Cystic Fibrosis Trust

Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis

Second edition

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Uniting for a life unlimited

The UK Cystic Fibrosis Trust Microbiology Laboratory Standards Working Group

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Cystic Fibrosis Trust

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Glossary

ABPA	Allergic bronchopulmonary aspergillosis
ACPCF	Association of Chartered Physiotherapists in Cystic Fibrosis
AFB	Acid-fast bacilli
AMR	Antimicrobial resistance
AMRHAI	Antimicrobial Resistance and Healthcare Associated Infections
ANI	Average nucleotide identity
AST	Antimicrobial susceptibility testing
BA	Blood agar
Bactericidal	An agent that destroys bacteria
Bacteriostatic	An agent that slows or inhibits bacterial growth
BAL	Bronchoalveolar lavage
BC	Bacitracin-chocolate agar
Bcc	*Burkholderia cepacia* complex
BCESM	*B. cepacia* epidemic strain marker
BCSA	*B. cepacia* selective agar
BMI	Body mass index
BSAC	British Society for Antimicrobial Chemotherapy
BTS	British Thoracic Society
CA	Chocolate agar
CCBA	Cefsulodin chocolate blood agar
CFF	Cystic Fibrosis Foundation
CFTR	Cystic fibrosis transmembrane conductance regulator
CFTRM	Cystic fibrosis transmembrane conductance regulator modulator
CFU	Colony-forming unit
CLSI	Clinical and Laboratory Standards Institute
CSA	CHROMagar *S. aureus* selective and differential agar
DDH	DNA-DNA hybridisation
DGGE	Denaturing gradient gel electrophoresis
DTT	Dithiothreitol
ECFS	European Cystic Fibrosis Society
EQA	External quality assessment

EUCAST	European Committee on Antimicrobial Susceptibility Testing
FEV_1	Forced expiratory volume in one second
GDP mannose	Guanosine diphosphate mannose
GNNF	Gram-negative non-fermenters
GNSA	Gram-negative selective agar
Gram stain	A staining technique to characterise bacteria based on their cell wall composition
HBBA	Haemin-bacitracin blood agar
IDSA	Infectious Diseases Society of America
IPC	Infection prevention and control
IV	Intravenous
LES	Liverpool epidemic strain (of *P. aeruginosa*)
LPA	Line probe assay
MABSC	*Mycobacterium abscessus* complex
MAC	*Mycobacterium avium* complex
MALDI-TOF MS	Matrix assisted laser desorption/ionisation time of flight mass spectrometry
MAN	Manchester epidemic strain (of *P. aeruginosa*)
MCBT	Multiple combination bactericidal antibiotic testing
Mdl1	Midlands 1 epidemic strain (of *P. aeruginosa*)
MDT	Multidisciplinary team
MGIT	Mycobacterial Growth Indicator Tube
MH	Mechanical homogenisation
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRSA	Meticillin-resistant *S. aureus*
MSA	Mannitol salt agar
MSSA	Meticillin-sensitive *S. aureus*
MTB	*M. tuberculosis*
NAD	Nicotinamide adenine dinucleotide
NAG	N-acetyl-D-glucosamine
NALC	N-acetyl L-cysteine
NICE	National Institute for Health and Care Excellence
NO	Nitric oxide

NTM	Nontuberculous mycobacteria
NTM-PD	NTM-pulmonary disease
OFPBL	Oxidative-fermentative polymyxin B-bacitracin-lactose
OPS	Oropharyngeal suction
Pathovars	A strain or set of strains with the same or similar characteristics
PIA	*Pseudomonas* isolation agar
PCN	*Pseudomonas* CN selective agar
POCT	Point of care test
PCR	Polymerase chain reaction
PD	Pharmacodynamic
PFGE	Pulsed-field gel electrophoresis
PIA	*Pseudomonas* isolation agar
PK	Pharmacokinetic
PVL	Panton-Valentine leukocidin
qPCR	quantitative PCR
RCT	Randomised controlled trial
Rep-PCR	Repetitive PCR
RFLP	Restriction fragment length polymorphism
RGM	Rapidly growing mycobacteria
RISA	Ribosomal intergenic spacer analysis
rMLST	Ribosomal MLST
SAFS	Severe asthma with fungal sensitisation
SCV	Small colony variant
SGA	Sabouraud glucose agar
SMI	UK Standards for Microbiology Investigations
ST	Sequence type
UKHSA	UK Health Security Agency
VIA	Vancomycin-imipenem-amphotericin B
VNTR	Variable-number tandem repeat
VOC	Volatile organic compound
WGS	Whole genome sequencing

Preface

I was diagnosed with cystic fibrosis at the age of 13 months. I remained relatively well despite living with the condition until in 2003, when I became the first CF patient in the UK diagnosed with *Mycobacterium abscessus*, an aggressive bacterial lung infection.

Little was known about this infection at the time, and it took several months of me being hospitalised to find a course of treatment that had any effect against the disease, during which time I was warned that my prospects of a positive outcome were extremely bleak.

However, thanks to my clinical team working collaboratively with other CF teams across the world, I survived. Indeed, my experience in 2003 paved the way for the development of treatment regimens that continue to be used in patients presenting with *Mycobacterium Abscessus* worldwide to this day.

Since 2003, amongst other things, I have had two children, and have recently celebrated both my twentieth wedding anniversary and my fiftieth birthday. I have also received a new pair of lungs.

Yet, without excellent clinical care, backed up by sound research and the availability of robust historic data retrieved from pathological samples, I would not have been afforded these opportunities, nor the additional lifetime I have enjoyed.

My case is just a single example of where the importance of the everyday work carried out in laboratories translates into a real impact on those living with CF. Such work is vital to identify an individual's infection status, so that appropriate care and treatment plans are instigated without delay, thus enhancing the chances of a positive outcome for the patient.

The importance of a unified approach to the diagnostics of key pathogens should not be under-estimated. Through using a common standard at diagnostic level, and the adoption of best practice by each laboratory carrying out this vital work, such approaches should ultimately be of benefit to all those living with CF worldwide.

Ultimately, it is through collaboration such as that evidenced by this document that ensures that real people such as me, can continue to live real lives.

Working together, while always keeping the patient at the focal point of any such research projects, will ensure that this continues to be the case.

Luke Twentyman, adult with cystic fibrosis

My introduction to CF microbiology came in 1970 when I was contacted by a CF paediatrician for advice on mucoid *P. aeruginosa*, a variant I had seldom encountered in non-CF infections. This collaboration led to a growing interest in CF microbiology. I soon appreciated the diagnostic importance in young CF individuals of distinguishing culture of commensal *H. influenzae* in small numbers from its pathogenic role when cultured in densities greater than 10^6 CFUs/ml sputum during pulmonary exacerbation.

Sputum homogenisation and dilution were identified as especially important for culture of *H. influenzae* in the presence of other respiratory microbes. The challenges of antibiotic therapy in CF individuals, and the potential consequences of the as yet unidentified lung microbiome, also became apparent. For example, failure to eradicate sensitive *H. influenza* with a beta-lactam if beta-lactamase-producing *P. aeruginosa* were co-cultured.

Advances in antimicrobial therapy, including an increased range of antimicrobials and use of aerosolised delivery contributed greatly to CF life expectancy and the role of adult CF centres. These advances were accompanied by recognition of the importance in CF adults of mucoid *P. aeruginosa*, mycobacteria, fungi and viruses. In the 1990s, a major new threat emerged with evidence of person-to-person transmission of species within the *Burkholderia cepacia* complex (Bcc), a highly resistant pathogen which, in some patients led to a fatal fulminating pneumonia, known as cepacia syndrome. The significance and laboratory diagnosis of each of these CF pathogens is covered within the guidelines.

For the CF community, the draconian infection control measures initiated in the 1990s to defend against the Bcc, which included strict segregation of CF individuals in clinical and social settings, are a powerful memory, and parallel ongoing measures to control the pandemic of SARS-CoV-2.

Cystic Fibrosis Trust and the Laboratory Standards working group are to be congratulated for completion of this 2nd edition of *Laboratory Standards*. Based on a wealth of published evidence, this edition provides a comprehensive state of the art account of CF microbiology. It is to their credit that the working group have provided recommendations which will ensure the highest standards for laboratory diagnosis and optimum treatment of CF lung infections. The guidelines cover a wide range of topics. The format, however, allows the reader to concentrate on areas of interest; for example, specific pathogens or controversial topics such as antimicrobial susceptibility testing. Overall, this 2nd edition emphasises the scope and complexity of CF microbiology. The introduction of section 7, which scans the horizon of CF microbiology, including the impact of CF gene modulators on reducing sputum production, is both informative and relevant for future reliance on sputum microbiology.

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Grading scheme for recommendations

Grading scheme for recommendations used in Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis

GRADE is a system developed by an international working group for rating the quality of evidence across outcomes in systematic reviews and guidelines; it can also be used to grade the strength of recommendations in guidelines. The system is designed for reviews and guidelines that examine management strategies or interventions, and these may include no intervention or current best management. The key difference from other assessment systems is that GRADE rates the quality of evidence for a particular outcome across studies and does not rate the quality of individual studies.

In order to apply GRADE, the evidence must clearly specify the relevant setting, population, intervention, comparator(s) and outcomes.

Before starting an evidence review, the GDG should apply an initial rating to the importance of outcomes, in order to identify which outcomes of interest are both 'critical' to decision-making and 'important' to patients. This rating should be confirmed or, if absolutely necessary, revised after completing the evidence review.

Box 1. The GRADE approach to assessing the quality of evidence for intervention studies

Box 1

The GRADE approach to assessing the quality of evidence for intervention studies

In the GRADE system, the following features are assessed for the evidence found for each 'critical' and each 'important' outcome from a systematic review:

- study limitations (risk of bias): assessing the 'internal validity' of the evidence
- inconsistency: assessing heterogeneity or variability in the estimates of treatment effect across studies
- indirectness: assessing the degree of differences between the population, intervention, comparator for the intervention and outcome of interest
- imprecision (random error): assessing the extent to which confidence in the effect estimate is adequate to support a particular decision
- publication bias: assessing the degree of selective publication of studies.

Other considerations (for observational studies only):

- effect size
- effect of all plausible confounding
- evidence of a dose–response relationship.

The quality of evidence is classified as high, moderate, low or very low (see Table 1).

Table 1. Quality of Evidence Grades

Quality of Evidence Grades	
Grade	Definition
High	We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate	We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low	Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very Low	We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

For more details about GRADE, see the **GRADE working group website**.

Summary of key recommendations

2. Sampling issues

- Respiratory sampling should be undertaken at each hospital visit and at times of respiratory exacerbation (low).
- Sputum is the recommended specimen for routine sampling (moderate).
- Sputum induction is superior to cough swabs for isolating CF pathogens in children (moderate). This is likely to be the same for adults (low).
- Sputum induction is equivalent to two-lobe bronchoalveolar lavage (BAL) in symptomatic children with CF (moderate). This is likely to be the same for adults (low).
- A cough swab should only be used if a patient cannot expectorate and the use of sputum induction is not feasible at that time of sampling (low).
- Cough swabs should not be used in preference to sputum or BAL for the isolation of nontuberculous mycobacteria (low). Expert opinion is that cough swabs will have a lower yield to detect fungi in the lower airways. However, if cough swabs are used, they are only suitable for culture directly on solid media and not in liquid culture systems (low).
- Cough swabs should not be used for culture-independent analysis (low).
- Sputum induction or BAL should be considered to identify lower airway infection in patients who are clinically deteriorating, particularly those with persistently negative cough swabs (low).
- Samples should be sent promptly to the laboratory and processed as soon as possible; there is insufficient evidence to judge whether refrigeration of samples produces better microbiological results than following storage at room temperature. However, current consensus is that if samples are delayed more than a few hours they should be refrigerated at 4°C (very low).
- Evidence of the impact on sample quality following submission by post is limited. Until further research improves our understanding it would seem prudent that the results of bacterial culture of respiratory samples submitted to the laboratory by post should be interpreted with caution (low).

3. Sampling processing

- There is insufficient evidence to support the routine use of Gram staining of sputum samples from people with CF, either as a marker of specimen quality or as a predictor of subsequent culture results (moderate).
- There is insufficient evidence to suggest that use of a selective medium enhances the yield of *P. aeruginosa* from the respiratory secretions of people with CF. However, the use of some selective media may assist in identification, particularly the appearance of pigmentation and the mucoid phenotype (moderate).
- The presence of *P. aeruginosa* should always be reported, irrespective of quantity (high).
- All CF respiratory samples must be cultured to a good quality Bcc selective agar and incubated as per manufactures instructions or by robust in-house evaluation (low).
- We would also recommend that all routine cultures are incubated as per the chosen selective agar manufacturer's recommendation or for a minimum of three days where guidance is not available (low).
- The presence of Bcc should always be reported, irrespective of quantity (high).
- A selective medium should be used to enhance the detection of *S. aureus* from respiratory samples of people with CF. Options include mannitol salt agar or a chromogenic medium that has been validated for isolation of *S. aureus* from CF samples (moderate).
- The presence of *S. aureus* should always be reported, irrespective of quantity (moderate).
- A selective medium should be used to enhance the recovery of *H. influenzae*. Options include chocolate agar supplemented with either bacitracin or cefsulodin (high).
- Sabouraud medium with additional antibiotics should be used to enhance the recovery of fungi from respiratory samples of people with CF (high).
- The use of high-volume sputum samples (≥100µl) increases the detection rate of moulds; the use of undiluted sample may potentially increase the yield (moderate).
- Plates should be incubated at 35–37°C in air and examined after overnight incubation followed

by every 24h for at least seven days (moderate). Extended incubation up to two weeks could be considered for individuals listed for lung transplant (low).

- Smear for acid-fast bacilli may be used along with culture for NTM screening (moderate).
- Respiratory tract samples should be cultured via the conventional method using both solid and liquid media and incubated for a minimum of six weeks (with consideration that that some slow growing mycobacteria can take up to 12 weeks) (moderate).
- The use of selective rapidly growing mycobacteria (RGM) agar can be considered as an adjunct direct inoculation (without decontamination) to recover *M. abscessus* when incubated at 30°C for 21–28 day (moderate).
- PCR assays are the gold standard technique for diagnosis of viral respiratory infection in CF and other viral pathogens should be conducted in line with current national standards (high).

4. Identification

- Biochemical methods are not reliable for identification of non-fermentative Gram-negative bacteria recovered from the sputum of people with CF. Hospital laboratories without access to MALDI-TOF should arrange to send Gram-negative non-fermenters (GNNF) to laboratories with this facility (high).
- Scores greater than, or equal to the threshold for both the Bruker and VITEK®-MS platforms are reliable to genus level (high).
- First isolates of GNNF of proven clinical importance which are difficult to reliably speciate by MALDI-TOF analysis, such as the Bcc, should undergo confirmatory testing by a validated method, such as *recA* sequencing (high).
- For subsequent patient isolates of Bcc with the same MALDI-TOF identification, *recA* sequencing is not essential unless there is concern about a new infection, or for surveillance purposes (moderate).
- *P. aeruginosa* with scores equal to, or above the threshold, can be reliably identified by both the Bruker MALDI-MS and VITEK®-MS platforms to species-level. Dual identifications (*P. aeruginosa/ Pseudomonas* sp.) occasionally occur on the VITEK®-MS. In these cases, species-level confirmation should be sought using an alternative method (moderate).
- For other CF-related GNNF, species-level identification by MALDI-TOF using existing databases is currently not reliable. The creation of in-house databases has been shown to be useful for improved species-level ID (moderate).
- If required, GNNF may be submitted to the reference laboratory for species-level identification (moderate).
- Laboratories are encouraged to use MALDI-TOF MS following robust in-house validation for the identification of NTM from CF respiratory samples (moderate).
- Clinical outcome with all *Burkholderia* species infection is variable in CF and all species potentially constitute an infection control risk, therefore accurate species identification is recommended (high).
- *recA* gene and MLST-based approaches are sufficient to place a strain within the Bcc and define the majority of species prevalent in CF, but may not accurately identify certain taxonomic groups such as those within taxon K (high).
- All first isolates of *Mycobacterium abscessus* complex (MABSC) should be referred for confirmation, subspeciation and typing using a robust molecular method (moderate).
- If the diagnostic laboratory does not have the facilities for molecular identification of the Bcc, the isolate should be sent to the reference laboratory (high).
- Confirmation, subspeciation and typing should be performed on all first isolates of MABSC; this is useful for infection control and monitoring treatment success or failure (moderate).
- Epidemic strain markers are not sufficiently reliable to determine the potential transmissibility of Bcc (high).
- All newly confirmed Bcc isolates should undergo molecular typing to establish whether they are members of ET-12 or other lineages (high).
- Molecular surveillance should be undertaken if there is concern about prevalence or increasing incidence, or if requested by the clinical team (high).
- If the hospital laboratory does not have the facilities for molecular typing, isolates may be submitted to an appropriate reference laboratory (high).
- Conventional identification of *Aspergillus* species should be done to differentiate *A. fumigatus* from non-*fumigatus* species in routine sputum samples (moderate).

- Conventional identification of *Aspergillus* species should be done if a specific request for fungal culture of sputum and BAL-fluid samples and antifungal therapy is considered (high).
- If non-*Aspergillus* species are cultured from sputum or BAL-fluids, consider sending to reference laboratory for further identification if deemed clinically relevant and antifungal therapy is considered (moderate).

5. Susceptibility testing

- Susceptibility testing should be performed on isolates of *P. aeruginosa* associated with early and intermittent colonisation (low).
- Susceptibility testing should be performed using a standardised and validated method (for instance, EUCAST) (moderate).
- Susceptibility testing of *P. aeruginosa* using automated devices cannot be recommended at this time (moderate).
- There is no evidence to support the routine use of biofilm testing methods (moderate).
- There is no evidence to support the routine use of multiple combination bactericidal antibiotic testing (MCBT) to select antibiotics for acute pulmonary exacerbations (high).
- Conventional susceptibility tests on *P. aeruginosa* isolates associated with chronic infection in CF are poorly reproducible and may not predict clinical response (high). This would also apply to susceptibility tests performed on newer agents such as ß-lactam/ß-lactam inhibitor combinations, such as ceftazidime-avibactam.
- Limiting testing to once annually on *P. aeruginosa* isolates associated with chronic infection does not adversely affect clinical outcomes for at least two years (moderate).
- There is insufficient evidence to make any specific recommendations regarding susceptibility testing of Bcc isolates. If laboratories do perform susceptibility testing a standardised method should be used (such as EUCAST disc diffusion) (low). There are no published breakpoints specifically for Bcc.
- In people with CF, isolates of *Aspergillus* spp. or other moulds, and in particular *A. fumigatus*, should be subjected to fungal susceptibility testing when antifungal treatment is being considered. Consider referral to a mycology reference laboratory (such as the UK Health Security Agency Mycology Reference Laboratory, Bristol) if local testing is not available (low).

6. Post-analytical processes: interfacing with clinical team

- CF clinicians and microbiologists should agree the structure, content and communication of laboratory reports (low).
- All new or suspected isolates of *P. aeruginosa,* methicillin-resistant *S. aureus (*MRSA), Bcc and *Mycobacterium* species should be communicated urgently to the CF clinical team (moderate).
- In addition to providing a laboratory service and advice on IPC, microbiologists should participate in regular multidisciplinary team (MDT) meetings to discuss the management of the service and of individuals with CF (low).

1. Microbiology and cystic fibrosis: an overview

1.1 Introduction

The quality of clinical care provided by a specialist CF centre for people with cystic fibrosis (CF) is dependent on good microbiological support. The pathogens someone with CF is infected by will determine their treatment, quality of life, prospects for transplantation and overall survival. Microbiological surveillance with accurate and prompt identification of respiratory pathogens is essential for timely commencement of eradication treatment, the use of appropriate long-term and rescue antibiotics for those with chronic bacterial infection and can also inform appropriate infection control measures.

1.2 Pathogens

The microbiology of the CF lung is complex and challenging. Historically, it was assumed that there was a limited spectrum of respiratory pathogens in CF, but the past decade has seen the recognition of many new pathogens and a broadening of our understanding of the complex balance of the microflora of the CF lung in health and disease. Newer techniques such as molecular testing have underpinned this growth in knowledge. The clinical significance of some organisms detected in CF respiratory samples is still unclear. A further area of uncertainty relates to the long-term impact of CFTR modulator therapy on the microbiology of CF lung disease.

1.2.1 *Staphylococcus aureus*

Staphylococcus aureus is a frequent isolate in respiratory samples from people with CF and may be cultured early in infancy. Small colony variants (SCVs) of *S. aureus* are well described in people with CF and have the potential to persist in the airways for prolonged periods of time. There is also concern that SCVs of *S. aureus* may be more tolerant to conventional anti-staphylococcal antibiotics.[1] In the UK, continuous anti-staphylococcal antibiotic prophylaxis is recommended for all children with CF under three years of age,[2] although a large multicentre UK study (CF START) is currently enrolling UK infants at diagnosis to test the safety and efficacy of this approach.

The overall prevalence of lower respiratory tract infection with MRSA among people with CF has increased significantly over the last decade in some countries.[3] The prevalence differs between specialist CF centres, possibly reflecting regional differences in prevalence of MRSA strains in local healthcare and community settings and differences in screening policies, infection control practices and use of eradication treatment.[4] Acquisition of MRSA is associated with a number of negative outcomes for individuals with CF including increased hospitalisation rates, increased use of oral, inhaled and intravenous antibiotic use and a greater rate of lung function decline.[5]

1.2.2 *Haemophilus influenzae*

Haemophilus influenzae is reported to be the most common CF pathogen at age one year.[6, 7] Since *H. influenzae* is a natural commensal of the upper respiratory tract infection must be distinguished from contamination / colonisation when *H. influenzae* is cultured from respiratory samples. *H. influenzae* responsible for CF lung infections are mostly non-capsulate and non-typeable and are not prevented by vaccines for capsule type b *H. influenzae*. Prevalence rises over time and the median age of acquisition in childhood estimated by the Australian AREST-CF studies was 3.1 years.[8] In the same cohort around 9% of annual lavage samples were positive for *H. influenzae*.[8]

1.2.3 *Streptococcus pneumoniae*

Streptococcus pneumoniae is occasionally isolated from young people with CF. The incidence of infection related to *S. pneumoniae* has dropped following the introduction of routine immunisation with pneumococcal conjugate vaccines.

1.2.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is the most common pathogen in CF and the prevalence increases with age.[7] In 2021, 20.2% of adults with CF in the UK had chronic *P. aeruginosa* infection, defined by the UK CF Registry as three or more isolates within the last twelve months, with a further 23.4% recorded as having intermittent infection.[7] Chronic *P. aeruginosa* infection in CF has been associated with more rapid lung function decline, exacerbation rates and mortality.[9] As a result, new *P. aeruginosa* infection in CF is typically treated with an intensive course of antibiotics aimed at eradicating the organism from the airways.

Options for such eradication therapy include inhaled, oral and IV antibiotics.[10] A large randomised controlled trial (RCT) published in 2020 did not show any significant advantage of IV over oral antibiotics when combined with inhaled colistimethate for twelve weeks.[11] Data from the UK CF Registry have shown a reduction in the proportion of people with CF who have chronic *P. aeruginosa* infection over recent years. In 2010, 13.5% of children and 56.2% of adults had chronic *P. aeruginosa* infection compared with 4.5% and 20.2%, respectively, in 2021.[7]

Some strains of *P. aeruginosa* are known to be transmissible between people with CF. Transmissible strains of *P. aeruginosa* have been associated with increased hospitalisation and IV antibiotic therapy.[12] A variety of microbiological techniques can be used to detect transmissible strains of *P. aeruginosa* as detailed in section 4.3.1 of this guideline. Previous Cystic Fibrosis Trust guidance has recommended regular surveillance and molecular typing of pathogens including *P. aeruginosa* to allow identification and prevention of cross-infection.[13]

1.2.5 *Burkholderia cepacia* complex and other *Burkholderia* species

The *Burkholderia cepacia* complex (Bcc) comprises a number of closely related species that, along with *B. gladioli*, can be significant pathogens for people with CF. Historically, there have been notable large-scale outbreaks of cross-infection with strains *B. cenocepacia*,[14] although cross-infection in CF has also been reported in other Bcc species.[15, 16] Strict infection control measures were implemented to address these cross-infection outbreaks. The most encountered Bcc in CF now is *B. multivorans*.[17]

The clinical outcome of infection with Bcc differs from species to species. *B. cenocepacia* is particularly virulent and has resulted in the premature deaths of many people with CF who acquired infection. *B. cenocepacia* infection is also associated with a much poorer outcome for lung transplantation and is regarded as a contra-indication for transplantation in many countries.

Cases of *B. pseudomallei*, a species closely related to the Bcc and the cause of the subtropical infection melioidosis, have been described in patients with CF returning from travel to areas where *B. pseudomallei* is endemic.[18] Cystic Fibrosis Trust has published a factsheet providing facts and advice on *B. pseudomallei* for individuals with CF planning travel to areas where *B. pseudomallei* may be encountered.[19]

1.2.6 Other bacterial species

While *P. aeruginosa* is by far the most common Gram-negative bacterial pathogen in CF, others are also encountered including species of *Pandoraea, Achromobacter, Ralstonia, Stenotrophomonas, Acinetobacter, Serratia, Chryseobacterium, Elizabethkingia, Cupriavidus* and *Inquilinus*. There are several potential reasons for more frequent recognition of these organisms among patients with CF, including greater detection through increased use of mass spectrometry technology (with broad identification databases) within most large diagnostic microbiology laboratories, and changes in taxonomy for microorganisms.

The prevalence of infection and clinical consequences differ from species to species, and not all have been proven to be pathogenic in CF. These organisms commonly display *in vitro* resistance to many antibiotics. They are commonly isolated from individuals who already have low baseline lung function. Initial infection can lead to chronic carriage, and for some of these species, cases are described with evidence of an associated host immune response and subsequent clinical deterioration.[20] There are no clinical studies to guide on whether eradication therapy is warranted. Case series of shared strains of some of these species suggesting potential cross-infection have also been reported at some CF centres.[20, 21]

The CF airways are not only colonised by aerobic species. There are steep oxygen gradients within CF lungs and relatively high levels of anaerobic bacteria can be readily identified using specialist culture and molecular identification methodologies.[22, 23] Growing microbiota evidence indicates that anaerobes are present in CF lung at high abundance, but the clinical significance of their presence remains unclear.[22, 24]

1.2.7 Nontuberculous mycobacteria

Nontuberculous mycobacteria (NTM) are a group of >150 species that are found in various environmental niches including water, soil, and other organic material. The NTM species which most commonly cause clinical disease in CF are those making up the *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex (MABSC). Three subspecies within the MABSC are currently recognised as: *M. abscessus* subsp. *bolletii, M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *abscessus*.[25] The prevalence of NTM infection in CF has been slowly rising over recent decades.[26–28] Data from the UK CF Registry in 2021 indicated that 3.9% of people with CF including 6.2% of adults had at least one respiratory sample positive for NTM.[7]

Over the last decade, evidence has emerged that suggests indirect person-to-person transmission of *M. abscessus* subsp. *massiliense* occurs among people with CF,[29–31] although several other studies have not identified evidence of cross-infection with *M. abscessus* in CF.[32–34] Cystic Fibrosis Trust has published infection control guidelines aimed at preventing the transmission of *M. abscessus* between people with CF,[35] and stringent infection control measures have been introduced at centres experiencing apparent outbreaks of *M. abscessus* infection.[29, 36] International consensus guidelines have been published on the management of NTM-pulmonary disease (NTM-PD) in CF.[25]

Treatment of NTM-PD is challenging and involves prolonged courses of multiple antibiotics in combination. There have no randomised controlled trials comparing treatment strategies for NTM-PD exclusively in CF.[25] *M. abscessus* infection is a contra-indication to lung transplantation in many transplant centres and countries.[25]

1.2.8 Fungi

Fungi are often isolated from the respiratory secretions of patients with CF. The most frequently isolated are *Aspergillus* and *Candida* species, but *Scedosporium, Exophiala* and *Trichosporon* species are also encountered. Outside of allergic bronchopulmonary aspergillosis, a hypersensitivity reaction associated with *A. fumigatus*, the clinical relevance of airway colonisation with fungi is often unclear. The concept of an aspergillus bronchitis is recognised,[37] but as with other fungal species there are no clinical studies that have demonstrated benefits of treatment intervention, other than small case studies. Many of these fungi may also cause complications at the time of lung transplantation.

1.2.9 Respiratory viruses

People with CF typically experience viral respiratory tract infections with the same frequency as the general population but the impact of these pathogens in CF can be more severe and more prolonged. Human rhinovirus is the most common respiratory virus detected in most observational studies in CF.[38] Other viruses that may be associated with pulmonary exacerbations include influenza, respiratory syncytial virus (RSV), coronavirus, parainfluenza, adenovirus and human metapneumovirus.

Treatment options for viral respiratory tract infections are limited with no randomised controlled trials conducted in CF. Currently available antivirals include neuraminidase inhibitors, such as oseltamivir and zanamivir, which are licensed for the treatment and prophylaxis of influenza. Prevention of viral respiratory infections centres on infection control measures, rigorous hand and respiratory hygiene and vaccination against influenza and SARS-CoV-2.

The threat from respiratory viruses for people with CF has been highlighted by the SARS-CoV-2 pandemic which emerged in late 2019. Between February and June 2020, 130 cases of SARS-CoV-2 infection in people with CF were reported to the European CF Registry .[39] Increased incidence was seen in lung transplant recipients and the overall case fatality rate was 3.8%. The SARS-CoV-2 pandemic has had a major impact on day-to-day life and the provision of usual healthcare services for people with CF, the consequences of which are yet to be fully determined. There will continue to be a risk of future pandemics from novel viruses, including influenza and coronaviruses, for which preparation and learning from the COVID-19 pandemic will be key.

1.2.10 CF microbiota

Studies utilising both non-culture-based and specialist culture methodologies have shown that CF airways are typically inhabited by a rich and diverse microbiota containing many bacterial, fungal and viral species that interact with each other. The microbiota in people with CF differs from that of healthy individuals, and furthermore in CF the diversity narrows with age and lung disease severity.[40] At present, the clinical relevance of these findings remains unclear and treatment decisions remain based on the isolation of individual pathogens, the most common of which are mentioned in the above sub-sections, and identified through standard culture.

2. Sampling issues

2.1 Sampling methods

Respiratory samples for culture or non-culture-based diagnosis can be obtained from people with CF who do not expectorate sputum with: cough swabs, cough plates, oropharyngeal culture, laryngeal or naso-pharyngeal aspirate, induced sputum following hypertonic saline, BAL and bronchoscopy brush specimens.

2.1.1. Comparison of yield

Several studies have compared yields of pathogens using different methods:

- Thomassen et al compared bacteriological cultures of sputum and specimens obtained at thoracotomy from 17 people with CF. All organisms found in the surgical specimens were also detected in the sputum. The authors concluded that the observed correlation of sputum and lung specimen culture results supports the value of sputum culture in the management of lung disease in CF.[41]
- A comparative study of oropharyngeal suction (OPS) cultures and bronchial sampling in 26 non-expectorating people with CF demonstrated a sensitivity and specificity of 70% and 83% for isolation of *P. aeruginosa*, and 80% and 91% for isolation of S. aureus respectively. OPS cultures yielding *P. aeruginosa* or *S. aureus* are highly predictive, but the absence of these organisms in such cultures does not rule out their presence in the lower airways.[42]
- Pathogens obtained by OPS and BAL were compared in 44 children with chronic lung infection (five had CF). Twenty seven of 44 (61%) BAL samples yielded a positive bacterial culture. The sensitivity of OPS in detecting the same pathogen as found in BAL was 89% (24/27 samples), the specificity was 94% (16/17 samples) and the predictive value was 91% (40/44 samples). The authors concluded that OPS is a simple non-invasive method which may be helpful in the diagnosis of chronic pulmonary infection.[43]
- The yield from throat swabs from 50 children with CF was studied using three different processing methods. Swabs were cultured directly or placed into either saline or Ringer's lactate, left for 30 minutes, and the liquid then inoculated onto solid media for culture. A total of 124 isolates not considered part of normal throat flora were obtained (*Candida albicans* (39); *P. aeruginosa* (36); *S. aureus* (22); coliforms (12); other Pseudomonads (5); *A. fumigatus* (5); *H. influenzae* (2); other *Candida* spp. (2)). No method cultured all 124 isolates. The three methods gave sensitivities of 91.9% (Ringer's lactate), 82.3% (saline), and 59.7% (direct culture), respectively.[44]
- Seventy-five infants with CF diagnosed by neonatal screening had simultaneous BAL and OPS samples collected for quantitative bacterial culture at a mean age of 17 months. Ten children undergoing bronchoscopy for stridor served as controls. *S. aureus* (19%), *P. aeruginosa* (11%), and *H. influenzae* (8%) were the most frequent isolates from BAL samples; the corresponding frequency from OPS were *S. aureus* (47%), *Escherichia coli* (23%), *H. influenzae* (15%), and *P. aeruginosa* (13%). The sensitivity, specificity, and positive and negative predictive values of OPS for pathogens causing lower respiratory infections was 82%, 83%, 41%, and 97%, respectively. When the same species was isolated from paired OPS and BAL cultures, pulsed-field gel electrophoresis (PFGE) showed some strains were unrelated. The authors concluded that oropharyngeal cultures do not reliably predict the presence of bacterial pathogens in the lower airways of young CF children.[45]
- In one study which enrolled 10 adults with CF, recovery of bacterial pathogens was 79% for induced sputum, 76% for spontaneously expectorated sputum, and 73% for BAL. There was perfect agreement between the three methods for recovery of Bcc and mucoid *P. aeruginosa* but not for non-mucoid *P. aeruginosa*, *H. influenzae*, and other non-fermenting Gram-negative bacilli.[46]
- Rosenfeld et al reviewed the results of bacterial cultures from three studies of simultaneous oropharyngeal and BAL sampling from 141 children with CF under five years old. In subjects less than 18 months of age oropharyngeal cultures had a sensitivity, specificity, positive and negative predictive value of 44%, 95%, 44% and 95% respectively in relation to isolating *P. aeruginosa* from BAL. They concluded that in this age range, a negative throat culture is helpful in ruling out lower airway infection but a positive culture does not reliably rule in the presence of *P. aeruginosa*.[47]
- Oropharyngeal, sputum and BAL samples were evaluated from 38 stable people with CF for the detection of *P. aeruginosa* and isolates were typed by PFGE. Sensitivity, specificity, negative and positive predictive values to

detect *P. aeruginosa* were 35.7%, 96.2%, 73.5% and 83.3% for oropharyngeal cultures in non-expectorating individuals and 91.7%, 100%, 94.1%, and 100% for sputum cultures from expectorating individuals, respectively. Molecular typing showed that genotypes of *P. aeruginosa* isolates recovered from oropharyngeal swabs and sputum differed from the strains recovered by bronchoscopy in 55% and 40%, respectively. Longitudinal variations in the genotype occurred in 62% of samples and were mostly evident in bronchoscopy samples only. The study concluded that sputum was of equal value as BAL for detection of *P. aeruginosa*, but cultures from the oropharynx are not suitable for characterizing bacterial conditions in the CF lung. Different genotypes within the same host and longitudinal genetic alterations are common and may be detectable in the BAL fluid exclusively.[48]

- Equi et al compared cough swabs and spontaneously expectorated sputum samples for 30 children with CF. Cough swabs yielded 12 isolates and the corresponding sputum samples 36 isolates, giving a specificity of 100% and sensitivity of 34% for cough swabs compared to sputum. No details of the identity of these isolates were provided. Positive and negative predictive values for cough swab were 100% and 21%, respectively.[49]
- Kabra et al compared cough swabs and throat swabs taken before and after physiotherapy with sputum samples; 387 samples were collected from 48 people with CF. Sensitivity for isolation of *P. aeruginosa* was 40%, 42% and 82% for cough swabs, throat swabs taken before physiotherapy and throat swabs taken after physiotherapy, respectively, and for *S. aureus* 57%, 50% and 100%. Specificity was high (99-100%) for all three sampling methods.[50]
- In a study of 43 children with CF, results of culture of sputum or OPS cough swab samples in those unable to expectorate sputum were compared with the results from induced samples taken following administration of 6% hypertonic saline solution (HTS). Sputum induction was tolerated in 41/43 subjects. Four subjects were able to expectorate sputum before and 19 after HTS induction. Four pre-HTS samples were positive when post-HTS samples were negative. Thirteen post-HTS samples were positive when pre-HTS samples were negative. The authors concluded that cultures on HTS-induced samples provide additional microbiological information.[51]
- Maiya et al carried out a randomised prospective study of respiratory sample collection involving 31 people with CF aged between eight and 16 years old and found that, of 20 who had a positive sputum culture, 16 were also positive by cough plate (sensitivity 80%); this compared with seven positive cultures each for moistened swab and dry swab (sensitivity 35%), respectively. *P. aeruginosa* was isolated from cough plates as effectively as from sputum but not from cough swabs. Conversely *Aspergillus* species were isolated from sputum samples but not from either cough plates or cough swabs.[52]
- The performance of BAL, protected brush specimens and spontaneously expectorated sputum as specimens in 12 adults with CF for bacteriological culture of the lung was evaluated by Aaron et al. *P. aeruginosa* was isolated from each of the samples from all 12 subjects. Genotyping of isolates showed full correspondence between the specimens, indicating that bronchoscopy did not offer any advantage in sensitivity.[53]
- The yield of induced sputum was compared to BAL samples collected from 11 non-expectorating children with CF aged 3–7.4 years old. Induced sputum was obtained from 10 subjects and eight of these yielded the same predominant bacterial pathogens as isolated from BAL.[54]
- A prospective, internally controlled, interventional study compared pathogen yield from cough swabs, sputum induction and single lobe, two-lobe and six-lobe BAL in 124 children with CF. One hundred and sixty-seven successful sputum induction procedures were paired with cough swabs. Sputum induction yielded significant pathogens on 63 (38%) occasions, compared to only 24 (14%) cough swabs. Thirty-five patients had 41 paired sputum induction and BAL procedures. Twenty-eight of the 41 were positive for at least one of the concurrent samples and 39 pathogens were isolated. Sputum induction identified 27 (68%) of the 39 pathogens, compared to 22 (56%) by single lobe, 28 (72%) by two-lobe and 33 (85%) by six-lobe BAL. None of these differences were statistically significant.[55]
- The comparative yield of nasal swabs, cough swabs and sputum were compared in 105 expectorating adults and children with CF. This study also compared nasal swab, cough swab and induced sputum in 30 non-expectorating individuals and nasal swab, cough swab and BAL in 39 individuals undergoing the latter. Nasal swabs had poor sensitivity for the detection of lower airways pathogens in all three groups. Although cough swabs yielded fewer pathogens than expectorated sputa, these differences were not statistically significant in those able to expectorate. Similarly, there were no

statistically significant differences in the yield of cough swabs compared to induced sputa and BAL in the non-expectorating and BAL patient groups. However, there were too few subjects with *P. aeruginosa* and *H. influenzae* in the non-expectorating group and too few subjects with *H. influenzae* in the BAL group for statistical analysis to be performed.[56]

- The comparative yield of 75 paired cough swab and sputum samples from 22 adults and eight children with CF was assessed both by conventional culture-based methods and next-generation sequencing. Culture-independent cough swabs showed poor consistency with culture-dependent/independent analysis of sputa. The authors concluded that cough swabs give an inaccurate diagnosis of lower airways colonisation and should not be used as a diagnostic test in CF.[57]
- De Bel et al evaluated two different methods of sample decontamination to aid recovery of NTM from respiratory samples collected from people with CF. They studied 795 samples from 312 patients with CF. None of the 145 cough swabs cultured NTM, compared to 18 of the 647 sputum samples. The authors concluded that cough swabs are low quality samples for the isolation of NTM.[58]
- Conversely, a study comparing RGM medium – a selective medium for nontuberculous mycobacteria – with *B. cepacia* selective agar (BCSA) medium showed that the proportion of deep pharyngeal swabs positive for NTM was 5.4% on RGM compared to only 0.6% on BCSA. Therefore, the use of an appropriate solid medium may enhance the detection of NTM from pharyngeal swabs. However, the proportion of sputum/tracheal aspirates and BAL growing NTM on RGM was 12.2% and 25% respective. Hence pharyngeal swabs were less sensitive than sputum or BAL, even when RGM medium was used.[59]
- Sequentially paired OPS followed by induced sputum was performed in children with CF aged under five attending a single centre in South Africa. Paired OPS and induced sputum samples were obtained in 98 (85%) of 113 attempts from 32 children. There was a statistically significant increased yield of *S. aureus* and *H. influenzae* (but not *P. aeruginosa*) from induced sputum samples. Induced sputum sampling was deemed feasible and safe in young children with CF.[60]
- A five-year prospective observational study followed 42 children with CF. Forty-one induced sputum samples were obtained at annual review from 29 patients during the study period, six of which yielded a nontuberculous mycobacterium which had not previously been identified. They also identified three new cases of *P. aeruginosa* as well as single new cases of Bcc and MRSA.[61]
- A study prospectively followed up 100 adults with CF over a 12-month period. Sputum, nose swabs and throat swabs were collected every two months and during every pulmonary exacerbation. These were examined for the presence of a range of respiratory viruses using PCR. Complete sets of samples were obtained on 469 visits and one or more viruses detected on 151 (32.2%) of occasions. Sputum samples identified 58% of these viruses, compared to 46.7% and 43.3% for nose and throat swabs, respectively. Sputum samples also identified a wider diversity of viruses.[62]

2.1.2 Other national and international guidance

The National Institute for Health and Care Excellence (NICE) Cystic Fibrosis: Diagnosis and Management guideline (October 2017) recommends taking respiratory secretion samples for microbiological investigations, using sputum samples if possible, or a cough swab/nasopharyngeal aspirate if not.[63] It advises thinking about BAL to obtain airways samples for microbiological investigation if the individual had not responded adequately to treatment and the cause could not be identified with non-invasive upper airway secretion sampling (including sputum induction). Sampling should be performed every eight weeks in children and young people and every three months in adults.

The Association of Chartered Physiotherapists in Cystic Fibrosis (ACPCF) has produced Standards of Care and Good Clinical Practice for Physiotherapy Management of Cystic Fibrosis.[64] It recommends induced sputum should be considered as a means of sampling the microbiome of the CF lung, particularly in those individuals who do not expectorate sputum. The guidance provides information on good practice points for carrying out a safe induced sputum procedure.

Recommendations

- Respiratory sampling should be undertaken at each hospital visit and at times of respiratory exacerbation (low).
- Sputum is the recommended specimen for routine sampling (moderate).
- Sputum induction is superior to cough swabs for isolating CF pathogens in children (moderate). This is likely to be the same for adults (low).

- Sputum induction is equivalent to two-lobe BAL in symptomatic children with CF (moderate). This is likely to be the same for adults (low).
- A cough swab should only be used if a patient cannot expectorate and the use of sputum induction is not feasible at that time of sampling (low).
- Cough swabs should not be used in preference to sputum or BAL for the isolation of nontuberculous mycobacteria (low). Expert opinion is that cough swabs will have a lower yield to detect fungi in the lower airways. However, if cough swabs are used, they are only suitable for culture directly on solid media and not in liquid culture systems (low).
- Cough swabs should not be used for culture-independent analysis (low).
- Sputum induction or BAL should be considered to identify lower airway infection in patients who are clinically deteriorating, particularly those with persistently negative cough swabs (low).
- Nasal swabs are not suitable for the detection of lower airways pathogens (moderate).
- Sputum samples are more sensitive than nose/throat samples for the detection of respiratory viruses by PCR (low).

2.2 Transport and storage of samples

Traditional teaching has been that processing the sample as soon as possible after collection will yield the most accurate microbiological results. Any delay in processing, particularly with storage at room temperature, was thought to increase the overgrowth of more-rapidly multiplying bacteria, which could mask true pathogens; conversely, preventing overgrowth by refrigeration could result in the death of fragile pathogenic organisms. The emphasis has therefore been on prompt processing to optimise analysis quality.

There are few studies evaluating the optimal environmental conditions for storing CF sputum specimens prior to microbiological analysis. There are more studies examining the survival of organisms in non-CF sputa under different storage conditions which may be extrapolated to the likely impact on the quality of results of CF samples.

2.2.1 Refrigeration

Some studies have reported negative effects of refrigeration on sputum cultures. Gould et al found that *H. influenzae, Moraxella catarrhalis* and *S. pneumoniae* were lost in 8.7% of samples kept at 4°C for 48h.[65]

Pye et al studied sputum samples from patients with bronchiectasis using a quantitative method, comparing counts obtained from immediate processing with those following storage at 4°C for 24h or following storage at 20°C for 24h, including the survival when samples were posted back by first-class mail. Fifteen of the 38 samples studied had *P. aeruginosa* as the predominant organism. There was at least a 10-fold loss in viability in 24% of samples stored at 4°C compared with only 8% stored at 20°C.[66]

There is also evidence that freezing of sputum from patients with non-CF bronchiectasis reduces the recovery of *P. aeruginosa*,[67] and Bcc isolates "die" (become dormant) when kept at 4°C.[68]

However, other studies have not demonstrated a negative impact of refrigeration on quality of sputum samples. Quantities of *S. aureus, P. aeruginosa, H. influenzae* or *S. pneumoniae* were unaffected by storage at 4°C for up to two days.[69] Williams et al also found that pneumococci survived refrigeration for several days.[70] A prospective study on 50 sputum samples (patient details were not provided) showed that, although refrigeration of sputum led to changes in the interpretation of the Gram stain, it did not lead to significant loss of bacterial growth after 20h of refrigeration.[71] Refrigeration at 2–4°C for up to 14 days was shown to minimise the decay in viability of mycobacteria.[72] During the same study, samples kept at 37°C saw significant increases in numbers of non-specific microflora and almost complete loss of mycobacteria between the fourth and eighth day.[72]

The impact of freezing sputum samples has also been studied. Sputum from 10 people with CF with chronic *P. aeruginosa* infection was collected at routine clinic visits. Samples were split so that half were cultured within three hours of collection and the other half were placed in a -20°C freezer for seven days to mimic home storage. After a week, the freezer aliquot was cultured, and colony counts of *P. aeruginosa* were compared with those obtained by culture on the day of collection. Freezing resulted in a 10^3 CFU/mL drop in quantities of *P. aeruginosa*. However, there was no difference in antimicrobial susceptibility or detection of four virulence factors (proteases, siderophore, haemolysis, rhamnolipids).[73]

Similarly, a study assessed the impact of delayed freezing on the culture-independent microbiological analysis of sputum expectorated from eight adults with CF. Sputum aliquots were stored at room temperature for increasing time periods up to 72h before being frozen at -80°C. Increases in time to freezing were associated with

substantial variation in samples with high diversity bacterial communities but had little impact on the variability of low diversity communities. Significant decreases in the abundance of anaerobes were noted after 12h at room temperature.[74]

2.2.2 Storage at room temperature

A 50% decrease in isolation rate of *H. influenzae* and *S. pneumoniae* was observed in 102 samples of sputum submitted by post from patients attending chest clinics. Samples were typically delayed by 24h to 30h prior to culture.[75] *S. pneumoniae* was shown to survive at room temperature for over two days but overgrowth occurred after 12h.[70] A study of 34,314 sputum samples submitted to a reference laboratory showed that fungi (including *Aspergillus* species) could still be isolated even after delays of up to three months after collection.[76]

The ability to grow mycobacteria from sputum specimens was reduced with storage time at room temperature in a tropical country although smear-positivity was unaffected for four weeks. Culture positivity declined from 88% at the time of storage to 83%, 68%, 22%, 13% and 0% after 3, 7, 14, 21, and 28 days, respectively. It was therefore recommended not to store samples beyond three days prior to culture.[77]

Another study compared the impact of storing sputum samples from people with CF at room temperature versus refrigeration on the metabolically active microbial community. Five samples were divided into two aliquots; one was immediately treated with a bacteriostatic agent (RNA*Later*) and stored at 4°C and the other was left at 20°C (room temperature) for a further 24h before being treated with the bacteriostatic agent. Treated samples were then subjected to mRNA extraction and 16S (bacterial) and 28S (fungal) PCR amplification, denaturing gradient gel electrophoresis (DGGE) and quantitative PCR. Results showed that there was no significant effect of storage conditions on the fungal content of samples. However, there was a doubling in the numbers of *Pseudomonas* species and a >50% decline in the numbers of *H. influenzae* in samples stored at room temperature.[78]

Another study of the effects of sample storage on culture-independent analyses of CF sputa showed less-pronounced effects. Zhao et al divided a single sample obtained routinely from a young adult with CF and stored it for 2h at 4°C before dividing into multiple aliquots. These were then either immediately frozen at -80°C, kept at room temperature (20°C), refrigerated at 4°C, or frozen at -20°C for one, two or four weeks respectively. Culture-independent analysis of all of the variously stored aliquots using 16S PCR pyrosequencing did show increased numbers of *P. aeruginosa* in samples stored at room temperature, although this was considered to be within the boundaries of inter- and intra-run variability. There was no significant difference in organism numbers observed over time.[79]

2.2.3 Transport and near-patient testing

The impact of submitting sputum samples by post was studied using 32 sputa from people with CF. Samples were cultured on the same day as expectorated and compared to aliquots of the same samples sent by post and cultured the following day. There were no differences in the range of pathogens, or the mean number of colony-forming units obtained.[80]

Another study compared 42 freshly processed samples from people with bronchiectasis with aliquots processed 24h after posting to the laboratory. There were no significant differences in overall total colony counts or in the total colony counts of *P. aeruginosa* (13 samples), *H. influenzae* (13 samples), *M. catarrhalis* (ten samples) or *S. pneumoniae* (four samples) obtained when processed 24h after posting.[66]

Deep nasal flocked swabs and anterior nares foam swabs after saline installation were collected in hospital and compared with two foam nasal swabs collected at home for the detection of respiratory viruses. Paired swabs were collected from 28 people with CF aged 6–18 years old. Nasal swabs collected at home were then posted to the laboratory for multiplex respiratory virus PCR. Home-collected swabs were obtained closer to symptom onset, on average 2.3 days earlier, and had a higher positivity than hospital-collected swabs. Rhinovirus accounted for 73% of virus detections. Home collection of nasal swabs for respiratory virus screening was well tolerated and feasible.[81]

2.2.4 National recommendations

The National Standard Method for Investigation advises that all respiratory specimens should be fresh and collected prior to commencing antibiotics.[82] It does not make specific recommendations regarding CF specimens. Samples should be transported and processed promptly to give the best opportunity to isolate pathogens and reduce the risk of overgrowth of contaminants. If processing is delayed more than 24h, refrigeration is preferable to storage at ambient temperature. If samples are not processed on the same day as collection, this should be noted on the report and the results interpreted with care. The American Society of Microbiology

Cumulative Techniques and Procedures in Clinical Microbiology document specific for Cystic Fibrosis Microbiology states that if the sample cannot be plated out at the site of collection, then specimens should be transported at 4°C; "*P. aeruginosa* survives this temperature for 24 hours, and most of the other pathogens of people with CF will survive this temperature for a minimum of one to two hours".[83]

The American Society of Microbiology Cumulative Techniques and Procedures in Clinical Microbiology document specific for Lower Respiratory Tract Infections is less specific than the above, stating only that a two-hour maximum delay between collection and processing is generally acceptable. If there are delays beyond two hours, protocols including sample refrigeration or transport of inoculated media should be developed.[84]

Recommendations

- Samples should be sent promptly to the laboratory and processed as soon as possible; there is insufficient evidence to judge whether refrigeration of samples produces better microbiological results than following storage at room temperature. However, current consensus is that if samples are delayed more than a few hours they should be refrigerated at 4°C (very low).
- Freezing of samples at -20°C prior to culture may result in a reduction in colony counts of *P. aeruginosa* (very low).
- Evidence of the impact on sample quality following submission by post is limited. Until further research improves our understanding it would seem prudent that the results of bacterial culture of respiratory samples submitted to the laboratory by post should be interpreted with caution (low).
- Nasal swabs taken at home and posted to the laboratory for respiratory virus PCR may have a higher positivity rate than hospital-collected swabs if taken earlier after symptom onset (low).

3. Sampling processing

This section reviews standards for processing respiratory samples from people with CF. Information on processing other samples types can be found in the national **Standards for Microbiology Investigations (SMI)**. Laboratories are encouraged to ensure they follow the national SMI as a minimal requirement.

3.1 Homogenisation

The viscosity and mucopurulent nature of sputum presents challenges in obtaining appropriate samples for microbial culture, and particularly when quantitative culture is required. Methodologies to liquefy or reduce viscosity include mechanical disruption by glass beads or chemically by mucolytic agents.

In the 2021 edition of the UK Standards for Microbiology Investigations, homogenisation with the addition of 0.1% solution of dithiothreitol (DTT) or N-acetyl L-cysteine (NALC) to sputum is recommended as a sample preparation step.[85] Chemical homogenisation with DTT was compared to homogenisation with saline or glass beads for recovery of bacteria from sputum samples obtained from 18 people with non-CF bronchiectasis.[86] The quantity of pathogen recovered was significantly higher from samples homogenised using DTT compared to either saline or glass beads, particularly of *H. influenzae*. DTT had no inhibitory effect on the recovery of *P. aeruginosa, H. influenzae, S. pneumoniae*, or *M. catarrhalis.*

Stokell et al reviewed nine expectorated sputum samples from one individual with CF and measured bacterial abundance in DTT-treated sputum to determine if added mechanical homogenisation (MH) decreases the variability in abundance of all bacteria. They found a significant difference between the mean bacterial abundances in aliquots that were subjected to only DTT treatment and those of the aliquots which included a MH step (all bacteria, P=0.04; *B. multivorans*, P=0.05). Though the results are not statistically significant they concluded that an additional MH step increases the homogeneity of bacteria in sputum.[87]

Studies predominantly looking at the quantification of bacterial counts from sputum treated with DTT noted DTT-treated sputum was not completely robust for isolating *S. aureus* and *C. albicans* but did not affect *P. aeruginosa*.[69, 88]

Recommendations

- The use of mucolytic agents, such as dithiothreitol (DTT), allows dilution and facilitates retrieval of microorganisms present in the sample as is the current national recommendation (moderate).
- There is insufficient evidence to currently recommend mechanical methods of homogenisation (high).

3.2 Microscopy

3.2.1 Gram stain

The utility of sputum Gram stain in assessing both salivary contamination and in predicting the presence of pathogens was investigated using 287 respiratory samples from 270 people with CF. Microscopy was performed by a single observer, assessing sample quality based on the ratio of leucocytes to squamous epithelial cells (a ratio of >5 indicated acceptable sample quality) and predicting culture results based on observed bacterial morphotypes. Overall, 220 (77%) of samples were of acceptable quality. The appearance of bacteria on direct microscopy of sputum had a positive predictive value for subsequent culture of 98% for *P. aeruginosa*, 84% for Bcc, 86% for *S. aureus*, and 100% for *H. influenzae*.[89]

This was further evaluated using sputum samples from 101 people with CF. Microscopy was performed by two different observers in a blinded fashion. Subjectively all but one sample appeared purulent but only 59 samples would have been accepted for culture using a quality score based upon leucocytes and squamous epithelial cells. In addition, there was agreement for the quality score between both observers for only 21 samples, suggesting poor reproducibility. All but four of the 41 samples that would have been rejected using the quality score grew CF-associated Gram-negative bacilli. The positive predictive value of large quantities of Gram-negative bacilli and subsequent growth of *P. aeruginosa* was 85%. However, the negative predictive value for absence of Gram-negative bacilli and negative culture was very poor at just 10%. Results suggested that routine Gram staining is not necessary for evaluating the quality or results of CF sputum samples.[90]

Recommendation

- There is insufficient evidence to support the routine use of Gram staining of sputum samples from people with CF, either as a marker of specimen quality or as a predictor of subsequent culture results (moderate).

3.2.2 Acid-fast staining for mycobacteria

The sensitivity of microscopy is greatly influenced by sample quality, mycobacterial species, and laboratory expertise, and therefore should not be used as the only indicator of bacterial load.[91–94] It does allow the direct evaluation of bacterial burden and may reveal false-negative culture results caused by excessive sample decontamination or overgrowth of conventional bacteria. Many CF culture-positive samples are microscopy negative. Microscopy cannot reliably distinguish NTM from *M. tuberculosis*. The limited available data on MABSC suggest that, in contrast to TB where people with smear-positive samples cause most transmission, smear-negative individuals may still contribute to cross-infection.[29] Given the comments above the utility of microscopy is uncertain. Nevertheless, microscopy can provide a useful adjunct to culture and may be helpful where full mycobacterial examination is undertaken. Best results are obtained by using auramine-phenol staining rather than the Ziehl-Neelsen method due to enhanced sensitivity.[91–94]

Recommendation

- Where microscopy is undertaken the preferred method is to use auramine-phenol staining (high).

3.3 Conventional Culture

3.3.1 *P. aeruginosa*

There are few data on the utility of specific selective media for isolation of *P. aeruginosa* from the respiratory secretions of people with CF.

A comparison of different selective and non-selective media on 258 respiratory tract secretions from an unknown number of people with CF was conducted at a single North American CF centre. All plates were incubated at 35°C in 5–7% CO_2 for 72h. Of the 137 isolates of *P. aeruginosa* obtained, all 137 (100%) grew on MacConkey agar, 136/137 (99.3%) were isolated on chocolate agar and 113 (82.5%) grew on cetrimide agar.[95]

A novel chromogenic agar for isolating and identifying *P. aeruginosa* (PS-ID) was compared against 5% horse blood agar and *Pseudomonas* CN selective agar (PCN) using 100 sputa from distinct people with CF. Plates were incubated at 37°C in air for 72 hours. *P. aeruginosa* was isolated from 62 samples after 72h incubation, with 59 samples (95.2%) positive on both PS-ID and PCN and 56 samples (90.3%) positive on BA. Almost all isolates were grown within 48h incubation. Colonies producing a purple colour on PS-ID had a positive predictive value of 98.3% for being *P. aeruginosa*.[96]

The expression of the mucoid phenotype on six different isolation media was examined using 15 *P. aeruginosa* isolates from people with CF. All 15 expressed the mucoid phenotype on *Pseudomonas* Isolation Agar (PIA), and 14 of 15 expressed it on MacConkey agar. Expression on four other non-selective, basal media was much more variable.[97] Govan and Deretic observed that if an isolate was non-mucoid on PIA it was always non-mucoid on other isolation media.[98]

Some constituents of selective media have been shown to be inhibitory for CF isolates of *P. aeruginosa*. Of 200 CF isolates tested, 22 were inhibited by 16mg/L Irgasan (the selective agent in PIA), and 45 and 15 were inhibited by 8mg/L nalidixic acid and 128mg/L cetrimide, respectively (the latter two being selective agents in *Pseudomonas* selective agar).[99]

Recommendations

- There is insufficient evidence to suggest that use of a selective medium enhances the yield of *P. aeruginosa* from the respiratory secretions of people with CF. However, the use of some selective media may assist in identification, particularly the appearance of pigmentation and the mucoid phenotype (moderate).
- Plates should be incubated at 35–37°C in air and examined after overnight incubation and after at least another 24h (low).
- The presence of *P. aeruginosa* should always be reported, irrespective of quantity (high).

3.3.2 *B. cepacia complex*

The use of a selective medium is essential for the isolation of Bcc and inhibition of other organisms commonly found in the respiratory secretions of people with CF. There have been many studies performed over time, comparing various selective media for the successful isolation of Bcc from CF respiratory samples.[100–104]

Preece et al, compared five Bcc selective agars including:[105]

- BCSA (bioMérieux, Basingstoke UK, or Nürtingen, Germany)
- Cepacia selective agar (bioMérieux, Basingstoke UK, or Nürtingen, Germany)
- *Burkholderia cepacia* agar (Oxoid Ltd., Basingstoke, UK).
- BD™ Cepacia Medium and (BD Diagnostic Systems, Oxford, UK)
- BD™ oxidation-fermentation polymyxin-bacitracin-lactose (OFPBL) medium (BD Diagnostic Systems, Oxford, UK)

A Bcc experimental strain panel (n=26) and clinical isolates (n=43) were inoculated onto all media.[106] None of the five brands of media were able to support the growth of every Bcc isolate within the standard incubation period of five days. Cepacia selective agar showed the highest sensitivity (93%), with only three isolates being inhibited (one *B. stabilis* isolate and two *B. multivorans* isolates), whereas the growth of seven isolates was inhibited on Oxoid *B. cepacia* agar (one *B. cenocepacia* isolate, five *B. multivorans* isolates, and one *B. stabilis* isolate). Extended incubation up to 10 days resulted in the recovery of three additional isolates on BD OFPBL medium but had no impact on other Bcc selective agars. The media were also challenged with non-*B. cepacia* organisms. All Bcc selective agars showed poor ability to inhibit the growth of fungi, particularly *Aspergillus* spp., which concurs with other studies.[106]

Naqvi et al, cultured 307 consecutive CF respiratory samples onto *B. cepacia* selective agar (Oxoid-ThermoFisher, UK) and compared the rates of isolation at day one to day five of incubation at 37°C. Thirteen cultures (4%) from eight adult and five paediatric specimens grew Bcc; all were recovered within 48h. It was inferred that prolonged incubation is not required for recovery of Bcc if selective medium containing gentamicin and polymyxin is used, consistent with the results of previous studies. In their study three isolates of *A. fumigatus* and two isolates of *Lomentospora prolificans* were recovered on prolonged incubation, after day five.[107]

The limitations of the study are that only one selective media was used, and it was not evaluated against a range of *B. cepacia* subspecies. In some countries, *B. contaminans* is the most prevalent species isolated. Martina et al used *recA*-PCR-based techniques to identify 120 Bcc isolates collected during 2004–2010 from 66 patients in two CF reference centres in Argentina. *B. contaminans* was the species most frequently recovered (57.6%) followed by *B. cenocepacia* (15%). Analysis of *B. contaminans* isolates revealed that 85% of the population carried the *recA*-ST-71 allele.[108] Clusters of *B. contaminans* have been reported in other countries.[109]

Our guidelines recommend that no less than 100µL of liquid sample should be inoculated onto Bcc agar for a minimum of three days. The addition of an anti-fungal would be of great advantage as cultures must otherwise be abandoned early. Naqvi et al recommended gentamicin and polymyxin as essential selective agents but each Bcc agar by various manufacturers have different formulae.[105, 107] The consistent component is polymyxin but each media has this incorporated at different concentrations.

Our previous recommendations were based on published studies that have compared various laboratory media for the successful isolation of Bcc from CF respiratory samples. Currently we cannot recommend a specific brand or manufacturer and further, more extensive studies are warranted.

Our recommendations remain as with previous guidelines, that laboratories should use a selective medium to enhance the detection of Bcc. The Bcc selective medium should have a high sensitivity and specificity for Bcc. All selective Bcc agars contain antibacterial agents, the addition of an antifungal would be advantageous.

Recommendations

- All CF respiratory samples must be cultured to a good quality Bcc selective agar and incubated as per manufactures instructions or by robust in-house evaluation (low).
- We would also recommend that all routine cultures are incubated as per the chosen selective agar manufacturer's recommendation or for a minimum of three days where guidance is not available (low).
- The presence of Bcc should always be reported, irrespective of quantity (high).

3.3.3 *S. aureus*

The use of mannitol salt agar (MSA) has been associated with improved detection of *S. aureus*. A collection of 60 sputum samples from 14 people with CF yielded 50 samples positive for *S. aureus* from 11 people. Plates had been incubated at 37°C in air for 48h. The inclusion of MSA increased the number of samples from which *S. aureus* was isolated from 21 to 50 when compared to non-selective media.[110]

A retrospective review of all culture results of CF respiratory samples during a single calendar year

in one US laboratory found 207 samples positive for *S. aureus*. More than 98% of those samples with greater than 1+ colony forming unit of growth of yellow colonies on MSA were ultimately confirmed as *S. aureus* on further testing.[111]

A comparative evaluation of MSA and CHROMagar Staph. aureus selective and differential agar (CSA) was conducted using 220 respiratory samples from an unknown number of people with CF. Plates were incubated at 37°C in 5–10% CO_2 for 72h. A total of 66 samples were positive for *S. aureus*, all of which were positive on CSA (100%) and 59 (89.4%) were positive on MSA. Incubation of plates for 48 hours resulted in an increase in *S. aureus* recovery of 8% for MSA and 11% for CSA compared to 24 hours incubation. No non-*S. aureus* isolates were misidentified on CSA, giving 100% specificity. The cost of CSA ($3.07 per plate) was higher than the cost of MSA ($1.31) in this study but could be offset by reducing the cost of additional subcultures ($1.03) and additional biochemical confirmatory tests such as slide coagulase.[112]

A comparative evaluation of CSA, MSA, and 5% sheep blood agar was conducted using 200 sputum samples from an unknown number of people with CF. Plates were incubated at 37°C in air for 72h. *S. aureus* was isolated from 50 samples. After 16–20h incubation 39 samples (78%) were positive on CSA compared to 30 samples (60%) and 29 samples (58%) for blood agar (BA) and MSA, respectively. Two (4%) *S. aureus* isolates did not grow after 48h incubation. After 72h incubation the sensitivities of CSA, MSA, and BA were 98%, 96%, and 96%, respectively (P=NS). The specificity of CSA was 99%.[113]

Recommendations

- A selective medium should be used to enhance the detection of *S. aureus* from respiratory samples of people with CF. Options include mannitol salt agar or a chromogenic medium that has been validated for isolation of *S. aureus* from CF samples (moderate).
- Selective plates for *S. aureus* should be incubated at 35–37°C in air and examined after overnight incubation and after at least another 24h (low).
- The presence of *S. aureus* should always be reported, irrespective of quantity (moderate).

3.3.4 *H. influenzae*

The recovery of *H. influenzae* from the respiratory secretions of people with CF has been enhanced by using medium supplemented with N-acetyl-D-glucosamine (NAG), haemin (X-factor), nicotinamide adenine dinucleotide (NAD; V-factor) and cefsulodin disks. The NAG medium was compared with chocolate agar with or without a bacitracin disk for isolating *H. influenzae* from 203 sputum samples obtained from 30 people with CF. Plates were incubated overnight at 37°C in 5% CO_2. *H. influenzae* was isolated from 86 samples (42%) from 24 people. The recovery rate on NAG was superior to chocolate with or without a bacitracin disk, with 84/86 (97.7%) isolates growing on NAG, compared to 46/86 (53.5%) and 62/86 (72.1%) growing on chocolate with or without a bacitracin disk, respectively.[114]

A comparative evaluation of two selective media, cefsulodin chocolate blood agar (CCBA) and haemin-bacitracin blood agar (HBBA), and non-selective chocolate agar (CA) was conducted on 73 respiratory samples from an unknown number of people with CF. CCBA plates were incubated overnight at 37°C in 5% CO_2. *H. influenzae* was isolated from 13 samples. All 13 were recovered on CCBA, ten on HBBA, and only three on non-selective CA.[115]

Recommendations

- A selective medium should be used to enhance the recovery of *H. influenzae*. Options include chocolate agar supplemented with either bacitracin or cefsulodin (high).
- Plates should be incubated at 35–37°C in 5% CO_2 and examined after overnight incubation and after at least another 24h (low).
- As isolation of *H. influenzae* could indicate contamination from the upper respiratory tract, it may be helpful to report the approximate amount present (very low).

3.3.5 Other Gram-negative bacilli

The group of largely non-fermenting Gram-negative environmental bacteria that can colonise the lungs and cause chronic infection that lowers lung function includes: *Stenotrophomonas maltophilia, Achromobacter* spp., *Ralstonia* spp., *Pandoraea* spp., *Cupriavidus* spp., *Inquilinus limosus* and others.[116]

A novel Gram-negative selective agar (GNSA) was evaluated using a three-stage process. Stage one tested its ability to grow a laboratory collection of organisms, comprised of 31 examples of CF-associated Gram-negative bacilli, 11 non-CF-associated Gram-negative bacilli, 13 Gram-positive organisms, and seven fungi. Stage two tested its ability to recover *P. aeruginosa, B. cenocepacia* and *S. maltophilia* from simulated sputum samples and stage three tested its ability to recover organisms from 12 sputum samples submitted from 12 people with CF. Plates were incubated at

37°C in air for 48h. GNSA supported the growth of all Gram-negative bacilli but not Gram-positive organisms or fungi. It was able to support the growth of organisms in simulated sputum samples at quantities in the range 1.5 x 10^2 to 6.7 x 10^3 CFU/mL. GNSA successfully recovered all Gram-negative bacilli from the 12 sputum samples from people with CF, including the isolation of *Achromobacter xylosoxidans* from two people that was not recovered using standard culture media (MacConkey agar, PIA and blood agar).[117]

A comparative evaluation of vancomycin-amphotericin B-imipenem (VIA) agar, a selective medium for *S. maltophilia*, against bacitracin-chocolate agar (BC) with an additional imipenem disk was conducted using 814 sputum samples from 87 people with CF. Plates were incubated at 37°C in air for 48h. Two hundred and thirty-five samples from 34 people with CF were positive for *S. maltophilia*. All 235 (100%) were positive on VIA but only 106/235 (45.1%; P<0.0001) were positive on BC. Other imipenem-resistant Gram-negative bacilli, including *P. aeruginosa* and Bcc, were isolated on VIA.[118]

Recommendation

- If there are particular clinical concerns then laboratories may wish to consider additional selective media for isolating *S. maltophilia, Achromobacter* spp and other Gram-negative bacilli (low).

3.3.6 Fungi

The ubiquitous nature of *Aspergillus* and other moulds in our environment means the presence of *Aspergillus* in sputum samples is not diagnostic and its clinical significance is still the subject of debate. Recovery of *Aspergillus* or other moulds from bronchoscopic specimens has a higher positive predictive value for indicating disease compared to colonisation.[119] The significance of growth of *Candida* spp. in respiratory samples is dubious and reflects oral-pharyngeal colonisation as they are rarely a cause of true infection of the lung.[120] Along with *Aspergillus* spp. and *Candida* spp. the other fungal species of interest include *Scedospotium apiospermium complex, Lomentospora prolificans* and *Exophiala dermatitidis* but the clinical significance of isolating them from the CF respiratory tract is also a matter of debate. A cross-sectional study using the Dutch national CF Registry (from five of the seven Dutch CF centres) between 2010 and 2013 observed that people with CF with and without *Scedosporium* spp. or *E. dermatitidis* were comparable in terms of BMI and lung function. This suggests that *Scedosporium* spp. and *E. dermatitidis* are probably not major CF pathogens on a population level.[121]

Recently Tewksbury et al evaluated the differences in clinical outcomes over 12 months between two groups of people with CF (21 with *E. dermatitidis* vs 105 without).[122] Their findings noted no significant differences between the two groups in changes in FEV_1 at the beginning of an exacerbation, at the end of IV antibiotic therapy or at subsequent clinic follow-up following the exacerbation. This further supports that the presence of *E. dermatitidis* is not associated with adverse clinical outcomes in CF.

Microscopy of bronchoscopy specimens is highly recommended but has not been systematically studied. The use of fluorescent dyes such as Calcofluor White™ or Blancophor™ are recommended to enhance the visualisation of fungal elements.[123]

Respiratory samples cultured on media specific for fungi have a higher yield than bacterial culture plates.[124] An investigation into the prevalence of *A. fumigatus* and other fungal species in adults with CF was conducted at a single specialist centre in Germany. Three hundred and sixty-nine sputum samples were received from 94 subjects and *A. fumigatus* was isolated from 109 (29.5%) samples from 43 subjects (45.7%). Of the samples positive for *A. fumigatus*, 85 (78%) grew on Sabouraud glucose agar (SGA) with additional antibiotics, 82 (75.2%) on SGA without antibiotics, 46 (42.2%) on chocolate agar and 25 (22.9%) on blood agar. All media had been incubated at 37°C in air for three days. The incorporation of antibiotics to the SGA significantly reduced contamination rates with *P. aeruginosa*.[125]

Two studies have shown the value of using 'high volume' sputum samples to detect the presence of *A. fumigatus* and other moulds. Vergidis et al compared cultures using an aliquot of homogenised, diluted sputum versus high volume undiluted sputum on Sabouraud agar. Two hundred and twenty-nine sputum samples from individuals with chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis/severe asthma with fungal sensitisation (ABPA/SAFS) and *Aspergillus* bronchitis were included. Positive cultures for *Aspergillus* spp. were obtained in 15.4% of the homogenised, diluted sputum sample versus 54.2% of the high-volume undiluted samples (P<0.001).[126] Engel et al evaluated the influence of mucolytic pre-treatment, increased inoculum volume (100µl) and a longer incubation time (three weeks) on the yield of *Aspergillus* spp. and other moulds from CF sputum samples. Detection of moulds increased from 42% to 76% (P<0.0001), with increased *A. fumigatus* detection from 36% to 57%. The mucolytic pre-treatment together with the increased inoculum volume was the most important variable increasing the detection of moulds in the sample. Sabouraud medium showed the highest detection rates for all moulds.[127]

Due to different growth velocities of pathogenic moulds at different temperatures, to improve the detection of individual moulds, an incubation temperature of 25–30°C in addition to 35–37°C should be used.[123] Two studies have shown that an incubation period of 2 weeks will detect most moulds present in the sample (>94%).[127, 128]

The association between pre-transplant fungal colonisation and post-transplant invasive pulmonary fungal infection is well-documented in *Aspergillus* spp. with most infections occurring in the six months after transplantation.[129] However, collective evidence on the significance of pre-transplant non-*Aspergillus* spp. mould colonisation in post lung transplant is still in its infancy, with growing case reports of invasive *E. dermatitidis* and *Scedosporium* spp. being published.[130, 131] Until further evidence is available, we recommend considering extended incubation up to two weeks for individuals listed for lung transplant.

Recommendations

- Sabouraud medium with additional antibiotics should be used to enhance the recovery of fungi from respiratory samples of people with CF (high).
- The use of high-volume sputum samples (≥100µl) increases the detection rate of moulds; the use of undiluted sample may potentially increase the yield (moderate).
- Plates should be incubated at 35–37°C in air and examined after overnight incubation followed by every 24h for at least seven days (moderate). Extended incubation up to two weeks could be considered for individuals listed for lung transplant (low).

3.3.7 Mycobacteria

3.3.7.1 Isolation of NTM in the CF respiratory tract

The recovery of NTM from the respiratory tract may indicate transient or persistent colonisation, or active infection characterised by progressive inflammatory lung damage.[25, 132] NTM-pulmonary disease (NTM-PD) is defined by the presence of specific microbiological, clinical and radiological features.[133] Although the isolation of NTM by culture does not provide a diagnosis for NTM-PD alone, it remains a cornerstone of diagnosis.

3.3.7.2 Decontamination

There is a balance to be struck between culturing for all mycobacteria, including slowly and rapidly growing mycobacteria in a diagnostic laboratory, and particularly *M. abscessus* and other RGM. Without measures to prevent overgrowth by other bacteria and fungi, isolation of mycobacteria from CF sputa is unlikely to be successful. Conventionally, solid and liquid mycobacterial media are inoculated after chemical decontamination to balance these demands, often at 35°C which is sub-optimal for some RGM.

International recommendations are largely consistent with more recently published recommendations by other expert groups. For example, the UK SMI, updated in 2021, recommends the use of N-acetyl cysteine-NaOH (NALC-NaOH) as the preferred method for digestion and decontamination of sputum samples as it is the least toxic to mycobacteria and provides the highest yield of positives.[85] The SMI provides detailed methods for performing decontamination using NALC-NaOH and also a method for using 5% oxalic acid for samples likely to be contaminated with *Pseudomonas* spp. (such as those from people with CF). The most recent guidelines for the management of NTM-PD published by the British Thoracic Society (BTS) argue that the ideal strategy for isolation of NTM is the use of a two-step approach with routine use of NALC-NaOH for all samples followed by 5% oxalic acid for persistently contaminated samples.[134, 135]

It was shown by Bange and Böttger that a high contamination rate of 45.1% in 920 specimens from 229 people with CF could be reduced to 7.3% when decontaminating with 0.5% NALC/2% NaOH followed by 5% oxalic acid, culturing on Lowenstein Jensen media and the BACTEC MGIT 960 system (Becton Dickinson).[135] An approach by Jordan et al used seeded sterilised sputum with known quantities of *M. abscessus*. Treatment with 3% oxalic acid or 2% NALC/5% NaOH oxalic acid could detect 760 CFU/mL but by using 4% NaOH the threshold was 11,000 CFU/mL on LJ slopes.[136]

Recently, Stephenson et al carried out a study comparing six chemical decontamination methods: NALC-NaOH (with 2% NaOH), 4% NaOH, 1% chlorhexidine, 0.5N sulfuric acid, 5% oxalic acid, and double decontamination with NALC-NaOH followed by 5% oxalic acid. Saline (0.85%) was used as a control. Following homogenisation, sputa were inoculated directly onto RGM agar (commercialised as NTM Elite) without pre-treatment or decontaminated and inoculated onto RGM agar. Plates were incubated at 30°C for up to 14 days for *M. abscessus* or 21 days for MAC. Mycobacteria identified by Bruker MALDI-TOF MS were identified in 41 samples from 52 individuals with known *M. abscessus* or MAC. Using the direct inoculation of RGM as the gold standard, all decontamination methods, particularly those using NaOH, lead to a reduction in the recovery of viable mycobacteria.[137]

This and other related papers illustrate the impact of decontamination on recovering viable mycobacteria, especially *M. abscessus*, and suggests that when seeking to isolate this species including an RGM-selective agar to aid recovery is advantageous. This study did not compare conventional media recovery incubated for longer times or at 35°C but is seems reasonable to consider that particularly methods using NaOH which are recommended in current guidelines may lead to reduced recovery of RGM. The clinical consequences of this have not been fully explored but could result in delayed diagnosis of NTM infection, impacting on timeliness of treatment and infection control measures.

On balance, we recommend a sample decontamination method that maximises recovery but does not suppress a major portion of mycobacteria present.

3.3.7.3 Culture

Optimal means of culturing mycobacteria generally require more than one media type, temperature and durations. Direct culture onto RGM agar at 30°C without prior decontamination aids the recovery of RGM, especially *M. abscessus* and, over three weeks, MAC. A meta-analysis by Cruciani et al assessed sensitivity and specificity of media for mycobacterial detection. Following decontamination, culture into a liquid culture medium such as the automated Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson), and a solid agar such as Lowenstein Jensen or Middlebrook 7H10 and 7H11 agar was found to increase the recovery of mycobacteria.[138] Combined liquid and solid culture methods maximised sensitivity and specificity at 87.7% and 89.7%, respectively. Time to detection was shorter in MGIT than solid media. This meta-analysis including 1,381 strains from 14,745 specimens, included culture for *M. tuberculosis* (MTB) and was not focused on CF.

3.3.7.4 The use of selective (agar-based) culture media

As an adjunct to the formal acid-fast bacilli (AFB) culture methods recommended by international guidelines, laboratories should consider enhanced surveillance using agar-based culture media, which can be particularly useful for enhanced isolation of MABSC. Several studies have demonstrated that alkali or acid used in the decontamination process can reduce the viability of NTM and particularly rapidly-growing species such as MABSC.[58, 135, 139–144]

Such agar media include Middlebrook 7H11 selective agar and RGM medium (commercialised as NTM Elite agar). The most recent BTS guidelines highlight the advantages of the use of solid media. These include the ability to differentiate different colony morphologies (facilitating the detection of mixed species of NTM) and the ability to detect NTM despite incomplete sample decontamination.[134]

RGM medium has the advantage that it can be used without chemical decontamination of sputum samples,[145] thus maximising recovery of RGM, and also allowing the culture of deep pharyngeal swabs (cough swabs).[59] This is particularly relevant for MABSC as a recent study by Stephenson et al demonstrated that decontamination with NALC 0.5%/NaOH 2% reduced the average number of viable of MABSC in sputum by 87%, and 4% NaOH reduced viability by 98%, when compared with direct culture on RGM medium. In contrast, MAC was not significantly affected.[137] One abstract has shown that RGM agar incubated at 30°C renders it safe to use in Containment level 2 facilities, as the lower temperature prevents any risk of isolating MTB.[146] Laboratories planning to introduce the RGM plate will have to undertake their own risk assessments until there is further published evidence to corroborate these findings.

Large studies in the US and the UK have both demonstrated a significantly higher yield of MABSC using RGM medium compared with standard AFB culture methods ($P<0.01$).[59, 147] The medium can also be useful for isolation of slow-growing NTM such as MAC but there is less evidence of any superiority.[59, 147] Agar-based culture media can be routinely applied to any respiratory sample from people with CF and cultures can be conveniently set up alongside cultures for other pathogens. As previously noted, MABSC is as significant as other pathogens in this setting,[148] such as Bcc, and may be isolated at higher frequency.[105] It has been argued that all respiratory samples from people with CF should be cultured for MABSC and that postponement of diagnosis might be a deciding factor in whether an early colonisation turns into chronic infection.[149]

When such culture plates are used without decontamination it is important to interpret results with caution as transient colonisation with small numbers of NTM can be detected, such as *M. llatzerense* and *M. chelonae*; such species are highly unlikely causes of NTM-PD in CF. As ever, strict criteria should be applied in making a diagnosis of NTM-PD.[133]

Esther et al demonstrated that extended incubation on BCSA increased the recovery rate of NTM and they recommended this as a practical method for culture of RGM from people with CF.[150] In two large studies comparing RGM medium with BSCA, the sensitivity of BCSA (after extended incubation) was 31–62.5% for detection of MABSC compared with

98–100% for RGM medium (P<0.0001).[59, 145] Extended incubation of BCSA is therefore not recommended for isolation of NTM as better methods are now available.

Recommendations

- The oxalic acid method is the preferred option for decontamination when compared particularly to methods using NaOH, as the latter has been shown to lead to a reduction in the recovery of viable mycobacteria (low).
- Smear for acid-fast bacilli may be used along with culture for NTM screening (moderate).
- Respiratory tract samples should be cultured via the conventional method using both solid and liquid media and incubated for a minimum of six weeks (with consideration that that some slow growing mycobacteria can take up to 12 weeks) (moderate).
- The use of selective RGM agar can be considered as an adjunct direct inoculation (without decontamination) to recover *M. abscessus* when incubated at 30°C for 21–28 days (moderate).

3.3.8 Viruses

A variety of methods for the diagnosis of respiratory virus infection are available, including culture, serology and antigen detection, but PCR assays are established as the gold-standard. Upper and lower airway samples are suitable for viral diagnostics including upper airway swabs, nasal aspirates, sputum and BAL.

There are currently no respiratory virus PCR assays that have been developed or validated specifically for use in CF. National standards outline good practice in performing PCR assays and ensuring effective quality control in the diagnostic virology laboratory.[85] A variety of commercial and in-house PCR assays are available for identification of respiratory viruses which should adhere to these standards. The UK government has published advice and analysis of the various diagnostic platforms that are available for the diagnosis of SARS-CoV-2 coronavirus infection.[151] In addition, the UK national virus reference laboratory offers additional services including SARS-CoV-2 sequencing, identification of novel influenza strains and antiviral resistance testing.

Point of care tests (POCTs) are available and in clinical use for both influenza and SARS-CoV-2 although data from cohorts with CF are lacking. These tests use either antigen detection or nucleic acid amplification techniques and allow more rapid turnaround times than laboratory-based PCR assays. Antigen detection tests typically have a sensitivity between 40–80% for influenza while newer nucleic acid amplification-based POCTs offer a sensitivity of >90%. Public Health England has published guidance on the use of POCTs for influenza.[152] POCTs using both antigen detection and nucleic acid amplification have been developed for SARS-CoV-2. Lateral flow devices for antigens have been widely distributed in the UK as part of the National Testing Programme and have a sensitivity of 40–80% compared with laboratory-based PCR assays.[151, 153–155] When positive, POCTs for SARS-CoV-2 require confirmation with a PCR test and are considered inadequate for the investigation of symptomatic patients.

Recommendations

- PCR assays are the gold standard technique for diagnosis of viral respiratory infection in CF and other viral pathogens should be conducted in line with current national standards (high).
- Point of care testing for SARS-CoV-2 should be conducted in line with the National Testing Programme (moderate).

4. Identification

4.1 Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF is a rapid, sensitive and cost-effective alternative to traditional phenotypic methods. It identifies isolates to genus and species level through the application of a laser-absorbing matrix to single colonies on a target plate, (or through direct testing on certain sample types such as blood and liquid culture), which are ionised by a pulsed laser beam within the MALDI-TOF MS instrument. The mass and charge of these peptides generates a species-specific spectrum.

The two most common MALDI-TOF MS instruments are manufactured by Bruker and bioMérieux (VITEK®-MS). Both may be used to test colonies directly without pre-treatment, although either a simplified formic acid protein extraction, or a full extraction using ethanol and formic acid may yield more accurate results for mucoid organisms and some fungi, and is recommended by the manufacturer for the identification of NTM.

For the Bruker instrument, results are presented on a log scale with a maximum of 3.0. For many organisms, a score of between 2.000 and 2.299 provides secure genus identification and probable species identification. A primary and secondary identification is provided for each isolate tested. For the VITEK®-MS scores are presented as a percentage, with scores of ≥60% indicative of species-level identification for many organisms. For both platforms a score below the confidence threshold may be given which may reflect sampling problems, for example mucoid organisms, or insufficient growth, growth that was not fresh or other factors relating to the organism being tested. The range of different species in the MALDI-TOF MS databases also has an important impact on the accuracy of results.

The advantage of MALDI-TOF MS is that it is widely available in many diagnostic laboratories, relatively inexpensive and quick to perform compared to current standard methods, which are mostly used by reference laboratories. Laboratories have reported increase in cost savings compared to other methods in addition to decrease in turn-around time for results.[156]

4.1.1 Identification of Gram-negative organisms using MALDI-TOF MS

4.1.1.1 *P. aeruginosa*

Both the Bruker and VITEK®-MS instruments have been shown to be reliable for the identification of *P. aeruginosa*. One study reported that of 413 *P. aeruginosa* isolates analysed using the Bruker MALDI-TOF, all were correctly identified with scores of ≥2.2.[157] Another study found that of 89 *P. aeruginosa* isolates, 16 of which were mucoid, 100% concordance was demonstrated between both Bruker and VITEK®-MS platforms, in comparison with identification by biochemical or molecular methods.[158]

4.1.1.2 Other *Pseudomonas* species

The *Pseudomonas* genus contains more than 200 described species, further subdivided into phylogenetic groups (*P. fluorescens* group, *P. putida* group) which are challenging to identify accurately to species-level by currently available methods including MALDI-TOF, due to the existence of many novel species. One study compared the VITEK®-MS with *rpoD* gene sequencing of 611 isolates of *Pseudomonas* spp. and found that although MALDI-TOF assigned many of the isolates to the correct phylogenetic group (*P. putida* group), it failed to identify many correctly to species-level.[159]

Although published reports of misidentifications between *P. aeruginosa* and other *Pseudomonas* species by MALDI-TOF are few, Bizzini et al reported that one of the 99 *P. aeruginosa* isolates analysed by the Bruker system was limited to genus-level agreement with the VITEK®-MS,[160] and Marko et al., reported a single incidence of a *Pseudomonas* sp. isolate identified as *P. aeruginosa* by VITEK®-MS.[158]

4.1.1.3 *B. cepacia* complex (Bcc)

Species-level identification within this complex is essential for patient management and cross-infection purposes as certain species, notably the ET-12 lineage of *B. cenocepacia* IIIA, are associated with poor outcome and are a contra-indication for lung transplant.[161] Although both the Bruker and VITEK®-MS instruments have been shown to be reliable to *B. cepacia* complex level, Bruker's restriction information states that their MALDI-TOF software cannot reliably separate species of the Bcc. One study used a threshold score of ≥2.3 on the Bruker MALDI following ethanol and formic acid extraction comparing these results with *recA*

sequence cluster analysis and found that *B. multivorans* was the only species which produced consistently reliable results, with only one false-positive identification for *B. pseudomultivorans* (score below 2.2). For other Bcc species, and for novel species not yet present on the Bruker database, misidentifications were found to occur, with isolates of the novel cluster from a single individual variably identified as *B. cepacia, B. diffusa, B. cenocepacia* and *B. vietnamiensis*, on two occasions with scores of 2.3.[17] A recent study comparing the VITEK®-MS and Bruker machines reported that to genus level, 100% and 97.0% of isolates were confidently identified to Bcc level by the Bruker and VITEK®-MS systems, respectively; with 26.0% and 67.0% of isolates correctly identified to the species level, respectively, in comparison to *recA* sequencing.[162]

4.1.1.4. *B. gladioli*

B. gladioli is closely related, but distinct from the Bcc. It has been associated with poor outcome in CF post-lung transplantation, with one report of systemic abscesses.[163, 164] *B. gladioli* comprises four historical disease "pathovars" (pv.) (*B. gladioli* pv. *agaricicola, B. gladioli* pv. *allicola, B. gladioli* pv. *gladioli* and *B. cocovenenans*). A recent study examining the whole genome sequences of 206 isolates from multiple geographic locations and sources found that representatives of CF isolates were found in each of the pathovars, but all constituted a single genomic species, *B. gladioli*.[165] Using phylogenomic analysis pathovar "*B. cocovenenans*" formed 3 evolutionary clades collectively designated *B. gladioli* group 1. These group 1 strains encoded the potent respiratory toxin bongkrekic acid and comprised 13% of the CF strains examined (194 in total).[165] It is not known whether this poison contributes to poor clinical outcome with *B. gladioli* infection in CF, but testing for the bongkrekic acid toxin gene by PCR is accurate if a case of rapid clinical decline is suspected.[165] Trimethoprim was shown to suppress production of bongkrekic in *B. gladioli*,[165] advocating a role for administration of this antibiotic even if the isolate lacks clinical susceptibility. Pathovars "*gladioli*" and "*agaricola*" formed a single phylogenomic group while "*allicola*" was a distinct genetic group within *B. gladioli*.

To date neither the Bruker nor VITEK®-MS systems have representatives of the pathovars on their databases, therefore differentiation to sub-group level is not possible. However, differentiation of *B. gladioli* from other *Burkholderia* species has been found to be reliable using the Bruker MALDI-TOF MS, which correctly identified 39 isolates of *B. gladioli* in comparison with *gyrB* gene sequencing, although 19 of these had scores of less than 2.3, with a range of 2.02–2.21.[17] Identification by VITEK®-MS found occasional misidentifications with other *Burkholderia* species. In one study, six isolates were correctly identified compared with *recA* sequencing, with one false positive for an isolate that was identified as a *B. lata* on *recA* sequencing.[162]

4.1.1.5 *B. pseudomallei*

B. pseudomallei is known to cause infection (melioidosis) in people with CF and is endemic in South-east Asia and Northern Australia.[166] It is classically described as causing a chronic infection in people with CF; this is not the case in non-CF populations where acute melioidosis can often present as a fulminant pneumonia and septic shock.[166, 167]

There are important laboratory considerations to be taken into account with *B. pseudomallei* as it is a Hazard Group 3 pathogen as defined by the Advisory Committee on Dangerous Pathogens 2021.[167] Therefore, dialogue is required with the clinical teams, especially if there is a strong travel history, to ensure samples are highlighted in advance and processed appropriately in a Containment level 3 laboratory with scientists wearing appropriate personal protective equipment (PPE). Confirmatory molecular identification is described in the Recommendations under 4.2.1.

4.1.1.6 *Stenotrophomonas* genus

The reclassification of certain species (such as *Stenotrophomonas africana*, formerly *Pseudomonas africana*) and the addition of others has led to many recent changes to the *Stenotrophomonas* genus which currently comprises at least 20 species. Formerly *S. maltophilia* was considered to represent a single species, but in recent years the use of molecular methods has led to the proposal of a "*S. maltophilia* complex or group" comprising several *Stenotrophomonas* species and species formerly designated part of the *Pseudomonas* genus (*S. maltophilia, S. pavanii, P. beteli, P. geniculata* and *P. hibisciola*).[168, 169] At the time of publication the Bruker and VITEK®-MS databases do not contain reference strains for many of these new species, therefore accurate identification to species-level may not be possible using this technology. However, both platforms have been shown to identify *Stenotrophomonas* accurately to genus level, with one study finding that both systems correctly identified 55 *Stenotrophomonas* isolates to genus level compared with a combination of the VITEK®-2/Phoenix and/or API NE/16S rRNA gene sequencing.[158] Another study reported that all 32 *Stenotrophomonas* isolates were correctly identified by Bruker

compared with API 20NE, combined with 16S rRNA gene sequencing.[170]

4.1.1.7 *Achromobacter, Pandoraea* and *Ralstonia* species

Updates to commercial MALDI-TOF databases are infrequent and do not always include all newly described species, which is the case for the *Achromobacter* (currently 27 species) and the *Pandoraea* genera (currently 28 species). Discrimination of these genera to species-level is therefore not accurate by MALDI-TOF and requires molecular methods. One study found that the Bruker MALDI-TOF correctly identified 147 isolates of *Achromobacter* to genus level. In comparison with *nrdA* sequencing, species-level identification was accurate for 84 of 89 *A. xylosoxidans* isolates. However, *A. ruhlandii* was misidentified as *A. xylosoxidans* in two of three cases and species not yet added to the Bruker database were misidentified as *A. xylosoxidans* and *A. ruhlandii*.[171] Another study comparing both the VITEK®-MS and Bruker instruments found that the Bruker MALDI-TOF identified 11 of 12 *Achromobacter* isolates correctly to genus-level, with one isolate giving no reliable identification, while the VITEK®-MS was only able to identify four of the 12 isolates correctly to genus level.[158] Studies detailing MALDI-TOF identification of *Ralstonia* and *Pandoraea* are limited but Marko et al found that both platforms were able to identify these genera correctly (albeit small numbers of each were tested), compared with 16S rRNA gene sequencing.[158] *gyrB* sequencing of both genera has also been found to agree with the Bruker MALDI-TOF to genus level (14 isolates of *Ralstonia* sp. screened and 18 of *Pandoraea* sp., respectively).[172, 173]

4.1.1.8 *Elizabethkingia* spp.

The *Elizabethkingia* genus currently comprises seven species (*E. meningoseptica, E. miricola, E. anophelis, E. ursingii, E. occulta, E. bruuniana* and *E. argenteiflava*). Their clinical significance in CF is still unclear although one case study suggested a pathogenic role for *E. miricola* in one person with CF,[174] and another found some evidence of shared strains.[175] In recent years Bruker have added *E. anophelis* to their database and have corrected their entry for *E. meningoseptica* for which the wrong reference had been used, therefore differentiation between *E. meningoseptica, E. miricola* and *E. anophelis* is possible using this platform. However, *E. ursingii, E. occulta, E. bruuniana* and *E. argenteiflava* have not yet been added to the Bruker database. The VITEK®-MS has also been shown to differentiate between *E. meningoseptica, E. miricola* and *E. anophelis*, with 27 of 28 isolates being correctly identified on comparison with whole genome sequencing (WGS), the one misidentification being between an *E. miricola* which was shown to be *E. bruuniana* by WGS.[176]

4.1.2 In-house expanded MALDI-TOF databases

The development of in-house databases to expand upon existing commercial databases has also been described. Although requiring some expertise and the inclusion of isolates that have been identified by an established method, this process has provided much greater accuracy for some genera. In one study this method correctly identified 58 of 64 *Achromobacter* spp. isolates to species-level on comparison with *nrdA* sequencing.[177] In-house databases have also been used by others for species-level discrimination within the Bcc and *Ralstonia*, for the former with partial resolution only.[170]

4.1.2.1 Challenging genera

Table 2 provides a list of organisms for which MALDI-TOF identification beyond genus level is often challenging, for which current molecular methods such as 16S rRNA gene sequencing are also often unable to differentiate to species level and/or for which no species-specific PCR assays are currently available.

Table 2 Examples of CF microbiota organisms that are difficult to identify beyond genus-level accurately by MALDI or through current routinely used molecular methods

***Chryseobacterium* spp.**	Comprises >100 species	*C. indologenes* and *C. gleum* are the only species that have been shown to be reliably identified on both VITEK®-MS and Bruker instruments (scores ≥2.3) in comparison with 16S rRNA gene sequencing.[178]
***Ochrobactrum* spp.**	Comprises at least 22 species, many of which have been reassigned to the *Brucella* genus.[179]	Isolates may exhibit scores of <2.0 on MALDI-TOF analysis and the databases have yet to be updated to include the changes in nomenclature.[180] Species are nevertheless difficult to separate due to highly conserved 16S rRNA genes.
***Sphingomonas* spp.**	Comprises >100 species	Currently no published comparative studies.
***Inquilinus* spp.**	*I. limosus* and *I. ginsengisoli*	Although there are currently only two valid species, these organisms may produce low scores if mucoid, even following full extraction. 16S rRNA gene sequencing suggests that both *I. limosus* and *I. ginsengisoli* are difficult to separate.

4.1.3 Identification of NTM

4.1.3.1 Background to NTM identification

The identification of colonies with the use of MALDI-TOF MS is now a well-established method for identification of clinically relevant bacteria and fungi, including NTM. Studies have compared this technology to traditional methods including HAIN Genotyping and WGS with comparable results for identification of liquid cultures such as MGIT™ and colonies from solid agars including pure growths on Middlebrook agars, blood agar, Lowenstein Jensen slopes and RGM agar (NTM-Elite; bioMérieux).[105, 181, 182]

The use of simple, inexpensive extraction methods for both liquid cultures and colonies from solid agar for the identification of NTM can enhance the accuracy of MALDI-TOF results, in addition to inactivating cultures.[182, 183]

Laboratories should perform in-house validation when introducing MALDI-TOF technology into routine practice. Some laboratories also create their own databases.[156]

Extraction methods

The accuracy of identification can be improved for both the Bruker Biotyper® and the bioMérieux VITEK®-MS by using a simple extraction method if initial identification of colonies fails with the addition of 70% formic acid. Extraction methods break the cell wall using chemical and mechanical methods and extract the bacterial proteins using formic acid and acetonitrile. Physical disruption of the cells is achieved using sonication, bead beating, or vortexing in the presence of silica beads. People with CF are very rarely infected with MTB but, should tuberculosis be suspected as a possibility, a heat or ethanol inactivation step should be performed under BSL3-CLS3 conditions prior to extraction.[183]

Bruker and bioMérieux recommend slightly different extraction protocol.[184, 185]

A simple method is to prepare a 0.5 to 1 McFarland standard in 0.5mL sterile distilled water using a swab for easier emulsification, in a small serum vial or similar tube. Add 1mL of absolute ethanol and vortex for two minutes. Centrifuge for two minutes at 13,000 rpm, pour off the alcohol and re-spin briefly then carefully remove the remaining alcohol with a fine pipette or tip. Leave in a warm place for a few minutes to ensure complete evaporation of ethanol. Add around ten 1mm glass beads and 50µL of acetyl nitrate and vortex for one minute. Add 50µL of freshly prepared 70% formic acid, vortex briefly then centrifuge for two minutes at 13,000 rpm. Pipette 1.5µL in triplicate to the MALDI tile, air dry then add 1.5µL of matrix solution. This method was validated in-house at the Freeman Hospital microbiology department, Newcastle upon Tyne for preparing NTM and difficult to identify bacterial cultures for identification using the Bruker MALDI-TOF; for one isolate this takes no more than 20 minutes.[186]

Interpreting results

For the Bruker system the score value accepted for 'high confidence identification' has been established at 1.8 instead of 2.0 and the cut-off value for 'low confidence identification' at 1.6 instead of 1.7. For the VITEK® MS system, the cut-off for reliable species-level identification has been established at >90%, although score values between 80% and 90% are accepted. Results between 60% and 80% are considered as 'low confidence' results and might be consistent only at genus level, while those below 60% are considered 'not reliable'.[156]

Updates and further information for the interpretation of results are available from both Bruker for the Biotyper® and bioMérieux for the VITEK®-MS.

4.1.3.2 *M. abscessus* complex

It is recommended that all first isolates of MABSC are referred for confirmation and typing by a robust molecular method. Further isolates identified by MALDI-TOF, from individuals with chronic colonisation, can be reported as MABSC without the need for further molecular confirmation. In some circumstances it is worth considering referring further isolates on the same patient, in discussion with the CF clinical team. Such instances include where there is evidence of treatment failure, relapse or re-infection, pre-transplant work up or there have been culture-negative samples prior to a further positive culture.

4.1.3.3 *M. avium* complex

MALDI-TOF MS is reliable for the accurate identification of *M. avium* but is unable to separate *M. chimaera* from *M. intracellulare*.[181] The treatment for any member of this complex is the same but for clinically significant isolates, formal identification using an established molecular method may be required. For individuals with chronic MAC infection, further isolates identified can be reported as MAC.

4.1.3.4 Other nontuberculous mycobacteria

There are many NTM such as the *M. fortuitum* complex and *M. chelonae* complex that are highly unlikely respiratory pathogens in CF and further identification of these and others should only be sought if thought to be clinically relevant. The databases for both VITEK®2 and Bruker Biotyper® are quite extensive and guide users on the confidence in their results.

Recommendations

- Biochemical methods are not reliable for identification of non-fermentative Gram-negative bacteria recovered from the sputum of people with CF. Hospital laboratories without access to MALDI-TOF should arrange to send GNNF to laboratories with this facility (high).
- Scores greater than, or equal to the threshold for both the Bruker and VITEK®-MS platforms are reliable to genus level (high).
- First isolates of GNNF of proven clinical importance which are difficult to reliably speciate by MALDI-TOF analysis, such as the Bcc should undergo confirmatory testing by a validated method, such as *recA* sequencing (high).
- For subsequent patient isolates of Bcc with the same MALDI-TOF identification, *recA* sequencing is not essential unless there is concern about a new infection, or for surveillance purposes (moderate).
- *P. aeruginosa* with scores equal to, or above, the threshold, can be reliably identified by both the Bruker MALDI-MS and VITEK®-MS platforms to species-level. Dual identifications (*P. aeruginosa/Pseudomonas* sp.) occasionally occur on the VITEK®-MS. In these cases, species-level confirmation should be sought using an alternative method (moderate).
- For other CF-related GNNF, species-level identification by MALDI-TOF using existing databases is currently not reliable. The creation of in-house databases has been shown to be useful for improved species-level identification (moderate).
- If required, GNNF may be submitted to the reference laboratory for species-level identification (moderate).
- Laboratories are encouraged to use MALDI-TOF MS following robust in-house validation for the identification of NTM from CF respiratory samples (moderate).
- All first isolates of MABSC should be referred for confirmation, subspeciation and typing using a robust molecular method (moderate).

4.2 Molecular identification

4.2.1 *B. cepacia* complex

Since its definition as a multi-species complex in 1997, molecular identification methods have been required to accurately identify Bcc species.[187] There are currently more than 20 validly named species within the Bcc. A recent study used genome sequencing-based comparisons to identify 22 named species and at least 14 additional putative

novel Bcc species groups.[188] In the context of CF lung infection, *B. cenocepacia* and *B. multivorans* are the most frequently encountered, with *B. multivorans* now being dominant within the US and UK populations.[17, 116] Other Bcc species are less common in CF, generally accounting for less than 10% of infections with this taxonomic complex.[17] Outside of the Bcc, *B. gladioli* is the most likely member of the genus to be encountered in CF.[17, 116]

For the Bcc, PCR using primers BCR1 and BCR2 that amplify the near full-length sequence of the *recA* gene remains an excellent molecular identification approach. Comparing amplified *recA* gene sequence between CF isolates to Bcc taxonomic reference strains provides accurate identification, but attention should be paid to the following: for the 22 named Bcc species examined genomically by Jin et al, the BCR1 and BCR2 PCR primers will amplify the *recA* gene.[188] However, testing of the original *recA* PCR on novel genomic species is required to validate that they can still be targeted;[188] extraction of the *recA* sequences from the genomes of these novel Bcc groups indicates the primers still match the target gene sufficiently for successful PCR.[189]

To improve identification, additional genes such as the *gyr*, which has the most sequence variation, or others from the expanded Bcc multilocus sequence typing (MLST) scheme should be considered.[190] The sequences can be compared at the curated **Bcc MLST database**. If allele matches for these MLST genes are not clear within the database, genome sequencing should be considered, and further analysis carried out using the ribosomal MLST (rMLST) scheme.[191] The MLST database hosts a straightforward genomic identification tool called **Species ID**, which uses rMLST to accurately determine the taxonomic identity of Bcc genomes.

Species within a subgroup of the Bcc called taxon K, have proven difficult to taxonomically differentiate using just *recA* or MLST-based approaches.[192] Taxon K contains *B. lata, B. contaminans* and potentially novel species groups. The species are best identified using genomic approaches such as Average Nucleotide Identity (ANI) and digital DNA-DNA hybridization (dDDH), which resulted in the definition of the new Bcc species *B. aenigmatica* sp. nov.[192]

For CF *Burkholderia* species outside of the Bcc such as *B. gladioli*, the near full length 16S rRNA gene sequence amplified using universal primers or the sequence of the genus specific *recA* PCR are sufficient for identification if required.[193] In addition, a range of PCRs targeting other genes were also successfully applied to identify the different non-Bcc species occasionally identified within the UK CF population.[17]

Recommendations

- Clinical outcome with all *Burkholderia* species infection is variable in CF and all species potentially constitute an infection control risk, therefore accurate species identification is recommended (high).
- *recA* gene and MLST-based approaches are sufficient to place a strain within the Bcc and define the majority of species prevalent in CF, but may not accurately identify certain taxonomic groups such as those within taxon K (high).
- Whole genome sequence-based analysis should be considered for CF isolates which cannot be accurately placed using *recA* or MLST (moderate).
- Non-Bcc species such as *B. gladioli* or *B. pseudomallei* can be accurately identified using a range of PCR and single gene sequence-based approaches (moderate).
- If the diagnostic laboratory does not have the facilities for molecular identification of the Bcc, the isolate should be sent to the reference laboratory (high).

4.2.2 *P. aeruginosa*

If required, there are several simple PCR assays that are both sensitive and specific for *P. aeruginosa*. PCR assays targeting the multiple species-specific genes *gyrB, ecfX* and *oprL* rather than a single gene have been found to display better sensitivity and specificity. Laboratories with appropriate structures in place to carry out quality assured molecular assays should be confident in the ability of these PCRs to confirm the identification of *P. aeruginosa*.

Recommendation

- A validated species-specific PCR should be used when molecular identification of *P. aeruginosa* is required (very low).

4.2.3 NTM

4.2.3.1 NTM ID methods

Speciation of NTM helps to guide likely clinical importance as species differ in their potential to cause human disease and their response to antimicrobials. Subspeciation may predict treatment response and potentially allow for targeted therapy, especially if drug susceptibility testing is not available.[194, 195] However, caution should be used when using using subspeciation alone in inferring macrolide resistance.[196]

In recent years mycobacterial subspeciation has moved from using primarily biochemical to

molecular techniques whilst MALDI-TOF MS as discussed above is now a realistic option as many diagnostic laboratories use this technology. In the UK WGS is increasingly used whilst line probe assays (LPAs) such as the Hain CM and AS are used in some centres, as well as the Hain GenoType NTM-DR which can differentiate *M. avium, M. intracellulare, M. chimaera, M. chelonae* and *M. abscessus* subspecies. The latter can also detect resistance to clarithromycin and amikacin as discussed in the susceptibility section.[197] Alternatively, PCR product restriction analysis is more frequently used in low resource settings and is comparable to LPAs.[198, 199]

Partial gene sequencing of *hsp65* and *rpoB* genes, for example, has also been successfully used, often giving a higher level of discrimination to subspecies level.[200–202]

4.2.3.2 Molecular Confirmation and Typing Methods of MABSC

In 2012, Blauwendraat published the first study that identified members of the MABSC from non-MABSC NTM in a UK cohort of mainly individuals with CF, by sequencing of three housekeeping genes: *hsp65, rpoB* and *sodA* of 81 isolates of NTM.[203] They were identified as 46 *M. abscessus ssp abscessus*, 20 *M. abscessus ssp massiliense*, five *M.abscessus ssp bolletii alongsidenine M. chelonae* and one *M. fortuitum*. By contrast, commercial line probe assays identified 21 isolates as *M. chelonae*, 59 as MABSC and one *M. fortuitum*.

Harris et al typed 41 MABSC isolates from 17 children with CF using a novel variable-number tandem repeat (VNTR) scheme and an automated repetitive-PCR (rep-PCR) system. Both VNTR and rep-PCR typing methods differentiated between members of the MABSC. The isolates from individuals were indistinguishable, and their data strongly suggested that people with CF are persistently infected with one strain and that different people with CF can harbour the same strain.[204]

Phylogenetic analysis of the *M. abscessus* subspecies *massiliense* from 11 individuals by Bryant et al revealed two clustered outbreaks of near-identical isolates, differing by less than 10 base pairs, strongly indicating between-patient transmission. Every person within these clusters had numerous opportunities for within-hospital transmission from other individuals.[29]

On the contrary, Doyle et al retrospectively sequenced the whole genomes of 145 *M. abscessus* isolates from 62 individuals, seen at four hospitals in two countries over 16 years with only one episode of possible direct patient-to-patient transmission and that was between a sibling pair. They found that people acquired unique *M. abscessus* strains even after spending considerable time on the same wards with other *M. abscessus*–positive individuals. WGS of *M. abscessus* isolates could determine subspecies, identify previously reported antimicrobial resistance (AMR) associated mutations, and provide common typing definitions in a single workflow.[205]

MLST has been a valuable tool for at least 20 years for identification, evolution, and surveillance of pathogenic bacteria and whilst WGS can provide more extensive information, the data presented in bioinformatics format, such as phylogenetic trees, is unfamiliar to most clinical microbiologists.

An MLST scheme was developed for *M. abscessus* sensu lato, based on the partial sequencing of seven housekeeping genes: *argH, cya, glpK, gnd, murC, pta* and *purH*.

Macheras et al used this scheme to characterise a collection of 227 isolates recovered between 1994 and 2010 in France, Germany, Switzerland and Brazil. One hundred different sequence types (STs) were identified, which were distributed into three groups on the tree obtained by concatenating the sequences of the seven housekeeping genes. The most prevalent STs were ST1 (CC5; 20 isolates) and ST23 (CC3; 31 isolates). Both STs were found in Europe and Brazil, and the latter was implicated in a large post-surgical procedure outbreak in Brazil. However, respiratory isolates from people with CF belonged to a large variety of STs.[206]

The PubMLST scheme for *M. abscessus* uses seven housekeeping genes: *argH, cya, gnd, murC, pta, purH*, and *rpoB*. Wuzinski et al extended this scheme and included eight new genes: *hsp65, erm(41), arr, rrs, rrl, gyrA, gyrB*, and *recA*.[207] The scheme, MAB-MLST, takes advantage of the increased discrimination that WGS can provide and presents results in a user-friendly tabular output. A major advantage is the addition of AMR genes in the MLST scheme which increases clinically relevant information by helping predict antibiotic resistance profiles.

For example, a functional *erm(41)* confers inducible resistance to macrolides in isolates that may initially appear susceptible to clarithromycin. This is important for *M. abscessus* subsp. *abscessus* and subsp. *bolletii* as they have functional *erm(41)* genes with unique sequences. On extended incubation, they can demonstrate resistance to the drug. Molecular examination does not currently reliably predict susceptibility and so phenotypic macrolide sensitivity should be carried out rather than depending on genotypics (see section 5). *M. abscessus* subsp. *massiliense* has a large deletion in *erm(41)* which produces a non-functional Erm protein.

Recommendations

- Confirmation, subspeciation and typing should be performed on all first isolates of MABSC; this is useful for infection control and monitoring treatment success or failure (moderate).
- Further isolates of MABSC on the same patient may be required to be sent for repeat subspeciation and typing, in certain clinical situations in dialogue with CF team, as per above (low).

4.3 Molecular typing of Gram-negative non-fermenters

4.3.1 *P. aeruginosa*

Surveillance of different strains of *P. aeruginosa* within CF clinics is useful to establish the prevalence of transmissible strains such as the Liverpool (LES), Manchester (MAN) and Midlands 1 (Mdl1) strains, and to monitor the prevalence of other shared strains for cross-infection purposes. People with CF with transmissible strains such as the LES have been found to have a poorer outcome than those with other strains and the LES has been associated with increased AMR and virulence.[208, 209] Other countries have also described CF-associated transmissible strains, such as DK1 in Denmark, the Australian AES-1 strain, the Canadian "Prairie" strain and ST406 from the Netherlands.[210–213] Studies have shown that a combination of genotyping and patient segregation have been successful in reducing the incidence of some of these strains.[212–214]

A multiplex PCR assay may be useful for preliminary screening for LES, Mdl1 and MAN.[215] Primers for the specific identification of *P. aeruginosa* can also be incorporated in this multiplex PCR allowing both the identification of *P. aeruginosa* and these transmissible strains. It should be noted, however, that the LES markers, PS21 and F9, are not unique to LES, nor are both present in all LES isolates. In addition, false positive results may occur for the MAN strain therefore isolates positive for any of these markers should undergo confirmatory testing by a validated typing method.[216]

Molecular typing is best performed using established methods such as VNTR analysis.[217] VNTR analysis generates a numerical code based on the number of repeats at selected loci in the genome. It is a rapid technique that is both sensitive and specific and allows direct comparison of isolates between laboratories. WGS may also be used, if available, as discussed in Chapter 7.

It is recommended that all specialist CF centres and clinics undertake pro-active surveillance at local and national level to ensure that evidence of cross-infection with *P. aeruginosa* is rapidly detected to enable appropriate measures to be put in place to limit spread.[218]

Recommendations

- The frequency of typing isolates may vary between centres depending on the local prevalence and laboratory provisions, but molecular typing/surveillance should be performed on all first isolates, on isolates from people with CF who are transitioning from paediatric to adult services, and if there is an increase in incidence of *P. aeruginosa* in a CF cohort. If centres are unable to perform their own typing, they may send isolates to UK Health Security Agency (UKHSA) Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit in Colindale, London (high).

4.3.2 *B. cepacia* complex

There have been several publications describing shared strains of species of the Bcc, most notably the ET-12 lineage of *B. cenocepacia* IIIA which is responsible for increased mortality by being able to cause fulminating, often fatal, pneumonia and septicaemia, in part due to the presence of cable-like pili (*cblA*) which increase binding to epithelial cells and pro-inflammatory responses.[219, 220] Multiple other globally-spread and virulent *B. cenocepacia* IIIA CF strains have also be identified with sequence type ST-32 being particularly prevalent in Europe.[221]

The introduction of IPC measures within CF clinics has resulted in a decline in the incidence of this strain over the past decade. Other examples of prominent strains include the US-associated PHDC and Mid-West clones of *B. cenocepacia* IIIB and the transmissible *B. dolosa* strain AU0158.[116, 222]

Epidemic strain markers are unreliable for accurate identification of Bcc with enhanced transmissibility such as ET-12. The *B. cepacia* Epidemic Strain Marker (BCESM) and the *cblA* gene responsible have been used for the identification of ET-12 as it appeared this lineage was unique in possessing both markers. However, neither of these markers are both consistently present in all ET-12 isolates, and *cblA*-positive isolates exist that are distinct from the ET-12 lineage.[223, 224] MLST of the Bcc is now very advanced as a molecular typing resource.[225] There are over 3,850 isolates (1,120 genomes) within the **public database** and this can be rapidly searched with an MLST profile or

individual allele sequences to find matching strains. There are also numerous examples of Bcc strains responsible for epidemic spread that contain neither *cblA* nor BCESM, and the evidence that of small clusters of *B. cenocepacia* IIIB and *B. multivorans* isolates in the UK,[17] suggesting that continued national surveillance of all species of the Bcc would be prudent.

Recommendations

- Epidemic strain markers are not sufficiently reliable to determine the potential transmissibility of Bcc (high).
- All newly confirmed Bcc isolates should undergo molecular typing to establish whether they are members of ET-12 or other lineages (high).
- Molecular surveillance should be undertaken if there is concern about prevalence or increasing incidence, or if requested by the clinical team (high).
- If the hospital laboratory does not have the facilities for molecular typing, isolates may be submitted to an appropriate reference laboratory (high).

4.3.3 Molecular typing of other GNNF

Depending on the prevalence of other GNNF within individual CF clinics, molecular typing may prove useful for investigating the presence of any common strains, particularly if there has been an increase in acquisition. Typing should be conducted using a validated method for the GNNF of interest, such as PFGE, MLST or WGS.

Recommendations

- Molecular typing of other GNNF may be helpful if there is concern about prevalence or increasing incidence, or if requested by the clinical team. Typing should be performed using validated methods for the organism of interest (moderate).
- If the hospital laboratory does not have the facilities for molecular typing, isolates may be submitted to appropriate reference laboratory (moderate).

4.3.4 Panton-Valentine leukocidin-positive *S. aureus*

Panton-Valentine leukocidin-positive *S. aureus* (PVL-SA) is described in CF literature. Elizur et al reviewed MRSA isolates from 40 children with CF. Fifteen percent had PVL-MRSA; these individuals were more likely to have focal chest X-ray findings including lung cavities, and significantly decreased FEV_1, higher white cell counts and were more likely to require admission for IV antibiotics.[226] A case report by Barry et al described PVL-SA causing severe pulmonary exacerbation in an adult with CF, with potential transmission from a healthy family member to the individual.[227] However, a concrete link between PVL-SA and its clinical relevance is not firmly established within the CF population.

A review of both methicillin-sensitive *S. aureus* (MSSA) and MRSA published in the Journal of Cystic Fibrosis in 2011 notes PVL as a virulence factor of concern and references the Elizur et al[226], but makes no firm recommendations on PVL-specific management in the context of CF.[228] Garbacz et al assessed the relatedness of 215 isolates of *S. aureus* from 107 people with CF (paediatric and adult). *S. aureus* spa typing and detection of a variety of toxin genes was performed. They did not find the PVL toxin gene in any of the isolates tested.[229]

A study from Brazil found slightly higher numbers of chronic MRSA isolates from children with CF as PVL-positive; out of 116 samples, 8.6% tested positive for the gene producing PVL toxin.[230] Another study analysing the genotypic diversity of *S. aureus* and interactions with *P. aeruginosa* demonstrated high detection rate of PVL within seven USA300 clone isolates, again very small numbers.[231] Interestingly, a molecular analysis of skin and soft tissue infections in 501 people without CF demonstrated a detection rate of 41.5% PVL-positivity from 169 MSSA isolates.[232]

Recommendations

- CF clinicians should discuss with CF microbiology teams if there are clinical concerns around potential of PVL-SA, (especially if associated with skin and soft tissue lesions and/or new lung cavities) and organising of appropriate molecular testing (low).
- If PVL-SA is identified, CF clinicians should discuss ongoing management strategies with the CF microbiology team and infection control. Liaising with Public Health bodies to assess household contacts may also be required (moderate).

4.4 Identification of fungi

The identification of *Aspergillus* spp. and other moulds is commonly based on macro- and microscopic characteristics of colonies grown on agar plates in the lab.[233] This conventional method is time consuming and requires experienced technicians highly skilled in mycology. Identification to the species senso stricto level cannot be obtained using the phenotypic

identification and requires DNA sequencing. Emergent rare non-*Aspergillus* moulds observed in samples from people with CF include *Lomentospora, Scedosporium* and *Exophiala* species.[234]

The use of fungal-specific PCRs has been reported to aid in the identification of fungal pathogens from respiratory samples. Most experience has been reported with use of various commercial *Aspergillus*-PCRs. Vergidis et al showed that *Aspergillus* PCR significantly increased the detection of *Aspergillus* spp. in conventional sputum cultures from 15.7% (culture only) to 49.2% in a cohort of people with chronic pulmonary aspergillosis, ABPA/SAFS and *Aspergillus* bronchitis.[126] Baxter et al showed that in a CF cohort, use of an *Aspergillus* real-time PCR (RT-PCR) increased the yield to 74% compared to 37% by conventional culture.[235]

A few commercially available *Aspergillus* RT-PCR are worth mentioning in this context, although reported experience in CF and chronic pulmonary aspergillosis is scarce. The AsperGenius® (PathoNostics) is multiplex RT-PCR and detects a limited number of *Aspergillus* species directly in BAL specimens and can detect mutations in the *CYP51A* gene associated with azole-resistance. It has reported sensitivity and specificity of 84% and 80% in haematological patients.

Singh et al used the AsperGenius® to test 160 BAL samples with chronic respiratory diseases in Delhi, India. Only 23% of samples were culture-positive compared to 83% positivity by *A. fumigatus* species PCR, highlighting concerns about the low yield of cultures. Notably, 25% of BAL samples (from 33/160 subjects) had azole resistance-associated mutations by direct detection using this PCR assay.[236]

Guegan et al prospectively processed 119 sputum samples from 87 people with CF for *Aspergillus* detection by means of mycological culture and four quantitative PCR (qPCR) assays, two in-house methods and two commercial multiplex RT-PCR assays simultaneously detecting *Aspergillus* and the most relevant *cyp51A* gene mutations (MycoGENIE® and AsperGenius®). The overall rate of *Aspergillus* detection with the four qPCR assays ranged from 47.9 to 57.1%, contrasting with 42/119 (35.3%) positive cultures with *A. fumigatus*.[237] The high sensitivity of PCR on sputum could then contribute to more effective grading of *Aspergillus* disease in CF.

The MycAssay™ Aspergillus (Myconostica) is a RT-PCR detecting 18 different *Aspergillus* species directly in BAL specimens, with a reported sensitivity and specificity of 80% and 97.1% in immunocompromised people.[238]

There are no specific PCRs available to identify the rarer moulds (*Lomentaspora, Scedosporium* and *Exophiala* species amongst others). MALDI-TOF MS is currently replacing traditional microbiological identification methods, especially in the field of bacteriology. For filamentous fungi, this new identification method is challenged by the intrinsic characteristics of eukaryotic organisms and reference libraries are not as comprehensive as the bacterial ones. Four different MALDI-TOF MS benchtop platforms are currently approved and commercialised in Europe for the routine identification of fungi in clinical microbiology laboratories: the Bruker Biotyper® (Bruker Daltonics, Bremen, Germany), VITEK® MS (bioMérieux, Marcy l'Etoile, France), Axima-SARAMIS (Shimadzu/AnagnosTec, Duisburg, Germany), and the Andromas system (Andromas SAS, Paris, France).[239] In a recent review, Wilkendorf et al discussed approaches for sample processing and growth conditions before analysis, and performance of commercially-available databases.[240]

Recommendations

- Conventional identification of *Aspergillus* species should be done to differentiate *A. fumigatus* from non-*fumigatus* species in routine sputum samples (moderate).
- Conventional identification of *Aspergillus* species should be done if a specific request for fungal culture of sputum and BAL-fluid samples and antifungal therapy is considered (high).
- If non-*Aspergillus* species are cultured from sputum or BAL-fluids, consider sending to reference laboratory for further identification if deemed clinically relevant and antifungal therapy is considered (moderate).
- *Aspergillus*-PCR (in-house or commercial) should be done on BAL-fluids if cause of pulmonary exacerbation and/or lung function decline is unclear and fungal culture of BAL-fluid is negative (low).
- 18S PCR (pan-fungal) is not recommended to be used on sputum and BAL-fluids as the technique is not appropriate for use on non-sterile samples (moderate).

5. Susceptibility testing

Before isolates obtained from CF respiratory samples are susceptibility tested it is important to understand the stage of infection, as this will have profound effects on interpretation of the results. This is particularly important for *P. aeruginosa*. The value of susceptibility testing in people with chronic *P. aeruginosa* infection is questionable – see Lee et al for definition.[241] It remains unclear whether the findings of these studies in chronic *P. aeruginosa* infection in CF translate to chronic infection with other CF-associated pathogens such as Bcc, *A. xylosoxidans*, and *M. abscessus* complex.

5.1 General antimicrobial susceptibility testing guidelines

Laboratory standards in the US recommend the use of agar diffusion methods for susceptibility testing.[242] However, a survey of the laboratory protocols of 150 of the 190 laboratories providing support to US CF centres found that only 52% of these were performing agar diffusion methods for susceptibility testing, such as disc diffusion and gradient test.[243] An external quality assessment (EQA) scheme for German laboratories processing CF respiratory samples revealed a wide variability in methods used for susceptibility testing and inconsistent performance.[244] Similarly, a Europe-wide EQA scheme revealed considerable variability in the methods of susceptibility testing used (including disc diffusion, minimum inhibitory concentration (MIC) gradient strips and automated devices), along with disparity in the number and range of antimicrobials tested.[245] A combination of automated devices and agar diffusion methods was also reported from a survey of CF Microbiology practices conducted in 17 Spanish laboratories.[246]

Historically many UK laboratories followed guidance on susceptibility testing methodologies from the British Society for Antimicrobial Chemotherapy (BSAC). However, since an update of the BSAC method in 2015 there has been a shift towards using standardised guidance and information from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to inform testing. The **most recent guidance** from EUCAST (published January 2022) noted that wild type phenotypes of *P. aeruginosa* without detectable resistance to the agents tested should now be reported as "Susceptible, increased exposure". Laboratories should not report such results as "Susceptible, standard dosing regimen". Instead, the report should encourage prescribers to use higher doses/frequencies of agents when treating *P. aeruginosa*.

All general, national and international methods are based on recommendations for the treatment of organisms causing acute infection and pharmacokinetics/pharmocodynamics (PK/PD) models using blood levels of antibiotics. These methods therefore do not address the particular challenges of treatment in the CF respiratory tract, with the exception of local deposition to lungs achieved by aerosolised therapy where antibiotic levels are generally higher. Nor are they designed to test organisms causing chronic infection which, in the case of *P. aeruginosa*, is associated with slow-growing bacterial populations within complex polymicrobial biofilms.

The EUCAST guidelines are appropriate for testing organisms associated with acute infections that grow overnight without the need for special growth requirements. Once bacteria cause chronic infection in CF, mixtures of colonial morphotypes are observed. At this stage, the susceptibility of different morphotypes and even the same morphotype within a single specimen can vary greatly.[247] Atypical forms can include slow growing variants. All these factors can make antimicrobial susceptibility testing (AST) difficult, particularly for aiding the choice of antibiotic(s) and forecasting clinical efficacy.

Some bacterial species cultured from CF samples are rarely encountered in respiratory tract infections in people without CF. For non-fermentative Gram-negative bacilli other than *P. aeruginosa*, specific EUCAST guidance is currently limited to trimethoprim-sulfamethoxazole and cefiderocol for *S. maltophilia* and piperacillin-tazobactam, meropenem and trimethoprim-sulfamethoxazole for *A. xylosoxidans*. There is no guidance in relation to other species, such as Bcc members, other *Burkholderia* spp., *Bordetella* spp., *R. pickettii, Pandorea* spp., or *I. limosus*. Although there are data on the distribution of MICs in wild type strains of Bcc,[248–250] and including some of the novel antimicrobials now available,[251–254] very little has been published on the correlation of *in vitro* antibiotic susceptibility with the clinical efficacy of treatment. In practice, treatment of such infections, including life-threatening 'cepacia syndrome' is usually empirical, and based on combinations of 3–4 antibiotics delivered by a variety of routes.[2, 255]

One study proposed revised breakpoints for tobramycin when testing *P. aeruginosa* isolates from people with CF receiving aerosolised tobramycin therapy.[256] The Spanish Antibiogram Committee (The Mensura Group) advised the

adoption of higher breakpoints for those receiving aerosolised rather than parenteral tobramycin. These breakpoints were susceptible ≤64µg/mL and resistant ≥128µg/mL, as compared to the normal Clinical and Laboratory Standards Institute (CLSI) breakpoints of susceptible ≤4µg/mL, intermediate 8µg/mL, and resistant ≥16µg/mL. Using the lower CLSI breakpoints susceptibility rates were 79% and 81% for agar dilution and gradient test methods, respectively. These rates increased to 95% using the proposed Mensura breakpoints. It is not known if these higher breakpoints correlate better with clinical outcomes.

5.2 Susceptibility testing guidelines

5.2.1 *P. aeruginosa*

A comparison was made between susceptibility testing of single *P. aeruginosa* morphotypes and a mix of all *P. aeruginosa* morphotypes using isolates obtained from 94 sputum samples from children with CF. Susceptibilities were performed using a broth microdilution method. Mixed morphotype testing correctly predicted susceptibility to a panel of seven agents on 90.4% of occasions but correctly predicted resistance on only 57% of occasions. Although the mixed morphotype method was quicker and cheaper than selecting individual morphotypes, it was less accurate.[257]

5.2.1.1 Conventional manual methods – disc diffusion, gradient strips, agar dilution, broth microdilution

A survey was conducted using 2,194 isolates of *P. aeruginosa* to assess the accuracy of the standardised BSAC disc diffusion method performed in 25 diagnostic laboratories compared to MIC determination in a central reference laboratory. Ninety-eight of the isolates were from people with CF. While resistance was over-reported by the BSAC method, results obtained were more accurate than previously obtained with the non-standardised Stokes' method.[258]

The correlation between disc diffusion, gradient test and a reference broth microdilution method for susceptibility testing of 12 antimicrobials was evaluated using 597 isolates of *P. aeruginosa* obtained from CF sputum samples. Disc diffusion was performed using a standardised CLSI method. CLSI breakpoints were used to interpret gradient test and broth microdilution results. Results of standardised disc diffusion and gradient test both correlated well with MICs obtained by broth microdilution. Major errors (calling an organism resistant when susceptible) and very major errors (calling an organism susceptible when resistant) were 1.1 and 0.4% for standardised disc diffusion and 2.2% and 0.1% for gradient test, respectively. Most problems were observed with mucoid strains, particularly when testing ceftazidime and piperacillin-tazobactam.[259]

A method of direct susceptibility testing on CF sputum samples was compared with testing of individual morphotypes using 316 sputum samples from people with CF. Gradient test strips for six different antibiotics (ticarcillin, ceftazidime, imipenem, aztreonam, ciprofloxacin and tobramycin) were placed directly onto agar plates inoculated with sputum. MICs were determined at 18h, 24h and 48h of incubation. *P. aeruginosa* was isolated from 303/316 (95.8%) samples, with a bacterial load of $\geq 10^5$ CFU/mL in 276 (91%) of these. Agreement between the direct method and testing of individual morphotypes occurred on 90.4% (ticarcillin) to 96.3% (imipenem) of tests. The direct susceptibility method was less able to detect *P. aeruginosa* if other Gram-negative bacilli, such as Bcc, or *S. aureus* were present in the sample and was particularly poor if the bacterial load of *P. aeruginosa* was $<10^5$ CFU/mL. No comment was made regarding the impact of this method on laboratory resources.[260]

A comparison was made of direct plating of 45 CF sputum samples onto conventional culture media for isolation of *P. aeruginosa* versus directly onto Iso-Sensitest agar plates containing one of 10 antimicrobials incorporated at a 'breakpoint' concentration.[261] A representative of each colonial type (morphotype) recovered from both routine media and selective media was tested for its susceptibility to 10 antimicrobials using a standard agar dilution MIC technique.

Of the samples shown to contain resistant strains, the proportion detected using routine media and selective media, respectively, was: 42% and 100% for amikacin, 57% and 100% for gentamicin, 54% and 100% for tobramycin, 88% and 77% for aztreonam, 62% and 90% for ceftazidime, 70% and 97% for meropenem, 61% and 100% for piperacillin/tazobactam, 90% and 86 for temocillin%, 66% and 100% for ticarcillin/clavulanic acid, and 80% and 90% for ciprofloxacin resistance. The increased rates of isolation on selective media were statistically significant ($P<0.05$) for amikacin, gentamicin, tobramycin, meropenem, piperacillin/tazobactam and ticarcillin/clavulanic acid. This study adds further weight to the hypothesis that the results of conventional susceptibility testing grossly underestimate the amount of AMR in *P. aeruginosa* isolated from CF sputa.[261]

The reproducibility of the BSAC disc diffusion method in ascertaining the susceptibility of *P. aeruginosa* isolates associated with chronic

infection in CF was studied using 101 sputum cultures. Susceptibility tests were performed by eight different workers in the same laboratory and then on the same sample by seven different laboratories. A mean of four different morphotypes of *P. aeruginosa* were identified in each sample, each morphotype yielding a mean of three different antibiograms. Wide variability in susceptibility test results was observed, whether performed by different workers in the same laboratory or between different laboratories testing the same sample. The authors questioned the role of antimicrobial susceptibility testing (AST) once chronic *P. aeruginosa* infection was established in the CF lung.[247]

Four different methods of testing susceptibility to colistin were compared using 25 isolates of *P. aeruginosa* and 12 isolates of *S. maltophilia* from people with CF. The four methods were agar dilution, broth microdilution, gradient test and disk diffusion. Although all four methods showed excellent repeatability there were significant levels of non-concordance between agar dilution and the other three methods, giving rise to concerns that broth microdilution, gradient tests and disk diffusion may all over-report levels of susceptibility to colistin.[262] However, microdilution is still the only recommended AST for colistin recommended by EUCAST.

The problems in measuring antibiotic susceptibility of *P. aeruginosa* were further illustrated by the results of the 2007 European Quality Assessment survey of diagnostic microbiology. An atypical *P. aeruginosa* from a person with CF was sent to 31 laboratories in Europe for susceptibility testing. Most laboratories used either disc diffusion or gradient tests. The results were described by the author as "close to random" for quinolones and tobramycin. For ciprofloxacin 36% of laboratories reported the organism as susceptible (S), 27% reported intermediate susceptibility (I) and 37% resistant (R). For tobramycin the results were 36% S, 27% I, and 36% R.[245]

5.2.1.2 Automated devices

The susceptibility results obtained by two automated devices were compared to those of a reference method using 498 isolates of *P. aeruginosa* obtained from people with CF.[263] The devices used were VITEK® (bioMérieux, Marcy l'Etoile, France) and MicroScan WalkAway (Dade-Behring, Sacramento, USA). More very major errors were seen using these devices than were previously encountered using broth microdilution, disc diffusion or gradient test methods.[259]

The Micronaut Merlin system was evaluated using 56 isolates of *P. aeruginosa* (22 were mucoid) obtained from people with CF. The system determines MICs using a broth microdilution method. The results generated by the system were compared to an in-house broth microdilution method and agar dilution using CLSI breakpoints as standards. There were more very major errors with the system compared to the in-house broth microdilution method, particularly for non-mucoid *P. aeruginosa*.[264] A follow-up study further evaluated the system using 405 *P. aeruginosa* isolates (109 were mucoid) from 154 people with CF. The system generally reported lower MICs than were reported with a standard agar dilution method. This resulted in fewer major errors but the rate of very major errors was high (19.2%). In this study, these very major errors were more likely with mucoid strains.[265]

5.2.1.3 Multiple combination bactericidal testing and biofilm models

Culture conditions may have a significant impact on the results of susceptibility testing. Twelve multi-resistant isolates of *P. aeruginosa* were obtained from sputum samples of six people with CF and grown either under planktonic conditions, as an adherent monolayer, or as a biofilm. MICs and multiple combination bactericidal antibiotic susceptibility testing (MCBT) were performed using CLSI criteria. MICs of ceftazidime and ciprofloxacin were significantly higher for adherent monolayer and biofilm-grown bacteria compared to planktonic bacteria but largely unchanged for aminoglycosides and meropenem. The MCBT tests were no different for planktonic, adherent, or biofilm-grown bacteria for three isolates but for the other nine isolates two- and three-drug regimens were less active against biofilm-grown bacteria than adherent bacteria, which in turn were less active than against planktonic bacteria. Antagonism was seen against biofilm-grown bacteria but not against planktonic bacteria.[266]

A similar study was conducted using 16 *P. aeruginosa* isolates from the sputa of 16 people with CF. MICs and MCBT to ten different agents were tested using CLSI criteria under aerobic and anaerobic planktonic conditions and when grown in biofilms. However, some results conflicted with those obtained by the previous study. Colistin was bactericidal against 100%, 75% and 25% of isolates under aerobic, anaerobic, and biofilm conditions, respectively. By contrast, meropenem was bactericidal against 69%, 31%, and 19% of isolates under the same conditions, respectively. MCBT results indicated significantly more bactericidal regimens (25/37) under aerobic conditions than either anaerobic (14/37) or biofilm (13/37) conditions. Antagonism was seen with 38%, 26%, and 36% of regimens under aerobic, anaerobic, and biofilm conditions, respectively.[267]

Another study of susceptibility testing under biofilm testing found that 29% of CF *P. aeruginosa* isolates were resistant to double-antibiotic combinations. Interestingly the addition of either azithromycin or rifampicin to effective antibiotic combinations frequently resulted in antagonism.[268]

Biofilm and conventional susceptibility tests were compared using isolates of *P. aeruginosa* obtained from 40 people with CF. Conventional susceptibilities were determined using a broth microdilution method. For chronic *P. aeruginosa* infections, the outcome of conventional testing was the inclusion of a ß-lactam antibiotic in all regimens. Conversely, biofilm testing resulted in the inclusion of a ß-lactam in only 42.5% of regimens, with 57.5% of regimens including azithromycin. There was significant discordance between the two susceptibility testing methods, with conventional and biofilm testing indicating the same combination on only 20% of occasions.[269]

MCBT does not measure classical synergy, but looks at the effect *in vitro* of antibiotic combinations at single concentrations. Classical synergy testing methods, such as checkerboard and time kill studies, have therefore been proposed as a way to find antibiotic combinations that may be effective against multi-resistant *P. aeruginosa*. A study of 44 resistant isolates of *P. aeruginosa* from nine people with CF compared the results of synergy testing by checkerboard and time kill with MCBT. The authors found that isolates with the same colonial morphotype or PFGE pulsotype from single sputum samples could produce different synergy results. The results also varied depending on the method used with no one method able to predict the outcome of treatment of an acute exacerbation.[270]

An artificial sputum medium model was developed to test *P. aeruginosa* in conditions more akin to those found in the CF lung. The medium consisted of amino acids, mucin and free DNA. *P. aeruginosa* formed self-aggregating biofilm structures within the medium. The assay was developed in a microtitre plate format. Fifteen isolates of *P. aeruginosa* had MICs of tobramycin determined. Big differences were noted when isolates were cultured in microaerophilic conditions with tobramycin MICs increasing up to >128-fold compared to isolates grown aerobically. However, this assay took over three days to perform and to date no study has been performed to ascertain whether the model is more predictive of clinical response compared to standard methods.[271]

The use of a biofilm model to perform susceptibility tests on *P. aeruginosa* greatly increased the level of resistance seen to cefepime, imipenem, gentamicin, tobramycin and ciprofloxacin but not azithromycin in comparison to results obtained with tests performed on planktonic bacteria.[272]

5.2.1.4 Correlation between susceptibility test results and clinical outcomes

A multi-centre, randomised double-blind controlled trial was performed in which 14 days of IV antibiotic therapy for *P. aeruginosa* was selected on the basis of biofilm or conventional susceptibility testing. There were 74 exacerbations in 39 subjects. A total of 46% of exacerbations in the conventional group achieved a 3-log reduction in *P. aeruginosa* sputum density compared to 40% in the biofilm group. There was no difference in improvements in lung function between the two groups.[273] The most recent Cochrane Review concluded that there was insufficient evidence to recommend choosing antibiotics on the basis of biofilm rather than conventional antimicrobial susceptibility tests in the treatment of *P. aeruginosa* pulmonary infections in people with CF.[274]

The relationship between conventional susceptibility testing results and clinical response to IV antibiotics (tobramycin plus ceftazidime) was evaluated in 77 people with CF with an acute exacerbation of chronic *P. aeruginosa* infection. MICs were determined (method not stated) and isolates categorised as susceptible or resistant on the basis of CLSI breakpoints. Fifty-four patients improved on therapy, nine worsened, and 14 remained unchanged. There was no correlation between susceptibilities to tobramycin and ceftazidime and clinical response to therapy for acute exacerbations of chronic *P. aeruginosa* infection in CF. The authors concluded that even if there was a correlation its impact would be small and not clinically relevant.[275]

A five-year retrospective review of 103 courses of antibiotics for pulmonary exacerbations of chronic *P. aeruginosa* infection in 52 children with CF categorised episodes according to antibiotics prescribed versus the susceptibility test results obtained. *P. aeruginosa* was fully susceptible, partially susceptible or fully resistant on 33%, 44.7% and 16.5% of episodes, respectively. There was no association between change in lung function, change in BMI or time to next exacerbation and the use of antibiotics to which the isolates were either fully or partially susceptible or fully resistant.[276]

Another study examined the associations between antimicrobial susceptibility test results and antimicrobial switching during pulmonary exacerbations of chronic *P. aeruginosa* infection in children with CF between 2011 & 2016. During this period susceptibility test results were available in 2,518 (39%) of 6,451 pulmonary exacerbations across 36 US hospitals. Whilst the availability of susceptibility test results significantly increased the odds of making an antimicrobial switch there was no evidence that these switches resulted in improved clinical outcomes or increased time to next exacerbation.[277]

A review of 3,820 pulmonary exacerbations in 413 people with CF with chronic *P. aeruginosa* infection treated between 1999 and 2018 was conducted in a single CF centre in the US. *P. aeruginosa* isolates were categorised as either 'fully' susceptible to at least one administered agent (62.6%), 'none' where isolates were not susceptible to any agent (8.9%), 'incomplete' where some but not all isolates were susceptible to at least one administered agent (2.4%) and 'indeterminate' where the results of susceptibility tests were unknown (26.2%). There was no association between improvements in respiratory function and weight gain and susceptibility of *P. aeruginosa* to the antimicrobials administered.[278]

A prospective, randomised, double-blind controlled clinical trial assessed whether MCBT improved clinical outcomes in people with CF experiencing acute infective exacerbations with multi-resistant bacteria, including *P. aeruginosa*. Two hundred and fifty-one people with CF were enrolled in the study. Each study participant submitted a sputum sample every three months for conventional culture and susceptibility testing and MCBT. After a study period of 4.5 years, 132 participants had experienced an acute infective exacerbation and were randomised to receive a 14-day course of two blinded IV antibiotics chosen either on the basis of conventional testing results or MCBT. Forty-three (67%) of those randomised to the MCBT group and 39 (57%) of those randomised to the control group grew *P. aeruginosa*. There were no significant differences between the two groups in clinical response rates or time to next exacerbation. The authors concluded that the additional complexity of MCBT did not result in improved clinical outcomes.[279]

One study has analysed the correlation between clinical outcomes and biofilm susceptibility testing by MCBT. Isolates from 110 acute exacerbations in people with CF were retrospectively subjected to biofilm susceptibility testing in addition to conventional MCBT. They included people chronically infected with *P. aeruginosa* (50 cases), Bcc (33), *A. xylosoxidans* (five), *S. maltophilia* (three) or various combinations of these four organisms (19). Sixty-six (60%) of 110 people were treated with combinations to which all their isolates were susceptible on conventional testing but only 24 (22%) were treated with combinations to which all isolates were susceptible on biofilm testing. There was no significant difference in treatment failure rates or time to next exacerbation comparing those treated with a combination to which at least one isolate was susceptible on biofilm testing to those treated with a combination to which none were susceptible, but there was a significant trend to shorter hospital stay (13.3 days versus 17.4, P=0.04). However, there was no difference in any outcome measure between patients with all isolates susceptible and those with at least one organism resistant by biofilm MCBT. This potential contradiction may be explained by study design and the complex mix of different species involved.[280] The most recent Cochrane Review concluded that there was insufficient evidence to determine the effect of choosing antibiotics on the basis of MCBT results as opposed to conventional susceptibility results for acute exacerbations of chronic *P. aeruginosa* infection in CF. The authors concluded that a large, international, multi-centre study would be needed to further investigate this.[281]

MCBT has been used to select surgical prophylaxis regimens in people with CF with chronic *P. aeruginosa* infection undergoing lung transplantation between 2000 and 2010. The incidence of post-transplant infection was compared between prophylaxis selected either on the basis of MCBT (50 individuals) or standard susceptibility testing (79 individuals). There were only two cases of sepsis in the MCBT group compared to 13 in the conventional group (P<0.05). However, almost half of the sepsis cases in the study were caused by organisms other than *P. aeruginosa*. Whilst there was a trend towards fewer cases of *P. aeruginosa* sepsis and empyema in the MCBT group, these reductions were not statistically significant. The study was also unable to measure the impact of MCBT prophylaxis selection on mortality.[282]

The impact of a new protocol reducing the number of routine susceptibility tests performed on isolates of *P. aeruginosa* associated with chronic infection in CF was assessed by comparing two time periods in 2005 and 2006. In the first period in 2005 susceptibility tests were performed on all *P. aeruginosa* isolates obtained from every sample. In the second time period in 2006 susceptibility tests were only performed if the sample had been taken at the commencement of IV antibiotics, if there was a clinical deterioration, or if no tests had been performed in the previous three months. The testing method was Stokes' disc diffusion. The number of tests performed in 2006 was reduced by 56%, saving an estimated 170 hours of laboratory time and €10,000 in consumables and salary costs. No significant differences in several clinical parameters were observed after the introduction of the new testing protocol, suggesting the omission of susceptibility tests had had no detrimental effect on clinical outcomes.[283]

Reducing susceptibility tests on *P. aeruginosa* isolates to just annually was evaluated in 44 adults with CF and chronic infection. Clinical parameters in the two years before and after introduction were compared. There were no significant differences in number of pulmonary exacerbations, hospital admissions, length of stay or rate of decline in lung

function in the two years following introduction of annual only susceptibility tests.[284]

Recently the Antimicrobial Resistance International Working Group in Cystic Fibrosis concluded that there is little evidence that antimicrobial susceptibility tests predict clinical responses in CF.[285] A further publication by the group recommended that clinicians should prioritise clinical response rather than *in vitro* susceptibility test results and not change antimicrobial therapy solely on the basis of these results if the individual is improving.[286]

Recommendations

- Susceptibility testing should be performed on isolates of *P. aeruginosa* associated with early and intermittent colonisation (low).
- Susceptibility testing should be performed using a standardised and validated method (for instance, EUCAST) (moderate).
- Susceptibility testing of *P. aeruginosa* using automated devices cannot be recommended at this time (moderate).
- There is no evidence to support the routine use of biofilm testing methods (moderate).
- There is no evidence to support the routine use of multiple combination bactericidal antibiotic testing (MCBT) to select antibiotics for acute pulmonary exacerbations (high).
- There is weak evidence that surgical prophylaxis selected on the basis of MCBT results may reduce the incidence of early post-transplant infections with *P. aeruginosa* (low).
- Conventional susceptibility tests on *P. aeruginosa* isolates associated with chronic infection in CF are poorly reproducible and may not predict clinical response (high). This would also apply to susceptibility tests performed on newer agents such as ß-lactam/ß-lactam inhibitor combinations, such as ceftazidime-avibactam.
- Limiting testing to once annually on *P. aeruginosa* isolates associated with chronic infection does not adversely affect clinical outcomes for at least two years (moderate).

5.2.2 *B. cepacia* complex

There is little published evidence specifically relating to susceptibility testing of Bcc isolates from people with CF.

An initial evaluation of the Micronaut Merlin system included 14 '*Burkholderia cepacia*-like organisms'. Species listed included *B. multivorans* and *B cenocepacia*. The number of major and very major errors was comparable to the in-house broth microdilution methods.[264]

Broth microdilution, agar dilution, gradient test and disk diffusion based on CLSI standards were compared for their performance in evaluating the susceptibility of 82 clinical isolates of Bcc.[387] Using broth microdilution as the 'gold standard', results were broadly comparable between this and agar dilution. However, gradient test and disk diffusion both showed unacceptably high major and minor error rates.

The accuracy and reliability of a range of different methods to determine susceptibility to minocycline, ciprofloxacin, trimethoprim-sulfamethoxazole, meropenem, ceftazidime and chloramphenicol was evaluated using 155 different strains of Bcc. Broth microdilution performed at both 30°C and 35°C was compared to agar dilution at the same temperatures, gradient strips and EUCAST standardised disk diffusion. Broth microdilution at 35°C showed a reproducibility ranging from 70% to 84.5% for all agents. Correlation of MICs obtained by broth microdilution at 30°C and 35°C ranged from 63%–85% and for agar dilution at 30°C and 35°C ranged from 32.9%–87%. Essential agreement between broth microdilution at 35°C and gradient strips ranged from 62.6% (trimethoprim-sulfamethoxazole) to 83.9% (minocycline). Significant numbers of major errors for trimethoprim-sulfamethoxazole were also seen when using disk diffusion. The authors concluded that no method was able to provide accurate and reliable determination of MICs for Bcc isolates.[287]

Problems were also encountered in measuring antibiotic susceptibility of Bcc in the 2007 and 2008 European Quality Assessment surveys of diagnostic microbiology. Most participating laboratories used agar diffusion or gradient test and reported varying susceptibility results for *B. cenocepacia* and *B. vietnamiensis*.[245] It is unclear if these differences were because the organism formed a heterogenous population, or were due to variations in methodology or because the breakpoints divided the distribution of MIC for the natural population.

Isolates from sputa of 110 people with CF were subjected to MCBT of 94 double- and triple-antibiotic combinations. Biofilm-grown isolates were significantly less susceptible to combinations than planktonic-grown bacteria. Fifty-nine percent of Bcc isolates were resistant to all double-antibiotic combinations tested. Triple-antibiotic regimens were significantly more active.[268]

A study using three different species from the Bcc revealed that biofilm inhibitory concentrations of meropenem and piperacillin-tazobactam were

considerably higher than MICs for planktonic-grown isolates. Such differences were not observed for either tobramycin or amikacin.[288]

The prospective, randomised, double-blind controlled clinical trial that assessed whether selecting antibiotic therapy based on MCBT improved clinical outcomes in people with CF also included people infected with Bcc. Twenty-five (39%) of those randomised to the MCBT and 29 (43%) randomised to the control group were infected with Bcc. There were no significant differences in outcome between the two groups.[279]

Recommendations

- There is insufficient evidence to make any specific recommendations regarding susceptibility testing of Bcc isolates. If laboratories do perform susceptibility testing a standardised method should be used (such as EUCAST disc diffusion) (low). There are no published breakpoints specifically for Bcc — see section 5.2.3.
- There is no evidence to support the routine use of multiple combination bactericidal antibiotic testing to select treatment regimens for Bcc (high).

5.2.3 Other Gram-negative bacilli

There are no CF-specific publications relating to susceptibility testing of *S. maltophilia*.

Disc diffusion and gradient test were compared with an agar dilution method using 70 isolates of *S. maltophilia* from a variety of clinical sites.[289] Methods were conducted using CLSI standards and the isolates were tested against chloramphenicol, doxycycline, gatifloxacin, trimethoprim-sulfamethoxazole, ticarcillin-clavulanate, polymyxin B and colistin. Good correlation between disc diffusion and gradient test with agar dilution was observed for chloramphenicol, doxycycline, gatifloxacin, trimethoprim-sulfamethoxazole and ticarcillin-clavulanate but not for polymyxin B or colistin. At that time BSAC recommendations for conducting susceptibility tests suggested that only trimethoprim-sulfamethoxazole can be reliably tested by disc diffusion or gradient test.[290]

There are difficulties in interpreting susceptibility results for many of the other unusual oxidase positive Gram-negative bacilli associated with CF (such as *A. xylosoxidans, Pandoraea* spp., *Ralstonia* spp.) as there is limited data on the distribution of MICs in the wild-type population and few have had breakpoints set by EUCAST. EUCAST has however listed non-species-specific "clinical" breakpoints based on pharmocokinetics (Monte Carlo simulation) that may be used to categorise the *in vitro* susceptibility of these bacteria.

Recommendations

- Susceptibility testing of *S. maltophilia* isolates should be guided by published recommendations, such as EUCAST, CLSI (low).
- EUCAST non-species-specific breakpoints may act a guide for interpretation of susceptibility results for other unusual Gram-negative bacilli (GNBs) associated with CF (very low).

5.2.4 *S. aureus* and *H. influenzae*

There are no CF-specific publications relating to susceptibility testing of *S. aureus* or *H. influenzae*.

Recommendation

- Susceptibility testing of *S. aureus* and *H. influenzae* isolates should be guided by published recommendations, such as EUCAST or CLSI (high).

5.2.5 NTM

The clinical value of *in vitro* susceptibility testing in the treatment of infection requires correlation between *in vitro* susceptibility to a drug by a defined method to clinically achievable drug concentrations at the site of infection and good outcome to treatment with that drug. The value of susceptibility testing for pulmonary NTM infection is mostly uncertain. EUCAST has no such agreed breakpoints, however there is CLSI guidance. It could be noted that in North America 'sensitivities' are required to recoup treatment costs. Possibly this may encourage the use of antibiotics where evidence of benefit is sparse. Using such standardised methodology can however provide a baseline for research and development.

In considering how to treat NTM infections with combinations of antimicrobials, factors such as antimicrobial synergy,[291–294] indifference and antagonism should be considered, as well as other potential drug interactions, allergies, and toxicities. The enthusiasm to 'do something' and the perception that 'more is better' should be tempered by the need to avoid harm. Although the European Committee on Antimicrobial Susceptibility Testing has presently no guidelines for susceptibility testing of NTM, the CLSI guidelines recommend testing using broth microdilution and provide breakpoint concentrations to interpret MICs as 'susceptible', intermediate' or 'resistant'.[295] However, these cut-offs have had very limited clinical validation. Although there is limited pharmacokinetic data available for MAC lung disease to support breakpoint concentrations,[296] there are no representative pharmacokinetic or pharmacodynamic data to guide treatment of other NTM infections.

Susceptibility testing for NTM is discussed in recent clinical guidelines.[25, 134, 297] Briefly, for MAC, macrolide and amikacin should be tested; for *M. kansasii*, rifampicin and clarithromycin should be tested; for *M. abscessus* species, macrolides and amikacin should be tested. *M. abscessus* subsp. *abscessus* and *bolletii* have an *erm(41)* erythromycin resistance methylase gene that results in inducible resistance to macrolides.[298] This can be detected by molecular means or extended incubation (up to 14 days) in microdilution trays.[299] The GenoType NTM-DR kit and WGS can also be used where available.[196, 197] It should be noted that obtaining consistent susceptibility test results is challenging, with EQA schemes evolving.[300]

Recommendations

- Where susceptibility testing is requested for clinically significant infection it should be performed in an appropriate specialist laboratory such as a Mycobacterium Reference Laboratory prior to initiation or for relapse/failure to guide but not dictate treatment (moderate).
- Results for macrolides and amikacin along with rifampicin in some instances should be reported as they impact on clinical outcomes (moderate).
- Laboratories should consider releasing MIC as well as qualitative SIR data on their reports to clinicians (low).

5.2.6 *Aspergillus* spp.

Correct identification of species of *Aspergillus* is important as some are resistant to amphotericin such as *A. versicolor, A. nidulans* and *A. lentulus*. Azole resistance has been recognised for more than two decades. Itraconazole-resistant *A. fumigatus* was first described in the late 1990s.[301] Multiple triazole resistance was reported more than 10 years ago,[302] as was resistance to echinocandins,[303] and evidence was already emerging at this time of increasing rates of resistance in UK isolates of *Aspergillus*.[304] The Infectious Diseases Society of America (IDSA) guidelines around that time therefore recommended that if a person had had prior treatment with azoles, susceptibility testing may be warranted.[305]

More recently though, there have been reports of increasing antifungal resistance in *Aspergillus* isolates, including some in CF. A study in the UK showed that azole resistance amongst *A. fumigatus* isolates had increased from 0.4% in the period 1998–2011 to 2.2% in the period 2015–2017.[306] Reduced susceptibility to caspofungin was also noted in the latter period, although not to amphotericin B. Isolates in this study came from people with a range of clinical backgrounds rather than specifically CF.

A study in Denmark analysed 340 mould isolates from 159 people with CF, most of which were either *A. fumigatus* (266; 78%) or *A. terreus* (26; 7.6%). Azole non-susceptibility rose from 4.5% in 2007–2009 to 10.5% in 2018. Of note was the identification of *cyp51A* gene mutations, such as TR_{34}/L98H, which are thought to be of environmental origin and selected by the extensive use of azoles in fungicides for plant protection.[307]

There are two standardised antifungal susceptibility testing guidelines available: the **EUCAST** microdilution method , and the **CLSI methodology**. Clinical breakpoints for interpretation of azole and amphotericin B MICs for *Aspergillus* are only provided by EUCAST. Clinical breakpoints for the rarer moulds are often not available.

A commercial microdilution method has been described for performing antifungal susceptibility tests on *Aspergillus* species. The Sensititre™ YeastOne™ (SYO) system (Thermo Fisher Scientific) was evaluated with 59 common and 27 uncommon *Aspergillus* species. Overall agreement between the SYO system and the CLSI reference method was 96.5% for itraconazole and posaconazole and 100% for voriconazole. All 10 isolates with *cyp51* resistance gene mutations were correctly identified as having MICs above recognised cut-offs for susceptibility.[308]

Recommendations

- In people with CF, isolates of *Aspergillus* spp. or other moulds, and in particular *A. fumigatus*, should be subjected to fungal susceptibility testing when antifungal treatment is being considered. Consider referral to a mycology reference laboratory (such as the UK Health Security Agency Mycology Reference Laboratory, Bristol) if local testing is not available (low).
- For moulds other than *A. fumigatus* for which antifungal susceptibility testing is indicated, it is recommended to contact a mycology reference laboratory (such as UK Health Security Agency Mycology Reference Laboratory, Bristol) for further guidance (low).

6. Post-analytical processes: interfacing with the clinical team

Appropriate microbiological sampling and analysis is critically important in providing CF clinicians with relevant data to deliver targeted infection management. However, the role of microbiology in providing care for people with CF crosses far beyond acting as a 'results reporting service'. Microbiology plays a crucial central role in the clinical interface, and the CF microbiologist is an essential part of the CF multidisciplinary team (MDT), advising on infection prevention and control (IPC) and individual and population-level infection treatments.[309]

6.1 Infection prevention and control (IPC)

The CF microbiologist plays a key role in working with local IPC teams and informing appropriate IPC protocols for the CF centre. Transmissible *P. aeruginosa*, MRSA and members of the Bcc are well recognised pathogens, associated with worse clinical outcomes and potential to spread between patients. At time of writing Cystic Fibrosis Trust is working towards re-iteration of IPC guidance with regards to these organisms.

More recently *M. abscessus* has been implicated as cause for IPC concern,[25, 35] and the 2013 Cystic Fibrosis Foundation guidance recognises potential IPC risk of other Gram-negative organisms (such as *Ralstonia* spp.) and also non-bacterial organisms such as respiratory viruses and fungi, such as *Aspergillus* spp.[310] At time of writing the COVID-19 pandemic brings developing and adhering to appropriate IPC processes for all CF centres and CF healthcare professionals into sharp focus.

The ECFS Standards of Care highlights the broad-reaching remit of the role played by the CF microbiologist with respect to IPC, including:[311]

- Develop a local IPC policy and procedures in line with expert national and international guidelines
- Management of individuals with transmissible infections, both in the community and in hospital, to prevent the spread of infection. This includes determining the transmission risk of key pathogens via different routes, for example direct and non-direct modes of transmission
- Outbreak management
- Surveillance and screening for transmissible infection, with knowledge of local epidemiology crucial to inform best practice for each centre
- Antimicrobial treatment to attempt clearance carriage of potentially transmissible microorganisms
- Guidelines for CF Health Care Workers with infections
- Advice around facilities for the CF centre and the outpatient department, include the cleaning and maintenance of equipment and built environment (such as water and ventilation standards), and involvement in any plans for refurbishment or rebuild of the department

6.2 Clinical Intervention

All clinical CF guidelines make recommendations on antibiotic therapy based on the pathogens identified in respiratory samples.[2, 63, 312, 313] This applies to both maintenance therapies, such as prophylactic inhaled and oral antibiotics, as well as the choice of antibiotics for treatment of acute pulmonary exacerbations. It is important to note, however, that identification of an organism in a respiratory sample does not necessarily indicate active infection, particularly for uncommon bacteria such as *Elizabethkingia* spp., *Chryseobacterium* spp. and many others.[314] Respiratory microbiology results in CF need to be interpreted in the wider clinical context.

The direct importance of microbiological investigations is perhaps clearest in the context of the first isolate of a new pathogen such as *P. aeruginosa*. There is evidence from randomised trials to support the use of eradication therapy with nebulised +/- oral antibiotics for new *P. aeruginosa* infection but no single regimen has been shown to be superior to others.[10] Long-term benefits of eradication therapy for early *P. aeruginosa* have yet to be demonstrated conclusively but international guidelines highlight the importance of prompt initiation of treatment following a first isolation of *P. aeruginosa*.[313]

In addition to facilitating timely eradication therapy and effective infection control strategies, clinical microbiology has other important roles to play in the management of CF. Surveillance of sputum microbiology may allow de-escalation of nebulised antibiotic therapy if a pathogen is no longer identified, for instance. Given that treatment burden is a major issue for people with CF,[315]

this may help improve wellbeing. Antimicrobial susceptibility testing may have a role to play in optimising antibiotic therapy in CF, although this is a contentious area as such tests have poor predictive power for clinical improvement with anti-pseudomonal therapy.[2]

The CF microbiologist must also be an advocate for judicial antimicrobial prescription. Some organisms identified will not be pathogenic and therefore not require antimicrobials. The challenges of clinically impactful AMR in CF are clearly significant, but defining what AMR is in relation to CF infections is not straightforward.[316] As knowledge and understanding in this area particular to CF infections grows, so will the importance of antimicrobial (and diagnostic) stewardship, to appropriately treat multi-drug resistant infections whilst conserving effective treatments for future use.

6.3 Multidisciplinary team working

In addition to providing a laboratory service and advice on IPC, microbiologists should participate in regular MDT meetings to discuss the management of the service and of individuals with CF. Technology for remote contact should be utilised to aid joint working where appropriate. The CF MDT and microbiology services (including the CF microbiologists and laboratory staff) should have a close working relationship and jointly undertake regular audit, quality improvement projects, teaching, and educational meetings such as journal clubs. They should work together to develop local policies for IPC, antibiotic stewardship and reporting of laboratory results, including pathways for rapid communication for results requiring immediate action, such as first/new growth of *P. aeruginosa*. The CF MDT and microbiology teams at large CF centres should also both provide advice and support to local CF and microbiology teams at shared care hospitals.

Recommendations

- CF clinicians and microbiologists should agree the structure, content and communication of laboratory reports (low).
- All new or suspected isolates of *P. aeruginosa*, MRSA, Bcc and *Mycobacterium* species should be communicated urgently to the CF clinical team (moderate).
- Regular surveillance of potentially transmissible pathogens (such as *P. aeruginosa*, MRSA, *M. abscessus*) including molecular typing, should be carried out as recommended by Cystic Fibrosis Trust Infection Control Group (moderate).
- Local surveillance should inform local infection control policy, and centres should be aware of the incidence and prevalence of pathogens amongst people with CF in their care (low).
- In addition to providing a laboratory service and advice on IPC, microbiologists should participate in regular MDT meetings to discuss the management of the service and of individuals with CF (low).
- CF MDT and microbiology teams should participate regularly in joint audit and quality improvement activity (low).

7. Scanning the horizon of CF microbiology

There are multiple knowledge gaps in our current understanding of the microbiology of CF lung disease. Here, we review current research which has improved understanding of CF microbiology over the last decade, and also explore what is likely to bring considerable change in the future.
Topics discussed include:

- The application of molecular and novel diagnostics to CF infection
- Insights from the fields of genomics and lung microbiota analysis
- CF pathogen taxonomy, with continual reclassification of several key pathogens
- The impact of the SARS-CoV-2 pandemic
- The introduction of CFTR modulator therapy
- Antimicrobial resistance and infection control in the context of CF lung disease

7.1 CF microbiology in the context of the lung infection microbiota

The polymicrobial nature of CF lung infection has been understood for some time and is a key factor for why specific microbiological guidelines are needed to isolate and diagnose organisms which are considered pathogens versus bystanders during chronic lung disease. The diversity of the microorganisms present in the lung is known as the microbiota, while the totality of the different functions they may bring to the site of infection is known as the microbiome. **Five major features of the CF microbiome stand out in the context of our understanding of CF lung infection:**

i. **The lung is not sterile.** Even from birth the lung is not a sterile environment, and a range of microorganisms may be found within it. Knowledge of the CF microbiota has expanded considerably in the last 20 years.[317, 318] It is not yet clear which of the additional microbiota beyond the clinical priority organisms should be considered as pathogens and treated to reduce disease.[319]

ii. **Anaerobic organisms are present.** Anaerobic bacteria persist in high numbers within this environment. Anaerobes such as *Prevotella, Veillonella* and the *Streptococcus milleri* group are nearly always present in the lungs of chronically-infected individuals with CF.[22, 320, 321] The anaerobic microbiota may interact with pathogens to drive lung disease and increase virulence, while others may be beneficial to lung health.

iii. **Microbiota diversity is lost as disease progresses during chronic CF lung infection.** A clear signature from the research is that once the lung microbiota becomes less diverse and dominated with pathogens such as *P. aeruginosa*, disease is severe with the lung becoming irreversibly damaged.[322–325] A measure used to understand this diversity of microorganisms in the microbiota is known as the Shannon index. Multiple studies have shown that a low Shannon index is associated with individuals with CF that have poor lung function, as defined by FEV_1, and severe disease.[322–325]

iv. **The CF lung microbiota varies considerably between individuals with CF.** Another feature from the extensive research studies of the CF microbiota is that it is frequently specific to individuals with CF.[322–325]

v. **Microbiota pathogens versus bystanders.** Finally, at the current time it is also unclear which additional members of the CF microbiota beyond specific known pathogens may drive exacerbation or disease progression. This is largely the reason why CF microbiome analysis has not progressed further in terms of use in routine diagnostics. Understanding the diversity of the microbiota has not been validated as a means to predict the best therapeutic options for infection. The pathogens which are the subject of the microbiology diagnostics reviewed in this guidance, remain the key organisms for which a role in disease progression and a response in terms of therapeutic administration have been historically linked for management of CF lung infection.

7.1.1 CF microbiota diagnostics and potential roles

Given the complexity associated with the CF microbiota and current inability for it to be harnessed as a diagnostic, what roles can microbiota diagnostics play? One role microbiota analysis can play is in exposing the weaknesses of conventional microbiology and to improve accuracy and efficiency for CF infection diagnostics. For example, certain pathogens

such as *P. aeruginosa* grow very rapidly in the laboratory overtaking other organisms that are present, and because it is such a well-known CF pathogen, microbiologists likely have an 'unconscious bias' for isolating it, despite other dominant microbiota being present during culture. Given the range of selective media that are applied to isolate pathogens from CF respiratory specimens, there ultimately has to be filtering in terms of what is detected or not, and this may not necessarily give a representative picture of the pathogens that are driving disease in individuals with CF.

The application of microbiota-based diagnostics reveals the difficulty in identifying nonfermenting Gram-negative pathogens such as *Burkholderia, Achromobacter, Stenotrophomonas*, and *Ralstonia*, which may be missed more than 10% of the time by conventional culture.[324] In this study, a simple, single PCR microbiota profiling method, Ribosomal Intergenic Spacer Analysis (RISA), was applied directly to total DNA extracted from CF sputum. The nucleic acid had been obtained using clinically accredited procedures that were part of standard virology diagnostics. This raises another interesting point in the context of CF microbiology, where virology has moved towards molecular culture-free diagnostic methods, while bacteriology remains largely culture-based and hence prone to bias and subjectivity. The RISA PCR profiling method was also able to differentiate individuals who had lost the diversity in their CF lung microbiome, and were pathogen dominated.[324] Hence its ability, and that of other microbiome methods such as diversity sequencing, to detect this loss of diversity signal as a biomarker for progressive lung disease could be useful in the context of future diagnostics.

Whilst it is clear that CF lung organisms live within a polymicrobial community in biofilm niches, understanding how they interact with each other and the host to cause infection is still unclear. Further research utilising -omics technologies is needed to more accurately describe these interactions within the clinical setting. As an example, transcriptomics is the study of pathogen genetic expression and can provide a link between the genome and cellular phenotype, and may allow exploration of these interactions. Welp and Bomberger reviewed bacterial community interactions in chronic respiratory disease and noted in the discussion that 'although many microbial interactions have been elegantly described, understanding how organisms interact in the host during [chronic respiratory disease] is still largely being elucidated'.

Furthermore, it is possible to design multi-omic studies (including genomics, proteomics, transcriptomics, among others) to gain further clarity. Mac Aogain and colleagues evaluated integrative microbiomics during bronchiectasis exacerbation, (utilising microbiome, mycobiome and metagenomics) to help described longitudinal interactome dynamics which was altered during exacerbations, before stabilising. The authors state that 'integrative microbiomics captures microbial interactions to determine exacerbation risk, which cannot be appreciated by the study of a single microbial group'.[388, 389]

Areas for future research

- The application of CF microbiota diagnostics has not been validated for predicting optimal therapeutic options for individuals with CF. However, considering the alignment of virology with bacteriology in relation to CF diagnostics, it may open up avenues to use the same respiratory sample nucleic acid derived from clinically accredited extraction procedures.
- Additionally, microbiota diagnostics can be useful to accurately identify pathogens which are difficult to isolate by culture, and for setting out baselines for understanding how the microbiota diversity in individuals with CF is altering over time.
- The application of culture-free molecular microbiota diagnostics which only require limited amounts of respiratory specimen may also become useful in the new era of CFTR modulator therapy as described below.
- The interactions of identifiable organisms within the CF lung need further elucidating (utilising a variety of -omics approaches, potentially in a multi format) in order to understand role of pathogens versus bystander or even protective organisms. This could help target antimicrobial strategies in a more personalised manner.

7.2 Rapid and novel diagnostics strategies for CF infections

7.2.1 Molecular detection direct from samples

A number of molecular assays for detecting CF-associated pathogens have now been reported but few studies have subjected these techniques to rigorous evaluations against conventional culture methods using respiratory samples taken from people with CF. PCR based methods are outlined below for key CF pathogens.

7.2.2 *P. aeruginosa* detection

Several PCR diagnostics for *P. aeruginosa* have been developed and tested in the context of CF infection. A multiplex PCR based on simultaneous amplification of two lipoprotein genes of *P. aeruginosa*, *oprI* and *oprL*, was evaluated using 49 sputum samples from people with CF.[326] Conventional culture was conducted using blood agar, chocolate-bacitracin agar and MacConkey agar with plates incubated at 35°C in air (chocolate-bacitracin in 5% CO_2) for 48h. All 40 samples positive for *P. aeruginosa* on conventional culture were PCR-positive. Four samples negative on culture for *P. aeruginosa* were also PCR-positive.

A PCR method targeting the *P. aeruginosa algD* GDP mannose gene was compared with conventional culture on cetrimide agar using 15 respiratory samples (ten sputum, five throat swabs) from an unspecified number of people with CF. The *P. aeruginosa algD* PCR was positive in seven samples, six of which were also positive on culture. One sample was culture-positive but PCR-negative.[327] Subsequently the same group compared the *algD* GDP mannose gene PCR with conventional culture and detection of serum antibodies against three *P. aeruginosa* antigens (elastase, alkaline protease, exotoxin A) for the early diagnosis of *P. aeruginosa* in 87 people with CF with a mean age of 9.7 years. Culture was positive in 42/87 (48.2%) whilst PCR was positive in 53/87 (60.9%; P=NS). Serology was positive in 38/87 (43.6%). The combination of PCR plus serology was superior to each single method, to PCR plus culture, and to culture plus serology.[328]

Molecular detection of *P. aeruginosa* using PCR to detect *oprL* and *exoA* was compared with conventional culture detection using a combination of blood agar, MacConkey agar, and PIA with incubation at 37°C in air for 48h.[329] Both methods were used on sputum samples submitted from 57 adults with CF. PCR was performed using *oprL* and *exoA* targets. Thirty sputum samples were positive for *P. aeruginosa* using conventional methods and 35 were positive by PCR. PCR for the *oprL* target was more sensitive than for the *exoA* target. Five adults with initially negative sputum samples on culture were PCR-positive and then subsequently became culture-positive a mean duration of 4.5 months later (range 4–17 months).[329]

More recently a qPCR assay specific for the *P. aeruginosa oprL* gene was used for the early detection of *P. aeruginosa* in stored sputa from 47 children with CF a year prior to culture positivity. The lower limit of detection for this assay was 10–6µg/mL. At this lower limit, the sensitivity compared with culture was 93.5%, and the specificity 71.5%. Of 312 samples tested 201 (64.4%) were negative by both culture and qPCR, two samples were culture-positive and qPCR-negative, and 80 (25.6%) samples were culture-negative but qPCR-positive.[330] A further study used qPCR to discriminate between chronic and intermittent *P. aeuginosa* infection. This involved detection of the *oprL* gene, followed by a second qPCR targeting the *ecfX* and *gyrB* genes which was performed on samples with a significant qPCR signal for the *oprL* gene but where *P. aeruginosa* could not be cultured. This dual qPCR approach offered a sensitivity of 100% with a threshold of 10 CFU/mL and a specificity of 100%. This study found a correlation between significant increases in the abundance of *P. aeruginosa* in throat swabs and sputum from people who were chronically infected compared to those with intermittent infection, and like the previous study, detected *P. aeruginosa* infection before they became culture-positive.[331]

7.2.3 *B. cepacia* complex detection

The following studies describe direct Bcc detection from samples by PCR in comparison with culture. A PCR method to detect the Bcc's 16S rRNA gene loci was compared to conventional culture using MacConkey agar supplemented with an unspecified concentration of polymyxin. Plates were incubated for five days. Both methods were applied to 219 sputum samples from adult (109 samples) and paediatric (110 samples) individuals with CF. In total 63/219 (28.8%) samples were culture-positive for Bcc, of which 60 (95.2%) were PCR-positive. Conversely there were seven culture-negative samples that were PCR-positive for Bcc.[332]

PCR amplification of the *recA* gene followed by restriction fragment length polymorphism (RFLP) analysis was compared with conventional culture on MAST selective agar incubated at 37°C in air for 48h using 100 sputum samples from people with CF. Seventeen samples were positive for Bcc on culture, all of which were PCR-positive, and there were no positive PCR results with culture-negative samples.[333] The addition of multiple novel species to the Bcc has however retrospectively shown that RFLP is no longer reliable for species-level identification,[334] although *recA* gene sequencing may be used in its place for the majority of species seen in CF infection.[17] Certain species groups within the Bcc such as taxon K, now require multiple tests and genomics to accurately identify species such as *B. lata, B. contaminans* and *B. aenigmatica*.[192]

A novel rRNA gene-based PCR assay successfully and rapidly identified 69 Bcc-positive sputa, irrespective of the Bcc species. The technique exhibited 100% sensitivity and specificity for the 17 Bcc species tested at the time of the study and

detected 10 CFU/mL when applied to sputum.[335] More recently a study described a multiplex real-time PCR assay which used species-specific probes to identify seven Bcc species (including *B. cenocepacia* and *B. multivorans*), and *B. gladioli*. The assay was found to exhibit 100% sensitivity for all eight species examined and detected between 102 and 103 CFU/mL in spiked sputum. Analysis of 200 CF sputa previously identified as Bcc-positive by culture on selective media and identified to species level by *recA* sequencing correlated fully with the real-time PCR assay results. Cross-reactions of probes between species other than those examined in the study were not examined but with further validation using *recA* sequencing this method may prove useful.[336]

Area for future research

- While these direct PCR diagnostic methods may have utility in a number of situations, given the extensive reclassification of Bcc species, the species-level identification performance has not been assessed for all species. Therefore, it is recommended that caution is applied for identification beyond Bcc level.

7.2.4 Direct molecular detection of NTM

Although molecular techniques to detect NTM in respiratory specimens are now commercially available, they are costly and remain less sensitive than conventional AFB culture. Examples of methods available for direct NTM detection include PCR restriction analysis of the *rpoB* gene,[337] and the GenoType Mycobacteria Direct test.[338, 339] To date, clinical validation of these tests in different cohorts wih CF and for different NTM species is incomplete and therefore their role in routine clinical practice remains to be determined. However, one very useful application of direct molecular detection is to rapidly differentiate MTB complex from NTM in smear-positive samples.[340]

Area for future research

- Until there is a more robust longitudinal study along with more standardised validated species-specific PCR there is currently insufficient data to recommend the routine application of molecular detection methods directly to samples in addition to routine culture methods.

7.2.5 Detection of volatile compounds for the identification of CF lung infection

There are several published studies that indicate *P. aeruginosa* can be detected on the breath or from the sputum of people with CF via volatile organic compounds (VOCs). These studies have used *in vitro* detection methods or sniffer dogs to detect VOCs. The compound 2-aminoacetophenone (2-AA) was found in 1996 to be responsible for the as the sweet "grape-like" odour produced by *P. aeruginosa* during growth on laboratory media and from infected wounds, and this has been used as an indicative VOC in several studies.[341] An extensive review of VOCs useful for the detection of *P. aeruginosa* infection in exhaled breath has been carried out by Kos et al.[341]

Kos et al identified 241 VOCs from a comprehensive review of the exhaled breath literature. Fifty six were further evaluated, and 13 could be detected in exhaled breath of 25 paediatric and 28 adult individuals with CF positive or negative for *P. aeruginosa* infection. Three of the 13 VOCs were significantly ($P<0.05$) different between *P. aeruginosa* groups in children; however none were different in adults. They concluded that targeted VOC analysis appears to discriminate children with and without *P. aeruginosa*, but that analysis of composite VOC fingerprints is required to distinguish potential infection with adults.[341]

Sniffer dogs have also been evaluated as means to detect *P. aeruginosa* infection in CF. Davies et al ran a series of pilot studies to evaluate their performance. The study confirmed that following training, dogs can detect the odour of *P. aeruginosa* in broth culture supernatants with high levels of sensitivity, distinguishing them from other bacteria commonly encountered in the CF lung. But as the mode of growth *P. aeruginosa* in broth is significantly different from that in the CF airway, the volatile signals generated compared to infection will likely differ. The study provided useful proof-of-principle for the concept and further work to develop a non-sputum, non-culture-based test which can be performed by people with CF on a frequent basis is ongoing. This could involve breath, cough/ huff tissues, exhaled breath condensate or even urine collected at home. They concluded that dogs may ultimately prove more sensitive or more affordable for screening lower airway infection in CF than other methods.[342]

7.2.6 Serological diagnosis of *P. aeruginosa* infection

The routine use of serological testing for the diagnosis of *P. aeruginosa* infection remains controversial, and its availability as a standardised clinical test is currently extremely limited across the UK. There have been a few studies that have demonstrated the usefulness of investigating for antibodies against *P. aeruginosa* when used as an adjunct in the assessment of eradication therapy and chronic *P. aeruginosa*.[343] Studies

where the testing bundle looked at multiple sero-markers have reported higher levels of diagnostic accuracy. Possible targets include: anti-alkaline protease and anti-Exotoxin A, *Pseudomonas*-CF-IgG ELISA (CF-IgG) using St-Ag:1–17, and crossed immunoelectrophoresis using water-soluble antigens (St-Ag:1–17). There remains the controversy in the consistency and standardisation of the levels of anti-*P. aeruginosa* antibody response, and whether this depends on age, the testing kit used or the host response.[344]

Area for future research

- Although controversial, there is evidence demonstrating there may be a place for serological diagnostics for *P. aeruginosa*, especially as it is likely that CFTR modulator therapy will lessen sputum production. Until there are more robust longitudinal studies along with more standardised serological testing and methodology, the use of *P. aeruginosa* antibodies cannot be currently recommended.

7.2.7 Detection of CF related pathogen evolution: *S. aureus* small colony variants

Multiple CF pathogens are known to evolve their phenotype during chronic lung infection. The switch of *P. aeruginosa* from non-mucoid to mucoid, and its association with poor clinical prognosis is one of the best characterised transitions. Recent studies have also shown that *S. aureus* SCVs are associated with persistence and disease in CF.[1, 345] A more recent large multi-centre observational study in the US using multivariable analysis found that children isolating *S. aureus* SCVs, particularly thymidine deficient strains, have lower lung function and increased exacerbation risk compared for those who did not isolate *S. aureus* SCVs. In contrast, no such association was found for *P. aeruginosa* or MRSA colonisation.[346]

The ability of two different chromogenic agars and four conventional media for isolating *S. aureus* SVCs was assessed using 53 well characterised strains, some of which had been obtained from the sputa of people with CF. The media used were *S. aureus* ID (SAID), CSA, MSA, Baird-Parker agar, tryptic soy agar and Columbia blood agar. After 72 hours of incubation all SCV isolates were recovered on Columbia blood agar. However, one isolate failed to grow on either SAID or Baird-Parker agar, three failed to grow on either CSA or MSA, and five failed to grow on tryptic soy agar.[347] Most SCVs do not exhibit colour change on chromogenic media, so for examination of non-pigmented colonies, subculture of possible SCVs onto Columbia blood agar should be considered.[1]

Areas for future research

- For optimal identification of *S. aureus* SCVs, a combination of selective media, with specific supplements to detect thymidine deficient strains and CBA, with culture up to 72 hours, is necessary. Currently many clinical laboratories do not examine for SCVs and efforts to standardise laboratory methodologies to achieve this should be considered.
- Longitudinal studies, such as data capture of people with CF colonised with *S. aureus* SCVs in National/International CF Registries, and investigating the relationships between presence and absence of *S. aureus* SCVs in clinical outcomes, should be considered.

7.2.8 Impact of remote monitoring and telemedicine

There has been increasing interest in recent years in remote, home monitoring for people with CF with the aim of early detection of exacerbations and clinical deterioration.[348, 349] This trend was rapidly accelerated by the SARS-CoV-2 pandemic in 2020 which led to rapid changes in the delivery of CF care. In the UK, people with CF were advised to shield at home for prolonged periods during the pandemic and there was a widespread move towards telemedicine and remote consultations. However, microbiological investigations pose a major challenge to remote monitoring and virtual clinics. The practice of sending respiratory samples to the microbiology laboratory by post has long been used in clinical practice, but further studies are required to determine fully the utility of such an approach (see Chapter 2 for an extended discussion of postal CF specimens). There is also a need to develop novel technologies which would allow near-patient detection of CF pathogens without the need for laboratory testing.

7.3 The impact of microbial genome sequencing on CF microbiology

The last decades have seen a massive increase in research determining the partial or complete genomes of multiple CF pathogens. These genomic resources have enabled a much greater understanding of the taxonomy and classification of certain pathogens, and the high-resolution nature of the analysis has enabled accurate strain typing and a detailed understanding of transmission between people with CF.

7.3.1 Genomic taxonomy and classification: implications for CF pathogens

Historically, a range of phenotypic and genetic characteristics of bacteria have been used as markers for their taxonomic classification. However, genome sequencing has led to the widespread use of genomic methods such as digital DNA-DNA hybridisation (DDH) and average nucleotide identity (ANI),[350–352] which help considerably in defining existing named species, as well as novel bacterial genomic species. In particular, a boundary of approximately 95% ANI at the genomic level is useful in defining a bacterial species, but for certain groups a higher threshold may be required.[350, 351] Examples of how these genetic and genomic methods bacterial classification have altered our understanding of CF pathogens are described below.

Application of genomic methods to the Bcc has proved to be highly valuable in revealing novel species classifications and subgroups. Genomic methods were influential in teasing apart species within taxon K, and resulted in the proposition of a new species *B. aenigmatica*,[192] alongside existing species of *B. lata* and *B. contaminans* which are part of this closely related group. It has also been shown that for these taxon K Bcc species, genomic taxonomy is more accurate than *recA* or MLST, and its particularly useful for differentiating *B. aenigmatica* from *B. lata*.[353] It is also clear from genomic methods that we have not reached an end to the potential reclassification and expansion of Bcc species. After analysis of 116 Bcc strain genomes, 22 genomic taxonomy clusters were predictive of known species, while an additional 14 novel species groups were identified.[188] Expanding on this, Mullins and Mahenthiralingam examined over 4,000 *Burkholderia* genomes and were able to define 26 novel genomic species groups within the Bcc.[354] In particular, five of these were closely related to *B. cenocepacia*,[354] which is one of the more dominant Bcc groups seen in CF.[17] Further examination of these novel groups may alter our understanding of their epidemiology and genesis in CF.

The phylogenetic division of *B. cenocepacia* using the *recA* gene alone had identified two key subgroups designated III-A and III-B. *B. cenocepacia* III-A isolates are the second most dominant Bcc species seen in the UK CF population,[17] and have been associated with severe clinical outcome in CF.[355, 356] In comparison, the *B. cenocepacia* III-B subgroup is more common than III-A within the US CF population.[27, 224] Recent genomic analysis of the III-B subgroup indicated that it meets the >95% ANI threshold for proposal as a novel genomic species based on both ANI, and also core gene phylogeny which increase resolution in terms of classification.[357] The authors put forward the name "*Burkholderia servocepacia*",[357] but this is considered an invalid name as they have not yet applied all the appropriate taxonomic criteria in their analysis and species designation. In addition, the prefix "*servo*" (meaning to guard or protect) was added because of the ability of certain environmental *B. cenocepacia* strains within the subgroup III-B to act as protective biological control agents. Unfortunately, this name completely ignores the fact that over 50% of the strains analysed within the study were derived from CF infection,[357] and hence cannot be considered protective in relation to their virulence and opportunistic pathogenicity in CF.

Beyond the Bcc, genomic classification has revealed the following in relation to other CF genes: *B. gladioli*, a problematic *Burkholderia* species encountered in both US and UK CF populations has also been shown to have at least three major genomic groups, encompassing five different evolutionary clades.[17, 27, 165] Although all of these subgroups can all be considered a single species by ANI (*B. gladioli*) , group 1 isolates were associated with the production of the lethal toxin, bongkrekic acid, and hence are worthy of differentiation using PCRs that can target the toxin gene.[165] The application of genomic analyses to *Achromobacter* species has also confirmed the ability of multiple species to cause CF infection such as *A. xylosoxidans, A. ruhlandii, A. insuavis, A. aegrifaciens, A. dolens*, and *A. insolitus*, with the studies also defining novel genomic species groups which require potential classification.[358, 359]

Phylogenomic analysis using thousands of core genes within *P. aeruginosa* genomes has shown that while it can be considered a single genomic species, it divides into two major genomic groups.[360, 361] Well-characterised reference strains such as PAO1 and the Liverpool epidemic CF strain LES B58, fall into group 1, while other reference strains such as PA14, as well as other isolates from CF infection fall into group 2.[361, 362] Whether these genomic sub-groupings within *P. aeruginosa* have phenotypic or clinical significance remains to be determined. Genomic analysis has played a major role in understanding the emergence of *M. abscessus* and closely-related NTM species groups as problematic CF pathogens in the last 10 years. In particular, genomics of NTM has provided evidence for their transmission in CF as noted below.

Area for future research

- With the large-scale application of genome sequencing to multiple CF pathogens, it is clear that there is genomic diversity within many species, and additional species groups

within pathogens such as *Burkholderia* and *Achromobacter*. Understanding if these evolutionary subgroups contribute to differing clinical outcome is a key area of future study, and to facilitate this, rapid cost-effective diagnostics for these genomic groups are required.

7.3.2 Strain typing, transmission and global molecular epidemiology in CF

Strain genotyping below the level of species identification is a key part of CF microbiology because of the known problems caused by transmissible strains of multiple pathogens (see Chapter 4). Patient-to-patient spread by socialisation as well as nosocomial acquisition is most notably associated with bacteria of the Bcc, but is now also accepted to occur for *P. aeruginosa*, MRSA, and *M. abscessus*.[310] Other antibiotic-resistant Gram-negative pathogens such as *S. maltophilia, Achromobacter, Ralstonia, Cupriavidus*, and *Pandoraea* spp. have also been associated with sharing among individuals with CF, although direct evidence for patient-to-patient transmission of these potentially epidemic species has only been shown to a limited extent or is now emerging via genomics.[310] Multiple epidemiological tools have been applied to genotype these transmissible pathogens below the species level, including VNTR analysis (see Chapter 4). The majority of research and analysis is now being performed using sequence-based methods such as MLST and WGS which have much greater resolution in terms of pinpointing strain relatedness down to the single nucleotide polymorphism level.[363]

A number of recent studies have shown the power of WGS as an epidemiological tool to study emerging transmissible CF pathogens. Evidence from WGS underpins the current evidence for transmission of *M. abscessus* in CF.[29] The transmission of *Achromobacter* species including *A. ruhlandii, A. xylosoxidans*, and *A. insuavis* was demonstrated by genomic methods in a study of 51 people with CF at a single treatment centre.[364] Shared strains were found in 35% of the individuals examined in this study suggesting that potential transmission of *Achromobacter* species may be more widespread than originally thought.[310]

MLST and the genomic resources associated with this public database, pubMLST,[191] are an incredibly helpful resource from which to identify different CF pathogen strains. Examination of the MLST database shows that molecular typing schemes and resources exist for *P. aeruginosa* (>1,352 isolates), Bcc (>2,092 isolates), *B. gladioli* (>53 isolates), *Achromobacter* species (>1,035 isolates), *S. maltophilia* (>994 isolates), *H. influenza* (>6,028 isolates), MABSC (>1,836 isolates), and *S. aureus* (>36,458 isolates).[191] The website also hosts a very straightforward species identification tool, **Species ID** which can interrogate and compare an uploaded draft genome by extracting the 53 ribosomal MLST loci.[365] Overall, the pubMLST database makes it very straightforward to understand the global epidemiology behind any single strain of a CF pathogen if researchers can obtain a genome sequence, partial or full MLST for it, and compare it to the isolate genotype profiles that are available.

Area for future research

- Moving the surveillance of transmissible CF pathogens towards routine genomic levels of understanding should also be a priority in order to build detailed and comparable knowledge of their microbiology.

7.4 Impact of CFTR modulators and other novel therapies on CF microbiology

CFTR modulator (CFTRM) therapy is a genuine game-changer for CF therapy. This is an ever-evolving landscape with newer generations and combinations of CFTRMs being produced and trialled; more information and research will undoubtedly emerge over the next few years. Currently most data concerning CFTRMs is based upon the use of ivacaftor, the first in the class of CFTRMs. These data suggest that CFTRMs are likely to impact on CF microbiology in two major ways: the effect of CFTRMs on sputum production, and the potential effect of CFTRMs on the CF lung microbiota itself.

7.4.1 Effect of CFTRMs on sputum production

Duckers et al published a systematic review of real-world outcome data on patients on ivacaftor. They found sustained improvements in lung function, pulmonary exacerbations, nutritional status and utilisation of healthcare.[366] Similar results were demonstrated in Registry data from the UK and USA.[367] A key implication for the microbiology laboratory going forward is that people with CF, because of CFTRM therapy, will produce less sputum, particularly individuals who do not have established severe bronchiectasis. As such, the lab may need to be agile enough to work with non-sputum samples, or possibly integrate non-culture identification techniques to limited sputa to

increase detection yield. In addition, the inherent variation of non-sputum samples such as throat and cough swabs needs to be taken into account.

7.4.2 Effect of CFTRMs on CF lung microbiota

CFTRMs may have potential effects (both directly and indirectly) on the microbial constituents within the CF lung, especially in people with less severe structural lung damage. These are summarised in recent reviews.[368, 369] As an example, ivacaftor has a quinolone ring and has been shown to inhibit Gram-positive pathogens such as *S. aureus* and *S. pneumonia*.[370] There is also data to suggest that combination of CFTRMs (ivacaftor and lumicaftor) with the anti-pseudomonal agent polymixin B is synergistic in decreasing *P. aeruginosa* growth after 24h.[371] Also, by their effects on improving lung function as described above, there is a theoretical benefit of reducing antimicrobial usage and therefore further influencing the lung microbiota by not creating selective pressure for antimicrobial resistant pathogens. However, as these therapies are currently still relatively new, there is lack of consistent and robust longitudinal data to support this at this point.

Bearing in mind emergent data, there is possibly an early signal that use of CFTRMs increase biodiversity and reduce *P. aeruginosa* burden. However, there are conflicting results between studies, as illustrated by data produced by the GOAL Investigators of the CFF Therapeutic Development Network. Heltshe et al conducted a longitudinal cohort study analysing microbiology in individuals a year before compared to a year after commencing ivacaftor treatment.[372] They reported significant reduction in *P. aeruginosa* culture-positivity following ivacaftor treatment, whereas a multi-centre cohort study by Harris et al did not demonstrate significant changes to the microbiota following 6 months of ivacaftor.[373]

7.4.3. Novel therapeutics in early stages of clinical use or under development

Therapeutic antibiotic guidance falls within Cystic Fibrosis Trust Antibiotic treatment for cystic fibrosis 2009 document. However, this section gives a flavour of some of the newer approaches which may come into clinical use.

7.4.3.1 Bacteriophages as novel CF infection therapeutics

There has been a resurgence of interest in using bacteriophages (viruses which attack bacteria) because of increasing antibiotic resistance, and the inability to treat certain chronic infections. In CF, the use of customised cocktails of bacteriophages have shown to be successful. Law et al reported successful treatment of MDR *P. aeruginosa* in a person with CF with respiratory failure and who suffered kidney failure due to antibiotic therapy. They were able to be stabilised with bacteriophage therapy, allowing subsequent successful lung transplantation.[374] Dedrick et al described the use of a customised cocktail of lytic phages for the treatment of post lung transplant *M. masssiliense* infection in a child. Sputum and serum were negative by culture after initiation of phage therapy but some skin nodules were culture positive at 121 days. The IV administration of the phage cocktail used in this study was also well tolerated and led to multiple improvements in the clinical condition of the individual.[375] Lebeaux et al reported the use of phage therapy for *A. xylosoxidans* lung infection in a 12-year-old lung transplant recipient with CF. They received two rounds of phage therapy because of persistent lung infection with pandrug-resistant *A. xylosoxidans*, and although initial BAL grew *A. xylosoxidans*, the individual's respiratory condition improved. After months of persistence, *A. xylosoxidans* was eventually cleared from and no re-colonisation occurred in the subsequent two years after phage therapy.[376] Overall, these initial case studies treating antimicrobial resistant persistent CF pathogens are highly encouraging, and phage therapy has the potential to be more widely applied in CF. Several phase I and II clinical trials are underway (clinicaltrials.gov NCT04596319; NCT05010577; NCT04684641).

7.4.3.2 Other novel infection therapeutics in the pipelines

Whilst phage therapy has attracted a lot of attention in recent times, other anti-infection options are under investigation. Some are described below; this is by no means an exhaustive list and for up-to-date overview see the CF Foundation's **Drug Development Pipeline**.

Antibacterial approaches

i. Gallium nitrate

Gallium nitrate disrupts bacterial iron metabolism and this compound has been studied in people with CF with chronic pseudomonal infection. The IGNITE Study was a phase II, multi-centre, randomized, placebo-controlled study of IV Gallium Nitrate in patients with CF, and was presented at the 2018 North American CF Conference.[132]

Whilst no significant difference in lung function was reported compared to the control arm, there was a decrease in *Pseudomonas* in sputum in those in the Gallium nitrate arm.

Studies assessing Gallium nitrate are ongoing, with the phase II ABATE-18-IP Study recruiting to assess efficacy of IV Gallium nitrate in people with CF with NTM (clinicialtrials.gov NCT04294043), and a phase II study of AR501 (inhaled Gallium) planned to follow a successful phase I of safety of inhaled Gallium in healthy volunteers (clinicaltrials.gov NCT03669614).

ii. Nitric Oxide (NO)
NO is well established as a complex molecular influencer of the immune system which, alongside being a blood vessel dilator, has non-specific broad antimicrobial activity against bacteria and viruses.[377] There is also evidence to suggest lower levels of NO in CF airways is associated with poorer outcomes.[378]

As it is active in the lung it has been of long-standing interest to the CF community. Miller et al reported effects of inhaled NO decreasing Pseudomonal burden in rat models,[379] and Deppisch et al demonstrated safety and feasibility of adjunctive NO in eight people with CF colonised with a range of organisms.[380]

Currently there are phase II clinical trials ongoing assessing efficacy of NO. One study focusses on efficacy versus NTM (clinicaltrials.gov NCT04685720), whereas another is a multicentre US study assessing lung function and bacterial load difference in those taking NO.

iii. ACG-701
This compound is a version of sodium fusidate, and a phase I study of its safety and efficacy in patients with CF is in the planning phase.

iv. Lefamulin
Lefamulin is a novel pleuromutilin antibiotic which inhibits bacterial protein synthesis. It has broad antibacterial activity, including against MRSA and common causative agents of community associated bacterial pneumonia. File et al demonstrated non inferiority of lefamulin versus moxifloxacin in a phase III clinical trial, LEAP1.[381]

A phase I study to test the safety and pharmacokinetics of lefamulin in adults with CF is currently underway (clinicaltrials.gov NCT05225805).

Antifungal approaches

i. Opelconazole
There are several newer antifungal agents coming to clinical trial including fosmanogepix, ibrexafungerp, olorofim, opelconazole, and rezafungin. Of these, opelconazole has been designed for inhalation so is extremely attractive to the CF community. It is a novel azole and has a broad range of antifungal activity, including *Aspergillus* spp and *Candida* spp.

A phase II study assessing safety and tolerability of opelconazole in lung transplant recipients with CF is being planned (clinicaltrials.gov NCT05037851).

Antibiofilm approaches

i. SNSP113
There is significant interest in disrupting the biofilm in which bacterial communities thrive in the CF lung. SNSP113 is an inhaled glycopolymer that can target and attack bacterial biofilms, as well as having generalized anti-inflammatory effects and affecting mucus viscosity by interacting with mucin polymers.[382,383]

Studies are planned to assess safety and efficacy of SNSP1134 in people with CF.

7.5 IPC developments that could impact microbiology services

A systematic review of IPC interventions in CF showed whilst there are several guidelines, most recommendations have little or no evidence to support them.[384] There are multiple areas of IPC where further research is needed. **A review from Saiman et al highlighted several areas at the time that needed further evidence to inform recommendation, such as:**[310]

- When should a person with CF who previously had *Burkholderia* species isolated from respiratory tract cultures be considered *Burkholderia*-free?
- Should CF clinics be routinely scheduled based on specific respiratory tract pathogens?
- Should people with CF who are infected with NTM be placed on 'Airborne Precautions' (negative pressure single room, more than 12 air exchanges per hour, air exhausted to outside)?
- Should solid organ transplant recipients with CF be placed in a 'Protective Environment' (a positive pressure room with HEPA filtration)?

An argument could be made for a number of these questions being posed for other emergent CF pathogens.

In 2018 Health Protection Scotland published **an extensive literature review** with an aim to inform national CF IPC guidance. This was a wide-ranging search, incorporating research questions pertinent to neonatal, paediatric and adult CF individuals. **Specific points were raised as future IPC research questions, including:**[385]

- Optimal methods for terminal decontamination

of a hospital room after an individual with MABSC is discharged.

- Studying new disinfectants for nebulisers
- Effective methods for cleaning airway clearance devices.

Interestingly, the NICE guidance on **diagnosis and management of CF** does not specifically set out any CF IPC research recommendations.[63] The ability to maintain safe healthcare infrastructure relies on several factors: broadly speaking there are requirements around ventilation, safe water and clean clinical areas, and equipment. There is a push to make healthcare premises as green and sustainable as possible, whilst providing care that fits IPC remit. This is demonstrated by the latest **HTM0301 guidance** on ventilation, which has sections on Net Zero Carbon ambitions.[386] Together with increased interest around airborne transmission of pathogens and the importance of adequate ventilation in hierarchy of IPC controls, the likely formation of Ventilation Safety Groups for healthcare premises could impact upon microbiology service resources and need for expert microbiological input. Specific guidance around NTM recommends that "any new facilities for patients with CF must take into account the potential for cross-infection with *M. abscessus*. This must include the provision of enhanced ventilation for both inpatient and outpatient care and adequate ventilation in other areas".[35]

Specialist IPC guidance specific to certain organisms (MRSA, *P. aeruginosa*, and Bcc) exists for UK CF healthcare providers through older Cystic Fibrosis Trust publications (https://www.cysticfibrosis.org.uk/the-work-we-do/resources-for-cf-professionals/consensus-documents). However, other organisms may also represent a transmission issue. Transmission of NTM has been extensively debated, with the greatest evidence coming from WGS to suggest possible indirect transmission between patients.[29] Whilst person-person transmission may still need delineating there is no doubt the impact of *M. abscessus* infection upon the people with CF. Even though this organism is ubiquitous in water; it is not mandatory to test for *M. abscessus* in healthcare water supplies. Inkster et al. demonstrated *M. chelonae* presence in hospital tap water, amongst other organisms.[390] If it became a requirement for CF centres to attempt detection within its water supply, this would have significant impact on food and water microbiology testing laboratories, as well as needing evidence-based guidance on any required actions when mycobacteria are found.

As previously described, personalised CF care has embraced the role of virtual medicine. As an example, virtual reality headsets are increasingly being used within and outside CF care, but there is little data currently available regarding optimal decontamination process of such equipment. This may not be an issue where items are single use and strictly not shared but could be problematic if they are being used by several individuals. This represents an IPC research gap as things stand. Therefore, there a number of IPC-related issues that need addressing in the future, which have the potential to affect diagnostic and reference microbiology services.

8. References

1 Kahl BC, Becker K, Löffler B. Clinical Significance and Pathogenesis of Staphylococcal Small Colony Variants in Persistent Infections. Clin Microbiol Rev 2016; 29: 401–427.

2 UK Cystic Fibrosis Trust Antibiotic Working Group. Antibiotic treatment for cystic fibrosis (3rd ed.). 2009.

3 Zobell JT, Epps KL, Young DC, Montague M, Olson J, Ampofo K et al. Utilization of antibiotics for methicillin-resistant Staphylococcus aureus infection in cystic fibrosis. Pediatr Pulmonol 2015; 50: 552–559.

4 The UK Cystic Fibrosis Trust Infection Control Working Group. Methicillin-resistant Staphylococcus aureus (MRSA). 2008.

5 Blanchard AC, Waters VJ. Microbiology of Cystic Fibrosis Airway Disease. Semin Respir Crit Care Med 2019; 40: 727–736.

6 Rosenfeld M, Gibson RL, McNamara S, Emerson J, Burns JL, Castile R et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. Pediatr Pulmonol 2001; 32: 356–366.

7 Cystic Fibrosis Trust. UK Cystic Fibrosis Registry Annual Data Report 2021. London, 2022.

8 Breuer O, Schultz A, Turkovic L, de Klerk N, Keil AD, Brennan S et al. Changing Prevalence of Lower Airway Infections in Young Children with Cystic Fibrosis. Am J Respir Crit Care Med 2019; 200: 590–599.

9 Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol 2002; 34: 91–100.

10 Langton Hewer SC, Smyth AR. Antibiotic strategies for eradicating Pseudomonas aeruginosa in people with cystic fibrosis. Cochrane Database Syst Rev 2014; : CD004197.

11 Hewer SCL, Smyth AR, Brown M, Jones AP, Hickey H, Kenna D et al. Intravenous versus oral antibiotics for eradication of Pseudomonas aeruginosa in cystic fibrosis (TORPEDO-CF): a randomised controlled trial. Lancet Respir Med 2020; 8: 975–986.

12 Jones AM, Dodd ME, Morris J, Doherty C, Govan JRW, Webb AK. Clinical Outcome for Cystic Fibrosis Patients Infected With Transmissible Pseudomonas aeruginosa : An 8-Year Prospective Study. Chest 2010; 137: 1405–1409.

13 Cystic Fibrosis Trust. Standards of Clinical Care for Children and Adults with Cystic Fibrosis in the UK. . London, 2011.

14 Govan JR, Brown AR, Jones AM. Evolving epidemiology of Pseudomonas aeruginosa and the Burkholderia cepacia complex in cystic fibrosis lung infection. Future Microbiol 2007; 2: 153–164.

15 Kalish LA, Waltz DA, Dovey M, Potter-Bynoe G, McAdam AJ, LiPuma JJ et al. Impact of Burkholderia dolosa on Lung Function and Survival in Cystic Fibrosis. Am J Respir Crit Care Med 2006; 173: 421–425.

16 Mahenthiralingam E, Baldwin A, Vandamme P. Burkholderia cepacia complex infection in patients with cystic fibrosis. J Med Microbiol 2002; 51: 533–538.

17 Kenna DTD, Lilley D, Coward A, Martin K, Perry C, Pike R et al. Prevalence of Burkholderia species, including members of Burkholderia cepacia complex, among UK cystic and non-cystic fibrosis patients. J Med Microbiol 2017; 66: 490–501.

18 Geake JB, Reid DW, Currie BJ, Bell SC. An international, multicentre evaluation and description of Burkholderia pseudomallei infection in cystic fibrosis. BMC Pulm Med 2015; 15: 116.

19 Cystic Fibrosis Trust. Melioidosis and world-wide travel. 2016.

20 Gade SS, Nørskov-Lauritsen N, Ridderberg W. Prevalence and species distribution of Achromobacter sp. cultured from cystic fibrosis patients attending the Aarhus centre in Denmark. J Med Microbiol 2017; 66: 686–689.

21 Degand N, Lotte R, Decondé Le Butor C, Segonds C, Thouverez M, Ferroni A et al. Epidemic spread of Pandoraea pulmonicola in a cystic fibrosis center. BMC Infect Dis 2015; 15: 583.

22 Tunney MM, Field TR, Moriarty TF, Patrick S, Doering G, Muhlebach MS et al. Detection of Anaerobic Bacteria in High Numbers in Sputum from Patients with Cystic Fibrosis. Am J Respir Crit Care Med 2008; 177: 995–1001.

23 Rogers GB, Carroll MP, Serisier DJ, Hockey PM, Jones G, Bruce KD. Characterization of Bacterial Community Diversity in Cystic Fibrosis Lung Infections by Use of 16S Ribosomal DNA Terminal Restriction Fragment Length Polymorphism Profiling. J Clin Microbiol 2004; 42: 5176–5183.

24 Guss AM, Roeselers G, Newton ILG, Young CR, Klepac-Ceraj V, Lory S et al. Phylogenetic and metabolic diversity of bacteria associated with cystic fibrosis. ISME J 2011; 5: 20–29.

25 Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann J-L, Nick JA et al. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. Thorax 2016; 71: i1–i22.

26 Roux A-L, Catherinot E, Ripoll F, Soismier N, Macheras E, Ravilly S et al. Multicenter Study of Prevalence of Nontuberculous Mycobacteria in Patients with Cystic Fibrosis in France. J Clin Microbiol 2009; 47: 4124–4128.

27 Salsgiver EL, Fink AK, Knapp EA, LiPuma JJ, Olivier KN, Marshall BC et al. Changing Epidemiology of the Respiratory Bacteriology of Patients With Cystic Fibrosis. Chest 2016; 149: 390–400.

28 Gardner AI, McClenaghan E, Saint G, McNamara PS, Brodlie M, Thomas MF. Epidemiology of Nontuberculous Mycobacteria Infection in Children and Young People With Cystic Fibrosis: Analysis of UK Cystic Fibrosis Registry. Clinical Infectious Diseases 2019; 68: 731–737.

29 Bryant JM, Grogono DM, Greaves D, Foweraker J, Roddick I, Inns T et al. Whole-genome sequencing to identify transmission of Mycobacterium abscessus between patients with cystic fibrosis: a retrospective cohort study. The Lancet 2013; 381: 1551–1560.

30 Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P et al. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. Science (1979) 2016; 354: 751–757.

31 Aitken ML, Limaye A, Pottinger P, Whimbey E, Goss CH, Tonelli MR et al. Respiratory Outbreak of Mycobacterium abscessus Subspecies massiliense in a Lung Transplant and Cystic Fibrosis Center. Am J Respir Crit Care Med 2012; 185: 231–232.

32 Bange F-C, Brown BA, Smaczny C, Wallace RJ, Bottger EC. Lack of Transmission of Mycobacterium abscessus among Patients with Cystic Fibrosis Attending a Single Clinic. Clinical Infectious Diseases 2001; 32: 1648–1650.

33 Lipworth S, Hough N, Weston N, Muller-Pebody B, Phin N, Myers R et al. Epidemiology of Mycobacterium abscessus in England: an observational study. Lancet Microbe 2021; 2: e498–e507.

34 Tortoli E, Kohl TA, Trovato A, Baldan R, Campana S, Cariani L et al. Mycobacterium abscessus in patients with cystic fibrosis: low impact of inter-human transmission in Italy. European Respiratory Journal 2017; 50: 1602525.

35 Cystic Fibrosis Trust Mycobacterium abscessus Infection Control Working Group. Mycobacterium abscessus. Recommendations for infection prevention and control. 2017.

36 Kapnadak SG, Hisert KB, Pottinger PS, Limaye AP, Aitken ML. Infection control strategies that successfully controlled an outbreak of Mycobacterium abscessus at a cystic fibrosis center. Am J Infect Control 2016; 44: 154–159.

37 Shoseyov D, Brownlee KG, Conway SP, Kerem E. Aspergillus Bronchitis in Cystic Fibrosis. Chest 2006; 130: 222–226.

38 Flight W, Jones A. The diagnosis and management of respiratory viral infections in cystic fibrosis. Expert Rev Respir Med 2017; 11: 221–227.

39 Naehrlich L, Orenti A, Dunlevy F, Kasmi I, Harutyunyan S, Pfleger A et al. Incidence of SARS-CoV-2 in people with cystic fibrosis in Europe between February and June 2020. Journal of Cystic Fibrosis 2021; 20: 566–577.

40 Cox MJ, Allgaier M, Taylor B, Baek MS, Huang YJ, Daly RA et al. Airway Microbiota and Pathogen Abundance in Age-Stratified Cystic Fibrosis Patients. PLoS One 2010; 5: e11044.

41 Thomassen MJ, Klinger JD, Badger SJ, van Heeckeren DW, Stern RC. Cultures of thoracotomy specimens confirm usefulness of sputum cultures in cystic fibrosis. J Pediatr 1984; 104: 352–356.

42 Ramsey BW, Wentz KR, Smith AL, Richardson M, Williams-Warren J, Hedges DL et al. Predictive Value of Oropharyngeal Cultures for Identifying Lower Airway Bacteria in Cystic Fibrosis Patients. American Review of Respiratory Disease 1991; 144: 331–337.

43 Avital A, Uwyyed K, Picard E, Godfrey S, Springer C. Sensitivity and specificity of oropharyngeal suction versus bronchoalveolar lavage in identifying respiratory tract pathogens in children with chronic pulmonary infection. Pediatr Pulmonol 1995; 20: 40–43.

44 Hoppe JE, Theurer-Mainka U, Stern M. Comparison of three methods for culturing throat swabs from cystic fibrosis patients. J Clin Microbiol 1995; 33: 1896–1898.

45 Armstrong DS, Grimwood K, Carlin JB, Carzino R, Olinsky A, Phenlan PD. Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. Pediatr Pulmonol 1996; 21: 267–275.

46 Henig NR. Sputum induction as a research tool for sampling the airways of subjects with cystic fibrosis. Thorax 2001; 56: 306–311.

47 Rosenfeld M, Emerson J, Accurso F, Armstrong D, Castile R, Grimwood K et al. Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis. Pediatr Pulmonol 1999; 28: 321–328.

48 Jung A, Kleinau I, Schönian G, Bauernfeind A, Chen C, Griese M et al. Sequential genotyping of Pseudomonas aeruginosa from upper and lower airways of cystic fibrosis patients. European Respiratory Journal 2002; 20: 1457–1463.

49 Equi AC. Use of cough swabs in a cystic fibrosis clinic. Arch Dis Child 2001; 85: 438–439.

50 Kabra SK, Alok A, Kapil A, Aggarwal G, Kabra M, Lodha R et al. Can throat swab after physiotherapy replace sputum for identification of microbial pathogens in children with cystic fibrosis? The Indian Journal of Pediatrics 2004; 71: 21–23.

51 Ho SA, Ball R, Morrison LJ, Brownlee KG, Conway SP. Clinical value of obtaining sputum and cough swab samples following inhaled hypertonic saline in children with cystic fibrosis. Pediatr Pulmonol 2004; 38: 82–87.

52 Maiya S. Cough plate versus cough swab in patients with cystic fibrosis; a pilot study. Arch Dis Child 2004; 89: 577–579.

53 Aaron SD. Sputum versus bronchoscopy for diagnosis of Pseudomonas aeruginosa biofilms in cystic fibrosis. European Respiratory Journal 2004; 24: 631–637.

54 Blau H, Linnane B, Carzino R, Tannenbaum E-L, Skoric B, Robinson PJ et al. Induced sputum compared to bronchoalveolar lavage in young, non-expectorating cystic fibrosis children. Journal of Cystic Fibrosis 2014; 13: 106–110.

55 Ronchetti K, Tame J-D, Paisey C, Thia LP, Doull I, Howe R et al. The CF-Sputum Induction Trial (CF-SpIT) to assess lower airway bacterial sampling in young children with cystic fibrosis: a prospective internally controlled interventional trial. Lancet Respir Med 2018; 6: 461–471.

56 Eyns H, Piérard D, de Wachter E, Eeckhout L, Vaes P, Malfroot A. Respiratory Bacterial Culture Sampling in Expectorating and Non-expectorating Patients With Cystic Fibrosis. Front Pediatr 2018; 6. doi:10.3389/fped.2018.00403.

57 Fenn D, Abdel-Aziz MI, Brinkman P, Kos R, Neerincx AH, Altenburg J et al. Comparison of microbial composition of cough swabs and sputum for pathogen detection in patients with cystic fibrosis. Journal of Cystic Fibrosis 2021. doi:10.1016/j.jcf.2021.08.031.

58 de Bel A, de Geyter D, de Schutter I, Mouton C, Wellemans I, Hanssens L et al. Sampling and Decontamination Method for Culture of Nontuberculous Mycobacteria in Respiratory Samples of Cystic Fibrosis Patients. J Clin Microbiol 2013; 51: 4204–4206.

59 Plongla R, Preece CL, Perry JD, Gilligan PH. Evaluation of RGM Medium for Isolation of Nontuberculous Mycobacteria from Respiratory Samples from Patients with Cystic Fibrosis in the United States. J Clin Microbiol 2017; 55: 1469–1477.

60 Zampoli M, Pillay K, Carrara H, Zar HJ, Morrow B. Microbiological yield from induced sputum compared to oropharyngeal swab in young children with cystic fibrosis. Journal of Cystic Fibrosis 2016; 15: 605–610.

61 Ahmed MI, Kulkarni H, Shajpal S, Patel D, Patel P, Claydon A et al. Early detection of non-tuberculous mycobacteria in children with cystic fibrosis using induced sputum at annual review. Pediatr Pulmonol 2019; 54: 257–263.

62 Flight WG, Bright-Thomas RJ, Tilston P, Mutton KJ, Guiver M, Morris J et al. Incidence and clinical impact of respiratory viruses in adults with cystic fibrosis. Thorax 2014; 69: 247–253.

63 NICE. Cystic fibrosis: diagnosis and management (NG78). 2017.

64 Cystic Fibrosis Trust. Standard of Care and Good Clinical Practice for the Physiotherapy Management of Cystic Fibrosis . London, 2020.

65 Gould FK, Freeman R, Hudson S, Magee J, Nelson D, Stafford R et al. Does storage of sputum specimens adversely affect culture results? J Clin Pathol 1996; 49: 684–686.

66 Pye A, Hill SL, Bharadwa P, Stockley RA. Effect of storage and postage on recovery and quantitation of bacteria in sputum samples. J Clin Pathol 2007; 61: 352–354.

67 Murray MP, Doherty CJ, Govan JRW, Hill AT. Do processing time and storage of sputum influence quantitative bacteriology in bronchiectasis? J Med Microbiol 2010; 59: 829–833.

68 Pitt T, Govan J. Pseudomonas cepacia in cystic fibrosis patients. PHLS Microbiol Digest 1993; 10: 69–72.

69 Wong K, Roberts MC, Owens L, Fife M, Smith AL. Selective media for the quantitation of bacteria in cystic fibrosis sputum. J Med Microbiol 1984; 17: 113–9.

70 Williams SG, Kauffman CA. Survival of Streptococcus pneumoniae in sputum from patients with pneumonia. J Clin Microbiol 1978; 7: 3–5.

71 Penn RL, Silberman R. Effects of overnight refrigeration on the microscopic evaluation of sputum. J Clin Microbiol 1984; 19: 161–3.

72 Traore I, Slosárek M. Survival of mycobacteria in sputum at different temperatures. Czech Med 1981; 4: 203–8.

73 Poonja A, Heirali A, Workentine M, Storey DG, Somayaji R, Rabin HR et al. Effect of freezing sputum on Pseudomonas aeruginosa population heterogeneity. Journal of Cystic Fibrosis 2017; 16: 353–357.

74 Cuthbertson L, Rogers GB, Walker AW, Oliver A, Hafiz T, Hoffman LR et al. Time between Collection and Storage Significantly Influences Bacterial Sequence Composition in Sputum Samples from Cystic Fibrosis Respiratory Infections. J Clin Microbiol 2014; 52: 3011–3016.

75 May JR, Delves DM. The survival of Haemophilus influenzae and pneumococci in specimens of sputum sent to the laboratory by post. J Clin Pathol 1964; 17: 254–256.

76 Hariri AR, Hempel HO, Kimberlin CL, Goodman NL. Effects of time lapse between sputum collection and culturing on isolation of clinically significant fungi. J Clin Microbiol 1982; 15: 425–8.

77 Paramasivan CN, Narayana ASL, Prabhakar R, Rajagopal MS, Somasundaram PR, Tripathy SP. Effect of storage of sputum specimens at room temperature on smear and culture results. Tubercle 1983; 64: 119–124.

78 Nelson A, de Soyza A, Bourke SJ, Perry JD, Cummings SP. Assessment of sample handling practices on microbial activity in sputum samples from patients with cystic fibrosis. Lett Appl Microbiol 2010; 51: 272–277.

79 Zhao J, Li J, Schloss PD, Kalikin LM, Raymond TA, Petrosino JF et al. Effect of Sample Storage Conditions on Culture-Independent Bacterial Community Measures in Cystic Fibrosis Sputum Specimens. J Clin Microbiol 2011; 49: 3717–3718.

80 Hoppe JE, Holzwarth I, Stern M. Postal Transport of Sputa from Cystic Fibrosis Patients Does Not Decrease the Microbiological Yield. Zentralblatt für Bakteriologie 1997; 286: 468–471.

81 Emerson J, Cochrane E, McNamara S, Kuypers J, Gibson RL, Campbell AP. Home Self-Collection of Nasal Swabs for Diagnosis of Acute Respiratory Virus Infections in Children With Cystic Fibrosis. J Pediatric Infect Dis Soc 2013; 2: 345–351.

82 Public Health England. Investigation of bronchoalveolar lavage, sputum and associated specimens. UK Standards for Microbiology Investigations. 2019; B 57.

83 Gilligan P, Kiska D, Appleman MD. Cumitech 43: cystic fibrosis microbiology. 2006.

84 Sharp S, Robinson A, Saubolle M, Santa Cruz M, Carroll K, Baselski V. Cumitech 7B Lower Respiratory Tract Infections. 2004.

85 UK Health Security Agency. Standards for microbiology investigations (UK SMI). 2021https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi (accessed 14 Feb2022).

86 Pye A, Stockley RA, Hill SL. Simple method for quantifying viable bacterial numbers in sputum. J Clin Pathol 1995; 48: 719–724.

87 Stokell JR, Khan A, Steck TR. Mechanical Homogenization Increases Bacterial Homogeneity in Sputum. J Clin Microbiol 2014; 52: 2340–2345.

88 Hammerschlag MR, Harding L, Macone A, Smith AL, Goldmann DA. Bacteriology of sputum in cystic fibrosis: evaluation of dithiothreitol as a mucolytic agent. J Clin Microbiol 1980; 11: 552–7.

89 Sadeghi E, Matlow A, MacLusky I, Karmali MA. Utility of gram stain in evaluation of sputa from patients with cystic fibrosis. J Clin Microbiol 1994; 32: 54–58.

90 Nair B, Stapp J, Stapp L, Bugni L, van Dalfsen J, Burns JL. Utility of Gram Staining for Evaluation of the Quality of Cystic Fibrosis Sputum Samples. J Clin Microbiol 2002; 40: 2791–2794.

91 Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006; 6: 570–581.

92 Ulukanligil M, Aslan G, Tasçi S. A comparative study on the different staining methods and number of specimens for the detection of acid fast bacilli. Mem Inst Oswaldo Cruz 2000; 95: 855–858.

93 Murray SJ. Optimisation of acid fast smears for the direct detection of mycobacteria in clinical samples. J Clin Pathol 2003; 56: 613–615.

94 Wright PW, Wallace RJ, Wright NW, Brown BA, Griffith DE. Sensitivity of Fluorochrome Microscopy for Detection of Mycobacterium tuberculosis versus Nontuberculous Mycobacteria. J Clin Microbiol 1998; 36: 1046–1049.

95 Doern G v, Brogden-Torres B. Optimum use of selective plated media in primary processing of respiratory tract specimens from patients with cystic fibrosis. J Clin Microbiol 1992; 30: 2740–2.

96 Laine L, Perry JD, Lee J, Oliver M, James AL, de La Foata C et al. A novel chromogenic medium for isolation of Pseudomonas aeruginosa from the sputa of cystic fibrosis patients. Journal of Cystic Fibrosis 2009; 8: 143–149.

97 Pugashetti BK, Metzger HM, Vadas L, Feingold DS. Phenotypic differences among clinically isolated mucoid Pseudomonas aeruginosa strains. J Clin Microbiol 1982; 16: 686–91.

98 Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid Pseudomonas aeruginosa and Burkholderia cepacia. Microbiol Rev 1996; 60: 539–574.

99 Fonseca K, MacDougall J, Pitt TL. Inhibition of Pseudomonas aeruginosa from cystic fibrosis by selective media. J Clin Pathol 1986; 39: 220–222.

100 Gilligan PH, Gage PA, Bradshaw LM, Schidlow D v, DeCicco BT. Isolation medium for the recovery of Pseudomonas cepacia from respiratory secretions of patients with cystic fibrosis. J Clin Microbiol 1985; 22: 5–8.

101 Tablan OC, Carson LA, Cusick LB, Bland LA, Martone WJ, Jarvis WR. Laboratory proficiency test results on use of selective media for isolating Pseudomonas cepacia from simulated sputum specimens of patients with cystic fibrosis. J Clin Microbiol 1987; 25: 485–487.

102 Carson LA, Tablan OC, Cusick LB, Jarvis WR, Favero MS, Bland LA. Comparative evaluation of selective media for isolation of Pseudomonas cepacia from cystic fibrosis patients and environmental sources. J Clin Microbiol 1988; 26: 2096–2100.

103 Henry D, Campbell M, McGimpsey C, Clarke A, Louden L, Burns JL et al. Comparison of Isolation Media for Recovery of Burkholderia cepacia Complex from Respiratory Secretions of Patients with Cystic Fibrosis. J Clin Microbiol 1999; 37: 1004–1007.

104 Wright RM, Moore JE, Shaw A, Dunbar K, Dodd M, Webb K et al. Improved cultural detection of Burkholderia cepacia from sputum in patients with cystic fibrosis. J Clin Pathol 2001; 54: 803–805.

105 Preece CL, Wichelhaus TA, Perry A, Jones AL, Cummings SP, Perry JD et al. Evaluation of Various Culture Media for Detection of Rapidly Growing Mycobacteria from Patients with Cystic Fibrosis. J Clin Microbiol 2016; 54: 1797–1803.

106 Mahenthiralingam E, Coenye T, Chung JW, Speert DP, Govan JRW, Taylor P et al. Diagnostically and Experimentally Useful Panel of Strains from the Burkholderia cepacia Complex. J Clin Microbiol 2000; 38: 910–913.

107 Naqvi S, Varadhan H, Givney R. Is prolonged incubation required for optimal recovery of Burkholderia cepacia complex in sputum from cystic fibrosis patients? Data versus dogma. Pathology 2020; 52: 366–369.

108 Martina P, Bettiol M, Vescina C, Montanaro P, Mannino MC, Prieto CI et al. Genetic Diversity of Burkholderia contaminans Isolates from Cystic Fibrosis Patients in Argentina. J Clin Microbiol 2013; 51: 339–344.

109 Savi D, Quattrucci S, Trancassini M, Dalmastri C, de Biase R v., Maggisano M et al. Impact of clonally-related Burkholderia contaminans strains in two patients attending an Italian cystic fibrosis centre: a case report. BMC Pulm Med 2019; 19: 164.

110 Sparham PD, Lobban DI, Speller DC. Isolation of Staphylococcus aureus from sputum in cystic fibrosis. J Clin Pathol 1978; 31: 913–918.

111 Sharp SE, Searcy C. Comparison of Mannitol Salt Agar and Blood Agar Plates for Identification and Susceptibility Testing of Staphylococcus aureus in Specimens from Cystic Fibrosis Patients. J Clin Microbiol 2006; 44: 4545–4546.

112 Flayhart D, Lema C, Borek A, Carroll KC. Comparison of the BBL CHROMagar Staph aureus Agar Medium to Conventional Media for Detection of Staphylococcus aureus in Respiratory Samples. J Clin Microbiol 2004; 42: 3566–3569.

113 D'Souza HA, Baron EJ. BBL CHROMagar Staph aureus Is Superior to Mannitol Salt for Detection of Staphylococcus aureus in Complex Mixed Infections. Am J Clin Pathol 2005; 123: 806–808.

114 Möller L v, van Alphen L, Grasselier H, Dankert J. N-acetyl-D-glucosamine medium improves recovery of Haemophilus influenzae from sputa of patients with cystic fibrosis. J Clin Microbiol 1993; 31: 1952–1954.

115 Smith A, Baker M. Cefsulodin chocolate blood agar: a selective medium for the recovery of Haemophilus influenzae from the respiratory secretions of patients with cystic fibrosis. J Med Microbiol 1997; 46: 883–5.

116 LiPuma JJ. The Changing Microbial Epidemiology in Cystic Fibrosis. Clin Microbiol Rev 2010; 23: 299–323.

117 Moore JE, Xu J, Millar BC, Courtney J, Elborn JS. Development of a Gram-negative selective agar (GNSA) for the detection of Gram-negative microflora in sputa in patients with cystic fibrosis. J Appl Microbiol 2003; 95: 160–166.

118 Denton M, Hall MJ, Todd NJ, Kerr KG, Littlewood JM. Improved isolation of Stenotrophomonas maltophilia from the sputa of patients with cystic fibrosis using a selective medium. Clinical Microbiology and Infection 2000; 6: 395–396.

119 Uffredi ML, Mangiapan G, Cadranel J, Kac G. Significance of Aspergillus fumigatus Isolation from Respiratory Specimens of Nongranulocytopenic Patients. European Journal of Clinical Microbiology & Infectious Diseases 2003; 22: 457–462.

120 Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases 2016; 62: e1–e50.

121 de Jong CCM, Slabbers L, Engel TGP, Yntema JB, van Westreenen M, Croughs PD et al. Clinical relevance of Scedosporium spp. and Exophiala dermatitidis in patients with cystic fibrosis: A nationwide study. Med Mycol 2020; 58: 859–866.

122 Tewkesbury DH, Looi E, Barry PJ, Edwards G, Green H, Smith M et al. Isolation of Exophiala dermatitidis is not associated with worse clinical outcomes during acute pulmonary exacerbations in cystic fibrosis. J Med Microbiol 2022; 71. doi:10.1099/jmm.0.001431.

123 Vyzantiadis T-AA, Johnson EM, Kibbler CC. From the patient to the clinical mycology laboratory: how can we optimise microscopy and culture methods for mould identification? J Clin Pathol 2012; 65: 475–483.

124 Horvath JA, Dummer S. The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. Am J Med 1996; 100: 171–178.

125 Bakare N, Rickerts V, Bargon J, Just-Nübling G. Prevalence of Aspergillus fumigatus and other fungal species in the sputum of adult patients with cystic fibrosis. Mycoses 2003; 46: 19–23.

126 Vergidis P, Moore CB, Novak-Frazer L, Rautemaa-Richardson R, Walker A, Denning DW et al. High-volume culture and quantitative real-time PCR for the detection of Aspergillus in sputum. Clinical Microbiology and Infection 2020; 26: 935–940.

127 Engel TGP, Tehupeiory-Kooreman M, Melchers WJG, Reijers MH, Merkus P, Verweij PE. Evaluation of a New Culture Protocol for Enhancing Fungal Detection Rates in Respiratory Samples of Cystic Fibrosis Patients. Journal of Fungi 2020; 6: 82.

128 Morris AJ, Byrne TC, Madden JF, Reller LB. Duration of incubation of fungal cultures. J Clin Microbiol 1996; 34: 1583–1585.

129 Danziger-Isakov LA, Worley S, Arrigain S, Aurora P, Ballmann M, Boyer D et al. Increased Mortality After Pulmonary Fungal Infection Within the First Year After Pediatric Lung Transplantation. The Journal of Heart and Lung Transplantation 2008; 27: 655–661.

130 Klasinc R, Riesenhuber M, Bacher A, Willinger B. Invasive Fungal Infection Caused by Exophiala dermatitidis in a Patient After Lung Transplantation: Case Report and Literature Review. Mycopathologia 2019; 184: 107–113.

131 Symoens F, Knoop C, Schrooyen M, Denis O, Estenne M, Nolard N et al. Disseminated Scedosporium apiospermum infection in a cystic fibrosis patient after double-lung transplantation. J Heart Lung Transplant 2006; 25: 603–7.

132 Martiniano SL, Sontag MK, Daley CL, Nick JA, Sagel SD. Clinical Significance of a First Positive Nontuberculous Mycobacteria Culture in Cystic Fibrosis. Ann Am Thorac Soc 2014; 11: 36–44.

133 Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F et al. An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases. Am J Respir Crit Care Med 2007; 175: 367–416.

134 Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF et al. British Thoracic Society Guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). BMJ Open Respir Res 2017; 4: e000242.

135 Bange F-C, Böttger EC. Improved decontamination method for recovering mycobacteria from patients with cystic fibrosis. Eur J Clin Microbiol Infect Dis 2002; 21: 546–8.

136 Jordan PW, Stanley T, Donnelly FM, Elborn JS, McClurg RB, Millar BC et al. Non-tuberculous mycobacterial infection in adult patients with cystic fibrosis: comparison of three decontamination methods. Br J Biomed Sci 2008; 65: 28–30.

137 Stephenson D, Perry A, Nelson A, Robb AE, Thomas MF, Bourke SJ et al. Decontamination Strategies Used for AFB Culture Significantly Reduce the Viability of Mycobacterium abscessus Complex in Sputum Samples from Patients with Cystic Fibrosis. Microorganisms 2021; 9: 1597.

138 Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-Analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without Solid Media, for Detection of Mycobacteria. J Clin Microbiol 2004; 42: 2321–2325.

139 Bange FC, Kirschner P, Böttger EC. Recovery of mycobacteria from patients with cystic fibrosis. J Clin Microbiol 1999; 37: 3761–3.

140 Ferroni A, Vu-Thien H, Lanotte P, le Bourgeois M, Sermet-Gaudelus I, Fauroux B et al. Value of the Chlorhexidine Decontamination Method for Recovery of Nontuberculous Mycobacteria from Sputum Samples of Patients with Cystic Fibrosis. J Clin Microbiol 2006; 44: 2237–2239.

141 Whittier S, Hopfer RL, Knowles MR, Gilligan PH. Improved recovery of mycobacteria from respiratory secretions of patients with cystic fibrosis. J Clin Microbiol 1993; 31: 861–4.

142 Buijtels PCAM, Petit PLC. Comparison of NaOH-N-acetyl cysteine and sulfuric acid decontamination methods for recovery of mycobacteria from clinical specimens. J Microbiol Methods 2005; 62: 83–88.

143 Ratnam S, Stead FA, Howes M. Simplified acetylcysteine-alkali digestion-decontamination procedure for isolation of mycobacteria from clinical specimens. J Clin Microbiol 1987; 25: 1428–1432.

144 Rothlauf M v, Brown GL, Blair EB. Isolation of mycobacteria from undecontaminated specimens with selective 7H10 medium. J Clin Microbiol 1981; 13: 76–79.

145 Preece CL, Perry A, Gray B, Kenna DT, Jones AL, Cummings SP et al. A novel culture medium for isolation of rapidly-growing mycobacteria from the sputum of patients with cystic fibrosis. Journal of Cystic Fibrosis 2016; 15: 186–191.

146 Vrignaud M, Patel P, Cantrell C, André E. Appropriateness of Utilization of NTM Elite agar for the Diagnosis of Nontuberculous Mycobacteria Outside Biosafety Level 3 Laboratories. Abstract #4705. . ASM Microbe. 2020. **https://virtualmeeting.ctimeetingtech.com/asm2020/attendee/eposter/poster/2452?s=a** (accessed 14 Feb 2022).

147 Stephenson D, Perry A, Appleby MR, Lee D, Davison J, Johnston A et al. An evaluation of methods for the isolation of nontuberculous mycobacteria from patients with cystic fibrosis, bronchiectasis and patients assessed for lung transplantation. BMC Pulm Med 2019; 19: 19.

148 Qvist T, Taylor-Robinson D, Waldmann E, Olesen HV, Hansen CR, Mathiesen IH et al. Comparing the harmful effects of nontuberculous mycobacteria and Gram negative bacteria on lung function in patients with cystic fibrosis. Journal of Cystic Fibrosis 2016; 15: 380–385.

149 Ravnholt C, Kolpen M, Skov M, Moser C, Katzenstein TL, Pressler T et al. The importance of early diagnosis of Mycobacterium abscessus complex in patients with cystic fibrosis. APMIS 2018; 126: 885–891.

150 Esther CR, Hoberman S, Fine J, Allen S, Culbreath K, Rodino K et al. Detection of Rapidly Growing Mycobacteria in Routine Cultures of Samples from Patients with Cystic Fibrosis. J Clin Microbiol 2011; 49: 1421–1425.

151 Public Health England. COVID-19: laboratory investigations and sample requirements for diagnosis. 2021 **https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/laboratory-investigations-and-sample-requirements-for-diagnosing-and-monitoring-wn-cov-infection** (accessed 14 Feb 2022).

152 Public Health England. Point of care tests for influenza and other respiratory viruses. Winter 2019 to 2020. 2019 **https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/846008/Point_of_care_tests_for_influenza_and_other_respiratory_viruses.pdf** (accessed 14 Feb 2022).

153 Department of Health & Social Care. Liverpool COVID-19 community testing pilot: interim evaluation report summary. 2021 **https://www.gov.uk/government/publications/liverpool-covid-19-community-testing-pilot-interim-evaluation-report-summary/liverpool-covid-19-community-testing-pilot-interim-evaluation-report-summary** (accessed 14 Feb 2022).

154 Pray IW, Ford L, Cole D, Lee C, Bigouette JP, Abedi GR et al. Performance of an Antigen-Based Test for Asymptomatic and Symptomatic SARS-CoV-2 Testing at Two University Campuses - Wisconsin, September-October 2020. MMWR Morb Mortal Wkly Rep 2021; 69: 1642–1647.

155 UK Health Security Agency. COVID-19: summary guidance for service providers on point of care tests for diagnosis and management. 2022.

156 APHL. Best Practices for Identification of Mycobacterium Species Using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. 2019 **https://www.aphl.org/aboutAPHL/publications/Documents/ID-2019Aug-MALDI-TOF-TB-Fact-Sheet.pdf** (accessed 14 Feb 2022).

157 Baillie S, Ireland K, Warwick S, Wareham D, Wilks M. Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry: rapid identification of bacteria isolated from patients with cystic fibrosis. Br J Biomed Sci 2013; 70: 144–148.

158 Marko DC, Saffert RT, Cunningham SA, Hyman J, Walsh J, Arbefeville S et al. Evaluation of the Bruker Biotyper and Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems for Identification of Nonfermenting Gram-Negative Bacilli Isolated from Cultures from Cystic Fibrosis Patients. J Clin Microbiol 2012; 50: 2034–2039.

159 Mulet M, Montaner M, Román D, Gomila M, Kittinger C, Zarfel G et al. Pseudomonas Species Diversity Along the Danube River Assessed by rpoD Gene Sequence and MALDI-TOF MS Analyses of Cultivated Strains. Front Microbiol 2020; 11. doi:10.3389/fmicb.2020.02114.

160 Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G. Performance of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Bacterial Strains Routinely Isolated in a Clinical Microbiology Laboratory. J Clin Microbiol 2010; 48: 1549–1554.

161 de Soyza A, Meachery G, Hester KLM, Nicholson A, Parry G, Tocewicz K et al. Lung transplantation for patients with cystic fibrosis and Burkholderia cepacia complex infection: a single-center experience. J Heart Lung Transplant 2010; 29: 1395–404.

162 Wong KSK, Dhaliwal S, Bilawka J, Srigley JA, Champagne S, Romney MG et al. Matrix-assisted laser desorption/ionization time-of-flight MS for the accurate identification of Burkholderia cepacia complex and Burkholderia gladioli in the clinical microbiology laboratory. J Med Microbiol 2020; 69: 1105–1113.

163 Murray S, Charbeneau J, Marshall BC, LiPuma JJ. Impact of Burkholderia Infection on Lung Transplantation in Cystic Fibrosis. Am J Respir Crit Care Med 2008; 178: 363–371.

164 Jones AM, Stanbridge TN, Isalska BJ, Dodd ME, Webb AK. Burkholderia gladioli:Recurrent Abscesses in a Patient with Cystic Fibrosis. Journal of Infection 2001; 42: 69–71.

165 Jones C, Webster G, Mullins AJ, Jenner M, Bull MJ, Dashti Y et al. Kill and cure: genomic phylogeny and bioactivity of Burkholderia gladioli bacteria capable of pathogenic and beneficial lifestyles. Microb Genom 2021; 7. doi:10.1099/mgen.0.000515.

166 McLeod C, Morris PS, Bauert PA, Kilburn CJ, Ward LM, Baird RW et al. Clinical Presentation and Medical Management of Melioidosis in Children: A 24-Year Prospective Study in the Northern Territory of Australia and Review of the Literature. Clinical Infectious Diseases 2015; 60: 21–26.

167 Hettiarachchi IT, Duckers J, Lau D, Dhillon R. The Brief Case: A Traveler's Tale-Burkholderia pseudomallei Infection in a Cystic Fibrosis Patient. J Clin Microbiol 2019; 57. doi:10.1128/JCM.00106-19.

168 Mercier-Darty M, Royer G, Lamy B, Charron C, Lemenand O, Gomart C et al. Comparative Whole-Genome Phylogeny of Animal, Environmental, and Human Strains Confirms the Genogroup Organization and Diversity of the Stenotrophomonas maltophilia Complex. Appl Environ Microbiol 2020; 86. doi:10.1128/AEM.02919-19.

169 Svensson-Stadler LA, Mihaylova SA, Moore ERB. Stenotrophomonas interspecies differentiation and identification by gyrB sequence analysis. FEMS Microbiol Lett 2012; 327: 15–24.

170 Degand N, Carbonnelle E, Dauphin B, Beretti J-L, le Bourgeois M, Sermet-Gaudelus I et al. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Nonfermenting Gram-Negative Bacilli Isolated from Cystic Fibrosis Patients. J Clin Microbiol 2008; 46: 3361–3367.

171 Coward A, Kenna DTD, Perry C, Martin K, Doumith M, Turton JF. Use of nrdA gene sequence clustering to estimate the prevalence of different Achromobacter species among Cystic Fibrosis patients in the UK. Journal of Cystic Fibrosis 2016; 15: 479–485.

172 Green HD, Bright-Thomas R, Kenna DT, Turton JF, Woodford N, Jones AM. Ralstonia infection in cystic fibrosis. Epidemiol Infect 2017; 145: 2864–2872.

173 Kenna DTD, Coward A, Perry C, Pike R, Schaefer U, Turton J et al. Investigation of a Pandoraea apista cluster common to adult and paediatric cystic fibrosis patients attending two hospitals in the same city. J Med Microbiol 2019; 68: 1081–1095.

174 Frost F, Nazareth D. Case Report: First report of Elizabethkingia miricola infection in a patient with cystic fibrosis. F1000Res 2018; 7: 440.

175 Kenna DTD, Fuller A, Martin K, Perry C, Pike R, Burns PJ et al. rpoB gene sequencing highlights the prevalence of an E. miricola cluster over other Elizabethkingia species among UK cystic fibrosis patients. Diagn Microbiol Infect Dis 2018; 90: 109–114.

176 Burnard D, Gore L, Henderson A, Ranasinghe A, Bergh H, Cottrell K et al. Comparative Genomics and Antimicrobial Resistance Profiling of Elizabethkingia Isolates Reveal Nosocomial Transmission and In Vitro Susceptibility to Fluoroquinolones, Tetracyclines, and Trimethoprim-Sulfamethoxazole. J Clin Microbiol 2020; 58. doi:10.1128/JCM.00730-20.

177 Papalia M, Figueroa-Espinosa R, Steffanowski C, Barberis C, Almuzara M, Barrios R et al. Expansion and improvement of MALDI-TOF MS databases for accurate identification of Achromobacter species. J Microbiol Methods 2020; 172: 105889.

178 Lin J-N, Teng S-H, Lai C-H, Yang C-H, Huang Y-H, Lin H-F et al. Comparison of the Vitek MS and Bruker Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems for Identification of Chryseobacterium Isolates from Clinical Specimens and Report of Uncommon Chryseobacterium Infections in Humans. J Clin Microbiol 2018; 56. doi:10.1128/JCM.00712-18.

179 Hördt A, López MG, Meier-Kolthoff JP, Schleuning M, Weinhold L-M, Tindall BJ et al. Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria. Front Microbiol 2020; 11. doi:10.3389/fmicb.2020.00468.

180 Gazolla Volpiano C, Hayashi Sant'Anna F, Ambrosini A, Brito Lisboa B, Kayser Vargas L, Passaglia LMP. Reclassification of Ochrobactrum lupini as a later heterotypic synonym of Ochrobactrum anthropi based on whole-genome sequence analysis. Int J Syst Evol Microbiol 2019; 69: 2312–2314.

181 Balada-Llasat JM, Kamboj K, Pancholi P. Identification of Mycobacteria from Solid and Liquid Media by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry in the Clinical Laboratory. J Clin Microbiol 2013; 51: 2875–2879.

182 Alcolea-Medina A, Fernandez MTC, Montiel N, García MPL, Sevilla CD, North N et al. An improved simple method for the identification of Mycobacteria by MALDI-TOF MS (Matrix-Assisted Laser Desorption- Ionization mass spectrometry). Sci Rep 2019; 9: 20216.

183 Alcaide F, Amlerová J, Bou G, Ceyssens PJ, Coll P, Corcoran D et al. How to: identify non-tuberculous Mycobacterium species using MALDI-TOF mass spectrometry. Clinical Microbiology and Infection 2018; 24: 599–603.

184 Bruker. MBT Mycobacteria RUO Module. **https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/microbial-identification/mbt-mycobacteria-ruo.html** (accessed 14 Feb 2022).

185 bioMérieux. VITEK MS Mass spectrometry microbial identification system. **https://www.biomerieux-diagnostics.com/vitekr-ms-0** (accessed 14 Feb 2022).

186 Perry A, Perry J. Personal communication. .

187 Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, Av-Gay Y et al. DNA-Based Diagnostic Approaches for Identification of Burkholderia cepacia Complex, Burkholderia vietnamiensis, Burkholderia multivorans,Burkholderia stabilis, and Burkholderia cepacia Genomovars I and III. J Clin Microbiol 2000; 38: 3165–3173.

188 Jin Y, Zhou J, Zhou J, Hu M, Zhang Q, Kong N et al. Genome-based classification of Burkholderia cepacia complex provides new insight into its taxonomic status. Biol Direct 2020; 15: 6.

189 Mullins A. Personal communication. Cardiff University . .

190 Spilker T, Baldwin A, Bumford A, Dowson CG, Mahenthiralingam E, LiPuma JJ. Expanded Multilocus Sequence Typing for Burkholderia Species. J Clin Microbiol 2009; 47: 2607–2610.

191 Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 2018; 3: 124.

192 Depoorter E, de Canck E, Peeters C, Wieme AD, Cnockaert M, Zlosnik JEA et al. Burkholderia cepacia Complex Taxon K: Where to Split? Front Microbiol 2020; 11. doi:10.3389/fmicb.2020.01594.

193 Payne GW, Vandamme P, Morgan SH, LiPuma JJ, Coenye T, Weightman AJ et al. Development of a recA Gene-Based Identification Approach for the Entire Burkholderia Genus. Appl Environ Microbiol 2005; 71: 3917–3927.

194 Koh W-J, Jeon K, Lee NY, Kim B-J, Kook Y-H, Lee S-H et al. Clinical Significance of Differentiation of Mycobacterium massiliense from Mycobacterium abscessus. Am J Respir Crit Care Med 2011; 183: 405–410.

195 Choi G-E, Shin SJ, Won C-J, Min K-N, Oh T, Hahn M-Y et al. Macrolide Treatment for Mycobacterium abscessus and Mycobacterium massiliense Infection and Inducible Resistance. Am J Respir Crit Care Med 2012; 186: 917–925.

196 Lipworth S, Hough N, Leach L, Morgan M, Jeffery K, Andersson M et al. Whole-Genome Sequencing for Predicting Clarithromycin Resistance in Mycobacterium abscessus. Antimicrob Agents Chemother 2019; 63. doi:10.1128/AAC.01204-18.

197 Huh HJ, Kim S-Y, Shim HJ, Kim DH, Yoo IY, Kang O-K et al. GenoType NTM-DR Performance Evaluation for Identification of Mycobacterium avium Complex and Mycobacterium abscessus and Determination of Clarithromycin and Amikacin Resistance. J Clin Microbiol 2019; 57. doi:10.1128/JCM.00516-19.

198 Devallois A, Goh KS, Rastogi N. Rapid identification of mycobacteria to species level by PCR-restriction fragment length polymorphism analysis of the hsp65 gene and proposition of an algorithm to differentiate 34 mycobacterial species. J Clin Microbiol 1997; 35: 2969–2973.

199 Telenti A, Marchesi F, Balz M, Bally F, Böttger EC, Bodmer T. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J Clin Microbiol 1993; 31: 175–8.

200 Kim K, Hong S-H, Kim B-J, Kim B-R, Lee S-Y, Kim G-N et al. Separation of Mycobacterium abscessus into subspecies or genotype level by direct application of peptide nucleic acid multi-probe- real-time PCR method into sputa samples. BMC Infect Dis 2015; 15: 325.

201 Ringuet H, Akoua-Koffi C, Honore S, Varnerot A, Vincent V, Berche P et al. hsp65 Sequencing for Identification of Rapidly Growing Mycobacteria. J Clin Microbiol 1999; 37: 852–857.

202 Macheras E, Roux A-L, Ripoll F, Sivadon-Tardy V, Gutierrez C, Gaillard J-L et al. Inaccuracy of Single-Target Sequencing for Discriminating Species of the Mycobacterium abscessus Group. J Clin Microbiol 2009; 47: 2596–2600.

203 Blauwendraat C, Dixon GLJ, Hartley JC, Foweraker J, Harris KA. The use of a two-gene sequencing approach to accurately distinguish between the species within the Mycobacterium abscessus complex and Mycobacterium chelonae. European Journal of Clinical Microbiology & Infectious Diseases 2012; 31: 1847–1853.

204 Harris KA, Kenna DTD, Blauwendraat C, Hartley JC, Turton JF, Aurora P et al. Molecular Fingerprinting of Mycobacterium abscessus Strains in a Cohort of Pediatric Cystic Fibrosis Patients. J Clin Microbiol 2012; 50: 1758–1761.

205 Doyle RM, Rubio M, Dixon G, Hartley J, Klein N, Coll P et al. Cross-transmission Is Not the Source of New Mycobacterium abscessus Infections in a Multicenter Cohort of Cystic Fibrosis Patients. Clinical Infectious Diseases 2020; 70: 1855–1864.

206 Macheras E, Konjek J, Roux A-L, Thiberge J-M, Bastian S, Leão SC et al. Multilocus sequence typing scheme for the Mycobacterium abscessus complex. Res Microbiol 2014; 165: 82–90.

207 Wuzinski M, Bak A, Petkau A, B. Demczuk W, Soualhine H, Sharma M. A multilocus sequence typing scheme for Mycobacterium abscessus complex (MAB-multilocus sequence typing) using whole-genome sequencing data. Int J Mycobacteriol 2019; 8: 273.

208 Al-Aloul M. Increased morbidity associated with chronic infection by an epidemic Pseudomonas aeruginosa strain in CF patients. Thorax 2004; 59: 334–336.

209 Salunkhe P, Smart CHM, Morgan JAW, Panagea S, Walshaw MJ, Hart CA et al. A Cystic Fibrosis Epidemic Strain of Pseudomonas aeruginosa Displays Enhanced Virulence and Antimicrobial Resistance. J Bacteriol 2005; 187: 4908–4920.

210 van Mansfeld R, de Been M, Paganelli F, Yang L, Bonten M, Willems R. Within-Host Evolution of the Dutch High-Prevalent Pseudomonas aeruginosa Clone ST406 during Chronic Colonization of a Patient with Cystic Fibrosis. PLoS One 2016; 11: e0158106.

211 Markussen T, Marvig RL, Gómez-Lozano M, Aanæs K, Burleigh AE, Høiby N et al. Environmental Heterogeneity Drives Within-Host Diversification and Evolution of Pseudomonas aeruginosa. mBio 2014; 5. doi:10.1128/mBio.01592-14.

212 Kevat A, Carzino R, Massie J, Harrison J, Griffiths AL. Elimination of Australian epidemic strain (AES1) pseudomonas aeruginosa in a pediatric cystic fibrosis center. Pediatr Pulmonol 2018; 53: 1498–1503.

213 Somayaji R, Lam JC, Surette MG, Waddell B, Rabin HR, Sibley CD et al. Long-term clinical outcomes of 'Prairie Epidemic Strain' Pseudomonas aeruginosa infection in adults with cystic fibrosis. Thorax 2017; 72: 333–339.

214 Ashish A, Shaw M, Winstanley C, Humphreys L, Walshaw MJ. Halting the spread of epidemic Pseudomonas aeruginosa in an adult cystic fibrosis centre: a prospective cohort study. JRSM Short Rep 2013; 4: 1–8.

215 Fothergill JL, Upton AL, Pitt TL, Hart CA, Winstanley C. Diagnostic multiplex PCR assay for the identification of the Liverpool, Midlands 1 and Manchester CF epidemic strains of Pseudomonas aeruginosa. Journal of Cystic Fibrosis 2008; 7: 258–261.

216 Fothergill JL, White J, Foweraker JE, Walshaw MJ, Ledson MJ, Mahenthiralingam E et al. Impact of Pseudomonas aeruginosa Genomic Instability on the Application of Typing Methods for Chronic Cystic Fibrosis Infections. J Clin Microbiol 2010; 48: 2053–2059.

217 Martin K, Baddal B, Mustafa N, Perry C, Underwood A, Constantidou C et al. Clusters of genetically similar isolates of Pseudomonas aeruginosa from multiple hospitals in the UK. J Med Microbiol 2013; 62: 988–1000.

218 Cystic Fibrosis Trust. Pseudomonas aeruginosa infection in people with cystic fibrosis. Suggestions for Prevention and Infection Control. London, 2004.

219 Goldberg JB, Ganesan S, Comstock AT, Zhao Y, Sajjan US. Cable Pili and the Associated 22 Kda Adhesin Contribute to Burkholderia Cenocepacia Persistence In Vivo. PLoS One 2011; 6: e22435.

220 Ganesan S, Sajjan US. Host Evasion by Burkholderia cenocepacia. Front Cell Infect Microbiol 2012; 1. doi:10.3389/fcimb.2011.00025.

221 Drevinek P, Mahenthiralingam E. Burkholderia cenocepacia in cystic fibrosis: epidemiology and molecular mechanisms of virulence. Clinical Microbiology and Infection 2010; 16: 821–830.

222 Roux D, Weatherholt M, Clark B, Gadjeva M, Renaud D, Scott D et al. Immune Recognition of the Epidemic Cystic Fibrosis Pathogen Burkholderia dolosa. Infect Immun 2017; 85. doi:10.1128/IAI.00765-16.

223 Turton JF, O'Brien E, Megson B, Kaufmann ME, Pitt TL. Strains of Burkholderia cenocepacia genomovar IIIA possessing the cblA gene that are distinct from ET12. Diagn Microbiol Infect Dis 2009; 64: 94–97.

224 LiPuma JJ, Spilker T, Gill LH, Campbell PW, Liu L, Mahenthiralingam E. Disproportionate distribution of Burkholderia cepacia complex species and transmissibility markers in cystic fibrosis. Am J Respir Crit Care Med 2001; 164: 92–6.

225 Baldwin A, Mahenthiralingam E, Thickett KM, Honeybourne D, Maiden MCJ, Govan JR et al. Multilocus Sequence Typing Scheme That Provides Both Species and Strain Differentiation for the Burkholderia cepacia Complex. J Clin Microbiol 2005; 43: 4665–4673.

226 Elizur A, Orscheln RC, Ferkol TW, Atkinson JJ, Dunne WM, Buller RS et al. Panton-Valentine Leukocidin-Positive Methicillin-Resistant Staphylococcus aureus Lung Infection in Patients With Cystic Fibrosis. Chest 2007; 131: 1718–1725.

227 Barry PJ, Isalska BJ, Jones AM. Panton-Valentine Leukocidin-positive Staphylococcus aureus: a potentially significant pathogen in cystic fibrosis. Paediatr Respir Rev 2014; 15: 22–25.

228 Goss CH, Muhlebach MS. Review: Staphylococcus aureus and MRSA in cystic fibrosis. Journal of Cystic Fibrosis 2011; 10: 298–306.

229 Garbacz K, Piechowicz L, Podkowik M, Mroczkowska A, Empel J, Bania J. Emergence and spread of worldwide Staphylococcus aureus clones among cystic fibrosis patients. Infect Drug Resist 2018; Volume 11: 247–255.

230 Rodrigues DCS, Lima DF, Cohen RWF, Marques EA, Leão RS. Molecular characterisation of methicillin-resistant Staphylococcus aureus from chronically colonised cystic fibrosis paediatric patients in Brazil. Epidemiol Infect 2020; 148: e149.

231 Bernardy EE, Petit RA, Raghuram V, Alexander AM, Read TD, Goldberg JB. Genotypic and Phenotypic Diversity of Staphylococcus aureus Isolates from Cystic Fibrosis Patient Lung Infections and Their Interactions with Pseudomonas aeruginosa. mBio 2020; 11. doi:10.1128/mBio.00735-20.

232 Zhao C, Liu Y, Zhao M, Liu Y, Yu Y, Chen H et al. Characterization of Community Acquired Staphylococcus aureus Associated with Skin and Soft Tissue Infection in Beijing: High Prevalence of PVL+ ST398. PLoS One 2012; 7: e38577.

233 de Hoog G, Guarro J, Gené J, Ahmed S, Al-Hatmi A, Figueras M et al. Atlas of Clinical Fungi. 4th ed. Hilversum, 2020.

234 Renner S, Nachbaur E, Jaksch P, Dehlink E. Update on Respiratory Fungal Infections in Cystic Fibrosis Lung Disease and after Lung Transplantation. Journal of Fungi 2020; 6: 381.

235 Baxter CG, Dunn G, Jones AM, Webb K, Gore R, Richardson MD et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. Journal of Allergy and Clinical Immunology 2013; 132: 560-566.e10.

236 Singh A, Sharma B, Mahto KK, Meis JF, Chowdhary A. High-Frequency Direct Detection of Triazole Resistance in Aspergillus fumigatus from Patients with Chronic Pulmonary Fungal Diseases in India. Journal of Fungi 2020; 6: 67.

237 Guegan H, Chevrier S, Belleguic C, Deneuville E, Robert-Gangneux F, Gangneux J-P. Performance of Molecular Approaches for Aspergillus Detection and Azole Resistance Surveillance in Cystic Fibrosis. Front Microbiol 2018; 9. doi:10.3389/fmicb.2018.00531.

238 Orsi CF, Bettua C, Pini P, Venturelli C, la Regina A, Morace G et al. Detection of Pneumocystis jirovecii and Aspergillus spp. DNa in bronchoalveolar lavage fluids by commercial real-time PCr assays: comparison with conventional diagnostic tests. New Microbiol 2015; 38: 75–84.

239 Bader O. MALDI-TOF-MS-based species identification and typing approaches in medical mycology. Proteomics 2013; 13: 788–799.

240 Wilkendorf LS, Bowles E, Buil JB, van der Lee HAL, Posteraro B, Sanguinetti M et al. Update on Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Identification of Filamentous Fungi. J Clin Microbiol 2020; 58. doi:10.1128/JCM.01263-20.

241 Lee TWR, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. Journal of Cystic Fibrosis 2003; 2: 29–34.

242 Saiman L, Siegel J, Cystic Fibrosis Foundation Consensus Conference on Infection Control Participants. Infection control recommendations for patients with cystic fibrosis: Microbiology, important pathogens, and infection control practices to prevent patient-to-patient transmission. Am J Infect Control 2003; 31: S1-62.

243 Zhou J, Garber E, Desai M, Saiman L. Compliance of Clinical Microbiology Laboratories in the United States with Current Recommendations for Processing Respiratory Tract Specimens from Patients with Cystic Fibrosis. J Clin Microbiol 2006; 44: 1547–1549.

244 Balke B, Schmoldt S, Häußler S, Suerbaum S, Heesemann J, Hogardt M. A German external quality survey of diagnostic microbiology of respiratory tract infections in patients with cystic fibrosis. Journal of Cystic Fibrosis 2008; 7: 7–14.

245 Hogardt M, Ulrich J, Riehn-Kopp H, Tümmler B. EuroCareCF Quality Assessment of Diagnostic Microbiology of Cystic Fibrosis Isolates. J Clin Microbiol 2009; 47: 3435–3438.

246 Caballero J de D, del Campo R, Tato M, Gómez G de la Pedrosa E, Cobo M, López-Causapé C et al. Microbiological diagnostic procedures for respiratory cystic fibrosis samples in Spain: towards standard of care practices. BMC Microbiol 2014; 14: 335.

247 Foweraker JE, Laughton CR, Brown DFJ, Bilton D. Phenotypic variability of Pseudomonas aeruginosa in sputa from patients with acute infective exacerbation of cystic fibrosis and its impact on the validity of antimicrobial susceptibility testing. Journal of Antimicrobial Chemotherapy 2005; 55: 921–927.

248 Nzula S, Vandamme P, Govan J. Influence of taxonomic status on the in vitro antimicrobial susceptibility of the Burkholderia cepacia complex. Journal of Antimicrobial Chemotherapy 2002; 50: 265–269.

249 Peeters E, Nelis HJ, Coenye T. In vitro activity of ceftazidime, ciprofloxacin, meropenem, minocycline, tobramycin and trimethoprim/sulfamethoxazole against planktonic and sessile Burkholderia cepacia complex bacteria. Journal of Antimicrobial Chemotherapy 2009; 64: 801–809.

250 Flamm RK, Shortridge D, Castanheira M, Sader HS, Pfaller MA. In Vitro Activity of Minocycline against U.S. Isolates of Acinetobacter baumannii-Acinetobacter calcoaceticus Species Complex, Stenotrophomonas maltophilia, and Burkholderia cepacia Complex: Results from the SENTRY Antimicrobial Surveillance Program, 2014 to 2018. Antimicrob Agents Chemother 2019; 63. doi:10.1128/AAC.01154-19.

251 Mazer DM, Young C, Kalikin LM, Spilker T, LiPuma JJ. In Vitro Activity of Ceftolozane-Tazobactam and Other Antimicrobial Agents against Burkholderia cepacia Complex and Burkholderia gladioli. Antimicrob Agents Chemother 2017; 61. doi:10.1128/AAC.00766-17.

252 van Dalem A, Herpol M, Echahidi F, Peeters C, Wybo I, de Wachter E et al. In Vitro Susceptibility of Burkholderia cepacia Complex Isolated from Cystic Fibrosis Patients to Ceftazidime-Avibactam and Ceftolozane-Tazobactam. Antimicrob Agents Chemother 2018; 62. doi:10.1128/AAC.00590-18.

253 Karlowsky JA, Hackel MA, Tsuji M, Yamano Y, Echols R, Sahm DF. In Vitro Activity of Cefiderocol, a Siderophore Cephalosporin, Against Gram-Negative Bacilli Isolated by Clinical Laboratories in North America and Europe in 2015-2016: SIDERO-WT-2015. Int J Antimicrob Agents 2019; 53: 456–466.

254 Massip C, Mathieu C, Gaudru C, Miaut V, Floch P, Oswald E et al. In vitro activity of seven ß-lactams including ceftolozane/tazobactam and ceftazidime/avibactam against Burkholderia cepacia complex, Burkholderia gladioli and other non-fermentative Gram-negative bacilli isolated from cystic fibrosis patients. Journal of Antimicrobial Chemotherapy 2019; 74: 525–528.

255 Grimwood K, Kidd TJ, Tweed M. Successful treatment of cepacia syndrome. Journal of Cystic Fibrosis 2009; 8: 291–293.

256 Morosini MI, García-Castillo M, Loza E, Pérez-Vázquez M, Baquero F, Cantón R. Breakpoints for Predicting Pseudomonas aeruginosa Susceptibility to Inhaled Tobramycin in Cystic Fibrosis Patients: Use of High-Range Etest Strips. J Clin Microbiol 2005; 43: 4480–4485.

257 Morlin GL, Hedges DL, Smith AL, Burns JL. Accuracy and cost of antibiotic susceptibility testing of mixed morphotypes of Pseudomonas aeruginosa. J Clin Microbiol 1994; 32: 1027–30.

258 Henwood CJ, Livermore D, James D, Warner M. Antimicrobial susceptibility of Pseudomonas aeruginosa: results of a UK survey and evaluation of the British Society for Antimicrobial Chemotherapy disc susceptibility test. Journal of Antimicrobial Chemotherapy 2001; 47: 789–799.

259 Burns JL, Saiman L, Whittier S, Larone D, Krzewinski J, Liu Z et al. Comparison of Agar Diffusion Methodologies for Antimicrobial Susceptibility Testing of Pseudomonas aeruginosa Isolates from Cystic Fibrosis Patients. J Clin Microbiol 2000; 38: 1818–1822.

260 Zebouh M, Thomas C, Honderlick P, Lemee L, Segonds C, Wallet F et al. Direct antimicrobial susceptibility testing method for analysis of sputum collected from patients with cystic fibrosis. Journal of Cystic Fibrosis 2008; 7: 238–243.

261 Perry JD, Laine L, Hughes S, Nicholson A, Galloway A, Gould FK. Recovery of antimicrobial-resistant Pseudomonas aeruginosa from sputa of cystic fibrosis patients by culture on selective media. Journal of Antimicrobial Chemotherapy 2008; 61: 1057–1061.

262 Moskowitz SM, Garber E, Chen Y, Clock SA, Tabibi S, Miller AK et al. Colistin susceptibility testing: evaluation of reliability for cystic fibrosis isolates of Pseudomonas aeruginosa and Stenotrophomonas maltophilia. Journal of Antimicrobial Chemotherapy 2010; 65: 1416–1423.

263 Burns JL, Saiman L, Whittier S, Krzewinski J, Liu Z, Larone D et al. Comparison of two commercial systems (Vitek and MicroScan-WalkAway) for antimicrobial susceptibility testing of Pseudomonas aeruginosa isolates from cystic fibrosis patients. Diagn Microbiol Infect Dis 2001; 39: 257–260.

264 Häussler S, Ziesing S, Rademacher G, Hoy L, Wei brodt H. Evaluation of the Merlin, Micronaut System for Automated Antimicrobial Susceptibility Testing of Pseudomonas aeruginosa and Burkholderia Species Isolated from Cystic Fibrosis Patients. European Journal of Clinical Microbiology & Infectious Diseases 2003; 22: 496–500.

265 Balke B, Hoy L, Weissbrodt H, Häussler S. Comparison of the Micronaut Merlin automated broth microtiter system with the standard agar dilution method for antimicrobial susceptibility testing of mucoid and nonmucoid Pseudomonas aeruginosa isolates from cystic fibrosis patients. European Journal of Clinical Microbiology & Infectious Diseases 2004; 23: 765–771.

266 Aaron SD, Ferris W, Ramotar K, Vandemheen K, Chan F, Saginur R. Single and Combination Antibiotic Susceptibilities of Planktonic, Adherent, and Biofilm-Grown Pseudomonas aeruginosa Isolates Cultured from Sputa of Adults with Cystic Fibrosis. J Clin Microbiol 2002; 40: 4172–4179.

267 Hill D, Rose B, Pajkos A, Robinson M, Bye P, Bell S et al. Antibiotic Susceptibilities of Pseudomonas aeruginosa Isolates Derived from Patients with Cystic Fibrosis under Aerobic, Anaerobic, and Biofilm Conditions. J Clin Microbiol 2005; 43: 5085–5090.

268 Dales L, Ferris W, Vandemheen K, Aaron SD. Combination antibiotic susceptibility of biofilm-grown Burkholderia cepacia and Pseudomonas aeruginosa isolated from patients with pulmonary exacerbations of cystic fibrosis. European Journal of Clinical Microbiology & Infectious Diseases 2009; 28: 1275–1279.

269 Moskowitz SM, Foster JM, Emerson JC, Gibson RL, Burns JL. Use of Pseudomonas biofilm susceptibilities to assign simulated antibiotic regimens for cystic fibrosis airway infection. Journal of Antimicrobial Chemotherapy 2005; 56: 879–886.

270 Foweraker JE, Laughton CR, Brown DF, Bilton D. Comparison of Methods To Test Antibiotic Combinations against Heterogeneous Populations of Multiresistant Pseudomonas aeruginosa from Patients with Acute Infective Exacerbations in Cystic Fibrosis. Antimicrob Agents Chemother 2009; 53: 4809–4815.

271 Kirchner S, Fothergill JL, Wright EA, James CE, Mowat E, Winstanley C. Use of Artificial Sputum Medium to Test Antibiotic Efficacy Against Pseudomonas aeruginosa in Conditions More Relevant to the Cystic Fibrosis Lung. Journal of Visualized Experiments 2012. doi:10.3791/3857.

272 Lozano C, López M, Rojo-Bezares B, Sáenz Y. Antimicrobial Susceptibility Testing in Pseudomonas aeruginosa Biofilms: One Step Closer to a Standardized Method. Antibiotics 2020; 9: 880.

273 Yau YCW, Ratjen F, Tullis E, Wilcox P, Freitag A, Chilvers M et al. Randomized controlled trial of biofilm antimicrobial susceptibility testing in cystic fibrosis patients. Journal of Cystic Fibrosis 2015; 14: 262–266.

274 Smith S, Waters V, Jahnke N, Ratjen F. Standard versus biofilm antimicrobial susceptibility testing to guide antibiotic therapy in cystic fibrosis. Cochrane Database of Systematic Reviews 2020; 2021. doi:10.1002/14651858.CD009528.pub5.

275 Smith AL, Fiel SB, Mayer-Hamblett N, Ramsey B, Burns JL. Susceptibility Testing of Pseudomonas aeruginosa Isolates and Clinical Response to Parenteral Antibiotic Administration. Chest 2003; 123: 1495–1502.

276 Hurley MN, Ariff AHA, Bertenshaw C, Bhatt J, Smyth AR. Results of antibiotic susceptibility testing do not influence clinical outcome in children with cystic fibrosis. Journal of Cystic Fibrosis 2012; 11: 288–292.

277 Cogen JD, Whitlock KB, Gibson RL, Hoffman LR, VanDevanter DR. The use of antimicrobial susceptibility testing in pediatric cystic fibrosis pulmonary exacerbations. Journal of Cystic Fibrosis 2019; 18: 851–856.

278 VanDevanter DR, Heltshe SL, Hilliard JB, Konstan MW. Pseudomonas aeruginosa antimicrobial susceptibility test (AST) results and pulmonary exacerbation treatment responses in cystic fibrosis. Journal of Cystic Fibrosis 2021; 20: 257–263.

279 Aaron SD, Vandemheen KL, Ferris W, Fergusson D, Tullis E, Haase D et al. Combination antibiotic susceptibility testing to treat exacerbations of cystic fibrosis associated with multiresistant bacteria: a randomised, double-blind, controlled clinical trial. The Lancet 2005; 366: 463–471.

280 Keays T, Ferris W, Vandemheen KL, Chan F, Yeung S-W, Mah T-F et al. A retrospective analysis of biofilm antibiotic susceptibility testing: A better predictor of clinical response in cystic fibrosis exacerbations. Journal of Cystic Fibrosis 2009; 8: 122–127.

281 Smith S, Ratjen F, Remmington T, Waters V. Combination antimicrobial susceptibility testing for acute exacerbations in chronic infection of Pseudomonas aeruginosa in cystic fibrosis. Cochrane Database of Systematic Reviews 2020; 2021. doi:10.1002/14651858.CD006961.pub5.

282 Haja Mydin H, Corris PA, Nicholson A, Perry JD, Meachery G, Marrs ECL et al. Targeted Antibiotic Prophylaxis for Lung Transplantation in Cystic Fibrosis Patients Colonised with Pseudomonas aeruginosa Using Multiple Combination Bactericidal Testing. J Transplant 2012; 2012: 1–5.

283 Etherington C, Hall M, Conway S, Peckham D, Denton M. Clinical impact of reducing routine susceptibility testing in chronic Pseudomonas aeruginosa infections in cystic fibrosis. Journal of Antimicrobial Chemotherapy 2007; 61: 425–427.

284 Ponce MC, Svendsen E, Steed L, Flume PA. Impact of a Reduction in Susceptibility Testing for Pseudomonas aeruginosa in a Cystic Fibrosis Program. Am J Respir Crit Care Med 2020; 201: 114–116.

285 Somayaji R, Parkins MD, Shah A, Martiniano SL, Tunney MM, Kahle JS et al. Antimicrobial susceptibility testing (AST) and associated clinical outcomes in individuals with cystic fibrosis: A systematic review. Journal of Cystic Fibrosis 2019; 18: 236–243.

286 Waters VJ, Kidd TJ, Canton R, Ekkelenkamp MB, Johansen HK, LiPuma JJ et al. Reconciling Antimicrobial Susceptibility Testing and Clinical Response in Antimicrobial Treatment of Chronic Cystic Fibrosis Lung Infections. Clinical Infectious Diseases 2019; 69: 1812–1816.

287 Wootton M, Davies L, Pitman K, Howe RA. Evaluation of susceptibility testing methods for Burkholderia cepacia complex: a comparison of broth microdilution, agar dilution, gradient strip and EUCAST disc diffusion. Clinical Microbiology and Infection 2021; 27: 788.e1-788.e4.

288 Caraher E, Reynolds G, Murphy P, McClean S, Callaghan M. Comparison of antibiotic susceptibility of Burkholderia cepacia complex organisms when grown planktonically or as biofilm in vitro. European Journal of Clinical Microbiology & Infectious Diseases 2007; 26: 213–216.

289 Nicodemo AC, Araujo MRE, Ruiz AS, Gales AC. In vitro susceptibility of Stenotrophomonas maltophilia isolates: comparison of disc diffusion, Etest and agar dilution methods. J Antimicrob Chemother 2004; 53: 604–8.

290 Andrews JM. BSAC standardized disc susceptibility testing method (version 8). Journal of Antimicrobial Chemotherapy 2009; 64: 454–489.

291 Heifets LB. Synergistic effect of rifampin, streptomycin, ethionamide, and ethambutol on Mycobacterium intracellulare. Am Rev Respir Dis 1982; 125: 43–8.

292 Hoffner SE, Svenson SB, Källenius G. Synergistic effects of antimycobacterial drag combinations onMycobacterium avium complex determined radiometrically in liquid medium. Eur J Clin Microbiol 1987; 6: 530–535.

293 Banks J, Jenkins PA. Combined versus single antituberculosis drugs on the in vitro sensitivity patterns of non-tuberculous mycobacteria. Thorax 1987; 42: 838–842.

294 van Ingen J, Totten SE, Helstrom NK, Heifets LB, Boeree MJ, Daley CL. In vitro synergy between clofazimine and amikacin in treatment of nontuberculous mycobacterial disease. Antimicrob Agents Chemother 2012; 56: 6324–7.

295 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, Nocardia spp, and other aerobic actinomyces. 3rd ed. Clinical and Laboratory Standards Institute, 2018.

296 van Ingen J, Egelund EF, Levin A, Totten SE, Boeree MJ, Mouton JW et al. The Pharmacokinetics and Pharmacodynamics of Pulmonary Mycobacterium avium Complex Disease Treatment. Am J Respir Crit Care Med 2012; 186: 559–565.

297 Daley CL, Iaccarino JM, Lange C, Cambau E, Wallace RJ, Andrejak C et al. Treatment of Nontuberculous Mycobacterial Pulmonary Disease: An Official ATS/ERS/ESCMID/IDSA Clinical Practice Guideline. Clinical Infectious Diseases 2020; 71: e1–e36.

298 Nash KA, Brown-Elliott BA, Wallace RJ. A Novel Gene, erm (41), Confers Inducible Macrolide Resistance to Clinical Isolates of Mycobacterium abscessus but Is Absent from Mycobacterium chelonae. Antimicrob Agents Chemother 2009; 53: 1367–1376.

299 Bastian S, Veziris N, Roux A-L, Brossier F, Gaillard J-L, Jarlier V et al. Assessment of Clarithromycin Susceptibility in Strains Belonging to the Mycobacterium abscessus Group by erm (41) and rrl Sequencing. Antimicrob Agents Chemother 2011; 55: 775–781.

300 Nikolayevskyy V, Maurer FP, Holicka Y, Taylor L, Liddy H, Kranzer K. Novel external quality assurance scheme for drug susceptibility testing of non-tuberculous mycobacteria: a multicentre pilot study. J Antimicrob Chemother 2019; 74: 1288–1294.

301 Denning D. Correlation between in-vitro susceptibility testing to itraconazole and in-vivo outcome of Aspergillus fumigatus infection. Journal of Antimicrobial Chemotherapy 1997; 40: 401–414.

302 Verweij PE, Mellado E, Melchers WJG. Multiple-Triazole–Resistant Aspergillosis. New England Journal of Medicine 2007; 356: 1481–1483.

303 Arendrup MC, Perkhofer S, Howard SJ, Garcia-Effron G, Vishukumar A, Perlin D et al. Establishing In Vitro-In Vivo Correlations for Aspergillus fumigatus: the Challenge of Azoles versus Echinocandins. Antimicrob Agents Chemother 2008; 52: 3504–3511.

304 Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC, Pasqualotto AC et al. Frequency and Evolution of Azole Resistance in Aspergillus fumigatus Associated with Treatment Failure1. Emerg Infect Dis 2009; 15: 1068–1076.

305 Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA et al. Treatment of Aspergillosis: Clinical Practice Guidelines of the Infectious Diseases Society of America. Clinical Infectious Diseases 2008; 46: 327–360.

306 Abdolrasouli A, Petrou MA, Park H, Rhodes JL, Rawson TM, Moore LSP et al. Surveillance for Azole-Resistant Aspergillus fumigatus in a Centralized Diagnostic Mycology Service, London, United Kingdom, 1998–2017. Front Microbiol 2018; 9. doi:10.3389/fmicb.2018.02234.

307 Risum M, Hare RK, Gertsen JB, Kristensen L, Johansen HK, Helweg-Larsen J et al. Azole-Resistant Aspergillus fumigatus Among Danish Cystic Fibrosis Patients: Increasing Prevalence and Dominance of TR34/L98H. Front Microbiol 2020; 11. doi:10.3389/fmicb.2020.01850.

308 Mello E, Posteraro B, Vella A, de Carolis E, Torelli R, D'Inzeo T et al. Susceptibility Testing of Common and Uncommon Aspergillus Species against Posaconazole and Other Mold-Active Antifungal Azoles Using the Sensititre Method. Antimicrob Agents Chemother 2017; 61. doi:10.1128/AAC.00168-17.

309 Ratnaraja NVDV, Davies AP, Atkins BL, Dhillon R, Mahida N, Moses S et al. Best practice standards for the delivery of NHS infection services in the United Kingdom. Clin Infect Pract 2021; 12: 100095.

310 Saiman L, Siegel JD, LiPuma JJ, Brown RF, Bryson EA, Chambers MJ et al. Infection Prevention and Control Guideline for Cystic Fibrosis: 2013 Update. Infect Control Hosp Epidemiol 2014; 35: s1–s67.

311 Conway S, Balfour-Lynn IM, de Rijcke K, Drevinek P, Foweraker J, Havermans T et al. European Cystic Fibrosis Society Standards of Care: Framework for the Cystic Fibrosis Centre. Journal of Cystic Fibrosis 2014; 13: S3–S22.

312 Flume PA, Mogayzel PJ, Robinson KA, Goss CH, Rosenblatt RL, Kuhn RJ et al. Cystic Fibrosis Pulmonary Guidelines. Am J Respir Crit Care Med 2009; 180: 802–808.

313 Castellani C, Duff AJA, Bell SC, Heijerman HGM, Munck A, Ratjen F et al. ECFS best practice guidelines: the 2018 revision. Journal of Cystic Fibrosis 2018; 17: 153–178.

314 Ademhan Tural D, Dogru Ersöz D, Emiralioglu N, Ozsezen B, Hazirolan G, Sunman B et al. Clinical characteristics of children with cystic fibrosis infected with unusual bacteria. Minerva Pediatrics 2021. doi:10.23736/S2724-5276.21.06189-2.

315 Rowbotham NJ, Smith S, Leighton PA, Rayner OC, Gathercole K, Elliott ZC et al. The top 10 research priorities in cystic fibrosis developed by a partnership between people with CF and healthcare providers. Thorax 2018; 73: 388–390.

316 Kidd TJ, Canton R, Ekkelenkamp M, Johansen HK, Gilligan P, LiPuma JJ et al. Defining antimicrobial resistance in cystic fibrosis. Journal of Cystic Fibrosis 2018; 17: 696–704.

317 Man WH, de Steenhuijsen Piters WAA, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol 2017; 15: 259–270.

318 de Koff EM, Groot KM de W, Bogaert D. Development of the respiratory tract microbiota in cystic fibrosis. Curr Opin Pulm Med 2016; 22: 623–628.

319 Rogers GB, Bruce KD, Hoffman LR. How can the cystic fibrosis respiratory microbiome influence our clinical decision-making? Curr Opin Pulm Med 2017; 23: 536–543.

320 Gilpin DF, Nixon KA, Bull M, McGrath SJ, Sherrard L, Rolain JM et al. Evidence of persistence of Prevotella spp. in the cystic fibrosis lung. J Med Microbiol 2017; 66: 825–832.

321 Sibley CD, Parkins MD, Rabin HR, Duan K, Norgaard JC, Surette MG. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. Proceedings of the National Academy of Sciences 2008; 105: 15070–15075.

322 Zhao J, Carmody LA, Kalikin LM, Li J, Petrosino JF, Schloss PD et al. Impact of Enhanced Staphylococcus DNA Extraction on Microbial Community Measures in Cystic Fibrosis Sputum. PLoS One 2012; 7: e33127.

323 Blainey PC, Milla CE, Cornfield DN, Quake SR. Quantitative Analysis of the Human Airway Microbial Ecology Reveals a Pervasive Signature for Cystic Fibrosis. Sci Transl Med 2012; 4. doi:10.1126/scitranslmed.3004458.

324 Flight WG, Smith A, Paisey C, Marchesi JR, Bull MJ, Norville PJ et al. Rapid Detection of Emerging Pathogens and Loss of Microbial Diversity Associated with Severe Lung Disease in Cystic Fibrosis. J Clin Microbiol 2015; 53: 2022–2029.

325 Goddard AF, Staudinger BJ, Dowd SE, Joshi-Datar A, Wolcott RD, Aitken ML et al. Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of upper-airway specimens can misrepresent lung microbiota. Proceedings of the National Academy of Sciences 2012; 109: 13769–13774.

326 de Vos D, Lim A, Pirnay JP, Struelens M, Vandenvelde C, Duinslaeger L et al. Direct detection and identification of Pseudomonas aeruginosa in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane lipoprotein genes, oprI and oprL. J Clin Microbiol 1997; 35: 1295–1299.

327 da Silva Filho LVF, Levi JE, Bento CNO, da Silva Ramos SRT, Rozov T. PCR identification of Pseudomonas aeruginosa and direct detection in clinical samples from cystic fibrosis patients. J Med Microbiol 1999; 48: 357–361.

328 da Silva Filho LVF, Tateno AF, Martins KM, Azzuz Chernishev AC, de Oliveira Garcia D, Haug M et al. The combination of PCR and serology increases the diagnosis of Pseudomonas aeruginosa colonization/infection in cystic fibrosis. Pediatr Pulmonol 2007; 42: 938–944.

329 Xu J, Moore JE, Murphy PG, Millar BC, Elborn JS. Early detection of Pseudomonas aeruginosa – comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). Ann Clin Microbiol Antimicrob 2004; 3: 21.

330 Blanchard AC, Rooney AM, Yau Y, Zhang Y, Stapleton PJ, Horton E et al. Early detection using qPCR of Pseudomonas aeruginosa infection in children with cystic fibrosis undergoing eradication treatment. Journal of Cystic Fibrosis 2018; 17: 723–728.

331 Boutin S, Weitnauer M, Hassel S, Graeber SY, Stahl M, Dittrich AS et al. One time quantitative PCR detection of Pseudomonas aeruginosa to discriminate intermittent from chronic infection in cystic fibrosis. Journal of Cystic Fibrosis 2018; 17: 348–355.

332 Whitby PW, Dick HL, Campbell PW, Tullis DE, Matlow A, Stull TL. Comparison of culture and PCR for detection of Burkholderia cepacia in sputum samples of patients with cystic fibrosis. J Clin Microbiol 1998; 36: 1642–5.

333 McDowell A, Mahenthiralingam E, Moore JE, Dunbar KEA, Webb AK, Dodd ME et al. PCR-Based Detection and Identification of Burkholderia cepacia Complex Pathogens in Sputum from Cystic Fibrosis Patients. J Clin Microbiol 2001; 39: 4247–4255.

334 Vandamme P, Dawyndt P. Classification and identification of the Burkholderia cepacia complex: Past, present and future. Syst Appl Microbiol 2011; 34: 87–95.

335 Brown AR, Govan JRW. Assessment of Fluorescent In Situ Hybridization and PCR-Based Methods for Rapid Identification of Burkholderia cepacia Complex Organisms Directly from Sputum Samples. J Clin Microbiol 2007; 45: 1920–1926.

336 Martinucci M, Roscetto E, Iula VD, Votsi A, Catania MR, de Gregorio E. Accurate identification of members of the Burkholderia cepacia complex in cystic fibrosis sputum. Lett Appl Microbiol 2016; 62: 221–229.

337 Kim S, Park EM, Kwon OJ, Lee JH, Ki C-S, Lee NY et al. Direct application of the PCR restriction analysis method for identifying NTM species in AFB smear-positive respiratory specimens. Int J Tuberc Lung Dis 2008; 12: 1344–6.

338 Franco-Alvarez de Luna F, Ruiz P, Gutiérrez J, Casal M. Evaluation of the GenoType Mycobacteria Direct assay for detection of Mycobacterium tuberculosis complex and four atypical mycobacterial species in clinical samples. J Clin Microbiol 2006; 44: 3025–7.

339 Seagar A-L, Prendergast C, Emmanuel FX, Rayner A, Thomson S, Laurenson IF. Evaluation of the GenoType Mycobacteria Direct assay for the simultaneous detection of the Mycobacterium tuberculosis complex and four atypical mycobacterial species in smear-positive respiratory specimens. J Med Microbiol 2008; 57: 605–611.

340 Perry MD, White PL, Ruddy M. Potential for Use of the Seegene Anyplex MTB/NTM Real-Time Detection Assay in a Regional Reference Laboratory. J Clin Microbiol 2014; 52: 1708–1710.

341 Kos R, Brinkman P, Neerincx AH, Paff T, Gerritsen MG, Lammers A et al. Targeted exhaled breath analysis for detection of Pseudomonas aeruginosa in cystic fibrosis patients. Journal of Cystic Fibrosis 2022; 21: e28–e34.

342 Davies JC, Alton E, Simbo A, Murphy R, Seth I, Williams K et al. Training dogs to differentiate Pseudomonas aeruginosa from other cystic fibrosis bacterial pathogens: not to be sniffed at? European Respiratory Journal 2019; 54: 1900970.

343 Daines C, VanDeVanter D, Khan U, Emerson J, Heltshe S, McNamara S et al. Serology as a diagnostic tool for predicting initialPseudomonas aeruginosa acquisition in childrenwith cystic fibrosis. Journal of Cystic Fibrosis 2014; 13: 542–549.

344 Taccetti G, Denton M, Hayes K, Drevinek P, Sermet-Gaudelus I, Bilton D et al. A critical review of definitions used to describe Pseudomonas aeruginosa microbiological status in patients with cystic fibrosis for application in clinical trials. Journal of Cystic Fibrosis 2020; 19: 52–67.

345 Wolter DJ, Emerson JC, McNamara S, Buccat AM, Qin X, Cochrane E et al. Staphylococcus aureus Small-Colony Variants Are Independently Associated With Worse Lung Disease in Children With Cystic Fibrosis. Clinical Infectious Diseases 2013; 57: 384–391.

346 Wolter DJ, Onchiri FM, Emerson J, Precit MR, Lee M, McNamara S et al. Prevalence and clinical associations of Staphylococcus aureus small-colony variant respiratory infection in children with cystic fibrosis (SCVSA): a multicentre, observational study. Lancet Respir Med 2019; 7: 1027–1038.

347 Kipp F, Kahl BC, Becker K, Baron EJ, Proctor RA, Peters G et al. Evaluation of Two Chromogenic Agar Media for Recovery and Identification of Staphylococcus aureus Small-Colony Variants. J Clin Microbiol 2005; 43: 1956–1959.

348 Lechtzin N, Mayer-Hamblett N, West NE, Allgood S, Wilhelm E, Khan U et al. Home Monitoring of Patients with Cystic Fibrosis to Identify and Treat Acute Pulmonary Exacerbations. eICE Study Results. Am J Respir Crit Care Med 2017; 196: 1144–1151.

349 Choyce J, Shaw KL, Sitch AJ, Mistry H, Whitehouse JL, Nash EF. A prospective pilot study of home monitoring in adults with cystic fibrosis (HOME-CF): protocol for a randomised controlled trial. BMC Pulm Med 2017; 17: 22.

350 Parks DH, Chuvochina M, Chaumeil P-A, Rinke C, Mussig AJ, Hugenholtz P. A complete domain-to-species taxonomy for Bacteria and Archaea. Nat Biotechnol 2020; 38: 1079–1086.

351 Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 2018; 9: 5114.

352 Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 2007; 57: 81–91.

353 Cunningham-Oakes E, Pointon T, Murphy B, Campbell-Lee S, Webster G, Connor TR et al. Genomics reveals the novel species placement of industrial contaminant isolates incorrectly identified as Burkholderia lata. Microb Genom 2021; 7. doi:10.1099/mgen.0.000564.

354 Mullins AJ, Mahenthiralingam E. The Hidden Genomic Diversity, Specialized Metabolite Capacity, and Revised Taxonomy of Burkholderia Sensu Lato. Front Microbiol 2021; 12. doi:10.3389/fmicb.2021.726847.

355 Mahenthiralingam E, Vandamme P, Campbell ME, Henry DA, Gravelle AM, Wong LTK et al. Infection with Burkholderia cepacia Complex Genomovars in Patients with Cystic Fibrosis: Virulent Transmissible Strains of Genomovar III Can Replace Burkholderia multivorans. Clinical Infectious Diseases 2001; 33: 1469–1475.

356 Zlosnik JEA, Zhou G, Brant R, Henry DA, Hird TJ, Mahenthiralingam E et al. Burkholderia Species Infections in Patients with Cystic Fibrosis in British Columbia, Canada. 30 Years' Experience. Ann Am Thorac Soc 2015; 12: 70–78.

357 Wallner A, King E, Ngonkeu ELM, Moulin L, Béna G. Genomic analyses of Burkholderia cenocepacia reveal multiple species with differential host-adaptation to plants and humans. BMC Genomics 2019; 20: 803.

358 Veschetti L, Sandri A, Patuzzo C, Melotti P, Malerba G, Lleo MM. Genomic characterization of Achromobacter species isolates from chronic and occasional lung infection in cystic fibrosis patients. Microb Genom 2021; 7. doi:10.1099/mgen.0.000606.

359 Gabrielaite M, Nielsen FC, Johansen HK, Marvig RL. Achromobacter spp. genetic adaptation in cystic fibrosis. Microb Genom 2021; 7. doi:10.1099/mgen.0.000582.

360 Weiser R, Green AE, Bull MJ, Cunningham-Oakes E, Jolley KA, Maiden MCJ et al. Not all Pseudomonas aeruginosa are equal: strains from industrial sources possess uniquely large multireplicon genomes. Microb Genom 2019; 5. doi:10.1099/mgen.0.000276.

361 Freschi L, Bertelli C, Jeukens J, Moore MP, Kukavica-Ibrulj I, Emond-Rheault J-G et al. Genomic characterisation of an international Pseudomonas aeruginosa reference panel indicates that the two major groups draw upon distinct mobile gene pools. FEMS Microbiol Lett 2018; 365. doi:10.1093/femsle/fny120.

362 Weisner AM, Chart H, Bush A, Davies JC, Pitt TL. Detection of antibodies to Pseudomonas aeruginosa in serum and oral fluid from patients with cystic fibrosis. J Med Microbiol 2007; 56: 670–674.

363 Maiden MCJ, van Rensburg MJJ, Bray JE, Earle SG, Ford SA, Jolley KA et al. MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol 2013; 11: 728–736.

364 Gabrielaite M, Bartell JA, Nørskov-Lauritsen N, Pressler T, Nielsen FC, Johansen HK et al. Transmission and Antibiotic Resistance of Achromobacter in Cystic Fibrosis. J Clin Microbiol 2021; 59. doi:10.1128/JCM.02911-20.

365 Jolley KA, Bliss CM, Bennett JS, Bratcher HB, Brehony C, Colles FM et al. Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. Microbiology (N Y) 2012; 158: 1005–1015.

366 Duckers J, Lesher B, Thorat T, Lucas E, McGarry LJ, Chandarana K et al. Real-World Outcomes of Ivacaftor Treatment in People with Cystic Fibrosis: A Systematic Review. J Clin Med 2021; 10: 1527.

367 Volkova N, Moy K, Evans J, Campbell D, Tian S, Simard C et al. Disease progression in patients with cystic fibrosis treated with ivacaftor: Data from national US and UK registries. Journal of Cystic Fibrosis 2020; 19: 68–79.

368 Yi B, Dalpke AH, Boutin S. Changes in the Cystic Fibrosis Airway Microbiome in Response to CFTR Modulator Therapy. Front Cell Infect Microbiol 2021; 11. doi:10.3389/fcimb.2021.548613.

369 Rogers GB, Taylor SL, Hoffman LR, Burr LD. The impact of CFTR modulator therapies on CF airway microbiology. Journal of Cystic Fibrosis 2020; 19: 359–364.

370 Reznikov LR, Abou Alaiwa MH, Dohrn CL, Gansemer ND, Diekema DJ, Stoltz DA et al. Antibacterial properties of the CFTR potentiator ivacaftor. Journal of Cystic Fibrosis 2014; 13: 515–519.

371 Schneider EK, Azad MAK, Han M-L, (Tony) Zhou Q, Wang J, Huang JX et al. An "Unlikely" Pair: The Antimicrobial Synergy of Polymyxin B in Combination with the Cystic Fibrosis Transmembrane Conductance Regulator Drugs KALYDECO and ORKAMBI. ACS Infect Dis 2016; 2: 478–488.

372 Heltshe SL, Mayer-Hamblett N, Burns JL, Khan U, Baines A, Ramsey BW et al. Pseudomonas aeruginosa in Cystic Fibrosis Patients With G551D-CFTR Treated With Ivacaftor. Clinical Infectious Diseases 2015; 60: 703–712.

373 Harris JK, Wagner BD, Zemanick ET, Robertson CE, Stevens MJ, Heltshe SL et al. Changes in Airway Microbiome and Inflammation with Ivacaftor Treatment in Patients with Cystic Fibrosis and the G551D Mutation. Ann Am Thorac Soc 2020; 17: 212–220.

374 Law N, Logan C, Yung G, Furr C-LL, Lehman SM, Morales S et al. Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant Pseudomonas aeruginosa infection in a cystic fibrosis patient. Infection 2019; 47: 665–668.

375 Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat Med 2019; 25: 730–733.

376 Lebeaux D, Merabishvili M, Caudron E, Lannoy D, van Simaey L, Duyvejonck H et al. A Case of Phage Therapy against Pandrug-Resistant Achromobacter xylosoxidans in a 12-Year-Old Lung-Transplanted Cystic Fibrosis Patient. Viruses 2021; 13: 60.

377 Bogdan C. Nitric oxide and the immune response. Nat Immunol 2001; 2: 907–916.

378 Keen C, Gustafsson P, Lindblad A, Wennergren G, Olin A-C. Low levels of exhaled nitric oxide are associated with impaired lung function in cystic fibrosis. Pediatr Pulmonol 2010; 45: 241–248.

379 Miller CC, Hergott CA, Rohan M, Arsenault-Mehta K, Döring G, Mehta S. Inhaled nitric oxide decreases the bacterial load in a rat model of Pseudomonas aeruginosa pneumonia. Journal of Cystic Fibrosis 2013; 12: 817–820.

380 Deppisch C, Herrmann G, Graepler-Mainka U, Wirtz H, Heyder S, Engel C et al. Gaseous nitric oxide to treat antibiotic resistant bacterial and fungal lung infections in patients with cystic fibrosis: a phase I clinical study. Infection 2016; 44: 513–520.

381 File TM, Goldberg L, Das A, Sweeney C, Saviski J, Gelone SP et al. Efficacy and Safety of Intravenous-to-oral Lefamulin, a Pleuromutilin Antibiotic, for the Treatment of Community-acquired Bacterial Pneumonia: The Phase III Lefamulin Evaluation Against Pneumonia (LEAP 1) Trial. Clinical Infectious Diseases 2019; 69: 1856–1867.

382 Garcia BA, McDaniel MS, Loughran AJ, Johns JD, Narayanaswamy V, Fernandez Petty C et al. Poly (acetyl, arginyl) glucosamine disrupts Pseudomonas aeruginosa biofilms and enhances bacterial clearance in a rat lung infection model. Microbiology (N Y) 2022; 168. doi:10.1099/mic.0.001121.

383 Langendonk RF, Neill DR, Fothergill JL. The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for Current Resistance-Breaking Therapies. Front Cell Infect Microbiol 2021; 11. doi:10.3389/fcimb.2021.665759.

384 Rowbotham NJ, Palser SC, Smith SJ, Smyth AR. Infection prevention and control in cystic fibrosis: a systematic review of interventions. Expert Rev Respir Med 2019; 13: 425–434.

385 Health Protection Scotland, Unzurrunzaga GR-A. Cystic Fibrosis Literature Review: Infection Prevention and Control for patients with Cystic Fibrosis. 2018 **https://hpspubsrepo.blob.core.windows.net/hps-website/nss/2679/documents/1_lr-cystic-fibrosis.pdf** (accessed 15 Feb 2022).

386 UK National Health Service. (HTM 03-01) Specialised ventilation for healthcare buildings. 2021 **https://www.england.nhs.uk/publication/specialised-ventilation-for-healthcare-buildings/** (accessed 15 Feb 2022).

387 Fehlberg LCC, et al. In vitro susceptibility of Burkholderia cepacia complex isolates: Comparison of disk diffusion, Etest®, agar dilution, and broth microdilution methods. Diagn Microbiol Infect Dis. 2016; 86(4): 422–427.

388 Welp AL and Bomberger JM. Bacterial community interactions during chronic respiratory disease. Front Cell Infect. Microbiolol. 2020; 10: 213. doi: 10.3389/fcimb.2020.00213.

389 Mac Aogain M, Narayana JK, Tiew PY et al. Integrative microbiomics in bronchiectasis exacerbations. Nature Medicine. 2021; 27: 688–689.

390 Inkster T, Peters C, Seagar AL, Holden MTG, Laurenson IF. Investigation of two cases of Mycobacterium chelonae infection in haemato-oncology patients using whole-genome sequencing and a potential link to the hospital water supply. J Hosp Infect. 2021; 114: 111–116. doi: 10.1016/j.jhin.2021.04.028.

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