Intravenous antibiotics given for 2 weeks do not eradicate persistent *Staphylococcus aureus* clones in cystic fibrosis patients

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Abstract

Staphylococcus aureus is the most commonly isolated pathogen in respiratory tract secretions from young patients with cystic fibrosis (CF), and several treatment strategies are used to control the infection. However, it is not known whether intensified treatment with antimicrobial agents causes eradication of *S. aureus* clones. We retrospectively determined the impact of intravenous (IV) antimicrobial agents on the suppression and eradication of *S. aureus* clones. One thousand and sixty-one *S. aureus* isolates cultured from 2526 samples from 130 CF patients during a 2-year study period were subjected to *spa* typing. Intervals between positive samples and the occurrence of clone replacements were calculated in relation to courses of IV antimicrobial agents. Of 65 patients chronically infected with *S. aureus*, 37 received 139 courses of IV antimicrobial agents with activity against *S. aureus* (mean duration, 15 days; range, 6–31 days). Administration of IV antibiotics increased the time to the next sample with growth of *S. aureus*: the mean interval between two positive samples was 68 days if IV treatment had been administered, in contrast to 49 days if no IV treatment had been administered (p 0.003). When *S. aureus* recurred in sputum after IV treatment, the isolate belonged to a different clone in 33 of 114 (29%) intervals, in comparison with 68 of 232 (29%) intervals where IV treatment had not been prescribed (OR 0.98, 95% CI 0.60–1.61). In conclusion, we show that 2 weeks of IV antimicrobial treatment can significantly suppress chronic staphylococcal infection in CF, but is not associated with the eradication of persistent bacterial clones.

Keywords: Antistaphylococcal therapy, colonization, fusidic acid, persistent bacterial clones, *spa* typing
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Introduction

Cystic fibrosis (CF) is a multi-organ disease characterized by dysfunction of exocrine glands. The most serious threat to this group of patients is progressive lung destruction elicited by a vicious cycle of bacterial colonization, infection, and inflammation [1]. *Staphylococcus aureus* is the most commonly isolated pathogen in respiratory tract secretions from young CF patients, often being replaced later by *Pseudomonas aeruginosa* [2–4]. The high prevalence of *S. aureus* in respiratory tract secretions from CF patients has led to different strategies for antimicrobial therapy, including monthly surveillance cultures combined with culture-directed antimicrobial therapy [5], the use of long-term antistaphylococcal therapy [6,7], and treatment depending on clinical symptoms [8].

When *S. aureus* is repeatedly cultured from a patient's specimens for a prolonged period of time, the patient is categorized as chronically infected. Cultivation of *S. aureus* from chronically infected patients may terminate for reasons that are incompletely understood; it may be related to competition between *S. aureus* and a new pathogen, such as *P. aeruginosa*, for the ecological niche in the lungs [9].

Studies examining persistence and strain replacement in CF patients have shown that the same clone of *S. aureus* may be recovered from individual patients for years; however, recovery of interspersed, solitary clones is common [10–12]. These studies differ in patient characteristics, typing methods, and sampling frequencies, and do not provide data on the antibiotic regimens employed or assess the impact of antimicrobial therapy.

It is not known whether intensified treatment with antimicrobial agents causes eradication of *S. aureus* clones and subsequent re-infection by new clones. To elucidate this, we characterized the colonization dynamics of *S. aureus* by *spa* typing isolates from a frequently sampled cohort of CF patients attending a single centre with a relatively strict antibiotic prescription policy.

Materials and Methods

Patients and sampling of respiratory tract specimens

Samples were collected from CF patients attending the CF centre at Aarhus University Hospital from January 2009 to December 2010. Most patients were seen monthly, and cultures were performed on sputum or, if the patient was unable to produce sputum, on airway secretions obtained by laryngeal aspiration. A total of 2526 respiratory tract samples from 154 patients, 87 males and 67 females, with an age range from 0 to 44 years were investigated (range, 1–33 samples per patient).

Identification and antimicrobial susceptibility testing of *S. aureus*

Samples were semiquantitatively plated on 5% horse blood agar, chocolate agar, MacConkey agar, Sabouraud agar (Statens Serum Institut (SSI), Copenhagen, Denmark), and *Burkholderia cepacia* selective agar (bioMérieux, Paris, France). A tentative identification of *S. aureus* was confirmed by use of an agglutination kit (Slidex Staph-PLUS; bioMérieux, France); colonies with atypical reactions in the agglutination kit were identified by the tube coagulase test (SSI), or by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing of all strains was performed by the disk diffusion technique according to the Swedish Reference Group for Antibiotics guidelines (www.srga.org) until November 2009, and thereafter according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (www.eucast.org). All isolates of *S. aureus* were stored at −80°C in 10% glycerol for subsequent typing.

spa and *agr* typing

The polymorphic X region of the staphylococcal protein A gene (*spa*) was amplified with the primers spa-1113f and spa-1514r, as previously described [13]. Sanger sequencing of PCR amplicons was performed at an external facility (GATC Biotech AB, Konstanz, Germany) by use of the forward primer. *spa* types were assigned by use of Ridom StaphType software (Ridom, Würzburg, Germany) [14]. Electropherograms were visually inspected if a change in *spa* type was encountered, and sequencing was repeated on both strands if the shift could be questioned. As shifts in *spa* types can represent mutational change rather than clone replacement [13], application of the BURP (Based Upon Repeat Patterns) algorithm implemented by the Ridom software was used to combine *spa* types into BURP clusters [15]. Accessory gene regulator (*agr*) typing was performed by amplifying the *agr* alleles I, II, III, and IV, as previously described [16].

Definition of chronic infection, clones, and short-term clone replacement

Chronic infection was defined as the isolation of the bacterium in question from more than half of the cultures during a calendar year, provided that the patient had been affiliated with the centre throughout the year and delivered four or more samples (modified 'Leeds criteria' for chronic infection with *P. aeruginosa*) [17]. *S. aureus* isolates from individual patients were considered to be clonally related if they belonged to the same BURP cluster. Solitary clones were recovered only once from individual patients during the study period, whereas persistent clones were detected two or more times. A short-term clone replacement was defined as the culture of an *S. aureus spa* type that differed from the previous isolate from the same patient (after adjustment by the BURP algorithm).

Antibiotic prescription policy and antistaphylococcal treatment regimens

Antibiotic treatment of *S. aureus* in the two Danish CF centres is based on monthly surveillance cultures, and the policy entails patients with positive cultures of *S. aureus* being treated regardless of symptoms [5,18]. The standard regimen consists of 2 weeks of oral treatment with dicloxacillin and fusidic acid, which is modified according to antimicrobial susceptibility testing. In the case of severe clinical deterioration attributed to staphylococcal infection, the patient is treated with intravenous (IV) dicloxacillin or cefuroxime for 2 weeks; the regimen is initiated in the outpatient clinic, and thereafter administered at home. In recent years, a number of chronically infected patients have been treated with IV cefuroxime at fixed