

# A survey of carbapenem- and/or colistin-resistant Enterobacteriaceae (CCRE survey)

Kliinilise mikrobioloogia sektsiooni seminar

Marika Jürna-Ellam

01.11.2019

# A survey of carbapenem- and/or colistin-resistant Enterobacteriaceae (CCRE survey)

- is being conducted within the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) in 2019.
- 10 carbapenem-non-susceptible and the following 10 carbapenem-susceptible isolates of the same species are collected by each of the participating local hospital laboratories.
- and forwarded to the national reference/expert laboratory (NRL) for confirmatory antimicrobial susceptibility testing (AST).
- forwarded to the central laboratory for whole genome sequencing (WGS).

# The primary public health objective of the CCRE survey is:

- to determine the occurrence,
- geographic distribution and,
- the population dynamics

of high-risk CCRE clones and/or transmissible resistance/genetic elements of critical public health importance in Europe.

## EURGen-Net EQA

- Thirty-eight NRLs representing 37 participating countries took part in the EQA.
- A panel of six strains of *Escherichia coli* and/or *Klebsiella pneumoniae* with varying susceptibilities to carbapenems and colistin were distributed to the NRLs.

## **Mandatory testing**

- Species identification
- Colistin MIC values determined by BMD and SIR category
- Meropenem MIC values determined by BMD and SIR category
- Meropenem disk diffusion, zone diameter
- Imipenem MIC values or disk diffusion zone diameter and SIR category
- Ertapenem MIC values or disk diffusion zone diameter and SIR category
- SIR category for following antibiotics:
  - Aminoglycosides: amikacin, tobramycin, gentamicin
  - Beta-lactams/penicillins: ampicillin
  - Beta-lactams/monobactams: aztreonam
  - Beta-lactams/cephalosporins: cefotaxime, cefepime, ceftazidime
  - Beta-lactam - beta-lactamase inhibitor combinations: amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime-avibactam
  - Fluoroquinolones: ciprofloxacin
  - Glycylcyclines: tigecycline
  - Others: trimethoprim-sulfamethoxazole, fosfomycin
- Identification of carbapenem-resistance mechanisms (OXA-48-like, NDM, KPC, and VIM)
- Identification of colistin-resistance mechanisms (*mcr*-genes)

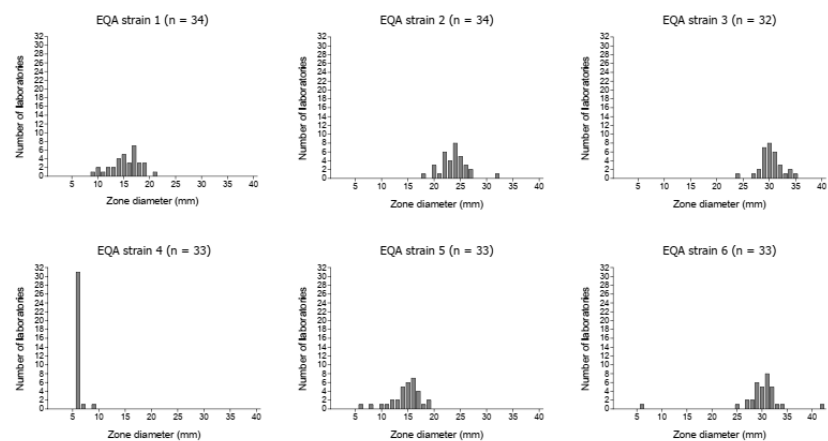
### 3.3 Carbapenems

#### 3.3.1 Meropenem

Table 3 Meropenem MIC

EQA strain	Expected MIC range (mg/L)	NRL MIC value (mg/L)	EA	Expected zone value (mm, range)	NRL zone value (mm)	Expected category	NRL category	CA	mE	ME	VME	NRL points	Category reported by participating laboratories (%)			
													S	I	R	n
1	4 -16	8	Yes	15 (13 - 17)	17	I	I	Yes				1	0	58	42	38
2	0.25 - 1	0.25	Yes	23 (22 - 25)	24	S	S	Yes				1	92	8	0	38
3	0.015 - 0.06	≤ 0.25	NA	30 (28 - 31)	31	S	S	Yes				1	100	0	0	38
4	> 16	64	NA	6	6	R	R	Yes				1	0	0	100	38
5	4 - 16	8	Yes	15 (13 - 17)	17	I	I	Yes				1	0	47	53	38
6	0.015 - 0.06	≤ 0.125	NA	30 (28 - 31)	33	S	S	Yes				1	97	0	3	38

Figure 1 Meropenem zone diameter distribution



### 3.3.2 Imipenem

Table 4 Imipenem MIC

EQA strain	Expected MIC range (mg/L)	NRL MIC value (mg/L)	EA	NRL zone value (mm)	Expected category	NRL category	CA	mE	ME	VME	NRL points	Category reported by participating laboratories (%)			
												S	I	R	n
1	2 - 8	8	Yes	20	I	<b>R</b>	No	Yes			0	24	53	24	38
2	1 - 4	2	Yes	23	S	S	Yes				1	87	13	0	38
3	0.06 - 0.24	≤ 1	NA	29	S	S	Yes				1	100	0	0	38
4	> 16	≥ 8	NA	6	R	R	Yes				1	0	0	100	38
5	2 - 8	4	Yes	21	I	I	Yes				1	26	47	26	38
6	0.06 - 0.24	≤ 1	NA	30	S	S	Yes				1	97	0	3	38

### 3.3.3 Ertapenem

Table 5 Ertapenem MIC

EQA strain	Expected MIC range (mg/L)	NRL MIC value (mg/L)	EA	NRL zone value (mm)	Expected category	NRL category	CA	mE	ME	VME	NRL points	Category reported by participating laboratories (%)			
												S	I	R	n
1	> 4	32	NA	6	R	R	Yes				1	0	0	100	37
2	0.25 - 2	0.38	Yes	21	S or R	S	Yes				1	46	0	54	37
3	0.06 - 0.24	0.125	Yes	30	S	S	Yes				1	95	0	5	37
4	> 4	≥ 32	NA	6	R	R	Yes				1	0	0	100	37
5	> 4	32	NA	8	R	R	Yes				1	0	0	100	36
6	0.06 - 0.24	0.094	Yes	30	S	S	Yes				1	92	0	8	37



### 3.4 Colistin

Table 6 Colistin MIC

EQA strain	Expected MIC range (mg/L)	NRL MIC value (mg/L)	EA	Expected category	NRL category	CA	mE	ME	VME	NRL points	Category reported by participating laboratories (%)			
											S	I	R	n
1	0.5 - 2	0.5	Yes	S	S	Yes				1	100	0	0	37
2	0.25 - 1	0.5	Yes	S	S	Yes				1	100	0	0	37
3	4 -16	8	Yes	R	R	Yes				1	0	0	100	36
4	16 - 64	32	Yes	R	R	Yes				1	0	0	100	37
5	0.5 - 2	0.5	Yes	S	S	Yes				1	83	3	14	36
6	4 - 16	4	Yes	R	R	Yes				1	0	0	100	36

### 3.5 Other antibiotics

Table 7 EQA strain 1 - other antibiotics SIR category

Antibiotic	Expected category	NRL category	Category reported by participating laboratories (%)			
			S	I	R	n
Amikacin	S	S	95	3	3	38
Gentamicin	S	S	100	0	0	38
Tobramycin	R	I	17	36	47	36
Ampicillin	R	R	3	0	97	37
Cefotaxime	R	R	0	0	100	37
Ceftazidime	R	R	0	0	100	38
Cefepime	R	R	0	0	100	34
Aztreonam	R	R	0	0	100	32
Piperacillin-tazobactam	R	R	0	0	100	37
Amoxicillin-clavulanic acid	R	R	0	0	100	38
Ceftazidime-avibactam	S	S	100	0	0	29
Ciprofloxacin	R	R	0	0	100	38
Fosfomycin	NT	R	10	0	90	29
Tigecycline	R	R	4	4	93	28
Trimethoprim-sulfamethoxazole	R	R	0	0	100	35

Table 11 EQA strain 5 - other antibiotics SIR category

Antibiotic	Expected category	NRL category	Category reported by participating laboratories (%)			
			S	I	R	n
Amikacin	S	S	89	3	8	37
Gentamicin	S	S	95	5	0	38
Tobramycin	R	I	11	36	53	36
Ampicillin	R	R	0	0	100	37
Cefotaxime	R	R	0	0	100	38
Ceftazidime	R	R	0	0	100	38
Cefepime	R	R	0	0	100	34
Aztreonam	R	R	3	0	97	33
Piperacillin-tazobactam	R	R	0	0	100	37
Amoxicillin-clavulanic acid	R	R	0	0	100	38
Ceftazidime-avibactam	S	S	97	0	3	29
Ciprofloxacin	R	R	0	0	100	38
Fosfomycin	NT	R	10	0	90	29
Tigecycline	R	R	7	7	86	28
Trimethoprim-sulfamethoxazole	R	R	8	0	92	37

Table 8 EQA strain 2 - other antibiotics SIR category

Antibiotic	Expected category	NRL category	Category reported by participating laboratories (%)			
			S	I	R	n
Amikacin	S	S	100	0	0	38
Gentamicin	S	S	100	0	0	38
Tobramycin	S	S	100	0	0	36
Ampicillin	R	R	0	0	100	37
Cefotaxime	S	S	100	0	0	37
Ceftazidime	S	S	100	0	0	38
Cefepime	S	S	97	0	3	35
Aztreonam	S	S	97	3	0	33
Piperacillin-tazobactam	R	R	0	0	100	37
Amoxicillin-clavulanic acid	R	R	0	0	100	38
Ceftazidime-avibactam	S	S	100	0	0	29
Ciprofloxacin	S	S	89	8	3	38
Fosfomicin	NT	S	90	0	10	30
Tigecycline	S	S	97	0	3	35
Trimethoprim-sulfamethoxazole	R	R	0	0	100	36

Table 9 EQA strain 3 - other antibiotics SIR category

Antibiotic	Expected category	NRL category	Category reported by participating laboratories (%)			
			S	I	R	n
Amikacin	S	S	66	21	13	38
Gentamicin	S	S	92	8	0	38
Tobramycin	R	R	0	0	100	35
Ampicillin	R	R	0	0	100	37
Cefotaxime	R	R	0	0	100	37
Ceftazidime	R	R	0	0	100	38
Cefepime	R	R	0	0	100	34
Aztreonam	R	R	0	0	100	33
Piperacillin-tazobactam	I	<b>S</b>	57	24	19	37
Amoxicillin-clavulanic acid	R	R	3	0	97	38
Ceftazidime-avibactam	S	S	100	0	0	28
Ciprofloxacin	R	R	3	0	97	38
Fosfomicin	NT	S	90	3	7	30
Tigecycline	S	S	97	0	3	34
Trimethoprim-sulfamethoxazole	R	R	6	0	94	36

Table 12 EQA strain 6 - other antibiotics SIR category

Antibiotic	Expected category	NRL category	Category reported by participating laboratories (%)			
			S	I	R	n
Amikacin	S	S	59	24	16	37
Gentamicin	S	S	97	3	0	38
Tobramycin	R	R	3	3	94	36
Ampicillin	R	R	0	0	100	37
Cefotaxime	R	R	0	0	100	38
Ceftazidime	R	R	0	0	100	38
Cefepime	R	R	0	0	100	34
Aztreonam	R	R	0	0	100	33
Piperacillin-tazobactam	I	<b>S</b>	51	35	14	37
Amoxicillin-clavulanic acid	R	R	0	0	100	38
Ceftazidime-avibactam	S	S	100	0	0	29
Ciprofloxacin	R	R	0	0	100	38
Fosfomicin	NT	S	87	0	13	30
Tigecycline	S	S	94	0	6	34
Trimethoprim-sulfamethoxazole	R	R	8	0	92	37

Table 10 EQA strain 4 - other antibiotics SIR category

Antibiotic	Expected category	NRL category	Category reported by participating laboratories (%)			
			S	I	R	n
Amikacin	R	R	5	5	89	37
Gentamicin	I	I	61	29	11	38
Tobramycin	R	R	6	0	94	36
Ampicillin	R	R	0	0	100	37
Cefotaxime	R	R	0	0	100	37
Ceftazidime	R	R	0	3	97	38
Cefepime	R	R	0	0	100	34
Aztreonam	R	R	0	0	100	33
Piperacillin-tazobactam	R	R	0	0	100	37
Amoxicillin-clavulanic acid	R	R	0	0	100	38
Ceftazidime-avibactam	S	S	100	0	0	29
Ciprofloxacin	R	R	0	0	100	38
Fosfomicin	NT	S	14	0	86	29
Tigecycline	S	S	93	3	3	29
Trimethoprim-sulfamethoxazole	S	S	97	0	3	36

### 3.6 Resistance mechanisms

Table 13 Carbapenem and colistin resistance mechanisms and carbapenemase-production

EQA strain	Expected resistance mechanism	NRL detected resistance mechanism	NRL detected carbapenemase-production
1			Yes
2	OXA-48	OXA-48-like	Yes
3	<i>mcr-1</i>	NT	No
4	KPC-2	KPC	Yes
5			Yes
6	<i>mcr-1</i>	NT	No

Table 1 EQA scoring system

Parameters	Points
Correct species	1
Correct categorical agreement (CA) <sup>a</sup>	1
No result reported	0
Minor error (mE) <sup>b</sup>	0
Major error (ME) <sup>c</sup>	-1
Very major error (VME) <sup>d</sup>	-2
<b>Maximum score</b>	<b>30</b>

<sup>a</sup> Categorical agreement: Correct assignment of SIR category for meropenem, imipenem, ertapenem, and colistin.

<sup>b</sup> Minor error: Categorical error from S to I or I to S or R to I or I to R

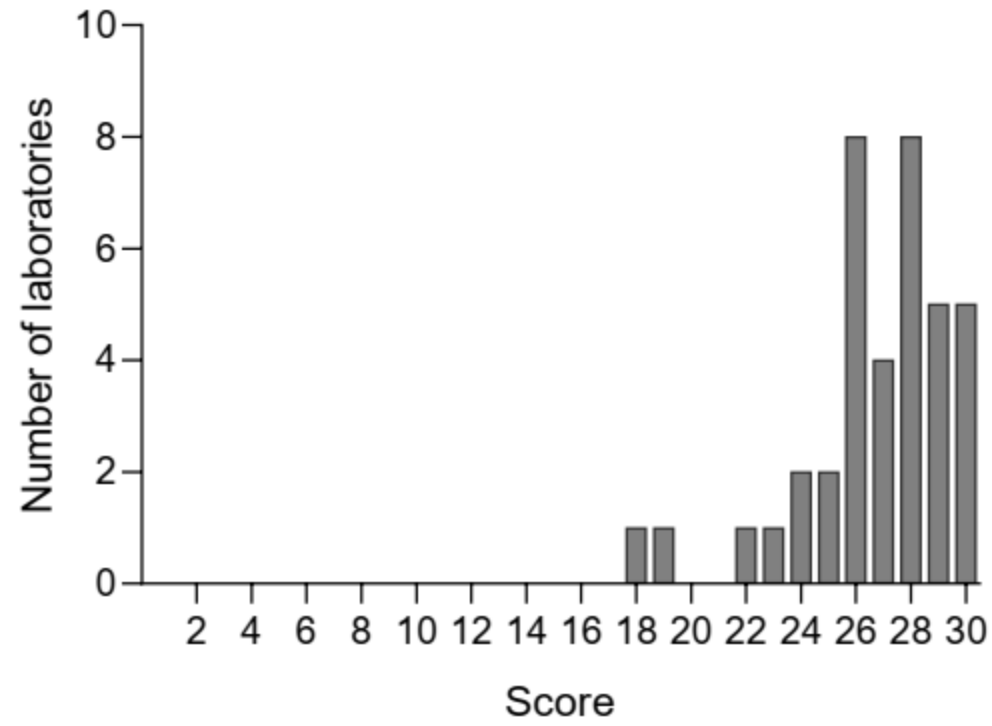
<sup>c</sup> Major error: Categorical error from S to R (false resistant)

<sup>d</sup> Very major error: Categorical error from R to S (false susceptible)

# Score

NRL score: 29

Figure 4 Score distribution





## EQA strain 1 and 5

EQA strains 1 and 5 were duplicates of the same isolate and prepared in one batch from culture to lyophilisation. The strain was an ESBL-producing *K. pneumoniae* that was meropenem susceptible increased exposure (I) and susceptible (S) to colistin. The reference MIC values for meropenem, imipenem, and ertapenem obtained at the EDL were 8, 4, and >4 respectively.

## EQA strain 2

EQA strain 2 was a meropenem non-wildtype, MIC above ECOFF (epidemiological cut-off) but still susceptible *E. coli* harbouring *bla*<sub>OXA-48</sub>. Reference MIC values for meropenem, imipenem, and ertapenem were 0.5, 2, and 0.5-1 (0.5 and 1 mg/L in three repeated tests each), respectively. The strain was colistin susceptible, reference MIC 0.5 mg/L.

## EQA strain 3 and 6

EQA strains 3 and 6 were duplicates of the same strain and prepared in one batch from culture to lyophilisation. The strain was an *E. coli* harbouring the *mcr-1* gene. The isolate was meropenem, ertapenem, and imipenem susceptible and colistin resistant. The results reported for EQA strains 3 and 6 showed high concordance.

## EQA strain 4

EQA strain 4 was a *K. pneumoniae* strain highly resistant to the carbapenems but susceptible to colistin. This strain harboured *bla*<sub>KPC-2</sub>. MIC values for meropenem, imipenem and ertapenem obtained at the EDL were >16, >16, and >4 respectively.

# Country specific comments

Species determination was performed correctly for all EQA strains.

For imipenem there was a categorical error for EQA strain 1 resulting in a minor error. The MIC value is within essential agreement. The expected MIC range spanned over two categories (S and I), which makes these results difficult to interpret. Upon extensive retesting at the EDL I would be the most likely result.

For EQA strains 1 and 5 minor errors were reported for tobramycin. For EQA strains 3 and 6 minor errors were found for piperacillin-tazobactam. Please see comment regarding piperacillin-tazobactam under general conclusions.

Detection of carbapenem resistance genes was performed using real-time PCR. The carbapenem resistance genes were not subtyped. Carbapenemase production was analysed using combination disc test and the MICRONAUT-S MDR MRGN Screening test.

No molecular method was available for detecting *mcr*-genes.

Plasmid mediated colistin resistance is of high concern and it would be advisable to set up such a method.

The susceptibility testing of piperacillin-tazobactam for this strain was difficult since the MIC (16 mg/L) falls within ATU (area of technical uncertainty). In the tests performed at the EDL the strain was categorised as S (MIC 8 mg/L) four times and I (MIC 16 mg/L) six times.



European Antimicrobial Resistance Genes Surveillance Network  
(EURGen-Net)



## CERTIFICATE OF PARTICIPATION

awarded to

**NORTH ESTONIA MEDICAL CENTRE, ESTONIA**

to attest completion of

### **EURGEN-NET EXTERNAL QUALITY ASSESSMENT SCHEME FOR THE IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF CARBAPENEM- AND COLISTIN-RESISTANT ENTEROBACTERIACEAE, 2019**

Covering the following:

- Species identification
- Carbapenem and colistin antimicrobial susceptibility testing
- Molecular detection of carbapenem and colistin resistance genes

Organised by:  
**European Centre for Disease Prevention and Control**  
Michael Catchpole, Chief Scientist,  
European Centre for Disease Prevention and Control

Performed under FWC: OJ/2017/OCS/7530 contract by:  
**EURGenCCRE consortium**  
Karin Tegmark-Wisell, Head of Microbiology department,  
Public Health Agency of Sweden