BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Medical Microbiology

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

Jaime Lagos[a], *Pedro Alarcón*[c], *Dona Benadof*[d], *Soledad Ulloa*[a], *Rodrigo Fasce*[b], *Javier Tognarelli*[a], *Carolina Aguayo*[a], *Pamela Araya*[c], *Bárbara Parra*[a], *Berta Olivares*[a], *Juan Carlos Hormazábal*[c], *Jorge Fernández*[a,*]

[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*

ARTICLE INFO

Article history:
Received 4 November 2014
Accepted 8 August 2015

Associate Editor: Agnes Marie Sá Figueiredo

Keywords:
Staphylococcus aureus
Catalase-negative
Catalase gene
katA

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,

☆ Sponsorships: Public Health Institute of Chile.

* *Corresponding author at*: Subdepto Genética Molecular, Instituto de Salud Pública de Chile, Marathon 1000, Ñuñoa, Santiago, Chile.
E-mail: jofernand@gmail.com (J. Fernández).

http://dx.doi.org/10.1016/j.bjm.2015.11.012

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.

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.,[18] and Kateete et al.[19]

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.[8,20] Staphylococci with a catalase-negative phenotype were thought to be less virulent but several cases of CNSA bacteremia, which have on occasion proven fatal, have been reported.[5]

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.[21] 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.[4] found a mutation at position 802 which changed the GAA codon to TAA which is a stop signal for translation. Ellis et al.[7] 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 must 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.

Acknowledgements

We thank María Ibáñez Aravena from Public Health Institute of Chile for excellent technical assistance. This study was supported by Public Health Institute of Chile.

REFERENCES

1. Lee N, Chang LC, Chiu CP. A case of carbuncle caused by a catalase-negative strain of *Staphylococcus aureus*. *Diagn Microbiol Infect Dis*. 1996;24:221–223.
2. Bertrand X, Huguenin Y, Talon D. First report of a catalase-negative methicillin-resistant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis*. 2002;43:245–246.
3. Ôver U, Tüç Y, Söyletir G. Catalase-negative *Staphylococcus aureus*: a rare isolate of human infection. *Clin Microbiol Infect*. 2000;6:681–682.
4. To KK, Cheng VC, Chan JF, et al. Molecular characterization of a catalase-negative *Staphylococcus aureus* subsp. aureus strain collected from a patient with mitral valve endocarditis and pericarditis revealed a novel nonsense mutation in the *katA* gene. *J Clin Microbiol*. 2011;49:3398–3402.
5. Del'Alamo L, d'Azevedo PA, Strob AJ, et al. An outbreak of catalase-negative methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect*. 2007;65:226–230.
6. Grüner BM, Han SR, Meyer HG, Wulf U, Bhakdi S, Siegel EK. Characterization of a catalase-negative methicillin-resistant *Staphylococcus aureus* strain. *J Clin Microbiol*. 2007;45:2684–2685.
7. Ellis MW, Johnson RC, Crawford K, Lanier JB, Merrell DS. Molecular characterization of a catalase-negative methicillin-susceptible *Staphylococcus aureus* subsp. aureus strain collected from a patient with cutaneous abscess. *J Clin Microbiol*. 2014;52:344–346.
8. Le Coustumier I, Brun Y, Be's M, Le Coustumier AM, Fleurette J, Etienne J. A stunning outbreak of infections due to catalase negative *Staphylococcus aureus*. In: *8th International Symposium of Staphylococci and Staphylococcal Infections*. 1996:28.
9. Piau C, Jehan J, Leclercq R, Daurel C. Catalase-negative *Staphylococcus aureus* strain with point mutations in the katA gene. *J Clin Microbiol*. 2008;46:2060–2061.
10. Becker K, Von Eiff C. Staphylococcus, Micrococcus and other catalase positive cocci. In: *Manual of Clinical Microbiology*. 10th ed. ASM Press; 2011.
11. Clinical Laboratory Standards Institute, Wayne, PA, USA *Performance Standards for Antimicrobial Disk Susceptibility Testing; Approved Standard, Document M02-A11*; 2012.
12. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*. 1991;173:697–703.
13. Toyama T, Kita-Tsukamoto K, Wakabayashi H. Identification of *Flexibacter maritimus*, *Flavobacterium branchiophilum* and *Cytophaga columnaris* by PCR targeted 16S ribosomal DNA. *Fish Pathol*. 1996;31:25–31.
14. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol*. 2000;38:1008–1015.
15. Sanz R, Marín I, Ruiz-Santa-Quiteria JA, et al. Catalase deficiency in *Staphylococcus aureus*. *Microbiology*. 2000;146:465–475.
16. Sakwinska O, Kuhn G, Balmelli C, et al. Genetic diversity and ecological success of *Staphylococcus aureus* strains colonizing humans. *Appl Environ Microbiol*. 2009;75:175–183.
17. Blumental S, Deplano A, Jourdain S, et al. Dynamic pattern and genotypic diversity of *Staphylococcus aureus* nasopharyngeal carriage in healthy pre-school children. *J Antimicrob Chemother*. 2013;68:1517–1523.
18. Shittu A, Lin J, Morrison D. Molecular identification and characterization of mannitol-negative methicillin-resistant

Staphylococcus aureus. *Diagn Microbiol Infect Dis*. 2007;57:93–95.
19. Kateete DP, Kimani CN, Katabazi FA, et al. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Ann Clin Microbiol Antimicrob*. 2010;9:23.
20. Millar M, Wilcock A, Sanderson Y, Kite P, McDonnell MK. Catalase negative *Staphylococcus aureus*. *J Clin Pathol*. 1986;39:695.
21. Rocha ER, Smith CJ. Biochemical and genetic analyses of a catalase from the anaerobic bacterium *Bacteroides fragilis*. *J Bacteriol*. 1995;177:3111–3119.