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Clonal dissemination of two MRSA strains in Germany

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SUMMARY

Clonal dissemination of two different MRSA strains, both clumping factor negative, has been observed in Germany for more than a year. Both strains possess the mec-A determinant and each exhibits a characteristic genomic DNA fragment pattern. One strain has spread in the north, the other in the south-west of Germany.

Intensive care units are mainly affected by MRSA-infections and probably play a special role in further intra- and inter-hospital spread.

INTRODUCTION

Whilst an increasing frequency of occurrence of multiply- and methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported from western European countries since the middle of the 1980s, multicentre studies in Germany in 1990 revealed frequencies between 1·1 and 3·7% relative to the total number of *S. aureus* from nosocomial infections [1, 2]. The main mechanism of methicillin-resistance is the possession of an additional low-affinity penicillin binding protein PBP2′ [3]. Based on a study of restriction site polymorphism in the neighbourhood of the corresponding mec-A gene, Kreiswirth and co-workers [4] concluded that a worldwide clonal dissemination had occurred of a distinct ancestor of the recently isolated MRSA which once had acquired the mec-A gene. MRSA can be differentiated by a number of conventional, for example phage typing [5], and molecular, for example ribotyping, genomic DNA fragment patterns etc. [6–8] methods. Results of typing indicate clonal dissemination of the most frequently isolated MRSA in western European countries [9–12]; MRSA isolated from infections in German hospitals were obviously different [1, 13]. However from autumn 1992 until now we have observed clonal dissemination of two MRSA strains, one in the north and one in the south-west of Germany.

MATERIALS AND METHODS

Phage typing was performed as described previously [5] by the use of two sets of experimental phages in addition to the International Basic Set for phage typing *S. aureus*.

Genomic DNA fragment patterns were obtained after digestion of genomic DNA by restriction endonuclease *Sma* I and subsequent pulsed-field electro-

phoresis using the CHEF-II-system of BioRad (pulse-scheme: 5–60 sec for 15 h and 60–90 sec for another 15 h; for details see [7, 14].

Resistance determinations: minimal inhibitory concentrations were determined by the microbroth dilution assay as recommended by DIN 58940 [15].

Demonstration of the mec-A determinant by PCR: for isolation of whole cellular DNA cells were grown in 10 ml Trypticase-Soy-Broth overnight. After pelleting by centrifugation they were washed once with TE-buffer (10 mM Tris HCl, 50 mM EDTA) and subjected to lysostaphin at 30 °C (50 units in 100 mM EDTA, 10 mM Tris HCl, 2% SDS, pH 7·5) until the mixture became viscous. Lysis buffer was added, the tube was gently mixed and immediately centrifuged at 37 °C (20000 g for 15 min). The supernatant was phenol/chloroform extracted and the DNA ethanol precipitated. About 20 ng of DNA served as template for PCR with 100 pmol of each of the primers (mecAI:5′-AAAATCGATGGTAAAGGTTGGC; mecAII::5′AGTTCTGCAGTACCGGATTTGC), 200 μg of each of the deoxynucleotides and 2·5 U of the Replithern® polymerase from Biozym. Following an initial denaturation at 94 °C for 5 min, the DNA was amplified during 30 cycles of PCR consisting of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min except the last cycle with an extension step of 4 min (Maxicycler PTC 100; Biozyme).

Demonstration of clumping factor: purified and stabilized human fibrinogen was used as previously described [16]. For tests of coagulase and deoxyribonuclease see [17].

RESULTS

Typing characteristics of the two epidemic MRSA

Table 1 shows the results of phage typing and other phenotypical traits; both of the strains were only partly typable by phages, exhibited characteristic phenotypes of multiresistance and genomic DNA fragment pattern. All isolates of strains examined were negative for the clumping factor but able to produce coagulase and deoxyribonuclease.

Amplification by PCR of the *mec*A region resulted in the expected 0·5 kb fragment (data not shown).

Clonal inter-hospital spread of an MRSA strain in the north of Germany

The occurrence of this strain was first recorded in the autumn of 1992 in university medical school 1 in the south of lower Saxony (Fig. 1). Between autumn 1992 and May 1993 19 cases of infections were recorded. This included an intensive care centre which also admits victims of car-accidents from neighbouring federal counties. Later the epidemic strain was isolated in eight further hospitals. For hospitals 5, 6 and 7 it is evident that this strain was spread by patients as a result of transfer to hospitals of their home area after therapy in two intensive care units (ICU) of hospital 1. In four of these hospitals further outbreaks of infections were recorded; these outbreaks were terminated at hospitals 2 and 4. By taking appropriate preventive measures in time a further intrahospital dissemination of the epidemic strain was prevented in hospitals 6 and 7. These hospitals had been warned before the admission of the infected patients from hospital 1. How the epidemic strain came into hospital 3 is not known. Analysis of this outbreak illustrates the epidemic spread and virulence of the disseminated strain in

Table 1. *Typing characteristics of two clonally disseminated MRSA*

Area of dissemination	Phage pattern*		Clumping factor	Coagulase	DNase	Crystal-violet type	Resistance phenotype*
I North of Germany	a, b, c NT only for strains of outbreak 1	100 RTD	−	+	+	C	PEN, OXA, GEN, ERY CLI, TMP, CIP, partly RIF
	in fig. 1						
	a 77	RTD					
	b 616, 617, 626	100 RTD					
	c 92	RTD					
II South-West of Germany	a, b, c NT	100 RTD	−	+	+	A	PEN, OXA, GEN, ERY, CLI, CIP

* Phage-pattern: a, International Basic Set for phage-typing; b, experimental phages 616–630; c = experimental phages 88 = 93.
† PEN, penicillin; OXA, oxacillin; GEN, gentamicin; ERY, erythromycin; CLI, clindamycin; TMP, trimethoprim-sulfamethozazole; CIP, ciprofloxacin; RIF, rifampicin.

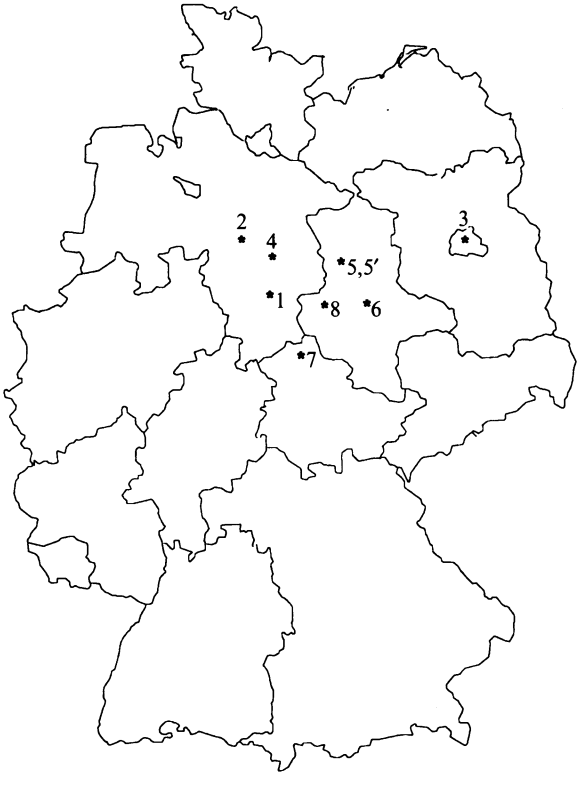

Fig. 1. Clonal dissemination of a MRSA-strain in the north of Germany. 1, autumn 1992–spring 1993 (19 cases of infection); 2, February–March 1993 (4 cases of infection); 3, April–July 1993 (14 cases of infection); 4, spring 1993 (outbreak, number of infections not communicated); 5, May–June 1993 (13 cases of infection); 5′, August–October 1993 (9 cases of infection); 6, April–May 1993 (1 infected patient from hospital 1; 2 further cases of infection); 7, April 1993 (1 infected patient from hospital 1); 8, July 1993 (3 cases of infection; 1 case of colonization).

Table 2. *Infections with three different MRSA in an intensive care unit of university hospital 3 with strains A, B, C*

ICU a	3 × A
ICU a	2 × C
ICU a	1 × B
Liver transplantation unit	2 × C
General surgery station b	3 × C
General surgery station c	1 × C
General surgery station d	2 × C
Haemodialysis unit	1 × C

Patterns are shown in Fig. 3.

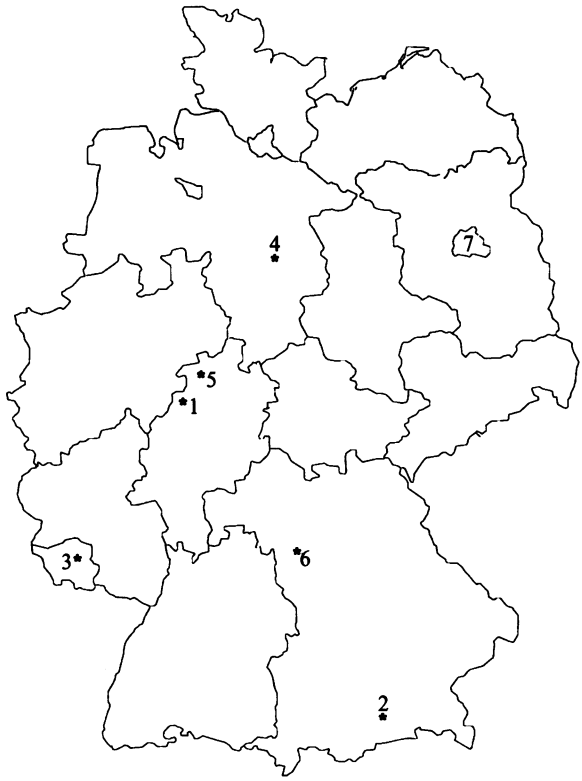

Fig. 2. Clonal dissemination of a MRSA-strain in the south-west of Germany. 1, January–October 1992 (12 cases of infection); 2, January–March 1993 (9 cases of infection); 3, January–April 1993 (7 cases of infection); 4, Spring 1993 (outbreak, number of infections not communicated); 5, October 1992–May 1993 (10 cases of infection); 6, March–May 1993 (36 cases of infection); 7, May 1993 (1 case of infection).

comparison to other MRSA. At the beginning of this outbreak three different MRSA strains were observed at ICU 8. Only strain C, which exhibited the genomic DNA fragment pattern of the epidemic strain, was spread to further wards of this clinic (Table 2).

Clonal spread of an MRSA strain among hospitals in the south-west of Germany

Occurrence and spread of the second epidemic strain is shown in Fig. 2. This strain was first observed in a clinic in town W; later this strain was isolated from outbreaks of infections in further hospitals.

Table 3. *Occurrence and dissemination of the MRSA in different hospital settings*

Hospital	Total	Intensive care	Surgery/ traumat-ology	Ortho-paedics	Neuro-surgery	Internal medicine	Paedi-atrics	Urology
A	10	6	1	1			2	
B	19	12			7			
C	9	4		1		2		2
D	4	3	1					
E	7	4	1				1	1
F	3	3						
G	13	7	3			3		
H	14	8	3		2	1		
I	36	15	12		2	4	3	

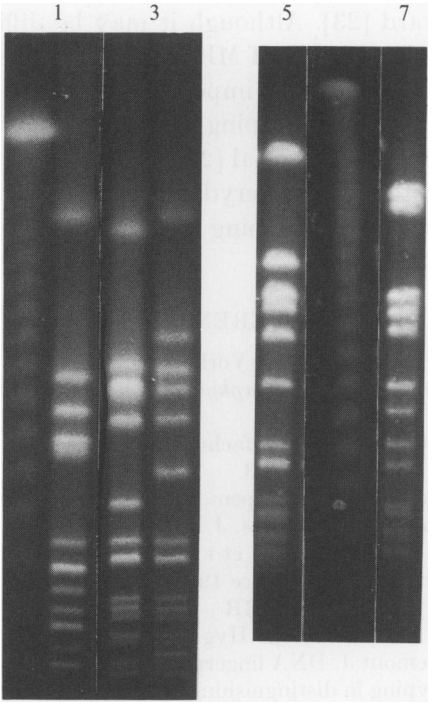

Fig. 3. Genomic DNA restriction patterns: Lane 1, lambda DNA; lanes 2–4, strains A, B and C from university hospital 3; lane 5, clonal pattern I; lane 6, lambda DNA; lane 7, clonal pattern II.

Intensive care units and MRSA

The significance of ICU for the dissemination of MRSA is evident from the data presented above. Table 3 shows for nine larger hospitals that in most cases the clonal disseminated MRSA strains had been isolated from ICUs.

DISCUSSION

Widespread dissemination of specific MRSA strains had already been reported from the middle of the 1980s, in England, demonstrated by phage-typing and restriction site polymorphism [9, 10], from Portugal, demonstrated by phage

typing and plasmid patterns [11], from Spain by genomic DNA fragment patterns [12], and from France by polymorphism of restriction sites flanking the aac6′-aph2″-determinant [18]. The main reason for inter-hospital spread of multi-resistant strains of *S. aureus* are obviously colonized or infected patients (see also [19, 20]). Before transmission of a patient between hospitals, the hospital of destination should be prewarned and measures taken to prevent further spread (for details see [21]). Although eradication of the carrier state in affected patients has been shown to be effective in cases of nasal colonization [22], eradication from other sites (e.g. wounds) is more difficult. As evident from previous studies [19] beside other risk factors the duration of antibiotic treatment as well as prolonged nasogastric intubation predispose to colonization and infection with MRSA. Thus intensive care units are preferentially affected by MRSA. ICUs can have a 'turntable' function for intra-hospital dissemination of MRSA when patients are transferred to another ward [23]. Although it may be difficult to carry out the well-established methods for control of MRSA outbreaks, elimination or at least reduction of MRSA in an ICU is very important.

An outbreak of infection with clumping-factor negative MRSA was described recently in a German university hospital [24]. Since the clumping factor reaction is the main species-characteristic in everyday diagnostics in routine laboratories, the widespread dissemination of clumping factor MRSA needs attention.

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