European Union Reference Laboratory Antimicrobial Resistance

DTU Food
National Food Institute

MRSA EQAS 2009

Isolation identification and typing of MRSA from dust samples

EURL workshop, April 8-9th, 2010

Lina Cavaco (licav@food.dtu.dk)

Background for this EQAS

- CC398 MRSA recent emergence in Europe
- MRSA considered an emergent zoonotic pathogen
- Need for knowledge on MRSA epidemiology
- Lack of laboratory experience on MRSA in animal/food labs
- Request for baseline study in EC Decision 2008/55/EC

MRSA EQAS 2009- Objectives

- Assess the performance of laboratories on the use of selective isolation procedures for detection of MRSA from dust samples in laboratories involved in the MRSA baseline studies determined by EC Decision
- Assess the identification and confirmation of MRSA isolates using molecular methods
- Assess performance of typing of MRSA by *spa* typing

Description of the MRSA EQAS 2009

- Prenotification and participation
  - All NRL's were notified 3 months before
  - NRL's not involved in baseline were invited to give the contact info on lab that performed baseline studies for their participation
  - Participant list was filled with NRL and new participants concerning MRSA- 25 participant laboratories
  - Database was constructed on password protected website for collection of results

Description of the MRSA EQAS 2009

- Selection of strains for preparation of samples
  - Eleven candidate strains tested initially
    - mecA status, identification by 16S DNA sequencing, susceptibility testing and *spa* typing
    - Verification by an additional lab
  - Choice of eight sample preparations containing MRSA, MSSA, CNS or blanks
  - Definition of expected results

Description of the MRSA EQAS 2009

- Preparation of samples
  - 8 Dust samples (about 0,1g of dust each) from MRSA-free pig stables
  - Samples were spiked to contain about 10^6 cfu of MRSA or other staphylococci (MSSA, MRCNS, CNS test strain and maintained background flora existing in the pig farm environment
  - After preparation (and shipping) the samples were tested continuously for homogeneity and stability

Shipping of parcels

- Shipping of parcels was performed immediately after preparation of samples and parcels were sent off 17-19th June 2009 containing:

– 8 samples containing the spiked dust samples

– Welcome letter including:

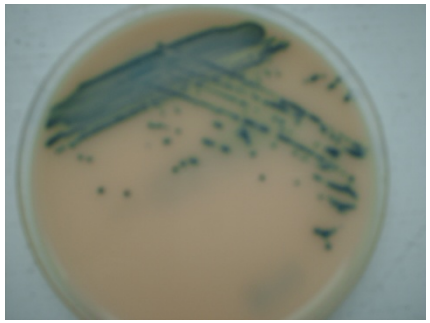
  - Instructions for procedure and link to detailed protocol for sample processing and data upload (available online)
  - Login and password information for database access

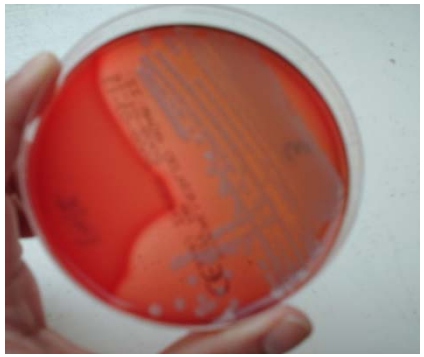
Description of the MRSA EQAS 2009

- Isolation procedures
  - Due to the type of sample, we have asked to **perform the isolation procedure immediately after the reception of the samples**
  - The protocol is based on the media and methods used in the baseline studies was posted online on the CRL-AR website (http://crl-ar.eu)

Isolation procedures

- Selective isolation procedure using
  - pre enrichment in Mueller Hinton Agar w 6,5%NaCl,
  - enrichment in TSB with 3,5 mg/L cefoxitin and 75 mg/L aztreonam
  - plating on Chromogenic Agar (Brilliance MRSA Agar) and blood agar
  - Isolation up to 5 colonies



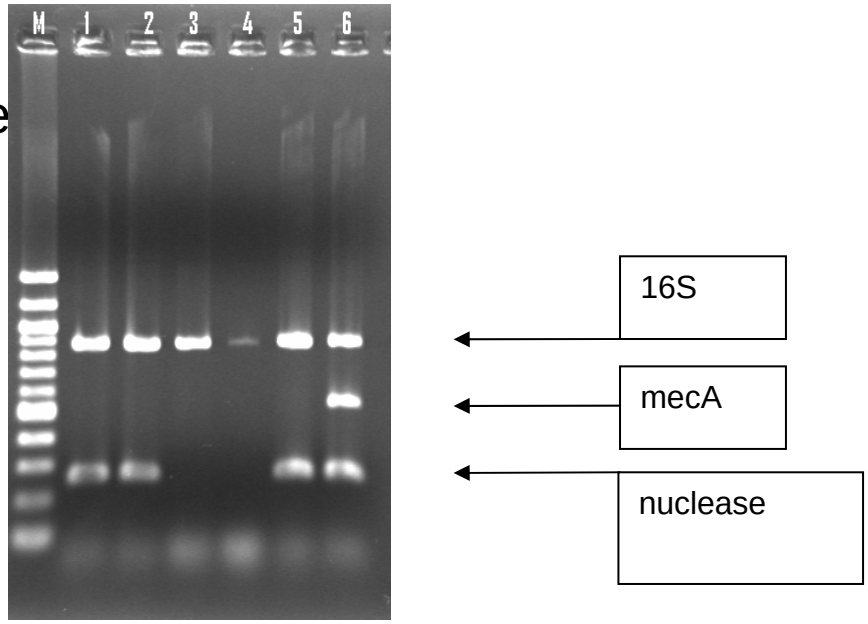


Description of the processing of samples for the MRSA EQAS 2009

- Confirmation of MRSA ID and presence of *mecA* gene
  - The detection of methicillin resistant *Staphylococcus aureus* (MRSA) must always be confirmed using molecular methods
    - Only isolates with confirmed ID as *Staphylococcus aureus* containing the *mecA* gene were considered MRSA
    - Other Staphylococci found and tested were reported as negative for MRSA, and described as: MSSA, MSCoNS,MRCoNS...

Confirmation of id and methicillin resistance status

- PCR 16S, *mecA* and *nuc* for MRSA ID
  - *16S*- confirms that the PCR works
  - *mecA* – Confirm methicillin resistance
  - *Nuc- confirm ID* (only positive in *Staphylococcus aureus)*



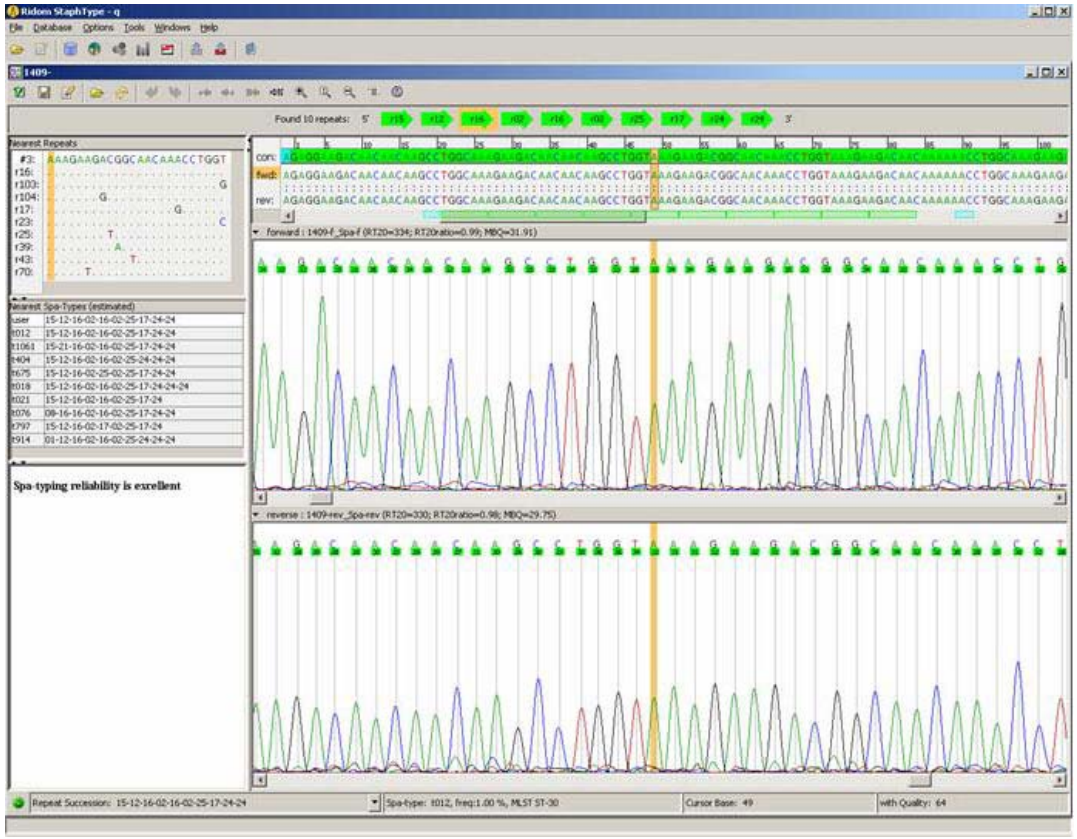
Description of the MRSA EQAS 2009

- Typing of MRSA
  - Labs were instructed to keep any MRSA frozen at -80ºC for re-testing (if needed)
  - *spa* typing (optional)

- Labs should keep records of all methods used and results obtained during the process

Typing of isolates

- Typing of strains
  - *Sequence based typing based on repeat sequences on the Staphylococcal protein A gene (Spa* typing) (Shopsin *et al*., 1999)



http://www.uniklinik-freiburg.de/iuk/live/molhyglabor/leistungskatalog/spa.jpg

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Preparation of database

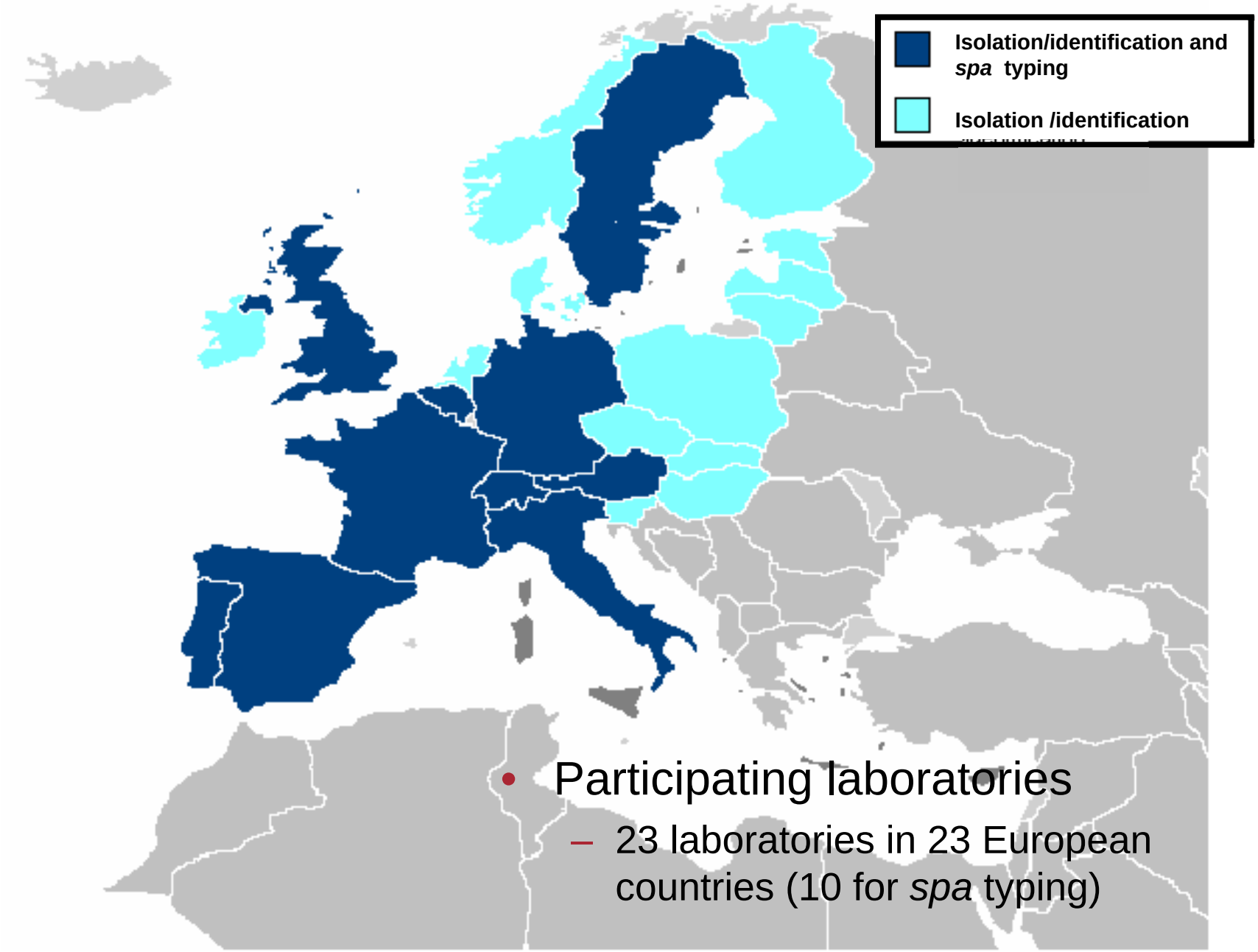
- The database was prepared on a password protected site
- MRSA EQAS forms included:
  - General questionnaire on MRSA related activities
  - Methods used
  - 8 individual test forms for results of MRSA detection and identification and spa typing (optional)
- Lab code for data – anonimity of results
- Expected results uploaded on database for evaluation of results
- Immediate generation of evaluation reports

Evaluation of data

- The sample isolation and detection procedure was evaluated only qualitatively, based on detection of the confirmed MRSA vs the expected result (Positive/negative)
- The intermediate results were used to describe the isolation process and detect possible dificulties or problems.
- The typing result was considered optional for the laboratories able to perform *spa* typing, however if reported it will be evaluated by comparison with the expected *spa* type.

Data analysis- Results

- As for other EQAS the data was subjected to a descriptive analysis
- No threshold for acceptance has been defined yet, since the MRSA EQAS is new



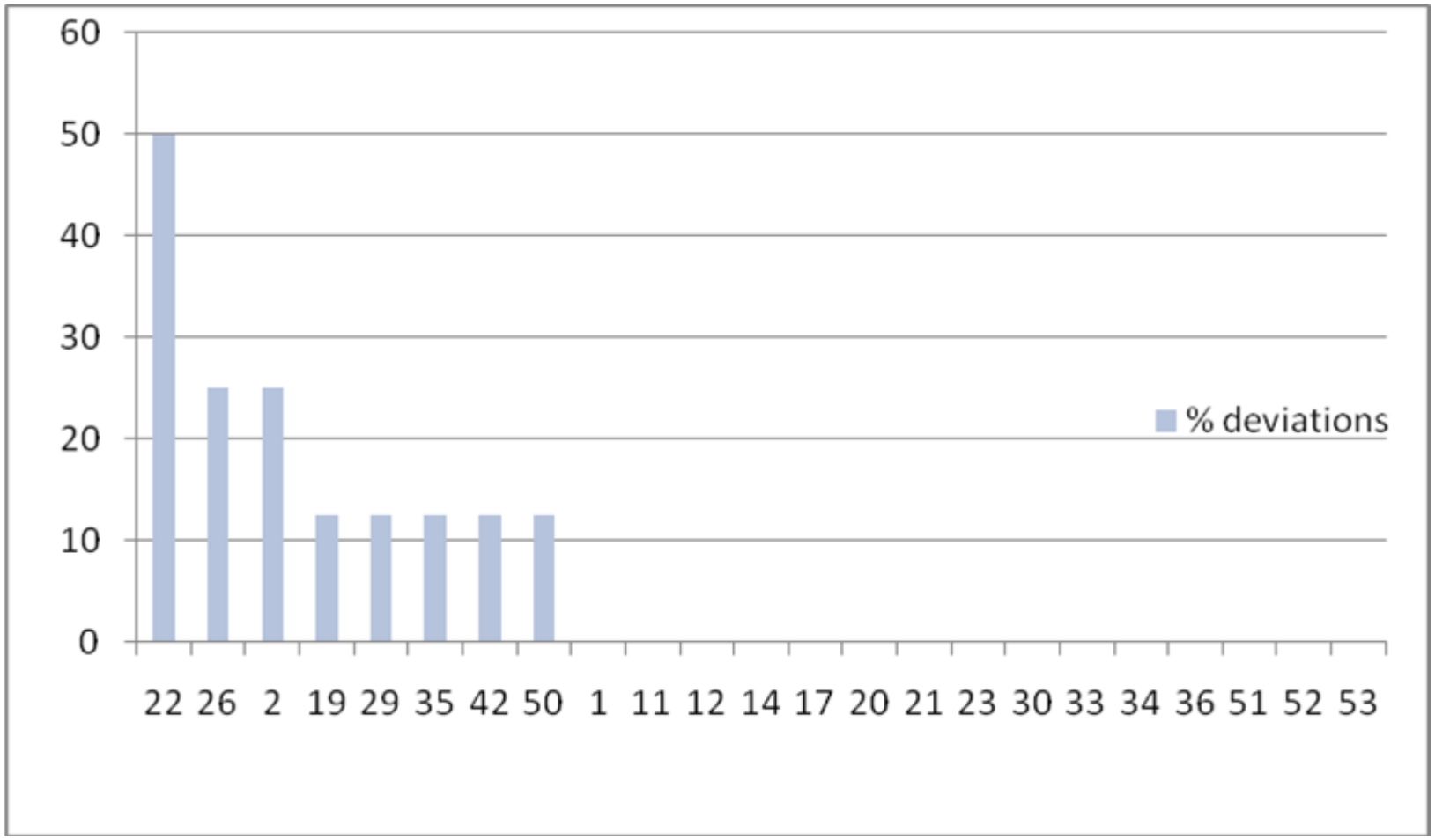
- Participating laboratories
  - 23 laboratories in 23 European countries (10 for *spa* typing)

Overall results MRSA detection

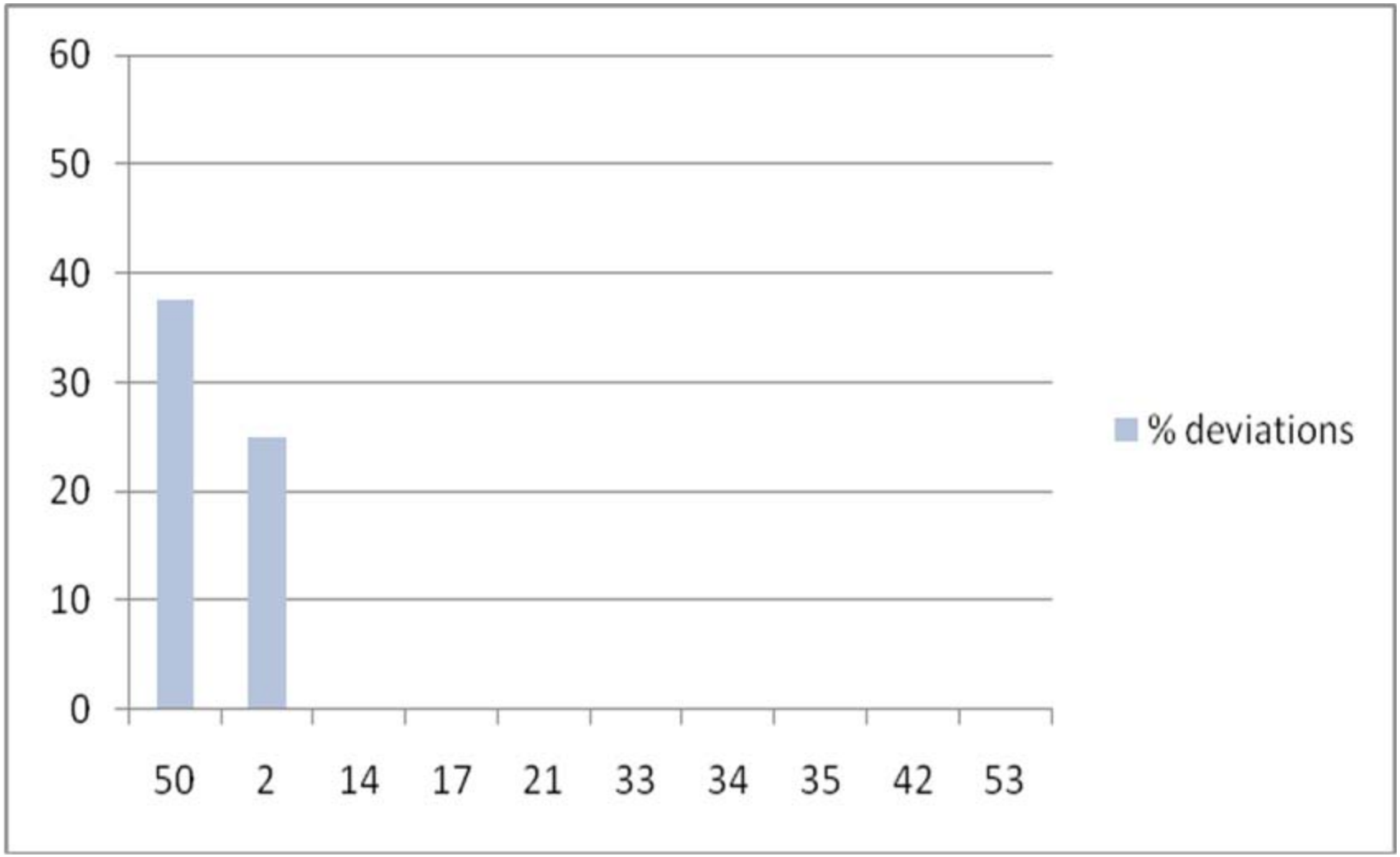
Table 1-. The overall performance of MRSA isolation and identification, 2009.

Isolation of MRSA from dust samples		**Correctly classified samples**	
Number of performed tests		Number of correct tests N(%)	
n	%	N	%
179	100	166	92.7
Number of expected negative tests		Number of correctly identified negative tests	
n	%	N	%
88	49.2	86	97.7
Number of expected positive tests		Number of correctly identified positive tests	
n	%	N	%
91	50.8	80	87.9%

Results per laboratory- detection of MRSA



Results per laboratory- *spa* typing of MRSA



Results per sample

Sample number	N part labs	expected repeat succession	expected *spa* type	correct	Deviating results
CRL MRSA 1. 1	5	None – *mecA* negative should not be isolated	N/A (t011)	4	t021
CRL MRSA 1. 2	3	None- S. *haemolyticus*, *spa* negative	N/A	3	-
CRL MRSA 1. 3	7	r11r19r21r21r12r21r17r34r24r34r22r25	t075	7	-
CRL MRSA 1. 4	4	None- S. *simulans*, *spa* negative	N/A	3	t034
CRL MRSA 1. 5	10	r08r16r02r25r02r25r34r24r25	t034	9	N/A
CRL MRSA 1. 6	10	r08r16r02r25r24r25	t108	9	t021
CRL MRSA 1. 7	3	None- Negative control	N/A	3	-
CRL MRSA 1. 8	10	r15r12r16r02r16r02r25r17r24	t021	9	t108

Conclusions

- Detection of MRSA
  - Overall results were good
  - some deviations due to lack of sensitivity of methods
  - Confirmatory testing of MRSA confirms that labs obtain reliable results of identification and *mecA* detection
  - Results per strain show differences regarding morphology that might have induced false negative results

Conclusions and perspectives

- *Spa* typing
  - Performed in 10 out of 23 labs
  - Ten labs participated but only few uploaded results for the eight samples
  - *Spa* typing showed reproducible and comparable results
  - Deviations caused by lack of detection and either contamination or switch between samples

- A report of the MRSA EQAS 2009 was approved and concluded in the end of 2009 (http://www.crl-ar.eu/203-reports.htm)
- MRSA EQAS 2010 in preparation

MRSA EQAS 2010

- Pre notification sent in March
- Participant list based on participants of 2009
  - Please communicate any changes
- New set of samples
- Methods will be the same
  - MRSA isolation /identification
  - *Spa* typing of isolates optional
- Database unchanged
- Expected shipping around middle June
- Deadline for result until September
- Analysis of results

Future perspectives

- Discussion
- EQAS for MRSA
- Samples/Methods
- Qualitative /quantitative approach
- Implement thresholds for acceptance of results?

Questions??


DTU
Report of the 1st External Quality Assurance System on Isolation, Identification and Typing of Methicillin resistant *Staphylococcus aureus* (MRSA) from Dust Samples, year 2009
Lina Maria Cavaco
Rene Hendriksen
Susanne Karlsmose
Frank Møller Aarestrup
DTU Food
National Food Institute