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CONCISE ARTICLE

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Healthcare-associated outbreaks and community-acquired infections due to MRSA carrying the Panton-Valentine leucocidin gene in southeastern Germany

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Abstract In response to several isolations of methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leucocidin gene (PVL-MRSA), the present study was conducted to document the spread of infection in a small region of southeastern Germany. During a 9-month period, two healthcare-associated outbreaks with PVL-MRSA occurred, affecting 83 patients, personnel and contacts of personnel, and 34 additional cases were detected in the community. The clinical spectrum ranged from colonization to skin infection and necrotizing pneumonia. The findings represent the largest number of PVL-MRSA cases detected in Germany so far, and demonstrate the potential of this emerging pathogen to spread within the community and in healthcare institutions.

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Introduction

Until recently, methicillin-resistant *Staphylococcus aureus* harboring the Panton-Valentine leucocidin gene (PVL-MRSA) was considered to be rare in Germany [1]. Worldwide, PVL-MRSA is recognized as an emerging community-acquired pathogen causing skin-related infections and necrotizing pneumonia in persons with no apparent risk factors, including children [2–4]. Transmission in hospitals has also been observed [5]. PVL-MRSA, also called community-acquired or community-associated MRSA, possesses a unique combination of pathogenicity and resistance factors. Among other virulence factors, Panton-Valentine leucocidin (*lukS/F-PV*), which causes lysis of granulocytes and monocytes as well as tissue necrosis, is considered to be of special importance [6]. Resistance to oxacillin is encoded by the *mec*A gene typically present

on a *mec* type IV [7] or type II staphylococcal cassette chromosome (SCC).

In Regensburg, the first isolate of PVL-MRSA related to this study was found in an 18-year-old woman in December 2003. In January 2004, the detection of PVL-MRSA in one nursing home resident and in one nursing home employee within a 7-day period prompted a systematic investigation of all residents and personnel in two nursing homes. The strain isolated from the patient was detected during routine hospital admission screening and that of the employee was the causative agent of community-acquired pneumonia. A second outbreak was observed in a neonatal care unit. During the time of the investigation, an unprecedented number of patients with community-acquired PVL-MRSA was also reported in the area under study. Described here are the clinical findings from 117 cases of healthcare-associated and community-acquired PVL-MRSA detected between December 2003 and August 2004.

Patients and methods

This observational study was conducted between December 2003 and August 2004 in a geographically well-defined region, measuring approximately 50 km in radius, around the city of Regensburg, in southeastern Germany. Six laboratories (Institute for Medical Microbiology, University Regensburg; Microbiology Laboratory of the St. Elisabeth-Krankenhaus, Straubing; Microbiology Laboratory of the Klinikum Fürth, Fürth; and three microbiology laboratories serving outpatients) participated in the study by testing for PVL-MRSA in screening samples obtained during two outbreaks and in routine samples obtained from patients suspected of having infection.

Patient characteristics and clinical data were obtained using a standardized questionnaire. On the basis of epidemiological data, patients with PVL-MRSA were classified as hospital-acquired or community-acquired cases. A case was considered hospital-acquired if the patient was (i) staying or working in a healthcare institution during the time of sampling, (ii) identified by screening during the investigation of outbreak I or II, or (iii) a close contact of a person identified by screening. In outbreak I, screening samples were obtained from all residents (anterior nares, groin, wounds if applicable) and personnel (anterior nares) in two nursing homes. In outbreak II, samples obtained from all patients (anterior nares, ear, groin, feces, wounds if applicable) and personnel (anterior nares) in a neonatal care unit were screened. A case was considered community-acquired, if the patient had not been treated as an inpatient within the last 6 months, and was not affiliated with a hospital, nursing home, or any other healthcare institution. All patients were encouraged to report close private contacts with symptoms or a history of recurrent skin infection, and close contacts were screened on a voluntary basis.

Routine clinical specimens were plated directly, without pre-enrichment, onto non-selective and selective media (Orsab agar; Oxoid, Basingstoke, UK). For screening, swabs were moistened with sterile saline before use, and a pre-enrichment step (thioglycolate broth, Oxoid) was included.

Susceptibility testing was performed according to the guidelines of the National Committee for Clinical Laboratory Standards [8], and resistance to fusidic acid was tested according to previously described procedures [9]. Results were confirmed by simultaneous real-time PCR-based detection of the *S. aureus*-specific marker *nuc* and the *mec*A gene [10]. *LukS*/*F*-PV and *hly*A genes were amplified by block-cycler PCR as described previously [3]. Typing of selected isolates was performed using *Sma*I-macrorestriction and multilocus sequence typing (http://www.MLST.net) [11].

Results and discussion

During a 9-month period, from December 2003 to September 2004, we observed 117 cases of PVL-MRSA colonization or infection in a small region of southeastern Germany (within a radius of 50 km around Regensburg). Eighty-three of the cases were found in two healthcare-associated outbreaks. Outbreak I occurred in a cluster of 10 healthcare institutions (2 hospitals, 5 nursing homes, 1 home for disabled persons, 1 hemodialysis outpatient clinic, and 1 patient transport service) in two closely positioned cities; it involved 52 patients and nursing home residents as well as 21 personnel and 2 private contact persons. This outbreak prompted screening of all residents (n=394) and personnel (n=192) in two nursing homes and revealed a PVL-MRSA carrier rate of 9.1% in residents and 9.7% in personnel (the carrier rates for MRSA isolates susceptible to fusidic acid and negative for the *lukS*/*F*-PV genes were 3.5% and 1%, respectively). Outbreak II occurred in a neonatal care unit and involved 5 of 20 patients and 3 of 131 personnel. During the same time period, 34 cases of community-acquired PVL-MRSA were found. Community-acquired cases of PVL-MRSA were identified, in part, due to referral of patients with recurrent subcutaneous abscesses after the first case of necrotizing pneumonia due to PVL-MRSA was reported in a local newspaper.

The demographic and clinical details of all cases are given in Table 1. Community-acquired cases had higher rates of clinical disease than the cases identified by screening in the healthcare-associated outbreaks. When patients and personnel carrying PVL-MRSA from outbreaks I and II were compared, the rate of clinical disease was 28.1% for patients and 16.6% for personnel. Spontaneous abscesses occurred at similar rates in both groups (12.3% in patients, 16.6% in personnel). Nearly all 117 PVL-MRSA isolates showed a uniform antimicrobial susceptibility pattern: All isolates were resistant to oxacillin and resistant or intermediately susceptible to fusidic acid (1 was susceptible) but susceptible to macrolides, tetracyclines (3 resistant), or quinolones (1 resistant). All were positive for the *lukS*/*F*-PV genes. Results of pulsed-field gel electrophoresis and multilocus sequence typing were in

Table 1 Characteristics and types of infection in 117 patients, personnel, and private contacts of personnel with PVL-MRSA

Characteristic	Patients (*n*)			Personnel (*n*)		Total (*n*)
	Community (34)	Outbreak I (52)	Outbreak II (5)	Outbreak I/II (24)	Private contacts[a] (2)	(117)
Age range in years (median)	<1–82 (30)	42–95 (83)	<1	21–59 (35)	2 and 16	<1–95 (55)
Female, *n*	17	29	3	22	1	72 (61.5%)
Colonisation, *n*	5	38	3	20	1	67 (57.2%)
Clinical disease, *n*	29	14	2	4	1	50 (42.8%)
Abscess	25	7	–	4	1	37 (31.6%)
Device-related	–	5	–	–	–	5 (4.2%)
Pneumonia	4[b]	2	–	–	–	6 (5.1%)
Fever	3	1	2	–	–	6 (5.1%)
Hospitalisation, *n*	11	10	2	–	–	23 (19.7%)

[a]Private contact persons were not screened systematically

[b]Three necrotizing pneumonia, two fatalities

accordance with epidemiological data from the patients and suggested a high degree of relatedness within the two groups of isolates from the two healthcare-associated outbreaks (outbreak I: ST22, *n*=33/33; outbreak II: ST80, *n*=8/8), whereas a variety of multilocus sequence types was found among the community-acquired cases (ST80, *n*=10/21; ST22, *n*=8/21; ST30, *n*=2/21; ST8, *n*=1/21).

Worldwide, PVL-MRSA is recognized as an emerging pathogen [12, 13]. The results of our study in southeastern Germany are new and noteworthy in several respects. The high number of PVL-MRSA isolates we found within a small geographical region (*n*=117) during a 9-month period far exceeds the previously reported and confirmed total number of 23 PVL-MRSA isolates found in Germany until the end of 2003 [14]. This raises the question as to what extent PVL-MRSA is currently underreported in Germany, especially in southeastern Germany.

In our investigation, PVL-MRSA isolates were detected in two distinct settings, community and healthcare. The two outbreaks in healthcare settings demonstrate the potential of PVL-MRSA to spread as a healthcare-associated pathogen. The similar rates of cutaneous infection found by screening patients and personnel in outbreaks I and II support the concept that no special risk factors are needed in order for an individual to contract a skin infection due to *S. aureus* carrying the PVL gene. The high rate of clinical manifestations we observed among the community-acquired cases is most likely due to detection bias. In our study, cases of community-acquired PVL-MRSA were not retrieved systematically, thereby making it impossible to determine whether PVL-MRSA has been increasing in our region recently. We cannot exclude that PVL-MRSA may have been prevalent in the region before and gone undetected. This is especially true for community-acquired cases, which are rarely subjected to microbiological testing.

Several aspects of PVL-MRSA might become a severe problem in the future. Compared to common *S. aureus* strains susceptible to oxacillin, PVL-MRSA are unique in terms of their virulence, antibiotic resistance, and tendency to spread. PVL-MRSA can cause significant infections in people with and without risk factors, and treatment options are limited due to the pathogen's resistance to all beta-lactam antibiotics. Obviously, selective pressure by antibiotics is not necessary for successful dissemination of PVL-MRSA in the community, and it easily occurs in the healthcare setting. In response to outbreak I, appropriate steps for the diagnosis, hygiene, and therapy of patients and personnel were defined in collaboration with the public health authorities. Further studies of the epidemiology of PVL-MRSA in the community and in healthcare institutions in southern Germany are warranted.

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