

Molecular characterization of *Staphylococcus epidermidis* strains isolated from a teaching hospital in Shanghai, China

Min Li,^{1†} Xing Wang,^{2†} Qian Gao² and Yuan Lu¹

Correspondence

Yuan Lu

yuanlu@hsh.stn.sh.cn

¹Department of Laboratory Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, 12 Central Urumqi Road, Shanghai, PR China

²Key Laboratory of Medical Molecular Virology, and Institutes of Medical Microbiology and Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai, PR China

Staphylococcus epidermidis is a leading cause of hospital-acquired infections, mostly associated with the use of medical devices in seriously ill or immunocompromised patients. Currently, the clonal characteristics of *S. epidermidis* in the hospital environment in China are unknown; neither is it known why these sequence types are easily disseminated in the hospital setting. In this study, multilocus sequence typing (MLST) was employed for the clonal analysis of 80 *S. epidermidis* isolates collected from patients with *S. epidermidis* infections. MLST revealed a total of 16 different sequence types among these isolates. ST2, which contained exclusively *ica*-positive, IS256-positive and biofilm-forming isolates, represented the majority of clinical strains tested. Of the *S. epidermidis* strains circulating in the hospital environment in China, as many as 96.25% are resistant to meticillin. Four staphylococcal chromosomal cassette *mec* (SCC*mec*) types were identified among the total 80 *S. epidermidis* isolates, none of the strains carried an SCC*mec* I cassette. All of the ST2 isolates carried the SCC*mec* type III cassette. Taken together, the combination of biofilm-forming ability and antibiotic resistance helps ST2 become successfully established within nosocomial environments, and promotes the device-related infection and bacteraemia.

Received 27 October 2008

Accepted 6 January 2009

INTRODUCTION

Staphylococci are one of the most important causes of nosocomial infections, from minor skin infections to life-threatening bacteraemia (Monk *et al.*, 2008; Francois *et al.*, 2008; Miragaia *et al.*, 2008). The two major opportunistic pathogens of this genus, *Staphylococcus aureus* and *Staphylococcus epidermidis*, colonize a sizeable proportion of the human population. Because *S. aureus* can produce a greater variety of exotoxins and enterotoxins compared to *S. epidermidis* it is a more aggressive pathogen, in recent years, a lot of work has been focused on this bacteria (Diep *et al.*, 2008; Diep & Otto, 2008; Otto, 2008). Compared to *S. aureus*, there is a scarcity of information concerning the molecular characteristics of *S. epidermidis*, especially in the hospital setting in China.

Biofilm formation is the most important factor for the establishment of *S. epidermidis* as a nosocomial pathogen

(Hansen *et al.*, 2007). Although the production of staphylococcal biofilm is dependent upon multiple regulatory proteins, an essential factor is the presence and expression of the four gene *icaADBC* operon (Li *et al.*, 2005). It was reported recently that an insertion sequence element called IS256 has the capacity to influence expression of the *ica* operon, and subsequent biofilm formation, by reversible insertion into the *ica* operon and its regulatory genes, as well as by chromosomal rearrangement (Valle *et al.*, 2007). *S. epidermidis* is a polymorphic species that was found to have a clonal population structure. With respect to the *ica* and IS256 operon, it is not currently known from where this genetic information originates, and there is uncertainty as to whether biofilm-forming strains disseminate clonally or by horizontal transfer of the *ica* and IS256 operon to different clonal lineages. Multilocus sequence typing (MLST), which has become established for the population analysis of many bacterial pathogens, including *S. aureus* and *S. epidermidis* (Enright & Spratt, 1999), is good method for addressing this lack of information. MLST is based on the comparison of the nucleotide sequences of seven housekeeping genes of a micro-organism. It can be used to elucidate relationships between strains and to identify ancestral genotypes, as well

†These authors contributed equally to this work.

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; CSF, cerebrospinal fluid; MLST, multilocus sequence typing; MRSE, meticillin-resistant *Staphylococcus epidermidis*; SCC*mec*, staphylococcal chromosomal cassette *mec*.

as to predict patterns of evolutionary divergence within groups of related genotypes. By using MLST, one sequence type, ST27, was identified in the hospitals of the USA, that contained exclusively *ica*-positive isolates and represented the majority of clinical strains (Ziebuhr *et al.*, 2006).

Meticillin resistance is the other very important factor in the establishment of *S. epidermidis* as a nosocomial pathogen. Resistance to meticillin in staphylococci is known to be associated with the presence of the *mecA* gene, which encodes a penicillin-binding protein with low affinity for β -lactam antibiotics (PBP2A). The *mecA* gene is carried by a genetic mobile element called the staphylococcal chromosomal cassette *mec* (SCC*mec*) (Ziebuhr *et al.*, 2006; Goering *et al.*, 2008). To date, five major SCC*mec* types have been identified, and SCC*mec* have been shown to be transferable among staphylococcal species. The predominant *ica*-positive *S. epidermidis* ST27 clone in the hospitals in the USA carried different SCC*mec* cassettes (Ziebuhr *et al.*, 2006).

Because of the abuse of antibiotics, the status of antimicrobial resistance in bacteria in the hospitals of China is very complicated. We are not clear yet of the level of meticillin-resistant *S. epidermidis* (MRSE) isolates nor the types of SCC*mec* MRSE harboured in the hospital environment in China. *ica*-positive ST27 was detected in different hospitals both in Europe and in the USA; the sequence types of *S. epidermidis* and the molecular characteristics of these strains in China are unknown. In the present study, we examined diversity/clonality by characterizing the population structure of 80 *S. epidermidis* isolates that were collected from inpatients from Shanghai Huashan Hospital, China, with *S. epidermidis* infections, using both molecular (the improved MLST scheme, SCC*mec* typing and the presence of *icaADBC* gene and IS256 element) and phenotypic (antibiotic resistance and biofilm-forming ability) methods.

METHODS

Bacterial isolates. A total of 80 *S. epidermidis* isolates were collected from January 2005 to December 2006 from different inpatients from Shanghai Huashan Hospital with *S. epidermidis* infections. This hospital is located in the centre of Shanghai, China, and is a large (1300 bed), teaching hospital that handles about 8000 admissions per day. The isolates were recovered from blood (36.25%), catheters (33.75%) and cerebrospinal fluid (CSF) (30%). *S. epidermidis* ATCC12228 and *S. epidermidis* RP62A, two reference strains for which the full-genome nucleotide sequences have been published, were also included in the study as controls. The identities of the *S. epidermidis* isolates were confirmed by classic microbiological methods: Gram staining, catalase testing and coagulase activity testing with rabbit plasma. *S. epidermidis* strains were further identified by biochemical characterization using the API Staph test (bioMérieux).

Antimicrobial resistance profiles. Antibiograms were determined by disc diffusion on Mueller–Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards) guidelines. The antimicrobial agents tested included gentamicin (10 μ g), penicillin (10 IU), cefoxitin (30 μ g), ampicillin (10 μ g) + sulbactam (10 μ g),

cefazolin (30 μ g), vancomycin (30 μ g), linezolid (30 μ g), erythromycin (15 μ g), clindamycin (2 μ g), sulfamethoxazole (23.75 μ g) + trimethoprim (1.25 μ g), fosfomicin (200 μ g), rifampicin (5 μ g), teicoplanin (30 μ g) and levofloxacin (5 μ g). The MIC of vancomycin was determined by agar dilution method according to CLSI guidelines. The interpretation of results was also carried out according to CLSI guidelines.

MLST. MLST was performed as described by Thomas *et al.* (2007). PCR of the seven housekeeping genes encoding carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), ABC transporter (*gtr*), DNA mismatch repair protein (*mutS*), pyrimidine operon regulatory protein (*pyrR*), triosephosphate isomerase (*tpiA*) and acetyl-CoA acetyltransferase (*yqiL*) was carried out using the primers described by Thomas *et al.* (2007). Nucleotide sequences were compared to known alleles for each locus via the MLST website (<http://www.mlst.net>).

Determination of SCC*mec* types. SCC*mec* typing was performed by a multiplex PCR approach with subsequent visualization of the amplified DNA fragment patterns by agarose gel electrophoresis and ethidium bromide staining. To distinguish between SCC*mec* types I to V, primers and conditions identical to those described by Miragaia *et al.* (2007) were used.

***ica* and IS256 specific PCRs.** Bacterial strains were tested for the presence of the *ica* gene and IS256 by PCR using the primers described by Kozitskaya *et al.* (2005). The PCR fragments were visualized by agarose gel electrophoresis and ethidium bromide staining. *S. epidermidis* RP62A and ATCC12228 served as controls.

Semiquantitative biofilm assay. Semiquantitative biofilm assays were performed as described elsewhere (Wang *et al.*, 2008). Subsequently, cells were either directly stained with safranin or fixed by Bouin's fixative. The fixative was removed after 1 h and wells were washed with PBS. Organisms in the wells were then stained with crystal violet, and the floating stain was washed off with slowly running water. After drying, the absorbance of the stained biofilm was measured with a MicroELISA autoreader (Bio-Rad) at 570 nm (crystal violet stain) or 490 nm (safranin stain).

Evaluation of the expression level of *icaB* gene by RT-PCR. For RNA isolation, cultures grown overnight were diluted 1:100 into 50 ml tryptic soy broth and incubated at 37 °C with shaking at 180 r.p.m. until the late exponential growth phase was reached. cDNA was synthesized from total RNA by using the SuperScript III first-strand synthesis system (Invitrogen) according to the manufacturer's instructions. Oligonucleotide primers (*icaB*) as described by Wang *et al.* (2008) were used. The resulting cDNA and negative control samples were amplified by use of LC-Fast Start reaction mix SYBR Green I (Roche Diagnostics). Reactions were performed in a LightCycler apparatus (Roche Diagnostics). Standard curves were determined for each gene, by use of purified chromosomal template DNA at concentrations of 0.005–50 ng ml⁻¹. The experiments were performed in triplicate, with 16S rRNA used as a control.

Statistical analysis. Statistical analysis was performed using GraphPad Prism version 4.0.

RESULTS

MLST profiles and SCC*mec* typing of the invasive *S. epidermidis* isolates

From 2005 to 2006, 80 *S. epidermidis* isolates, which were recovered from blood, catheters and CSF from different

inpatients with *S. epidermidis* infections, were collected. These isolates were considered to be invasive *S. epidermidis* isolates. The key characteristics of the 80 *S. epidermidis*-infected patients are provided in Table 1. All of the *S. epidermidis* isolates we investigated in this study were hospital-associated *S. epidermidis*. MLST revealed a total of 16 different sequence types among these isolates (Table 2), the majority of them (75 %, 60/80) were clustered into 6 sequence types: ST2, ST5, ST66, ST23, ST6 and ST20. A total of 3 of these 16 STs corresponded to single isolates, while 7 STs included 2 or 3 isolates. The most represented sequence type was ST2, which comprised 31.25 % of all clinical isolates (25/80 isolates), with an antimicrobial resistance profile as follows: gentamicin (77.8 %), penicillin (100 %), cefoxitin (100 %), ampicillin + sulbactam (100 %), cefazolin (100 %), vancomycin (intermediate resistance 4 %), linezolid (0 %), erythromycin (72.2 %), clindamycin (55.6 %), sulfamethoxazole + trimethoprim (61.1 %), fosfomycin (61.1 %), rifampicin (44.4 %), teicoplanin (0 %), levofloxacin (61.1 %). All of the ST2 isolates harboured a SCCmec type III gene. Five SCCmec types have been identified in staphylococci. Of the 80 *S. epidermidis* isolates tested, 77 (96.25 %) were *mecA* positive. SCCmec II was detected in 18 isolates, SCCmec III in 31 isolates, SCCmec IV in 3 isolates and SCCmec V in 25 isolates, none of the strains carried an SCCmec I cassette (Table 2).

Association of biofilm-forming ability and the presence of the *ica* gene and IS256

By semiquantitative biofilm assay, 43 clinical isolates (53.8 %) were found to be biofilm positive and displayed 14 different STs; 36 (45.0 %) isolates were *ica* positive and displayed 6 different STs; 52 (65.0 %) were IS256 positive and displayed by 9 STs (Table 2). The predominant clone, ST2, contained 68 % (17/25) of the biofilm-forming, and 100 % of the *ica*- and IS256-positive, isolates. In our study, IS256-carrying strains were detected in four STs, which contained *ica*-positive and biofilm-forming isolates, and ST2 represented the group containing the most IS256-positive, *ica*-positive and biofilm-forming isolates. Biofilm-negative isolates were also found in 32 % (8/25) of ST2

isolates. In the present study, *ica* operon expression was detected using RT-PCR in biofilm-positive and biofilm-negative isolates of ST2, but we did not find any difference in *ica* expression between two group of isolates ($P > 0.05$) (Fig. 1).

Distribution of STs among clinical samples

Of the 80 *S. epidermidis* isolates tested, 29 isolates were recovered from blood, 27 were from catheters and 24 were from CSF. Fig. 2 shows the distribution of the 16 sequence types among these three infection sites. Interestingly, of the 25 ST2 isolates, 10 were from blood, 14 were from catheters and just 1 isolate was from CSF. This result suggests that the predominant sequence type, ST2, which contained exclusively *ica*-positive, IS256-positive and biofilm-forming isolates associated with device-related infection and bacteraemia.

DISCUSSION

S. epidermidis epidemiological studies have been limited historically, due to the fact that *S. epidermidis* strains are often considered to be contaminants, as opposed to the disease-causing organism (Wang *et al.*, 2003; Herwaldt *et al.*, 1996). However, *S. epidermidis* infections are an increasing cause of concern, due to the high distribution of methicillin resistance amongst the isolates and their persistence on indwelling devices, often resulting in replacement of the device, which causes more trauma and is costly (Hakim *et al.*, 2000). In this investigation, 96.25 % of isolates from hospitals in China were methicillin resistant. By using MLST, we examined the molecular characteristics of these clinical *S. epidermidis* isolates, which were recovered from blood, catheters and CSF of patients with *S. epidermidis* infections. In our study, the percentage of patients from whom *S. epidermidis* was isolated from within 48 h of admission was 10 %, but all of these patients had been exposed to the healthcare setting before. So all of *S. epidermidis* isolates we investigated in this study could be considered as clones circulating in the hospital. Isolates

Table 1. Key characteristics of *S. epidermidis*-infected patients in Shanghai Huashan Hospital, China

Sex/no. (%)	Age (years)/no. (%)	Sample collection day*/no. (%)	Infection site/no. (%)
F/ 26 (32.5)	≤20/ 4 (15.4)		
	20–40/ 8 (30.8)	≤2/ 3 (11.5)	Blood/ 7 (26.9)
	40–60/ 10 (38.5)	4–30/ 21 (80.8)	CSF/ 9 (34.6)
	60/ 4 (15.4)	30/ 2 (7.7)	Catheter/ 10 (38.5)
M/ 54 (67.5)	≤20/ 8 (14.8)		
	20–40/ 8(14.8)	≤2/ 5 (9.3)	Blood/ 22 (40.7)
	40–60/ 25 (46.3)	4–30/ 40 (74.1)	CSF/ 15 (27.8)
	60/ 13 (24.1)	30/ 9 (16.7)	Catheter/ 17 (31.5)

F, Female; M, male.

*The number of days after the patient's admission to hospital that had elapsed when the samples were collected.

Table 2. Characteristics of *S. epidermidis* clones isolated from patients with *S. epidermidis* infections in Shanghai Huashan Hospital, China

MLST	MLST profile	No. of isolates	SCC <i>mec</i> type (no.)	Biofilm positive	IS256 positive	<i>icaADBC</i> positive
ST2	7-1-2-2-4-1-1	25	III (25)	68.0 % (17/25)	100 % (25/25)	100 % (25/25)
ST5	1-1-1-2-2-1-1	10	V (10)	50.0 % (5/10)	70.0 % (7/10)	0 % (0/10)
ST66	12-3-5-5-7-14-11	7	V (6) MSSE (1)	14.3 % (1/7)	85.7 % (6/7)	0 % (0/7)
ST23	7-1-2-1-3-3-1	7	II (7)	57.1 % (4/7)	42.9 % (3/7)	57.1 % (4/7)
ST6	1-1-2-2-2-1-1	6	II (2) V (4)	66.7 % (4/6)	33.3 % (2/6)	33.3 % (2/6)
ST20	1-1-2-2-1-1-3	5	III (5)	40.0 % (2/5)	40.0 % (2/5)	40.0 % (2/5)
ST10	1-1-1-1-3-1-1	3	IV (3)	33.3 % (1/3)	100 % (3/3)	0 % (0/3)
ST59	2-1-1-1-2-1-1	3	II (3)	66.7 % (2/3)	0 % (0/3)	0 % (0/3)
ST71	3-1-5-5-3-1-11	3	II (2) MSSE (1)	33.3 % (1/3)	66.7 % (2/3)	0 % (0/3)
ST130	1-1-1-2-1-1-1	2	II (2)	0 % (0/2)	0 % (0/2)	0 % (0/2)
ST21	2-1-1-2-1-1-1	2	III (1) MSSE (1)	50 % (1/2)	100 % (2/2)	0 % (0/2)
ST16	2-1-2-2-15-1-1	2	II (2)	100 % (2/2)	0 % (0/2)	100 % (2/2)
ST110	1-1-1-6-2-1-1	2	V (2)	50.0 % (1/2)	0 % (0/2)	0 % (0/2)
ST133	1-2-1-2-1-1-1	1	V (1)	0 % (0/1)	0 % (0/1)	0 % (0/1)
ST89	1-1-2-1-2-1-1	1	V (1)	100 % (1/1)	0 % (0/1)	0 % (0/1)
ST152	1-1-2-6-2-1-1	1	V (1)	100 % (1/1)	0 % (0/1)	100 % (1/1)

that were recovered from sputum or urine were not included, because they were often considered to be contaminants. The isolates recovered from blood/catheters may also not represent infections. We should note here that because of the ubiquitous prevalence of *S. epidermidis* as a commensal bacterium, it is very difficult for a clinician to decide whether an isolate represents the causative agent of an infection. The MLST result showed that the most represented sequence type was ST2, which comprised 31.25 % of all clinical isolates in our study. ST2 has also been detected in different hospitals in Europe, but this is

believed to be the first report that the ST2 clone has spread in the hospital environment in China.

Why is the ST2 clone easy to disseminate in the hospital setting? As biofilm formation and meticillin resistance are the most important factors for the establishment of *S. epidermidis* as a nosocomial pathogen, in the present study, a semiquantitative biofilm assay was used to detect the biofilm-forming ability of all the clinical isolates; the presence of the *ica* gene, IS256 and different types of SCC*mec* cassette were tested by PCR. In previous studies, a correlation was shown between the presence of the *ica* operon, biofilm formation and the detection of the insertion sequence IS256 in clinical *S. epidermidis* isolates (Johansson *et al.*, 2006; Spare *et al.*, 2003; Kozitskaya *et al.*, 2005). In our study, the predominant clone ST2, 100 % *ica* and IS256 positive, contained 68.0 % of the biofilm-forming isolates, so ST2 represented the group containing the most IS256 positive, *ica*-positive and biofilm-forming isolates. These data suggest that *ica*, IS256 and biofilm-forming ability occur jointly in specific *S. epidermidis* clones and spread preferentially in the hospital environment. Recombination is the major mechanism for the divergence of *S. epidermidis* strains (Wisplinghoff *et al.*, 2003). We hypothesized that by recombination, ST2 generates novel phenotypic and genotypic variants, such as *ica* and IS256, which makes it easily able to spread in the hospital environment, and causes device-related infection and bacteraemia. But our study was based on a limited number of *S. epidermidis* isolates, to get a firmer conclusion, further study will be needed. *S. epidermidis* sequence type ST27, which represents the majority of clinical strains in hospitals in the USA, also contained exclusively *ica*- and IS256-positive isolates. ST2, with 100 % *ica*- and IS256-positive isolates, also contained 32.0 % of the biofilm-negative isolates. We hypothesized that the

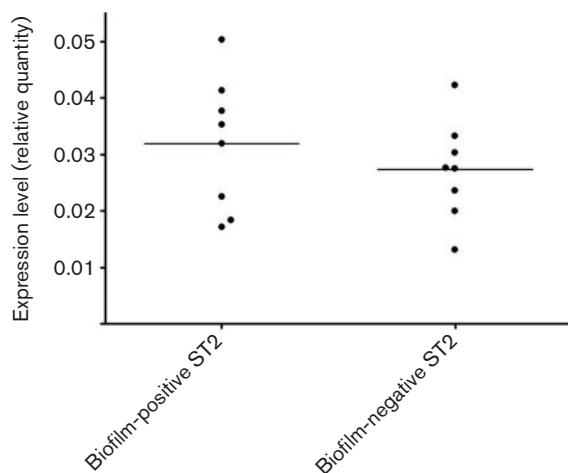


Fig. 1. Detection of *ica* operon expression level by RT-PCR. Eight isolates of ST2 MRSE with biofilm-forming ability and eight isolates without biofilm-forming ability were randomly selected for *ica* operon expression level detection.

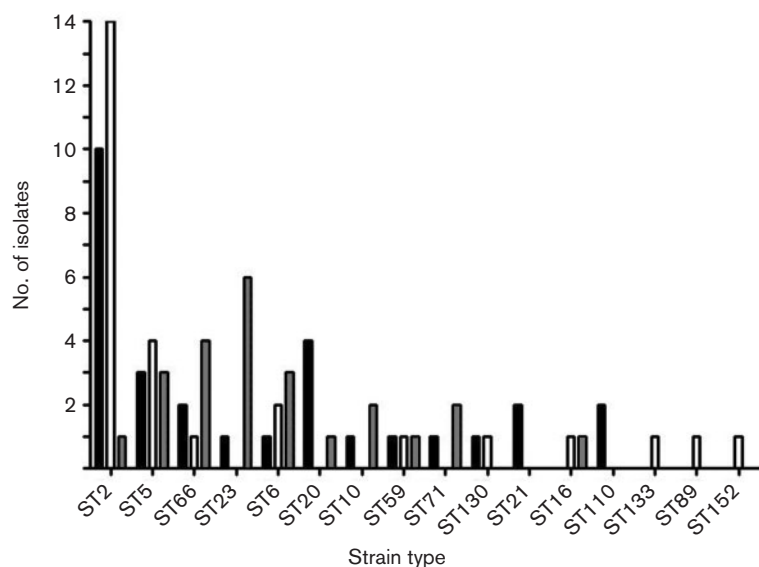


Fig. 2. Distribution of different sequence types isolated from different infection sites. Black bars, blood isolates; grey bars, CSF isolates; white bars, catheter isolates.

reason was the difference in the *ica* operon expression level in the biofilm-positive and biofilm-negative isolates of ST2, but we did not find any difference in the *ica* expression between the two groups of ST2. The biofilm formation of staphylococci is a very complicated process, and the real reason remains to be elucidated.

Four *SCCmec* types were identified amongst the total of 80 *S. epidermidis* isolates, none of the strains carried an *SCCmec* I cassette. All of the isolates that belonged to ST2 carried the *SCCmec* type III cassette. The result of the vancomycin MIC test showed that one isolate of ST2 MRSE was vancomycin intermediate ($8 \mu\text{g ml}^{-1}$), but this isolate was negative for the *vanA/vanB* gene by PCR. We found the biofilm formed by this isolate was thicker than most other *S. epidermidis* isolates. It is reported that bacteria organized in biofilms are more resistant to antibiotics (Ziebuhr *et al.*, 2006). Maybe the biofilm thickening is responsible for the development of intermediate resistance to vancomycin.

It is reported that ST27, the sequence type represented in USA hospitals, and other *ica*-positive clones are rarely found outside of medical facilities (Ziebuhr *et al.*, 2006). It is very likely that they are highly adapted to the hospital environment and differ from commensal *S. epidermidis* in the community. We are not clear yet if the molecular characteristics of *S. epidermidis* from the hospital setting in China are different to those of isolates from the community; future work needs to be done to clarify this.

ACKNOWLEDGEMENTS

This work was supported by grants from the Shanghai Medical Key Discipline, the Chinese National Natural Science Foundation (30670108 and 30872259) and the Science Foundation for Youth of Fudan University (08FQ38)

REFERENCES

- Diep, B. A. & Otto, M. (2008). The role of virulence determinants in community-associated MRSA pathogenesis. *Trends Microbiol* **16**, 361–369.
- Diep, B. A., Palazzolo-Balance, A. M., Tattavin, P., Basuino, L., Braughton, K. R., Whitney, A. R., Chen, L., Kreiswirth, B. N., Otto, M. & other authors (2008). Contribution of Pantone-Valentine leukocidin in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. *PLoS One* **3**, e3198.
- Enright, M. C. & Spratt, B. G. (1999). Multilocus sequence typing. *Trends Microbiol* **7**, 482–487.
- Francois, P., Hochmann, A., Huyghe, A., Bonetti, E. J., Renzi, G., Harbarth, S., Klingenberg, C., Pittet, D. & Schrenzel, J. (2008). Rapid and high-throughput genotyping of *Staphylococcus epidermidis* isolates by automated multilocus variable-number of tandem repeats: a tool for real-time epidemiology. *J Microbiol Methods* **72**, 296–305.
- Goering, R. V., Shawar, R. M., Scangarella, N. E., O'Hara, F. P., Amrine-Madsen, H., West, J. M., Dalessandro, M., Becker, J. A., Walsh, S. L. & other authors (2008). Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from global clinical trials. *J Clin Microbiol* **46**, 2842–2847.
- Hakim, A., Rossi, C., Kabanda, A., Deplano, A., De Gheldre, Y. & Struelens, M. J. (2000). Ommaya-catheter-related *Staphylococcus epidermidis* cerebritis and recurrent bacteremia documented by molecular typing. *Eur J Clin Microbiol Infect Dis* **19**, 875–877.
- Hansen, S. K., Rainey, P. B., Haagenen, J. A. & Molin, S. (2007). Evolution of species interactions in a biofilm community. *Nature* **445**, 533–536.
- Herwaldt, L. A., Geiss, M., Kao, C. & Pfaller, M. A. (1996). The positive predictive value of isolating coagulase-negative staphylococci from blood cultures. *Clin Infect Dis* **22**, 14–20.
- Johansson, A., Koskiniemi, S., Gottfridsson, P., Wiström, J. & Monsen, T. (2006). Multiple-locus variable-number tandem repeat analysis for typing of *Staphylococcus epidermidis*. *J Clin Microbiol* **44**, 260–265.
- Kozitskaya, S., Olson, M. E., Fey, P. D., Witte, W., Ohlsen, K. & Ziebuhr, W. (2005). Clonal analysis of *Staphylococcus epidermidis*

- isolates carrying or lacking biofilm-mediating genes by multilocus sequence typing. *J Clin Microbiol* **43**, 4751–4757.
- Li, H., Xu, L., Wang, J., Wen, Y., Vuong, C., Otto, M. & Gao, Q. (2005). Conversion of *Staphylococcus epidermidis* strains from commensal to invasive by expression of the *ica* locus encoding production of biofilm exopolysaccharide. *Infect Immun* **73**, 3188–3191.
- Miragaia, M., Thomas, J. C., Couto, I., Enright, M. C. & De Lencastre, H. (2007). Inferring a population structure for *Staphylococcus epidermidis* from multilocus sequence typing data. *J Bacteriol* **189**, 2540–2552.
- Miragaia, M., Carriço, J. A., Thomas, J. C., Couto, I., Enright, M. C. & De Lencastre, H. (2008). Comparison of molecular typing methods for characterization of *Staphylococcus epidermidis*: proposal for clone definition. *J Clin Microbiol* **46**, 118–129.
- Monk, A. B., Boundy, S., Chu, V. H., Bettinger, J. C., Robles, J. R., Fowler, V. G., Jr & Archer, G. L. (2008). Analysis of the genotype and virulence of *Staphylococcus epidermidis* isolates from patients with infective endocarditis. *Infect Immun* **76**, 5127–5132.
- Otto, M. (2008). Targeted immunotherapy for staphylococcal infections: focus on anti-MSCRAMM antibodies. *BioDrugs* **22**, 27–36.
- Spare, M. K., Tebbs, S. E., Lang, S., Lambert, P. A., Worthington, T., Lipkin, G. W. & Elliott, T. S. (2003). Genotypic and phenotypic properties of coagulase-negative staphylococci causing dialysis catheter-related sepsis. *J Hosp Infect* **54**, 272–278.
- Thomas, J. C., Vargas, M. R., Miragaia, M., Peacock, S. J., Archer, G. L. & Enright, M. C. (2007). Improved multilocus sequence typing scheme for *Staphylococcus epidermidis*. *J Clin Microbiol* **45**, 616–619.
- Valle, J., Vergara-Irigaray, M., Merino, N., Penadés, J. R. & Lasa, I. (2007). σ^B regulates IS256-mediated *Staphylococcus aureus* biofilm phenotypic variation. *J Bacteriol* **189**, 2886–2896.
- Wang, X. M., Noble, L., Kreiswirth, B. N., Eisner, W., McClements, W., Jansen, K. U. & Anderson, A. S. (2003). Evaluation of a multilocus sequence typing system for *Staphylococcus epidermidis*. *J Med Microbiol* **52**, 989–998.
- Wang, L., Li, M., Dong, D., Bach, T. H., Sturdevant, D. E., Vuong, C., Otto, M. & Gao, Q. (2008). SarZ is a key regulator of biofilm formation and virulence in *Staphylococcus epidermidis*. *J Infect Dis* **197**, 1254–1262.
- Wisplinghoff, H., Rosato, A. E., Enright, M. C., Noto, M., Craig, W. & Archer, G. L. (2003). Related clones containing SCCmec type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. *Antimicrob Agents Chemother* **47**, 3574–3579.
- Ziebuhr, W., Hennig, S., Eckart, M., Kränzler, H., Batzilla, C. & Kozitskaya, S. (2006). Nosocomial infections by *Staphylococcus epidermidis*: how a commensal bacterium turns into a pathogen. *Int J Antimicrob Agents* **28** (Suppl.1), S14–S20.