Staphylococcus aureus in Dermatology Outpatients with Special Emphasis on Community-Associated Methicillin-Resistant Strains

Uta Jappe[1], Dagmar Heuck[2], Birgit Strommenger[2], Constanze Wendt[3], Guido Werner[2], Doris Altmann[4] and Wolfgang Witte[2]

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged as a community-associated pathogen (CA-MRSA) in the past 6 years. This prospective study investigated dermatology outpatients with inflammatory skin diseases, leg ulcers, and skin infections for Panton-Valentine leukocidin (PVL)-positive *S. aureus*, often associated with deep skin infection. In case of PVL positivity, molecular typing and PCR demonstration of resistance genes were performed. Out of 248 patients, 130 carried *S. aureus*, 24 being *luk*S-PV *luk*F-PV positive. Eighteen were MRSA, 11 of them belonging to the multilocus sequence typing clonal complex (CC)5, 1 to CC45, and 2/18 to CC8. Out of 18 patients, 4 were CA-MRSA containing *luk*S-PV *luk*F-PV as an important trait of CA-MRSA. Out of four CA-MRSA isolates, two were of type ST080 containing *far-1* coding for fusidic acid (FUS) resistance and two were FUS sensitive (ST152 and ST001). The FUS-sensitive CA-MRSA, which corresponded to the CA-MRSA of ST001 from the United States, was detected in Germany for the first time, indicating that dermatologists are first in line to detect CA-MRSA. In contrast to CA-MRSA from other continents, *far-1*-coded FUS resistance represents a typical marker for the widespread CA-MRSA ST080 in Europe, especially in Germany. The significant risk factor for the acquisition of CA-MRSA was visits to foreign countries and/or professional or private contacts with foreigners.

JID JOURNAL CLUB ARTICLE: For questions, answers, and open discussion about this article, please go to http://network.nature.com/group/jidclub

Journal of Investigative Dermatology (2008) **128,** 2655–2664; doi:10.1038/jid.2008.133; published online 3 July 2008

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified as a nosocomial pathogen (health-care-associated MRSA (HA-MRSA)). Risk factors for acquisition are diabetes, hemodialysis, peripheral malperfusion, immunosuppression, systemic and topical antibiotic treatments during 6 months before admission, previous surgery, hospitalization, admission to a nursing home, and contact with MRSA carriers (reviewed by Lowy, 1998; von Baum *et al.*, 2002). Until 1987 no MRSA was isolated in a dermatology outpatient clinic (McBride *et al.*, 1989), whereas a gradual increase in infections with MRSA from 1.5% of all *S. aureus* in 1988 to 11.9% in 1996 in the same dermatology outpatient facilities was observed, and the prevalence is still increasing (Price *et al.*, 1998). From the early 1990s onward, MRSA infections were reported in otherwise healthy young individuals, and the respective strains named ''community-associated MRSA (CA-MRSA)'' (Embil *et al.*, 1994; Moreno *et al.*, 1995). CA-MRSA is distinct from HA-MRSA (reviewed by Millar *et al.*, 2007). HA-MRSA corresponds to definite predominant clonal lineages of the *S. aureus* population with some of them having pandemic dissemination (Oliveira and De Lencastre 2002), whereas CA-MRSA strains usually represent different lineages. So far, CA-MRSA ST008 (USA 300) is most frequent in the United States but has also spread to Europe (Witte *et al.*, 2007) and to Asia (Ho *et al.*, 2007; Zaraket *et al.*, 2007). CA-MRSA ST080 is frequent in Europe. HA-MRSA is prevalent in health-care settings and nursing homes affecting immunocompromized patients, whereas CA-MRSA affects young and healthy individuals in the community and settings such as combat and ball sports, military, and prisons. The molecular characteristics of HA-MRSA differ from those of CA-MRSA in that HA-MRSA predominantly possesses the type I–IV staphylococcal cassette chromosome *mec* (SCC*mec*), which confers resistance to currently available β-lactam antibiotics and also many other non-β-lactam antibiotics, whereas CA-MRSA mainly possesses SCC*mec*A type IV or V. HA-MRSA exhibits multidrug resistance

[1]*Department of Dermatology, University of Heidelberg, Heidelberg, Germany;* [2]*National Reference Centre for Staphylococci (NCS), Robert-Koch-Institute, Wernigerode Branch, Wernigerode, Germany;* [3]*Institute of Hygiene, University of Heidelberg, Heidelberg, Germany and* [4]*Department of Infectious Diseases and Epidemiology, Robert-Koch-Institute, Berlin, Germany*

Correspondence: Dr Uta Jappe, Paul-Ehrlich-Institut, Paul-Ehrlich Strasse 51–59, D-63225 Langen, Germany. E-mail: japut@pei.de

Abbreviations: CA-MRSA, community-associated MRSA; FUS, fusidic acid; HA-MRSA, health-care-associated MRSA; MLST, multilocus sequence typing; MRSA, methicillin-resistant Staphylococcus aureus*; NCS, National Reference Centre for Staphylococci; OTE, oxytetracycline; PVL, Panton-Valentine leukocidin; SCC*mec*, staphylococcal cassette chromosome* mec*;* Sma*I,* Serratia *macrorestriction enzyme I;* spa *typing,* S. aureus *protein A typing*

Received 1 August 2007; revised 28 March 2008; accepted 2 April 2008; published online 3 July 2008

to drugs such as clindamycin, gentamicin, and fluorochinolones (Naimi *et al.*, 2003). CA-MRSA, susceptible to most groups of antibiotic agents, displays a variable resistance to fusidic acid (FUS) (often associated with the *far-1* gene coding for a ribosome protection mechanism), oxytetracycline (OTE), and ciprofloxacin in European isolates (Witte *et al.*, 2004, 2005), and the majority of CA-MRSA has the Panton-Valentine leukocidin (PVL) gene, which is very rare in HA-MRSA (Millar *et al.*, 2007). PVL is a bicomponent (*luk*S-PV and *luk*F-PV), pore-forming exotoxin that targets cells of the immune system such as polymorphonuclear neutrophils. PVL-producing strains including CA-MRSA have preferentially been isolated from furuncles, cutaneous abscesses, and severe necrotic skin infections, suggesting the participation of PVL in the pathological process (Cribier *et al.*, 1992; Couppie *et al.*, 1994; Prevost *et al.*, 1995; Miller *et al.*, 2005; Yamasaki *et al.*, 2005). Although most PVL-related infections are uncomplicated skin infections, they bear the risk of developing severe systemic infections such as bacterial endocarditis (Al-Tawfiq and Aldaabil, 2005; Bahrain *et al.*, 2006). Some are primarily fatal owing to necrotizing pneumonia (Lina *et al.*, 1999; Gillet *et al.*, 2002) and necrotizing fasciitis (Miller *et al.*, 2005) for both adults and children (Fridkin *et al.*, 2005). Owing to the confusion concerning clear definitions of MRSA detected outside the hospital setting, there are several limitations of the current data on CA-MRSA (Salgado *et al.*, 2003).

Therefore, a study on outpatients in the Department of Dermatology, University of Heidelberg, Germany, was designed to investigate (1) the frequency of skin-colonizing or infectious *S. aureus*, (2) their phenotypic antibiotic resistance patterns (focusing on the MRSA status and FUS resistance), (3) whether they contained the PVL gene, and (4) their molecular characteristics.

RESULTS

Study population and *S. aureus* positivity

The study was performed from September 2003 to November 2005. A total of 248 patients of the dermatology outpatient clinic with deep skin infections or superficial erosions and ulcers due to other underlying skin diseases were enrolled. Both sexes were nearly equally represented (53% female and 47% male patients). *S. aureus* was isolated from 130/248 (52.4%) patients. In 54 individuals both sites (anterior nares as well as lesional skin), in 64 only lesional skin, and in 12 only nares were sampled. In 37 (69%) of the 54 patients, of whom both sites were sampled, the *S. aureus* isolates were of the same phage type. The *S. aureus*-positive patients ($n=130$) were aged between 1 and 92 years (median: 46 years), and those individuals with *luk*S-PV *luk*F-PV-positive *S. aureus* ($n=24$) were found to be in nearly the same group of age (3–90 years, median: 37 years). However, only two patients were older than 50 years. Approximately one-third of patients positive for *S. aureus* (36/130; 27.7%) had deep skin infections and other *S. aureus* were collected from superficial erosions, ulcers, and nares.

Antibiotic resistance. Eighteen out of 130 (13.8%) *S. aureus* isolates were MRSA. CA-MRSA are defined as being present or incubating at the time of admission (within 48 hours after hospital admission) and were not acquired during health care during the 12 months prior to actual sampling (Naimi *et al.*, 2003; Salgado *et al.*, 2003). However, as these definitions are under discussion and the period of 12 months no longer holds true for most of the detected CA-MRSA, CA-MRSA in our study was suspected when sampled in an outpatient setting or before the actual hospitalization. CA-MRSA was diagnosed if the following additional criteria were present: PVL positivity, characteristic multilocus sequence typing (MLST)/*S. aureus* protein A (*spa*) type, and SCC*mec* type (IV, V). This was the case for 4/130 (3%) strains (Table 1 and Figure 1). All four CA-MRSA proved to have a typically narrow resistance pattern. Both CA-MRSA ST080 isolates were distinguishable from the other CA-MRSA by *far-1*-encoded FUS resistance and mostly showing OTE resistance as is well known for this genotype (RKI, 2004a). In this study, OTE resistance is found in 17% of the MRSA strains (Table 1). In general, 6% of German MRSA strains sent to the National Reference Centre for Staphylococci (NCS, Wernigerode, Germany) are resistant to OTE (NCS, personal communication). The CA-MRSA ST152 and ST001 only show resistance to two and three antibiotics, respectively. While comparing the percentages of resistance phenotypes of patients with atopic dermatitis with those of other skin diseases, no apparent differences were found. In 26.2% (34/130) of patients with a topical FUS treatment history, *S. aureus* was detected (Table 2). Four of these 34 isolates were FUS resistant (11.7 vs 7.3% of the remaining 96 patients without a FUS history). One of them was a CA-MRSA ST080 containing the FUS resistance-encoding *far-1* gene. The three others (one MRSA ST617, one methicillin-sensitive *Staphylococcus aureus* (MSSA) ST101, and one MSSA ST121) were *far-1* negative, indicating the existence of alternative resistance mechanisms. Both groups (with history for FUS treatment vs no FUS treatment) had one patient positive for a CA-MRSA with FUS resistance (2.9% of 34 vs 1% of 96). These data did not reveal a statistically significant difference between FUS-treated patients and those without treatment, concerning the prevalence of FUS-resistant *S. aureus* in general ($P=0.477$) and of CA-MRSA in particular ($P=0.456$).

PVL positivity of the collected S. aureus strains. All isolates from deep and partly from superficial skin infections were investigated by PCR for the *luk*S-PV *luk*F-PV gene. Twenty-four *S. aureus* isolates, including four CA-MRSA isolates, were *luk*S-PV *luk*F-PV positive. They were sampled from areas of 20 deep skin infections (14 abscesses and 6 furuncles), 2 were collected from superficial infections (pustules), and 2 strains from colonized lesional skin and/or nares. They belong to different clonal complexes (CCs) (Figure 1). Substantial numbers of *luk*S-PV *luk*F-PV-positive *S. aureus* or CA-MRSA were isolated from patients with deep skin infections (20/36; 55.6%), whereas only four patients with superficial wounds including venous leg ulcers (72 tested/94 sampled isolates) had *luk*S-PV *luk*F-PV-positive isolates (5.6%) (Table 3).

Table 1. MRSA in dermatology outpatients (*n*=18/130 *S. aureus* patients; 13.8%)

Strain	Underlying disease	Lesion	*Spa* typing	Epidemic type	*lukS–lukF*	*far-1*	FUS resistance	FUS therapy	PEN	OXA	CIP	ERY	CLI	CMP	SXT	OTE	GEN	MFL	MUP
03-02884	Asthma, hypertension	Venous leg ulcer	t008	ST008[1]	–	ND	No	No	+	+	+	–	–	–	–	–	–	+	–
04-00194-2	Diabetes, hypertension	Venous leg ulcer	t003	ST225[1]	–	ND	No	No	+	+	+	+	+	+	–	–	–	+	–
04-00239-1	Peripheral malperfusion	Venous leg ulcer	t003	ST225[1]	–	ND	No	No	+	+	+	+	+	+	–	–	–	+	–
04-00410	Peripheral malperfusion, neoplasm	Venous leg ulcer	t008	ST008[1]	–	ND	No	No	+	+	+	+	–	–	+	–	+	+	–
04-00411	Diabetes	Venous leg ulcer	t003	ST225[1]	–	ND	No	No	+	+	+	+	+	+	–	–	–	+	–
05-01932-2	Resolved tuberculosis	Venous leg ulcer	t003	ST225[1]	–	ND	No	No	+	+	+	+	+	–	–	–	–	+	–
04-00413	Peripheral malperfusion	Venous leg ulcer	t1282	ST005[1]	–	ND	No	Yes	+	+	+	+	+	–	–	–	–	+	–
04-01730	Diabetes, peripheral malperfusion	Venous leg ulcer	t003	ST225[1]	–	ND	No	No	+	+	+	+	+	+	–	–	–	+	–
03-02595	Lymphoma, atopic dermatitis	Superficial wound, cellulitis	t003	ST225[1]	–	–	Yes	No	+	+	+	+	+	+	–	–	–	+	–
04-00244-1	Atopic Dermatitis	Superficial wound erosion	t003	ST225[1]	–	ND	No	No	+	+	+	+	+	+	–	–	–	+	–
03-02594	Hemodialysis, anemia	Superficial wound chronic erosion	t002	ST005	–	–	Yes	No	+	+	+	i	–	–	–	–	–	+	–
04-02054	Atopic dermatitis, bronchitis	Superficial wound pustule	t355	CA-MRSA, ST152	+	ND	No	No	+	+	i	–	–	–	–	–	+	–	–
03-02773	Acne inversa	Abscess	t175	CA-MRSA, ST001	+	–	No	Yes	+	+	i	–	–	–	–	–	–	–	–
05-00941	Diabetes, neoplasm	Abscess	t003	ST225	–	–	No	No	+	+	+	+	+	+	–	–	–	+	–
05-01354	Acne inversa	Abscess	t305	ST617[1]	–	–	Yes	Yes	+	+	+	+	–	–	–	+	+	+	–
04-00114-2	Diabetes, furunculosis	Furuncle	t002	ST005	–	ND	No	No	+	+	+	+	–	–	–	–	–	+	–
04-00117	Superinfected insect bite	Furuncle	t044	CA-MRSA, ST080[1]	+	+	Yes	No	+	+	i	–	–	–	–	+	–	+	–
04-00187	Furunculosis	Furuncle	t044	CA-MRSA, ST080[1]	+	+	Yes	Yes	+	+	i	–	–	–	–	+	–	–	–

BURP, Based Upon Repeat Pattern; CA-MRSA, community-associated MRSA; CIP, ciprofloxacin; CLI, clindamycin; CMP, chloramphenicol; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; MFL, moxifloxacin; MRSA, methicillin-resistant *Staphylococcus aureus*; MUP, mupirocin; ND, not determined; OTE, oxytetracycline; OXA, oxacillin; PEN, penicillin; SXT, trimethoprim/sulfamethoxazol.
ST, MLST sequence type; +, resistant to antibiotics; i, intermediate resistant.
[1]As deduced from *spa* typing in combination with BURP grouping.

Molecular characteristics of the collected S. aureus strains. Figure 1 summarizes the molecular characteristics of all PVL-positive strains and MRSA of our study. The dendrogramm visualizes clustering of the isolates based on their macro-restriction profiles in comparison with reference strains. Cluster analysis reveals the existence of eight distinct clusters. The dendrogramm on the left side of the figure shows the capacity of *Sma*I (*Serratia* macrorestriction enzyme I) macro-restriction patterns to discriminate between different clonal lineages within the two subpopulations of CA-MRSA and HA-MRSA. This is confirmed by *spa* typing in connection with grouping by Based Upon Repeat Pattern algorithm. Typing of SCC*mec* revealed that 13/18 MRSA strains were SCC*mec* type II, four had SCC*mec* type IV, and one had SCC*mec* type V (Figure 1). Eleven out of 18 belonged to the CC5, including ST005 and ST225, which is endemic in the region of Heidelberg, Germany. Two MRSA isolates belonged to CC8, which is of more international significance. There were three PVL-negative MRSA isolates (ST005, ST225, and ST617, the latter belonging to CC45) sampled from deep skin infections (two abscesses and one furuncle) (Table 1). Of the four CA-MRSA isolates, three types were found to be

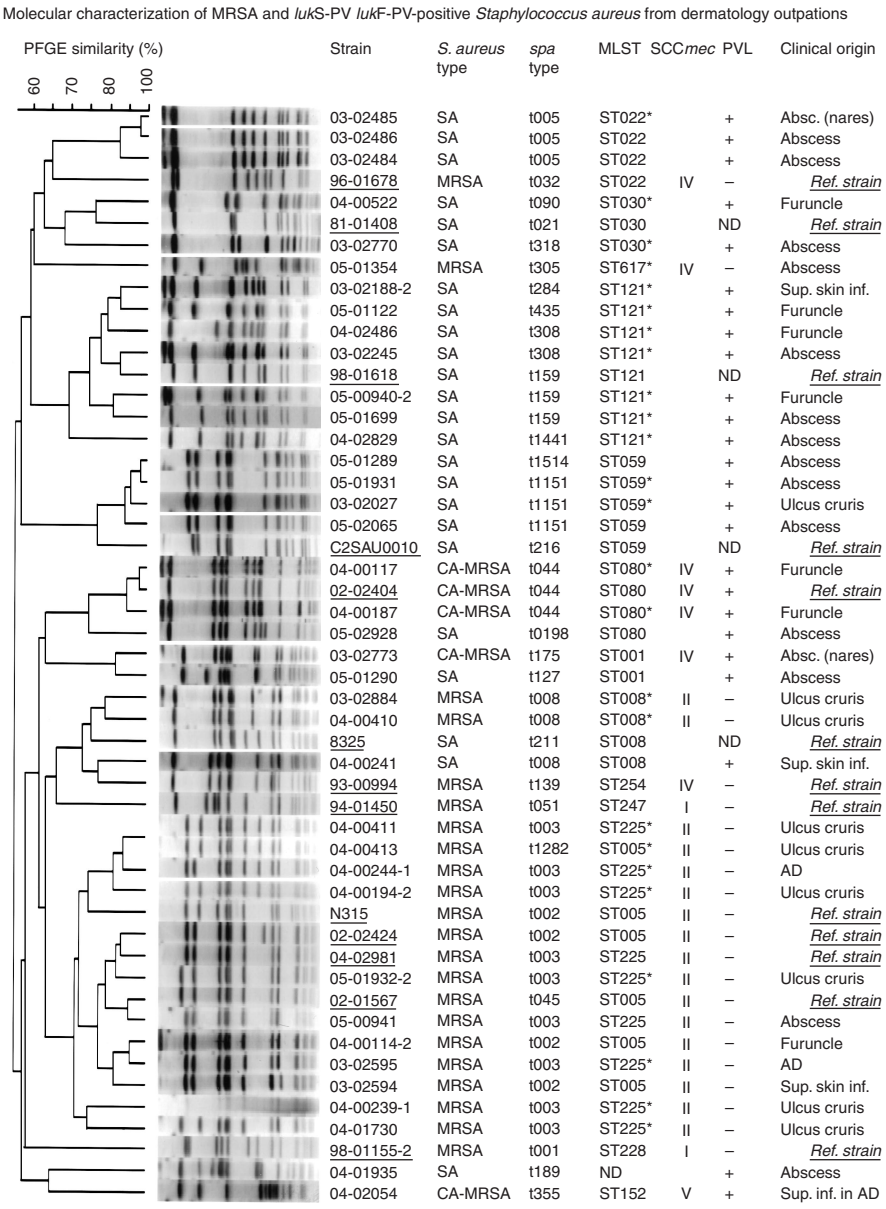


Figure 1. PFGE pattern and phylogenetic tree of MRSA and *luk*S-PV *luk*F-PV-positive *S. aureus*/MRSA of dermatology outpatients with superficial wounds or deep skin infections via *Sma*I-macrorestriction patterns expressed as a dendrogramm. The scale indicates the level of pattern similarity. PFGE, pulsed field gel electrophoresis; SA, *luk*S-PV *luk*F-PV-positive *S. aureus*; MLST, multilocus sequence typing; PVL, Panton-Valentine leukocidin gene (*luk*S-PV *luk*F-PV); SCC*mec*, staphylococcal cassette chromosome *mec*; *Spa*, *S. aureus* protein A; ND, not determined; AD, atopic dermatitis; Absc., abscess; Sup. skin inf., superficial skin infection; BURP, Based Upon Repeat Pattern. *As deduced from *spa* typing in combination with BURP grouping.

different among each other. Two strains from furuncles (04-00117 and 04-00187) were identical with regard to their microbiological and molecular characteristics and belong to the most frequent CA-MRSA type in Germany (see Figures 1 and 2, lanes 2 and 3).

Risk factor analysis. The risk factor analysis revealed that the majority of the patients carrying PVL-positive *S. aureus* (19/24; 79.2%; $P=0.033$) reported visits to foreign countries and/or professional or private contacts with foreigners, in contrast to patients with PVL-negative strains (Table 4). However, only for one strain, the single case of the North American CA-MRSA (Figure 2), could close contact with foreigners in a childcare unit be verified. The second major risk factor for the carriage of PVL-positive *S. aureus*—although statistically not significant—was previous antibiotic treatment (17/24; 71%; $P=0.302$) (Table 4). Only a few (7/24; 29%) patients with PVL-positive strains had been treated with FUS, and 7/24 (29.2%) patients reported hospitalization during the 6 months before admission. From this study, there is evidence that patients with atopic dermatitis do not have a statistically significant increased risk of acquiring PVL-positive *S. aureus*/

Table 2. Occurrence of FUS-resistant *S. aureus* and CA-MRSA in dermatology outpatients

	No. of patients with			
	S. aureus	**FUS-resistant *S. aureus* (including MRSA and CA-MRSA)**	**CA-MRSA**	**FUS-resistant CA-MRSA**
Patients with *S. aureus* (total number)	130	11 (8.5 %)	4 (3.0 %)	2 (1.5 %)
Patients with FUS treatment	34	4 (11.7 %)[1]	2 (5.8 %)	1 (2.9 %)[2]
Patients without FUS treatment	96	7 (7.3 %)[1]	2 (2.0 %)	1 (1.0 %)[2]

CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; FUS, fusidic acid.
[1]Difference statistically nonsignificant (Fisher's exact test: P=0.477).
[2]Difference statistically nonsignificant (Fisher's exact test: P=0.456).

Table 3. Clinical origin of *S. aureus* isolates (skin infection and skin colonization*) and their capacity of PVL production (n=108)

	***lukS*-PV *lukF*-PV**			
	Positive (n=24)		**Negative (n=84)**	
Clinical origin	**MRSA (n=4)**	**MSSA (n=20)**	**MRSA (n=14)**	**MSSA (n=70)**
Abscesses (25)	1	13	2	9
Furuncles (11)	2	4	1	4
Impetigo (4)	0	0	0	4
Superficial skin infections (27)	1	1	3	22
Atopic dermatitis* (13)	0	1	0	12
Venous leg ulcer* (28)	0	1	8	19

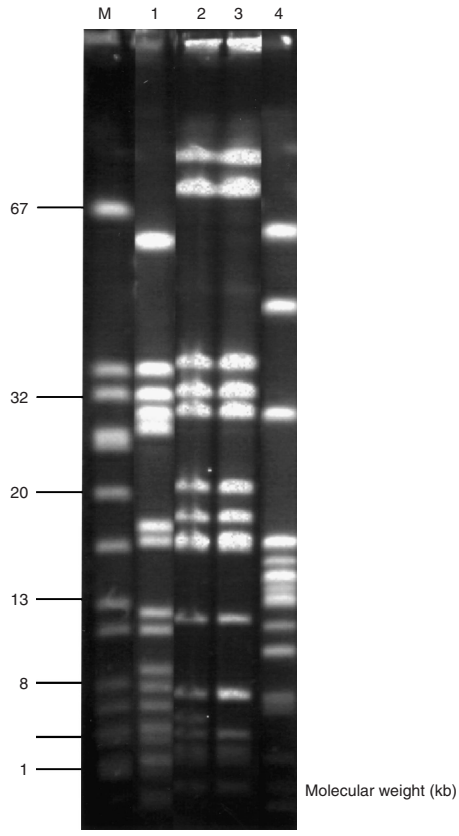
MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PVL, Panton-Valentine leukocidin.

CA-MRSA (Table 4). In the MRSA group, 13/18 had at least one well-known risk factor for MRSA colonization: diabetes mellitus, hemodialysis, venous leg ulcer, atopic dermatitis, and other chronic erosions. The investigation of the sources of MRSA in the 18 patients revealed previous hospitalization in 6 cases, 4 of them with surgery and 5 of them additionally suffered from diabetes. Two patients were admitted to a nursing home, one patient had hemodialysis, and two had atopic dermatitis. Patients with diabetes mellitus (5/16; 31%) had a nearly threefold increased statistically significant risk (P=0.012) of acquiring MRSA when compared to non-diabetic patients (13/114; 11.4%), none of them had CA-MRSA. So, in 72% of patients these risk factors can be made partly responsible for causing colonization with MRSA, particularly when considering the fact that 11 of these patients carried an endemic strain of the CC5, including ST005 and ST225 (Figure 1). In diabetic patients, *S. aureus* was sampled in 63% from venous leg ulcers and only in 13% from deep skin infections. The comparison of colonization and/or infection with MRSA including CA-MRSA of patients with atopic dermatitis and of those with other skin diseases revealed a similar percentage of carriage in both groups: 10.7% (3/28 of atopic dermatitis patients) vs 14.7% (15/102) remaining patients with other skin diseases. Concerning antibiotic treatment, 19/34 (56%) patients with a history of previous FUS treatment and who were positive for *S. aureus* had additionally received antibiotics during the 6 months before sampling. Eleven out of 34 had a positive history for deep skin infection. In 13 patients (4 with venous leg ulcers and 9 with atopic dermatitis), the collected strains were colonizing lesional skin.

DISCUSSION

Although the significance of MRSA in dermatology is obvious, the prevalence of multiresistant strains in skin lesions and their molecular characteristics have not been investigated thoroughly. Despite the worldwide increase in prevalence of MRSA, very few studies on surveillance of MRSA of dermatology outpatients exist. Preliminary investigations on MRSA in dermatology outpatients, shown to be HA-MRSA consisting of MLST CC5 (formerly named ''Rhine–Hesse'' epidemic strain) or local epidemic strains, revealed that chronic ulcers and erosions represented significant additional risk factors, pointing to a considerable impact of dermatological diseases in this particular context (Jappe *et al.*, 2004). The first prospective study on the prevalence of CA-MRSA in dermatology was performed in France nearly in parallel to the project presented here, exclusively investigating *S. aureus* strains sampled from 207 primary and secondary skin infections in 197 patients for their MRSA status, strictly following the definition of CA-MRSA and HA-MRSA, provided by Salgado *et al.* (2003) (Del Giudice *et al.*, 2006). Our study, in contrast, investigated not only primary and secondary skin infections in 248 outpatients but also skin colonization. In addition, we focused on PVL-positive strains without MRSA status and risk factors associated with HA-MRSA and CA-MRSA.

One hundred and thirty patients carried *S. aureus*, approximately one-third suffering from deep skin infections. Eighteen strains were MRSA, 11 endemic strains with a broad phenotypic resistance pattern, and 4 strains proved to be CA-MRSA. The CA-MRSA-detection rate of 3% was comparable to that in the French study. In contrast to the French study (Del Giudice *et al.*, 2006), where the CA-MRSA had similar pulsed field gel electrophoresis types comparable to the French CA-MRSA reference strain, we detected different



Lane	Strain	Lesion	Risk factors	Antibiotic treatment	Occurrence
1	03-02773	Abscess (acne inversa)	Professional contact (kindergarten) to a US American child who had recently immigrated	FUS, other antibiotics, steroids	Endemic in North America
2	04-00117	Furuncle	Native African, immigration to Germany 3 months prior to visit; clinic symptoms since immigration	Antibiotics	Germany, France, Scotland, Norway, Switzerland
3	04-00187	Furuncle	Journey to Turkey 5 months prior to first symptoms; two children with furunculosis; one child known MRSA carrier	FUS, other antibiotics, steroids	Germany, France, Scotland, Norway, Switzerland
4	04-02054	Pustule (atopic dermatitis)	Native Yugoslavian; last visit there 12 months prior to admission; chronic bronchitis	Topical antibiotics	Slovenia (Mueller-Premru *et al.*, 2005), Switzerland

Figure 2. Clinical characteristics of CA-MRSA isolates detected in this prospective study on *S. aureus* from dermatology outpatients with colonized superficial wounds or deep skin infections.

Table 4. Analysis of risk factors for *S. aureus*/MRSA acquisition in patients (*n*=108) with MRSA compared with patients with MSSA

	Patients with MRSA (*n*=18)		Patients with MSSA (*n*=90)	
Associated factors during the previous 6 months	**PVL+ (*n*=4)**	**PVL− (*n*=14)**	**PVL+ (*n*=20)**	**PVL− (*n*=70)**
Visits to foreign countries/contact to foreigners	4	4	15	24
Hospitalization	0	6	7	15
Topical FUS	2	2	5	18
Antibiotics	4	11	13	45
Atopic dermatitis	1	2	2	18
Glucocorticosteroids	2	2	5	25
Diabetes mellitus	0	5	0	8

FUS, fusidic acid; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PVL, Panton-Valentine leukocidin.

CA-MRSA strains. One originating from North America (ST001) has been detected in Germany for the first time (Witte *et al.*, 2005). It is the second most prevalent CA-MRSA in the United States. CA-MRSA ST152 isolated from a patient with atopic dermatitis is widely spread in the southeast of Europe (Mueller-Premru *et al.*, 2005). The PVL-negative MRSA strain ST225 was dominant in our study and has increasing significance in central Europe (RKI, 2007). MRSA ST005 is endemic in the region from which our patients were collected (Rhine–Hesse strain). ST008 is a well-established strain in health-care settings.

Twenty of 36 patients with deep skin infections had PVL-positive strains (55.6% (55% of furuncles and 56% of abscesses)). There were even MRSA sampled from two abscesses and one furuncle, lacking characteristic CA-MRSA traits. Similarly designed studies had revealed results on PVL-positive strains from deep skin infections with rates between 35.2 and 70.8% (Nolte *et al.*, 2005; Yamasaki *et al.*, 2005). From our as well as the above mentioned data, it is obvious that not everyone with invasive *S. aureus* infections carries PVL-positive (methicillin-resistant) strains, which contradicts the significance of PVL positivity as a *conditio sine qua non* (Couppie *et al.*, 1994; Lina *et al.*, 1999) for the development of deep skin infection. Our findings are additionally supported by recent investigations on the pathogenic mechanism of PVL providing evidence that PVL is not the major virulence determinant of CA-MRSA (Said-Salim *et al.*, 2005; Voyich *et al.*, 2006; Labandeira-Rey *et al.*, 2007; Bubeck Wardenburg *et al.*, 2007). Although there are contradicting findings on the role of PVL in the pathogenesis

of invasive *S. aureus* infections and although high expression of small cationic peptides with the capacity for recruitment and destruction of neutrophilic granulocytes seems to be more important (Wang *et al.*, 2007), there is a strong epidemiological association of the PVL genes with CA-MRSA (Lina *et al.*, 1999), suggesting PVL to be a highly informative epidemiological marker. The genetic determinants of *luk*S-PV *luk*F-PV are associated with particular lines of *S. aureus*, which have an unequivocal relation to particular isolates associated with deep skin infections: MLST type 121 and ST030 (see Figure 1), the former being dominant in this study. *S. aureus* ST121 caused an outbreak of furunculosis in a village in Germany (Wiese-Posselt *et al.*, 2007). Strains belonging to the CC ST030 had been isolated from furunculosis patients in the early 1940s after World War II. The same group was also associated with mastitis puerperalis and severe newborn infections during the 1950s known then as phage type 80/81 (Robinson *et al.*, 2005). However, nowadays these strains can be found in patients with furunculosis and tropical pyomyositis (Cuny *et al.*, 1996). All together, the PVL-positive *S. aureus* strains of our study collected from deep skin infections have a narrow resistance pattern and do not show the selection of particular antibiotic resistance profiles, which may be due to the lack of selective pressure, because abscesses and furuncles are treated surgically and afterward antiseptically rather than with systemic antibiotic therapy in the dermatology department of the University of Heidelberg, Heidelberg, Germany.

HA-MRSA and CA-MRSA carriers were investigated for risk factors. A significant factor for the acquisition of CA-MRSA was visits to foreign countries and/or professional or private contacts with foreigners, which is in accordance with previous reports (Maier *et al.*, 2005; Helgason *et al.*, 2008). It also proved to be a major risk factor for the acquisition of PVL-positive *S. aureus* strains in general, together with previous antibiotic treatment. The majority (72%) of MRSA-carrying patients in the presented study had well-known risk factors for HA-MRSA acquisition. The risk of patients with diabetes mellitus to acquire HA-MRSA is statistically significant and increased by nearly threefold when compared to non-diabetic patients, possibly having acquired these strains in regular ''ambulatory care.'' Interestingly, diabetic patients had no PVL-positive *S. aureus*. They were mostly elderly patients, whereas PVL-positive strains mainly were sampled from much younger individuals and are only rarely found in hospital settings, which is in accordance with the literature. From our data, there is no evidence that patients with atopic dermatitis are prone to be infected by MRSA, neither CA-MRSA nor HA-MRSA.

The frequency for FUS resistance detected in our study is 8.5% (*S. aureus*, including MRSA, of 11/130 dermatology outpatients) and, therefore, twice as high as the frequency of 3–4% of all MRSA sent to the NCS from all hospital disciplines (RKI, 2004b) of the last 8–10 years. FUS resistance was found in one *luk*S-PV *luk*F-PV-positive MSSA of the clonal group ST30 without *far-1* positivity, indicating that FUS resistance had developed independently of the *far-1* gene, probably due to a mutation or other unknown resistance mechanisms. Data on FUS resistance of *S. aureus* in dermatology generally are controversial. The community isolates are composed of a heterogeneous mixture of strains. According to Turnidge and Collignon (1999), the selection of FUS-resistant strains does not occur at high frequency in clinical practice, an observation supported by investigations in children with impetigo, not even after long-term treatment (Koning *et al.*, 2002). This is, however, in contrast to a Dutch group that observed an increase of FUS resistance in *S. aureus* strains isolated from atopic dermatitis in-patients from 9.7 to 23.4% between 1995 and 2001 (Peeters *et al.*, 2002). Ravenscroft *et al.* (2003) and Shah and Mohanraj (2003) described even higher rates of FUS resistance in patients with atopic dermatitis, which may be due to the adhesion of *S. aureus* to the skin of patients with this particular condition.

Our results do not show significant differences between patients with and without topical FUS treatment concerning the emergence of FUS-resistant *S. aureus* in general and CA-MRSA in particular. However, such a development cannot completely be excluded considering the relatively low number of treated patients in our study, which is due to the fact that in the Heidelberg dermatology department a policy on a most restricted use of topical antibiotics has been followed for years. Furthermore, our data are the result of a one-point analysis at the day of admission rather than a time course investigation. To strengthen our observation, future analysis of the isolates before and after FUS treatment should be performed with regard to FUS susceptibility.

Previous studies as well as our results demonstrate that dermatologists are first in line to detect the spread of virulent resistant *S. aureus* strains, thereby surveying the changes occurring in *S. aureus* epidemiology, relevant for antibiotic resistance policies as well as clinical symptoms. The primary transmission route of PVL-positive *S. aureus*/CA-MRSA is skin-to-skin contact with no skin effraction and indirect contact of contaminated objects in close communities (for example, family, sports, daily childcare, and health-care facilities) (Osterlund *et al.*, 2002; Linde *et al.*, 2005; Lu and Holtom, 2005; Mueller-Premru *et al.*, 2005). Clinicians should be aware of the major high-risk groups for CA-MRSA. Outbreaks of any pyoderma, soft tissue abscesses, and recurrent skin infections justify the investigation for PVL-positive *S. aureus*/CA-MRSA (Fleming *et al.*, 2006), especially with the patient's history containing information as mentioned above. Although there is no definite proof for PVL being the ultimate cause of deep skin infections, it seems to be a highly informative epidemiological marker for severe infections and, in some cases, CA-MRSA. Suspected infection and/or colonization with CA-MRSA needs particular hygiene procedures, as the affected individuals and settings as well as the vehicles differ from those of HA-MRSA-associated circumstances. Treatment of CA-MRSA-related abscesses and furuncles includes early and adequate incision and drainage; antibiotic therapy is of secondary importance if cellulitis or bacteremia is not a concern. Together with the previous two prospective studies (Maier *et al.*, 2005; Del Giudice *et al.*, 2006), our data provide evidence for the necessity to adopt the existing guidelines for management of

MRSA carriers in hospitals, nursing homes, and outpatient clinics. Obtaining a culture and resistance profile of all skin diseases holding the risk of PVL-positive *S. aureus* and CA-MRSA colonization should be encouraged to become routine. Our results demonstrate the occurrence of CA-MRSA in dermatology outpatients also in Germany. With increasing prevalence of CA-MRSA as expected, the empiric use of β-lactam antimicrobials such as cephalosporins or penicillins without wound culturing is hazardous.

MATERIALS AND METHODS

The study was performed in the dermatology outpatient clinic of the Department of Dermatology and Venerology, University of Heidelberg, Heidelberg, Germany. The outpatient clinic is attended by 100–120 patients a day.

Study population and sampling

A study population of 248 dermatology outpatients with venous leg ulcers, wounds, erosions accompanying inflammatory skin diseases, deep skin infections (abscesses and furuncles) as well as superficial skin infections such as impetigo, superficial wound infections, or pyoderma participated in the study. Swabs were taken from lesional skin and in nearly 50% of the patients additionally from anterior nares using a commercially available transportation medium (Trans-swab, Mast, Rheinfeld, Germany). The fact that not in any case nasal swabs were taken is due to the logistic fact that swabs were taken by medical personnel not always acquainted with the study protocol (for example, in the surgery). The data on potential risk factors for MRSA acquisition were abstracted from the medical history and recorded by a standardized questionnaire. If a patient was sampled more than once, the patient was counted only once.

Bacterial strains

All isolates were from primary skin and soft tissue infections and from colonized lesions of patients with erosive inflammatory skin diseases and venous leg ulcer—sometimes additionally from the nares. A set of representative reference strains was included in the study (Figure 1).

All swabs were cultured on blood agar plates containing 8 mg l^{-1} polymyxin to suppress the growth of Gram-negative bacteria. *S. aureus* was identified based on colony morphology and using plasma coagulation.

Antimicrobial susceptibility testing

All available isolates were tested for susceptibility to the following antibiotics: penicillin, oxacillin, moxifloxacin, erythromycin, clindamycin, OTE, gentamicin, chloramphenicol, rifampicin, fosfomycin, vancomycin, teicoplanin, linezolid, trimethoprim/sulfamethoxazole, ciprofloxacin, quinupristin/dalfopristin, FUS, and mupirocin. Mupirocin had been included because it is widely used for MRSA eradication in the nares. Susceptibility testing was performed by broth microdilution according to DIN 58940 (Deutsches Institut für Normung, 2004) using *S. aureus* strains NCTC 6571, SA403, SA159, and 1309/80 as reference strains for internal quality assurance. For oxacillin resistance detection, an additional special tube test was performed (about 10^7 colony-forming units were inoculated into 1 ml ISO Sensitest broth containing 2% NaCl and 2 μg ml^{-1} oxacillin and 8 μg ml^{-1} sulbactam) (Cuny *et al.*, 1999). Oxacillin-resistant isolates were screened for the *mec*A gene by PCR. Criteria for infections were as defined by Centers for Disease Control (CDC) guidelines (Garner *et al.*, 1988). Isolates not associated with infections were classified as colonizing.

Strain typing

Initially, all isolates were typed by phage typing for a first grouping (Witte *et al.*, 1988). All *S. aureus* obtained from deep skin infections (abscesses and furuncles) and all *luk*S-PV *luk*F-PV-positive isolates, as well as *S. aureus* phage group II known as particular line for causing deep skin infections, and isolates with oxacillin and FUS resistance were characterized by *Sma*I macrorestriction analysis, according to the standardized HARMONY protocol (Murchan *et al.*, 2003). Resulting band patterns were analyzed using BioNumerics (Applied Maths, Sint Martens-Latem, Belgium). Similarity values were computed using the Dice coefficient. Clustering was performed based on unweighted pair group arithmetic averaging. Additionally, the polymorphic X-region of *spa* gene—coding for a surface compound of *S. aureus*—was investigated. The X-region of the protein A gene consists of direct repeats exhibiting an extensive polymorphism. The number of repeats and repeat succession define the *spa* type. Sequence data were analyzed according to Harmsen *et al.* (2003) using the software Ridom StaphType (Ridom GmbH, Würzburg, Germany; see http://www.spaserver.de). *Spa* typing in connection with the new clustering algorithm Based Upon Repeat Pattern integrated into the software for grouping related *spa* types together allows classification of bacterial strains with a wide congruence to *Sma*I macrorestriction analysis (pulsed field gel electrophoresis)—the current ''gold standard'' (Strommenger *et al.*, 2006b). Selected isolates, which were grouped ambiguously by *Sma*I macrorestriction and *spa* typing, were investigated via MLST, allowing an assignment to clonal lineages. The MLST primers and PCR conditions were chosen as described by Enright *et al.* (2002). Sequencing reactions were carried out using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA). Allele types and resulting sequence types were assigned at the *S. aureus* MLST database (http://www.mlst.net).

Characterization of strains by PCR

For all MRSA strains, staphylococcal genomic DNA was extracted from 2 ml overnight culture with the DNeasy Tissue kit (Qiagen, Hilden, Germany) by using lysostaphin (100 mg l^{-1}; Sigma, Taufkirchen, Germany) to achieve bacterial lysis.

The *mec*A gene, which codes for the additional penicillin binding protein PBP2a mediating methicillin resistance, was detected as described previously (Witte *et al.*, 1994). All *S. aureus* isolates, obtained from deep skin infections and partly from superficial wounds, and all FUS-resistant *S. aureus* were investigated for the genetic determinants for PVL production and FUS resistance, *luk*S-PV *luk*F-PV and *far-1*, respectively. The presence of the corresponding genes was investigated by PCR as described previously (Witte *et al.*, 2005). Typing of SCC*mec* was performed as described previously (Strommenger *et al.*, 2006a).

As the specimens and information on potential risk factors were sampled for infection control following the infection control guideline of the university hospital, which is based on the recommendations of the NCS (Robert-Koch-Institute, Germany)

and the Protection against Infection Act (Infektionsschutzgesetz); formal ethical approval was not required (Ethics Committee, University of Heidelberg, Germany).

Statistics

A univariate comparison of data was performed using Fisher's exact test for categorical data (SPSS for Windows; SPSS Inc., Chicago, IL), and *P*-values <0.05 were considered significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The assistance of the nurses and doctors of the dermatology outpatient clinic and the dermatology surgery unit is gratefully acknowledged. We also thank Dr Jansen, dermatologist in practice, for providing samples from patients treated with FUS, B Pasemann and H Illiger for their invaluable technical assistance, and Dr M an der Heiden for statistical analysis.

REFERENCES

Al-Tawfiq JA, Aldaabil RA (2005) Community-acquired MRSA bacteremic necrotizing pneumonia in a patient with scrotal ulceration. *J Infect* 51:241–3

Bahrain M, Vasiliades M, Wolff M, Younus F (2006) Five cases of bacterial endocarditis after furunculosis and the ongoing saga of community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Scand J Infect Dis* 38:702–7

Bubeck Wardenburg J, Bae T, Otto M, DeLeo FR, Schneewind O (2007) Poring over pores: alpha-hemolysin and Panton-Valentine leukocidin in *Staphylococcus aureus* pneumonia. *Nat Med* 13:1405–6

Couppie P, Cribier B, Prevost G, Grosshans E, Piemont Y (1994) Leukocidin from *Staphylococcus aureus* and cutaneous infections: an epidemiologic study. *Arch Dermatol* 130:1208–9

Cribier B, Prevost G, Coupie P, Finck-Barbancon V, Grosshans E, Piemont Y (1992) *Staphylococcus aureus* leukocidin: a new virulence factor in cutaneous infections? An epidemiological and experimental study. *Dermatology* 185:175–85

Cuny C, Pasemann B, Witte W (1999) Detection of oxacillin resistance in *Staphylococcus aureus* by screening tests. *Eur J Clin Microbiol Infect Dis* 18:834–6

Cuny C, Zappletal C, Langenscheidt PH, Witte W (1996) Genomic typing of *Staphylococcus aureus* from pyomyositis in Uganda. *Med Microbiol Lett* 5:124–32

Del Giudice P, Blanc V, Durupt F, Bes M, Martinez JP, Counillon E *et al.* (2006) Emergence of two populations of methicillin-resistant *Staphylococcus aureus* with distinct epidemiological, clinical and biological features, isolated from patients with community-acquired skin infections. *Br J Dermatol* 154:118–24

Deutsches Institut für Normung (2004) DIN 58940. Medical microbiology-susceptibility testing of pathogens to antimicrobial agents. Part 8: microdilution. General method specific requirements. In: *DIN-Taschenbuch 222: Medizinische Mikrobiologie und Immunologie—Diagnostische Verfahren* (DIN Deutsches Institut für Normung e.V., ed), Berlin: Beuth-Verlag, 342–53

Embil J, Ramotar K, Romance L, Alfa M, Conly J, Cronk S *et al.* (1994) Methicillin-resistant *Staphylococcus aureus* in tertiary care institutions on the Canadian prairies 1990–1992. *Infect Control Hosp Epidemiol* 15:646–51

Enright M, Robinson D, Randle G, Feil EJ, Grundmann H, Spratt BG (2002) The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci USA* 99:7687–92

Fleming SW, Brown LH, Tice SE (2006) Community-acquired methicillin-resistant *Staphylococcus aureus* skin infections: report of a local outbreak and implications for emergency department care. *J Am Acad Nurse Pract* 18:297–300

Fridkin SK, Hagemann JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA *et al.* (2005) Methicillin-resistant *Staphylococcus aureus* disease in three communities. *N Engl J Med* 352:1436–44

Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM (1988) CDC definitions for nosocomial infections. *Am J Infect Control* 16:129–40

Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M *et al.* (2002) Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 359:753–9

Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D *et al.* (2003) Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J Clin Microbiol* 41:5442–8

Helgason KO, Jones ME, Edwards G (2008) Panton-Valentine leukocidin-positive *Staphylococcus aureus* and foreign travel. *J Clin Microbiol* 46:832–3

Ho PL, Cheung C, Mak GC, Tse CW, Ng TK, Cheung CH *et al.* (2007) Molecular epidemiology and household transmission of community-associated methicillin-resistant *Staphylococcus aureus* in Hong Kong. *Diagn Microbiol Infect Dis* 57:145–51

Jappe U, Petzoldt D, Wendt C (2004) Methicillin-resistant *Staphylococcus aureus* colonization in inflammatory versus non-inflammatory skin diseases: who should be screened? *Acta Derm Venereol* 84:1–7

Koning S, van Suijlekom-Smit LWA, Nouwen JL, Verduin CM, Bernsen RMD, Oranje AP *et al.* (2002) Fusidic acid cream in the treatment of impetigo in general practice: double-blind randomised placebo controlled trial. *BMJ* 324:203–6

Labandeira-Rey M, Couzon F, Boisset S, Brown E, Bes M, Benito Y *et al.* (2007) *Staphylococcus aureus*Panton Valentine leukocidin causes necrotizing pneumonia. *Science* 315:1130–3

Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V *et al.* (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 29:1128–32

Linde H, Wagenlehner F, Strommenger B, Drubel I, Tanzer J, Reischl U *et al.* (2005) Healthcare-associated outbreaks and community-acquired infections due to MRSA carrying the Panton-Valentine leucocidin gene in southeastern Germany. *Eur J Clin Microbiol Infect Dis* 24:1–5

Lowy FD (1998) *Staphylococcus aureus* infections. *New Engl J Med* 339:520–32

Lu D, Holtom P (2005) Community-acquired methicillin-resistant *Staphylococcus aureus*, a new player in sports medicine. *Curr Sports Med Rep* 4:265–70

Maier J, Melzl H, Reischl U, Drubel I, Witte W, Lehn N *et al.* (2005) Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* in Germany associated with travel or foreign family origin. *Eur J Clin Microbiol Infect Dis.* 24:637–9

McBride ME, Schaefer D, Rudolph AH, Aldama S, Wolf JE Jr (1989) Evaluation of antibacterial sensitivity testing methods for methicillin-resistant *S. aureus* in a dermatology outpatient population. *South Med J* 82:165–8

Millar BC, Loughrey A, Elborn JS, Moore JE (2007) Proposed definitions of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *J Hosp Infect* 67:109–13

Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS (2005) Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. *N Engl J Med* 352:1445–53

Moreno F, Crisp C, Jorgenson J, Patterson J (1995) Methicillin-resistant *Staphylococcus aureus* as a community organism. *Clin Infect Dis* 21:1308–12

Mueller-Premru M, Strommenger B, Alikadic N, Witte W, Friedrich AW, Seme K *et al.* (2005) New strains of community-acquired methicillin-resistant *Staphylococcus aureus* with Panton-Valentine leukocidin causing an outbreak of severe soft tissue infection in a football team. *Eur J Clin Microbiol Infect Dis* 24:848–50

Murchan S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, Zinn CE *et al.* (2003) Harmonization of pulsed-field gel electrophoresis protocols for

epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus:* a single approach developed by consensus of 10 European laboratories and its application for tracing the spread of related strains. *J Clin Microbiol* 41:1574–85

Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J *et al.* (2003) Comparison of community- and health care associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 290: 2976–84

Nolte O, Haag H, Zimmerman A, Geiss HK (2005) *Staphylococcus aureus* positive for Panton-Valentine leukocidin genes but susceptible to methicillin in patients with furuncles. *Eur J Clin Microbiol Infect Dis* 24:477–9

Oliveira D, De Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 46:2155–61

Osterlund A, Kahlmeter G, Bieber L, Runehagen A, Breider JM (2002) Intrafamilial spread of highly virulent *Staphylococcus aureus* strains carrying the gene for Panton-Valentine leukocidin. *Scand J Infect Dis* 34:763–4

Peeters KA, Mascini EM, Blok HE, Sanders CJ (2002) Increase in rate of resistance to fusidic acid among *Staphylococcus aureus* isolates from patients admitted with atopic dermatitis. *Ned Tijdschr Geneeskd* 146:2100–1

Prevost G, Couppie P, Prevost P, Gayet S, Petiau P, Cribier B *et al.* (1995) Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. *J Med Microbiol* 42:237–45

Price MF, McBride ME, Wolf JE (1998) Prevalence of methicillin-resistant *Staphylococcus aureus* in a dermatology outpatient population. *South Med J* 91:369–71

Ravenscroft JC, Layton AM, Eady EA, Murtagh MS, Coates P, Walker M *et al.* (2003) Short-term effects of topical fusidic acid or mupirocin on the prevalence of fusidic acid resistant (FusR) *Staphylococcus aureus* in atopic eczema. *Br J Dermatol* 148:1010–7

Robert Koch-Institut Wernigerode (2004a) Community acquired MRSA weltweit und in Deutschland. *Epid Bull* 5:33–6

Robert Koch-Institut Wernigerode (2004b) Zur MRSA-Situation in Deutschland im Jahr 2003. *Epid Bull* 42:358–61

Robert Koch Institut (2007) Zur MRSA-Situation in Deutschland 2005 und 2006. *Epid Bull* 6:41–7

Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G *et al.* (2005) Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. *Lancet* 365:1256–8

Said-Salim B, Mathema B, Braughton K, Davis S, Sinsimer D, Eisner W *et al.* (2005) Differential distribution and expression of Panton-Valentine leucocidin among community-acquired methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol* 43:3373–9

Salgado CD, Farr BM, Calfee DP (2003) Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. *Clin Infect Dis* 36:131–9

Shah M, Mohanraj M (2003) High levels of fusidic acid-resistant *Staphylococcus aureus* in dermatology patients. *Br J Dermatol* 148:1018–20

Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J, Witte W *et al.* (2006a) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. *J Antimicrob Chemother* 57:461–5

Strommenger B, Kettlitz C, Weniger T, Harmsen D, Friedrich AW, Witte W (2006b) Assignment of *Staphylococcus* isolates to groups by spa typing, *Sma*I macro-restriction analysis, and multilocus sequence typing. *J Clin Microbiol* 44:2533–40

Turnidge J, Collignon P (1999) Resistance to fusidic acid. *Int J Antimicrobial Agents* 12(Suppl 2):S35–44

von Baum H, Schmidt C, Svoboda D, Bock-Hensley O, Wendt C (2002) Risk factors for methicillin-resistant *Staphylococcus aureus* carriage in residents of German nursing homes. *Infect Control Hosp Epidemiol* 23:511–5

Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D *et al.* (2006) Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? *J Infect Dis* 194:1761–70

Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M *et al.* (2007) Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med* 13:1510–4

Wiese-Posselt M, Heuck D, Draeger A, Mielke M, Witte W, Ammon A *et al.* (2007) Successful termination of a furunculosis outbreak due to *lukS-lukF*positive, methicillin-susceptible *Staphylococcus aureus* in a German village by stringent decolonization, 2002–2005. *Clin Infect Dis* 44:e88–95

Witte W, Braulke C, Cuny C, Strommenger B, Werner G, Heuck D *et al.* (2005) Emergence of methicillin-resistant *Staphylococcus aureus* with Panton-Valentine leukocidin genes in central Europe. *Eur J Clin Microbiol Infect Dis* 24:1–5

Witte W, Cuny C, Halle E, Mauch H, Wagner J (1994) Methicillin resistance in an epidemic *Staphylococcus aureus* strain with genomic fingerprints corresponding to those. *Med Microb Lett* 3:388–95

Witte W, Cuny C, Strommenger B, Braulke C, Heuck D (2004) Emergence of a new community-acquired MRSA strain in Germany. *Euro Surveill* 9:1–2

Witte W, Richardson JF, Marples RR (1988) Complex typing of methicillin-resistant *Staphylococcus aureus* (MRSA). *Zbl Bakt Hyg A* 270:26876–82

Witte W, Strommenger B, Cuny C, Heuck D, Nuebel U (2007) Methicillin-resistant *Staphylococcus aureus* containing the Panton-Valentine leucocidin gene in Germany in 2005 and 2006. *J Antimicrobial Chemother* 60:1258–63

Yamasaki O, Kaneko J, Morizane S, Akiyama H, Arata J, Marita S *et al.* (2005) The association between *Staphylococcus aureus* strains carrying Panton-Valentine leukocidin genes and the development of deep-seated follicular infection. *Clin Infect Dis* 40:381–5

Zaraket H, Otsuka T, Saito K, Dohmae S, Takano T, Higuchi W *et al.* (2007) Molecular characterization of methicillin-resistant *Staphylococcus aureus* in hospitals in Niigata, Japan: divergence and transmission. *Microb Immunol* 51:171–6