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Original Article

Effects of postage on recovery of pathogens from cystic fibrosis sputum samples

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ABSTRACT

Background: Regular surveillance microbiology of sputum is used in cystic fibrosis (CF) to monitor for new pathogens and target treatments. A move to remote clinics has meant greater reliance on samples collected at home and posted back. The impact of delays and sample disruption caused by posting has not been systematically assessed but could have significant implications for CF microbiology.
Methods: Sputum samples collected from adult CF patients were mixed, split, and either processed immediately or posted back to laboratory. Processing involved a further split into aliquots for culture-dependant and-independent microbiology (quantitative PCR [QPCR] and microbiota sequencing). We calculated retrieval by both approaches for five typical CF pathogens: *Pseudomonas aeruginosa, Burkholderia cepacia complex, Achromobacter xylosoxidans, Staphylococcus aureus* and *Stenotrophomonas maltophilia*.
Results: 93 paired samples were collected from 73 CF patients. Median interval between sample posting and receipt was 5 days (range 1–10). For culture, overall concordance for posted and fresh samples was 86% across the five targeted pathogens (ranging from 57 to 100% for different organisms), with no bias towards either sample type. For QPCR, overall concordance was 62% (range 39–84%), again with no bias towards fresh or posted samples. There were no significant differences in culture or QPCR for samples with short (≤3days) versus extended (≥7days) postal delays. Posting had no significant impact on pathogen abundance nor on microbiota characteristics.
Conclusions: Posted sputum samples reliably reproduced culture-based and molecular microbiology of freshly collected samples, even after prolonged delays at ambient conditions. This supports use of posted samples during remote monitoring.
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1. Introduction

Surveillance microbiology of airway samples is a cornerstone of modern cystic fibrosis (CF) care [1–3]. Routine culture of sputum or cough swab samples typically occurs every time a patient attends a CF review clinic or when admitted for treatment of an exacerbation [3]. Pathogen surveillance guides targeted treatment, provides an indication of the effectiveness of treatment against pre-existing infection, and enables discovery of recently acquired infections [2]. In the case of *Pseudomonas aeruginosa* in particular this facilitates timely eradication therapy to prevent chronic infection, with its association with poorer long-term clinical outcomes [4]. Other organisms where chronic infection is associated with adverse outcomes include MRSA and some members of the *Burkholderia cepacia* complex [5]. In these and other cases, surveillance microbiology also

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allows cohorting of patients to reduce opportunities for transmission to other CF patients [3].

The use of posting samples collected at home back to clinical laboratories for surveillance microbiology has recently become a much more topical and widespread issue. Concerns about COVID-19 infection and reducing hospital exposure have led to a reduction of patient visits to CF centres and an increase in remote healthcare delivery, including video consultations, home spirometry, and use of postal samples for surveillance microbiology [6,7]. Many of these developments were available prior to COVID-19 but the pandemic has provided a catalyst to implementation of telehealth and remote monitoring in CF [7–9]. There are however a paucity of data on how remote and clinic-based microbiological surveillance compare [6,10,11], and no up-to-date and realistic assessment of the impact of posting respiratory samples on pathogen detection and surveillance using microbiological culture [12–15]. Posting samples involves exposing them to a range of delays and temperature fluctuations that are unpredictable and hard to control for, but which inevitably are outside of those recommended for sputum storage and transport [16]. Furthermore, with the availability of increasingly sensitive molecular techniques to either target specific pathogens using quantitative PCR (QPCR) [17], or sequencing the entire bacterial lung microbiota present in a respiratory sample [18], there is now an additional need to assess the effect of posting respiratory samples on these molecular-based microbiological approaches.

We hypothesized that posting samples would have significant effects on microbiological retrieval. The aims of this study were therefore to:

1. Assess delays between sample collection and processing caused by postal return.
2. Evaluate the impact of delayed culture on microbiological classification and identification of canonical CF pathogens from paired fresh and posted samples.
3. Assess the impact on CF pathogens along with the wider lung microbiota of delayed freezing on subsequent molecular identification through pathogen-targeted QPCR and 16S rRNA gene-targeted sequencing.

2. Methods

2.1. Study participants and design

Patients were recruited from those attending routine review at the Manchester Adult CF Centre. There were no restrictions based on lung function or microbiology. The only exclusion was of patients consistently unable to spontaneously expectorate sputum. Samples were collected in between July 2018 and July 2019, prior to widespread introduction of CFTR modulators. All patients provided written informed consent. This study was reviewed and approved by the NHS Research Ethic Committee (Ref 20/NW/0302).

Spontaneously expectorated sputum samples were collected at the time of clinic visits. Spirometry was performed by the usual clinical team, using Easyone spirometers (NDD, Zurich, Switzerland). Normal ranges for spirometry were those from the Global Lung Initiative [19].

Sputum samples were transported to the lab within 3 hrs for processing. All samples were weighed and mixed, using sterile forceps and petri dish, before splitting into two equal aliquots. Each aliquot was then split again into two: half was frozen at −80 °C for subsequent molecular analysis, and half was sent to the clinical microbiology lab for usual sample processing and microbiology. The other aliquot was placed into a tightly sealed sputum container and placed within a UK Royal Mail Safebox (Royal Mail, London, UK) designed for sending biological specimens and samples by post. This was posted back to the research lab, delivered through the usual national and hospital post systems, without temperature control or sample insulation. Samples returned to the hospital on Saturdays would not get processed until the following Monday. Date and time of posting and return of sample to the lab were recorded. On receipt, this sample was re-weighed, split into two and, as with the first aliquot, half was stored immediately, and half sent to the clinical microbiology laboratory. Each sputum sample therefore yielded two clinical samples and two stored samples. Control samples, consisting of 5 ml of purified water, were mixed using the same methods as sputum (including sputum pot, sterile forceps, and petri dish), stored and frozen at −80 °C. Repeat sampling was performed on a single occasion up to 6 months after the first sample was collected.

2.2. Diagnostic microbiology

Diagnostic culture-based microbiology data were provided by the Manchester University NHS Foundation Trust microbiology service in line with international guidance and standards [1,20].

2.3. Sample preparation and DNA extraction

DNA from dead or damaged cells, along with extracellular DNA (which could bias final molecular analysis) was excluded from analysis via crosslinking with propidium monoazide, as previously described [17]. Bacterial DNA extraction was then performed on sputum samples as previously described [17].

2.4. Quantitative PCR

All QPCR was performed on a Bio-Rad CFX connect machine (Bio-Rad, Deeside, UK), as previously described [21]. In brief, each plate contained a blank control consisting of the master mix, probes, primers and water. In addition, pure strains of each pathogen were run on each plate in a ten-fold dilution as both a positive control and a detection standard. Primers (Life Technologies Ltd, Paisley, UK), probes (Eurofins Genomics UK Ltd, Wolverhampton, UK), reaction mix and cycling conditions for each specific bacterial species are shown in Table S1.

2.5. Targeted amplicon sequencing

Following DNA extraction, approximately 20 ng of template DNA was amplified using Q5 high-fidelity DNA polymerase (New England Biolabs, Hitchin, UK) using a paired-end sequencing approach targeting the bacterial 16S rRNA gene region (V5-V6). Primers and PCR conditions can be found in the online supplement. Pooled barcoded amplicon libraries were sequenced on the Illumina MiSeq platform (V3 chemistry). Mock communities, DNA extract and PCR negative controls were included in each sequencing run.

2.6. Sequence processing and analysis

Sequence processing and analysis were carried out in R (Version 4.0.1), utilising the package DADA2, as previously described [22]. Raw sequence data have been deposited in the European Nucleotide Archive under study accession number PRJEB52183.

2.7. Statistical analysis

Comparisons of categorical pathogen detection data were calculated using odds ratios, 95% confidence intervals, and Fisher's exact test using the ImerTest package in R (Version 4.0.1) [23]. The odds ratio approach was used to determine if there was a disparity in instances of pathogen detection between (1) fresh- and

Table 1
Summary of patient characteristics. Data are presented as mean and standard deviation (SD) or number and percent (%) unless otherwise stated.

Number of patients	73
Number of paired samples	93
Patients with two sets of paired samples	20
Sex (Male:Female)	46:27
Mean age (years) at first or only sample (±SD)	32.1 (±11.1)
Minimum and maximum age (years)	19 to 70
CFTR Genotype:	
Homozygous Phe508del (%)	35 (48%)
Heterozygous Phe508del (%)	30 (41%)
Non-Phe508del (%)	8 (11%)
Mean%FEV_1 at sampling (±SD)	45.4 (±15.5)
CF Therapies	
Patients on CFTR modulator therapy (%)	13 (18%)
Patients on any inhaled antibiotic (%)	54 (74%)
Patients on any long-term oral antibiotic	71 (96%)
Other clinical features	
Liver transplant	2 (3%)
Diabetes on insulin	28 (38%)

%FEV_1 – percentage forced expiratory volume in 1 second.

postal-samples and (2) between early- and late-returned samples. Mixed effect models were used to compare pathogen abundances, as determined by QPCR, as well as microbiota characteristics between paired fresh- and postal-samples using the lmerTest in R [23]. High variability is an inherent feature in microbiology measures between CF patients [17,18,24]. These models allowed for the inclusion of both fixed (fresh or postal) and random effects (patients), and therefore variation between patients was accounted for in each model [24]. All data were log-transformed prior to mixed effects modelling.

Bacterial taxa in the microbiota in fresh samples were partitioned into either core taxa or satellite taxa, based upon their prevalence and relative abundance across samples, as previously described [18,22]. Regression analyses were performed using XLSTAT v2018.1 (Addinsoft, Paris, France). Richness, Fisher's alpha index of diversity, Sørensen indices of similarity were calculated in PAST v4.04 (https://www.nhm.uio.no/english/research/resources/past/). Analysis of similarities (ANOSIM) with Bonferroni correction, used to test for significant differences in microbiota composition, was performed in PAST.

3. Results

One hundred and one adult patients were recruited to the study, but 4 were unable to produce sputum samples. A further 83 samples from 23 patients were not included as there was insufficient sample for a postal aliquot. Analysis was therefore restricted to 93 paired fresh and postal sputum samples from 73 patients (Fig. 1). Clinical characteristics of these are summarised in Table 1, with characteristics of individual patients presented in Table S2. Overall, 54 (74%) of patients were on inhaled antibiotics 71 (96%) were on long-term oral antibiotics (the majority of these, $n = 66$, were on azithromycin).

3.1. Time spent by samples in postal system

Samples were typically posted towards the end of the working day, reflecting the timing of CF clinics. Median interval between date of collection and date of laboratory processing was 5 days, interquartile range (IQR) 2.5 – 7 days, range 1–10 days. These times include any delays to processing caused by the national mail service, the hospital mail distribution, as well as any delays in lab processing (Figure S1).

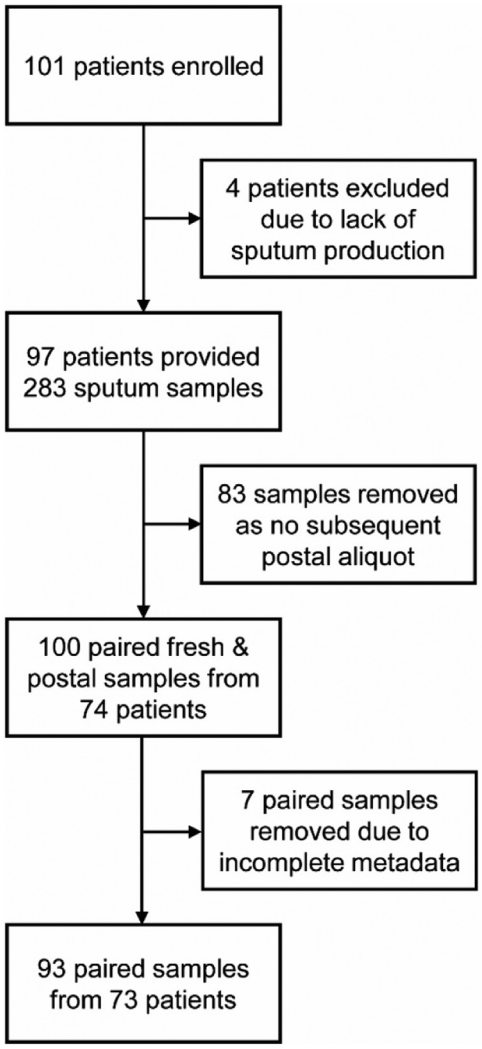

Fig. 1. Flow diagram detailing patient and sample selection process.

3.2. Pathogen detection in fresh and posted samples

By culture, *A. xylosoxidans* were detected in 14 samples overall (15%) and showed concordance (i.e. cultured in both fresh and posted paired samples) in 12 (86%) cases. *Burkholderia* species were detected in 19 samples (20%), with 100% concordance. *P. aeruginosa* was detected in 60 samples (65%), with 85% concordance. *S. aureus* was detected in 30 samples in total (32%), with 87% concordance. Finally, *S. maltophilia* was detected in 7 samples overall (8%), with 57% (Fig. 2 and Figure S2). Overall concordance across all five pathogens was 86%, with 6% cultured only in fresh samples and 8% in posted samples.

By QPCR, there were higher rates of detection of all pathogens than by microbiological culture. *A. xylosoxidans* were detected in 65 samples overall (70%), of which 51% were concordant. *Burkholderia* species were detected in 88 samples overall (95%), of which 59 were concordant. *P. aeruginosa* was detected in 81 samples overall (87%), with 84% concordance. *S. aureus* was detected in 76 samples overall (82%), with 59% concordance. Finally, *S. maltophilia* was detected in 59 samples overall (63%) with 39% concordance (Fig. 2 and Figure S2). Overall concordance across all five pathogens was 62%, with 20% found only in fresh samples and 18% only in posted samples. There were no significant differences in detection for any pathogen using culture or by targeted QPCR ($P > 0.05$ in all instances, Fig. 2).

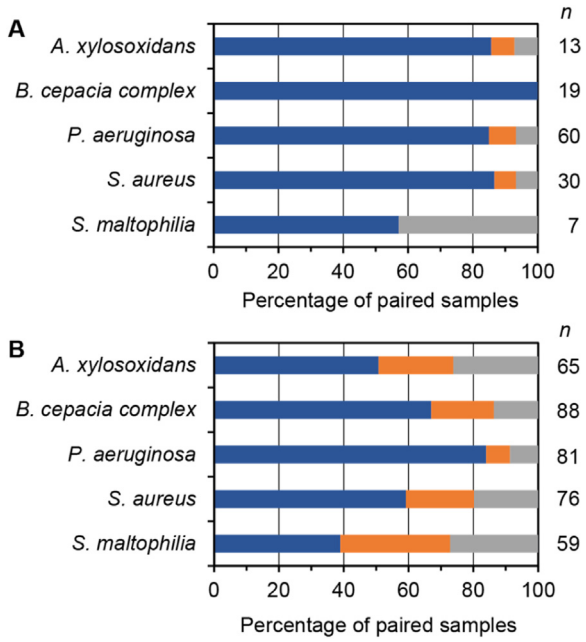

Fig. 2. Pathogen detection in fresh and posted respiratory samples determined by (A) microbiological culture and (B) targeted QPCR. Blue bars indicate the percentage of paired samples where a pathogen was detected in both the fresh and corresponding postal sample. Orange and grey bars indicate percentage of paired samples where a pathogen was detected in only the fresh or posted samples, respectively. Given in each instance is the number of paired samples (n) that a pathogen was detected within by culture or QPCR across all paired samples ($N = 93$). Odds Ratio (OR) results from (A) culture-based detection: *A. xylosoxidans* (OR = 1; 95% confidence intervals (CI) = 0.06, 17.75; significance (P) = 1), *B. cepacia* complex (OR = 1; CI 0.02, 52.98; $P = 1$), *P. aeruginosa* (OR = 1.27; CI = 0.32, 4.99; $P = 0.729$), *S. aureus* (OR = 1; CI = 0.13, 7.60; $P = 1$), and *S. maltophilia* (OR = 0.09; CI = 0.01, 2.07; $P = 0.131$). OR results from (B) QPCR-based detection: *A. xylosoxidans* (OR = 0.85; CI = 0.38, 1.88, $P = 0.684$), *B. cepacia* complex (OR = 1.52, CI = 0.68, 3.40, $P = 0.312$), *P. aeruginosa* (OR = 0.85, CI = 0.27, 2.64, $P = 0.773$), *S. aureus* (OR = 1.08, CI = 0.49, 2.39, $P = 0.841$), and *S. maltophilia* (OR = 1.38, CI = 0.63, 3.03, $P = 0.425$). See Supplemental Figures S2 and S3 for culture-based and QPCR-based pathogen detection in paired fresh and postal samples from individual patients.

In order to explore the impact of length of delays, samples returned in ≤3 days (accounting for 28% of all samples) were compared to those returned from 7 days (26% of all samples). Time taken to return samples by post did not significantly affect instances of pathogen detection by microbiological culture or by targeted QPCR ($P > 0.05$ in all instances) (Figure S1).

3.3. Effect of posting on bacterial abundance of canonical CF pathogens

There were no statistically significant differences in abundance between fresh and posted samples for any of the CF pathogens when compared using mixed-effects modelling ($P > 0.05$ in all instances)(Fig. 3). Mean (±standard deviation [SD]) pathogen abundances in paired fresh and postal samples respectively were: *A. xylosoxidans* 2.21×10^7 ($\pm 2.77 \times 10^5$) vs 3.49×10^7 ($\pm 2.39 \times 10^5$) colony forming units (CFU) ml^{-1} equivalents; *B. cepacia complex* 1.46×10^8 ($\pm 3.81 \times 10^4$) vs 1.08×10^8 ($\pm 1.80 \times 10^5$) CFU ml^{-1} equivalents; *P. aeruginosa* 6.57×10^8 ($\pm 2.58 \times 10^4$) vs 1.10×10^9 ($\pm 1.84 \times 10^4$) CFU ml^{-1} equivalents; *S. aureus* 8.85×10^7 ($\pm 1.13 \times 10^5$) vs 1.24×10^8 ($\pm 2.57 \times 10^5$) CFU ml^{-1} equivalents; and *S. maltophilia* 5.96×10^7 ($\pm 6.96 \times 10^5$) vs 2.48×10^7 ($\pm 1.39 \times 10^6$) CFU ml^{-1} equivalents (Fig. 3 and Figure S4).

3.4. Effect of posting on wider microbiota

Effects of postage on characteristics of the wider bacterial microbiota were investigated by partitioning the whole microbiota into core and satellite taxa (Figure S4). Bacterial taxa richness and Fisher's alpha index of diversity of the whole, core, and satellite microbiota were then compared between fresh and posted samples, using mixed-effects modelling (Fig. 4). No significant differences in richness or diversity were found between the paired sample groups ($P > 0.05$ in all instances) (Fig. 4 and Figures S5 & S6). Finally, ANOSIM tests revealed no significant differences in the composition of the whole, core, and satellite microbiota resulting from stage of sputum samples (Fig. 5).

4. Discussion

This is the largest systematic comparison of posted versus fresh sputum microbiology in CF, exploring the impact on both conventional culture as well as molecular identification. We have reproduced the real-life scenario by posting samples back to the laboratory, thereby exposing samples to the full range of delays, physical disruption and temperature fluctuations experienced in postal transport. Samples were mixed beforehand and split from the same source to ensure that they were as homogeneous before posting as possible. What we have discovered is that clinical microbiology for the majority of key CF pathogens is well recovered in posted samples. For *A. xylosoxidans, Burkholderia* spp., *P. aeruginosa* and *S. aureus*, recovery was concordant in 85–100% of cases, with no clear bias in favour of either fresh or posted samples from the discordant cases. In the case of *S. maltophilia*, there was a much lower rate of concordance (57%), with more samples positive after posting (43% detected by postal-only), but this result is likely confounded by the far lower numbers of *S. maltophilia* positive samples overall ($n = 7$). We also saw no difference in rates of pathogen retrieval between samples returned to the lab early (i.e. ≤3 days) and those returned late (≥7 days). For microbiological culture therefore, the results of this study are reassuring and support the use of posted samples. This result is in contrast with our original hypothesis that posting would result in significant change pathogen detection.

Previous comparable studies have produced mixed results, even though the practice of posting sputum samples from home has been common in CF clinics for several years. Some authors have reported loss of viability of important respiratory pathogens with samples stored at 4 °C [15] whilst others have failed to show significant deterioration after up to 2 days of refrigeration [25,26]. Sputum samples stored at room temperature have also been shown to result in significant reduction in growth of *Haemophilus influenzae* [27] and mycobacteria [28]. For this reason, national guidelines in the UK [1] and US [29] have recommended rapid processing and caution in interpreting postal samples. An important difference between this study and previous work is that we used real-life as opposed to simulated postage delays, which turned out to be much longer than those described in earlier studies. Processing of the posted samples was delayed by 5 days or more for over half of the samples returned, compared to 48 hrs for lab-simulated delays, and 1–6 days for posted samples in previous studies [13,15]. This may reflect the current state of UK national mail transit times or be a reflection of more local issues due to receipt and processing by the hospital mail system, but even with these increased delays we were still able to show reliable recovery of important CF pathogens from posted samples.

Given the growing prominence of molecular techniques to identify pathogens in CF sputum [18,21], it is also important to understand the potential impact of postage delays on these more sensitive analyses. In tuberculosis and viral infections, reliable recovery of genetic material can be obtained from dried sputum samples stored at ambient temperatures for prolonged periods [30,31]. CF infections however represent a more complex challenge, containing CF-specific pathogens embedded within a diverse and interacting

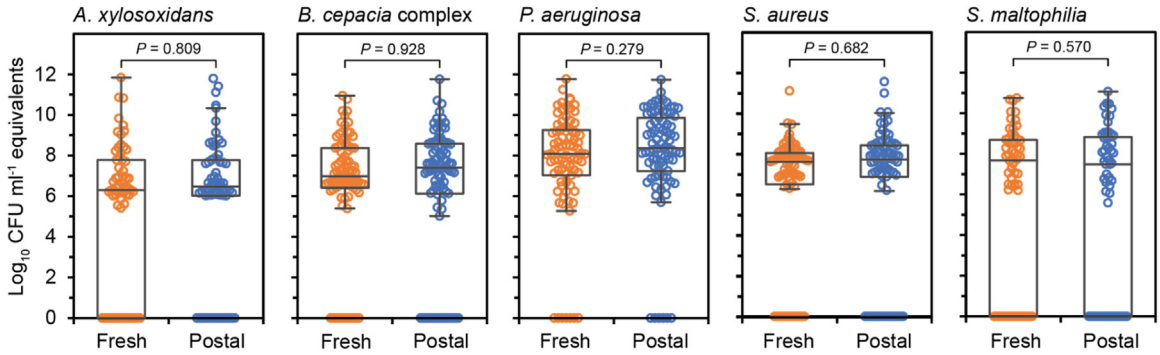

Fig. 3. Comparison of pathogen abundances between fresh and postal samples with mixed-effects modelling. Given for each pathogen is the change in abundance, expressed as colony forming units per ml equivalents derived from targeted QPCR, between fresh and posted paired samples. Orange and grey circles denote pathogen abundances in individual fresh and postal samples, respectively. Grey boxplots show 25–75th interquartile (IQR) range with whiskers showing 1.5 times IQR. Parameters are extracted from mixed-effects models for each pathogen with significance (*P*) displayed in each figure panel. Mixed-effects model results: *A. xylosoxidans*, degrees of freedom (*df*) = 128, *F*-test statistic = 0.06, *P* = 0.809; *B. cepacia* complex, *df* = 84, *F* = 0.01, *P* = 0.928; *P. aeruginosa, df* = 81, *F* = 1.19, *P* = 0.279; *S. aureus, df* = 150, *F* = 0.17, *P* = 0.682; and *S. maltophilia, df* = 112, *F* = 0.33, *P* = 0.570. See Supplemental Figure S3 for QPCR-derived pathogen abundances in paired fresh and postal samples from individual patients.

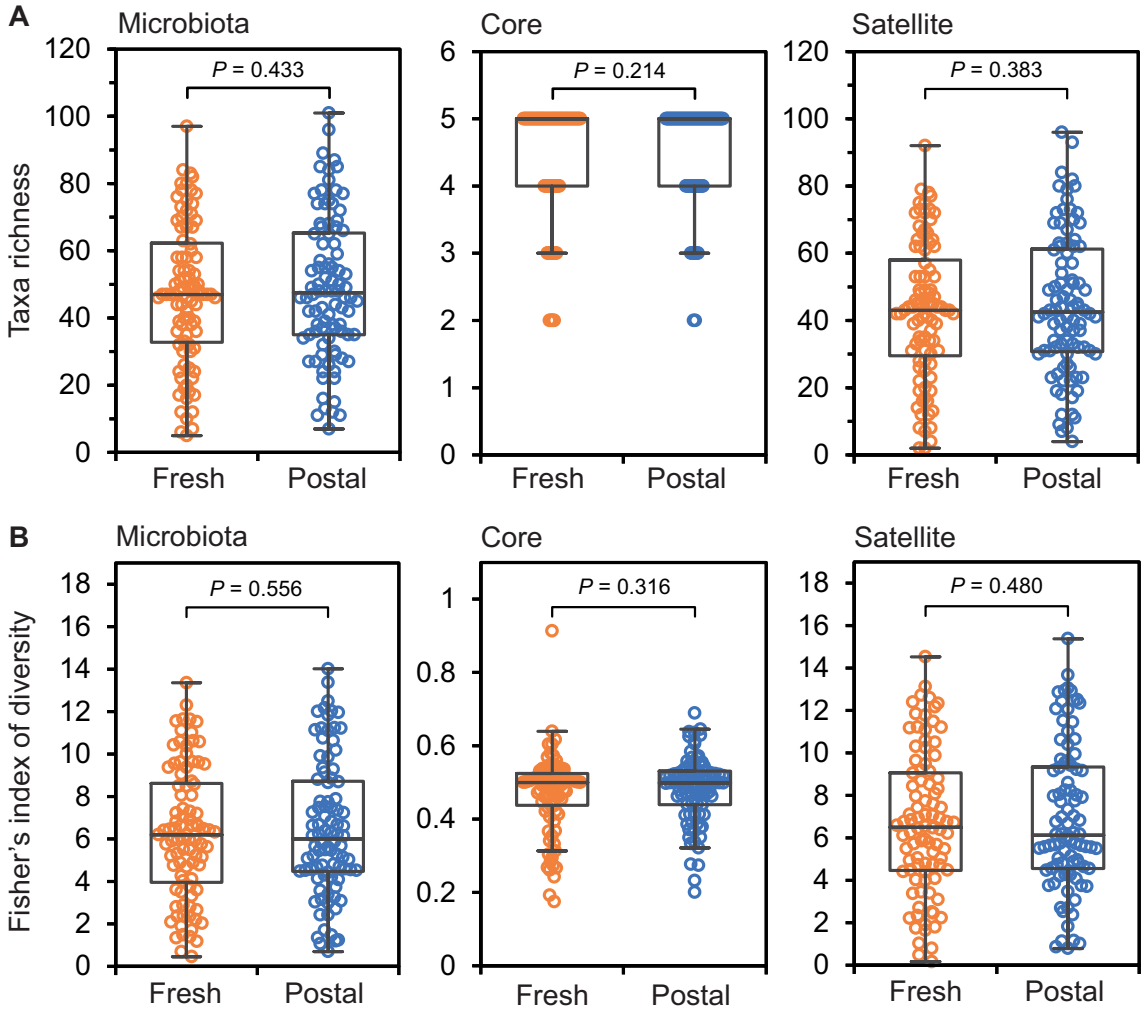

Fig. 4. Comparison of (A) bacterial taxa richness and (B) Fisher's alpha index of diversity in fresh and postal samples with mixed-effects modelling. Given are richness and diversity comparisons for the whole microbiota and the core and satellite taxa groups. Orange and grey circles denote either richness or diversity in individual fresh and postal samples, respectively. Grey boxplots show 25–75th interquartile (IQR) range with whiskers showing 1.5 times IQR. Parameters are extracted from mixed-effects models for each pathogen with significance (*P*) displayed in each figure panel. Mixed-effects model results: (A) Microbiota richness, *F*-test statistic = 0.62, significance (*P*) = 0.433; Core microbiota richness, *F* = 1.57, *P* = 0.214; Satellite microbiota, *F* = 0.77, *P* = 0.383; and (B) Microbiota diversity, *F* = 0.35, *P* = 0.556; Core microbiota diversity, *F* = 1.02, *P* = 0.316; Satellite microbiota *F* = 0.504, *P* = 0.480. Degrees of freedom = 92 in all instances. See Supplemental Figures S5 and S6 for taxa richness and diversity in paired fresh and postal samples from individual patients.

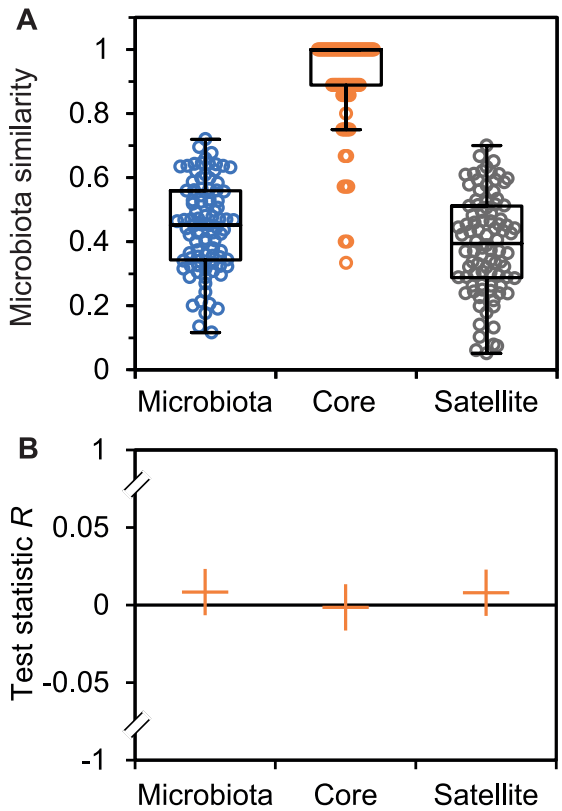

Fig. 5. Microbiota composition similarities between paired fresh and postal samples with analysis of similarities (ANOSIM). (A) Compositional similarity between paired fresh and postal samples measured by Sørensen index of similarity for the whole microbiota (blue circles) and the core (orange) and satellite (grey) taxa groups. Black boxplots show 25–75th interquartile (IQR) range with whiskers showing 1.5 times IQR. (B) ANOSIM results, where orange crosses denote the ANOSIM test statistic (R) in each instance. R scales from $+1$ to -1. $+1$ indicates that all the most similar samples are within the same groups. $R = 0$ occurs if the high and low similarities are perfectly mixed and bear no relationship to the group. A value of -1 indicates that the most similar samples are all outside of the groups. Note data breaks in y-axis. Bonferroni corrected significance (P) for microbiota = 0.073, core = 0.593, satellite = 0.075. ANOSIM R and P values were generated using the Sørensen index of similarity. See Supplemental Figure S7 for Sørensen index of similarities in paired fresh and postal samples from individual patients.

lung infection microbiota [18]. Two small studies (up to 8 patients) previously looked at the impact of delays of up to 24–72 h for sample stabilisation on QPCR-derived pathogen abundance and microbiota composition [32,33]. Both observed changes in microbiota composition related to time taken to stabilise samples. In contrast, we did not see any consistent change in pathogen retrieval due to posting. There was more variability than seen with culture, with concordance between 39 and 84%, but no bias in favour of either freshly stored or posted samples. We also did not see any significant change in bacterial abundance on QPCR, and no change in microbiota characteristics. These differences may be because our study included a much greater number of patients and samples than previous reports. Moreover, our analyses also controlled for the high inter-patient variation inherent in CF microbiota as a potential confounding factor [24]. However, the molecular methods used here and in prior reports are all research-based and not standardised for clinical use, so there will be additional variation between laboratories in DNA extraction, amplification, and sequencing protocols. We would therefore recommend that centres adopting posted samples for molecular-based analyses carry out their own local validation first.

There are some limitations to this study that deserve consideration. The samples were collected before widespread availability of CFTR modulators, and it is possible that if modulated patients had reduced bacterial densities this might make the effects of sample delays more significant. Minimum sputum volume for the processing and splitting protocols was 0.5 ml. Inevitably we have looked at patients with predominantly chronic infection, in whom it is expected that bacterial burden is already high, and this may therefore reduce applicability to identification of emergent infections at low density. We have only looked at classical CF pathogens and have not included *Haemophilus influenzae*, non-tuberculous mycobacteria, notable pathogen phenotypes (e.g. methicillin-resistant *Staphylococcus aureus* [MRSA] or multi-drug resistant *Pseudomonas aeruginosa* [MDR-PA]), or fungi. The postage delays described here are greater than those from previous studies, but deliberately reflect the real-world experience of posting back samples via non-priority post, as well as no temperature control or sample insulation. For rarer pathogens, or different circumstances (e.g. in geographical regions where temperature fluctuations are likely to be more severe) these studies may need repeating to confirm the findings. Finally, there was a sex imbalance within the recruited patients (Table 1), however, we do not believe this was likely to affect our findings or conclusions.

5. Conclusions

The last two years has seen two highly significant changes in CF clinical practice which have challenged our conventional approaches to microbiological surveillance. A move to remote clinics, hastened by the COVID-19 pandemic, has reduced the number of sputum samples that can be collected in clinic and processed rapidly. In addition, the widespread availability of CFTR modulators has significantly reduced the volumes of sputum that people with CF can reliably expectorate on demand. The importance of this issue was highlighted in the recent James Lind Alliance CF research priorities refresh, where the issue of how to diagnose lung infection in the absence of easily obtainable sputum samples was the second highest research priority. At present, this often means relying on home-based sample collection. Our data support this for culture-based microbiological surveillance, even with very extended postage times. Moreover, this was also found to be the case for the culture-independent approaches, with overall retrieval being robust for typical CF pathogens. These are important observations, and in an era where remote clinics are also being deployed for other chronic respiratory diseases, provide reassurance that samples posted from home can be relied upon for research and clinical interpretation.

Author contributions

The authors would like to thank all the patients and their families who took part in this study, and the CF clinical team at Wythenshawe Hospital. AH, CvdG, and PB conceived the study. LH, BB, MS, HG, and DR performed sample processing and analysis. LH, DR, PB, AH, and CvdG performed data and statistical analysis. BB, MS, AJ, and AH were responsible for sample collection, clinical care records and documentation. LH, AH, and CvdG were responsible for the creation of the original draft of the manuscript. CvdG and AH are guarantors of this work. All authors read and approved the final manuscript.

Declaration of Competing Interest

Outside of the submitted work, AH reports personal fees for advisory services (Mylan Pharmaceuticals) and educational and presentation activities (Vertex Pharmaceuticals), and CvdG and DR report grants from Vertex Pharmaceuticals.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2023.03.008.

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