MRSA EQAS 2010

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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
- Reccomended routine monitoring.

MRSA EQAS 2010- Objectives

- Assess the performance of laboratories on the use of selective isolation procedures for detection of MRSA from swab 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 2010

- Prenotification and participation
  - All NRL's were notified 3 months before for the trial in June and again before the second trial in October
  - 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- 23 participant laboratories
  - Database was constructed on password protected website for collection of results

Description of the MRSA EQAS 2010

- 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
  - –Sample preparation protocol

Description of the MRSA EQAS 2010

- Preparation of samples (only October trial)
  - 8 swab samples
  - Samples were spiked to contain about 10^6 cfu of MRSA or other staphylococci (MSSA, MRCNS, CNS test strain and added background flora containing *Staphylococcus* spp, *Enterococcus faecalis* and *Escherichia coli*
  - After preparation (and shipping) the samples were tested continuously for homogeneity and stability during 2,5 months.

Shipping of parcels

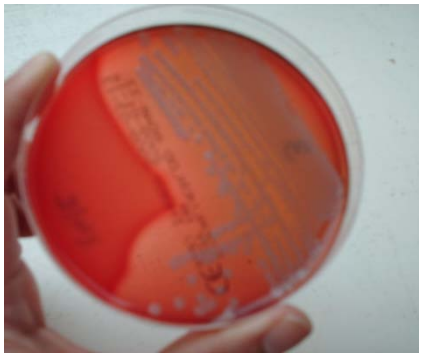
- Shipping of parcels was performed immediately after preparation of samples and parcels were sent off 26-27th October 2010 containing:
  - 8 swab samples in transport media
  - Welcome letter including:
    - Instructions for procedure and link to detailed protocol for sample processing and data upload (available online)
    - Login and password information for database access

Description of the MRSA EQAS 2010

- 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 EURL-AR website (http://eurl-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) or equivalent and on blood agar
  - Isolation up to 5 colonies

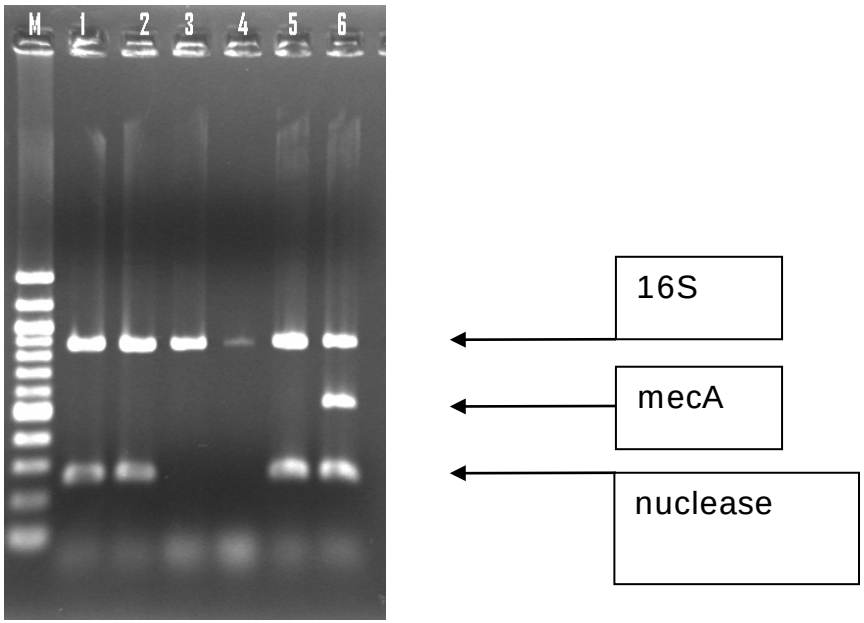


Description of the processing of samples for the MRSA EQAS 2010

- 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)*



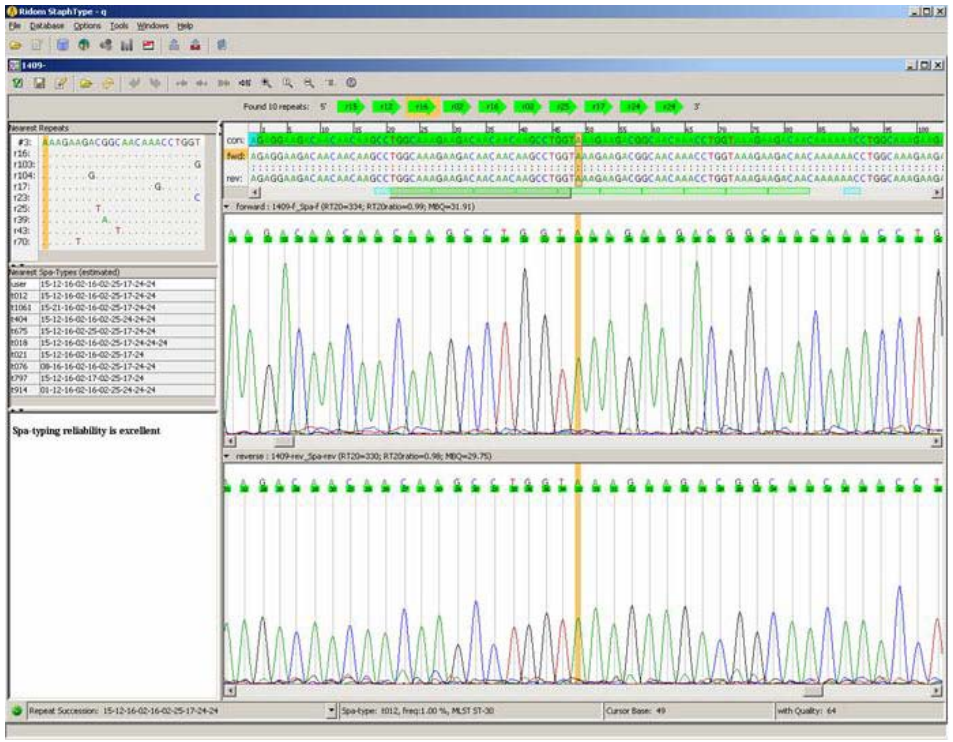
Description of the MRSA EQAS 2010

- Typing of MRSA
  - Labs were instructed to keep any MRSA frozen at -80ºC for eventual re-testing
  - *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

Preparation of database

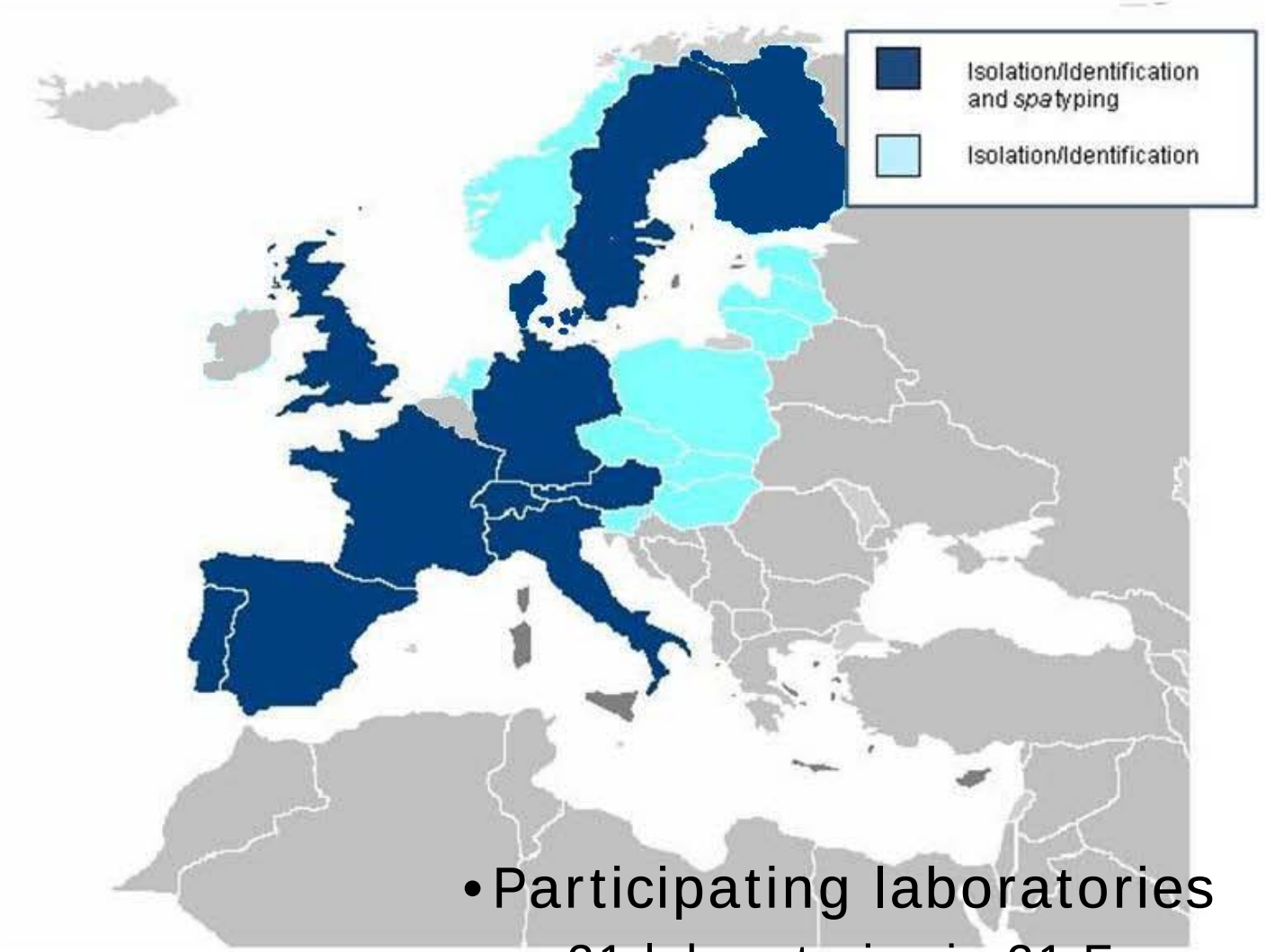
- The database was prepared on a password protected site
- MRSA EQAS forms included:
  - General questionnaire on MRSA related activities
  - Methods used
  - 8 individual test forms for results of MRSA detection and identification and *spa* typing (optional)
- Lab code for data – anonimity of results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



- Participating laboratories
  - 21 laboratories in 21 European countries (12 for *spa* typing)

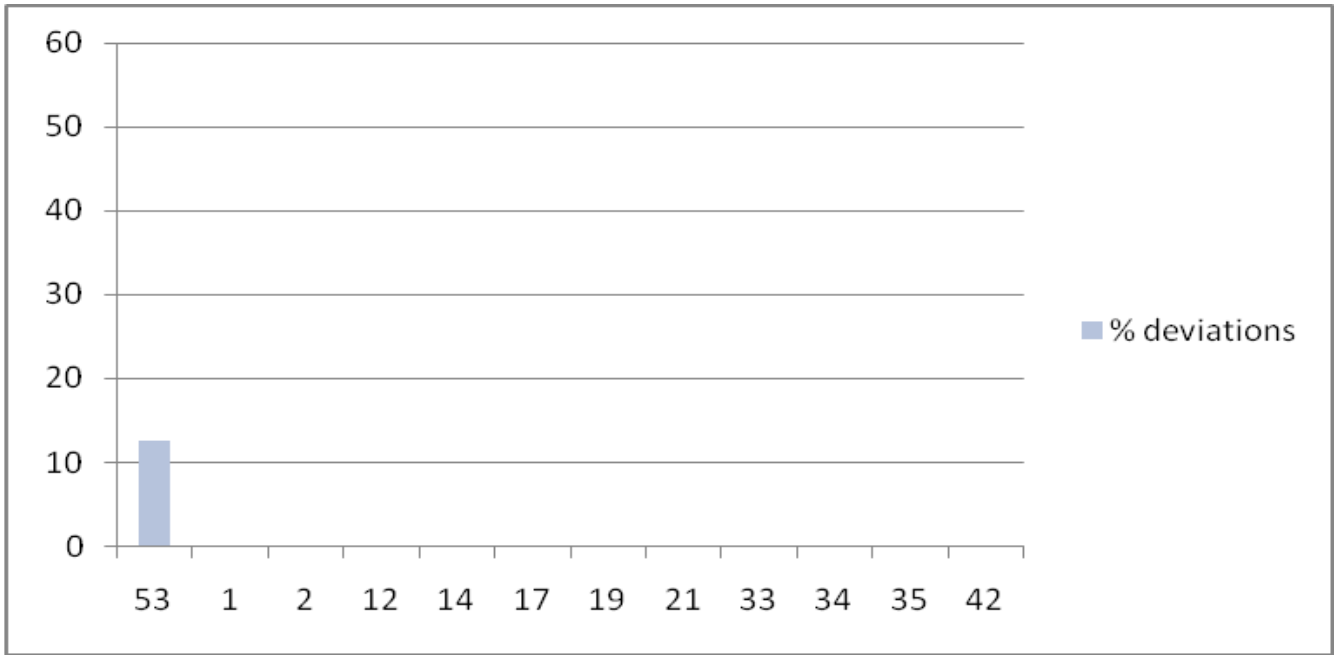
Overall results MRSA detection

Isolation of MRSA from swab samples		**Correctly classified samples**	
Number of performed tests		Number of correct tests N(%)	
n	%	N	%
168	100	168	100
Number of expected negative tests		Number of correctly identified negative tests	
n	%	N	%
63	37,5	63	100
Number of expected positive tests		Number of correctly identified positive tests	
n	%	N	%
105	62,5	105	100

Results per laboratory- detection of MRSA

- No deviations were observed!!!
- Excellent performance by all participants

Results per laboratory- *spa* typing of MRSA



Results per sample

Sample number	N part labs	expected repeat succession	expected *spa* type	correct	Deviating results
EURL-MRSA 2B.1	7	None- negative control	N/A	7	None
EURL-MRSA 2B.2	8	None- S. *haemolyticus*, *spa* negative	N/A	8	None
EURL-MRSA 2B.3	12	08-16-02-25-02-25-34-24-25	t034	12	None
EURL-MRSA 2B.4	12	08-16-02-25-24-25	t108	12	None
EURL-MRSA 2B.5	12	07-16-23-02-34	t899	12	None
EURL-MRSA 2B.6	7	None – *mecA* negative should not be isolated	N/A (t3855)	7	None
EURL-MRSA 2B.7	12	11-19-21-21-12-21-17-34-24-34-22-25	t075	11	**t899**
EURL-MRSA 2B.8	11	08-16-02-25-34-24-25	t011	11	None

Conclusions

- Detection of MRSA
  - Overall results were excellent
  - No deviations due to lack of sensitivity of methods
  - Confirmatory testing of MRSA confirms that labs obtain reliable results of identification and *mecA* detection

Conclusions and perspectives

- *Spa* typing
  - ring trial performed in 12 out of 21 labs
  - Twelve labs participated but only few uploaded results for the eight samples
  - *Spa* typing showed reproducible and comparable results
  - Only one deviation caused either contamination or switch between samples

- A report of MRSA EQAS 2010 has been circulated and will be concluded after this workshop
- MRSA EQAS 2011 is in preparation for expected shipment in October

MRSA EQAS 2011

- Pre notification will be sent out
- Participant list based on participants of 2010
  - 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 October
- Deadline for result until December
- Analysis of results/reporting spring 2012

Future perspectives

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


Thank you!