**TITLE:** Cystic fibrosis related diabetes is not independently associated with increased *Stenotrophomonas maltophilia* infection: longitudinal data from the UK CF Registry

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**Abstract**

Introduction:

*Stenotrophomonas maltophilia* is common in the sputum of people with cystic fibrosis related diabetes (CFRD), raising the question as to whether this is a risk factor for its acquisition. We investigated this at a population level.

Methods:

We analysed national Cystic Fibrosis Registry data 2011-2015 for 8047 people with CF >age 6 years, looking at demographics, diagnosis of CFRD, lung function and sputum microbiology; using descriptive and multivariate strategies to establish independent predictors *for* *S.* maltophilia culture and associated outcomes.

Results:

*S. maltophilia* was present in 1148 (14.1%). Although univariate analysis confirmed it was more prevalent in those with CFRD, when adjusted for other clinical parameters there was no longer a relationship. Markers of more severe lung disease were independent risk-factors for *S. maltophilia*.

Conclusion:

Although *S. maltophilia* is more common in people with CFRD, it is not an independent risk-factor for *S. maltophilia* acquisition.

# Introduction

Treatment for people with cystic fibrosis (CF) has advanced dramatically over the last few decades and as survival improves the spectrum of organisms implicated in pulmonary infection is also shifting. The increasing prevalence of organisms such as *Stenotrophomonas maltophilia, Achromobacter spp,* and Non-Tuberculous *Mycobacteria spp* (NTM) has led them being grouped together as potential “emerging pathogens”. (1)

*S. maltophilia* (previously known as *Pseudomonas maltophilia and Xanthomonas maltophilia*) is a Gram-negative, obligate aerobic non-fermenting rod with intrinsic multi-drug resistance. (2) Its prevalence in people with CF varies from centre to centre but has been reported as high as 31%. (3) The clinical significance of *S. maltophilia* remains controversial as no clear evidence for more rapid pulmonary decline following *S. maltophilia* acquisition has been found. However, strains isolated from people with CF have increased biofilm forming ability and a specific immune response has been observed and associated with increased exacerbations, suggesting *S. maltophilia* has potential to be more than a simple coloniser of the lungs in CF. (4-6)

It is therefore of interest to understand which factors affect the acquisition of *S. maltophilia* and recently CF-related diabetes (CFRD) has been implicated. Lehoux Dubois *et al* (7) reported increased *S. maltophilia* in respiratory samples of patients with dysglycaemia compared to their normoglycaemic comparators. CFRD is modifiable and optimisable and therefore any potential association with *S. maltophilia* warrants further investigation. Equally, should an association between CFRD and *S. maltophilia* exist; *S. maltophilia* “first growth” would dictate further investigation for CFRD. With this in mind, we sought to use data from the national UK CF Registry to investigate the relationship between CFRD and *S. maltophilia*.

# Methods

We utilised the UK Cystic Fibrosis Registry which collects pseudo-anonymised longitudinal data from over 10000 people with CF in the UK. With patient consent, data are inputted annually at every UK specialist CF centre including baseline demographics, co-morbidities, spirometry, blood test results, medications, intravenous antibiotics days and respiratory sample microbiology results. The registry has excellent coverage of the CF population in the UK (> 99%). The UK CF Registry Research Steering Committee approved the application and subsequent release of data for this study.

## Data extraction

Data were extracted for all 10552 individuals with records in the registry for the study period 2011-2015 to look for an association between CFRD and *S. maltophilia,* where those with any growth in the study period were considered to bepositive (SM+).

## Missing data and standardisation

Although missing data accounted for only 5.9% of all data points, it occurred more frequently in those ≤6 years age than the remainder (1.7 vs. 0.55 missing data-points per person respectively), so the younger age group were removed from the analysis. In the remaining 8047 individuals missing data accounted for only 3.6% of all data-points (see table 1). Hba1c values were standardised by conversion to the International Federation of Clinical Chemistry (IFCC) reference as per published formulae. (8) Hba1c values were missing in the majority of patients and we therefore did not include Hba1c in any multivariate analyses. BMI (adults) and BMI percentile (children) are automatically calculated by the registry based on the height and weight entered. In order to include BMI in a population wide multivariate analysis, we standardised adult and paediatric BMI into “underweight”, “normal”, “overweight” and “obese” based on WHO criteria. (9). The registry does not formally define diagnostic criteria for CFRD and therefore for the purposes of the study a diagnosis of CFRD was considered to be made if the local CF team had indicated accordingly in the annual review proforma. During the study period UK guidelines recommended CFRD was a clinical diagnosis with OGTT and/or home blood glucose monitoring taken into consideration. (10) Lung function was taken as the % predicted forced expiratory volume in 1 second (FEV1) recorded at the time of the annual review, calculated using the Global Lung Initiative reference formulae. (11)

## Statistical analysis

Categorical and dichotomous variables were summarised as absolute number and percentages and non-normally distributed continuous data as median and interquartile ranges (IQR). The prevalence of missing data in the registry for baseline and clinical measurements reported. Where categorical comparisons were made between groups the χ2 test was used; for similar comparisons between continuous variables, the Wilcoxon rank-sum test was used.

To examine the relationship between patient demographics and risk-factors with *S. maltophilia*, a logistic regression model for the presence of *S. maltophilia* was created. A purposeful model selection strategy was used to fit the final model. Variables were selected for inclusion in the model when univariate *p-*value was <0.2 or where there was biologic plausibility. Continuous variables age, FEV1, IV days were separated into clinically meaningful dummy variables so that each strata of the variable could be assessed independently. The final model included the following independent demographic and risk-factor variables: age <18 years, female gender, underweight BMI, overweight BMI , obese BMI, CFRD, pancreatic supplements, FEV1 50-79 % predicted, FEV1 <50 % predicted, Phe508del homozygous genotype, ‘other’ genotype, >14 IV days, presence of *S. aureus*, *P. aeruginosa*, *Aspergillus* spp. or *Burkholderia cepacia* complex (BCC).

Statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC). In all cases, *p*<0.05 was considered statistically significant. Categorical data are presented as count (%), averages are presented as mean (SD) or median (IQR) as appropriate depending on normality. Odd ratios are presented with 95% confidence intervals. Unadjusted *p*-values are presented throughout.

# Results

Patient characteristics

Cohort demographics are presented in table 1. Briefly, mean ± SD age was 22 ± 14 years, mean FEV1 76.0 ± 21.1 %predicted, 1815 (22.6%) had a diagnosis of CFRD, 3635 (45.2%) had documented *P. aeruginosa* (Pa) growth, and 3736 (46.4%) were female. There were 1138 cases (14.1%) with a growth of *S. maltophilia* in the study period.

*S. maltophilia* rates

*Annual S. maltophilia* prevalence was observed to rise over the course of the study period with a peak in 2014 at 6.9%. Overall those with a documented growth of *S. maltophilia* (SM+) were younger than those without (SM-) (20.2 ± 13.2 yrs vs. 22.1 ± 14.2 yrs, p<0.001) However, *S. maltophilia* prevalence appeared to have a bi-modal distribution with a peak prevalence in adolescence before a trough during early-mid adulthood and subsequent increasing prevalence in those aged 45 and above, see Figure 1.

Univariate analysis

Univariate analysis was performed to assess for plausible associations with *S. maltophilia* growth and the results are presented in table 1. The SM+ cohort had higher prevalence of CFRD (25.0% vs. 22.2%, p=0.03), were more likely to be female (52.0% vs. 45.5% respectively, p<0.001) and demonstrated evidence of more severe disease with poorer lung function (68.6 ± 23.8% vs. 76.0 ± 24.7% predicted FEV1, p<0.001), more annual IV antibiotics days (10 [0-31] days vs. 0 [0-28], p<0.0001), and higher rates of *Pa* colonisation (48.2% vs. 44.7%, p=0.029) than SM- cohort. They also had higher prevalence of *S. aureus* (45.0% vs. 34.9%, p<0.001) and *Aspergillus* spp.(32.9% vs. 9.3%, p<0.001), but overall no significant associations were seen for BCC.

Multivariate

A multivariate logistic regression model was then created to test for independent association between covariates and SM+ (Figure 2). Age <18 years (OR 1.62 [1.38-1.89], p<0.001), female sex (OR 1.24 [1.08-1.42],p=0.002), severe lung function (FEV1 <50% predicted OR 1.35 [1.12-1.64]. p=0.002), pancreatic supplementation (OR 1.352 [1.09-1.67], p=0.006), >14 annual IV days (OR 2.00 [1.73-2.32], P<0.001), *S. aureus* growth (OR 1.57 [1.37-1.80], p<0.001) and *Aspergillus* spp. growth (OR 4.55 [3.89-5.31], p<0.001) were all independently associated with an increased likelihood of *S. maltophilia* growth, whereas BCCwas associated with reduced likelihood of *S. maltophilia* growth (OR 0.64 [0.43-0.94, p=0.02]. However CFRD was not found to be independently associated with *S. maltophilia* growth (OR 1.08 [0.92-1.28]).

Sensitivity analyses

To test the robustness of our findings we performed two sensitivity analyses. Firstly, we performed the same analysis but excluded those individuals where *S. maltophilia* was seen in only one year, thereby only including those with recurrent growths in the SM+ group, See Table S1 and Figure S1. Secondly, we examined whether inclusion of young children <10 years of age (below the age of recommended screening) impacted the outcomes of our primary analysis, See Table S2 and Figure S2. Both sensitivity analyses were consistent with the primary analyses.

# Discussion

We utilised UK CF Registry data to investigate the association between CFRD and *S. maltophilia*. Although higher rates of CFRD occurred in people with *S. maltophilia*, when adjusted for other variables there was no evidence of an independent association.

Increased *S. maltophilia* in people with CFRD has been reported in a number of studies. Stanojevic *et al* (12) found significantly higher rates of CFRD in their SM+ group when investigating factors that influence the acquisition of *S. maltophilia*. Marshall *et al* (13) noted increased *S.* maltophilia in people with CFRD and Vidigal *et al* (14) reported a higher prevalence of CFRD in SM+ subjects. Conversely, a number of studies have not observed a relationship between CFRD and *S. maltophilia*, although the aims of these studies were often targeted towards outcomes of *S. maltophilia* infection rather than its acquisition. (2, 4, 5) All previous studies have been limited by sample size, the largest including only 150 cases with *S. maltophilia* (12).

By utilising national registry data we were able to apply multivariable logistic regression analysis to a large CF population which included over 1000 cases of *S. maltophilia*. Although we confirmed the apparent increased prevalence of *S. maltophilia* in people with CFRD, this association disappeared once adjusted for markers of severe lung disease including poorer lung function and increased IV antibiotic exposure. These findings are in keeping with those recently reported by Lehoux Dubois *et al* (7) but go a step further in suggesting the increased *S. maltophilia* seen in CF people patients with dysglycaemia is associated with their poorer clinical condition rather than the dysglycaemia itself.

In addition to the association between pulmonary function and IV days, we observed associations between *S. maltophilia* and other organisms. For example, we observed increased *S. aureus* and *Aspergillus* spp.in the *S. maltophilia* population and less BCC*.* Interestingly the SM+ population had over 4 times the odds of an *Aspergillus* spp*.* growth than their SM- counterparts and this relationship persisted on multivariate analysis. A similar relationship was previously reported by Marchac *et al* (15) and whilst it could be inferred that a synergism or probiosis exists between the two organisms there is also recent evidence *S. maltophilia* and *Aspergillus* spp.are antagonistic of each other in mixed biofilms. (16)

Although we have shown that younger age was an independent risk factor for *S. maltophilia* growth (those SM+ were on average 2 years younger), there was a bi-modal distribution of *S. maltophilia* prevalence across the age groups and to our knowledge this is the first time this has been reported. A recent study of the US CF registry found older patients to be at significantly higher risk of NTM (17) and hence as life expectancy increases, further work may be required to investigate the risk factors for acquisition of the emerging pathogens and other organisms in the older CF population, which may be different particularly in the presence of a “survivor effect”.

There are several limitations to this study. Firstly, the retrospective observational design means we cannot exclude a confounder that exists outside our model. For example, *S. maltophilia* and *Aspergillus* may each thrive in a particular niche in the lung such as cavities or severe bronchiectasis which we are unable to account for in our multivariate analysis.

Secondly, SM+ individuals not classified as CFRD may still have early glucose handling abnormalities. The registry does not define diagnostic criteria for CFRD and in practice there may be heterogeneity between centres as to how CFRD is classified and/or treated. Lehoux Dubois *et al* (7) noted increased *S. maltophilia* not only in those with CFRD based on the oral glucose tolerance test (OGTT) but also those with impaired glucose tolerance that did not meet the OGTT criteria. Therefore, those with early dysglycaemia (often characterised only by post-prandial glucose excursions and missed by OGTT) (18) may have increased prevalence of *S. maltophilia* but would not be classified as CFRD in the registry. Further work is required to understand the impact of early glucose abnormalities on *S. maltophilia* growth.

Thirdly, if hyperglycaemia were related to *S. maltophilia* acquisition then anti-diabetic agents such as insulin may have an antagonistic effect and given no data is available on insulin prescriptions, dosing, or adherence we cannot exclude a confounding treatment effect.

Fourthly, we excluded children under the age of 6 from our analysis due to increased missing data, and although the generalizability of our results to that cohort is uncertain, CFRD increases with age and is uncommon in young children. Although screening is recommended from the age of 10, many centres screen for CFRD from a younger age, (19) a sensitivity analysis confirmed that our results were robust even when the 6-10 years age-group were excluded.

Finally, the number of annual positive respiratory samples or type of sample that was positive for *S. maltophilia* (and other emerging pathogens) was not recorded in the UK CF Registry during the study period and hence misclassification of those individuals with no sputum samples as SM- may falsely inflate that population and equally the SM+ population may be falsely inflated by one-off growths or misclassification. Indeed, the overall 14.1% prevalence of *S. maltophilia* we report is higher than 9.9% and 10.4% in the historical studies reported by Goss *et al* (20) and Waters *et al* (21) respectively. However, given the increasing detection of *S. maltophilia* globally as well as the introduction of novel technologies such as MALDI-TOF for microbial identification, the prevalence data in our contemporaneous study is unlikely to be significantly inflated. Furthermore, our sensitivity analysis confirmed our primary analysis was not unduly affected by those with just a single year of documented *S. maltophilia* growth.

In conclusion, this is by far the largest study looking at the association between *S. maltophilia* and CFRD, where multiple datasets in many thousands of individuals were compared over a number of years. We have shown that the previously documented association between *S. maltophilia* and CFRD is no longer apparent once adjustment for other clinical parameters has been made.

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