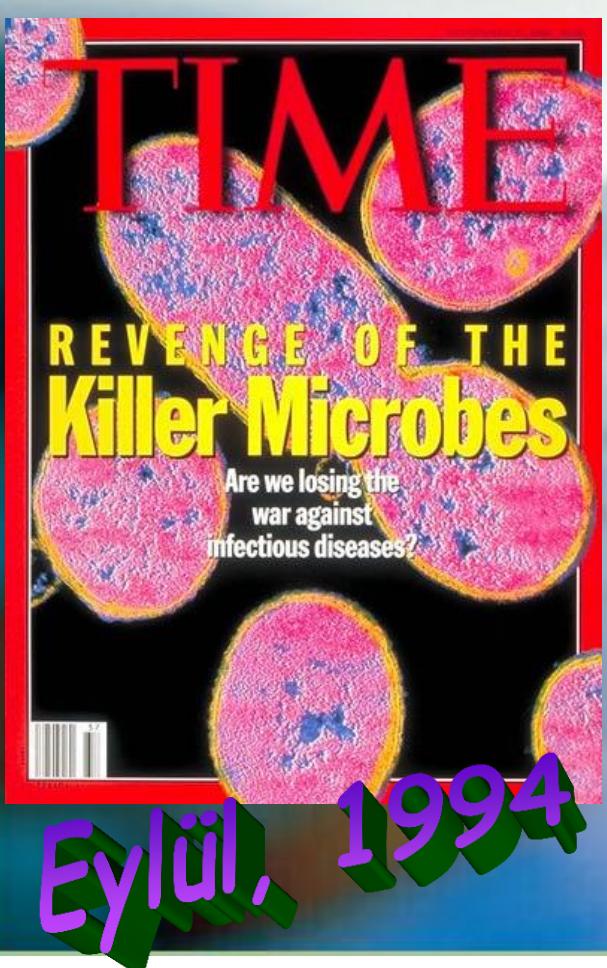
4 - 7 Haziran 2014  
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Organizing Committee  
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Anadolu University  
Gazi University  
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Marmara University  
Veterninary Faculty  
Marmara University

ÖZGEN ESER  
HACETTEPE TIP FAKÜLTESİ  
TİBBİ MİKROBİYOLOJİ ANABİLİM DA

# BAD BUGS, NO DRUGS

As Antibiotic Discovery Stagnates ...  
A Public Health Crisis Brews



## Estimating Health Care-Associated Infections and Deaths in U.S. Hospitals, 2002

- 1.7 milyon hastane enfeksiyonu
- 100,000 ölüm/yıl, çok ilaca dirençli patojen ile
- Antibiyotiklere dirençli enfeksiyon maliyeti 5 milyar USD/yıl

Bad Bugs, No Drugs." IDSA. 2004

- 25,000 ölüm/yıl, çok ilaca dirençli patojen ile
- Antibiyotiklere dirençli enfeksiyon maliyeti 1.5 milyar Euro/yıl

European Centre for Disease Prevention and Control. Antimicrobial Resistance Surveillance in Europe 2011.



## ESCAPE PATOJENLERİ

- Enterokok (Vankomisin dirençli)
- *Staphylococcus aureus* (Metisilin dirençli)
- *Clostridium difficile*
- *Acinetobacter baumannii* (MDR)
- *Pseudomonas aeruginosa* (MDR)
- *Enterobacteriaceae*

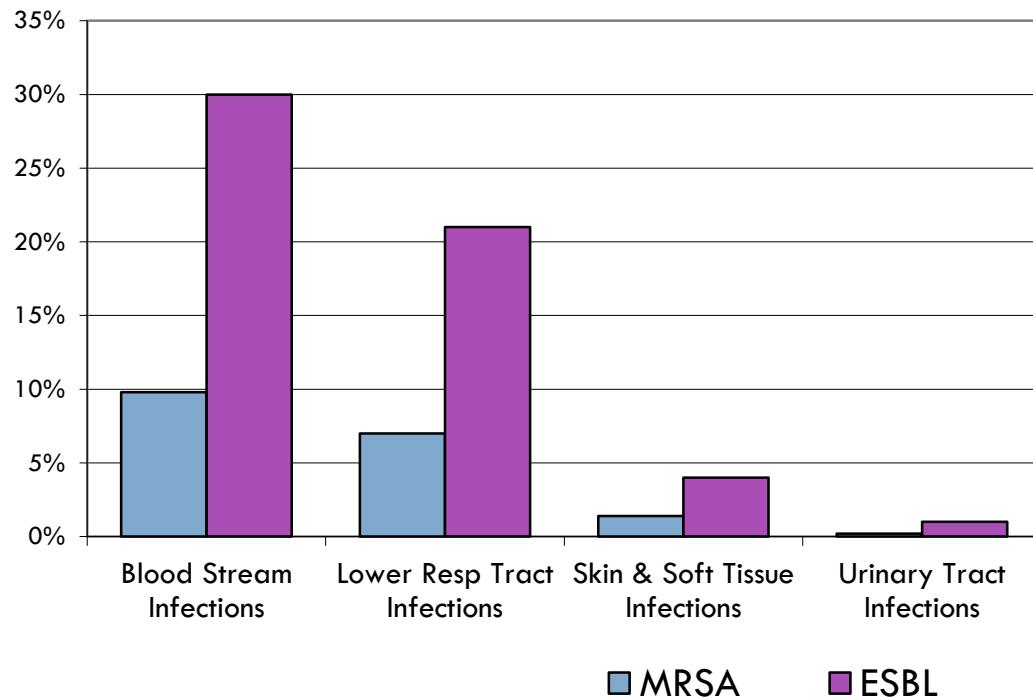
# Antibiyotik direncinin mortalite, hastanede yatis süresi ve sağlık harcamalarına yansımıası

Enfeksiyon ve etken	Ölüm riski	Hastanede yatis süresi (gün)	Maliyet (USD)
MRSA enfeksiyonu	1.9	2.2	6916
MRSA cerrahi enfeksiyonu	3.4	2.6	13901
VRE enfeksiyonu	2.1	6.2	12766
Dirençli <i>Pseudomonas aeruginosa</i> enfeksiyonu	1.8-5.4	5.7-6.5	11981-32949
Dirençli <i>Enterobacter</i> enfeksiyonu	5.0	9.0	29379
Dirençli <i>Acinetobacter</i> enfeksiyonu	2.4-6.2	5-13	37758
GSBL veya KPC üreten <i>E.coli</i> veya <i>Klebsiella</i> enfeksiyonu	3.1	11.7	1.7 milyon

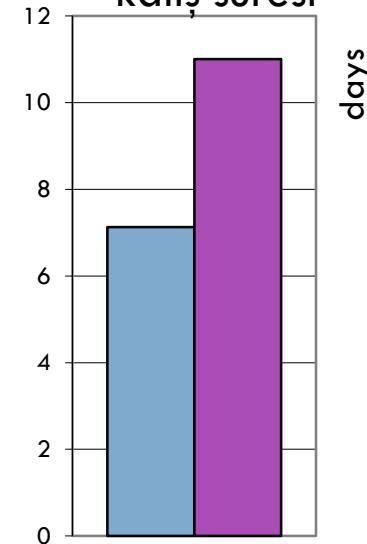
7000-38000 USD

# Dirençli bakteri enfeksiyonlarında klinik durum

Atfedilen Mortalite



Hastanede daha uzun kalış süresi



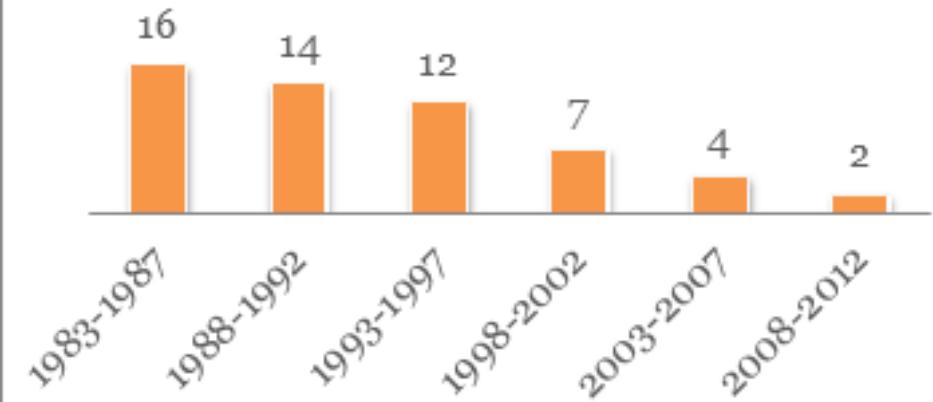
- MRSA den daha fazla sıklıkta
- Gelecek 10 yıl içinde MDR gram negatiflerde artış

## MDR gram negatiflerde tedavi maliyeti

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- Ek hastane masrafı: **29.3%**
- Hastanede kalış süresinde uzama: **23.8%**
- GSBL'a bağlı hastane maliyetinde artış: **9,620 USD**
- Hasta tedavisinde karbapenem kullanım maliyeti: > **700 USD**

## Total # of new antimicrobial agents approved by the FDA for humans



- Yeni ilaç keşfi
  - 10-20 yıl süre,
  - 800-1.700 milyon USD bütçe

Bad Bugs, No Drugs. IDSA, 2004

- Quinupristin/dalfopristin 1999
- Moksifloksasin 1999
- Linezolid 2000
- Ertapenem 2001
- Gemifloksasin 2003
- Daptomisin 2003
- Tigeciklin 2005
- Doripenem 2007
- Telavansin 2008
- Seftarolin 2010

# Antimikrobiyal dirençle başa çıkmaının 8 yolu

- Endüstriyel alanda, hayvanlarda antibiyotik kullanımını durdurmak,
- Antimikrobiyal Direnç Yönetimini uygulamak,
- Veri toplamak
- Uygunuz antibiyotik kullanımını kısıtlamak,
- **Hızlı tanı testlerine geçmek,**
- Yeni ilaç geliştirmek,
- Antibiyotik direncine karşı girişimlerini sağlık reformuna dönüştürmek,
- Ulusal bir plan yürütmek..

of such antibiotics in animals only for those uses that are considered necessary  
for assuring animal health; and having veterinary oversight for such an  
use in animals

# Enfeksiyon Acilleri ve Antimikrobiyal Direncin Belirlenmesi

- **Kan kültürleri**
  - Konvansiyonel yöntemler
  - Otomatize kan kültür sistemleri
    - Pozitif sinyal verme 48 saat içinde
    - Pozitif sinyal sonrası test süresi 24-48 saat
    - Duyarlılık test süresi 24-48 saat
- **Mikrobiyoloji Lab antibiyotik duyarlılık test sonlandırma süresi**
  - Ort 40 saat
- **Uygunsuz antibiyotik tedavisi başlanması halinde ölüm riski**
  - 1.88

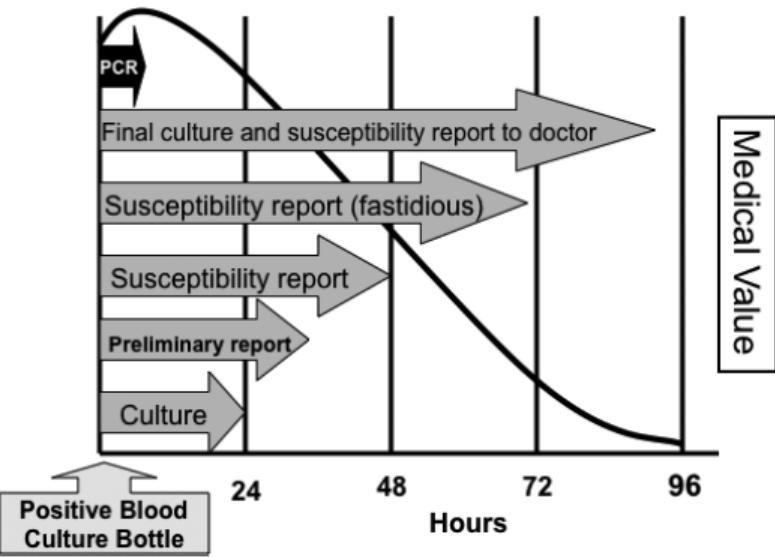
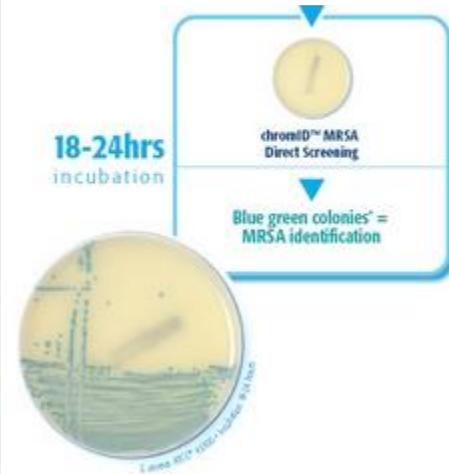


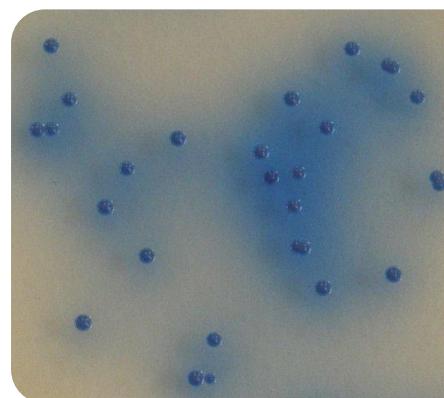
Figure 1. Time required to deliver final results of routine bacterial culture and antimicrobial susceptibility testing versus polymerase chain reaction (PCR) assays.

# MRSA

## Konvansiyonel yöntemler vs Kromojenik besiyeri



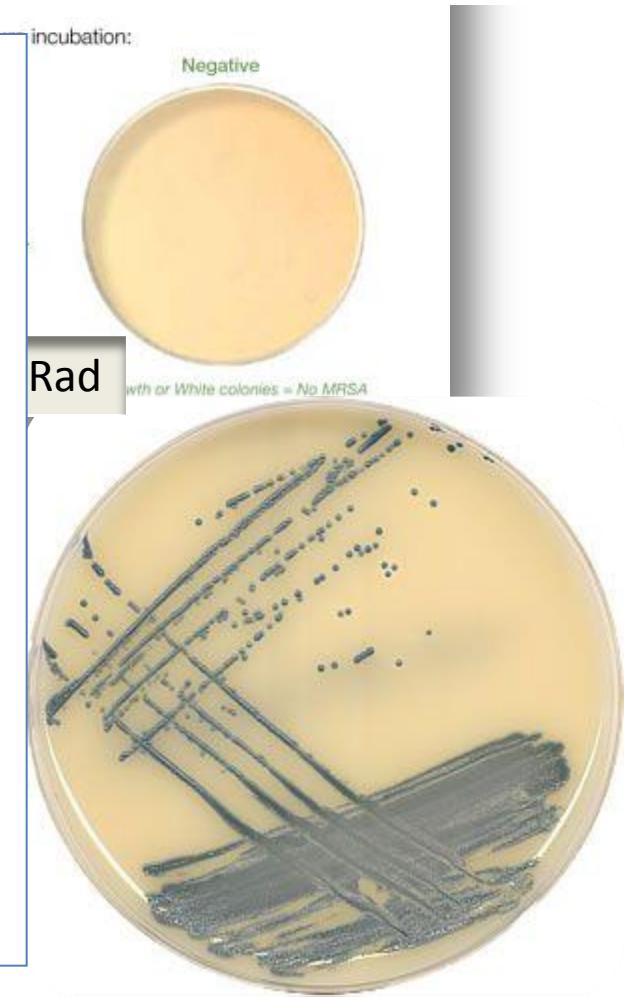
ChromID MRSA, bioMe



ORSAB, Oxoid

- Maliyet
  - 2-13 kat pahalı
- Tanımlama Süresi
  - 1.39 gün azalma
- Duyarlılık/Özgüllük
  - %96-100

Chromagar MRSA, Chromagar



Chromogenic MRSA/  
Denim blue Agar, Oxoid

# MRSA

## Moleküler Hızlı Tanı Testleri

TABLE 3. Overview of currently available molecular assays for MRSA detection

Assay	Direct inoculation or OE	References	Gold standard or comparators	Sampling sites	% Sensitivity	% Specificity	% PPV	% NPV	Total assay time (h)
Hyplex StaphyloResist	Direct OE	Daeschlein et al., 2006 (11)	Conventional culture	Nose	91.1	90.0	31.4	99.5	4.5–6
		Wagenvoort et al., 2007 (41)	Conventional culture and ORSAB	Nose, throat, perineum, and wounds	97.6	83.7	37.4	99.7	—
		Michiels et al., submitted	Conventional culture and in-house real-time PCR	Nose, throat, perineum, and wounds	98.0	96.0	83.0	99.0	3.5
LC <i>Staphylococcus</i> kit and LC MRSA detection kit	OE	Levi and Towner, 2005 (25)	Conventional culture	Nose, axilla, perineum, and others	95.7	90.8	75.9	98.6	— <sup>b</sup>
IDI-MRSA/GeneOhm MRSA	Direct	Paule et al., 2007 (31)	Conventional culture and in-house PCR	Nose	98.0	96.0	77.4	99.7	2
		Bishop et al., 2006 (4)	Conventional culture	Nose	90.0	91.7	56.3	98.8	2.5
	Rossney et al., 2007 (35)			Groin	83.3	90.2	46.9	98.1	2.5
				Pooled nose/groin	88.0	91.6	61.1	98.1	2.5
			Direct culture on MRSA Select	Nose	89.0	89.0	63.0	97.0	2.5
			Culture on MRSA Select after OE	Nose	81.0	91.0	71.0	95.0	2.5
			Culture on MRSA Select with and without OE	Throat	89.0	99.0	98.0	97.0	2.5
	van Hal et al., 2007 (39)		MRSA ID, MRSA Select, and CHROMagar	Groin/perineum	88.0	99.0	93.0	98.0	2.5
			MRSA ID, MRSA Select, and CHROMagar	Nose	94.0	94.0	94.0	94.0	2–4
		Zhang et al., 2007 (44)	MRSA Select	Groin/axilla	80.0	97.0	90.0	94.0	2–4
	Genotype MRSA Direct	NA <sup>a</sup>		Nose	100.0	96.8	69.2	100.0	—
			Conventional culture	Rectum	93.1	95.4	61.4	99.4	—
		OE	Characterized clinical isolates	Open chronic wounds and exit sites	100.0	95.0	60.0	100.0	—
			Conventional culture	Characterized clinical isolates	94.0	64.0	71.0	92.0	NA
		Direct	Pooled nose/rectum	Pooled nose/rectum	96.0	96.0	90.0	98.0	—
			Conventional culture	Nose, throat, skin, groin, axilla, and wounds	94.6	98.7	85.4	99.6	4
GeneXpert MRSA assay	Direct	van Hal et al., 2007 (39)	MRSA ID, MRSA Select, and CHROMagar	Nose	70.0	96.0	95.0	73.0	6
			Conventional culture	Groin/axilla	68.0	96.0	85.0	90.0	—
		Francois et al., 2007 (17)	IDI-MRSA and conventional culture	Characterized clinical isolates	90.0	53.0	66.0	84.0	—
		Mehta et al., 2007 (29)		Nose	98.5	90.4	—	—	—

<sup>a</sup> NA, not applicable.

<sup>b</sup> =, data not available.

# Hyplex Staphylo Resist



- Multipleks PCR
- mecA ve mec (mecLGA251) geni metisilin direnci
- ileS geni mupirosin direnci
- CA-MRSA; lukS, lukF geni ile PVL ekzotoksin
- Test süresi 3 sa 25 dk
- Duyarlılık: %91.6 Özgüllük: %83.7

Eur J Clin Microbiol Infect Dis (2006) 26:155–160  
DOI 10.1007/s10096-007-0269-5

## ARTICLE

### Comparison of an enrichment broth-enhanced commercial PCR procedure versus bacteriological culture for separating non-colonized from suspected or colonized MRSA individuals

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C. T. M. Meuwissen · J. M. H. Gronenschild ·  
E. I. G. B. De Brauwer

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**Abstract** The aim of the study presented here was to evaluate an enrichment broth-enhanced commercial PCR procedure for excluding the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) from patient samples in less than 24 h. The results of the direct sample detection have been successfully compared with the Dutch search-and-destroy policy. However, PCR facilitates more rapid screening for MRSA than traditional culture. One commercial PCR option is the hyplex *StaphyloResist*<sup>®</sup> PCR assay (Biologische Analysesystemen GmbH, Lich, Germany), which detects *Staphylococcus aureus* and the *mecA* gene in MRSA as well as in coagulase-negative staphylococci (CoNS). Since 2001, a total of 936 specimens obtained from 346 individuals during resolution of all discrepancies, the prevalence, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for all separate specimens were 9.0, 97.6, 83.7, 37.4 and 99.7%, respectively, and for specimens grouped according to daily episode submitted per individual, they were 7.5, 97.4, 77.2, 26.2 and 99.7%, respectively. These results led to the introduction of this PCR into the hospital laboratory's routine for the purpose outlined above.

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## Introduction

In The Netherlands, any epidemic with methicillin-resistant *Staphylococcus aureus* (MRSA) occurring to date has been successfully controlled with the Dutch search-and-destroy policy [1, 2]. Since 2001, 12 epidemics have occurred at the Atrium Medical Center, Parkstad, which is an 800-bed hospital serving the Limburg Parkstad Rhine-Meuse Euregion; each epidemic resulted in closure of the affected department for more than 1 month. This severely interfered with the hospital's normal patient-care routine and led to hospital costs of circa EUR 250,000 per episode [3]. Since 2001, no MRSA outbreak outside of The Netherlands was found for the patients involved, it seems that MRSA not only spreads during hospital epidemics, it also spreads in the communities within our region [4]. Intra-family transmission has also been documented, particularly with multi-locus sequence type (MLST) 5 [3]. Consequently, there is an obvious need for a quick and sensitive method of identifying MRSA or excluding it as a causative agent.

PCR can screen for MRSA more rapidly than traditional culture. The processing time with a PCR assay varies from 5 h using directly sampled swabs to 1.5 days when an overnight enrichment broth is employed. In comparison, culture averages 5 days for MRSA detection. This rapid processing time of PCR has been demonstrated by several in-house assays, and sensitivity and specificity values of up to and exceeding 90% have been reported [5, 6]. However, use of these tests is generally limited to local settings. The worldwide prevalence of MRSA has highlighted the need for rapid tests and new commercial PCR

# LightCycler® MRSA Research Use Only Detection Kit

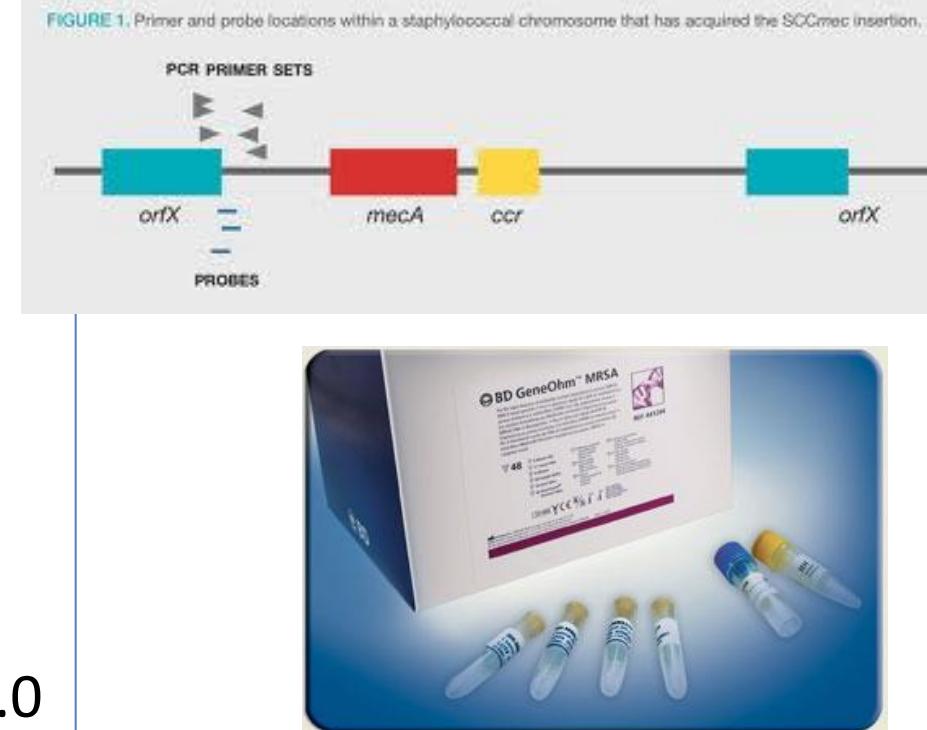


- MagNa Pure LC/LightCycler 2.0 Real-time PCR
- mecA geni ile metisilin direnci
- Örnek; burun, aksilla, perine
- 48 örnek
- Test süresi 3 sa
- Duyarlılık: %95.7 Özgüllük: %90.8
- Araştırma temelli, rutin kullanımı yok



# IDI-MRSA Assay/BD GeneOhm MRSA

- Real-time multipleks PCR
- Smart Cycler
- SCCmec tip I-V ve IVa, orfX geni
- Toplam 6 primer
- Test süresi 60-75 dk
- Direkt klinik örnek
- Kan kültürü
- Burun (FDA onaylı)
- 14 örnek
- Duyarlılık: %98.0 Özgüllük: %96.0

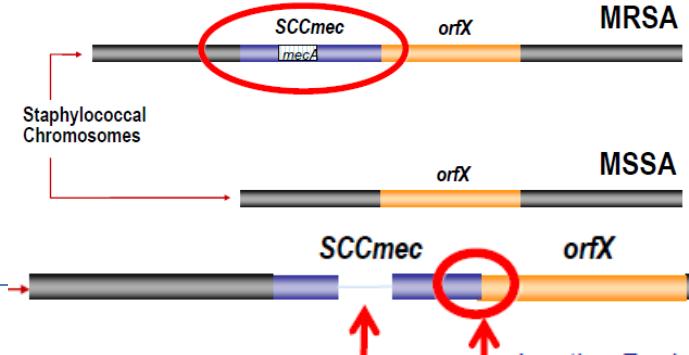


# Evaluation of the BD GeneOhm StaphSR Assay for Detection of Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Isolates from Spiked Positive Blood Culture Bottles<sup>▽</sup>

Sabine Gröbner,<sup>1</sup> Mireille Dion,<sup>2</sup> Mélanie Plante,<sup>2</sup> and Volkhard A. J. Kempf<sup>3\*</sup>

Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany<sup>1</sup>; BD Diagnostics GeneOhm, Québec, Canada<sup>2</sup>; and Institute of Medical Microbiology and Infection Control, University of Frankfurt am Main, Frankfurt am Main, Germany<sup>3</sup>

- *Staphylococcus aureus* (n:90); MSSA (n:45), MRSA(n:45)
- BD GeneOhm Staph SR ile MRSA saptamada
  - Duyarlılık %95.6 ve özgüllük %95.3
  - 3 izolat yanlış pozitif (MRSA)
    - SCCmec pozitif *mecA* neg izolatlar “*mecA dropout*”
  - 2 izolat yanlış negatif (MSSA)
    - Hedeflenmiş bölgenin suşu tanımaması



# GeneXpert MRSA Assay

- Real-time multipleks PCR
- Smart Cycler
- SCCmec tip I-V ve IVa, orfX, spa, nuc genleri (*S.aureus* ve CoNS ayrımı)
- mecA geni
- Test süresi 66 dk
- Direkt klinik örnek
- Kan kültürü, burun
- Duyarlılık: %98.0 Özgüllük: %96.0
- Piyasadan çekilmiş durumdadır.



# An Antimicrobial Stewardship Program's Impact with Rapid Polymerase Chain Reaction Methicillin-Resistant *Staphylococcus aureus/S. aureus* Blood Culture Test in Patients with *S. aureus* Bacteremia



Karri A. Bauer,<sup>1</sup> Jessica E. West,<sup>3</sup> Joan-Miquel Balada-Llasat,<sup>2</sup> Preeti Pancholi,<sup>2</sup> Kurt B. Stevenson,<sup>3</sup> and Debra A. Goff<sup>1</sup>

Departments of <sup>1</sup>Pharmacy and <sup>2</sup>Pathology, The Ohio State University Medical Center, <sup>3</sup>Division of Infectious Diseases, College of Medicine, The Ohio State University, Columbus, Ohio

- 156 *S. aureus* bakteriyemisi olan hasta
- Pre-PCR dönem 1 Eylül 2008-31 Aralık 2008
- Post-PCR dönem 10 Mart 2009-30 Haziran 2009
- GeneXpert MRSA ve kültür ile doğrulama
- Duyarlılık ve özgüllük %100
  - Optimal antibiyotik başlama süresinde **1.7 gün** azalma
  - Hastanede yatış süresinde **6.2 gün** kısalma
  - Hastane masraflarında **21.387 USD** azalma/episod başına

## Laboratory Evaluation of the BD MAX™ MRSA Assay

Sample Identification	Description	MREJ Type	Xpert® MRSA	BD MAX™ MRSA
			Assay	Assay
1	MRSA	MRSA i	Positive	Positive
2800	MRSA	MRSA ii	Positive	Positive
9	MRSA	MRSA iii	Positive	Positive
11	MRSA	MRSA iv	Positive	Positive
16	MRSA	MRSA v	Positive	Positive
19	MRSA	MRSA vii	Positive	Positive
ATCC 2312	MRSA ( <i>mecC</i> )	MRSA xxi	Negative	Negative
3097	MSSA (empty cassette)	N/A	Positive	Positive
ATCC 29213	MSSA	N/A	Negative	Negative
ATCC 14990	MSSE	N/A	Negative	Negative

- 239 burun sürüntüsü örneği
- BD Max ve Xpert MRSA karşılaştırması
- Her iki yöntemde %97.9 uyum
- BD Max ile test süresi daha kısa

# Verigene BC-GP



## “nanoparticle probe technology”

- Nanogold teknoloji,
- “Microarray” üzerinde hibridizasyon
- ELISA’dan 2-3 kat duyarlı
- Gram pozitif koklar ve Listeria türleri
- mecA, VanA, VanB genleri
- Kan kültürü (FDA onaylı)
- Araştırma amaçlı kullanım
- Duyarlılık: %97, Özgüllük: %100

Test sonlanması: <3 saat

# Rapid Detection of Gram-Positive Organisms by Use of the Verigene Gram-Positive Blood Culture Nucleic Acid Test and the BacT/Alert Pediatric FAN System in a Multicenter Pediatric Evaluation

K. V. Sullivan,<sup>a,b</sup> N. N. Turner,<sup>a</sup> S. S. Roundtree,<sup>a</sup> S. Young,<sup>c</sup> C. A. Brock-Haag,<sup>c</sup> D. Lacey,<sup>c</sup> S. Abuzaid,<sup>c</sup> D. L. Blecker-Shelly,<sup>a</sup> C. D. Doern<sup>c,d</sup>

The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA<sup>a</sup>; Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA<sup>b</sup>;

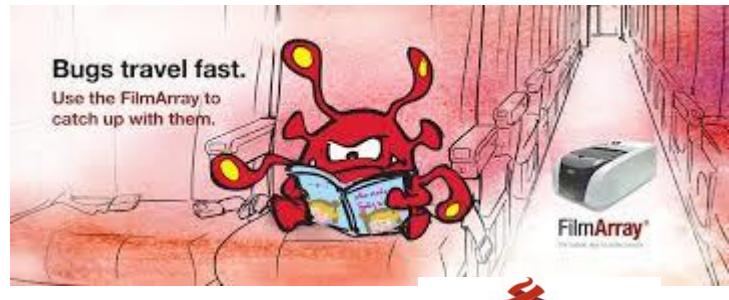
TABLE 3 Performance of Verigene BC-GP in mixed cultures

Blood culture no.	Organisms isolated	BC-GP result	BC-GP error
CHOP093	<i>S. aureus</i> (methicillin-susceptible) and <i>Klebsiella oxytoca</i>	<i>S. aureus, meca-negative</i>	None
CHOP148	<i>S. epidermidis</i> (methicillin-resistant) and <i>S. hominis</i> (methicillin-resistant)	<i>S. epidermidis, meca-positive</i>	None
CHOP149	<i>S. epidermidis</i> (methicillin-resistant) and <i>S. hominis</i> (methicillin-resistant)	<i>S. epidermidis, meca-positive</i>	None
CMC027	<i>S. bovis</i> and <i>Salmonella</i> spp.	<i>Streptococcus</i> spp.	None
CMC036	<i>S. agalactiae</i> and <i>S. aureus</i> (methicillin-susceptible)	<i>S. aureus, meca-negative</i>	<i>S. agalactiae</i> not detected
CMC041	<i>E. faecalis</i> and <i>E. faecium</i>	<i>E. faecium</i>	<i>E. faecalis</i> not detected
CMC073	<i>S. aureus</i> (methicillin-susceptible), <i>S. saprophyticus</i> (methicillin-resistant), and <i>S. hominis</i> (methicillin-susceptible)	<i>S. aureus, meca-positive</i>	False-positive <i>meca</i> result
CMC082	<i>S. mitis/S. oralis</i> and <i>Abiotrophia</i>	<i>Streptococcus</i> spp.	None
CMC088	<i>S. aureus</i> (methicillin-susceptible) and <i>S. epidermidis</i> (methicillin-susceptible)	<i>S. aureus, meca-negative</i>	<i>S. epidermidis</i> not detected
CMC108	<i>E. faecium, Streptococcus lutetiensis</i> , and <i>Escherichia coli</i>	<i>E. faecium</i> and <i>Streptococcus</i> spp.	None

- Polimikroiyal örneklerde saptamada %30 hata
- Bazı türleri tanımlamada hata (*Rothia* spp, *Micrococcus* spp)
- VRE ve MRSA/CoNS tanımlama oranı yüksek

# Film Array Kan Kültür Tanımlama Paneli

- Nested multipleks real-time PCR
- 15 cins, 11 tür düzeyinde tanımlama
- mecA, vanA, vanB direnç genleri
- Kan kültürü tanımlama paneli
- Solunum yolu ve gastrointestinal patojen panelleri
- FDA onaylı
- Duyarlılık: %88-100, Özgüllük:%100



BIO FIRE<sup>TM</sup>  
DIAGNOSTICS, INC.

Test sonlanması: <1 saat

# MRSA

## Kromojenik Besiyeri vs Moleküler Yöntemler

Ürün	Duyarlılık	Özgüllük	PPV	NPV
BBL Chromagar MRSA	93.5-95.2	96.7-99.7	84.4	99.9
MRSA Select	94-96	98-99	97	73
Spectra MRSA	95.2-95.4	99.1-99.7	93.4-99.8	98.2-99.8
ChromID MRSA	94.2	97.2	91.1	98.2
BD GeneOhm MRSA	88-96.1	93.5-99	61.1-94	97-99
GeneXpert MRSA	69.2-96.5	90.4-98	78.3-90.4	96.3-99.6

# MRSA

## Kromojenik Besiyeri vs Moleküler Yöntemler

Ürün	Yöntem	Maliyet USD	Teknik süre Dakika	Test süresi Saat	Testin Zorluğu
BBL Chromagar MRSA	Kültür	6.90	1-2	20-48	Yüksek
MRSA Select	Kültür	8.40	1-2	18-28	Yüksek
Spectra MRSA	Kültür	7.89	1-2	24	Yüksek
ChromID MRSA	Kültür	3.00	1-2	24	Yüksek
BD GeneOhm MRSA	Real-time PCR	29.5	5-9	1-1.25	Yüksek
GeneXpert MRSA	Real-time PCR	42.0	1	1.25	Orta

# VRE

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2007, p. 3360–3365

0095-1137/07/\$08.00+0 doi:10.1128/JCM.01458-07

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Vol. 45, No. 10

## Comparison of the BD GeneOhm VanR Assay to Culture for Identification of Vancomycin-Resistant Enterococci in Rectal and Stool Specimens<sup>▼</sup>

Paul D. Stamper,<sup>1,2</sup> Mian Cai,<sup>1</sup> Clara Lema,<sup>3</sup> Kim Eskey,<sup>3</sup> and Karen C. Carroll<sup>1,3\*</sup>

*Department of Pathology, The Johns Hopkins University School of Medicine,<sup>1</sup> Department of International Health Program in Disease Prevention and Control, The Johns Hopkins Bloomberg School of Public Health,<sup>2</sup> and Division of Medical Microbiology, The Johns Hopkins Hospital,<sup>3</sup> Baltimore, Maryland*

- 502 rektal sürüntü ve gaita örneği
- 147 VRE kültür pozitif örnek
- 142 VRE, BD GeneOhm VanR Assay ile
- Duyarlılık %96.6, Özgüllük %87
- Duyarlılık rektal sürüntüde gaitadan, VanA'da VanB'den daha fazla

## Performance Characteristics of the Cepheid Xpert *vanA* Assay for Rapid Identification of Patients at High Risk for Carriage of Vancomycin-Resistant Enterococci

N. Esther Babady,<sup>a</sup> Kathleen Gilhuley,<sup>a</sup> Diane Ciancimino-Bordelon,<sup>a</sup> and Yi-Wei Tang<sup>a,b</sup>

Clinical Microbiology Service, Department of Laboratory Medicine,<sup>a</sup> and Infectious Disease Service, Department of Medicine,<sup>b</sup> Memorial Sloan-Kettering Cancer Center, New York, New York, USA

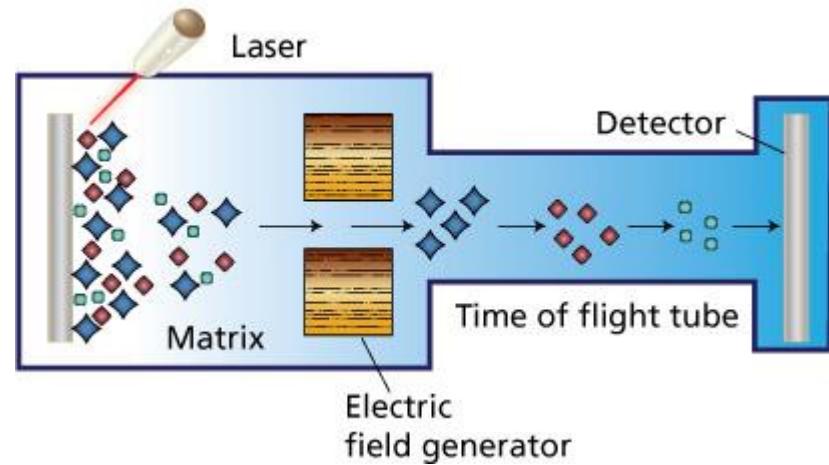
- 300 rektal sürüntü, 162 hasta
- 101 VRE pozitif, 27 VRE negatif örnek
- Duyarlılık %100, Özgüllük %96.9
- 100 cfu/ml üzerini tanıyalıme
- VRE taşıyıcılığı yüksek riskte hastalar için rutin taramalarda öneri

## Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry To Identify Vancomycin-Resistant Enterococci and Investigate the Epidemiology of an Outbreak

Paul M. Griffin,<sup>a</sup> Gareth R. Price,<sup>b</sup> Jacqueline M. Schooneveldt,<sup>a</sup> Sanmarié Schlebusch,<sup>a</sup> Martyn H. Tilse,<sup>a</sup> Tess Urbanski,<sup>a</sup> Brett Hamilton,<sup>b</sup> and Deon Venter<sup>a,b</sup>

Microbiology Division, Mater Pathology, Brisbane, Australia,<sup>a</sup> and Omics Laboratory, Mater Pathology, Brisbane, Australia<sup>b</sup>

- VanB pozitif *Enteroccus faecium*'u ayırır.
- Duyarlılık %92.4, Özgüllük %85.2
- Hızlı (dakika vs gün)
- Ucuz (0.10 vs 23 USD)



# Evaluation of Vancomycin Resistance 3 Multiplexed PCR Assay for Detection of Vancomycin-Resistant Enterococci from Rectal Swabs

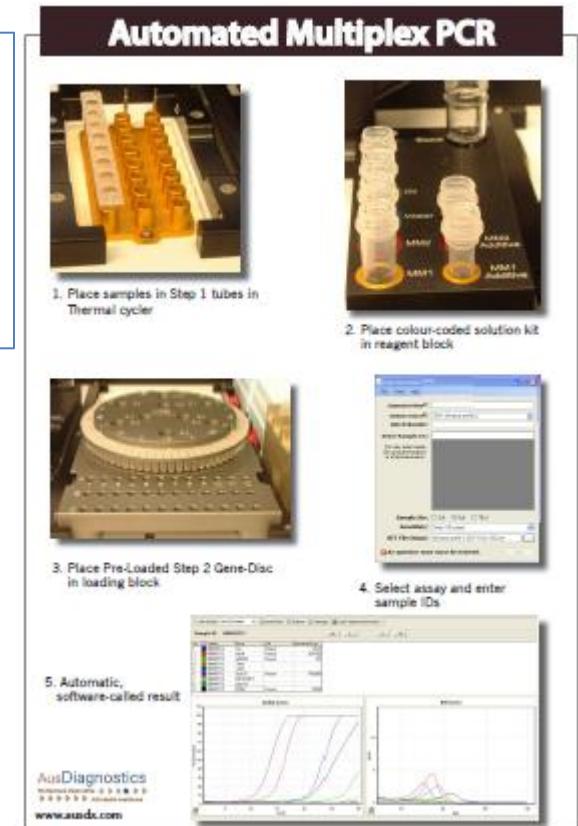
Yesim Cekin, M.D.<sup>1</sup>, Aylin Erman Daloğlu, M.D.<sup>2</sup>, Dilara Öğünç, M.D.<sup>2</sup>, Betil Özhak Baysan, M.D.<sup>2</sup>, Duygu Dağlar, M.D.<sup>3</sup>, Dilara İnan, M.D.<sup>4</sup>, Derya Mutlu, M.D.<sup>2</sup>, Gözde Öngüt, M.D.<sup>2</sup>, and Dilek Çolak, M.D.<sup>2</sup>

Clinical Microbiology<sup>1</sup>, Antalya Research and Training Hospital; Department of Medical Microbiology<sup>2</sup>, Akdeniz University Faculty of Medicine; Clinical Microbiology<sup>3</sup>, Serik State Hospital; Department of Infectious Diseases and Clinical Microbiology<sup>4</sup>, Akdeniz University Faculty of Medicine, Antalya, Turkey

- Multipleks real-time PCR, Easy Plex System
- 90 dk, 24 örnek
- VanA ve VanB



- 211 rektal sürüntü örneği
- Duyarlılık %84, Özgüllük %98.8
- PCR inhibitörlerinden dolayı çalışma dışı örnek %8.5
- VanB için düşük özgüllükte



# *Clostridium difficile*

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3478–3481

0095-1137/09/\$12.00 doi:10.1128/JCM.01133-09

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Vol. 47, No. 11

## Comparison of a Rapid Molecular Method, the BD GeneOhm Cdiff Assay, to the Most Frequently Used Laboratory Tests for Detection of Toxin-Producing *Clostridium difficile* in Diarrheal Feces<sup>▼</sup>

Gabriella Terhes,<sup>1\*</sup> Edit Urbán,<sup>1</sup> József Sóki,<sup>1</sup> Enikő Nacsá,<sup>2</sup> and Elisabeth Nagy<sup>1</sup>

*Institute of Clinical Microbiology, Albert Szent-Györgyi Medical and Pharmaceutical Center, H-6725 Szeged, Hungary,<sup>1</sup> and Department of Infectious Diseases, Albert Szent-Györgyi Medical and Pharmaceutical Center, H-6725 Szeged, Hungary<sup>2</sup>*

- Real-time PCR
- *Clostridium difficile* tcdB genini belirler.
- Test süresi 75-90 dakika
- Enzim Immün Assay (EIA), sitotoksisite ve kültür yöntemlerine göre
  - Duyarlılık / Özgüllük yüksek
  - Test süresi kısa

# *Clostridium difficile*

## Evaluation of the Fully Automated BD MAX Cdiff and Xpert *C. difficile* Assays for Direct Detection of *Clostridium difficile* in Stool Specimens

Alexander H. Dalpke,<sup>a</sup> Marjeta Hofko,<sup>a</sup> Markus Zorn,<sup>b</sup> Stefan Zimmermann<sup>a</sup>

Department of Infectious Diseases, Medical Microbiology and Hygiene<sup>a</sup> and Department of Internal Medicine I and Clinical Chemistry,<sup>b</sup> University Hospital Heidelberg, Heidelberg, Germany

### BDMax Cdiff

- Real-time PCR
- tcdB geni
- <3 sa



### Xpert Cdifficile Assay

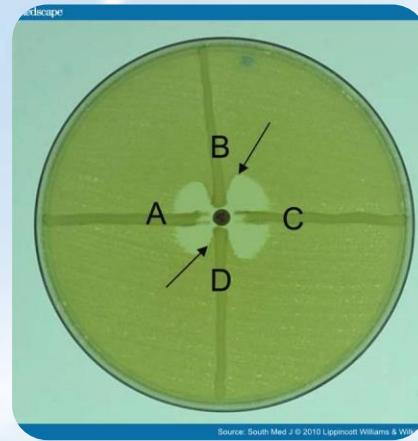
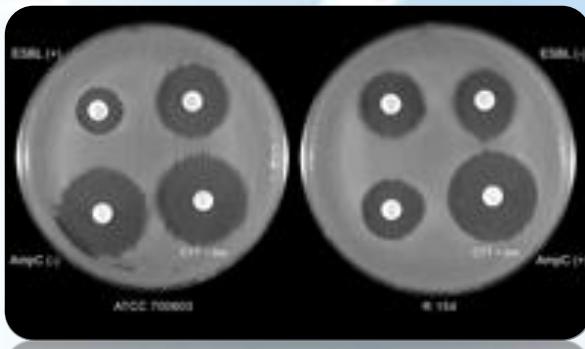
- Real-time PCR
- tcdB, cdt, tcdC mutasyon geni
- <3 sa



- 448 gaita örneği, 86 kültür pozitif örnek
- 74 örnek toksin pozitif *C. difficile*
- Her iki yöntem duyarlılık ve özgüllüğü yüksek
- Test süresi; BD max 110 dk, Xpert *C. difficile* 65 dk
- Dezavantajları:
  - Kolonizasyon ile enfeksiyon ayırmaları zayıf

# Enterobacteriaceae

## Dirence neden olan mekanizmalar



- Genişlemiş spektrumlu beta-laktamazlar (GSBL)
- Karbapenemazlar (KPC, VIM, IMP, NDM, OXA-48, ..)
- AmpC tipi beta-laktamazlar

# GSBL

## Kültür Temelli Otomatize Sistemler



Vitek2, bioMerieux



Phoenix, BD



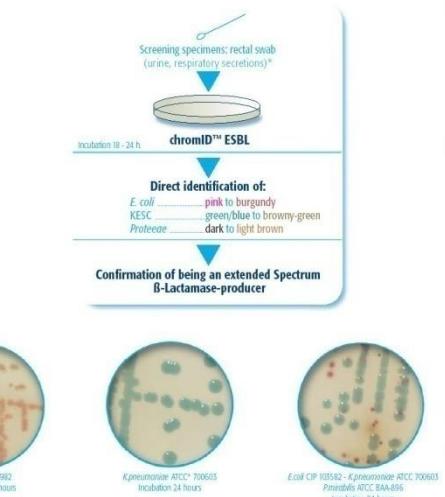
- Phoenix, BD
  - GSBL saptama: duyarlılık %94.2, özgüllük %100
- Vitek 2, bioMerieux
  - GSBL saptamada uyumsuzluk: Phoenix ile **%23.6**

# GSBL

## Kültür Temelli Hızlı Testler

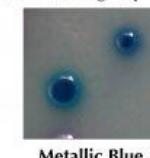
Seftazidim/seftazidim-klavulanik asit/BA  
Seftriakson/seftriakson-klavulanik asit/BA  
Sefotaksim/sefotaksim-klavulanik asit/BA

EbSA, Cepheid



ChromID, BioMerieux

Klebsiella, Enterobacter,  
Citrobacter group -ESBL

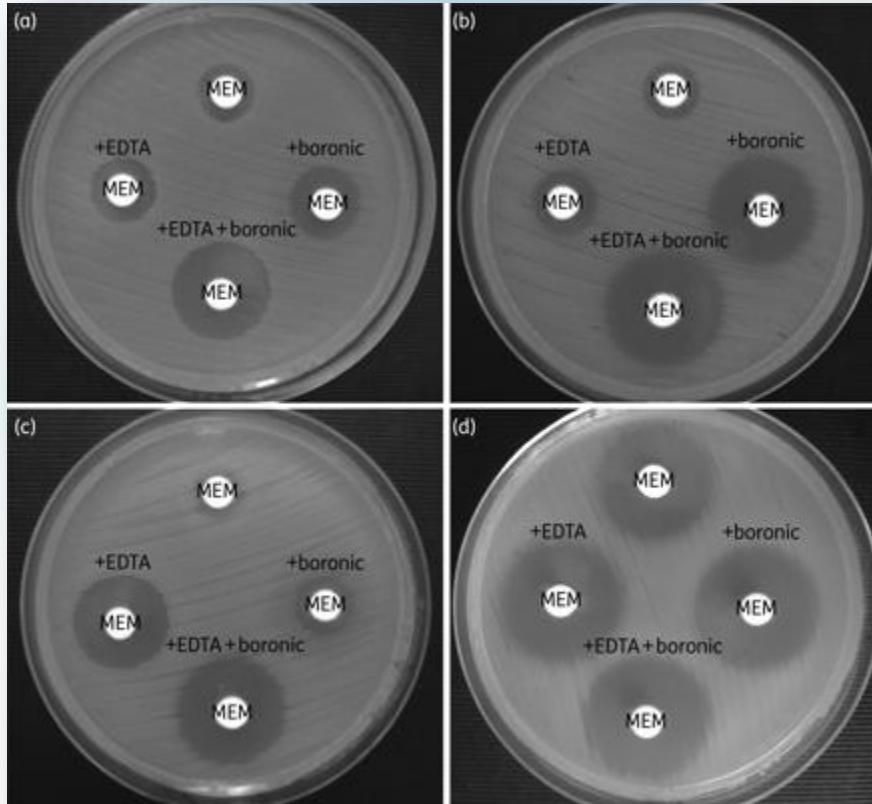


Metallic Blue

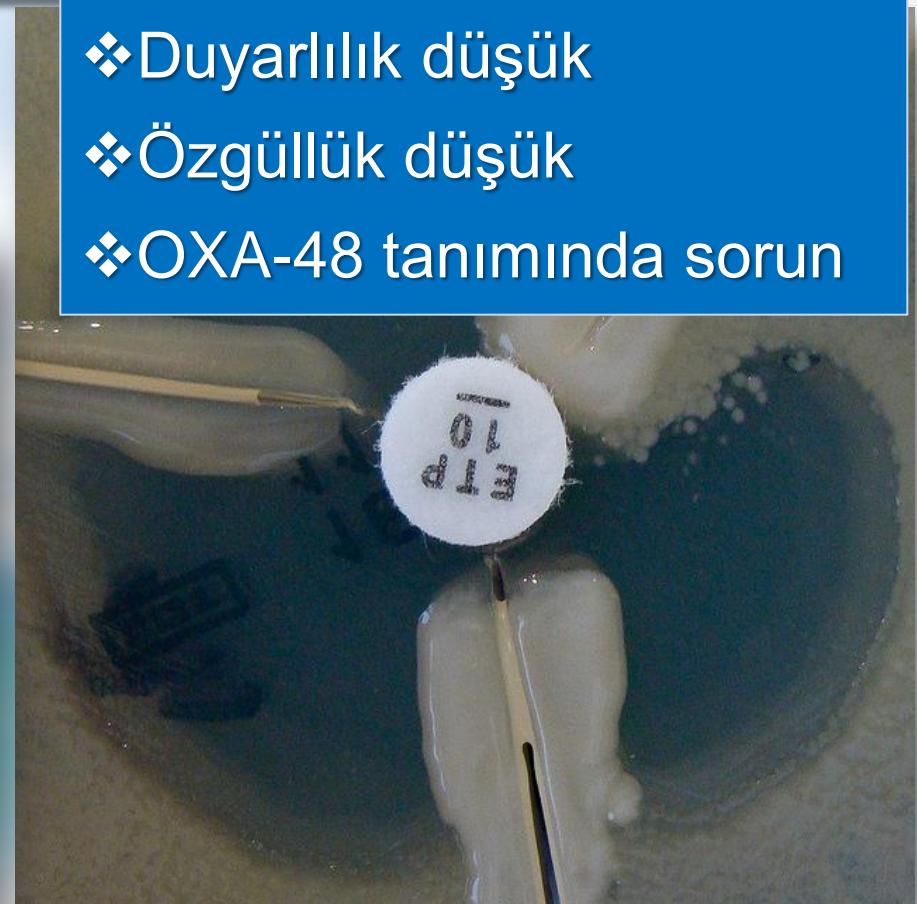
# Karbapenemaz

## Fenotipik testler

- Fenilboronik asit
- EDTA



- ❖ Duyarlılık düşük
- ❖ Özgüllük düşük
- ❖ OXA-48 tanımında sorun



Modifiye Hodge Test

# Karbapenemaz Kültür temelli hızlı testler



- ❖ Duyarlılık yüksek
- ❖ Özgüllük yüksek
- ❖ OXA-48 yapımına bağlı direnci tanımda güçlük
- ❖ Tanıda 18 saatlik gecikme



CHROMAgar KPC,



Brilliance CRE, Oxoid

# Check-Points

## Check-MDR ESBL Real-Time PCR

## Check-MDR Carba

## Check-Direct CPE

### “Real-time PCR”

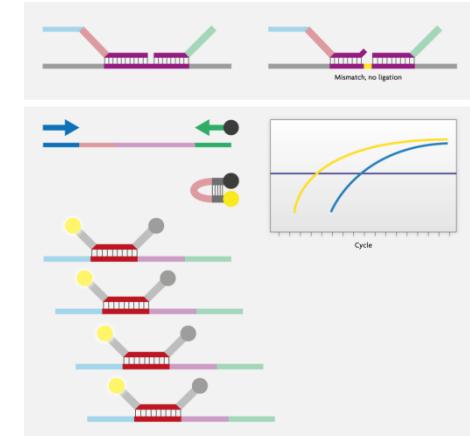
- CTX-M
- SHV
- TEM

- KPC
- OXA-48
- NDM
- VIM
- IMP

- Kültür temelli
- Duyarlılık %100
- Özgüllük %100



- KPC
- NDM
- VIM
- OXA-48



Test sonlanması: 4 saat

# Check-Point

Ticari Kit

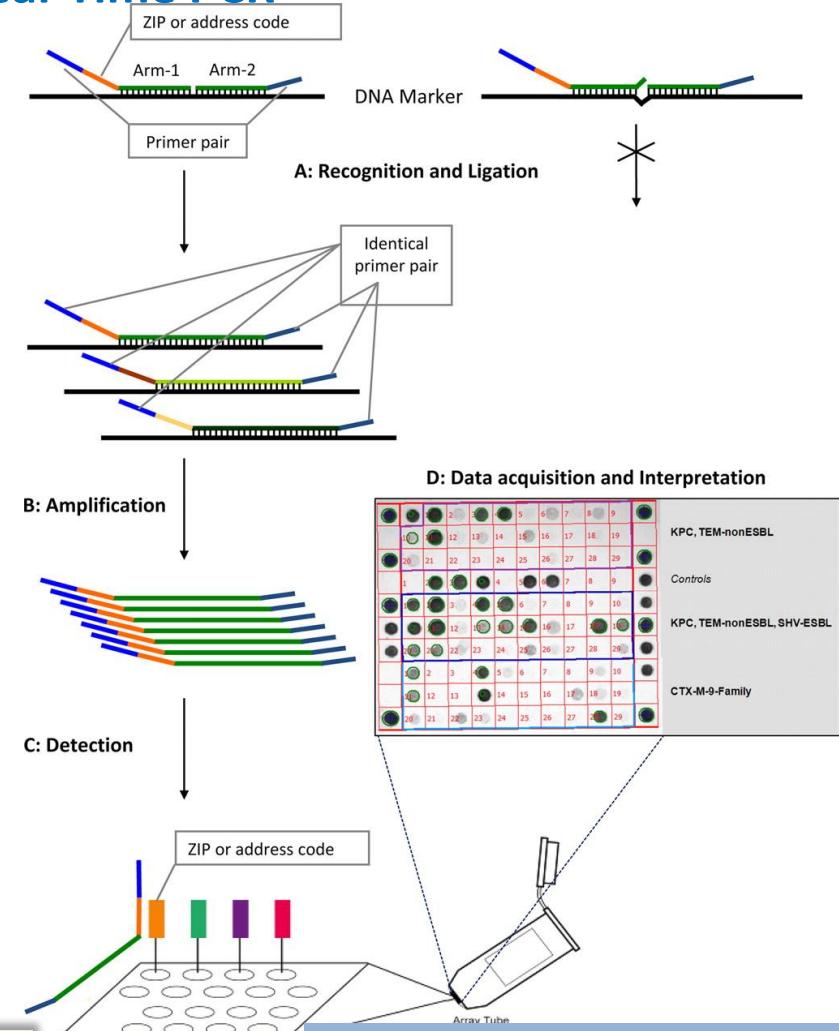
## Check-MDR CT103XL Real-Time PCR

### Check-MDR CT103 Real-Time PCR

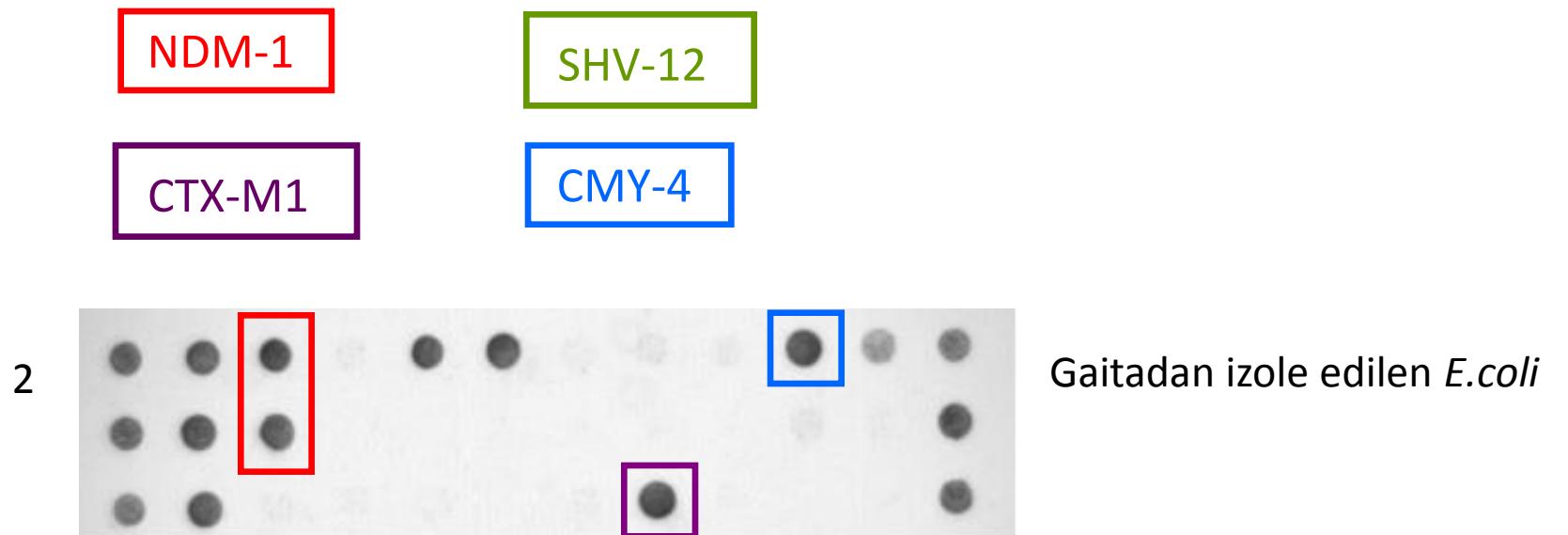
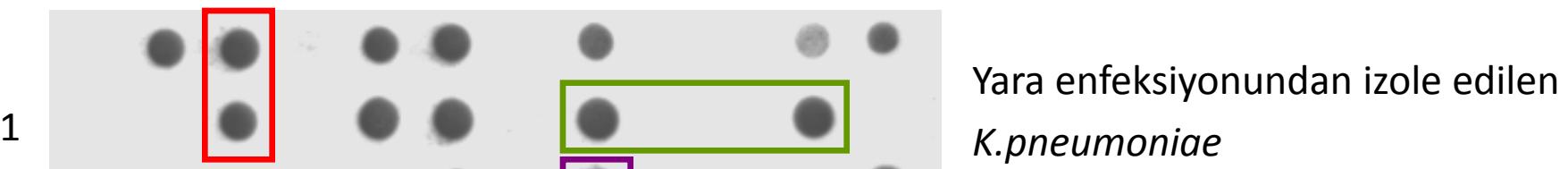
### Check-MDR CT101 Real-Time PCR

“Real-time PCR ve  
Microarray”

- NDM, VIM, IMP,
- OXA
- KPC
- AmpC
- CTX-M
- SHV
- TEM



# Check-Points molecular microarray



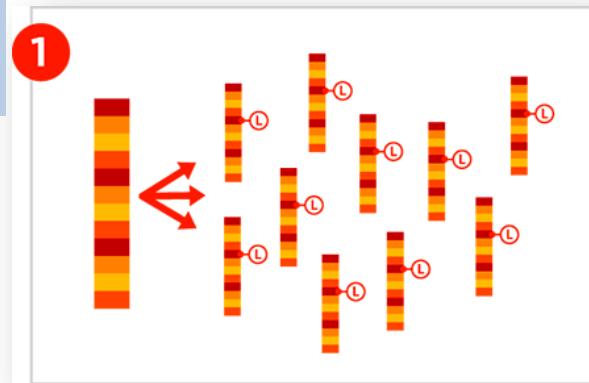
# Identibac AMR-ve

“Multipleks PCR  
ve Microarray”

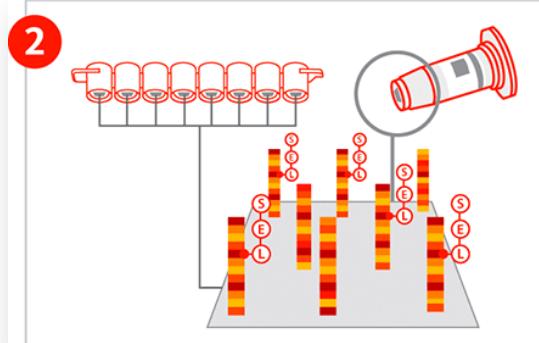
57 farklı direnç geni

- TEM, SHV, CTX-M
- VIM
- OXA
- DHA
- CMY
- aac
- qnr
- Kütür temelli
- Duyarlılık %98.8
- Ticari kit

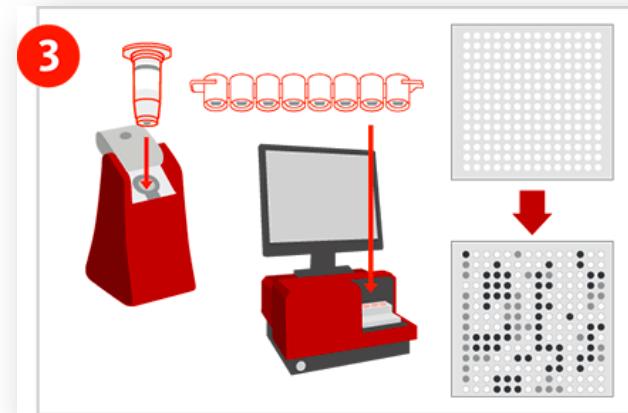
Test sonlanması: 8 saat



“Amplifikasyon”



“Hibridizasyon”



“Saptama”



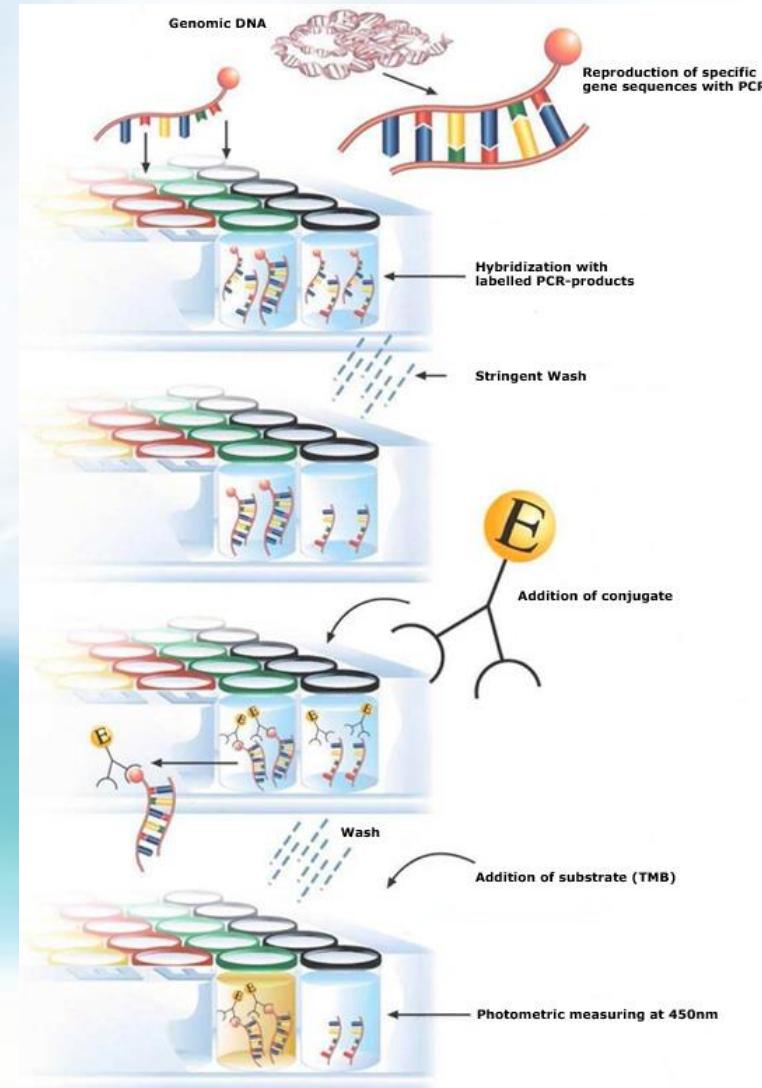
“Analiz”

# Hyplex, Multiplex PCR ELISA

## “Multipleks PCR ve ELISA”

- GSBL
- KPC
- MBL
- OXA

- Hyplex, Multiplex PCR ELISA**
- DNA ekstraksiyonu
  - Multipleks PCR
  - ELISA temelli hibridizasyon
  - Duyarlılık (%98)
  - Özgüllük (%99)
  - Klinik örnekte uygulanım



# Evaluation of Phenotypic and Genotypic Approaches for the Detection of Class A and Class B Carbapenemases in *Enterobacteriaceae*

Simone Ambretti,\* Paolo Gaibani,\* Andrea Berlingeri, Miriam Cordovana, Maria Vittoria Tamburini, Gloria Bua, Maria Paola Landini, and Vittorio Sambri

TABLE 1. SUMMARY OF RESULTS OBTAINED ON 108 ISOLATES OF *ENTEROBACTERIACEAE* CLASSIFIED BY PCR AS PRODUCERS OF CLASS A AND CLASS B CARBAPENEMASES, ES $\beta$ L, AND AMPC, TESTED BY MHT [ $\pm$  ZnSO<sub>4</sub>], Rosco SYNERGIC TEST, AND HYPLEX PCR-ELISA

Strains (No. of studied)	MHT No. of positive		Rosco disc synergic assay No. of positive			Hyplex PCR assay No. of positive		
	[− ZnSO <sub>4</sub> ]	[+ ZnSO <sub>4</sub> ]	Boronic acid	Dipicolinic acid	Cloxacillin	KPC	NDM	VIM
Class A carbapenemases	50/50	50/50	50/50	—	—	50/50	—	—
KPC								
<i>Klebsiella pneumoniae</i> (50)	50	50	50	—	—	50	—	—
Class B carbapenemases	11/20	17/20	—	20/20	—	—	8/8	12/12
NDM								
<i>K. pneumonia</i> (7)	2	5	—	7	—	—	7	—
<i>Escherichia coli</i> (1)	1	1	—	1	—	—	1	—
VIM								
<i>K. pneumonia</i> (6)	2	5	—	6	—	—	—	6
<i>Citrobacter freundii</i> (3)	3	3	—	3	—	—	—	3
<i>Enterobacter cloacae</i> (2)	2	2	—	2	—	—	—	2
<i>Klebsiella oxytoca</i> (1)	1	1	—	1	—	—	—	1
<i>Aeromonas</i>	1/10a	1/10a	1/10	—	—	—	—	—

# “MALDI-TOF MS”

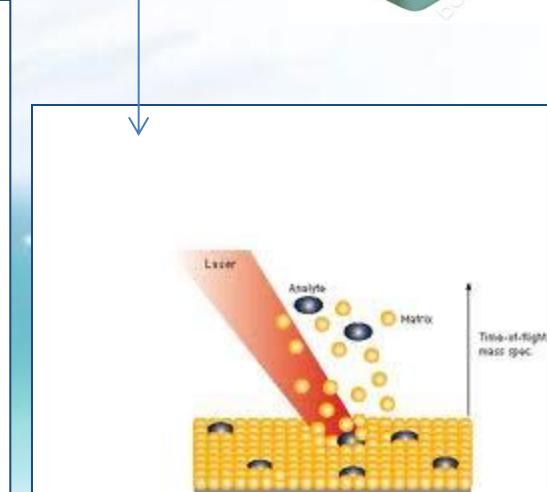


## Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 Carbapenemases by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Jaroslav Hrabák,<sup>a</sup> Vendula Študentová,<sup>a</sup> Radka Walková,<sup>a</sup> Helena Žemličková,<sup>b</sup> Vladislav Jakubů,<sup>b</sup> Eva Chudáčková,<sup>a</sup> Marek Gniadkowski,<sup>c</sup> Yvonne Pfeifer,<sup>d</sup> John D. Perry,<sup>e</sup> Kathryn Wilkinson,<sup>e</sup> and Tamara Bergerová<sup>a</sup>

Department of Microbiology, Faculty of Medicine and University Hospital in Plzen, Charles University in Prague, Plzen, Czech Republic<sup>a</sup>; National Reference Laboratory for Antibiotics, National Institute for Health, Prague, Czech Republic<sup>b</sup>; National Medicines Institute, Warsaw, Poland<sup>c</sup>; Robert Koch Institute, Nosocomial Infections, Wernigerode, Germany<sup>d</sup>, and Microbiology Department, Freeman Hospital, Newcastle upon Tyne, United Kingdom<sup>e</sup>

- Proteomik profile göre tanımlama
- Karbapenemaz varlığı
- Meropenem yıkım ürününü saptar
- Dakikalar içinde sonuç verir
- Duyarlılık %100, Özgüllük %100
- Sistem son derece pahalı ancak test ucuz
- Kültüre dayalı





## Detection of Colonization by Carbapenemase-Producing Gram-Negative Bacilli in Patients by Use of the Xpert MDRO Assay

Fred C. Tenover,<sup>a</sup> Rafael Canton,<sup>b</sup> JoAnn Kop,<sup>a</sup> Ryan Chan,<sup>a</sup> Jamie Ryan,<sup>a</sup> Fred Weir,<sup>a</sup> Patricia Ruiz-Garbajosa,<sup>b</sup> Vincent LaBombardi,<sup>c\*</sup>  
David H. Persing<sup>a</sup>

Cepheid, Sunnyvale, California, USA<sup>a</sup>; Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain<sup>b</sup>; Microbiology Laboratory, Mt. Sinai Medical Center, New York, New York, USA<sup>c</sup>

- Multipleks real-time PCR
- Klinik örnek; rektal sürüntü
- Duyarlılık %100, Özgüllük %99.4
- Test süresi <1 sa
- FDA onaylı

- IMP-1
- VIM
- NDM
- KPC
- OXA-48

## Detection of carbapenemases by real-time PCR and melt-curve analysis on the BD MAX™ System

- 65 karbapenemaz pozitif, 87 negatif izolat
- Syber green real-time PCR, BD Max Sistem ile
- IMP1/2, GES, KPC, VIM1/2, OXA 23 ve 48, NDM
- Duyarlılık %91 (IMP) , Özgüllük %100

# Verigene BC-GN (Nanosphere)

## Evaluation of an Automated Rapid Diagnostic Assay for Detection of Gram-Negative Bacteria and Their Drug-Resistance Genes in Positive Blood Cultures

Masayoshi Tojo<sup>1,2</sup>, Takahiro Fujita<sup>3</sup>, Yusuke Ainoda<sup>3</sup>, Maki Nagamatsu<sup>1,2</sup>, Kayoko Hayakawa<sup>1</sup>, Kazuhisa Mezaki<sup>4</sup>, Aki Sakurai<sup>5</sup>, Yoshinori Masui<sup>6</sup>, Hirohisa Yazaki<sup>7</sup>, Hiroshi Takahashi<sup>8</sup>, Tohru Miyoshi-Akiyama<sup>2</sup>, Kyoichi Totsuka<sup>3</sup>, Teruo Kirikae<sup>2\*</sup>, Norio Ohmagari<sup>1</sup>

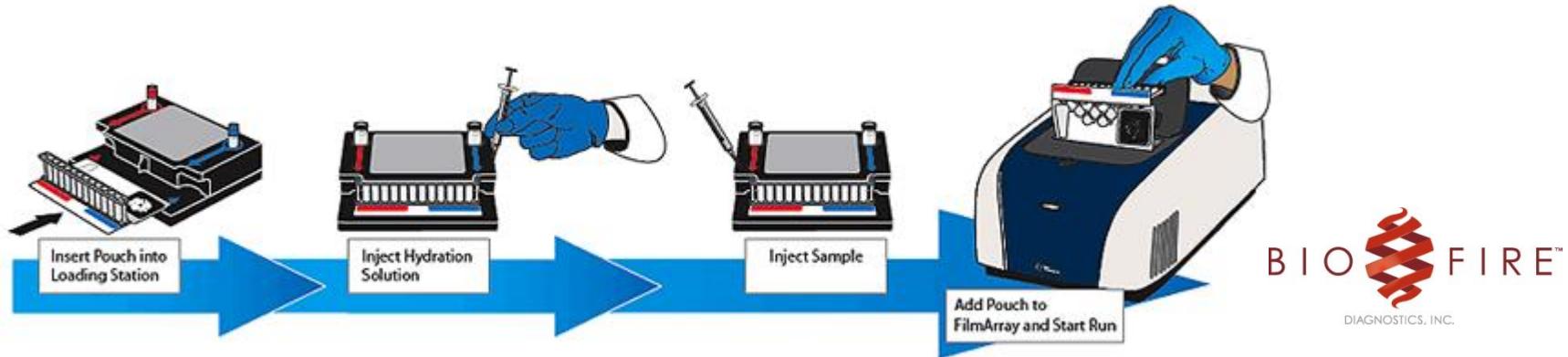
- 102 klinik, 295 simule kan kültür örneği
- Bakteri tanımlamada uyum %96.3
- Antibiyotik direnç genleri saptama %100
- Test süresi 2 sa, teknik destek 5 dk

Table 2  
Verigene Gram-negative (BC-GN) blood culture assay targets (in development)

Genus-Level	Species-Level	Antibiotic Resistance
<i>Acinetobacter</i> spp	<i>Escherichia coli</i> / <i>Shigella</i> spp	<i>bla</i> <sub>KPC</sub>
<i>Proteus</i> spp	<i>Klebsiella pneumoniae</i>	<i>bla</i> <sub>NDM</sub>
<i>Citrobacter</i> spp	<i>Klebsiella oxytoca</i>	<i>bla</i> <sub>CTX-M</sub>
<i>Enterobacter</i> spp	<i>Pseudomonas aeruginosa</i>	<i>bla</i> <sub>VIM</sub>
	<i>Serratia marcescens</i>	<i>bla</i> <sub>IMP</sub>
		<i>bla</i> <sub>Oxa</sub>

# FilmArray™

The fastest way to better results.



- KPC
- Van A
- Van B
- mec A

- Nested multipleks PCR
- Kan kültürü tanımlama paneli
- 27 tür (gram pozitif, gram negatif, maya)

Test sonlanması: 1 saat

- TEM
- SHV
- CTX-M
- DHA
- EBC
- OXA-51
- KPC
- İntegron
- Kinolon direnci
- Makrolid direnci



- Multipleks PCR
- Prob üzerine hibridizasyon sonrası membran üzerine aktarma
- Solunum yolu örnekleri
- 18 bakteri, 22 antibiyotik direnç geni

Evaluation of Curetis Unyvero<sup>TM</sup>, a multiplex PCR-based testing system, for rapid detection of bacteria and antibiotic resistance and its impact on the management of severe nosocomial pneumonia

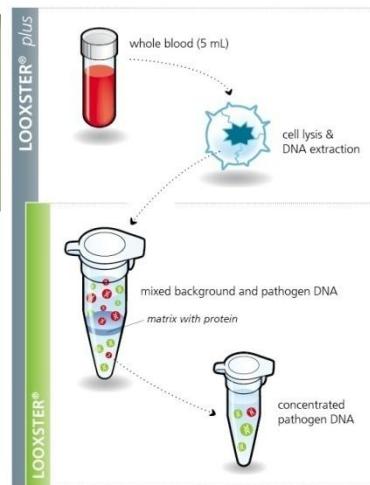
# Multipleks ve Real-time PCR temelli hızlı yöntemler



- Septi-Fast
- Septi-Test

## “Microarray” temelli hızlı yöntemler

- Looxter/VYOO
- Prove-it Sepsis



## Proteomik temelli hızlı yöntemler

- ESI-MS
- Maldi-Biotyper





# DİRENÇ TAYİNİNDE HIZLI MOLEKÜLER TANI YÖNTEMLERİ

- Testin “antimikroiyal yönetim programı” ile birlikte uygulanımı
- Morbidite ve mortalitede azalma oranlarına etkisi
- Akılçıl antibiyotik kullanımında artış
- Hastanede yatış süresinde kısalma
- Hasta maliyetinde azalma

# DİR HIZLI MOLEKİ

EMLERİ



- Moleküler temelli testler pahalı, kompleks
- Tecrübeli personele gereksinim var
- Fenotipik testler ile uyumsuzluk sorunu
- Testin tekrarlanabilirlik süresi
- Sınırlı sayıda patojeni, sınırlı sayıda direnç genini tanıtmakta, direcin düzeyini belirleyemekte

# DİRENÇ TAYİNİNDE AMAÇ

- Tanı koyma süresini kısaltan,
- Tedavi kalitesini artıran,
- Tedavi maliyetini düşüren,
- Hızlı, güvenilir, doğru ve basit testlerin geliştirilmesi olmalıdır.





## Antimikrobiyal Yönetim

- Antimikrobiyallerin uygun ve akıllı kullanımı
  - Antimikrobiyalın doz, süre ve seçiminin en uygun şekilde yapılması
  - Direncin ortaya çıkışının engellenmesi

# Enfeksiyon Hastalıklarında Uzman Yaklaşımı



Teşekkür ederim