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Guidelines for Reporting Novel *mecA* Gene Homologues

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Methicillin-resistant staphylococci are disseminated all over the world and are frequent causes of health care- and community-associated infections. Methicillin-resistant strains typically carry the acquired *mecA* gene that encodes a low-affinity penicillin-binding protein (PBP), designated PBP2a or PBP2′. In most strains, *mecA* is part of a chromosomally integrated mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*). The *mecA* gene is widely disseminated among *Staphylococcus aureus* and other staphylococcal species, and its expression is essential for the methicillin-resistant phenotype.

Recently, *mecA* gene homologues that are only distantly related to *mecA* have been identified in the genomes of staphylococci and some related bacterial species (Table 1). So far, four groups of *mecA* homologues have been described based on their degree of homology to the earliest identified *mecA* gene.

We believe that this diversity warrants a new naming system based on phylogenetic principles which can also serve as a guideline for the reporting of additional novel *mecA* homologues that may be identified in the future.

OVERVIEW OF *mecA* GENE HOMOLOGUES

The *mecA* gene originally identified in methicillin-resistant *S. aureus* (MRSA) (2, 9, 12) encodes a PBP of 668 amino acid residues which is responsible for beta-lactam resistance (8, 13, 17, 19). The *mecA* gene is carried by SCC*mec* and has been identified in various staphylococcal species, such as *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, and *Staphylococcus fleurettii* (18). The *mecA* genes present in these species have >98% sequence identity with the *mecA* carried by the first fully sequenced prototype MRSA strain N315 (10). The first divergent *mecA* gene homologues were identified on the chromosomes of *Staphylococcus sciuri* subsp. *sciuri*, *S. sciuri* subsp. *rodentius*, and *S. sciuri* subsp. *carnaticum.* These homologues are very similar to each other and have approximately 80% nucleotide sequence identity to *mecA* of N315 (3, 20, 21). A second group of *mecA* gene homologues identified in *Staphylococcus vitulinus* have about 90% nucleotide identity to *mecA* of N315 (15).

A third group of *mecA* gene homologues are located on the chromosome and plasmids of *Macrococcus caseolyticus*, a member of a genus that is phylogenetically closely related to *Staphylococcus*.

TABLE 1 List of *mecA* homologues

Strain	Reported gene name	Proposed new name	Size (bp)	% identity with the *mecA* gene in *S. aureus* N315
S. aureus N315[a]	*mecA*	*mecA*	2,007	100
Staphylococcal strains that carry *mecA*	*mecA*	*mecA*	2,007	98.3–100
S. sciuri K11[a]	*mecA* (*mecA1*)	*mecA1*	2,001	79.1
S. sciuri ATCC 700061	*mecAs*	*mecA1*	2,001	80.2
S. vitulinus CSBO8[a]	*mecA*	*mecA2*	2,007	91
M. caseolyticus JCSC5402[a]	*mecAm*	*mecB*	2,025	61.6
S. aureus LGA251[a]	$mecA_{LGA251}$	*mecC*	1,998	68.7

[a] Prototype strains representing each *mec* gene: *S. aureus* N315 for *mecA*, *S. sciuri* K11 for *mecA1*, *S. vitulinus* CSBO8 for *mecA2*, *M. caseolyticus* JCSC5402 for *mecB*, and *S. aureus* LGA251 for *mecC*.

The *mecA* homologues carried by these strains have 62% nucleotide sequence identity to *mecA* of N315 (1). The fourth *mecA* gene homologue most recently identified in *S. aureus* strain LGA251 shows 69% identity to *mecA* of N315 (5, 16). The phylogenetic relationship of these genes is illustrated in Fig. 1. A detailed comparison of nucleotide sequences is shown in Tables S1 and S2 in the supplemental material.

PROPOSED SCHEME OF CLASSIFICATION

Several of the best-studied antimicrobial resistance genes have been classified based on differences in deduced amino acid sequences (e.g., tetracycline resistance, macrolide resistance, and

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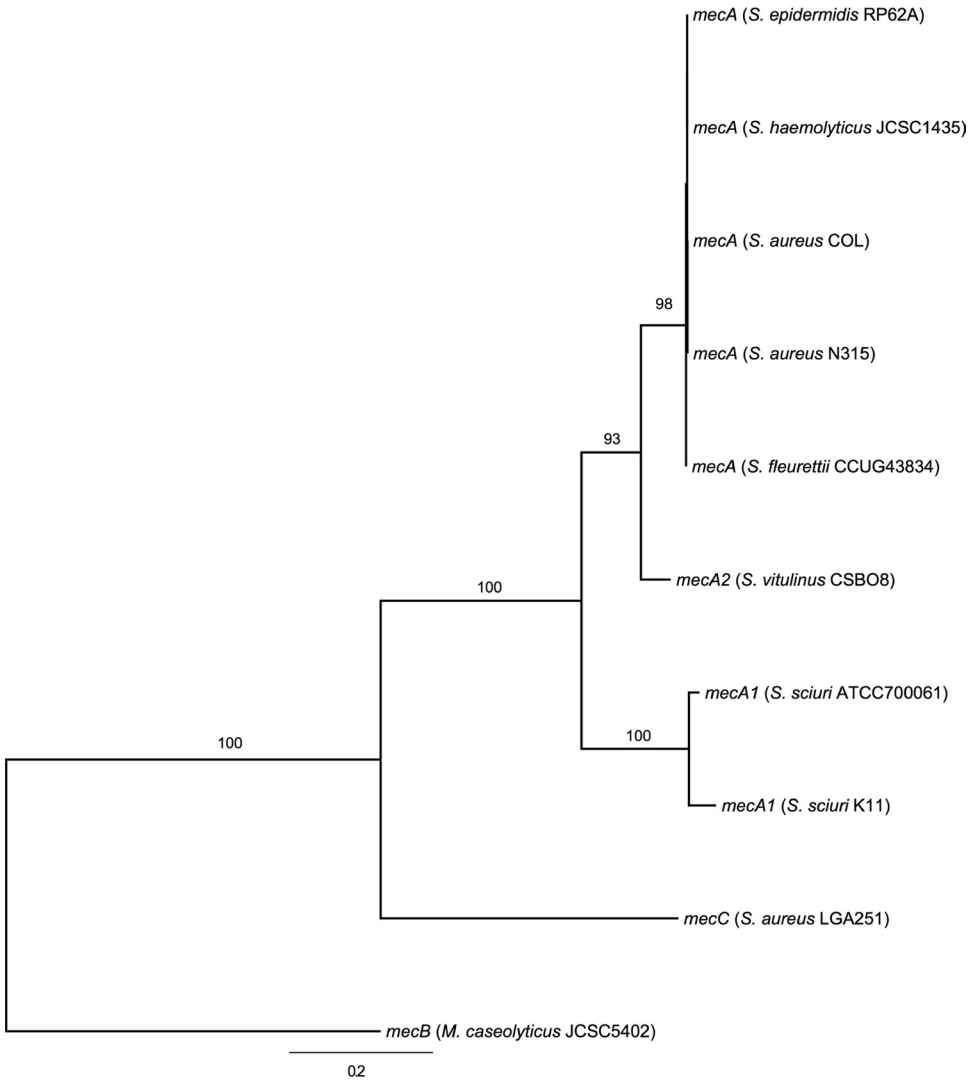

FIG 1 Phylogenetic relationships of *mecA* homologues. Maximum likelihood phylogenetic tree constructed using Seaview (4) with nucleotide sequences deposited in the EMBL/GenBank/DDBJ databases. Sequences were aligned using MUSCLE (6), and the tree was built with PhyML (7) using a general time-reversible (GTR) model assuming a gamma distribution of among-site substitution rates. The numbers at the tree branches are percentage bootstrap values indicating the confidence levels. The bar length indicates the number of substitutions per site (bar, 20 per 100 sites). Nucleotide sequences of *mecA* and its homologues were found under the following accession numbers: *S. aureus* COL, NC_002951; *S. aureus* N315, D86923, NC_002745; *S. epidermidis* RP62A, NC_0029765; *S. haemolyticus* JCSC1435, NC_0071685; *S. fleurettii* CCUG43834, AB546266; *S. vitulinus* CSBO8, AM048810; *S. sciuri* ATCC 700061, AB547235; *S. sciuri* K11, Y13094; *S. aureus* LGA251, FR821779.1; *M. caseolyticus* JCSC5402, NC_011996.1.

β-lactamase genes), and the breakpoint for classification is 80% amino acid identity (11, 14). Based on extensive discussion within the International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements (IWG-SCC) (http://www.sccmec.org), especially including the authors who first described and reported the four *mecA* gene homologues described above, we herein propose a classification and naming system for *mecA* gene homologues based on a combination of nucleotide sequence similarity and the chronological order of their discovery, i.e., the date of publication. In this way, we can more easily discern phylogenetic relationships among *mecA* genes which have been identified in various bacterial species of human as well as animal origin. This method will also help to identify the transfer of the methicillin resistance genes among human and animal commensals, independent of their antimicrobial resistance phenotype.

The *mec* gene is defined as a determinant that encodes a PBP similar to PBP2a or PBP2′ that is composed of three structural domains, a characteristic N-terminal structure, a transpeptidase domain, and a nonbinding domain.

A *mec* gene type encompasses *mec* genes sharing ≥70% nucleotide sequence identity with their respective prototype. The types are referred to as *mecA*, *mecB*, *mecC*, etc., which reflects the chronological order of their discovery. We suggest that the following prototype *mec* genes should be used in the definition of new types: *mecA* of *S. aureus* N315, *mecB* of *M. caseolyticus*, and *mecC* of *S. aureus* LGA251. Sequence identities among *mecA* homologues should be determined by creating a similarity matrix and a phylo-

genetic tree. Since *mecA* and most of its homologues are associated with mobile DNA elements, they are likely to be found across barriers of species or genera. Therefore, we do not limit the *mec* nomenclature system to the genus *Staphylococcus*.

The *mec* gene types are divided into allotypes, where each allotype encompasses a group of *mec* genes that share ≥70% but <95% nucleotide sequence identity to *mecA* of *S. aureus* N315, *mecB* of *M. caseolyticus* JCSC5402, or *mecC* of *S. aureus* LGA251. The allotypes for the class *mecA*, for example, are referred to as *mecA1*, *mecA2*, *mecA3*, etc., with the numeral based on the chronological order of discovery. The same applies for the classes *mecB*, *mecC*, etc.

According to the proposed new nomenclature, the *mecA* gene homologues described to date are renamed as follows (Table 1).

(i) The *mecA* homologues formerly called *mecAm* in *M. caseolyticus* and $mecA_{LGA251}$ of *S. aureus* strain LGA251 are renamed chronologically as *mecB* and *mecC*, respectively, to reflect the order of publication.

(ii) *mecA* genes that have nucleotide sequence identities to the original *mecA* gene of ≥95% are referred to as *mecA*, signifying that they are members of the allotype represented by the original *mecA* gene. Those with nucleotide sequence identities to the original *mecA* of ≥70% but <95% are regarded as belonging to other allotypes of *mecA*. Accordingly, the *mecA* homologues detected in *S. sciuri*, which have a nucleotide sequence identity to the original *mecA* gene of approximately 80%, are designated *mecA1*. The *mecA* homologues in *S. vitulinus* that have nucleotide sequence identities to the original *mecA* gene of approximately 90% are designated *mecA2*.

With the continued selective pressure of beta-lactam use and the increasing number of whole-bacterial-genome sequences becoming available, many more *mecA* gene homologues may be discovered in the future. We hope that the proposed classification and naming system will help to facilitate a better understanding of the transfer of methicillin resistance determinants among commensal and pathogenic bacteria.

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