Detection of ESBL and carbapenemases in Gram-negatives

Hiba ALMIR, PhD
Technical University of Denmark
16 May 2024

The Fleming Fund
Regional Grants

UKaid
from the British people

MOTT MACDONALD

DTU

International Vaccine Institute

Faculty of Veterinary Science
Chulalongkorn University

Outline

- Background
- ESBL detection methods
- CRE detection methods
- Q&A
- Post-webinar assignment

Background

- ESBL: Extended-Spectrum Beta-Lactamase
- AmpC: Beta-lactamase of the AmpC-type
- Carbapenemase

…ase = suffix to form enzyme name
(added to the end of the substrate name)

Background

- Which bacteria carry beta-lactamases?
  - Found in many types of bacteria – mainly gram-negative. Some examples are:

Enterobacterales	**Non-Enterobacterales**	**Gram-positive**
• *Escherichia coli* • *Salmonella enterica* • *Klebsiella pneumoniae* • *Klebsiella oxytoca* • *Citrobacter freundii* • *Enterobacter* spp. • *Proteus mirabilis* • *Serratia marcescens*	• *Pseudomonas aeruginosa* • *Pseudomonas putida* • *Acinetobacter* spp. • *Neisseria gonorrheae* • *Haemophilus influenzae* • *Campylobacter* spp.	• *Staphlococcus aureus* -MRSA • Others…

Background

- Beta-lactam antibiotics:

Class	Group	Effect	Examples (generation)
Beta-lactams	Penicillins	Narrow spectrum	Benzylpenicillin (1. gen) Oxacillin (2. gen)
		Extended spectrum	Amoxicillin (3. gen) Piperacillin (4. gen)
	Cephalosporins (Cephamycins) (Cephems)		Cefalotin (1. gen)/ Cefoxitin (2. gen)
		Extended spectrum	Cefotaxime, Ceftazidime (3. gen)
			Cefepime (4. gen)
		MRSA	Ceftaroline (5. gen)
		MDR	Cefiderocol (Others - siderophore)
	Monobactams		Aztreonam
	Combinations with beta-lactam inhibitors	ESBL	Cefotaxime or ceftazidime with clavulanic acid
	Carbapenems	MDR	Meropenem

Background

- Carbapenem antibiotics:
  - Carbapenems are β -lactam antibiotics – similar to penicillin
    - meropenem, imipenem, ertapenem, doripenem
  - Carbapenems are highly resistant to most β-lactamases
  - Broad spectrum of activity
    - Both Gram-positive and Gram-negative bacteria

	Strep spp. & MSSA	*Enterobacteriales*	Pseudomonads, *Acinetoobacter* spp	Anaerobes
Meropenem	+	+	+	+
Imipenem	+	+	+	+
Ertapenem	+	+	Limited activity	+
Doripenem	+	+	+	+

Background

- Which bacteria carry Carbapenemases?
  - Carbapenemases are mainly found in Gram-negative bacteria

Enterobacteriales	**Non-*Enterobacteriales***
• *Klebsiella pneumoniae* • *Klebsiella oxytoca* • *Escherichia coli* • *Citrobacter freundi* • *Enterobacter aerogenes* • *Enterobacter cloacae* • *Enterobacter gergoviae* • *Proteus mirabilis* • *Salmonella enterica* • *Serratia marcescens*	• *Pseudomonas aeruginosa* • *Pseudomonas putida* • *Acinetobacter* spp.

Background

- Carbapenemase-producing *Enterobacteriaceae* (CPE):
  - Gram negative bacteria that can live in human and animal gut
    - *Klebsiella* spp., *E. coli*, *Enterobacter* spp.
  - Involved in a variety of infections
    - Bacteremia, urinary tract infections, intra-abdominal infections and pneumonia
    - High mortality rates associated with CPE infections as high as 57%*
  - Urgent need of new treatment strategies for treating CPE infections

*Luke D. Hughes et al, 2019

ESBL Detection methods

- Screening methods
  - Broth/agar dilution or disk diffusion method
  - Selective media
- Phenotypic confirmatory methods
- Genotypic confirmatory methods

ESBL screening methods

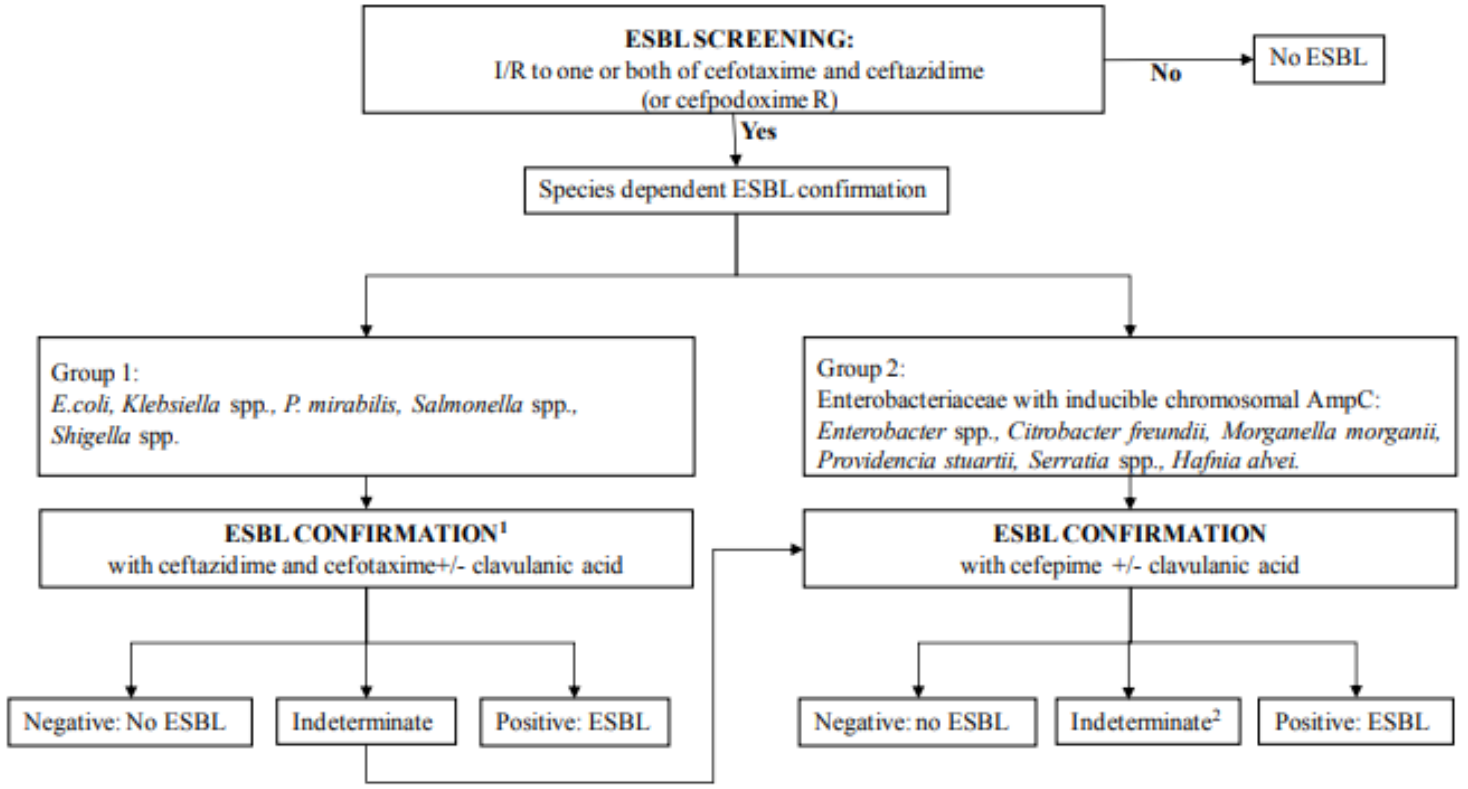
Method	Antibiotic	Conduct ESBL-testing if
Broth or agar dilution[1]	Cefotaxime/ceftriaxone AND Ceftazidime	MIC >1 mg/L for either agent
	Cefpodoxime	MIC >1 mg/L
Disk diffusion[1]	Cefotaxime (5 µg) or	Inhibition zone <21 mm
	Ceftriaxone (30 µg)	Inhibition zone <23 mm
	AND Ceftazidime (10 µg)	Inhibition zone <22 mm
	Cefpdoxime (10 µg)	Inhibition zone <21 mm

[1] With all methods test either (i) cefotaxime or ceftriaxone AND ceftazidime OR (ii) cefpodoxime alone.

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

ESBL detection

Figure 1. Algorithm for phenotypic detection of ESBLs



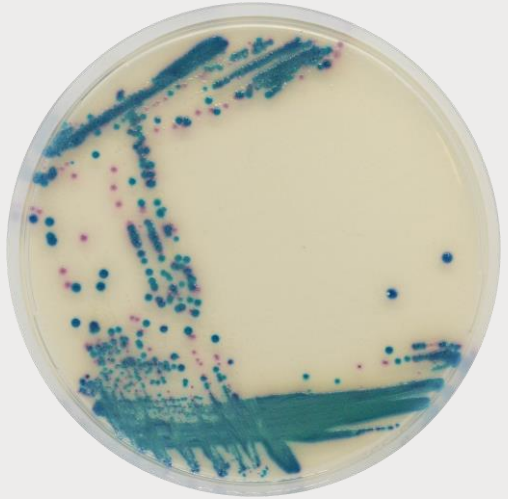
[1] If cefoxitin has been tested and has an MIC >8 mg/L, perform cefepime+/- clavulanic acid confirmation test

[2] Cannot be determined as either positive or negative (e.g. if a gradient diffusion strip cannot be read due to growth beyond the MIC range of the strip or there is no clear synergy in combination-disk and double-disk synergy tests). In confirmation with cefepime +/- clavulanic acid is still indeterminate, genotypic testing is required.

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

ESBL screening methods

- Culture media in-house (with cefotaxime and/or ceftazidime)
- Commercial ready-to-use media plates
- Chromogenic media (with cefpodoxime)

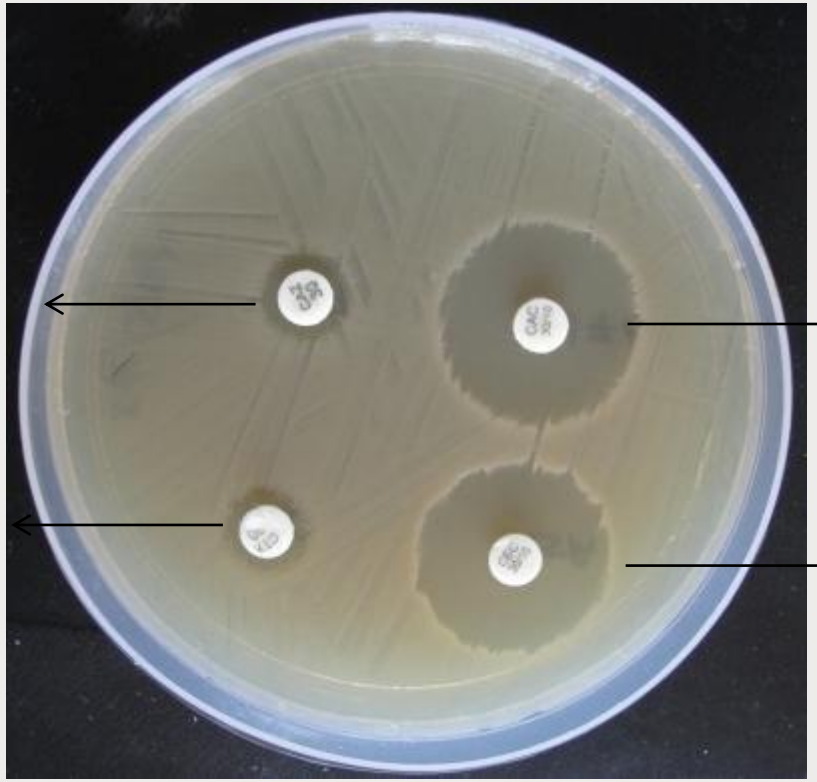


ESBL Phenotypic confirmatory methods

1. Combination disk test (CDT)
2. Double-disk synergy test (DDST)
3. Gradient method – Etest ESBL
4. Broth microdilution

Combination disk test (CDT)

- Disk containing cephalosporin alone (cefotaxime, ceftazidime, cefepime) and in combination with clavulanic acid
- Compare inhibition zones

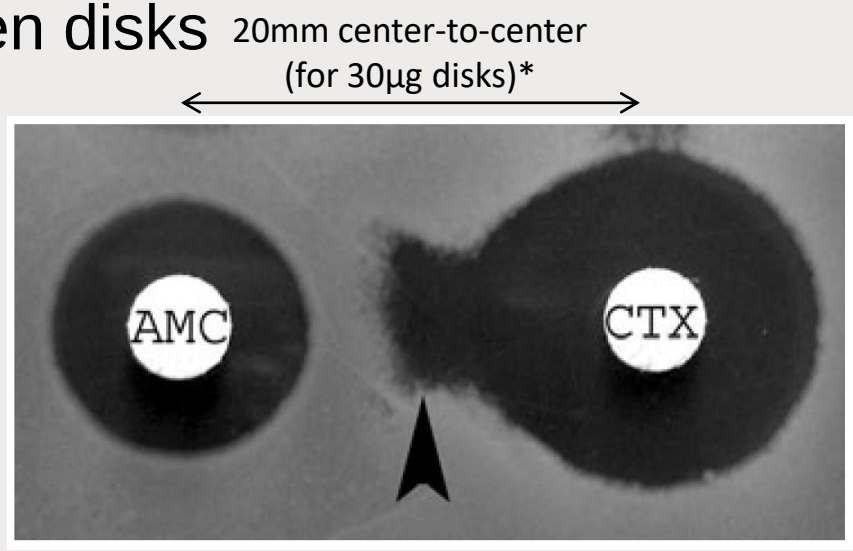


- positive if ≥5 mm increase in inhibition zone with clavulanic acid

DOI: 10.20546/ijcmas.2016.505.045

Double-disk synergy test (DDST)

- Disk containing cephalosporin (cefotaxime, ceftazidime, cefepime) is applied next to disk with clavulanic acid (amoxicillin-clavulanic acid)
- Shorter distance between disks



*: May be reduced to 15mm or expanded to 30mm if high or low resistance level.
EUCAST uses lower disk content → re-evaluation needed!

- positive if inhibition zone around cephalosporin disk is enhanced in the direction of the disk with clavulanic acid

Drieux; Clin Microbiol Infect 2008; 14:0-103

Gradient method – Etest ESBL

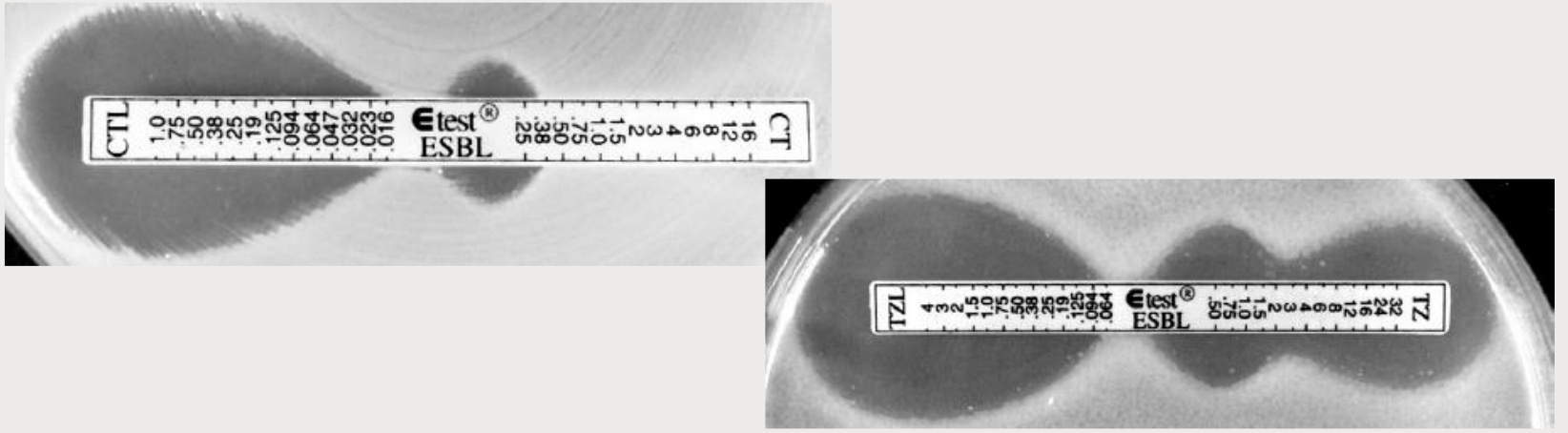
- 2-sided strips containing gradients of cefotaxime, ceftazidime or cefepime either alone or in combination with clavulanic acid
- according manufacturer's instruction

MIC ratio: CT/CTL = 1.5/0.047 = 32

- positive if MIC ratio ≥ 8 (MIC reduced by three doubling dilution steps)

Gradient method – Etest ESBL

- according manufacturer's instruction
- 2-sided strips containing gradients of cefotaxime, ceftazidime or cefepime either alone or in combination with clavulanic acid



- positive if MIC ratio ≥ 8 (MIC reduced by three doubling dilution steps)
- or phantom zone/deformed ellipse is present
- indeterminate if growth beyond the MIC range of the strip

False negative results

- ESBL presence **masked** due to **high-level expression of AmpC β-lactamases**
  - clear resistance to 3rd generation cephalosporins + to cephamycins e.g. cefoxitin MIC > 8mg/L (except ACC AmpC β-lactamases)

Perform **confirmatory test** with **cefepime** as indicator cephalosporin or use **cloxacillin**

Cefepime: usually not hydrolyzed by AmpC β-lactamases → CDT, DDST, Etest, broth microdilution

Cloxacillin: good inhibitor of AmpC enzymes → CDT with disk containing cloxacillin or CDT/DDST with agar supplemented with cloxacillin

- ESBL presence **masked** by **carbapenemases such as MBLs or KPCs** (not OXA's) and/or severe **permeability defects**

False positive results

- *Klebsiella oxytoca* with hyperproduction of chromosomal K1 or OXA-like β-lactamases

- *Proteus vulgaris, Citrobacter koseri, Kluyvera* spp due to chromosomal β-lactamases inhibited by clavulanic acid

- Strains with hyperproduction of SHV-1, TEM-1 or OXA-1-like broad-spectrum β-lactamases combined with alterered permeability

ESBL Genotypic confirmatory methods

- PCR (and sequencing) → epidemiological information is needed to decide which genes to target
- Microarrays → limitations in the number of resistance genes targeted
- WGS → well curated and complete databases are needed

ESBL Genotypic confirmatory methods

- Beta-lactamases are numerous!
- **ESBLs**
  - CTX-M
  - TEM
  - SHV
  - (OXA)
  - …..
- **AmpCs**
  - cAmpC
  - CMY
  - ACC
  - DHA
  - FOX
  - …..
- Each type of enzyme is further divided into variants: E.g. CTX-M-1, CTX-M-2…
- You can find a database of beta-lactam genes here: http://bldb.eu/

Correct nomenclature:	
Gene	Protein
bla$_{\text{CTX-M-1}}$	CTX-M-1
bla$_{\text{CMY-2}}$	CMY-2
etc…	etc…

CRE Detection methods

- Screening methods
  - Broth/agar dilution or disk diffusion method
  - Selective media
- Phenotypic confirmatory methods
- Genotypic confirmatory methods

Broth/agar dilution or disk diffusion method

	MIC breakpoint (mg/L)			Zone diameter breakpoint (mm) with 10µg disks		
	S≤	R>		S≥	R<	
Ertapenem	0.5	0.5		23	23	
Imipenem	2	4		22	19	
Meropenem	2	8		22	16	

Broth/agar dilution or disk diffusion method

	MIC breakpoint (mg/L)			Zone diameter breakpoint (mm) with 10µg disks		
	S≤	R>	cut-off	S≥	R<	cut-off
Ertapenem	0.5	0.5	**> 0.125**	25	25	**< 25**
Meropenem	2	8	**> 0.125**	22	16	**< 28***

Use **ECOFF** values

Ertapenem: excellent sensitivity but poor specificity, especially in species such as *Enterobacter spp.* (relative instability to ESBLs and AmpC in combination with porin loss

→ not recommended for routine use

Imipenem: the separation between the wild-type and carbapenemase-producers is relatively poor

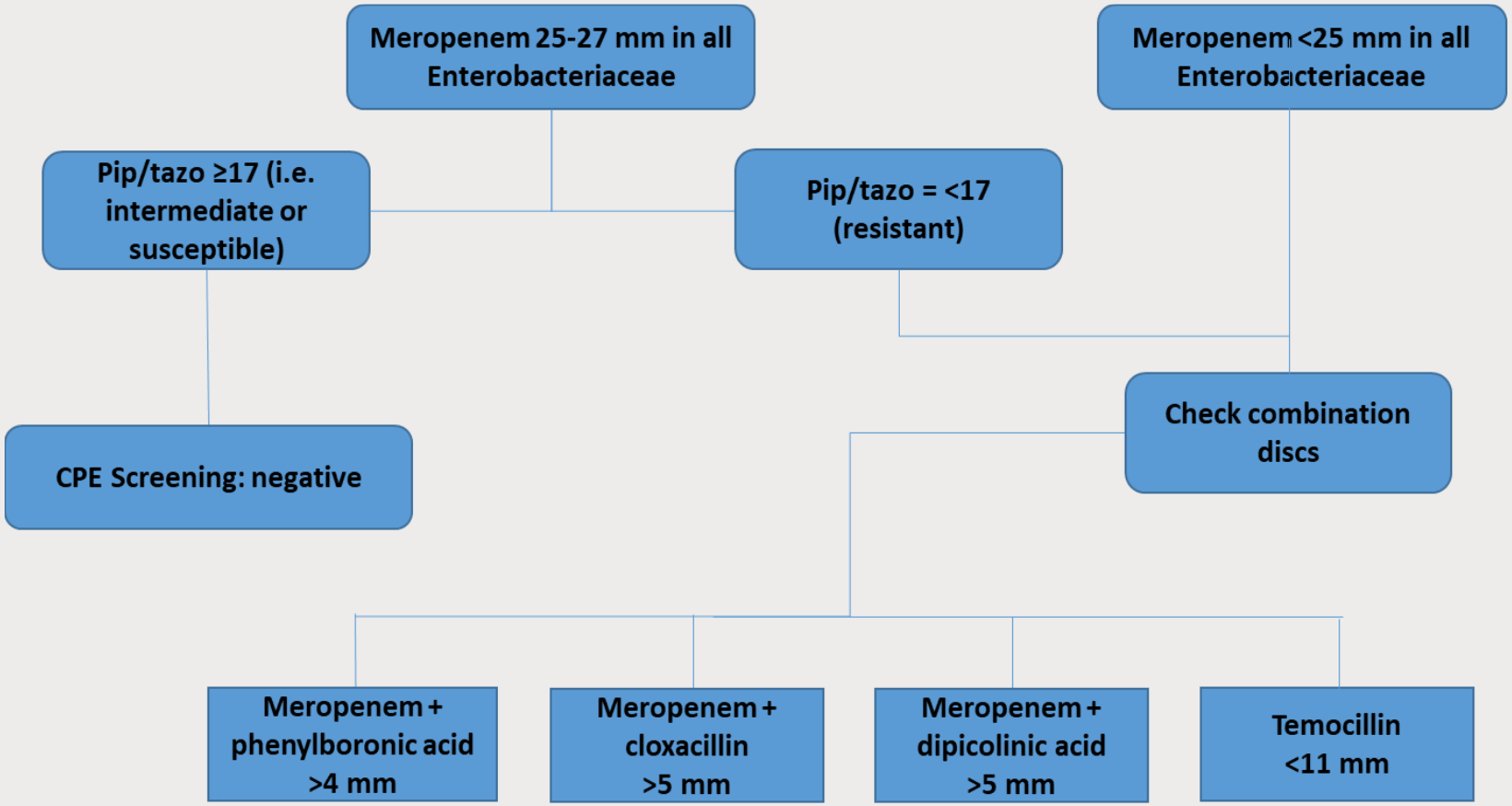
→ not recommended for use as a stand-alone screening test compound

Meropenem: best balance of sensitivity and specificity

* Isolates with 25-27 mm only need to be investigated for carbapenemase production if they are resistant to piperacillin/tazobactam and/or temocillin

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

CRE screening



EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

CRE screening methods

- Culture media in-house (with meropenem)
- Commercial ready-to-use media plates
- Chromogenic media

Combination disk method

- meropenem (10µg) +/- various inhibitors

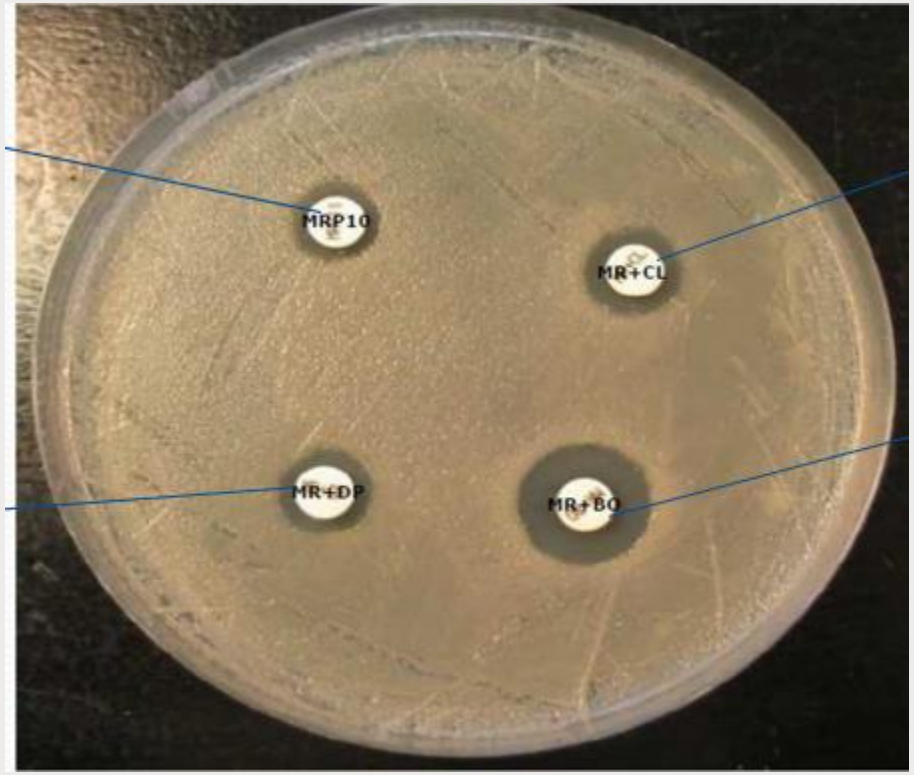
Table 2. Interpretation of phenotypic tests (carbapenemases in **bold type**) by diffusion methods with disks or tablets. The exact definitions of synergy are provided in package inserts for the various commercial products.

B-lactamase	Synergy observed as increase in zone diameter (mm) with 10 µg meropenem disk/tablet				Temocillin MIC >128 mg/L or zone diameter <11 mm
	DPA/EDTA	APBA/PBA	DPA+APBA	CLX	
MBL	+	-	-	-	Variable[1]
KPC	-	+	-	-	Variable[1]
MBL + KPC[2]	Variable	Variable	+	-	Variable[1]
OXA-48-like	-	-	-	-	Yes
AmpC + porin loss	-	+	-	+	Variable[1]
ESBL + porin loss	-	-	-	-	No

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

Combination disk method

- meropenem (10µg) +/- various inhibitors

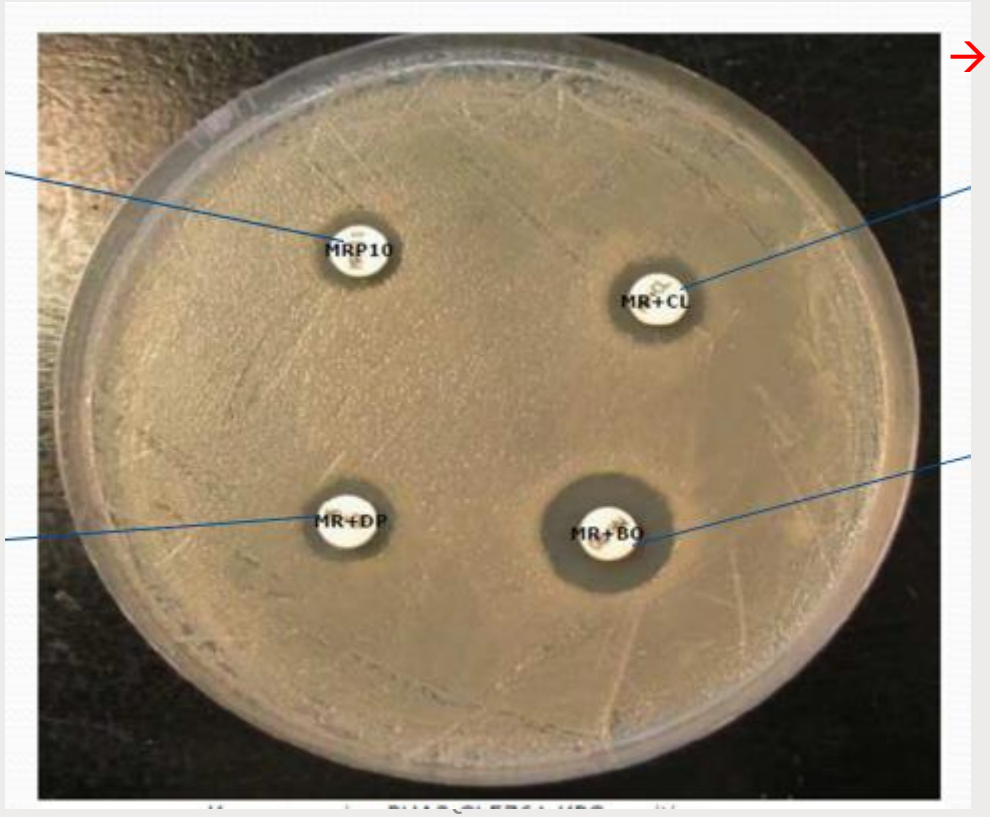


EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

Combination disk method

Interpretation criteria?

Compare inhibition zone diameter of meropenem with meropenem + inhibitor



Meropenem 10µg

Meropenem 10µg + DPA 1000µg

≥ 5mm

→ **Synergy/positive result**

Meropenem 10µg + cloxacillin 750µg

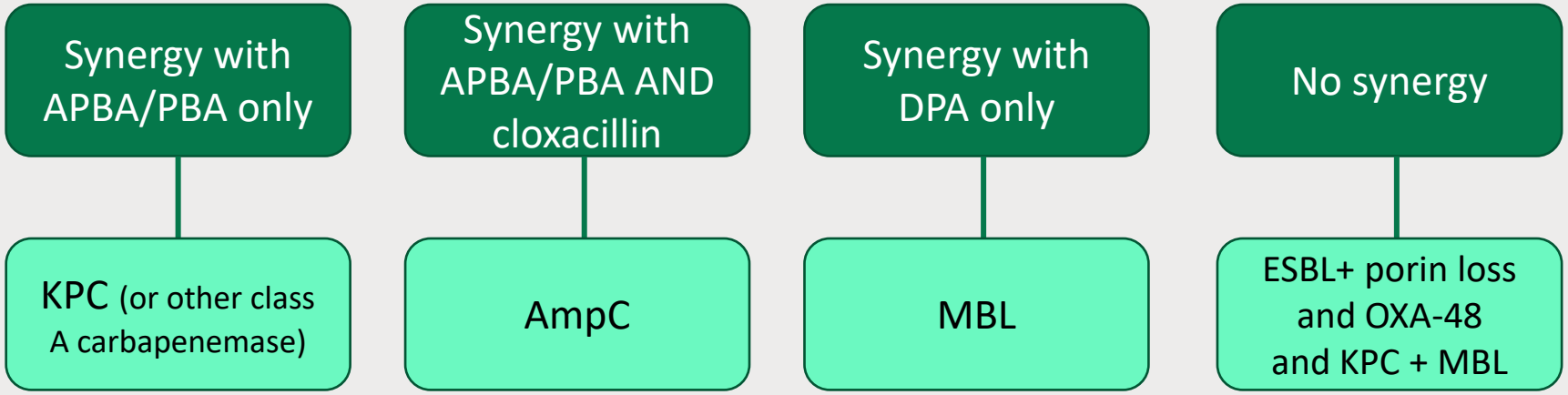
≥ 5mm

Meropenem 10µg + APBA/PBA 600µg

≥ 4mm

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

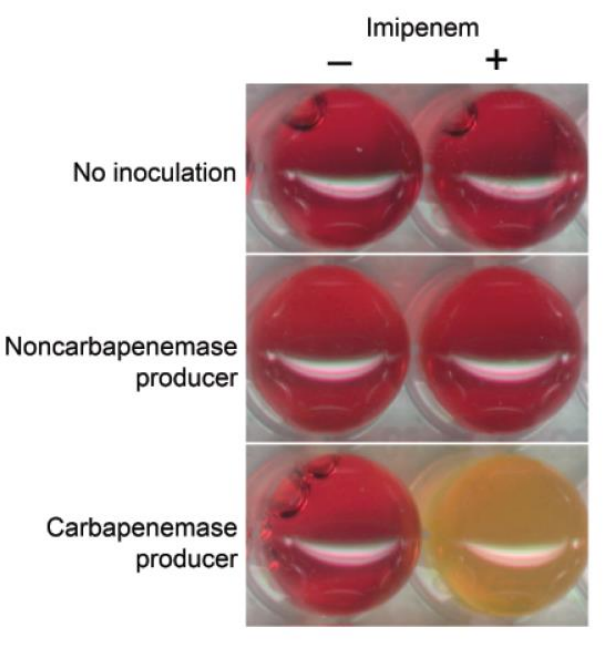
Combination disk method

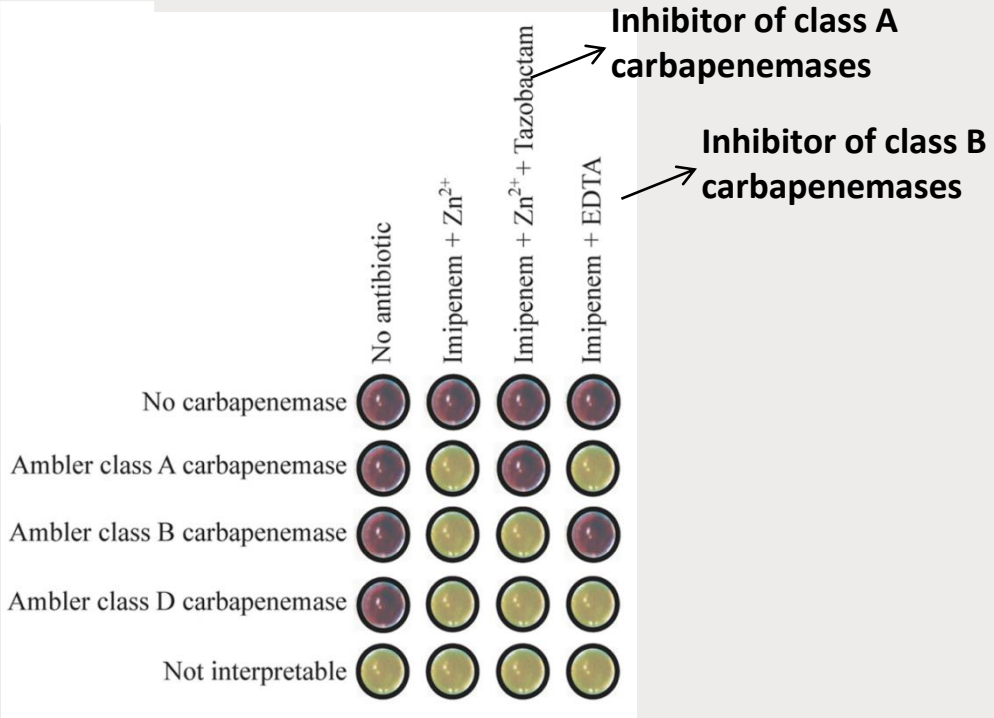


EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

Carba NP test

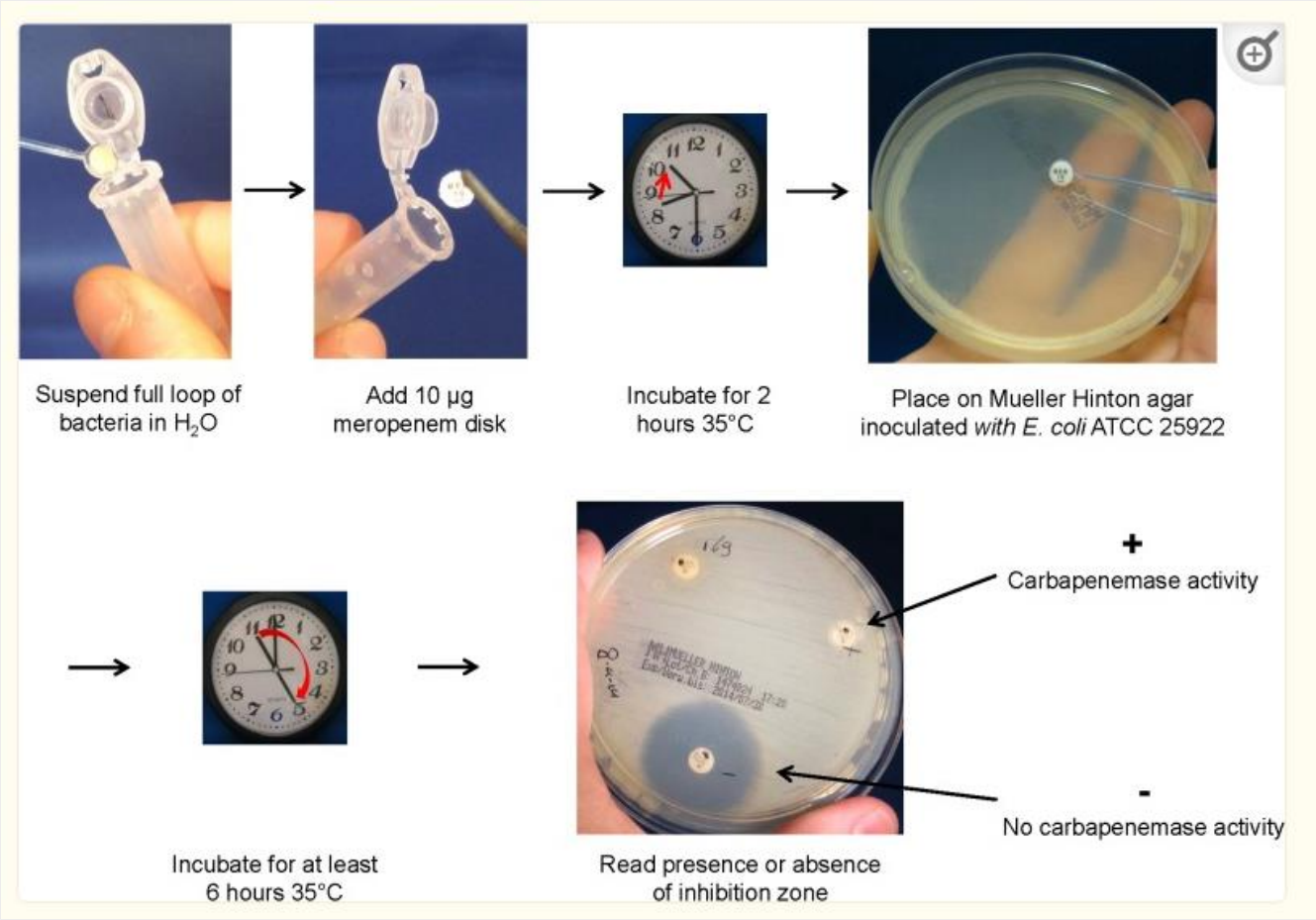
- rapid (2 hours), cheap, high sensitivity and specificity biochemical test
  - change of phenol red color (red to yellow) after hydrolysis of imipenem by carbapenemase





Carbapenem Inactivation Method (CIM)

- Low-cost alternative to the CarbaNP test



PLoS One. 2015; 10(3): e0123690.

CRE Genotypic confirmatory methods

- Multiplex PCR
- Real-time PCR
- Cepheid GeneXpert Carba-R
  - Detection and differentiation of KPC, NDM, VIM, IMP-1, and OXA-48 in 48 minutes
- Whole-genome sequencing

CRE Genotypic confirmatory methods

- Sequencing of entire gene or genome might be needed to differentiate between variants and derivatives
  - For example, to differentiate between OXA-48 (true carbapenemase) and OXA-163 (weak carbapenemase)

- Screening of sequenced genome against a database of known carbapenemase genes using bioinformatics tools
  - Continuously updated tools and databases such as ResFinder, AMRFinderPlus, and CARD

- Both known and unknown variants can be detected with WGS

The Fleming Fund
Regional Grants

Q&A

UKaid from the British people

MOTT MACDONALD

DTU

International Vaccine Institute

Faculty of Veterinary Science • Chulalongkorn University

Post-webinar assignment

- All participants in this webinar will receive an email with a link and brief instructions for the assignment

- 6 cases. Case background provided, and you'll answer related questions.

- **Deadline for submitting results: 30th of May 2024**

- Should you have any questions or need further clarification, feel free to reach out to us at eqasia@food.dtu.dk

The Fleming Fund
Regional Grants

Q&A

UKaid from the British people

MOTT MACDONALD

DTU

International Vaccine Institute

Faculty of Veterinary Science • Chulalongkorn University