What are Beta-lactamases?

Proteins degrading Beta-lactam's

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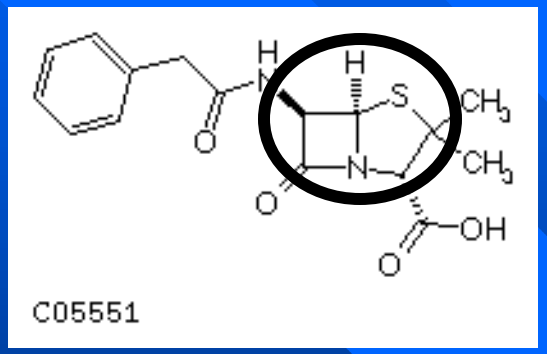
The Beta-lactam antibiotics

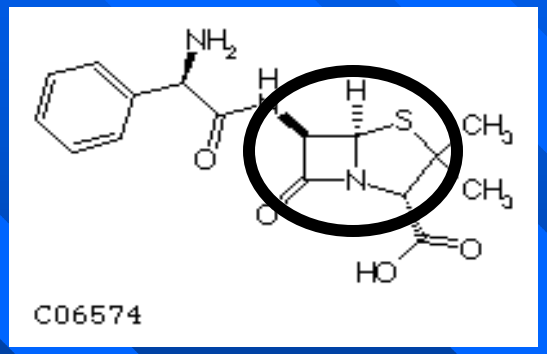
- Isolated from *Penicillium chrysogenum*
- App. 50 % of the antibiotics used worldwide
- The Beta-lactam group is constantly expanding
- Is now being produced semi-synthetically
- Kills growing cells by interfering with the cell-wall synthesis
- One of the most important human antibiotics.

Penicillins

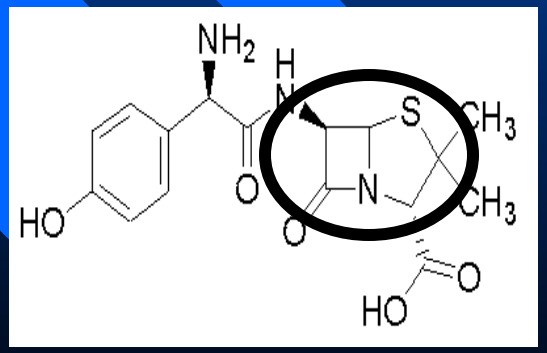
Penicillin G



Ampicillin (AMP)

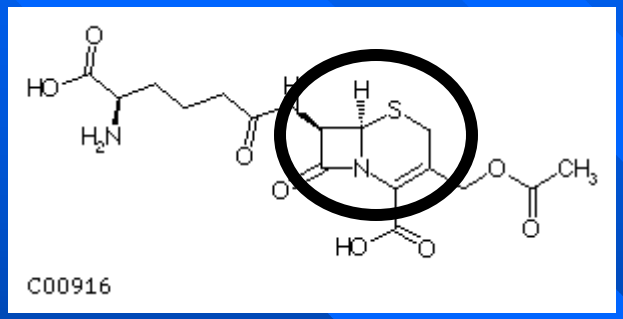


Amoxicillin

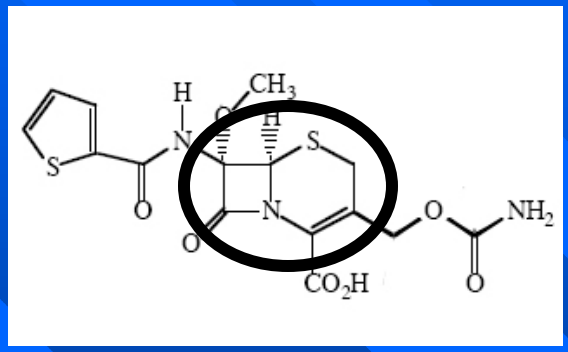


Cephalosporin's

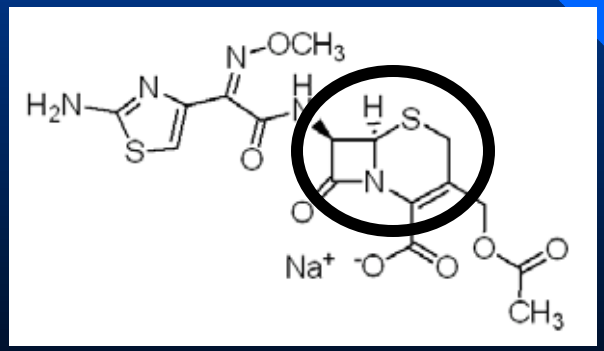
Cephalosporin C
(1. gen. Cephalosporin**)**



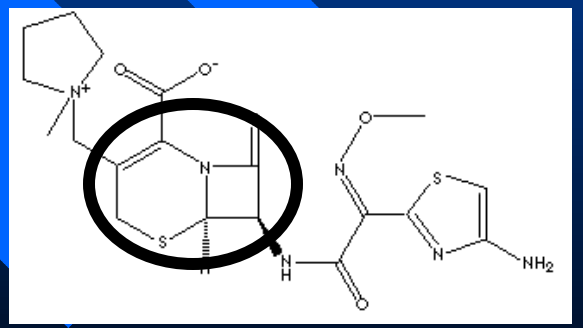
Cefoxitin (FOX)
(2. gen. cephamycin**)**



Cefotaxime (CTX)
(3. gen. Cephalosporin**)**

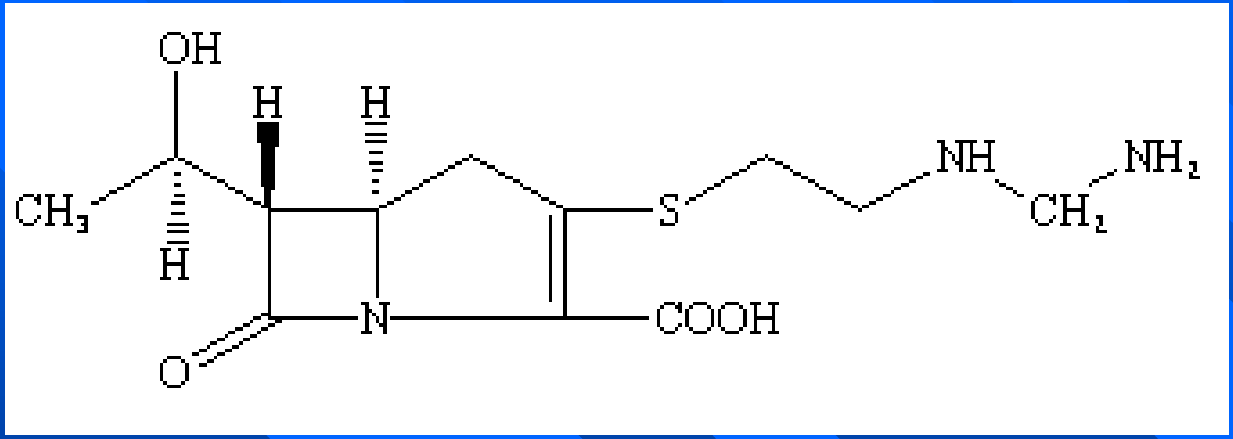


Cefepime (FEB)
(4. gen. cephalosporin**)**

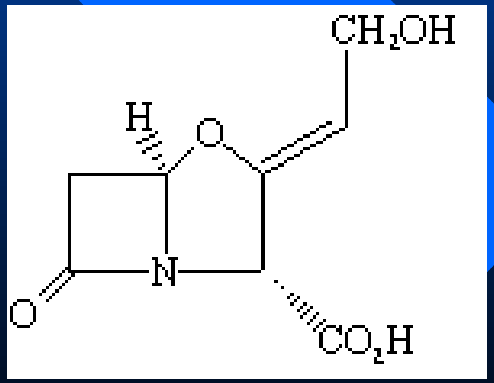


Carbapenem's og inhibitors

Imipenem (IMP)
Carbapenem



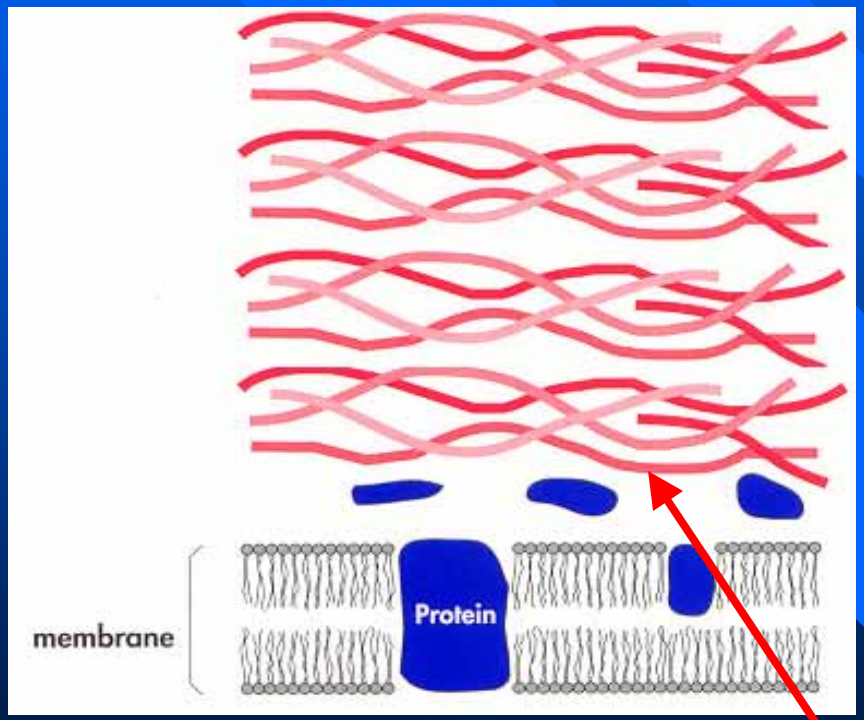
Clavulanic acid (CLA)
Inhibitor

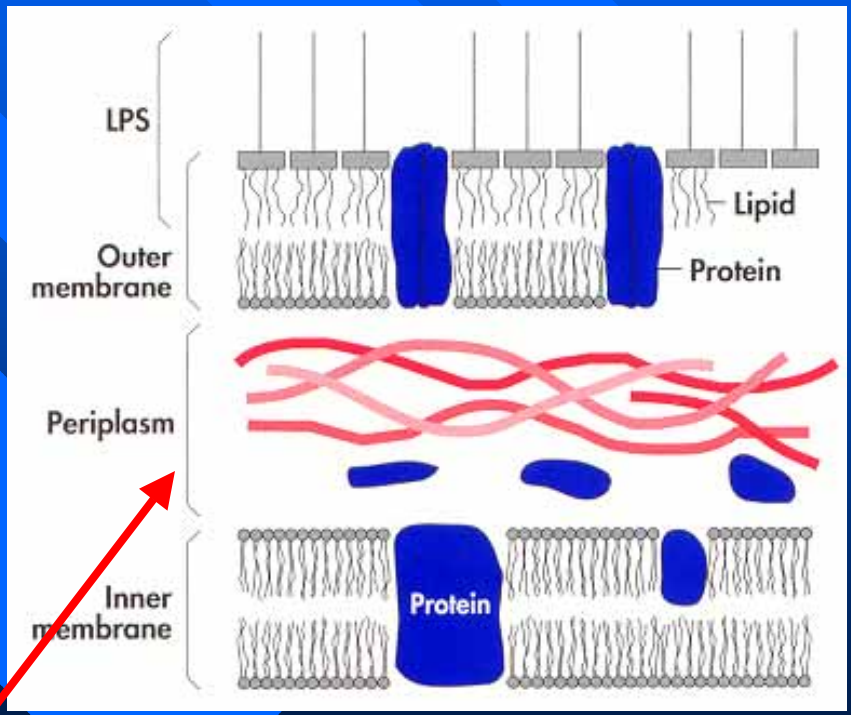


Bakterial cellwall

Gram positive

Gram negative





Peptidoglycan

Narrow spectrum vs. Extended spectrum Beta-lactam's

Narrow and moderate spectrum BL's

- Penicillin G and V (PEN)
- Methicillin (MET)
- amoxicillin (AMOX) and ampicillin (AMP)
- Cephalotin (CEP)

Broad and Extended spectrum BL's

- Cefoxitin (FOX)
- Cefotaxime (CTX) and Ceftazidime (CAZ)
- Cefepime (FEB)
- Imipenem (IMI)

What are ESBL's then?

- Able to degrade Broad and extended spectrum beta-lactam's
- Divided into: **ampC's,** "True **ESBL"** and **Metallo-BL's.**
- First identified 22 years ago (SHV-2).
- Different affinities to different beta-lactam's.
- ESBL and plasmidic ampC's mainly i *Enterobacteriaceae.*
- Metallo-BL mainly i *Pseudomonas.*
- now > 200 different genes.
- Approximately 20 different groups.
- Big difference in homology.
- Seen in all environments where Extended spectrum beta-lactam's are used.

Beta-lactamase genes so far….

TEM CMY FOX VEB KLU
SHV MOX DHA CME FEC
OXA PSE ACC GES LAT
CTX FOX PER TLA …….

Pitfalls:

- Reduced susceptibility can be caused by up-regulated efflux-pumps or defective influx pumps.
- *E. coli* carries a down-regulated *ampC* beta-lactamase, which can be activated (up-regulated) by two mutations.

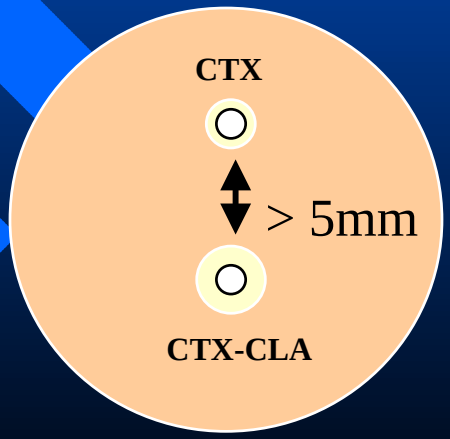
Plasmidic AmpC's	ESBL	MBL
CMY	**TEM**	**IMP**
ACC	**SHV**	**VIM**
DHA	**OXA**	SPM
FOX	**CTX-M**	GIM
BIL	VEB	
MIR	PER	
ACT	CME	
KLU	SFO	
	FEC	
	GES	

Genes in yellow indicate most prevalent types!

The three different ESBL groups

The 'True' ESBL's:

- Often located on transferable plasmids/elements
- often found in bacteria lacking a chromosomal AmpC's
- rarely resistant to inhibitors (results in the 'synergy effect')
- resistant to both 3. and 4. generation ceph's
- Inducible by beta-lactams.

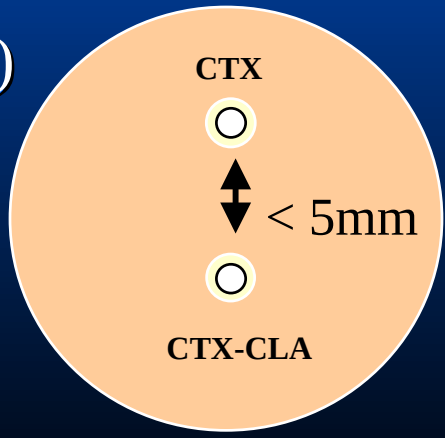


Synergy!

The three different ESBL groups

AmpC's

- Often located on chromosomes (*E. coli*, *Citrobacter*, *Enterobacter*)
- ...or on plasmids but originating from chromosomal versions
- confers resistance to beta-lactam inhibitors (thus no '*synergy*')
- confers resistance to cefoxitin (FOX); a 2. gen. cephamycin)
- sensitive to 4. gen. ceph's (like cefepime (FEB))
- Not inducible by beta-lactams.



No synergy!

The three different ESBL groups

Metallo beta-lactamases:

- Can be inhibited by metal chelators (like EDTA)
- mainly found in Pseudomonas
- confers resistance to all generations of ceph's
- confers resistance to carbaphenems like Imipenem
- rarely found in Enterobacteriaceae.

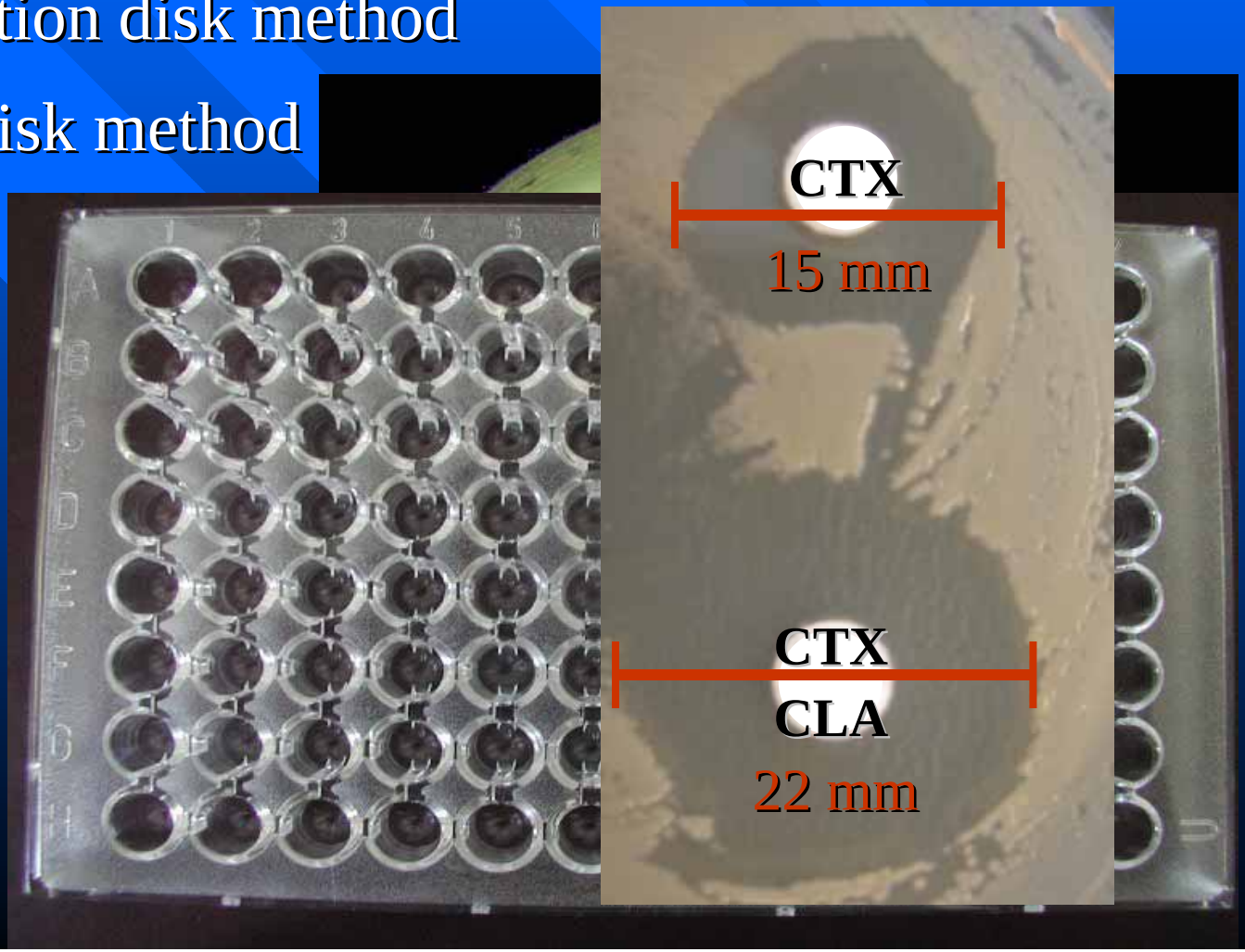
Phenotypic differences of the groups

Phenotype	ampC	ESBL	MBL
Resistance to 3. Gen. Ceph's?	YES	YES	YES
Inhibited by Clavulanic acid?	NO	YES[1]	NO
Inhibited by EDTA?	NO	NO	YES
Resistance to cephamycins (cefoxitin)?	YES	NO	YES
Resistance to 4. Gen. Ceph's?	NO	YES	YES
Resistance to carbaphenem's?	NO	NO	YES

[1]Some (especially TEM's) are inhibitor resistant!

How to detect ESBL I?

- Phenotypically:
  - Combination disk method
  - Double disk method
  - MIC test
  - E-test



How to detect ESBL II?

Method (CLSI 2007)	Initial Screen Test	Phenotypic Confirmatory Test
Antimicrobial concentration	Cefpodoxime 4 µg/ml or Ceftazidime 1 µg/ml or Aztreonam 1 µg/ml or Cefotaxime 1 µg/ml or Ceftriaxone 1 µg/ml (The use of more than one antimicrobial agent for screening will improve the sensitivity of detection).	Ceftazidime 0.25-128 µg/ml Ceftazidime+clav. 0.25/4-128/4 µg/ml **And** Cefotaxime 0.25-64 µg/ml Cefotaxime+clav. 0.25/4-64/4 µg/ml (Confirmatory testing requires use of both cefotaxime and ceftazidime alone and in combination with clavulanic acid).

Breakpoints (CLSI): Ceftazidime: S: ≤ 8; I: 32; R: ≥ 32

Cefotaxime: S: ≤ 8; I: 16-32; R: ≥ 64

Breakpoints (EFSA): Cefotaxime: R: ≥ 0.5

Synergy (CLSI):A ≥ 3 two-fold reduction in MIC (e.g. from 8 to 1 µg/ml)

Screening for ESBL at the CRL

Primary screening : (MIC)

- Ampicillin
- Amoxicillin + clavulanic acid
- Cephalothin (1. generation cephalosporin)

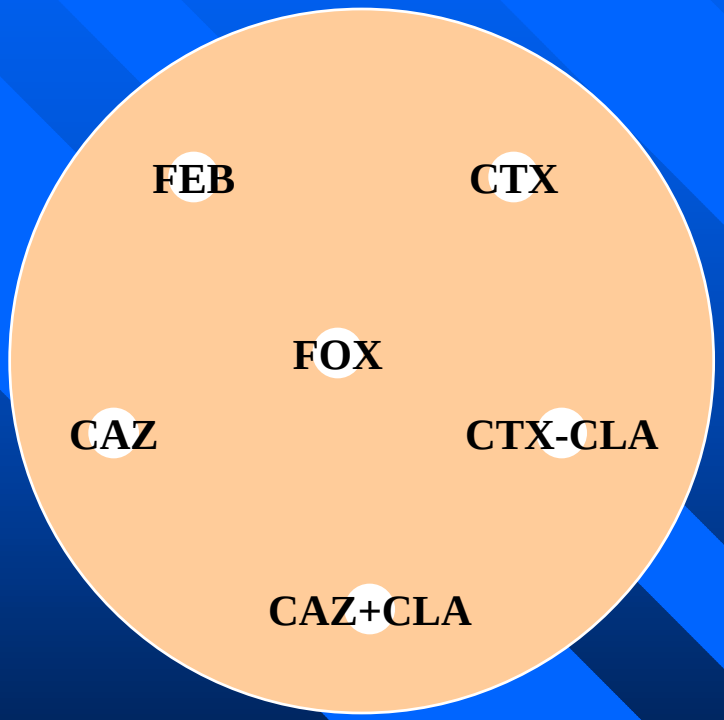
- Cefpodoxime (3. generation cephalosporin)
- Ceftiofur (3. generation cephalosporin)

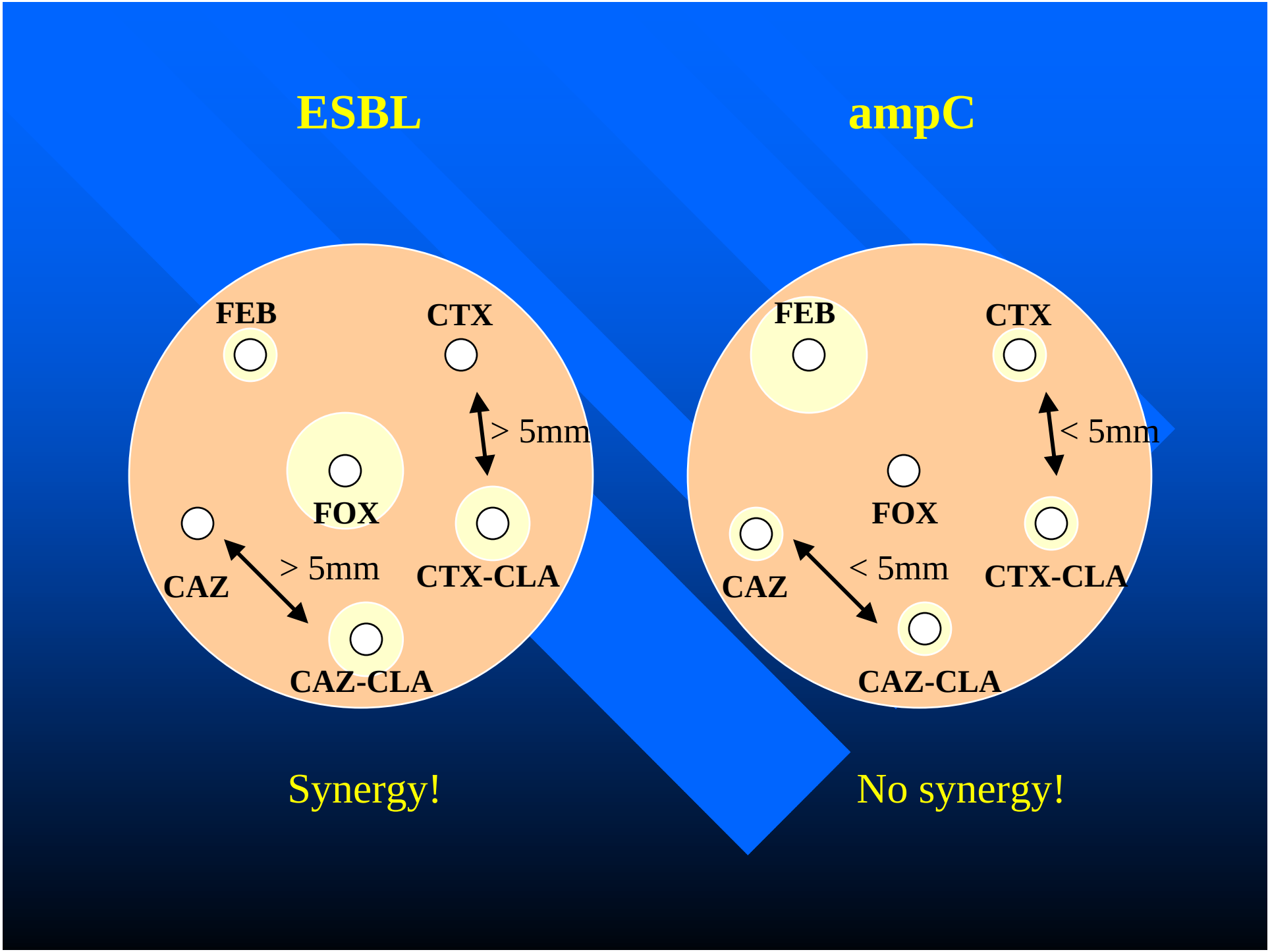
↓

Secondary : screening (Disc's)

- Ceftazidime (CAZ - 3. gen.)
- CAZ + CLA (CAZ-CLA + inhibitor)
- Cefotaxime (CTX - 3. gen.)
- CTX + CLA (CTX-CLA + inhibitor)
- Cefoxitin (FOX – 2. gen. cephamycin)
- Cefepime (PER - 4. gen.)

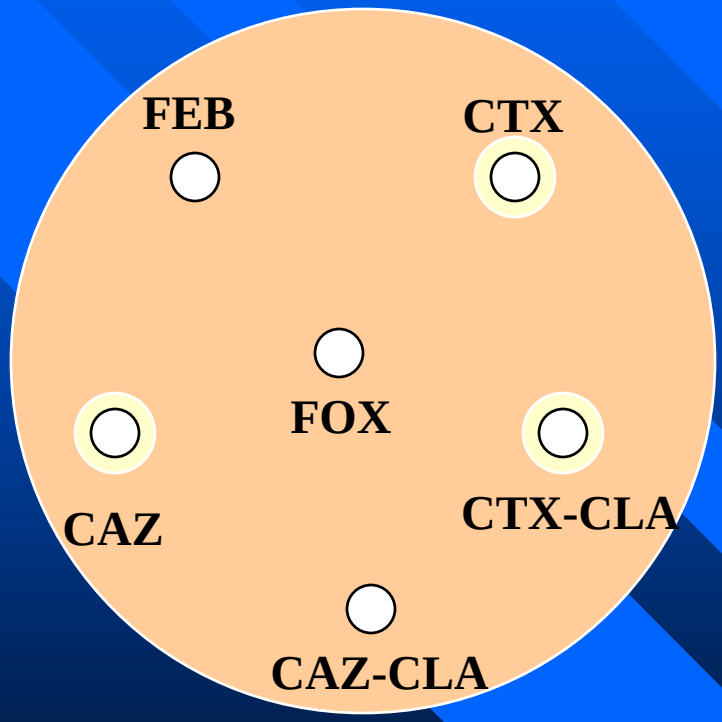
ESBL-tablet assay




ESBL
ampC
FEB
CTX
> 5mm
FOX
CAZ
> 5mm
CTX-CLA
CAZ-CLA
FEB
CTX
< 5mm
FOX
CAZ
< 5mm
CTX-CLA
CAZ-CLA
Synergy!
No synergy!

BUT BUT BUT…….

Different beta-lactamases can have different affinity towards different Beta-lactams!



And one strain can easily have more than one beta-lactamase!

Table 3

Strain S 1.3 **Strain S 1.4** **Strain S 1.6**

Cephalosporines / strains	Strain #3				Strain #4				Strain #5			
	ESBL not detected		ESBL detected		ESBL not detected		ESBL detected		ESBL not detected		ESBL detected	
	Number, n:	Percentages, %	Number, n:	Percentages, %	Number, n:	Percentages, %	Number, n:	Percentages, %	Number, n:	Percentages, %	Number, n:	Percentages, %
CTX, CAZ, XNL	1	17%	5	83%	0	0%	6	100%	0	0%	6	100%
CTX, CAZ	4	50%	4	50%	2	25%	6	75%	0	0%	8	100%
CTX, XNL	2	33%	4	67%	0	0%	5	100%	0	0%	5	100%
CTX	1	33%	2	67%	0	0%	4	100%	0	0%	4	100%
XNL	0	0%	4	100%	0	0%	4	100%	0	0%	5	100%
CTX/CI:CTX	2	33%	4	67%	1	17%	5	83%	0	0%	6	100%
CAZ/CI:CAZ	0	0%	0	0%	2	33%	4	67%	0	0%	6	100%

CTX-M-9 **CTX-M-14** **CTX-M-1**

Future project on phenotypic detection and characterzation of ESBL

- ≈250 well-characterized *E. coli* and *Salmonella* isolates
  - 50 AMP^S *E. coli* and 50 AMP^S *Salmonella* isolates
  - 25 AMP^R *E. coli* and 25 AMP^R *Salmonella* isolates
  - 25 *ampC* up-regulated *E. coli*
  - 75 geno-typed ESBL resistant *E. coli* and *Salmonella*
- 8 relevant veterinary and human cephalosporin's
- Long-rang MIC testing as well as disc diffusion testing
- Two different laboratories (FOOD-DTU and CIDC-Lelystad)

Future project on phenotypic detection and characterzation of ESBL

	MIC (TREK)	Disc's (BD)
Cefoperazone	[0.06-128]	75 µg
Cefotaxime	[0.015-32]	30 µg
Ceftiofur	[0.06-128]	30 µg
Ceftriaxone	[0.015-32]	30 µg
Cefquinome	[0.015-32]	30 µg
Cefuroxime	[0.12-128]	30 µg
Cefpodoxime	[0.06-64]	10 µg
Ceftazidime	[0.03-32]	30 µg

REQUEST: Please inform us, in case you have ESBL resistant *E. Coli* or *Salmonella* isolates with KNOWN resistance genes!

Genotypic detection of the groups

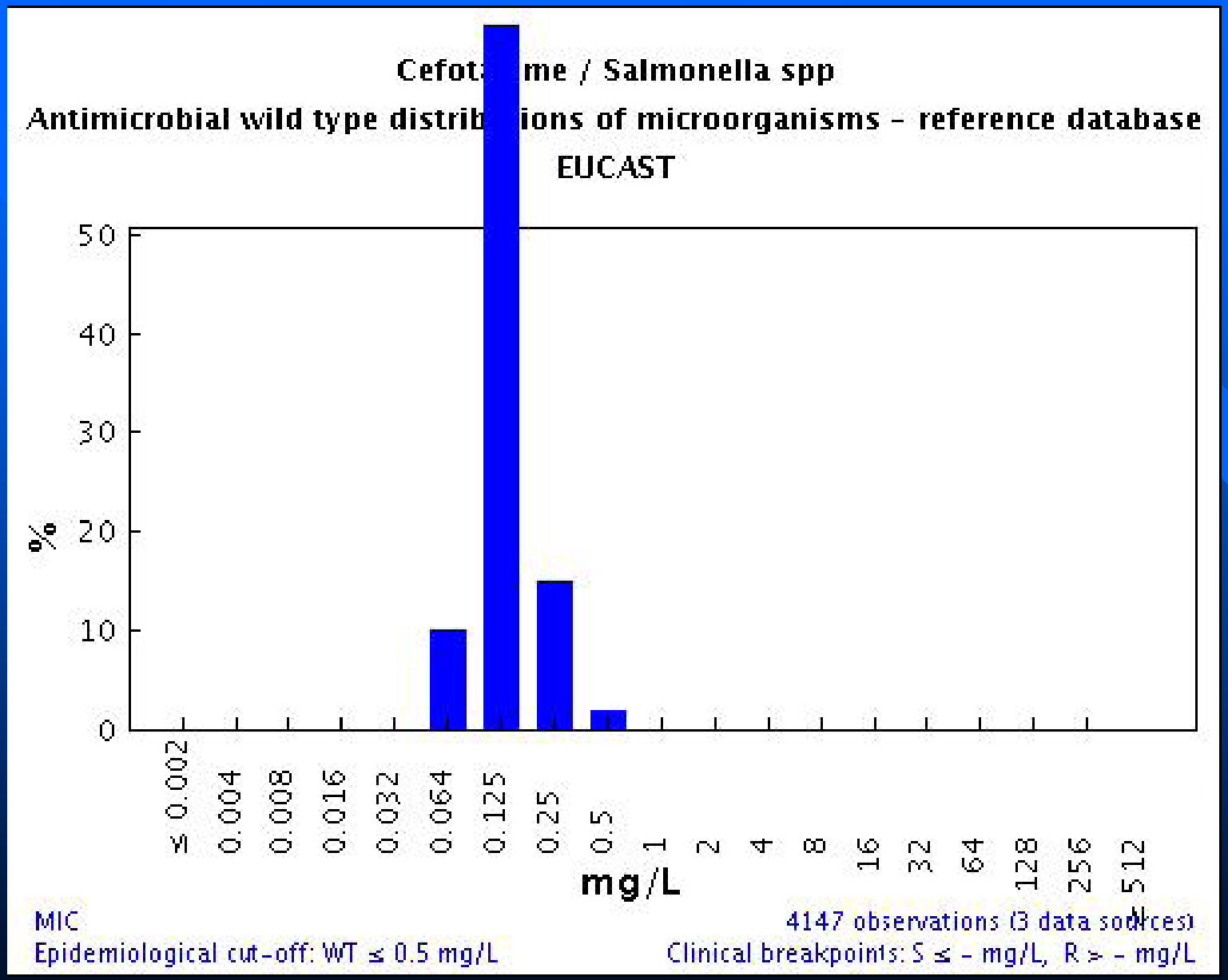
- PCR
- Southern blotting (RLB)
- Microarray
- Cloning and sequencing

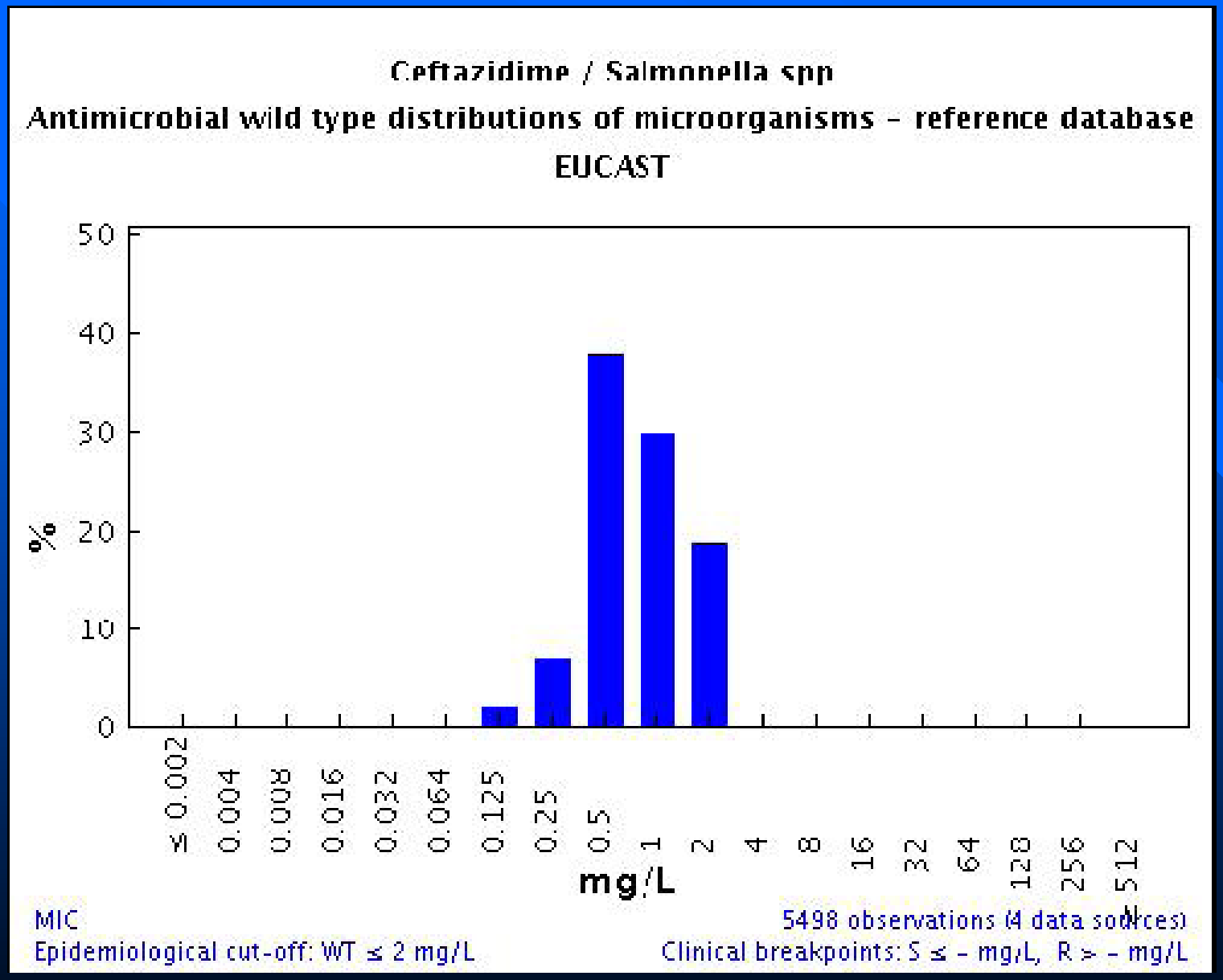
E. Coli from Danish veterinary submissions in 2006

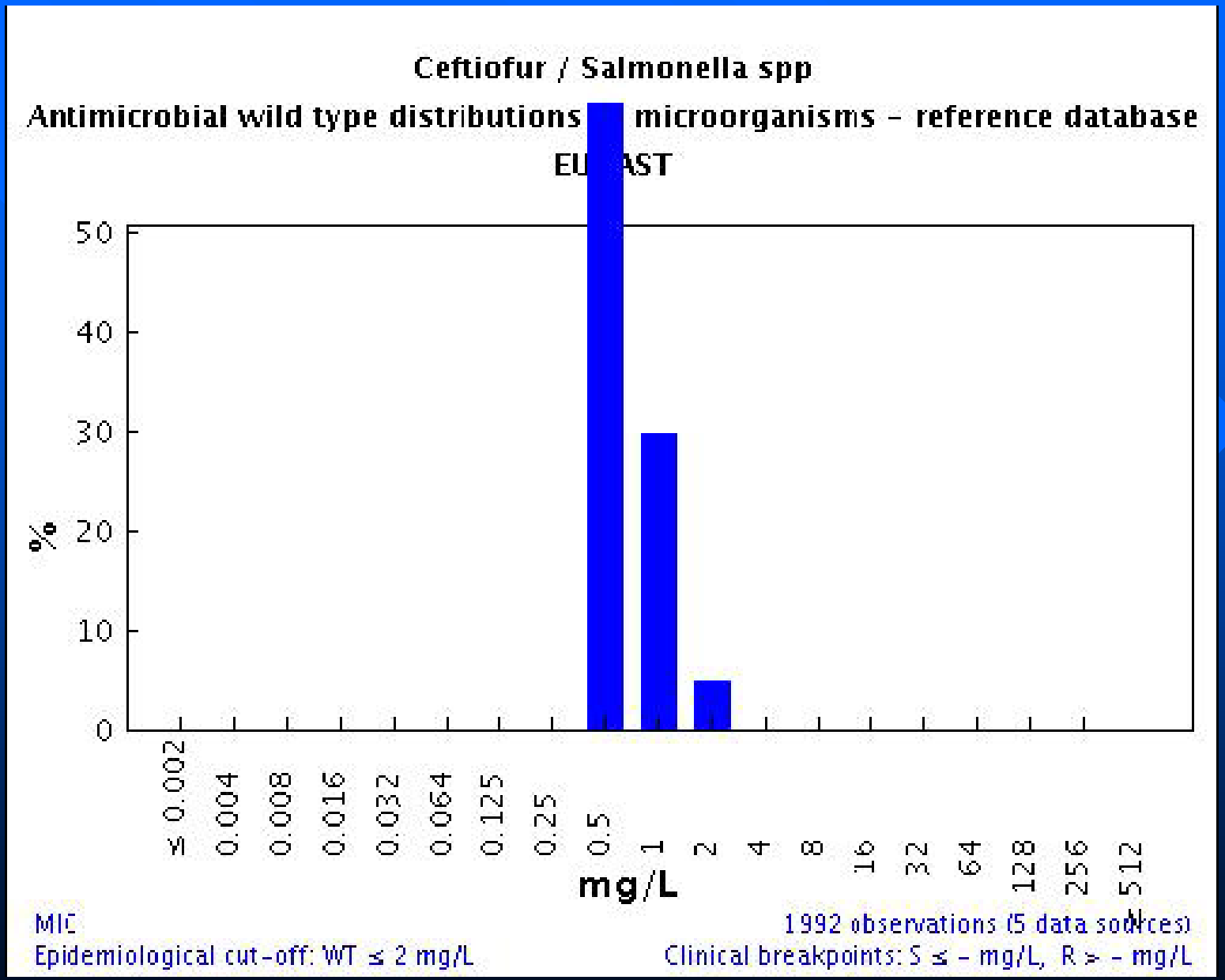
	Cefpodoxime (µg/ml)	Ceftiofur (µg/ml)	Amox / clav. (µg/ml)	Cefotaxime (Disc's)	Ceftazidime (Disc's)
Type 1 (n=11)	>4	=8 / ≥8	4/2 → 16/8	R	S / I / R
Type 2 (n=9)	>4	1 / 2	≥ 32/16	S	S / I / R

Type 1: "True ESBL's" (CTX-M1, CTX-M-2 or CTX-M-9)

Type 2: Chromosomal *ampC* up-regulators.


Cefot me / Salmonella spp
Antimicrobial wild type distrib ions of microorganisms - reference database
EUCAST
%
50
40
30
20
10
0
≤ 0.002
0.004
0.008
0.016
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
32
64
128
256
512
mg/L
MIC
Epidemiological cut-off: WT ≤ 0.5 mg/L
4147 observations (3 data sources)
Clinical breakpoints: S ≤ - mg/L, R > - mg/L
N


Ceftazidime / Salmonella spp
Antimicrobial wild type distributions of microorganisms - reference database
EUCAST
50
40
30
20
10
0
%
≤ 0.002
0.004
0.008
0.016
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
32
64
128
256
512
mg/L
MIC
Epidemiological cut-off: WT ≤ 2 mg/L
5498 observations (4 data sources)
Clinical breakpoints: S ≤ - mg/L, R > - mg/L


Ceftiofur / Salmonella spp
Antimicrobial wild type distributions microorganisms - reference database
EU AST
50
40
30
20
10
0
%
≤ 0.002
0.004
0.008
0.016
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
32
64
128
256
512
mg/L
MIC
Epidemiological cut-off: WT ≤ 2 mg/L
1992 observations (5 data sources)
Clinical breakpoints: S ≤ - mg/L, R > - mg/L