

Short Communication

Isolation and Molecular Characterization of Catalase-Negative *Staphylococcus aureus* from Sputum of a Patient with Aspiration Pneumonia

Kazuki Horiuchi¹, Takehisa Matsumoto^{1*}, Eiko Hidaka¹, Eriko Kasuga¹, Mitsutoshi Sugano¹, Kozue Oana², Yoshiyuki Kawakami², and Takayuki Honda¹

¹Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto 390-8621; and

²Department of Biomedical Laboratory Sciences, School of Health Sciences, Shinshu University School of Medicine, Matsumoto 390-8621, Japan

(Received June 11, 2012. Accepted July 5, 2012)

SUMMARY: *Staphylococcus aureus* produces various virulence factors. The catalase enzyme, in particular, is considered to be involved in oxidative stress resistance, and catalase activity is an important criterion for differentiating staphylococci from streptococci. In this report, we describe the catalase-negative *S. aureus* strain SH3064, which was isolated from the sputum of a patient with aspiration pneumonia. To evaluate the causes of the lack of catalase activity in *S. aureus* SH3064, we analyzed the sequence of *katA* gene encoding the catalase enzyme in this strain. We amplified the complete sequence of *katA* gene of *S. aureus* SH3064 by polymerase chain reaction using 2 sets of primers. The *katA* sequence showed 99.6% sequence identity (1512/1518 bp) with that of *S. aureus* ATCC 12600. We detected 2 mutations in the *katA* gene from *S. aureus* SH3064, an A217T substitution leading to a threonine 73-to-serine substitution and a single-base pair deletion (c.637delG) resulting in a frameshift mutation. The lack of catalase activity in this strain was attributed to the shift of the nucleotide reading frame.

Catalase is a virulence factor of *Staphylococcus aureus* and has been implicated in oxidative stress resistance (1–3). Catalase produced by *S. aureus* consists of 4 subunits encoded by the *katA* gene; it has a 1518-base pair (bp) open-reading frame and encodes a protein with 505 amino acids (4). Catalase activity is an important criterion for differentiating staphylococci from streptococci, as all *Staphylococcus* spp. produces catalase, except for *S. aureus* subspecies *anaerobius* and *S. saccharolyticus*. Several isolates of *S. aureus* that do not produce catalase have been previously reported and described (5,6). In a few cases, mutations in the *katA* gene have been described for isolates lacking catalase activity. For example, Grüner et al. (7) reported that the loss of catalase enzymatic activity in a clinical isolate was associated with a 5-bp deletion, leading to a frameshift in the *katA* gene. Piau et al. (8) identified a point mutation in the *katA* gene associated with the loss of catalase activity, and To et al. (9) isolated a catalase-negative *S. aureus* with a nonsense mutation in the *katA* gene.

In this study, we report an *S. aureus* clinical isolate SH3064 with a mutated *katA* gene, which was isolated from a patient with aspiration pneumonia. To the best of our knowledge, this is the first report on this particular strain in Japan and the fourth globally.

A 61-year-old man with diabetes mellitus was admit-

ted to the Shinshu University Hospital for cardiac arrest. He was retained in the intensive care unit and supported with mechanical ventilation. Two days after admission of the patient, purulent sputum was observed, and he was diagnosed with mechanical ventilation-associated aspiration pneumonia. He was treated with a combination of ampicillin and sulbactam for 7 days, followed by treatment with ampicillin for 3 days. The patient was fully recovered 12 days after administration of the antibiotic and sulbactam.

Gram staining of the sputum revealed several clusters of Gram-positive cocci phagocytosed by neutrophils. Creamy beta-hemolytic colonies, which are typical of *S. aureus*, were observed on 5% sheep blood agar, and a highly positive slide-coagulase test result was obtained. However, the result of the catalase test performed with 3% H₂O₂ was negative. The isolate was identified as *S. aureus* with 99.99% probability, and it was found to be sensitive to all the antimicrobial agents as determined using the MicroScan Pos Combo 6.1J (Siemens Healthcare Diagnostics, Tokyo, Japan).

The complete *katA* gene of *S. aureus* SH3064 was amplified by polymerase chain reaction using 2 sets of primers (KatDIR1-KatREV1 and KatDIR2-KatREV2) (8) and sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA). The *katA* gene of *S. aureus* SH3064 showed 99.6% sequence homology (1512/1518 bp) with that of *S. aureus* ATCC 12600 (accession no. AJ000472). Two mutations were detected in the *katA* sequence; one of these was an A217T substitution leading to a threonine 73-to-serine substitution, and the other was a single-bp deletion (c.637delG) that resulted in a frameshift muta-

*Corresponding author: Mailing address: Department of Laboratory Medicine, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto 390-8621, Japan. Tel: +(81) 263 37 3493, Fax: +(81) 263 34 5316, E-mail: ggatcc@shinshu-u.ac.jp

A

		190	200	210	220	230	240	
							
ATCC 12600	181	GGTGCATTTGGGACATTTACTGTA	ACTAAAGATATAACAAAATATACGAATGCTAAAATA					240
		G A F G T F T V T K D I T K Y T N A K I						
SH3064		GGTGCATTTGGGACATTTACTGTA	ACTAAAGATATATCAAAAATATACGAATGCTAAAATA					
		G A F G T F T V T K D I S K Y T N A K I						

B

		610	620	630	640	650	660	
							
ATCC 12600	601	TACTCTATGTATAATGATTCTGGTGAACGTGTTTGGGTTAAATTCATTTTAGAACGCAA						660
		Y S M Y N D S G E R V W V K F H F R T Q						
SH3064		TACTCTATGTATAATGATTCTGGTGAACGTGTTTGG-TTAAATTCATTTTAGAACGCAA						
		Y S M Y N D S G E R V W L N S I L E R N						
		670	680	690	700	710	720	
							
ATCC 12600	661	CAAGGTATTGAAAACCTAACTGATGAAGAAGCTGCTGAAATTATAGCTACAGATCGTGAT						720
		Q G I E N L T D E E A A E I I A T D R D						
SH3064		CAAGGTATTGAAAAC TAA -----						
		K V L K T						

Fig. 1. Nucleotide alignments and deduced amino acid sequences of the *katA* gene in *S. aureus* SH3064 and *S. aureus* ATCC 12600. The numbers indicate the nucleotide position in the *katA* gene. (A) The *katA* gene nucleotide sequence region from 181 to 240 bp. A217T/threonine 73-to-serine substitution is indicated in boldface type. (B) The *katA* gene nucleotide sequence region from 601 to 720 bp (based on nucleotide positions of the *katA* gene in *S. aureus* ATCC 12600). The single-base pair deletion (c.637delG) and the following frameshift mutations are indicated in boldface type. The stop codon of the *katA* gene of *S. aureus* SH3064 is enclosed in a box.

tion (Fig. 1).

The complete nucleotide sequence of the *katA* gene from *S. aureus* strain SH3064 reported in this study has been submitted to the GenBank and EMBL databases under the accession no. AB665064.

Catalase is considered to be a virulent factor in *S. aureus*; however, several pathogenic catalase-negative *S. aureus* isolates have been previously reported (5,6). In addition, previous studies using mouse models have shown that catalase-positive *S. aureus* strains were not more virulent than catalase-negative strains (10,11). In this study, *S. aureus* SH3064 was isolated from a patient with aspiration pneumonia. Gram staining of the sputum sample showed Gram-positive cocci phagocytosed by neutrophils, and the isolates grew well on a 5% sheep blood agar plate. Therefore, we concluded that the patient had aspiration pneumonia caused by a catalase-negative *S. aureus* strain, and the lack of catalase activity, in this case, did not negatively affect the virulence of *S. aureus*.

We have reported 2 mutations in the *katA* gene of *S. aureus* SH3064 in this study. We assessed the potential impact of these mutations on the catalase activity of the isolate. Because both serine and threonine are polar, non-charged amino acids, we predicted that the A217T

mutation had weakly influenced catalase activity. In contrast, the deletion mutation c.637delG resulted in a shift of the nucleotide-reading frame, truncating the KatA protein to 225 amino acids. It is likely that this frameshift mutation resulted in the production of a non-functional enzyme in *S. aureus* SH3064, as the C-terminal region of KatA is important for catalase activity.

Sanz et al. (4) reported that His 56, Ser 95, and Asn 129 residues of the catalase enzyme of *S. aureus* are necessary for peroxide binding, whereas the proximal heme-binding sites are located at Pro 317, Arg 335, and Tyr 339, and the distal heme-binding sites, at Met 55, Arg 93, Thr 95, Phe 134, and Phe 142. A deletion mutation at 1338 bp, leading to premature termination of translation at 1368 bp, and a missense mutation resulting in proline 317-to-serine substitution, which forms a part of the proximal heme-binding site, were both reported in the *katA* gene of catalase-negative *S. aureus* subspecies *anaerobius*. However, the mutation directly associated with loss of catalase activity has not been identified.

Grüner et al. (7) reported that the *katA* gene in the catalase-negative *S. aureus* strain had a 5-bp deletion from 1388 to 1392 bp, inducing a premature stop codon at 1418 bp. They suggested that this deletion in the C-

terminal region of the catalase protein hindered enzymatic activity. In the present study, we proposed that the identified *S. aureus* SH3064 did not produce catalase with its enzymatic activity owing to a truncation of the C-terminal region of the KatA protein, including the loss of the proximal heme-binding site.

Catalase-negative *S. aureus* with mutated KatA proteins have rarely been reported in clinical specimens; thus, it was difficult to identify this particular strain as staphylococci using routine laboratory techniques. However, it is important to note that despite the loss of catalase activity, staphylococci are virulent pathogens and can impact on human health. Therefore, more efforts to improve methods for the detection of these isolates are warranted. For example, the identification of creamy beta-hemolytic colonies on 5% sheep blood agar can be used as a potential indicator of *S. aureus* and can thus facilitate additional testing for this pathogen. In general, our findings demonstrate that it is critical to be aware of the potential presence of catalase-negative strains of *S. aureus*.

Conflict of interest None to declare.

REFERENCES

1. Das, D. and Bishayi, B. (2009): Staphylococcal catalase protects intracellularly survived bacteria by destroying H₂O₂ produced by the murine peritoneal macrophages. *Microb. Pathog.*, 475, 57–67.
2. Das, D., Saha, S.S. and Bishayi, B. (2008): Intracellular survival

of *Staphylococcus aureus*: correlating production of catalase and superoxide dismutase with levels of inflammatory cytokines. *Inflamm. Res.*, 57, 340–349.

3. Mandell, G.L. (1975): Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. In vitro and in vivo studies with emphasis on staphylococcal—leukocyte interaction. *J. Clin. Invest.*, 55, 561–566.
4. Sanz, R., Marín, I., Ruiz-Santa-Quiteria, J.A., et al. (2000): Catalase deficiency in *Staphylococcus aureus* subsp. *anaerobius* is associated with natural loss-of-function mutations within the structural gene. *Microbiology*, 146, 465–475.
5. Del’Alamo, L., d’Azevedo, P.A., Strob, A.J., et al. (2007): An outbreak of catalase-negative methicillin-resistant *Staphylococcus aureus*. *J. Hosp. Infect.*, 65, 226–230.
6. Over, U., Tüç, Y. and Söyletir, G.. (2000): Catalase-negative *Staphylococcus aureus*: a rare isolate of human infection. *Clin. Microbiol. Infect.*, 6, 681–682.
7. Grüner, B.M., Han, S.R., Meyer, H.G., et al. (2007): Characterization of a catalase-negative methicillin-resistant *Staphylococcus aureus* strain. *J. Clin. Microbiol.*, 45, 2684–2685.
8. Piau, C., Jehan, J., Leclercq, R., et al. (2008): Catalase-negative *Staphylococcus aureus* strain with point mutations in the *kata* gene. *J. Clin. Microbiol.*, 46, 2060–2061.
9. To, K.K.W., Cheng, V.C.C., Chan, J.F.W., et al. (2011): 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.*, 49, 3398–3402.
10. Martínez-Pulgarín, S., Domínguez-Bernal, G., Orden, J.A., et al. (2009): Simultaneous lack of catalase and beta-toxin in *Staphylococcus aureus* leads to increased intracellular survival in macrophages and epithelial cells and to attenuated virulence in murine and ovine models. *Microbiology*, 155, 1505–1515.
11. Messina, C.G., Reeves, E.P., Roes, J., et al. (2002): Catalase negative *Staphylococcus aureus* retain virulence in mouse model of chronic granulomatous disease. *FEBS Lett.*, 518, 107–110.