Author’s response to reviews

Title: Air pollution exposure is associated with MRSA acquisition in young U.S. children with cystic fibrosis

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Author’s response to reviews:

Thank you for your detailed and thoughtful review of this manuscript by the Editors and Reviewers. The comments and suggestions provided have helped us to significantly improve the content and style of the manuscript. We have addressed each of the comments as detailed point by point below:

Editor Comments:

I am willing to consider you manuscript for publication. However, I would like you to address the comments raised by the three reviewers (and especially reviewer n. 2). Also see my further comments below. Please submit both a clean version and a version with all the changes done highlighted.

ABSTRACT:

- the term “other” respiratory pathogen/infection may be a typo (page 2, lines 17 and 41)

We have revised the Background section of the Abstract and it now reads, “We recently demonstrated that chronic PM2.5 exposure is associated with an increased risk of initial Pseudomonas aeruginosa acquisition in young children with CF. The purpose of this study was to determine whether PM2.5 exposure is a risk factor for acquisition of other respiratory pathogens in young children with CF.”
- “initial” (line 17) should be placed closer to “acquisition” (line 21).

We have revised the sentence in the Methods section of the Abstract so that “initial” and “acquisition” are closer together; it now reads “We conducted a retrospective study of initial acquisition of methicillin susceptible and methicillin resistant Staphylococcus aureus (MSSA and MRSA), Stenotrophomonas maltophilia and Achromobacter xylosoxidans in U.S. children <6 years of age with CF using the CF Foundation Patient Registry, 2003-2009.”

METHODS

- Page 5, line 47: was the mean annual concentration calculated for the 365 day-period before date of birth (thus including pregnancy duration)? Or did you consider the previous calendar year (e.g. the whole 2002 for a child born on October 2003)?

The mean annual concentration was taken as the previous calendar year and we have clarified this in the Methods section as, “The primary exposure of interest was the mean annual concentration of PM2.5 in the calendar year prior to birth for each child.”

- Page 6, lines 7-12: how many monitoring stations were active throughout the period? How many are not considered because of the exclusion criteria?

We have included the following description of the monitoring stations and those that were active during the study duration in the Methods section, “Over the course of the study, 1574 monitoring stations came on-line and went off-line; only data from those monitors in operation for an entire year with no more than a 45-day gap between measurements, which ranged from 73% of monitors in 2003 to 81% in 2002 and 2007, were utilized for analyses.”

- Page 7, lines 4-12: can you clarify how is time of incident events calculated? Did you assume that an event occurred at the mid-point between two consecutive cultures (a negative and a positive culture)?

Time to incident acquisition was taken as the time interval (not an exact date), defined as the date of positive culture and the date of previous negative culture. Our analysis method (Weibull regression with interval censored outcomes) explicitly accounted for the interval of acquisition as the outcome, precluding the need to “input” a date of acquisition or having to take acquisition as the date of positive culture.
- Page 7, line 41 (and table 3): when mentioning “diagnosis” (age at diagnosis, diagnosis by newborn screening) please report you refer to diagnosis of CF.

We have clarified that that “diagnosis” refers to “age at diagnosis of CF” in both the Methods section and in Table 3 of the manuscript.

- I am wondering whether age at diagnosis and diagnosis by newborn screening are collinear and if you can include both in the same model. How is their joint distribution?

There would in fact be a relationship between age at diagnosis (continuous) and newborn screening (dichotomous) as those diagnosed by newborn screening would be diagnosed at an earlier age (on average) than those not identified by newborn screening, which would include a mixture of time of diagnoses based on a number of factors occurring both post-pregnancy and in early life.

However, this will not impact the interpretation of our models as we were interested in only making inference upon the PM2.5 covariate in the model(s), after adjusting for age at diagnosis and identification by newborn screening (yes/no). Collinear variables and any resultant increase in standard errors of the variable coefficients will not impact the PM2.5 estimates or inference obtained in the models. We would be concerned about potential variance inflation only if we were making inference on either the age at diagnosis of CF or the diagnosis by newborn screening covariates (or both).

RESULTS

- a flowchart would be very useful to describe the selection of subjects for the different analyses; this would also make the number of prevalent cases evident.

We have included a flowchart as Figure 1 that describes the inclusion of subjects for each analysis.

- page 8, line 40: something wrong here? I think your data show that a SMALLER proportion of children were identified by newborn screening among those who acquired the pathogens.

Thank you for noticing this error. Yes, a smaller proportion of patients who were identified by newborn screening acquired each pathogen compared to those that did not acquire during follow-up. We have revised the statement in the manuscript as follows, “Children acquiring each
pathogen were more likely to have severe CFTR mutations and less likely to be diagnosed by newborn screening than those remaining pathogen-free.”

- page 9 and table 3: you should clarify that you study the association between exposure and risk (and not time). Otherwise a RR>1 would be protective, implying an association between greater exposure and longer time free from disease.

We have edited this sentence, to read “Results of the Weibull regression evaluating the association of PM2.5 exposure and risk of respiratory infection are presented in Table 3.”

We have also modified the Abstract to read, “Multivariable Weibull regression with interval-censored outcomes was used to evaluate the association of PM2.5 concentration in the year prior to birth and risk of acquisition of each organism.”

TABLE 1

line 44: I guess that the correct coding of the “residual” group is “children for which ONE allele…” Could you check this?

The CFTR functional clas is defined as follows in the footnote to Table 1: “Severe, both CFTR mutations result in minimal CFTR function (class 1, 2, or 3), including F508 del; Residual, at least one allele with a mutation resulting in partial CFTR function (class 4 or 5); Unclassified, both alleles with unknown functional class, or one allele with minimal CFTR function and the second with unknown functional class.

TABLE 2

“linear” seems not very appropriate. Maybe “Nearest monitor”?

We have replaced “linear” with “nearest monitor” in Table 2, Table 3, the corresponding Table footnotes, as well as, in the Results section.

TABLE 3

“Rural urban commuting area” is not defined among adjustment variables in the Methods.

We have included this variable, its classification and the reference in the Methods section as this variable was included in multivariable analyses, “urban/rural status using the Rural Urban
Reviewer reports:

Reviewer 1: Thank you for the possibility to review the manuscript by Psoter et al.

This is an interesting study, and contributes to the discussion about exposure to pollutants and the increasing susceptibility to respiratory infection in children with cystic fibrosis.

The authors reported in a recent original article a similar association between PM2.5 exposure and risk of Pseudomonas aeruginosa acquisition in the same cohort.

However, I had some concerns as follows:

My minor comments

The authors evaluated the mean annual concentration of PM2.5 in the year prior to birth. This period encompasses the gestational period.

1. There is an association between prenatal exposure to air pollution and prematurity and low birth weight. Also, premature birth and/or low birth weight are considered as risk factors for a worse respiratory outcome later in life in children. Could these aspects influence on acquisition of CF pathogens?

This is a very important point. Air pollution exposure has been demonstrated to adversely affect pregnancy outcomes in different study populations with evidence supporting a causal link between increased air pollution and low birth weight. Unfortunately, information regarding low birth weight is not captured in the Cystic Fibrosis National Patient Registry; therefore, we are unable to explore these potential associations further.

However, the absence of this information should not impact the present study. Although there may be observed associations between both: 1) air pollution exposure and pregnancy outcome and 2) pregnancy outcome and respiratory outcomes, in our analyses we would not want to adjust for pregnancy outcome(s) in the model (i.e. as a confounder) as pregnancy outcome would be in the casual pathway between air pollution exposure and respiratory outcomes.

While these factors may not be confounders in the present study, they may in fact mediate the observed associations found in the present study. To address this issue, we have included the following in the Discussion section, “In the general population, prenatal exposure to air pollution is a risk factor for low birth weight [27, 28], which in turn is a risk factor for adverse respiratory
outcomes in early childhood [29]. In addition, post-natal air pollution exposure has been associated with an increased risk of bronchiolitis and recurrent wheeze in infants and young children [30, 31]. Our findings of increased risk of MRSA acquisition in CF patients may be in part mediated by these same risk factors, particularly since PM2.5 exposure was measured in the year prior to birth.”

2- Would the factors related to pregnancy and perinatal (maternal occupational exposure, maternal education, household incomes, preterm birth …) be associated with CF respiratory infection? Would they be a limitation of the study?

Perinatal factors may be associated with the risk of respiratory infections (outcome) and exposure to PM2.5 (exposure) in young children with CF; thereby, confounding the association between PM2.5 exposure and risk of pathogen acquisition. Similarly, pregnancy outcomes may mediate the observed associations.

We have included the following in the Limitations section of the Discussion, “Third, information on environmental tobacco smoke exposure, preterm birth/low birth weight, or maternal occupational exposures, potentially important variables, were not available.”

3- table 1: age at diagnosis ..month?

We have clarified that the age at diagnosis of CF is in months.

4- table 3: line 40. Change "are" to "were"

We have changed “are” to “were”.

Reviewer 2: Thank you very much for asking me to review this interesting analysis from North America based on the Cystic Fibrosis Patient Registry. The authors are experts in analysis and have contributed significantly to the understanding of links between the environment, climate and air pollution and cystic fibrosis past decade or more.

Overall I think that it is an interesting analysis. I do have some questions for the authors:

1. Biological plausibility. Overall the discussion is tight and interesting however it is unclear to me what the biological plausibility of a link between MRSA and measures of air pollution
and this should be further clarified. Furthermore, extra discussion about the potential links between bacteria, particularly Staphylococcus aureus and MRSA in the general population and climate (if there are any data) would be most useful. This is absent in the discussion as far as I can see.

To address the question of biological plausibility, we have included the following in the Discussion section of the manuscript, “The mechanism by which exposure to PM2.5 may increase the risk of MRSA acquisition among young CF patients deserves further exploration. As there are no data to suggest that components of air pollution directly increase exposure to MRSA or other pathogens, it seems more likely that adverse effects of PM2.5 exposure on the CF airway increases susceptibility to MRSA infection. Limited in vitro studies exist regarding the biologic mechanisms by which exposure to fine particulate matter adversely affects the CF airway. Kamdar and colleagues demonstrated that PM2.5 increases oxidative stress and mitochondrial signaling-mediated apoptosis in CF human bronchial epithelial cells [23] which in turn could increase airway inflammation. Further, Gelser et al. demonstrated higher uptake of inhaled nanoparticles by alveolar epithelial cells and increased inflammatory response of CFTR mutant mice compared to wild type [24]. The effects of gaseous air pollutants on the CF airway are less well understood; however, increased ozone levels have been shown to downregulate CFTR function in human bronchial epithelial cells [25].”

In regards to a link between S. aureus and MRSA and environmental factors, we note that, to our knowledge, no studies have described the association between climatic and environmental factors and MSSA and MRSA nasal colonization or acquisition in the general population. We have included the following in the Discussion section to address this concern, ” Environmental factors are associated with approximately 50% of the population variability in lung function [32] and P. aeruginosa acquisition [33] in CF patients. Many of the CF-related pathogens are naturally occurring in the environment, although few studies have investigated specific environmental factors that may contribute to pathogen acquisition [34]. P. aeruginosa is the most widely studied CF-related bacterium with seasonal variations in acquisition [16] and differential geographic residual relative risk [13] reported. These same factors may contribute to MRSA acquisition in CF patients.”

2. Some discussion about the types of MRSA. I am aware that there has been a significant increase in MRSA rates particularly in children with cystic fibrosis in the U.S. over the past 15 years with prevalence rates approaching ¼ of the population in some CF centres. Are these so called "community acquired" or "hospital acquired" infections and how might this be further analysed as presumably a significant proportion of people with CF in the U.S. acquire MRSA in the hospital environment. How does this link with the biological plausibility?
This is a very important consideration and recently more attention has focused on MRSA acquisition and infection in the CF population. We have greatly expanded our description in the Discussion section as follows, “Chronic infection with MRSA is associated with poorer clinical outcomes and survival in CF patients [17-19]. Of concern, MRSA prevalence among U.S. CF patients has increased steadily from 9% in 2002 to 27% in 2012 [20]. About 70% of MRSA isolates among children with CF in the US are “health care–associated” (SCCmec II) versus “community-associated” (SCCmec IV) strains [21], though the prevalence of SCCmecIV relative to SCCmecII strains has increased over the last decade. Known risk factors for MRSA acquisition in CF patients include colonization with P. aeruginosa [21], more frequent clinic visits [21] and higher mean ambient temperature [22]. To our knowledge, the association of air pollution exposure and risk of MRSA acquisition has not previously been evaluated.

The mechanism by which exposure to PM2.5 may increase the risk of MRSA acquisition among young CF patients deserves further exploration. As there are no data to suggest that components of air pollution directly increase exposure to MRSA or other pathogens, it seems more likely that adverse effects of PM2.5 exposure on the CF airway increases susceptibility to MRSA infection.”

And included the following in the Limitations section, “Fourth, information on MRSA strains (SCCmec type or Panton Valentine Leukocidin status) was unavailable so we were not able to distinguish community vs. healthcare associated strains. It is possible that the risk of MRSA acquisition associated with PM2.5 exposure may differ by subtypes.”

3. Analysis of children who may have relocated during the term of the study. I may have missed this but was there an analysis of children who relocated during the term of the study? This should be explicitly stated that patients were analysed in one place.

The Cystic Fibrosis Foundation Patient Registry includes the zip code of residence collected on a yearly basis, distinct from culture results which are collected at each patient encounter. During follow-up, approximately 10% of children reported a change in zip code of residence. As we were interested in evaluating long-term exposure to air pollution, we did not perform an analysis that considered time-varying PM2.5 exposure. We note that the approach taken in this study is consistent with previous studies in this patient population.

We have included the following in the limitations section that “Furthermore, as air pollution exposure was determined in the calendar year prior to birth, change in zip code of residence during follow-up may not accurately represent long-term PM2.5 exposure.”
4. Limitation of using upper airway sampling techniques. There should be some discussion
about the limitation of using upper airway sampling techniques particularly as
Staphylococcus aureus/MRSA can be resident in the upper airway in healthy individuals.

We agree that there are limitations in the use of oropharyngeal swabs for identification of
respiratory pathogens. We have included the following in the Limitations section of the
manuscript, “Finally, respiratory cultures were performed primarily on oropharyngeal swabs in
the non-expectorating young cohort. The specificity and, even more, the sensitivity of
oropharyngeal cultures in comparison to lower respiratory samples is limited [35]; results of this
study may not be generalizable to lower airway colonization. Nonetheless, oropharyngeal swabs
are standard of care for assessment of respiratory cultures in pre-expectorating patients in the
U.S., and acquisition of pathogens in the upper airway is generally considered an important
clinical outcome”.

5. Parallelism between MRSA and Pseudomonas aeruginosa infection in two separate papers.
   It is interesting that there is a parallelism between MRSA and Pseudomonas aeruginosa
   infection in two separate papers. I am not sure as to how one would approach the analysis
   but it would be interesting to see if it was the same patients that had increased risk of
   P.aeruginosa and MRSA. In the analysis of the two, were the periods overlapping and were
   the same cohorts analysed. This should be explicitly stated at some point in the study.

The study population in this investigation is comprised of a similar group of individuals that
were previously analyzed by our group. However, they are distinct cohorts in that the eligibility
to enter each pathogen-specific analysis were different with prevalent conditions at first
encounter being excluded from analyses. We have clarified the study population by including
Figure 1 which described the analytic population for each of the pathogen-specific analyses and
included the following in the Introduction, “The purpose of this study was to evaluate early life
fine particulate matter exposure and risk of initial acquisition of other CF pathogens, including
methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant Staphylococcus
aureus (MRSA), Stenotrophomonas maltophilia, and Achromobacter xylosoxidans in the same
population of young children with CF.”

Both the issues of concomitant infection and predisposition to other pathogen acquisition are of
interest; however, this was beyond the scope of the present study and an area of active research
within the CF community.

6. Links between other pathogenic organisms and air pollution outside the CF population in the
general community. As outlined previously, the discussion covers links between health and
air pollution and is particularly focused on CF. What is not so well covered is out-with
cystic fibrosis patients in the general community and in other disease states, what are the links being shown for bacterial and other pathogenic organisms and air pollution?

Thank you for this point. Unfortunately, there is a dearth of information regarding air pollution and its effects on bacterial respiratory infection in young children. In contrast, a link between increased air pollution exposure and viral respiratory infections in children has been reported by multiple researchers. In the Discussion section we have included the following, “In addition, post-natal air pollution exposure has been associated with an increased risk of bronchiolitis and recurrent wheeze in infants and young children [30, 31].”

Reviewer 3: The manuscript "Air pollution exposure is associated with MRSA acquisition in young U.S. children with cystic fibrosis" by Psoter et al. investigates exposure of increased fine particular matter (PM) and the acquisition of several CF-related pathogens such as methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant S. aureus (MRSA), Stenotrophomonas maltophilia and Achromobacter xylosoxidans in young children with CF. To do so, the authors use data from the US CF Foundation National Patient Registry from 2003 until 2009 and data from the US Environmental Protection Agency Air Quality System. In this analysis there was only an association of MRSA and increased PM2.5 exposure, but no significant association of the other pathogens and PM2.5 exposure.

The same analysis was conducted by the same group of authors, in which they showed that there was an association between increased exposure to PM2.5 and earlier acquisition of Pseudomonas aeruginosa.

There are major concerns about this analysis and the conclusions drawn by the authors.

1. Staphylococcus aureus is neither an airborne-transmitted nor an environmental pathogen. There might be other factors that could explain the association of increased PM2.5 exposure and earlier MRSA acquisition. Did the authors control for social status? In areas with higher PM2.5 exposure the social status and the cultural background could be very different from the overall CF-population. An association of social status, cultural background and acquisition of MRSA is well-known. Are the areas with high PM2.5 data areas, where the overall prevalence of MRSA is higher in the healthy population already compared to other areas, which might explain the increased acquisition of MRSA in the young CF-children?

We appreciate the reviewer’s concern. In our analysis, we adjusted for both individual level socioeconomic status using insurance (any public vs. private insurance), which has been previously shown to be associated with poorer clinical outcomes in young children with cystic fibrosis and for place of residence using the Rural Urban Commuting Areas (RUCA). Therefore
these factors have been considered as thoroughly as possible given the limitations of registry data in our models. While it is possible that residual confounding exists, we think it is unlikely to explain the entire observed association. The most likely mechanistic explanation for the observed association is that exposure to PM2.5 renders the CF respiratory epithelium more susceptible to infection with pathogens such as P. aeruginosa and MRSA.

2. In the conclusion the authors suggest to perform further studies on the acquisition of other CF pathogens. However, in their analysis they already showed that there was no association with other important pathogens such as MSSA, Stenotrophomonas and Achromobacter. Therefore, it is very unlikely that further studies will reveal any important data.

We agree with the reviewer. In the Conclusion we have clarified that, “Additional studies that investigate the impact of air pollution on other CF-related outcomes in young children are recommended. Given the morbidity associated with CF chronic respiratory infections and lack of strategies available to patients to prevent them, future studies that can elucidate other risk factors for these infections are needed.”

We believe that 1) increased exposure to air pollution may affect other clinical outcomes in young children with CF and 2) investigation of other exposures (i.e. environmental factors) may be warranted.

3. The authors should discuss that the effects of increased exposure to PM2.5 might sensitize for earlier acquisition of MRSA due to the increased inflammatory response.

We have expanded the following in the Discussion section of the manuscript to specifically address the biological plausibility as it relates to an inflammatory response, “Limited in vitro studies exist regarding the biologic mechanisms by which exposure to fine particulate matter adversely affects the CF airway. Kamdar and colleagues demonstrated that PM2.5 increases oxidative stress and mitochondrial signaling-mediated apoptosis in CF human bronchial epithelial cells [23] which in turn could increase airway inflammation. Further, Gelser et al. demonstrated higher uptake of inhaled nanoparticles by alveolar epithelial cells and increased inflammatory response of CFTR mutant mice compared to wild type [24].”

4. The authors relate to literature, which should describe the influence of environmental factors on non-CF MRSA infections. However, the articles, which the authors refer to (31) only show an association of "Seasonal and temperature-associated increases in gramnegative bloodstream infections..." and an association of S. aureus and skin- and soft tissue infection but not S. aureus pneumonia and seasonality (32), however this was only one study, which
investigated this association, but did not find any no seasonal variation (Watanakunakorn et al.).

We thank the reviewer for raising this important point and agree that the literature cited describing the associations between geographic and environmental/climatic factors with non upper airway respiratory infections and/or colonization is not pertinent to the discussion of the results from this investigation. As such, we have deleted that sentence and corresponding references and revised the Discussion section in the following manner, “Environmental factors are associated with approximately 50% of the population variability in lung function [32] and P. aeruginosa acquisition [33] in CF patients. Many of the CF-related pathogens are naturally occurring in the environment, although few studies have investigated specific environmental factors that may contribute to pathogen acquisition [34]. P. aeruginosa is the most widely studied CF-related bacterium with seasonal variations in acquisition [16] and differential geographic residual relative risk [13] reported. These same factors may contribute to MRSA acquisition in CF patients.”