

# **NOVEL PHENOTYPIC AST METHODS**

**NORDICAST, MALMÖ, MAY 10, 2023**

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**CLINICAL MICROBIOLOGY, VÄXJÖ/KARLSKRONA  
SWEDEN**

# OUTLINE

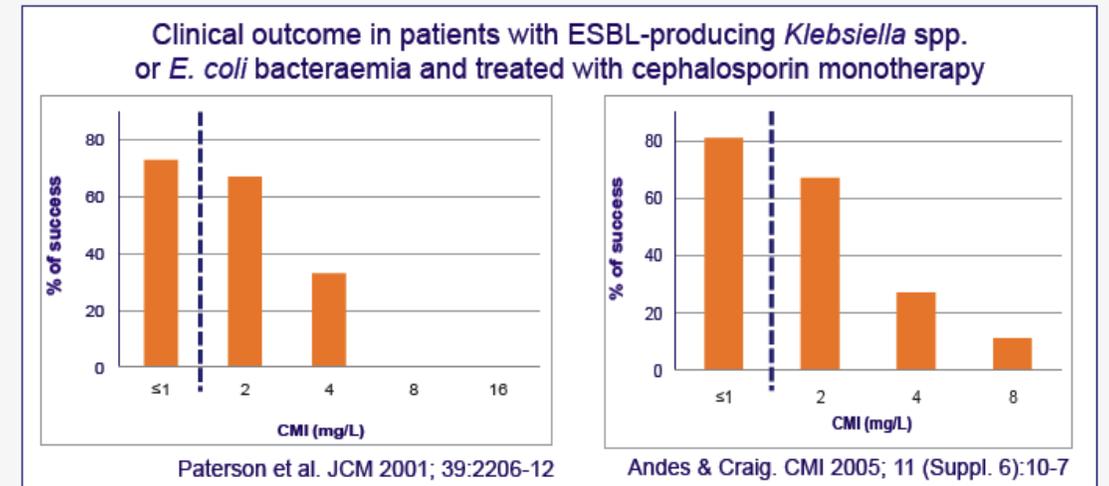
- Why is there a need for new AST methods?
- Validation criteria
- Common principles of novel AST methods
- Challenges and hurdles
- Is there an added value of AST results from novel methods?

# ALWAYS AND EVERYWHERE THIS MIC...

- Cons:
  - Decided by convention rather than a biological fact
  - Incubation time, temperature, medium and inoculum are critical
- Pros:
  - Scientifically proven correlation to outcome and survival
  - Seems to separate wild-type isolates from those with acquired mechanisms of resistance in a reliable way

## Enterobacteriaceae – 3<sup>rd</sup> & 4<sup>th</sup> gen .cephalosporins

- Clinical outcome decreased in isolates with ESBLs when MIC values of cefotaxime or ceftriaxone are > 2 mg/L



# STANDARDS FOR THE EVALUATION OF NEW AST METHODS

- ISO 20776-2
  - BMD MIC = reference
  - Categorical agreement (CA)
    - VME – very major error – R erroneously called as S
    - ME – major error – S erroneously called as R
    - mE – minor error – errors between S and I or I and R
  - Essential agreement (EA)
    - For quantitative methods – MIC within +/- 1 dilution step from BMD results
- ISO 20776-2; 2021
  - Sensitivity and specificity
  - Quantitative methods with more than three MIC steps: EA, bias, precision

# WHY IS THERE A NEED FOR NOVEL AST METHODS?

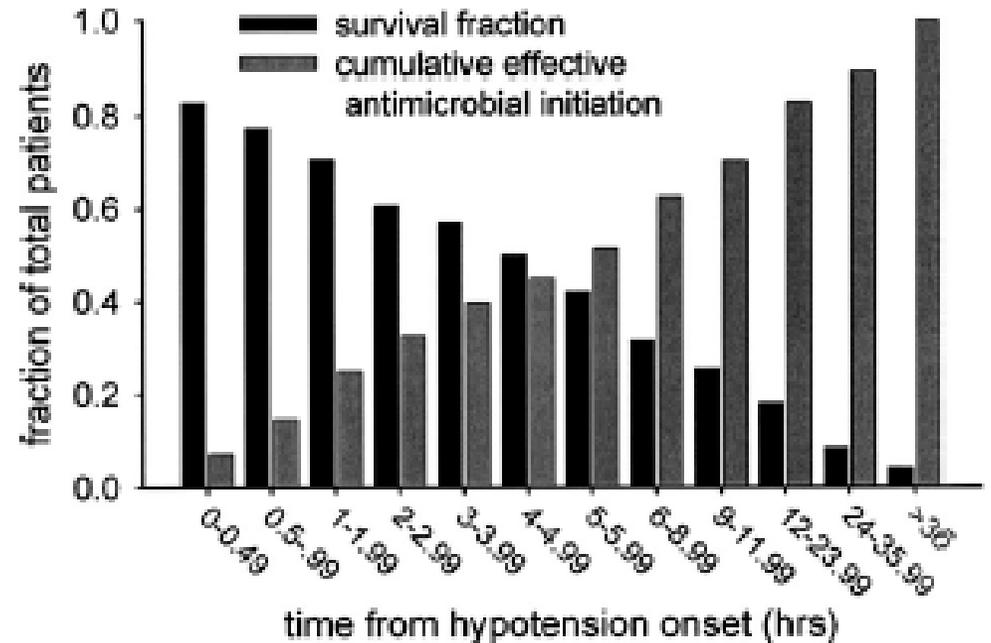
Time to results

Added clinical  
value of  
reported results

Cost

# THE NEED FOR EARLY ADEQUATE THERAPY IN SEPSIS

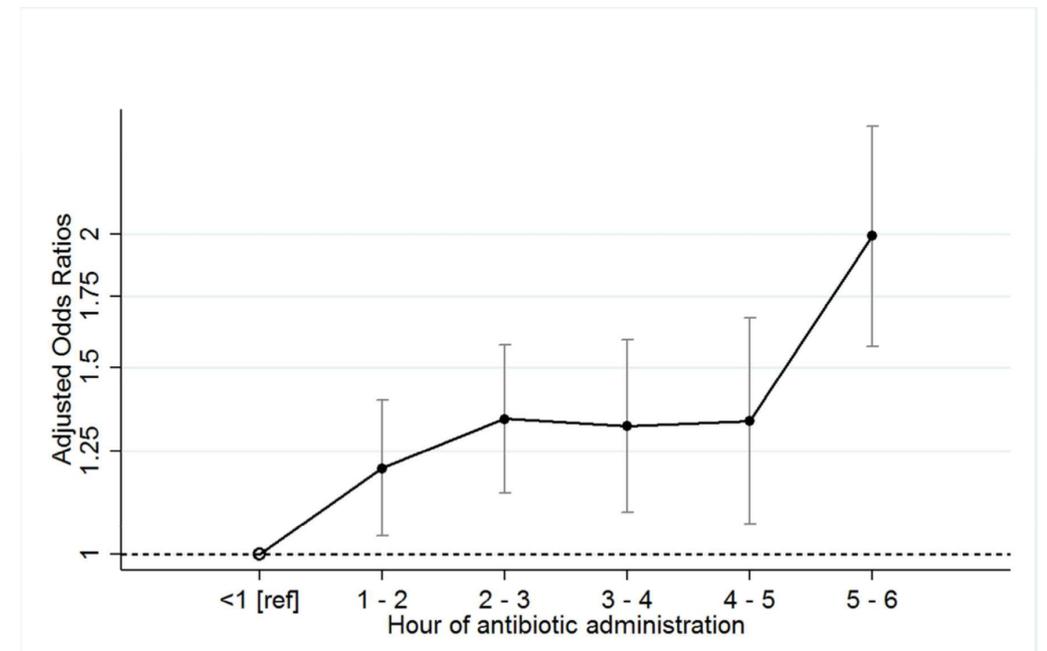
- Retrospective cohort 1989-2004, septic shock, n=2731 out of which 2154 received adequate therapy after debute of hypotension
- Median time to adequate therapy 6 h
- 8% increase of mortality for every hour



# THE NEED FOR EARLY ADEQUATE THERAPY IN SEPSIS

- Retrospective study of 35 000 patients with septicemia, severe septicemia (n=18210) and septic shock (n=4 668) in emergency departments i California
- Absolute increase in mortality per hour delay
  - 0,3% septicemia
  - 0,4% severe septicemia
  - 1,8% septic shock

Figure 2. Adjusted odds ratios for hospital mortality comparing patients within each hourly antibiotic administration group with the reference group of patients given antibiotics in <1 hour. Y-axis is on logarithmic scale.



# **ASSAYS MEASURING EXPRESSION OF RESISTANCE MECHANISMS**

**”PSEUDOGENOTYPIC” TESTS**

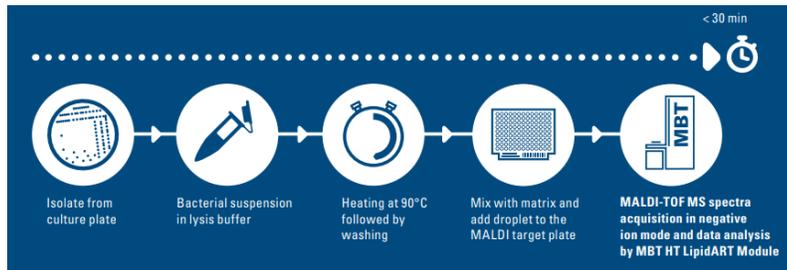
# MALDI-ToF profiling of lipid A as a diagnostic tool for the detection of colistin resistance in *Escherichia coli*,

## *Klebsiella pneumoniae* and *Acinetobacter baumannii*

F. Nilsson <sup>1</sup>, S. Lockwood <sup>1</sup>, Å. Johansson <sup>1</sup>, M.A. Klokhammer Hetland <sup>2, 3</sup>,  
I. Høyland Löhr <sup>2</sup>, O. Ekelund <sup>1</sup>, S. Somajo <sup>4, 1</sup>



<sup>1</sup>Department of Clinical Microbiology, Region Kronoberg (Sweden), <sup>2</sup>Department of Medical Microbiology, Stavanger University Hospital - Stavanger (Norway), <sup>3</sup>Department of Biological Sciences, Faculty of Mathematics and Natural Sciences, University of Bergen - Bergen (Norway), <sup>4</sup>Linnæus University, Department of Chemistry and Biomedical Sciences - Kalmar (Sweden)



**Table 1.** MALDI-ToF lipid A profiling performance for detection of colistin resistance in terms of specificity and sensitivity (95%CI), categorical agreement (CA), major (ME) and very major errors (VME), as compared to S/R categorization by reference BMD.

	Total (n)	Sensitivity (%)	Specificity (%)	CA (%)	ME (n)	VME (n)
<i>E. coli</i>	26	100 (68-100)	94 (74-100)	96	1	0
<i>K. pneumoniae</i>	56	100 (87-100)	90 (75-97)	95	3	0
<i>A. baumannii</i>	20	100 (65-100)	62 (36-82)	75	5	0
<b>TOTAL</b>	<b>102</b>	<b>100 (91-100)</b>	<b>85 (75-92)</b>	<b>91</b>	<b>9</b>	<b>0</b>

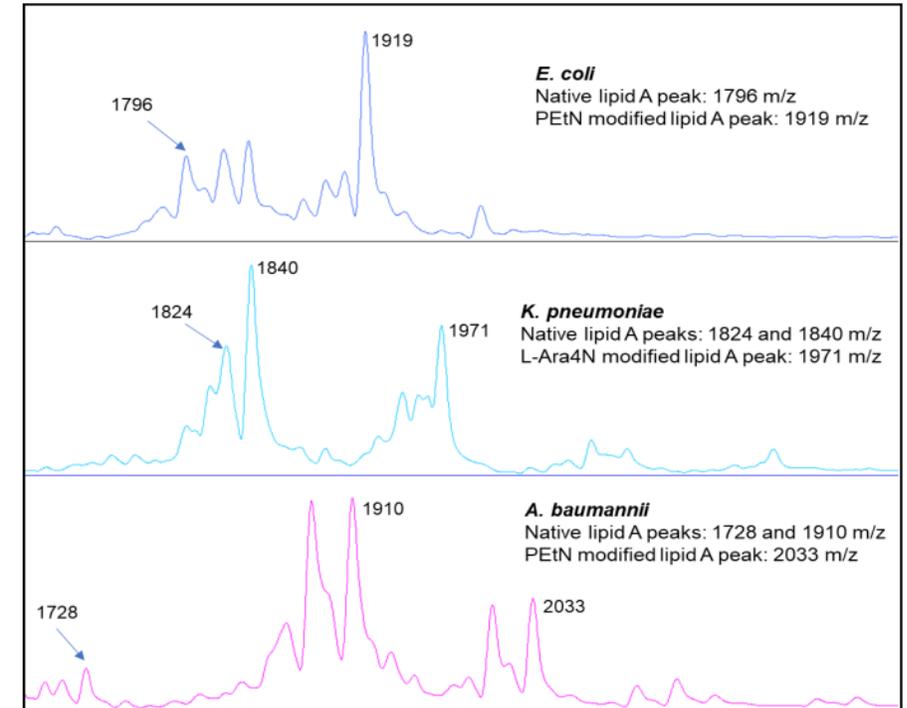


Figure 1. Spectra with native and modified lipid A peaks for each species.

30 min from colonies



# Fast-track identification of CTX-M-extended-spectrum-β-lactamase- and carbapenemase-producing *Enterobacterales* in bloodstream infections: implications on the likelihood of deduction of antibiotic susceptibility in emergency and internal medicine departments

Matteo Boattini<sup>1</sup> · Gabriele Bianco<sup>1</sup> · Marco Iannaccone<sup>1</sup> · Davide Ghibaudò<sup>1</sup> · André Almeida<sup>2,3</sup> · Rossana Cavallo<sup>1</sup> · Cristina Costa<sup>1</sup>



**Table 1** Accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of the fast-track blood cultures workflow for the detection of CTX-M ESBL- and carbapenemase-producers in patients admitted in emergency and internal medicine departments

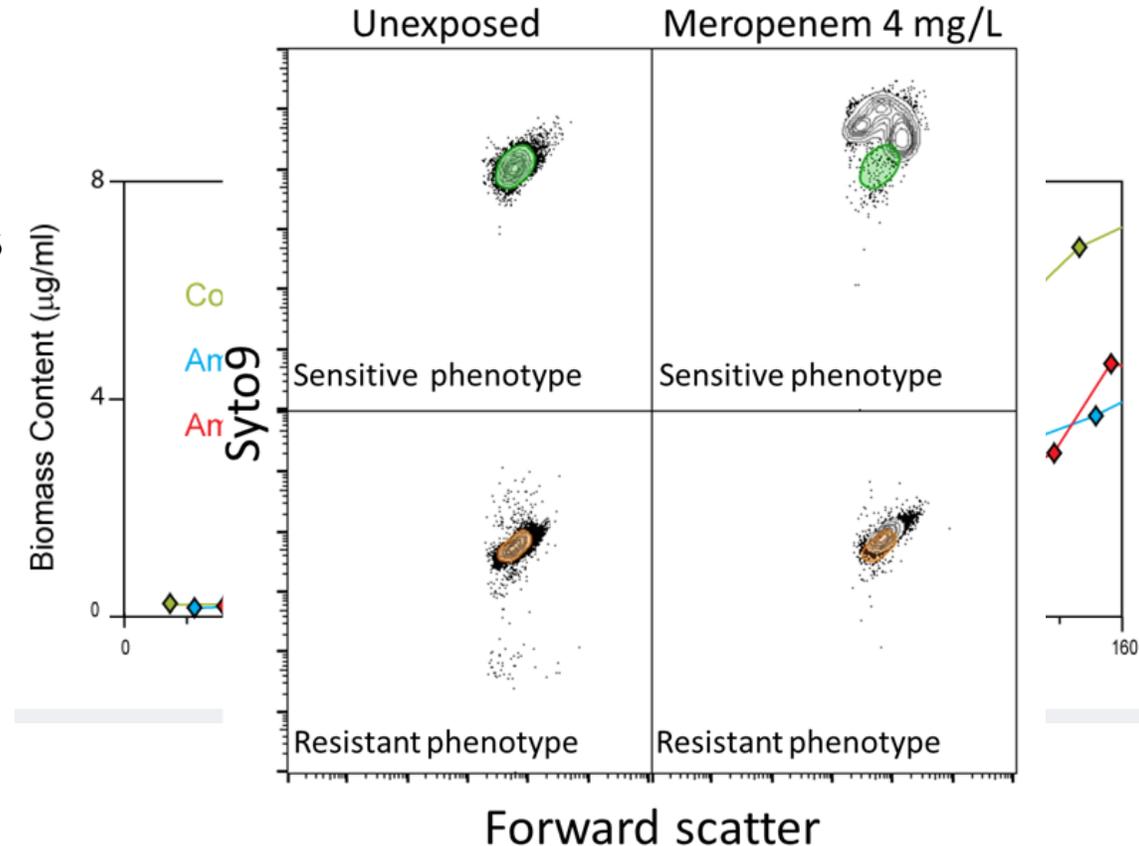
Fast-track workflow results		Conventional phenotypic routine results		Accuracy, % (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
		Positive	Negative					
CTX-M-p	Positive	43	0	100 (98.5–100)	100 (91.8–100)	100 (98.1–100)	100	100
	Negative	0	193					
CA-p	Positive	8	0	99.6 (97.7–100)	88.9 (51.8–99.7)	100 (98.4–100)	100	99.6 (97.3–99.9)
	Negative	1	227					
CTX-M-CA-np	Positive	184	1	99.6 (97.7–100)	100 (98–100)	98.1 (89.7–100)	99.5 (96.4–99.9)	100
	Negative	0	51					

1 + 1 + 15 min  
from pos BC

*Abbreviations:* PPV positive predictive value, NPV negative predictive value, CTX-M-p CTX-M-producer, CA-p main-carbapenemases-producer, CTX-M-CA-np CTX-M-and-main-carbapenemases-non-producer

# COMMON PRINCIPLES OF NOVEL PHENOTYPIC AST METHODS

- Short exposure to antibiotics
- High-sensitive detection of anti-bacterial effects
  - On a population level
    - Flow cytometry – cell count
    - Microcalorimetry
    - Mean microbe mass
  - On a single-cell level
    - Flow cytometry – cell morphology/fluorescens
    - Time-lapse microscopy – morfology/biomass



# COMMERCIALLY AVAILABLE PLATFORMS

## Accelerate PhenoTest BC Kit

FDA approved

7 h to AST results, incl. species ID (FISH)

Streptococci: only ID, no AST

Ullberg et al (2020):

Reliable species ID

CA 86-100% for tested antibiotics (update)

No data on CPO and ESBLs

7 h from pos BC



## QuantaMatrix dRAST

CE-IVD

6 h to AST results, no species ID

Does not include streptococci

Wong et al. (2022):

Overall agreement: 95.5%

MDR isolates: 87.2%

Pip/tazo, clindamycin

6 h from pos BC



Ullberg, Öcenzi. *Eur J Clinical Microbiol & Inf Dis* (2020) 39:139–149  
Wong et al. *Microbio Spectrum*, 2022 vol 10(2), e02107-21

6 h from pos BC

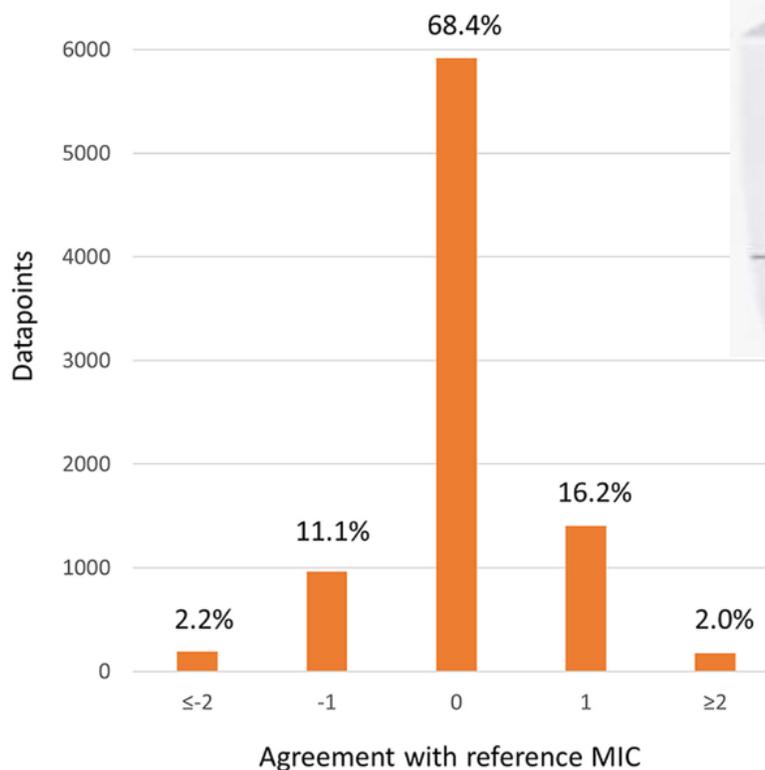


# Performance of a System for Rapid Phenotypic Antimicrobial Susceptibility Testing of Gram-Negative Bacteria Directly from Positive Blood Culture Bottles

J. Göransson<sup>a</sup>, M. Sundqvist<sup>b</sup>, E. Ghaderi<sup>c</sup>, J. G. Lisby<sup>d</sup>, Y. Molin<sup>a</sup>, E. Eriksson<sup>a</sup>, S. Carlsson<sup>a</sup>, A. Cederlöf<sup>a</sup>, L. Ellis<sup>a</sup>, J. Melin<sup>a</sup>

Antimicrobial agent	EA (%)	CA (%)
Ampicillin	233/241 (96.7)	237/241 (98.3)
Amoxicillin-clavulanic acid <sup>a</sup>	341/357 (95.5)	332/357 (93.0)
Piperacillin-tazobactam <sup>b</sup>	416/436 (95.4)	426/436 (97.7)
Cefazolin	276/286 (96.5)	262/286 (91.6)
Cefepime	440/452 (97.3) <sup>e</sup>	435/441 (98.6) <sup>e</sup>
Cefotaxime	422/443 (95.3)	438/443 (98.9)
Ceftazidime	389/399 (97.5)	387/399 (97.0)
Ceftazidime-avibactam <sup>c</sup>	393/429 (91.6)	422/429 (98.4)
Ceftolozane-tazobactam <sup>b</sup>	416/426 (97.7)	418/426 (98.1)
Ceftriaxone	429/444 (96.6)	440/444 (99.1)
Cefuroxime	282/294 (95.9)	285/294 (96.9)
Ertapenem	391/413 (94.7)	412/413 (99.8)
Meropenem	455/481 (94.6)	461/481 (95.8)
Aztreonam	421/427 (98.6)	421/427 (98.6)
Ciprofloxacin	431/447 (96.4)	429/447 (96.0)
Levofloxacin	466/475 (98.1)	459/475 (96.6)
Amikacin	413/448 (92.2)	442/448 (98.7)
Gentamicin	412/431 (95.6)	423/431 (98.1)
Tobramycin	428/451 (94.9)	448/451 (99.3)
Tigecycline	189/196 (96.4)	195/196 (99.5)
Colistin	237/251 (94.4)	251/251 (100)
Trimethoprim-sulfamethoxazole <sup>d</sup>	403/423 (95.3)	410/423 (96.9)
<b>Total</b>	<b>8,283/8,650 (95.8)</b>	<b>8,433/8,639 (97.6)</b>

Q-LINEA



# Evaluation of the Speed, Accuracy and Precision of the QuickMIC Rapid Antibiotic Susceptibility Testing Assay With Gram-Negative Bacteria in a Clinical Setting

Christer Malmberg<sup>1,2</sup>, Jessie Torpner<sup>2†</sup>, Jenny Fernberg<sup>2†</sup>, Håkan Öhrn<sup>2</sup>, Jonas Ångström<sup>2</sup>, Cecilia Johansson<sup>2</sup>, Thomas Tängdén<sup>3</sup> and Johan Kreuger<sup>1\*</sup>



2-4 h from pos BC

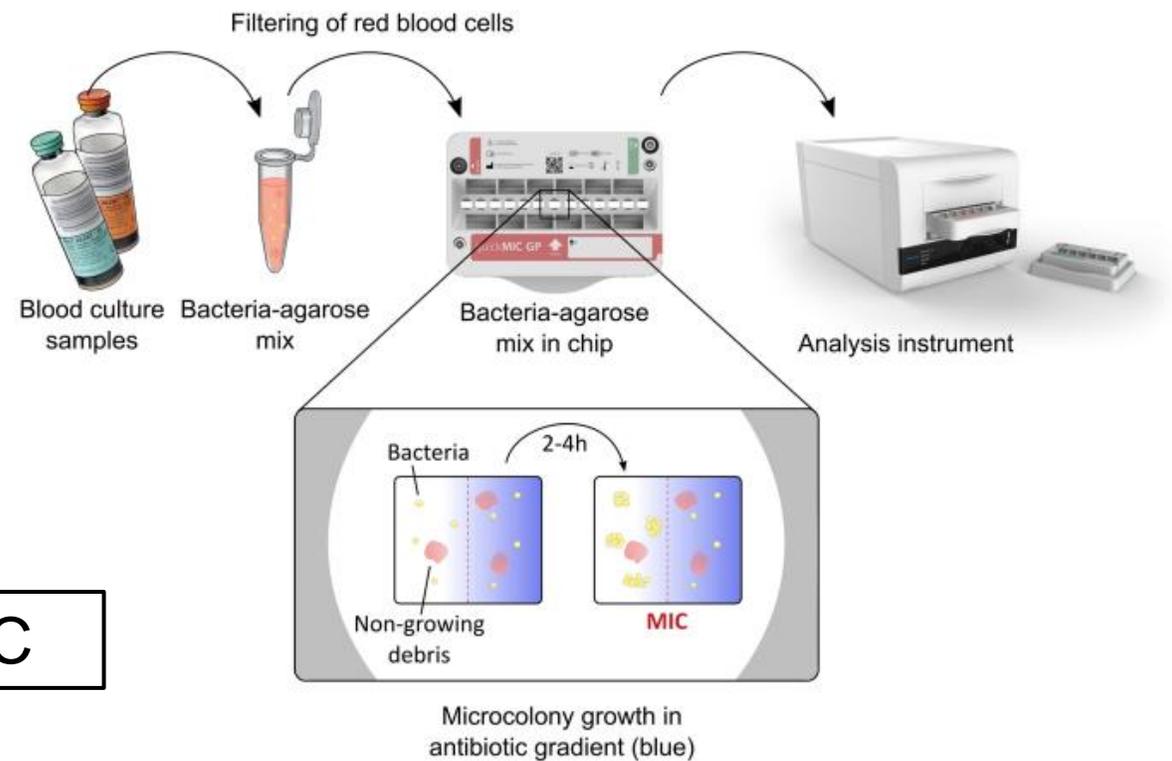


TABLE 3A | Essential agreement and categorical agreement between QuickMIC and BMD for the reference strains, by tested antibiotic.

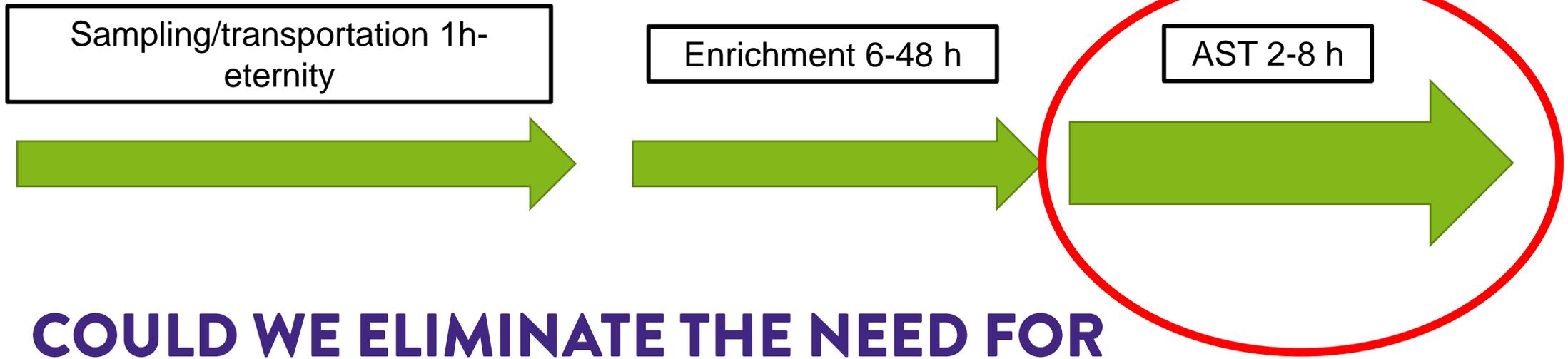
n =	Antibiotic												
	AMI (100)	CEP (141)	CIP (147)	COL (149)	CTA (137)	CTV(131)	CTZ (144)	GEN (147)	MER (152)	PIT (141)	TIG (145)	TOB (137)	Total (1671)
EA (%)	70.8	84.9	91.0	71.4	86.0	89.3	75.5	91.7	84.1	83.1	87.7	82.0	83.4
CA (%)	94.9	81.7	91.8	92.6	86.9	99.1	72.5	96.9	84.9	78.5	57.6	89.8	87.4
MID (%)	0.0	14.3	6.1	0.0	4.0	0.0	16.7	0.0	10.5	14.0	0.0	0.0	5.9
MD (%)	0.0	4.0	1.4	0.7	5.1	0.0	8.3	0.8	0.7	5.8	30.3	2.9	3.2
VMD (%)	5.1	0.0	0.7	6.7	4.0	0.9	2.5	2.3	3.9	1.7	12.1	7.3	3.4

Malmberg C, et al (2022). *Front. Cell. Infect. Microbiol.* 12:758262. doi: 10.3389/fcimb.2022.758262



# COMMERCIAALLY AVAILABLE PLATFORMS

- Low number of peer-reviewed publications
- Results mainly presented as posters
- Even fewer independent evaluations
- Strain collections - lack of transparency
- Problematic drug/bug combinations – pip/tazo, colistin
- Cost



**COULD WE ELIMINATE THE NEED FOR ENRICHMENT OR AT LEAST SHORTEN THE INCUBATION TIME SIGNIFICANTLY?**

# A rapid workflow for bacterial isolation and phenotypic AST directly from blood

Julia Pärssinen<sup>1</sup>, Aram Kadoom<sup>2</sup>, Cyrine Mestiri<sup>2</sup>, Emma Davies<sup>3</sup>, Amanda Åman<sup>3</sup>, Jenny Fernberg<sup>3</sup>, Linnea Flinkfeldt<sup>3</sup>, Jessie Torpner<sup>3</sup>, Johan Bergqvist<sup>3</sup>, Håkan Öhrn<sup>3</sup>, Jonas Ångström<sup>3</sup>, Daniel Lockhart<sup>2</sup>, Cecilia Johansson<sup>3</sup>, William Mullen<sup>2</sup>, Pernilla Lagerbäck<sup>1</sup>, Thomas Tängdén<sup>1</sup>, Christer Malmberg<sup>1,3</sup>

<sup>1</sup>: Uppsala University, Department of Medical Sciences, Uppsala (Sweden) <sup>2</sup>: Momentum Bioscience Ltd., Oxford (United Kingdom), <sup>3</sup>: Gradientech AB, Uppsala (Sweden)

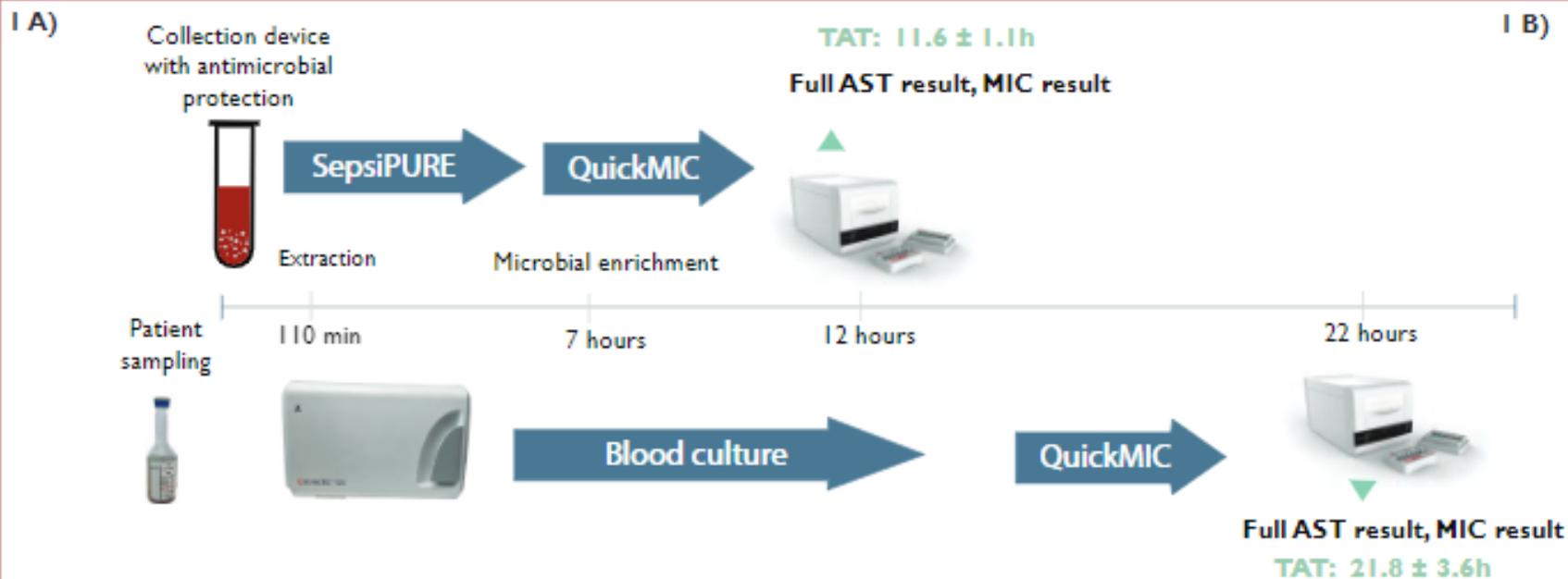
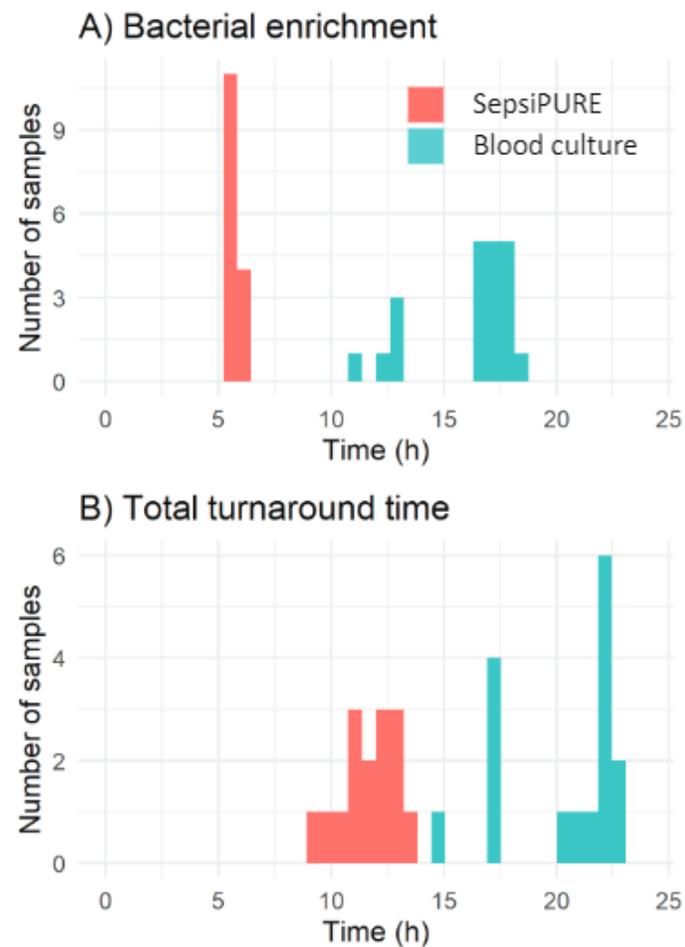
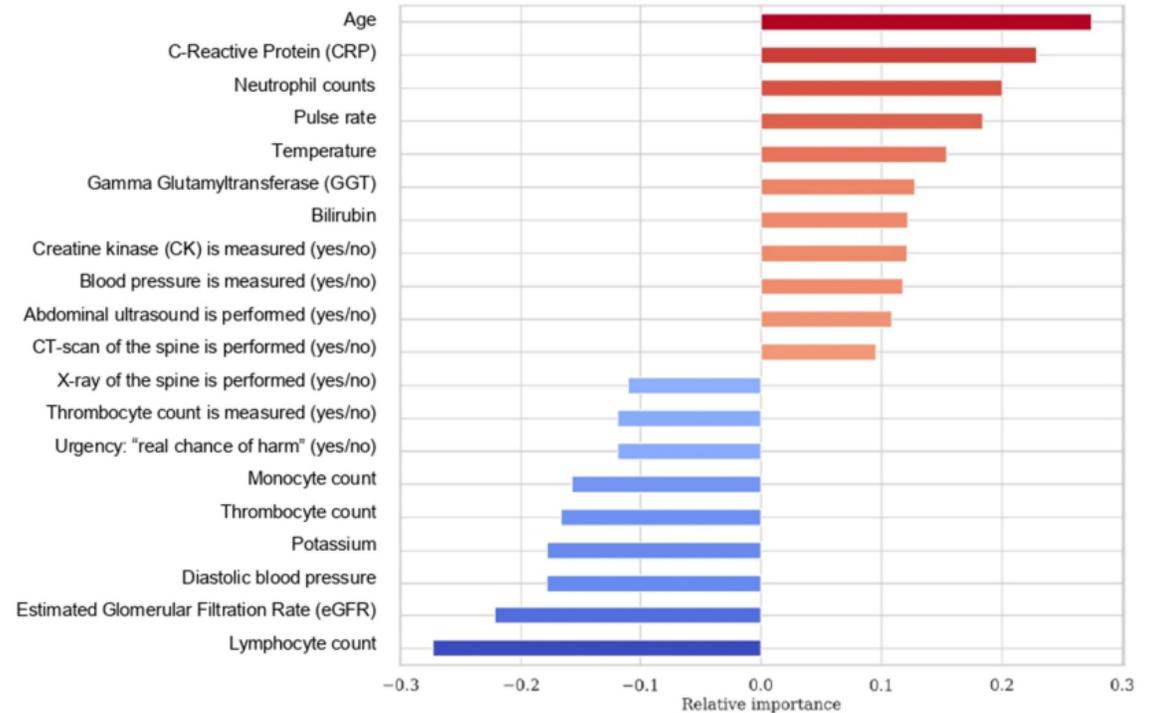
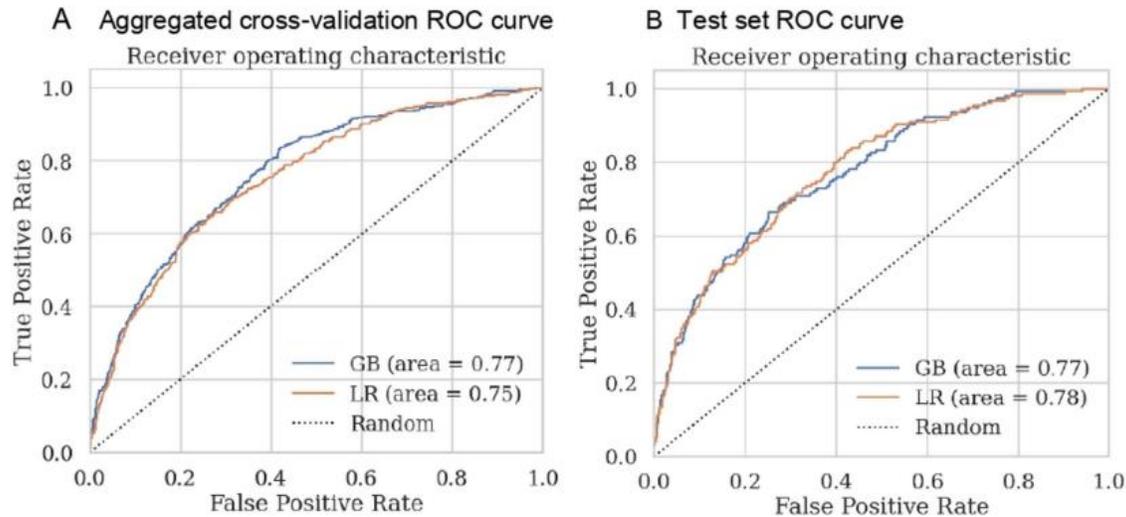


Figure 1: A) The Sepsipure and QuickMIC workflow was significantly faster than the traditional QuickMIC workflow with blood culture. B) The growth of bacterial microcolonies can be visually inspected during the QuickMIC run.

# BMJ Open Using machine learning to predict blood culture outcomes in the emergency department: a single-centre, retrospective, observational study

Anneroos W Boerman,<sup>1,2</sup> Michiel Schinkel,<sup>1,3</sup> Lotta Meijerink,<sup>4</sup> Eva S van den Ende,<sup>1</sup> Lara CA Pladet,<sup>1</sup> Martijn G Scholtemeijer,<sup>4</sup> Joost Zeeuw,<sup>4</sup> Anuschka Y van der Zaag,<sup>1</sup> Tanca C Minderhoud,<sup>1</sup> Paul W G Elbers,<sup>5</sup> W Joost Wiersinga,<sup>3,6</sup> Robert de Jonge,<sup>2</sup> Mark HH Kramer,<sup>7</sup> Prabath W B Nanayakkara <sup>1</sup>



**Figure 3** Feature importances of the logistic regression model. The 20 most important features in the logistic regression model are shown. The features for which a high value is predictive of a positive BC are shown in red and those predictive of a negative culture in blue. The X-axis presents the relative importance of these features.

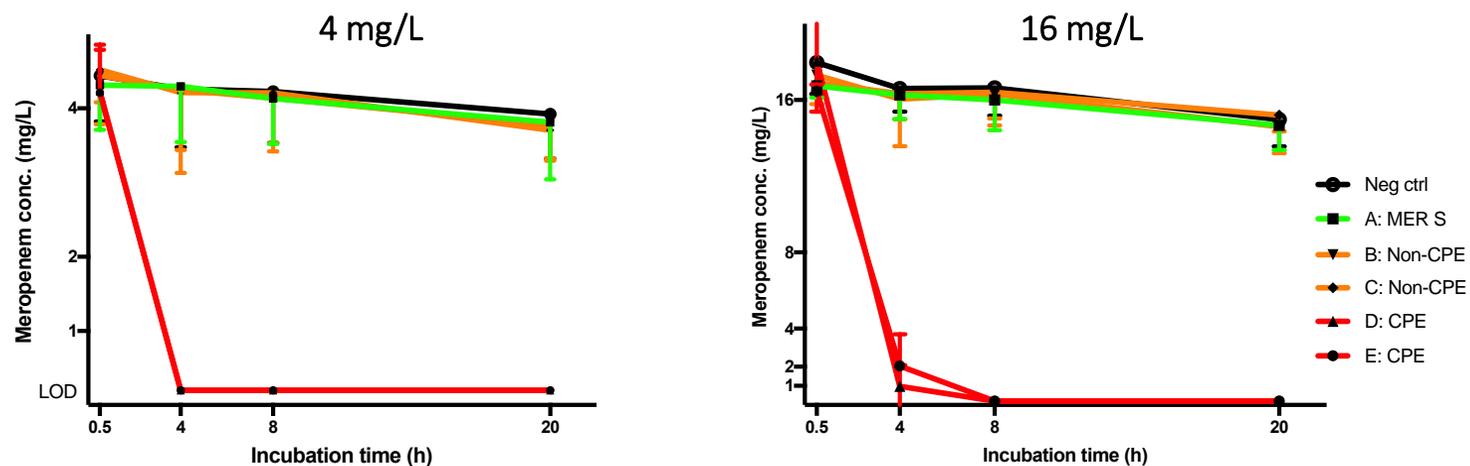
*“The optimal threshold in the gradient boosted tree model would predict 69% of BCs in the test set to be negative, with a negative predictive value of over 94%”*

# **ADDED VALUE OF RESULTS REPORTED BY NOVEL PHENOTYPIC METHODS FOR AST?**

# EXAMPLE 1 – CARBAPENEMASE PRODUCING K. PNEUMONIAE AND MEROPENEM

Meropenem mg/l	TID (h)											
	0,5	1	2	3	4	5	6	7	8	9	12	24
0	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
2	97%	83%	94%	38%	0%	0%	86%	44%	93%	49%	104%	98%
4	90%	56%	14%	1%	2%	2%	11%	10%	37%	0%	104%	94%
8	69%	8%	0%	0%	0%	0%	0%	0%	1%	0%	30%	81%
16	35%	1%	0%	0%	0%	0%	1%	0%	0%	0%	1%	48%
32	16%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	30%
64	8%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
128	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Resistant strain (MIC 64 mg/L). Observation over 24 h, cell count.

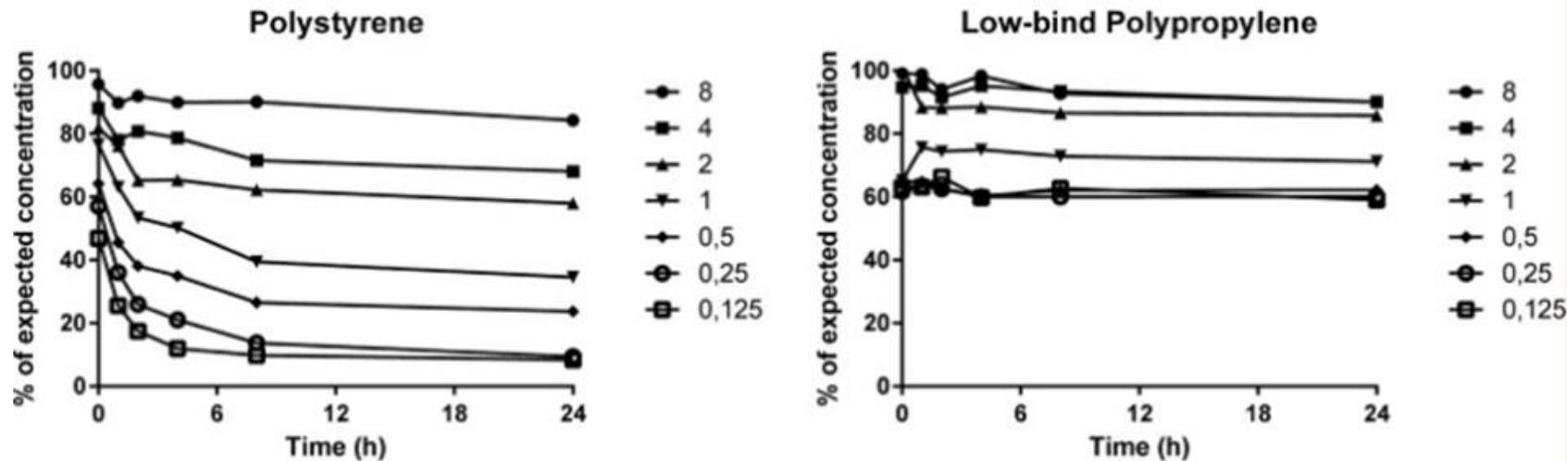


Unpublished data.

# EXAMPLE 2 - K. PNEUMONIAE AND COLISTIN

## Colistin Is Extensively Lost during Standard *In Vitro* Experimental Conditions

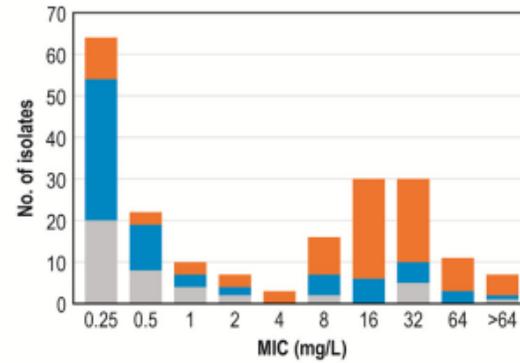
Matti Karvanen,<sup>a</sup> Christer Malmberg,<sup>a</sup> Pernilla Lagerbäck,<sup>a</sup> Lena E. Friberg,<sup>b</sup>  
Otto Cars<sup>a</sup>



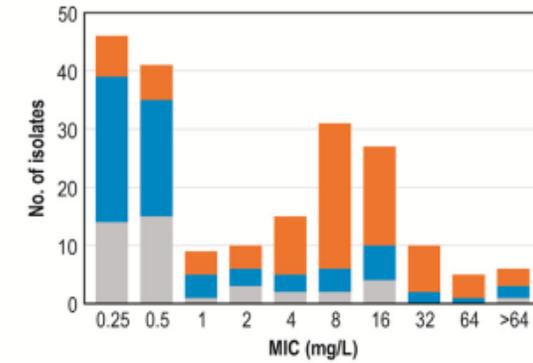
# Media for colistin susceptibility testing does not improve the detection of *Klebsiella pneumoniae* isolates carrying MgrB disruption and other mutation driven colistin resistance mechanisms

Mariana Castanheira<sup>1</sup>, Timothy B Doyle<sup>2</sup>, Cecilia G Carvalhaes<sup>2</sup>, Brianna M Roth<sup>2</sup>, Paul R Rhomberg<sup>2</sup>, Rodrigo E Mendes<sup>2</sup>

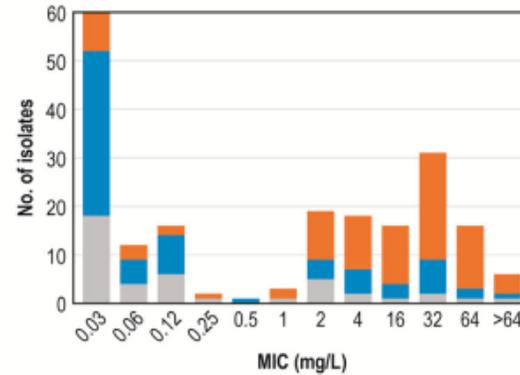
▶ A. Colistin retest



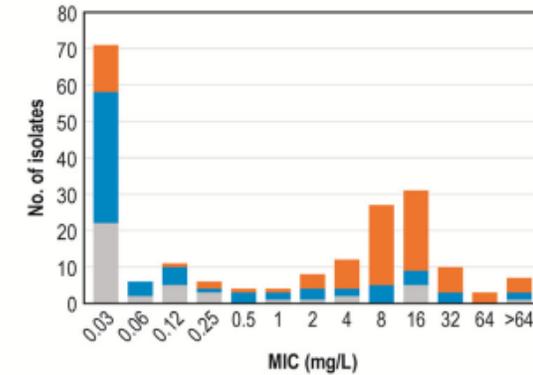
▶ D. Polymyxin B



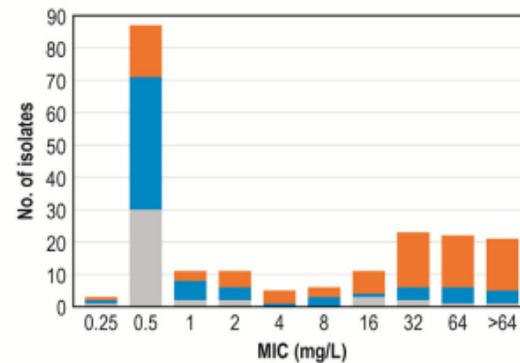
▶ B. Colistin tested with 0.02% polysorbate 80



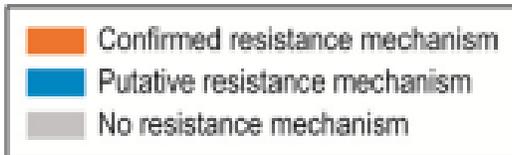
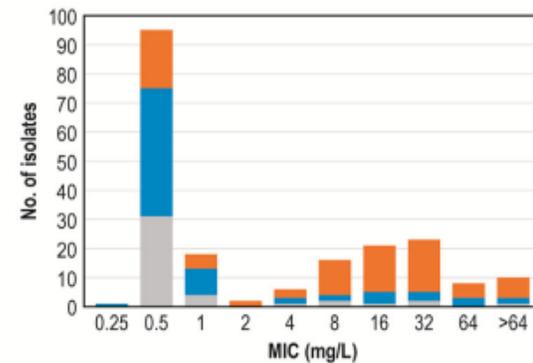
▶ E. Polymyxin B tested with 0.02% polysorbate 80



▶ C. Colistin tested with 5 mM Ca<sup>+2</sup>



▶ F. Polymyxin B tested with 5 mM Ca<sup>+2</sup>

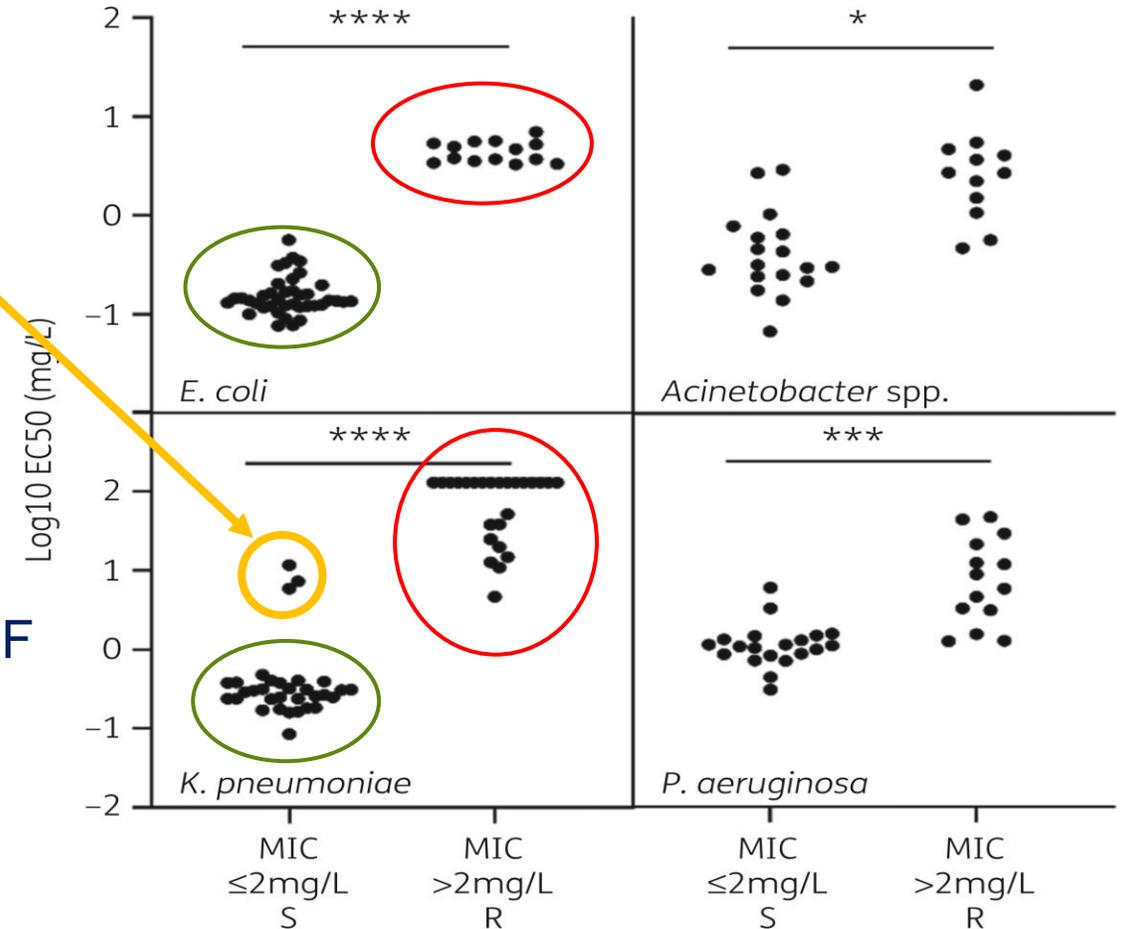
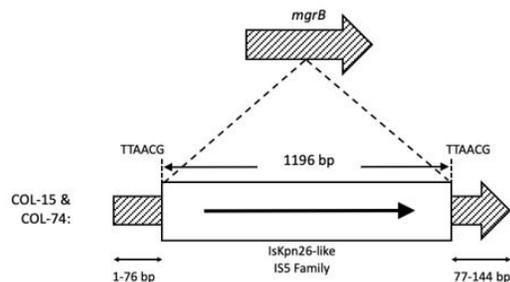


# EXAMPLE 2 - K. PNEUMONIAE AND COLISTIN

Flow cytometry, 30 min exposure

## Discrepancy analysis

- Three isolates erroneously called as resistant by FC
- All three exhibited genetic changes (*mgrB*)
- All three grew on colistin-containing agar
- 2 out of 3 were positive in Rapid polymyxin NP
- All three exhibited altered lipid A using MALDI-TOF



# CONCLUSIONS

- Speed!
- Novel rapid phenotypic AST methods – short incubation, high-sensitive detection. Promising, but need for more independent evaluations.
- Targeted rapid assays (CPOs, colistin) may be warranted depending on your local AMR epidemiology
- Most available AST methods are still culture-dependent = bottle neck for reducing time to results
- Measuring the immediate response to antibiotic exposure could potentially be of added clinical value
- EUCAST RAST = new standard for rapid AST. Should be the primary comparator in future evaluations of new rapid methods.

# THANK YOU

Sofia Somajo

Frida Nilsson

Sam Lockwood

Gunnar Kahlmeter



**REGION  
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