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Novel nonsense mutation in the katA gene of a catalase-negative Staphylococcus aureus strain

Jaime Lagos, Pedro Alarcón, Dona Benadof, Soledad Ulloa, Rodrigo Fasce, Javier Tognarelli, Carolina Aguayo, Pamela Araya, Bárbara Parra, Berta Olivares, Juan Carlos Hormazábal, Jorge Fernández

a Molecular Genetics Subdepartment, Chile
b Virology Subdepartment, Chile
c Infection Disease Subdepartment, Chile
d Public Health Institute, Clinical Laboratory, Hospital Roberto del Río, Chile

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A B S T R A C T
We report the first description of a rare catalase-negative strain of Staphylococcus aureus in Chile. This new variant was isolated from blood and synovial tissue samples of a pediatric patient. Sequencing analysis revealed that this catalase-negative strain is related to ST10 strain, which has earlier been described in relation to S. aureus carriers. Interestingly, sequence analysis of the catalase gene katA revealed presence of a novel nonsense mutation that causes premature translational truncation of the C-terminus of the enzyme leading to a loss of 222 amino acids. Our study suggests that loss of catalase activity in this rare catalase-negative Chilean strain is due to this novel nonsense mutation in the katA gene, which truncates the enzyme to just 283 amino acids.

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Staphylococcus aureus, one of the most important pathogens known to mankind, is a prominent member of the genus Staphylococcus. Most members of this genus are catalase-positive with notable exceptions being S. saccharolyticus and S. aureus subsp. anaerobius, both of which are catalase-negative and are known to grow vigorously in anaerobic conditions. Presence or absence of catalase enzyme is widely used to facilitate identification of the organism at the genus level and some authors have even suggested that catalase might be an important contributor to virulence given its ability to decompose hydrogen peroxide, a reactive oxygen intermediate indispensable for the bactericidal activity of phagocytes.

Although several cases of human infection caused by catalase-negative S. aureus (CNSA) have been reported, the molecular basis for loss of catalase activity remains poorly studied.

A number of studies have suggested that the loss of catalase enzymatic activity is linked to either a 5-bp deletion or a point mutation in the katA gene. In the present study,
we report the isolation and characterization of a catalase-negative clinical strain of *S. aureus* collected from blood and synovial tissue samples of a pediatric patient.

A 2-year and 7-month old child complaining of a sore knee was admitted to the Hospital Pediátrico Roberto del Río in Santiago, Chile. The preliminary diagnosis was one of the infectious arthritis; therefore, both blood and synovial tissue samples were analyzed using routine microbiological techniques.  

The isolated Staphylococcal strain was received at the Public Health Institute of Chile and analyzed as per routine microbiological laboratory protocols. The studies undertaken included an analysis of specific culturing conditions, macroscopic and microscopic appearance as well as catalase activity assay. Antibiotic susceptibility testing was conducted on Mueller-Hinton agar using the disk diffusion method as per guidelines issued by CLSI. The 16S rRNA gene of the novel strain was amplified by PCR using the primer pair: BH48 5’-CGGTCAGAGGTTCGATCTGGCTCCAG 3’ and 1500R 5’ GGTTACCTTGTTACGACTT 3’, as described earlier in the literature. MLST was performed on the 16S rRNA, katA and MLST housekeeping genes were purified and sequenced on an ABI 310 DNA automated sequencer (Applied Biosystems). For sequencing the complete katA gene sequence, the following set of primers was used: (a) cat1; (b) cat2; (c) CAT-FW 5’ GTGCCCGAGCACCAAGCCAACCAATTACA 3’; and (d) CAT-RV 5’ TCAGGCACGTCAACCTGTCGAG 3’. All the sequence data generated were assembled and edited electronically using Biedit 7.2.0 and Chromas Lite 2.1.1 software. DNA sequences of the housekeeping genes were submitted to the MLST database (http://saureus.mlst.net/) in order to obtain the sequence type (ST).

The isolated strain (denominated CHI-2609) was grown on 5% sheep blood trypticase soy agar (TSA) under both aerobic as well as anaerobic conditions. Positive culture results were obtained under aerobic atmospheric conditions. Opaque, smooth, creamy and β-hemolytic golden-yellow colonies were observed following overnight incubation in the culture medium. Gram staining of the culture preparations showed that the isolated strain was containing of Gram-positive cocci clusters. Interestingly, the catalase tests, both the 3% *H₂O₂* slide test as well as the nutrient broth tube test with 30% *H₂O₂*, were repeatedly negative. The isolate was positive for coagulase activity when tested using the rabbit plasma coagulase test. CHI-2609 differs from *S. saccharolyticus* and *S. aureus* subsp. *anaerobius* by virtue of its clumping factor, positive urea and Voges Proskauer test as well as with respect to acid production from trehalose, mannose, sucrose and maltose. CHI-2609 strain is also deficient in the ability to ferment mannitol, and is sensitive to methicillin, vancomycin, erythromycin, and clindamycin.

To confirm that the isolated strain, CHI-2609, was *S. aureus* subsp. *aureus*, nucleotide analysis of the 16S rRNA gene was conducted. The results demonstrated 100% sequence identity with the 16S RNA sequence of ATCC 12600 (GenBank accession number AJ000472) type strain. The MLST typing revealed a new allele and ST that had never been reported earlier. The novel ST was submitted to the MLST database and was given the designation ST3145. The relationship between ST3145 and previously known MLST database STs was examined using concatenated sequences of the seven MLST loci so as to enable the construction of a neighbor-joining tree (data not shown). ST3145 was observed to cluster with ST10, ST145, ST1162, ST1936, ST1951 and ST2289, all of these are known to differ from each other at only a single MLST locus. ST10 has previously been described by Sakwinska et al., and Blumenthal et al., in *S. aureus* carriers.

In order to decipher the mechanism responsible for the loss of catalase activity, the complete katA gene of CHI-2609 was amplified and sequenced (Genbank accession number KM036425). Nucleotide sequence analysis demonstrated 98% identity with the corresponding sequence of the *S. aureus* subsp. *aureus* type strain ATCC 12600 (GenBank accession number AJ000472). The two sequences differed in 30 nucleotides of which 25 were synonymous mutations (Table 1). Of the remaining 5 nucleotides, 4 were missense mutations identified as: (a) C322T substitution leading to a R108C change; (b) C923T substitution leading to a T308M change; (c) C1165T substitution leading to a H389Y change; and (d) A1442G substitution leading to a K481R change. Of great interest was the 5th mutation: a single-base substitution of G>A at position 852, which resulted in a TGG to TGA alteration at the 284th codon leading to a premature termination of the translation. The amplification and sequencing steps were repeated thrice so as to confirm the presence of the unusual G852A mutation beyond doubt. Sequence identity between the CHI-2609

| Table 1 – Nonsynonymous and synonymous nucleotide substitution of katA gene. |
|-----------------|-----------------|-----------------|
| **Synonymous**  | **Nonsynonymous** | **Amino acid change** |
| T30A            | C322T           | R108C           |
| T33A            | G852A           |                 |
| A75G            | C923T           | T308M           |
| G78A            | C1165T          | H389Y           |
| T90A            | A1442G          | K481R           |
| T93A            | C110T           |                 |
| T114C           |                 |                 |
| T302A           |                 |                 |
| G318A           |                 |                 |
| G321A           |                 |                 |
| G357A           |                 |                 |
| G366A           |                 |                 |
| A393G           |                 |                 |
| T421C           |                 |                 |
| G444T           |                 |                 |
| G447T           |                 |                 |
| A480T           |                 |                 |
| T507G           |                 |                 |
| A596G           |                 |                 |
| T576C           |                 |                 |
| T594A           |                 |                 |
| T712C           |                 |                 |
| T882G           |                 |                 |
| C1164T          |                 |                 |

* Stop codon.
catalase gene and the katA sequences of S. aureus MSSA476, COL, NCTC 8325 and USA300 was 99.6% for the protein sequence and between 98 and 98.2% for the nucleotide sequence.

Catalase production is a biochemical feature that has traditionally been associated with Staphylococcus spp. since long. It is universally used as a screening test to distinguish between two major species of Gram-positive cocci: Staphylococcus and Streptococcus. In the case of an atypical isolate such as ours, use of the catalase test may lead to an incorrect identification, especially when the ability to ferment an important sugar such as mannitol is absent as described by Shittu et al., and Kateete et al. Catalase is an oxidoreductase that allows bacteria to withstand intracellular hydrogen peroxide. Production of catalase has been hypothesized to be a virulence factor in S. aureus but its exact role in that respect needs to be further elucidated. Staphylococci with a catalase-negative phenotype were thought to be less virulent but several cases of CNSA bacteraemia, which have on occasion proven fatal, have been reported.

The C-terminal region of catalase appears to be essential for enzymatic activity. The studies carried out on the Bacteroides fragilis and S. aureus subsp. anaerobius catalase genes have demonstrated that catalytic activity is observed to be lost when the last 21 and 50 amino acids, respectively, are deleted. The occurrence of a premature translation termination in the katA gene of S. aureus subsp. aureus has been reported in two studies. To et al. found a mutation at position 802 which changed the GAA codon to TAA which is a stop signal for translation. Ellis et al. reported the insertion of thymine after position 31 which also leads to the insertion of a premature stop codon at position 64. In our study, on CHI-2609 isolates, the insertion of a premature translation termination codon in the katA gene resulted in an 849-bp open reading frame that encoded a polypeptide of just 283 amino acids suggesting that the loss of the last 222 amino acids of the C-terminus gene are responsible for the lack of catalase activity.

To conclude, our study is the first report of a rare catalase-negative strain of S. aureus in Chile. In addition, we have also identified a hitherto unreported premature translational truncation of catalase that leads to a loss of 222 amino acids from the C-terminus. In spite of the possibility that catalase could function as a potential virulence factor, it appears in our case that lack of enzymatic activity did not impair the ability of the isolate to cause septic arthritis. Finally, it is imperative that clinical microbial laboratory staff be made aware of the existence of such catalase-negative S. aureus isolates, whose incidence and clinical implications remain unknown.

**Conflicts of interest**

The authors declare no conflicts of interest.

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