

Direkt resistensbestämning

(Direct Antimicrobial Susceptibility Testing, D-AST)

Martin Sundqvist
Laboratoriemedicinska Länskliniken, Klinisk Mikrobiologi,
Universitetssjukhuset, Örebro

Varför?

- Ökande resistens
- Antibiotic stewardship (NNT 64, Klein Breteler SJID 2011)
- Kostnadsbesparande (Garcia-Vazquez SJID 2013)
- För att matcha snabb artbestämning
- För patienten



Två nivåer av D-AST

1. Direkt från provmaterial

- a) Urin
- b) Sår (Malmö modellen)
- c) Sputum (Kontopidou et al Int J Antimicrobial Agents 2011)
- d) Likvor?
- e) Alla material med total dominans av en art?
- f) Kammarvätska? (NEJ, Gupta et al Retina 2004)

2. Direkt från anrikningsbuljong/blododling

- a. Disk Diffusion
- b. Gradient-tester
- c. Automatiskt

Hur kan D-AST användas?

- Som preliminär metod
 - Begränsat antal antibiotika
 - Man gör standardiserad AST dag 2
- + Ger snabbare information
- + Kräver mindre validering
- Ger mer jobb
- Ökad kostnad?
- Kan innebära utmaning för LIS

Hur kan D-AST användas?

- Som definitiv metod
 - Fler antibiotika testas
 - Ingen ny resistensbestämning
- + Ger snabbare information
- + Ger alla AST data direkt
 - Måste valideras noggrant
 - Kan innebära slöseri

Vad säger EUCAST om D-AST

- Ett icke standardiserat inokulat
- Risk för Blandkultur och pH effekter
- EUCAST rekommenderar inte DAST
- Om man gör det ska man
 - Validera
 - Upprepa från ren koloni
 - Om tunn växt rapportera inte S
 - Inget stöd för kort inkuberingstid

Grattis Danmark!



D-AST från blodkultur

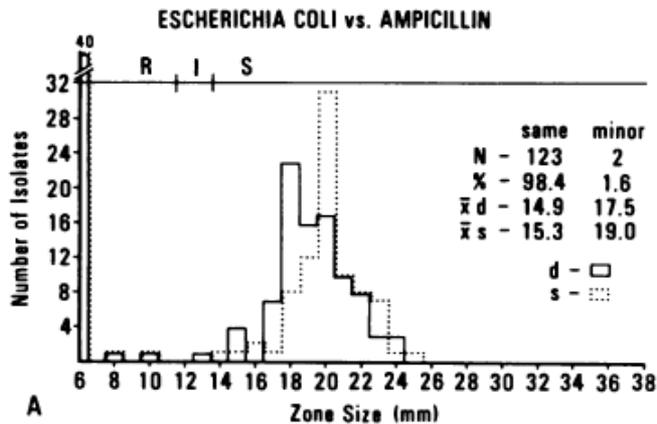
- De flesta lab i Sverige gör AST direkt från pos blododlingsflaska.
- Snabb D-AST (inkubation 6-8h) gör 5 av 25 svenska lab (sept 2012)



Vilket stöd har vi för D-AST från positiv odlingsflaska

Direkt från odlingsflaska

- Mirrett JCM 1979
 - 1:1 från flaskan inkuberades 1h, sedan spädning till 0.5 McF



Early reading

- Coyle et al JCM 1984
 - Utan spädning, 4 och 6 h

TABLE 2. Discrepancies from direct tests compared with standardized tests

| Isolate type, time incubated | No. of tests | No. of discrepancies | | | Overall agreement (%) |
|------------------------------|--------------|----------------------|-----------|------------|-----------------------|
| | | Very major (%) | Major (%) | Minor (%) | |
| Gram positive | | | | | |
| 4 h | 216 | 1 (0.5) | 3 (1.4) | 32 (14.8) | 83.3 |
| 6 h | 494 | 0 | 3 (0.6) | 39 (7.9) | 91.4 |
| Overnight | 1,307 | 1 (0.07) | 8 (0.6) | 65 (5.0) | 94.3 |
| Gram negative | | | | | |
| 4 h | 361 | 0 | 17 (4.7) | 114 (31.6) | 63.7 |
| 6 h | 438 | 0 | 3 (0.7) | 59 (13.5) | 85.8 |
| Overnight | 762 | 0 | 6 (0.8) | 73 (9.6) | 89.6 |

- Etest, (Leegaard et al ECCMID 2008?)
- DD MH-F (Sundqvist et al ECCMID 2008, 2012)

EUROSTAR RAPID DISK

Metod

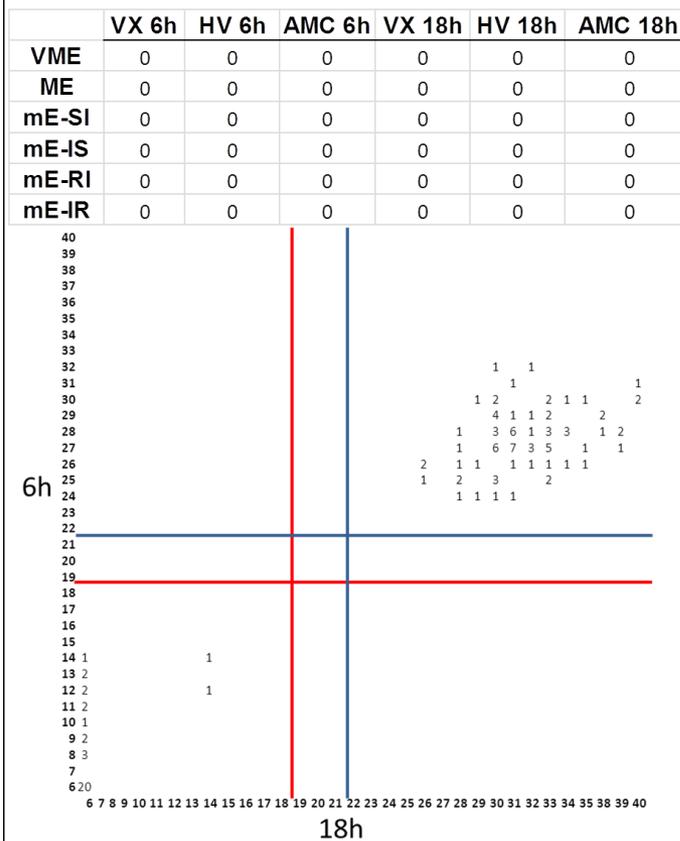
- 0.5 McF, kort inkubation
- 6h
 - E.coli
 - K.pneumoniae
 - Enterokocker
- 8h
 - P.aeruginosa
 - S.aureus
- 93% av isolaten kunde läsas efter 6 el 8 h

Lab

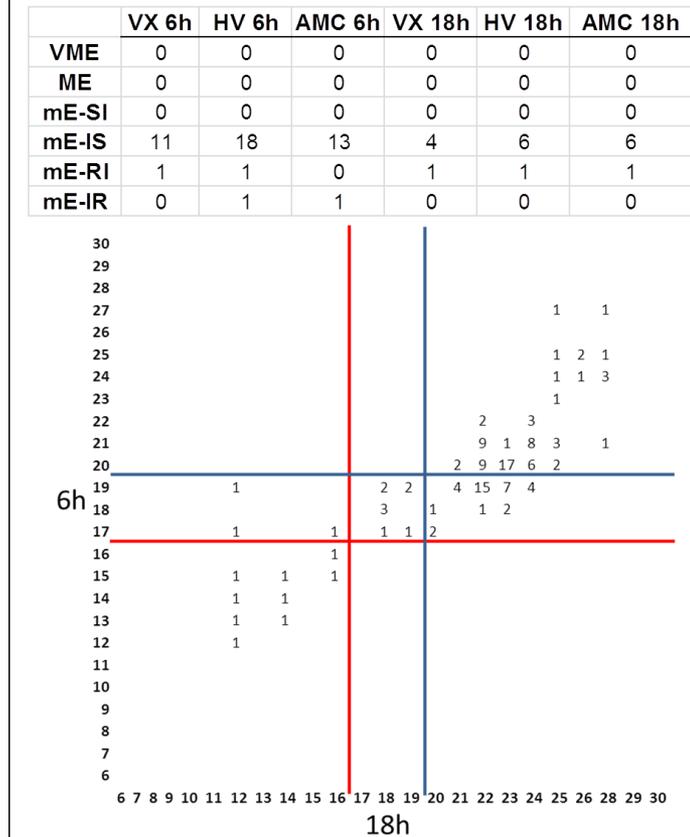
- Växjö
 - manuell avläsning
- Hvidovre
 - kamera med justering
- AMC
 - kamera med justering.
Avläsning varje timma.

Tidig avläsning, 6h vs 18h

E.coli, ciprofloxacin (S≥22, R<19)



K.pneumoniae, Pip-Taz (S≥20, R<17)



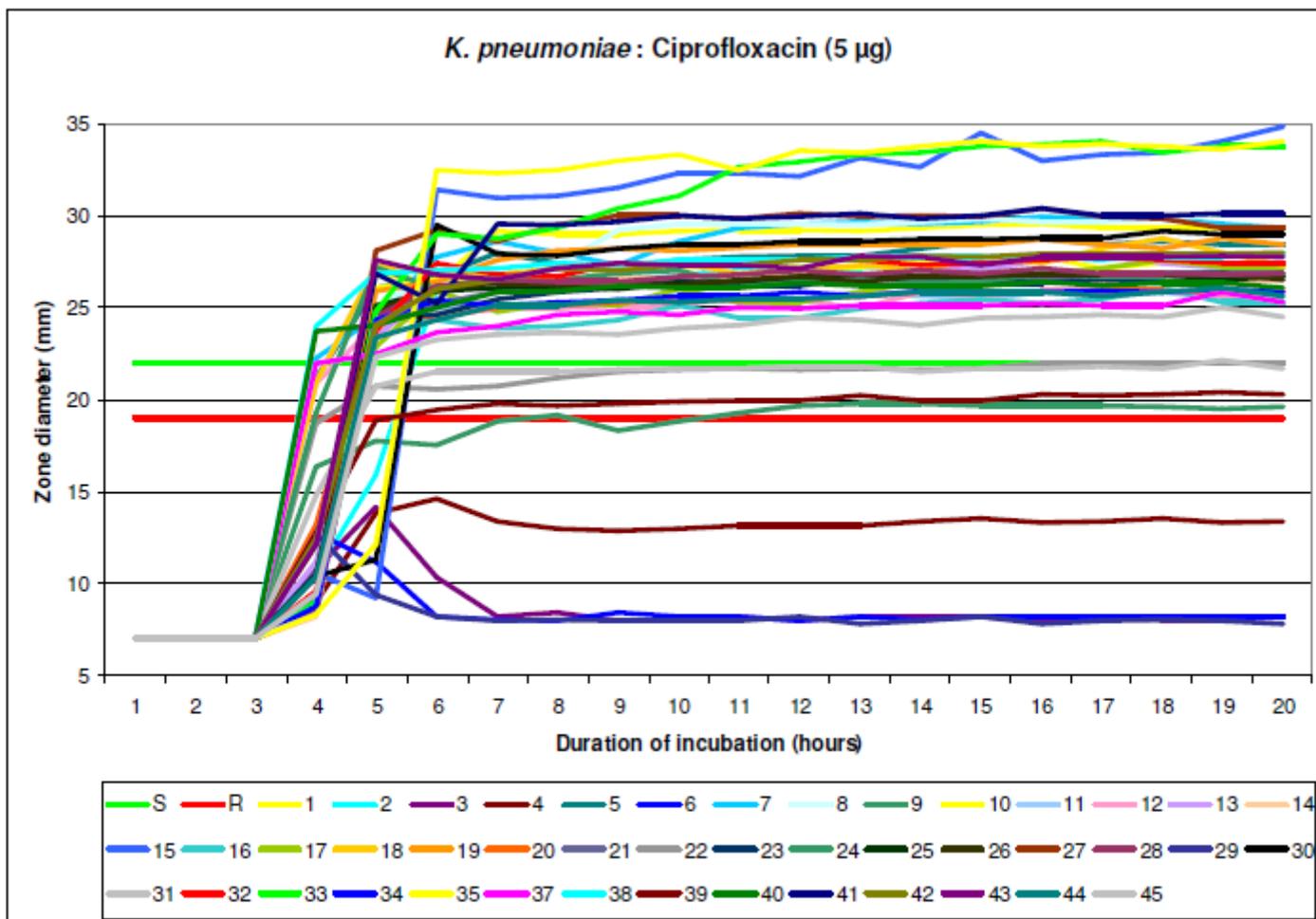
Konklusion

A method for rapid susceptibility testing (6 or 8h) was calibrated to EUCAST disk diffusion methodology and EUCAST clinical breakpoints.

MRSA (8h) and resistance to 3rd generation cephalosporins due to ESBLs in *E.coli* and *K.pneumoniae* (6h) were readily detected by all three laboratories.

A panel of ciprofloxacin, cefotaxime, ceftazidime, meropenem and gentamicin in Enterobacteriaceae and cefoxitin, erythromycin, clindamycin in *S.aureus* can be used for rapid susceptibility testing with disk diffusion.

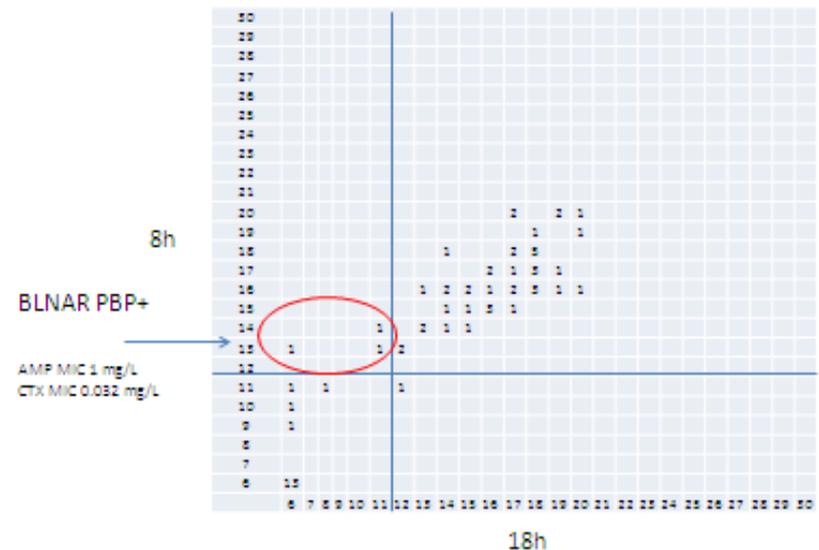
Hur utvecklas en zon?



På gång

- *Haemophilus influenzae*
- Pneumokocker
- Validera direkt från Blod
- Testa nytt protokoll tillsammans med MALDI
 - Lysering
 - Centrifugering
 - Inoculum-beredning

H. Influenzae, PcG 1 unit, 8 h vs 18 h F (n=68/74)
S_≥12



Anna Åkerlund
2013

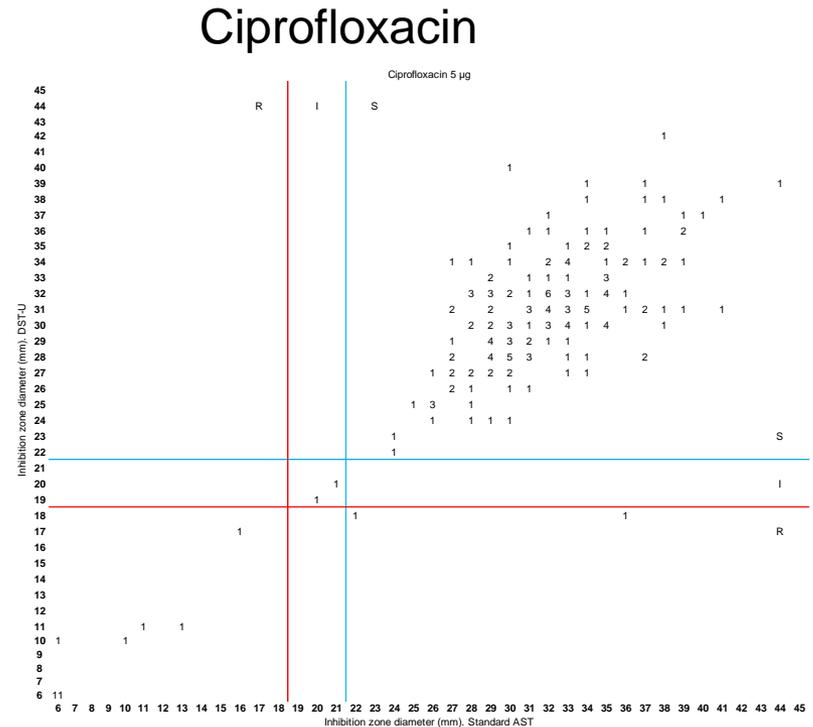
D-AST från urin

- Många gör detta
- Stöd i litteraturen
 - Olika screening
 - Relativt små serier (100-200 pat)
 - Johnsson JCM 1995
- Kombinerat med direkt artbestämning med MALDI?



D-AST Urin

- $\geq 10^5$ cfu/mL
- Renkultur
- 177 prover, 123 E.coli
- EUCAST BP
- DAST från urin gav fler resultat än från pellet



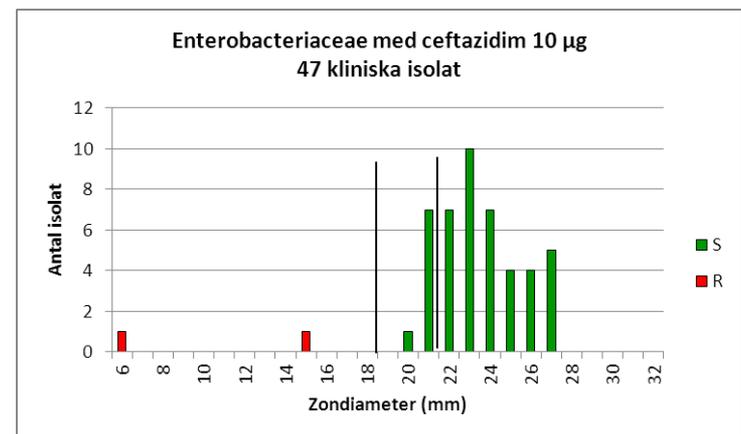
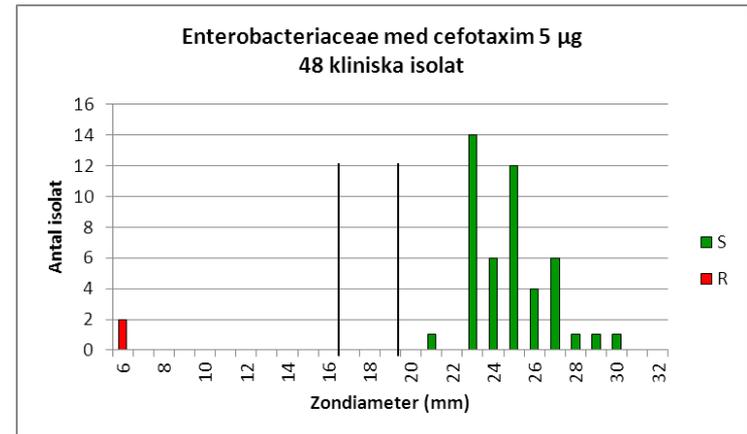
D-AST urin i praktiken

Örebro 470 prover april 2013

- 470 prov
 - 53% negativa
 - Gram pos 9%
 - 10% blandflora
 - Direktresistens avläst på 25%
- 98 *E.coli* (87%)
 - 2 ME (TRI och MEC)
 - 2/2 ESBL hittades
 - 10 *Proteus* spp
 - 1 mE
 - Övriga koliforma bakt
 - Inga fel
 - Snabb artbestämning viktig!

Snabb D-AST från Urin, pilot

- $\geq 10^5$ cfu/mL
- Renkultur
- 72 prover
- 68 Synlig växt på Uriselect (29 med färg)
- Zoner kunde läsas på 48 (42 E.coli) efter 6h på MH



Vad är viktigt att tänka på?

DAST

- Hitta screeningmetod för att få fram de sannolikt positiva
- Allvarliga fel är ovanliga om man:
 - Endast accepterar renkultur
 - Är hård mot inokulatet

Kort inkubation

- Registrera zonstorlek
- Alla bakterie/antibiotika kombinationer fungerar inte
- Resultat precis intill brytpunkten bör tolkas med försiktighet.

Vad krävs för att få ut mest klinisk nytta av D-AST?

- Patientfokus
- Snabb artbestämning
(Chromogena medier, MALDI-TOF)
- Elektroniska svar
- Förlängda öppettider
- Blododlingsskåp tillgängliga 24/7
- Sjukvård som är beredd på snabbare svar



Tack

Växjö



- **Emma Jonasson**
- **Gunilla Cederbrandt**
- Robert Smyth
- Stina Bengtsson
- Jonas Olafsson
- Liselott Byhlén
- Åsa Johansson
- Erika Matuscheck
- Gunnar Kahlmeter

Hvidovre

Pia Littauer

Jenny Dahl Knudsen

AMC

Caspar Hodiament

Caroline Visser

Nijmegen

Johan Mouton

BD/Kiestra

Gerard Muller

Martin van der Kaap

“...if the density of the suspension used are not measured by some fixed standards, exact repetition either by the experimenter himself or by those who try to repeat his work will be impossible”

Vad behövde vi göra?

- Skapa QC-gränser

- *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853
- 40 to 70 test per stam
- target value (median) och range (min-max) beräknad



Results part 2 (ATCC)

| <i>Escherichia coli</i> ATCC 25922 | Eurostar (6h) | | EUCAST (18h) | |
|---|----------------------|--------------|---------------------|--------------|
| | Target | Range | Target | Range |
| (n=44/48) | | | | |
| Cefotaxime 5 | 22 | 20-24 | 28 | 25-31 |
| Ciprofloxacin 5 | 27 | 25-29 | 35 | 30-40 |
| Meropenem 10 | 25 | 23-27 | 31 | 28-34 |
| Ceftazidime 10 | 22 | 20-24 | 26 | 23-29 |
| Piperacillin/tazobactam 30-6 | 20 | 18-22 | 24 | 21-27 |
| Trimethoprim 5 | 22 | 20-24 | 25 | 21-28 |
| Nitrofurantoin 100 | 20 | 17-22 | 20 | 17-23 |
| Tobramycin 10 | 19 | 17-21 | 22 | 18-26 |
| Gentamicin 10 | 19 | 17-21 | 23 | 19-26 |

| <i>Enterococcus faecalis</i> ATCC 29212 | Eurostar (6h) | | EUCAST (18h) | |
|--|----------------------|--------------|---------------------|--------------|
| | Target | Range | Target | Range |
| (n=55/56) | | | | |
| Ampicillin 2 | 16 | 14-18 | 18 | 15-21 |
| Vancomycin 5 | 14 | 13-15 | 13 | 10-16 |
| Gentamicin 30* | 16 | 14-18 | 15 | 12-18 |
| Linezolid 10 | 22 | 20-24 | 22 | 19-25 |
| Nitrofurantoin 100 | 20 | 18-22 | 21 | 18-24 |

* 39/48 tested isolates

Results part 2 (ATCC)

| <i>Ps. aeruginosa</i> ATCC 29213 | Eurostar (8h) | | EUCAST (18h) | |
|---|----------------------|--------------|---------------------|--------------|
| (n=46/46) | Target | Range | Target | Range |
| Piperacillin/tazobactam 30-6 | 22 | 20-24 | 26 | 23-29 |
| Tobramycin 10 | 21 | 19-23 | 22 | 19-25 |
| Meropenem 10 | 25 | 23-27 | 30 | 27-33 |
| Ceftazidime 10 | 20 | 19-23 | 24 | 21-27 |
| Imipenem 10 | 21 | 19-23 | 24 | 20-28 |
| Ciprofloxacin 5 | 24 | 22-26 | 29 | 25-33 |
| Gentamicin 10 | 20 | 18-22 | 19 | 16-21 |

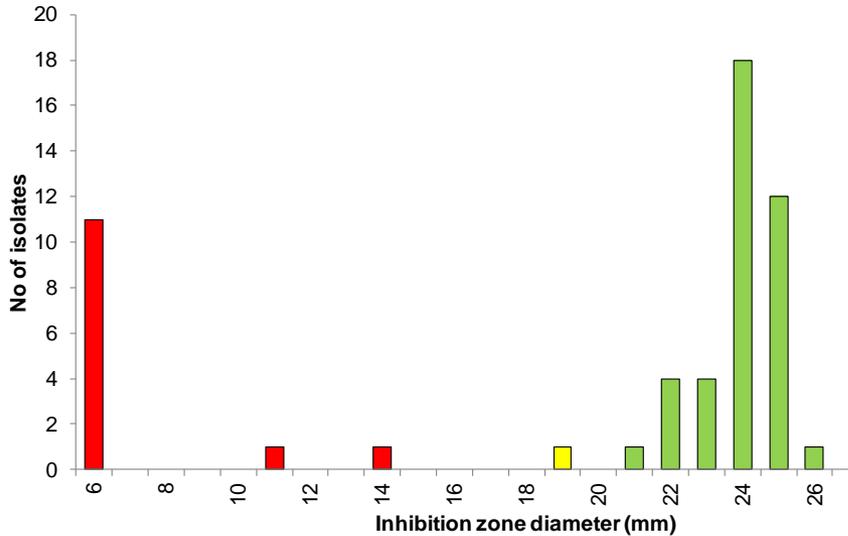
| <i>Staphylococcus aureus</i> ATCC 29213 | Eurostar (8h) | | EUCAST (18h) | |
|--|----------------------|--------------|---------------------|--------------|
| (n=46/46) | Target | Range | Target | Range |
| Clindamycin 2 | 24 | 22-26 | 26 | 23-29 |
| Erythromycin 15 | 25 | 23-27 | 26 | 23-29 |
| Linezolid 10 | 26 | 24-28 | 24 | 21-27 |
| Norfloxacin 10 | 20 | 18-22 | 21 | 18-24 |
| Cefoxitin 30 | 23 | 21-25 | 27 | 24-30 |

Vad behöver vi göra?

- Skapa QC-gränser
 - *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853
 - 40 to 70 test per stam
 - target value (median) och range (min-max) beräknad
- Skapa vildtypsdistributioner (med enstaka resistent isolat)
 - Kliniskt betydelsefulla antibiotika
 - 50 välkarakteriserade *E. coli*, *E. faecalis* /*E. faecium*, *S.aureus* och *P.aeruginosa*
 - Jämföra både SIR kategorisering och zon-zon korrelerat med EUCAST metoden (16-20h incubation).

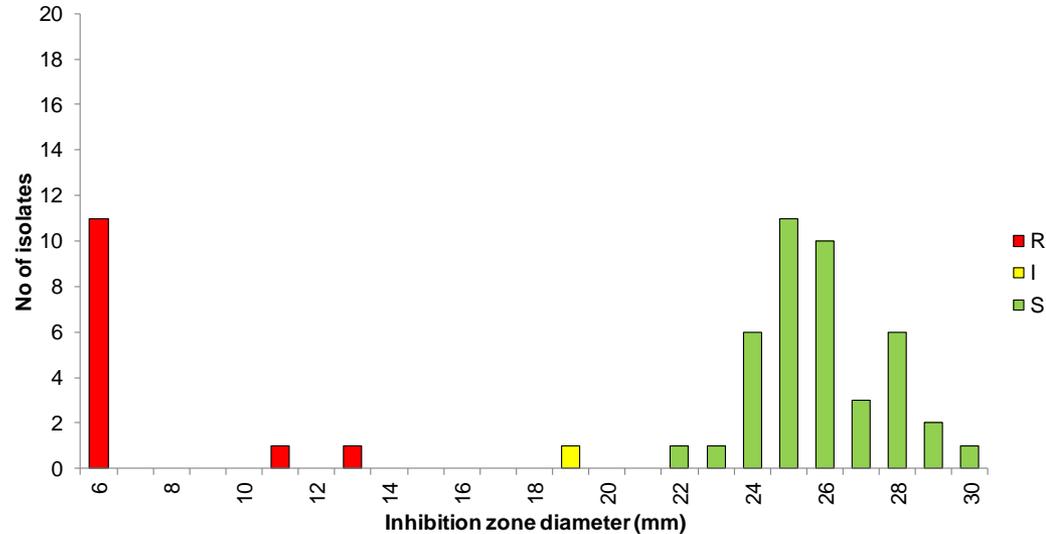
E. coli and cefotaxime 5 μ g

Cefotaxime 5 μ g, 6 hours incubation
E. coli, 55 clinical isolates

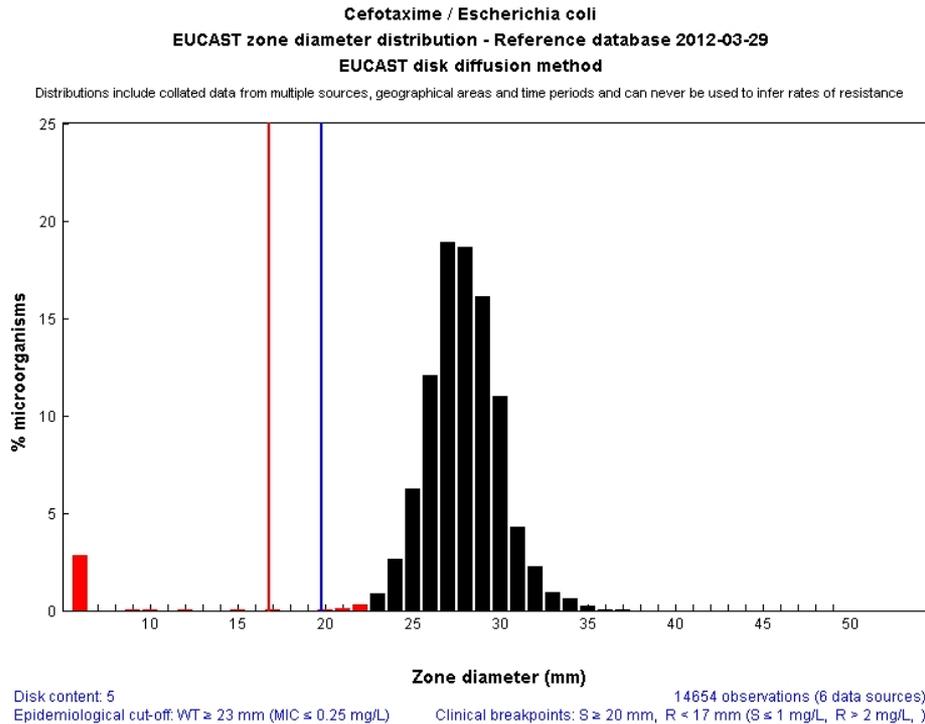
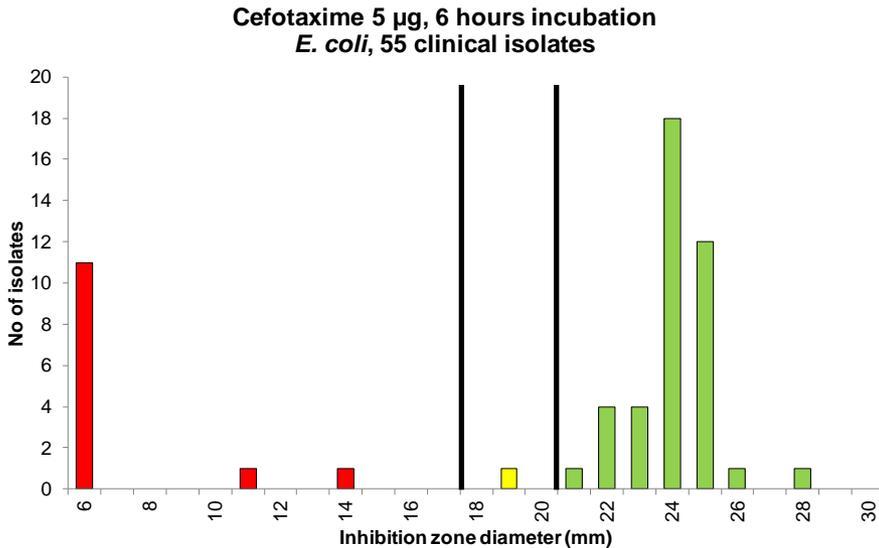


18h CBP
 $S \geq 21$
 $R < 18$

Cefotaxime 5 μ g, 8 hours incubation
E. coli, 55 clinical isolates



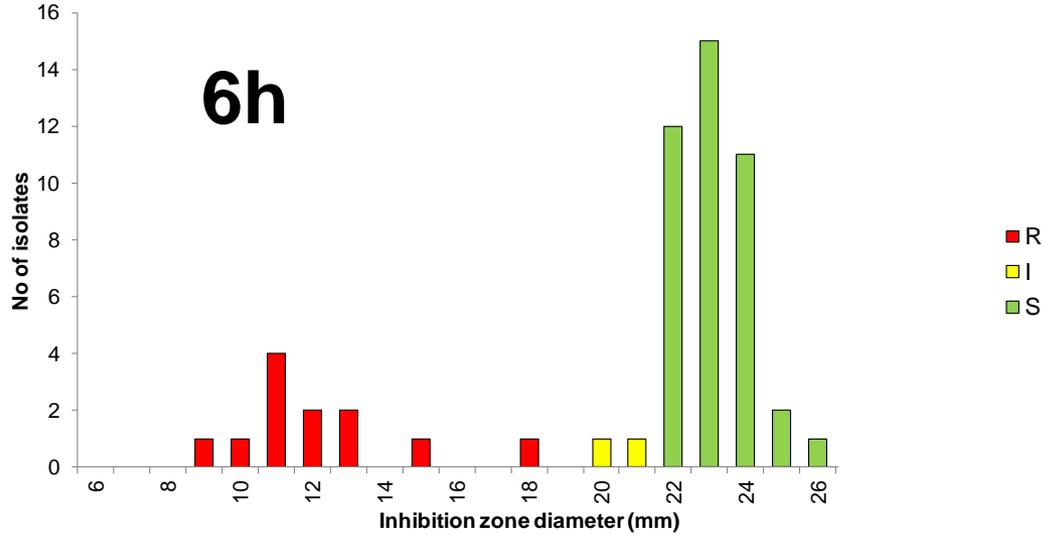
E. coli and cefotaxime 5 μ g



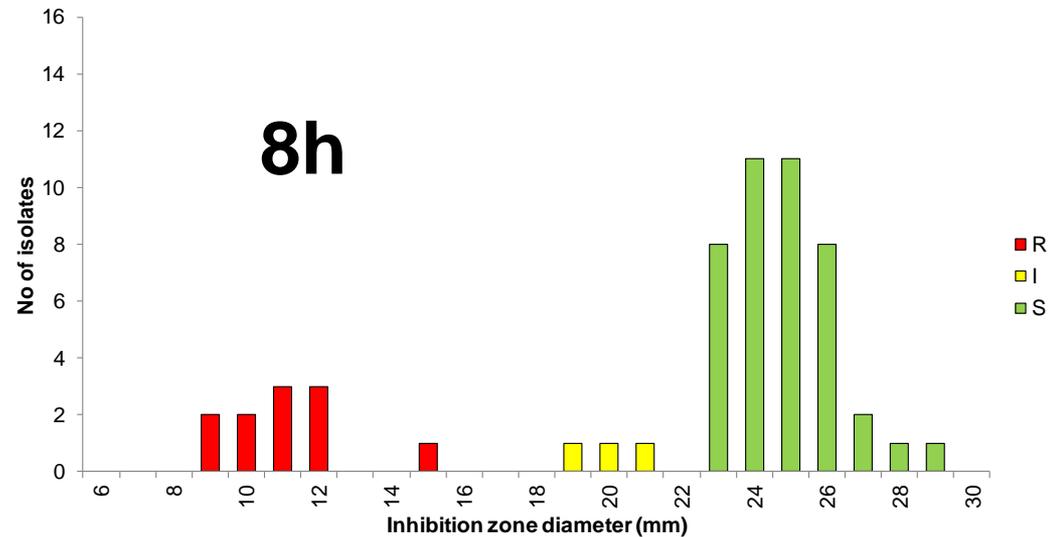
18h CBP
S \geq 21
R $<$ 18

E. coli and ceftazidime 10 μ g

Ceftazidime 10 μ g, 6 hours incubation
E. coli, 55 clinical isolates



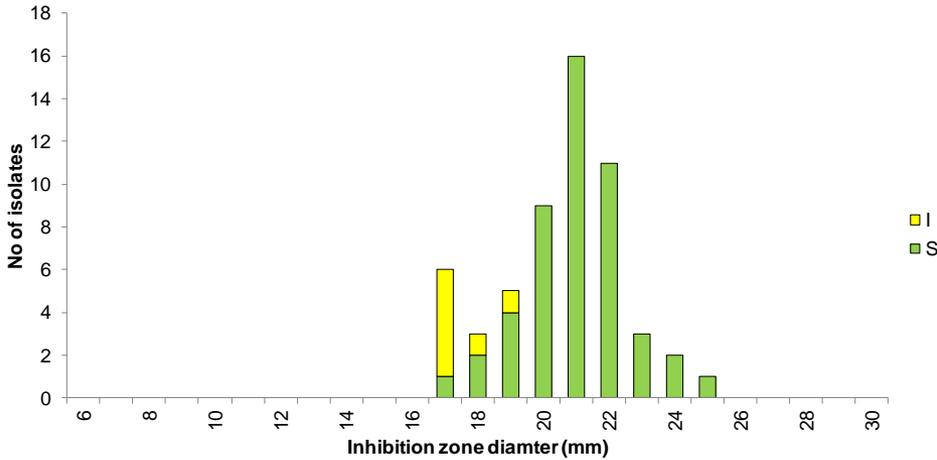
Ceftazidime 10 μ g, 8h incubation
E. coli, 55 clinical isolates



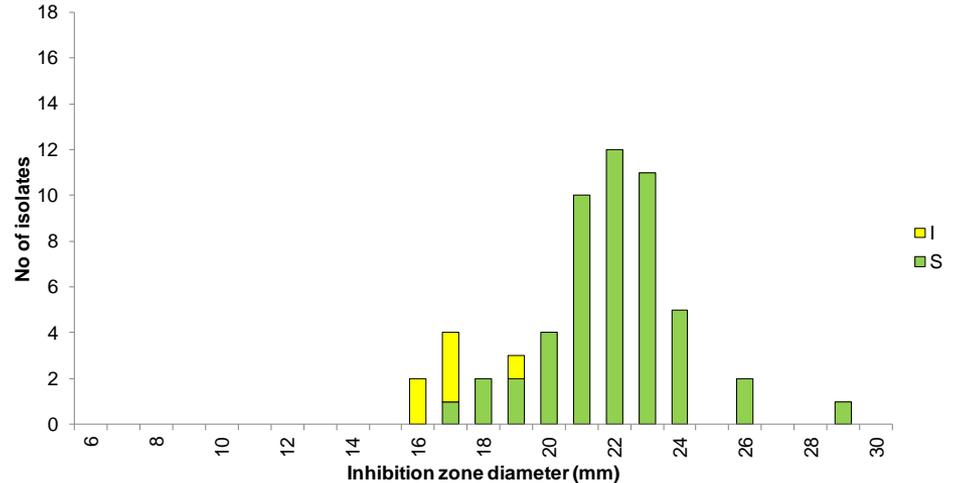
18h CBP
 $S \geq 22$
 $R < 19$

E. coli and piperacillin-tazobactam

Piperacillin-tazobaktam 36 µg, 6 hours incubation
E. coli, 56 clinical isolates



Piperacillin-tazobaktam 36 µg, 8 hours incubation
E. coli, 56 clinical isolates



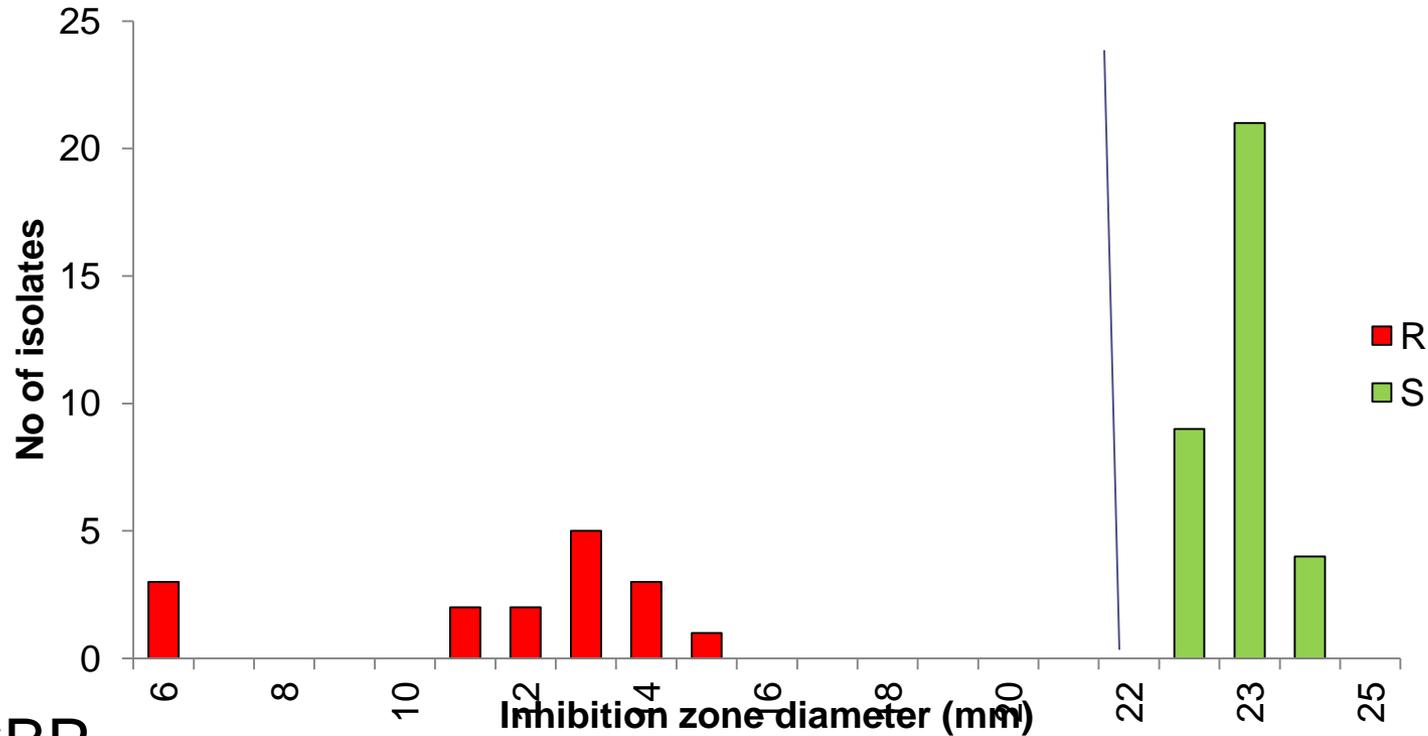
18h CBP
 $S \geq 18$
 $R < 15$

Vad behöver vi göra?

- Skapa QC-gränser
 - *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853
 - 40 to 70 test per stam
 - target value (median) och range (min-max) beräknad
- Skapa vildtypsdistributioner (med enstaka resistent isolat)
 - Kliniskt betydelsefulla antibiotika
 - 50 välkarakteriserade *E. coli*, *E. faecalis* /*E. faecium*, *S. aureus* och *P. aeruginosa*
 - Jämföra både SIR kategorisering och zon-zon korrelerat med EUCAST metoden (16-20h incubation).

S. aureus och cefoxitin 30 μ g

Cefoxitin 30 μ g, 8 hours incubation
S. aureus, 50 clinical isolates



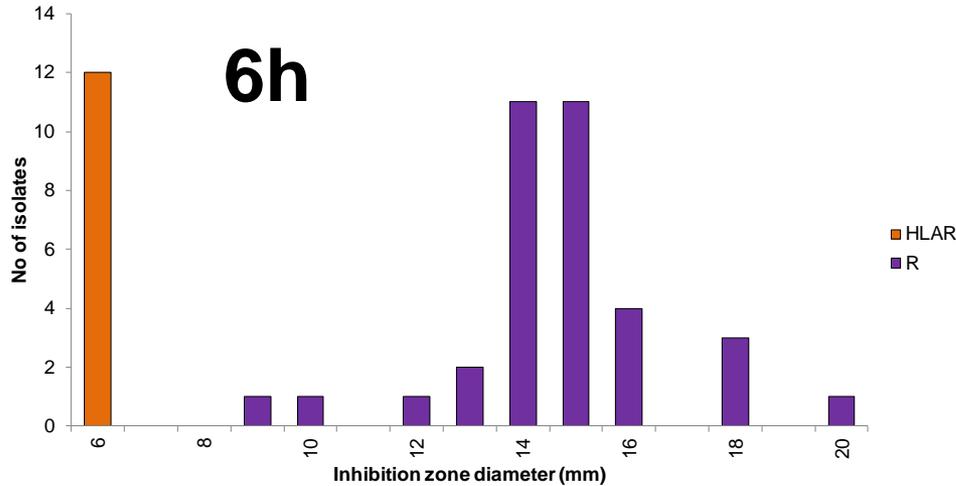
18h CBP

S \geq 22

R<22

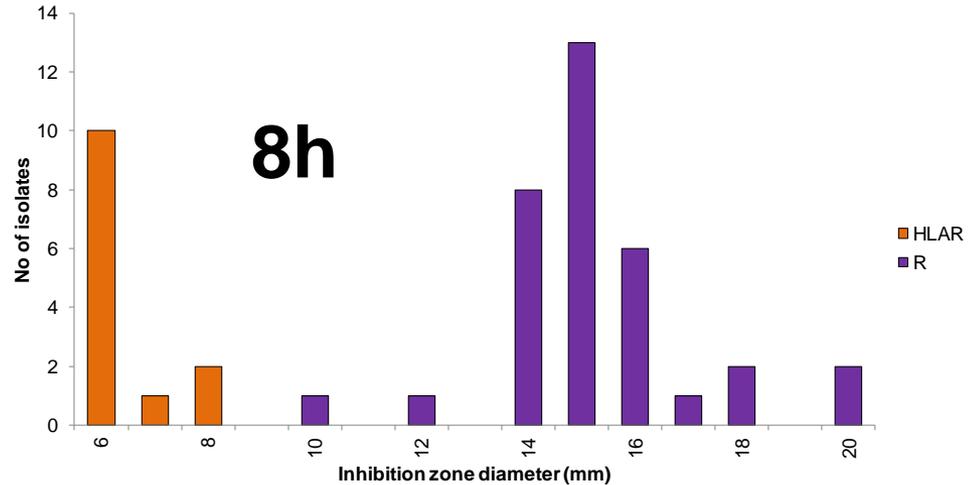
E. faecalis/faecium och gentamicin 30 µg

Gentamicin 30 µg, 6h incubation
Enterococcus, 47 isolates



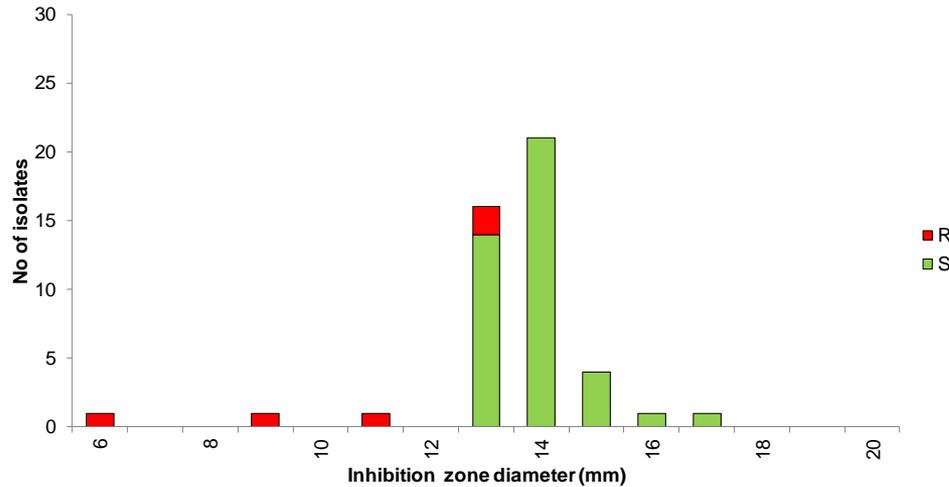
HLAR 6 mm 18h

Gentamicin 30 µg, 8h incubation
Enterococcus, 47 isolates

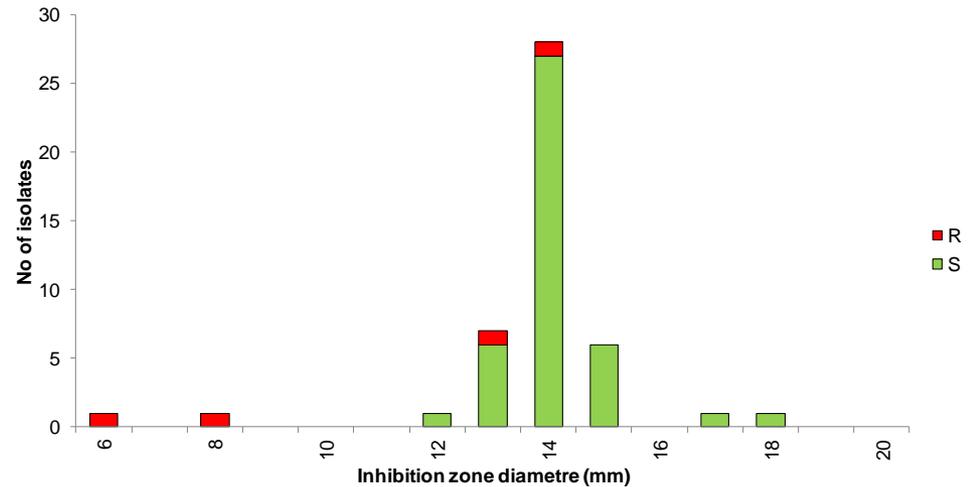


E. faecalis/faecium and vancomycin

Vancomycin 5 µg, 6h incubation
Enterococcus, 46 isolates



Vancomycin 5 µg, 8 h incubation
Enterococcus, 48 isolates



18h CBP

$S \geq 12$

$R < 12$

Slutsats

- Det är möjligt att standardisera resistensbestämning på 6h (E.coli och Enterokocker) eller 8h (S.aureus och P.aeruginosa) för de flesta antibiotika
- Vissa antibiotika/resistensmekanismer kommer inte att passa
- Fler specifika resistenskombinationer ska testas.
- Större kollektion av konsekutiva kliniska isolat ska testas

Structure

1. Pilot studies

2. Quality control strains

- *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853
- 40 to 70 repeated tests
- target value (median) and range (min-max)

3. Clinical isolates

- 50 well characterised isolates of *E. coli* and *E. faecalis* / *E. faecium*
- Compared to SIR categorisation, using EUCAST methodology (16-20h incubation) and breakpoints.

Pilot studies (1)

- Reading from front and back of the plates. (n=90)
- Reading direct after incubation and after storage in fridge. (n=98)
- Preincubated plates. (n=45)

Results (1)

- Reading from the front (open lid) were a prerequisite for short incubation
- Pre-heating not decrease the time to readable zones
- No significant difference between direct measurements and after storage in fridge

Conclusions (1)

- It is possible to get valid QC of the methodology based on
 - 6 hours of incubation for *E. coli* and *E. faecalis*
 - 8 h incubation for *S. aureus* and *P. aeruginosa*
- The target values were 0-8 mm lower than standardised EUCAST QC targets
- The range was typically 5 mm (EUCAST 6-10).

Methods (2)

- Isolates from overnight cultures.
- Suspension of McFarland 0,5-0,6 in NaCl.
- Rotator (Biomerieux) used for spread.
- Two suspensions prepared
 - 6 and 8h
 - 18h
- Plates read directly or within 2 days after storage in fridge.

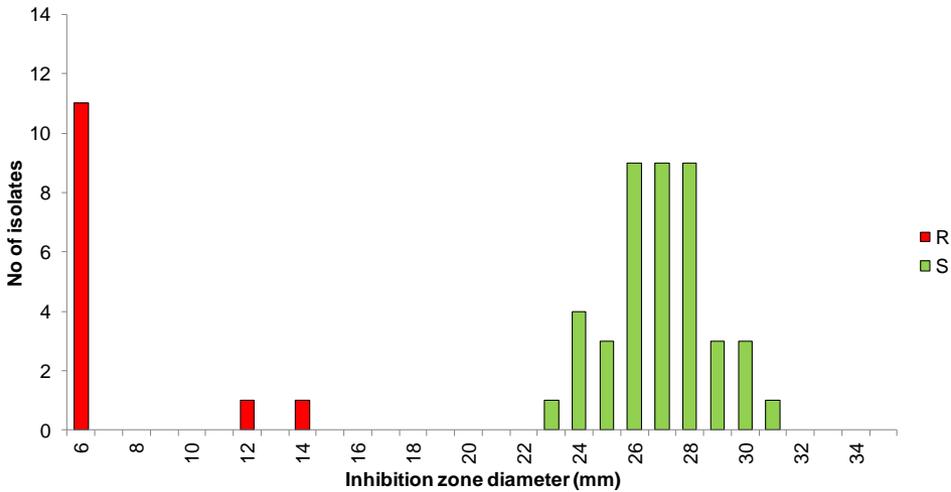
Methods part 3

(EUCAST collection)

- Isolates from overnight cultures
- McF 0.5-0.6
- Rotator
- Zone measurements by two technicians from the front using a slide gauge (direct or after storage in fridge)
- Good light and dark background

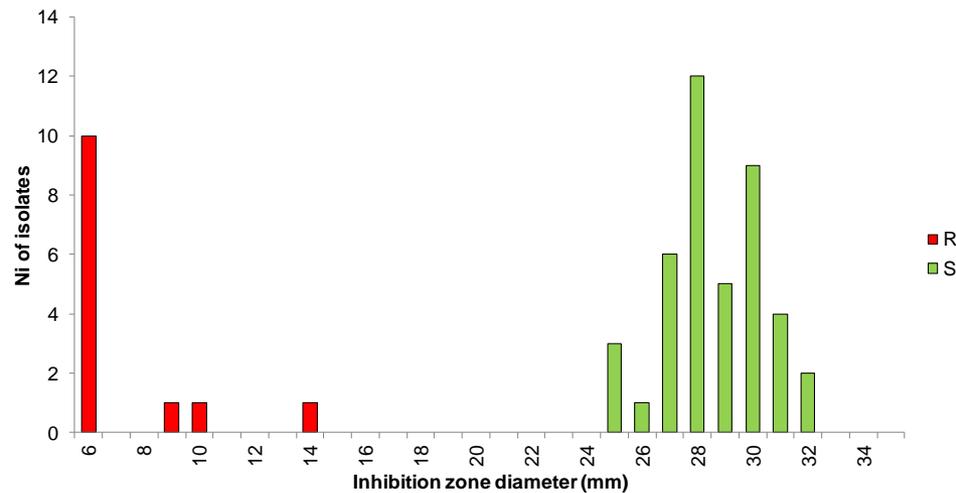
E. coli and ciprofloxacin 5 μ g

Ciprofloxacin 5 μ g, 6 hours incubation
E. coli, 55 clinical isolates



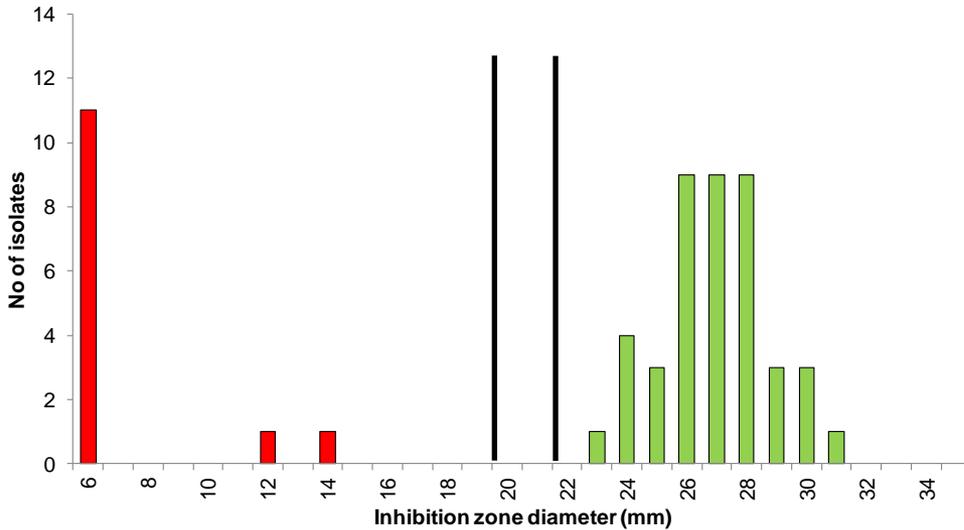
18h CBP
S \geq 21
R<19

Ciprofloxacin 5 μ g, 8 hours incubation
E. coli, 55 clinical isolates

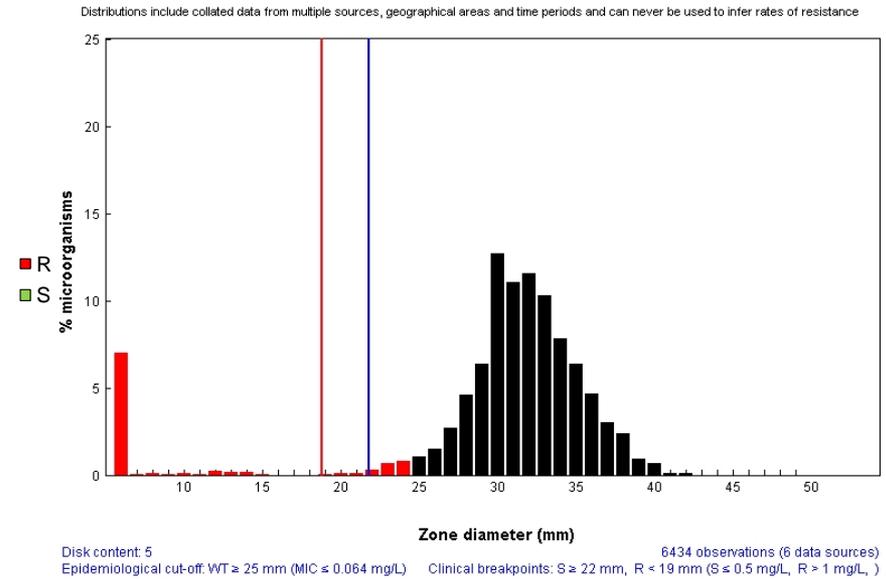


E. coli and ciprofloxacin 5 μ g

Ciprofloxacin 5 μ g, 6 hours incubation
E. coli, 55 clinical isolates

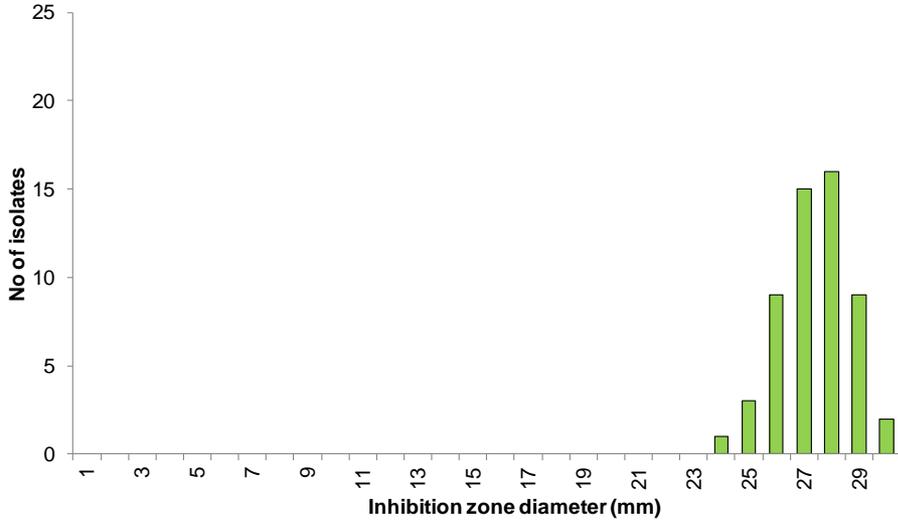


Ciprofloxacin / *Escherichia coli*
EUCAST zone diameter distribution - Reference database 2012-03-29
EUCAST disk diffusion method

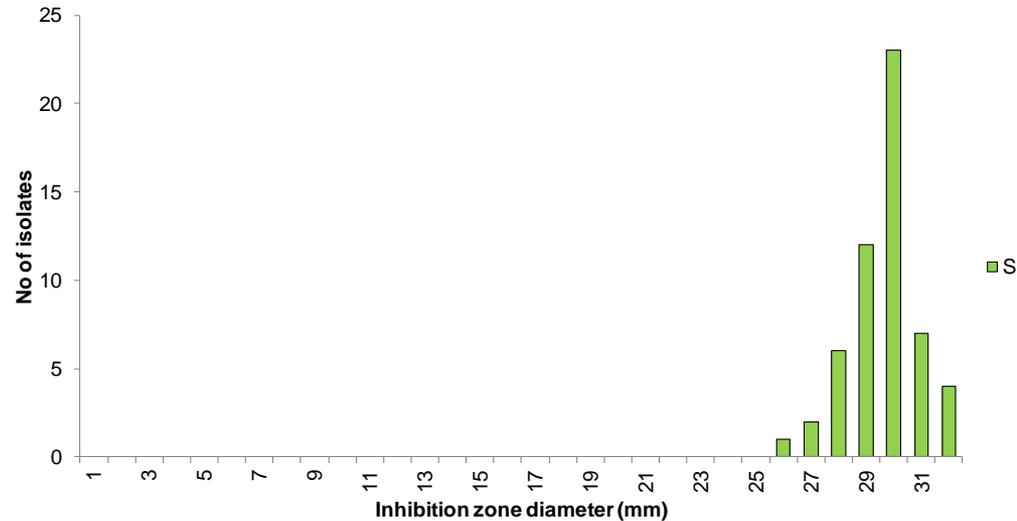


E. coli and meropenem

Meropenem 10 µg, 6 hours incubation
E. coli, 55 clinical isolates



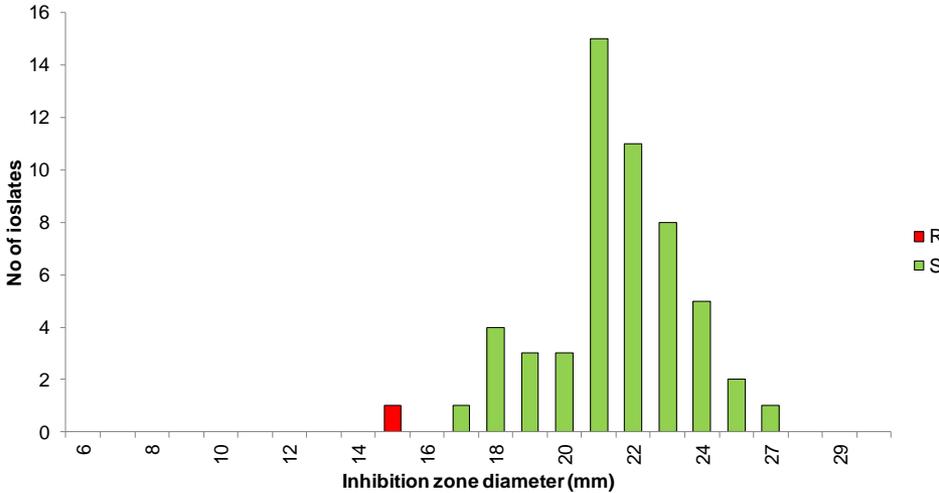
Meropenem 10 µg, 8 hours incubation
E. coli, 55 clinical isolates



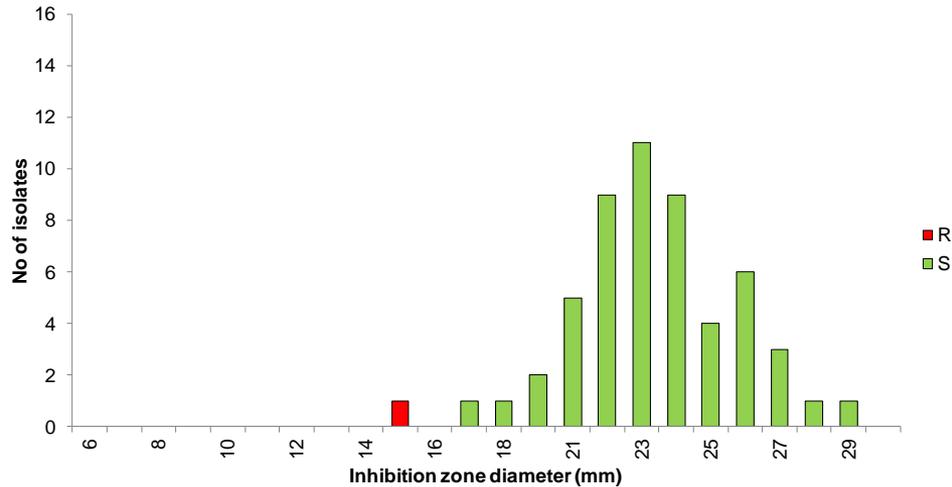
18h CBP
 $S \geq 22$
 $R < 16$

E. coli and mecillinam

Mecillinam 10 µg, 6 hours incubation
E. coli, 54 clinical isolates



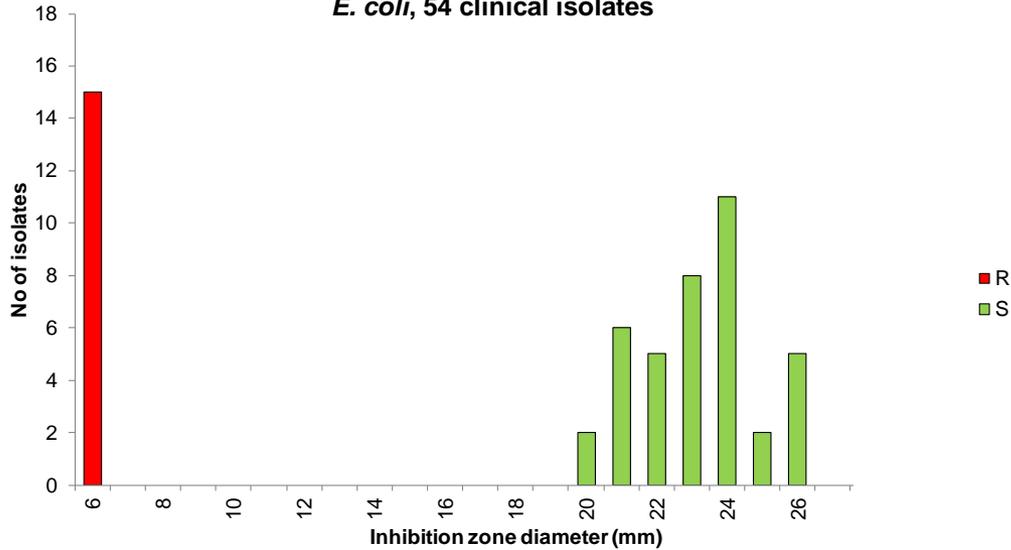
Mecillinam 10 µg, 8 hours incubation
E. coli, 54 clinical isolates



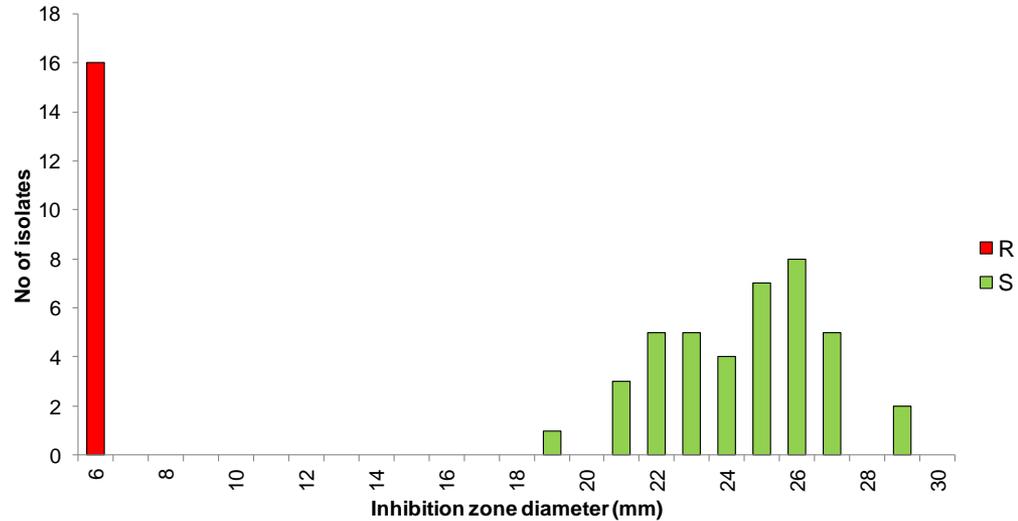
18h CBP
 $S \geq 15$
 $R < 15$

E. coli and trimethoprim

Trimethoprim 5 µg, 6 hours incubation
E. coli, 54 clinical isolates



Trimethoprim 5 µg, 8 hours incubation
E. coli, 54 clinical isolates



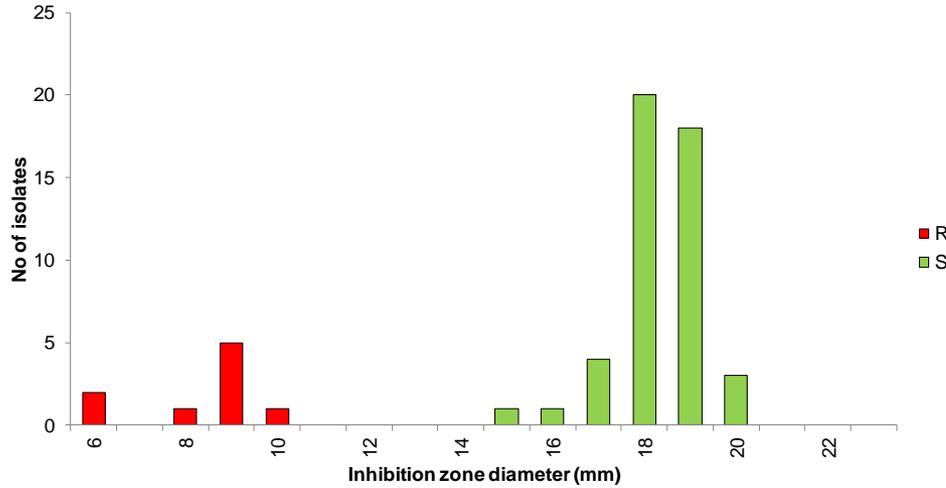
18h CBP

$S \geq 18$

$R < 15$

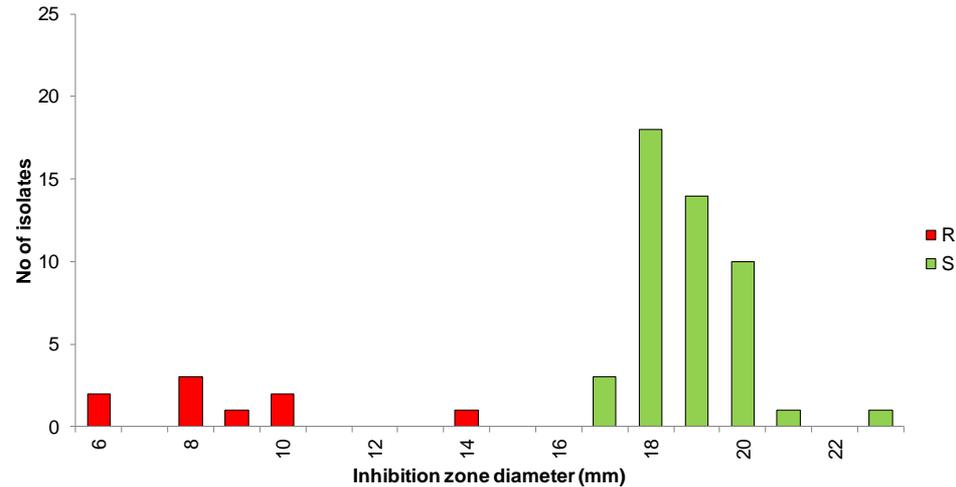
E. coli and gentamicin

Gentamicin 10 µg, 6 hours incubation
E. coli, 56 clinical isolates



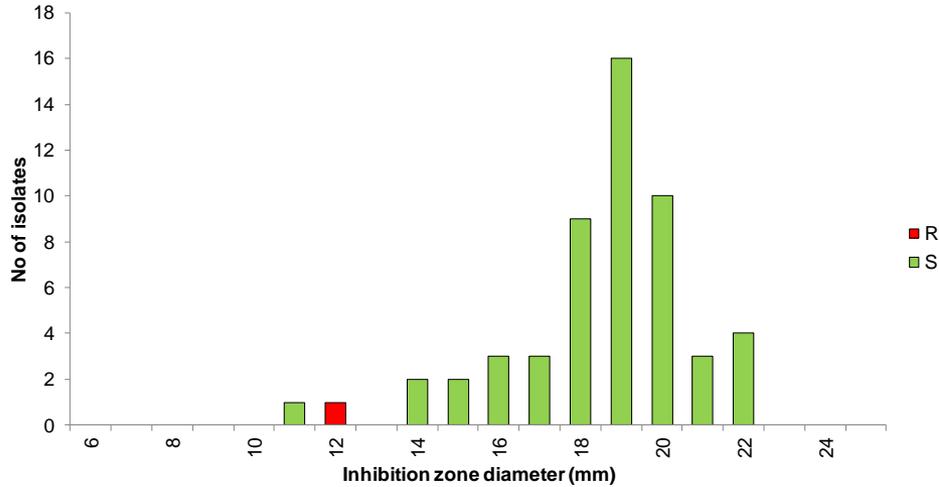
18h CBP
 $S \geq 17$
 $R < 14$

Gentamicin 10 µg, 8 hours incubation
E. coli, 56 clinical isolates

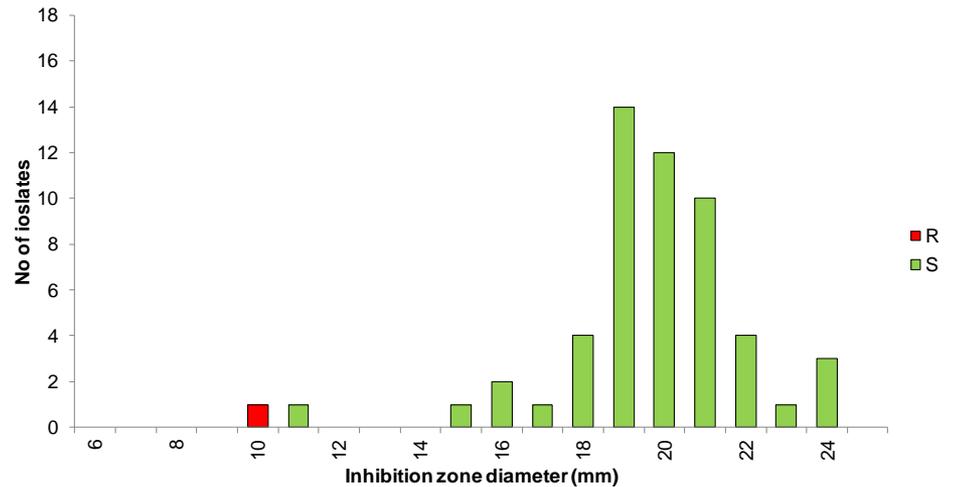


E.coli and nitrofurantion

Nitrofurantoin 100 µg, 6 hours incubation
E. coli, 54 clinical isolates



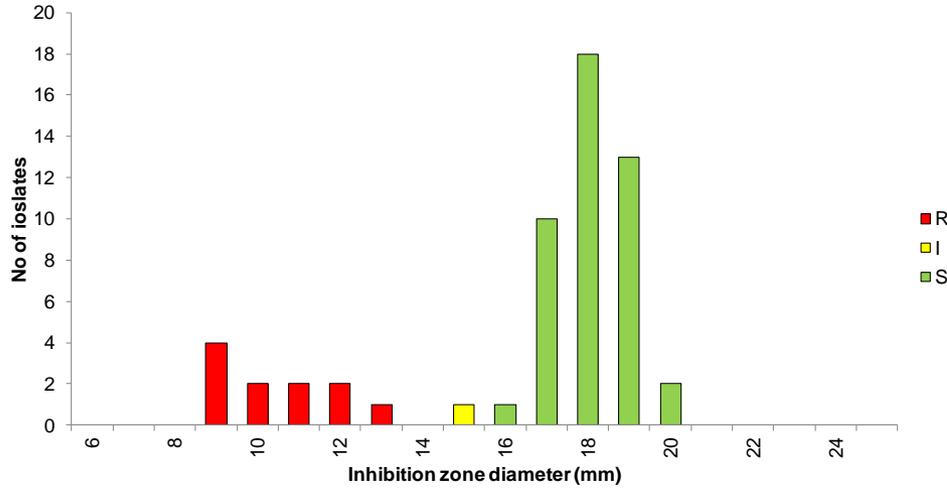
Nitrofurantoin 100 µg, 8 hours incubation
E. coli, 54 clinical isolates



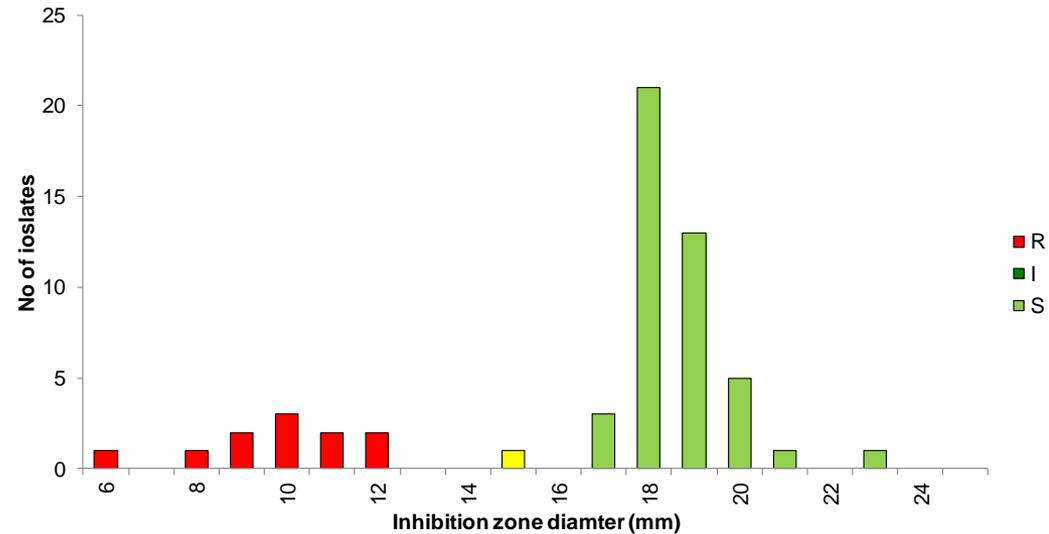
18h CBP
 $S \geq 11$
 $R < 11$

E. coli and tobramycin

Tobramycin 10 µg, 6 hours incubation
E. coli, 56 clinical isolates



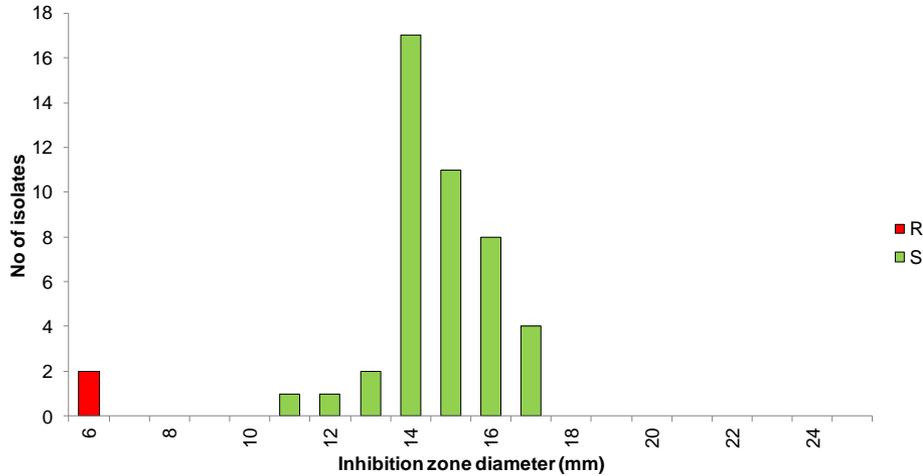
Tobramycin 10 µg, 8 hours incubation
E. coli, 56 clinical isolates



18h CBP
 $S \geq 16$
 $R < 13$

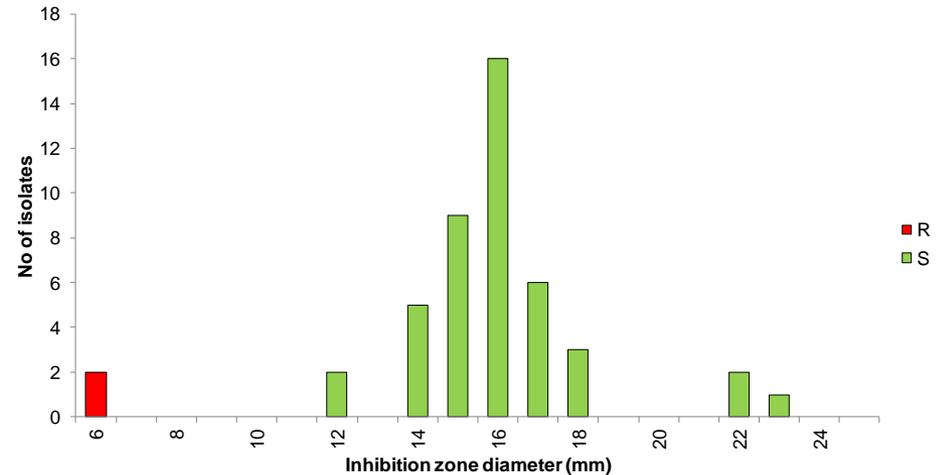
E. faecalis/faecium and ampicillin

Ampicillin 2 µg, 6h incubation
Enterococcus, 46 isolates



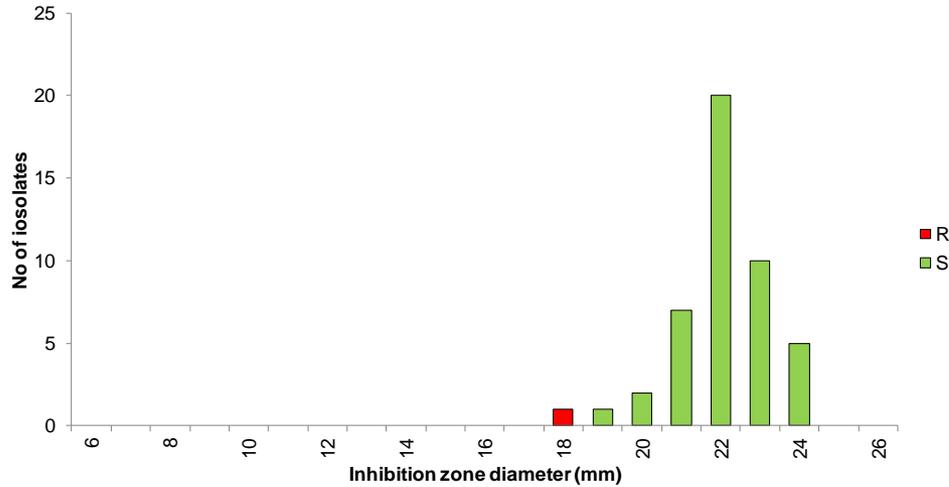
18h CBP
S \geq 10
R<8

Ampicillin 2 µg, 8 h incubation
Enterococcus, 46 isolates



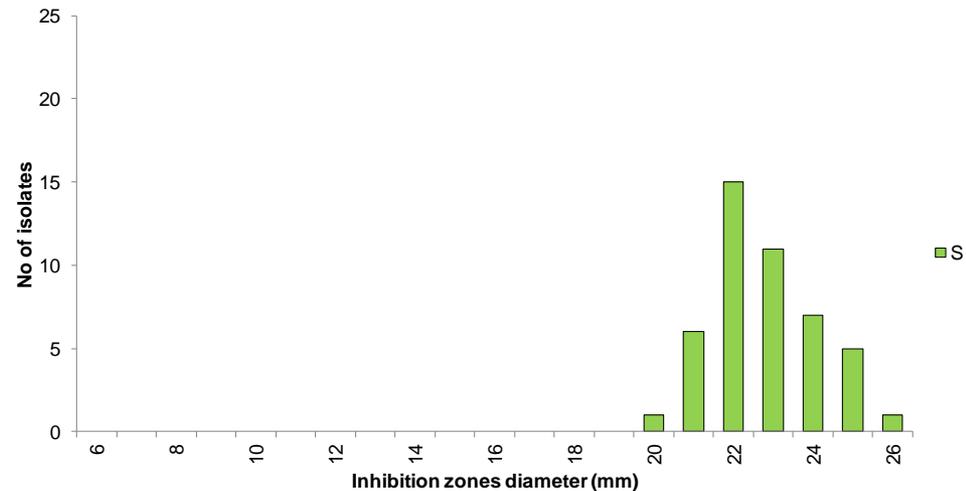
E. faecalis/faecium and linezolid

Linezolid 10 µg, 6h incubation
Enterococcus, 46 isolates



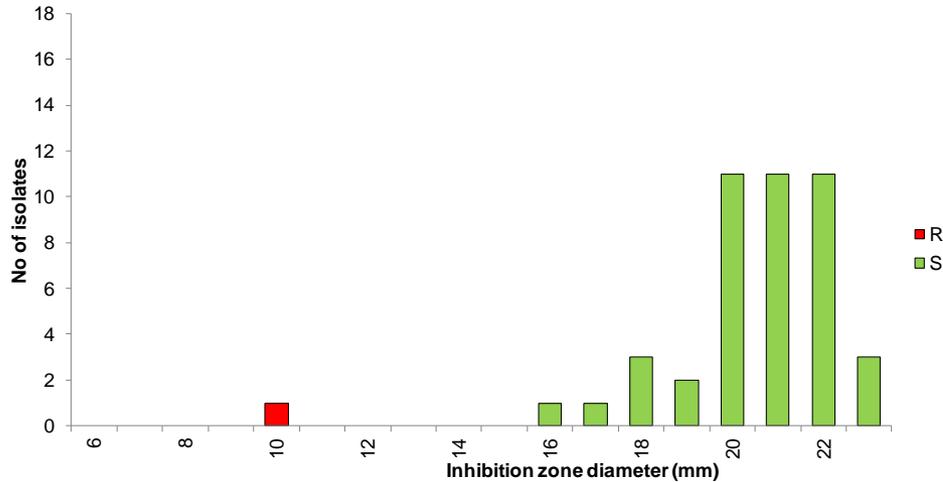
18h CBP
 $S \geq 19$
 $R < 19$

Linezolid 10 µg, 8h incubation
Enterococcus, 46 isolates



E. faecalis/faecium and nitrofurantoin

Nitrofurantoin 10 µg, 6h incubation
Enterococcus, 46 isolates



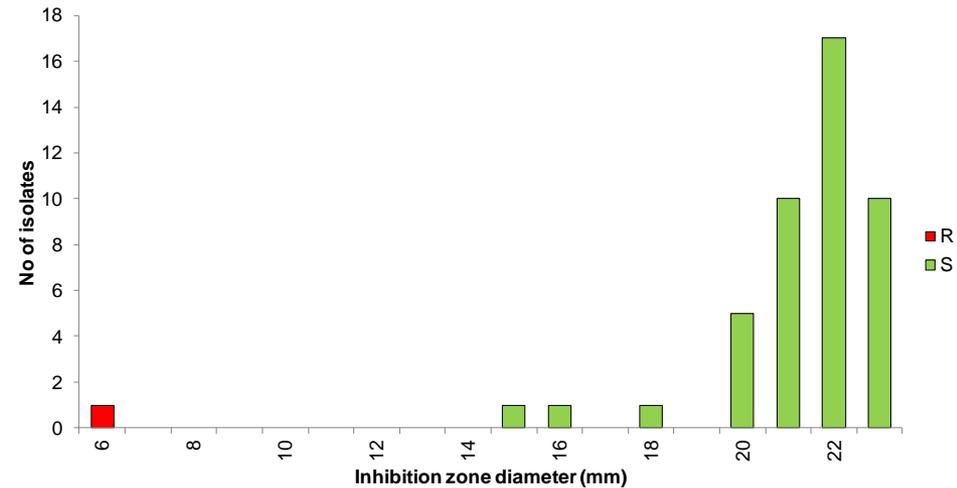
nitrofurantoin

18h CBP

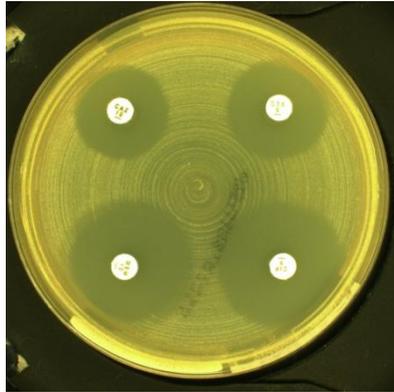
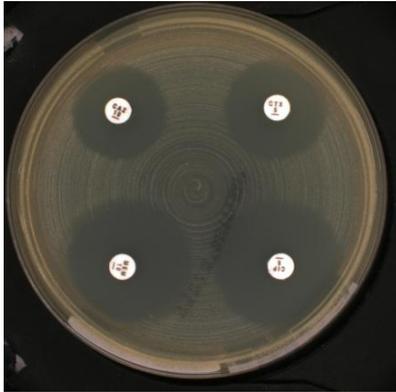
$S \geq 15$

$R < 15$

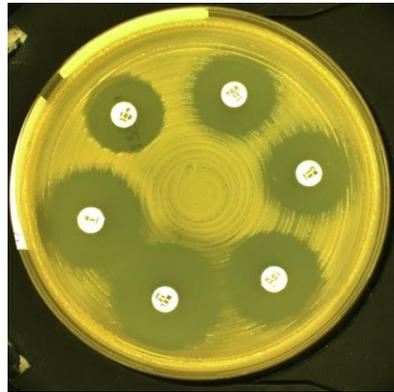
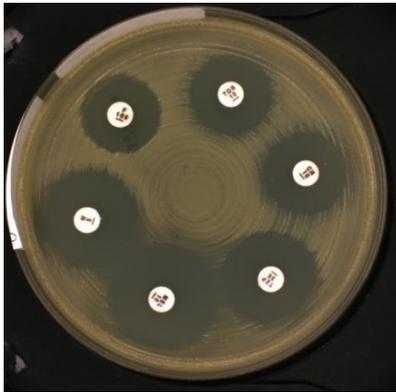
Nitrofurantoin 100 µg, 8h incubation
Enterococcus, 46 isolates



6 h vs 18 h incubation

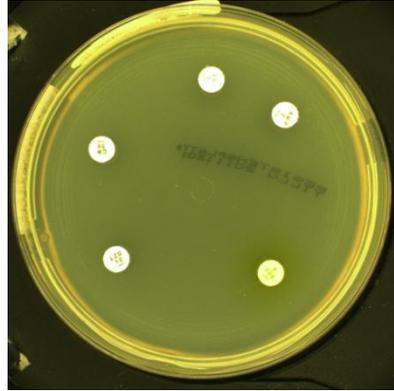
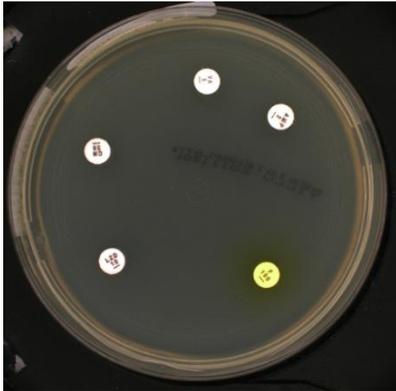


Escherichia coli ATCC 25922 after 6h incubation.

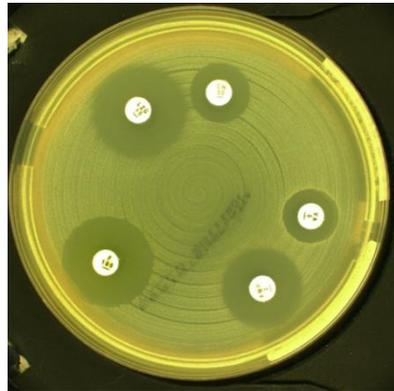
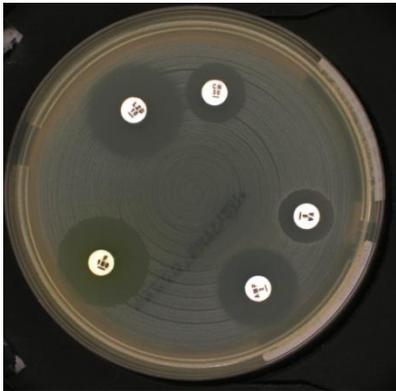


Escherichia coli ATCC 25922 and 18h incubation.

6 h vs 18 h incubation



Enterococcus faecalis ATCC 29212 after 6h incubation.



Enterococcus faecalis ATCC 29212 after 18h incubation.

ImagA -results

- ATCC 25922
- ATCC 29213
- Very unstable reading with the camera
- Our conclusion
 - It is a software problem not a cameraproblem
 - Manual adjustment of zones using the same pictures gives OK results

Conclusion

“It seems possible to standardise AST for 6-8 hours on MH agar. All species/antimicrobial combinations will probably not respond equally to a shorter incubation time and time-related zone diameter breakpoints might be needed.

A standardised rapid AST method is now developed within the Eurostar project.”