Antimicrobial susceptibility testing of *Klebsiella pneumoniae*

Alessandra Cornacchia, WOAH CC FH IZS-Teramo, Italy

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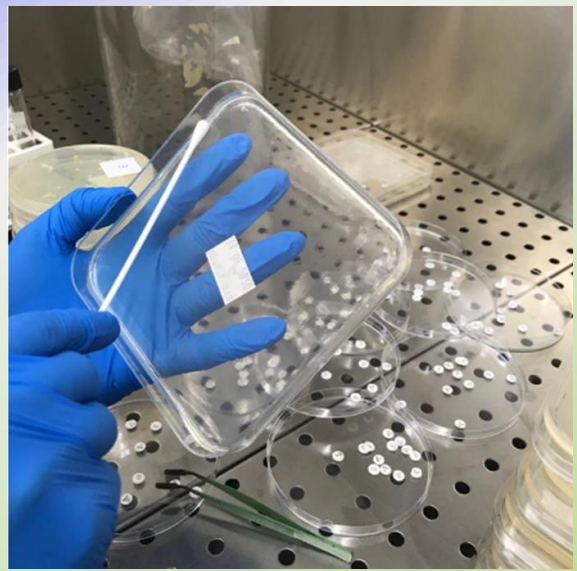
*"*Detection and characterization of *Listeria monocytogenes*, *Klebsiella pneumoniae* and *Salmonella* spp.*"*

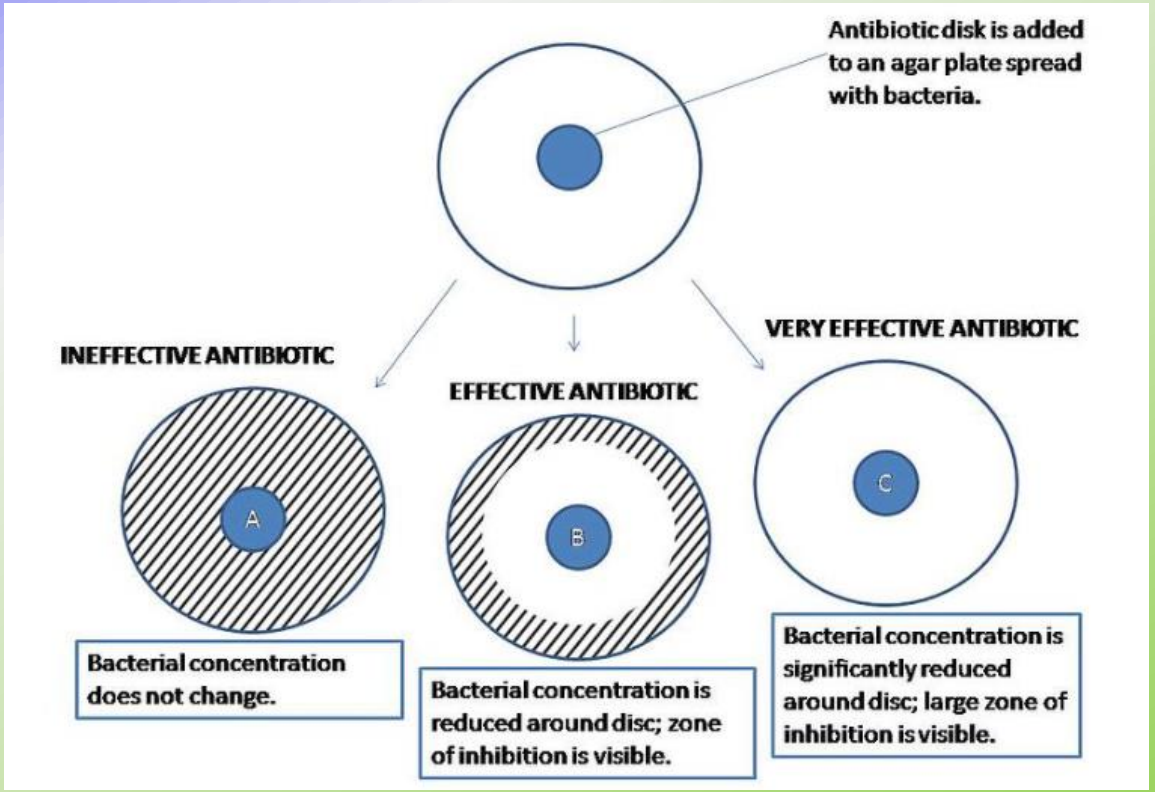
Phenotipic characterization of AMR
Disk Diffusion Method

- Bacterial inoculum equivalent to 0.5 Mc Farland standard,
- Streak the suspension on Mueller-Hinton agar (MHA) using a cotton swab;
- Apply disks on agar;
- Incubation at 37 °C for 16-18 h.

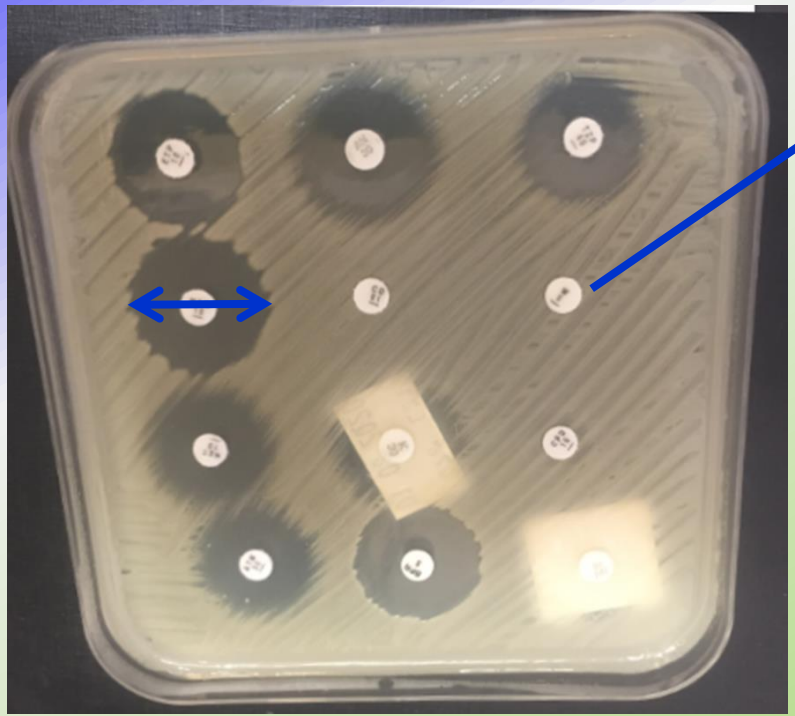


Phenotipic characterization of AMR
Disk Diffusion Method

After incubation measure each zone diameter of complete inhibition (including the diameter of the disk) with a ruler



Phenotipic characterization of AMR
Disk Diffusion Method



Ineffective antibiotic
The strain is Resistant

Interpret zone diameters into susceptibility categories according to EUCAST breakpoint tables to define S, I or R strains

S= Susceptible
I= Intermediate
R= Resistant

Phenotipic characterization of AMR
Disk Diffusion Method

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 12.0, valid from 2022-01-01

This document should be cited as "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org."

Phenotipic characterization of AMR
Disk Diffusion Method

Enterobacterales *

Expert Rules and Intrinsic Resistance Tables

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

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 (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		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[6]	Note[8]	
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
Ticarcillin	8	16		75	23	20	
Ticarcillin-clavulanic acid	8[3]	16[3]		75-10	23	20	
Temocillin (infections originating from the urinary tract), *E. coli*, *Klebsiella* spp. (except *K. aerogenes*) and *P. mirabilis*	0.001	16		30	50[C]	17[C]	
Phenoxymethylpenicillin	-	-			-	-	
Oxacillin	-	-			-	-	
Cloxacillin	-	-			-	-	
Dicloxacillin	-	-			-	-	
Flucloxacillin	-	-			-	-	

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.

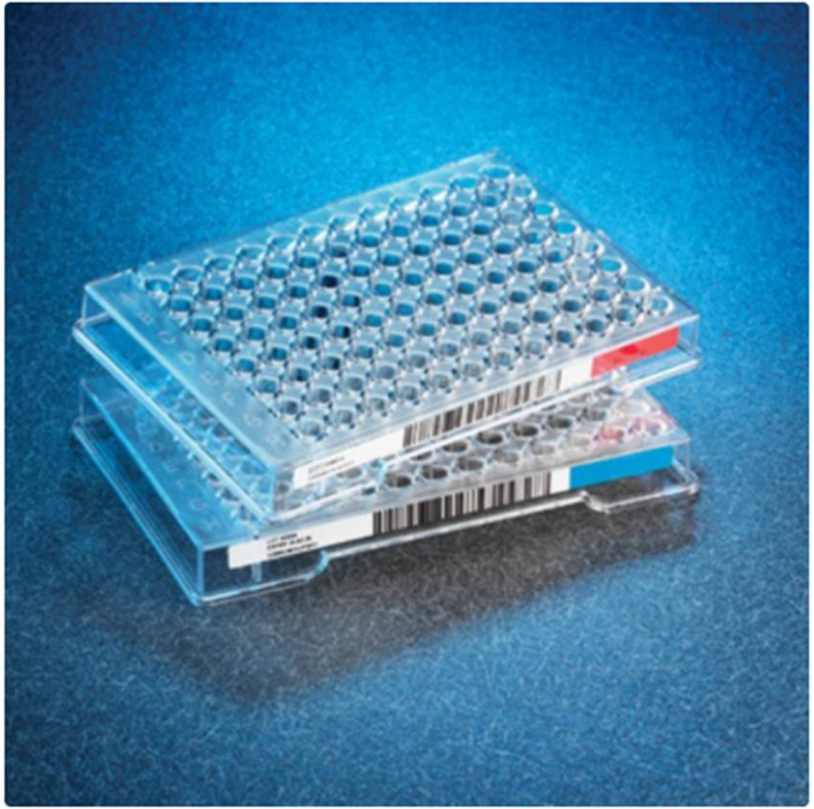
A. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars.
B. Susceptibility inferred from ampicillin.
C. Ignore isolated colonies within the inhibition zone.

AST Broth Microdilution

- 3-5 colonies picked and suspended in 5 ml of **demineralized water** (Sensititre) to reach a final turbidity equal to 0.5 McFarland Standard
- 10 µl of inoculum was added to a tube containing **Cation Adjusted Mueller–Hinton Broth CAMHB** (Sensititre)
- 50 µl of CAMHB was dispensed to a U-bottom 96-well microtiter **GN3F plate** containing the dried serially diluted antimicrobials.

AST Broth Microdilution

U-bottom 96-well microtiter **GN3F plate**



Sensititre™ Gram Negative MIC Plate

Incubation of plates at 37°C for 20-24 hours

AST Broth Microdilution

SENSITITRE™ GRAM NEGATIVE PLATE FORMAT

Plate Code: **GN3F**

	1	2	3	4	5	6	7	8	9	10	11	12
A	AMI	A/S2	FAZ	CEP	ETP	GEN	P/T4	SXT	TAZ	TGC	AXO	TET
	64	32/16	32	16	16	16	128/4	4/76	32	8	64	16
B	AMI	A/S2	FAZ	CEP	ETP	GEN	P/T4	SXT	TAZ	TGC	AXO	TET
	32	16/8	16	8	8	8	64/4	2/38	16	4	32	8
C	AMI	A/S2	FAZ	CEP	ETP	GEN	P/T4	SXT	TAZ	TGC	AXO	TET
	16	8/4	8	4	4	4	32/4	1/19	8	2	16	4
D	AMI	A/S2	FAZ	CEP	ETP	GEN	P/T4	SXT	TAZ	TGC	AXO	TET
	8	4/2	4	2	2	2	16/4	0.5/9.5	4	1	8	2
E	AMP	AZT	FEP	MERO	FUR	CIP	FOX	POD	TAZ	TIM2	AXO	TET
	32	32	32	8	32	4	32	16	2	64/2	4	1
F	AMP	AZT	FEP	MERO	FUR	CIP	FOX	POD	TAZ	TIM2	AXO	TET
	16	16	16	4	16	2	16	8	1	32/2	2	0.5
G	AMP	AZT	FEP	MERO	FUR	CIP	FOX	POD	TOB	TIM2	AXO	NEG
	8	8	8	2	8	1	8	4	8	16/2	1	CON
H	AMP	AZT	FEP	MERO	FUR	CIP	FOX	POD	TOB	POS	POS	POS
	4	4	4	1	4	0.5	4	2	4	CON	CON	CON

ANTIMICROBICS

AMI	Amikacin
AMP	Ampicillin
A/S2	Ampicillin/sulbactam 2:1 ratio
AZT	Aztreonam
FAZ	Cefazolin
FEP	Cefepime
CEP	Cephalothin
MERO	Meropenem
ETP	Ertapenem
FUR	Cefuroxime
GEN	Gentamicin
CIP	Ciprofloxacin
P/T4	Piperacillin / tazobactam constant 4
FOX	Cefoxitin
SXT	Trimethoprim / sulfamethoxazole
POD	Cefpodoxime
TAZ	Ceftazidime
TOB	Tobramycin
TGC	Tigecycline
TIM2	Ticarcillin / clavulanic acid constant 2
AXO	Ceftriaxone
TET	Tetracycline
NEG	Negative Control
POS	Positive Control

AST Broth Microdilution

The minimal inhibitory concentration (MIC) values were read using the Sensititre OptiRead Automated Fluorometric Plate Reading System

AST Broth Microdilution

Rapporto multi-isolato

Campione	:	15700/1
Specimen Date/Time	:	30/08/2019 08:50:41
Materiale	:	QUALSIASI FONTE-LUOGO
Isolato	:	1
Organismo	:	Klebsiella pneumoniae

Antimicrobico			
Amoxicillin/Clavulanic Acid		2	S
Ampicillin		32	R
Azithromycin		8	NI
Cefoxitin		8	S
Ceftiofur		1	NI
Ceftriaxone	<=	0.25	S
Chloramphenicol		8	S
Ciprofloxacin		0.03	S
Gentamicin		0.5	S
Nalidixic Acid		2	NI
Streptomycin		4	NI
Sulfisoxazole		32	NI
Tetracycline	<=	4	S
Trimethoprim/Sulfamethoxazole	<=	0.12	S

We could obtain an accurate minimum inhibitory concentration (MIC) based on the actual growth of the organism.
When comparing MIC results against the latest clinical breakpoints from EUCAST and CLSI, we can define the susceptibility profile of strains and to track emerging resistance.

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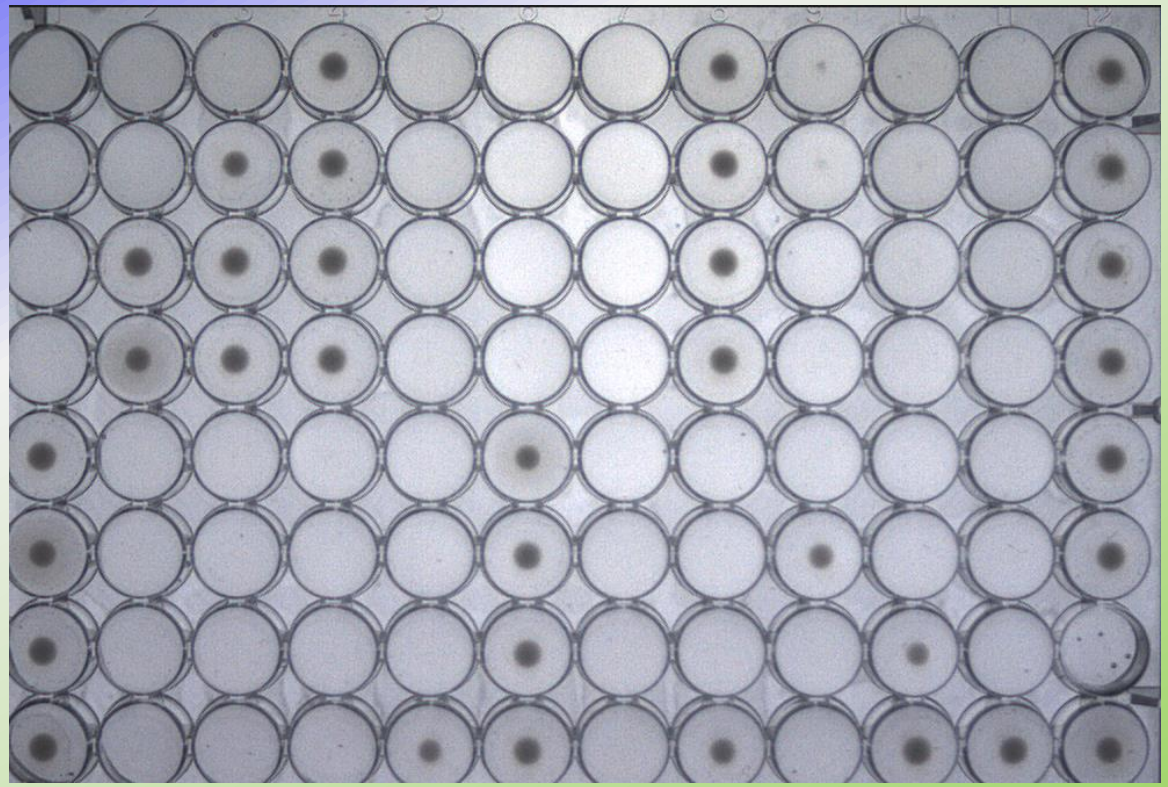
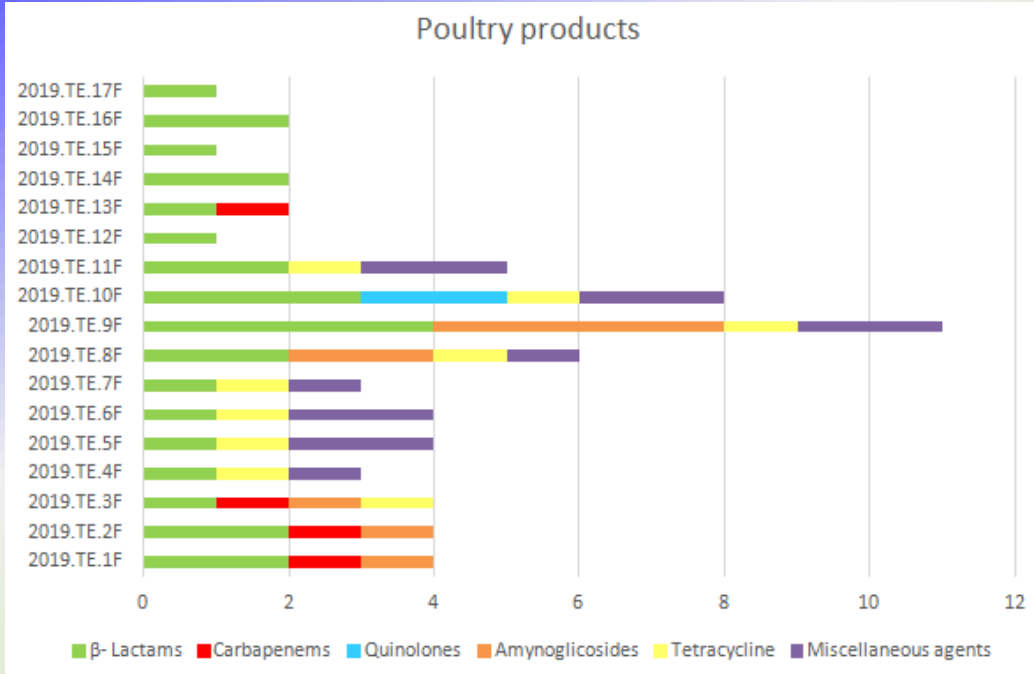
AST Broth Microdilution



IZS TERAMO
WOAH Collaborating Centre for animal production food safety
Reference Centre
World Organisation for Animal Health
Founded as OIE

Kp Phenotypic resistance: some results

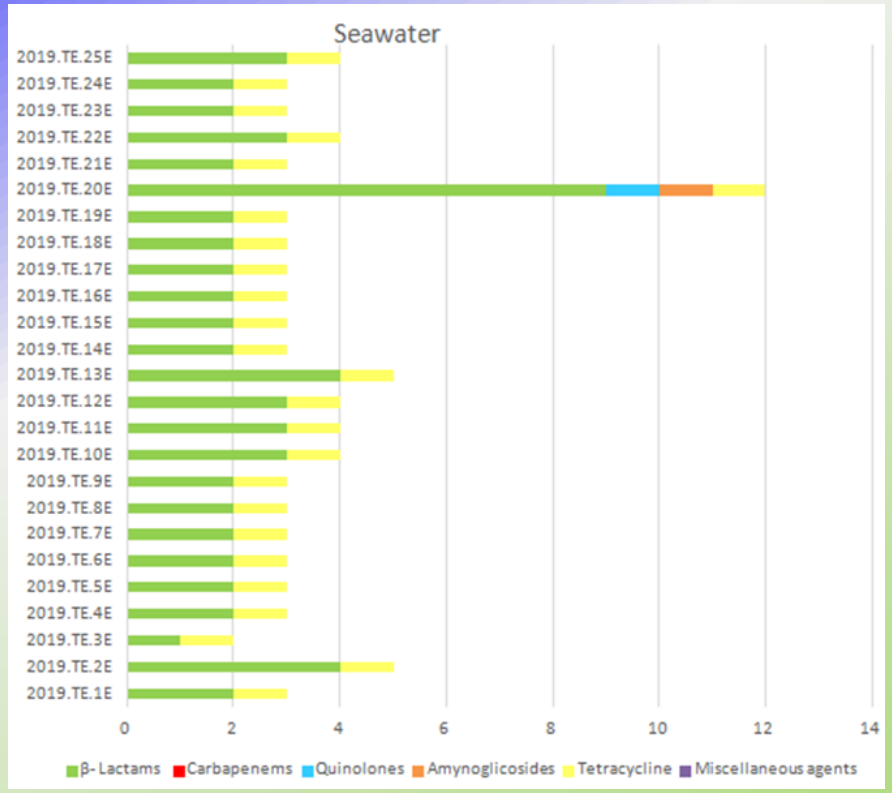


- 9 strains out of 17 (52.9 %) are MDR showing resistant to 3 or more classes of antimicrobials.

- 10 strains out of 17 resistant to β-lactams (58.8%) e 5 strains out of 17 (29.4%) resistant to aminoglycosides.

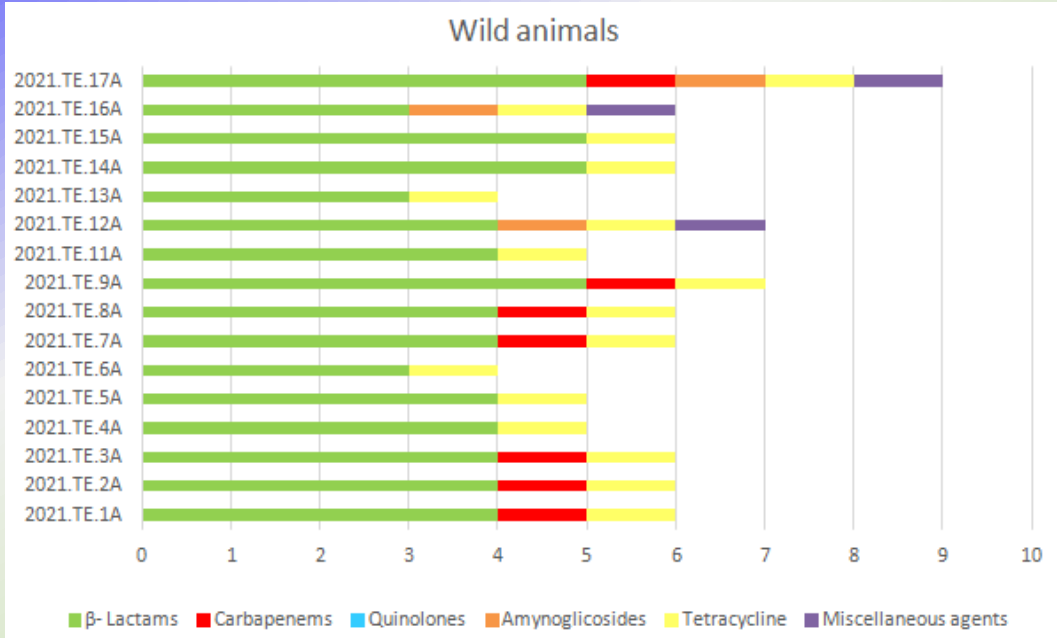
- 9 strains resistant to TET
- 5 strains resistant to TMP
- 4 strains resistant to MEM

Kp Phenotypic resistance: some results



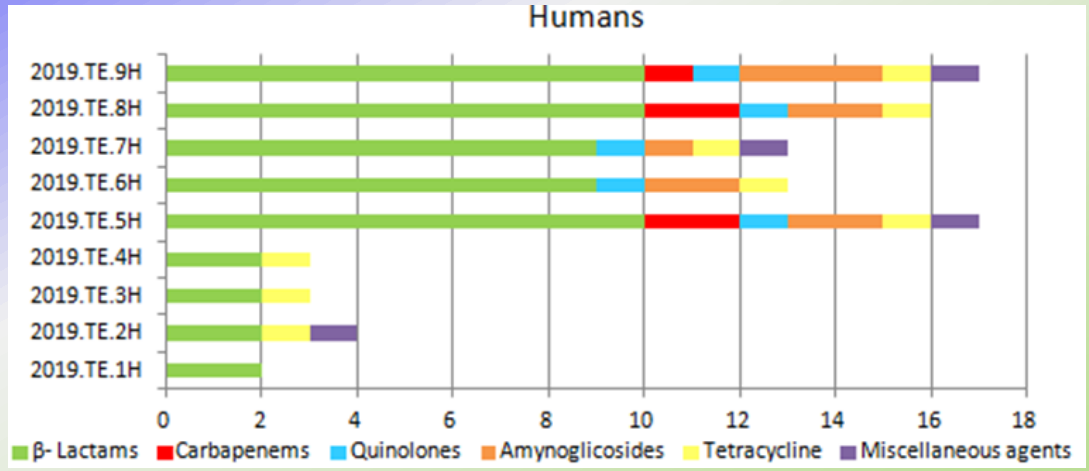
- Just one MDR strain
- All resistant to TET and β-Lactams

Kp Phenotypic resistance: some results



- Nine MDR strain
- All resistant to TET and β-Lactams
- Seven strains resistant to carbapenem ETP or MERO.

Kp Phenotypic resistance: some results



Most of the strains were resistant to all classes of antimicrobials

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THANK YOU FOR YOUR ATTENTION!

Alessandra Cornacchia
a.cornacchia@izs.it