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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▽

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We report a case of endocarditis and pericarditis caused by catalase-negative *Staphylococcus aureus*. Molecular characterization revealed a novel nonsense mutation in the *katA* gene, leading to a loss of 238 amino acids (47% of the wild-type catalase protein), including the heme-binding site, NADPH-binding region, and Tyr-337, essential for catalysis.

CASE REPORT

A 15-year-old boy presented to the Accident and Emergency Department with a 2-day history of fever, rhinorrhea, vomiting, and colicky abdominal pain. His past health was good except for a repaired cleft lip. His body temperature was 37.7°C, with blood pressure of 98/37, a pulse rate of 122 beats per minute, and oxygen saturation of 98% while breathing ambient air. With a clinical diagnosis of upper respiratory tract infection, the patient was not admitted to the hospital. One day later, he presented to the Accident and Emergency Department again for left-side chest pain and generalized bone pain. Electrocardiography showed ST segment elevation in leads I, II, III, and V2 to V6. The level of troponin was 0.03 μg/liter, and the level of creatinine kinase was 135 IU/liter. Chest radiography was normal. After admission, he complained of neck pain and stiffness and was found to be confused. Physical examination revealed vesicles on the palms and feet and oral ulcers. Computed tomography of the brain was unremarkable. Lumbar puncture was performed, and analysis of the cerebrospinal fluid revealed a white cell count of 2 per mm^3, with glucose and protein within their normal ranges. Bacterial culture of the cerebrospinal fluid was negative. Further questioning revealed that one of the patient's classmates had recently been diagnosed to have hand, foot, and mouth disease. Due to the clinical suspicion of severe hand, foot, and mouth disease, intravenous immunoglobulin was given on the day of admission. Paired sera, collected 14 days apart, revealed a rise in the titer of antibody to coxsackievirus B virus type 4 from <10 to 40, compatible with acute infection. Viral culture and reverse transcriptase PCR for enterovirus/coxsackievirus on the nasopharyngeal aspirate, throat swab, rectal swab, and cerebrospinal fluid were all negative. However, blood culture taken on days 1, 3, 6, and 7 of admission grew Gram-positive cocci in clusters (Bactec 9240 blood culture system; Becton, Dickinson, MD). After incubation at 37°C in 5% CO_2 for 24 h, 2-mm creamy white colonies were seen. The bacterium was negative for catalase, but both the slide and tube coagulase tests using rabbit plasma were positive. It was also positive with Slidex Staph Plus (bioMérieux). A BD Phoenix PID panel (Becton, Dickinson and Company, Sparks, MD) identified the bacterium as *Staphylococcus aureus*, while the Vitek system Gram-positive identification (GPI) test (bioMérieux, Durham, NC) could not identify the organism. According to the 2010 Clinical and Laboratory Standards Institute interpretive criteria, the isolate was susceptible to cloxacillin, cotrimoxazole, minocycline, and gentamicin but resistant to clindamycin and erythromycin. A transthoracic echocardiogram revealed a 1.5-cm vegetation at the medial leaflet of the mitral valve associated with mitral regurgitation, a dilated left atrium and left ventricle, and pericardial effusion of up to 2 cm. The patient was initially given vancomycin and ceftriaxone on admission but was switched to intravenous cloxacillin and gentamicin on day 5 of admission. Gentamicin was stopped after a 5-day course, and cloxacillin was continued. Blood culture was negative on day 8 of admission. The patient developed mild right-side weakness with right-hand intention tremor on day 6 of admission, and computed tomography of the brain with contrast on day 7 of admission revealed a loss of corticomedullary differentiation at the anterior and posterior aspects of the left parietal lobe, suggestive of ischemic changes. On day 9 of admission, mitral valve replacement was performed. Both pericardial

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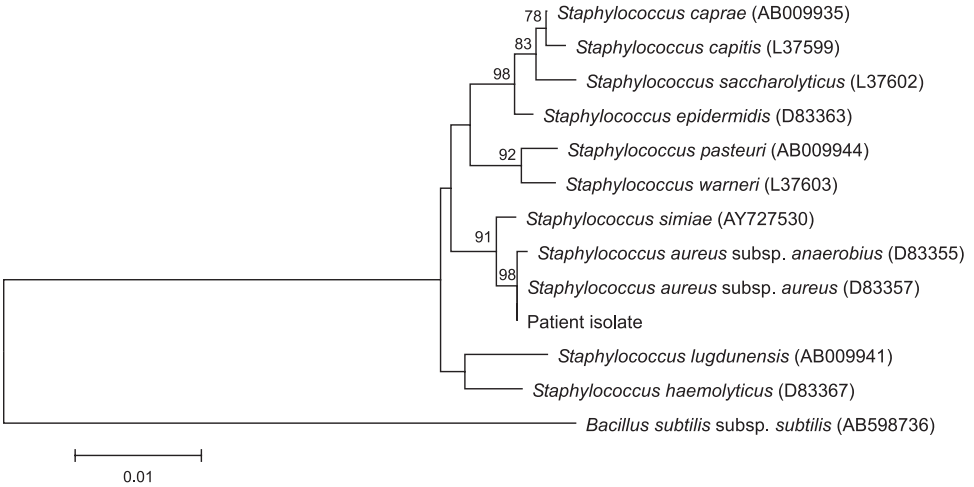

FIG. 1. Phylogenetic tree showing the relationships of the patient's isolate to related species. The tree was inferred from 16S rRNA data by the neighbor-joining method and rooted using the 16S rRNA gene sequence of *Bacillus subtilis* subsp. *subtilis* (GenBank accession number AB598736). Bootstrap values were calculated from 1,000 trees. The scale bar indicates the estimated number of substitutions per 50 bases. Names and accession numbers are given as cited in the GenBank database.

fluid and mitral valve tissue culture yielded the same Gram-positive cocci. Histological examination of the excised mitral valve revealed numerous Gram-positive cocci within the fibrinous material, which was admixed with lymphocytes and neutrophils. Because of a suspected drug fever, cloxacillin was switched to vancomycin from day 19 to day 23. The patient then developed a drug rash, and hence, vancomycin was switched to cefazolin on day 23 of admission, which was then continued to day 42.

16S rRNA gene sequencing. Bacterial DNA extraction, PCR amplification, and DNA sequencing of the 16S rRNA gene were performed according to our previous publications (21–23), using LPW2632, 5′-GTTAGCGGCGGACGGGTGA-3′, and LPW2633, 5′-GGTTACTCYACCGRCTTCGG-3′ (Sigma-Proligo, Singapore), as the PCR and sequencing primers. The sequences of the PCR products were compared with 16S rRNA gene sequences of closely related species in GenBank by multiple-sequence alignment using Clustal X 1.83 (19). Phylogenetic relationships were determined using the neighbor-joining method. Sequencing of the 16S rRNA gene of the isolate showed that there was no base difference between the 16S rRNA gene sequence of the isolate and that of *S. aureus* subsp. *aureus* strain ATCC 12600 (GenBank accession no. D83357), 1 (0.08%) base difference between the 16S rRNA gene sequence of the isolate and that of *S. aureus* subsp. *anaerobius* (GenBank accession no. D83355), 4 (0.3%) base differences between the 16S rRNA gene sequence of the isolate and that of *Staphylococcus simiae* (GenBank accession no. AY727530), 15 (1.2%) base differences between the 16S rRNA gene sequence of the isolate and that of *Staphylococcus caprae* (GenBank accession no. AB009935) and *Staphylococcus epidermidis* (GenBank accession no. D83363), 17 (1.3%) base differences between the 16S rRNA gene sequence of the isolate and those of *Staphylococcus capitis* (GenBank accession no. L37599) and *Staphylococcus pasteuri* (GenBank accession no. AB009944), and 18 (1.4%) base differences between the 16S rRNA gene sequence of the isolate and that of *Staphylococcus saccharolyticus* (GenBank accession no. L37602), indicating that the isolate was most compatible with being a strain of *S. aureus* (Fig. 1).

***katA* gene sequencing.** PCR amplification and DNA sequencing of the complete *katA* gene were performed as described above, using two sets of primers, LPW16705, 5′-GCG AGTATAGCGCCTCC-3′, and LPW16706, 5′-CAGCAGCTT CTTCATCAG-3′, and LPW16707, 5′-CGTCATATGCATGG GTTCG-3′, and LPW16708, 5′-GCCACATTCTGTGCATG C-3′ (Sigma-Proligo, Singapore), as the PCR and sequencing primers according to a previously published protocol (17). The sequences of the PCR products were compared with closely related sequences in GenBank by multiple-sequence alignment using Clustal X 1.83 (19). Sequencing of the *katA* gene of the isolate showed that there were 20 base differences between the *katA* gene sequence of the isolate and that of *S. aureus* subsp. *aureus* type strain ATCC 12600 (GenBank accession no. AJ000472). These included 15 silent mutations (A8G, C15T, C54T, T114C, A153T, A156G, A162T, G366A, T507G, A570G, T576C, T594A, A689T, T708A, and T717C), a nonsense mutation at position 802, and four additional mutations (C923T, T1005C, C1164T, and C1165T) downstream of the nonsense mutation (Fig. 2).

The production of catalase is a characteristic of most *Staphylococcus* species, except *S. aureus* subsp. *anaerobius* and *Staphylococcus saccharolyticus*. The presence of catalase can be tested by the addition of 3% hydrogen peroxide; this is a rapid test routinely employed in the identification of *S. aureus* in clinical microbiology laboratories. Catalase-negative *S. aureus* subsp. *aureus* (CNSA) strains are rare, and cases of CNSA have been summarized previously (6, 15). However, most of these reports did not perform molecular testing for the definite identification of *S. aureus* subsp. *aureus*. For this case report, we used a polyphasic approach, including determination of phenotypic characteristics and 16S rRNA gene sequencing, to

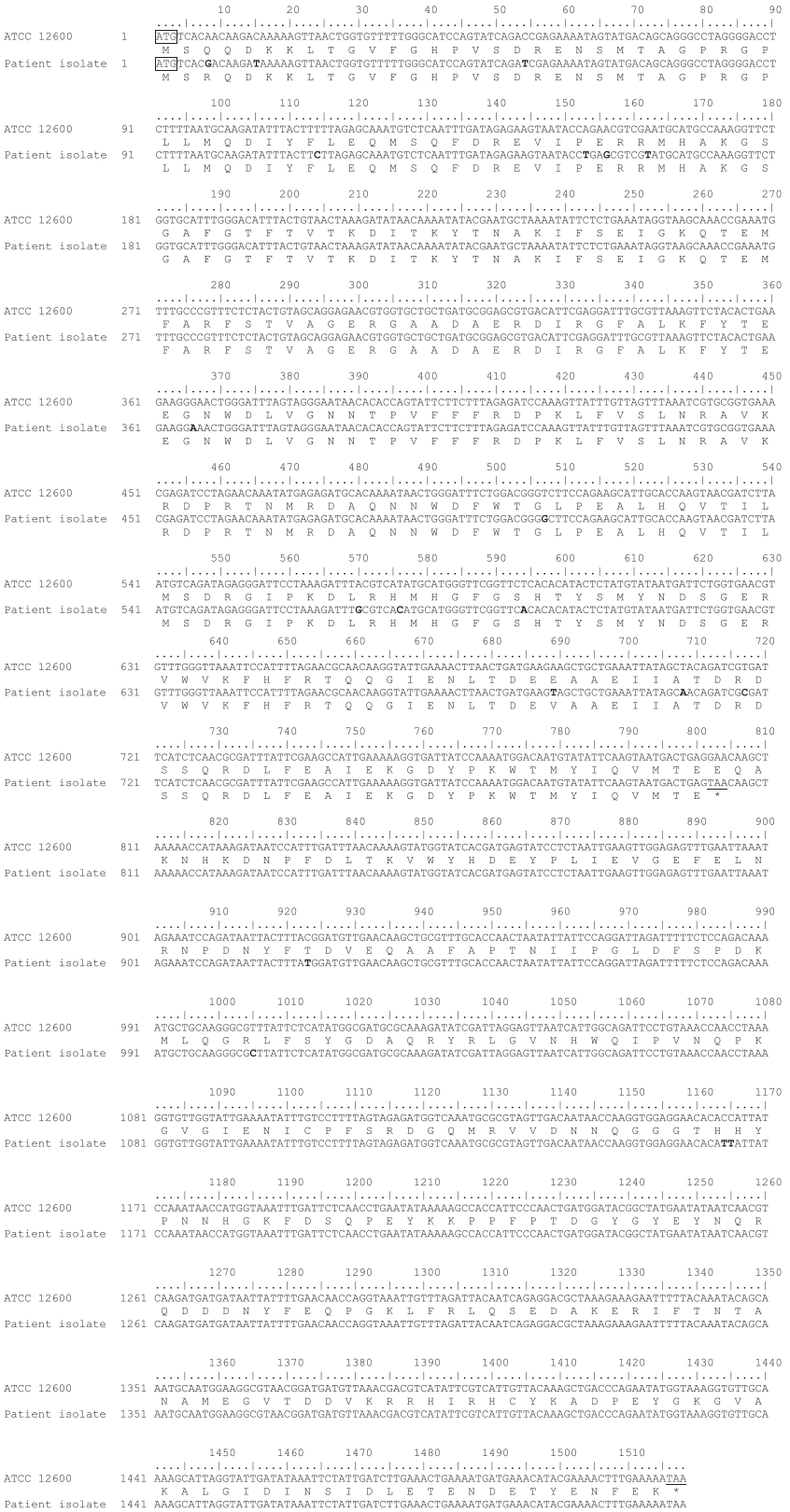

FIG. 2. Alignment of the nucleotide sequence of the *katA* gene of the patient's isolate and the deduced amino acid sequence of its product with those of *S. aureus* subsp. *aureus* ATCC 12600. The deduced amino acid sequence is designated in the single-letter code. Single-base substitutions are in boldface. The start codons are boxed, and stop codons are underlined. Numbers indicate relative nucleotide positions in the *katA* gene.

confirm the identity of *S. aureus* subsp. *aureus*. 16S rRNA gene sequencing showed that the isolate from our patient was most compatible with *S. aureus*, whereas its aerobic growth and positive slide coagulase test distinguished it from *S. aureus* subsp. *anaerobius*, which is microaerophilic, and *S. aureus* subsp. *saccharolyticus*, which is strictly anaerobic, confirming our isolate to be *S. aureus* subsp. *aureus*.

Catalase has been suggested to be a virulence factor because it can decompose hydrogen peroxide, a reactive oxygen intermediate responsible for the bactericidal activities of phagocytes (5, 11). Strains which do not produce catalase are thought to be less virulent. The importance of catalase production by *S. aureus* is especially apparent in patients with chronic granulomatous disease (20). However, animal studies have shown that CNSA strains have levels of virulence similar to that of catalase-positive *S. aureus* in a mouse model (13). Our case, which is the first report of CNSA-related endocarditis and pericarditis, has clearly demonstrated the high virulence of this CNSA isolate, even in the absence of an obvious predisposing factor for *S. aureus* infection. A previously reported case of CNSA-related endocarditis is in a patient with catheter-related bacteremia (6). Other invasive infections due to CNSA have also rarely been reported (1, 24). Catalase has also been postulated to be important in nasal colonization and environmental persistence (4, 16). However, an outbreak of catalase-negative methicillin-resistant *S. aureus* has been reported in Brazil (6).

We believe that this is the first report of a clinical strain of CNSA due to a nonsense mutation in the *katA* gene. The molecular mechanism that accounted for the lack of catalase production has been reported in only 2 studies. Piau et al. reported point mutations T172C and G636A, resulting in histidine 58-to-tyrosine and arginine 212-to-histidine substitutions, respectively (17), while Grüner et al. found a frameshift mutation due to a 5-base deletion upstream of the initiation codon of the *katA* gene (8). In our isolate, the mutation at position 802 led to a change of the codon GAA to TAA, which is a stop codon. This premature translation termination in the isolate's *katA* gene thus resulted in an 801-bp open reading frame that encoded a polypeptide of 267 amino acids. This constituted only 53% of the wild-type catalase with 505 amino acids. The three-dimensional structures of several bacterial monofunctional heme-containing catalases, including those from *Helicobacter pylori*, *Enterococcus faecalis*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas syringae*, *Vibrio salmonicida*, and *Proteus mirabilis*, have been determined (2, 3, 7, 9, 10, 14, 18). It was shown that they consist of four identical monomers, each with a heme group buried deep inside, and can bind NADPH in some catalases. Each monomer is composed of four distinct structural regions, including the N-terminal arm (residues 1 to 55, *Proteus mirabilis* numbering), an antiparallel eight-stranded β-barrel (residues 55 to 301), an extended wrapping loop (residues 302 to 416), and a helical domain at the carboxy terminus (residues 417 to 484) (12). In a comparison of the deduced amino acid sequence of the catalase from our isolate with those of other monofunctional catalases whose structures have been determined, only the N-terminal arm of the catalase from our isolate was predicted to be conserved, while the other regions were either disrupted or lost due to the premature translation termination. The lack of these regions could affect the maintenance of the heme-binding site and NADPH-binding region. In addition, among the three residues (His-54, Asn-127, and Tyr-337, *Proteus mirabilis* numbering) that are considered to be essential in catalysis (12), only His-56 and Asn-129 (our isolate's catalase numbering) are present, whereas Tyr-337 is lost. Therefore, it is highly unlikely that the catalase from our isolate would be functional, which is compatible with its loss of catalase activity by phenotypic testing.

Nucleotide sequence accession numbers. The 16S rRNA and *katA* gene sequences of the isolate have been deposited in the GenBank database under accession numbers JF746996 and JF740689.

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REFERENCES

1. **Alvarez-García, P., M. García-Campello, A. Pascual, and E. Alemparte.** 2003. First case of acute pericarditis due to catalase-negative *Staphylococcus aureus*. Enferm. Infecc. Microbiol. Clin. **21:**601–602.
2. **Bravo, J., et al.** 1995. Crystal structure of catalase HPII from *Escherichia coli*. Structure **3:**491–502.
3. **Carpena, X., et al.** 2003. Structure of the clade 1 catalase, CatF of *Pseudomonas syringae*, at 1.8 A resolution. Proteins **50:**423–436.
4. **Cosgrove, K., et al.** 2007. Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in *Staphylococcus aureus*. J. Bacteriol. **189:**1025–1035.
5. **Das, D., and B. Bishayi.** 2009. Staphylococcal catalase protects intracellularly survived bacteria by destroying H_2O_2 produced by the murine peritoneal macrophages. Microb. Pathog. **47:**57–67.
6. **Del'Alamo, L., et al.** 2007. An outbreak of catalase-negative methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **65:**226–230.
7. **Gouet, P., H. M. Jouve, and O. Dideberg.** 1995. Crystal structure of *Proteus mirabilis* PR catalase with and without bound NADPH. J. Mol. Biol. **249:**933–954.
8. **Grüner, B. M., et al.** 2007. Characterization of a catalase-negative methicillin-resistant *Staphylococcus aureus* strain. J. Clin. Microbiol. **45:**2684–2685.
9. **Håkansson, K. O., M. Brugna, and L. Tasse.** 2004. The three-dimensional structure of catalase from *Enterococcus faecalis*. Acta Crystallogr. D Biol. Crystallogr. **60:**1374–1380.
10. **Loewen, P. C., et al.** 2004. Structure of *Helicobacter pylori* catalase, with and without formic acid bound, at 1.6 A resolution. Biochemistry **43:**3089–3103.
11. **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.
12. **Messerschmidt, A.** 2001. Handbook of metalloproteins. Wiley, Chichester, NY.
13. **Messina, C. G., E. P. Reeves, J. Roes, and A. W. Segal.** 2002. Catalase negative *Staphylococcus aureus* retain virulence in mouse model of chronic granulomatous disease. FEBS Lett. **518:**107–110.
14. **Murshudov, G. N., et al.** 1992. Three-dimensional structure of catalase from *Micrococcus lysodeikticus* at 1.5 A resolution. FEBS Lett. **312:**127–131.
15. **Over, U., Y. Tuc, and G. Soyletir.** 2000. Catalase-negative *Staphylococcus aureus*: a rare isolate of human infection. Clin. Microbiol. Infect. **6:**681–682.
16. **Park, B., V. Nizet, and G. Y. Liu.** 2008. Role of *Staphylococcus aureus* catalase in niche competition against *Streptococcus pneumoniae*. J. Bacteriol. **190:**2275–2278.
17. **Piau, C., J. Jehan, R. Leclercq, and C. Daurel.** 2008. Catalase-negative *Staphylococcus aureus* strain with point mutations in the *katA* gene. J. Clin. Microbiol. **46:**2060–2061.
18. **Riise, E. K., et al.** 2007. The first structure of a cold-active catalase from *Vibrio salmonicida* at 1.96 A reveals structural aspects of cold adaptation. Acta Crystallogr. D Biol. Crystallogr. **63:**135–148.
19. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:**4876–4882.
20. **van den Berg, J. M., et al.** 2009. Chronic granulomatous disease: the European experience. PLoS One **4:**e5234.

21. **Woo, P. C., A. S. Leung, K. W. Leung, and K. Y. Yuen.** 2001. Identification of slide coagulase positive, tube coagulase negative *Staphylococcus aureus* by 16S rRNA gene sequencing. Mol. Pathol. **54:**244–247.
22. **Woo, P. C., et al.** 2002. *Streptococcus sinensis* sp. nov., a novel species isolated from a patient with infective endocarditis. J. Clin. Microbiol. **40:**805–810.
23. **Woo, P. C., et al.** 2011. Automated identification of medically important bacteria by 16S rRNA gene sequencing using a novel comprehensive database, 16SpathDB. J. Clin. Microbiol. **49:**1799–1809.
24. **Yilmaz, M., G. Aygun, T. Utku, Y. Dikmen, and R. Ozturk.** 2005. First report of catalase-negative methicillin-resistant *Staphylococcus aureus* sepsis. J. Hosp. Infect. **60:**188–189.