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Outbreak of Group A Streptococci in a Burn Center: Use of Pheno- and Genotypic Procedures for Strain Tracking

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In a burn center, an outbreak of group A streptococci (GAS) colonizations involving 13 patients and two staff members occurred. Adverse events due to GAS, loss of skin graft after initial take (secondary loss) and partial take, occurred in patients who underwent surgery before the colonization was detected. GAS isolates from nine patients and one staff member were stored and subsequently pheno- and genotyped by T serotyping, gas chromatography, M genotyping, and random amplified polymorphic DNA typing. The outbreak was caused by two types of GAS, identified as T4/28-M48 and T13-M77 by T serotyping and M genotyping. Random amplified polymorphic DNA typing and gas chromatography distinguished both clusters accurately. One subcluster indicated by gas chromatography could be linked to the acquisition of GAS from a roommate. The T13-M77 cluster was characterized by rapid spread through the center compared with the T4/28-M48 cluster. One patient contracted the T13-M77 strain while in protective isolation, indicating a role for staff members in the transmission. Our standard GAS control policy, consisting of twice weekly screening of all burned patients and immediate isolation and treatment, proved efficacious in preventing further spread of GAS. Reporting by staff members of signs and symptoms compatible with GAS infection was reenforced.

Group A streptococci (GAS) have been recognized as major contributors to morbidity and mortality in burned patients for more than 50 years (3). Extracellular products of GAS are thought to cause loss of skin grafts, although there is no certainty of which factor(s) is responsible. Methods to distinguish different strains of GAS are indispensable for elucidating outbreaks (8). New methods, including random amplified polymorphic DNA (RAPD) analysis (19) and M genotyping (6), have recently been introduced and compared with the standard T and M serotyping of GAS (13, 14). In this report we describe an outbreak in the Rotterdam burn center and the typing methods used to investigate it. The outbreak, involving 13 patients and two staff members, occurred in the period from December 1993 to January 1994.

MATERIALS AND METHODS

Burn center. The Rotterdam burn center was moved to a new building in 1987. This facility, separate from the rest of the hospital, has 20 beds, a resuscitation room, a dressing room with an immersion bath, an operating theatre, a family room, and a playroom for children. The center has 5 double and 10 single rooms, the latter equipped with mass airflow facilities. The single rooms can be used for intensive care treatment. All rooms have negative-pressure air locks, with facilities for hand washing.

The center handles approximately 180 admissions per year, of which 130 are admitted within 48 h of the burn accident (primary admissions) and the remainder are admitted more than 48 h after the injury (secondary admissions). Elective corrective surgery late after burn injuries is also performed in the center. Patients with extensive burns are assigned to single rooms. Whenever these patients have their wound dressings changed, the medical and nursing staffs don disposable gowns, caps, and masks in the air lock, prior to entering the room. On leaving, the gowns, caps, and masks are disposed of in the air lock. The patients can, thus, be considered to be in protective isolation. Mobile patients are allowed to move throughout the unit. While in the center, all patients are cultured (throat, nose, perianal region, and all burn sites), twice weekly while in intensive care and once weekly thereafter, with the first cultures taken within 48 h after admission. Personnel and visitors are not routinely screened. For the years 1982 to 1991, 0.9 to 4.2% per year of both primary and secondary admissions had detectable GAS in their wounds or elsewhere at the time of admission. During the same time period, the percentage of acquisitions of GAS by patients while in the center varied from 0 to 3.5% per year. Any patient with GAS is isolated as soon as the culture results become available. The GAS control policy distinguishes three categories of isolated patients: (category 1) patients having GAS in a wound, (category 2) patients having GAS in the throat, and (category 3) patients who have shared a room with a patient with GAS. Patients in categories 1 and 2 are treated with oral amoxicillin and clavulanate (500 and 125 mg, respectively, three times a day for 7 days). This combination is given to overcome β-lactamases produced by other microorganisms. Isolation is lifted for patients in category 1 if subsequent wound cultures are negative for GAS on two consecutive occasions, for patients in category 2 after 48 h of treatment, and for patients in category 3 when surveillance cultures, both throat and wounds, are negative for GAS.

Patients and health-care workers. All patients initially identified with GAS in one or more cultures during the outbreak of December 1993 to January 1994 were included in the study. On 7 January 1994, staff members were encouraged to report sore throats. Fifteen of the 50 staff members did so and were cultured for GAS.

Microbiological methods. In order to detect GAS, swabs were plated onto 5% sheep blood agar and incubated aerobically and anaerobically for 48 h. GAS were initially identified by colony morphology and serology. As soon as the increased incidence of colonization by GAS was recognized, all available GAS isolates (10 patients' isolates and 1 staff member's isolate) were stored at −70°C in brain heart infusion (BHI) broth with 16% glycerol. Also, 11 strains of beta-hemolytic streptococci from patients from other clinical and outpatient departments of the hospital were likewise stored. The outbreak isolates were subjected to T serotyping, M genotyping, and gas chromatographic typing (National Institute of Public Health and Environmental Protection [RIVM], Bilthoven, The Netherlands). T serotyping was performed according to previously described methods (5), by using 5 polyvalent and 19 monovalent T serum samples commercially obtained from Denka Seiken (Tokyo, Japan).

M typing consisted of PCR amplification of the M protein gene from crude lysates of GAS strains and subsequent hybridization with M-type-specific probes in a reverse line blotting system (6). M types detectable with this method are M1 to 6, 8, 9, 12, 13, 18, 22, 24, 26, 28, 29, 33, 41, 46, 49, 52, 57, 60, and 61. In addition, six oligonucleotide probes based on distinct sequences of M (or M-like) genes from GAS strains with unknown M serotypes were used in the assay. These oligonucleotide probes are designated RM100, RM101, RM103, RM104, and RM105.

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TABLE 1. Identification of streptococcal isolates used in this study

Streptococcus strain type group	Lane[a]	Strain no.	Source of isolate[b]	T serotype	M genotype	RAPD pattern with[d]:	
						ERIC1	ERIC2
A (epidemic)	1	A94/25	V	T4/28	ND[c]	A	A
	2	A94/35	VI	T4/28	ND	A	A
	3	A94/27	VIII	T4/28	M48	A	A
	4	A94/28	IX	T4/28	M48	A	A
	6	A94/30	X	T13	ND	B	B
	7	A94/31	XII	T13	ND	B	B
	8	A94/32	VII	T13	ND	B	B
	9	A94/33	II	T13	M77	B	B
	10	A94/34	III	T13	M77	B	B
	11	A94/26	Nurse	T13	ND	B	B
	12	BWC012	VIII (hand)	ND	ND	A	A
	13	BWC013	VIII (wrist)	ND	ND	A	A
	14	BWC014	VIII (groin)	ND	ND	A	A
A (controls)	15	BWC015		ND	ND	C	C
	19	BWC019		ND	ND	B	G
	20	BWC020		ND	ND	B	B
	21	BWC021		ND	ND	B	B
	22	BWC022		ND	ND	F	H
	23	BWC023		ND	ND	G	I
	24	A94/86		B3264	M9	H	J
	32	44984-1		ND	ND	H	J
	25	A94/97		T1	M1	I	K
	31	45322-9		ND	ND	M	P
B	26	43892-2		ND	ND	J	L
	29	44689-4		ND	ND	J	L
	27	44010-0		ND	ND	K	M
	28	45458-3		ND	ND	J	N
	30	44804-6		ND	ND	L	O
C	17	BWC017		ND	ND	E	E
G	16	BWC016		ND	ND	D	D
	18	BWC018		ND	ND	D	F

[a] Lane numbers refers to the lanes in Fig. 3. The sample for lane 5 was eliminated for this study.
[b] Roman numerals indicate individual patients from whom isolates were obtained. Patient VIII experienced recurrent infection at three body sites (indicated in parentheses) 6 weeks after the initial colonization. The isolate of patient XI was *S. milleri* and was not further typed.
[c] ND, not done.
[d] Each unique RAPD pattern was arbitrarily assigned a letter for identification and comparisons.

For fatty acid gas chromatographic profile typing, bacteria were harvested after 48 h of aerobic incubation at 35°C on BHI agar supplemented with 5% yeast extract, 1 g of hemin type III per liter, and 1 g of vitamin K1 per liter. Saponification, methylation, and extraction were performed as described by Sasser (10). The gas chromatography system, operating parameters, and the identification of fatty acids on the basis of equivalent chain length data have been described previously (7, 10). Clustering was done by the unweighed-pair group method for arithmetic averages (UPGMA) as described by Romsburg (9) by using the relative (percentages of the total) areas under named peaks. Differences between the individual clusters are expressed in euclidian distances.

RAPD typing, by using primers based on enterobacterial repetitive intergenic consensus sequences (ERIC), was performed for all beta-hemolytic streptococci including the control strains. DNA was prepared from cultures grown on blood agar. Harvested bacteria were washed in 20 mM Tris-HCl (pH 8.0). Spheroplasts were prepared in 25 mM Tris-HCl (pH 8.0)–10 mM EDTA–50 mM glucose (TEG buffer) containing lysozyme (20 mg/ml; Sigma Chemical Co., St. Louis, Mo.) and incubated at 37°C for 1 h. DNA was isolated according to previously described procedures (1). Reaction mixtures for PCR contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM deoxynucleoside triphosphates, 50 pmol of a primer, and 0.2 U of Tth DNA polymerase (Sphaero Q, Leiden, The Netherlands); for amplification, the primers ERIC1 and ERIC2 were used (19). PCR conditions consisted of 40 cycles of consecutive denaturation, annealing, and DNA chain extension (1 min at 94°C, 1 min at 25°C, and 4 min at 74°C) in a Biomed (Theres, Germany) model 60 thermocycler. Fragments were separated on agarose gels (1.5%). DNA banding patterns were interpreted by two independent individuals. Capital letters were arbitrarily assigned to unique patterns (Table 1).

RESULTS

Description of outbreak. An increase in the number of patients colonized with GAS in the burn center was recognized on 2 January 1994. The start of the outbreak was retrospectively determined to be 20 December 1993, and it lasted until 11 January 1994, since on that day the last patient with detectable GAS was discharged. During the outbreak, six male and seven female patients were infected with GAS. The median age of the patients was 33 years (range, 9 months to 78 years), with five children under the age of 3 years. The total amounts of burned body surface of the 11 patients ranged from 2 to 18%, and 1 patient had an additional inhalation trauma. One patient (IV) had toxic epidermal necrolysis, and another (XI) underwent elective corrective surgery. All patients were treated with amoxicillin-clavulanate, except patient IV, who was given erythromycin. Patient VIII became GAS culture positive again 6 weeks after treatment. Typing of the newly isolated strain (see below) showed that this strain was identical to the last isolate, possibly indicating that the patient had been colonized persistently to a level not detected by routine culture. Alternatively, he may have been recolonized from an-

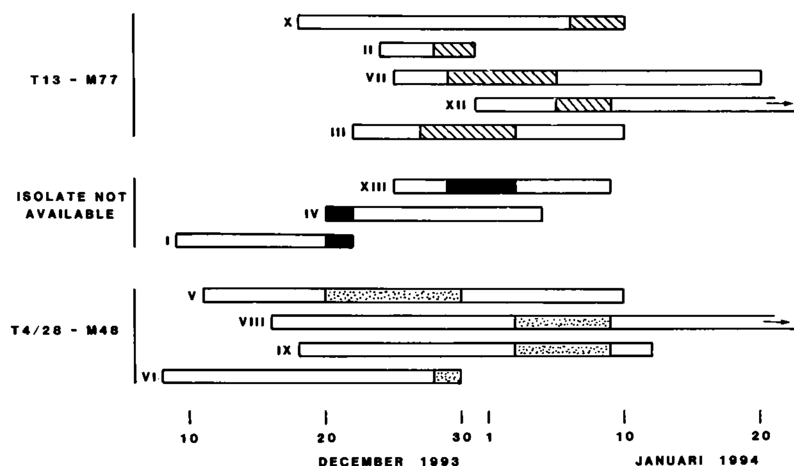

FIG. 1. Admission and discharge dates of patients (indicated by roman numerals) involved in GAS outbreak. Arrows indicate discharge dates beyond 20 January 1994. Shading indicates colonization by GAS.

other source. Of the 13 patients initially included in the outbreak, 9 underwent surgery: 1 patient (XI) was colonized with *Streptococcus milleri* (see below) and 8 were colonized with GAS. Of the latter, three patients (V, VII, and XII) had GAS colonization detected before they had surgery. These patients were treated before their skin-graft procedures were performed, and no GAS were detected thereafter. Five patients underwent surgery before GAS colonization was detected. Four of these five initially had partial graft take, and two experienced secondary loss as well. On clinical grounds, excluding surgical causes, the contribution of GAS infection to the loss of skin grafts was strongly suspected in three patients (VI, VIII, and X). One of 15 symptomatic staff members, a nurse, had a GAS-positive throat culture. Another nurse reported that she had a GAS throat infection diagnosed by her general practitioner while she was on leave. This GAS was cultured in another laboratory, and the culture was not saved.

T serotyping. T serotyping of 10 isolates of the GAS outbreak revealed 4 T4/28 and 6 T13 isolates. The isolate of patient XI was *S. milleri* with a serologically detectable group A antigen. As only one isolate (T13) was available from the two staff members with detectable GAS, the hypothesis that staff members played a role in the spread of the GAS could not be fully confirmed. Admission and discharge dates, as well as the time periods during which patients had GAS-positive cultures, are indicated in Fig. 1 for the patients with both GAS types and for the patients whose GAS isolates were not saved. Patient XI was not included. It can be noted that four of five patients with serotype T13 had positive cultures within 8 days of admission. These four patients were admitted to single rooms, and three were initially in intensive care. One patient (XII) became GAS T13 culture positive while in intensive care, despite the above-described routine preventive measures; in this case, a role of the staff in the spread of the T13 strain seems likely. Two of these four patients had surgery, but not before they were treated for GAS infection. To investigate the distribution of both strains by time and place, and the possible transmission from patient to patient, a map showing room numbers and the times that patients involved in the outbreak were located there was made. By doing this, transmission from patient to patient by sharing a room was highly probable for patients IX and VI, both with T4/28, and likely for patients X (T13) and XIII (no isolate available).

Phenotyping by gas chromatography. Gas chromatography and subsequent cluster analyses also revealed two major clusters matching the T typing results (Fig. 2). Within the T4/28 group, the isolates from patients VI and IX are very similar according to the clustering (euclidian distance less than 1). These patients shared a room for a week prior to their isolation. Within the T13 cluster, isolates from patients II and X are similar. The fact that the strains were isolated at more than a 1-week interval makes interpretation of the significance of the results difficult. These patients, 9 months and 13 years of age, respectively, might have spend time together in the playroom.

Genotyping. Because no hybridization signals were obtained with the outbreak strains and the current panel of probes, the PCR fragments were sequenced directly, by using fluorescent dye terminators in a Biosystems cycling sequencing system. The sequences obtained were compared with the sequences in a data bank to determine the M genotype. Sequencing of the M protein genes of two representatives of both the T13 and T4/28 clusters showed that these were homologous to M77 (accession no. U11961) and M48 (accession no. U11991), respectively.

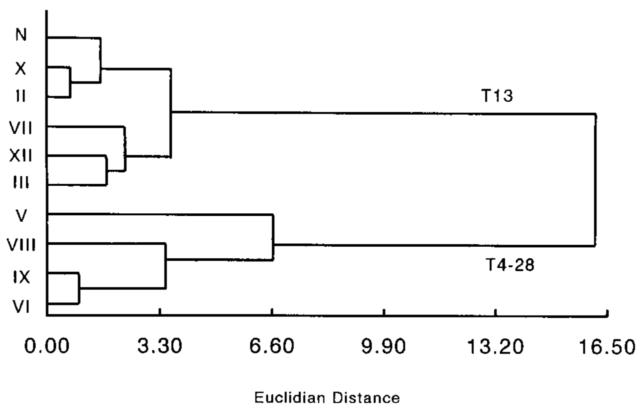

FIG. 2. Cluster analysis of epidemic GAS isolates typed by gas chromatography. Roman numerals, individual patients (sources of the isolates); N, nurse (staff member). Euclidian distance is the relative area of each named peak in gas chromatography, considered as the attribute of each object (isolate). The dissimilarity coefficients are calculated as the literal (euclidian) distance between objects, viewing these object points in an *n*-dimensional space formed by their attributes. The clustering method used was unweighted-pair group method for arithmetic averages (9).

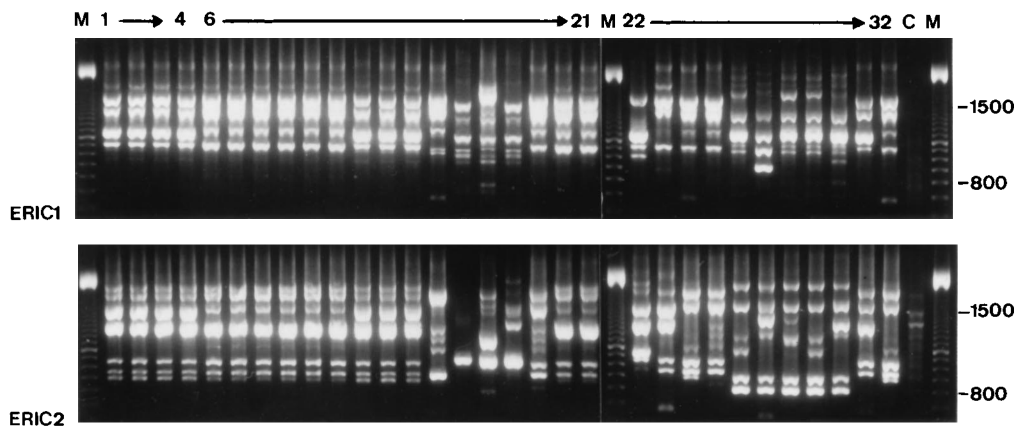

FIG. 3. Gel electrophoresis of PCR products obtained with primers ERIC1 (upper panel) and ERIC2 (lower panel). Lanes: 1 to 4 and 6 to 14, epidemic strains; 15 to 26, control strains from Zuiderziekenhuis; 27 to 32, control strains from University Hospital Rotterdam; C, control; M, markers (with sizes in daltons indicated on the right). The sample intended for lane 5 (data not shown) did not produce amplificated DNA; this isolate proved to be *S. milleri.*

RAPD analysis was done for 10 isolates from outbreak patients (Fig. 3 lanes 1 to 4 and 6 to 11) and for the isolates (three sites, lanes 12 to 14) from patient VIII, who had a recurrence of GAS T13 colonization following treatment. Lane 5 was omitted because no amplification product was obtained with the isolate that proved to be *S. milleri*. To control for the discriminating power of this method, an additional 18 strains (lanes 15 to 32) of beta-hemolytic streptococci of groups A, B, C, and G were typed as well. As can be seen in Fig. 3, the patterns obtained with either primer ERIC1 or ERIC2 allowed good discrimination. All T4/29-M48 isolates have RAPD pattern type AA with both primers ERIC1 and ERIC2, respectively (Table 1) whereas all T13-M77 isolates have RAPD pattern type BB. The patterns in lanes 20 and 21 are identical to that of the epidemic type BB. We therefore assume that this epidemic strain was prevalent among the outpatients of the Zuiderziekenhuis.

DISCUSSION

The Rotterdam burn center experienced an outbreak of GAS infection both in patients and staff. Thirteen patients and two staff members were initially identified as cases. Fortunately, no serious clinical disease developed as a consequence of GAS colonization. However, patients that had skin graft procedures before they became colonized with GAS, as indicated by surveillance cultures, experienced partial graft take and secondary skin graft loss. Several planned skin graft procedures had to be postponed. Typing of GAS isolates from burn patients in our center revealed that the outbreak was caused by two distinct GAS types. This finding is in line with a report by Ridgway and Allen, who studied GAS outbreaks in a burn unit during an 11-month period (8). Their burn unit experienced several smaller outbreaks in which several serotypes of GAS were involved. GAS found in burn units may reflect the GAS found in the community (2). The only data on the prevalent serotypes of GAS in The Netherlands are available from the surveillance for invasive GAS infections (11), but these strains do not represent all currently circulating GAS strains. There are two other interesting similarities to the Ridgway report. Firstly, a low incidence of throat carriage of GAS was encountered among staff members. Secondly, it appears that patients may experience relapses, despite apparently successful treatment with beta-lactam antibiotics. It is known that pharyngeal carriage of GAS is not always cleared by penicillin treatment (15).

T serotyping and gas chromatography proved important to resolving this outbreak. Both methods identified the two clusters of isolates, and gas chromatography allowed some discrimination within both clusters. One couple of similar isolates could be linked to patients who shared a room for a week prior to isolation. No other obvious links of gas chromatographic subclusters to epidemiological data could be found. We noted that the T13 isolates were more similar to each other than were those of the T4/28 cluster, and at the same time the T13 isolates showed more pronounced clustering over a period of time. In our opinion gas chromatography is worth considering when evaluating GAS outbreaks for which other typing procedures do not offer enough differentiation.

We were able to demonstrate the usefulness of molecular methods in typing GAS as well. Genetic M typing, as performed in this study, is used in GAS surveillance in The Netherlands, and 80% of the GAS strains isolated can be typed with the currently available number of probes (12). This method may enable more laboratories to do this typing, and it has the advantage of reference to the vast amount of information on M types. Whereas the M-serotyping system is technically demanding and requires a large number of serum samples, which are difficult to obtain, M genotyping is easy to perform. M genotyping does not require elaborate isolation of DNA and utilizes a hybridization procedure that does not require advanced equipment or skills. RAPD typing lacks the advantages of having a long history as do T and M typing, but it is feasible for any modern laboratory. It was recently shown that even the use of a single primer in a RAPD test allowed appropriate recognition of a large number of GAS subtypes (13). Primers ERIC1 and ERIC2 have proved useful for the typing of several bacteria and fungi (4, 16–18). If appropriate DNA extraction methods are used, RAPD typing with these primers provides a powerful tool in resolving epidemiologic matters in a diverse range of clinical settings.

The control policy for GAS in our burn center has been in force for several years now. One patient in intensive care who was culture negative on admission acquired an epidemic strain, most likely transmitted by a staff member. With this experience, the rule that any physical complaint of a staff member that might be due to GAS must be reported to the head of the

center has been reenforced. We conclude that the current policy offers sufficient protection for this vulnerable category of patients without excessively restricting their mobility during convalescence.

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