

# Genotypic approaches for rapid AST on positive blood cultures

NordicAST workshop 2023

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# The Role of Microbiology laboratory in BSI

- Blood culture is
  - the golden standard for detection and identification of microbes in bloodstream
  - a critical diagnostic test for identification of certain infections (ie. endocarditis)
  - the only way to produce phenotypic AST-results for microbes in bloodstream.
- Time is of essence!
- High mortality and morbidity in BSIs
- In septic shock time to effective treatment correlates with mortality
- Microbiological diagnosis → antimicrobial treatment escalation / de-escalation

[Review](#) > [Clin Microbiol Infect.](#) 2013 Jun;19(6):501-9. doi: 10.1111/1469-0691.12195.

Epub 2013 Mar 8.

## Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe

M Goto <sup>1</sup>, M N Al-Hasan

[Multicenter Study](#) > [Antimicrob Agents Chemother.](#) 2012 Jan;56(1):472-8.

doi: 10.1128/AAC.00462-11. Epub 2011 Oct 17.

## Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: a propensity score-based analysis

Pilar Retamar <sup>1</sup>, María M Portillo, María Dolores López-Prieto, Fernando Rodríguez-López,

> [N Engl J Med.](#) 2017 Jun 8;376(23):2235-2244. doi: 10.1056/NEJMoa1703058. Epub 2017 May 21.

## Time to Treatment and Mortality during Mandated Emergency Care for Sepsis

Christopher W Seymour <sup>1</sup>, Foster Gesten <sup>1</sup>, Hallie C Prescott <sup>1</sup>, Marcus E Friedrich <sup>1</sup>, Theodore J Iwashyna <sup>1</sup>, Gary S Phillips <sup>1</sup>, Stanley Lemeshow <sup>1</sup>, Tiffany Osborn <sup>1</sup>, Kathleen M Terry <sup>1</sup>, Mitchell M Levy <sup>1</sup>

# Goals to set

## Bloodstream infections – Standard and progress in pathogen diagnostics

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ESCMID Study Group for Bloodstream Infections, Endocarditis and Sepsis (ESGBIES)

**Table 1**  
State-of-the-art in microbiological diagnostics of bloodstream infections

Diagnostic procedure	State-of-the-art <sup>a</sup>	Comment	Reference
Blood sampling Skin preparation	The contamination rate is <3%, optimally <1%	Contamination is defined as a microorganism recovered from BC bottle that is indeed absent in the patient blood. Most often, contaminations are bacteria from the skin microbiota (e.g. coagulase negative staphylococci, coryneforms) Contamination rate is defined as the number of contaminated samples (whatever the number of positive bottles) per 100 samples (whatever the number of bottles collected). Contamination rate is reduced by compliance with skin preparation best practice, diversion technique, single sampling strategy and prior careful disinfection of bottle's septum	[12]
Bottle-filling (adult patient) Number of bottles collected per episode (adult patient)	The volume of blood per bottle is 8–10 mL 4–6 bottles are collected (4 acceptable only if all bottles properly filled) Rate of solitary BC (2 bottles) below 10%	These two parameters result in a total volume of blood cultured of at least 40 mL to ensure optimal BC sensitivity For children, refer to article in the current theme review [77]	[10,11,16,78]
Specimen transport <u>Species identification</u>	<4 hr for the majority of BC <u>The result of species identification is available on the same day of BC positivity</u>	With decentralized BC cabinets 2 hr should be aimed for <u>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</u>	[9,61]
<u>AST</u>	<u>The preliminary or final AST report is available on the same or at latest next day of BC positivity</u>	<u>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</u>	[36]
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	[10]

AST, antimicrobial susceptibility testing; BC, blood culture; MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry.

<sup>a</sup> State-of-the-art statements represent authors' opinion on what is currently feasible and what should be implemented as requirement for timely and qualitative diagnostics of bloodstream infections.

# Principle of genotypic approach for AST is simple

positive bloodculture

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graph TD; A[positive bloodculture] --> B[1 resistance gene detected = resistance to 1 or several agents]; B --> C[Staphylococcus aureus + MecA gene = MRSA]; C --> D[Inference of likely phenotype]; D --> E[Modification of empirical therapy]; E --> F[Possible Infection Prevention Control measures];
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1 resistance gene detected = resistance to 1 or several agents

Staphylococcus aureus + MecA gene = MRSA

Inference of likely phenotype

Modification of empirical therapy

Possible Infection Prevention Control measures

# Methods

- From positive blood culture:
  - PNA-FISH: identification only
  - NAATs (Real-time PCR, multiplex PCR, microarray)
- Detection of 1 gene only
- Syndromic panels:
  - Gram staining mandatory before running or not
  - identification of species level or taxonomic rank
  - Resistance genes (molecular antibiogram)

▪ MecA/C	-> MRSA
▪ VanA/B	-> VRE
▪ CTX	-> ESBL
▪ Carbapenemases	-> CPE, MDR-P. aeruginosa
- KPC	
- OXA 48	
- NDM	
- VIM	
- IMP	

- (Metagenomics)

Xpert® MRSA/SA Blood Culture

**BIOFIRE® FILMARRAY®  
SYSTEMS**

ePlex® Blood Culture  
Identification Panels

**MOLECULAR MOUSE**  
SEPSIS PANEL

Molecular diagnostics for genotypic detection of antibiotic resistance:  
current landscape and future directions

Banerjee R. et al JAC 2023

# Advantages of genotype-based diagnostics

- Analytical
  - From positive bloodcultures/cultures/clinical specimens
  - Rapid
  - Sensitive
  - multimicrobial samples
- Organizational
  - easy to use
  - short hands on time
  - simplification of process
  - do not require necessarily PCR lab facilities, decentralized laboratory

# Does it work well?

Yes generally, good test performance

but there are challenges...

- Identification: BC bottles not DNA free?
- streetlight effect: detect only a subset of resistance markers
- Genotype-phenotype discrepancies
- does not provide MIC
- Cannot detect new resistance mechanisms
- Provide only preliminary results, must always be confirmed by phenotypic AST
- High Cost
- Probably more studies needed to evaluate the real clinical impact



# Case 1

- First positive bloodculture
- Gram staining: gram positive cocci in cluster

## Molecular test

Staphylococcus sp.  
*S. aureus*  
*S. epidermidis*  
mecA/C and MREJ  
(MRSA)

## Preliminary results

1) *S. aureus*

Based on a rapid test from the blood culture bottle the strain is suspected to be MRSA. Efficacy of beta-lactam antibiotics might be compromised. Preliminary identification. Definitive identification and AST reported later.

2) *S. epidermidis*

## Phenotypic AST

1) MSSA (cefoxitin S)

2) *S. epidermidis* (cefoxitin R)

3) *S. hominis* (cefoxitin S)

No gene detected



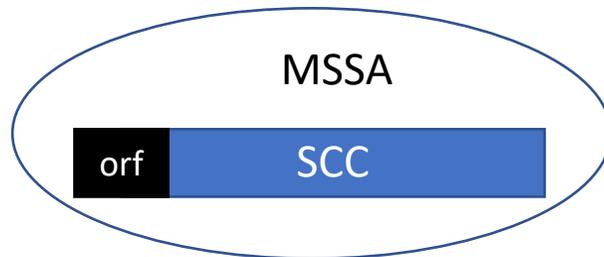
= MSSA (wild type)

= MRSA (orf + SCC + MEC)

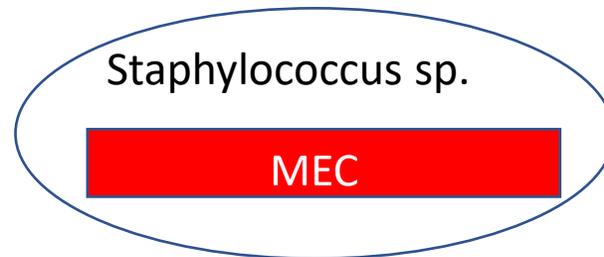
= MSSA (orf + SCC)

= MSSA (orf + SCC + unfunctional MEC)

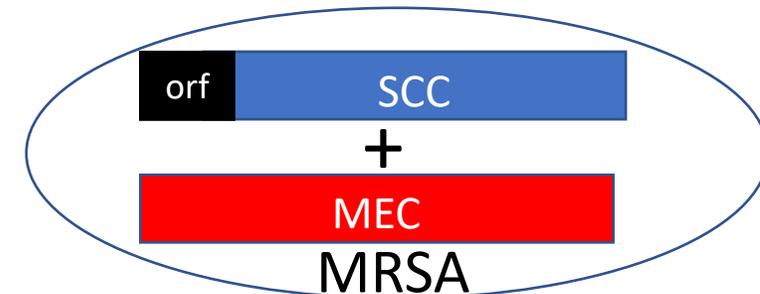
= Staphylococcus sp. (MEC) non aureus



+



=



# Case 2

- Bacteremic Hospital Acquired Pneumonia, general ICU
- Empiric treatment: meropenem
- Positive blood culture Gram negative bacilli
- Molecular test from blood culture: K. pneumoniae group + KPC detected
- Expected phenotype

Antibiotic	Expected AST	Phenotypic AST
BLICs: amox/clav, pip/tazo	R	R
3rd gen cephalosporins	R	R
Carbapenems	R	S
Ceftolozane/tazo	R	-
Ceftazidime/avibactam	S	R
Meropenem/vaborbactam	S	S
Imipenem/relebactam	S	-
Cefiderocol	S	-

Modification of empirical treatment:  
mero+varbactam (not cefta/avibactam  
because pneumonia)

Strain producing a KPC variant (KPC-31)

Carbapenemases: does genotype trump phenotype?

# Genotype vs phenotype AST

Discrepancy reason	Misleading predictions
<b>Unexpressed/silenced gene</b> (i.e Enterococcus sp and silent VanA)	<b>Resistance</b> to the concerned agents
<b>Nonfunctional gene</b> (i.e truncated KPC)	<b>Resistance</b> to the concerned agents
<b>Mutated target with altered phenotypes</b> (i.e KPC-3)	<b>Resistance</b> or <b>susceptibility</b> depending of the mutated target
<b>Off-panel genes</b> (i.e carbapenemases IMI, SME, SPM, GIM ESBL and TEM/SHV)	<b>Susceptibility</b> to the concerned agents
<b>Alternative resistance mechanisms</b> (i.e AmpC, porin mutations, efflux pumps)	<b>Susceptibility</b> to the concerned agents
<b>Mixed infections</b> (i.e MSSA and MecA Staphylococcus sp.)	<b>Resistance</b>

# The Genotype-to-Phenotype Dilemma: How Should Laboratories Approach Discordant Susceptibility Results?

**TABLE 1** Examples of genetic alterations resulting in genotypic and phenotypic susceptibility discrepancies<sup>a</sup>

Organism	Genotype	Phenotype	Description	Discrepancy reason	Reference(s)
<i>Staphylococcus aureus</i>	<i>mecA</i> detected	Methicillin susceptible	Met-to-Ile change at amino acid residue 483	Elimination of PBP2a transpeptidase activity	38
	PBP2a detected, <i>mecA</i> not detected	Methicillin susceptible	Divergent SCC <i>mec</i> element	Remnant not functional	39
	SCC <i>mec-orfX</i> detected	Methicillin susceptible	SCC <i>mec-orfX</i> junction variants	Low specificity of proprietary target	40
	SCC <i>mec</i> detected	Methicillin susceptible	SCC <i>mec</i> remnant with <i>ccr</i> and partial <i>mecA</i> genes	Remnant not functional	41
	SCC <i>mec</i> detected	Methicillin susceptible	<i>mecA</i> deletions within SCC <i>mec</i>	Detectable by molecular methods, but remnant not functional	43
	SCC <i>mec</i> detected	Methicillin susceptible	Amplification of segments on right-extremity junction sequence of SCC <i>mec</i>	Retained segments do not contain the actual <i>mecA</i> gene	42
	<i>mecA</i> detected	Methicillin susceptible	Heterogeneously resistant large inoculum led to resistant phenotype; oxacillin induction increased MIC	Heterogeneously resistant large inoculum led to resistant phenotype	38
<i>mecA</i> not detected	Methicillin resistant	Presence of <i>mecA</i> homologue (LGA251)	Not detectable by molecular target	43	
<i>Enterococcus</i> species	<i>vanA</i> detected	Vancomycin susceptible	Nucleotide deletions in Tn546	Nonfunctional <i>vanA</i>	44
	<i>vanA</i> detected	Vancomycin variable <sup>b</sup>	Silenced <i>vanA</i> ( <i>vanHAX</i> ) gene	Unexpressed <i>vanA</i>	45
	<i>vanAB</i> not detected	Vancomycin resistant	Presence of <i>vanC</i>	Not detectable by molecular target	46
	<i>vanAB</i> not detected	Vancomycin resistant	Presence of <i>vanM</i>	Not detectable by molecular target	24
Enterobacterales	<i>bla</i> <sub>KPC</sub> detected	Carbapenem susceptible	Truncated <i>bla</i> <sub>KPC</sub>	Nonfunctional carbapenemase	26
	<i>bla</i> <sub>CTX-M</sub> not detected	Cefotaxime or ceftriaxone resistant	<i>bla</i> <sub>TEM/SHV</sub> -derived ESBLs	Not detectable by molecular target	16, 17
	<i>bla</i> <sub>OXA-48</sub> detected	Carbapenem variable <sup>b</sup>	<i>bla</i> <sub>OXA-48</sub> and other mechanisms (e.g., other $\beta$ -lactamases)	Alternative resistance mechanisms	17
	Carbapenemase not detected	Carbapenem resistant	Alternative carbapenem resistance mechanism (e.g., AmpC production combined with porin mutations and/or efflux)	Alternative carbapenem resistance mechanism	47
	Carbapenemase not detected	Carbapenem resistant	Test (e.g., Carba-R) cannot detect <i>bla</i> <sub>OXA-48</sub> -like and some other carbapenemases	Not detectable by molecular target	48
	Carbapenemase not detected	Imipenem resistance	Intrinsic low-level resistance ( <i>Morganella</i> , <i>Proteus</i> spp., and <i>Providencia</i> spp.)	Intrinsic low-level resistance	49
	NA	Cefepime variable <sup>b</sup>	ESBL-carrying strains	Alternative resistance mechanisms	50
NA	$\beta$ -Lactam/inhibitor combination variable <sup>b</sup>	ESBL-carrying strains	Alternative resistance mechanisms	51	
NA	Cephalosporin variable <sup>b</sup>	Presence of multiple $\beta$ -lactamases	Alternative resistance mechanisms	17	

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

<sup>b</sup>Variable describes potentially resistant, intermediate resistant, and susceptible interpretations.

# Clinical impact and implementation considerations

The clinical impact depends on

- test performance
- availability (24/7 vs limited times)
- subgroup of patients vs all patients
- comprehensive laboratory reports: explicit comments
- association with antimicrobial stewardship: more appropriate antibiotic therapy decisions
- local epidemiology

Timbrook TT, CID 2016

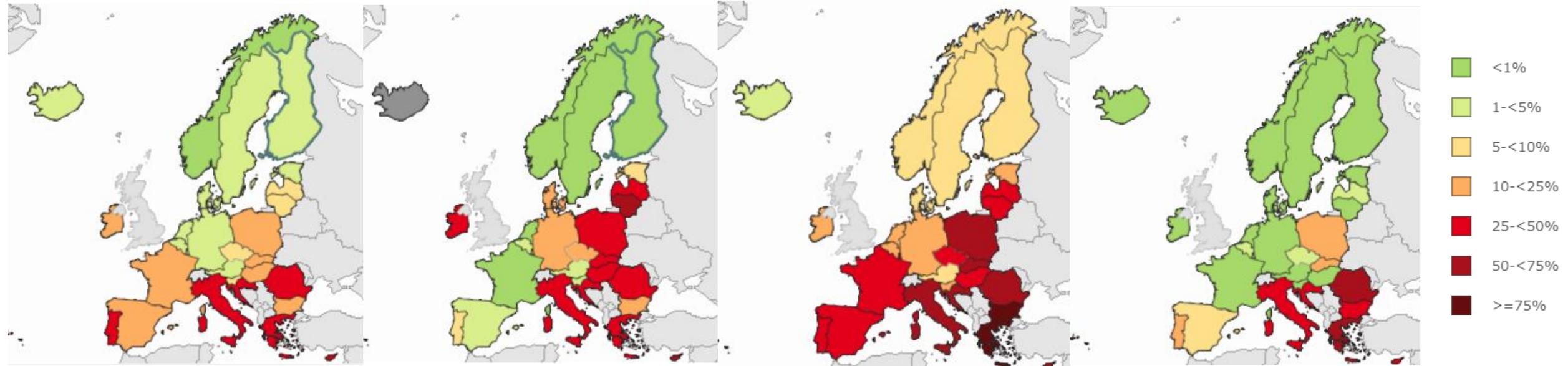
Simner PJ, CID 2023

Satlin MJ et al CID 2022

Banerjee R et al CID 2020

Rivard KR et al Eur J Clin Microbiol Infect Dis 2017

# Higher impact in settings with high resistance rates



MRSA

VRE  
(*E. faecium*)

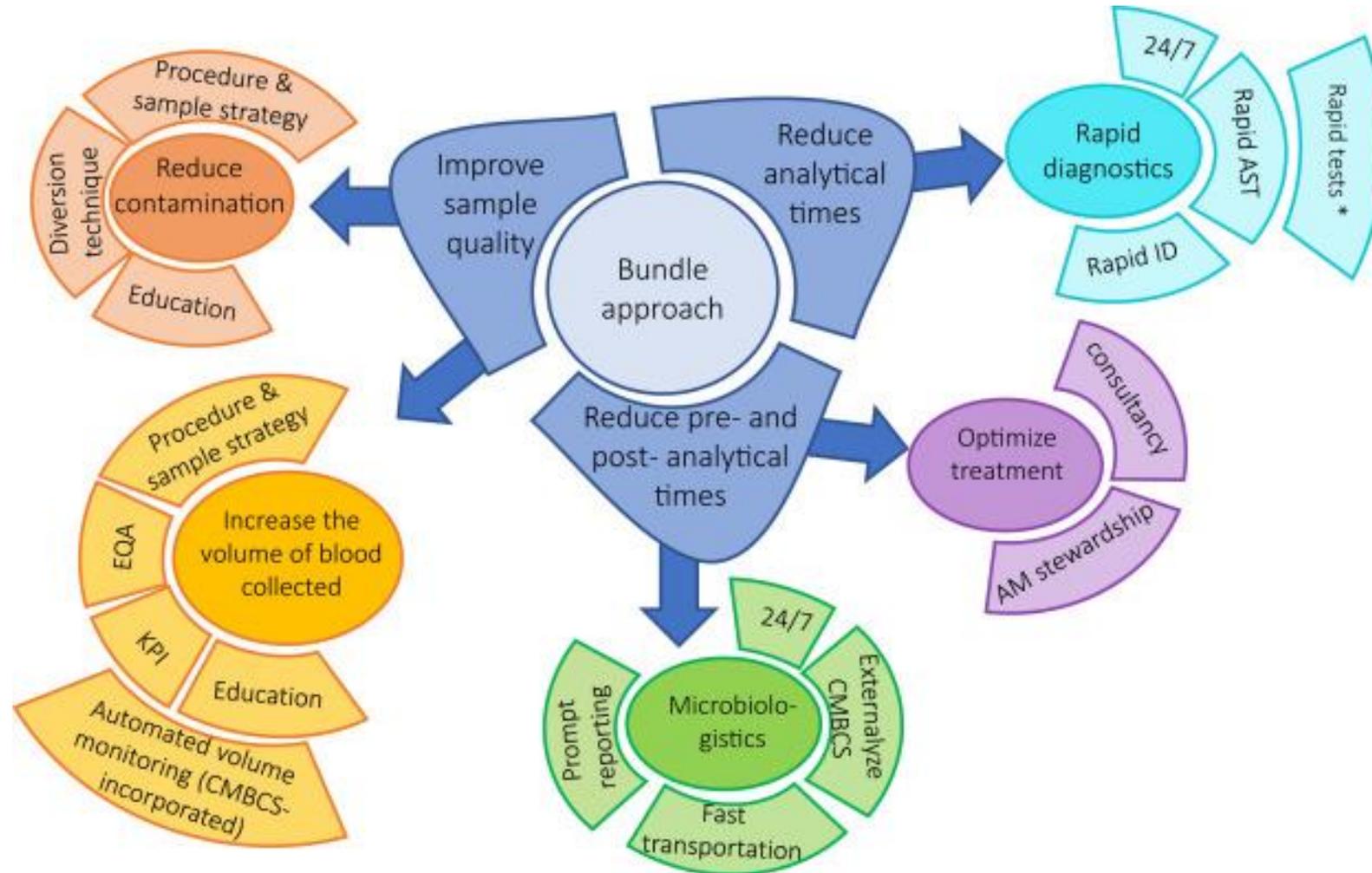
*K. pneumoniae*  
3rd gen. cephalosporin R

*K. pneumoniae*  
carbapenem R

ECDC 2021 data from bloodcultures and cerebral fluid

## Bloodstream infections – Standard and progress in pathogen diagnostics

Brigitte Lamy<sup>1,2,3,\*</sup>, Martin Sundqvist<sup>4</sup>, Evgeny A. Idelevich<sup>5</sup>, on behalf of the ESCMID Study Group for Bloodstream Infections, Endocarditis and Sepsis (ESGBIES)



# In 2022 in Helsinki

- Opening hours 24/7
- 1 central laboratory with MALDI-TOF
- 6 decentralized with BC incubators
- 2 laboratories in different regions



- Previously: MALDI-TOF on short sub-culture on solid medium
- Implementation of molecular method = 1 test for each new case
- smoothens the workflow
- easy to use
- costly: increase of overall BC cost

- Around 85-90% of isolates identified, 2 *P. aeruginosa* cases non identified
- MRSA: all detected (n=23), 2 false positive
- ESBL: 74 detected, total of 79 cases

Species	CTX-M detected (n)	CTX-M non detected (n)
<i>E. coli</i>	66	1
<i>K. Pneumoniae</i>	6	0
<i>K. Oxytoca</i>	1	1
<i>E. Cloacae</i>	0	2
<i>Salmonella typhi</i>	1	0
Total	74 (95%)	4 (5%)

- 2 OXA48 cases both detected (*E. coli*, *P. mirabilis*)
- MDR-*P. aeruginosa*: IMP (detected)
- no VRE, no MDR-Acinetobacter

# In conclusion

- Genotypic approach for rapid AST on blood culture bottles can be **useful** (rapid results, appropriate antibiotic therapy )
- Higher impact if associated with **antimicrobial stewardship** and in settings of **higher resistance rates**
- Challenges due to **genotype-phenotype discrepancies**
- Should always be **confirmed by phenotypic AST**
- **High cost**

Thank you  
for your attention!

