NOVEL PHENOTYPIC METHODS FOR AST AND DETECTION OF RESISTANCE

ESCMID POSTGRADUATE COURSE, TALLINN
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EU-RL for AMR

WHO Collaborating Centre
for Standardisation of Antimicrobial
Susceptibility Testing of Bacteria

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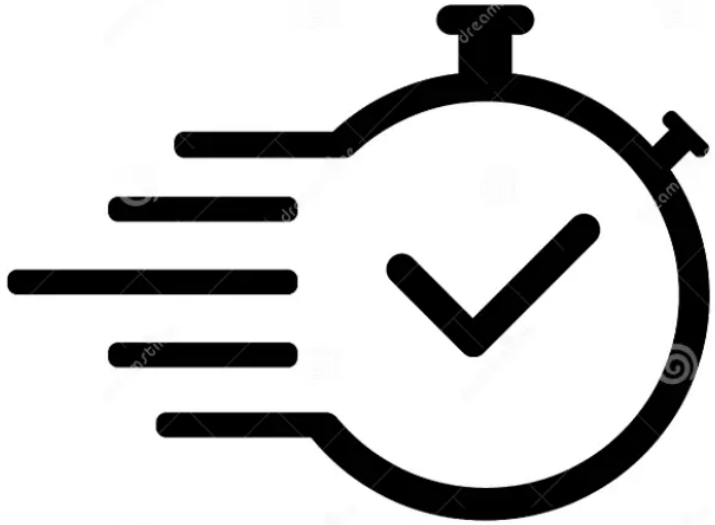

Novel = Rapid

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OUTLINE

- How important is it to shorten the time to adequate antibiotic therapy?
- Is there evidence for an added value of rapid AST in clinical practice?
- Are there certain requirements for making rapid AST worthwhile?
- Different approaches – pros and cons
- Challenges and hurdles
- Is there an added value of novel AST methods apart from speed?

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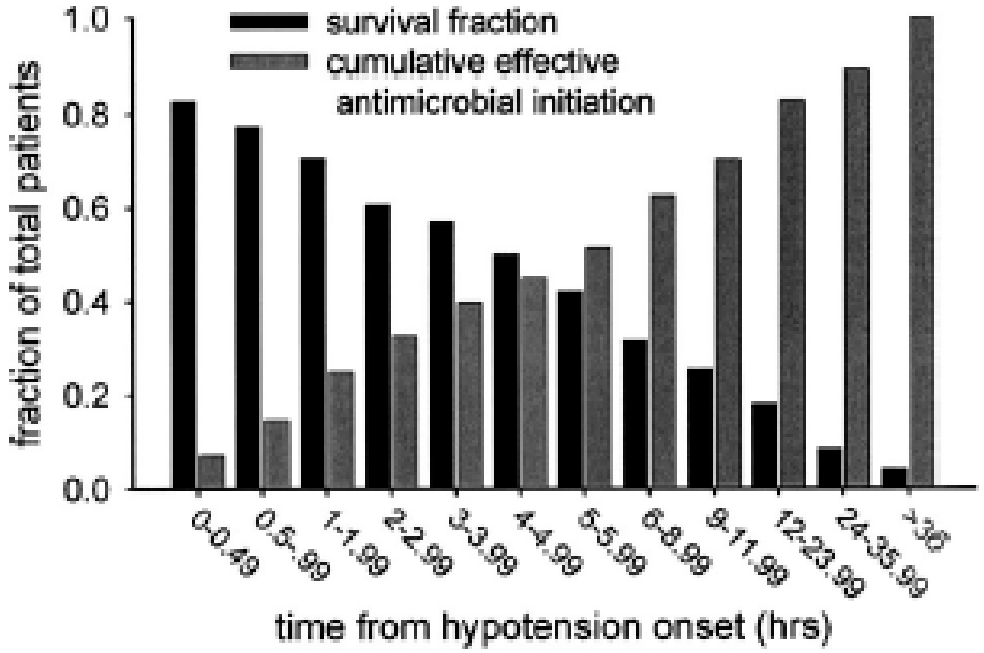
THE NEED FOR EARLY ADEQUATE THERAPY IN SEPSIS

- 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



Kumar A et al. Crit Care Med. 2006 Jun;34(6):1589-96.

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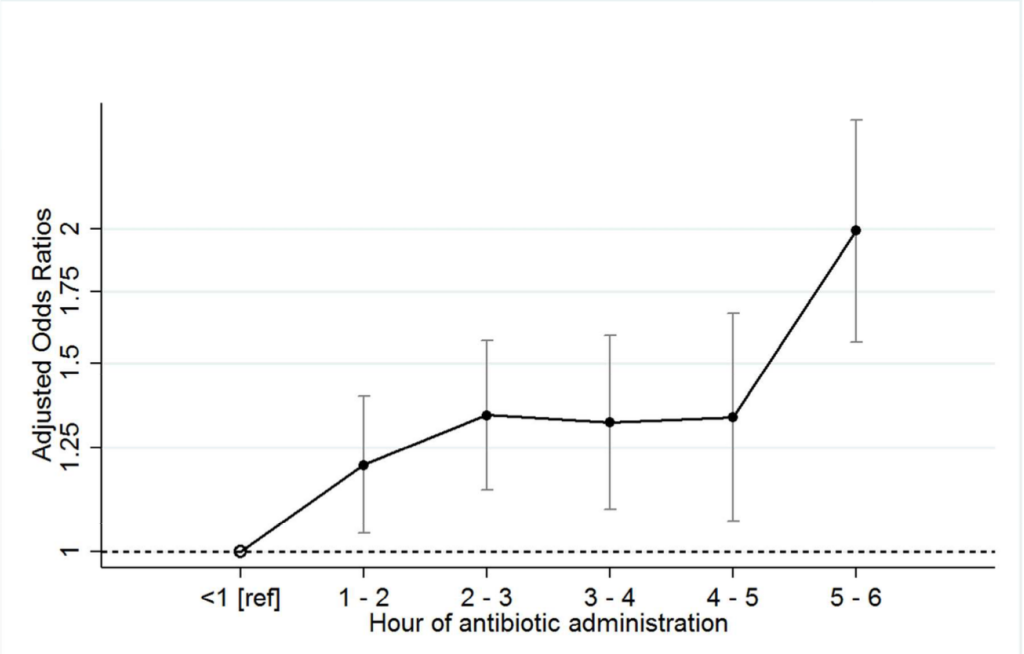
THE NEED FOR EARLY ADEQUATE THERAPY IN SEPSIS

- 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.



Liu VX, et al. Am J Respir Crit Care Med. 2017 Oct 1;196(7):856-863.

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Clinical Infectious Diseases

MAJOR ARTICLE

Association Treatment Bloodstrea

Jasper Van Heuverswyn,[1,a]

Results. We inc mortality was 11.8%. No association in favo t the 1, 3 and 6 hours landmark after bloc reatment (adjusted odds ratio 1.17 [95% con 72 hours. Stratifying by high or low SOFA s

Landmark time	Inappropriate therapy Events (Episodes)	Appropriate therapy Events (Episodes)	Risk of mortality Adjusted odds ratio (95% CI)
Total cohort			
1 hour	750 (7022)	447 (3266)	0.83 (.72 - .95)
3 hours	530 (4699)	631 (5346)	1.00 (.87 - 1.15)
6 hours	392 (3404)	730 (6458)	1.05 (.91 - 1.22)
12 hours	323 (2594)	752 (7129)	1.17 (1.01 - 1.37)
24 hours	227 (1755)	776 (7837)	1.24 (1.04 - 1.47)
48 hours	150 (1092)	784 (8461)	1.41 (1.15 - 1.74)
72 hours	99 (657)	768 (8908)	1.67 (1.30 - 2.15)
Low SOFA score			
1 hour	133 (2716)	49 (957)	0.90 (.63 - 1.29)
3 hours	100 (1858)	79 (1721)	1.01 (.73 - 1.39)
6 hours	78 (1374)	96 (2151)	1.06 (.76 - 1.46)
12 hours	66 (1009)	108 (2479)	1.20 (.86 - 1.68)
24 hours	43 (617)	120 (2823)	1.23 (.84 - 1.82)
48 hours	33 (385)	124 (3064)	1.52 (.98 - 2.34)
72 hours	18 (239)	129 (3230)	1.29 (.73 - 2.27)
High SOFA score			
1 hour	617 (4306)	398 (2309)	0.81 (.70 - .95)
3 hours	430 (2841)	552 (3625)	0.99 (.85 - 1.16)
6 hours	314 (2030)	634 (4307)	1.05 (.89 - 1.24)
12 hours	257 (1585)	644 (4650)	1.17 (.98 - 1.39)
24 hours	184 (1138)	656 (5014)	1.24 (1.01 - 1.51)
48 hours	117 (707)	660 (5397)	1.36 (1.07 - 1.73)
72 hours	81 (418)	639 (5678)	1.78 (1.34 - 2.35)

Clin Infect Dis. 2023 Feb 8;76(3):469-478. doi: 10.1093/cid/ciac727.

Mortality impact of further delays in active targeted antibiotic therapy in bacteraemic patients that did not receive initial active empiric treatment: results from the prospective, multicentre cohort PROBAC.

Sandra De la Rosa Riestra , Pedro María Martínez Pérez-Crespo ,
María Teresa Pérez Rodríguez , Adrián Sousa ,
Josune Goikoetxea , José María Reguera Iglesias ,
Carlos Armiñanzas , Inmaculada López-Hernández ,
Luis E. López-Cortés , Jesús Rodríguez-Baño , the PROBAC group

Delay in active therapy	Adjusted OR for mortality	P value
Day 3 or after	1.53	0.006
Day 4 or after	2.26	<0.001
Day 5 or after	4.33	<0.001
Day 6 or after	11.38	<0.001

We conclude that delayed administration of active targeted antibiotic treatment in patients is associated with a deleterious impact in the prognosis of patients; these results reinforce the importance of rapid reporting of blood culture results and of specialized advice in the management of BSI.

DOI: https://doi.org/10.1016/j.ijid.2024.107072

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[Intervention Review]

Rapid versus standard antimicrobial susceptibility testing to guide treatment of bloodstream infection

Vanesa Anton-Vazquez[1], Paul Hine[2], Sanjeev Krishna[1], Marty Chaplin[2], Timothy Planche[3]

[1]Institute of Infection and Immunity, St George's University of London, London, UK. [2]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK. [3]Medical Microbiology Department, SouthWest London Pathology, Jenner Wing St George's Hospital, London, UK

Main results

We included six trials, with 1638 participants. For rapid antimicrobial susceptibility testing compared to conventional methods, there was little or no difference in mortality between groups (RR 1.10, 95% CI 0.82 to 1.46; 6 RCTs, 1638 participants; low-certainty evidence). In subgroup analysis, for rapid genotypic or molecular antimicrobial susceptibility testing compared to conventional methods, there was little or no difference in mortality between groups (RR 1.02, 95% CI 0.69 to 1.49; 4 RCTs, 1074 participants; low-certainty evidence). For phenotypic rapid susceptibility testing compared to conventional methods, there was little or no difference in mortality between groups (RR 1.37, 95% CI 0.80 to 2.35; 2 RCTs, 564 participants; low-certainty evidence).

Authors' conclusions

The theoretical benefits of rapid susceptibility testing have not been demonstrated to directly improve mortality, time-to-discharge, or time-to-appropriate antibiotic in these randomized studies. Future large prospective studies should be designed to focus on the most clinically meaningful outcomes, and aim to optimize blood culture pathways.

Cochrane Database of Systematic Reviews 2021, Issue 5. Art. No.: CD013235.
DOI: 10.1002/14651858.CD013235.pub2.

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COMMENTS ON THE COCHRANE METAANALYSIS

- Only 25-49% of patients were on inadequate antibiotic therapy
- 4 of 6 trials excluded Gram-negative pathogens
- 4 of 6 trials focused on targeted genotypic testing in Gram-positive BSI (e.g. *mecA*, *vanA*)
- All trials did not include an antimicrobial stewardship (AMS) program

- **Rapid species ID + rapid AST + AMS program: *”…may have played a role in reducing time-to-appropriate antibiotics…*”**

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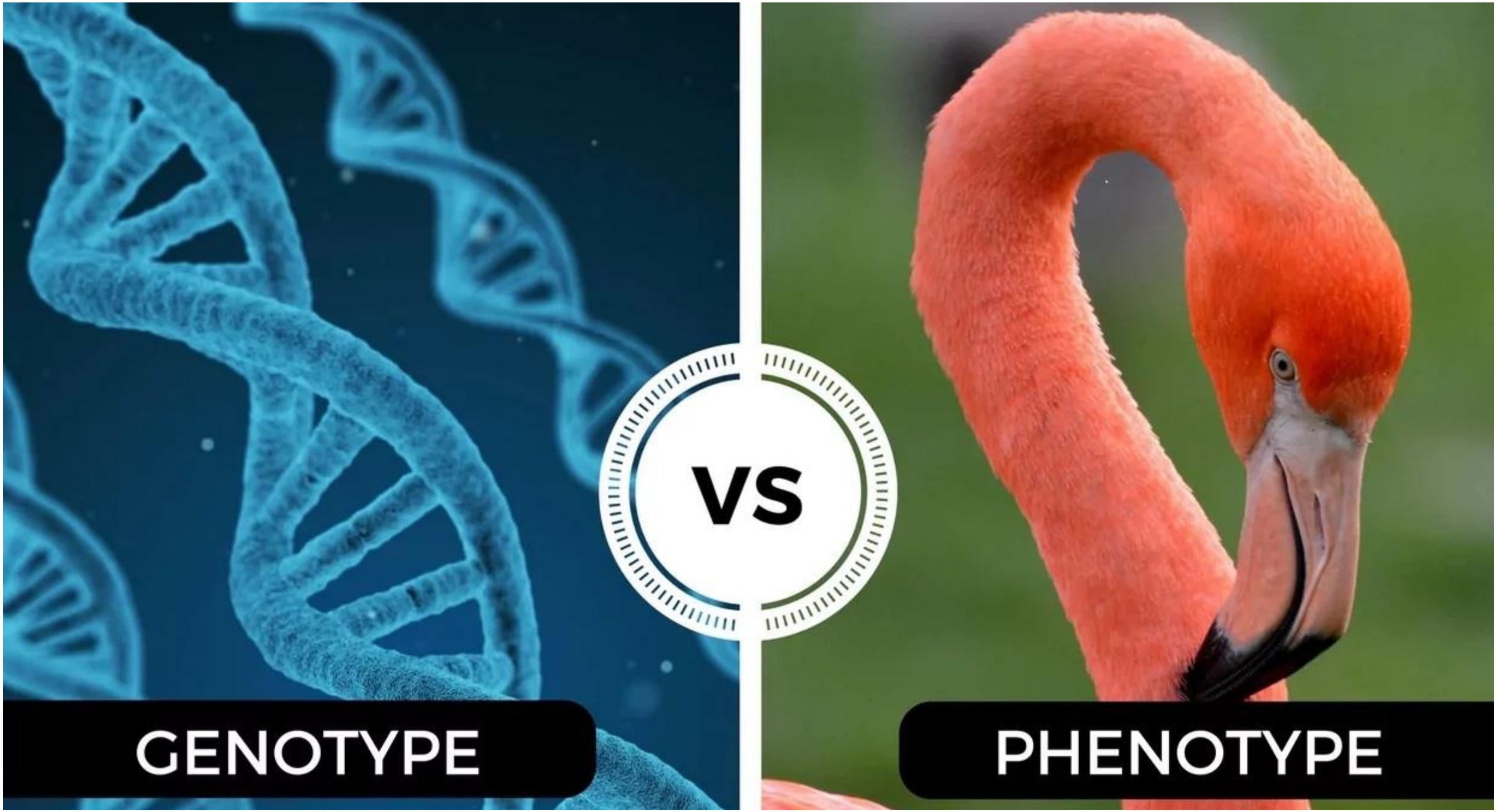

VS
GENOTYPE
PHENOTYPE

European Journal of Clinical Microbiology & Infectious Diseases (2021) 40:1495–1501
https://doi.org/10.1007/s10096-021-04192-8

ORIGINAL ARTICLE

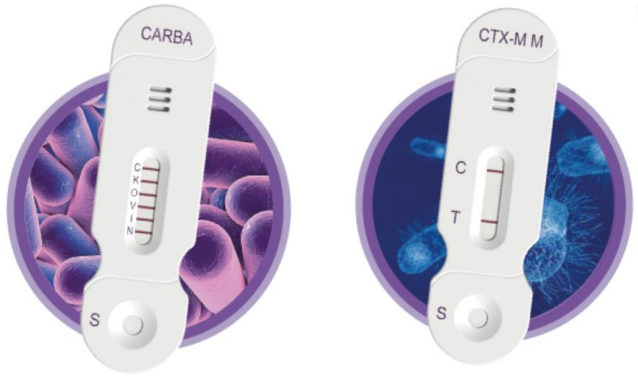
Fast-track identification of CTX-M-extended-spectrum-β-lactamase- and carbapenemase-producing *Enterobacterales* in bloodstream infections: implications on the likelihood of deduction of antibiotic susceptibility in emergency and internal medicine departments

Matteo Boattini[1] · Gabriele Bianco[1] · Marco Iannaccone[1] · Davide Ghibaudo[1] · André Almeida[2,3] · Rossana Cavallo[1] · Cristina Costa[1]

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					

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



GENOTYPIC TESTS / LATERAL FLOW TESTS

- Very rapid (15 - 60 min)
- Can only detect known mechanisms of resistance
- Hence, can at best guarantee resistance but never susceptibility

PHENOTYPIC AST

- Slower (4-8 h)
- Independent of mechanisms of resistance
- Can potentially guarantee both resistance **and** susceptibility
- **Generic - has the potential to be used for all classes of antibiotics**

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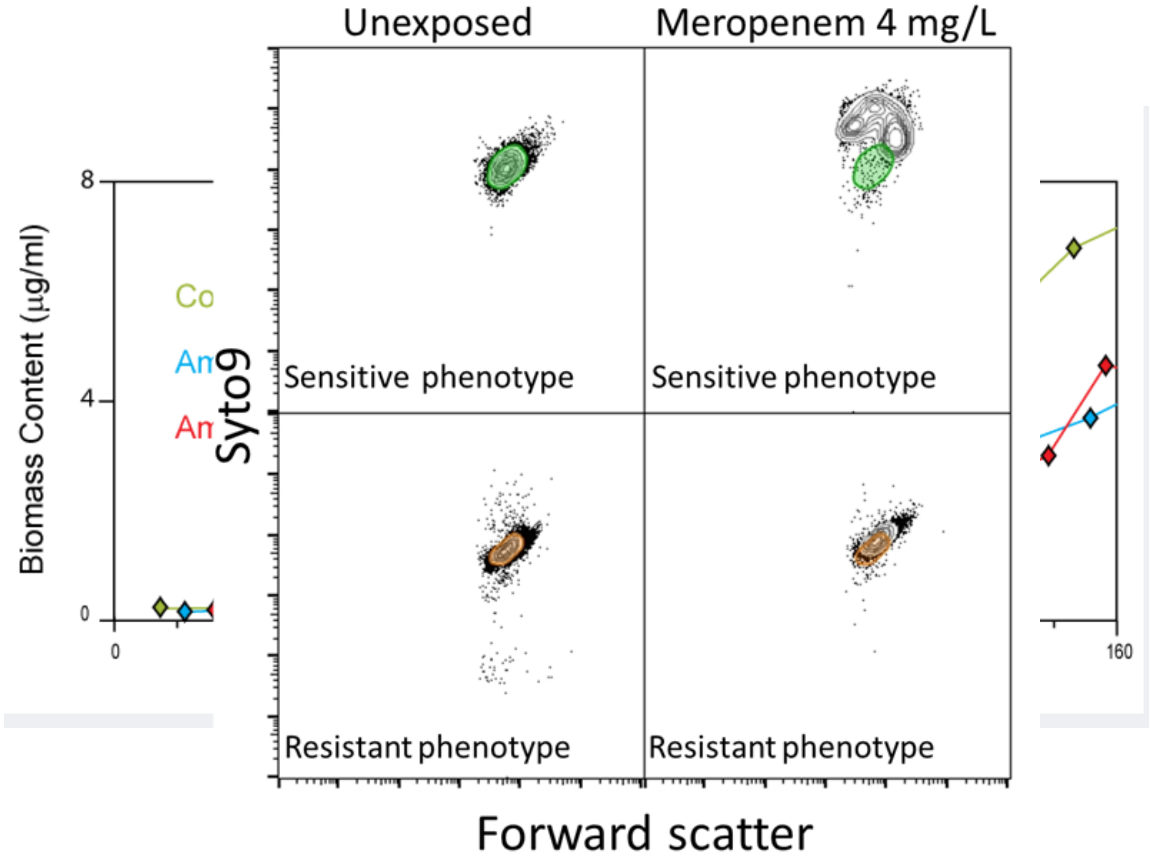
COMMON PRINCIPLES OF NOVEL PHENOTYPIC AST METHODS

- 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 singel-cell level
    - Flow cytometry – cell morfology/fluorescens
    - Time-lapse microscopy – morfology/biomass



Mulroney, Ekelund et al.

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BACTERIOLOGY
March 2023 Volume 61 Issue 3 e01525-22
https://doi.org/10.1128/jcm.01525-22

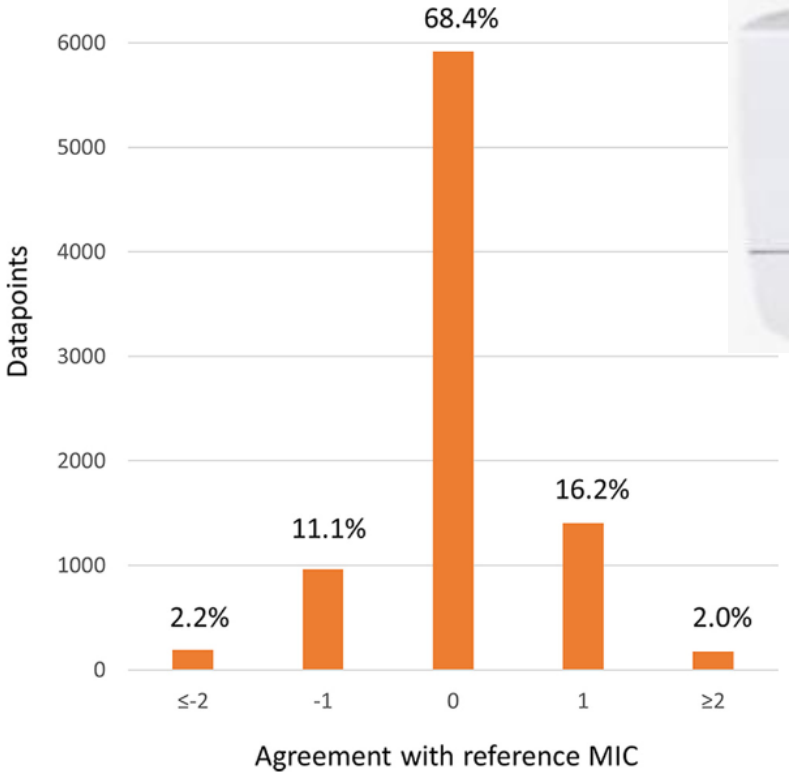
6 h from pos BC

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

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

Antimicrobial agent	EA (%)	CA (%)
Ampicillin	233/241 (96.7)	237/241 (98.3)
Amoxicillin-clavulanic acid[a]	341/357 (95.5)	332/357 (93.0)
Piperacillin-tazobactam[b]	416/436 (95.4)	426/436 (97.7)
Cefazolin	276/286 (96.5)	262/286 (91.6)
Cefepime	440/452 (97.3)[e]	435/441 (98.6)[e]
Cefotaxime	422/443 (95.3)	438/443 (98.9)
Ceftazidime	389/399 (97.5)	387/399 (97.0)
Ceftazidime-avibactam[c]	393/429 (91.6)	422/429 (98.4)
Ceftolozane-tazobactam[b]	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[d]	403/423 (95.3)	410/423 (96.9)
Total	8,283/8,650 (95.8)	8,433/8,639 (97.6)

Q-LINEA



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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[1,2], Jessie Torpner[2†], Jenny Fernberg[2†], Håkan Öhrn[2], Jonas Ångström[2], Cecilia Johansson[2], Thomas Tängdén[3] and Johan Kreuger[1]*

gradientech

2-4 h from pos BC

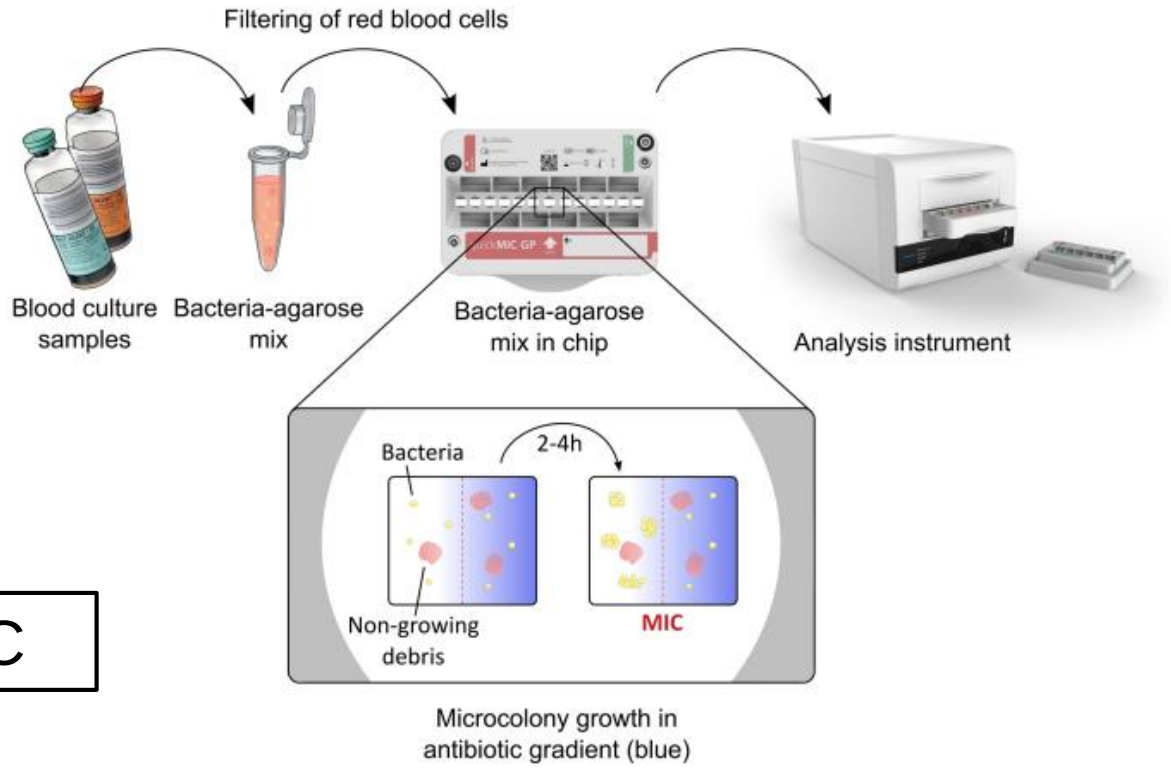

TABLE 3A | Essential agreement and categorical agreement between QuickMIC and BMD for the reference strains, by tested antibiotic.

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

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COMMERCIALLY 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

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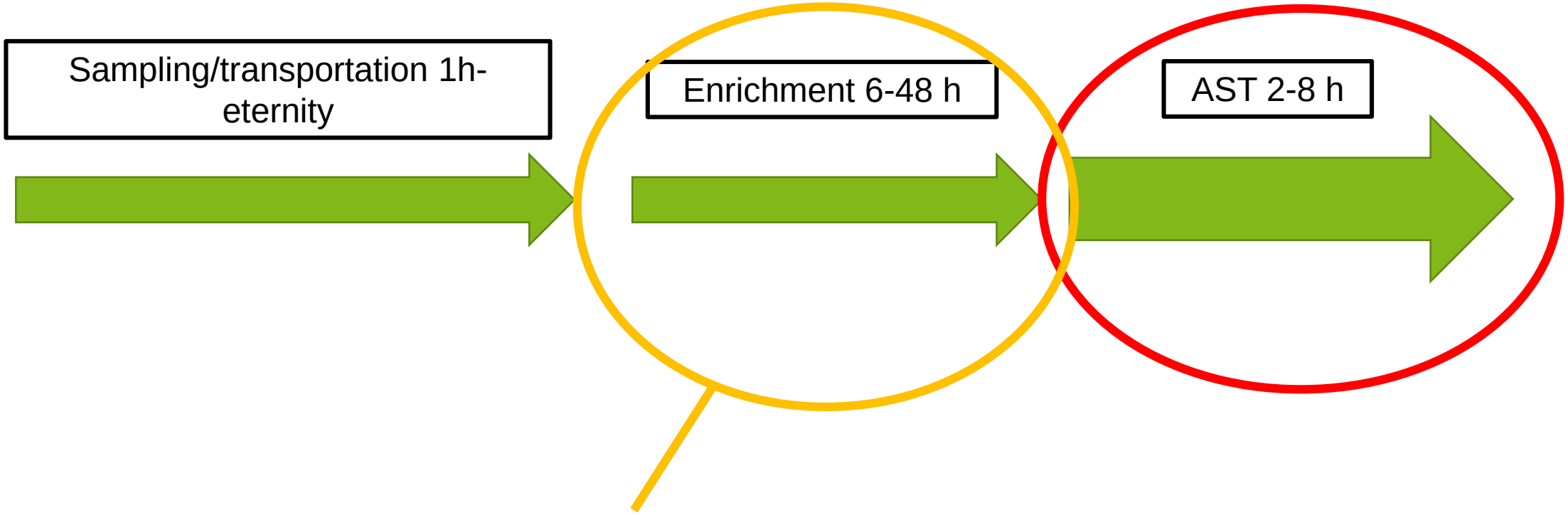

CAN WE SIGNIFICANTLY SHORTEN OR EVEN ELIMINATE THE NEED FOR ENRICHMENT?

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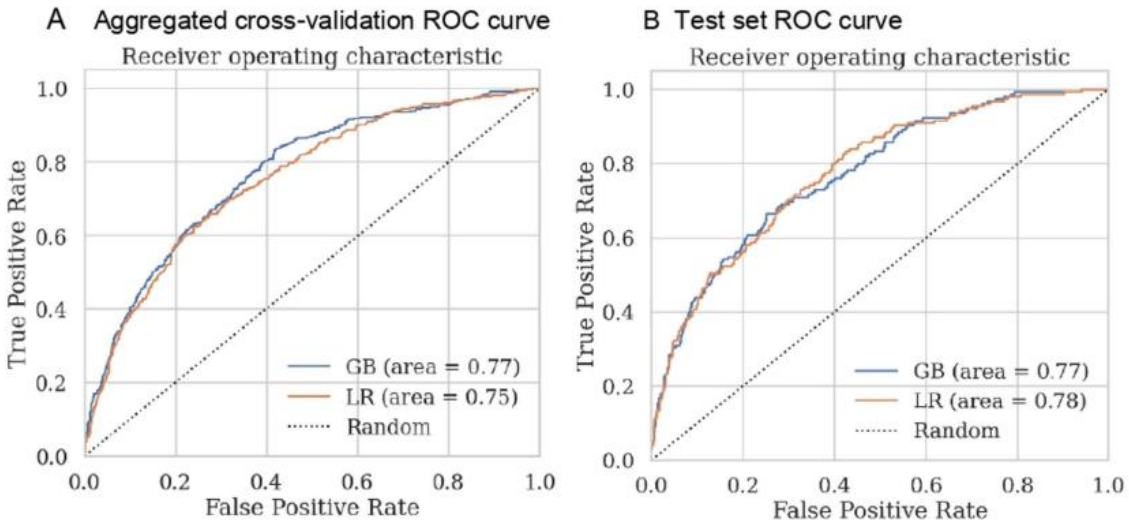
BMJ Open

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

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



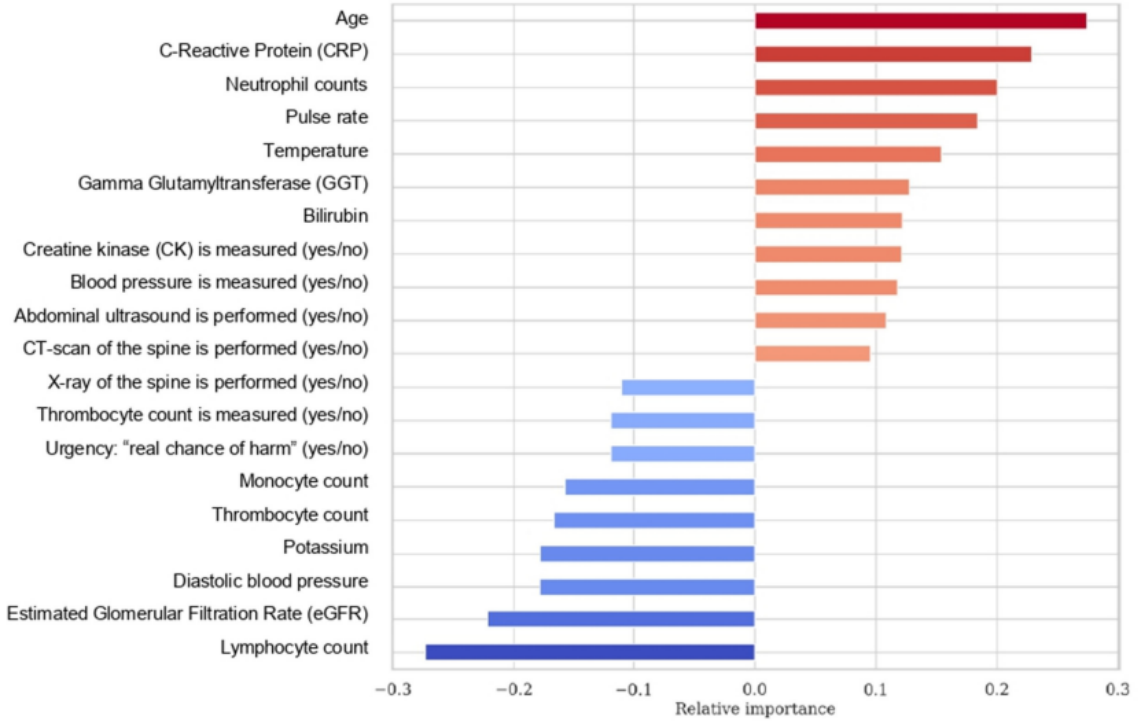


Figure 3 Feature importances of the logistic regression model. The 20 most important features in the logistic regression model are shown. The features for which a high value is predictive of a positive BC are shown in red and those predictive of a negative culture in blue. The X-axis presents the relative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

Boerman AW, *et al. BMJ Open* 2022;**12**:e053332. doi:10.1136/bmjopen-2021-053332

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

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

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

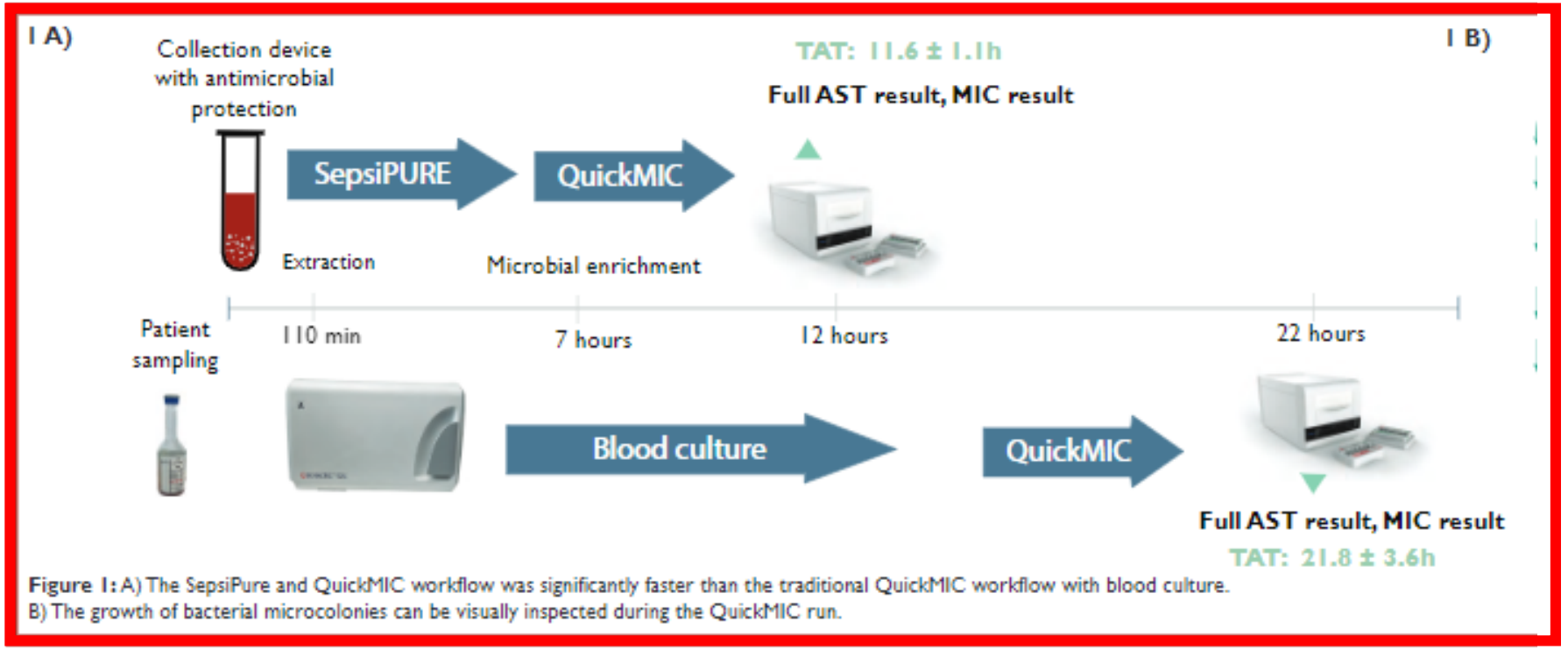
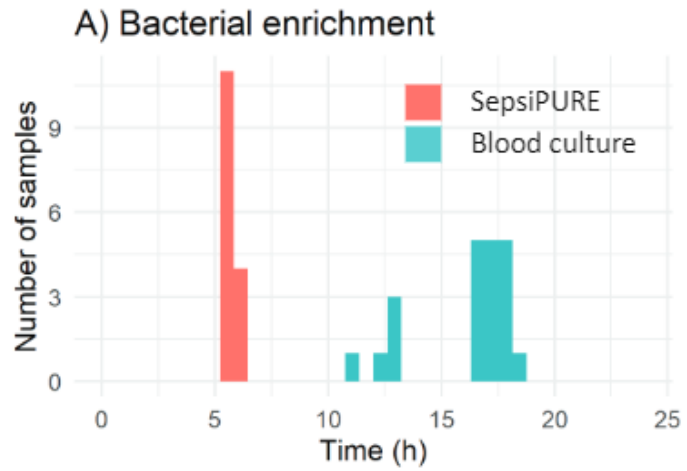
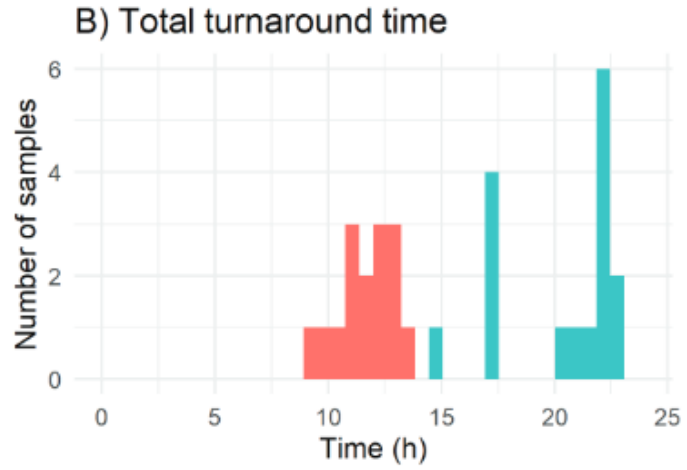


Figure 1: A) The SepsiPure and QuickMIC workflow was significantly faster than the traditional QuickMIC workflow with blood culture. B) The growth of bacterial microcolonies can be visually inspected during the QuickMIC run.





Pärssinen et al. A rapid workflow for bacterial isolation and phenotypic AST directly from blood. ECCMID 2023, Copenhagen. Poster P0394.
Momentum Bioscience https://momentumbio.co.uk

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Toward Rapid Detection of Viable Bacteria in Whole Blood for Early Sepsis Diagnostics and Susceptibility Testing

Sharath Narayana Iyengar,[⊥] Jiri Dietvorst,[⊥] Amparo Ferrer-Vilanova, Gonzalo Guirado, Xavier Muñoz-Berbel,* and Aman Russom*

Cite This: *ACS Sens.* 2021, 6, 3357−3366

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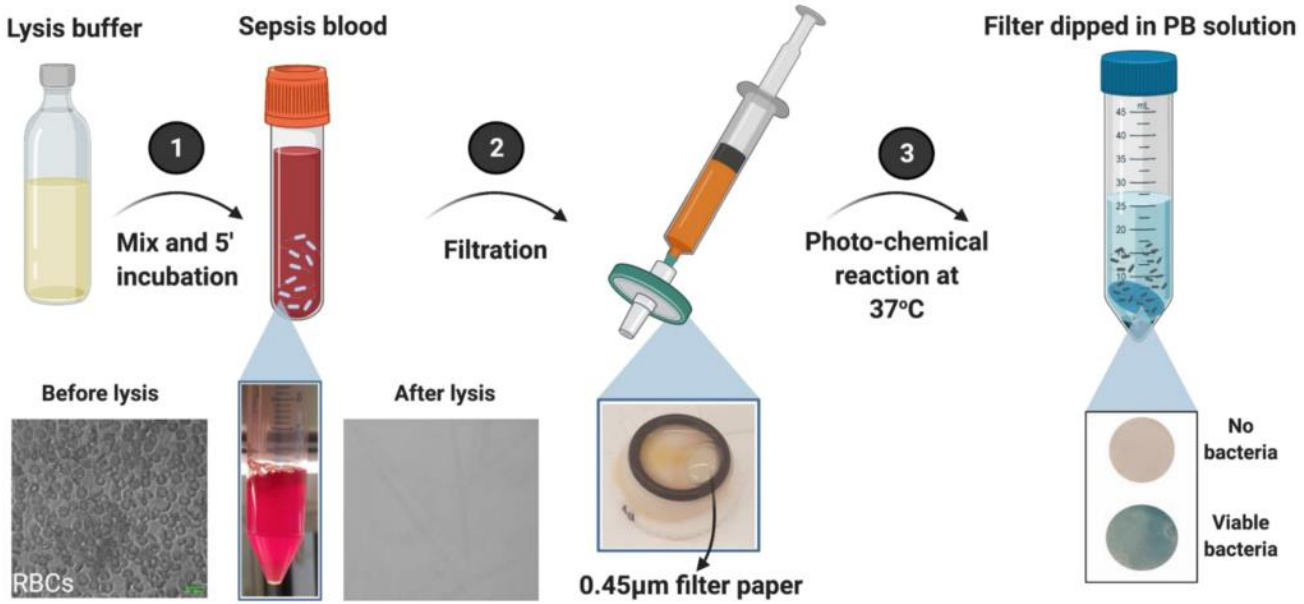

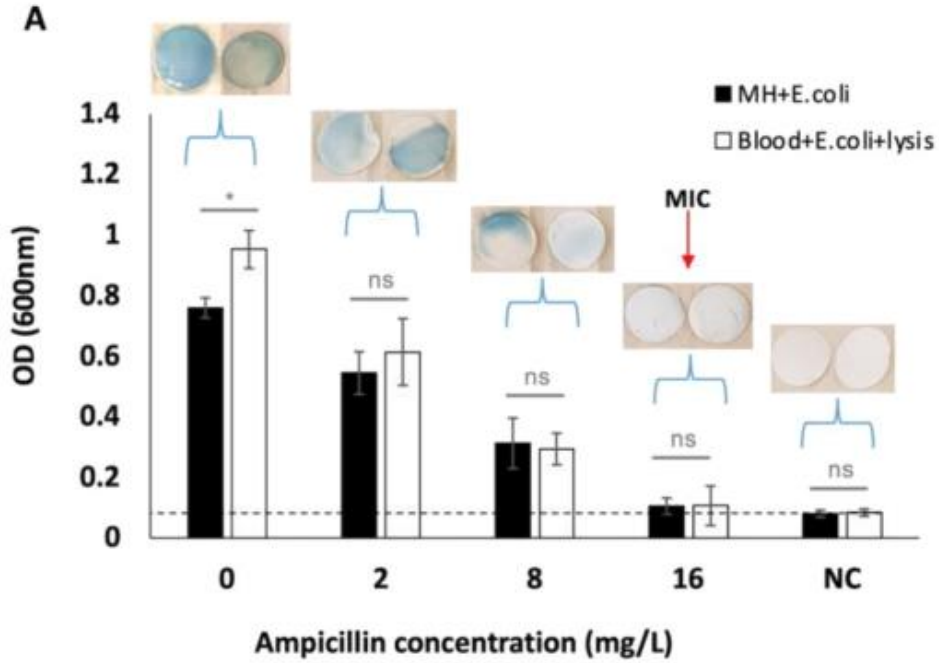

ACS Sens. 2021 Sep 24;6(9):3357-3366. doi: 10.1021/acssensors.1c01219.

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ADDED VALUE APART FROM SPEED?

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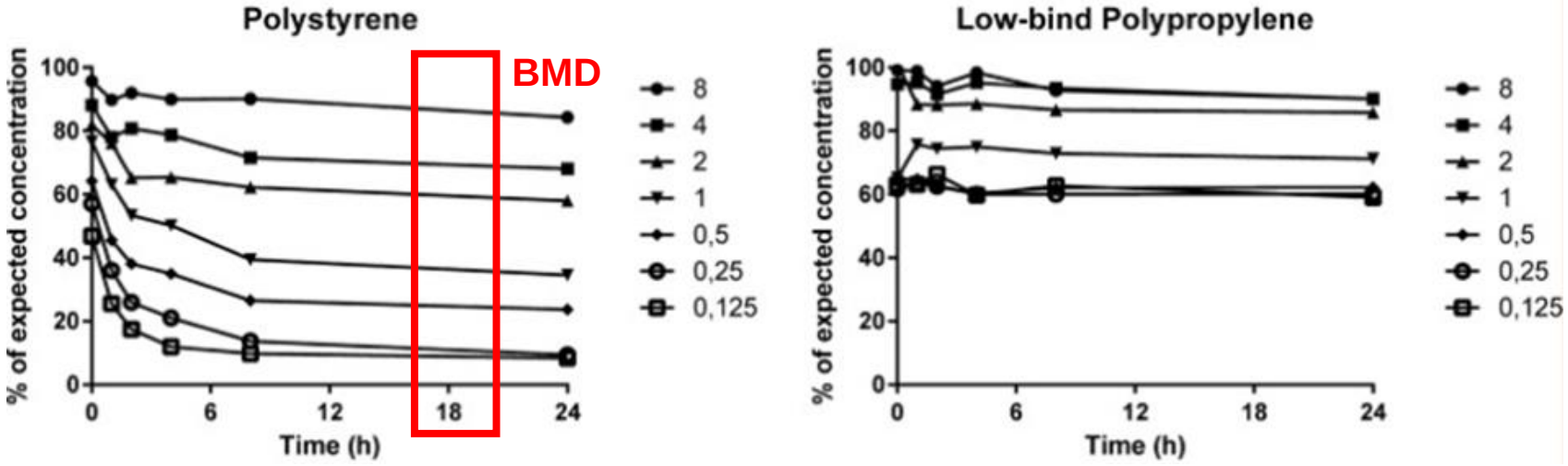
K. PNEUMONIAE AND COLISTIN

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

Matti Karvanen,[a] Christer Malmberg,[a] Pernilla Lagerbäck,[a] Lena E. Friberg,[b] Otto Cars[a]



Karvanen et al. Antimicrob Agents Chemother. 2017 Oct 24;61(11)

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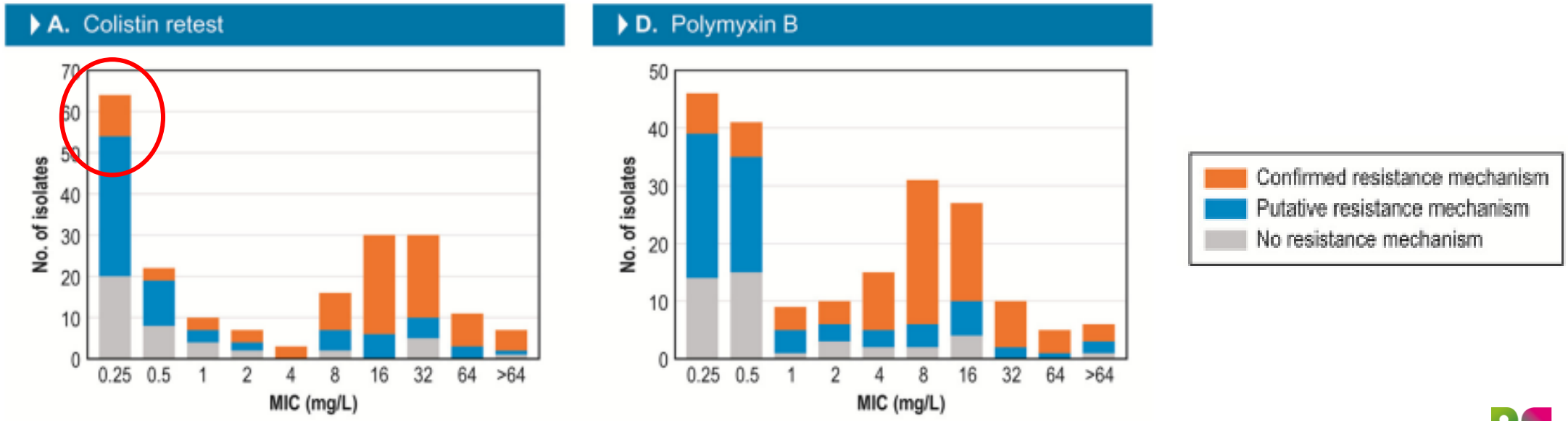
› Diagn Microbiol Infect Dis. 2020 Sep;98(1):115077. doi: 10.1016/j.diagmicrobio.2020.115077.
Epub 2020 May 7.

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 [1], Timothy B Doyle [2], Cecilia G Carvalhaes [2], Brieanna M Roth [2], Paul R Rhomberg [2], Rodrigo E Mendes [2]



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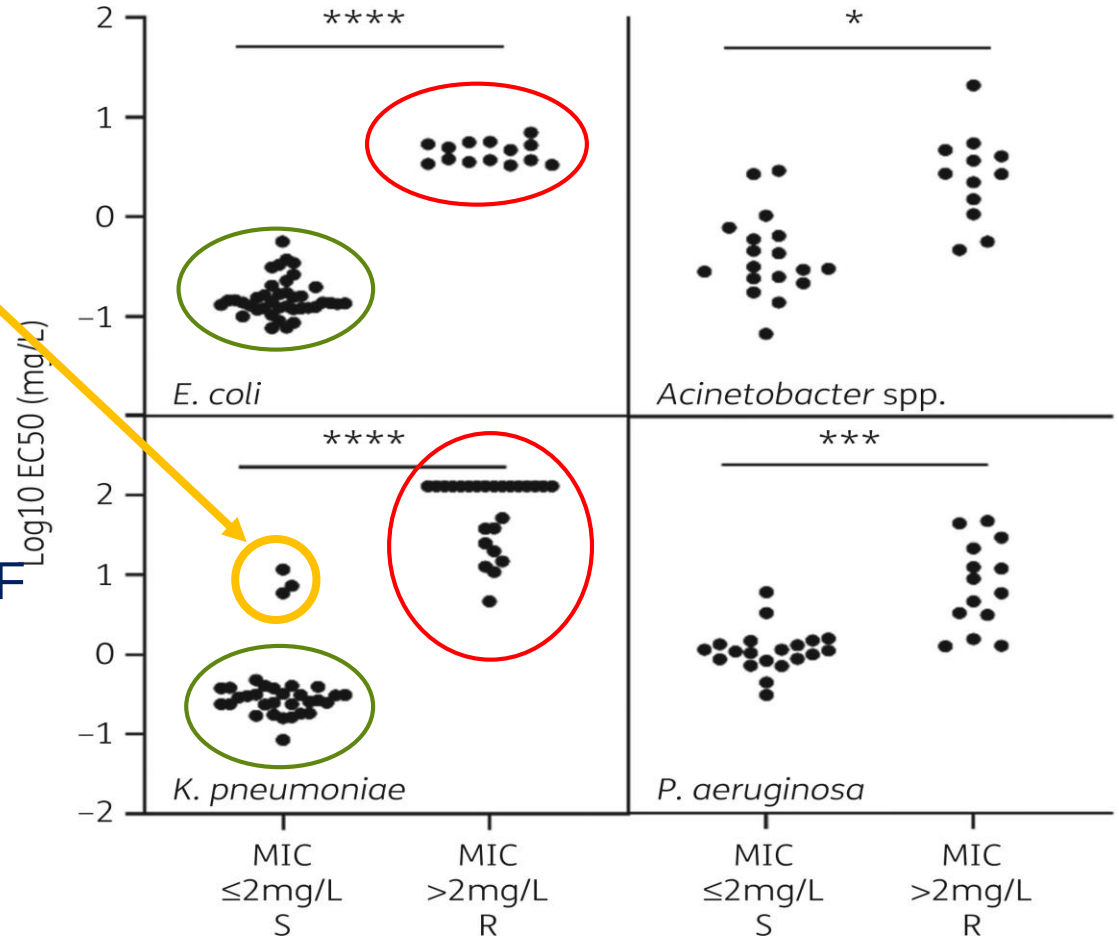
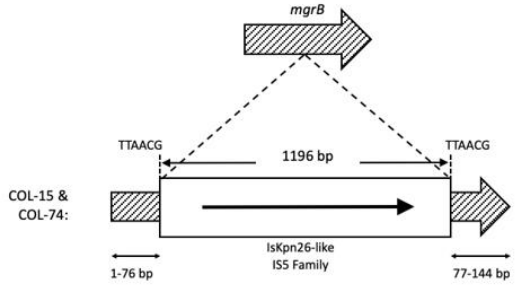
K. PNEUMONIAE AND COLISTIN

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
- All three exhibited altered lipid A using MALDI-TOF





Ekelund et al. J Antimicrob Chemother. 2021 Nov 12;76(12):3183-3191

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CONCLUSIONS

- Delaying adequate antibiotics increases mortality – strong evidence
- Insufficient evidence that the introduction of rapid AST (alone) affects patient outcomes
- Reasonable to believe that a combination of rapid species ID + rapid AST + antimicrobial stewardship program may improve patient outcomes
  - Local epidemiology
  - Local guidelines for empirical therapy
- Genotypic AST may be of value especially in certain epidemiological situations
- Many new techniques for rapid phenotypic AST are introduced – huge validation work to do!
- Eliminating the enrichement step would dramatically reduce time to results

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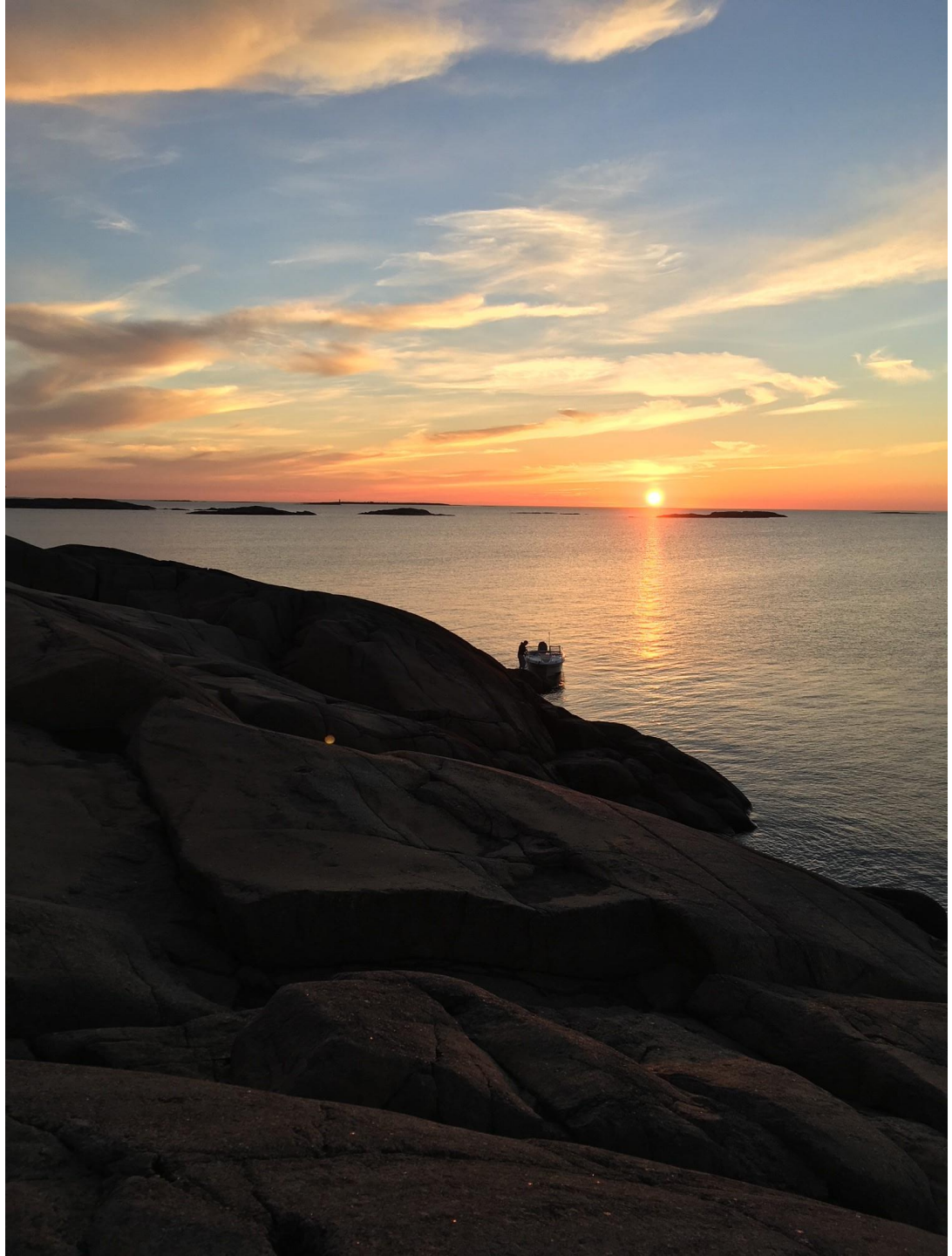
THANK YOU



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