

# NordicAST/EUCAST RAST Multi-laboratory study

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## A standardized RAST method directly from positive blood culture bottles

- Proposed by and based on EUCAST standard disk diffusion methodology (MH/MH-F plates, atmosphere, disk content)

BUT with...

- Modified inoculum (100-150  $\mu$ L positive blood culture)
- Shortened incubation time (4, 6 and 8h)
  
- Evaluated and validated in a multi-lab study, 2017

And this is how it went....

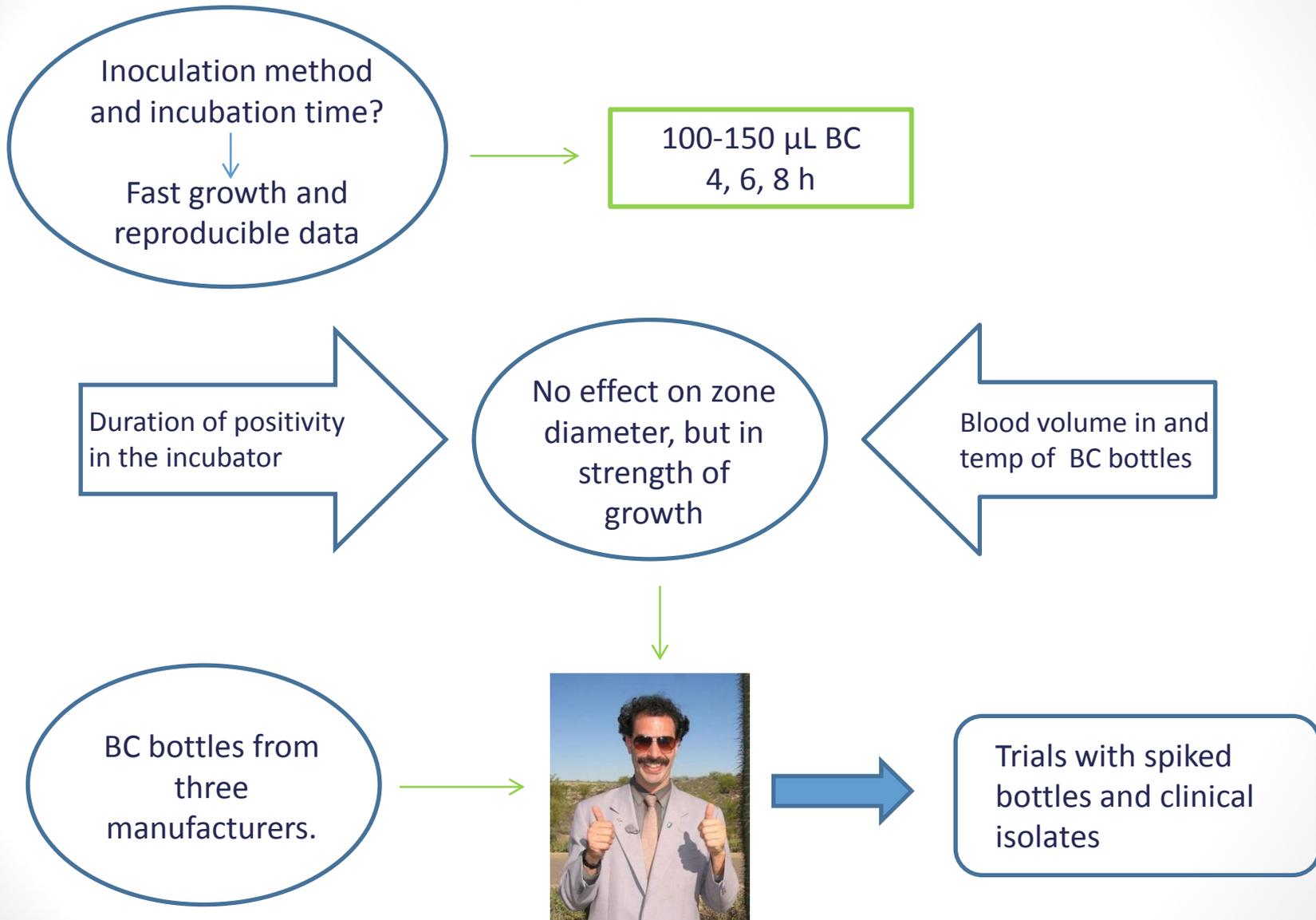


# First - repetition of abbreviations...

- Categorical agreement = S/R in new method congruent with S/R in reference method
- Definition of error:
  - VME = Very Major Error
    - ✓ False susceptibility (RAST = S, Standard = R)
  - ME = Major Error
    - ✓ False resistance (RAST = R, Standard = S)
  - mE = minor Error
    - ✓ Categorized as susceptible (S) or resistant (R) with RAST when intermediate (I) with standard method.
- Wild type (wt)
  - ✓ No acquired or mutational resistance mechanisms to the agent.
- Non-wild type (non-wt)
  - ✓ Harbor acquired or mutational resistance mechanism/s to the agent.



# Short review of how we got there...



# What about the breakpoints?

Shortened incubation time



Smaller zones  
lesser separation wt/non-wt  
→ New breakpoint needed

We can not expect better result than with standard method

## New RAST breakpoints

Specific for the species/antibiotic agent combination  
and incubation time

Set to avoid VME  
and to ensure susceptibility and resistance

Introduction of a category of uncertainty (ATU) prevents errors  
If ATU → read again or reanalyse with standard incubation

# The multi-laboratory study

- 40 participating laboratories  
(Denmark n=3, Finland n=3, Iceland n=1, Ireland n=1, Norway n=11, Sweden n=21)
- Blood culture systems:
  - BD; BACTEC n=17
  - bioMerieux; BacT/ALERT n=23
- 4 Muller-Hinton and 4 Disc manufactures
- Clinical isolates tested and compiled in this first evaluation:
  - E. coli*: 436
  - S. aureus*: 270



# The antimicrobials

most important sepsis agents in Europe

<i>E.coli</i>	<i>S. aureus</i>
Piperacillin-tazobactam	Cefoxitin
Cefotaxime	Norfloxacin
Ceftazidime	Gentamicin
Meropenem	Erytromycin
Ciprofloxacin	Clindamycin
Amikacin	
Gentamicin	
Tobramycin	

# Reading zones

- After 4 and 6h of incubation ( $\pm 5$  minutes)  
Reincubated within 10 minutes
- After 8h of incubation (if possible)
- All plates read from the front
- Confluent growth and distinct zone edge  
Disregard thin growth within the zone

# Reference method

- Standard AST (disk diffusion)
  - all isolates
- Broth microdilution/Mec PCR
  - every third *S. aureus* / fourth *E.coli* per lab



No difference in categorical agreement using either **standard disk diffusion** or BMD as reference

# Results

- No systematic difference between the BC systems
- Variable duration of positivity in the incubator (0-24h)  
- no effect on the result

Readable zones after 4, 6 and 8h incubation

	4h	6h	8h
<i>S. aureus</i>	66%	93%	96%
<i>E. coli</i>	91%	99%	99%

- Number of isolates in the ATU decreased with incubation time (Pip-tazo)

# Accuracy of the laboratories

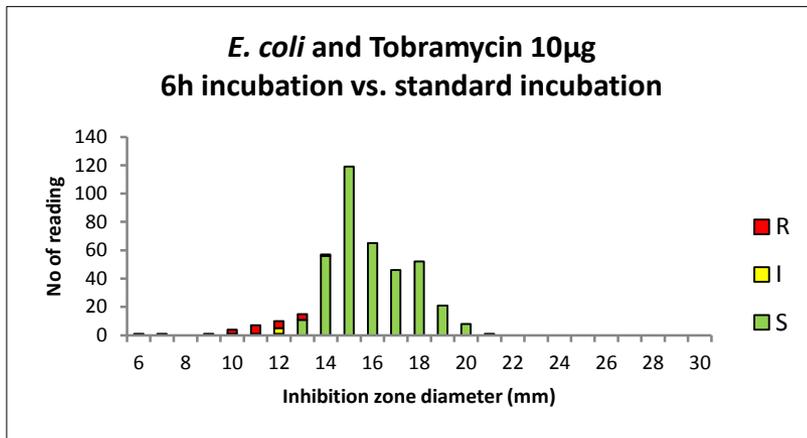
34/40 lab showed excellent reading with  $\leq 3\%$  errors

	No errors no (%)	$\leq 1\%$ errors no (%)	$\leq 2\%$ errors no (%)	$\leq 3\%$ errors no (%)	$\leq 5\%$ errors no (%)
<i>E. coli</i> (40 lab)	19 (48)	29 (73)	31 (78)	35 (88)	38 (95)
<i>S. aureus</i> (39 lab)	17 (44)	19 (49)	24 (62)	31 (79)	34 (87)
<i>S. aureus</i> (39 lab) 4 h excluded	28 (72)	28 (72)	31 (79)	34 (87)	38 (97)
<i>E. coli</i> and <i>S. aureus</i>	12 (30)	26 (65)	31 (78)	34 (85)	37 (93)

Mainly *S. aureus* (4h)

(Some of the labs  $>3\%$  -  
diverged from the method.  
Also disk related?)

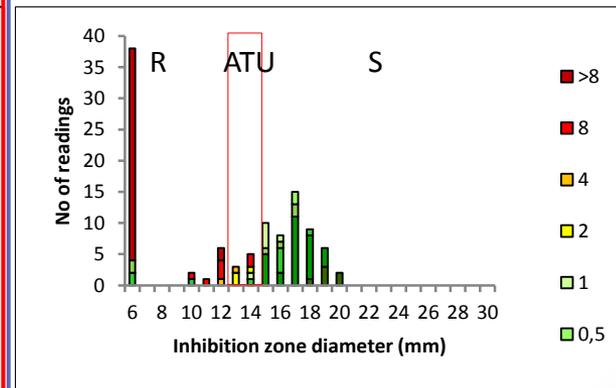
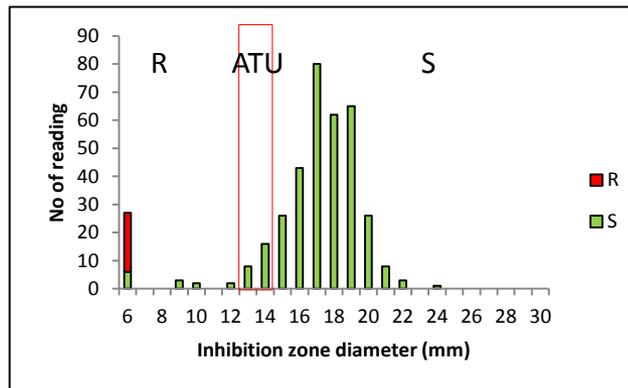
# Explanation of graphs



Zone diameter distribution from RAST with SIR interpretations from standard incubation (in colour).

- Resistant by EUCAST standard disk diffusion zone breakpoint
- Intermediate by EUCAST standard disk diffusion zone breakpoint
- Susceptible by EUCAST standard disk diffusion zone breakpoint

**NordicAST/EUCAST  
Multilab study  
Zon RAST vs  
Zon standard**



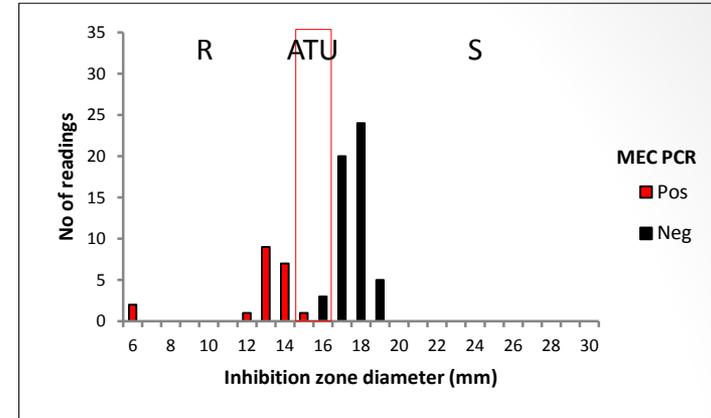
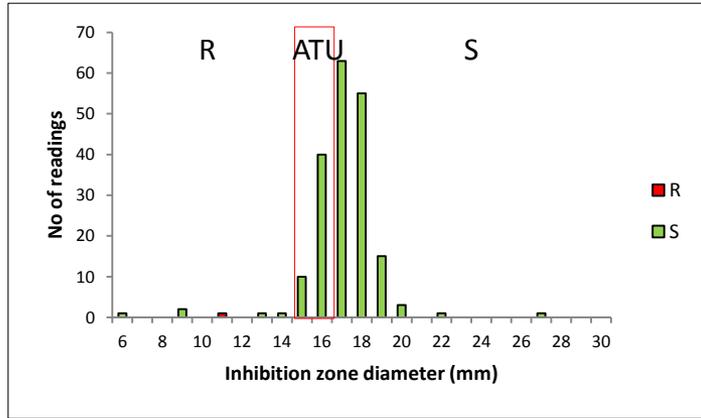
**Spiked  
Blood culture  
bottles  
Zon RAST vs  
MIC (BMD)**

*S. aureus*

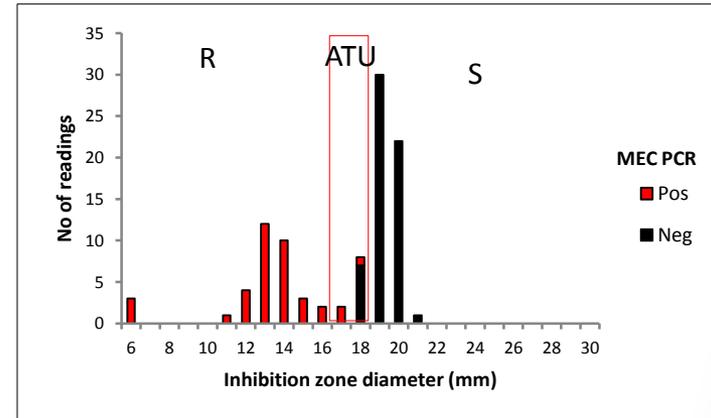
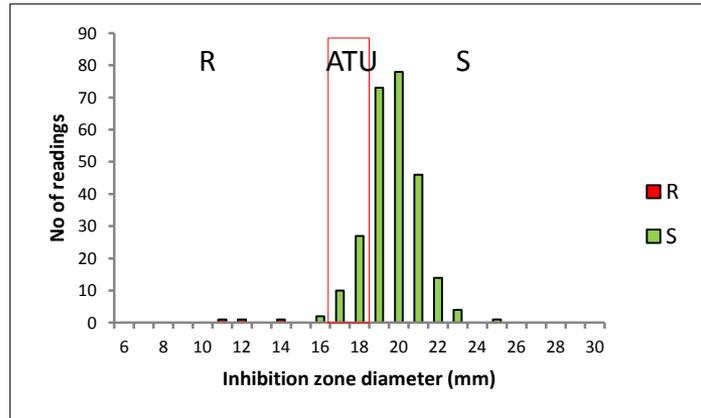


# *S. aureus* and Cefoxitin 30 µg

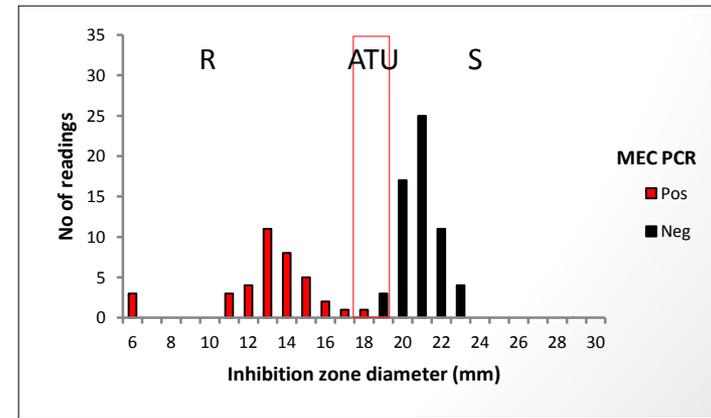
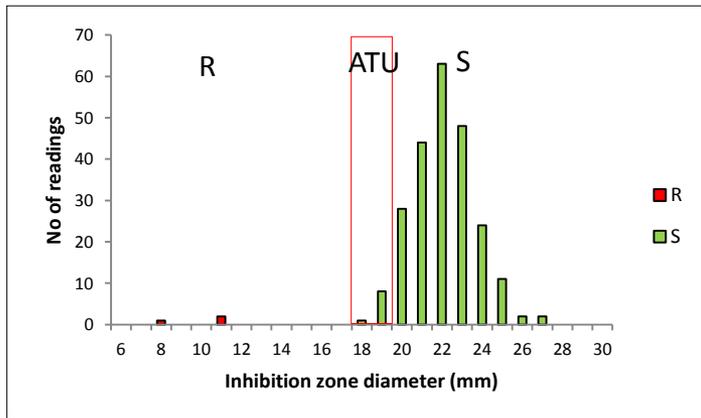
4h



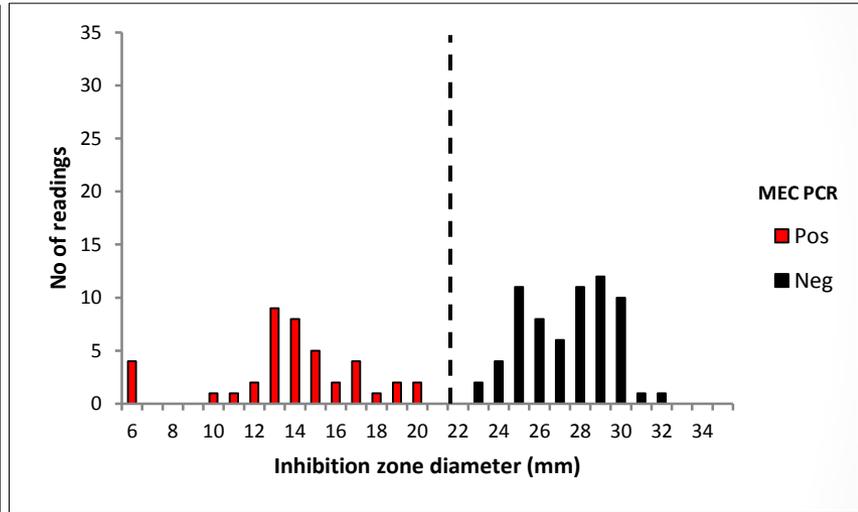
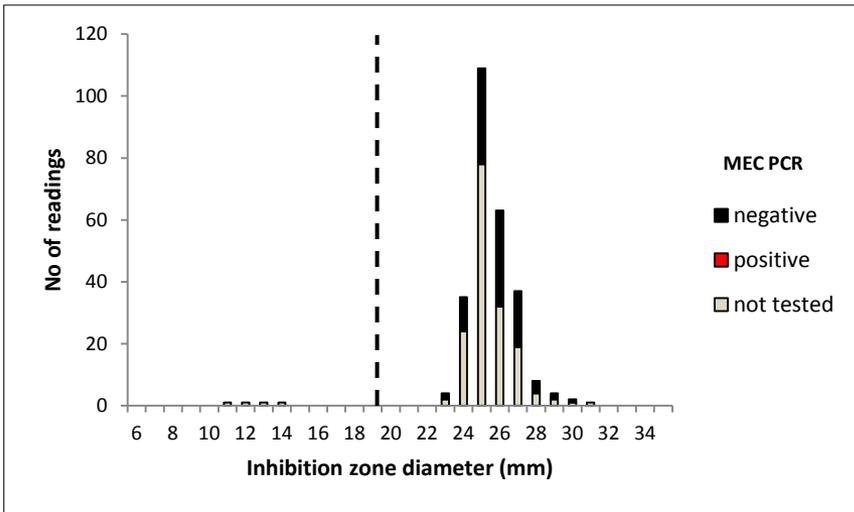
6h



8h



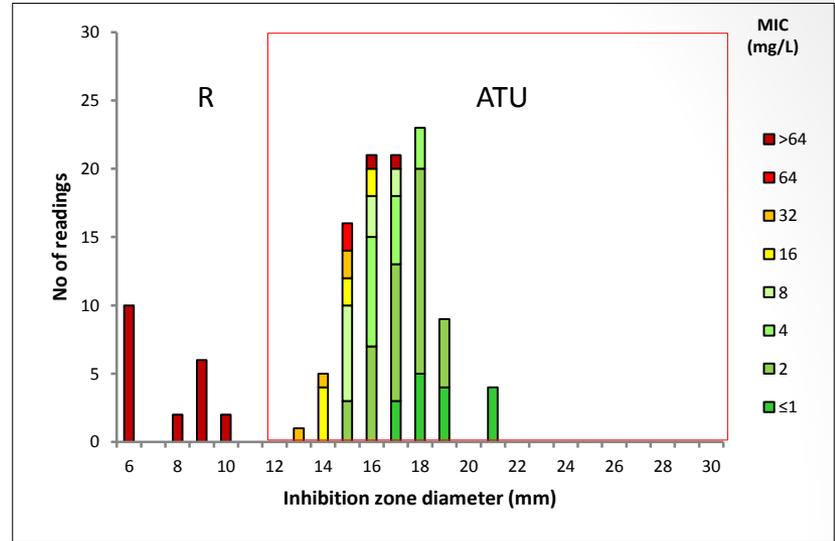
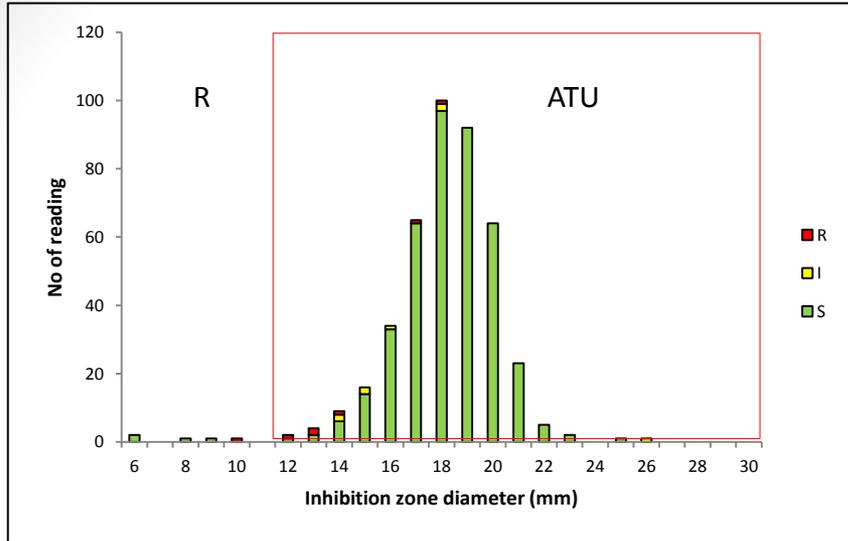
### *S. aureus* and Cefoxitin 30 µg vs MEC PCR 18h incubation



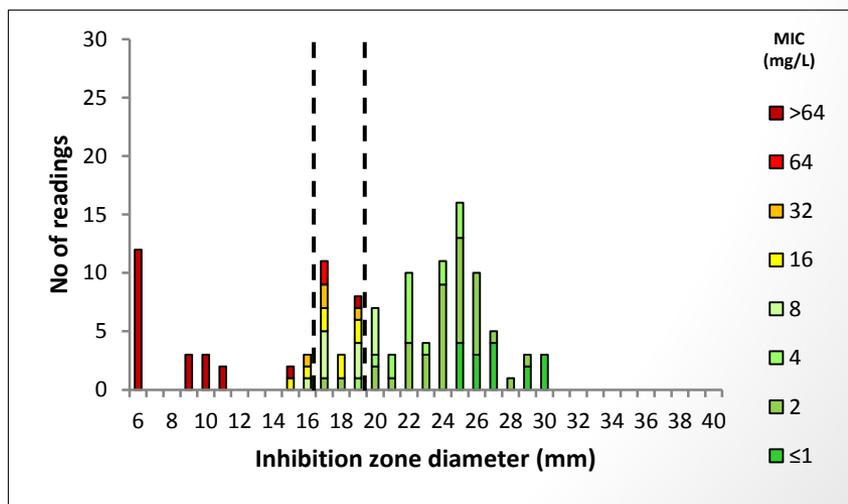
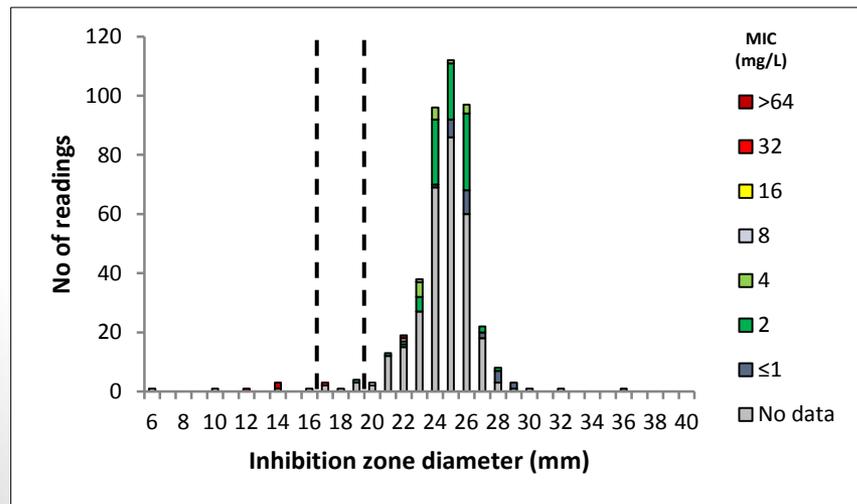
*E. coli*



## *E. coli* and Piperacillin-tazobactam 30-6µg 6h incubation



## 18h incubation



# Major Errors

(false resistance)



	<i>S. aureus</i> (n=267)			<i>E. coli</i> (n=429)		
Number of tests for which a zone could be measured	687	976	912	3 065	3 341	3 014
	Categorical agreement, n (%)			Categorical agreement, n (%)		
Correct	465 (68)	875 (90)	866 (95)	2393 (78)	2703 (81)	2508 (83)
mE	0	0	0	8 (0.3)	3 (0.1)	3 (0.1)
ME	<b>53 (7.7)</b>	<b>5 (0.5)</b>	<b>3 (0.3)</b>	<b>50 (1.6)</b>	<b>14 (0.4)</b>	<b>9 (0.3)</b>
VME	1 (0.1)	3 (0.3)	4 (0.4)	3 (0.1)	4 (0.1)	5 (0.2)
ATU	168 (24)	93 (9.5)	39 (4.3)	611 (20)	617 (18)	489 (16)

*S. aureus*

Incubation time	Tot	ME			
		FOX	NOR	GEN	ERY
4h	53 (7,7%)	5	0	11	<b>37</b>
6h	5	2	0	1	2
8h	3	0	0	0	3

# Very Major errors

(false susceptibility)



- $\leq 0.5\%$  false susceptible results (VME) – few resistant isolates

## Multi laboratory study

Antibiotic agent	Resistant isolates (no)	VME		
		4h	6h	8h
PTZ	7			
CTX	24	0	0	0
AK	0	0	0	0
GEN	29	0	2	2
TOB	25	0	0	1
CAZ	17	0	1	0
CIP	58	3	1	2
MER	0	0	0	0

*E. coli*

## Spiked blood culture bottles

Antibiotic agent	Resistant isolates (no)	VME		
		4h	6h	8h
PTZ	28			
CTX	50	0	0	0
AK	2	0	0	0
GEN	34	0	0	0
TOB	40	1	0	0
CAZ	40	0	0	0
CIP	42	0	0	0
MER	6	0	0	0

Antibiotic agent	Resistant isolates (no)	VME		
		4h	6h	8h
FOX	4	0	0	0
NOR	15	1	3	4
GEN	0	0	0	0
ERY	4	0	0	0

*S. aureus*

Antibiotic agent	Resistant isolates (no)	VME		
		4h	6h	8h
FOX	42	0	0	0
NOR	34	2	3	1
GEN	16	0	0	0
ERY	30	0	0	0

# Conclusion

- Few VME  
No more errors with additional resistant isolates (spiked bottles)
- Area of Technical Uncertainty (ATU) prevents most VME and ME
- Some adjustments of the breakpoints and width of ATUs have been and will be done

- A few outliers shows the great importance of:
  - calibrate your disk diffusion method to the standardized (QC)
  - thoroughly verification
  - follow the methodology
  - read zones only when readable.
- After publication on EUCAST website - the EUCAST RAST method can be implemented in clinical laboratories with blood culture systems from BD and bioMérieux
- It is valid to use for *E.coli* and *S. aureus* without confirming standardized

# And now what...?

- Further validation in labs with higher resistance level in south of Europe
- RAST heading on EUCAST homepage later this year:
  - Methodology
  - QC-table
  - Breakpoint table } Incubation time-related
- Further breakpoints to wait for:
  - *Klebsiella spp*
  - *P. aeruginosa*
  - *E. faecium/faecalis*
  - *S. pneumoniae*

# Many thanks to the 40 participating laboratories!

- ✓ Denmark (n=3): Herlev, Esbjerg and Odense
- ✓ Finland (n=3): Helsinki, Pori and Turku
- ✓ Iceland (n=1): Reykjavik
- ✓ Ireland (n=1): Waterford
- ✓ Norway (n=11): Bergen, Kristiansand, Tønsberg, Sarpsborg, Lørenskog, Oslo, Haugesund, Levanger, Lillehammer, Stavanger and Tromsø
- ✓ Sweden (n=21): Borås, Gävle, Uppsala, Västerås, Örebro, Eskilstuna, Göteborg, Jönköping, Karlskrona, Karlstad, Luleå, Lund, Skövde, Unilabs Stockholm, Karolinska Solna Stockholm, Karolinska Huddinge Stockholm, Sundsvall, Trollhättan, Umeå, Linköping and Växjö



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