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CASE REPORT

# Molecular Characterization of a Catalase-Negative *Staphylococcus aureus* Blood Culture Isolate

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**Here we report a catalase-negative methicillin-sensitive *Staphylococcus aureus* isolate collected from a blood culture. Sequencing through the gene encoding catalase, *katA*, demonstrated a 2-bp insertion. The resulting frameshift mutation generates a protein that has lost 26 amino acids (aa) at its C-terminal domain.**

## CASE REPORT

An 86-year-old nondiabetic male with chronic renal failure had a prosthetic arteriovenous (AV) polytetrafluoroethylene (PTFE) graft inserted in the right thigh for dialysis. The operation was complicated by superficial wound infection and immediate graft thrombosis, which was managed conservatively. The graft was left *in situ*. Subsequently, he presented with purulent discharge from the operative site and was started on vancomycin empirically. He underwent immediate graft removal, and frank pus was noted along the whole length of the graft intraoperatively. Postoperatively, the patient recovered uneventfully.

The local ethics committee deemed that ethics review was not required for this case report (National Healthcare group Domain Specific Review Board application number 2015/00590).

Aerobic cultures of the infected graft grew no bacteria. Blood cultures taken at the same time as the graft removal gave positive results. The positive blood culture was plated on Trypticase soy agar with 5% sheep blood. After 24 h of incubation at 35°C, smooth and creamy β-hemolytic colonies were seen. Gram staining of the culture preparations showed clusters of Gram-positive cocci characteristic of staphylococci. The routine procedure on the blood culture bench entails performing two supplementary phenotypic tests (tube coagulase and catalase production) in addition to matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) to ensure the most accurate identification. The isolate gave a coagulase-positive test result suggestive of *S. aureus* but was repeatedly negative in the catalase slide test performed with 3% $H_2O_2$. MALDI-TOF MS identified the isolate as *S. aureus* with a high level of confidence. Antibiotic susceptibility was determined using the Etest (bioMérieux, Marcy l'Etoile, France), and breakpoints were defined according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The isolate was susceptible to oxacillin, cefoxitin, linezolid, daptomycin, vancomycin, clarithromycin, erythromycin, gentamicin, doxycycline, and rifampin. The molecular identification of *S. aureus* was confirmed with *rpoB* sequencing (1), with the isolated strain having 99% identity to *S. aureus* MSHR1132 (GenBank accession number FR821777.2). A species-specific PCR also confirmed that the isolate was *S. aureus* (2).

Full-length catalase gene (*katA*) sequencing was performed using the primer set comprising 5′-ATGTCACAACATGATAAAA A-3′ and 5′-TTATTTTTTAAAGTTTTCGTA-3′. The primers were designed using *S. aureus* MSHR1132 (GenBank accession number FR821777.2) catalase as a reference. This yielded an amplicon of 1,518 bp. Sequencing analysis revealed the presence of 7 silent mutations, A75T, A78G, T306A, T477C, G708A, A732T, and A951T, and a 2-bp insertion (CA) after nucleotide position 1157 compared to the *katA* of *S. aureus* MSHR1132. The insertion is predicted to create a frameshift resulting in the production of a truncated protein of 479 aa instead of the full-length enzyme of 505 aa.

Multilocus sequence typing (MLST) was performed using modified primers for *aroE* (3), *glpF*, *gmk*, *tpi*, and *yqiL* (4). Allele and sequence type (ST) assignments were made by comparisons to the *S. aureus* MLST database (http://saureus.mlst.net/). The isolate was of ST2250, belonging to clonal complex 75 (CC75). Genome sequencing of staphylococci of this lineage has shown them to be phylogenetically divergent from typical *S. aureus* strains (5). Initial descriptions of CC75 isolates came from analyses of *S. aureus* skin and soft tissue infections in indigenous communities in the Northern Territory of Australia (6). *spa* sequence typing was performed using the Ridom StaphType *spa* sequencing protocol (http://www.ridom.de/staphtype/spa_sequencing.shtml). The isolate was assigned a spa type of t5078 at the Ridom SpaServer (http://www.spaserver.ridom.de/). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was carried out as previously described (7). No SCC*mec* elements were detected, consistent with its methicillin sensitivity.

The susceptibility of *S. aureus* to $H_2O_2$ was determined (8). Methicillin-sensitive NCTC (National Collection of Type Cultures) *S. aureus* 10788 was used as a catalase-positive control. Increased sensitivity to $H_2O_2$ was observed in the catalase-negative isolate, with kill curves indicating that the isolate had an approx-

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**TABLE 1** Key characteristics of catalase-negative *Staphylococcus aureus* specimens reported between years 2007 and 2015

| Report no. | Specimen | Type of mutation | Effect of the mutation on catalase | Reference or source |
|---|---|---|---|---|
| 1 | Tracheal secretion | Frameshift | Truncated protein of 462 aa | 11 |
| 2 | Sputum | Frameshift | Truncated protein of 225 aa | 12 |
| 3 | Abscess | Frameshift | Truncated protein of 21 aa | 13 |
| 4 | Excised mitral valve | Nonsense | Truncated protein of 267 aa | 14 |
| 5 | Ulcer | Missense | Loss of active site residing at His58 | 15 |
| 6 | Blood | Frameshift | Truncated protein of 479 aa | This study |

imately 60% survival rate compared to the catalase-positive counterpart (data not shown).

For most clinical microbiology laboratories, MALDI-TOF MS is the dominant method of bacterial identification. However, as no diagnostic test is perfectly accurate, phenotypic tests are still relied upon to complement and corroborate identifications made by MALDI-TOF MS. As such, catalase production remains an important supplementary biochemical test for the differentiation of staphylococci from streptococci (catalase negative). All *Staphylococcus* species produce catalase except for *S. aureus* subsp. *anaerobius* and *S. saccharolyticus* (9). Catalase-negative *S. aureus* strains have been sporadically reported and represent an atypical minority of isolates implicated in human infection. The earliest observation of catalase-negative *S. aureus* was in 1976 (10), but it was only in the last decade, through molecular studies, that deficiencies in catalase production were correlated to mutations in *katA*. Table 1 summarizes the *katA* mutations detected in various catalase-negative isolates. The mutations identified are seemingly random, with no particular bias toward a particular mutation type.

A typical catalase is formed by four identical monomeric subunits, each containing in its active center a heme group and NADPH (16). Each monomer is composed of four distinct structural regions, including the N-terminal arm (approximately residues 1 to 55), an antiparallel β-barrel forming the core of the subunit (approximately residues 55 to 301), a wrapping domain (approximately residues 302 to 416) that links the β-barrel, and the α-helical domain of the C terminus (approximately residues 417 to 484) (16). Functional analysis of *Escherichia coli* catalase via site-directed mutagenesis demonstrates that a complete C-terminal domain is indispensable for efficient folding into an active and stable enzyme (17). We hypothesize that the requirement of the C terminus for activity can also be extended to the *S. aureus* catalase. In our case, the truncation of the protein to 479 aa eliminates the C terminus and hence is likely the cause of loss of catalase activity.

Catalase is critical for oxidative stress resistance. It protects the bacterium from oxidative damage by reactive oxygen species (ROS) by catalyzing the decomposition of hydrogen peroxide ($H_2O_2$) to water and oxygen. Consequently, catalase is postulated to be a virulence factor in bacterial pathogens that operates by conferring protection from ROS generated by host phagocytes. There is clear evidence that staphylococcal catalase protects the bacteria from $H_2O_2$-mediated killing of macrophages, thereby contributing to intracellular persistence (18). Catalase has also been demonstrated to be essential for the intracellular survival of bacteria such as *Mycobacterium tuberculosis* (19), *Helicobacter pylori* (20), and *Leptospira interrogans* (21).

The role of *S. aureus* catalase in virulence is less clear. Some researchers have observed a correlation between virulence and catalase activity (8, 22, 23), and yet others have not found any evidence of such a correlation (24, 25). Nevertheless, clinical isolates of catalase-negative *S. aureus* appear to have a role in human infections (11–15, 26). Clonal outbreaks of catalase-negative *S. aureus* infections have been reported, suggesting that such strains are as virulent as wild-type strains (27, 28).

## REFERENCES


1. **Drancourt M, Raoult D.** 2002. *rpoB* gene sequence-based identification of Staphylococcus species. J Clin Microbiol **40:**1333–1338. http://dx.doi.org/10.1128/JCM.40.4.1333-1338.2002.
2. **Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG.** 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus. J Clin Microbiol **36:**618–623.
3. **Ruimy R, Armand-Lefevre L, Barbier F, Ruppé E, Cocojaru R, Mesli Y, Maiga A, Benkalfat M, Benchouk S, Hassaine H, Dufourcq JB, Nareth C, Sarthou JL, Andremont A, Feil EJ.** 2009. Comparisons between geographically diverse samples of carried Staphylococcus aureus. J Bacteriol **191:**5577–5583. http://dx.doi.org/10.1128/JB.00493-09.
4. **Ng JW, Holt DC, Lilliebridge RA, Stephens AJ, Huygens F, Tong SY, Currie BJ, Giffard PM.** 2009. Phylogenetically distinct Staphylococcus aureus lineage prevalent among indigenous communities in northern Australia. J Clin Microbiol **47:**2295–2300. http://dx.doi.org/10.1128/JCM.00122-09.
5. **Holt DC, Holden MT, Tong SY, Castillo-Ramirez S, Clarke L, Quail MA, Currie BJ, Parkhill J, Bentley SD, Feil EJ, Giffard PM.** 2011. A very early-branching Staphylococcus aureus lineage lacking the carotenoid pigment staphyloxanthin. Genome Biol Evol **3:**881–895. http://dx.doi.org/10.1093/gbe/evr078.
6. **McDonald M, Dougall A, Holt D, Huygens F, Oppedisano F, Giffard PM, Inman-Bamber J, Stephens AJ, Towers R, Carapetis JR, Currie BJ.** 2006. Use of a single-nucleotide polymorphism genotyping system to demonstrate the unique epidemiology of methicillin-resistant Staphylococcus aureus in remote aboriginal communities. J Clin Microbiol **44:**3720–3727. http://dx.doi.org/10.1128/JCM.00836-06.
7. **Chen L, Mediavilla JR, Oliveira DC, Willey BM, de Lencastre H, Kreiswirth BN.** 2009. Multiplex real-time PCR for rapid Staphylococcal cassette chromosome mec typing. J Clin Microbiol **47:**3692–3706. http://dx.doi.org/10.1128/JCM.00766-09.
8. **Messina CG, Reeves EP, Roes J, Segal AW.** 2002. Catalase negative staphylococcus aureus retain virulence in mouse model of chronic granulomatous disease. FEBS Lett **518:**107–110. http://dx.doi.org/10.1016/S0014-5793(02)02658-3.
9. **Baron S (ed).**1996. Medical microbiology, 4th ed. University of Texas Medical Branch at Galveston, Galveston, Texas.
10. **Tu KK, Palutke WA.** 1976. Isolation and characterization of a catalase-negative strain of Staphylococcus aureus. J Clin Microbiol **3:**77–78.
11. **Grüner BM, Han SR, Meyer HG, Wulf U, Bhakdi S, Siegel EK.** 2007. Characterization of a catalase-negative methicillin-resistant Staphylococcus aureus strain. J Clin Microbiol **45:**2684–2685. http://dx.doi.org/10.1128/JCM.02457-06.
12. **Horiuchi K, Matsumoto T, Hidaka E, Kasuga E, Sugano M, Oana K, Kawakami Y, Honda T.** 2012. Isolation and molecular characterization of catalase-negative Staphylococcus aureus from sputum of a patient with aspiration pneumonia. Jpn J Infect Dis **65:**439–441. http://dx.doi.org/10.7883/yoken.65.439.

13. **Ellis MW, Johnson RC, Crawford K, Lanier JB, Merrell DS.** 2014. Molecular characterization of a catalase-negative methicillin-susceptible Staphylococcus aureus subsp. aureus strain collected from a patient with cutaneous abscess. J Clin Microbiol **52:**344–346. http://dx.doi.org/10.1128/JCM.02455-13.
14. **To KK, Cheng VC, Chan JF, Wong AC, Chau S, Tsang FH, Curreem SO, Lau SK, Yuen KY, Woo PC.** 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. http://dx.doi.org/10.1128/JCM.00849-11.
15. **Piau C, Jehan J, Leclercq R, Daurel C.** 2008. Catalase-negative Staphylococcus aureus strain with point mutations in the katA gene. J Clin Microbiol **46:**2060–2061. http://dx.doi.org/10.1128/JCM.02300-07.
16. **Nicholls P; Fita I; Loewen PC.** 2000. Enzymology and structure of catalases. Adv Inorg Chem **51:**51–106. http://dx.doi.org/10.1016/S0898-8838(00)51001-0.
17. **Sevinc MS, Switala J, Bravo J, Fita I, Loewen PC.** 1998. Truncation and heme pocket mutations reduce production of functional catalase HPII in Escherichia coli. Protein Eng **11:**549–555. http://dx.doi.org/10.1093/protein/11.7.549.
18. **Das D, Bishayi B.** 2009. Staphylococcal catalase protects intracellularly survived bacteria by destroying $H_2O_2$ produced by the murine peritoneal macrophages. Microb Pathog **47:**57–67. http://dx.doi.org/10.1016/j.micpath.2009.04.012.
19. **Manca C, Paul S, Barry CE, III, Freedman VH, Kaplan G.** 1999. Mycobacterium tuberculosis catalase and peroxidase activities and resistance to oxidative killing in human monocytes in vitro. Infect Immun **67:**74–79.
20. **Basu M, Czinn SJ, Blanchard TG.** 2004. Absence of catalase reduces long-term survival of Helicobacter pylori in macrophage phagosomes. Helicobacter **9:**211–216. http://dx.doi.org/10.1111/j.1083-4389.2004.00226.x.
21. **Eshghi A, Lourdault K, Murray GL, Bartpho T, Sermswan RW, Picardeau M, Adler B, Snarr B, Zuerner RL, Cameron CE.** 2012. Leptospira interrogans catalase is required for resistance to $H_2O_2$ and for virulence. Infect Immun **80:**3892–3899. http://dx.doi.org/10.1128/IAI.00466-12.
22. **Kanafani H, Martin SE.** 1985. Catalase and superoxide dismutase activities in virulent and nonvirulent Staphylococcus aureus isolates. J Clin Microbiol **2:**607–610.
23. **Mandell GL.** 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. http://dx.doi.org/10.1172/JCI107963.
24. **Martínez-Pulgarín S, Domínguez-Bernal G, Orden JA, de la Fuente R.** 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. http://dx.doi.org/10.1099/mic.0.025544-0.
25. **Cosgrove K, Coutts G, Jonsson IM, Tarkowski A, Kokai-Kun JF, Mond JJ, Foster SJ.** 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. http://dx.doi.org/10.1128/JB.01524-06.
26. **Yilmaz M, Aygun G, Utku T, Dikmen Y, Ozturk R.** 2005. First report of catalase-negative methicillin-resistant Staphylococcus aureus sepsis. J Hosp Infect **60:**188–189. http://dx.doi.org/10.1016/j.jhin.2004.11.012.
27. **Del'Alamo L, d'Azevedo PA, Strob AJ, Rodríguez-Lopez DV, Monteiro J, Andrade SS, Pignatari AC, Gales AC.** 2007. An outbreak of catalase-negative methicillin-resistant Staphylococcus aureus. J Hosp Infect **65:**226–230. http://dx.doi.org/10.1016/j.jhin.2006.12.005.
28. **Lee HK, Kim JB, Kim H, Jekarl DW, Kim YR, Yu JK, Park YJ.** 2014. Clonal spread of catalase-negative ST5/SCCmec II Staphylococcus aureus carrying the staphylococcal enterotoxin A (sea), staphylococcal enterotoxin b (seb), and toxic shock toxin (tst) virulence genes. Ann Clin Lab Sci **44:**394–398.