Rapid susceptibility testing: new phenotypic and non-WGS genotypic approaches

BSAC Spring Conference 2018
Next generation Antimicrobial Susceptibility Testing of Bacteria

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

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Competing interest

Nothing to declare

High tech
Novel
Fascinating

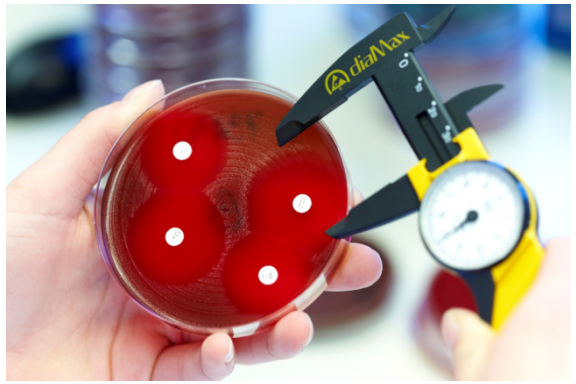
Low tech
Well validated
Not as exciting

How rapid is rapid?

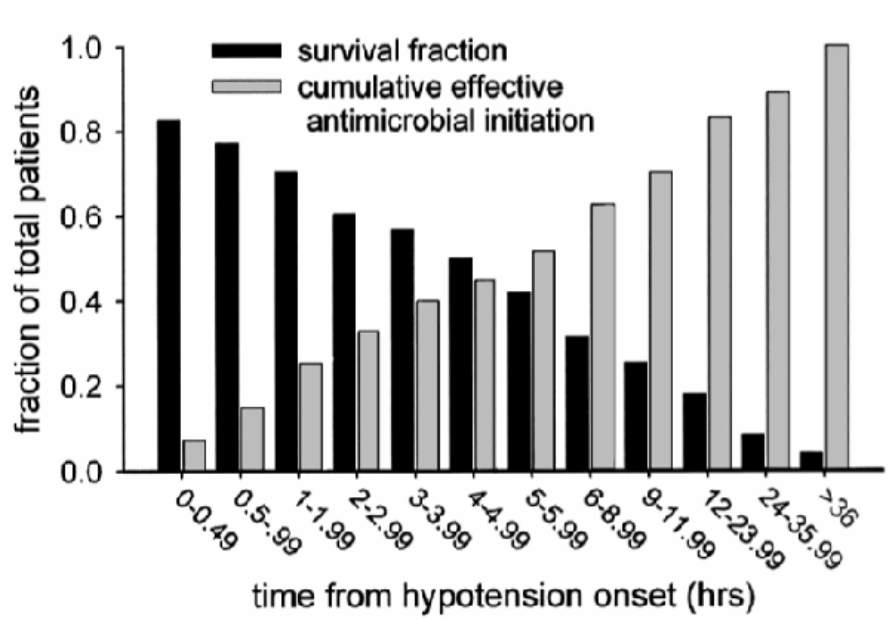
Empiric therapy?

Clinical chemistry

Clinical microbiology



Time is critical – Rapid species ID



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

Blood Culture Assays
PNA-FISH
QuickFISH
MALDI-TOF
Gene Xpert MRSA/SA BC
Verigene blood culture
FilmArray

Whole blood assays
Light Cycler SeptiFast
SeptiTest
T2 Candida

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Rapid genotypic AST

Very rapid ≈ 1 hour

Detection of a limited number of known mechanisms of resistance (*mecA*, *vanA/B*, *KPC*, *NDM*…)

Can at best predict resistance

Can never guarantee susceptibility

Global AMR - endemic	**Local AMR - epidemic**
Of limited use	Useful

New phenotypic approaches

Common features:

Short or very short exposure to the antibiotic in question

High-sensitive detection of growth or changes in phenotype

- Calorimetry
- Mean microbe mass
- Two-photon excitation fluorescence
- Flow cytometry
- Time-lapse microscopy

< 8 h

Accelerate PhenoTest™ BC

Sample: positive blood culture bottles
Technique: time-lapse microscopy

AST results 7 h + blood culture incubation time

Pros:
FDA approved
Several recently published peer-reviewed papers
Good cathegorical agreement with BMD

Cons:
Single sample
Cost
Performance in difficult isolates?
Colistin

Pantel et al. JAC 2018 (Epub ahead of print)
Charnot-Katsikas et al. JCM Jan 2018
Pancholi et al. JCM 2018 Jan 5.

QuantaMatrix dRAST™

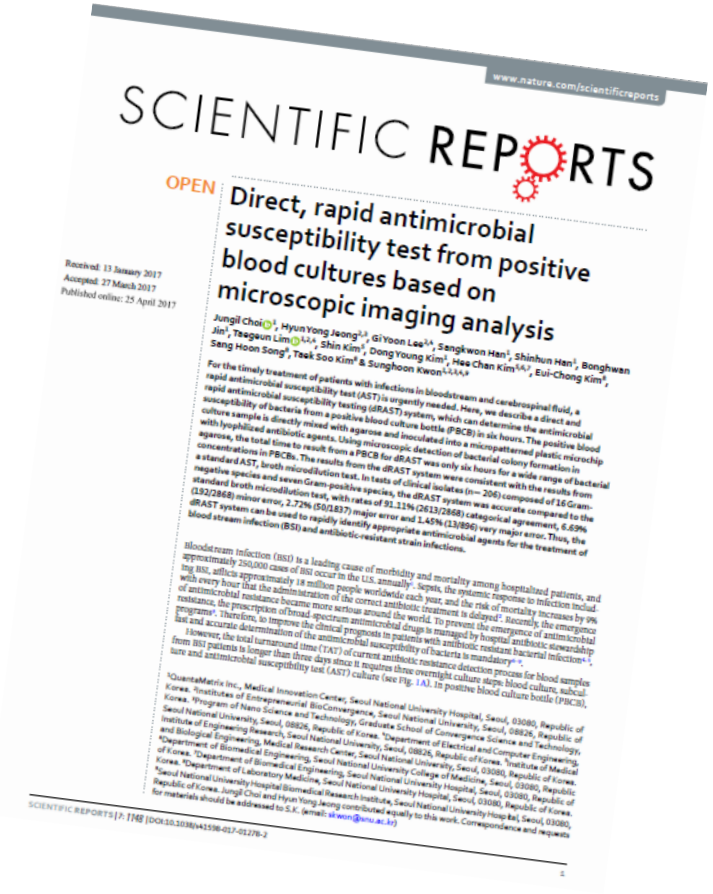
Sample: positive blood culture bottles
Technique: time-lapse microscopy

AST results 6 h + blood culture incubation time

Pros:
22 different antibiotics
Some peer-reviewed papers
Good cathegorical agreement with BMD

Cons:
Single sample
Cost?

Performance in difficult isolates?



Choi et al. Sci Rep April 2017

Rapid AST directly from positive blood culture bottles using disk diffusion

A EUCAST standard method. Implementation 2018

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EUCAST RAST blood culture field trial

44 laboratories signed up for the study

40 participated and delivered results and all strains

Denmark (3), Finland (3), Iceland (1), Ireland (1),
Norway (11) and Sweden (21)

Blood culture systems:

BD BACTEC n=17
bioMerieux BacT/ALERT n=23

Disk manufacturers: 4

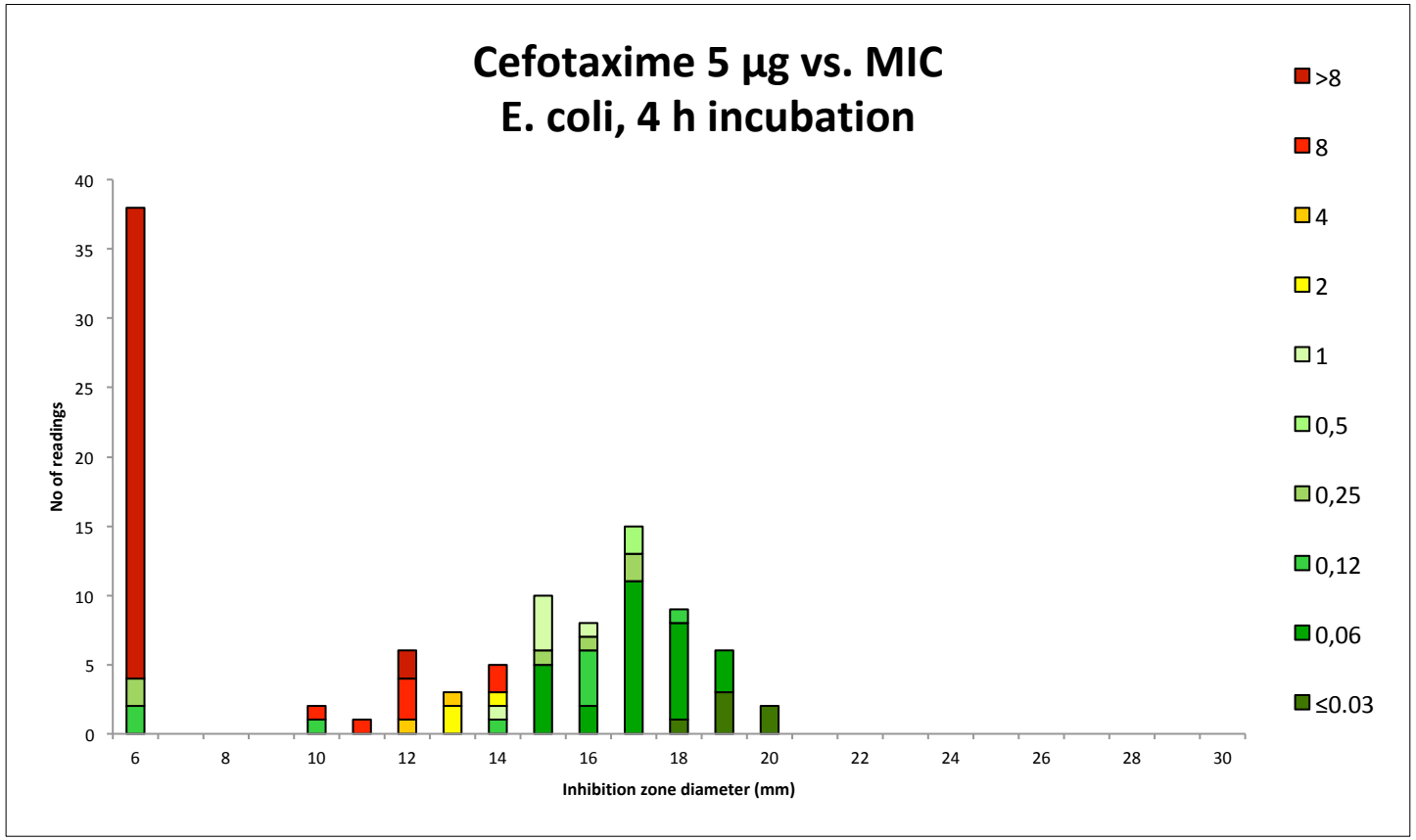
MH manufacturers: 6

Reference: BMD + standard EUCAST disk diffusion (16-20h)

Species	Number
E. coli	436
K. pneumoniae	64
Ps. aeruginosa	37
Other gram negatives	52
S. aureus	270
Coagulasnegative staph.	357
S. pneumoniae	35
Total number	**1251**

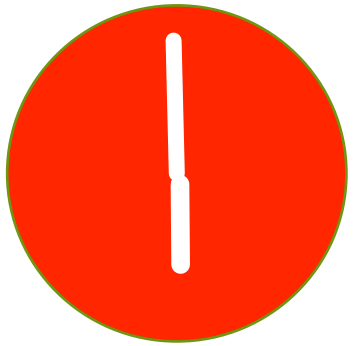
Rapid Disk Diffusion – RAST EUCAST

4 h

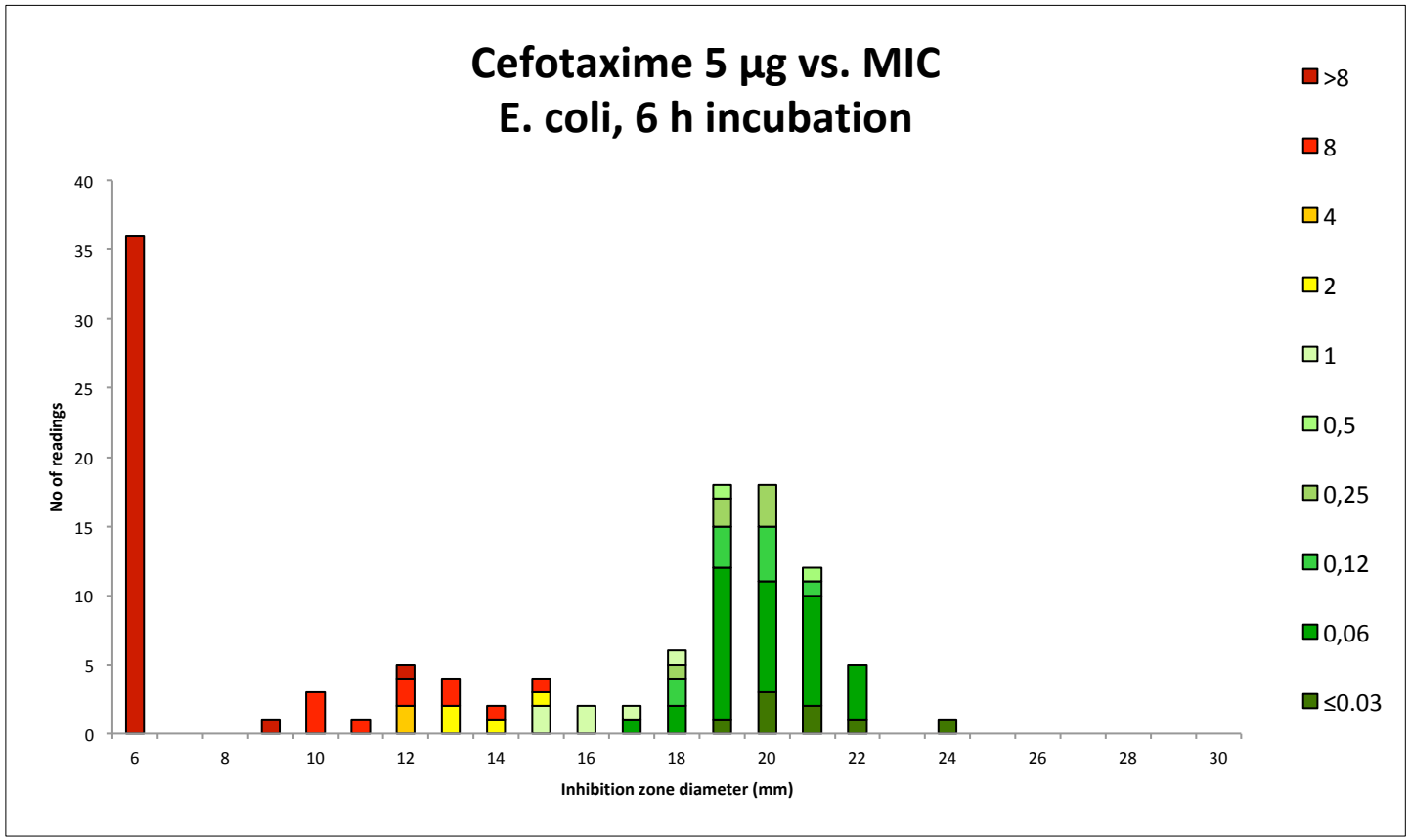


Unpublished data. Jonasson, Matuschek, Kahlmeter.

Rapid Disk Diffusion – RAST EUCAST



6 h



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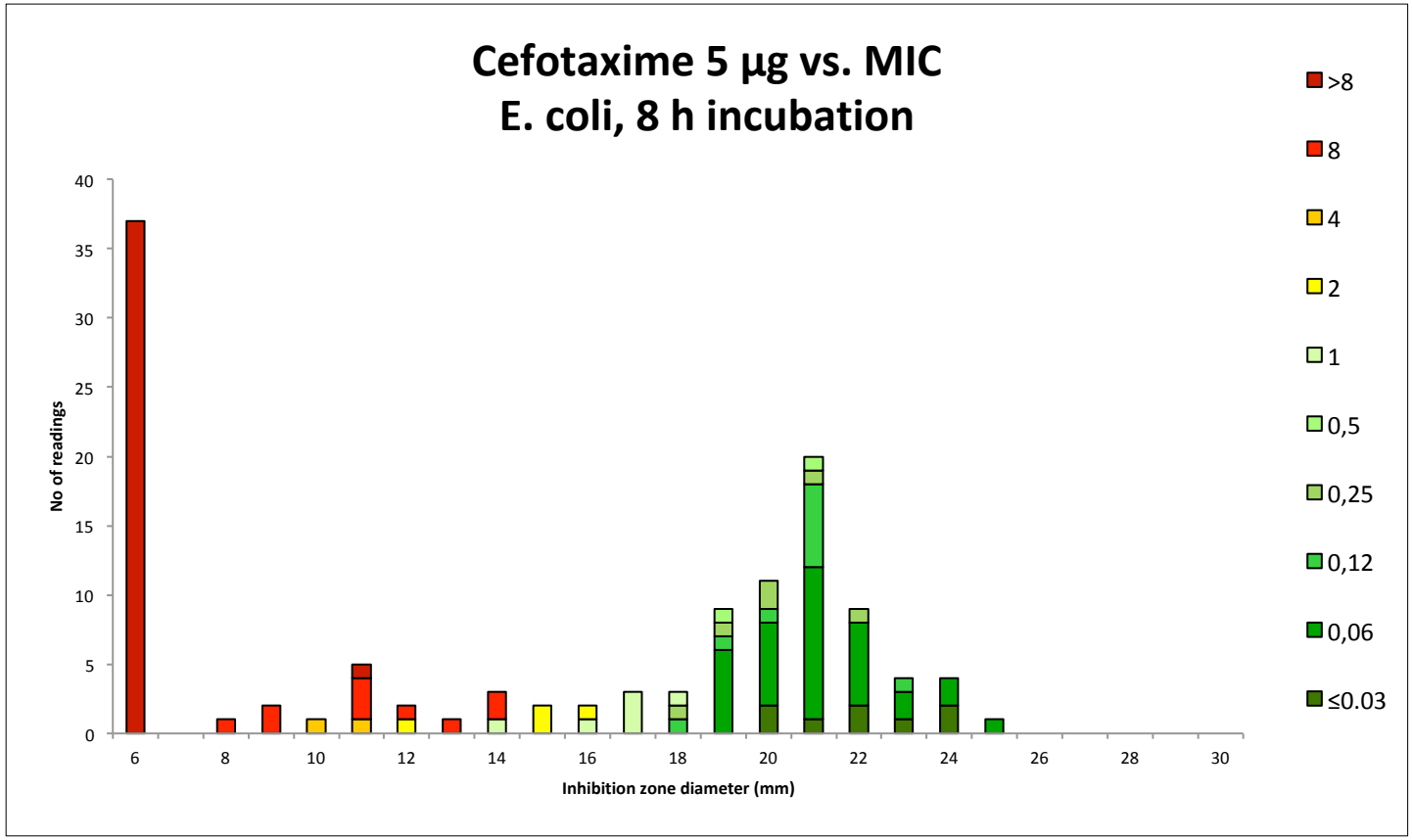
Rapid Disk Diffusion – RAST EUCAST

8 h



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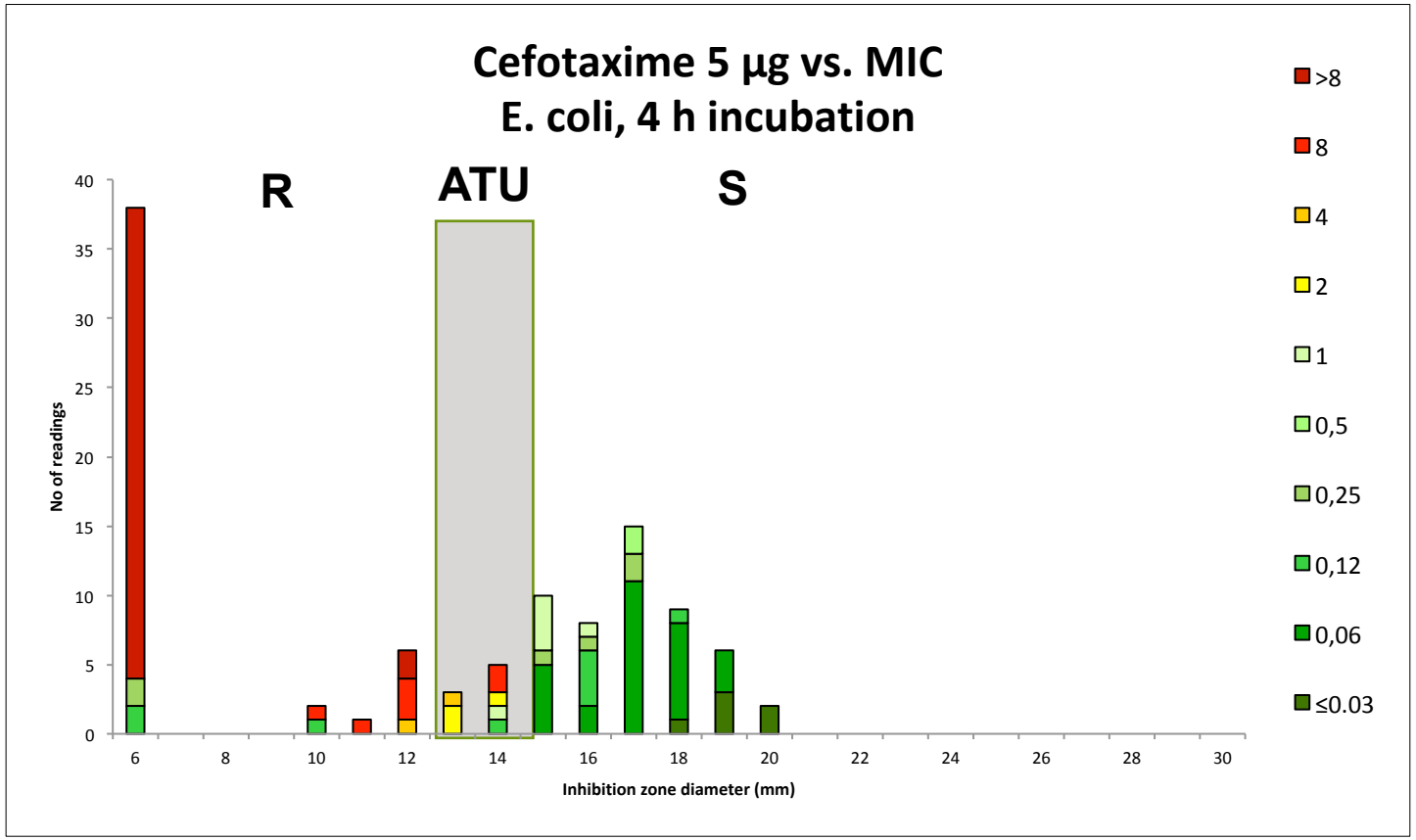
4 h



Only **S** or **R**, no intermediate results
ATU - Area of technical uncertainty

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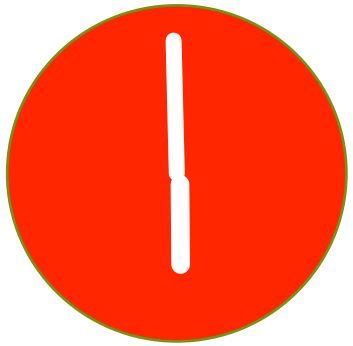
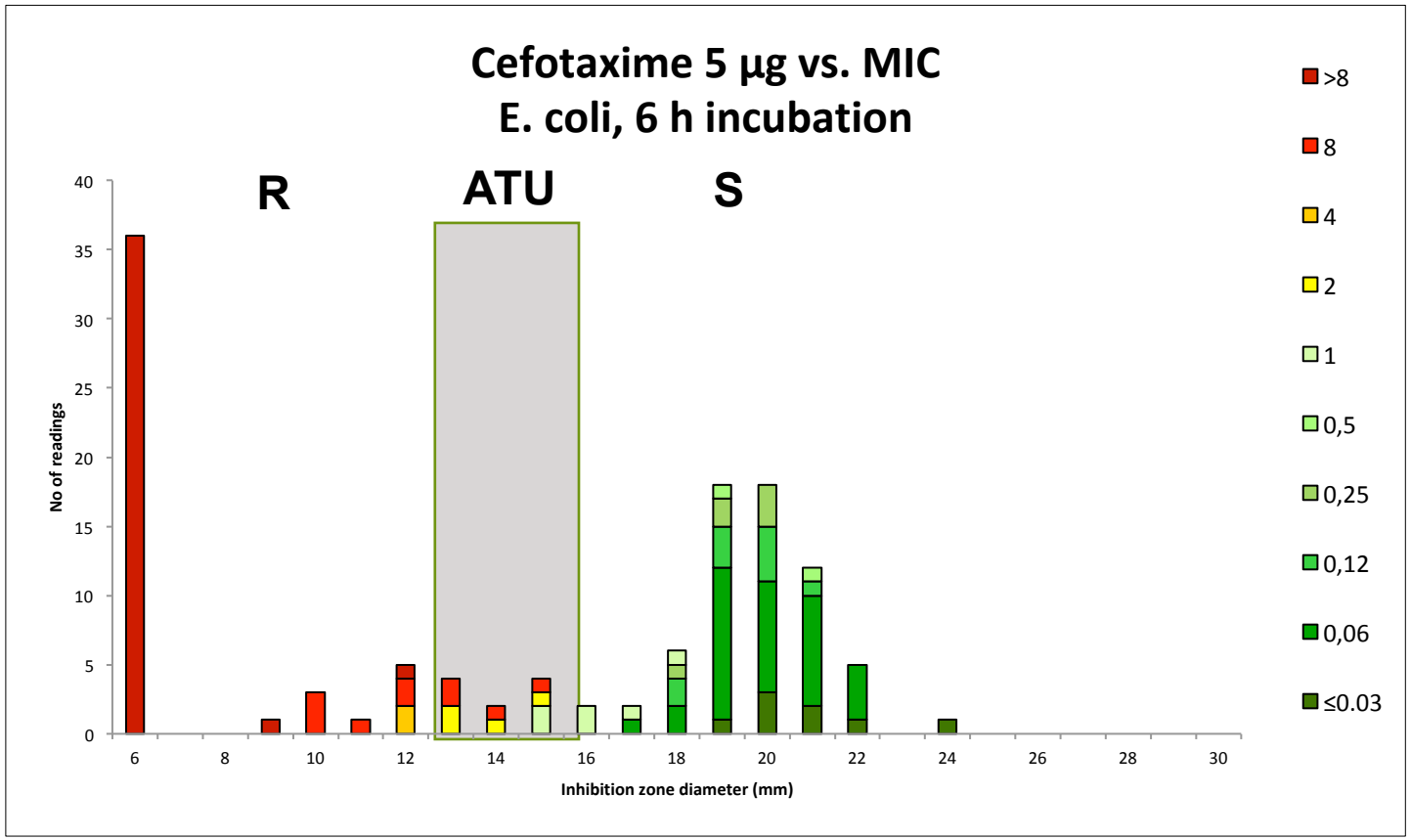
Unpublished data. Jonasson, Matuschek, Kahlmeter.

Rapid Disk Diffusion – RAST EUCAST



6 h



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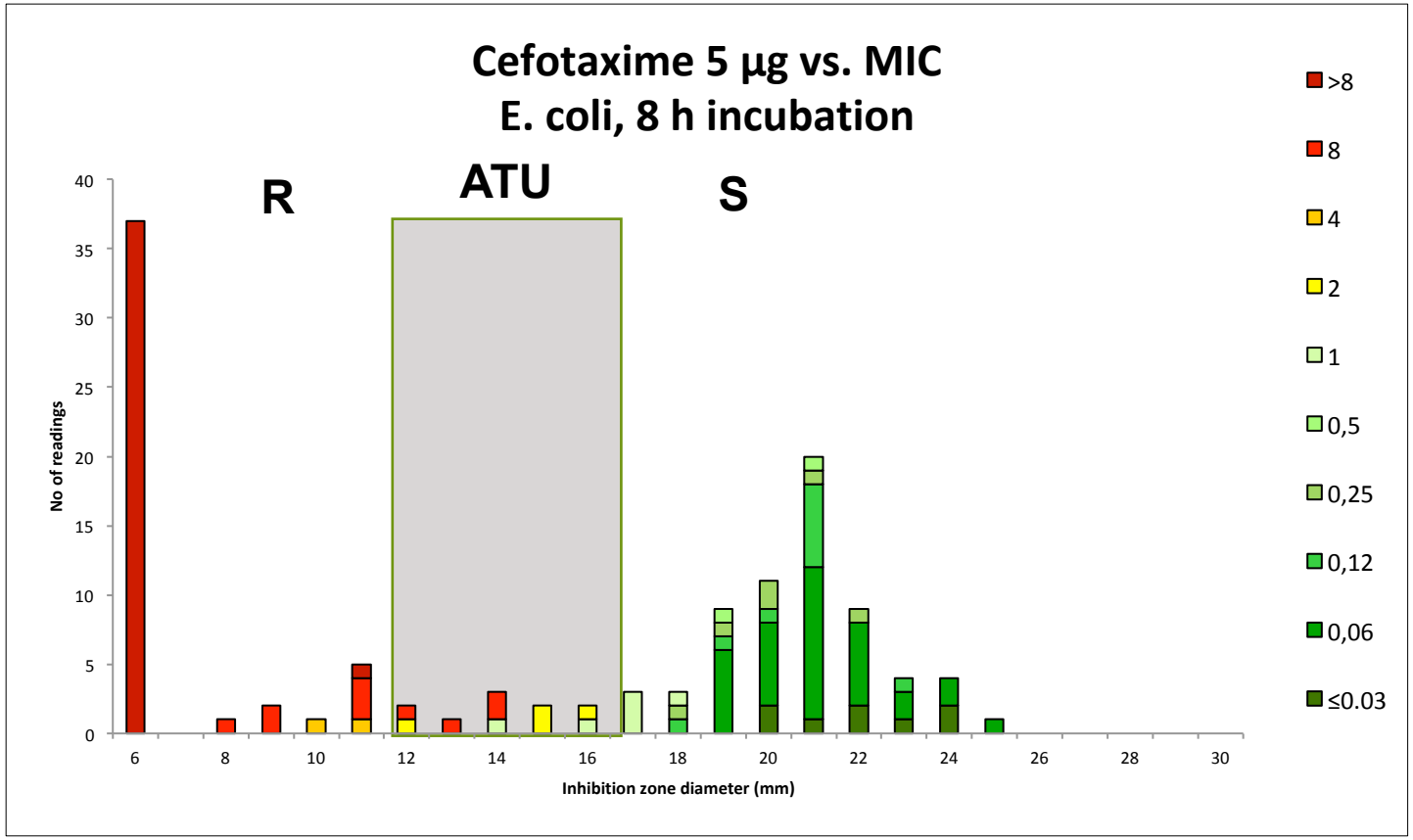

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RAST vs. standard disk diffusion - *E. coli*

	E. coli **(n=386)** Cefotaxime, ceftazidime, piperacillin-tazobactam, meropenem, ciprofloxacin,			***E. coli*** **(n=386)** Piperacillin-tazobactam excluded		
Incubation time	**4h**	**6h**	**8h**	**4h**	**6h**	**8h**
Number of possible tests[a]	3 088	3 088	3 088	2 702	2 702	2 702
Number of performed tests[b]	3 034	3 027	2 768	2 651	2 645	2 419
Number of zones registered[c]	2 756	2 993	2 752	2 415	2 613	2 404
	Categorical agreement (%)					
Correct	77	81	84	88	93	95
mE	0.3	0.1	0.1	0.2	0.2	0.1
ME	1.6	0.4	0.2	1.8	0.5	0.3
VME	0.1	0.1	0.1	0.1	0.1	0.1
ATU	20	18	16	10	6.2	4.0

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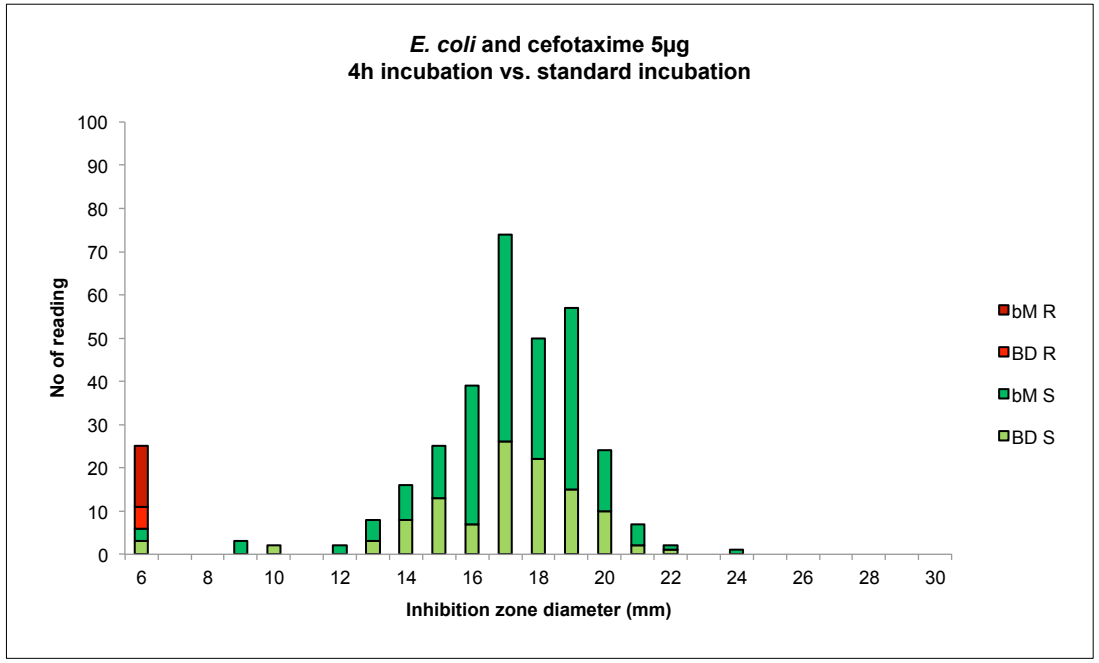
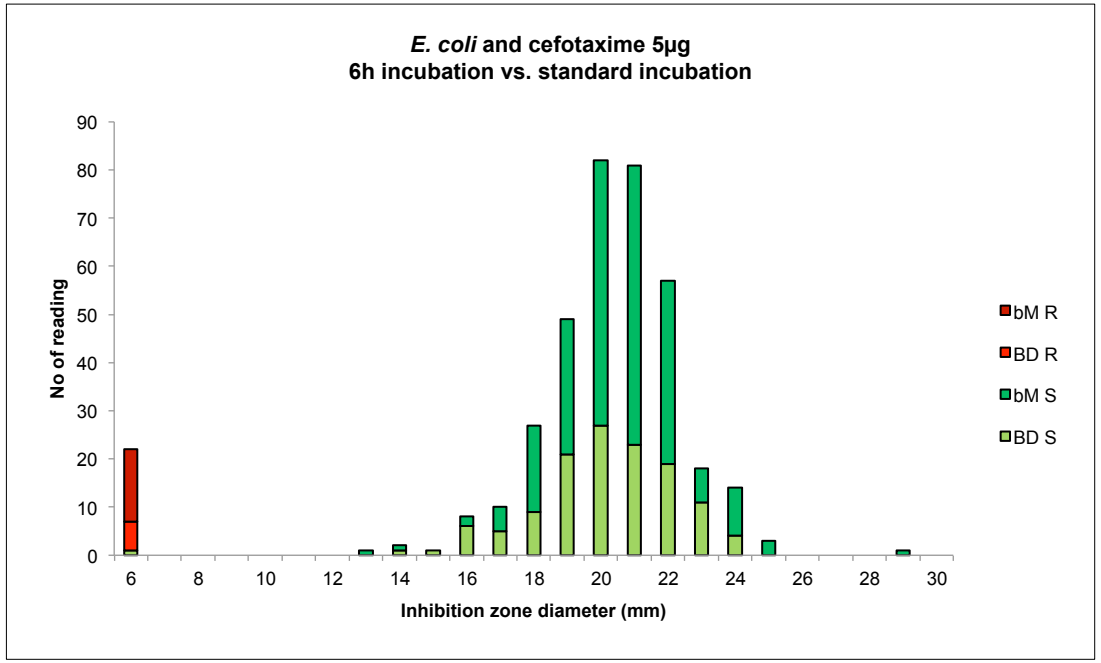
Unpublished data. Jonasson, Matuschek, Kahlmeter.

Two different blood culture systems





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Unpublished data. Jonasson, Matuschek, Kahlmeter.

RAST vs. standard disk diffusion - S. aureus

	S. aureus (n=242) Cefoxitin, norfloxacin, erythromycin, gentamicin		
Incubation time	**4h**	**6h**	**8h**
Number of possible tests[a]	968	968	968
Number of performed tests[b]	952	956	892
Number of zones registered[c]	623	880	844
	Categorical agreement (%)		
Correct	66	92	95
mE	0.0	0.0	0.0
ME	8.5	0.3	0.4
VME	0.2	0.3	0.5
ATU	25	7.2	4.0

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Unpublished data. Jonasson, Matuschek, Kahlmeter.

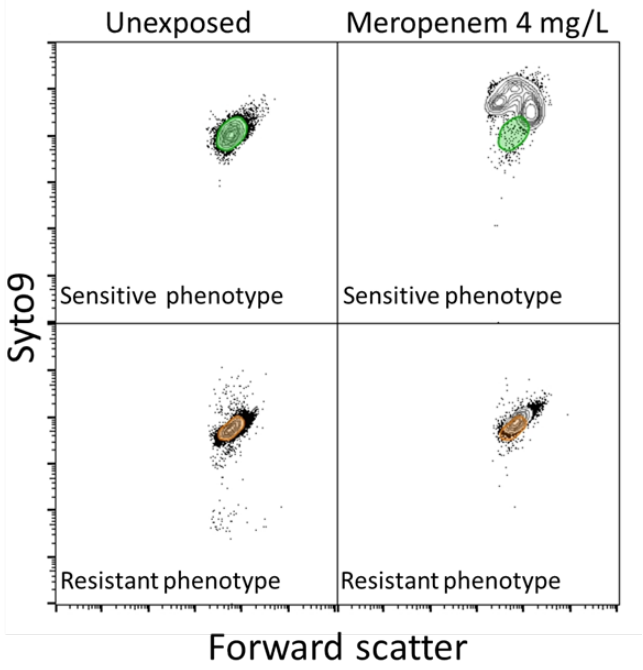
< 4 h

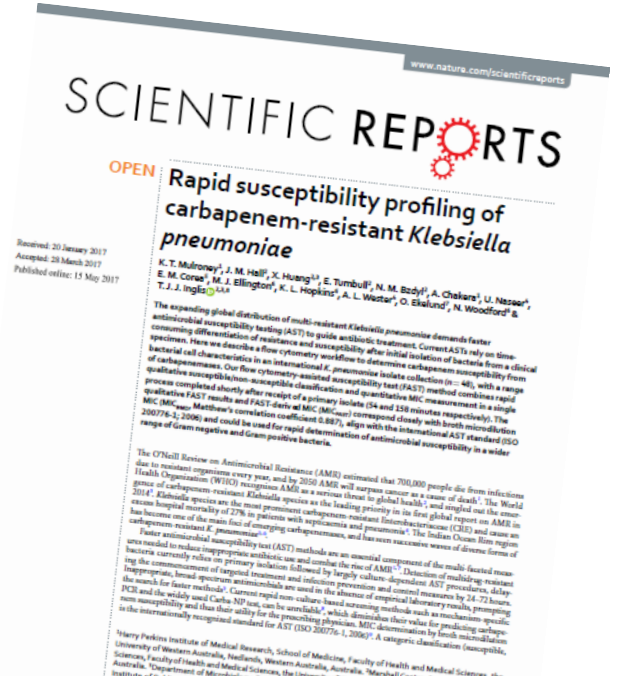
Flow cytometry AST - FAST

Meropenem, *K. pneumoniae*
Exposure time = 30 min

MIC determination
Based on morphological changes

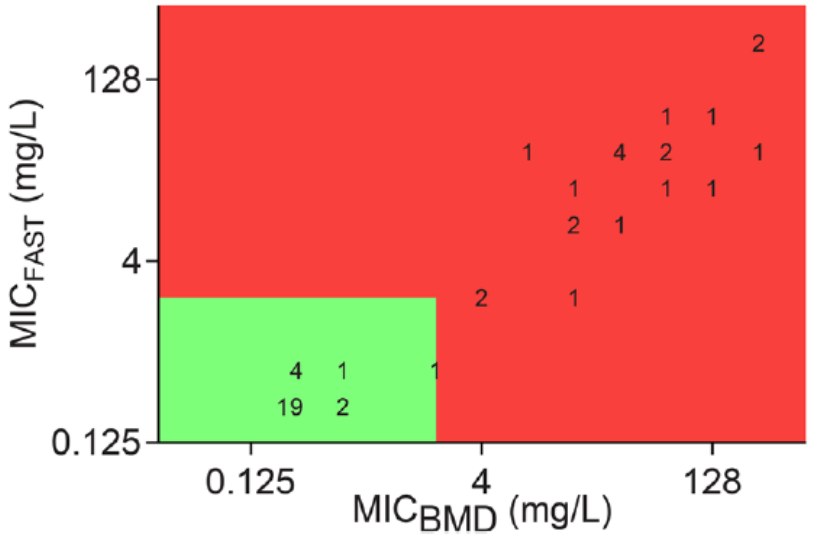
Total time to results
= 90-150 min




SCIENTIFIC REPORTS

OPEN

Rapid susceptibility profiling of carbapenem-resistant *Klebsiella pneumoniae*



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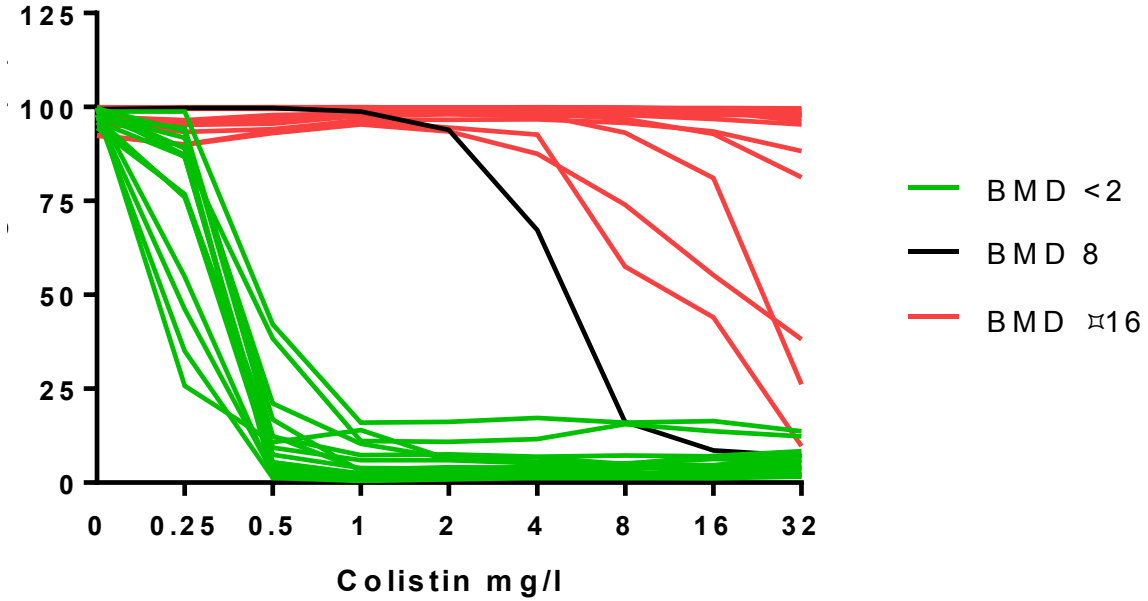
Mulroney et al. Sci Rep 2017.

Flow cytometry AST - FAST

Colistin, *K. pneumoniae*

Exposure time = 30 min



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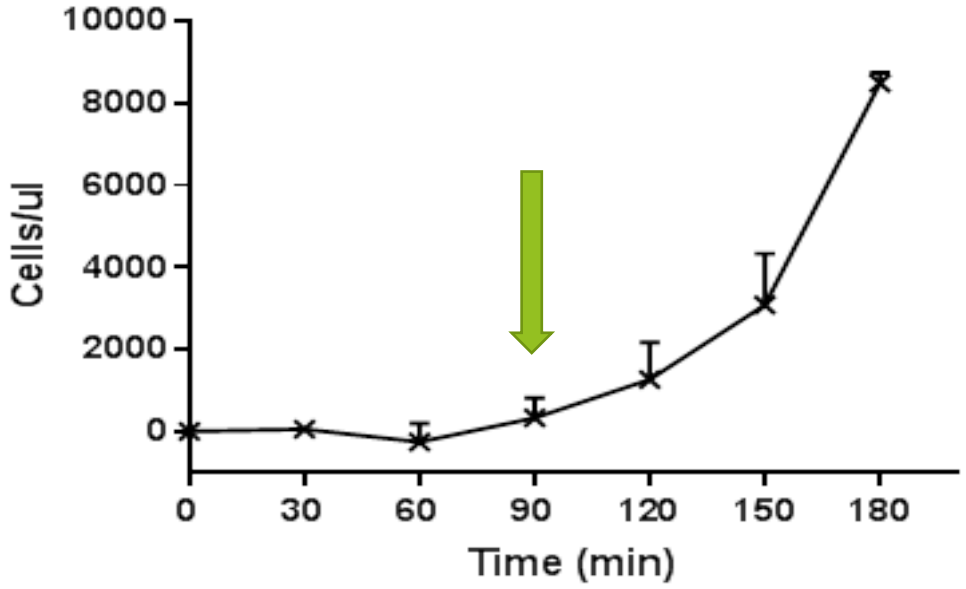
Unpublished data. Ekelund, Somajo.

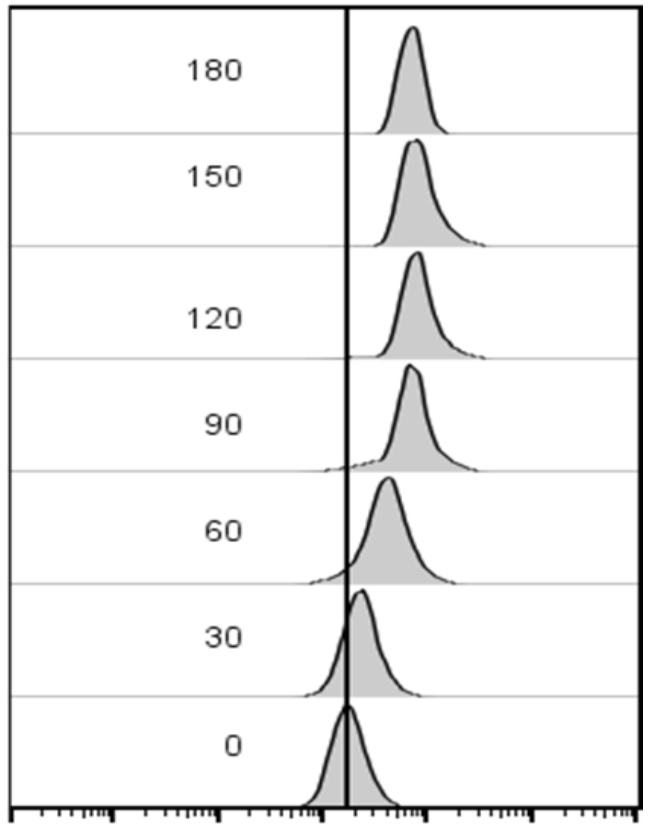
Implementation of new phenotypic methods in clinical diagnostics

- what challenges are we facing?

Challenge 1 - lag / log phase



Cell count



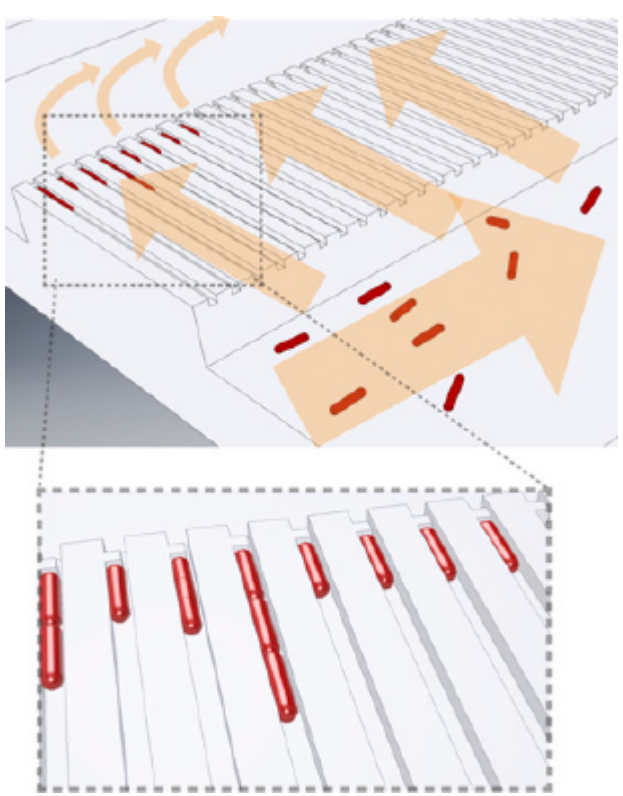
Cell size

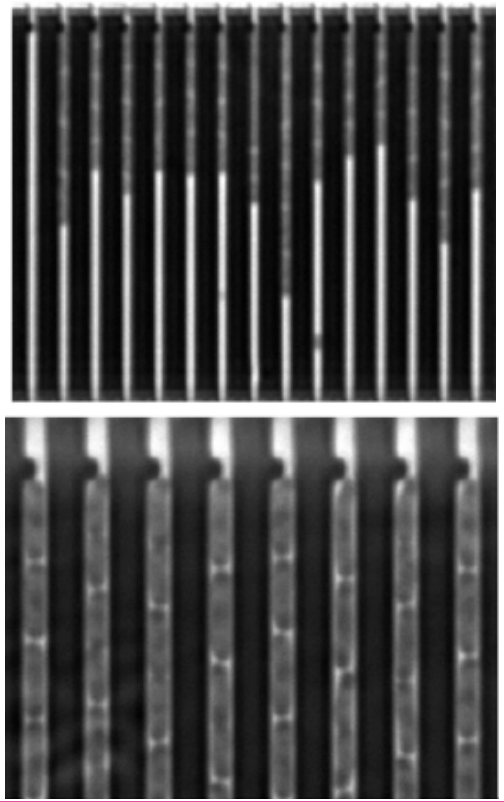
Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging

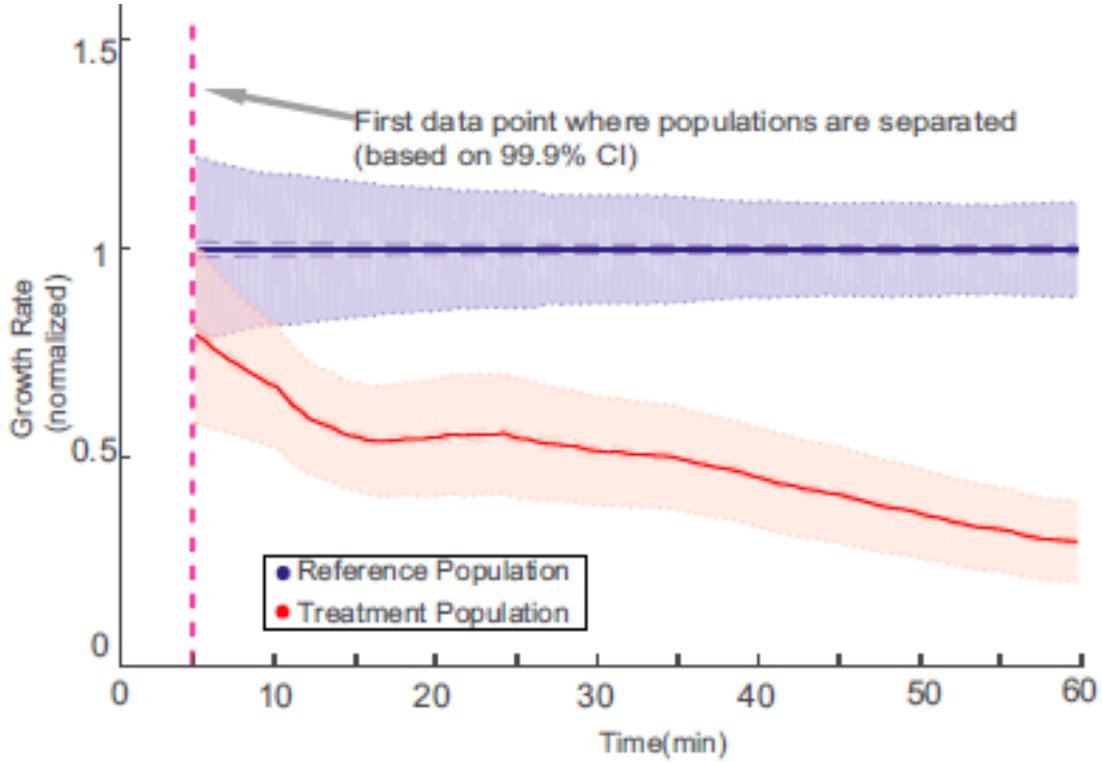
Özden Baltekin[a], Alexis Boucharin[a], Eva Tano[b], Dan I. Andersson[c], and Johan Elf[a,1]

[a]Department of Cell and Molecular Biology, Science for Life Laboratory, Uppsala University, SE-751 24 Uppsala, Sweden; [b]Department of Medical Sciences, Uppsala University, SE-751 85 Uppsala, Sweden; and [c]Department of Medical Biochemistry and Microbiology, Uppsala University, SE-751 23 Uppsala, Sweden

Edited by Nancy E. Kleckner, Harvard University, Cambridge, MA, and approved July 7, 2017 (received for review May 23, 2017)







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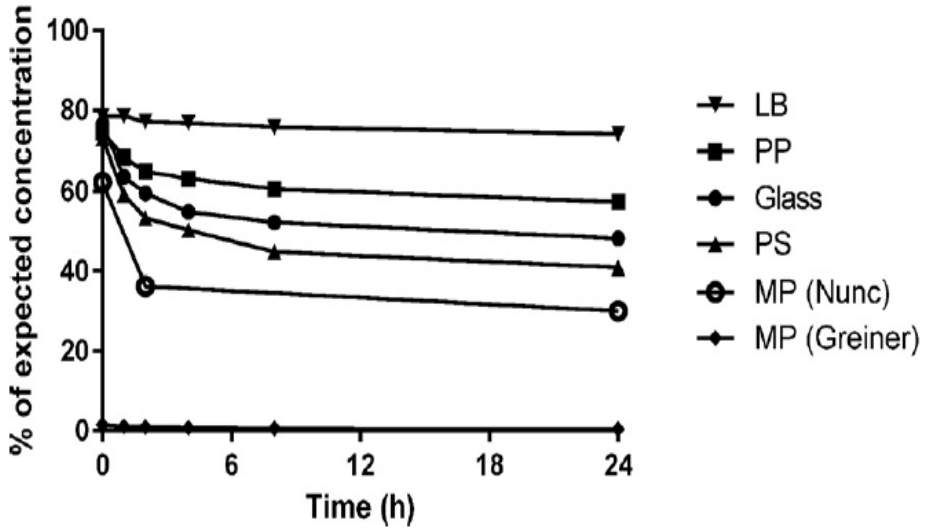
Baltekin et al. PNAS Aug 22, 2017.

Challenge 2 – difficult drug / bug combinations

Colistin



Combinations

Piperacillin / tazobactam
Ampicilin / sulbactam
Co-amoxiclav

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Karvanen et al. AAC Sept 2017.

Challenge 3 – polymicrobial samples

Sterile fluids

Blood, CSF

Non-sterile samples

Urine, respiratory samples

Challenge 4 – the need for large scale validation

Multi-center approach needed

Categorical agreement not enough

Borderline isolates must be included

Huge number of drug-bug combinations!

Thank you for your attention!

Acknowledgements

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