Detection of *Staphylococcus aureus* Small Colony Variants in Chronic Wounds

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ABSTRACT

*Staphylococcus aureus* Small Colony Variants (SA-SCVs) are a novel colony phenotype of *Staphylococcus aureus*. SA-SCVs differ from wild-type *Staphylococcus aureus* (SA) by the formation of smaller colonies and decreased growth rate, hemolysis, pigmentation, and antibiotic susceptibility. SA-SCVs are induced under stressful microbial environmental conditions, such as co-infection with *Pseudomonas aeruginosa*, common in chronic wound specimens. Differences in SA-SCV morphology and biochemical reactions from SA are responsible for errors in SA-SCV detection, which can contribute to delayed wound healing and treatment failure. In a clinical setting, SA-SCVs present difficulties such as decreased antibiotic susceptibility and reoccurring infections. This study was conducted to evaluate the prevalence and consequence of SA-SCVs in chronic wound specimens by obtaining and screening samples for the presence of SA-SCVs. Suspected SA-SCVs were assessed for their biochemical reaction and identification via routine diagnostic testing. Our results suggest that chronic wound specimens harbor a previously unrecognized burden of SA-SCVs, which has implications for diagnostic accuracy and treatment failure.

INTRODUCTION

SA is often found as transient colonizer on the human body, particularly as part of the nasal microflora in approximately 30% of the population (Krismer et al., 2008). Despite this, SA will oftentimes act as an opportunistic pathogen, ultimately contributing to a wide range of human infections, both acute and chronic. A novel phenotype of SA called SA-SCVs has been identified as being largely associated with chronic and recurrent infections such as soft-tissue infections, foreign-body associated infections, cystic fibrosis, and sepsis via altered antimicrobial susceptibility, intracellular persistence, and increased biofilm formation (Neut et al., 2007). Due to specific nutritional requirement deficiencies, called auxotrophy, for the chemicals hemin, menadione, and thymidine, SA-SCVs are unable to produce key components for a functional electron transport chain, characterizing the phenotype as metabolically dormant (Garcia, et al., 2007). Auxotrophic and antimicrobial treatment, and interaction with *Pseudomonas aeruginosa* (PA) contribute to induction of the SA-SCV phenotype (Kahl et al., 2014). SA-SCVs exhibit decreased pigmentation, decreased hemolysis, and a slower growth rate, which require a longer optimal incubation period around 48-72 hours. This phenotype arises spontaneously, induced by environmental stressors, such as harsh conditions, antimicrobial treatment, and polymicrobial interactions. WT-SA and other organisms overgrow SA-SCVs in co-culture, making detection increasingly difficult in a polymicrobial environment. Characteristic biochemical tests are also different for SA-SCVs in comparison to tests for WT-SA. In addition to being non-hemolytic on Columbia Blood Agar (CBA) and mannitol negative, SA-SCVs are also negative for catalase, coagulase, and latex agglutination tests. Many clinical laboratories are unfamiliar with this phenotype, and altered drug resistance profiles, biochemical tests, and abnormal morphological characteristics can result in SA-SCVs going undetected or misidentified as commensal organisms. The major clinical implication of SA-SCVs is chronic or recurrent infection leading to patient mortality due to the inability of clinicians to detect, identify, and effectively treat SA-SCV infections. This stresses the importance of familiarizing the clinical laboratory with the characteristics, mechanisms of action, and implications of SA-SCVs in order for new detection methods to be incorporated into routine diagnostic procedures, allowing for new treatment considerations.

EXPERIMENTAL DESIGN

**RESULTS**

To date, thirteen chronic wound specimens have been evaluated. Of these, eight samples initially screened for suspected SA-SCVs. One specimen did not proceed through the workflow; therefore 7/13 samples (53.8%) screened positive for suspected SA-SCVs colonies that proceeded through the workflow outlined previously. Of these seven samples, one confirmed SA-SCVs, upon initial screening by culture-based microbial ID and AST (from CMC) direct from the tissue specimens, only three specimens (3/7, 42.8%) were found to harbor SA; ID and AST results are described in Table 1. Given that all specimens initially screened positive for SA via qPCR at the RTL per patient inclusion criteria, it is suspected that some specimens may harbor SA-SCV, but due to their unusual morphology (Figures 1 and 2) and/or biochemical reactions, they were not detected as WT-SA. Of the four specimens that did not initially screen for SA, species such as coagulase-negative *Staphylococcus* (CNS), diaphorosis, and Gram-negative rods were detected (Table 1). Given the unusual morphology and biochemical reactions of SA-SCVs compared to WT-SA, we suspected that SA-SCVs may be misidentified as CNS and/or diaphorosis. Further confirmatory testing will need to be conducted to evaluate this suspicion.

**CONCLUSIONS**

Because many biochemical reactions of SA-SCVs are altered compared to WT-SA, automated systems have not traditionally been utilized to identify SA-SCVs. Pure cultures of suspected SA-SCVs were submitted to CMC for a NGS-based microbial identification and, if *S. aureus* is detected, AST was performed; results are shown in Table 1. This experiment was performed to determine that if SA-SCVs are present in chronic wound specimens, an automated system be able to reliably and correctly identify SA-SCVs, based on biochemical reactions. None of the pure cultures of suspected SA-SCVs resulted in the detection of *S. aureus* from culture-based and biochemical-based identification (Table 2); other species of microorganisms such as *Staphylococcus warneri* and *Corynebacterium jeikeium* were detected. Given that SA-SCVs have altered biochemical reactions compared to WT-SA (Table 2), and that culture-based microbial ID is based from biochemical reactions, it is not surprising that our suspected SA-SCVs were not identified as *S. aureus*. The observed differences in the biosynthesis between WT-SA and SA-SCV exemplify that not only the morphological changes, but also any physiological changes, which are induced in WT-SA to result in SA-SCVs. As WT-SA is put under the pressure of aminoglycosides, such as gentamicin, the induced SA-SCV phenotype may exhibit inherent characteristics that allow them to, for example, thrive as a latex-agglutination negative phenotype. Of all the biochemical tests performed, latex agglutination was found to be negative in all seven of the WT-SA species, such as coagulase-negative *Staphylococcus* (CNS), diaphorosis, and Gram-negative rods, not *Pseudomonas aeruginosa* (no AST performed). SA-SCV 10 Diphosphates (no AST performed).

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This work has the potential to develop an NGS protocol for the detection of SA-SCVs from clinical samples that could be widely adopted in diagnostic laboratories.

**REFERENCES**

To date, we have evaluated 13 chronic wound samples, and have found suspected SA-SCV colonies in 7 specimens (53.8%). We will continue with patient recruitment and sampling until a minimum of 25 clinical samples have been evaluated for the presence of SA-SCVs in collaboration with Nick Sanford at the Southwest Regional Wound Care Center (Lubbock, TX) Suspected SA-SCVs from our 7 samples that screened positive were evaluated via culture-based microbial ID and AST and biochemical reactions. These results imply that traditional culture-based microbial ID and AST is not optimized to detect clinical SA-SCVs. To determine that our pure cultures of suspected SA-SCVs are indeed *S. aureus*, we will conduct qPCR utilizing *S. aureus*-specific analytics in regions that are not associated with mutations in genes resulting in SA-SCVs, and should be conserved across WT-SA and SA-SCVs. Confirmatory qPCR testing will be conducted in collaboration with RTGenomics (Lubbock, TX), a next-generation sequencing company specializing in microbial identification.

**FUTURE DIRECTIONS**

To further confirm the presence of SA-SCVs in our clinical specimens, as well as to determine mutations in which gene resulted in the SA-SCV phenotype, next-generation sequencing (NGS) of our tissue samples will be conducted in collaboration with Klara C. Keim and Alexander Horsell at the Department of Microbiology and Immunology, University of Colorado. This work has the potential to develop an NGS protocol for the detection of SA-SCVs from clinical samples that could be widely adopted in diagnostic laboratories.

**WORKS CITED**

[Reference List: 10-20 references, including all cited sources]

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