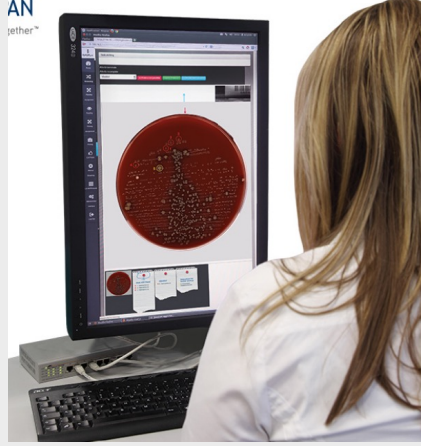


Klinik Mikrobiyoloji Laboratuvarında Otomatize Sistemlerin Klinik Karara Etkileri

Dr. Banu Bayraktar

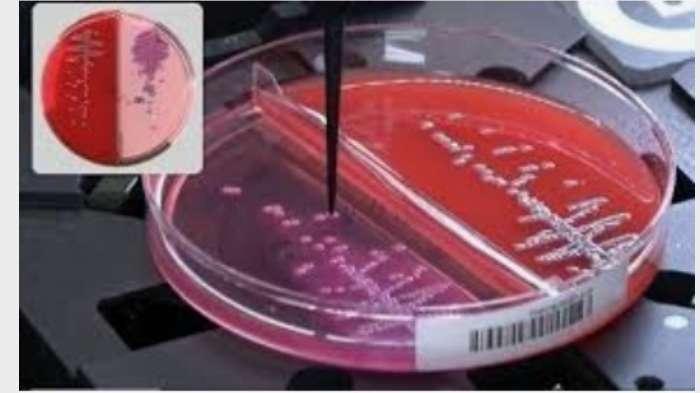
Sağlık Bilimleri Üniversitesi, Şişli
Hamidiye Etfal SUAM



TIBBİ MİKROBİYOLOJİ
LABORATUVARLARININ SON ON YIL İÇİNDE
GEÇİRMEKTE OLDUĞU BÜYÜK DEĞİŞİM

SUNUM PLANI

- MALDI-TOF MS
- Otomatize kan kltr sistemleri
- Otomatize antibiyotik duyarlılık testleri
- Total laboratuvar otomasyonu
- Kliniēe yansımaları



MALDI-TOF MS

- Matriks aracılı lazer dezorpsiyon/ionizasyon- uçuş zamanlı kütle spektrometresi
- ***Matrix Assisted Laser Desorption/Ionization—Time of Flight Mass Spectrometry (MALDI-TOF MS)***

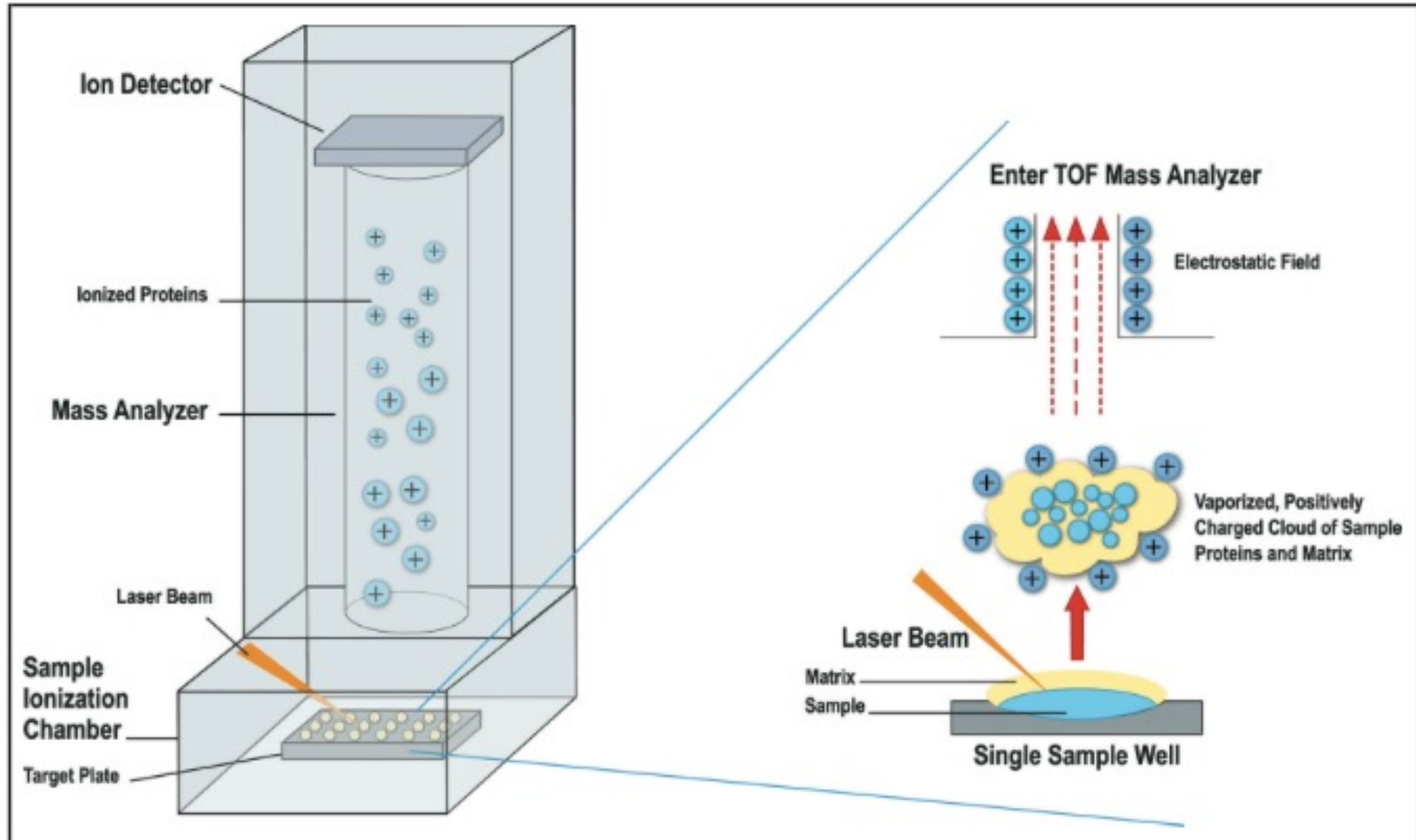


MALDI-TOF MS mikrobiyoloji laboratuvarlarında paradigma değiştiren teknoloji

Tek bir bakteri kolonisinden identifikasyon süresi 4 dakika

MALDI-TOF MS

Çalışma prensibi



Her bakteri türü kendine özel
kütle piklerinden oluşan profile
sahip

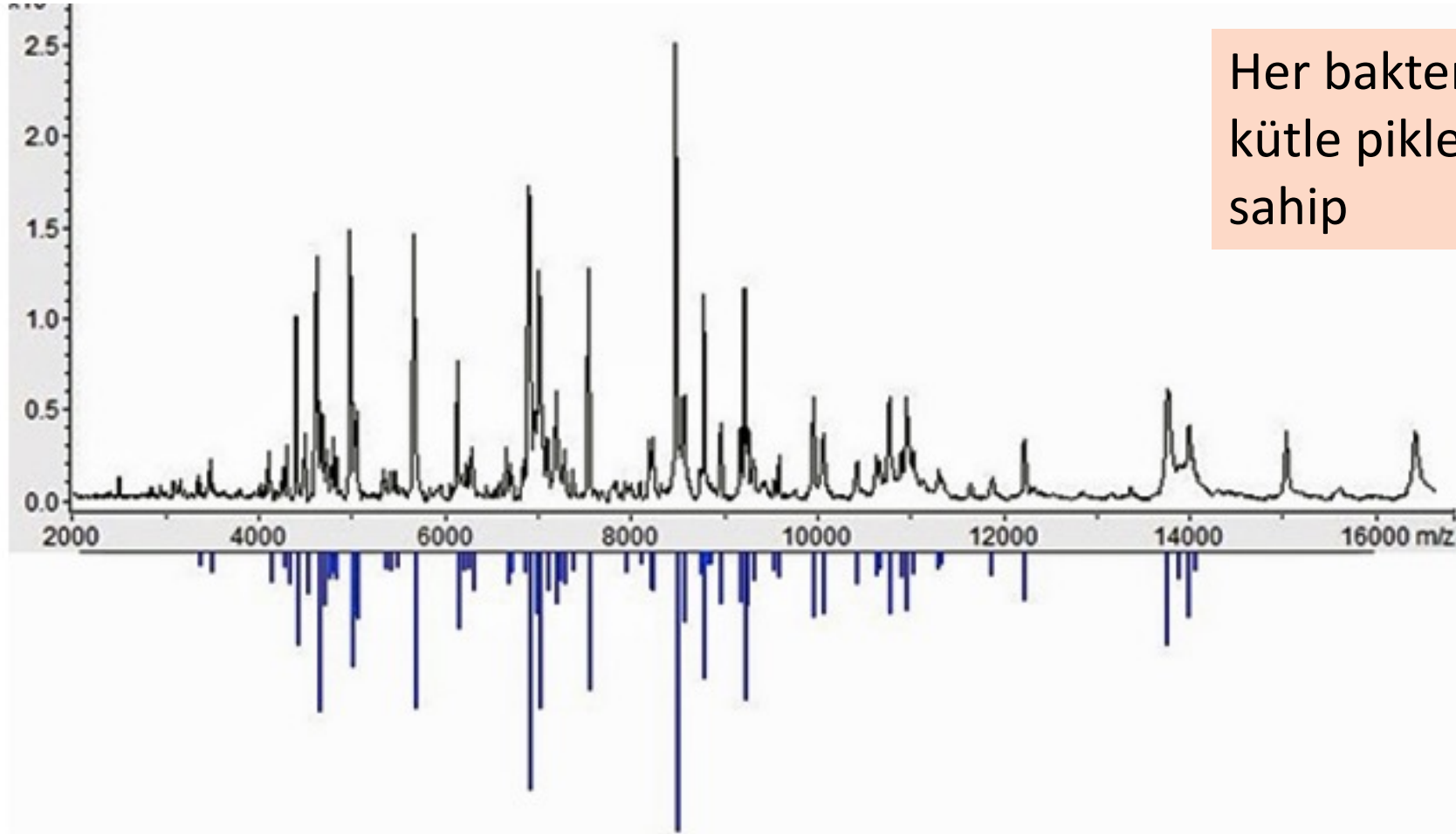
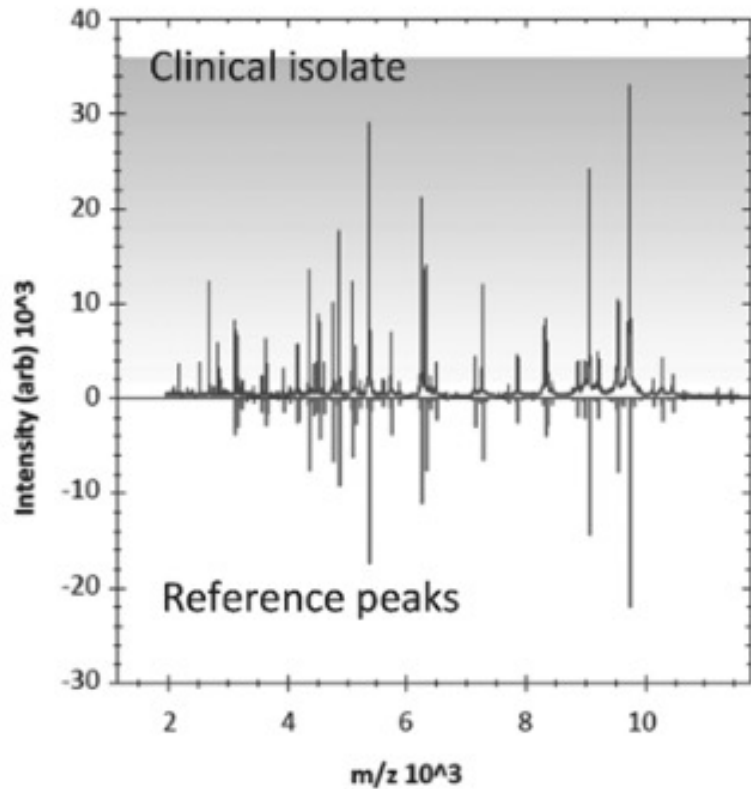


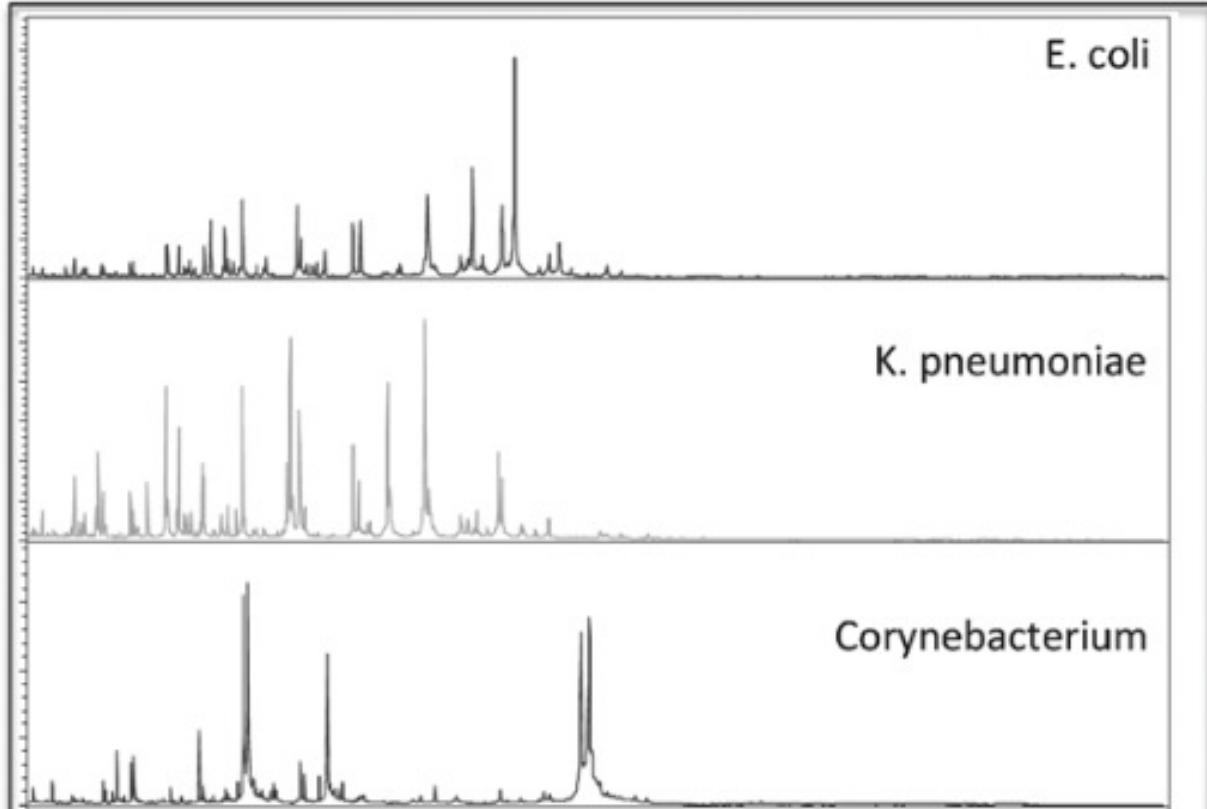
Illustration 3: Typical mass spectrum (black) and the resulting calculated reduced spectrum

MALDI-TOF MS

Çalışma Prensibi



3. Identification of peaks e.g. *E. coli*



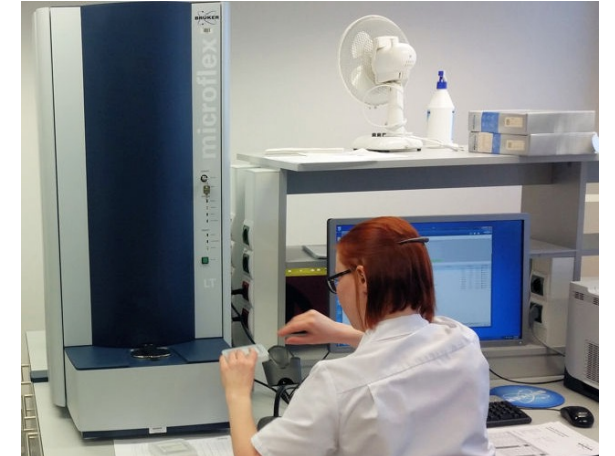
4. Comparison of peak spectra of bacterial isolates

MALDI-TOF MS

- 2000-17.000 daltonluk proteinleri analiz ediyor
- Primer olarak ribozomal proteinler
- Ribozomal proteinler türler arasında yüksek çeşitlilik gösterir
- Bu nedenle hem identifikasyon hem de tiplendirme için ideal



VITEK MS (Biomerieux, Fransa)



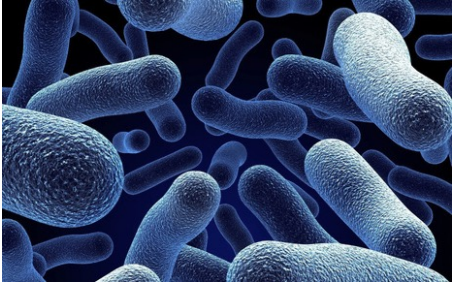
Microflex/MALDI Biotyper (Bruker Daltonics, Almanya)

MALDI-TOF MS

Mevcut cihazların performansı birbirine benzer

Veri tabanları farklı

Eğer bir bakteri MALDI-TOF ile isimlendirilemezse, konvansiyonel metodlarla da genellikle tanımlanamıyor



Tür Tanısının önemi

Bakteri ve mantarların hızlı güvenilir tanısı, hedefe yönelik antimikrobiyal tedavinin erken başlatılması açısından kritik öneme sahip !!!



Antimikrobiyal tedavide **gecikme** mortalite/ morbitide için temel risk faktörü



Doğru **tedaviye başlangıç süresinin kısalması** mortalite, hastanede kalış süresi, maliyeti azaltır



Olası kontaminant bakteri (ör:KNS) tanımlanması, uygunsuz antibiyotik kullanımını azaltır (öz:Vankomisin)

MALDI-TOF MS & Tür Tanısının önemi

Gram-pozitif çomaklar

Non-fermentatif gram-negatif çomaklar

Koagülaz negatif stafilokoklar

Anaerop bakterileri

Mantar

Mikobakteriler





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Clinical Significance of Commensal Gram-Positive Rods Routinely Isolated from Patient Samples

Sixto M. Leal, Jr., Melissa Jones, Peter H. Gilligan

Clinical Microbiology-Immunology Laboratories, University of North Carolina Hospitals, Chapel Hill, North Carolina, USA

- 2013-2015 verilerini retrospektif değerlendirmişler
- 762 izolat/ 13 cins/ 41 tür
- %18'i gerçek enfeksiyon etkeni, %82 kontaminant
- *Actinomyces* türleri → Yaralarda
- *Propionibacterium* türleri → Eklem sıvısı ve BOS da
- *Corynebacterium kroppenstedtii* → Meme
- *Corynebacterium striatum* → birçok vücut bölgesinde

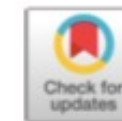
difteroidler
deri florası
orofarengeal flora
ürogenital flora



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Clinical Characteristics and Outcome of Lung Transplant Recipients with Respiratory Isolation of *Corynebacterium* spp.

● Ibai Los-Arcos,^{a,g} ● Oscar Len, Judith Sacanell,^f Antonio Romár

^aInfectious Diseases Department, Hospital I

^bMicrobiology Department, Hospital Unive Spain

^cInternal Medicine Department, Complejo

^dLung Transplant Unit, Hospital Universitar Spain

^eThoracic Surgery Department, Hospital Ur Barcelona, Spain

^fCritical Care Department, Hospital Unvers Spain

^gDepartment of Medicine, Universitat Autò

ABSTRACT Although chronic respiratory disease and immunosuppression are risk factors for *Corynebacterium* species respiratory infection, data are scarce regarding this disease in lung transplantation. Our aim was to describe the clinical characteristics and outcomes of lung transplant recipients (LTR) with respiratory isolation of *Corynebacterium* spp. This was a retrospective observational study performed at a referral center in Barcelona, Spain (2014 to 2016). We included all LTR in whom *Corynebacterium* spp. were isolated in at least one good-quality lower respiratory tract specimen. Overall, 24 of 527 (4.6%) LTR at risk during the study period were included. The main epidemiological, clinical, and microbiological data were analyzed. The most frequently isolated species were *C. striatum* (11/24), *C. pseudodiphtheriticum* (3/24), and *C. amycolatum* (3/24). All 19 (76%) patients who underwent bronchoscopy showed abnormalities, mainly mucosal plaques at the bronchial suture and purulent secretions. Clinical cure was achieved in 8/12 (67%) patients who fulfilled the CDC definition of lower respiratory tract infection (LRTI). To assess the clinical relevance of *Corynebacterium* spp., only patients with monomicrobial isolation ($n = 18$) were evaluated. LRTI was diagnosed in 9, and a nonsignificant association was found with a significant number of *Corynebacterium* sp. CFU/ml (7/9 LRTI versus 2/9 non-LRTI, $P = 0.057$). Persistent infection was associated with metallic bronchial stent implantation (4/4 versus 2/14, $P = 0.005$). The isolation of *Corynebacterium* spp. in respiratory specimens of lung transplant recipients may herald a respiratory tract infection or bronchial suture damage. Bronchial stent implantation is a risk factor for the persistence of *Corynebacterium* species infection.

MALDI-TOF MS & Anaerop bakteriler



- Anaerop bakteriler yavaş üüyor
- Klasik metodlar çok zaman alabiliyor. Otomatize fenotipik yöntemler için büyük inokulum gerekebiliyor
- Çoğu anaerop bakteri biokimyasal testlerde inaktif
- Klasik identifikasyonda üreyen bakterinin anaerop olduğunu doğrulamak için + bir gün gerekiyor
- Direnç belirlenmesi zor

- MALDI-TOF MS için büyük biyo kütleye ihtiyaç yok
- Konvansiyonel metodlarla isimlendirebildiğimizden çok daha fazlasını isimlendirebiliyoruz
- Primer ekimden identifikasyon yapmak mümkün
- Tür adını koymak dirençle ilgili bir fikir verebiliyor



Identifying Anaerobic Bacteria Using MALDI-TOF Mass Spectrometry: A Four-Year Experience

Luis Alcalá^{1,2}, Mercedes Marín^{1,2,3}, Adrián Ruiz^{1,2}, Lidia Quiroga^{1,2},
Maribel Zamora-Cintas¹, María Antonia Fernández-Chico¹, Patricia Muñoz^{1,2,3,4}
and Belén Rodríguez-Sánchez^{1,2*}

- Toplam 4094 izolat (190 tür ve 50 cins'e ait)
- Bruker MALDI-TOF MS ile identifiye edilmiş
- Örnek türü dağılımı: %32,7 abse, %23.2 yumuşak doku biyopsisi, %12,5 yara akıntısı, %8,4 kan, %8.2 peritoneal sıvı ve %15 diğer
 1. %95.7'si (n=3916) tür düzeyinde,
 2. %2.3'ü (n=94) cins düzeyinde isimlendirilirken
 3. %2.1'i için güvenilir sonuç elde edilememiş
- Son iki kategori için 16S rRNA sekanslama
- Ayrıca ilk kez izole ettikleri 237 tür için doğrulama amaçlı 16S rRNA sekanslaması yapılmış.
- Bu suşların çoğunun, *Bacteroides*, *Fusobacterium*, *Prevotella*, *Actinomyces*, *Clostridium*, *Lactobacillus* ve *Propionibacterium* cinslerinden olduğu saptanmış
- MALDI-TOF MS'in anaerop bakterilerin isimlendirilmesinde güvenilir bir araç olduğu;
- ***Klinisyenlerin antibiyotik tedavisini en uygun şekilde yapmalarını olanaklı kıldığı*** vurgulanmış.



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Case report

Polymicrobial anaerobic meningitis caused by *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Fusobacterium necrophorum* and *Slackia exigua* in a patient with mastoiditis following otitis media



G.N. Kalay^{a,*}, N. Dalgic^a, T. Bozan^c, N. Ulger- Toprak^c, B. Bayraktar^b, G. Soyletir^c

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ABSTRACT

We have reported a case of mastoiditis which progressed to meningitis in a 16-year old male patient. CSF (cerebrospinal fluid) anaerobic culture revealed four species of isolated anaerobic bacteria. This is the first case in the literature in which a patient survived childhood polymicrobial anaerobic meningitis diagnosed by MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry).

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Our patient had a mixed infection involving only anaerobic agents considered originating from microbiota of the patients. Our case serves as an example of how the early diagnosis and initiation of the appropriate antibiotic treatment as early as possible can be life-saving [9,12,13]. Introduction of the MALDI-TOF MS systems made identification of anaerobic bacteria very easy in the clinical practice [15,16]. Starting the appropriate treatment within 72 hours raised our treatment success rate in our case. The level of antibiotic resistance may vary depending on species [14,15].

2021-2022'de tanımlanan anaerop bakteriler	Sayısı
<i>Actinomyces neuui</i>	3
<i>Actinomyces radingae</i>	1
<i>Bacteroides fragilis</i>	15
<i>Bacteroides vulgatus</i>	2
<i>Bacteroides thetaiotaomicron</i>	1
<i>Clostridium clostridioforme</i>	2
<i>Clostridium paraputrificum</i>	2
<i>Clostridium perfringens</i>	6
<i>Clostridium butyricum</i>	3
<i>Cutibacterium acnes</i>	4
<i>Eggerthella lenta</i>	1
<i>Facklamia hominis</i>	1
<i>Flavonifractor plautii</i>	1
<i>Fusobacterium necrophorum</i>	4
<i>Fusobacterium nucleatum</i>	1
<i>Paeniclostridium sordellii</i>	1
<i>Paracoccus yeei</i>	1
<i>Peptoniphilus asaccharolyticus</i>	1
<i>Parvimonas micra</i>	1
<i>Terrisporobacter glycolicus</i>	2
<i>Trueperella bernardiae</i>	1
TOPLAM	54

Cost Savings Realized by Implementation of Routine Microbiological Identification by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Anthony Tran,^{a*} Kevin Alby,^{a*} Alan Kerr,^a Melissa Jones,^a Peter H. Gilligan^{a,b}

Clinical Microbiology-Immunology Laboratories, University of North Carolina Health Care,^a and Departments of Pathology-Laboratory Medicine and Microbiology-Immunology,^b Chapel Hill, North Carolina, USA

Organism	Traditional Identification Methodologies										
	Agglutination Tests (\$0.57)	API Strips (\$8.93)	Bile Esculin Agar (\$0.71)	CHROMagar Candida (\$0.97)	Lancefield Serology (\$4.57)	Molecular Sequencing (\$61.39)	Oxidase (\$0.10)	Presumptive (A, P-disk & CAMP) (\$0.23-0.31)	PYR Disk (\$1.94)	RAT Testing (\$8.24)	Spot Indole (\$0.10)
<i>Enterobacteriaceae</i>											
<i>Enterococcus</i> spp.			X						X		X
GNF											
<i>Staphylococcus</i> spp.	X						X				X
<i>Streptococcus</i> spp.				X				X	X		
Yeast		X	X							X	

FIG 1 Traditional methods required for identification, by organism category. GNF, Gram-negative glucose nonfermenters.

Organism	No. of samples
<i>Enterobacteriaceae</i>	7,503
<i>Enterococcus</i> spp.	1,454
GNF ^a	3,489
<i>Staphylococcus</i> spp.	5,790
<i>Streptococcus</i> spp.	2,332
Yeast	1,362
Total	21,930

- Teknisyenin harcadığı zaman ve bakım ücretleri dahil edildiğinde MALDI-TOF kullanarak sağladıkları bir yıllık kazanç \$73,646.18 (51.7%).
- Başlangıç maliyetini de 3 yılda çıkartabiliyorlar.

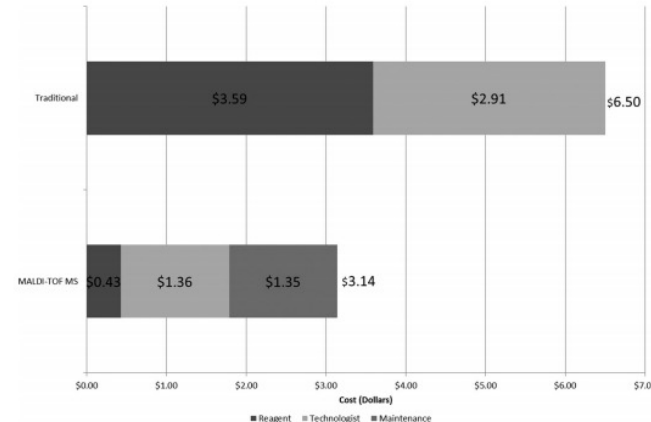


FIG 3 Mean reagent and total costs (with and without maintenance costs) per identification of isolates by traditional and MALDI-TOF MS methods.

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: a Fundamental Shift in the Routine Practice of Clinical Microbiology

Andrew E. Clark,^a Erin J. Kaleta,^b Amit Arora,^c Donna M. Wolk^{d,e,f}

Department of Veterinary Science and Microbiology, University of Arizona, Tucson, Arizona, USA^a; Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA^b; Department of Surgery, University of Arizona, Tucson, Arizona, USA^c; Department of Pathology, University of Arizona Medical Center, Tucson, Arizona, USA^d; The BIO5 Institute, University of Arizona, Tucson, Arizona, USA^e; Geisinger Health Systems, Danville, Pennsylvania, USA^f



MALDI-TOF MS mikrobiyoloji laboratuvarlarında patojen mikroorganizmaların identifikasyonu için büyük bir devrim!



Rutin laboratuvar pratiğinde yer etmiş durumda



Basit, hızlı, güvenilir, maliyet etkin

Modern Blood Culture

Management Decisions and Method Options



Mark D. Gonzalez, PhD^a, Timothy Chao, MD, PhD^b,
Matthew A. Pettengill, PhD^{c,*}

KEYWORDS

• Blood culture • Bloodstream infections • Contamination • Sepsis

KEY POINTS

- Considerable improvements can be made in preanalytical aspects of blood culture collection that impact patient care.
- There are newer methods developed in recent years to improve time to reporting blood culture isolate identification and antibiotic susceptibility patterns.
- The successful utilization of rapid identification and antimicrobial susceptibility results requires coordination with antimicrobial stewardship programs.

INTRODUCTION

One of the most important functions of the clinical microbiology laboratory is the detection and characterization of organisms causing bloodstream infections. Preanalytical considerations have a considerable impact on the success of blood cultures. The laboratory, with input from key stakeholders, must select media types and provides guidance on collection method, the volume of blood to be collected, and downstream processing of blood cultures. This article provides an update on management decisions in each of these areas.

SELECTION OF MEDIA AND ADDITIVE TYPES FOR BLOOD CULTURE

Most modern media formulations are similar, a base of soybean casein digest (tryptic case soy broth) with sodium polyanethol sulfonate (SPS) as an anticoagulant. The

KEY POINTS

- Considerable improvements can be made in preanalytical aspects of blood culture collection that impact patient care.
- There are newer methods developed in recent years to improve time to reporting blood culture isolate identification and antibiotic susceptibility patterns.
- The successful utilization of rapid identification and antimicrobial susceptibility results requires coordination with antimicrobial stewardship programs.

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Otomatize kan kültürü



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Otomatize kan kültürü

- Virtuo kan kültürü sisteminin sonuçları uzun zamandan beri kullanılan BacT/Alert 3D sistemi ile karşılaştırılabilir bulunmuş
- Virtuo kan kültürü sisteminin yaklaşık 2 saat daha kısa sürede pozitiflik saptadığı belirlenmiş
- BacT/Alert 3D için pozitifleşme süresi ortalaması yaklaşık 17.7 saatken, Virtuo için 15.9 bulunmuş
- Bu durum organizma grupları ile ilişkili
 - enterik Gram-negatif basiller (3.6 saat daha kısa)
 - enterokoklar (2.3 saat daha kısa)

Jacobs MR, Mazzulli T, Hazen KC, Good CE, Abdelhamed AM, Lo P, Shum B, Roman KP, Robinson DC. Multicenter Clinical Evaluation of BacT/Alert Virtuo Blood Culture System. J Clin Microbiol. 2017 Aug;55(8):2413-2421. doi: 10.1128/JCM.00307-17. Epub 2017 May 24. PMID: 28539343; PMCID: PMC5527419.





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Evaluation of Optimal Blood Culture Incubation Time To Maximize Clinically Relevant Results from a Contemporary Blood Culture Instrument and Media System

Eric M. Ransom,^a Zahra Alipour,^a Meghan A. Wallace,^a Carey-Ann D. Burnham^{a,b,c,d}

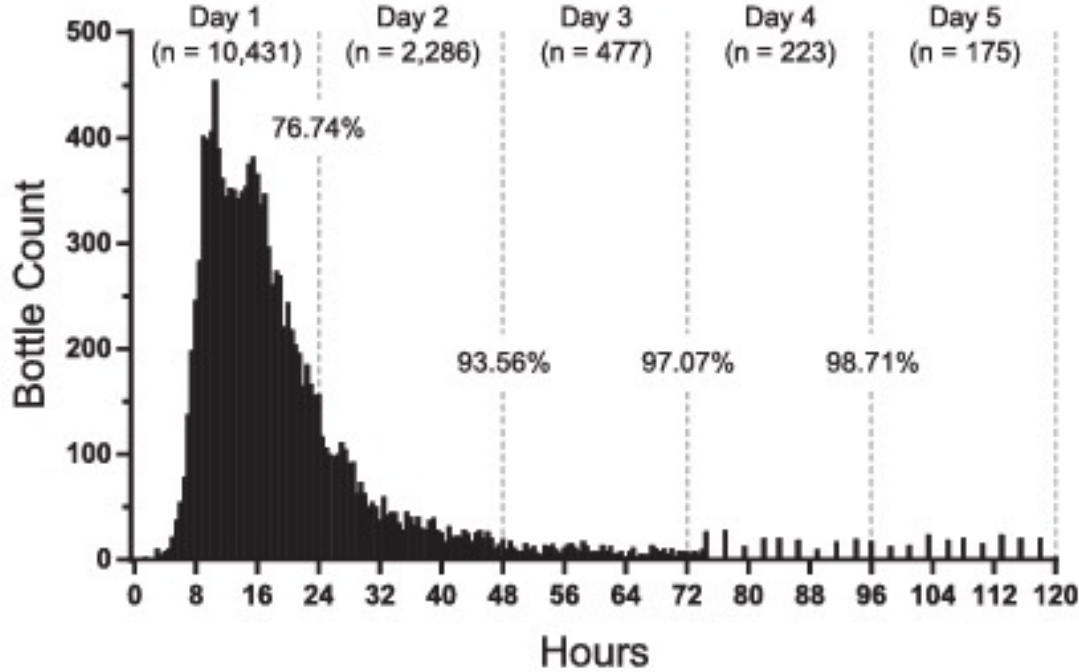
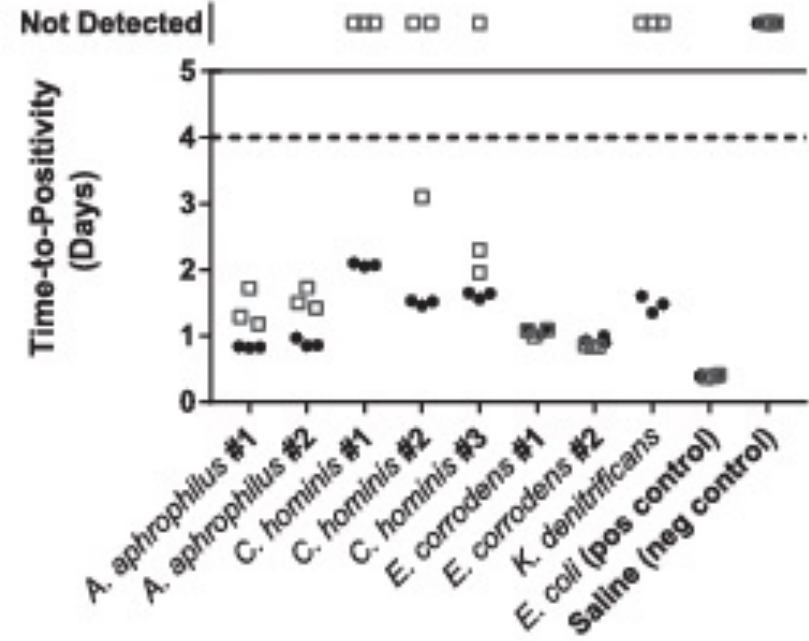


FIG 1 Time to positivity for all positive blood culture bottles. Positive bottles ($n=13,592$) were plotted in 30-min intervals. Of note, the BacT/Alert Virtuo system screens for positivity continuously for the first 74.4 h and every 144 min thereafter, resulting in greater column interspersion at later time points. After 4 days of incubation, 98.71% of all bottles flagged positive.



HACEK: *Aggregatibacter aphrophilus*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella denitrificans*, and *Kingella kingae*.

SONUÇ:

BacT/Alert Virtuo sistemi 4 günlük inkübasyonla mikroorganizmaların çoğunluğunu saptayabiliyor. Bu durumun mikrobiyoloji iş akışı yanında hasta bakımını da iyileştirecektir.



Narrative review

Bloodstream infections – Standard and progress in pathogen diagnostics

Brigitte Lamy ^{1,2,3,*}, Martin Sundqvist ⁴, Evgeny A. Idelevich ⁵, on behalf of the ESCMID Study Group for Bloodstream Infections, Endocarditis and Sepsis (ESGBIES)

AMAÇ:

- Standart prosedürler ve KDI patojenlerinin tanısındaki gelişmeler için literatürün gözden geçirilmesi ve tartışılması
- Gelişmiş tanı akışına ulaşmak için yeni bir bakış açısı önermek

Kan dolaşımı enfeksiyonları patojenlerinin tanısında demet yaklaşımı öneriyor

- pre-analytical parametrelerin optimizasyonu
- İnkübasyonun hızla başlatılması
- Hızlı metodların kullanımı
- Tekrar organize olmak (ör: 24/7 transport hizmeti)
- Antimikrobiyal stewardship takımları ile yakın çalışma

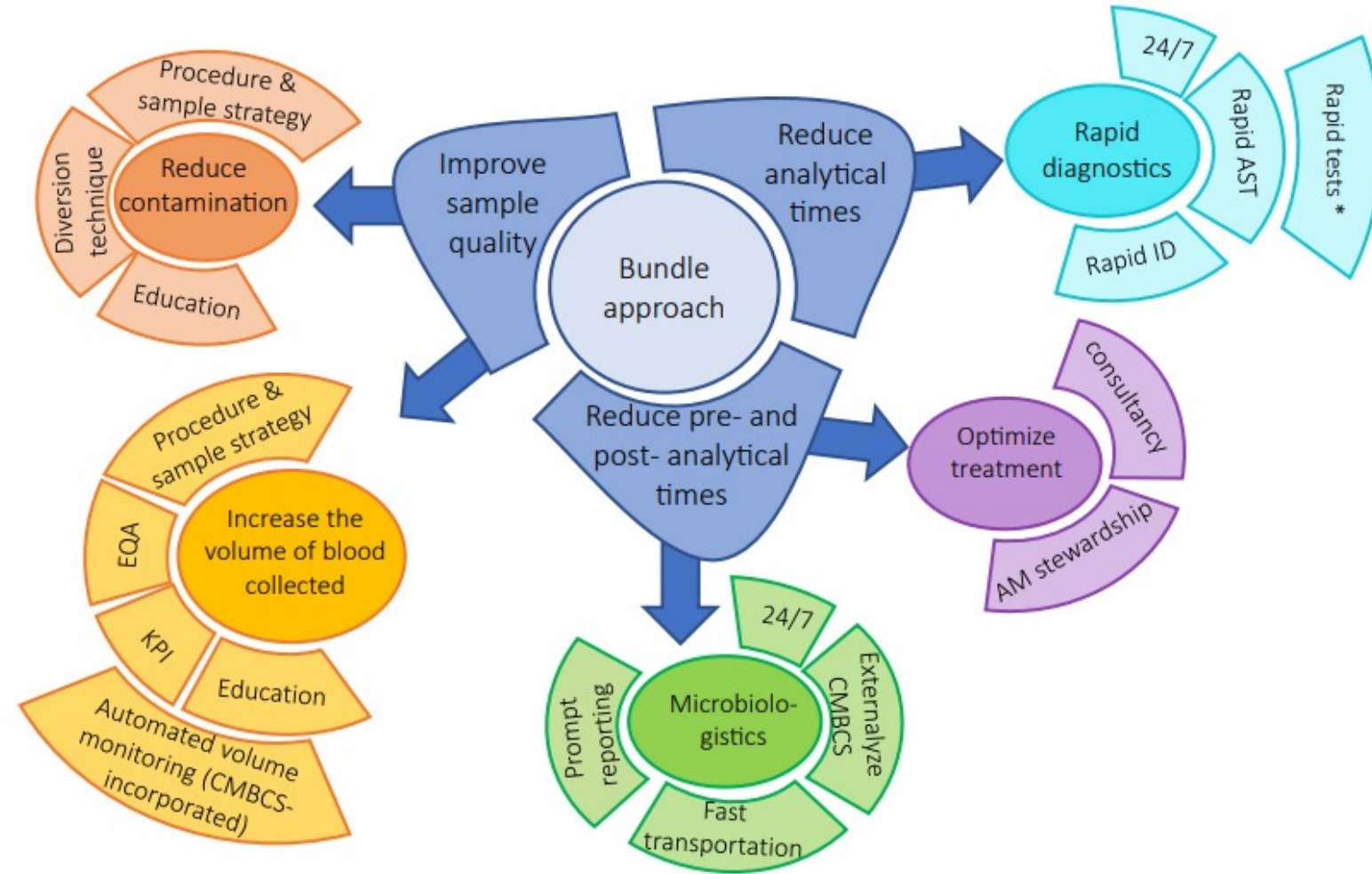
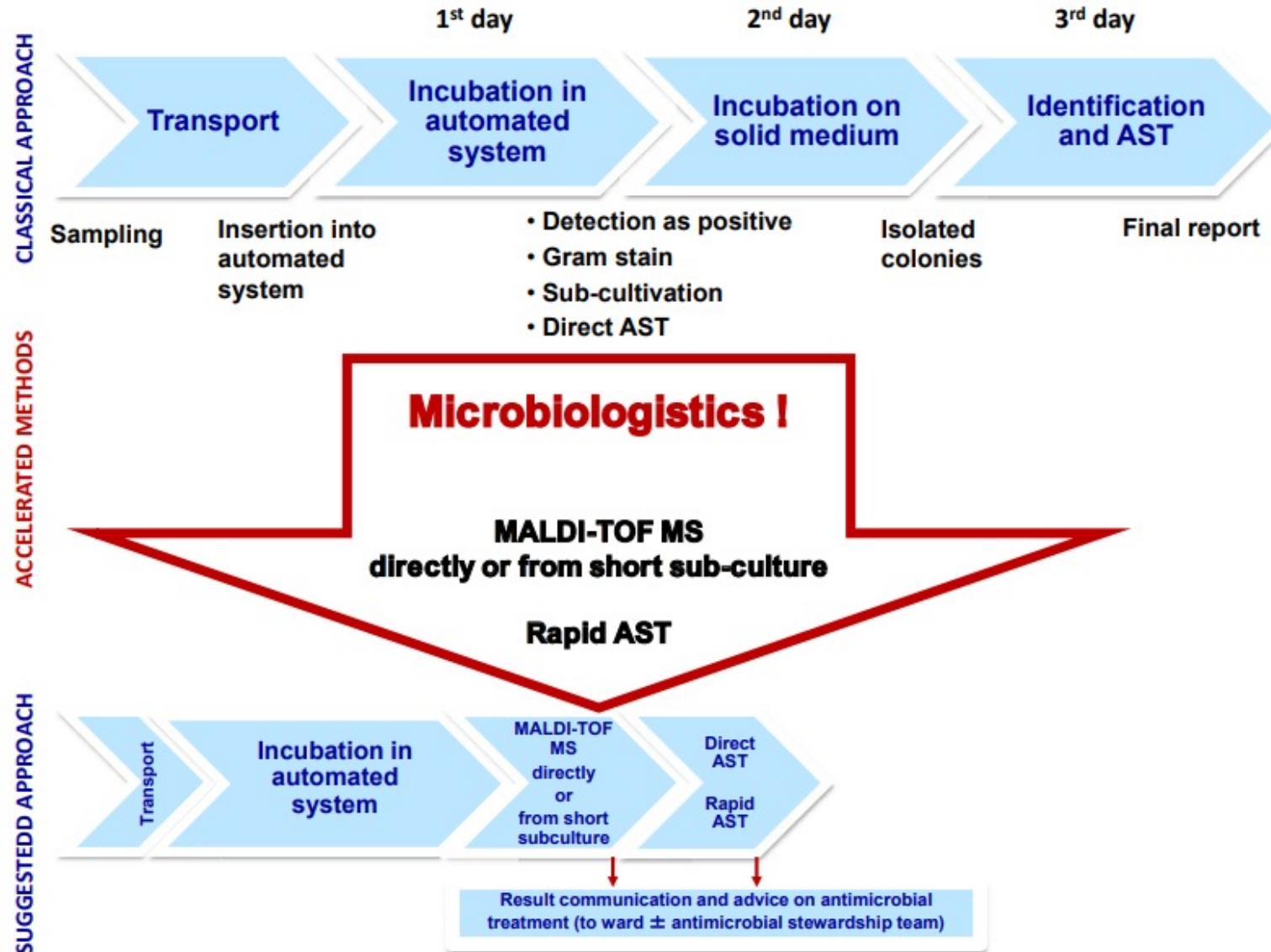


Fig. 1. Summary of all the actions to improve the bloodstream infection pathogen diagnostics. Types of actions belong to three complementary axes and actions aim to manage sample quality, times before and after analysis and analytical times. Each action per se is associated with a limited improvement but combination of several actions significantly improves diagnosis. Improvement is maximum when programme include actions on sampling quality, rapid diagnostics and logistics. KPI, key performance indicator; EQA, external quality assessment; CMBCS, continuous-monitoring blood culture system; AM stewardship, antimicrobial stewardship. *Rapid tests (e.g. *mecA* detection) may be needed in area of high level of resistance.



Species identification	The result of species identification is available on the same day of BC positivity	Achievable with MALDI-TOF MS performed directly from positive blood cultures or from short sub-cultures on solid medium. Also achievable with genotypic rapid methods for the most frequent pathogens
AST	The preliminary or final AST report is available on the same or at latest next day of BC positivity	Achievable by AST initiation on the same day from positive BCs (inoculation of short sub-cultures on solid medium, of microbial pellet, or directly of positive BC broth) instead of application of overnight colonies
Reporting of positive results	100% of new positive episodes, both identification and AST, are promptly reported to clinicians	By an active and prompt communication of the index information (e.g. positive flagged BC, Gram stain result or result of the direct identification) through a phone call or an electronic alert. Subsequent information (obtained on Day 1) can be reported using electronic report, except when new information is identified as being critical to patient care (e.g. AST result that shows prompt treatment change is required due to ineffective antibiotic regimen) Index information should preferably be also communicated to an Antibiotic Stewardship Team or an Infectious Diseases department

İDENTİFİKASYON KK
POZİTİFLEŞTİĞİ
GÜN VERİLMELİ

ADT SONUÇLARI (ön
veya son hali) AYNI
GÜN VEYA EN GEÇ
BİRGÜN SONRA
VERİLMELİ

YENİ KDI
EPİZOTLARININ
%100'ü KLİNİSYENE
HABER VERİLMELİ
(ID ve ADT)

Direct MALDI-TOF MS identification from positive blood cultures		MALDI-TOF MS identification from positive blood cultures using short sub-culture on solid medium		Molecular methods for identification from positive blood cultures	
ADVANTAGES	LIMITATIONS	ADVANTAGES	LIMITATIONS	ADVANTAGES	LIMITATIONS
Very rapid identification Easy Low additional cost	Identification difficulties with some species Confirmation might be needed Mixed cultures	Rapid identification Very easy Easy to integrate in lab workflow Confirmation usually not needed	Slow-growing organisms Mixed cultures	Rapid identification Easy	High cost Confirmation needed Limited pathogen panels

DNA-based identification from whole blood

ADVANTAGES

Time advantage compared to culture

Advantage in pathogen detection under antibiotics

LIMITATIONS

Limited number of pathogens in panel assays

Relatively low sensitivity (only 1-5 ml blood sampled)

Resistance detection ≠ susceptibility testing

Clinical relevance of DNAemia ?

High workload

Highly experienced staff required

High cost

Analytical cut-offs set by some manufacturers

Does not substitute culture-based testing

Otomatize kan kültürü & MALDI-TOF MS



Rapid Identification of Bacteria Directly from Positive Blood Cultures by Use of a Serum Separator Tube, Smudge Plate Preparation, and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Yan Chen,^a Vanessa Porter,^b Samira Mubareka,^{a,b} Leona Kotowich,^b Andrew E. Simor^{a,b}

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We analyzed the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) of smudge plate growth for bacterial identification from 400 blood cultures. Ninety-seven percent of Gram-negative bacilli and 85% of Gram-positive organisms were correctly identified within 4 h; only eight isolates (2.0%) were misidentified. This method provided rapid and accurate microbial identification from positive blood cultures.

Direct Rapid Identification from Positive Blood Cultures by MALDI-TOF MS: Specific Focus on Turnaround Times

Hazan Zengin Canalp¹, Banu Bayraktar¹



Kısa süreli inkübasyon sonrası MALDI-TOF ile identifikasyon (SIRID) ile direk şişeden MALDI-TOF ile hızlı identifikasyon (RID) yöntemlerini karşılaştırdık



Çalışmayı haftaiçinde ve sinyal verme zamanlarına göre örnekleri iki gruba ayırarak yaptık.
Gündüz sinyal grubu (06:00- 16:00),Gece sinyal grubu (16:00-06:00)



Kısa süreli inkübasyon (SIRID) için plaklarımızı 09:00 ve 14:00 da kontrol ettik



Sonuç verme sürelerini (TAT) karşılaştırdık.

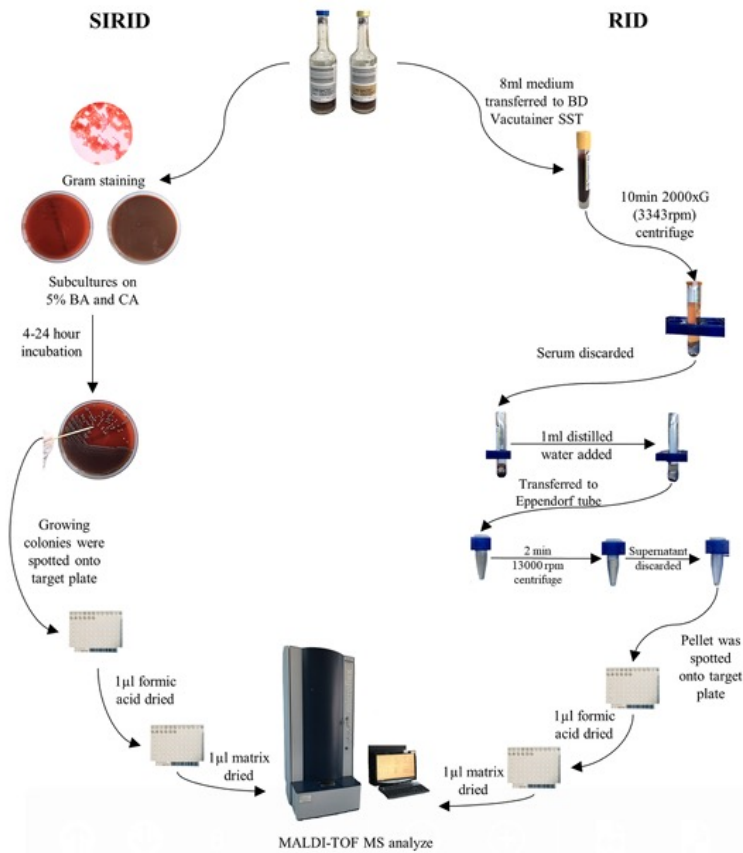


FIG 3 Flow chart of short-term incubation routine identification (SIRID) and rapid identification (RID) methods. SST, serum separator tube; BA, Blood agar; CA, Chocolate agar.

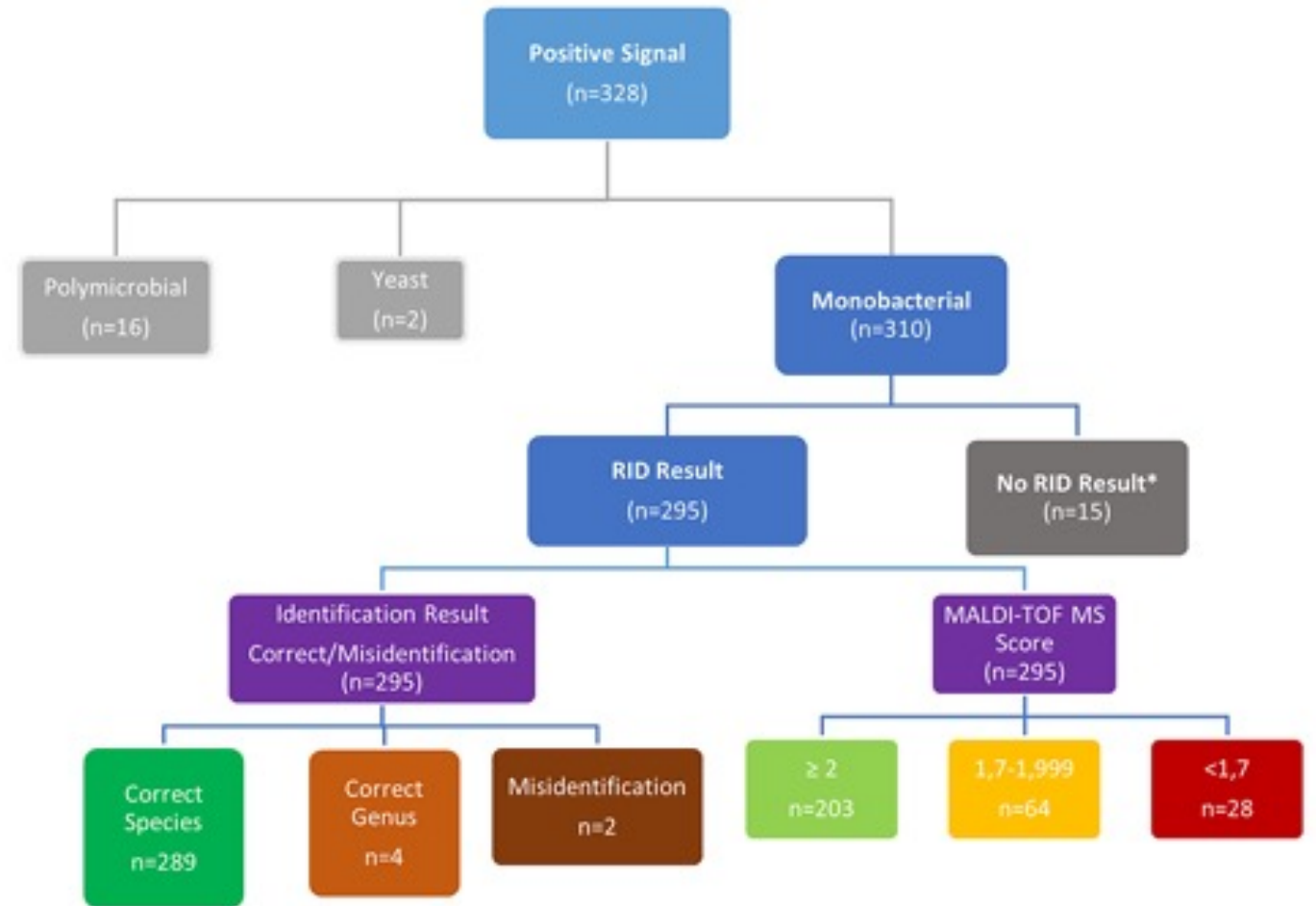


FIG 1 Rapid identification (RID) method results of positive signaling blood cultures. *, No peak in MALDI-TOF MS analysis of the RID method.



GSG'da RID (mean: 2.86 saat; 95% CI: 2.65 to 3.07) ile sonuç verme süresi SIRID (mean: 19.49 saat; 95% CI: 18.08 to 20.89) ile karşılaştırıldığında anlamlı ölçüde düşük bulundu ($p < 0.001$)



Doğru identifikasyon oranları:

Gram-negatiflerde %100

Gram-pozitiflerde %88.9

Tüm bakterilerde %93.2



TAT'daki göze çarpan kısalmanın empirik tedavinin gözden geçirilmesinde ve dolayısıyla hasta yönetiminde etkili olabileceği kanaatine varıldı.

**Effect of Matrix-Assisted Laser
Desorption Ionization–Time of Flight
Mass Spectrometry (MALDI-TOF MS)
Alone versus MALDI-TOF MS Combined
with Real-Time Antimicrobial
Stewardship Interventions on Time to
Optimal Antimicrobial Therapy in
Patients with Positive Blood Cultures**

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J Clin Microbiol. 2017 May;55(5):1437-1445.

- Pozitif kan kültürü olup hastanede yatmakta olan, tekbaşına MALDI-TOF MS sonucu verilen hasta grubu ile MALDI-TOF MS + antibiyotik stewardship (AMS) müdahalesi yapılan ikinci hasta grubu karşılaştırılmış
- Primer çıktı: Optimal tedaviye kadar geçen süre (TTOT)
- Bunun yanında hastanede kalış süresi, yoğun bakımda kalış süresi, tedavi süresi vb. etkisi araştırılmış

TABLE 2 AMS interventions

Intervention type	No. (%) of Interventions ^a
Narrowed coverage	25 (33.3)
Discontinued therapy	24 (32)
Initiated/broadened coverage	15 (20)
Other	11 (14.7)
Total	75 (100)

^aInterventions were accepted 88% of the time ($n = 66$).

TABLE 3 Primary endpoint outcomes

Category	Mean TTOT (h) $\pm$ SD (n)		P value
	Preintervention ($n = 126$)	Intervention ($n = 126$)	
Overall	75.17 $\pm$ 59.5	43.06 $\pm$ 35.3	<0.001
Gram-positive infection	64.04 $\pm$ 63.3 (43)	41.61 $\pm$ 44.9 (35)	0.082
Gram-positive contaminant	48.21 $\pm$ 37.1 (13)	11.75 $\pm$ 23.7 (43)	<0.001
Gram-negative infection	71.83 $\pm$ 61.5 (59)	35.98 $\pm$ 30.9 (46)	<0.001

TABLE 4 Secondary endpoint outcomes

Outcome	Preintervention	Intervention	P value
Mean TTET (h) $\pm$ SD (n)	16.8 $\pm$ 19.59 (113)	12.15 $\pm$ 17.2 (83)	0.082
No. of in-hospital deaths from any cause/total no. of patients (%)			
Overall	12/116 (10.3)	15/123 (12.2)	0.805
Gram-positive infection	3/116 (2.6)	8/123 (6.5)	0.256
Gram-negative infection	6/116 (5.2)	7/123 (5.7)	0.860
Mean time (h) to microbiologic clearance $\pm$ SD			
Overall	55.07 $\pm$ 45.6	42.49 $\pm$ 46.2	0.059
Gram-positive infection	58.49 $\pm$ 56.1	53.94 $\pm$ 62.8	0.595
Gram-negative infection	51.13 $\pm$ 31.2	34.51 $\pm$ 26.5	<0.001
Mean hospital LOS (days) $\pm$ SD			
Overall	15.03 $\pm$ 22.7	9.02 $\pm$ 7.3	0.021
Gram-positive infection	14.64 $\pm$ 10.5	10.31 $\pm$ 7.89	0.002
Gram-negative infection	15.40 $\pm$ 30.1	7.90 $\pm$ 6.7	0.027
Mean ICU LOS (days) $\pm$ SD			
Overall	4.30 $\pm$ 14.0	1.22 $\pm$ 3.8	0.053
Gram-positive infection	1.43 $\pm$ 4.2	1.32 $\pm$ 3.5	0.846
Gram-negative infection	5.55 $\pm$ 18.3	1.19 $\pm$ 4.13	0.035
No. (%) of patients with recurrence of same bacteremia			
Overall	4 (3.5)	1 (1.2)	0.255
Gram-positive infection	0	0	
Gram-negative infection	4 (3.5)	1 (1.2)	0.255
Mean length (days) of antimicrobial therapy $\pm$ SD			
Overall	18.57 $\pm$ 11.95	15.93 $\pm$ 11.11	0.117
Gram-positive infection	24.30 $\pm$ 16.0	18.97 $\pm$ 14.8	0.018
Gram-negative infection	14.25 $\pm$ 5.5	13.20 $\pm$ 4.5	0.156
Avg direct costs ^a	\$28,677	\$15,784	0.010

^aDifference between preintervention and intervention groups, \$12,893. Projected annual cost savings, \$6,291,784.



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The impact of blood culture identification by MALDI-TOF MS on the antimicrobial management of pediatric patients



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- İdentifikasyon süresi; 41 saatten -11 saate
- Duyarlılık test süresi; 50.8 saatten- 37.7 saate
- De-eskalasyon süresi; 58 saatten- 23 saate
- Gereksiz antibiyotiğin kesilme süresi; 49 saatten- 20 saate
- Yatış süresi;10.5 günden 8.37 güne düşmüş (p=0.006)

(p=<0.0001)

antibiotic treatment for pediatric inpatients.

Fenotipik hızlı Direnç Testleri

- Hızlı identifikasyon testlerini takiben hızlı direnç testleri uyguluyoruz
- Bu testler üremiş kolonilerden veya hızlı tanı için hazırlanmış pelletten çalışıyor.

❖ Beta-LACTA (BioRad)

❖ CarbaNP direkt (Laboratuvarımızda hazırladığımız)

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EPIDEMIOLOGY

Comparison of Carba NP-Direct, Carbapenem Inactivation Method, and β -CARBA Tests for Detection of Carbapenemase Production in *Enterobacteriaceae*

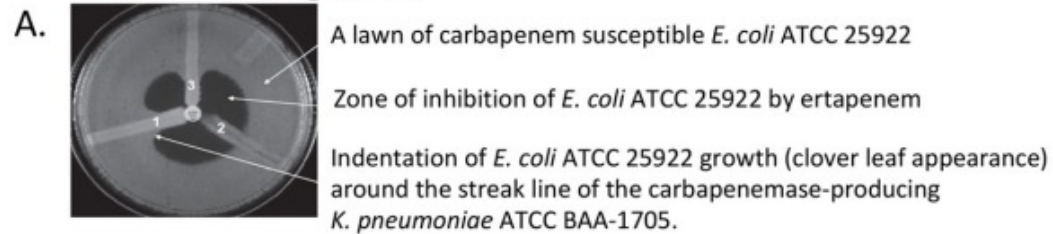
Banu Bayraktar,¹ Ayşe Barış,¹ Gülşah Malkoçoğlu,² Duygu Erdemir,¹ and Nur Kına¹

Rapid and accurate detection of carbapenemase-producing isolates are extremely important for management of antimicrobial therapy and the implementation of infection control measures. We evaluated the performance of Carba NP-direct, carbapenem inactivation method (CIM), and the commercial β -CARBA tests for detection of carbapenemase production in *Enterobacteriaceae*. *Enterobacteriaceae* isolates with previously characterized carbapenemase types ($n = 110$) and non-carbapenemase-producing *Escherichia coli* ($n = 15$) isolates were tested. Sensitivities of Carba NP-direct, CIM, and β -CARBA tests were 99.0%, 92.7%, and 93.6%, respectively, while specificity was 100% for all three tests. For β -CARBA test, a 60-min incubation time instead of 30 increased the sensitivity to 98.1%, and lessened false negativity, particularly with OXA-48-like producers. Our results showed that Carba NP-direct, CIM, and β -CARBA tests are useful tools for the reliable detection of carbapenemase activity in enterobacterial isolates. Carba NP-direct is a simple, rapid, and low-cost test for routine use.

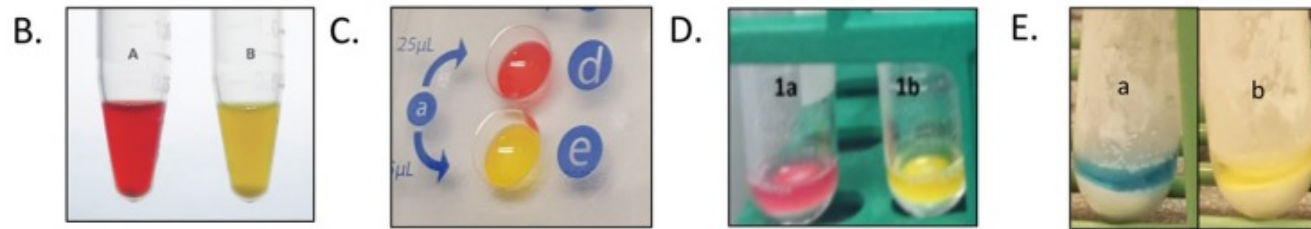
in-house multiplex PCR method, blaKPC, blaNDM, blaOXA-48, blaIMP, and blaVIM

Fenotipik direnç testleri

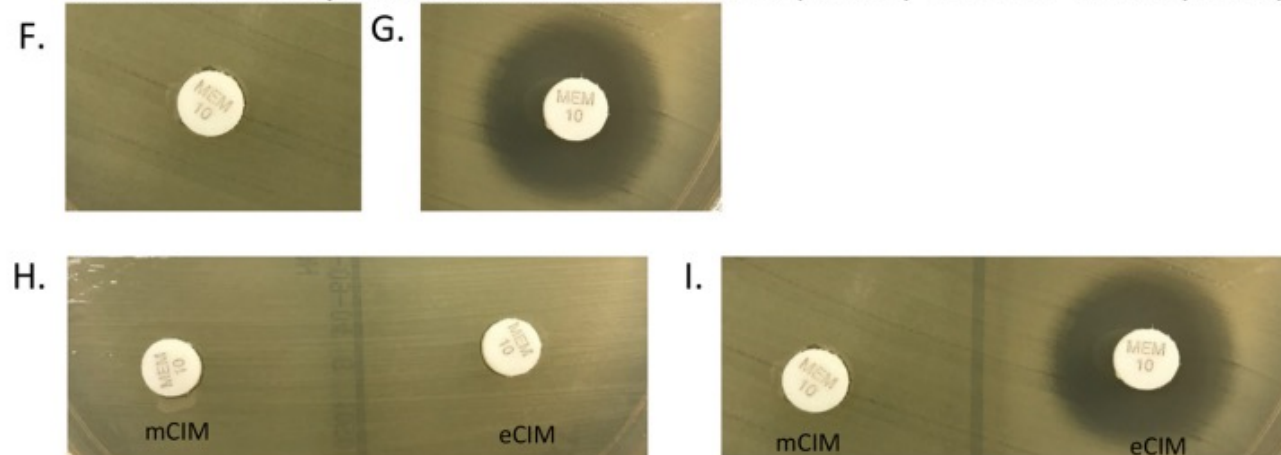
Modified Hodge Test



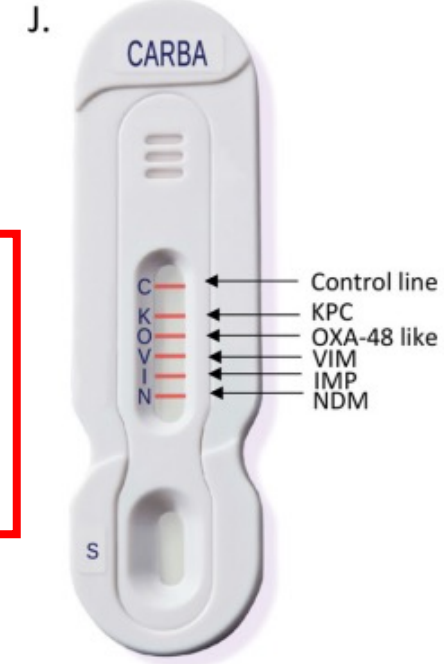
Carba NP and variants



Modified Carbapenem Inactivation Method (mCIM) & EDTA- mCIM (eCIM)



Lateral Flow Immunoassay



B-Carba NP test (Bio-Rad, France) B
Rapidec Carba NP test (bioMérieux, France) C
Neo-Rapid Carb (Rosco Diagnostica, Denmark) D
Rapid Carb Blue screen (Rosco Diagnostica) F

Otomatize kan kltr- Hızlı Antibiyotik Testleri

- Accelerate Pheno Sistemi
- Otomatize antibiyotik duyarlılık test platformları (BD Phoenix, VITEK 2)
- EUCAST hızlı AST (RAST) metodu
 - Pozitif kan kltrlerinden MH veya MH fastidious agarda yapılan direk disk diffzyon metodu (dDD)





Rapid antibiotic susceptibility testing in blood culture diagnostics performed by direct inoculation using the VITEK®-2 and BD Phoenix™ platforms

Steffen Höring¹ • Alain Sami Massarani¹ • Bettina Löffler¹ • Jürgen Rödel¹

- VITEK 2 ve Phoenix karşılaştırılmış
- iki farklı metod uygulanmış.
 - Serum seperatör tüp metodu (SST)
 - İki kez santrifüj metodu (2XC)
- Standart koloniden yapılan yöntemle karşılaştırılmış
- Örnek sayısı 98, Gram-negatif bakterilerle yapılmış
- 66 *Escherichia coli*, 7 *Enterobacter spp.*, 5 *Klebsiella pneumoniae*, 8 *Proteus spp.*, 1 *Serratia marcescens*, 1 *Achromobacter xylosoxidans*, 1 *Leclercia adecarboxylata*, 1 *Citrobacter koseri*, 1 *Aeromonas hydrophila*, ve 7 *Pseudomonas aeruginosa*.

Table 2 Error rates for conventional AST compared to confirmatory test

	No. of very major errors (%)	No. of major errors (%)	No. of minor errors (%)
VITEK®-2	5 (0.45)	4 (0.36)	10 (0.9)
BD Phoenix™	6 (0.54)	6 (0.54)	14 (1.3)

- İki sistemin sonuçları arasında istatistiksel olarak anlamlı fark olmasa da VITEK 2 ile alınan sonuçların daha iyi olduğunu görmüşler
- MALDI-TOF MS gibi hızlı identifikasyon yapılan bir yöntemle birlikte kullanılmalı

- Ek bir cihaz gerektirmediğinden kaynakları kısıtlı laboratuvarlarda da kullanılabilir
- Rutinde uzun süreli performansı ve sonuçların erken raporlamasının klinik etkisi araştırılmalı

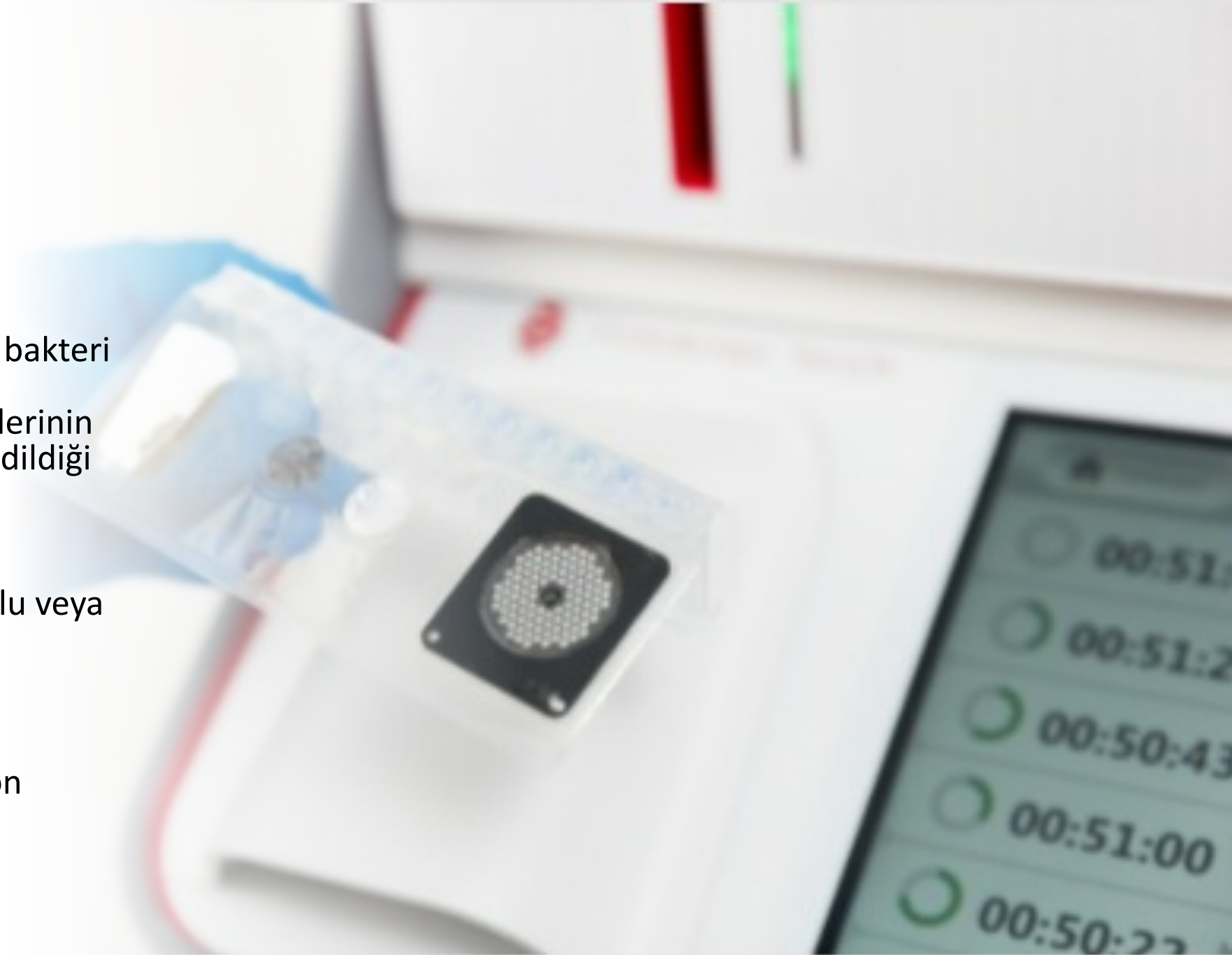
Table 5 Direct comparison of both platforms for direct inoculation (BSI core panel)

Method	Platform	Piperacillin-tazobactam	Ceftriaxone/cefotaxime	Ceftazidime	Meropenem	Ciprofloxacin	Gentamicin
SST	VITEK®-2	96.7% (3/93)	100.0% (0/88)	94.5% (91/5)	100.0% (0/96)	99.0% (1/95)	100.0% (0/95)
	Phoenix™	87.1% (9/70)	97.4% (2/76)	93.3% (75/5)	94.7% (4/76)	96.1% (3/76)	96.0% (3/76)
	p value	0.038*	0.219	0.766	0.041*	0.329	0.092
2XC	VITEK®-2	98.16% (92/2)	100% (0/88)	91.8% (6/73)	100.0% (0/95)	98.9% (1/94)	100.0% (0/94)
	Phoenix™	95.68% (72/6)	98.7% (1/76)	94.5% (5/91)	98.7% (1/78)	96.0% (3/75)	98.7% (1/79)
	p value	0.142	0.467	0.517	0.454	0.328	0.460

*Significant *p* values

Sendromik Paneller

- Klinik sendromlarla ilişkili bakteri türlerinin ve seçilmiş antimikrobiyal direnç genlerinin saptanmasının kombine edildiği testlerdir.
- Kan dolaşımı, solunum yolu veya gastrointestinal sistem enfeksiyonları gibi
- PCR and DNA hibridizasyon temelli bir teknoloji.



Otomatize kan kültürü- Sendromik Paneller

Commercial assays for the detection of acquired carbapenemases

Table 7. List of syndromic assays

Assay	Assay coverage*	Additional equipment required	Workflow
BIOFIRE® Blood Culture Identification 2 / BIOFIRE® FILMARRAY® Pneumonia plus Panel Biomérieux 	KPC, OXA-48-like, NDM, VIM, IMP	BIOFIRE® FILMARRAY® Multiplex Real-Time PCR systems	Sample in FILMARRAY sample buffer then injected in FILMARRAY pouch
Unyvero® Curetis AG 	KPC, OXA-48-like, NDM, VIM, IMP	None	Sample in Unyvero Sample tube, sample tube inserted in the Unyvero Lysator, sample tube and mastermix in the Unyvero cartridge, cartridge in the Unyvero Analyzer

Assay	Assay coverage*	Additional equipment required	Workflow
ePlex® BCID-GN panel GenMarkDx 	KPC, OXA-48-like, NDM, VIM, IMP	None	Blood culture sample loaded in cartridge
Verigene® Gram-negative blood culture test (BC-GN) Nanosphere Luminex 	KPC, OXA-48-like, NDM, VIM	none	Blood culture sample loaded in cartridge
T2Resistance Panel T2Biosystems®	KPC, OXA-48-like, NDM/VIM/IMP	None	Blood culture sample loaded in sample tube and reagent tray and snapped onto cartridge, inserted in the T2Dx® Instrument

The BioFire BCID2 Panel Menu

GRAM-NEGATIVE BACTERIA:

- *Acinetobacter calcoaceticus-baumannii* complex
- *Bacteroides fragilis**
- *Enterobacteriales*
 - *Enterobacter cloacae* complex
 - *Escherichia coli*
 - *Klebsiella aerogenes**
 - *Klebsiella oxytoca*
 - *Klebsiella pneumoniae* group
 - *Proteus*
 - *Salmonella**
 - *Serratia marcescens*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia**

ANTIMICROBIAL RESISTANCE GENES:

- **Carbapenemases**
 - IMP*
 - KPC
 - OXA-48-like*
 - NDM*
 - VIM*
- **Colistin Resistance**
 - *mcr-1**
- **ESBL**
 - CTX-M*
- **Methicillin Resistance**
 - *mecA/C*
 - *mecA/C* and MREJ (MRSA)*
- **Vancomycin Resistance**
 - *vanA/B*

GRAM-POSITIVE BACTERIA:

- *Enterococcus faecalis**
- *Enterococcus faecium**
- *Listeria monocytogenes*
- *Staphylococcus*
 - *Staphylococcus aureus*
 - *Staphylococcus epidermidis**
 - *Staphylococcus lugdunensis**
- *Streptococcus*
 - *Streptococcus agalactiae*
 - *Streptococcus pneumoniae*
 - *Streptococcus pyogenes*

YEAST:

- *Candida albicans*
- *Candida auris**
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Cryptococcus neoformans/gattii**

Total Laboratuvar Otomasyonu

- WASPLab (Biomérieux)
- Kiestra (Becton Dickenson)



Total Laboratuvar Otomasyonu

- Ön işlem
 - Besiyeri seçimi
 - Hasta bilgilerinin uygulanması ve barkotlama
 - Besiyerlerine ekim
- Akıllı inkübatör
- Petri görüntüleme

- Preamanalitik hatalar önleniyor
- Koloniler tek tek düşüyor. Tekrar pasaj alınarak zaman kaybı olmuyor
- İnkübatörlerden petriler çıkarılmadığı için sabit bir ısıda ve atmosferde kalıyor. Bu nedenle bakteri ve mayalar daha hızlı üüyor
- Petri görüntüleme sayesinde teknologlar bilgisayar ekranında büyütülmüş görüntüler ile çalışabiliyorlar

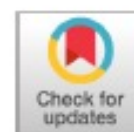
- Ayrıca dijital görüntüleme ve makine öğrenmesi ile verim ve kaliteyi artırmak mümkün
- Segregation yazılımları var. Üreme olmayan idrar kültürlerini ayırabiliyor.
- Koloni sayımı yapabiliyor



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Benefits Derived from Full Laboratory Automation in Microbiology: a Tale of Four Laboratories

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TABLE 1 Laboratory instrumentation and workflow overview

Condition	Instrumentation or workflow overview for: ^a			
	WKL	TC	HRMLP	SHSL
Pre-FLA instrumentation	None	None	None	2 WASP
Post-FLA instrumentation	2 WASP, 1 track line, 3 incubators	1 WASP, 2 incubators	2 WASP, 1 track line, 3 incubators	3 WASP, 2 track lines, 6 incubators
Specimen processing	Pre-FLA, 24/7 with 70% of volume processed off-site; post-FLA, 24/7 with 100% volume processed on-site	Pre-FLA, 24/7; post-FLA, 24/7	Pre-FLA, 24/7; post-FLA, 24/7	Pre-FLA, 24/7; post-FLA, 24/7
Culture workup	Pre-FLA, 08:00–19:00; post-FLA, 24/7	Pre-FLA, workup 07:00–00:30; post-FLA, 05:30–02:30	Pre-FLA, 08:00–17:00; post-FLA, 08:00–23:00 (positive blood cultures: 24/7)	Pre-FLA, 24/7; post-FLA, 24/7
Specimens/collections processed by WASP/FLA (percent of laboratory volume)	Urine, MRSA, ESwab, GBS, body fluid, positive blood culture (87% FLA)	Urine, MRSA, ESwab, respiratory, GAS, body fluid, disc diffusion AST (90% WASP; 81% FLA)	Urine, MRSA, VRE, GAS, CSF, positive blood culture (88% WASP; 80% FLA)	Urine, MRSA, GAS, GBS, body fluid, stool (96% FLA)
Segregation algorithms	No	Yes: urine	Yes: urine, MRSA, VRE, MRSA/VRE biplate	Yes: urine, MRSA
Years between pre- and post-FLA analysis	3	5	8	3

^aWKL, Willis-Knighton Laboratory; TC, TriCore Reference Laboratories; HRMLP, Hamilton Regional Medicine Laboratory Program; SHSL, Sutter Health Shared Laboratory; WASP, Walk-Away specimen processor; FLA, full laboratory automation; MRSA, screening culture for MRSA; GBS, screening culture for group B *Streptococcus*; GAS, screening culture for group A *Streptococcus*; AST, antimicrobial susceptibility testing; VRE, screening culture for VRE; ESwab, specimens collected in ESwab devices; CSF, cerebrospinal fluid.

TABLE 2 Pre- and post-FLA metrics

Lab and metric ^a	Pre-FLA					Post-FLA				
	FTE	Total labor cost/day (\$)	Specimen vol: peak day	Specimens/FTE (productivity)	Labor cost/ specimen (\$)	FTE	Total labor cost/day (\$)	Specimen vol: peak day	Specimens/FTE (productivity)	Labor cost/ specimen (\$)
WKL										
Processing	2.9	317.81	222	77	1.43	2	219.18	325	163	0.67
Culture Work up	5	958.90	222	44	4.32	4	767.12	325	81	2.36
Total	7.9	1,276.71	222	28	5.75	6	986.30	325	54	3.03
TC										
Processing	7	1,150.68	872	125	1.32	4	657.52	1,083	271	0.61
Culture Work up	9	2,219.18	872	97	2.54	12	2,794.51	1,083	90	2.58
Total	16	3,369.86	872	55	3.86	16	3,452.03	1,083	68	3.19
HRMLP										
Processing	5	821.92	957	191	0.86	7	1,150.68	1,565	224	0.74
Culture Work up	18	4,438.36	957	53	4.64	17	4,191.78	1,565	92	2.68
Total	23	5,260.27	957	42	5.50	24	5,342.47	1,565	65	3.41
SHSL										
Processing	13	2,315.07	1,125	87	2.06	13	2,315.07	1,648	87	2.06
Culture Work up	17	5,775.34	1,125	66	5.13	23.5	7,720.62	1,648	70	4.68
Total	30	8,090.41	1,125	38	7.19	36.5	10,035.69	1,648	45	6.09

^aSome laboratories provided actual FTE salaries while others preferred to use a national average of \$90,000/year for a clinical laboratory scientist and \$60,000/year for a laboratory assistant.

FTE: full time equivalent number
Verim artmış, iş gücü maliyeti düşmüş

TAT Frequency Distribution with WASP and FLA



FIG 1 Turnaround times for TC through evaluation period. Pre-WASP 2015, no automation in the clinical laboratory; WASP 2015, initial implementation of the WASP alone; FLA 2016, initial integration of FLA; FLA 2018, implementation of algorithm-assisted FLA.

Evaluation of WASPLab Software To Automatically Read chromID CPS Elite Agar for Reporting of Urine Cultures

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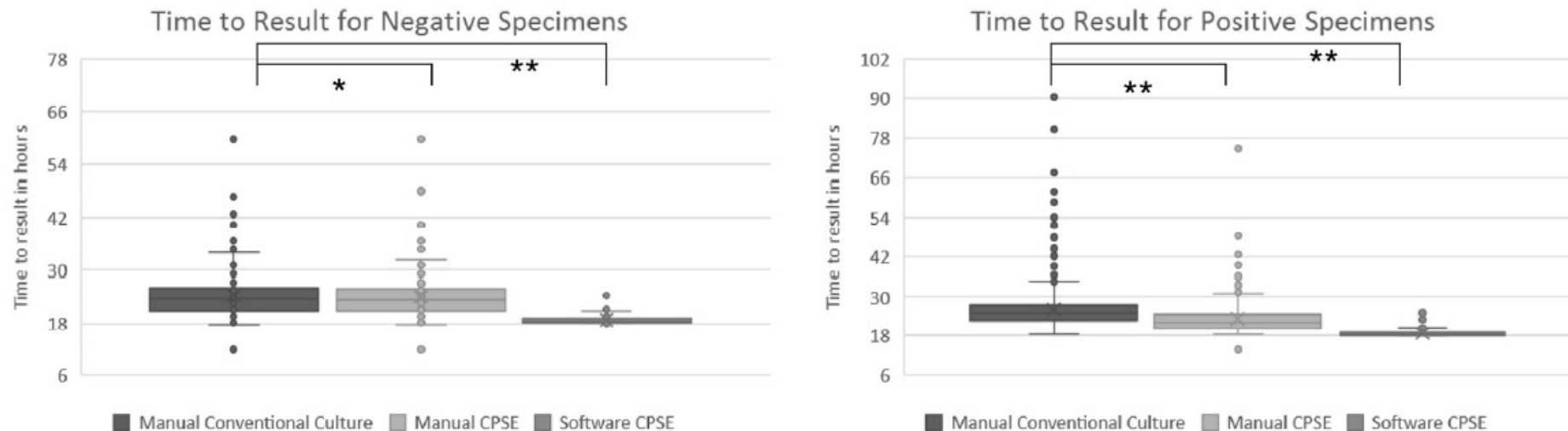
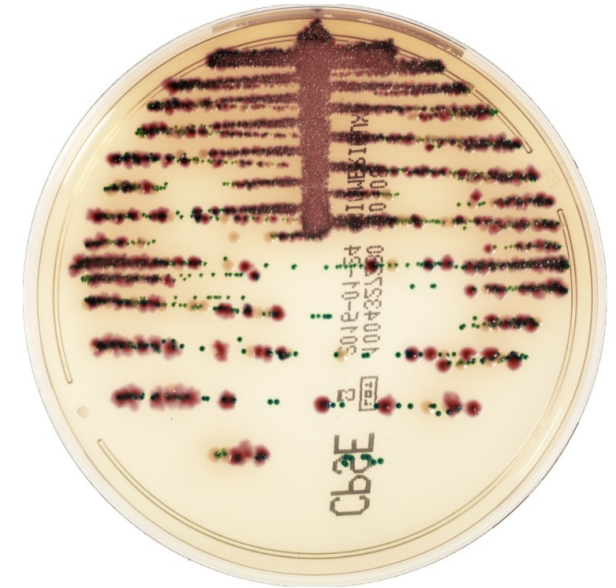


FIG 3 Distribution of time of result differences for negative and positive specimens between standard of care, manual CPSE, and software analysis. Significance was determined by a 2-tailed paired Student's *t* test (*, $P = 0.046$; **, $P < 0.001$).

TABLE 1 Performance of WASPLab software compared to manual read for 10 or more colonies

Analysis	No. with result of ^a :				% (95% CI) for measure of:	
	MP/AP	MN/AN	MN/AP	MP/AN	Sensitivity	Specificity
Real-time results	1,013	396	170	2	99.8 (99–100)	69.9 (66–74)
Postdiscrepant analysis	1,013	485	54	2	99.8 (99–100)	90.0 (87–92)

^aMP/AP, manual positive/automation positive; MN/AN, manual negative/automation positive; MN/AP, manual negative/automation positive; MP/AN, manual positive/automation negative. *n* = 1,581.

TABLE 2 Discrepant analysis of manual-negative/automation-positive specimens

Discrepancy category	No. of plates
Counting microcolonies	116
Counts near the limit of detection	43
Counts with a >50-CFU difference	11



Evaluation of EUCAST rapid antimicrobial susceptibility testing (RAST) for positive blood cultures in clinical practice using a total lab automation

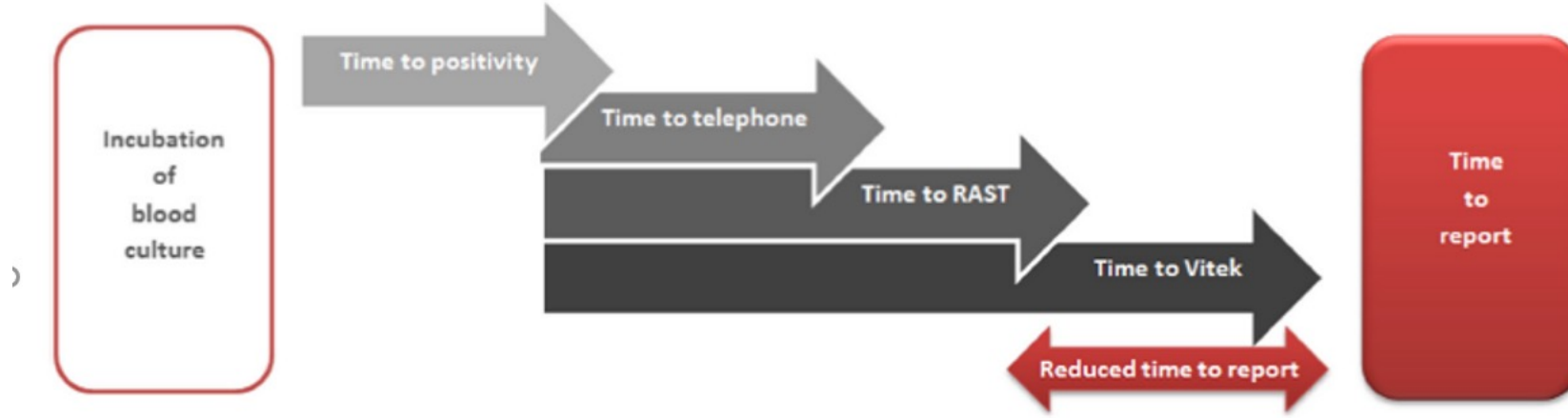
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Abstract

Our objective was to evaluate EUCAST's 'rapid antimicrobial susceptibility testing' (RAST) directly from positive blood culture that delivers antimicrobial results within 6 h for *Staphylococcus aureus*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, using total lab automation. Zone diameters from RAST were compared with MIC results. Furthermore, its influence on time to report was investigated. RAST was performed to all positive aerobic and anaerobic blood culture bottles by subculturing them, i.e. onto Mueller-Hinton agar and adding six antibiotic discs covering Gram-negative and Gram-positive therapy (cefoxitin, ampicillin, vancomycin, piperacillin/tazobactam, meropenem and ciprofloxacin). RAST was automatically imaged after 6 h. Zone sizes were measured using a TLA software tool and interpreted according to EUCAST clinical breakpoints. Bacteria were identified using MALDI-TOF MS and MIC results were determined using Vitek2 panels. Categorial agreement between agar diffusion and MIC results was investigated. Additionally, time to RAST and time to Vitek were compared for 100 isolates (20 per species). Between November 2018 and April 2019, 3313 positive mono-bacterial blood culture bottles were collected of which 894 bottles with RAST-validated species were investigated. Among these bottles, 2029 individual antibiotic measurements were compared with MIC results from Vitek2 and 14 very major, 28 major and 12 minor errors were found. A median reduction of 17:30 h in time to report was observed. Introduction of RAST with automatic TLA imaging function could reduce time to report by 17:30 h. Excellent accordance between zone diameter and MIC results, particularly for cefoxitin, vancomycin and meropenem, was observed, but drawbacks due to ATU were seen.

- RAST- *S.aureus*, *Enterococcus* spp., *E.coli*, *K. Pneumoniae*, *P.aeruginosa* 6 saatte değerlendirmişler
- Gr(+) ve Gr(-) kapsayacak şekilde bir plak düzenlemişler
- Sefoksitin, ampisilin, vankomisin, piperasillin+tazobaktam, meropenem ve siprofloksasin disklerini yerleştirmişler
- Zon çapları ölçümü TLA yazılımı aracılığıyla 6.saatte yapılmış
- VITEK 2 panellerinin sonuçları ile kategorik uyum araştırılmış
- Ayrıca 100 izolat için sonuç verme süreleri karşılaştırılmış



Raporlama ortalanca deęerini TLA otomatik g r nt leme sistemini kullanarak 17:30 saat azaltabilmiřler (Bařka hastanelerden gelen  rnekler dahil)

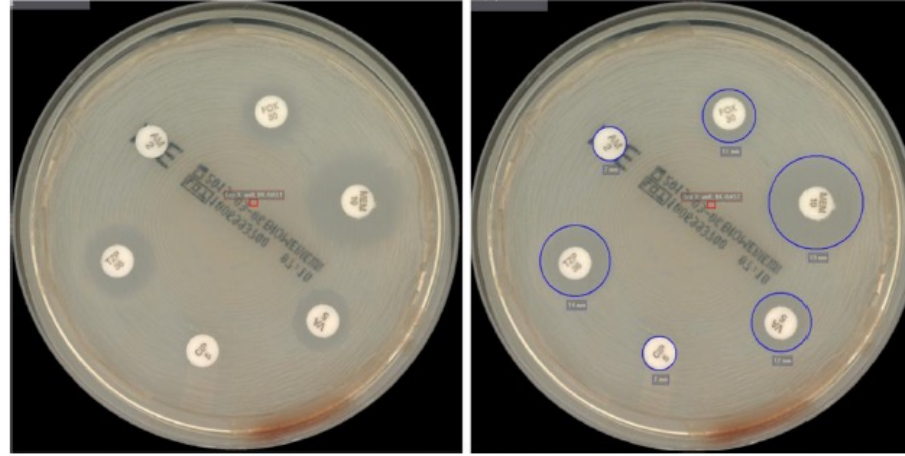


Fig. 1 Images of RAST plate of methicillin-resistant *Staphylococcus aureus* (MRSA) without (left) and with (right) measured inhibition zones automatically taken with the photo lab function of the total lab automation (TLA, BD Kiestra™) at the Department for Infectious Diseases at the University Hospital Heidelberg, Germany. The inhibition zone for

cefoxitin (FOX) is measured with 11 mm. According to the EUCAST zone diameter breakpoints for RAST directly from blood culture bottles, this zone diameter is within resistant category and, hence, indicates an MRSA

- Hızlı ve güvenilir antibiyotik duyarlılık test sonucu verme hedeflerine ulaşmışlar, özellikle MRSA, VRE ve CRE için

- TLA ile kombine edilmiş RAST'ın hasta yararına olduğu kadar kan kültürü prosedürlerini de hızlandığını söylemişler

Sonuç

- Mikrobiyoloji laboratuvarlarında otomasyon son 10 yıl içinde çok hızlanmış durumda
- Bu durum sonuçların kalitesini artırıyor
- Aynı zamanda sonuç verme sürelerini de kısaltıyor
- Ancak, hızlı sonuçların hasta yönetimine katkı sağlayabilmesi için Antibiyotik stewardship mutlak gerekli
- Değişim devam ediyor. Makine öğrenmesi ve yapay zeka uygulamaları ile tüm laboratuvar süreçlerinin giderek daha da otomatize olması kaçınılmaz gözüküyor



TEŞEKKÜRLER...