Characterization of a Catalase-Negative Methicillin-Resistant
Staphylococcus aureus Strain

B. M. Grüner, 1† S.-R. Han, 1,*† H.-G. Meyer, 2 U. Wulf, 1 S. Bhakdi, 1 and E. K. Siegel 1

Institute of Medical Microbiology, Johannes Gutenberg University, D-55101 Mainz, 1 and Department of Health and Infection Prevention, Rheinland-Pfalz Center for Laboratory Investigation, D-56068 Koblenz, 2 Germany

Received 7 December 2006/Returned for modification 21 February 2007/Accepted 11 June 2007

We describe an unusual clinical strain of catalase-negative methicillin-resistant Staphylococcus aureus sensu stricto. Sequence analysis of its catalase gene showed 99.60% identities to the catalase genes of the reference strains. A 5-base deletion, however, led to a shift of the nucleotide reading frame and a loss of the enzymatic activity.

Production of catalase is considered to be a virulence determinant in Staphylococcus aureus, allowing bacteria to better resist intra- and extracellular killing by hydrogen peroxide (4, 5). Staphylococcus species are catalase positive and facultatively anaerobic, except for S. aureus subsp. anaerobius and S. saccharolyticus, which are catalase negative and anaerobic. The latter is generally considered to be apathogenic. S. aureus catalase is encoded by the katA gene, which has a 1,518-bp open reading frame and encodes a protein with 505 amino acids (9). S. aureus subsp. anaerobius harbors a mutated gene, designated katB, which is 1,368 bp long and encodes a polypeptide of 455 amino acids. Compared to the nucleotide sequence of katA, that of katB showed six missense mutations and a single-base-pair deletion, located at bp 1338 upstream from the initiation codon, which causes a shift of the nucleotide reading frame and premature translation termination at bp 1368 (9).

Facultatively anaerobic, catalase-negative S. aureus strains have been reported. However, none of them has been characterized with molecular methods (1, 2, 7, 10, 12). We here describe a catalase-negative methicillin-resistant S. aureus (MRSA) isolate that was characterized by amplification and sequencing of the putative catalase gene. To our knowledge, this is the first molecular description of a catalase-negative S. aureus subsp. aureus strain.

A 65-year-old male was admitted to the intensive care unit of the Surgery Department of the Mainz University Hospital. He was multimorbid, suffering from alcohol-toxic liver cirrhosis, arterial hypertension, coronary artery disease, heart failure (class II according to the New York Heart Association classification), diabetes mellitus, and chronic obstructive pulmonary disease. A tracheal secretion sample was taken for routine microbiological investigation, without signs of any infection being apparent at that time.

The tracheal secretion sample was processed conventionally, and creamy beta-hemolytic colonies, typical of S. aureus, were observed on 5% sheep blood agar. The isolate repeatedly tested negative for catalase after either aerobic or anaerobic incubation, even at the fifth subculture. It grew well both aerobically and anaerobically. The results of both the slide coagulase test and the DNase test were strongly positive. The BBL CRYSTAL system for gram-positive bacteria (Becton Dickinson Company, Sparks, MD) and the API Staph system (bioMérieux, Marcy l’Étoile, France) identified the isolate as S. aureus with 98.6% and 97.8% probabilities (profile codes 0064773465 and 6736153), respectively. DNA sequence analysis of the 16S rRNA gene confirmed the strain as S. aureus subsp. aureus. This strain was deposited in the DSMZ (German Collection of Microorganisms and Cell Cultures) under the number DSM 18827.

Antibiotic susceptibility was determined by disk diffusion on Mueller-Hinton agar based on CLSI (Clinical and Laboratory Standards Institute) guidelines. The strain was resistant to penicillin, oxacillin, cefaclor, cefuroxime, erythromycin, clindamycin, and ciprofloxacin but sensitive to gentamicin, rifampin, sulfamethoxazole-trimethoprim, vancomycin, teicoplanin, and linezolid. This represents the typical phenotype of endemic MRSA strains observed in our institute. The strain was further confirmed as MRSA by using PCR to identify mecA and S. aureus-specific genes as described previously (6).

The nucleotide sequence of the S. aureus catalase gene in the catalase-negative MRSA strain was amplified by PCR using primers described previously (9), and both strands were sequenced using dye terminator chemistry with an ABI PRISM 3700 DNA analyzer. The sequence analysis revealed 99.60% identity to that of the catalase gene katA of MRSA strain Mu50 or N315 (Gen Bank accession no. BA000017 or BA000018, respectively), with a difference in 6 nucleotides at positions 1152 and 1388 to 1392 upstream from the initiation codon. The single-base substitution (T) located at bp 1152 upstream from the initiation codon represented a silent mutation. However, deletion of five bases (AAACG) (bp 1388 to 1392 upstream from the initiation codon) led to a shift of the nucleotide reading frame, with the consequence of replacement of consecutive amino acids and premature translation termination at bp 1418. Similarly, our catalase gene sequence was 99.54% identical to the katA sequences of S. aureus subsp. aureus strains MSSA476, COL, NCTC 8325, USA300, and MW2, of

* Corresponding author. Mailing address: Institute of Medical Microbiology, Johannes Gutenberg University, Hochhaus am Augustusplatz, D-55101 Mainz, Germany. Phone: (49) 6131 3937341. Fax: (49) 6131 3932359. E-mail: srhan@uni-mainz.de.
† B. M. Grüner and S.-R. Han contributed equally to the paper.
‡ Published ahead of print on 20 June 2007.
which complete genomes were also sequenced, with repeated confirmation of the same 5-base deletion.

This mutation thus differed fundamentally from mutations that have been described for the catalase gene katB of catalase-negative S. aureus subsp. anaeroebius strains (GenBank accession No. AJ000471) (9). However, the consequences of a deletion in the C-terminal region of catalase seemed to be the same, i.e., a shift of the nucleotide reading frame, an early termination codon, and a loss of the enzymatic activity.

Two Staphylococcus species, S. aureus subsp. anaeroebius and S. saccharolyticus, are known not to produce catalase. Our strain differs from these species on the basis of its ability to grow well under aerobic conditions, its expression of clumping factor, its production of acid from trehalose and lactose, and its 16S rRNA sequence.

Catalases, or more correctly, hydroperoxidases, are enzymes involved in degradation of hydrogen peroxide (generated during cellular metabolism or encountered during host infection) to water and molecular oxygen. Catalase has long been implicated as a virulence determinant in S. aureus. The importance of in vivo expression of the oxidative stress enzymes catalase and superoxide dismutase has been suggested through the analysis of clinical isolates with reduced levels of expression of these enzymes (4, 5). S. aureus subsp. anaeroebius is very closely related to S. aureus sensu stricto and shares with it the ability to produce extracellular toxins and enzymes but is endowed with much lower pathogenic potential than S. aureus (9). Both intracellular survival and extracellular multiplication play important roles in the pathogenesis of S. aureus infections. Intracellular survival in neutrophils, endothelial cells, epithelial cells, and osteoblasts has been described to occur in S. aureus and thereby requires that bacteria can withstand oxidative stress (3). Catalase is a critical component for maintaining viability during long-term starvation, an ability important for the nosocomial transmission of S. aureus or MRSA (11). Finally, the production of catalase is an important mechanism allowing S. aureus to coexist with microorganisms which generate hydrogen peroxide in an aerobic environment like the upper respiratory tract. The bactericidal activity of Streptococcus pneumoniae toward S. aureus is apparently mediated by hydrogen peroxide, providing a possible mechanistic explanation for the interspecies interference observed in epidemiological studies (8).

The clinical relevance of catalase-negative S. aureus strains requires study. In previous reports, the catalase-negative S. aureus strains were isolated from blood samples, catheters, bronchial secretion samples, ulcers, and other wounds associated with infections or nosocomial endemias (1, 2, 7, 10, 12). Although few, these reports do provide evidence that catalase is not an absolute requirement for pathogenicity. However, it is possible that pathogenicity or transmission efficiency is decreased. In our case, the catalase-negative MRSA strain was repeatedly isolated from the same patient but never from other patients of the intensive care unit or the university hospital. There were no indications that the strain had caused an infection. The antibiotic resistance pattern suggested a common origin for the catalase-negative MRSA strain and other local MRSA strains. There are few, if any, reports of catalase-negative MRSA that have been isolated from a patient without apparent disease from the isolate. This paper presents a unique report in this sense. This is also the first report of a catalase-negative S. aureus subsp. aureus strain characterized with molecular methods.

**Nucleotide sequence accession number.** The catalase gene sequence identified in this study was submitted to GenBank under accession no. EF140590.

**REFERENCES**