

# NordicAST 2016 CPE-study - an update

NORDICAST WORKSHOP GÖTEBORG

MAY 23 2017

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Norwegian National Advisory Unit on  
Detection of Antimicrobial Resistance





# AIM

- To examine the performance of the EUCAST disk diffusion method and other supplementary methods to detect carbapenemase producing Enterobacteriaceae (CPE)
- A multicenter format in which all Nordic clinical microbiology laboratories were invited to participate



# Strain collection $n=29$

		MIC			MIC			MIC			MIC
Class A (Serine)		mg/L	Class B (MBL)		mg/L	Class D (OXA-48 like)		mg/L	CPE negative		mg/L
KPC (n=3)	<i>K. pneumoniae</i>	1	NDM (n=5)	<i>K. pneumoniae</i>	>16	(n=8)	<i>K. variicola</i>	0,5	(n=8)	<i>K. pneumoniae</i>	4
	<i>E. cloacae cplex</i>	>16		<i>K. pneumoniae</i>	>16		<i>K. variicola</i>	0.5		<i>E. coli</i>	8
	<i>E. cloacae cplex</i>	8		<i>P. mirabilis</i>	0.25		<i>K. variicola</i>	0.5		<i>K. pneumoniae</i>	4
IMI (n=1)	<i>E. cloacae cplex</i>	1		<i>E. coli</i>	2		<i>K. pneumoniae</i>	2		<i>E. cloacae cplex</i>	0.25
				<i>Citrobacter sp.</i>	4		<i>K. pneumoniae</i>	0.5		<i>E. coli</i>	8
			VIM (n=3)	<i>E. coli</i>	1		<i>E. coli</i>	1		<i>P. mirabilis</i>	0.5
				<i>K. pneumoniae</i>	2		<i>E. coli</i>	0.5		<i>E. aerogenes</i>	8
		<i>K. pneumoniae</i>		>16	<i>K. pneumoniae</i>		2	<i>P. rettgeri</i>		2	
			IMP (n=1)	<i>E. coli</i>	1						

+ *E. coli* ATCC 25922 *E. coli*



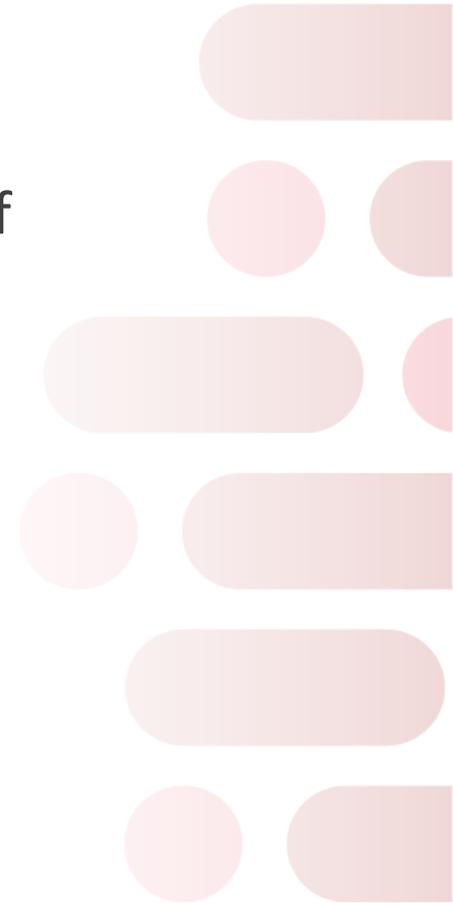
# Mandatory methods

- Bacterial identification at the species level
- AST of all strains by the agar disk diffusion method as recommended by EUCAST
  - Meropenem, Pip-tazobactam, Cefotaxime and Ceftazidime
- Meropenem MIC-determination using broth microdilution (BMD) and/or a MIC gradient strip test
- Automated AST device : report the result for meropenem (and other carbapenems)
- Report manufacturer and lot number of antimicrobial susceptibility testing material



# Optional methods

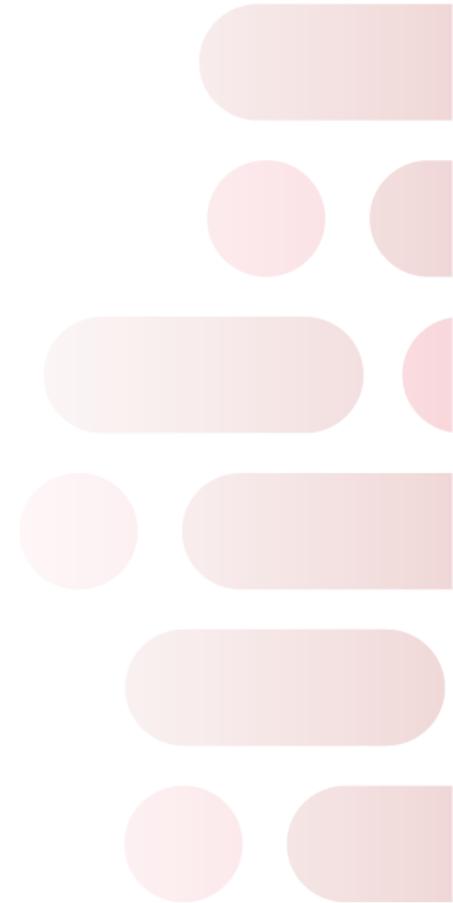
- Phenotypic/biochemical method for detection of carbapenemase activity
  - CarbaNP, CIM, modified Hodge etc
- Phenotypic combination disc system
  - ROSCO MBL/KPC/OXA-48 kit  
(provided along with the strain collection)





# Participation

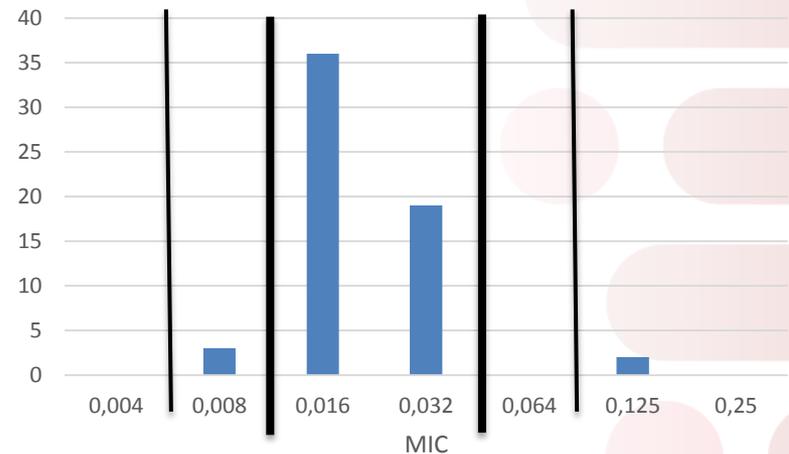
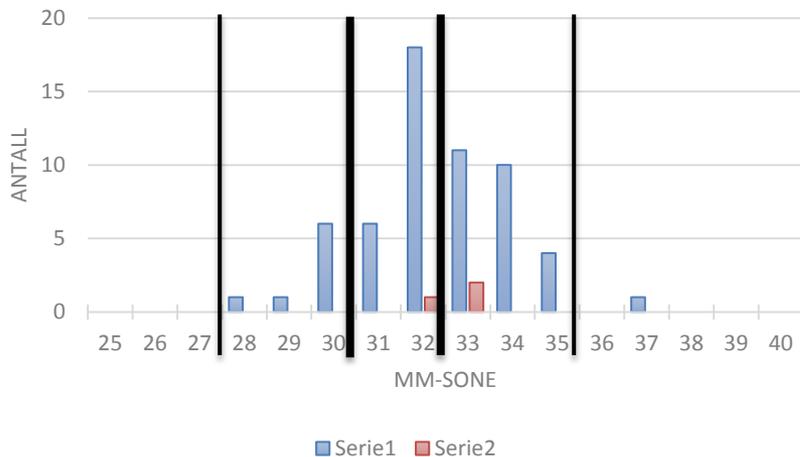
- 61 laboratories (73 %)
  - Denmark  $n= 9$  (82 %)
  - Finland  $n= 15$  (63 %)
  - Iceland  $n= 1$  (100 %)
  - Sweden  $n= 22$  (85 %)
  - Norway  $n= 14$  (64 %)





# Identification and QC AST and MIC Meropenem ATCC 25922

- All isolates correctly identified on genus level
  - Exception: One *K. pneumoniae* reported as *E. cloacae*



Disk manufacturer	Oxoid	BD	Mast	Rosco
Norway	9	3	2	
Denmark/Iceland	6		1	3
Finland	13		2	
Sweden	22			

Gradient strip manufacturer	Oxoid	BioMerieux	Liofilchem	MBD-in house	Sensititre
Norway			14		
Denmark/Iceland	2	7		1	
Finland	2	9	2		1
Sweden	2	18	2		



# Disk diffusion method Meropenem

## ESBL-CARBA positive

		MEM MIC mg/L	Zone diameter distribution using meropenem discs from Oxoid, BD and Mast (n =58)																									
			S ≥ 22 mm R < 16 mm. Screeningbreakpoint < 27 mm																									
			6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28			
<b>Class A</b>	n = 4																											
KPC (n =3)	<i>K. pneumoniae</i>	1			1		1	1	2	1	5	6	9	13*	11	4	2		1					1				
	<i>E. cloacae cplex</i>	>16	57*	1																								
	<i>E. cloacae cplex</i>	8	7			4	3	2	4	4	14*	12	3	4	1													
IMI (n =1)	<i>E. cloacae cplex</i>	1	12		2		1	3	4	2	1	1	2		5*	5	7	3	7	2		1						
<b>Class B</b>	n = 9																											
NDM (n =5)	<i>K. pneumoniae</i>	>16	57*	1																								
	<i>K. pneumoniae</i>	>16	9		4	3	16*	16	6	2	2																	
	<i>P. mirabilis</i>	0.25								1				1	3	3	11	6	12*	6	9	4	1	1				
	<i>E. coli</i>	2	9	1	1		2	1	3	3	5	8*	11	6	5	3												
	<i>Citrobacter sp.</i>	4	3		1	1		2	6	6	4	10*	8	4	1	5	1	2		2								
VIM (n =3)	<i>E. coli</i>	1								3	1	1	5	6	18*	10	8	5		1								
	<i>K. pneumoniae</i>	2	1								3	5	7	9	8*	8	2	4	7	2	2							
	<i>K. pneumoniae</i>	>16	53*	1	4																							
IMP (n =1)	<i>E. coli</i>	1								1	1		2	3	12	15*	14	6	2	2								
<b>Class D</b>	n = 8																											
(OXA-48 like)	<i>K. variicola</i>	0.5														1	3	2	9	11	19*	9	4					
	<i>K. variicola</i>	0.5	1												1		1	7	10	13*	13	9	3					
	<i>K. variicola</i>	0.5														1	3	3	6	13	11*	12	5	2	2			
	<i>K. pneumoniae</i>	2	3				2					2	2	1	7	7	17*	13	2	2								
	<i>K. pneumoniae</i>	0.5															2	2	7	12	15*	9	8	3	3			
	<i>E. coli</i>	1							1		1					6	6	15	18*	8	2	2						
	<i>E. coli</i>	0.5														2	5	6	8	18*	13	5	1	1				
	<i>K. pneumoniae</i>	2						1	1						2	1	4	11	12*	15	8	2	1					

\* median



# Disk diffusion method Meropenem ESBL-CARBA negative

mm-distribution Oxoid, BD, Mast (n=58)

	MIC mg/L	mm-distribution Oxoid, BD, Mast (n=58)																																				
		R < 16 mm														I							S ≥ 22 Screenings bp < 27 mm															
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35							
<i>K. pneumoniae</i>	4										1	1	4	4	2	15	22*	9																				
<i>E. coli</i>	8					1	4	6	4	9	9*	8	5	3	6	3																						
<i>K. pneumoniae</i>	4	1					1	2	3	6	12	23*	6	2	1	1																						
<i>E. cloacae cplex</i>	0.25													1							2	1	1	3	5	8	15*	15	6	1								
<i>E. coli</i>	8	1					1				2	8	3	14	7*	6	10	3	1	1																	1	
<i>P. mirabilis</i>	0.5																																					
<i>E. aerogenes</i>	8	1									5	6	7	17*	5	6	3	6	1	1																		
<i>P. rettgeri</i>	2	1									1	3	2	8	7	12*	13	9	2																			

\* Median



# AST Meropenem Suspect ESBL-CARBA?

		MEM MIC mg/L	Zone diameter distribution using meropenem discs from Oxoid, BD and Mast (n=58) S ≥ 22 mm R < 16 mm. Screeningbreakpoint < 27 mm																								Suspect CPE %
			6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		
<b>Class A</b>	n = 4																										
KPC (n=3)	<i>K. pneumoniae</i>	1			1		1	1	2	1	5	6	9	13*	11	4	2		1				1			98	
	<i>E. cloacae cplex</i>	>16	57*	1																						100	
	<i>E. cloacae cplex</i>	8	7			4	3	2	4	4	14*	12	3	4	1											98	
IMI (n=1)	<i>E. cloacae cplex</i>	1	12		2		1	3	4	2	1	1	2		5*	5	7	3	7	2		1				89	
<b>Class B</b>	n = 9																										
NDM (n=5)	<i>K. pneumoniae</i>	>16	57*	1																						100	
	<i>K. pneumoniae</i>	>16	9		4	3	16*	16	6	2	2															100	
	<i>P. mirabilis</i>	0.25								1				1	3	3	11	6	12*	6	9	4	1	1	75		
	<i>E. coli</i>	2	9	1	1		2	1	3	3	5	8*	11	6	5	3										100	
	<i>Citrobacter sp.</i>	4	3		1	1		2	6	6	4	10*	8	4	1	5	1	2		2						98	
VIM (n=3)	<i>E. coli</i>	1								3	1	1	5	6	18*	10	8	5		1						98	
	<i>K. pneumoniae</i>	2	1								3	5	7	9	8*	8	2	4	7	2	2					98	
	<i>K. pneumoniae</i>	>16	53*	1	4																					100	
IMP (n=1)	<i>E. coli</i>	1								1	1		2	3	12	15*	14	6	2	2						97	
<b>Class D</b>	n = 8																										
(OXA-48 like)	<i>K. variicola</i>	0.5														1	3	2	9	11	19*	9	4			82	
	<i>K. variicola</i>	0.5	1												1		1	7	10	13*	13	9	3			84	
	<i>K. variicola</i>	0.5														1	3	3	6	13	11*	12	5	2	2	80	
	<i>K. pneumoniae</i>	2	3				2					2	2	1	7	7	17*	13	2	2						98	
	<i>K. pneumoniae</i>	0.5															2	2	7	12	15*	9	8	3		80	
	<i>E. coli</i>	1							1							6	6	15	18*	8	2	2				89	
	<i>E. coli</i>	0.5														2	5	6	8	18*	13	5	1			87	
	<i>K. pneumoniae</i>	2						1	1						2	1	4	11	12*	15	8	2	1			85	

\* median



# Suspect CPE?

## Different screening criteria used

- 44 laboratories (72 %)
  - < 27 mm cut-off
- 8 laboratories (13 %)
  - < 25 mm cut-off
- 7 laboratories (11 %)
  - Clinical breakpoints
- 2 laboratories did not report their screening criteria

MIC 0.25 or 0.5 mg/L:

**2-11 % did not suspect CPE**

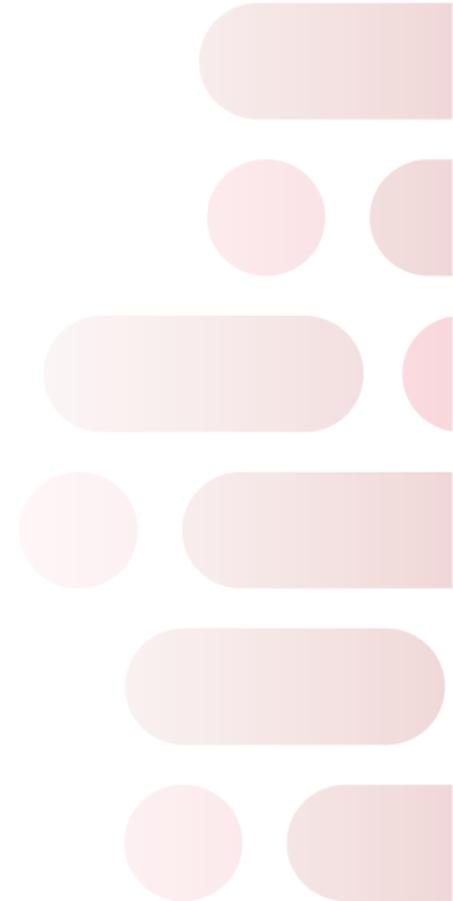
**13-38 % did not suspect CPE**

**57-86 % did not suspect CPE**



# Automated AST

- VITEK2 AST ( $n= 20$ )
  - Finland: AST-N230 ( $n= 6$ )
  - Sweden: AST-N218 ( $n= 5$ )
  - Norway: AST-N209 ( $n= 9$ )
- Phoenix AST ( $n= 3$ )
  - NMIC-93 ( $n= 2$ )
  - 25NMIC/ID90 ( $n= 1$ )





# Overall categorical agreement between BMD and VITEK2

VITEK2

Total		579 observations							VITEK2	
	>=16	20	2	78	42	12	20	<b>80</b>	Equal:	<b>30</b>
	8				1	8	<b>7</b>			
	4		19	4	37	<b>38</b>	41		Over:	<b>55</b>
VITEK	2		22	3	<b>7</b>	1	9		Under:	<b>15</b>
	1		49	<b>15</b>	13		2			
	0.5	3	<b>8</b>							
	<=0.25	<b>17</b>	20				1			
		0.25	0.5	1	2	4	8	>=16		
					BMD					

VITEK2	579 observations			
MIC	VME	ME (n)	mE (n)	No Error (n)
0.25		20		20
0.5		2	19	99
1		78	4	18
2		42	38	20
4			13	46
8			32	48
>=16				80
		142	106	331
<b>%</b>		<b>25</b>	<b>18</b>	<b>57</b>

Very major error:

Major error:

Minor error:

VME: R → S

ME: S → R

mE: R ↔ I, S ↔ I



# Overall categorical agreement between BMD and Phoenix

Phoenix

	Total	87 observations								Phoenix
	>=16			5	6		1	<b>12</b>		%
	8		1			4	<b>6</b>		Equal:	47
	4	2		3	3	<b>4</b>	4		Over:	39
Phoenix	2		2	1	<b>4</b>				Under:	14
	1		6	<b>4</b>	2		1			
	0,5		<b>7</b>	2		1				
	<=0,25	<b>4</b>	2							
		0.25	0.5	1	2	4	8	>=16		
										BMD

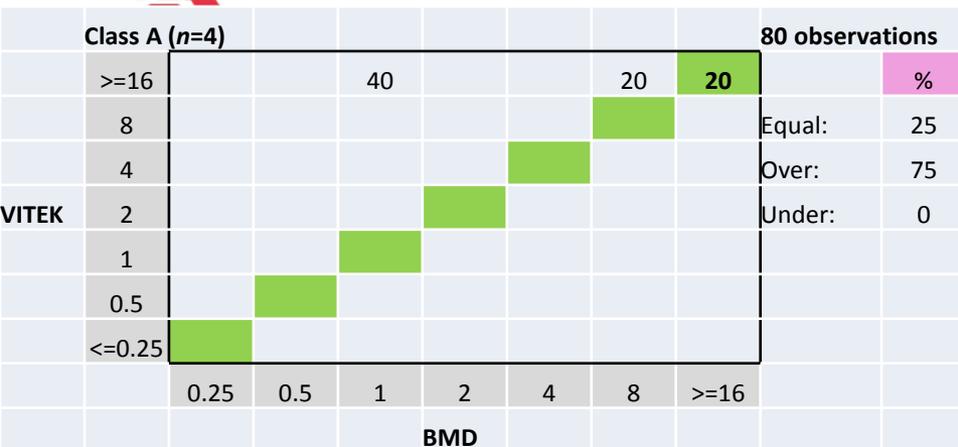
Phoenix	87 observations			
MIC	VME	ME (n)	mE (n)	No Error (n)
0.25			2	4
0.5			1	17
1		5	3	7
2		6	3	6
4			1	8
8			2	10
>=16				12
		11	12	64
%		<b>12,5</b>	<b>14</b>	<b>73,5</b>

Very major error:  
Major error:  
Minor error:

VME: R → S  
ME: S → R  
mE: R ↔ I, S ↔ I

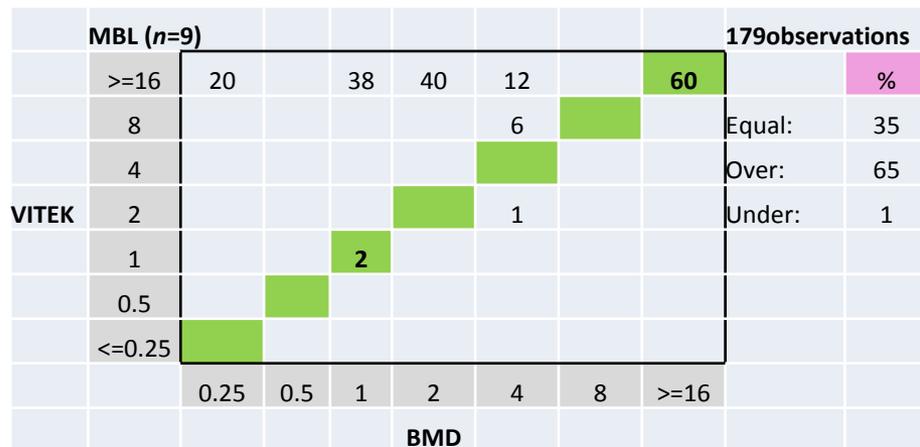


### CLASS A - serine



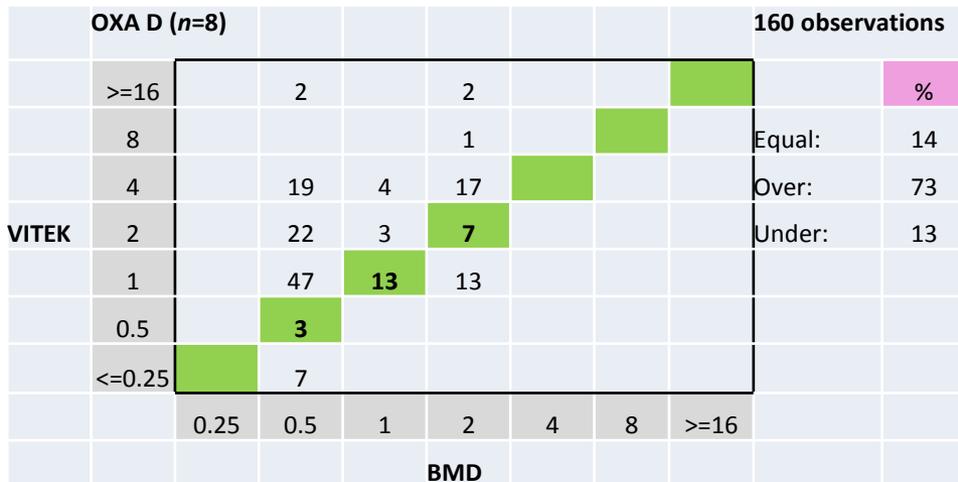
	ME	mE	No Error
%	50	25	25

### CLASS B - MBL



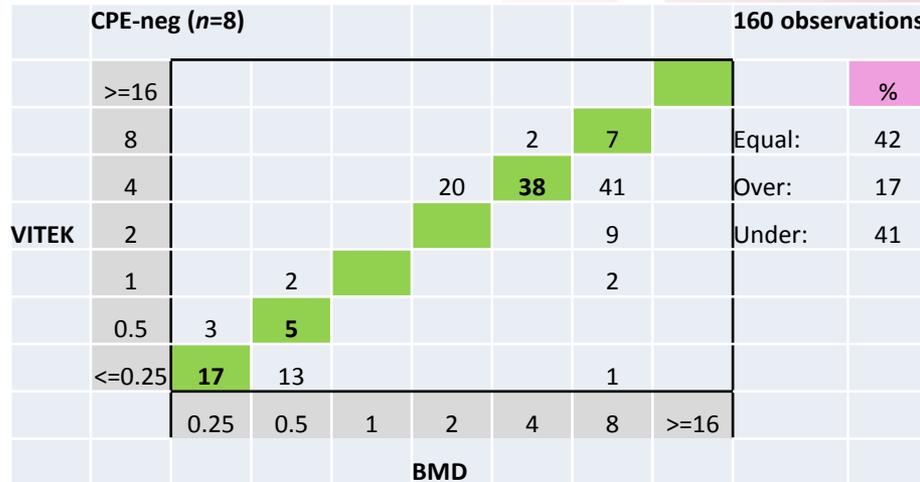
	ME	mE	No Error
%	55	7	38

### CLASS D - OXA



	ME	mE	No Error
%	2.5	25.5	72

### CPE - negative



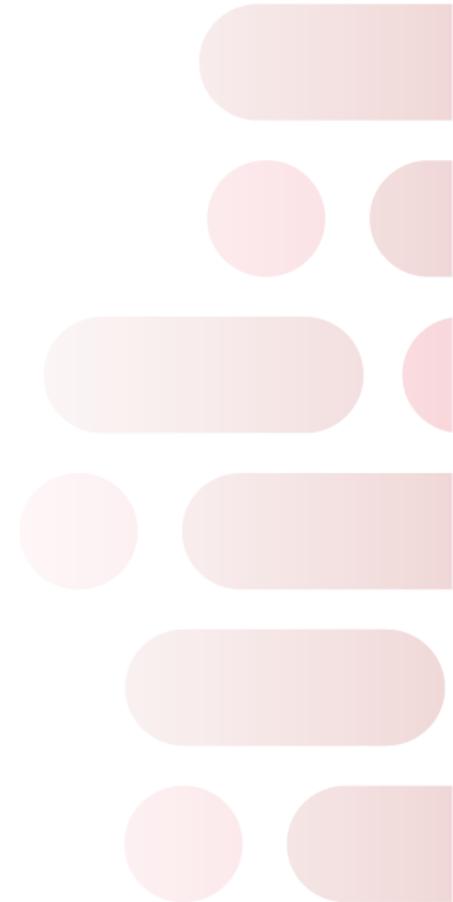
	ME	mE	No Error
%	20	80	



## Optional

# Phenotypic/biochemical method for detection of carbapenemase activity

- Rapidec Carba-NP bioMerieux  $n=4$
- Carba-NP in-house  $n=1$
- CIM  $n=1$
- Modified Hodge test  $n=5$
- UV-spectrophotometric assay  $n=1$
- Maldi Hydrolysis assay  $n=1$
- Carbapenemase test Rosco dobbeldisk  $n=1$
- Mast CAT-ID  $n=1$
- In-house MBL/KPC, temocillin  $n=1$
- Gradient test
  - MRP/MRD  $n=1$
  - MP/MPI  $n=1$
  - MP/MPI + Temocillin  $n=1$



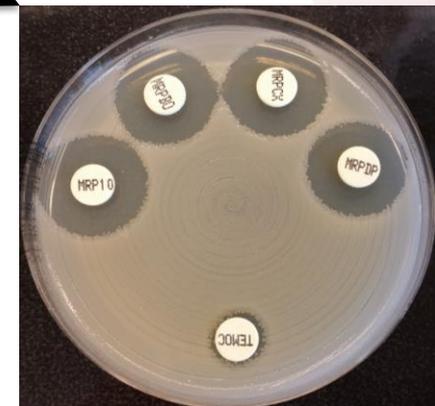
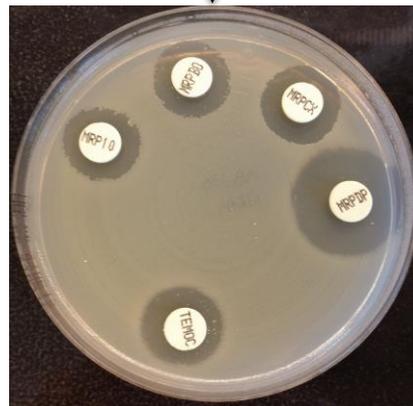
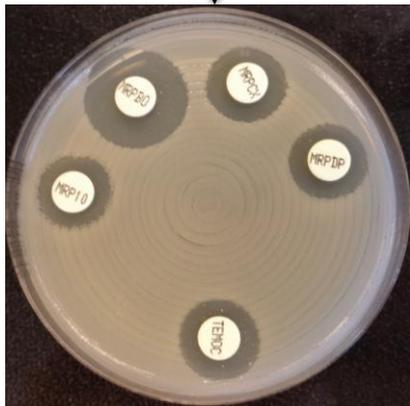
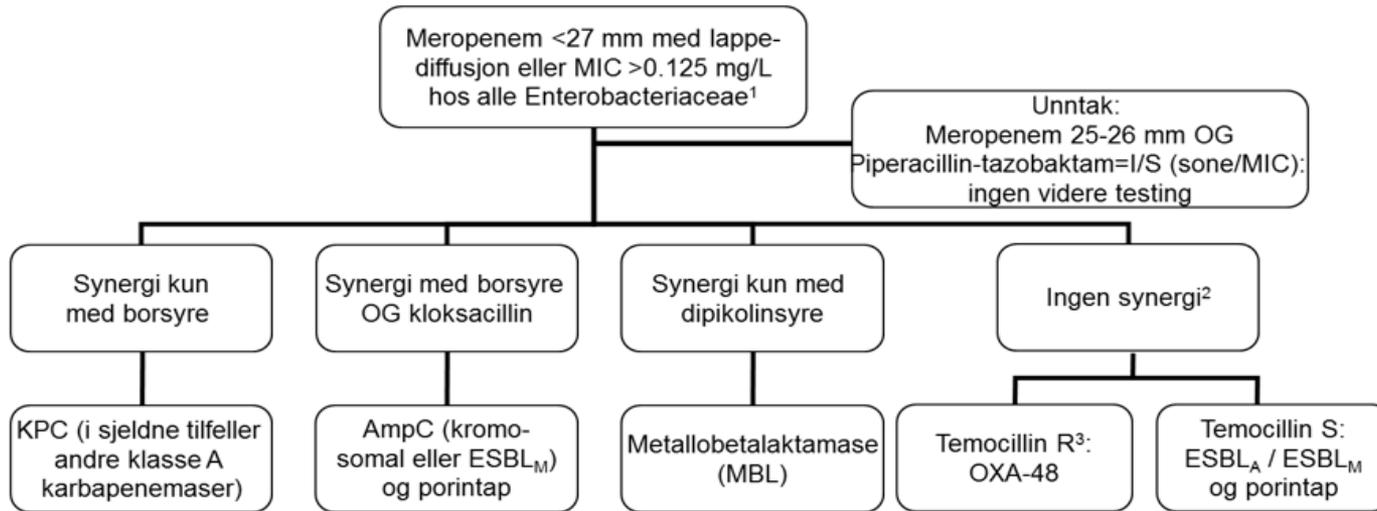


# ROSCO MBL/KPC/OXA-48 kit

Enterobacteriaceae

NordicAST v. 7.0, 2017-01-01

## ALGORITME FOR PÅVISNING AV KARBAPENEMASER (ESBL<sub>CARBA</sub>)





# ROSCO MBL/KPC/OXA-48 kit

	<i>n</i>	First time to use the kit	Previous experience	Use the kit in daily routine
Denmark	8	1		7
Finland	5	5		
Iceland	1			1
Sweden	17	11		6
Norway	11	7	2	2

42/47 laboratories reported results incl. interpretations



# ROSCO MBL/KPC/OXA-48 kit

## Interpretation

		MEM MIC	Correct interpretation	
Class A (Serine)		mg/L	<i>n</i>	%
KPC (n=3)	<i>K. pneumoniae</i>	1	41	98
	<i>E. cloacae</i> complex	>16	42	100
	<i>E. cloacae</i> complex	8	40	95
IMI (n=1)	<i>E. cloacae</i> complex	1	31	74
<hr/>				
Class B (MBL)				
NDM (n=5)	<i>K. pneumoniae</i>	>16	37	88
	<i>K. pneumoniae</i>	>16	42	100
	<i>P. mirabilis</i>	0.25	12	29
	<i>E. coli</i>	2	41	98
	<i>Citrobacter</i> sp.	4	40	95
VIM (n=3)	<i>E. coli</i>	1	37	88
	<i>K. pneumoniae</i>	2	40	95
	<i>K. pneumoniae</i>	>16	41	98
IMP (n=1)	<i>E. coli</i>	1	30	71
<hr/>				
Class D (OXA-48 like) n=8				
	<i>K. variicola</i>	0.5	31	74
	<i>K. variicola</i>	0.5	35	83
	<i>K. variicola</i>	0.5	35	83
	<i>K. pneumoniae</i>	2	42	100
	<i>K. pneumoniae</i>	0.5	32	76
	<i>E. coli</i>	1	41	98
	<i>E. coli</i>	0.5	41	98
	<i>K. pneumoniae</i>	2	41	98



# ROSCO MBL/KPC/OXA-48 kit Interpretation

	MIC mg/L	Correct interpretation	
Gruppe 4 CPE neg (n= 8)		n	%
<i>K. pneumoniae</i>	4	29	69
<i>E. coli</i>	8	36	86
<i>K. pneumoniae</i>	4	35	83
<i>E. cloacae complex</i>	0.25	41	98
<i>E. coli</i>	8	39	93
<i>P. mirabilis</i>	0.5	38	90
<i>E. aerogenes</i>	8	14	33
<i>P. rettgeri</i>	2	39	93



		MIC mg/L	Correct interpretation <i>n</i>	Previous experience %	<b>N (n=11)</b> 4/11 <b>36 %</b>	<b>DK (n=8)</b> 7/8 <b>87.5 %</b>	<b>FI (n=5)</b> 0/5 <b>0 %</b>	<b>SE (n=17)</b> 6/17 <b>35 %</b>
<b>Class A (Serine)</b>								
KPC (n=3)	<i>K. pneumoniae</i>	1	41	98				
	<i>E. cloacae</i> complex	>16	42	100				
	<i>E. cloacae</i> complex	8	40	95				
IMI (n=1)	<i>E. cloacae</i> complex	1	31	74	82 %	62.5 %	60 %	76 %
<b>Class B (MBL)</b>								
NDM (n=5)	<i>K. pneumoniae</i>	>16	37	88				
	<i>K. pneumoniae</i>	>16	42	100				
	<i>P. mirabilis</i>	0.25	12	29	36 %	62.5 %	20 %	12 %
	<i>E. coli</i>	2	41	98				
	<i>Citrobacter</i> sp.	4	40	95				
VIM (n=3)	<i>E. coli</i>	1	37	88				
	<i>K. pneumoniae</i>	2	40	95				
	<i>K. pneumoniae</i>	>16	41	98				
IMP (n=1)	<i>E. coli</i>	1	30	71	82 %	62.5 %	60 %	76 %
<b>Class D (OXA-48 like)</b>								
	<i>K. variicola</i>	0.5	31	74	82 %	87.5 %	80 %	65 %
	<i>K. variicola</i>	0.5	35	83				
	<i>K. variicola</i>	0.5	35	83				
	<i>K. pneumoniae</i>	2	42	100				
	<i>K. pneumoniae</i>	0.5	32	76	82 %	87.5 %	100 %	65 %
	<i>E. coli</i>	1	41	98				
	<i>E. coli</i>	0.5	41	98				
	<i>K. pneumoniae</i>	2	41	98				



Gruppe 4 CPE neg	MIC	Correct	Previous experience	N (n=11)	DK (n=8)	FI (n=5)	SE (n=17)
	mg/L	interpretation					
<i>K. pneumoniae</i>	4	29	69	64 %	25 %	80 %	88 %
<i>E. coli</i>	8	36	86				
<i>K. pneumoniae</i>	4	35	83				
<i>E. cloacae</i> complex	0.25	41	98				
<i>E. coli</i>	8	39	93				
<i>P. mirabilis</i>	0.5	38	90				
<i>E. aerogenes</i>	8	14	33	45 %	25 %	20 %	35 %
<i>P. rettgeri</i>	2	39	93				



# Conclusions

- Regarding QC, the laboratories performed very well
- The EUCAST disk diffusion method is a robust method to detect CPE
  - Isolates with low meropenem MIC are challenging
  - Some species (*P. mirabilis* + NDM) and mechanisms (OXA-48) cause challenges
- Automated AST overestimates MIC to meropenem, particularly isolates with class A and B carbapenemases
- The Rosco KPC/MBL/OXA-48 kit shows promising results in classification of CPE



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