



EQAS 2010

Enterococci, Staphylococci and *E. coli*

EURL workshop, April 4, 2011

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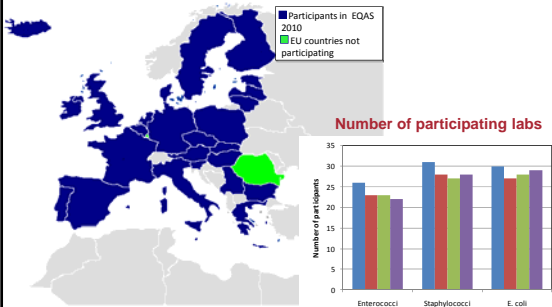
Main objectives of the EURL EQAS's

- To improve the comparability of antimicrobial susceptibility testing (AST) data
- To harmonise the breakpoints/epidemiological cut off values
- To assess the quality of AST in European laboratories and identify possible barriers
- To support laboratories in performing, evaluating and if necessary improving the quality of AST



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Participants in the enterococci, staphylococci and *E. coli* EQAS, 2009



Methods for EQAS 2009

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- Eight strains of enterococci, staphylococci and *E. coli*, respectively were selected
- New participants were provided with the reference strains, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 for QC testing
- AST guidelines were set according to the CLSI. MIC results were interpreted using the epidemiological cut off values set by EUCAST (www.eucast.org), recommended by EFSA and described in the protocol
- Participants using disk diffusion were advised to interpret the results according to their individual breakpoints
- Results were categorized as resistant or susceptible



Analysis of data based on these agreements

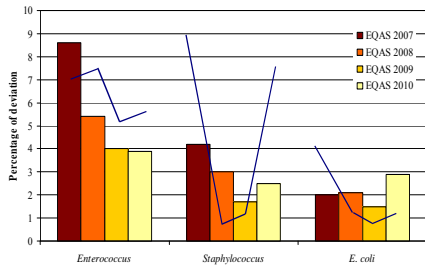
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- During the passed EURL-AR Workshop (2008) the network agreed upon the following decisions for EQAS 2009:
 - The accepted deviation for each laboratory is set up at 5%
 - Results should be further analysed (possibly ignored) when more than 25% are incorrect (strain/antimicrobial combination)
 - AST data that the MS report to EFSA is based on the interpretation of the results, the EQAS evaluates interpretation

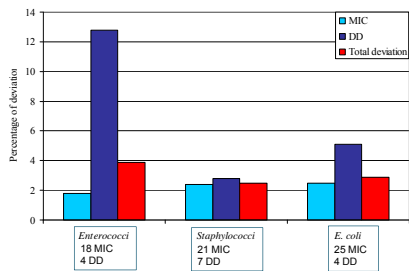


EQAS 2009 versus previous EQAS

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Deviation by species comparing the AST methods



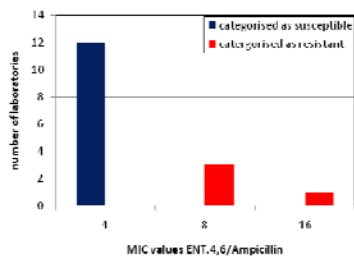
• Significant differences were observed in the results obtained depending of the AST method used ($p < 0.01$)



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Enterococci trial - results

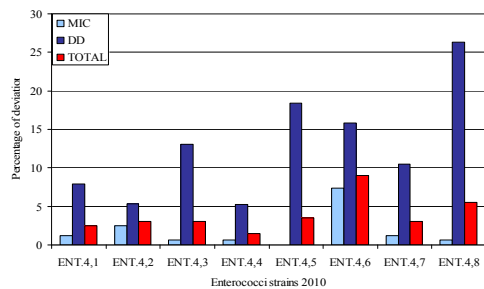
• Results that have 75% missed from the evaluation



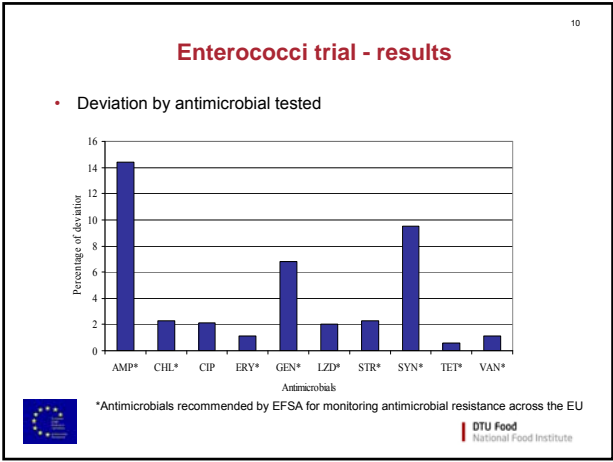
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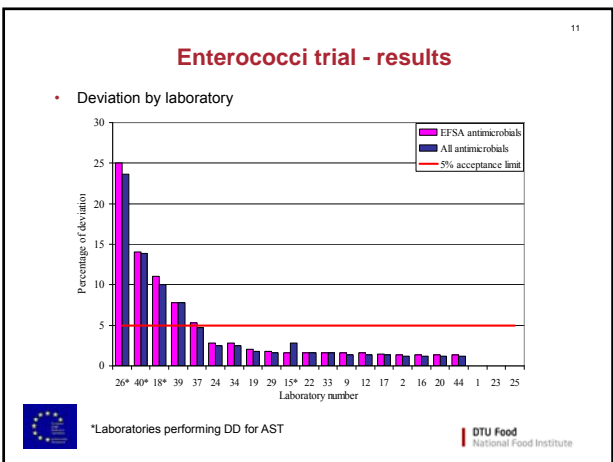
Enterococci trial - results

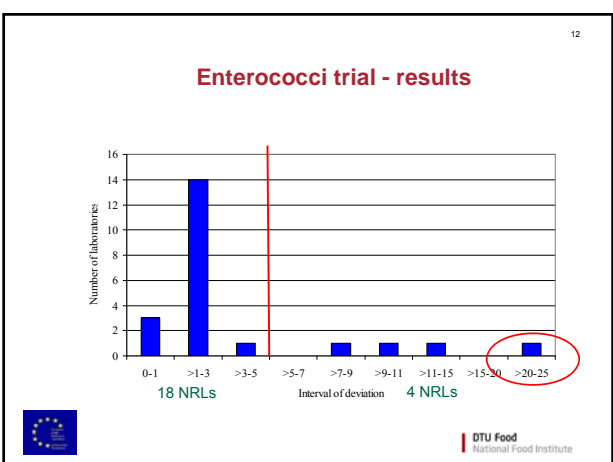
• Deviation by strain and AST method



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QC- STRAIN MIC

<i>E. faecalis</i> ATCC 29212				
Antimicrobial	MIC deviations /Total no. of tests	QC range MIC	Min value	Max value
Ampicillin	0/16	0.5 - 2	0.5	2
Chloramphenicol	0/16	4 - 16	4	8
Ciprofloxacin	0/12	0.25 - 2	0.5	2
Erythromycin	0/17	1 - 4	1	4
Gentamicin	0/17	4 - 16	4	≤128
Linezolid	0/14	1 - 4	1	2
Streptomycin	0/17	0-256	32	128
Synacid	0/9	2 - 8	4	8
Tetracycline	0/17	8 - 32	8	32
Vancomycin	0/17	1 - 4	2	4

- 17 participants
- 152 correct tests performed



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Summarizing enterococci trial

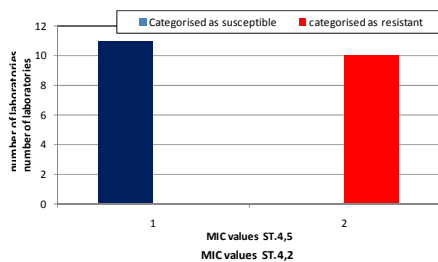
- For the first time, the total deviation for the enterococcal trial falls below 4%
 - 3/4 laboratories performing disk diffusion obtained deviations higher than the 5%
- 3/9 antimicrobials recommended by EFSA failed to produce 100% of correct results
 - Ampicillin: ENT.4,6/ampicillin, ECOFF ampicillin 4 mg/L, the expected MIC =8 mg/L
 - Synacid: #26 and #18 performing disk diffusion
 - Gentamicin: #26
- Deviations were mainly caused by laboratories performing DD for AST
- One participant clustered in the interval between 20%-25% deviation and was considered an outlier



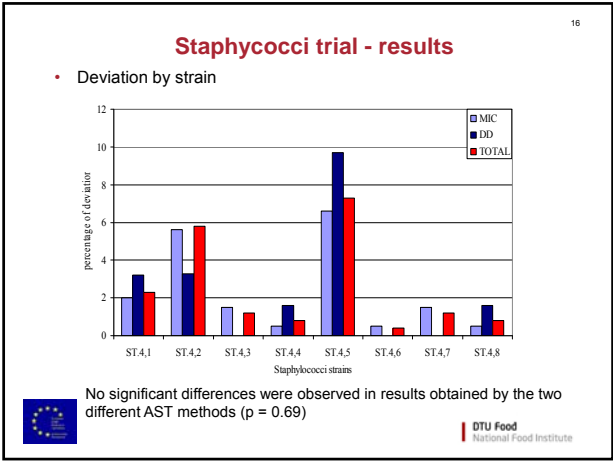
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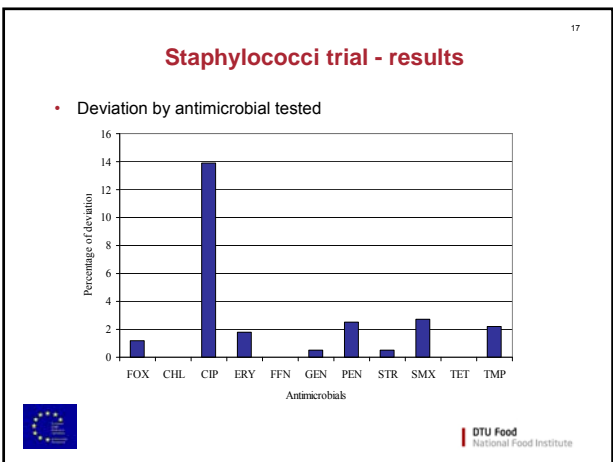
Staphylococci trial - results

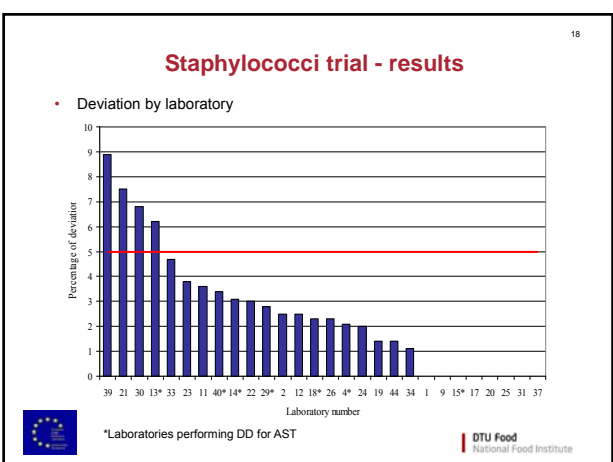
- Results that have NOT been omitted from the evaluation



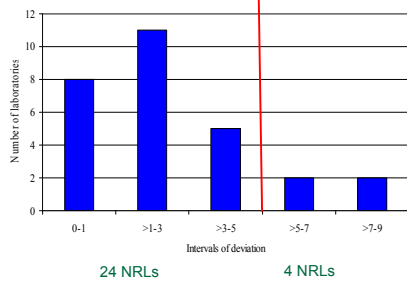
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Staphylococci trial - results



Methicillin Resistant *S. aureus* (MRSA)

- ST.4, 1, ST.4,4 and ST.4,5 were confirmed to be methicillin resistant
- 100% correct results
 - Participant #39 did not perform the test



QC strain - *S. aureus* ATCC 25923 by DD

Antimicrobial	Deviation/Total no. of tests	QC range	Min value	Max value
Cefoxitin	1/5	23-29	26	32
Chloramphenicol	0/3	16-26	18	26
Ciprofloxacin	0/5	22-30	23	29
Erythromycin	0/5	22-30	22	28.5
Florfenicol	0/3	None	20	29
Gentamicin	1/5	19-27	19	30
Penicillin	0/5	26-37	30	37
Streptomycin	0/4	14-22	14	22
Sulfisoxazole	0/3	24-30	24	30
Tetracycline	0/4	24-34	24	33
Trimethoprim	0/3	19-26	20	26

A total of 43 correct tests performed in this strain out of 45



S. aureus ATCC 25913 by MIC

Antimicrobial	Deviation/Total no. of tests	QC range	Min value	Max value
Cefoxitin	0/13	1-4	2	4
Chloramphenicol	2/18	2-8	4	16
Ciprofloxacin	0/17	0.12-0.5	0.12	0.5
Erythromycin	0/20	0.25-1	0.25	1
Florfenicol	0/9	2-8	4	8
Gentamicin	0/19	0.12-1	0.25	≤2
Penicillin	0/18	0.25-2	0.25	2
Sulfisoxazole	0/8	32-128	32	128
Tetracycline	0/20	0.12-1	0.5	
Trimethoprim	1/15	1-4	0.5	4

Total number of test was 172 of which 3 were incorrect



Summarizing staphylococci trial

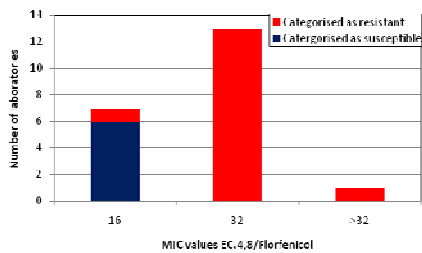
- For the first time in this staphylococci iteration, no significant differences were observed between the two AST methods.
- All of the strains and antimicrobials tested presented deviations below 2.3% except the combinations ST.4,2/ciprofloxacin and ST.4,5/ciprofloxacin
- Four laboratories clustered outside the 5%, most of the participants grouped in the deviation interval between 1% and 3%.
- All the laboratories identify correctly the MRSA strains except one that didn't performed the test
- Laboratories performing DD on *S. aureus* ATCC 25923 produced a deviation of 4.4% whereas laboratories performing MIC obtained 1.7%.

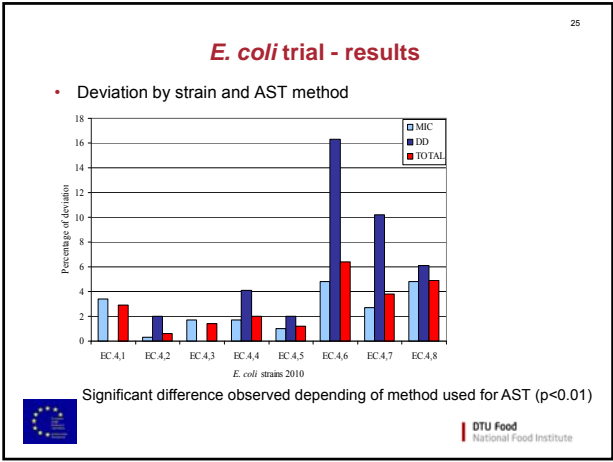


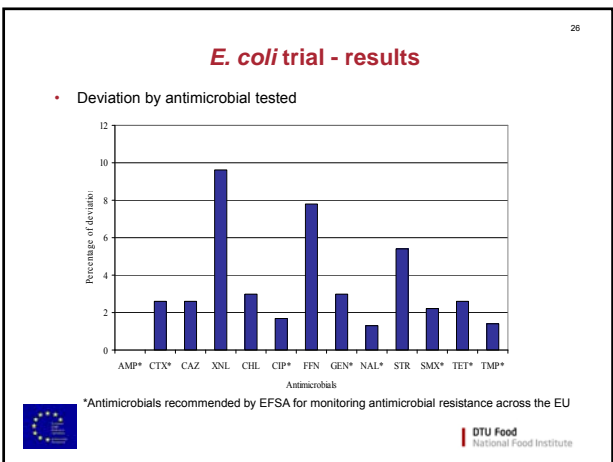
E. coli trial - results

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- Results that have NOT been omitted from the evaluation







Cephalosporin resistant strains

EC.4.5	<i>bla</i> _{CTX-M-1}
EC.4.8	<i>bla</i> _{CTX-M-15}
EC.4.7	<i>bla</i> _{CMV-2}

- #39 did not perform any of the confirmatory tests
- 2/28 labs failed to identify ESBL producing organisms
- #32 obtained MIC value for cefotaxime ≤ 0.12 mg/L instead of 4 mg/L and ceftazidime ≤ 0.25 mg/L instead of 32 mg/L
- #2 obtained MIC for cefotaxime 0.12 mg/L instead of 4 mg/L, they performed the two confirmatory tests on the strain, both of them were negative for ESBL production

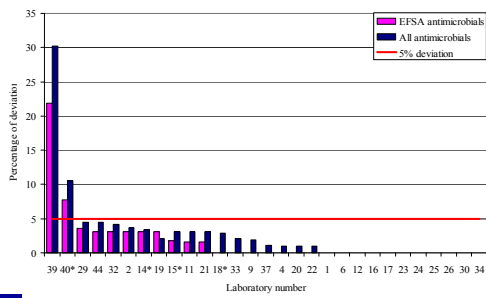
AmpC strain

- 7/28 labs failed to identify the ampC strain EC.4,7
- #40 obtained susceptible values for all cephalosporins tested
- #29 susceptible value for ceftazidime, resistant for CTX and CAZ and did not find synergy (CTX/CL:CTX)
- #15, #22 and #32 identified the strain as ESBL and ampC
 - #15 did not perform confirmatory test for ESBL
 - #22 obtained an increase in the diameters (≥ 5 mm) for the two confirmatory tests (CAZ:CAZ/CL and CTX:CTX/CL)
 - #32 reported increase in the MIC ratio only for one of the confirmatory test (CAZ/CL:CAZ)
- #24 and #30 performed all tests and got correct results even for ceftazidime but fail to interpret them correctly
- #44 identified strain EC.4,1 as an ampC. They obtained MICs of 1 mg/L, 2 mg/L and >16 mg/L for ceftazidime, cefotaxime and ceftazidime/ceftioxcid



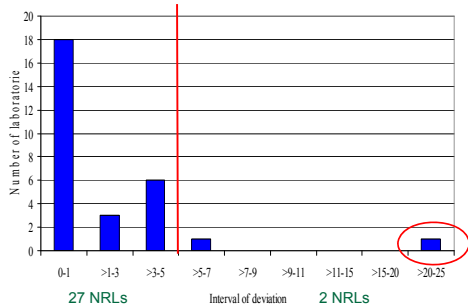
E. coli trial - results

- Deviation by laboratory



*Laboratories performing DD for AST

E. coli trial - results



QC strain - *E. coli* ATCC 25922 by DD

-48 tests and 8 were incorrect (16.6% deviation)

Antimicrobial	Deviation/Total no of tests	QC range	Min value	Max value
Ampicillin	0/2	16-22	18	20
Cefotaxime	1/4	29-35	32	40
Cefoxitin	1/3	23-29	25	30
Ceftazidime	1/3	25-32	27	33
Ceftiofur	1/3	26-31	27	33
Chloramphenicol	1/3	21-27	22	28
Ciprofloxacin	0/4	30-40	34	40
Florfenicol	1/2	22-28	23	33
Gentamicin	0/4	19-26	20	24.4
Imipenem	1/2	26-32	29	40
Nalidixic acid	0/4	22-28	25	27
Sulfisoxazole	0/2	15-23	18	23
Tetracycline	0/3	18-25	20	25
Trimethoprim	1/4	21-28	17	26



QC strain - *E. coli* ATCC 25922 by MIC

- 288 test performed of which 7 were incorrect (deviation 2.4%)

Antimicrobial	Deviation/Total no of tests	QC range	Min value	Max value
Ampicillin	1/25	2-8	2	16
Cefotaxime	3/25	0.03-0.12	0.06	4
Cefoxitin	0/6	2-8	2	4
Ceftazidime	0/20	0.06-0.5	0.12	0.25
Ceftiofur	0/3	0.25-1	0.25	0.5
Chloramphenicol	0/24	2-8	4	8
Ciprofloxacin	2/25	0.004-0.016	0.008	0.03
Florfenicol	0/21	2-8	4	8
Gentamicin	1/25	0.25-1	0.25	2
Imipenem	0/4	0.06-0.25	0.12	0.25
Nalidixic acid	0/24	1-4	1	4
Streptomycin	0/23	4-16	4	8
Sulfisoxazole	0/17	8-32	16	
Tetracycline	0/24	0.5-2	1	2
Trimethoprim	0/22	0.5-2	0.5	1



Summarizing *E. coli* trial

- Deviations in EFSA recommended antimicrobials remained lower than 3%
- Deviations were mainly caused by laboratories performing DD for AST
- They majority clustered in the interval of deviation between 0% and 1%
- Two laboratories obtained deviations above the 5% acceptance limit and one of them clustered has been identified as an outlier
- Deviations for ESBL and ampC detection are still high
- For *E. coli* ATCC 25922 the percentage of results within range for all tests performed by disk diffusion was 83.3% compared to the 97.6% obtained by MIC



Conclusions

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- Performance has improved for the enterococci trial
- There is still a significant difference in the quality of results obtained by NRLs performing MIC when compared to those performing DD
- 100% correct results in detection of MRSA
- the number of laboratories failing to identify the strains resistant to cephalosporins has been remarkably high, especially for the ampC strain
- Main cause of deviations
 - Strains with expected MIC values close to the epidemiological cut off values to define them as resistant
 - Laboratories performing disk diffusion
- two outliers have been identified, one for enterococci trial and one for the *E. coli* trial



Thank you for your attention