

Original Article

Prevalence and impact on FEV₁ decline of chronic methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in patients with Cystic Fibrosis

A single-center, case control study of 165 patients

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3 May 2011; 11 August 2011; 14 August 2011

Available online 9 September 2011

Abstract

Background: Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) in Cystic Fibrosis (CF) and the impact on CF disease progression are still under debate.

The objectives of this study were to determine clinical variables associated with MRSA colonization and examine impact on FEV₁ evolution in CF patients.

Methods: A retrospective case–control study using the University Hospital of Brussels CF clinic patient registry from 2002 to 2010, comparing clinical variables and decline of FEV₁ of MRSA positive patients with age and sex matched controls, chronically colonized with *S. aureus*.

Results: Thirty of the 165 CF patients, chronically colonized with *S. aureus*, had cultures positive for MRSA (18.2%). Excluding patients under 4 years, the prevalence became 15.2% (23/151). Chronic colonization (i.e., three or more consecutive positive cultures) was found in 19/151 (12.6%).

The MRSA positive group showed a higher proportion of patients with genotype F508del, less pancreas sufficient patients, more bronchiectasis and more frequent hospitalization.

The FEV₁ recorded one year prior to, and at the moment of MRSA acquisition, was lower but not significantly different from that obtained in controls (72.9%±26.6 vs 84.3±21.8 and 68.2%±27.1 vs 81.4%±24.3 respectively, $p>0.1$). However, FEV₁ decline over 2- and 6-year periods, were significantly greater in the chronic MRSA group than in the controls (−5%±5.5 vs −2.5±2.3 over 2 years ($p=0.043$) and −1.8%±4.6 vs −1.0%±1.9 over a 6-year period ($p=0.026$)).

Conclusion: In our center the prevalence of MRSA in CF patients, chronically colonized with *S. aureus* and over the age of 4 years, was 15.2% (12.6% chronic infection). MRSA colonization was shown to be associated with a genotype F508del, presence of bronchiectasis and hospitalization. Our spirometric data also show that a MRSA episode entails an FEV₁ decline that is almost double that predicted for CF patients who can remain unaffected by MRSA.

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Keywords: Bronchiectasis; Cystic fibrosis disease progression; MRSA infection; FEV1 decline

1. Introduction

Cystic Fibrosis (CF) remains one of the most common lethal autosomal recessive disorders. Repeated lung infection results in

progressive respiratory damage, which almost inevitably leads to respiratory failure and death. With increasing survival due to improvements of care, an increased frequency of pulmonary infections with new and resistant pathogens has been identified [1]. In particular, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in respiratory cultures of CF patients has increased over the past decade. Previous reports have documented the prevalence of MRSA in CF patients, some

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reporting as little as 3% but ranging up to 22% [2–9]. A Belgian national survey performed in nine CF centers found a prevalence of MRSA colonization of 5% (range 0% to 17%) [10].

Little is known about the risk factors for acquisition of MRSA; and the impact of chronic MRSA colonization on lung function and exacerbation rate remains uncertain. On the one hand, the presence of MRSA in any given patient could be an indication of more severe lung disease, making the patient more prone to MRSA infection. This predisposition to MRSA infection could be related to one or several aspects of disease severity in CF, such as presence of bronchiectases and/or grossly impaired lung function. On the other hand, the possibility of enhanced lung function decline subsequent to MRSA colonization remains controversial. Previous studies have failed to reach consensus on these issues of risk and impact of MRSA in CF [2,3,5,6,11,12]. Very recently, Dasenbrook et al. [11] showed a worse survival in patients with MRSA colonization of the respiratory tract, even after controlling for several factors associated with severity of illness.

To understand the risk factors for acquisition of MRSA in CF patients will aid the expansion of effective preventive strategies. Hence, we conducted a retrospective case–control study, to first assess prevalence of MRSA in a cohort of patients, chronically colonized with *S. aureus*, having visited our CF clinic over the past decade. The second goal was to characterize these patients in terms of various aspects of disease severity at the time of MRSA diagnosis, and compare those to matched CF controls, chronically colonized with *S. aureus*, but who never acquired MRSA. The third goal was to investigate short and long term evolution of spirometry in the chronic MRSA group, considering an observation period starting from one year prior to MRSA acquisition.

2. Patients and methods

We conducted a retrospective case–control study using the patient registry from the CF Clinic of the University Hospital of Brussels spanning the years 2002–2010 (a total of 247 patients were followed-up in our center in this time period). Approval was obtained from local ethics committee. From the 2002–2010 patient registry, all CF patients known with *S. aureus* positive cultures were retrieved. MRSA status was determined from respiratory tract cultures (obtained from sputum or throat swabs). Chronic infection was defined as having three or more MRSA cultures during at least 6 months follow-up. From this MRSA subgroup, all patients under the age of 4 were excluded to avoid unreliable spirometric results for the purpose of this study. A control group was then sought from the same pool of CF patients with *S. aureus* sensitive to methicillin, and matching each MRSA patient for gender and age. We also verified that all relevant data for each age and gender matched control patient were available for a time point which differed by no more than 2 months from the corresponding time of MRSA diagnosis in the MRSA group.

We determined the patient's body mass index (BMI), retrieved all relevant data on genotype, pancreatic function (where pancreatic insufficiency was defined by pancreatic enzyme treatment and fecal elastase below 15 $\mu\text{g/g}$ feces), and presence of CF related diabetes (CFRD) mellitus for all patients (or equivalent

time point in the control group). We also inspected the patient files for *Pseudomonas aeruginosa* colonization and bronchiectasis already present at the time of MRSA diagnosis. Finally, we checked whether patients had been hospitalized in the year preceding MRSA diagnosis. None of the patients were chronically colonized with *Burkholderia cepacia* complex. Transplant and death in the period subsequent to MRSA (or equivalent time in controls) was also documented.

In order to quantify short and long term longitudinal variations in spirometry, we considered FEV₁ measurements along the following timeline: 1 year prior to MRSA diagnosis, at time of MRSA diagnosis, 1 year after MRSA diagnosis, and after maximal follow-up time (and equivalent times in the control patients). Note that maximal follow-up time differed between patients for a variety of reasons, such as: lung transplant, death, or patients having acquired MRSA towards the end of the registry period under consideration.

2.1. Statistical analysis

All data were analyzed with non-parametric tests, using MedCalc (v10.4; Mariakerke; Belgium). Mann Whitney U tests were performed to test for differences in BMI and FEV₁ between MRSA and control groups. The significance of any changes in FEV₁ within each group was tested with Wilcoxon tests. Categorical parameters (CFRD, pancreatic function, bronchiectasia, genotype, chronic *P. aeruginosa* infection) were examined using the Fisher exact test. Statistical significance was accepted at $p < 0.05$.

3. Results

From the 2002–2010 CF patient registry, 165 patients could be retrieved on basis of chronic *S. aureus* colonization. Of these 165 patients (age range 4–57 years), 30 (18.2%) were identified as having respiratory cultures positive for MRSA at some point in time. When excluding all patients younger than 4 years for the purpose of the present study, the CF population with *S. aureus* decreased to 151 patients, and MRSA prevalence in this subgroup became 15.2% (23/151). Of the 23 CF patients constituting the MRSA group, 19 had chronic infection (three or more MRSA cultures during at least 6 months follow-up); thus, prevalence of chronic colonization in the patient population aged over 3 years amounted to 12.6%. This subgroup of 19 patients with chronic MRSA infection was used for further analysis.

Table 1 summarizes the characteristics of these 19 patients. While the control patients were matched to the MRSA patients only for age and gender, body mass index turned out to not be significantly different between both groups either. The most apparent differences between the groups were: a higher proportion of patients in the MRSA group of genotype F508del (c(1679+94–2619+986del8118)), none of the MRSA patients were pancreas sufficient, and more MRSA patients had bronchiectasis. Although we saw a higher proportion of CFRD and chronic *P. aeruginosa* infection, the observed differences between the two groups, did not reach statistical

Table 1
Characterization of all patients with chronic MRSA and a group of age – gender matched controls.

	MRSA group n=19	Control group n=19	P value
M/F	9/10	9/10	
Mean age	20.3±12.3	20.3±12.3	>0.1
Body mass index	18.4±3.0	20.3±5.1	>0.1
Pancreatic insufficiency	19	13	0.020
CFRD	7	4	>0.1
<i>Pseudomonas aeruginosa</i>	12	8	>0.1
F508del	15	8	0.045
Bronchiectasis	19	12	0.008
Hospitalization the year preceding MRSA	15	8	0.045
Death after MRSA diagnosis	2	0	>0.1
Transplant after MRSA diagnosis	2	2	>0.1
FEV ₁ one year prior to MRSA diagnosis	72.9% ±26.6%	84.3% ±21.8%	>0.1
FEV ₁ at MRSA diagnosis	68.2% ±27.1%	81.4% ±24.3%	>0.1
FEV ₁ 1-year post MRSA diagnosis	65.2% ±23.2%	80.6% ±24.8%	0.016
Short term FEV ₁ change (over 2 years)	–5.0% ±5.5%	–2.5% ±2.3%	0.043

significance. Over the course of the year prior to diagnosis, patients from the MRSA group had been subject to hospitalization more frequently than the control group.

Despite the fact that average FEV₁ one year prior to MRSA diagnosis, and average FEV₁ at the moment of MRSA diagnosis, were more than 10%pred lower in the chronic MRSA group compared to the control group, the FEV₁ differences between both groups did not reach statistical significance (72.9% ±26.6 vs 84.3±21.8 and 68.2%±27.1 vs 81.4%±24.3 respectively; $p>0.1$). However, one year after MRSA diagnosis, FEV₁ was significantly lower in the chronic MRSA versus control group (65.2%±23.2 vs 80.6%±24.8; $p=0.016$).

The short and long term decline in FEV₁ can be better appreciated from Fig. 1, where individual time-course is plotted versus actual time prior to, or following, MRSA diagnosis ($t=0$ in the plot). In order to cancel out FEV₁ inter-subject variability in either group at baseline (considered here as one year prior to MRSA diagnosis), data are represented as an individual change in FEV₁ with respect to each patient's baseline value, i.e., ΔFEV_1 (in %predicted) equals FEV₁ obtained at any point in time, minus FEV₁ obtained one year prior to diagnosis. Solid lines are the short term changes in the 2-year time frame spanning one year before to one year after MRSA diagnosis. Within each group, Fig. 1 shows considerable heterogeneity of FEV₁ change between one year prior to and the time of diagnosis. The short term FEV₁ change, being the FEV₁ decline per year in the 2-year time frame around MRSA diagnosis, was –5.0%pred per year in the chronic MRSA group, versus only –2.5%pred per year in the control group ($p=0.043$; Mann Whitney U test).

From visual inspection of the time evolution of FEV₁ recorded over periods exceeding 1.5 years after MRSA diagnosis (dashed lines in Fig. 1), it can be seen that overall decline is greater in the MRSA group. Average time interval over which longer

term FEV₁ changes could be considered was 6.1 ± 2.1 (SD) years in the MRSA group and 6.0 ± 2.1 (SD) years in controls. When computing individual long term FEV₁ change as the FEV₁ decline per year in the time frame spanning one year before to the longest follow-up FEV₁ measurement on each patient, long term FEV₁ decline amounted to –1.8%pred per year in the chronic MRSA group, and –1.0%pred per year in the controls; the rate of FEV₁ decline in the chronic MRSA group versus control was significant ($p=0.026$; Mann Whitney U test).

None of the MRSA positive patients received eradication treatment during the study period. Nevertheless, when hospitalized for infectious exacerbation, the patients received standard anti-MRSA treatment (i.e. glycopeptides as vancomycin 1 g twice daily intravenously or linezolid 600 mg twice daily orally in case of vancomycin resistance or intolerance). Until now we did not use decolonization therapies (in case of nasal carriage) with topical antibiotics to reduce the risk of self-infection or transmission in our center.

Over the course of the observation period two patients died of respiratory insufficiency, both of these in the MRSA group (indicated by crosses in Fig. 1A). Furthermore, in both groups two patients underwent bilateral lung transplantation (indicated by open circles in Fig. 1A and B).

4. Discussion

The prevalence of MRSA positive cultures in our population, known with *S. aureus* colonization, was 15.2%; and chronic colonization (i.e., three or more consecutive cultures) was found in 12.6% of our population. It has been observed that MRSA prevalence in CF has increased in recent years, from 0.1% in 1995 to 22% in 2007 according to one report [7], and that prevalence is greater in CF patients over 18 years old (16%) than in younger patients (10%) [6]. It must be highlighted that the 22% prevalence noted in the Annual Report of the Cystic Fibrosis Foundation is derived from a different population than in our study (whole group of US Cystic Fibrosis patients, not only those colonized with MSSA) [7]. The 13% chronic MRSA prevalence value found here in CF population chronically colonized with *S. aureus* ranging 4–57 years, is in line with those prevalence values reported by others that relate to observation periods after 2000. This prevalence ranged 10–19% [5–7,13] as opposed to much smaller numbers in studies examining CF patients prior to 2000, showing a rather consistent 3% [2,3,14]. There is one report of a similarly low prevalence despite being derived from a recent CF population (3%), but the patient characteristics of this study are not specified [13]. A Belgian national survey, performed in nine specialized CF centers (including ours), found widely varying prevalences from 0 to 17% [10]. Surely, variability in prevalence numbers will also be influenced by local hospital policies (e.g., use of antibiotics, eradication protocols, segregation measures, barrier nursing), and geographic differences in antibiotics susceptibility.

As could be expected, acquisition and chronic colonization of MRSA was associated with hospitalization in the year previous to the MRSA infection (Table 1), as also shown by Nadeslingam et al. [15] and Ren et al. [6]. Although MRSA is still mainly

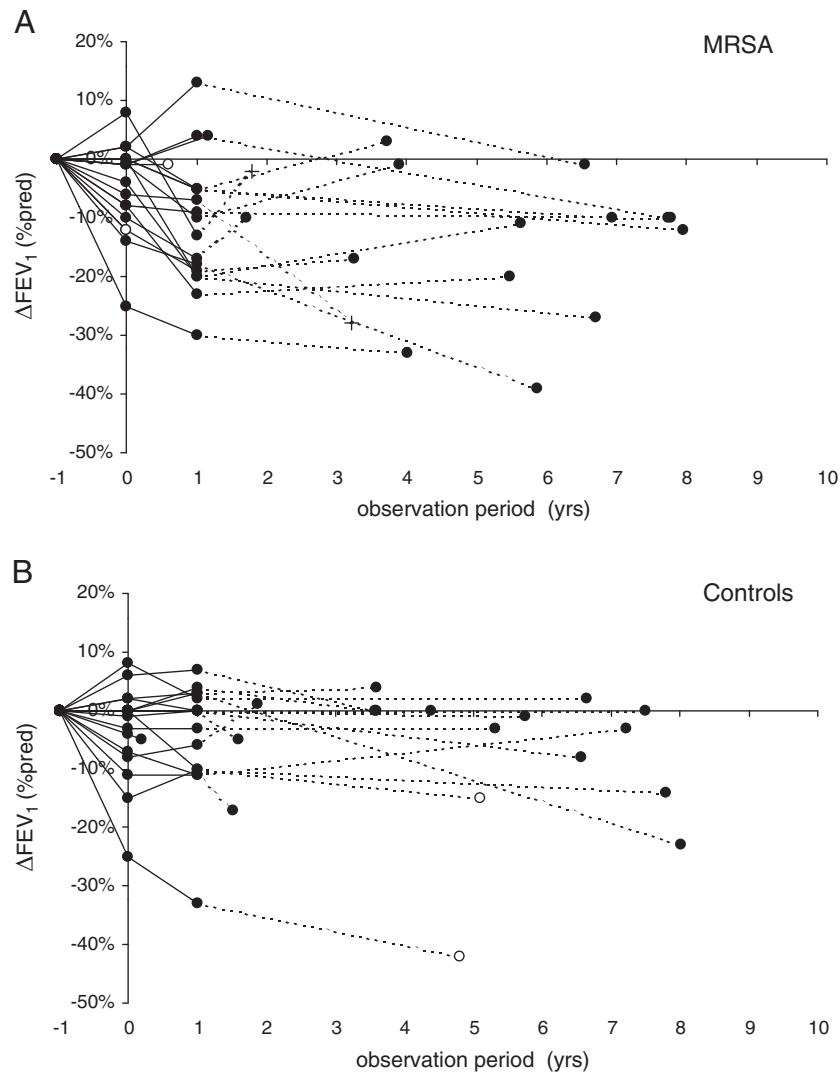


Fig. 1. Short and long term evolution of each patient's FEV₁ change versus individual baseline FEV₁ in the chronic MRSA group (panel A) and controls (panel B) (see text for details). All data points are plotted versus actual time prior to, or following the time of MRSA acquisition (which corresponds to t=0). Solid lines show the short term changes in the 2-year frame spanning one year before to one year after MRSA diagnosis. Dotted lines connect the 1-year post MRSA values with those obtained after the longest follow-up period available for each patient. Open circles and crosses indicate patients who underwent bilateral lung transplantation or died after the last FEV₁.

perceived as a hospital acquired infection, there are a number of different strains of MRSA. Some are known to cause outbreaks in hospital setting, referred to as Epidemic MRSA or EMRSA. Community-acquired MRSA (CA-MRSA) is defined as any MRSA acquired by a person who has had no contact with hospitals or has no other healthcare-associated risk factors. Yet, this does not exclude the possibility that this strain is in fact a hospital strain that has been spread between people in the community. Therefore a molecular definition of CA-MRSA is more reliable to confirm its origin. The *mecA* gene is carried on a mobile genetic element, the Staphylococcal Cassette Chromosome (SCC) *mec*. If the strain is SCC *mec* type IV or V and is genetically unrelated to known hospital strains it is classified as CA-MRSA (Kluytmans-VandenBergh and Kluytmans) [16,17]. Recent new strains from the community have emerged, typically expressing the virulence factor, Pantone-Valentine Leucocidin (PVL) and

causing fatal necrotizing pneumonias [18]. We did not perform molecular analysis on the MRSA strains used in this study.

Our CF patients in the MRSA group were also characterized by two factors which clearly distinguished them from the control patients: genotype F508del and the presence of bronchiectasis (Table 1). It could be argued that both are associated with more severe lung disease. Indeed, patients with the F508del genotype generally present with a more severe CF phenotype [19]. The presence of bronchiectasis is also a known marker of more severe lung destruction, possibly making these patients more at risk for colonization with resistant bacteria. Our patients known with chronic *S. aureus* colonization are treated routinely with narrow spectrum antibiotics, such as flucloxacillin, whenever *S. aureus* grows from respiratory cultures.

In summary of the clinical findings, we could state that time spent in hospital, genotype, pancreatic insufficiency and pre-

existing bronchiectasis are identified here as possible risk factors for acquisition of MRSA. Although we also saw a higher proportion of CFRD and chronic *P. aeruginosa* colonization, the observed differences between the two groups did not reach statistical significance.

In the past, several studies have examined possible risk factors for MRSA acquisition, yet have failed to draw firm conclusions, possibly because of the underlying variability in patient characteristics and the time periods under study. Boxerbaum et al. [2] found no differences in clinical score or hospitalization frequency between CF patients with or without MRSA. Miall et al. [3] found no difference in clinical score or use of antibiotics in the year prior to MRSA infection, but did obtain a statistical significant difference in radiological score and use of antibiotics in the following year after acquisition of MRSA (this cohort consisted of children under the age of 16 years). Giron et al. [5] showed no difference in pancreatic function, clinical or radiological scores between the patients with or without MRSA, but noted significantly more exacerbations in the year prior to the infection. Finally, Ren et al. [6] demonstrated more hospitalization and use of antibiotics in the year prior to the infection, and also observed a significantly lower FEV₁ in the MRSA infected patients (of the order of 10%pred lower). The magnitude in FEV₁ differences is very similar to the differences in average FEV₁ observed here between MRSA and control groups (Table 1), which did not reach statistical significance one year prior to, nor at the time of, MRSA diagnosis.

We did not observe a statistical significant difference in FEV₁ values between MRSA and control groups one year prior to MRSA infection, which may not be surprising given the heterogeneity of the CF patient population in general in terms of FEV₁ and the small number of patients. Hence, on basis of our results in a study population that was limited in number, it seems unlikely that FEV₁ will provide any predictive value of which CF patient is more likely to get infected with MRSA. There was a considerable heterogeneity in FEV₁ change between one year prior to and the time of diagnosis. However, one year after MRSA diagnosis, FEV₁ had become significantly lower in the chronic MRSA versus the control group. We therefore quantified the rate of FEV₁ decline, which is an important outcome measure because it is closely related to morbidity and mortality in CF [20].

The impact of chronic MRSA infection on FEV₁ has also been studied by Dasenbrook et al. [12], who suggested that it is associated with a more rapid decline in FEV₁ in patients aged 8–21 years. Determined over an average follow-up period of 5.3 years, the rate of FEV₁ decline was 2.1%pred per year in children with chronic MRSA and 1.4%pred per year in controls. These data can be directly compared to our long term FEV₁ declines determined over 6 years, i.e., 1.8%pred per year in the chronic MRSA group and 1.0%pred per year in the control group. In fact, we observed here that both the short term and long term FEV₁ decline, determined over respectively 2 and 6 years, were significantly greater in the chronic MRSA group. Dasenbrook et al. could also demonstrate a clear association between MRSA and worse survival in patients with CF, even after controlling for several factors

associated with disease severity [11]. Taken together our results suggest an association between chronic MRSA colonization and lung function decline.

The degree of FEV₁ decline found in chronic MRSA patients of the present study clearly indicates that the pathogenesis by which MRSA could lead to more rapid lung destruction deserves more study. Staphylococci are known to be associated with virulence factors, including membrane-damaging toxins and invasins that promote bacterial spread in tissues. In one study, only the PVL+ MRSA was associated with a higher rate of decline of FEV₁ [18]. An important limitation to our study is that we did not perform molecular analysis on our MRSA strains, hence we could not differentiate between hospital-acquired and community-acquired MRSA.

Given the impact of MRSA on lung function decline and survival, it seems reasonable to support the need for all CF centers to adopt a policy of segregation, infection control and eradication. In 2008 the Cystic Fibrosis Trust published a comprehensive report with specific recommendations for the management of MRSA in CF centers including addressing segregation, hygiene and surveillance [7]. Segregation and eradication have been shown to be successful in keeping the prevalence of MRSA infection low [21], and minimize the likelihood of cross-infection. None of the MRSA positive patients under study received eradication treatment, but they did receive standard anti-MRSA treatment (vancomycin intravenously or linezolid orally) when they were hospitalized for any infectious exacerbation. We also obtain surveillance samples to detect carriers of MRSA and use segregation methods to minimize transmission of MRSA between patients. Patients also have well-ventilated single rooms with their own nebulizer compressor system and oxygen delivery supplies.

Until now, eradication regimens to clear the MRSA positive cultures are not routinely performed in our CF center. Based on our findings and other recent reports it is probably justified to start eradication therapy as soon as MRSA grows from respiratory cultures.

To the best of our knowledge this study, based on data from the CF center of the University Hospital of Brussels, is the first European study on this matter. We recognize the limitation that our data originate from a relatively small sample of CF patients and were collected retrospectively. Hence, generalization of these results should be handled with caution, also because strains and prevalence of MRSA may vary between countries. This study may also have been affected by some biases that should be taken into account when comparing with other studies. For instance, the two patient groups were matched for chronic *S. aureus* colonization, age and gender; but not for several other potential risk factors such as pancreatic insufficiency, genotype, chronic *P. aeruginosa* colonization, or CFRD. Concerning those last two variables, we found no statistically significant difference between the MRSA and the control group; but still these characteristics could play a role in the outcome on lung function parameters. Misclassification of patients was avoided by strict inclusion criteria (multiple MRSA negative cultures in the year before entering the control group), and microbiologic laboratory misclassification was avoided by using

selective media for *S. aureus* cultures. Finally, as mentioned before, we did not perform molecular analyses on the MRSA strains. To fully delineate the impact of MRSA colonization on lung function, larger prospective longitudinal studies are needed, which also link molecular characteristics of MRSA to clinical outcomes.

In conclusion, we retrospectively analyzed 165 CF patients with chronic positive *S. aureus* cultures over an 8-year period to assess the effect of MRSA infection in CF on lung function. Chronic colonization of MRSA amounted to 12.6%, and MRSA acquisition was shown to be associated with time spent in hospital, pancreatic insufficiency, genotype, and pre-existing bronchiectasis. In addition, we observed consistent trends of FEV₁ decline over 2- to 6-year time intervals in MRSA infected CF patients, indicating that an episode of MRSA can entail a FEV₁ decline that is almost double that predicted for CF patients who can remain unaffected by MRSA. The present results prompt us to support the need for a more pro-active policy of MRSA management of CF patients which may include enhanced segregation, infection control and eradication.

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