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Multicenter Evaluation of Arbitrarily Primed PCR for Typing of *Staphylococcus aureus* Strains

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Fifty-nine isolates of *Staphylococcus aureus* and a single strain of *Staphylococcus intermedius* were typed by arbitrarily primed PCR (AP-PCR). To study reproducibility and discriminatory abilities, AP-PCR was carried out in seven laboratories with a standardized amplification protocol, template DNA isolated in a single institution, and a common set of three primers with different resolving powers. The 60 strains could be divided into 16 to 30 different genetic types, depending on the laboratory. This difference in resolution was due to differences in technical procedures (as shown by the deliberate introduction of experimental variables) and/or the interpretation of the DNA fingerprints. However, this did not hamper the epidemiologically correct clustering of related strains. The average number of different genotypes identified exceeded those of the more traditional typing strategies (F. C. Tenover, R. Arbeit, G. Archer, J. Biddle, S. Byrne, R. Goering, G. Hancock, G. A. Hebert, B. Hill, R. Hollis, W. R. Jarvis, B. Kreiswirth, W. Eisner, J. Maslow, L. K. McDougal, J. M. Miller, M. Mulligan, and M. A. Pfaller, J. Clin. Microbiol. 32:407–415, 1994). Comparison of AP-PCR with pulsed-field gel electrophoresis (PFGE) indicated the existence of strains with constant PFGE types but variable AP-PCR types. The reverse (constant AP-PCR and variable PFGE patterns) was also observed. This indicates additional resolution for combined analyses. It is concluded that AP-PCR is well suited for genetic analysis and monitoring of nosocomial spreading of staphylococci. The interlaboratory reproducibility of DNA-banding patterns and the intralaboratory standardization need improvement.

Numerous procedures for comparison of bacterial isolates have been developed (for reviews, see references 2, 11, 13, and 15). These procedures are important in investigations of strain origin, clonal relatedness among strains, and epidemiology. For *Staphylococcus aureus*, it has been demonstrated that most of the typing procedures can be applied successfully to obtain epidemiologically useful data. Tenover et al. (22) compared 12 typing strategies and concluded that DNA-based typing methods and immunoblotting are best suited for epidemiological analyses. With the exception of biotyping, which appeared to produce too many subtypes, no single technique proved overtly superior or inferior. When all procedures were compared, unrelated strains were grouped with differing frequencies. This comparative analysis of typing procedures provides a reference scheme for rating novel typing strategies against the more established methods.

Recently, a large number of reports describing the use of PCR for genetic typing of medically important microorganisms (for surveys, see references 23 and 26) have appeared. By arbitrarily amplifying variable regions in the bacterial genome (arbitrarily primed PCR [AP-PCR]), isolate-specific DNA fingerprints can be obtained in a rapid and reproducible manner. In most cases, these analyses are not accompanied by detailed comparisons with the results of alternative typing procedures. For *S. aureus*, for instance, several studies have compared AP-PCR with only a single other technique (20, 21, 24, 25).

The present study was undertaken to determine the reproducibility and discriminatory abilities of AP-PCR when compared with other staphylococcal typing procedures. To this end, the *S. aureus* strains that were studied previously by Tenover et al. (22) were typed in multiple AP-PCR assays with three different arbitrary primers, guided by a standard amplification protocol and performed independently in seven different laboratories.

MATERIALS AND METHODS

Bacterial strains and description of isolates. Fifty-nine isolates of *S. aureus* were included in this study. All isolates were identified and confirmed to be *S. aureus* by standard biochemical methods (12). The strains have been described before (22), and 40 of them were derived from five well-documented outbreaks. The 19 additional isolates are epidemiologically unlinked. A single isolate of *S. intermedius* was included.

The 60 strains were divided into three groups (SA, SB, and SC), some of whose key features are summarized below (Table 1). Group SA contains the strains involved in outbreaks that occurred in two nursing homes (strains labeled NH1 and NH2). Strain SA-04 is ATCC 12600 (American Type Culture Collection,

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TABLE 1. General survey of phenotypic and genotypic typing data in comparison with the results from the multicenter study on AP-PCR-mediated DNA fingerprinting for 60 *Staphylococcus* strains

Strain[a]	Out-break[b]	Ox[rc]	Phage type	Anti-biogram	Bio-type	AP-PCR datasets[d]								Plas-mid[e]	Ribo-type[f]	PFGE	FIGE	Im-muno-blot	MLEE	IS[g]	Coagu-lase PCR[h]	RFLP type
						Ia	Ib	II	III	IV	V	VI	VII									
SA-16	NO	S	NR	I	INT[i]	HEH	FEF	HEG	GDH	FDF	FDG	GFG	DDC	NP	de	I	VII	K	F	NH	0.0	NH:NH:NH:NH
SA-04	NO	S	6/47/54/75	B	A2B	BBB	BBB	BBB	BBB	BBB	BBC	BCC	BBA2	B	fi	E	IV	D	E	NH	2.1	NH:X:4:NH
SA-12	NO	R	47/54/75/77/83A	G	A3B	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA1	NP	bb	J	IC2	A	A5	C	9.0	I:A:1:NH
SA-18	NO	R	47/54/75/77/83A	J	A3B	AAA	AAA	AAA	AAA	AAA	AAA	ABA	AAA1	I	bb	J	IC3	A2	A3	C	9.0	I:A:1:NH
SA-20	NO	R	47/54/75/77/83A	K	A3B	AAA	AAA	AAA	AAA	AAA	AAA	ABB	EAA1	J	bb	J	IC1	A1	A1	C	9.0	I:A:1:NH
SA-06	NO	I	NR	C	A3B	CAC	AAC	CAC	CAC	AAA	AAB	ABB	AAA1	C	aa	C	III	A4	A4	B	9.0	II:NH:1:a
SA-07	NO	S	53/+	D	H4	DAD	AAC	DAC	AAD	CAA	AAD	CBA	AAA1	D	bc	B	V	C	A2	NH	9.0	NH:NH:1:NH
SA-08	NO	R	54/75/77/81	E	I2B	ECE	CCD	ECD	DBE	DBC	CBE	DDD	CB′B	E	ed	G	IIA	E1	D1	D	7.0	I:NH:6:NH
SA-11	NO	R	NR	F	A2B	ECF	CCD	ECE	DBF	DBD	CBF	DDE	CB′B	E	gd	F	IIB	E2	D2	G	7.0	II:NH:6:NH
SA-01	NH1	R	54/77	A1	A1B	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA1	A	aa1	K1	IB	A1	A1	A	9.0	I:A:5:a
SA-09	NH1	R	54/77	A	A1B	AAA	AAA	AAA	AAA	AAA	AAD	ABA	AAA1	NP	aa1	K2	IB	A1	A1	A	9.0	I:A:5:a
SA-03	NH1	R	47/54/75/77	A2	A3B	AAA	AAA	AAA	AAA	AAA	AAB	ABB	AAA1	NP	aa	A	IA	A	A1	C	9.0	I:A:1:NH
SA-13	NH1	R	54/77	A3	A1B	AAG	AAA	AAA	AAA	AAA	AAA	AAA	AAA1	G	aa	A	IA	A3	A2	A	9.0	I:A:1:a
SA-14	NH1	S	54/75/77	H	B1B	GDG	EDE	GDF	FCG	-CE	ECA	FEF	ACA3	H	ci	H	VI	E3	C	NH	9.0	NH:NH:1:NH
SA-19	NH1	R	54/77	A4	G1B	AAA	AAA	AAA	AAA	AAA	AAB	ABB	EAA1	A	aa1	K3	IB	A1	A1	A	9.0	I:A:1:a
SA-17	NH2	R	54/75/77	A	C3B	AAA	AAA	AAA	AAA	AAA	AAB	ABB	EAA1	A	aa	A	IA	A	A1	A	9.0	I:A:1:a
SA-02	NH2	R	75/77	A	A3B	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA1	A	aa	A	IA	A	A1	A1	9.0	I:A:1:b
SA-15	NH2	R	77	A	A3B	AAA	AAA	AAA	AAA	AAA	AAA	AAB	AAA1	A	aa	A	IA	A1	A5	A1	9.0	I:A:1:a
SA-05	NH2	R	77	A	A3B	AAA	AAA	AAA	AAA	AAA	AAB	ABB	AAA1	A	aa	A	IA	A	A1	A	9.0	I:A:1:a
SA-10	NH2	R	77	A	A3B	FAA	DAA	FAA	EAA	EAA	DAA	EAA	JAA1	A	aa	D	ID	A1	B	A	9.0	I:A:1:a
SB-07	NO	S	6/47/54/75	C	A2B	BBB	BBB	BBB	JBB	BBH	-BC	BCC	BBA1	D	ci	D	IIB3	D′	B3	NH	2.1	NH:X:4:NH
SB-03	I	R	75/+	A	C4	AAA	AAA	AFA	HEA	AE-	AEA	HGA	EEA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
SB-05	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEA	AEA	HGA	EEA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
SB-10	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEA	AEK	HGA	HEA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
SB-12	I	R	75/+	A	C4	AAA	AAA	AFA	HEA	AEL	AEK	HGA	AAA1	C	aa	A1	IA	A6	A1	E	9.0	I:A:1:a
SB-15	I	R	75/77/83	A	C4	AAA	AAA	AFA	HEA	AEL	AEA	HGA	AAA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
SB-19	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEL	AEA	HGA	AAA1	C	aa	A	IA	A5	A1	E	9.0	I:A:1:a
SB-20	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEL	AEK	HGA	AAA1	C	aa	A	IA	A5	A1	E	9.0	I:A:1:a
SB-01	NO	R	75/77	A	A4	AAA	AAA	AFA	HEA	AEA	AEA	HGA	EEA1	A	aa	A1	IB1	A5	A1	E	9.0	I:Y:1:a
SB-16	NO	R	75/77/83A	A	A4	AAA	AAA	AFA	HEA	AEL	AEA	HGA	AAA1	A	aa	A1	IB1	A5	A1	E	9.0	I:Y:1:a
SB-18	NO	R	75/+	A	C4	AAA	AAA	AFA	HEA	AEL	AEA	HGA	AAA1	J	aa	A	IA	A7	A1	E1	9.0	I:A:1:a
SB-17	NO	I	96	E	B3B	LDL	JIM	NJM	OCQ	LCO	KCM	NKM	ACA6	I	fj	E	IV	G	A2	NH	6.0	NH:NH:1:NH
SB-14	NO	R	47/54/75/77/83A	A1	A3B	AAG	AHL	AAL	HAP	AAN	AAA	AAF	AAA3	H	ea	A2	IB2	A5	A3	D	9.0	I:A:1:NH
SB-08	NO	S	95	B1	C4	JGJ	HBI	JHI	KGM	IGI	IGI	JII	GFG	E	dd1	F	III	E5	C	NH	2.0	NH:NH:1:NH
SB-02	II	S	3A/55	B	B1B	IFI	GFH	IGH	IFK	GFG	HFH	IHH	FBD	B	bb	B	IIA	D1	B1	NH	6.0	NH:NH:7:NH
SB-04	II	S	3A/55	B	D1B	IFI	GFH	IGH	IFL	HFG	-FH	IHH	FBD	B	bb	B	IIA	D1	B1	NH	6.0	NH:NH:7:NH
SB-06	II	S	3A/55	B	B1B	IFI	GFH	IGH	IFK	HFG	HFH	IHH	FBD	B	bb	B	IIA	D1	B1	NH	6.0	NH:NH:7:NH
SB-11	II	S	3A/55	B	B3B	FFK	GFK	LGK	MFO	KHK	JFL	LHK	ABE	G	b1b	C	IIB2	D2	B1	NH	14.0	NH:NH:7:NH
SB-09	NO	S	3A	D	D3B	BFI	IDJ	KIJ	LBN	JBJ	BBJ	KJJ	HBE	F	bb	B	IIA	D1	B1	NH	6.0	NH:Z:7:NH
SB-13	NO	S	3A	B2	D3B	KFI	GGJ	MIJ	NBN	RBM	BBJ	MIL	ABF	G	bb	B1	IIB1	E6	B2	NH	6.0	NH:NH:7:NH

SC-03	NO	S	6/47/54/75	C	A2B	BBB	BJB	BBB	JBB	OBP	LBC	BCP	ABA4	C	ai	C	III	D	B	NH	2.1	NH:NH:4:NH
SC-01	III	R	75	A	A1B	MFB	BJB	OBB	PBB	MBP	LBB	OLN	ABA4	A	ab	A	IA	F	A1	F	10.0	I:A:4:a
SC-04	III	R	75	A	A1B	MFB	BJB	OBB	PBB	MBP	LBB	OLN	ABA4	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
SC-05	III	R	NR	A1	A1B	MFB	BJB	OBB	PBB	-BP	LBB	OLN	ABA4	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
SC-09	III	R	75	A	A1B	MFB	BJB	OBB	PBB	MBP	LBN	OCN	IBA5	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
SC-11	III	R	75	E	A1B	MFB	BJB	OBB	PBB	MBP	LBN	OCN	IBA5	NP	ab	A	IB	F	A1	NH	10.0	I:A:4:NH
SC-12	III	R	75	A2	A1B	MFB	BJB	OBB	PBB	MBP	LBN	OLN	IBA5	A	ab	A	IA	F	A1	F	10.0	I:A:4:a
SC-14	III	R	75	A2	B2B	MFB	BJB	OBB	PBB	MBP	LBN	OLN	IBA5	A	ab	A	IA	F	A2	F	10.0	I:A:4:a
SC-15	III	R	75	A	A1B	MFB	BJB	OBB	P-B	MBP	LBN	OCN	IBA5	D	b2b	A	IA	F	A1	F	10.0	I:A:4:a
SC-17	III	R	75	A	A1B	MFB	BJB	OBB	P-B	MBP	LBN	JIN	IBA5	A	ab	A	IA	F	A1	F	10.0	I:A:4:a
SC-20	III	R	75	A	A1B	MFB	BJB	OBB	PB-	MBP	LBN	JIN	IBA5	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
SC-08	NO	S	NR	B	B3A	JHD	HKI	JHI	KGM	PII	MGI	JIH	GFG	E	b1g	B1	II	E7	A3	NH	2.0	NH:NH:1:NH
SC-02	IV	S	52/52A/80/47/54 83A/84/95	B	E1B	NGJ	HKI	JHI	KGM	NII	MGI	JIO	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
SC-06	IV	S	95	B	J1B	JHD	HKI	JHI	KGM	PJI	MGI	JIH	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
SC-07	IV	S	95	D	I1A	JHD	HKI	JHI	KGM	PJI	MGI	JIQ	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
SC-10	IV	S	52A/79/80/47/54 75/77/83A/95	B	I2A	JHD	HKI	JHI	KGM	-II	MGI	JIH	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
SC-13	IV	S	95	B1	I1B	JHD	HKI	JHI	KGM	NII	MGI	JII	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
SC-16	IV	S	95	B1	I1B	JHD	HKI	JHI	KGM	PJI	MGI	OLI	GFG	B	ag	B	II	H	D1	NH	2.0	NH:NH:1:NH
SC-18	IV	S	95	F	I3B	JHD	HKI	JHI	KGM	PJI	MGI	OLI	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
SC-19	IV	S	95	B1	D1A	JHD	HKI	JHI	KGM	PII	MGI	JOI	GFG	B	bg	B	II	E7	D2	NH	2.0	NH:NH:1:NH
No. of types[j]		3	19	11	15	20	16	18	21	30	21	28	19	10	15	11	7	7	6	8	8	17

[a] The strain numbers that are underlined are three isolates of a single ATCC strain.
[b] NO, not in epidemiologically related cluster; I to IV, outbreak number; NH1/2, nursing home pseudo-outbreak.
[c] Ox^r, oxacillin susceptibility test result.
[d] Columns numbered Ia through VII give surveys of the AP-PCR data as determined in the different institutions. The three-letter code summarizes the typing results per primer used (first digit, primer 1; second digit, primer 7; third digit, primer E2). Data represented by a capital letter given in a certain column may be different from the same character in another column. Underlining in the AP-PCR datasets indicates minor differences in DNA staining intensities.
[e] Plasmid, plasmid restriction profile. NP, no plasmids.
[f] Ribotyping results obtained with *Hin*dIII and *Cla*I, respectively.
[g] IS, insertion sequence. NH, no hybridization.
[h] Coagulase PCR, coagulase gene PCR typing.
[i] INT, *S. intermedius* biotype.
[j] In the cumulative number of types, subnumbers are counted as a single type number.

TABLE 2. Survey of experimental variables with respect to PCR fingerprinting performed in the different participating laboratories

Variable	Value for laboratory:							
	Ia	Ib	II	III	IV	V	VI	VII
Incubation vol (μl)	100	100	100	50	100	100	50	50
Polymerase type[a]	*Tth*	*Tth*	*Tth*	*Tth*	*Taq*	*Thp*	*Taq*	*Taq*
Amt of polymerase (u)	0.20	0.20	0.25	0.10	1.25	0.25	1.00	0.25
Mg^{2+} concn (mM)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
dNTP concn (μM)	40	40	40	100	200	40	200	200
KCl concn (mM)	50	50	50	50	50	*[b]	50	50
PCR machine	Biomed	Biomed	Biomed	Hybaid	Biomed	P.E.	P.E.	P.E.
Sample size (%)[c]	30	30	30	50	25	30	30	30
Polaroid[d]	57/3,000	57/3,000	52/400	665/80	665/80	667/3,000	667/3,000	665/80
Type of agarose	Pron.	Pron.	Pron.	MP	Pron.	MP	UP	MP
% agarose	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Voltage	150	150	200	100	100	100	130	150
Migration (cm)	17	10	10	8	10	10	8–9	8–10
Ethidium bromide[f]	−	−	+	+	+	+	−	+

[a] *Thp* (Thermoperfect DNA polymerase; Integro, Zaandam, The Netherlands); *Tth* (Supertaq; Sphaero Q, Leiden, The Netherlands); *Taq* (*Taq* polymerase; Cetus, Emeryville, Calif.).
[b] *, in this case no KCl was present; instead, 50 mM $(NH_4)_2SO_4$ was included.
[c] The sample size indicates the amount of the amplification reaction that has been separated electrophoretically.
[d] The description of the photographs includes the type and the sensitivity in ASA values.
[e] Pron. (Pronarose; Hispanagar, Burgos, Spain); MP (multipurpose agarose; Boehringer Mannheim); UP (Ultra Pure Agarose, Gibco/BRL, Breda, The Netherlands).
[f] The presence (+) or absence (−) of ethidium bromide during the electrophoresis is indicated.

Rockville, Md.). This set also contains a number of independent isolates from the Centers for Disease Control and Prevention, the *S. intermedius* strain, and three isolates of phage type 47/54/75/77/83A. These latter strains were isolated in three different American states during three different years.

Group SB contains strains from outbreaks I and II, eight unrelated strains, and, again, *S. aureus* ATCC 12600 (SB-07). Strains from outbreak I are methicillin resistant and were isolated at the Iowa Veterans Affairs Medical Center (18). Outbreak II was related to a contaminated anesthetic (6).

Group SC contains strains from outbreaks III and IV, an unrelated control, and ATCC 12600 (SC-03). Outbreak IV was again anesthetic related (6), although it differed from outbreak II. Outbreak III was caused by 10 methicillin-resistant strains in the Sepulveda Veterans Affairs Medical Center, Sepulveda, Calif. (9).

Bacterial typing studies. All isolates were typed previously by a number of procedures (22). Antibiograms and biotypes were determined, and bacteriophage sensitivity was assayed. Restriction fragment length polymorphisms (RFLP) were screened by using enzymatic digestion of plasmid DNA, variable-gene probes, or DNA probes derived from insertion elements (IS mapping). DNA macrorestriction fragments were separated by field inversion gel electrophoresis (FIGE) and pulsed-field gel electrophoresis (PFGE). Multilocus enzyme electrophoresis (MLEE) and immunoblotting were also performed, as were ribotyping and restriction enzyme analysis of PCR fragments derived from the staphylococcal coagulase gene.

PCR multicenter study design. Participants were from seven institutions: two Belgian institutes (Hôpital Erasme, Brussels; and University Hospital of Ghent, Ghent), and five Dutch hospitals (University Hospital Nijmegen, Nijmegen; University Hospital Utrecht, Utrecht; Diagnostic Centre SSDZ, Delft; Free University Hospital, Amsterdam; and University Hospital Dijkzigt, Rotterdam). The study was coordinated at the Dijkzigt Hospital, where the AP-PCR assays were performed in duplicate by two individuals following slightly different experimental protocols. All participants had experience in performing AP-PCR. This guarantees intralaboratory reproducibility of the assays. For this reason, the participants were also allowed to process the *S. aureus* DNA samples according to their own, optimized AP-PCR protocol. Results are presented anonymously, and datasets are numbered from I through VII (sets Ia and Ib derive from the coordinating laboratory).

To prevent interlaboratory variation due to different DNA extraction protocols, bacterial DNA, and not the organisms, was distributed from the coordinating center to the participating laboratories. Primers were aliquoted in Rotterdam as well and shipped together with the DNA preparations. In this way, the variables of bacterial cultivation, DNA isolation, and primer quality were controlled. This implies, however, that the results obtained during this study may differ from those that would have been obtained if bacterial strains, rather than DNA, had been distributed. DNA amplification was performed in the different laboratories with the locally available equipment and PCR ingredients. Gels containing the amplified DNA were photographed, and the results were interpreted locally according to the researchers' individual standards. Generally, differences in the number of bands indicated a novel type. Variations in band-staining intensities were disregarded. Interpretation was performed without knowledge of epidemiological relatedness. The fingerprint types were transformed in a cumulative three-letter code (one letter per type per primer) and sent to Rotterdam, where a comparative analysis was carried out. Results were studied with respect to reproducibility of the DNA fingerprints (and the accompanying interpretation and strain grouping), relation to the results obtained by other typing procedures, and epidemiological value. When possible, data were further analyzed with Gelcompar Software (Applied Maths, Kortrijk, Belgium). Pictures were digitized with a Hewlett-Packard HP ScanJet IIc document scanner. After conversion and visual normalization, the data were analyzed. Degrees of homology were determined by Dice comparisons, and clustering correlation coefficients were calculated by the unweighted pair group method with arithmetic averages (UPGMA).

AP-PCR. A description of the three PCR-related procedures is given below. This protocol served as a reference manual. Specific deviations from this protocol are summarized per institute in Table 2.

(i) Cultivation of bacteria and isolation of DNA. Bacteria were grown in suspension in brain heart infusion broth for 18 h at 37°C. Approximately 100 μl of a bacterial pellet was suspended in 150 μl of 25 mM Tris · HCl (pH 8.0)–50 mM glucose–10 mM EDTA. Lysostaphin (75 μl of a 100-μg/ml solution) was added, and the mixture was incubated at 37°C for 1 h. Spheroplasts were lysed by the addition of 1 ml of 4 M guanidinium isothiocyanate–50 mM Tris · HCl (pH 6.4)–3 mM EDTA–1% (wt/wt) Triton X-100. To immobilize and purify the DNA, 50 μl of a Celite suspension (0.2 g/ml; Janssen Pharmaceuticals, Beerse, Belgium) was added. The entire mixture was shaken for 15 s and incubated at room temperature for 10 min. After centrifugation, the supernatant was discarded; the pellet was washed once with 1 ml of lysis buffer, twice with lysis buffer without EDTA and Triton X-100; twice with 70% ethanol in water, and, finally, once with acetone. The Celite pellet was dried in vacuo. Between 100 and 400 μl of 10 mM Tris · HCl (pH 8.0)–1 mM EDTA was added, and DNA was eluted by incubation at 56°C for 10 min, interrupted by short periods of vortexing. The supernatant containing the DNA was separated from the Celite by centrifugation. The DNA concentration was determined by spectrophotometry at 260 nm, and the DNA was stored at −20°C. Stock solutions of bacterial DNA were adjusted to a concentration of 5 ng/μl.

(ii) PCR. When *Tth* polymerase (SuperTaq; HT Biotechnology, Cambridge, United Kingdom) was used, the amplification conditions described below guaranteed optimal performance for this particular enzyme. The use of other enzymes usually required modification of the buffer conditions used during PCR (Table 2) and may lead to different AP-PCR results. PCR was performed with a buffer system containing 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 (dNTPs), 50 pmol of primer, and 0.2 U of the *Tth* polymerase, to which DNA was added (50 ng per amplification). The PCR mixtures were overlaid with 100 μl of mineral oil. Cycling was performed in Biomed PCR machines (Model 60) and consisted of the following steps: predenaturation at 94°C for 4 min followed by 35 cycles of 1 min at 94°C, 1 min at 25°C, and 2 min at 74°C. Amplified DNA was stored at −20°C. The primers used to discriminate *S. aureus* strains were 1 (GGTTGGGTGAGAATTGCACG), 7 (GTGGATGCGA), and ERIC2 (E2) (AAGTAAGTGACTGGGGTGAGCG) (24, 25, 28).

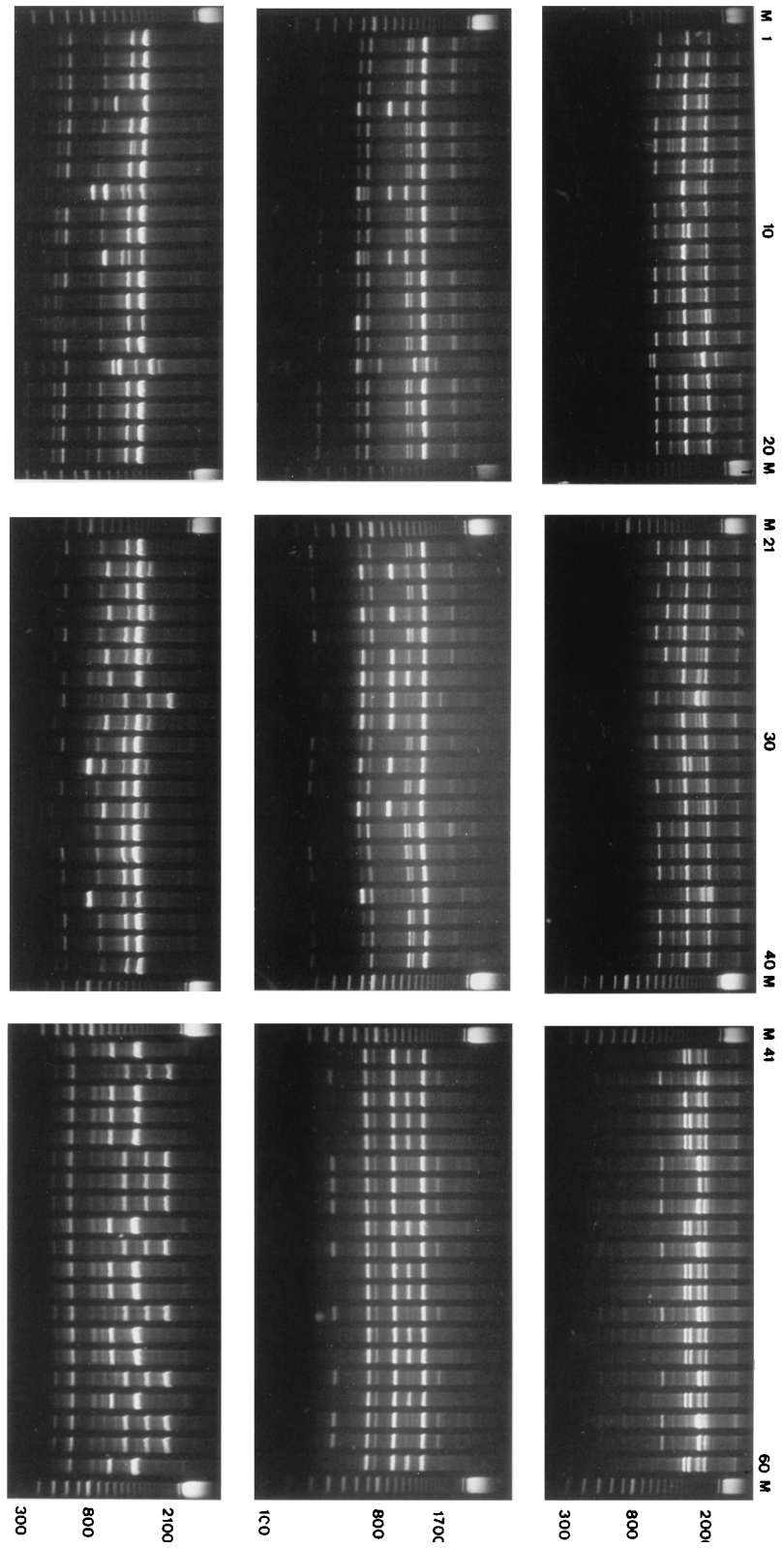

FIG. 1. AP-PCR for the 60 staphylococcal strains used in this study. This figure is a survey of the experimental results belonging to dataset Ia. From left to right, the strains are numbered as in Table 1. Different primers are used in the three different rows. The outer lanes in all nine panels contain molecular length markers, whose sizes are indicated on the right. The interpretation of the banding patterns is given in Table 1 in column Ia.

TABLE 3. Number of genetic variants detected with the individual PCR primers as independently documented by the seven participating laboratories

Participating center	No. detected with primer:			Overall no.	No. of unique strains
	1	7	E2		
Ia	14	8	11	20	13
Ib	10	11	11	16	9
II	15	10	13	18	12
III	16	7	17	21	16
IV	17	10	16	30	15
V	13	7	14	21	11
VI	15	12	17	28	15
VII	10	7	12	19	11
Mean	14	9	14	22	13

(iii) Electrophoresis. Amplification products were separated by electrophoresis in 5-mm-thick 1.5% agarose gels (Hispanagar; Sphaero Q, Leiden, The Netherlands). Gels were run in 0.5× Tris-borate-EDTA (TBE) at a constant current of 100 mA for 2 h. Prior to electrophoresis, samples were mixed with a fivefold-concentrated layer mix consisting of 50% glycerol in water and 0.8 mg of bromophenol blue per ml. Then 35 μl of the amplified material was loaded on the gel, and a molecular weight marker was run in parallel with the AP-PCR samples. Gels were stained after electrophoresis by addition of 10 μl of ethidium bromide (10 mg/ml) to a total volume of 300 ml of 0.5× TBE. The gels were photographed with a Polaroid MP4 Landcamera and Polaroid 57 High Speed films, with an exposure time of 0.125 to 0.25 s (diaphragm F5.6). Table 2 surveys the differences among the electrophoresis conditions as applied in the different laboratories.

RESULTS

PCR fingerprinting. An overview of the typing results is given in Table 1. The AP-PCR data are displayed in separate columns, one per participating research center, except for the coordinating laboratory, where the assays were performed in duplicate (Ia and Ib). Figure 1 gives an example of a complete set of gel pictures obtained for the 60 strains with the three AP-PCR primers. Table 3 displays the number of genotypes that were detected in the participants' laboratories. When primer 1 was used, the overall number of types varied from 10 to 17, with a mean of 14 types. The mean numbers for primers 7 and E2 were 9 and 14, respectively. With this set of strains, the discriminatory power of primers 1 and E2 is over 60% higher than that of primer 7.

The overall number of DNA bands generated per primer does not correspond to the number of detectable genotypes. Dataset IV, displaying 30 different genocodes (Tables 1 and 3) was produced from a relatively small number of DNA fragments synthesized: seven, five, and seven fragments for primers 1, 7, and E2, respectively. These are smaller numbers than those found by the group describing the smallest number of genotypes (dataset Ib, with 16 types deduced from fingerprints consisting of 9, 9, or 11 bands for individual fingerprints). The maximum number of bands was observed when primer 1 was used. The mean score for this primer, averaged among the groups, is 11.4 DNA fragments. For primers 7 and E2, these numbers are 6.6 and 9.6 fragments, respectively. There is no apparent variation in the average length of the fragments, as demonstrated by a survey of the cumulative results obtained by application of primer 1 (Fig. 2). Although most of the types were found in all the laboratories, some additional bands gave rise to additional genotypes.

Discordant results can be observed. The data obtained with primer E2 seemed to be the most variable (results not shown). When the overall number of combined genocodes is considered, major differences among laboratories are encountered. The number of types varies between 16 and 30, with a mean of 22 types identified.

When the lengths of the DNA fragments generated by the individual PCR tests were investigated, primer 1 was found to generate amplicons with an overall length of approximately 11,000 bp. For primers 7 and E2, these values are 4,000 and 5,500 bp, respectively. These differences are not reflected in the overall number of detectable PCR types (which is 14 for both primers 1 and E2). Primer 7, which detected the smallest number of types, is also associated with the shortest cumulative length of the DNA fragments synthesized. There is an apparent variation in the number and size of fragments generated per primer species. For primer 1, this number varies from 6.4 to 11.4 on average. The numbers for primers 7 and E2 are 3.7 to 6.6 and 6.0 to 9.6, respectively. In general, fragments vary in length from 0.15 to over 2 kbp.

Epidemiological considerations. Analysis of the strains from outbreak IV illustrates that the data obtained by five of seven laboratories group these isolates into a homogeneous genotype that is not encountered in the rest of the collection, with the exception of a single strain (SC-08). These data are similar to those obtained by oxacillin susceptibility testing, plasmid typing, ribotyping, PFGE, FIGE, immunoblotting, IS mapping, PCR typing and RFLP mapping (22). Two of the seven laboratories detected three to six different types in this group of eight bacterial isolates. The results collected for the strains from outbreak III are similar. In this case, four of seven datasets demonstrated the homogeneity of this subgroup. Three participants identified two or three different types. Interestingly, in two of these laboratories, where the same subtypes are established, the differences were limited to data obtained by only one of the PCR primers. Again, the PCR data are in general agreement with those obtained by the other typing techniques. The four strains from outbreak II are split into two types: three are identical (five of seven laboratories) or very similar (two of seven laboratories), whereas a single strain (SB-11) appears to be different. The other datasets confirm this observation. Results with strains from outbreak I and the nursing home (NH1 and NH2) conform to those of the other typing procedures. Since 19 non-outbreak-related strains are included in the collection, this implies that the resolution of PCR fingerprinting varies from approximately 50% to nearly 90%, since between 9 and 16 unique types were identified depending on the institution. It is assumed that all 19 non-outbreak-related strains are indeed genetically independent.

Typeability and reproducibility. All strains were typeable by PCR. Four of seven laboratories obtained 100% typeability; negative results seen by other participants were due to technical inadequacy not related to bacterial genome structure. The overall mean level of typeability was 99.5%. This makes PCR-mediated typing preferable in principle over phage typing, plasmid typing, some of the RFLP approaches, and IS mapping, which all leave an appreciable percentage of strains untyped.

Discriminatory power. PCR fingerprinting was not able to detect differences between strain SA-12, SA-18, and SA-20. These strains are also identical by phage typing, ribotyping, PFGE, and IS mapping. Since these strains were derived from diverse origins, it seems likely that certain clones of *S. aureus* spread easily and remain genotypically constant. On the other hand, several other strains belonging to the same phagovar are differentiated by the PCR tests. As has been argued previously (25), PCR fingerprinting provides additional discrimination over that provided by phage typing.

The participants who detected the smallest number of types

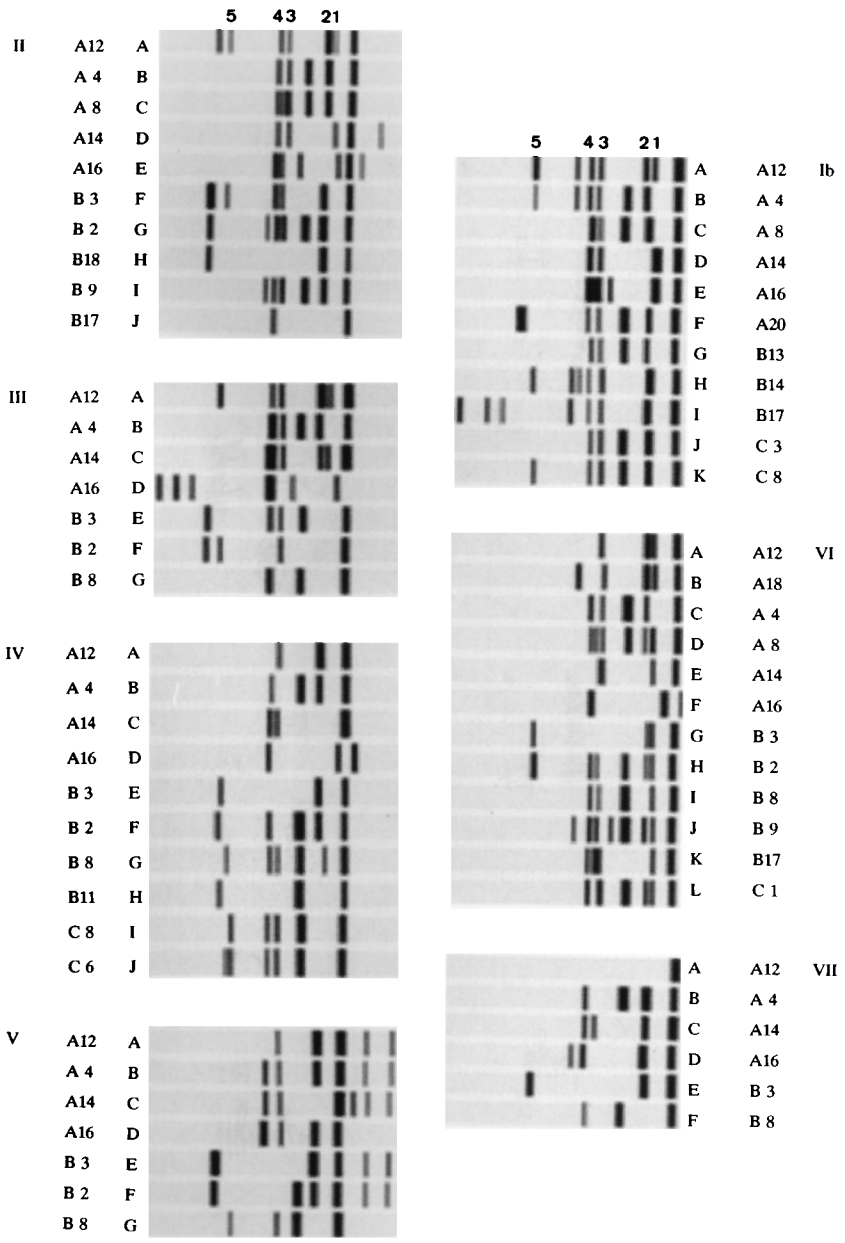

FIG. 2. Survey of unique PCR fingerprints as generated by amplification of staphylococcal DNA by primer 7. In panels Ib through VII, a survey of the unique banding patterns as observed by the various participants is represented schematically. Strain numbers and single-character PCR genocodes are indicated alongside the separate panels, as in Table 1. The migration distance of the DNA fragments in panels Ib, VI, and VII is different from those in panels II to V. For reasons of comparison, some of the common bands are identified with a number (1 through 5) above the panels. Dataset Ia has not been included in this comparison; in dataset VII, pattern B′ has been omitted because of its similarity to pattern B.

(n = 16) clustered 37 of 40 epidemiologically linked strains (including the NH1 and NH2 strains) correctly; however, 8 of the 20 unrelated isolates could not be distinguished from strains within the outbreak groups. When the dataset displaying the maximal number of PCR types (n = 30) was evaluated, 29 of 40 strains were clustered. This is not an improvement when compared with the least discriminative data. Among the 20 unrelated strains, six genotypes were detected which were also found among the epidemiologically linked strains. Apparently, the rise in the absolute number of detectable PCR genotypes adversely affects the correlation with the epidemiological data.

Comparison with PFGE. PFGE is currently considered to be one of the most reliable and reproducible typing procedures, allowing the detection of a high degree of DNA polymorphism (15). PCR and PFGE data were compared; the results are described in Table 4. First, the PCR codes for the two groups detecting the largest (dataset IV) and the smallest (dataset Ib) number of types were simplified. The three-letter code was condensed into a single digit, and new types were defined only when more than one individual AP-PCR assay gave a different result. In case of a single change (from AAA to AAC, for instance) subtypes were defined. The results for set Ib were rearranged into 11 types and 5 subtypes, and the data for set IV

TABLE 4. Comparison of PCR fingerprinting and PFGE on the basis of simplified genetic codes for the PCR assay[a]

Strain	PCR code		Simplified code		PFGE code
	Ib	IV	Ib	IV	
A16	FEF	FDF	1	1	I
A4	BBB	BBB	2	2	E
A12	AAA	AAA	3	3	J
A18	AAA	AAA	3	3	J
A20	AAA	AAA	3	3	J
A6	AAC	AAA	3a	3	C
A7	AAC	CAA	3a	3a	B
A8	CCD	DBC	4	4	G
A11	CCD	DBD	4	4a	F
A1	AAA	AAA	3	3	K1
A9	AAA	AAA	3	3	K2
A3	AAA	AAA	3	3	A
A13	AAA	AAA	3	3	A
A14	EDE	-CE	5	5	H
A19	AAA	AAA	3	3	K3
A17	AAA	AAA	3	3	A
A2	AAA	AAA	3	3	A
A15	AAA	AAA	3	3	A
A5	AAA	AAA	3	3	A
A10	DAA	EAA	3b	3b	D
B7	BBB	BBH	2	2a	D
B3	AAA	AE-	3	3c	A
B5	AAA	AEA	3	3c	A
B10	AAA	AEA	3	3c	A
B12	AAA	AEL	3	6	A1
B15	AAA	AEL	3	6	A
B19	AAA	AEL	3	6	A
B20	AAA	AEL	3	6	A
B1	AAA	AEA	3	3c	A1
B16	AAA	AEL	3	6	A1
B18	AAA	AEK	3	6	A
B17	JIM	LCO	6	7	E
B14	AHL	AAN	7	3d	A2
B8	HBI	IGI	8	8	F
B2	GFH	GFG	9	9	B
B4	GFH	HFG	9	9a	B
B6	GFH	HFG	9	9a	B
B11	GFK	KHK	9a	10	C
B9	IDJ	JBJ	10	11	B
B13	GGJ	RBM	11	12	B1
C3	BJB	OBP	2a	13	C
C1	BJB	MBP	2a	13a	A
C4	BJB	MBP	2a	13a	A
C5	BJB	-BP	2a	13a	A
C9	BJB	MBP	2a	13a	A
C11	BJB	MBP	2a	13a	A
C12	BJB	MBP	2a	13a	A
C14	BJB	MBP	2a	13a	A
C15	BJB	MBP	2a	13a	A
C17	BJB	MBP	2a	13a	A
C20	BJB	MBP	2a	13a	A
C8	HKI	PII	2a	14	B1
C2	HKI	NII	8a	14a	B
C6	HKI	PJI	8a	15	B
C7	HKI	PJI	8a	15	B
C10	HKI	-II	8a	14a	B
C13	HKI	NII	8a	14a	B
C16	HKI	PJI	8a	15	B
C18	HKI	PJI	8a	15	B
C19	HKI	PII	8a	14	B

[a] The datasets Ib and IV have been simplified by changing three-letter codes into one-letter codes. New types were defined when at least two characters from the three-letter code had changed; single assay changes result in subtyping (a through d). Strain numbering is as in Table 1.

defined 15 types and 9 subtypes. PFGE recognized 11 types (A through K) and 5 subtypes, equaling the numbers detected in the set Ib experiments. Nearly full epidemiological agreement exists between these latter data and PFGE results. The only difference occurs for the NH1 outbreak: PFGE found three instead of the expected two types (isolate 14 is also recognized as a deviant type by other procedures). The set IV data subdivide strains from outbreak I and IV and, as such, give rise to an overestimation of the number of types.

Gelcompar analysis. Gelcompar analysis of the results was disappointing. Of eight datasets, only four were accessible to scanning reproduction. Of these four datasets, only one could be used for successful phylogenetic analysis. The other three composite pictures could not be analyzed because of lack of contrast, excessive smiling of the gels, and low-resolution photography. In the single instance in which an interpretable phylogenetic tree could be constructed, it appeared that the result was in agreement with visual inspection and epidemiological data (Fig. 3). The four sets of outbreak-related strains were clustered with homology percentages from 79 up to 93%, when data gathered with the three primers were combined. Clearly, Gelcompar analysis is heavily influenced by electrophoretic and photographic artifacts.

DISCUSSION

Approximately 5 years ago, PCR-mediated procedures enabling genome scanning by random amplification of polymorphic DNA were discovered (29, 31). AP-PCR can be used for genetic characterizations and comparisons even among closely related bacterial species and isolates (1, 20, 21, 24–26, 28, 30). The procedure is used with increasing frequency, facilitated by general applicability and high speed. However, only a limited number of studies have compared the effectiveness of AP-PCR typing with that of other microbiological typing procedures (4, 7, 16, 19–21, 24, 25). In the field of staphylococcal typing, numerous studies describe conventional or molecular elucidation of clonality or epidemiological relatedness. Recently, this was combined in a comparative study on typing of a large panel of *S. aureus* isolates (22).

The overall conclusion from the present data is that AP-PCR adequately clusters strains isolated from given outbreaks. On the other hand, considerable differences between the results from different laboratories have been encountered. This is reflected by the number of isolates that are identified by a unique genotype. This number varies from 9 to 16, and participants who detect more than one type among epidemiologically clustered strains score relatively highly in this respect. It has to be emphasized that during this study, several of the experimental parameters were standardized. In this respect, it is noteworthy that a relatively high degree of heterogeneity between laboratories was encountered as a result of this limited number of additional variables (Table 2). Including the DNA isolation protocol in the multicenter approach would most probably have led to an even lower degree of interlaboratory reproducibility.

In the single multicenter AP-PCR typing study that has been described to date (17), the time- and cost-effectiveness of PCR typing were investigated. This study demonstrated that successful AP-PCR depends heavily on the optimal use of PCR protocols (see, for instance, reference 5). For this reason, it was decided not to study the intralaboratory reproducibility of the AP-PCR tests. These items have been addressed in previous studies (7, 21, 23–26). However, the fact that epidemiological clusters of strains generate identical DNA-banding patterns upon DNA amplification is evidence of at least a reasonable

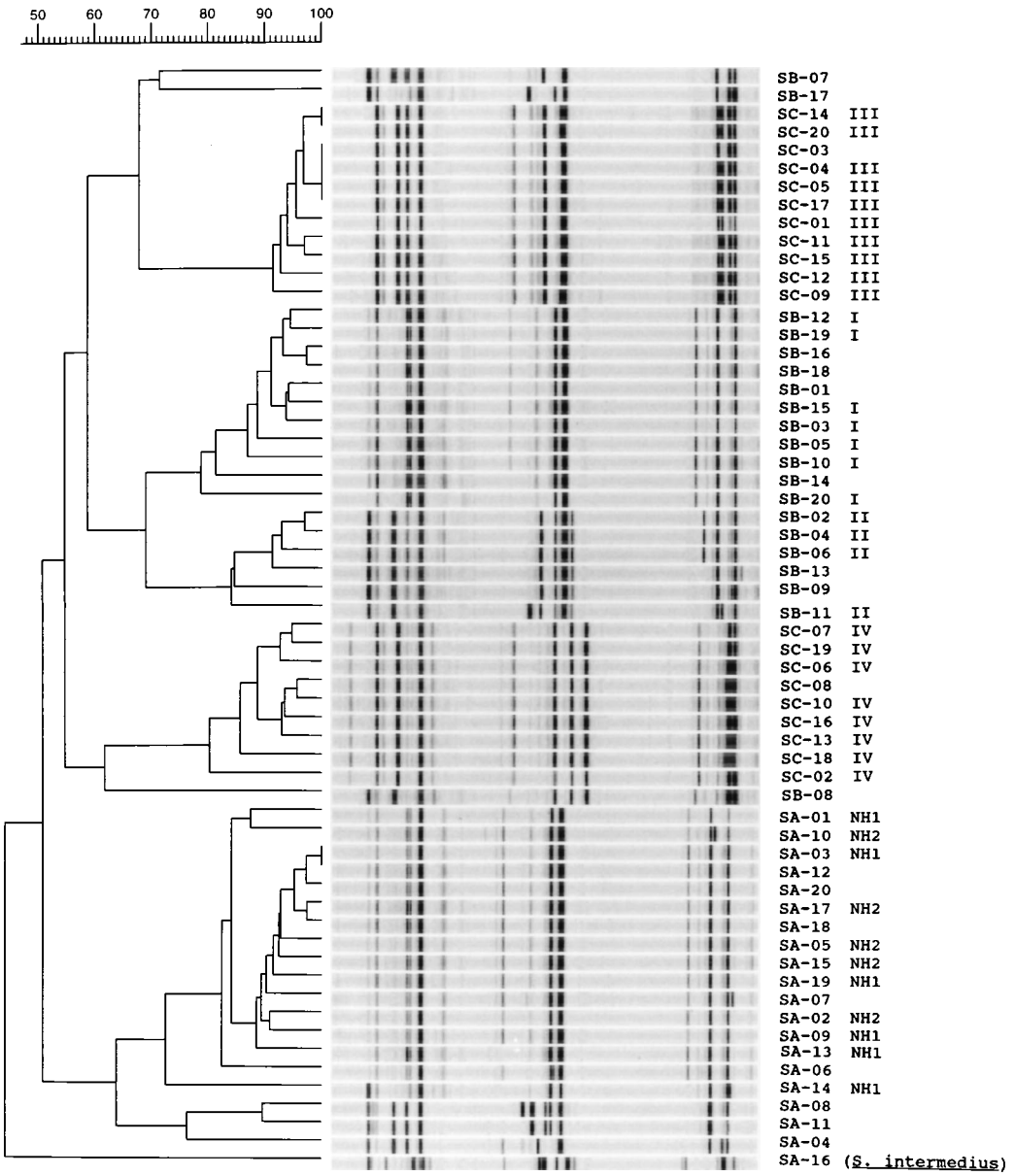

FIG. 3. Gelcompar analysis of dataset Ia. The pictures shown in Fig. 1 have been digitized by scanning procedures. All three AP-PCR DNA-banding patterns have been combined into one single lane. The degree of homology was subsequently calculated by Dice comparisons, and correlation coefficients were determined by the unweighted pair group method with arithmetic averages. The strain code as presented in Table 1 is shown on the right, together with the deduced three-digit genocode (column Ia, Table 1) and the epidemiological clustering.

degree of intralaboratory reliability. Upon reamplification of some of the DNA samples, as performed in two of the participating laboratories, AP-PCR profiles appeared to be reproducible.

Reproducibility was affected by the nature of the primer used and the identity of the intratube thermoprofile. Isolates SA-04, SB-07, and SC-03, all of which were *S. aureus* ATCC 12600, were included in the three sets of strains to evaluate the reproducibility of AP-PCR. Only one of the participating laboratories unequivocally identified all three strains to belong to a single genotype. It must be emphasized that the other typing procedures also detected gross differences among these three particular isolates. It has been demonstrated previously that genetic variability as measured by PCR can be a consequence of repeated conservation and "reviving" of strains; this is probably due to replication defects or the absence or presence of lytic phages (3). This might be an explanation for the extensive variability encountered among the ATCC strains. PFGE, for instance, corroborates the PCR data in six of seven PCR datasets by designating genotypes E, D, and C. Plasmid types are also very different: B, D, and C are the indexes. This, in combination with other experimental results, may be indicative of intrastrain heterogeneity or sampling error. Computerized correction of AP-PCR artifacts is currently under development (13, 14). It is particularly important to implement this approach, which takes reproducibility and erroneous amplification into account, when multicenter studies are performed. However, on the basis of the results of the present study, it is

expected that interinstitute standardization will be very hard to achieve.

PCR typing is currently restricted to laboratories with appropriate equipment and experimental expertise. In this respect, the applicability of AP-PCR is as yet limited. It is clear from the present study that generation and interpretation of PCR data are likely to vary among laboratories. The percentage of variant types identified can be on the order of 27 to 50%, based on the application of three PCR tests and a single DNA-processing protocol. The variables that still exist between laboratories (Table 2) must be responsible, at least in part, for these large discrepancies. From the duplicate experiments performed in Rotterdam, it was concluded that gel electrophoresis is a major cause of experimental variability; this has been confirmed by a recent report (10). An increase in electrophoresis time led to improved separation, which in turn enabled successful digitization and Gelcompar analysis. It is also acknowledged that the present study suffers from the fact that DNA isolation and primer quality were standardized. If this had not been the case, differences between laboratories may have been even larger.

AP-PCR shares characteristics with the genome-scanning capacities of electrophoretic techniques such as PFGE and FIGE. These last two procedures identify epidemiological relations for staphylococci that are in good agreement with the present data (Table 4). Recently, guidelines for interpretation of PFGE patterns for outbreak investigations were proposed by an American working group (8). Since these rules have been used to define staphylococcal subtypes in the collection used in the previous and present studies (22), our PCR data may enable the development of similar rules for the definition of PCR subtypes. The rules should be based either on differences within the banding pattern generated during a single PCR or on differences in composite genocodes as presented in Table 1 and simplified in Table 4. The present study indicates that differences in the combined results of multiple AP-PCR assays are better indicators of genetic variability than are the results of individual assays. It is clear, however, that PFGE subtypes do not fully match the subtypes as defined by PCR. It is advisable to perform model studies starting with PFGE-uniform strains on the one hand and PCR-uniform strains on the other hand. For studies such as these, it is also important to start the analysis with a collection comparable to the set of strains in this study: it should provide a mixture of unique, solitary isolates together with epidemiologically well-defined clusters.

Our data demonstrate that PCR fingerprinting deserves a position among the procedures that are well suited for the epidemiological analysis of *S. aureus*. The procedure seems particularly appropriate for the high-speed typing of nosocomial isolates. This conclusion was also drawn previously (2); on the basis of theoretical considerations, it was suggested that AP-PCR is a cost-effective procedure as well. It is necessary to test multiple primers, since differences in discriminatory power are to be expected. Strain-specific amplicons can be generated quite easily, even among clonally related isolates of *S. aureus*. With the exclusion of subtype numbering for the other typing strategies, AP-PCR generates the largest number of individual types. It exceeds the resolution of PFGE, which detects 11 types and 5 subtypes. Only phage typing and RFLP mapping approach the average number of types detectable by AP-PCR. It must be emphasized, however, that the generation of excessive numbers of types introduces the possibility that the relationship between the AP-PCR data and epidemiological characteristics will start to deteriorate.

Finally, we recommend establishing collections like the one used in this and the previous study (22) for other microorganisms as well. The availability of these strains enables the individual researcher to establish the value of newly developed typing tools or to use these strains as internal controls in typing studies. Well-documented collections or experimental protocols can be used for standardization of typing procedures (27), an initiative important for the development of international standards on genetic relatedness or clonality among pathogenic microorganisms.

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