Recalcitrant coagulase-negative methicillin-sensitive *Staphylococcus aureus* in an extremely low-birth-weight pre-term infant with thrombocytopaenia


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Introduction: Infections due to *Staphylococcus aureus* are cumbersome to treat and control. Virulent *S. aureus* is commonly coagulase positive, whilst non-virulent isolates are coagulase negative. Literature on coagulase-negative variants of *S. aureus* and their role in clinical disease in neonates is seldom available.

Case presentation: We report a case of an extremely low-birth-weight pre-term infant who had persistent recalcitrant infection with coagulase-negative methicillin-sensitive *S. aureus*, which resulted in clinical sepsis, thrombocytopaenia and persistence in blood cultures for 7 weeks. The API STAPH-Ident system (Bio-Merieux) and 16S rRNA gene sequencing were used to confirm identity. Parallel use of blood culture yielded an isolate with the same phenotypic identity.

Conclusion: Prompt identification of uncommon phenotypes of *S. aureus* also requires the use of appropriate molecular diagnostics to necessitate timely treatment of life-threatening systemic infections in neonates.

Keywords: 16S rRNA gene sequencing; coagulase negative; neonatal infections; sepsis.

**Introduction**

*Staphylococcus aureus* represents a common resident flora of the anterior nares and skin of healthy individuals (Aswani & Shukla, 2011; Bannerman & Peacock, 2007). Infections due to *S. aureus* are cumbersome to treat and control. Virulent *S. aureus* is reported to be coagulase positive, whilst non-virulent isolates are coagulase negative (Tan et al., 2008). Coagulase-negative staphylococci (CoNS) and coagulase-negative *S. aureus* represents the most commonly colonizing bacterial species of the digestive tract of infants especially during the first 3 weeks (Jacquot et al., 2011). Accumulating evidence suggests that methicillin-sensitive *S. aureus* (MSSA) accounts for a substantial number of infectious manifestations in newborns (van der Mee-Marquet et al., 2007, 2009). The role of skin carriage of CoNS by healthcare workers in neonatal intensive care units (NICUs) causing cross-contamination and infection among hospitalized patients has also been described (Hira et al., 2010). However, there are few reports in the literature on coagulase-negative *S. aureus* and its role in clinical disease in neonates (Vandenesch et al., 1993). Here, we report a neonate presenting with persistent recalcitrant infection due to coagulase-negative MSSA, which resulted in clinical sepsis, thrombocytopaenia and persistence of the organism in blood cultures for 7 weeks prior to successful final eradication.

**Case report**

A 27-week-old extremely low-birth-weight (980 g) female infant was delivered via assisted breech delivery. The mother presented in labour with leaking liquor, a bloody show and placental abruption. There was no prior evidence of maternal sepsis. Written consent was obtained from the mother. One dose of antenatal dexamethasone was administered ~2 h prior to delivery. The infant had poor
respiratory effort and was limp at birth, requiring resuscitation. The Apgar score was 4 at 1 min and 6 at 5 min, and surfactant was administered at 1 h after birth at the NICU. She had umbilical artery and vein catheters inserted at birth, and was subsequently extubated to nasal continuous airway pressure following 38 h of invasive ventilation. She was started empirically on intravenous crystalline penicillin and gentamicin. Both blood culture and an admission surveillance ear swab at birth demonstrated heavy growth of group B streptococci. Blood counts at birth were suggestive of a perinatal infection, as evidenced by a low leukocyte count of 2000 cells μl⁻¹, absolute neutrophil count of 360 μl⁻¹ and thrombocytopenia of 59 μl⁻¹ (Palis & Segel, 2010). A chest X-ray was consistent with features of moderate respiratory distress syndrome. A peripherally inserted central catheter (PICC) was inserted the following day following removal of the central umbilical lines. Underlying thrombocytopenia had resulted in difficulties in achieving homeostasis at the site of insertion of the PICC. There was no initial evidence of bacterial colonization, as noted by negative cultures from the central catheter tips, including the first PICC following removal.

In view of the persistent thrombocytopenia and potential sepsis owing to the underlying thrombocytopenia, a repeat septic work-up was performed on day 7. Blood culture showed evidence of bacteraemia with coagulase-negative MSSA. A peripheral blood smear demonstrated hypochromic microcytic anaemia with occasional red cells, polychromasia and fragmentation with spherocytes. There was neutropenia with left-shift maturation, some neutrophils showing toxic granulation and cytoplasmic vacuolation. Thrombocytopenia with large platelets suggestive of sepsis was also seen. Therefore, the treatment regimen was changed to vancomycin and cefotaxime. On receipt of the sensitivity report of a methicillin-sensitive organism, vancomycin was replaced with cloxacillin 3 days later. Her clinical course reflected an extremely low-birthweight pre-term infant with an ongoing moderate-grade infection. There was no episode of low perfusion or hypotension to signify profound sepsis or septic shock. Despite successful extubation to nasal continuous airway pressure after 38 h of invasive ventilation, she remained fairly tachypnoic with recurrent apnoea and bradycardias requiring stimulation and 60 days of dependence on non-invasive ventilation. The persistence of MSSA led us to optimize her cloxacillin dose to 200 mg kg⁻¹ day⁻¹, changed again to vancomycin due to failure of eradication. In addition, she developed multiple episodes of feeding intolerance, receiving only trophic feeds for up to 6 days before the feeding increment was eventually successful, necessitating a prolonged requirement for total parenteral nutrition of 4 weeks. She had two more PICC lines inserted, each of which became colonized with coagulase-negative MSSA. Her surveillance throat swab demonstrated colonization with Stenotrophomonas maltophilia from day 12 of hospitalization in the NICU.

On day 21 after birth, she developed an episode of vomiting associated with a distended abdomen and was kept at nil orally again. There was no other evidence of necrotizing enterocolitis. She was re-intubated on day 23 and the antibiotics were revised. Cefotaxime was replaced with meropenem and vancomycin was continued. Intravenous fluconazole was also commenced on presumption of a possible concurrent fungal infection. Her urine grew Candida tropicalis. During this period, blood cultures repeatedly grew coagulase-negative MSSA. On day 34, she developed two small abscesses over the dorsum of her right hand and her left foot, appearing several days apart, which resolved after bedside incision and drainage followed by topical application of fusidic acid. Apart from osteopaenia of prematurity, X-rays of her limbs did not indicate any evidence of septic arthritis. A cranial ultrasound, which showed evidence of a left grade I intraventricular haemorrhage on day 3, did not demonstrate any evidence of bleeding or an intra-abdominal abscess. The lowest platelet level was a mere 6000 μl⁻¹. Despite clinical sepsis, the highest C-reactive protein level was 3.3 mg l⁻¹. Vancomycin was kept as the preferred antibiotic, despite sensitivity of S. aureus to methicillin in view of the organism’s persistence.

The first negative blood culture was documented by week 6 after admission. A new onset of clinical sepsis was noted on day 65 as evidenced by lethargy and mild tachypnoea. This was supported by a slight reduction in platelet numbers again, albeit with a C-reactive protein level of 2 mg l⁻¹. Blood cultures at this time did not grow any organism. Intravenous antibiotics were finally stopped on day 75 upon documentation of consequent negative blood cultures, as well as determining that the infant was clinically sepsis free. The infant was discharged at a corrected age of almost 39 weeks with a weight of 1900 g. There was a dilemma when processing the blood culture on day 31. The strain isolated had a typical morphology of Gram-positive cocci in clusters showing golden-yellow and β-haemolytic colonies on blood agar. The isolate was confirmed as S. aureus by a positive DNase test, although the tube coagulase test was negative. The isolate was tested for pyrrolidonyl arylamidase and ornithine decarboxylase, as these are part of identification tests for Staphylococcus lugdunensis [3]. However, both tests were negative. The API STAPH-Ident system (Bio-Merieux) and 16S rRNA gene sequencing were done to confirm the identity of the isolate as S. aureus. Another set of blood cultures repeated on day 38 yielded an isolate with the same phenotypic identity.

Discussion

There are a number of possible reasons for the false-negative coagulase results. Staphylokinase production by some staphylococcal isolates may lyse the clot following prolonged incubation. Furthermore, the potential presence of contaminants also could have contributed to the false-negative results after prolonged incubation. There have been reports of recovery of some uncommon strains of S.
aureus, which required longer incubation periods to yield a positive coagulase reaction (Bannerman & Peacock, 2007). Although uncommon in clinical specimens, there have been reports of CoNS isolates showing a weakly positive DNase test (Bannerman & Peacock, 2007). S. lugdunensis might produce colonies with a yellow-white hue and that are β-haemolytic, resembling the colonial morphology of S. aureus. Our isolate showed a negative coagulase test and positive DNase test. Staphylococci grow well aerobically and in a CO₂-enriched atmosphere. Isolates of S. aureus are β-haemolytic when grown aerobically, whilst CoNS are non-haemolytic. It is important that the plates are retained for 2–3 days for the possible emergence of small-colony variants, especially in cases of protracted and difficult-to-treat chronic infections (Que & Moreillon, 2010). Our isolate tested positive for DNase, and the API STAPH-Ident system and 16S rRNA gene sequencing were finally used to confirm the identity. It appears that there might be discrepancies with the use of conventional biochemical tests due to discrepancies in incubation period, substrate concentrations or indicator activities (Kloos & Wolfshohl, 1982). There have been studies reporting the isolation of coagulase-variant forms of S. aureus (Fung et al., 1984; Młynarczyk et al., 1998). A strain of CoNS isolated from a 20-year-old heroin user was treated as infective endocarditis (Fung et al., 1984). This strain maintained its coagulase-negative properties after 20 subcultures. Our report suggests that a combination of biological properties should be used for identification of clinically important Staphylococcus isolates that are coagulase negative but suspected to be an atypical strain of S. aureus. Among the tests that were found to be useful in this study to differentiate S. aureus and CoNS were mannitol fermentation, extracellular protein A, DNase and thermostable endonuclease, for which most S. aureus strains gave a positive result, but for which other non-S. aureus coagulase-negative strains are negative (Fung et al., 1984). To rule out contamination and to confirm the aetiology of CoNS in clinical sepsis, there are several assessment algorithms suggested such as that based on quantitative culture (Craft & Finer, 2001) and an alternative one based on time to positivity (Haimi-Cohen et al., 2002), which could help prompt and definitive diagnosis.

In conclusion, here we have reported a case of coagulase-negative MSSA causing infection that resulted in clinical sepsis, thrombocytopenia and persistent bacteraemia in a neonate presenting with infection for 7 weeks prior to final eradication. Because of diagnostic problems in the initial phases of infection, prompt identification of uncommon phenotypes of S. aureus requires the use of molecular methods to necessitate timely treatment of life-threatening infections in neonates.

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References


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