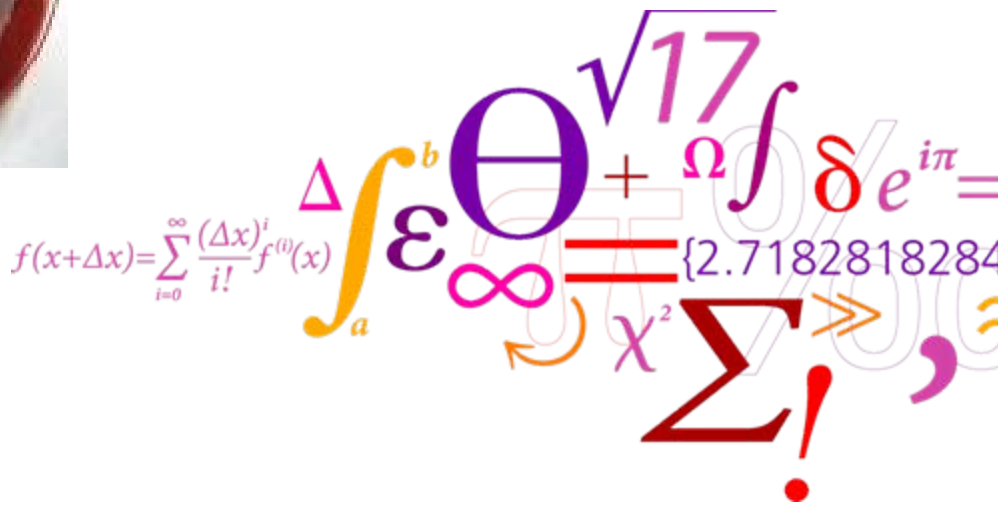
Methicillin resistant *Staphylococcus aureus* (MRSA)- Background and analysis

Community Reference Laboratory Antimicrobial Resistance



DTU Food
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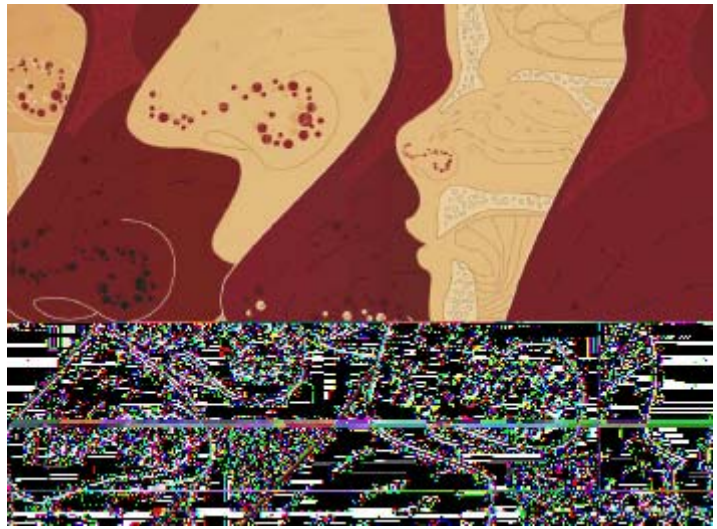
Outline

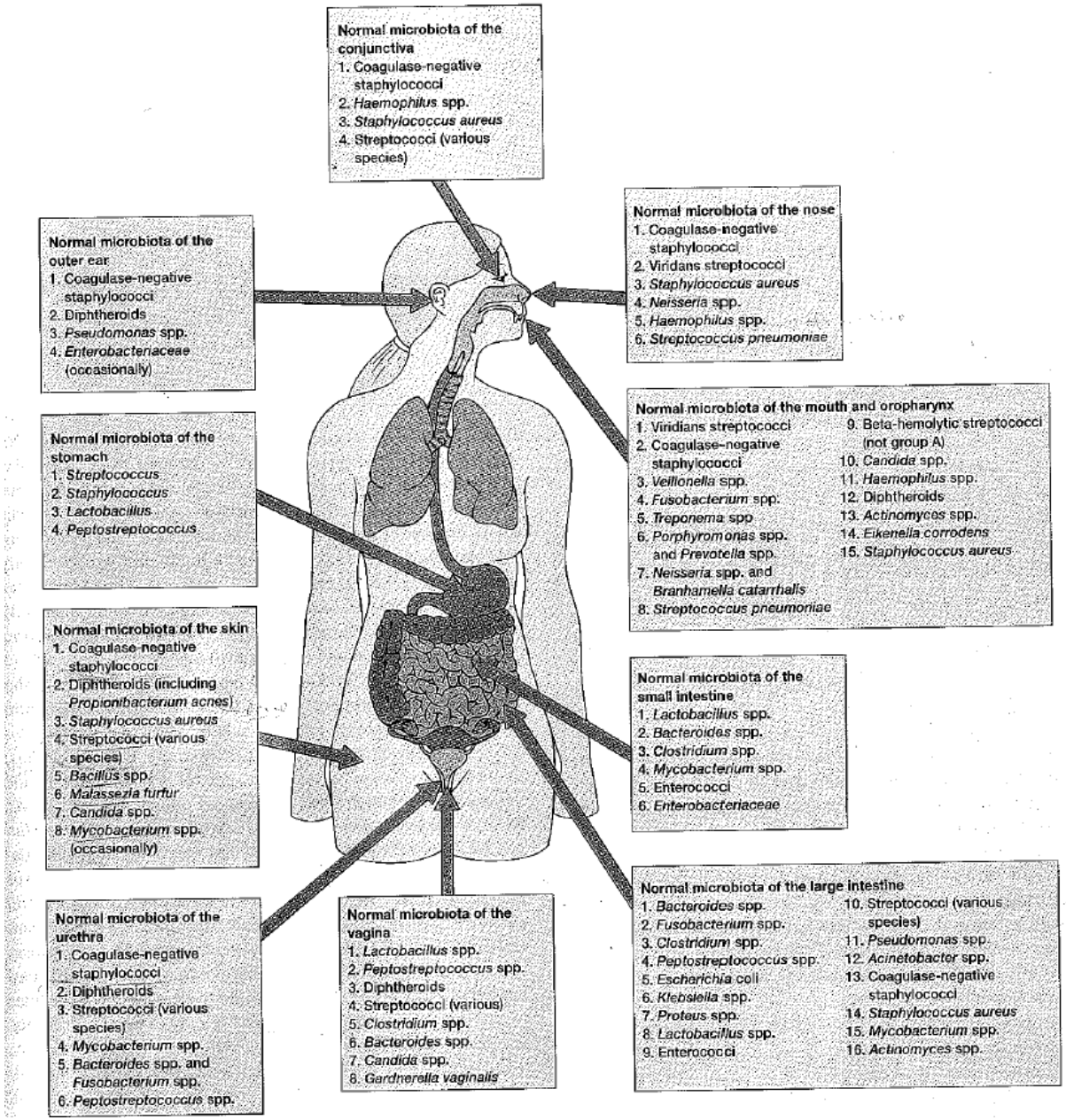
- *Staphylococcus aureus*
  - Classification
  - Ecology
  - Commensal vs pathogenic *S. aureus*

- MRSA
  - Definition
  - Resistance determinant and mechanism
  - MRSA detection
  - MRSA typing
  - MRSA ST398
    - Background
    - Importance
  - Epidemiologyl
    - Human clones
    - MRSA CC398
- Protocol for isolation from dust samples- Baseline study

Staphylococcus aureus

- Gram positive cocci
- Catalase positive
- Coagulase postive
- Inhabitants of skin and mucosa in animals and man
  - Principal carriage site- nasal cavity
  - Perineum
  - Axillae
- Oportunistic pathogens



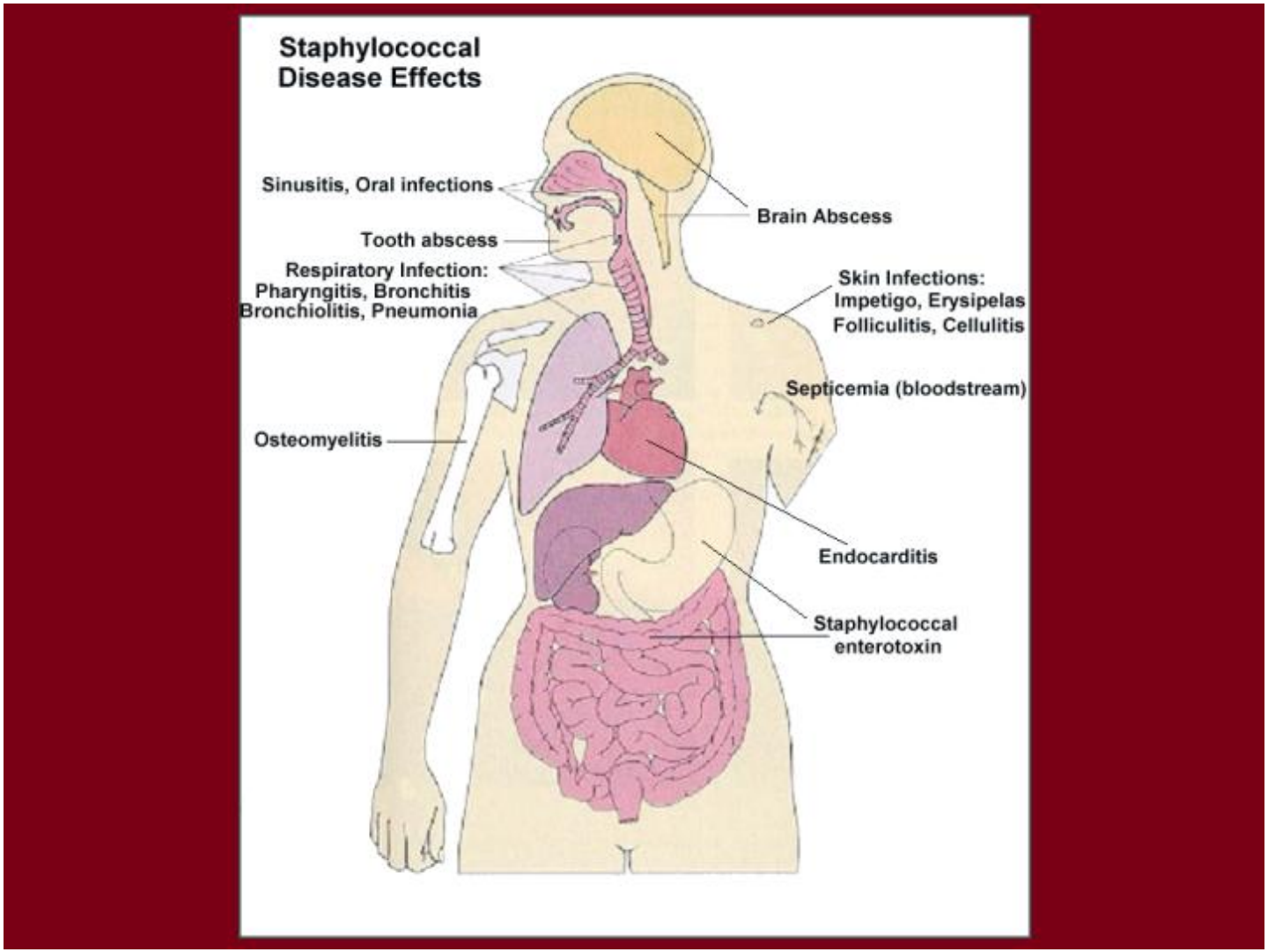


Prescott et al, 1996

Staphylococcus aureus as pathogen

- Humans
  - Skin infections
  - Wound infections
  - Soft tissue infections
  - Enterotoxinogenic strains- diarrhoea
  - Necrotizing pneumonia
  - Septicaemia

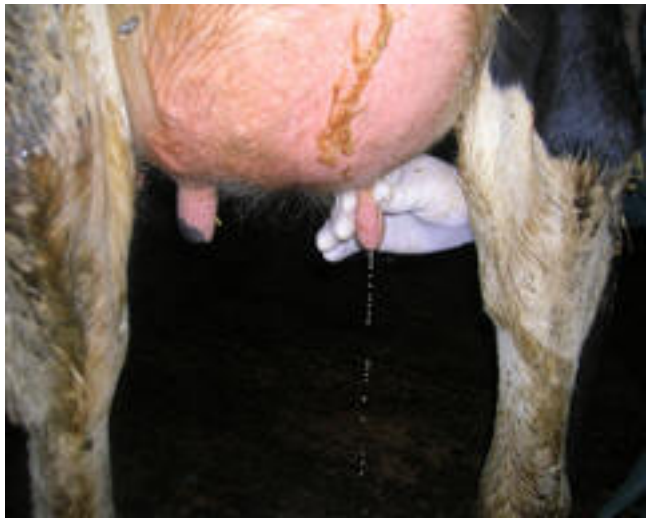
- Mostly Hospital acquired infections
- Some community acquired infections (increasing trend)



Source: http://www.freewebs.com/ccabral21/

Staphylococcus aureus as pathogen

- Animals
  - Mastitis in cows
  - Skin and soft tissue infections in pets
  - Skin infections in production animals



MRSA

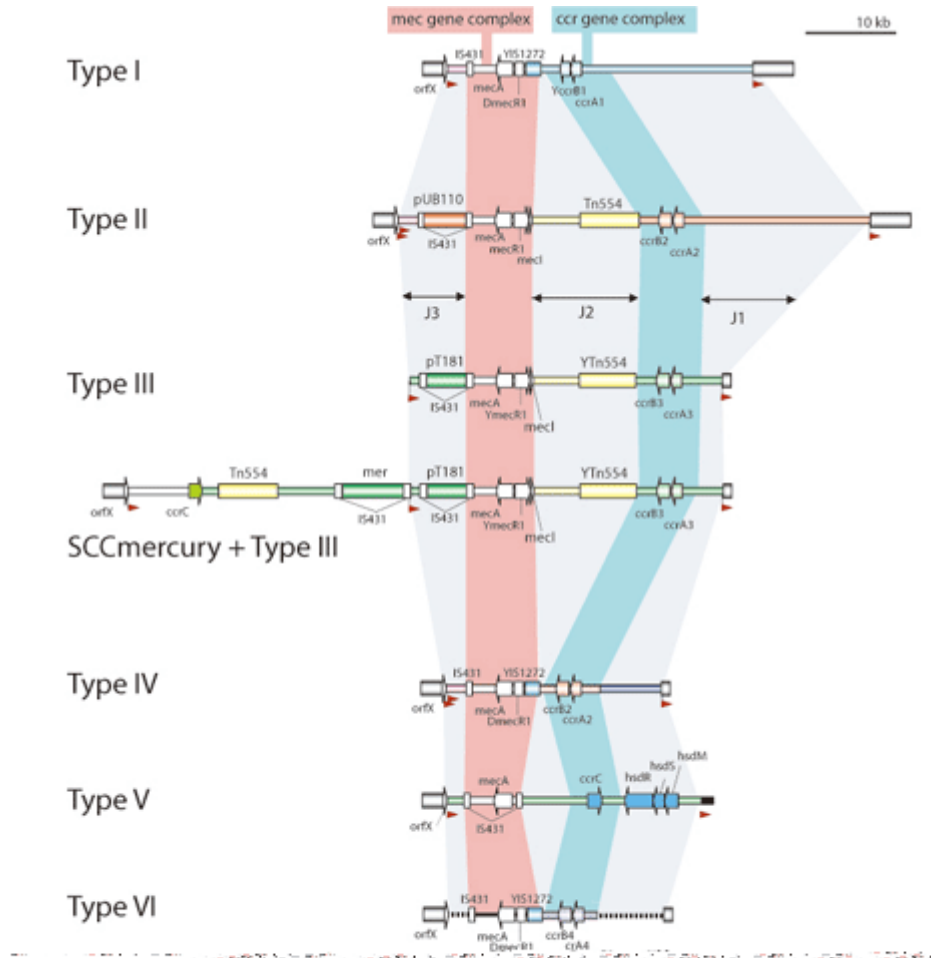
- Definition of MRSA

Methicillin resistant *Staphylococcus aureus*- derives from the first antistaphylococcal drug, methicillin and defines resistance to all beta-lactam drugs

- Resistance determinant:
    - *mecA* -PBP2a
    - mecA is part of the SCC mec cassette and inserted in the cromossome
    - Res penicillins, antistaphylococcal penicillins and cephalosporins

Resistance mechanism

- *mecA* target replacement PBP2a
  - PBP2a has low affinity to beta lactam drugs
  - Inibition of drug activity
- *mecA* located in the Staphylococcal cassette chromossome mec (SCCmec)
  - Large genetic element
  - several different SCCmec cassettes found in MRSA



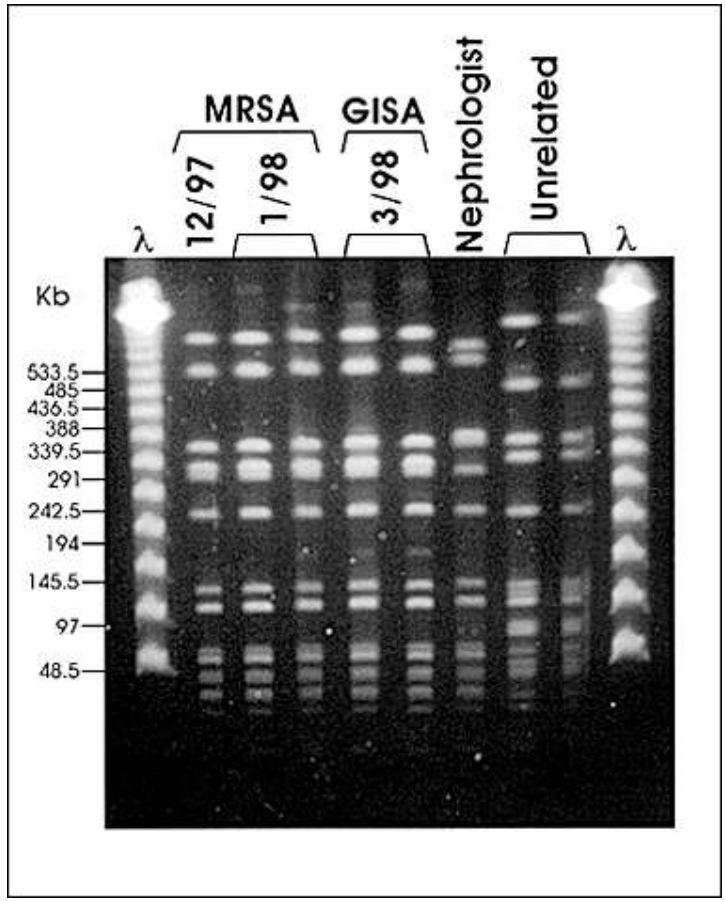
Chongtrakool *et al.*, 2006

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

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.

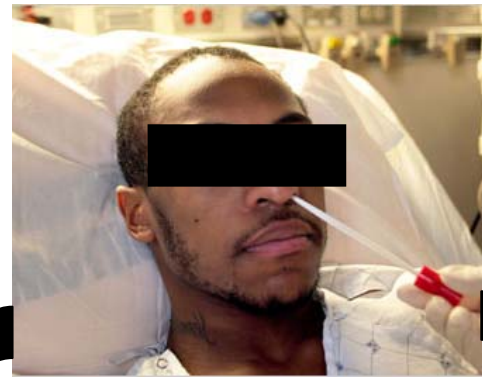
Epidemiology of MRSA

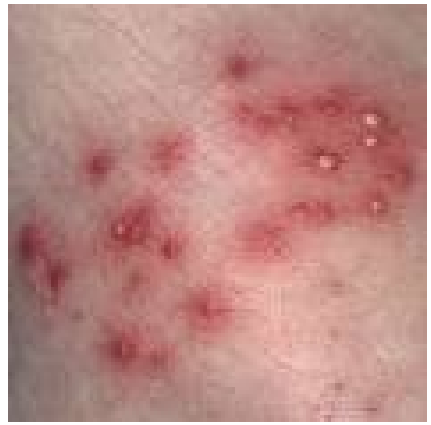
– Human clones

Companion animals

Transmission

Human carriers:





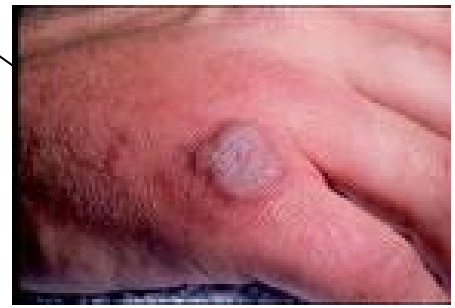
Epidemiology of MRSA

– CC398

Production animals

Transmission

Human carriage occupational:

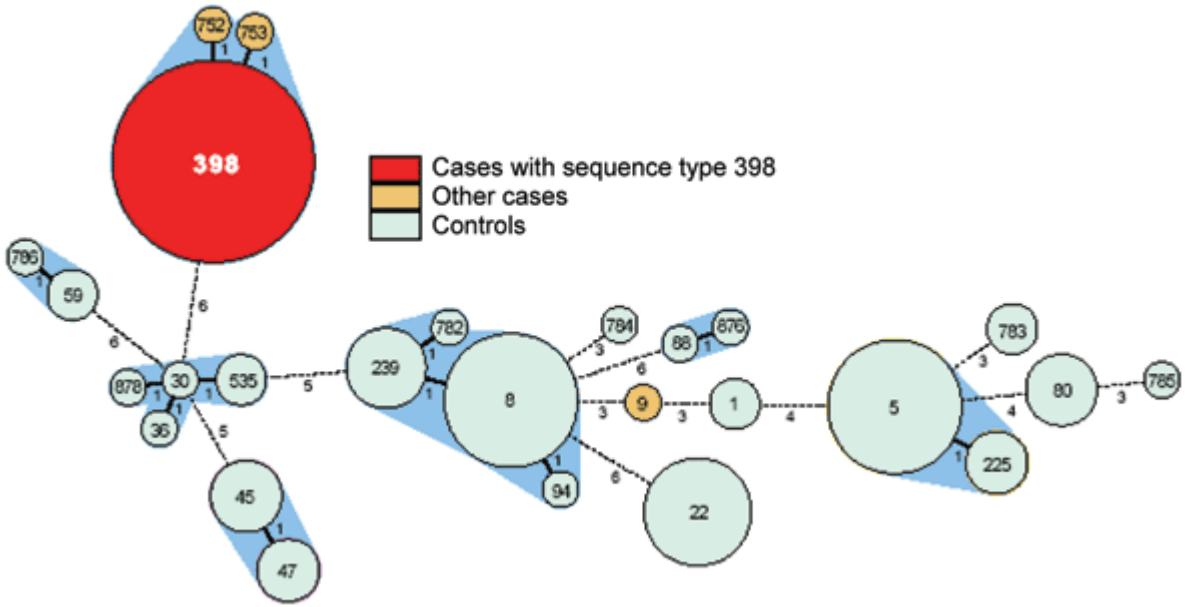


Particularities of MRSA CC398

- Seems to be mostly a ”pig strain”
- Adapted to animal hosts- not only pig but found in other species
- Non typable by PFGE with *Sma*I
- Widespread: several countries in Europe, Singapore, Canada
- Some strains PVL positive- more pathogenic
- Human carriage and or infections related to occupation

MRSA CC398

- CC 398 the designation of the clonal complex obtained by MLST typing
- ST398 is the predominant type



Source: Van Loo *et al*, 2007.

Recent developments

- Dutch studies indicate a large prevalence of MRSA in pig farms both in **dust** or **nasal swabs** in healthy animals
- Humans with contact to farms have increased risk of carriage
- Human related clinical cases are emerging
- Found also in exsudative epidermitis in swine
- MRSA ST398 detected in pork

Large reservoir for Community acquired (CA) MRSA infections even in Countries with low prevalence of MRSA

MRSA ST398 Possible implications

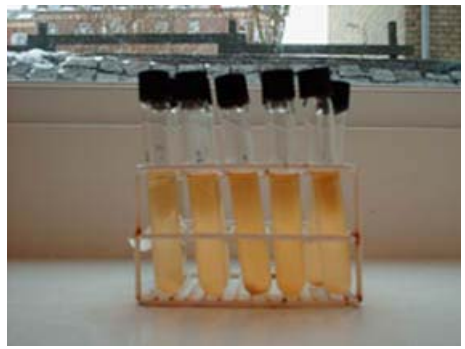
- Risk of human infections due to direct or indirect contact with animals
- Occupational hazard
- Need for data about epidemiology
- Assessment of Risk
- Evaluation of possibilities to combat spread with correct management options

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 days after sample collection

Protocol for selective enrichment of MRSA from dust samples

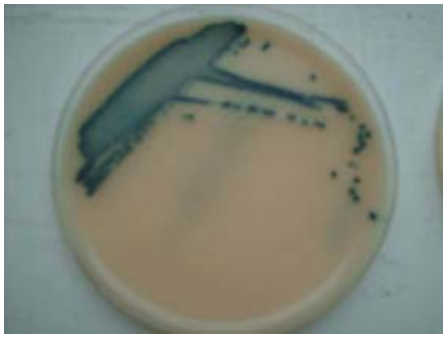
- Pre-enrichment:
  - The 5 Pooled dust swabs are unpacked and placed in sterile containers
  - 300ml of Mueller Hinton Broth with 6,5% NaCl is added
  - Samples are incubated 16-20h at 37C
- Selective enrichment
  - 1ml of the previous culture is added into
  - Tryptone Soya Broth supplemented with:
    - 3,5 mg/L cefoxitin and 75mg/L aztreonam
  - Incubate 16-20h at 37C



Protocol for isolation of MRSA from dust samples

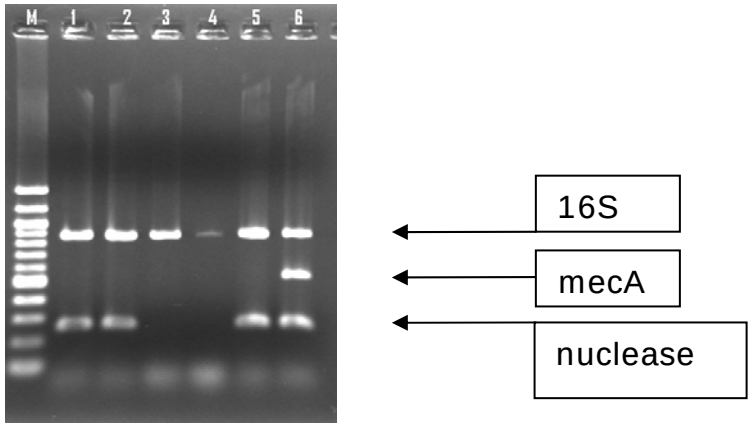
- Selective plating:
  - Plate 10ul (one blue loop) of the previous selective enrichment onto:
    - Brilliance MRSA Chromogenic agar (Oxoxid)
    - Blood agar
  - Incubate 24h at 37C

- 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)



Questions