Methicillin resistant Staphylococcus aureus (MRSA)

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**Staphylococcus aureus**

- Gram positive cocci
- Catalase positive
- Coagulase postive
- Inhabitants of skin and mucosa in animals and man
- Circa 25-30% carriers of *Staphylococcus aureus*, less than 2% carry MRSA
  - Principal carriage site- nasal cavity
  - Perineum
  - Axillae
- Opportunistic pathogens
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)
**Staphylococcus aureus** as pathogen

- **Animals**
  - Mastitis in cows
  - Skin and soft tissue infections in pets
  - Skin infections in production animals
  - Emergence also of MRSP (Methicillin Resistant *Staphylococcus pseudointermedius*)
Resistance in *S. aureus*

The first treatment:
penicillin (1930); end 40’s: 50% *S. aureus*
resistant)

1959: introduction methicillin; after 3 months 3/5000
R

2009: high frequent resistance against penicillines (by
enzyme penicillinase/beta-lactamase) => clavulanic
acid!)

Resistance against other groups of antimicrobials “not
extreme”
**S. aureus becomes MRSA.....**

- Methicillin resistant *Staphylococcus aureus* - derives from the first antistaphylococcal drug, methicillin and defines resistance to all beta-lactam drugs
- *contains mecA gene* in Staphylococcal Chromosome Cassette - SCCmec
- Resistant against all beta-lactam antimicrobials - penicillines, cephalosporines
  (beta-lactamase inhibitors like clavulanic acid are not active)
Resistance mechanism

- *mecA* target replacement
  - PBP2a
  - PBP2a has low affinity to beta lactam drugs
  - Inhibition of drug activity
- *mecA* located in the Staphylococcal cassette chromosome mec (SCCmec)
  - Large genetic element
  - Several different SCCmec cassettes found in MRSA
- A new *mecA* homologue was found recently in a novel cassette called SCCmec XI

Chongtrakool et al. 2006
MRSA (definition of classification)

- **Hospital acquired (HA)-MRSA**
  - Most infections that become clinically evident **after 48 hours of hospitalization**.
  - Infections that occur after the patient is discharged from the hospital can be considered healthcare-associated if the organisms were acquired during the hospital stay.
  - Causes bacteriæmia, pneumonia, surgery wound infections...
  - Well characterized hospital clones harbouring large SCCmec cassettes (I-III)
MRSA (definition of classification)

- **Community acquired (CA)-MRSA**
  - Diagnosis of MRSA was made in the outpatient setting or by a culture positive for MRSA **within 48 hours after admission to the hospital**.
    - No medical history of MRSA infection or colonization.
    - No medical history in the past year of:
      - Hospitalization
      - Admission to a nursing home, skilled nursing facility, or hospice
      - Dialysis
      - Surgery
    - No permanent indwelling catheters or medical devices that pass through the skin into the body.
  - Mostly causes skin and soft tissue infections, but can be more serious
  - Strains fit into diverse backgrounds harbouring small SCCmec cassettes (IV and V)
  - Some contain associated virulence factors such as PVL
MRSA (definition of classification)

- **LA-MRSA**
  - Associated to clones adapted to livestock (ST398, ST97)
    - Mostly pigs but other species found as carriers (veal calfs, poultry, horses, pets)
  - Carried by healthy and also sick animals
  - Human carriage and/or infection associated to direct or indirect contact to animal sources
  - Found in environmental samples (dust) in farms
  - Found in meat, even though considered as low risk for food handlers and consumers
Epidemiology of MRSA in humans

- Hospital-acquired infections influenced by:
  - Distribution of HA-clones
  - Infection control measures
  - Antimicrobial usage
  - Search and destroy policies

- Community-acquired infections
  - Sporadic cases but also some outbreaks
  - Associated to specific clones (USA300) but also other sporadic clones

- Very different prevalences between EU countries
Epidemiology of MRSA in animals

• **Pets and companion animals**
  - Most human strains
  - Sporadic cases
  - More problematic Vet health issue: MRSP

• **Cows**: Mastitis

• **Pigs, veal calves, poultry**
  - ST398 main LA-MRSA clone
  - Animal carriers, sporadic infections
  - Widespread in Europe

Figure 7: Prevalence of MRSA positive production holdings, MRSA EU baseline survey in breeding pigs, 2008(a)
Epidemiology of MRSA

- Human clones

Companion animals

Transmission

Human carriers:
CC398 LA MRSA with broad host range

Contact with animals is main risk factor for humans
Potential Risk factors for CC398

- Vertical dissemination from breeding holdings
- Holding size
- Trade
- Environmental contamination
- Antimicrobial use
- Selection/ co-selection

- Still lacking knowledge on how factors interplay!

Figure 4: Prevalence of MRSA-positive breeding holdings in 2008 (EFSA, 2009) and intra-Community trade of breeding pigs in 2007.
New MRSA carrying the $mecA_{LGA251}$ gene

- Firstly found in the UK
  - Mastitis isolate
  - Phenotypic resistance
  - $mecA$ not detected, PBP2a negative
  - Overexpressed betalactamase test negative
  - Full genome sequenced

**SCCmec type XI containing divergent $mecA_{LGA251}$**


New $\textit{mecA}_{\text{LGA251}}$ gene distribution in the UK-human and animal isolates and their $\text{spa}$ and CC types

So far the new $meca_{LGA251}$ homologue has been found in animal and human isolates from:

Detection of MRSA

- Routine susceptibility testing
  - Screening tests (oxacillin, cefoxitin)
- Selective procedures for isolation
- Confirmation of presence of resistance determinant
  - Molecular detection of mecA (PCR) (different PCR primers for new homologue gene)
  - Detection of PBP2a with immunoaassays (rapid agglutination tests) (not suitable for detection of new PBP)
- The genotypic confirmation is compulsory for MRSA identification
Phenotypic detection

- Oxacillin screening test – CLSI guidelines describe procedure in Appendix B.

- Cefoxitin testing
  - Disk diffusion
    - ≤21mm
  - Broth dilution
    - >4mg/L

- Due to new mecA homologue genotypic detection by mecA PCR might no longer be sufficient
Selective isolation procedures (Baseline method)

- 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 MRSA 2) or equivalent
    and on blood agar
  - Isolation up to 5 colonies
Confirmation of id and methicillin resistance status - PCF we used until now

- 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*)
Need for new methods to detect new \textit{mecA} homologue

\begin{itemize}
\item With the possibility of this new gene, it is not sufficient ot test for \textit{mecA}!
\item Agglutination tests directed to PBP2a fail in detecting resistance
\item Hyperproduction of beta-lactamase not detected in these isolates as cause of resistance
\item Phenotype more important for detection!
\item Need to implement new PCR methods (you will try one in this course, which is still under implementation)
\end{itemize}
New mecA- need to change methods!!

- PCR for mecA, spa, pvl and mecA_{Lga251} will be used in the practical part of this course

  - mecA – Confirm classic methicillin resistance
  - mecA_{LGA251} – new gene
  - Spa confirm ID (only positive in *Staphylococcus aureus*) and used for spa typing directly
  - PVL- Determination of the presence of the Panton Valentine Leucocidin gene

Published protocol from:

with new update that will be published soon:
Additional amplification of mecA_{LGA251} – 138bp
Typing of MRSA - PFGE

• Typing of strains
  – PFGE with Smal most frequently (except for ST398 which is not typable with PFGE with Smal) - very discriminatory method

• Subjective but very discriminatory, useful for outbreak investigation

Rotun et al., 1999.
Typing of isolates- *spa*- typing

• Typing of strains
  – *Single-locus sequencing*

• *Spa*-typing (Shopsin *et al.*, 1999)
  – Easy and reproducible method with good correlation to MLST (CC), some exceptions

http://www.uniklinik-freiburg.de/iuk/live/molhyqlabor/leistungskatalog/spa.jpg
Typing of isolates - MLST

- Multi locus sequence typing (MLST) based on sequencing 7 housekeeping genes and determination of their allelles (Enright et al., 2000)
  - Large-scale epidemiological linkage
Typing of isolates - SCC\textit{mec}

• Typing of Staphylococcal chromossomal cassette mec (SCC\textit{mec})
  – Typing of the SCC element responsible for resistance-
    • Origin of SCC\textit{mec}
    • Longer trend epidemiology and evolution
      – SCC\textit{mec} acquisition into strain background

  – SCC\textit{mec} subtyping
    • Determine SCC\textit{mec} subtypes
    • Direct repeat unit (DRU)- typing
    • \textit{ccrB} and \textit{ccrC} typing

  – Generally performed by multiplex or simple PCR methods and/or sequencing
**SCCmec**

- Staphylococcal Chromossomal Cassette:
  - Integrated in the chromosome
  - Variable in length
  - Different types in MRSA
  - Some SCC cassettes without mec
  - Some SCCmec cassettes are composites of different cassettes
  - At present- types I-XI
Main concerns regarding MRSA in animal sector

- Studies indicate a large prevalence of MRSA in healthy animals in several countries – widespread in Europe and other parts of the world
- LA-MRSA found adapted to animal hosts
- Finding in food products, even though considered low risk for consumption and handling
- Environmental persistence and colonization
- Selection and co-selection factors not completely known (Antimicrobials, metals, desinfectants?)
- Increasing diversity of strains backgrounds and diversity of SCCmecs
- New mecA homologue in S. aureus from cattle UK

Large reservoir for Community acquired (CA) MRSA infections even in Countries with low prevalence of MRSA
Reference laboratory activities on MRSA by EURL-AR

- Verification of strains from other European countries
- Provide courses including detection and typing of MRSA for European labs
- Provide counselling to countries involved in MRSA studies required by EC
- Proficiency testing
  - MRSA EQAS ring trial on swab samples
  - EQAS Staph- compulsory MRSA detection
- Advisory tasks
- Follow up on activities depending on EC decisions
Thank you!
Any questions???