

mecA-blaZ Corepressors in Clinical *Staphylococcus aureus* Isolates

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The presence and nucleotide sequences of the two *mecA* repressors, *mecI* and *blaI*, were assessed in 73 clinical *Staphylococcus aureus* isolates. Isolates with *mecI* mutations were grouped into unique clonal types based on their *spa* nucleotide repeat patterns. Forty-three of the 45 (96%) isolates with mutant *mecI* or with a deletion of *mecI* contained *blaI*, while *blaI* was present in only 21 of 28 (78%) isolates with wild-type *mecI* ($P < 0.05$). Among 22 additional isolates that did not contain *blaI*, all had wild-type *mecI* sequences. We conclude that oxacillin-resistant *S. aureus* must have at least one of the two functional *mecA* regulators.

Transcription of *mecA*, the gene mediating oxacillin resistance in staphylococci, is regulated by a repressor, *mecI*, that is divergently transcribed from *mecA* as the second gene in a two-gene operon that also includes *mecR1* (1, 11, 21). More than 90% of staphylococcal isolates also produce β -lactamase, the product of *blaZ*, and contain *blaZ* regulatory sequences (*blaI* and *blaR1*) that are similar in sequence and function to *mecA* regulators (9, 10). In addition to regulating *blaZ* transcription, BlaI also binds to *mecA-mecR1* promoter-operator (P-O) sequences and regulates their transcription (9, 13, 14, 19). Coregulation of *mecA* by both MecI and BlaI has been demonstrated in defined laboratory strains (14), but neither the presence nor the nucleotide sequences of the two coregulators have been investigated in clinical isolates.

Mutations and deletions in *mecI* and deletions of both *mecI* and *mecR1* appear to be common in clinical *Staphylococcus aureus* isolates (1, 12, 16, 21, 22). In contrast, while there has been no systematic assessment of *blaI* and *blaR1* in clinical isolates, these regulators have been found to be intact in sequence or function whenever they have been examined. To explore the extent of MecI and BlaI coregulation of *mecA*, we examined a collection of geographically and temporally diverse oxacillin-resistant *S. aureus* isolates, most of which were also shown to have genetic diversity. We also correlated the clonal type with regulator sequence by using the number of unique nucleotide repeat sequences in the protein A (*spa*) gene as a typing tool as previously described (20).

Source of isolates. The oxacillin-resistant *S. aureus* isolates examined in this study came from three sources. The first source was the Public Health Research Institute (PHRI) collection, from which 40 isolates were chosen. These isolates were selected because 37 of the 40 isolates had different *spa* repeat patterns (20), indicating clonal diversity. They were of unknown *mecI* status, came from five different countries and 19 cities, and were isolated between 1960 and 1997. The second

source was the Virginia Commonwealth University (VCU) collection, from which 33 isolates were chosen. These isolates had been previously shown to have sequences that hybridized with a *mecI* probe (1) and were recovered from patients in 25 different cities in four countries between 1968 and 1993. The final source was the SCOPE collection (6), from which 208 oxacillin-resistant *S. aureus* isolates were examined for β -lactamase production. These isolates were recovered from the blood of patients in 38 contributing hospitals in the United States between 1997 and 2000. All isolates were identified as *S. aureus* by standard criteria and were shown to have the *mecA* gene by PCR amplification of genomic DNA.

Identification and characterization of repressors. Chromosomal DNA was prepared by using a Qiagen (Chatsworth, Calif.) genomic DNA preparation kit according to the manufacturer's directions. PCR amplification of DNA sequences was performed to confirm the presence of *mecA*, *mecI*, *mecA-mecR1* P-O sequences, *blaZ*, and *blaI* by use of the corresponding primers shown in Table 1. A Qiagen Taq PCR master mix kit was used in the amplification reaction, and the recommended thermocycling conditions were achieved according to the manufacturer's directions. The sequences of *mecI*, *blaI*, and *mecA* P-O from different *S. aureus* isolates were determined from specific amplified PCR products, and sequence analysis was performed by using the automated laser fluorescence technique employing fluorescein-labeled oligonucleotides (Applied Biosystems, Foster City, Calif.). Sequencher DNA sequencing analysis software (Gene Codes Corporation, Ann Arbor, Mich.) was used to compare sequences for each isolate with the database sequences for these genes from *S. aureus* N315 and the β -lactamase plasmid pI258. The production of β -lactamase was assessed with nitrocefin disks (Becton Dickinson, Cockeysville, Md.) according to the manufacturer's instructions and as previously described (4).

The *mecI* gene was detected in 56 of the 73 VCU and PHRI isolates (77%) by hybridization and subsequent PCR amplification. The other 17 isolates contained the identical deletion of *mecI* and the 3' 783 nucleotides of *mecR1*, with insertion of a truncated copy of *IS1272*, as has been previously described (1, 2). Examination of the nucleotide sequences of the *mecI* PCR

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TABLE 1. Primers used in this study

Gene and primer	Nucleotide sequence
<i>mecA</i>	
566.....	5'-TCATAGCGTCATTATTCC-3'
570.....	5'-ATCACTGGTATATCTTCACC-3'
<i>mecA-mecRI</i>	
P-O	
234.....	5'-CCAAACCCGACAACACTAC-3'
247.....	5'-CGTGTACAGATACATTTTCG-3'
<i>mecI</i>	
205.....	5'-CCGGAATTCGCATATGGATTTCAC-3'
251.....	5'-GATGGTTCGTAGGTTATGTTG-3'
214.....	5'-CGGATCCGAAATGGAATTAATATAATG-3'
215.....	5'-CGGAATTCGACTTGATTGTTTCT-3'
<i>blaZ</i>	
048.....	5'-TCAAACAGTTCACATGCC-3'
049.....	5'-TTCATTACTCTGGCG-3'
<i>blaI</i>	
422.....	5'-CCCAATGGGTGTTTTAAATGGCCAA-3'
453.....	5'-AATGGTATTCTGTACTCT-3'

products showed that 27 of the 56 isolates (48%) contained the wild-type gene, identical to sequences shown to provide maximal repression of *mecA* transcription (14, 19). The other 29 isolates had genetic alterations in *mecI*. In addition, the *mecA-mecRI* intergenic region, containing the *mecA* and *mecRI* promoters as well as the MecI binding site, was PCR amplified in all 56 isolates having *mecI*; all sequences were identical to wild type.

β -lactamase was produced by 64 of the 73 VCU and PHRI isolates, and all of these 64 isolates also had PCR products amplified by *blaZ*- and *blaI*-specific primers. None of the nine β -lactamase-negative isolates had sequences that could be amplified by *blaI* primers. The *blaI* nucleotide sequence was determined for all isolates having a *mecI* mutation or deletion (45 isolates). For all but two of these isolates, the *blaI* sequences were the same as those published previously or in the database. Seven of the nine isolates with no *blaI* contained *mecI* with wild-type sequence. Twenty-two of the 208 (11%) oxacillin-resistant *S. aureus* isolates from the SCOPE collection were β -lactamase- and *blaI*-negative, and each of the 22 contained wild-type *mecI*.

The genetic alterations in *mecI* were nonsense (13 isolates), frameshift (4 isolates), or missense (11 isolates) mutations. The nonsense and frameshift mutations introduced translational stop codons into the sequence at positions that were predicted to truncate the protein, rendering it inactive. The effect of missense mutations on repressor function could not be reliably predicted from examination of the predicted amino acid sequence, but in each case the nucleotide change resulted in substitution of an amino acid. Sixteen of the 17 isolates with a deletion of *mecI* and 27 of the 28 isolates with *mecI* mutations (43 of 45 isolates in all [98%]) contained *blaI* with sequence identical to that of published, functional repressors. The *mecI* mutation in the single isolate of this group that was *blaI* negative was a missense mutation (Leu₄₈ → Phe), resulting in a conservative substitution. In contrast, only 21 of 28 (75%)

TABLE 2. Number of *S. aureus* isolates containing *mecA* repressors

<i>mecI</i> genotype	No. (%) of isolates with <i>blaI</i>	
	Present	Absent
Mutant (nonsense)	13 (100)	0 (0)
Mutant (frameshift)	4 (100)	0 (0)
Mutant (missense)	10 (92)	1 (8)
Deletion	16 (94)	1 (6)
Wild type	21 (75)	7 (25)
Total	64 (88)	9 (12)

isolates with intact *mecI* contained *blaI* (43 of 45 versus 21 of 28; $P < 0.05$, Fisher's exact test) (Table 2). Thus, among 281 isolates examined in this study, 31 (11%) were β -lactamase and *blaI* negative, and 29 of these 31 (94%) had wild-type *mecI* sequences. In contrast, among the 65 β -lactamase-positive isolates in which *mecI* was examined, only 21 (32%) contained wild-type *mecI* sequences ($P < 0.001$, Fisher's exact test).

Relationship between repressor content and clonal type.

Some of the isolates with the same *spa* type were also examined by pulsed-field gel electrophoresis (PFGE) of genomic DNA, digested with the restriction enzyme *SmaI*, and electrophoresed to visualize both large and small restriction fragments, as previously described (7). The isolates with *mecI* mutations and deletions were clustered into clonal groups, and those with the same mutation were in the same clonal group. As shown in Table 3, 12 isolates had the same mutation at nucleotide 202 (Gln₆₈ → stop) and all 12 had the identical *spa* pattern. Seven of the 12 isolates containing this nonsense mutation came from different cities and were isolated in different years, and 9 of the 12 had unique PFGE patterns (>3-band difference). Three isolates and two isolates had the same missense mutations (Ala₁₁ → Val and Leu₁₁₆ → Ser, respectively). The *spa* types were the same for the latter two isolates and were the same or closely related for the former three. All five of these isolates had unique PFGE types. Thus, the *spa* and *mecI* types seem to designate stable clones that have disseminated over time and place while PFGE analysis identifies more recent genetic alterations that reflect local environmental pressures.

Among the 45 isolates containing a *mecI* mutation or deletion, only 3 had a *spa* type that was found among the 27 isolates with wild-type *mecI*; among the 28 with wild-type sequence, only 2 had a type related to those isolates with mutations (Table 3). Thus, *spa* typing identified unique clonal groups that might be more likely to develop a mutant or deleted *mecI*. It is possible that the presence of *mecI* mutations defines oxacillin-resistant *S. aureus* clones with a mutator phenotype that could result in mutations, deletions, or insertions of other genes that were not sought in this study.

Conclusion. The retention of functional *mecA* and *blaZ* repressors may not be due solely to a need to modulate the quantity of regulated gene products in response to the presence of β -lactam antibiotics in the environment. It has been shown that the expression of oxacillin resistance (high level [homotypic] or low level [heterotypic]) has little correlation with *mecA* transcription (8, 17, 18). Furthermore, 90% of staphylococci retain *blaZ* and its regulatory genes despite the narrow substrate specificity of the β -lactamase (5), providing

TABLE 3. Clonal distribution of *S. aureus* isolates relative to *mecA* repressor genotype

<i>mecI-blaI</i> genotype	No. of isolates	<i>spa</i> type(s)	Related profile ^a
<i>mecI</i> mutation (Gln ₆₈ →stop)	12	Type 3 (12 isolates)	WGKAOMQ
<i>mecI</i> mutation (Glu ₁₁₅ →stop)	1	Type 16 (1 isolate)	WGKAKAOMQQQ
<i>mecI</i> mutation (Ala ₁₁ →Val)	3	Type 245 (2 isolates) Type 7 (1 isolate)	YHFMBQBLO YHGCMQBLO
<i>mecI</i> mutation (Leu ₁₁₆ →Ser)	2	Type 1 (2 isolates)	YGFMBQBLO
Different <i>mecI</i> mutations (frame-shifts, missense, and deletions)	27	Types 1 (3 isolates), 48, 7 (2 isolates), 1, and 4 Type 3 Types 5, 6, 9, 10, 13, 15, 17, 19, 20, 21, 27, 31, 32, 34, 43, and 55 Types 2, 14, and 28	YHGFMBQBLO related WGKAOMQ Unrelated to any other types TJMBMDMGMK related ^b
<i>mecI</i> wild type	28	Types 2 (13 isolates), 11, 12, 23, 24, 26, 29, and 41 Type 123 (2 isolates) Types 8, 43, 49, 50, 56, and NEW	TJMBMDMGM related WGKKKAOM ^c Unrelated to any other types

^a Related, ≥80% of repeats in the same order; unrelated, ≤20% of repeats in the same order.

^b Isolates with mutant *mecI* having *spa* types found among isolates with wild-type *mecI*.

^c Isolates with wild-type *mecI* having *spa* types found among isolates with mutant *mecI*.

no protection against most of the β-lactam antibiotics that are prevalent in hospitals. Therefore, the retention of β-lactamase and *mecA* regulatory sequences may be necessary to prevent the deleterious effects on the cell of unregulated protein production. The target of regulation is either PBP 2a or penicillinase, both of which can be produced in large quantities in the absence of induction without any obvious consequences to the cell (3, 15). However, *mecR1* and *blaR1* are also regulated by *mecI* and *blaI* as a consequence of autoregulation of the two-gene operon. Overproduction of *mecR1* on an expression vector in the absence of β-lactam induction markedly retards cell growth (A. Rosato, unpublished observations). Both *blaZ* and *mecA* encode penicillin-binding proteins, which, like all penicillin-binding proteins, are products of evolution from a common ancestor (11). However, PBP2a and penicillinase are less closely related, by comparison of amino acid sequences, than are their regulators. Thus, it is tempting to speculate that the regulators evolved from an ancestor different from that of at least one of the regulated genes and that, in the case of *mecA*, regulation has persisted not so much to allow fine modulation of the regulated gene product as to prevent toxic overproduction of one of the proteins.

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