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

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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 determin-
tant in Staphylococcus aureus, allowing bacteria to better resist intra- and extracellular killing by hydrogen peroxide (4,
5). Staphylococcus species are catalase positive and faculta-
tively 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 cata-
lase 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 charac-
terized 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 classi-
fication), 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 aer-
obically and anaerobically. The results of both the slide coag-
ulase test and the DNase test were strongly positive. The BBL
CRYSTAL system for gram-positive bacteria (Becton Dickin-
son Company, Sparks, MD) and the API Staph system (bio-
Mérieux, Marcy l’Etoile, France) identified the isolate as S. aureus
with 98.6% and 97.8% probabilities (profile codes 0064773465 and 6736153), respectively. DNA sequence analy-
sis of the 16S rRNA gene confirmed the strain as S. aureus
subsp. aureus. This strain was deposited in the DSMZ (Ger-
man 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, clinda-
mycin, 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 us-
ing 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 con-
secutive 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

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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 Staphylococcus aureus subsp. anaerobius 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, Staphylococcus aureus subsp. anaerobius and Staphylococcus 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 Staphylococcus 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). Staphylococcus aureus subsp. anaerobius is very closely related to Staphylococcus aureus sensu stricto and shares with it the ability to produce extracellular toxins and enzymes but is endowed with much lower pathogenic potential than Staphylococcus aureus (9). Both intracellular survival and extracellular multiplication play important roles in the pathogenesis of Staphylococcus aureus infections. Intracellular survival in neutrophils, endothelial cells, epithelial cells, and osteoblasts has been described to occur in Staphylococcus 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 Staphylococcus aureus or MRSA (11). Finally, the production of catalase is an important mechanism allowing Staphylococcus 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 Staphylococcus 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 Staphylococcus aureus strains requires study. In previous reports, the catalase-negative Staphylococcus aureus strains were isolated from blood samples, catheters, bronchial secretion samples, ulcers, and other wounds associated with infections or nosocomial endemics (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 Staphylococcus 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**