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**

A 68-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% H2O2. 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, gentamicin, doxycycline, and rifampin. The molecular identification of *S. aureus* was confirmed with tpeB sequencing (1), with the isolated strain having 99% identity to *S. aureus* NCTC 10788 (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′-TTATTATTTAAAGTTTTTCGTA-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* (*SCCmec*) typing was carried out as previously described (7). No *SCCmec* elements were detected, consistent with its methicillin sensitivity.

The susceptibility of *S. aureus* to H2O2 was determined (8). Methicillin-sensitive NCTC (National Collection of Type Cultures) *S. aureus* 10788 was used as a catalase-positive control. Increased sensitivity to H2O2 was observed in the catalase-negative isolate, with kill curves indicating that the isolate had an approx...
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


