MRSA EQAS 2011

European Union Reference Laboratory Antimicrobial Resistance

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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
- Recommended routine monitoring

MRSA EQAS 2011- Objectives

- Assess the performance of laboratories on the use of selective isolation procedures for detection of MRSA from swab samples in laboratories in the EURL-AR network
- 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 2011

- Prenotification and participation
  - All NRL's were notified 3 months before the trial in October
  - Participant list was filled with NRL and new participants concerning MRSA- 29 participant laboratories registered and 24 uploaded results
  - Database was constructed on password protected website for collection of results

Description of the MRSA EQAS 2011

- 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 2011

- Preparation of samples
  - 8 swab samples (only seven shipped out)
  - Samples were spiked to contain about 10^5 or 10^3 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 at least 5 weeks.

Shipping of parcels

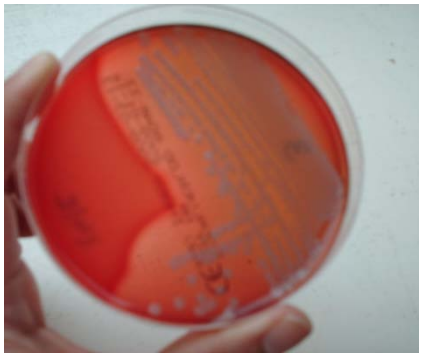
- Shipping of parcels was performed immediately after preparation of samples and parcels were shipped on the 24th October 2011 containing:
  - 7 swab samples in transport media
  - Welcome letter including:
    - Instructions for procedure and link to detailed protocol for sample processing and data upload (available online)
    - Login and password information for database access

Description of the MRSA EQAS 2011

- 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

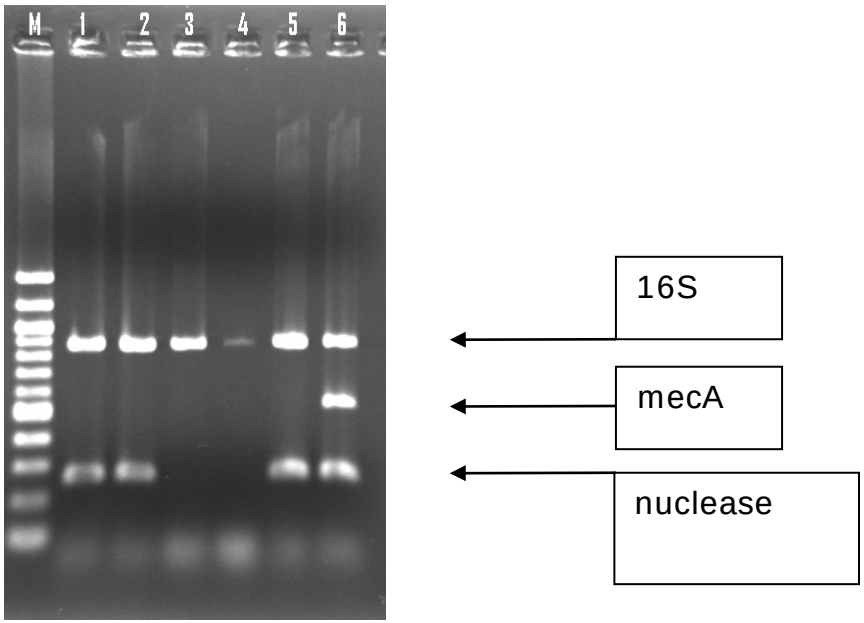


Description of the processing of samples for the MRSA EQAS 2011

- 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)*



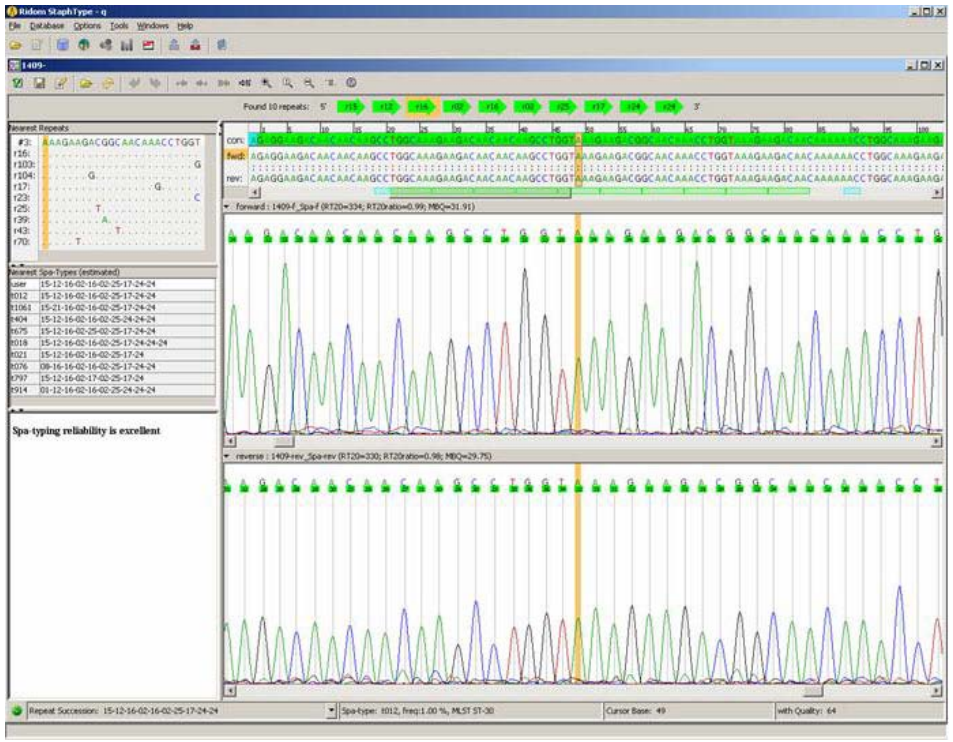
Description of the MRSA EQAS 2011

- 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

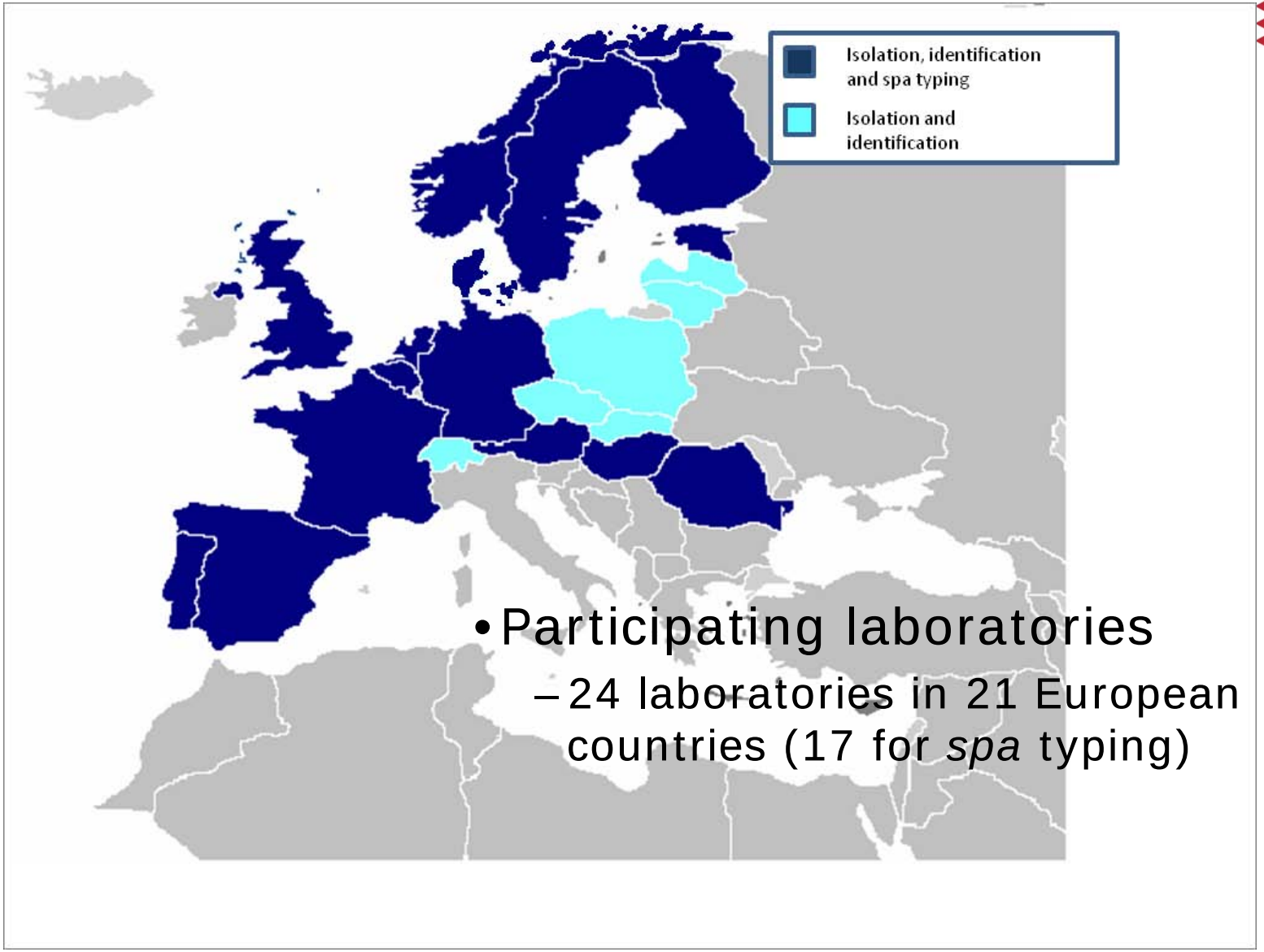
- The database was prepared on a password protected site
- MRSA EQAS forms included:
  - General questionnaire on MRSA related activities
  - Methods used
  - 8 individual test forms for results of MRSA detection and identification and *spa* typing (optional)
- Lab code for data – anonimity of results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

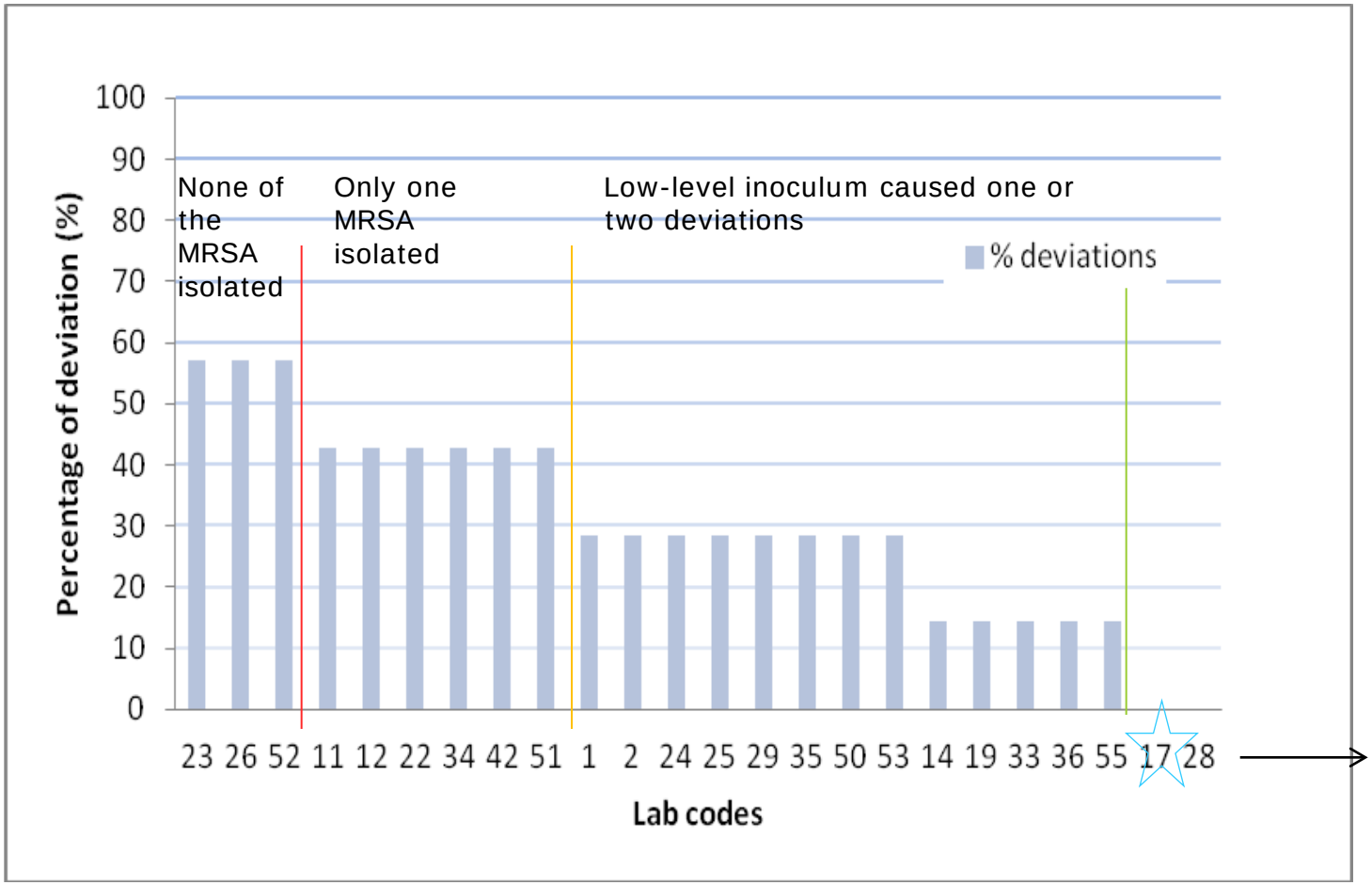


- Participating laboratories
  - 24 laboratories in 21 European countries (17 for *spa* typing)

Overall results MRSA detection

Isolation of MRSA from swab samples		**Correctly classified samples**	
Number of performed tests		Number of correct tests N(%)	
n	%	N	%
163	100	112	82
Number of expected negative tests		Number of correctly identified negative tests	
n	%	N	%
69	42	69	100
Number of expected positive tests		Number of correctly identified positive tests	
n	%	N	%
94	58	43	46

Results per laboratory- MRSA isolation and identification

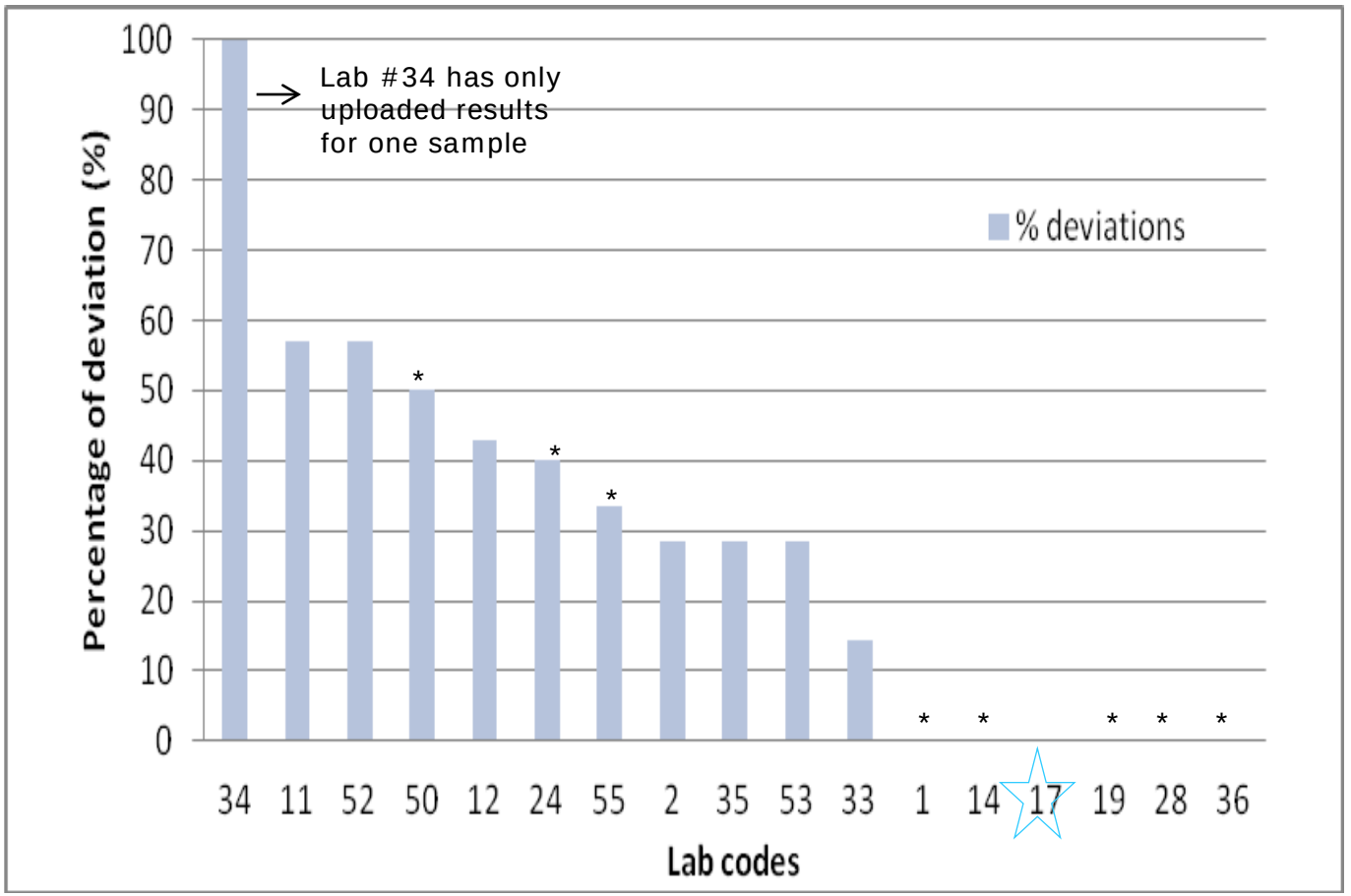


Lab #28 only reported results for two samples

Results per laboratory- detection of MRSA

- Good specificity
- Deviations due to low sensitivity, mostly observed for the two low-inoculum samples
- Six labs had one additional deviation
- Three labs were not able to recover MRSA from the samples

Results per laboratory- *spa* typing of MRSA



* Results uploaded for less than seven samples

Results per sample

Sample number	N participating laboratories	Repeat succession	expected *spa* type	correct	Deviating results (number of deviations)
EURL-MRSA 3.1	9	None	N/A	9	None
EURL-MRSA 3.2	9	None	N/A	9	None
EURL-MRSA 3.3	12	07-16-23-02-12-23-02-34	t1430	5	N/A (7)
EURL-MRSA 3.4	9	None	N/A	9	None
EURL-MRSA 3.5	10	08-16-02-25-02-25-34-24-25	t034	2	N/A (8)
EURL-MRSA 3.6	15	08-16-02-25-34-24-25	t011	12	N/A (2) t108 (1)
EURL-MRSA 3.7	16	08-16-02-25-24-25	t108	11	N/A (3) t021 (1) t1430 (1)
EURL-MRSA 3.8	None *	11-19-21-21-12-21-17-34-24-34-22-25	t075	None *	-

*sample not shipped due to lack of stability

Conclusions

- Detection of MRSA
  - Overall results were ok regarding specificity
  - A large number of deviations due to lack of sensitivity of methods/low inoculum in the samples
    - Reduced recovery either due to low sensitivity of the methods and/or to reduced stability which was noticed after 1-2 weeks
  - In some labs recovery of MRSA from samples was not achieved

Conclusions and perspectives

- *Spa* typing
  - Ring trial performed in 17 out of 24 labs
  - Seventeen labs participated but only few uploaded results for the seven samples
  - *Spa* typing showed reproducible and comparable results
  - Deviations caused mostly because lack of sensitivity, few deviations may be explained by contamination or possible switch between samples
- A report of MRSA EQAS 2011 has been circulated and will be concluded after this workshop
- MRSA EQAS 2012 is in preparation for expected shipment in October

MRSA EQAS 2012

- Pre notification will be sent out
- Participant list based on participants of 2011
  - Please communicate any changes
- New set of samples
- Methods will be changed slightly:
  - MRSA isolation /identification- new PCR multiplex recomended to detect both *mecA* and $mecA_{LGA251}$
  - Might include *S. pseudintermedius*
  - *Spa* typing of isolates optional
- Expected shipping around middle October
- Deadline for result until December
- Analysis of results/reporting spring 2013

Future perspectives

- Discussion
- Future of EQAS for MRSA?
- Samples/Methods


Thank you!