Author/s:
Carzino, R;Hart, E;Sutton, P;King, L;Ranganathan, S

Title:
Lack of small colony variants of Staphylococcus aureus from lower respiratory tract specimens

Date:
2017-05-01

Citation:
Carzino, R., Hart, E., Sutton, P., King, L. & Ranganathan, S. (2017). Lack of small colony variants of Staphylococcus aureus from lower respiratory tract specimens. Pediatric Pulmonology, 52 (5), pp.632-635. https://doi.org/10.1002/ppul.23699.

Persistent Link:
https://hdl.handle.net/11343/292684

Original Article: Cystic Fibrosis

LACK OF SMALL COLONY VARIANTS OF STAPHYLOCOCCUS AUREUS FROM LOWER RESPIRATORY TRACT SPECIMENS

Rosemary Carzino[1], Emily Hart[1,] Philip Sutton[1,2], Louise King[1,] Sarath Ranganathan[1,2], on behalf of AREST CF.

Murdoch Children's Research Institute, The Royal Children's Hospital, Melbourne, Australia[1] and The University of Melbourne, Parkville, Australia [2]

Corresponding author:

Rosemary Carzino

Respiratory Diseases, Murdoch Children's Research Institute

Royal Children's Hospital

Flemington Red, Parkville VIC 3052

Rosemary.Carzino@mcri.edu.au

+613 9345 4670

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ppul.23699

ABSTRACT

Background: Small-colony variants (SCVs) *of Staphylococcus aureus* are associated with worse lung disease in children with Cystic Fibrosis (CF), exhibit a higher resistance to antibiotics and co-colonize more commonly with *Pseudomonas aeruginosa* compared to the normal phenotype. The prevalence of SCVs in lower airway specimens from children with CF is largely unknown.

Methods: Each visible morphotype of *S. aureus* was subcultured onto horse blood agar (HBA) to enable identification of SCVs.

Results: Sixty-one samples from 41 children (mean age 11.7 (SD 5.3) years) were identified with a positive *S. aureus* culture from lower respiratory tract specimens collected in 2014-2015. None of the differing morphotypes isolated were identified as *S. aureus* SCVs.

Conclusion: In a centre where anti staphylococcal prophylaxis is adopted, *S. aureus* SCVs were not isolated from the lower airways specimens in young children with CF indicating that acquisition of small colony variant *S. aureus* may not be a significant clinical problem in young children with CF.

Key words: Cystic fibrosis, *Staphylococcus aureus*, small colony variants

INTRODUCTION

Lung disease associated with chronic airway inflammation and infection is the major cause of morbidity and mortality in people with cystic fibrosis (CF). *Staphylococcus aureus* (*S. aureus*) is one of the first bacteria isolated from the CF airways, and is the most common cause of respiratory infections among infants and young children. Previous studies have found that infection with *S. aureus* is associated with inflammation [1], development of bronchiectasis [2], poorer nutritional status [3] and overall decline in lung function [4] in infants and young children with CF.

Although respiratory infections caused by *S. aureus* early in life are known to negatively affect health outcomes of individuals with CF, the role of small-colony phenotypic variants (SCVs) in disease progression is still to be defined. Recent studies have reported that these variants have been associated with worse respiratory outcomes in children with CF compared to those that have only normal *S. aureus* phenotype cultured [5-7]. However many of the samples studied from young children were isolated from oral pharyngeal swabs which are not a reliable predictor of lower airway infection [5, 8].

S. aureus SCVs can be typically found in association with many chronic and or persistent infections other than CF, such as chronic osteomyelitis and skin and soft tissue infections [9, 10], and are found to be selected for by long-term exposure to antibiotics as well as co-culture with *Pseudomonas aeruginosa* [5, 6, 11]. *S. aureus* SCVs exhibit distinct metabolic defects that confer resistance to antibiotics and contribute to slower growth on most laboratory media. Typically they are thymidine dependent or have deficient electron transport resulting in auxotrophisms to haemin and/or menadione. They cleverly adapt to their environment by entering into a survival mode of slow growth and increased antibiotic

resistance [12]. As a consequence, *S. aureus* SCVs are difficult to detect and the prevalence of this potential pathogen among many CF patient populations is largely unknown.

Currently, most clinical laboratories do not use culture methods required to detect *S. aureus* SCVs or actively look for these isolates which are therefore not routinely reported. Previous studies that have investigated the prevalence of *S. aureus* SCVs in their CF centres have reported differing rates of infection, ranging from 8-72% [5-7, 13-15]. These differences may reflect the particular cohort being studied; age range, disease severity and antibiotic treatment centre practices.

This study aimed to establish the prevalence of *S. aureus* SCVs in lower airway respiratory specimens in children with CF attending an Australian CF treatment centre at The Royal Children's Hospital, Melbourne in order to inform the need for future surveillance of *S. aureus* SCVs.

METHODS

Method for identification of *S.aureus* SCVs were previously published by Wolter etal. 2013 [5]. Briefly, Mannitol salt agar (MSA) plates were used for all CF sputum, bronchoalveolar lavage (BAL) and cough swab specimens processed between September 2014 and May 2015 as part of clinical care. Multiple samples were collected during this period from individual patients with CF. Each visible morphotype was subcultured from MSA plate onto HBA to enable identification of *S. aureus* SCVs, followed by confirmation by PCR amplification of the *S. aureus*-specific gene, *nuc*.

Culture results were obtained from the hospital's online medical reporting system, CLARA and *S. aureus* antibiotic sensitivity results obtained from The Royal Children's Hospital Microbiology Diagnostic Service. *S. aureus* infection status was then classified according to the following definition: negative (no *S. aureus* growth within the 12 months preceding the sample collection), intermittent (*S. aureus* culture in ≤50% of samples within the 12 months preceding the sample collection) or persistent (persistent presence of *S. aureus* in >50% of samples within the 12 months preceding the sample collection). Subjects with less than 4 samples within 12 months preceding the sample reviewed were excluded.

Information pertaining to the patients' antibiotic treatment regime within 12 months preceding the sample reviewed was accessed through the hospital's Electronic Medical Record System.

This research project was supported and approved by The Royal Children's Hospital Human Research Ethics Committee, Melbourne (HREC 36050A)

RESULTS

During this study period, there were 277 lower airway samples collected from 124 CF patients for microbiology analysis. Sixty-one (22%) of these samples were positive for normal phenotype of *S. aureus*, however no SCV of *S. aureus* were detected. Of the sputum, BAL and cough swab samples collected, the majority of *S. aureus* isolates were cultured from Sputum samples (77%) and 13% of these were co-infected with *Pseudomonas aeruginosa.*

Sixty-one of these samples from forty-one patients with CF were studied. Twelve out of the forty-one patients had more than one *S.aureus* positive sample collected during the study period. The characteristics of the patients with positive *S.aureus* samples and nature of the

samples from which isolates were obtained are shown in Table 1. The mean age of patients was 11.7 (SD 5.3) years.

Nearly half of the *S. aureus* isolates were resistant to only Penicillin (49%) and 31% were resistant to all three: Penicillin, Clindamycin, and Erythromycin. Of the remaining resistant *S. aureus* isolates, Methicillin accounted for 13%, Trimethoprim sulfamethoxazole (6%) and both Methicillin Trimethoprim and sulfamethoxazole (3%) (Table 2).

Reviewing the antibiotic treatment given to the 41 patients over three months preceding positive S.aureus cultures showed that the majority of patients (39.3%), were treated with a combination of anti-staphylococcal antibiotics followed by (27.8%) with Augmentin and approximately one quarter (24.6%) with Trimethoprim sulfamethoxazole.Samples collected from patients 12 months preceding positive *S.aureus* culture identified that the majority of patients had an intermittent history of *S. aureus* infection (40.5%), followed by persistent infection (35.1%) and no previous *S. aureus* infection for 24.3% of patients (Table 4).

DISCUSSION

We conducted a small study to ascertain the prevalence of *S. aureus* SCVs in children with CF in our centre following a previously published protocol [5]. Our intention, was to examine metabolic defects of *S. aureus* SCV isolates in the presence of haemin, menadione, thymidine and CO_2 and further use pulse field gel electrophoresis (PFGE) to genotype paired normal and *S. aureus* SCV isolates. *S. aureus* SCVs were however not identified in any of the 61 samples from 41 patients obtained via cough swab, sputum or BAL, suggesting that this phenotype is not likely to be common at our institution.

The majority of these *S. aureus* isolates were sensitive to anti-staphylococcal antibiotics and is therefore possible that they were successfully treated with these antibiotics, potentially not evolving resistant strains such as SCV. Over a third (35%) of these patients had a persistent infection with *S. aureus*; whether or not this was genotypically the same strain is unknown. Persistent infection with *S. aureus* has been associated with the emergence of SCVs and increased antibiotic resistance. Studies have shown the clonality between *S. aureus* SCVs and simultaneously isolated normal *S. aureus* strains [5, 13, 14], indicating *in vivo* selection rather than patient to patient transmission. A six year study by Kahl *et al*. [14], cultured *S. aureus* from 185 of 391 (47.3%) throat swabs from 25 patients with a median age of 9 years and from 272 of 518 sputum samples (52.5%) of 22 patients with a median age of 20.5 years. Persistent infection with *S. aureus* (isolated for more than 6 months) was observed in 52 of 72 patients (72.2%); 24 patients (33%) were infected with SCV *S.aureus* and 22 patients were continuously treated for more than 18 months with trimethoprim-sulfamethoxazole. The majority of CF patients "33 of 52 (63%)" harboured a distinct single clone of *S. aureus* and 17 (33%) patients had two or more clones, most likely the result of persistent *S. aureus* infection in the airways.

In our clinic, routine anti–staphylococcal antibiotic prophylaxis treatment has been implemented from 2004 for children with CF under 2 years of age. Eighteen (44%) of the subjects in this cohort had received anti-staphylococcal prophylaxis and 3 subjects (<2 years old) were still being treated. Typically a combination of two antibiotics was the favoured treatment for the majority of patients in this study, followed by amoxicillin/clavulanic acid alone. Treatment with the antibiotic trimethoprim-sulfamethoxazole has been reported in previous studies to promote the selection of SCV within CF airways [5, 13, 15], although this was a treatment for less than a quarter of patients in this study [5]. Similarly while *S. aureus*

and *P. aeruginosa* co-infection of the CF airways appears to be a selective pressure for *S. aureus* SCVs to evolve [11], in this study, only 8 (13%) samples were co-infected with *P. aeruginosa* and no *S. aureus* SCVs were identified. There were only 3 subjects treated with trimethoprim-sulfamethoxazole at the time of co-infection with *P. aeruginosa.*

S. aureus SCVs are more likely to be cultured from older patients with CF [13]. Some studies have reported median ages of 13 years [15], 14.4 years [13], 21 years [6]. These patients typically have worse lung disease, chronic persistent infection with *S. aureus*, co-infection with *P. aeruginosa* and long term antibiotic treatment perhaps explaining the difference between their reports and our findings in younger subjects.

Kahl et al [15] also reported a higher prevalence of both *S. aureus* (n= 53/78, 68%) and *S. aureus* SCVs (n=27/53, 49%) in their cohort (0.5-43 years) with a median age of 13 years. The reason for the relatively high prevalence of both the normal phenotypic *S. aureus* and *S. aureus* SCVs is not clear. The cultures were isolated mainly from bronchial secretion specimens, or in young patients, when no secretions were produced, by oropharyngeal swabs (OP). However, *S. aureus* cultured from OP swabs are not a reliable predictor of lower airway infection [16, 17].

The limitation of this report is that it was conducted in a single centre with low prevalence of *S. aureus* infection in general. In addition, there is no gold standard method for the detection of *S. aureus* SCVs. The techniques used in this study were similar to previous studies [5] that were able to identify *S.aureus* SCV's , however improved methods may yet be developed to ensure that *S. aureus* SCV are not being missed. However, the likelihood of finding *S. aureus* SCVs in the lower airway of young children with CF, appeared to be low in our centre. In

addition, young children with CF in our centre are less likely to be chronically infected or co-infected with *P. aeruginosa* or treated with mainly Trimethoprim-sulfamethoxazole all of which have been associated with the emergence of *S. aureus* SCVs.

CONCLUSION

Small colony variant S. *aureus* isolates were not found in the lower airways of children with CF in our centre and, if these findings are replicated at other centres, suggest that small colony variant *S. aureus* is not a significant problem in young children with CF.

Acknowledgements

RCH Bacteriology Department

Contributors

Conception of design and study: SR, RC, EH

Acquisition of data, analysis and interpretation: RC, LK, SR

Statistical analysis of data: RC, LK

Writing of manuscript: RC, LK, SR

Revision of manuscript for intellectual content and approval before submission: SR, EH

Competing interest

None

Funding

Sources of grants: This study has been funded by grants from the Murdoch Children's Research Institute Cystic Fibrosis Research Fund and National Health and Medical Research Council (APP1043768).

REFERENCES

1. Gangell, C., et al., *Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis.* Clin Infect Dis, 2011. **53**(5): p. 425-32. http://dx.doi.org/10.1093/cid/cir399
2. Sly, P.D., et al., *Lung Disease at Diagnosis in Infants with Cystic Fibrosis Detected by Newborn Screening.* American Journal of Respiratory and Critical Care Medicine, 2009. **180**(2): p. 146-152. http://dx.doi.org/10.1164/rccm.200901-0069OC
3. Ranganathan, S.C., et al., *Evolution of pulmonary inflammation and nutritional status in infants and young children with cystic fibrosis.* Thorax, 2011. **66**(5): p. 408-413. http://dx.doi.org/10.1136/thx.2010.139493
4. Pillarisetti, N., et al., *Infection, Inflammation, and Lung Function Decline in Infants with Cystic Fibrosis.* American Journal of Respiratory and Critical Care Medicine, 2011. **184**(1): p. 75-81. http://dx.doi.org/10.1164/rccm.201011-1892OC
5. Wolter, D.J., et al., *Staphylococcus aureus small-colony variants are independently associated with worse lung disease in children with cystic fibrosis.* Clin Infect Dis, 2013. **57**(3): p. 384-91. http://dx.doi.org/10.1093/cid/cit270
6. Besier, S., et al., *Prevalence and clinical significance of Staphylococcus aureus small-colony variants in cystic fibrosis lung disease.* J Clin Microbiol, 2007. **45**(1): p. 168-72. http://dx.doi.org/10.1128/jcm.01510-06
7. Schneider, M., et al., *Clinical characteristics associated with isolation of small-colony variants of Staphylococcus aureus and Pseudomonas aeruginosa from respiratory secretions of patients with cystic fibrosis.* J Clin Microbiol, 2008. **46**(5): p. 1832-4. http://dx.doi.org/10.1128/JCM.00361-08
8. Armstrong, D.S., et al., *Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis.* Pediatr Pulmonol, 1996.

21(5): p. 267-75. http://dx.doi.org/10.1002/(sici)1099-0496(199605)21:5<267::aid-ppul1>3.0.co;2-k

9. Cervantes-Garcia, E., et al., *Staphylococcus aureus small colony variants in diabetic foot infections.* Diabet Foot Ankle, 2015. **6**: p. 26431. http://dx.doi.org/10.3402/dfa.v6.26431

10. von Eiff, C., et al., *Recovery of Small Colony Variants of Streptococcus aureus Following Gentamicin Bead Placement for Osteomyelitis.* Clinical Infectious Diseases, 1997. **25(**5): p. 1250-1251. http://dx.doi.org/10.1086/516962

11. Hoffman, L.R., et al., *Selection for Staphylococcus aureus small-colony variants due to growth in the presence of Pseudomonas aeruginosa.* Proc Natl Acad Sci U S A, 2006. **103(**52): p. 19890-5. http://dx.doi.org/10.1073/pnas.0606756104

12. von Eiff, C., *Staphylococcus aureus small colony variants: a challenge to microbiologists and clinicians.* International Journal of Antimicrobial Agents, 2008. **31**(6): p. 507-510.DOI: http://dx.doi.org/10.1016/j.ijantimicag.2007.10.026

13. Yagci, S., et al., *Prevalence and genetic diversity of Staphylococcus aureus small-colony variants in cystic fibrosis patients.* Clin Microbiol Infect, 2013. **19**(1): p. 77-84. http://dx.doi.org/10.1111/j.1469-0691.2011.03742.x

14. Kahl, B.C., et al., *Population dynamics of persistent Staphylococcus aureus isolated from the airways of cystic fibrosis patients during a 6-year prospective study.* J Clin Microbiol, 2003. **41**(9): p. 4424-7.

15. Kahl, B., et al., *Persistent infection with small colony variant strains of Staphylococcus aureus in patients with cystic fibrosis.* J Infect Dis, 1998. **177**(4): p. 1023-9.

16. Rosenfeld, M., et al., *Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis.* Pediatric Pulmonology, 1999. **28**(5): p. 321-328.

http://dx.doi.org/10.1002/(SICI)1099-0496(199911)28:5<321::AID-PPUL3>3.0.CO;2-V

17. Armstrong, D.S., et al., *Bronchoalveolar lavage or oropharyngeal cultures to indentify lower respiratory pathogens in infants with cystic fibrosis.* Pediatric Pulmonology, 1996. **21**(5): p. 267-275. http://dx.doi.org/10.1002/(SICI)1099-0496(199605)21:5<267::AID-PPUL1>3.0.CO;2-K

Table 1 **- Characteristics of Patients with positive *S. aureus* culture**

Characteristics	**N (%)**
Samples	61
Patients	41
Mean age years ±SD	11.7 ± 5.3
Sex	20 (49.0)
Males	21 (51.0)
Females	
CFTR genotype	16 (39.0)
P.Phe508del homozygous	17 (41.0)
P.Phe508del heterozygous	8 (20.0)
Other	
Pancreatic insufficiency	35 (85.0)

Table 2 **- Proportion of sample types and description with positive *S. aureus* culture**

Sample types/description	**N samples (%)**
Sputum	47 (77.0)
BAL	9 (14.8)
Cough swab	5 (8.2)
Samples infected with Small colony variant *S. aureus*	0
Samples co- infected with *P. aeruginosa* (3 patients)	8 (13.0)
Samples infected with Methicillin-Resistant *S. aureus* (MRSA) (7 patients)	8 (13.0)
Samples with Trimethoprim sulfamethoxazole resistant *S. aureus* isolates (2 patients)	4 (6.0)
Samples infected with Methicillin and Trimethoprim sulfamethoxazole Resistant *S. aureus* (1 patient)	2 (3.0)
Samples with Penicillin resistant only *S. aureus* isolates (24 patients)	30 (49.0)
Samples with Penicillin, clindamycin and erythromycin resistant *S. aureus* isolates (10 patients)	19 (31.0)
Samples antibiotic sensitive *S. aureus* isolates (2 patients)	2 (3.0)

Table 3 **- Antibiotic use in patients with positive *S. aureus* culture during 3 months prior to specimen collection**

Antibiotic use	**N (%)**
Combination therapy*	24 (39.3)
Augmentin	17 (27.8)
Trimethoprim sulfamethoxazole**	15 (24.6)
Tobramycin	2 (3.3)
Colistin	1 (1.6)

**Variable cycling combination of 2 or more antibiotics.*

***3 subjects treated with Trimethoprim sulfamethoxazole had co-culture positive sample with P. aeruginosa*.

***Table 4* - *S. aureus* infection in patients 12 months preceding positive culture sample reviewed.**

S. aureus infection12 months preceding culture	N (%)
None	9 (24.3)
Intermittent	15 (40.5)
Persistent*	13 (35.1)

**Persistent infection is defined by more than 50% of positive samples, minimum of 4 tested within the 12 months*