# Staphylococci #8

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RESEARCH LETTER

## Genome sequence of *Staphylococcus lugdunensis* N920143 allows identification of putative colonization and virulence factors

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### Abstract

*Staphylococcus lugdunensis* is an opportunistic pathogen related to *Staphylococcus aureus* and *Staphylococcus epidermidis*. The genome sequence of *S. lugdunensis* strain N920143 has been compared with other staphylococci, and genes were identified that could promote survival of *S. lugdunensis* on human skin and pathogenesis of infections. *Staphylococcus lugdunensis* lacks virulence factors that characterize *S. aureus* and harbours a smaller number of genes encoding surface proteins. It is the only staphylococcal species other than *S. aureus* that possesses a locus encoding iron-regulated surface determinant (Isd) proteins involved in iron acquisition from haemoglobin.

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FEMS Microbiol Lett **322** (2011) 60–67

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# Introduction - *Staphylococcus*

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Gram positive coccus

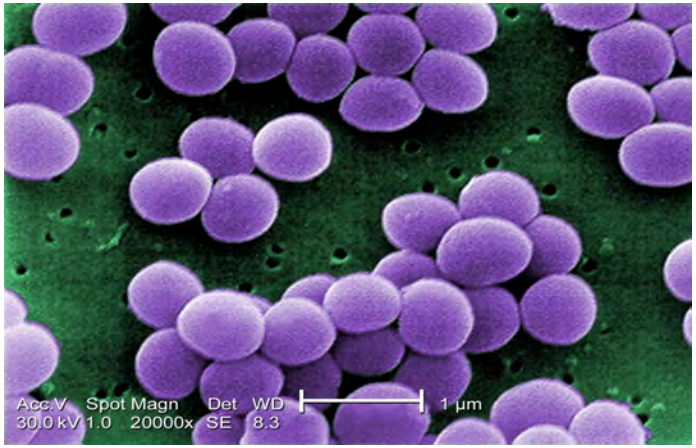
Various species (>40), often linked to specific mammalian hosts

About 10 species colonize skin and mucous membranes of humans

Two major types:

coagulase negative species and

coagulase positive species




About 30% of healthy humans carry *S. aureus* in their nose

*S. aureus* is the major human pathogen within the genus, generally causing minor skin infections, bacteraemia, endocarditis, pneumonia, abcess, osteomyelitis as well as Toxic-shock-syndrome (tampon syge)

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# Introduction



Estimation: 2% of all surgical incisions lead to infection with Staphylococci

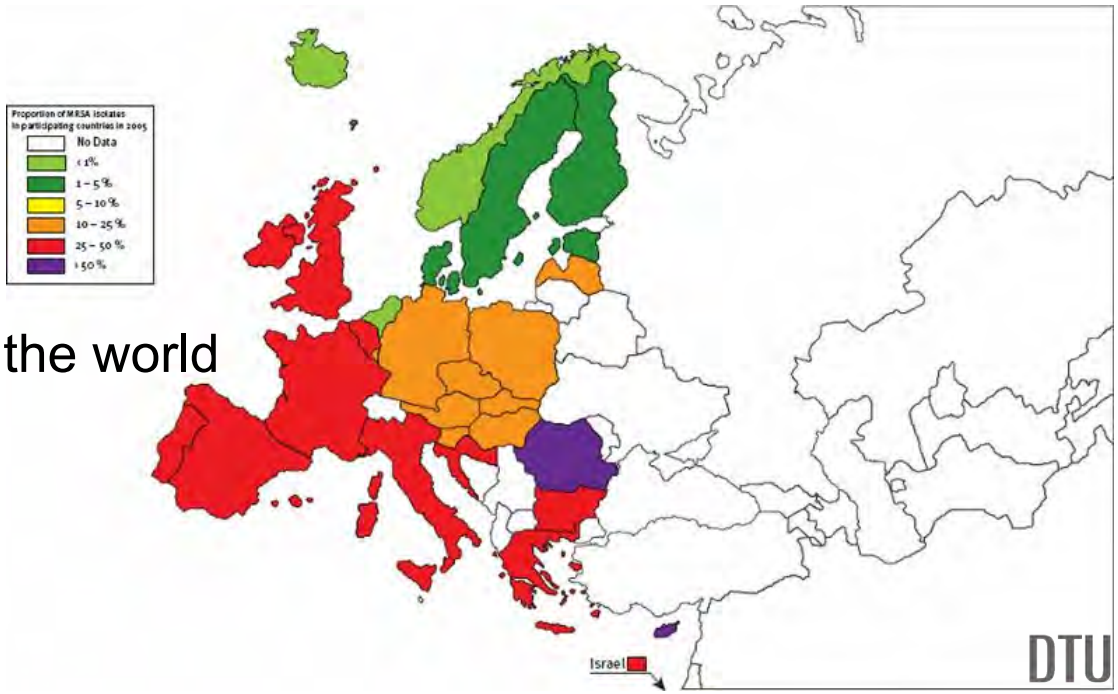
*S. aureus* causes 20 thousand cases of bacteraemia/year in the UK and >1500 cases in DK/year

Resistance towards meticillin emerged in the early 1960s ➔ MRSA

MRSA levels vary:

2% in DK

Up to 50% in major parts of the world




10 X more expensive to treat and 2 X mortality rate

# *Staphylococcus lugdunensis*

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Coagulase negative species

Commensal of human skin, most frequently on lower parts of the body, in moist areas, but is also considered as an opportunistic pathogen

Causes relative rare but often severe infections (brain abcesses, infective endocarditis, sepsis, etc.)

Easily mistaken with *S. aureus*

1. same colony morphology
2. same haemolytic activity
3. same ability to agglutinate latex particles coated with fibrinogen

Only one genome fully sequenced and published (strain HKU09-01) yet

Annotation incomplete

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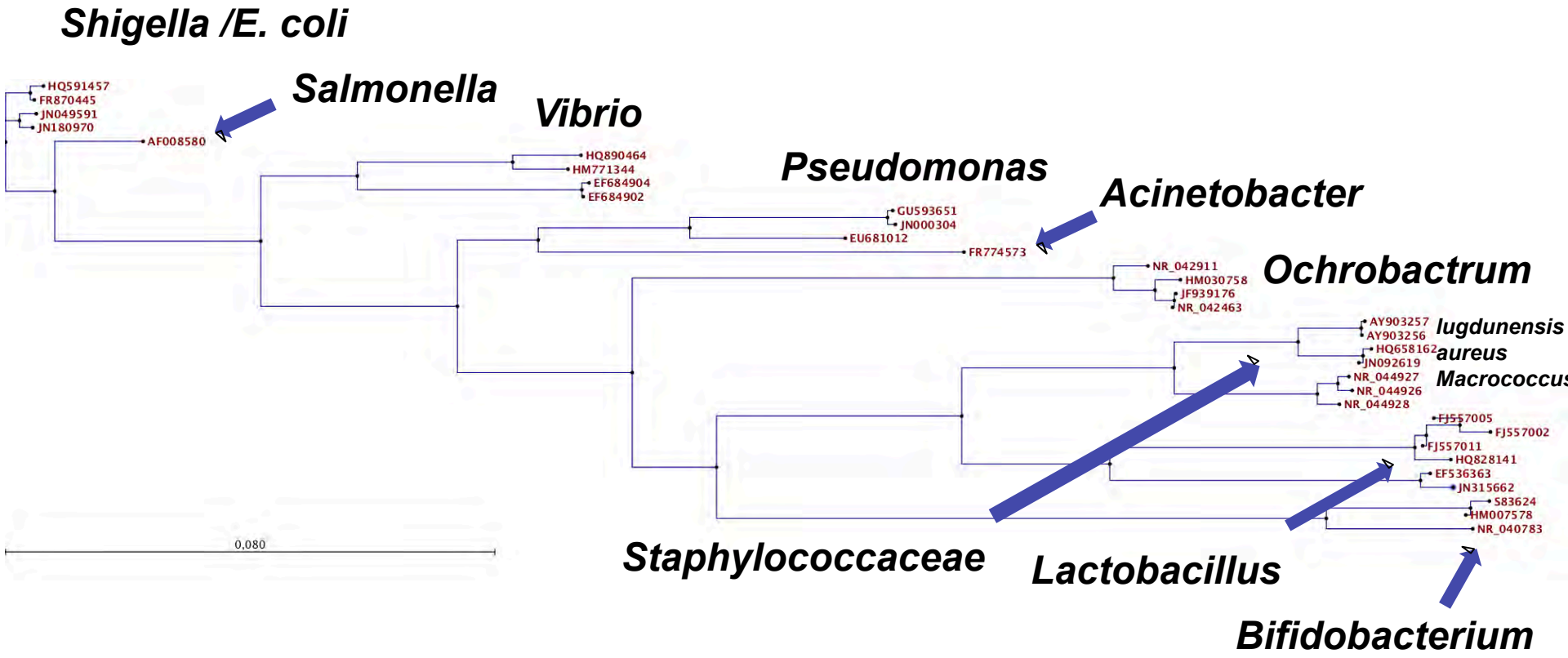
# Introduction






**16S, partial CDS, CLUSTALW**

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# Materials & Methods

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*S. lugdunensis* isolate from breast abcess (1992)

Genome and draft genome assembly:

Illumina (PE 54 bp) and 454 (250 bp) data

Combined assembly incl Sanger seq: 9 scaffolds

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# Materials & Methods

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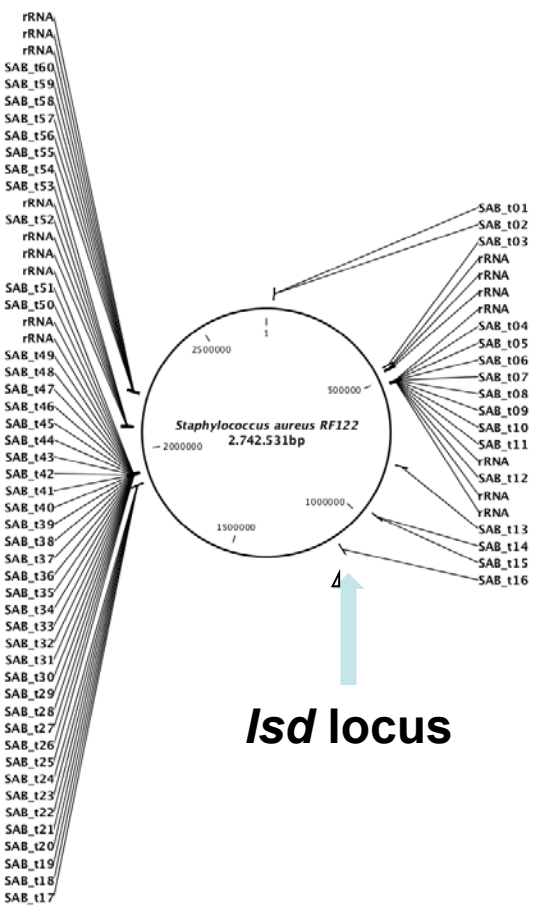
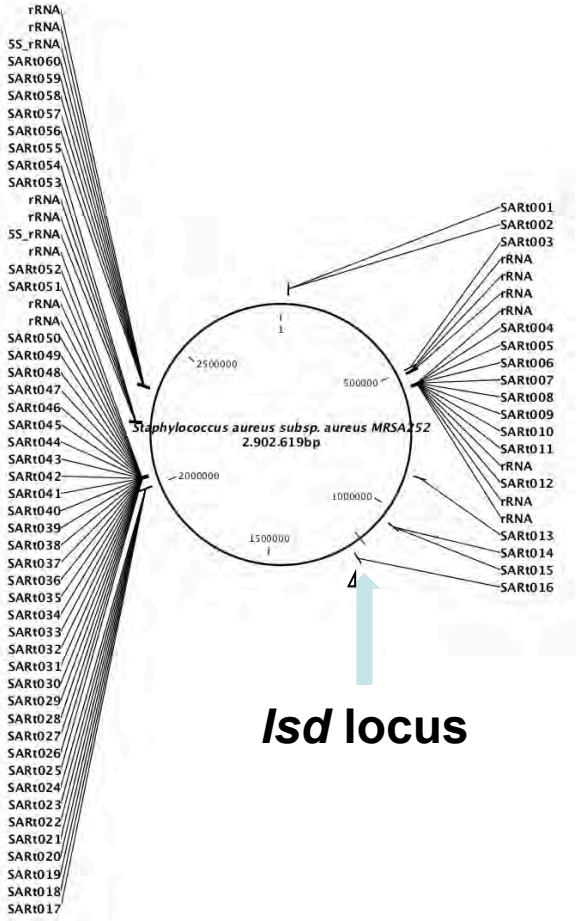
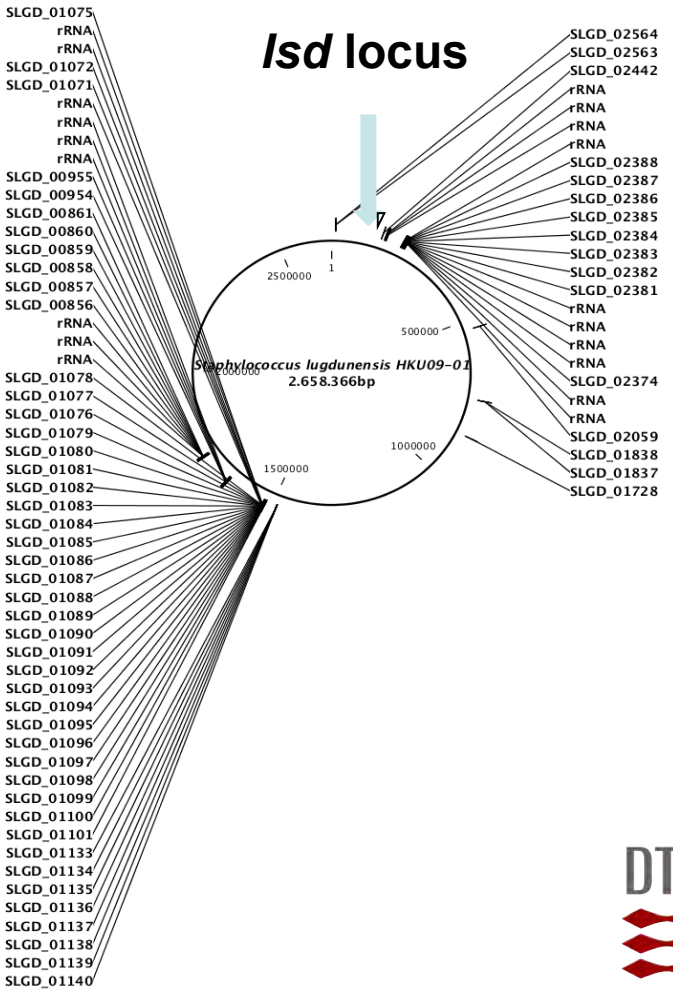
## Assembly approach:

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# Materials & Methods

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Annotation using Artemis with manual curation

Compare *S. lugdunensis* to *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. carnosus* and *Macrococcus caselyticus*

Two of the 5 genus in the *Staphylococcaceae* family

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# Results

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Genome ~ 2.6 Mbp

- gaps in rRNA and repetitative region in surface protein
- Aligned using HKU09-01 *S. lugdunensis* genom
- 2450 CDS (125 unique compared to HKU09-01)

MGE content of interest:

- one prophage (φSL1), no integrated or replicating plasmids

16S analysis place *S. lugdunensis* in the *Staphylococcus* clade

Comparative genomics reveal

- 78% of CDS matches to *S. aureus* MRSA252
- 75% to *S. epidermidis*
- 78% to *S. haemolyticus*

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# *Staphylococcus* genome comparison



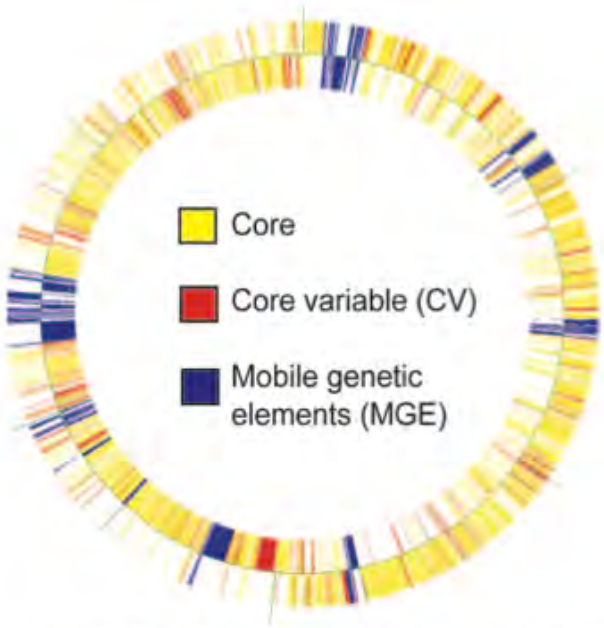
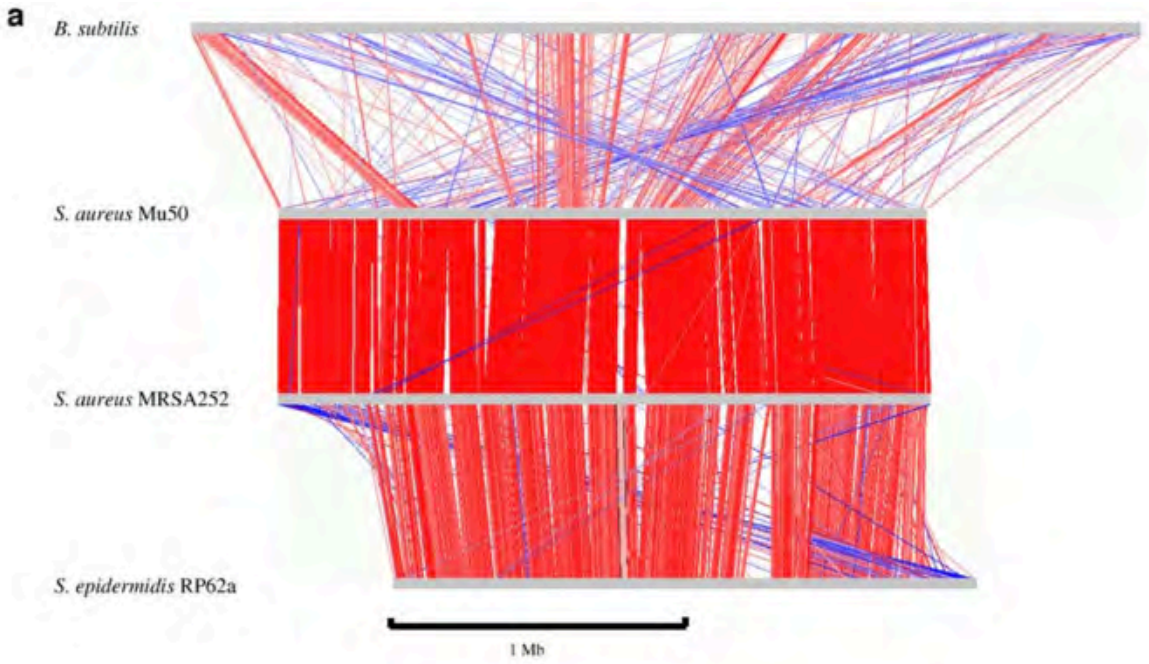



FIG. 3. Representation of the MRSA252 genome with each gene colored according to whether it is a core gene or CV gene or whether it is found on an MGE. The figure was generated in GeneSpring using the same lists used for identifying CV genes. The outer circle represents genes on the forward coding strand, and the inner circle represents genes on the complementary strand.




**Lindsay *et al*., Journal of Bacteriology (2006)**

**Lindsay *et al*., Functional integrated Genomics (2006)**

# Results

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Comparison between the two *S. lugdunensis* genomes:

- 95,4% conserved
- HKU09-01 contains two transposons encoding B-lactamase and regulatory proteins, a putative GI encoding cadmium resistance
- A 32 kb duplication of an iron regulated surface determinant (*Isd*)

Putative virulence and colonication factors:

- Accessory gene regulator (agr)
- System for changing cell envelope charge (defence against AMP)
- Contain small CRISPR region, putative explanation for low number of MGEs

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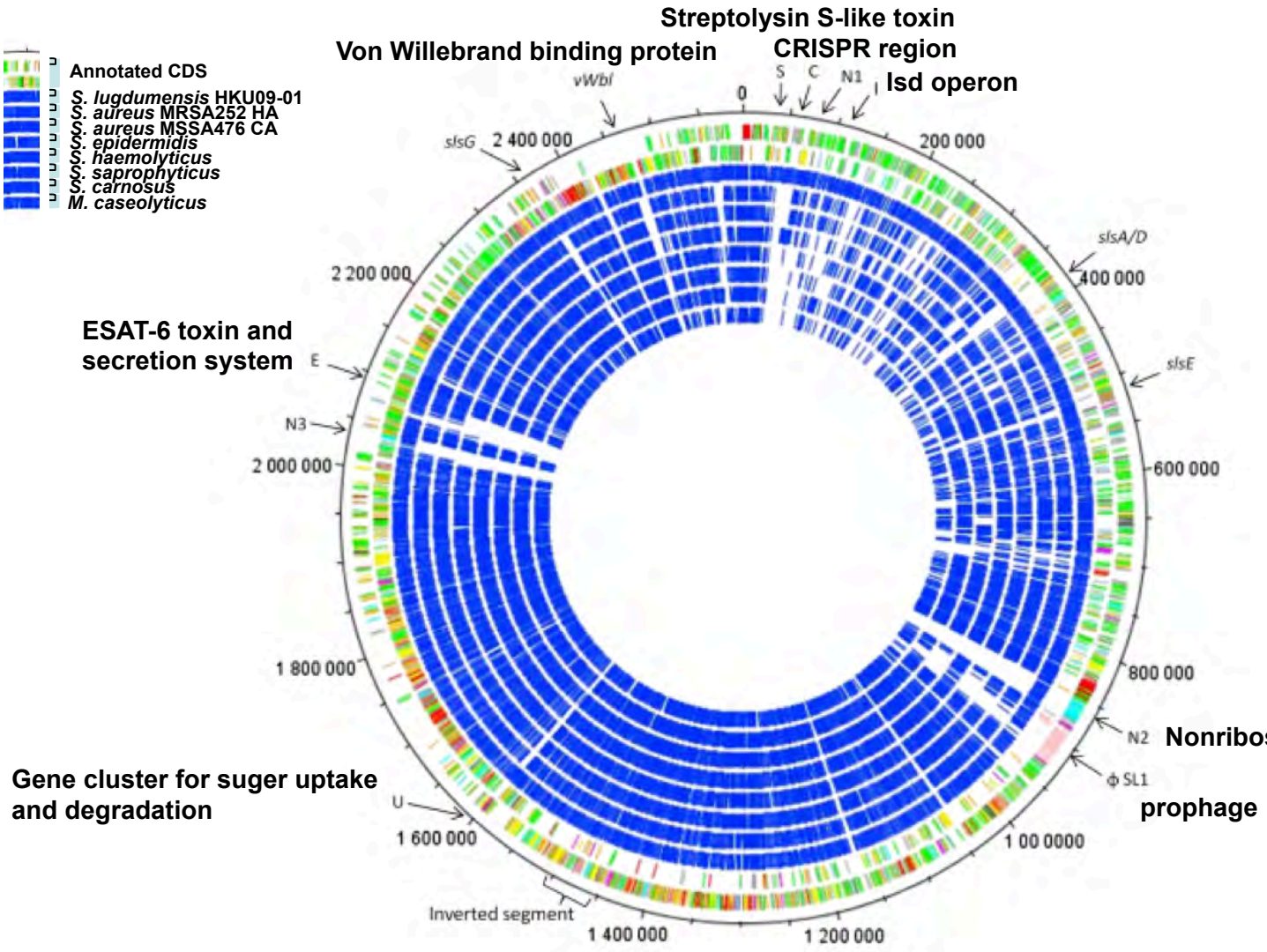
# Genome comparison






CDS functions: neon green, pathogenicity/adaptation; dark gray, energy metabolism; red, information transfer; dark green, surface associated; sky blue, degradation of large molecules; dark pink, degradation of small molecules; yellow, central/intermediary metabolism; pale green, unknown; pale blue, regulators; orange, conserved hypothetical; brown, pseudogenes; pink, phage and IS elements; gray, miscellaneous. Arrows indicate regions of

# *Isd* locus

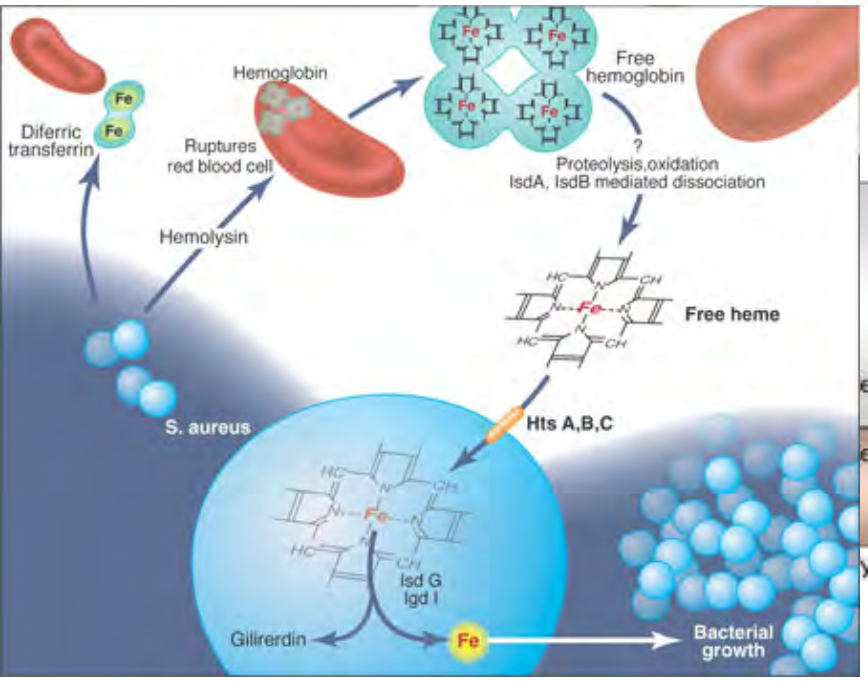
*Isd* locus is unique to *S. lugdunensis* among CoNS

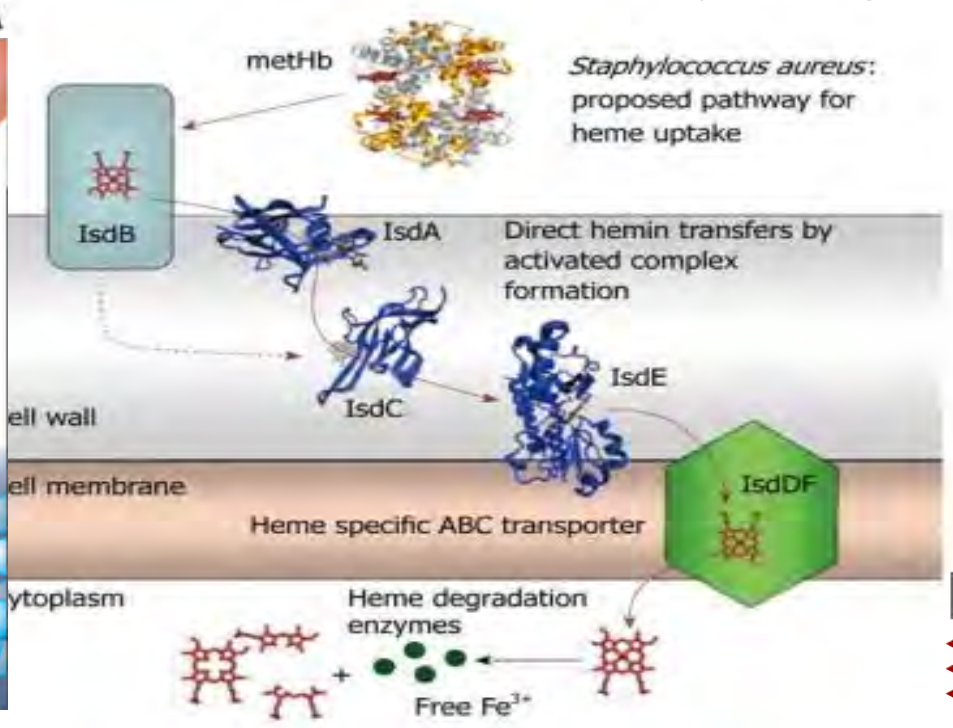
Isd proteins: Anchored in cell wall

Responsible for extracting haem from haemoglobin

ABC transporter tranfers haem in the cytoplasm

Haem monoxygenase cleaves Fe from porphyrin ring



# *Isd* locus



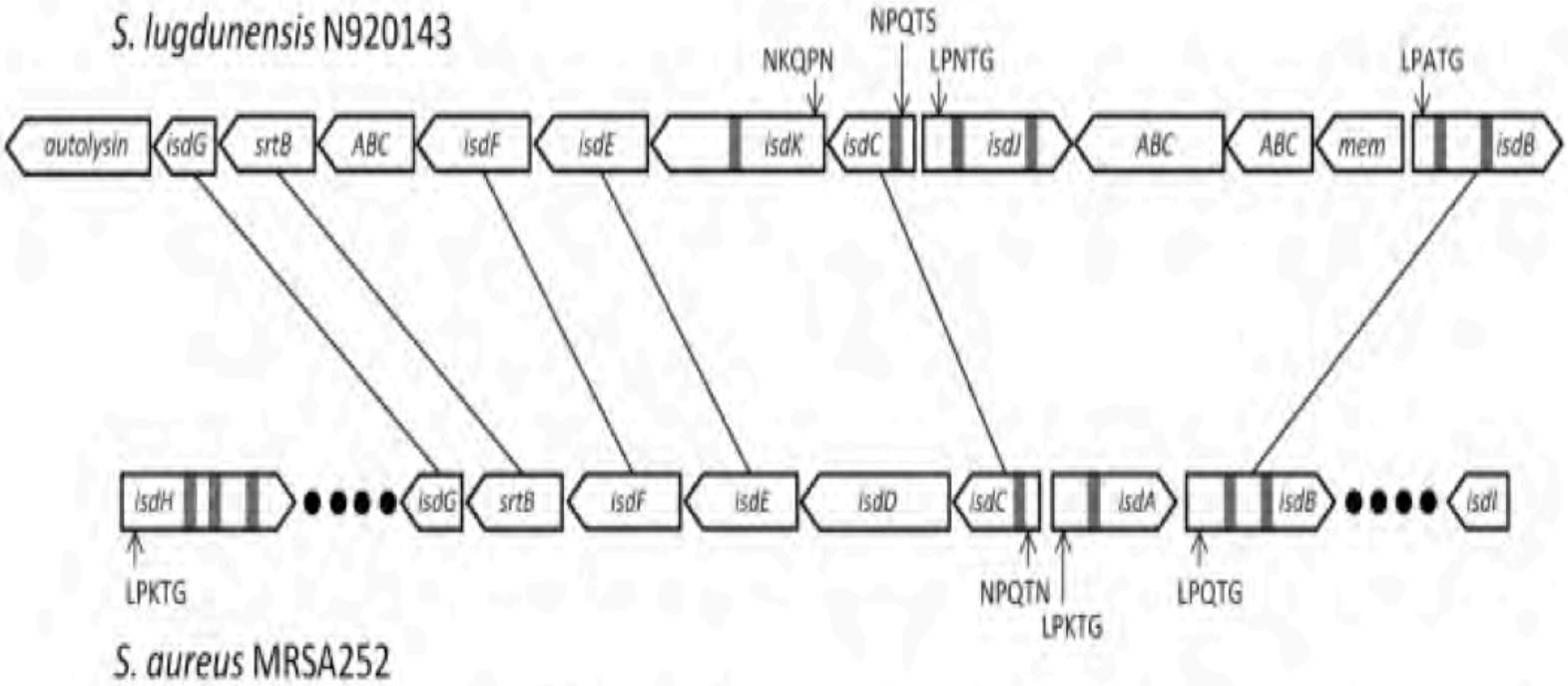


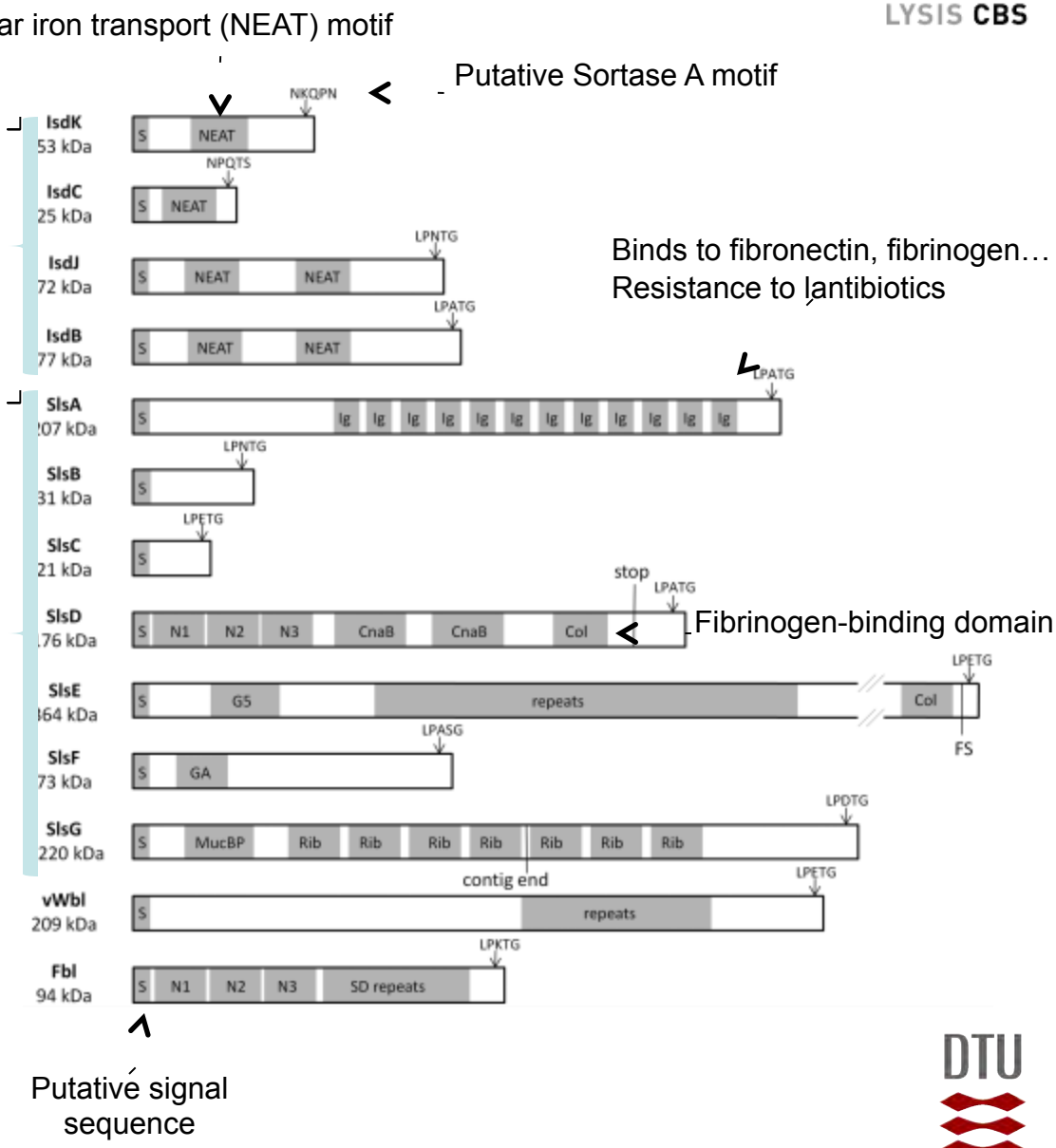

**Fig. 2.** Comparison of the *isd* loci of *Staphylococcus aureus* and *Staphylococcus lugdunensis*. A schematic diagram of the *isd* loci is shown. The open boxes denote individual genes and the arrows the direction of their transcription. Encoded NEAT motifs are shown as small black boxes. Orthologous genes are linked by thin black lines. The % identities between the encoded proteins are as follows: IsdB 36.8%, IsdC 57.6%, IsdE, 74.7%, IsdF 57.7%, SrtB 58.2%, IsdG 68.2%. Cell wall sorting signals are indicated. The *isdH* and *isdI* genes of *S. aureus* are located outside the locus as indicated by black circles.

# Results

MSCRAMMs of *S. lugdunensis* with important properties:

Iron regulated surface-determinant locus

*S. lugdunensis* surface proteins



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## Sub-conclusion

These surface proteins provide good survival and competition abilities on human skin.

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# Discussion



*S. lugdunensis* is similar to *S. aureus*

- *S. lugdunensis* shares a few toxins with *S. aureus*:
  - Streptolysin S-like toxin (cytolytic toxin).
  - ESAT-6 system (potent T-cell antigen).
  - *Isd* locus is also important for pathogenicity.

- *S. lugdunensis* established on a heart valve or in an abscess, it is as deadly as *S. aureus*.

- Generally, *S. aureus* mobile elements are not present in *S. lugdunensis*.

# Discussion

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## The *isd* locus

- Iron is difficult for the bacterium to get inside mammals.
- Suggested evidence for covergent evolution in *S. aureus* and *S. lugdunensis* towards invasiveness.

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# Summary

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- They present a high quality draft genome of *S. lugdunensis.*
- Analysis indicates genes/gene clusters related to colonization and invasive abilities on human skin.
- The *lsd* locus in *S. aureus* and *S. lugdunensis* share similar genetic makeup.

**Provide a scaffold for further investigation of skin colonisation mechanisms and pathogenesis of *S. lugdunensis*.**

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# Perspective

- Clinical relevance of *isd* locus duplication.

- Two *S. lugdunensis* genomes, both infective – do they resemble generel colonization isolates?

# Critics



- Good article
- Informative figures
- They don’t over interpret the used data
- Annotation quality?
- GI content in tRNA?
- Plasmid content?

# Results

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**Table 1.** Summary of notable *Staphylococcus lugdunensis* features and distribution of orthologues in other staphylococci

| | *S. lugdunensis* N920143 | *S. lugdunensis* HKU09-01 | *S. aureus* MRSA252 | *S. epidermidis* RP62a | *S. haemolyticus* | *S. saprophyticus* | *S. carnosus* | *M. caseolyticus* |
|---|---|---|---|---|---|---|---|---|
| NRPS 1 | + | + | + | + | – | – | – | – |
| NRPS 2 | + | + | – | – | – | – | – | – |
| NRPS 3 | + | + | – | – | – | – | – | – |
| *isd* locus | + | Duplicated | + | – | – | – | – | – |
| *sst* locus | Duplicated | Duplicated | + | + | + | + | + | + |
| *cap* locus (PS capsule) | + | + | + | – | + | + | + | + |
| *cap* locus (PGA capsule) | + | + | – | + | + | + | – | – |
| *esx* locus | + one gene FS | + one gene FS | + | – | – | – | – | – |
| Streptolysin S-like toxin | + one gene FS | + one gene FS | – (RF122) | – | – | – | – | – |
| Lantibiotic resistance locus | + | + | – | – | – | – | – | – |
| *agr* locus | + | + | + | + | + | + | + | – |
| *ica* locus | + | + | + | + | – | + | – | + |
| *mprF/dlt* | + | + | + | + | + | + | + | + |
| CRISPR region | + | + | – | + | – | – | – | – |
| β-Haemolysin | + | + | + | + | – | – | – | – |
| Putative haemolysin III | + | + | + | + | + | + | + | + |

FS, frameshift; NRPS, nonribosomal peptide synthetase; PGA, polyglutamic acid; PS, polysaccharide; RF122, sequenced *Staphylococcus aureus* bovine isolate.

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**Thank you for your attention!**

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