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Saarland University
Medical Center

Staphylococcus aureus CC398: Factors promoting host adhesion and immune evasion

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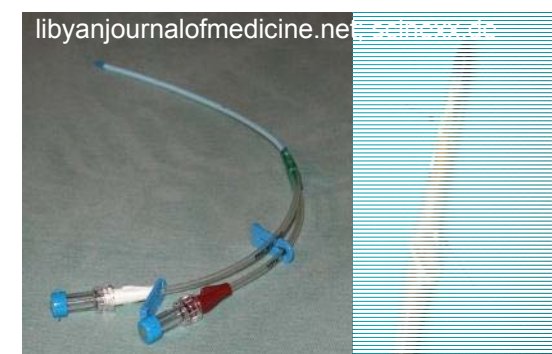
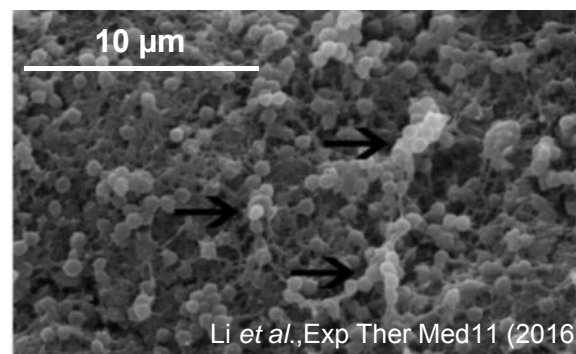
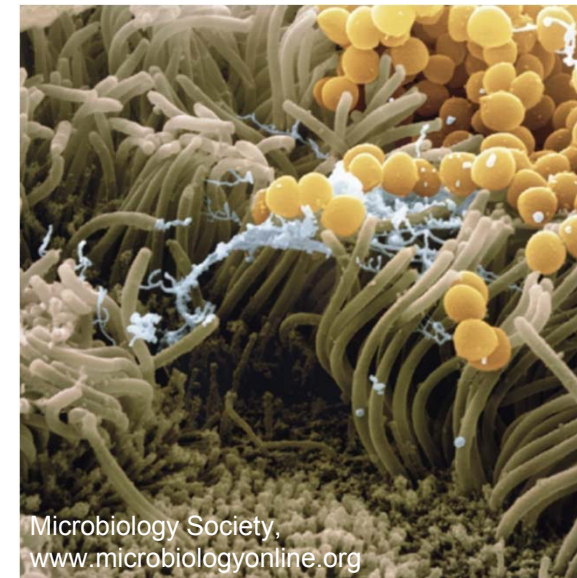
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Staphylococcus aureus: host adhesion

- Colonizer of mammal skin and nares
- Opportunistic pathogen
- Local skin infection (carbuncle, furuncle)
- Life-threatening systemic infections (sepsis, endocarditis)
- Formation of biofilms on biotic surfaces, medical devices
- Host adhesion is the basic condition for infections

Voss *et al.*, EJCMID 13 (1994)

Lowy, F.D., N Engl J Med 339 (1998)





Staphylococcus aureus: specific host adhesion

MSCRAMM (microbial surface components recognizing adhesive matrix molecules)

covalently bound to the bacterial cell wall by LPXTG motif (Sortase A) Schneewind *et al.*, EMBO J 12 (1993)

| MSCRAMM | Binding target / function |
|--|---|
| Protein A | IgG antibodies |
| Fibronectin binding proteins A and B Fnb A/B | Fibronectin, Plasma-Fibronectin |
| Clumping factors A and B Cif A/B | Fibrinogen, involved in blood clotting, adhesion to nasal epithelial cells |

Heilmann, Adv Exp Med Biol 715 (2011), J Wann *et al.*, Biol Chem 275 (2000), McDevitt *et al.*, Eur J Biochem 247 (1997)

SERAM (engl. secretable expanded repertoire adhesive molecules)

Secreted by bacterial cell, adapter between bacterial cell and host

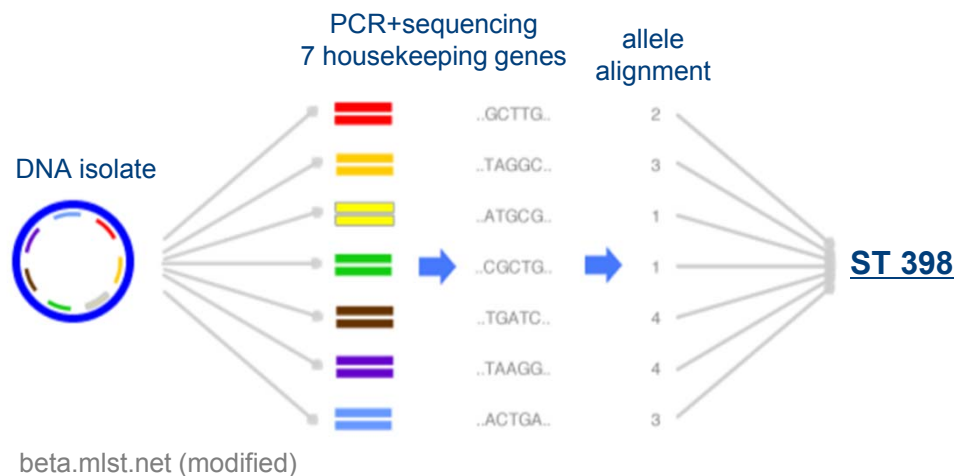
| SERAM | function |
|--|---|
| Extracellular adherence protein Eap | adhesion, immune modulation, internalization into host cells |
| Extracellular matrix protein Emp | adhesion to extracellular matrix |

Heilmann, Adv Exp Med Biol 715 (2011), Chavakis *et al.*, Thromb Haemost 94 (2005), Haggart *et al.*, J Infect Dis 192 (2005)

Staphylococcus aureus: Molecular epidemiology

genotyping of *S. aureus* isolates; common PCR- based methods:

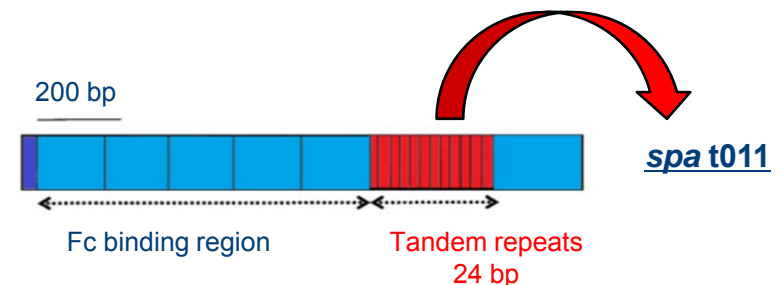
Multilocus sequence typing (MLST):



➔ Assignment of sequence types (ST);
Clonal Complexes (CC)

Single Locus sequence typing (SLST):

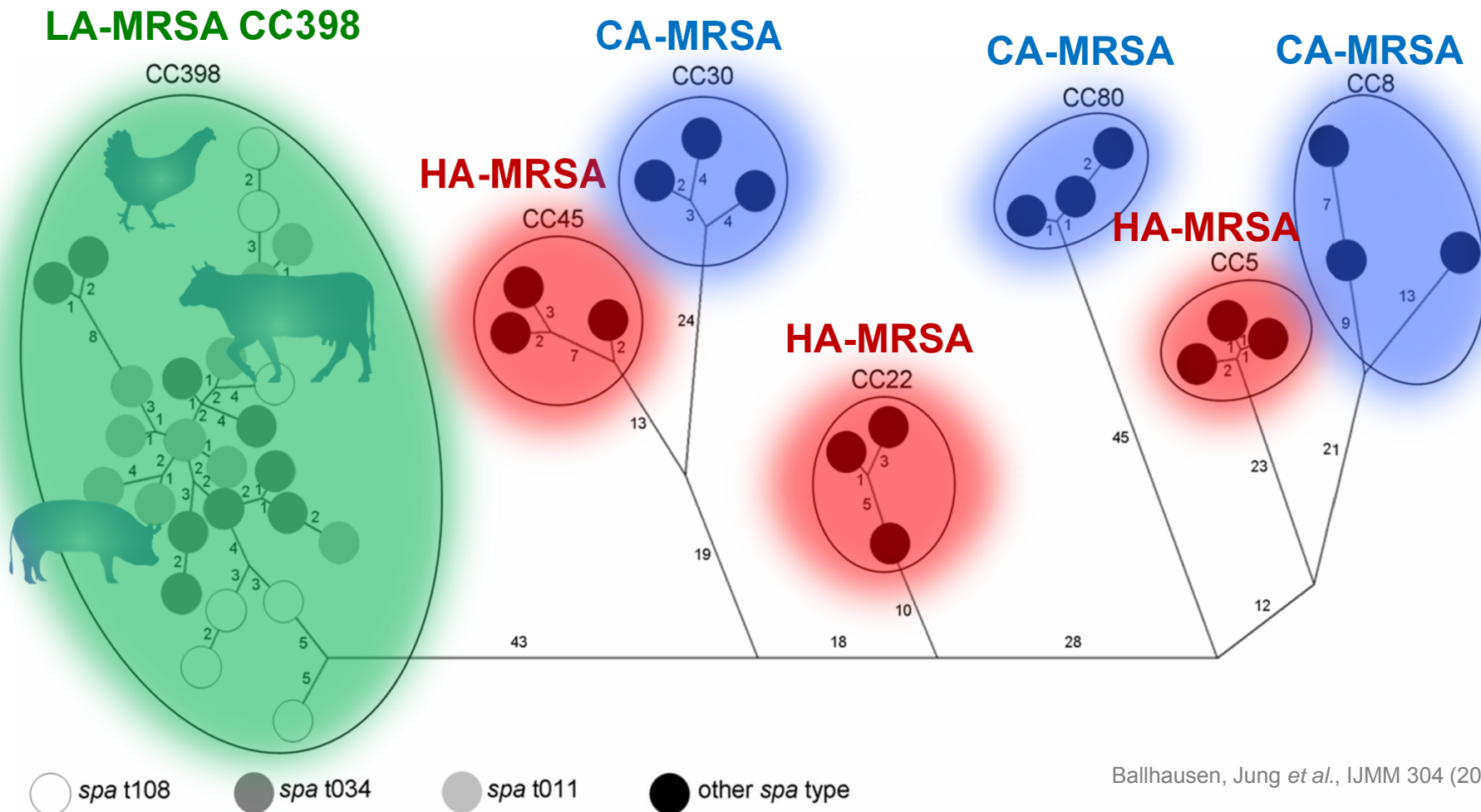
sequence von 24 bp tandem repeats of the *spa* gene (Protein A)



Cuny and Witte, EP1903116 A1 (2008) (modified)

➔ Assignments of *spa*-types

Genotyping of *S. aureus* isolates; The Clonal Complex (CC) 398

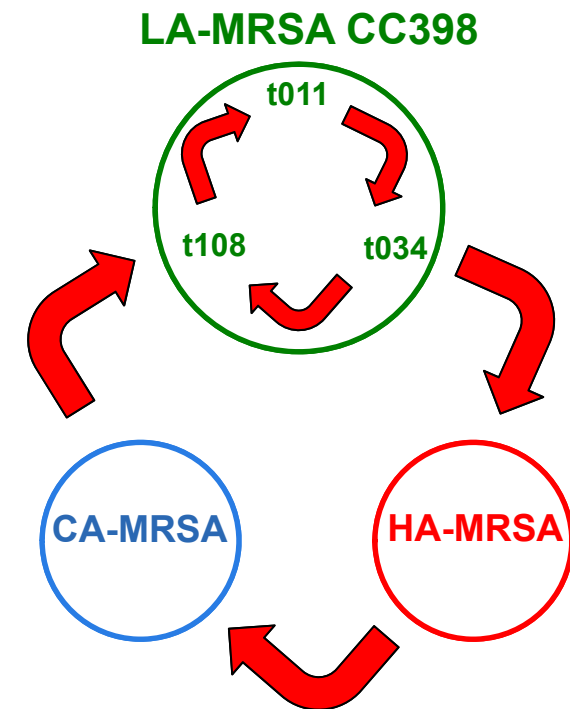


Ballhausen, Jung *et al.*, IJMM 304 (2014)

S. aureus isolates of the Clonal Complex (CC)398:

Adhesion to human keratinocytes

- **LA-MRSA CC398** (n=10)
- human **MSSA CC398**, spa t571 (n=10)



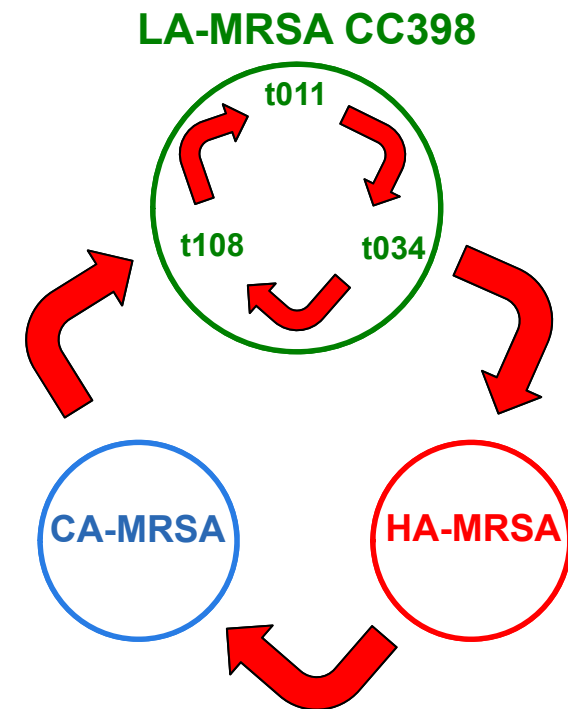
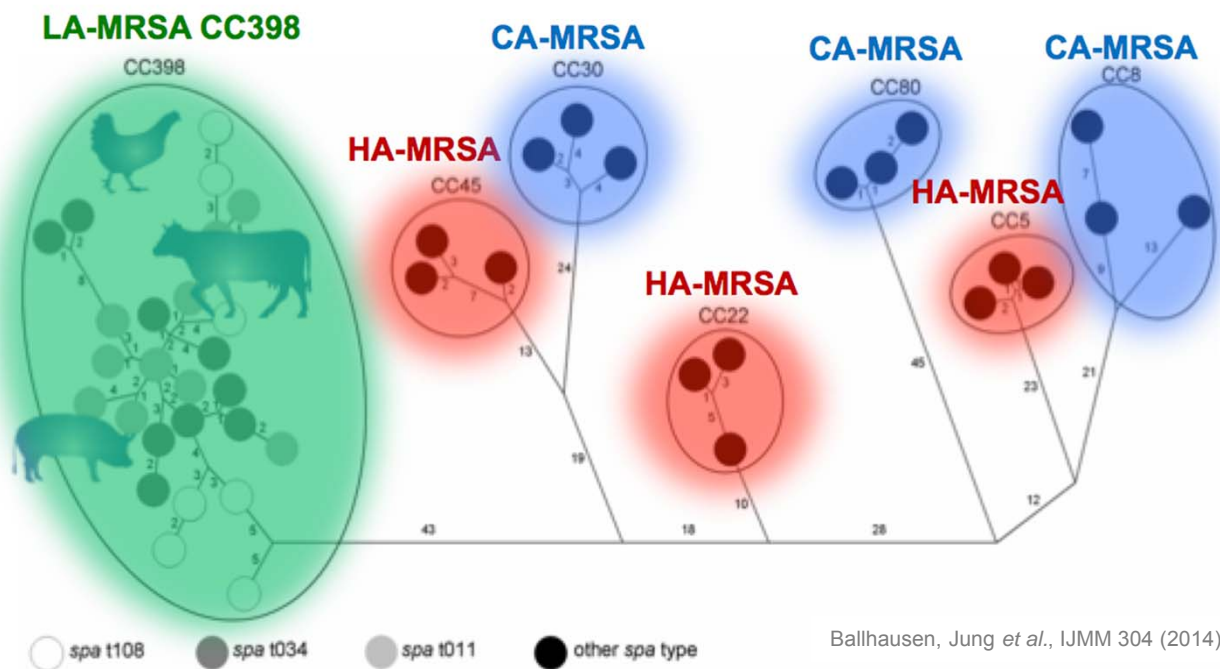
RESEARCH ARTICLE
Uhlenmann *et al.*, mBio Volume 3 Issue 2 (2012)

Identification of a Highly Transmissible Animal-Independent *Staphylococcus aureus* ST398 Clone with Distinct Genomic and Cell Adhesion Properties

Anne-Catrin Uhlemann,^a Stephen F. Porcella,^b Sheetal Trivedi,^a Sean B. Sullivan,^a Cory Hafer,^a Adam D. Kennedy,^c Kent D. Barbian,^b Alex J. McCarthy,^d Craig Street,^e David L. Hirschberg,^a W. Ian Lipkin,^a Jodi A. Lindsay,^d Frank R. DeLeo,^c and Franklin D. Lowy^a

ABSTRACT A methicillin-resistant *Staphylococcus aureus* (MRSA) clone known as ST398 has emerged as a major cause of acute infections in individuals who have close contact with livestock. More recently, the emergence of an animal-independent ST398 methicillin-sensitive *S. aureus* (MSSA) clone has been documented in several countries. However, the limited surveillance of MSSA has precluded an accurate assessment of the global spread of ST398 and its clinical relevance. Here we provide evidence that ST398 is a frequent source of MSSA infections in northern Manhattan and is readily transmitted between individual households. This contrasts with the limited transmissibility of livestock-associated ST398 (LA-ST398) in rural areas and smaller than that of the LA-ST398 MRSA reference strain S0398. Our whole-genome sequence analysis revealed that the chromosome of the LA-ST398 MSSA isolates harbored a distinct *scn*. While most of the

The Clonal Complex (CC)398: Common *spa* types

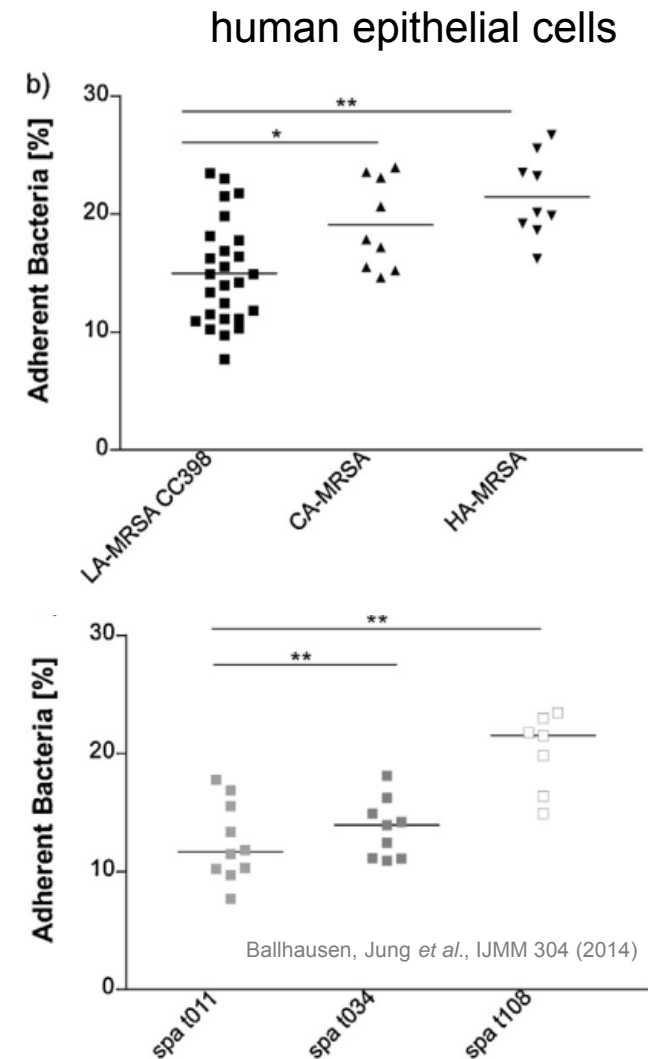
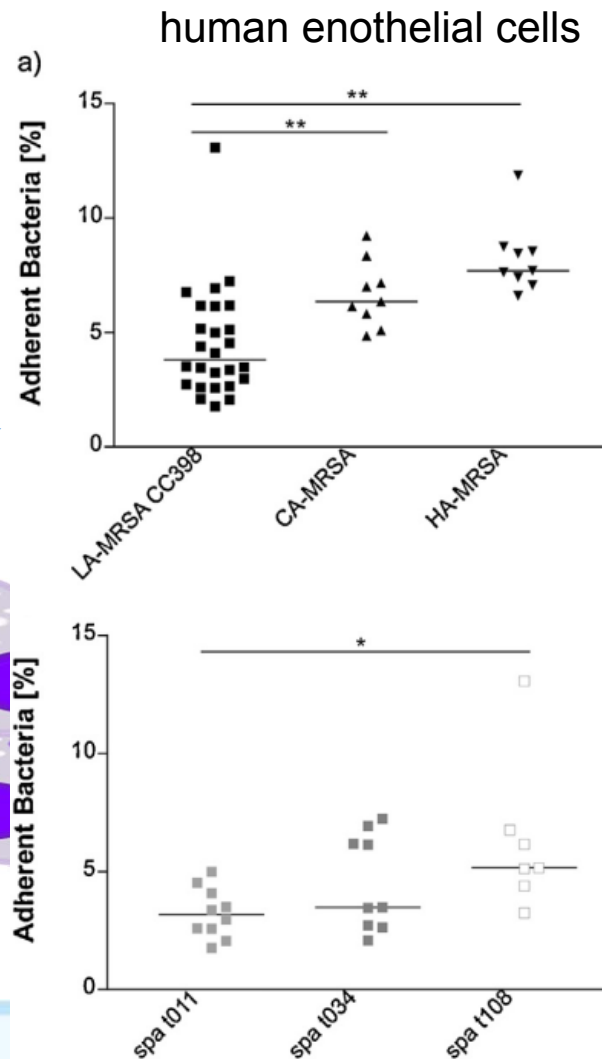
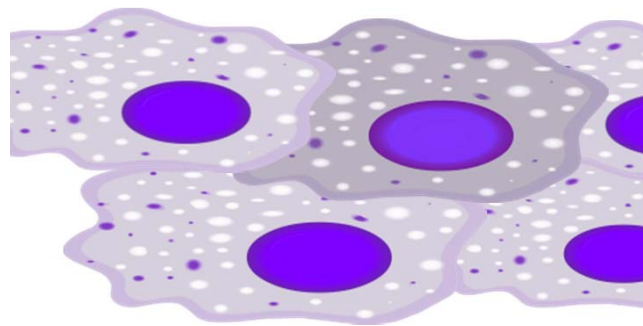
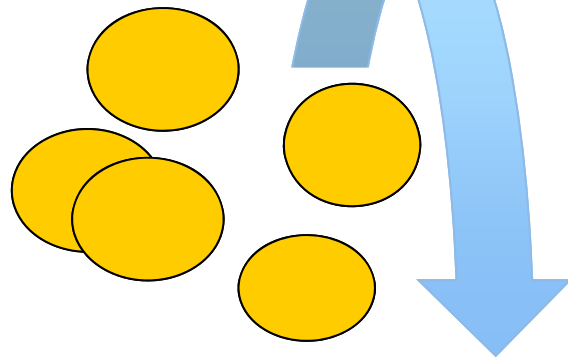


set of 44 MRSA isolates

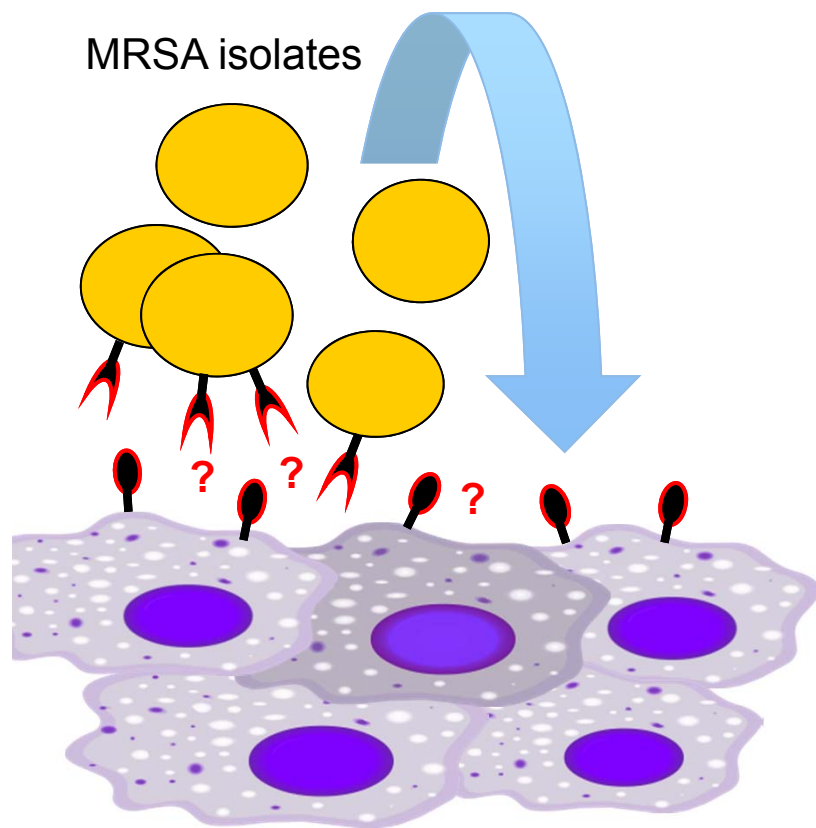
- 26 LA-MRSA CC398 (*spa* t011, *spa* t034, *spa* t108)
- 9 CA-MRSA
- 9 HA-MRSA

LA-MRSA CC398: Adhesion to human endothelial and epithelial cells

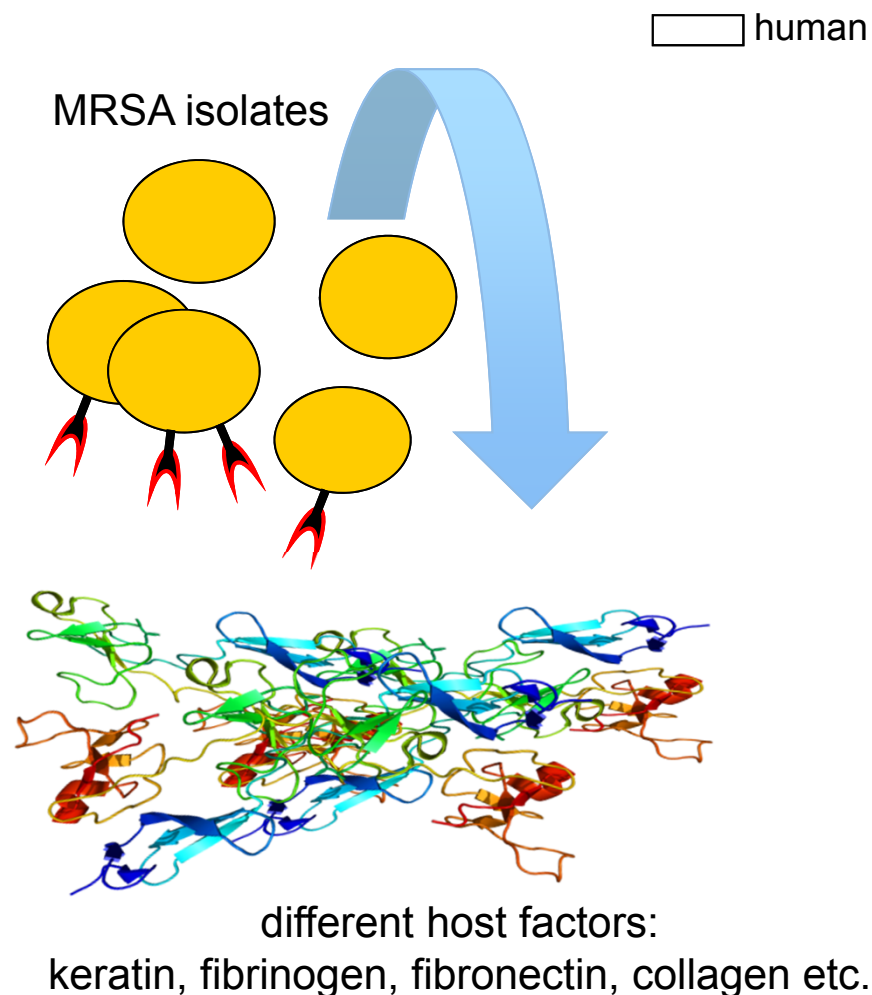
MRSA isolates



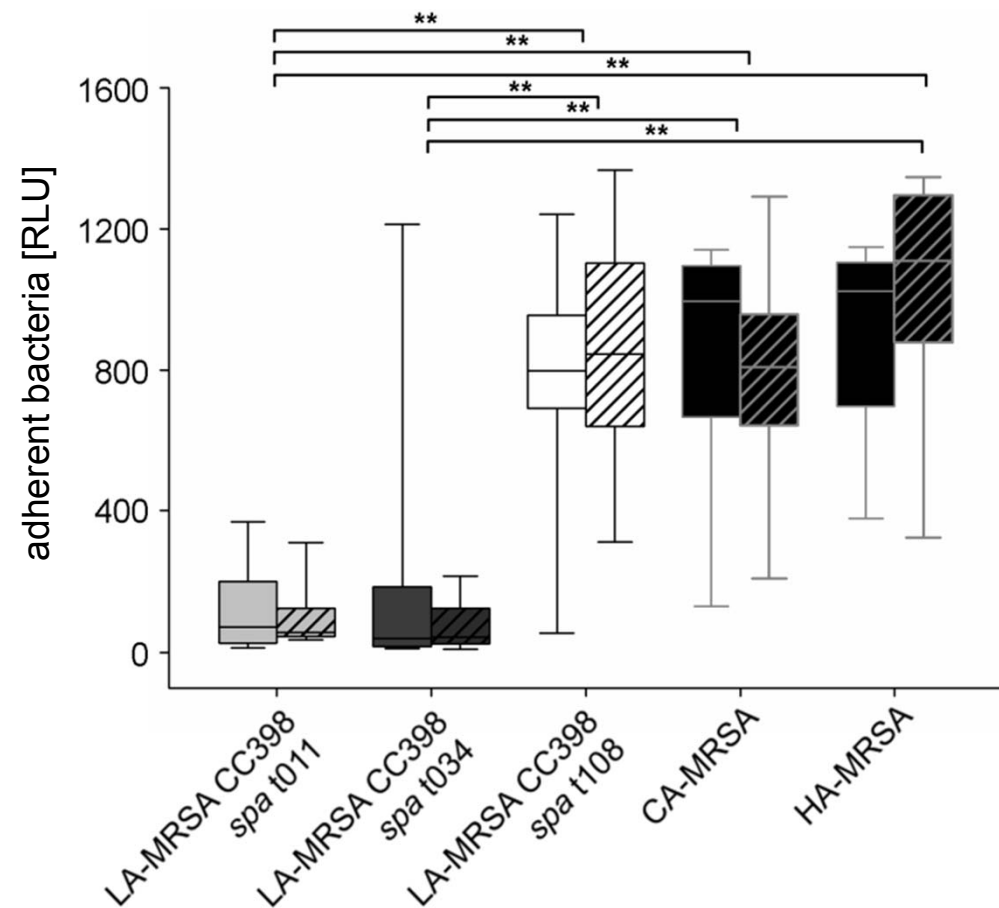
LA-MRSA CC398: Adhesion to specific host factors



LA-MRSA CC398: Adhesion to specific host factors



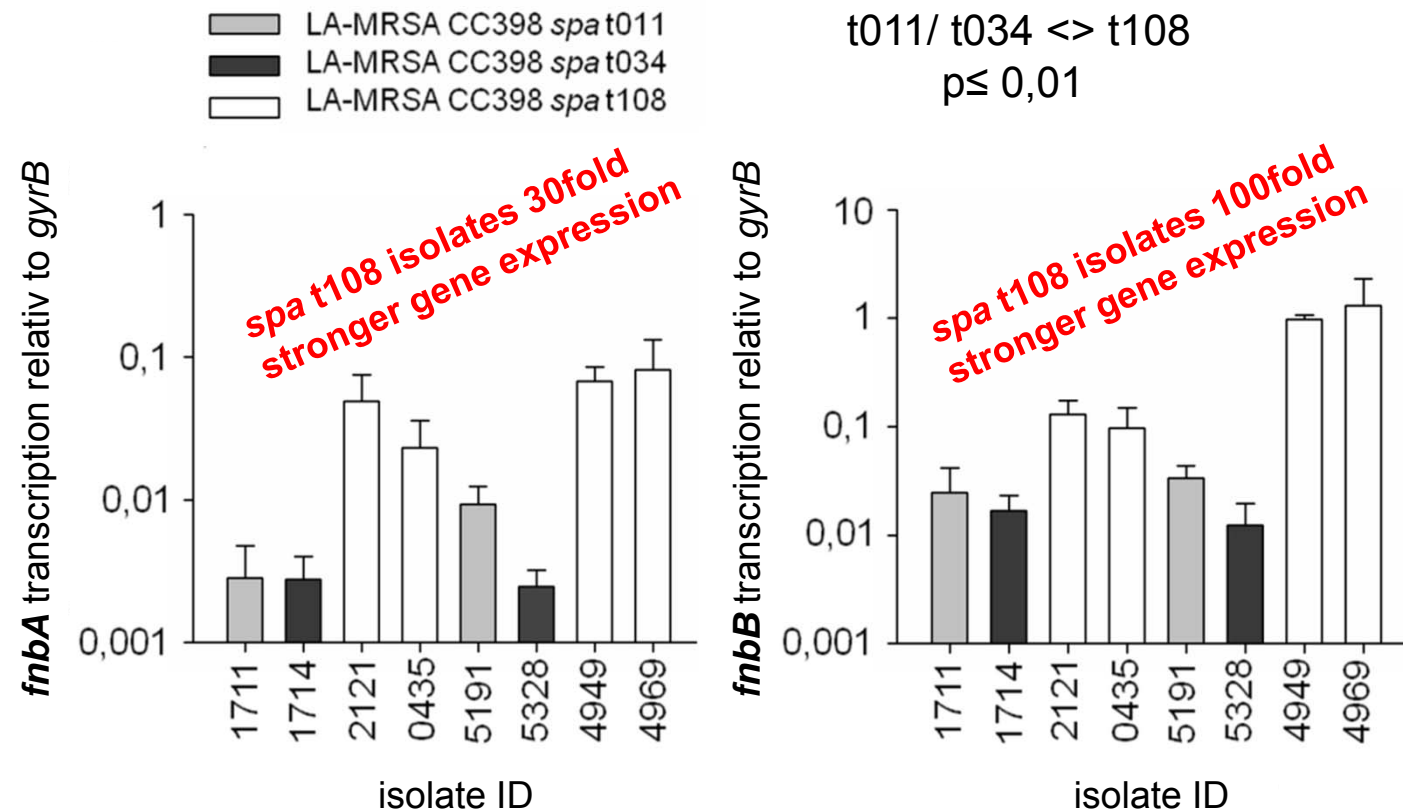
□ human plasma fibronectin ▨ bovine plasma fibronectin



Ballhausen, Jung *et al.*, IJMM 304 (2014)

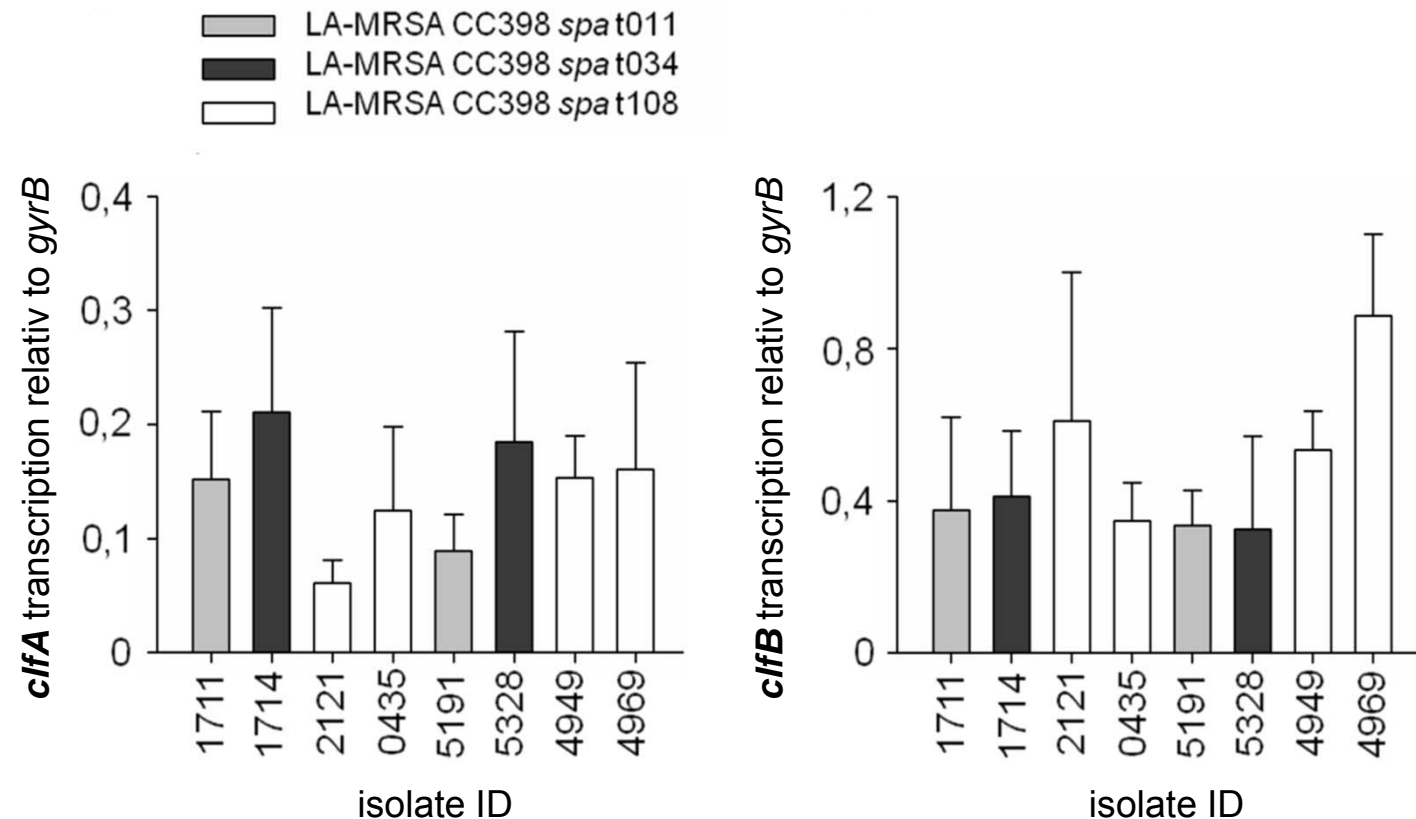
LA-MRSA CC398: transcription of bacterial adhesins

fnb A/B transcription (genes encoding the fibronectin binding proteins)

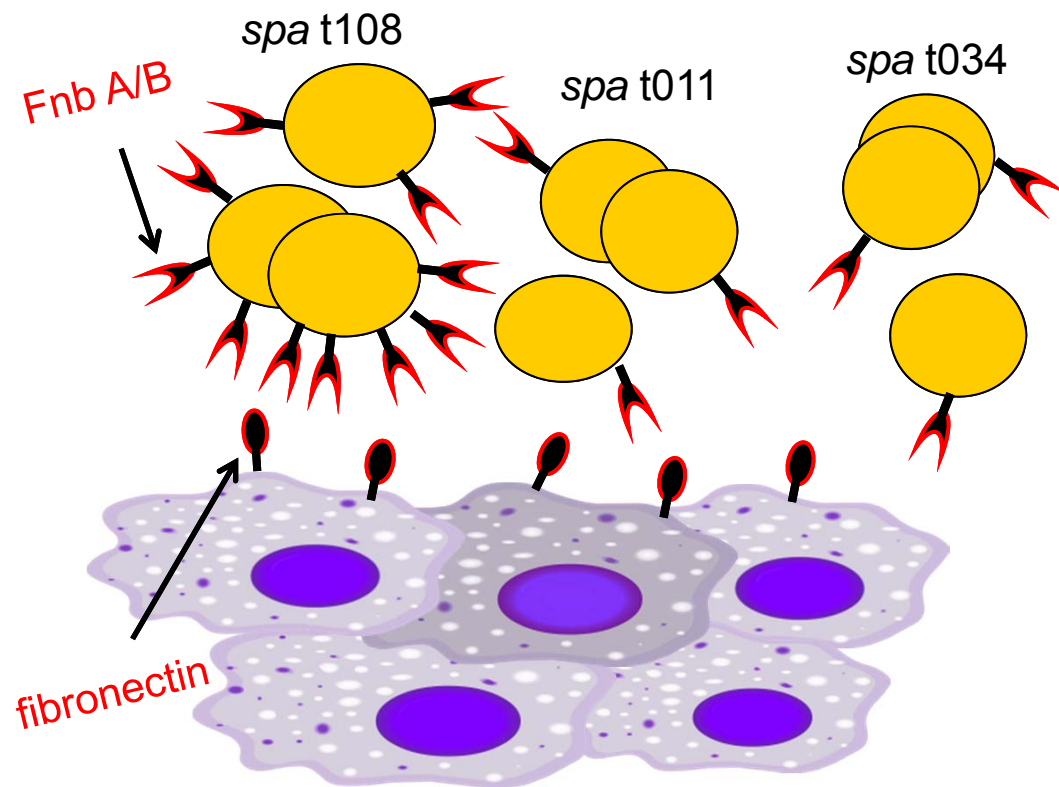


LA-MRSA CC398: transcription of bacterial adhesins

clf A/B transcription (genes encoding the clumping factors A and B)



LA-MRSA CC398: Adhesion to specific host factors



- CC398: reduced host adhesion compared to HA-, CA-MRSA
- High intra-lineage diversity: *spa* type t108 isolates stronger host adhesion
- Host cell adhesions characteristics might be explained with adhesion to fibronectin
- *spa* type t108 enhanced transcription of fibronectin binding proteins

fnb B point mutation leading to premature stop codon (AA 372):

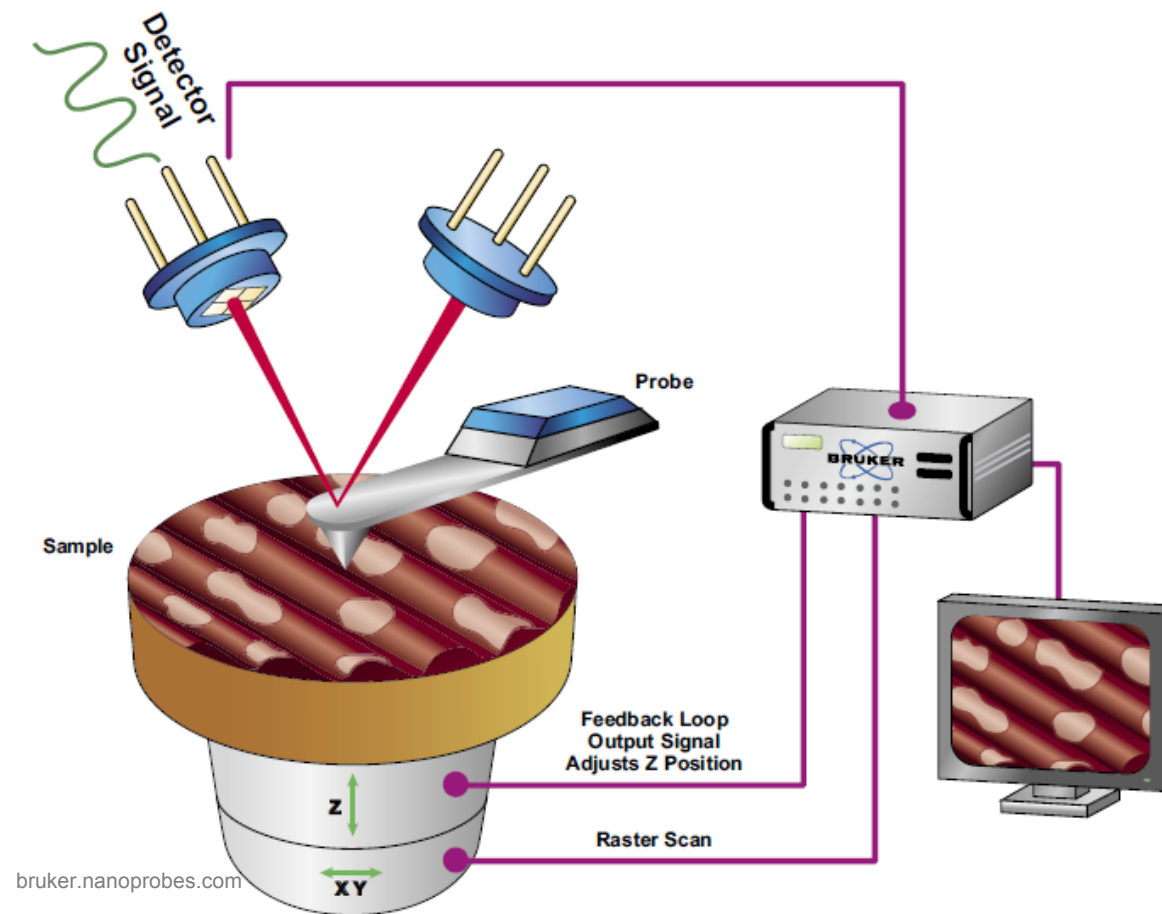
spa t011 / t034:

+ 4 of 4 isolates tested

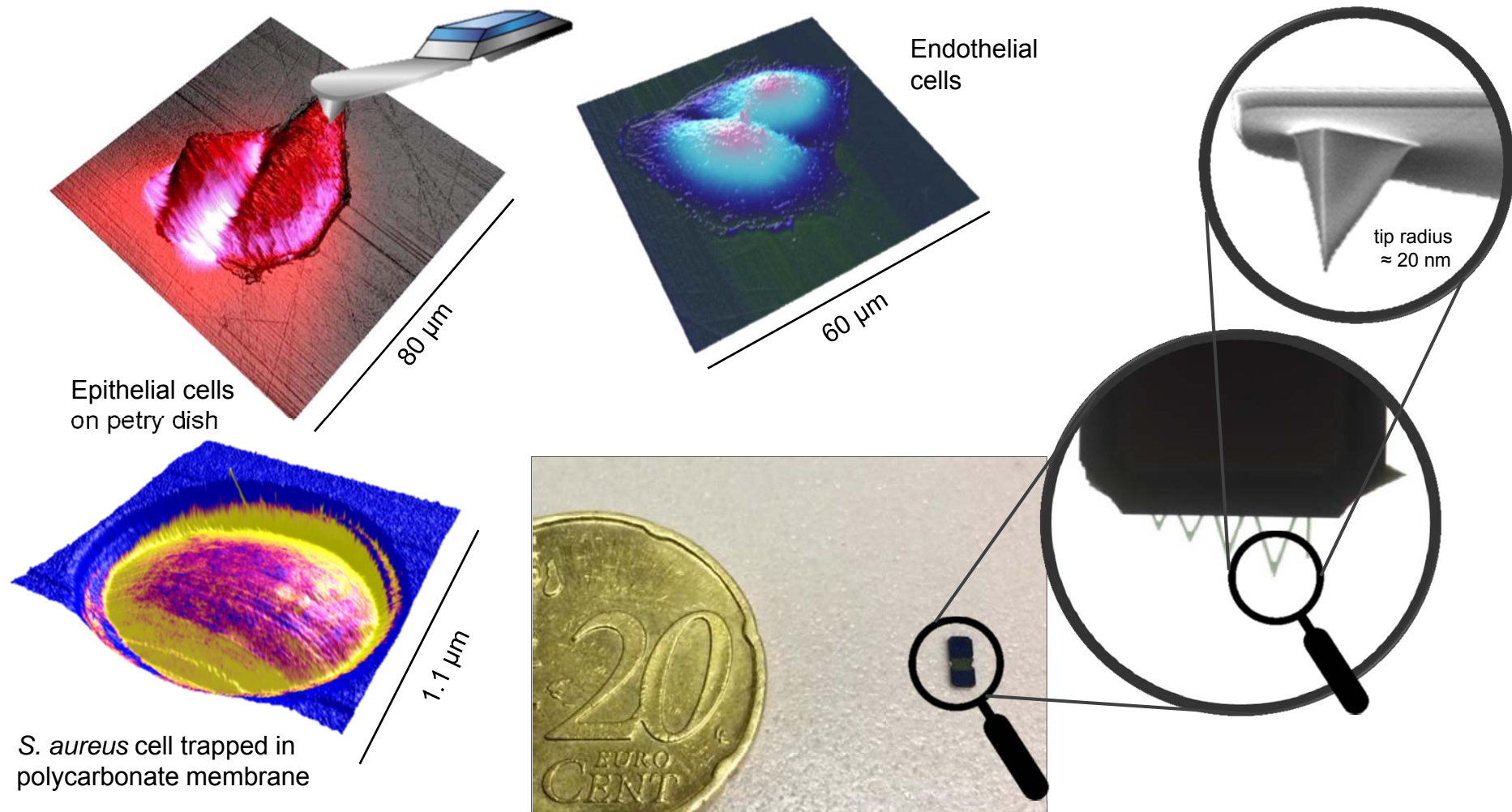
spa t108:

+ 1 of 4 isolates tested

Atomic Force Microscopy: Opportunities in Microbiology

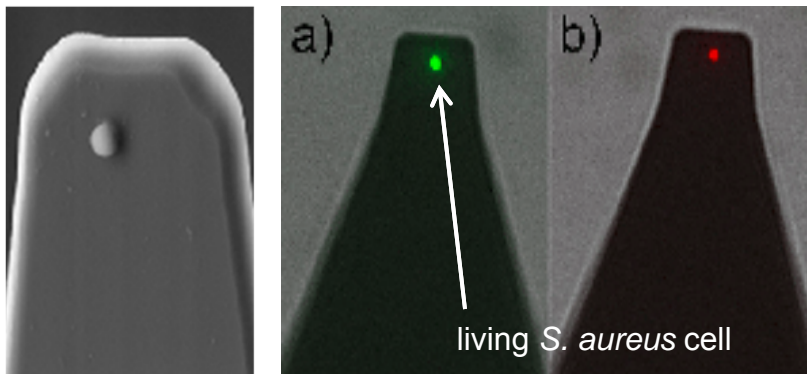


Atomic Force Microscopy: Opportunities in Microbiology



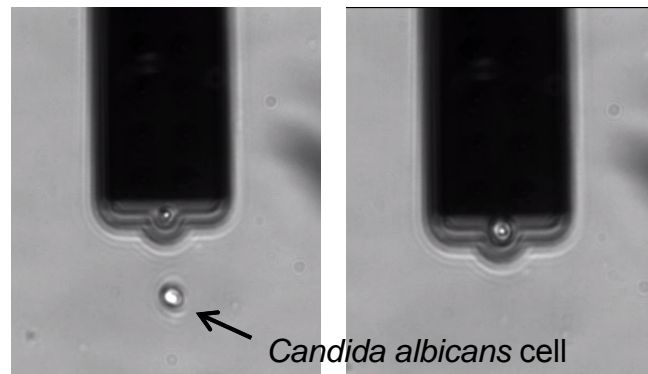
Atomic Force Microscopy: Opportunities in Microbiology

Single cell force spectroscopy:



fixation by gluing

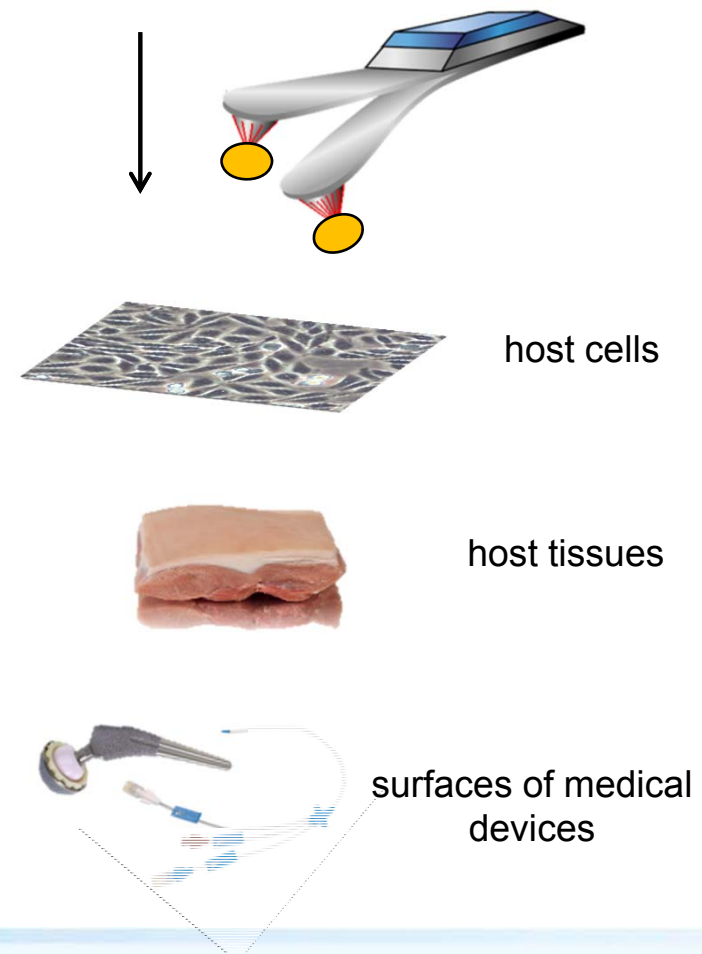
Thewes *et al.*, Eur. Phys. J. E 38 (2015)



fixation by negative pressure

Candida albicans cell

Single cell/molecule force spectroscopy



host cells

host tissues

surfaces of medical devices

Staphylococcus aureus pathogenicity

- Colonizer of mammal skin and nares
- Opportunistic pathogen
- Local skin infection (carbuncle, furuncle)
- Life-threatening systemic infections (sepsis, endocarditis)
- Host adhesion is the basic condition for infections

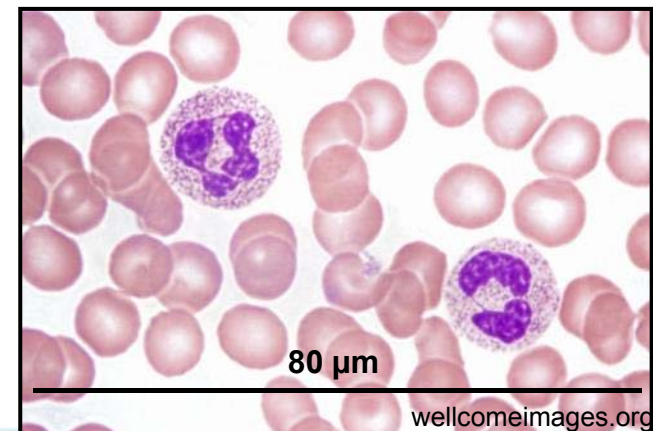
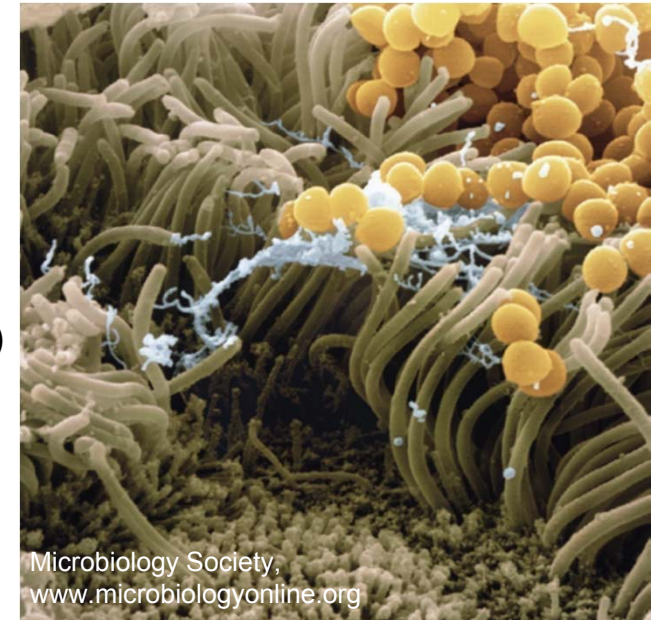
Voss *et al.*, EJCMI 13 (1994)

Lowy, F.D., N Engl J Med 339 (1998)

- Immune evasion (protection against phagocytosis)
- Exchange of Mobile Genetic Elements (MGE)

Lowy, F.D., N Engl J Med 339 (1998)

Lindsay, IJMM 304 (2014)





S. aureus immune evasion: The ϕ *Saint3* coded Immune Evasion Cluster

- Exchange of MGE by Horizontal Gene Transfer
- Horizontal Gene Transfer important role in host adaptation
- Bacteriophage *Saint3* -coded MGE:

Immune Evasion Cluster (IEC)

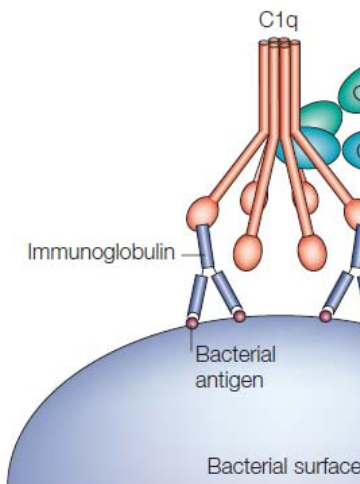
- ***chp***: chemotaxis inhibitory protein,
- ***scin***: staphylococcal complement inhibitor
- ***sak***: staphylokinase

- ϕ *Saint3* inserts in the β -hemolysin coding gene *hly* (*hly* function is lost)
- Publications suggests that the IEC factors acts human specifically

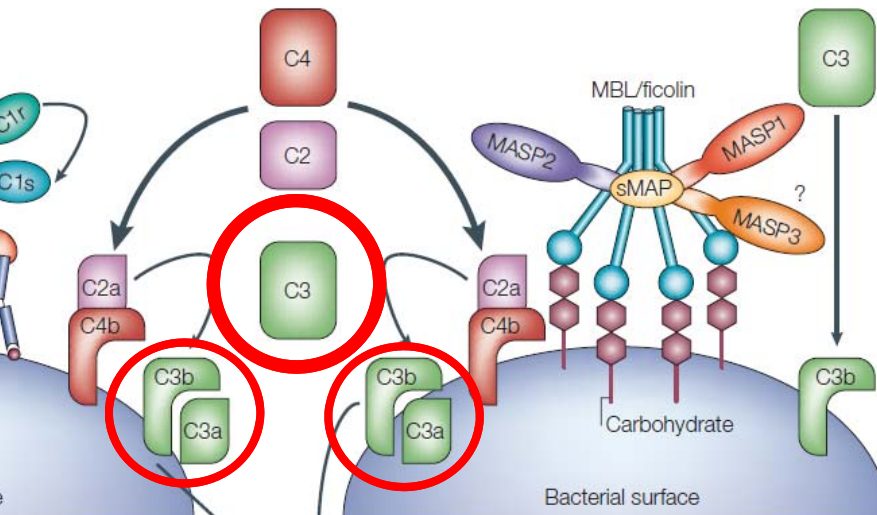
van Wamel *et al.* J Bac Vol. 188 (2005); Mc Carthy *et al.*, Appl. Environ. Microbiol. 78 (2012)

S. aureus immune evasion: Inhibition of the complement system

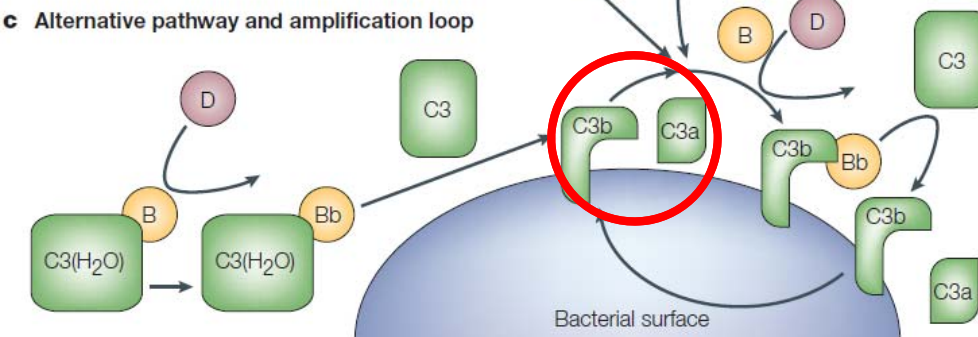
a Classical pathway



b Lectin pathway

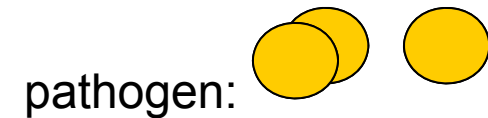


c Alternative pathway and amplification loop



facilitate phagocytosis

- chemotaxis
- opsonization



avoid phagocytosis:

CHIP: blocking the C5a receptor
 SCIN: inhibiting the C3 convertase-complex
 SAK: inactivating cell wall bound C3b, antibodies

Foster, Nat Rev Microbiol 3 (2005)

Impact of bacteriophage *Saint3* carriage on the protection against phagocytosis

Set: 20 *S. aureus* CC398 isolates

10x ϕ *Saint3* positive
10x ϕ *Saint3* negative

(human, pig, horse, poultry)

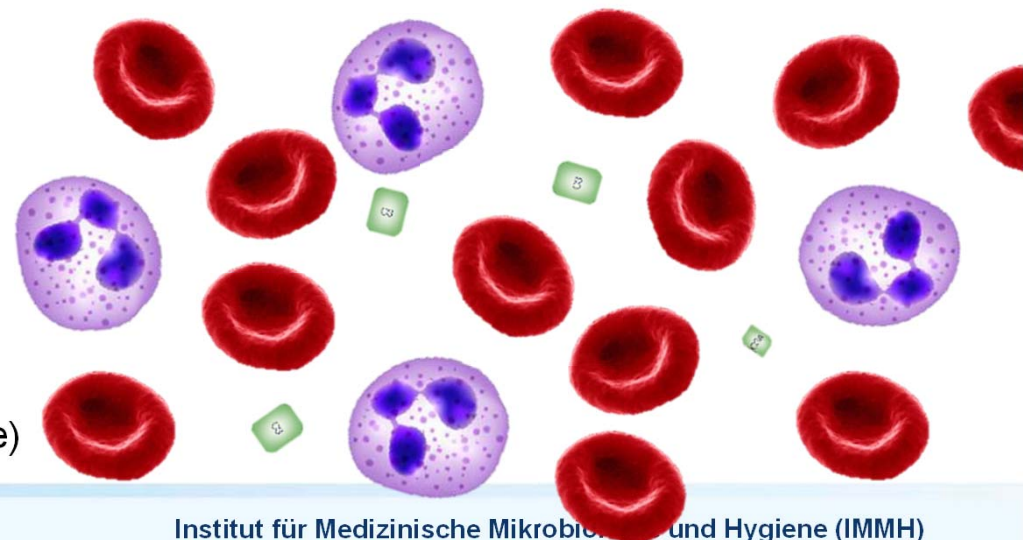
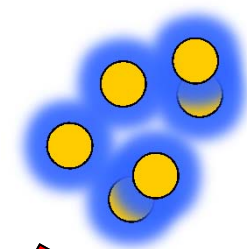
3 ϕ *Saint3* negative isolates transduced
from ϕ *Saint3* positive CC398 donor

**3 ϕ *Saint3*-positive / negative isolate
pairs**



flow cytometer

fluorescence stained
S. aureus isolates

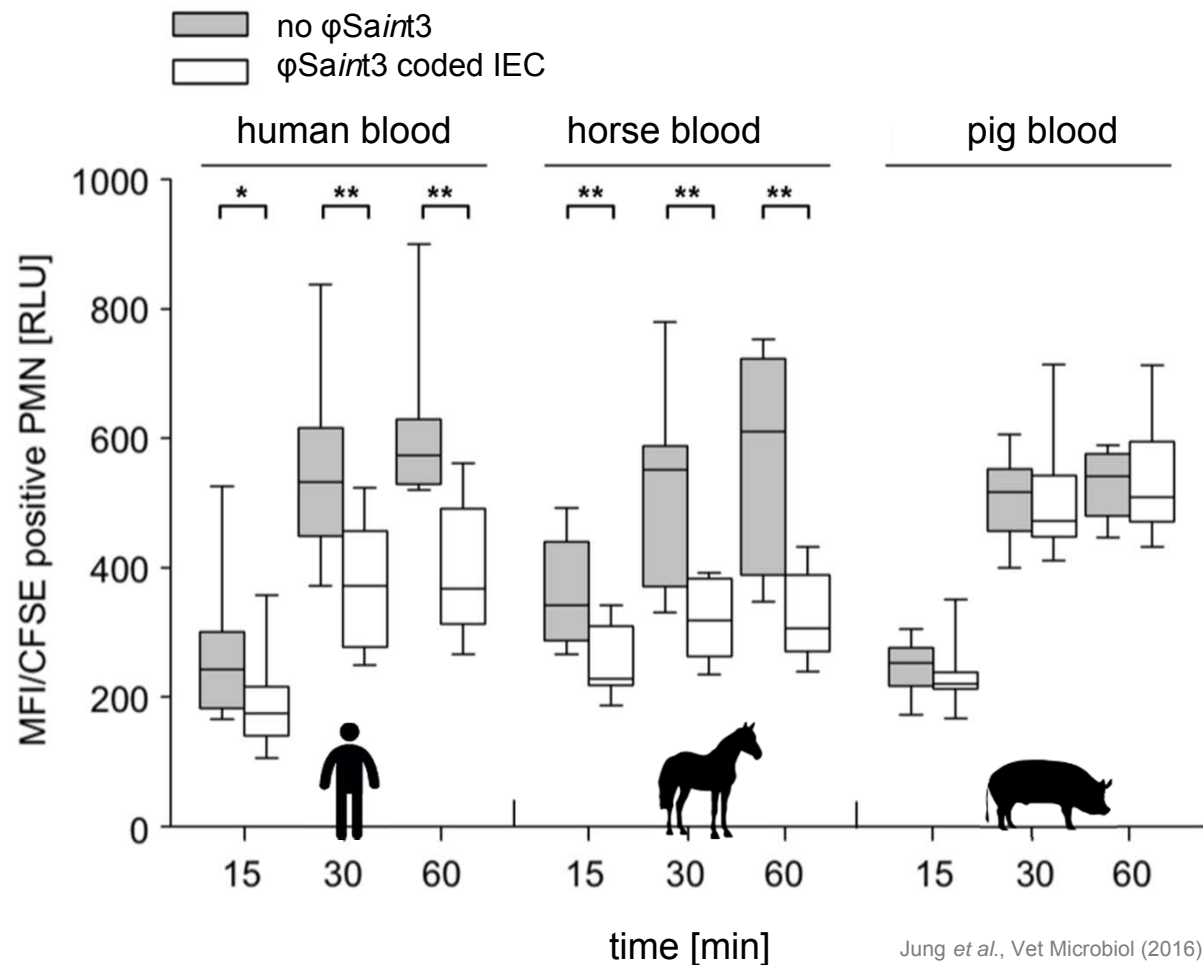


whole blood
(human, pig, horse)

Impact of bacteriophage *Saint3* carriage on the protection against phagocytosis

ϕ Saint3 coded Immune Evasion Cluster (IEC)

- **chp**: chemotaxis inhibitory protein,
- **scin**: staphylococcal compl. inhibitor
- **sak**: staphylokinase,

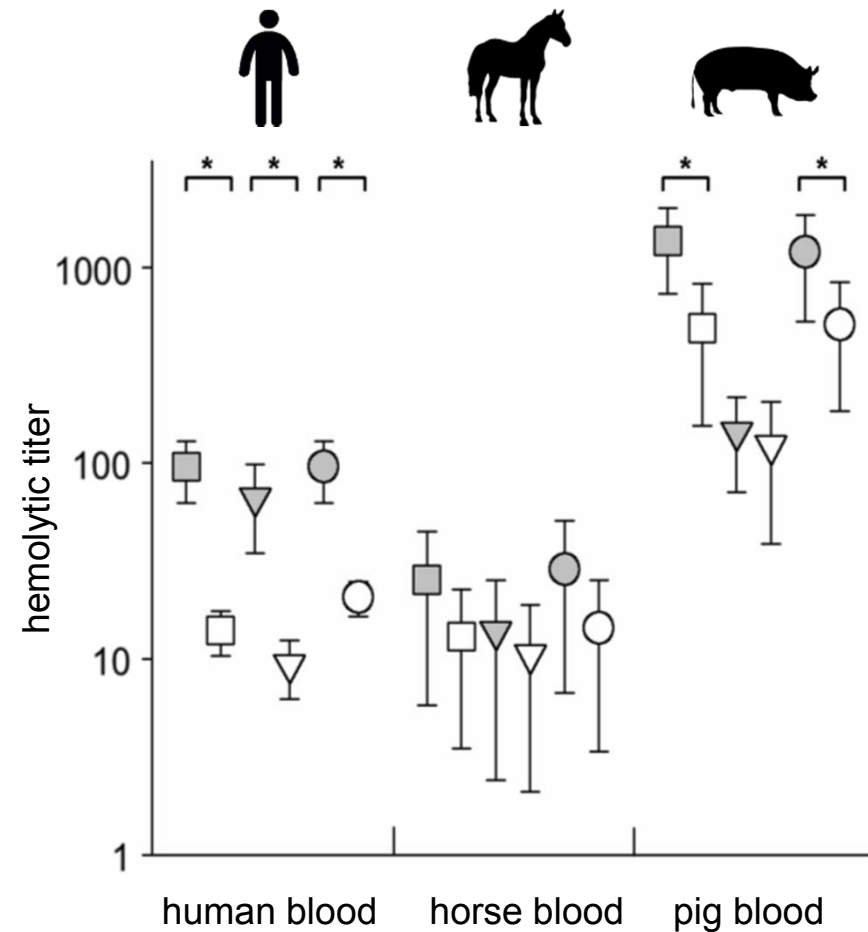
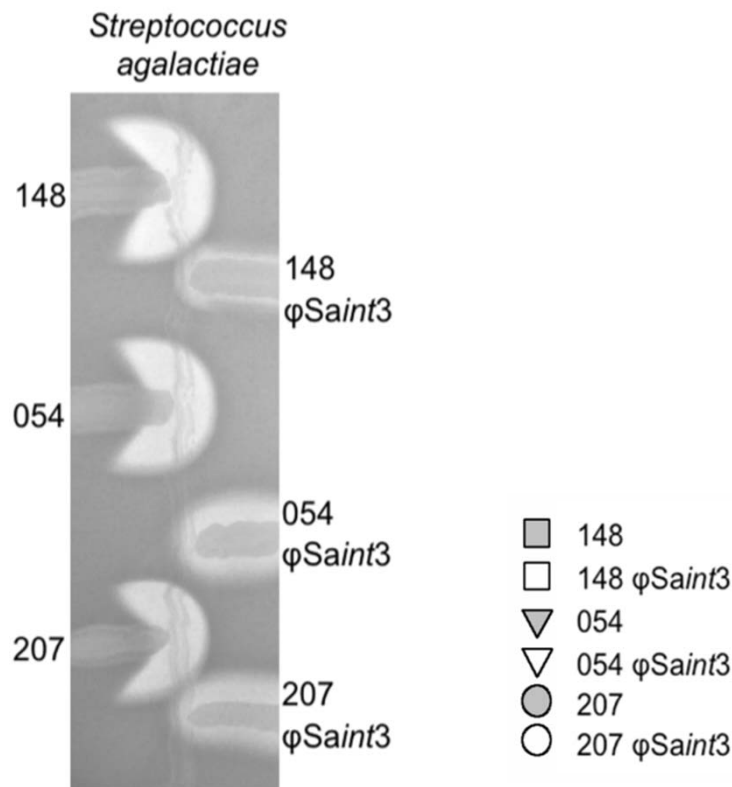


Impact of bacteriophage ϕ Saint3 carriage on the hemolytic potential

Set:

3x ϕ Saint3-negative isolates

3x ϕ Saint3 positive transduced isolates

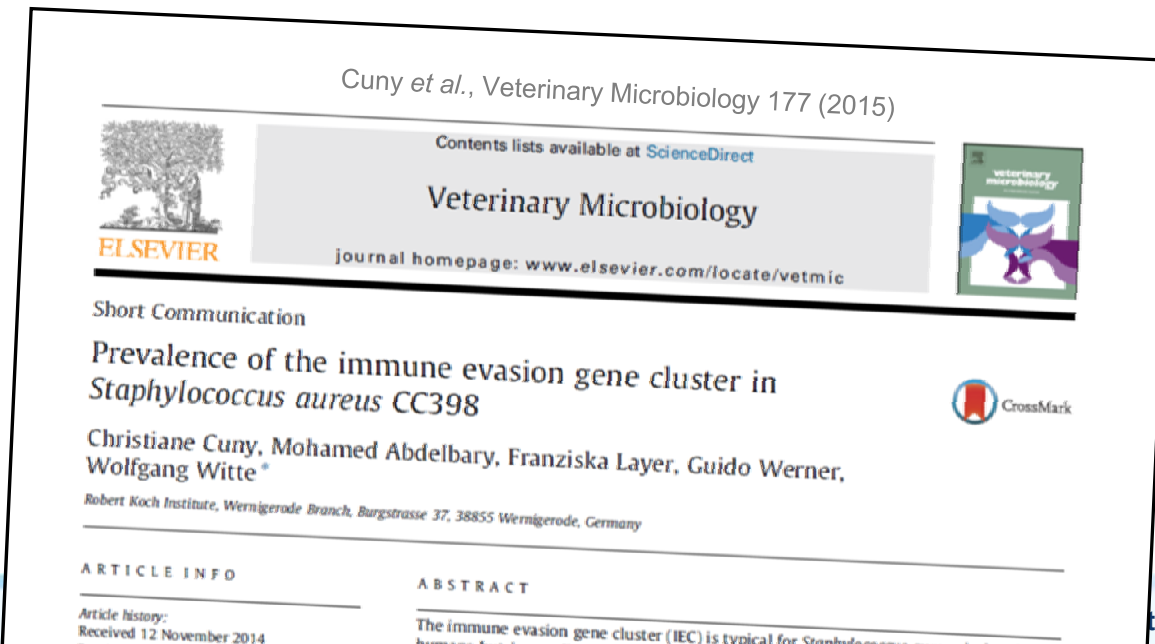


Jung *et al.*, Vet Microbiol (2016)



Impact of ϕ Saint3 carriage on CC398 isolates: Summary

- Significant protective effect of ϕ Saint3 coded IEC against phagocytosis by human, equine neutrophils, but not porcine neutrophils
- Loss of the Hlb function effects the hemolytic potential of CC398 isolates, on human and porcine erythrocytes, while equine erythrocytes are not affected
- Host specificity of the IEC components might be broader than currently assumed



- CC398 colonization pigs: **0/94**
- CC398 infection horses: **6/61**
- CC398 colonization veterinarians treating horses : **4/64**



Thank You!!!

- Partners from the MedVet-Staph Network for sharing isolates and data!
- colleagues and students for help and commitment!
- BMBF for six years of funding!
- Thank You for your attention!

Institute of Medical Microbiology and Hygiene



Impact of bacteriophage ϕ Saint3 carriage on the hemolytic potential

Aufnahme durch PMN in Vollblut:

3x ϕ Saint3-negative Mutterisolate

3x ϕ Saint3-positive Isolatderivate

