

## Food-Initiated Outbreak of Methicillin-Resistant *Staphylococcus aureus* Analyzed by Pheno- and Genotyping

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**An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) involving 27 patients and 14 health-care workers (HCW) was studied. The outbreak started in the hematology unit of the University Hospital Rotterdam, Dijkzigt, The Netherlands, and spread to the surgical unit. Twenty-one patients (77.8%) developed clinical disease, and five died. Subsequently, MRSA was detected in food and in the throat of one of the HCW who prepared food for hematology patients. Food contaminated by an HCW most likely caused the first case of MRSA septicemia. This route of transmission has not been described before. The outbreak strain was probably transmitted to the surgical unit by a colonized nurse, where it caused an explosive outbreak. Airborne MRSA transmission played an important role in disseminating the organism. The outbreak was controlled within 6 months by intensifying surveillance, temporarily closing the affected wards, treating carriers, and instituting an MRSA ward outside the hospital. Phage typing, insertion sequence probing, protein A gene typing, and DNA fingerprinting by PCR revealed that all outbreak-related isolates were identical. By pulsed-field gel electrophoresis, all but one of the outbreak-related isolates were determined to be identical. Protein A gene typing identified numerous (11) repeat units in all outbreak-related isolates, which supports the suggestion that the outbreak strain may have been more virulent and more transmissible than other MRSA strains. Pheno- and genotyping studies underlined the value of DNA fingerprinting methods for investigation of MRSA epidemiology. Optimal discriminatory power was achieved by combining the results of four genotyping methods.**

In Europe, the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals varies considerably from country to country. Generally, the incidence of MRSA increases from north to south. In a pan-European surveillance, the incidence was found to be highest in Spain, France, and Italy (30.3 to 34.4%) and lowest in Denmark, Sweden, and The Netherlands (0.1 to 1.5%) (34). Despite the low incidence in Dutch hospitals, outbreaks of MRSA occur regularly, especially in the larger teaching hospitals. Most outbreaks are due to the import of strains by patients transferred from hospitals in countries with increased MRSA incidence (33). Outbreaks in low-prevalence settings offer unique opportunities to determine the value of various typing methods, since the clonal relatedness among the MRSA isolates involved is obvious.

Scientists still debate whether one clone of MRSA has spread throughout the world (1, 17, 21). However, numerous investigations have demonstrated that the phenotype (19, 21, 30) and genotype (7, 13, 14, 17, 28) of MRSA vary. These procedures are particularly useful for comparing bacterial isolates involved in nosocomial outbreaks. Recently, it has been shown that especially the genotypic assays possess excellent discriminatory power and exhibit subtle resolution capabilities (29).

This report describes the epidemiological aspects of an MRSA outbreak which occurred in the University Hospital

Rotterdam, Dijkzigt, The Netherlands. The initial reservoir for the outbreak strain was probably a dietary worker, who carried MRSA in his throat and prepared food for patients on the hematology unit. Transmission of MRSA by contaminated food has not been described before.

### MATERIALS AND METHODS

**Isolation and processing of MRSA strains.** Between November 1992 and April 1993, an outbreak of MRSA occurred in the University Hospital Rotterdam. MRSA strains were isolated from 27 patients (Table 1), 14 health-care workers (HCW), and environmental samples. Surveillance cultures were routinely made for patient specimens from the nares, throat, perirectal area, and, if present, skin lesions (including wounds) and catheter insertion sites. For patients who had urinary catheters, urine was also cultured. Surveillance cultures from HCW consisted routinely of swabs from the nares and from skin lesions if present.

The initial cultures were inoculated on Columbia agar base supplemented with 5% sheep blood and mannitol salt agar. The plates were incubated at 37°C for 48 h and inspected after 18 to 24 h and after 42 to 48 h. All isolates were identified on the basis of colony morphology, positive catalase slide test, and positive coagulase tube test. Additionally, biochemical analysis was performed with Vitek (BioMerieux, Lyon, France). Methicillin resistance was determined by inoculation of strains on Mueller-Hinton agar (Oxoid CM 337; Brunswick, Amsterdam, The Netherlands) with a disk containing 5 µg of methicillin (Oxoid; Brunswick). Plates were incubated at 30°C for 24 h, and inhibition zones were measured (3). Isolates with inhibition zones smaller than 17 mm in diameter were considered methicillin resistant. All strains were grown as monocultures in brain heart infusion broth and stored as lyophilized powder.

A sample of 17 outbreak-related isolates, including isolates from patients, HCW, and the environment, were evaluated by several typing methods (outbreak group; see Table 2). Thirteen epidemiologically unrelated strains were included in the typing studies as comparison strains: five isolates were obtained in the same hospital from patients transferred from foreign hospitals (control group 1), and eight isolates with the same phageovar as the outbreak-related isolates were obtained from the Dutch National Institute for Public Health and Environmental Protection, Bilthoven, The Netherlands (control group 2).

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TABLE 1. Clinical, geographical, and bacteriological data for patients colonized or infected with MRSA

Patient no.	Ward	MRSA isolation date (mo/day/yr)	Wk of outbreak	Clinical diagnosis or surgery	Presence of infection <sup>a</sup>	Site of infection	Prosthetic vascular graft	Amputation	Outcome <sup>b</sup>
1	Hematology	11/12/92	1	Acute myeloid leukemia	Y	Blood	N	N	Death
2	Vascular surgery	11/22/92	3	Atherosclerotic vascular disease	Y	Wound	Y	N	Death
3	Vascular surgery	11/24/92	4	Atherosclerotic vascular disease	Y	Wound	Y	Y	Survival
4	Vascular surgery	11/26/92	4	Atherosclerotic vascular disease	Y	Wound	N	N	Survival
5	Vascular surgery	11/26/92	4	Atherosclerotic vascular disease	Y	Wound	N	Y	Survival
6	Vascular surgery	11/26/92	4	Atherosclerotic vascular disease	Y	Wound	N	Y	Survival
7	Vascular surgery	11/26/92	4	Atherosclerotic vascular disease	Y	Wound	Y	Y	Survival
8	Vascular surgery	11/26/92	4	Aneurysm of aorta	N		Y	N	Survival
9	Vascular surgery	11/28/92	4	Atherosclerotic vascular disease	Y	Wound	Y	Y	Survival
10	Vascular surgery	12/01/92	5	Atherosclerotic vascular disease	Y	Wound	Y	N	Survival
11	Vascular surgery	12/03/92	5	Aneurysm of aorta	N		Y	N	Survival
12	Surgical ICU	12/03/92	5	Peritonitis	Y	Wound	N	N	Survival
13	General surgery	12/03/92	5	Osteomyelitis	Y	Wound	N	N	Survival
14	Vascular surgery	12/07/92	6	Atherosclerotic vascular disease	Y	Wound	N	Y	Survival
15	Vascular surgery	12/07/92	6	Aneurysm of aorta	Y	Wound	Y	N	Survival
16	Vascular surgery	12/07/92	6	Atherosclerotic vascular disease	Y	Wound	Y	Y	Survival
17	Surgical ICU	12/21/92	8	Colon surgery for obstruction	Y	Wound	N	N	Death
18	Surgical ICU	03/01/93	18	Liver transplantation	Y	Wound	N	N	Death
19	General surgery	03/03/93	18	Esophageal cancer	Y	Wound	N	N	Survival
20	Vascular surgery	03/03/93	18	Aneurysm of aorta	Y	Wound	Y	N	Survival
21	General surgery	03/06/93	18	Colon cancer	Y	Wound	N	N	Survival
22	General surgery	03/10/93	19	Breast cancer	Y	Wound	N	N	Survival
23	Vascular surgery	03/15/93	19	Atherosclerotic vascular disease	Y	Wound	Y	N	Survival
24	General surgery	03/17/93	20	Colonic diverticulosis	Y	Wound	N	N	Survival
25	Vascular surgery	03/22/93	20	Atherosclerotic vascular disease	Y	Wound	Y	Y	Death
26	General surgery	03/30/93	22	Colon cancer	Y	Wound	N	N	Survival
27	General surgery	03/31/93	22	Gastric cancer	N		N	N	Survival

<sup>a</sup> Y, yes; N, no.<sup>b</sup> Defined as mortality 1 year after MRSA was first isolated. In patients 1, 2, and 18, mortality was directly related to infection with MRSA.

**Antimicrobial susceptibility testing.** In addition to susceptibility to methicillin, the susceptibilities of the strains to ciprofloxacin, erythromycin, oxacillin, rifampin, tetracycline, phosphomycin, tobramycin, co-trimoxazole, clindamycin, vancomycin, and fusidic acid were determined. All assays were performed by the method of Bauer et al. (3), using breakpoints according to National Committee for Clinical Laboratory Standards guidelines (22). Resistance was classified as 0, intermediate susceptibility was classified as 1, and full susceptibility was classified as 2.

**Phage typing.** Phage typing was performed at the Dutch National Institute of Public Health and Environmental Protection by application of the international set of typing phages and a set of typical Dutch phages (18, 23). Different phage patterns were given a type designation.

**PCR-mediated DNA fingerprinting (arbitrary primed PCR [AP-PCR]).** AP-PCR was performed essentially as described previously (30). DNA was isolated from overnight cultures by lysostaphin treatment, guanidine isothiocyanate lysis (5), and subsequent DNA affinity chromatography. Approximately 5 ng of DNA was included per PCR mixture. The PCR mixture consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.01% gelatin, and 0.1% Triton X-100. Deoxyribonucleotide triphosphates were present at 0.2 mM in the reaction mixture, and 0.5 U of *Taq* DNA polymerase (Sphaero Q, Leiden, The Netherlands) was included. Five different primers were included in the typing assays. The designations and sequences of the primers were as follows: 186, 5'-GGTTGGGTGAGAATTGACG-3'; 188, 5'-AAGAGCCCGT-3'; 192, 5'-GTGGATGCGA-3'; ERIC1, 5'-ATGTAAGCTCCTGGGGATTAC-3'; and ERIC2, 5'-AAGTAAGTACTGGGGTGACG-3'. All primers were applied in separate assays (50 pmol of primer per PCR mixture). The amplification was performed in a model 60 thermocycler (Biomed, Thores, Germany) by a program consisting of 40 repeated cycles of 1 min at 94°C, 1 min at 25°C, and 2 min at 74°C. Prior to cycling, the mixtures were denatured at 94°C for 4 min, and postcycling, the mixtures were incubated at 74°C for an additional 10 min. PCR samples were analyzed by agarose gel electrophoresis. The gels, containing 2% agarose in 40 mM Tris-borate (pH 7.8)-2 mM EDTA (0.5× TBE), were run in the presence of ethidium bromide. After photography (Polaroid high-speed sheet film 57), DNA fingerprints were compared by visual inspection.

The results for each primer were indexed by numbering, thereby defining the number of different DNA fingerprints (i.e., amplicon banding patterns) that could be distinguished by single assays. One- and two-band differences between two DNA fingerprints derived from different strains were classified as subclonal variation, indicated by adding a prime to the index character (see Table 2). The overall PCR type was defined by a combination of the results obtained with the

five individual primers. If subclonal variations were detected with individual primers, this was indicated by subnumbering of the PCR type.

**PFGE.** Pulsed-field gel electrophoresis (PFGE) was performed at the University of Iowa, Iowa City. Contour-clamped homogeneous electric field (CHEF) analysis was performed with the CHEF DR-II (Bio-Rad Laboratories, Richmond, Calif.). Bacteria were embedded in low-melting-temperature agarose (FMC Corp., Philadelphia, Pa.) and treated with lysostaphin and proteinase K-sodium dodecyl sulfate (SDS) according to established protocols (24, 28, 29). DNA was digested *in situ* by *Sma*I (New England Biolabs, Beverly, Mass.) according to the manufacturer's instructions. Agarose gels (1%) were run in 0.5× TBE at 13°C and at 6 V/cm. Switching times were ramped from 10 to 90 s for a total run time of 24 h for optimal separation of DNA fragments between 50 and 500 kb in size. Strain differences were based on the detection of restriction fragment length polymorphism (RFLP). One- and two-band differences were designated subclonal variations, indicated by subnumbering; differences of three or more bands were interpreted as indicating different strains. Comparisons and calculation of the percent homology were performed manually. Dendrograms were constructed by the unweighted pair group method using arithmetic averages (UPGMA) subroutine of the PHYLIP software (9, 11).

**Detection of protein A gene polymorphisms by PCR.** Length polymorphisms in the staphylococcal protein A gene were determined by PCR essentially as described elsewhere (12). In short, the repetitive region within the protein A gene was amplified by using oligonucleotide primers with the following DNA sequences: 5'-TGTAACACGACGCGCAGTGCTAAAAAGCTAAACGATG C-3' and 5'-CAGGAAACAGCTATGACCCACCAATACAGTTGTACC-3'. After PCR, DNA was cleaved with the restriction enzyme *Rsa*I (Boehringer GmbH, Mannheim, Germany) and RFLPs were determined by electrophoresis in 2% agarose gels run in 0.5× TBE. The number of repetitive units present in the genes' variable region was estimated by comparisons with molecular weight markers (100-bp marker; Pharmacia, Gouda, The Netherlands).

**Insertion sequence probing.** Insertion sequence probing was performed at the Dutch National Institute of Public Health and Environmental Protection. For RFLP analysis, extraction of chromosomal DNA and Southern blotting were performed as described previously (15). Genomic DNA was extracted from 1.5 ml of overnight culture by using lysostaphin, SDS, and proteinase K. DNA was purified further by extraction with phenol-chloroform and ethanol precipitation. Finally, the DNA was dissolved in 100 µl of TE (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). *Cla*I (Boehringer) was used to digest DNA according to the manufacturer's instructions (4). The digested DNA was analyzed by electrophoresis in a 1% agarose gel at 25 V overnight in TBE and stained in ethidium bromide (0.5

µg/ml). Southern blots were prepared on Hybond N<sup>+</sup> membranes (Amersham International, Amersham, United Kingdom) by using a vacuum miniblott system (Millipore Corp., Bedford, Mass.) and stored at 4°C until use. For hybridization, a probe based on the IS431 sequence, an insertion-like element which is found frequently among staphylococci (2), was used. The probe, designed to amplify an 800-bp sequence from IS431, was produced by PCR using two primers: primer 1, 5'-TACATCATGTTAATAAGGG-3'; and primer 2, 5'-TTGCGTGAGTGTG GCGAAGC-3'. The target for amplification was 5 µl of *S. aureus* suspension which was also used for digestion as described above. Amplification was performed as described previously (25) for 35 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C using a thermocycler (Perkin-Elmer). After amplification, the PCR products were divided into aliquots of 10 µl and purified by agarose gel electrophoresis using low-melting-temperature agarose (preparative grade; Bio-Rad) (26). After the DNA fragments were separated, agarose blocks containing DNA with the expected molecular weight were cut from the gel and directly used for labelling or stored at -20°C until they were processed. Nonradioactive labelling of probe DNA with horseradish peroxidase was performed directly in the agarose blocks by using the enhanced chemiluminescence gene detection system (Amersham International) according to the manufacturer's instructions. One agarose block containing labelled DNA was used for hybridization of one blot. Southern blot hybridization was performed and recorded as described previously (15).

## RESULTS

**Epidemiological surveillance.** The first patient involved in the MRSA outbreak was identified in the hematology unit, where she was treated for acute myeloid leukemia. Because of neutropenia (granulocyte count, <500 × 10<sup>9</sup>/liter), she was nursed in a private room with laminar-airflow high-efficiency particulate air filtration, as were all other neutropenic patients on this unit. The patient was given oral ciprofloxacin prophylactically, as was the unit's routine. Subsequently, she developed sepsis with MRSA of which she died within 3 days, despite immediate treatment with vancomycin. The surveillance cultures, which were obtained twice weekly, were negative until the patient developed the bacteremia. Also, two sets of surveillance cultures which were obtained for all other patients and the HCW and other contacts on the hematology unit were all negative for MRSA. No environmental source was identified.

Ten days after the first patient was identified, additional patients who carried MRSA were identified on the surgical unit, which was located far from the hematology unit. A nurse on the surgical unit was colonized by an MRSA strain (strain 6; Table 2) that was identical to the outbreak strain. This nurse probably transmitted the outbreak strain of MRSA to the surgical unit, as she had been transferred from the hematology unit to surgery shortly after the first patient was identified. After MRSA was detected on the surgical unit, surveillance cultures were done repeatedly for all patients and HCW (nurses, physicians, and paramedical employees) on this unit. A total of 26 patients and 13 HCW (including the nurse who came from the hematology unit) carried MRSA. Of the 26 surgical patients colonized with MRSA, 20 (77%) developed a clinically proven MRSA infection. Four of these individuals died (25%); two of these deaths were directly related to the MRSA infection. The clinical diagnoses and geographical and bacteriological data for the 27 MRSA-positive patients are detailed in Table 1.

Figure 1 shows the incidence of MRSA-positive patients during the outbreak period. The first episode of the outbreak involved primarily patients who had had vascular surgery who had necrotic wounds or had prosthetic vascular grafts that had been implanted recently. Most of these patients developed clinically significant wound infections.

To control this outbreak, the vascular surgery ward was closed and the patients who were colonized with MRSA were transferred to an MRSA cohort isolation facility which was created outside the hospital in week 10 after the outbreak

began. A separate team of nurses and doctors took care of these patients. The surgical ward, including the floors, walls, ceilings, and furniture, was cleaned completely and disinfected with 4-chlor-2-benzylphenol (2% [wt/vol]) and *ortho*-phenylphenol (2% [wt/vol]). HCW who were colonized with MRSA were treated with nasal mupirocin ointment (Bactroban; SmithKline Beecham) and sent home until MRSA carriage was eradicated. As can be seen in Fig. 1, the first episode was rapidly controlled. In week 13, only a single patient remained in the isolation facility. It was then decided to close this facility and treat the last patient in a regular hospital department, in strict isolation (private room with negative air pressure and gloves, gowns, and masks required for everyone who entered the room, and the patient confined to his room as much as possible). Despite strict isolation, this particular patient served as a source for the second episode of the outbreak. This episode involved fewer patients and HCW but more surgical wards. Most patients probably acquired MRSA in the surgical intensive care unit (ICU), where they were treated for a few days postoperation. However, MRSA carriage was not detected until after the patients were transferred to other surgical wards. The second episode of the outbreak was controlled by intensifying bacteriological surveillance of patients and HCW and by reinstating the isolation facility.

Twenty-two weeks after the outbreak began, routine bacterial cultures of food prepared for neutropenic patients in the hematology unit revealed MRSA in a piece of banana, which had been peeled by a dietary worker who worked in this unit. Therefore, all personnel who prepared food for patients on the hematology unit were screened for MRSA carriage. MRSA was cultured repeatedly from throat swabs but not from the nares or the perirectal area of the dietary worker. This HCW was working on the hematology unit at the time the first patient was detected but never contacted directly patients or HCW with MRSA. The MRSA strains cultured from the hematology patient, the piece of banana, and the dietary worker were identical (Table 2, strains 1a/b, 2, and 3, respectively). The dietary worker was treated successfully by application of mupirocin nasal ointment twice a day and daily washing of body and hair with disinfecting soap for 5 consecutive days.

**Antibiotic sensitivity of MRSA.** All outbreak strains that were evaluated had similar antibiograms (Table 2). Except for isolate 17, all outbreak strains (1 through 17) were resistant to methicillin. Also, all isolates were resistant to tobramycin, erythromycin, ciprofloxacin, rifampin, clindamycin, and tetracycline. All were susceptible to vancomycin and co-trimoxazole. More variability was found regarding susceptibility to phosphomycin and fusidic acid. Isolate 17 was sensitive to methicillin as determined by the disk diffusion assay. This isolate was obtained from a patient who also carried methicillin-resistant isolates. The antibiograms of the isolates in control group 1 (Table 2, isolates 18 to 22) were more heterogeneous. The antibiograms of the isolates in control group 2 (Table 2, isolates 23 to 30) were generally similar to the outbreak strains.

**Phage typing.** All outbreak-related isolates, including the methicillin-susceptible isolate 17, were phage type III29. None of the isolates in control group 2 were of this phage type.

**AP-PCR and PFGE.** AP-PCR, based on a combination of several primer-dependent genocodes, revealed genetic homogeneity in the group of outbreak-related isolates, all of which were type A (Table 2 and Fig. 2). Three subtypes (A1 to A3) were identified within the outbreak-related isolates. As determined by PFGE analysis, all strains but one (isolate 17) were type A, although subclonal variation was observed (Table 2 and Fig. 3). All strains identified as PFGE type A had a similarity index of more than 90%, as determined by UPGMA

TABLE 2. Comparison of results from phenotypic and genotypic typing methods

Group and isolation date (mo/yr)	Site or source <sup>a</sup>	Ward	Isolate no. <sup>b</sup>	Antibiotic susceptibility <sup>c</sup>	Phage type	Protein A type <sup>d</sup>	IS431 type	Type determined by PCR primer species <sup>e</sup> :					AP-PCR type <sup>f</sup>	PFGE type <sup>f</sup>	Combined PFGE-AP-PCR type <sup>g</sup>	Combined PFGE-AP-PCR-protein A-IS431 type <sup>g</sup>
								A	B	C	D	E				
<b>Outbreak group</b>																
11/92	Blood	Hematology	1a/b	20020002020	III29	11	1	1'	1	1'	1	1	A3	A1/A1	1'	1'
03/93	Banana	Hematology	2	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
03/93	HCW	Hematology	3	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
11/92	ASP	Vascular surgery	4	00020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
11/92	Nose	Vascular surgery	5a/b	00020002000	III29	11	1	1'	1	1'	1	1	A3	A1/A1	1'	1'
11/92	HCW	Vascular surgery	6	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
11/92	HCW	Vascular surgery	7	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
03/93	Wound	Vascular surgery	8	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
12/92	HCW	Surgical ICU	9	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
12/92	Wound	Surgical ICU	10	20020002020	III29	11	1	1	1	1	1	1	A1	A3	1''	1''
12/92	Wound	Surgical ICU	11	00020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
01/93	IVC	Surgical ICU	12	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
01/93	Nose	Surgical ICU	13	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
03/93	Bile	Surgical ICU	14	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
12/92	Blood	General surgery	15	00020002000	III29	11	1	1'	1	1'	1	1	A3	A1	1'	1'
01/93	Wound	General surgery	16	20020002020	III29	11	1	1	1	1	1	1	A1	A2	1	1
04/93	Wound	General surgery	17	02020002020	III29	11	1	1	1'	1	1	1	A2	C	2	2
<b>Control group 1</b>																
08/92	Nose	Surgery	18	20020002020	III95	9	3	1'	2	1	1	2	B	D	3	3
12/92	Urine	Plastic surgery	19	10022022220	E1	7	2	2	3	2	1	3	C	E1	4	4
05/93	Wound	Dermatology	20	20002022200	Z74	9	2	1'	4	3	1	1	D	F	5	5
11/86	Sputum	Cardiac surgery	21a/b	20022022220	E1	7	5	2	3	2	1	3	C	E1/E2	4/4'	6/6'
12/86	Feces	Internal medicine	22	20022002220	E1	7	5	2	3	2	1	3	C	E2	4'	6
<b>Control group 2<sup>h</sup></b>																
05/92	NA		23	20020002220	III29	10	1	3	1	1'	1	1	E	B2	6	7
01/92	NA		24	20020002021	III29	11	1	4	1	4	1	1	F	A1	7	8
02/91	NA		25	20020002020	III29	6	6	5	5	5	1	4	G	A1	8	9
04/91	NA		26	20020002020	III29	10	6	6	6	6	2	5	H	A1	9	10
06/91	NA		27	20020002020	III29	ND <sup>i</sup>	6	3	1	1'	1	1	E	A1	10	11
06/91	NA		28	20020002220	III29	11	1	3	1'	1'	1	1	E'	G	11	12
08/91	NA		29	20020002020	III29	11	ND	3	1	1'	1	1	E	A4	10	11
11/91	NA		30	20020002220	III29	11	4	3	1	1'	1	1	E	B1	6	13

<sup>a</sup> ASP, air-settling plate; IVC, intravascular catheter; NA, not available.

<sup>b</sup> a/b, strain typed in duplicate by PFGE.

<sup>c</sup> The values shown are for, from left to right, phosphomycin, oxacillin, tobramycin, co-trimoxazole, ciprofloxacin, erythromycin, clindamycin, vancomycin, rifampin, fusidic acid, and tetracycline. In the antibiogram, 0 indicates resistance, 2 indicates susceptibility, and 1 indicates intermediate susceptibility.

<sup>d</sup> Number of repetitive units present in the protein A gene.

<sup>e</sup> A, primer 186; B, primer 188; C, primer 192; D, primer ERIC1; E, primer ERIC2. Primes indicate one- or two-band differences.

<sup>f</sup> Subnumbering indicates subclonal relationships. Prime indicates one- or two-band differences.

<sup>g</sup> Primes indicate subclonal variation.

<sup>h</sup> Control group 2 strains were derived from a collection of MRSA strains from various Dutch hospitals in the Dutch National Institute of Public Health and Environmental Protection.

<sup>i</sup> ND, not determined.

analysis. Strain 17 was discriminated from the other outbreak-related isolates (similarity index of approximately 80%). Both AP-PCR and PFGE distinguished all control group 1 isolates from the outbreak-related isolates. Also, there was full agreement between PFGE and AP-PCR within this group of strains. Both AP-PCR and PFGE detected genetic heterogeneity among control group 2 strains. AP-PCR discriminated four types and PFGE detected three types, when subclonal variation is disregarded. However, some results of AP-PCR and PFGE typing were discordant. For instance, AP-PCR did not discriminate among isolates 23 and 27 to 30, whereas PFGE identified three different types (A, B, and G) within this group. On the other hand, PFGE did not discriminate among isolates 24 to 27 and 29 (PFGE type A), but AP-PCR identified four different types (E through H).

AP-PCR discriminated eight types and PFGE discriminated

seven types in the total group of 30 strains. The combined results of AP-PCR and PFGE differentiated 11 types.

**PCR-mediated protein A typing.** Protein A gene typing revealed 11 repeats in all outbreak-related isolates (Table 2 and Fig. 4). In contrast, only seven or nine repeats were identified in isolates from control group 1. Four of eight isolates from control group 2 also contained 11 repeats.

**IS431 probing.** Table 2 shows the results of IS431 typing. All outbreak-related isolates and three of eight isolates from control group 2 were IS431 type 1, whereas none of the isolates in control group 1 were of this type.

**Combination of genotypic methods.** Together, the genotyping methods (AP-PCR, PFGE, protein A, and IS431) discriminated 13 different types among all isolates tested. All outbreak strains but one (the methicillin-susceptible isolate 17) were similar when subclonal variation was disregarded. Eleven dif-

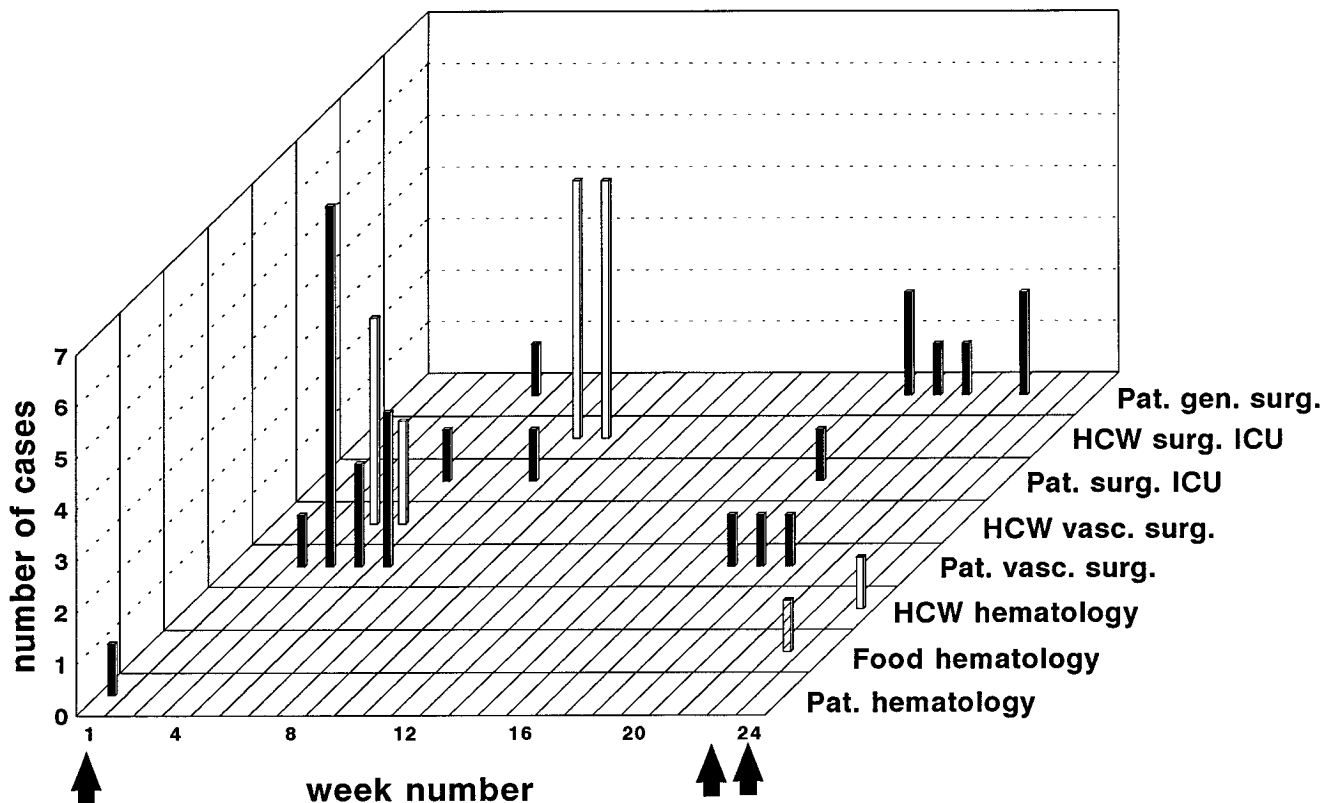


FIG. 1. Incidence of MRSA in patients (Pat.), HCW, and food during the outbreak in the hematology, vascular surgery (vasc. surg.), and general surgery (gen. surg.) units and the surgical ICU (surg. ICU). The number of new cases per week (bars) is shown. The three strains isolated from the dietary worker, from food, and from the patient in the hematology unit (arrows) are indicated. Patients are indicated by solid bars, HCW by open bars, and food by hatched bars.

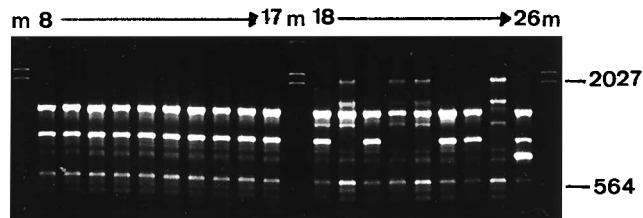
ferent strains were identified among the 13 isolates from control groups 1 and 2. The two control group 1 strains that could not be distinguished from each other (21 and 22) were isolated from patients who, within a 3-week period, were transferred to our hospital from the same foreign hospital.

DISCUSSION

Despite the low incidence (<5%) of MRSA in Dutch hospitals, this organism regularly causes outbreaks (33). In order to prevent outbreaks and decrease the endemic incidence of MRSA, we need to define the mechanisms by which this multiresistant microorganism is transmitted. Numerous investigators have used traditional epidemiological and molecular typing methods to identify risk factors for acquisition of MRSA,

the reservoirs occupied by MRSA, and the means by which it is transmitted, yet we have much to learn about the epidemiology of this important pathogen.

We used several pheno- and genotyping methods to investigate an unusual explosive outbreak of MRSA in the Univer-



ERIC 2

FIG. 2. Representative examples of the results of PCR-mediated DNA fingerprinting using primer ERIC2. Isolate numbers (in accordance with Table 2) are indicated above the lanes, and molecular weight markers (m) (lambda DNA cut with *HindIII*) are indicated on the right (in base pairs).

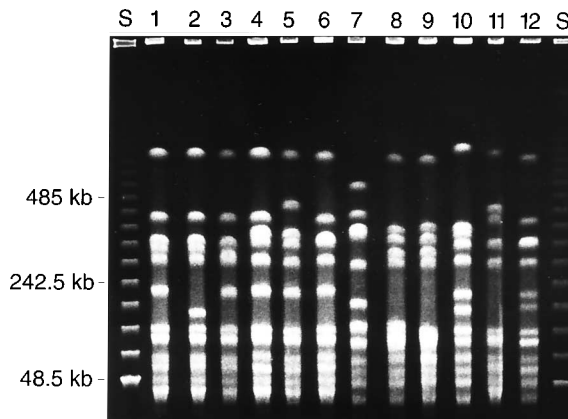


FIG. 3. PFGE-mediated DNA typing of MRSA strains. DNA was digested with restriction enzyme *SmaI*. Lanes S, concatemeric forms of lambda DNA, differing in size from 48.5 to 485 kb, as indicated on the left; lanes 1 to 12, DNA from the following strains and PFGE types: strain 4, type A2 (lane 1); strain 17, type C (lane 2); strain 10, type A3 (lane 3); strain 5a, type A1 (lane 4); strain 23, type B2 (lane 5); strain 29, type A4 (lane 6); strain 18, type D (lane 7); strain 19, type E1 (lane 8); strain 21, type E2 (lane 9); strain 20, type F (lane 10); strain 28, type G (lane 11); and strain 30, type B1 (lane 12).

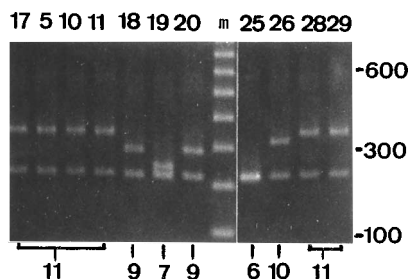


FIG. 4. Typing of protein A gene polymorphisms by PCR and *RsaI* RFLP analysis. Shown are representative examples of all types that were encountered during the analysis. Isolate numbers are displayed above the lanes. Lane m, molecular weight markers (Pharmacia 100-bp ladder). On the right, the lengths of some of the markers are indicated (in base pairs). Below the lanes, the length of the variable fragment is indicated (in numbers of repeat units).

sity Hospital Rotterdam. The likely source of this outbreak was a dietary worker who prepared food for patients on the hematology unit. We identified this individual, who had no direct contact with patients, because we found the epidemic strain in a routine surveillance culture of a banana which the dietary worker had peeled. A subsequent culture of the dietary worker's nares revealed that he carried the epidemic strain. In contrast, the index patient's surveillance cultures were negative for 3 weeks before she developed MRSA sepsis. To our knowledge, this is the first time investigators have implicated contaminated food in the transmission of MRSA. In general, ingestion of MRSA should not lead to subsequent infection because gastric acid and the normal gastrointestinal flora should prevent this organism from colonizing the gastrointestinal tract. Moreover, the immune system usually eliminates transient invading microorganisms. The index patient, however, was severely immunocompromised, and she had taken both antacids, which would neutralize the gastric acid, and oral ciprofloxacin, which may have selected the ciprofloxacin-resistant outbreak strain. We postulate that the index patient ingested food contaminated by the outbreak strain, which then colonized the patient's gastrointestinal tract. Subsequently, while the patient was neutropenic, the outbreak strain translocated through the gastrointestinal mucosa into the bloodstream, causing the patient's fatal infection.

A nurse who initially worked on the hematology unit probably transmitted the outbreak strain between wards when she was transferred to the vascular surgery unit. In the latter unit, the outbreak strain caused an explosive MRSA epidemic. By using stringent infection control measures, we terminated the outbreak within 6 months, and we eliminated the outbreak strain from our hospital. Although many infection control experts consider instituting an isolation facility outside the hospital an extreme measure, we feel that in this particular outbreak it was better than other systems of isolation. First, because the outbreak strain was airborne and hence contaminated the environment extensively, we think the isolation unit enabled us to control this outbreak more quickly than we could have if the patients had been cared for within the hospital. Second, because all patients in the isolation facility had MRSA, the patients were not kept in strict isolation. The patients were able to move about the ward as they desired, which may have enhanced their recovery. It should be remembered that in The Netherlands there is a national guideline for the control of MRSA, aimed at total elimination of MRSA and not confinement to a low endemic level.

The ratio of MRSA-infected to MRSA-colonized patients in this outbreak (78%) was much higher than that observed in

previous outbreaks (up to 40%) (20). The increased frequency of infection and the high mortality rate (5 of 27; 18.5%) may have been related in part to the severity and nature of the patients' underlying diseases. The index patient was severely immunocompromised, and the patients on the vascular surgery unit had large, poorly healing wounds and many had prosthetic vascular grafts. In addition, we think the outbreak strain might have been more virulent than other MRSA strains. For methicillin-susceptible *S. aureus*, it has been shown that there are differences in virulence between strains (36). Because protein A modulates opsonization and chemotaxis (35), the outbreak strain, which had more copies of the protein A gene than did many control isolates, might have evaded host defenses more readily than other strains.

We routinely evaluate the ability of all patients with MRSA to disperse the organism into the air and hence to contaminate the environment. Of the 20 patients with MRSA who were transferred to our hospital from foreign hospitals before the outbreak, none dispersed MRSA to the extent that we observed in this epidemic. Air-settling plates for 16 of those 20 patients were negative and for 4 patients contained <5 CFU per plate. In contrast, air-settling plates in the rooms of several patients from this outbreak contained >100 CFU. Furthermore, despite the patients being kept in strict isolation, air-settling plates in the corridors outside patients' rooms were also positive for the outbreak strain. We think that the positive air pressure in the patients' rooms carried MRSA from the contaminated rooms into the hallway. Although staff who carried the outbreak strain may have contaminated the settling plates in the corridors, we do not think that this was the likely mechanism. We made cultures from specimens from the staff twice weekly to identify those who acquired MRSA, and we tested all positive staff members (16) to determine whether they dispersed the organism. We did not identify any staff members who dispersed MRSA during their tests.

Particular characteristics of the affected patients and the outbreak strain may have facilitated the profuse environmental contamination noted in this outbreak. Boyce et al. previously reported that patients who had MRSA in a wound or in their urine contaminated the environment more frequently than did patients with MRSA in the nares or sputum (34 versus 2% of the surfaces cultured were positive, respectively) (6). Therefore, our patients who had large, poorly healing wounds may have been more likely to disperse MRSA.

Other investigators have observed differences in the transmissibility of *S. aureus* strains (8, 10). Recently, Frenay et al. proposed that the number of repetitive elements in the spacer region of the staphylococcal protein A gene correlated with the ability of MRSA strains to cause outbreaks (12). They argued that higher numbers of repeats enhanced the accessibility of the immunoglobulin G binding region of protein A and thereby contributed to epidemic spread of the organism. Our outbreak strain had 11 copies of the protein A gene, which is within the epidemic range (more than 7 copies) defined by Frenay et al. (12).

In summary, we hypothesize that patients who had wounds that were colonized or infected with the outbreak strain dispersed MRSA into the air. The airborne organisms subsequently contaminated many surfaces in the patients' rooms and corridors. HCW who touched these contaminated surfaces and who did not subsequently wash their hands transmitted the epidemic strain to other patients. As in the study of Boyce et al. (6), HCW may also have contaminated their clothing and transmitted the outbreak strain in this manner.

Because the incidence of MRSA in The Netherlands is extremely low, isolates obtained during outbreaks are likely to be

clonally related. Therefore, we evaluated isolates from our outbreak by several molecular typing methods, and we compared the results of the different methods. AP-PCR and PFGE most clearly discriminated outbreak-associated isolates from control isolates. In our study, PFGE identified more subclones than did PCR. Saulnier et al. noted similar results (27), but further analysis determined that the discriminatory abilities of PFGE and PCR depended, respectively, on the number of restriction enzymes and the number of primers used (31). Furthermore, as demonstrated in Table 2, some PCR primers detected more variability than did others; four primers identified five or more types, whereas primer ERIC1 identified only two types. To date, no one has determined which primers and how many primers should be used to evaluate MRSA isolates.

At present, we do not know why MRSA isolates obtained during the course of an outbreak had minor genomic differences. However, Van Belkum et al. noted similar variability among *Legionella pneumophila* isolates (32). Perhaps this variability reflects the intrinsic genetic flexibility of the microbes or their ability to adapt to a host.

Both PFGE and PCR were more discriminatory than protein A typing or IS431 typing. We identified the most strain variability when we combined the results of these four genotyping methods. Using the combined results, we considered all but one of the outbreak-related isolates (isolate 17) to be the same strain. We did not identify the outbreak strain among the control isolates.

Only PFGE and methicillin susceptibility discriminated isolate 17 from the other outbreak isolates. Isolate 17 possessed the *mecA* gene (documented by PCR) but did not express methicillin resistance. At present, we do not know the mechanism by which this isolate remains susceptible to methicillin.

None of the typing methods distinguished between two isolates in control group 1 (isolates 21 and 22). We subsequently learned that these isolates were obtained from two patients who, within a short period, were transferred to our hospital from the same foreign hospital. Hence, the patients may have acquired the same strain while they were in the other hospital.

In summary, our MRSA outbreak displayed several unusual features, including transmission of the outbreak strain by food and by air and a very high infection-to-colonization ratio. We think the patients' wounds and particular characteristics of the outbreak MRSA strain allowed that strain to spread explosively on our vascular surgery unit. Furthermore, we think the isolation unit helped us terminate the outbreak expeditiously. Finally, PFGE and AP-PCR discriminated the outbreak-related isolates from control isolates, and protein A typing suggested that the outbreak strain might be more transmissible and virulent than other MRSA isolates.

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