

# NordicASTs workshop TIRSDAG 27. maj 2014

Aulan på NHV, Nya varvet byggnad 25

GRUNDLÆGGENDE OM RESISTENS HOS GRAMNEGATIVE STAVE  
(enterobakterier: betalaktamer, kinoloner og aminoglycosider)

## **AGENDA:**

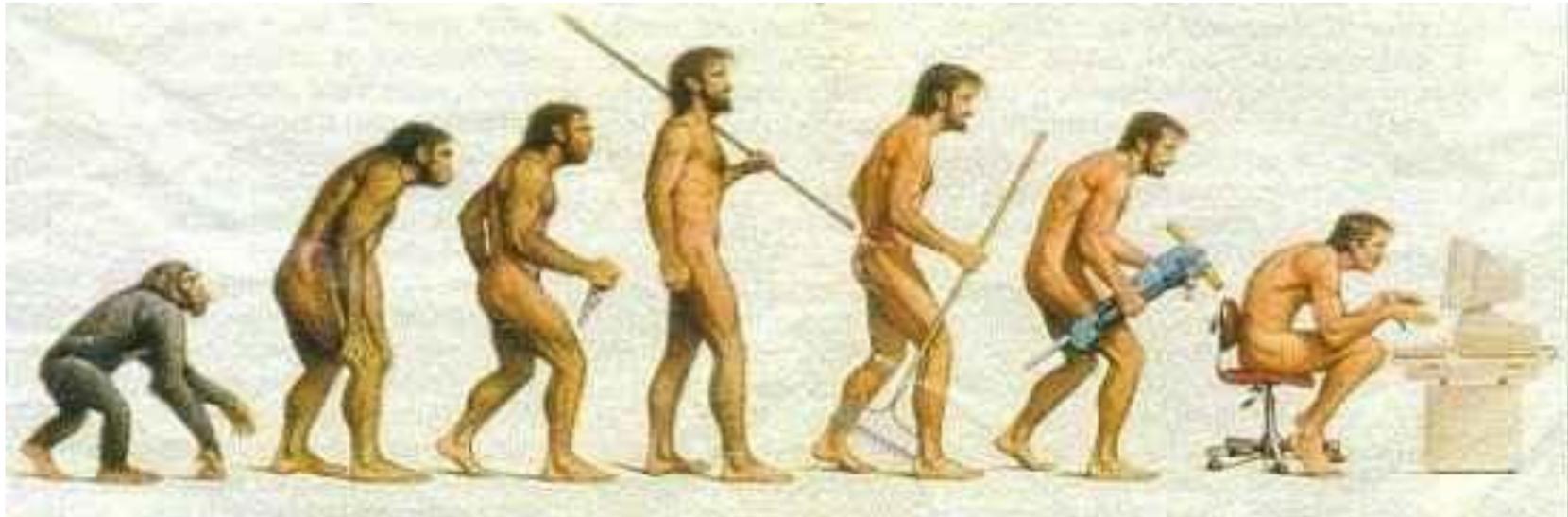
10.00 – 10.20 Betalaktamaser, ESBL og AmpC (Dennis Hansen)

10.20 – 10.30 Karbapenemaser (Mikala Wang)

10.30 – 10.45 Kinoloner (Mikala Wang)

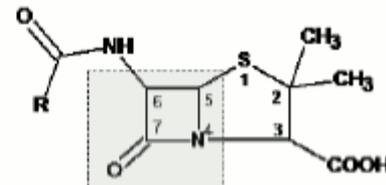
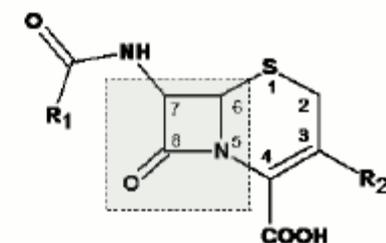
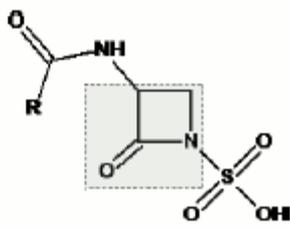
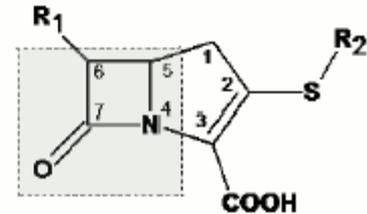
10.45 – 11.00 Aminoglycosider (Ørjan Samuelson)

# Grundlæggende om beta-laktam resistens, ESBL og AmpC

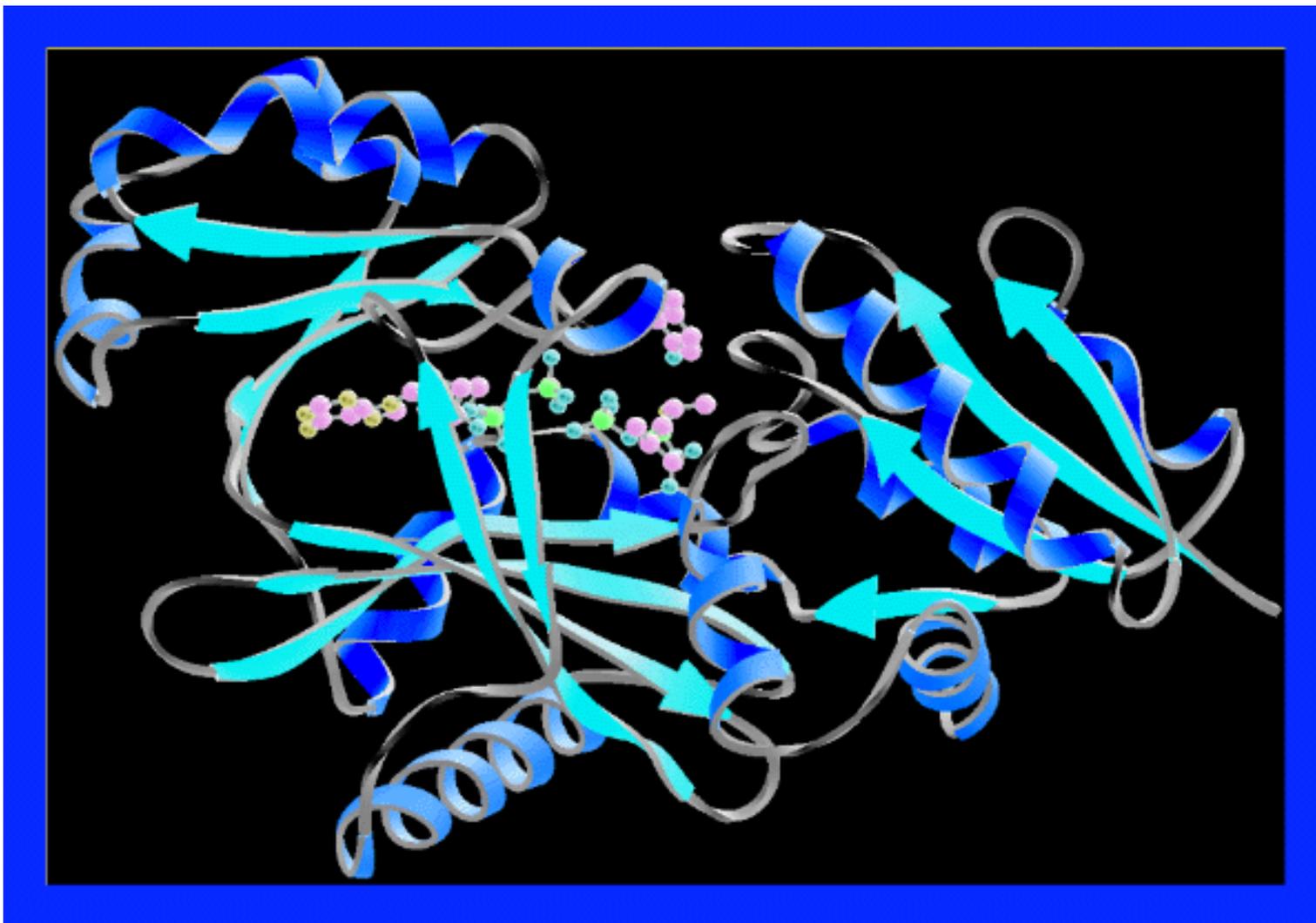


Dennis Hansen  
Klinisk mikrobiologisk afdeling  
Herlev Hospital

For alle  $\beta$ -laktamer...

Group	Structure
Penicillins	 <p>The diagram shows the penam nucleus of penicillins, consisting of a fused four-membered beta-lactam ring and five-membered thiazolidine ring. The beta-lactam ring is numbered 4, 5, 6, 7, with a carbonyl group at C6 and an NH group at C5. The thiazolidine ring is numbered 1, 2, 3, 4, with a sulfur atom at C1, a methyl group at C2, and a carboxylic acid group at C3. A side chain R is attached to the nitrogen of the beta-lactam ring.</p>
Cephalosporins	 <p>The diagram shows the cephalosporin nucleus, consisting of a fused four-membered beta-lactam ring and six-membered dihydrothiazine ring. The beta-lactam ring is numbered 4, 5, 6, 7, with a carbonyl group at C6 and an NH group at C5. The dihydrothiazine ring is numbered 1, 2, 3, 4, with a sulfur atom at C1, a carboxylic acid group at C3, and a side chain R2 at C4. A side chain R1 is attached to the nitrogen of the beta-lactam ring.</p>
Monobactams	 <p>The diagram shows the monobactam nucleus, consisting of a four-membered beta-lactam ring fused to a five-membered imidazolidinone ring. The beta-lactam ring is numbered 4, 5, 6, 7, with a carbonyl group at C6 and an NH group at C5. The imidazolidinone ring is numbered 1, 2, 3, 4, with a sulfur atom at C1 and a carboxylic acid group at C3. A side chain R is attached to the nitrogen of the beta-lactam ring.</p>
Carbapenems	 <p>The diagram shows the carbapenem nucleus, consisting of a fused four-membered beta-lactam ring and five-membered dihydrothiazine ring. The beta-lactam ring is numbered 4, 5, 6, 7, with a carbonyl group at C6 and an NH group at C5. The dihydrothiazine ring is numbered 1, 2, 3, 4, with a sulfur atom at C1, a carboxylic acid group at C3, and a side chain R2 at C4. A side chain R1 is attached to the nitrogen of the beta-lactam ring.</p>

# ...er der en $\beta$ -laktamase



- vigtigste årsag til resistens overfor  $\beta$ -laktamer

# The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens

Kevin J. Forsberg,<sup>1\*</sup> Alejandro Reyes,<sup>1\*</sup> Bin Wang,<sup>1,2</sup> Elizabeth M. Selleck,<sup>3</sup>  
Morten O. A. Sommer,<sup>4,5†</sup> Gautam Dantas<sup>1,2†</sup>

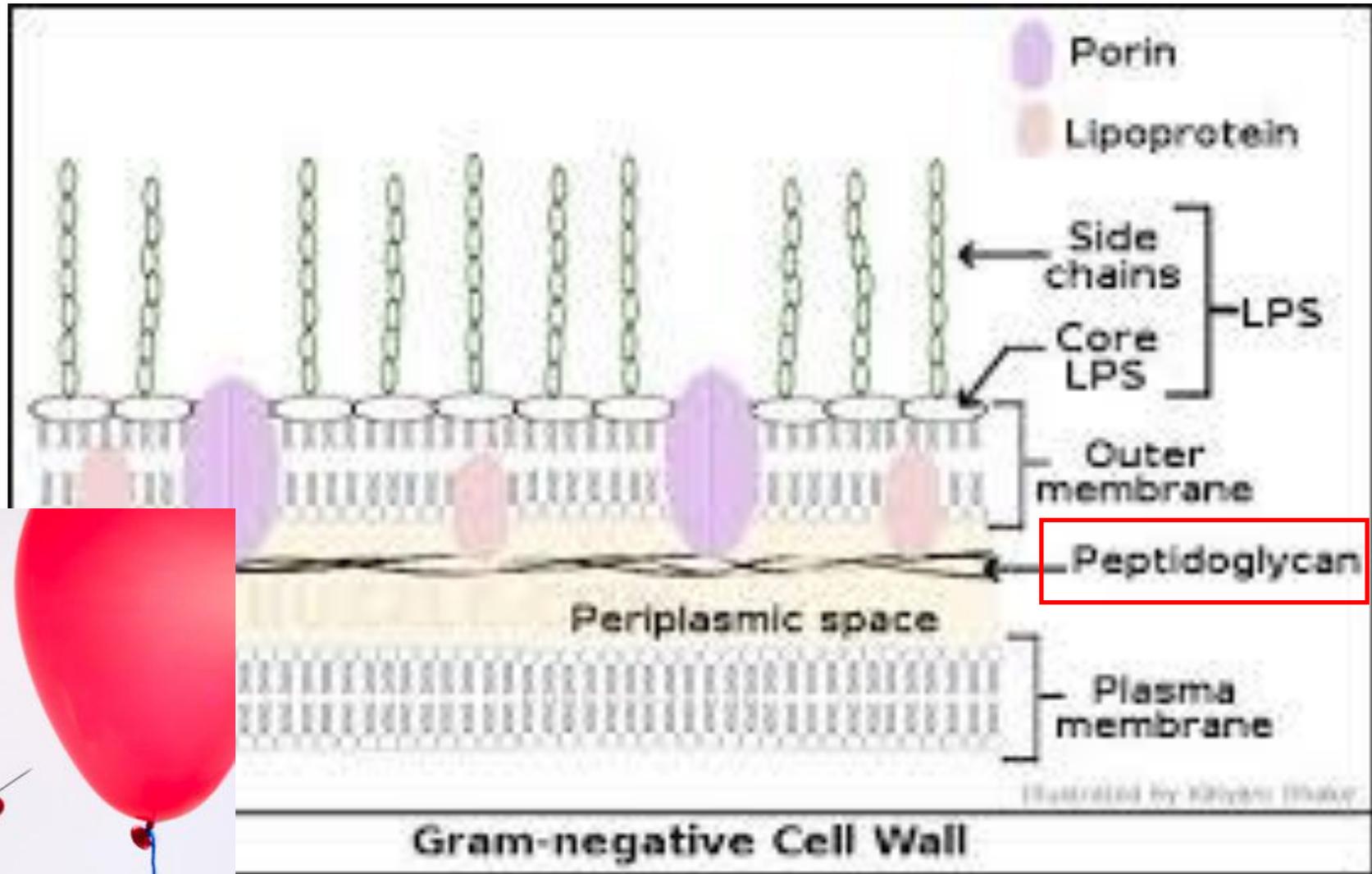
Soil microbiota represent one of the ancient evolutionary reservoirs of antibiotic resistance genes proposed as a reservoir of resistance genes available for

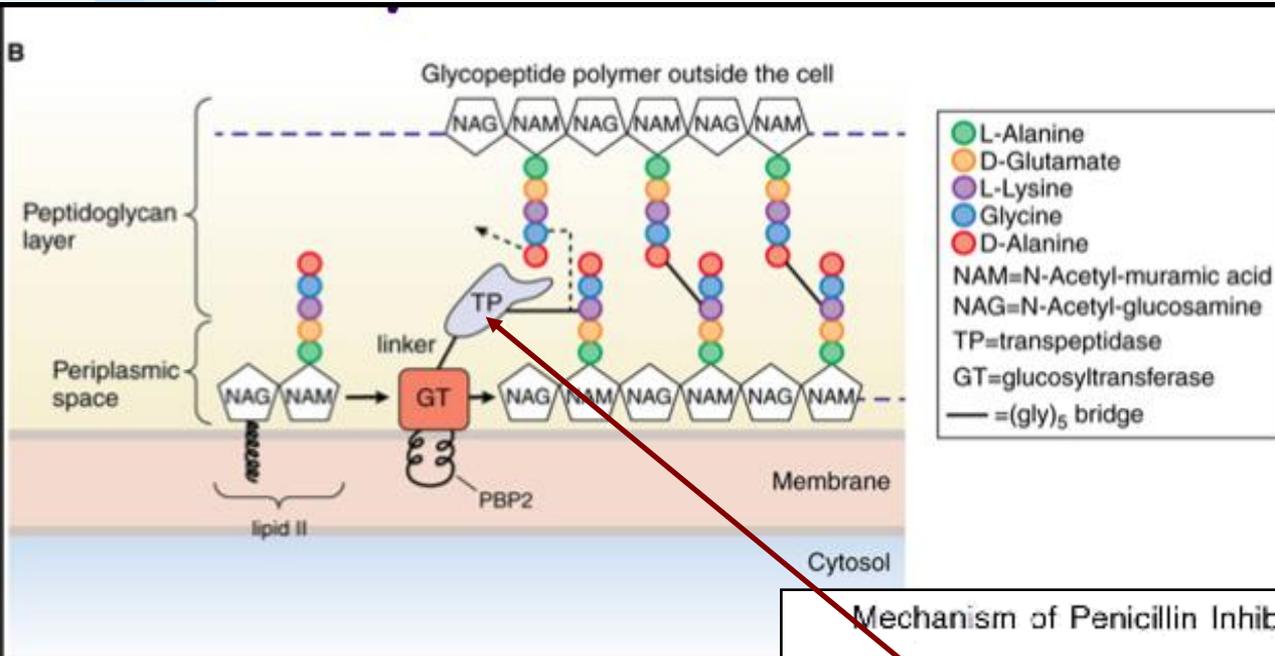
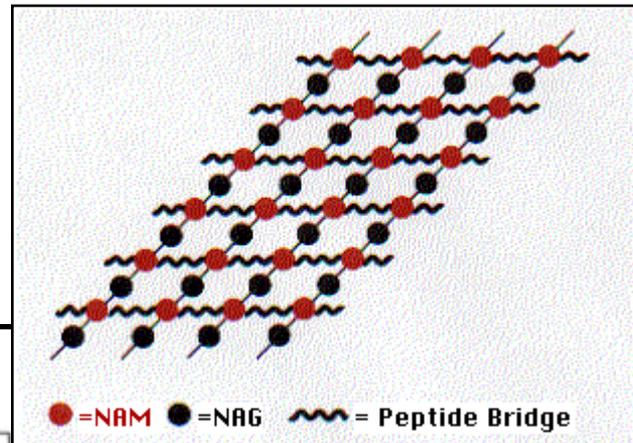
are rare (4, 5). The two documented examples are of *Kluyvera* and *Shewanella* isolates, which are found free-living in environmental settings (5, 6) yet have resistance genes (CTX-M  $\beta$ -lactamase and *qnrA* genes, respectively) with high identity (100% identity in clinical *Kluyvera* isolates) to those of pathogens (4, 5). The limited examples of

**Table 1.** Nonredundant antibiotic resistance genes with 100% identity to known human pathogens.

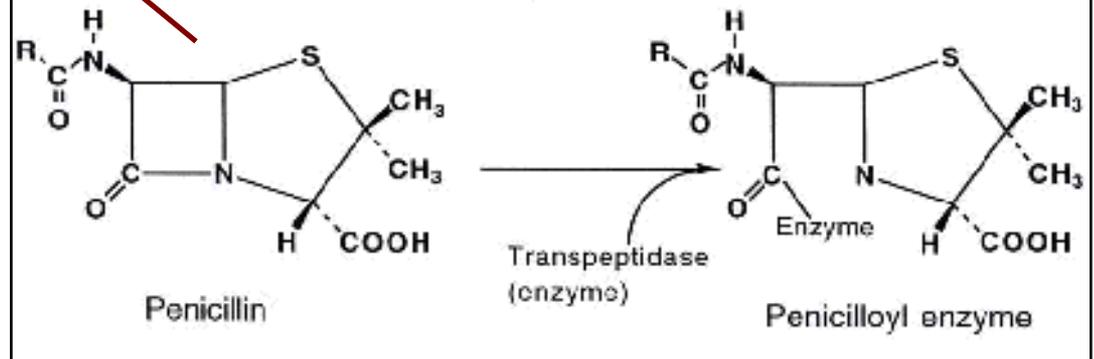
Gene name	GenBank ID	Number of selections*	Antibiotic class	Annotation [mechanism]	Pathogens hit (GI number)
AB95_PI_68.1	JX009363	4	$\beta$ -lactam	blaP1 [enzymatic degradation]	<i>A. baumannii</i> (94960156), <i>K. pneumoniae</i> (114147191), <i>P. aeruginosa</i> (117321883), <i>S. typhimurium</i> (12719011), <i>P. mirabilis</i> (157674381)†
AB95_CH_13.1	JX009364	1	Amphenicol	Chloramphenicol efflux [efflux]	<i>A. baumannii</i> (169147133), <i>P. aeruginosa</i> (260677483)
AB95_TE_2.2	JX009366	3	Tetracycline	tetA(G) [efflux]	<i>A. baumannii</i> (169147133), <i>S. typhimurium</i> (12719011)
AB95_TE_1.1	JX009365	3	Tetracycline	tetA [efflux]	<i>A. baumannii</i> (169147133), <i>E. coli</i> (312949035), <i>K. pneumoniae</i> (290792160), <i>S. typhimurium</i> (37962716)†
AB95_GE_3.3	JX009367 JX009373	2	Aminoglycoside	aadB [covalent modification]	<i>E. cloacae</i> (71361871), <i>K. pneumoniae</i> (206731403), <i>P. aeruginosa</i> (37955767), <i>S. typhimurium</i> (17383994)†
AB95_GE_3.1	JX009368 JX009374	2	Sulfonamide	sul1 [target modification]	<i>C. diphtheriae</i> (323714042) <i>E. cloacae</i> (71361871), <i>K. pneumoniae</i> (206731403), <i>P. aeruginosa</i> (37955767), <i>S. typhimurium</i> (17383994), <i>Yersinia pestis</i> (165913934)†
AB95_CH_21.1	JX009369	1	Aminoglycoside	aacA4 [covalent modification]	<i>A. baumannii</i> (164449567), <i>K. pneumoniae</i> (238865601), <i>P. aeruginosa</i> (219872982), <i>S. typhi</i> (34014739)†

\*Number of selections in which the entirety of a given gene was captured. †More pathogens exist for which 100% nucleotide identity was observed than listed



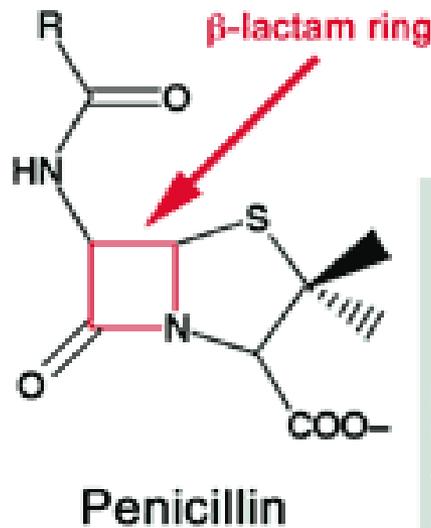


Mechanism of Penicillin Inhibition of Transpeptidase Enzyme



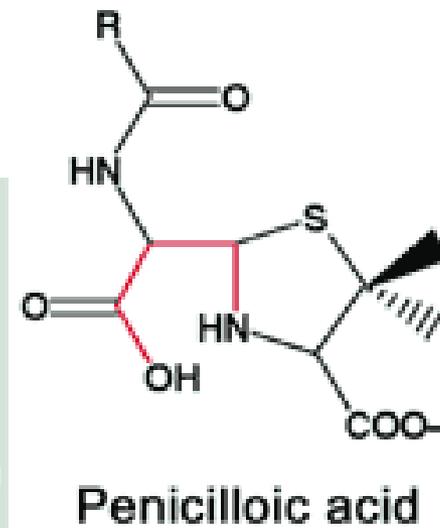


## Penicillin Resistance



$\beta$ -lactamase

$\beta$ -lactamase breaks a bond in the  $\beta$ -lactam ring of penicillin to disable the molecule. Bacteria with this enzyme can resist the effects of penicillin and other  $\beta$ -lactam antibiotics.



# ESBL ved punktmutation



©/MBP

**SHV-1**

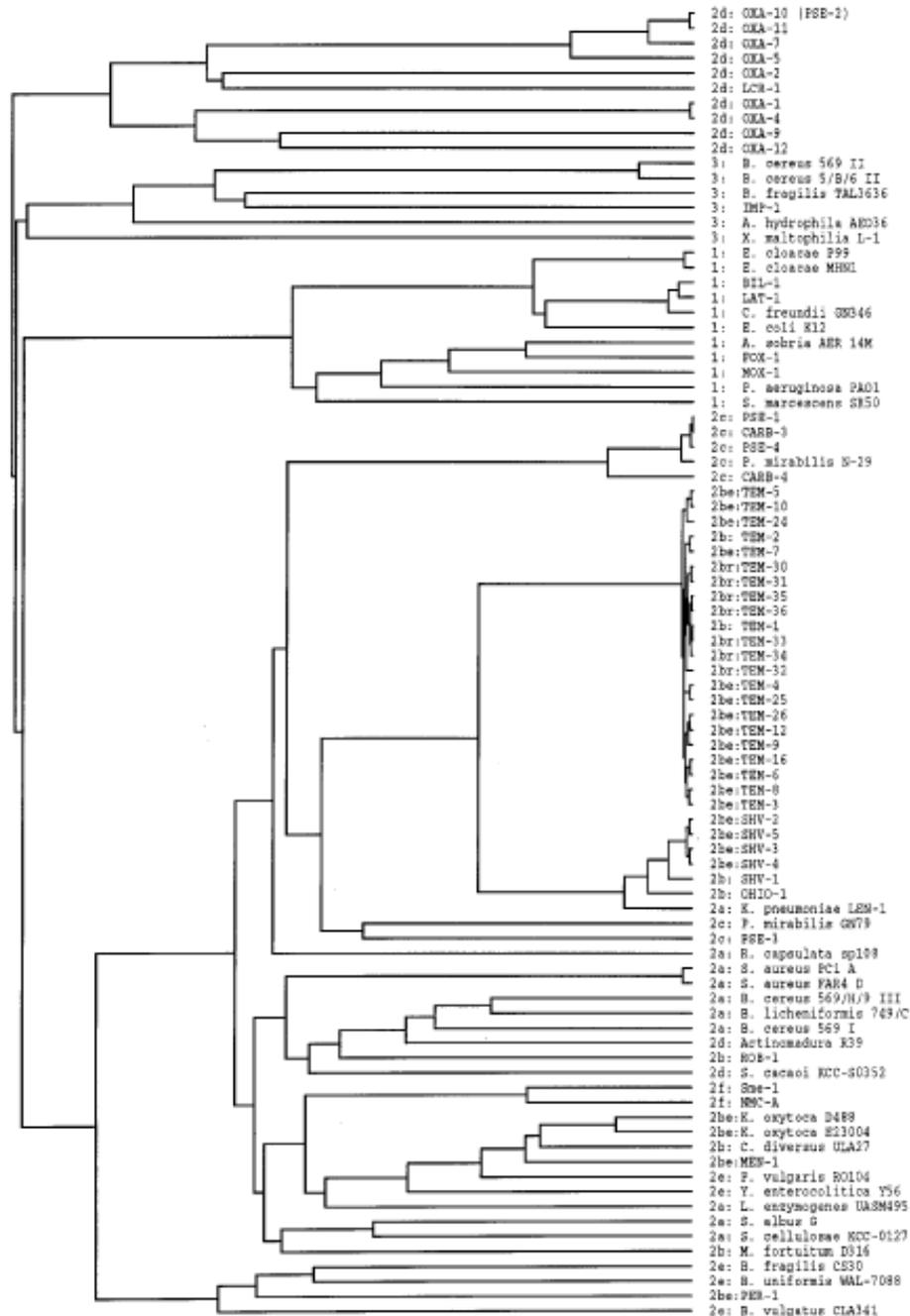
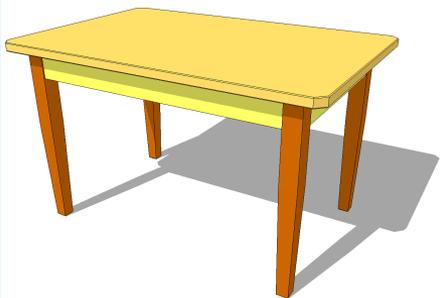
- ampicillin

**Mutation**(glycin til serin  
på position 238)**SHV-2**

- ampicillin
- cephalothin
- cefuroxim
- cefotaxim
- ceftazidim
- ceftriaxon
- aztreonam



©/MBP



Bush K, AAC  
 1995;39:1211-33

FIG. 1. Dendrogram showing relationships among  $\beta$ -lactamases clustered on the basis of structural similarities and their functional classification.

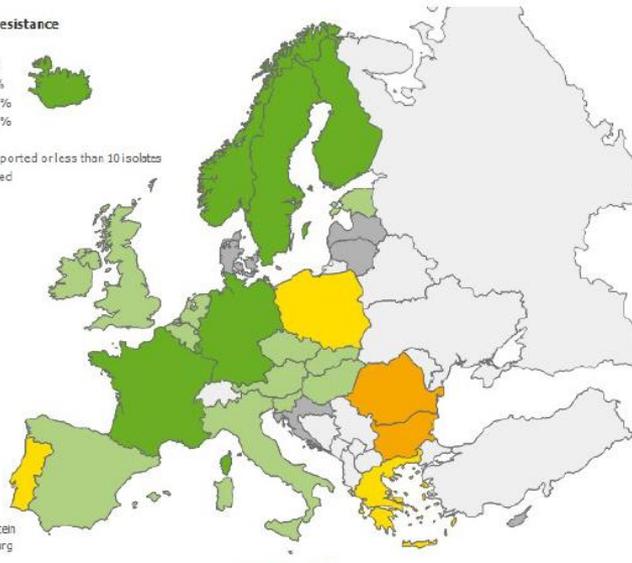
## β-lactamase classification

↓	↓												
Ambler	Bush	PEN	CARB	OXA	LOR	CTX	AZT	IMP	CA	AZT	EDTA	Note	
A	2a	+++	+	-	+/-	-	-	-	++	-	-	G+, S.aureus	
A	2b	+++	+	+	++	-	-	-	++	-	-	K.pneumoniae	
<b>A</b>	<b>2be</b>	+++	+	+	++	++	++	-	++	-	-	<b>ESBL</b>	
A	2br	++	+	+	+	-	-	-	-	-	-	IRT-1 (TEM-31), m.fl.	
A	2c	++	+++	+	+	-	-	-	+	-	-	A.hydrophila, P.mirabilis	
A	2e	++	++	-	++	++	++	-	++	-	-	P.vulgaris, C.diversus	
A	2f	++	+	?	+	+	++	++	+	-	-	Carbapenemase	
<b>C</b>	<b>1</b>	++	+/-	-	+++	+	-	-	-	++	-	<b>AmpC</b>	
D	2d	++	+	+++	+	-	-	-	V	-	-	A.sobria, OXA-enz.	
?	4	++	++	++	V	V	-	-	-	-	-	Penicillinase	
B	3	++	++	++	++	++	-	++	-	-	++	A.hydrophila, B.cereus	

### Proportion of 3rd gen. cephalosporins Resistant (R) *Escherichia coli* Isolates in Participating Countries in 2002

Percentage resistance

- < 1%
- 1 to < 5%
- 5 to < 10%
- 10 to < 25%
- 25 to < 50%
- ≥ 50%
- No data reported or less than 10 isolates
- Not included

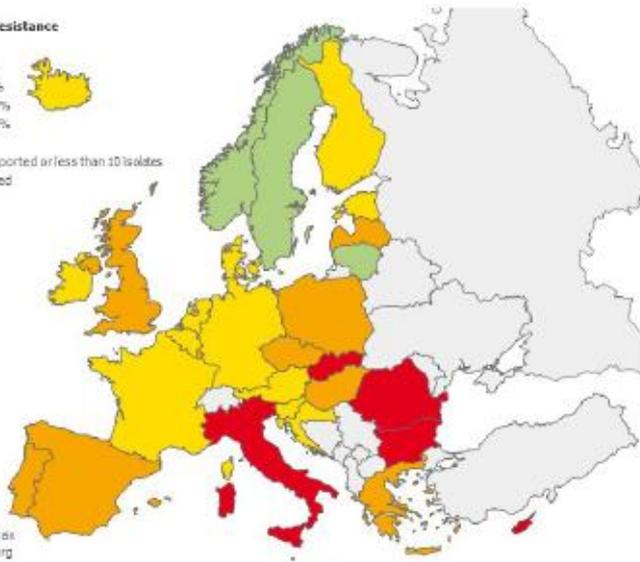


■ Liechtenstein  
■ Luxembourg  
■ Malta

### Proportion of 3rd gen. cephalosporins Resistant (R) *Escherichia coli* Isolates in Participating Countries in 2012

Percentage resistance

- < 1%
- 1 to < 5%
- 5 to < 10%
- 10 to < 25%
- 25 to < 50%
- ≥ 50%
- No data reported or less than 10 isolates
- Not included

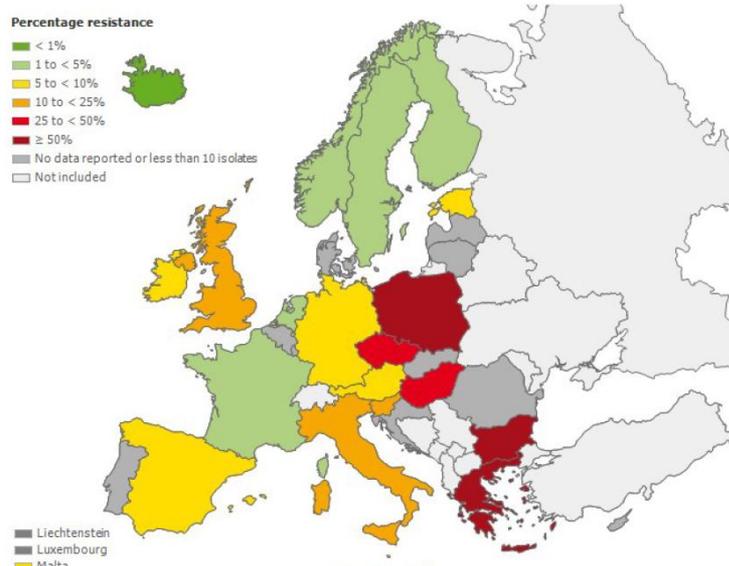


■ Liechtenstein  
■ Luxembourg  
■ Malta

### Proportion of 3rd gen. cephalosporins Resistant (R) *Klebsiella pneumoniae* Isolates in Participating Countries in 2005

Percentage resistance

- < 1%
- 1 to < 5%
- 5 to < 10%
- 10 to < 25%
- 25 to < 50%
- ≥ 50%
- No data reported or less than 10 isolates
- Not included

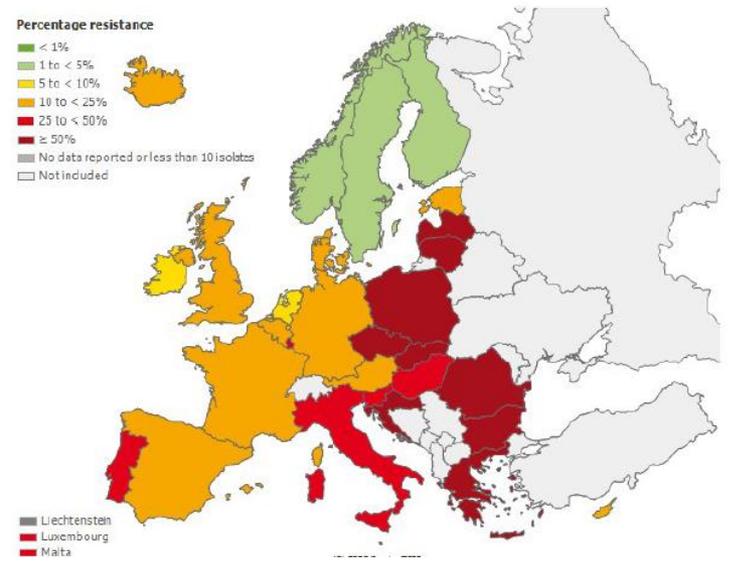


■ Liechtenstein  
■ Luxembourg  
■ Malta

### Proportion of 3rd gen. cephalosporins Resistant (R) *Klebsiella pneumoniae* Isolates in Participating Countries in 2012

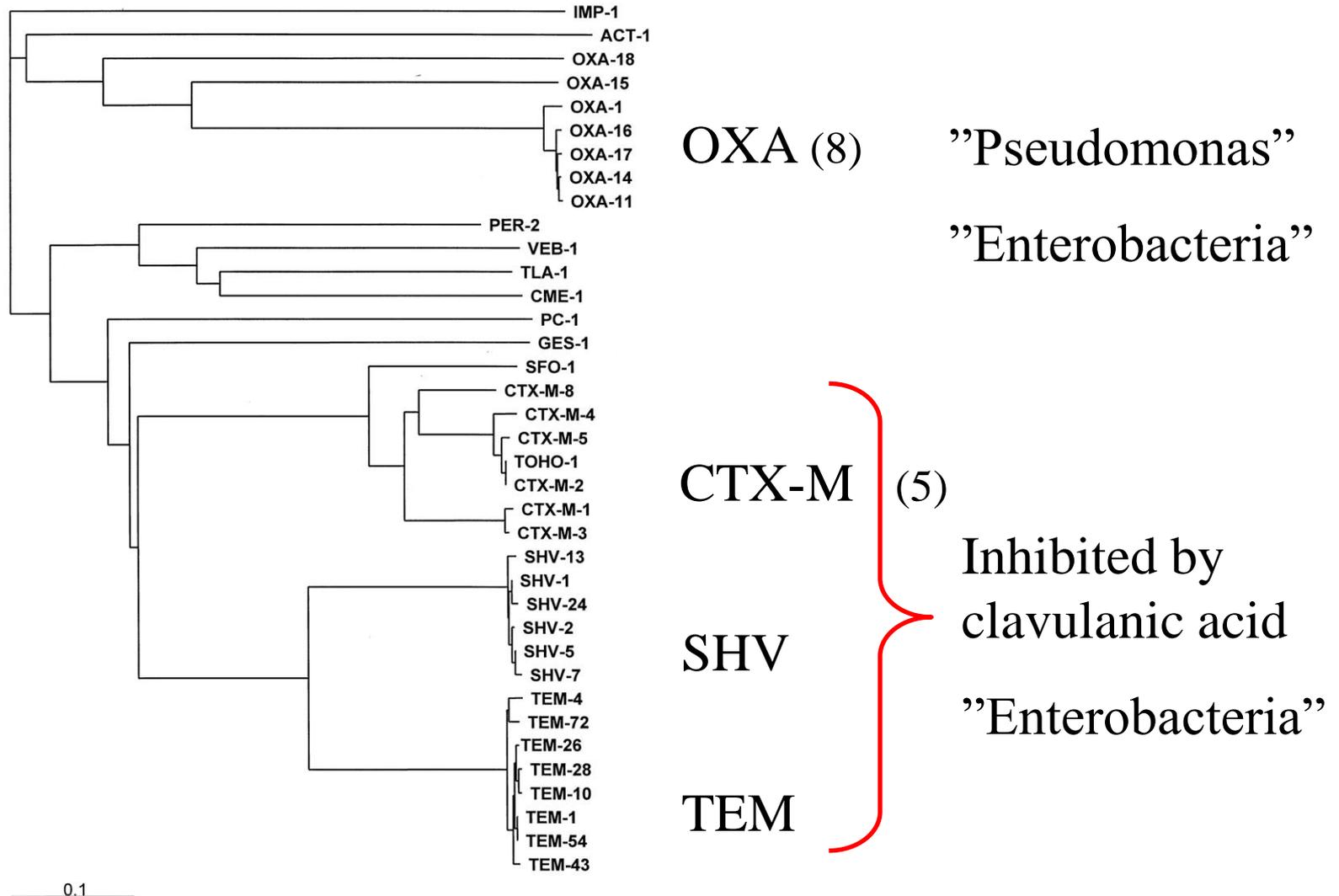
Percentage resistance

- < 1%
- 1 to < 5%
- 5 to < 10%
- 10 to < 25%
- 25 to < 50%
- ≥ 50%
- No data reported or less than 10 isolates
- Not included



■ Liechtenstein  
■ Luxembourg  
■ Malta

# ESBL phylogeny



## Number of $\beta$ -lactamases (ESBLs) (22-10-13 [www.lahey.org/studies](http://www.lahey.org/studies))

TEM	215	
SHV	182	
OXA	161	(18 ESBLs & carbapenemases)
CTX-M	150	
CMY	109	(AmpC)
IMP	47	(metallo)
VIM	39	(metallo)
KPC	15	(carbapenemases)
NDM	10	(metallo)

Smaller number of many other  $\beta$ -lactamases  
Approximately 50% with ESBL activity

# Global EPIDEMIOLOGY of ESBLs

**1982 → 2000**

SHV & TEM

EU, Frankrig, USA

Hospitalized patients

***K. pneumoniae***

Gentamicin and MR

**2000 →**

CTX-M

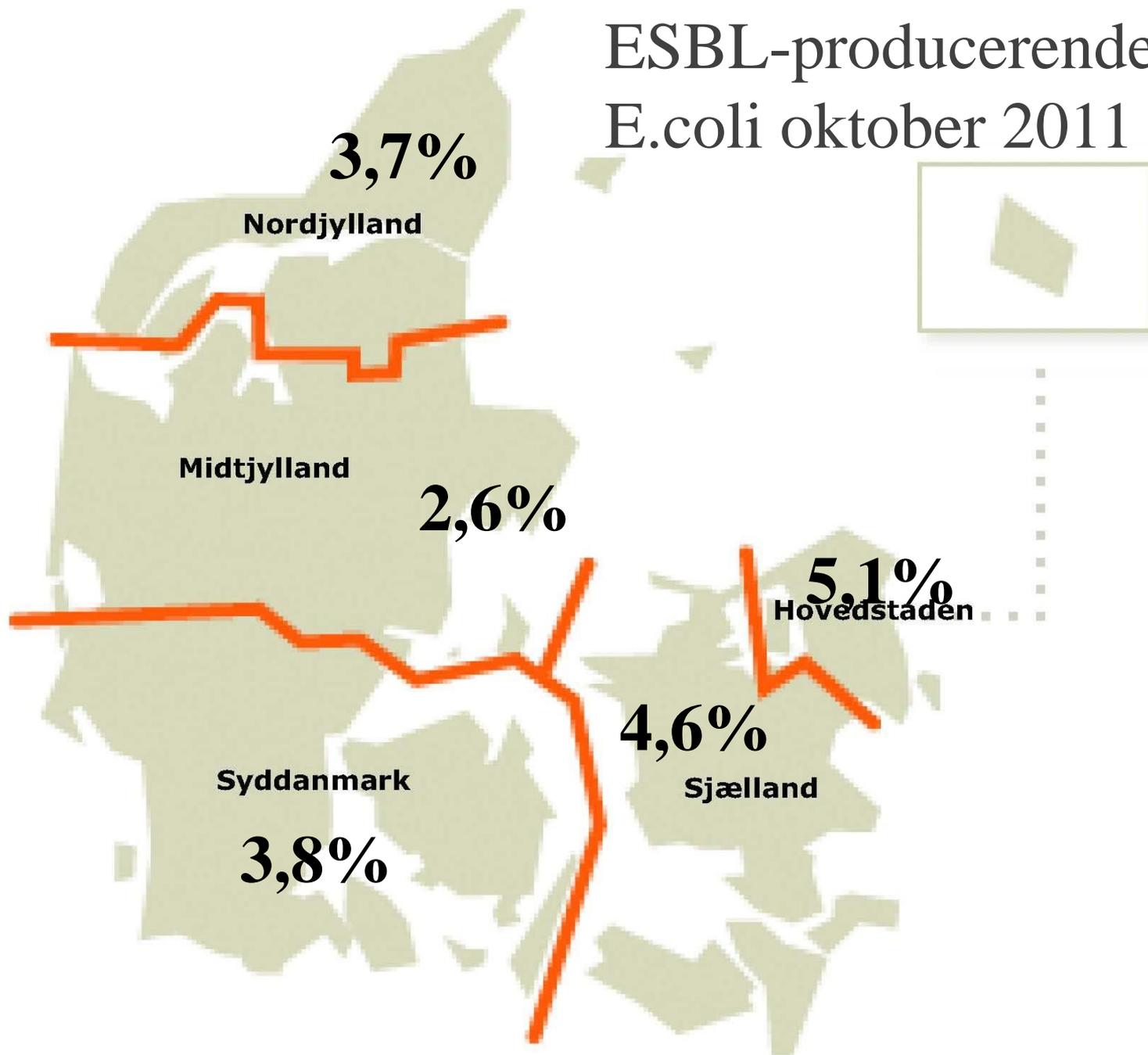
Sydamerika, WW

Community acquired

***E. coli***

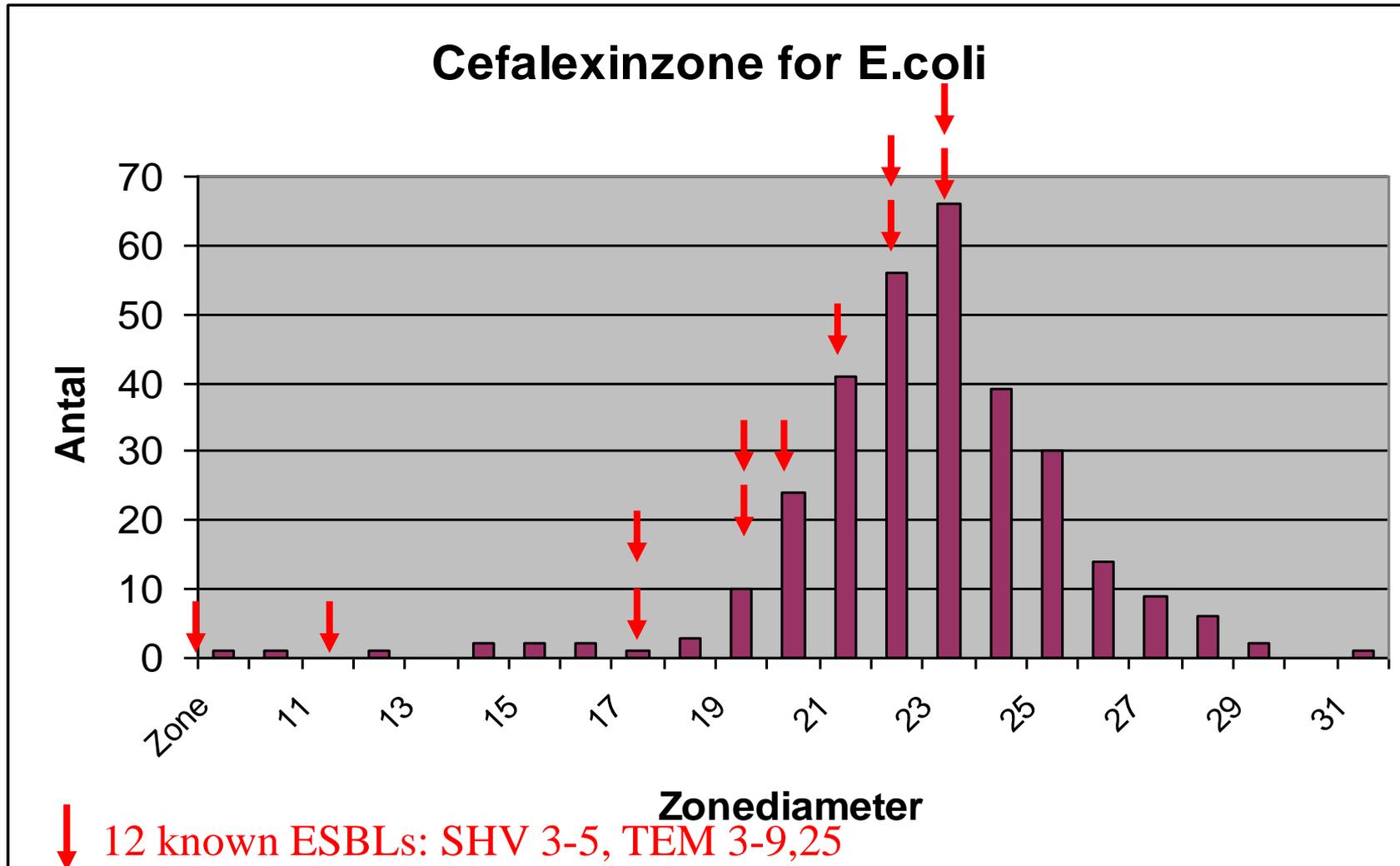
Less co-resistance

# ESBL-producerende E.coli oktober 2011



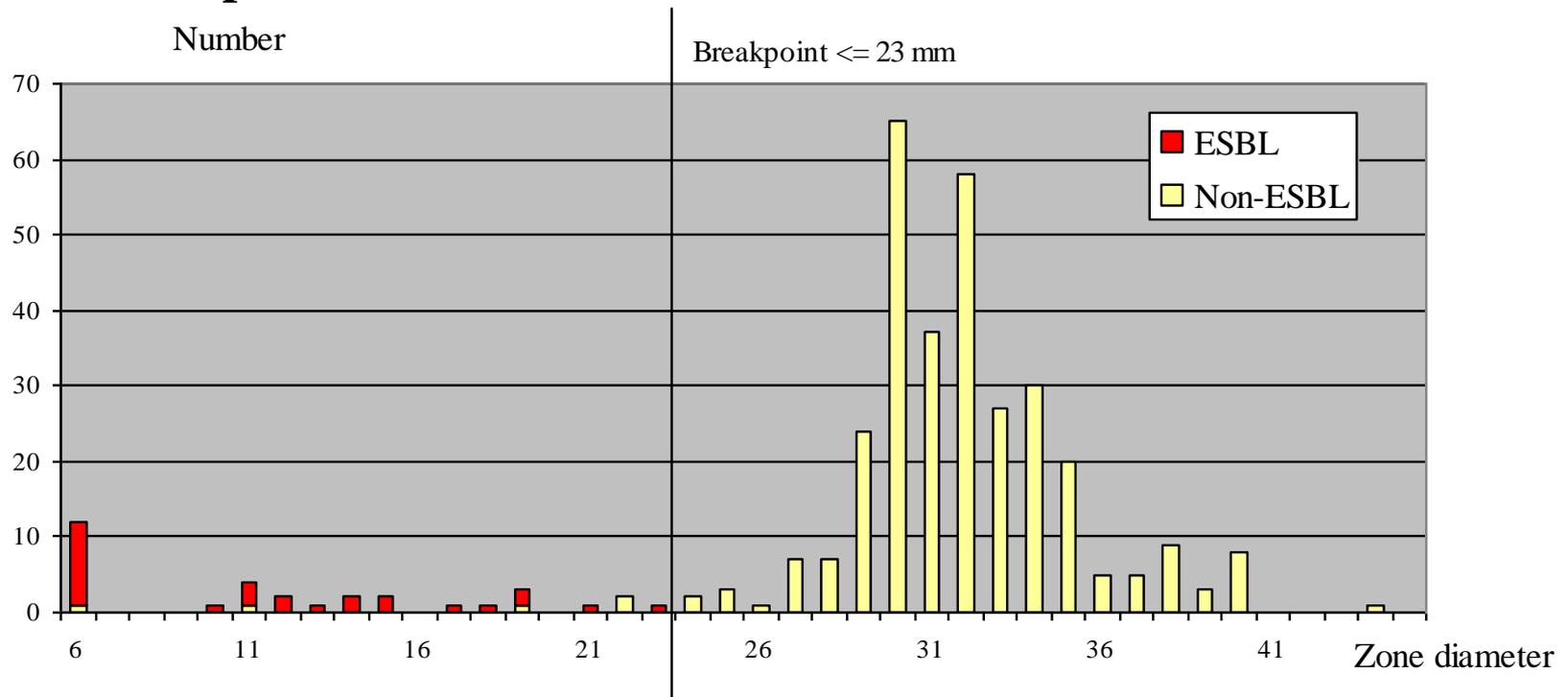
<b>ESBL type</b>	<b>E.coli</b>	<b>K.pneumoniae</b>	<b>P.mirabilis</b>	<b>Antal</b>
CTX-M-15	105	10		115
CTX-M-15 + SHV-28	1	24		25
CTX-M-15 + SHV-32	1	14		15
CTX-M-15 + SHV-11		9		9
CTX-M-1	19		1	20
CTX-M-14	15			15
CTX-M-27	7			7
CTX-M-3	4			4
CTX-M +/- diverse	7	13	1	21
CIT/CMY-2/DHA	6	1	1	8
Ec pheno AmpC	19			19
KPC-2 + SHV-12		1		1
SHV-2/5/11/12	3	6		9
TEM ESBL	3			3
ESBL phenotype	8	1		9
ND	5			5
WT	20	11		31
<b>Hovedtotal</b>	<b>223</b>	<b>90</b>	<b>3</b>	<b>316</b>

## Screening for ESBL 1/2



## Screening for ESBL 2/2

## Cefpodoxime zones for 318 E.coli and 28 ESBL strains



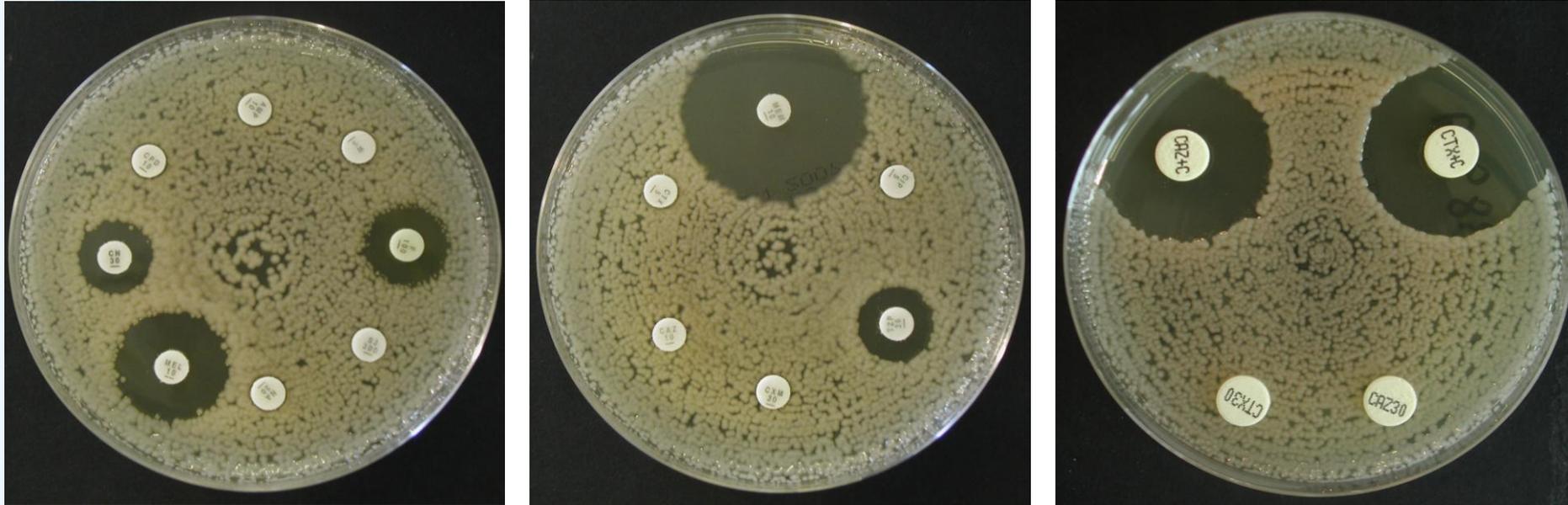
SRGA methodology, Isosensitest agar without blood, CPD 10  $\mu$ g Oxoid disc

*K. pneumoniae* with 2 positive confirmatory tests:

- 1) double disk (synergism)
- 2) combination disc test with Roco Neosensitabs



# Antibiogram for CTX-M-15 producerende K.pneumoniae



**Disc codes:** Ampicillin (AMP10), Cefpodoxime (CPD10), Gentamicin (CN30), Mecillinam (MEL10), Nalidixic acid (NA30), Sulfamethoxazole (S3), Nitrofurantoin (F100), Trimethoprim (W5), Ceftazidime (CAZ10), Cefuroxime (CXM30), Pip/tazobactam (TZP36), Ciprofloxacin (CIP5), Meropenem (MEM10), Cefotaxime + Clavulanic acid (CTX+C), Cefotaxime (CTX30), Ceftazidime + Clavulanic acid (CAZ+C), Ceftazidime (CAZ30).

# Phylogeny of chromosomal and plasmid-encoded AmpC $\beta$ -lactamases (Philippon AAC 2002;46:1-11)

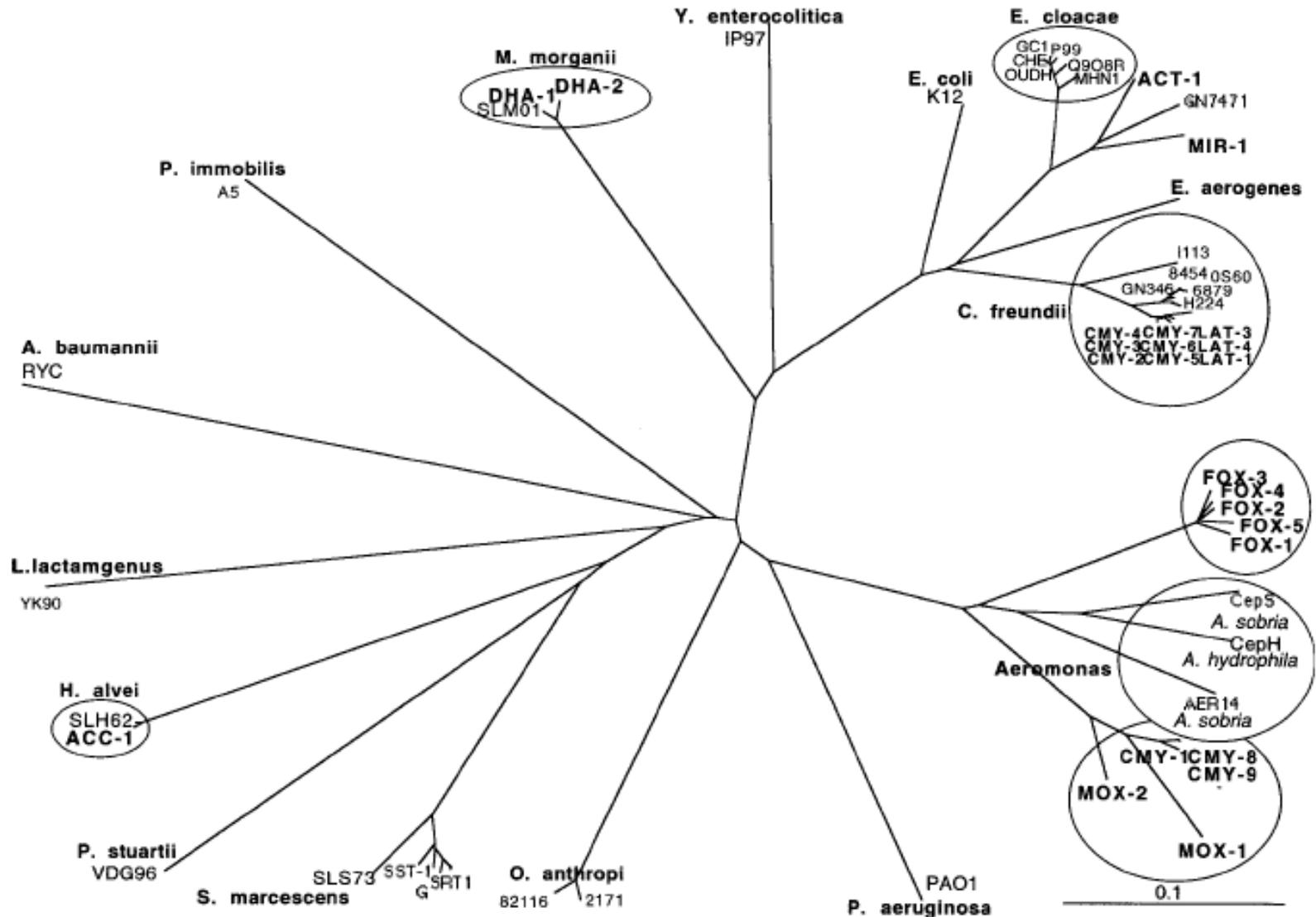
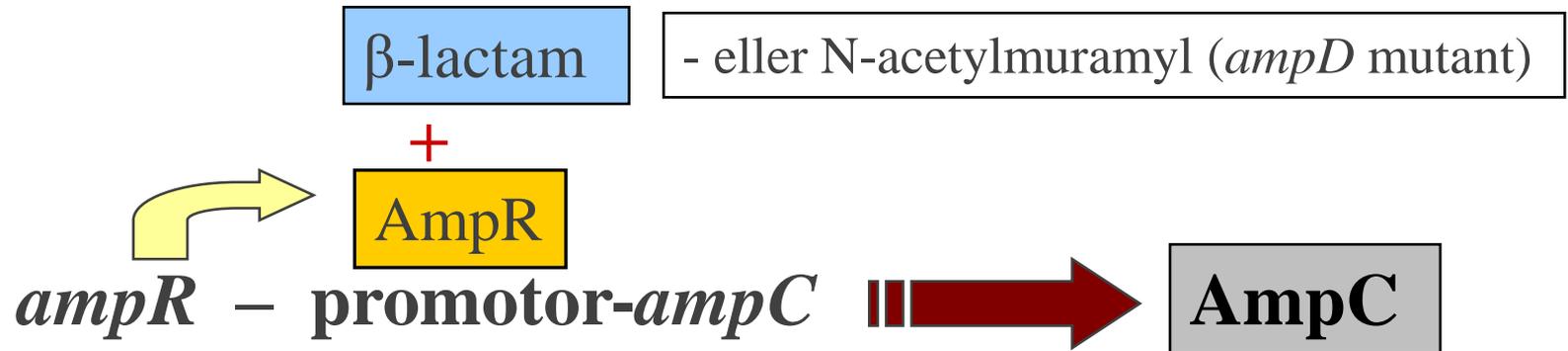


FIG. 1. Dendrogram for chromosomal and plasmid-encoded AmpC  $\beta$ -lactamases, calculated by Clustal X (34) and the neighbor-joining method (73). Branch lengths are proportional to the number of amino acid exchanges. GenBank accession numbers for *bla*<sub>AmpC</sub> can be found at [http://www.rochester.edu/College/BIO/labs/HallLab/AmpC\\_Phylo.html](http://www.rochester.edu/College/BIO/labs/HallLab/AmpC_Phylo.html).

# AmpC subgroups

Group	Plasmid located
C.freundii	LAT-1, LAT-3, LAT-4, CMY-2, CMY-3, CMY-4, CMY-5, CMY-6, CMY-7
Enterobacter	MIR-1, ACT-1 (i)
M.morganii	DHA-1 (i), DHA-2 (i)
H.alvei	ACC-1 (cefox I/S)
Aeromonas I	MOX-1, MOX-2, CMY-1, CMY-8, CMY-9
Aeromonas II	FOX-1, FOX-2, FOX-3, FOX-4, FOX-5

# AmpC induction og derepressed mutants



- Derepressed mutanter opstår m frekvens på  $10^5 - 10^8$
- Selekteres let af aminothiazolyl cephalosporiner men mindre let af ureidopenicilliner
- Selektion hos 20-30% af pneumonier/bakteriæmier med *Enterobacter/citrobacter* som behandles med aminothiazolyl cephalosporiner

# Arter med og uden kromosomal AmpC

+

Enterobacter spp. (cloacae, aerogenes, aspuria)

Citrobacter freundii

Serratia marcescens

Escherichia coli

Shigella sonnei

Hafnia alvei

Buttiauxella

Morganella morganii

Ochrobactrum anthropi

Proteus rettgeri

Providencia stuartii

Yersinia enterocolitica

Pseudomonas aeruginosa

Acinetobacter spp.

Aeromonas spp. (sobria, hydrophila)

Chromobacterium violaceum

÷

Klebsiella spp. (pneumoniae, oxytoca)

Salmonella spp

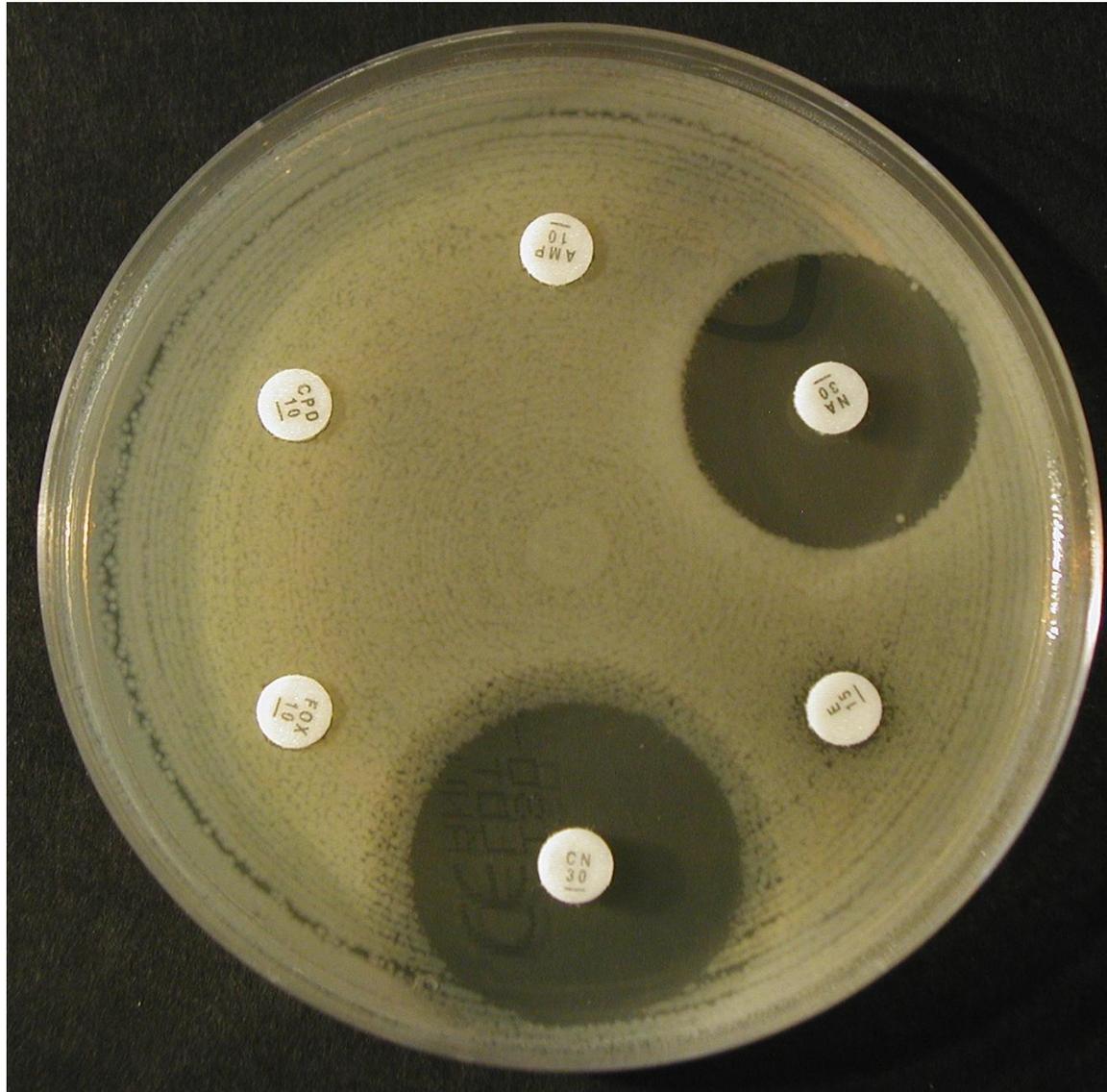
Shigella flexneri

Shigella dysenteriae

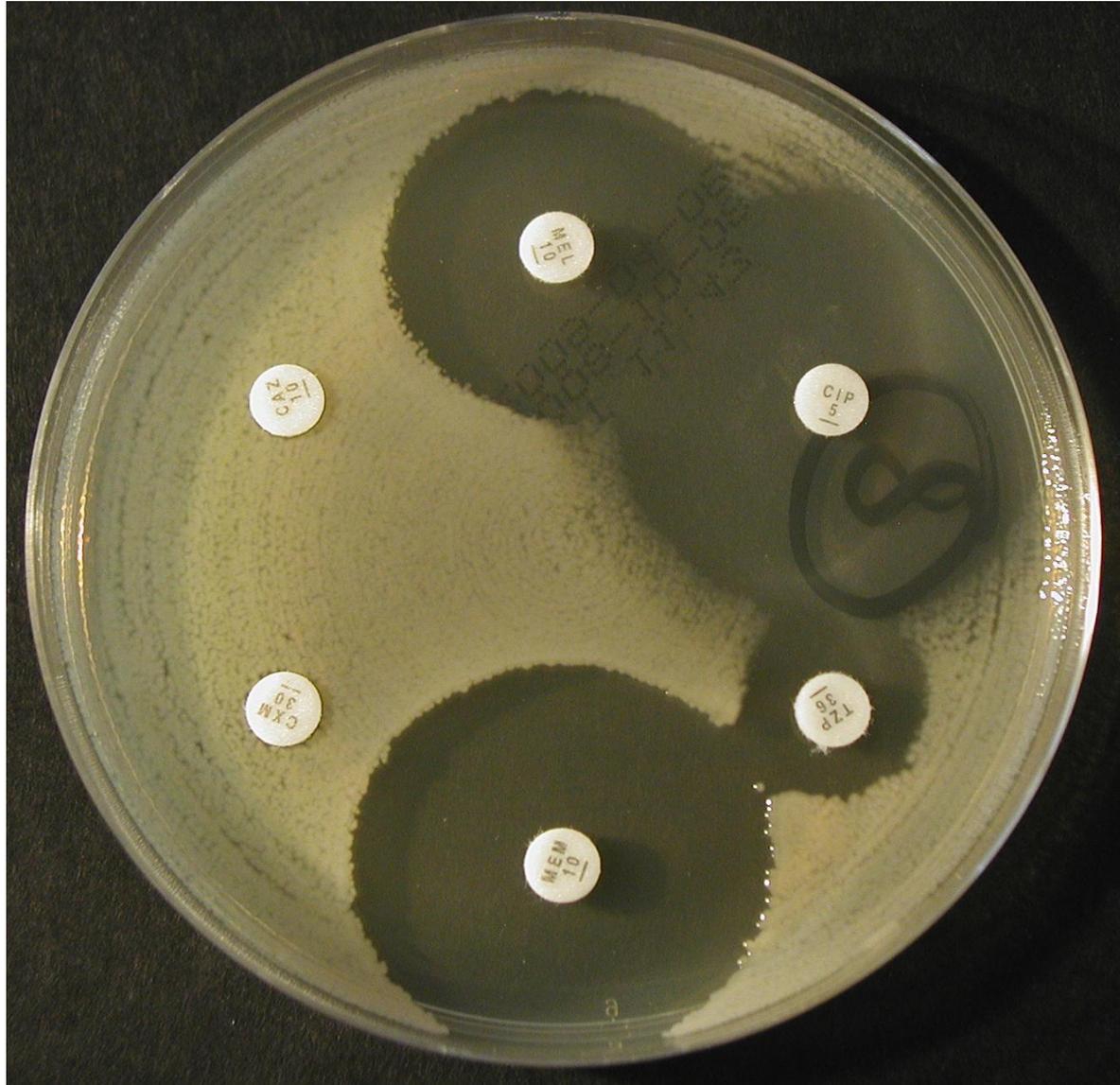
P.mirabilis

P.vulgaris

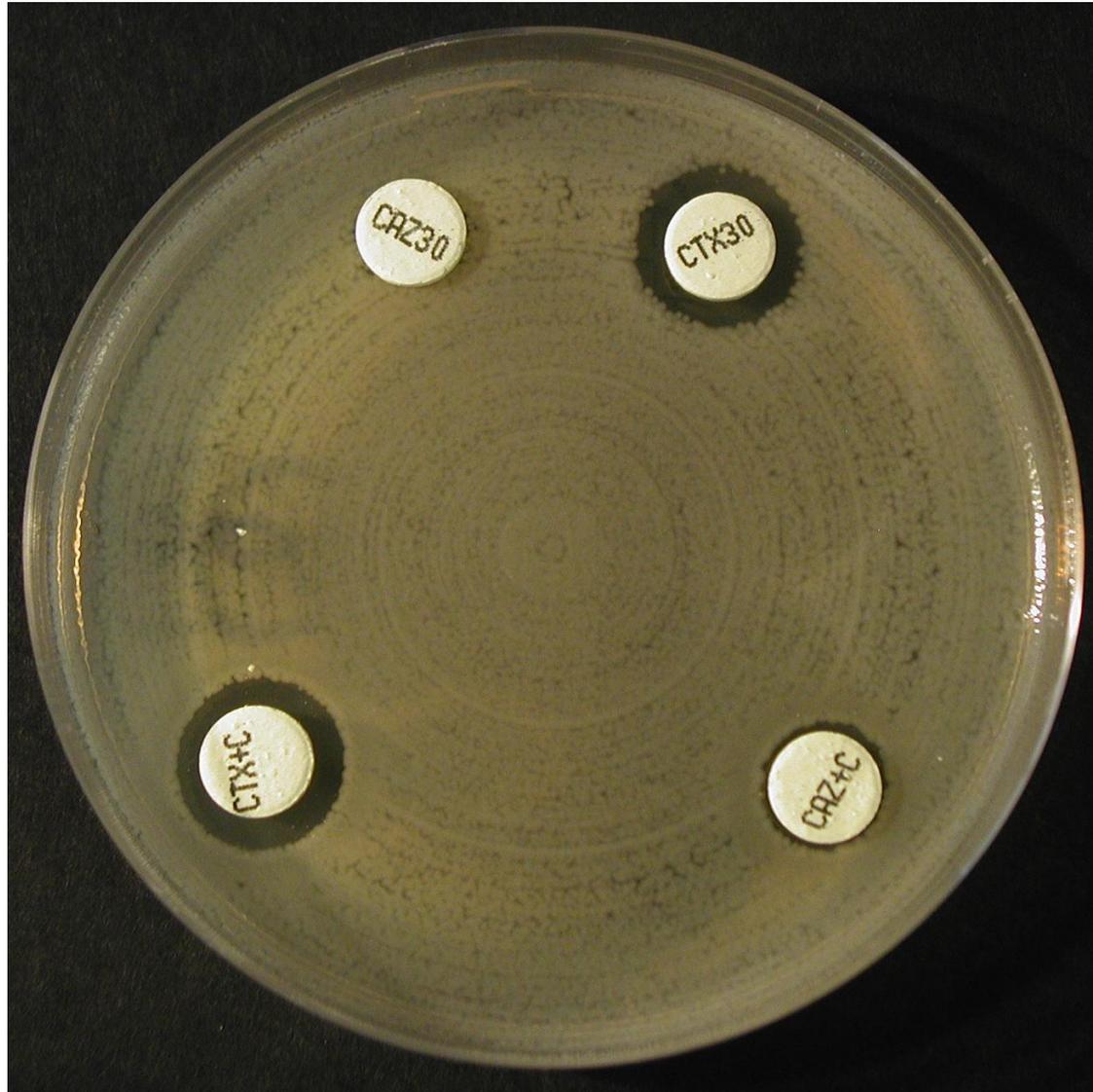
# C.freundii



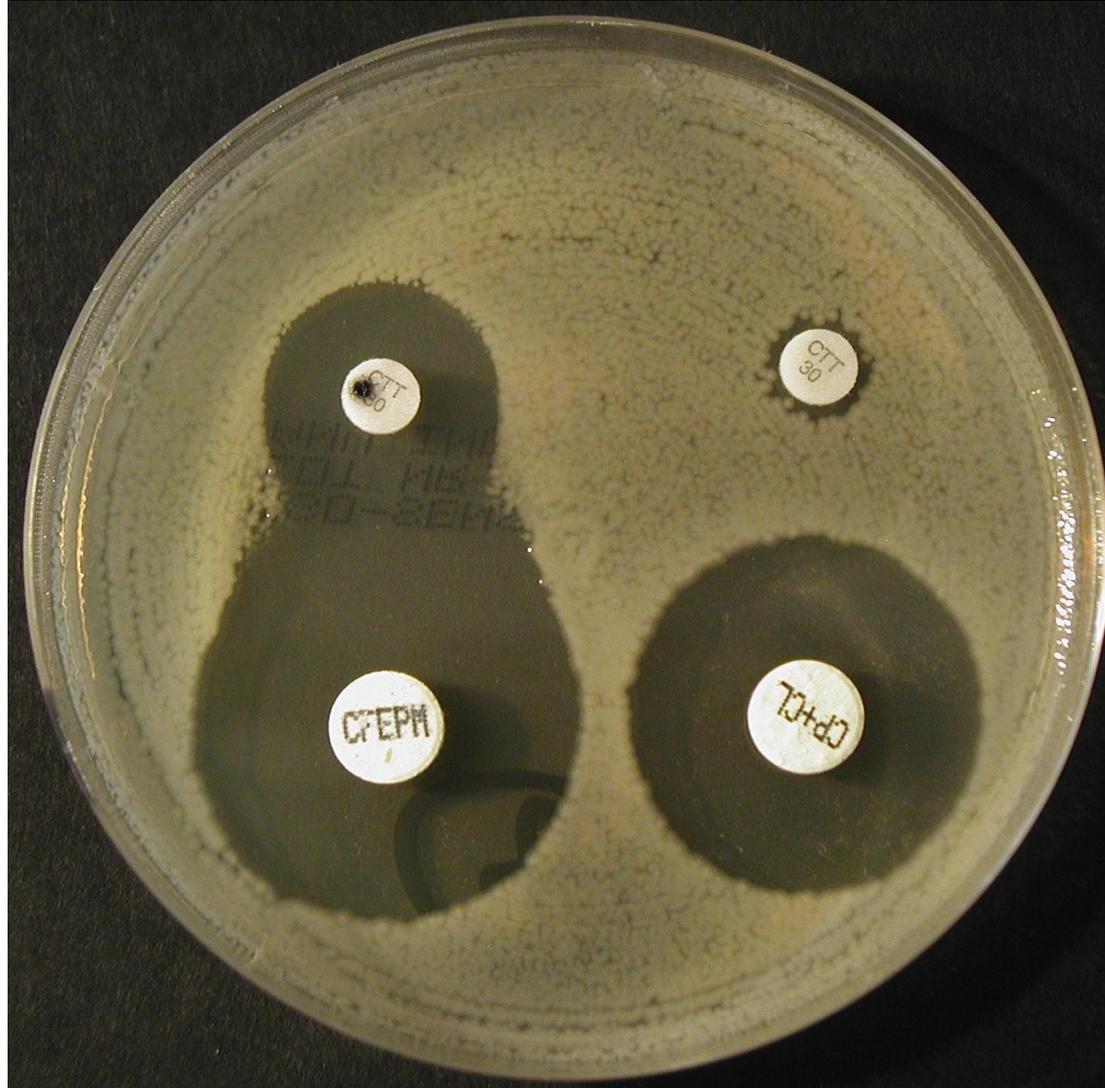
# C.freundii



# C.freundii

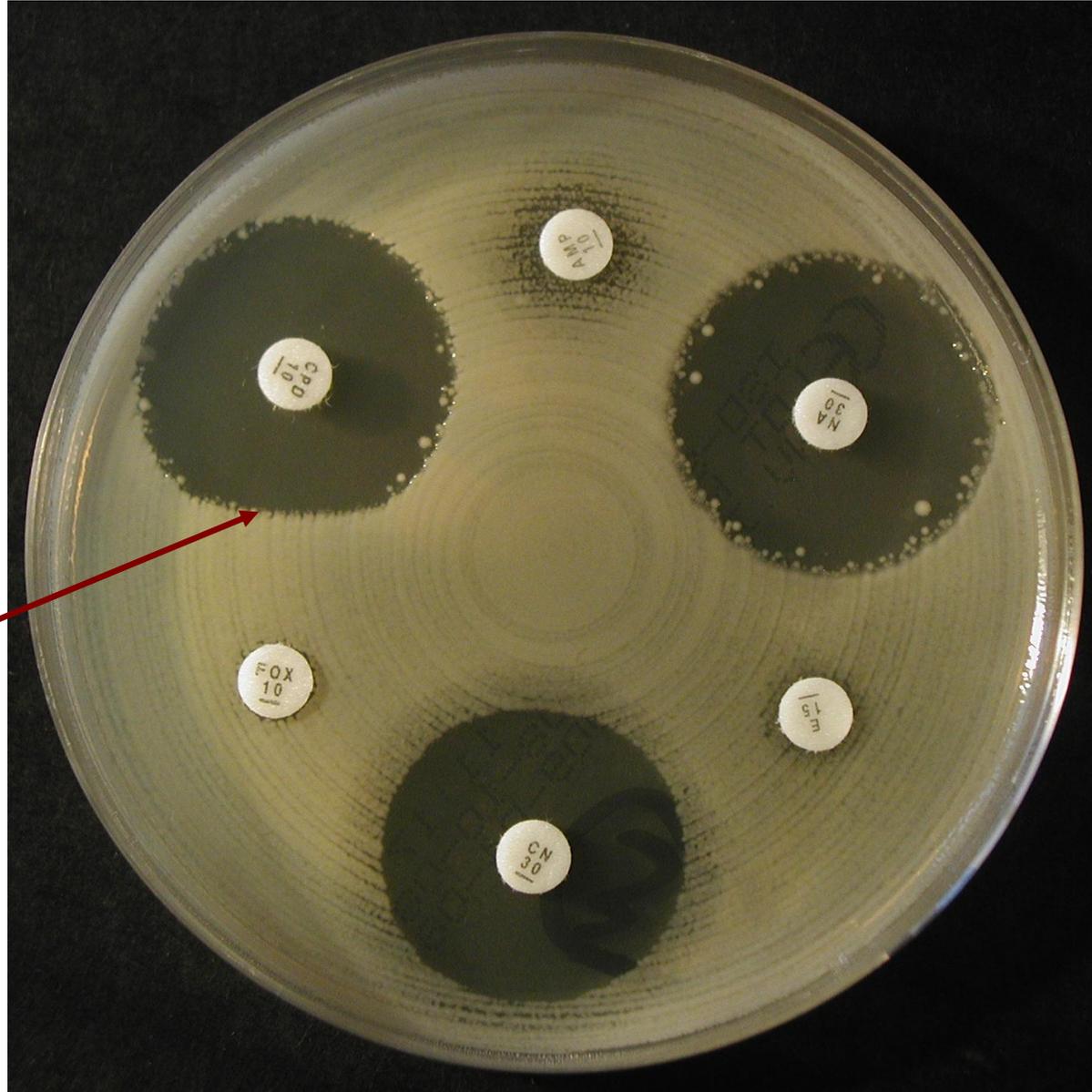


# C.freundii

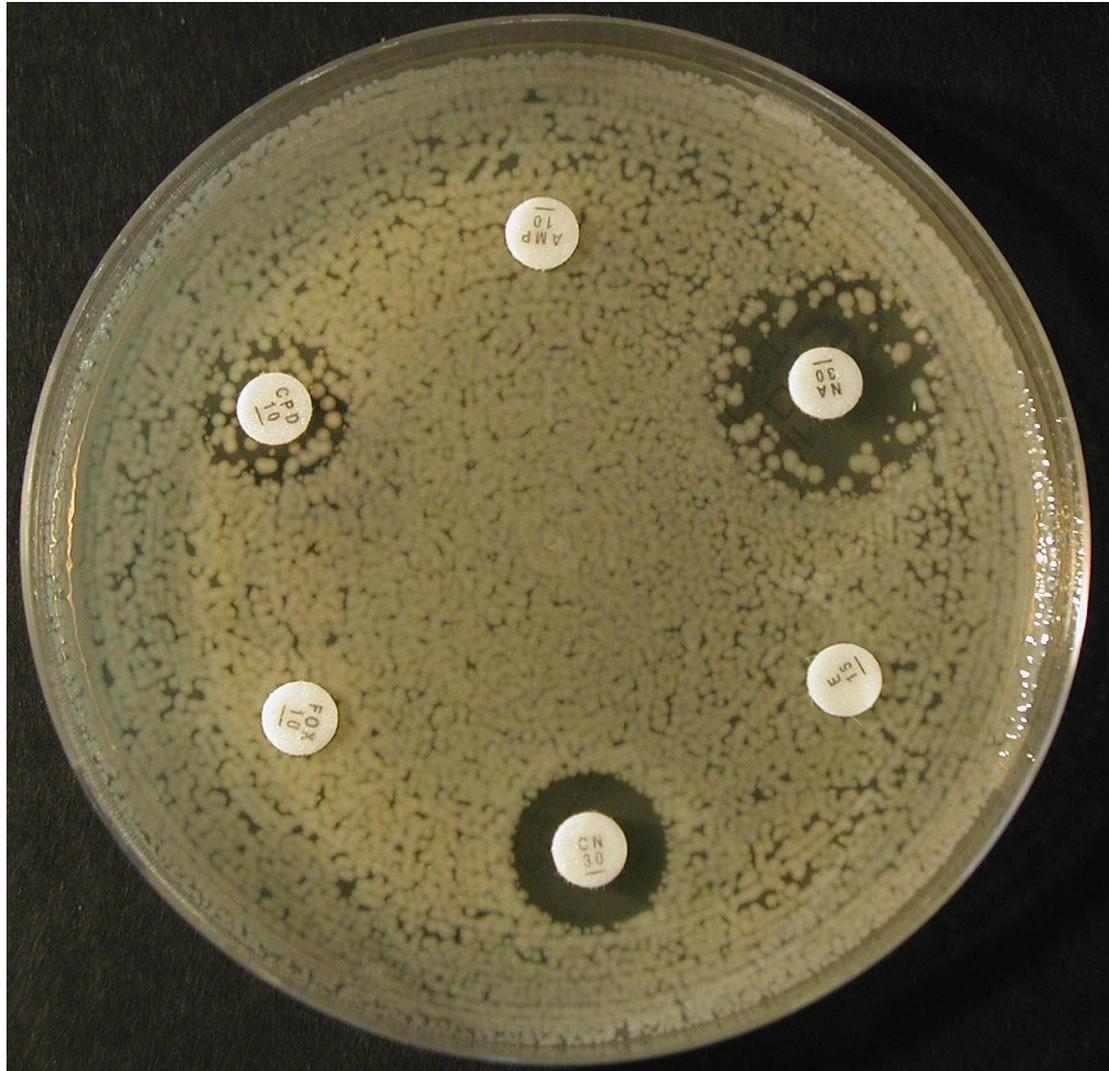


# E. cloacae

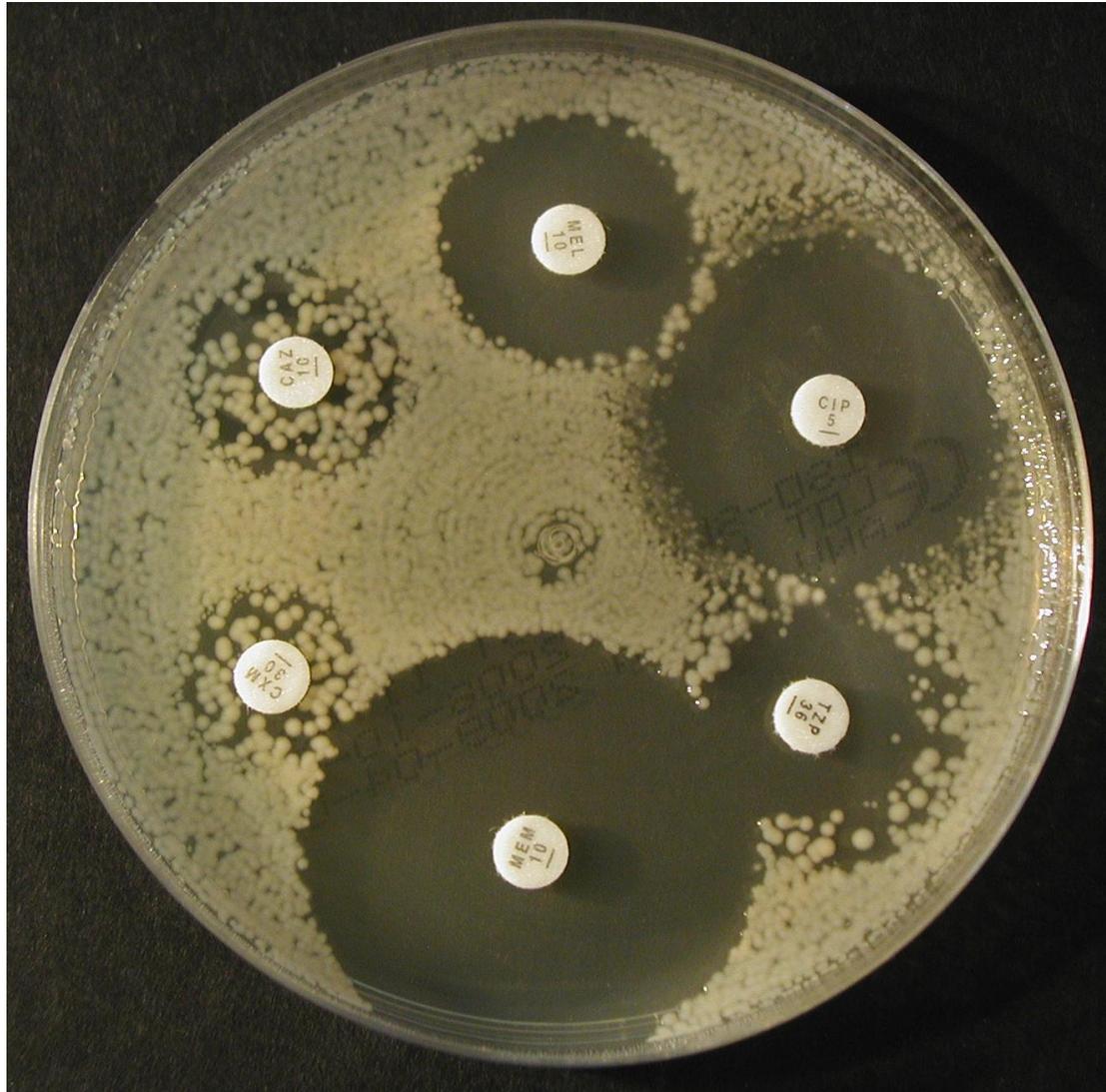
Induktion



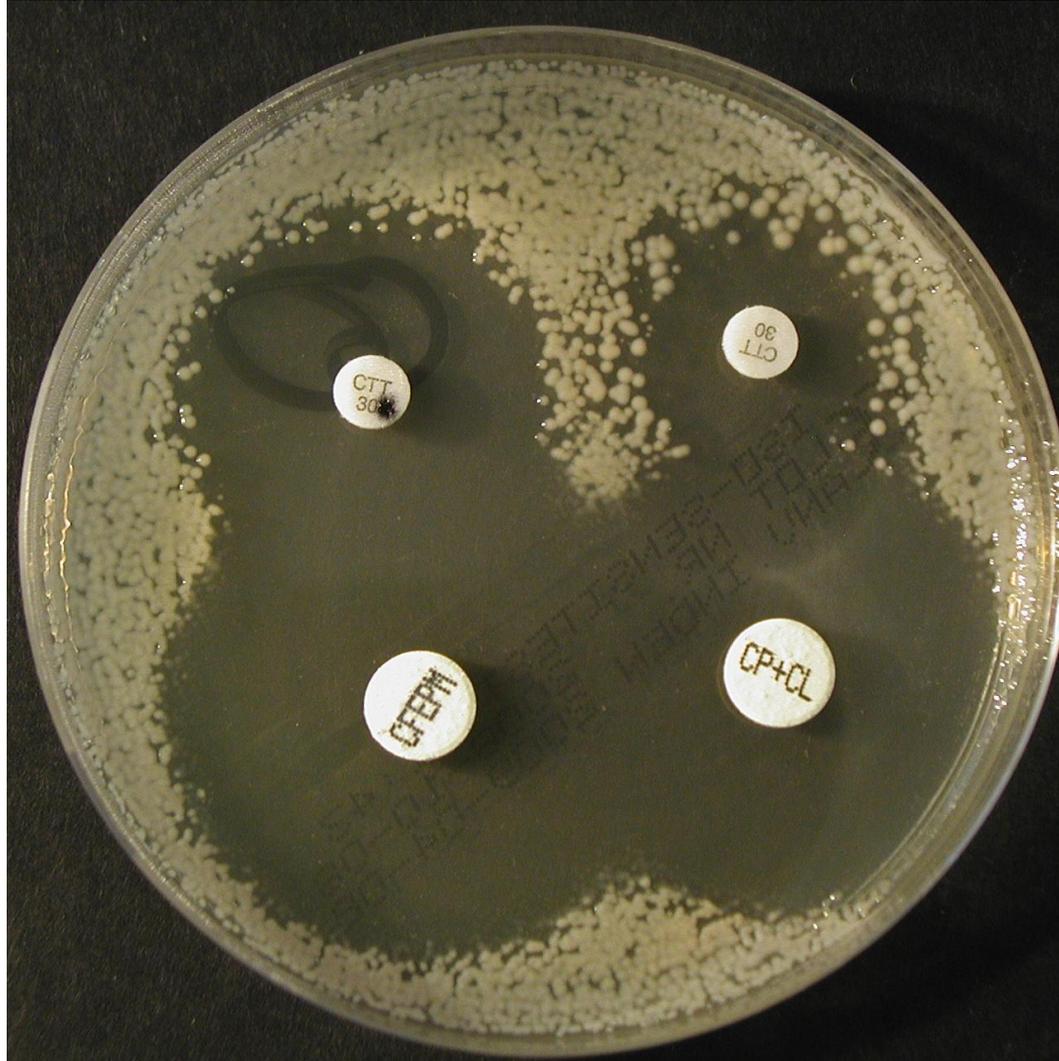
# E.coli AmpC



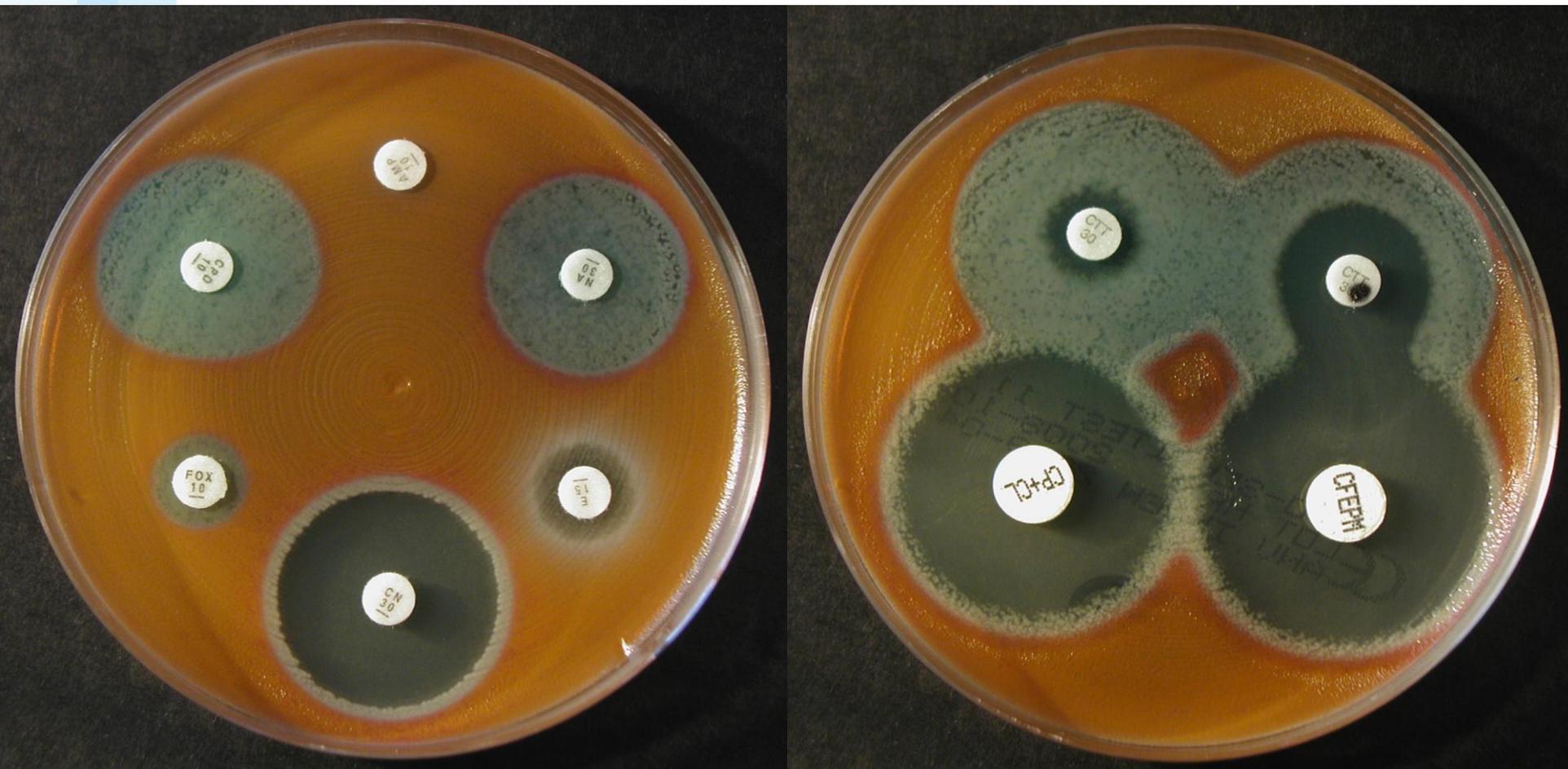
# E.coli AmpC



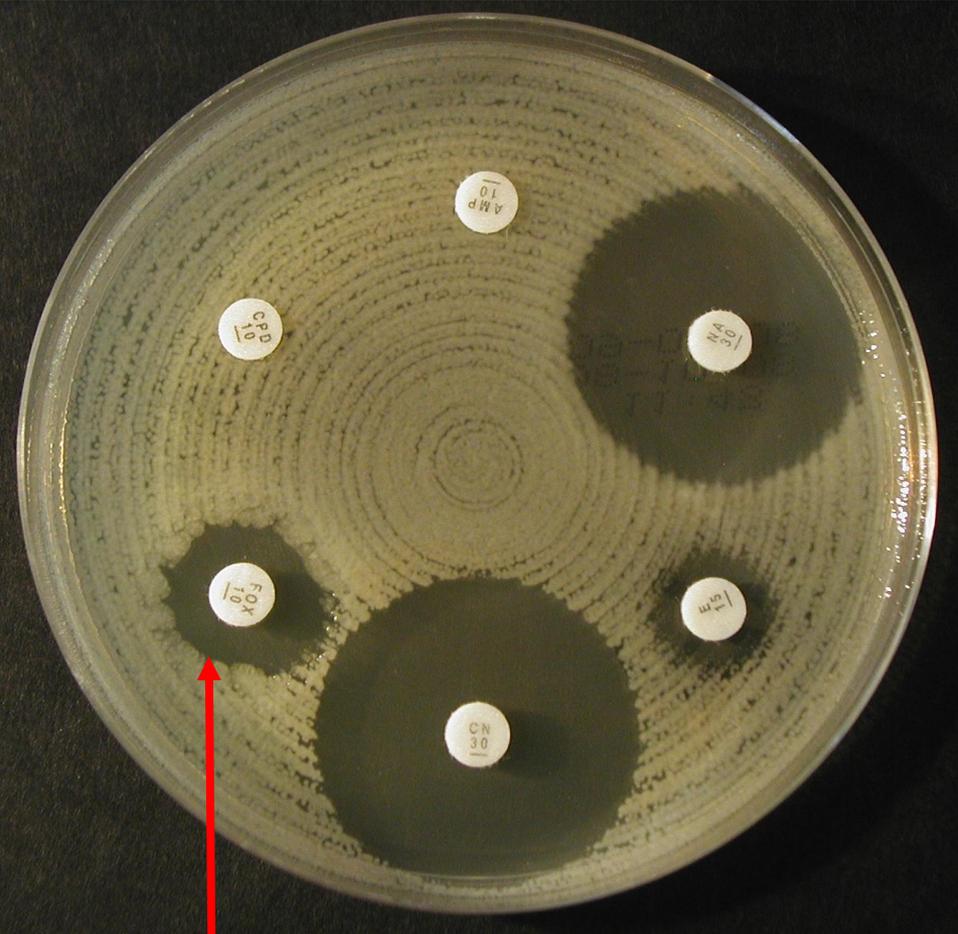
# E.coli AmpC



# S. marcescens



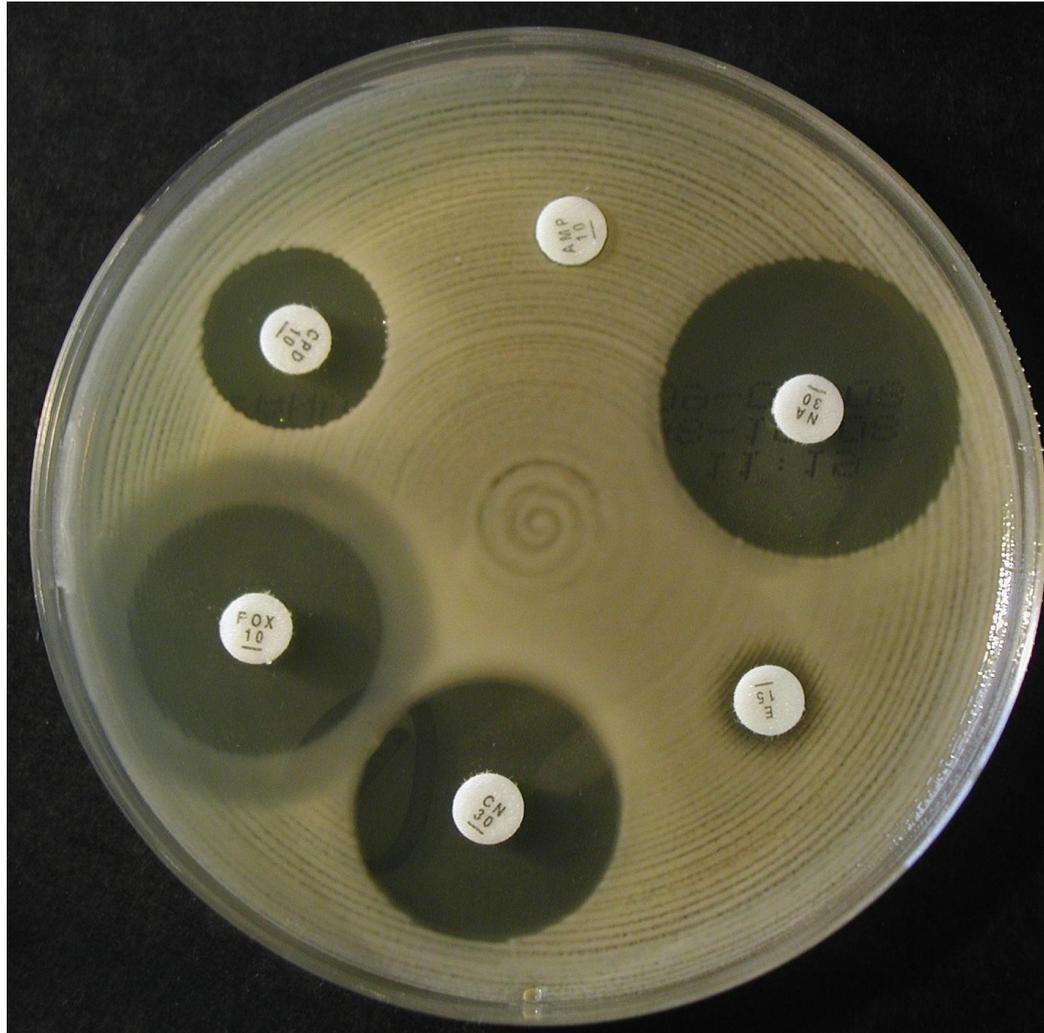
# H.alvei



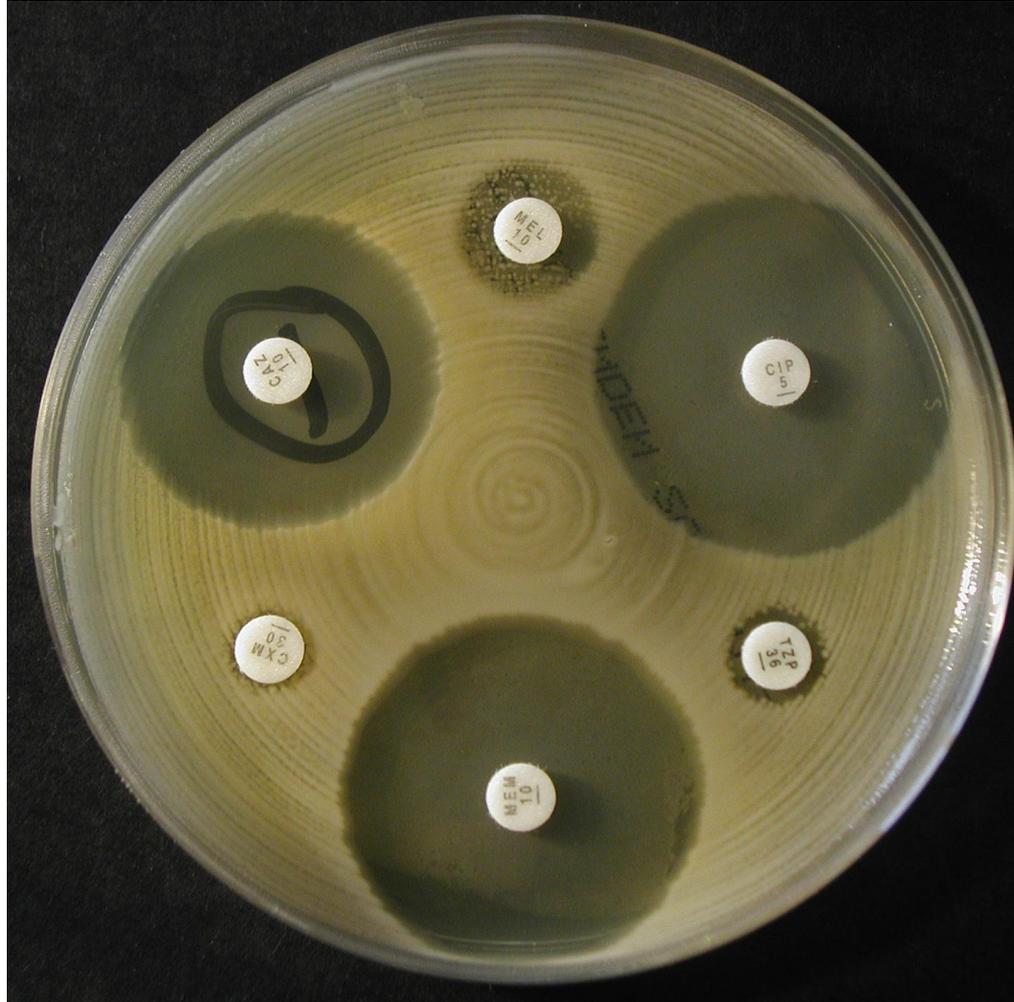
Cefoxitin I

**2 eksempler...**

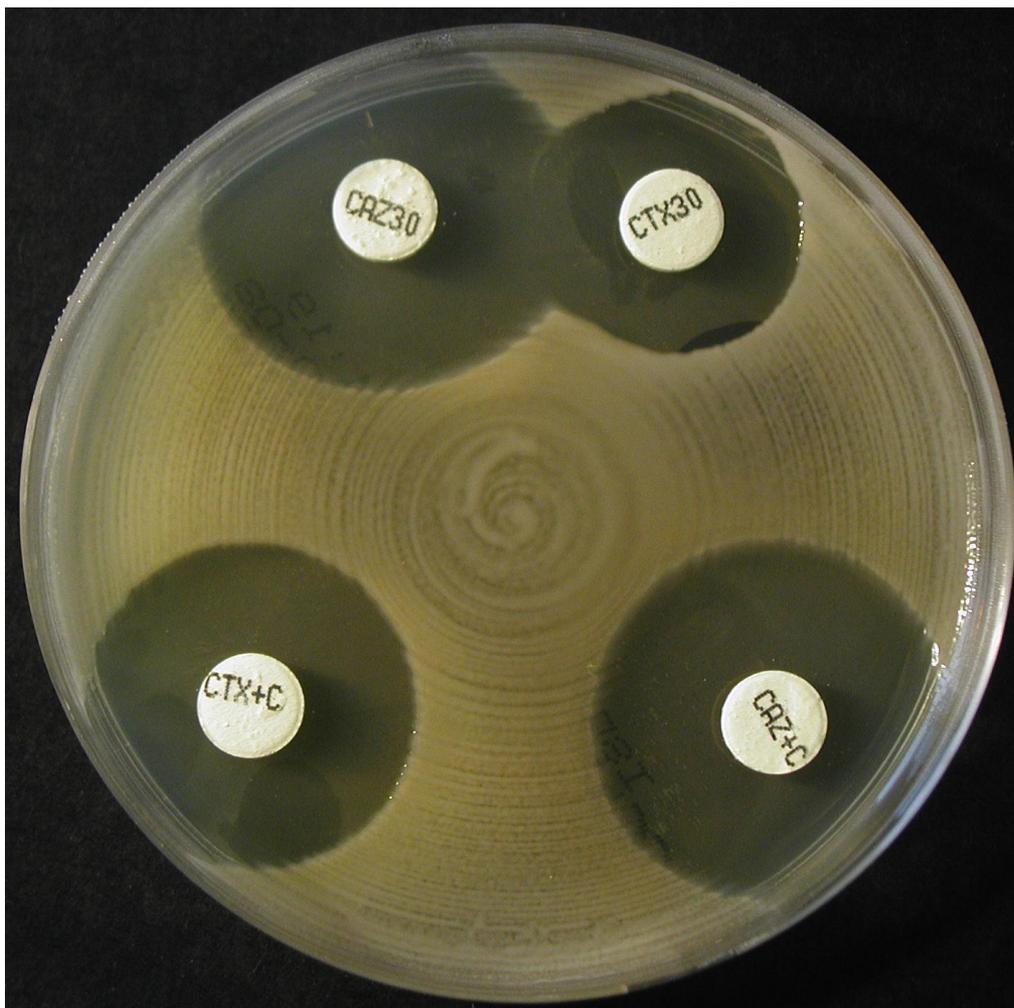
# K.oxytoca ↑K1



# K.oxytoca ↑K1

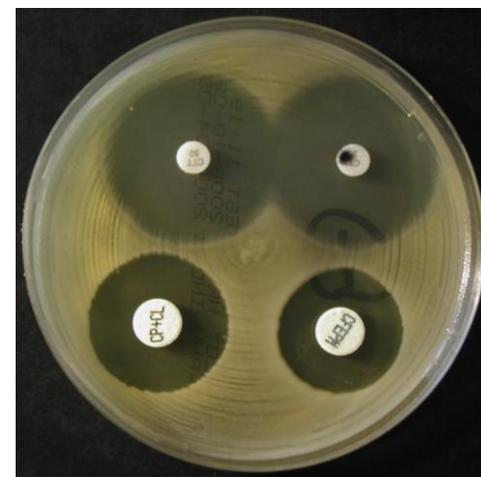


# K.oxytoca ↑K1

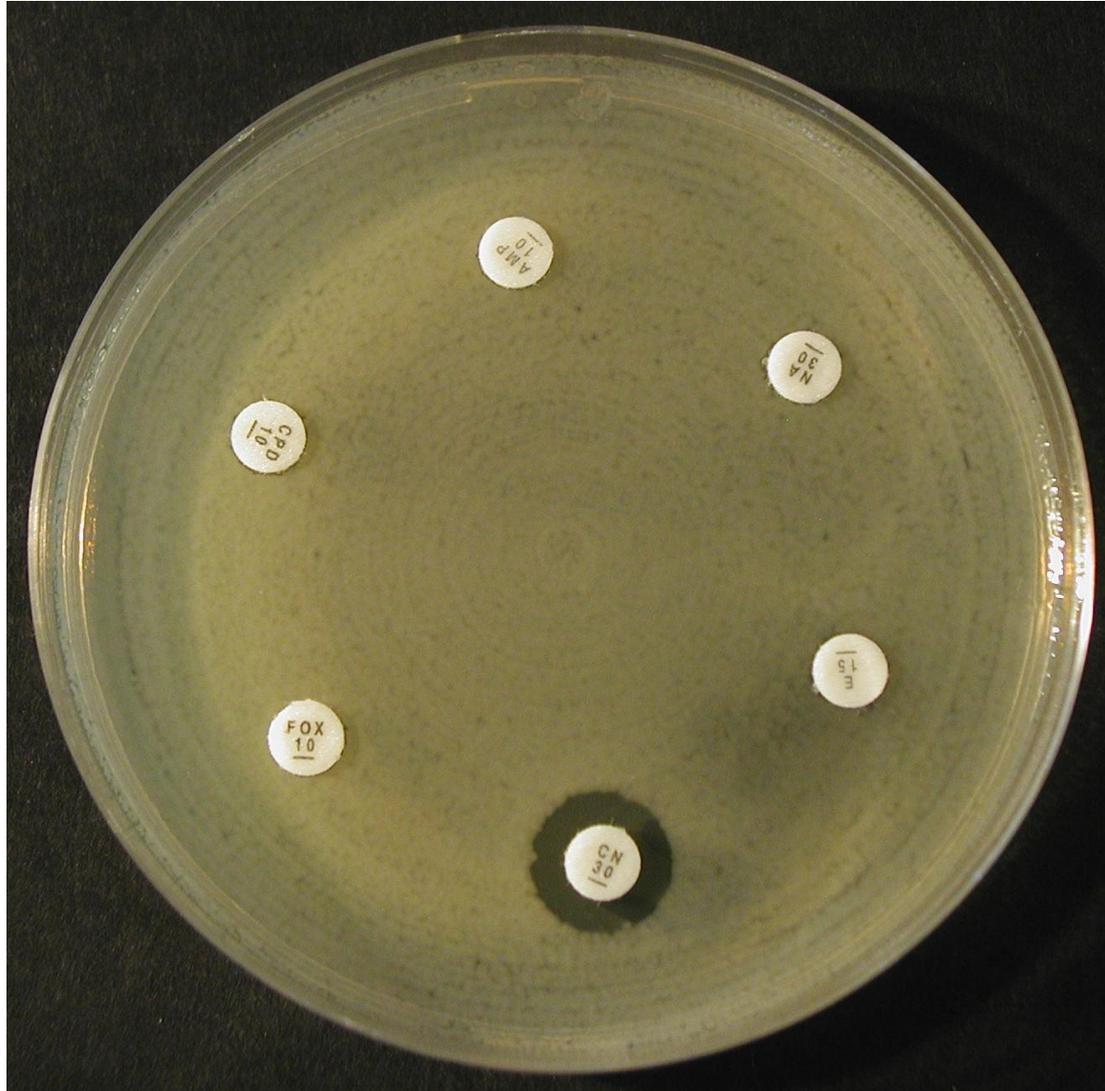


1) Falsk pos ESBL-test  
for CTX+/- CA

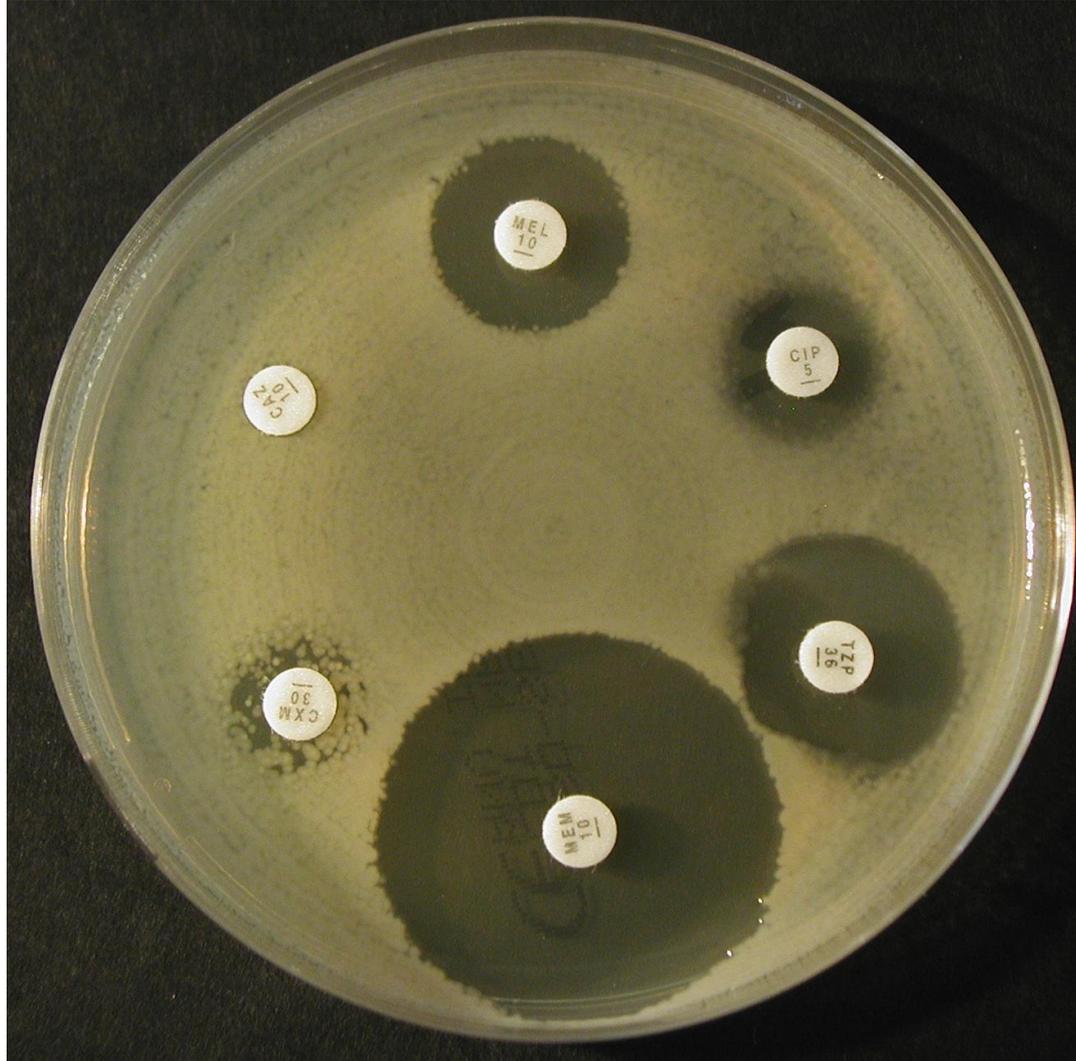
2) CAZ > CTX



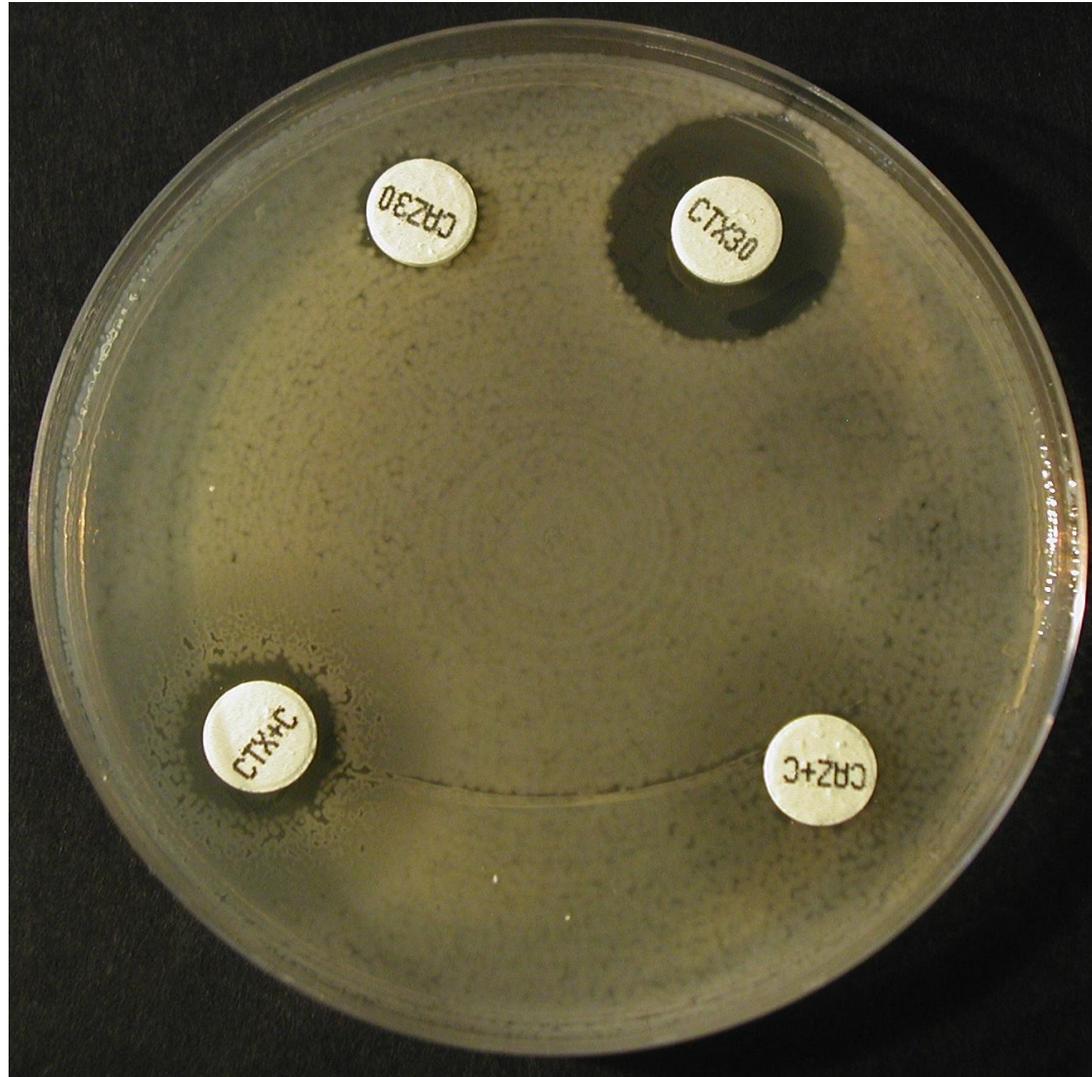
# C.freundii 2



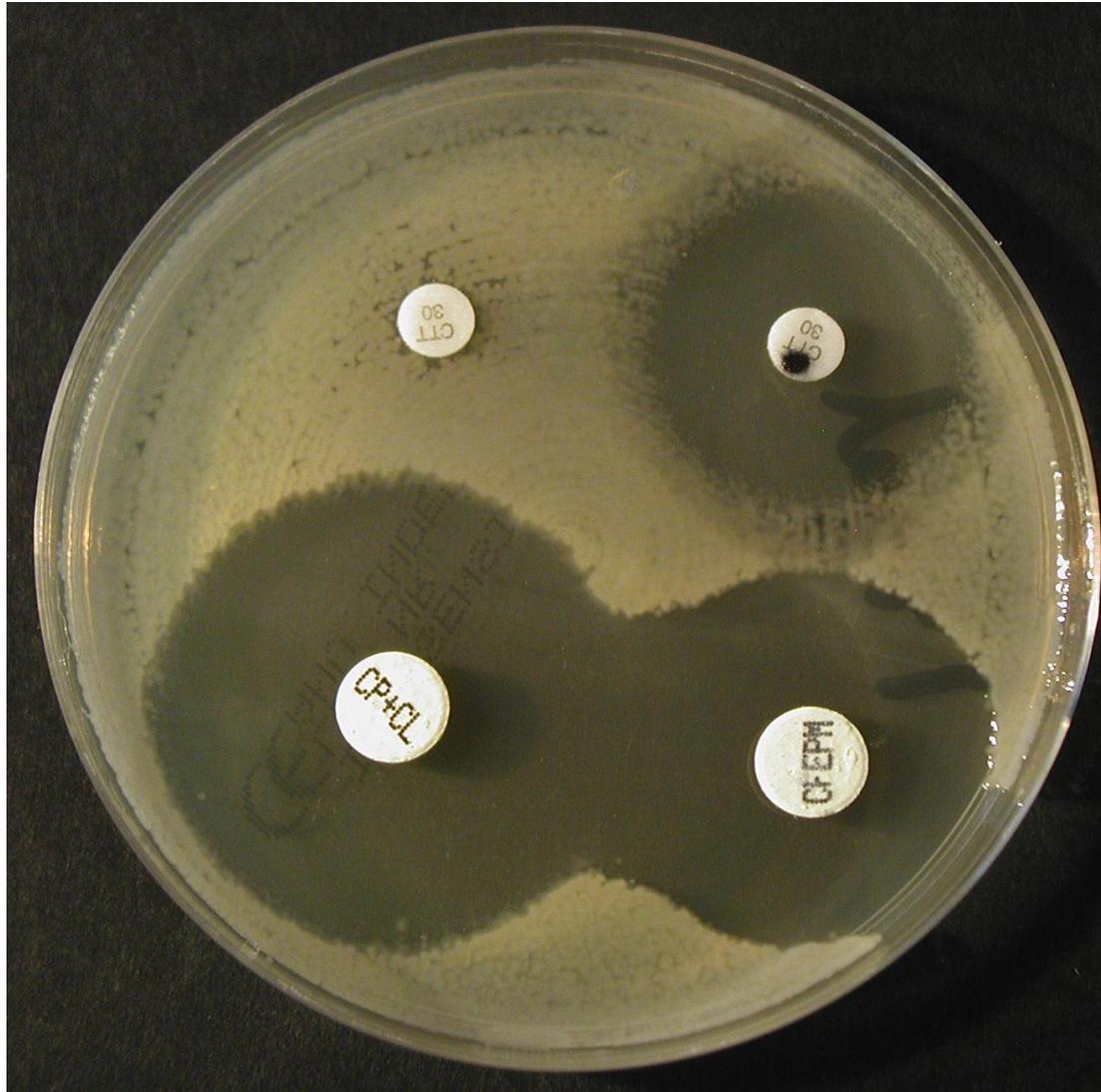
# C.freundii 2



# C.freundii 2



# C.freundii 2



CTT+B – CTT  
= 21 mm

CP+CL – CFEPM  
= 8 mm (SHV-12)