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Case Report

Small colony variants of *Staphylococcus aureus* causing chronic respiratory infections in a dog

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ABSTRACT: *Staphylococcus aureus* produces a colonial variant called small colony variant (SCV), responsible for chronic infections in immunocompromised animals and humans. This variant contributes to recurrent infections via increased biofilm formation, altered antimicrobial susceptibility and intracellular persistence to evade host defence mechanisms. These infections are an emerging problem because of the difficulty in laboratory diagnosis, a tendency among clinicians and technologists to dismiss these colonies as commensal organisms, and their resistance to antibiotics that target bacterial cell walls and aminoglycosides. This study reports a case of recurrent and persistent respiratory infections in a 5-year-old female dog caused by SCV of *S. aureus.*

Keywords: Auxiotrophicity, antimicrobial resistance, small colony variants chronic infections, vitamin K.

INTRODUCTION

Staphylococcus aureus can cause a variety of infections such as pneumonia, osteomyelitis, toxic shock syndrome, and subcutaneous infections (Kahl, 2014). It is usually found as a colonizer in the animal body, particularly as part of the nasal microflora (Kaspar *et al*., 2016). These variants have gained more attention in human medicine but have been underestimated and overlooked in veterinary medicine. SCVs are persistent and often remain undetected or undiagnosed in the clinical laboratory because they have low metabolism and slow growth, which means they can only be detected after about 48 – 72 hours of incubation (Kahl, 2014). These infections are problematic because they are difficult to diagnose and treat due to antimicrobial resistance, including multidrug resistance, and the chronic nature of the infections (Suwantarat *et al*., 2017). This study reports report a case of recurrent and persistent respiratory infections in a dog (bitch) caused by SCV of *S. aureus.*

CASE REPORT

A 5-year-old female Caucasian dog was presented to a private clinic in Makurdi, Benue State, with respiratory distress, mucopurulent nasal discharges, fever, and anorexia. A review of the records showed that the patient had been vaccinated with all the required vaccines and was up to date with the boosters. The client also revealed that this was a recurring problem, and the dog had received multiple doses of antibiotics (gentamicin and neomycin). Clinical examination revealed respiratory rales on auscultation of the lungs. A pharyngeal swab was taken and submitted for bacterial culture, and doxycycline along with multivitamins was administered to the dog.

RESULTS

Culture on 5% sheep blood agar revealed small (about 0.1 mm) colonies that were non-haemolytic following incubation at 37ºC for 72 hours. There was no growth on MacConkey agar. Small, colourless colonies were observed after subculturing on nutrient agar and incubation for 48 hours. Colonies were catalase-positive, oxidase-negative, and coagulase-negative (Table 1). Gram stain revealed Gram-positive cocci of varying sizes

Table 1. Biochemical Reactions of SCVs.

Biochemical test	Results/reactions
Mannitol fermentation	negative
Coagulase test	negative
Oxidase test	negative
Catalase test	positive

arranged in clusters. Colonies were incubated in nutrient broth until a turbidity of 0.5% McFarland's standard was obtained. The broth was then streaked on nutrient agar with vitamin K added at the rate of 40 µg/ml of agar. After incubation for 24 hours at 37ºC, large colonies that were catalase-positive, oxidase-negative and Gram-positive cocci in clusters were observed, confirming auxiotrophy and a reversal to the wild-type *S. aureus* morphotype.

DISCUSSION

Small colony variants of staphylococci were first described decades ago from *S. aureus* and coagulase-negative *Staphylococcus* species (Kahl *et al*., 2016). Since then, many studies and observations have been published linking recurrent persistent staphylococcal infections to this special phenotype. SCVs isolated from a dairy cow with chronic mastitis have been shown to possess similar phenotypic properties to those of humans (Proctor. *et al*., 2006). *Staphylococcus aureus* SCVs are characterized by their small colony size, slow growth, and downregulated virulence genes, while genes important for biofilm formation and adhesion are mostly upregulated (Proctor *et al*., 2006). The uncommon physiological, metabolic, and morphological features of SCVs are challenging for routine diagnostic laboratories. In fact, they are difficult to recover, identify, and store (Kahl *et al*., 2016).

In this report, we identified these colonial variants of *S. aureus* by their typical colonial characteristics, which included a missing β-haemolytic zone and their auxotrophy for vitamin K. SCVs are very rapidly overgrown in mixed cultures, particularly in enrichment broths. Careful treatment of the specimens and meticulous examination of the agar plate are prerequisites for SCV diagnostics. Diagnosis is usually achieved by reversal to wild-type *Staphylococcus aureus* colonies in the presence of vitamin K.

SCVs of *S. aureus* are often mistaken for coagulase-negative staphylococci unless there is proper suspicion. Electron microscopy as well as Gram staining shows the SCVs to be slightly irregular in size due to incomplete separation after cell division, which adds to the diagnostic dilemma (Kahl *et al*., 2003).

Treatment usually consists of a combination of antibiotics with rifampicin for prolonged periods (Morellion *et al*., 2005). There are a few reports of isolation of SCVs from clinical specimens, Bhattacharyya *et al*. (2011) reported a case of endocarditis in a patient. It can be inferred that the SCV morphotype was selected because the patient was on an aminoglycoside antibiotic, and there were studies showing that SCVs are selected from wild-type *S. aureus* as early as 24 hours after initiation of aminoglycoside therapy (Samuelsen *et al*., 2005).

It would not be unreasonable to infer that SCV of *S. aureus* were responsible for causing chronic respiratory infections in this patient. Therefore, further research was recommended to determine a standardized laboratory approach to diagnose and treat these SCV *S. aureus* infections.

CONFLICT OF INTEREST

The authors of this case report declare no conflict of interest concerning its publication.

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