HADEA SERVICE CONTRACT 20197409

Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

FWD AMR·
RefLabCap

Introduction to ESBL, AmpC and carbapenemase producing Salmonella

Phenotypic testing and interpretation of test

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FWD AMR· RefLabCap

ANTIMICROBIAL RESISTANCE…?

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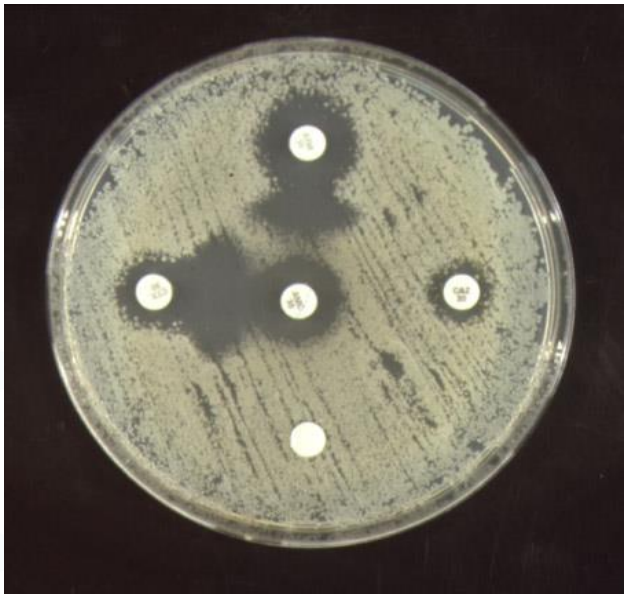
- Different concepts?
- Different definitions??
- Technicians, microbiologists, clinicians…



THE CONCEPT OF RESISTANCE … CLINICIANS

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- **Absence of an adequate clinical outcome** when using an antibiotic with a standard dose/schedule for an infection due to a specific organism that normally responds to this treatment

  - it can be predicted in the microbiology laboratory using **clinical breakpoints** that interpret as **clinically resistant** MIC values obtained in susceptibility testing (antibiogram)

  - when the microbiology laboratory reports **resistant (R),** clinicians normally avoid the use of the antimicrobial for which this result has been obtained

THE CONCEPT OF RESISTANCE… MICROBIOLOGISTS

- The microorganism has a "**resistance determinant" (gene)** that express a **"resistance mechanism"** and consequently …
  - there is an **absence of killing or an inhibitory effect** with the antimicrobial when confronted in vitro with the **bacteria**
  - the MIC obtained in susceptibility testing is higher than that obtained with an organism that does not present and/or express a resistance determinant related with the drug
- The **epidemiological cut off (ECOFF)* value** can be used to separate isolates with no resistance mechanism (wild type isolate) from those that have a resistance mechanism

*http://www.eucast.org

STANDARDS FOR PHENOTYPIC AST METHODS

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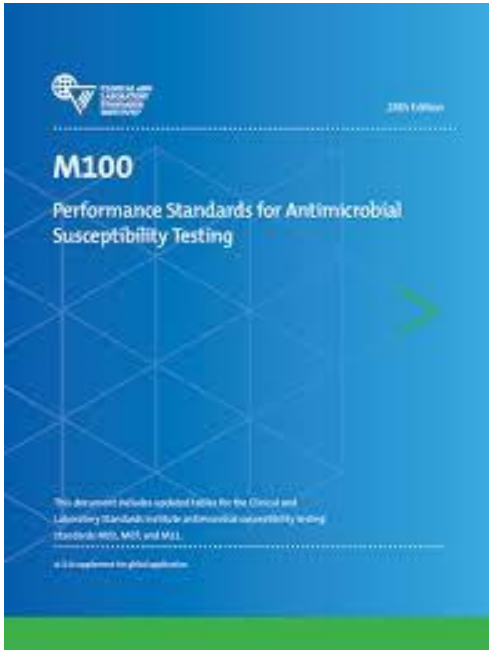

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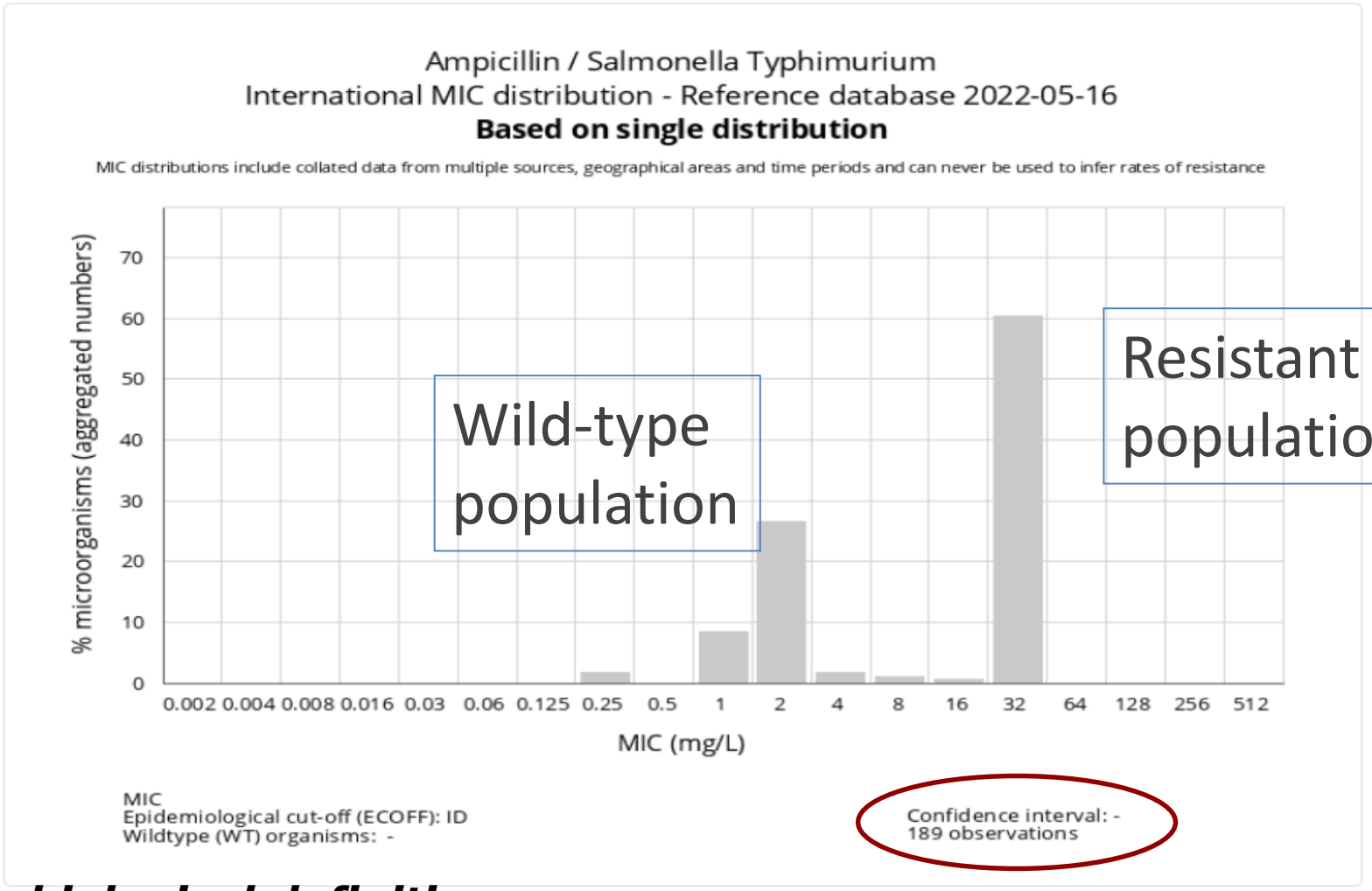
…and for interpretation of results

EXAMPLE

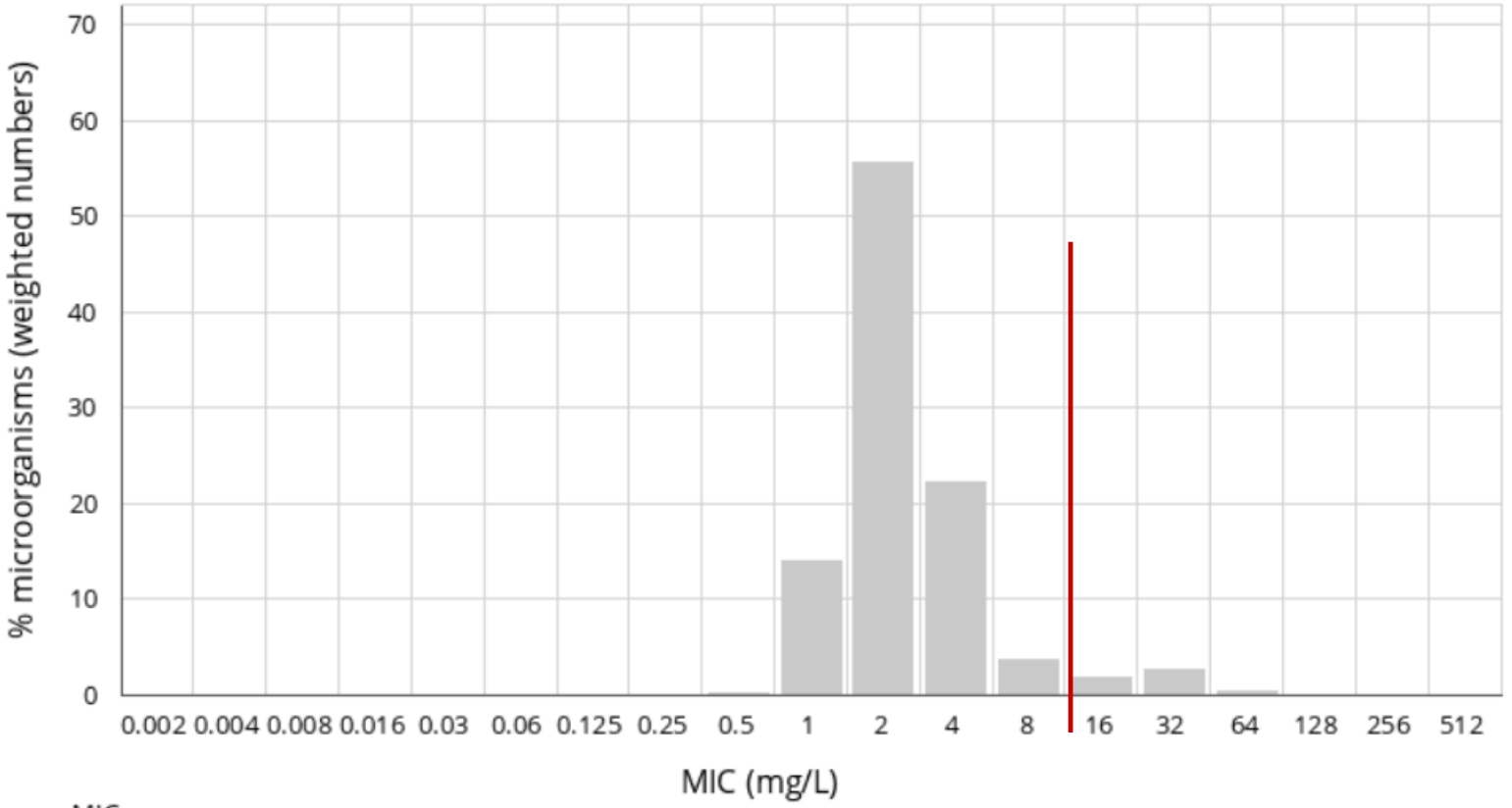


Microbiological definition

Resistance is the property of bacterial strains to survive at higher antibiotic concentrations compared with the wild-type population

Cefoxitin / Salmonella Typhimurium
International MIC distribution - Reference database 2022-05-16
Based on aggregated distributions where each distribution has equal weight *

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 (ECOFF): -
Wildtype (WT) organisms: -

Confidence interval: -
5242 observations (2 data sources)

* individual distributions were converted to percentages of their individual total and then aggregated

ENTEROBACTERALES – 3RD & 4TH GEN CEPHALOSPORINS

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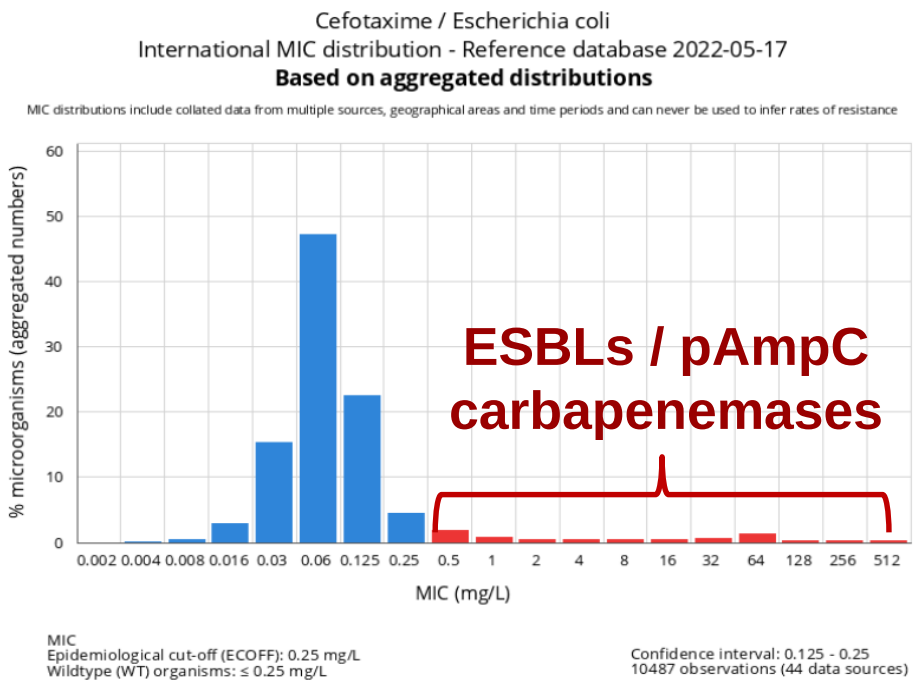

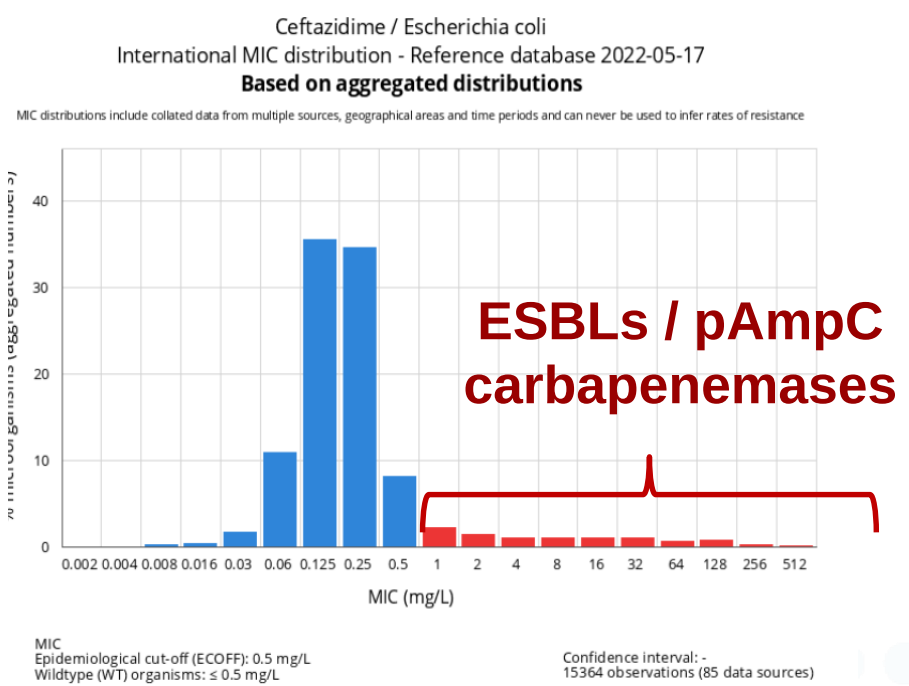

These breakpoints will detect all clinically important resistance mechanisms (including ESBLs and plasmid mediated AmpCs). Some isolates that produce β-lactamases are susceptible or intermediate to 3rd / 4th gen. cephalosporins and should be **reported as tested**, i.e. the presence or absence of an ESBL does not in itself influence the categorisation of susceptibility.

EUCAST CLINICAL BREAKPOINTS

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*Enterobacterales**

EUCAST Clinical Breakpoint Tables v. 12.0, valid from 2022-01-01

Expert Rules and Intrinsic Resistance Tables

An MIC breakpoint of S ≤ 0.001 mg/L is an arbitrary, "off scale" breakpoint (corresponding to a zone diameter breakpoint of "S ≥ 50 mm") which categorises wild-type organisms (organisms without phenotypically detectable resistance mechanisms to the agent) as "Susceptible, increased exposure" (I). For these organism-agent combinations, never report "Susceptible, standard dosing regimen" (S).

MIC determination (broth microdilution according to ISO standard 20776-1 except for mecillinam and fosfomycin where agar dilution is used)
Medium: Mueller-Hinton broth (for cefiderocol, see https://www.eucast.org/eucastguidancedocuments/)
Inoculum: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1°C, 18±2h
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth. See "EUCAST Reading Guide for broth microdilution" for further information.
Quality control: *Escherichia coli* ATCC 25922. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor combinations, see EUCAST QC Tables.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1°C, 18±2h
Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light. See "EUCAST Reading Guide for disk diffusion" for further information.
Quality control: *Escherichia coli* ATCC 25922. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor-combination disks, see EUCAST QC Tables.

*** Recent taxonomic studies have narrowed the definition of the family Enterobacteriaceae. Some previous members of this family are now included in other families within the Order *Enterobacterales*. Breakpoints in this table apply to all members of the *Enterobacterales*.**

Penicillins[1]	MIC breakpoints (mg/L)			Disk content	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU	(µg)	S ≥	R <	ATU
Benzylpenicillin	-	-			-	-	
Ampicillin[1]	8	8		10	14[A]	14[A]	
Ampicillin-sulbactam[1]	8[2]	8[2]		10-10	14[A]	14[A]	
Amoxicillin[1]	8	8		-	Note[B]	Note[B]	
Amoxicillin-clavulanic acid[1]	8[3]	8[3]		20-10	19[A]	19[A]	19-20
Amoxicillin-clavulanic acid (uncomplicated UTI only)	32[3]	32[3]		20-10	16[A]	16[A]	
Piperacillin	8	8		30	20	20	
Piperacillin-tazobactam	8[4]	8[4]	16	30-6	20	20	19

Notes
Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1. Aminopenicillin breakpoints in *Enterobacterales* are based on intravenous administration. For oral administration the breakpoints are relevant for urinary tract infections only. Breakpoints for other infections are under review.
2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
3. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
4. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
5. Agar dilution is the reference method for mecillinam MIC determination.

A. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars.
B. Susceptibility inferred from ampicillin.
C. Ignore isolated colonies within the inhibition zone.

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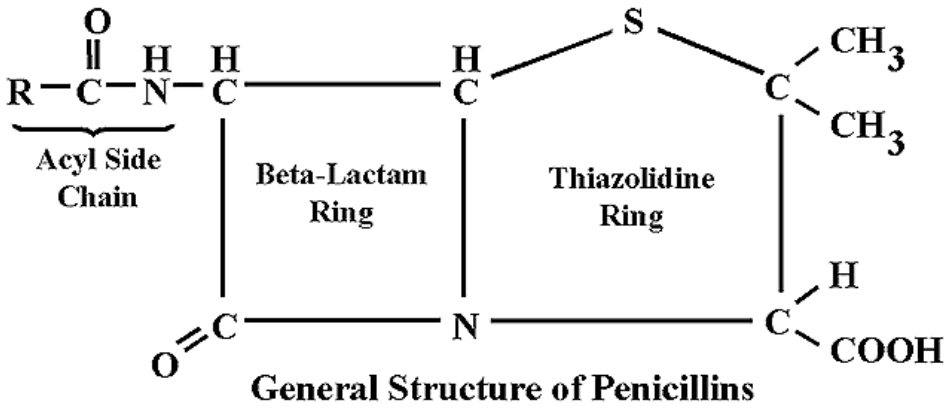
EUCAST Expert Rules v 3.2 — Salmonella — June 2019

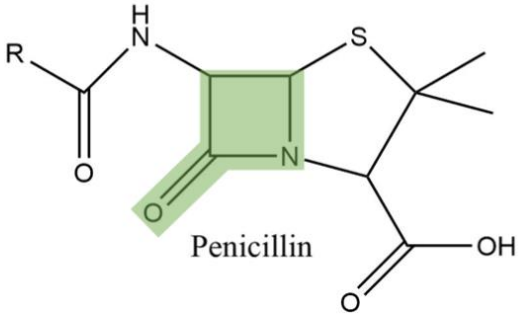
Rule No.	Organisms	Indicator Agent	Agents affected*	Rule	Remarks	Grade	References
1	*Salmonella* spp.	2nd generation cephalosporin	2nd generation cephalosporin	IF tested susceptible to a 2nd generation cephalosporin THEN report as resistant or not at all	There have been animal studies and limited clinical reports saying that the cure rate with 1st and 2nd generation cephalosporins is considerably lower than with alternative agents. However, other publications describe success with cefazolin or cefuroxime	B	Uwaydah, 1976 Bonina et al., 1990; Deshpande, Joshi, Lal, Cooverji, & Ajay, 1996; Takkar, Kumar, Khurana, & Takkar, 1994

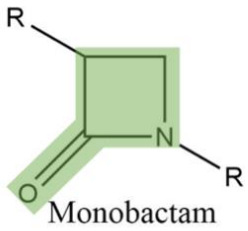
INTRODUCTION TO B-LACTAM RESISTANT SALM.

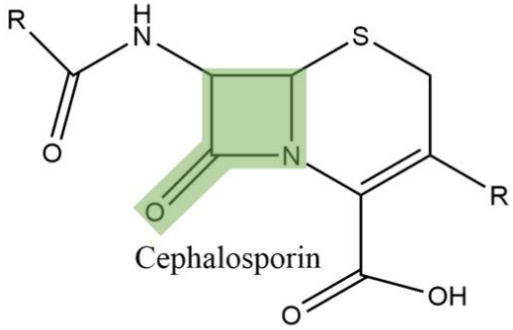
- B-lactams is a broad group of antibiotics

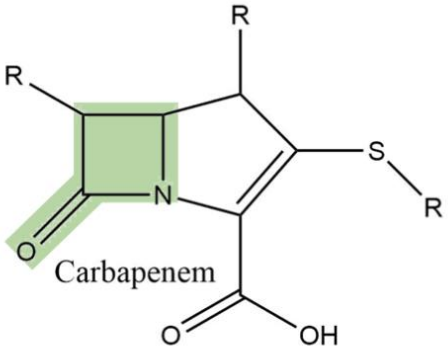
  - Penicillins
  - Cephalosporins
  - Carbapenems











Targeted by a broad group of B-lactamases!

EXTENDED SPECTRUM B-LACTAMASES

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- Some B-lactamases give resistance only to penicillin, ampicillin, piperacillin etc. and are not ESBL relevant

- ESBL
  - Resistance to 3rd/4th generation cephalosporins in Enterobacterales

EUCAST Clinical Breakpoint Tables v. 12.0, valid from 2022-01-01

	MIC breakpoints (mg/L)		Zone diameter breakpoints (mm)	
Cephalosporins	S ≤	R >	S ≥	R<
Cefotaxime	1	1	20	20
Ceftriaxone	1	2	25	22
Ceftazidime	1	4	22	19
Cefepime	1	4	27	24
Cefoxitin	8*	8*	19	19

*screening only

Examples of bla-genes:
SHV
TEM
CTX
OXA
…..

CARBAPENEMASE PRODUCING ENTEROBACTERALES

- Carbapenemase producing Enterobacterales
  - Examples of clinical breakpoints

EUCAST Clinical Breakpoint Tables v. 12.0, valid from 2022-01-01

	MIC breakpoints (mg/L)		Zone diameter breakpoints (mm)	
Carbapenems	S ≤	R >	S ≥	R<
Doripenem	1	2	24	21
Ertapenem	0.5	0.5	25	25
Imipenem	2	4	22	19
Meropenem	2	8	22	16

Examples of carba-genes:
VIM
NDM
KPC
OXA(-48)
IMP

Additional genes: IMI, GES, VEB, SMB, SME, VMB, GIM, SIM, SPM, JOHN…..

WHAT ABOUT AMPC?

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- AmpC is a special type of ESBL resistance

- Plasmid mediated by
  - CMY genes
- Chromosomally encoded
  - Upregulation of AmpC - mutations in promoter region

- Clinically they are often regarded as ESBL and treated like this
  - but there are differences in phenotypes

Examples of AmpC type genes:
CMY
DHA
FOX
ACT
Point mutations in promoter region

AMPC PHENOTYPES

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- Active on penicillins and cephalosporins
- Hydrolyses cephamycins (like cefoxitin)
- Low hydrolysis rate for cefepime and carbapenems (generally)
  - both CMY-2 genes and upregulations **can give an elevated ertapenem MIC** (but not meropenem)
  - No synergy with ESBL inhibitors like clavulanic acid
  - **susceptibility to cefepime**
    - Low MIC in AmpC/High in ESBL
  - **resistance to cefoxitin (current indicator**)
    - High MIC in AmpC/Low MIC in ESBL)

HOW DO WE TEST FOR ESBL (DD)

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- Both ESBL and AmpC organisms are resistant to cephalosporins
  - for example either Cefotaxime/Ceftazidime: FOT/TAZ
- For true ESBL we see a synergy when adding the beta-lactamase inhibitor clavulanic acid for AmpC, we don't
- The synergy means that by adding the clavulanic acid, a beta-lactamase inhibitor, we see an inhibitory effect of the antimicrobial combination

- The synergy can be determined by the lab results

- For **disk diffusion**: Synergy is present if we have a ≥ 5 mm increase in the zone diameter for FOT/TAZ alone compared to for FOT/TAZ + clavulanic acid. It is enough if this is the case for either FOT or TAZ

- For confirmation of AmpC, the interpretation of a cefoxitin-R (FOX <19 mm) is indication of AmpC

HOW DO WE TEST FOR ESBL (E-TEST)

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- For **E-test ESBL (gradient strip)** we test both cefotaxime (FOT; CT/CTL) and ceftazidime (TAZ; TZ/TZL) in combination with clavulanic acid

- Determine the MIC for all 4 compounds/combinations and calculate MIC ratio:
- ESBL positive:
- CT ≥ 0.5 and CT/CTL ratio ≥ 8 OR
- TZ ≥ 1 and TZ/TZL ≥ 8 OR
- Phantom zone

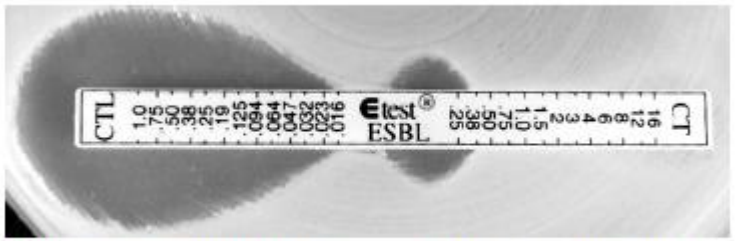

Figure 5. A "rounded" phantom inhibition zone below CT indicative of ESBL.

HOW DO WE TEST FOR ESBL (MIC)

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- **For MIC:** Synergy is present if you have **≥ 3 two-fold** concentration decrease in the MIC for FOT/TAZ + clavulanic acid compared to for FOT/TAZ alone. It is enough if this is the case for either FOT or TAZ

- For confirmation of AmpC, the interpretation of a cefoxitin FOX MIC > 8 is indication of AmpC

- EFSA criteria:
- **ESBL+ AmpC** phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);

Criteria for interpretation of *E. coli* and *Salmonella*, panel 2 results

SUSCEPTIBLE
CTX - CAZ – FOX- MEM ≤ ECOFF

1. ESBL-Phenotype
- CTX or CAZ > 1 mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX ≤ 8 mg/L AND
- SYN CTX/CLA and/or CAZ/CLA

2. AmpC-Phenotype
- CTX or CAZ > 1 mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- NO SYN CTX/CLA nor CAZ/CLA
- (Not excluded presence of ESBLs)

3. ESBL + AmpC-Phenotype
- CTX or CAZ > 1 mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- SYN CTX/CLA and/or CAZ/CLA

4. Carbapenemase-Phenotype
- MEM > 0.12 mg/L
- Needs confirmation
- (Not excluded presence of ESBLs or AmpC)

5. Other Phenotypes

1) IF CTX or CAZ > 1 mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX ≤ 8 mg/L AND
- NO SYN CTX/CLA nor CAZ/CLA
- Not excluded CPs (consult EURL)

3) IF CTX and CAZ ≤ 1 mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L
*cAmpCs could be included here

2) IF CTX and/or CAZ ≤ 1 mg/L AND > ECOFF AND
- MEM ≤ 0.12 mg/L AND
- FOX ≤ 8 mg/L AND

4) IF MEM ≤ 0.12 mg/L BUT
- ETP > ECOFF AND/OR
- IMI > ECOFF
- Not excluded CPs, needs confirmation (consult EURL)

5) Any other combinations not described in previous boxes (consult EURL)

Presumptive ESBL-producers include isolates exhibiting Phenotype 1 or 3.
Presumptive AmpC producers include isolates exhibiting Phenotype 2 or 3.

Please refer to: EFSA (European Food Safety Authority) and ECDC 2021. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal 2021;19(4):6490. https://doi.org/10.2903/j.efsa.2021.6490 (Annex A).

HOW DO WE TEST FOR CARBAPENEMASES

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- Resistance to carbapenems (meropenem)
  - Keep in mind that AmpC type ESBL can be ertapenem non-susceptible
- Confirmed by PCR of typical carba-genes
  - Microarray for ESBL and carba-genes
- CLSI – confirmation of carbapenemase activity
  - CarbaNP test
  - CIM test
    - (mCIM/eCIM)
    - differentiation of enzyme type

Figure 2B. mCIM Test Interpretation

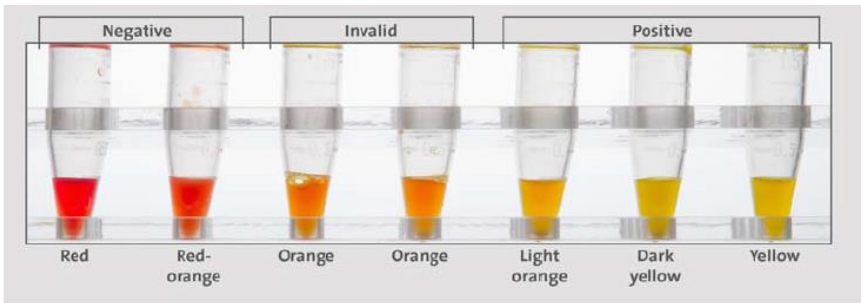

Figure 1. Interpretation of Color Reactions

EMERGENCE OF RESISTANT CLONES IN EUROPE

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JOINT ECDC-EFSA RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of monophasic *Salmonella* Typhimurium sequence type (ST) 34 linked to chocolate products

12 April 2022

International Spread of an Epidemic Population of *Salmonella enterica* Serotype Kentucky ST198 Resistant to Ciprofloxacin

Simon Le Hello,[1] Rene S. Hendriksen,[2] Benoît Doublet,[3] Ian Fisher,[4] Eva Møller Nielsen,[5] Jean M. Whichard,[6] Brahim Bouchrif,[7] Kayode Fashae,[8] Sophie A. Granier,[9] Nathalie Jourdan-Da Silva,[10] Axel Cloeckaert,[3] E. John Threlfall,[4] Frederick J. Angulo,[6] Frank M. Aarestrup,[2] John Wain,[4] and François-Xavier Weill[1]

Carbapenemase-producing *Salmonella enterica* isolates in the UK FREE

Martin R. Day, Danièle Meunier, Michel Doumith, Elizabeth de Pinna, Neil Woodford, Katie L. Hopkins ✉

Journal of Antimicrobial Chemotherapy, Volume 70, Issue 7, July 2015, Pages 2165–2167, https://doi.org/10.1093/jac/dkv075

Published: 19 March 2015

Table 1. Carbapenemase-encoding genes and plasmid location in non-typhoidal serovars of *Salmonella enterica* from humans, animals, and food.

Gene	Serovar	Plasmid	Inc [a]	Origin	Country	Reference
Humans						
bla_{KPC-2}	Cubana	+	ukn	Feces	USA	[20]
	Typhimurium	nt	na	Blood	Colombia	[21]
bla_{IMP-4}	Waycross	+	ukn	Urine/Feces	Australia	[22]
bla_{NDM-1}	Senftenberg	+	L/M	Perirectal swab	United States [Tv (India)]	[23,24]
	Westhampton [b]	nt	na	Perirectal swab	Reunion Island [Tv (India)]	[25]
	Stanley	+	A/C	Feces	China	[26,27]
	Senftenberg	+	X3	Feces	United Kingdom	[28]
	Senftenberg	+	A/C	Feces	India	[29]
	Agona	nt	na	Feces	Pakistan	[30]
bla_{NDM-5}	1,4,[5],12:i:-	+	FII	Feces	China	[31]
bla_{VIM-2}	Kentucky	+	W(UT)	Urine/blood	Morocco	[32]
bla_{OXA-48}	Saintpaul	+	L/M	Blood/Feces	France [Tv (Egypt)]	[32]
	Kentucky	+	ukn	Feces	France [Tv (Egypt)]	[32]
	Kentucky	+	L/M	Perianal swab	Switzerland [Tv (Libya)]	[33]
	Paratyphi B	+	L/M	Feces	United Kingdom [Tv (Africa)]	[28]
	Typhimurium	+	L/M	Feces	United Kingdom	[28]
Animals and Food						
bla_{VIM-1}	Infantis	+	HI2	Swine and poultry farms	Germany	[34,35]
	Infantis	+	HI2	Sick piglet	Germany	[36]
	Infantis	+	HI2	Minced pork meat	Germany	[36]
bla_{NDM-1}	Indiana	+	HI2	Chicken carcass	China	[37]
	Corvallis	+	A/C	Wild bird	Germany	[38]
bla_{IMP-4}	Typhimurium	+	HI2	Cats	Australia	[39]

[a] Inc, incompatibility group; + present; ukn, unknown; nt, not tested; na, not applicable; UT, untypeable; [b] NDM-1 production was assumed based on the Indian provenance of the patient, but not demonstrated; [Tv] known travel history.

Review

Resistance to Carbapenems in Non-Typhoidal *Salmonella enterica* Serovars from Humans, Animals and Food

Javier Fernández [1,2], Beatriz Guerra [3] and M. Rosario Rodicio [2,4,*]

doi:10.3390/vetsci5020040

- Thank you for the attention!
- Questions/Comments?