

#O416

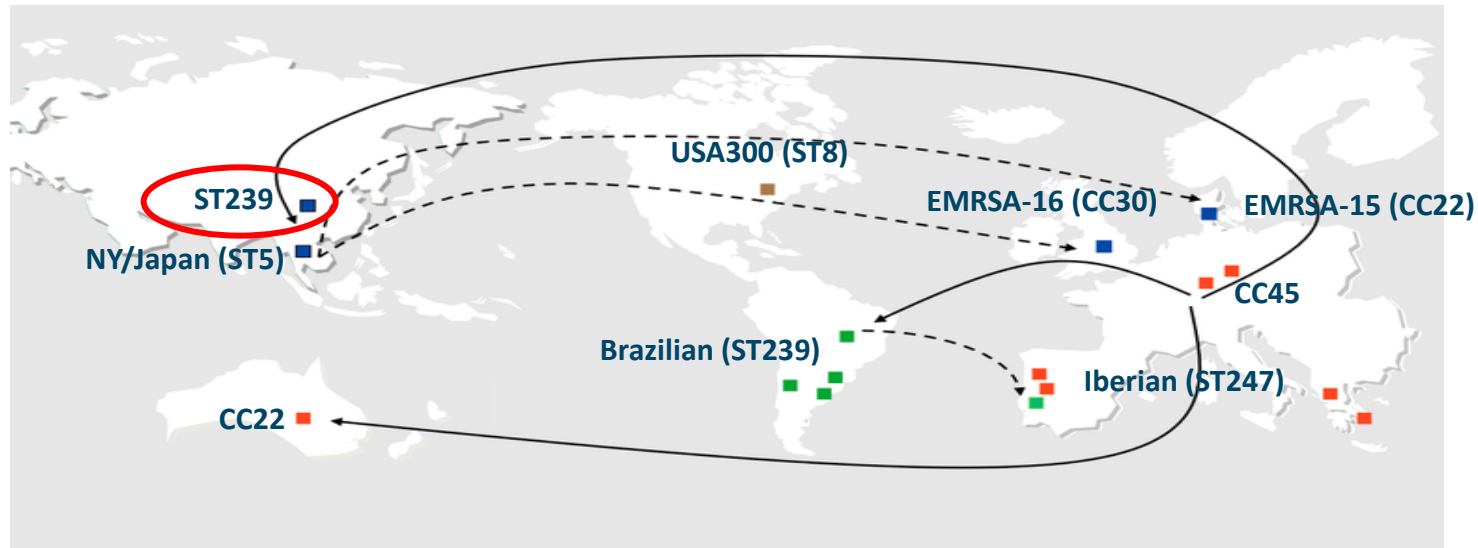
# Genomic context of a colonization and pathogenicity determinant, *sasX*, in methicillin resistant *Staphylococcus aureus*

**De Backer, S.<sup>1</sup>**, Xavier, B. B.<sup>1</sup>, Vanjari, L.<sup>2</sup>, Coppens, J.<sup>1</sup>, Lammens, C.<sup>1</sup>, Vemu, L.<sup>2</sup>, Tacconelli, E.<sup>3</sup>, Schrenzel, J.<sup>4</sup>, Empel, J.<sup>5</sup>, Hryniewicz, W.<sup>5</sup>, Goossens, H.<sup>1</sup>, & Malhotra-Kumar, S.<sup>1#</sup>

1 – Department of Medical Microbiology, Vaccine & Infectious Diseases Institute, University of Antwerp, 2610 Wilrijk, Belgium. 2 – Department of Microbiology, Nizam's Institute of Medical Sciences, Panjagutta, Hyderabad 500082, Telangana, India. 3 – Department of Infectious Diseases, Università Cattolica Sacro Cuoro, 00168 Rome, Italy. 4 – Genomic Research Laboratory, Service of Infectious Diseases, University Hospitals of Geneva, 1211 Geneva-14, Switzerland. 5 – National Institute of Public Health, 00-725 Warsaw, Poland.

# Corresponding author mailing address: Department of Medical Microbiology, Campus Drie Eiken, University of Antwerp, Building S, Universiteitsplein 1, B-2610 Wilrijk, Belgium. Phone: 32-3-265-27-52. Fax: 32-3-265-26-63. E-mail: surbhi.malhotra@uantwerpen.be.

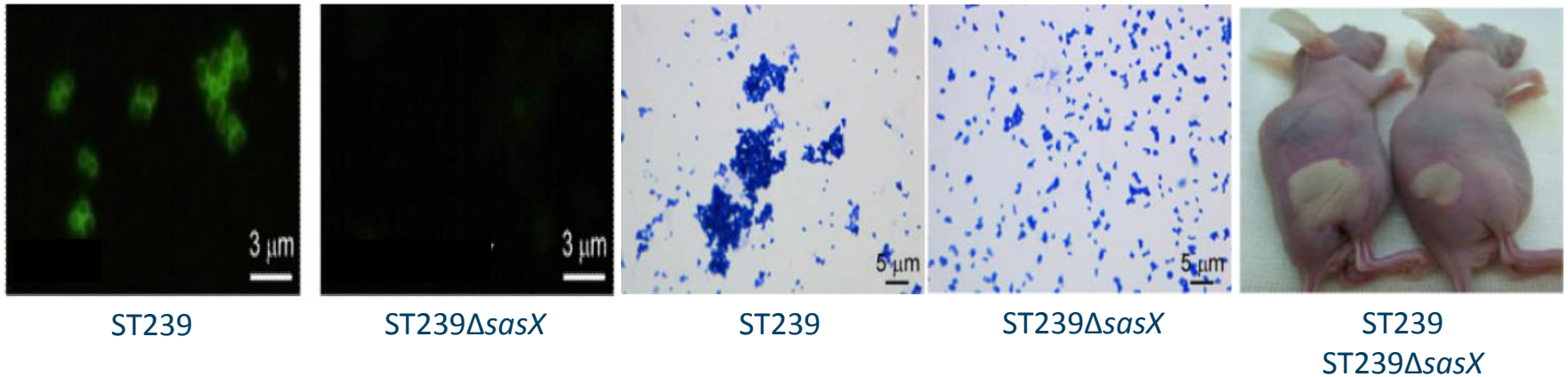
# Evolutionary dynamics of methicillin-resistant *S. aureus* ST239



- MRSA infectious outbreaks occur in ‘**epidemic waves**’.
- Molecular factors potentially underlying the epidemiological success are poorly understood!
- **ST239** into focus: originated in Europe and most abundant ST recovered from hospital infections in Asia.
- TW20 associated with vascular access device-related bacteremia: **increased virulence capacity?**

REF: Harris, S. R., Feil, E. J., Holden, M. T., Quail, M. A., Nickerson, E. K., Chantratita, N., Gardete, S., Tavares, A., Day, N., Lindsay, J. A., Edgeworth, J. D., De Lencastre, H., Parkhill, J., Peacock, S. J., & Bentley, S. D., (2010). 'Evolution of MRSA during hospital transmission and intercontinental spread,' Science, 327(5964): 469-74. Chatterjee SS, Otto M. 2013. Improved understanding of factors driving methicillin-resistant Staphylococcus aureus epidemic waves. Clin Epidemiol 5:205-217. Uhlemann AC, Otto M, Lowy FD, DeLeo FR. 2014. Evolution of community- and healthcare-associated methicillin-resistant Staphylococcus aureus. Infect Genet Evol 21:563-574.

# Identification of the TW20 $\phi$ SP $\beta$ -like prophage encoded surface protein sasX

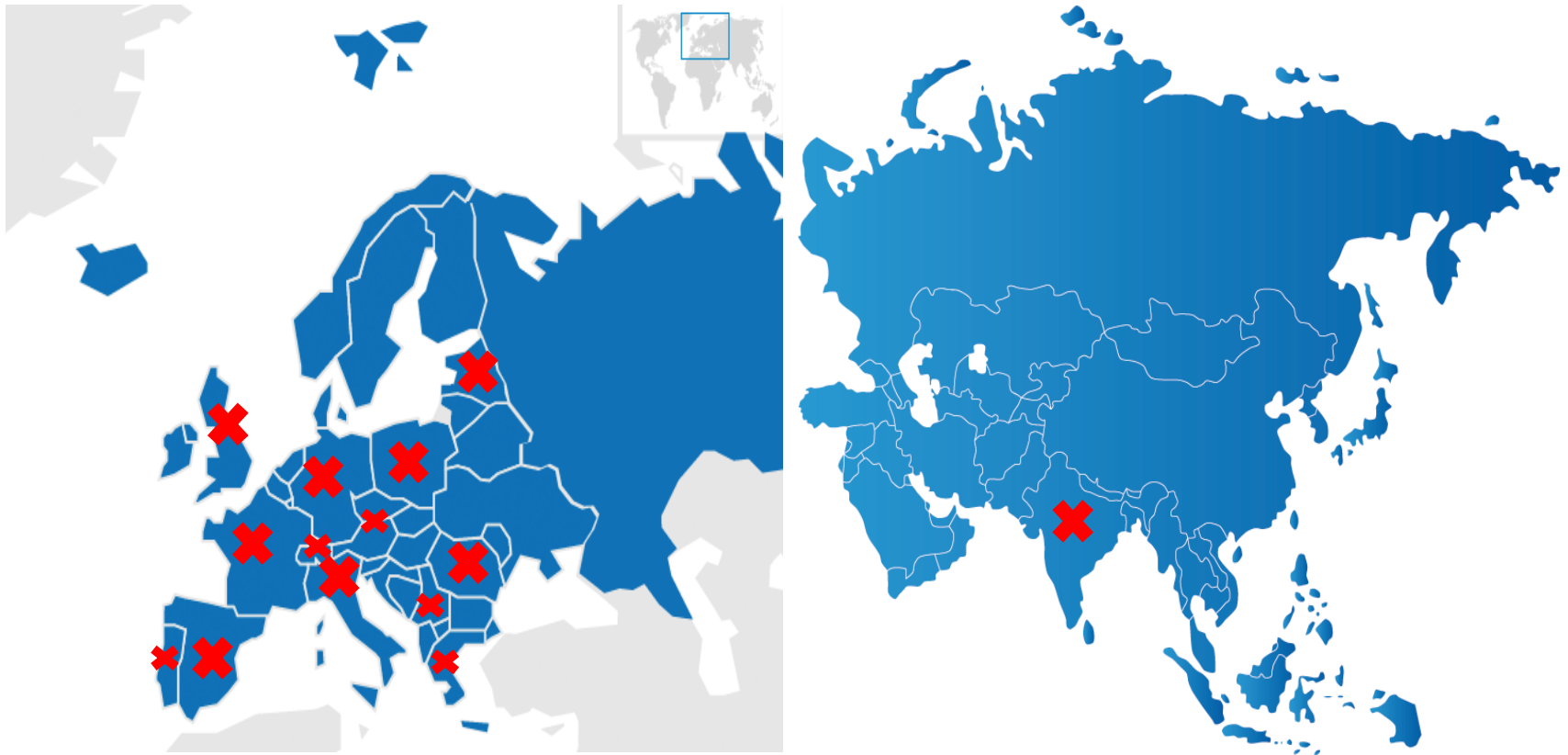


- Presence of a  $\phi$ SP $\beta$ -like prophage in TW20.
- Increased virulence: **LPXTG motif-harboring predicted surface protein** encoded close to the 3' end of the phage.
- *Staphylococcus aureus* surface protein **sasX**.
- Epidemiological success due to *sasX*:
  - Increased **nasal colonization**
  - Large **bacterial aggregation**
  - Enhanced **pathogen survival**

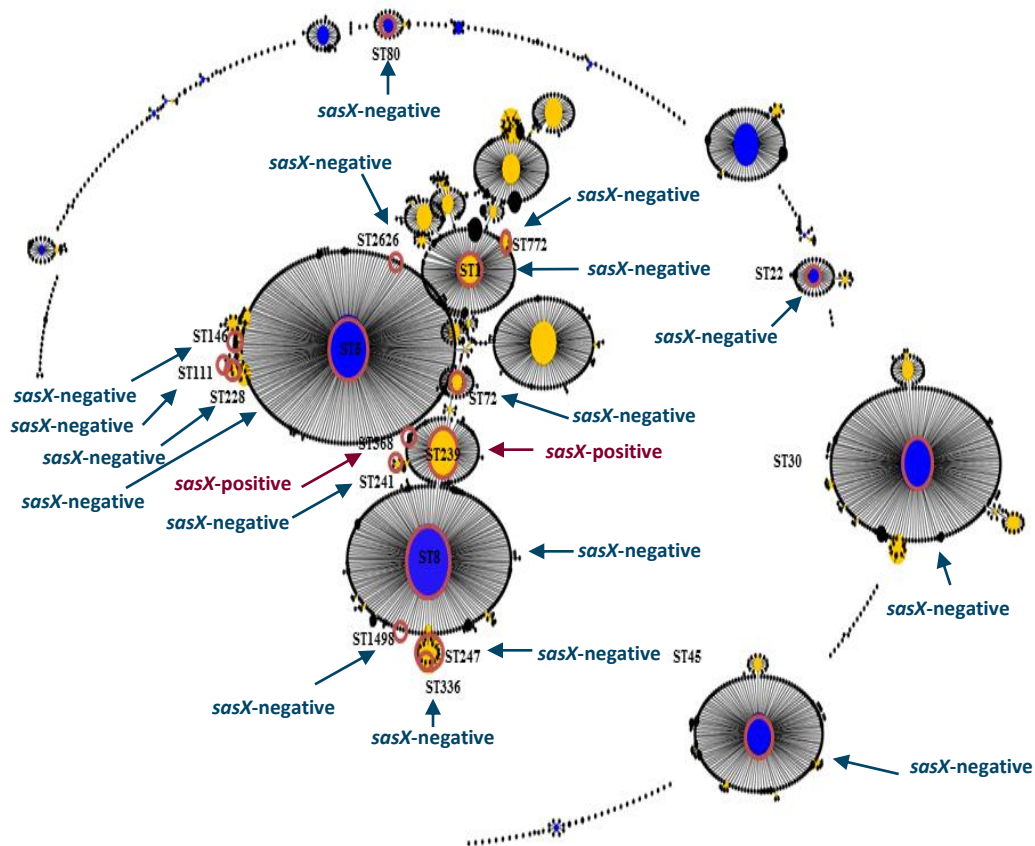
REF: Li M, Du X, Villaruz AE, Diep BA, Wang D, Song Y, Tian Y, Hu J, Yu F, Lu Y, Otto M. 2012. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. Nat Med 18:816-819.

## Aim of the study

This study employs *in vitro* studies using optimized shear-flow and static biofilm models, as well as powerful comparative genomics with whole genome sequencing to observe the dissemination and genomic context of *sasX* in divergent clonal and geographical backgrounds of *S. aureus*.



# Clonality profiling of *sasX*-positive and -negative *S. aureus* isolates



**Figure:** Analysis of European and Indian *S. aureus* isolates with eBURST as a population snapshot based on all unique STs of the entire *S. aureus* MLST database. Isolates within this study are highlighted in red and their corresponding ST is indicated. Primary founders are depicted in blue, secondary founders are depicted in yellow (De Backer, S., et al., in preparation).

## Clonality profiling of *sasX*-positive and *sasX*-negative *S. aureus* isolates

Table: Characteri

- Indian isolates

Name	ST
IN_ST239_b	239
IN_ST368	368
IN_ST239_c	239
IN_ST239_a	239
IN_ST161	161

- European isolates (n=

EU_ST239_1_6	239
EU_ST239_7	239

### SUMMARY:

- SasX* was exclusively found among **ST239** in Europe, while the frequency of *sasX* considerably increased among isolates of several **other STs** in India.
- European *sasX*-carrying were only isolated in **Poland**.
- Only strains belonging to **CC8**, and **SLVs of ST239**, proved to carry *sasX*.
- Most common infections associated with *sasX*: **wound infections, osteomyelitis and sepsis**.

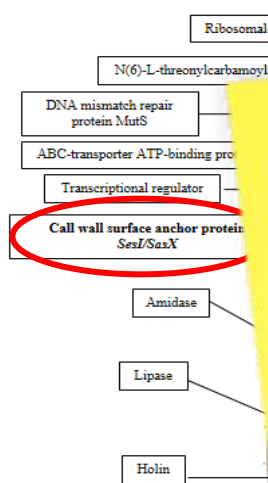
### Remark

patient in orthopaedic leg debridement  
 patient in neuro surgery osteomyelitis skull  
 patient in orthopaedic hemiarthoplasty  
 patient in orthopaedic ward intramedullary nailing of tibia  
 patient in orthopaedic ward dynamic compression of femur

men

men

# Characterization of the $\phi$ SP $\beta$ -like prophage



## SUMMARY:

- Horizontal gene transfer of *sasX* is linked to the  $\phi$ SP $\beta$ -like prophage:
  - Conserved phage insertion site.
  - Occassional phage deletions.
- Desintegration of the  $\phi$ SP $\beta$ -like prophage and chromosomal integration of *sasX*:
  - Original *B. Subtillus*  $\phi$ SP $\beta$ -like prophage: 172,2 kb in size.
  - *B. Subtillus*  $\phi$ SP $\beta$ -like prophage in Europe: 122 kb in size.
  - *B. Subtillus*  $\phi$ SP $\beta$ -like prophage in India: 60-80 kb in size.

**Figure:** Structure of the constructed in CGView position 1557651..1638 EU\_ST239\_7, respectively

REF: Brussow H, Canchaya C, Hardt WD. 2004. Phage evolution and virulence of bacterial pathogens. Virulence. Fortier LC, Sekulovic O. 2013. Importance of prophages to MRSA epidemic linked to a quickly spreading colonization and virulence determinant. Nat Med 18:816-819.



# Comparison of *sasX*- and *sesI*-harbouring *S. aureus* and *S. epidermidis* strains

A

IN\_S  
IN  
XN108  
TW20  
IN  
IN\_ST  
IN\_ST  
EU\_ST2  
EU\_ST2  
Cons  
Conserv  
Sequence

## SUMMARY:

- *S. aureus sasX* was acquired from clinical *S. epidermidis* causing invasive infections by horizontal gene transfer.

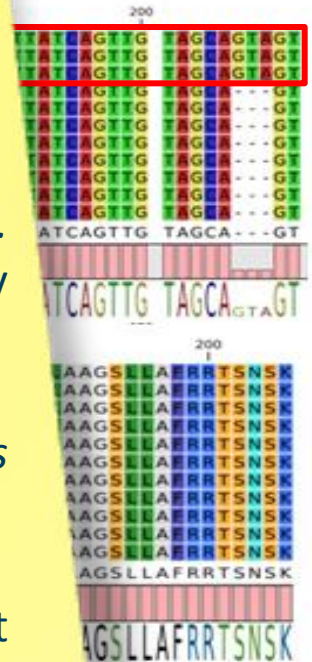
B

IN\_ST  
IN\_S  
XN108-S  
TW20-S  
IN\_ST  
IN\_ST2  
EU\_ST23  
EU\_ST23  
Conserv  
Conserv  
Sequence

- Presence of a **native *sesI*-homologue** in *S. aureus* showing 100% homology to *sesI* in *S. epidermidis*.
- Proof for **primary horizontal gene transfer event (*sasX/sesI*)**:

- Inherited multiple times?
- Alterations within gene have yet to occur?

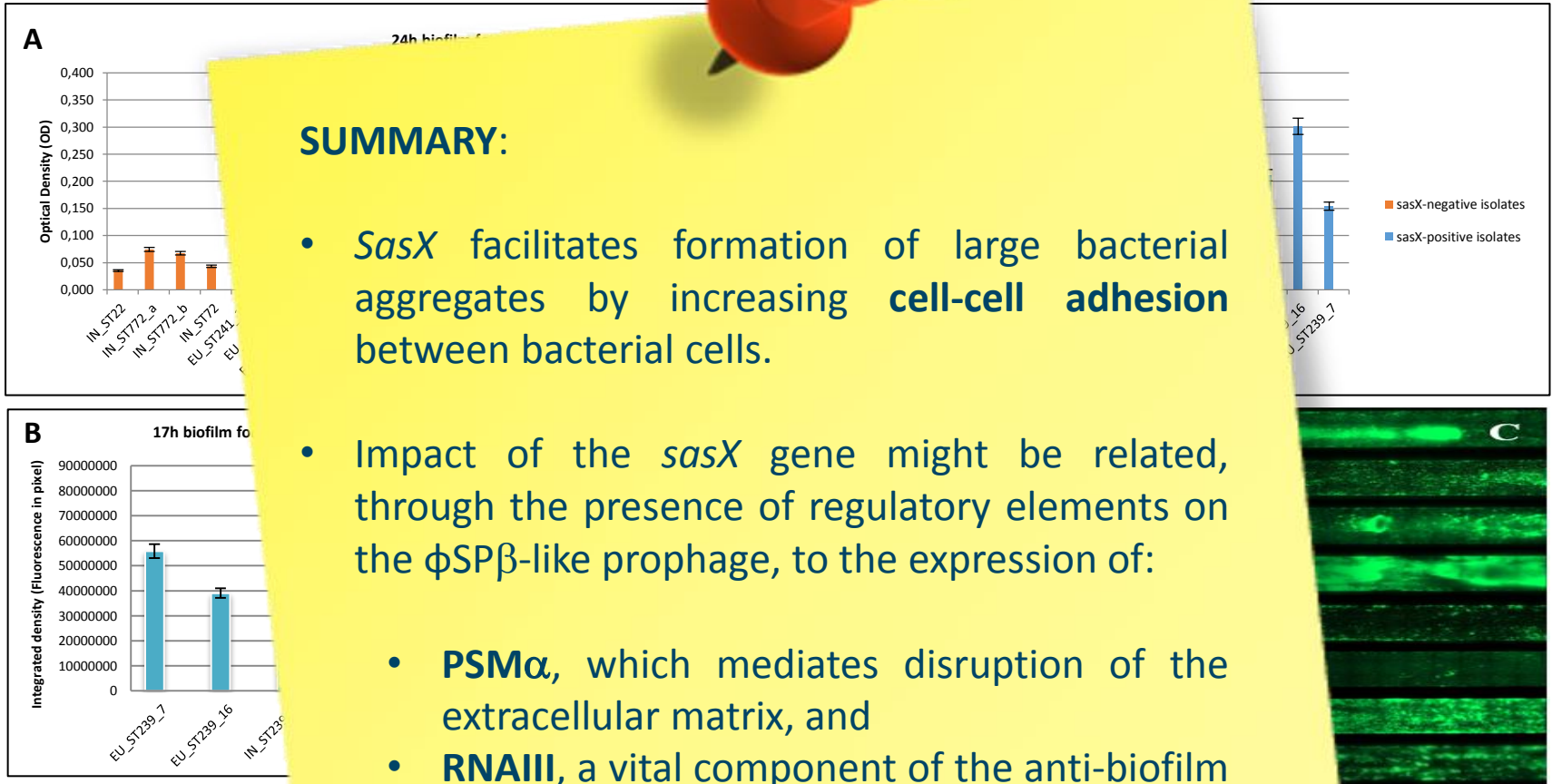
**Figure: (A)** *SasX* alignment with significant differences compared to *S. aureus* preparation).



REF: Wang, Z., Gerstein, M., & Snyder, M., (2006). The human genome. *Nature* 444:839-849. Soderquist B, Andersson M, Nilsson M, Nilsdotter-Augustinsson A, Persson L, Friberg O, Jacobsson S. 2009. Staphylococcal infection: Staphylococcal commensal species such as *S. epidermidis* and *S. aureus* are recognized as important sources of genes promoting MRSA colonization and virulence. *Bioessays* 35:4-11. Kriegeskorte A, Peters G. 2012. Horizontal gene transfer boosts MRSA spreading. *Nat Med* 18:662-663. Otto M. 2013. Molecular insight into how MRSA is becoming increasingly dangerous. *Virulence* 3:521-523.



# The impact of *sasX* on biofilm formation by *S. aureus*



**Figure:** (A) Formation of biofilms by *S. aureus* strains ST623 and ST670; (B) Formation of biofilms by *S. aureus* strains ST623 and ST670; (C) Fluorescence microscopy images of biofilms.

## SUMMARY:

- SasX* facilitates formation of large bacterial aggregates by increasing cell-cell adhesion between bacterial cells.
- Impact of the *sasX* gene might be related, through the presence of regulatory elements on the  $\phi$ SP $\beta$ -like prophage, to the expression of:
  - PSM $\alpha$** , which mediates disruption of the extracellular matrix, and
  - RNAIII**, a vital component of the anti-biofilm promoting *agr* system.

## Conclusions: horizontal gene transfer as the culprit for the rise of new MRSA clones?

- Occurrence of *sasX* in Indian and European **ST239** and its dissemination to **other ST's** in India.
  - Europe: *sasX*-positive strains limited to Poland
  - India: *sasX*-positive strains as SLVs of ST239
- **Desintegration** of the  $\phi$ SP $\beta$ -like prophage and **chromosomal integration** of *sasX* in India.
- *SasX* is responsible for spread and predominance of ST239 in the **hospital environment**.
- **Horizontal gene transfer** is a major mechanism enhancing epidemiological success of MRSA clones.
  - Prevalence of *sasX* and native *sesI*-homologue in *S. aureus*
  - Evolutionary selection pressure or multiple inheritance events?
- **Coagulase-negative staphylococci** as an important reservoir of genes.

## Aknowledgements



‘Impact on Specific Antibiotic Therapies on the prevalence of hUman host ResistaNt bacteria’, 2008.



‘Mastering hOSpital Antimicrobial Resistance and its spread into the Community’, 2007.

