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Outbreak of nosocomial infections with two different MRSA-strains involved: significance of genomic DNA fragment patterns in strains otherwise difficult to type

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SUMMARY

Methicillin-resistant *Staphylococcus aureus* isolates from an outbreak of 17 cases of wound infection in a municipal hospital were typed by conventional methods, phage typing by three sets of phages, reverse phage typing and plasmid profiles, as well as by genomic DNA fragment patterns obtained after *Sma*-I digestion and pulsed-field electrophoresis. These isolates were non-typable by phages, only some were typable by reverse phage typing and were not uniform in plasmid profile. Only the genomic DNA fragment patterns resulted in a clear discrimination of 2 strains (12 isolates for the first and 7 isolates for the second). Both strains were disseminated in different wards of the same hospital and one strain had obviously spread to another clinic in the same city.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* have become important nosocomial pathogens worldwide. Until recently they have not been frequent (2–3%) in German hospitals [1]. As described previously [2], several nosocomial outbreaks with MRSA which were also resistant to quinolones have been recorded during the last 3 years. As shown for representative strains from 10 outbreaks in the whole of Germany by phage typing, plasmid-profiles and *Sma*-I fragment patterns of genomic DNA, these strains were different and the outbreaks obviously unrelated.

This paper reports an outbreak of infection with two different MRSA. Until recently, multiply resistant *S. aureus* strains in Germany were typable by phages of the International Basic Set. The use of an additional phage set established by Richardson and co-workers [3] was found to be very supportive for strain discrimination especially for MRSA [4]. The strains involved in the outbreak reported here were non-typable by phages and only partly typable by 'reverse' phage typing (lysis profile of induced phages). In this situation the significance of plasmid profiles and restriction fragment patterns of genomic DNA have been investigated.

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Table 1. *Origin of the investigated* S. aureus *strains*

Isolate no.	Wards	Origin
1	B14	Blood culture; patient was a soldier from the Gulf War admitted with this infection
2	A34	Wound swab
3	Otorhinolaryngological ambulatory	Wound swab
4	B44	Wound swab
5	B44	Wound swab
6	B44	Wound swab
7	B44	By-pass wound, swab
8	B14	Tracheal secretion (taken via tubus in connection with artificial ventilation)
9	B14	Blood culture
10	K23	Throat swab
11	A43	Wound swab
12	B44	Nasal swab, nurse D.B.
13	B14	Nasal swab, nurse D.M.
14	Ophthalmological ambulatory	Skin swab, physician S.R.
15	K33	Swab from a fistula, left
16	B14	Wound swab
17	A53	Throat swab
18	B14	Tracheal swab
19	B44	Wound swab
20	A12	Sputum
21	Rheumatology clinic I	Wound swab

MATERIALS AND METHODS

Staphylococcal strains: the origin of the wild strains is given in Table 1; strain 8325-4 is a prophage-free derivative of strain 8325 used in this study for detection of prophages released from wild-type strains after mitomycin C-induction.

Phage typing: performed according to the method of Blair and Williams [5] using the International Basic Set for phage typing *S. aureus* and of two sets of additional phages (a: 88, 89, 99, 91, 92, 93 and b: 616, 617, 618, 620, 622, 623, 625, 626, 629, 630 as described).

Reverse phage typing: induction of prophage was achieved by the method of de Saxe and Notly [6]. The lysates were spotted on strain 8325-4 as well as on the propagating strains of the International Basic Set of phages as in phage-typing. Results were read as in phage-typing.

Crystal-violet type: determined by the staining pattern of macrocolonies on agar-medium containing crystal violet [7].

Resistance phenotype: minimal inhibitory concentrations were determined by microbroth-dilution assay according to the protocol of DIN 58940, part 8 [8]. Resistance to ethidium bromide (as representative for quaternary ammonium ions) was also determined as a marker; MIC's $> 32\ \mu g/ml$ were regarded as resistant.

Plasmid profiles: plasmid DNA was isolated as described previously [4] and subjected to horizontal agarose-gel electrophoresis.

Fragment patterns of genomic DNA after *Sma*-I digestion: embedding staphylococci into LMP-agarose, incubation of these agarose blocks in lysis-solution containing lysostaphin, deproteinization with proteinase K1 (1 mg/ml) and DNA restriction (incubation with 20 U/ml of *Sma*-I overnight at 25 °C) were performed as described previously [9]. For separation of DNA fragments pulsed-field electrophoresis was applied by use of the CHEFII-system from BioRad. Conditions were: 1% agarose-gel (Sigma, medium EEO), 0·5 M tris-borate buffer pH 8·0 and two periods of ramped pulses from 5 to 60 sec for 15 h and 60–90 sec for another 15 h. Concatemers of the genome of bacteriophage λ with different molecular masses were used as molecular mass standards.

RESULTS

Methicillin (oxacillin)-resistant *S. aureus* strains additionally resistant to quinolones (QR-MRSA) have been isolated from infections in nine different wards of a large municipal hospital during a period of 5 months. QR-MRSA were also isolated in two out-patient departments and from one patient in the rheumatology clinic of the same city (details in Table 1). The first QR-MRSA isolate was obtained from a blood culture of a soldier in the Gulf War who was already infected on admission to hospital (ward B14). In order to confirm our assumption of intra-hospital spread of this strain, all of the QR-MRSA strains were subjected to typing as described below.

Typing by conventional methods: these data are put together in Table 2. All multiply resistant isolates are non-typable by phages of the International Basic Set and by two sets of additional phages used.

In reverse phage-typing the isolates 1, 2, 4, 6, 11, 15, 17, 18, 19 and 21 released phages which lysed strains 8325-4, 83A and 85. Isolates 3, 5, 7, 8, 9, 10 and 13 gave no reaction. Both groups of strains also differed in the crystal-violet type.

The first group of isolates exhibited a unique plasmid profile with molecular masses clearly different from those of the second group. Among these isolates, differences in plasmid profile were found to correspond with definite resistance characters: resistance to fusidic acid and presence of the 43 kb plasmid; resistance to ethidium bromide and presence of the 29 kb-plasmid. Typing by genomic DNA fragment patterns: Fig. 1 shows the results of pulsed-field electrophoresis after digestion of genomic DNA with restriction endonuclease *Sma*-I.

Isolates 1, 2, 4, 6, 11, 15, 17, 18, 19 and 21 exhibited indistinguishable patterns; isolate 16 differed from them in two fragments. This strain lacked resistance to trimethoprim/sulphonamide. Different from the cluster of strains mentioned above, but indistinguishable amongst themselves were isolates 3, 5, 7, 8, 9, 10 and 13. Strains 12 and 14 from carriers among the personnel and also strain 20 from a patient's sputum were clearly different.

The results of typing revealed that isolates of two clonally related QR-MRSA strains have been disseminated in the hospital. This is illustrated in more detail by Table 3. QR-MRSA strain I was introduced to ward B14 of the hospital already in August 1991 by patient A.N.-M. who later died (October 1991). Ward B14 is a surgical intensive care unit from which patients are transferred to other wards during the course of further treatment. Two episodes of spread to other wards

Table 2. *Results of typing by conventional methods*

Strain no.	Phage pattern			Reaction pattern in reverse phage typing	Crystal violet type	Resistance phenotype	Plasmid-profile (Md)
1,2,4,6,11,	a NT	100	RTD	8325-4, 83A, 85, +	C	Pn,Ox,Em,Tc,Sm,Gm,Tp/Sa,	17·5; 2
15,17,18,	b NT	100	RTD			Cp,Eb	
19,21	c NT	100	RTD				
16	a NT	100	RTD	8325-4, 83A, 85, +	C	Pn,Ox,Em,Sm,Gm,Cp,Eb	17·5; 2
	b NT						
	c NT	100	RTD				
3,5,8,9,	a NT	100	RTD	NT	A	Pn,Ox,Em,Sm,Gm,Cp,Eb,Fs	28; 19
10	b NT	100	RTD				
	c NT	100	RTD				
7	a NT	100	RTD	NT	A	Pn,Ox,Em,Sm,Gm,Cp,Eb	19
	b NT	100	RTD				
	c NT	100	RTD				
13	a NT	100	RTD	NT	A	Pn,Ox,Em,Sm,Gm	No plasmids
	b NT	100	RTD			Cp	detectable
	c NT	100	RTD				
12	a 95,+		RTD	n.d.	C	Pn	17
	b 616,617,622,		100				
	623,626,629		RTD				
	c 91,92,+		RTD				
14	a 29,52,80		RTD	n.d.	C	Pn,Em	21
	b NT	100	RTD				
	c NT	100	RTD				

Abbreviations: NT, non typable; RTD, routine test dilution; a, International Basic Set; b, additional phages 616, 617, 618, 620, 622, 623, 625, 626, 630; c, additional phages 88, 89, 90, 91, 92, 93; Pn, penicillin; Ox, oxacillin; Em, erythromycin; Fs, fusidic acid; Tc, oxytetracycline; Sm, streptomycin; Gm, gentamicin; Tp/Sa, trimethoprim/sulphonamide; Cp, ciprofloxacin; Eb, ethidium bromide.

n.s. = not determined.

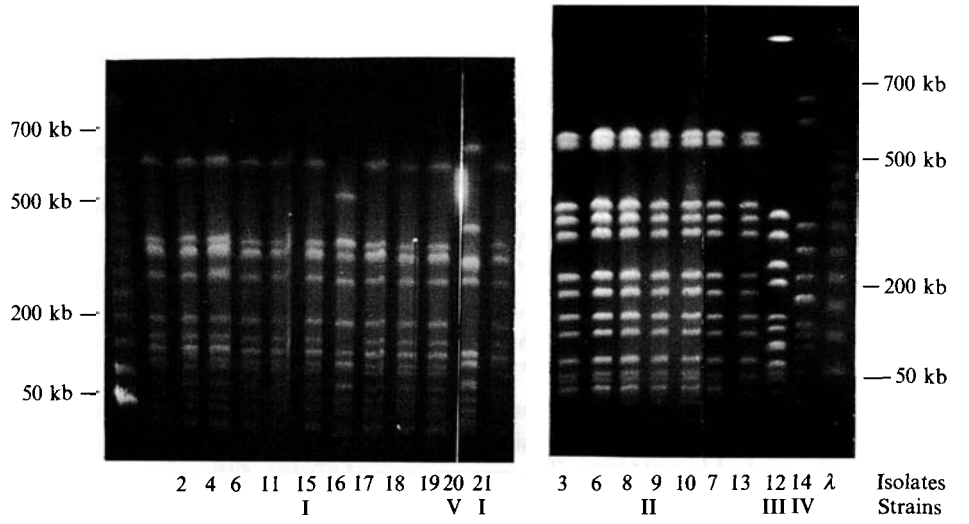
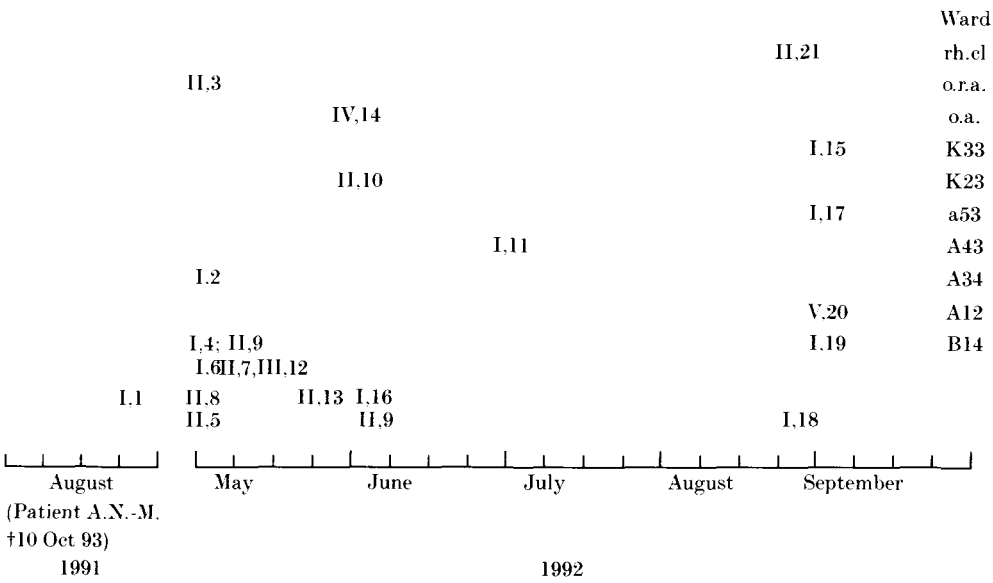


Fig. 1. Fragment pattern of genomic DNA of *S. aureus*: MRSA strains I and II with several isolates from infections in different wards and strains III, IV and V from carriers among the personnel.

Table 3. *Kind of MRSA strains, isolate no. and date of isolation on different wards at municiple hospital W. According to Table 1, strains are designated by Roman, isolates by arabic figures. o.a., ophthalmological ambulatory; o.r.a., otorhinolaryngological ambulatory; rh.cl., rheumatology clinic*



followed: one in May and another at the end of August 1992. In May and at the beginning of June a second QR-MRSA strain was isolated from infections from patients in ward B14 and also in other wards. The origin of this strain remains open.

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DISCUSSION

Although the majority of multi-resistant *S. aureus* strains from nosocomial infections are typable by phages, isolates which are only typable at 100 RTD or non-typable may occur [10]. As shown in this study, reverse phage-typing can also give a negative result if no phages are induced. Although plasmid profiles, the first DNA-based typing method in staphylococcal epidemiology [11], have been reported to be superior to phage typing of MRSA [12, 14], plasmids can be gained or lost in the course of an outbreak. This variability was observed in this study with the second QR-MRSA strain for 43 kb plasmid. The absence of these plasmids was in parallel to the lack of resistance of fusidic acid and to ethidium bromide respectively.

Typing by genomic DNA-fragment analysis after *Sma*-I digestion clearly discriminated between two strains with several clonally related isolates of each giving indistinguishable patterns. The presence or absence of 'large' plasmids was without influence on the *Sma*-I fragment patterns. DNA fragments with molecular masses of at least 20 kb can be detected under the conditions of pulsed-field electrophoresis used. The lack of influence of the presence or absence of large plasmids on the *Sma*-I restriction patterns can be only explained by a lack of recognition sites for this enzyme on these particular plasmids. Thus the data presented confirm the use of *Sma*-I patterns of genomic DNA of *S. aureus* as an epidemiological tool [15–17]. As evident from the *Sma*-I fragment of isolate 16, *S. aureus* strains can undergo rearrangements of their genomic DNA. In parallel with the absence of a fragment of 160 kb and a shortening of the largest fragment this strain also lost its resistance to trimethoprim sulphonamide, probably by excision of a trimethoprim sulphonamide-resistance transposon [18].

In the outbreak described here the source of one QR-MRSA strain could be traced to a definite patient; the source of the other strain remains unclear; nurse D.M. as the carrier cannot simply be accused of being a reservoir for infection. The dissemination of the MRSA strains among different wards can be explained by transfer of patients from the ICU to different wards. The outbreak could be stopped by application of a complex of control measures such as isolation nursing of affected patients and control of the disinfection regimes. The ICU was closed for some days and all rooms were disinfected completely. Carriers of MRSA-strains among the personnel were treated with fusidic acid and later with mupirocin nasal ointment [19]. Patients with MRSA infections were treated with vancomycin. Strains 12 and 14 from hospital personnel are not identical with the two epidemic strains. Only the combination of hygienic and therapy measurements ended the outbreak.

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