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

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ABSTRACT

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.

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.[1,2] 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.[3,4] Although several cases of human infection caused by catalase-negative *S. aureus* (CNSA) have been reported,[4–7] 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.[7–9] In the present study,

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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.[10]

The isolated Staphylococcal strain was received at the Public Health Institute of Chile and analyzed as per routine microbiological laboratory protocols.[10] The studies undertaken included an analysis of specific culturing conditions, macroscopic and microscopic appearance as well as catalase activity assay.[10] Antibiotic susceptibility testing was conducted on Mueller-Hinton agar using the disk diffusion method as per guidelines issued by CLSI.[11] The 16S rRNA gene of the novel strain was amplified by PCR using the primer pair: Belt4 5′ CGGTCGACAGAGGTTTGATCCTGGCTCAG 3′ and 1500R 5′ GGTTACCTTGTTACGACTT 3′, as described earlier in the literature.[12,13] MLST was performed using a standardized international scheme as described by Enright et al.[14] For amplification of the complete *katA* gene sequence, the previously enumerated primers, cat1 and cat2, were employed.[15] Amplicons of 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′ GTGCCCGAGCAACACCCACCCATTACA 3′; and (d) CAT-RV 5′ TCAGCGCACGTCGAACCTGTCGAG 3′. All the sequence data generated were assembled and edited electronically using Bioedit 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% H_2O_2 slide test as well as the nutrient broth tube test with 30% H_2O_2, 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.[10] 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.,[16] and Blumental et al.,[17] 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	R103C
T33A	G852A	[a]
A75G	C923T	T308M
G78A	C1165T	H389Y
T90A	A1442G	K481R
T93A		
C110T		
T114C		
T302A		
G318A		
G321A		
T357A		
G366A		
A393G		
T421C		
G444T		
G447T		
A480T		
T507G		
A546G		
T576C		
T594A		
T712C		
T882G		
C1164T		

[a] Stop codon.