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Optical Fingerprinting in Bacterial Epidemiology: Raman Spectroscopy as a Real-Time Typing Method[▽]

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Hospital-acquired infections (HAI) increase morbidity and mortality and constitute a high financial burden on health care systems. An effective weapon against HAI is early detection of potential outbreaks and sources of contamination. Such monitoring requires microbial typing with sufficient reproducibility and discriminatory power. Here, a microbial-typing method is presented, based on Raman spectroscopy. This technique provides strain-specific optical fingerprints in a few minutes instead of several hours to days, as is the case with genotyping methods. Although the method is generally applicable, we used 118 *Staphylococcus aureus* isolates to illustrate that the discriminatory power matches that of established genotyping techniques (numerical index of diversity [*D*] = 0.989) and that concordance with the gold standard (pulsed-field gel electrophoresis) is high (95%). The Raman clustering of isolates was reproducible to the strain level for five independent cultures, despite the various culture times from 18 h to 24 h. Furthermore, this technique was able to classify stored (−80°C) and recent isolates of a methicillin-resistant *Staphylococcus aureus*-colonized individual during surveillance studies and did so days earlier than established genotyping techniques did. Its high throughput and ease of use make it suitable for use in routine diagnostic laboratory settings. This will set the stage for continuous, automated, real-time epidemiological monitoring of bacterial infections in a hospital, which can then be followed by timely corrective action by infection prevention teams.

Hospital-acquired infections (HAI) are among the most pressing problems in modern health care. Up to 10% of all admitted patients become infected while hospitalized, with incidences of 30% being reported in intensive-care units (8, 23). These infections cause significant increases in morbidity and mortality and also have considerable impact on the costs of health care (4, 5, 16, 21). Direct medical costs per patient of between $27,000 and $35,000 have been reported (5).

Up to 30% of HAI are caused by transmission and can therefore be prevented by means of infection control strategies (6). Several studies have emphasized the importance of active screening of patients in order to identify transmission of microorganisms. This detects possible cases of cross-transmission and thereby indicates the infection control measures to be taken, including isolation of patients (1, 2). Rapid assessment of microbial clonal relationships (typing) enables tracking of the spread of pathogens, which may be used to significantly decrease the number of HAI and additional costs, even in high-risk situations (14, 17).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of HAI, and numerous DNA-based typing methods have been developed for the organism. Therefore, we used MRSA as an example to demonstrate the capabilities of a new typing method.

Pulsed-field gel electrophoresis (PFGE) is generally the most preferred typing method (9), followed by multilocus sequence typing (MLST) and *spa* typing. All these techniques require dedicated facilities, are labor-intensive, and have a long turnaround time (48 to 72 h when starting from a pure culture) and a low sample throughput. Therefore, they are unsuitable for routine use in hospitals and, consequently, are mostly applied in retrospective analysis of outbreak situations.

An ideal typing method should be rapid and simple and have high throughput and good discriminatory power and should provide results that are in concordance with the epidemiological data (19). We present a novel approach to meet these requirements, based on Raman spectroscopy. Raman spectra of bacteria are representations of their overall molecular composition and can be used as highly specific spectroscopic fingerprints. We used four different collections of methicillin-sensitive *S. aureus* (MSSA) and MRSA isolates to demonstrate the effectiveness of Raman spectroscopy as a typing tool that could be used in epidemiological surveillance studies and concluded that this technique is an easy-to-use and rapid alternative in the battle against MRSA.

MATERIALS AND METHODS

Bacterial strain collections. Four different strain collections were used in this study.

Collection I was used as a reference collection and contained 20 well-charac-

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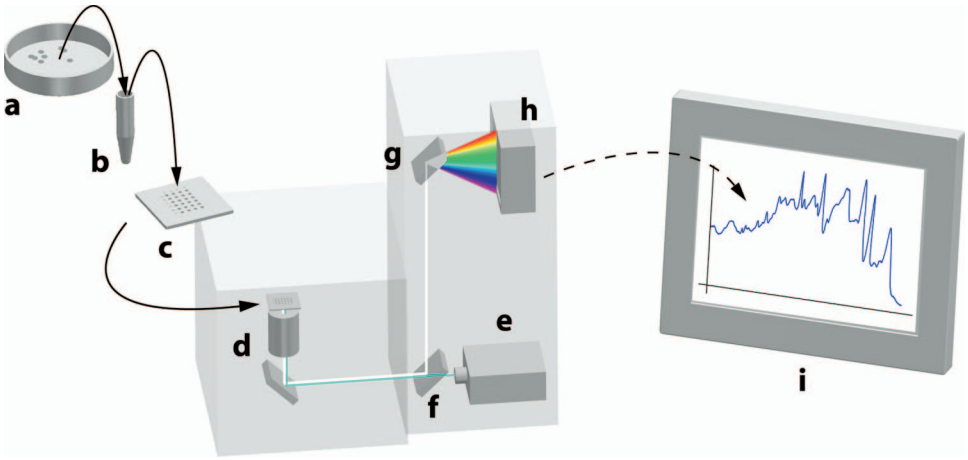

FIG. 1. Overview of Raman procedure and spectrometer. Biomass from a bacterial culture (a) on TSA medium is collected using a 1-μl inoculation loop and suspended in 5 μl of demineralized water (b). After a brief centrifugation step to remove air bubbles, the wet pellet is transferred onto a fused silica slide (c), where it is allowed to dry (a typical slide holds 24 samples). The slide with the dried biomass is placed in the measurement stage (d), where the samples are illuminated with laser light (e). The Raman signal generated is collected along the same optical path and separated from the laser light using an optical filter (f) that only reflects light of a higher wavelength than the laser. The laser light is passed through. The wavelength of the Raman signal is dispersed on an optical grating (g) and collected using a near-infrared-optimized charge-coupled device detector (h). The Raman spectra are gathered, stored, and analyzed on a personal computer (i).

terized MRSA isolates. These isolates have previously been analyzed using multiple typing techniques (22) and have been used to determine the interlaboratory reproducibility of PFGE in a multicenter study (20). This collection contains 5 isolates with identical PFGE patterns, 5 isolates with related PFGE patterns (patterns that differ by ≤3 bands), and 10 unrelated isolates (with patterns that differ by >3 bands).

Collection II was obtained from the Department of Medical Microbiology and Infectious Diseases of the Erasmus University Medical Center, Rotterdam, The Netherlands (Erasmus MC), and included all 78 MRSA isolates collected in the year 2002 in this tertiary-care Dutch hospital. The isolates were retrieved from patients, as well as from health care workers. All of the isolates have been analyzed independently using PFGE at the Department of Medical Microbiology at the Erasmus MC, as well as at the Dutch National Institute for Public Health and the Environment (RIVM).

Collection III was obtained from the Department of Microbiology at the University Hospital Maastricht, Maastricht, The Netherlands, and contained five isolates retrieved from a MRSA-colonized member of the hospital staff collected over a period of 18 months and four isolates obtained from different patients during an MRSA contact screening.

Collection IV contained four MRSA isolates (one from a patient and three from a colonized staff member) and seven MSSA isolates obtained during an MRSA contact screening in January 2007 at the Erasmus MC.

Storage and culture. The isolates were stored at −80°C in brain heart infusion broth (Becton Dickinson, Franklin Lakes, NJ) containing 10% glycerol until they were used.

For Raman measurements, all isolates were grown overnight on Trypticase soy agar (TSA) (Becton Dickinson, Franklin Lakes, NJ). After this first culture was checked for purity, three to five colonies were picked to fill a calibrated 1-μl loop and suspended in 20 μl of sterilized demineralized water (aquadest, prepared in house). This suspension was diluted 100 times in aquadest, and 20 μl was plated on a TSA plate. These plates were incubated for 20 h at 35°C to obtain a confluent bacterial layer.

PFGE. PFGE was performed according to the Harmony protocol (3). Briefly, a suspension of bacteria was mixed with 1% InCert agarose (FMC Bioproducts). The agarose plugs were incubated with lysostaphine (Sigma-Aldrich, Zwijndrecht, The Netherlands), and spheroplasts were lysed using proteinase K (Sigma-Aldrich). DNA was digested by SmaI (Fermentas, St. Leon-Rot, Germany). Macro-restriction fragments were separated using a Bio-Rad Chef Mapper (Bio-Rad, Veenendaal, The Netherlands) with a total run time of 20 h. PFGE patterns were assigned based on unique patterns (19).

***spa* typing.** Real-time amplification of the *spa* locus, followed by sequencing, was performed as described previously (15). The *spa* types were clustered into *spa* clonal complexes (CCs) using the algorithm Based upon Repeat Pattern (BURP) with the Ridom StaphType version 1.5 software package (http://www.ridom.de). The default settings recommended by the manufacturer were used. The associated CCs, as determined by MLST, were allocated through the Ridom SpaServer (http://spaserver.ridom.de).

Sample preparation for Raman spectroscopy. For sample preparation, a calibrated 1-μl loop was filled with biomass and suspended in 5 μl of demineralized water. After a centrifugation step for 1 min at 12,000 × *g* to remove air bubbles, the pellet was resuspended and transferred to a fused silica slide (Hellma Benelux BV, Aartselaar, Belgium). On this slide, a removable silicone isolator (Sigma-Aldrich, Zwijndrecht, The Netherlands) containing 24 wells was placed. Samples were allowed to dry for 20 min at 35°C.

Raman spectroscopy. A scheme of the custom-built Raman setup that was used for measuring Raman spectra of the bacterial samples is shown in Fig. 1. The instrument consisted of a Model 2500 High Performance Raman Module (River Diagnostics BV, Rotterdam, The Netherlands) coupled to a custom-built measurement compartment. The measurement compartment was equipped with an automated *x*- and *y*-axis-positioning stage, which held the fused silica slide with bacterial samples and a custom-designed near-infrared-optimized microscope objective (numerical aperture, 0.7). The objective was used to focus laser light emitted by the Model 2500 High Performance Raman Module in the samples on the fused silica slide and to collect Raman scattered light from the samples. Approximately 220 mW of laser light at 785 nm was used to illuminate the samples (Sacher Tiger; Sacher Lasertechnik, Marburg, Germany). The spectrometer was calibrated according to the manufacturer's guidelines. In each sample, 10 to 60 spectra at a 1-s collection time were measured at different positions.

Data analysis. (i) Signal preprocessing. The measured spectra contained three sources of noninformative signal variance: the signal contribution from the fused silica substrate, a varying broadband background signal of the Raman spectra of the bacteria, and various signal contributions obtained from the carotenoids in the *S. aureus* cells. The elimination of this noninformative signal variance is described in detail elsewhere (18). It is based on the use of extended multiplicative scattering correction (12) and also uses the sensitivity to photodecomposition of carotenoids. The resulting spectra all had identical spectral backgrounds and carotenoid signal intensities.

The software scripts for spectrum pretreatment and data analysis were written in MATLAB version 7.1 (Mathworks, Natick, MA).

(ii) Calculation of similarities between spectra. The similarity between pairs of spectra was calculated using the squared Pearson correlation coefficient (R^2) and multiplied by 100 to be expressed as percentages.

(iii) Hierarchical cluster analysis. Cluster analysis of sets of spectra was performed using the pairwise similarities as a distance matrix, in combination with Ward's cluster algorithm. This resulted in a dendrogram in which each node represented the lowest correlation coefficient (or similarity) between all isolates combined in the cluster defined by this node.

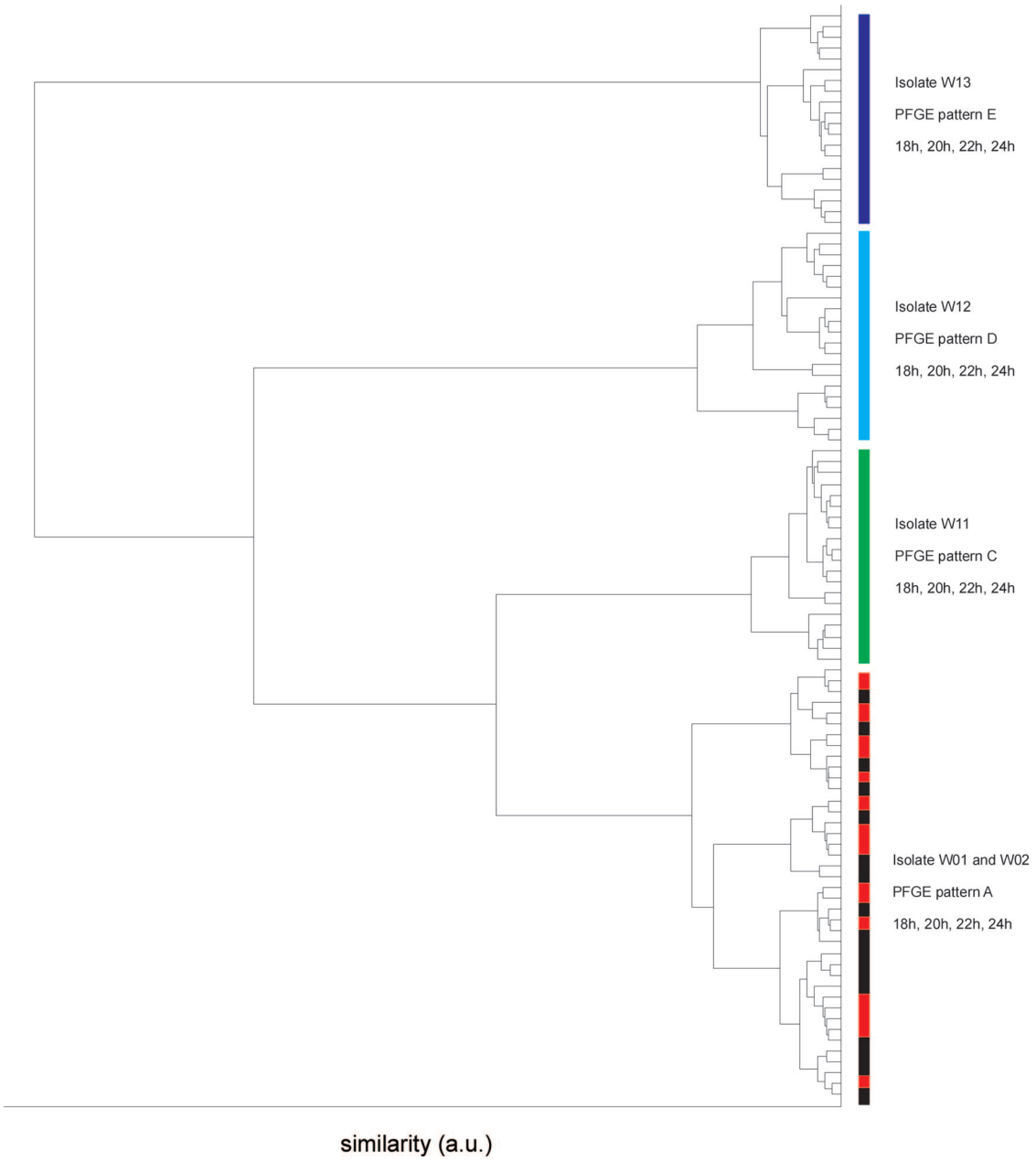

FIG. 2. Reproducibility of Raman spectroscopy. Shown are the results of hierarchical cluster analysis of the repeated measurements of five MRSA isolates measured after different culturing times. Each isolate is indicated with a colored label. a.u., arbitrary units.

For each isolate collection, the five genetically identical isolates of collection I were measured and analyzed simultaneously and used to indicate clusters, or Raman types.

The individual steps of this process were as follows. In step 1, the node where the five identical reference isolates of collection I combined in the dendrogram was defined. In step 2, the correlation coefficient represented by this node was used as a cutoff value in the dendrogram. In step 3, all isolates were combined in a cluster in which, if the value of the node was higher than the correlation coefficient found for the reference, the isolates were considered to be indistinguishable.

All clusters defined by this procedure could then be validated using previously known typing results of the isolates, epidemiological data, or a collection of reference isolates.

(iv) Discriminatory power. The discriminatory power of a typing method is defined as its ability to assign a different type to two unrelated strains (19). To quantify the discriminatory power of Raman spectroscopy, the numerical index of diversity (*D*) was calculated as described by Hunter and Gaston (7).

Experiments applied to evaluate Raman spectroscopy. (i) Reproducibility and influence of culturing time. Raman spectra represent the overall molecular composition of bacterial cells. Since differences in culture conditions may influence the metabolic pathways and therefore the molecular makeup of bacterial cells, changes in growth conditions may lead to changes in spectra and negatively affect the reproducibility of the Raman procedure.

Two MRSA isolates with identical PFGE patterns and three MRSA isolates with unique PFGE patterns were selected from collection I to evaluate the reproducibility of the Raman technique. All isolates were cultured in five independent sessions, and in each session, samples were prepared and measured after 18 h, 20 h, 22 h, and 24 h of incubation time, yielding a total of 20 samples for each isolate.

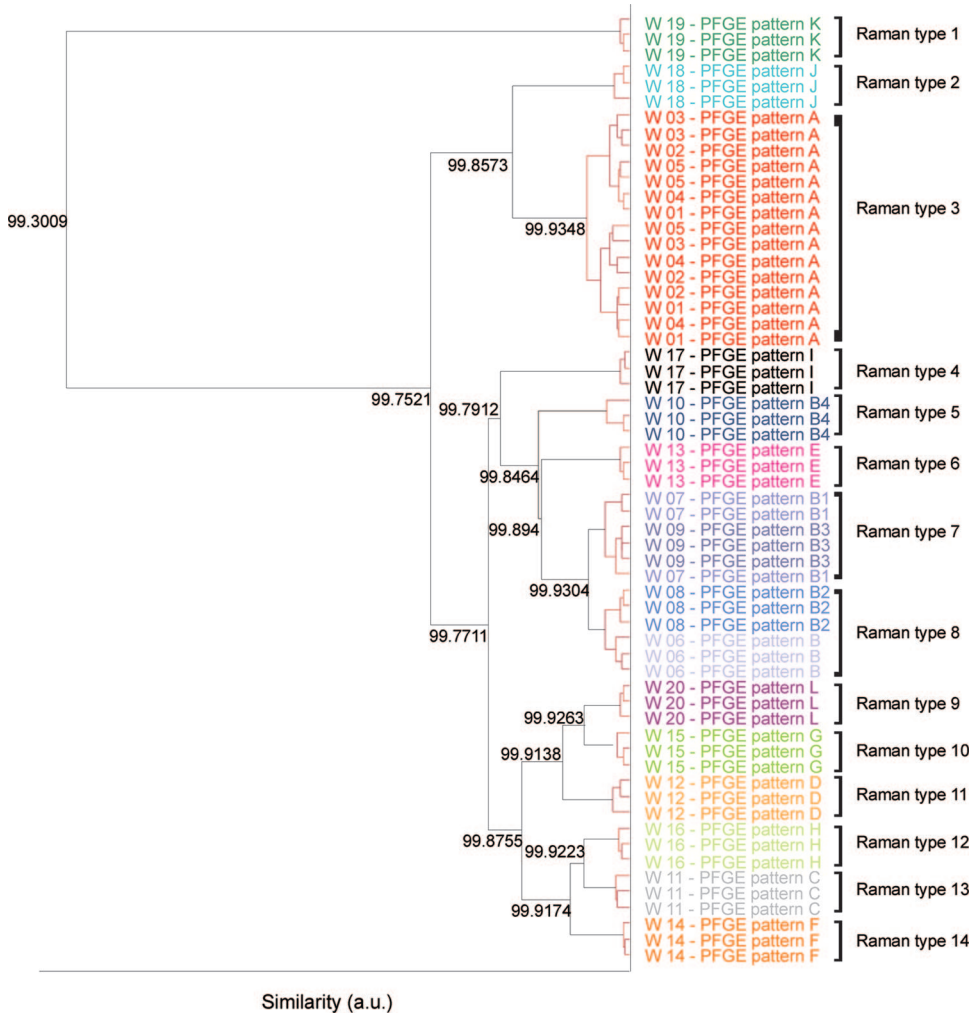

FIG. 3. Hierarchical cluster analysis of the MRSA isolates of collection I. Clusters are indicated in red and are based on the correlation coefficient of the five isolates with identical PFGE patterns (pattern A). For each node with a correlation coefficient lower than the cutoff, the corresponding value is indicated. a.u., arbitrary units.

(ii) Reproducibility and concordance with other typing techniques. PFGE is considered the gold standard for typing of *S. aureus* isolates. Therefore, the 20 MRSA isolates of reference collection I were used to compare the results of the Raman typing with previously obtained typing results (22).

Experiments to evaluate the application of Raman spectroscopy in epidemiological surveillance. (i) Retrospective study: concordance with clinical epidemiology. The 78 clinical MRSA isolates of collection II were used to validate the concordance of Raman typing with clinical epidemiological data.

(ii) Historical isolates. Collection III contains MRSA isolates obtained from a staff member that were collected over a period of 18 months. These isolates were used to evaluate the ability of Raman spectroscopy to recognize historical and recent isolates from a colonized person.

(iii) Prospective study: comparative typing. Collection IV represents isolates obtained during an MRSA contact screening and was used to validate Raman spectroscopy to assess relatedness within a small set of isolates (comparative typing) in a possible outbreak situation.

RESULTS

Experiments were carried out to test the reproducibility of Raman spectroscopy; its discriminatory power in comparison with established typing techniques, such as PFGE and *spa* typing; and its concordance with epidemiological data.

Evaluation of Raman spectroscopy. (i) Reproducibility and influence of culturing time. To evaluate the influence of culturing time on the reproducibility of Raman spectra, five isolates of reference collection I were cultured five times and spectra were measured after 18 h, 20 h, 22 h, and 24 h of incubation time.

As expected, based on their PFGE patterns, isolates W01 and W02 were found in one distinct cluster, indicating that they were indistinguishable using Raman spectroscopy (Fig. 2). For the unrelated isolates W11, W12, and W13, Raman clustering was isolate specific, since three clusters containing the spectra of only one isolate were formed. For all isolates, the Raman clustering was equal for each of the five repeats and independent of culturing times that varied from 18 h to 24 h.

(ii) Reproducibility and concordance with PFGE. In Fig. 3 are shown the results of a hierarchical cluster analysis performed on the Raman spectra of the 20 MRSA isolates of reference collection I. One distinct cluster was found containing the repeated measurements of the PFGE-identical isolates W01 to W05, indicating that they were also indistinguishable

TABLE 1. Results of the Raman-based typing and PFGE analysis of the 78 isolates of collection II

Raman type	No. of isolates	PFGE pattern	
		MMB[a]	RIVM[b]
1	1	BG	cl 29a
2	2	AM	cl 29
3	1	AZ	cl 144
4	1	AZ1	cl 144
5	13	C (n = 11)	cl 38 (n = 11)
		AN (n = 2)	cl 37 (n = 2)
6	1	BE	cl 168
7	1	AT	cl 86
8	1	AU	cl 67a
9	1	J	cl 68
10	1	Q	cl 24
11	1	AP2	cl 30
12	3	AK	cl 34
13	1	N	I-67
14	1	A	cl 16c
15	1	L	cl 20b
16	1	U	cl 84
17	3	M	cl 15
18	1	BH	cl 209
19	1	BA	cl 122
20	1	AJ	cl 35a
21	3	J1	cl 22
22	8	R	cl 16
23	1	AO	XI-27
24	1	AP1	cl 18
25	2	AC	cl 23
26	1	J	cl 23
27	1	BJ	cl 218a
28	1	AP1	cl 30
29	23	AP1	cl 18

[a] PFGE analysis was performed at the Department of Microbiology of the Erasmus MC.
[b] PFGE analysis was performed at the RIVM.

using Raman spectroscopy. The lowest correlation coefficient between spectra in this cluster was 99.9348. Taking this value as a cutoff to determine Raman types, the PFGE-related isolates W07 and W09 were both classified as Raman type 7, and the PFGE-related isolates W06 and W08 were classified as Raman type 8. The remaining PFGE-related isolate, W10, and the 10 unrelated isolates were found in separate clusters and were therefore assigned unique Raman types. In total, 14 different Raman types were assigned in this collection compared to 16 unique PFGE patterns or 11 PFGE types based on ≤3 band differences between patterns (20).

For this collection, D was calculated according to the method of Hunter and Gaston (7). Although the number of isolates was low, we see that Raman spectroscopy scored high (D = 0.989).

Application of Raman spectroscopy. (i) Retrospective study: concordance with clinical epidemiology. A collection (collection II) of 78 MRSA isolates for which the clinical and epidemiological data were known was evaluated. All isolates were analyzed in house using a standardized PFGE protocol (13). PFGE results for the same isolates were obtained from the RIVM. Both the Raman and PFGE results are summarized in Table 1.

Raman spectroscopy resulted in 29 different Raman types among the 78 isolates of this collection. According to the in-house PFGE analysis, these isolates could be divided into 27 unique patterns, while the RIVM found 27 different PFGE patterns. Raman spectroscopy differentiated 72 out of 78 isolates identical to the in-house PFGE (92%) and 74 out of 78 isolates identical to the PFGE results of the RIVM (95%). These findings are comparable to the concordance found between the PFGE results obtained at the two different institutions (75 out of 78 isolates, or 96%).

In total, three discrepancies were documented between the different typing methods. The first discrepancy involved isolates from a patient and a staff member that shared the same PFGE pattern according to the in-house PFGE (both pattern J) but had unique Raman types (type 9 and type 26), as well as unique RIVM-PFGE patterns (cl23 and cl68). No epidemiological connection between these two people was documented. Although this does not exclude the possibility that these patients were colonized or infected with the same MRSA strain, as suggested by the in-house PFGE data, the epidemiological data are in agreement with both the Raman typing and the RIVM-PFGE results.

Thirteen isolates obtained from 11 patients and two staff members were found to share a single Raman type (type 5), but two different PFGE patterns were defined for these isolates (pattern AN/cl37 and pattern C/cl38). No epidemiological information is available to establish a relationship between these two groups.

The last discrepancy involved two staff members with Raman types 24 and 28. According to PFGE, their isolates belonged to a large group of isolates with type AP1 or cl18. The epidemiological data also suggested that these isolates were obtained during the same outbreak, but each had a unique Raman type.

(ii) Historical isolates. During a contact screening at the University hospital Maastricht, one staff member and four patients were found to be positive for MRSA. The staff member was known to carry MRSA for a longer period of time, and isolates from an 18-month period were included in the study. The isolates were previously analyzed using *spa* typing and belonged to the same *spa* CC. MLST data were associated through the SpaServer and revealed that all the isolates also belonged to the same MLST CC (Table 2).

One Raman cluster was found that contained all of the spectra of the patients and the staff member.

(iii) Prospective study: comparative typing. During a contact screening at the Erasmus MC, four MRSA isolates (one from the index patient and three from colonized staff member 1) and seven MSSA isolates were obtained. As shown in Table 2, the MRSA isolate from the index patient was defined as Raman type 8, while the three MRSA isolates from staff member 1 were defined as Raman type 9. These findings suggested that there was no transmission between the index patient and the staff member. PFGE confirmation of these results was obtained 4 days later (Fig. 4).

DISCUSSION

An easy-to-use, high-throughput, fast, and reliable typing system that allows real-time monitoring of the spread of bacterial isolates is essential for the epidemiological surveillance of microorganisms. Two important quality requirements for a

TABLE 2. Result of the Raman based typing of the MRSA and MSSA isolates of collections III and IV[a]

Collection	Isolate	Patient	Isolate type	Raman type	PFGE pattern		*spa* type[d]	*spa* CC[d]	MLST CC[e]
					MMB[b]	RIVM[c]			
III	6	Staff member	MRSA, historical isolate	1			t447	1	5
	8	Staff member	MRSA, historical isolate	1			t447	1	5
	25	Staff member	MRSA, historical isolate	1			t447	1	5
	BL-1	Staff member	MRSA, recent isolate	1			t447	1	5
	BL-2	Staff member	MRSA, recent isolate	1			t447	1	5
	BL-6	Patient 1	MRSA	1			t447	1	5
	BL-8	Patient 2	MRSA	1			t447	1	5
	BL-9	Patient 3	MRSA	1			t447	1	5
	BL-10	Patient 4	MRSA	1			t2738	1	5
IV	00028	Patient 1	MRSA, historical isolate	8	NT[f]	NT			
	00014	Staff member 1	MRSA, historical isolate	9	A				
	00015	Staff member 1	MRSA, historical isolate	9	A	cl 34			
	00016	Staff member 1	MRSA, recent isolate	9	A				
	00017	Patient 2	MRSA, recent isolate	2	B				
	00018	Staff member 2	MRSA	3	B1				
	00019	Staff member 2	MRSA	3	B1				
	00020	Staff member 3	MRSA	4	B2				
	00023	Patient 3	MRSA	5	C				
	00024	Staff member 4		6	D				
	00025	Staff member 5	MSSA	7	E				

[a] For both collections, the previous results obtained by PFGE or *spa* typing are indicated.
[b] PFGE analysis was performed at the Department of Microbiology of the Erasmus MC.
[c] PFGE analysis was performed at the RIVM.
[d] *spa* typing was performed at the University Hospital Maastricht.
[e] MLST CCs that are associated with the *spa* types as indicated by the Ridom SpaServer.
[f] NT, nontypeable.

typing system are high reproducibility and good discriminatory power (19). The results presented here, obtained using different collections of *S. aureus* isolates, illustrate that Raman spectroscopy meets these requirements. Multiple independent measurements of the same isolate resulted in identical results. For reference collection I, for example, we found that all replicate measurements of an isolate combined in the same Raman cluster and were assigned a single Raman type. The *D* value calculated for this collection was 0.989. This value is above the generally accepted minimal value of 0.95 for a highly discriminatory technique (19).

Previous research showed that data obtained with Raman spectroscopy can be seen as spectroscopic fingerprints and used for identification to the species and subspecies levels (10, 11). Recently, we transformed Raman spectroscopy into a rapid and easy-to-use typing technique. The current progress entails simplification of the instrument and improved sensitivity and robustness of the spectrometer. Combined with the use of more powerful lasers, this has resulted in a significant reduction in signal collection time. Previously, a signal collection time of 30 s per spectrum was needed; now, a spectrum of the same quality can be obtained with a signal collection time of 1 s. Furthermore, a general procedure has been developed that is similar to the procedures used today in diagnostic microbi-

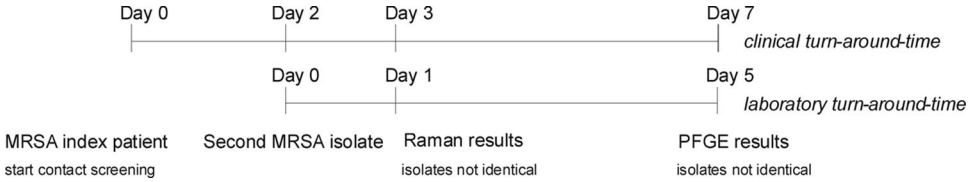

FIG. 4. Contact screening of January 2007. Directly after the first MRSA isolate was isolated, the contact screening was started. Two days later, a second MRSA isolate was found and sent to the RIVM for analysis. At the same time, all isolates were analyzed using Raman spectroscopy. Within 24 h, the Raman results showed that the two MRSA isolates were not identical. This was confirmed by PFGE 4 days later.

ology laboratories. The protocols can be applied to a wide range of organisms, since there is no need for specific labels or dyes or special laboratory equipment, other than the Raman spectrometer. Starting from positive microbial cultures, sample preparation of 24 isolates takes approximately 45 min, including 30 min hands-on time. Raman spectra are collected automatically, and this usually takes 10 s to 1 minute per sample.

To validate typing techniques, a combination of typing data with epidemiological data is needed to confirm an outbreak situation. The Raman clustering obtained for the isolates of collection II revealed good agreement with the epidemiological data. In 2002, five outbreaks were reported in the Erasmus MC that were confirmed by PFGE. Raman spectroscopy recognized these five clusters of isolates and identified the majority of unique PFGE isolates as unique Raman types. Both techniques classified a number of isolates as identical, although no epidemiological link could be found.

One of the main reasons for typing is outbreak investigation. Dutch hospitals use an active "search-and-destroy" policy for MRSA control (24). During such a contact screening, a considerable number of samples have to be screened for the presence of MRSA. Typing methods play an important role in this strategy in order to confirm transmission of isolates (comparative typing). The results obtained with collection III show that Raman spectroscopy enabled historical isolates from an MRSA-colonized individual to be classified as identical, strongly demonstrating a long-time colonization of the staff member and a possible epidemiological link between the staff member and four different patients. Furthermore, both collections III and IV show that, for the contact screenings described here, Raman spectroscopy yielded the same results as established genotyping techniques, but several days earlier.

We conclude that Raman spectroscopy is a highly reproducible, easy-to-use, and rapid alternative to the currently used typing techniques that is suitable for real-time typing in clinical diagnostic laboratories. Such a technique will give infection prevention teams a tool for the continuous monitoring of isolates in their hospitals and will alert them immediately when corrective actions should be taken. This will lead to accurate real-time, rather than retrospective, surveillance and a new approach to combating HAI.

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REFERENCES

1. **Bootsma, M. C., O. Diekmann, and M. J. Bonten.** 2006. Controlling methicillin-resistant *Staphylococcus aureus*: quantifying the effects of interventions and rapid diagnostic testing. Proc. Natl. Acad. Sci. USA **103:**5620–5625.
2. **Clancy, M., A. Graepler, M. Wilson, I. Douglas, J. Johnson, and C. S. Price.** 2006. Active screening in high-risk units is an effective and cost-avoidant method to reduce the rate of methicillin-resistant *Staphylococcus aureus* infection in the hospital. Infect. Control Hosp. Epidemiol. **27:**1009–1017.
3. **Cookson, B. D., D. A. Robinson, A. B. Monk, S. Murchan, A. Deplano, R. de Ryck, M. J. Struelens, C. Scheel, V. Fussing, S. Salmenlinna, J. Vuopio-Varkila, C. Cuny, W. Witte, P. T. Tassios, N. J. Legakis, W. van Leeeuwen, A. van Belkum, A. Vindel, J. Garaizer, S. Haeggman, B. Olsson-Liljequist, U. Ransjo, M. Muller-Premru, W. Hryniewicz, A. Rossney, B. O'Connell, B. D. Short, J. Thomas, S. O'Hanlon, and M. C. Enright.** 2007. Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant *Staphylococcus aureus* (MRSA)—the HARMONY collection. J. Clin. Microbiol. **45:**1830–1837.
4. **Cosgrove, S. E., Y. Qi, K. S. Kaye, S. Harbarth, A. W. Karchmer, and Y. Carmeli.** 2005. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect. Control Hosp. Epidemiol. **26:**166–174.
5. **Gould, I. M.** 2006. Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and its control. Int. J. Antimicrob. Agents **28:**379–384.
6. **Harbarth, S., H. Sax, and P. Gastmeier** 2003. The preventable proportion of nosocomial infections: an overview of published reports. J. Hosp. Infect. **54:**258–266.
7. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
8. **Klevens, R. M., J. R. Edwards, C. L. Richards, Jr., T. C. Horan, R. P. Gaynes, D. A. Pollock, and D. M. Cardo.** 2007. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep. **122:**160–166.
9. **Maquelin, K., B. Cookson, P. Tassios, and A. van Belkum for the European Society for Microbiology and Infectious Diseases Study Group on Epidemiological Markers.** 2007. Current trends in the epidemiological typing of clinically relevant microbes in Europe. J. Microbiol. Methods **69:**222–226.
10. **Maquelin, K., L. Dijkshoorn, T. J. van der Reijden, and G. J. Puppels.** 2006. Rapid epidemiological analysis of *Acinetobacter* strains by Raman spectroscopy. J. Microbiol. Methods **64:**126–131.
11. **Maquelin, K., C. Kirschner, L. P. Choo-Smith, N. A. Ngo-Thi, T. van Vreeswijk, M. Stammler, H. P. Endtz, H. A. Bruining, D. Naumann, and G. J. Puppels.** 2003. Prospective study of the performance of vibrational spectroscopies for rapid identification of bacterial and fungal pathogens recovered from blood cultures. J. Clin. Microbiol. **41:**324–329.
12. **Martens, H., and E. Stark.** 1991. Extended multiplicative signal correction and spectral interference subtraction: new preprocessing methods for near infrared spectroscopy. J. Pharm. Biomed Anal. **9:**625–635.
13. **Murchan, S., M. E. Kaufmann, A. Deplano, R. de Ryck, M. Struelens, C. E. Zinn, V. Fussing, S. Salmenlinna, J. Vuopio-Varkila, N. El Solh, C. Cuny, W. Witte, P. T. Tassios, N. Legakis, W. van Leeuwen, A. van Belkum, A. Vindel, I. Laconcha, J. Garaizar, S. Haeggman, B. Olsson-Liljequist, U. Ransjo, G. Coombes, and B. Cookson.** 2003. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J. Clin. Microbiol. **41:**1574–1585.
14. **Nulens, E., E. Broex, A. Ament, R. H. Deurenberg, E. Smeets, J. Scheres, F. H. van Tiel, B. Gordts, and E. E. Stobberingh.** 2008. Cost of the methicillin-resistant *Staphylococcus aureus* search and destroy policy in a Dutch university hospital. J. Hosp. Infect. **68:**301–307.
15. **Nulens, E., E. E. Stobberingh, H. van Dessel, S. Sebastian, F. H. van Tiel, P. S. Beisser, and R. H. Deurenberg.** 2008. Molecular characterization of *Staphylococcus aureus* bloodstream isolates in a Dutch university hospital between 1999 and 2006. J. Clin. Microbiol. **46:**2438–2441.
16. **Perencevich, E. N., P. W. Stone, S. B. Wright, Y. Carmeli, D. N. Fisman, S. E. Cosgrove, and the Society for Healthcare Epidemiology of America.** 2007. Raising standards while watching the bottom line: making a business case for infection control. Infect. Control Hosp. Epidemiol. **28:**1121–1133.
17. **Peterson, L. R., and G. A. Noskin.** 2001. New technology for detecting multidrug-resistant pathogens in the clinical microbiology laboratory. Emerg Infect. Dis. **7:**306–311.
18. **Scholtes-Timmerman, M., D. Willemse-Erix, T. C. Bakker Schut, A. van Belkum, G. J. Puppels, and K. Maquelin.** 2009. A novel approach to correct specific variations in Raman spectra due to bleachable cellular components. Analyst. doi:10.1039/b811596f.
19. **van Belkum, A., P. T. Tassios, L. Dijkshoorn, S. Haeggman, B. Cookson, N. K. Fry, V. Fussing, J. Green, E. Feil, P. Gerner-Smidt, S. Brisse, M. Struelens, and the European Society of Clinical Microbiology and Infectious Diseases Study Group on Epidemiological Markers.** 2007. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin. Microbiol. Infect. **13**(Suppl. 3)**:**1–46.
20. **van Belkum, A., W. van Leeuwen, M. E. Kaufmann, B. Cookson, F. Forey, J. Etienne, R. Goering, F. Tenover, C. Steward, F. O'Brien, W. Grubb, P. Tassios, N. Legakis, A. Morvan, N. El Solh, R. de Ryck, M. Struelens, S.**

Salmenlinna, J. Vuopio-Varkila, M. Kooistra, A. Talens, W. Witte, and H. Verbrugh. 1998. Assessment of resolution and intercenter reproducibility of results of genotyping *Staphylococcus aureus* by pulsed-field gel electrophoresis of SmaI macrorestriction fragments: a multicenter study. J. Clin. Microbiol. **36:**1653–1659.

21. **Vandijck, D. M., M. Depaemelaere, S. O. Labeau, P. O. Depuydt, L. Annemans, F. M. Buyle, S. Oeyen, K. E. Colpaert, R. P. Peleman, S. I. Blot, and J. M. Decruyenaere.** 2008. Daily cost of antimicrobial therapy in patients with intensive care unit-acquired, laboratory-confirmed bloodstream infection. Int. J. Antimicrob. Agents **31:**161–165.
22. **van Leeuwen, W., C. Libregts, M. Schalk, J. Veuskens, H. Verbrugh, and A. van Belkum.** 2001. Binary typing of *Staphylococcus aureus* strains through reversed hybridization using digoxigenin-universal linkage system-labeled bacterial genomic DNA. J. Clin. Microbiol. **39:**328–331.
23. **Vincent, J. L., M. Chierego, M. Struelens, and B. Byl.** 2004. Infection control in the intensive care unit. Exp. Rev. Anti-Infect. Ther. **2:**795–805.
24. **Wertheim, H. F., M. C. Vos, H. A. Boelens, A. Voss, C. M. Vandenbroucke-Grauls, M. H. Meester, J. A. Kluytmans, P. H. van Keulen, and H. A. Verbrugh.** 2004. Low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) at hospital admission in The Netherlands: the value of search and destroy and restrictive antibiotic use. J. Hosp. Infect. **56:**321–325.