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 *S. aureus* sensu stricto. Sequence analysis of its catalase gene showed 99.60% identity to that of the reference strains. A five-base deletion, however, led to a shift of the nucleotide reading frame and a loss of the enzymatic activity.

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Production of catalase is considered to be a virulence determinant in *S. 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. *anaerobicus* and *S. saccharolyticus*, which are catalase negative and anaerobic. The latter are generally considered to be apathogenic. *S. aureus* catalase is encoded by the *katA* gene, which has a 1518 bp open reading frame and encodes a protein with 505 amino acids (9). *S. aureus* subsp. *anaerobicus* harbors a mutated gene, designated as *katB*, which is 1368 bp long and encodes a polypeptide of 455 amino acids. Compared to the nucleotide sequence of *katA*, *katB* showed six mis-sense mutations and a single base-pair deletion, located at 1338 bp from the initiation codon, which causes a shift of the nucleotide reading frame and premature translation termination at 1368 bp (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, which 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 New York Heart Association classification), diabetes mellitus, and COPD. Tracheal secretion was taken for routine microbiological investigation without signs of any infection being apparent at that time.

Tracheal secretion was processed conventionally and creamy β-hemolytic colonies, typical of *S. aureus* were observed on 5% sheep blood agar. The isolate was repeatedly negative for the catalase test after either aerobic or anaerobic incubation, even at the fifth subculture. It grew well both
aerobically and anaerobically. Both the slide coagulase test and the DNAse test were strongly positive. The BBL CRYSTAL for Gram-positive (Becton Dickinson Company, Sparks, Maryland) and the API Staph (bioMérieux, Marcy-l'Etoile, France) identified the isolate as *S. aureus* with 98.6% and 97.8% probability (Profile code 0064773465 and 6736153), respectively. DNA sequence analysis of the 16S ribosomal RNA 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, rifampicin, 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 gene as described (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 and BA000018, respectively), with a difference in six nucleotides at positions 1152 and 1388-1392 from the initiation codon. The single base substitution (T) located at 1152 bp from the initiation codon represented a silent mutation. However, deletion of five bases (AAACG) (1388-1392 bp 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 1418 bp. Similarly, our catalase gene sequence was in 99.54% identical to the *katA*
sequence of *S. aureus* subsp. *aureus* strains MSSA476, COL, NCTC 8325, USA300 and MW2, of
which complete genomes were also sequenced, with repeated confirmation of the same five-base
deletion.

This mutation thus differed fundamentally from mutations that have been described in the catalase
gene *katB* of catalase-negative *S. aureus* subsp. *anaerobius* strains (Gen Bank accession No.
AJ000471) (9). However, the consequences of a deletion in the C-terminal region of catalase
seemed to be 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. *anaerobius* and *S. saccharolyticus*, are known not to
produce catalase. Our strain differs from these species by its ability to grow well under aerobic
condition, expression of clumping factor, by production of acid from trehalose and lactose, and on
the basis of 16S ribosomal DNA 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. *anaerobius* 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 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 the aerobic environment like the upper respiratory tract. 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, catheter, bronchial secretion, 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 ICU or the University Hospital. There were no indications that the
strain had caused an infection. The antibiotic resistance pattern suggested a common origin of the
catalase-negative MRSA strain with 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


