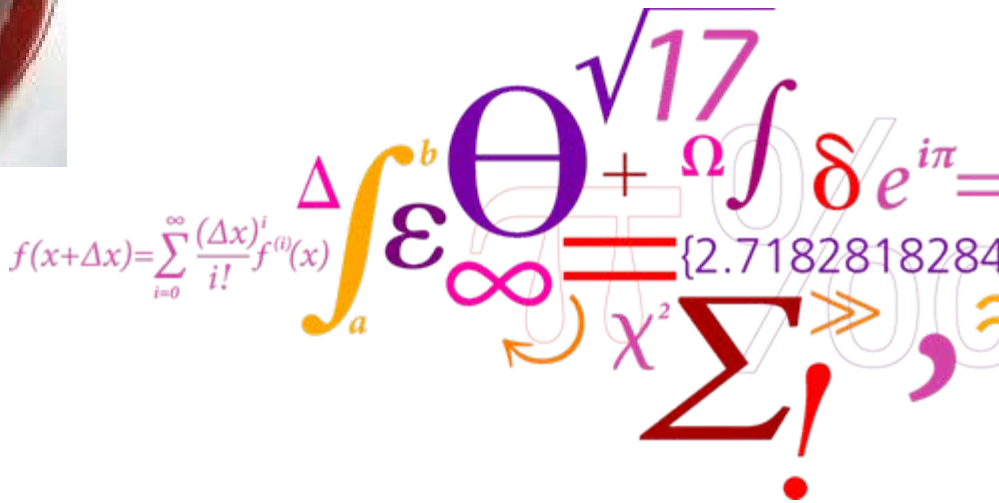
Methicillin resistant *Staphylococcus aureus* (MRSA)- Laboratory analysis of dust samples (Baseline methods)

Community Reference Laboratory Antimicrobial Resistance



DTU Food
National Food Institute

Detection of MRSA

- Routine susceptibility testing
  - Screening tests (oxacillin, cefoxitin)
  - Confirmation of presence of resistance determinant
    - Molecular detection of *mecA* (PCR)
    - Detection of PBP2a with imunoassays (rapid agglutination tests)
- Selective procedures for isolation

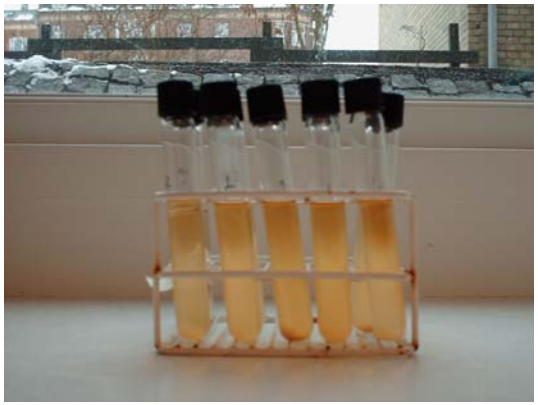
MRSA ST398- Sampling used in baseline study

- Samples: dust samples collected in swabs taken at the farms
  - Pooled sample of 5 soiled swabs of 500cm^2
  - Keep in a sterile and identified plastic bag
  - Analysis should start max 13 dys after sample collection

Protocol for selective enrichment of MRSA from dust samples

- Pre-enrichment:
  - The 5 Pooled dust swabs are unpacked and placed in sterile containers
  - 300ml of Mueller Hinton Broth with 6,5% NACl is added
  - Samples are incubated 16-20h at 37C

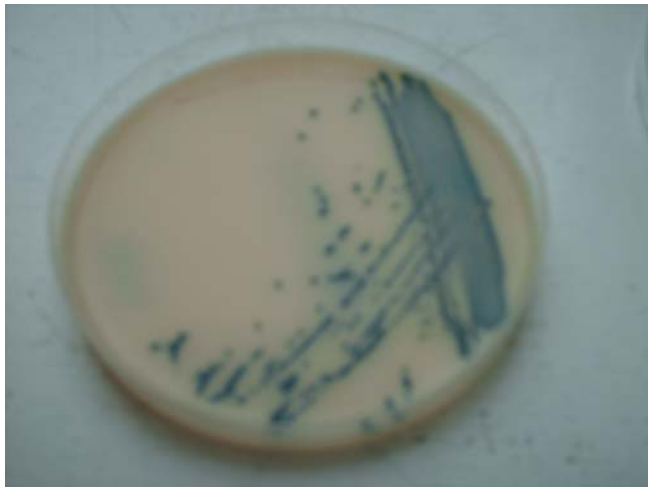
- Selective enrichment
  - 1ml of the previous culture is added into
  - Tryptone Soya Broth supplemented with:
    - 3,5 mg/L cefoxitin and 75mg/L aztreonam
  - Incubate 16-20h at 37C



Protocol for isolation of MRSA from dust samples

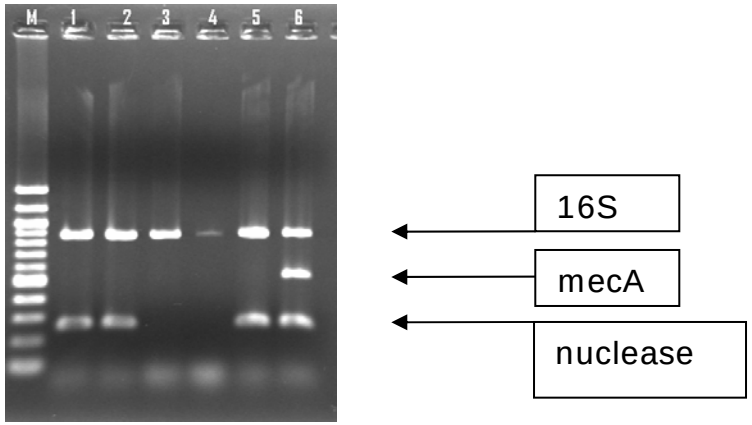
- Selective plating:
  - Plate 10ul (one blue loop) of the previous selective enrichment onto:
    - Brilliance MRSA Chromogenic agar (Oxoxid)
    - Blood agar
  - Incubate 24h at 37C

- Interpretation of results and isolationt
  - Check for **blue colonies** on the chromogenic MRSA plate and for typical hemolytic colonies on the blood agar.
  - Isolate 4 blue colonies onto a new blood agar plate
  - Incubate 24h at 37C



Confirmation of MRSA

- Extract DNA of the suspected MRSA isolates
- Confirm the *mecA* gene presence by PCR (multiplex is the recommended)
  - *16S*- confirm the PCR works
  - *mecA* – Confirm methicillin resistance
  - *Nuc- confirm ID* (only positive in *Staphylococcus aureus)*
- Chracterization of strains
  - Typoing
    - S*p*a typing
    - MLST typing (sample)
  - Susceptibility testing (optional)



Typing of MRSA

- Typing of strains
  - PFGE with *Sma*I most frequently (except for ST398 which is not typable with PFGE with *Sma*I) - very discriminatory method
  - Sequence based methods:
    - MLST typing
    - *spa* typing

- Typing of resistance determinant
  - *SCCmec* typing

Rotun *et al.,* 1999.

Questions