



Er Borsa borte?

*mec*<sub>lga251</sub>/*mecC*

NordicAST workshop 2012

Robert Skov

# Borsa

- *mecA* negative borderline resistant *S. aureus* -i.e. low level resistant
  - Majority: seen using oxacillin (1µg) disk diffusion
    - Most often “Screening phenomenon” ie normal oxacillin MIC
  - Few isolates: elevated oxacillin MIC – but most often cephalosporin “susceptible”
  - Extremely few isolates: elevated oxacillin **and** elevated cephalosporins

# Novel methicillin resistance gene

*mecA*<sub>LGA251</sub>

Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study



*Laura García-Álvarez, Matthew T G Holden, Heather Lindsay, Cerian R Webb, Derek F J Brown, Martin D Curran, Enid Walpole, Karen Brooks, Derek J Pickard, Christopher Teale, Julian Parkhill, Stephen D Bentley, Giles F Edwards, E Kirsty Girvan, Angela M Kearns, Bruno Pichon, Robert L R Hill, Anders Rhod Larsen, Robert L Skov, Sharon J Peacock, Duncan J Maskell, Mark A Holmes*

*Lancet Infect Dis* 2011;  
11: 595-603

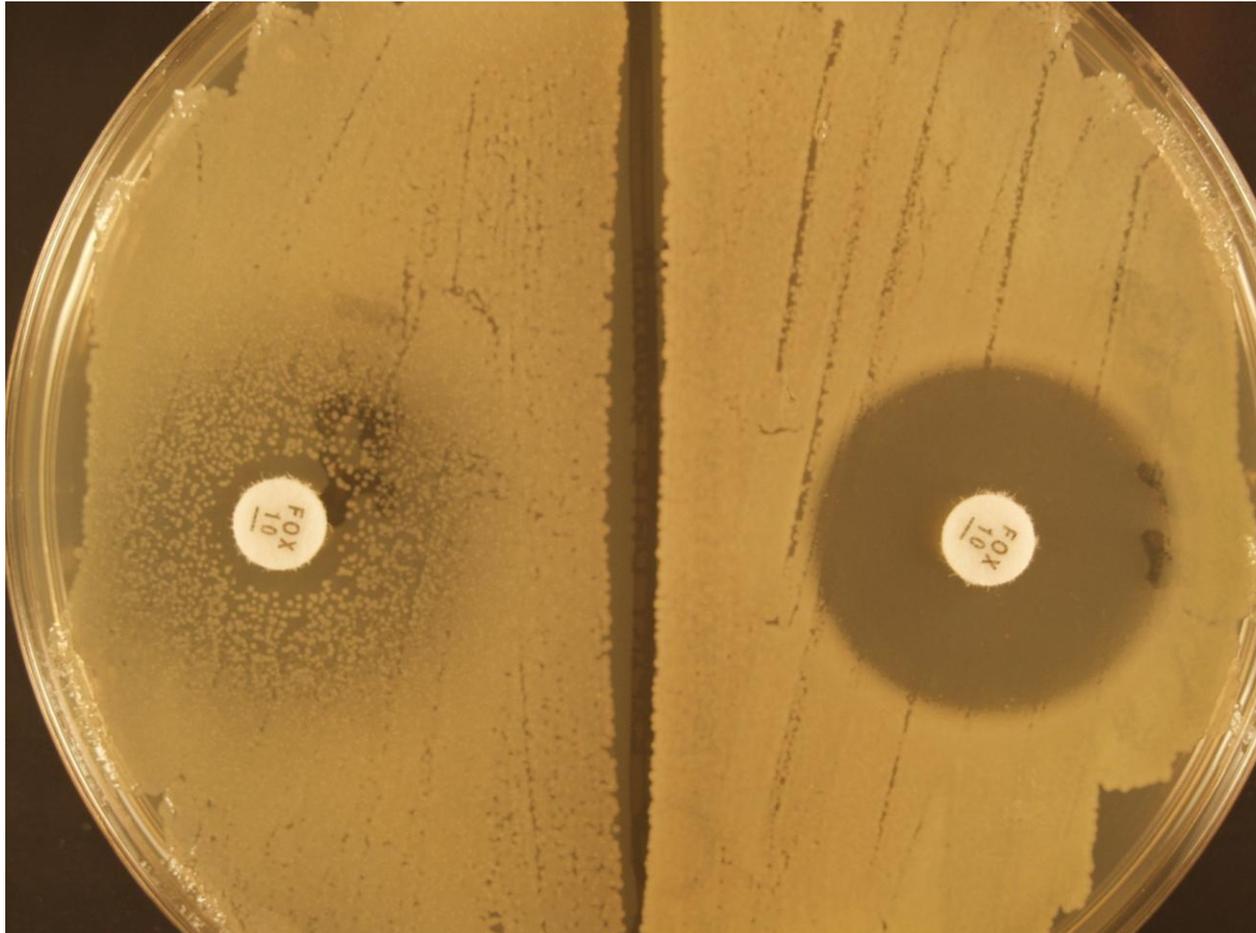


# Epidemiology UK

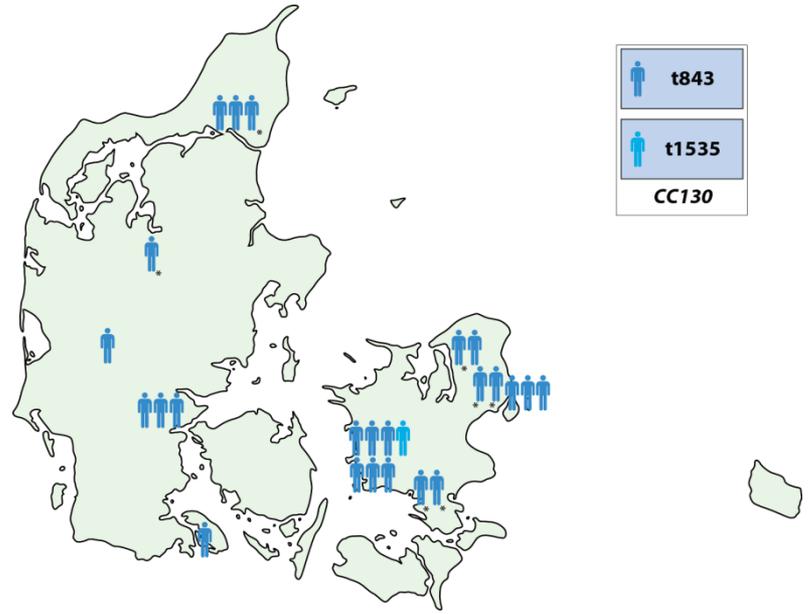
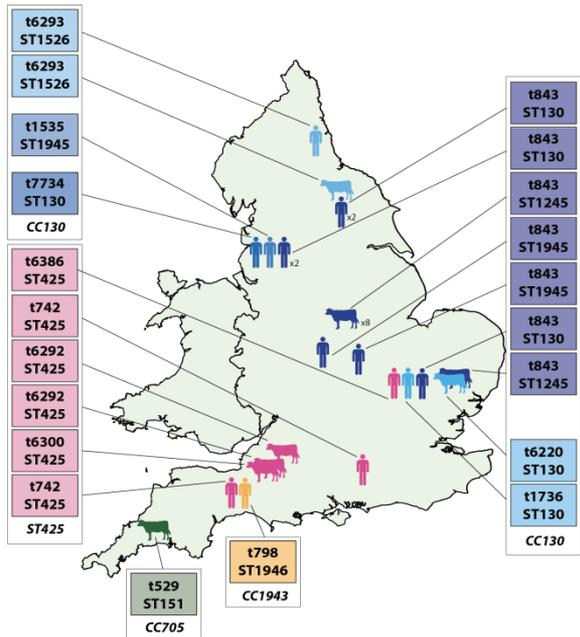
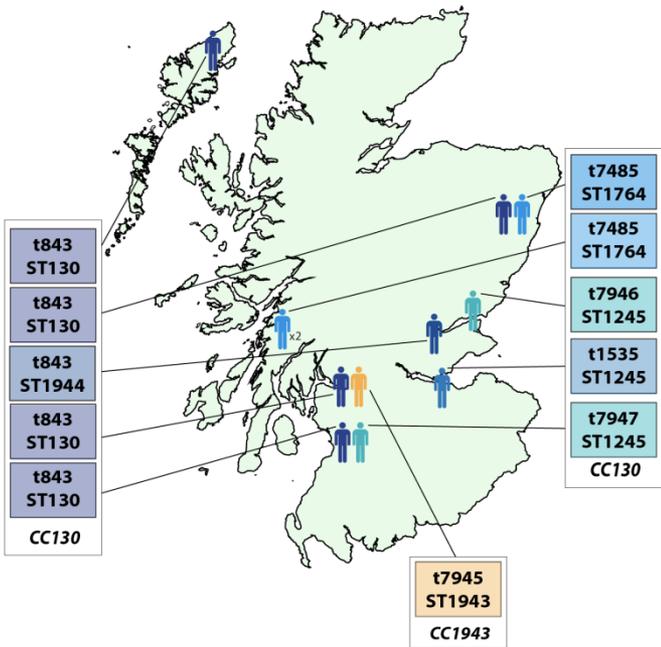
- Initially discovered in 2007 England in a milk sample
  - oxacillin MIC = 16 mg/l
  - cefoxitin MIC = 32 mg/l
  - ST425, PCR for *mecA* - negative
    - WGS – variant of the *mecA* gene
  - In a larger survey of cows in UK
    - 13 had the new *mecA* variant
- Few humane isolates from UK reference laboratories

**LGA251**

**MSSA**

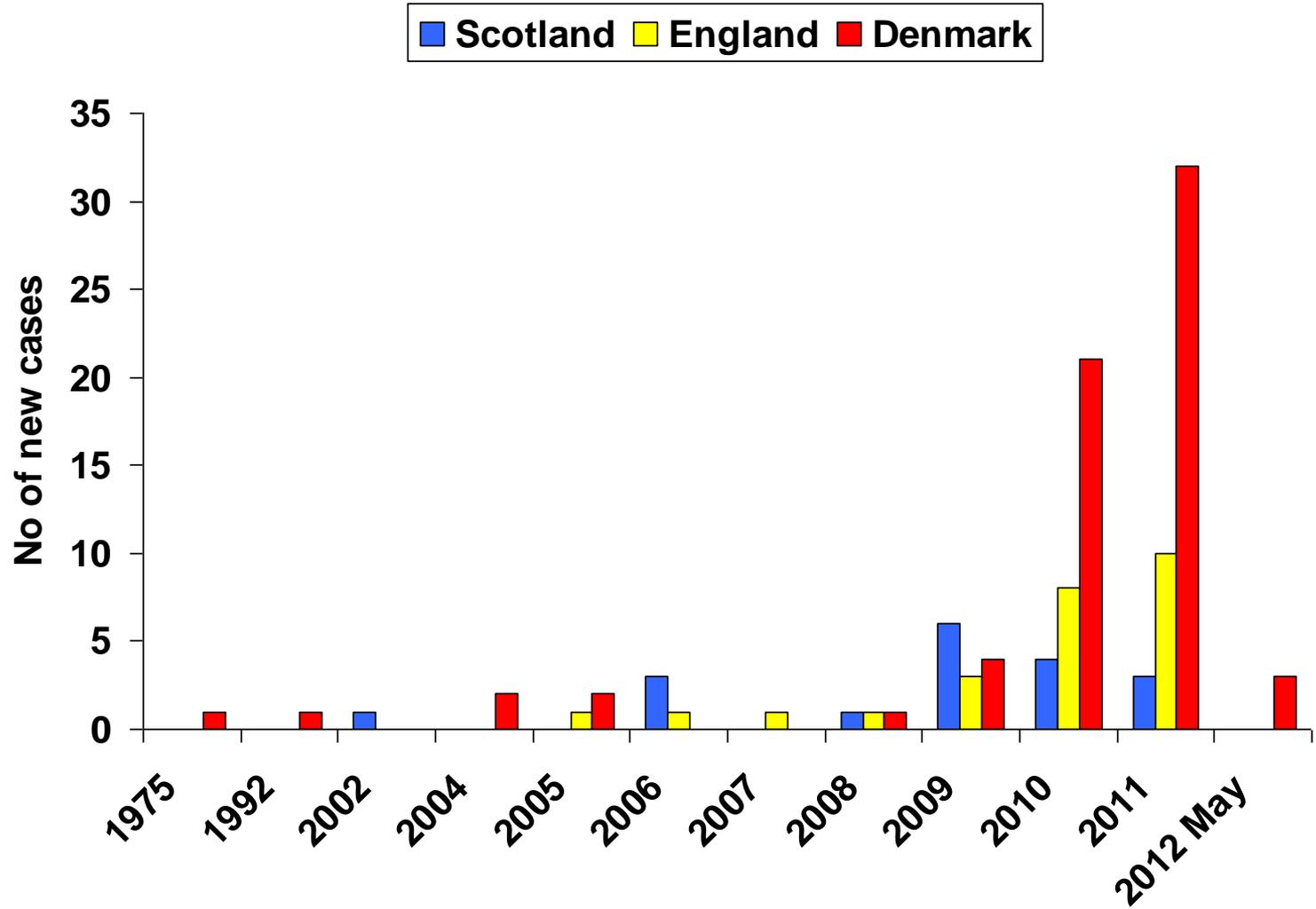


Courtesy Mark Holmes



Alvarez LG et al. LID, Aug.2011

# No. of new human cases per year

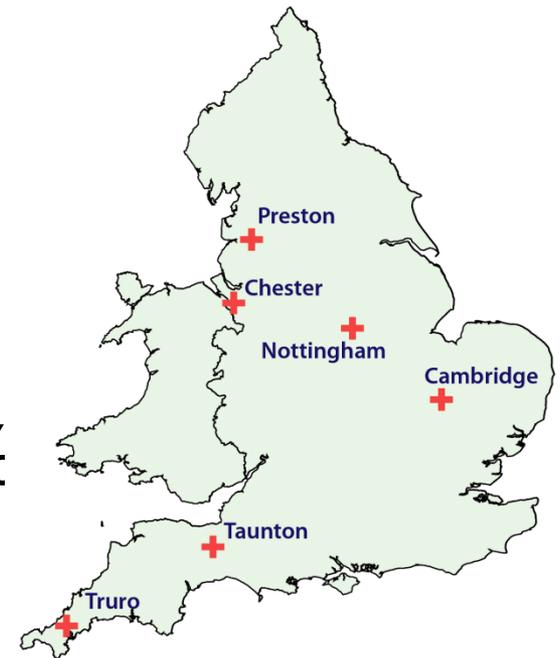


# Epidemiology Denmark

- Retrospective screening of all resistant - *mecA* negative isolates since 1970, prospective screening
- Old isolates from 1975 and 1992!
  - Reappearance in 2004, increasing numbers
    - 21 in 2010, 32 in 2011
    - Patients are older than patients with traditional CA-MRSA; primarily from the countryside
  - Clinical information available for 84 cases
    - 74 had infection (59 skin and soft tissue, **8 bacteremia**)
  - Found in cows and sheep

# Human prevalence in England

- 6 hospitals (plus Scotland)
- 6 x 350 MRSA isolates (2100, all unique isolations, up to 1 yr)
- Able to detect  $0.2\% \pm 0.19\%$  (expecting about 0.5%)
- **Currently 0.6% (4/707)**



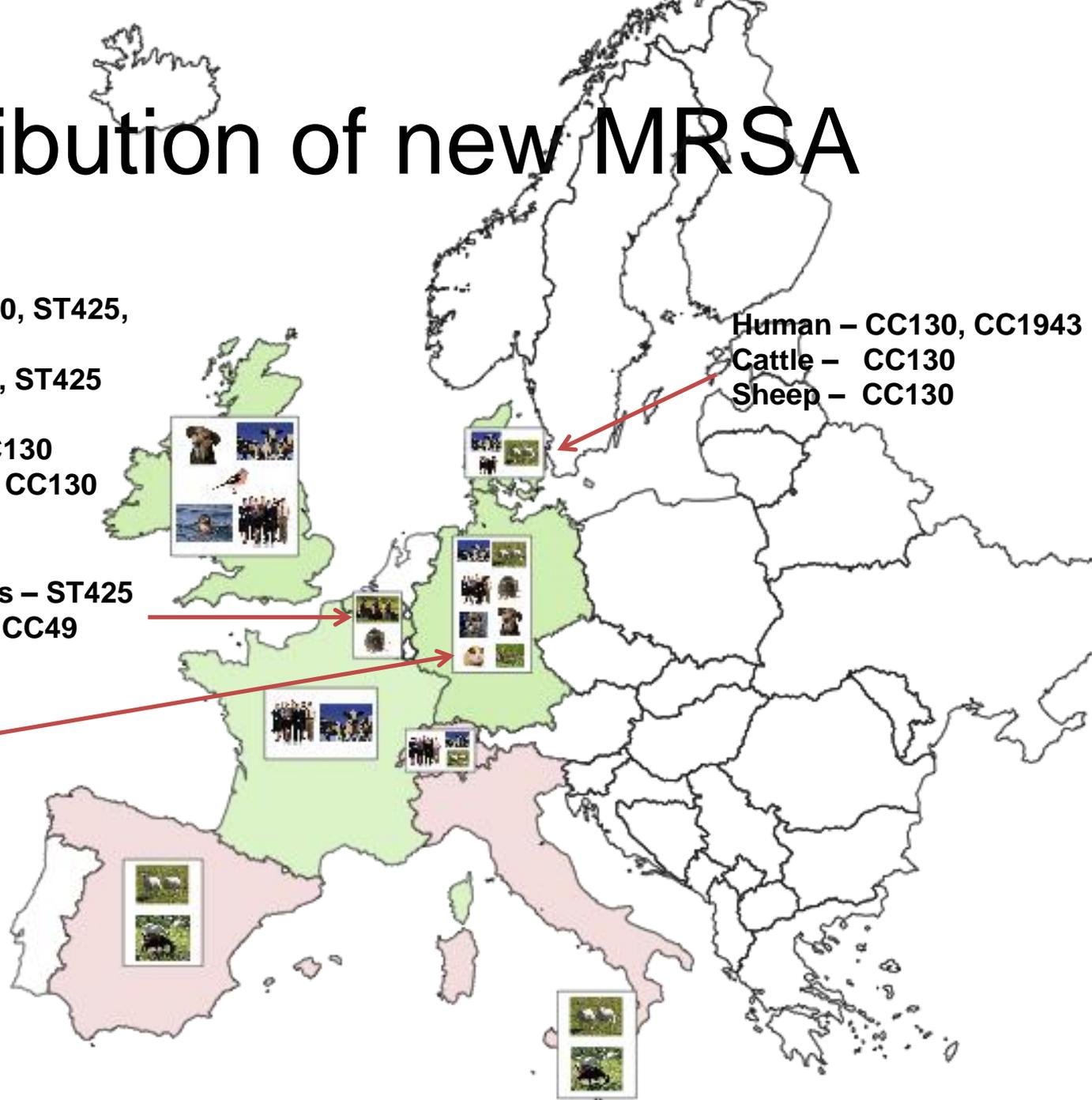
# Distribution of new MRSA

Human – CC130, ST425,  
CC1943  
Cattle – CC130, ST425  
Dog – CC130  
Chaffinch – CC130  
Harbour seal – CC130

Human – CC130, CC1943  
Cattle – CC130  
Sheep – CC130

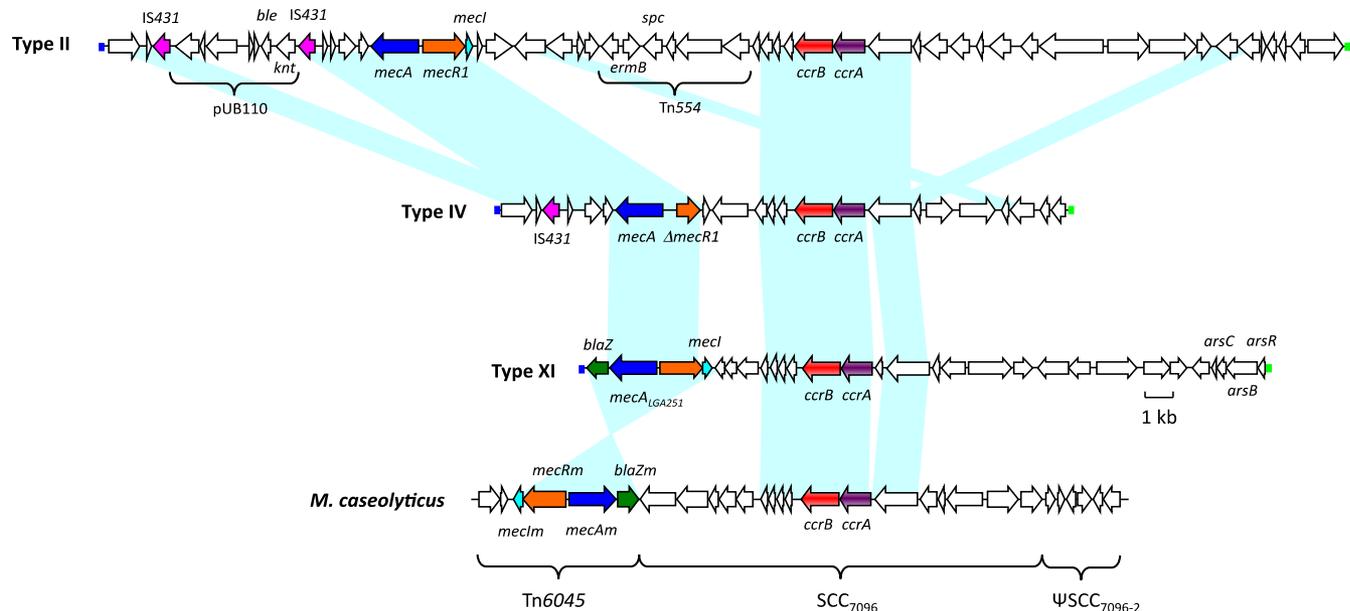
Rabbits – ST425  
Rats – CC49

Humans – CC130  
Cows – CC130  
Sheep – CC130  
Rats – CC130  
Cats – CC130, CC599  
Dogs – CC599  
Guinea Pig – CC130  
Roe Deer – CC130  
Horse – CC130

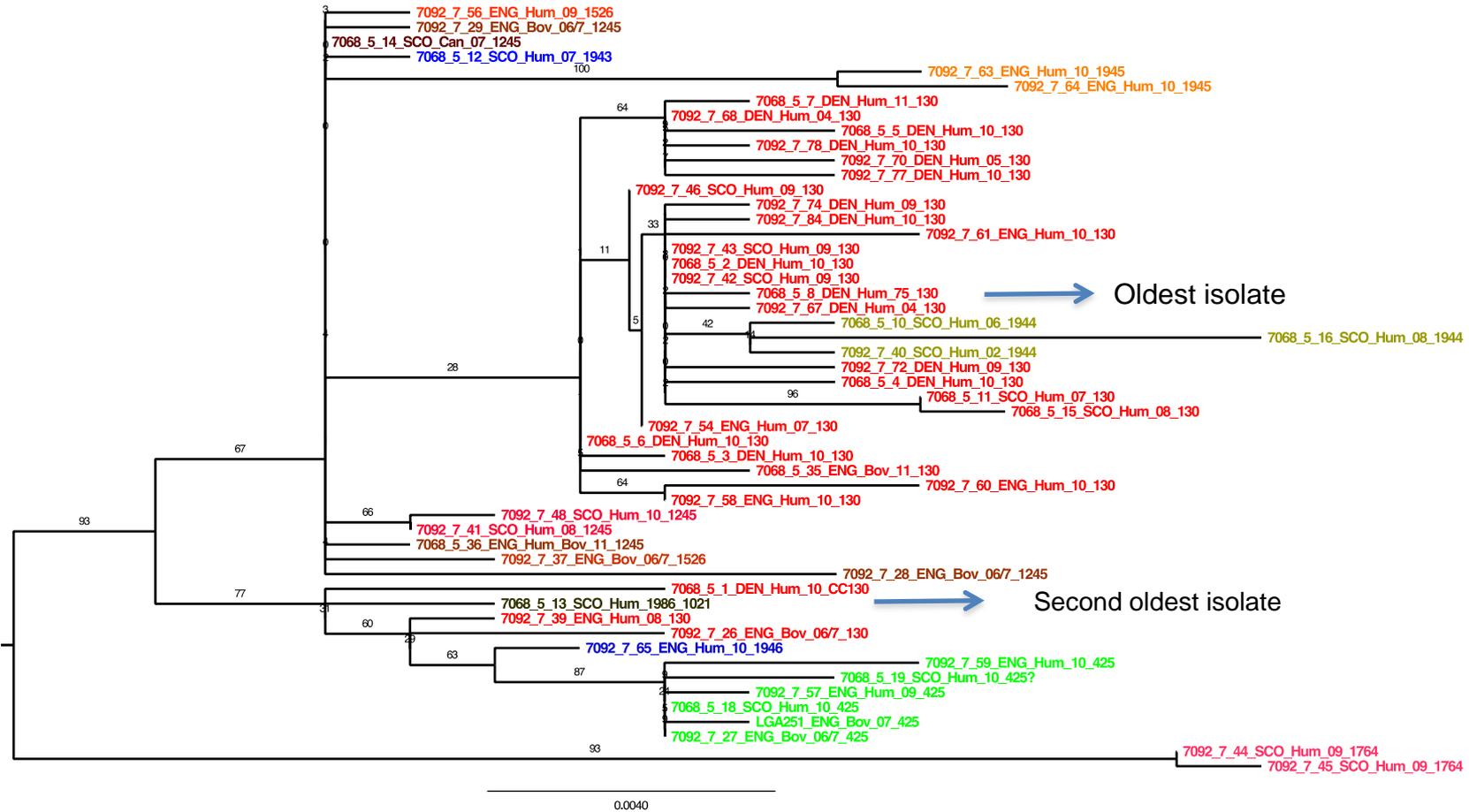


# Genetics

- Novel *mecA* gene only 70% homology to the known *mecA* gene
- New SCC*mec* XI (29,4 Kb)



In general *SCCmec* Type XI phylogeny matches with sequence type and core genome SNP phylogeny – suggesting single entries into ancestor and diversification

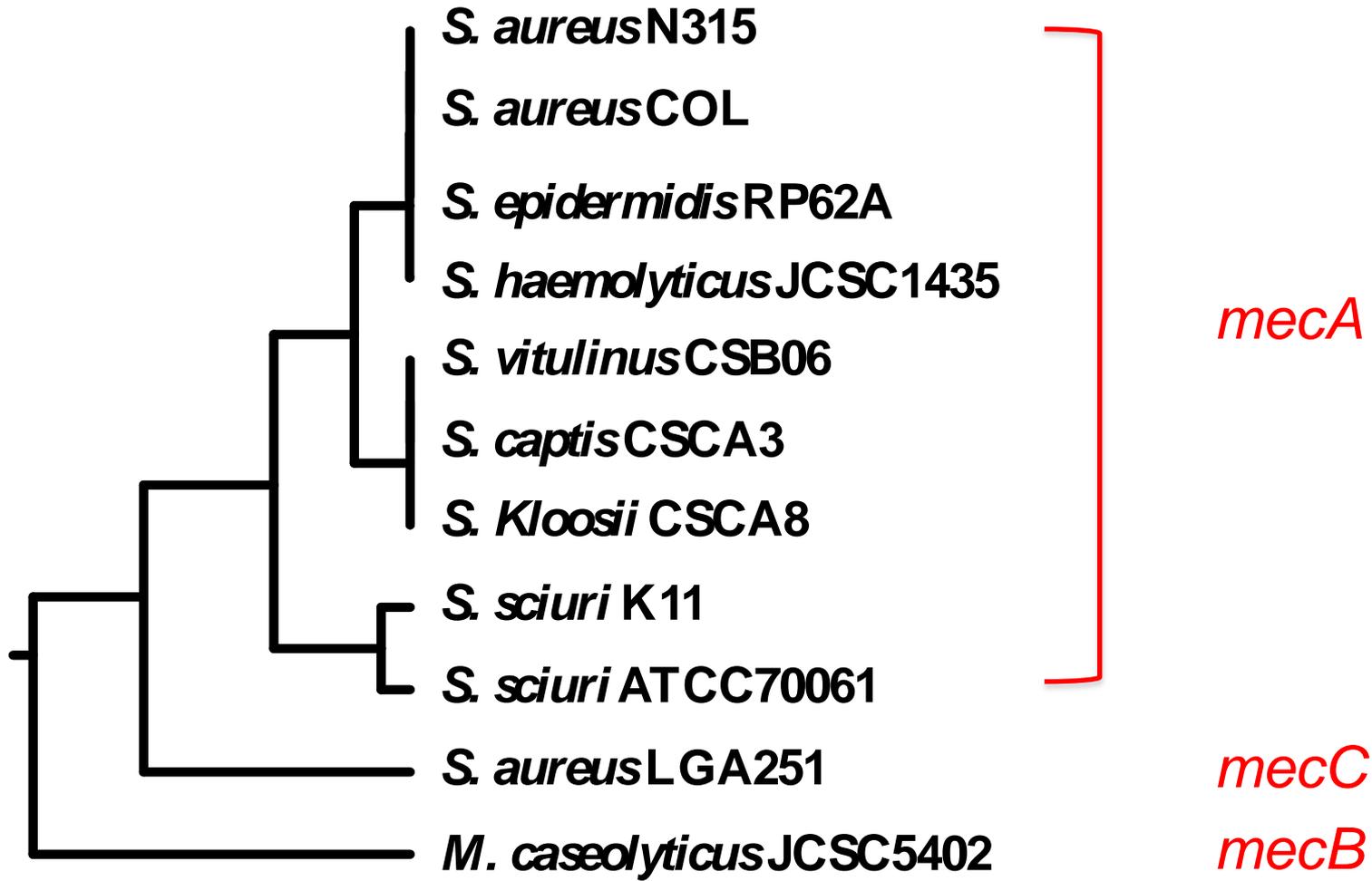


Likely *SCCmec* Type XI transferred from CC130 strains possible via ST1946 into ST425

ML tree with GTR and 1000 bootstraps. Values on branches are bootstrap support values. Identical sequences removed from analysis

Courtesy Mark Holmes

# *mec* nomenclature



# Detection

- Isolates
  - 111 isolates containing the mecA variant
- Methods (not all 111 isolates have been tested in all methods)
  - Phenotypic
    - MIC oxacillin and ceftazidime
      - (BMD, agardilution, Etest)
    - Disk diffusion (oxacillin and ceftazidime)
    - Chromogenic agars MRSA
      - Brilliance (Oxoid), Chrome ID (bioMerriex), Chrome agar BD, MRSA select
    - Vitek (bioMerriex), Phoenix (BD), Microscan (Siemens)
    - PBP2a agglutination, Clearview

# Detection

- Methods – genotypic
  - In house as well as commercial PCR
    - GeneOhm™ StaphSR (BD), GeneXpert™ MRSA (Cepheid) og NucliSENS EasyQ MRSA (bioMérieux)

# Results

- BMD /AD
  - Cefox 8-64 mg/L
  - Oxacillin 1-64 mg/L / 2-32 mg/L (4 / 6 isolates  $\leq$  2 mg/L)
- Etest
  - Cefox 1-48 mg/L (1 isolate  $>$ 6 mg/L)
  - Oxacillin 0.75-24 mg/L (5 isolates  $\leq$  2 mg/L)
- Disk diffusion
  - Cefox 10-20 mm
  - Oxacillin 6-18 mm 5 isolates  $>$  12 mm)

# Results

- Chromogenic agars MRSA – growth of (24h)
  - Brillance (Oxoid)
    - 108 of 111
  - Chrome ID (bioMerrieux),
    - 110 of 111
  - Chrome agar (BD)
    - 88 of 111
  - MRSA select (Biorad)
    - 70 of 111

# Results

- Automated systems

- Vitek

- cefoxitin screen 108 of 111
    - Oxacillin ( $\geq 4$  mg/L) 21 of 111

- Phoenix

- cefoxitin ( $> 4$  mg/L) 83 of 111
    - oxacillin ( $> 2$  mg/L) 17 of 111
    - Latamoxcef ( $>16$  mg/L) 99 of 111

- Microscan

- cefoxitin ( $> 4$  mg/L) 106 of 110
    - oxacillin ( $> 2$  mg/L) 0 of 110
    - Cefotaxime ( $> 4$  mg/L) 108 of 110

# Detection

- Agglutinations assay
  - PBP2a detection – negative
    - Some are aberrant positive
- Clearview
  - Without induction                      10 of 111
  - With induction                              111 of 111

# Detection

- Genotypic methods - All negative!
  - Commercial methods:
    - GeneOhm™ StaphSR (BD), GeneXpert™ MRSA (Cepheid) og NucliSENS EasyQ MRSA (bioMérieux)
  - Novel PCRs
    - *Spa, mecA, mecA<sub>LGA251</sub>, pvl* – *Direct spa seq.* (Stegger, CMI, online)
    - Real Time PCR (Pichon B. et al, JAC, accepted)

# Conclusions

- No Borsas have not disappeared (entirely)
  - Relatively seldom – obs may represent novel *mec* genes
- *mecC*
  - First divergent *mec* gene found in *S. aureus*
  - Broad host range
  - Been present in human samples for more than 3 decades
    - Incidence/prevalence seems to be low!

# Acknowledgements

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  - **Scottish MRSA reference lab:** Giles Edwards

