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# Comparison of MIDI Sherlock System and Pulsed-Field Gel Electrophoresis in Characterizing Strains of Methicillin-Resistant *Staphylococcus aureus* from a Recent Hospital Outbreak

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**An outbreak of methicillin-resistant *Staphylococcus aureus* infections at the University of Utah Health Sciences Center occurred over a 7-month period. While the isolates phenotypically appeared to be similar in gross morphology and have similar Vitek antibiotic susceptibility patterns, two additional methods of strain characterization were evaluated to enhance the epidemiological investigation: pulsed-field gel electrophoresis and gas chromatography with the MIDI Sherlock system. Sherlock uses gas chromatography to qualitatively and quantitatively analyze the cellular fatty acid composition of organisms and creates two-dimensional plots based on principal-component analysis to define groups of closely related organisms. All isolates were also evaluated by digesting their chromosomal DNAs with the low-frequency-cutting enzyme *Sma*I and separating the restriction fragments by contour-clamped homogeneous electric field gel electrophoresis. Sample preparation for this pulsed-field gel electrophoresis included a novel cell lysis procedure involving achromopeptidase, greatly reducing the turnaround time. Isolates tested were recovered from the following: 45 suspected outbreak patients, 6 hospitalized patients believed to be unrelated to the outbreak, 6 patients from outside the hospital, and one health care practitioner implicated in the outbreak. Of 45 phenotypically similar suspect strains, 43 clustered tightly on the Sherlock two-dimensional plot. All outbreak patient isolates were also identical by pulsed-field gel electrophoresis with the exception of the same two outliers identified by Sherlock. In this epidemiologic investigation, we found an excellent correlation between the Sherlock and pulsed-field gel electrophoresis results for strain characterization of methicillin-resistant *S. aureus*.**

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important cause of nosocomial infections (13). Nationally, 5 to 10% of all hospitalized patients in a given facility become colonized or infected with MRSA (14). Outbreaks of MRSA in an institution may be difficult to evaluate because of MRSA endemicity and limited evolutionary diversity (11).

Over a 7-month period, the University of Utah Health Sciences Center (UUHSC) experienced an outbreak of MRSA infections. Isolates were available from 45 patients in the medical and surgical intensive care, burn, and rehabilitation units. Traditional tracking methods involving antibiograms were strongly indicative of single-strain involvement. Therefore, the outbreak was thought to be well defined and an excellent opportunity to evaluate methods of epidemiologic typing.

Although analysis of cellular fatty acids (CFA), especially by the MIDI Sherlock system, is well established as a tool for identification of unusual and novel isolates in the clinical laboratory (5, 26, 30, 35), we believed Sherlock's capacity to compare large numbers of isolates to be an underutilized advantage. We decided to challenge the system's strain tracking ability with isolates from this well-defined outbreak.

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Numerous investigators have demonstrated the superiority of macrorestriction analysis by using pulsed-field gel electrophoresis (PFGE) for strain characterization compared with techniques such as plasmid profiling (9), restriction enzyme analysis of plasmid DNA (3), ribotyping (23), amplification of randomly primed DNA (27), or zymotyping, phage typing, and capsular typing (28). These techniques are summarized in reviews by Maslow et al. (15) and Tenover et al. (32). We have developed a time-efficient method, applicable to a clinical laboratory's work flow, for preparing *S. aureus* lysates for PFGE. This method was chosen as a reference standard.

## MATERIALS AND METHODS

**Bacterial isolates.** The hospital epidemiology staff performed an epidemiologic investigation involving the medical, surgical, neurosurgical, and burn-trauma intensive care units and the general surgery, psychiatry, and rehabilitation units. They identified the initial body site of colonization for each of the patients thought to be associated with the outbreak. Isolates from the following specimens were chosen for study: sputum ($n = 20$), blood ($n = 2$), surgical wounds ($n = 1$), intravenous catheter tips ($n = 3$), decubitus ulcers ($n = 2$), urine ($n = 3$), peritoneal fluid ($n = 2$), and, as the outbreak progressed into the burn unit, Rodac cultures from a variety of body sites ($n = 12$). Isolates were identified as *S. aureus* by the Staphaurex test (Murex Diagnostics Limited, Dartford, England). Six known MRSA isolates from patients thought not to be associated with the outbreak were also included. Six isolates from patients other than those at the UUHSC were used as a control group, including three patients from elsewhere in the Salt Lake Valley and three from areas outside Utah (Arizona, Hawaii, and Kansas). One isolate was cultured from the hands of a respiratory therapist suspected to be involved in the spread of the outbreak.

**Antibiograms.** All patient isolates were initially screened with Vitek GPS-SA

TABLE 1. Results of Kirby-Bauer susceptibility testing of all 58 isolates

| Isolate source | Zone of inhibition (mm)[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AN | CZ | OFX | CC | E | GM | OX | P | Te | SXT | NN | VA |
| Outbreak | 14 (13–18)[b] | | | | | 9 (6–12)[b] | | | 20 (18–24)[b] | | | 19 (17–20)[b] |
| UUHSC employees | 13 | | | | | 8 | | | 25 | | | 18 |
| UUHSC outpatient clinic | 20 | | | | | 11 | | | 31 | | | 19 |
| Non-outbreak-associated UUHSC site | 14 | | | | | 18 | | | 24 | 29 | | 18 |
| Non-outbreak-associated UUHSC site | 13 | 18 | | | | 21 | | | 29 | 31 | | 18 |
| Non-outbreak-associated UUHSC site | 14 | 10 | | | | 20 | | | 25 | 28 | | 18 |
| Non-outbreak-associated UUHSC site | 14[c] | | | | | 8 | | | 21 | | | 19 |
| Non-outbreak-associated UUHSC site | 21 | 28 | 23 | 25 | 27 | 22 | 17[d] | 13 | 26 | 27 | 22 | 21 |
| Non-outbreak-associated UUHSC site | 14 | | 25 | 27 | 7 | 22 | | | 27 | 32 | | 19 |
| Non-UUHSC sites | | | | | | | | | | | | |
| Salt Lake City physician's laboratory | 14 | | | | | 19 | | | 25 | 31 | | 19 |
| Salt Lake City hospital | 14 | | | | | 19 | | | 26 | 30 | | 18 |
| Arizona | 19 | 24 | 25 | 24 | 22 | 19 | 16[d] | 12 | 26 | 37 | 20 | 18 |
| Hawaii | 19 | | 21 | 23 | 24 | 19 | | | 25 | 26 | 20 | 18 |
| Kansas | 18 | | | | | 8 | | | 12 | 26 | 10 | 20 |
| Possibly epidemiologically associated UUHSC site | 16 | | 7 | | | 20 | | | 25 | 30 | | 19 |
| Possibly epidemiologically associated UUHSC site | 18 | 24 | 25 | 24 | 24 | 20 | | 20[e] | 26 | 28 | 21 | 18 |

[a] Boxed areas indicate no zone of inhibition. Shading indicates hazy growth up to OX disk. Antibiotic disk abbreviations: AN, amikacin; CC, clindamycin, OX, oxacillin; SXT, trimethoprim-sulfamethoxazole; CZ, cefazolin; E, erythromycin; P, penicillin; NN, tobramycin; OFX, ofloxacin; GM, gentamicin, TE, tetracycline; VA, vancomycin.

[b] Median and range of values for 43 outbreak isolates.

[c] Sherlock and PFGE results indistinguishable from those of outbreak strains.

[d] β-Lactamase-hyperproduction; light growth on oxacillin agar screening plate.

[e] β-Lactamase (nitrocefin) negative at 1 h.

cards (Bio-Merieux Vitek, Inc. Hazelwood, Mo.) and on Mueller-Hinton agar supplemented with 2% NaCl and 6 μg of oxacillin per ml. The Vitek AMS instrument automatically edits the results obtained with β-lactam antibiotics to report resistance when oxacillin resistance is detected. The Kirby-Bauer disk diffusion method was also performed in accordance with National Committee for Clinical Laboratory Standards guidelines (20) to obtain a more complete susceptibility profile of each isolate. Mueller-Hinton agar for Kirby-Bauer testing was purchased from BBL, Cockeysville, Md. Disks were from Becton-Dickinson, Cockeysville, Md. The antibiotics tested are listed in Table 1.

**Sherlock.** Sherlock uses gas chromatography to qualitatively and quantitatively analyze the CFA composition of organisms. All 58 isolates were sent to MIDI for analysis. The samples were labeled with stock numbers only, essentially blinding the collection for MIDI.

Instrument configuration and organism growth and hydrolysis were those described in the Sherlock protocol (the latest version of the previously described [17] MIDI Microbial Identification System). Briefly, one heaping 4-mm loopful of overnight growth was used to coat the bottom of a glass screw-cap tube (13 by 100 mm). All reagents for saponification, methylation, extraction, and washing were dispensed with autopipets into this same tube, making the hands-on time minimal. Up to 45 samples can be prepared in approximately 3.5 h. The instrument can classify one sample every 30 min and is totally automated; i.e., the samples are generally left to run overnight.

Peak naming and organism identification were similar to those previously described (35), except that Sherlock utilizes the Hewlett-Packard 3365 GC ChemStation for sample handling and analysis. Cluster analysis of the data generated was performed with the MIDI Library Generation Software, which includes a principal-component two-dimensional plot program based on components responsible for the greatest degree of variability among the isolates tested (18, 25).

**PFGE. (i) Plug preparation.** Isolates to be tested were recovered from frozen stocks and subcultured twice on Columbia agar with 5% sheep erythrocytes (BBL). A single colony was selected and grown overnight in tryptic soy broth at 37°C with agitation. A 500-μl aliquot of the broth growth (adjusted to equal a 3.0 McFarland density standard) was pelleted by centrifugation and washed in 1 ml of TEN (0.1 M Tris, 0.1 M EDTA, 0.15 M NaCl, pH 7.5) and then in 1 ml of TN (10 mM Tris, 10 mM NaCl, pH 8.0). The final pellet was resuspended in 100 μl of TN and mixed with 100 μl of TN containing 2% InCert agarose (FMC BioProducts, Rockland, Maine) and 6 μl of achromopeptidase (ACP; 20 U/μl; Wako BioProducts, Richmond, Va.). This suspension was sufficient to fill two wells of a Bio-Rad disposable plug mold (Bio-Rad Laboratories, Hercules, Calif.). The plugs were kept at 4°C for 10 min, expelled into 300 μl of TN, transferred to a 50°C water bath, and kept there for 30 min. The lysed plugs were washed twice in 2 ml of TE (10 mM Tris, 1 mM EDTA, pH 7.5) at room temperature with agitation for 30 min each time. Plugs were stored at 4°C in TE or processed immediately for restriction enzyme digestion.

**(ii) Restriction endonuclease digestion.** One-hundred-microliter plugs were cut in half, resulting in 50-μl agarose blocks of approximately 2.5 by 10 mm. Alternately, large numbers of isolates could be processed by cutting the plugs in fourths. The blocks were transferred to a 200-μl aliquot of NEBuffer 4 (New England BioLabs, Beverly, Mass.) containing 2 μl (20 U) of the enzyme *Sma*I (New England BioLabs). A previously prepared and lysed plug (or a plug purchased from Bio-Rad) containing *S. aureus* NCTC 8325 was included as a control for the endonuclease. Digestion was complete in 2 h at room temperature.

**(iii) Contour-clamped homogeneous electric field gel electrophoresis.** Sample blocks were placed in 1 ml of 0.5× TBE (50 mM Tris, 50 mM $H_3BO_3$, 0.5 mM EDTA) for at least 30 min before being embedded in a 1.2% GTG agarose gel (FMC). Bacteriophage lambda DNA concatemers (Lambda Ladder PFG Marker; New England BioLabs) were embedded in a similar fashion and used as size standards. Restriction fragments were separated over a size range of approximately 50 to 700 kb by contour-clamped homogeneous electric field gel electrophoresis in 0.5× TBE on a CHEF Mapper (Bio-Rad). The CHEF Mapper was programmed with a voltage gradient of 6 V/cm and a switch time ramped linearly from 15 to 55 s over 22 h. Gels were stained with ethidium bromide and imaged by UV transillumination to Polaroid film.

## RESULTS

The results of Kirby-Bauer susceptibility testing are in Table 1. With the exception of slight variability in amikacin zone sizes that straddled the interpretative breakpoints, the outbreak strains were very similar. Resistance to trimethoprim-sulfamethoxazole evolved as a marker for this group. Two strains originally thought to be epidemiologically associated clearly showed discrepant susceptibility patterns, one due primarily to trimethoprin-sulfamethoxazole susceptibility. Two isolates not known to be associated had patterns consistent with the out-

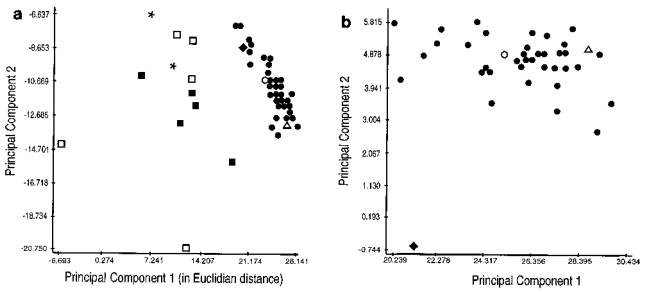


FIG. 1. Clustering of MRSA isolates by Sherlock two-dimensional plot. Graph a shows the complete collection of 59 isolates. Graph b shows a re-evaluation (see the explanation in the text) of the upper right cluster shown in graph a. Note that several outbreak strains were superimposed on the two-dimensional plot; therefore, the number of ● symbols is less than the expected 43. Symbols: ●, epidemiologically associated isolates from UUHSC; ◆, closely related clinic isolate, not epidemiologically associated; ○, isolate originally not thought to be epidemiologically associated and indistinguishable by PFGE and Sherlock; △, hospital employee; ∗, isolates thought to be epidemiologically associated and proved dissimilar by PFGE and Sherlock; □, isolates from outside clients (not UUHSC); ■, UUHSC isolates not epidemiologically associated.

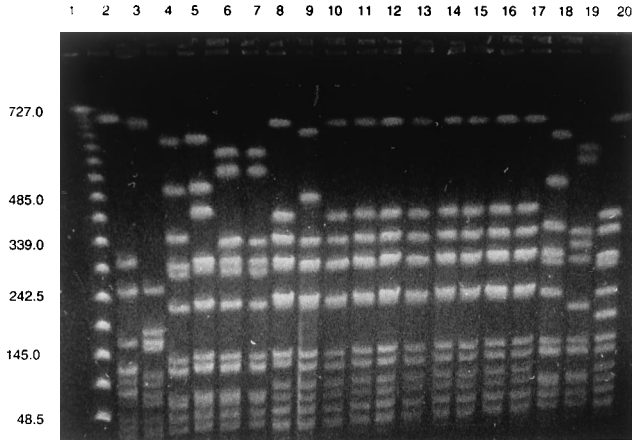


FIG. 2. Photograph showing a representative selection of isolates on PFGE. Lane numbers: 1, lambda ladder marker (48.5-kb bacteriophage lambda DNA concatemers); 2 to 7, isolates not epidemiologically associated; 8, isolate originally not thought to be epidemiologically associated and indistinguishable by PFGE and Sherlock; 9, closely related clinic isolate not epidemiologically associated; 10, hospital employee; 11 to 17, epidemiologically associated isolates from UUHSC (surgical intensive care unit; lanes 11 and 12; medical intensive care unit, lanes 13 and 14; burn unit, lanes 15 and 16; rehabilitation unit, lane 17); 18 to 19, isolates thought to be epidemiologically associated and proved dissimilar by PFGE and Sherlock; 20, *S. aureus* NCTC 8325.

break (one from a nonassociated inpatient and one from an outpatient clinic.)

The Sherlock clustering, based on principal-component analysis of the CFA of the isolates, is shown in Fig. 1a and b. Figure 1a shows the complete collection of 58 isolates. The cluster in the upper right quadrant represents the suspect outbreak strains (●), with the exception of the two mentioned above as having discrepant susceptibility patterns. The previously nonsuspect patient (○) is in that cluster along with the isolates from the UUHSC outpatient clinic (◆) and the hospital employee (△).

Figure 1b shows a re-evaluation of the upper right cluster shown in Fig. 1a. This graph was generated by Sherlock by manually turning off all of the datum points except those represented by the symbols ●, ○, ◆, and △. The program then recalculated the principal components of this smaller set of data and replotted their variability on the basis of the new principal components 1 and 2, measured in Euclidian distance (ED). MIDI defines strain level similarity as points covering an area on the plot of less than 30 $ED^2$. The one obvious outlying point (◆) represents the outpatient clinic strain and is outside the 30 $ED^2$ necessary for strain identity. All other isolates, including the one previously epidemiologically nonassociated inpatient and the health care worker, continued to appear similar.

A representative example of PFGE results is shown in Fig. 2. The patterns of all outbreak strains were identical, with the exception of the same two strains identified as outliers by Sherlock. The same previously nonsuspect patient which clustered with the outbreak strains by Sherlock was also identified by PFGE. The outpatient strain that was similar by Sherlock showed differences in two bands by PFGE and would be considered distinct. All outliers varied by two or more bands.

## DISCUSSION

Nosocomial infections are a major cause of morbidity, involving more than 2 million patients annually at an estimated cost of $4.5 billion in 1992 (4). Infection control measures have been shown to effectively reduce nosocomial infections (14). In recent years, epidemiologic investigations have utilized a variety of typing techniques to augment classical clinical analyses (7, 8, 21, 31, 33). Since the UUHSC did not have a high prevalence of MRSA, the outbreak afforded us the opportunity to evaluate two laboratory methods in a clinically well-defined epidemic.

The Kirby-Bauer susceptibility patterns of this group of organisms were eventually shown to correlate with their PFGE and CFA types. Several outlying strains, however, varied by only one drug and would have been difficult to identify as truly different (32). Enhanced discriminatory power would be more essential in centers with a high prevalence of MRSA.

Macrorestriction analysis of MRSA by PFGE has been evaluated in recent years and appears to be more sensitive than other, more commonly utilized methods. Although PFGE has been shown to provide more information than traditional typing methods, it is also time consuming and costly to run (2, 16). Recent reports have shown some of the original steps to be unnecessary, such as the use of proteinase K and the toxic compound phenylmethylsulfonyl fluoride for its removal (6). Our laboratory initiated PFGE by using more traditional approaches (22). The method presented herein evolved from an effort to make PFGE more applicable to the work flow of a busy clinical laboratory.

To our knowledge, this is the first report of the use of ACP alone for lysis of staphylococci in agarose plugs. The lysis is visually obvious and rapid. Murakami and Minamide (19) have previously reported the use of this enzyme for lysis of *S. aureus* prior to PCR detection of the *mecA* gene. We incubated the lysis mixture at 50°C on the basis of their recommendation. The manufacturer's application notes, however, cite procedures in which lysis occurs with ACP at 37°C (29, 34). Our own experience has shown the plugs to be transparent when removed from the refrigerator, illustrating the enzyme's activity over a very broad temperature range. The manufacturer's notes (34) state that ACP is most active in 0.01 M Tris HCl buffer (pH 8.0) containing 0.01 M NaCl. Because ACP is very sensitive to ionic strength (34), we found that when it was suspended in this solution, it provided excellent clearing of organisms. It produced poor or no clearing with the more conventional TE/SDS (19) or EC (22) lysis solution. Even with the rapid ACP lysis process, the PFGE procedure takes at least 48 h.

The Sherlock method for CFA analysis and clustering is rapid, easy to use, and after the initial instrument purchase, cost effective. Although there is limited experience with the use of CFA for comparison of clinical specimens (1, 10, 12, 18), pharmaceutical and food industry quality control operations have recently shown interest in using the Sherlock method to track the source of contaminating organisms (24). As this method appears promising, we decided to evaluate Sherlock with our clinical outbreak.

Both Sherlock and PFGE rendered similar results which correlated with the antibiograms. These typing methods both identified the same two isolates, which were previously thought to be epidemiologically associated, as different from the clustered strains. Retrospectively, the Infection Control Practitioner felt that these two patients were, in fact, questionably associated with the outbreak. Similarly, both methods identified a single isolate, not previously known to be involved with the epidemic, that appeared to be identical to the clustered strains. Again, the initial clinical impression of the association had been unclear. The additional typing information provided better definition of the outbreak. In addition, one of the known outside isolates, from a UUHSC outpatient clinic, although distinct, was surprisingly similar by PFGE, Sherlock, and antibiogram to the outbreak isolates. PFGE differed in the position of only two bands. It is still not clear whether this represents an example of the limited genetic diversity of MRSA or whether this patient acquired the hospital strain on one of his many visits to various UUHSC hospital clinics.

In our laboratory (Associated Regional and University Pathologists, Inc.), we foresee the combined use of the two systems. The Sherlock system has the ability to screen large numbers of isolates and would easily accommodate duplicate subcultures of the same isolate to ensure purity and reproducibility. In a recent study, 200 isolates were processed by a single individual in 5 days (1). In the current effort, the unexpected outliers were retested from the original stock cultures and from intermediate subcultures with very similar results. One obviously contaminated or mislabeled sample was also identified (data not shown). The original sample extracts were prepared by three different individuals, showing good batch-to-batch reproducibility.

Assuming that Sherlock could first identify gross outliers, the laboratory could then be selective with the more costly and labor-intensive PFGE as a confirmatory test for only the most similar strains. For laboratories that have already invested in the Sherlock system for microbial identification, the ability to use the instrument for epidemiologic typing could be an added advantage.

The information provided by these laboratory methods was useful in focusing infection control efforts. The discriminatory power, or the ability to differentiate among unrelated strains, appeared to be comparable for both Sherlock and the better-evaluated PFGE method with this single, well-defined outbreak. The Sherlock method appears to be promising as a typing system, although more rigorous evaluation with adequate numbers of epidemic and sporadic isolates is needed.

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