DTU Food
National Food Institute

EQAS 2014

Salmonella and E. coli

EURL-AR workshop, April 23-24th, 2015

Participation

Salmonella

32 countries

35 sets of *Salmonella* results

One set excluded due to lack of full-range dilution tests

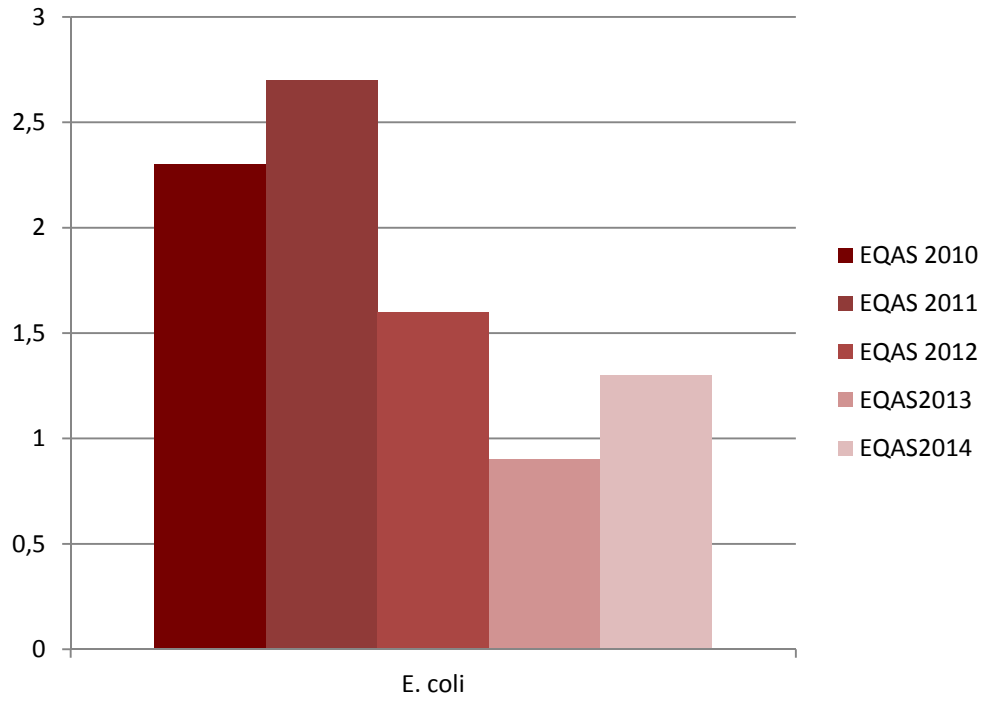
E. coli

31 countries

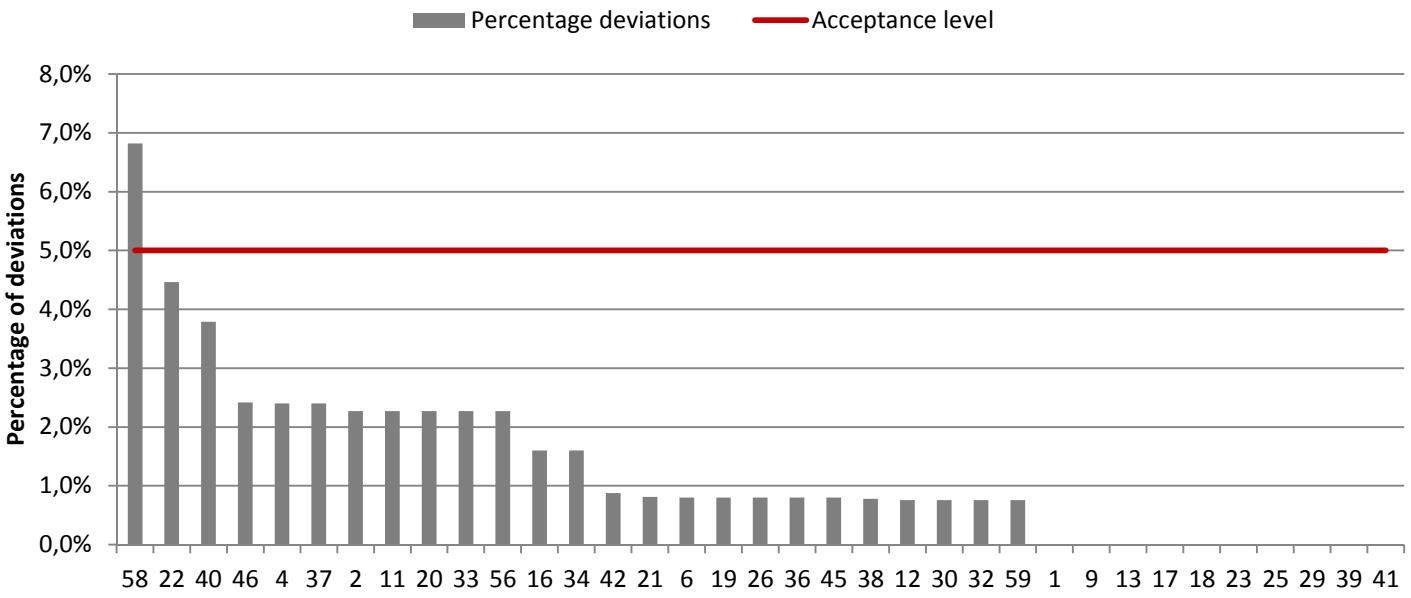
35 sets of *E. coli* results included

2 sets excluded due to DD data

Comparison to former EQASs – *E. coli*



E. coli results – pr. lab



One participant (lab #58) had 6.8 % deviations

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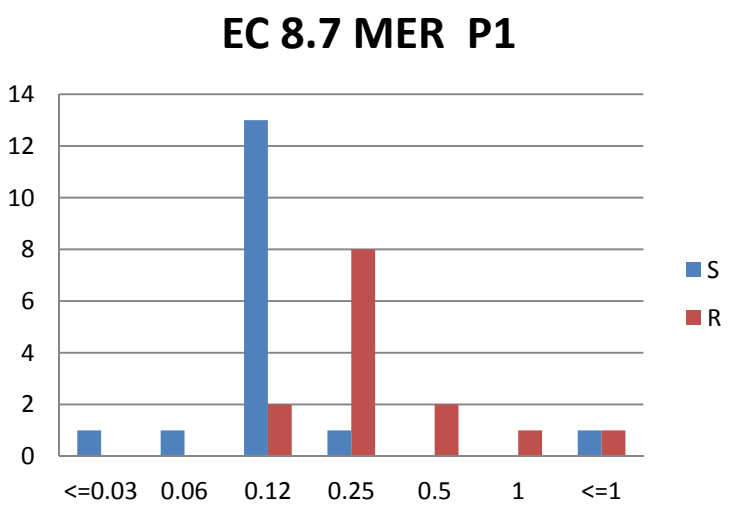
ESBL-producing test strains

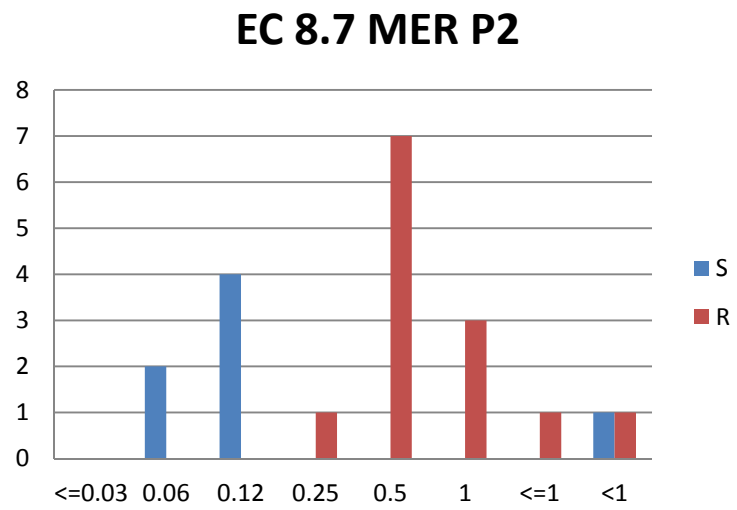
ESBL-detection is mandatory in the *E. coli* EQAS

Percentage reporting correct ESBL-results		EC 8.2	EC 8.3	EC 8.4	EC 8.7
ESBL-genes harboured in the test strain		KPC-2	CTX-M-1	CMY-2	OXA-48
ESBL-, pAmpC- and carbapenemase-producing strain – expected results		carbapenemase	ESBL	AmpC	carbapenemase
Obtained results	Presumptive ESBL-producer		34/36 (94.4%)	-	
	Presumptive pAmpC-producer	2/36 (5.6%)	1/36 (2.8%)	32/36 (88.9%) -	
	Presumptive ESBL+pAmpC	4/36 (11.1%)	1/36 (2.8%)	4/36 (11.1%)	-
	Presumptive carbapenemase-producer	21/36 (58.3%)	-	-	16/36 (44.4%)
	unusual phenotype	9/36 (25%)	-	-	3/36 (8.3%)
	Not ESBL-, pAmpC- or carbapenemase-producing	-	-	-	17/36 (47.2%)

Labs #54 did not provide results for all strains

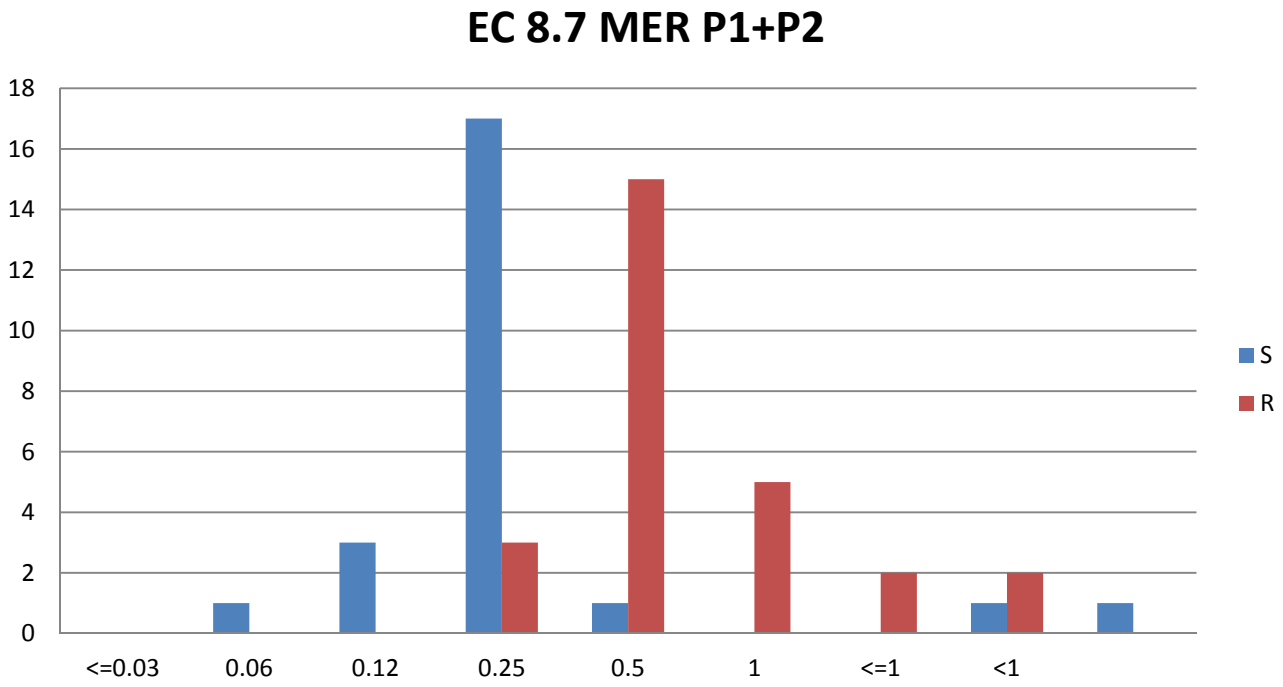
EC 8.7/meropenem
54.8% deviations in panel 1 and 35% in panel 2





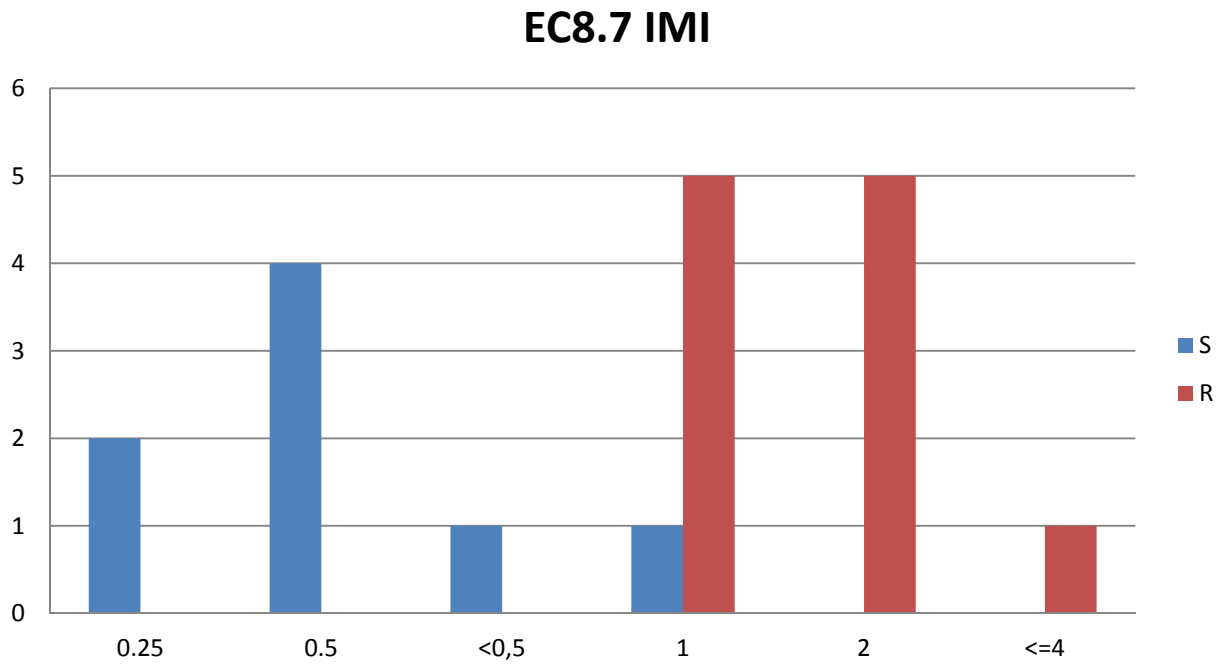
Participants had slightly better results in second panel; probably due to considering the results in relation to other carbapenems in the panel. Expected MIC value: 0, 25 mg/l ; R

EC 8.7 meropenem both panels total deviation 47%



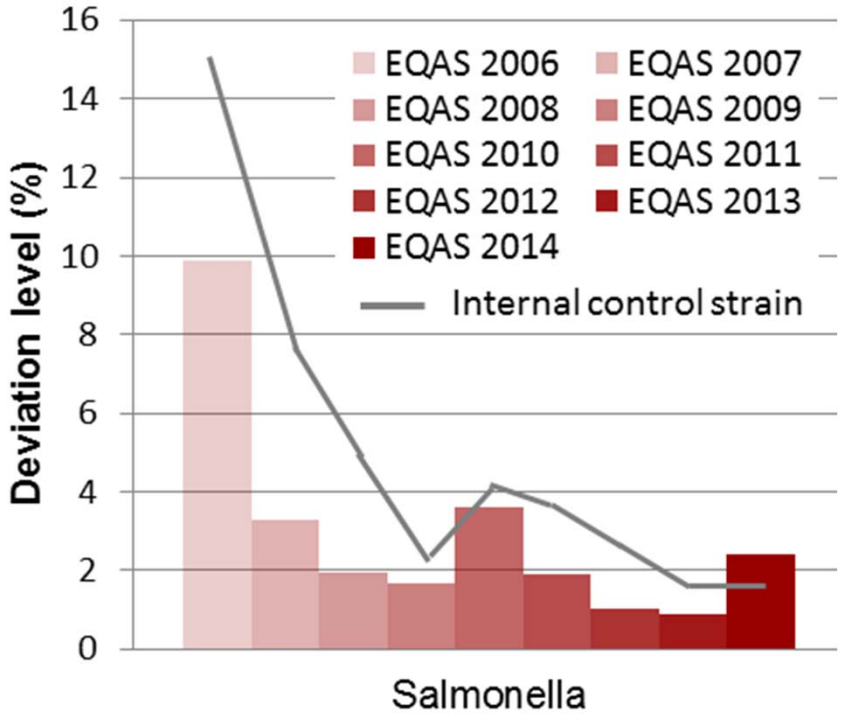
Because the issues with this strain/antimicrobial combination are related to the OXA-48 gene phenotype the data was kept in the report. Expected MIC value: 0, 25 mg/l ; R

EC 8.7/imipenem
total deviation 42.1%

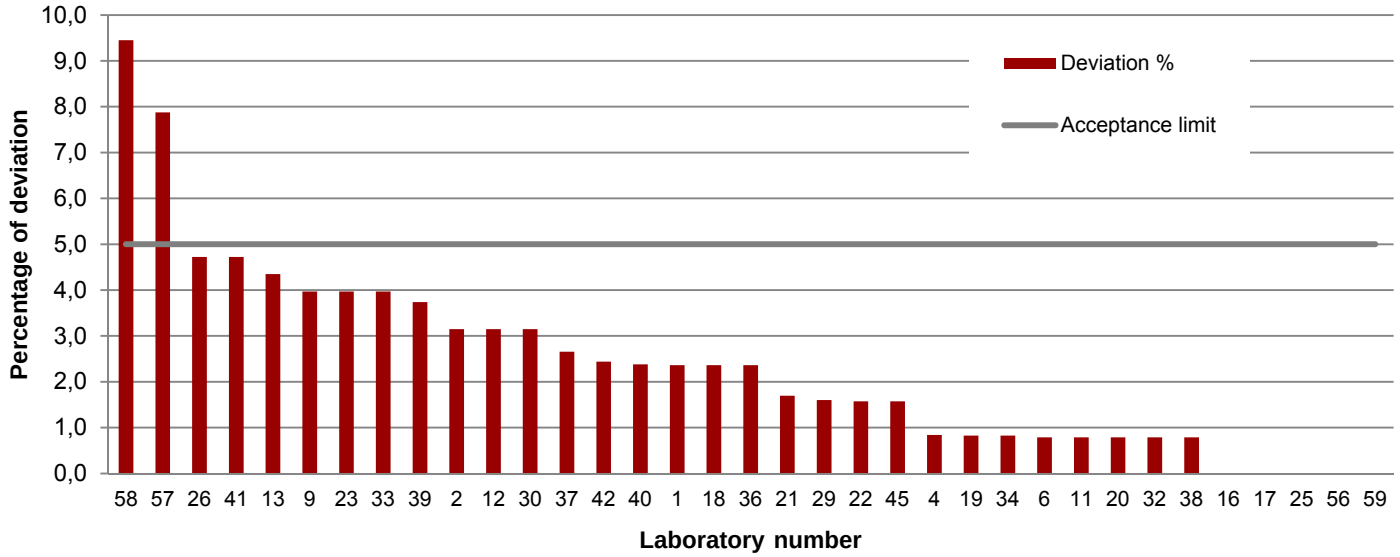


The issues with this strain/ antimicrobial combination are related to the expected OXA-48 gene phenotype, therefore the data was kept in the report. Expected MIC value: 1 mg/l ; R

Comparison to former EQASs - *Salmonella*



Salmonella results – pr. lab



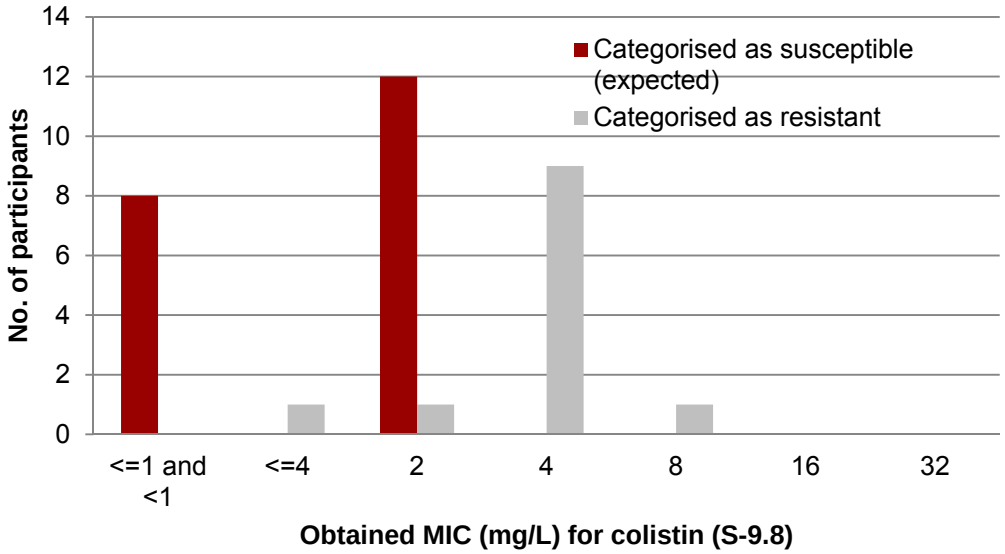
ESBL-producing test strains

ESBL-detection is mandatory in the *Salmonella* EQAS

		Strain S-9.3	Strain S-9.4	Strain S-9.5	Strain S-9.6
ESC-genes harboured in the test strain		***bla*$_{CMY-2}$**	***bla*$_{CTX-M-9}$** ***bla*$_{TEM-1}$**	***bla*$_{VIM-2}$** ***bla*$_{TEM-1}$**	***bla*$_{OXA-48}$**
ESBL-, pAmpC- and carbapenemase-producing strain – expected results		pAmpC	ESBL	carbapenemase	carbapenemase
Obtained results	Confirmed ESBL-producer	-	32/35 (91%)	-	-
	Confirmed ESBL + pAmpC-producer	*3/35 (9%)*	-	-	-
	Confirmed pAmpC-producer	32/35 (91%)	-	*4/35 (11%)*	-
	Confirmed carbapenemase-producer	-	-	28/35 (80%)	29/35 (83%)
	Confirmed unusual phenotype	-	*2/35 (6%)*	*3/35 (9%)*	*4/35 (11%)*
	Not ESBL-, pAmpC- or carbapenemase-producing	-	*1/35 (3%)*	-	*2/35 (6%)*

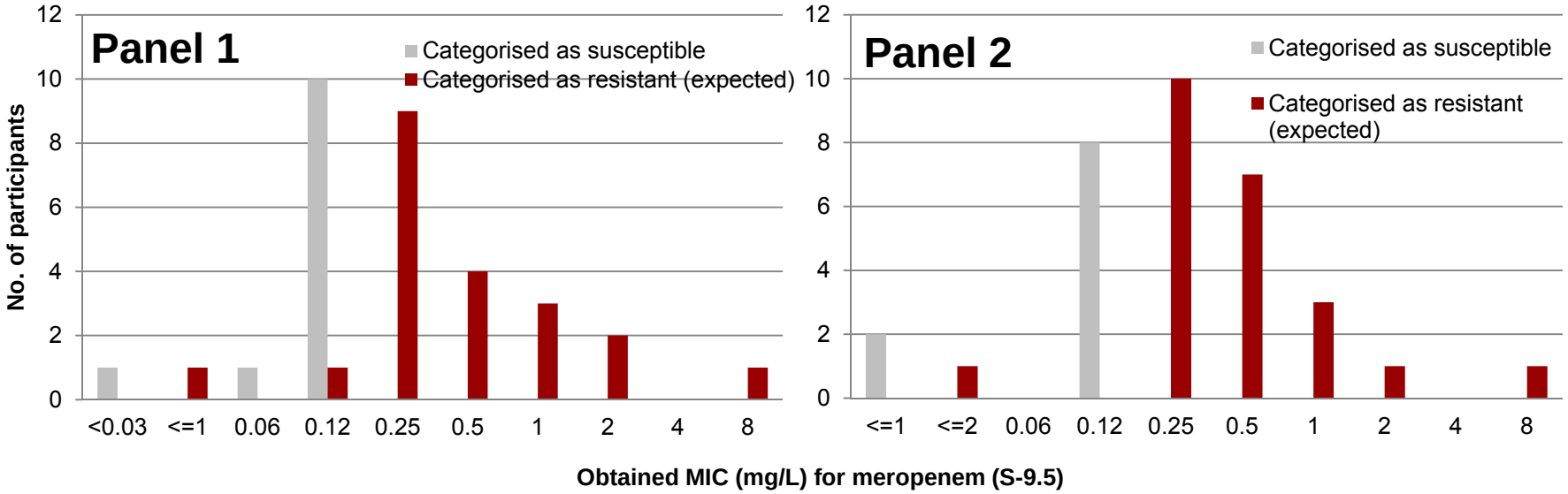
Marked in grey: incorrect results

S9.8/colistin excluded
36% deviations



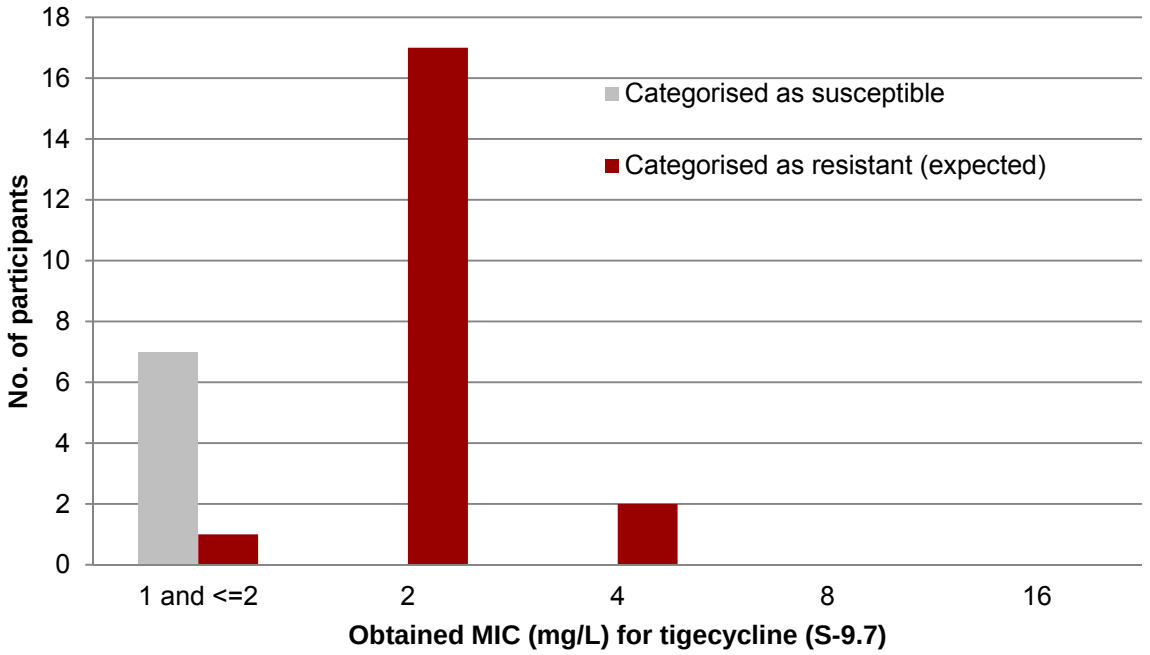
High level of deviation could not be explained
=> results excluded from evaluation
Expected MIC value: 2 mg/l ; S

S-9.5/meropenem (not excluded)
36% deviations in panel 1 and 30% in panel 2



High level of deviation
Meropenem => results not excluded from evaluation
Expected MIC value: 1 mg/l ; R

S-9.7/tigecycline (not excluded)
29% deviations



High level of deviation.
New antimicrobial => results not excluded from evaluation
Expected MIC value: 2 mg/l ; R

NRL-AR - Czech Republic - point of view

Our results of problematic strains and antibiotic combination :

- **EC 8.7** MER 0,25 mg/l (P1,P2); IMP 1 mg/l – fits to expected
- **S 9.5** MER 0,25mg/l (P1,P2) – expected (1; Rmg/l)
- **S 9.7** TGC 1 mg/l (P1) – expected (2; R mg/l)
  Result fits with ISO 20776 – one dilution step below only
- **S 9.8** COL 4 mg/l (P1) – expected (2; S mg/l) –
  Result fits with ISO 20776 – one dilution step up only

NRL- AR - Czech Republic - point of view

Analysis of the possible causes of results deviation:

Suspensions solutions (demineralised water, CAMHB)

- Home made
- Original brand (TREK)

Primary inoculum density - 0,5 McF (1 x 10^8 CFU/ml)

- Calibrated densitometer - TRECK 0,5 McF density control
- Spectrometric control - 0,08-0,13 (625 nm)

Volume of suspension which was transferred into the CAMHB

- Manufacturer instruction 10 µl (1 x 10^5 CFU/ml)
- EURL protocol 50 µl (5 x 10^5 CFU/ml) **Major hazard point**
- ISO 20776-1 [5 x 10^5 CFU/ml (2-8x 10^5)]

Final transfer of 50 µl CAMHB into the MIC plate wells

- Multichannel pipette inoculation
- SENSITITRE autoinoculator

Quality control with the E. coli ATCC 25922 strain **Major hazard point**

- Lowest and highest ranges of antimicrobial MIC which is valid for QCS
- Lowest and highest dilution range of antimicrobials to the MIC plate

NRL-AR - Czech Republic - point of view

Analysis of the possible causes of results deviation:

Final density of inoculum in the wells: 5 x 105 CFU/ml (2-8x 10^5):

o Semiquantitative checking 10µl from PGC >> 9.9 ml saline >> 100 µl cultivate onto the plate >> grow 20-80 colonies (ISO 20776-1,CLSI)

o Quantitative checking by ISO 4833-1 (direct number of CFU)

Our opinion:

o Transferring 10 µl of 0.5 McF suspension into CAMHB only, may be resulting of the lower final concentrations (9 x 10^4 – 2 x 10^5)

Quality control check by the E. coli ATCC 25922:

o Lowest dilution of antimicrobials in the EUVSEC plate does not fit with the lowest QCS range of some antimicrobials

Our opinion:

o In some cases we can not check of lowest QCS border of antimicrobials

		MIC (mg/l)											
	Range	0,008	0,015	0,03	0,06	0,12	0,25	0,5	1	2	4	8	16
MER	QCS												
	PLATE												
TGC	QCS												
	PLATE												
COL	QCS												
	PLATE												

NRL-AR - Czech Republic - conclusion

System error input material or workflow >>>system error of achieved MIC result:

- Lowest inoculum density >>> lowest MIC (MER, TGC may correspond)
- But our result of COL (higher than expected) defies of this rule

Our deviations in described cases are not alone:

- MER 36%, TGC 29%, COL 36%

There may be other unmentioned reasons for such a high percentage deviations appointed by antimicrobials ???

- Variability of antimicrobial activity of the bacterial population of the test samples in time and space
- Another unnamed and unexpected influences (reasons)

DISCUSSION