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Use of Coagulase Gene (*coa*) Repeat Region Nucleotide Sequences for Typing of Methicillin-Resistant *Staphylococcus aureus* Strains

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Coagulase gene (*coa*) short sequence repeat region sequencing was used to measure relatedness among a collection of temporally and geographically diverse methicillin-resistant *Staphylococcus aureus* isolates. The results show that *coa* polymorphism is free of strong selective pressure and has a low index of variation that may be useful for long-term epidemiological investigations. *coa* typing is a useful addition to *spa* typing for analysis of *S. aureus*, including methicillin-resistant strains.

Recently, a method for sequencing and analyzing the polymorphic region of the protein A gene of *Staphylococcus aureus* (*spa* typing) was described for use in outbreak investigations (13). Although *spa* typing is appropriate for the study of short-term epidemiology, additional genetic markers with a slower rate of evolution are needed to help relate clonal groups of methicillin-resistant *S. aureus* (MRSA) isolates from temporally and geographically diverse locations (1, 12). Toward this end, we examined the utility of a second sequence target, the coagulase gene (*coa*) variable region, for use in conjunction with *spa* sequencing for the strain typing of MRSA.

The coagulase protein is an important virulence factor of *S. aureus*. Like *spa*, *coa* has a polymorphic repeat region that can be used for differentiating *S. aureus* isolates (Fig. 1). The variable region of *coa* is comprised of 81-bp tandem short sequence repeats (SSRs) (16) that are variable in both number and sequence (2), as determined by restriction fragment length polymorphism analysis of PCR products (2–4).

To determine the suitability of *coa* variability for molecular typing of MRSA, the nucleotide sequences of the *coa* repeat regions of 54 archived MRSA isolates were determined. Eighteen MRSA isolates (group 1) were chosen for their geographic as well as temporal diversity (6). Another 36 isolates (group 2) represented the breadth of observed *spa* type diversity seen in MRSA isolates from New York City hospitals (Table 1) (13). Strain discrimination and the grouping of isolates determined by *coa* and *spa* typing were compared.

coa and *spa* sequencing and analysis were performed as described previously (13). The forward and reverse primers used for amplification and direct sequencing of the *coa* variable-region were *coa*F (5′-TGCTGGTACAGGTATCCGTG AAT-3′) and *coa*R (5′-AGAAGCACATAGAATGCATGA-3′), respectively. Primers were designed from available sequences (5) (GenBank accession no. X167457). *coa* (and *spa*) repeats were analyzed using the program FINDPATTERNS from Genetics Computer Group Wisconsin Package 9.1. The following ambiguous search sequence identified all unique coagulase variable region repeats in the isolates analyzed: GCN{4}CCN{8}ANAANNCN{5}ANANNAANGCNNA NAANGNNACNACNNAN{5}ANGGN{11}ANNGN. A repeat type, designated by an alphabetical code, was assigned to each of the identified repeats (e.g., repeat A1, B1, C2. . .). *coa* strain types were then defined by the string of repeat types (from the 5′ to the 3′ ends of the repeat region) that represented the organization of the *coa* SSR region of each of the strains [e.g., *coa* repeat types A(1)+B(1)+C2+C2+E3 = *coa* strain type (#)-ABC2C2E3; to make the notation shorter, letter codes ending in 1 have been simplified by removing the number]. All unique *coa* strain types were assigned a numerical code.

In the *coa* variable regions of group 1 and 2 isolates, sequence determination and analysis identified 36 different *coa* repeat types, representing 18 amino acid sequence combinations (Fig. 1). *spa* SSRs showed less diversity, with 24 unique repeat types identified (*spa* repeat sequences not shown).

Since the *coa* gene product is a phenotypic trait that may have a role in virulence, selective forces could affect the rate of *coa* repeat evolution, uncoupling it from that of the overall *S. aureus* genome and obscuring genetic relationships based on *coa* variation. To evaluate the selective forces acting on repeat units, synonymous and nonsynonymous substitution rates were determined using the GCG program DIVERGE, which analyzes pairwise, codon-by-codon comparisons of protein coding sequences based on the method by Li et al. (7). Substitution rates were calculated from aligned repeat sequences (Fig. 1), which were created using the GCG program PILEUP. *coa* repeats had a higher number of synonymous substitutions per synonymous site (ds = silent) relative to nonsynonymous substitutions per nonsynonymous site (dn = amino acid altering), indicating an average ds/dn ratio of 6.3 for *coa*. A ds/dn ratio near 1 (the neutral expectation) suggests that *coa* repeats are subject to weak conservation. In agreement with this hypothesis, amino acid replacements maintained the same hydrophobicity, indicating that polymorphisms may be selectively neutral. Thus, as with *spa* (13), *coa* repeats do not evolve from positive selection, i.e., variability is likely to be uncoupled from environmental selection.

As mentioned above, the organization of *coa* and *spa* repeats in the SSR regions of each isolate was used to determine a *spa* or *coa* strain type. A total of 13 unique *coa* and 33 *spa* types were identified, and *coa* polymorphic regions varied in size from three to seven repeats (Table 1). Together, *spa* and *coa* typing resulted in 33 strain types (the same as *spa* alone). Thus, *coa* typing did not distinguish isolates with the same *spa* type. Even though *spa* has fewer repeat motifs (or greater repeat conservation), its variable region is more polymorphic and therefore more discriminatory than that of *coa*, apparently due

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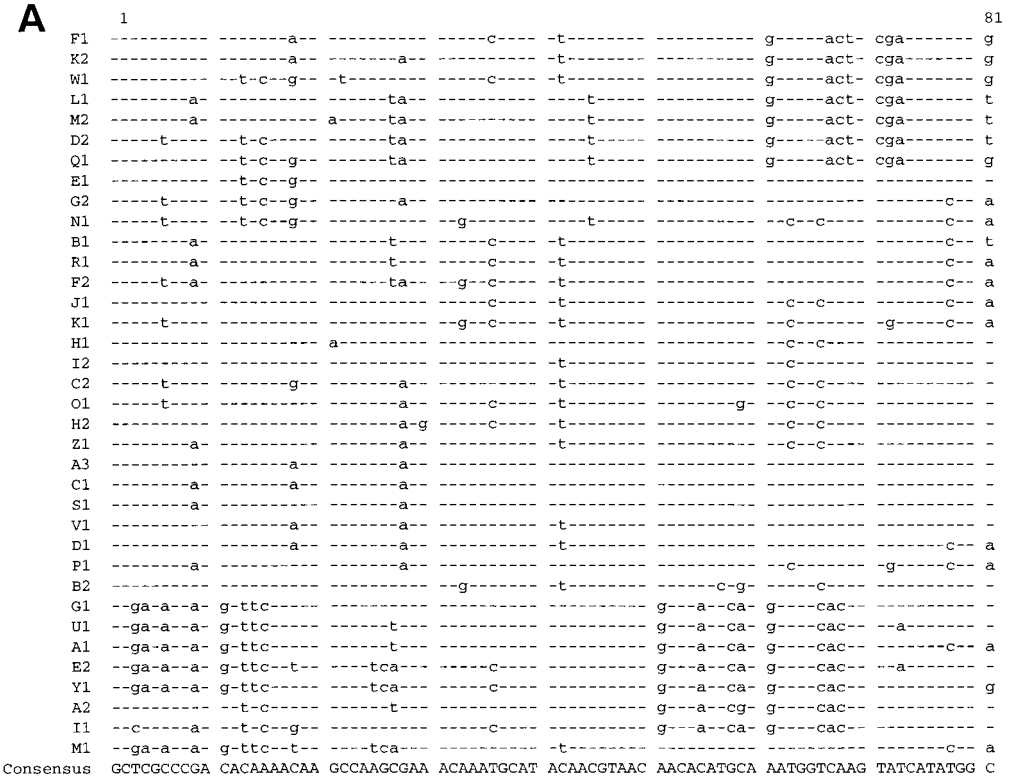
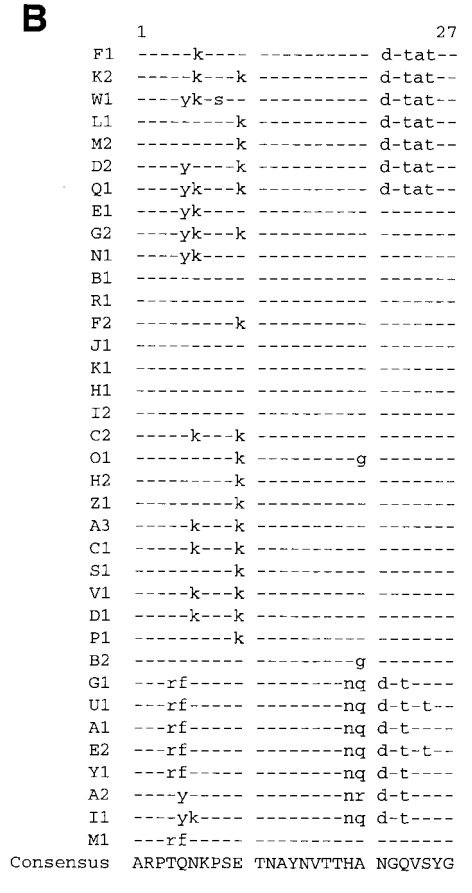


FIG. 1. DNA (A) and the deduced amino acid (B) sequences of *coa* repeats. Identical residues are identified by dashes.



to a greater rate of repeat recombination. Each *spa* type was associated with only one *coa* type, which, given the temporal and geographic diversity of the isolates, suggests that in MRSA, primary differentiation of *coa* is for the most part followed by polymorphism of *spa*.

Distinguishing MRSA isolates for outbreak investigation requires the analysis of rapidly evolving markers, which can obscure relationships among clones that have had more time to diverge (15). The slower rate of change (clock speed) associated with *coa* typing (it has less discrimination than *spa* typing [13]) is more appropriate for answering questions of global epidemiology. Thus, *coa* typing can be used to enhance the value of *spa* typing by providing more supported inferences on strain lineage and clonality among isolates with similar or identical *spa* repeat organization, since congruence between different markers is an indication of linkage disequilibrium or clonality (*coa* and *spa* are estimated to be approximately 100 kb apart and are therefore unlikely to be coinherited in a single transfer event (Steve Gill [TIGR] personal communication). The use of more than one genetic marker for relating strains is desirable (14) and likely to become increasingly important because recombination will eventually diversify MRSA to the extent that clonal types within a given region can no longer be distinguished by a single locus.

To determine congruence between *coa* and *spa* typing results among the population sampled, isolates were organized based on the similarity of their *coa* and *spa* repeat regions (Table 1), which placed 53 of 54 isolates into four major groups (labeled groups A, B, C, and D). This association was based on the assumption that accumulated point mutations are consistent between strains and thus may be an indication of more

TABLE 1. Characteristics of group 1 and 2 isolates

Group	City (borough)	State or province[a]	Country	Yr(s) of isolation	*spa*[b]		*coa*[b]		*spa* and *coa* group[c]
					Type	Repeats	Type	Repeats	
II	New York	NY	United States	1996	20	YHB2CMBQBLO	11	MNPQ	C
I	San Antonio	TX	United States	1986–1987	7	YHGCMBQBLO	11	MNPQ	C
II	New York (Queens)	NY	United States	1996	7	YHGCMBQBLO	11	MNPQ	C
II	New York (Brooklyn)	NY	United States	1996	7	YHGCMBQBLO	11	MNPQ	C
II	New York	NY	United States	1996	7	YHGCMBQBLO	11	MNPQ	C
I	London	Ontario	Canada	1997	40	YFGFMBQBLO	3	MNOPQ	C
I	London		England	1989	55	YGFMBQBLQBLPO	3	MNOPQ	C
I			Denmark	1960s	4	YHFGFMBQBLO	3	MNOPQ	C
I	Toronto	Ontario	Canada	1986	4	YHFGFMBQBLO	3	MNOPQ	C
II	New York	NY	United States	1996	4	YHFGFMBQBLO	3	MNOPQ	C
I	London		England	1960s	1	YHGFMBQBLO	3	MNOPQ	C
I	New York	NY	United States	1986–1987	1	YHGFMBQBLO	3	MNOPQ	C
II	New York	NY	United States	1996	1	YHGFMBQBLO	3	MNOPQ	C
I	New York	NY	United States	1990	48	YHGFMBQBLO	3	MNOPQ	C
I			Denmark	1960s	59	YHGFMBQBLO	3	MNOPQ	C
I	Dublin		Ireland	1991	46	YMBQBLO	3	MNOPQ	C
I	New York	NY	United States	1994	4	YHFGFMBQBLO	3	MNOPQ	C
I	Dublin		Ireland	1991	1	YHGFMBQBLO	3	MNOPQ	C
I	Geneva		Switzerland	1962	1	YHGFMBQBLO	3	MNOPQ	C
I	Cairo		Egypt	1961	1	YHGFMBQBLO	3	MNOPQ	C
I			Uganda	1966	1	YHGFMBQBLO	3	MNOPQ	C
I	New York (Brooklyn)	NY	United States	1990	12	TJMGMK	3	MNOPQ	C
I	Iowa City	IA	United States	1986–1987	8	UJ	8	USVDW	
I	San Francisco	CA	United States	1986–1987	9	UGFMEEBBPB	12	E2F2G2H212J2K2	
I	Toronto	Ontario	Canada	1996	30	XKAKAOM	14	GHIJKM2	B
II	New York (Staten Island)	NY	United States	1996	19	XKAKAOMQ	14	GHIJKM2	B
I			Japan	1981	43	WGKAKAOMQ	2	GHIJKL	B
I	New York (Brooklyn)	NY	United States	1990	3	WGKAOMQ	2	GHIJKL	B
I	Davis[d]	CA	United States	1978	3	WGKAOMQ	2	GHIJKL	B
I	London		England	1989	3	WGKAOMQ	2	GHIJKL	B
II	New York (Brooklyn)	NY	United States	1996	3	WGKAOMQ	2	GHIJKL	B
I	Montreal	Quebec	Canada	1989	44	WGMQ	2	GHIJKL	B
I	New Orleans	LA	United States	1986–1987	6	A2AKBEKBKB	10	YZA2B2C2D2	D
I	Toronto	Ontario	Canada	1996	31	XKAKBBEBKB	10	YZA2B2C2D2	D
I	Toronto	Ontario	Canada	1996	15	A2AKEEMBMK	18	YZD2	D
I	London	Ontario	Canada	1997	15	A2AKEEMBMK	18	YZD2	D
I	Memphis	TN	United States	1986–1987	10	TIMBMDMGMK	4	AREDEF	A
I	Dublin		Ireland	1990	47	TMDMGMK	21	ARCDEF	A
I	Winnepeg	Manitoba	Canada	1989	56	SMBDMGMK	6	ABSDEF	A
I	Edmonton	Alberta	Canada	1989	45	TJMBDMGMK	6	ABSDEF	A
II	New York (Bronx)	NY	United States	1996	29	TJMBMDMGGMK	1	ABCDEF	A
II	New York	NY	United States	1996	23	TJMBMDMGK	1	ABCDEF	A
I	New York	NY	United States	1990	2	TJMBMDMGMK	1	ABCDEF	A
I	Davis	CA	United States	1976	2	TJMBMDMGMK	1	ABCDEF	A
II	New York	NY	United States	1996	2	TJMBMDMGMK	1	ABCDEF	A
II	New York	NY	United States	1996	2	TJMBMDMGMK	1	ABCDEF	A
II	New York (Bronx)	NY	United States	1996	2	TJMBMDMGMK	1	ABCDEF	A
II	New York	NY	United States	1996	2	TJMBMDMGMK	1	ABCDEF	A
I	New York (Brooklyn)	NY	United States	1991	14	TJMBMDMGMKK	1	ABCDEF	A
I	Memphis	TN	United States	1986–1987	11	TJMBMDMMK	1	ABCDEF	A
II	New York (Brooklyn)	NY	United States	1996	26	TJMBMGMK	1	ABCDEF	A
II	Stony Brook	NY	United States	1996	25	TJMDMGMK	1	ABCDEF	A
II	New York	NY	United States	1996	28	TKJMBMDMGMKK	1	ABCDEF	A
II	Westchester	NY	United States	1996	27	TMDGMMK	15	UA3DW	A

[a] NY, New York; CA, California; TN, Tennessee; LA, Louisiana; IA, Iowa.

[b] *spa* and *coa* types are arbitrarily assigned for each unique sequence identified. Repeat codes are derived from organization of individual repeats (random alphabetical code). To make the notation shorter, letter codes ending with the digit "1" have been simplified by removing the number. It should be noted that two isolates were assigned separate *spa* types (48 and 59), despite an identical repeat pattern. They contained a sequence polymorphism in a flanking region that is normally conserved (not shown).

[c] *spa* and *coa* groups are organized based on sequence alignment and congruence of the *spa* and *coa* repeat similarity. The assignment of a particular *spa* or *coa* group (A, B, C, and D) was based on the reported *spa* repeat similarity groupings of New York City MRSA (Shopsin et al. [13]).

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recent common ancestry (13). Because of the high sequence identity among *coa* repeat regions in our study isolates, the same major groupings may be obtained by dendrograms constructed from progressive, pairwise alignments of *coa* sequences (not shown). However, since more extensive duplication or deletion of repetitive units obviates comparison using algorithms based on alignments, visual analysis of repeat organization is a more simple and reliable means of relating strains.

The apparent nonrandom association between genetic markers (linkage disequilibrium) among isolates from diverse temporal and geographic locations suggests only four predominant lineages among MRSA strains. This striking observation may

be explained by at least three hypotheses. First, there may simply be four primary lineages of the *coa* gene and/or *S. aureus*. Second, the predominance of particular clones may result from a longer association of the *mec* resistance element with the chromosomes of particular isolates (9) and the possibility of a low-rate of horizontal transfer of *mec* DNA among *S. aureus* lineages. A third but not mutually exclusive hypothesis is that not all MRSA clones are equally fit.

In addition, it appears that local (New York City) MRSA populations can contain the full extent of genetic variation that exists worldwide. The global recovery of the same genotypes is an indication of restricted recombination, possibly related to the lack of natural transformability of *S. aureus* (10, 11). Recovery of the breadth of global clonal types, however, may only be common in urban regions. Elsewhere, geographic isolation probably creates even more homogenous hospital populations, which are difficult to discriminate. Thus, the effectiveness of typing for determining the likelihood of strain transmission will have strong regional variation.

In summary, current methods for tracking the spread of MRSA over large geographic areas commonly require the use of multiple techniques that lack many of the inherent advantages in terms of interpretation, databasing, and portability of results afforded by sequence-based methods. The multilocus sequence typing technique combines sequence information from several housekeeping genes (approximately seven to eight genes) in a manner similar to multilocus enzyme electrophoresis (8). However, because of the polymorphism associated with repeat regions, SSR sequencing can discriminate isolates with a minimum of loci (as well as nucleotides), and strain types can be described using a simple numerical format and alphabetical repeat designation that lends itself to use in a wide range of laboratories (13). *coa* typing now expands the role of SSR sequence typing from infection control and outbreak investigation (via the *spa* locus) by providing the clinical microbiologist and researcher with the opportunity to use a simple, rapid, and practical method to monitor variation in MRSA populations and relate strains from divergent regions and time periods.

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REFERENCES

1. **Ayliffe, G. A.** 1997. The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. **24**(Suppl. 1)**:**S74–S79.
2. **Goh, S. H., S. K. Byrne, J. L. Zhang, and A. W. Chow.** 1992. Molecular typing of Staphylococcus aureus on the basis of coagulase gene polymorphisms. J. Clin. Microbiol. **30:**1642–1645.
3. **Hookey, J. V., V. Edwards, B. D. Cookson, and J. F. Richardson.** 1999. PCR-RFLP analysis of the coagulase gene of *Staphylococcus aureus*: application to the differentiation of epidemic and sporadic methicillin-resistant strains. J. Hosp. Infect. **42:**205–212.
4. **Hookey, J. V., J. F. Richardson, and B. D. Cookson.** 1998. Molecular typing of *Staphylococcus aureus* based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. J. Clin. Microbiol. **36:**1083–1089.
5. **Kaida, S., T. Miyata, Y. Yoshizawa, H. Igarashi, and S. Iwanaga.** 1989. Nucleotide and deduced amino acid sequences of staphylocoagulase gene from *Staphylococcus aureus* strain 213. Nucleic Acids Res. **17:**8871.
6. **Kreiswirth, B., J. Kornblum, R. D. Arbeit, W. Eisner, J. N. Maslow, A. McGeer, D. E. Low, and R. P. Novick.** 1993. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. Science **259:**227–230.
7. **Li, W. H., C. I. Wu, and C. C. Luo.** 1985. A new method for estimating synonymous and nonsynonymous rates of nucleotide substitution considering the relative likelihood of nucleotide and codon changes. Mol. Biol. Evol. **2:**150–174.
8. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K.Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
9. **Musser, J., and R. Selander.** 1990. Genetic analysis of natural populations of *Staphylococcus aureus*. VCH, New York, N.Y.
10. **Musser, J. M., J. S. Kroll, E. R. Moxon, and R. K. Selander.** 1988. Evolutionary genetics of the encapsulated strains of *Haemophilus influenzae*. Proc. Natl. Acad. Sci. USA **85:**7758–7762.
11. **O'Rourke, M., and E. Stevens.** 1993. Genetic structure of *Neisseria gonorrhoeae* populations: a non-clonal pathogen. J. Gen. Microbiol. **139**(Pt. 11)**:** 2603–2611.
12. **Roberts, R. B., A. M. Tennenberg, W. Eisner, J. Hargrave, L. M. Drusin, R. Yurt, and B. N. Kreiswirth.** 1998. Outbreak in a New York City teaching hospital burn center caused by the Iberian epidemic clone of MRSA. Microb. Drug Resist. **4:**175–183.
13. **Shopsin, B., M. Gomez, S. O. Montgomery, D. H. Smith, M. Waddington, D. E. Dodge, D. A. Bost, M. Riehman, S. Naidich, and B. N. Kreiswirth.** 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. **37:**3556–3563.
14. **Tenover, F. C., R. Arbeit, G. Archer, J. Biddle, S. Byrne, R. Goering, G. Hancock, G. A. Hebert, B. Hill, R. Hollis et al.** 1994. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. J. Clin. Microbiol. **32:**407–415.
15. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed- field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
16. **van Belkum, A., S. Scherer, L. van Alphen, and H. Verbrugh.** 1998. Short-sequence DNA repeats in prokaryotic genomes. Microbiol. Mol. Biol. Rev. **62:**275–293.