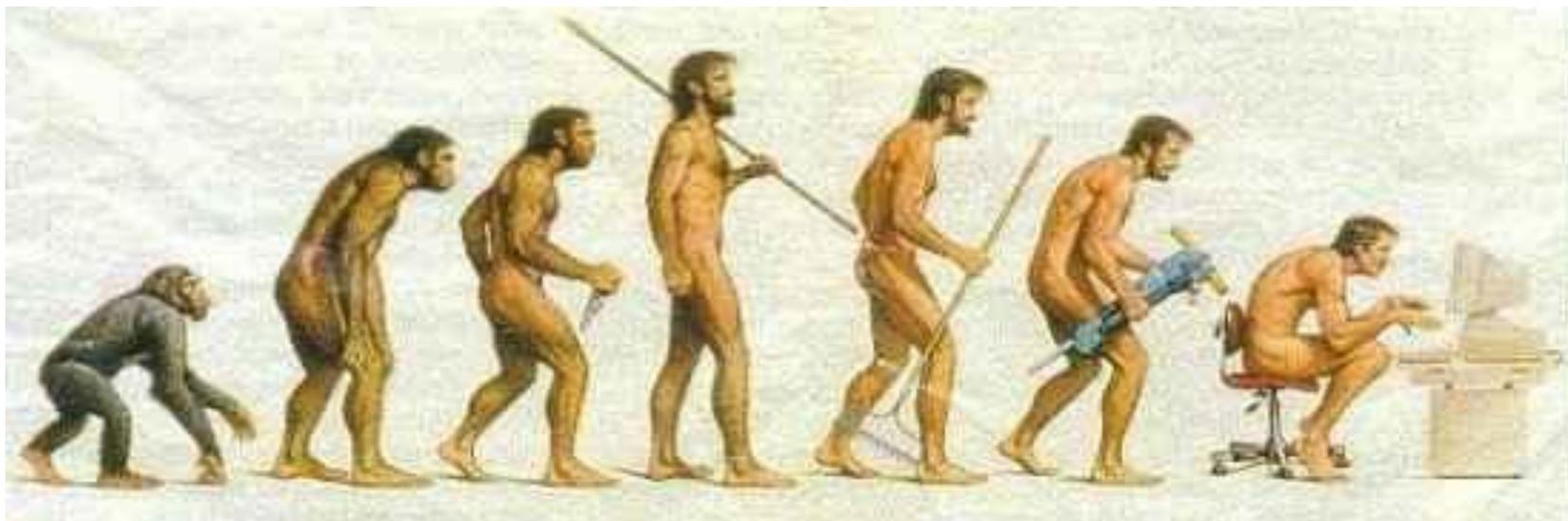


NordicAST Göteborg 12. + 13. maj 2015

Karbapenemaser – laboratorie detektion



Dennis Hansen
Klinisk mikrobiologisk afdeling
Herlev Hospital

Number of β -lactamases

(10-05-2015 www.lahey.org/studies)

TEM	223	
SHV	191	
CTX-M	168	
CMY	134	(AmpC)
OXA	488	(127 carbapenemases)
IMP	52	(metallo)
VIM	46	(metallo)
KPC	23	(carbapenemases)
NDM	16	(metallo)

Smaller number of many other β -lactamases

Approximately 50% with ESBL activity

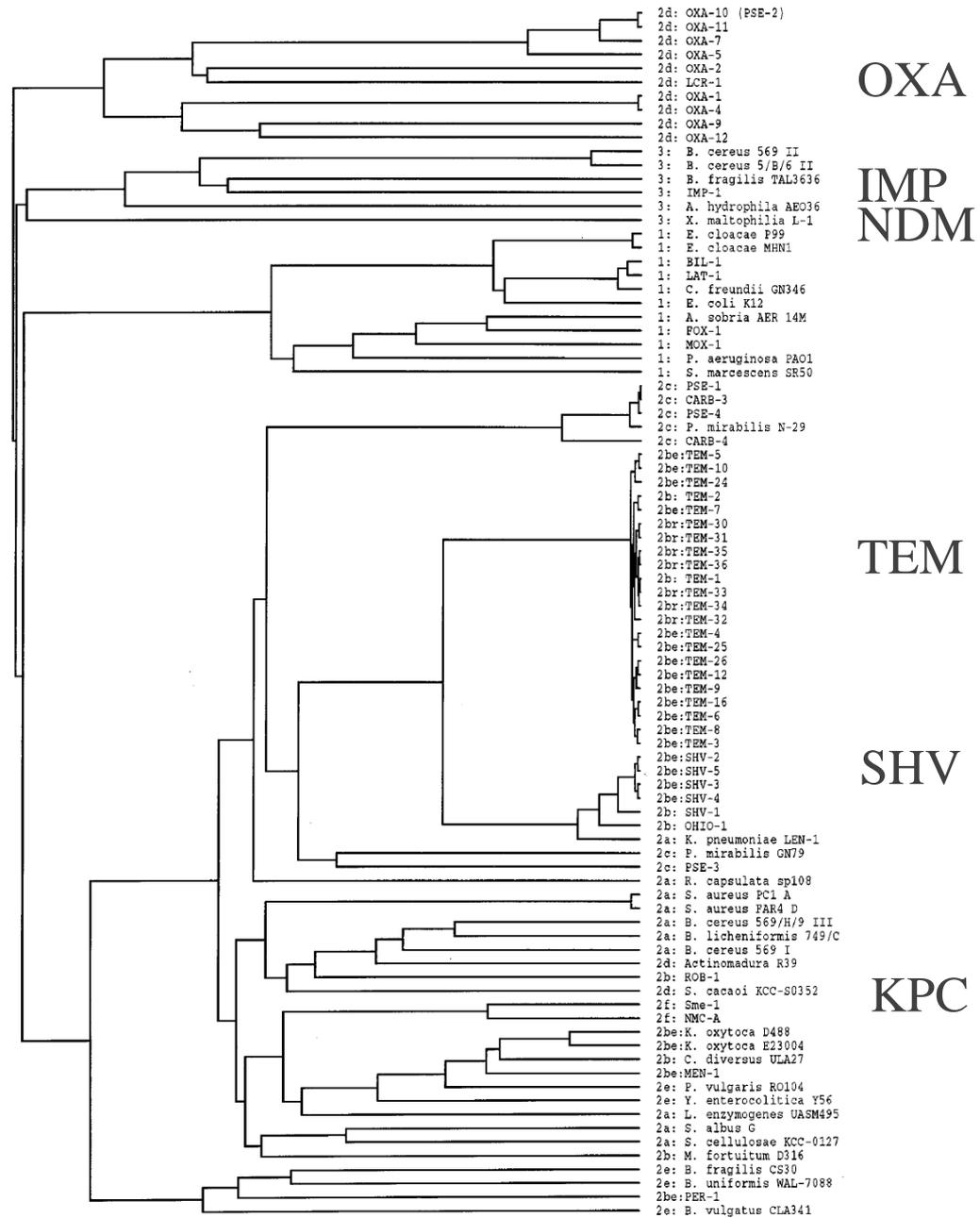


FIG. 1. Dendrogram showing relationships among β -lactamases clustered on the basis of structural similarities and their functional classification.

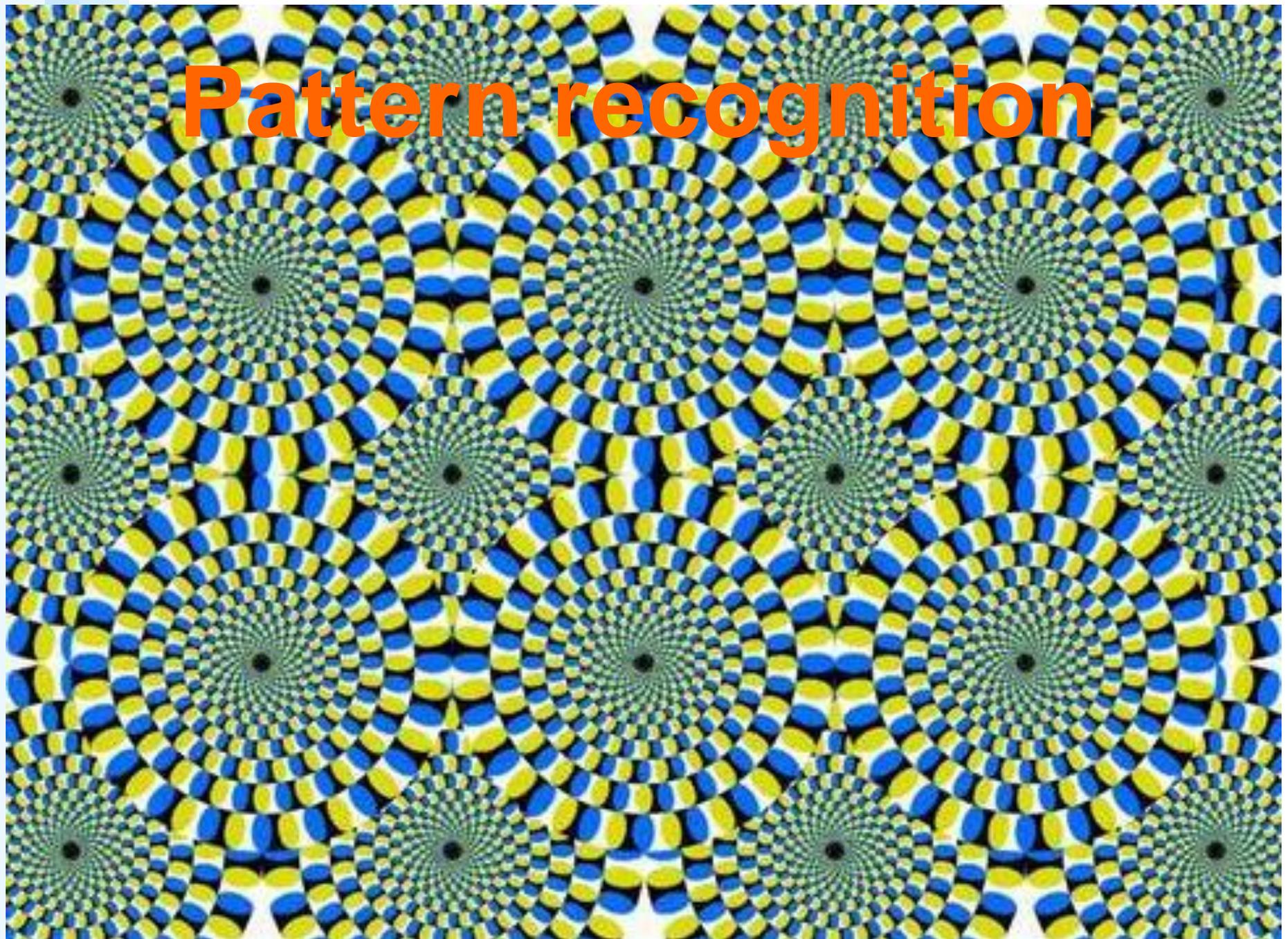
β -laktamase klassifikation og substratprofilTABLE 1. Classification schemes for bacterial β -lactamases, expanded from Bush et al. (16)

Bush-Jacoby group (2009)	Bush-Jacoby-Medeiros group (1995)	Molecular class (subclass)	Distinctive substrate(s)	Inhibited by		Defining characteristic(s)	Representative enzyme(s)
				CA or TZB ^a	EDTA		
1	1	C	Cephalosporins	No	No	Greater hydrolysis of cephalosporins than benzylpenicillin; hydrolyzes cephamycins	<i>E. coli</i> AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
1e	NI ^b	C	Cephalosporins	No	No	Increased hydrolysis of ceftazidime and often other oxyimino- β -lactams	GC1, CMY-37
2a	2a	A	Penicillins	Yes	No	Greater hydrolysis of benzylpenicillin than cephalosporins	PC1
2b	2b	A	Penicillins, early cephalosporins	Yes	No	Similar hydrolysis of benzylpenicillin and cephalosporins	TEM-1, TEM-2, SHV-1
2be	2be	A	Extended-spectrum cephalosporins, monobactams	Yes	No	Increased hydrolysis of oxyimino- β -lactams (cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam)	TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1
2br	2br	A	Penicillins	No	No	Resistance to clavulanic acid, sulbactam, and tazobactam	TEM-30, SHV-10
2ber	NI	A	Extended-spectrum cephalosporins, monobactams	No	No	Increased hydrolysis of oxyimino- β -lactams combined with resistance to clavulanic acid, sulbactam, and tazobactam	TEM-50
2c	2c	A	Carbenicillin	Yes	No	Increased hydrolysis of carbenicillin	PSE-1, CARB-3
2ce	NI	A	Carbenicillin, cefepime	Yes	No	Increased hydrolysis of carbenicillin, cefepime, and ceftiprome	RTG-4
2d	2d	D	Cloxacillin	Variable	No	Increased hydrolysis of cloxacillin or oxacillin	OXA-1, OXA-10
2de	NI	D	Extended-spectrum cephalosporins	Variable	No	Hydrolyzes cloxacillin or oxacillin and oxyimino- β -lactams	OXA-11, OXA-15
2df	NI	D	Carbapenems	Variable	No	Hydrolyzes cloxacillin or oxacillin and carbapenems	OXA-23, OXA-48
2e	2e	A	Extended-spectrum cephalosporins	Yes	No	Hydrolyzes cephalosporins. Inhibited by clavulanic acid but not aztreonam	CepA
2f	2f	A	Carbapenems	Variable	No	Increased hydrolysis of carbapenems, oxyimino- β -lactams, cephamycins	KPC-2, IMI-1, SME-1
3a	3	B (B1) B (B3)	Carbapenems	No	Yes	Broad-spectrum hydrolysis including carbapenems but not monobactams	IMP-1, VIM-1, CcrA, IND-1
3b	3	B (B2)	Carbapenems	No	Yes	Preferential hydrolysis of carbapenems	L1, CAU-1, GOB-1, FEZ-1 CphA, Sfh-1
NI	4	Unknown					

^a CA, clavulanic acid; TZB, tazobactam.^b NI, not included.

Function	Class	Cephalosporin	Monobactam	Carbapenem	CA	EDTA	BA	Clox	Enzyme	Organism
2be	A	+	+	(+)	+	0	0	0	TEM, SHV, CTX-M	enterobacteria
1	C	+	+	(+)	0	0	+	+	CMY, FOX, ACT	enterobacteria
2df	D	0	0	+	v	0	0	0	OXA-48/162/181	enterobacteria
2f	A	+	+	+	v	0	+	0	KPC, IMI	enterobacteria
3a	B	+	0	+	0	+	0	0	IMP, VIM, NDM	enterobacteria
3a	B3	+	0	+	0	+	0	0	L1,	S.maltophilia
3a	B1	+	0	+	0	+	0	0	CcrA	B.fragilis
3b	B2	+	0	+	0	+	0	0	CphA	A.hydrophila

Pattern recognition



Synergy tests

Double Disk Test and Combination Disc Test

Clavulan syre

Borsyre

Cloxacillin

EDTA

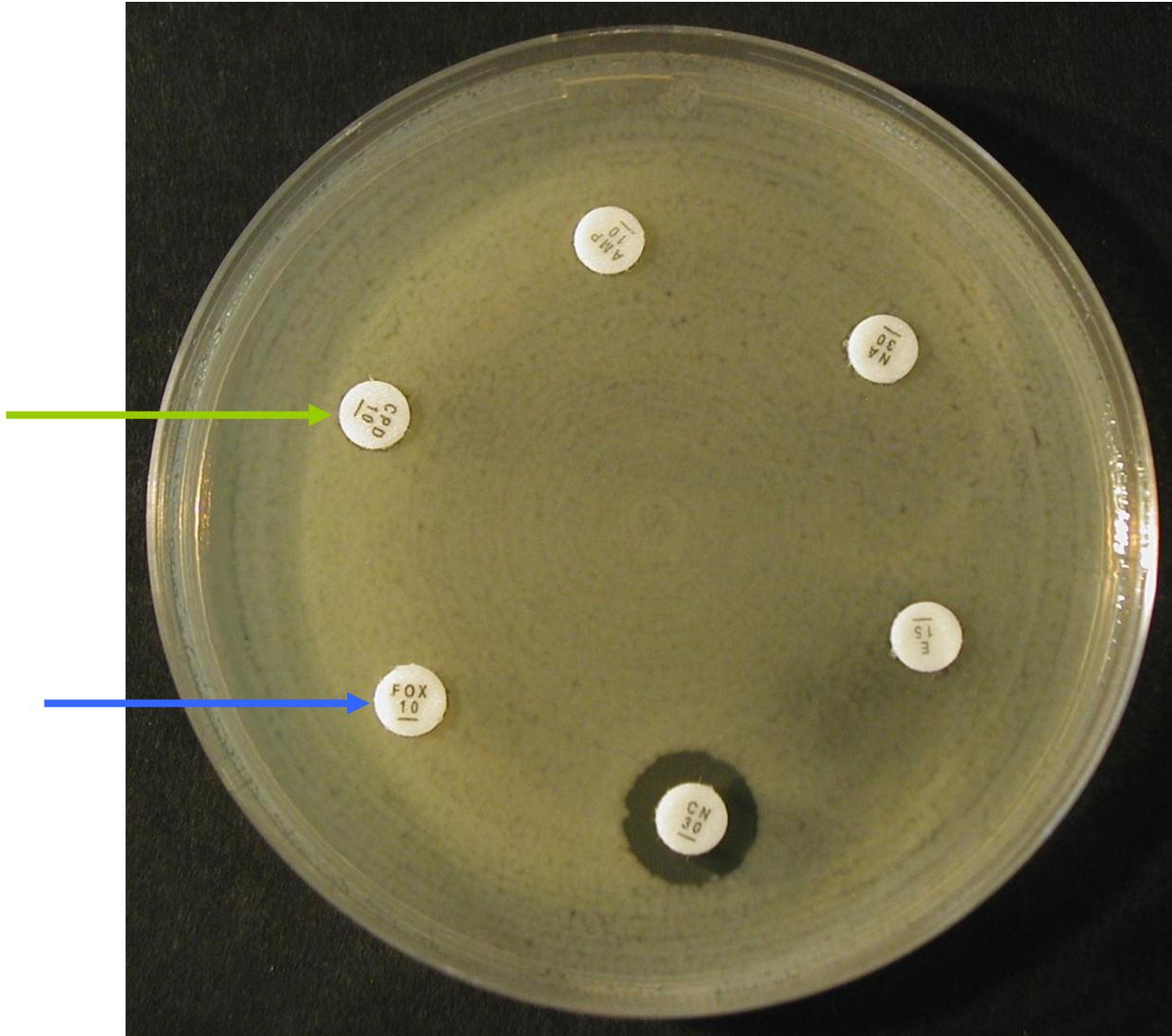
Dipicolinsyre

ESBL

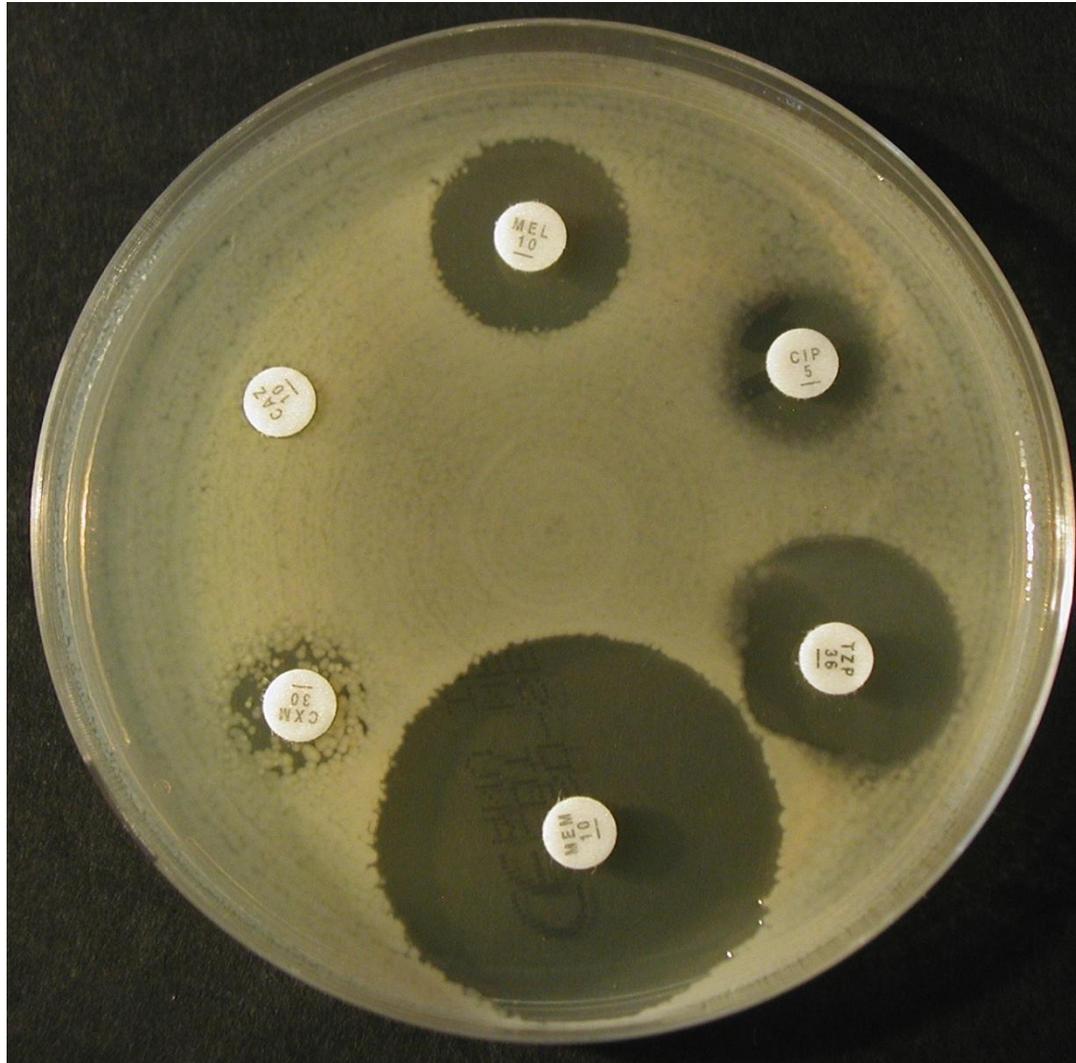
K. pneumoniae



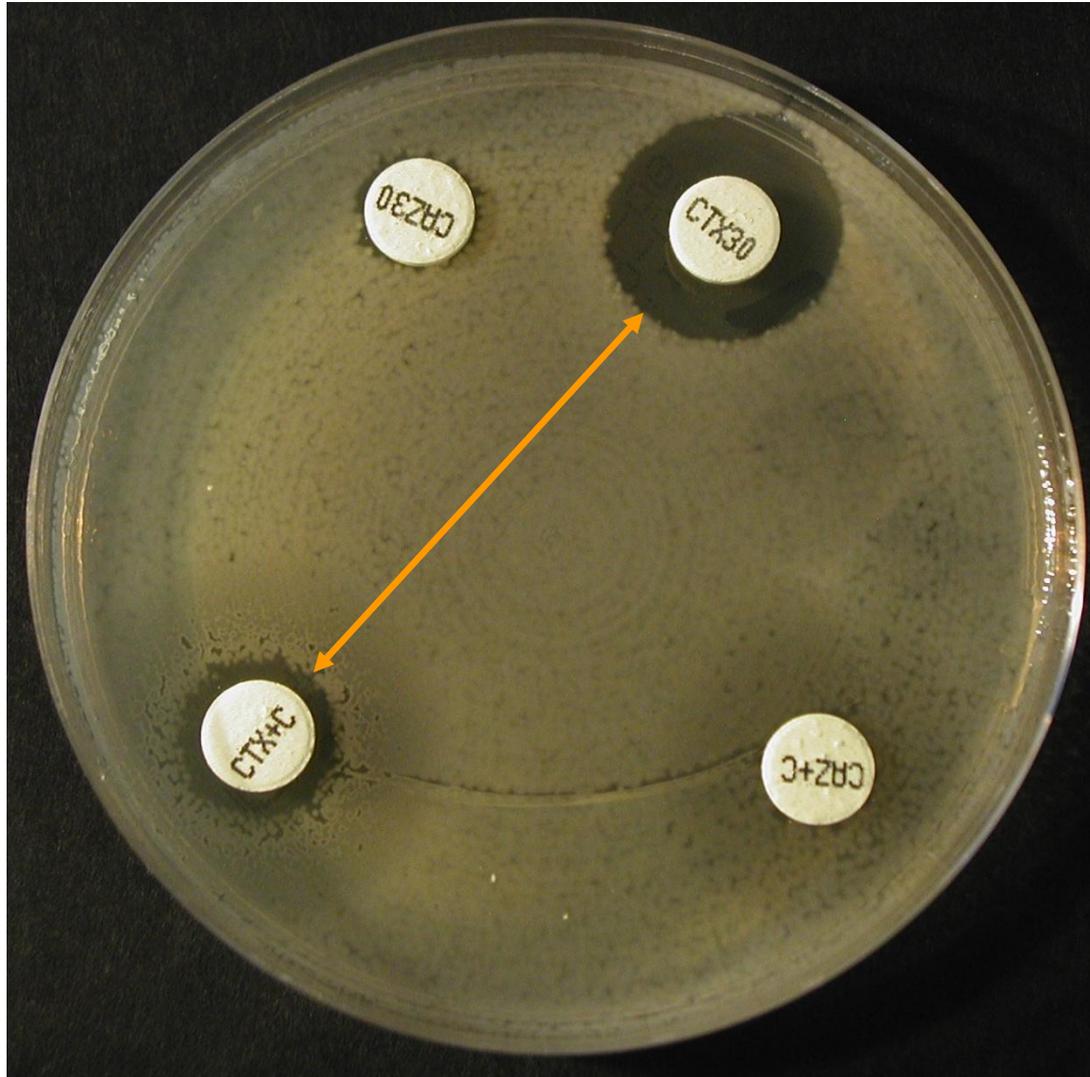
C.freundii 2



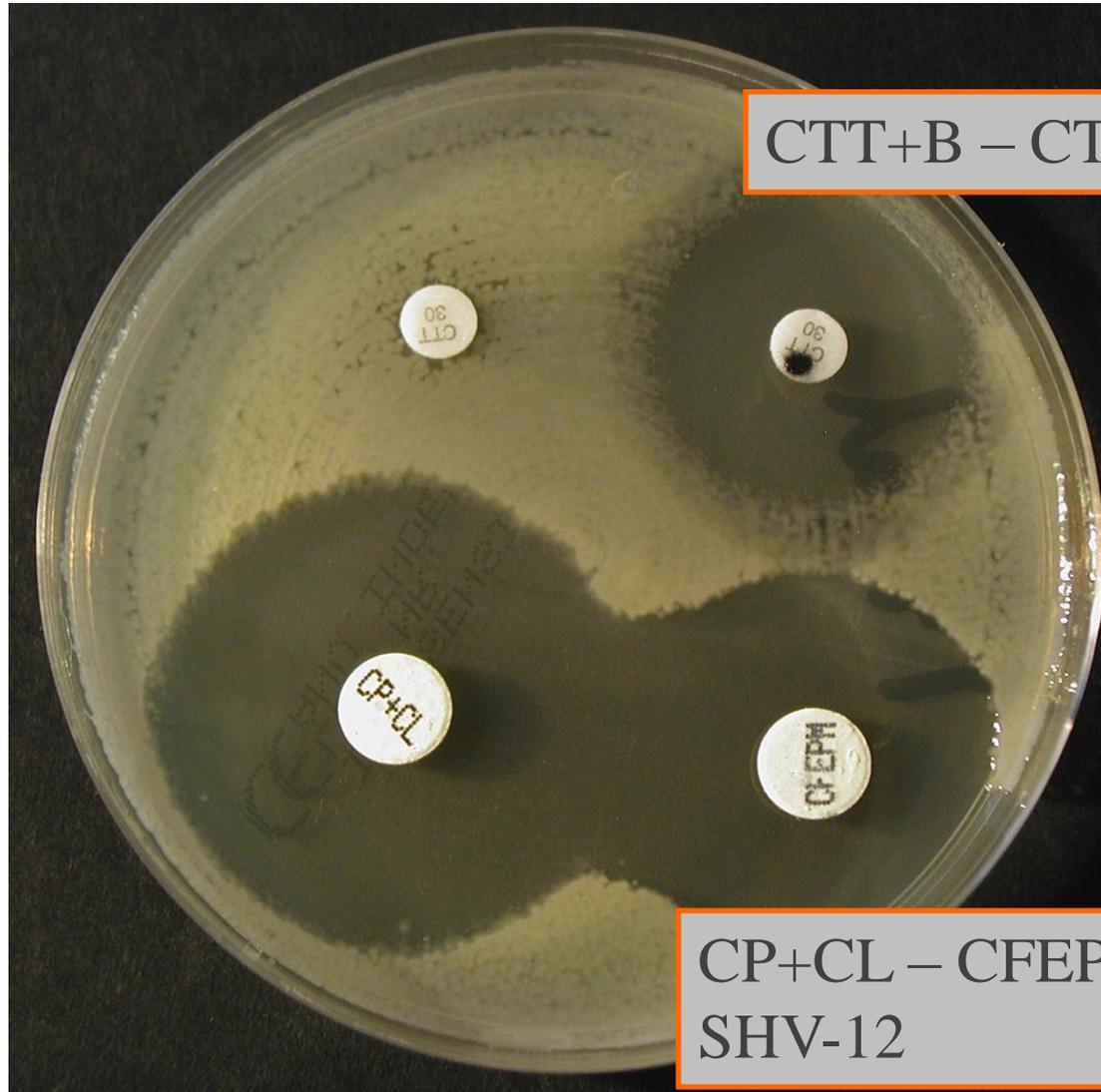
C.freundii 2



C.freundii 2



C.freundii 2

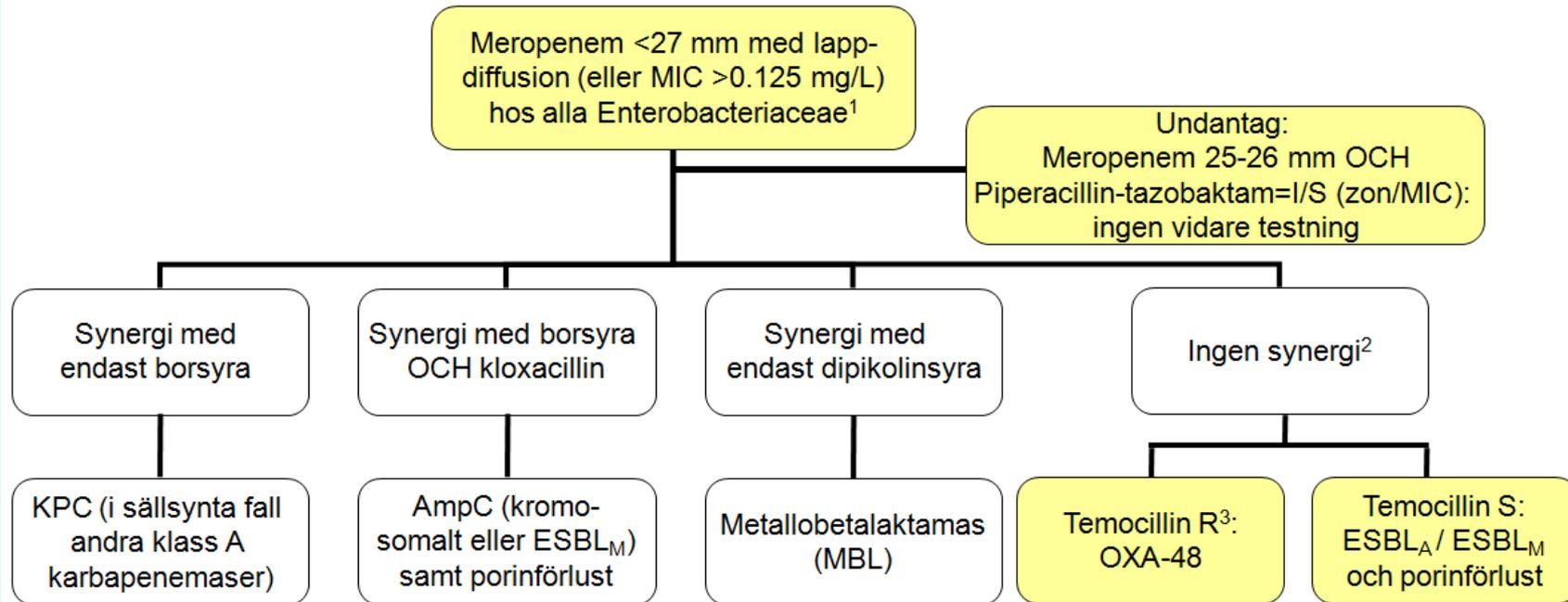


Screening for karbapenemaser

Enterobacteriaceae

ALGORITM FÖR DETEKTION AV KARBAPENEMASER

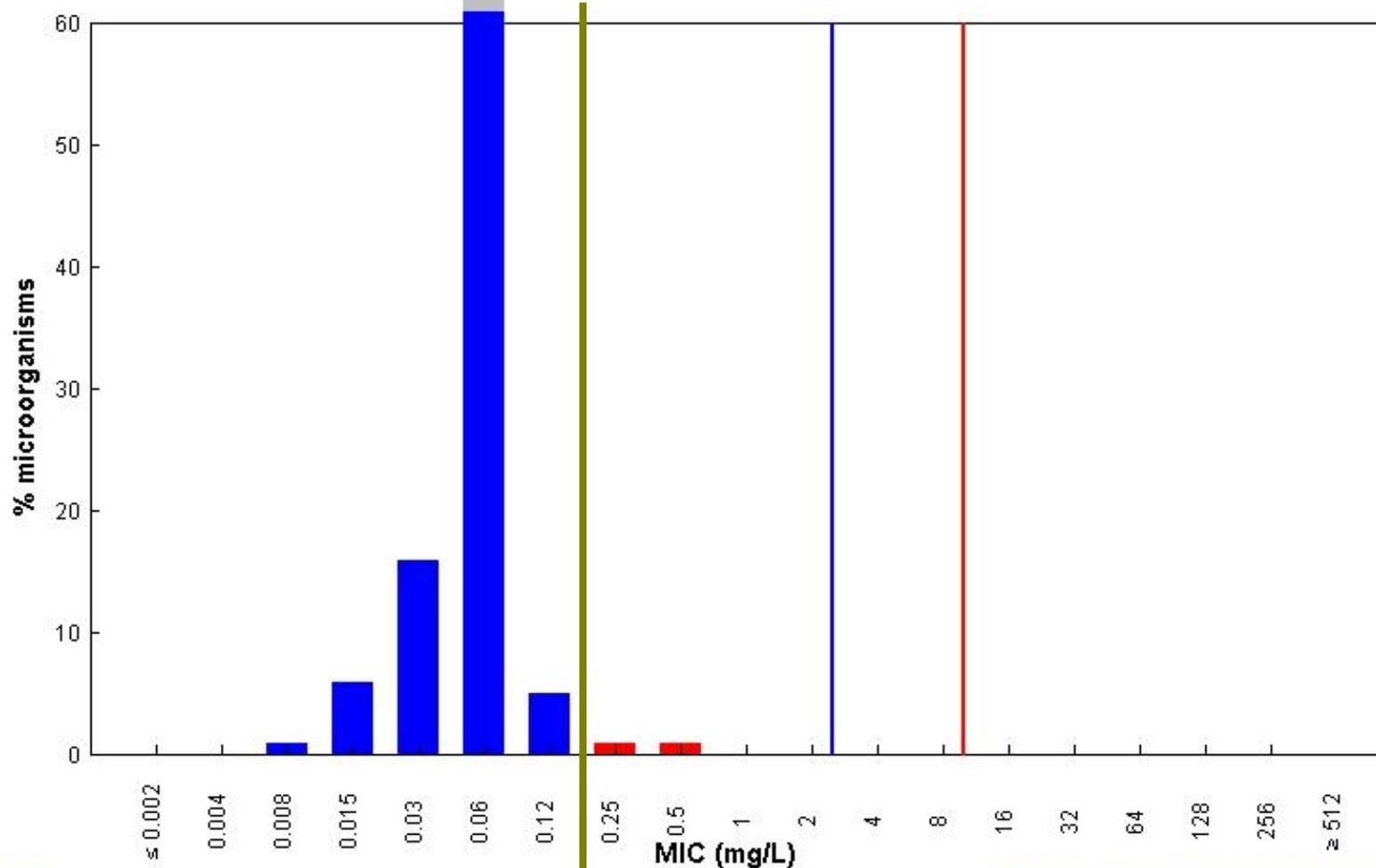
NordicAST v. 5.1, 2015-04-20



3 Högradig temocillinresistens (>128 mg/L, zondiameter <12 mm) är en fenotypisk indikator på OXA-48. OXA-48-producerande isolat är även resistenta mot piperacillin-tazobaktam och amoxicillin-klavulansyra.

Meropenem / *Klebsiella pneumoniae* EUCAST MIC Distribution - Reference Database 2012-02-28

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC

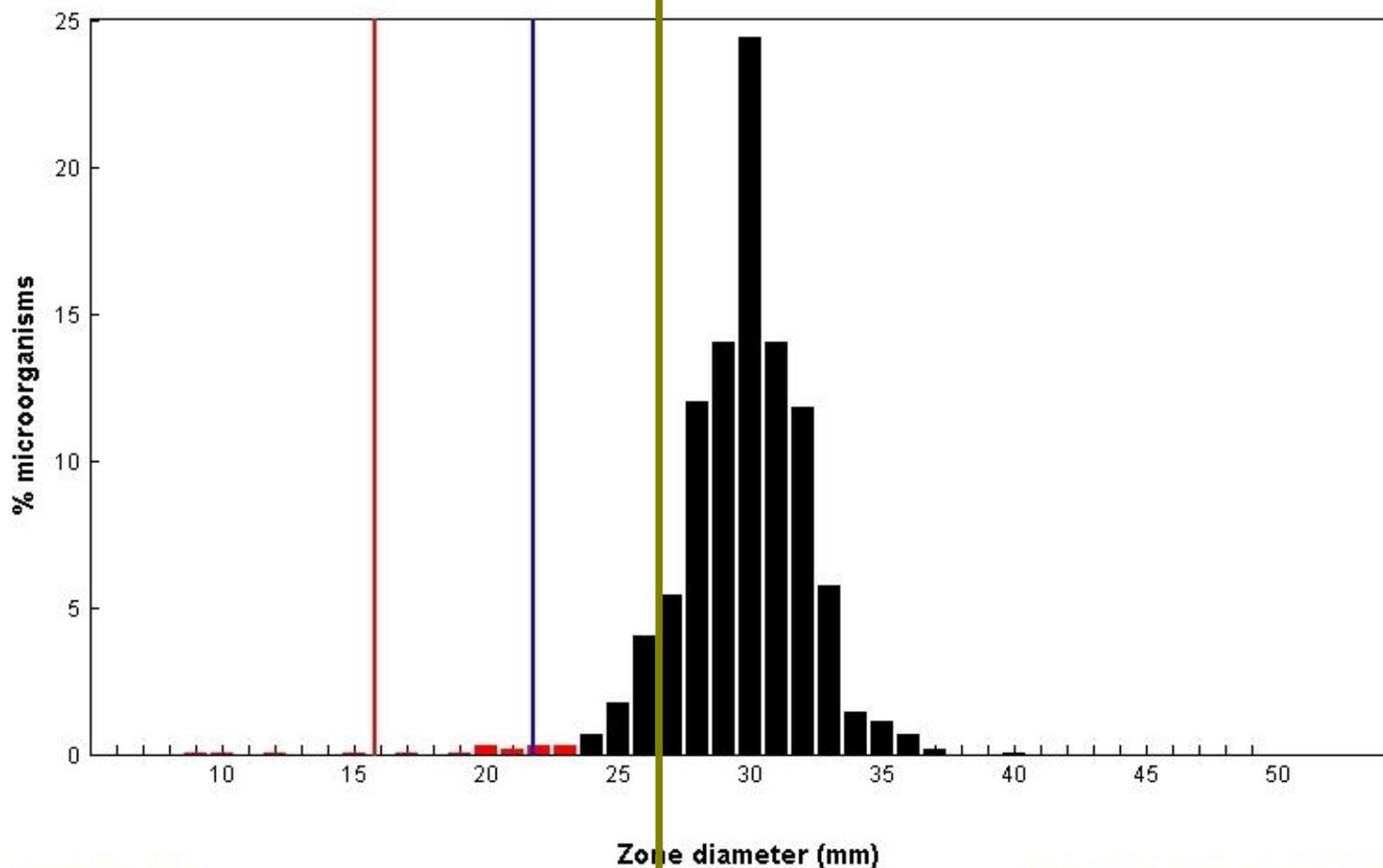
Epidemiological cut-off: WT ≤ 0.125 mg/L

18171 observations (67 data sources)

Clinical breakpoints: S ≤ 2 mg/L, R > 8 mg/L

Meropenem / *Klebsiella pneumoniae*
EUCAST zone diameter distribution - Reference database 2012-02-28
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 10

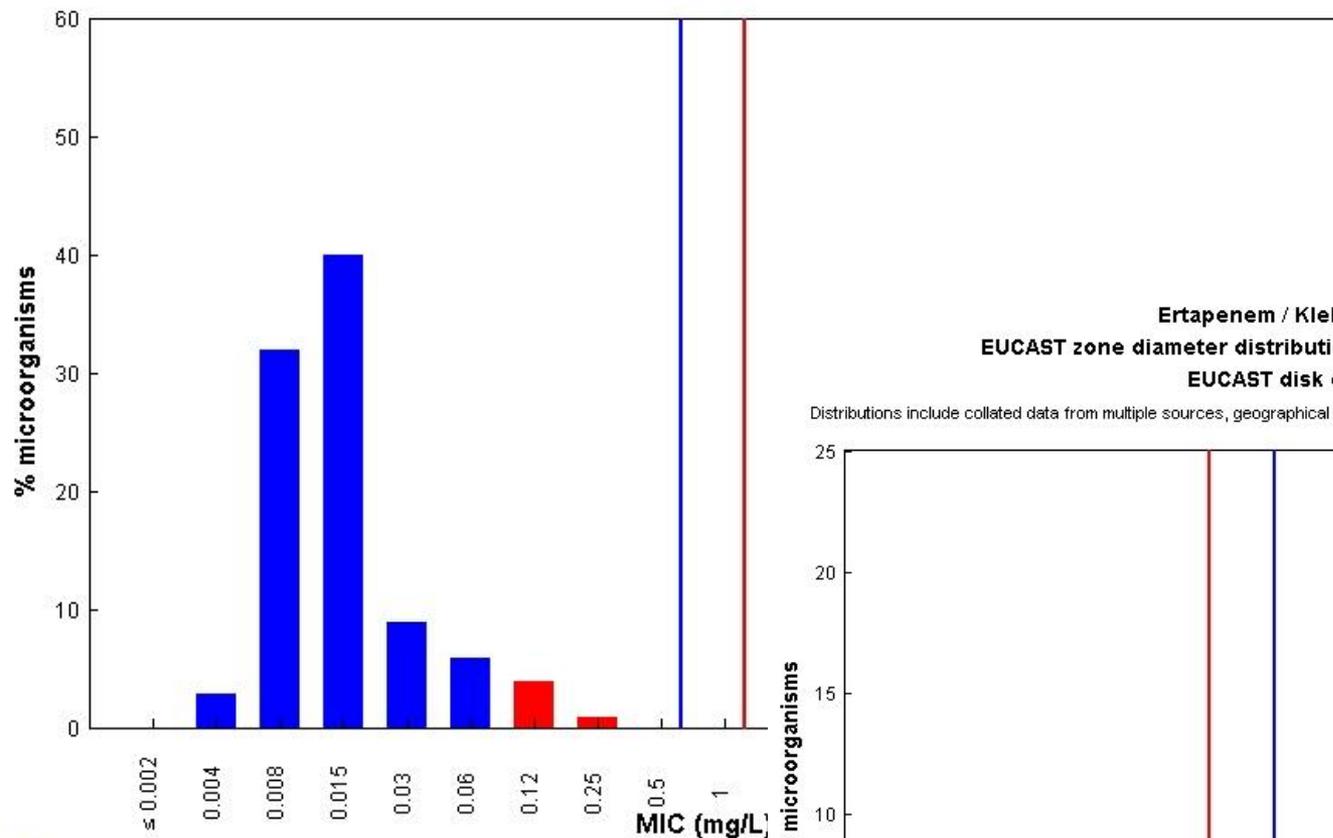
Epidemiological cut-off: WT \geq 24 mm (MIC \leq 0.125 mg/L)

936 observations (6 data sources)

Clinical breakpoints: S \geq 22 mm, R < 16 mm (S \leq 2 mg/L, R $>$ 8 mg/L,)

Ertapenem / *Klebsiella pneumoniae*
EUCAST MIC Distribution - Reference Database 2012-02-28

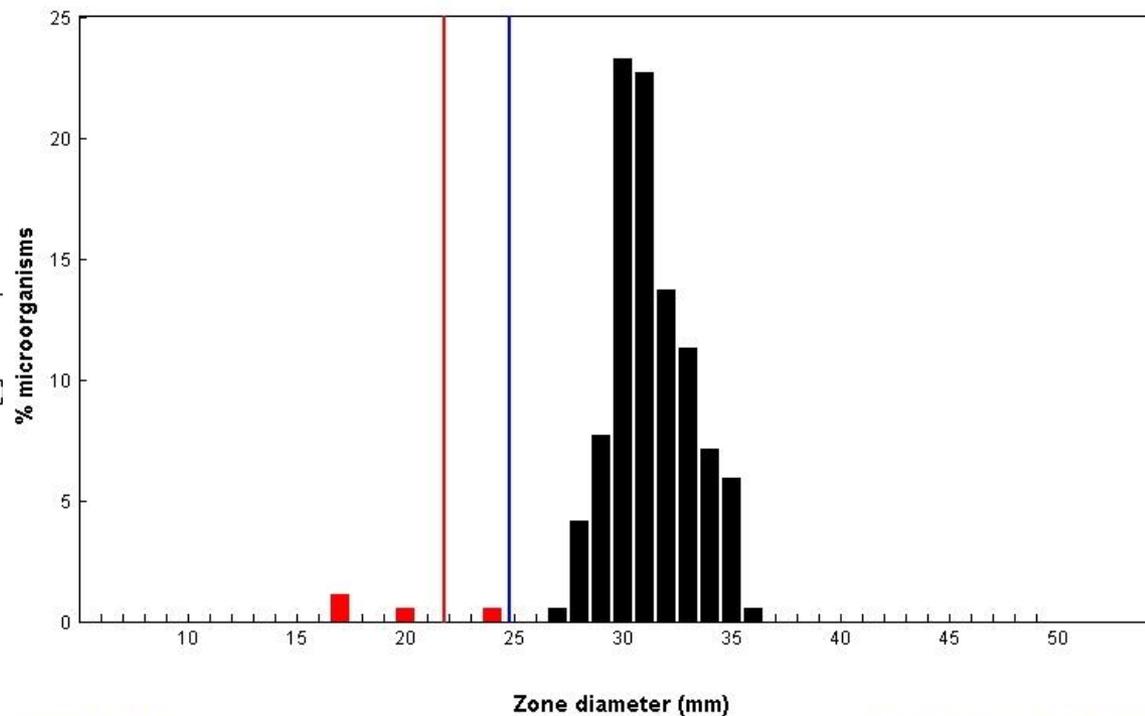
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
 Epidemiological cut-off: WT ≤ 0.064 mg/L

Ertapenem / *Klebsiella pneumoniae*
EUCAST zone diameter distribution - Reference database 2012-02-28
EUCAST disk diffusion method

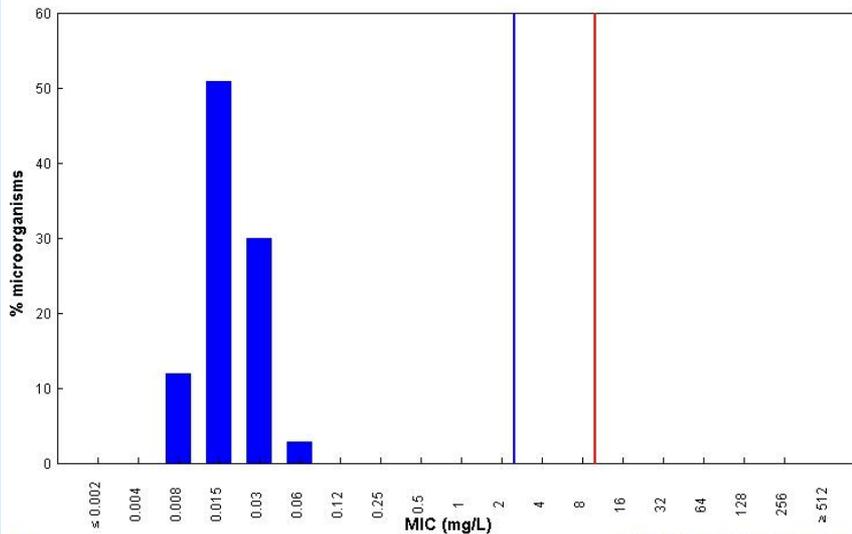
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 10
 Epidemiological cut-off: WT ≥ 25 mm (MIC ≤ 0.064 mg/L) Clinical breakpoints: S ≥ 25 mm, R < 22 mm (S ≤ 0.5 mg/L, R > 1 mg/L,)
 167 observations (2 data sources)

Meropenem / Escherichia coli
EUCAST MIC Distribution - Reference Database 2012-02-28

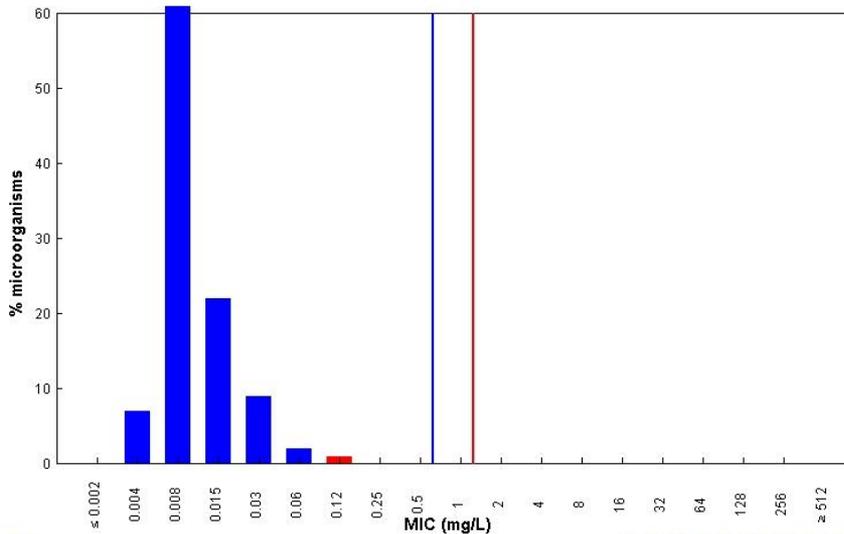
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC 8005 observations (68 data sources)
 Epidemiological cut-off: WT ≤ 0.125 mg/L
 Clinical breakpoints: S ≤ 2 mg/L, R > 8 mg/L

Ertapenem / Escherichia coli
EUCAST MIC Distribution - Reference Database 2012-02-28

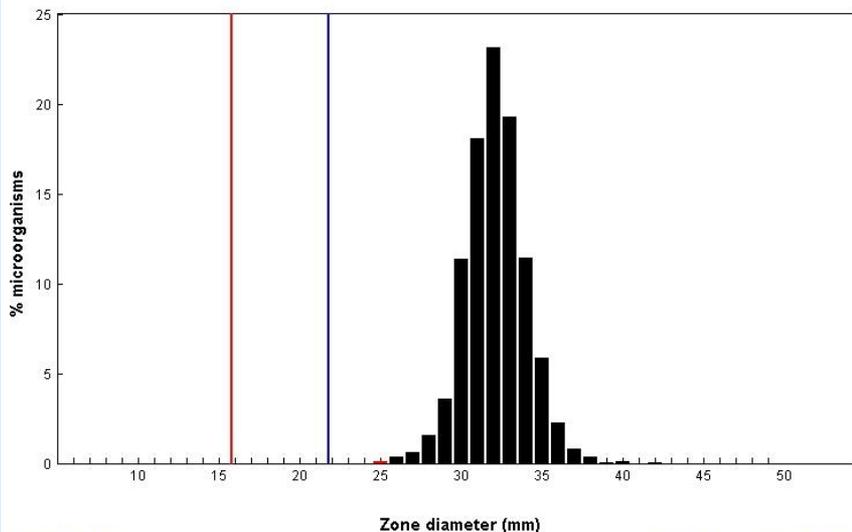
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC 2181 observations (11 data sources)
 Epidemiological cut-off: WT ≤ 0.064 mg/L
 Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

Meropenem / Escherichia coli
EUCAST zone diameter distribution - Reference database 2012-02-28
EUCAST disk diffusion method

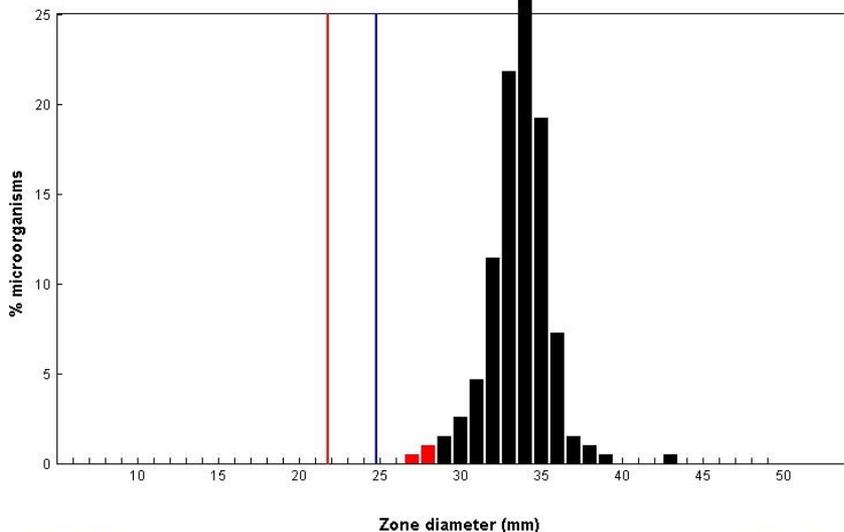
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 10 7027 observations (7 data sources)
 Epidemiological cut-off: WT ≥ 26 mm (MIC ≤ 0.125 mg/L)
 Clinical breakpoints: S ≥ 22 mm, R < 16 mm (S ≤ 2 mg/L, R > 8 mg/L)

Ertapenem / Escherichia coli
EUCAST zone diameter distribution - Reference database 2012-02-28
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

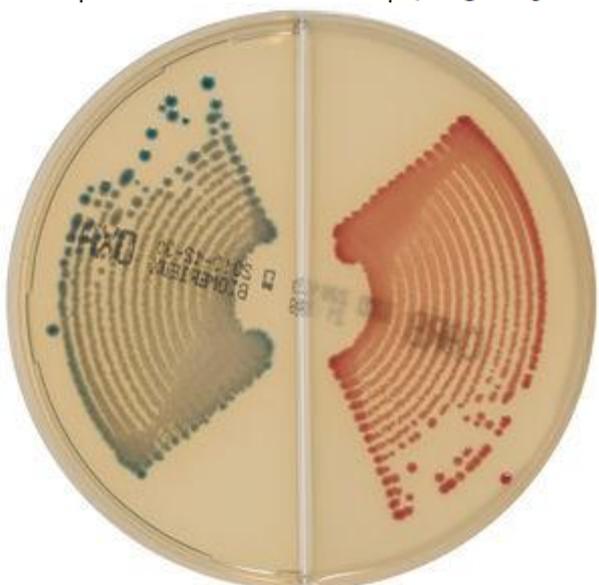


Disk content: 10 192 observations
 Epidemiological cut-off: WT ≥ 29 mm (MIC ≤ 0.064 mg/L)
 Clinical breakpoints: S ≥ 25 mm, R < 22 mm (S ≤ 0.5 mg/L, R > 1 mg/L)

Screening for karbapenemaser



Tabel 1: Test af selektive medier til detektion af carbapenemaser hos *Enterobacteriaceae*

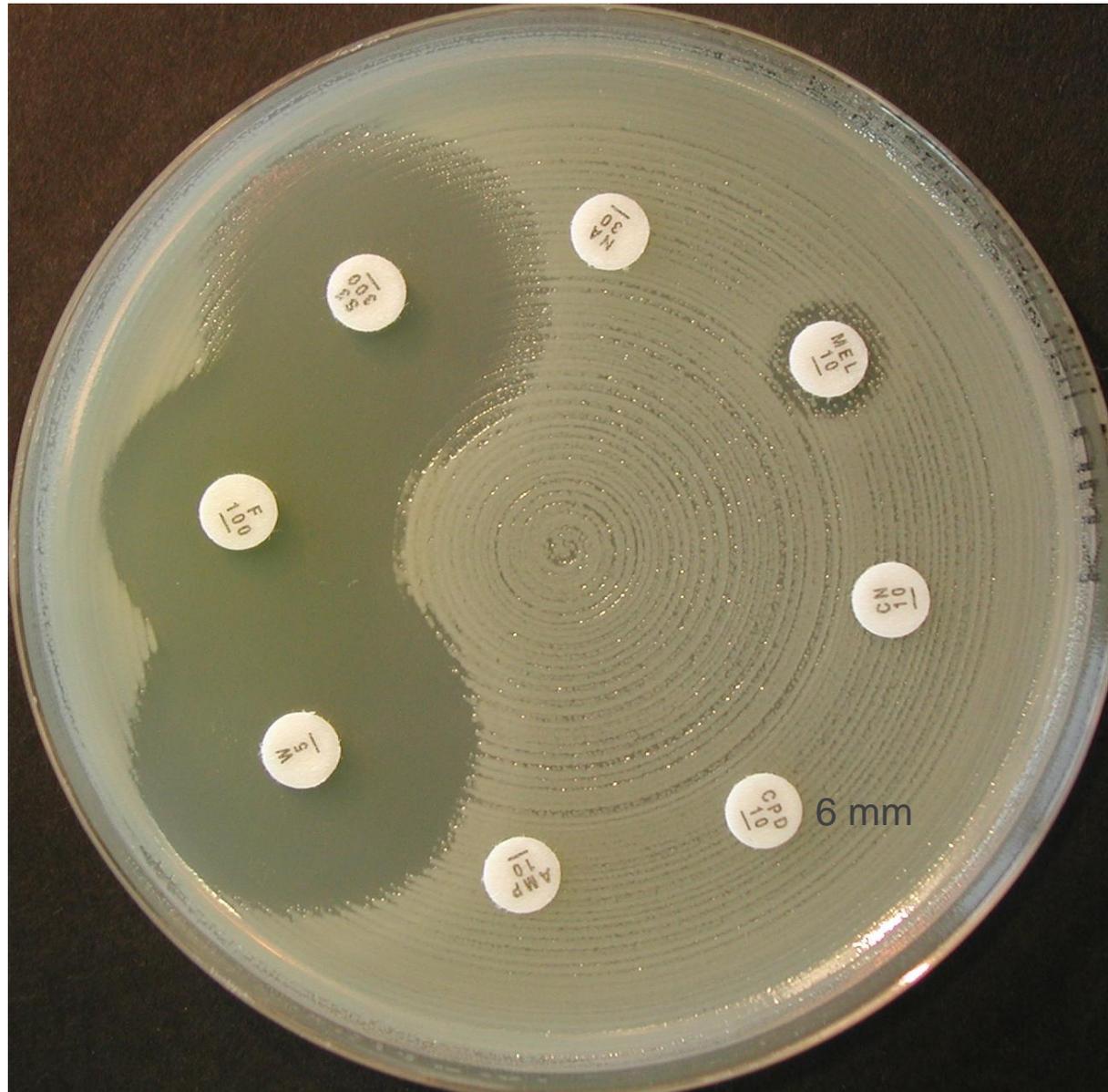
Reference	Medie	Sensitivitet (%)	Specificitet (%)	Test materiale	Kommentarer
Girlich et al, Clin Microbiol Infect 2014 (ref 1)	ChromID ESBL	90 (uden opformering) 100 (opformering)	29,9	77 rektalpodninger 10 OXA-48	Opformering i BHI med 0,25 mg/L ertapenem
	Brilliance CRE	80 (uden opformering) 100 (opformering)	71,6		
	SUPERCARBA	80 (uden opformering) 100 (opformering)	52,2		
Papadimitriou-Olivgeris et al, Eur J Clin Microbiol Infect Dis 2014 (ref 2)	ChromID CARBA	96,5	91,2		
	MacConkey m. imipenem disk	89,5	31,9		
	TSB m. ertapenem (2 mg/L)	98,8	80,2		
Girlich et al, Diagn Microbiol Infect Dis 2013 (ref 3)	SUPERCARBA	96,5	60,7		
	Brilliance CRE	76,3	57,1		
	CHROMagar KPC	43	67,8		
Girlich et al, Diagn Microbiol Infect Dis 2013 (ref 4)	SUPERCARBA	96,1	52,5		
	ChromID OXA-48	70,1	100 (for OXA-48)		
	ChromID CARBA	40,3	67,5		
	ChromID OXA-48 & CARBA	94,8	67,5		

... ”Med henblik på detektion af alle typer af carbapenemaser inkl. OXA-48-producerende isolater med lave carbapenem MIC-værdier, opnår SUPERCARBA samt chromID® CARBA SMART, BioMérieux (CARBA & OXA-48 splitplade) højest sensitivitet og specificitet i de hidtil publicerede test.”

Karbapenemaser – fænotypisk identifikation

- Metallo
- KPC
- ESBL + OMP-forandringer
- OXA-48

Stamme 5: VIM-1 + CTX-M + TEM, meropenem MIC = 0,75 µg/ml



E. coli

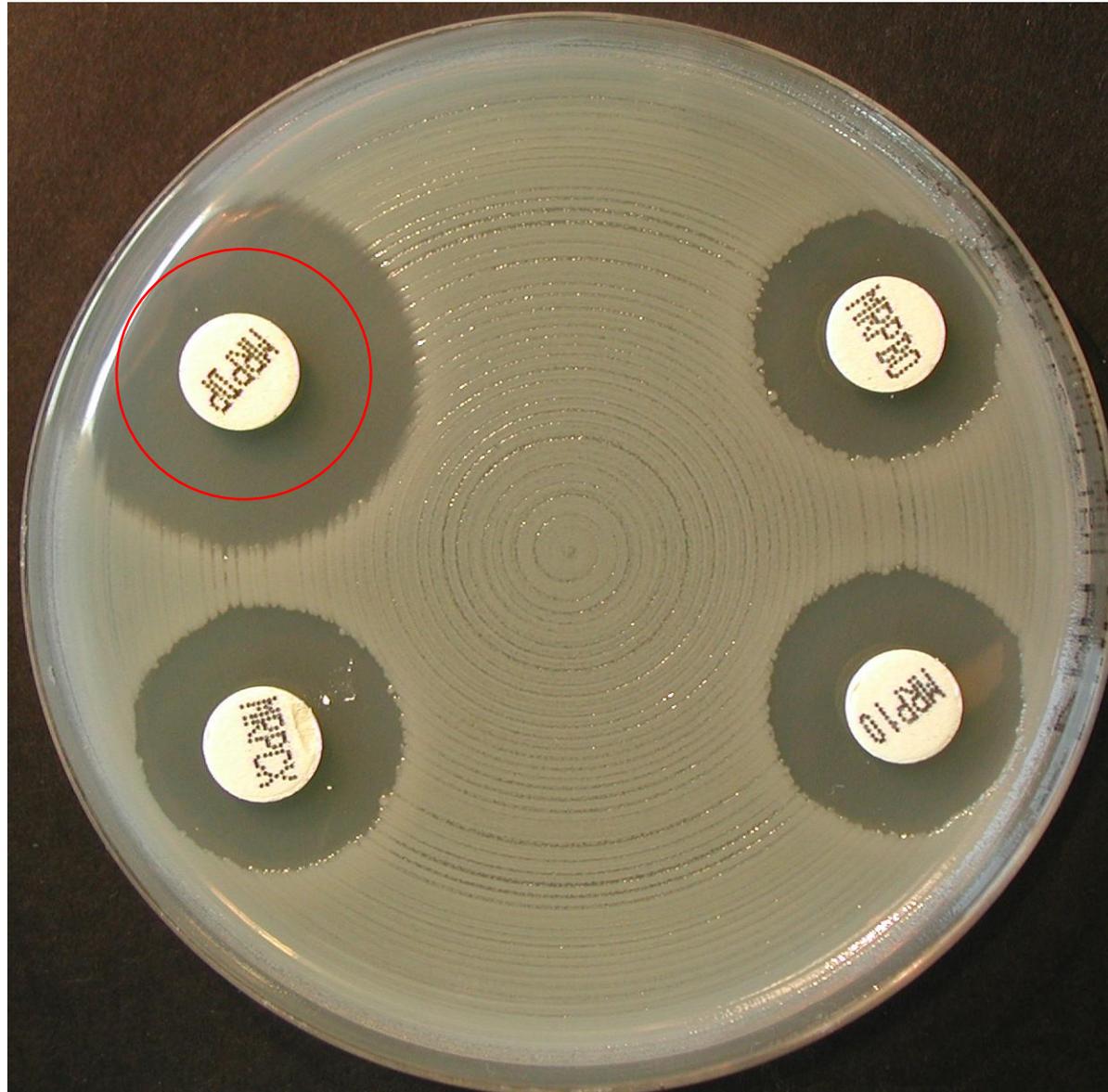
Stamme 5: VIM-1 + CTX-M + TEM, meropenem MIC = 0,75 µg/ml



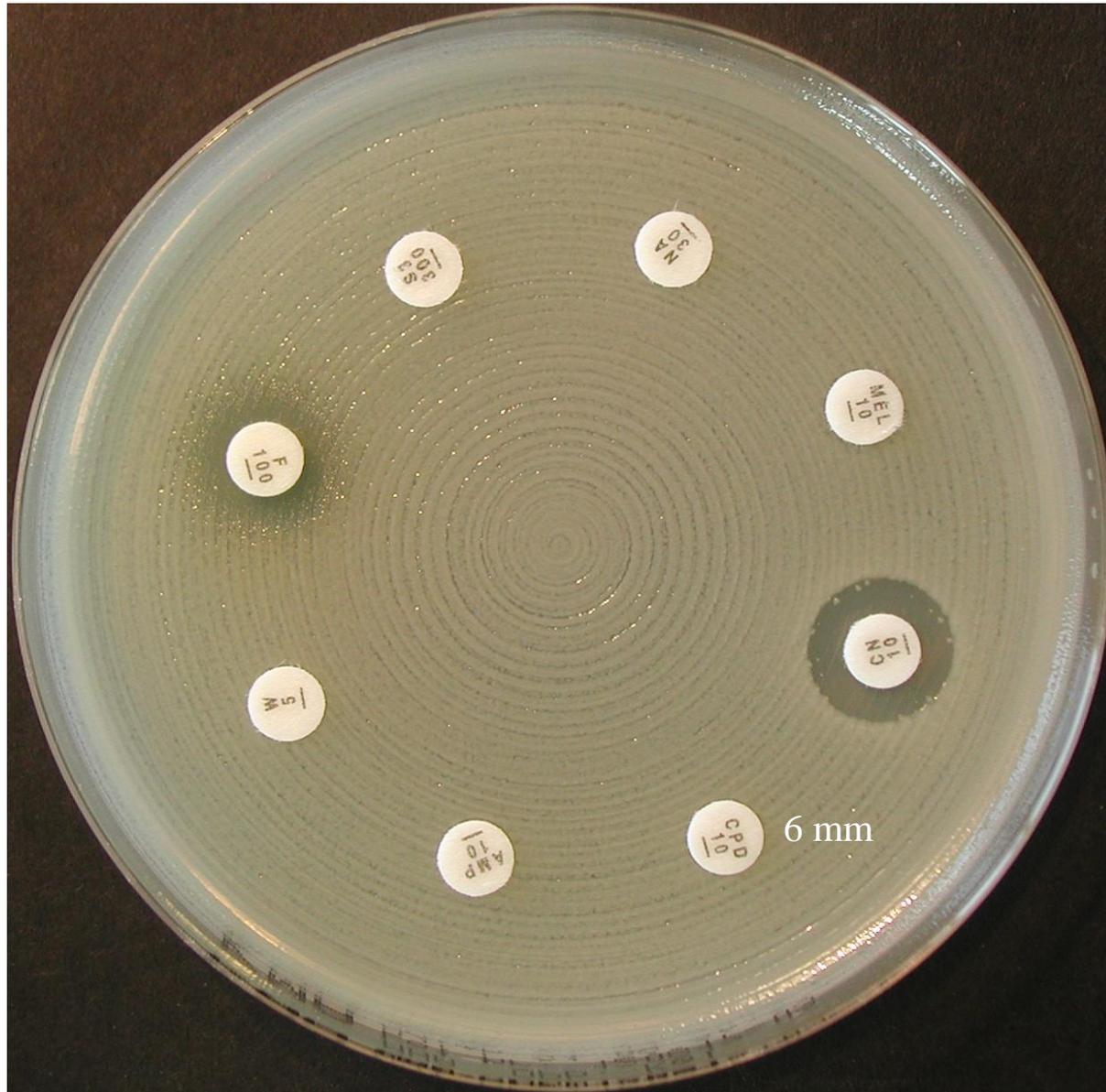
Stamme 5: VIM-1 + CTX-M + TEM, meropenem MIC = 0,75 µg/ml



Stamme 5: VIM-1 + CTX-M + TEM, meropenem MIC = 0,75 µg/ml

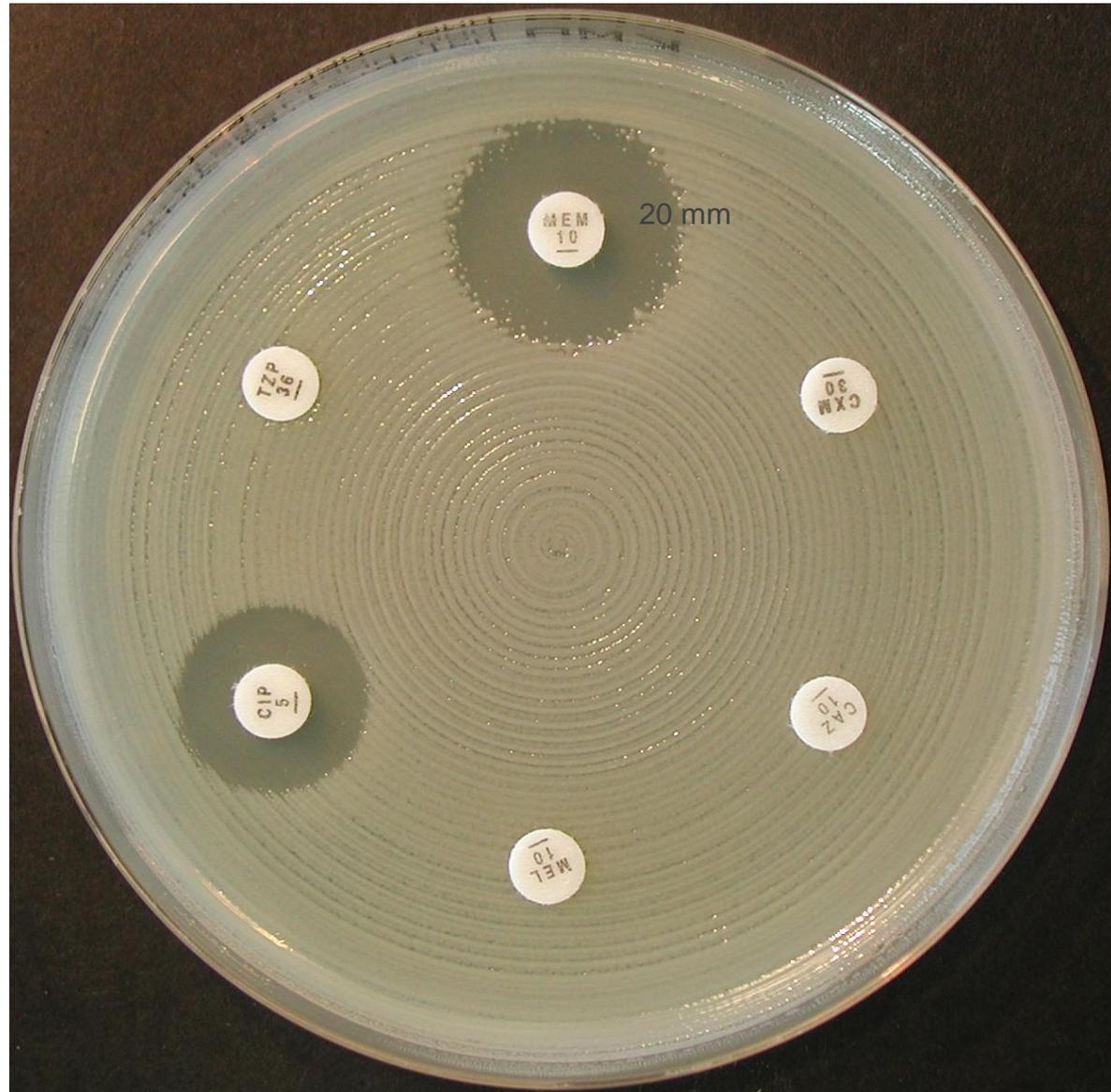


Stamme 7: KPC-3 + SHV + TEM, meropenem MIC = 1,0 µg/ml

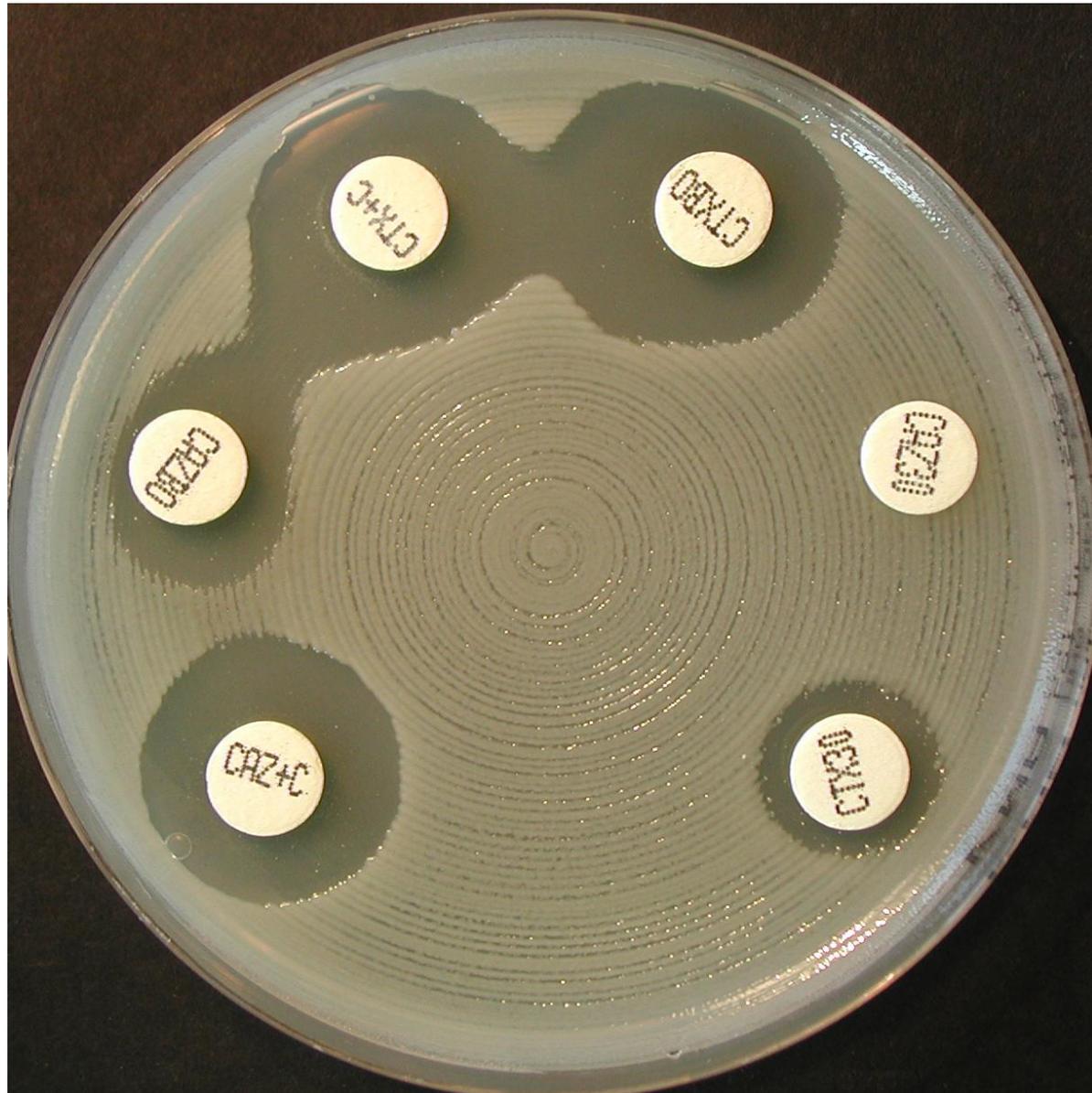


Kpn

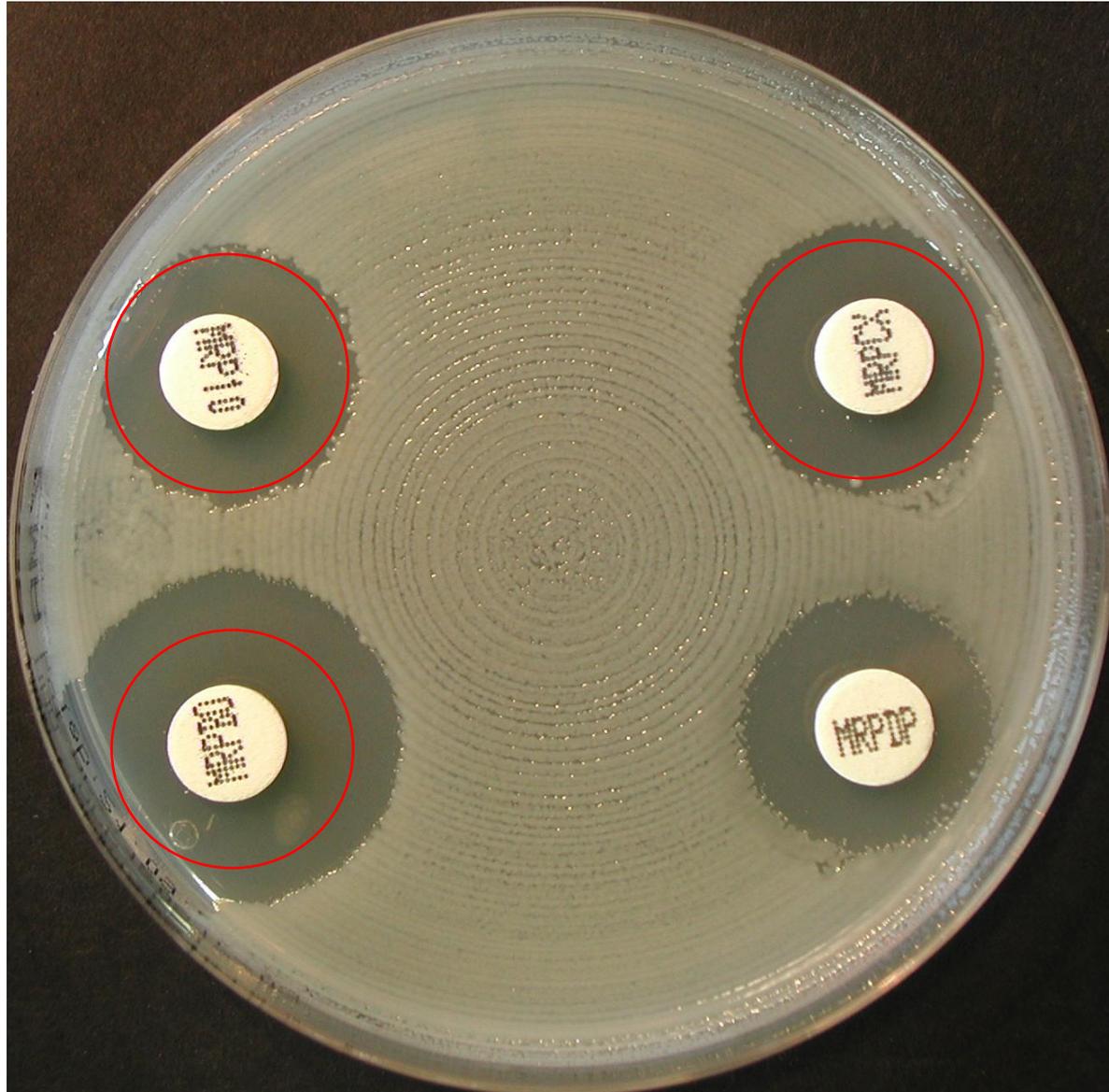
Stamme 7: KPC-3 + SHV + TEM, meropenem MIC = 1,0 µg/ml



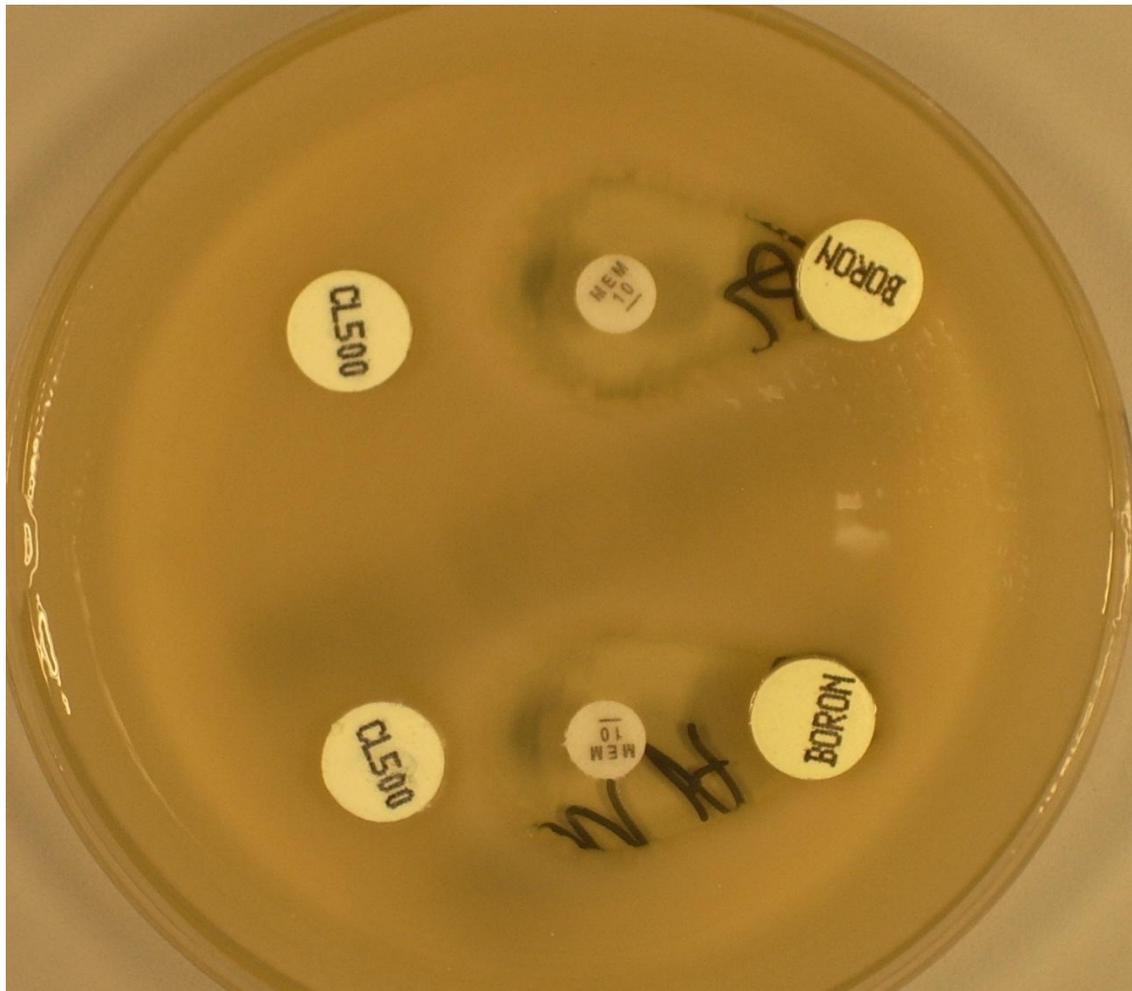
Stamme 7: KPC-3 + SHV + TEM, meropenem MIC = 1,0 µg/ml



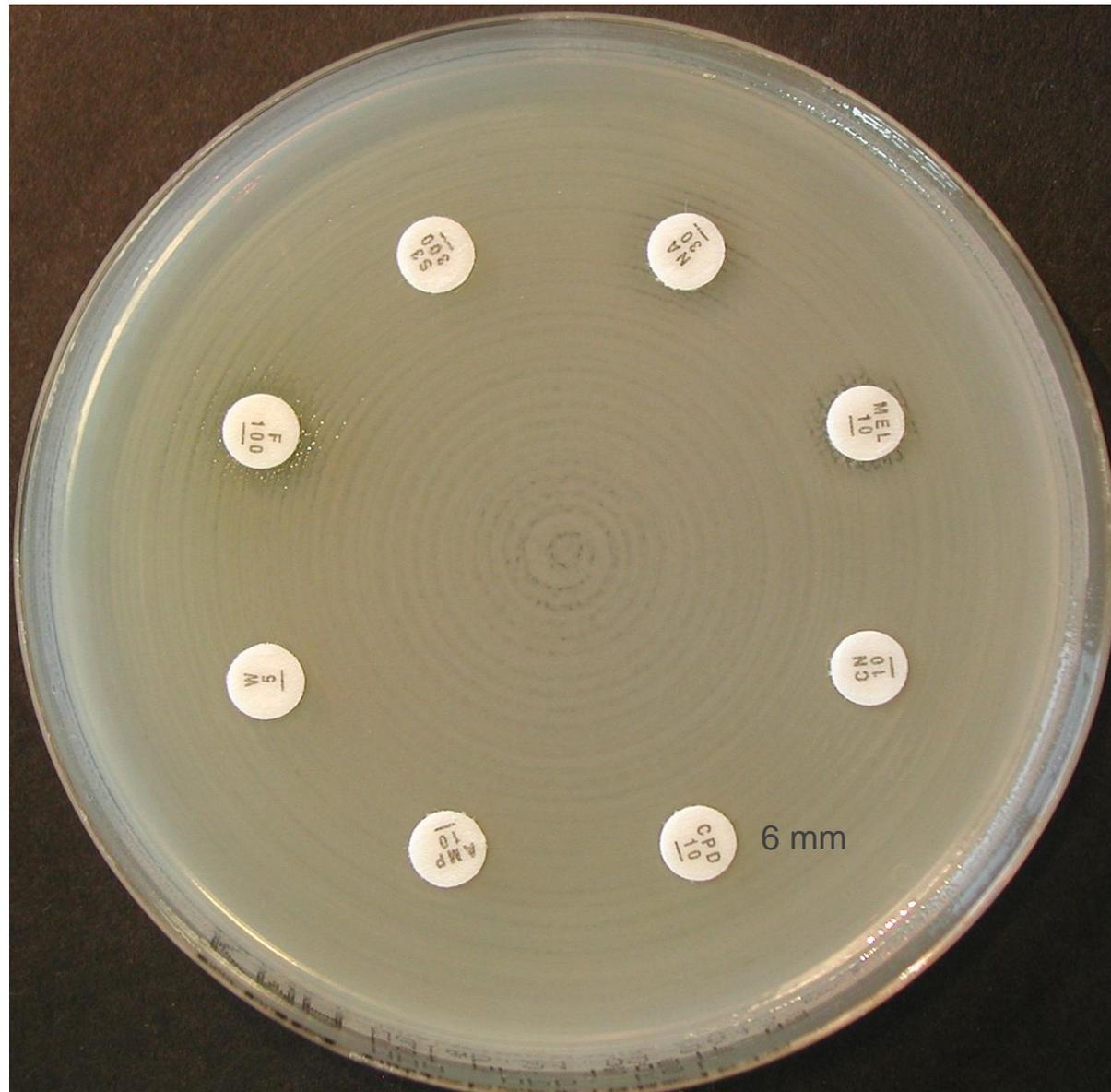
Stamme 7: KPC-3 + SHV + TEM, meropenem MIC = 1,0 µg/ml



KPC fundet på Esbjerg KMA – patient fra Grækenland

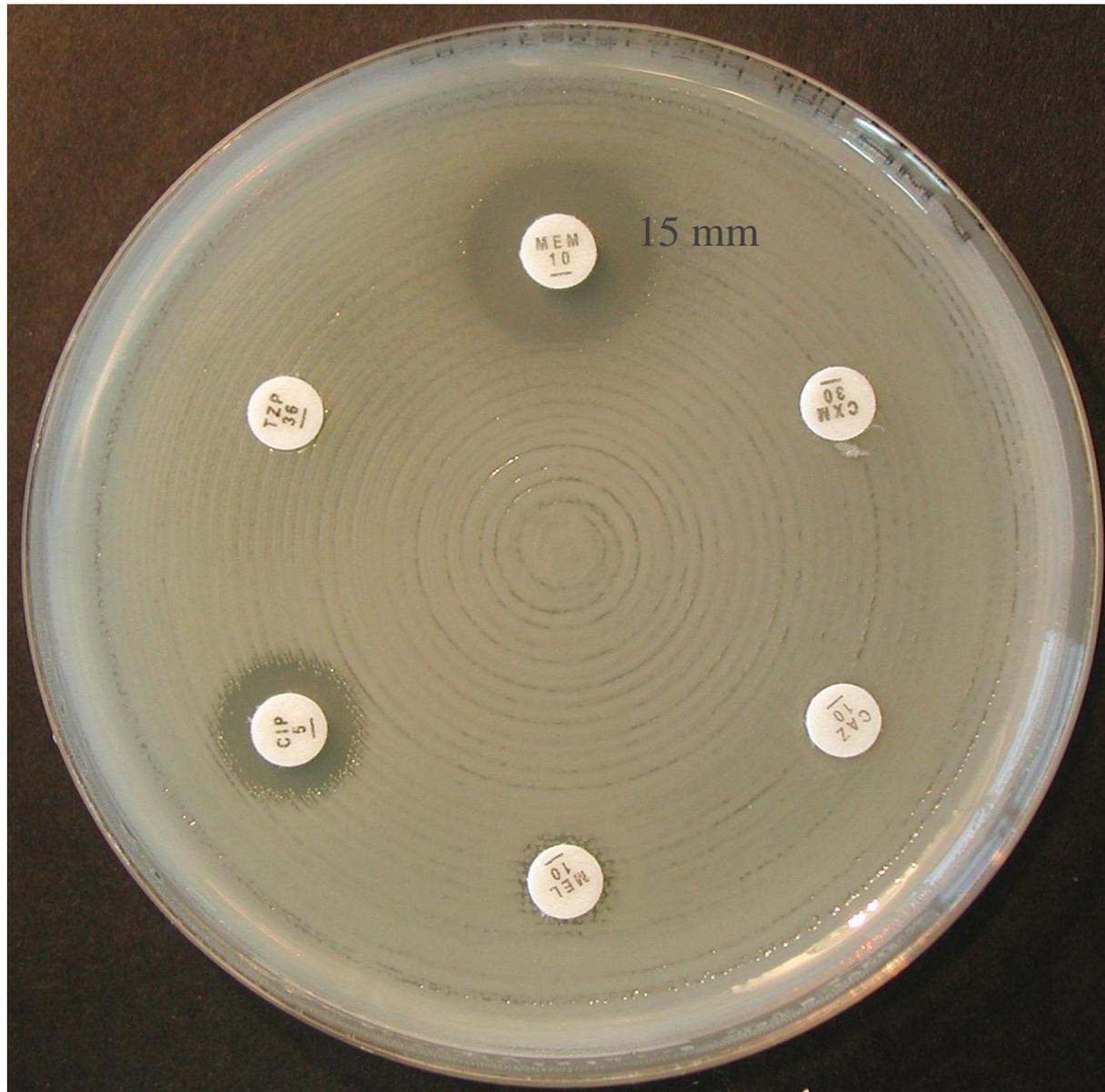


Stamme 8: CTX-M-15 + SHV-32 + imp., meropenem MIC = 6 µg/ml



Kpn

Stamme 8: CTX-M-15 + SHV-32 + imp., meropenem MIC = 6 µg/ml

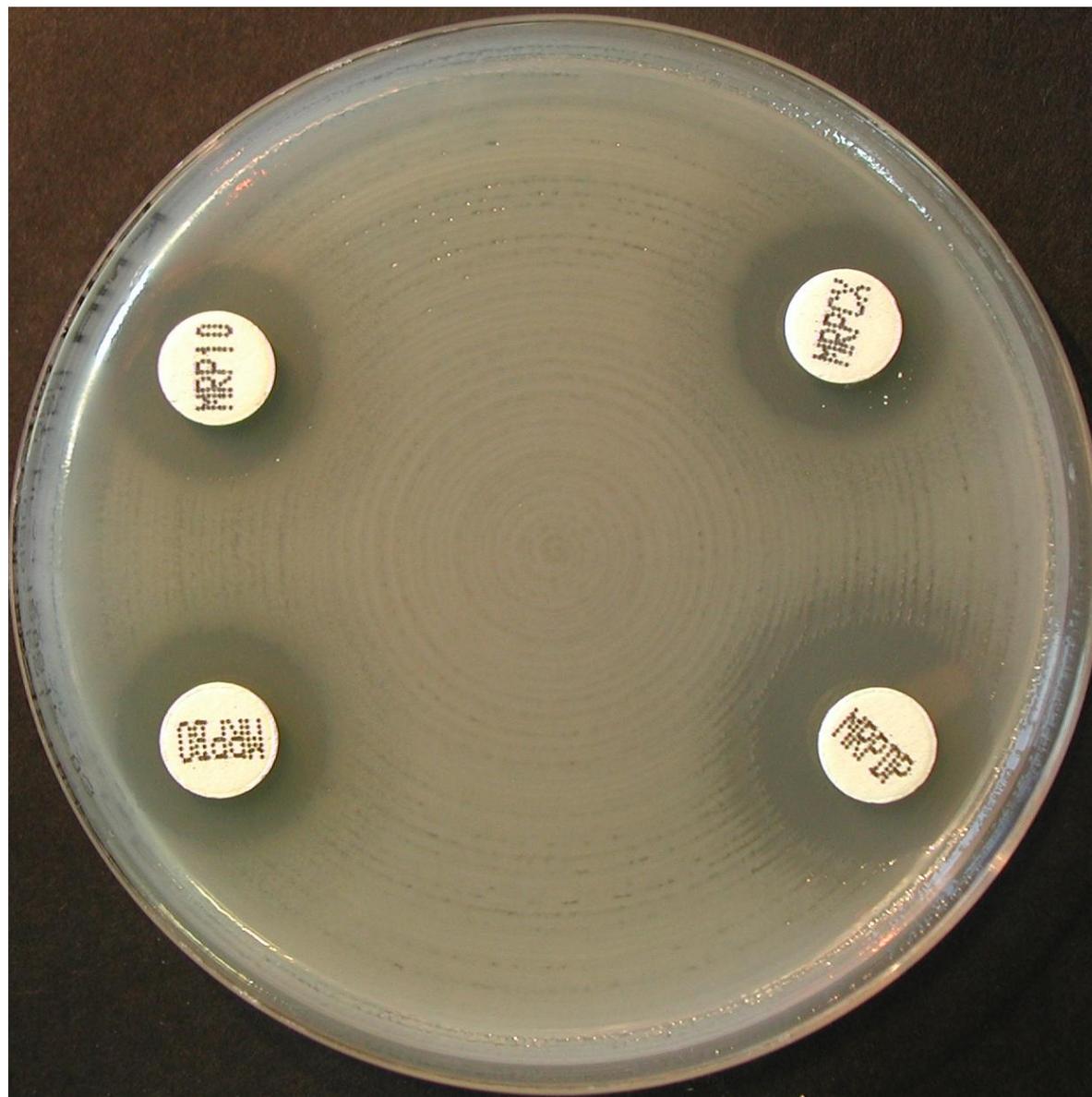


Stamme 8: CTX-M-15 + SHV-32 + imp., meropenem MIC = 6 µg/ml

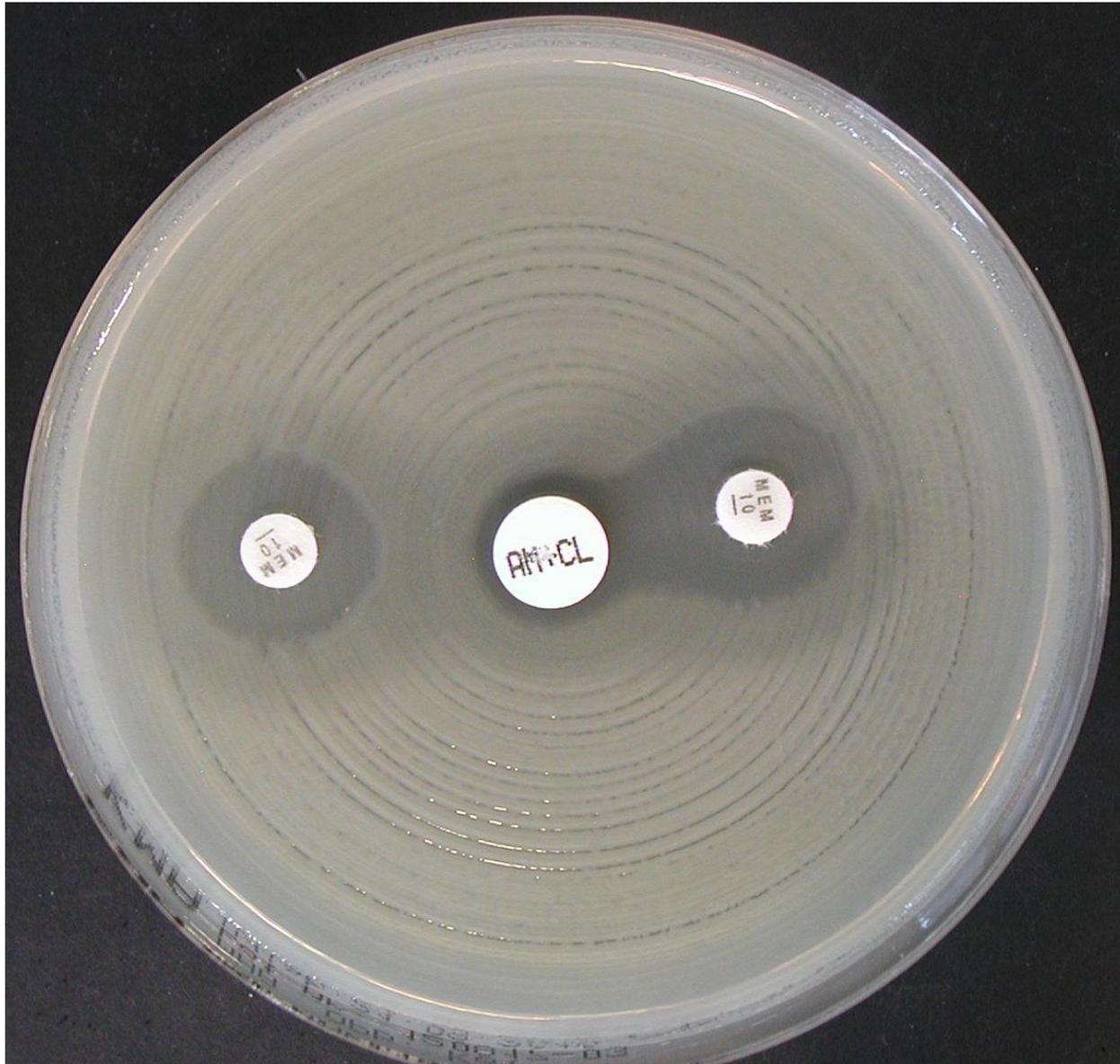


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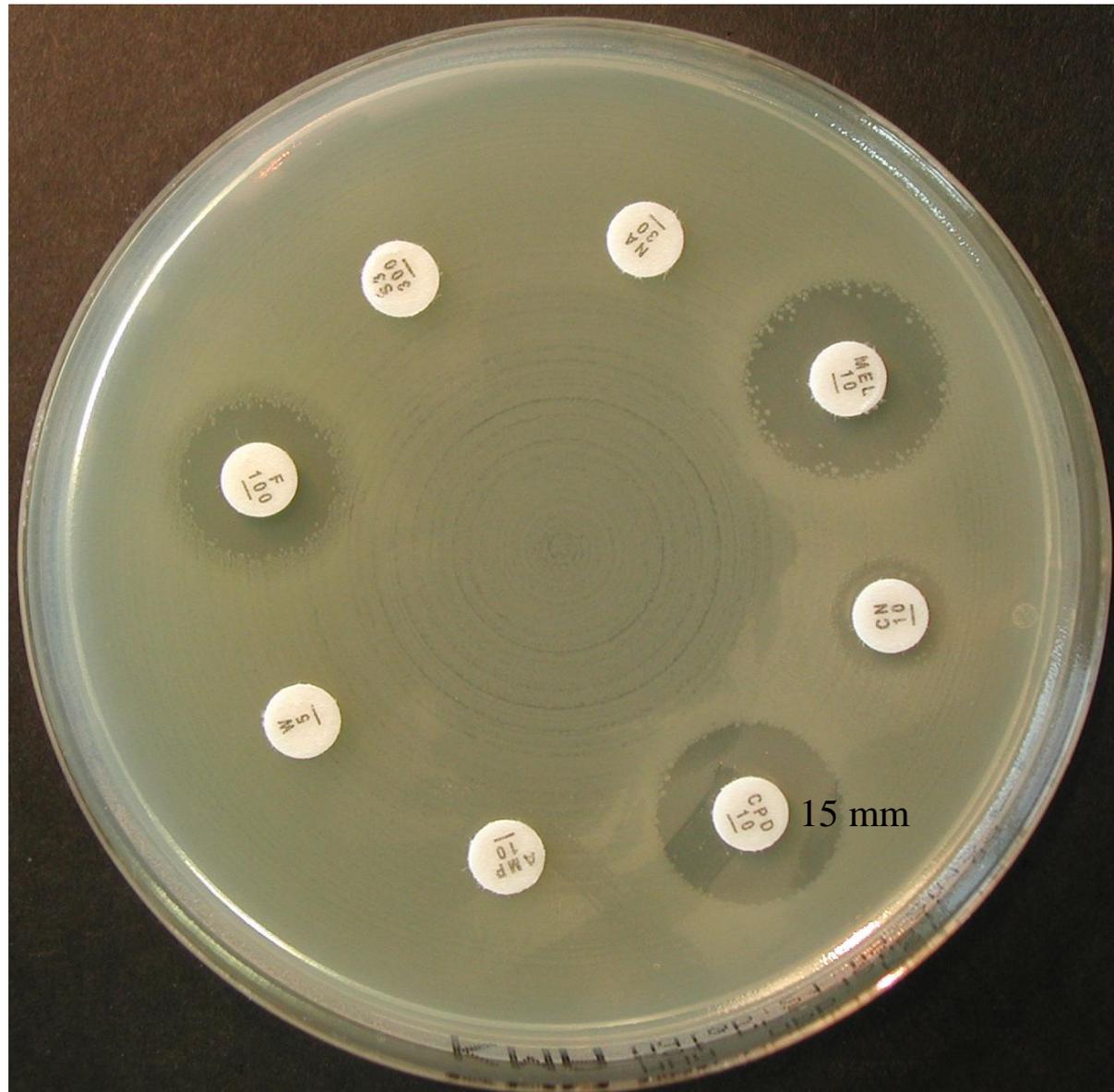
– hvilken
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Stamme 8: CTX-M-15 + SHV-32 + imp., meropenem MIC = 6 µg/ml

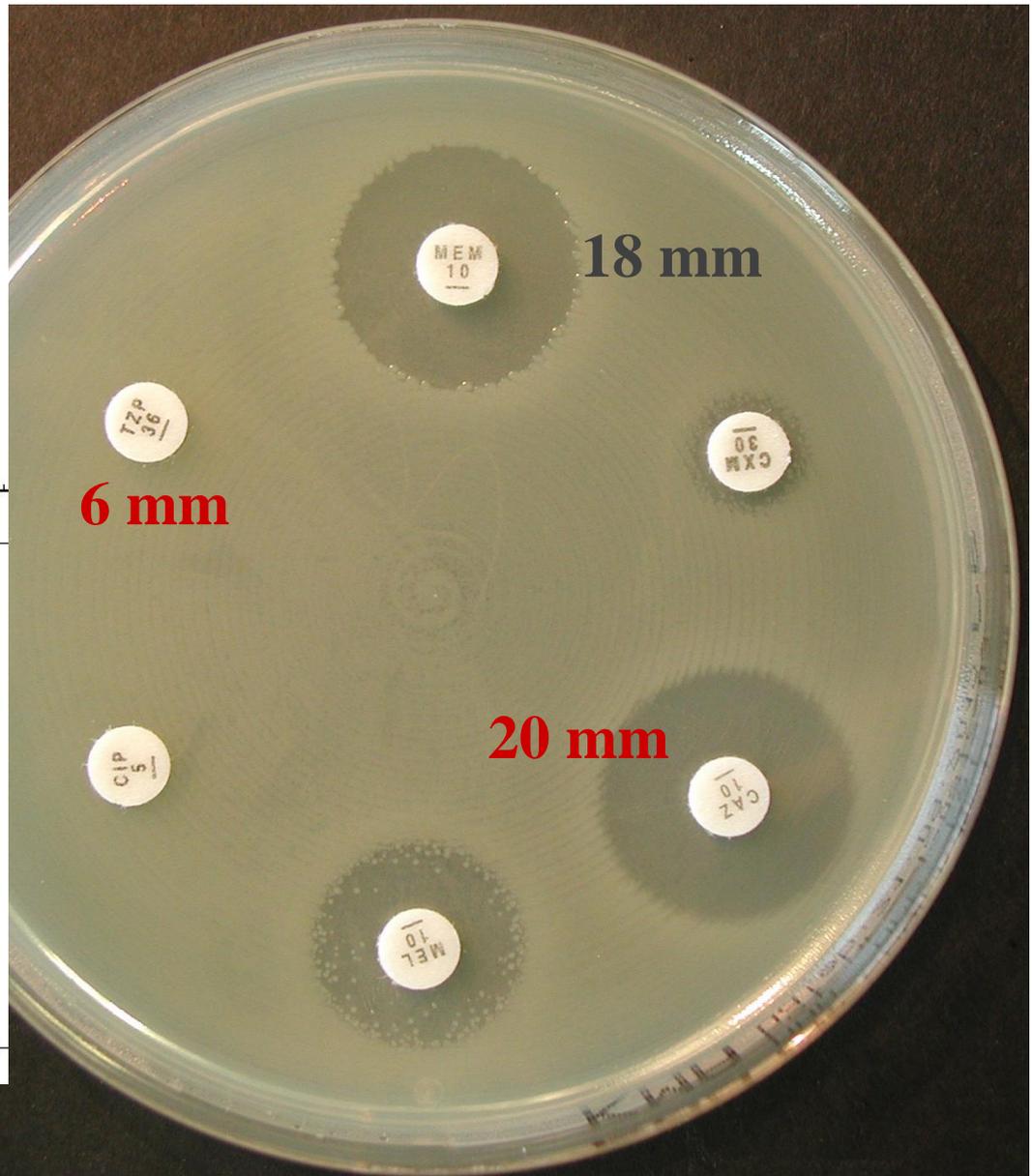
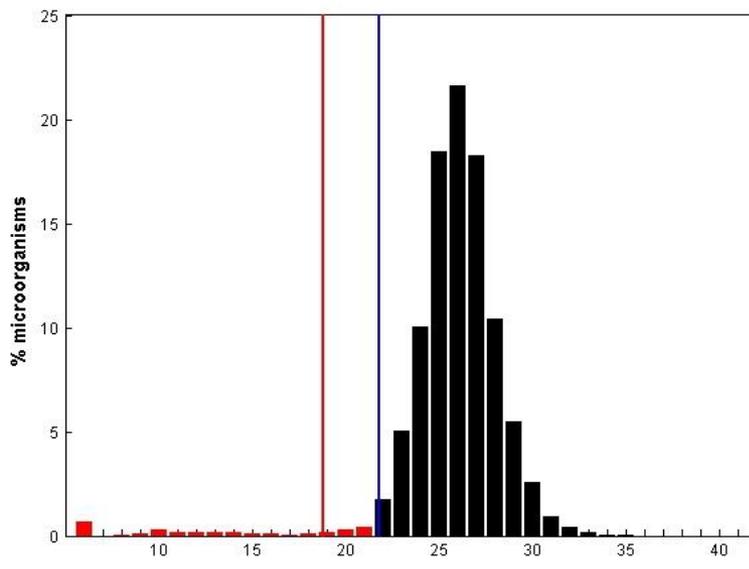
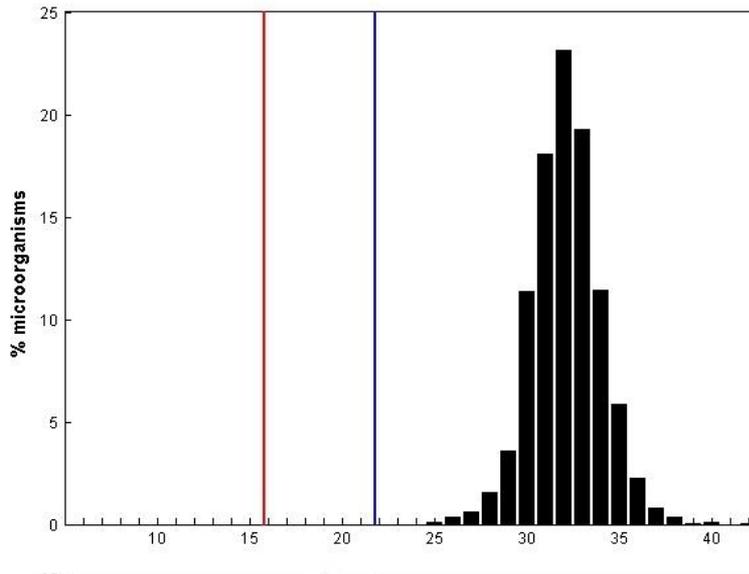


Stamme 2: OXA-48 + TEM, meropenem MIC = 1,5 µg/ml

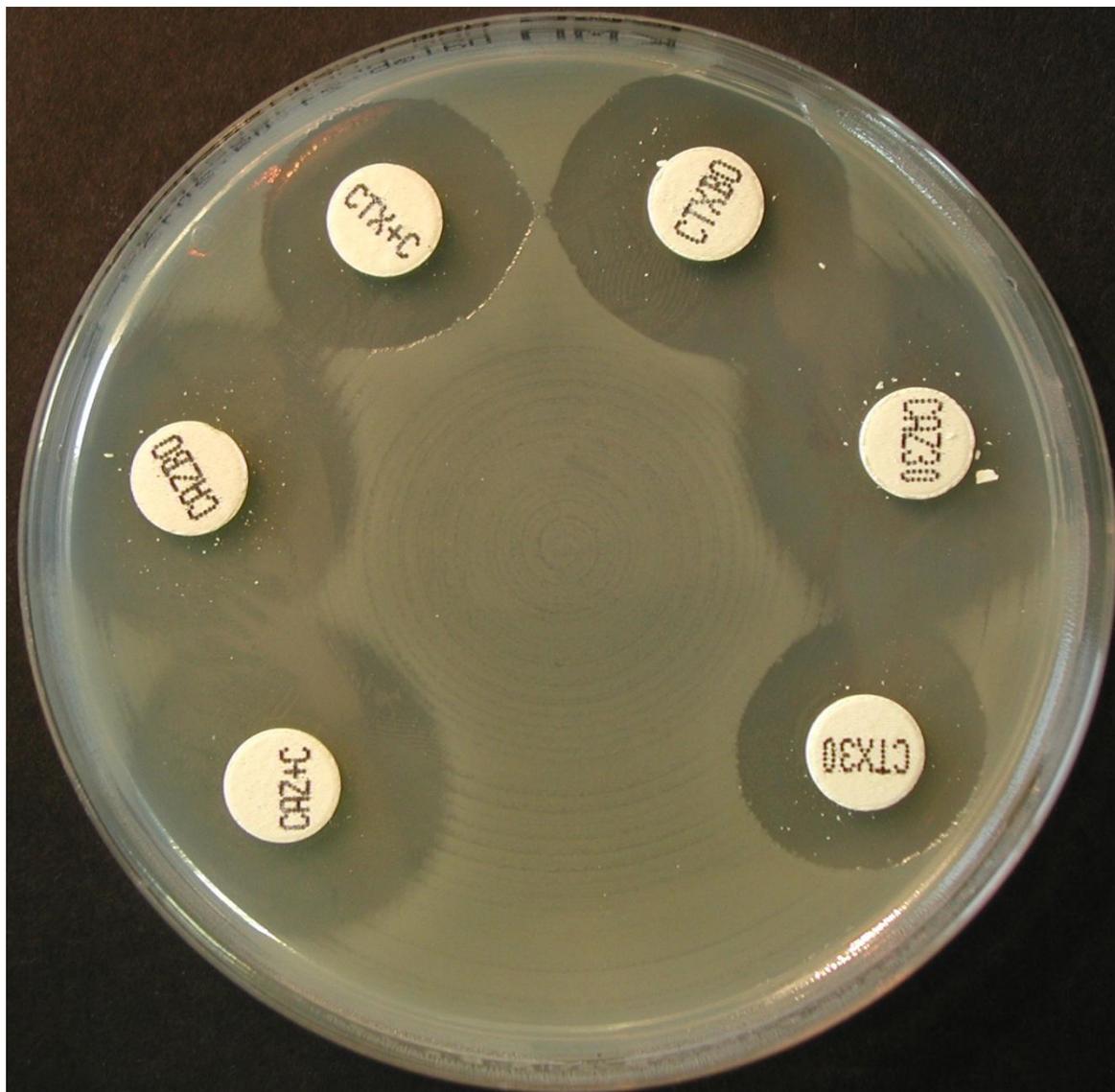


E.coli

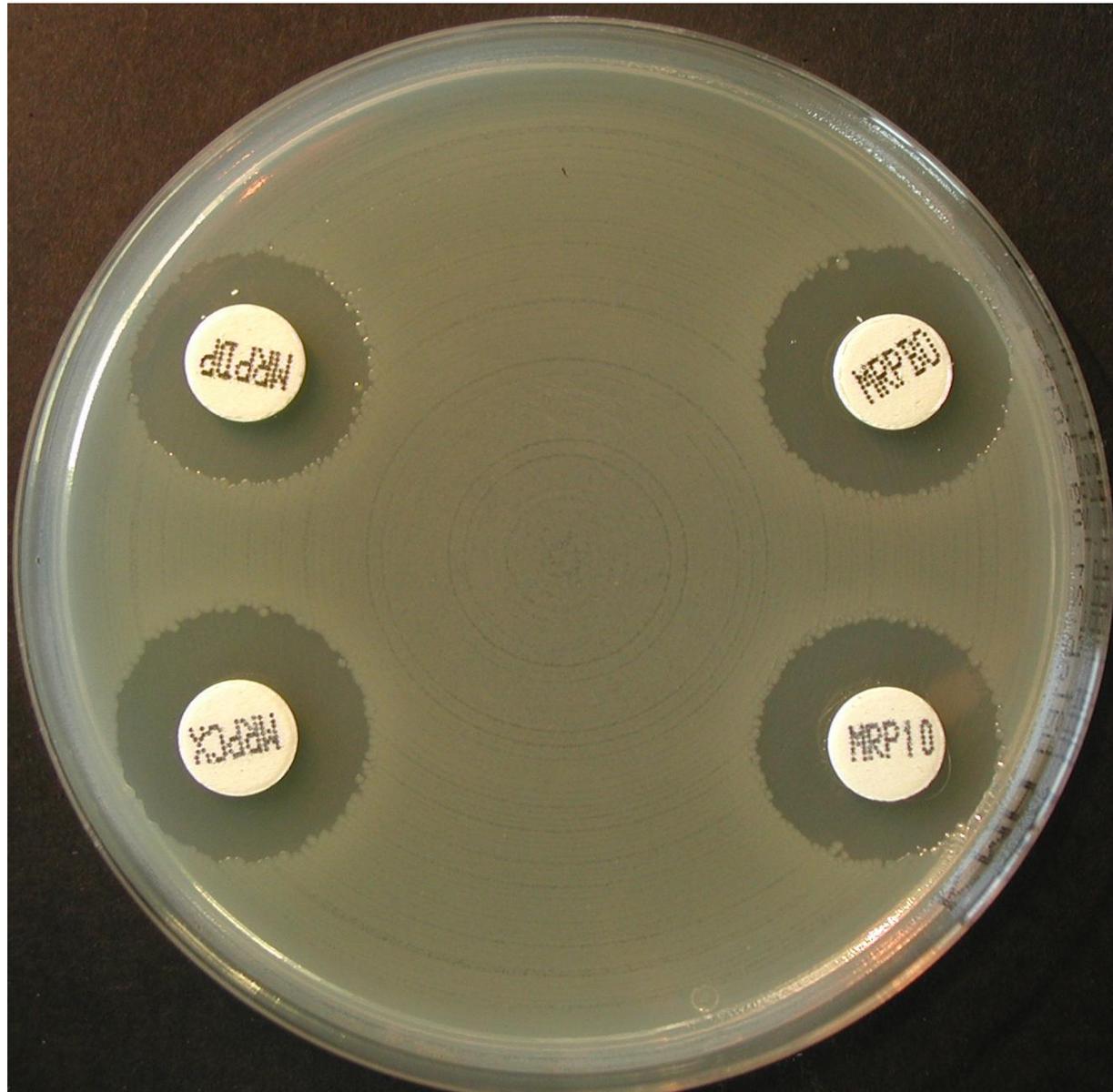
Stamme 2: OXA-48 + TEM, meropenem MIC = 1,5 µg/ml



Stamme 2: OXA-48 + TEM, meropenem MIC = 1,5 µg/ml



Stamme 2: OXA-48 + TEM, meropenem MIC = 1,5 µg/ml



Use of ChromID Extended-Spectrum β -Lactamase Medium for Detecting Carbapenemase-Producing *Enterobacteriaceae*[∇]

TABLE 1. Sensitivity of detection of ChromID ESBL medium and CHROMagar KPC medium for 28 carbapenemase- and/or ESBL-producing enterobacterial isolates

Isolate	Carbapenemase	ESBL	MIC ($\mu\text{g/ml}$) of drug ^a :					Lowest limit of detection (CFU/ml)	
			CTX	CAZ	IMP	ETP	MP	ChromID ESBL	CHROMagar KPC
<i>Citrobacter freundii</i>	KPC-2		16	32	4	2	3	1×10^1	4.4×10^1
<i>Serratia marcescens</i>	KPC-2		>256	>256	>32	>32	>32	1×10^1	1.4×10^1
<i>Enterobacter cloacae</i>	KPC-2		>256	>256	4	6	2	1×10^1	2.5×10^1
<i>Enterobacter cloacae</i> HPTU	KPC-2		6	4	1	1.5	1	1×10^1	1×10^4
<i>Escherichia coli</i>	KPC-2		6	4	0.5	0.5	0.5	1×10^1	1×10^4
<i>Klebsiella pneumoniae</i> A28006	KPC-2	CTX-M-2	>256	16	16	24	32	1×10^1	8.3×10^1
<i>Klebsiella pneumoniae</i> 588	KPC-2		16	48	24	32	16	1×10^1	1.4×10^1
<i>Klebsiella pneumoniae</i> 633	KPC-2	CTX-M-12	>256	>256	>32	>32	4	1×10^1	1.6×10^1
<i>Klebsiella pneumoniae</i> 2303	KPC-2		>256	>256	>32	>32	>32	8×10^1	2.5×10^1
<i>Klebsiella pneumoniae</i> GR	KPC-2		>256	>256	12	24	6	1×10^1	1.5×10^1
<i>Escherichia coli</i>	VIM-1	CTX-M-3	>256	>256	1.5	0.5	0.5	1×10^1	2×10^5
<i>Escherichia coli</i>	IMP-1		>256	>256	0.5	3	0.5	1×10^1	2×10^5
<i>Serratia marcescens</i>	IMP-1		>256	6	4	>32	4	1×10^1	7×10^1
<i>Klebsiella pneumoniae</i> 6852	IMP-1		>256	>256	1	2	8	1×10^1	1.7×10^1
<i>Klebsiella pneumoniae</i>	VIM-1	CTX-M-3	>256	>256	1	2	1	3×10^1	2×10^4
<i>Klebsiella pneumoniae</i> 149	IMP-4	SHV-12	>256	>256	2	4	2	2×10^1	1×10^3
<i>Klebsiella pneumoniae</i>	VIM-1	SHV-5	>256	>256	>32	>32	>32	1×10^1	5.7×10^1
<i>Enterobacter cloacae</i>	VIM-1	CTX-M-3	>256	>256	3	4	2	1×10^1	1.5×10^1
<i>Klebsiella pneumoniae</i> BIC	OXA-48		0.5	0.5	0.5	2	0.5	$>2 \times 10^7$	5×10^6
<i>Klebsiella pneumoniae</i> I	OXA-48	CTX-M-15	>256	32	24	24	16	1×10^1	1×10^1
<i>Klebsiella pneumoniae</i> B	OXA-48	CTX-M-15	>256	>256	16	>32	32	2×10^1	5×10^3
<i>Klebsiella pneumoniae</i> BEL	OXA-48		0.5	1	1	4	1	$>2 \times 10^7$	1×10^6
<i>Klebsiella pneumoniae</i> LIB	OXA-48		2	1	16	16	16	$>2 \times 10^7$	5×10^4
<i>Klebsiella pneumoniae</i> EG	OXA-48	CTX-M-15	64	256	2	3	2	2×10^1	1×10^5
<i>Klebsiella pneumoniae</i> 11978	OXA-48	SHV-2a	64	256	>32	>32	>32	2×10^1	1×10^1
<i>Providencia rettgeri</i>	OXA-48	TEM-101	64	256	>32	>32	>32	6×10^1	1×10^2
<i>Citrobacter freundii</i>	OXA-48	VEB-1	64	256	>32	>32	>32	3×10^1	2×10^7
<i>Enterobacter cloacae</i>	OXA-48	SHV-5	>256	>256	0.5	0.5	0.5	2×10^1	1×10^5

^a CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; ETP, ertapenem; MP, meropenem.

Karbapenemaser – fænotypisk identifikation

- Detektion af karbapenemase enzym
- Detektion af OXA-48

Biokemisk enzym detektion

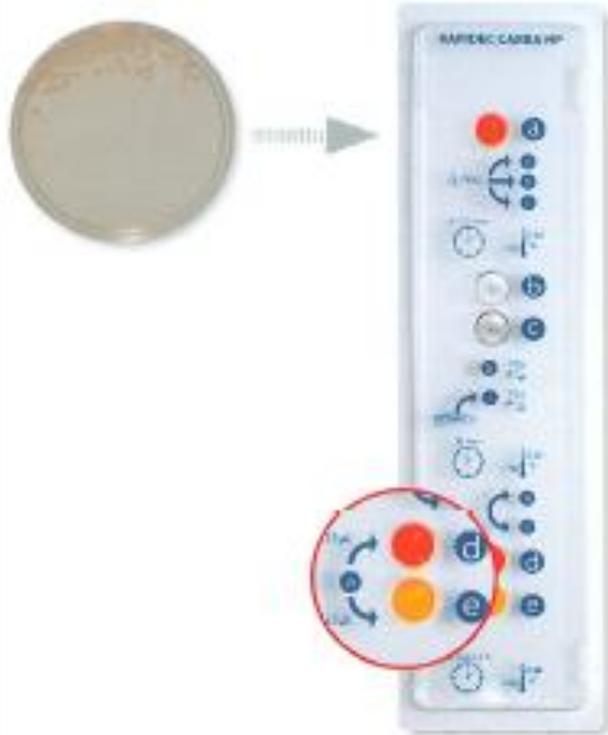
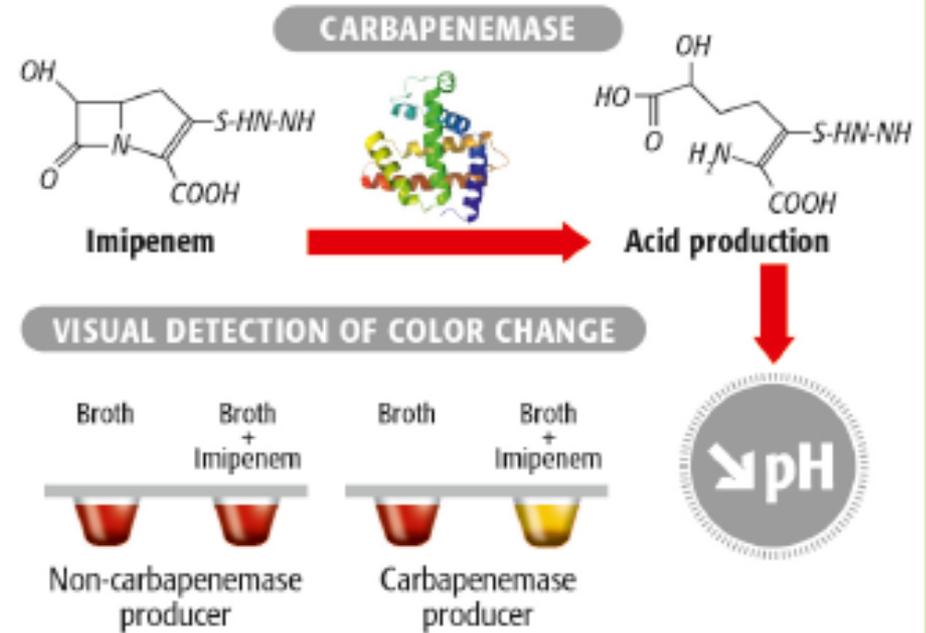


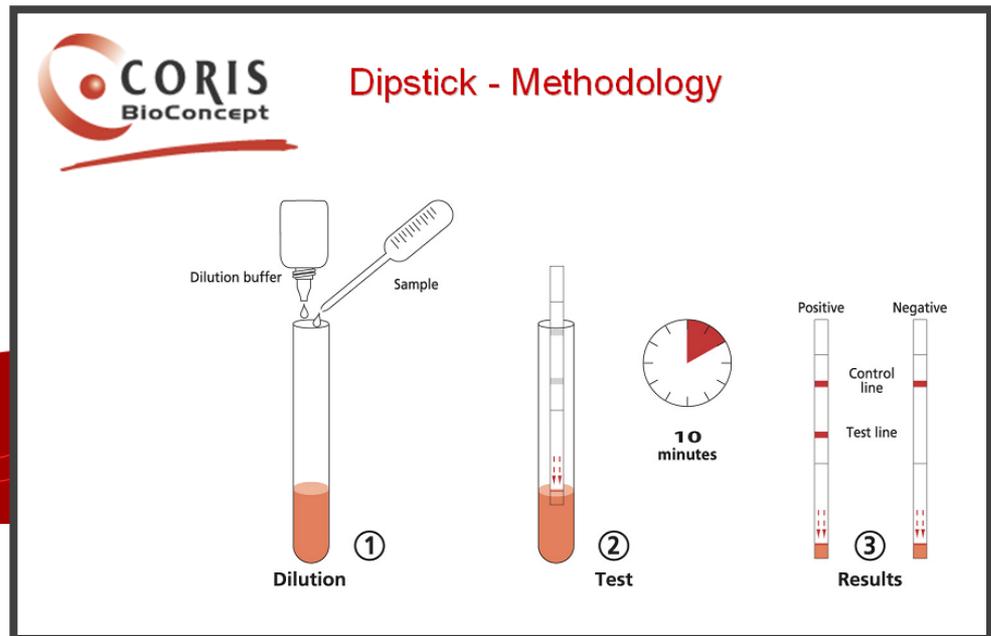
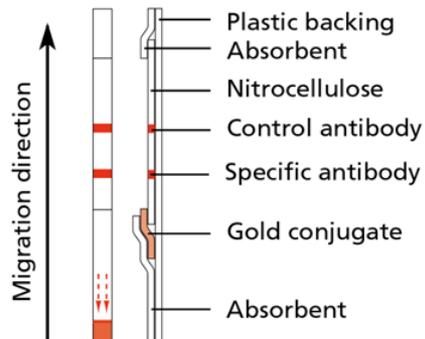
Figure 6: The principle of colorimetric detection of carbapenemase activity

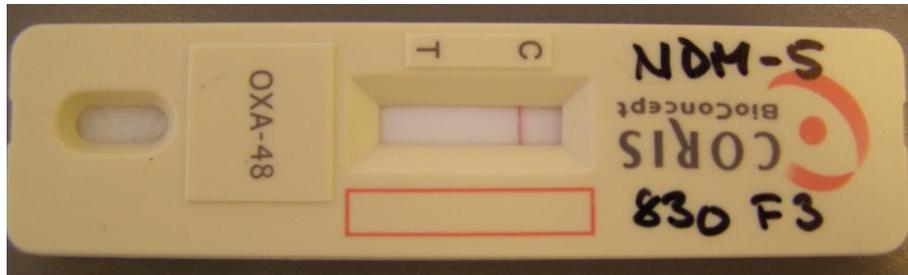
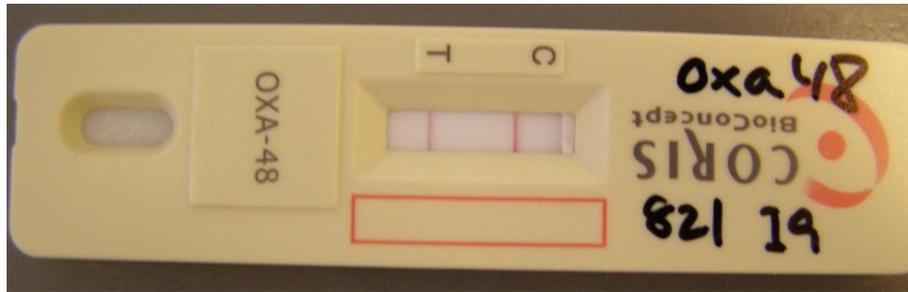
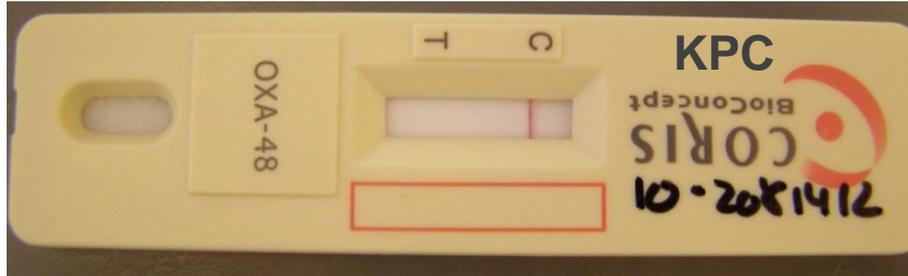
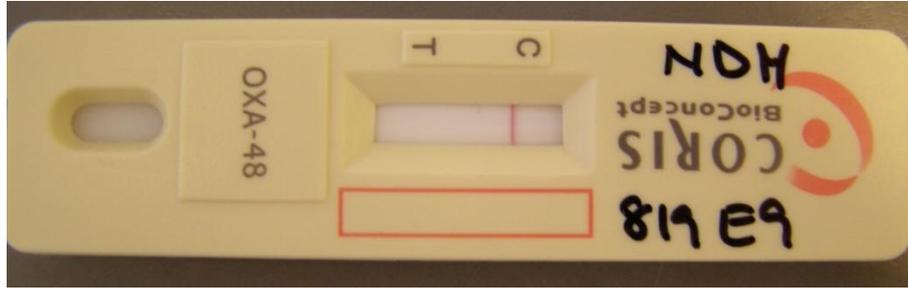




Rapid membrane test

Coris BioConcept has developed mainly rapid kits to detect human enteric and respiratory pathogens. Tests are suitable for single sample analysis, require minimal sample manipulation and give unequivocal diagnostic within 5 to 15 minutes for positive samples. No sophisticated extra material is required to perform the test.

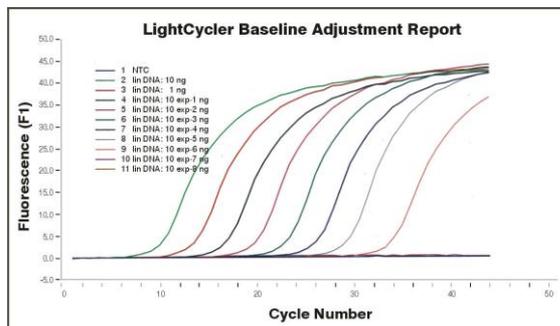




PCR.....



TEM	223	
SHV	191	
CTX-M	168	
CMY	134	(AmpC)
OXA	488	(127 carbapenemases)
IMP	52	(metallo)
VIM	46	(metallo)
KPC	23	(carbapenemases)
NDM	16	(metallo)



.....sekventering

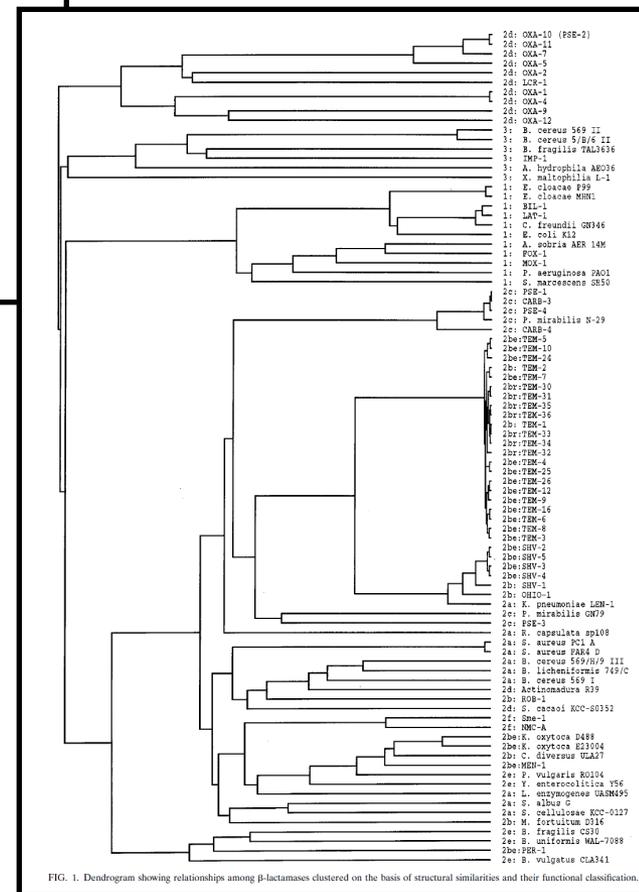
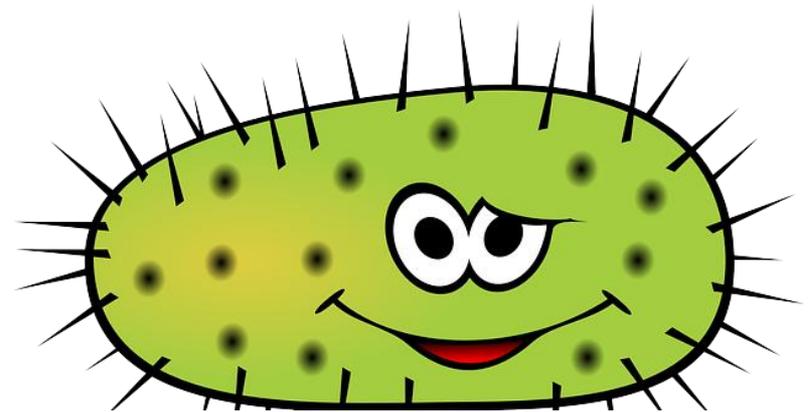
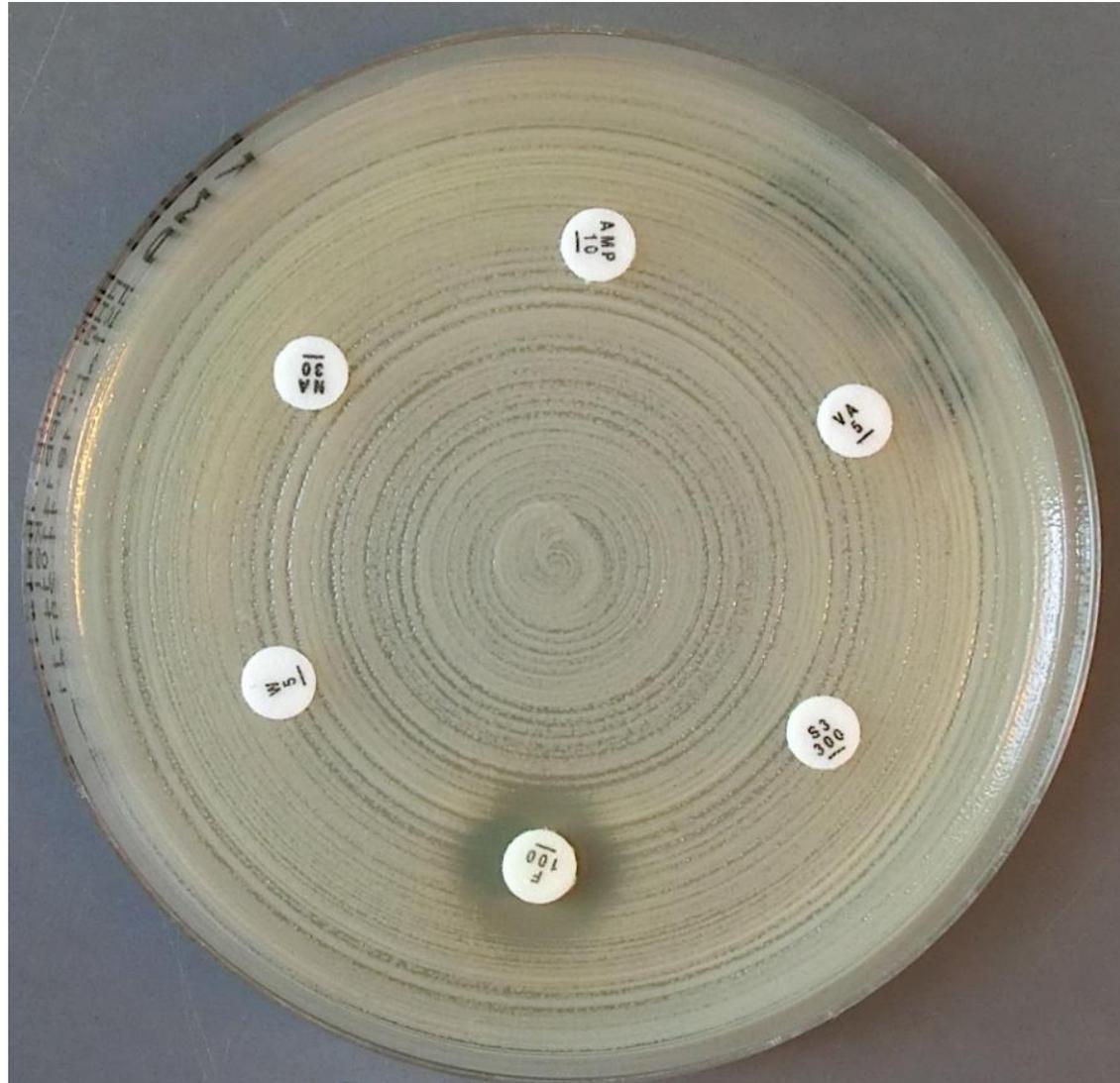


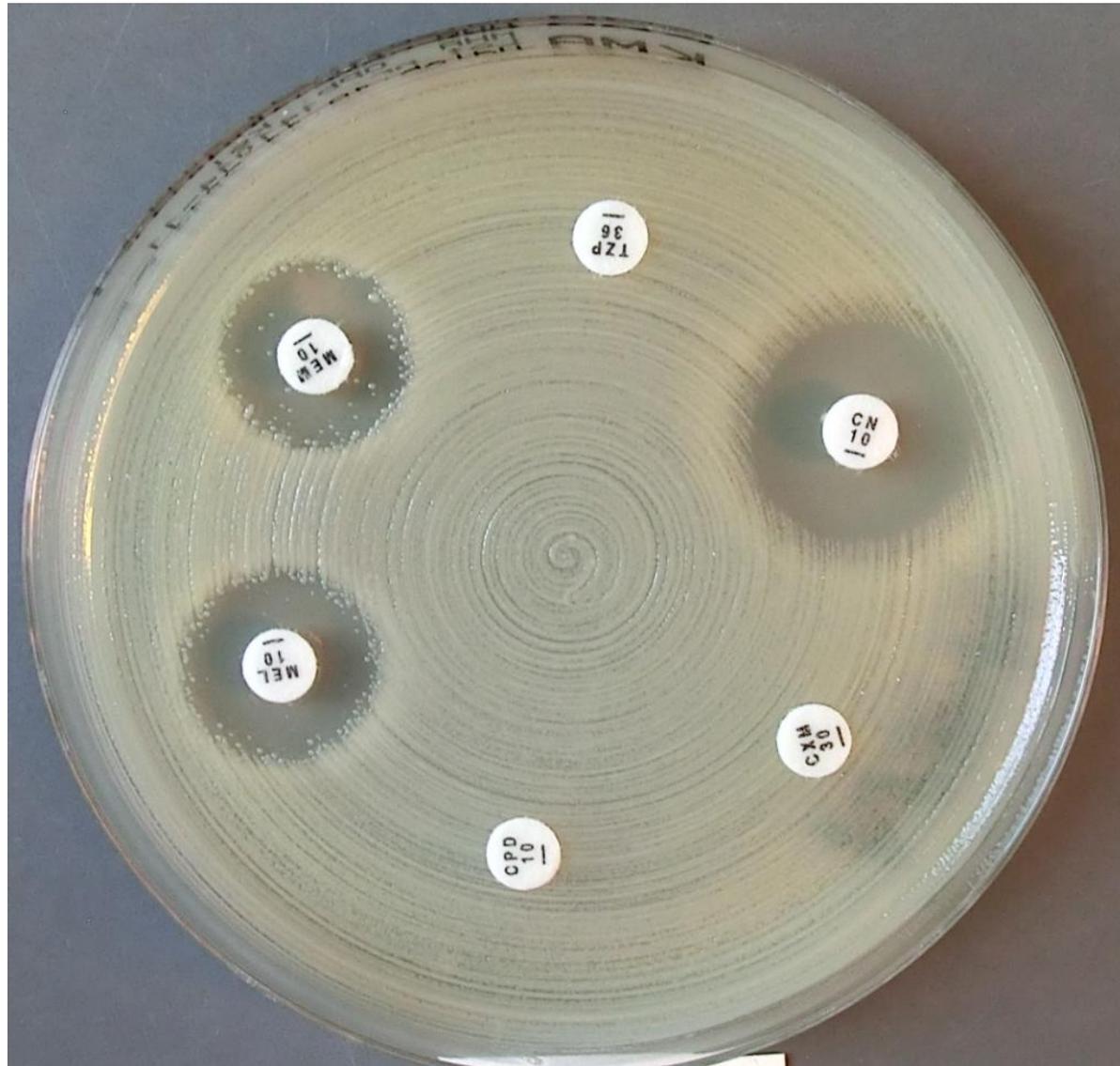
FIG. 1. Dendrogram showing relationships among beta-lactamases clustered on the basis of structural similarities and their functional classification.

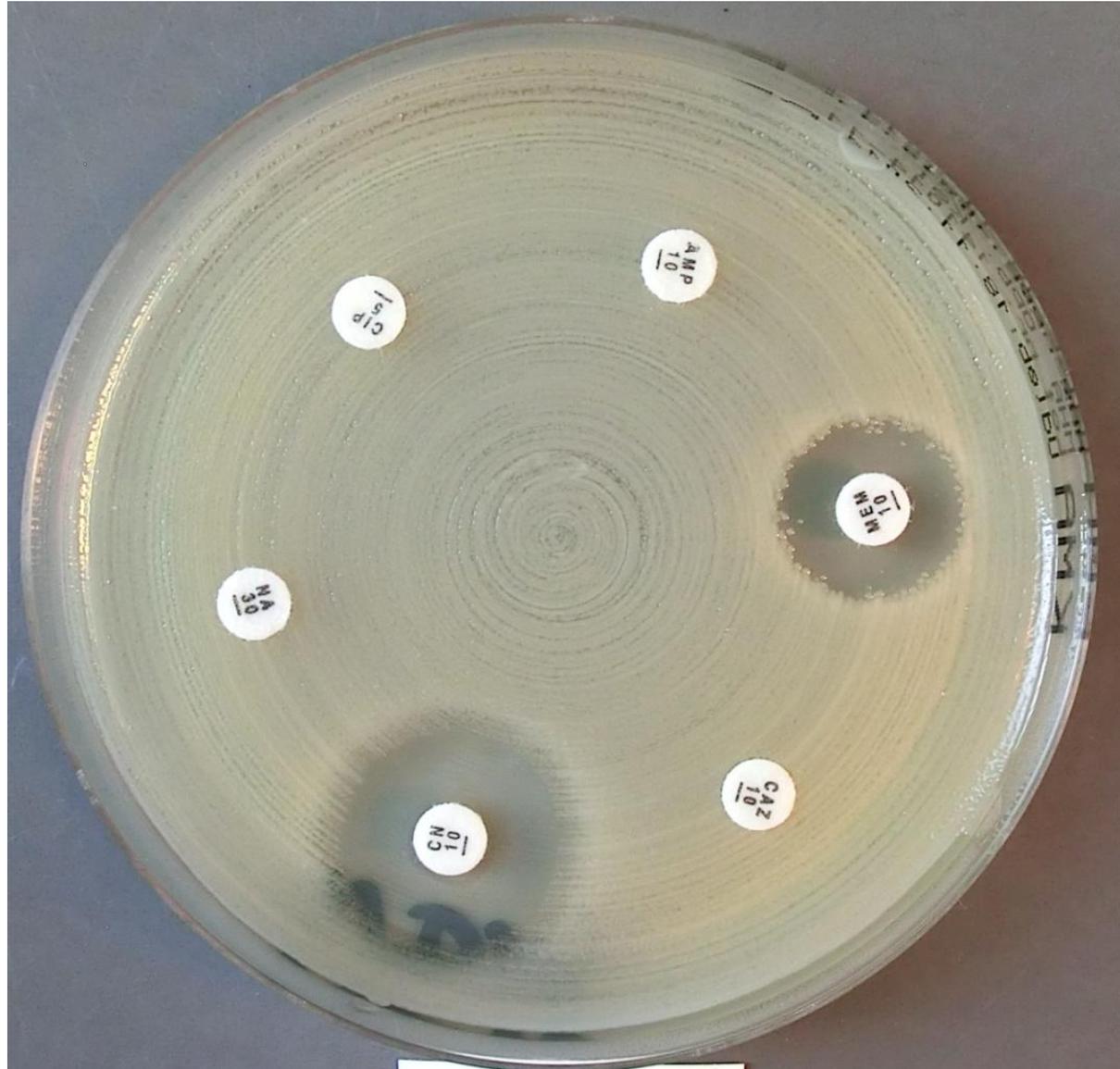
Den store karbapenemase test...

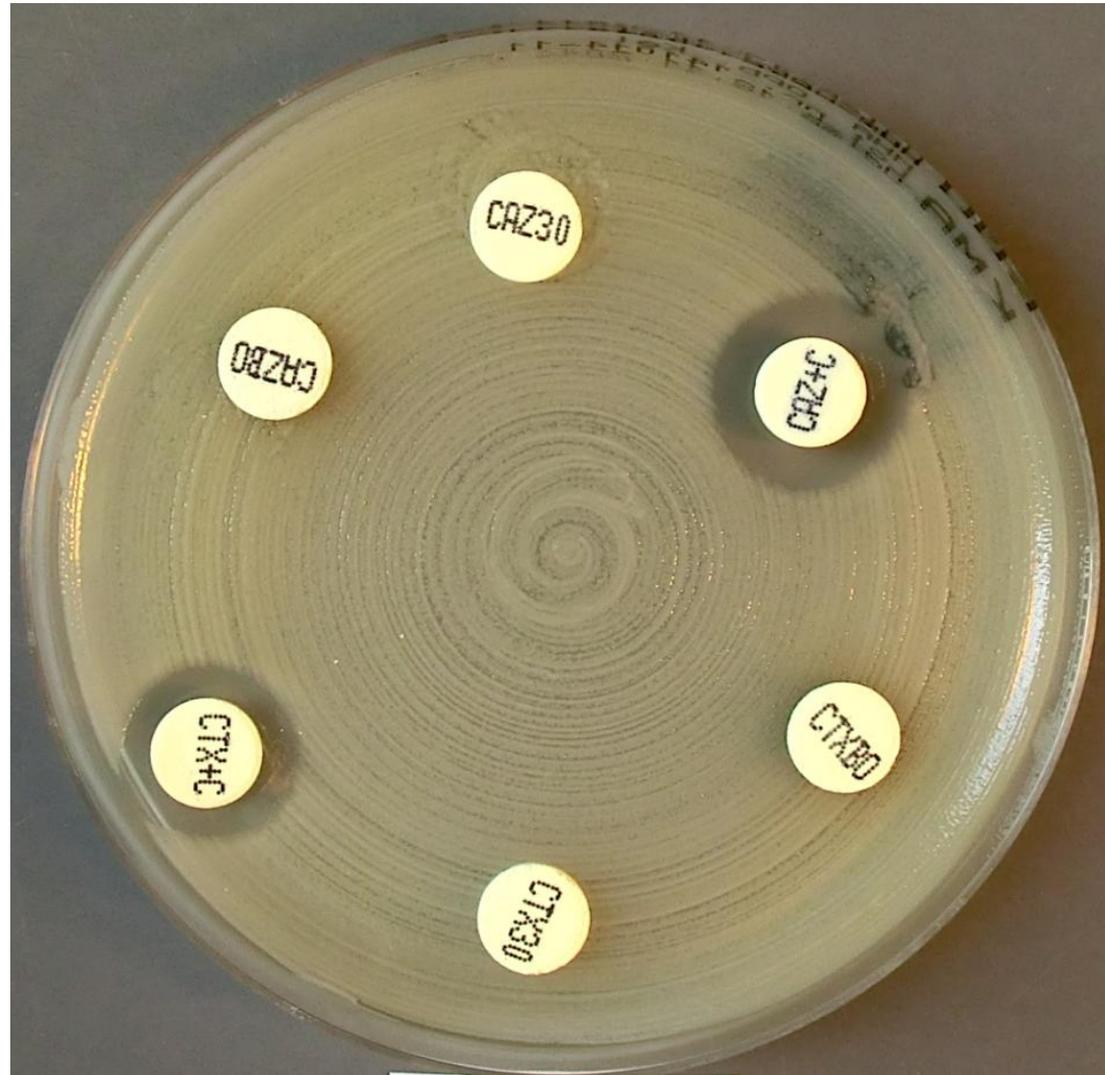
(to stammer fra ITA)



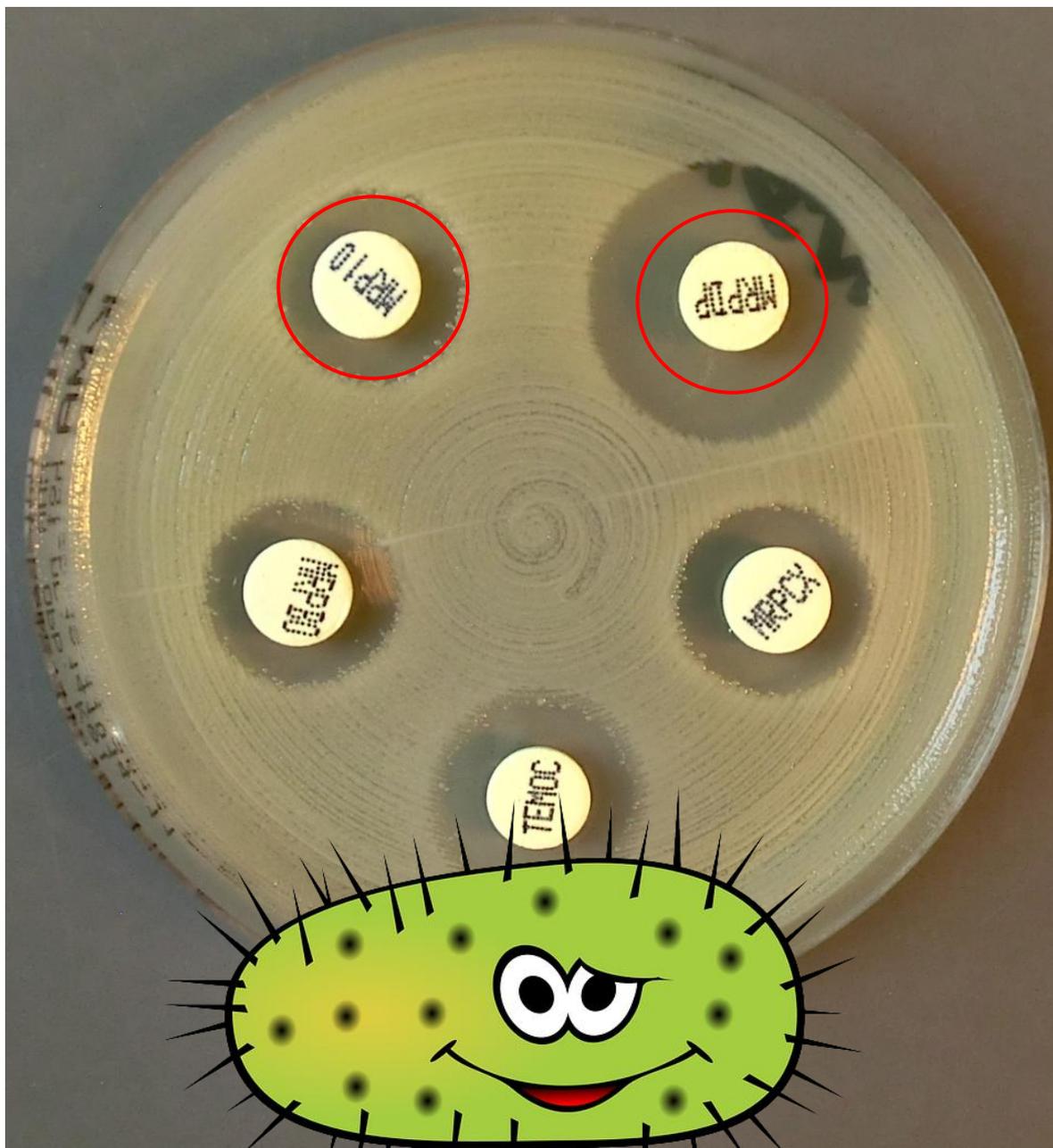
Urindyrkning fra ITA med $10E5$ K.pneumoniae

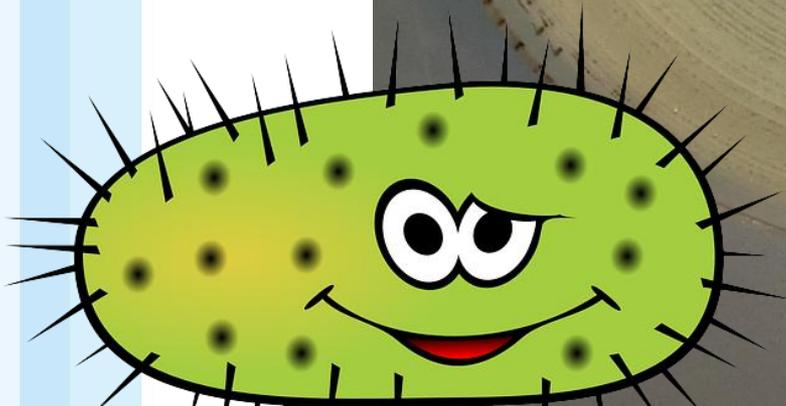
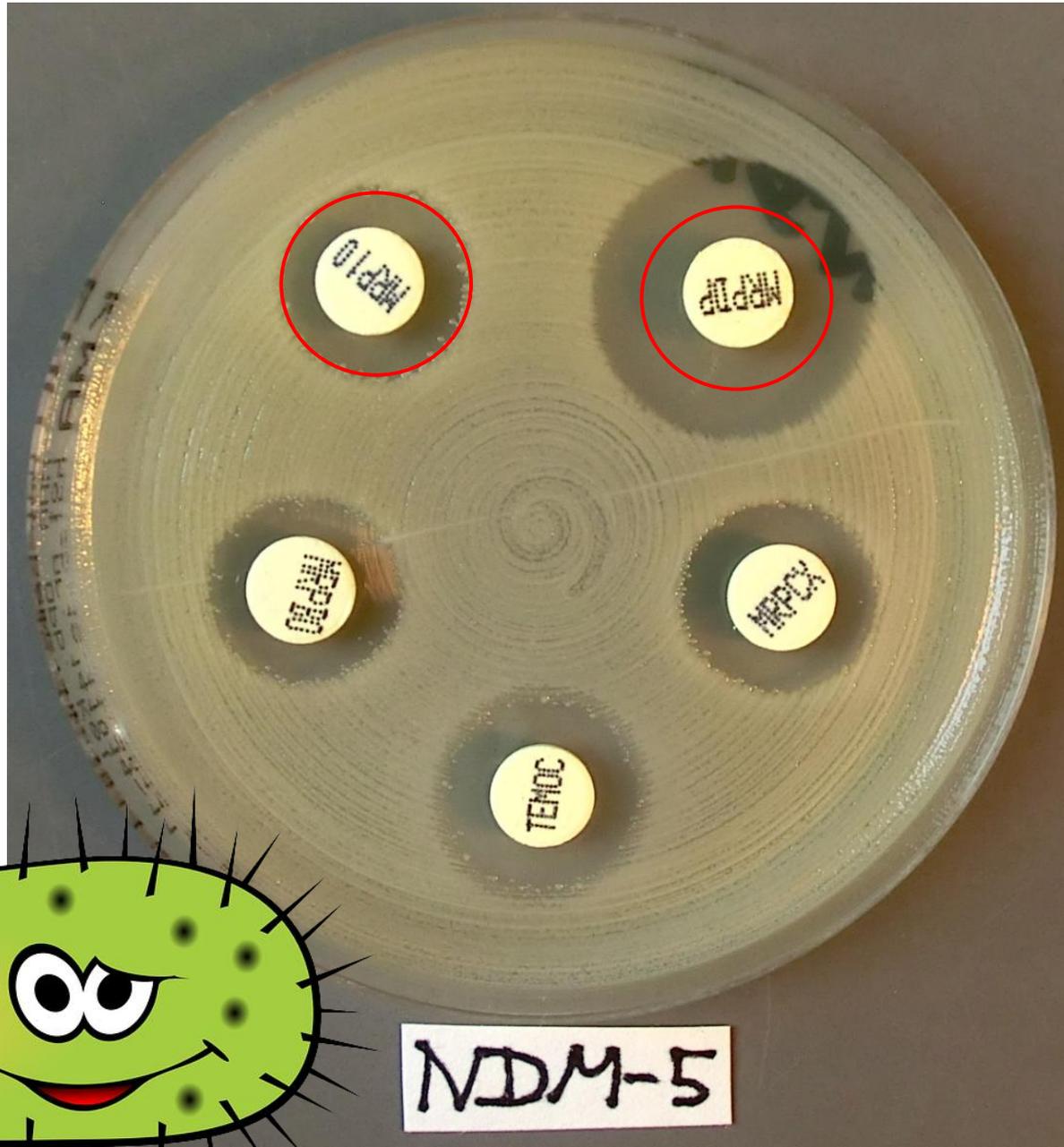
Urindyrkning fra ITA med $10E5$ *K.pneumoniae*

Urindyrkning fra ITA med $10E5$ *K.pneumoniae*.

Urindyrkning fra ITA med $10E5$ K.pneumoniae

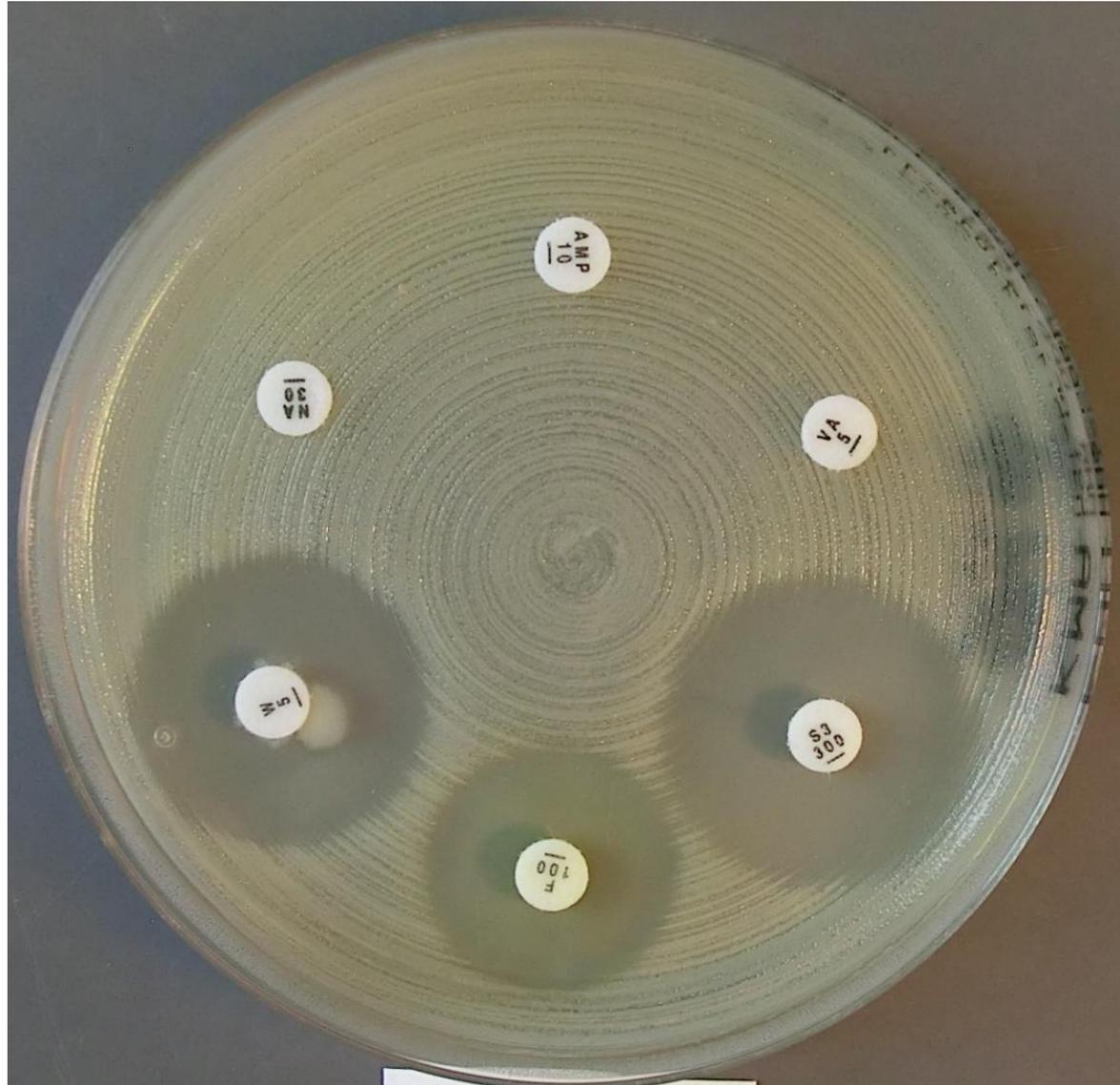
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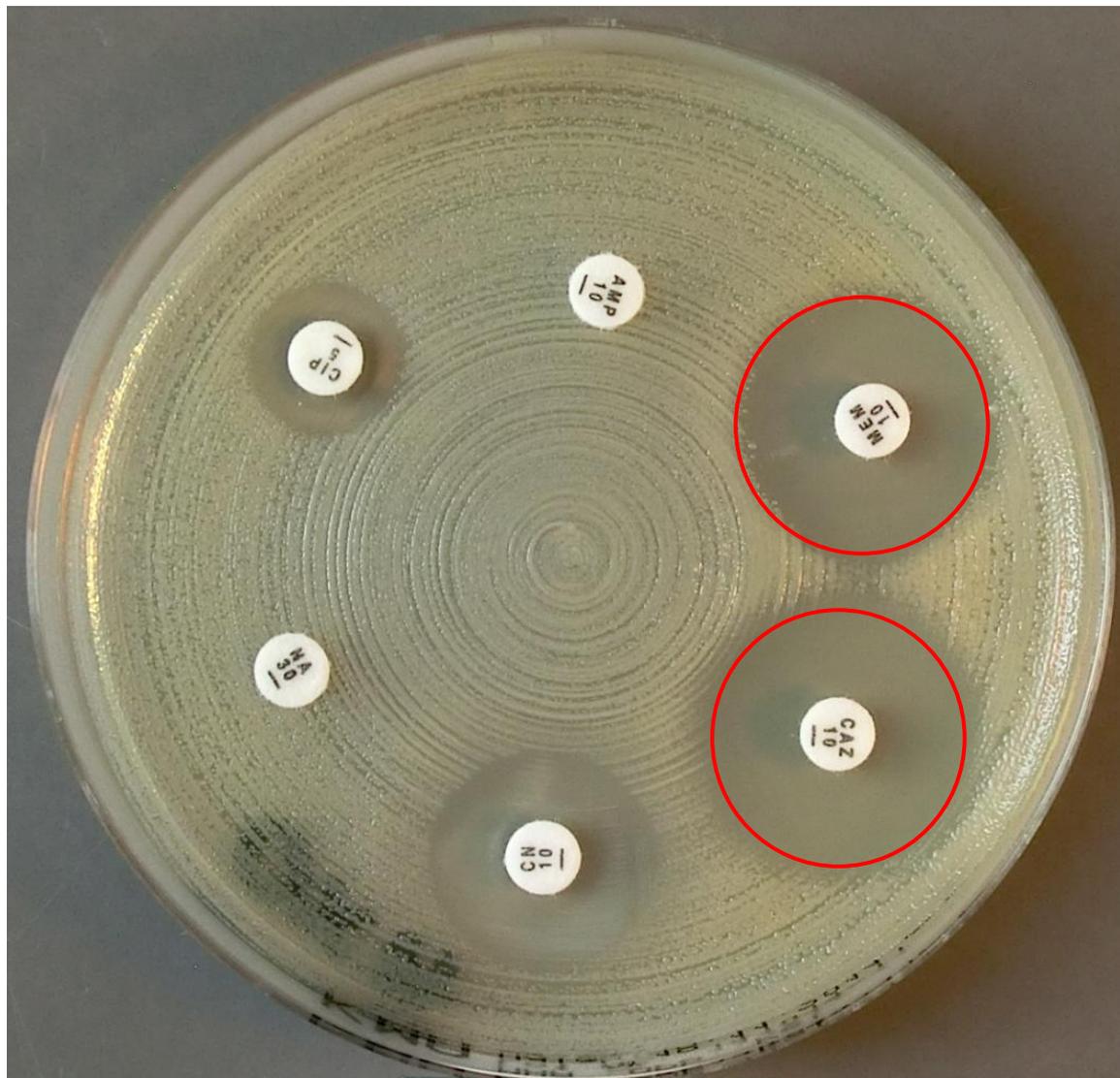
Urindyrkning fra ITA med $10E5$ K.pneumoniae

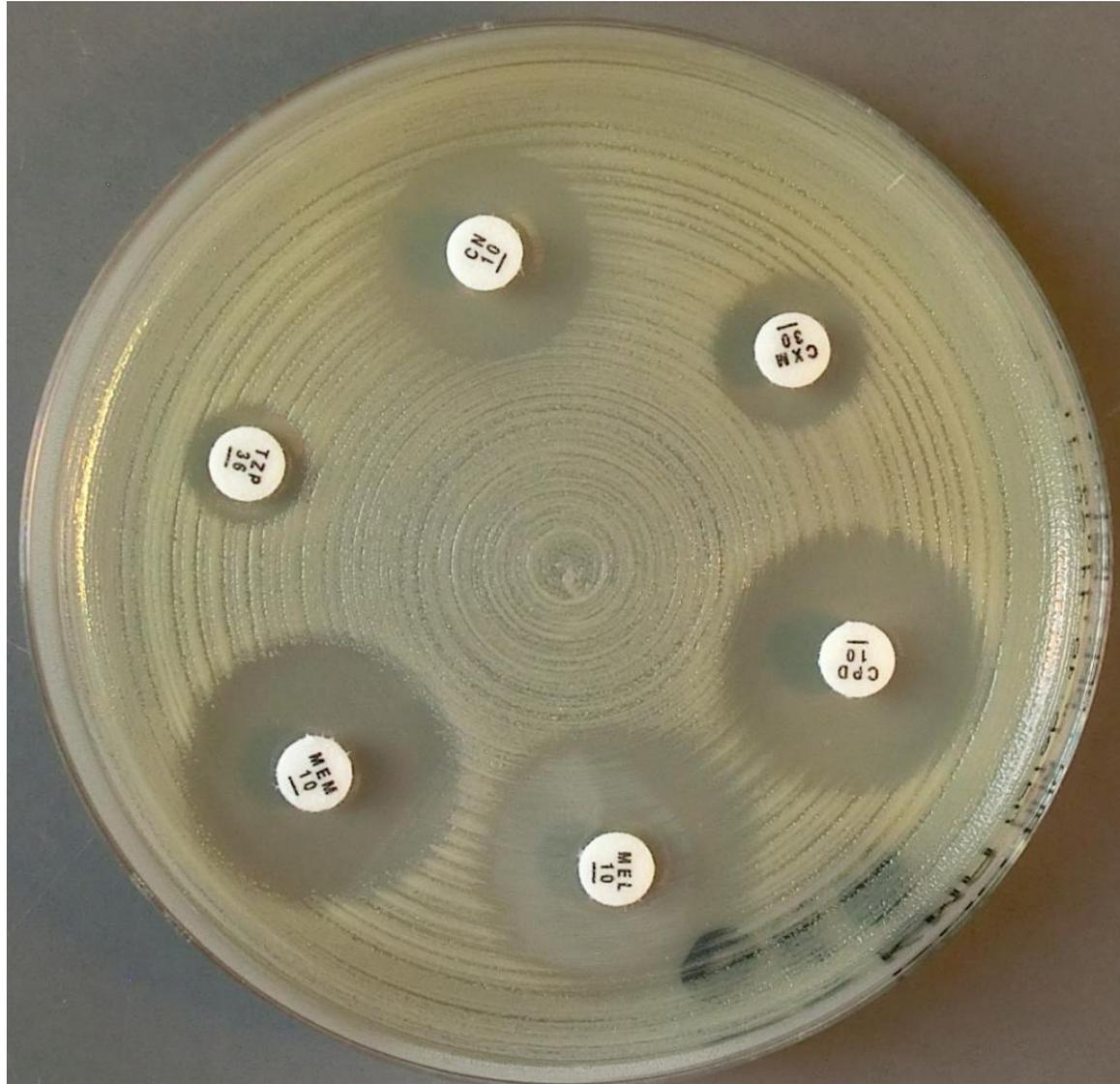
Urindyrkning fra ITA med $10E5$ K.pneumoniae

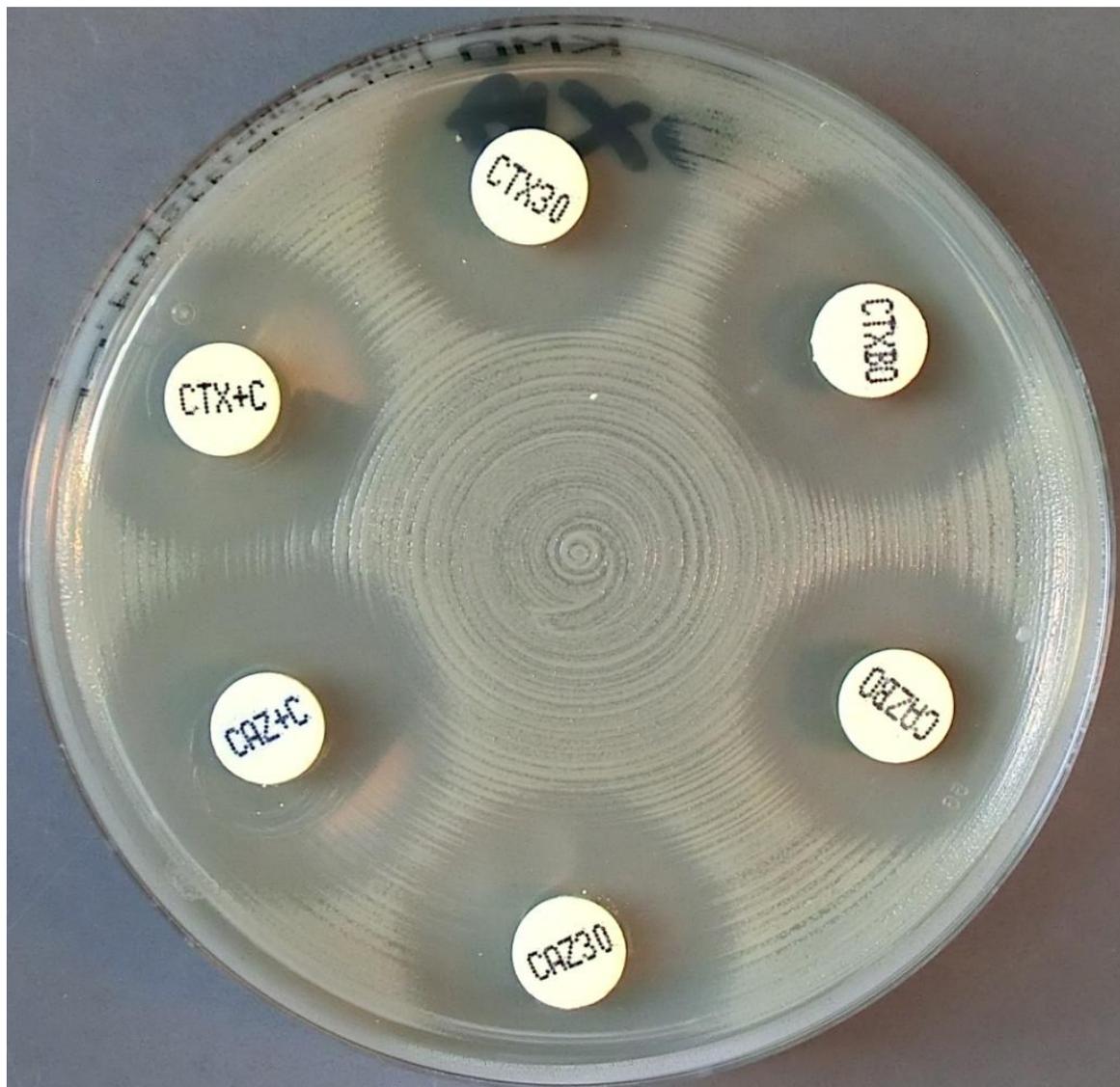
NDM-5

Urindyrkning fra ITA med 10E5 K.oxytoca

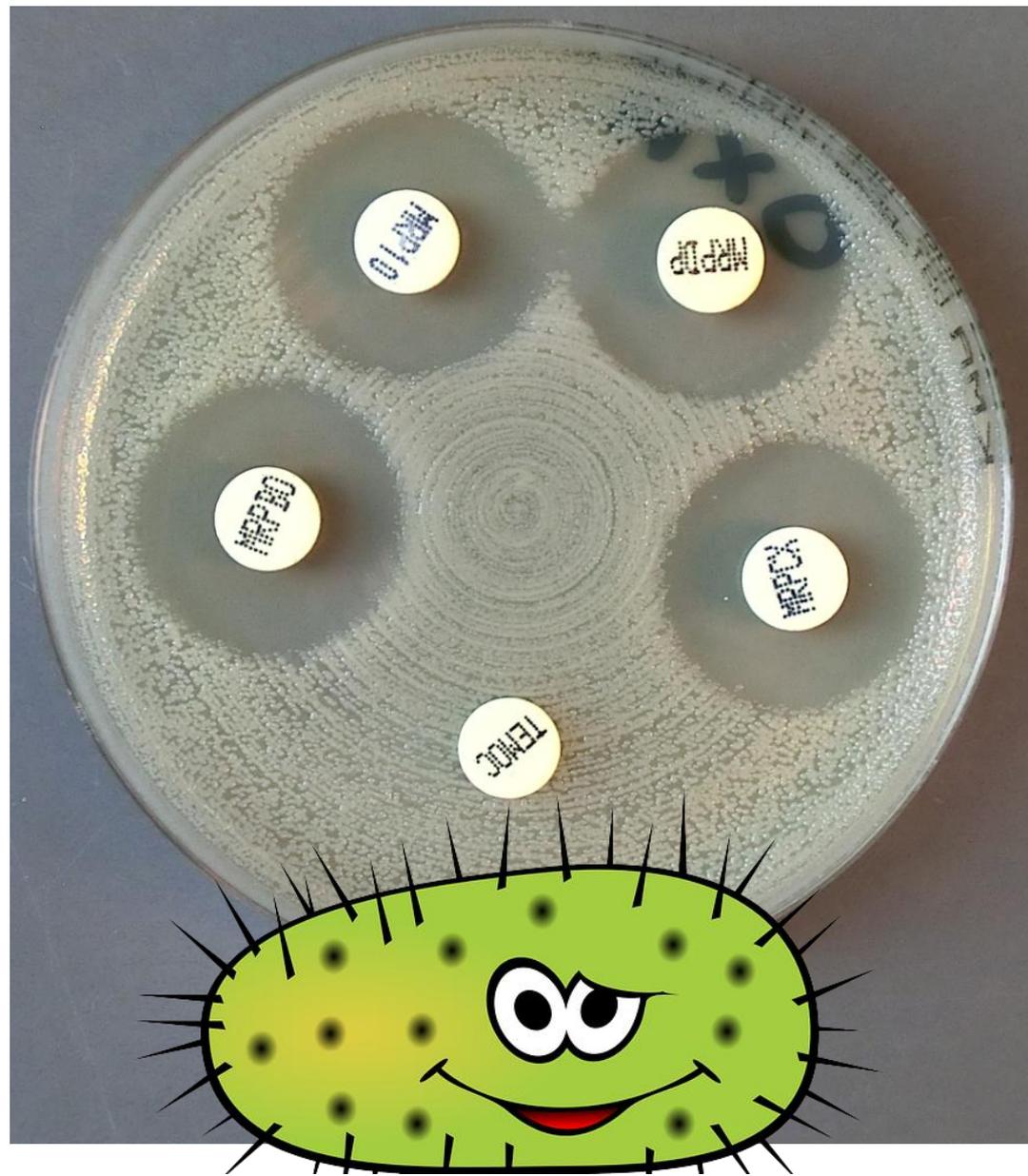


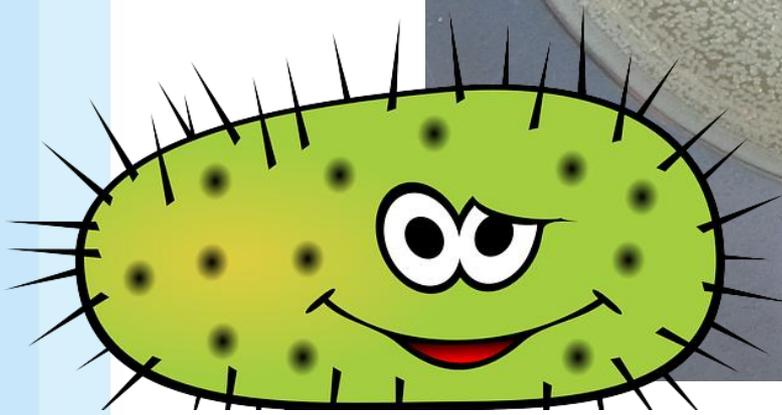
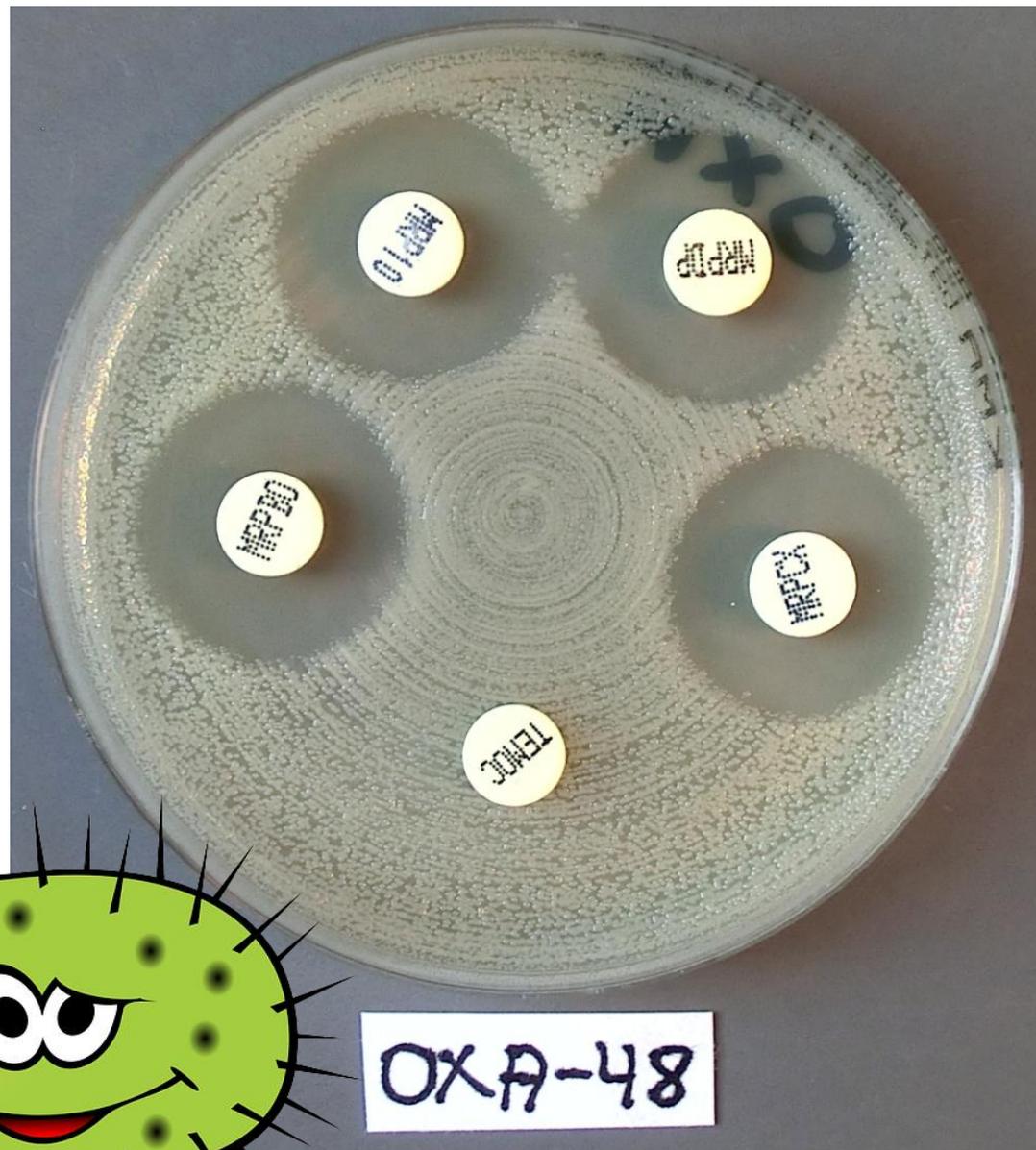
Urindyrkning fra ITA med $10E5$ K.oxytoca

Urindyrkning fra ITA med $10E5$ K.oxytoca

Urindyrkning fra ITA med $10E5$ K.oxytoca

Urindyrkning fra ITA med 10E5 K.oxytoca



Urindyrkning fra ITA med $10E5$ K.oxytoca

OX-A-48

