Catalase-Negative *Staphylococcus lugdunensis* Strain with a Novel Point Mutation in the Catalase Gene Isolated from a Patient with Chronic Suppurative Otitis Media

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This report describes the results of the sequence analysis of a methicillin-susceptible strain of catalase-negative *Staphylococcus lugdunensis*. Molecular characterization of the deduced sequence revealed a novel point mutation in the catalase gene. To our knowledge, this is the first report of a catalase-negative *S. lugdunensis* strain, although catalase-negative isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* have been previously reported.

**CASE REPORT**

A 22-year-old woman presented to the Department of Otolaryngology with chronic suppurative otitis media. A pus sample was collected from her ear canal for routine microbial cultivation. The sample was inoculated in sheep blood agar and chocolate agar and incubated anaerobically and aerobically at 37°C for 24 h. After incubation, 1- to 2-mm creamy white colonies without hemolytic reaction were detected on sheep blood agar and chocolate agar plates maintained under aerobic atmospheric conditions. The bacterium was found to be catalase negative on analysis with 3% H2O2, and Gram staining showed the presence of Gram-positive staphylococcal clusters. The bacterium was again tested for the next 2 generations for catalase activity after subculturing on sheep blood agar and chocolate agar; the results remained negative for each generation. In addition, the isolate showed negative results for the slide and tube coagulase tests (performed with EDTA-human plasma) with the primary and subculturing cultures. In the tube coagulase test, the isolate tested negative at every time point (1, 2, 3, 4, 6, 8, 10, 12, and 24 h). *Staphylococcus aureus* (ATCC 25923) was used as the positive control. Some isolates produced clumping factor, which generated a positive result in the slide coagulase test (1).

The Vitek 2 Gram-positive identification card (GP) identified the isolate as *Staphylococcus lugdunensis* (NBL01). Positive results were obtained with the ornithine decarboxylase (ODC) and pyrrolidonyl arylamidase (PYR) (bioMérieux) tests. *S. lugdunensis* could be differentiated from other coagulase-negative staphylococci on the basis of its ability to produce ODC and could be differentiated from other coagulase-negative species. The sample was inoculated in sheep blood agar and chocolate agar plates maintained under aerobic atmospheric conditions. The bacterium was found to be catalase negative on analysis with 3% H2O2, and Gram staining showed the presence of Gram-positive staphylococcal clusters. The bacteria was also tested for catalase activity after subculturing on sheep blood agar and chocolate agar; the results remained negative for each generation. In addition, the isolate showed negative results for the slide and tube coagulase tests performed with EDTA-human plasma with the primary and subculturing cultures. In the tube coagulase test, the isolate tested negative at every time point (1, 2, 3, 4, 6, 8, 10, 12, and 24 h). *Staphylococcus aureus* (ATCC 25923) was used as the positive control. Some isolates produced clumping factor, which generated a positive result in the slide coagulase test (1).

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**Catalase (katA) gene sequencing.** The entire catalase gene (1,506 bp) was PCR amplified using the primers NBL3 (5′-ATGTCCAAAAGAGATTTAAAATTAATGCATC-3′) and NBL4 (5′-CTATTCAATGCTCTCATCATTTCATT-3′); the design of the primers was based on the *S. lugdunensis* HKU09-01 chromosome from the complete genome (GenBank accession no. NC_013893.1). The PCR product was cloned into a pMD18-T vector and submitted for sequencing to Sunny Biotechnology (Shanghai, China). The resulting DNA sequences were analyzed by BLAST. DNA analysis showed that the deduced sequences of the isolate harbored 5 single-nucleotide changes relative to the sequences of *S. lugdunensis* HKU09-01 (GenBank accession no. NC_013893.1) and *S. lugdunensis* N920143 (GenBank accession no. NC_017353.1). These changes included two sense and two silent mutations (C26G, G28C, T705A, and T942C) and one nonsense mutation at C1180T (CAA-TAA).

*S. lugdunensis* is a coagulase-negative staphylococcus commonly found as part of the normal human skin flora and the perineal region (4); this species was first described by Freney et al. (5). Infections caused by this bacterium include acute postoperative endophthalmitis (6), acute necrotizing sinusitis (7), prosthesis joint infections (8), urinary tract infections (9), and skin and soft tissue infections (4). Most importantly, *S. lugdunensis* has...
been associated with native valve endocarditis, an aggressive infection that is associated with a high mortality risk (2, 10).

Most Staphylococcus species show positive results with the catalase biochemical test; this assay is routinely used in clinical microbiology laboratories with 3% H₂O₂. The catalase reaction generally occurs in 2 steps. First, the Fe(III) heme reacts with H₂O₂ to form an oxoferryl intermediate (compound I). Second, compound I reacts with another molecule of H₂O₂ and the enzyme returns to its resting state (11, 12). To date, Staphylococcus aureus subsp. anaerobius is the only subspecies that has naturally occurring catalase deficiency that has been documented. Sanz et al. (13) showed that catalase deficiency in S. aureus subsp. anaerobius is associated with loss-of-function mutations within the structural gene. However, it has recently been reported that other catalase-negative Staphylococcus isolates, including S. aureus and Staphylococcus epidermidis, harbor mutations within key amino acids and/or codons (11, 14–17). In this report, the phenotypic characteristics of S. lugdunensis were further confirmed by sequence analysis of the catalase gene.

Several studies have suggested that catalase serves as a virulence factor (18, 19) due to its ability to cleave hydrogen peroxide, a reactive oxygen intermediate responsible for the bactericidal activities of phagocytes. As a result, strains that do not produce catalase have reduced virulence. It has also been suggested that catalase plays a role in nasal colonization and environmental persistence (20).

To our knowledge, this is the first reported case of a catalase-negative S. lugdunensis strain that harbors a nonsense mutation in its catalase gene. The genetic associations with the lack of catalase activity have been previously reported elsewhere. Grüner et al. reported a frameshift mutation due to a 5-base deletion upstream of the initiation codon of the catalase gene (16), whereas a T172C substitution, leading to a histidine 58-to-tyrosine change, and a G636A substitution, leading to an arginine 212-to-histidine change, have been reported by Piau et al. (11). To et al. (21) identified 15 silent mutations; a critical nonsense mutation at position 802, leading to a change from codon GAA to TAA (stop codon); and 4 additional mutations downstream of the nonsense mutation. In this study, we detected a nonsense mutation at position C1180T, which caused a codon change from CAA to TAA (stop codon). We therefore hypothesize that a single codon mutation is responsible for the loss of catalase activity in this bacterium, similar to what has been previously described (21). Sequence analysis showed that the N-terminal arm of the catalase gene in our isolate was conserved, whereas the rest of the gene was either disrupted or lost due to the premature termination of the translation process. Deletion of the critical regions of the catalase gene may thus have been responsible for the disruption of the heme- and NADPH-binding regions. Because of these structural gene rearrangements, our S. lugdunensis isolate is unlikely to show catalase activity when tested using biochemical assays. However, it may still be possible for certain small-colony variants (i.e., S. aureus, Staphylococcus haemolyticus, and S. epidermidis) to phenotypically show reduced catalase activity (22).

The results of this study thus emphasize the importance of proper identification of bacterial strains, especially when these microorganisms are constantly evolving over time. Extensive characterization of a newly isolated bacterium should be performed to fully establish its identity and taxonomic classification.

Nucleotide sequence accession numbers. The 16S rRNA and catalase gene nucleotide sequences determined in this study have been submitted to the GenBank database under accession numbers JX629460 to JX987891.

REFERENCES


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ERRATUM

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Volume 51, no. 4, p. 1310–1312, 2013. Page 1311, column 2, line 3: "JX629460 to JX987891" should read "JX629460 and JX987891."

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