DTU Food
National Food Institute

Laboratory Protocols

MRSA Training Course

Isolation of MRSA from dust samples

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Background

A new strain of MRSA (ST398) has recently been detected in production animals in several Member States of EU.

In particular, pigs have been recognised as an important source of infection for pig farmers or their relatives by direct contact with pigs. Infection with the new strain may also enter hospitals as previously MRSA did in several Member States.

In order to increase awareness and to assess whether it is necessary to take measures to detect and control MRSA in order to reduce their prevalence and the risk they pose to public health, comparable data on the percentage of MRSA (ST398) infected holdings of breeding pigs in the Member States are needed. A European baseline screening has been performed in 2008 to gather information on prevalence of MRSA in pig farms in Europe

References

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:014:0010:0025:EN:PDF

http://www.crl-ar.eu/mrsa_baseline_study.html

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Procedure

Theory/comments

Sample collection

1. Five dust samples shall be gathered using five dry sterile swabs of about 500 cm^2 each from five of the 10 pens.

By taking dust samples from several pens the samples will represent the herd.

2. These five pens shall be chosen in a way that breeding pigs in different production stages are included.

3. For each pen dorsal surfaces of pen partition walls shall be swabbed using clean gloves.

Always use gloves in sample collection and processing. It is important that human carriers do not transmit MRSA to the samples.

4. In case there is not enough dust present, then ventilator ducts etc. shall be sampled in addition.

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5. After use, the soiled swab shall be placed in a sterile plastic bag.

6. Each sample and its sample form shall be labelled with a unique number, which shall be used from sampling to testing

7. The creation of aerosol in the building shall be avoided during sampling.

Procedure	**Theory/comments**
Transport of samples	
1. Keep samples at constant temperature between + 2 °C and 25 °C (room temperature) and free of external contamination during storage and transportation.	
2. The samples shall be sent to the laboratory as quickly as possible and reach the laboratory no later 10 days after sampling.	The MRSA can survive for at least two weeks in dust samples
Receipt of samples	
1. Samples arriving 10 days after sampling shall be discarded unless bacteriological examination can be started within 13 days.	It is not necessary to keep the samples refrigerated
2. At the laboratory, samples shall be kept at a constant temperature between + 2 °C and 25 °C until bacteriological examination, which shall be carried out within 13 days after sampling.	

Procedure

Sample analysis

1. Enrichment: In the laboratory the five dust swabs shall be pooled in a 300 ml of Mueller-Hinton broth supplemented with 6,5 % NaCl and incubated at 37 °C for 16-20 h.
2. One ml of the enriched M-H broth is inoculated into 9 ml Tryptone Soya Broth + 3,5 mg/l cefoxitin and 75 mg/l aztreonam and incubated for a further 16-20 h at 37 °C.
3. One loop-full (blue 10ul loop) of TSB shall then be spread onto an Oxoid Brilliance MRSA Agar and a blood agar plate and incubated for 24-48 h at 37 °C.
4. Based on colony morphology and colour - presumptive MRSA colonies will be denim blue on the Brilliance chromogenic agar (Oxoid)- subcultivate up to five colonies indicative for being MRSA on blood agar.
5. If in doubt, the colonies can be analysed for peroxidase activity by dripping a drop of 3 % H_2O_2 on a smear of the colony onto an glass slide. Presumptive MRSA colonies will produce tiny bubbles.
6. Presumptive MRSA shall at this stage either be stored under appropriate conditions (–80 °C) for later identification and characterisation or processed immediately.

Theory/comments

It can be necessary to use more than 300 ml broth. The dust swabs shall be submerged in the broth.

A positive and negative control shall be analysed with the samples.

See appendix 1 for a photograph of the colony morphology.

Some enterococci strains can survive incubation in the selective enrichment broths and form small (denim)blue colonies on the Oxoid Brilliance MRSA Agar. They will be excluded by the peroxidase test.

Procedure

Identification of M R S A

1. Presumptive MRSA strains need always to be identified by the presence of the resistance determinant, the mecA gene, using PCR

2. To limit the amount of work it can be suitable to identify only one of the five presumptive MRSA isolates initially. If this isolate is identified as MRSA, it shall be stored.

3. No further testing of the remaining four isolates is required if the first isolate is identified as MRSA and they can be discarded.

4. If the first isolate is not identified as MRSA, the next of the initial five isolates shall be tested.

5. This process shall continue until one MRSA has been identified or all five isolates have been discarded

6. Alternatively, identification by PCR as a first step can be done on a pool of the five presumptive colonies from a sample.

7. In case of a positive PCR, the analysis shall be repeated on individual colonies to identify a positive colony

Theory/comments

PROTOCOL:
Multiplex PCR for the detection of the *mec*A gene

In practise it can be less work demanding (but more expensive) to analyse the five presumptive MRSA colonies at first by PCR.

APPENDIX 1

Composition and preparation of culture media and reagents

The media and reagents are available from several companies including Oxoid, BD, Merck and Difco. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also, the media should be prepared according to the manufacturers description if it differs from the description given here. Refer to Appendix 2 for a colour presentation of growth of MRSA on selective agar media and on BA.

Müller Hinton Broth with 6,5% NaCl

Formula	Gm/litre
Dehydrated infusion from Beef	300
Casein hydrolysate	17.5
Starch	1.5
Sodium Chloride	65
pH 7.3 +/- 0.1	

Tryptone Soya Broth

Formula	g/litre
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium Chloride	5.0
Dibasic potassium phosphate	2.5
Glucose	2.5
pH 7.3 +/- 0.2	

Selective Supplements

Formula	mg/ml
1. Cefoxitin in water	3.5
2. Aztreonam in dimethylformamide/methanol 1/1	75

Dispense 10µl of supplement 1 and 2 to each test tube with 9 ml TSB

Oxoid *Brilliance*™ MRSA Agar

Prepare according to the manufacturers description.

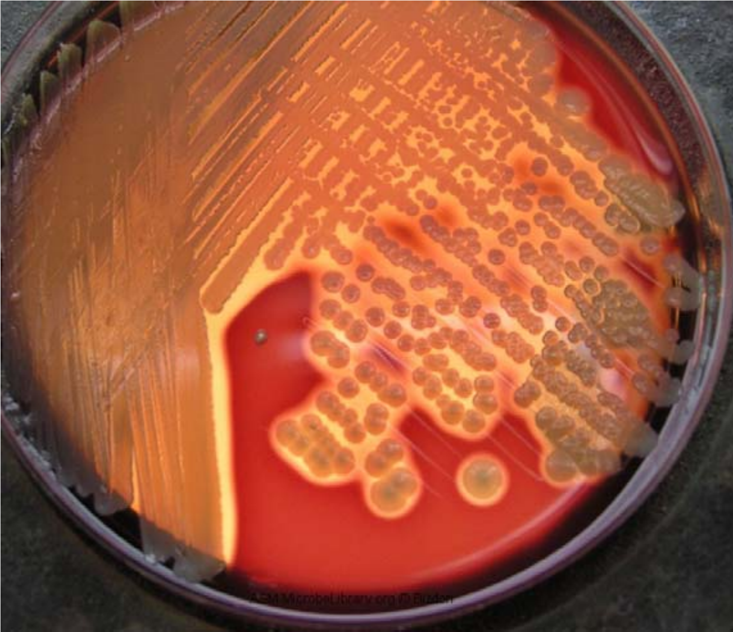
Blood Agar

Formula	g/litre
"Lab-Lemco" powdwer	10.0
Peptone neutralised	10.0
Sodium chloride	5.0
Agar	15.0
PH 7.3 +/- 0.2	

After cooling to 50°C add 7% of defibrinated sheep or horse blood.

APPENDIX 2

MRSA on Brilliance MRSA AGAR



S. aureus on BA

APPENDIX 3

Flow Diagram

Flow diagram for detection MRSA from dust samples