Report of the 2nd External Quality Assurance System on Isolation, Identification and Typing of Methicillin resistant *Staphylococcus aureus* (MRSA) from Swab Samples, year 2010

Lina Maria Cavaco
Rene Hendriksen
Susanne Karlsmose
Frank Møller Aarestrup

DTU Food
National Food Institute

Community Reference Laboratory Antimicrobial Resistance

DTU Food
National Food Institute

European Union Reference Laboratory – Antimicrobial Resistance

REPORT OF THE 2ND EXTERNAL QUALITY ASSURANCE SYSTEM ON ISOLATION, IDENTIFICATION AND TYPING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM SWAB SAMPLES, YEAR 2010

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National Food Institute
Technical University of Denmark
Kemitorvet 204, st
DK-2800 Kgs. Lyngby

Index

1. Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) have recently emerged in food producing animals. The emergence of this potential zoonotic pathogen in animals has raised the concerns of potential transmission to humans from the animal reservoirs and consequences on Public Health. In 2008, the European Commission has taken a decision of screening the population of pig farms in Europe for this new emerging pathogen, taking this screening into the framework of the baseline screening for *Salmonella* in pig farms (European Commission Decision 2008/55/EC). This screening has been performed in most European Countries by laboratories appointed by the national authorities and according to a common protocol defined by the EC.

The European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) has taken part in the development and support of the laboratories in this task by providing assistance in setting up the methods for isolation, identification and typing of MRSA, and providing training in the specific methods on individual basis and on an MRSA training course that was organized in April 2009.

In June 2009, the EURL-AR in addition to the External Quality Assurance System (EQAS) already performed, launched an MRSA specific EQAS, to enhance the capacity of the laboratories in MRSA isolation, identification and typing. This EQAS aimed at the identification of potential problems and identification of focus areas for training/education that might improve data quality in future European studies focusing on MRSA epidemiology.

In June 2010, the EURL-AR launched an MRSA EQAS trial on dust samples. However, due to stability issues, the EQAS was cancelled on the 28th of June and no results were collected. Therefore, a second trial was prepared in October, using swab samples. All information contained in this report concerns only the October MRSA EQAS trial.

This was the second EQAS performed on MRSA isolation, identification and typing. As the previous EQAS, this included isolation steps besides the identification and also a molecular component; detection of methicillin resistance. The detection of the *mecA* gene was included as a mandatory part for confirmation of MRSA. An optional sequence based typing module based on *spa* typing was also offered. The *spa* typing trial aimed at increasing the capacity of laboratories by performing a standard typing method which can be easily performed, and which results are comparable and useful to assess the epidemiology of MRSA in Europe.

At the starting point of this EQAS, the laboratories should have set up the methods for its performance. In this view, the EQAS results may also be used to assess, although retrospectively, the quality of the performances of the laboratories on the Baseline survey in 2008. Therefore, the EQAS, may be used to assess the quality of data provided to EFSA, but also to prepare the laboratories for performing correctly the isolation, identification and molecular detection of MRSA in any future studies.

No thresholds have been set in advance to evaluate the performance of the participating laboratories, nor classify the results of this EQAS. This discussion will be taken at the annual workshop, if the European Commission decide to continue the monitoring of MRSA in 2012.

The MRSA EQAS was organized by the National Food Institute (DTU Food), Lyngby, Denmark and the verification/confirmation of the strains used in the preparation of the test samples was performed at the Statens Serum Institute in Copenhagen, Denmark.

The Individual laboratory data is kept confidential and is only known to the participating laboratory, the EQAS Organizer (DTU Food) and the European Commission. All summary conclusions are made public.

2. Materials and Methods

2.1 Participants

A pre-notification (App 1) inviting the participants to the MRSA EQAS 2010 was issued by e-mail to the EURL-AR network on October 5, 2010 and National Reference laboratories (NRL) that have participated in the baseline studies on MRSA. Additionally, the laboratories that participated in the MRSA baseline studies in 2008 and participated in the MRSA EQAS in 2009 were invited to participate using the same pre-notification.

All participants were included in a participant list (App 2) before preparation and shipping of the samples. Participation was free of charge but each laboratory was expected to cover expenses associated with their own analysis.

2.2 Samples

Eight swab samples were prepared and dispatched for isolation of MRSA, identification, and typing of the obtained isolates. The samples were artificially

prepared to contain either methicillin-resistant, methicillin-susceptible *S. aureus* and/or coagulase negative staphylococci strains, besides a mix of *S. aureus*, *Enterococcus faecalis* and *Escherichia coli* which was intended to mimic the normal flora that would be present in a nasal swab collected from an animal.

2.2.1 Preparation of samples:

The background flora was prepared by using 0,5 Mc Farland bacterial suspensions of *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 which were diluted to 10^{-3} in saline and mixed in equal parts. The swabs were dipped into this mixture before preparation of the samples. The expected amount of each bacterial culture in the mix was about 10^5 CFU/ml and was confirmed by performing serial dilution and colony forming unit counts in duplicate from all bacterial suspensions.

The MRSA isolates chosen for the MRSA EQAS (EURL-MRSA 2B.1- EURL-MRSA 2B.8) were prepared in advance and subcultured in week 40 and re-subcultured on the day before sample preparation. For the sample standardization, suspensions equal to McFarland 0.5 were prepared in saline tubes of the relevant isolates to contain about 10^8 CFU/ml. The suspensions were further diluted 1:10 to 10^7 CFU/ml of which 100 µl were used to inoculate the transport media contained in the tubes with the swab samples. The expected inoculum was about 10^6 CFU per sample and was confirmed by performing serial dilution and colony forming unit counts in duplicate from all isolate suspensions.

The strains used for the EQAS were selected from the strain collections at DTU-Food and the identification were confirmed by PCR and 16S rDNA. Furthermore, the *spa* type of the MRSA isolates was determined by PCR and sequencing and assigned according to the *spa* server using Bionumerics (App 3). Identification and the *spa* type of the selected isolates were verified and confirmed by parallel analysis conducted at SSI, Copenhagen, Denmark. The results obtained after preparation of the contaminated swab samples were confirmed at DTU-Food, by testing (isolation, identification and typing) of three sample sets just after preparation. Stability testing was performed in one set of eight samples per week, during one month. Further tests were performed after 5 weeks and 2.5 months using the recommended protocol for isolation and identification of MRSA, confirming that the results of the isolation/identification of MRSA from the eight EQAS samples were repeatable.

2.3 Isolation and identification of MRSA from swab samples

The protocol for isolation and identification of the MRSA isolates contained in the swab samples was made available on the EURL website (www.EURL-ar.eu) (App.4).

2.4 Typing

The isolates used for the sample preparation were typed by a single locus sequence typing method - *spa* typing (Shopsin *et al.* 1999) and the results were verified by parallel *spa* typing conducted at the SSI. Furthermore, the *spa* typing results were verified a third time in the isolates obtained from prepared samples.

The laboratories with capacity to perform *spa* typing were invited to include also the *spa* typing results to the MRSA EQAS database, which were evaluated for accuracy against the expected *spa* types.

2.5 Distribution

The samples were prepared just before shipment to avoid problems with stability and were kept at room temperature. The tubes containing the swab samples in transport media were enclosed in double pack containers (class UN 6,2) and sent to the selected laboratories according to the International Air Transport Association (IATA) regulations as “Biological Substance category B” classified UN3373. The parcels were dispatched from DTU-Food October 26, 2010 to October 27.

2.6 Procedure

The laboratories were instructed to download the protocol and test forms (App. 4 and 5), from http://www.eurl-ar.eu

The EQAS protocol was based on the EC baseline protocol for isolation of MRSA from dust samples, however, with slight changes in the volume of the media needed in the enrichment steps, since the samples were sent as swab samples in a tube containing transport medium and not large dust swabs. The method recommended for the identification of the isolates was the EURL multiplex PCR which would allow identification of *S. aureus* and also identify the *mecA* gene, mandatory for the classification of isolates as MRSA (App 4).

After completion of the tests, the laboratories were requested to enter the obtained results into an electronic record sheet in the EURL-AR web based database through a secured individual login, or alternatively send the record sheets from the enclosed

protocol by fax to DTU Food (App 5). The database was activated 26 October, 2010 and closed January 17, 2011.

The samples were categorised as positive or negative in function of the isolation and identification of MRSA isolates and no quantitative variable was introduced. Negative samples could be subdivided into different categories (negative sample, MSSA, MRCNS, CNS), however they would be grouped as negative. Final interpretations of the results obtained were compared to the expected results and classified as correct or incorrect, without further classification of the deviation.

Optionally, results of *spa* types were requested for typing the positive MRSA samples. In this case, the participants would be invited to choose the obtained *spa* type from a pick list, and the typing result would be evaluated against the expected *spa* type and classified into correct or incorrect.

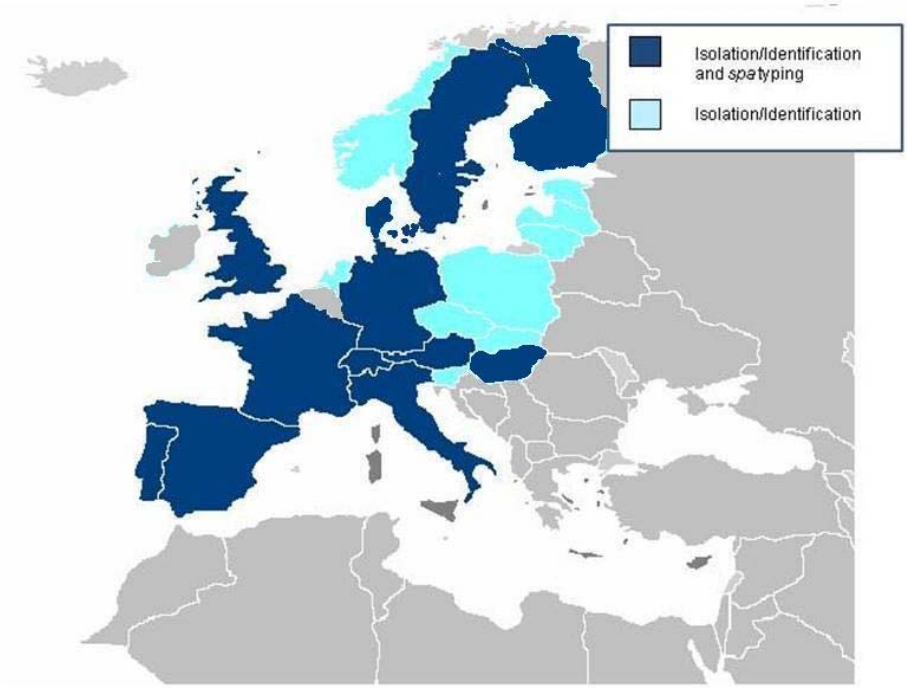
3. Results

A total of 23 laboratories, of which 17 were NRLs, six were non-NRLs and laboratories from Switzerland and Norway responded to the pre-notification, and were enrolled in the EQAS. Additionally, two NRLs which did not participate in the baseline study and two additional laboratories(which are not NRLs and did not participate in the baseline studies) participated in the EQAS, however, the results obtained at these particular laboratories were excluded from this report and will not be further mentioned.

When the deadline for submitting results was reached, 21 laboratories in 21 European countries (including Switzerland and Norway) had uploaded data (one of the NRLs, and one of the non, NRLs was unable to perform the testing and did not upload data due to technical problems).

The following countries provided data to at least one of the EQAS components (also shown below in Figure 1): Austria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, the Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and UK.

Figure 1- Participating Countries



Participating countries are marked in light blue for laboratories participating in MRSA isolation and identification and dark blue for those additionally participating in the *spa* typing.

3.1 Methods used by EQAS-participants

Of the 21 laboratories submitting results all (100%) of the laboratories participated in the MRSA isolation and identification module whereas only twelve (57%) participated in the optional *spa* typing module offered.

Information on the methods used for isolation, identification and typing was collected from the participants through the database.

Most laboratories (n=15) reported that isolation has been performed according to the protocol that was provided by the EURL for this EQAS and any changes in media, concentrations of antibiotics, etc. were noted. The differences reported by some

laboratories from the method described in the original protocol were: use of phenol red mannitol broth with 5 mg/l ceftizoxime en 75 g/l aztreonam in one lab and use of different selective plates for the selective isolation, including CHROMagar MRSA (Mast Diagnostica), BD MRSA Chrom Agar (BD), Oxacillin Resistance Screening Agar (ORSAB) (Oxoid); Chrom ID MRSA Agar (bioMérieux) and Baird Parker with a Penicillin disk, were reported. The species identification of *S. aureus* was performed using the EURL recommended PCR in most laboratories and the other referred methods for identification included other published PCR and RT-PCR methods (n=7) and/or biochemical reactions (n=4). The detection of the *mecA* gene was mostly performed by PCR, using the EURL recommended PCR primers (n=11), and using other published or In-house PCR methods in the remaining laboratories (n=10).

3.2 MRSA isolation and identification

A total of 168 tests (all laboratories have reported results for all the eight samples) have been performed and the overall result indicate that all 168 tests were correct, corresponding to 100% and no deviations were observed in any of the laboratories.

Table 1-. The overall performance of MRSA isolation and identification, 2010.

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

The individual laboratory results of this year's MRSA EQAS on isolation and identification of MRSA from swab samples, in general showed an excellent performance by all laboratories. In fact, all laboratories were able to correctly assign all

the tested samples as either MRSA positive or negative. Therefore we conclude that the samples did not cause any specific problems in this EQAS trial.

An internal control was common to both MRSA EQAS 2009 and 2010 trials. The internal control was sample EURL-MRSA 2B.7, which contained the same MRSA *spa* t075 strain sent the previous year as CRL MRSA 1.3. In 2009, we observed five deviations for the isolation and identification from the dust sample containing this strain. The deviations in 2009 were related to the failure in detection of this isolate from the dust sample possibly related to a slightly different morphology (smaller and light-blue colonies on Brilliance selective agar). However, the same strain was detected in the swab samples sent out in the MRSA EQAS 2010, indicating that even though the morphology on selective agar plates was slightly different the strain was isolated for further testing and confirmed as a MRSA. This indicates an improvement on the sensitivity of the isolation methods, even though direct comparison cannot be made since different sample types (dust vs swabs) were tested in the EQAS trials

3.3 *spa* typing of the MRSA strains

The *spa* typing module accounted participation of 12 laboratories, which have uploaded *spa* typing data (Lab #1, #2, #12, #14, #17, #19, #21, #33, #34, #35, #42, #53).

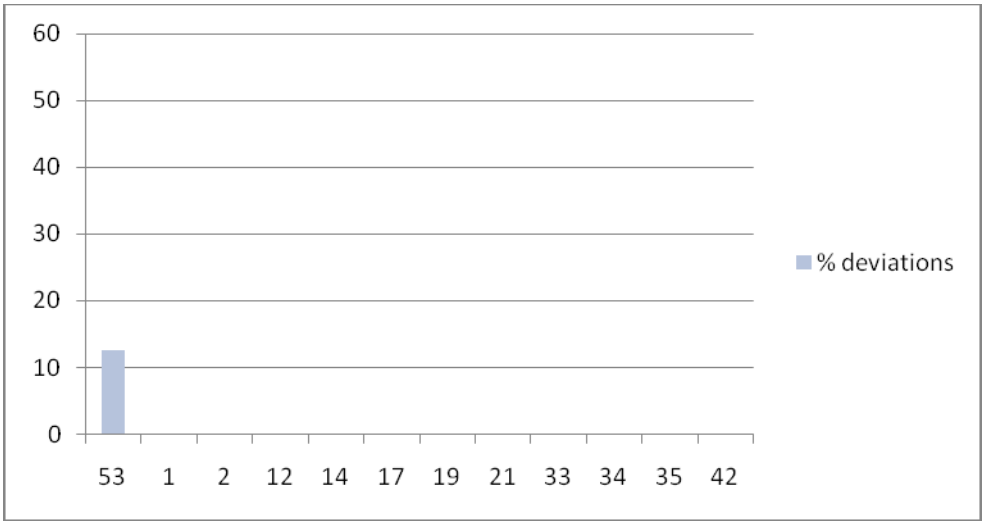
The reported results for *spa* typing included 81 tests and the general results show that 49 (98.8%) of results were considered correct whereas one (1.2%) deviation was observed. The observed deviation was caused by incorrect *spa* type assignment (n=1).

In this module, samples EURL-MRSA 2B.3, 2B.4, 2B.5, 2B.7 and 2B.8 were expected to be identified positive for MRSA .Therefore, optional *spa* typing was offered for the respective isolates found, whereas the remaining negative samples EURL-MRSA 2B.1, 2B.2 and 2B.6 would not render a positive MRSA isolate for typing and be therefore classified as N/A.

Most of the laboratories have only reported the results regarding the sample previously found positive for MRSA, whereas no more than five have reported N/A (not applicable) results for all the samples considered MRSA negative, as required. So, even though all participating labs have participated and correctly assigned the 8 samples in the isolation and identification module, the optional *spa* typing results were uploaded for 5-8 samples, in each participating lab.

By laboratory, the results show that the laboratories uploading *spa* typing data have in general obtained excellent results with eleven laboratories without any deviations and one laboratory with only one deviation (Lab #53).

Graph 1- Deviating results per participating laboratory. The laboratories were ranked by decreasing percentage of deviation.



The single deviation observed for sample EURL-MRSA 2B.7 (Lab #53) was due to reporting incorrect *spa* type. It is interesting to note that the reported *spa* type t899 would be correct if reported for sample EURL-MRSA 2B.5 suggesting a switch or contamination between samples.

Table 2. Results of the *spa* typing trial 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

4. Discussion

4.1 MRSA isolation and identification

In the 2010 EURL MRSA EQAS trial, the samples were prepared with strains selected based on recent findings and the quantity inoculated was rather high to facilitate the recovery of the MRSA isolates in the positive samples which reflects in the excellent overall results with no deviation.

The samples were sent out as swab samples containing background flora (around $3X10^4$ CFU/sample) and MRSA, MSSA and or CNS in an amount equivalent to 10^6 CFU/sample. All laboratories have been able to recover MRSA from the positive samples and correctly assign the sample status.

The results revealed that the isolation method was sensitive and the confirmation of MRSA was highly specific, independently on the use of the recommended methods or

modifications introduced in the selective isolation, identification or *mecA* detection steps.

4.2 *spa* typing

For *spa* typing, twelve laboratories have participated in the trial and the results were excellent, with eleven of the twelve laboratories showing no deviation and only one laboratory with one deviation. Four laboratories uploaded the results of the five positive MRSA, and three labs uploaded results for seven samples, whereas only five laboratories have submitted data for all eight samples.

The deviations observed do not demonstrate any problems with the *spa* typing method, but reflect only a small issue concerning possible cross contamination or sample management that might have caused the deviation observed in one *spa* type found incorrect.

5. Conclusion

In general, the results of the second MRSA EQAS 2010 demonstrate that most participating labs have set up the methods and are able to isolate MRSA from swab samples in a reliable fashion. Furthermore the identification of MRSA using molecular methods is performed successfully in all the participating laboratories, with excellent sensitivity and specificity.

The problems observed in the first trial regarding sensitivity of the isolation procedure on dust samples were overcome and excellent results were obtained with the swab samples tested in this trial. There is certainly a difference related to the type of sample which we should consider in this comparison, as it is known that the dust samples are more difficult to test and less stable, which might have lead to the lack of sensitivity observed in the 2009 trial.

The participation in the *spa* typing module has increased indicating that more labs have this method available among the laboratories in Europe. However, there were still nine laboratories participating in the MRSA EQAS 2010 which did not take part in this optional module. This might indicate that these laboratories have difficulties in performing *spa* typing and/or have not set up this methodology.

The results of this module show that *spa* typing is as expected a reproducible method which did not cause problems relative to the execution or interpretation in the participating laboratories. The only deviation observed was due to possible switching of samples or cross contamination, which again shows the need of controlling the analysis process in every step, to obtain a reliable final result.

6. References

European Commission, 2008. COMMISSION DECISION of 20 December 2007 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs to be carried out in the Member States. Decision 2008/55/EC in: http://ec.europa.eu/food/food/biosafety/salmonella/impl_reg_en.htm

Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol. 1999 Nov;37(11):3556-63.

Appendix 1- EURL-AR MRSA EQAS 2010 pre-notification

EXTERNAL QUALITY ASSURANCE SYSTEM (EQAS) 2010 FOR DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN SWAB SAMPLES

The EURL are pleased to announce the launch of the second MRSA-EQAS trial. The EQAS provides the opportunity for proficiency testing, which is considered an important tool for the production of reliable laboratory results of consistently good quality. This EQAS offers detection, identification and typing of MRSA from eight samples which contain a background flora and of which some will contain MRSA.

We would like to draw attention to the fact that due to stability problems with the dust samples we have modified the sample format, and in this trial we will send out eight samples consisting in swabs that will be sent out in tubes containing transport medium, which contain a background flora and may contain MRSA.

In the same way as in the previous round of this trial in June 2010, this EQAS was designed specifically to the laboratories which in the EU baseline studies performed the selective isolation and identification of MRSA from pig farms. Therefore, if your laboratory took part in the MRSA-EQAS 2009, you are automatically regarded as participant of the MRSA-EQAS 2010. You may contact the EQAS-coordinator, if you wish to inform of changes regarding the contact information.

If no laboratory from your country has participated in the MRSA EQAS 2009, you are welcome to contact us to sign up or to indicate another laboratory for the participation in this EQAS. Participation is free of charge for all NRL's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

Please remember to provide the EQAS coordinator with import permits or other information that can ease the parcel's way through customs (eg. specific text that should be written on the invoice). As means of avoiding passing the deadline we ask you to send us this information already at this stage. For your information, the contents of the parcel are UN3373 "Biological Substance Category B": Eight tubes containing swabs in transport media that might contain MRSA. The parcels are expected to arrive at your laboratory in the end of October 2010.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The samples will be shipped in October 2010. The new version of the protocol (adapted to the new sample format) will be provided via our website. This protocol, as in the previous trials will be based on the protocol used in the baseline studies and therefore we would like to ask you to have the same media referred in the baseline protocol and any materials or reagents needed for the selective isolation procedure, the confirmatory PCR method and *spa* typing (optional) readily available for processing the samples which will need to be processed right upon arrival.

Returning of results: Results must be returned to the National Food Institute, by December 31st, 2010. When you enter your results via a password-protected website, an evaluation report of your results will be generated immediately.

EQAS report: When the EQAS is concluded, the data will be collected in an overall report in which it is possible to see all participants' results in comparison. In the report the laboratories will be coded, thus ensuring full anonymity; only the National Food Institute and the EU Commission will be given access to un-coded results.

Any comments regarding the EQAS, please contact me by e-mail (licav@food.dtu.dk) or by fax (+45 32 88 63 41).

Sincerely, Lina Cavaco (**MRSA EQAS-Coordinator)**

Appendix 2- Participant list

reg	MRSA	*spa*	Institute	Country
x	x	x	Austrian Agency for Health and Food Safety	Austria
x			Veterinary and Agrochemical Research Centre	Belgium
x			NRL Antimicrobial resistance on food, Nacional Diagnostic and Research Veterinary Institute	Bulgaria
x	x		SVI Olomouc	Czech Republic
x	x	x	The National Food Institute	Denmark
x	x		Estonian Veterinary and Food Laboratory	Estonia
x	x	x	Finnish Food Safety Authority EVIRA	Finland
x	x	x	ANSES (French Agency for Food, Environmental and occupational Health & Safety).	France
x	x	x	Federal Institute for Risk Assessment	Germany
x	x	x	Central Agricultural Office, Veterinary Diagnostical Directorate	Hungary
x	x	x	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
x	x		National Diagnostic Centre of Food and Veterinary Service	Latvia
x	x		National Veterinary Laboratory	Lithuania
x	x		Animal Health Service Deventer	Netherlands
x	x		Veterinærinstituttet	Norway
x	x		National Veterinary Research Institute	Poland
x	x	x	Faculdade de Medicina Veterinária- UTL	Portugal
x	x		State Veterinary and Food Institute (SVFI)	Slovakia
x	x		National Veterinary Institute	Slovenia
x	x	x	Complutense University of Madrid	Spain
x	x	x	National Veterinary Institute, SVA	Sweden
x	x	x	Vetsuisse faculty Bern, Institute of Veterinary Bacteriology	Switzerland
x	x	x	The Veterinary Laboratory Agency	United Kingdom

Appendix 3- expected results

Sample ID	*mecA*	*spa* type	MRSA status sample
EURL-MRSA 2B.1	na	na	**Negative (blank)**
EURL-MRSA 2B.2	pos	na	**negative (MRCNS)**
EURL-MRSA 2B.3	pos	t034	**positive**
EURL-MRSA 2B.4	pos	t108	**positive**
EURL-MRSA 2B.5	pos	t899	**positive**
EURL-MRSA 2B.6	neg	t3855	**negative (MSSA)**
EURL-MRSA 2B.7	pos	t075	**positive**
EURL-MRSA 2B.8	pos	t011	**positive**

Appendix 4- Protocol for the MRSA EQAS

Protocol for MRSA EQAS 2010

For selective isolation, detection and typing of methicillin resistant *Staphylococcus aureus* (MRSA) from swab samples.

Introduction

One of the tasks as the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR)is to organise and conduct External Quality Assurance Systems (EQAS's). Usually the EQAS's have been focused on susceptibility testing, however, due to the recent concern about FA-MRSA (farm acquired methicillin resistant *Staphylococcus aureus*) detected in European farms and the recently performed baseline screening performed aiming at the detection of FA-MRSA in pig farms, we have now decided to continue the MRSA EQAS to evaluate the proficiency of the laboratories on the selective isolation procedures for detection of MRSA, including their confirmatory testing and *spa* typing.

As referred in the pre-notification, this EQAS is designed specifically for the laboratories involved in the EU baseline studies which performed the selective isolation and identification of MRSA from dust samples originated from pig farms and therefore these laboratories should perform the analysis of the

samples sent in this EQAS, using the same media and similar methods to those that have been used for sample analysis in the baseline studies.

In this MRSA EQAS the dust samples have been replaced by swab samples, which should be processed in a similar way, according to the following description in this protocol.

Objectives

The main objective of this EQAS is to support laboratories in assessing and if necessary improve the quality of selective isolation for detection, confirmatory testing and *spa*-typing of MRSA. Furthermore, to assess and improve the comparability of the baseline screening data reported to EFSA by different laboratories within the EU.

Outline of the MRSA EQAS 2010

Shipping, receipt and storage of strains

In October 2010 all EU appointed National Reference Laboratories (or the alternative laboratories that have processed the MRSA baseline sample analysis) will receive a parcel from the EURL-AR containing eight swab samples which contain a background flora and might contain MRSA (some samples will be negative, or contain other methicillin susceptible or resistant *Staphylococcus* strains).

NOTE: The samples should be received and processed immediately upon arrival, or as quickly as possible to assure the stability of the samples

Detection and identification of MRSA from swab samples

Selective enrichment and isolation

1. In the laboratory, open carefully the tubes containing the swab samples in transport medium (in a laminar air flow bench and using protective gloves) and inoculate each swab into a tube containing 10 ml of Mueller-Hinton broth supplemented with 6.5 % NaCl. Mix thoroughly.
2. Incubate at 37 °C for 16-20 h.

3. Take one millilitre of this first pre-enrichment culture and then inoculate into 9 ml Tryptone Soya Broth with 3.5 mg/L cefoxitin and 75 mg/L aztreonam. Mix thoroughly.
4. Incubate for 16-20 h at 37 °C.
5. Take one loop-full (10µl loop) of the latter selective enriched culture and streak onto a Brilliance MRSA Chromogenic Agar plate (Oxoid) and a blood agar plate.
6. Incubate 24-48 h at 37 °C.
7. Observe the colony morphology (size and coloration) of the colonies obtained on the Brilliance MRSA Chromogenic Agar plate, choose up to five blue colonies indicative for being MRSA and isolate them **from the MRSA selective plate** onto a new blood agar plate. The additional blood agar plate obtained from inoculation of the selective broth is used for parallel observation with the MRSA plate to double-check the colony morphology (to observe typical *S. aureus* morphology and haemolysis), but should not be used for isolation.
8. Incubate the new blood agar plates containing the isolates during 24h at 37ºC.
9. Observe the colony morphology of the isolates on the blood agar plate (colour, appearance, haemolysis). Check for purity and re-isolate if necessary.
10. Presumptive isolates of methicillin resistant *Staphylococcus aureus* should at this stage either be stored under appropriate conditions (–80°C) for later identification and characterisation or processed immediately.

Identification of MRSA

Presumptive MRSA isolates should be confirmed as *Staphylococcus aureus* isolates carrying the *mecA* gene by PCR. There is no need to perform other screening methods (such as screening with either oxacillin or cefoxitin) if the isolates have been obtained from selective isolation, thus, the presence of the *mecA* gene can be directly confirmed by PCR amplification. The species identification is simultaneously confirmed by using a multiplex PCR protocol including the amplification of 16S rDNA (internal control of the PCR reaction), nuclease (specific for *Staphylococcus aureus* species) and *mecA*-gene, which should be performed according to the EURL-AR recommended protocol (http://www.eurl-ar.eu/data/images/meca-pcr_protocol%2006.02.08.pdf).

Spa typing

Spa typing of the MRSA isolates may be performed additionally if the laboratory has the capacity to perform and analyse the *spa*- typing data. In case you decide to include *spa* types in the data submitted, these will be evaluated on the accuracy of the *spa* typing.

Reporting of results and evaluation

Fill in your results in the enclosed test form. Please enter your results into the interactive web database. Please read the detailed description below before entering the web database. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print an evaluation report of your results. Please submit results by latest December 31st 2010.

If you do not have access to the Internet, or if you experience difficulties entering the data, please return results by e-mail, fax or mail to the EURL-AR.

All results will be summarized in a report which will be made available to all participants. The data in the report will be presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions are public.

If you have any questions, please do not hesitate to contact the MRSA EQAS Coordinator:

Lina Cavaco

The National Food Institute

Technical University of Denmark

Kemitorvet Building 204 Ground floor,

DK-2800 Kongens Lyngby

Denmark

Tel: +45 3588 6269

Fax: +45 3588 6341

E-mail: licav@food.dtu.dk

How to enter results in the interactive database

Please read this passage before entering the web page. Before you go ahead, you need your test form.

You are able to browse back and forth by using the forward and back keys or click on the EURL logo.

You enter the EURL-AR EQAS 2010 start web page (http://thor.dfvf.dk/crl) then write your username and password in low cases and press enter. Your username and password is the same as in the previous EQAS's arranged by The National Food Institute. If you have problems with the login please contact us.

Click on “MRSA tests” to start entering your data regarding the MRSA EQAS.

Please read carefullly the instructions on the webpage and start by answering to the questionnaire on the work performed in your laboratory relative to MRSA by clicking on “General MRSA questionnaire”.

Please choose the options that more correctly describe your work on MRSA and before you leave this page click on “Save page” which will take you back to the previous menu.

Then, we will ask you to fill in the methods used in a second page which you will reach by clicking on “ Methods for MRSA test samples.”

In the next page you navigate to fields with the Tab-key and mouse.

Fill in what kind of method you have used for the selective isolation and confirmation of MRSA in this EQAS.

Click on "Save and go to the next page”

In the data entry pages for each sample “EURL-MRSA 2B.1, …”, you enter the obtained results for each of the MRSA EQAS samples.

If you wish so, you will have the option to include the *spa*-typing results.

If you did not perform *spa* typing leave the field blank , in case you have obtained an isolate but it is not a methicillin resistant *Staphylococcus aureus*, choose “not applicable (N/A)”, otherwise if you have performed the *spa* typing, choose the *spa* type from the list. Click on "save and go to next page" to navigate to the next sample results, until you finish to upload all your data.

From the last result sheet you get into the general menu, from where you can review the input pages, approve your input and finally see and print the evaluated results:

Browse through the pages and make corrections if necessary. Remember to save a page if you make any corrections. If you save a page without changes, you will see an error screen, and you just have to click on "back" to get back to the page and "go to next page" to continue.

At the end, approve your input. Be sure that you have filled in all the results before approval, as YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database, but allows you to see the evaluated results.

See the evaluated results. You can print each page. *You may have to choose a smaller text* size to print the whole screen on one piece of paper. In the Internet Explorer (or the Internet program you may have), you click on "view", "text size" and e.g. "smallest".

Appendix 5 – Example of test forms MRSA EQAS 2010

Test forms MRSA EQAS 2010

Test form- MRSA questionnaire

Name:
Name of laboratory:
Name of institute:
City:
Country:
E-mail:
Fax:

Did you participate in the EU- baseline study for MRSA?

Yes ☐ /No ☐

How many samples did you process in 2009 for MRSA detection? (Choose only one option)

☐ less than 50
☐ 50-100
☐ 101- 200
☐ 201-400
☐ more than 400

Which kind of samples did you process in your laboratory for MRSA detection in 2009?

☐ dust swabs
☐ nasal swabs
☐ skin swabs
☐ faecal samples
☐ other matrices Specify:

Which was the origin of the samples processed for MRSA detection in 2009?

☐ from humans
☐ environmental
☐ from animals if you check this answer, please answer the next question
☐ other . Specify:

The samples processed from animals included samples obtained from which species:

☐ pigs
☐ cattle
☐ poultry
☐ pets
☐ other . Specify:

Any other Comments:

TEST FORM – MRSA methods

Method used for selective isolation of MRSA in this EQAS:

☐ Selective isolation procedure using the exact protocol

☐ different pre-enrichment

Salt concentration used in the Müller Hinton broth: %

Other medium:

☐ different selective enrichment

Cefoxitin concentration: mg/L

Aztreonam concentration: mg/L

Other modifications: medium antimicrobials or concentrations:

☐ different selective agar plates

Different agar brand**:**

☐ different incubation conditions

Specify: pre enrichment °C/ h;

selective enrichment °C/ h;

selective plating °C/ h

Method used for confirmatory testing of MRSA (choose only one option)

☐ PCR using the EURL recommended multiplex PCR protocol
☐ modified protocol but same multiplex PCR primers
☐ other published PCR method
☐ in- house PCR method

Describe if you did not use the EURL recommended method and justify your choice**:**

Method used for confirmation of species identification (choose only one option)

☐ PCR using the EURL recommended multiplex PCR protocol
☐ biochemical methods
☐ other published PCR method
☐ in-house PCR method

Describe if you did not use the EURL recommended method and justify your choice**:**

TEST FORM

Entry data Sample EURL-MRSA 2B.1

Please describe the results you have observed regarding this swab sample:

Growth in pre-enrichment:

Yes ☐/ No☐

Growth in selective enrichment:

Yes ☐/ No ☐

Growth in MRSA selective plates:

Yes ☐ / No ☐

Please describe the growth observed on MRSA selective plates? (choose only one option)

☐ Mixed culture containing typical colonies
☐ Mixed culture without typical colonies
☐ Pure culture of typical colonies
☐ Pure culture without typical colonies
☐ No growth

Results of confirmatory PCR and species identification: (choose only one option)

☐ no isolates tested, sample negative
☐ *mecA* positive, but not *S . aureus* (MRCNS)
☐ *mecA* negative and not *S . aureus* (CNS)
☐ *mecA* negative, *S. aureus* (MSSA)
☐ *mecA* positive, *S. aureus* (MRSA)

***Spa* Typing (optional): *spa* type** (choose only one option)

☐ Not performed
☐ Not applicable (N/A)
☐ t011
☐ t021
☐ t034
☐ t075
☐ t108
☐ t127
☐ t337
☐ t524
☐ t571
☐ t899
☐ t1333
☐ t1730
☐ t1793
☐ t2510
☐ t2922
☐ t899

National Food Institute
Technical University of Denmark
Kemitorvet, 204
DK - 2800 Kgs. Lyngby

Tel. 35 88 70 00
Fax 35 88 70 01

www.food.dtu.dk

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