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Multilocus Sequence Typing Compared to Pulsed-Field Gel Electrophoresis for Molecular Typing of *Pseudomonas aeruginosa*▽

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For hospital epidemiologists, determining a system of typing that is discriminatory is essential for measuring the effectiveness of infection control measures. In situations in which the incidence of resistant *Pseudomonas aeruginosa* is increasing, the ability to discern whether it is due to patient-to-patient transmission versus an increase in patient endogenous strains is often made on the basis of molecular typing. The present study compared the discriminatory abilities of pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) for 90 *P. aeruginosa* isolates obtained from cultures of perirectal surveillance swabs from patients in an intensive care unit. PFGE identified 85 distinct types and 76 distinct groups when similarity cutoffs of 100% and 87%, respectively, were used. By comparison, MLST identified 60 sequence types that could be clustered into 11 clonal complexes and 32 singletons. By using the Simpson index of diversity (*D*), PFGE had a greater discriminatory ability than MLST for *P. aeruginosa* isolates (*D* values, 0.999 versus 0.975, respectively). Thus, while MLST was better for detecting genetic relatedness, we determined that PFGE was more discriminatory than MLST for determining genetic differences in *P. aeruginosa*.

Pseudomonas aeruginosa is a common pathogen that causes nosocomial infections in intensive care units (ICUs) (1, 28). Resistant *P. aeruginosa* is an emerging threat to patients (15, 19, 23). Several schemes for the molecular typing of *P. aeruginosa* have been proposed to determine the relatedness of nosocomial pathogens. These include pulsed-field gel electrophoresis (PFGE), ribotyping (5), and PCR-based fingerprinting (7). For hospital epidemiologists, determining the best type of discriminatory molecular genetic analysis is essential for measuring the effectiveness of infection control measures. Also, in situations in which the incidence of resistant *P. aeruginosa* is increasing, the ability to discern whether it is due to patient-to-patient transmission versus an increase in patient endogenous strains is often made on the basis of molecular typing. Although other studies have compared numerous molecular typing schemes (2, 13, 26), PFGE is still considered by most to be the "gold standard."

Recently, multilocus sequence typing (MLST), which is based on the allelic differences among housekeeping genes, has become a popular bacterial typing method (9). MLST typing schemes for determination of the relatedness of numerous microorganisms, including *Staphylococcus aureus* (8), *Salmonella enterica* (11), *Enterococcus faecalis* (20), and *Vibrio cholerae* (18), have been developed. Recently, Curran and colleagues developed a multilocus sequence typing scheme that discriminates *P. aeruginosa* isolates by differences in the sequences of the following seven loci: *acsA*, *aroE*, *guaA*, *mutL*, *noD*, *ppsA*, and *trpE* (4).

In the study described here, we set out to determine the discriminatory abilities and potential utilities of PFGE and MLST for the genetic characterization of *P. aeruginosa*. In order to compare these two methods, we analyzed 90 *P. aeruginosa* isolates obtained from perirectal surveillance swab specimens from 90 unique patients among a cohort of ICU patients. We believe that this is the first study to have compared MLST and PFGE by the use of a large cohort of *P. aeruginosa* isolates.

MATERIALS AND METHODS

Bacterial isolates. Surveillance perirectal swab specimens taken from a cohort of patients admitted to the medical and surgical ICUs at the University of Maryland Medical Center between 1 September 2001 and 1 September 2004 were used. Perirectal swab specimens for culture were obtained from the patients on admission, weekly, and upon discharge. Non-lactose-fermenting, oxidase-positive isolates were identified as *P. aeruginosa* if they did not produce an acid reaction in triple sugar iron agar (Becton Dickinson, Franklin Lakes, NJ), grew at 42°C, and produced blue-green pigment on *Pseudomonas* P agar (Remel, Lenexa, KS). Nonfermenters not matching these characteristics were identified by using API 20NE system (BioMerieux Inc., Durham, NC) test strips. Antimicrobial susceptibility testing was done by disk diffusion according to the CLSI guidelines (3). Forty-six imipenem-resistant isolates (24 isolates susceptible and 22 isolates resistant to piperacillin-tazobactam) and 44 imipenem-susceptible *P. aeruginosa* isolates (4 isolates susceptible and 40 isolates resistant to piperacillin-tazobactam) from unique patients were randomly chosen to undergo typing by PFGE and MLST.

PFGE. PFGE was performed as described previously (6). All isolates were digested with SpeI, and the resulting fragments were separated by electrophoresis in 1% agarose gels with a CHEF DR apparatus (Bio-Rad Laboratories, Hercules, CA) for 24 h, with the switch times ranging from 2 s and 40 s in Tris-borate EDTA buffer containing 50 μM thiourea (25). Photographic images of the gels were saved digitally with Geldoc EQ software (Bio-Rad Laboratories) and saved as TIFF files for gel analysis with Fingerprinting II software (Bio-Rad Laboratories). The band patterns were compared by use of the Dice coefficient by using the unweighted pair group method to determine band similarity and the criteria established by Tenover et al. to define the pulsed-field type clusters (27). Isolates with band patterns that were 100% identical were considered to be of

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TABLE 1. PFGE types compared to MLST types of 90 clinical *P. aeruginosa* isolates

Isolate no.	PFGE no.	PFGE group	MLST ST	Burst group (CC)	Susceptibility[a]	
					Imipenem	Piperacillin-tazobactam
1	PT01		282	UM08	R	S
2	PT02		235	UM05	R	R
3	PT03		444	UM09	S	S
4	PT04		111	UM02	R	R
5	PT05		232	UM04	S	R
6	PT06		471	UM04	S	S
7	PT07		257	Singleton	S	S
8	PT08		460	Singleton	S	S
9	PT09		469	Singleton	R	S
10	PT10		155	UM03	R	S
11	PT11		252	Singleton	S	S
12	PT12		17	UM01	S	S
13	PT13		442	Singleton	R	S
14	PT14		390	Singleton	S	S
15	PT15		244	UM06	S	S
16	PT16		270	Singleton	S	R
17	PT17		17	UM01	S	S
18	PT18		439	Singleton	R	S
19	PT19		444	UM08	R	R
20	PT20		282	UM08	R	S
21	PT21	A	253	Singleton	S	S
22	PT22	A	253	Singleton	R	S
23	PT23		321	UM01	R	S
24	PT24		253	Singleton	R	S
25	PT25	B	444	UM08	S	S
26	PT26	B	444	UM08	R	S
27	PT27		282	UM08	S	S
28	PT28		282	UM08	R	S
29	PT29		282	UM08	R	S
30	PT30		458	Singleton	R	S
31	PT31		ND[b]	Singleton	R	S
32	PT32		466	UM08	R	R
33	PT33		455	Singleton	S	S
34	PT34		274	UM08	S	S
35	PT35		453	Singleton	R	S
36	PT36	C	111	UM02	R	R
37	PT37	C	111	UM02	S	R
38	PT38	C	111	UM02	R	R
39	PT38	C	111	UM02	R	R
40	PT39	C	111	UM02	R	R
41	PT39	C	111	UM02	R	R
42	PT40	C	111	UM02	S	R
43	PT40	C	464	UM02	R	R
44	PT41		111	UM02	R	R
45	PT42		445	UM01	S	S
46	PT43		111	UM02	R	R
47	PT44		463	Singleton	R	S
48	PT45		282	UM08	R	S
49	PT46		446	UM10	S	S
50	PT47		190	Singleton	S	S
51	PT48		385	UM09	S	S
52	PT49		461	UM03	R	S
53	PT50	D	441	UM06	S	S
54	PT51	D	462	UM06	R	S
55	PT52		244	UM06	R	S
56	PT53		ND	UM04	R	R
57	PT54		457	UM05	R	R
58	PT55		235	UM05	R	R
59	PT56		459	Singleton	S	S
60	PT57		443	UM09	S	S
61	PT58		235	UM05	S	S
62	PT59		27	UM01	S	S
63	PT60		468	Singleton	S	S
64	PT61	E	449	UM01	R	S
65	PT62	E	27	UM01	R	S

Continued on facing page

TABLE 1—*Continued*

Isolate no.	PFGE no.	PFGE group	MLST ST	Burst group (CC)	Susceptibility[a]	
					Imipenem	Piperacillin-tazobactam
66	PT63		452	Singleton	S	S
67	PT64		27	UM01	S	S
68	PT65		348	Singleton	R	R
69	PT66		164	Singleton	S	S
70	PT67		450	Singleton	S	S
71	PT68		244	UM06	S	S
72	PT69		440	UM07	S	S
73	PT70		267	UM07	S	S
74	PT71	F	447	UM11	R	R
75	PT71	F	447	UM11	R	R
76	PT72	F	448	UM11	R	R
77	PT73		299	UM03	S	S
78	PT74		179	Singleton	R	R
79	PT75		470	Singleton	S	S
80	PT76		281	Singleton	R	S
81	PT77		111	UM02	R	R
82	PT78		467	Singleton	S	S
83	PT79		465	Singleton	S	S
84	PT80		456	Singleton	S	S
85	PT81		454	UM10	S	S
86	PT82		17	UM01	S	S
87	PT83		275	Singleton	S	S
88	PT84		451	Singleton	S	S
89	PT85		111	UM02	R	R
90	ND		ND	Singleton	R	S

[a] R, resistant; S, susceptible.
[b] ND, not determined.

identical PFGE types, and isolates that had band patterns with ≥87% similarity were considered to be genetically related.

MLST. MLST was performed by previously published protocols (4). Briefly, genomic DNA was isolated by using a Prepman Ultura apparatus (Applied Biosystems, Foster City, CA), according to the manufacturer's guidelines. Standard DNA amplification and sequencing of the seven housekeeping genes (*acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE*) were performed for all isolates. The nucleotide sequences of both strands were determined by using previously published primers and were compared to existing sequences in the MLST database (www.pubmlst.org/paeruginosa) for assignment of allelic numbers. The isolates were assigned a sequence type (ST) number according to their allelic profiles. The eBURST algorithm (http://pubmlst.org/analysis/) was used for phylogenetic analysis, and isolates that were identical at five or more alleles were considered to be part of a clonal complex (CC).

Data analysis. Simpson's index of diversity (*D*) was used to compare the discriminatory powers of PFGE and MLST by use of the following formula (16):

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j(n_j - 1)$$

where N is the total number of strains in the sample population, S is the total number of types described, and n_j is the number of strains belonging to the jth type.

RESULTS

PFGE. SpeI digestion of the 90 *P. aeruginosa* isolates from different patients identified 85 unique patterns (designated pattern types [PTs] PT1 to PT85) (Table 1). When the genetic relatedness (defined as ≥87% band identity) of the isolates was compared with that determined by MLST, PFGE revealed 19 isolates that formed into six groups with two or more isolates each, denoted groups A to F (Table 1), and there were 70 unique PFGE patterns with one isolate each. The group with the largest number of isolates of the same PT was group C, which consisted of eight isolates with five related PFGE patterns. All isolates in this group were resistant to piperacillin-tazobactam, six isolates were resistant to imipenem, and two isolates were susceptible to imipenem. The isolates in three of the other five groups were all susceptible to piperacillin-tazobactam but had different susceptibilities to imipenem. However, for the four groups that had more than one isolate with identical PTs, only PT40 contained isolates with different patterns of resistance. Therefore, PFGE did not group the organisms by their susceptibilities. One isolate was not digested by SpeI; however, the isolate was digested with XbaI (data not shown) and was not designated a PFGE type.

MLST. MLST of the isolates was performed to compare the discriminatory power of MLST to that of PFGE. MLST revealed 60 different STs (Table 2). Among the 60 STs, 36 were not previously submitted to the *P. aeruginosa* database (17). Fifty-eight STs could be clustered by analysis with the eBURST program into 11 different CCs, while the remaining 32 STs were classified as singletons. The three largest CCs, denoted UM01, UM02, and UM08 (Tables 1 and 2), contained 9, 13, and 12 STs, respectively. In 7 of 12 CCs (CCs UM01, UM02, UM03, UM04, UM05, UM06, and UM08) there were combinations of resistance and susceptibility to piperacillin-tazobactam and imipenem among the isolates. In six of eight cases in which more than one isolate had the same ST, the isolates did not have the same patterns of susceptibility to piperacillin-tazobactam and imipenem. MLST also did not group the isolates by their resistance to these two antimicrobial agents.

TABLE 2. MLST types and the seven locus STs[a]

CC	MLST type	No. of isolates	ST[a]						
			acsA	*aroE*	*guaA*	*mutL*	*nuoD*	*ppsA*	*trpE*
UM01	17	3	11	5	1	7	9	4	7
UM01	27	3	6	5	6	7	4	6	7
UM01	321	1	6	5	1	7	4	4	7
UM01	445	1	54	5	1	7	9	4	7
UM01	449	1	6	5	1	7	4	6	7
UM02	111	12	17	5	5	4	4	4	3
UM02	464	1	17	5	5	4	11	4	7
UM03	155	1	28	5	36	3	3	13	7
UM03	299	1	17	5	36	3	3	7	3
UM03	461	1	28	5	36	3	3	13	3
UM04	232	1	17	5	11	64	4	4	2
UM04	471	1	17	5	11	5	4	4	2
UM05	ND	1	38	11	3	13	1	2	ND
UM05	235	3	38	11	3	13	1	2	4
UM05	457	1	38	11	10	13	1	2	4
UM06	441	1	17	5	12	76	14	4	7
UM06	462	1	17	5	12	76	14	4	2
UM06	244	3	17	5	12	3	14	4	7
UM07	440	1	19	5	5	11	11	4	14
UM07	267	1	19	5	12	11	11	4	14
UM08	274	1	23	5	11	7	1	12	7
UM08	282	6	6	5	11	7	3	12	19
UM08	444	4	6	5	75	7	3	12	19
UM08	466	1	6	5	11	7	1	12	7
UM09	443	1	15	5	5	5	50	4	1
UM09	385	1	15	5	5	5	50	4	14
UM10	446	1	18	4	5	3	1	17	13
UM10	454	1	38	4	10	3	1	17	13
UM11	447	2	40	5	11	3	1	47	60
UM11	448	1	40	5	11	3	1	47	8
Singleton	164	1	1	5	1	11	4	10	10
Singleton	179	1	36	27	28	3	4	13	7
Singleton	190	1	6	3	1	3	2	14	1
Singleton	252	1	6	28	4	3	3	4	7
Singleton	253	3	4	4	16	12	1	6	3
Singleton	257	1	35	24	36	11	4	15	14
Singleton	270	1	22	3	17	5	2	10	7
Singleton	275	1	11	5	20	5	4	4	52
Singleton	281	1	11	57	1	5	4	4	2
Singleton	348	1	22	20	11	3	3	3	7
Singleton	390	1	39	5	1	3	4	46	56
Singleton	439	1	6	68	20	11	4	4	7
Singleton	442	1	6	5	10	1	1	12	1
Singleton	450	1	28	5	11	77	3	4	1
Singleton	451	1	17	22	11	3	4	4	7
Singleton	452	1	35	5	36	11	4	10	7
Singleton	453	1	16	5	11	7	1	7	61
Singleton	455	1	6	14	12	11	1	4	20
Singleton	456	1	28	68	5	11	3	15	44
Singleton	458	1	13	8	9	3	1	17	3
Singleton	459	1	28	24	10	5	1	6	4
Singleton	460	1	40	57	11	3	4	28	37
Singleton	463	1	6	5	5	3	1	6	3
Singleton	465	1	9	54	78	72	4	20	7
Singleton	467	1	6	5	11	18	4	12	3
Singleton	468	1	5	67	76	3	1	4	62
Singleton	469	1	17	66	5	77	3	4	7
Singleton	470	1	6	6	1	5	27	4	7
Singleton	ND	1	32	8	5	3	5	6	ND
Singleton	ND	1	15	5	77	72	3	6	ND

[a] ND, not determined.

Comparison of PFGE and MLST. The discriminatory abilities of PFGE and MLST were compared by the number of unique STs or patterns determined by each method and by the number of related groups. PFGE determined 85 unique types, with 19 of the isolates grouped into six groups, defined by isolates that were genetically related because they had ≥87% band identity. MLST determined 60 unique STs and grouped the isolates into 11 CCs because of their sequence identity at five or more alleles. Simpson's *D* value showed that when isolates with identical (or unique) patterns were compared, PFGE had a slightly higher discriminatory power than MLST (*D* values, 0.999 and 0.975, respectively). The clustering of the isolates by PFGE and MLST was correlated globally, but the details differed. All 32 singleton MLST STs had unique PFGE patterns. The isolates in each PFGE group were found to be members of the same MLST CC and, in most cases, the same ST. Two of the four pairs of isolates with the same PFGE patterns had identical STs. Similarly, for all eight of the STs

with multiple isolates, the isolates with the same ST did not have the same PFGE patterns. When PFGE and MLST were compared, although the isolates with similar PTs had different STs, they were grouped in the same CC. Exceptions were PT groups A and B, which contained two isolates with the same MLST type. When multiple isolates with identical MLST types were analyzed, the isolates often had different unrelated PFGE patterns.

DISCUSSION

In this study we compared two different methods, PFGE and MLST, for the molecular typing of *P. aeruginosa* using 90 isolates obtained from surveillance perirectal swab specimens from patients in ICUs. Using Simpson's *D* value, we determined that PFGE had a greater discriminatory ability than MLST, with *D* values of 0.999 and 0.975, respectively. Although both of these methods have high discriminatory abilities, PFGE distinguished more types (85 versus 60 types distinguished by MLST). Neither method grouped the isolates according to their resistance to the antimicrobials imipenem and piperacillin-tazobactam.

Many schemes for the typing of *P. aeruginosa* have been developed. These include PFGE, ribotyping (5), and PCR-based fingerprinting (5). Although other typing schemes have been developed and show a variety of discriminatory powers, PFGE is known to be the gold standard for the molecular typing of *P. aeruginosa*. Our study has shown that PFGE is highly discriminatory when it is used for the typing of *P. aeruginosa*; however, MLST is also highly discriminatory and could be used as a method for the typing of *P. aeruginosa*. These findings are similar to those of a recent study by Giske et al. that compared multiple methods, including PFGE and MLST, for the molecular typing of 10 multidrug-resistant *P. aeruginosa* isolates and that determined that PFGE had the greater discriminatory ability (12). MLST is a new typing technique that is becoming popular due to the ease of data analysis, but some studies have shown that MLST had a lower discriminatory ability than PFGE (10, 14, 18, 22, 24). However, other studies have shown that MLST has a greater discriminatory ability than PFGE for *Vibrio cholerae* and other bacteria (18, 21). Therefore, we believe that our conclusions are specific to *P. aeruginosa* and not universal for all bacterial species. Comparison studies must be performed with each bacterial species to determine which molecular typing method should be used.

Although our study showed that PFGE had a higher discriminatory ability than MLST, MLST has the advantage that it gives information about the clonal relationships of isolates that PFGE does not. In our study, MLST showed that among the 90 isolates of *P. aeruginosa* tested, 58 of the isolates fell into 11 CCs. We observed eight STs among the isolates showing different and often unrelated PTs, suggesting that for *P. aeruginosa*, the PT evolves more rapidly than the ST. In other words, PFGE detects changes in the molecular clock better than MLST does. Giske et al. analyzed 10 VIM-1-like metallo-β-lactamase-producing *P. aeruginosa* isolates and showed that PFGE was more discriminatory but concluded that the MLST method and the clonal relationship data provided by that method were more advantageous for the typing of *P. aeruginosa* (12).

In this study we have compared PFGE and MLST to determine their discriminatory abilities and the potential utilities of PFGE and MLST in determining the relatedness of *P. aeruginosa* isolates. We determined that PFGE is more discriminatory than MLST, and although MLST has the power to establish clonal relationships between the isolates, for eight STs, PFGE discriminated the isolates further than MLST did. Future studies analyzing *P. aeruginosa* isolates from the same patient or from patients who are known to have epidemiological links are needed to compare PFGE and MLST for their abilities to distinguish isolates that are similar.

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